Samejima et al., http://www.jcb.org/cgi/content/full/jcb.201202155/DC1

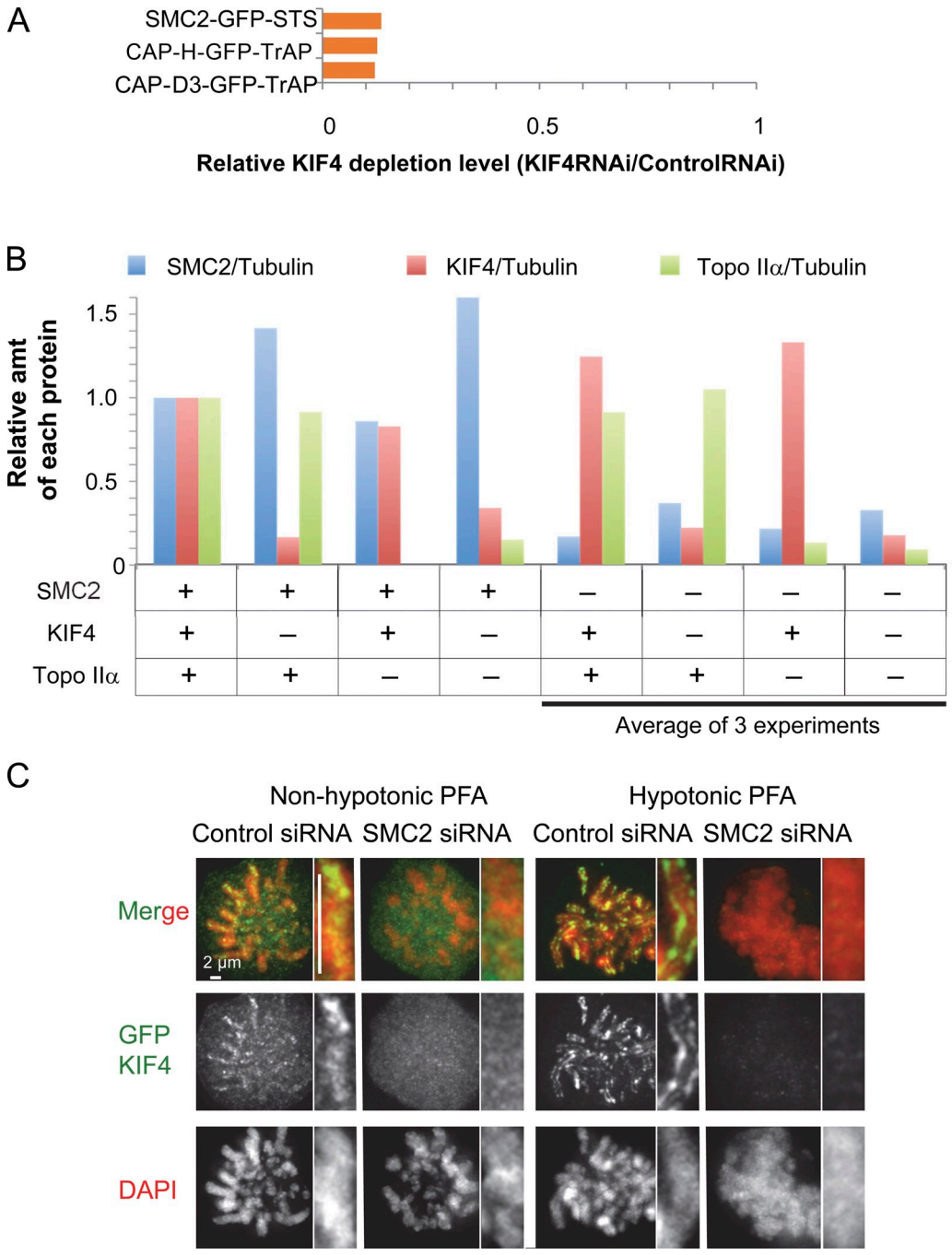

Figure S1. **Quantification of KIF4 depletions and dependency of KIF4 localization on SMC2.** (A) Quantification of immunoblotting after depletion of KIF4 by transfection of KIF4siRNA. Cells were harvested 20 h after transfection and blots were quantitated using an Odyssey system (LI-COR Biosciences). Quantification of the KIF4 depletion was completed once for these particular cell lines, but similar levels of depletion were observed when the experiment was repeated more than three times with similar DT40 cell lines. (B) Quantification of immunoblotting after single/double/triple depletion of