Supplementary Materials for

Nanoscale 3D DNA tracing reveals the mechanism of self-organization of mitotic chromosomes

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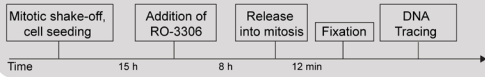[4] Max Planck Institute of Biochemistry, Martinsried, Germany

Contents:

A

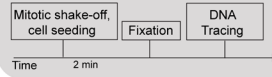**Synchronisation for mitotic entry**
(pro-/prometaphase)

Mitotic shake-off, cell seeding		Addition of RO-3306		Release into mitosis		Fixation		DNA Tracing

Time   15 h   8 h   12 min

Synchronisation for metaphase stage
(suitable for all mitotic stages except prophase)

Mitotic shake-off, cell seeding		Fixation		DNA Tracing

Time   2 min

B

Before FISH   After FISH

Denaturing FISH   Non-denaturing FISH   Control

Rigid registration

Affine registration

5 µm

Pearson correlation coefficient

Denaturing FISH
Non-denaturing FISH
Control

Rigid registration   Affine registration

C

Interphase   Metaphase

Trace completeness (%)

Chr2   Chr5   Chr14   Chr2   Chr14   Chr18   Chr2   Chr14

1.2 Mb
12 kb resolution

10 Mb
200 kb resolution

Full chromosome
1 Mb resolution

D

Interphase   Metaphase

12 kb spots   30 kb spots

Relocalization precision [nm]
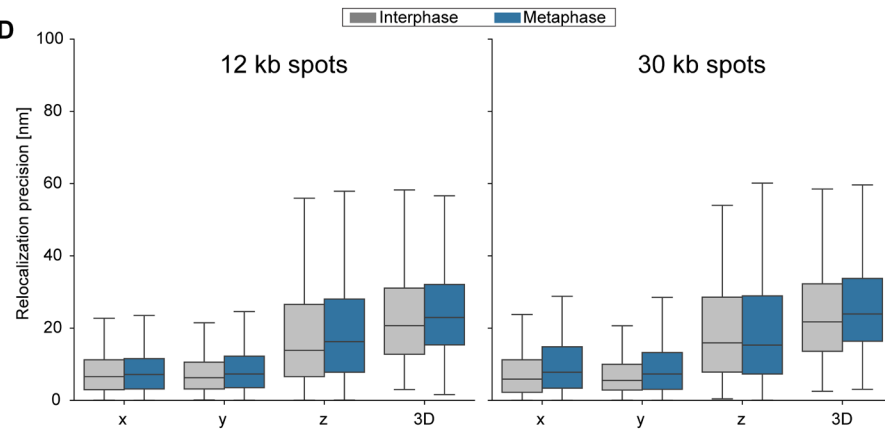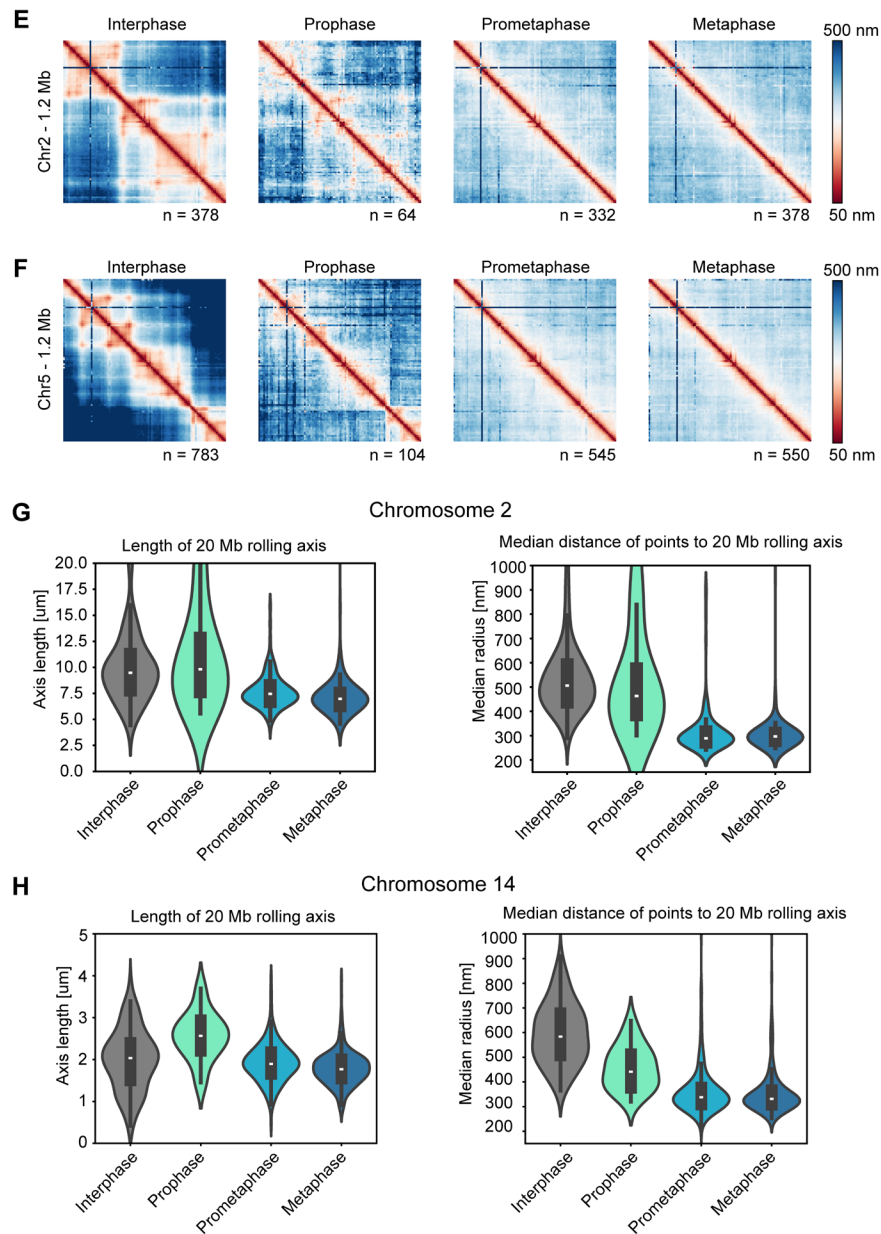
x   y   z   3D   x   y   z   3D

