Supporting Information

Lee et al. 10.1073/pnas.1304103110

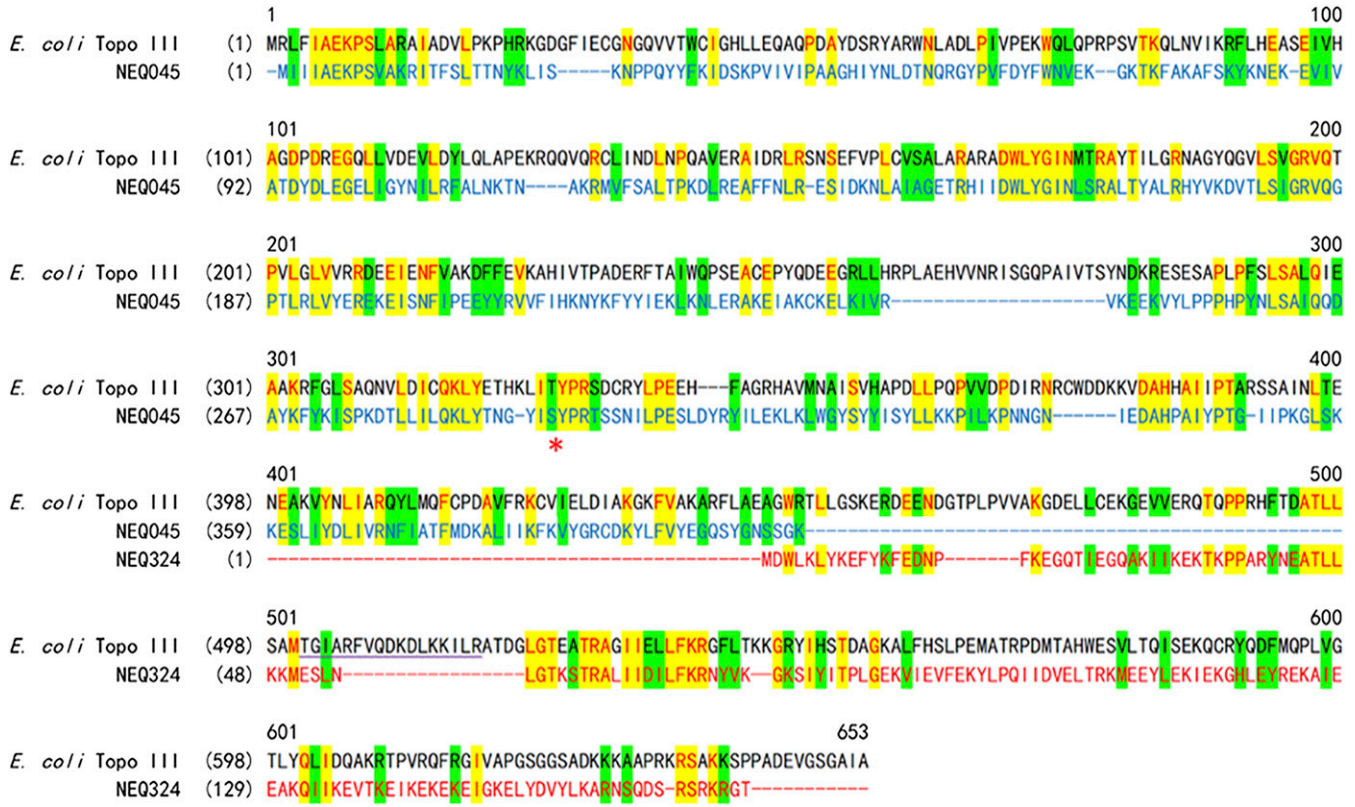
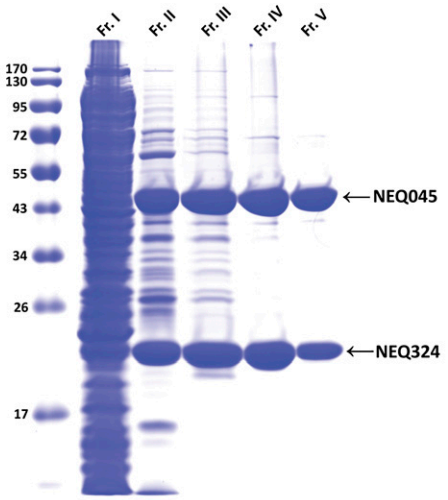


