

Materials and Methods

Cell culture

Chicken DT40 (B-cell lymphoma) cells were cultured in RPMI 1640 medium supplemented with 10 % fetal bovine serum and 1% chicken serum at 39°C in 5% CO₂ in air.

Stable transfection into DT40 cells was performed as described previously (23). Transient transfection into DT40 cells was performed using a Neon transfection system from ThermoFisher Scientific. 2-4 million cells suspended in 100 µl 1X buffer R were mixed with plasmid DNA (4-10 µg), then electroporated at setting 5.

Doxycycline (BD) dissolved in water (1mg/ml) was added to a final concentration of 0.5 µg/ml. For G₂ arrest, 1NM-PP1 dissolved in DMSO (10 mM) was added to cultures at a final concentration of 2 µM. Degradation of AID-containing proteins was induced by addition of a 50 mM solution of Indole-3-acetic acid (auxin, Fluka) dissolved in ethanol to a final concentration of 125 µM. To prevent cells from entering anaphase, Nocodazole (Sigma-Aldrich) dissolved in DMSO at 1 mg/ml was added to some cultures to a final concentration of 0.5 µg/ml (see details below, **Table S1**).

Cell lines

A summary of the cell lines described below can be found in **Table S3**.

SMC2-AID-GFP cells: SMC2-AID-GFP cells were derived from a previously described SMC2 conditional (doxycycline induced) knockout cell line (54). Plasmids coding SMC2-mAID-GFP and OsTIR1 were randomly integrated into the DT40 genome. A 3.8 kb fragment of the endogenous GgSMC2 promoter (54) drove expression of the GgSMC2 cDNA fused with a minimal AID (mAID) tag (AtIAA1765-132) and GFP tag (SMC2-mAID-GFP) at the C-terminus. A CMV promoter drove the expression of plant-specific F-box protein OsTIR1 linked to a MmDHFR cDNA by a T2A peptide (synthesized at ThermoFisher Scientific and cloned into pCDNA3). 10 µM Methotrexate (MTX) was used to select cells expressing high levels of MmDHFR and OsTIR1. The resultant cell lines (named SMC2-AID-GFP) were cultured continuously in the presence of doxycycline (0.5 µg/ml) to suppress the expression of non-tagged GgSMC2 protein.

CAP-H-AID-GFP cells: Plasmids encoding CAP-H-mAID-GFP and plant specific F-box protein OsTIR1 were randomly integrated into the genome of a CAP-H conditional (doxycycline induced) knockout cell line (61). A CMV promoter drove the expression of the GgCAP-H-mAID-GFP. A CMV promoter drove the expression of OsTIR1 linked to a MmDHFR cDNA by a T2A peptide (synthesized at ThermoFisher Scientific and cloned into pCDNA3). 10 µM Methotrexate (MTX) was used to select cells expressing high levels of MmDHFR and OsTIR1. The resultant CAP-H-AID-GFP cell lines were cultured continuously in the presence of doxycycline (0.5 µg/ml) to suppress the expression of non-tagged GgCAP-H protein.

CAP-H2-AID-GFP cells: CAP-H2-AID-GFP cells were constructed starting with wild type DT40 cells. A codon-optimized GgCAP-H2 cDNA was synthesized (ThermoFisher Scientific) and cloned into pAID2.3C so that CAP-H2 was tagged with mAID and GFP at its C-terminus. A CMV promoter drove the co-expression of OsTIR1 and CAP-H2-mAID-GFP, which are linked by a P2A peptide in the pAID2.3C-CAP-H2 plasmid. The pAID2.3C-CAP-H2 plasmid was randomly integrated into the DT40 genome. The endogenous GgCAP-H2 gene was then

inactivated by transient transfection of plasmids encoding hCas9 cDNA and GgCapH2 guide RNA (ThermoFisher Scientific). The target sequence of guide RNA was CCTATACTCGCTGGTCTACCAGG.

CDK1as cells: A CMV promoter drove the expression of a XI₁Cdk1as cDNA (22) linked to a puromycin or zeocin resistance gene via a T2A peptide. This construct was integrated at random in the genome of the desired target cell line. The endogenous GgCdk1 gene was then inactivated by transient transfection of plasmids encoding hCas9 cDNA (Addgene #41815) and GgCdk1 guideRNA (based on Addgene #41824). The target sequence of the guide RNA was AAAATACGTCTAGAAAGTG. SMC2-AID-GFP cells, CAP-H-AID-GFP cells and CAP-H2-AID-GFP cells were individually converted into CDK1as cell lines by this method. Wild type CDK1as cells were a gift of Helfrid Hochegger (University of Sussex) (22).

Detailed protocols to establish DT40-AID/CDK1as cell lines are available upon request to Dr. Kumiko Samejima.

Time course experiments

Typically $1-3 \times 10^7$ CDK1as wild type cells/sample were treated with 1NM-PP1 (final concentration: 2 μ M) for 10 h, after which over 95% were in G₂ (**Table S1**). G₂-arrested cells were collected prior to 1NM-PP1-washout. To release cells from the G₂ arrest, 1NM-PP1 was washed out by collecting the cells by centrifugation (1300 rpm, 2-5 min - depending on the volume of cultured cells - at RT) and suspending the cells in fresh media (10-20 ml). Cells were washed twice over a period of 10-20 minutes (this varied depending on the number of cultures being processed simultaneously). These cells were suspended in fresh media and divided into flasks (one flask for each time point, typically $1-3 \times 10^7$ cells per flask) and returned to the incubator (counted as t = 0 minutes). At each time point (0, 2.5, 5, 7.5, 10, 30, 45, 60, 75 minutes), a flask was removed from the incubator and cells with media were transferred to a 50 ml Falcon tube, which was topped up with fresh media to 45 ml.

Three ml of 16% formaldehyde solution was added to the 50 ml tube to obtain a final concentration of 1%. The content was quickly mixed by inverting the tube twice and left at RT for 10 min for cross-linking. 2.5 ml of 2.5 M glycine was then added to the tube to stop cross-linking. The content was quickly mixed by inverting the tube twice and left at RT for 5 min, and then on ice for at least 15 min. Subsequently, cells were collected by centrifugation (2000 rpm, 10 min), and the cell pellet was frozen on dry ice and then stored at -80°C.

Modifications to the time course experiment

Auxin-induced protein depletion

Where required, auxin was added after 10 h 1NM-PP1 treatment and cells (SMC2-mAID-GFP cells, CAP-H-mAID-GFP cells, CAP-H2-mAID-GFP cells) were further incubated for 3 hours to deplete AID-tagged proteins. Cells were then released from the G₂ arrest as described above, except that auxin was kept in the 1NM-PP1 washout media and in the media during all further incubations. Note that the G₂ cells for CAP-H-mAID-GFP and CAP-H2-mAID-GFP are either GFP-negative (degron active) or GFP-positive (degron not activated), depending on auxin addition (indicated in **Tables S1, S2**).

Cell sorting to isolate cells depleted of target proteins

As a control, in some cases (indicated as “sorted”) SMC2-AID-GFP cells were FACS-sorted after cross-linking to exclude residual GFP-positive cells, which had not responded fully

to auxin. Some wild type cells were also sorted. Sorting did not alter the Hi-C data as compared to unsorted cells, indicating that degran-mediated protein degradation was very efficient (**Fig. 4A, Fig.S8 (set #4) and Fig.S20A (set #1)**).

Nocodazole addition to prevent anaphase entry

Nocodazole was added for 30 minutes prior to 1NM-PP1 washout only for the $t \geq 30$ minute time points (Replicate 1 of WT) or for $t \geq 60$ minutes samples (all other time courses; **Table S1**). Nocodazole was present in 1NM-PP1 washout media and in the media during further incubations. No nocodazole was added for cells collected at any of the other time points.

Microscopy on fixed cells

Fixation of chromosomes from mitotic cells

Cells in media (200 μ l) were directly fixed in 1 ml of ice-cold Methanol/Acetic acid solution (3:1) for > 30 minutes. Fixed Cells were centrifuged at 2000 g for 1 min, applied on slides, air dried, and stained with DAPI in Vectashield antifade mounting medium (H-1200, Vectashield). Images were taken by DeltaVision microscopy.

Lamin B1 and Histone H3 Phos-Ser10 staining

Cells were fixed in pre-warmed 4% Formaldehyde/PBS for 10 min and permeabilized with 0.15% triton for 5 minutes. The following steps were then performed at RT. Cells were blocked with 5% BSA/PBS for 30 minutes. Anti-lamin B1 mouse monoclonal antibody (1:100, Zymed) and Anti-histone H3 phospho-Ser10 rabbit polyclonal antibody (1:500, Millipore) diluted in the blocking buffer were applied to the cells for 1 hour. Cells were washed with PBS 3x for 5 minutes. Secondary antibodies (Molecular Probes, ThermoFisher Scientific) anti-mouse and anti-rabbit coupled with Alexa Fluor 488 and 594, respectively and diluted 1:500 and 1:1000 in the blocking buffer, were applied to the cells for 30 minutes. Cells were washed with PBS three times for 5 minutes. DNA was stained with Hoechst 33452 and mounted with Prolong diamond (Molecular Probes, ThermoFisher Scientific). Images were taken by DeltaVision microscopy.

DeltaVision microscopy

3D datasets were acquired using a cooled CCD camera (CoolSNAP HQ; Photometrics) on a wide-field microscope (DeltaVision Spectris; Applied Precision) with a 100 \times NA 1.4 Plan Apochromat lens. The datasets were deconvolved with softWoRx (Applied Precision), converted to Quick Projections in softWoRx, exported as TIFF files, and imported into Adobe Photoshop for final presentation.

Chromosome length measurements

Samples were prepared essentially by the same method used for Hi-C samples. WT/CDK1as cells were treated with 1NMPP1 for 10 hours. After 1NM-PP1 washout, cells were plated on poly-L-lysine coated coverslips and incubated with the corresponding media for either 30 or 60 minutes. Then the cells were rinsed with PBS and fixed with 1% formaldehyde in PBS. Note: Nocodazole was added to the culture 30 minutes prior to 1NM-PP1 washout and kept in the culture thereafter. MG132 was added to the culture at $t = 30$ minutes after release and cells were fixed 30 minutes after that. Ten or more pictures were taken for each condition using the Zeiss Airyscan microscope and analysed using IMARIS.

We measured the length and width of the longest chromosomes in DT40 cells fixed with 1% formaldehyde solution as used in the preparation of Hi-C specimens. It was not possible to measure all chromosomes in cells fixed under these Hi-C conditions, so by choosing the longest chromosome in each cell, we could be confident that we were measuring and comparing the same chromosome (chromosome 1). In order to identify chromosome 1 in each mitotic cell, we measured the length of several candidate chromosomes in each cell using IMARIS to track that chromosome through the three-dimensional data set. The measurement reported was for the longest chromosome. In order to calculate the chromosome width in each cell, avoiding centromeres and telomeres, we measured the width of the longest chromosome at 5 different positions.

Visualization of a repeating structure on chicken mitotic chromosomes

Asynchronously growing WT/CDK1as, SMC2-mAID-GFP/CDK1as, CAP-H-mAID-GFP/CDK1as, CAP-H2-mAID-GFP/CDK1as, CAP-H-GFP/CDK1as cells were treated with colcemid (100 ng/ml) for 2.5 hours. Cells were treated with 75 mM KCl for 5 minutes then fixed with ice-cold Methanol/Acetic acid. Cells were spread on coverslips, mounted with vectashield containing DAPI (Vectashield). 3D datasets were acquired using a cooled CCD camera (CoolSNAP HQ; Photometrics) on a wide-field microscope (DeltaVision Spectris; Applied Precision) with a 100× NA 1.4 Plan Apochromat lens. The datasets were deconvolved with softWoRx (Applied Precision), converted to Quick Projections in softWoRx, exported as TIFF files, and imported into Adobe Photoshop for final presentation.

This hypotonic treatment and spreading procedure caused a reorganization of the chromosome structure that appears to reveal the underlying helical organization of the chromosome scaffold. This helical organization is not seen in minimally perturbed chromosomes fixed with formaldehyde, as used for our Hi-C studies, and where the chromatin distribution appears to be uniform. In the chromosome spreads, we could unambiguously identify chromosomes 1 and 2 and could count the number of gyres, and therefore calculate the amount of DNA per gyre. This number is almost certainly an overestimate, because we did not see gyres at centromeres or telomeres. For Chromosome 1 we counted 10.5 ± 3.8 gyres, yielding 18.7 MB/gyre (n = 19 chromosomes). For chromosome 2 we counted 8.5 ± 2.4 gyres, yielding 17.7 Mb/gyre (n = 17 chromosomes). We note that these chromosomes had been arrested in mitosis for up to 2.5 hours with a colcemid block, so they would be expected to be more compacted than the 60 minute time point analyzed by Hi-C.

Helical distribution of GFP signal in single sister chromatids

SMC2-mAID-GFP/CDK1as, CAP-H-mAID-GFP/CDK1as, CAP-H2-mAID-GFP/CDK1as, CAP-H-GFP/CDK1as cells were treated with 2 μ M 1NM-PP1 for 4h without auxin. Cells were plated on poly-lysine coated coverslips immediately after 1NM-PP1 washout and incubated for 40 minutes. The cells were then fixed with 4% Formaldehyde/PBS for 10 minutes, permeabilized with 0.15 % Triton/PBS for 5 minutes, stained with Hoechst solution, mounted with Prolong Diamond antifadant and imaged using the Airyscan module on a Zeiss LSM 880 confocal, using a x100 alpha Plan-Apochromat objective. Hoechst 33342 was detected using a 405 nm Diode laser and a 420-480 nm bandpass emission filter, Alexa488 was detected using the 488 nm line of an Argon laser and a 495-550 nm bandpass emission filter, Step size for Z stacks was set to 0.145 μ m. 3D datasets were visualized and analyzed using Imaris V8.4 (Bitplane, Oxford Instruments, Oxfordshire, UK). Images show single sections from three-dimensional data stacks

and are representative of two biological replicate experiments. Cells had been arrested in mitosis for up to 2.5 hours with a colcemid block, so they would be expected to be more compacted than the 60 minute time point analyzed by Hi-C.

Immunoblot analysis

Typically $0.5-1 \times 10^6$ cells were loaded in each lane of a 7.5 % SDS-PAGE gel. Proteins were transferred to PVDF membranes at 200 mA for 2.5 hours in transfer buffer containing 119 mM Tris (pH 8.5), 40 mM glycine, 0.1% SDS, and 20% Methanol. Membranes were blocked with 5% skimmed milk in PBS (154 mM NaCl, 1.5 mM KH_2PO_4 , 5.5 mM Na_2HPO_4) for 30 minutes, then incubated with relevant primary antibodies recognizing α -tubulin (1:2000, DM1A, Sigma-Aldrich; as a loading control), SMC2 (1:1000) (17), or GFP (1:1000) (gift from Simona Saccani, University of Edinburgh) with 1% skimmed milk in PBS/tween20 (1%) for 3 hours up to overnight. Membranes were washed three times for 5 minutes with PBS/tween20 (1%). Membranes were incubated with IRDye-labeled secondary antibodies diluted in 1% skimmed milk in PBS/tween20 (1%) (1:10,000) for 45 minutes followed by three 5 minute washes with PBS/tween20 (1%). Membranes were kept in PBS until fluorescence intensities were determined using a CCD scanner (Odyssey; LI-COR Biosciences) according to the manufacturer's instructions.

Flow cytometry analysis

DNA content: Cells were suspended overnight in ice-cold 70% ethanol. The next morning, cells were rinsed with PBS then re-suspended in PBS containing 100 $\mu\text{g}/\text{ml}$ RNase A and 5 $\mu\text{g}/\text{ml}$ propidium iodide. Samples were then analyzed using a FACSCalibur flow cytometer following the manufacturer's instructions. Data was analyzed using FlowJo V10.3. WT cells were gated for viability based on forward and side scatter (FSC/SSC), from which single cells were selected based on FSC height (H) and width (W). These gates were applied to all samples and DNA content was plotted as a histogram of FSC-H.

Chromatin Enrichment for Proteomics (ChEP)

Synchronized Cdk1as cells were crosslinked with 1% formaldehyde at specified time points in mitosis and chromatin was extracted from the crosslinked cells by the ChEP protocol (58). Briefly, 5×10^7 cells were fixed for 10 minutes by addition of formaldehyde to the cell culture. Formaldehyde was quenched by 125 mM glycine for 5 minutes. Crosslinked cells were washed with TBS (20 mM Tris pH 7.4, 150 mM NaCl) and frozen in liquid nitrogen. Cells were lysed on ice in Lysis buffer (25 mM Tris pH 7.4, 0.1% Triton X-100, 85 mM KCl, protease inhibitors: 1 mM PMSF, 1 $\mu\text{g}/\text{ml}$ each of chymostatin, leupeptin, antipain, pepstatin A). Cells were washed with Lysis buffer and the pellet was resuspended in 300 μl SDS buffer (50 mM Tris pH 7.4, 10 mM EDTA, 4% SDS, protease inhibitors as above) for 10 minutes at room temperature. Next, 3 volumes of Urea buffer (10 mM Tris pH 7.4, 1 mM EDTA, 8M Urea) were added and samples centrifuged at 16,000 $\times g$ for 30 minutes. The pellet was again resuspended in 1 volume of SDS buffer, then 3 volumes of Urea buffer was added before centrifugation again. The pellet was washed with SDS buffer before resuspension in ice cold storage buffer (10 mM Tris pH 7.4, 1 mM EDTA, 25 mM NaCl, 10% glycerol). Chromatin DNA was sheared by sonication. The solubilized chromatin was mixed with 3x SDS sample buffer (6% SDS, 150 mM Tris pH 6.8, 30% glycerol) and heated at 95°C for 30 minutes to reverse the crosslinks. 10% of the sample was electrophoresed in a NuPAGE Bis-Tris gel (NP0335, Invitrogen). The de-crosslinked

chromatin proteins were in-gel digested with 2µg trypsin overnight (> 16hours). The tryptic peptides were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Data was analyzed with MaxQuant 1.5.7.4 (85). The abundance of proteins was estimated using the iBAQ algorithm (86), and normalized by the copy number of histone H4 (87).

ChIP-seq data re-analysis.

We used the publicly available CAPH ChIP-seq dataset obtained in DT40 cells (GSE45552). We processed the raw sequences following the steps of the ChIP-seq pipeline of the ENCODE consortium (88), available at <https://github.com/ENCODE-DCC/chip-seq-pipeline/>.

Hi-C protocol

Chromosome conformation capture was performed as described previously (89). Briefly, 10-20x10⁶ cells were cross-linked in 1% formaldehyde for 10 minutes and quenched in 125 mM glycine. Cells were snap-frozen and stored at -80°C before cell lysis.

Cells were lysed for 15 minutes in ice cold lysis buffer (10 mM Tris-HCl pH8.0, 10 mM NaCl, 0.2% Igepal CA-630) in the presence of Halt protease inhibitors (Thermo Fisher, 78429) and cells were disrupted by homogenization with pestle A for 2x 30 strokes. Chromatin was solubilized in 0.1% SDS at 65°C for 10 minutes, quenched by 1% Triton X-100 (Sigma, 93443) and chromatin was digested with 400 units of HindIII (NEB, R0104) overnight at 37°C.

Fill-in of digested overhangs by DNA polymerase I, large Klenow fragment (NEB, M0210) in the presence of 250 nM biotin-14-dCTP (Life Technologies, 19518-018) for a minimum of 90 minutes was performed prior to 1% SDS based enzyme inactivation and dilute ligation with T4 DNA ligase (Life Technologies, 15224) for 4 hours at 16°C. Cross-links of ligated chromatin were reversed overnight by proteinase K (Life Technologies, 25530-031) incubation at 65°C. DNA was isolated with 1:1 phenol:chloroform, followed by 30 minutes of RNase A incubation.

Biotin was removed from unligated ends by incubation with 15 units of T4 DNA polymerase in the presence of 25 nM dATP/dGTP. DNA was sheared using an E220 evolution sonicator (Covaris) and size selected with Agencourt AMPure® XP (Beckman Coulter) to 150-350 bps. After end repair in a mixture of T4 polynucleotide kinase (25 units; NEB, M0201), T4 DNA polymerase (7.5 units; NEB, M0203L) and DNA polymerase I, large (Klenow) fragment (2.5 units; NEB, M0210) at 20°C for 30 minutes, dATP was added to blunted ends using 15 units of polymerase I, large fragment (Klenow 3' → 5' exo-) (NEB, M0212L) at 37°C for 30 minutes.

Biotinylated DNA was collected by incubation in the presence of 10 µl of streptavidin coated myOne C1 beads (Life technologies, 650.01) and Illumina paired-end adapters were added by ligation with T4 DNA ligase (Life technologies) for 2 hours at room temperature. A PCR titration (primers PE1.0 and PE2.0) was performed prior to a production PCR to determine the minimal number of PCR cycles needed to generate a Hi-C library. Primers were separated from the library using Ampure size selection prior to 50 bp paired-end sequencing on an Illumina HiSeq sequencer (Life Technologies).

Hi-C data analysis

Briefly, we processed Hi-C data as described in (36), with multiple modifications, including using the galGal5 genome assembly. All scripts mentioned below to be from the Dekker Lab c-world pipeline are available at a GitHub repository (90). cMapping at

<https://github.com/dekkerlab/cMapping>; iterative correction (balancing) at <https://github.com/dekkerlab/balance> and analysis scripts at <https://github.com/dekkerlab/cworld-dekker>. The Mirny Lab software, including the *cooler* library for storage and analysis of Hi-C data is available at <https://github.com/mirnylab>.

