Kawamura et al., http://www.jcb.org/cgi/content/full/jcb.200910085/DC1

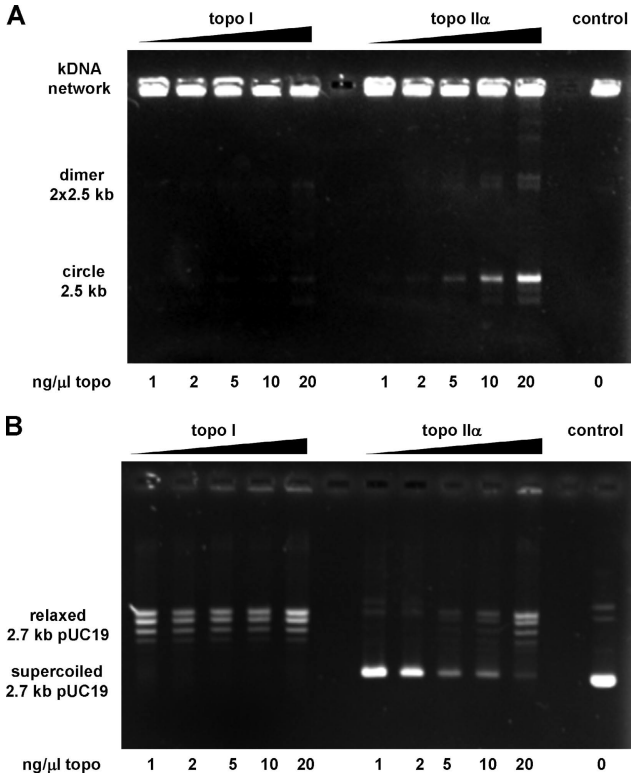

Figure S1. **Relative decatenation and supercoil relaxation activities of topo I and topo IIα.** (A) Results of agarose gel analysis of decatenation activities of topo I (left) and topo IIα (right) as a function of enzyme concentration. Catenated networks of kinetoplast DNA (network of linked 2.5-kb circles; 10 ng/μl) cannot run out of the well (top) of a 1% agarose gel in the absence of enzyme (right, no-enzyme control). 30-min reactions in topo I AB with up to 20 ng/μl topo I release essentially no isolated circles into the gel. By comparison, 30-min reactions with the same concentration range of topo IIα + 1 mM ATP in topo II AB release individual 2.5-kb circles for topo IIα concentrations >10 ng/μl. Given that the topo IIα dimer has a molar weight exceeding that of topo I, we conclude that either per molecule or per mass of molecule, topo IIα is able to decatenate DNA, whereas topo I is unable to decatenate DNA in accord with previous studies. (B) Results of agarose gel analysis of supercoil relaxation activities of topo I (left) and topo IIα (right) as a function of enzyme concentration. Supercoiled 2.7 kb pUC19 (10 ng/μl) runs quickly (right, no-enzyme control), but after 30-min reaction with topo I in topo I AB (left; 1–20 ng/μl), the supercoiling is relaxed. Relaxation is essentially complete with even 1 ng/μl enzyme (left). Similar 30-min experiments with topo IIα (right) in topo II AB with 1 mM ATP do not relax supercoiled pUC19 until concentrations of ~20 ng/μl are used. We conclude that topo I has a much higher supercoil-relaxing activity than topo II and that sufficient quantities of topo IIα are able to relax supercoiled DNA in accord with previous data (Habermeyer et al., 2005).

References

Habermeyer, M., J. Fritz, H.U. Barthelmes, M.O. Christensen, M.K. Larsen, F. Boege, and D. Marko. 2005. Anthocyanidins modulate the activity of human DNA topoisomerases I and II and affect cellular DNA integrity. *Chem. Res. Toxicol.* 18:1395–1404. doi:10.1021/tx050039n

