Supplementary Material

Condensin Controls mitotic chromosome stiffness and stability without forming a mechanically contiguous scaffold

Quantification of Western blot measurements of condensin subunits

Western blot band intensities were calibrated against a calibration blots from a gel where a series of dilutions of whole-cell extracts in PBS (from 100% to 5% cell extracts by volume) were run. Given the known concentrations relative to the undiluted case, this allowed corrections to be made for nonlinearity of band intensities as a function of protein concentration. The results were used to estimate the degree of protein knockdown in the siRNA experiments, and also to introduce slight corrections for variation of degree of knockdown to the micromechanical data via linear extrapolation (compare Fig. 5D and Fig. S9, which show essentially the same quantitative effect before and after this correction).

Quantification of condensin level in WT, colchicine-, and nocodazole-treated cells

We investigated the mechanism of how condensin level on chromosomes is up-regulated in metaphase-stalled cells. We examined the total condensin fluorescence in whole cells, for mitotic cells from WT and colchicine-treated cultures. In brief, WT and colchicine treated (14 hours) cell cultures were fixed in 0.37% formaldehyde in PBS, and permeabilized with 0.1% Triton X-100 in PBS. Condensin was immunostained with primary and secondary antibodies described in Materials and Methods. Mitotic cells from each condition were then imaged and the total fluorescence intensity were analyzed and normalized against WT cells. The total condensin fluorescence levels are

comparable between WT and colchicine-treated cells (99.89% confidence level), suggesting the expression of condensin for those two conditions is comparable (Fig. S10A).

We then examined the condensin fluorescence level on mitotic chromosomes isolated from WT and colchicine treated cells without fixation. Mitotic chromosomes were microsurgically removed from either WT or colchicine treated cells, then immunolabeled using microspray, as described previously. Total condensin fluorescence on each chromosome was analyzed, and normalized against WT chromosomes (Fig. S10B). Chromosomes from colchicine treated cells have a significantly higher level of condensins despite the size of each chromosome (2.9 ± 0.7), compared with that of WT (1 ± 0.3), with a P-value of 0.004.

To further investigate if the overloading of condensin is caused by one isoform of condensin, the ratio of condensin I and II was analyzed. In brief, isolated chromosomes were simultaneously labeled with condensin I and condensin II, and the ratio of fluorescence intensity for condensin I and II was plotted and normalized against WT chromosomes. The ratio of condensin I to condensin II was comparable, and only slightly elevated in chromosomes from colchicine treated cells (1.3 ± 0.2) (Figure S10C).

Finally, to test whether the results for overloading of SMC2 were specific to colchicine treatment, we carried out a series of 3 trials using nocodazole to stall HeLa cells in metaphase. We found that for similar stall times (14 hours) we had a similar overloading of SMC2 onto metaphase chromosomes with nocodazole compared to the colchicine results (Fig. S11).

