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Supplemental Information

Purified Smc5/6 Complex Exhibits DNA Substrate Recognition and Compaction

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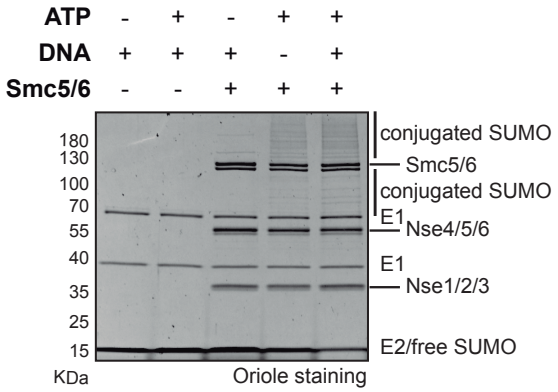
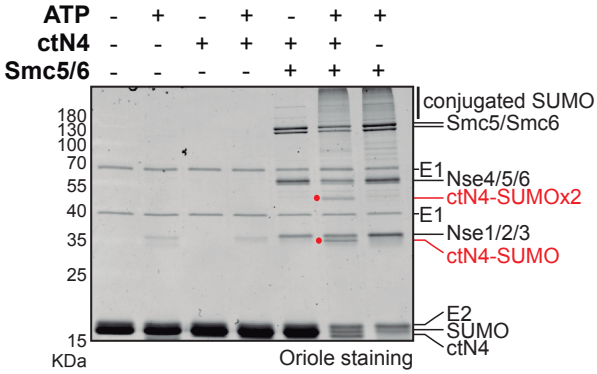
Supplementary Fig. 1