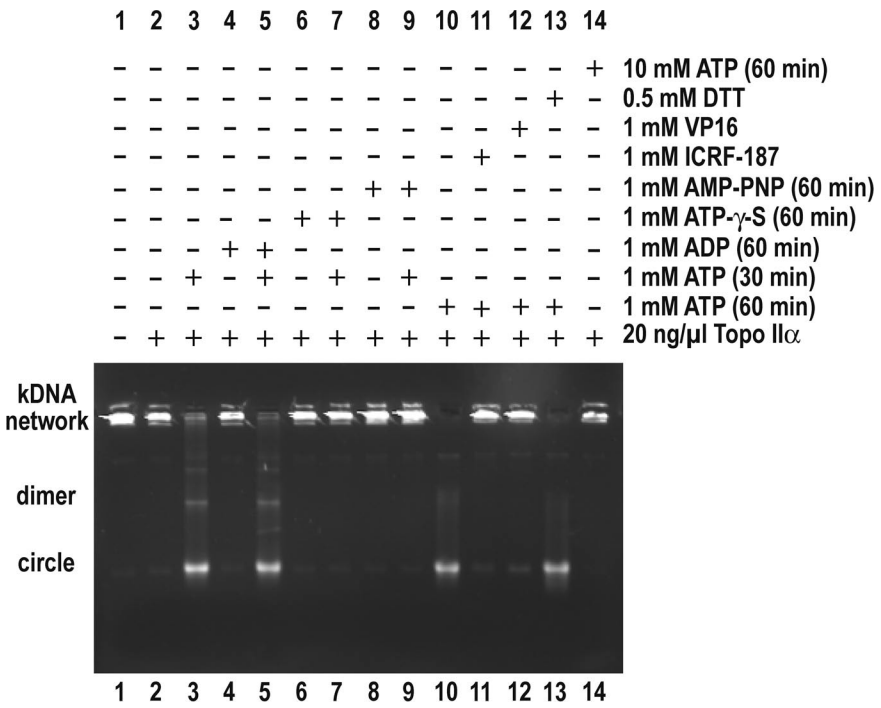

Figure S2. **Effects of nucleotide analogues and ICRF-187 on decatenation of kinetoplast DNA by topo IIα.** Each lane contains 100 ng kinetoplast DNA incubated with reagents in a 10 µl vol. Lane 1 shows kinetoplast DNA incubated in AB but without topo II or ATP added; the kinetoplast DNA network is not decatenated and stays in the well (top). Lane 2 shows the result of incubation with topo IIα without ATP (30-min reaction); again, no decatenation is observed. Lane 3 shows the result of incubation with topo IIα + 1 mM ATP (30-min reaction); decatenation occurs (lowest bands are individual, circular 2.5-kb DNAs released from the kinetoplast DNA network). Lane 10 shows the same experiment but with 60-min incubation; decatenation is observed. Lane 4 shows the result of incubation with topo IIα + 1 mM ADP (60-min reaction); no decatenation occurs, indicating that topo IIα decatenation activity is not stimulated by ADP. Lane 5 shows the result of incubation first with topo IIα + ADP (60 min) followed by addition of 1 mM ATP (30 min); decatenation is observed, indicating that the initial ADP exposure does not eliminate subsequent ATP-stimulated decatenation activity. Lane 6 shows the result of incubation with topo IIα + 1 mM ATPγS (60 min; Sigma-Aldrich); no decatenation is observed. Lane 7 shows the result of incubation first with topo IIα + 1 mM ATPγS (60 min) followed by addition of 1 mM ATP; no decatenation occurs, indicating that binding of the nonhydrolyzable ATPγS poisons topo IIα's decatenation activity, presumably by simply staying bound to the enzyme. Lanes 8 and 9 show the same behavior to occur for AMP-PNP; it acts as an effective topo II poison. Lanes 11 and 12 show the result of topo IIα + ATP reactions as in lane 10 (60-min incubation) but with topo II poisons ICRF-187 and VP16 added, respectively; in both cases, no decatenation is observed. Lane 13 also shows a decatenation reaction as in lane 10 but with DTT added; decatenation is observed. Finally, lane 14 shows a decatenation reaction (60 min) run with topo IIα + 10 mM ATP; no decatenation is observed, indicating that high ATP concentrations act to suppress DNA decatenation by topo IIα.

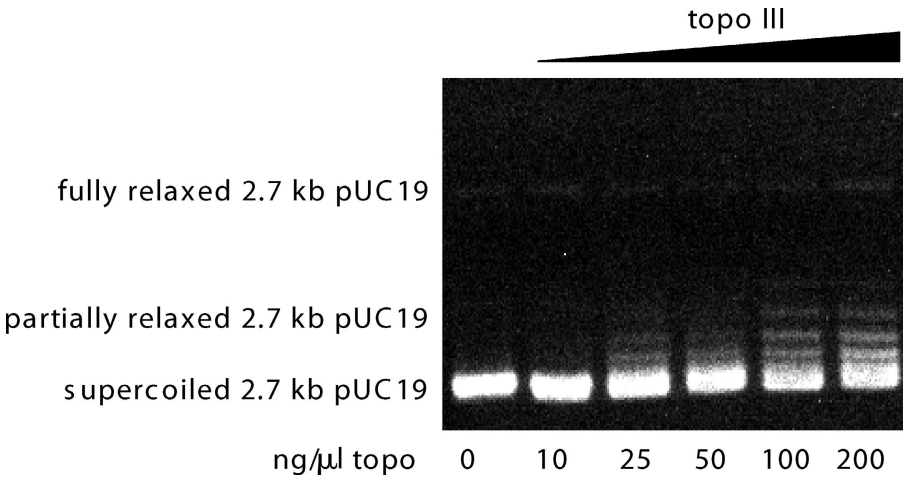

Figure S3. **Agarose gel analysis of supercoil relaxation by topo III.** Supercoiled 2.7 kb pUC19 (10 ng/µl) runs quickly in absence of enzyme treatment (left); treatment by successively larger concentrations of topo III in T3AB (no ATP) for 30 min leads to partial relaxation (right), which saturates for enzyme concentrations ~100 ng/µl. Note that topo I more completely relaxes supercoiled DNA (Fig. S1 B), as topo III requires single-stranded regions to relax supercoiled DNA.