KIF4, SMC2, and Topo IIα using the Odyssey system. Data were normalized against α-tubulin. (Left) One representative experiment out of two repeats. (Right side) Average of three experiments. (C) KIF4 depends on SMC2 for its localization of chromatid axis. GFP-KIF4^{wt} expressing KIF4^{OFF} cells were treated with either SMC2 siRNA or control siRNA, which were then fixed with either PFA/PBS or PFA/75 mM KCl and stained with DAPI. Bars, 2 μm.

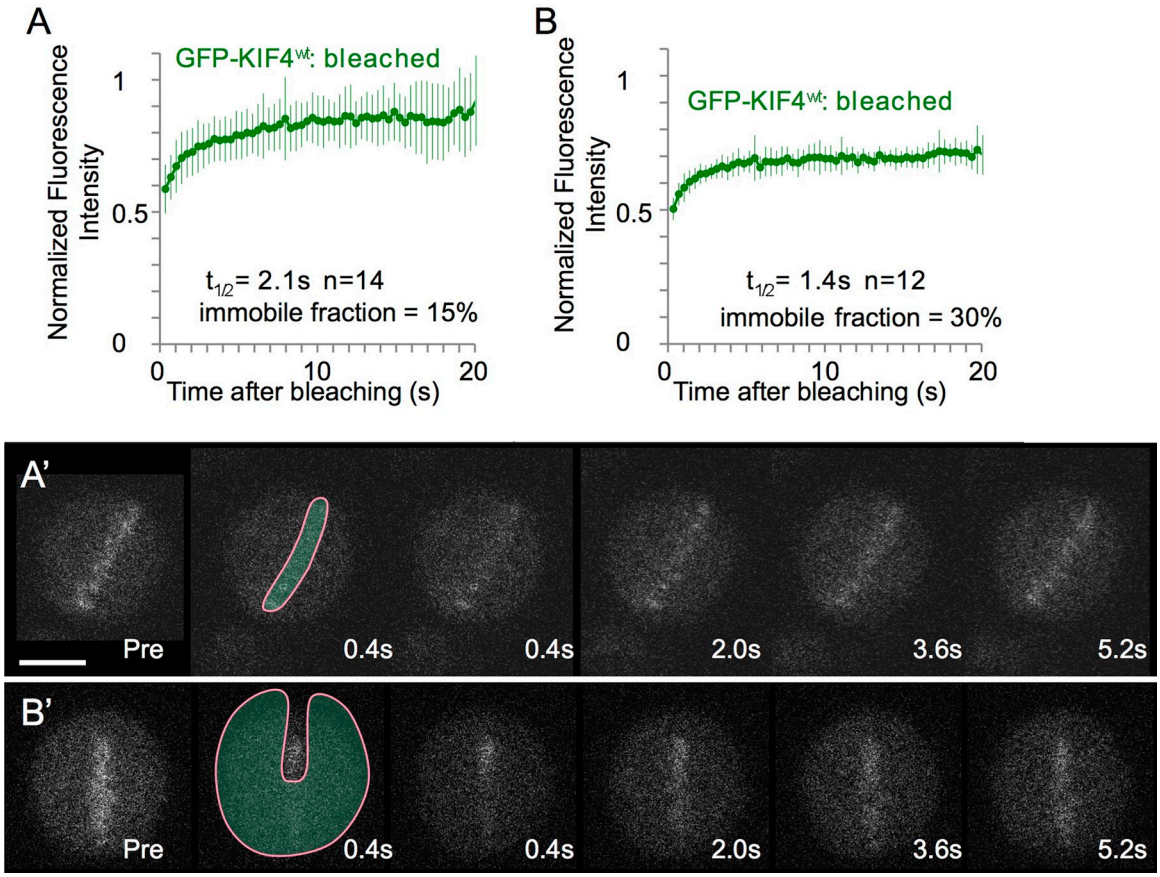

Figure S2. **FRAP experiments of GFP-KIF4^{wt} expressed in KIF4^{OFF} cells.** (A) The entire metaphase plate was bleached. (B) The entire cell except for half of metaphase plate was bleached. Bar, 5 µm. Error bars show SD.

