JCB

XMAP215 promotes microtubule catastrophe by disrupting the growing microtubule end

Veronica Farmer, Göker Arpağ, Sarah Hall, and Marija Zanic

Corresponding Author(s): Marija Zanic, Vanderbilt University

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February 25, 2021

Re: JCB manuscript #202012144

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 "XMAP215 promotes microtubule catastrophe by disrupting the growing microtubule end". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

As you will see, the reviewers have differing opinions regarding the suitability of your study for JCB. I agree with reviewer #2 that there is a lack of mechanistic insight, however, an extended study to explore the mechanism for the phenomenon described here would require an article format, rather than a short report. As your study carefully identifies what is potentially an important observation - that the manner in which microtubules grow can ultimately influence their destruction, as defined by the probability of a catastrophe event, editorially I find your study suitable as a Report in JCB.

In revising, please address the reviewers comments as outlined:

Reviewer #1: The authors should carefully address all comments by reviewer #1.

Reviewer #2: The authors should also carefully address all comments by reviewer #2, and incorporate explanations and caveats as appropriate. In addition, please note the following regarding reviewer #2 comments:

- As noted above, I feel that the novelty and the level of mechanistic insight is appropriate for a report format.

- Given that the concentration of EB1 is kept constant for the experiments in the manuscript, I do not agree that effect of EB1 on microtubule end stability is problematic, as noted by reviewer #1.

- Reviewer #2 is also concerned about the synergism between EB1 and XMAP215 activities at the growing microtubule ends. This could be a concern if nonlinear biochemical effects were observed, for example in Fig. 3A. However, the response of EB1 comet length with increasing XMAP concentrations appears to be nearly linear in Fig. 3A, suggesting that the synergism between EB1 and XMAP215 may not be a nonlinear biochemical effect, but rather a result of changes in microtubule tip structure, as is explicitly explored in this manuscript. Therefore, I also disagree with the concern regarding synergism between EB1 and XMAP215.

- Reviewer #2 notes that the conclusions are vague. I do agree that the take-home message from this manuscript should be clarified. The authors note that "our results demonstrate that XMAP215 simultaneously promotes microtubule growth and catastrophe frequency without accelerating the GTP-hydrolysis rate, or otherwise decreasing the mean GTP-cap size". However, the data from Figures 4 provides insight into an explanation for how this may occur. A final cartoon to summarize the author's ideas regarding the mechanism to explain their results, along with a paragraph in the discussion, would help to clarify the take-home message for the paper, and to clarify what exactly the title "XMAP215 promotes microtubule catastrophe by disrupting the growing microtubule end" means.

- The authors may wish to rethink Fig. 5 in light of reviewer #2 comments - I agree that the interpretation for Fig. 5 is unclear. One idea may be to eliminate the current Fig. 5, and replace it with a cartoon that demonstrates the take home message from the paper, based on Figs 1-4.

- Reviewer #2's minor points should all be addressed, with the exception of performing new experiments to remove the EB1 His-tag. This seems unnecessary given that the concentration of EB1 is held constant in the paper experiments. However, the authors may wish to comment on this issue.

Further suggestions:

Figure 2B and Figure 2C: Please add legends onto the graphs themselves, as it is difficult to search the legend to understand the graphs - eg, define dotted lines, different colored points, and fitted lines on the graphs themselves.

Figure 4B, right: perhaps avoid the acronym "SSR" which is not widely understood, and replace with complete text.

Figure 4D - It would be good to add p-values here, which can be calculated for proportions - one idea may be to report a p-value comparing the proportion of intact (full) comets vs disrupted (split+curled) comets, with and without XMAP, to provide a more definitive conclusion that XMAP is altering tip structure.

Figure 5 - As noted above by reviewer #2, Fig. 5 does not currently seem well connected to the remainder of the paper, and I would suggest dropping it. If it is not dropped, a clear connection between Fig. 4 and Fig. 5 should be described, as well as the contribution of Fig. 5 to the final conclusions in the paper.

New Figure: As noted above, a final cartoon to clarify the model put forward here, and to clarify the paper title, should be included.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:
Text limits: Character count for a Report is < 20,000, 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: Reports may have up to 5 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. 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.

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.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

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 in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Melissa Gardner
Monitoring Editor

Andrea L. Marat
Senior Scientific Editor

Journal of Cell Biology

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

This is a very solid paper on the effects of the Microtubule polymerase XMAP215 on microtubule growth and catastrophe. The principal finding is that although XMAP215 increases the growth rate

and increases GTP cap size, it increases the catastrophe frequency. The interpretation, which is well supported by the data is that the microtubule lattice is defective when grown in XMAP. I thought the writing was clear and the presentation logical. I have some cosmetic comments.

1) The authors should be clearer about comet intensity. In some cases it is the intensity of the peak pixel if I understand it correctly, whereas in other places it is integrated intensity. It seems laid out pretty well in the methods, but the authors should be clearer in the results what they're referring to by intensity (perhaps rename peak pixel intensity or something like that).

