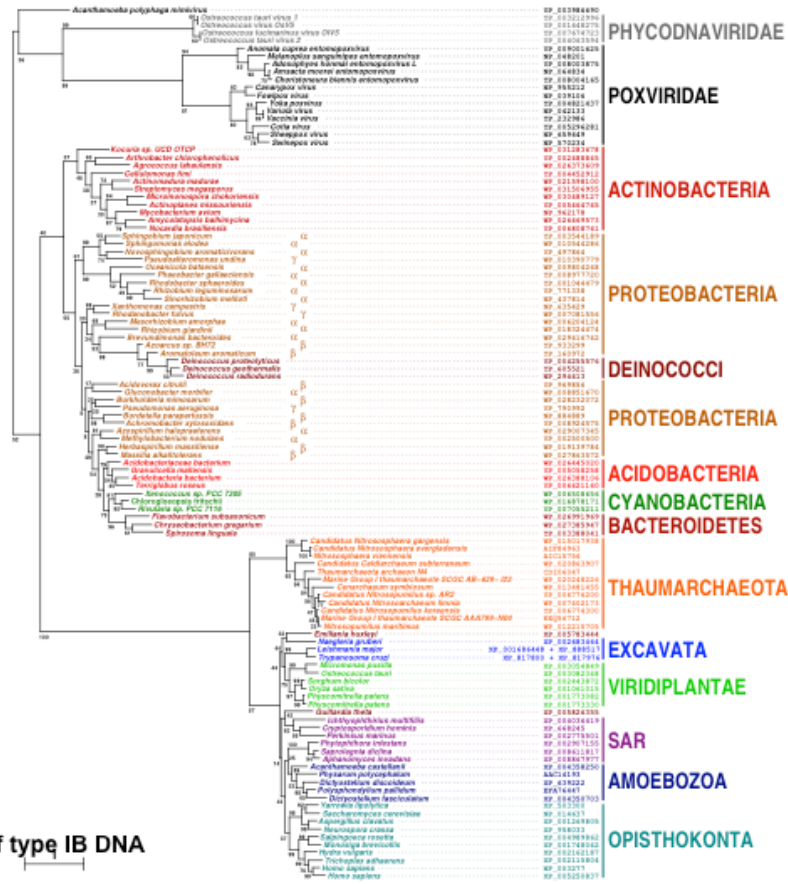
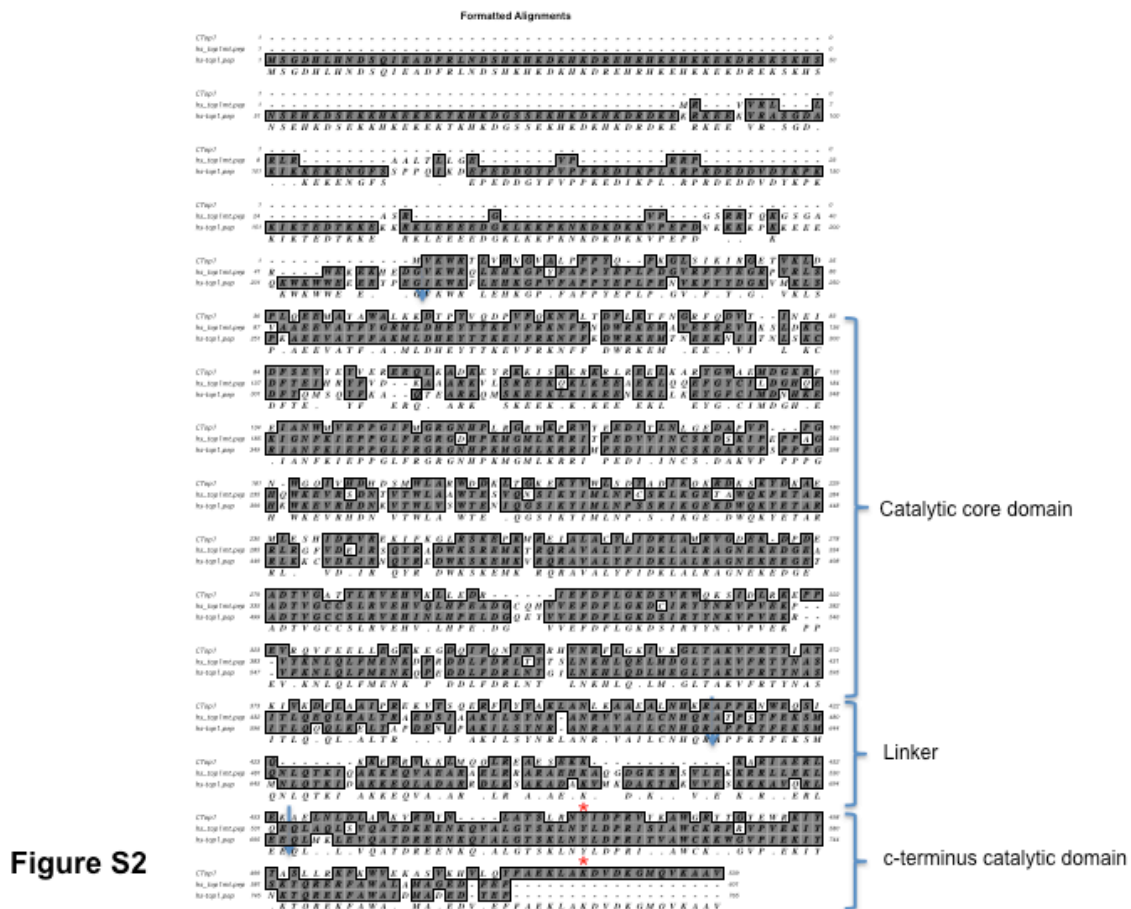


