Ryu et al., http://www.jcb.org/cgi/content/full/jcb.201004033/DC1

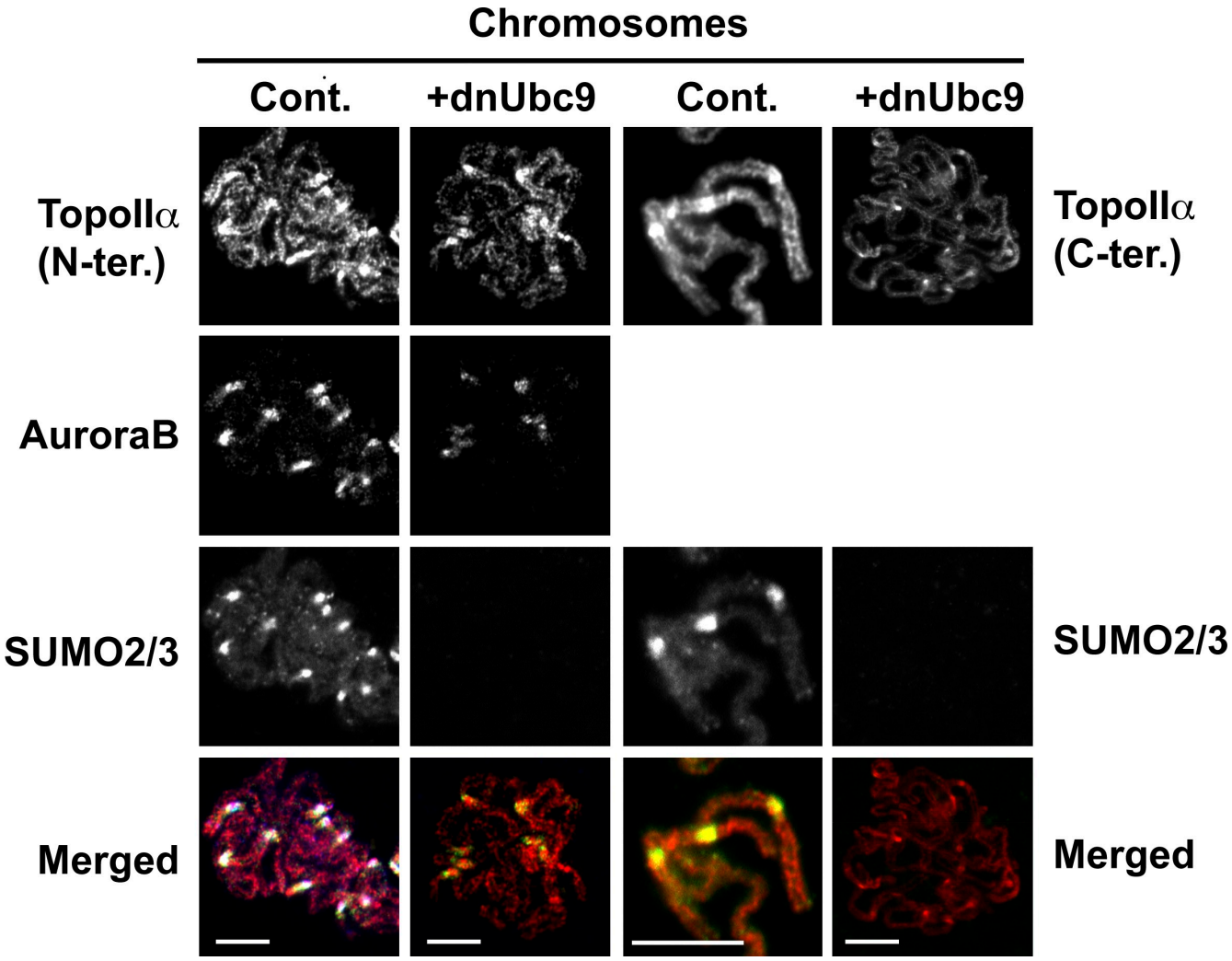


Figure S1. **TopoIIα colocalizes with Aurora B on the centromere of mitotic chromosomes.** The mitotic chromosomes were prepared as in Fig. 1, and samples were analyzed by immunostaining as indicated. (left) Monoclonal TopoIIα (N-ter.) is shown in red, Aurora B is shown in green, and SUMO2/3 is shown in blue in merged panel. (right) polyclonal TopoIIα (C-ter.) is shown in red and SUMO2/3 is shown in green. Both monoclonal and polyclonal TopoIIα antibodies showed indistinguishable staining. Bars, 10 µm.

