Supporting Information

Dixit *et al.* 10.1073/pnas.0807614106

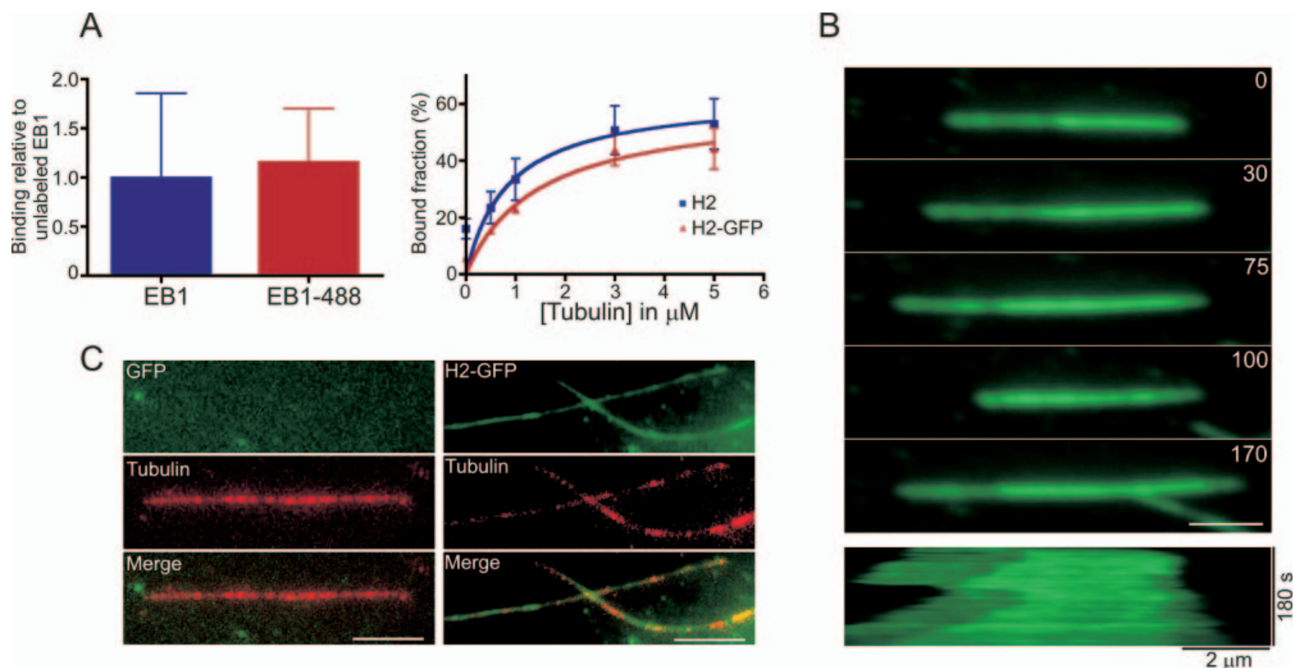


Fig. S1. Microtubule binding and labeling of +TIP proteins. (*A*) The bar graph on the left shows the average (±SD) from 3 or more replicates of the microtubule bound fraction of 3 μM unlabeled EB1 and EB1–Alexa 488 proteins at 5 μM microtubules normalized to the extent of binding observed for unlabeled EB1. The binding curves on the right show the percentage of microtubule bound fraction (±SD) from 3 independent experiments with 3 μM unlabeled CLIP-170(H2) and CLIP-170(H2)–GFP proteins at increasing microtubule concentrations. These data were fit to a K_d of 0.9 ± 0.5 μM for unlabeled CLIP-170(H2) and 1.5 ± 0.6 μM for CLIP-170(H2)–GFP. In both cases, there is no statistical difference between the microtubule binding activities of unlabeled protein and fluorescently tagged protein. (*B*) The montage at the top shows the microtubule labeling pattern of 150 nM CLIP-170(H2). At the bottom is a kymograph of the same microtubule over the duration of the observation period. At this relatively high concentration, CLIP-170(H2) shows indiscriminate labeling of the entire microtubule length during both the polymerization and depolymerization phases of microtubule dynamics. The numbers in each frame indicate time (in seconds). (*C*) Comparison of the microtubule-binding activity of 150 nM GFP alone (*Left*) and 150 nM CLIP-170(H2)–GFP (*Right*). GFP alone, even at relatively high concentrations, exhibits no detectable binding to the microtubule lattice under our experimental conditions. (Scale bar, 2 μm.)

