Supplemental Information Appendix

Supplemental information for:

"Topoisomerase IIα is essential for maintenance of mitotic chromosome structure".

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Additional supplementary material for this manuscript includes:

- Supplementary movies S1-S22

Supplemental figures and legends

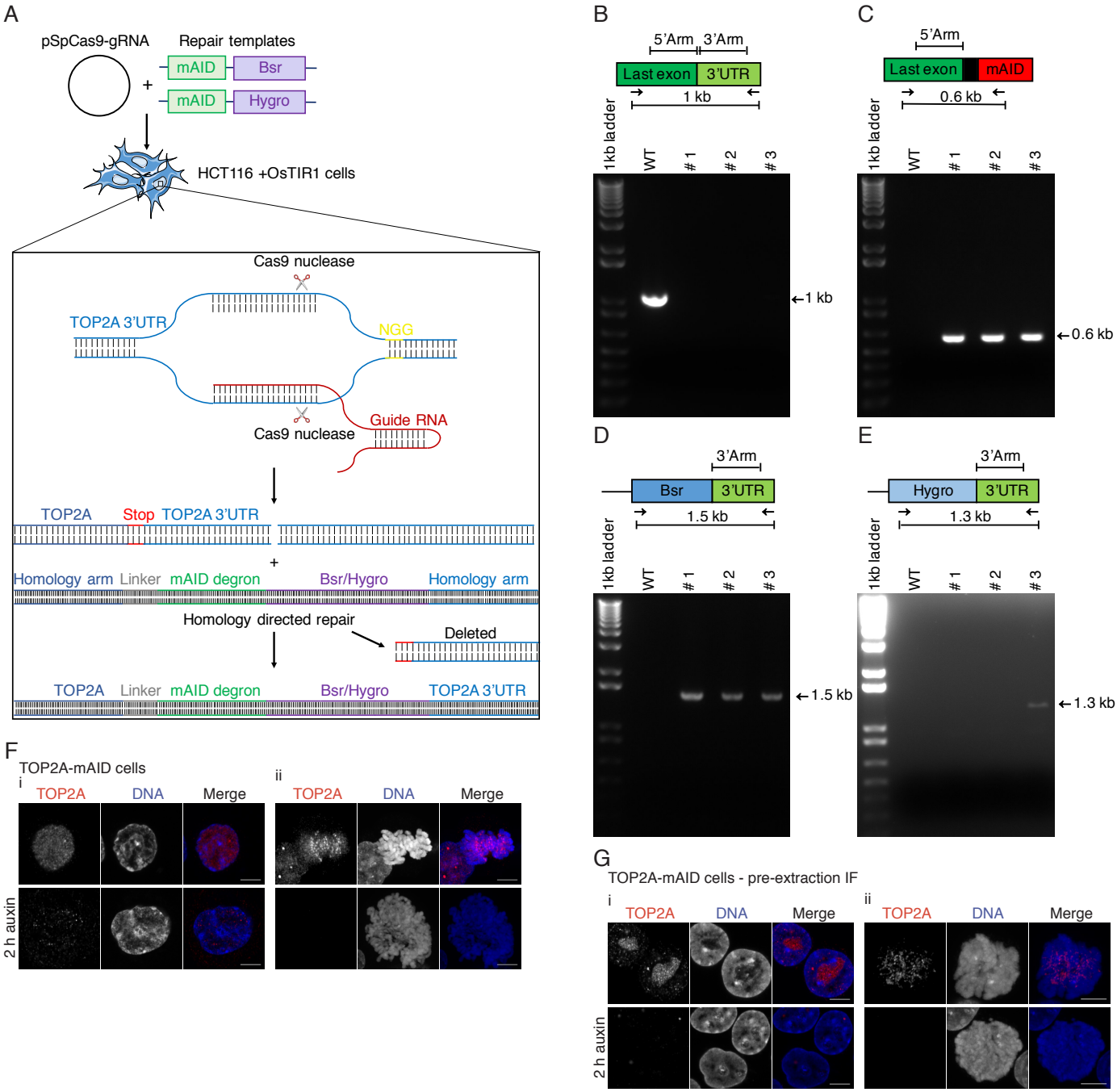

Fig. S1.

Cloning and validation of TOP2A-mAID cells. (A) Schematic detailing the cloning strategy for introducing mAID at the C-terminus of both alleles of the TOP2A gene. (B-E) Schematics of loci and binding sites of primers and genomic DNA PCRs testing successful knockin of mAID, and Bsr and Hygro antibiotic resistance genes. WT denotes genomic DNA from parental HCT116 OsTIR1 cells. Arrows in the schematics represent forward and reverse primers. (B) PCR of the area around the TOP2A stop codon and 3'UTR using "TOP2A ext. forward" and "TOP2A ext. reverse" primers. The primers bind outside the homology arms used as templates for HDR. In clones #1-3 both alleles appear to be disrupted. (C) PCR testing mAID knock in using "TOP2A ext. forward" and "mAID reverse" primers. Clones #1-3 all have the mAID insertion. (D) PCR testing knock in of the Bsr resistance gene using "Bsr forward" and "TOP2A ext. reverse" primers. Clones # 1-3 have integration of Bsr. (E) PCR testing knock in of the Hygro resistance gene using "Hygro forward" and "TOP2A ext. reverse" primers. Only clone # 3 have integration of the Hygromycin gene. (F) Immunofluorescent staining of TOP2A and DAPI staining of DNA in TOP2A-mAID cells grown with or without auxin and visualized by Airyscan super-resolution microscopy. Representative interphase (i) and mitotic cells are shown (ii). (G) Representative Airyscan super-resolution images of TOP2A-mAID cells with or without auxin. Following pre-extraction, cells were immunofluorecently stained for TOP2A (red) and DNA (blue) was stained with DAPI.

