

Supplementary Table 1: Codon-optimized genes

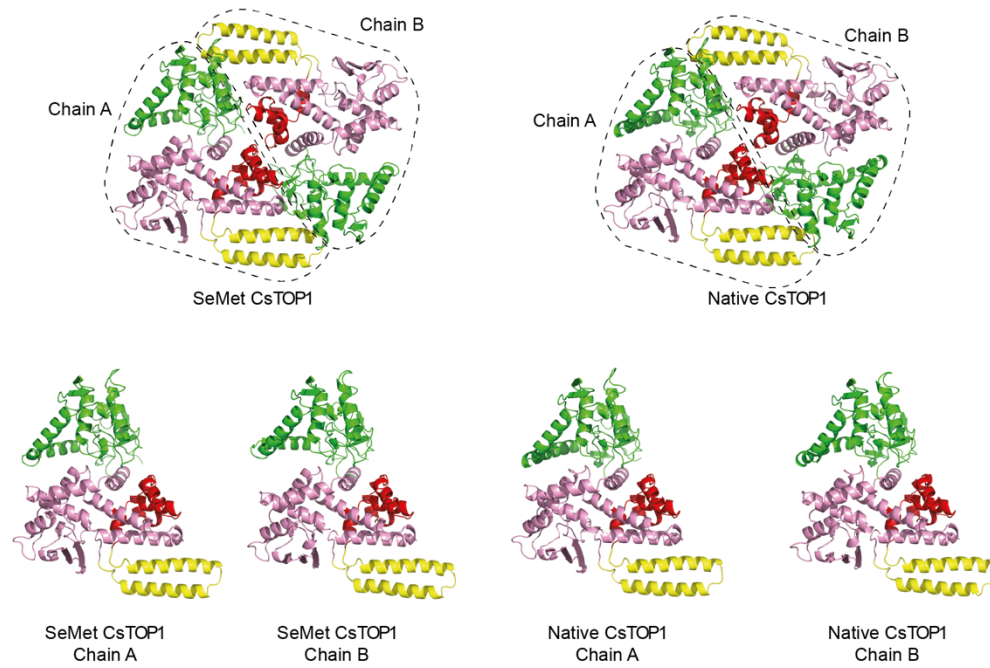
Sequence of the codon-optimized genes used in this study. The sequences of the His-tag and the cleavage site (blue) and the sequence of the gene (black) are shown.

