Supplemental Materials

Molecular Biology of the Cell

Lawrence et al.

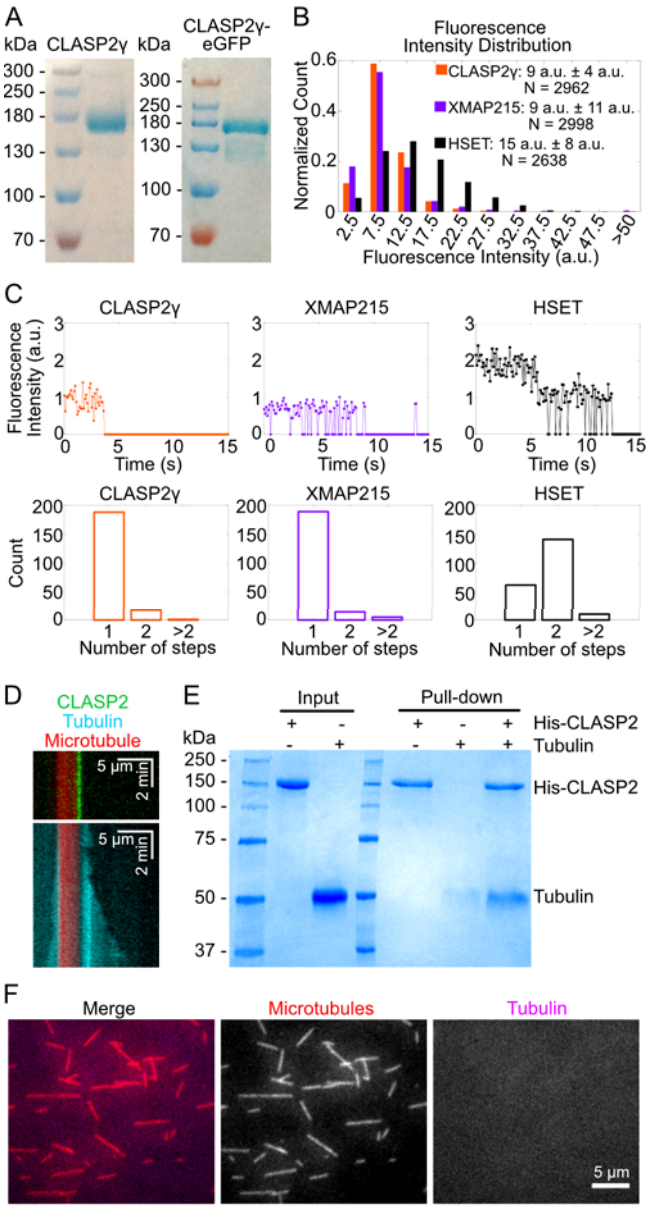

Figure S1. CLASP2γ is a monomer and binds to the plus-tips of GMPCPP-stabilized microtubules. (A) SDS-PAGE gels showing purified His-CLASP2γ proteins and His-CLASP2γ-eGFP-Strep. (B) First-frame fluorescence intensity distribution of CLASP2γ-eGFP, XMAP215-eGFP and eGFP-HSET at single molecule concentrations. Reported values are mean fluorescence intensities with standard deviations, N indicates the number of particles. (C) Example fluorescence intensity traces over time (top row) and stepwise photobleaching analysis (bottom row) of single molecules of CLASP2γ-eGFP, XMAP215-eGFP andeGFP-HSET. (D) A representative kymograph showing CLASP2γ recognizing one tip of a GMPCPP-stabilized microtubule. CLASP2γ binding preference was determined by assessing the microtubule polarity from the growth rate of microtubule extensions polymerized using 12 µM Cy5-labeled tubulin (12 µM). (E) Pull-down experiments to detect direct binding between His-CLASP2γ and soluble tubulin. (F) Representative fields of view of GMPCPP-stabilized microtubules incubated with 1 µM Cy5-labeled soluble tubulin.