2) Line 160: N=0?

3) The authors should better denote the different tubulin concentrations in Figure 3. For instance, in Fig 3B put tubulin on x-axis as well. As is, a casual reader might infer no change in growth rate from XMAP215 addition.

4) The authors should consider discussing Chretien's work "Mechanical Stress Induced Mechanism of MicrotubuleCatastrophes", J Mol Bio 2005. This is relevant for the catastrophe question.

Addendum to Review:
Reviewer 2 brings up the following point:
"The use of EB1 protein as an indicator of the GTP cap size is common in the field and may be appropriate in some cases. It is worth remembering, however, that EB1 has a very strong effect on microtubule end stability. This important fact will not be obvious to most readers, although this effect is clearly visible from carefully comparing Figs. 1B and S1B, which have vastly different scales. Moreover, as nicely shown by Zanic et al., 2013, there is a strong synergism between EB1 and XMAP215 activities at the growing microtubule ends. Thus, many of the conclusions in the current manuscript could be questioned because of this ill-understood synergism and the strong destabilizing effect by EB1."

Response to Reviewer 2:
I see that at 20 uM tubulin the catastrophe frequency is around 0.1/s in Fig 1B. In Fig S1B, which is at 20 uM tubulin, the zero XMAP215 value (yellow dotted line) is about 0.07/s. These seem to agree to me.

These experiments were carried out with 200 nM EB1-GFP in all conditions. In Zanic 2013, the Fig 1e catastrophe data used 5 uM tubulin and 400 nM EB1, and there was a clear enhancement of catastrophe frequency by EB1. The differences between the current and older data are worth pointing out by the authors, but I don't agree that these details undermine the study or make the current data questionable.

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

Farmer and colleagues use in vitro assays to investigate XMAP215 activity at the growing microtubule end. Previous work, including by this group, found that XMAP215 is a strong polymerization factor that has little effect on catastrophe frequency. This seems counterintuitive because in a simplified model of microtubule stability, catastrophe frequency is determined by the size of the GTP cap, which is known to increase with increasing rate of microtubule polymerization.

However, it is also well established that other factors, including structure of the growing microtubule end, can also influence the frequency of microtubule catastrophe. Current work, which uses EB1 protein to visualize microtubule plus ends growing in the presence of XMAP215, reinforces these views. The authors report the XMAP215 increases complexity of the microtubule ends structure (they are often curled), resulting in elevated fluctuations in the microtubule growth rate and uncoupling of the correlation between the GTP cap size and catastrophe frequency.
Understanding the mechanisms of microtubule dynamic instability and how it is controlled by MAPs is a very challenging and exciting area of cytoskeletal research. Despite my enthusiasm for this topic, and in general high quality of imaging and quantifications in the current study, I am concerned that its results do not provide significantly novel mechanistic insights that would be of interest to the general readership of JCB. First two figures in the paper establish important methodological background for this study, but the results reported in these figures are hardly novel. The rest of the figures provide more interesting contribution. However, the interpretation of these data is in my opinion problematic. The use of EB1 protein as an indicator of the GTP cap size is common in the field and may be appropriate in some cases. It is worth remembering, however, that EB1 has a very strong effect on microtubule end stability. This important fact will not be obvious to most readers, although this effect is clearly visible from carefully comparing Figs. 1B and S1B, which have vastly different scales. Moreover, as nicely shown by Zanic et al., 2013, there is a strong synergism between EB1 and XMAP215 activities at the growing microtubule ends. Thus, many of the conclusions in the current manuscript could be questioned because of this ill-understood synergism and the strong destabilizing effect by EB1. There is not enough data to define contribution of these complicating factors on the main results (end morphology and rate fluctuations). Although it is well established by prior work and in the current manuscript that XMAP215 increases microtubule growth rate without suppressing catastrophe frequency even in the absence of EB1, the main findings described in this work could well be affected by EB1 presence.

Other significant criticism.

1) Some of the kymographs show unexplained peculiarities in microtubule dynamics.

Fig 1A. There is a very high rescue frequency for microtubule grown at low tubulin concentration, while with more EB1 this effect is gone. Generally, microtubules show little or no rescue at these tubulin concentrations (if highly competent tubulin is used). The high rescue frequency and its apparent dependence on tubulin concentration require additional examination and reasonable explanation
Fig 2A. Microtubule disassembly rate with no/low XMAP215 appears to be much lower than in other in vitro studies.

I am worried that there are some hidden (synergistic?) effects or technical deviations in this experimental system that need to be explained. Furthermore, these behaviors are very different from previous data by the same group (compare current Fig 1A with Fig 3B in Lawrence et al., 2018).

