

CLASPs stabilize the pre-catastrophe intermediate state between microtubule growth and shrinkage

Elizabeth Lawrence, Saptarshi Chatterjee, and Marija Zanic

Corresponding Author(s): Marija Zanic, Vanderbilt University

Review Timeline:

Submission Date:	2021-07-06
Editorial Decision:	2021-08-23
Revision Received:	2022-12-03
Editorial Decision:	2023-01-19
Revision Received:	2023-04-11

Monitoring Editor: Samara Reck-Peterson

Scientific Editor: Lucia Morgado-Palacin

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. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: <https://doi.org/10.1083/jcb.202107027>

August 23, 2021

Re: JCB manuscript #202107027

Dr. Marija Zanic
Vanderbilt University
Cell and Developmental Biology
465 21st Avenue South
4120 MRB3 Biosciences Building
Nashville, TN 37232

Dear Dr. Zanic,

Thank you for submitting your manuscript entitled "CLASP is a nucleotide-dependent microtubule depolymerase". Your manuscript has been assessed by expert reviewers, whose comments are appended below. Although the reviewers express potential interest in this work, significant concerns unfortunately preclude publication of the current version of the manuscript in JCB.

As you will see, reviewers #1 and #3 feel enthusiastic about the study and recognize the data is of high quality. However, all three reviewers note major mechanistic caveats that would need to be addressed with new experimental data to actually determine the relevance of the work and pinpoint the distinct function of CLASP from XMAP215. In particular, they request further investigation of the nucleotide-dependent microtubule depolymerase activity of CLASP, including to examine the required concentrations of microtubule ends, GTP, and free soluble tubulin needed to modulate the depolymerizing activity of CLASP (rev #1 paragraph 5 & rev #2 paragraphs 3, 5 & rev #3 p1). In addition, whether hydrolysis of GTP to GDP is required for CLASP-dependent microtubule depolymerization and whether CLASP depolymerization is enhanced or inhibited with a transition state analog should need to be tested (rev #1 paragraph 3, 4 & rev #3 p2). We agree with reviewers that these are all needed and reasonable requests, and every effort should be made to address them with new data. We also hope that you will be able to address each of the reviewers' other issues as well.

Please let us know if you are able to address the major issues outlined above and wish to submit a revised manuscript to JCB. Note that a substantial amount of additional experimental data likely would be needed to satisfactorily address the concerns of the reviewers. Given the breadth of the mechanistic extension requested, it may be necessary to extend your manuscript to a full Research Article. In the interest of time, we would encourage you to consider sending us a revision plan so we can give you feedback on the suitability of your proposed path for addressing the mechanistic shortcoming during revision. As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing and shelter in place measures that limit spread of COVID-19 also pose challenges to scientific researchers. Lab closures especially are preventing scientists from conducting experiments to further their research. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors once your lab has reopened to decide on an appropriate time frame for resubmission. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

If you choose to revise and resubmit your manuscript, please also attend to the following editorial points. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:

Text limits: Character count for a Report is < 20,000; a full Research Article is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: A Report may include up to 5 main text figures; a full Research Article may have up to 10 main text figures. To avoid delays in production, figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, <https://jcb.rupress.org/site/misc/ifora.xhtml>. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Reports may have up to 3 supplemental figures; a full Research Article may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

If you choose to resubmit, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

Regardless of how you choose to proceed, we hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised. You can contact the journal office with any questions, cellbio@rockefeller.edu.

Thank you for thinking of JCB as an appropriate place to publish your work.

Sincerely,

Samara Reck-Peterson, PhD
Monitoring Editor
Journal of Cell Biology

Lucia Morgado-Palacin, PhD
Scientific Editor
Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

In the manuscript by Lawrence and Zanic entitled "CLASP is a nucleotide-dependent microtubule depolymerase" the authors use live microtubule assays of purified components in conjunction with TIRF microscopy to demonstrate that the TOG domain possessing protein CLASP robustly disassembles GMP-CPP stabilized microtubules from both ends in the presence of excess GTP. This surprising observation contrasted with ch-TOG which was capable of depolymerizing GMP-CPP microtubules and did not appreciably improve when the microtubules were supplemented with GTP. I find the data to be of high quality. The findings are worthy of publication and essential to understand the interplay of +TIP regulators of microtubule dynamics. This study provides more foundation to understand the regulation of microtubule dynamics by TOG domain proteins.

The principal puzzling aspect of this study concerns the lattice nucleotide state of GMP-CPP microtubules in the presence of other nucleotides. Presumably the GTP and GDP can exchange with GMP-CPP as has been shown for the terminal dimers of the plus end (Mitchison 1994). But the data in this paper point toward more widely distributed exchange, possibly within the lattice and possibly at the minus end (at least for GTP). This leads to some confusion (at least in my mind) as to what is going on mechanistically that serves as the core explanation for CLASP's depolymerizing activity in GTP and GDP.

The authors state that GTP hydrolysis is not necessary for depolymerization. Superficially this appears to contradict the observation that CLASP cannot depolymerize microtubules composed of GMP-CPP tubulin (which is widely thought to mimic GTP). Does, for example, GDP exchange with GMP-CPP in the lattice? Does GTP exchange with GMP-CPP and then become hydrolyzed? If this is occurring it would contradict the conclusion that GTP hydrolysis is not necessary.

The data appear to support the conclusion that the presence of GDP in the microtubule lattice may be required for CLASP to depolymerize microtubules robustly. It may be that when GTP exchanges for GMP-CPP, it is then hydrolyzed. This would be more compatible with the widely accepted idea that GMP-CPP mimics the GTP-bound state. I believe this might be testable by assessing GTP-exchanged microtubules on a PEI-cellulose plate and scoring the mobility of the nucleotides released from the tubulin. Washed GMP-CPP microtubules that have been incubated in GTP or GDP could be submitted to lithium chloride chromatography on PEI cellulose. This could be performed on simple PEI-cellulose plates. GDP is easily distinguished from GTP and GMP-CPP by UV light. If GTP, once exchanged for GMP-CPP, is hydrolyzed in the tubulin lattice it would contradict the idea that GTP hydrolysis is not necessary for depolymerization and clarify this interesting result. The fascinating observation that minus-end depolymerization can only be effected with GTP might, in turn, be explained by enhanced efficiency of exchange for GTP versus GDP for GMP-CPP at the minus end. This would not be distinguishable by chromatography but a test of GDP versus GTP would serve as a good first step toward quantifying how much GTP or GDP exchanges on MTs of a defined length and concentration. A defined length would enable the authors to calculate the concentration of end-bound tubulin dimers versus lattice-bound.