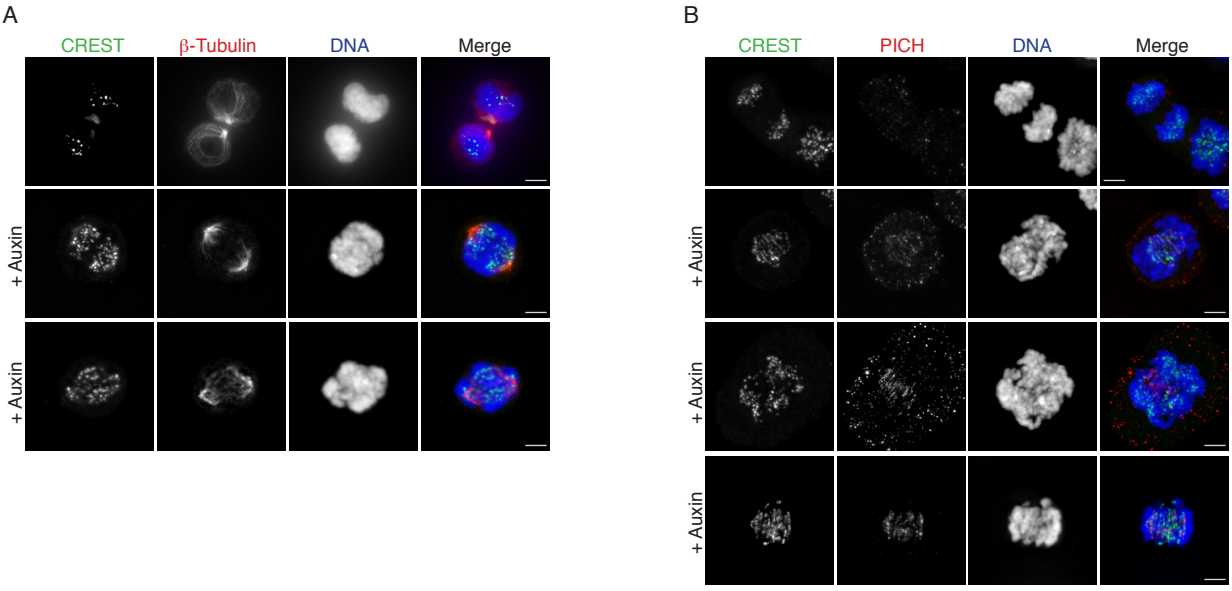

Fig. S2.

Examples of cells with severe entanglement. (A-B) Representative images of TOP2A-mAID cells grown like in Figure 2E. Scale bars are 5 microns. (A) Cells were stained immunofluorescently for CREST (green), β-tubulin (red) and DNA stained with DAPI (blue). (B) Cells were stained immunofluorescently for CREST (green), PICH (red) and DNA stained with DAPI (blue).

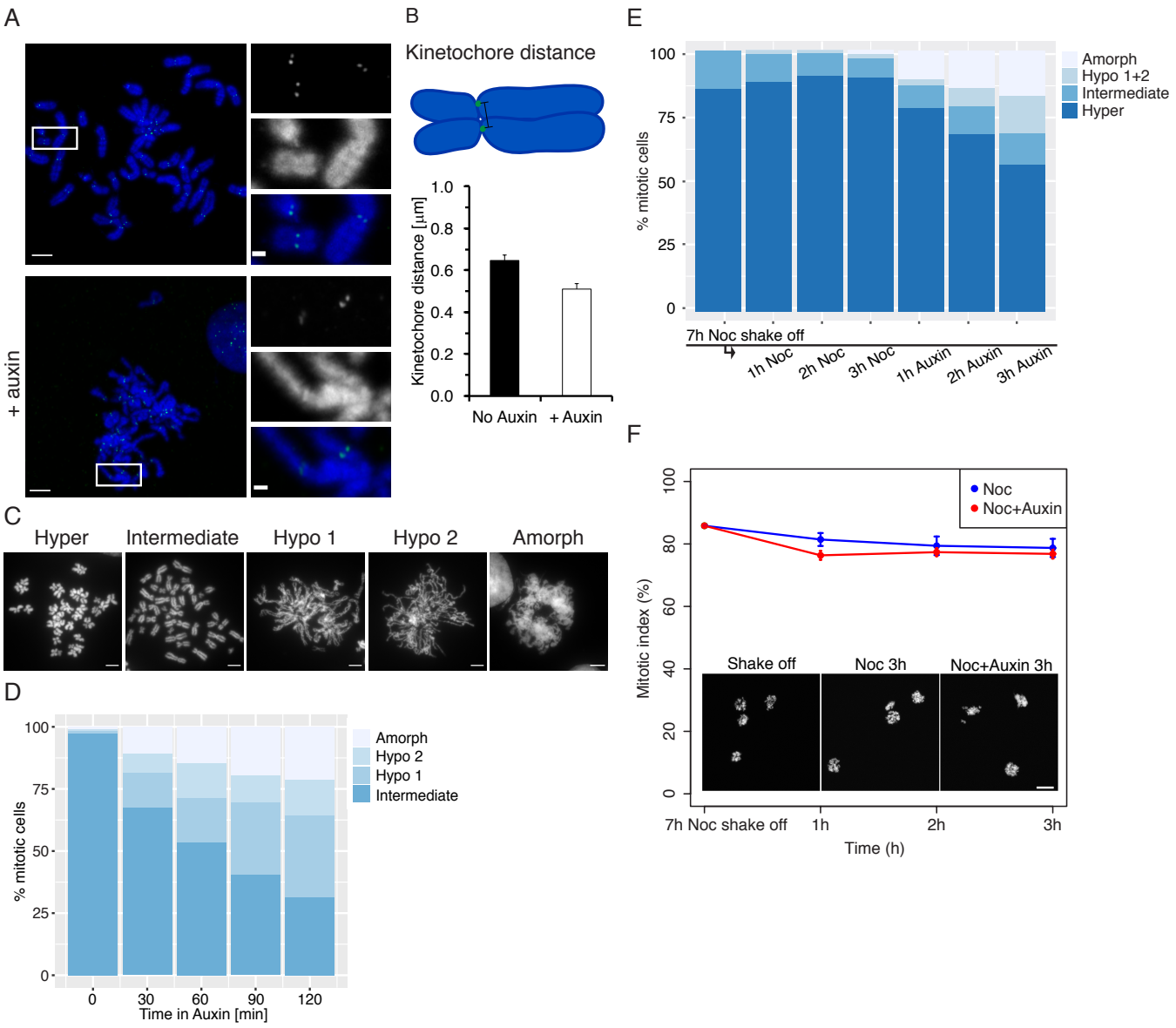

Fig. S3.