2) It is difficult to follow the authors' interpretations for findings illustrated with Fig. 5. It is known from prior work that microtubules grow much faster in the presence of XMAP215. Is it surprising that they undergo catastrophes at higher growth rates? The second conclusion drawn by the authors is that catastrophes take place with "more EB1". This is hardly evident from these data, which show almost similar levels of EB1 at catastrophe. More striking feature of the disassembling XMAP215 end is a strong drop in EB1 intensity over a relatively long time (20 s) prior to catastrophe. The authors should discuss these various features and seek mechanistic explanations. One concern is that the loss of EB1 could affect XMAP215 activity (through a loss of

synergistic interactions), leading to this unusual behavior, as already explained above.

More minor points:

3) Line 54: "early work demonstrated that increasing the microtubule growth rate by increasing the tubulin concentration in vitro is accompanied by a decrease in the catastrophe frequency (Walker et al., 1988)." This sentence does not do justice to a highly complex and controversial subject. Others found much smaller decrease (e.g. Gardner et al., 2011, Odde et al., 1995). It is also worth pointing out that Walker et al. findings at high tubulin concentration are based on a small data set, so referencing other similar studies would be appropriate.

4) Fig. 4. EB1 blobs and curling at the microtubule plus end are certainly indicative of some abnormal morphology, but why does this Figure title refer to these ends as "tapered" (which usually means gradual thinning of the tip)?

5) According to Zhu et al., 2009, His-tagged EB1, as used in the current work, has somewhat different properties than EB1 with no charged His extension. Have authors confirmed that the His-tag does not impact their results?

Rebuttal Letter

Farmer*, Arpag*, Hall & Zanic. XMAP215 promotes microtubule catastrophe by disrupting the growing microtubule end.

Response to the Editor

As you will see, the reviewers have differing opinions regarding the suitability of your study for JCB. I agree with reviewer #2 that there is a lack of mechanistic insight, however, an extended study to explore the mechanism for the phenomenon described here would require an article format, rather than a short report. As your study carefully identifies what is potentially an important observation - that the manner in which microtubules grow can ultimately influence their destruction, as defined by the probability of a catastrophe event, editorially I find your study suitable as a Report in JCB.

Thank you for your comments. We appreciate the suggestions and concerns raised by both reviewers and the editor, and have addressed them, as outlined below. Please note that in the revised manuscript document, we have highlighted the specific changes made as a response to the editor and reviewers in purple (also denoted by line numbers below).

In revising, please address the reviewers comments as outlined:

Reviewer #1: The authors should carefully address all comments by reviewer #1.

We have addressed all of Reviewer #1's comments, as detailed below.

Reviewer #2: The authors should also carefully address all comments by reviewer #2, and incorporate explanations and caveats as appropriate.

We have addressed all of Reviewer #2's comments, as detailed below.

In addition, please note the following regarding reviewer #2 comments:
- As noted above, I feel that the novelty and the level of mechanistic insight is appropriate for a report format.
- Given that the concentration of EB1 is kept constant for the experiments in the manuscript, I do not agree that effect of EB1 on microtubule end stability is problematic, as noted by reviewer #1.
- Reviewer #2 is also concerned about the synergism between EB1 and XMAP215 activities at the growing microtubule ends. This could be a concern if nonlinear biochemical effects were observed, for example in Fig. 3A. However, the response of EB1 comet length with increasing XMAP concentrations appears to be nearly linear in Fig. 3A, suggesting that the synergism between EB1 and XMAP215 may not be a nonlinear biochemical effect, but rather a result of changes in microtubule tip structure, as is explicitly explored in this manuscript. Therefore, I also disagree with the concern regarding synergism between EB1 and XMAP215.

We agree with the Editor on the above points and have provided a detailed response to these concerns raised by Reviewer #2 below.

- Reviewer #2 notes that the conclusions are vague. I do agree that the take-home message from this manuscript should be clarified. The authors note that "our results demonstrate that

XMAP215 simultaneously promotes microtubule growth and catastrophe frequency without accelerating the GTP-hydrolysis rate, or otherwise decreasing the mean GTP-cap size". However, the data from Figures 4 provides insight into an explanation for how this may occur. A final cartoon to summarize the author's ideas regarding the mechanism to explain their results, along with a paragraph in the discussion, would help to clarify the take-home message for the paper, and to clarify what exactly the title "XMAP215 promotes microtubule catastrophe by disrupting the growing microtubule end" means.
- The authors may wish to rethink Fig. 5 in light of reviewer #2 comments - I agree that the interpretation for Fig. 5 is unclear. One idea may be to eliminate the current Fig. 5, and replace it with a cartoon that demonstrates the take home message from the paper, based on Figs 1-4.

Based on the Reviewers' and Editor's comments, we realized that our motivation for previous Figure 5 (now Figure 4), interpretation of the presented results, and the proposed mechanism have not been sufficiently clear. To address this issue, we have now significantly revised previous Figure 5 (now Figure 4), added a cartoon schematic outlining our proposed model (new Figure 5), as well as clarified and elaborated our discussion, as suggested. We think that these revisions have significantly improved the clarity of our manuscript.

- Reviewer #2's minor points should all be addressed, with the exception of performing new experiments to remove the EB1 His-tag. This seems unnecessary given that the concentration of EB1 is held constant in the paper experiments. However, the authors may wish to comment on this issue.

