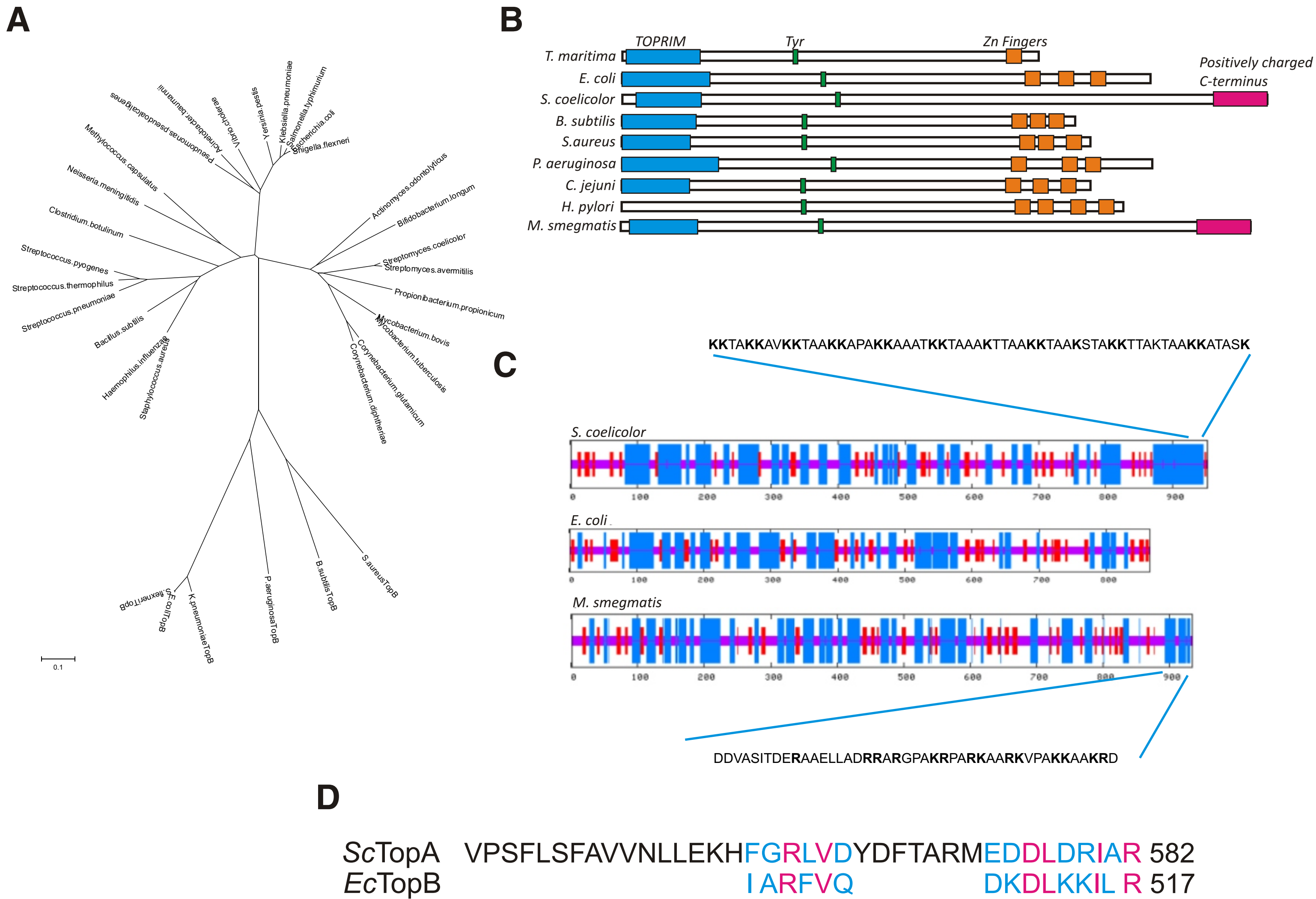

Fig. S1 (A) Phylogenetic tree of bacterial TopA and TopB proteins. (B) Comparison of the domain organisation of bacterial TopA proteins. Conservative TOPRIM (blue) region and catalytic tyrosine residues (green) are indicated. The C-terminal domain is highly variable and contains different numbers of Zn-finger motifs (orange) or the stretch of positively charged amino acids (pink). (C) Secondary structure prediction for *Sc*TopA, *Ms*TopA and *Ec*TopA with SOPMA algorithm reveals the presence of putative alfa-helix at C-terminal end of *Sc*TopA and *Ms*TopA rich in lysine and alanine residues. (D) Comparison of *Ec*TopB region