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MATCAP1 preferentially binds an expanded tubulin conformation to generate detyrosinated and ΔC2 α-tubulin

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Review #1

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

Summary

This paper presents data evaluating the microtubule binding and activity of MAPCAP1, a metallopeptidase that cleaves the C-terminus of alpha tubulin when incorporated into microtubules (MT), and that both binding and activity are dependent on the expanded vs compacted state of the MT lattice. Known modifications of the terminus are removal of the terminal tyrosine (ΔY), or the terminal two residues (ΔC2), and authors show that MATCAP1 can perform both of these modifications, unlike the other known carboxypeptidase, VASH(1/2) / SVBP (hereafter: VASH), which only produces ΔY, and which was evaluated in a previous publication by the same authors. Authors use studies of cells (HeLa and engineered HeLa), as well as invitro studies with purified proteins, to show that MATCAP1 preferentially binds to the expanded MT lattice with long dwell time, and producing both ΔY and ΔC2, in contrast to VASH, which binds to both expanded and compacted lattices, binds with short dwell time and produces only ΔY.

Major comments

In general, the work is well thought out and well done. The context and background are clearly spelled out and well referenced. It is well-presented and doesn't go beyond the data (but see small point below). The methods are remarkably detailed and would be easy to follow, if one had the reagents that authors do.

Minor comments

Prior studies are well referenced. The text and figures are well composed and easy to follow.
.
A small point relates to Figure 6D, in which the production of ΔY is evaluated from MT from HeLa cells treated with DMSO or Taxol, in the absence or presence of EpoY, a VASH1/SVBP inhibitor. The point of the figure is that EpoY doesn't prevent all ΔY formation, therefore there must be another enzyme (i.e. MATCAP1) present to perform this. The problem is that it isn't clear, at first glance, that EpoY did anything. Closer inspection shows that the band for ΔY is dimmer in the lane with EpoY, but I missed it on first read. A further sample with cells lacking MATCAP1 would show that in that case the EpoY should knock out the ΔY completely.
I estimate that this will take less than one month to accomplish, if authors have a ΔMATCAP1 cell line.

2. Significance:

Significance (Required)

This paper is very well done - it clearly lays out the relevant questions and the progress of experiments, and then presents them clearly and interprets them clearly and in context.

It will be of interest to those who study MT structure, regulation, and post-translational modifications (PTM), such as myself. It will also be of interest to those who are concerned with the broader point of how enzyme activity is regulated by structural details of binding surfaces.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Less than 1 month

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No

Review #2

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

The manuscript by Yue et al. investigates the molecular mechanisms by which MATCAP1, a metalloprotease enzyme, selectively modifies microtubules through detyrosination and ΔC2 modifications of α-tubulin. Using sophisticated microscopy-based enzymatic assays with newly developed fluorescent Fab probes specific for ΔY- and ΔC2-tubulin modifications, the authors

demonstrate that MATCAP1 preferentially binds to microtubules in an expanded conformational state, achieved through preventing β-tubulin GTP hydrolysis (GMPCPP), Taxol treatment, or active kinesin-1 stepping, and exhibits remarkably long dwell times (>30 seconds) on these substrates. Through real-time visualization experiments in both cellular and reconstituted in vitro systems, they show that MATCAP1 sequentially removes amino acids from the α-tubulin C-terminal tail, generating ΔY modifications faster than ΔC2 modifications, and can operate independently of cytosolic carboxypeptidases. The findings significantly advance our understanding of tubulin post-translational modification regulation by revealing that microtubule lattice conformation serves as a critical gating mechanism for enzymatic activity. This work fits into the broader "tubulin code" framework, where specific combinations of post-translational modifications create functionally distinct microtubule populations that selectively recruit different motor proteins and microtubule-associated proteins. The discovery that MATCAP1's activity is conformationally gated provides a molecular explanation for how cells might spatially and temporally control tubulin modifications, complementing recent structural studies and adding to the growing understanding of how mechanical and conformational states of the cytoskeleton influence biochemical processes.
The paper should be of high interest to the cytoskeleton and cell biology communities and I support publication after the authors address a few concerns below.

Major points:

1. There is no explicit demonstration that the Fab probes do not bind to microtubules in the absence of MATCAP1 or VASH1/SVBP. It is critical, particularly for the ΔC2 Fab647 probe, to show that incubation for 10 minutes with the Fab but without added modifying enzyme results in negligible labeling. This would rule out two key confounders: (a) pre-existing ΔY or ΔC2 PTMs in the tubulin preparation, and (b) non-specific Fab probe binding.
2. Figure 3: The non-saturation of ΔC2-Fab647 signal over time suggests potential issues with probe specificity and/or Fab probe affinity. Controls in which the ΔC2 probe is incubated with microtubules lacking any enzyme treatment should be shown, ideally on the same batch/preparation of tubulin as used in experimental samples. The specificity and occupancy properties of these probes (especially ΔC2-Fab647) should be clarified.
3. It is unclear when and why the authors add MATCAP1 or VASH1/SVBP enzymes as cell lysates rather than as purified proteins. This raises some concerns
- Lysates can contain endogenous enzymes, nucleases, or additional PTM-modifying activities that may confound the interpretation of results. It would be more rigorous to demonstrate key findings with purified MATCAP1 and VASH1/SVBP proteins, at least as a confirmatory step.
- The justification for using lysates rather than purified proteins should be clearly stated in the Results and rationales provided in the Methods. If purified proteins were limiting, this should be acknowledged, and the purity and concentration of MATCAP1 in lysates should be quantified and controlled across experiments.
4. The major conclusions of the paper rest on rate measurements for fluorescently labeled MATCAP1 binding to microtubules with different PTM states. However, inspection of the kymographs shows that most particles appear stably bound before imaging starts, leading to some confusion. The authors must clarify in both the Results and Methods whether only particles landing de novo during image acquisition were counted when calculating landing rates. If so, the precise criteria used to define a "landing event" versus pre-bound molecules should be detailed.