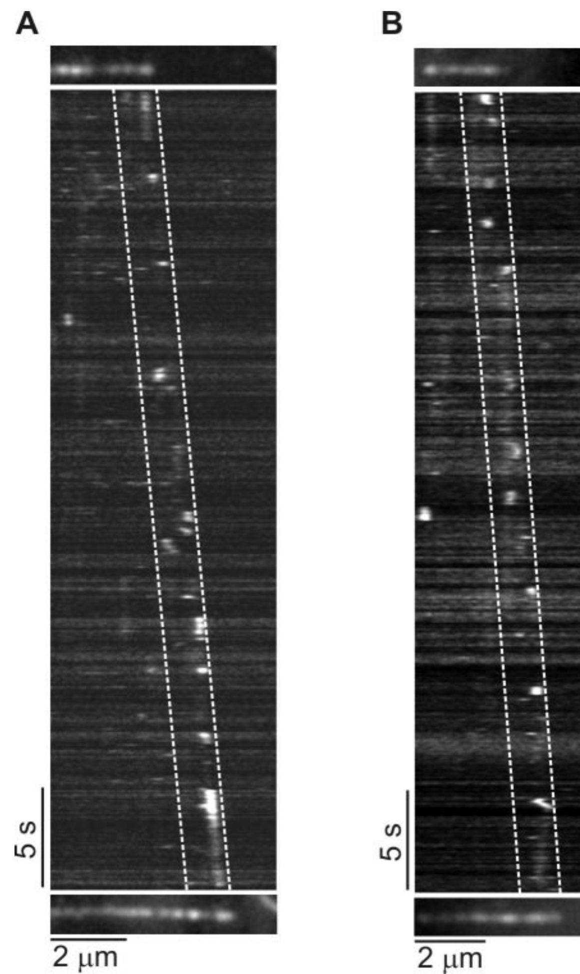
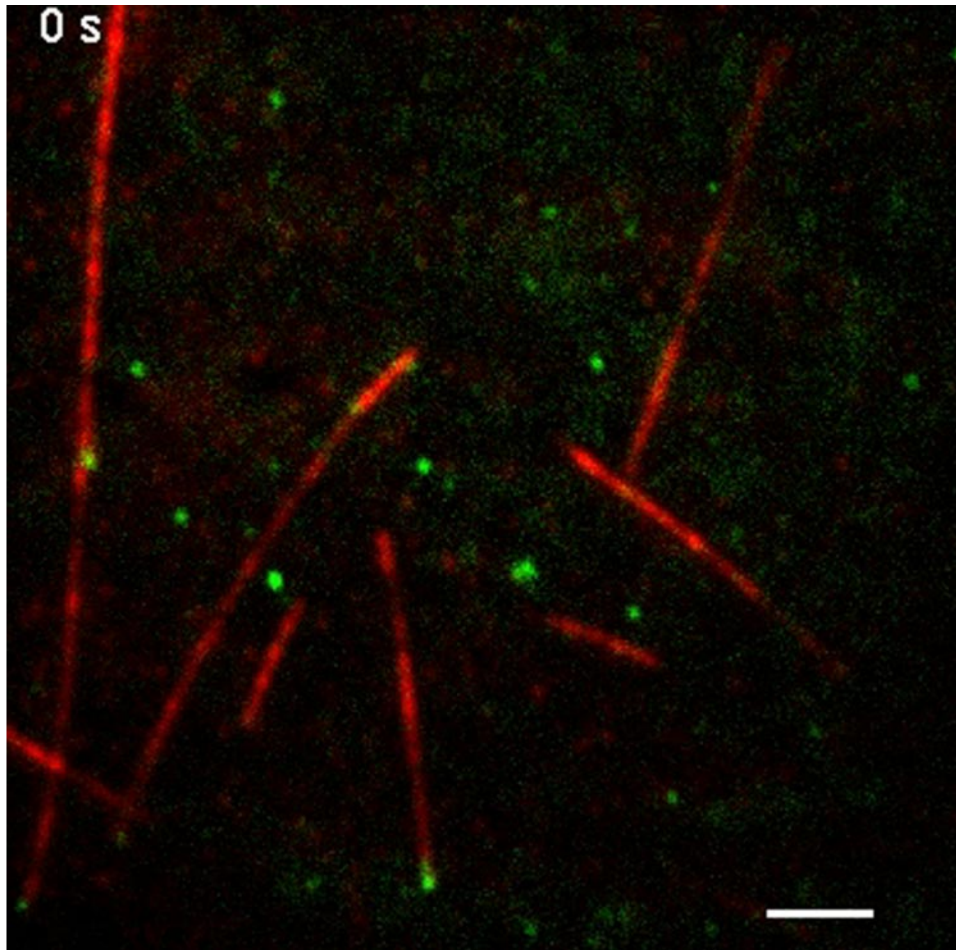