A

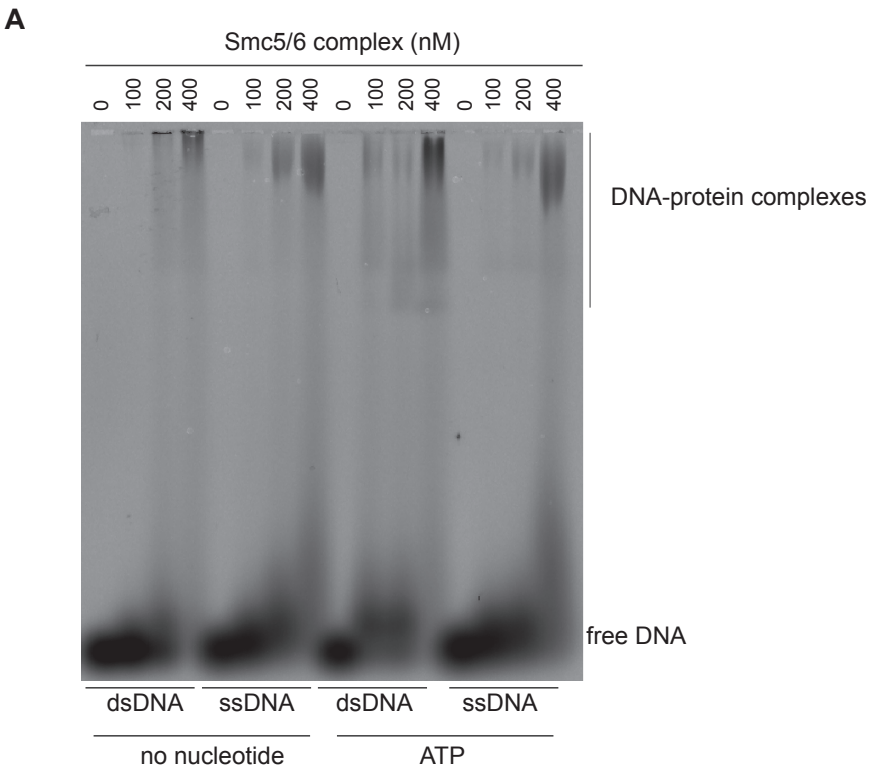


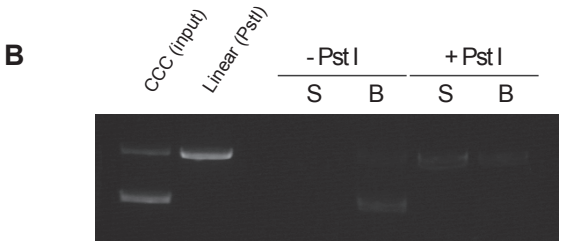
B



Supplementary Figure 1. Purified Smc5-6 complexes contain an active SUMO ligase. Related to Figure 1. (A) Oriole staining of SUMOylation reactions described in Figure 1D containing 150 nM E1, 100 nM E2 and 16 µM SUMO. Reactions were started by addition of 2 mM ATP and allowed to proceed for 15 min before being stopped by addition of SDS-PAGE loading buffer and boiling. Where indicated (+), Smc5/6 and DNA were added to 165 nM and 10 mM, respectively. **(B)** Oriole staining of ctN4 (c-terminal domain of Nse4) SUMOylation in the presence and absence of the Smc5/6 complex. Reactions were started by addition of 2mM ATP and allowed to progress for 30 minutes. Note that mono and di-sumoylated ctN4 species accumulate to a higher extent in the presence of Smc5/6 complex. ctN4-SUMO=monosumoylated ctN4; ctN4-SUMOx2= disumoylated ctN4. Red dots mark the position of sumoylated ctN4 species.

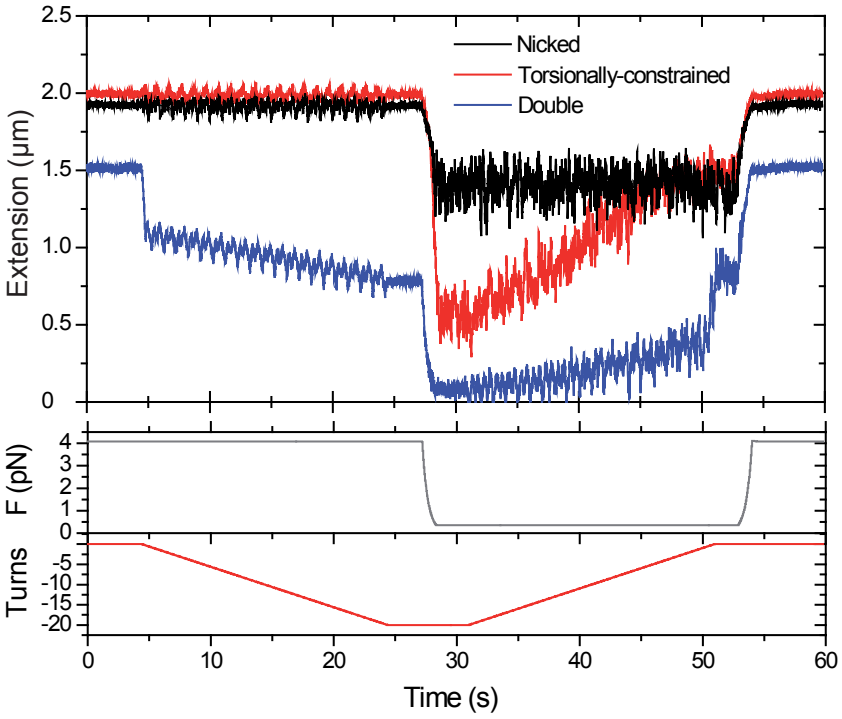
Supplementary Fig. 2





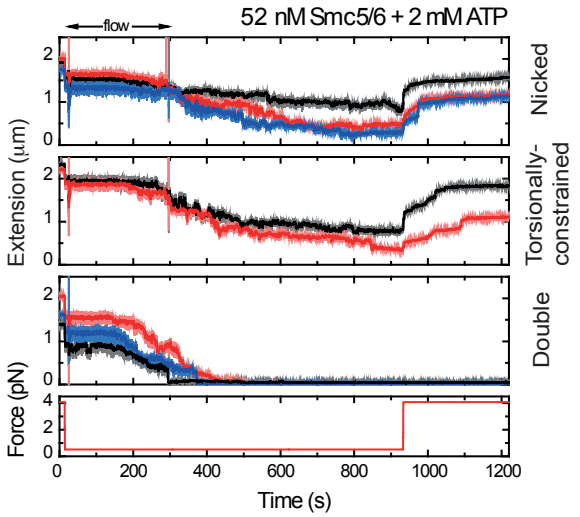
Supplementary Figure 2. DNA binding of Smc5/6 complex using an electrophoretic mobility shift assay. Related to Figure 4. (A) 50nM of 6-carboxyfluorescein–labelled ssDNA and dsDNA were incubated with increasing concentrations of Smc5/6 complex, as indicated. **(B)** Agarose gel electrophoresis showing recovered DNA after Smc5/6 complex loading and immunoprecipitation in the absence and presence of digestion with the restriction enzyme *PstI* to linearise circular DNA bound so Smc5/6 complex.