We have addressed all of Reviewer #2's minor points, as detailed below.

Further suggestions:

Figure 2B and Figure 2C: Please add legends onto the graphs themselves, as it is difficult to search the legend to understand the graphs - eg, define dotted lines, different colored points, and fitted lines on the graphs themselves.

Please note that previous Figure 2 is now Figure 1; it has been revised as suggested.

Figure 4B, right: perhaps avoid the acronym "SSR" which is not widely understood, and replace with complete text.
Figure 4D - It would be good to add p-values here, which can be calculated for proportions - one idea may be to report a p-value comparing the proportion of intact (full) comets vs disrupted (split+curled) comets, with and without XMAP, to provide a more definitive conclusion that XMAP is altering tip structure.

Please note that previous Figure 4 is now Figure 3; it has been revised as suggested, and a p-value has been added in the caption, as well as the main text (line 202).

Figure 5 - As noted above by reviewer #2, Fig. 5 does not currently seem well connected to the remainder of the paper, and I would suggest dropping it. If it is not dropped, a clear connection between Fig. 4 and Fig. 5 should be described, as well as the contribution of Fig. 5 to the final conclusions in the paper.

Based on the reviewers' and editor's feedback, we realized that the point of the previous Figure 5 was not clear in the original version of the manuscript. We have now significantly revised

previous Figure 5 (now Figure 4), its description in the text, as well as clarified its contribution to the final conclusions of the manuscript (lines 206-235).

New Figure: As noted above, a final cartoon to clarify the model put forward here, and to clarify the paper title, should be included.

We have added the final cartoon as a new Figure 5, as suggested by the Editor.

Response to the Reviewers

Reviewer #1 (Comments to the Authors (Required)):
This is a very solid paper on the effects of the Microtubule polymerase XMAP215 on microtubule growth and catastrophe. The principal finding is that although XMAP215 increases the growth rate and increases GTP cap size, it increases the catastrophe frequency. The interpretation, which is well supported by the data is that the microtubule lattice is defective when grown in XMAP. I thought the writing was clear and the presentation logical. I have some cosmetic comments.

We thank the Reviewer for the constructive evaluation of our manuscript. We have addressed all of the Reviewer's comments, as summarized below.

1) The authors should be clearer about comet intensity. In some cases it is the intensity of the peak pixel if I understand it correctly, whereas in other places it is integrated intensity. It seems laid out pretty well in the methods, but the authors should be clearer in the results what they're referring to by intensity (perhaps rename peak pixel intensity or something like that).

Thank you for raising this important point. This comment inspired us to rethink our EB1 comet measurement approaches. We have now revised our methods for EB1 comet measurements, and significantly clarified what is being reported in each figure. Specifically, to allow direct comparison of EB1 comets between tubulin/EB1 and tubulin/EB1/XMAP215 conditions, we are now reporting EB1 comet decay lengths, instead of absolute intensities, in Figures S1 and 2. To determine EB1 comet decay lengths we adapted the previously-published approach from the Surrey lab (Bieling et al., 2007). While the absolute intensity measurements are highly dependent on imaging conditions (e.g. laser power, exposure time, TIRF angle), the EB1 comet decay length is an independent variable, which reflects the size of the GTP-cap, as set by the GTP hydrolysis rate and the microtubule growth rate. Thus, we think that the use of EB1 comet decay length is the most appropriate measurement for determining differences in the size of the GTP-cap between different experimental conditions. We now report EB1 intensity only at the moment of catastrophe in Figure 4, where the two experimental conditions (0 vs 3.13 nM XMAP215) are performed on the same day with the same imaging setup. In this case, our goal is to directly compare the relative amounts of EB1 remaining at the microtubule end at the onset of catastrophe, in order to test the hypothesis that there is a universal critical threshold of EB1 binding sites that needs to be reached to induce microtubule catastrophe (see Duellberg et al., 2016). Here, our methods adopt the previously-published approaches from the Surrey lab (Duellberg et al., 2016).

We have accordingly revised the figure legends, main text and the Methods to clarify our approaches.

2) Line 160: N=0?

The numbers originally reported in lines 159/160 referred to the total number of catastrophes observed in growth-rate-matching experiments in the absence vs. the presence of XMAP215 during the total time spent in growth for 20 microtubule kymographs analyzed for each condition We have updated the text accordingly to clarify this point, now reporting both the number of catastrophes observed, as well as the number of growth events analyzed (lines 162-164).

3) The authors should better denote the different tubulin concentrations in Figure 3. For instance, in Fig 3B put tubulin on x-axis as well. As is, a casual reader might infer no change in growth rate from XMAP215 addition.

Thank you for this suggestion. We have updated the figure (now Figure 2) to reflect tubulin concentrations, as suggested by the Reviewer.

4) The authors should consider discussing Chretien's work "Mechanical Stress Induced Mechanism of MicrotubuleCatastrophes", J Mol Bio 2005. This is relevant for the catastrophe question.