Given the use of cell lysates with fluorophore-conjugated Halo ligand, a negative control is essential: add Halo ligand to lysates from cells never transfected with (or expressing) MATCAP1, then perform the same TIRF binding and landing analysis. This would control for non-specific labeling of cellular proteins and verify that any observed binding is genuinely due to fluorescent MATCAP1, not other endogenous or labeled species. Without this clarification, it is difficult to interpret the main results of lattice preference as measured by landing rates, although some of this concern is alleviated by average fluorescence measurements as in Fig. 5.

Minor comments:

1. Figure S4 examines MATCAP1 detachment from microtubules in response to buffer changes, but critical technical details are missing. The basis for calculating ionic strength (IS) across the different buffer conditions should be documented, including the formula used and all constituent ions considered. The abbreviation "IS" is not defined in the figure or legend and should be added.
2. The interpretation that ATP-bound KIF5C can promote an expanded microtubule lattice, enhancing MATCAP1 binding, is important to the authors' conclusions. Yet, there are no data presented to demonstrate that the purified KIF5C used is functionally active as a motor. The manuscript should report a direct activity assay showing that the KIF5C preparation is capable of ATP-driven stepping along microtubules, e.g., via gliding or single-molecule motility assays. If not performed, a statement should be added to justify the assumption of activity.

2. Significance:

Significance (Required)

This is a nice paper that should be of interest to a broad community of cell biologists and biochemists interested in the cytoskeleton and tubulin code. The reagents and assays developed will also be of interest to this community.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 3 and 6 months

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Yes

Review #3

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

This manuscript examines the mechanism of MATCAP1, a tubulin-modifying enzyme that removes residues from the C-terminal tails. The authors find that MATCAP1 generates both dY and dC2 tubulin using a combination of cell-based assays, in vitro assays with cell lysates, and in vitro assays with purified proteins. They find that MATCAP1 acts preferentially on microtubules in the expanded conformation, which is consistent with this team's recently-published work on VASH/SVBP, a tubulin-modifying assay that generated dY tubulin, suggesting a general mechanism for regulation of the dY and dC2 modifications. Overall, the manuscript is excellent, with high-quality data that strongly supports the central conclusions.

Concerns / Areas for Improvement

- The paper discusses the kinetics of MATCAP enzymatic activity in a few places. For example, in Figure 2, the authors show that the binding of their dY probe to microtubules is relatively fast (Fig. 2D), while the binding of their dC2 probe to microtubules is relatively slow (Fig. 2I). One obvious interpretation of this data is that the reaction is sequential: MATCAP first cleaves the Y (quickly) and then cleaves the E to generate dC2 (slowly). The Abstract and Introduction use the phrase "sequentially", although the authors do not make an explicit link between their data in Figure 2 and the concept of sequential cleavage. This is a minor writing issue. A more significant issue is how the data in Figure 2 interacts with the data in Figure 4. In Figure 4F, the binding of the dC2 probe is identical regardless of whether the microtubules were Y or dY to start with. I'm confused by this finding. If the microtubules are Y-MTs, then they need to first be cleaved to dY, then cleaved to dC2, and then the probe can bind. The data in Figure 4F can be explained a few ways. Option 1 is that the cleavage of the Y to generate the dY microtubules is really really really fast, such that it's not detectable in the binding kinetics of the dC2 probe. But this option is not consistent with Figure 2D and Figure 3D, which shows that the dY probe takes 10's to 100's of seconds to reach saturation. In other words, cleavage of the Y is fast, but it's not THAT fast. Option 2 to explain Figure 4F could be that the binding of the dC2 probe is actually rather slow, such that the kinetics of dC2 probe binding are a measure only of the probe's binding kinetics,

not the rate of generation of the epitope. However, if that's the case, then the data in Figure 2D and 3D is not sufficient to demonstrate the "MATCAP1 generates dY-MTs faster than dC2-MTs in vitro", because that conclusion interprets the slow binding of the dC2 probe as evidence of slow generation of epitope. In Figure S2, the authors measure the binding kinetics of the probes to pre-cleaved MTs. Indeed, the binding of the dC2 probe does appear slower than binding of the dY probe -- but also that the dC2 probe never reaches saturation, even after 1 hour of incubation.

I hope the paragraph above makes sense -- it's hard to describe kinetics w/out just writing down a reaction scheme. But I do believe there is an issue to resolve. Either the dC2 probe is very slow and can't reach saturation for some reason--in which case the title of Figure 3 is not support. Alternatively, the binding of the probe is not very slow, in which case Figure 4F doesn't make sense to me. Perhaps the authors would benefit from writing down the kinetic scheme for a sequential cleavage reaction, including probe binding. The curves they have generated (e.g. in Figure S2B) might then be sufficient to see what's going on.
- Some experiments in the paper are performed with MATCAP1 in cell lysates while other experiments used purified proteins. The rationale for which experiments used which system were unclear to me. At key points, the switch to purified protein was described as a way to exclude potential artifacts that come from the lysate (e.g. another MAP that is present, etc). But why is that a concern in some experiments but not others? Maybe the lysate is actually advantageous? The paper would benefit from a clearer rationale and explanation for the switch between the different systems.
- Similarly, the authors use a catalytically-inactive MATCAP late in the paper (Figure 5)--could the E281Q construct be used elsewhere to measure, e.g., in the experiments using Y vs. dY-MTs? This suggestion is not essential but might strengthen the idea that the Y-status of the lattice is not important to recruitment.
- In Figure 5D, the results show that MATCAP binding to GMPCPP microtubules is higher relative to Taxol-stabilized microtubules, even though (presumably) both microtubules are fully expanded. Does this finding suggest there are other structural features besides expansion that are at work? What could those features be?