containing the decatenation loop with corresponding ScTopA region reveals the presence of conserved residues in *Sc*TopA (pink - identical residues, blue - highly similar residues) separated with amino acids not present in *Ec*TopA.

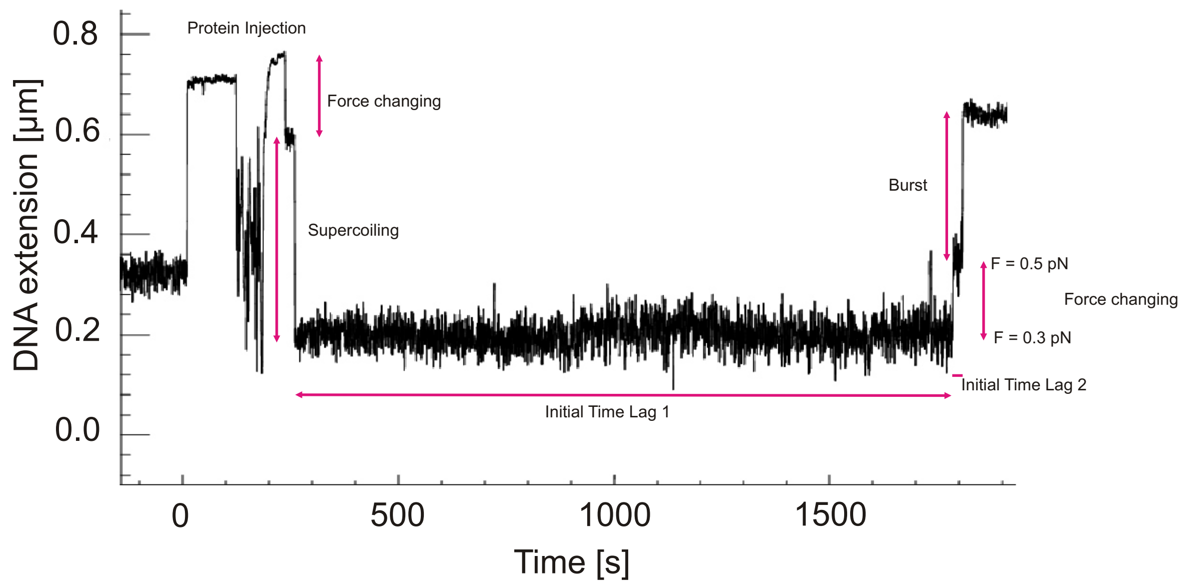

Fig. S2 Relaxation of negatively supercoiled 2-kb DNA in a single-molecule experiment at two stretching forces (F = 0.3 and 0.5 pN). The supercoiled DNA in the presence of 5 nM TopA at low stretching forces remained intact for 1000 sec (Initial Time Lag 1). After changing the stretching force the same DNA molecule was completely relaxed in short time (Initial Time Lag 2) in a single burst.