Codon-Optimised gene	Sequence
CsTop1	<p>ATGAAACATCACCATCACCATCACCCCATGAGCGATTACGACATCCCCACTACTGAGAATCTTTATTTTCAGGGC GCCATGGTGAAATGGCGTACCCTGGTCCACAATGGTGTGCGACTGCCGCCGCGTATCAACCGAAAGGTCTGTCA ATCAAAAATCCGTGGTGAACCCGTGAAACTGGATCCGCTGCAGGAAGAAATGGCGTATGCCCTGGGCACTGAAGAAA GATACGCCGTACGTGCAGGATCCGGTTTTTCAGAAAACTTTCTGACCGATTTTCTGAAAACGTTCAACGGCCGC TTTCAGGACGTTACCATCAACGAAATCGATTTTCAGCGAAGTCTACGAATACGTGGAACGTGAACGCCAACTGAAA CGGGATAAAGAATACCGTAAGAAAATTTCTGCCGAACGTAACCGCTGCGTGAAGAACTGAAAGCGCGCTACGGC TGGCCGAAATGGATGGTAAACGTTTCGAAATTCGAACTGGATGGTTGAACCCGCGGGCATCTTTATGGGTCGT GGTAATCATCCGCTGCGTGGTTCGTTGAAACCGCGTGTCTATGAAGAAGACATTACCCTGAATCTGGGTGAAGAT GCCCGGTCCCGCCGGTAATTGGGGTCAGATCGTGCATGATCAGACTCAATGTGGCTGGCCCGCTGGGATGAC AACTGACCGGTAAGAAAAATATGTGTGGCTGAGCGATACCGCAGATATTAACAGAAACCGCAGAAAAAGCAAA TAGCAGAAAGCAGAAATGCTGAAAACCATATTGATCGTGTGCGGAGAAAAATCTTTAAAAGGTCTGCGTAGCAAA GAACCGAAAAATGCGTGAATTGCCTGCGCTGTTATCTGATTGATCGTCTGGCAATGCGTGTGGTGATGAAAA GATCCGGATGAAGCAGATACCGTTGGTGAACCCACCTGCGTGTGAACATGTTAAACTGCTGGAAGATCGCATC GAGTTGATTTTCTGGGTAAAGTAGCGTTCTGTTGGCAGAAAAGCATTGATCTGCGTAAATGAACCTCCGGAGTT CGGCAGGTTTTTGAAGAAGTTCGGAAGGTAAAAAAGAAAGCGATCAGATTTTCCAGAAACATCAATAGCGGTCAT GTGAATCGTTTTCTGGGCAAAATGTTAAAGGCCCTGACCGCAAAAGTGTTCGTACCTATATCGCAACCAAAATC GTCAAAGATTTCCGCGCAGCAATTCGCGGTGAAAAAGTTACCAGCCAAGAAAAATTCATCTATTACGCCAAACTG GCCAATCTGAAAGCAGCAGAAGCACTGAATCATAAACGTGCACCGCCTAAAAATTTGGGAACAGAGCATCCAGAAA AAAGAGGAACGCGTTAAAAAACTGATGCAGCAGCTGCCGGAAGCCGAAAGCGAGAAAAAAAAGCACGTAATTGCA GAACGCTCGGAAAAAGCAGAAGCACTGAACCTGGATCTGGCAGTTAAAGTTTCGTGATTACAATCTGGCGACCAGCCTG CGTAACATATATTGATCCGCGTGTGTATAAAGCATGGGGTTCGTTATACCGGTTATGAATGGCGTAAAAATCTATACC GCAAGCCTGCTGCGTAAATTCAAATGGGTTGAAAAAGCCAGCGTTAAACATGTGCTGCAGTATTTTCCGCAAAAA CTGGCAAAAGATGTGGATAAAGGTATGCGAGTTAAAGCAGCCGTTTAA</p>
CsTOP1 ^{HsLinker-} Cter	<p>ATGAAACATCACCATCACCATCACCCCATGAGCGATTACGACATCCCCACTACTGAGAATCTTTATTTTCAGGGC GCCGTGAAATGGCGTACCCTGGTCCACAATGGTGTGCGACTGCCGCCGCGTATCAACCGAAAGGTCTGTCAATC AAAATCCGTGGTGAACCCGTGAAACTGGATCCGCTGCAGGAAGAAATGGCGTATGCCCTGGGCACTGAAGAAAGAT ACGCCGTACGTGCAGGATCCGGTTTTTCAGAAAACTTTCTGACCGATTTTCTGAAAACGTTCAACGGCCGCTTT CAGGACGTTACCATCAACGAAATCGATTTTCAGCGAAGTCTACGAATACGTGGAACGTGAACGCCAACTGAAAGCG GATAAAGAATACCGTAAGAAAATTTCTGCCGAACGTAACCGCTGCGTGAAGAACTGAAAGCGCGCTACGGCTGG GCCGAAATGGATGGTAAACGTTTCGAAATTCGAACTGGATGGTTGAACCCGCGGGCATCTTTATGGGTCGTGGT AATCATCCGCTGCGTGGTGGTTGAAACCCGCTGTCTATGAAGAAGACATTACCCTGAATCTGGGTGAAGATGCG CCGGTCCCGCCGGTAATTGGGGTCAGATCGTGCATGATCAGACTCAATGTGGCTGGCCCGCTGGGATGACAAA CTGACCGGTAAAGAAAAATATGTGTGGCTGAGTGTACGGCAGACATCAAAACAGAAACGTGATAATCCAAATAC GACAAAGCTGAAATGCTGAAAAATCATATTGATCGCGTTTCGTGAAAAATCTTCAAAGGCCCTGCGCTCGAAAGAA CCGAAAAATGCGTGAATTTGCTCTGGCGTGTACCTGATCAGCCGCTGCGGATGCGTGTGGCGATGAAAAAGAT CCGGACGAAGCAGACACCGCTCGGTGCTACCACGCTGCGCGTGAACACGTTAAAGTCTGGAAGATCGTATTGAA TTTGACTTCTGGGTAAAGTAGCGTGCCTGGCAGAAATCTATCGATCTGCGCAACGAACCGCGGAAGTTCGT CAAGTCTTTGAAGAACTGCTGGAAGGCAAGAAAGAAAGGCGATCAGATTTTCCAAAACATCAATAGTCGCCATGTT AATCGTTTTCTGGGCAAAATGTTAAAGTCTGACCGCAAAAGTCTTCCGCACCTACATCGCTACGAAAAATCGTG AAAGATTTTCTGGCGCCATTCCGCGTAAAAAGTTACGAGTCAGGAAAAATTCATCTATTACGCAAACTGGCT AACCTGAAAGCAGCTGAAGCGCTGAATCACCAGCGCGCACCGCCGAAACCTTTGAAAAATCCATGATGACCTG CAGACGAAAAATCGATGCGAAGAAAGAACTGGCCGATGACCGTGCAGCTGCGGACCTGAAATCAGCTAAAGCGGACGCC AAAGTGTGAAAGATGCGAAACCAAGAAAGTGGTTGAAAGCAAAAAGAAAGCGTCCAGCGCCCTGGAAGAACAA CTGATGAAACTGGAAGTGCAGGCAACGGATCGTGAAGAAAAACAACAAATTTGCTCTGGGCACCTCAAACCTGAAT TATCTGGACCCGCTATCAGGTTGGCTGGTGTAAAAATGGGGTGTTCGGATTGAAAAATCTACAATAAAACG CAGCGTAAAAAATTTGCTTGGGCTATTGATATGGCGGATGAAGACTATGAATTTCAA</p>