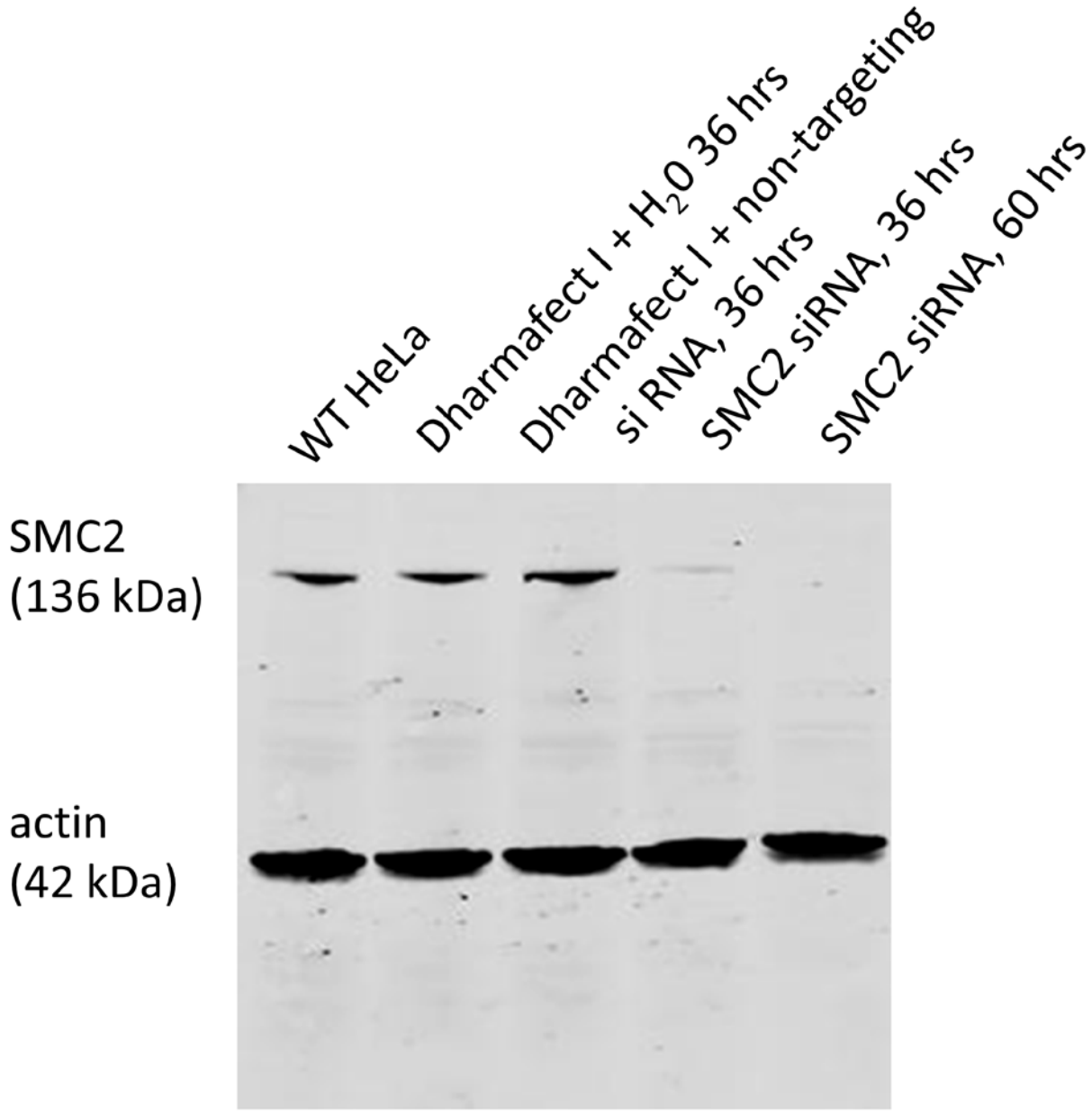

Figure S1. Western Blot of SMC2 in WT HeLa cells, controls, and SMC2 RNAi depleted cells. Controls were done by either using transfection reagent (Dharmafect I + water, lane 2) or transfection of non-targeting siRNA (Thermo Scientific, non-targeting siRNA sequence 1, lane 3). Western blot of actin was used as a loading control. SMC2 protein knockdown was quantified to be 90 ± 10% for cells after 36 hours of SMC2 siRNA transfection (Lane 4), via comparison of band intensities to that obtained from serial dilution of whole-cell extracts.

