Supplementary information for

Guiding functions of the C-terminal domain of topoisomerase IIa advance mitotic chromosome assembly

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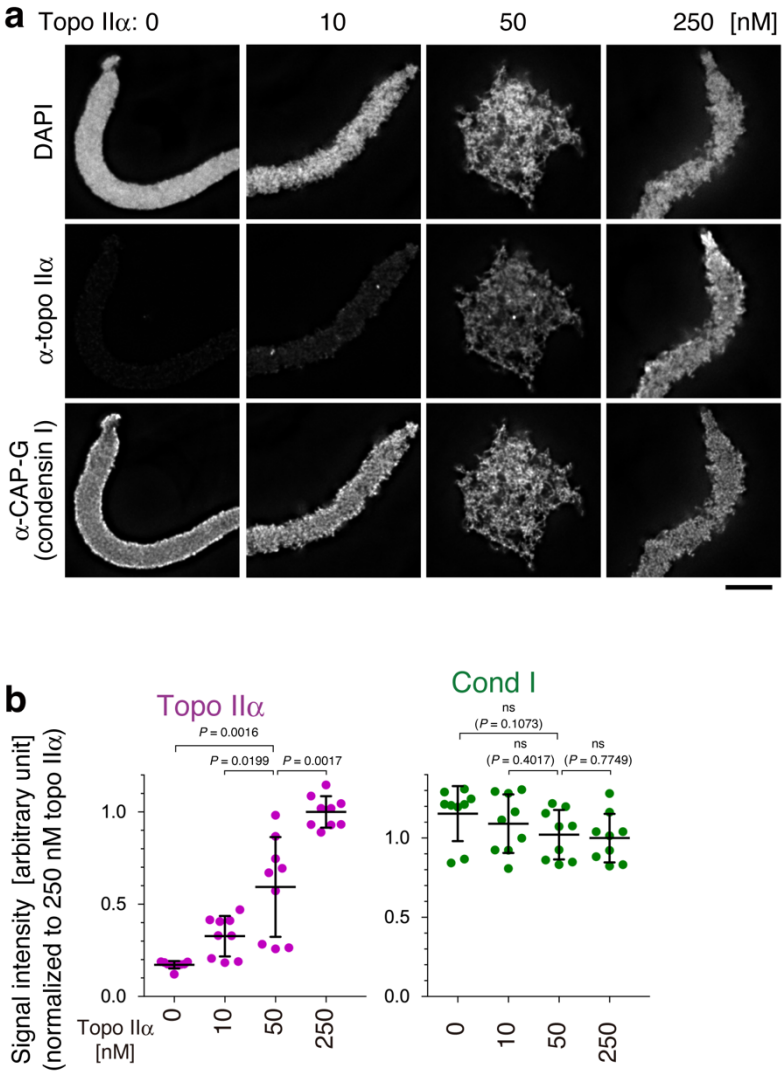
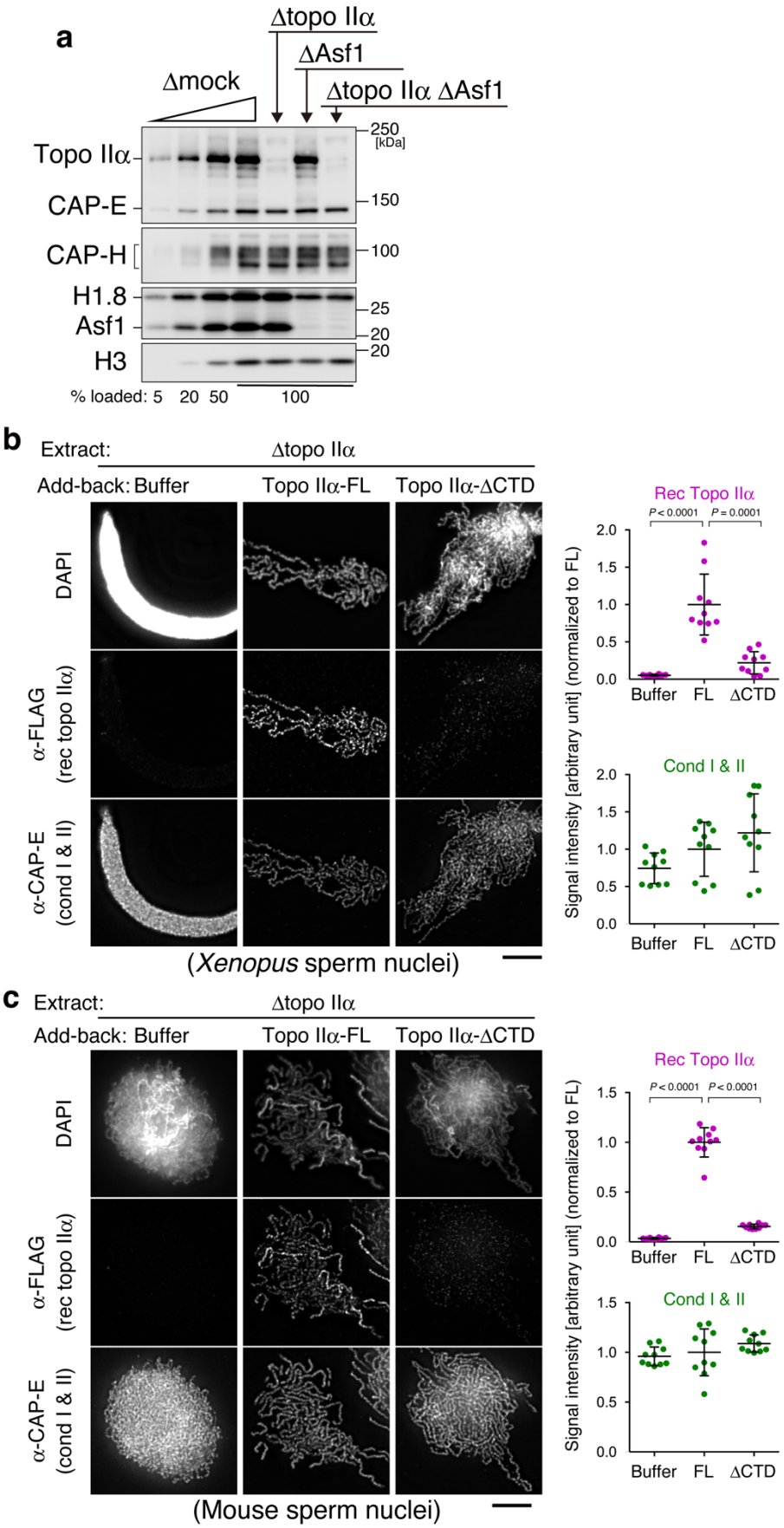
Supplementary Figures 1-3