Interkinetochore distance and chromosome spreads. (A) Representative super-resolution images of chromosomes from TOP2A-mAID cells grown in media +/- auxin for 2 h. Chromosomes were spread and stained immunofluorescently for CENP-C (green) and DNA stained with DAPI (blue). Zoom panels show enlargements of individual centromere regions. (B) The schematic describes how kinetochore distance was measured. Green dots symbolize CENP-C foci. The bar chart displays quantification of kinetochore distance on chromosomes performed like in (B). (C) Representative chromosome condensation categories quantified in (D) and (E). (D) Quantification of chromosome condensation in cells treated like in Figure 3A. (E) Quantification of chromosome hypercondensation in cells treated like in Figure 3E. (F) Quantification of mitotic index of cells treated like in Figure 3E.

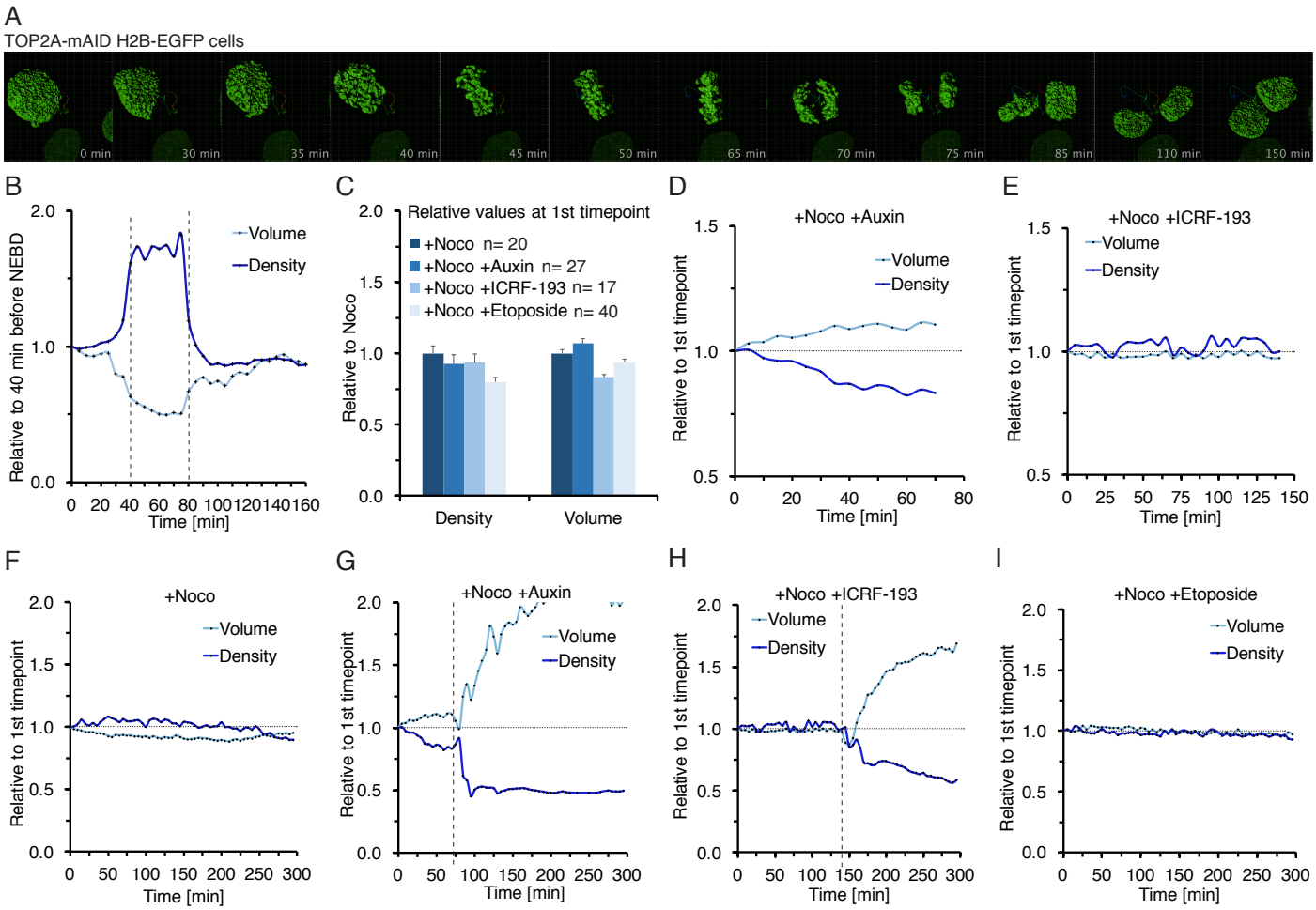

Fig. S4.

3D modelling of chromatin compaction. (A) 3D volume rendering of the chromatin of a TOP2A-mAID +H2B-EGFP cell imaged by live cell imaging over time going through mitosis. (B) Quantification of chromatin volume and mean fluorescence intensity of the cell from (A). Dashed lines mark nuclear envelope breakdown (NEB) and reformation (NER). (C) Chromatin volume and density values at t=0 relative to nocodazole treated control cells. (D, E) Representative plots of relative chromatin volume and density values quantified before mitotic exit from cells arrested in prometaphase with nocodazole and treated with auxin (D) or ICRF-193 (E). (F-I) Chromatin volume and density profiles for representative cells arrested in prometaphase with nocodazole and treated with DMSO (F), auxin (G), ICRF-193 (H) or etoposide (I). The profiles correspond to the images in Fig. 6 (B-E). (E) and (F) are full profiles of (D) and (E). (D-I) Dotted horizontal lines denote starting value. Dashed vertical lines denote last quantified time point before mitotic exit. (C-I) See Fig. 6A for experimental conditions.