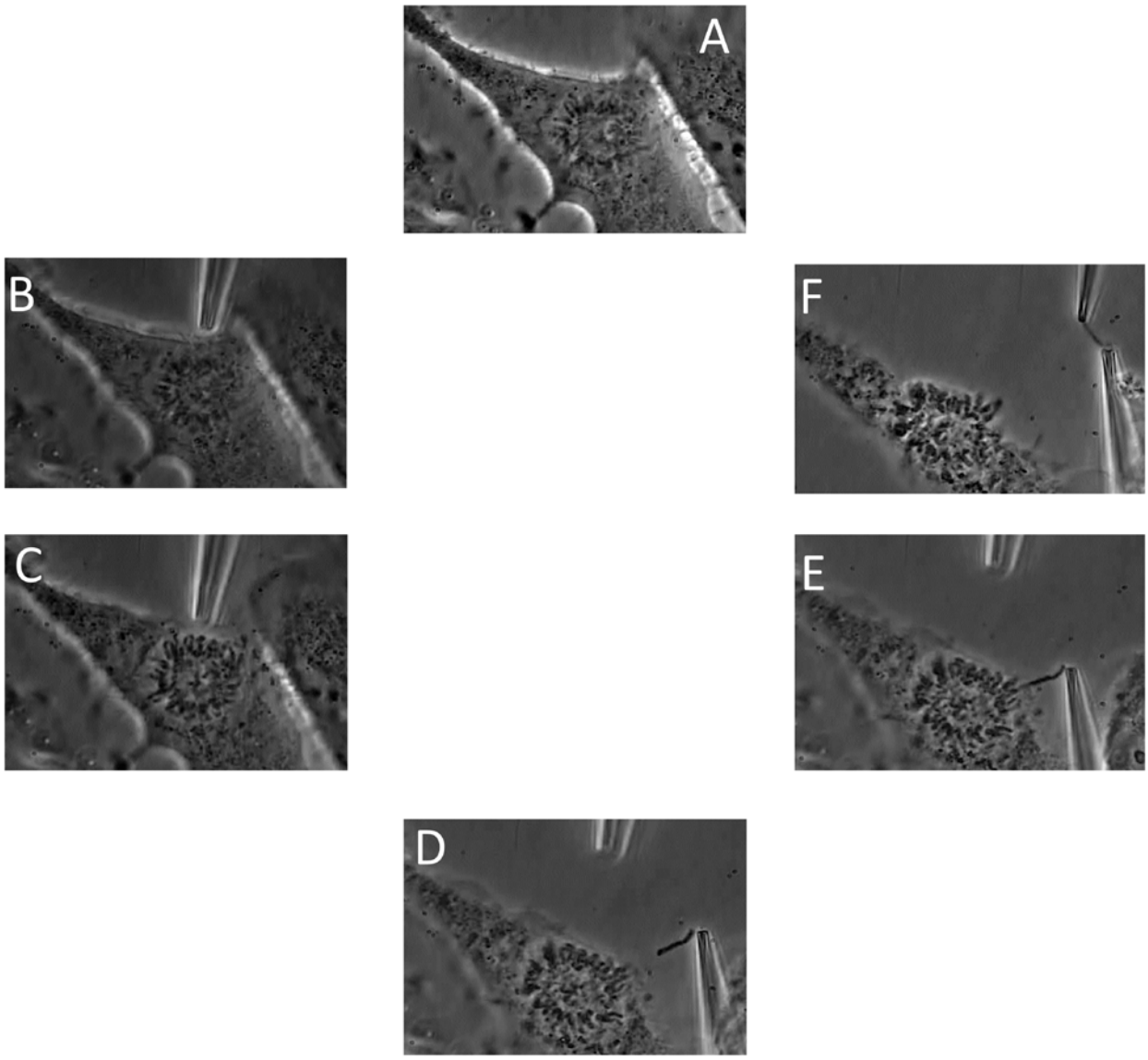

Figure S2. Chromosome isolation. Cells were identified using phase-contrast imaging (A). (B-C) A spray pipette filled with PBS with 0.1% Triton X-100 was introduced into the flow cell to gently disrupt the cell membrane locally. (D-E) A second pipette was introduced to capture one end of a single chromosome. (F) A force pipette is introduced to capture the second end of the chromosome.

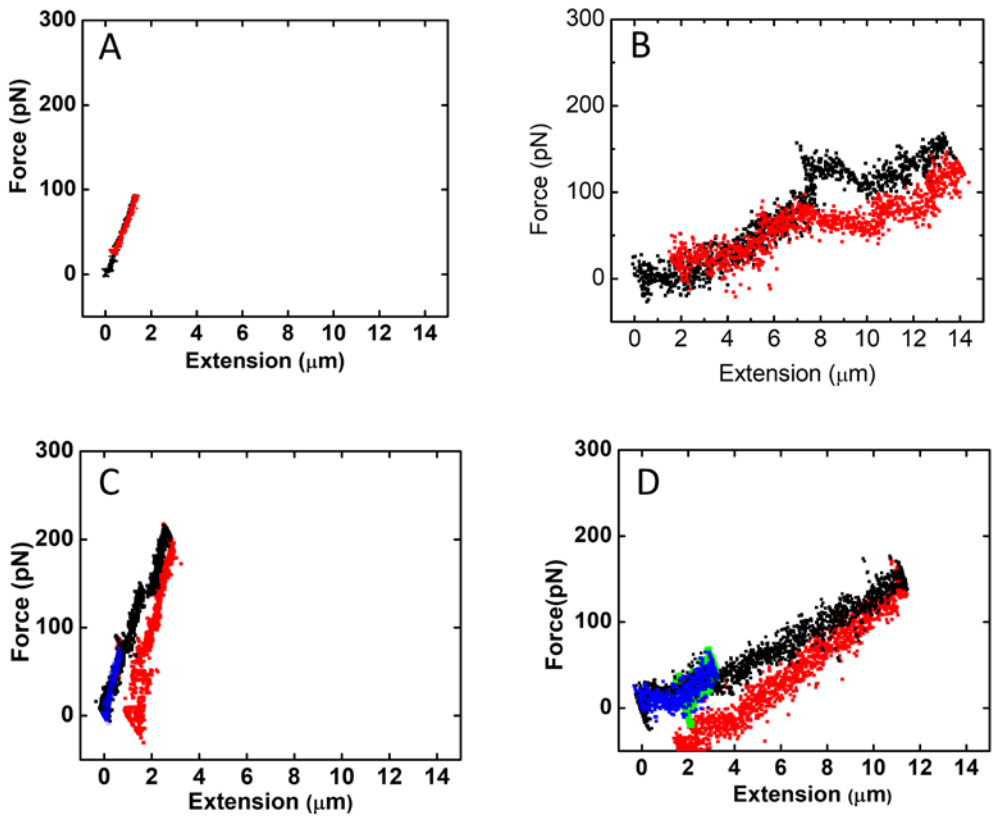

Figure S3. Force-extension curves for WT and condensin-subunit-depleted chromosomes, plotted on same extension and force scales for comparison.

(A) WT (untreated) chromosome elastic response in low-force-reversible force regime (data of Fig. 3Ad).

(B) Condensin-depleted (SMC2 siRNA) chromosome with high extension and stretching force showing irreversible force response. Black dots represent increasing extension and red dots represent the subsequent decrease in extension. The strong lack of irreversibility of the measurement is likely due to a combination of elastic failure of the very soft chromosome, combined with instrumental drift for the few-minute-long extension-retraction cycle, which leads to force measurement errors in the ~50 pN range.

(C) Condensin I-depleted (hCAP-G siRNA) chromosome force response (data of Fig. 5B).

(D) Condensin II-depleted (hCAP-G2 siRNA) chromosome force response (data of Fig. 5C).