Fig. S2. Single-molecule kinetics of EB1–Alexa 488 and CLIP-170-GFP at growing microtubule plus-ends. In vitro reconstitution experiments were conducted with either 10 nM EB1–Alexa488 (*A*) or 5 nM CLIP-170(H2)–GFP together with 250 nM unlabeled EB1 (*B*) at a temporal resolution of 10 frames/s. The kymographs show binding and unbinding events of single EB1–Alexa488 (*A*) and CLIP-170(H2)–GFP (*B*) molecules at the plus-end of a growing microtubule (shown above and below the kymograph). Dwell times were determined for binding events occurring within a 1-μm region along the growing microtubule tip (dotted lines).

Table S1. Dynamic instability properties of in vitro polymerized microtubules in the presence and absence of +TIPs

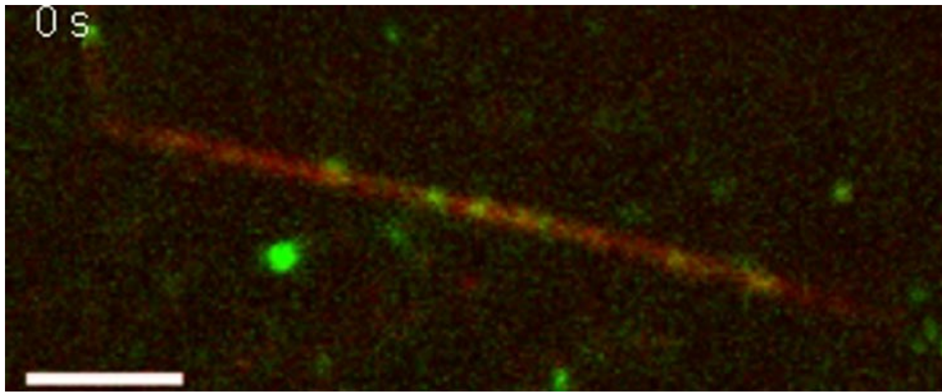
	V_g, μm/min	V_s, μm/min)	f_{rescue}, s^{-1}	$f_{catastrophe}$, s^{-1}
Tubulin	1.75 ± 0.52 (45)	6.27 ± 1.33 (21)	0.056 ± 0.039 (23)	0.008 ± 0.002 (21)
H2 alone	1.81 ± 0.34 (31)	8.02 ± 2.91 (29)	0.075 ± 0.039 (20)	0.008 ± 0.001 (21)
EB1 alone	1.83 ± 0.32 (52)	7.11 ± 1.09 (44)	0.059 ± 0.037 (36)	0.007 ± 0.003 (41)
H2 + EB1	1.79 ± 0.71 (55)	6.98 ± 2.31 (50)	0.046 ± 0.034 (23)	0.009 ± 0.004 (20)