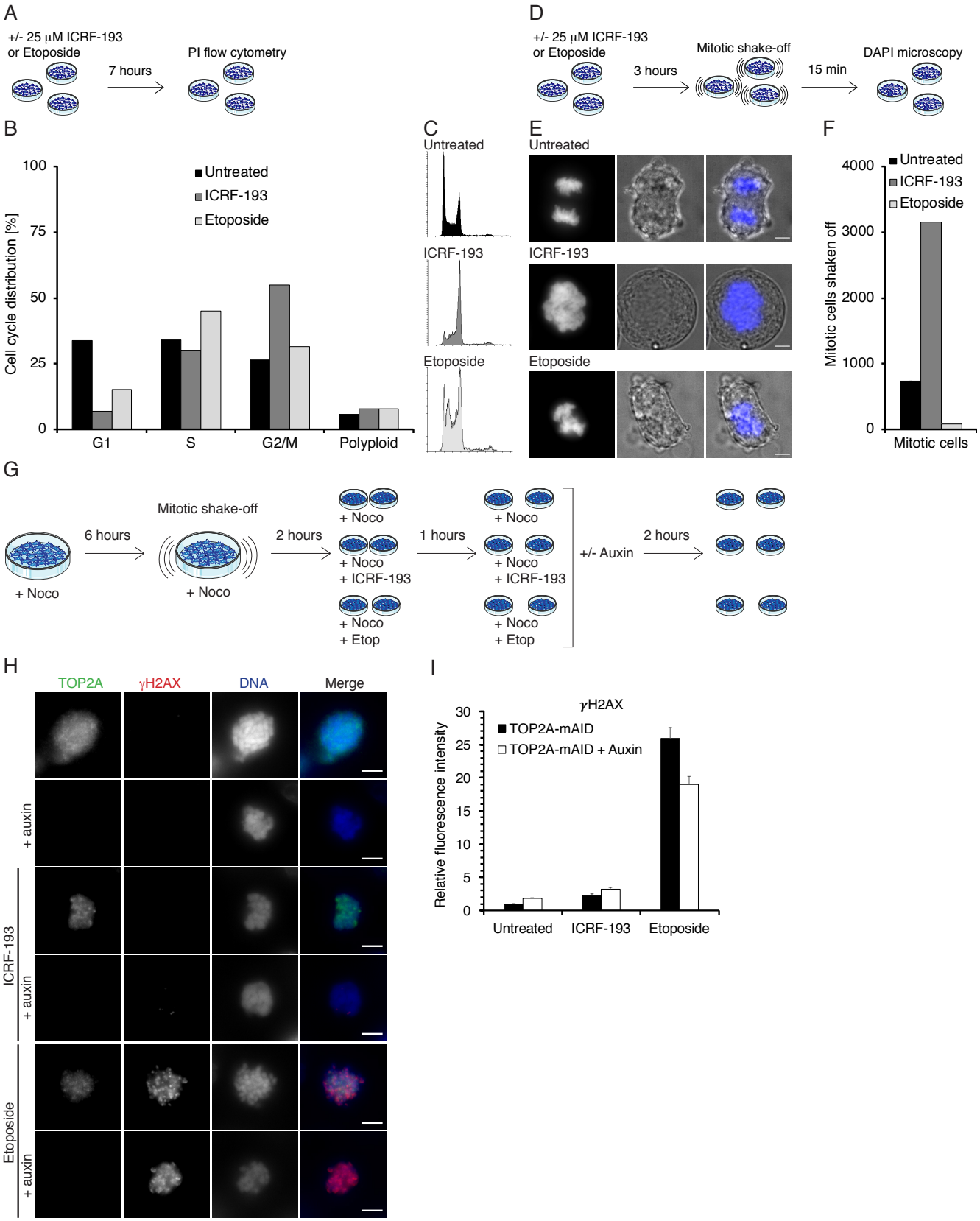

Fig. S5.

Control experiments for ICRF-193 and etoposide treatments. (A) Experimental conditions for (B-C). TOP2A-mAID cells were grown +/- 25μM ICRF-193 or etoposide for 7 h and harvested for either propidum iodide (PI) flow cytometry. (B) Quantification of cell cycle profiles of TOP2A-mAID cells +/- ICRF1-93 or etoposide. Polyploid is defined as non-aggregates with more than G2/M DNA content. (C) Flow cytometry profiles quantified in (B). (D) Experimental conditions for (E-F). TOP2A-mAID cells were grown +/- 25μM ICRF-193 or etoposide for 3 h, mitotic cells shaken off and grown for 15 min. before harvest for DAPI microscopy. (E) Representative mitotic cells. (F) Quantification of mitotic cells when treated as in (D). (G) Diagram of treatment conditions of TOP2A-mAID cells for (H-I). (H) Immunofluorescent staining of TOP2A (green) and γH2AX (red) following ICRF-193 or etoposide pre-treatment of TOP2A-mAID cells subsequently grown +/- auxin. DNA was stained with DAPI (blue). Scale bars are 5 μm. (I) Total chromatin γH2AX fluorescence

intensity as fold of the untreated (no auxin) condition. Error bars denote SEM. Stars denote P values from parametric students t-tests *$P < 0.05$ and **$P < 0.01$.

