Li et al., http://www.jcb.org/cgi/content/full/jcb.201206101/DC1

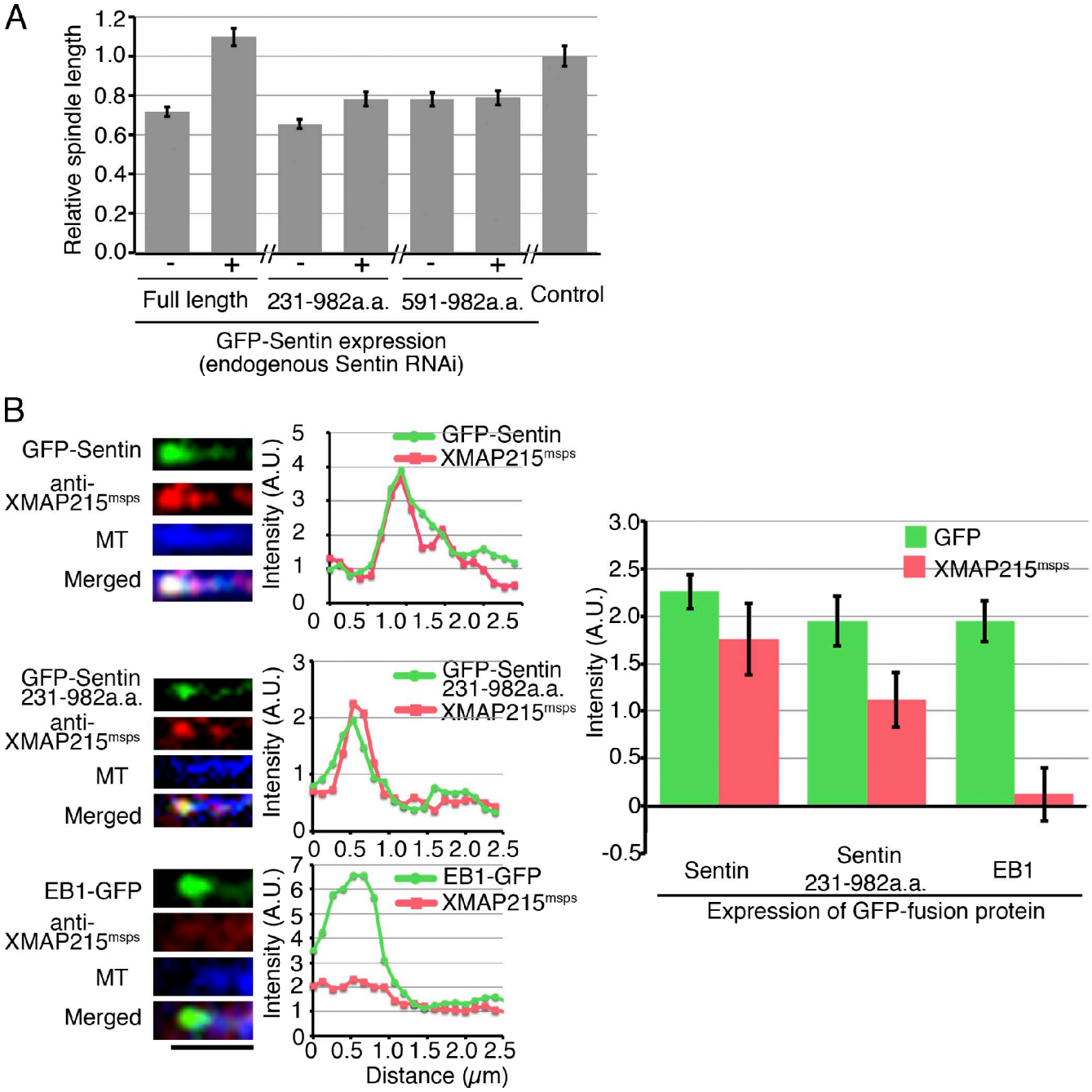


Figure S1. **Sentin is not a simple XMAP215^{msps} recruitment factor.** (A) The Sentin fragment that lacked the first 230 or 590 aa could not fully restore the spindle length when endogenous Sentin was knocked down. Control is the parental S2 cell line after control RNAi. The error bars represent SEM (n = 12–31). (B) Quantitative immunofluorescence using the anti-XMAP215^{msps} antibody. In this experiment, endogenous Sentin was knocked down by RNAi in cells expressing truncated forms of GFP-Sentin or EB1-GFP. Growing MT ends were identified by detecting the GFP comet signals. The position with maximum GFP intensity within the GFP comet was recorded, and the intensity of the XMAP215^{msps} signals at the corresponding position was measured. The EB1-GFP cell line was used as a control. For each cell line, >20 MTs from more than five cells were analyzed. GFP-Sentin (231–982 aa) restored XMAP215^{msps} plus-end accumulation ($P < 0.02$ vs. EB1-GFP). The data shown are from a single representative experiment out of two repeats. A.U., arbitrary unit. Bar, 2 µm.