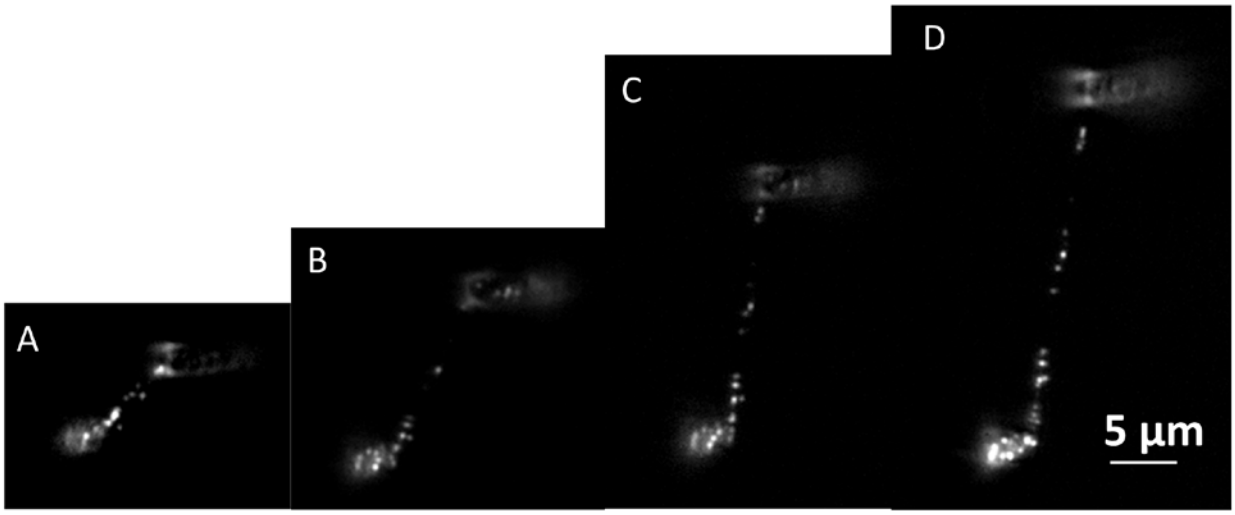

Figure S4. SMC2 staining pattern using antibodies diluted in 100% PBS. Bar = 5µm.

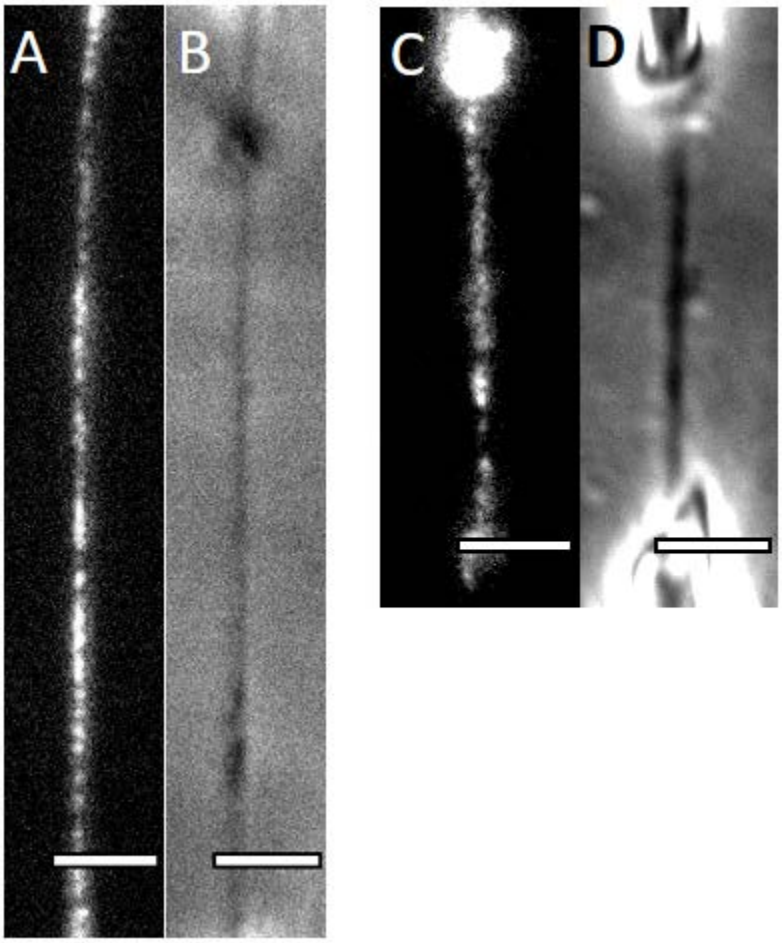

Figure S5. Patterning of anti-SMC2 binding is modulated for pre-stretched chromosomes, and for chromosomes labeled with SMC2 Fab fragments. (A-B) A chromosome stretched before spraying full-length anti-SMC2 and secondary antibodies. (C-D) Staining of SMC2 with Fab fragmented primary and secondary antibodies. Bars = 5μm.

