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Microtubule detyrosination by VASH1/SVBP is regulated by the conformational state of tubulin in the lattice

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SUMMARY

Tubulin, a heterodimer of α - and β -tubulin, is a GTPase that assembles into microtubule (MT) polymers whose dynamic properties are intimately coupled to nucleotide hydrolysis. In cells, the organization and dynamics of MTs are further tuned by post-translational modifications (PTMs), which control the ability of MT-associated proteins (MAPs) and molecular motors to engage MTs. Detyrosination is a PTM of α -tubulin, wherein its C-terminal tyrosine residue is enzymatically removed by either the Vasohibin (VASH) or MATCAP peptidases. How these enzymes generate specific patterns of MT detyrosination in cells is not known. Here, we use a novel antibody-based probe to visualize the formation of detyrosinated MTs in real-time and employ single-molecule imaging of VASH1 bound to its regulatory partner SVBP to understand the process of MT detyrosination *in vitro* and in cells. We demonstrate that the activity, but not binding, of VASH1/SVBP is much greater on mimics of GTP-MTs than on GDP-MTs. Given emerging data showing that tubulin subunits in GTP-MTs are in expanded conformation relative to tubulin subunits in GDP-MTs, we reasoned that the lattice conformation of MTs is a key factor that gates the activity of VASH1/SVBP. We show that taxol, a drug known to expand

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AUTHOR CONTRIBUTIONS

Y.Y., T.H., K.V., and R.O. conceived the project. Y.Y. analyzed the behavior and activity of VASH1/SVBP on MTs using TIRF microscopy. Y.Y. also analyzed the effect of CAMSAP2 on MT detyrosination *in vitro*. T.Higaki analyzed the effect of taxol on MT detyrosination by deep learning computation. T.H. generated the MT detyrosination Δ Y-Fab⁴⁸⁸ probe, and examined MT detyrosination in cells. K.V. and T.H. analyzed the effect of CAMSAP proteins on MT detyrosination in cells. Y.Y., T.H., K.V., and R.O. wrote the paper with input from all authors.

DECLARATION OF INTERESTS

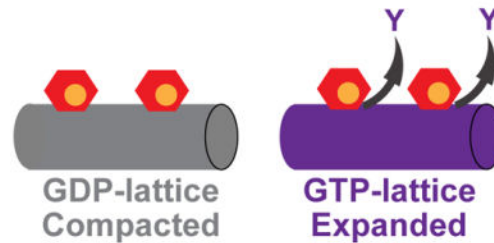
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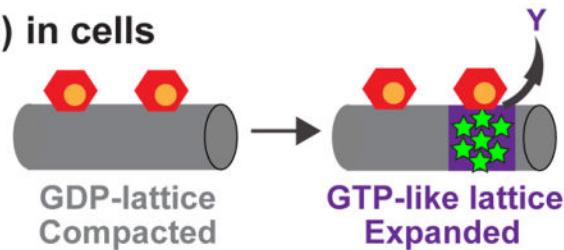
the MT lattice, promotes MT detyrosination and that CAMSAP2 and CAMSAP3 are two MAPs that spatially regulate detyrosination in cells. Collectively, our work shows that VASH1/SVBP detyrosination is regulated by the conformational state of tubulin in the MT lattice, and that this is spatially determined in cells by the activity of MAPs.

Graphical Abstract

1) on reconstituted microtubules



2) in cells



eTOC Blurp

Yue *et al.* develop a biosensor for detyrosinated microtubules to study how the enzyme VASH1/SVBP is regulated. They show that VASH1/SVBP has higher detyrosination activity on microtubules in a GTP-bound state and that in cells, a drug (taxol) or protein (CAMSAP2/3) causes GDP-microtubules to mimic a GTP-bound state, thereby inducing detyrosination.

Keywords

Microtubule; detyrosination; post-translational modification; Vasohibin; VASH/SVBP; CAMSAP

INTRODUCTION

A hallmark of the microtubule (MT) cytoskeleton is its ability to assemble and disassemble on a timescale of minutes. This property is driven by dynamic instability of MTs, where MTs switch stochastically between states of growth and shortening¹. Growth and shortening occur by addition or loss, respectively, of α,β -tubulin subunits at MT ends. The key to dynamic instability lies in the hydrolysis of GTP that is bound to β -tubulin. In solution and upon incorporation at MT ends, β -tubulin is bound to GTP. However, after assembly into the MT polymer, the GTP is rapidly hydrolyzed to successively yield β -tubulin-GDP-Pi

and β -tubulin-GDP. MTs composed of β -tubulin-GTP (GTP-MTs) are stable and promote MT growth, but GDP-MTs are unstable and depolymerize². Improvements in cryo-electron microscopy (cryo-EM) have provided insight into how the nucleotide state affects MT dynamics by showing that the helical lattice of vertebrate MTs twists and shortens by ~1–2 Å upon GTP hydrolysis and/or phosphate release^{3–7}. These observations suggest that MT dynamics are driven by compaction-dependent changes in tubulin subunit interactions within a protofilament as well as between protofilaments.

In cells, the properties of MTs are further modulated by post-translational modifications (PTMs)^{8,9}. Detyrosination was the first-identified tubulin PTM^{10,11}, and it occurs through enzymatic removal of the C-terminal tyrosine (Y) of α -tubulin. Mysteriously, detyrosination only occurs on a subset of MTs, appearing on MTs in the cytoplasm of interphase cells, the cilium, mitotic spindle, and cytokinetic midbody^{12–14}. The mechanism by which specific MTs are selected for modification is a major knowledge gap in the field. Detyrosination is associated with stable MTs and is likely to exert this effect by preventing MT association with destabilizing factors such as the kinesin-13s¹⁵. Tyrosinated α -tubulin, on the other hand, recruits a different set of MT-associated proteins (MAPs), *e.g.*, the dynactin component p150^{glued} and the WD40 repeat protein EML2-S^{16,17}. In the case of p150^{glued}, this increases the on-rate of dynein-dynactin to tyrosinated MTs (Y-MTs), which is thought to ensure that retrograde transport initiates at the cell periphery¹⁸. EML2-S is a regulator of MT dynamics that decreases the MT growth-to-shortening transition (catastrophes) and increases the shortening-to-growth transition (rescue)¹⁶.

Further progress in understanding the functions of detyrosinated MTs (Δ Y-MTs) have been hampered by a lag in the identification of enzymes that detyrosinate MTs and a lack of tools to visualize the biogenesis of MT detyrosination in real time. Recently, two classes of MT detyrosinating enzymes have been discovered: Vasohibins (VASH1 and VASH2), transglutaminase-like cysteine proteases which bind to the regulatory protein small-vasohibin binding protein (SVBP)^{19,20}, and MT-associated tyrosine carboxypeptidase (MATCAP)²¹. Deletion of both proteins in mice abolishes the production of Δ Y-MTs, suggesting that they are the only enzymes capable of MT detyrosination activity in mammals²¹. In this work, we set out to characterize the biophysical features of VASH1/SVBP, under the premise that its intrinsic properties underlie its ability to pattern Δ Y-MTs in cells. To overcome the barrier to visualizing MT detyrosination in real time, we developed an antibody-based fluorescent probe that is specific for Δ Y-MTs. Through our work, we discovered that VASH1/SVBP prefers to detyrosinate mimics of GTP-MTs, which is consistent with the well-known ability of taxol to stimulate MT detyrosination in cells. Intriguingly, the ability of VASH1/SVBP to preferentially detyrosinate GTP-MTs cannot be explained by a difference in VASH1/SVBP's ability to associate with GTP *versus* GDP-MTs. We propose that the nucleotide or conformational state of MTs is related to the ability of VASH1/SVBP to interact productively with the α -tubulin C-terminal tail (CTT), providing an explanation for why most MTs in cells, which exist in a GDP-bound state, are not selected for modification.

RESULTS

Generation of a fragment antigen-binding (Fab) probe that marks detyrosinated MTs

To develop a probe that can spatially and temporally recognize and mark sites of detyrosinated (ΔY) α -tubulin within the MT lattice, we started by generating a rabbit monoclonal antibody that is highly specific for ΔY - α -tubulin (clone RM444). The antibody was non-specifically labelled with fluorescent Alexa⁴⁸⁸ dye and then an antigen-binding fragment (Fab) was generated by papain digestion (Figure 1A). When added to flow cells containing taxol-stabilized brain MTs, which contain a mixture of Y- and ΔY - α -tubulins, the ΔY -Fab⁴⁸⁸ probe efficiently labeled the MT lattice (Figure 1B).

To show that the probe is sensitive to the Y- *versus* ΔY -state of the α -tubulin CTT, we first verified that the ΔY -Fab⁴⁸⁸ probe shows the same selectivity for ΔY - α -tubulin as the RM444 monoclonal antibody by western blotting (Figure 1C). We then compared the ability of the probe to label Y-MTs *vs* ΔY -MTs *in vitro*. To generate Y-MTs, we purified tubulin from HeLa cells which predominantly contain Y- α -tubulin^{16,22}. To generate ΔY -MTs, we incubated HeLa tubulin with carboxypeptidase A (CPA), an approach that is standard in the field for generating ΔY - α -tubulin^{16,22}. The ΔY -Fab⁴⁸⁸ probe did not bind to HeLa MTs that were untreated by CPA but did bind to HeLa MTs treated by the peptidase (Figure 1D). Since HeLa MTs are not recognized by our ΔY -Fab⁴⁸⁸ probe, we hereafter refer to HeLa MTs as Y-MTs.

We noticed that some ΔY -MTs generated by CPA treatment were heavily decorated by ΔY -Fab⁴⁸⁸, while others were not (Figure 1D and S1A). It is possible that CPA treatment removes additional amino acids from the C terminus of α -tubulin as well as β -tubulin²³, which could affect recognition by the ΔY -Fab⁴⁸⁸ probe. Thus, to verify that the ΔY -Fab⁴⁸⁸ labeling is due to removal of the C-terminal tyrosine of α -tubulin, we generated ΔY -MTs by incubating HeLa MTs with VASH1/SVBP. We found that both SiR-tubulin and the ΔY -Fab⁴⁸⁸ probe homogeneously decorated ΔY -MTs generated by VASH1/SVBP treatment (Figure 1D). The fluorescence intensity of the ΔY -Fab⁴⁸⁸ probe along ΔY -MTs generated by VASH1/SVBP treatment was higher than that along the ΔY -MTs generated by CPA treatment (Figures 1D and S1B), consistent with the possibility that CPA treatment removes more than the C-terminal tyrosine from α -tubulin. Collectively, our results demonstrate that the ΔY -Fab⁴⁸⁸ probe specifically recognizes ΔY - α -tubulin *in vitro*.

To determine if the ΔY -Fab⁴⁸⁸ probe detects ΔY -MTs in live cells, we examined MT labeling by ΔY -Fab⁴⁸⁸ in a HeLa cell line that expresses mCherry-VASH1/SVBP in a doxycycline-inducible manner. We used bead-loading²⁴ to introduce the ΔY -Fab⁴⁸⁸ into these cells and found that ΔY -Fab⁴⁸⁸ did not label MTs in the absence of doxycycline (*i.e.*, no VASH1/SVBP expression). However, ΔY -Fab⁴⁸⁸ decorated filamentous structures in the presence of doxycycline (*i.e.*, with VASH1/SVBP expression), demonstrating that ΔY -Fab⁴⁸⁸ specifically labels ΔY -MTs in living cells (Figure 1E).

VASH1/SVBP rapidly detyrosinates MTs

We next tested whether the ΔY -Fab⁴⁸⁸ probe can be used to detect the detyrosinase activity of VASH1/SVBP by monitoring the appearance of detyrosinated α -tubulin in real-time. To

do this, we adhered taxol-stabilized HeLa Y-MTs to the surface of a flow cell and then exposed them to VASH1/SVBP and the ΔY -Fab⁴⁸⁸ (Figure 2A). In optimizing the conditions for our assay, we initially compared the binding of single molecules of fluorescently-labeled VASH1/SVBP to MTs in buffers with different ionic strengths. We observed that VASH1/SVBP exhibited stronger binding to MTs in low ionic strength buffers (Figure S2A), consistent with recent work by Ramirez-Rios *et al.* [25]. Using the ΔY -Fab⁴⁸⁸ probe to detect ΔY -MTs, we found that VASH1/SVBP also displays higher detyrosination activity in low ionic strength buffers (Figure S2B). Together, these results indicate that an increase in ionic strength reduces the interaction of VASH1/SVBP with MTs, resulting in a significant reduction in enzymatic activity. Thus, we used the low ionic strength buffer P12 for the following experiments.