2. Significance:

Significance (Required)

Overall, only the first of the concerns above is important relative to the experimental support for the authors' conclusions, namely the conclusion stated in the title of Figure 3. Otherwise, it's an outstanding paper that will make an important contribution to our understanding of how the tubulin code is regulated.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 1 and 3 months

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Yes

We thank the reviewers for their time and effort in reviewing our manuscript and for their many helpful suggestions for improving the strength of our conclusions as well as the presentation of the data. Below we provide a point-by-point response to the reviewers comments with our response in blue text. Changes to the manuscript text are also indicated by blue text.

Reviewer #1

Evidence, reproducibility and clarity

Summary
This paper presents data evaluating the microtubule binding and activity of MAPCAP1, a metallopeptidase that cleaves the C-terminus of alpha tubulin when incorporated into microtubules (MT), and that both binding and activity are dependent on the expanded vs compacted state of the MT lattice. Known modifications of the terminus are removal of the terminal tyrosine (ΔY), or the terminal two residues (ΔC2), and authors show that MATCAP1 can perform both of these modifications, unlike the other known carboxypeptidase, VASH(1/2) / SVBP (hereafter: VASH), which only produces ΔY, and which was evaluated in a previous publication by the same authors. Authors use studies of cells (HeLa and engineered HeLa), as well as invitro studies with purified proteins, to show that MATCAP1 preferentially binds to the expanded MT lattice with long dwell time, and producing both ΔY and ΔC2, in contrast to VASH, which binds to both expanded and compacted lattices, binds with short dwell time and produces only ΔY.

Major comments
In general, the work is well thought out and well done. The context and background are clearly spelled out and well referenced. It is well-presented and doesn't go beyond the data (but see small point below). The methods are remarkably detailed and would be easy to follow, if one had the reagents that authors do.

Response: We thank the reviewer for these positive comments.

Minor comments
Prior studies are well referenced. The text and figures are well composed and easy to follow.

A small point relates to Figure 6D, in which the production of ΔY is evaluated from MT from HeLa cells treated with DMSO or Taxol, in the absence or presence of EpoY, a VASH1/SVBP inhibitor. The point of the figure is that EpoY doesn't prevent all ΔY formation, therefore there must be another enzyme (i.e. MATCAP1) present to perform this. The problem is that it isn't clear, at first glance, that EpoY did anything. Closer inspection shows that the band for ΔY is dimmer in the lane with EpoY, but I missed it on first read. A further sample with cells lacking MATCAP1 would show that in that case the EpoY should knock out the ΔY completely. I

estimate that this will take less than one month to accomplish, if authors have a ΔMATCAP1 cell line.

Response: The reviewer raises an important point concerning the activity of EpoY. As we currently do not have cell lines lacking MATCAP1, we considered an alternative approach to address the reviewer's concern. Instead of EpoY to abolish the activity of VASH1 and VASH2, we used CHL-1 cells in which the expression of both VASH1 and VASH2 has been eliminated [ΔVASH1/2 double knockout (DKO) cells]. Although detyrosination activity was dramatically decreased in the ΔVASH1/2 DKO cells, a small but observable increase in ΔY-microtubules was observed after treatment with Taxol for 1 and 18 h (**new Figure 6D**). This result indicates that microtubule expansion mediated by Taxol treatment regulates the detyrosination activity of MATCAP1.

This result is consistent with previous work showing that the VASH enzymes are major contributors to the detyrosination of microtubules in CHL-1 cells (Niewenhuis et al 2017). This result is also consistent with previous work showing that Taxol treatment causes a small by measurable increase in the levels of ΔY-microtubules in ΔVASH1/2 double knockout cells but not in ΔVASH1/2 and ΔMATCAP1 triple knockout cells (Landskron et al., 2022).

In the revised manuscript, we have included the new data, rewritten the Results section, and added supporting references.

Significance

This paper is very well done - it clearly lays out the relevant questions and the progress of experiments, and then presents them clearly and interprets them clearly and in context.

It will be of interest to those who study MT structure, regulation, and post-translational modifications (PTM), such as myself. It will also be of interest to those who are concerned with the broader point of how enzyme activity is regulated by structural details of binding surfaces.

Reviewer #2

Evidence, reproducibility and clarity

The manuscript by Yue et al. investigates the molecular mechanisms by which MATCAP1, a metalloprotease enzyme, selectively modifies microtubules through detyrosination and ΔC2 modifications of α-tubulin. Using sophisticated microscopy-based enzymatic assays with newly developed fluorescent Fab probes specific for ΔY- and ΔC2-tubulin modifications, the authors demonstrate that MATCAP1 preferentially binds to microtubules in an expanded conformational state, achieved through preventing β-tubulin GTP hydrolysis (GMPCPP), Taxol treatment, or active kinesin-1 stepping, and exhibits remarkably long dwell times (>30 seconds) on these substrates. Through real-time visualization experiments in both cellular and reconstituted in vitro systems, they show that MATCAP1 sequentially removes amino acids from the α-tubulin C-terminal tail, generating ΔY modifications faster than ΔC2 modifications, and can operate independently of cytosolic carboxypeptidases. The findings significantly advance our

understanding of tubulin post-translational modification regulation by revealing that microtubule lattice conformation serves as a critical gating mechanism for enzymatic activity. This work fits into the broader "tubulin code" framework, where specific combinations of post-translational modifications create functionally distinct microtubule populations that selectively recruit different motor proteins and microtubule-associated proteins. The discovery that MATCAP1's activity is conformationally gated provides a molecular explanation for how cells might spatially and temporally control tubulin modifications, complementing recent structural studies and adding to the growing understanding of how mechanical and conformational states of the cytoskeleton influence biochemical processes.