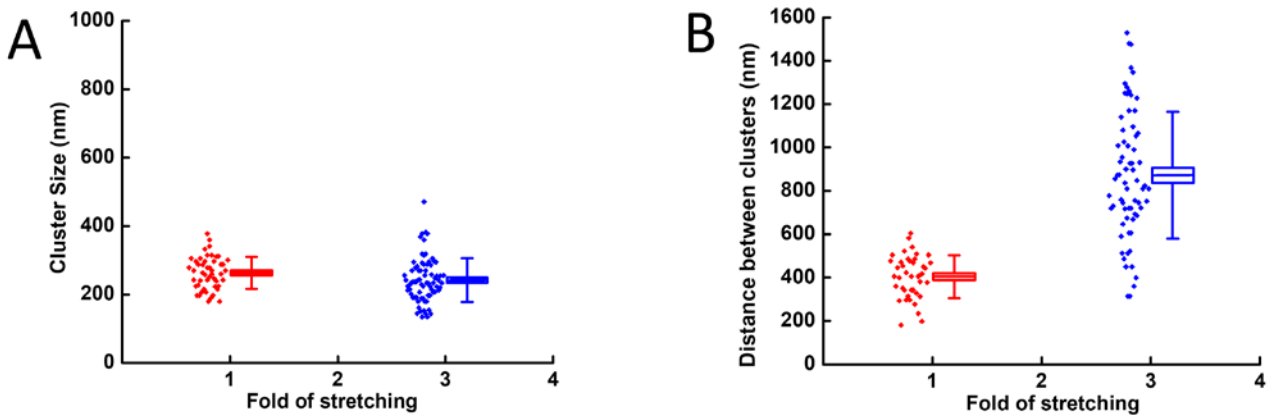

Figure S6. The size of condensin clusters and distances between the adjacent clusters as a function of chromosome stretching. (A) Size of clusters measured by the diameter of dots labeled by condensin staining at native chromosome length and those when the chromosomes were stretched to 3 fold of their native length. The average cluster sizes were 260 ± 7 nm and 240 ± 7 nm, at native chromosome length and 3 fold stretching of the native length, respectively. Boxes indicate standard errors while bars indicate standard deviations. (B) Distances between adjacent clusters as a function of chromosome stretching. Average distances were 400 ± 20 nm and 860 ± 40 nm, at native chromosome length and 3 fold times the native length, respectively. The overall chromosome stretching is measured by measuring the end to end distance of the whole chromosome.