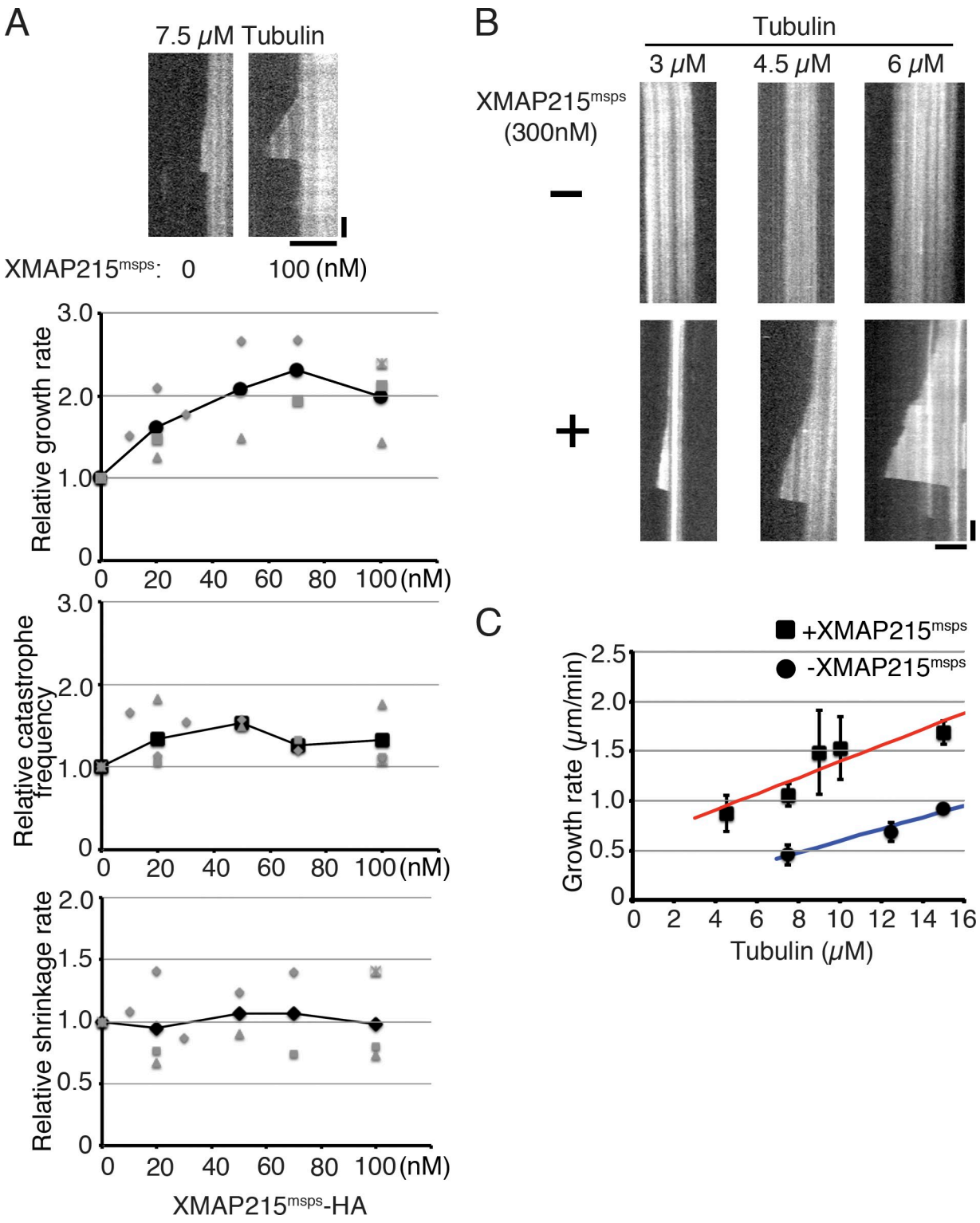


Figure S2. **Growth promotion by XMAP215^{msps}.** (A) Parameters of MT polymerization dynamics in the presence of 7.5 µM tubulin and various concentrations of XMAP215^{msps}-HA. Relative values are plotted in these graphs, whereas the actual values are presented in Table S2. The mean values of each experiment are marked in gray, whereas the mean values of all the experiments are indicated by black marks. Representative kymographs are shown on the top. Data from a single experiment are marked with the same shape. (B) Growth promotion by 300 nM XMAP215^{msps}-HA at 3, 4.5, and 6 µM tubulin. Representative kymographs of MTs are shown. (C) Growth rate at various tubulin concentrations in the presence or absence of XMAP215^{msps}. All the experimental data were combined in this graph (error bars represent SEM). In the absence of XMAP215^{msps} (dots, blue line), the growth rate increased linearly (R^2 = 0.97; 14–53 events from 10–20 MTs were analyzed in each experiment; *n* = 2 experiments). In the presence of 100 nM XMAP215^{msps} (squares, red line), the growth rate also increased when tubulin concentration was increased (R^2 = 0.82; 11–131 events from 10–20 MTs were analyzed in each experiment; *n* = 8 experiments). Bars: (horizontal) 5 µm; (vertical) 1 min.