Thank you for pointing out this omission. We have now included the relevant references in our revised Conclusions section. Specifically, we have added the following text (lines 270-275):

"…Notably, a variety of growing-end configurations have been observed by structural studies (Mcintosh et al., 2018)(Gudimchuk et al., 2020)(Chrétien et al., 1995)(Guesdon et al., 2016)(Atherton et al., 2018) (Mandelkow et al., 1991)(Reid et al., 2019), and it has been previously proposed that some of these end configurations may indeed be energetically unfavorable, leading to microtubule catastrophe (Chrétien and Fuller, 2000; Hunyadi et al., 2005)…"

Addendum to Review:
Reviewer 2 brings up the following point:
"The use of EB1 protein as an indicator of the GTP cap size is common in the field and may be appropriate in some cases. It is worth remembering, however, that EB1 has a very strong effect on microtubule end stability. This important fact will not be obvious to most readers, although this effect is clearly visible from carefully comparing Figs. 1B and S1B, which have vastly different scales. Moreover, as nicely shown by Zanic et al., 2013, there is a strong synergism between EB1 and XMAP215 activities at the growing microtubule ends. Thus, many of the conclusions in the current manuscript could be questioned because of this ill-understood synergism and the strong destabilizing effect by EB1."
Response to Reviewer 2:
I see that at 20 uM tubulin the catastrophe frequency is around 0.1/s in Fig 1B. In Fig S1B, which is at 20 uM tubulin, the zero XMAP215 value (yellow dotted line) is about 0.07/s. These seem to agree to me.
These experiments were carried out with 200 nM EB1-GFP in all conditions. In Zanic 2013, the Fig 1e catastrophe data used 5 uM tubulin and 400 nM EB1, and there was a clear enhancement of catastrophe frequency by EB1. The differences between the current and older data are worth pointing out by the authors, but I don't agree that these details undermine the study or make the current data questionable.

Thank you for this Addendum. We have addressed all of the Reviewer 2's comments and concerns, as detailed below.

Reviewer #2 (Comments to the Authors (Required)):
Farmer and colleagues use in vitro assays to investigate XMAP215 activity at the growing microtubule end. Previous work, including by this group, found that XMAP215 is a strong polymerization factor that has little effect on catastrophe frequency. This seems counterintuitive because in a simplified model of microtubule stability, catastrophe frequency is determined by the size of the GTP cap, which is known to increase with increasing rate of microtubule polymerization. However, it is also well established that other factors, including structure of the growing microtubule end, can also influence the frequency of microtubule catastrophe. Current work, which uses EB1 protein to visualize microtubule plus ends growing in the presence of XMAP215, reinforces these views. The authors report the XMAP215 increases complexity of the microtubule ends structure (they are often curled), resulting in elevated fluctuations in the microtubule growth rate and uncoupling of the correlation between the GTP cap size and catastrophe frequency.
Understanding the mechanisms of microtubule dynamic instability and how it is controlled by MAPs is a very challenging and exciting area of cytoskeletal research. Despite my enthusiasm for this topic, and in general high quality of imaging and quantifications in the current study, I am concerned that its results do not provide significantly novel mechanistic insights that would be of interest to the general readership of JCB.

We thank the Reviewer for the critical evaluation of our manuscript. We have addressed the raised concerns point-by-point, as outlined below.

First two figures in the paper establish important methodological background for this study, but the results reported in these figures are hardly novel. The rest of the figures provide more interesting contribution. However, the interpretation of these data is in my opinion problematic. The use of EB1 protein as an indicator of the GTP cap size is common in the field and may be appropriate in some cases. It is worth remembering, however, that EB1 has a very strong effect on microtubule end stability. This important fact will not be obvious to most readers, although this effect is clearly visible from carefully comparing Figs. 1B and S1B, which have vastly different scales. Moreover, as nicely shown by Zanic et al., 2013, there is a strong synergism between EB1 and XMAP215 activities at the growing microtubule ends. Thus, many of the conclusions in the current manuscript could be questioned because of this ill-understood synergism and the strong destabilizing effect by EB1. There is not enough data to define contribution of these complicating factors on the main results (end morphology and rate fluctuations). Although it is well established by prior work and in the current manuscript that XMAP215 increases microtubule growth rate without suppressing catastrophe frequency even in the absence of EB1, the main findings described in this work could well be affected by EB1 presence.