Mapping sequenced Hi-C reads

Fastq files were mapped and binned using the c-world mapping pipeline. Briefly, 50 bp paired end reads obtained as fastq files were truncated to 25 bp starting at the 5' end. Then, they were iteratively mapped (33) to the Red Jungle Fowl laboratory line chicken genome (UCSC Genome Browser assembly ID: galGal5, Dec. 2015, Accession ID: GCF_000002315.4; NCBI genome/111 (*Gallus gallus*)(91). Uniquely mapped, paired reads were kept and assigned to a HindIII restriction fragment assigned through its 5' position. Mapped reads were filtered for same fragment ends and uniqueness, excluding PCR duplicates (defined as sequence matches with the exact same start and end).

Hi-C based karyotyping

Genome wide interaction matrices from Hi-C were binned at 1 Megabase resolution. For each bin, we summed up the interactions of this bin with all other bins. Chromosome normalization was obtained by dividing these sums with the total number of interactions per chromosome, corrected for the chromosome size (in Megabases). Genome wide normalization was obtained by dividing each bin sum by the genome wide number of counts (matrix total). The difference between sums normalized by chromosome and matrix indicates copy number alterations and was displayed for each chromosome on a log₂ scale.

Contact probability (P(s)) curves

We used the mapped reads to calculate the functions of contact frequency P(s) vs genomic separation s. We split all genomic distances between 1 kb and 1Gb into bins of exponentially increasing widths, such that the upper edge of every bin was 1.12 larger than the lower edge. For every such separation bin, we found the number of observed cis-interactions within this range of separations and divided it by the number of all loci pairs, separated by such distances in cis.

Binning and balancing of Hi-C data

Unique valid pair reads were binned to 10kb, 50 and 100 kb bins, using cooler package, and noisy or low signal bins were excluded prior to balancing using the MADmax filter: we removed all bins, whose coverage was 7 genome-wide median deviations below the median bin coverage. Matrices were balanced by iterative correction (IC), equalizing the sum of every row/column to 1.0 (33), using cooler (<https://github.com/mirnylab/cooler>).

Compartment analysis

Compartments were quantified using principal component analysis on 100 kb binned data using the matrix2compartment perl script in our c-world pipeline. The largest eigenvector (eig1) typically represents the compartment profile(3, 32, 92). When compartment signals were low or absent, we checked consecutive eigenvectors to confirm loss of compartments. Per convention A/B-compartments were assigned by gene density, so that the A-compartment was more gene-dense than the B-compartment.

In order to measure the strength of compartmentalization, we used the observed/expected Hi-C maps, which we calculated from 100 kb iteratively corrected interaction maps of cis-interactions by dividing each diagonal of a matrix by its chromosome-wide average value. In each observed/expected map, we rearranged the rows and the columns in the order of increasing eigenvector value. Finally, we aggregated the rows and the columns of the resulting matrix into 30 equally sized aggregated bins, thus obtaining a compartmentalization plot (“saddle plot”).

Insulation analysis (TADs)

TAD calling was performed on 50 kb binned data exactly as described previously (32). We first determined the optimal insulation square for TAD calling with our c-world pipeline perl script `matrix2insulationRange`. We used a range of insulation squares from 50 kbp (`istart` variable) to 1 Mb (`iend` variable) with a 50 kb (bin size) step (`istep` variable) to sweep through the insulation topology. From this sweep, we determined to use a 250x250 kb sliding square (`is` = 250000) along the matrix diagonal without smoothing (`ss` = 50000) for capturing the aggregate TAD signal for *galGal5* (**Fig. S5**). For 40 kb binned HeLaS3 (ATCC CCL2.2) Hi-C data, we detected TADs using a 520x520 kb sliding square (`is` = 520000) without smoothing (`ss` = 40000). These settings were used as input variables for insulation square analysis with the `matrix2insulation` perl script from our c-world pipeline. Briefly, the inter quartile (IQR) mean signal within the square (`im` = `iqrMean`) was assigned to the diagonal bin and this process was repeated for all bins. The insulation score was then normalized relative to average score of the insulation scores across each chromosome by calculating the log₂ ratio of each bin’s insulation score and the mean of all insulation scores, as described previously (32). Minima along the normalized insulation score vector represent loci of high local insulation and are interpreted as TAD boundaries. For aggregate insulation score pileups, bed files with TAD boundary positions from G2 arrested cells were used as input for each mutant cell line separately (`ebf` variable). TAD boundary pileups were generated for 50 kb steps (`mindist` = 50000) covering 500 kb upstream and 500 kb downstream from TAD boundaries (`ezs` = 500000) with a maximum interaction distance of 500 kb (`maxdist` = 500000) using our `elementPileUp` c-world perl script.

Coarse-grained model of contact probability decay in mitotic chromosomes

In the coarse-grained model loops are regularly placed along the axis of the chromosome (z-axis) with a spacing Δ_z . Each loop is represented by a cylindrical wedge, where loci that belong to this loop are distributed normally along the z directions, with mean at the base of the loop and the standard deviation (σ), and normally in the angular position with the mean at the orientation of the loop and the standard deviation ϕ . Orientation of each loop depends on the model and can be uniformly random (*Randomly oriented uncorrelated loops*), correlated with its neighboring loops, forming an angular random walk (*Loops with correlated orientation*), or form a random walk while having a preferred orientation (*Loops with spiral staircase orientation*).

Below, we derive an approximate expression for the probability of a contact between two loci $P_c(s)$, separated by genomic distance s , in a chromosome compacted into an array of consecutive loops. Naturally, this expression has two regimes: (a) the intra-loop regime at shorter genomic separations, where the two loci are located on the same loop, and (b) the between-loop regime at larger separations, when loci are located on different loops. If the lengths of loops are random and distributed exponentially, the transition between the regimes occurs around the genomic separation comparable to the average loop length l_{avg} :

$$P_c(s) = e^{-s/l_{\text{avg}}} P_c^{\text{in}}(s) + (1 - e^{-s/l_{\text{avg}}}) P_c^{\text{between}}(s) \quad (1)$$

Contacts within loops

The simplest model of individual loop conformations is a random walk in 3D. In this case, the contact probability decays as a power-law with a -3/2 exponent, assuming poor solvent conditions in the melt of loops, and neglecting correction for the closed loop:

$$P_c^{\text{in}}(s) = C_{\text{in}} s^{-1.5} \quad (2)$$

This scaling is consistent with $P_c(s)$ observed even at larger distances during interphase in very large oocyte nuclei (93). Below, we will not attempt to improve this simple model, and instead focus on long-distance behavior of $P_c(s)$, which reflects the mutual arrangement of loops.

Contacts between loops

By definition, a contact between two loci occurs when they have similar coordinates, either in Cartesian or cylindrical coordinates:

$$P_c^{\text{between}}(s) = C_{\text{between}} P(x_i = x_j, y_i = y_j, z_i = z_j) = C_{\text{between}} P(r_i = r_j, \theta_i = \theta_j, z_i = z_j)$$

For two loci located on different loops, we can simplify this expression drastically by assuming that the probabilities of overlap at each coordinate are independent of each other:

$$P_c^{between}(s) = C_{between} P(r_i = r_j) P(\theta_i = \theta_j) P(z_i = z_j) \quad (3)$$

Randomly oriented uncorrelated loops

In order to estimate the probability of contact between loci on different loops, we have to make extra assumptions on the mutual orientations of these loops. In the simplest case, each loop is randomly oriented and the orientations of consecutive loops are not correlated with each other. In this case, both $P_c(r_i = r_j)$ and $P_c(\theta_i = \theta_j)$ are independent of the separation between the loops and thus integrate to a constant, leaving:

$$P_c^{uncorr}(s) = C_{between} P(z_i = z_j) = C_{between} P_c^z(s)$$

We can calculate this remaining expression easily if we assume that the loci within each loop are normally distributed along the z-axis, each around the base of its loop:

$$P_c^z(s) = C \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} N(z_i, \bar{z}_i, \sigma) N(z_j, \bar{z}_j, \sigma) \delta(z_i - z_j) dz_i dz_j$$

In this expression, $N(x, \text{mean}, \text{s.d.})$ is the PDF of the normal distribution, i and j are the genomic coordinates of the two loci, z_i and z_j are the axial coordinates in the 3D genomic structure, \bar{z}_i and \bar{z}_j are the expected axial coordinates of the i -th and j -th loci (which, by our assumption, are equal to the axial coordinates of their corresponding loop bases), σ is the measure of the spread of loop along the axis of the chromosome, i.e. the high of each loop, and $\delta(z_i - z_j)$ is the delta function that limits the integral only to the conformations where the particles have the same axial coordinate. Taking this integral gives us:

$$P_c^z(s) = C N(\bar{z}_i - \bar{z}_j, 0, \sqrt{2}\sigma)$$

Finally, if the loops are regularly places along the axis of the chromosome with a spacing Δ_z , than the expected axial distance between the bases of two loops is equal the expected number of loops between these two loci, multiplied by Δ_z :

$$P_c^z(s, l_{avg}, \Delta_z, \sigma)(s | l_{avg}, \Delta_z, \sigma) = C N\left(\Delta_z \frac{s}{l_{avg}}, 0, \sqrt{2}\sigma\right) = C' N\left(\frac{s}{l_{avg}}, 0, \frac{\sqrt{2}\sigma}{\Delta_z}\right) \quad (4)$$

$P_c^z(s)$ describes the decay of contact probability due to the decreasing axial overlap of more distant loops and, as illustrated in **Fig. 2B**, produces a characteristic drop-off in log-log representation of $P_c(s)$. The drop takes place at when the number of loop s/l_{avr} exceeds $\sim 2\sqrt{2}\sigma / \Delta_z$ the ratio of the loop high and the separation between loop.

For example, at prophase a drop at 4.5Mb for loops of the average size $l_{avr} = 80\text{Kb}$ requires 56 loops per layer. Hence $2\sqrt{2}\sigma / \Delta_z = 56$; then for $\sigma = 200\text{nm}$ of loop high, one gets $\Delta_z = 7.5\text{nm}$ separation between neighboring loops. This is a tight packing at the scaffold, but feasible with

10nm fiber emanating from condensins, possibly requiring the scaffold to wiggle inside the chromosome. For prometaphase, a drop at 10Mb and 80Kb loops leads to 125 loops per layer, and the spacing of 3nm, which is smaller than protein domains of condensin, necessitating nested loops and considerable winding of the scaffold inside the chromosome.

Loops with correlated orientation

$P_c^z(s)$ alone does not fully explain the experimentally observed $P_c(s)$ curves: it explains the origin of a drop in contact probability at longer distances, but does not explain the origin of a gradual decay before the drop. Equation (3) then suggests that this extra gradual decay might be due to the angular overlap $P(\theta_i = \theta_j)$. Specifically, if the angular orientations of consecutive loops are somehow correlated, forming the angular (1D) random walk, this would increase the frequency of angular overlaps for loci in adjacent loops and make it smaller for loci that are contained in distant loops.

$$P_c^{corr}(s) = C_{between} P(z_i = z_j) P(\theta_i = \theta_j) = C_{between} P_c^z(s) P_c^{ang.corr}(s) \quad (5)$$

We can derive the frequency of angular overlaps explicitly:

$$P_c^{ang.corr}(s) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} N(\theta_i, 0, \phi) N(\theta_j, \bar{\theta}_j, \phi) N(\bar{\theta}_j, 0, \gamma \sqrt{s/l_{avg}}) \sum_{k=-\infty}^{\infty} \delta(\theta_i - \theta_j + 2\pi k) d\theta_i d\theta_j d\bar{\theta}_j$$

Here, θ_i and θ_j are the angular coordinates of the two loci, which are distributed normally around the mean angular orientation of their loops (0 and $\bar{\theta}_j$, correspondingly) with the standard deviation ϕ . The correlations in the orientations of adjacent loops lead to the fact that the mean angular orientation of the loop with the j-th locus, $\bar{\theta}_j$, is normally distributed itself around the mean of 0 (which we picked to be the angular orientation of the loop with the i-th locus, without a loss of generality) and the standard deviation of $\gamma \sqrt{s/l_{avg}}$. The latter is calculated from the assumption the angles of two consecutive loops on average differ by γ ; thus, between the i-th and j-th locus there are s/l_{avg} loops, each taking a random step of γ , thus producing a 1D random walk with the standard deviation of $\gamma \sqrt{s/l_{avg}}$. Taking all integrals gives us:

$$P_c^{ang.corr}(s) = \int_{-\infty}^{\infty} \sum_{k=-\infty}^{\infty} N(\bar{\theta}_j + 2\pi k, 0, \sqrt{2}\phi) N(\bar{\theta}_j, 0, \gamma \sqrt{s/l_{avg}}) d\bar{\theta}_j$$

$$P_c^{ang.corr}(s, l_{avg}, \phi, \gamma)(s | l_{avg}, \phi, \gamma) = C \left(\gamma^2 \frac{s}{l_{avg}} + 2\phi^2 \right)^{-0.5} \quad (6)$$

$P_c^{\text{ang,corr}}$ describes the decay of contact probability with distance due to gradually decaying correlations of more distant loops. For a range of parameters, it can produce a characteristic -0.5 power-law decay of $P_c(s)$, before the random walk fills the whole 2π . In combination with the drop-off decay due to decreasing axial co-localization, P_c^z , these two terms produce a prophase-like three-regime decay of contact probability, as illustrated in **Fig. 2B**.

Interestingly, when the angular random walk fills a full circle the angular part of the contact probability becomes constant and independent of s , i.e. $P_c^{\text{ang,corr}}(s) \sim s^{-0.5}$, while the angular displacement $\gamma\sqrt{s/l_{\text{avg}}} < 2\pi$. For more loops per layer $P_c(s) = \text{const}$ is expected to appear. Since we don't see this regime in the data, it means that $\sqrt{s/l_{\text{avg}}} < 2\pi$ for s within a layer. Hence for a drop at 10Mb and 80Kb loops, we get 125 loops per layer and $\gamma < 2\pi/11$, so the angle between loops should be below or around 30 degrees.

Loops with spiral staircase orientation

Finally, we build a model that could explain the non-monotonic dependency of contact probability with separation, observed in Hi-C of prometaphase chromosomes. This non-monotonicity could be explained by spiralization of chromosomes, previously observed with an electron microscope. In our model, we can describe this spiralization as periodicity of the angular orientations of loops. In order to agree with the experimental data, we have, however, to combine this periodicity at larger scales with random correlated angular loop orientations at shorter separations.

These properties could all be achieved when the angular orientations of loops follow a 1D random walk under the constraint that each loop cannot deviate too much from its preferred orientation, set by the spiral, i.e., linearly proportional to its index. The probability of an angular overlap between loci on two loops can be calculated as the probability of a return of such 1D random walk to its initial position after s/l_{avg} steps. These random walks are known under the name of the Ornstein-Uhlenbeck (OU) processes, and their properties, including the return probability, have been studied in the theory of stochastic processes (94):

$$P_c^{\text{OU}}(s) = C_{\text{between}} P(z_i = z_j) P(\theta_i = \theta_j) = C_{\text{between}} P_c^z(s) P_c^{\text{ang,OU}}(s)$$

$$P_c^{\text{ang,OU}}(s) = \sum_{k=-\infty}^{\infty} N(2\pi k, \Delta_\theta s/l_{\text{avg}}, \sqrt{2\phi^2 + \gamma^2 n_{\text{return}}(1 - e^{s/l_{\text{avg}}/n_{\text{return}}})})$$

Here, Δ_θ is the systematic shift of the angle per loop (which produces the spiral phenotype). n_{return} is the parameter of the Ornstein-Uhlenbeck process characterizing the variability of the angular loop orientations around their optimal orientation: the loops deviate on average by $\sqrt{n_{\text{return}}}\gamma$ from their optimal angular orientation and the n_{return} consecutive loops have correlated orientations. We also modified the standard formula for the return probability of the trending OU process to account for the fact that, in angular coordinates, two points overlap when they have the same angle modulo 2π .

Bridging the in-loop and between-loop regimes

Note that both expressions of P_c^{in} and P_c^{between} contain arbitrarily defined coefficients C_{in} and C_{between} . In order to obtain a unified expression that describes the contact probability at all length scales, we need adjust these coefficients, such that $P_c^{\text{in}}(s)$ and P_c^{between} could be directly compared to each other. Both of these coefficients are arbitrary and their ratio only affects the narrow transitional region at $s \sim 1-3 l_{\text{avg}}$. In the calculations presented in the paper, we calculate these coefficients by matching the two curves at the separation of two average loop sizes:

$$P_c^{\text{in}}\left(\frac{2s}{l_{\text{avg}}}\right) = P_c^{\text{between}}\left(\frac{2s}{l_{\text{avg}}}\right)$$

Polymer models of mitotic chromosomes

The design of Langevin molecular dynamics simulations of chromosomes

In order to test the proposed architectures of prophase and prometaphase chromosomes against our Hi-C data, we simulate polymer models of chromosomes using Langevin molecular dynamics. The overall design of these simulations is similar to that in (8, 24), with a few major differences. We model 10nm ‘beads-on-string’ chromatin fiber of nucleosomes as a chain of 10nm particles, each representing one nucleosome with 200bp of DNA. These particles are connected by springs with a using a harmonic potential with an equilibrium length of 10nm and the stiffness coefficient of 1 kbT/nm². We simulated repulsion between spatially overlapping nucleosomes using the following force potential:

$$U = 5 \left(-1 + \left(\frac{0.105 \cdot r}{\sqrt{6/7}} \right)^{12} \cdot \left(\left(\frac{0.105 \cdot r}{\sqrt{6/7}} \right)^2 - 1 \right) \cdot \frac{823543}{46656} \right) k_b T$$

This potential is designed to be constant of 5.0 kbT until $r = 7-8\text{nm}$, and then rapidly go to zero around 10.5 nm. The limited maximum overlap energy of 5.0 kbT allowed chromatin fibers to pass through each other occasionally and thus accounted for the strand-passing activity topoisomerase II. In all of our simulations, we additionally compact chromosomes using cylindrical constraints to impose the high chromatin density of one nucleosome per 11nm x 11nm x 11nm box, as observed in EM images of mitotic chromosomes. The particular geometric parameters of the cylindrical constraint varied between simulations. We imposed this constraint using a harmonically increasing potential with $k=0.1$ kbT when monomer crossed the boundaries of the constraining cylinder. We perform Langevin dynamics polymer simulations using OpenMM, a high-performance GPU assisted molecular dynamics API (95, 96). We used the following parameters of the Langevin dynamics – variable time step to achieve the relative accuracy of 0.001, the friction coefficient of 0.01, particle mass of 1.0 and the temperature of 300K. We ran the simulations until the simulated $P(s)$ curves (see below) stopped changing at logarithmic time scales, which occurred after $1.5e7$ timesteps in prophase simulations and after $3e6$ timesteps in prometaphase simulations. We visualized the resulting chromosome structures using Pymol (DeLano, Warren Lyford. "PyMOL." (2002)).

Simulations of prophase and CAP-H2-depleted chromosomes

We simulated prophase chromosomes as arrays of consecutive loops compacted into a cylindrical shape with a high chromatin density. As in (8), we first randomly selected a subset of particles to be bases of consecutive loops, such that the loop lengths were exponentially distributed with an average of l_{avg} . We modelled loop-forming condensins by imposing extra harmonic bonds between the particles representing loop bases. Finally, we tethered the first and the last particles of the chromosome to the ends of the compacting cylinder using a harmonic attraction potential along z-coordinate with $k=0.15 \text{ kbT/nm}^2$. We equilibrated the each simulation for $1.5e7$ steps to allow for slow large-scale rearrangement of the flexible chromosome scaffold. To match the simulations with the WT prophase and CAP-H2 prometaphase experiments, we systematically varied the two model parameters: (1) the average loop length, l_{avg} , from 20 kb and 100 kb, with a step of 10 kb and (2) the average linear density of loops along the axis of the constraining cylinder, N_{loops}/L , from 50 loops/ μm to 350 loops/ μm , with a step of 50 loops/ μm . For each parameter set, we modeled a chromosome containing 500 average loop lengths.

Simulations of prometaphase and CAP-H-depleted chromosomes

We simulated prometaphase chromosomes as helical arrays of consecutive nested loops compacted into a cylindrical shape with a high chromatin density. As in the prophase simulations above, we first imposed an array of consecutive loops with the average loop length of l_{avgouter} . To account for the action of condensin I, we further split each of these “outer” loops into an array of smaller exponentially distributed “inner” loops, with an average length l_{avginner} . To test if the Hi-C data is consistent with helical winding of prometaphase chromosomes, we fixed the bases of the “outer” loops along a 3-dimensional helical path winding around the axis of the constraining cylinder. We imposed this helical path by tethering each loop base to its specific position along the helix with a harmonic potential with $k=0.04 \text{ kbT/nm}^2$. We equilibrated each simulation only for $1e6$ steps, since the motion of the backbone was suppressed and only individual loops had to equilibrate. To match the simulations with the prometaphase experiments, we varied four parameters of the model: (1) the average inner loop size, l_{avginner} , from 20 kb to 100 kb every 20 kb; (2) the average outer loop size l_{avgouter} as a multiple of the average inner loop size l_{avginner} , from 1 to 11 inner loops, with a step of 2; (3) the average amount of DNA per helical turn, P , from 2 to 15 Mb, with a step of 1 Mb; (4) the pitch of the helical scaffold, b , from 50 to 300nm, with a step of 50nm. As above, for each parameter set, we modeled a chromosome containing 500 l_{avgouter} . For each parameter set, the length of the constraining cylinder matched the axial length of the helical scaffold, and the radius of the cylinder was adjusted to reach the density of 1 particle per $11 \text{ nm} \times 11 \text{ nm} \times 11 \text{ nm}$ box.