When taxol-stabilized HeLa Y-MTs were incubated with 1 nM VASH1/SVBP in cell lysate, there was little decoration of Y-MTs by the ΔY -Fab⁴⁸⁸ at the start of the assay (0 min), but the probe fully decorated MTs within 10 min, indicating that detyrosination had occurred (Figures 2B and S2C,D). In contrast, treatment of taxol-stabilized Y-MTs with untransfected cell lysate resulted in very little MT decoration by ΔY -Fab⁴⁸⁸, indicating that detyrosination was driven by the overexpressed VASH1/SVBP enzyme and not other factors in cell lysate (Figures 2B, C and D). In addition, the VASH inhibitor EpoY¹⁹ blocked MT labeling by ΔY -Fab⁴⁸⁸, further indicating that detyrosination was largely driven by VASH1/SVBP (Figures 2B, C and D). Furthermore, the ΔY -Fab⁴⁸⁸ probe rapidly decorated ΔY -MTs previously detyrosinated by VASH1/SVBP, indicating that the amount of ΔY -Fab⁴⁸⁸ probe is not limiting under the conditions of our experiments (Figures 2B and 2C). Under these imaging conditions (1 frame/5s), we measured a near-linear increase in the amount of MT detyrosination over the first five minutes of incubation of HeLa Y-MTs with VASH1/SVBP (Figure 2D), indicating that VASH1/SVBP has high detyrosination activity *in vitro*.

To further study the mechanism of MT detyrosination by VASH1/SVBP, we imaged the reaction at a faster frame rate (5 frames/s) and simultaneously monitored the binding of Halo^{JFX554}-tagged VASH1/SVBP to MTs and its activity using the ΔY -Fab⁴⁸⁸ probe. VASH1/SVBP-Halo^{JFX554} underwent frequent, short interactions with taxol-stabilized Y-MTs (Figures 2E and 2F). In most cases, multiple interactions between VASH1/SVBP-Halo^{JFX554} and the MTs occurred before the ΔY -Fab⁴⁸⁸ probe bound statically to the MTs (Figures 2E and 2F), suggesting that VASH1/SVBP can undergo non-productive interactions with the MT lattice. Overall, our results demonstrate that frequent short interactions of VASH1/SVBP with taxol-stabilized Y-MTs result in rapid detyrosination of the entire MT lattice.

VASH1/SVBP binds to MTs independently of the MT detyrosination state

The ability of VASH1/SVBP to rapidly detyrosinate MTs in *in vitro* assays contrasts with the limited amounts of ΔY -MTs typically observed in cultured mammalian cells. In principle, increased association of VASH1/SVBP with ΔY -MTs would set up a positive feedback loop, creating a mechanism that would locally increase the levels of ΔY -MTs. We thus investigated whether the binding of VASH1/SVBP to MTs is influenced by the Y/ ΔY state of the MT. We measured the single-molecule behavior of VASH1/SVBP-

Halo^{JFX554} protein (Figure 3A) on taxol-stabilized HeLa Y-MTs, Δ Y-MTs generated by cell lysate containing VASH1/SVBP, and MTs assembled from brain tubulin (mix of Y- and Δ Y-tubulin). Our results show that VASH1/SVBP-Halo^{JFX554} binds similarly to all three types of MTs (Figure 3B, Video S1). The landing rate of VASH1/SVBP-Halo^{JFX554} was not statistically different on Y-MTs, Δ Y-MTs, and brain MTs [146.0 ± 23.2 , 130.4 ± 25.0 , and 132.2 ± 6.7 events $\cdot\mu\text{m}^{-1}\cdot\text{min}^{-1}\cdot\text{nM}^{-1}$, respectively (mean $\pm$ SD)] (Figure 3C). Likewise, the dwell time of VASH1/SVBP-Halo^{JFX554} was not statistically different on Y-MTs, Δ Y-MTs, and brain MTs (1.0 s [0.7 s, 1.5 s], 0.9 s [0.7 s, 1.4 s], and 1.0 s [0.7 s, 1.6 s], respectively (median [quartiles])) (Figure 3D). Our results demonstrate that VASH1/SVBP binds equally well to taxol-stabilized MTs regardless of their detyrosination state.

VASH1/SVBP detyrosination activity depends on the nucleotide-bound state of β -tubulin

We next hypothesized that the difference between VASH1/SVBP's high activity in *in vitro* assays and its low activity in cells is due to VASH1/SVBP regulation by the nucleotide state of β -tubulin within the MT lattice. To test this, we generated Y-MTs with tubulin in a GTP-like state by polymerizing HeLa tubulin in the presence of GMPCPP and generated Y-MTs with tubulin in the GDP-state by polymerizing HeLa tubulin in GTP (which is converted to GDP upon polymerization) and then stabilized the GDP-MTs by addition of GMPCPP-tubulin to create a GTP-like cap. We used the single-molecule VASH1/SVBP binding assay (Figure 3A) to investigate the MT binding behavior of VASH1/SVBP-Halo^{JF554} on GMPCPP-MTs vs capped GDP-MTs. VASH1/SVBP-Halo^{JF554} underwent frequent interactions of short duration with both GMPCPP-Y-MTs and capped GDP-Y-MTs (Figure 4A). Quantification of these data showed that there was no significant difference in the landing rate or dwell time of VASH1/SVBP-Halo^{JF554} on GMPCPP-Y-MTs vs capped GDP-Y-MTs [landing rate: 152.1 ± 17.8 and 140.2 ± 24.9 events $\cdot\mu\text{m}^{-1}\cdot\text{min}^{-1}\cdot\text{nM}^{-1}$, respectively (mean $\pm$ SD, Figure 4B); dwell time: 1.0 s [0.7 s, 1.6 s] and 1.0 s [0.7 s, 1.5 s], respectively (median [quartiles], Figure 4C)]. Our results demonstrate that VASH1/SVBP binds equally to MTs regardless of the β -tubulin nucleotide state.

We then examined whether the detyrosination activity of VASH1/SVBP differs on GMPCPP-Y-MTs vs GDP-Y-MTs using the Δ Y-Fab⁴⁸⁸ probe and our MT detyrosination assay (Figure 2A). At the beginning of the assay (0 min), there was little decoration of either GMPCPP-Y-MTs or capped GDP-Y-MTs by the Δ Y-Fab⁴⁸⁸ probe but after 5 min, much higher probe decoration was observed for the GMPCPP-Y-MTs, suggesting that VASH1/SVBP has a higher activity on GTP-like MTs (Figure 4D, Videos S2 and S3). We thus monitored Δ Y-Fab⁴⁸⁸ probe binding over time and found a dramatically larger detyrosination activity on GMPCPP-Y-MTs (Figures 4E and 4F). These results demonstrate that although VASH1/SVBP binds equally to GMPCPP-MTs and GDP-MTs, it has higher detyrosinase activity on GMPCPP-MTs.

VASH1/SVBP detyrosination activity is stimulated by taxol-mediated expansion of tubulin in the lattice

Recent cryo-EM structures have reported that tubulin within the MT lattice is in an expanded state with GTP mimics and in a compacted state with GDP bound³. It is thus possible

that VASH1/SVBP activity is regulated not by the nucleotide state of tubulin in the lattice but by the conformational state. To test whether VASH1/SVBP activity is regulated by the expanded vs compacted state of tubulin, we took advantage of the fact that taxol can reverse the compacted state of GDP-MTs into a GTP-tubulin-like state³. To do this, we carried out a real-time visualization of MT detyrosination with the ΔY -Fab⁴⁸⁸ probe in COS-7 cells treated with 10 μ M taxol. The flat nature of COS-7 cells enables high signal to noise (S/N) imaging of MTs by total internal reflection fluorescence microscopy (TIRF-M). The ΔY -Fab⁴⁸⁸ was delivered into cells by bead loading²⁴, and then imaged by time-lapse TIRF-M. DMSO (vehicle control) or taxol were added to the imaging media 5 min after the onset of imaging, and cells were then filmed for an additional 55 min. We observed only cytoplasmic fluorescence before taxol addition, but filamentous structures were evident 10 minutes following exposure to taxol and these became progressively brighter and more numerous with time (Figure 5A, Videos S4 and S5). Quantification demonstrated a clear increase in ΔY -Fab⁴⁸⁸-decorated filaments in taxol-treated cells (Figures 5B, 5C and S3A) and immunoblotting with the anti- ΔY antibody confirmed the accumulation of ΔY - α -tubulin upon taxol treatment (Figure 5D). Experiments in fixed cells (Figure S3B) revealed that although taxol treatment increases total MT intensity over time, ΔY - α -tubulin increases to a much greater extent (nearly 5-fold), suggesting that the observed increase in ΔY - α -tubulin detection in both live and fixed cells reflects an actual increase in α -tubulin detyrosination rather than increased MT density.

To directly test whether VASH1/SVBP activity is regulated by the expanded vs compacted state of tubulin in the lattice, we utilized the *in vitro* MT detyrosination assay (Figure 2A) but used 25% glycerol instead of a GMPCPP-cap to stabilize GDP-Y-MTs from depolymerization²⁵. We observed brighter ΔY -Fab⁴⁸⁸ signal along GDP-MTs in the presence of 80 μ M taxol than the vehicle (DMSO) control (Figure 5E). Real time imaging of ΔY -Fab⁴⁸⁸ binding to glycerol-stabilized GDP-Y-MTs confirmed that VASH1/SVBP has a higher α CTT detyrosination activity on expanded MTs (Figures 5F and 5G). Similar results were obtained using a GMPCPP-cap rather than glycerol to stabilize GDP-Y-MTs (Figure S4).

CAMSAPs are cellular factors that promote MT detyrosination

Our finding that VASH1/SVBP has a higher detyrosination activity on MTs with tubulin subunits in an expanded state provides a molecular explanation for why VASH1/SVBP displays high detyrosination activity in *in vitro* assays (*e.g.*, on GMPCPP-MTs or taxol-stabilized GDP-MTs) but has low detyrosination activity in cells (compacted GDP-MTs). We thus hypothesized that detyrosination in cells requires local alteration of tubulin's conformational state to generate substrate for VASH1/SVBP. Segments of MTs in cells can display an expanded state²⁶ but how these regions are generated and whether they correspond to ΔY -MTs is not known.

To identify factors that could locally promote tubulin detyrosination, we turned to the original screen that identified the vasohibin enzymes as tubulin detyrosinases²⁰ and noted that several MAPs including MAP2, MAP4, and CAMSAP2 displayed a phenotype consistent with positive regulation of detyrosination in cells²⁰. We focused on CAMSAPs

since i) CAMSAP2 and CAMSAP3 localize to MT minus ends^{27,28}, ii) detyrosinated MTs often terminate with a CAMSAP2 stretch²⁹, iii) CAMSAP2 is required for the generation of Δ Y-MTs in U-2-OS cells²⁹, and iv) CAMSAP3 can expand the lattice of GDP-MTs³⁰.

To investigate whether CAMSAP proteins can promote VASH1/SVBP detyrosination activity, we first expressed GFP-tagged CAMSAP1, CAMSAP2, or CAMSAP3 (Figure 6A) in HeLa cells. CAMSAP2 and CAMSAP3 localized to MT minus ends at the centrosome whereas CAMSAP1 showed both cytosolic localization and faint decoration of MTs. Importantly, CAMSAP2 and CAMSAP3 expression resulted in a dramatic increase in Δ Y-MTs which co-localized with the CAMSAP-decorated MT stretches (Figures 6B and S5A). When mean intensities of total MTs and Δ Y-MTs were measured for each cell, the increase in Δ Y-MTs was statistically significant in CAMSAP3-expressing cells but not in CAMSAP2-expressing cells (Figure 6C), perhaps due to the more spatially-restricted localization of CAMSAP2 and Δ Y-MTs near the nucleus in HeLa cells (Figure 6B). Similar results were obtained upon expression of CAMSAP1, CAMSAP2, and CAMSAP3 in COS-7 cells (Figures S5B and S5C).