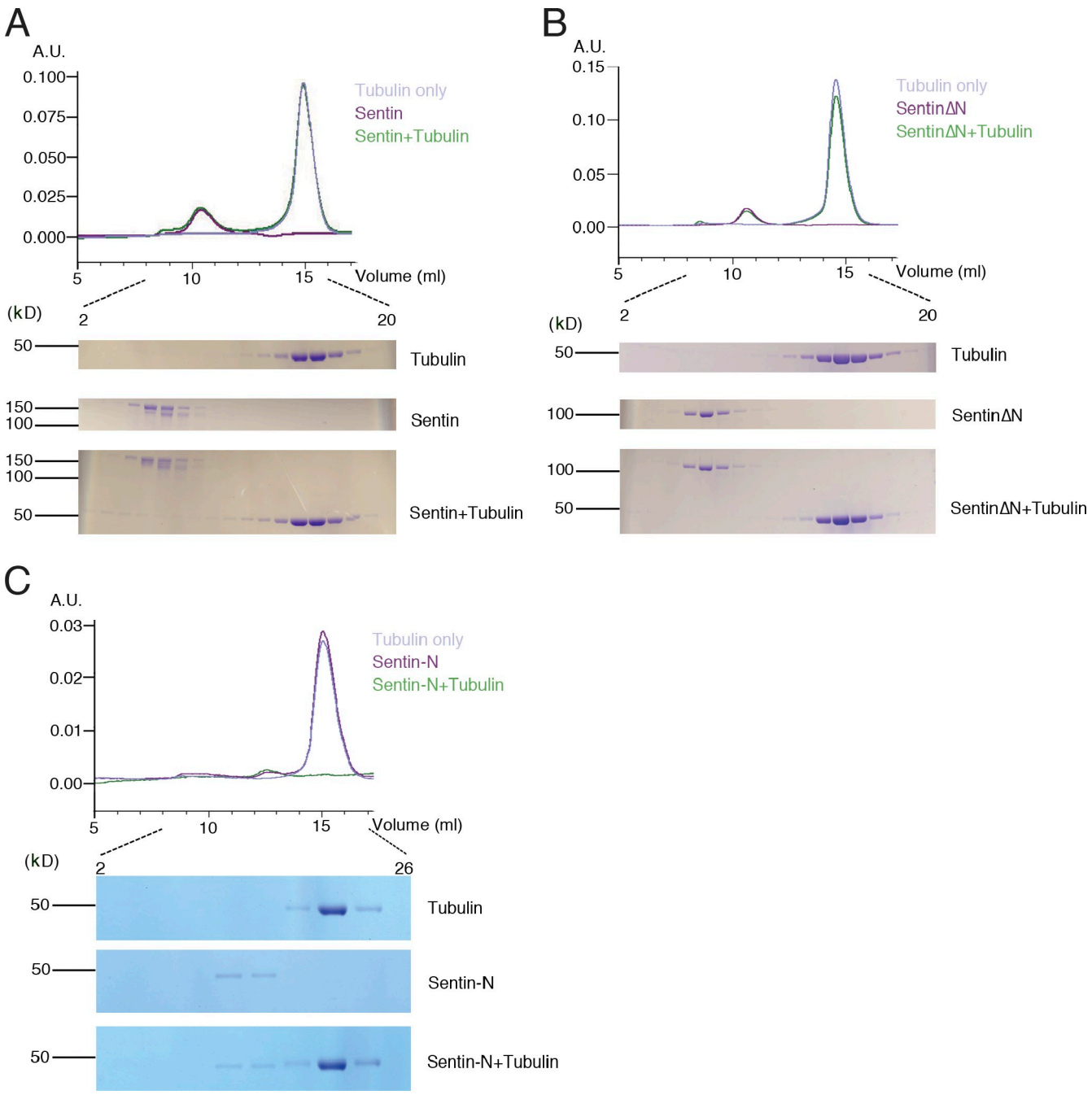


Figure S3. **No tubulin binding was detected for Sentin.** (A–C) Gel filtration chromatography was performed for purified full-length 3.5 µM GFP-Sentin (A), 4.5 µM GFP-Sentin-ΔN (231–982 aa; B), and untagged 5 µM Sentin-N (1–440 aa; C) in the presence or absence of tubulin (17.5, 22.5, and 15 µM, respectively) followed by Coomassie staining of each fraction. Cofractionation of Sentin and tubulin was not detected. The data shown are from a single representative experiment out of two to six repeats. A.U., arbitrary unit.