The data represent the mean ± SD (with the number of microtubules in parentheses) for the plus-ends of in vitro polymerized microtubules in the presence or absence of 25 nM CLIP-170(H2) and 250 nM EB1. V_g is the microtubule growth rate, V_s is the microtubule shrinkage rate, f_{rescue} is the frequency of microtubule rescue events, and $f_{catastrophe}$ is the frequency of microtubule catastrophe events.



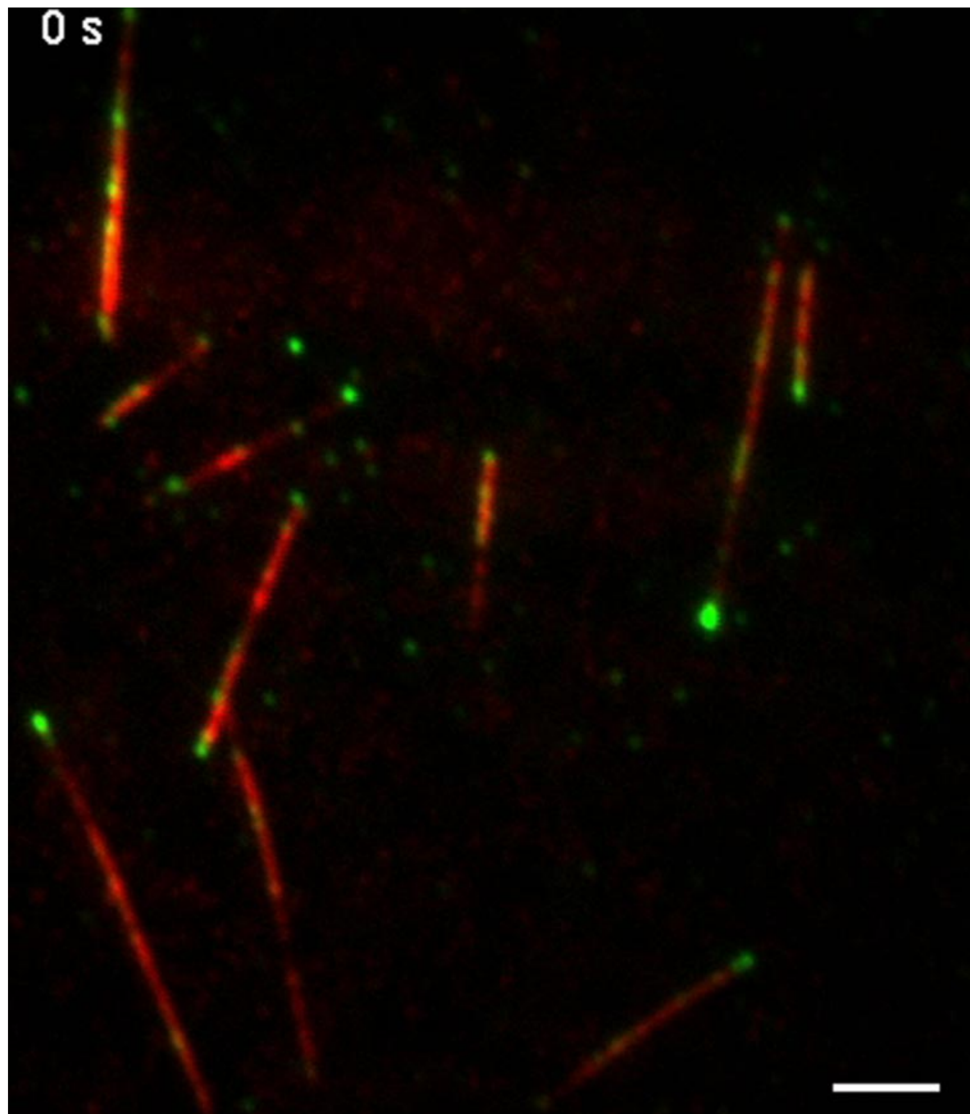
Movie S1. +TIP activity of Alexa 488–tagged EB1 protein. The movie shows pseudocolored overlaid images of rhodamine-labeled microtubules (red) and Alexa 488–tagged EB1 protein (green). EB1–Alexa 488 specifically decorates growing microtubule plus-ends and is lost during microtubule shortening phases. (Scale bar, 2 μm.)