Simulated Hi-C and $P(s)$ calculation for polymer models

We then used our polymer models to perform in-silico Hi-C. For each polymer model, we picked the chromosome structure at the end of the simulation and found all pairs of contacting monomers, i.e. pairs of monomers separated by less than 51nm in 3D space. As in the experimental Hi-C, we used these pairs to calculate the contact frequency vs separation $P(s)$ curves.

Model selection via comparison of simulated and experimental $P(s)$ curves

To find which simulations produce the best agreement with the experimental data, we systematically tried all possible combination of the model parameters. For each parameter set, we performed a simulation, generated a $P(s)$ curve and calculated the root mean square (r.m.s.) difference with the experimental $P(s)$ in the log10-log10 scale, after normalizing both of the curves at the specific distance (see the table below). Our simulations cannot predict $P(s)$ both at very short separations, below a loop size, and at very long separations, after the drop-off; for that reason, we limit our $P(s)$ discrepancy computations to ranges shown in the table below. Finally, we equalize the contribution of the short- and long-distance parts of the $P(s)$ (**Table S4**) by calculating the r.m.s. $P(s)$ difference in each of these regions separately and reporting the mean of the two.

$P(s)$ estimation for prophase models with sister chromatid cohesion

In order to understand how the presence of a spatially proximal sister chromatid could change the $P(s)$ curves we performed a mock-up simulation. We considered the 3D structure of the best-fitting prophase model and added to it its identical copy shifted along the direction perpendicular to the chromatid axis by distance Δ_x^{sister} . We then calculated the $P(s)$ for this structure, under the assumption that our *in-silico* Hi-C procedure could not tell apart the corresponding identical loci on the two chromatids. We then repeated this procedure for different values of Δ_x^{sister} , from 0 (a complete overlap between the two chromatids) to 2 chromatid radii (a complete segregation of the chromatids).

Estimation of the rate of loop formation at condensin binding sites.

We calculated the average contact frequency around condensin binding sites at 10kb resolution, considering interactions formed by loci located up to 1Mb away from the condensin sites. We then normalized these average maps by the distance-dependent decay of contact frequency $P(s)$, thus obtaining average “observed-over-expected” maps of contact enrichments around condensin binding sites.

We used the simulated 3D models of prometaphase chromosomes at 60 minutes to calculate the average contact map around a loop anchor. We simulated a Hi-C map for the whole model at 10kb resolution and averaged its segments around 1000 randomly chosen loop anchors. As with the experimental maps, we then normalized it by the decay of contact frequency with distance from the same model.

The average map of contacts formed by loop anchors in our 60 minute model showed a “plus-sign” pattern, corresponding to the enrichment of contacts between loop anchors and their neighboring loci. In our experimental Hi-C data, the condensin binding sites displayed a similar contact pattern, albeit noticeably weaker, suggesting that condensin binding sites harbor a loop anchor only in a fraction of sampled cells.

We then quantified the strength of these “plus-sign” patterns in our experimental and simulated maps as the median observed-over-expected enrichment of contacts formed between the sites in question (condensin binding sites, for experimental data, and a loop anchor, for simulated data) and their neighboring loci, divided by the median observed-over-expected of contacts between all pairs of neighboring loci. Comparing the strengths of “plus-signs” at condensin sites to the strength of such patterns at loop anchors in our simulations allowed us to estimate how often condensin sites harbor a loop in the sampled population of cells.

Simulations of nested loop formation by loop extrusion.

To test if the process of loop extrusion can form nested loops, we performed lattice loop extrusion simulations as previously described in (25). Briefly, such simulations aim to model the positions and sizes of loops produced through the process of loop extrusion and do not explicitly consider the 3D chromosome conformation. In these simulations, a chromosome is divided into a 1D lattice of sites and the condensin-extruded loops are represented by a pair of sites, loop anchors, which are brought together at the base of a loop. We simulate loop extrusion by step-wise shifting of the two loop anchors of each condensin away from each other over time. To simulate the chromatin binding/unbinding dynamics of condensins, we occasionally remove them from the lattice and put back to a randomly chosen location, where they start a new loop. The code for these simulations is available at <https://github.com/golobor/looplib/>.

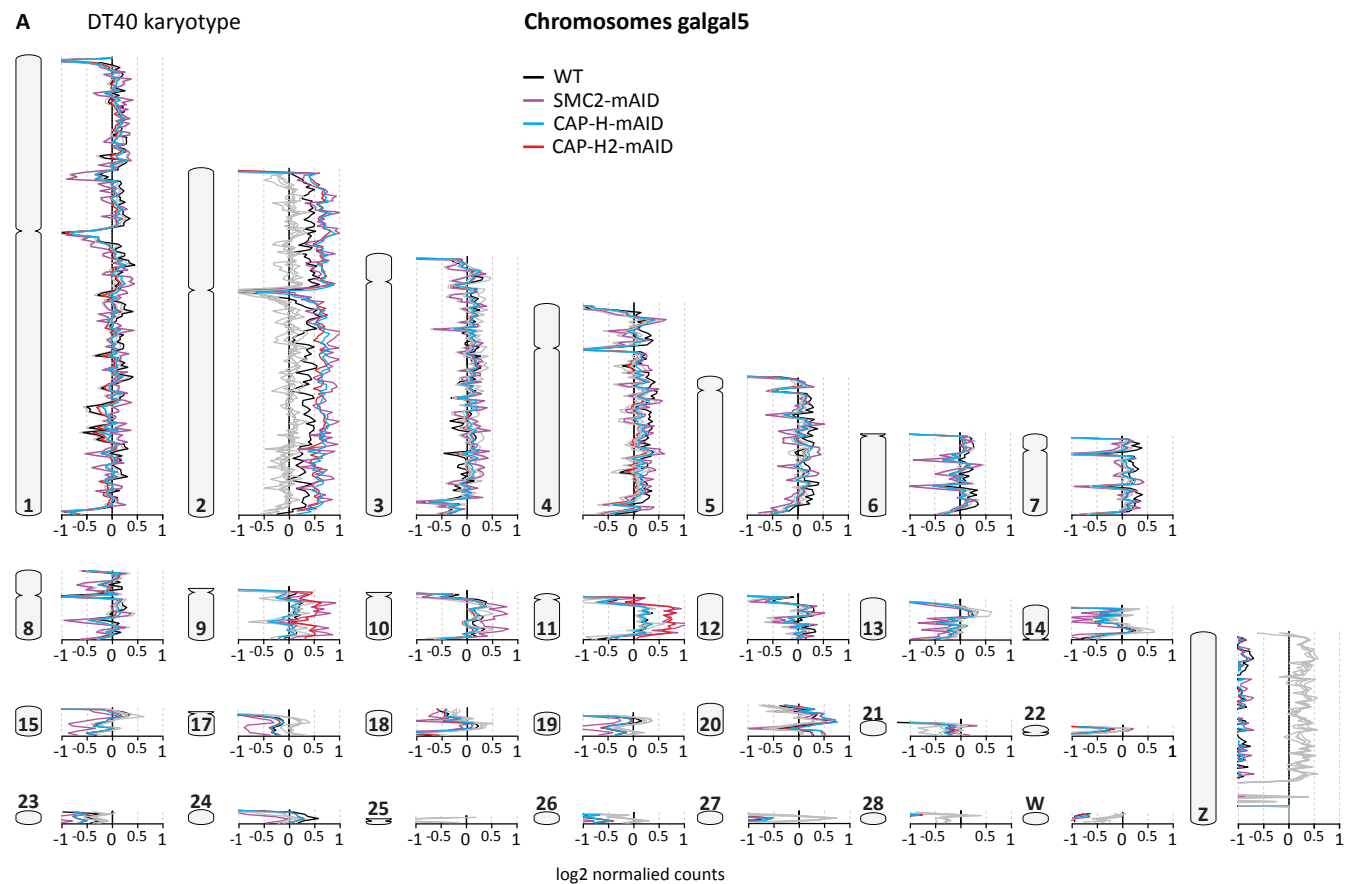
To simulate extrusion of loops by condensins I and II, we considered 20Mb of chromatin fiber, divided into a lattice of 100,000 sites of 200bp each. We initialized the system by randomly placing condensins I (one per 40kb) and II (one per 160kb) along the lattice, such that the two loop anchors of each condensin were located on the adjacent lattice sites (i.e. they have not yet extruded any loop). We then simulated the process of loop extrusion at the average speed of 10 sites per second at each loop anchor. The average residence time was 2 minutes for condensins I and 120 minutes for condensins II. We ran simulations for 2 hours of experimental time.

Simulations of suppression of TADs and compartments by loop extrusion in prophase

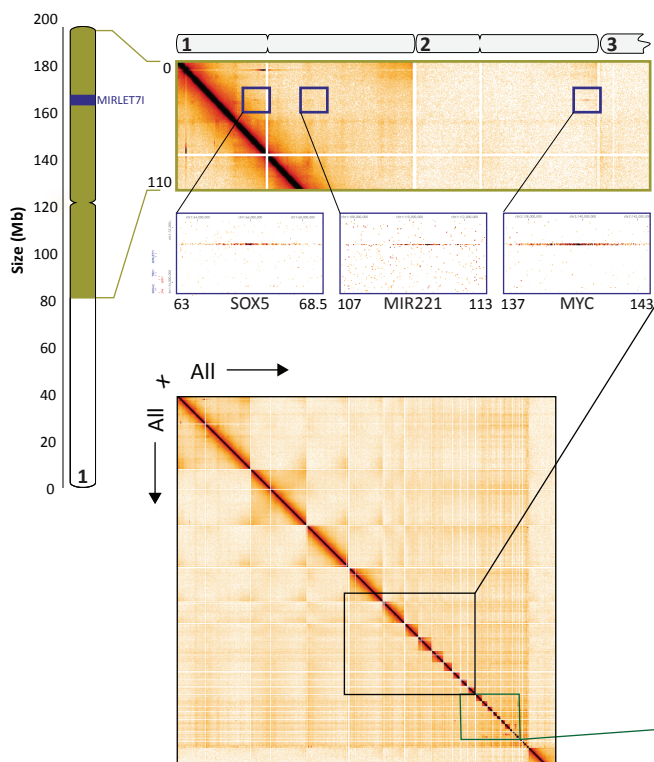
Polymer simulations of an 8.3 Mb fiber with loop extrusion and compartmentalization are performed as described in (24, 97) We chose parameters to reproduce loop length estimates in interphase / 5min prophase / 7.5min prophase: Loop extrusion factor (LEF) processivity/separation was 250kb/500kb = 1/2 (interphase), 125kb/31.3kb = 4 (5 min prophase), 250kb/25kb = 10 (7.5 min prophase). Other simulation parameters are: coarse graining = 400bp/monomer, LEF speed = 580 bp/s on each LEF side, boundary stopping probability=90%, monomer volume density=0.15 (bead diameter)³, compartmental interaction parameter=0.12 kT, repulsive core strength=3 kT, mean distance between CTCF sites=430 kb, crosslinking radius for simulated Hi-C = 4 bead diameters. Hi-C maps, insulation tracks, Eigenvectors, and insulation pileups are binned at 50 kb, compartmentalization plots at 100 kb. Example configurations are colored linearly along the fiber from red to blue.

Figures S1-S27

Fig.S1



B Integration sites



C Translocations

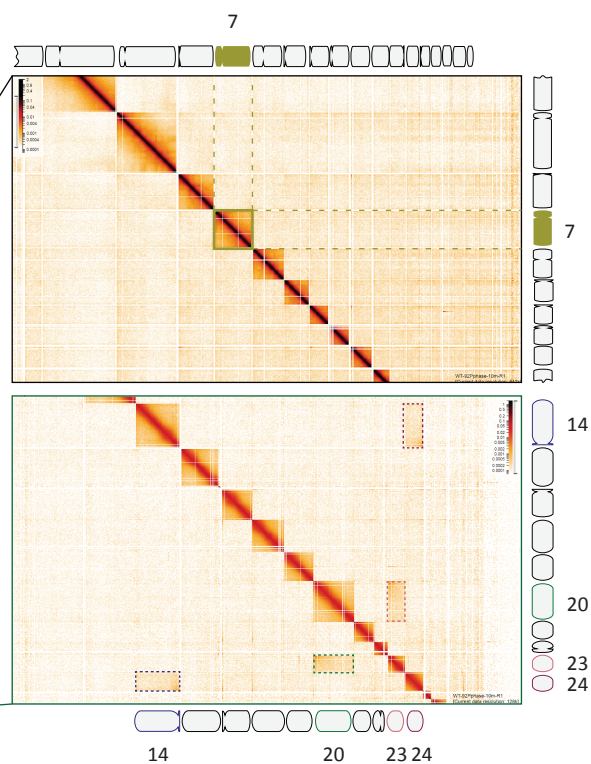


Fig.S1

Hi-C based karyotyping of cell lines. (A) Karyotype plots derived from 1Mb binned Hi-C matrices. The sum of interactions for each bin with every other bin is normalized both genome wide (gray lines) and by chromosome (colored by cell line) and displayed on the x-axis (log₂ normalized counts) for each individual chromosome (y-axis) from Hi-C data obtained from cells at t = 10 minutes (wild-type (black), SMC2-mAID (magenta), CAP-H2-mAID (red) and CAP-H-mAID (blue)). A relative increase in chromosome normalized counts in comparison to genome wide normalized counts indicates copy number gains, whereas a decrease shows copy number loss. (B) Integration sites. Translocations are visible from Hi-C maps as an increased normalized read counts at the intersection of the original locus and the site of translocation. The integration of SOX5 near the MYC gene occurred at the generation of the DT40 cells line and has been described previously (98). Our Hi-C maps show that MYC, SOX5 and MIR221 have translocated to a single integration site on chromosome 1 (shown in green box), just upstream of MIRLET7I (blue). (C) Translocations. Translocations show as an increase in Hi-C signal between different chromosomes. We observed translocations between chromosomes 14 and 24 as well as 20 and 23 (dotted boxes), but not for the other chromosomes including chromosome 7 (green), which was used as our model chromosome throughout this manuscript.

Fig.S2

FACS analysis after release from synchronization

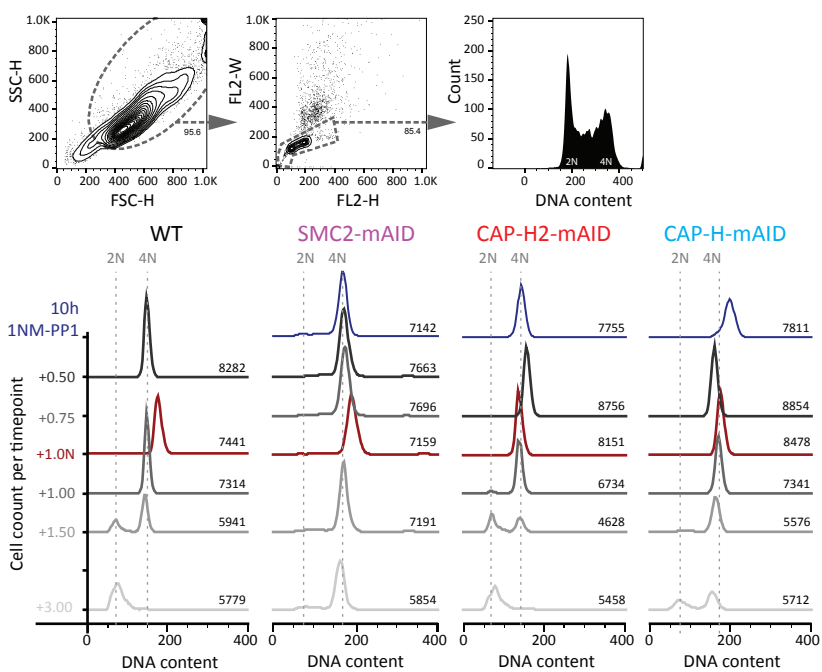


Fig.S2

FACS analysis for synchronized cell cultures. FACS is shown for auxin induced and CDK1 as synchronized (10 hr 1NM-PP1) cell cultures. Gating was applied for live cells (top-left) and singlets (top-middle) and gated cells were plotted as a histogram for DNA content (top-right). The bottom plot shows the DNA content (propidium iodide) on the x-axis and time after 1NM-PP1 release (in hours after release from G₂ arrest) on the y-axis (top to bottom) for each auxin induced cell line. Cells were not blocked at prometaphase with nocodazole except for the samples indicated with red lines (1.0N). This analysis allows determination of the timing for exit from mitosis as a loss of cells in G₂/M (4N peak) and a subsequent increase in cells in the G₂ peak (2N).

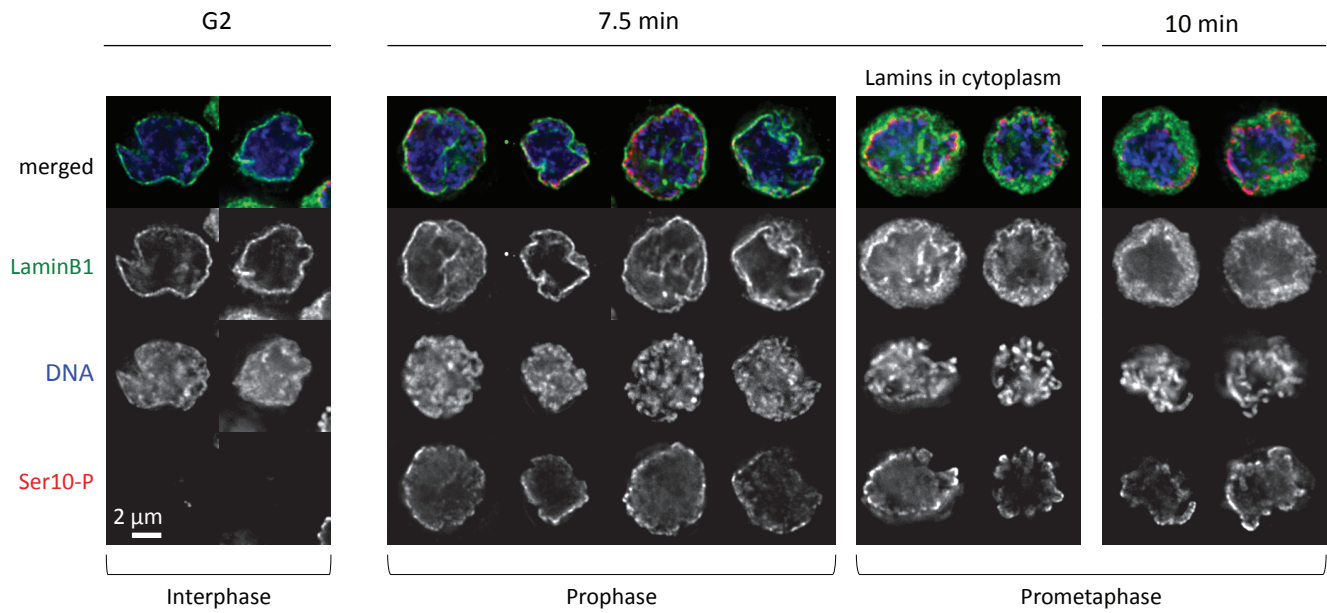
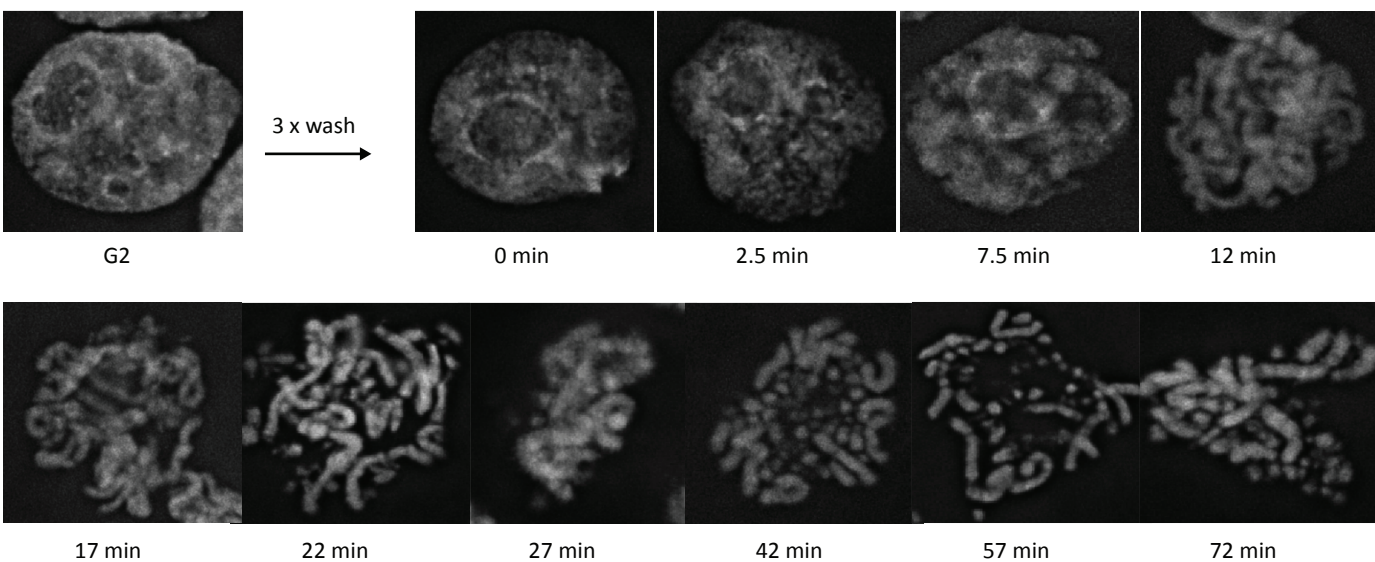


Fig.S3

Nuclear envelope breakdown (NEBD) in CDK1as synchronized DT40 cells determined by Lamin B1 staining. Lamin disassembly and chromosome condensation gradually occur at nuclear breakdown. Lamin B1 localizes at the nuclear periphery (green pseudocolor) before and throughout the cytoplasm after NEBD. Serine 10 of histone H3 becomes phosphorylated during mitosis (H3K10ph, red). DNA stained by DAPI is shown in blue (pseudocolored).