Finally, the observation that cooperativity is required for robust depolymerization suggests that 200nM CLASP may be insufficient for depolymerization at the minus end for a variety of reasons (ranging from reduced GDP exchange at the minus end to other, more complicated, structural constraints). A concentration series of CLASP tested against a consistent concentration of microtubule ends would be enlightening to understand the differences in the nucleotide requirement for plus and minus end depolymerization by CLASP. Regardless, a more thorough discussion of lattice occupancy of nucleotide is well within the expertise of the Zanic lab and would be welcomed by this reader.

Reviewer #2 (Comments to the Authors (Required)):

Regulation of microtubule dynamic polymerization is critical for cell division and development. The polymerization of microtubules is regulated by a variety of conserved classes of microtubule regulatory proteins. These regulators have unique functional roles in modulating the dynamics of microtubule polymerization and depolymerization. The XMAP215 and CLASPs represent unique and conserved microtubule regulators which utilize TOG domains to modulate the dynamics of microtubules by binding soluble tubulin with their TOG domains. XMAP215 proteins are microtubule polymerases, while CLASPs inhibit catastrophe and promote microtubule rescues, which are reversals of microtubule depolymerization back to polymerizing states. Early studies of XMAP215 demonstrate that in the absence of soluble tubulin, these proteins would utilize their TOG domains to promote a potent microtubule depolymerase for microtubules assembled with the non-hydrolyzable analog GMPCP. This microtubule depolymerase activity was shown to be biochemical reversal of their native microtubule polymerase function due to their high affinity to bind tubulins and displace them from microtubule ends.

In the study by Lawrence and Zanic, the authors explore the latter experiment to compare the activity of CLASP to that of XMAP215. The authors show that the MT depolymerase activity is GTP and GDP nucleotide dependent and little microtubule depolymerase activity is observed in the absence of nucleotide. They further demonstrate that in the presence of GTP CLASP forms a depolymerase at both ends, while in the presence of GDP it depolymerizes only microtubule plus ends and not minus ends. The authors further show that CLASP localization to microtubule plus ends is dramatically stabilized in the absence of GTP but not in its absence. This study suggests that the unique CLASP nucleotide dependent microtubule depolymerase activity relates to its functional activity as a microtubule catastrophe inhibitor and promoter of microtubule rescue, and contributes to its uniqueness as microtubule regulator from the XMAP215 family.

This study addresses a relevant and interesting topic. However, there are serious and major concerns about the experiments that puts into question the value of the studies presented. The fundamental concern about the experiments relates to lack of studies of regulating the CLASP microtubule depolymerase and its relationship to the native function. Studies for the role of tubulin in reversing the microtubule depolymerase to the native microtubule rescue function is completely absent. CLASP and XMAP215 proteins are present in vivo at low concentrations and thus in the physiological setting the high concentration of soluble tubulin (5-10 micromolar) plays a critical role in driving the direction of the biochemical equilibrium towards the facilitation of microtubule polymerization. In the case of XMAP215 it was clearly shown by Brouhard et al 2008, that XMAP215 microtubule depolymerase can easily be reversed by adding a stoichiometric concentration of soluble tubulin (100 nanomolar). Much higher soluble tubulin concentration and the presence of GTP causes XMAP215 to revert to being a microtubule polymerase.

The nature of the nucleotide dependence in the microtubule depolymerase reaction is interesting but lacking any context without the experiment of adding soluble tubulin and studying its impact on the microtubule depolymerase. The relationship of the biochemical microtubule rescue and catastrophe inhibition to the nucleotide dependent microtubule depolymerase is remains unknown and is completely undefined. The authors spend much time exploring this biochemical detail without performing this crucial experiment to relate their newly identified activity to the microtubule rescue function of CLASPs. In every experiment presented in this manuscript, the authors could have studied how the microtubule depolymerase can be influenced by soluble tubulin to explore role of the soluble tubulin in the nucleotide dependence depolymerase, localization at microtubule ends, and its impact on unique regions of the protein. At this point these experiments remain interesting experiments that fully lack any functional context.

In its current form, I believe the manuscript does not add much mechanistic insight to the field and presents a very minor detail that may or may not represent relevant advance to understanding the mechanisms of CLASP proteins as regulators of microtubule rescue. The lack of relationship between the native functions of CLASPs to soluble tubulin leaves a major gap in explaining this biochemical finding and its impact on differentiating CLASP from XMAP215. The GTP dependence for the CLASP microtubule depolymerase activity may tubulin as the target, however it has not been demonstrated and nucleotide could be potentially bound by CLASP itself? It is not even clear what concentration of GTP modulates the reaction and if a higher or lower concentration has an effect on the CLASP microtubule depolymerase. I suggest the authors perform titrations of soluble tubulin and nucleotide to explore its impact on this nucleotide dependent microtubule depolymerase. The authors should explore how the concentration of nucleotide (0-1 nM) or the concentration of soluble tubulin (0-1 micromolar) may tune the experiments presented in Figure 1, Figure 3 and Figure 5.

Reviewer #3 (Comments to the Authors (Required)):

This manuscript from Lawrence and Zanic investigates the microtubule tip regulator, CLASP, which has an array of TOG domains. Prior work from multiple labs investigating CLASP family activity using microtubule dynamics reconstitution assays found that CLASP suppresses catastrophe and promotes rescue and that TOG2 was the prime mediator of this activity. These prior reconstitution assays were conducted in the presence of free, unpolymerized tubulin. In the current submission, Lawrence and Zanic analyzed CLASP activity in vitro using GMPCPP-stabilized microtubules in the absence of free tubulin. While

CLASP1 did not affect the stability of these GMPCPP-microtubules, the addition of either GTP or GDP (which is expected to exchange for GMPCPP at the exposed microtubule ends) led to CLASP1-dependent depolymerization. While the microtubule polymerase chTOG also showed this depolymerization activity, chTOG's activity was nucleotide-independent, suggesting that CLASP uses a distinct depolymerization mechanisms that is nucleotide-sensitive. The rate of CLASP-dependent microtubule depolymerization is fastest at the microtubule plus end as compared to the rate of depolymerization at the minus end, and the rate of plus end depolymerization is fastest in the presence of GDP, rather than GTP. In contrast, minus end depolymerization, albeit slower than plus end depolymerization, occurs faster in the presence of GTP rather than GDP. Lawrence and Zanic go on to show that CLASP paralogs (CLASP2 alpha and gamma) can also depolymerize microtubules in the absence of tubulin, and that a CLASP2alpha TOG2 domain construct that includes flanking linker regions is sufficient for this activity. Microtubule co-localization assays show that CLASP1 as well as the CLASP2alpha TOG2 construct preferentially localize to MT ends in the GMPCPP state, but the addition of GTP or GDP leads to the loss of CLASP's preferential localization to microtubule ends. The authors suggest that CLASP dissociates with GTP or GDP-bound tubulin at the microtubule ends.

Overall, the manuscript presents unique observations of CLASP activity on microtubule ends. The experimental work is solid, well written, and the findings will be of interest to the field of cytoskeletal dynamics. My primary concerns are centered on the way the findings are caged and presented, as well as some key, critical experiments the authors are poised to perform that would help lead insight into the mechanism behind the unique observations.