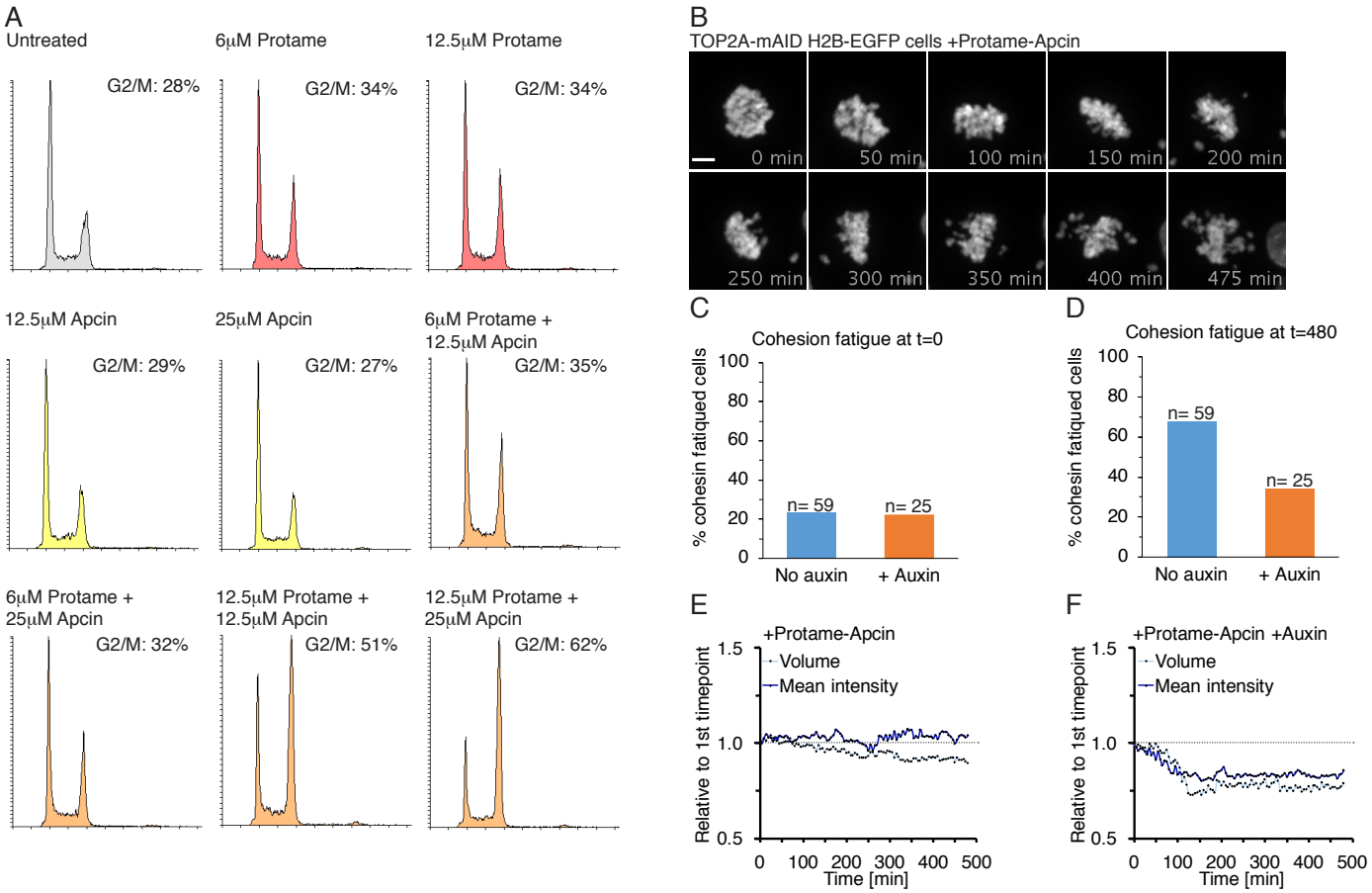

Fig. S6.

Metaphase arrest using APC/C inhibitors Protame and Apcin. (A) PI flow cytometry cell cycle profiles of cells treated with the indicated concentrations of Protame and Apcin for 5 hours. Quantified G2/M content is denoted on the graphs. (B) Representative images of a cell arrested in metaphase with Protame and Apcin and undergoing cohesion fatique. (C-D) Quantification of cohesion fatique at t=0 (C) and t=480 min (D), defined as the first timepoint where bipolar chromatids escape the chromatin mass. (E-F) Representative chromatin volume and density profiles of cells treated as depicted in Fig. 7A without auxin (E) or with auxin (F). The profiles correspond to the images in Fig. 7B I (E) and Fig. 7C III (F).