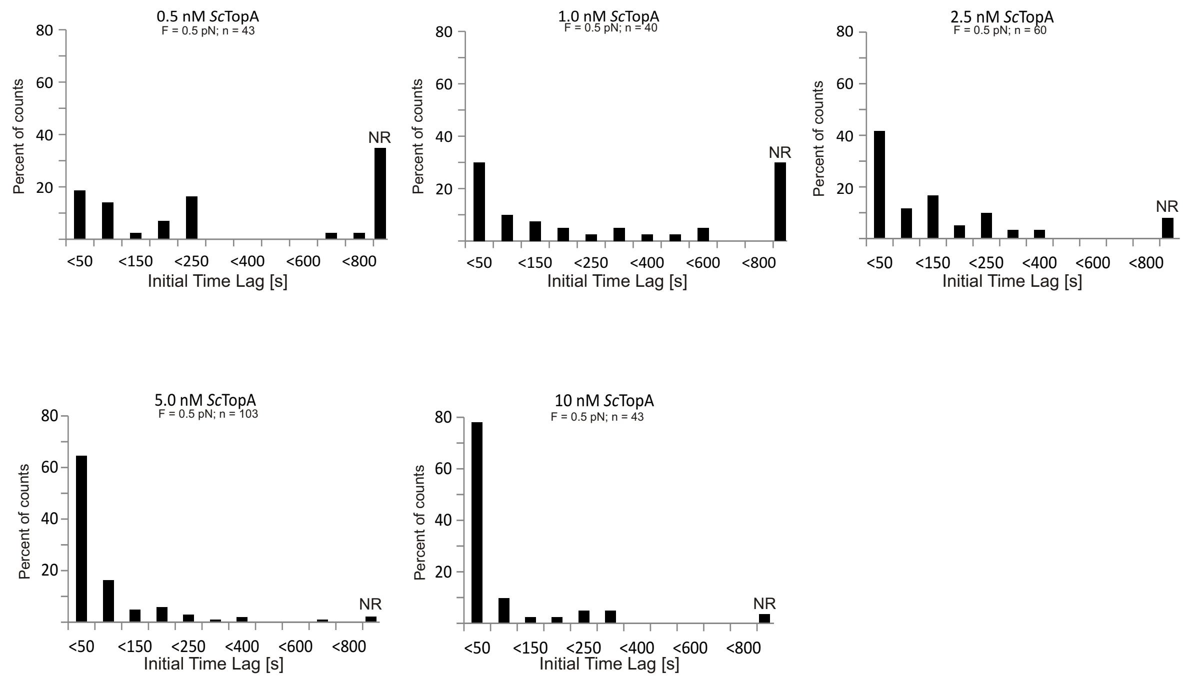

Fig. S3 Distribution of initial time lags (ITL) and non relaxed beads (NR) measured under different *Sc*TopA concentrations and used for plotting 1/ITL versus *Sc*TopA concentration.

