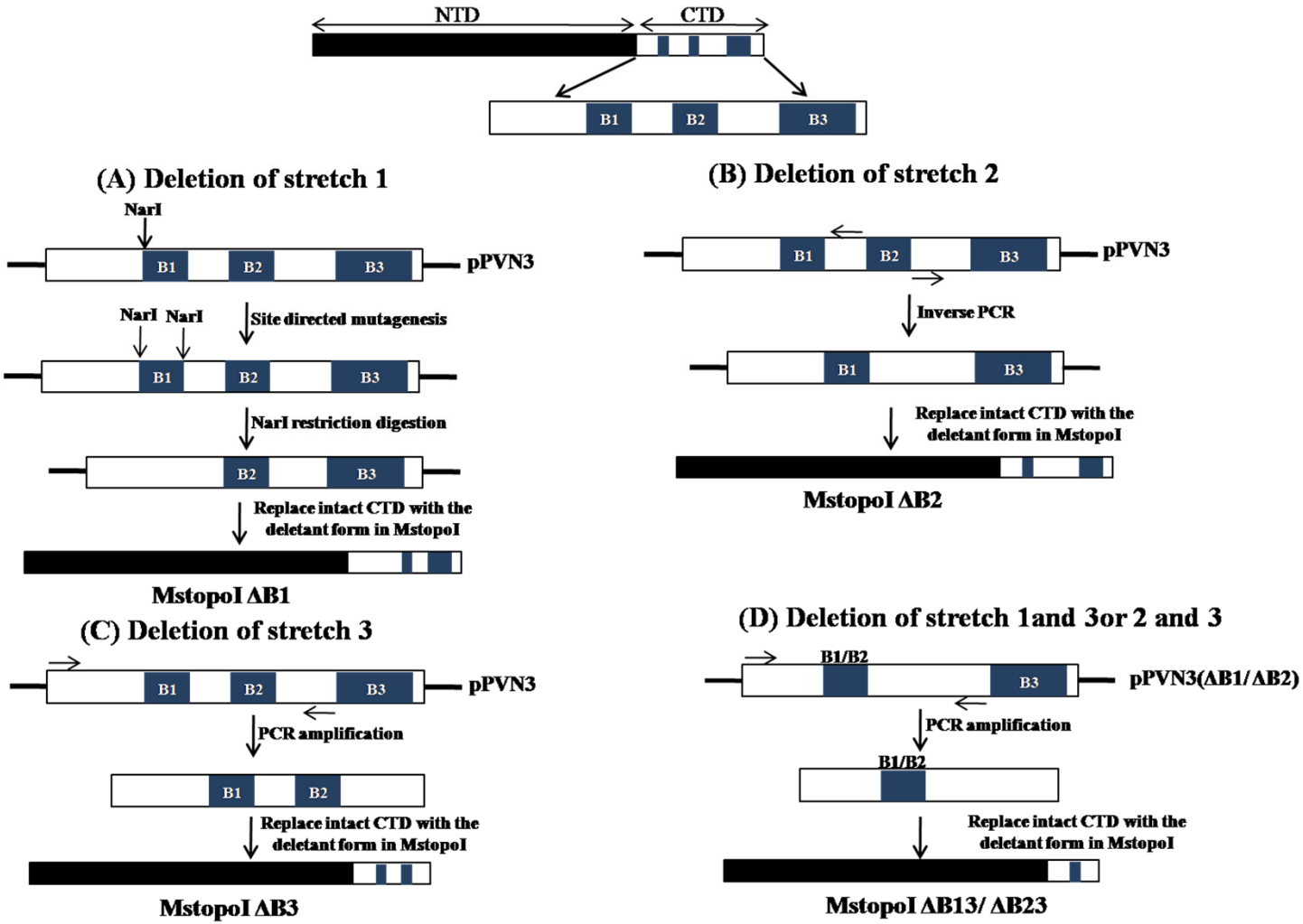
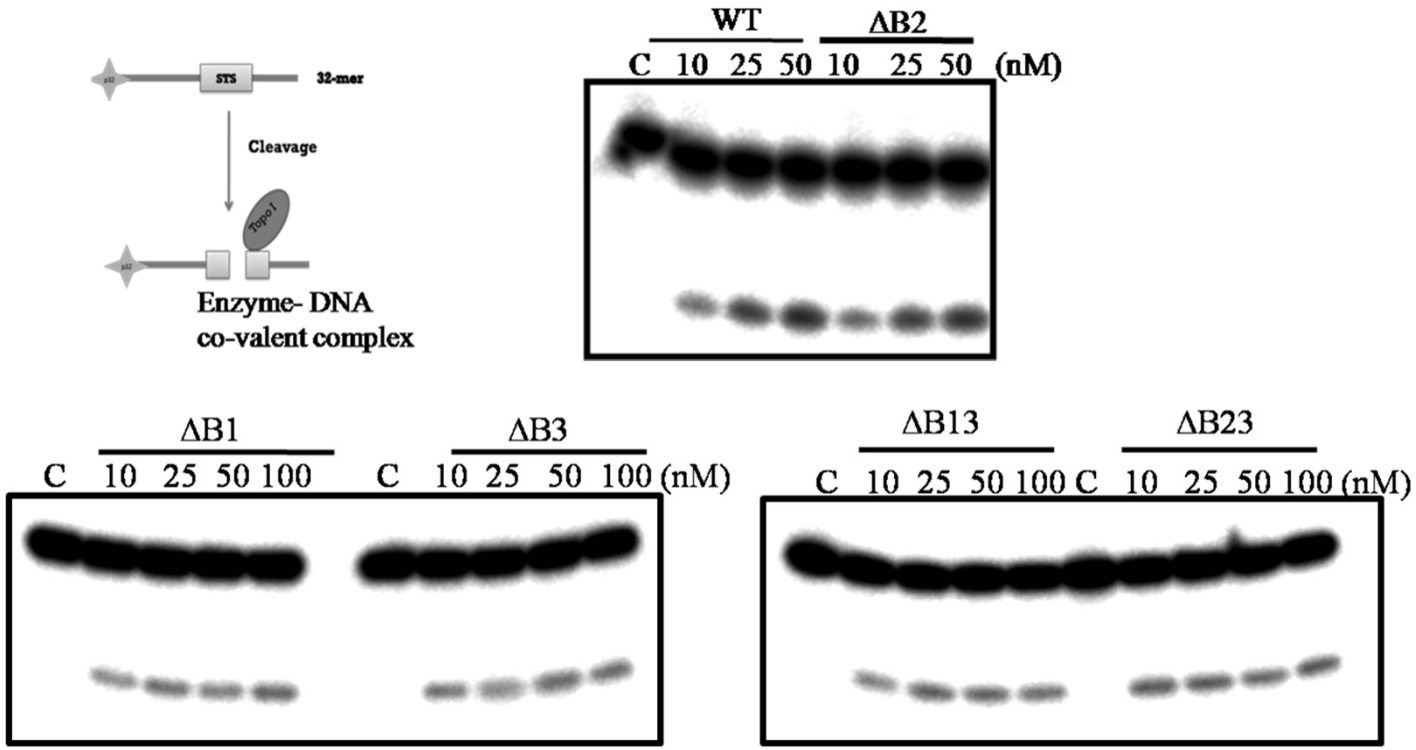
Fctd1	AGCTGGATCCATCGCGCGCGCCGGAG
Rctd1	CGCCGCCGCGGCGGCCGGCTCGGCGTAGATCTTGAGT
Fctd3	GCCGCCGCGGCGGCGCAGGCCGCGTCGGCTCCGCCGC
Rctd3	AGCTGGTACCCTAGAGGGCCTTCTTGGCGGCG
Rctd2	AGCTGGTACCCTAGTCGGCCAGCAGTTCGGAGGCA