1. It would strengthen the manuscript to present - in the introduction, and as part of the discussion - what an experiment, conducted in the absence of free tubulin, can inform about a reaction coordinate that occurs biologically in the presence of free tubulin. Specifically, the manuscript's observations indicate that a nucleotide-dependent state/conformation of the tubulin or lattice at microtubule ends is recognized by CLASP, potentially in a transition state. Normally, if free tubulin is present, the reaction proceeds in a forward direction, leading to rescue or anti-catastrophe behavior. However, if the reaction is conducted in the absence of free tubulin, the reaction leads to depolymerization. Introducing the general concept of what the experiment informs (insight into a structural transition state) will help the readership see value in the work and best be able to interpret the findings, rather than think that the manuscript is investigating a process unique to in vitro conditions. These is effort from the authors to convey this, but it should be strengthened to best promote the impact of the work.

2. Figures 1 and 2 investigate CLASP-dependent depolymerization activity on GMPCPP microtubules when either GTP or GDP is added. Because depolymerization occurs in the presence of GTP and GDP, the authors state that no nucleotide hydrolysis is required. Since depolymerization occurs in the presence of GDP, the gamma phosphate is not required. But that doesn't mean that the GTP provided is not hydrolyzed. Indeed, the authors discuss that CLASP may function as a GAP. To better investigate whether hydrolysis of GTP to GDP is required for CLASP-dependent microtubule depolymerization, or if CLASP depolymerization is enhance or inhibited with a transition state analog, the authors should investigate how transition state analogs (GTPgammaS, GDP + high phosphate, or GDP-AlFx or GDP-BeFx) affect CLASP-dependent depolymerization.

3. In Figures 1 and 2, the authors measure the "microtubule depolymerization rate". In Figure 3, they polarity label microtubules and measure the microtubule depolymerization rates for the plus and minus ends separately. These collective measurements don't seem to match. For example, in the presence of GDP, the plus end depolymerizes at an average rate of 11.2 nm/sec. The minus end depolymerizes at a rate of 0.21 nm/sec. This would total a "microtubule depolymerization rate" of 11.4 nm/sec. However, the authors present a microtubule depolymerization rate of 6 nm/sec (half the expected value). Are the authors intending to present the average depolymerization rate per end of a given microtubule, or the rate that the total microtubule depolymerizes at (the sum of the polymerization rates at both ends). The discrepancy also exists for the GTP data (9.2 + 3.6 does not equal 6.7).

Minor points:

4. The authors presented average values for the depolymerization rates except for the data in Figure 4C. These rates should be included (at least in the figure caption).

5. Figure 4C: the authors should measure statistical significance between the 200 nM CLASP2alpha condition and the 200 nM TOG2 condition. This would inform whether or not the eGFP-L-TOG2-S construct is sufficient for the full depolymerization activity observed with the FL molecule.

6. Figure 4D: It would be beneficial to readers to report the values from the Hill fit, as the authors did in Figure 1D (concentration for half max depolymerization, and Hill coefficient).

7. For Movie 2, in the control (left panel, no nucleotide added) - there appears to be microtubule annealing that is not observed in the conditions with nucleotide added. The authors might want to note this in the caption so that the general readership is not confused by the events.

Rebuttal for Lawrence et al. "CLASPs stabilize an intermediate state between microtubule growth and shrinkage to regulate microtubule dynamics"

We are very grateful to the reviewers for their incredibly insightful and valuable critique of our original manuscript. Based on the reviewers' comments, we have performed a significant revision and expansion of our original submission. We now demonstrate a direct link between CLASP's ability to depolymerize stabilized microtubules and its physiological role as a regulator of microtubule dynamics. Our results show that CLASP specifically stabilizes a nucleotide-dependent intermediate state of the microtubule end as it transitions from growth to shrinkage. This mechanism explains CLASP's anti-catastrophe and pro-rescue activities in cells.

As pointed out by Reviewer 2, CLASPs function in the presence of cytosolic tubulin in cells. To assess the specific role of soluble tubulin in CLASP's mechanism, we investigated CLASP's activity in the presence of 0-8 μ M soluble tubulin (new Figure S4). Notably, we observed instances where CLASP simultaneously depolymerized the stabilized microtubule seeds and promoted continuous growth of microtubule extensions at tubulin concentrations far exceeding the CLASP concentration (i.e. 8 μ M soluble tubulin and 200 nM CLASP1). These experiments revealed that, unlike XMAP215, the switch between CLASP's depolymerase activity and anti-catastrophe activity does not operate through binding to soluble tubulin.

The question of what determines whether CLASP acts as a microtubule depolymerase vs. growth stabilizer led us to undertake a comprehensive new investigation of CLASP's activity on microtubule substrates with different stabilities (new Figure 5). Following microtubule growth, we induced microtubule destabilization by tubulin dilution, in the presence or absence of CLASP and nucleotides. Strikingly, while tubulin removal from solution triggers rapid microtubule catastrophe with GTP alone, we found that CLASP freezes the microtubules in a pre-catastrophe state in the presence of GTP. Furthermore, we discovered that CLASP drives all microtubule substrates into the same slowly-depolymerizing state in the presence of GTP and the absence of tubulin, regardless of their inherent stability. We interpret this state as the intermediate state between growth and shrinkage. Thus, stabilizing the intermediate state underlies CLASP's ability to prevent microtubule catastrophe, as well as promote rescue of dynamic microtubules.

In addition to this new line of investigation, we also pursued a range of experiments inspired by reviewers' comments, as detailed below. These include titration studies of CLASP and nucleotides (GTP and GDP) on polarity-marked microtubules to distinguish plus and minus ends, as requested by the reviewers (new Figure 3). This analysis revealed that microtubule plus ends are more sensitive to both CLASP and nucleotides than minus ends. This finding is consistent with a previous report that the intermediate state between growth and shrinkage differs significantly at plus versus minus ends (Tran et al. JCB 1997). Furthermore, we used nucleotide analogues to probe the role of nucleotide hydrolysis, which led us to conclude that a post-hydrolysis tubulin nucleotide state is required for CLASP's depolymerase activity (new Figure S3). We further strengthened our findings that CLASP operates through a microtubule-end specific mechanism (as opposed to lattice) through a computational modeling approach (new Figure S2).

Taken together, we think that our revised manuscript presents significant new insights into the molecular mechanisms by which CLASPs regulate microtubule dynamics.