We agree with the reviewer that many previous studies have reported the dependence of microtubule growth rate and microtubule catastrophe frequency on tubulin concentration *in vitro* (see e.g. O'Brien et al., 1990, Drechsel et al., 1992, Odde et al., 1995, Gardner et al., 2011, Piedra et al., 2016, Chaaban et al., 2018, Strothman et al., 2019, Arpağ et al., 2020). Additionally, the size of the EB1/Mal3 comets was previously shown to increase with the microtubule growth rate when achieved by increasing tubulin concentrations (Bieling et al., 2007)(Duellberg et al., 2016)(Strothman et al., 2019). However, the relationship between microtubule catastrophe frequency and the EB1 comet size over a range of tubulin concentrations has, to our knowledge, never been reported previously. Our results in the previous Figure 1 (now Figure S1) for the first time directly establish the canonical relationship between the GTP-cap size (as determined by the EB1 comet length) and the microtubule catastrophe frequency, in conditions where the tubulin concentration is modulated. This

relationship presents the basis of the standard model of dynamic instability (i.e. larger GTP-cap leads to prolonged microtubule lifetime), which is subsequently challenged by our observations in the presence of XMAP215, and thus we think that it warrants an independent figure. In the revised manuscript, we present this result in the supplemental Figure S1. This restructuring allowed us to add a model cartoon as a new Figure 5, as suggested by the Editor.

Our results presented in the original Figure 2 (now Figure 1) demonstrate that the addition of XMAP215 increases microtubule catastrophe frequency, in spite of its well-known effect of strongly increasing the microtubule growth rate. Although simultaneous promotion of growth and catastrophe has been previously observed in the presence of XMAP215 and EB1 (Zanic et al., 2013), the increase in catastrophe was primarily attributed to the effects of EB1, which is indeed a known catastrophe factor *in vitro*. Here, we perform a full titration in XMAP215 concentration in the background of fixed EB1 concentration and show that the addition of XMAP215 on its own causes an increase in microtubule catastrophe frequency. We demonstrate that XMAP215-driven increase in catastrophe frequency is observed both in the presence (now Figure 1) and in the absence of EB1 (now Figure S2). Importantly, unlike the synergistic effect of XMAP215 and EB1 on microtubule growth (where the combined effects of the two proteins greatly exceed the product of their individual effects), the extent of the catastrophe frequency increase by XMAP215 in the presence of EB1 (~ 2-fold, Figure 1) is not larger than the XMAP215-induced increase in catastrophe frequency in the absence of EB1 (2 to 4-fold, Figure S2). Taken together, these results demonstrate that the observed effect of XMAP215 on catastrophe frequency is a direct consequence of XMAP215 alone, rather than a synergistic effect dependent on EB1. Moreover, the observed functional dependence of catastrophe frequency on XMAP215 concentration (both in the presence and in the absence of EB1) is clearly distinct from the dose-dependent effects of XMAP215 on microtubule growth rate, suggesting that the mechanisms driving the observed XMAP215-mediated effects on microtubule catastrophe are distinct from those driving the growth acceleration (both alone and in synergy with EB1).

Other significant criticism.
1) Some of the kymographs show unexplained peculiarities in microtubule dynamics.
Fig 1A. There is a very high rescue frequency for microtubule grown at low tubulin concentration, while with more EB1 this effect is gone. Generally, microtubules show little or no rescue at these tubulin concentrations (if highly competent tubulin is used). The high rescue frequency and its apparent dependence on tubulin concentration require additional examination and reasonable explanation.

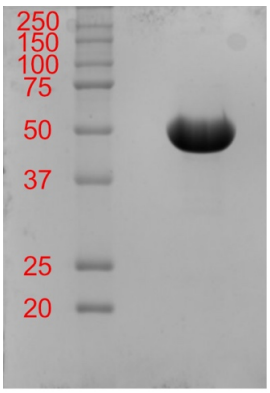
Thank you for raising this concern. Our previous analysis had focused solely on microtubule catastrophe and growth rates, as this is the subject of the manuscript. We have now additionally measured microtubule rescue frequencies and shrinkage rates in our experiments, to compare with previously-published data. In addition to the tubulin concentration, it is well known that buffer conditions, including KCl and glycerol concentrations, significantly impact microtubule dynamics (see e.g. Wieczorek et al., 2013, Schilstra et al., 1991, O'Brien et al., 1990, Stewart et al., 1990). To that end, please note that our buffer conditions for tubulin titration in previous Figure 1 (now Figure S1) included 17 mM KCl and 2.5% glycerol (to adequately maintain the composition of the XMAP215 storage buffer, even in conditions where XMAP215 was not used), which is significantly different from e.g. Lawrence et al., 2018. In Lawrence et al., 2018, where 50-100 mM KCl and no glycerol were used, rescue frequency was ~ 1 event/minute of shrinkage at 8 μM tubulin. Here, for 12 μM tubulin condition growing at 17 nm/s (the lowest concentration used in this study), our measured rescue frequency is 3.9 ± 0.8 events/minute of shrinkage (total of 26 rescues observed over 20 microtubule kymographs, 300 minutes of observed dynamics, of which 400 seconds spent in shrinkage). Note that the classic Walker et

al., 1988 study reported plus-end rescue frequencies in the range of 2-5 min^{-1} over a range of 7-16 μM tubulin. Similarly, our recent study (Arpağ et al., 2020) reported plus-end rescue frequencies in the range of 2-8 min^{-1} over a range of 5-12 μM tubulin. Thus, our rescue frequency measured here, even with the distinct buffer composition, is consistent with previously published studies.