60 mer	GCCCTGTCAACTCTGTATAAAAAACACCCCGCGAAACGAGCGCATATAGAAAACGACGAT
32 B (non-STS)	TATTGGGATGTCAAGCGGAAGCTCGCTCACTG
24 mer	TATTGGGATGTCAAGCGGAAGCTC
11 mer	GAGCTTCCGCT
32 mer	CAGTGAGCGAGCTTCCGCTTGACATCCCAATA

Supplementary Table 1. Oligonucleotides used in this study

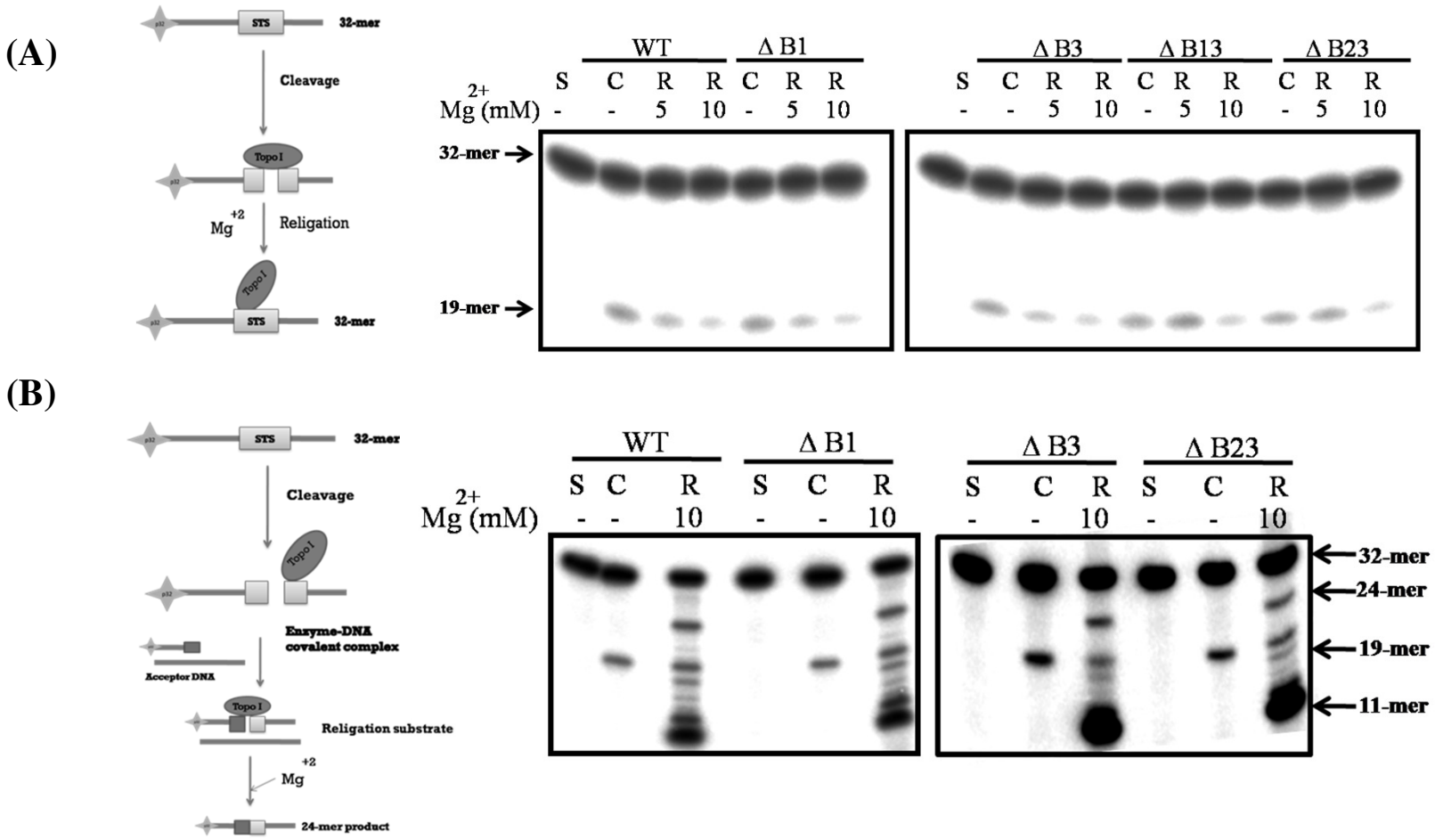
Supplementary Figure S1. Multiple sequence alignment of CTD of topoI from different mycobacterial species. To illustrate the conserved stretches of basic amino acids (displayed in boxes) the amino acid sequences were retrieved form pubmed and the alignment was performed with ClustalW.