2

Supplementary Figure S1

A Synchronization schemes for cells enriched in pro-/prometaphase (left) and metaphase (right).

B Representative images (left) and pixel-wise Pearson correlation coefficient (right) of PFA-fixed mitotic cells labelled with DAPI and imaged before (magenta) and after (green) treatment with heat-denaturation FISH, non-denaturing/RASER-FISH or simulated FISH (non-denaturing FISH without library hybridization, control). Two registration algorithms were tested, with affine registration showing improved overlay accuracy as uniform scaling was better compensated. Data from one experiment with n= 92 cells (denaturing FISH), n=78 cells (non-denaturing FISH) and n=72 cells (control).

C Trace completeness as measured by the percentage of possible spots detected per trace across different libraries in interphase and metaphase. Data from 13455 traces in 1712 cells in 5 independent experiments. HeLa cells contain a truncated copy of chromosome 2, which reduces the completeness of whole chromosome 2 traces compared to other regions. Median, quartiles and whiskers (1.5*IQR) are shown in the plots.

D Tracing precision as measured by the absolute deviation in fit position after drift correction when the same genomic position was re-labelled in a different imaging cycle. Data from n=373 (interphase, 12 kb),
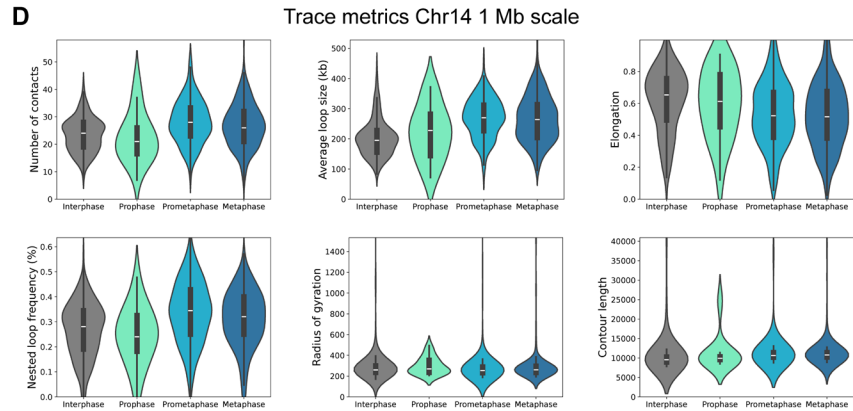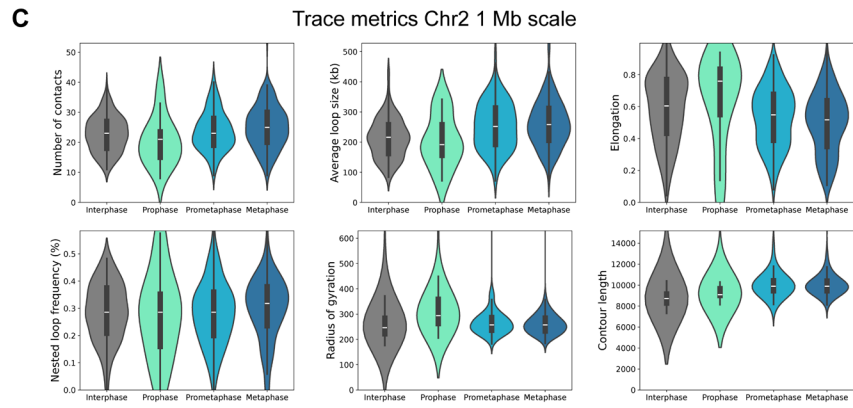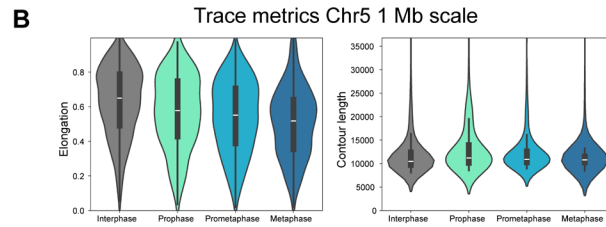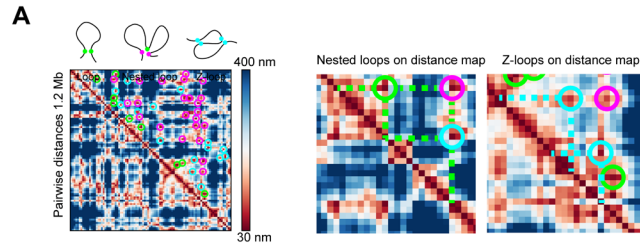
n=395 (metaphase, 12 kb), n=198 (interphase, 30 kb) and n=250 (metaphase, 30 kb) traces in one representative experiment of 2 independent experiments.
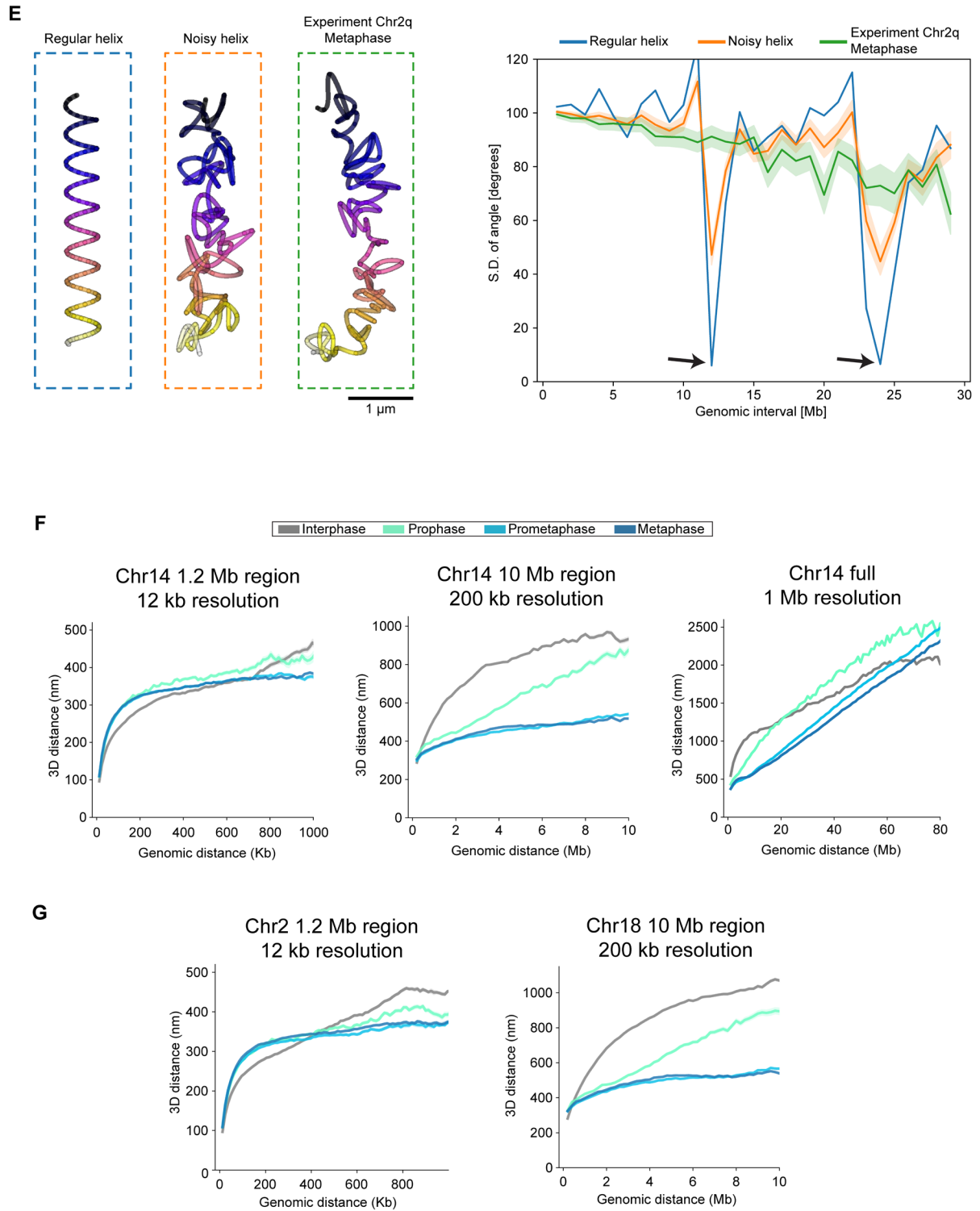
E Median pairwise distance maps of a 1.2 Mb region (Chr2:191110000-192309940) traced at 12 kb resolution. Number of traces as indicated from in total 666 cells in 2 independent experiments.

