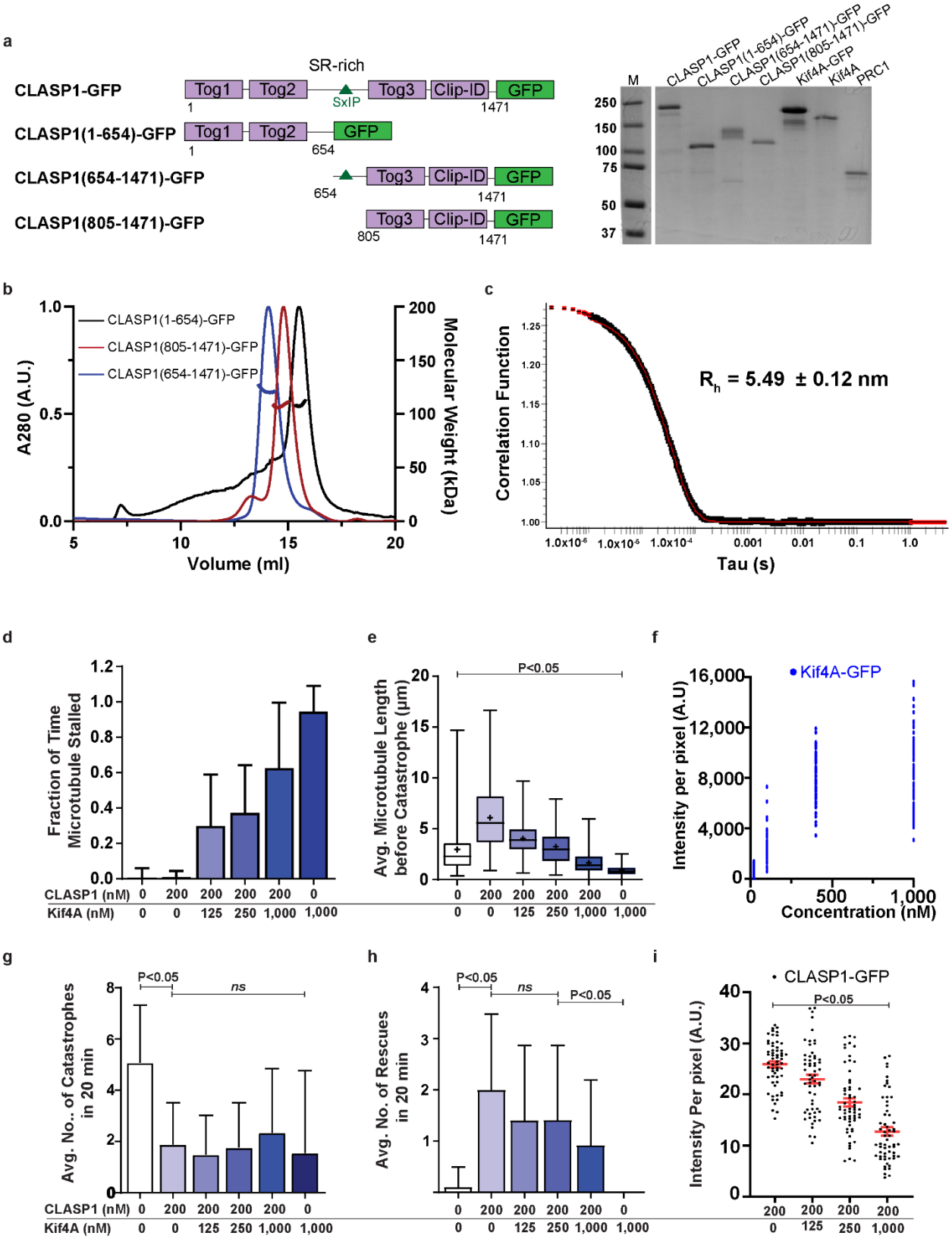
Supplementary Fig. 1



Supplementary Fig. 1. *Related to Fig. 1*

a. Domain diagram of CLASP1 constructs used in this study (left), along with SDS-PAGE gel of purified proteins (right). SR-rich: Serine Arginine rich, Clip-ID: Clip-Interacting Domain, M: molecular weight marker, with corresponding masses in kDa on the side.