We then tested whether CAMSAP2 or CAMSAP3 could promote VASH1/SVBP detyrosination activity in our *in vitro* MT detyrosination assay (Figure 2A). In this assay, tagged versions of CAMSAP2 in COS-7 cell lysates readily bound to glycerol-stabilized GDP-MTs whereas CAMSAP3 showed limited binding to MTs (Figures S6A and S6B). We thus used cell lysates expressing superTagRFP-tagged CAMSAP2 or untransfected cell lysates to pre-treat glycerol-stabilized GDP-Y-MTs, and then performed the MT detyrosination assay using VASH1/SVBP cell lysates and the Δ Y-Fab⁴⁸⁸. We observed a brighter Δ Y-Fab⁴⁸⁸ signal along MTs with CAMSAP2 pre-treatment than the vehicle control (Figure 6D). Real-time imaging of detyrosination by Δ Y-Fab⁴⁸⁸ binding showed that CAMSAP2 pre-treatment increased VASH1/SVBP-mediated α -tubulin detyrosination *in vitro* (Figures 6E and 6F). Importantly, MT detyrosination activity by VASH1/SVBP was most prominent at the CAMSAP2-decorated MT stretches (Figure S6C), consistent with the results observed in cells (Figures 6B and S5C).

Finally, we asked if CAMSAP-induced MT detyrosination occurs through MT lattice expansion in cells. At least two MT-binding domains are known for CAMSAPs: 1) a C-terminal CKK domain responsible for localizing CAMSAPs to the MT minus end^{29,31–33}, and 2) a central MT-binding domain (MBD;²⁹ called the D2 domain in³⁰). The MBDs of CAMSAP2 and CAMSAP3 preferentially bind to GMPCPP-MTs²⁹ and the MBD of CAMSAP3 can expand the lattice at high concentrations *in vitro*³⁰. We hypothesized that the MBD would localize along all MTs rather than be spatially restricted to the MT minus ends and thereby promote detyrosination throughout cells. Indeed, a superTagRFP-CAMSAP2-MBD decorated cytosolic MTs and resulted in a dramatic and significant increase in Δ Y-MTs throughout HeLa cells (Figures 6G and 6H). Similar results were obtained upon expression of superTagRFP-CAMSAP2-MBD in COS-7 cells (Figure S5D). Quantification of these results demonstrates that the increase in Δ Y-MTs cannot be simply due to an increase in MT bundling (Figures S5D and S5E). We conclude that CAMSAP2 and CAMSAP3 are MAPs that promote MT detyrosination by expanding the MT lattice, thus generating robust substrates for VASH1/SVBP.

DISCUSSION

The mechanisms by which patterns of MT PTMs are established within cells and how these patterns differ among cell types are largely unknown but are important for understanding how MTs contribute to many physiological processes including neuronal development, the beating of cardiomyocytes, intracellular trafficking, and cell division^{8,9,34}. A major roadblock has been a lack of probes to detect the formation of PTMs in real time, thus impeding an analysis of how a MT's history may relate to its selection for modification. As examples, PTMs may appear when MTs age^{13,35,36} or in response to cell stress^{37–40} or environmental cues^{41–43}. Biosensors would also better equip the field to reveal how certain PTMs, *e.g.*, α -tubulin detyrosination and K40 acetylation, become spatially correlated within the cell^{36,44}. Previous attempts have developed tools that detect unmodified tubulin. For example, screening of various synthetic hyperstable human scfv (single-chain variable fragment) libraries only identified anti-tubulin scfv probes^{45,46}. Similarly, screening of a yeast peptide display library merely identified a probe that binds to the CTT of tyrosinated (native) α -tubulin and thus recognizes unmodified MTs in live cells⁴⁷. Here, we developed an antibody-based probe, a fluorescently-labeled Fab, that can spatially and temporally mark detyrosinated MTs both *in vitro* and in live cells. To our knowledge, this probe is the first biosensor to any MT PTM.

By analyzing single molecules of the detyrosinase VASH1/SVBP on MTs, we observed that the enzyme binds equally well to MTs that differ in their tyrosination/detyrosination state. These results agree well with recently published work⁴⁸ and rule out a model in which VASH1/SVBP progressively detyrosinates a MT based on positive feedback caused by increased affinity of VASH1/SVBP for ΔY -MTs. Our data also indicate that the interaction of VASH1/SVBP with MTs is not affected by the nucleotide state of tubulin: VASH1/SVBP binds equally well to MTs that mimic the GTP- and GDP-bound states. A molecular explanation for how VASH1/SVBP can bind to MTs regardless of the nucleotide bound to β -tubulin or the tyrosination/detyrosination state of α -tubulin comes from recent cryo-electron microscopy (cryo-EM) structures which show that VASH1/SVBP and VASH2/SVBP interact with the α -tubulin subunits of two adjacent protofilaments^{48,49} in a manner that does not engage with β -tubulin or the tyrosination/detyrosination state of the α -tubulin CTT.

Unexpectedly, however, we found that VASH1/SVBP only detyrosinates MTs that are mimics of GTP-MTs, *e.g.*, GMPCPP-stabilized MTs. This finding provides the first molecular explanation for why VASH1/SVBP does not detyrosinate most MTs in cells. Specifically, the majority of MTs in cells are in the GDP-state which we now show are not good substrates for VASH1/SVBP. We speculate that the nucleotide state of β -tubulin drives a conformational change in α -tubulin that could directly affect lattice-bound VASH1/SVBP. To address this question, it will be important to compare the structures of VASH1/SVBP on GDP- vs GTP-MTs. The recent work by Ramirez-Rios *et al.* (2023) and Li *et al.* (2020) provides a starting point by determining the structure of VASH/SVBP bound to GMPCPP-MTs^{48,49}.

Our work further shows that VASH1/SVBP activity is not driven by the nucleotide-bound state of tubulin in the MT lattice *per se*, but rather by the conformation of tubulin in the MT lattice. Importantly, taxol causes the lattice of GDP-MTs to expand, both *in vitro* and in cells^{3,26,50}. Thus our demonstration that VASH1/SVBP has higher enzymatic activity on taxol-GDP-Y-MTs *vs* GDP-Y-MTs provides the first molecular explanation for the long-standing observation that taxol treatment increases MT detyrosination in cells⁵¹. Furthermore, our results indicate that taxol's ability to increase tubulin PTMs is not merely a consequence of increasing MT stability. At 10 μ M taxol, detyrosination was observed within 10–15 minutes (Figures 5A, 5C, S3B and S3C), despite the fact that binding of a fluorescent taxol derivative to MTs is much faster⁵² and causes MT dynamics of mitotic cells to cease within 5 minutes⁵³. We thus speculate that taxol induces an accumulation of Δ Y-MTs by affecting the structural state rather than the stability of the MT lattice. In the future, we will test this hypothesis more rigorously using taxol-like molecules that expand the MT lattice without stabilizing the polymer (*e.g.*, baccatin III⁵⁴).

How then is VASH1/SVBP able to detyrosinate cellular MTs which are in a GDP- and compacted state? We show that overexpression of CAMSAP2 and CAMSAP3, but not CAMSAP1, causes MT detyrosination in cells. Moreover, CAMSAP2 promotes detyrosination of GDP-MTs by VASH1/SVBP *in vitro*. Finally, the CAMSAP2 MBD alone promotes detyrosination by VASH1/SVBP, presumably through lattice expansion. In this regard, the CKK domain of CAMSAP2 (and presumably CAMSAP3) is important because it spatially restricts lattice expansion and MT detyrosination to minus ends that are located near the centrosome. Collectively, our work demonstrates that lattice expansion is a mechanism that promotes MT detyrosination, and identifies cellular factors that generate robust MT substrates for VASH1/SVBP. We propose the following model for how CAMSAP2 and CAMSAP3 regulate MT detyrosination in cells (Figure 6I) : 1) MTs are nucleated at centrosomes *via* γ TuRCs⁵⁵; 2) CAMSAP displaces γ TuRCs from the MT minus ends⁵⁶; 3) CAMSAP causes local expansion of tubulin subunits within the lattice³⁰; and finally, 4) VASH1/SVBP detyrosinates MTs with an expanded lattice. The fact that Δ Y-MTs are not always restricted to locations that are perinuclear, as in HeLa and COS-7 cells that we utilized for our work (Figure S5B), suggests that MAPs other than CAMSAPs are involved in the regulation of MT detyrosination through lattice expansion, particularly in cells with highly stereotyped patterns of post-translationally modified MTs, *e.g.*, muscle cells and neurons⁵⁷.

Our finding that MT detyrosination is gated by the conformation of the MT lattice expands recent findings in the field showing that lattice spacing can influence the binding of MAPs and molecular motor proteins. For example, kinesin-1 binds preferentially to GTP-like MTs⁵⁸ whereas tau has a higher affinity for GDP-like MTs⁵⁹. Likewise, the binding of MAPs and motors can change the conformational state of tubulin in the lattice, *e.g.* kinesin-1 binding transiently converts GDP-MTs to the expanded state^{58,60} and EB1 promotes the compacted state⁶. To our knowledge, this work is the first example that the conformational state of tubulin in the MT lattice can affect the enzymatic activity of a bound MAP. It will be interesting to look at other enzymes in future experiments, *e.g.*, are the activities of other MT detyrosinating enzymes, *i.e.*, VASH2/SVBP and MATCAP, gated in a manner similar

to VASH1/SVBP? Future work will also focus on how the conformational state of the MT lattice impacts enzymes that drive other MT PTMs such as acetylation and glutamylation.

STAR Methods

RESOURCE AVAILABILITY

LEAD CONTACT—Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Ryoma Ohi (oryoma@umich.edu).

MATERIALS AVAILABILITY

Plasmids and cell lines generated in this study are available upon request.

DATA AND CODE AVAILABILITY

- Original western blot images and microscopy data reported in this paper will be shared by the lead contact upon request.
- This work did not utilize newly developed code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

E. coli strain DH5 α (Invitrogen, 18258–012) was used throughout the molecular cloning. For the production of recombinant GST-CTT proteins, *E. coli* strain Rosetta 2 (DE3) pLysS (Millipore, Cat# 71403–3) was used. *E. coli* cells were cultured in standard LB medium supplemented with appropriate antibiotics at 37°C. COS-7 (monkey kidney fibroblast) cells were obtained from ATCC (RRID: CVCL_0224) and cultured in DMEM (Gibco) with 10% (vol/vol) Fetal Clone III (HyClone) and 1% GlutaMAX (Gibco). HeLa cells were cultured in DMEM medium (Thermo Fisher Scientific, Cat# 11965118) supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals, Cat# S11150) and penicillin-streptomycin (Thermo Fisher Scientific, Cat# 15140122). HeLa knock-in cell line co-expressing VASH1-mCherry and SVBP-FLAG in a doxycycline inducible manner was maintained in DMEM containing 10% fetal bovine serum, and antibiotics, and 1 mg/mL puromycin (Sigma-Aldrich, Cat# P8833). Cells were maintained in the presence of 5% CO₂ at 37°C. Expression of transgene(s) in knock-in cells was initiated by the addition of 2 μ g/ml doxycycline (Thermo Fisher Scientific, Cat# BP26531). Cells were not authenticated and are negative for mycoplasma contamination.

METHOD DETAILS

Plasmids—For the VASH1-Halo-IRES-SVBP construct, the Halo tag was amplified by PCR and swapped with the GFP tag of pmEGFP-N1-VASH1-GFP-IRES-SVBP⁶¹ by NEBuilder HiFi DNA assembly cloning kit. Similarly, VASH1-mCherry-IRES-SVBP construct was made and the entire coding sequence was transferred into the pEM791 vector, resulting pEM791-VASH1-mCherry-IRES-SVBP. Then this was used to generate a knock-in HeLa cell line that expresses VASH1-mCherry and SVBP-FLAG in a doxycycline inducible manner using recombination mediated cassette exchange (Khandelia et al 2011).

pEGFP-C1-CAMSAP1 and pEGFP-C1-CAMSAP2 were generated by inserting ORFs of human CAMSAP1 and CAMSAP2 into EcoRI/KpnI sites of pEGFP-C1 vector. pEGFP-C1-CAMSAP3 was generated by inserting a human CAMSAP3 cDNA fragment (Horizon Discovery, Cat# MHS1010–202694847; 3,777 bp) into EcoRI-linearized pEGFP-C1 vector by Gibson assembly. psTagRFP-C1-CAMSAP vectors were constructed by inserting human CAMSAP ORFs into EcoRI-linearized psTagRFP-C1 vector where EGFP was replaced with sTagRFP (super-TagRFP;⁶²) in pEGFP-C1 vector. Similarly, CAMSAP2 MBD (positions 922–1034 within the 1489 amino acid sequence of human CAMSAP2; Jiang et al 2014) was amplified and assembled into the psTagRFP-C1 vector. All plasmids were verified by DNA sequencing.

Cell culture transfection—HeLa (HeLa Kyoto) and COS-7 cells were transfected with either Lipofectamine 2000 (ThermoFisher) or Trans-IT LT1 (Mirus), according to the manufacturer's instructions. Halo-tagged protein was fluorescently labeled by the inclusion of 50 nM JFX554 Halo ligand (Tocris Bioscience) in the medium.