F Median pairwise distance maps of a 1.2 Mb region (Chr5:149500723-150699922) traced at 12 kb resolution. Number of traces as indicated from in total 874 cells in 3 independent experiments.

G Length and width of mitotic chromosomes, estimated by length of a 20 Mb rolling average axis and median distance of each point to the closest rolling average from chromosome 2 full chromosome library (1 Mb resolution). Data from 117 interphase, 19 prophase, 168 prometaphase and 212 metaphase chromosomes from 3 independent experiments. Median, quartiles and whiskers indicated are indicated in the plots.

H Data corresponding to **G** for Chromosome 14). Data from 112 interphase, 21 prophase, 292 prometaphase and 273 metaphase chromosomes from 3 independent experiments.

A

Nested loops on distance map   Z-loops on distance map

B

Trace metrics Chr5 1 Mb scale

C

Trace metrics Chr2 1 Mb scale

D

Trace metrics Chr14 1 Mb scale

5

E

F

G

Supplementary Figure S2

A Exemplary pairwise distance map showcasing the classification of loops. Nested loops (magenta) emerge when the bases of two loops (green) merge to form a loop contact with the size of the two base loops. Z-loops (cyan) overlap with each other partially.

B Additional trace metrics for chr5:149500723-150699962. Elongation indicates the ratio of minor to major axis of an ellipsoid fit to the trace, while contour length measures the cumulative point-to-point length of the trace. The similar contour length for the different phases indicates that the other structural metrics that change during mitosis are well sampled at this genomic resolution. Data from

n=278 (510), n=22 (44), n=124 (297) and n=152 (398) inter-, pro-, prometa- and metaphase cells (traces) from 3 independent experiments. Median, quartiles and whiskers are shown in the plots.

C Trace metrics for high resolution tracing in chromosome 2:191110000-192309940. Data from n=171 (246), n=13 (19), n=100 (180) and n=134 (248) inter-, pro-, prometa- and metaphase cells (traces) from 2 independent experiments.

D Trace metrics for high resolution tracing in chromosome 14:50923646-52104342. Data from n=136 (189), n=9 (15), n=94 (160) and n=118 (210) inter-, pro-, prometa- and metaphase cells (traces) from one experiment.

E Representative examples of a simulated regular helix (left), a regular helix with added gaussian noise (center) and experimental data from chromosome 2q (right), and plotted estimation of helical regularity. Helical regularity was measured by the standard deviation in radial angle between points separated by different genomic intervals. The radial angle was measured compared to a 20 Mb rolling average axis. Local minima in the standard deviation indicating helical regularity are indicated by arrows. Data from 100 generated regular or noisy helices and the 100 best resolved chromosome 2q traces from 3 independent experiments. Mean and 95% confidence interval (estimated by bootstrapping) shown.

F Pairwise distance scaling plots for chr14:50923646-52104342 (12 kb resolution), chr14:45200003-56429971 (200 kb resolution) and chr14:20000036-105029664 (1 Mb resolution). Data from n=677 (1243) cells (traces), 2 independent experiments; n=645 (1201) cells (traces), 2 independent experiments and n=543 (1039) cells (traces), 2 independent experiments, respectively. Plots show median ± standard error of the mean.