A Images corresponding to samples for condensin ChEP data

WT



ChEP data

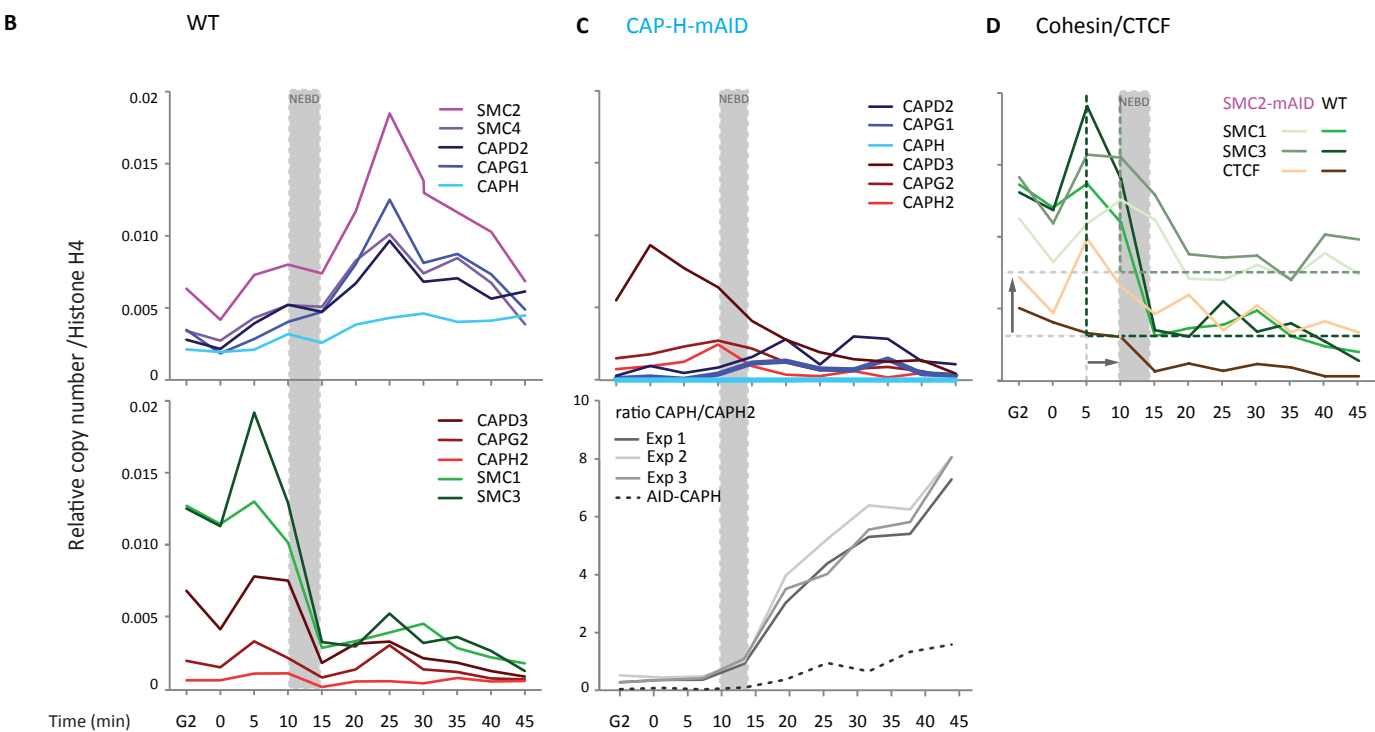
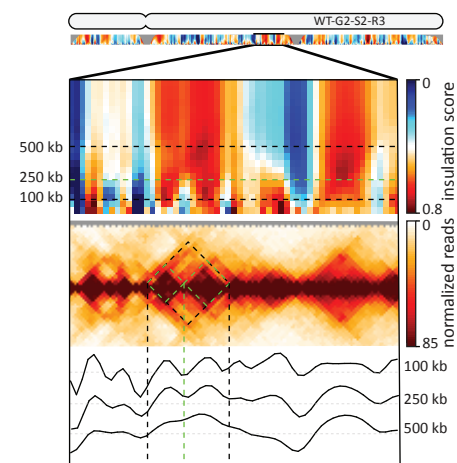


Fig.S4

Chromatin enriched proteomics (ChEP) data for CDK1as synchronized DT40 cells. Chromatin enrichment for proteomics (ChEP) (58) identifies proteins associated with the DNA (see Supplementary Methods). **(A)** Microscopy (DAPI staining of chromosomes) of cells at different time points in mitosis. **(B)** The relative quantitation of chromatin bound condensin I, II and cohesin subunits in CDK1as synchronized cells through WT prophase and prometaphase. Numbers on the y-axis are arbitrary MaxQuant units (in millions), normalized for Histone H4 levels. Timing was made comparable to samples used for microscopy and Hi-C libraries based on nuclear and chromosomal morphology in **(A)**. **(C)** ChEP from CAP-H depleted (CAP-H-mAID) cells indicates a loss of chromatin bound condensin I subunits after auxin mediated depletion (top). The bottom plot shows the ratio of chromatin binding for CAP-H over CAP-H2 subunits after CAP-H depletion (dotted line) in comparison to 3 replicate control synchronized time course experiments wherein CAP-H was not depleted. **(D)** ChEP for cohesin and CTCF binding to chromatin in SMC2-depleted cells (auxin induced SMC2-mAID) and condensin containing (un-induced) cells. Condensin depletion results in delayed loss of cohesin and CTCF (indicated by the horizontal arrow) and retention of more cohesin and CTCF binding (indicated by the vertical arrow).

Fig.S5

A Insulation analysis at varying length scales



B Insulation score variance

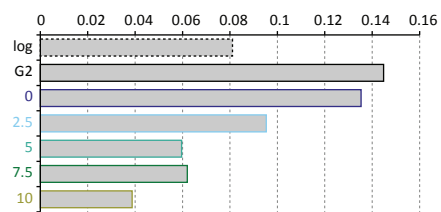


Fig.S5

Insulation parameter settings for TAD analysis. **(A)** Insulation at different length scales. Hi-C matrices were subjected to insulation analysis (depicted in interaction heatmap below). An insulation score is calculated for each bin by summing all interactions occurring between loci located up to a defined distance upstream and downstream of the bin (indicated by the diamond squares in the bottom panel). This method is described in detail in (32). Bins with high insulation (at a TAD boundary) have a low insulation score, as measured by fewer interactions occurring across it. Bins with low insulation or boundary activity (the middle of a TAD) have a high insulation score. Minima along the insulation profile are potential TAD boundaries. We determined the optimal square size from a range of 50kb to 1Mb to be 250 kb. The large black dotted square (chromatin domain) contains 2 smaller domains structures, indicated by green squares. The specific insulation analyses using 100, 250 and 500 kb windows below the interaction heatmap reveal that small insulation square sizes (<100kb) pick up small scale fluctuations in insulation, whereas larger squares (>500 kb) miss weaker boundaries (green dotted line derived from square above). The line graph in the bottom shows the fluctuation in insulation score for each tested square size (100, 250, 500kb) for the same chromosomal region as the interaction maps above. **(B)** Insulation score variance calculated with a square size of 250 kb for Hi-C data obtained at different times during prophase. The loss of variance in insulation score as prophase progresses indicates loss of TAD structure (8).

Fig.S6 Contact frequency $P(s)$ plots for all individual chromosome arms

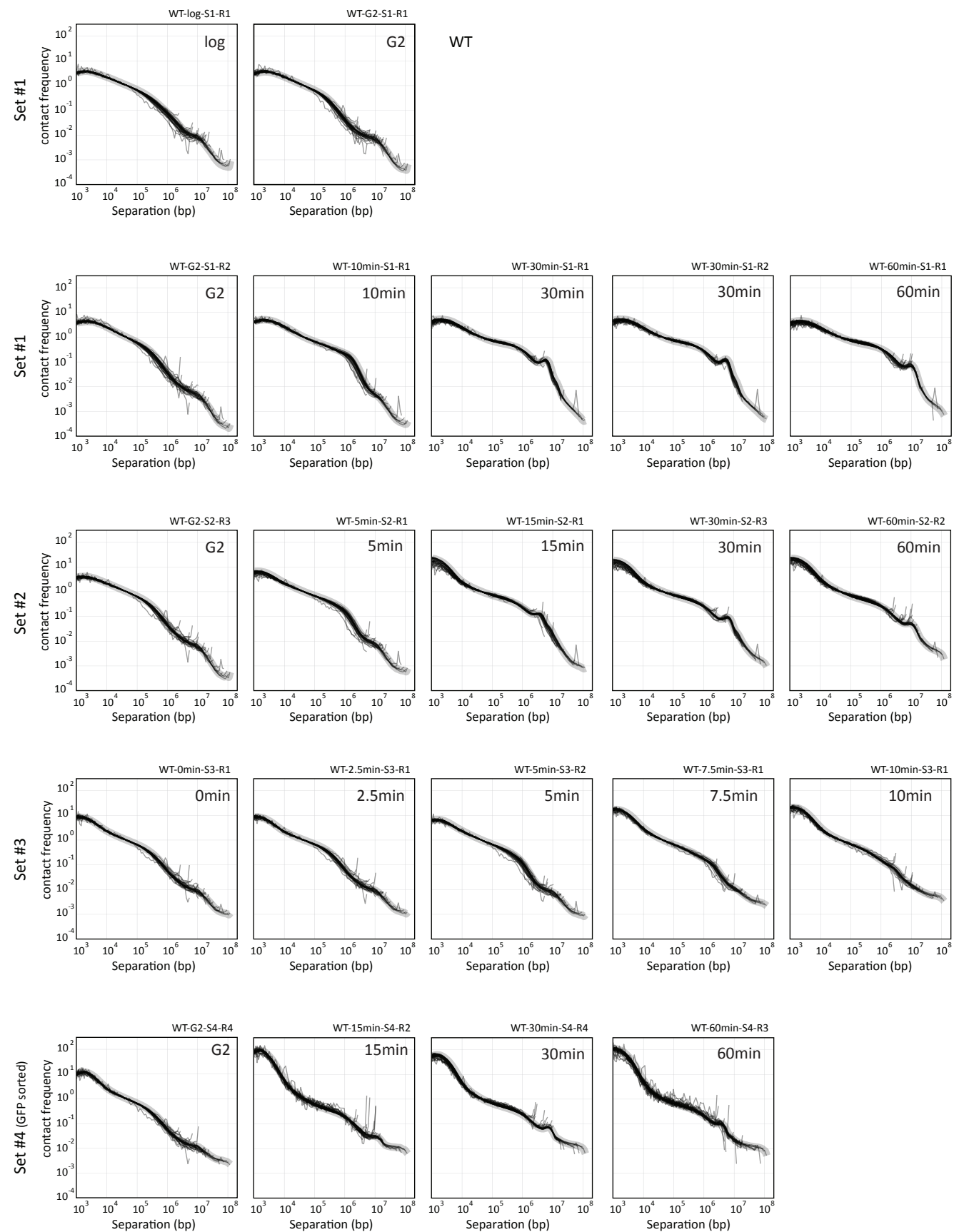
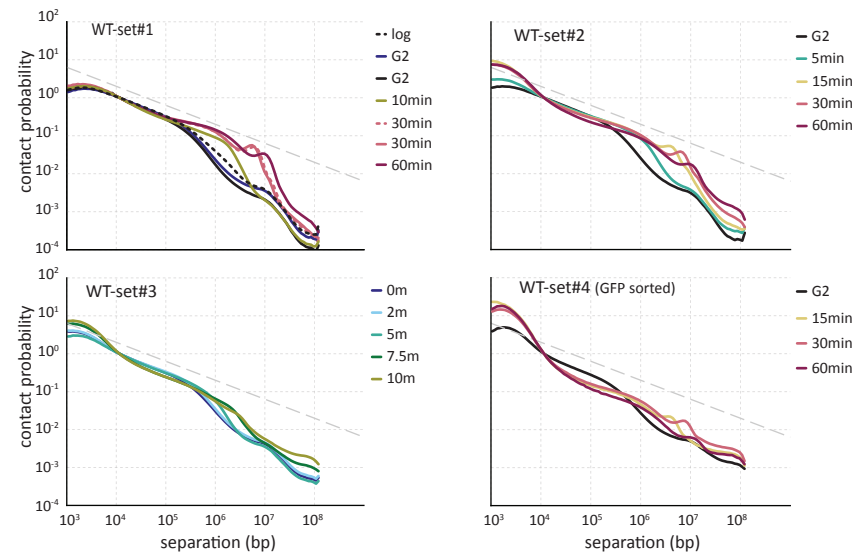


Fig.S6

Contact frequency $P(s)$ derived from Hi-C data obtained from CDK1 as synchronized DT40 cells. Contact frequency $P(s)$ plots for all 4 sets of WT Hi-C libraries. Plots for individual chromosome arms are shown in gray; the deviation from the mean decay is shown in light gray. **Table S2** describes each sample in detail.

A Contact frequency $P(s)$ plots for all WT chromosome arms combined



B Scaling derivative plots for all WT chromosome arms combined

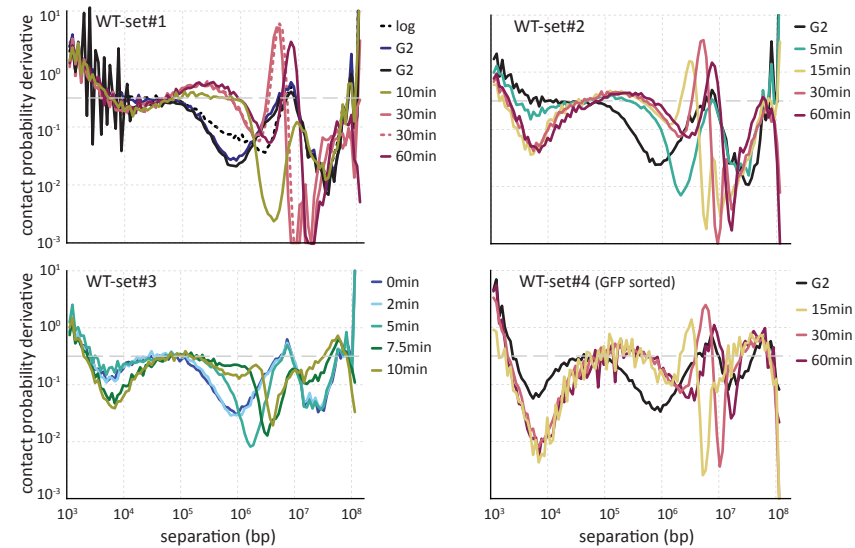


Fig.S7

P(s) plots and their corresponding derivative plots. *P(s)* plots and their corresponding derivative plots for 4 separate timecourse sets are shown for Hi-C datasets obtained from WT CDK1as cells at indicated time points after release from G₂ arrest.

Fig.S8 Chromosome 7 Hi-C interaction maps from all individual libraries for WT

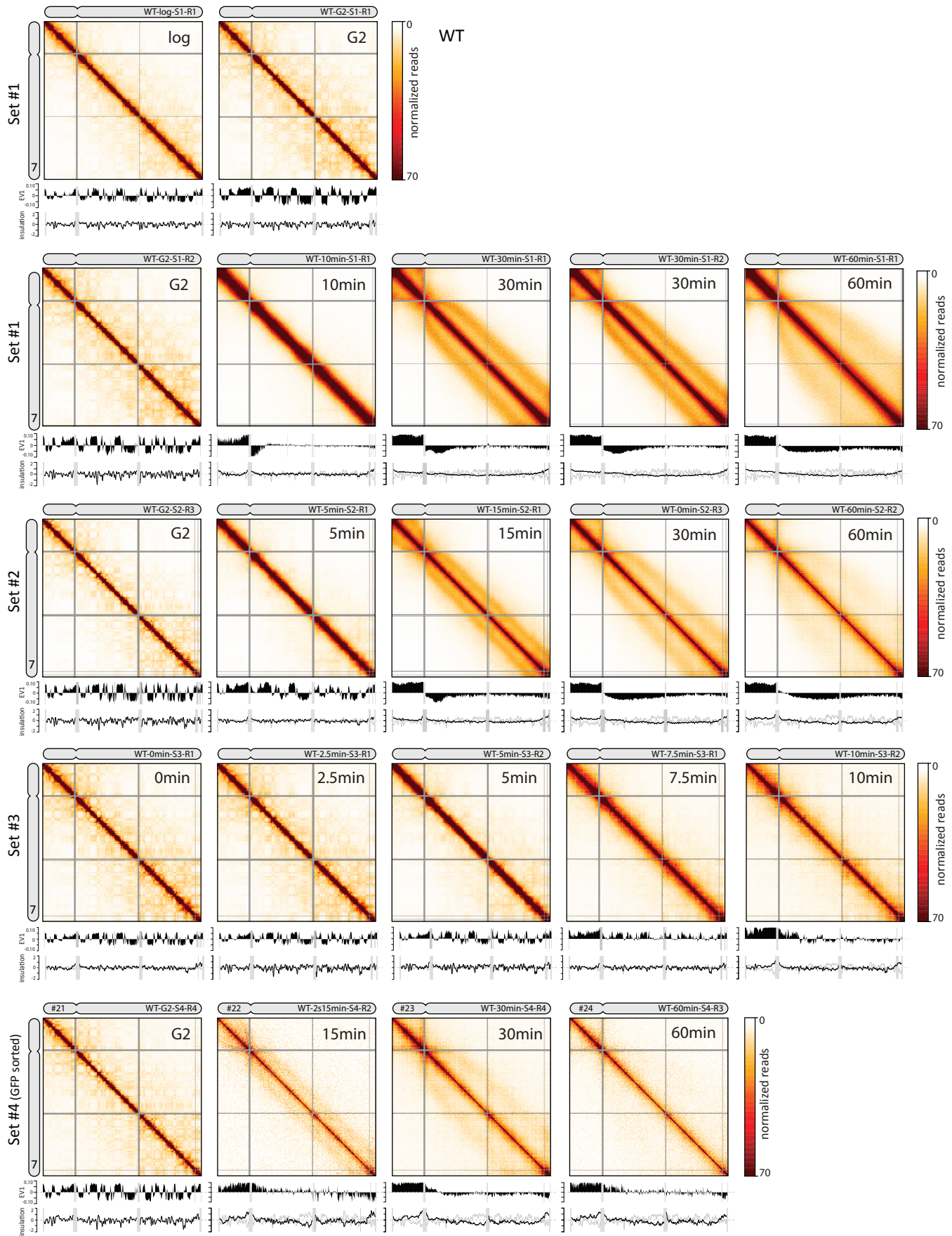


Fig.S8

Hi-C data obtained from CDK1 as synchronized DT40 cells at different time points in mitosis. Hi-C interaction maps were generated for chromosome 7 normalized to 1,000,000 interactions. Library names are indicated in the chromosome drawing on top right. **Table S2** describes each sample in detail. The top plot below each Hi-C interaction map displays compartment signal (Eigenvector 1). The bottom graph shows insulation score (TADs). Data for 4 independent sets of Hi-C data obtained from independent time courses are shown.