Reviewer #1 (Comments to the Authors (Required)):

In the manuscript by Lawrence and Zanic entitled "CLASP is a nucleotide-dependent microtubule depolymerase" the authors use live microtubule assays of purified components in conjunction with TIRF microscopy to demonstrate that the TOG domain possessing protein CLASP robustly disassembles GMP-CPP stabilized microtubules from both ends in the presence of excess GTP. This surprising observation contrasted with ch-TOG which was capable of depolymerizing GMP-CPP microtubules and did not appreciably improve when the microtubules were supplemented with GTP. I find the data to be of high quality. The findings are worthy of publication and essential to understand the interplay of +TIP regulators of microtubule dynamics. This study provides more foundation to understand the regulation of microtubule dynamics by TOG domain proteins.

The principal puzzling aspect of this study concerns the lattice nucleotide state of GMP-CPP microtubules in the presence of other nucleotides. Presumably the GTP and GDP can exchange with GMP-CPP as has been shown for the terminal dimers of the plus end (Mitchison 1994). But the data in this paper point toward more widely distributed exchange, possibly within the lattice and possibly at the minus end (at least for GTP). This leads to some confusion (at least in my mind) as to what is going on mechanistically that serves as the core explanation for CLASP's

depolymerizing activity in GTP and GDP.

The authors state that GTP hydrolysis is not necessary for depolymerization. Superficially this appears to contradict the observation that CLASP cannot depolymerize microtubules composed of GMP-CPP tubulin (which is widely thought to mimic GTP). Does, for example, GDP exchange with GMP-CPP in the lattice? Does GTP exchange with GMP-CPP and then become hydrolyzed? If this is occurring it would contradict the conclusion that GTP hydrolysis is not necessary.

The data appear to support the conclusion that the presence of GDP in the microtubule lattice may be required for CLASP to depolymerize microtubules robustly. It may be that when GTP exchanges for GMP-CPP, it is then hydrolyzed. This would be more compatible with the widely accepted idea that GMP-CPP mimics the GTP-bound state. I believe this might be testable by assessing GTP-exchanged microtubules on a PEI-cellulose plate and scoring the mobility of the nucleotides released from the tubulin. Washed GMP-CPP microtubules that have been incubated in GTP or GDP could be submitted to lithium chloride chromatography on PEI cellulose. This could be performed on simple PEI-cellulose plates. GDP is easily distinguished from GTP and GMP-CPP by UV light. If GTP, once exchanged for GMP-CPP, is hydrolyzed in the tubulin lattice it would contradict the idea that GTP hydrolysis is not necessary for depolymerization and clarify this interesting result.

The fascinating observation that minus-end depolymerization can only be effected with GTP might, in turn, be explained by enhanced efficiency of exchange for GTP versus GDP for GMP-CPP at the minus end. This would not be distinguishable by chromatography but a test of GDP versus GTP would serve as a good first step toward quantifying how much GTP or GDP exchanges on MTs of a defined length and concentration. A defined length would enable the authors to calculate the concentration of end-bound tubulin dimers versus lattice-bound.

Finally, the observation that cooperativity is required for robust depolymerization suggests that 200nM CLASP may be insufficient for depolymerization at the minus end for a variety of reasons (ranging from reduced GDP exchange at the minus end to other, more complicated, structural constraints). A concentration series of CLASP tested against a consistent concentration of microtubule ends would be enlightening to understand the differences in the nucleotide requirement for plus and minus end depolymerization by CLASP. Regardless, a more thorough discussion of lattice occupancy of nucleotide is well within the expertise of the Zanich lab and would be welcomed by this reader.

We agree with the Reviewer that the question of whether CLASP mediates nucleotide exchange throughout the microtubule lattice or specifically acts at microtubule ends is important. To that end, we first attempted the suggested TLC experiments. Unfortunately, the low overall concentration of nucleotides within microtubule polymers in our assays (micromolar range for the lattice, and nanomolar for the ends), combined with the low sensitivity of TLC with UV detection, did not allow us to distinguish the end vs. lattice nucleotide exchange by this method. We thus decided to pursue another line of investigation to address the potential lattice nucleotide exchange mechanism. We developed a computational model simulating nucleotide exchange at either ends alone, or at both ends and the lattice (new Figure S2). Our results demonstrated that the observed minus-end depolymerization cannot be explained through an overall lattice-nucleotide exchange mechanism. Specifically, we found that lattice nucleotide exchange would change the microtubule depolymerization profile from a uniform, linear depolymerization (as observed in experiments) to an acceleration of depolymerization over time, as more lattice-exchanged sites become exposed. In other words, significant destabilization through lattice nucleotide exchange would result in the GMPCPP-stabilized lattice depolymerizing from the ends through catastrophe-like events. Two additional lines of evidence argue against the lattice-based mechanism. Our single-molecule analysis revealed that CLASP1 preferentially binds to microtubule ends and that CLASP's end-binding preference is sensitive to nucleotides in solution (Figure 4). Furthermore, the microtubule depolymerization rates in the presence of CLASP are similar regardless of the nucleotide composition of the microtubule lattice substrates, which on their own have vastly different depolymerization rates (new Figure 5, Figure 1). We thus conclude that it is the state of the microtubule end, rather than the microtubule lattice, that defines microtubule stability in the presence of CLASP.

To determine the nucleotide-dependence of CLASP's activity, we have now investigated CLASP's effects on stabilized microtubules in the presence of GTPyS (an analogue of the post-hydrolysis GDP-Pi state) in addition to GTP, GDP, GMPCPP and no nucleotide (new Figure S3). We found that CLASP depolymerizes microtubules in the presence of GTP, GTPyS and GDP, but not with GMPCPP. Taken together, we conclude that the mechanism employed by CLASP depends on a post-hydrolysis nucleotide state at the microtubule end. Given that CLASP can depolymerize stabilized microtubules with GDP, we conclude that the energy of GTP hydrolysis is not strictly required for CLASP's activity. Furthermore, the finding that CLASP stabilizes GTP-grown microtubules in the presence of GMPCPP even in the absence of soluble tubulin, whereas GMPCPP alone in solution does not (new Figure 5),

suggests that CLASP facilitates the nucleotide exchange at the microtubule end. In this way, CLASP helps return the microtubule end from a polymerization-incompetent pre-catastrophe intermediate to a polymerization-competent (GTP-like) microtubule end, even without tubulin in solution.

Finally, to further elucidate potential differences between microtubule plus and minus ends, we have now performed full titration of CLASP1, as well as GTP and GDP with polarity-marked microtubules (new Figure 3). We found that plus ends are more sensitive to both CLASP and nucleotides. We note that an earlier study reported that GTP nucleotide exchange also occurs at minus ends, albeit at a much slower rate than at plus ends (Tran et al. JCB 1997). The overall sensitivity of plus ends was lower with GDP as compared to GTP. Notably, CLASP1 did not depolymerize minus ends at any of the GDP concentrations tested. Our results suggest that the nucleotide exchange is less efficient at minus ends, even in the presence of CLASP.

