TopA, the *Sulfolobus solfataricus* topoisomerase III, is a decatenase.

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SUPPLEMENTARY DATA

Figure legends

Figure S1. Purification of *S. solfataricus* recombinant TopA. Coomassie-stained SDS-PAGE of the purified enzyme. Approximately 1 µg of the protein was loaded onto the gel.

Figure S2. Activity of *Sso*TopA as a function of temperature. TopA was incubated 8 min with a protein/pTZ18R DNA molar ratio of 2:1 in the standard conditions and at the temperature indicated above each corresponding lane. The DNA control (C) was incubated for 8 min at 75 °C in the absence of TopA. The reaction was performed at 100 mM of NaCl. The reaction products were analysed by one dimensional gel electrophoresis: OC indicates open circular (nicked) DNA, Rel, the relaxed topoisomers and -SC the negatively supercoiled DNA.

Figure S3. Activity of *Sso*TopA as a function of the magnesium concentration. TopA was incubated 30 min at 75°C with a protein/pTZ18R DNA molar ratio of 1:1 in the standard conditions with the concentration of $MgCl_2$ indicated above. The reaction products were analysed by one dimensional gel electrophoresis: OC indicates open circular (nicked) DNA, Rel, the relaxed topoisomers and -SC the negatively supercoiled DNA.

Figure S4. Annealing properties of the FI** produced by *Sso*TopA. SsoTopA was incubated with pTZ18R DNA (molar ratio of 1:1) for 5 min at 95°C in the standard conditions with 15 mM $MgCl_2$. After incubation, the samples were quickly cooled and kept at 4°C except when indicated and finally 0.5% SDS (final concentration) was added (lanes 2-5). SDS was added before the incubation as a control (lane 1). An additional incubation at 65°C for 15 min was realized for lanes 4 and 5. For all the samples, 25 mg/ml bromophenol blue, and 15% sucrose (final concentrations) were added. Samples corresponding to lanes 2 and 4 were further equilibrated at room temperature for 15 min while samples corresponding to lanes 3 and 4 were incubated 2 min at 95°C just prior loading. The reaction products were analysed by agarose gel electrophoresis (2%) in TEP buffer. OC indicates open circular (nicked) DNA and -SC the negatively supercoiled DNA and the particular FI* and FI** forms.

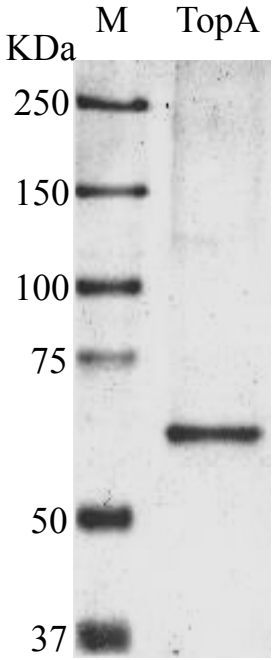
Figure S5. Decatenation of multi-linked DNA circles by *Sso*TopA. 600 ng of the multi-linked DNA substrate was incubated with *Sso*TopA for 20 min at 90 °C then cooled at 4°C (lane 1). The samples were further incubated for 30 min at 37°C in the presence of Nb.BbvCI (lane 2) or NdeI (lane 3). The panel B is a zoom of the squared zone from panel A. The DNAs ran in a 2% agarose gel at about 2.5 $V.cm^{-1}$ for 3 hours. The different DNA bands were attributed according to their size and topological forms : PP corresponds to the parental plasmid, LC to the decatenated large circle, and mC to the minicircle while OC is the open circular form, Lin the linear, Rel the relaxed and –SC the negatively supercoiled. M corresponds to the GeneRuler 1kb ladders.

Figure S6. DNA extension fluctuation in the absence of *Sso*TopA. (A) Schematic representation of the single molecule assay using magnetic tweezers explaining the

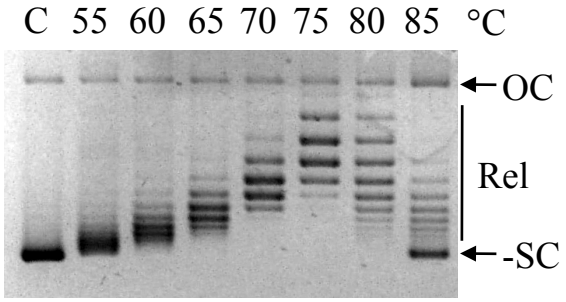
occurrence of DNA extension fluctuation. (B) Time-trace of the 3 kb DNA extension variation at 45 °C at an extended force of 0.45 pN in the absence of *Sso*TopA (control of the figure 6). The variation of extension is indicated as "fluctuation" in green. (C) Same extension fluctuation using the DNA containing the permanent single-stranded bubble region. (D) Corresponding time-trace of the DNA extension variation without *Sso*TopA (control of Figure 7).

