

Figure S1. Dynamic parameters are consistent between different preparations of recombinant human α 1A/ β III tubulin (related to Figures 1 and 3). (A) SDS-polyacrylamide gel (5 µgs) of recombinant human α 1A/ β III from two different growths and purifications. (B) Plus-end dynamic parameters of α 1A/ β III microtubules at 6 µM from the two different preparations shown in (A). From left to right: Growth rates in box-whisker plot (whiskers represent minimum to maximum); n= 256 and 95 growth phases for preparation #1 and preparation #2, respectively. Catastrophe frequencies; n= 29 and 12 microtubules for preparation #1 and preparation #2, respectively. Catastrophe frequencies; n= 256 and 95 growth phases for preparation #1 and preparation #2, respectively. Microtubule mean lifetime and mean length; n= 256 and 95 growth phases for preparation #1 and preparation #2, respectively. Error bars represent S.D. (C) Microtubule plus-end mean length at 4 µM tubulin concentration; n= 181 and 424 for α 1A/ β III and α 1A Δ -tail/ β III, respectively. Error bars represent S.D.; **** denotes *p* < 0.0001 as determined by a Mann-Whitney test.

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Figure S2. Sequence alignment of α -tubulin tails (related to Figures 1 and 3). (A) Sequence alignment of the C-termini of eight human α -tubulin isotypes. Uniprot ID for each sequence indicated in brackets. The length of the H12 helix is based on the cryo-EM structure of human recombinant α 1A/ β III microtubules (PDB ID: 5JCO; (Vemu, et al., 2016)). (B) Sequence alignment of tubulin α 1A isotypes from various species.

Α



Figure S3 (related to Figures 2 and 3).

(A) Schematics of MD models used to examine the interactions between the tail of α -tubulin and its body. Red outline marks the α -tubulin subunit used in the analyses. (B) A snapshot of a simulation with a GTP-tubulin dimer, showing the α -tubulin tail interacting with positively charged residues at the α -tubulin longitudinal polymerization interface colored on a gradient from yellow to red according to the probability of its contact with the α -tubulin tail. Residue S439 where the tail originates is shown in blue. (C) Distribution of conditional contact probabilities for residues in the α -tubulin tail with the longitudinal polymerization interface, provided that at least one residue of the α -tubulin tail contacts the interface. Calculated from 5 independent one-microsecond-long simulations for GTP-tubulin.





tions of the tail at three times are schematized. Bias potential accelerates displacement of the tail to enhance sampling of the configuration space. (B) A cloud of bias potentials (purple dots) added throughout the 2 μ s-long metadynamics simulation. (C) Visualization of the positions where the highest densities of bias potentials were added to steer the C-terminal tip of the α -tubulin tail. The spatial distribution of these spots illustrates that the tail is predominantly located at the longitudinal polymerization interface. Residue S439 where the tail originates is shown in blue.



Figure S5 (related to Figure 4)

(A) TIRF microscopy images of GFP-EB1 (100 nM, green) on recombinant tyrosinated (100%) and detyrosinated (0%) microtubules. (B) Averaged fluorescence intensity profiles of GFP-EB1 (100 nM) on 15 μM tyrosinated (100%; n=124) or detyrosinated (0%; n=96) microtubules. (C) Comet profiles parameters. Comet profiles were fitted using Gaussian (-1.5 to 0 μm) and single exponential functions (0 to 1.5 μm; STAR Methods). (D, E) Plus-end dynamic parameters of 6 μM tyrosinated (100% Tyr) or detyrosinated (0% Tyr) microtubules in the presence of 300 nM EB1 and 0/75/300 nM GFP-H2. (D) Growth rates; n= 223, 82, 426, 309, 934, and 106 growth phases for 100%Tyr/EB1, 0%Tyr/EB1, 100%Tyr/EB1/H2 (75 nM), 0%Tyr/EB1/H2 (75 nM), and 0%Tyr/EB1/H2 (300 nM), respectively. Whiskers represent minimum and maximum. (E) Catastrophe frequencies; n= 39, 17, 63, 50, 63, and 21 microtubules for 100%Tyr/EB1, 0%Tyr/EB1, 100%Tyr/EB1/H2 (75 nM), 0%Tyr/EB1/H2 (300 nM), and 0%Tyr/EB1/H2 (300 nM), respectively. Error bars represent S.D.



Figure S6. CLIP-170 preferentially increases the rescue rates of tyrosinated microtubules (related to Figure 4). (A) Representative IRM kymographs of 6 µM 100% (left) or 0% (right) tyrosinated microtubules with 300 nM GFP-H2. (B) Representative IRM (top) and TIRF microscopy (bottom) kymographs of 6 µM tyrosinated (100% Tyr; left) or detyrosinated (0% Tyr; right) microtubules with 300 nM GFP-H2 (green).