Supplementary Fig. 3



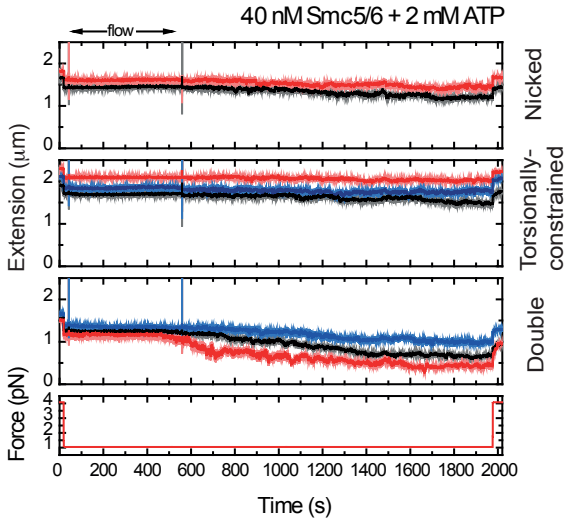
Supplementary Figure 3. Mechanical response of nicked, torsionally constrained and double DNA molecules. Related to Figure 5. Example of a characterization experiment carried out in buffer, before the addition of proteins and ATP.

Supplementary Fig. 4



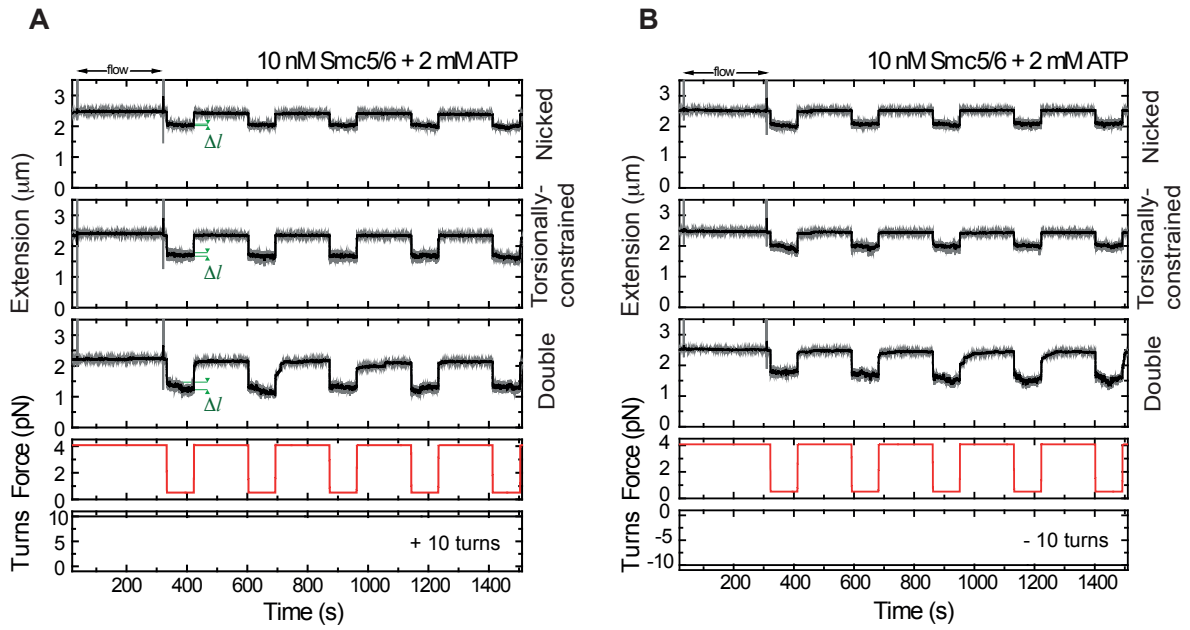
Supplementary Figure 4. DNA compaction experiment at 52 nM Smc5/6 and 0.5 pN. Related to Figure 5. A sample containing 52nM of Smc5/6 and 2 mM ATP is introduced at 0.5 pN while monitoring the extension of different DNA tethers at the same time. A stepwise compaction (total or partial) of the tethers is observed. At the end of the experiment, the force increases to 4 pN and the initial DNA extension is only partially recovered.