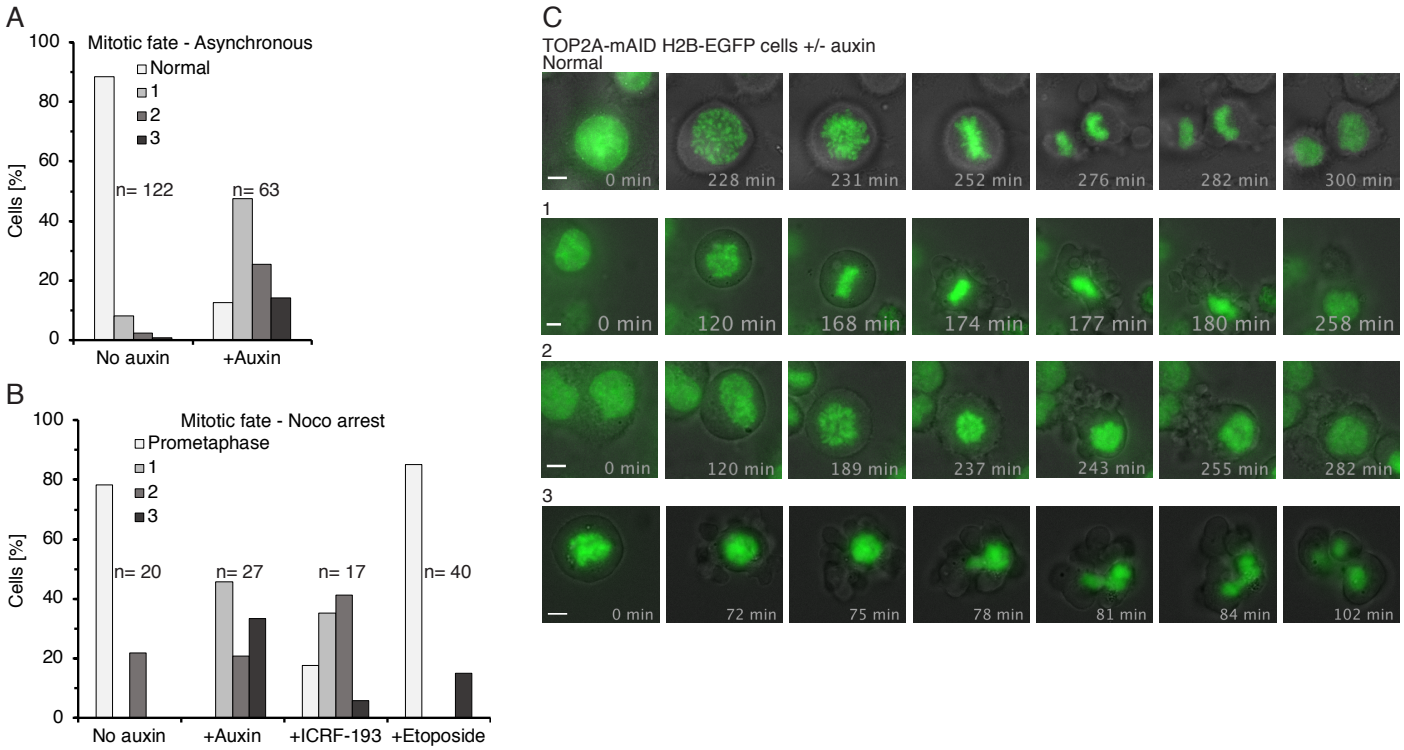

Fig. S7.

Mitotic exit phenotypes. (A) Quantification of the mitotic exit phenotypes of cells treated like in (Fig. 8A). (B) Quantification of the mitotic exit phenotypes of cells treated like in (Fig. 8C).

(C) Live cell imaging of TOP2A-mAID +H2B-EGFP cells. EGFP channel is merged with DIC images visualizing the plasma membrane. Scale bars are 5 μm. (1) A cell going through normal mitosis and cytokinesis. (1-3) Cells undergoing premature mitotic exit phenotypes 1-3.

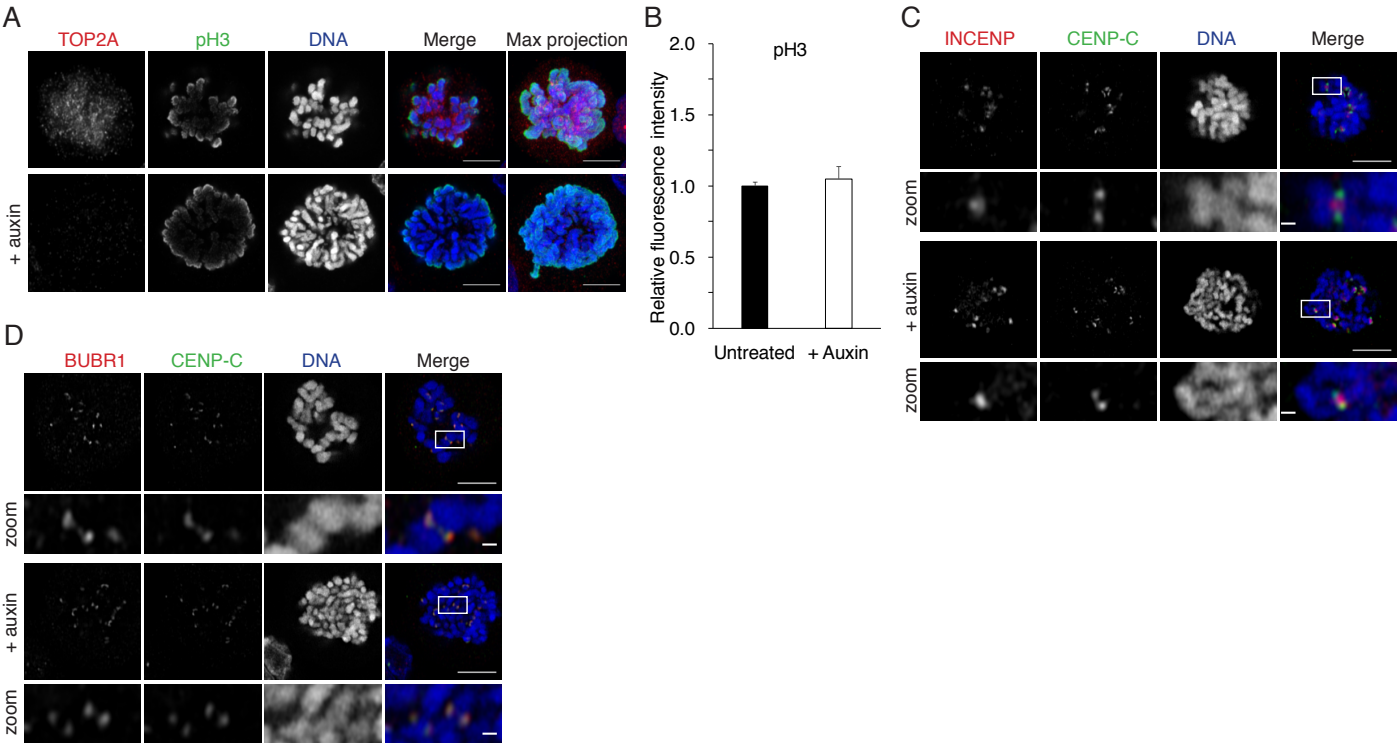

Fig. S8.