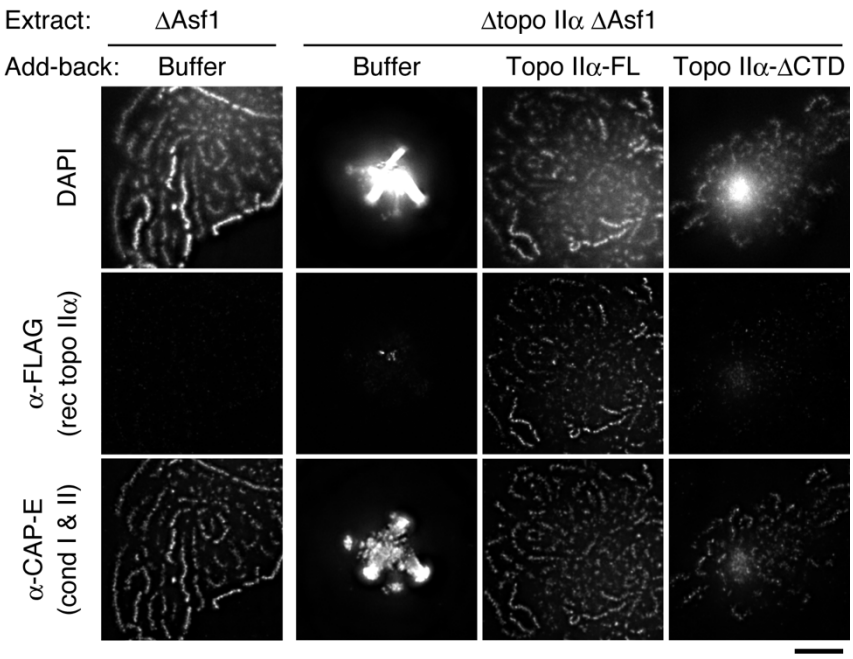
Supplementary Table 1



Supplementary Fig 1. **Titration of recombinant *Xenopus* topo IIα in the mitotic chromatid reconstitution assay. a, b.** Chromatid reconstitution assays were performed using increasing concentrations of *Xenopus* topo IIα and fixed concentrations of the other five proteins (condensin I, a truncation version of histone H2A.XF-H2B dimer, Npm2, Nap1, and FACT). After a 150-min incubation, the resultant structures were fixed and immunolabeled with antibodies against topo IIα and CAP-G. DNA was counterstained with DAPI (**a**). Quantitative analyses of the signal intensities were carried out as in Fig. 1. The mean ± s.d. is shown (*n*=9 clusters of chromatin). *P* values were assessed by two-tailed Welch's *t*-test (ns, not significant) (**b**). Bar, 5 μm.


a
Δtopo IIα
ΔAsf1
Δmock
Δtopo IIα ΔAsf1
250
[kDa]
Topo IIα
CAP-E
150
CAP-H
100
H1.8
25
Asf1
20
H3
20
% loaded: 5 20 50 100
b
Extract: Δtopo IIα
Add-back: Buffer
Topo IIα-FL
Topo IIα-ΔCTD
DAPI
α-FLAG (rec topo IIα)
α-CAP-E (cond I & II)
(Xenopus sperm nuclei)
Rec Topo IIα
P < 0.0001
P = 0.0001
Signal intensity [arbitrary unit] (normalized to FL)
Buffer
FL
ΔCTD
Cond I & II
c
Extract: Δtopo IIα
Add-back: Buffer
Topo IIα-FL
Topo IIα-ΔCTD
DAPI
α-FLAG (rec topo IIα)
α-CAP-E (cond I & II)
(Mouse sperm nuclei)
Rec Topo IIα
P < 0.0001
P < 0.0001
Signal intensity [arbitrary unit] (normalized to FL)
Buffer
FL
ΔCTD
Cond I & II