Supplementary Table 2: primer sequences

Sequence of the DNA oligonucleotides used in this study.

Name	Sequence	Construct
CsFL154R	5'-TAATCATCCGAAGCGTGGTCGTTG-3'	L154K point mutation in CsTop1
CsRL154R	5'-CCACGACCCATAAAGATG-3'	
CsFL399RL402R	5'-CGCTTCAGCTGCTTTCCGGTTAGCCCGTT TTGCGTAATAGAT-3'	L399R L402R point mutations in CsTop1
CsRFL399L402R	5'-ATCTATTACGAAAACGGGCTAACCGGA AAGCAGCTGAAGCG-3'	
CsFHsCat	5' CTACTGGATCCGCGTGTGTATAAAAG-3'	CsTOP1 ^{HsTyr} point mutations
CRFHsCat	5' TTCAGTTTGCTGGTCGCCAAGATTGTA-3'	
Q5SDM_12/7/20 16 F	5'-GAAATGGTGGGAAGAAGAGCGCTATCCT GAAGGCATCGTGAAATGGCGTACCCTG-3'	CsTOP1 ^{HsNter} insertion
Q5SDM_12/7/20 16 R	5'- CACTTCTGTTCCTCTTCTTTCTTCGGCTTC TTTTTCTTGATGGTGTATGGTGTATG-3'	
Q5SDM_4/13/20 19 F	5'-CCCTAGCGCAGATATTAACAGAAACG- 3'	Hinge-1 point mutations (MLNPS)
Q5SDM_4/13/20 19 R	5'-TTCAGCATCACATATTTTTCTTACC GG-3'	
Q5SDM_4/13/20 19 F	5'-CCTCCCGCAGATATTAACAGAAACG-3'	Hinge-2 point mutations (WLSPP)
Q5SDM_4/13/20 19 R	5'-GCTCAGCCACACATATTTTTCTTACC-3'	
CsTop1WLSGGf wd	5'- AAAAATATGTGTGGCTGAGCGGTGGCGC AGATATTAACAGAAACG-3'	Hinge-3 point mutations (WLSGG)
CsTop1WLSGGr ev	5'- CGTTTCTGTTAATATCTGCGCCACCGCT CAGCCACACATATTTTT-3'	
CsTop1Y207Afw d	5'- CTGACCGGTAAAGAAAAAGCTGTGTGG CTGAGCGATAC-3'	Y207A point mutation in CsTop1
CsTop1Y207Arev	5'-GTATCGCTCAGCCACACAGCTTTTTCTTT ACCGGTCAG-3'	