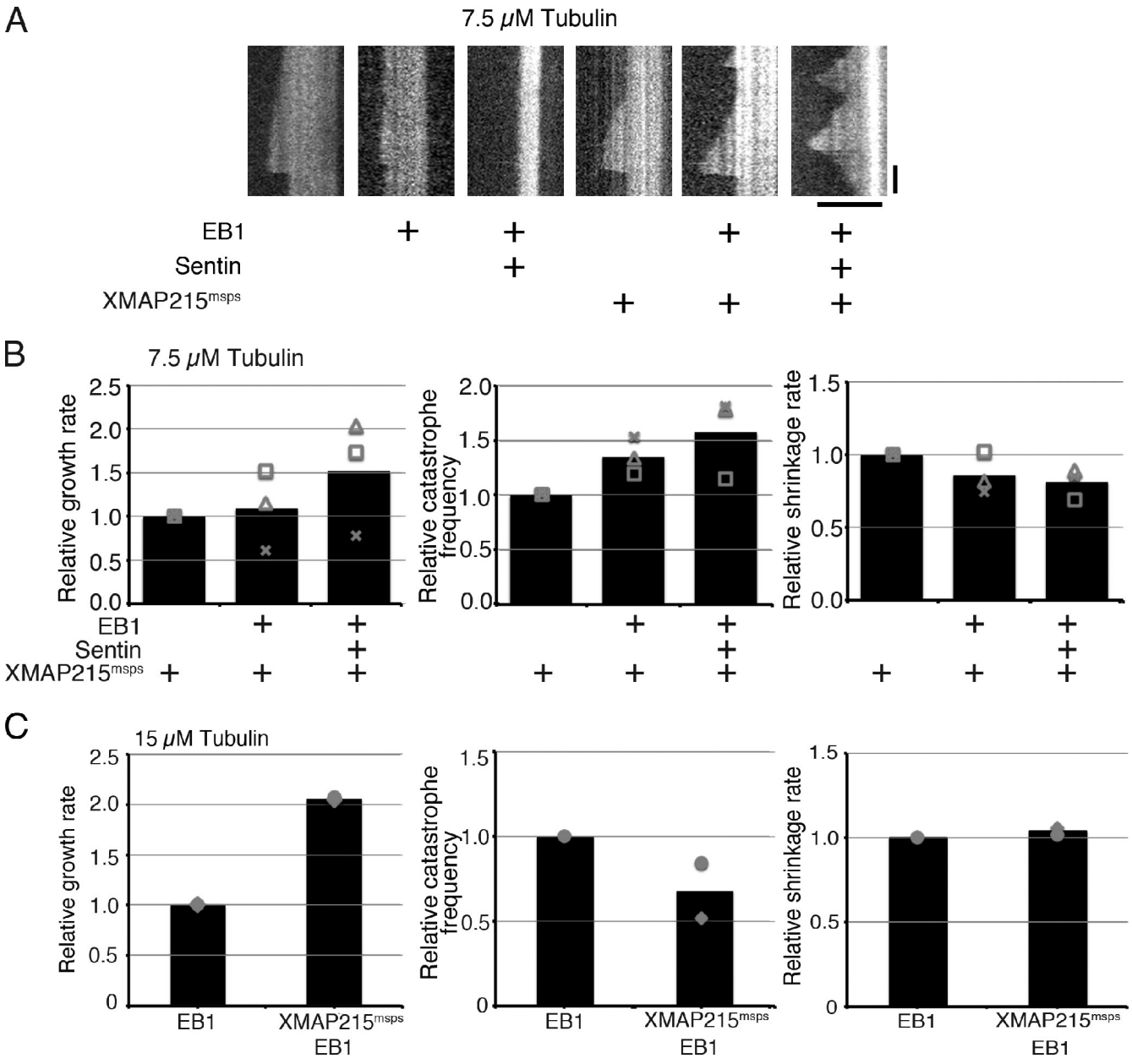


Figure S4. **Growth acceleration by XMAP215^{msps}, EB1, and Sentin at low tubulin concentration and the effects of EB1/ XMAP215^{msps}.** (A) Kymographs showing MT polymerization dynamics with various combinations of EB1, Sentin, and XMAP215^{msps} in the presence of 7.5 µM tubulin. Bars: (horizontal) 5 µm; (vertical) 1 min. See also Video 1. (B) Parameters of MT polymerization dynamics. The mean values of each experiment are marked in gray, whereas the mean values of all the experiments have been indicated by black bars. The relative values are shown in these graphs, whereas the actual values are presented in Table S6. 400 nM EB1, 200 nM Sentin, and 100 nM XMAP215^{msps}-HA were added to 7.5 µM tubulin. Some of the data (e.g., tubulin + XMAP215^{msps}-HA) overlapped