Fig.S9 Saddle plots for all individual chromosome arms from WT

WT

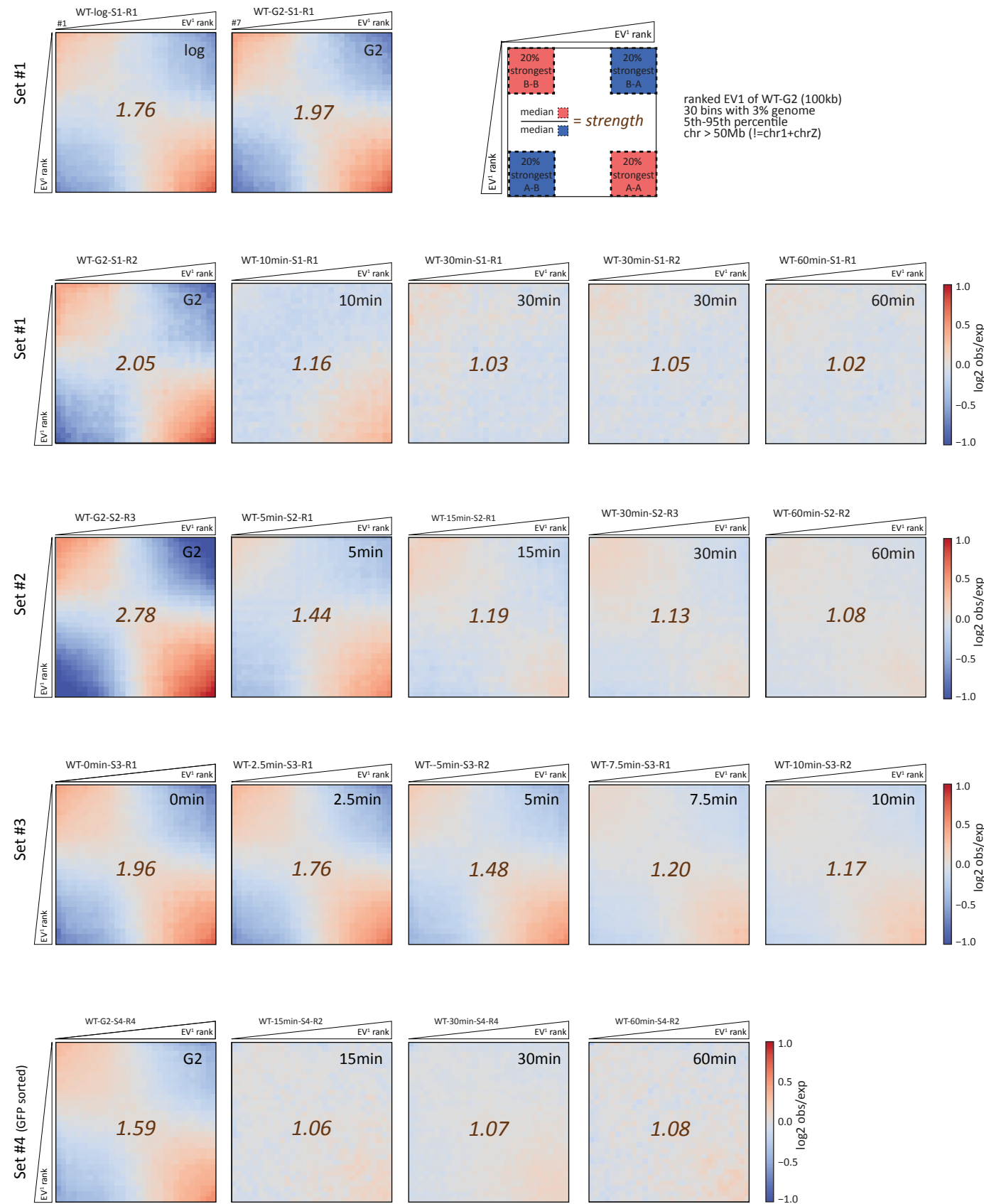
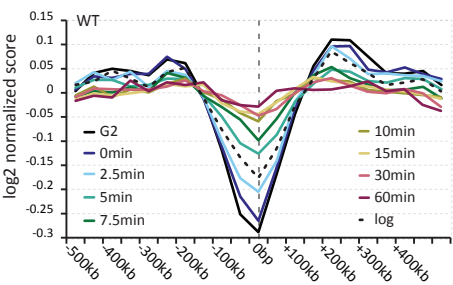


Fig.S9

Quantification of compartment interactions by “saddle plots”. The heatmaps show the average distance-normalized cis interaction frequencies between pairs of 100kb bins as a function of their compartment state (defined as the genome-wide rank of their eigenvector 1 value). The upper left corner of the map shows the interactions between pairs of bins, where both belong to the inactive compartment (i.e. compartment B); the lower right corner shows the interactions between pairs of bins from the active compartment (compartment A). The number on each heatmap indicates the overall strength of compartmentalization, defined as the median signal from the top 20% of A-A and B-B interactions, divided by the top 20% of A-B interactions $((AA+BB)/2AB)$. Library names are located on the top left. **Table S2** describes each sample in detail.

Fig.S10

Normalized insulation



Schematic of pileup at TAD borders

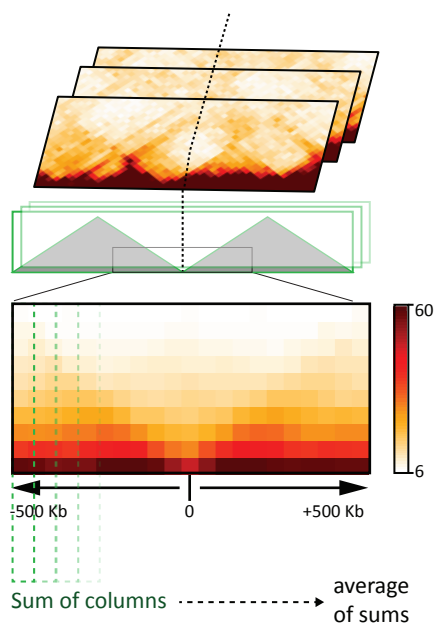


Fig.S10

Average insulation profiles around WT G₂ TAD boundaries on chromosome 7 at different time points in mitosis The schematic on the right outlines how average insulation plots are calculated around TAD boundaries. The insulation plot on the left shows the sum of the interactions for each 50kb bins located 500kb upstream or downstream of a TAD border, normalized by the average interactions for each timepoint. The presence of a minimum at 0 kb indicates a TAD boundary. As cells progress through mitosis the minimum disappears indicating loss of insulation and loss of the TAD boundary.

Heatmaps from WT-20150609-R1 libraries showing 2nd Diagonal at similar length scales for chromosomes of a range of sizes

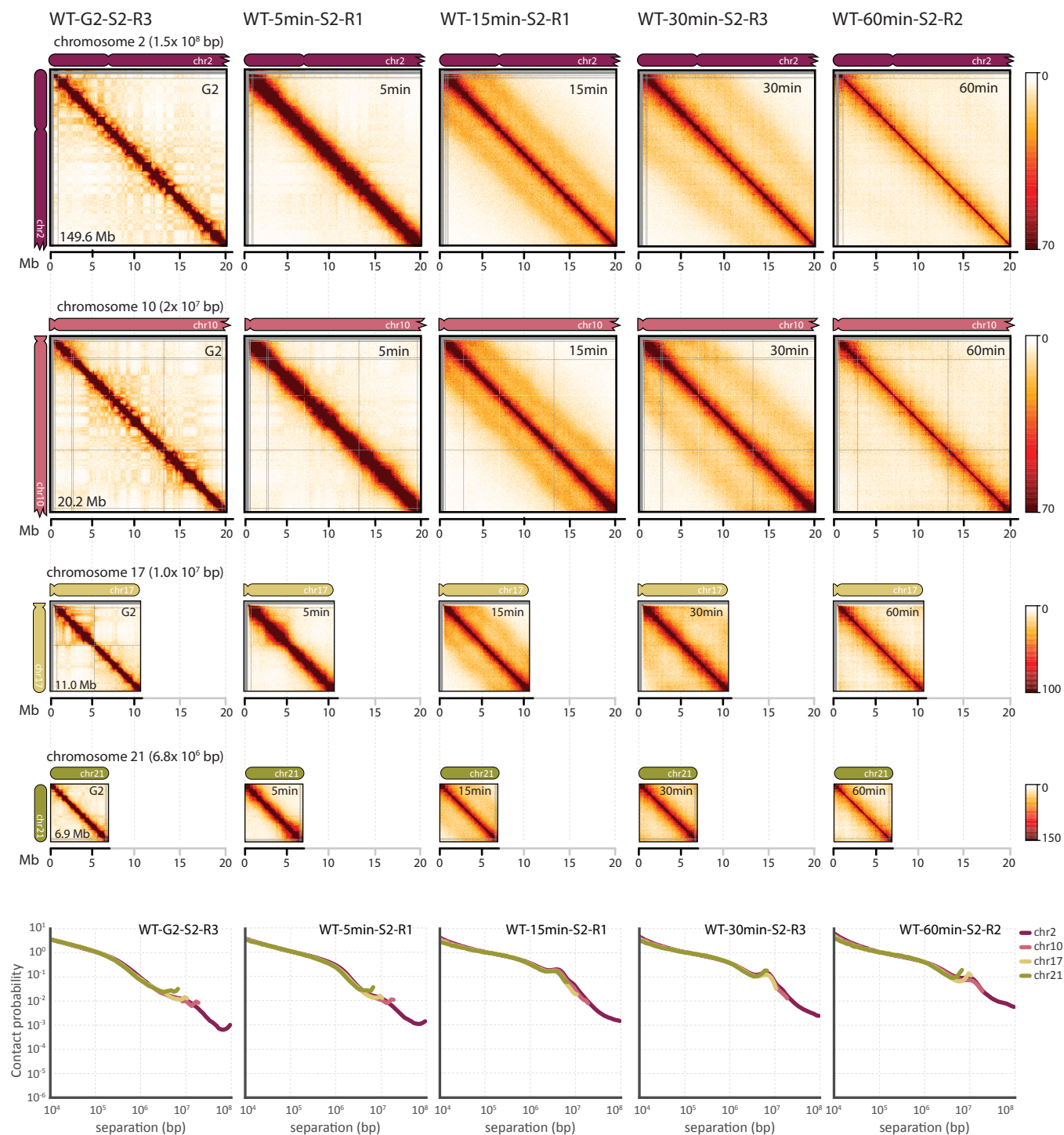
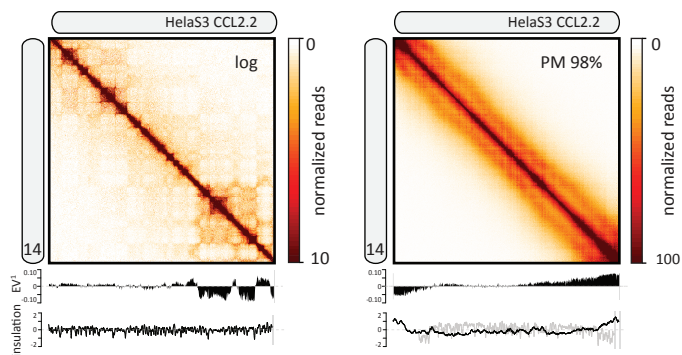


Fig.S11

Position of the second diagonal is independent of chromosome size. We compared contact frequency plots ($P(s)$) for wild-type chromosomes 2 (150 Mb), 10 (20 Mb), 17 (11 Mb) and 21 (6.8 Mb) to illustrate that the position of the second diagonal does not depend on chromosome size. At the onset of mitosis (G2) and in prophase ($t = 5$ min), there is no second diagonal for any chromosome. In early prometaphase ($t = 15$ min), a second diagonal (increase in contact frequency) can be seen for all chromosome sizes and in each Hi-C interaction map. At $t = 15$ minutes the position of the second diagonal is smaller than the size of each of these chromosome. At $t = 30$ minutes, the position of the second diagonal moved to larger genomic distance (~ 7 Mb). This distance exceeds the size of chromosome 21 (> 6.8 Mb), and leads to a disappearance of a visible increase in contact frequency for this chromosome only (as is observed in in both the Hi-C interaction map and contact frequency plot below). At $t = 60$ minutes the position of the second diagonal (~ 12 Mb) exceeds the size of chromosome 17, so that the second diagonal is no longer detected along that chromosomes. $P(s)$ plots (bottom) illustrate that positions of the second diagonals are the same for all chromosomes, at different timepoints in prometaphase.

A Heatmaps, compartments and TADs for HeLaS3



B Contact frequency $P(s)$ plot for HeLaS3

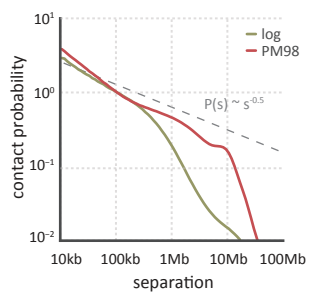


Fig.S12

Hi-C interaction maps from HeLaS3 cells arrested in late prometaphase display a second diagonal. **(A)** Hi-C interaction maps for HeLaS3 (ATCC CCL-2.2). Hi-C was performed on logarithmically growing HeLaS3 cells (log) and HeLaS3 cells arrested in late prometaphase with nocodazole, resulting in 98% prometaphase (PM 98%). Nocodazole synchronization was performed for 3 hrs following a double thymidine block, as described before (8). The compartment and insulation signals below the interaction frequency heatmaps are as in **Fig. S8**. **(B)** $P(s)$ for HeLaS3 logarithmically growing cells (log) and for prometaphase arrested cells (PM98) for chromosome 14, as in **Fig. 2**. The dotted line indicates $P(s) \sim s^{-0.5}$ reported before for mitotic chromosomes (8). The local peak in $P(s)$ around 10 Mb corresponds to the second diagonal band visible in the Hi-C interaction map **(A)**.

Fig.S13

Average Hi-C maps around condensin binding sites and loop anchor in models

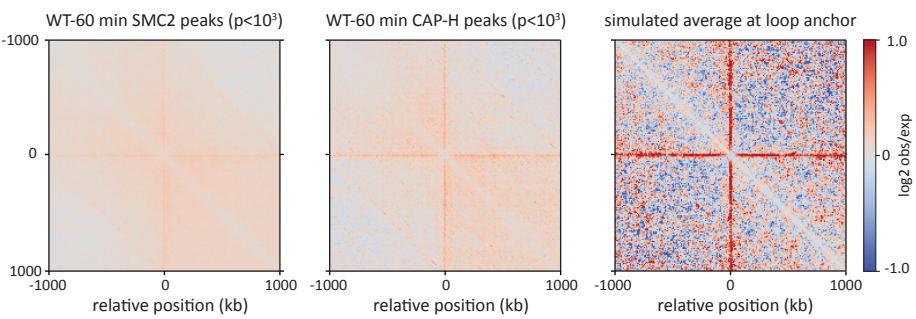
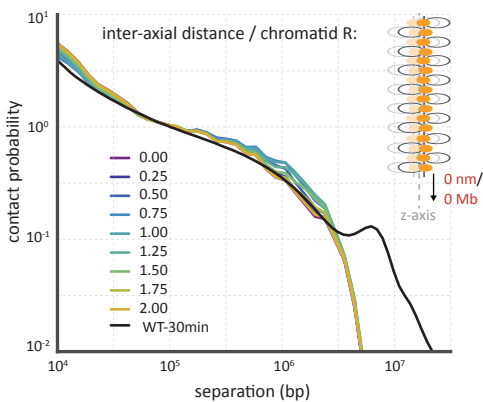


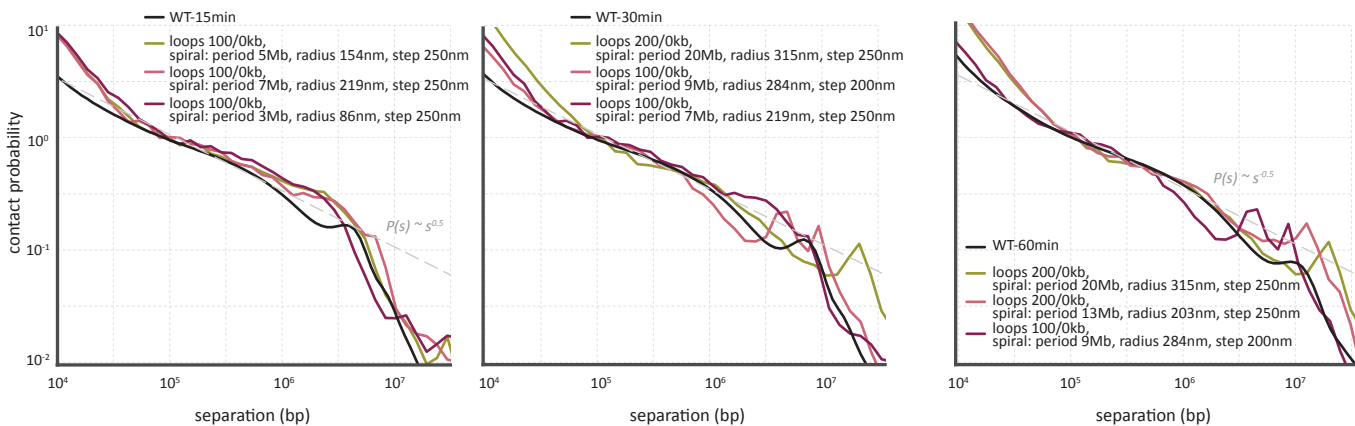
Fig.S13

The Hi-C contact footprint of condensin binding in experimental and simulated Hi-C maps. (A-B) The Hi-C maps of 60-min mitotic chromosomes averaged over 2Mb regions around (A) 13120 SMC2 and (B) 4674 CAPH ChIP-seq peaks. (C) The simulated Hi-C map of the 60-min mitotic chromosome model averaged over 2Mb regions around 1000 loop anchors. All three maps were normalized for the distance-dependent contact frequency decay in the corresponding dataset. See Supplementary Materials for description and interpretation of this analysis.

A Second diagonal not explained by sister overlap



B Without nested loops it does not fit



C An external helix does not fit

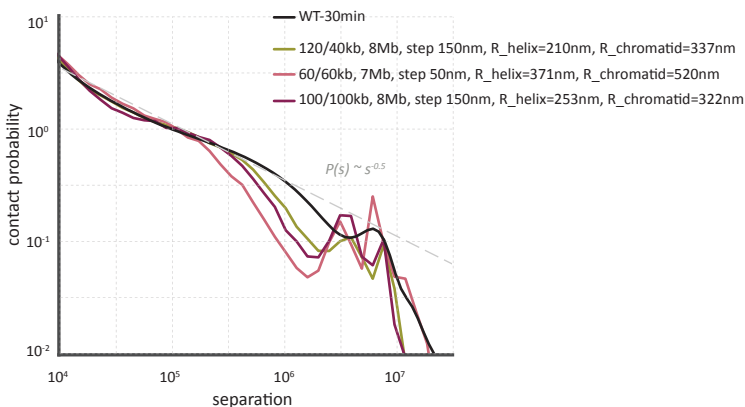
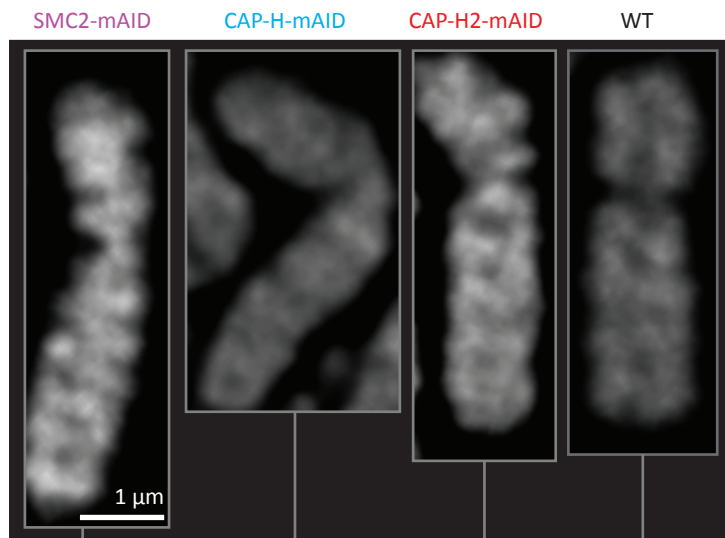


Fig.S14

Rejected polymer models of prometaphase chromosomes. **(A)** Interactions between sister chromatids do not produce a second diagonal in Hi-C interaction maps. Plot shows $P(s)$ of a model of sister chromatid interactions, where two identical prophase models are overlaid on top of each other in 3D space. The $P(s)$ in this model was calculated under the assumption that Hi-C would not be able distinguish between the two copies of the same locus on the two sister chromatids. $P(s)$ plots were calculated for a different levels of sister chromatid overlap. **(B)** Without nested loops polymer models do not reproduce experimental Hi-C data. Plots show $P(s)$ of prometaphase models without nested loops. Left-right: the panels show the best fitting models for each time point ($t = 15, 30$ and 60 minutes) in prometaphase. **(C)** Simulation of an external helix does not produce $P(s)$ that corresponds to experimental prometaphase Hi-C data. The $P(s)$ curves of examples of prometaphase models with wide spiraling of the scaffold (i.e. the radius of the scaffold $> \frac{1}{2}$ of the chromatid radius). All panels: black lines - experimental Hi-C data (dataset indicated in the legend); colored lines - $P(s)$ plot obtained by simulations with different parameters (indicated in the legend).