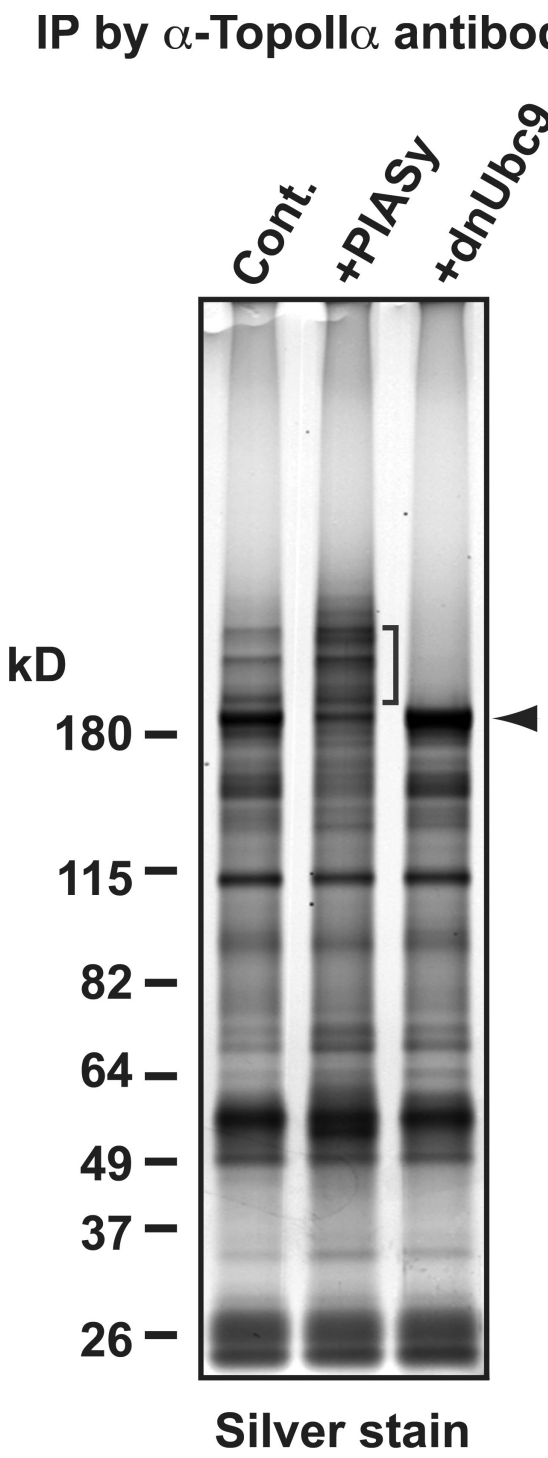
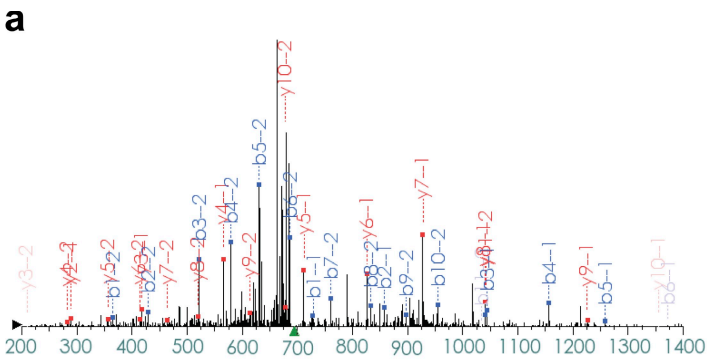


Figure S2. **Isolation of SUMOylated TopoIIα for mapping SUMOylation sites by mass spectrometry.** Chromosomal proteins were isolated from mitotic chromosomes that were assembled in CSF extracts (Cont.), CSF extracts with exogenous PIASy (+PIASy), or dnUbc9 (+dnUbc9) using SDS-PAGE sample buffer. After samples were renatured with buffer containing thesit, the extracted fractions were immunoprecipitated with affinity-purified anti-TopoIIα antibody. Immunoprecipitated fractions were further separated by SDS-PAGE and silver stained (Owl/Daiichi). Both SUMOylated (bracket) and non-SUMOylated TopoIIα (arrowhead) were subjected to MS/MS analysis: in brief, the samples were double digested with trypsin and chymotrypsin. The chymotrypsin digestion provides the QQQTGG signature tag on the modified lysine. The MS/MS analysis, with around 50% TopoIIα sequence coverage, indicated lysine 660 as a candidate site for SUMOylation (detailed LC-MS/MS analysis data are shown in Fig. S3). Positions of molecular mass standards (kD) are indicated on the left.



