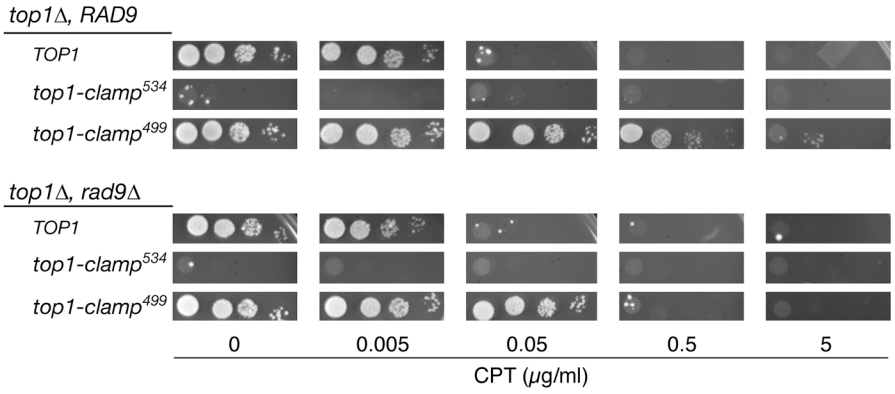

FIG. 1S. **The Rad9 DNA damage checkpoint protects cells from CPT poisoning of Top1-clamp^{499}.** Exponential cultures of congenic *top1*Δ yeast cells, wild-type for *RAD9* or *rad9*Δ, transformed with the indicated YCpGAL1-top1 vector, were serially 10-fold diluted. Aliquots of 5 µl were spotted onto SC-uracil supplemented with galactose, 25 mM HEPES, pH 7.2, the indicated concentration of CPT, and 0.125% Me2SO. Cell viability was assessed after incubation at 30ºC.

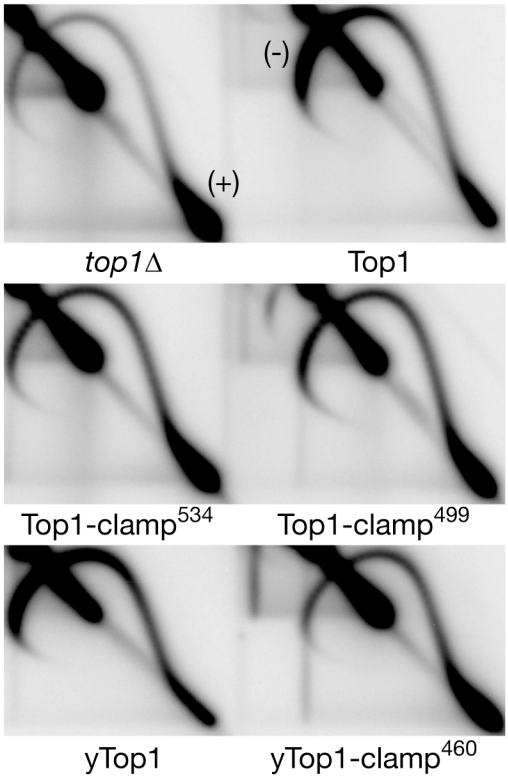

FIG. 2S. ***In vivo* Top1 activity assays.** The *in vivo* activity of the Top1-clamp proteins was assayed in 2-D agarose gels following purification of 2µm DNA topoisomers (1). Exponential cultures of *top1*Δ, *top2ts* cells, co-transformed with YEpGPD-topA (which constitutively expresses bacterial DNA topoisomerase I) and the indicated YCpGAL1-top1 vector (for galactose induced expression of yeast or human Top1), were grown at 26˚C in SC-uracil, tryptophan media supplemented with 2% dextrose, diluted 100-fold into media supplemented with 2% raffinose and incubated at 26˚C to an OD595=1. The cultures were then induced with a final 2% galactose for 5 hours. Half of the each culture was then shifted to nonpermissive temperature (36˚C) for an additional 3 hours. An equal volume of pre-chilled toluene/ethanol mix in 20 mM TE (pH8.0) was addeded to prevent any alterations in DNA topology during plasmid purification. The purified plasmid DNA topoisomers were then resolved by 2-D agarose gel electrophorosis. In the first dimension, the gels were run in TBE buffer supplemented with 0.5 µg/ml chloroquine. After pre-soaking the gel in TBE buffer supplemented with 2.5 µg/ml chloroquine for 3 hours, the gels were electrophoresed in the same buffer in the second dimension. Plasmid topoisomers were visualized by southern blotting using ^{32}P-labled 2µm plasmid probes followed by autoradiography. The relative position of negatively and positively supercoiled DNAs along the topoisomer arc are indicated by (-) and (+), respectively.

In the absence of a functional DNA topoisomerase I and II (*top1*Δ), the bacterial type I enzyme will selectively catalyze the relaxation of negatively supercoiled DNA generated by divergent transciption units within the 2µm plasmid (2). The net result is the accumulation of positively supercoiled DNA topoisomers [(+) in panel *top1*Δ]. However, if a catalytically active human type I enzyme (Top1) or yeast Top1 (yTop1) is also expressed in these cells, this enzyme also relaxes (+) supercoiled DNA (3), as evidenced by a shift in topoisomer distribution seen as an increase of (-) in panel Top1 and yTop1. Human Top1-clamp^{534} and Top1-clamp^{499}, as well as yTop1-clamp^{460}, are all active in vivo, albeit at lower levels than that observed for the corresponding wild-type enzymes.

1. Hann, C.L., Carlberg, A.L., and Bjornsti, M-A. (1998) *J Biol Chem* 273, 31519-31527.
2. Giaever, G.N. and Wang, J.C. (1988) *Cell* 55, 849-856.
3. Knab, A.M., Fertala, J., and Bjornsti, M-A. (1993) *J Biol Chem* 268, 22322-22330.