Furthermore, please note that our tubulin titration in previous Figure 1 (now Figure S1) was performed in the background of a constant EB1 concentration (200 nM EB1), so the data do not represent any EB1-concentration-dependent effects on rescue frequency. As far as the rescue dependence on tubulin concentration is concerned, our titration used relatively high concentrations of tubulin, and rescue frequencies become increasingly difficult to measure at tubulin concentrations of 20 μM and above because at these tubulin concentrations very few catastrophe events (and thus possibilities for rescues) are observed. We did manage to measure the rescue frequency in our 20 μM tubulin condition and determined it to be 4.2 ± 1.6 min^{-1} (total of 7 rescues observed over 20 microtubule kymographs, 300 minutes of observed dynamics, of which 101 seconds spent in shrinkage), which is not significantly different from our 12 μM tubulin measurement. Based on these limited data, we cannot make any meaningful comments on tubulin-concentration dependence of rescue frequency, but we do note that our data are consistent with previous studies showing no obvious dependence of plus-end rescue frequency on tubulin concentration (see e.g. Walker et al., 1988, Fees and Moore, 2019, Arpağ et al., 2020).

Notably, in light of our new rescue frequency measurements, we realized that one of the kymographs originally shown in Figure 1A (now Figure S1A) was not representative with respect to the rescue frequency, and have thus replaced it with a kymograph representative of all dynamics parameters measured in this condition.

Finally, we are also including here an overloaded SDS-PAGE gel of our purified tubulin showing no significant bands other than the tubulin itself.



SDS-Page gel stained with coomassie. 200 μg of purified tubulin loaded

Fig 2A. Microtubule disassembly rate with no/low XMAP215 appears to be much lower than in other in vitro studies.
I am worried that there are some hidden (synergistic?) effects or technical deviations in this experimental system that need to be explained. Furthermore, these behaviors are very different from previous data by the same group (compare current Fig 1A with Fig 3B in Lawrence et al., 2018).

Please note that the imaging conditions in previous Figure 2A (now Figure 1A) used an acquisition rate of 5 frames per second (fps), which is significantly (25x) faster than the acquisition rate used in Figure 3 of Lawrence et al., 2018 (0.2 fps). Consequently, the aspect ratio of the kymograph is very different, clearly showing the full duration of the microtubule shrinkage occurring over several imaging frames, unlike in Figure 3 of Lawrence et al., 2018, as well as most other studies, where slow acquisition rate (typically 0.2 fps = 1 frame every 5 seconds) does not allow time-resolved imaging of microtubule shrinkage. To clarify this point, we have now added additional details of the imaging conditions in the Methods. Furthermore, we have now also measured the microtubule shrinkage rates for our experiments shown in previous Figure 2A (now Figure 1A), and found the mean shrinkage rates to be 230 ± 40 nm/s (SD, N=6 independent experimental repeats, 739 total shrinkage events analyzed) in the absence of XMAP215, and 370 ± 80 nm/s (SD, N=3 independent experimental repeats, 656 total shrinkage events analyzed) in the presence of low XMAP215 concentration (6.25 nM XMAP215). These rates are consistent with the previously-published microtubule shrinkage rates (see e.g. Lawrence et al., 2018, Walker et al., 1988, Arpağ et al., 2020). The observed increase in shrinkage rate with XMAP215 is also consistent with previous reports of XMAP215's effects on microtubule shrinkage (Vasquez et al., 1994)(Zanic et al., 2013). Thus, we conclude that our data show no deviations from the expected microtubule disassembly rates, as based on the previous literature.

2) It is difficult to follow the authors' interpretations for findings illustrated with Fig. 5. It is known from prior work that microtubules grow much faster in the presence of XMAP215. Is it surprising that they undergo catastrophes at higher growth rates? The second conclusion drawn by the authors is that catastrophes take place with "more EB1". This is hardly evident from these data, which show almost similar levels of EB1 at catastrophe. More striking feature of the disassembling XMAP215 end is a strong drop in EB1 intensity over a relatively long time (20 s) prior to catastrophe. The authors should discuss these various features and seek mechanistic explanations. One concern is that the loss of EB1 could affect XMAP215 activity (through a loss of synergistic interactions), leading to this unusual behavior, as already explained above.