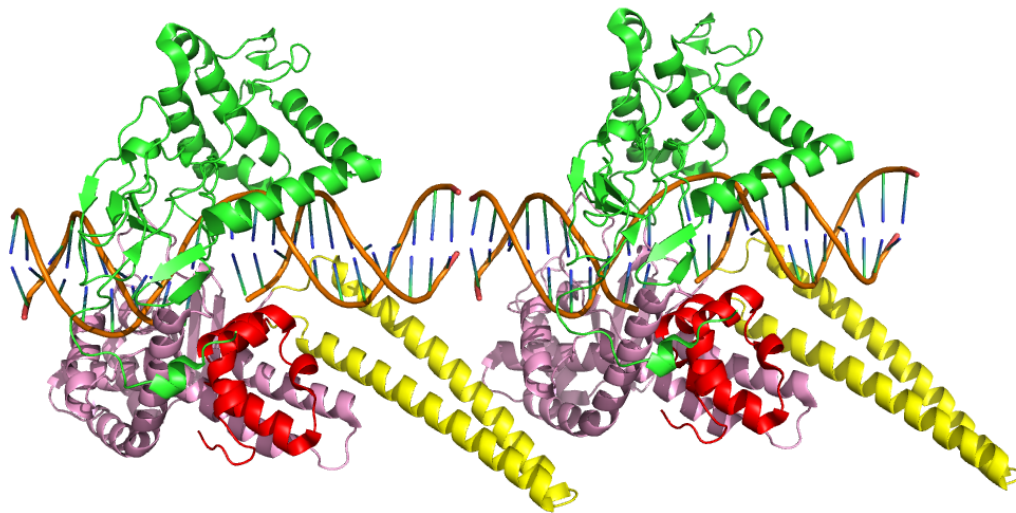
Supplementary Figure 1: CsTOP1 structure in one asymmetric unit.

Cartoon representation of the two CsTOP1 chains found in one asymmetric unit in SeMet CsTOP1 and native CsTOP1 crystals. The different subunits were further superposed. Coloring scheme is the same as Fig. 1.