Figure S7. Decatenation of kDNA by TopA of *S. solfataricus*. (A) TopA is added (0.5 ng) just before the incubation at indicated temperature for 30 min. (B) TopA (0.5 ng) is incubated at 85°C for 30 min in the presence of the indicated amount of SSB protein (ng). The lane T corresponds to kDNA incubated without TopA. The reactions were stopped and kDNA products were separated by a 2% agarose gel electrophoresis.

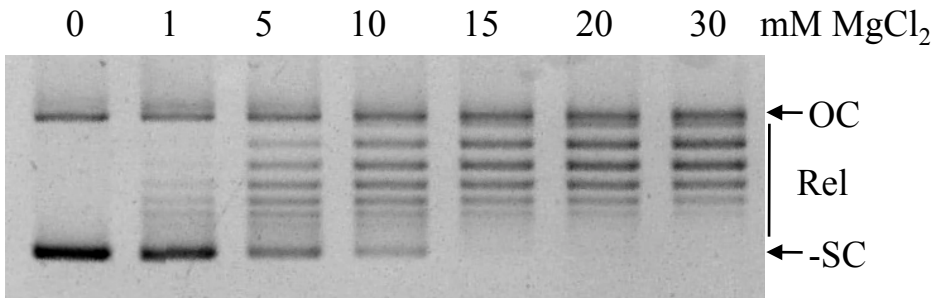
Supplementary Figure S1



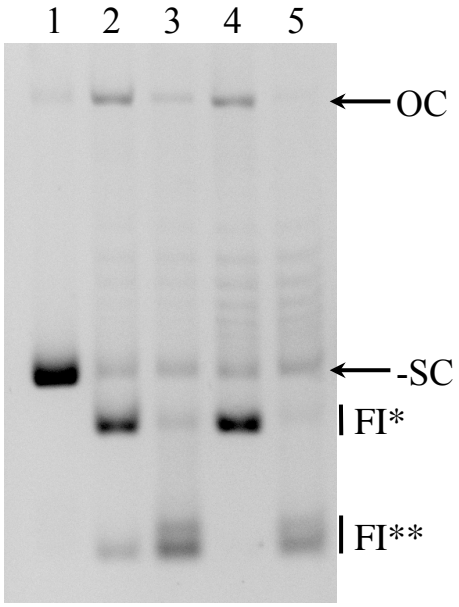
Supplementary Figure S2



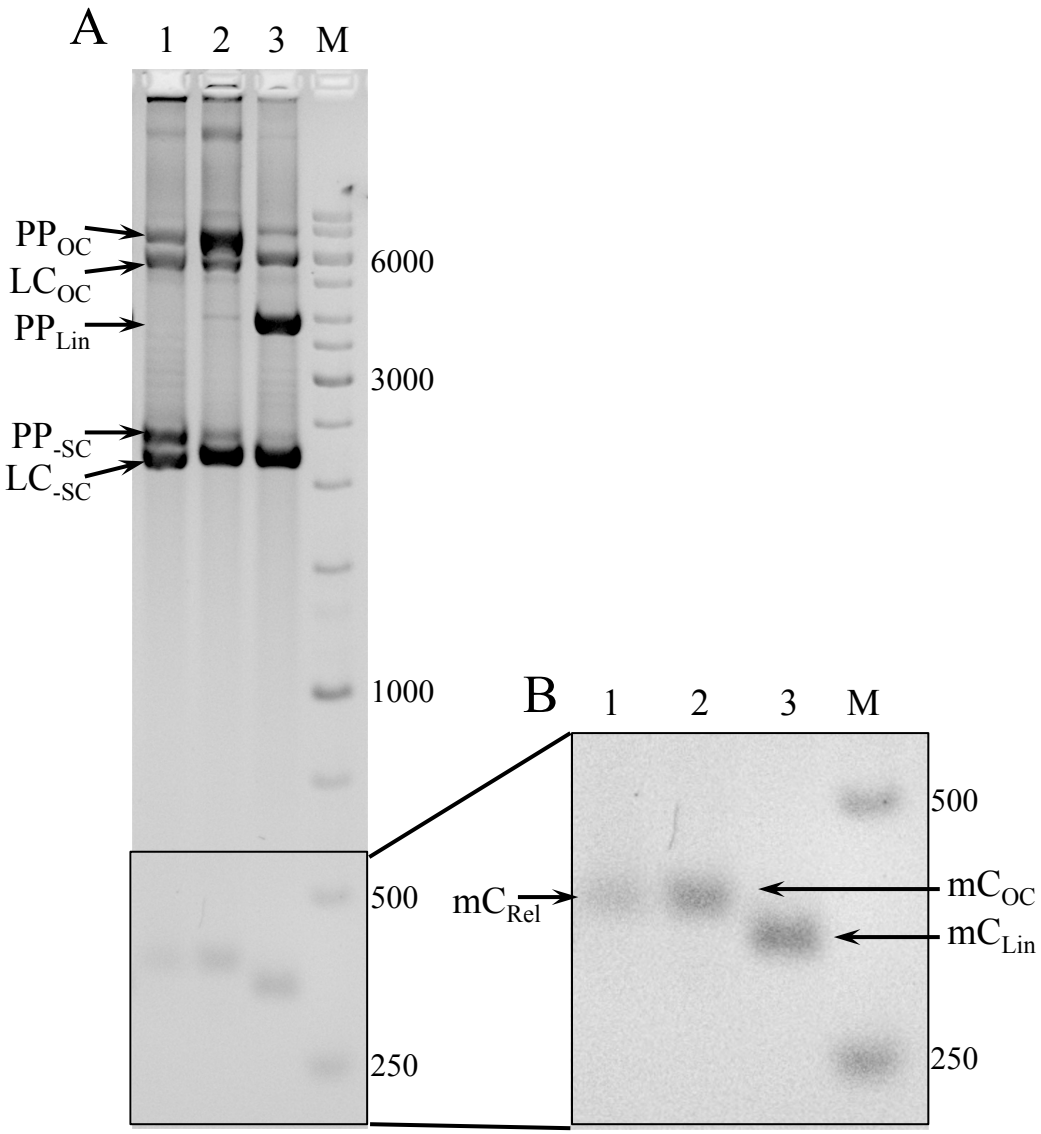
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6