Based on the Reviewers' and Editor's comments, we realized that our original motivation for and interpretation of the results in the original Figure 5 (now Figure 4) was not clear. The goal of this figure is to test the hypothesis that a specific threshold in the GTP-cap size needs to be reached as a prerequisite for catastrophe. The Reviewer is absolutely correct that microtubules will grow much faster in the presence of XMAP215. In the absence of XMAP215, it has been shown that microtubules undergo a significant slowdown period prior to catastrophe (see e.g. Maurer et al., 2014). This slowdown allows for the decay of the GTP-cap, and it has been proposed that a minimal cap density is required for microtubule stability (Duellberg et al., 2016). This GTP-cap threshold was shown to be independent of the growth rate measured during the microtubule growth phase significantly prior to the onset of catastrophe. Thus, regardless of how fast the microtubule initially grew, once the GTP-cap decay crosses the minimal threshold, catastrophe ensues (Duellberg et al., 2016). Our results show that the presence of XMAP215 changes this process, such that a microtubule can undergo catastrophe without having to slow down to the level necessary in the absence of XMAP215. Furthermore, we show that catastrophe in the

presence of XMAP215 occurs with significantly more EB1 sites still occupied, when compared to the EB1 levels observed in the absence of XMAP215. Thus, our results demonstrate that the presence of XMAP215 destabilizes the microtubule end and increases the minimal cap threshold needed for catastrophe.

To clarify these findings, we have now modified the previous Figure 5 (now Figure 4), as well as significantly clarified the accompanying text. Specifically, we have focused our figure on the last 10 seconds of microtubule growth prior to catastrophe, to draw attention that we are comparing instantaneous growth rates at catastrophe (i.e. measured only during the last 1 second prior to the catastrophe onset). We are now analyzing and showing traces obtained at the lowest XMAP215 concentration (3.13 nM), at which the effects of XMAP215 on growth rate acceleration are minimal, while the effects on catastrophe frequency have already reached their full magnitude (please see current Figure 1). As we originally demonstrated with higher XMAP215 concentrations, even with the lowest XMAP215 concentration we see that the instantaneous growth rate and the EB1 levels at the time of catastrophe are significantly larger in the absence of XMAP215 ($p < 0.001$ for instantaneous growth rate, and $p = 0.003$ for EB1 levels, unpaired Welch's t-test). We think that the ability of XMAP215 to induce microtubule catastrophe with a larger GTP-cap provides a significant insight into its molecular mechanism of action. To further illustrate our proposed model, we have now included a cartoon schematic as a new Figure 5.

More minor points:
3) Line 54: "early work demonstrated that increasing the microtubule growth rate by increasing the tubulin concentration in vitro is accompanied by a decrease in the catastrophe frequency (Walker et al., 1988)." This sentence does not do justice to a highly complex and controversial subject. Others found much smaller decrease (e.g. Gardner et al., 2011, Odde et al., 1995). It is also worth pointing out that Walker et al. findings at high tubulin concentration are based on a small data set, so referencing other similar studies would be appropriate.

Thank you for pointing this out; we very much appreciate the complexity of the subject. We have now added additional text and references to address this point (lines 53-58):

"Although the exact functional dependence and the extent of catastrophe suppression with increasing tubulin concentration have varied between subsequent reports, the finding that increase in tubulin concentration correlates with a decrease in catastrophe frequency remains generally supported (O'Brien et al., 1990)(Drechsel et al., 1992)(Odde et al., 1995)(Gardner et al., 2011)(Bowne-Anderson et al., 2013)(Piedra et al., 2016) (Chaaban et al., 2018)(Strothman et al., 2019)(Arpağ et al., 2020)…"

4) Fig. 4. EB1 blobs and curling at the microtubule plus end are certainly indicative of some abnormal morphology, but why does this Figure title refer to these ends as "tapered" (which usually means gradual thinning of the tip)?

We appreciate this concern and have put a lot of thought into what would be the most appropriate terminology for our observations. Our TIRF data demonstrate subsets of protofilaments protruding ahead of the core microtubule lattice to produce split and/or curled EB1 comets. This phenomenon, where some protofilaments protrude ahead of others has been widely referred to as 'tapered' ever since the early cryo-EM observations by Mandelkow et al., 1991 (see e.g. Aher and Akhmanova, 2018 for a more extensive review on this topic). Therefore, we think that the use of the term 'tapered' is most consistent with the existing literature on the topic.

5) According to Zhu et al., 2009, His-tagged EB1, as used in the current work, has somewhat different properties than EB1 with no charged His extension. Have authors confirmed that the His-tag does not impact their results?

We are aware of the Zhu et al., 2009 study, which suggested potential concentration-dependent effects of His-tagged EB1 proteins in bulk assays using very high EB1 concentrations (2-20 μM). In our study, we use a constant concentration of EB1-GFP (200 nM) across all conditions involving EB1. The main goal of our study is to investigate the effects of XMAP215 on microtubule catastrophe, and our data demonstrate that these do not depend on the presence of EB1. Overall, we do not think that studying the potential effects of His-tag on EB1 will contribute to the results of our study, as agreed by the editor.

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June 30, 2021

RE: JCB Manuscript #202012144R

Dr. Marija Zanic
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Dear Dr. Zanic:

Thank you for submitting your revised manuscript entitled "XMAP215 promotes microtubule catastrophe by disrupting the growing microtubule end". Reviewer #1 now supports publication, and while reviewer #2 was not available to re-review I have carefully read your responses to both reviewers and agree that you did an excellent job with the revisions. Therefore, we would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

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