Supporting Information

Sirikantaramas *et al.* 10.1073/pnas.0801038105

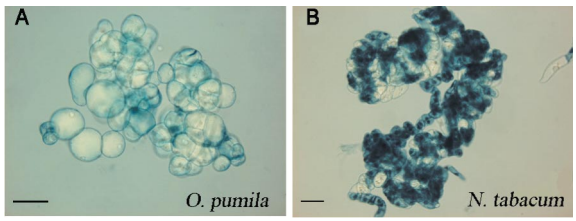

Fig. S1. Trypan blue exclusion cell-viability assay of CPT-producing *Ophiorrhiza pumila* (*A*) and CPT-nonproducing *Nicotiana tabacum* (tobacco BY-2) (*B*) cocultured with CPT (10 μM) for 24 h. Whereas the *O. pumila* cells were resistant, the tobacco cells were sensitive to CPT. (Scale bars: 0.2 μm.)

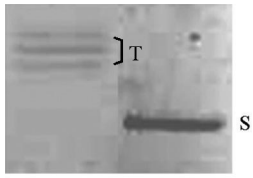

Fig. S2. Top1 activity of the *Op*Top1 recombinant protein toward pUC19 DNA. Relaxation of the supercoiled plasmid (S) pUC19 by recombinant *Op*Top1 from *Escherichia coli*. Topoisomers (T) of the plasmids were generated when the latter were incubated with recombinant *Op*Top1 that possesses Top1 activity.

