Supplementary Material to

Immunodetection of Human Topoisomerase I-DNA Covalent Complexes

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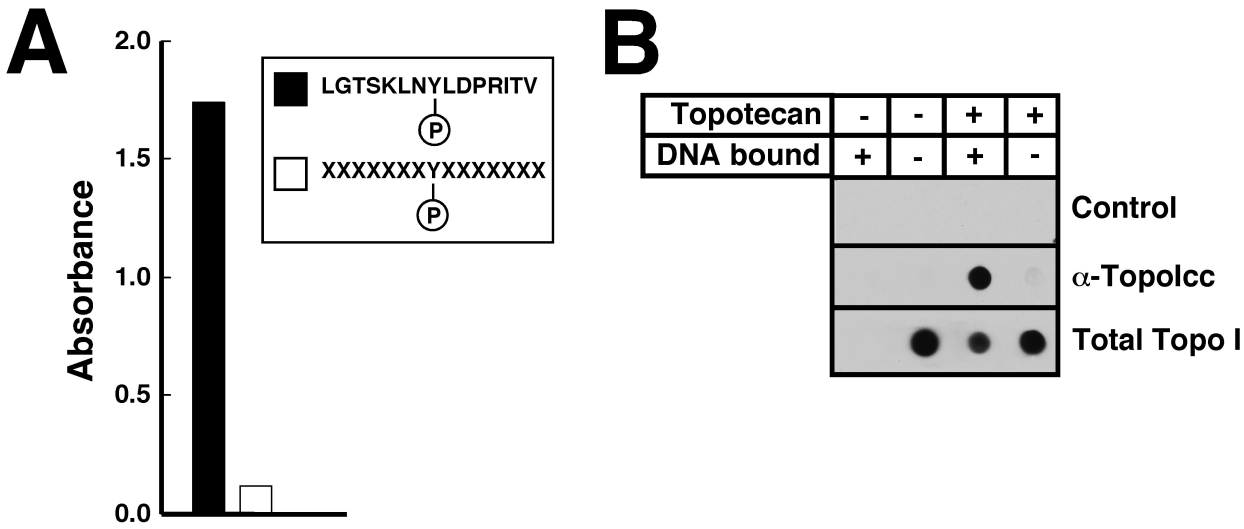

Figure S1. Results of screening that yielded α-TopoIcc. **A,** results of primary screening ELISA against the indicated peptides. **B,** for secondary screening, pooled fractions containing free topo I and DNA-bound topo I from cesium chloride gradients (see Fig. 2A, TPT treatment) or the corresponding fractions from diluent treated cells were subjected to blotting with hybridoma supernatant or C-21 anti-topo I. α-TopoIcc was unique among positive clones in its ability to react with topo I-DNA covalent complexes but not free topo I.

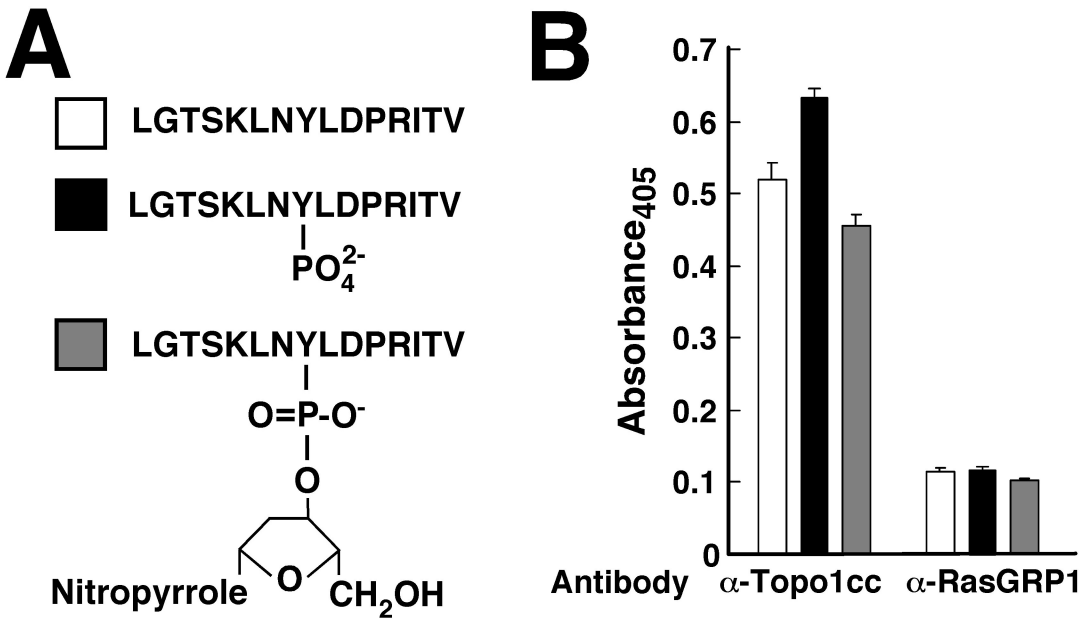

Figure S2. Extended testing of α-TopoIcc in ELISA assays. **A**, Peptides used in ELISA assays. **B**, results obtained with α-TopoIcc or previously described anti-RasGRP1 antibody (43) at 0.5 μg/ml. Error bars in B, ± SD of 3 replicate wells in one of two independent ELISA assays. Note that addition of the universal nucleoside analogue 1-(2'-deoxy-β-D-ribofuranosyl)-3-nitropyrrole to the phosphorylated topo I peptide failed to increase the reactivity with α-TopoIcc, suggesting that the epitope recognized by the antibody is more complicated than just the topo I-phosphate-deoxyribose moiety.