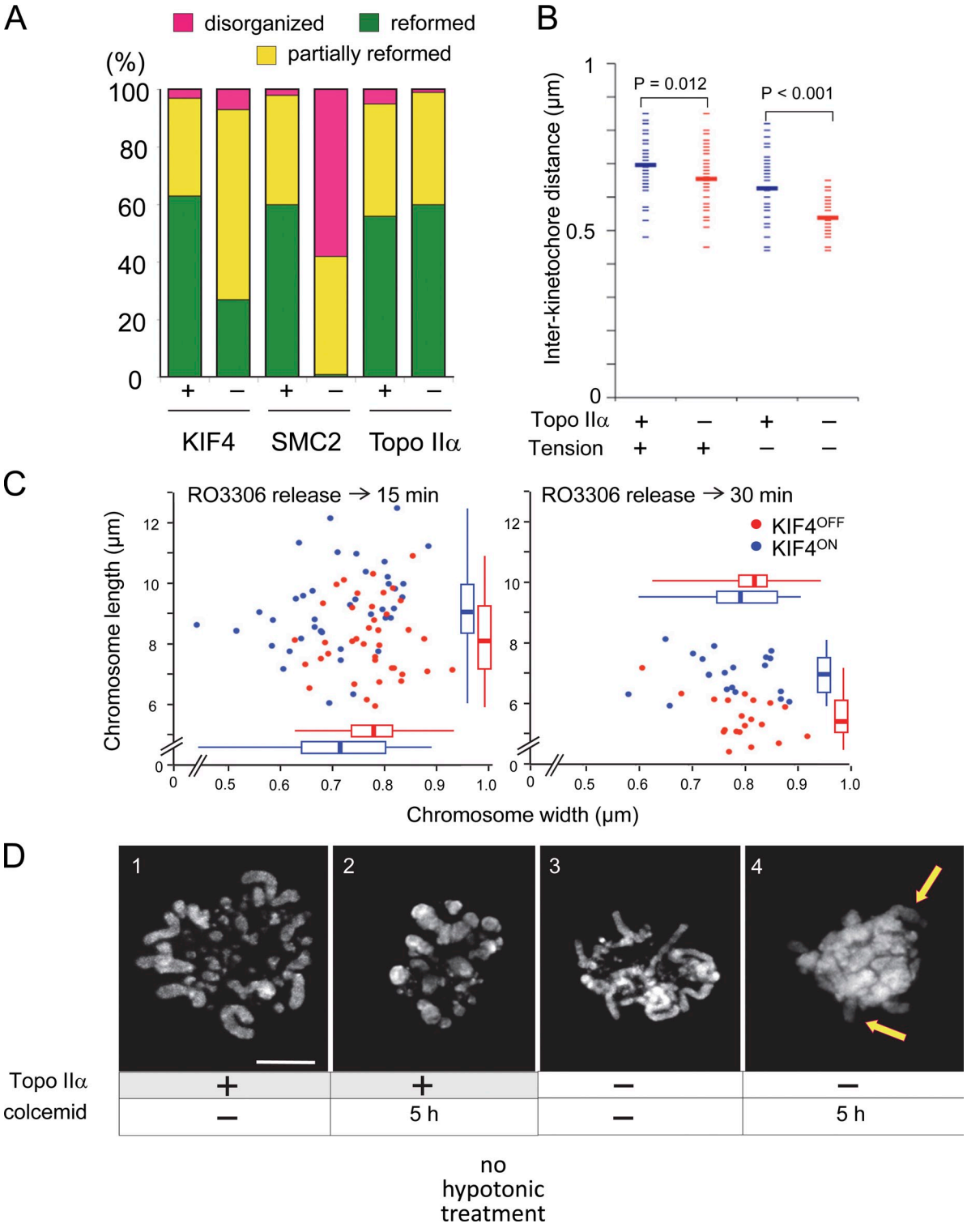

Figure S3. **Further characterization of the chromosomal phenotype after KIF4 depletion.** (A) IMS assay using KIF4$^{ON/OFF}$, SMC2$^{ON/OFF}$, Topo IIα$^{ON/OFF}$ cell lines. 100 cells were scored for each sample. The data shown are from a single representative experiment out of two repeats. (B) Inter-kinetochore distance. Topo IIα $^{ON/OFF}$ cell lines were treated either with MG132 (tension +) or with colcemid (tension −) for 3 h, fixed in 4% PFA/PBS, and stained with anti–CENP-T antibody. More than fifty kinetochore pairs were measured per cell line. P-values were based on student's *t* test. $n = 50$ from a single experiment. (C) Length of the third longest chromosome and width of chromosomes of KIF4$^{ON/OFF}$ cells. Cells were treated with RO3306 for 2–2.5 h released in to fresh media. 15 or 30 min later, cells were treated with 75 mM KCl for 5 min, then fixed with cold methanol/acetic acid. $n = 40$ (left), $n = 20$(right) from a single experiment. (D) Chromosome morphologies in the presence of absence of Topo IIα and plus and minus colcemid. Topo IIα–inducible shRNA cell lines were treated with/without doxycycline for 43 h (1 and 3), then further treated with colcemid for 5 h (2 and 4). The cells were subsequently washed with PBS and fixed in methanol/acetic acid. Arrows (4) point to protruding chromosome arms. Bar, 5 μm.