Fig. S1. Sequence alignment of *Nanoarchaeum equitans* split topoisomerase III (Top3) and *Escherichia coli* Top3. NEQ045 (*blue*) and NEQ324 (*red*) are aligned to the N- and C- terminal segments of *Escherichia coli* Top3, respectively. Identical residues are highlighted in yellow, and similar residues are highlighted in green. NEQ045 and the N-terminal segment of *E. coli* Top3 have 39% similarity (25% identity), whereas there is 32% similarity (18% identity) between Neq324 and the C-terminal segment of *E. coli* Top3. Catalytic tyrosine is marked by a red asterisk, and the decatenation loop of *E. coli* Top3 is underlined in purple.



	Volume (ml)	Protein (mg)	Activity (U)	Specific activity (U/mg)	Purification factor	Yield (%)
Fr. I (lysate)	72	2.1×10^3	7.2×10^5	343	1	100
Fr. II (Ni-NTA)	38	13.59	3.8×10^5	2.8×10^4	82	53
Fr. III (Heparin)	10	7.96	2.9×10^5	3.6×10^4	105	40
Fr. IV (Mono S)	5	5.22	2.0×10^5	3.8×10^4	111	28
Fr. V (SEC)	12	4.58	1.8×10^5	3.9×10^4	114	25

Fig. S2. Purification of the protein NeqTop3. (*Upper*) Coexpression of NEQ045 and NEQ324 in *E. coli* allows the purification of recombinant NeqTop3 through four chromatographic steps [fractions (Fr.) II–V; see *Materials and Methods* for details]. Proteins were run in 12% SDS/PAGE and were detected by Coomassie blue staining. (*Lower*) Purification table for NeqTop3. The assay conditions and activity units are described in *Materials and Methods*.

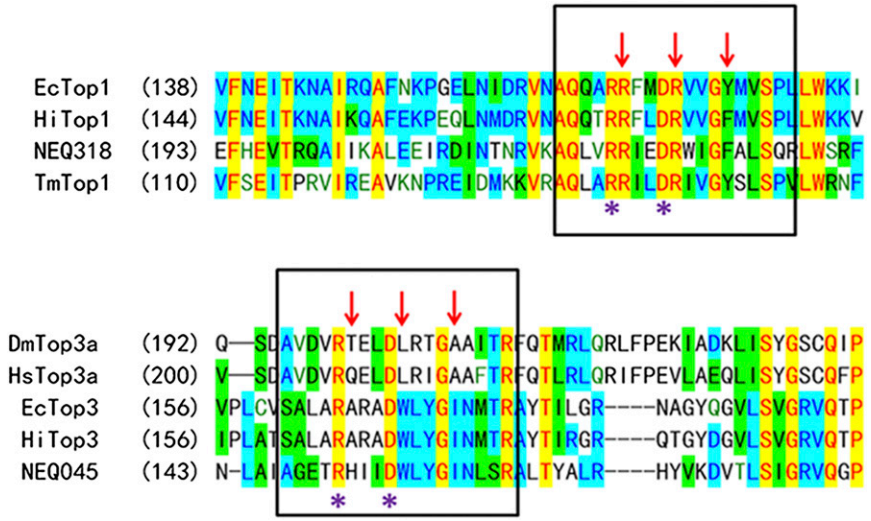
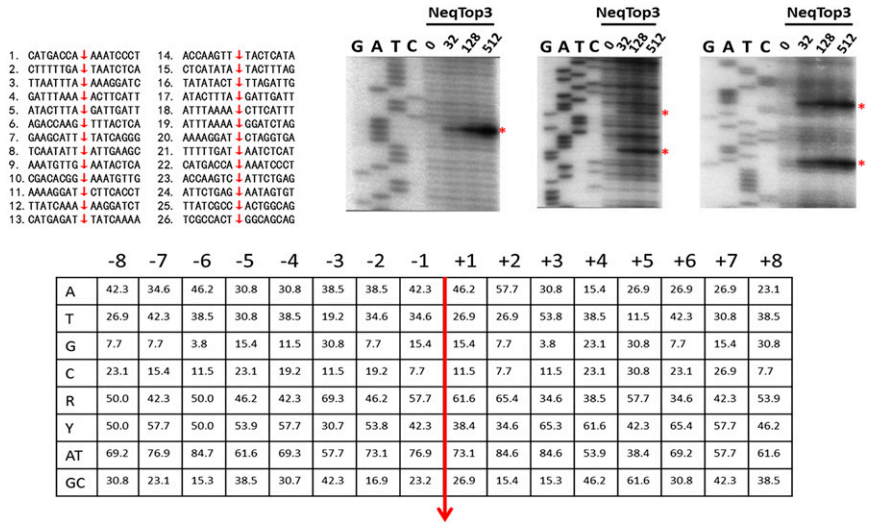