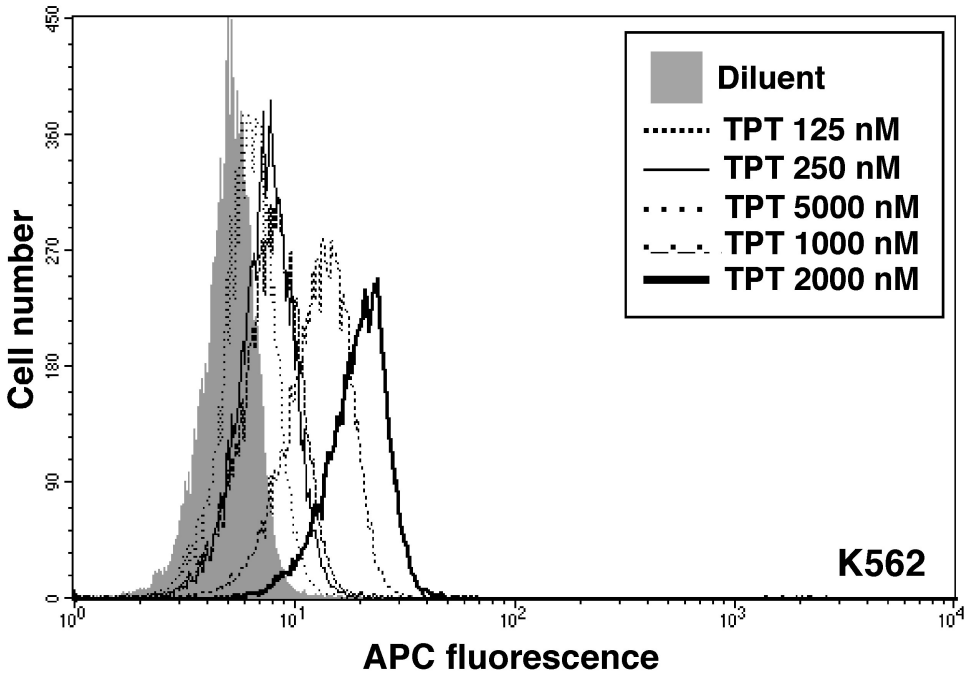

Figure S3. Detection of topo I-DNA covalent complexes in topotecan-treated K562 cells. Cells were treated for 1 h with the indicated concentration of topotecan, fixed, permeabilized with Triton X-100, incubated with SDS, and stained as described in the Methods.