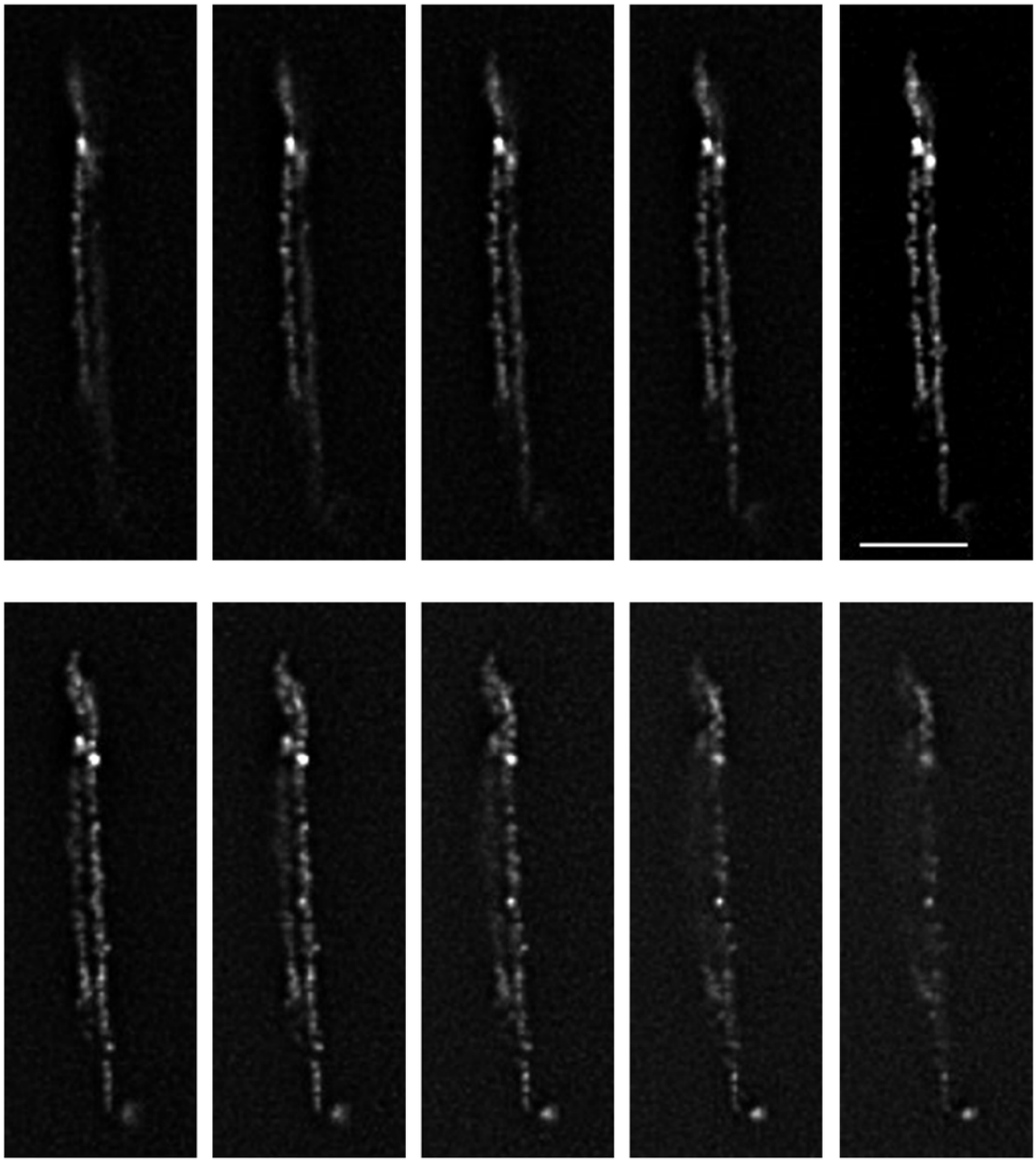

Figure S7. Z-stack of SMC2 staining on a stretched chromosome. Left to right, z-stack from top to bottom, distance between each slice is 200 nm. Bar = 5µm.

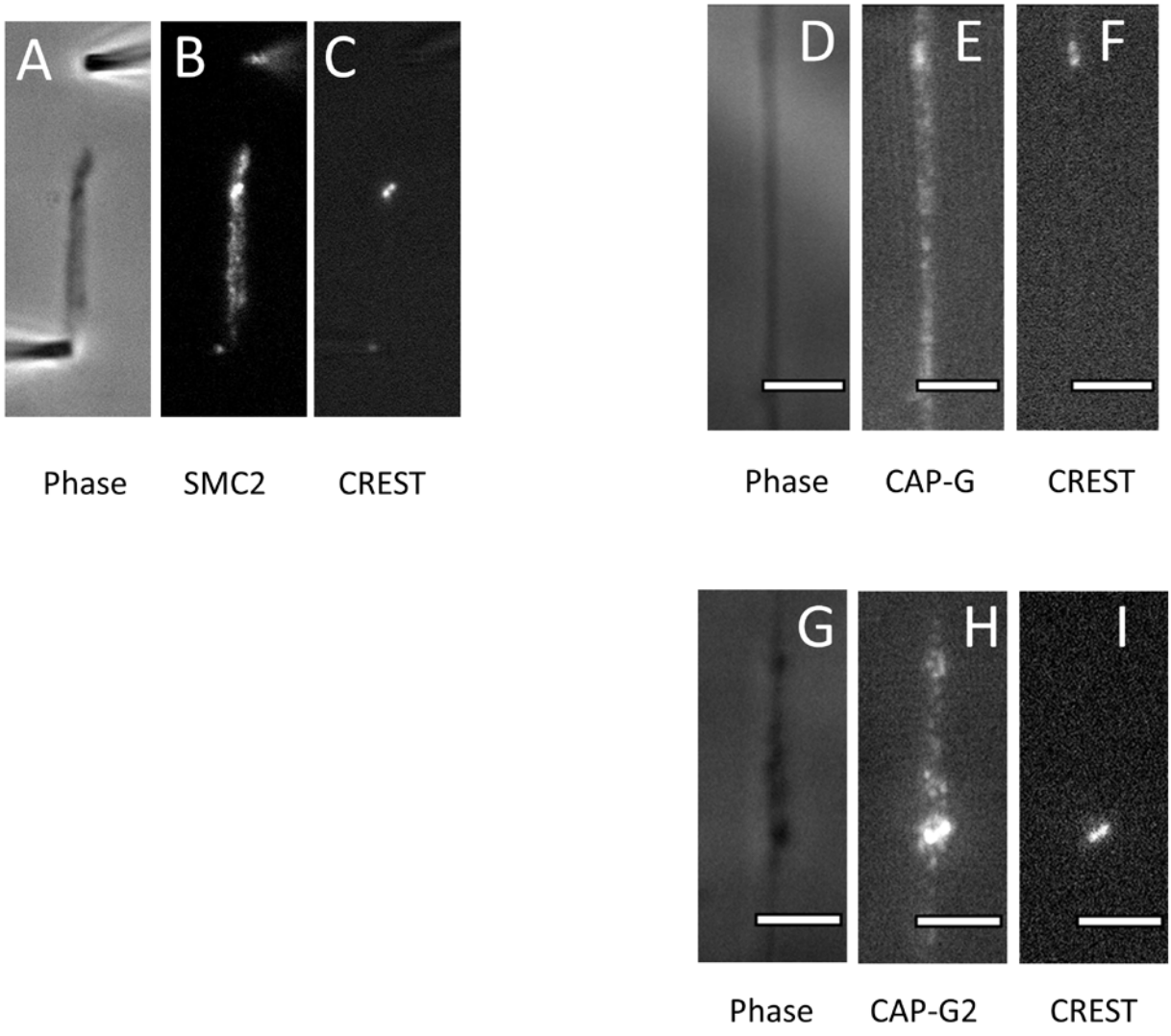

Figure S8. Centromere is enriched in condensin I and II. (A-C) Centromere is enriched in condensin. A single mitotic chromosome held between two pipettes imaged with phase-contrast (A), spontaneously stained and imaged with Alexa 488 labeled SMC2 (B), and with Texas-red conjugated CREST to label the centromere (C). hCAP-G (D-F) and hCAP-G2 (G-I) are concentrated in centromere region. Bar = 5μm.