Cell lysate preparation for *in vitro* assays—COS-7 cells were collected 16h post-transfection. The cells were harvested by low-speed centrifugation at $1,500 \times g$ for 5 min at 4°C. The pellet was rinsed once in PBS and resuspended in ice-cold lysis buffer (25 mM HEPES/KOH, 115 mM potassium acetate, 5 mM sodium acetate, 5 mM MgCl₂, 0.5 mM EGTA, and 1% Triton X-100, pH 7.4) freshly supplemented with 1mM ATP, 1 mM phenylmethylsulfonyl fluoride and protease inhibitors (P8340; Sigma-Aldrich). After the lysate was clarified by centrifugation at $20,000 \times g$ for 10 min at 4°C, aliquots of the supernatant were snap-frozen in liquid nitrogen and stored at –80°C until further use.

The concentration of VASH1/SVBP-Halo in the lysates was measured by a dot-blot, in which 1 µl of COS-7 lysates expressing VASH1/SVBP-Halo and a series of KIF5C(1–560)-Halo protein with the known concentrations were spotted onto a nitrocellulose membrane. The membrane was air-dried for 1 h and immunoblotted with a primary antibody to Halo tag (Promega, G9281) at room temperature for 1 h and subsequently with a secondary antibody 680nm anti-rabbit (Jackson ImmunoResearch Laboratories Inc.) at room temperature for 30 min. The fluorescence intensity of the spots on the nitrocellulose membrane was detected by Azure 600 (Azure Biosystems) and quantified based on the standard curve of known concentration of KIF5C(1–560)-Halo protein using Fiji/ImageJ (NIH).

Immunofluorescence—Immunofluorescence was performed as described previously⁶¹. To monitor tubulin detyrosination in Taxol-treated COS-7 cells, cells were cultured in DMEM supplemented with 10 µM taxol or 0.3% DMSO (vehicle control) for 0–60 min. To visualize tubulin detyrosination in COS-7 and HeLa cells transiently expressing EGFP-CAMSAPs or superTagRFP-CAMSAP2 MBD, cells were transfected with the plasmids and cultured overnight. Cells were fixed with cold methanol and stained with monoclonal anti-ΔY α-tubulin (RM444, RevMAb biosciences) followed by anti-rabbit Alexa⁵⁹⁴ (Taxol and the full length CAMSAP experiments) or anti-rabbit Alexa⁴⁸⁸ (CAMSAP2 MBD experiment) and FITC-conjugated-DM1α (Taxol experiment) or Alexa⁶⁴⁷-conjugated DM1α (full length CAMSAP and MBD experiments). DNA was

counter stained with Hoechst 33342 for 5 min and coverslips were mounted with Prolong diamond (ThermoFisher). Images were acquired with a DeltaVision microscope equipped with an Olympus Plan Apo 60X objective with 1.59x magnification optics. Deconvolution was performed on SoftWorx. Images of single focal plane were generated, and intensity was adjusted linearly on imageJ.

To quantify the total α -tubulin and ΔY α -tubulin within cells, the cell boundary was manually defined. For HeLa cells, the mean fluorescence intensity was then measured by dividing the integrated fluorescence intensity by cell area. The background fluorescence intensity, averaged from three independent circular regions (diameter = 3 μ m) in each image, was subtracted. The value of the control sample was set as 100. For COS-7 cells, the integrated fluorescence intensity of each cell was quantified and the background fluorescence (area of the cell x mean background fluorescence intensity) was subtracted to yield the corrected cell total fluorescence. To calculate relative abundance of ΔY - α -tubulin, the ΔY - α -tubulin intensity was normalized against the total α -tubulin intensity. The value of the control was set as 1.0.

Preparation of ΔY -Fab⁴⁸⁸ probe—Recombinant monoclonal anti-detyrosinated α -tubulin antibody (clone RM444, RevMAb Biosciences) was labeled with Alexa⁴⁸⁸ dye using Fluorescent Protein Labeling Kit (Thermo Fisher, Cat# A10235). Fab preparation was performed using Fab Preparation Kit (Thermo Fisher, Cat# 44985). Briefly, labeled IgG was digested with papain for 8 hours at 37°C followed by negative purification with Protein A column. Flow-through fractions from the Protein A column were combined and concentrated using Amicon Ultra-4 10K ultrafiltration device (Millipore, Cat# UFC801024). Half the concentrated Fab fraction (dissolved in PBS) was snap frozen and used for the bead-loading into living cells. The other half was further buffer-exchanged to BRB80 (80 mM PIPES, 1 mM EGTA, 1 mM MgCl₂ pH6.8) and used for the *in vitro* experiments. From 1 mg of unlabeled anti- ΔY IgG, approximately 0.25 mg of Fab was recovered. The degree of labeling (DOL) was 3.5.

Western blotting analysis—To validate the specificity of ΔY -Fab⁴⁸⁸ probe against detyrosinated α -tubulin CTT, GST-CTT fusion proteins were generated where GST was fused to various lengths of the human TubA1A CTT peptide (Y = SVEGEGEEEGEEY; ΔY = SVEGEGEEEGEE; $\Delta 2$ = SVEGEGEEEGE; and $\Delta 3$ = SVEGEGEEEG). The GST-CTT proteins were bacterially expressed and purified as described previously^{16,63}. GST-CTT proteins (200 ng) were separated by SDS-PAGE and transferred on to a nitrocellulose membrane.

To detect ΔY tubulin in COS-7 cell lysates, cells were treated with DMSO (0.3%, vehicle control) or 10 μ M taxol for 1 hour, lysed in NP-40 buffer (6 mM Na₂HPO₄, 4 mM NaH₂PO₄, 2 mM EDTA, 150 mM NaCl, 1% NP40 and protease inhibitors) and sonicated. After clarification, protein concentration was measure by Bradford protein assay using BSA as standard. Each 15 μ g of protein was separated by SDS-PAGE and subjected to western blotting. Membranes were blocked with 5% skim milk in PBS supplemented with 0.05% tween-20 (PBST) for 1 hour at room temperature followed by incubation with primary antibodies or 1 μ g/ml ΔY -Fab⁴⁸⁸ at 4°C overnight. After 3 times of wash with PBST,

membranes except for the ΔY -Fab⁴⁸⁸ blot were incubated with secondary antibodies for 1 hour at room temperature followed by another 3 times of wash. Fluorescent signals of secondary antibodies or ΔY -Fab⁴⁸⁸ were detected with Azure c600 (Azure Biosystems).

TIRF microscopy—Live cell imaging for Taxol-treated COS-7 cells and all *in vitro* assays were performed on an inverted Nikon Ti-E/B total internal reflection fluorescence (TIRF) microscope with a perfect focus system, a 100×1.49 NA oil immersion TIRF objective, three 20 mW diode lasers (488 nm, 561 nm, and 640 nm) and EMCCD camera (iXon⁺ DU879; Andor). Image acquisition was controlled using Nikon Elements software. The cells were imaged at 37°C in a temperature-controlled and humidified live-imaging chamber (Tokai Hit) mounted on TIRF microscope. The angle of illumination was adjusted for maximum penetration of the evanescent field into the cells. A flow cell (~10 μ l volume) was assembled by attaching a clean #1.5 coverslip (Fisher Scientific) to a glass slide (Fisher Scientific) with two strips of double-sided tape. All *in vitro* assays were performed in the flow cells at room temperature.

Live cell imaging—For the live imaging of ΔY -microtubules in HeLa cells expressing VASH1-mCherry and SVBP-FLAG, cells were seeded on glass bottom dishes (MatTek, Cat# P35GC-1.5–14-C) and cultured in DMEM medium with or without 2 μ g/ml doxycycline a day before imaging. On the day of imaging, ΔY -Fab⁴⁸⁸ probe (dissolved in PBS) was loaded into cells using glass beads as previously described²⁴. Briefly, 1–2 μ l of ΔY -Fab⁴⁸⁸ (1.09 mg/ml) was thawed and mixed with DPBS (Gibco, Cat# 14190–144) to make a total of 4 μ l Fab solution. Culture medium (~2 ml) was removed from the dish and kept in a tube. Fab solution was pipetted on top of the cells and pre-cleaned glass beads (Sigma, Cat# 64649) were placed over the cells. The dish was then firmly tapped 12 times. Excess beads were washed out using 500 μ l of culture medium kept above and then remaining ~1.5 ml medium was placed back on the cells. After 2 h of incubation at 37°C, culture medium was replaced with 1.5 ml of L-15 (Gibco, Cat# 21083–027) supplemented with 10% FBS (Atlanta Biologicals, Cat# S11150), Penicillin/streptomycin (Thermo Fisher, Cat# 15140122) and 7 mM Hepes (imaging medium) and cells were observed with a DeltaVision microscope equipped with an Olympus Plan Apo N 60x/1.42 oil immersion lens. Images of deconvolved single optical sections were presented.

For the time-lapse imaging of Taxol-treated COS-7 cells, Fab probes were bead loaded exactly the same way as described for HeLa cells except for the incubation time after the bead loading was shortened to 2 min. The dish was placed on a temperature-controlled TIRF microscope (see above) and cells exhibiting similar levels of fluorescence were virtually identified and subjected to the time-lapse imaging (exposure time, 100 msec; interval, 2 min; total imaging time, 60 min). Between the 3rd and 4th frame acquisition (~5 min after the time-lapse began), 150 μ l of warm imaging medium with 2 μ l of DMSO or 10 mM taxol (final concentration, 12.1 μ M) was gently added. Images were linearly processed in Fiji/ImageJ for data presentation.

To quantitatively evaluate tubulin polymerization from the time-lapse imaging data, we measured the density of microtubules with an image processing scheme summarized in Figure S3A. First, in order to enhance the microtubule signal, the raw microscopical

images were applied with a deep learning-based 2-D segmentation function of an image analysis software AIVIA (DRVision, Bellevue, WA, United States)⁶⁴. The deep learning model was trained using our training dataset consisting of 162 raw images extracted from the first and the last frames of all time-lapse data and corresponding binary images in which microtubules were manually segmented (Figure S3A, denoted as “Training dataset”). Then, the enhanced images were skeletonized using the ImageJ plug-in LpxLineExtract⁶⁵. Parameters used are as follows: giwsIter = 10, mdnmsLen = 15, shaveLen = 5, and delLen = 5. To determine the cell regions, raw images were filtered with a Gaussian filter (Sigma = 10 pixels) and binarized with Otsu’s thresholding using ImageJ. Finally, the occupancy (microtubule density inside cells) was calculated as a pixel ratio of the skeletonized region against the cell area.

Microtubule polymerization—Cow brain tubulin was purified by high-molarity PIPES method described previously⁶⁶. Y- and Δ Y-tubulin were purified from HeLa cells using TOG affinity chromatography as described¹⁶. To obtain Δ Y-tubulin, HeLa cell lysate was treated with carboxypeptidase A (Sigma, Cat# C9268) for 20 min on ice prior to the TOG chromatography¹⁶.

Taxol-stabilized MTs were polymerized from 60 μ M brain tubulin [unlabeled or including 10% HiLy647-labeled porcine brain tubulin (Cytoskeleton, Cat# TL670M)], 30 μ M HeLa Y-tubulin, or 28 μ M HeLa Δ Y-tubulin (CPA-treated HeLa tubulin) in BRB80 buffer (80 mM Pipes/KOH pH 6.8, 1 mM $MgCl_2$, and 1 mM EGTA) supplemented with 2.5 mM GTP and 4 mM $MgCl_2$ at 37°C for 35 min. A 5x volume of prewarmed BRB80 buffer containing 10 μ M taxol was added and incubated at 37°C for an additional 35 min to stabilize MTs. The MTs were centrifuged at 15,000 rpm for 10 min at room temperature. The pellet was resuspended with the same 5x volume of prewarmed BRB80 buffer containing 10 μ M taxol. MTs were stored in the dark at room temperature for further use.

To make the unlabeled taxol-stabilized Δ Y-MTs (VASH1), taxol-stabilized Y-MTs were infused into a flow cell and incubated for 3 min at room temperature for nonspecific adsorption to the coverslips. After being washed by blocking buffer [1mg/ml casein in P12 buffer (12 mM Pipes/KOH pH 6.8, 1 mM $MgCl_2$, 1 mM EGTA)], the flow cell was infused with P12 buffer supplemented with 3 mg/ml casein and cell lysate containing 1 nM unlabeled VASH1/SVBP and incubated for 6 min to generate the Δ Y-MTs. The Δ Y-MTs was confirmed by the Δ Y-Fab⁴⁸⁸ probe.