The paper should be of high interest to the cytoskeleton and cell biology communities and I support publication after the authors address a few concerns below.

Response: We thank the reviewer for these positive comments.

Major points:

1. There is no explicit demonstration that the Fab probes do not bind to microtubules in the absence of MATCAP1 or VASH1/SVBP. It is critical, particularly for the ΔC2 Fab647 probe, to show that incubation for 10 minutes with the Fab but without added modifying enzyme results in negligible labeling. This would rule out two key confounders: (a) pre-existing ΔY or ΔC2 PTMs in the tubulin preparation, and (b) non-specific Fab probe binding.

Response: We thank the reviewer for this important comment. We believe that our original Figure 2D provides this critical control data for the ΔY-Fab, demonstrating that the Fab does not bind to microtubules in the absence of the detyrosination enzymes. In the revised version, we have carried out the requested experiments for the ΔC2-Fab. We now show that the ΔC2-Fab does not bind to microtubules in the absence of the MATCAP1 enzyme (**new Figure 2I**).

To make these critical controls more explicit, we have added representative images and kymographs showing negligible binding of both ΔY-Fab and ΔC2-Fab probes to microtubules in untransfected cell lysate controls (**new Figure EV2**). These data rule out the possibility of pre-existing PTMs in our tubulin preparation and non-specific Fab binding confounding our results and interpretations.

2. Figure 3: The non-saturation of ΔC2-Fab647 signal over time suggests potential issues with probe specificity and/or Fab probe affinity. Controls in which the ΔC2 probe is incubated with microtubules lacking any enzyme treatment should be shown, ideally on the same batch/preparation of tubulin as used in experimental samples. The specificity and occupancy properties of these probes (especially ΔC2-Fab647) should be clarified.

Response: As addressed in our response to the reviewer's point #1 (above), the controls in which Fab probes were incubated with microtubules lacking any enzyme treatment (using untransfected cell lysates) are provided in Figure 2D, the **new Figure 2I**, and the **new Figure**

EV2. We note that the HeLa tubulin used for both control and experimental samples was from the same batch and preparation and has been validated to lack pre-existing ΔY and ΔC2 PTMs (see Figure 10 of Thomas et al., 2025).

The specificity of the ΔY-Fab probe for ΔY-α-tubulin can be found in Yue et al 2023 and the specificity of the ΔC2-Fab probe for ΔC2-α-tubulin can be found in Figure 2F.

The occupancy and affinity of the Fab probes are illustrated in the supplemental figures. Figure EV4A,B (previously Figure S3A,B) shows that the presence of the ΔY-Fab probe does not affect the occupancy of the ΔC2-Fab probe. Figure EV4C,D (previously Figure S3C,D) shows that both probes display rapid binding to and unbinding from modified microtubules at the single molecule level. Figure EV4E-J (previously Figure S3E,F) shows that both ΔY-Fab and ΔC2-Fab probes rapidly saturate pre-modified microtubules. Thus, the non-saturation of the DC2-Fab probe when incubated with microtubules and low concentrations of MATCAP1 or for shorter periods of time reflects the enzymatic activity of MATCAP1 rather than probe binding kinetics.

3. It is unclear when and why the authors add MATCAP1 or VASH1/SVBP enzymes as cell lysates rather than as purified proteins. This raises some concerns - Lysates can contain endogenous enzymes, nucleases, or additional PTM-modifying activities that may confound the interpretation of results. It would be more rigorous to demonstrate key findings with purified MATCAP1 and VASH1/SVBP proteins, at least as a confirmatory step.

- The justification for using lysates rather than purified proteins should be clearly stated in the Results and rationales provided in the Methods. If purified proteins were limiting, this should be acknowledged, and the purity and concentration of MATCAP1 in lysates should be quantified and controlled across experiments.

Response: We thank the reviewer for raising this important point. Cell lysates are much easier to obtain than purified proteins, and their use in in vitro assays is well-established in the field (e.g. Cai et al 2007, Cai et al 2009, Norris et al 2014, Fenton et al 2021, Jijumon et al 2022, Torvi et al 2022, Yue et al 2023). However, we agree that their usage comes with some concerns. We started this study using cell lysate to examine MATCAP1 activity to validate of the probes, compare MATCAP1 activity to VASH1/SVBP, and examine MATCAP1 binding to Y-microtubules vs ΔY-microtubules (Figures 1-4). Even in these experiments, we considered the potential for lysate-induced artifacts. For example, in comparing the enzymatic properties of MATCAP1 to VASH1/SVBP, we included untransfected cell lysate as a control (e.g., Figures 2D, 4B, and **new Figures 2I and EV2**). We were careful to quantify the amount of MATCAP1 or VASH1/SVBP in the lysates and to report the concentration of enzyme added to each experiment.

Once we obtained preliminary data indicating that MATCAP1 binding is sensitive to the expanded vs compacted state of the microtubule lattice, we proceeded to demonstrate these key findings with purified MATCAP1 protein (Figure 5 and Figure EV6).

4. The major conclusions of the paper rest on rate measurements for fluorescently labeled MATCAP1 binding to microtubules with different PTM states. However, inspection of the kymographs shows that most particles appear stably bound before imaging starts, leading to some confusion. The authors must clarify in both the Results and Methods whether only particles landing de novo during image acquisition were counted when calculating landing rates. If so, the precise criteria used to define a "landing event" versus pre-bound molecules should be detailed. Given the use of cell lysates with fluorophore-conjugated Halo ligand, a negative control is essential: add Halo ligand to lysates from cells never transfected with (or expressing) MATCAP1, then perform the same TIRF binding and landing analysis. This would control for non-specific labeling of cellular proteins and verify that any observed binding is genuinely due to fluorescent MATCAP1, not other endogenous or labeled species. Without this clarification, it is difficult to interpret the main results of lattice preference as measured by landing rates, although some of this concern is alleviated by average fluorescence measurements as in Fig. 5.

