

Fig. S1. Bridges link the axes of separated sister chromatids.

*A*. **Bridges occur along the shared axis interface of separated sister chromatids.** Paths of intensity-weighted axis centroids (red, blue), total chromatin centroids (green, white) and interaxis bridges between sister chromatids (dotted lines), shown for two prometaphase chromosomes. Red/blue and white/green denote regions of left/right-handed helicity.

*B.* Bridges are evenly spaced. Distribution of distances between adjacent bridges at late prophase (left) and prometaphase/metaphase (right) with best-fit gamma distribution. Average inter-bridge distance is 0.4  $\mu$ m in both cases. Degree of evenness, given by shape parameter of the gamma distribution, is n=6-10. (*A*–*B* reprinted from ref. S1, with permission from Elsevier.)

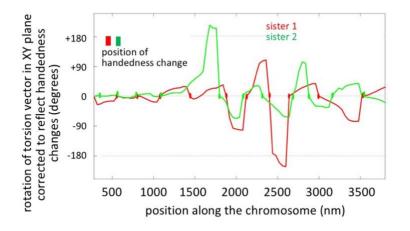


Fig. S2. Restricted torsion along a chromosome axis.

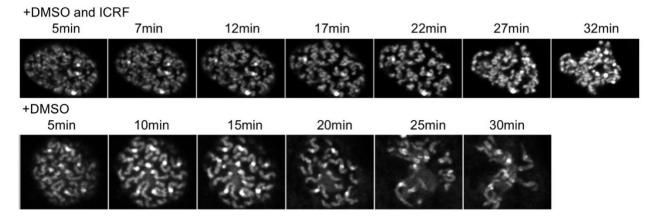
Rotation  $\theta$  of the curve along the long axis of the chromosome is plotted versus length for the chromatid in text Fig. 1*D left* (red) and its sister chromatid (green). Rotation is measured separately for left- or right-handed segment of centroid path (demarked by bars). The path is defined in cylindrical coordinates (r,  $\theta$ , h) as discussed in the methods section that describes determination of centroid helicity in (1). These results show that torsion is restricted: often, neighboring segments have alternating handedness with torsion vector making less than 2pi rotations in alternating directions.



#### Fig. S3. Perversions in a physical system.

Half-helical segments of alternating handedness linked by multiple perversions as predicted by mathematical modeling of twisting along an elastic rod of finite length with initial curvature and clamped ends. (Republished with permission of World Scientific Publishing Co., Inc., from ref. S2; permission conveyed through Copyright Clearance Center, Inc.)

#### LLC-Pk EGFP-TopIIa live cells



## Fig. S4. ICRF treatment of mid-prophase chromosomes results in hyper-deformation.

LLC-Pk EGFP-TopII $\alpha$  cells in mid-prophase were treated with DMSO, with or without ICRF-193, and imaged at indicated time points thereafter. Images in text Fig. 2*D* are t=22min (DMSO+ICRF) and t=25min (DMSO) after treatment.

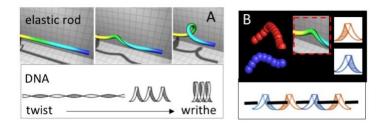


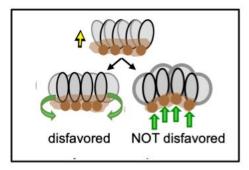
Fig. S5. Deformations from torsional stress along an elastic rod.

*A*. Increasing torsional stress along an elastic rod can create helicity by conversion of twist to writhe, as illustrated for a physical system (top) and for DNA (bottom). (Top) The right end of the rod is restrained in rotation but is allowed to translate towards the left end. (Bottom) Conformations that arise during topological conversion from twist to writhe along a DNA duplex. (*Top* adapted with permission from ref. S3. *Bottom* adapted with permission from ref. S4.)

**B.** Half-helical axis subsegment resembles that observed during initial deformation in the two systems shown in A.

# **Supplementary References**

- S1. L. Chu *et al.*, The 3D Topography of Mitotic Chromosomes. *Molecular Cell* in press. (2020).
- S2. G. Domokos, T. J. Healey, Multiple helical perversions of finite, intristically curved rods.
  *Int. J. Bifurc. Chaos* 15, 871-890 (2005).
- S3. S. Goyal, L. Todd, N. C. Perkins, E. Meyhofer, Cable dynamics applied to long lengthscale mechanics of DNA. *arXiv:physics/0702197* (2007).
- S4. N. R. Cozzarelli, J. C. Wang, DNA Topology and Its Biological Effects. (Cold Spring Harbor Laboratory Press, New York, 1990), pp.480.



## Fig. S6. Axis bending as constrained by chromatin compression.

Top: the chromosome axis (brown) in the visual loop/axis plane (yellow arrow). Bottom left: bending of the axis perpendicular to the visual loop/axis plane results in compression of loops on the inner surface of the bend (bottom left). Such compression is disfavored because DNA/chromatin intrinsically resists confinement (Ref. 14). Bottom right: In contrast, bending of the axis in the visual loop/axis plane does not result in compression of chromatin loops (bottom right).

The overall effect will be a tendency for bending (and thus helicity) to occur in the visual loop/axis plane, as is observed (text).