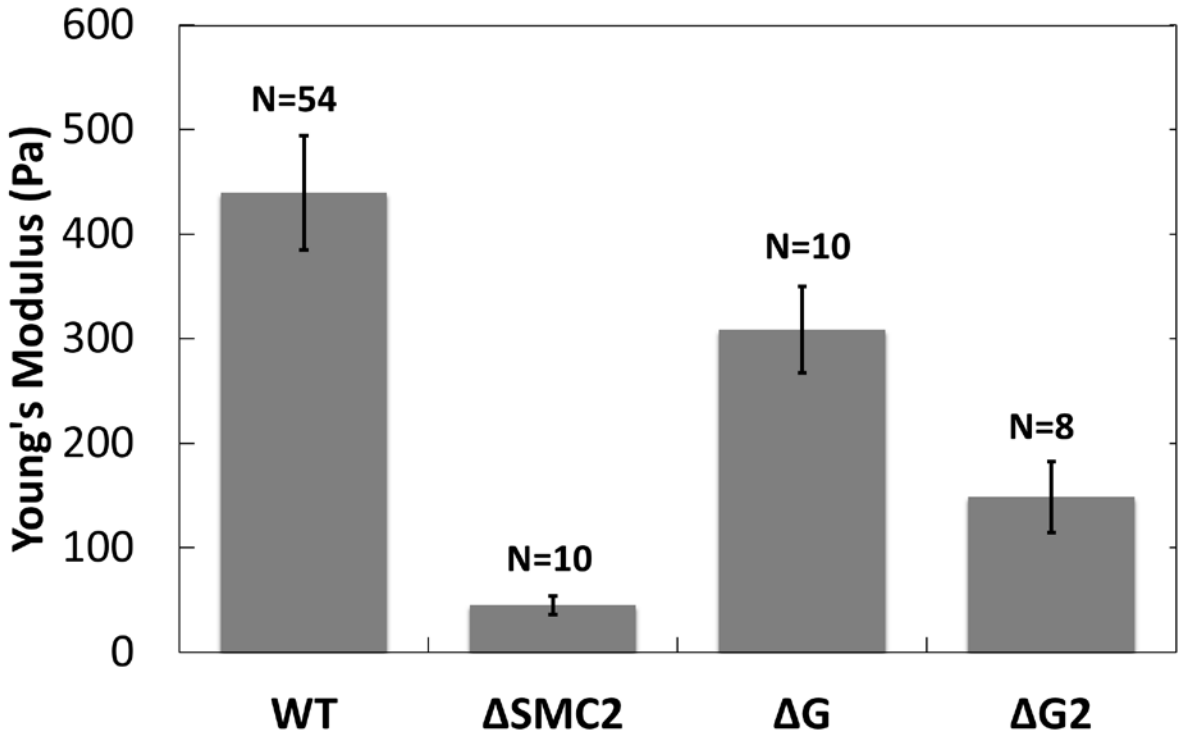

Figure S9. Corrected Young's moduli of WT chromosomes and condensin-depleted chromosomes. The protein knockdown levels estimated from Western blot were 90 ±10 %, 90±10 % and 77± 15 % for ΔSMC2, ΔG and ΔG2, respectively. The Young's moduli were extrapolated to 100% protein knockdown.