Response: We thank the reviewer for these suggestions.

With respect to the suggestion that "The authors must clarify in both the Results and Methods whether only particles landing de novo during image acquisition were counted when calculating landing rates. If so, the precise criteria used to define a "landing event" versus pre-bound molecules should be detailed.": We have clarified in the Results and Methods sections that the landing rate includes all MATCAP1 binding events observed on the microtubules, specifically those which occurred before image acquisition and those that occurred during image acquisition.

With respect to the suggestion that "Given the use of cell lysates with fluorophore-conjugated Halo ligand, a negative control is essential: add Halo ligand to lysates from cells never transfected with (or expressing) MATCAP1, then perform the same TIRF binding and landing analysis.": We have carried out the requested experiment (**new Figure 4B**). We find that no microtubule binding was observed when using untransfected cell lysate containing Halo JFX554 ligand.

Minor comments:

1. Figure S4 examines MATCAP1 detachment from microtubules in response to buffer changes, but critical technical details are missing. The basis for calculating ionic strength (IS) across the different buffer conditions should be documented, including the formula used and all constituent ions considered. The abbreviation "IS" is not defined in the figure or legend and should be added.

Response: We thank the reviewer for raising this point and apologize for the omission of technical details in the original manuscript. In the revised manuscript, we have added the details of the ionic strength calculation to the Methods section. The definition of the abbreviation 'IS' has been added in the figure legend of Figure EV5 (previously Figure S4).

2. The interpretation that ATP-bound KIF5C can promote an expanded microtubule lattice, enhancing MATCAP1 binding, is important to the authors' conclusions. Yet, there are no data presented to demonstrate that the purified KIF5C used is functionally active as a motor. The manuscript should report a direct activity assay showing that the KIF5C preparation is capable of ATP-driven stepping along microtubules, e.g., via gliding or single-molecule motility assays. If not performed, a statement should be added to justify the assumption of activity.

Response: We thank the reviewer for this comment. Per the reviewer's suggestion, we carried out microtubule gliding assays with the same preparation of purified KIF5C(1-560)-Halo-TwinStrep protein that was used in Figure 5. The data demonstrate that the purified KIF5C is an active motor (**new Figure EV7**).

Significance

This is a nice paper that should be of interest to a broad community of cell biologists and biochemists interested in the cytoskeleton and tubulin code. The reagents and assays developed will also be of interest to this community.

Reviewer #3

Evidence, reproducibility and clarity (Required)):

This manuscript examines the mechanism of MATCAP1, a tubulin-modifying enzyme that removes residues from the C-terminal tails. The authors find that MATCAP1 generates both dY and dC2 tubulin using a combination of cell-based assays, in vitro assays with cell lysates, and in vitro assays with purified proteins. They find that MATCAP1 acts preferentially on microtubules in the expanded conformation, which is consistent with this team's recently-published work on VASH/SVBP, a tubulin-modifying assay that generated dY tubulin, suggesting a general mechanism for regulation of the dY and dC2 modifications. Overall, the manuscript is excellent, with high-quality data that strongly supports the central conclusions.

Concerns / Areas for Improvement

- The paper discusses the kinetics of MATCAP enzymatic activity in a few places. For example, in Figure 2, the authors show that the binding of their dY probe to microtubules is relatively fast (Fig. 2D), while the binding of their dC2 probe to microtubules is relatively slow (Fig. 2I). One obvious interpretation of this data is that the reaction is sequential: MATCAP first cleaves the Y (quickly) and then cleaves the E to generate dC2 (slowly). The Abstract and Introduction use the phrase "sequentially", although the authors do not make an explicit link between their data in Figure 2 and the concept of sequential cleavage. This is a minor writing issue.

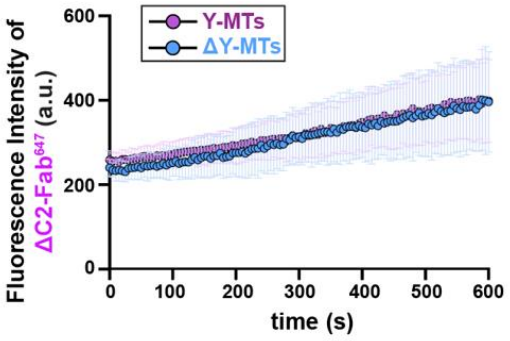
Response: We thank the reviewer for these thoughtful and insightful comments. We have added new text (last paragraph on p. 6) to make an explicit link between the data in Figure 2

and the concept of sequential cleavage. We have also added a schematic of the sequential cleavage reaction (**new Figure EV3A**).

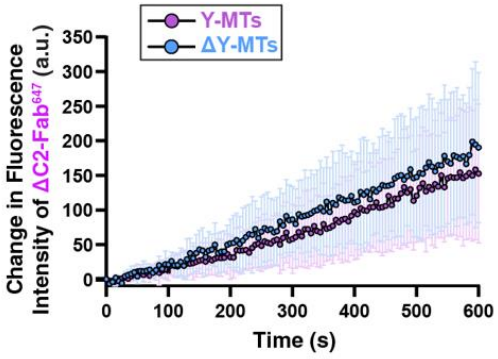
A more significant issue is how the data in Figure 2 interacts with the data in Figure 4. In Figure 4F, the binding of the dC2 probe is identical regardless of whether the microtubules were Y or dY to start with. I'm confused by this finding. If the microtubules are Y-MTs, then they need to first be cleaved to dY, then cleaved to dC2, and then the probe can bind.