with those in Fig. S2 A. (C) Parameters of MT polymerization dynamics with or without 100 nM XMAP215^{msps}-HA in the presence of 400 nM EB1 and 15 µM tubulin. The mean values of each experiment are marked in gray, whereas the mean values of all the experiments are indicated by black bars. Some data are overlapped with those presented in Fig. 2 D. The relative values are shown in these graphs, whereas the actual values are presented in Table S5. The data in B and C indicate that XMAP215^{msps} and EB1 accelerate MT growth and that EB1 increases catastrophe frequency in the absence of Sentin, a possible connector of XMAP215^{msps} and EB1. Data from a single experiment are marked with the same shape.

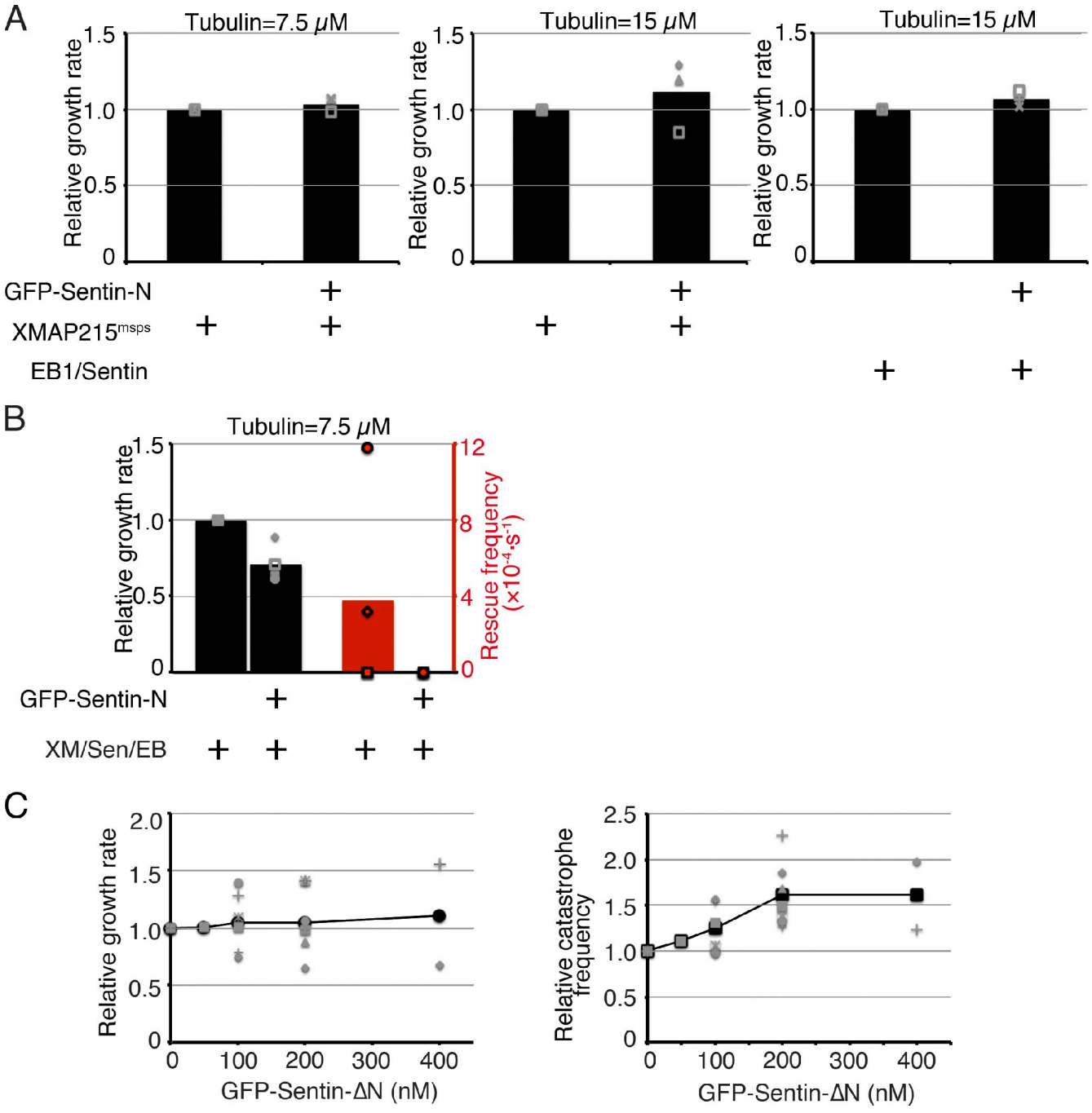
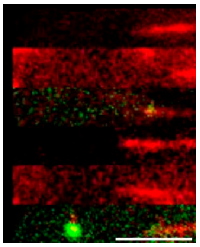


