SUPPLEMENTAL DATA

Legends to Supplemental Movies and Figures

<u>Supplemental Movies.</u> Representative streaming movies (200 msec exposures) of CLIP-170 fragments, acquired as described by Folker *et al.* (1).

<u>Supplemental Movie 1.</u> GFP-CLIP-170 $H1^{1-350}$ —As previously reported, the complete CLIP-170 $H1^{1-350}$ head domain highlights the growing plus-ends of microtubules (MTs) with bright, robust comets.

<u>Supplemental Movie 2.</u> GFP-CLIP-170 $H1^{58-300}$ —Removing the first and third serine-rich regions does not abolish the plus-end tracking activity, although the comets are slightly less bright and robust when compared to $H1^{1-350}$.

<u>Supplemental Movie 3.</u> GFP-CLIP-170 $H1^{203-350}$ —While not nearly as bright or robust as $H1^{1-350}$, the distal portion of CLIP-170 consisting of CAP-Gly-2 domain and third serine-rich region of $H1^{203-350}$ clearly has MT plus-end tracking ability.

<u>Supplemental Figure 1.</u> Fluorescence microscopy visualization of the dynamic polymer morphology formed in the presence of stabilized MT seeds, tubulin (5.0 μ M) and CLIP-170 fragments. Samples were prepared as described in Fig. 3*D*-*F* with some modifications. Briefly, bovine brain tubulin dimly labeled with TAMRA (Invitrogen) was incubated with brightly labeled seeds and either unlabeled H1¹⁻³⁵⁰ (120 nM) or unlabeled other fragments (240 nM) for 15-20 min at 37 °C and then samples were visualized (200 msec exposures) by fluorescence microscopy. (A) The control sample in the absence of CLIP-170 fragments; (B) H1¹⁻³⁵⁰; (C) H1¹⁻¹⁵⁵; (D) H1¹⁵⁶⁻³⁵⁰. Images shown are representative.

<u>Supplemental Figure 2.</u> Fluorescence microscopic analysis of the glutaraldehyde fixed MT polymers formed in the absence of exogenous MT seeds. (A) Tubulin alone (12 μ M); (B) H1¹⁻³⁵⁰; (C) H1⁵⁸⁻²¹¹; (D) H1⁵⁸⁻³⁰⁰; (E) H1¹²²⁻³⁰⁰; (F) H1²⁰³⁻³⁵⁰. Images shown are representative.

<u>Methods used for Supplemental Figure 1.</u> Tubulin was prepared by the high-molarity buffer approach of Castoldi and Popov (2). Images were captured with a 100x 1.3NA objective on a Zeiss Axioplan 2 microscope and a Photometrics CoolSNAP camera. Images were optimized for visualization of the MTs by Adobe Photoshop.

<u>Methods used for Supplemental Figure 2.</u> Samples were prepared, fixed, and diluted as described in Fig. 3A-C, the only modification being that 10% of the tubulin was labeled with TAMRA. They were then mounted for microscopy by compressing 2 μ L of the diluted sample between a slide and a 22mm coverslip so that the entire sample was visible in one plane. Images were captured (150 msec exposures) on a TE2000 Nikon inverted microscope with a Cascade 512 B camera and processed for visualization by Adobe Photoshop.

REFERENCES

- 1. Folker, E. S., Baker, B. M., and Goodson, H. V. (2005) Mol. Biol. Cell 16, 5373-5384
- 2. Castoldi, M., and Popov, A. V. (2003) Protein Expr. Purif. 32, 83-88.







Supplemental Figure 1.



Supplemental Figure 2.