G Pairwise distance scaling plots for chr2:191110000-192309940 (12 kb resolution), chr18: 50000077-62829816 (200 kb resolution). Data from n=711 (1280) cells (traces), 2 independent experiments and n=633 (1134) cells (traces), 2 independent experiments, respectively. Plots show median ± standard error of the mean.
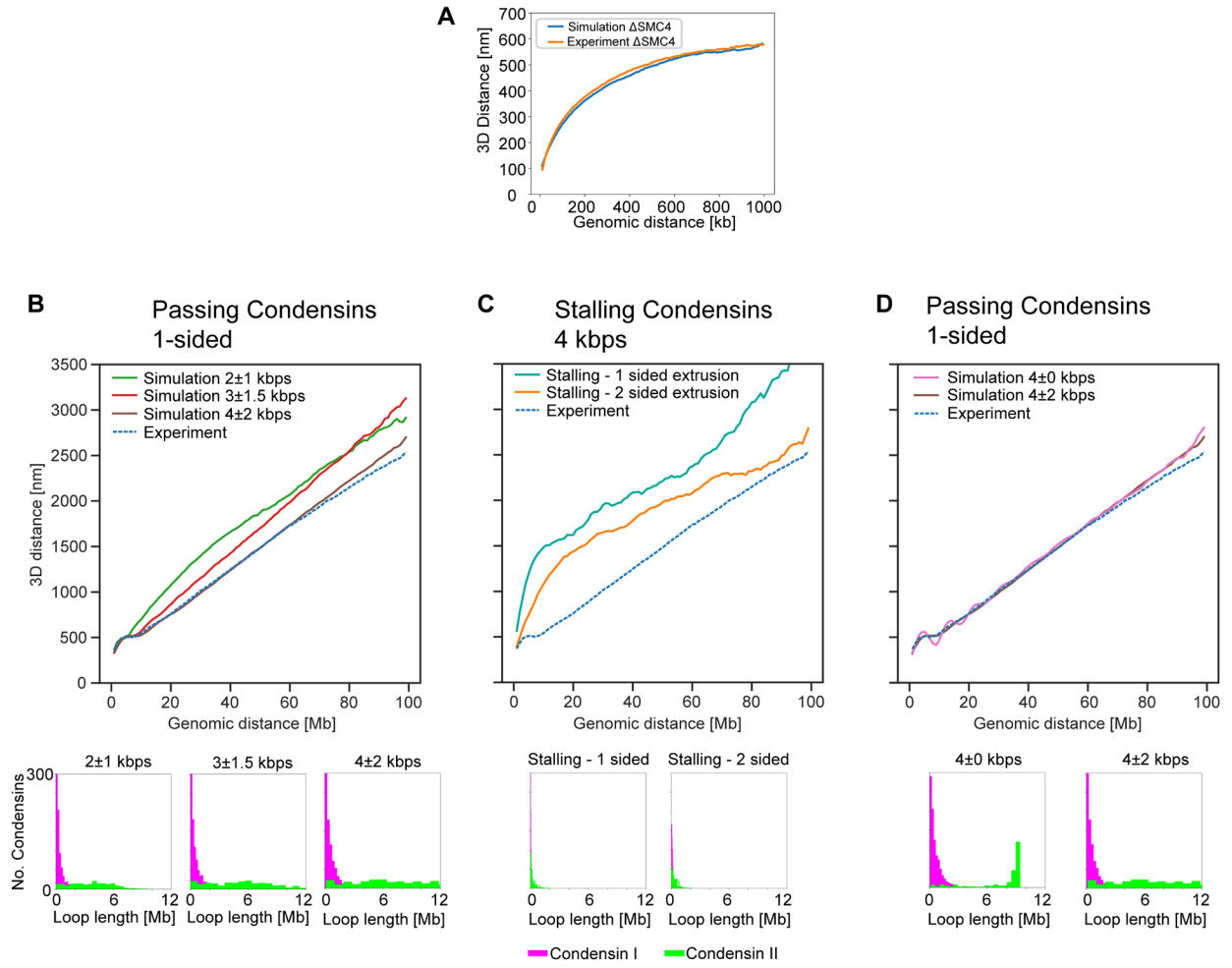
A Condensin depletion (ΔSMC4) & mitotic synchronization
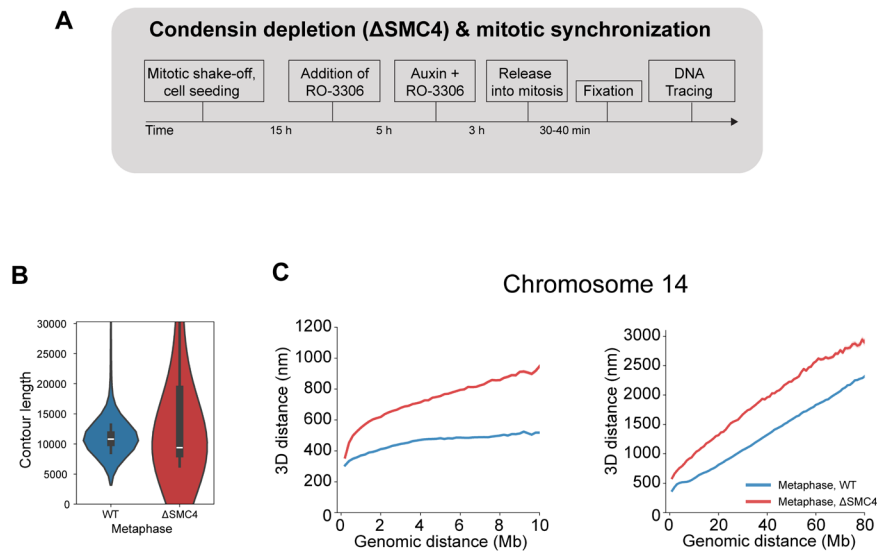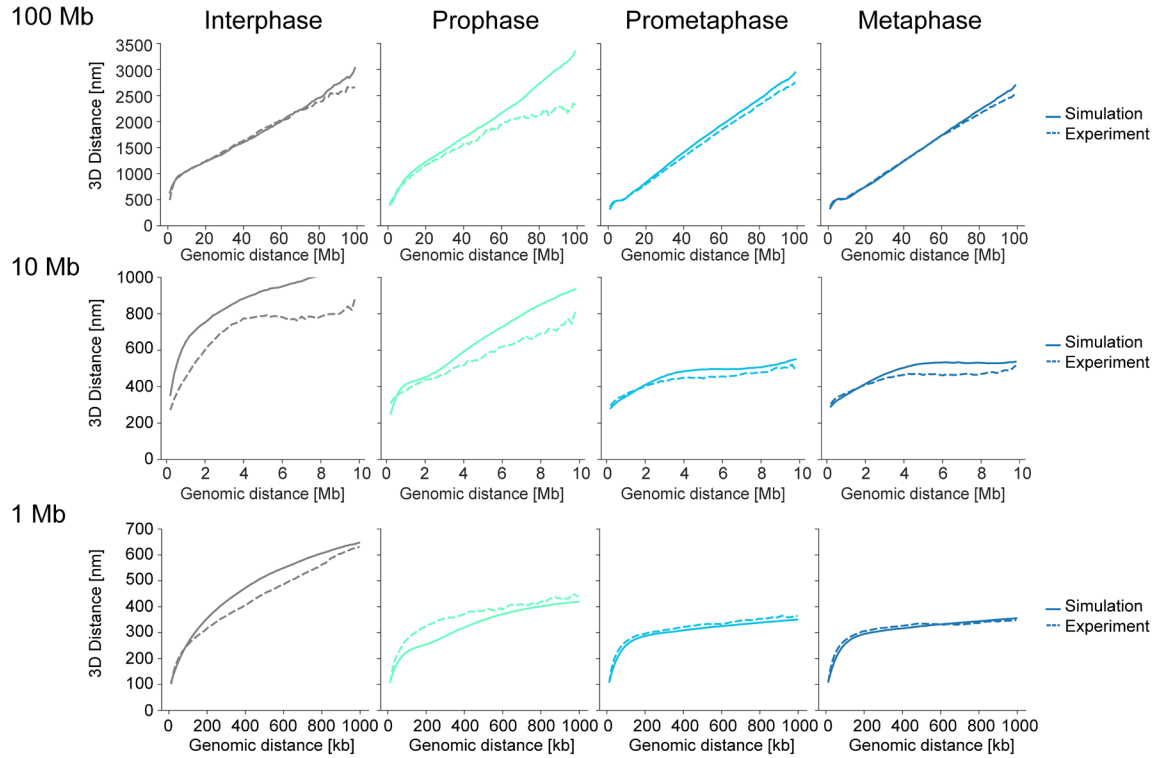
Supplementary Figure S3