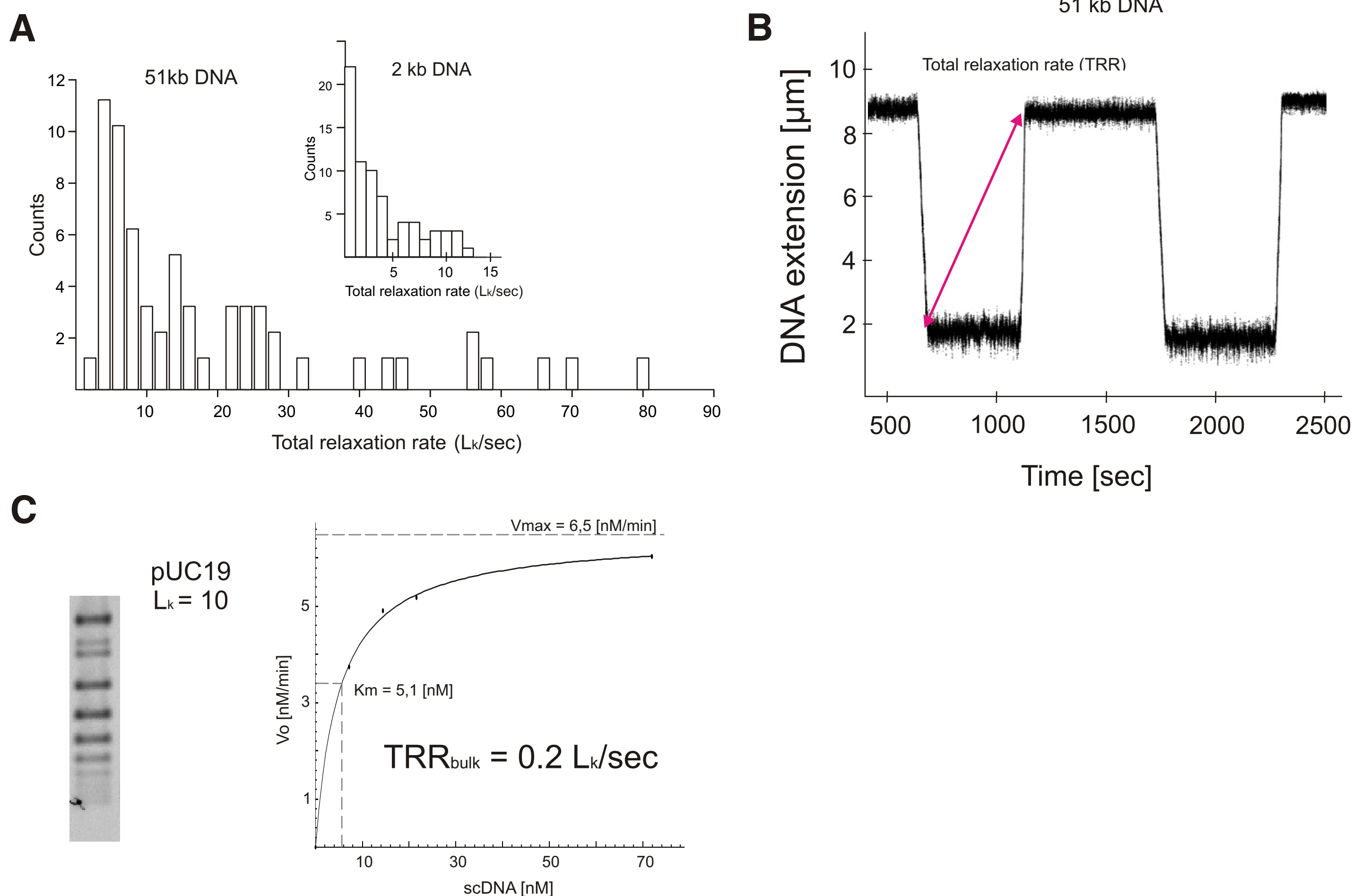

Fig. S4 ScTopA relaxes DNA with comparable velocities (0.1-0.4 Lk/sec) independently of substrate length. Total relaxation rates (TRRs) calculated for two DNA substrates 51-kb (A) and 2-kb (inset), respectivel. The typical record used to estimate TRR value (B). TTR_{bulk} (total relaxation rate for bulk experiment) value estimated based on fitting to the Michaelis-Menten equation is 0.2 Lk/sec for negtively supercoiled ($L_k = 10$) pUC19 plasmid as a substrate.