b

Seq	#	+1 b	+1 y	+1	Seq	#	+2 b	+2 y	+2
K#	1	728.37	---	11	K#	1	364.69	---	11
E	2	857.41	1355.61	10	E	2	429.21	678.31	10
W	3	1043.49	1226.57	9	W	3	522.25	613.79	9
L	4	1156.58	1040.49	8	L	4	578.79	520.75	8
T	5	1257.62	927.40	7	T	5	629.32	464.21	7
N	6	1371.67	826.36	6	N	6	686.34	413.68	6
F	7	1518.73	712.31	5	F	7	759.87	356.66	5
M*	8	1665.77	565.25	4	M*	8	833.39	283.13	4
Q	9	1793.83	418.21	3	Q	9	897.42	209.61	3
D	10	1908.86	290.15	2	D	10	954.93	145.58	2
R	11	---	175.12	1	R	11	---	88.07	1

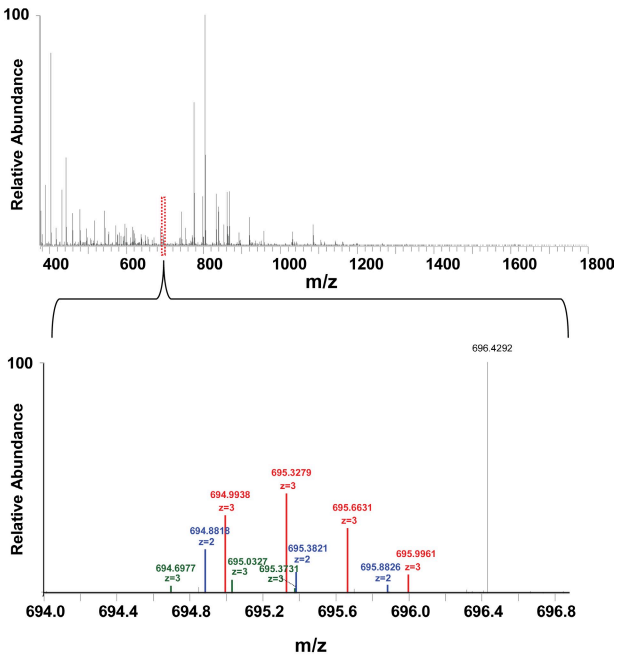


Figure S3. **SUMOylation of TopoIIα at K660 was identified by LC-MS/MS after a trypsin/chymotrypsin double digest.** (a and b) Precursor of 695.33 m/z was isolated and fragmented, with a series of b and y ions matching the sequence K#EWLTNFM*QDR. The asterisk refers to oxidized methionine. The SUMO2/3 signature of QQQTGG is denoted by #. The measured m/z of the monoisotopic precursor ion (694.9938) matched the expected within 0.3 ppm. (c) Multiple ion species, denoted by interlaced isotopic envelopes (blue, green) were found co-eluting within the isolation window of the SUMOylated TopoIIα peptide (red).

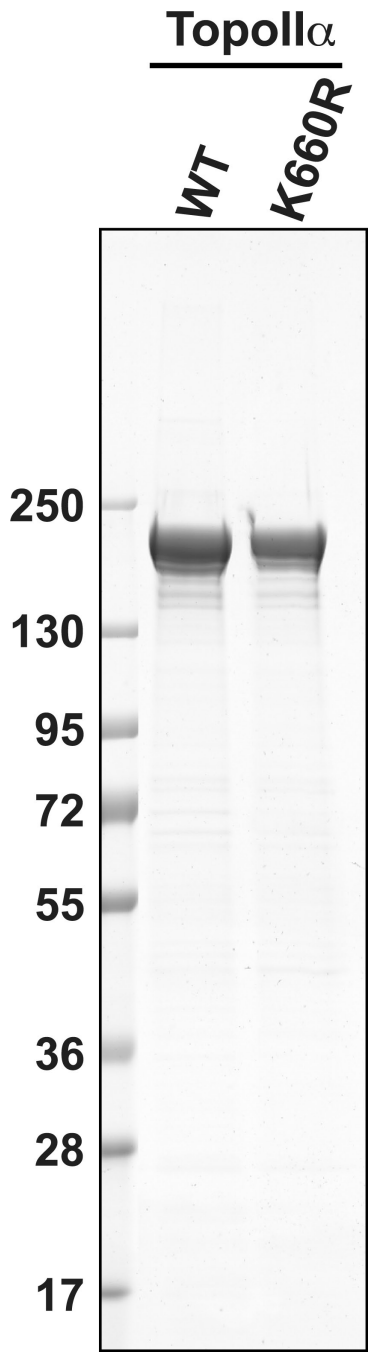


Figure S4. **The purity of TopoIIα WT and K660R proteins.** Recombinant proteins of TopoIIα were expressed and purified as described in Materials and methods, and 200 ng of each protein was resolved on a 8–16% gradient gel and Coomassie blue stained to determine the purity of the proteins. WT and K660R show comparable quality. Positions of molecular mass standards (kD) are indicated on the left.