A Experimental scheme for Condensin-depleted mitotic chromosomes, using the HK SMC4-mAID cell line (Schneider et al., 2022).

B Trace metric from chr5:149500723-150699962 (1.2 Mb, 12 kb resolution) for WT and ΔSMC4 cells. Data from 152 (398) WT cells (traces), 3 independent experiments, and 153 (265) ΔSMC4 cells, 2 independent experiments.

C Distance scaling plots for chromosome 14 10 Mb and whole chromosome scales. Data from Chr14, 10 Mb: 218 (434) WT cells (traces), 2 independent experiments and 217 (419) ΔSMC4 cells (traces), 2 independent experiments; Chr14, whole: 174 (357) WT cells (traces), 2 independent experiments and 78 (146) ΔSMC4 cells (traces), one experiment.

A Simulation ΔSMC4, Experiment ΔSMC4

B Passing Condensins 1-sided

Simulation 2±1 kbps
Simulation 3±1.5 kbps
Simulation 4±2 kbps
Experiment

2±1 kbps, 3±1.5 kbps, 4±2 kbps

C Stalling Condensins 4 kbps

Stalling - 1 sided extrusion
Stalling - 2 sided extrusion
Experiment

Stalling - 1 sided, Stalling - 2 sided

D Passing Condensins 1-sided

Simulation 4±0 kbps
Simulation 4±2 kbps
Experiment

4±0 kbps, 4±2 kbps

Condensin I   Condensin II

E 100 Mb, 10 Mb, 1 Mb

Interphase   Prophase   Prometaphase   Metaphase

Simulation, Experiment

F

**G** Metaphase radial distribution

Supplementary Figure S4

A Distance scaling plot of an unconstrained polymer to experimental ΔSMC4 metaphase high-resolution tracing data (12 kb resolution, Chr5) gave 12 nm/kb monomer and 1 k_bT repulsive potential as optimal polymer simulation parameters. Experimental data from 212 (608) ΔSMC4 cells (traces), 2 independent experiments.