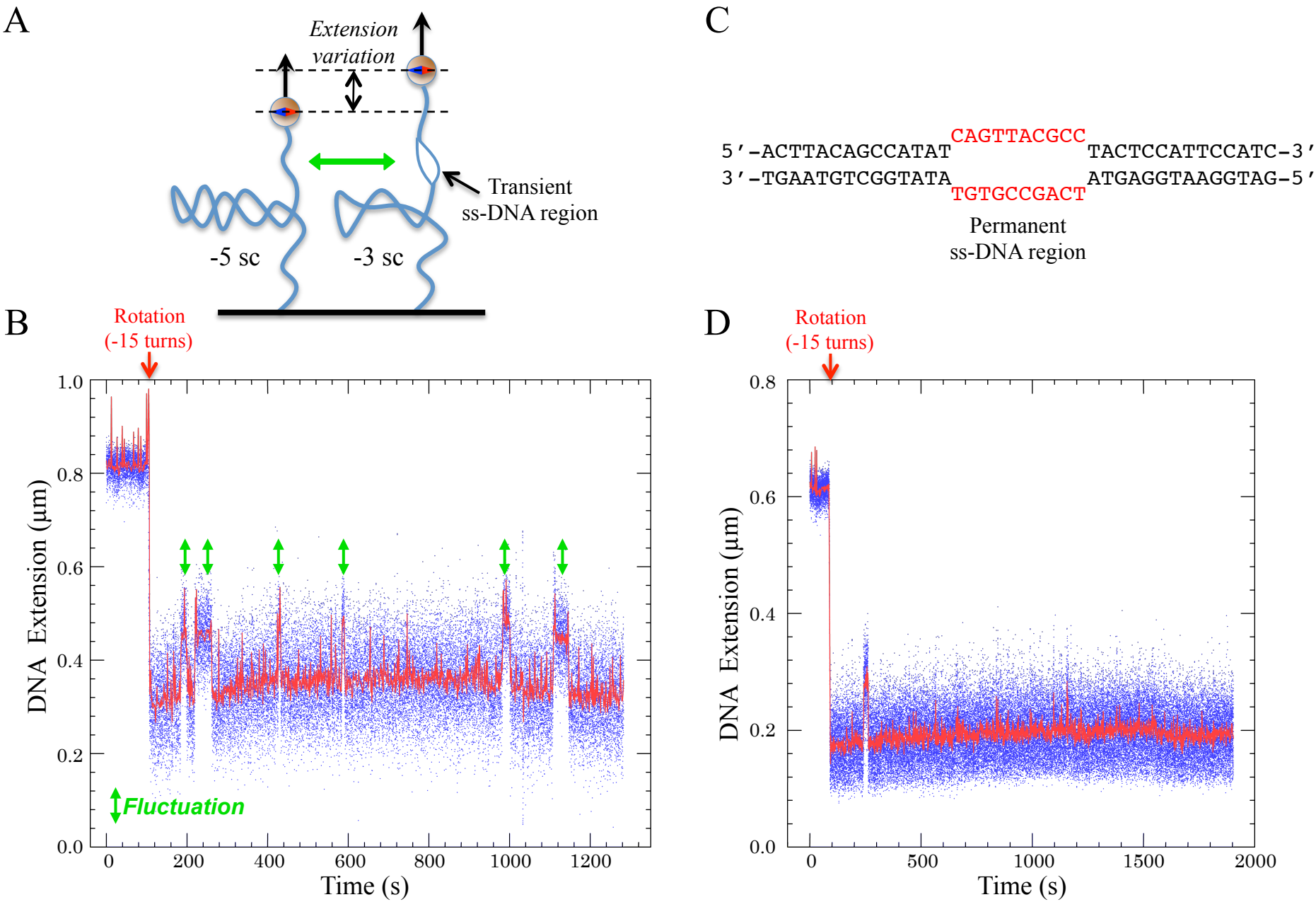


Figure S7