Reviewer #2 (Comments to the Authors (Required)):

Regulation of microtubule dynamic polymerization is critical for cell division and development. The polymerization of microtubules is regulated by a variety of conserved classes of microtubule regulatory proteins. These regulators have unique functional roles in modulating the dynamics of microtubule polymerization and depolymerization. The XMAP215 and CLASPs represent unique and conserved microtubule regulators which utilize TOG domains to modulate the dynamics of microtubules by binding soluble tubulin with their TOG domains. XMAP215 proteins are microtubule polymerases, while CLASPs inhibit catastrophe and promote microtubule rescues, which are reversals of microtubule depolymerization back to polymerizing states. Early studies of XMAP215 demonstrate that in the absence of soluble tubulin, these proteins would utilize their TOG domains to promote a potent microtubule depolymerase for microtubules assembled with the non-hydrolyzable analog GMCPP. This microtubule depolymerase activity was shown to be biochemical reversal of their native microtubule polymerase function due to their high affinity to bind tubulins and displace them from microtubule ends.

In the study by Lawrence and Zanic, the authors explore the latter experiment to compare the activity of CLASP to that of XMAP215. The authors show that the MT depolymerase activity is GTP and GDP nucleotide dependent and little microtubule depolymerase activity is observed in the absence of nucleotide. They further demonstrate that in the presence of GTP CLASP forms a depolymerase at both ends, while in the presence of GDP it depolymerizes only microtubule plus ends and not minus ends. The authors further show that CLASP localization to microtubule plus ends is dramatically stabilized in the absence of GTP but not in its absence. This study suggests that the unique CLASP nucleotide dependent microtubule depolymerase activity relates to its functional activity as a microtubule catastrophe inhibitor and promoter of microtubule rescue, and contributes to its uniqueness as microtubule regulator from the XMAP215 family.

This study addresses a relevant and interesting topic. However, there are serious and major concerns about the experiments that puts into question the value of the studies presented. The fundamental concern about the experiments relates to lack of studies of regulating the CLASP microtubule depolymerase and its relationship to the native function. Studies for the role of tubulin in reversing the microtubule depolymerase to the native microtubule rescue function is completely absent. CLASP and XMAP215 proteins are present in vivo at low concentrations and thus in the physiological setting the high concentration of soluble tubulin (5-10 micromolar) plays a critical role in driving the direction of the biochemical equilibrium towards the facilitation of microtubule polymerization. In the case of XMAP215 it was clearly shown by Brouhard et al 2008, that XMAP215 microtubule depolymerase can easily be reversed by adding a stoichiometric concentration of soluble tubulin (100 nanomolar). Much higher soluble tubulin concentration and the presence of GTP causes XMAP215 to revert to being a microtubule polymerase.

The nature of the nucleotide dependence in the microtubule depolymerase reaction is interesting but lacking any context without the experiment of adding soluble tubulin and studying its impact on the microtubule depolymerase. The relationship of the biochemical microtubule rescue and catastrophe inhibition to the nucleotide dependent microtubule depolymerase is remains unknown and is completely undefined. The authors spend much time exploring this biochemical detail without performing this crucial experiment to relate their newly identified activity to the microtubule rescue function of CLASPs. In every experiment presented in this manuscript, the authors could have studied how the microtubule depolymerase can be influenced by soluble tubulin to explore role of the soluble tubulin in the nucleotide dependence depolymerase, localization at microtubule ends, and its impact on unique regions of the protein. At this point these experiments remain interesting experiments that fully lack any functional context.

In its current form, I believe the manuscript does not add much mechanistic insight to the field and presents a very minor detail that may or may not represent relevant advance to understanding the mechanisms of CLASP proteins as regulators of microtubule rescue. The lack of relationship between the native functions of CLASPs to soluble tubulin leaves a major gap in explaining this biochemical finding and its impact on differentiating CLASP from XMAP215. The GTP dependence for the CLASP microtubule depolymerase activity may tubulin as the target, however it has not been demonstrated and nucleotide could be potentially bound by CLASP itself? It is not even clear what concentration of GTP modulates the reaction and if a higher or lower concentration has an effect on the CLASP microtubule depolymerase. I suggest the authors perform titrations of soluble tubulin and nucleotide to explore its impact on this nucleotide dependent microtubule depolymerase. The authors should explore how the concentration of nucleotide (0-1 nM) or the concentration of soluble tubulin (0-1 micromolar) may tune the experiments presented in Figure 1, Figure 3 and Figure 5.

We appreciate the reviewer's concern regarding the omission of soluble tubulin in our original manuscript. To address this important issue, we performed a titration of soluble tubulin from 0 μ M to 8 μ M in the presence of 200 nM CLASP1 and 1 mM GTP (new Figure S4). Our results showed a concentration-dependent decrease in the rate of microtubule depolymerization. At tubulin concentrations higher than 6 μ M microtubule depolymerization was inhibited and microtubules grew extensions that did not undergo catastrophe or shrinkage during the time course of the experiment. Therefore, CLASP1 activity switches from depolymerizing, when the tubulin concentration is below the critical concentration for templated nucleation, to stabilizing when the tubulin concentration is above the critical concentration. Notably, we observed instances where CLASP simultaneously depolymerized the stabilized microtubule seeds and promoted continuous growth of microtubule extensions at tubulin concentrations far exceeding the CLASP concentration (i.e. 8 μ M soluble tubulin and 200 nM CLASP1, new Figure S4C). Since CLASP depolymerized microtubules even at very high tubulin concentrations, and its depolymerase activity was abolished only once the tubulin concentration exceeded the critical concentration for growth, we conclude that CLASP's mechanism does not operate through the interaction with soluble tubulin. This is in contrast to XMAP215, whose depolymerase to polymerase activity is switched by soluble tubulin. Rather, CLASP must operate by recognizing a specific configuration of the microtubule end.

To determine how CLASP's depolymerase activity relates to its anti-catastrophe activity, we performed a comprehensive new investigation of CLASP's effects on microtubule substrates with different stabilities (new Figure 5). To study the transition to microtubule catastrophe, following the phase of microtubule growth, we induced microtubule depolymerization by dilution of soluble tubulin. As expected, dynamic microtubules underwent catastrophe and rapid depolymerization upon tubulin dilution in the presence of GTP. In contrast, in the presence of CLASP1 and GTP microtubules did not undergo catastrophe, but remained in a prolonged slowly-depolymerizing state. We found that CLASP drove all investigated microtubule substrates into the same slowly-depolymerizing state in the presence of GTP. We interpret this state as the intermediate state between microtubule growth and shrinkage. Notably, CLASP with GMPCPP (but not GMPCPP alone) completely stabilized microtubule ends on all investigated substrates. We conclude that CLASP facilitates nucleotide exchange at the microtubule end, allowing the growth-incompetent pre-catastrophe state to return to a GTP-like end, competent for growth. By acting on an intermediate state between growth and shrinkage, CLASP thus both suppresses microtubule catastrophe and promotes microtubule rescue.