B-D Distance scaling plots and histograms of Condensin I (magenta) and Condensin II (green) loop lengths resulting from alternative loop extrusion models, including (**B**) altered extrusion speed, assuming one-sided extrusion and no stalling, (**C**) One- and two-sided extrusion at 4±2 kbps with full stalling, and altered extrusion speed distributions assuming one-sided extrusion and no stalling. Simulation positions were sampled as experimental data (100 kb spots, 1 Mb resolution) ten times per 100 Mb chromosome with offset genomic positions. Simulated data from 10-20 dynamically simulated metaphase (40 minutes) chromosomes per condition. Experimental data from Chr2q, 100 Mb: n=212 (686) cell (traces), 3 independent experiments.

E Distance scaling plots of simulated chromosomes, assuming one-sided extrusion at 4±2 kbps and no stalling, and corresponding experimental data. Simulated traces were spatially sampled as experimental data (12 kb tiled probes, 30 kb probes with 200 kb resolution and 100 kb probes with 1 Mb resolution). Sampling timepoints were 0 min, 10 min, 32 min and 40 min for inter-, pro-, prometa- and metaphase, respectively. Simulated data from n=20 dynamically simulated 100 Mb chromosomes. Experimental data from HeLa WT cells, see Fig. 3 and Fig. S3 for detailed information.

F Ground-truth positions of Condensin I (magenta) and Condensin II (green) in simulated chromatin (2 kb sampling in light grey, 1 Mb rolling average in dark grey), corresponding to the example in Fig. 5A. **G** Radial distribution of Condensin I, II and chromatin in simulated 100 Mb chromosomes. Radial distances were calculated compared to a 20 Mb rolling average axis for 20 dynamically simulated chromosomes sampled at metaphase (40 minutes), assuming one-sided extrusion at 4±2 kbps and no Condensin stalling.
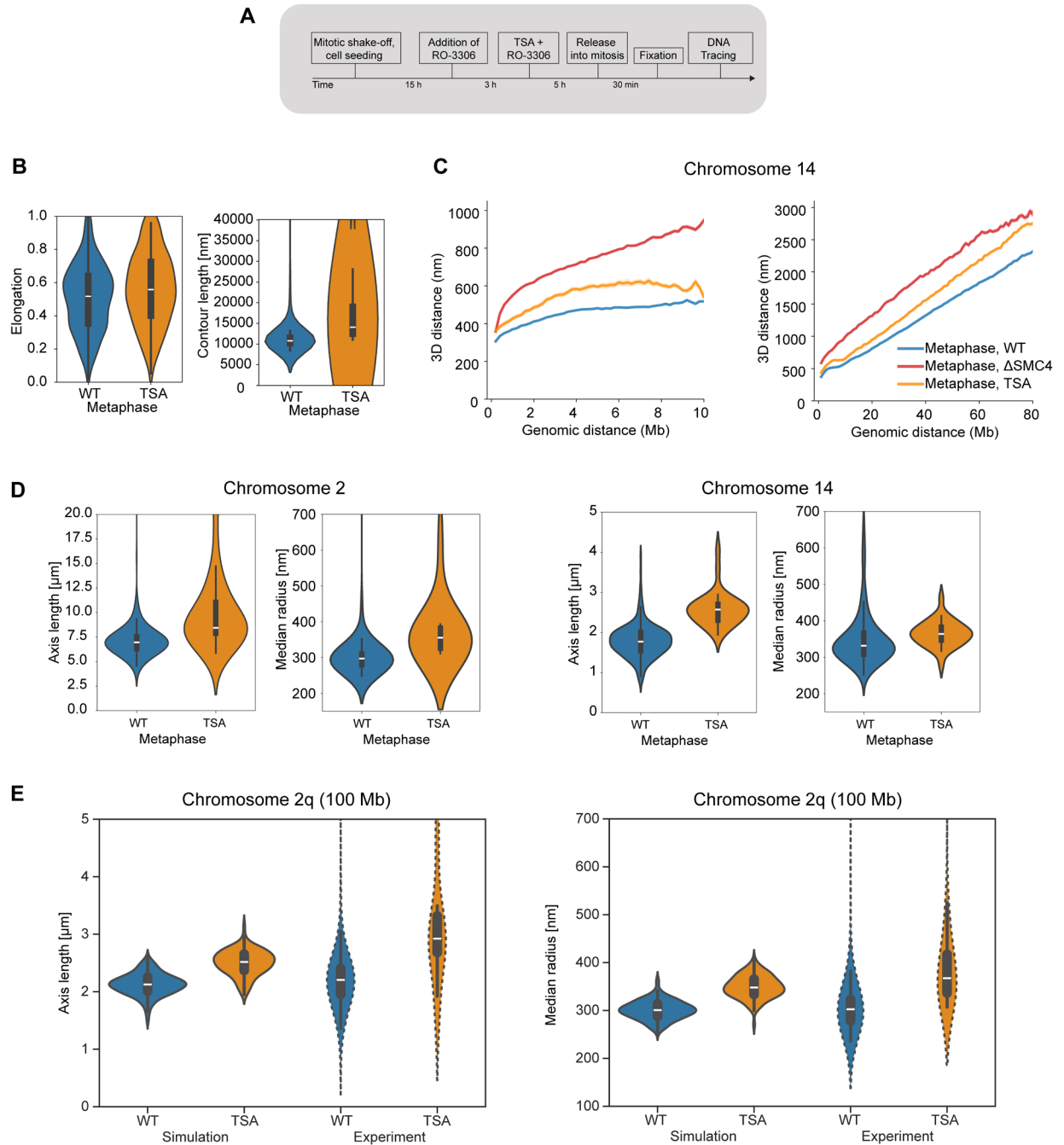
Supplementary Figure S5