b. SEC-MALS profiles showing elution volumes of CLASP1 constructs from a Superose-6 column, and their corresponding molecular weights in solution. *Calculated Molecular weight (expected weight of monomer):* CLASP1(1-654)-GFP (black) 108.8 ± 0.8 kDa (101.5 kDa); CLASP1(805-1471)-GFP (red) 107.6 ± 10.8 kDa (102.2 kDa); CLASP1(654-1471)-GFP (blue):128.7 ± 0.3 kDa (120 kDa).

c. Auto-correlation function for apex of eluting peak of CLASP1(805-1471)-GFP shown in (**b**), obtained through dynamic light scattering studies. Calculated hydrodynamic radius, R_h= 5.49 ± 0.12 nm.

d. Bar graph of fraction of time microtubule stalled in the presence of CLASP1-GFP and Kif4A at varying concentrations. Error bars indicate standard deviation. Assay conditions: tubulin control (0.0 ± 0.0, n = 59), 200 nM CLASP1 (0.0 ± 0.0, n = 67), 200 nM CLASP1 + 125 nM Kif4A (0.3 ± 0.3, n = 67), 200 nM CLASP1 + 250 nM Kif4A (0.4 ± 0.3, n = 73), 200 nM CLASP1 + 1000 nM Kif4A (0.6 ± 0.4, n = 73) and 1000 nM Kif4A (0.9 ± 0.1, n = 39).

e. Box and whisker plot of average length of microtubule before catastrophe in the presence of CLASP1-GFP and Kif4A at indicated concentrations. Plus-sign indicates mean. Horizontal lines within box indicate the 25th, median and 75th percentile. Error bars indicate minimum and maximum range. Mean and standard deviation for assay conditions: Tubulin control (2.9 ± 2.4 μm, n = 241), 200 nM CLASP1 (6.1 ± 3.2 μm, n = 150), 200 nM CLASP1 + 125 nM Kif4A (4.0 ± 1.9 μm, n = 82), 200 nM CLASP1 + 250 nM Kif4A (3.2 ± 1.8 μm, n = 107), 200 nM CLASP1 + 1000 nM Kif4A (1.7 ± 1.0 μm, n = 152) and 1000 nM Kif4A (0.9 ± 0.6 μm, n = 24). $P < 0.05$ for 1000 nM Kif4A compared to tubulin control.

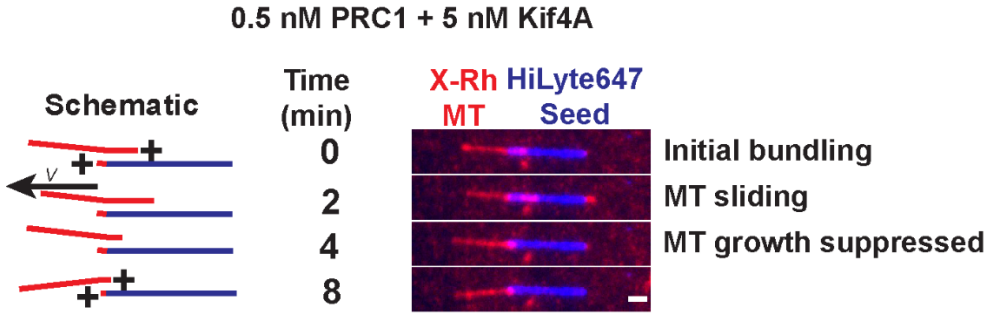
f. Scatter plot of Kif4A-GFP intensity per pixel on taxol-stabilized microtubules in the presence of 150 μM ATP (n = 70 microtubules). Concentrations refer to monomeric Kif4A. Mean and standard deviation of intensity for assay conditions with Kif4A-GFP: 20 nM (504.2 ± 395.8, n=54), 100 nM (2556 ± 1182, n=69), 400 nM (7823 ± 1961, n=68), 1000 nM (8276 ± 3386, n=69).

g. Bar graph of the average number of catastrophes in 20 minutes in the presence of CLASP1-GFP and Kif4A at indicated concentrations. Error bars indicate standard deviation. Assay conditions: tubulin control (5.1 ± 2.2, n = 65), 200 nM CLASP1 (1.9 ± 1.6, n = 76), 200 nM CLASP1 + 125 nM Kif4A (1.5 ± 1.5, n = 69), 200 nM CLASP1 + 250 nM Kif4A (1.8 ± 1.7, n = 73), 200 nM CLASP1 + 1000 nM Kif4A (2.3 ± 2.5, n = 73) and 1000 nM Kif4A (1.6 ± 3.2, n = 38). $P < 0.05$ for (i) tubulin control to 200 nM CLASP1. P is not significant for 200 nM CLASP1 to (i) 200 nM CLASP1 + 125 nM Kif4A, (ii) 200 nM CLASP1 + 250 nM Kif4A, (iii) 200 nM CLASP1 + 1000 nM Kif4A and to (iv) 1000 nM Kif4A.