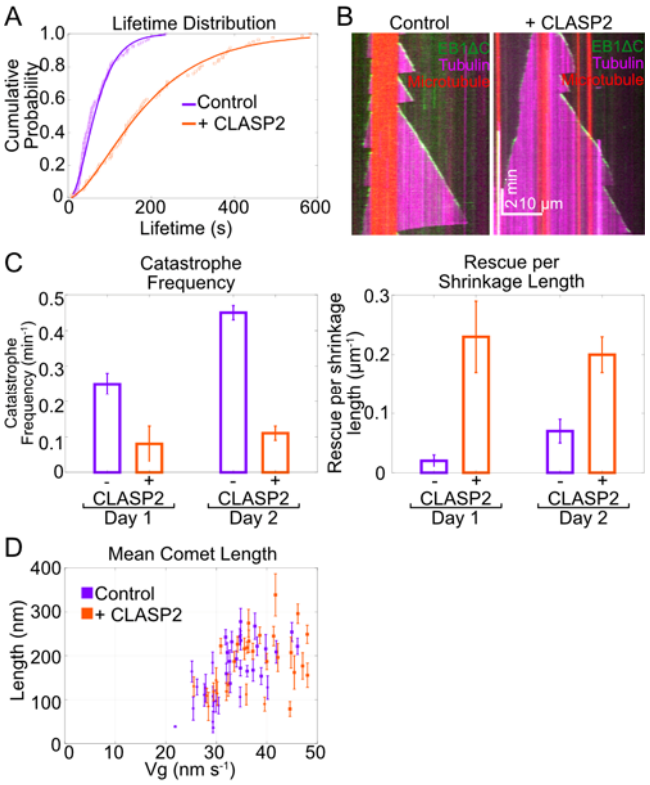

Figure S2. CLASP2γ suppresses catastrophe without increasing EB1ΔC comets.(A) Cumulative distribution of lifetimes of microtubules grown with 10 µM Cy5-labeled tubulin and 50 nM EB1 in the presence and absence of 12 nM CLASP2γ-eGFP. Lines are best fits to gamma distribution function (control: step = 2.5, 95%CI: [2.0, 3.2], rate= 0.035 s^{-1}, 95%CI: [0.027 s^{-1}, 0.046 s^{-1}], N=112; CLASP2: step = 1.9, 95%CI: [1.4, 2.5], rate= 0.009 s^{-1}, 95%CI: [0.007 s^{-1}, 0.013 s^{-1}], N=83). (B) Representative kymographs showing microtubules grown with 12 µM Alexa 647-labeled tubulin and 200 nM EB1ΔC-eGFP in the presence and absence of 1 µM CLASP2γ. (C) Quantification of catastrophe frequency and rescue per shrinkage length of microtubules grown under the conditions described in B. Error bars represent SE. (D) Quantification of EB1ΔC-eGFP comet decay length on microtubules grown with 12 µM Alexa 647-labeled tubulin and 200 nM EB1ΔC-eGFP with and without 1 µM CLASP2γ. Data are means with 95% CI; circles and squares represent data from two independent experimental days. Mean comet length: 163 nm ± 11 nm for control (SE, N=35), and 182 nm ± 12 nm for CLASP2γ (SE, N=30); p=0.2, Welch's unpaired t-test.

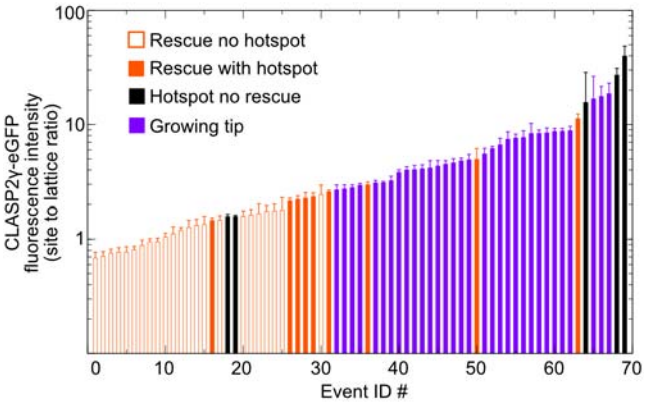

Figure S3.CLASP2γ-eGFP fluorescence intensity does not correlate with rescue. Site-to-lattice intensity ratio of CLASP2γ-eGFP fluorescenceat 32 rescue sites (orange), 5 CLASP2γ hotspots without rescue (black) and 32 growing tips (purple). Solid orange bars (9 out of 32) indicate rescues that occurred at CLASP2γ hotspots (see Methods).