Unlabeled GMPCPP-stabilized Y-MTs were polymerized from 5 μ M HeLa Y-tubulin in BRB80 buffer supplemented with 4 mM nonhydrolyzable GTP analog GMPCPP (Jena Bioscience) and 4 mM $MgCl_2$ at 37°C for 60 min. A 5x volume of prewarmed BRB80 buffer containing 1 mM DTT was added and incubated at 37°C for an additional 60 min to stabilize MTs. The MTs were centrifuged at 15,000 rpm for 10 min at room temperature. The pellet was resuspended with the 2.5-time volume of prewarmed BRB80 buffer containing 1 mM DTT. MTs were stored in the dark at room temperature for further use.

To make the capped GDP-Y-MTs, GMPCPP-MT seeds were generated by incubating 20 μ M Hela Y-tubulin, 2 μ M biotinylated-porcine brain tubulin (Cytoskeleton Inc, Cat# T333P), and 2 μ M X-Rhodamine porcine brain tubulin (Cytoskeleton Inc, Cat# TL620M), 2 mM GMPCPP (Jena Bioscience, Cat# NU405S) and 2.5 mM MgCl_2 in BRB80 buffer for 35 min at 37 °C. A 5x volume of prewarmed BRB80 buffer was then added. The seeds were centrifuged at 90,000 rpm for 5 min at 25 °C (Beckman Coulter). The microtubule pellet was resuspended with a 2x volume of warm BRB80 buffer and microtubule seeds were stored in the dark at room temperature. GMPCPP-MT seeds were then attached to the surface of a flow cell by sequential incubation with (1) 1 mg/ml BSA-biotin (Sigma, Cat# A8549) for 3 min, (2) blocking buffer (1mg/ml BSA in BRB80 buffer), (3) 0.5 mg/ml NeutrAvidin (Thermo, Cat# 31000) for 3 min, (4) blocking buffer, (5) biotinylated GMPCPP-MT seeds for 3 min, and (6) blocking buffer. GDP-Y-MTs were grown from the GMPCPP-MT seeds by incubating with 20 μ M Y-tubulin (additional 5% HiLyte647-labeled tubulin was added for MT detyrosination assays) and 1 mM GTP in reaction buffer [BRB80 buffer supplemented with 0.1% methylcellulose, 1 mg/ml casein, 3 mM MgCl_2 , 6 mM DTT] for 10 min at 37°C. Finally, GDP-Y-MTs were capped by incubating with 10 μ M unlabeled Y-tubulin, and 1 mM GMPCPP in reaction buffer for 5 min at 37°C.

Unlabeled glycerol-stabilized GDP-Y-MTs were polymerized from 60 μ M HeLa Y-tubulin in BRB80 buffer supplemented with 2.5 mM GTP and 5 mM MgCl_2 at 37°C for 35 min. A 5x volume of prewarmed BRB80 buffer containing 25% glycerol and 1 mM GTP was added. The MTs were centrifuged at 15,000 rpm for 10 min at room temperature. The pellet was resuspended with the same 5-time volume of BRB80 buffer containing 25% glycerol and 1 mM GTP. MTs were stored in the dark at room temperature for further use.

SiR-tubulin and Δ Y-Fab⁴⁸⁸ probe staining *in vitro*—Taxol-stabilized MTs and 500 nM SiR-tubulin (Cytoskeleton, Cat# CY-SC002) were incubated in BRB80 buffer for 5 min at room temperature. The labeled MTs were then infused into the flow cell and incubated for 3 min at room temperature for nonspecific adsorption to the coverslips. Subsequently, blocking buffer (1mg/ml casein in P12 buffer) was infused, followed by adding the imaging buffer [3 mg/ml casein and oxygen scavenging (1 mM DTT, 1 mM MgCl_2 , 10 mM glucose, 0.2 mg/ml glucose oxidase, and 0.08 mg/ml catalase) in P12 buffer] supplemented with 1.5 ng/ μ l Δ Y-Fab⁴⁸⁸ probe to the flow cell and incubated for 5 min at room temperature. After being washed by blocking buffer, the flow cell was infused with imaging buffer. The flow cell was sealed with molten paraffin wax. A snapshot of the microtubules was acquired by a TIRF microscope.

Single-molecule assays *in vitro*—To examine MT binding behavior of VASH1/SVBP, taxol-stabilized and GMPCPP-stabilized Y-MTs were polymerized as described above, added into a flow cell, and incubated for 3 min at room temperature for nonspecific adsorption to the coverslips. Capped GDP-Y-MTs were polymerized in a flow cell as described above. After being washed by blocking buffer (1 mg/ml casein in P12 buffer), the flow cell was infused with imaging buffer [3 mg/ml casein in P12 buffer supplemented with oxygen scavenging, and cell lysate containing 100 pM VASH1/SVBP-Halo^{JFX554}]. The flow cell was sealed with molten paraffin wax. To examine the binding of single molecules of

VASH1/SVBP to MTs in buffers with different ionic strengths, the P12 buffer was replaced with either BRB40 (40 mM Pipes/KOH pH 6.8, 1 mM MgCl₂, and 1 mM EGTA), BRB40 supplemented with 50 mM KCl, or BRB80. The ionic strength of each buffer was calculated based on the molar concentration of each ion and its counterion based on [BioMol.net](http://www.biomol.net/en/tools/buffercalculator.htm) (<http://www.biomol.net/en/tools/buffercalculator.htm>). Images were acquired continuously every 100 ms for 300 frames by a TIRF microscope. Maximum-intensity projections were generated, and kymographs were produced by drawing along MTs (width= 3 pixels) using Fiji/ImageJ2. The mean dwell time was determined for those events whose dwell time was more than 0.35 s. The landing rate was calculated as the number of MT binding events per minute per nanomolar protein per micrometer of MT.

To examine MT binding behavior of CAMSAPs, glycerol-stabilized HiLy647-labeled brain MTs or glycerol stabilized Y-MTs were polymerized as described above, added into a flow cell, and incubated for 3 min at room temperature for nonspecific adsorption to the coverslips. Then the blocking buffer (1 mg/ml casein, 25% glycerol, and 1 mM GTP in P12 buffer) was infused. Subsequently, the reaction buffer (5 μ l cell lysates expressing CAMSAPs, 5 μ l 10 mg/ml casein in P12 buffer, 30 μ l 25% glycerol in P12 buffer, and 1 mM GTP) was added to the flow cell and incubated for 10 min at room temperature. After being washed by blocking buffer, the flow cell was infused with imaging buffer (15 μ l 10 mg/ml casein in P12 buffer and 30 μ l 25% glycerol in P12 buffer supplemented with 1 mM GTP and oxygen scavenging). Snapshot were acquired by a TIRF microscope immediately.

MT detyrosination assay *in vitro*—To test whether the Δ Y-Fab⁴⁸⁸ probe can detect the formation of MT detyrosination in real-time, taxol-stabilized Y-MTs were polymerized as described above, added into a flow cell, and incubated for 3 min at room temperature for nonspecific adsorption to the coverslips. After being washed by blocking buffer (1 mg/ml casein in P12 buffer), the flow cell was infused with imaging buffer [15 μ l 10 mg/ml casein in P12 buffer and 30 μ l 10 μ M taxol in P12 buffer supplemented with oxygen scavenging (1 mM DTT, 1 mM MgCl₂, 10 mM glucose, 0.2 mg/ml glucose oxidase, and 0.08 mg/ml catalase), cell lysate containing 1 nM VASH1/SVBP-Halo^{JFX554} and 592 pg/ μ l Δ Y-Fab⁴⁸⁸ probe]. For the negative control, the imaging buffer contained additional 100 μ M EpoY. For the positive control, Δ Y-MTs were made by unlabeled VASH1/SVBP-Halo in a flow cell as described above. After being incubated with blocking buffer supplemented with 100 μ M EpoY for 1 min to inactivate the VASH1/SVBP, the flow cell was washed by blocking buffer and infused with imaging buffer (15 μ l 10 mg/ml casein in P12 buffer and 30 μ l 10 μ M taxol in P12 buffer supplemented with oxygen scavenging and 592 pg/ μ l Δ Y-Fab⁴⁸⁸ probe). The movie was acquired every 5 s for 10 min (slow imaging) or every 200 ms for 3 min (fast imaging) by a TIRF microscope immediately.

To compare VASH1/SVBP detyrosination activity on GMPCPP-stabilized Y-MTs and capped GDP-Y-MTs, GMPCPP-stabilized Y-MTs were polymerized as described above, added into a flow cell, and incubated for 3 min at room temperature for nonspecific adsorption to the coverslips. Capped GDP-Y-MTs were polymerized in a flow cell as described above. After being washed by blocking buffer (1 mg/ml casein in P12 buffer), the flow cell was infused with imaging buffer (3 mg/ml casein in P12 buffer supplemented with

oxygen scavenging, cell lysate containing 1 nM VASH1/SVBP-Halo and 74 pg/μl ΔY-Fab⁴⁸⁸ probe). The movie was acquired every 5 s for 5 min by a TIRF microscope immediately.

To examine VASH1/SVBP detyrosination activity on the glycerol-stabilized GDP-Y-MTs with DMSO and taxol treatment, glycerol-stabilized Y-MTs were polymerized as described above, added into a flow cell, and incubated for 3 min at room temperature for nonspecific adsorption to the coverslips. After being washed by blocking buffer (1 mg/ml casein, 25% glycerol, and 1 mM GTP in P12 buffer), the flow cell was infused with imaging buffer (15 μl 10 mg/ml casein in P12 buffer and 30 μl 25% glycerol in P12 buffer supplemented with oxygen scavenging, 1 mM GTP, 2 nM VASH1/SVBP-Halo cell lysate, 592 pg/μl ΔY-Fab⁴⁸⁸ probe, and 80 μM taxol/DMSO). The movie was acquired every 5 s for 10 min by a TIRF microscope immediately.

To compare VASH1/SVBP detyrosination activity on capped GDP-Y-MTs without or with taxol treatment, capped GDP-Y-MTs were polymerized in a flow cell as described above. After being washed by blocking buffer (1 mg/ml casein in P12 buffer), the flow cell was infused with imaging buffer (30 μl P12 buffer without or with 10 μM taxol and 15 μl 3 mg/ml casein in P12 buffer supplemented with oxygen scavenging, cell lysate containing 2 nM VASH1/SVBP-Halo and 74 pg/μl ΔY-Fab⁴⁸⁸ probe). The movie was acquired every 5 s for 5 min by a TIRF microscope immediately.

To investigate VASH1/SVBP detyrosination activity on the glycerol-stabilized Y-MT by pre-treatment with cell lysates expressing with superTagRFP-CAMSAP2 or untransfected cell lysates, glycerol-stabilized Y-MTs were polymerized as described above, added into a flow cell, and incubated for 3 min at room temperature for nonspecific adsorption to the coverslips. Then the blocking buffer (1 mg/ml casein, 25% glycerol, and 1 mM GTP in P12 buffer) was infused. Subsequently, the reaction buffer (5 μl cell lysates expressing CAMSAPs, 5 μl 10 mg/ml casein in P12 buffer, 30 μl 25% glycerol in P12 buffer, and 1 mM GTP) was added to the flow cell and incubated for 10 min at room temperature. After being washed by blocking buffer, the flow cell was infused with imaging buffer (15 μl 10 mg/ml casein in P12 buffer and 30 μl 25% glycerol in P12 buffer supplemented with 1 mM GTP, cell lysate containing 2 nM VASH1/SVBP-Halo, 592 pg/μl ΔY-Fab⁴⁸⁸ probe and oxygen scavenging). The movie was acquired every 5 s for 10 min by a TIRF microscope immediately.

For the quantification of the fluorescence intensity of the ΔY-Fab⁴⁸⁸ along the MTs over time, the mean fluorescence intensity of the ΔY-Fab⁴⁸⁸ probe along the MT in each frame was measured by drawing the line along the MT (width= 3 pixels) using Fiji/ImageJ2. The Normalization was made by subtracting the initial fluorescence intensity of the ΔY-Fab⁴⁸⁸ probe along the same MT (t= 0 min).

QUANTIFICATION AND STATISTICAL ANALYSIS

For the quantification of MT detyrosination assays *in vitro* (Figures 2D, 4F, 5G, 6F, S1B, S2D, and S4B), statistical analyses were performed with GraphPad Prism 8 (GraphPad Software). The mean and standard error, the number of values examined, the experimental replicates, and the statistical tests applied are described in the figure legends. Statistical

significance was determined as ****, $p < 0.0001$ and n.s., not significant using a two-tailed t -test (Figures S1B and S2D).