Response: We also expected there to be a delay in ΔC2-Fab decoration of Y-microtubules compared to ΔY-microtubules and thus we also struggled with understanding the data in the previous Figure 4F (**now Figure 4H**). The reviewer's comments urged us undertake a careful review and re-analysis of our data. The previous data showed the mean fluorescence intensity of ΔC2-Fab on Y- and ΔY-microtubules over time. However, our review of the data showed that the results were confounded by day-to-day background variation and experimental noise. To address this, we re-analyzed the data to normalize of the mean fluorescence intensity of the ΔC2-Fab in each frame against the mean intensity measured in the first frame. This brings the analysis in line with the way the data are collected, analyzed, and reported in Figure 3D. Our **new Figure 4H** shows that the increase in mean fluorescence intensity of the ΔC2-Fab along Y-microtubules exhibits a slight lag compared to ΔY-microtubules in the presence of MATCAP1:

Original Figure 4F



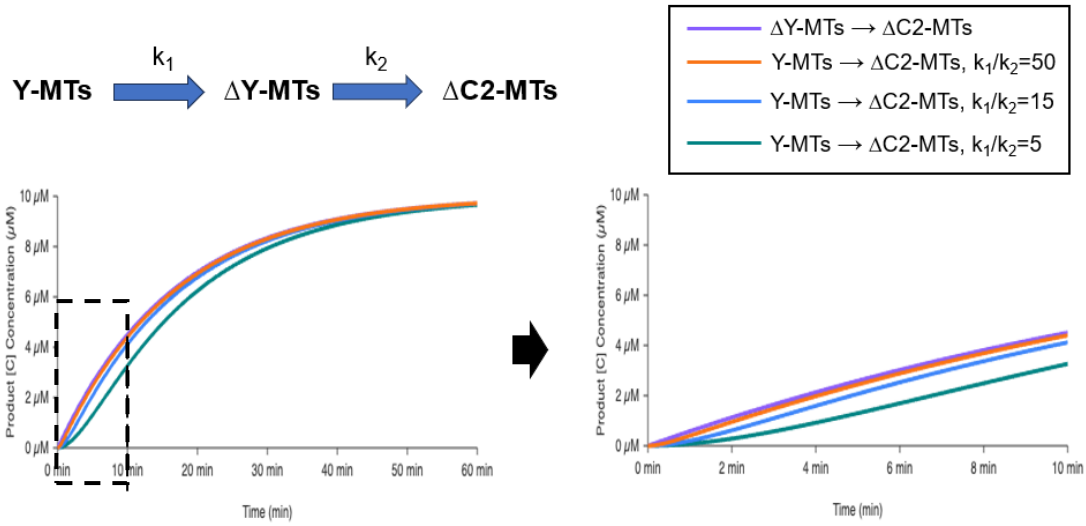
Revised Figure 4H



The data in Figure 4F can be explained a few ways. Option 1 is that the cleavage of the Y to generate the dY microtubules is really really really fast, such that it's not detectable in the binding kinetics of the dC2 probe. But this option is not consistent with Figure 2D and Figure 3D, which shows that the dY probe takes 10's to 100's of seconds to reach saturation. In other words, cleavage of the Y is fast, but it's not THAT fast.

To investigate this, we performed a simulation of the sequential enzymatic reactions (see figure below). Here, k_1 is the rate constant for the first reaction (Y → ΔY), and k_2 is the rate constant for the second reaction (ΔY → ΔC2). If the first reaction is much much faster than the second reaction (k_1/k_2 ratio = 50), then the curve for MATCAP1 activity starting with Y-MTs (Y → ΔC2, orange line) is similar to the curve for MATCAP1 activity starting with ΔY-MTs (ΔY → ΔC2, purple line). However, if the first reaction is only a bit faster than the second reaction (k_1/k_2 ratio

= 5), then the curve for MATCAP1 activity starting with Y-MTs (Y → ΔC2, green line) shows a significant delay compared to the curve for MATCAP1 activity starting with ΔY-MTs (ΔY → ΔC2, purple line).



We used our data shown in Figure EV3B,C (previously Figure S2) to approximate the k_1/k_2 ratio. In this assay, the levels of ΔY and ΔC2 modifications on HeLa microtubules were measured following the addition of high concentrations (4.2 nM) of MATCAP1 during 1 h of imaging. The fluorescence intensity of the ΔY-Fab plateaus at around 5 min, while the fluorescence intensity of the ΔC2-Fab continues to increase throughout the 60 min imaging period. These results suggest that the k_1/k_2 ratio is greater than 12. If we use a k_1/k_2 ratio = 15 in the simulations, then the curve for MATCAP1 activity starting with Y-MTs (Y → ΔC2, blue line) shows a slight delay compared to the curve for MATCAP1 activity starting with ΔY-MTs (ΔY → ΔC2, purple line). Thus, the simulation results support our experimental findings (revised Figure 4H) showing that there is only a slight delay in the generation of ΔC2-microtubules when starting from ΔY-microtubules as compared to Y-microtubules.

Option 2 to explain Figure 4F could be that the binding of the dC2 probe is actually rather slow, such that the kinetics of dC2 probe binding are a measure only of the probe's binding kinetics, not the rate of generation of the epitope. However, if that's the case, then the data in Figure 2D and 3D is not sufficient to demonstrate the "MATCAP1 generates dY-MTs faster than dC2-MTs in vitro", because that conclusion interprets the slow binding of the dC2 probe as evidence of slow generation of epitope. In Figure S2, the authors measure the binding kinetics of the probes to pre-cleaved MTs. Indeed, the binding of the dC2 probe does appear slower than binding of the dY probe -- but also that the dC2 probe never reaches saturation, even after 1 hour of incubation.