Spindle assembly checkpoint proteins and plasma membrane blebbing during mitotic exit. (A) Representative super-resolution images of TOP2A-mAID cells grown in media +/- auxin for 2 h and stained immunofluorescently for TOP2A (red) and pH3 (green) with DNA stained with DAPI (blue). (B) Quantification of total chromatin pH3 fluorescence intensity of mitotic cells grown for 2 h in media +/- auxin. Error bars denote SEM. (C) Representative super-resolution images of TOP2A-mAID cells grown in media +/- auxin for 2 h and stained immunofluorescently for INCENP (red) and CENP-C (green) with DNA stained with DAPI (blue). Zoom panels show enlargements of individual centromere regions. (D) Representative super-resolution images of TOP2A-mAID cells grown in media +/- auxin for 2 h and stained immunofluorescently for BUBR1 (red) and CENP-C (green) with DNA stained with DAPI (blue). Zoom panels show enlargements of individual centromere regions.

Tab. S1.

Guide DNAs and primers

gDNA name	**Sequence (-PAM) 5'-3'**
TOP2A guideDNA (-)	GGTAACTTTAAAACCAGTCT (-TGG)
Primer name	**Sequence 5'-3' (upper case: priming)**
TOP2A 5' arm forward	cctcgaggtcgacggtatcgataagcttgatatcgCACGTAACTAAGCCATTGCTCTT
TOP2A 5' arm reverse	caagcactcttctccttggcgcctgcaccggatccAAACAGATCATCTTCATCTG
TOP2A 3' arm forward	gttattaggtccctcgaagaggttcactaggatccGGTTTTAAAGTTACCTGAAGCTCT
TOP2A 3' arm reverse	ccgctctagaactagtggatcccccgggctgcaggTGGCACATAAGAGGCTGAGT
TOP2A ext. forward	AAGCAAGGTGAGTGTTGATCCTA
TOP2A ext. reverse	AGTGTTTTTCTTCGGCCTCTG
mAID reverse	ACCGCTTGATTTTTGGCAGG
Bsr forward	GTGGGAGCGGCAATTCGT
Hygro forward	GACCGATGGCTGTGTAGAAGT

Supplemental movie legends

Movie S1
Time lapse movie of an untreated TOP2A-mAID cell going through mitosis.

Movie S2
Time lapse movie of a TOP2A-mAID cell treated with 500μM auxin, going through mitosis.

Movie S3
3D rendering of an untreated TOP2A-mAID cell going mitosis.

Movie S4
Time lapse movie of a TOP2A-mAID cell arrested in prometaphase by nocodazole treatment.

Movie S5
Time lapse movie of a TOP2A-mAID cell arrested in prometaphase by nocodazole treatment and then treated with 500μM auxin.

Movie S6
Time lapse movie of a TOP2A-mAID cell arrested in prometaphase by nocodazole treatment and then treated with 25μM ICRF-193.

Movie S7
Time lapse movie of a TOP2A-mAID cell arrested in prometaphase by nocodazole treatment and then treated with 25μM etoposide.

Movie S8
Time lapse movie of a TOP2A-mAID cell arrested in metaphase by proTAME-Apcin treatment undergoing cohesion fatique.

Movie S9
Time lapse movie of a TOP2A-mAID cell arrested in metaphase by proTAME-Apcin treatment.

Movie S10
Time lapse movie of a TOP2A-mAID cell arrested in metaphase by proTAME-Apcin treatment.

Movie S11
Time lapse movie of a TOP2A-mAID cell arrested in metaphase by proTAME-Apcin treatment.

Movie S12
Time lapse movie of a TOP2A-mAID cell arrested in metaphase by proTAME-Apcin treatment and then treated with 500μM auxin.

Movie S13
Time lapse movie of a TOP2A-mAID cell arrested in metaphase by proTAME-Apcin treatment and then treated with 500μM auxin.

Movie S14
Time lapse movie of a TOP2A-mAID cell arrested in metaphase by proTAME-Apcin treatment and then treated with 500μM auxin.

Movie S15
Time lapse movie of an untreated TOP2A-mAID cell going through mitosis.

Movie S16
Time lapse movie of a TOP2A-mAID cell treated with 500μM auxin, entering mitosis and exiting prematurely without chromosome segregation.

Movie S17
Time lapse movie of a TOP2A-mAID cell treated with 500μM auxin, entering mitosis and exiting prematurely without chromosome segregation.

Movie S18
Time lapse movie of a TOP2A-mAID cell treated with 500μM auxin, entering mitosis and exiting prematurely without chromosome segregation.

Movie S19
Time lapse movie of a TOP2A-mAID cell arrested in prometaphase by nocodazole treatment.

Movie S20
Time lapse movie of a TOP2A-mAID cell arrested in prometaphase by nocodazole treatment, and then treated with 500μM auxin, eventually exiting mitosis prematurely without chromosome segregation.

Movie S21
Time lapse movie of a TOP2A-mAID cell arrested in prometaphase by nocodazole treatment, and then treated with 500μM auxin, eventually exiting mitosis prematurely without chromosome segregation.

Movie S22
Time lapse movie of a TOP2A-mAID cell arrested in prometaphase by nocodazole treatment, and then treated with 500μM auxin, eventually exiting mitosis prematurely without chromosome segregation.

Supplemental methods

Cell culture and treatments

HCT116 cells were grown in RPMI medium supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37°C in a humidified atmosphere incubator containing 5% CO_2. Unless otherwise stated, cells were treated with the following drugs at the final concentration: 500 μM auxin/Indole-3-acetic acid sodium salt (Sigma or Santa Cruz), 50ng/mL nocodazole (Sigma), 25 μM ICRF-193 (Sigma), 25 μM Etoposide (Sigma), 12.5 μM proTAME (R&D Systems), and 25 μM apcin (Sigma) Mitotic shakeoff was done by shaking plates on orbital shaker at 200 rpm for 2 mins Supernatant was collected, cells rinsed briefly with PBS and the rinse solution collected as well.