Gyre visualization and quantification for chromosome 2

A



Note:

No auxin, asynchronous cells
 100ng/ml cocemid for 2.5h
 75 mM KCl for 5 min
 Me/Ac fixation
 drop/dry on slides
 Vectashield with DAPI

B

chromosome 2
 10 gyres
 149.6 Mb
 =15 Mb/gyre
 length: 5.6 μ m

chromosome 2
 10 gyres?
 149.6 Mb
 =15 Mb/gyre
 length: 6.0 μ m

chromosome 2
 9 gyres
 149.6 Mb
 = 16.6 Mb/gyre
 length: 5.2 μ m

chromosome 2
 6 gyres?
 149.6 Mb
 = 24.9 Mb/gyre
 length: 4.5 μ m

C

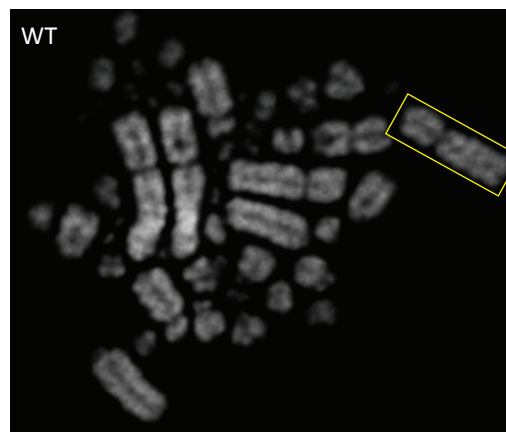
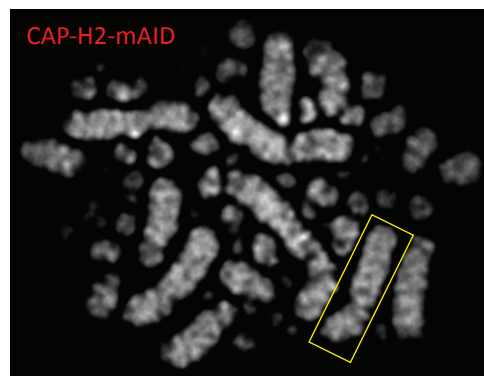
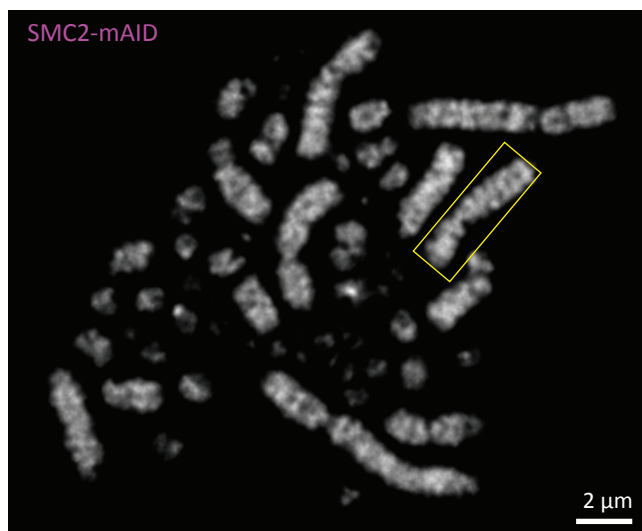


Fig.S15

Gyre visualization and quantification for chromosome 2. (A) Visualization (by DAPI) of a repeating structure on DT40 mitotic chromosomes following hypotonic treatment and spreading of indicated DT40 cell lines treated with colcemid for 2.5 hours. (B) Gyre count and size per gyre (Mb) estimates for chromosome 2. (C) Chromosome spreads of indicated cell lines. Boxed chromosomes are chromosome 2 based on size and centromere position, used for measurements in (A) and (B).

GFP expression of condensins

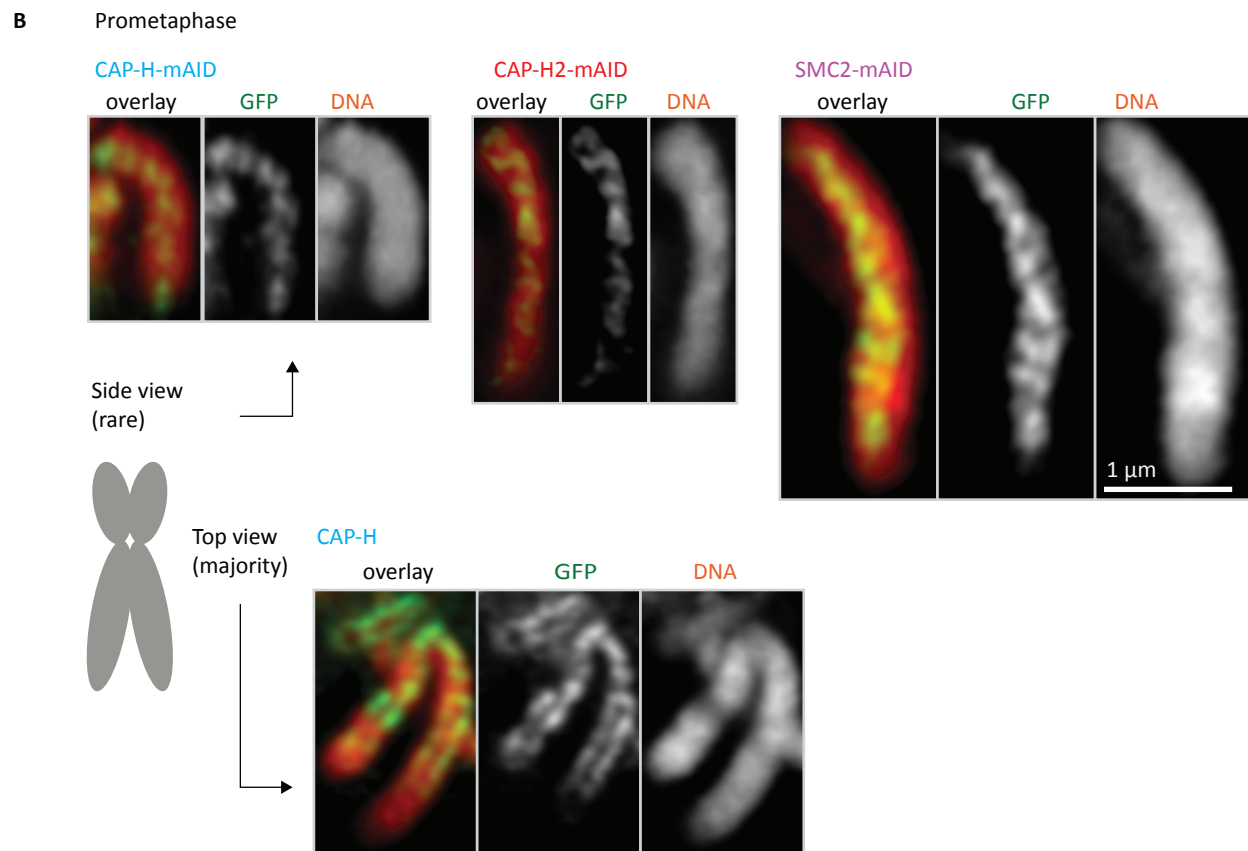
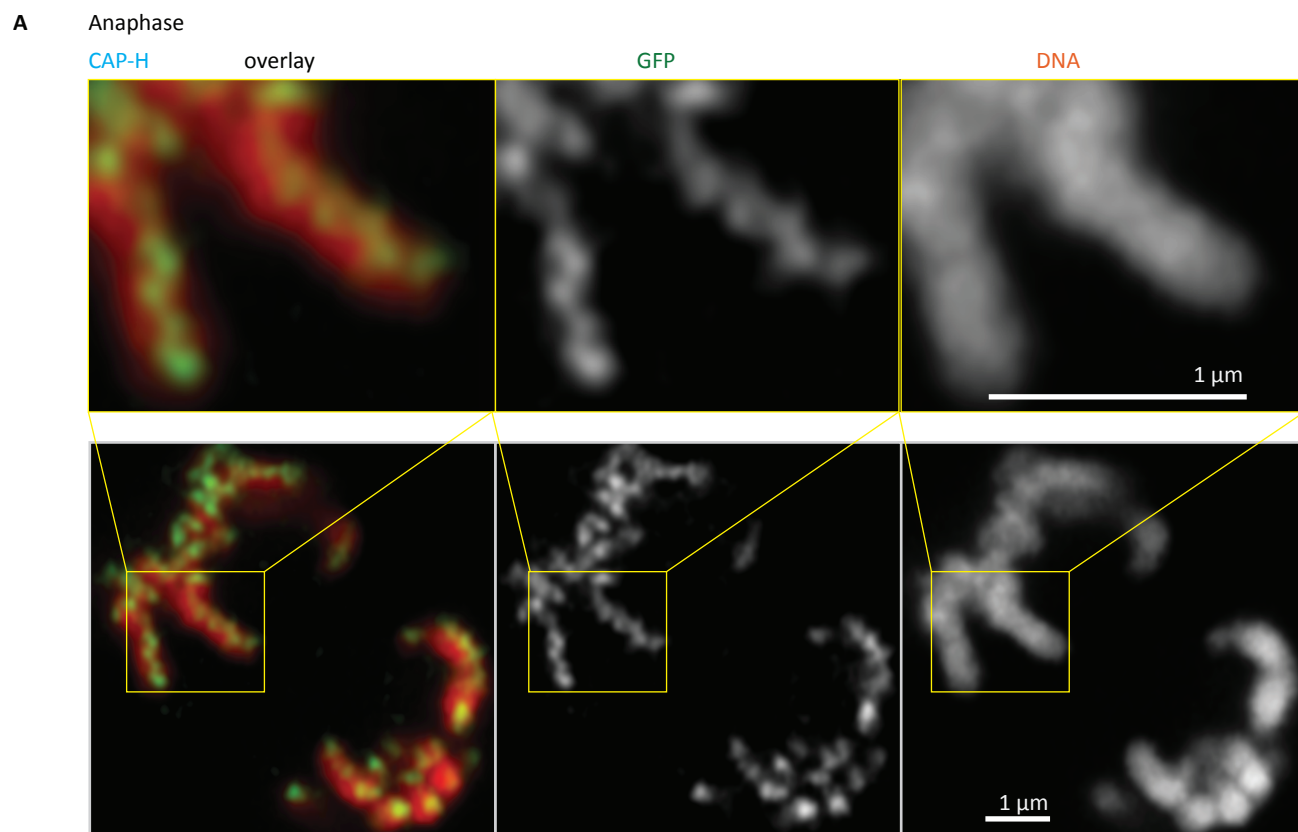


Fig.S16

Helical distribution of SMC2-mAID-GFP, CAP-H-mAID-GFP and CAP-H2-mAID-GFP in single sister chromatids. (A) Distribution of condensin components (as visualized by GFP) in anaphase chromosomes where only a single sister chromatid is present (this simplifies interpretation of the image). (B) Distribution of condensin components in rare views of prometaphase chromatids in which the two sister chromatids were oriented normal to the surface of the slide. This enabled us to use optical sectioning to resolve a single sister chromatid. Scale bars 1 μm . DNA is visualized by DAPI staining.

Fig.S17

Chromosome length and width measurements

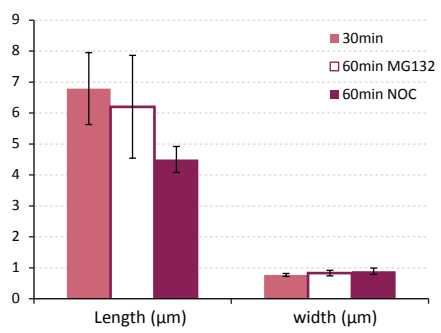


Fig.S17

Chromosome length and width measurements. Measurements of the length and width of chromosome 1 (196 Mb) in DT40 cells prepared under the conditions used for Hi-C experiments, at different timepoints in prometaphase.

Fig.S18

Prophase and prometaphase fits
for loop length and density

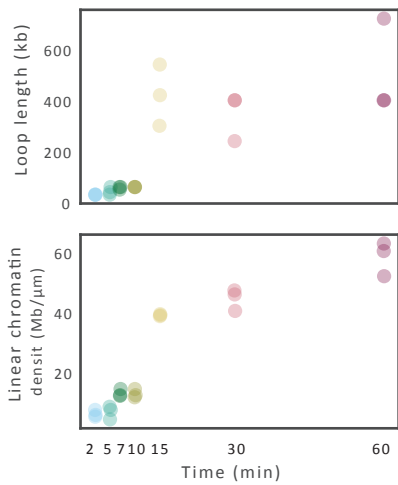


Fig.S18

Parameters of the best fitting models as a function time after release from G2 arrest. Chromatin density and loop length increase linearly from prophase through prometaphase.

Fig.S19

Western blots for auxin-treated condensin mutants

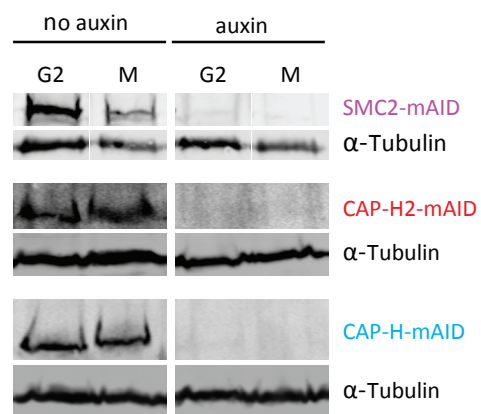
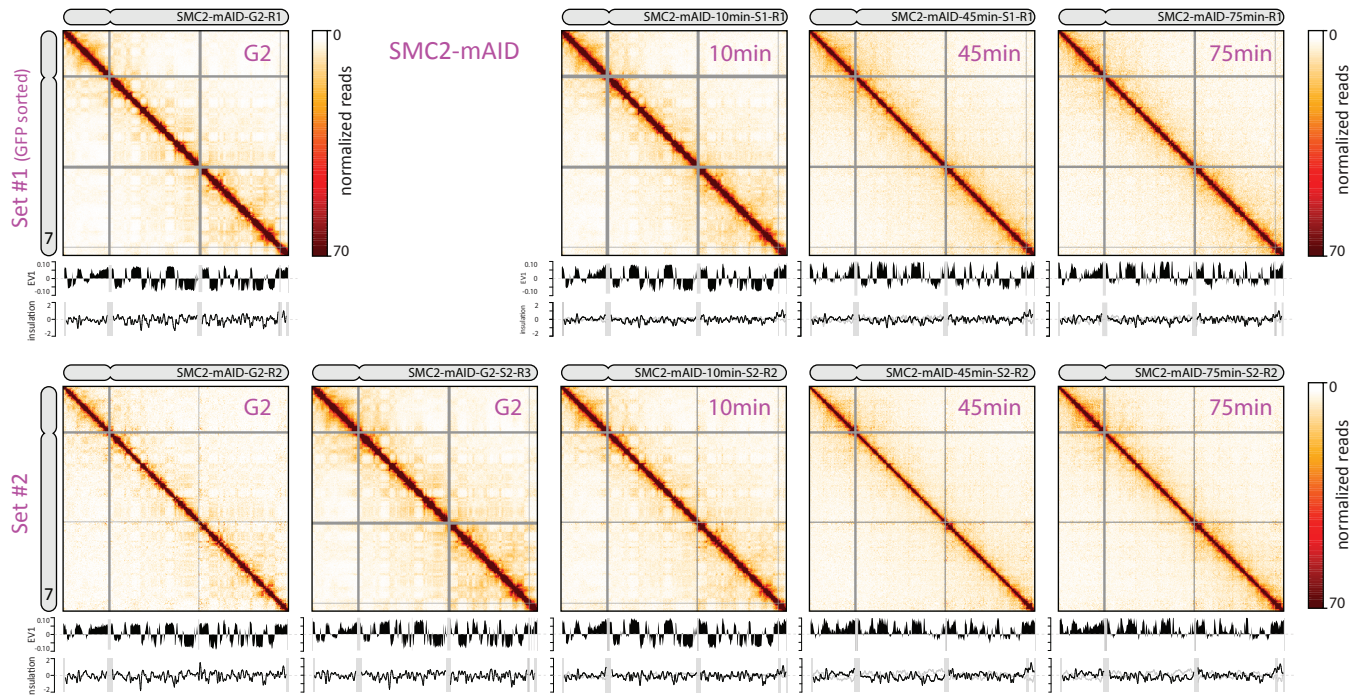


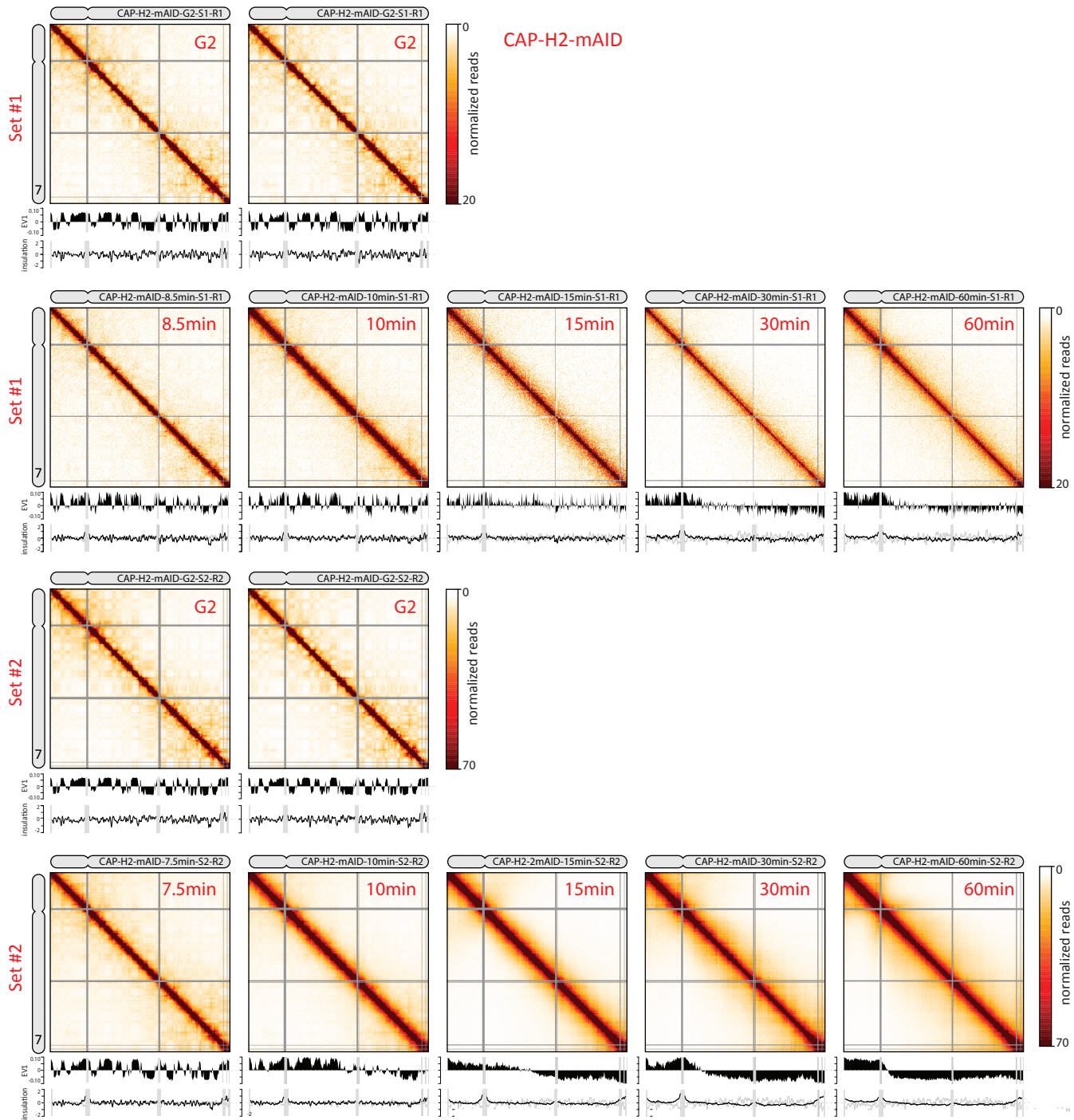
Fig.S19

Western blots showing protein levels of SMC2-mAID, CAP-H-mAID and CAP-H2-mAID for corresponding cell lines treated with or without auxin induction. Western blots indicate SMC2-mAID, CAP-H2-mAID and CAP-H-mAID are efficiently depleted after 3 hours of auxin addition.