Response: In the previous Figure S2 (now Figure EV3B,C), we did not use pre-cleaved microtubules. Rather, this experiment used HeLa microtubules and both probes to directly compare the ability of MATCAP1 to generate the ΔY and ΔC2 modifications. The point of Figure

EV3B,C is to examine DC2 generation at higher levels (4.2 nM) and longer times (60 min) of MATCAP1 enzyme than is shown in Figure 3 (1 nM of MATCAP1 over 10 min).

In the previous Figure S3 (now Figure EV4C-J), we measured the binding kinetics of the probes to pre-cleaved microtubules. On microtubules with low levels of modifications, both probes under rapid binding and unbinding events to their respective microtubules (Figure EV4C,D). On microtubules with high levels of modifications, both probes rapidly (within 2 min) saturate binding to their respective microtubules (Figure EV4I,J).

In the revised manuscript, we have rewritten the statement to highlight this property of the Fab probes and have included representative images showing Fab labeling of the pre-cleaved ΔY- and ΔC2-microtubules at 0 min and 10 min after Fab probe addition for clarification (new Figure EV4E,F).

I hope the paragraph above makes sense -- it's hard to describe kinetics w/out just writing down a reaction scheme. But I do believe there is an issue to resolve. Either the dC2 probe is very slow and can't reach saturation for some reason--in which case the title of Figure 3 is not support. Alternatively, the binding of the probe is not very slow, in which case Figure 4F doesn't make sense to me. Perhaps the authors would benefit from writing down the kinetic scheme for a sequential cleavage reaction, including probe binding. The curves they have generated (e.g. in Figure S2B) might then be sufficient to see what's going on.

Response: We again thank the reviewer for these thoughtful and insightful comments. In the revised manuscript, we have included a kinetic scheme illustrating the sequential cleavage reaction of MATCAP1 on microtubules including probe binding (**new Figure EV3A**).

- Some experiments in the paper are performed with MATCAP1 in cell lysates while other experiments used purified proteins. The rationale for which experiments used which system were unclear to me. At key points, the switch to purified protein was described as a way to exclude potential artifacts that come from the lysate (e.g. another MAP that is present, etc). But why is that a concern in some experiments but not others? Maybe the lysate is actually advantageous? The paper would benefit from a clearer rationale and explanation for the switch between the different systems.

Response: We thank the reviewer for raising this important point. Cell lysates are much easier to obtain than purified proteins, and their use in in vitro assays is well-established in the field (e.g. Cai et al 2007, Cai et al 2009, Norris et al 2014, Fenton et al 2021, Jijumon et al 2022, Torvi et al 2022, Yue et al 2023). However, we agree that their usage comes with some concerns. We started this study using cell lysate to examine MATCAP1 activity to validate of the probes, compare MATCAP1 activity to VASH1/SVBP, and examine MATCAP1 binding to Y-microtubules vs ΔY-microtubules (Figures 1-4). Even in these experiments, we considered the potential for lysate-induced artifacts. For example, in comparing the enzymatic properties of MATCAP1 to VASH1/SVBP, we included untransfected cell lysate as a control (e.g., Figures 2D, 4B, and **new Figures 2I and EV2**). We were careful to quantify the amount of MATCAP1 or

VASH1/SVBP in the lysates and to report the concentration of enzyme added to each experiment.

Once we obtained preliminary data indicating that MATCAP1 binding is sensitive to the expanded vs compacted state of the microtubule lattice, we proceeded to demonstrate these key findings with purified MATCAP1 protein (Figure 5 and Figure EV6).

- Similarly, the authors use a catalytically-inactive MATCAP late in the paper (Figure 5)--could the E281Q construct be used elsewhere to measure, e.g., in the experiments using Y vs. dY-MTs? This suggestion is not essential but might strengthen the idea that the Y-status of the lattice is not important to recruitment.

Response: We thank the reviewer for this suggestion. We carried out new experiments to examine the microtubule binding of WT and E281Q mNG-MATCAP1 (**new Figure 4D,E**). We find that both WT and E281Q mNG-MATCAP1 show similar affinities for Y-microtubules and ΔY-microtubules. We agree that these results strengthen our conclusion that the microtubule binding behavior of MATCAP1 is not affected by the tyrosination state of the lattice.

- In Figure 5D, the results show that MATCAP binding to GMPCPP microtubules is higher relative to Taxol-stabilized microtubules, even though (presumably) both microtubules are fully expanded. Does this finding suggest there are other structural features besides expansion that are at work? What could those features be?

Response: The reviewer raises an excellent point. We have added text to the Discussion noting that several differences have been reported between GMPCPP microtubules and Taxol-stabilized microtubules that could affect the binding and activity of microtubule-modifying enzymes. For example, GMPCPP microtubules are more expanded, contain more protofilaments, and exhibit a more extensive degree of positive twist (Alushin et al 2014, Zhang et al 2015, LaFrance et al 2019). All of these features could potentially affect MATCAP1 binding. Further investigation is needed to determine whether these properties act synergistically with lattice expansion to contribute to the observed differences in MATCAP1 microtubule affinity.

Reviewer #3 (Significance (Required)):

Overall, only the first of the concerns above is important relative to the experimental support for the authors' conclusions, namely the conclusion stated in the title of Figure 3. Otherwise, it's an outstanding paper that will make an important contribution to our understanding of how the tubulin code is regulated.

Literature Cited

Alushin, G. M., Lander, G. C., Kellogg, E. H., Zhang, R., Baker, D. and Nogales, E. (2014). High-resolution microtubule structures reveal the structural transitions in alpha/beta-tubulin upon GTP hydrolysis. *Cell*, 157, 1117-1129.