Per the Reviewer's request, we have also performed a full titration of both GTP and GDP nucleotides, which provided additional insight into the nucleotide sensitivity of the observed CLASP activity (new Figure 3, please also see the response to Reviewer 1).

Reviewer #3 (Comments to the Authors (Required)):

This manuscript from Lawrence and Zanic investigates the microtubule tip regulator, CLASP, which has an array of TOG domains. Prior work from multiple labs investigating CLASP family activity using microtubule dynamics reconstitution assays found that CLASP suppresses catastrophe and promotes rescue and that TOG2 was the prime mediator of this activity. These prior reconstitution assays were conducted in the presence of free, unpolymerized tubulin. In the current submission, Lawrence and Zanic analyzed CLASP activity in vitro using GMPCPP-stabilized microtubules in the absence of free tubulin. While CLASP1 did not affect the stability of these GMPCPP-microtubules, the addition of either GTP or GDP (which is expected to exchange for GMPCPP at the exposed microtubule ends) led to CLASP1-dependent depolymerization. While the microtubule polymerase chTOG also showed this depolymerization activity, chTOG's activity was nucleotide-independent, suggesting that CLASP uses a distinct

depolymerization mechanisms that is nucleotide-sensitive. The rate of CLASP-dependent microtubule depolymerization is fastest at the microtubule plus end as compared to the rate of depolymerization at the minus end, and the rate of plus end depolymerization is fastest in the presence of GDP, rather than GTP. In contrast, minus end depolymerization, albeit slower than plus end depolymerization, occurs faster in the presence of GTP rather than GDP. Lawrence and Zanic go on to show that CLASP paralogs (CLASP2 alpha and gamma) can also depolymerize microtubules in the absence of tubulin, and that a CLASP2alpha TOG2 domain construct that includes flanking linker regions is sufficient for this activity. Microtubule co-localization assays show that CLASP1 as well as the CLASP2alpha TOG2 construct preferentially localize to MT ends in the GMPCPP state, but the addition of GTP or GDP leads to the loss of CLASP's preferential localization to microtubule ends. The authors suggest that CLASP dissociates with GTP or GDP-bound tubulin at the microtubule ends.

Overall, the manuscript presents unique observations of CLASP activity on microtubule ends. The experimental work is solid, well written, and the findings will be of interest to the field of cytoskeletal dynamics. My primary concerns are centered on the way the findings are caged and presented, as well as some key, critical experiments the authors are poised to perform that would help lead insight into the mechanism behind the unique observations.

1. It would strengthen the manuscript to present - in the introduction, and as part of the discussion - what an experiment, conducted in the absence of free tubulin, can inform about a reaction coordinate that occurs biologically in the presence of free tubulin. Specifically, the manuscript's observations indicate that a nucleotide-dependent state/conformation of the tubulin or lattice at microtubule ends is recognized by CLASP, potentially in a transition state. Normally, if free tubulin is present, the reaction proceeds in a forward direction, leading to rescue or anti-catastrophe behavior. However, if the reaction is conducted in the absence of free tubulin, the reaction leads to depolymerization. Introducing the general concept of what the experiment informs (insight into a structural transition state) will help the readership see value in the work and best be able to interpret the findings, rather than think that the manuscript is investigating a process unique to in vitro conditions. There is effort from the authors to convey this, but it should be strengthened to best promote the impact of the work.

We completely agree with the Reviewer's point that our characterization of CLASP's activity in the absence of tubulin provides fundamental insights into the CLASP's mechanisms, bearing direct relevance for microtubule regulation in cells. We have significantly revised the introduction and discussion sections to more effectively convey the relevance of our investigations of CLASP activity in the absence of soluble tubulin. Our expanded results and analysis (particularly our findings in new Figure 5) fully support the mechanism by which CLASP stabilizes a metastable, intermediate state of the microtubule end in a nucleotide-dependent but tubulin-independent manner, which underlies its function as an anti-catastrophe and rescue factor.

2. Figures 1 and 2 investigate CLASP-dependent depolymerization activity on GMPCPP microtubules when either GTP or GDP is added. Because depolymerization occurs in the presence of GTP and GDP, the authors state that no nucleotide hydrolysis is required. Since depolymerization occurs in the presence of GDP, the gamma phosphate is not required. But that doesn't mean that the GTP provided is not hydrolyzed. Indeed, the authors discuss that CLASP may function as a GAP. To better investigate whether hydrolysis of GTP to GDP is required for CLASP-dependent microtubule depolymerization, or if CLASP depolymerization is enhanced or inhibited with a transition state analog, the authors should investigate how transition state analogs (GTPgammaS, GDP + high phosphate, or GDP-AlFx or GDP-BeFx) affect CLASP-dependent depolymerization.

Our expanded investigation of CLASP's activity in the presence of different nucleotides and nucleotide analogues, including GTPgammaS, which mimics a post-hydrolysis state, demonstrated that CLASP is able to depolymerize stable microtubules in the presence of GTP, GDP or GTPgammaS (new Figure S3). In contrast, CLASP does not depolymerize stabilized microtubules in the presence of GMPCPP. We thus conclude that, although the energy of GTP hydrolysis might not be strictly necessary, CLASP's mechanism relies on the recognition of a post-hydrolysis nucleotide state.

3. In Figures 1 and 2, the authors measure the "microtubule depolymerization rate". In Figure 3, they polarity label microtubules and measure the microtubule depolymerization rates for the plus and minus ends separately. These collective measurements don't seem to match. For example, in the presence of GDP, the plus end depolymerizes at an average rate of 11.2 nm/sec. The minus end depolymerizes at a rate of 0.21 nm/sec. This would total a "microtubule depolymerization rate" of 11.4 nm/sec. However, the authors present a microtubule depolymerization rate of 6 nm/sec (half the expected value). Are the authors intending to present the average depolymerization rate per end of a given microtubule or the rate that the total microtubule depolymerizes at (the sum of the polymerization rates at both ends). The discrepancy also exists for the GTP data (9.2 + 3.6 does not equal 6.7).

We thank the reviewer for pointing this out. The reason for the discrepancy is that in Figure 1, the microtubules are attached to coverslips all along the length of their lattices through coverslip-bound anti-rhodamine binding to the rhodamine dye used for fluorescent tubulin labeling. In contrast, for our polarity-marked microtubule assays, Alexa-488-tubulin extensions are grown from stabilized rhodamine-labeled seeds (Figures 3 and 5 in the revised submission). Thus, only the seed is attached to the coverslips, while the extensions are not. We reason that the surface-attached protofilaments impede microtubule depolymerization, thus lowering the observed depolymerization rates in Figure 1. We have now included schematics of our assay design for each of our depolymerization assays used in the manuscript (new Figure 1A, new Figure 3A, and new Figure 5A) to better explain the different assays and how surface attachment may affect depolymerization.