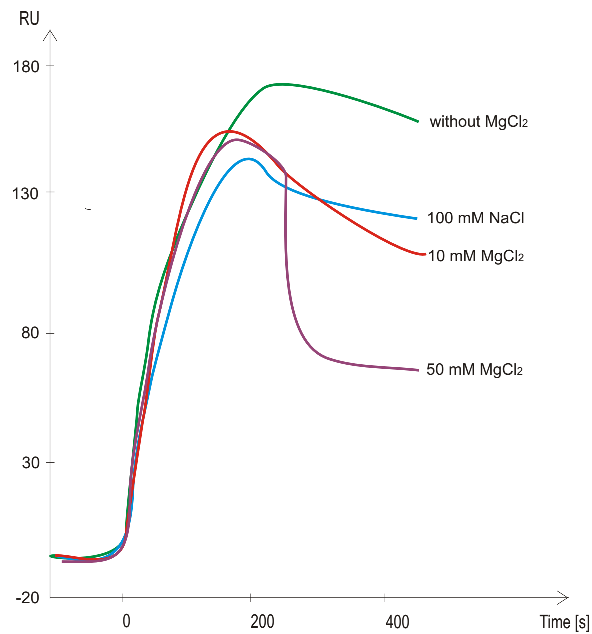

Fig. S5 Binding of *Sc*TopA to double-stranded DNA (dsDNA) observed using surface plasmon resonance (SPR). *Sc*TopA binds to dsDNA in phosphate buffer (green). Addition magnesium ions to phosphate buffer triggers ScTopA dissociation (red and magenta). In control experiment the ion strength was increased by addition of 100 mM (blue) however the dissociation remained weak. In the experiment the *Sc*TopA protein (80 nM in 25mM NaH_2PO_4 pH 8.0 and 150mM NaCl) was bound to the biotynylated 350-bp *S. coelciolor* DNA fragment immobilized on the chip surface.

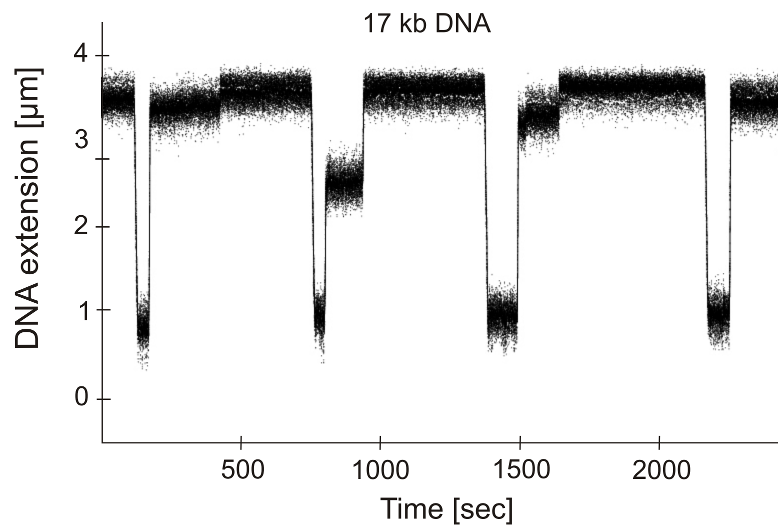

Fig. S6 The real-time record obtained for 17-kb DNA shows the relaxation of negatively supercoiled DNA in highly processive bursts (1-3) at single-moleule level.