Cai, D., Verhey, K. J., & Meyhöfer, E. (2007). Tracking single Kinesin molecules in the cytoplasm of mammalian cells. *Biophysical journal*, *92*(12), 4137–4144.

Cai, D., McEwen, D. P., Martens, J. R., Meyhofer, E., & Verhey, K. J. (2009). Single molecule imaging reveals differences in microtubule track selection between Kinesin motors. *PLoS biology*, *7*(10), e1000216.

Fenton, A. R., Cason, S. E., & Holzbaur, E. L. F. (2023). Single-Molecule Studies of Motor Adaptors Using Cell Lysates. *Methods in molecular biology 2623*, 97–111.

Hammond, J. W., Cai, D., Blasius, T. L., Li, Z., Jiang, Y., Jih, G. T., Meyhofer, E., & Verhey, K. J. (2009). Mammalian Kinesin-3 motors are dimeric in vivo and move by processive motility upon release of autoinhibition. *PLoS biology*, *7*(3), e72.

Jijumon, A. S., Bodakuntla, S., Genova, M., Bangera, M., Sackett, V., Besse, L., Maksut, F., Henriot, V., Magiera, M. M., Sirajuddin, M., & Janke, C. (2022). Lysate-based pipeline to characterize microtubule-associated proteins uncovers unique microtubule behaviours. *Nature cell biology*, *24*(2), 253–267.

LaFrance, B. J., Roostalu, J., Henkin, G., Greber, B. J., Zhang, R., Normanno, D., McCollum, C. O., Surrey, T. and Nogales, E. (2022). Structural transitions in the GTP cap visualized by cryo-electron microscopy of catalytically inactive microtubules. *Proc Natl Acad Sci U S A*, 119, e2114994119.

Landskron, L., Bak, J., Adamopoulos, A., Kaplani, K., Moraiti, M., van den Hengel, L. G., Song, J. Y., Bleijerveld, O. B., Nieuwenhuis, J., Heidebrecht, T., Henneman, L., Moutin, M. J., Barisic, M., Taraviras, S., Perrakis, A. and Brummelkamp, T. R. (2022). Posttranslational modification of microtubules by the MATCAP detyrosinase. *Science*, 376, eabn6020.

Nieuwenhuis, J., Adamopoulos, A., Bleijerveld, O. B., Mazouzi, A., Stickel, E., Celie, P., Altelaar, M., Knipscheer, P., Perrakis, A., Blomen, V. A. and Brummelkamp, T. R. (2017). Vasohibins encode tubulin detyrosinating activity. *Science*, 358, 1453-1456.

Norris, S. R., Soppina, V., Dizaji, A. S., Schimert, K. I., Sept, D., Cai, D., Sivaramakrishnan, S., & Verhey, K. J. (2014). A method for multiprotein assembly in cells reveals independent action of kinesins in complex. *The Journal of cell biology*, *207*(3), 393–406.

Thomas, E. C., Yue, Y., Pimm, M. L., Hotta, T., Ohi, R. and Verhey, K. J. (2025). Purification, fluorescent labelling, and detyrosination of mammalian cell tubulin for biochemical assays. *Cytoskeleton,* 1–19.

Torvi, J. R., Wong, J., Serwas, D., Moayed, A., Drubin, D. G., & Barnes, G. (2022). Reconstitution of kinetochore motility and microtubule dynamics reveals a role for a kinesin-8 in establishing end-on attachments. *eLife*, *11*, e78450.

Yue, Y., Hotta, T., Higaki, T., Verhey, K. J. and Ohi, R. (2023). Microtubule detyrosination by VASH1/SVBP is regulated by the conformational state of tubulin in the lattice. *Curr Biol*, 33, 4111-4123.

Zhang, R., LaFrance, B. and Nogales, E. (2018). Separating the effects of nucleotide and EB binding on microtubule structure. *Proc Natl Acad Sci U S A*, 115, E6191-E6200.

Dear Kristen,

Thank you for submitting your fully revised Review Commons manuscript to The EMBO Journal. I apologise for the delays in the assessment process due to delays in reviewer report submission and the standard revision checks that we have to perform on revised manuscripts at the pre-decision stage.

I have now received positive re-assessment from the original reviewer #2. I have gone through your responses to the comments by other reviewers and find them generally reasonable. Therefore, there now remain only a few editorial points that need to be addressed before I can extend official acceptance of the manuscript:

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With kind regards,

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Referee #2:

I am satisfied that the authors have fully addressed my concerns regarding experimental rigor and reproducibility. The addition of the 'no-enzyme' negative controls explicitly demonstrates the specificity of the Fab probes, and the new kinetic data on pre-cleaved microtubules effectively resolves the ambiguity regarding signal saturation, confirming that the probes are reliable reporters of enzymatic activity. Furthermore, the use of purified proteins to validate the lattice-preference mechanism significantly strengthens the study's central conclusions. The manuscript is now very solid in my opinion, and I support its publication in the EMBO J.

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Rev_Com_number: RC-2025-03171
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Corr_author: Verhey
Title: MATCAP1 preferentially binds an expanded tubulin conformation to generate detyrosinated and ΔC2 a-tubulin

The authors addressed the remaining editorial issues.

Dear Kristen,

Thank you for incorporating the final editorial and formatting requests in the manuscript. I apologise for the slow process from our side due to the large number of revised submissions that we currently receive. I am now pleased to inform you that your manuscript has been accepted for publication. Congratulations with a nice study!

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Best wishes,

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Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Yes	MIBBI information is available in the Materials and Methods section. ICMJE information is available in the Author Contributions and Disclosure and Competing Interests Statement sections.
For **tumor marker prognostic studies,** we recommend that you follow the **REMARK** reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
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Data Availability

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