Supplementary Fig. 5



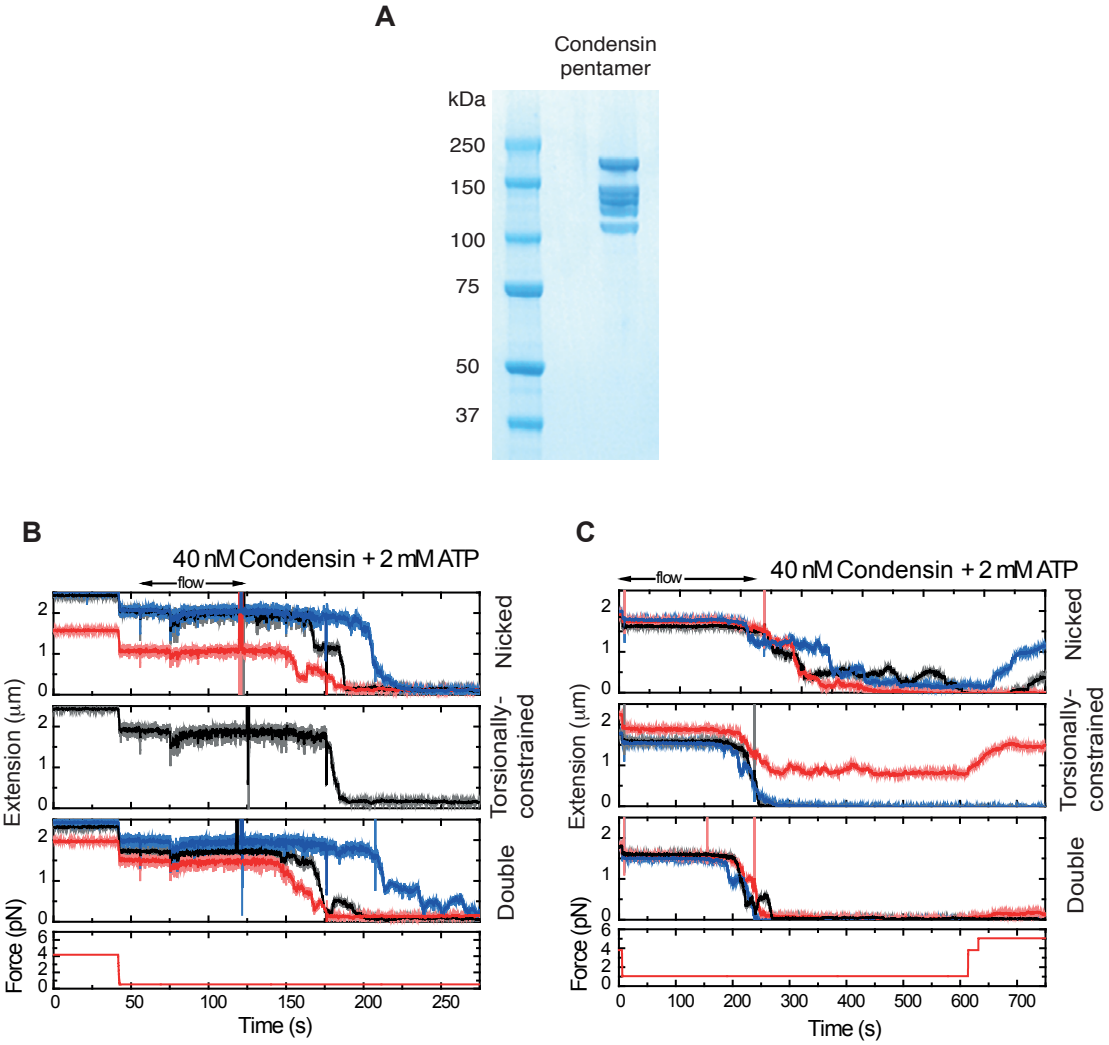
Supplementary Figure 5. DNA compaction experiment at 40 nM Smc5/6 and 1 pN. Related to Figure 5. Example of an experiment where a sample containing 40 nM Smc5/6 with 2 mM ATP is first introduced at 1 pN while monitoring the DNA extension. Slow condensation of the tethers is observed. At the end of the experiment, the force increases to 4 pN and the initial DNA extension is only partially recovered.

Supplementary Fig. 6



Supplementary Figure 6. DNA condensation as function of topological state of DNA. **Related to Figure 6.** Illustrative example of individual condensation traces of single nicked, single torsionally constrained and double tethers when the initial topological state of the DNA is altered by +10 **(A)** or -10 **(B)** turns. We allowed DNA compaction by 10 nM Smc5/6 at a low force (0.5 pN) for 90 seconds and the force was afterwards raised to 4 pN for 180 seconds. The cycle was repeated 5 times per experiment. DNA compaction was quantified by comparing the initial extension of the molecule to the minimum extension value in each step (Δl).

Supplementary Fig. 7



Supplementary Figure 7. Condensation curves of *S. cerevisiae* condensin complex. Related to Figure 6. **(A)** SDS-PAGE and Coomassie Blue staining of purified condesin pentameric complex. **(B)** and **(C)** Magnetic tweezers experiments where 40 nM condensin and 2 mM ATP are added at 0.5 pN **(B)** and 1 pN **(C)** while monitoring the DNA extension. Robust condensation of the DNA tethers is observed in both cases.

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