Figure S4. **The motor domain is required to fully rescue chromosomal functions of KIF4.** (A) Immunoblotting of KIF4^{OFF} cell lines expressing GFP-KIF4^{wt} or GFP-KIF4$^{510-1226}$. The expression level of GFP-KIF4 chimeric proteins was similar to that of KIF4 in wild-type cells. α-Tubulin was used as a loading control. (B) Localization of GFP-KIF4 mutants in KIF4^{OFF} cells. Constructs expressing the indicated GFP-KIF4 chimeric proteins were transiently transfected into KIF4^{OFF} cells, fixed with PFA/75 mM KCl, and stained with DAPI. Bar, 5 µm. (C) Sister chromatid spacing. SMC2$^{ON/OFF}$ LacO–bearing cells expressing LacI-GFP were treated with KIF4 siRNA or Control siRNA and then as in Fig. 5 C. D- and P-values were obtained by KS test. Compared with SMC2^{ON} control siRNA (−tension), D = 0.3, P = 0.33 (SMC2^{ON} KIF4 siRNA −tension); D = 0.6, P = 0.001 (SMC2^{OFF} control siRNA −tension); and D = 0.5 P = 0.01 (SMC2^{OFF} KIF4siRNA −tension). Compared with SMC2^{ON} control siRNA (+tension), D = 0.3, P = 0.33 (SMC2^{ON} KIF4 siRNA +tension); D = 0.55, P = 0.0005 (SMC2^{OFF} control siRNA +tension); and D = 0.55 P = 0.02 (SMC2^{OFF} KIF4 siRNA +tension). The data shown are from a single representative experiment out of two repeats.