h. Bar graph of the average number of rescues in 20 minutes in the presence of CLASP1-GFP and Kif4A at indicated concentrations. Error bars indicate standard deviation. Assay conditions: tubulin control (0.1 ± 0.4, n = 60), 200 nM CLASP1 (2.0 ± 1.5, n = 67), 200 nM CLASP1 + 125 nM Kif4A (1.4 ± 1.5, n = 67), 200 nM CLASP1 + 250 nM Kif4A (1.4 ± 1.5, n = 73), 200 nM CLASP1 + 1000 nM Kif4A (0.9 ± 1.3, n = 73) and 1000 nM Kif4A (0.0 ± 0.0, n = 37). $P < 0.05$ for (i) tubulin control to 200 nM CLASP1 and for 200 nM CLASP1 + 250 nM Kif4A to (ii) 200 nM CLASP1 + 1000 nM Kif4A and to (iii) 1000 nM Kif4A. P is not significant for 200 nM CLASP1 to (i) 200 nM CLASP1 + 125 nM Kif4A and to (ii) 200 nM CLASP1 + 250 nM Kif4A.

i. Scatter plot of CLASP1-GFP fluorescence intensity per pixel on single microtubules in the presence of CLASP1-GFP and Kif4A at indicated concentrations. Error bars indicate standard error of mean. Assay conditions: 200 nM CLASP1 (25.9 ± 4.6, n = 59), 200 nM CLASP1 + 125 nM Kif4A (23.0 ± 6.5, n = 59), 200 nM CLASP1 + 250 nM Kif4A (18.4 ± 6.3, n = 60) and 200 nM CLASP1 + 1000 nM Kif4A (12.8 ± 6.3, n = 59). $P < 0.05$ for 200 nM CLASP1 when compared to (i) 200 nM CLASP1 + 250 nM Kif4A and (ii) 200 nM CLASP1 and 1000 nM Kif4A.

Supplementary Fig. 2



Supplementary Fig. 2. *Related to Fig. 2*

Schematics and montages of microtubule bundles (red) grown from microtubule seeds (blue) in the presence of 5 nM Kif4A and 0.5 nM PRC1. Schematics indicate the plus end (+) of the microtubules within the bundle. Velocity arrow indicates direction of microtubule sliding. X-Rh MT: X-Rhodamine microtubules. Scale bar represents 2 μm.

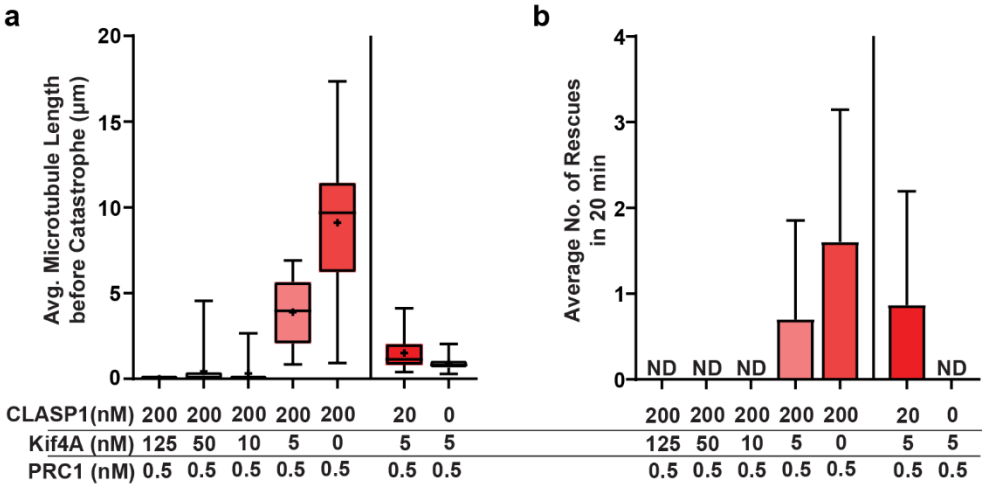
Supplementary Fig. 3

CLASP1 (nM)	200	200	200	200	200	20	0
Kif4A (nM)	125	50	10	5	0	5	5
PRC1 (nM)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dynamic Instability (%)	0	0	0	56	86	90	70
No Growth (%)	100	70	78	0	0	8	15
Only Growth (%)	0	30	22	44	14	2	15
Total Number of Events	30	33	36	50	63	61	53
Complete Catastophes (%)	0	0	0	7	2	64	100
Rescue (%)	0	0	0	93	98	36	0