Fig. S3. Alignment of NeqTop3 with type IA topoisomerases in the region critical for −4C selectivity. Two residues in domain 1, arginine and aspartate (purple asterisks), which are required for DNA substrate positioning, are strictly conserved in all type IA enzymes including NeqTop3 (R151 and D155 in NEQ045). However, two arginines and an aromatic residue (red arrows), presumed to be responsible for −4C selectivity, are conserved only in Top1 and reverse gyrase (NEQ318) but not in Top3.



	-8	-7	-6	-5	-4	-3	-2	-1	+1	+2	+3	+4	+5	+6	+7	+8
A	42.3	34.6	46.2	30.8	30.8	38.5	38.5	42.3	46.2	57.7	30.8	15.4	26.9	26.9	26.9	23.1
T	26.9	42.3	38.5	30.8	38.5	19.2	34.6	34.6	26.9	26.9	53.8	38.5	11.5	42.3	30.8	38.5
G	7.7	7.7	3.8	15.4	11.5	30.8	7.7	15.4	15.4	7.7	3.8	23.1	30.8	7.7	15.4	30.8
C	23.1	15.4	11.5	23.1	19.2	11.5	19.2	7.7	11.5	7.7	11.5	23.1	30.8	23.1	26.9	7.7
R	50.0	42.3	50.0	46.2	42.3	69.3	46.2	57.7	61.6	65.4	34.6	38.5	57.7	34.6	42.3	53.9
Y	50.0	57.7	50.0	53.9	57.7	30.7	53.8	42.3	38.4	34.6	65.3	61.6	42.3	65.4	57.7	46.2
AT	69.2	76.9	84.7	61.6	69.3	57.7	73.1	76.9	73.1	84.6	84.6	53.9	38.4	69.2	57.7	61.6
GC	30.8	23.1	15.3	38.5	30.7	42.3	16.9	23.2	26.9	15.4	15.3	46.2	61.6	30.8	42.3	38.5

Fig. S4. Mapping the consensus sequence of NeqTop3 cleavage. The NeqTop3 cleavage sequences were determined by the primer extension method using NeqTop3 cleavage products as template. The polymerization reactions terminated at the NeqTop3 cleavage sites, which are highlighted with red asterisks. The sequences surrounding cleavage sites were collected and the statistics show that NeqTop3 has no strictly conserved recognition sequence except for preferring AT-rich regions.