For the quantification of single-molecule assays *in vitro* (Figures 3C, 3D, 4B, and 4C), statistical analyses were performed with GraphPad Prism 8 (GraphPad Software). The mean and median with quartiles, the number of values examined, the experimental replicates, and the statistical tests applied are described in the figure legends. Statistical significance was determined as n.s., not significant using a two-tailed t -test.

For the quantification of dephosphorylation in the live imaging experiment (Figure 5C), images were processed with a deep learning-based 2-D segmentation prior to the quantification of the filament density. Scheme is summarized in Figure S3A and detailed information is described in the Method details section. Statistics are described in the legends.

For the quantification of dephosphorylation in fixed and stained cells (Figures 6C and H, S3C and S5C), statistical analyses were performed with GraphPad Prism 9 (GraphPad Software) using one-way ANOVA (Figures 6C, S3C and S5C) or a two-tailed t -test (Figure 6H). Statistical significance was determined at $P < 0.05$. Data were obtained for numbers indicated in each graph from three independent experiments unless otherwise noted. Detailed information is described in the legends.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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INCLUSION AND DIVERSITY

All authors of this study work to be inclusive, recruit team members from diverse backgrounds, and carry out research in an equitable manner.

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Highlights

- A biosensor that detects microtubule detyrosination in real-time is developed.
- The enzyme VASH1/SVBP preferentially detyrosinates mimics of GTPmicrotubules.
- Taxol likely promotes microtubule detyrosination by inducing a GTP-mimic state.
- The microtubule-associated proteins CAMSAP2/3 promote microtubule detyrosination.

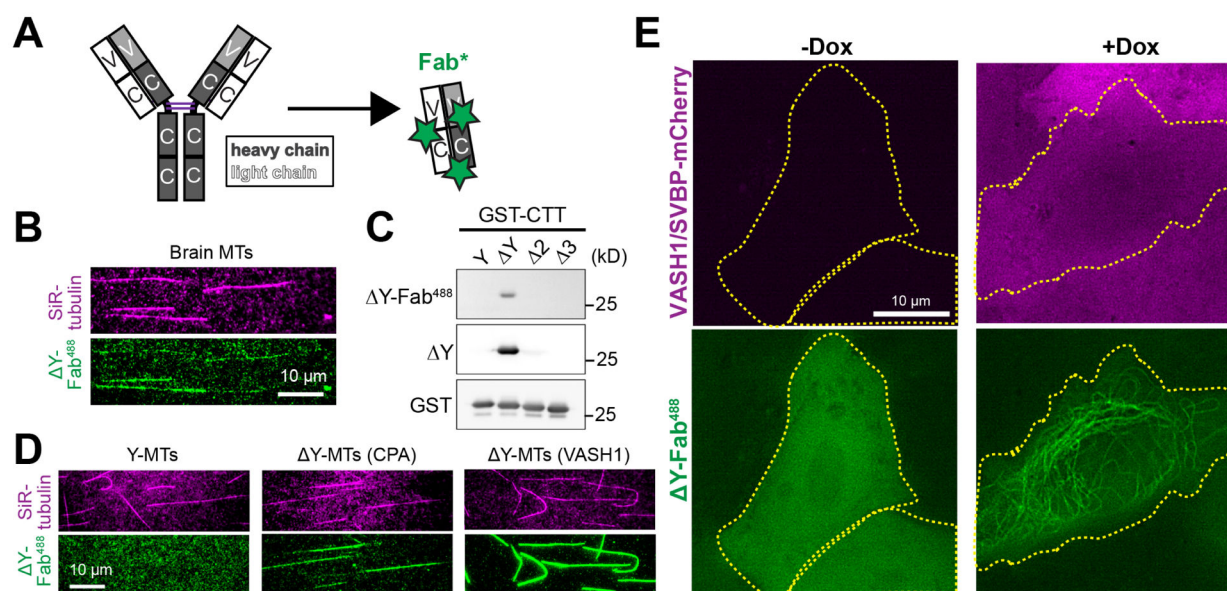


Figure 1: The ΔY -Fab⁴⁸⁸ probe is a biosensor ΔY -MTs.

(A) Schematic of probe development. A monoclonal antibody raised against ΔY - α -tubulin was non-specifically labelled with Alexa⁴⁸⁸ dye and then a Fab fragment was produced by papain digestion.

(B) Representative images of taxol-stabilized brain MTs stained with the ΔY -Fab⁴⁸⁸ probe (green) and SiR-tubulin (magenta). Scale bar, 10 μ m.

(C) Purified GST proteins fused to various α -tubulin C-terminal tail sequences were probed by western blotting with the ΔY -Fab⁴⁸⁸ probe (top), the RM444 monoclonal ΔY - α -tubulin antibody (middle), or antibody against GST (bottom). The α -tubulin CTT sequences are full-length (Y), missing the C-terminal tyrosine (ΔY), missing the C-terminal two amino acid residues ($\Delta 2$), or missing the C-terminal three amino acid residues ($\Delta 3$).

(D) Representative images of MTs assembled from HeLa tubulin either untreated (Y-MTs, left) or treated with carboxypeptidase A (CPA, middle) or VASH1/SVBP (VASH1, right) to generate ΔY -MTs. The MTs were stained with ΔY -Fab⁴⁸⁸ probe (green) and SiR-tubulin (magenta). Scale bar, 10 μ m.

(E) Representative images of VASH1/SVBP-mCherry-inducible HeLa cells loaded with the ΔY -Fab⁴⁸⁸ probe (green) in the absence (uninduced) or presence (induced) of doxycycline (Dox). Magenta, VASH1/SVBP-mCherry. Yellow dotted lines outline cells that have taken up the ΔY -Fab⁴⁸⁸ probe. Scale bar, 10 μ m. See also Figure S1.

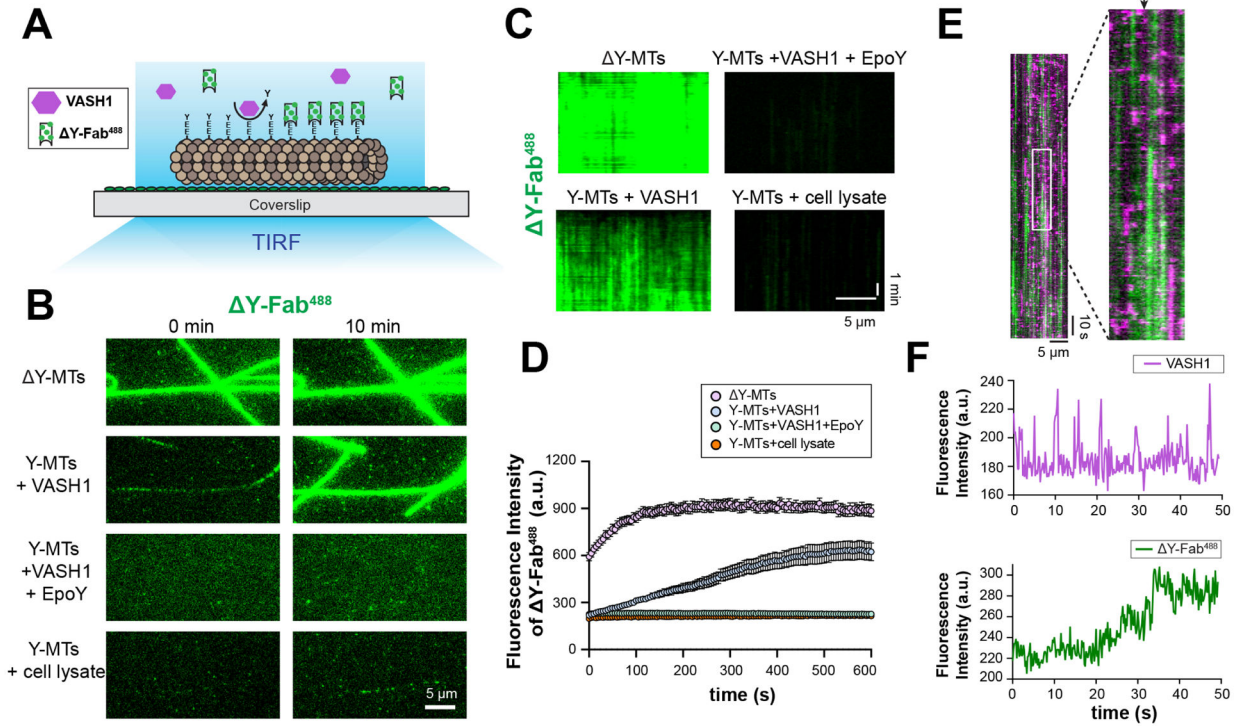


Figure 2: Frequent short interactions of VASH1/SVBP with MTs result in rapid detyrosination of the entire MT lattice.

(A) Schematic of the *in vitro* MT detyrosination assay. VASH1/SVBP molecules (magenta) detyrosinate Y-MTs and the resulting Δ Y-MT is bound by the Δ Y-Fab⁴⁸⁸ probe (green).

(B-D) VASH1/SVBP detyrosination activity assay. (B) Representative images of the Δ Y-Fab⁴⁸⁸ probe (green) at the start (0 min) and after 10 min of: Δ Y-MTs generated by previous incubation with VASH1/SVBP cell lysate (top row), Y-MT incubated with cell lysate containing 1 nM VASH1/SVBP (second row), Y-MTs incubated with cell lysate expressing 1 nM VASH1/SVBP and 100 μ M EpoY (third row), and Y-MTs incubated with untransfected cell lysate (bottom row). Scale bar, 5 μ m.

(C) Representative kymographs of Δ Y-Fab⁴⁸⁸ probe (green) labeling over time. Vertical scale bar, 1 min; horizontal scale bar, 5 μ m. The video was acquired at 1 frame/5s.

(D) Quantification of the mean fluorescence intensity of the Δ Y-Fab⁴⁸⁸ probe over time.

Mean $\pm$ Standard Error (S.E.M) across ≥ 14 MTs from three independent experiments.

(E and F) Live imaging of VASH1/SVBP binding and detyrosination activity. (E) Representative kymograph showing VASH1/SVBP-Halo^{JFX554} (magenta) and Δ Y-Fab⁴⁸⁸ probe (green) binding to taxol-stabilized Y-MTs over time. The video was acquired at 5 frames/s. Vertical scale bar, 10 sec; horizontal scale bar, 5 μ m. The boxed region indicates the area magnified on the right. The arrow indicates the position on the MT used to generate (F) line scans of the fluorescence intensity of VASH1/SVBP-Halo^{JFX554} (magenta) and Δ Y-Fab⁴⁸⁸ (green) over time.

See also Figure S2.

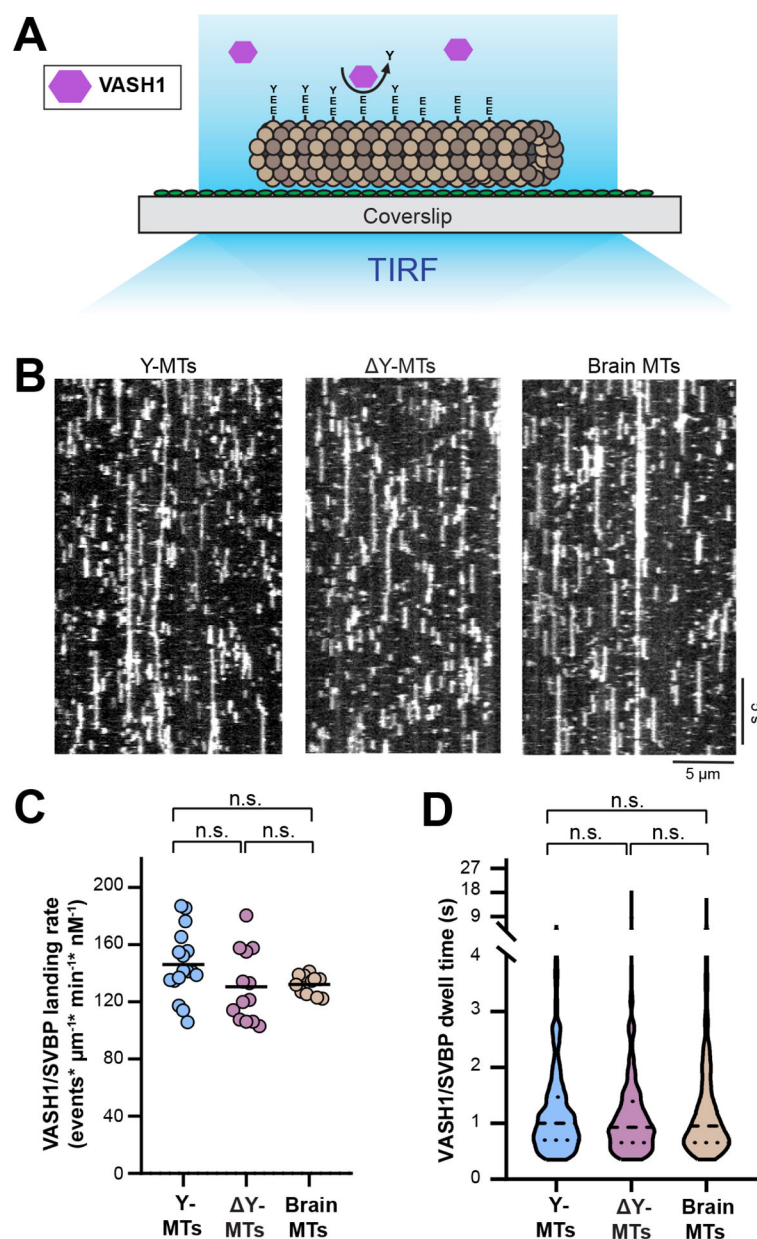


Figure 3: VASH1/SVBP's MT binding is not sensitive to the MT detyrosination state.