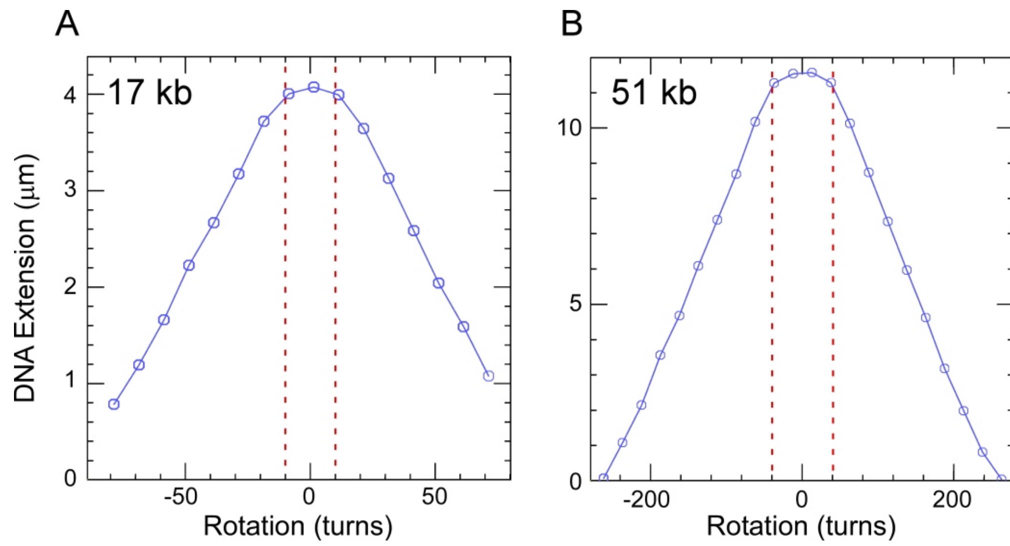

Fig. S7 Extension vs. supercoiling curves obtained at F = 0.3 pN for 17 kb and 51 kb DNA molecules. The dashed lines indicate the number of rotations for which DNA buckles and begins to form plectonemic supercoils., and correspond to $|\sigma| \sim 0.01$ as discussed in the main text.

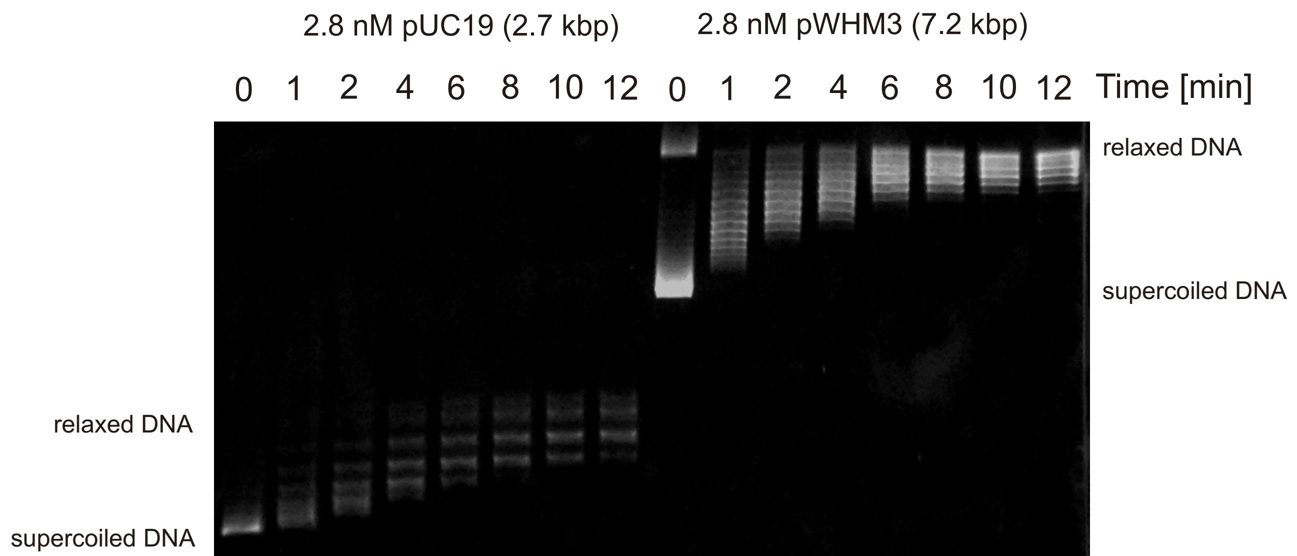

Fig. S8 Topoisomerase activity assays. 2.8 nM concentration of negatively supercoiled plasmids pUC19 (2.7 kb) and pWHM3 (7.2 kb) were relaxed in the presence of 25 nM *Sc*TopA. The reactions were stopped by addition of EDTA after 0-12 min of incubation. Products of reaction were subsequently resolved in 0.8% agarose gel and stained with ethidium bromide.