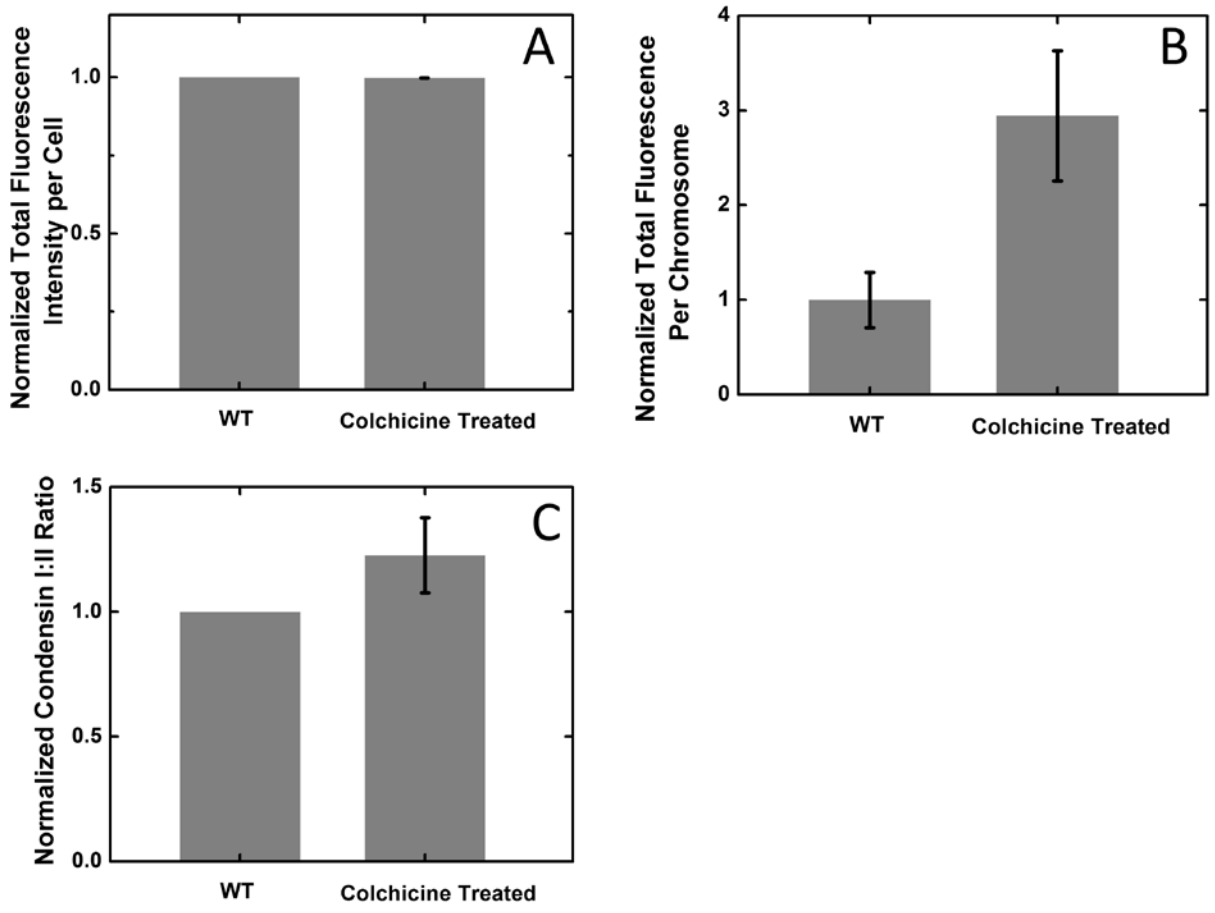

Figure S10. Condensin protein level analysis in WT and colchicine-treated HeLa cells. (A) Total SMC2 immunofluorescence of fixed whole mitotic cells from WT and colchicine-treated HeLa cultures, normalized against WT fluorescence level. (B) Normalized condensin fluorescence per chromosome. Condensin SMC2 fluorescence on native isolated chromosomes by microspray was measured for each chromosome for WT and colchicine treated cells. (C) Condensin I to condensin II ratio measured by hCAP-G and hCAP-G2 fluorescence on isolated native chromosomes for WT and colchicine-treated cells.

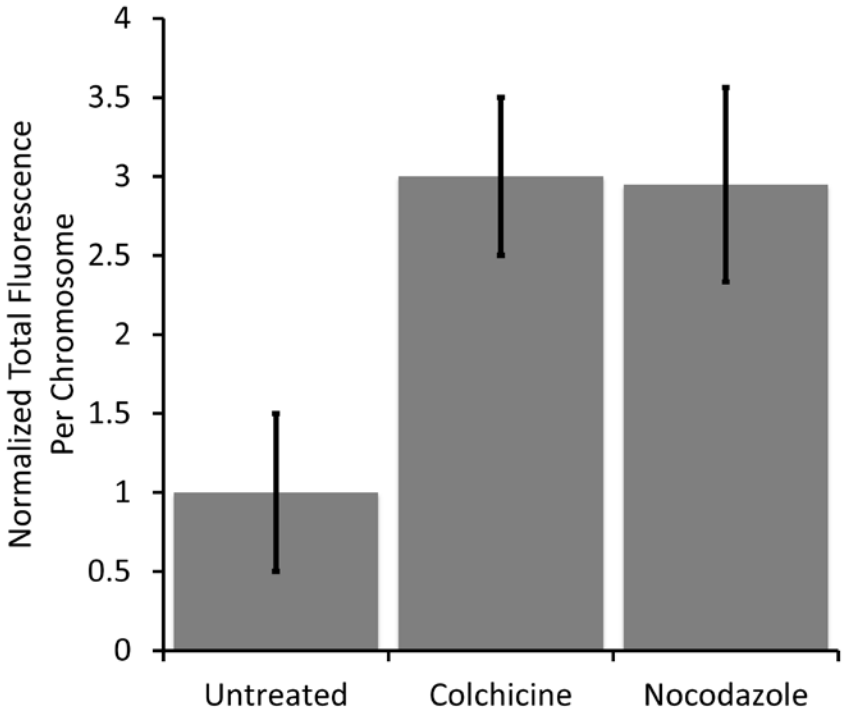

Figure S11. Condensin protein level analysis in WT and colchicine-treated HeLa cells. Total SMC2 immunofluorescence of fixed whole mitotic cells comparing WT He La (untreated cells), colchicine-treated HeLa cells, and nocodazole-treated HeLa cells, normalized against WT fluorescence level. The fluorescence level for metaphase chromosomes from colchicine- and nocodazole-treated cells are comparable, and about three-fold greater than the WT case.