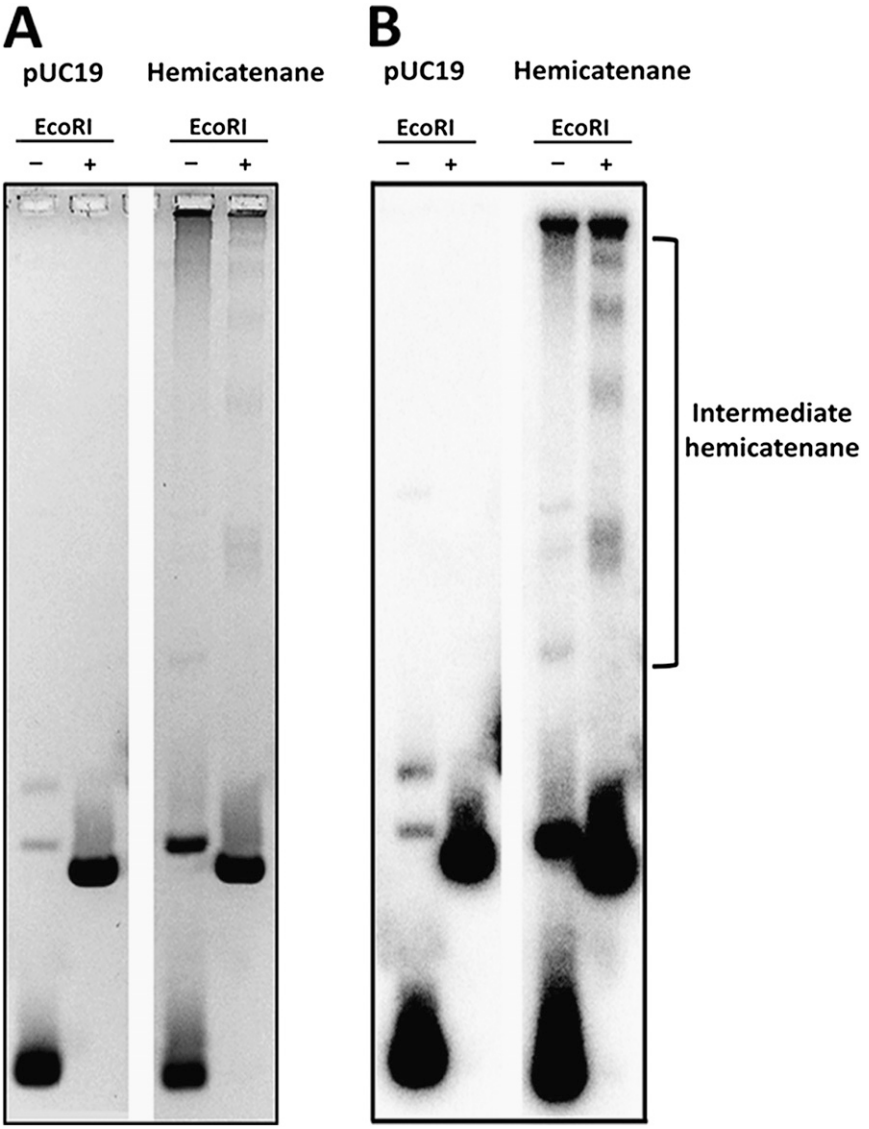


Fig. S5. Restriction assays of hemicatenanes with less complexity. (*A*) (*Right*) The DNA networks created by NeqTop3 with lower concentrations (hemicatenanes with less complexity) were treated with restriction endonuclease to linearize the DNA, analyzed by gel electrophoresis, and stained with ethidium. Linearization can liberate only part of the hemicatenane networks. (*Left*) Control experiment with plasmid DNA. (*B*) The same gel was analyzed by Southern blot hybridization to highlight the presence of hemicatenane intermediates after linearization.