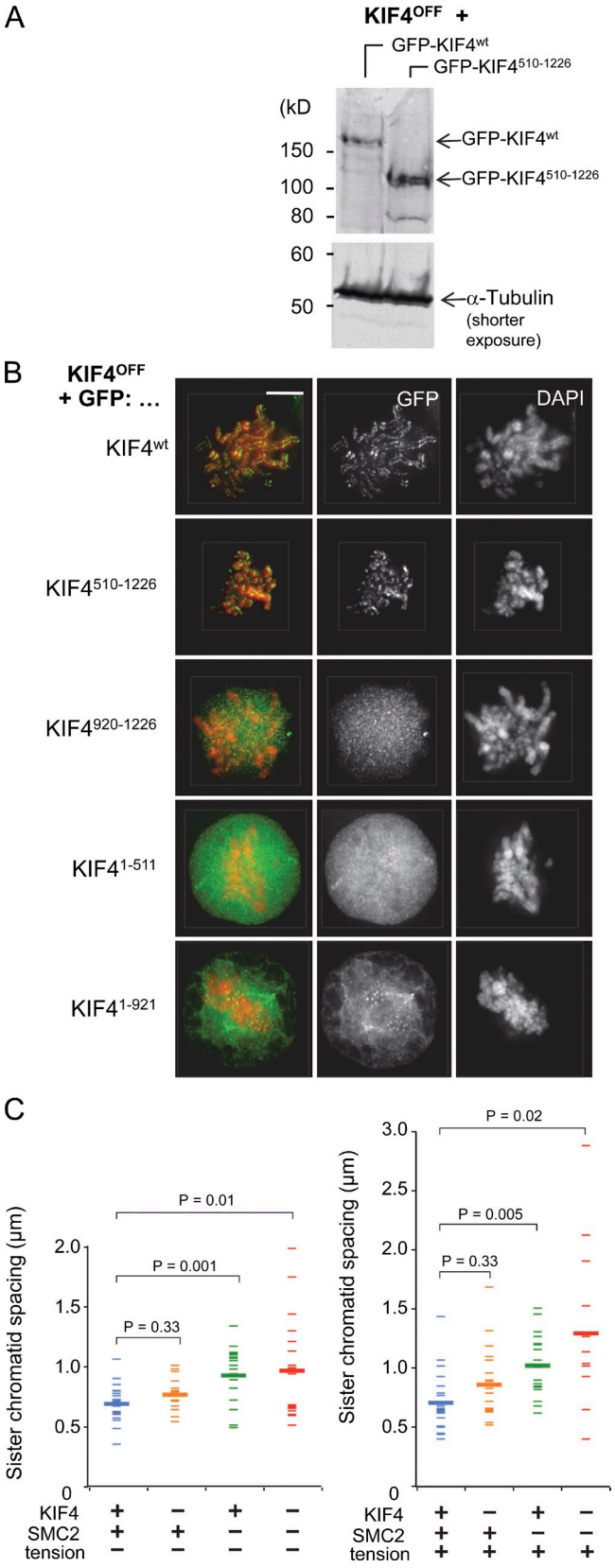

Table S1. **Cell pretreatment and fixation conditions for the figures**

Figure	Panel	Synchronization	Cell pretreatment before fixation	Fixation conditions	Key
1	A	Colcemid 3 h	75 mM KCl 5 min	MeOH/HOAc	H,N
1	B–G	None	None	PFA/PBS	N,N
2	A–D	Colcemid 3 h	75 mM KCl 5 min	MeOH/HOAc	H,N
3		MG132 1-3 h	(Live cells)	None	
5	A	None	75 mM KCl 5 min	PFA/75 mM KCl	H,H
5	B–D	± MG132 or colcemid 3 h	None	PFA/PBS	N,N
5	E	Colcemid 12 h	See Materials and methods	PFA/RSB	
7	A1–6	None	None	MeOH/HOAc	N,N
7	A7–12	None	75 mM KCl 5 min	MeOH/HOAc	H,N
6	A1 and 3	None	75 mM KCl 5 min	MeOH/HOAc	H,N
6	A2 and 4	Colcemid 5 h	75 mM KCl 5 min	MeOH/HOAc	H,N
6	D	None	None	PFA/PBS	N,N
7	B1–4	None	None	MeOH/HOAc	N,N
7	B 5–12	None	75 mM KCl 5 min	MeOH/HOAc	H,N
8	C	MG132 3 h	None	PFA/PBS	N,N
8	B	None	75 mM KCl 5 min	PFA/75 mM KCl	H,H
8	D	Colcemid 12 h	See Materials and methods	PFA/RSB	
S1	C, left	None	None	PFA/PBS	N,N
S1	C, right	None	75 mM KCl 5 min	PFA/75 mM KCl	H,H
S2		MG132 1-3 h	(Live cells)	None	
S3	A	Colcemid 12 h	See Materials and methods	PFA/RSB	
S3	B	± MG132 or colcemid 3 h	None	PFA/PBS	N,N
S3	C	R03306 2-2.5 h - release	75 mM KCl 5 min	MeOH/HOAc	H,N
S3	D1 and 3	None	None	MeOH/HOAc	N,N
S3	D2 and 4	Colcemid 5 h	None	MeOH/HOAc	N,N
S4	B	None	75 mM KCl 5 min	PFA/75 mM KCl	H,H
S4	C	± MG132 or colcemid 3 h	None	PFA/PBS	N,N

Key: (Left) H = hypotonic swelling of cell before fixation and permeabilization; N = cell under physiological conditions before fixation and permeabilization. (Right) H = fixation in hypotonic buffer; N = fixation in isotonic buffer or methanol/acetic acid.