A Experiment scheme for the generation of hyperacetylated mitotic chromosomes using TSA treatment during interphase.

B Trace metric from chr5:149500723-150699962 (1.2 Mb, 12 kb resolution) for WT and TSA-treated cells. Data from 152 (398) WT cells (traces), 3 independent experiments, and 67 (168) TSA-treated cells (traces), 2 independent experiments.

C Distance scaling plots from Chr 14, 10 Mb and whole chromosome scales for WT, ΔSMC4 and TSA-treated cells at metaphase. Chr14, 10 Mb: 69 (133) TSA-treated cells (traces), 2 independent experiments; Chr14, whole: 15 (30) TSA-treated cells (traces), one experiment. See Fig. S4 for details on WT and ΔSMC4 data.

D Length and width of WT and TSA-treated metaphase chromosomes 2 and 14, estimated by length of a 20 Mb rolling average axis and median distance of each point to the closest rolling average point from

chromosome 2 whole chromosome traces (1 Mb resolution). Data from 130 (212) WT cells (traces), 3 independent experiments and 19 (31) TSA-treated cells (traces), 2 independent experiments.
E Length and width of 100 Mb simulated chromosomes under WT and TSA-treated conditions, and corresponding experimental data from chromosome 2 q-arm (100 Mb). Data from 20 dynamically simulated 100 Mb chromosomes per conditions, sampled at metaphase (40 minutes). Experimental data detailed in **D**.

Supplementary Table S1:

Loop extrusion simulation parameters

Parameter	Value	Reference
Condensin-I abundance	9.6/Mb	9,22
Condensin-II abundance	2.4/Mb	9,22
Condensin-I residence time (prophase)	0 s	9
Condensin-I residence time (prometa-/metaphase)	150 s	9
Condensin-II residence time (pro-/prometa-/metaphase)	3600 s	9
Extrusion rate	1-4 ± 0-2 kbps, see main text	32
(A)symmetric extrusion	One- or two-sided, see main text	8,32
Stall probability	0-100%, see main text	32
Duration prophase	10 min	9,31
Duration prometa- and metaphase	30 min	9,31
Simulation time-step	1 s	This work

Polymer simulation parameters

Parameter	Value	Reference
Number of monomers	100 000 (defined to 1kb/monomer)	This work
Chain/loop bond type	Harmonic bond	Polychrom documentation
Chain bond distance	1 (scaled to 12 nm in analysis)	This work
Chain bond standard deviation	10 % of chain bond	Polychrom documentation
Loop bond distance	50 % of chain bond	Polychrom documentation
Loop bond standard deviation	20 % of chain bond	Polychrom documentation
Periodic boundary conditions	Not used	This work
Collision rate	0.03	Polychrom documentation
Error tolerance	0.01	Polychrom documentation
Angle force	1.5	Polychrom documentation
Repulsive force type	Polynomial repulsive	Polychrom documentation
Repulsive force WT	1	Polychrom documentation
Repulsive radius WT	1.05	Polychrom documentation
Repulsive force TSA	10	This work
Repulsive radius TSA	1.75	This work
Initial polymer equilibration steps	100 000 steps	This work
MD steps per loop extrusion step	20 000 steps	This work
Sampling frequency of loop extrusion steps	Every 5 seconds	This work