Figure S5. **Activities of Sentin-N and Sentin-ΔN fragments.** (A) GFP-Sentin-N (1–440 aa) did not show growth acceleration activity in the presence of XMAP215^{msps} or EB1 (n = 2–3 experiments, 11–35 events from 10–20 MTs were analyzed in each experiment). (B) GFP-Sentin-N interfered with growth acceleration and rescue induced by XMAP215^{msps}, EB1, and full-length Sentin (7.5 μM tubulin). The mean values of each experiment are marked, and the mean values of all the experiments are indicated by black or red bars. The relative values are shown for the growth rate in these graphs. For MT growth rate, 14–48 events from 10–20 MTs were analyzed in each experiment. Rescue events were detected for 9 of 119 or 0 of 88 shrinking MTs (n = 4 experiments). (C) GFP-Sentin-ΔN showed catastrophe promotion activity but not growth acceleration activity in the presence of 400 nM EB1 (seven independent experiments were performed). Data from a single experiment are marked with the same shape.



Video 1. **In vitro MT polymerization assay with purified tubulin, XMAP215^{msps}, EB1, and Sentin.** Images were acquired by total internal reflection fluorescence microscopy (Nikon) every 3 s. EB1, 400 nM. XMAP215^{msps}, 100 nM. GFP-Sentin, 200 nM. Red, rhodamine-labeled tubulin. Green, GFP-Sentin. Bar, 5 µm.

Table S1. **Kinetic parameters of MT polymerization dynamics in vitro in the presence of various concentrations of XMAP215^{msps}-HA (0–100 nM) at 15 µM tubulin**

XMAP215^{msps} concentration	*N*	Growth rate	Shrink rate	Catastrophe frequency (×10^{-3})	Rescue frequency (×10^{-3})
nM		*µm/min*	*µm/min*	s^{-1}	s^{-1}
0	4	1.0 ± 0.2 (108)	18.0 ± 2.2 (93)	5.7 ± 3.6	0.0 ± 0.0 (0)
25	2	1.5 ± 0.4 (37)	20.9 ± 2.2 (29)	4.6 ± 1.6	0.0 ± 0.0 (0)
75	2	1.7 ± 0.2 (47)	19.6 ± 4.7 (44)	5.4 ± 0.8	0.0 ± 0.0 (0)
100	3	1.5 ± 0.2 (45)	19.5 ± 0.1 (33)	4.0 ± 3.6	0.0 ± 0.0 (0)

Mean ± SD among experiments (number of total events). *N* represents the number of experiments.

Table S2. **Kinetic parameters of MT polymerization dynamics in vitro in the presence of various concentrations of XMAP215^{msps}-HA (0–100 nM) at 7.5 µM tubulin**

XMAP215^{msps} concentration	*N*	Growth rate	Shrink rate	Catastrophe frequency (×10^{-3})	Rescue frequency (×10^{-3})
nM		*µm/min*	*µm/min*	s^{-1}	s^{-1}
0	4	0.5 ± 0.1 (132)	12.8 ± 2.9 (125)	5.2 ± 1.5	0.0 ± 0.0 (0)
20	3	0.9 ± 0.2 (63)	13.3 ± 5.1 (58)	6.0 ± 1.0	0.0 ± 0.0 (0)
50	2	1.2 ± 0.4 (75)	14.5 ± 3.0 (68)	6.1 ± 0.9	0.0 ± 0.0 (0)
70	2	1.2 ± 0.3 (126)	15.1 ± 5.4 (117)	6.7 ± 2.2	0.0 ± 0.0 (0)
100	3	1.0 ± 0.1 (67)	11.4 ± 1.3 (63)	6.9 ± 0.4	0.0 ± 0.0 (0)

Mean ± SD among experiments (number of total events). *N* represents the number of experiments.