Supplementary Fig. 3. *Related to Fig. 3*

Summary of dynamics of PRC1 cross-linked microtubules, showing percentage of microtubules exhibiting dynamic instability, no growth or only growth in black. For all kymographs, the total number of events, percentage of events that are complete catastrophes and rescues in the presence of CLASP1-GFP, PRC1 and Kif4A at varying concentrations are shown in blue.

Supplementary Fig. 4.



Supplementary Fig. 4. *Related to Fig. 4*

a. Box and whisker plot of average microtubule length before catastrophe of cross-linked microtubules in the presence of indicated CLASP1-GFP, Kif4A and PRC1 concentrations. Plus-sign indicates mean. Horizontal lines within box indicate the 25th, median and 75th percentile. Error bars indicate minimum and maximum range. Mean and standard deviation for assay conditions: 200 nM CLASP1 + 0.5 nM PRC1 + 125 nM Kif4A (0.0 ± 0.0 nm, n = 30), 200 nM CLASP1 + 0.5 nM PRC1 + 50 nM Kif4A (0.4 ± 0.9 nm, n = 39), 200 nM CLASP1 + 0.5 nM PRC1 + 10 nM Kif4A (0.3 ± 0.7 nm, n = 36), 200 nM CLASP1 + 0.5 nM PRC1 + 5 nM Kif4A (3.9 ± 1.9 nm, n = 28), 200 nM + 0.5 nM PRC1 (9.1 ± 3.6 nm, n = 53), 20 nM CLASP1 + 0.5 nM PRC1 + 5 nM Kif4A (1.5 ± 0.9 nm, n = 55) and 0.5 nM PRC1 + 5 nM Kif4A (0.9 ± 0.4 nm, n = 37).

b. Bar graph of the average number of rescues in cross-linked microtubule in 20 minutes in the presence of varying CLASP1-GFP, Kif4A and PRC1 concentrations. Error bars indicate standard deviation. Assay conditions: 200 nM CLASP1 + 0.5 nM PRC1 + 125 nM Kif4A (*Not Determined*, n = 30), 200 nM CLASP1

+ 0.5 nM PRC1 + 50 nM Kif4A (*Not Determined*, n = 39), 200 nM CLASP1 + 0.5 nM PRC1 + 10 nM Kif4A (*Not Determined*, n = 36), 200 nM CLASP1 + 0.5 nM PRC1 + 5 nM Kif4A (0.7 ± 1.2, n = 37), 200 nM + 0.5 nM PRC1 (1.6 ± 1.5, n = 33), 20 nM CLASP1 + 0.5 nM PRC1 + 5 nM Kif4A (0.9 ± 1.3, n = 23) and 0.5 nM PRC1 + 5 nM Kif4A (0.0 ± 0.0, n = 20).

Supplementary Video 1 *Related to Fig. 2*

The collective activity of CLASP1 and PRC1 promotes the elongation of single and cross-linked microtubules: Shown in blue: GMPCPP-polymerized, HiLyte647 labeled microtubule seeds, and in red: GMPCPP-polymerized X-Rhodamine-labeled microtubule seeds and X-Rhodamine labeled tubulin. Single and cross-linked microtubules are indicated by white and yellow arrows respectively. The movie was taken over 20 min (120 frames) and displayed at a rate of 20 frames/sec. Assay conditions: 200 nM CLASP1-GFP and 0.5 nM PRC1. Scale bar represents 2 μm.

Supplementary Video 2 *Related to Fig. 2*

The collective activity of CLASP1, Kif4A and PRC1 differentially regulates dynamics of single and cross-linked microtubules: Blue: GMPCPP-polymerized, HiLyte647 labeled microtubule seeds, Red: GMPCPP-polymerized X-Rhodamine-labeled microtubule seeds and X-Rhodamine labeled tubulin. Single and cross-linked microtubules are indicated by white and yellow arrows respectively. The movie was taken over 20 min (120 frames) and displayed at a rate of 20 frames/sec. Assay conditions: 200 nM CLASP1-GFP, 0.5 nM PRC1 and 10 nM Kif4A.Scale bar represents 2 μm.