Minor points:

We have addressed the Minor Points as recommended and detailed below:

4. The authors presented average values for the depolymerization rates except for the data in Figure 4C. These rates should be included (at least in the figure caption).

We have included the mean rates in the figure caption.

5. Figure 4C: the authors should measure statistical significance between the 200 nM CLASP2 α condition and the 200 nM TOG2 condition. This would inform whether or not the eGFP-L-TOG2-S construct is sufficient for the full depolymerization activity observed with the FL molecule.

A one-way ANOVA followed by a post hoc Tukey HSD test revealed that all CLASP conditions were statistically significant from the control. The pair-wise comparison also revealed statistical significance between CLASP2 α versus TOG2 but not for CLASP2 β versus TOG2. Thus, TOG2 is not sufficient to recapitulate full-length CLASP2 α activity and suggests that the TOG1 domain from CLASP2 α may contribute to CLASP2's depolymerizing activity. We have now also included this new insight into the main text result for Figure 2 in the revised manuscript (original Figure 4).

6. Figure 4D: It would be beneficial to readers to report the values from the Hill fit, as the authors did in Figure 1D (concentration for half max depolymerization, and Hill coefficient).

We have now performed a careful analysis of the microtubule depolymerization rates at microtubule plus and minus ends across a range of CLASP1 concentrations as suggested by the reviewers. After separating the microtubule plus and minus end analysis in this way, the titration data fitted well to Michaelis-Menten revealing no cooperativity at either plus and minus ends (new Figure 3). Therefore, we do not report Hill coefficients in the revised manuscripts and have modified the main text, figures and figure legends accordingly.

7. For Movie 2, in the control (left panel, no nucleotide added) - there appears to be microtubule annealing that is not observed in the conditions with nucleotide added. The authors might want to note this in the caption so that the general readership is not confused by the events.

We have included a new version of this movie to avoid any confusion (new Movie 2).

January 19, 2023

RE: JCB Manuscript #202107027R

Dr. Marija Zanic
Vanderbilt University
Cell and Developmental Biology
465 21st Avenue South
4120 MRB3 Biosciences Building
Nashville, TN 37232

Dear Dr. Zanic:

Thank you for submitting your revised manuscript entitled "CLASPs stabilize the intermediate state between microtubule growth and catastrophe". The three original reviewers have now assessed your revised manuscript and, as you can see, they are overall satisfied with revisions. However, reviewers #2 and #3 would like you to further clarify some aspects of the paper with text edits where appropriate. Thus, we would be happy to publish your paper in JCB pending final revisions to address the lingering concerns of these two reviewers. In your final revision, please be sure to comply with our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully. Please go through all the formatting points paying special attention to those marked with asterisks.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, <https://jcb.rupress.org/submission-guidelines#revised>.

****Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.****

1) Text limits: Character count for Articles and Tools is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, and acknowledgments. Count does not include materials and methods, figure legends, references, tables, or supplemental legends.

2) Figures limits: Articles and Tools may have up to 10 main text figures.

***** Please note that main text figures should be provided as individual, editable files.**

3) Figure formatting:

Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

Scale bars must be present on all microscopy images, including inset magnifications.

Also, please avoid pairing red and green for images and graphs to ensure legibility for color-blind readers. If red and green are paired for images, please ensure that the particular red and green hues used in micrographs are distinctive with any of the colorblind types. If not, please modify colors accordingly or provide separate images of the individual channels.

4) Statistical analysis:

***** Error bars on graphic representations of numerical data must be clearly described in the figure legend. Please, describe error bars in Figs 3C, 3E-F.**

The number of independent data points (n) represented in a graph must be indicated in the legend. Please, indicate whether 'n' refers to technical or biological replicates (i.e. number of analyzed cells, samples or animals, number of independent experiments).

If independent experiments with multiple biological replicates have been performed, we recommend using distribution-reproducibility SuperPlots (please, see Lord et al., JCB 2020) to better display the distribution of the entire dataset, and report statistics (such as means, error bars, and P values) that address the reproducibility of the findings.

*** Statistical methods should be explained in full in the materials and methods in a separate section.

For figures presenting pooled data the statistical measure should be defined in the figure legends.

*** Please also be sure to indicate the statistical tests used in each of your experiments (both in the figure legend itself and in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.).

*** As you used parametric tests in your study (i.e. t-tests), you should have first determined whether the data was normally distributed before selecting that test. In the stats section of the methods, please indicate how you tested for normality. If you did not test for normality, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

5) Abstract and title:

The abstract should be no longer than 160 words and should communicate the significance of the paper for a general audience.

The title should be less than 100 characters including spaces. Make the title concise but accessible to a general readership.

6) Materials and methods:

Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. The text should not refer to methods "...as previously described."

Also, the materials and methods should be included with the main manuscript text and not in the supplementary materials.

7) For all cell lines, vectors, constructs/cDNAs, etc. - all genetic material: please include database / vendor ID (e.g., Addgene, ATCC, etc.) or if unavailable, please briefly describe their basic genetic features, even if described in other published work or gifted to you by other investigators (and provide references where appropriate).

Please be sure to provide the sequences for all of your oligos: primers, si/shRNA, RNAi, gRNAs, etc. in the materials and methods.

You must also indicate in the methods the source, species, and catalog numbers/vendor identifiers (where appropriate) for all of your antibodies, including secondary. If antibodies are not commercial, please add a reference citation if possible.

8) Microscope image acquisition:

The following information must be provided about the acquisition and processing of images:

- a. Make and model of microscope
- b. Type, magnification, and numerical aperture of the objective lenses
- c. Temperature
- d. imaging medium
- e. Fluorochromes
- f. Camera make and model
- g. Acquisition software
- h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

9) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

10) Supplemental materials:

There are strict limits on the allowable amount of supplemental data. Articles/Tools may have up to 5 supplemental figures. There is no limit for supplemental tables.

*** Please note that supplemental figures and tables should be provided as individual, editable files.

*** A summary of all supplemental material should appear at the end of the Materials and Methods section (please see any recent JCB paper for an example of this summary).

11) Video legends:

*** Video legends should describe what is being shown, the cell type or tissue being viewed (including relevant cell treatments, concentration and duration, or transfection), the imaging method (e.g., time-lapse epifluorescence microscopy), what each color represents, how often frames were collected, the frames/second display rate, and the number of any figure that has related video stills or images.

12) eTOC summary:

*** A ~40-50 word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person. It should begin with "First author name(s) et al..." to match our preferred style.

13) Conflict of interest statement:

*** JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the following statement: "The authors declare no competing financial interests."

14) Author contribution:

*** A separate author contribution section is required following the Acknowledgments in all research manuscripts. All authors should be mentioned and designated by their first and middle initials and full surnames and the CRediT nomenclature is encouraged (<https://casrai.org/credit/>).

15) ORCID IDs: ORCID IDs are unique identifiers allowing researchers to create a record of their various scholarly contributions in a single place. At resubmission of your final files, please consider providing an ORCID ID for as many contributing authors as possible.