1 **Supplementary material**

2 **Supplementary Figure S1. Phylogenetic analysis of type IB DNA topoisomerases**



11 **Supplementary Figure S2. Alignment of the Ctop1 with the hs_top1mt.pep and the hs-**
 12 **top1.pep using MacVector.** Polypeptide sequence alignment for Cs-TopIB (upper lines) and
 13 the human topoisomerase IB, Top1mt (mitochondrial) and Top1 (nuclear). Identical matches
 14 are in uppercase, bold, and shaded; similar amino acids are in uppercase but not shaded. The
 15 unmatched amino acids are in lowercase. The catalytic tyrosines, Y723 for Top1, Y559 for
 16 Top1mt and Y477 for Cs-TopIB are indicated by red asterisks.



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23 **Supplementary Figure S3. Cs-TopIB characterization.** A) Purification of Cs-TopIB. Coumassie
 24 Blue stained SDS/PAGE gel showing the purification steps of *Ca. C. subterraneum* TopIB protein
 25 expressed in *E. coli*. MW: Molecular Weight; FT: Flow Through; W1 and W2: wash; E: Eluted
 26 fractions. B) Optimum salt concentration: Assays were carried out at 65°C for 10 min with varying
 27 KCl concentrations in presence of 30 nM Cs-TopIB and 200 ng of pBR322 as negatively supercoiled
 28 substrate. C) Relaxation activities of wild type Cs-TopIB (TopIB_{wt}) and of the mutant Y477F
 29 (TopIB_{Y477F}) at 30°C for 30 min. The varying concentration of TopIB enzymes used in the assays are
 30 indicated in nM. 200 ng of pBR322 were used as a negatively supercoiled DNA substrate. D)
 31 Decatenation and supercoiling relaxation assays. Decatenation assays were carried out for 10 min at
 32 65°C with 130 nM of Cs-TopIB protein and 200 ng of kDNA as a DNA substrate. (Cat. kDNA:
 33 Catenated kinetoplastid DNA; Dcat: decatenated kinetoplastid DNA). Supercoiling relaxation assays
 34 were carried out for 10 min at 65°C for 10 min with 125 nM of Cs-TopIB protein and 200 ng of
 35 relaxed pBR322 as a DNA substrate. (R: relaxed DNA).