Supplementary Fig 2. **Topo IIα-ΔCTD is proficient in chromatid individualization, but is deficient in chromatid thickening in *Xenopus* egg cell-free extracts. a.** Mitotic egg extracts were immunodepleted with a control rabbit IgG (Δmock), anti-topo IIα antibody (Δtopo IIα), ant-Asf1 antibody (ΔAsf1), or a combination of anti-topo IIα and anti-Asf1 (Δtopo IIα ΔAsf1). To estimate the efficiency of depletion, an aliquot of each extract along with decreasing amounts of the Δmock extract was analyzed by immunoblotting with the antibodies indicated. Note that neither condensin subunits (CAP-E and CAP-H) nor histone H3 was depleted after each depletion while a sizable portion (50~80%) of the linker histone H1.8 was co-depleted with anti-Asf1 antibody (i.e., in the ΔAsf1 and Δtopo IIα ΔAsf1 extracts). This experiment was repeated independently at three times with similar results. **b.** *Xenopus* sperm nuclei were added to egg extracts depleted of topo IIα (Δtopo IIα) that had been supplemented with buffer, topo IIα-FL, or topo IIα-ΔCTD. After a 150-min incubation, the samples were fixed and processed for immunofluorescence and analyzed. Quantitative analyses of the signal intensities were carried out as in Fig. 1. The mean ± s.d. is shown (n=12 clusters of chromatin). P values were assessed by two-tailed Welch's t-test. **c.** Mouse sperm nuclei were used in the same experiment as shown above. The mean ± s.d. is shown (n=12 clusters of chromatin). P values were assessed by two-tailed Welch's t-test. Bars, 5 μm.



Supplementary Fig 3. **Topo IIα-FL, but not topo IIα-ΔCTD, can fully rescue the defects observed in the extracts depleted of both topo IIα and Asf1 (topo IIα and CAP-E labeling).** Mouse sperm nuclei were added to egg extracts depleted of topo IIα and Asf1 (Δtopo IIα ΔAsf1) that had been supplemented with buffer, topo IIα-FL, or topo IIα-ΔCTD. After a 150-min incubation, the samples were fixed and processed for immunolabeling with the antibodies indicated. As a reference, nucleosome-depleted chromatids were assembled in an extract depleted of Asf1 (ΔAsf1) that contained endogenous topo IIα. This experiment was repeated independently at three times with similar results. Bar, 5 μm.

Supplementary Table 1. The list of primers used in this study.

Identifier	Sequence	Note
KB87	GACGATGACAAGCCCGAGACCGAACC TTTGCAGCCTC	PCR (topo IIα-FL and -ΔCTD), forward, overlapping sequence A for Gibson assembly
KB88	CAGAACTTCCAGCCCAAAGTCATCGT CAGAATCCTCCAGG	PCR (topo IIα-FL), reverse, overlapping sequence B for Gibson assembly
KB89	CAGAACTTCCAGCCCAATTTCCTGCA ACTGCATTTTC	PCR (topo IIα-ΔCTD), reverse, overlapping sequence B for Gibson assembly
KA28	AAATGATAACCATCTCGC	Sequencing (pXR504, 505, and 506)
KA29	GAAATTTGTGATGCTATTGC	Sequencing (pXR504, 505, and 506)
KU27	AAGATTAAGCCATTTGAGGG	Sequencing (pXR505 and 506)
KU28	GTCGTGGCTGAGCAATACGC	Sequencing (pXR505)
KU31	CAACATCATCAAGATCGTGGG	Sequencing (pXR505 and 506)
KU32	TTCCAAAATGAATCTGGCTTGG	Sequencing (pXR505 and 506)
KU33	ATGTCTGCATACCATCATGGTG	Sequencing (pXR505 and 506)
KU34	TGTCGAGCACAGACACCTCACC	Sequencing (pXR505 and 506)
KU35	GTAAAGAAAATGCAGTTGCAGG	Sequencing (pXR505 and 506)
KU36	CTTTATGCAGGACATAGTTGGG	Sequencing (pXR505 and 506)
KU37	GATACTCCCATCATGATCAGTGC	Sequencing (pXR505)
KB90	GAGTACCACACTGATACCAC	Sequencing (pXR505 and 506)
KB91	TCGCTTCTTGGCTGCTGCAG	Sequencing (pXR505)
KB92	CCCTGCGGTCTTGCATGAAG	Sequencing (pXR505 and 506)
KB93	GTGGGAATACACCATAGCG	Sequencing (pXR505 and 506)
KB94	GATCATTCACCTGCTCGTGC	Sequencing (pXR505 and 506)