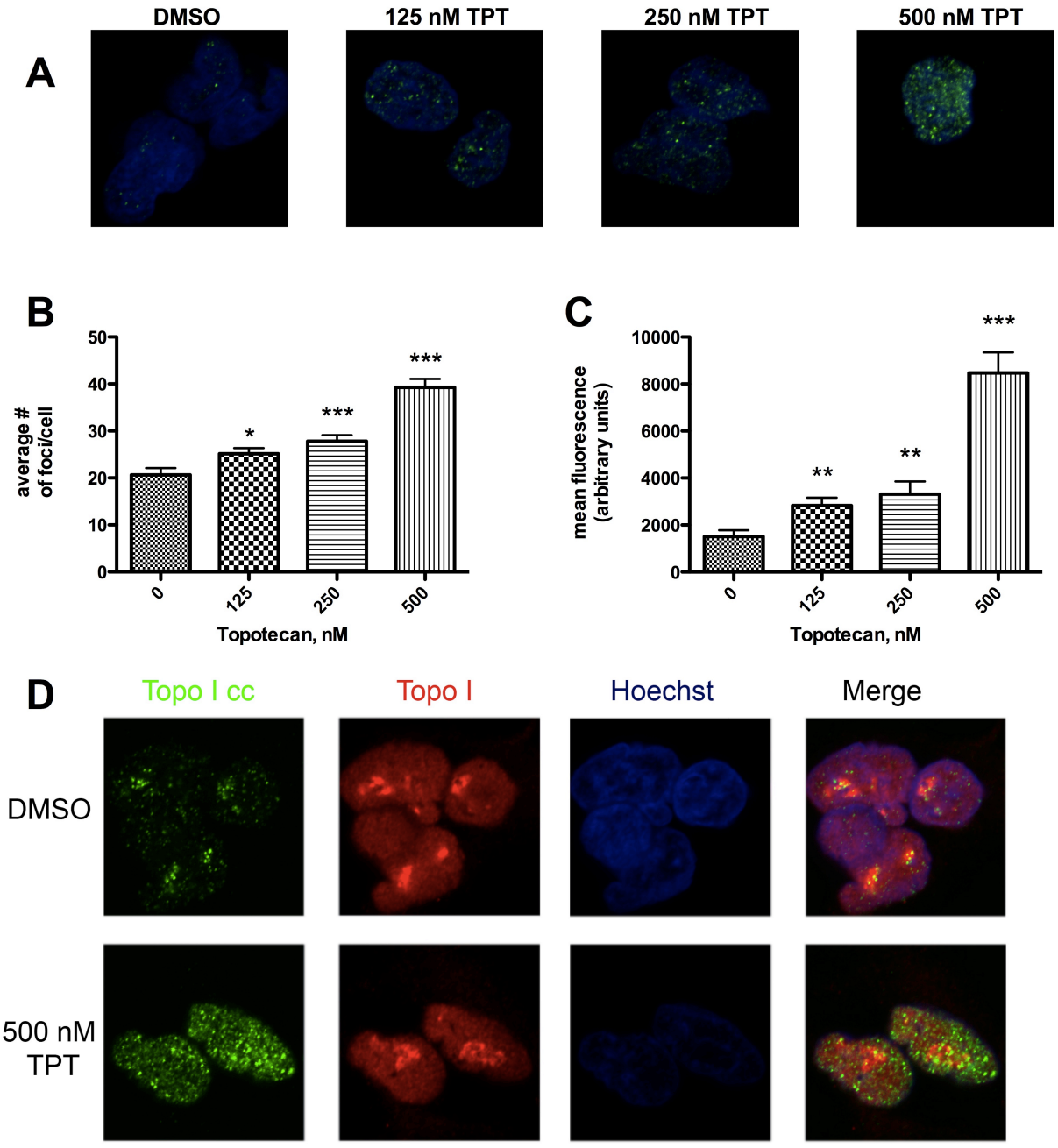

Figure S4. Quantitation of topo I-DNA covalent complexes in intact cells after fluorescence microscopy. **A**, A549 cells were stained with α-TopoIcc antibody (green) and Hoechst 33258 (blue). **B**, Quantitation of foci numbers for at least 30 cells. **C**, Quantitation of maximal mean fluorescence intensity for 25 cells. Error bars in B and C, ± SEM of 3 independent experiments. **D**, dual staining of topo I-DNA covalent complexes (green) and topo I (red) in A549 cells.

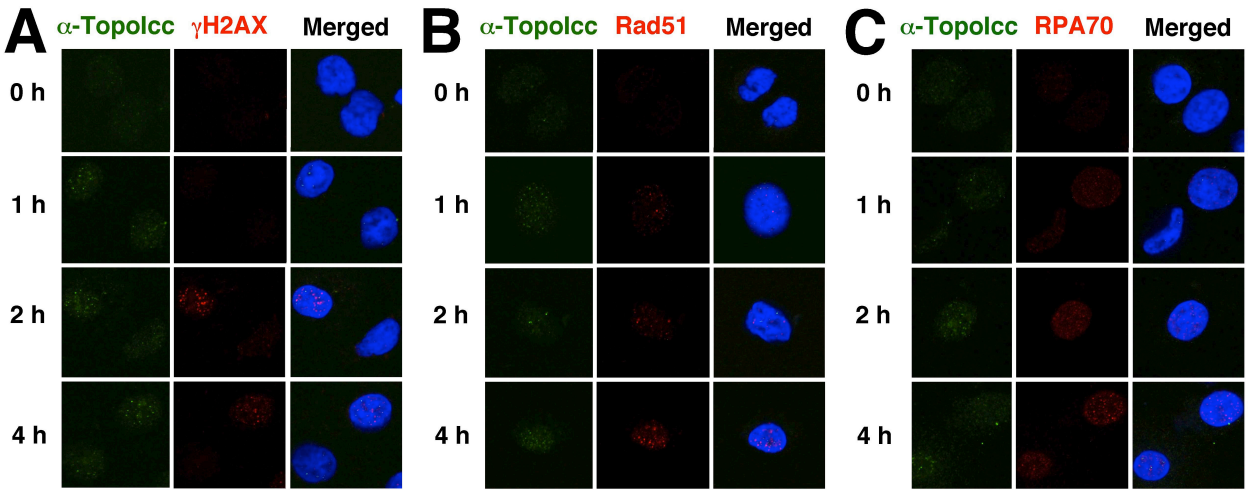

Figure S5. Examination of topo I-DNA covalent complexes after treatment with 25 nM TPT. A549 cells treated for up to 4 h with 25 nM TPT were fixed and stained with α-TopoIcc along with anti-phospho-Ser^{139}-H2AX (**A**), anti-Rad51 (**B**) or anti-RPA70 (**C**).