Table S3. **Kinetic parameters of MT polymerization dynamics in vitro in the presence of various concentrations of *Drosophila* EB1 (0–800 nM) at 15 µM tubulin**

EB1 concentration	*N*	Growth rate	Shrink rate	Catastrophe frequency (×10^{-3})	Rescue frequency (×10^{-3})
nM		*µm/min*	*µm/min*	s^{-1}	s^{-1}
0	4	1.0 ± 0.2 (114)	24.6 ± 7.3 (93)	2.6 ± 1.6	0.0 ± 0.0 (0)
100	2	1.0 ± 0.0 (46)	28.4 ± 8.5 (41)	3.3 ± 2.0	0.0 ± 0.0 (0)
200	2	1.2 ± 0.1 (66)	30.8 ± 5.4 (52)	2.4 ± 0.1	0.0 ± 0.0 (0)
400	4	1.3 ± 0.2 (140)	23.3 ± 3.7 (119)	3.3 ± 0.7	0.0 ± 0.0 (0)
800	3	1.3 ± 0.1 (62)	26.0 ± 11.6 (66)	2.6 ± 0.2	0.0 ± 0.0 (0)

Mean ± SD among experiments (number of total events). *N* represents the number of experiments.

Table S4. **Kinetic parameters of MT polymerization dynamics in vitro in the presence of 400 nM EB1 and various concentrations of GFP-Sentin (0–200 nM) at 15 μM tubulin**

Sentin concentration	*N*	Growth rate	Shrink rate	Catastrophe frequency ($\times 10^{-3}$)	Rescue frequency ($\times 10^{-3}$)
nM		*μm/min*	*μm/min*	s^{-1}	s^{-1}
0	6	1.1 ± 0.2 (243)	17.7 ± 4.8 (215)	5.9 ± 2.9	0.0 ± 0.0 (0)
50	3	1.3 ± 0.1 (109)	17.8 ± 7.0 (93)	6.4 ± 4.3	0.0 ± 0.0 (0)
100	3	1.4 ± 0.3 (216)	16.6 ± 5.1 (197)	8.4 ± 5.1	0.0 ± 0.0 (0)
200	6	1.5 ± 0.2 (410)	13.9 ± 4.1 (383)	9.0 ± 3.0	0.0 ± 0.0 (0)

Mean ± SD among experiments (number of total events). *N* represents the number of experiments.

Table S5. **Kinetic parameters of MT polymerization dynamics in vitro in the presence or absence of 400 nM EB1, 200 nM GFP-Sentin, and 100 nM XMAP215^{msps}-HA at 15 μM tubulin**

XMAP215^{msps}	EB1	Sentin	*N*	Growth rate	Shrink rate	Catastrophe frequency ($\times 10^{-3}$)	Rescue frequency ($\times 10^{-3}$)	Catastrophe length
				μm/min	*μm/min*	s^{-1}	s^{-1}	*μm*
−	−	−	3	0.9 ± 0.2 (91)	21.3 ± 7.2 (82)	4.1 ± 1.0	0.0 ± 0.0 (0)	3.3 ± 1.7
−	+	+	3	1.5 ± 0.1 (176)	14.0 ± 2.9 (169)	10.3 ± 2.3	0.0 ± 0.0 (0)	2.0 ± 0.6
+	−	−	2	1.5 ± 0.3 (35)	19.5 ± 0.2 (24)	2.1 ± 1.9	0.0 ± 0.0 (0)	8.9 ± 6.0
+	+	+	3	3.6 ± 0.8 (126)	17.1 ± 6.4 (120)	9.9 ± 2.5	0.8 ± 0.3 (10)	5.0 ± 2.2
+	−	+	2	1.5 ± 0.1 (39)	22.1 ± 5.3 (29)	2.0 ± 0.7	0.1 ± 0.1 (1)	8.6 ± 1.2
−	+	−	2	1.0 ± 0.1 (62)	15.0 ± 2.7 (58)	9.0 ± 0.7	0.0 ± 0.0 (0)	1.5 ± 0.2
+	+	−	2	2.0 ± 0.2 (75)	15.6 ± 2.4 (68)	6.0 ± 1.6	0.1 ± 0.1 (1)	4.5 ± 0.8

Mean ± SD among experiments (number of total events). Plus and minus signs indicate that the protein was added or not added to the reaction, respectively. *N* represents the number of experiments.