Scoring of DNA bridging

UFBs were defined as PICH positive and DAPI negative DNA bridges in anaphase B. Chromatin bridges (CBs) as DAPI positive DNA bridges in anaphase B. Cytokinesis bridges were defined as PICH or DAPI positive DNA bridges during cytokinesis. Anaphase B was defined as cells with condensed DNA masses separated completely and no ingression of the PM. Cytokinesis was defined as cells with separated global DNA masses and beginning or complete ingression of the PM. Severe entanglement was as defined as cells with separated centrosomes and microtubules but continuous DNA masses with intertwining PICH fibers.

Immunofluorescence

Cells at different conditions as described in Fig. legends were fixed in 4% PFA for 10 min, and permeabilised with 0.25% Triton X-100 for 5 min, then washed with 3% BSA in PBS and incubated with primary antibodies diluted in 3% BSA in PBS overnight at 4°C. Samples were incubated with secondary anti-rabbit or mouse Alexa Fluor 488, 568 or 594 (1:1000, Molecular probes) for 30 min and stained with DAPI (10 μg/mL). The antibodies and concentrations for immunofluorescence were TOP2A (1:100, sc-166934, Santa Cruz), PICH (1:100, 8886S Cell Signalling or 04-1540 Merck), CAP-H (1:200), CAP-H2 (1:200), SMC2 (1:200), KIF4A (1:400) (CAP-H, H2, SMC2 and KIF4A antibodies were kind gifts from Professor William C. Earnshaw, Edinburgh), Ki67 (1:400, sc-7844 Santa Cruz), PH3 (1:400, 3377 Cell Signalling), BubR1 (1:100, ab183496 Abcam), γH2AX (1:500, ab11174 Abcam), INCENP (1:200, ab36453 Abcam), β-tubulin (1:600, T4026, Sigma-Aldrich) and CENPC (1:200, sc-11286 Santa Cruz). CREST serum was used to detect centromeres (1:100, HCT-0100, Immunovision). For pre-extraction, cells were incubated with 0.25% triton-X in PBS for 2 mins before fixation with 4% PFA. Chromosome spreads were performed as previously described in Zhang et al. (2016)(7). In brief, cells were hypotonically swollen in 75mM KCl 37°C for 5 mins, and fixed with methanol: acetic acid (3:1) before dropping onto slides for immunofluorescence staining.

Microscopy, live cell imaging and image analysis

Immunofluorescence staining images were taken using a DeltaVision widefield deconvolution microscope (Applied Precision, GE Healthcare). After deconvolution, images were projected in 2D using SoftWoRx 4.1. For superresolution images were taken using an LSM 880 Airyscan Fast, upright confocal microscope (Zeiss). Images were Airyscan processed using Zen software (Zeiss). Timelapse live cell imaging was performed in 37C chamber humidity controlled chambers with 5% CO2 on motorized stages. Images were taken using either a Dragonfly spinning disc confocal microscope (Andor) (Fig. 5, 6 and S4A), a DeltaVision widefield deconvolution microscope (Applied Precision, GE Healthcare) (Fig. S8E) or a 3i spinning disc confocal imaging system equipped with

a Plan-Apochromat 63x/1.4NA differential interference contrast oil objective mounted on an inverted Zeiss Axio Observer Z1 microscope (Marianas Imaging Workstation from Intelligent Imaging and Innovations Inc. (3i), USA), equipped with a CSU-X1 spinning-disk confocal head (Yokogawa Corporation of America) (3i) (Fig. 7 and S7). For the Dragonfly microscope, fusion software (Andor) was used for imaging and deconvolution. Analysis by Imaris 3D modelling was used for H2B-EGFP live cell images to quantify chromatin volume and mean fluorescence intensity and for immunofluorescently stained images to quantify total chromatin fluorescence intensity of TOP2A, Ki67, γH2AX and PH3. In the analysis of mitotic phases, prophase was defined as the time from visible DNA condensation to nuclear envelope breakdown (NEB). The presence of the nuclear envelope could be clearly distinguished by the smoothness of the 3D chromatin mass or by increasing contrast and brightness. Prometa/metaphase was defined as time from NEB to the time point prior to the metaphase-to-anaphase transition. Anaphase was defined as the time from metaphase-to-anaphase transition to beginning ingression of the plasma membrane (PM). PM shape could be distinguished from background EGFP by increasing brightness. Telophase was defined as the time from PM ingression till complete chromatin decondensation.

Flow cytometry

Flow cytometry was performed as described in Nielsen *et al.* (2015) (76) . In brief, cells with or without auxin treatment were harvested and fixed with 70% ethanol at -20˚C overnight. Cells were washed with 1% BSA in PBS, then treated with RNase (1 mg/mL), stained with propidium iodide (50 µg/mL) at 37˚C for 30 mins Cell cycle profiles were obtained by flow sorting with FACSCalibur (Becton Dickinson) and analysed by Flowing Software (Turku Bioimaging).

Immunoblotting

Immunoblotting was performed as previously described by Zhang *et al.* (2016) (7). In brief, cell pellets were incubated with a cocktail of protease inhibitors (Roche) on ice for 30 mins Samples were then ultrasonicated on ice and centrifuged at 120,000 × *g* for 10 min at 4°C. Protein concentration was determined using the Bradford assay (Bio-rad). For each well, 40 *µ*g protein was loaded for immunoblotting. Primary antibodies were mouse monoclonal against TOPA2 (1:500, sc-166934 Santa Cruz) and mouse polyclonal against α-tubulin (1:2000, Sigma). Secondary HRP antibodies was goat anti-mouse (1:5000, Cell Signalling).

Statistics

All error bars represent standard error of the mean (SEM). Two-tailed, parametric Students t-tests for unpaired observations were performed to calculate statistical significance. All statistically analysed data sets were normal distributed. Asterisks on charts denote levels of significance. E.g. *: $p< 0.05$, **: $p< 0.01$ and ***: $p< 0.001$.