Caldiarchaeum_subterraneum	-----MVKWRTLHNGVALPPYQPKGLSIRGETVTKLDPLQEEMAYAWALK	48	
Nitrososphaera_viennensis	MEEVAAAPQPQARIRWSSLVHRGVAFPEHQPRGITISIKGEKVALNADQELVYAWAKK	60	
Crenarchaeota_archaeon	-----MKWKTLOHNGILFPFAFESQGIKIKVKEKVLDDLQEMIIYQWAKK	47	
Heimdallarchaeota_archaeon	-----MTKIEQLIHNGVLLSE-IPYLELSIEINGKVKHLEKSEQMAIAWVRK	47	
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Caldiarchaeum_subterraneum	KDTPYVQDPVFQKNFLTDFLKTFNFRQ--DVTINEIDFSEVYEVVERERQLKA-----	100	
Nitrososphaera_viennensis	KDTHYVQDPVFQSNFLDLKPLPEKPRKADLKI SDPDFSQAFLADEKMMKEREKERI	120	
Crenarchaeota_archaeon	KDTPYAQDKVFNFTADFAKTLDPKFK--GIKYEDIDFSEAFKVVDEKDLKE-----	99	
Heimdallarchaeota_archaeon	LSTVYVEDP IFCNKYFKDFGEEI----GDASLTDNDIDFSEVIAVVEKQKEIRD-----	97	CAP domain
	. * . : * : * . * : * : : . . : : * * : . . : : : :		
Caldiarchaeum_subterraneum	---DKEYRKKISAERKRLREELKARYGWAEMDGKRFEIANWVPEPPIFMGRGNHPLRGR	157	
Nitrososphaera_viennensis	KNLPREKKMAEAKKAERERLALYGVAVDQEVDIANWLVPEPGLFMGRGHPLRGR	180	
Crenarchaeota_archaeon	-MMTKEEKSLAAKRKLEKMKYKAVMDGQEVVANYMAEPPGIFIGRGEHPLRGR	158	
Heimdallarchaeota_archaeon	-AKTKEQKQEREERKVIREFQLKEKYGATLDGAQVEISNYTAEPSIFMGRGEHPMRGK	156	
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Caldiarchaeum_subterraneum	WKPRVYEEDITLNLGEDAPVPPGNWQIVHDHDSMWLARWDDKLTGKEKYVWLSDTADIK	217	
Nitrososphaera_viennensis	WKPRVQPQDVTLNLGEDAPVPEGEWKEIVHDHTS T W L A T M E N L T E K R K Y V W L H D S S E L R	240	
Crenarchaeota_archaeon	WKKRVTPEVDVILNLGDAKVPVPGWGI I H D Q N S M W L A S W M D D L T Q R K R Y V W L A D T A G L K	218	
Heimdallarchaeota_archaeon	WKEGPSKADIVLNLSPAPRPEGEWLEIVWEPECLWIAKWTDKLSGKTKYVWLSDTTPIK	216	Hinge
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Caldiarchaeum_subterraneum	QKRDKSKYDKAEMLENHIDRVREKIFKGLRSKEPKMREIALACYLIDRLAMRVGDEKDPD	277	
Nitrososphaera_viennensis	QGNDKAKYDKALNLAQQLGKVEKEIMRKMKSND---KAATAAYLIFKLAMRVGDEKDPD	297	
Crenarchaeota_archaeon	QERDQAKYDKATKLAKETEKIKNSIVKDMKSKDKLSRIATACYLIYRTAMRVGDEKDPD	278	
Heimdallarchaeota_archaeon	QDREIEKYDKANLVGDNLETIRNQIMSAISGSDKRNKKVATACYIIDLHNI RVGDEKDPD	276	
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Caldiarchaeum_subterraneum	EADTVGATTLRVEHVKLE---DRIEFDLKGDSVVRQKSIDLRNEP-PEVRQVFEELL	332	
Nitrososphaera_viennensis	EADTVGASTLRVEHIKFPQNGKQYIEFNFLGKDSVPWQKTLVNSDTRALYDNLRFNM	357	
Crenarchaeota_archaeon	EADTVGATTLRKEHIKLTG---KAI E F D L G K D S V R W Q E T V P A E G Q D - K Q F Y D N L E K L I	333	
Heimdallarchaeota_archaeon	EADTVGATTLRPEHIKING---STVIFDFLKGDSVVEWHKEREFFEIFVNSLQELINEAN	332	CAT domain
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Caldiarchaeum_subterraneum	EGKKEGDIQFNINSRHVNRFLGKIVKGLTAKVFTYIATKIVKDFLAAIPRE--KVTSQ	390	
Nitrososphaera_viennensis	KGKPKDQIFDIDINSRKVNFAFFTVMPLTAKVFTCIATKVVQALVNPPIKVDNRNSQE	417	
Crenarchaeota_archaeon	ANKKPKDEIFDGITSRHVNAIYSSIVKGLTAKVFTYLA T V N S Y L K N H D N V -- K S K S A	391	
Heimdallarchaeota_archaeon	ESGNDKQIFSDIGSRHVNAFDEIVDGLTAKVFTYHATTVVRNFLDESVD--PADPD	390	
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Caldiarchaeum_subterraneum	EKFIIYAKLANLKAEEALNHKRAPPKN-----WEQSIQKKEE-----	427	
Nitrososphaera_viennensis	SDKVYVAKSANLKAIECNHKKGVDPKNPAAKKAAEKFEAVAKRNQ-----	464	
Crenarchaeota_archaeon	NEKLYHAKMANLAAKMCNHRKRTI PKT-----YETALQKKE-----	428	
Heimdallarchaeota_archaeon	FAKKEAAMNREAAVECTHKKQEPKN-----WENRMQKYRERKLGQERIEKAN	440	
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Caldiarchaeum_subterraneum	-----RVKKLMQO--L-----	436	
Nitrososphaera_viennensis	-----ATAELEKQVAA-----	475	
Crenarchaeota_archaeon	-----TLKKAKES--T-----	437	
Heimdallarchaeota_archaeon	DNQTKREERIEELKGNLKLRRKQVREQEFLAQTKEDLAVIKNMSTNFATQREKDRHKNA	500	Linker
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Caldiarchaeum_subterraneum	-----REAESEK-----KK---ARIAERLEKAEINLNDLA	462	
Nitrososphaera_viennensis	-----GNWKTET-----QE---KRLKERLAKLKMQLKLQ	501	
Crenarchaeota_archaeon	-----PKTEK-----QK---EKLKDRIEKLKIQIDLQ	461	
Heimdallarchaeota_archaeon	IVKAKKKIETVKKKVVAEKRVETAKNQVERGKNSVGTAKERVYKAKLALKKIESQEKIS	560	
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Caldiarchaeum_subterraneum	VKVRDYNLATSRLNYIDPRVYKAWGRYTGVEWRKIYASLLRKFVWEKASVKHV--LQY	520	
Nitrososphaera_viennensis	QETRDYNLGTSLRNYIDPRVMKAWLNVLDLWTKVYATLQKRFKWEVGYEKNY--SRF	559	
Crenarchaeota_archaeon	EKTQDYNLGTSLRNYIDPRVFAWTEVGAWEKLYTAALQKFLVWKSADVWVKDISQQ	521	
Heimdallarchaeota_archaeon	KKTKTNLGTSLKSYIDPRVYDVGKVEDYDWRNYYSNALQRKFSWVERDADE-----	613	C-terminal
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Caldiarchaeum_subterraneum	FAEKLAKVDKGMQVKAIV	539	
Nitrososphaera_viennensis	YP-----	561	
Crenarchaeota_archaeon	Y-----	522	
Heimdallarchaeota_archaeon	-----	613	