Table S6. **Kinetic parameters of MT polymerization dynamics in vitro in the presence 100 nM XMAP215^{msps}-HA alone or additionally 400 nM EB1 and 200 nM GFP-Sentin at 7.5 μM tubulin**

XMAP215^{msps}	EB1	Sentin	*N*	Growth rate	Shrink rate	Catastrophe frequency ($\times 10^{-3}$)	Rescue frequency ($\times 10^{-3}$)
				μm/min	*μm/min*	s^{-1}	s^{-1}
+	−	−	3	0.9 ± 0.3 (50)	11.7 ± 1.1 (48)	6.9 ± 0.6	0.0 ± 0.0 (0)
+	+	−	3	1.0 ± 0.3 (68)	10.0 ± 0.7 (65)	9.3 ± 0.7	0.0 ± 0.0 (0)
+	+	+	3	1.4 ± 0.7 (67)	9.6 ± 2.1 (66)	10.9 ± 2.5	0.2 ± 0.4 (2)

Mean ± SD among experiments (number of total events). Plus and minus signs indicate that the protein was added or not added to the reaction, respectively. *N* represents the number of experiments.

Table S7. **Kinetic parameters of MT polymerization dynamics in vitro in the presence of 400 nM EB1 and various concentrations of GFP-Sentin (0–200 nM) and XMAP215^{msps}-HA (0–300 nM) at 15 μM tubulin**

XMAP215^{msps}-HA	GFP-Sentin	Growth rate	Shrink rate	Catastrophe frequency ($\times 10^{-3}$)	Rescue frequency ($\times 10^{-3}$)
nM	*nM*	*μm/min*	*μm/min*	s^{-1}	s^{-1}
300	0	2.2 ± 0.5 (27)	22.7 ± 10.4 (22)	2.5	0.2 (2)
300	100	3.0 ± 0.8 (38)	23.0 ± 11.6 (36)	4.5	0.4 (3)
300	200	2.8 ± 1.3(56)	16.1 ± 7.6 (52)	7.8	1.2 (8)
100	200	2.6 ± 0.4 (49)	16.2 ± 8.5 (48)	9.1	0.4 (2)
0	200	1.5 ± 0.4 (38)	18.2 ± 15.5 (34)	8.0	0.0 (0)

Mean ± SD in one representative experiment (number of total events).

Table S8. **Plasmids used in this study**

Plasmid name	Gene	Promoter
pWJ168	GST-EB1	T7
pMW154	GST-EB1-GFP	tac
pMW147	GST-GFP-Sentin-N (1–440 aa)	tac
pWJ230	His-Sentin	Polyhedrin
pGG650	His-mGFP-Sentin	Polyhedrin
pWJ246	His-mGFP-Sentin (1–440 aa)	Polyhedrin
pGG777	His-mGFP-Sentin (231–982 aa)	Polyhedrin
pGG816	XMAP215^{msps} -mGFP-His	Polyhedrin
pGG699	XMAP215^{msps} -HA-His	Polyhedrin
pWJ104	Sentin-mCherry	Metallothionein
pWJ223	mRFP-Sentin (231–982 aa)	Metallothionein
pWJ237	mRFP-Sentin (591–982 aa)	Metallothionein

Table S9. **Primer sequences for RNAi**

Gene	CG number	Primer sequence
Sentin	9028	GAGCTGATCACTTCCTCCGCCGC (3′UTR)
		TTATGGTTCGTAAGGCAAAGTTC (3′UTR)
		AGGTGACAGCAGAGATTGATCC (exon)
		TTGGATTTAATACCCATAGGGC (exon)
XMAP215^{msps}	5000	AGACAATGAGGACGATGATGG
		ATCCAAGGTGAATCATAATGCC
Cdc27	8610	GATGGGACTCAAGAAACAATCG
		TCTTCATGTAGAATTGCATGGC
Control	(pBS)	TAAATTGTAAGCGTTAATATTTTG
		AATTCGATATCAAGCTTATCGAT
Klp61F	9191	CAAAAATAGCATCAGCATGTCC
		GGATAAACGAAATCCCTTTTGG

T7 sequences were added at the 5′ end.