(A) Schematic of single-molecule VASH1/SVBP MT-binding assay.

(B) Representative kymographs of 100 pM VASH1/SVBP-Halo^{JFX554} in cell lysate on taxol-stabilized HeLa Y-MTs, HeLa ΔY-MTs generated by prior incubation with VASH1/SVBP cell lysate, and brain MTs. Vertical scale bar, 5 s; horizontal scale bar, 5 μm.

(C,D) Quantification of the (C) landing rate and (D) dwell time of VASH1/SVBP on taxol-stabilized Y-MTs, ΔY-MTs, and brain MTs. In (C), each spot indicates the events on a single MT and the horizontal line indicates the mean value across ~15 MTs from two independent experiments.

In (D), the violin plot indicates the median values with quartiles for $n \geq 430$ events from two independent experiments. n.s., not significant as determined by two-tailed t test.

See also Video S1.

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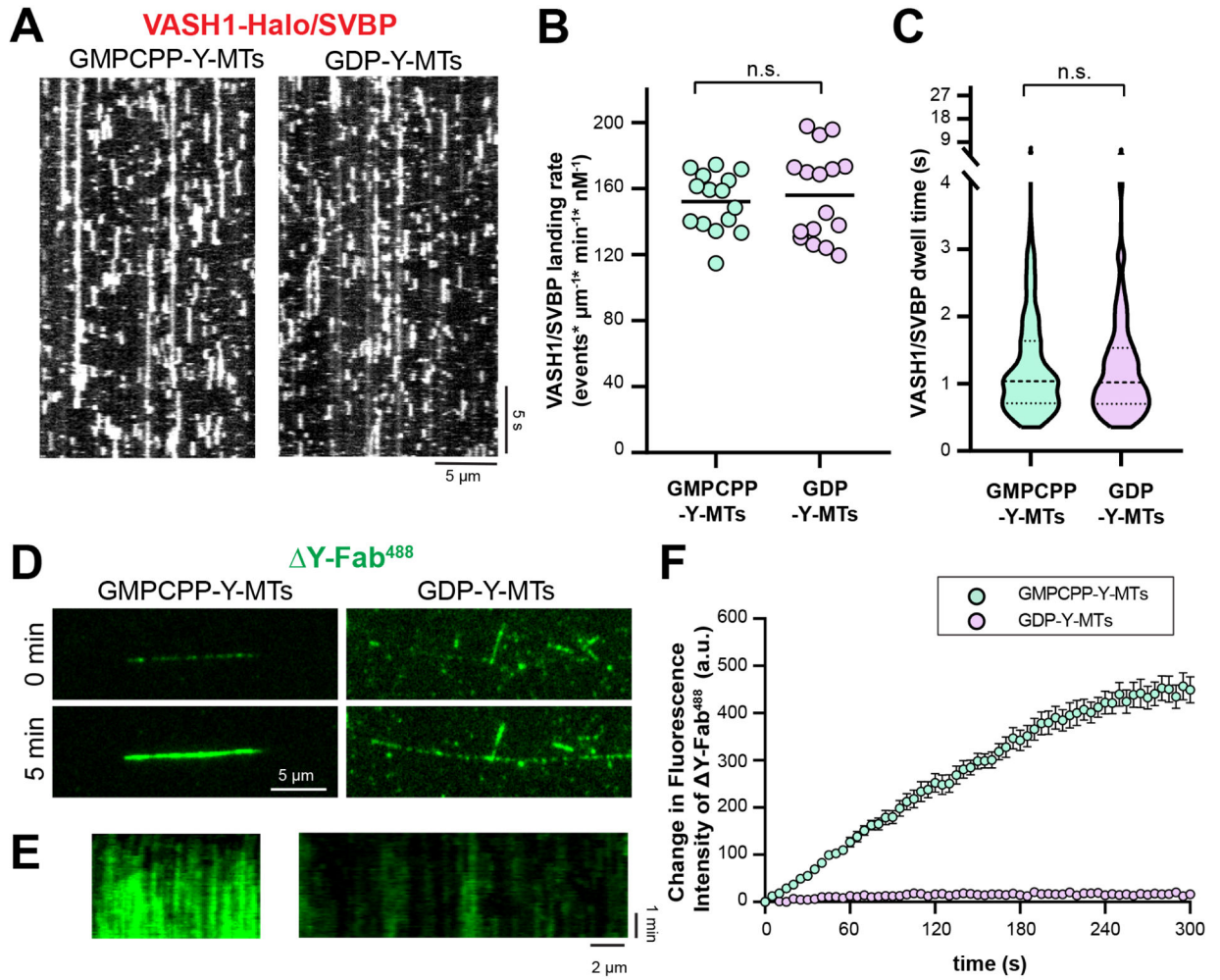


Figure 4: VASH1/SVBP activity is sensitive to the tubulin nucleotide state.

(A-C) Single-molecule VASH1/SVBP MT-binding assay. (A) Representative kymographs of 100 pM VASH1/SVBP in cell lysate on GMPCPP-Y-MTs or capped GDP-Y-MTs. Vertical scale bar, 5 sec; horizontal scale bar, 5 μ m. (B,C) Quantification of the (B) landing rate and (D) dwell time of VASH1/SVBP on GMPCPP-Y-MTs and GDP-Y-MTs. In (B), each spot indicates the number of events on a single MT and the horizontal line indicates the mean value across ~ 15 MTs from two independent experiments. In (C), the violin plot indicates the median values with quartiles for $n \geq 430$ VASH1/SVBP molecules from two independent experiments. n.s., not significant as determined by a two-tailed t -test.

(D-F) MT detyrosination assay as measured by Δ Y-Fab⁴⁸⁸ probe (green) labeling. (D) Representative images the start (0 min) and after 5 min of incubation of 1 nM VASH1/SVBP in cell lysate with GMPCPP-Y-MTs or capped GDP-Y-MTs. Scale bar, 5 μ m. (E) Representative kymographs of Δ Y-Fab⁴⁸⁸ probe (green) labeling over time. Vertical scale bar, 1 min; horizontal scale bar, 2 μ m. (F) Quantification of mean fluorescence intensity of the Δ Y-Fab⁴⁸⁸ probe along GMPCPP-Y-MTs or GDP-Y-MTs over time. Mean $\pm$ S.E.M. across $n=16$ GMPCPP-Y-MTs from four independent experiments and $n=9$ GDP-Y-MTs from three independent experiments.

See also Videos S2 and S3.

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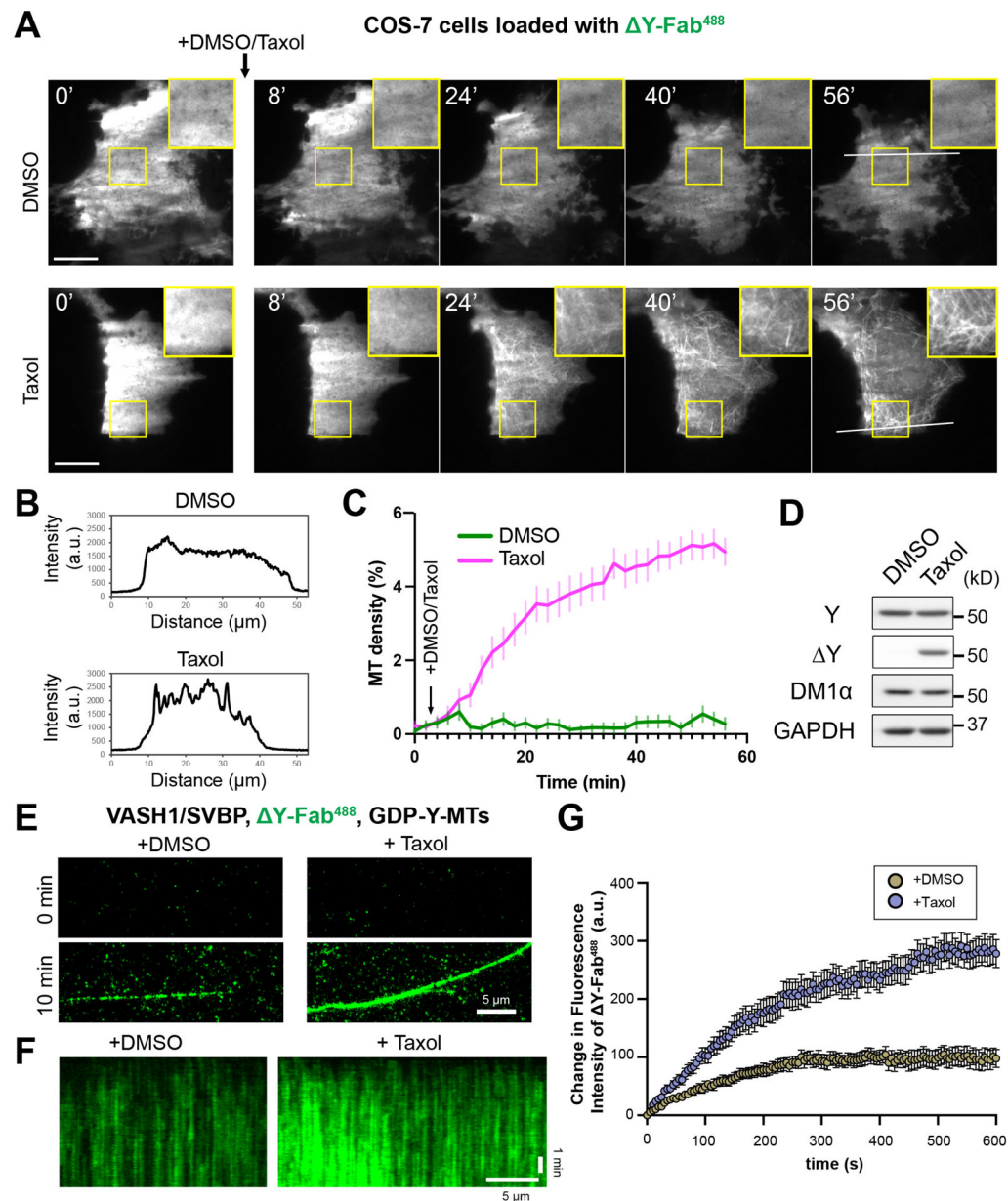


Figure 5. VASH1/SVBP activity is stimulated by taxol treatment both in cells and *in vitro*.

(A-D) Taxol increases VASH1/SVBP activity in cells. (A) Representative time-lapse images showing MT decoration by the $\Delta Y\text{-Fab}^{488}$ probe upon taxol treatment of COS-7 cells. Arrow indicates the time point for DMSO (vehicle control) or taxol addition. The yellow boxed regions are enlarged in the insets. Scale bars, 20 μm .

(B) Line profile of the $\Delta Y\text{-Fab}^{488}$ fluorescence intensity along the white lines in (A).

(C) Quantification of the density of $\Delta Y\text{-Fab}^{488}$ -decorated filaments over time in COS-7 cells treated with DMSO or taxol. Error bar, S.E.M. across 42 (DMSO) and 39 (Taxol) images from three independent experiments.

(D) Immunoblot analysis of lysates prepared from COS-7 cells treated with DMSO or taxol for 1 hour.