Mycobacterium tuberculosis (Mt), *Mycobacterium bovis* (Mb), *Mycobacterium leprae* (Ml), *Mycobacterium avium* (Ma), *Mycobacterium avium* subsp. *paratuberculosis* (Ma(ptb)), *Mycobacterium smegmatis* (Ms).



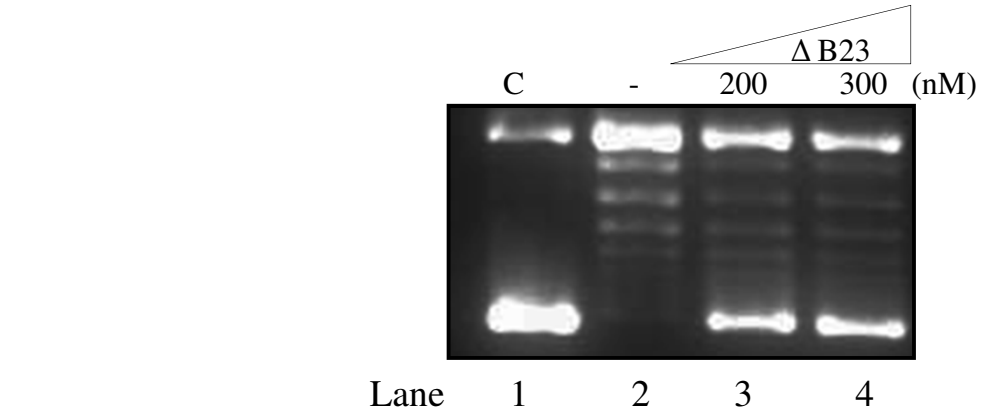
Supplementary Figure S2. Strategy for the deletion of basic stretches in the CTD of MstopoI



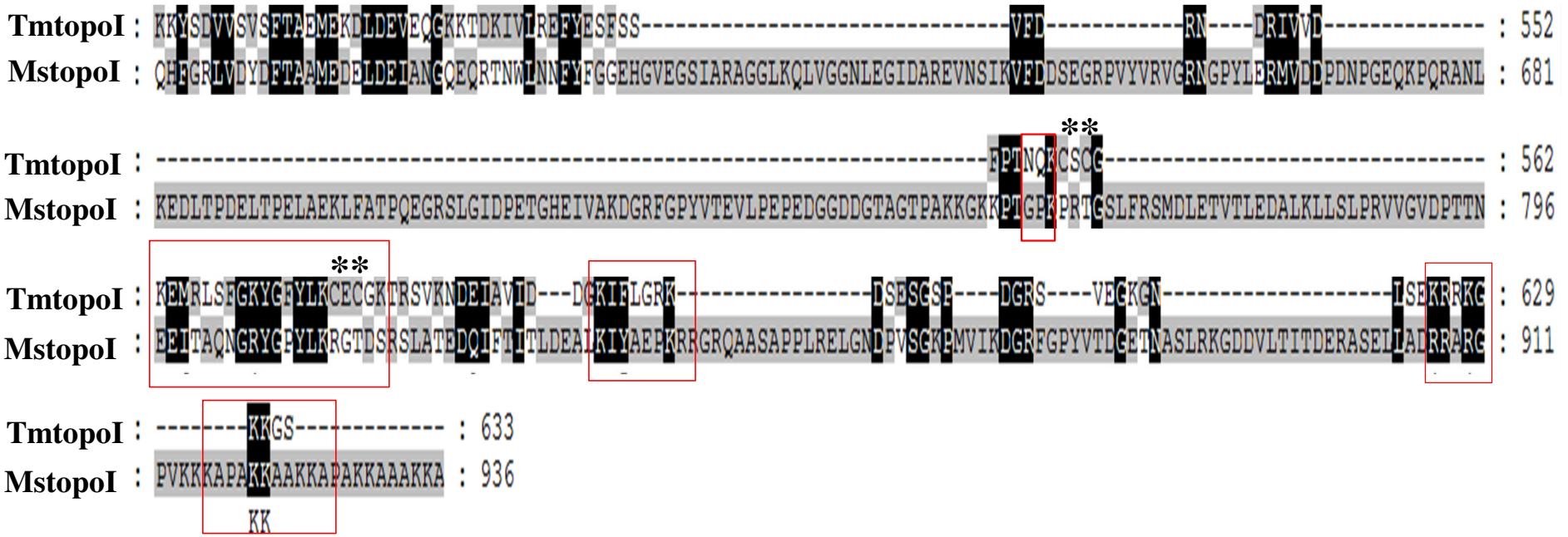
Supplementary Figure S3. DNA cleavage by the MstopoI deletants. 5′-end-labeled 32-mer oligonucleotide containing STS was used as cleavage substrate to yield a 19-mer-labeled product (schematic on the top left). Cleavage at different enzyme concentrations was monitored by arresting the reaction at 30 min. C: Oligonucleotide without enzyme.



Supplementary Figure S4. (A) Intramolecular religation. Schematic is depicted in the left panel. The religation reactions were carried out in the presence of 5'-end-labeled 32-mer oligonucleotide containing STS and 100 nM of WT or its deletant enzymes. S: 32-mer STS containing oligonucleotide without enzyme; C: cleavage with 100 nM enzyme and no Mg^{2+} ; R: Religation reaction with 5 and 10 mM Mg^{2+} ; **(B)** Intermolecular religation with MstopoI and its deletants. The experimental design is depicted