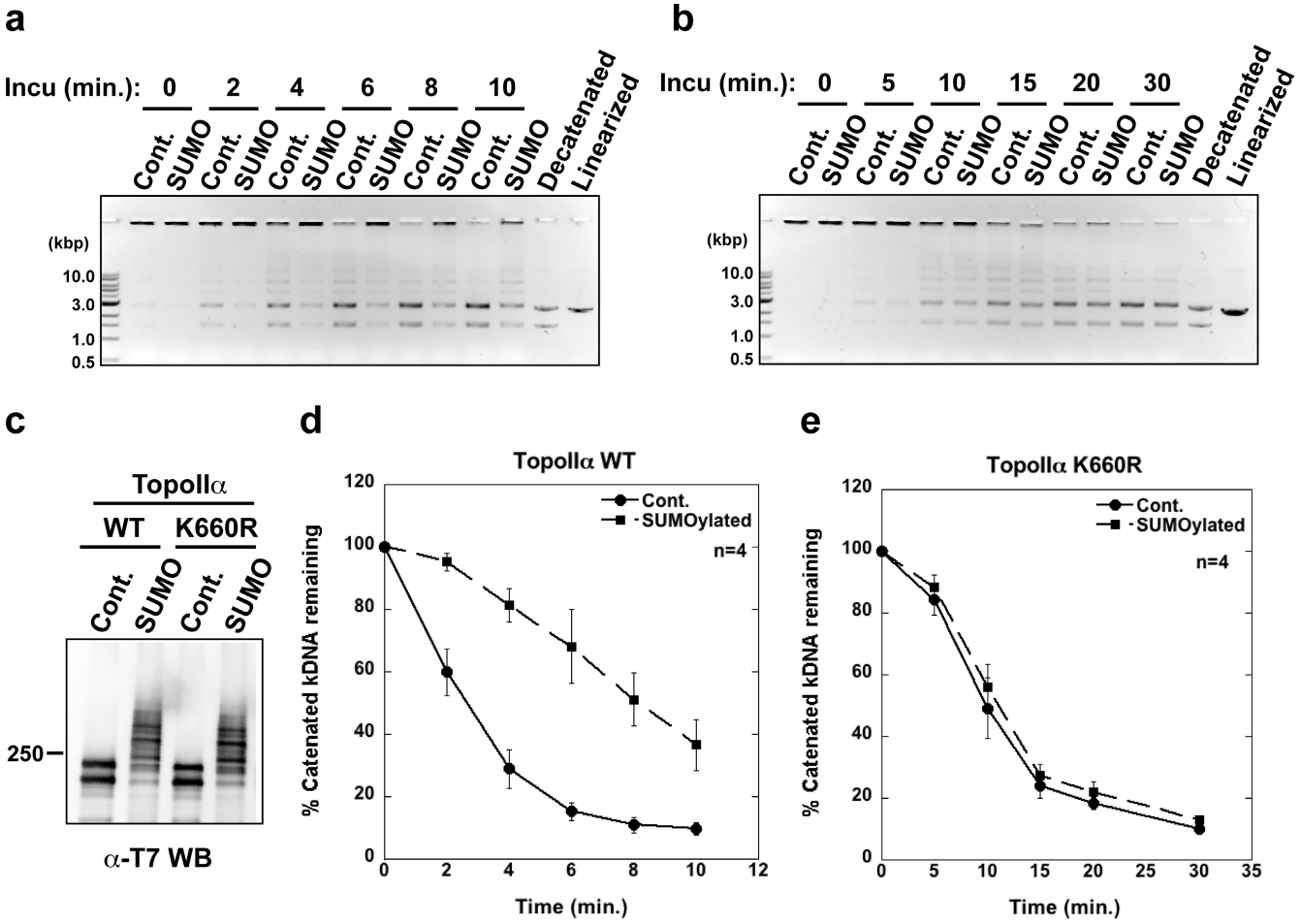


Figure S5. **The elimination of the SUMOylation at K660 abolishes the SUMOylation-mediated inhibition of TopoIIα activity.** TopoIIα proteins that were fused to the T7 tag and ZZ tag at the N and C terminus, respectively, were purified by ZZ affinity using an IgG sepharose column (GE Healthcare). The ZZ tag was removed by PreScission protease (GE). Further purified T7-TopoIIα was applied to the SUMOylation–decatenation coupled assay as in Fig. 5. (a) Representative result of SUMOylation–decatenation coupled assay with TopoIIα WT. (b) Representative result of SUMOylation–decatenation coupled assay with TopoIIα K660R. (c) Representative samples of in vitro SUMOylated TopoIIα WT and K660R. In vitro samples were analyzed by Western blotting for the T7 tag. Control (Cont.) contained SUMO2-G instead of SUMO2-GG. Positions of molecular mass standards (kD) are indicated on the left. (d and e) Results from four independent experiments performed as in panel a are presented as the mean of the percentage of catenated DNA remaining with standard error (error bars). The different preparations of TopoIIα showed the same results as in Fig. 5.