(E-G) Taxol increases VASH1/SVBP activity *in vitro*. (E) Representative images of ΔY -Fab⁴⁸⁸ probe (green) labeling of glycerol-stabilized GDP-Y-MTs at the start (0 min) or 10 min after the addition of cell lysate expressing 2 nM VASH1/SVBP with DMSO (vehicle control) or 80 μ M taxol. Scale bar, 5 μ m.

(F) Representative kymographs and (G) quantification of ΔY -Fab⁴⁸⁸ probe (green) labeling glycerol-stabilized GDP-Y-MTs over time upon the addition of 2 nM VASH1/SVBP with DMSO or taxol. In (F), vertical scale bar, 1 min; horizontal scale bar, 5 μ m. In (G), mean $\pm$ S.E.M across n=8 GDP-Y-MTs with DMSO treatment from four independent experiments and n=13 GDP-Y-MTs with taxol treatment from three independent experiments.

See also Figures S3 and S4, and Videos S4 and S5.

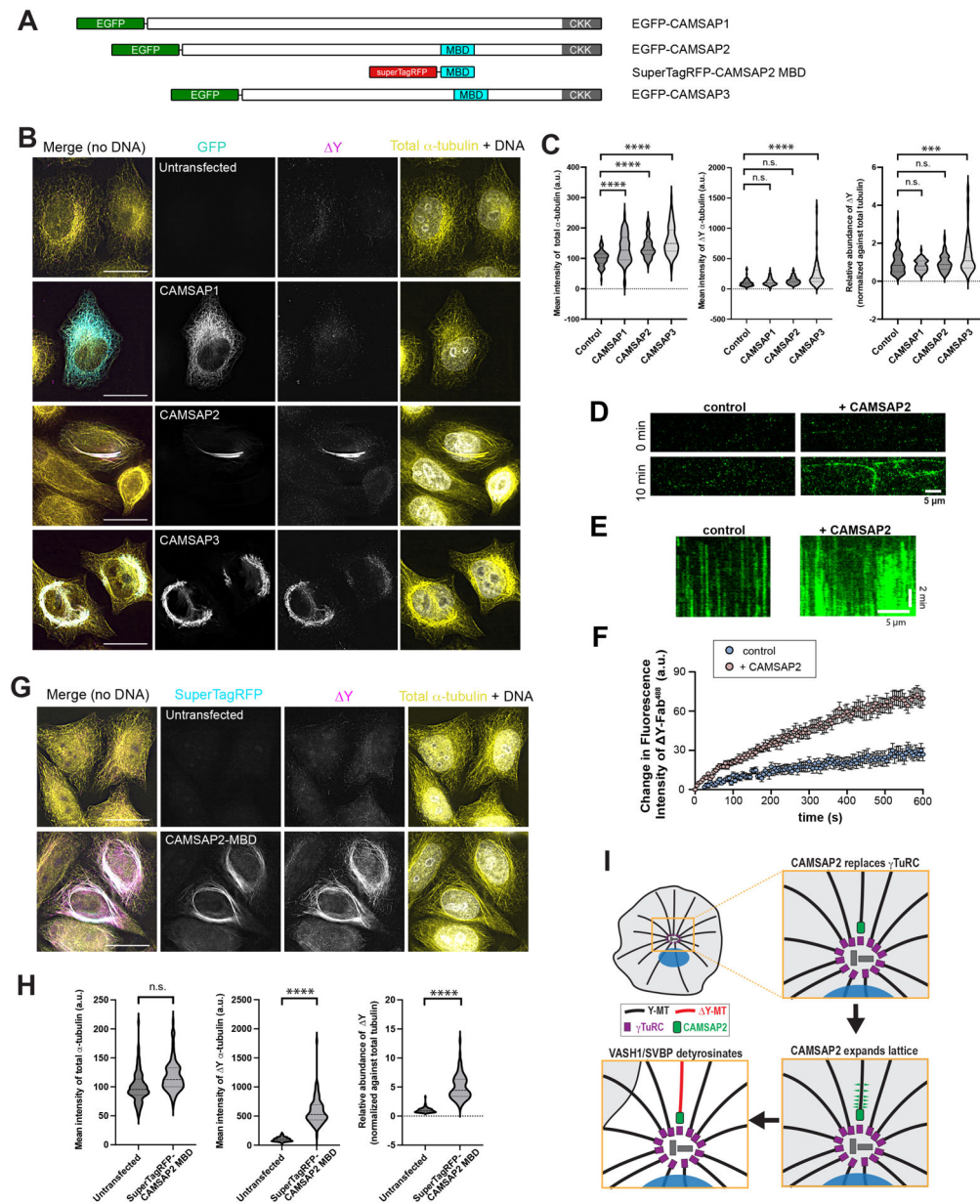


Figure 6. CAMSAPs promote MT dytrosination.

(A-C) CAMSAPs promote MT dytrosination in cells. (A) Schematic of human CAMSAP constructs.

(B) HeLa cells expressing the indicated GFP-tagged CAMSAPs were fixed and processed for immunofluorescence. Cells that were not transfected served as a negative control (untransfected). In merged images, total α -tubulin is shown in yellow, CAMSAPs in cyan, ΔY - α -tubulin in magenta, and DNA in gray. Scale bars, 20 μ m.

(C) Quantification of dytrosination in CAMSAP-expressing HeLa cells. Violin plots of mean fluorescence intensity of total α -tubulin and ΔY - α -tubulin, and relative abundance of ΔY - α -tubulin normalized against total α -tubulin (ΔY - α -tubulin/ total α -tubulin). n (number of cells analyzed) = 80 (untransfected control), 65 (GFP-CAMSAP1), 70 (GFP-CAMSAP2)

and 75 (GFP-CAMSAP3) from 3 independent experiments. n.s., not significant; *** $p < 0.01$; **** $p < 0.001$ as determined by one-way ANOVA followed by Dunnett's multiple comparison test.

(D-F) MT detyrosination assay *in vitro* using HeLa GDP-Y-MTs pre-treated with untransfected cell lysate (control) or CAMSAP2 cell lysate. (D) Representative images at start (0 min) and 10 min after incubation with 2 nM VASH1/SVBP in cell lysate and the ΔY -Fab⁴⁸⁸ probe (green). Scale bar, 5 μm . (E) Representative kymographs and (F) quantification of ΔY -Fab⁴⁸⁸ probe (green) labeling over time. Vertical scale bar, 2 min; horizontal scale bar, 5 μm . Mean $\pm$ S.E.M. across $n=19$ GDP-Y-MTs pre-treated by cell lysates overexpressing CAMSAP2, and $n=13$ MTs pre-treated with untransfected cell lysates from three independent experiments.

(G,H) The CAMSAP2 MBD promotes MT detyrosination. (G) Immunofluorescence staining of HeLa cells expressing superTagRFP-CAMSAP2 MBD. In merged images, total α -tubulin is shown in yellow, CAMSAP2 MBD in cyan, ΔY - α -tubulin in magenta, and DNA in gray. Scale bars, 20 μm .

(H) Quantification of mean fluorescence intensity of total α -tubulin and ΔY - α -tubulin, and relative abundance of ΔY - α -tubulin normalized against total α -tubulin (ΔY - α -tubulin/total α -tubulin). n (number of cells analyzed) = 104 (untransfected control) and 91 (superTagRFP-CAMSAP2 MBD) from 3 independent experiments. n.s., not significant; **** $p < 0.001$ as determined by a two-tailed *t*-test.

(I) Model showing how CAMSAP2 promotes MT detyrosination by VASH1/SVBP in cells. See also Figures S5 and S6.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Halo tag	Promega	Cat#G9281
680nm anti-rabbit	Jackson ImmunoResearch Laboratories Inc.	Cat#711-625-152
Anti-detyrosinated α -tubulin, rabbit monoclonal, clone RM444	RevMab Biosciences	Cat# 31-1335-00
Anti-tyrosinated α -tubulin, rat monoclonal, clone YL1/2	Accurate Chemical and Scientific	Cat# YSRTMCA77G
Anti- α -tubulin , mouse monoclonal, clone DM1 α	Millipore Sigma	Cat# 05-829
Anti- α -tubulin conjugated with FITC, mouse monoclonal, clone DM1 α	Sigma-Aldrich	Cat# F2168
Anti- α -tubulin conjugated with Alexa Fluor 647, mouse monoclonal, clone DM1 α	Sigma-Aldrich	Cat# 05-829-AF647
Anti-GAPDH mouse monoclonal, clone G-9	Santa Cruz	Cat# sc-365062
Anti-GST mouse monoclonal	Nacalai USA	Cat# 04435-26
Anti-rabbit Alexa Fluor 488, goat	Thermo Fisher	Cat# A-11034
Anti-rabbit Alexa Fluor 594, goat	Thermo Fisher	Cat# A-11012
Anti-rat Alexa Fluor 680, goat	Thermo Fisher	Cat# A-21096
Anti-rabbit IRDye 800CW, goat	LI-COR	Cat# 926-32211
Anti-mouse Alexa Fluor 700, goat	Thermo Fisher	Cat# A-21036
Bacterial and virus strains		
DH5 α	Invitrogen	Cat# 18258-012
Rosetta2(DE3)pLysS	Millipore	Cat# 71403-3
Biological samples		
x-Rhodamine tubulin	Cytoskeleton	Cat#TL620M
Biotinylated-porcine brain tubulin	Cytoskeleton	Cat#T333P
HiLyte647 tubulin	Cytoskeleton	Cat#TL670M
Bovine brain tubulin	R. Ohi lab	lab purified
Chemicals, peptides, and recombinant proteins		
Dulbecco's Modified Eagle Medium (DMEM)	Gibco	Cat# 11960
Fetal Clone III serum	HyClone	Cat# SH30109.03
GlutaMAX supplement	Gibco	Cat# 35050061
Janelia Fluor X 554 (JFX554) Halo ligand	Janelia Farms	Cat# JFX554
Trans-IT LT1	Mirus	Cat# MIR2305
Protease inhibitors	Sigma-Aldrich	Cat# P8340
Taxol	Cytoskeleton	Cat# TXD01
Bovine serum albumin	Sigma	Cat# A9647
Casein	Sigma	Cat# C8654

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Glucose oxidase	Sigma-Aldrich	Cat# G7141-10KU
Catalase	Sigma	Cat# C3515
GTP	Sigma	Cat# G8877
GMPCPP	Jena Bioscience	Cat# NU405S
BSA-biotin	Sigma	Cat# A8549
NeutrAvidin	Thermo	Cat# 31000
SiR-tubulin	Cytoskeleton	Cat# CY-SC002
EpoY	Vahlteich Medicinal Chemistry Core at University of Michigan	N/A
carboxypeptidase	Sigma	Cat# C9268
L-15	Gibco	Cat# 21083-027
FBS	Atlanta Biologicals	Cat# S11150
Penicillin/streptomycin	Thermo Fisher	Cat# 15140122
DPBS	Gibco	Cat# 14190-144
Critical commercial assays		
Fluorescent Protein Labeling Kit	Thermo Fisher	Cat# A10235
Fab Preparation Kit	Thermo Fisher	Cat# 44985
Deposited data		
Experimental models: Cell lines		
COS-7	ATCC	RRID: CVCL_0224
HeLa Kyoto	Shuh Narumiya	RRID: CVCL_1922
Knock-in HeLa Kyoto cell line co-expressing PA-VASH1-mCherry and SVBP-FLAG	This study	N/A
Experimental models: Organisms/strains		
Oligonucleotides		
Recombinant DNA		
VASH1-Halo-IRES-SVBP	This study	N/A

REAGENT or RESOURCE	SOURCE	IDENTIFIER
pEM791-VASH1-mCherry-IRES-SVBP	This study	N/A
pEGFP-C1-CAMSAP1	This study	N/A
pEGFP-C1-CAMSAP2	This study	N/A
pEGFP-C1-CAMSAP3	This study	N/A
psuperTagRFP-C1-CAMSAP2	This study	N/A
psuperTagRFP-C1-CAMSAP3	This study	N/A
psuperTagRFP-C1-CAMSAP2-MBD	This study	N/A
Software and algorithms		
Fiji/ImageJ2	Schindelin et al. ⁶⁷	https://imagej.net/software/fiji
Prism (v8.0.0 (224) and v9.5.1 (528))	GraphPad Software	https://www.graphpad.com
AIVIA	DRVision	https://www.aivia-software.com
Other		
#1.5 coverslip	Fisher Scientific	Cat#2850-18
Glass slide	Fisher Scientific	Cat#12-544-3
glass bottom dishes	MatTek	Cat# P35GC-1.5-14-C
glass bead	Sigma	Cat# 64649