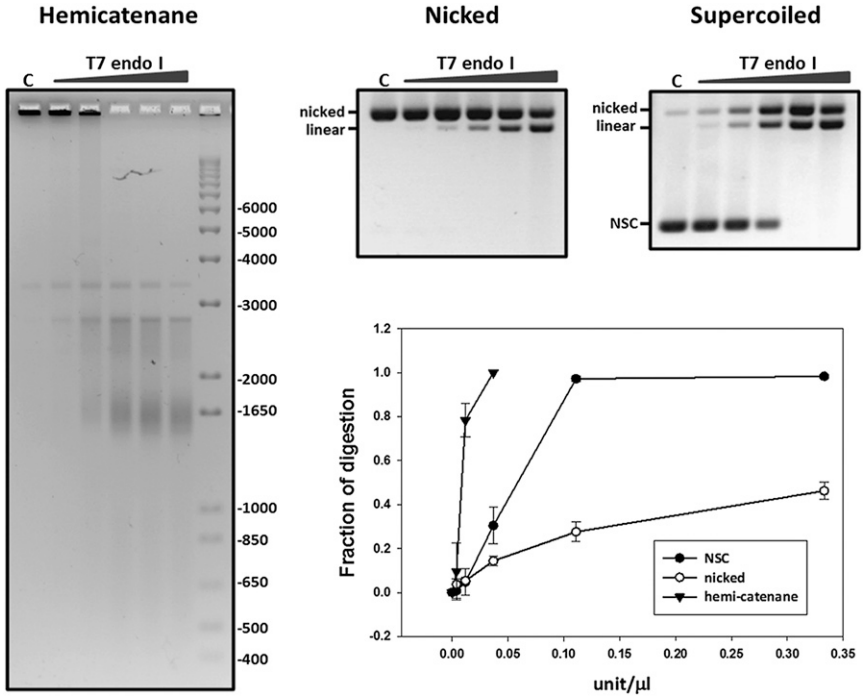


Fig. S6. Hemicatenanes are exquisitely sensitive to T7 endonuclease I treatment. Supercoiled and nicked pUC19, and hemicatenanes were treated with T7 endonuclease I. Hemicatenane networks can be resolved by low levels of the enzyme, and its products are able to migrate into gel. Hemicatenanes are more sensitive to this structure-specific endonuclease that is capable of resolving Holliday junctions, suggesting a Holliday junction-like structure.

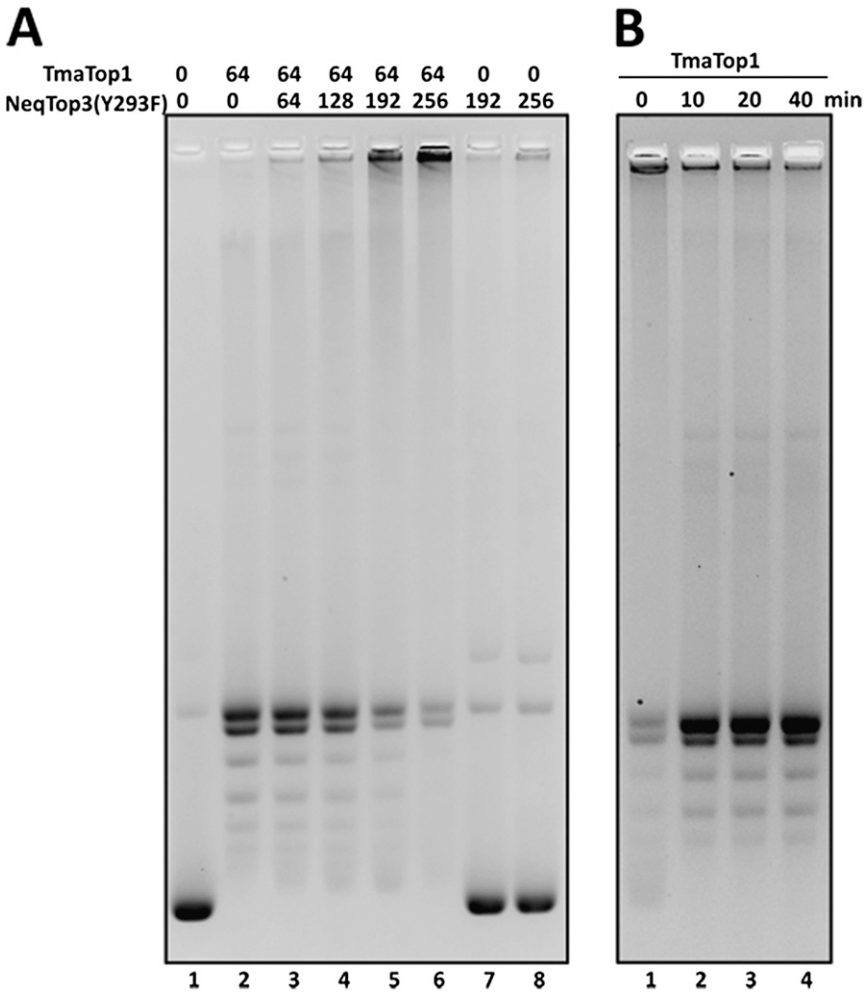


Fig. S7. *Thermotoga maritima* topoisomerase I (TmaTop1) can mediate the formation and dissolution of hemicatenanes. (*A*) In the presence of increasing amounts of a condensing agent NeqTop3(Y293F), TmaTop1 can generate more hemicatenanes (lanes 2–6). NeqTop3(Y293F) alone is inactive in hemicatenation (lanes 7 and 8). The protein concentrations used in these reactions are given above each lane (in nM). (*B*) The hemicatenanes produced under conditions like those shown in lane 6 in *A* were purified and treated with 32 nM of TmaTop1. Reaction products at various time points were analyzed (lanes 1–4). Increasing amounts of hemicatenanes can be dissolved during incubation.