16) Materials and data sharing:

All animal and human studies must be conducted in compliance with relevant local guidelines, such as the US Department of Health and Human Services Guide for the Care and Use of Laboratory Animals or MRC guidelines, and must be approved by the authors' Institutional Review Board(s). A statement to this effect with the name of the approving IRB(s) must be included in the Materials and Methods section.

*** As a condition of publication, authors must make protocols and unique materials (including, but not limited to, cloned DNAs; antibodies; bacterial, animal, or plant cells; and viruses) described in our published articles freely available upon request by researchers, who may use them in their own laboratory only. All materials must be made available on request and without undue delay. We strongly encourage to deposit all the cell lines/strains and reagents generated in this study in public repositories.

All datasets included in the manuscript must be available from the date of online publication, and the source code for all custom computational methods, apart from commercial software programs, must be made available either in a publicly available database or as supplemental materials hosted on the journal website. Numerous resources exist for data storage and sharing (see Data Deposition: <https://rupress.org/jcb/pages/data-deposition>), and you should choose the most appropriate venue based on your data type and/or community standard. If no appropriate specific database exists, please deposit your data to an appropriate publicly available database.

17) Please note that JCB now requires authors to submit Source Data used to generate figures containing gels and Western blots with all revised manuscripts. This Source Data consists of fully uncropped and unprocessed images for each gel/blot displayed in the main and supplemental figures. The Source Data files will be directly linked to specific figures in the published article.

If your paper includes cropped gel and/or blot images, please be sure to provide one Source Data file for each figure that contains gels and/or blots along with your revised manuscript files. File names for Source Data figures should be alphanumeric without any spaces or special characters (i.e., SourceDataF#, where F# refers to the associated main figure number or SourceDataFS# for those associated with Supplementary figures). The lanes of the gels/blots should be labeled as they are in the associated figure, the place where cropping was applied should be marked (with a box), and molecular weight/size standards should be labeled wherever possible.

Source Data Figures should be provided as individual PDF files (one file per figure). Authors should endeavor to retain a minimum resolution of 300 dpi or pixels per inch. Please review our instructions for export from Photoshop, Illustrator, and

PowerPoint here: <https://rupress.org/jcb/pages/submission-guidelines#revised>

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and MP4 video files: See our detailed guidelines for preparing your production-ready images, <https://jcb.rupress.org/fig-vid-guidelines>.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

****It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.****

****The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements before choosing the appropriate license.****

Additionally, JCB encourages authors to submit a short video summary of their work. These videos are intended to convey the main messages of the study to a non-specialist, scientific audience. Think of them as an extended version of your abstract, or a short poster presentation. We encourage first authors to present the results to increase their visibility. The videos will be shared on social media to promote your work. For more detailed guidelines and tips on preparing your video, please visit <https://rupress.org/jcb/pages/submission-guidelines#videoSummaries>.

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days. If complications arising from measures taken to prevent the spread of COVID-19 will prevent you from meeting this deadline (e.g. if you cannot retrieve necessary files from your laboratory, etc.), please let us know and we can work with you to determine a suitable revision period.

Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Samara Reck-Peterson
Monitoring Editor
Journal of Cell Biology

Lucia Morgado-Palacin, PhD
Scientific Editor
Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

I am satisfied with the comprehensive revision of this manuscript. I believe it provides a sufficient advance toward an improved understanding of the role of +TIP proteins, such as CLASP, in regulating microtubule assembly dynamics at microtubule ends.

Reviewer #2 (Comments to the Authors (Required)):

The authors' revised manuscript addresses most of the comments by the reviewers, but still falls short in interpreting their data in a general mechanistic manner that would be relevant to the field.

As a reviewer, I remain concerned about the data interpretation of CLASP's biochemical activity in binding soluble or exposed tubulins at microtubule ends as means of promoting microtubule regulation. The main discovery in the study- CLASP promotes nucleotide dependent microtubule depolymerization- is very narrowly interpreted to ignore significant understanding made for TOG domain-tubulin binding interactions and its role in the depolymerization process. This should be revised to enhance the impact of the work for the field as whole. The CLASP microtubule depolymerase /regulator activity is fundamentally linked its TOG domain tubulin binding activity, as depolymerization is directly resulting from the absence of soluble tubulin. The statement "CLASP's anti-catastrophe mechanism does not require soluble tubulin" (line 1012), is inaccurate and a misinterpretation of the data. Although the authors describe conditions in which CLASP promotes microtubule minus end depolymerization while polymerization at plus ends occurs, these could be impact for equilibria of CLASP-tubulin interaction interfaces which are generally weaker, but not fundamentally different than XMAP215 in binding soluble tubulin, except in its nucleotide dependence. The data presented do not suggest what the authors conclude in the above statement. Furthermore, data shown in Figure S4-A, middle panel, indicate that CLASP recruit soluble tubulins to microtubules in the intermediate concentrations of soluble between its regulation of polymerization and depolymerization suggesting recruitment of tubulin is a part of its regulatory activity, similar to an observation initially made by other groups on other clasp orthologs over a decade ago.

Furthermore, the recent work by Luo et al 2023 on this exact topic directly addresses the role of TOG-tubulin interface in the nucleotide dependent regulation of microtubules by CLASP. The work demonstrates that its TOG-tubulin interaction interface is fundamentally critical for microtubule nucleotide dependent regulation.

The authors must revise the discussion to address a soluble tubulin intermediate is a fundamental part of their depolymerase cycle and it is mediated by TOG-tubulin interaction interface. The authors should also revise to compare their results to the recent work published on the same topic, Luo et al 2023.

I strongly urge the authors to make some revisions to the discussion, so that this beautiful study will unify the field, rather than cause more confusion.

Reviewer #3 (Comments to the Authors (Required)):

1) I wonder if the authors can clarify an effect in the manuscript that does not seem internally consistent: In comparing figures 3 and 5, it is clear that CLASP can depolymerize the GMPCPP seed (which is antibody linked to the coverslip) in figure 3, for both GTP and GDP conditions, but this does not seem to be the case in Figure 5. Is this a CLASP concentration-dependent effect, or perhaps due to differing concentrations of antibody used in the two experiments? Explaining this to the reader would help make the paper internally consistent.

2) In figure 4, the authors examine the dwell time of CLASP single molecules on the MT lattice, and speculate that this dwell time is on par with the depolymerization rate. As depolymerization is occurring at the MT ends, while the dwell time is measured (what appears to be) for binding to the MT lattice wall (not the ends) - the authors should simply add the caveat that the dwell time is measuring lattice association, not the dwell time of binding to tubulin at the end of the lattice (which in Fig 4C - left panel - appears to have a quite different dwell time).

3) The last paragraph of the results section, cites figures 5G-H, but these panels are not present in the figure. Please update to cite the correct panels.