Fig.S20 A Chromosome 7 Hi-C interaction maps from all individual libraries for SMC2-mAID plus auxin



B Chromosome 7 Hi-C interaction maps from all individual libraries for CAP-H2mAID plus auxin



Chromosome 7 Hi-C interaction maps from all individual libraries for CAP-H-mAID plus auxin

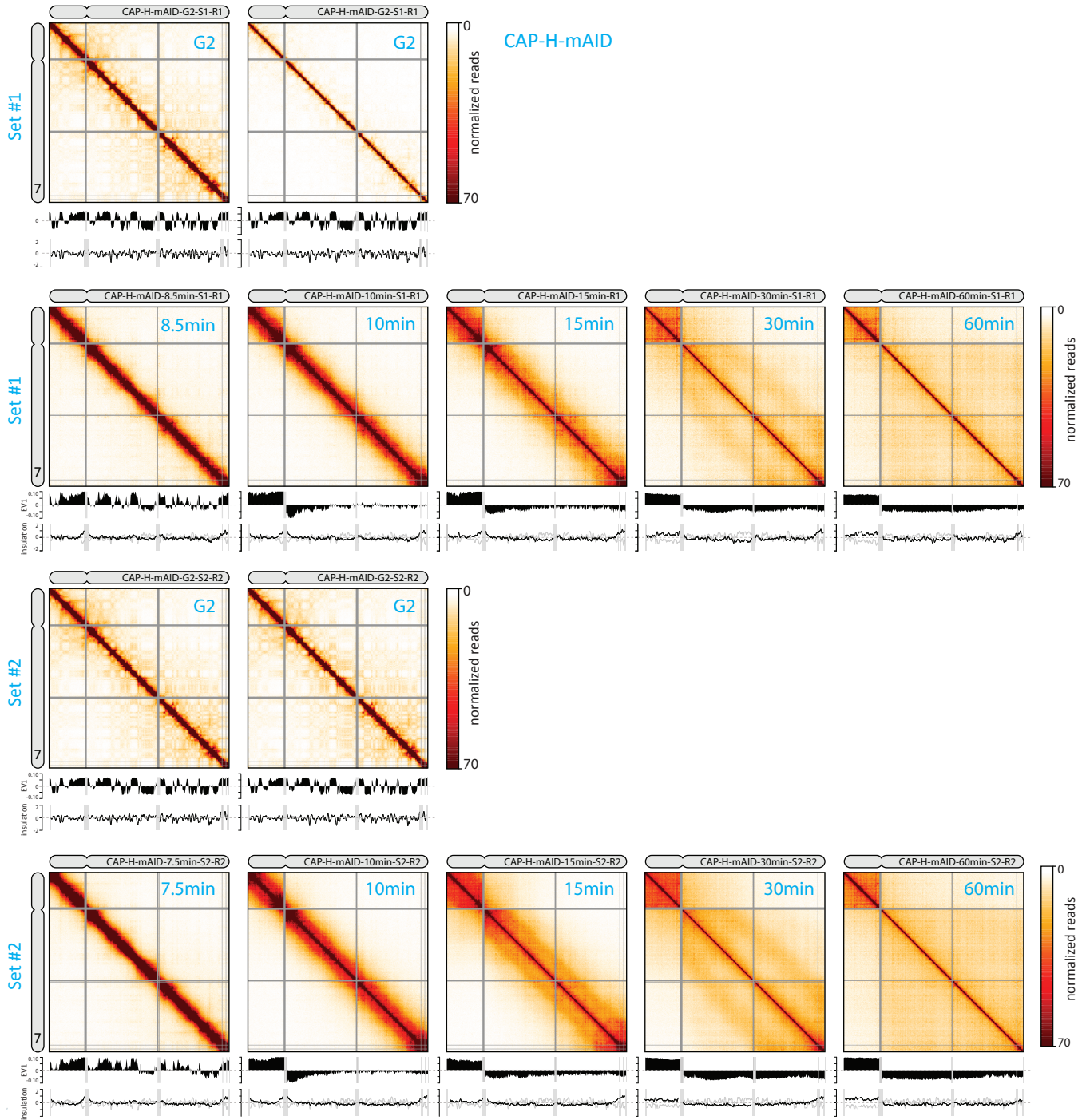


Fig.S20

Hi-C interaction maps obtained from SMC2, CAP-H and CAP-H2 depleted cells at different timepoints in mitosis. Hi-C interaction maps were generated for chromosome 7, normalized to 1,000,000 interactions. Dataset names are indicated in the chromosome drawing on top. **Table S2** describes each sample in detail. **(A)**: SMC2-mAID cells; **(B)**: CAP-H2-mAID cells; **(C)**: CAP-H-mAID cells. The top plot below each Hi-C interaction map displays compartment signal (Eigenvector 1; with percentage signal explained by first eigenvector). The bottom graph shows insulation score (TADs). Data for two independent time courses are shown.

Fig.S21

DAPI stain for synchronized cells

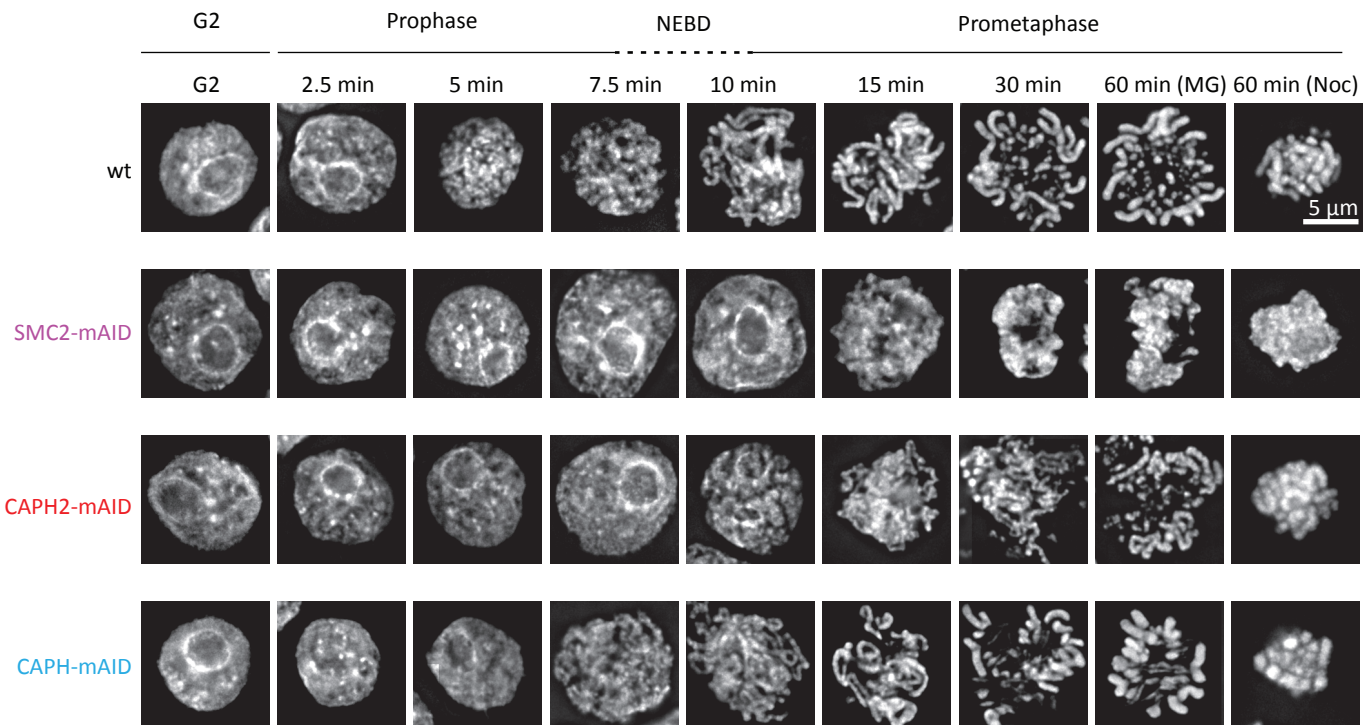
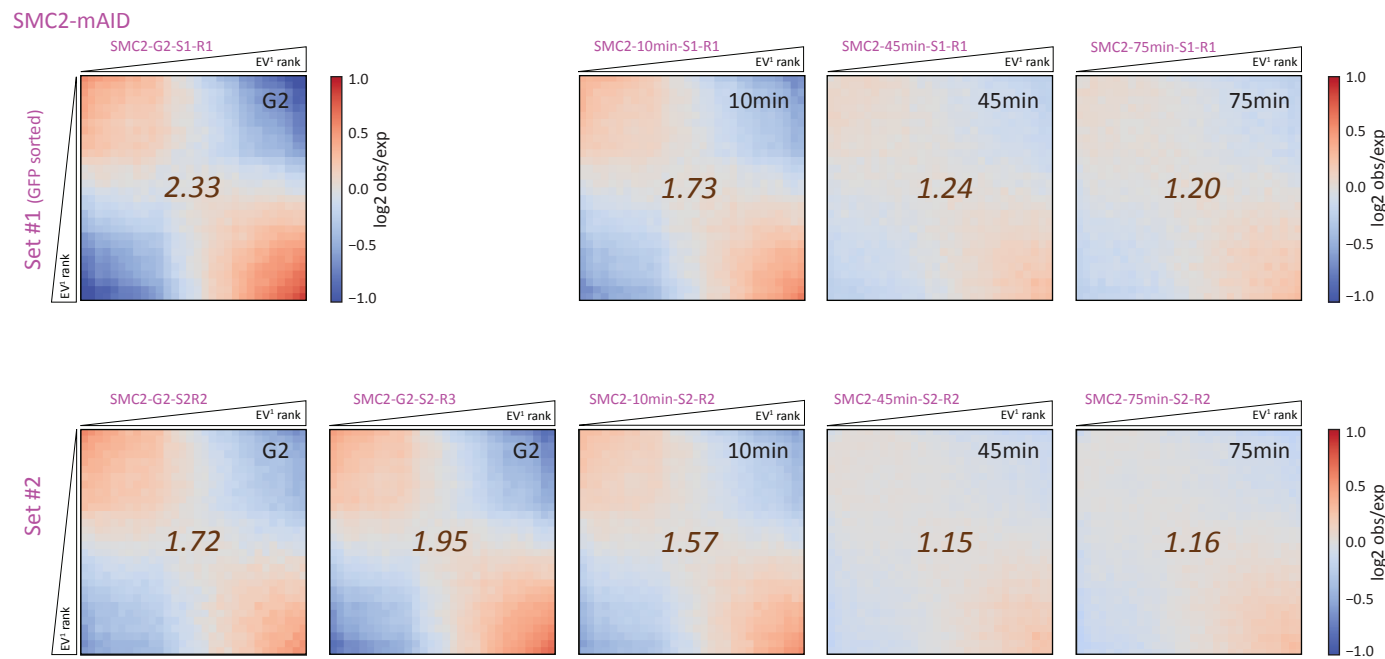


Fig.S21

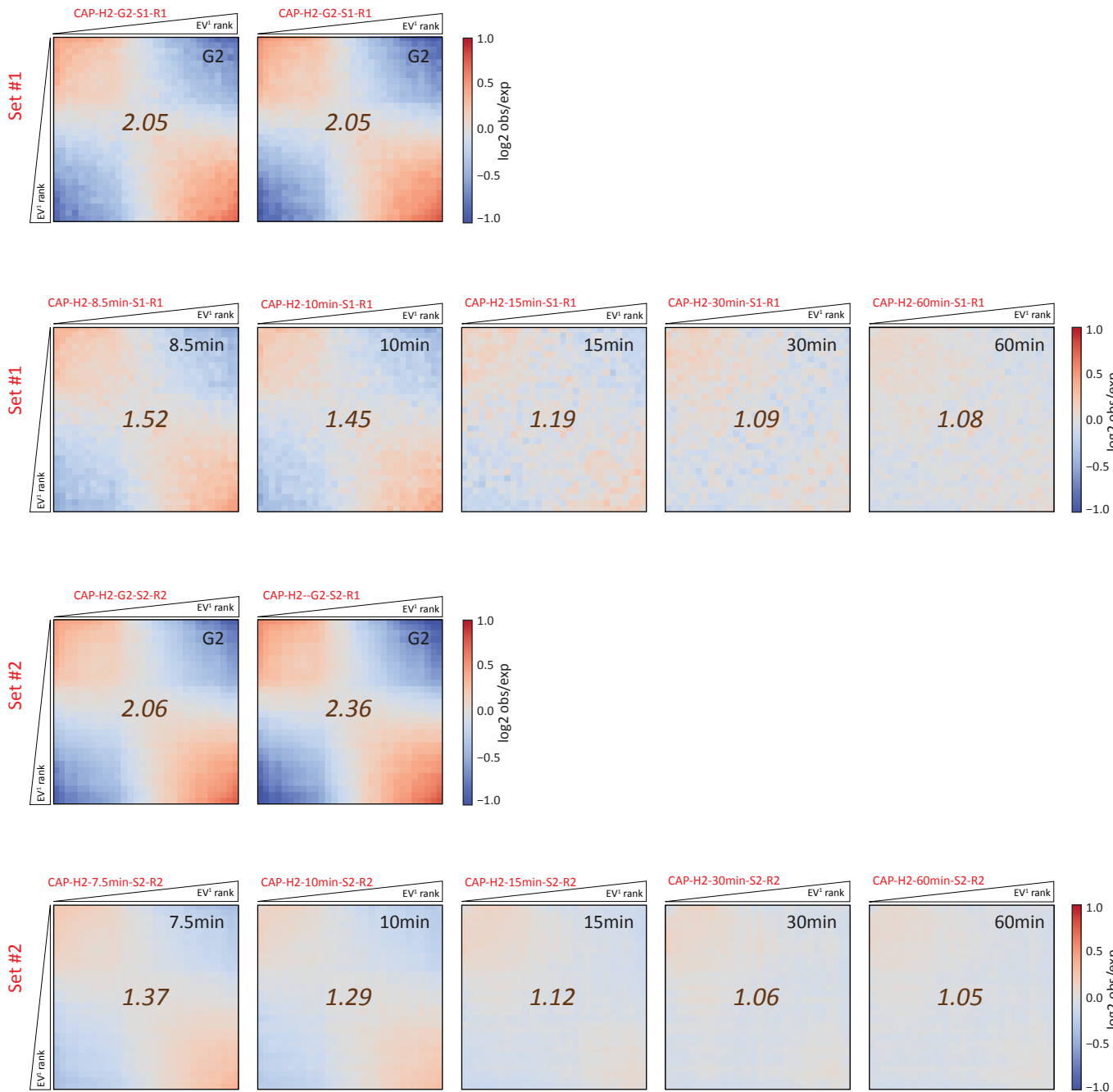
Microscopy analysis of chromosome morphology for wild-type (WT) cells and SMC2, CAP-H and CAP-H depleted cells. Chromosomes were stained with DAPI. Samples are ordered by time of harvest after release from G2 arrest. NEBD was used a marker for the prophase-prometaphase transition. Cells were prevented from entering anaphase by addition of nocodazole or MG132. Scale bar indicates 5 micron. Noc: nocodazole; MG: MG132.

A Saddle plots for all individual chromosome arms from SMC2-AID plus auxin



B Saddle plots for all individual chromosome arms from CAP-H2-mAID plus auxin

CAP-H2-mAID



C

Saddle plots for all individual chromosome arms from CAP-H-mAID plus auxin

CAP-H-mAID

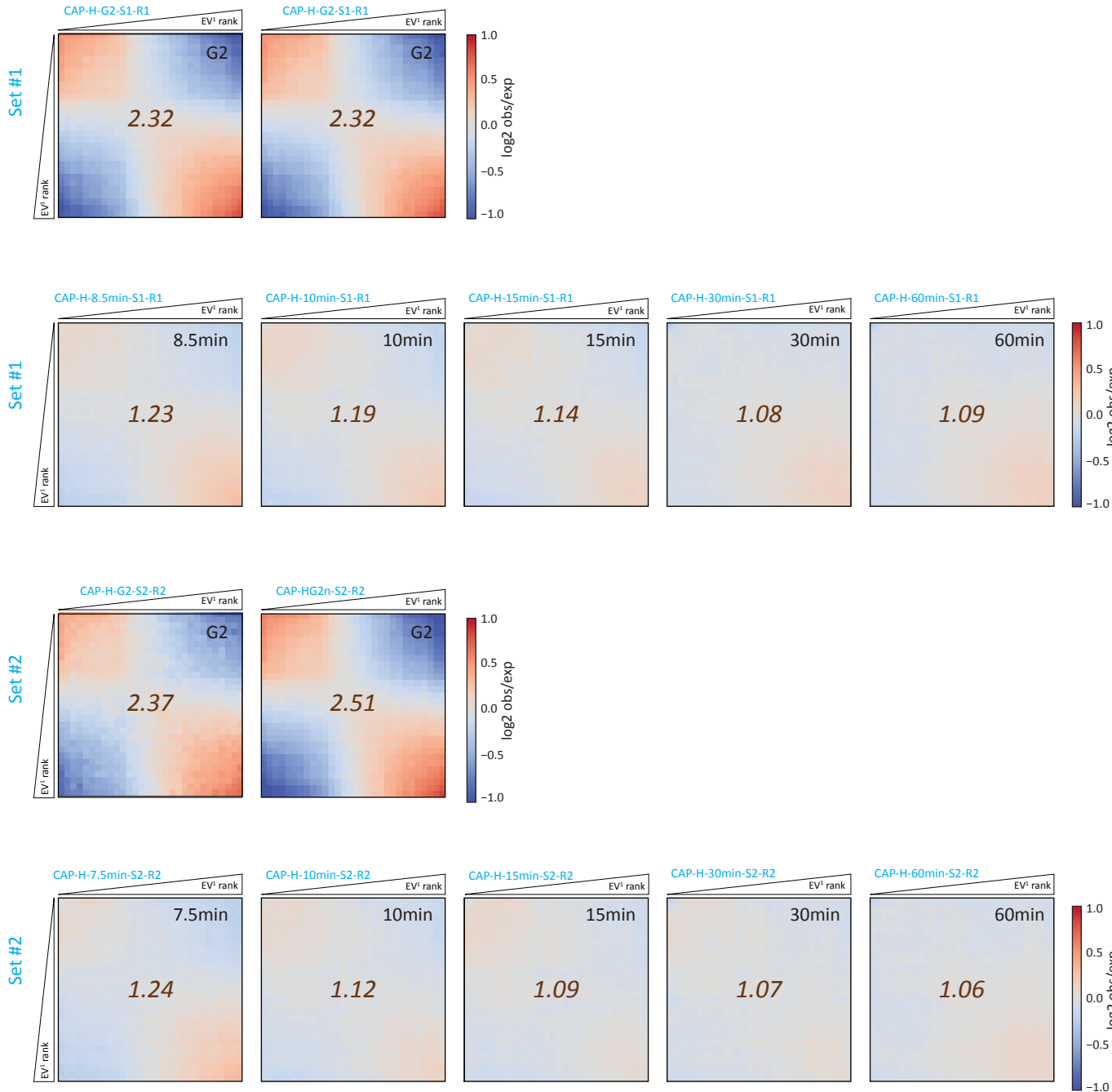


Fig.S22

Quantification of compartment interactions in SMC2, CAP-H and CAP-H2 depleted cells by saddle plots. (A): SMC2-mAID cells; (B): CAP-H2-mAID cells; (C): CAP-H-mAID cells. See supplementary Materials and legend of **Fig. S9** for description of saddle plots.

Normalized insulation at TAD boundaries, chromosome 7 in condensin mutants plus auxin