in left panel. The 5′-end-labeled 32-mer ssDNA is the substrate that yields 5′-end-labeled 19-mer and 13-mer covalent enzyme adduct as products. The 13-mer covalent adduct is the substrate for religation that is complementary to the pre-annealed 24-mer and 5′- end labeled 11-mer partial duplex to generate labeled 24-mer as religation product. Religation was carried out with 100 nM of enzyme in the presence of 10 mM Mg^{2+}. S: control oligonucleotide; C: cleavage control; R: religation.



Supplementary Figure S5. Deletant can compete with WT enzyme for DNA relaxation . 50 nM of MstopoI was incubated with 500 ng of supercoiled pUC18 at 37 °C (Lane 2).The WT enzyme was competed/ chased out with increasing concentrations of ΔB23 (Lane 3,4) and the relaxation reactions were carried out at 37 °C for 30 min. C: pUC18 DNA with no enzyme.



Supplementary Figure S6. Multiple sequence alignment of CTD of topoisomerase I from *Mycobacterium smegmatis* and *Thermotoga martima.* To illustrate the basic amino acids, sequences were retrieved from pubmed and the alignment was carried out with ClustalW version 2.0 and displayed by GeneDoc software. Basic amino acids in the CTD are displayed in boxes. ∗: cysteine motif in the TmtopoI.