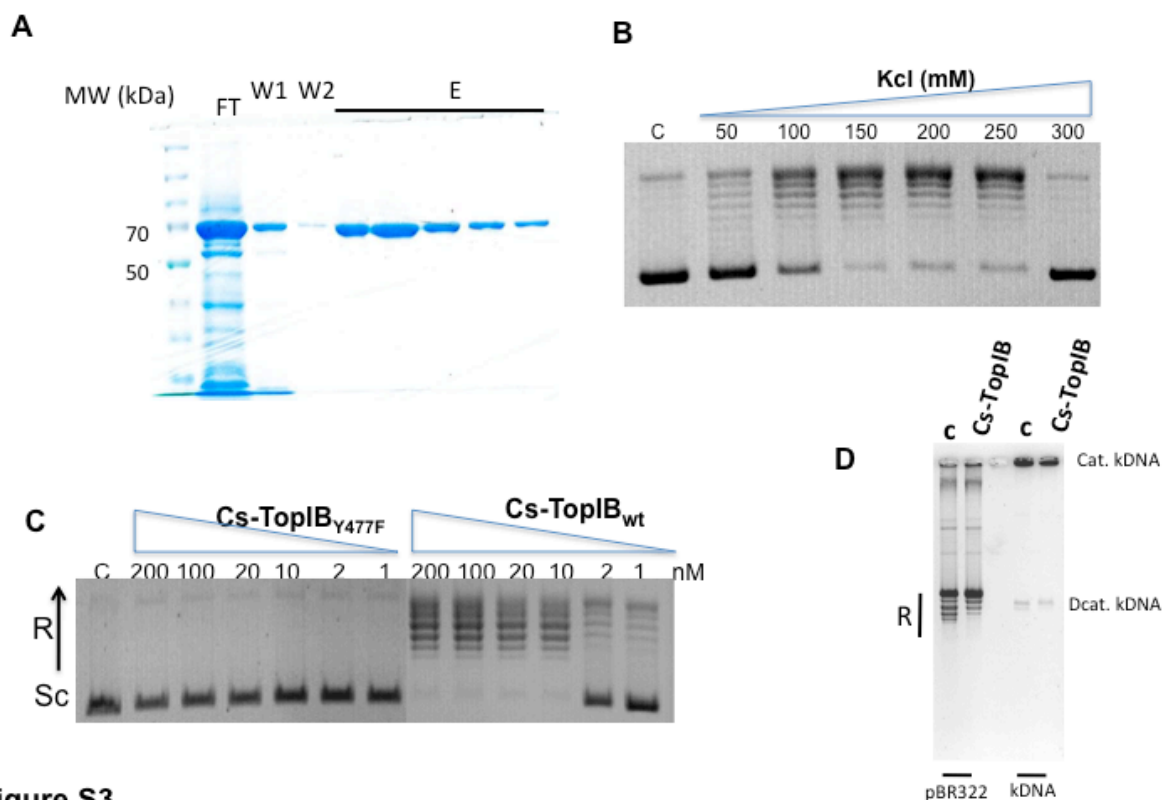


Figure S3

37 **Supplementary Figure S4. Cs-TopIB DNA time-course relaxation assays on negatively**
 38 **and positively supercoiled DNA plasmids. A)** Assays were carried out at 30°C for varying
 39 times (min) with 5 nM of Cs-TopIB protein and 200 ng of pBR322 as positively or negatively
 40 supercoiled DNA substrates. Sc: negatively or positively supercoiled DNA; R: relaxed DNA.
 41 **B)** Graphical depiction of the results, where relaxation activity is calculated as the relative
 42 loss of supercoiled pBR322 substrate, 100% representing the substrate after 2 min of
 43 incubation with Cs-Top1B enzyme.

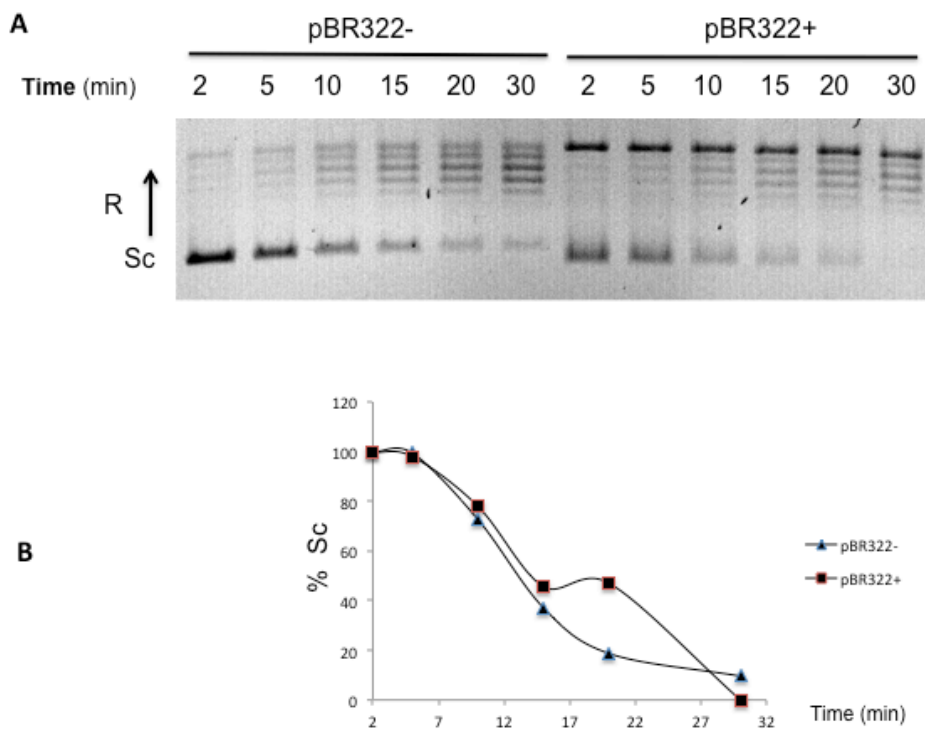


Figure S4

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