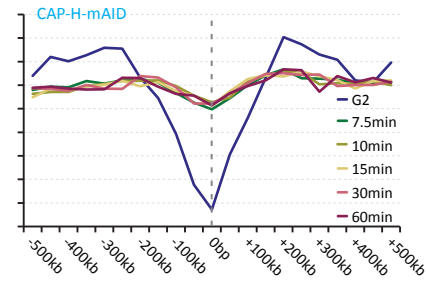
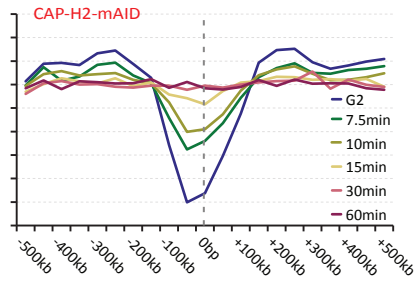
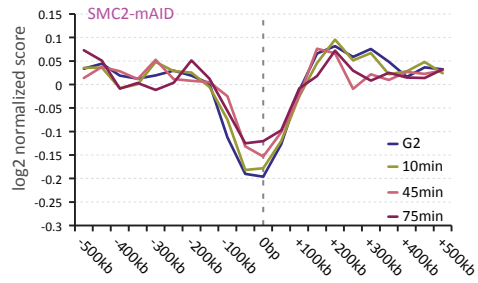
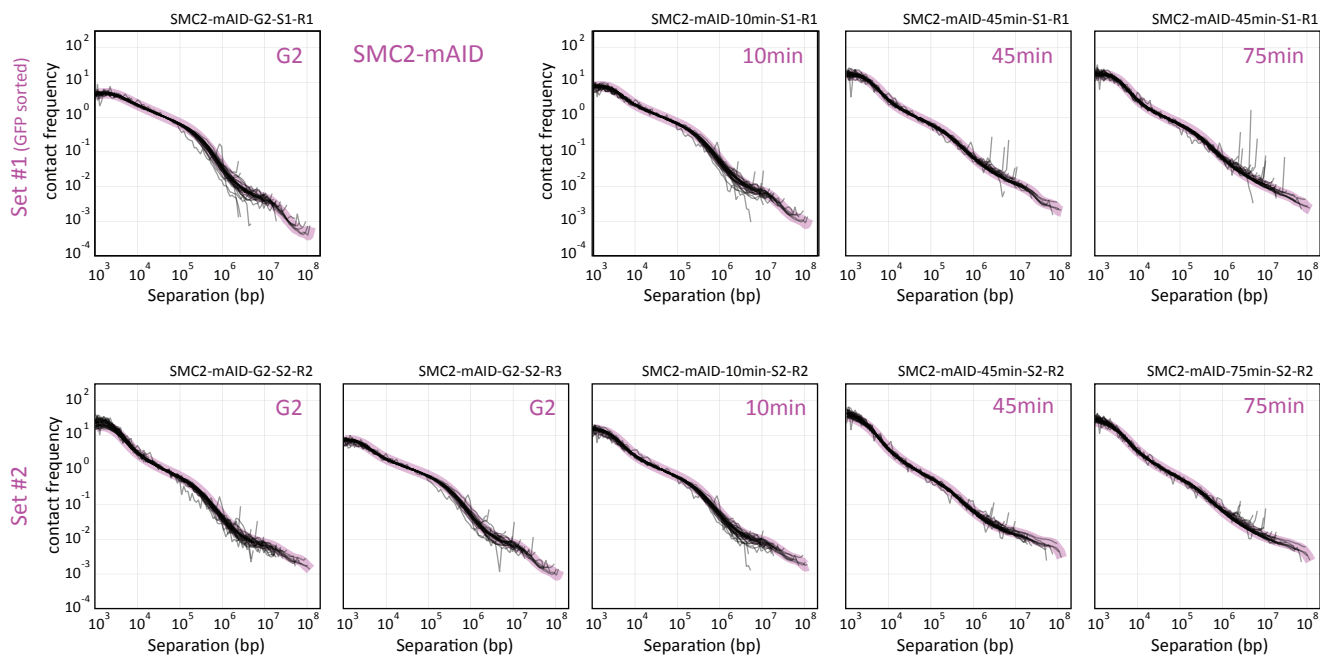
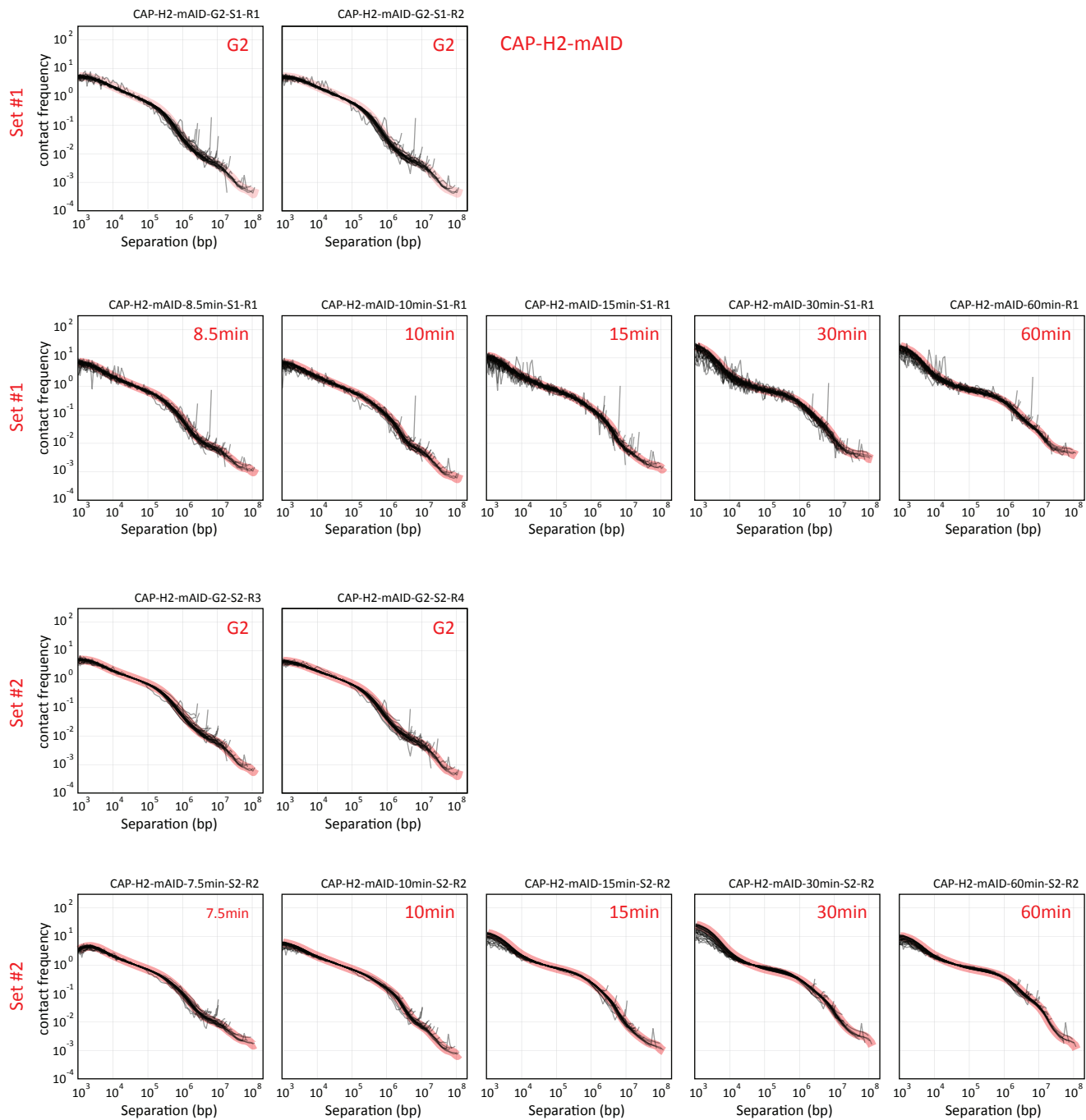


Fig.S23

Average insulation profiles around G_2 TAD boundaries on chromosome 7 for cells depleted for SMC2, CAP-H2 or CAP-H at different timepoints in mitosis. Plots show the sum of the interactions for each 100 kb bin 500kb upstream or downstream of the TAD normalized by the average sum of interactions for each time point. The presence of a minimum at 0 kb indicates a TAD boundary. SMC2 depletion prevents complete loss of the minimum, indicated maintenance of TAD boundaries. Depletion of CAP-H2 leads to slower loss of TAD boundaries as compared to WT (**Fig. S10**). Depletion of CAP-H does not prevent efficient loss of TAD boundaries.

A Contact frequency $P(s)$ plots for all individual chromosome arms auxin

BCAP-H2-mAID contact frequency $P(s)$ plots for all individual chromosome arms plus auxin

C

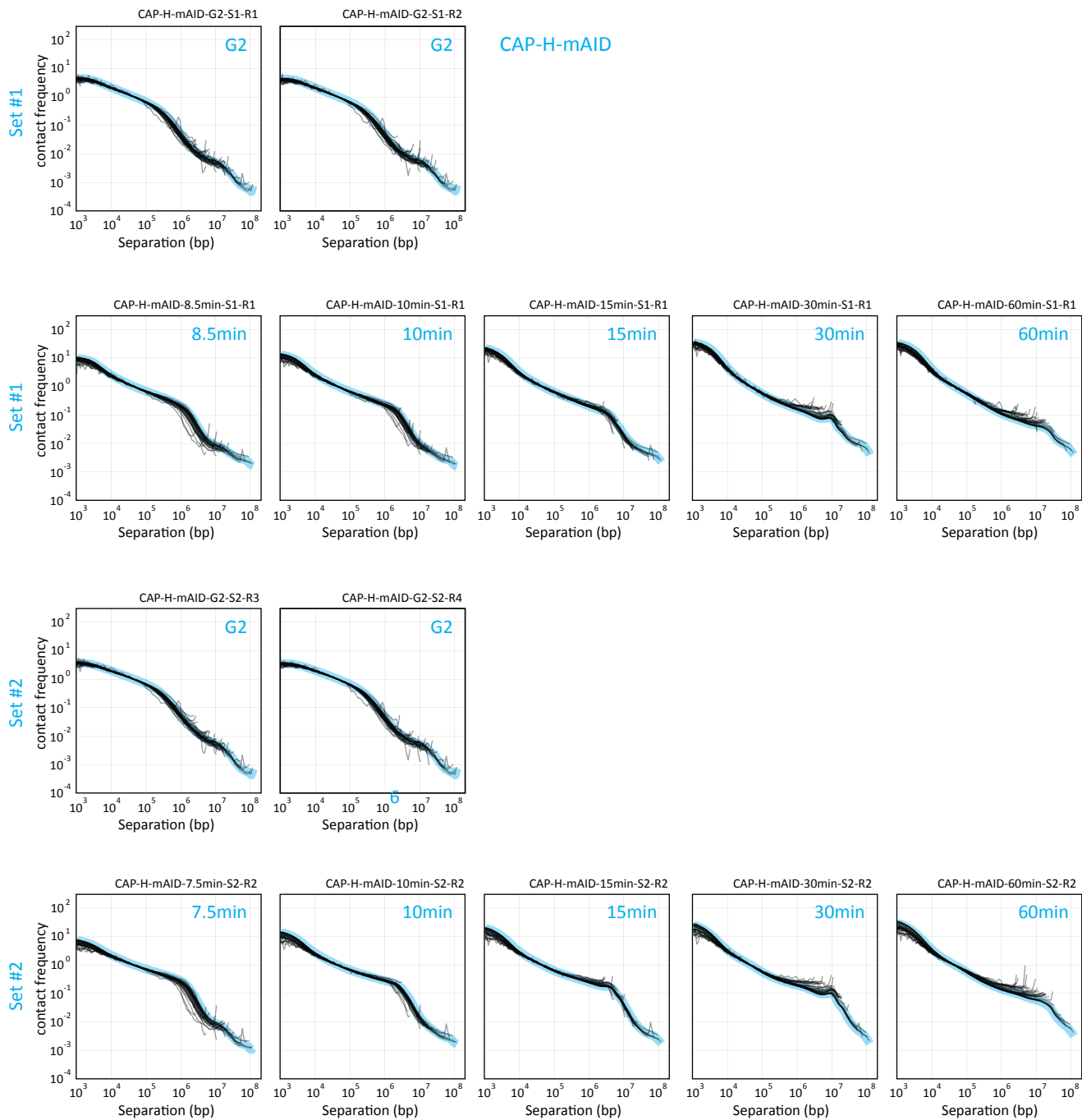
CAP-H contact frequency $P(s)$ plots for all individual chromosome arms plus auxin

Fig.S24

Contact frequency ($P(s)$) derived from Hi-C data obtained from CDK1 as synchronized DT40 cells depleted for SMC2, CAP-H2 or CAP-H. (A): SMC2-mAID cells; (B): CAP-H2-mAID cells; (C): CAP-H-mAID cells. Contact frequency $P(s)$ plots are shown for all Hi-C datasets (two independent time courses). Plots for individual chromosome arms are shown in gray; the deviation from the mean decay is shown in magenta (SMC2), red (CAP-H2-mAID) or blue (CAP-H-AID).

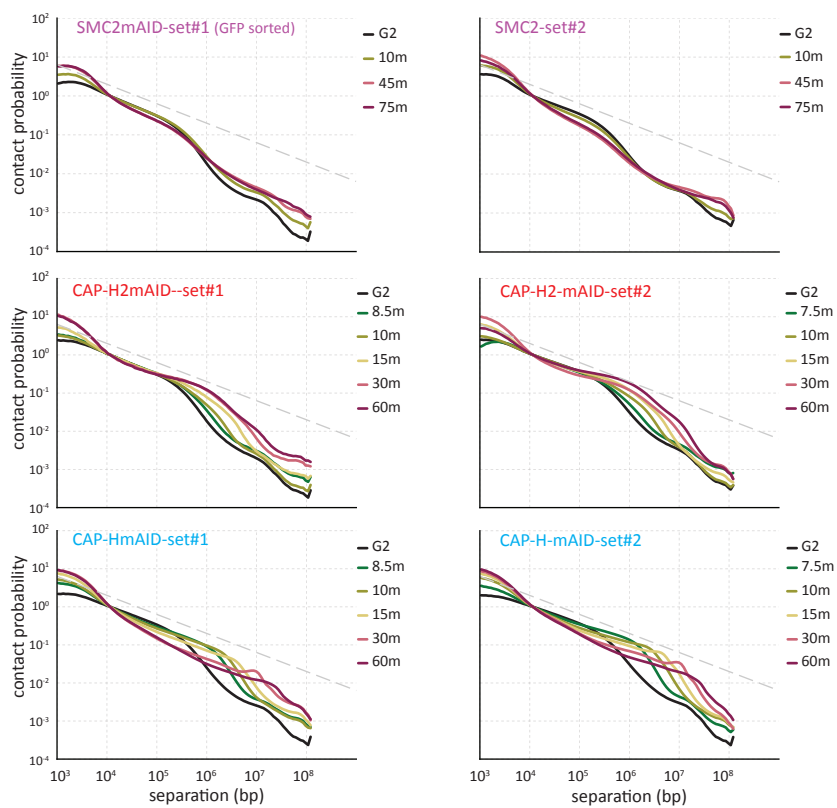
Contact frequency $P(s)$ plots for all chromosome arms combined from condensin mutants plus auxin

Fig.S25

P(s) plots for SMC2-mAID, CAP-H2-mAID and CAP-H-mAID DT40 cells at different time after degron induction and release from G2 arrest. *P(s)* plots for Hi-C datasets corresponding to different timepoints in each time course experiment are plotted in one graph.

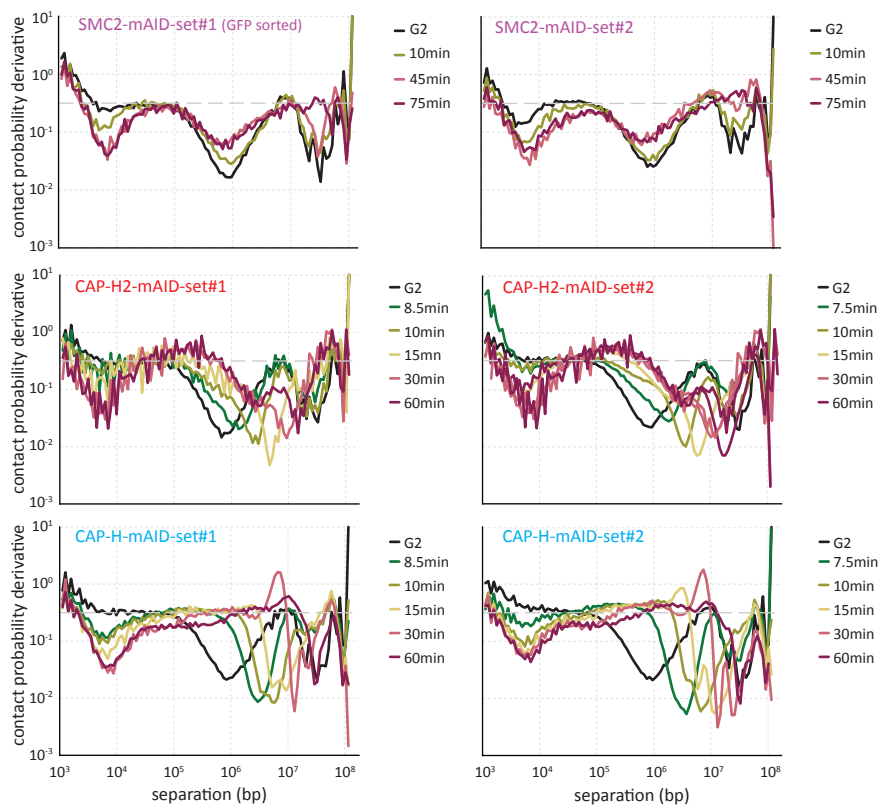
Contact frequency $P(s)$ derivative plots for all chromosome arms combined from condensin mutants plus auxin

Figure S26

Derivatives of $P(s)$ plots for SMC2, CAP-H2 or CAP-H depleted cells. Plots show derivative of $P(s)$ plots for Hi-C datasets obtained from SMC2-mAID, CAP-H2-mAID and CAP-H-mAID CDK1as cells at indicated time points after auxin-induced protein depletion and release from G₂ arrest.

Fig.S27

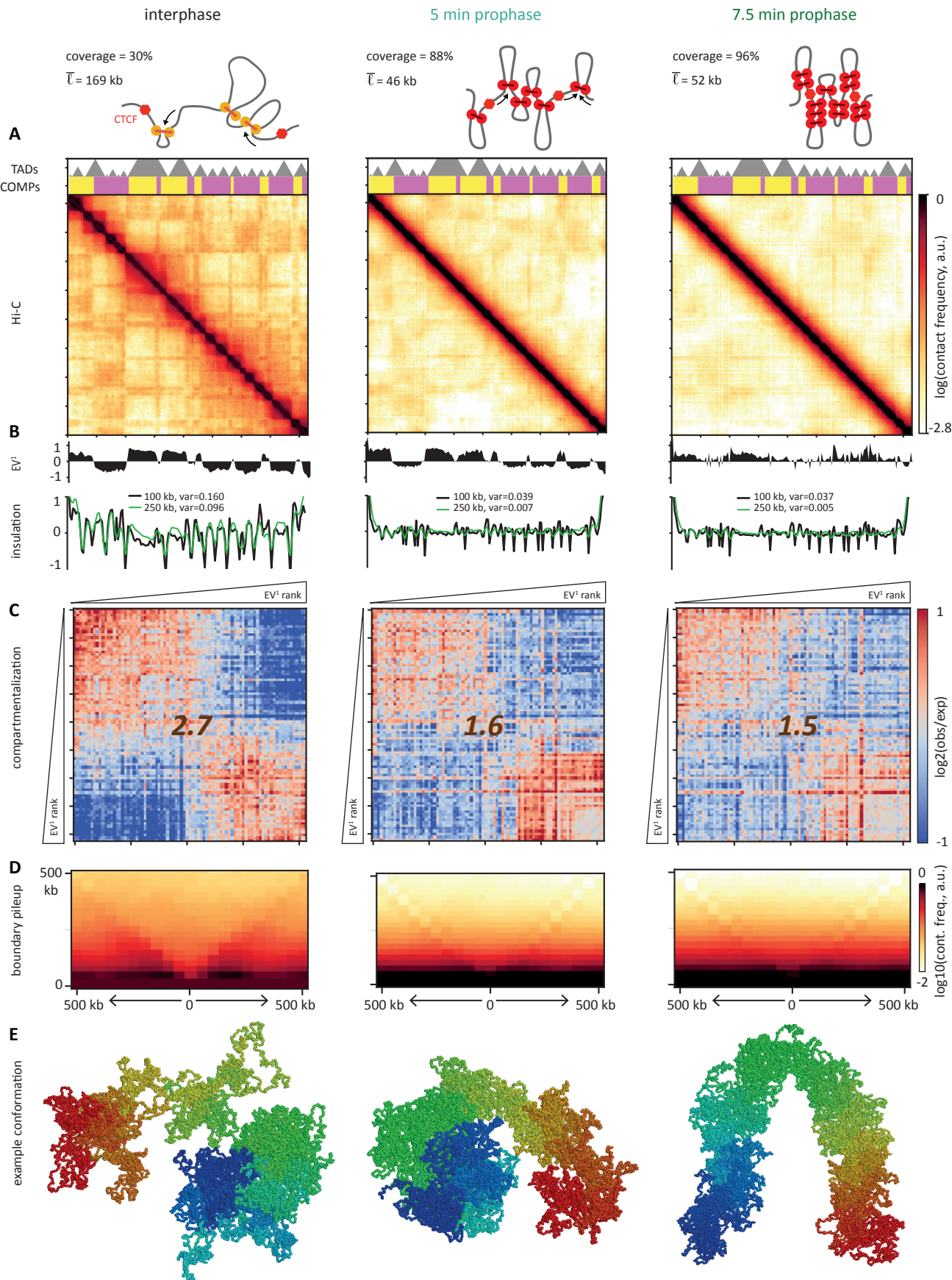


Figure S27

Chromosome compaction by loop extrusion suppresses TADs and compartments even in the presence of CTCF. A possible mechanism for the loss of TADs (gray rectangles in **(A)** and insulation signal in **(B)**) and A/B compartments in prophase is revealed by polymer simulations with loop extrusion and compartmental interaction. In interphase, the chromatin fiber is locally compacted into TADs by sparse loop extrusion factors (LEFs, yellow). Compartmentalization (yellow/magenta COMPs in **(A)** and EV^1 in **(B, C)**) emerges from preferential interactions between loci of the same compartment type. In prophase, coverage of chromosomes by LEFs (red) increases strongly. According to this model, TADs disappear in prophase because CTCF sites are now just one of many loci where loops are stacked up against each other and contacts across them are as frequent as between them (TAD boundary pileup in **(D)**), compartmentalization is suppressed (compartmentalization plots in **(C)**), because the bottlebrush imposes linear ordering and reduces fiber flexibility. Example conformation renderings are shown in **(E)**.

Tables S1-S3

Table S1

Sample and FACS summary. All samples with their cell type used for Hi-C libraries (name_galGal5) are listed by the names used throughout all figures. For each cell collection (time), the main cell cycle phase, treatments and drugs used are listed. FACS analysis was used to determine AID-GFP presence (and level of protein degradation). A subset of cells was sorted (sorted) for GFP positivity or negativity respectively, as indicated.

Table S2

Hi-C dataset overview. The names used corresponds to those used in figures and **Table S1**. Each harvested cell sample was submitted to Hi-C as part of a set (Set#) and the sequencing results and mapping statistics for galGal5 (gg5) are listed. Valid pair: reads mapped for both ends; NRVP (non-redundant valid pairs): valid pairs with duplicate reads removed (see Supplementary Methods); %Dangling: percentage of dangling ends (inward reads [\rightarrow | \leftarrow] derived from false biotin incorporation); %cis: percentage of intra-chromosomal interactions; %redundant: percentage of exact (PCR) duplicate reads (used to determine NRVP).

Table S3

DNA constructs used for generation of cell lines. Mutant: name used throughout the manuscript; background: cell line used to generate modified cell line; construct: vector used to generate mutants; FBOX: the specific FBOX protein used to degrade the AID-fused mutant; endogenous: the modification to the endogenous locus using homologous recombination (HR) or CRISPR; alleles: the number and location of altered alleles in DT40 mutants; selection: antibiotic resistance used for mutant selection; publication: previous work using these mutants.

Table S4

Short- and long-distance parts of the $P(s)$. Equalization of the contribution of the short- and long-distance parts of the $P(s)$ was calculated using the r.m.s. $P(s)$ difference in each of these regions separately and reporting the mean of the two.

Movie S1

The emergence of nested loop architecture in loop extrusion lattice simulations of slow- and fast-exchanging condensin populations. The video shows the simulated dynamics of loops extruded by condensins I and II on a 2Mb chromatin segment during two hours of simulated time. The positions of loops formed by fast-exchanging condensins I are shown as blue semi-transparent arcs and those formed by slow-exchanging condensin II are shown in red. The detailed description of simulations is available in **Supplemental Materials and Methods**.

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