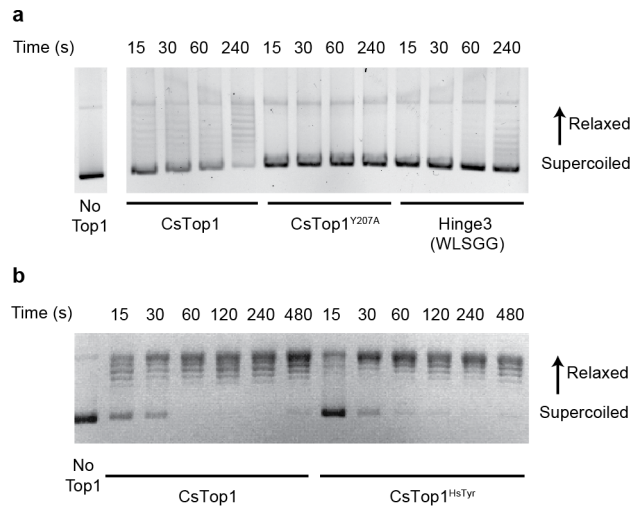
Supplementary Figure 2: Alignment of archaeal TOP1 sequences.

Alignment of four archaeal TOP1 sequences from different phyla including *Caldiarchaeum subtarreneum* (Aigarchaeota), *Nitrososphaera viennensis* (Thaumarchaeota), *Crenarchaeota* archaeon (Crenarchaeota) and *Heimdallarchaeota* archaeon (Heimdallarchaeota, Asgard).



Supplementary Figure 3: Packing artifact of HsTOP1-DNA.

Cartoon representation of two complexes within the packing of HsTop1-DNA crystal structure (PDB 1K4T). The linker domain (yellow) of one TOP1 is pushed away from the DNA by the CAT domain (pink) of a neighboring TOP1 molecule. Coloring scheme is the same as Fig. 1.



Supplementary Figure 4: Relaxation of supercoiled DNA by mutated CsTop1.

a) Relaxation assay with CsTop1, CsTop1(Y207A) and CsTop1 (Hinge3, WLSGG). **b)** Relaxation assay with CsTop1 and CsTop1(HsTyr). The kinetics of relaxation is shown for each construct. Both experiments were done twice independently.