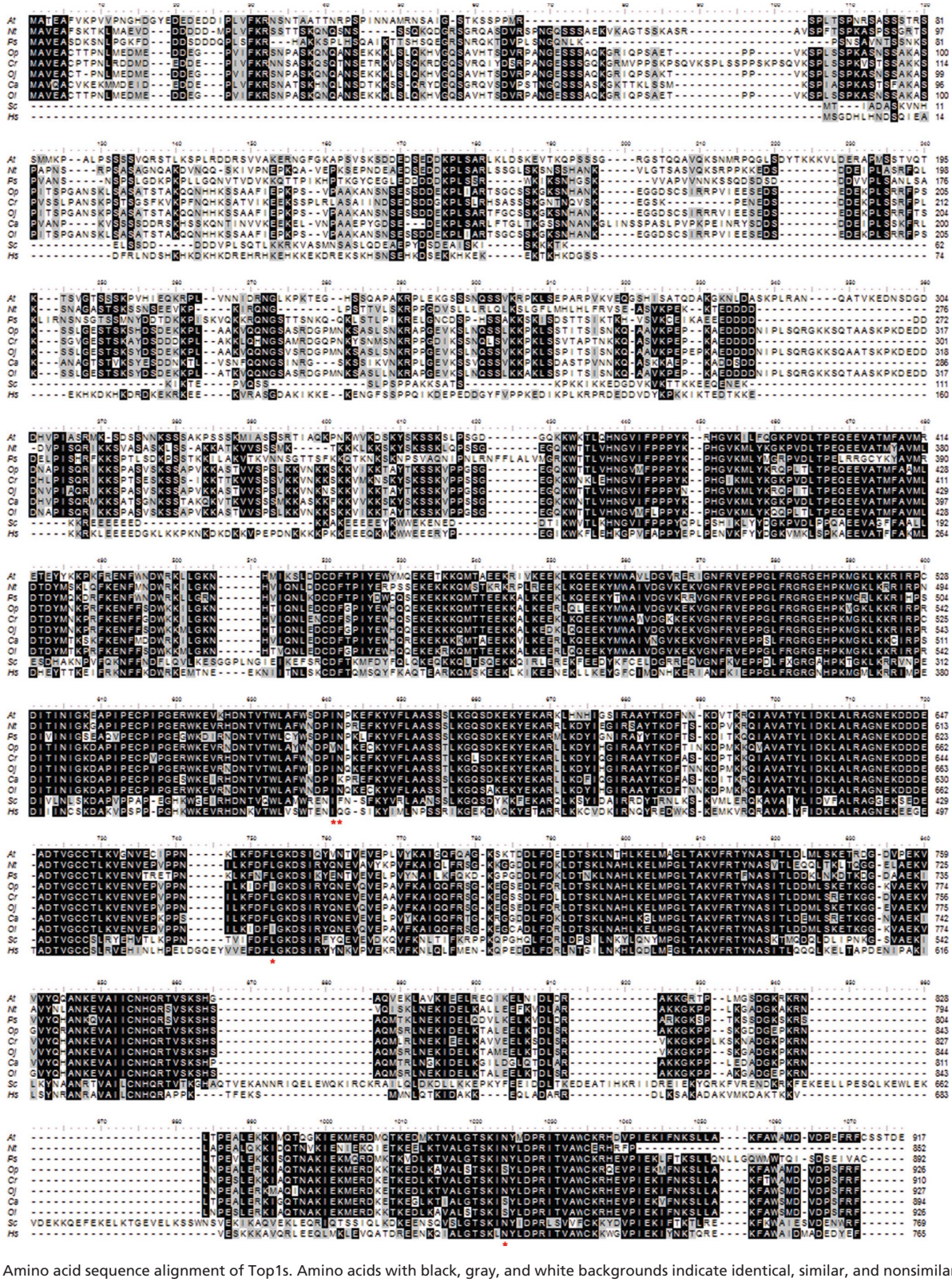

Fig. S3. Amino acid sequence alignment of Top1s. Amino acids with black, gray, and white backgrounds indicate identical, similar, and nonsimilar residues, respectively. The amino acid substitutions at position 420, 421, 530, and 722 are marked with red asterisks. *At*, *Arabidopsis thaliana*; *Nt*, *Nicotiana tabacum*; *Ps*, *Pisum sativum*; *Op*, *Ophiorrhiza pumila*; *Cr*, *Catharanthus roseus*; *Oj*, *Ophiorrhiza japonica*; *Ca*, *Camptotheca acuminata*; *Ol*, *Ophiorrhiza liukiuensis*; *Sc*, *Saccharomyces cerevisiae*; *Hs*, *Homo sapiens*.

Table S1. Quantitative CPT sensitivity assay in *Saccharomyces cerevisiae* mutant RS190

Gene	Relative number of viable cells at the CPT concentration indicated*		CPT sensitivity
	0 μg/ml	5 μg/ml	
*Op*Top1	1.00	0.96	R
*Op*Top1;Ile530Leu,Ser722Asn	1.00	0.77	S/R
*Oj*Top1	1.00	0.52	S/R
*Cr*Top1	1.00	0.02	S

S. cerevisiae cells expressing plant Top1s as indicated were cultured in liquid medium containing CPT at 0 (0.25% Me_2SO) or 5 μg/ml. At 8 h, aliquots were diluted, plated on YPD medium, and incubated at 30°C. The number of viable cells forming colonies was counted. R, CPT-resistant Top1; S/R, partially CPT-resistant Top1; S, CPT-sensitive Top1. Note that Top1 of CPT-producing *O. pumila* is resistant to CPT. *Op*Top1 mutant;Ile530Leu and Ser722Asn has become partially sensitive to CPT, suggesting the importance of these two residues in CPT resistance. Although neither *O. japonica* nor *C. roseus* produces CPT, *Oj*Top1 exhibits partial resistance to CPT compared with that of *C. roseus*, suggesting the concept of the CPT pre-resistance of Top1.

*Average number obtained from three experiments. The number of viable forming colony was divided by the number of colonies obtained on plates with no CPT.

Table S2. Oligonucleotide primers used to clone cDNAs encoding Top1

Gene	Primer	Sequence
OpTop1	Full-length/F	5′-***AAAAAGCAGGCTTA***ATGGCTGTTGAGGCCTGTACTACTC-3′
	Full-length/R	5′-***AGAAAGCTGGGTA***TCAAAATCTGAAGCTGGGATCAACAT-3′
OlTop1	5′ RACE	5′-ATTCAACCGTGACATTTGTGCACT-3′
	Full-length/F	Same as the *OpTop1* full-length/F primer
	Full-length/R	Same as the *OpTop1* full-length/R primer
OjTop1	5′ RACE	Same as the *OlTop1* 5′ RACE primer
	3′ RACE	5′-GAAGGAACTCATGCCTGGTCTTAC-3′
	Full-length/F	5′-***AAAAAGCAGGCTTA***ATGGCTGTTGAGGCCTGTACTCCT-3′
	Full-length/R	Same as the *OpTop1* full-length/R primer
CaTop1	5′ RACE	5′-ATTGCAACCTCCTTGTTTGCAT-3′
	3′ RACE	5′-GATGATCTTTTTGACAAGCTGGATAC-3′
	Full-length/F	5′-***AAAAAGCAGGCTTA***ATGGCTGTTCAGGCTTGTGTCAAA-3′
	Full-length/R	5′-***AGAAAGCTGGGTA***TCAGAATCTGAAGCTGGGATCAACA-3′
CrTop1	5′ RACE	5′-CTTGGATACAGTCCGCTGATGATTAC-3′
	3′ RACE	5′-TGAGGTTGAGGTTGAAGCTGCTG-3′
	Full-length/F	5′-***AAAAAGCAGGCTTA***ATGGCTGTTGAGGCTTGTCCAACAC-3′
	Full-length/R	5′-***AGAAAGCTGGGTA***TCAGAATCTGAAACTGGGATCAACAT-3′

The regions of primers with introduced Gateway *att*B1 forward or *att*B2 reverse primers are in boldface italics.