Movie S1 (MOV)



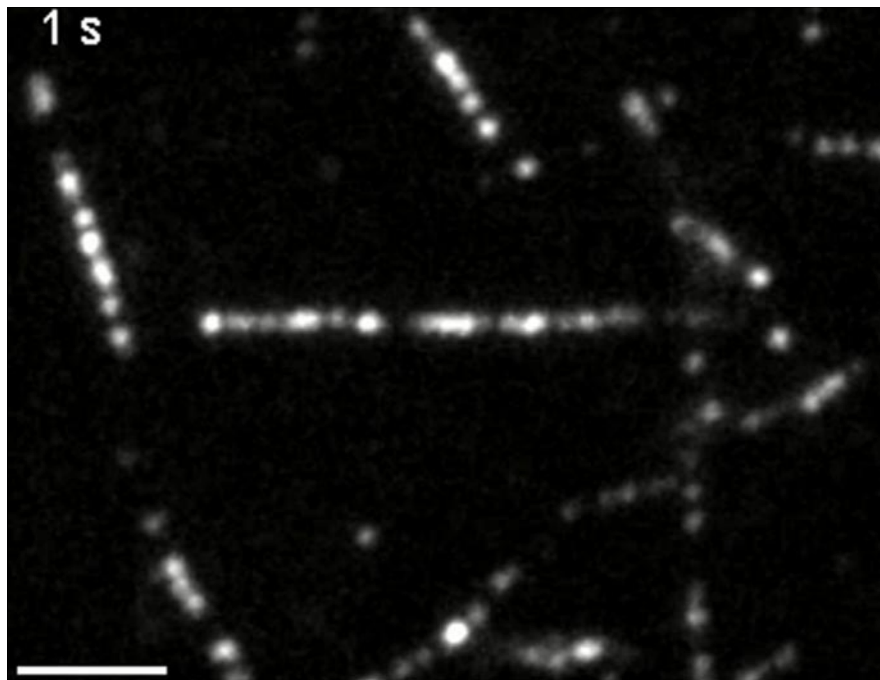
Movie S2. Lattice diffusion of CLIP-170(H2)–GFP protein. The movie shows pseudocolored overlaid images of rhodamine-labeled microtubules (red) and GFP-tagged CLIP-170(H2) protein (green). In the absence of EB1, CLIP-170(H2) binds along the length of the microtubule lattice and exhibits diffusive movement (blue arrowhead). (Scale bar, 2 μm.)

Movie S2 (MOV)



Movie S3. +TIP activity of CLIP-170(H2)–GFP in the presence of EB1 protein. The movie shows pseudocolored overlaid images of rhodamine-labeled microtubules (red) and GFP-tagged CLIP-170(H2) protein (green). In the presence of unlabeled EB1, CLIP-170(H2)–GFP specifically localizes to growing microtubule plus-ends and is lost during microtubule shortening phases. (Scale bar, 2 μm.)

Movie S3 (MOV)



Movie S4. GMPCPP abolishes +TIP activity of the CLIP-170(H2)–EB1 complex. The movie shows the localization of CLIP-170(H2)–GFP molecules on GMPCPP microtubules in the presence of unlabeled EB1. In the absence of GTP hydrolysis, the CLIP-170(H2)–EB1 complex does not specifically target growing microtubule plus-ends, but instead binds along the length of the microtubule lattice. (Scale bar, 2 μm.)

Movie S4 (MOV)