Figure S1: **Topo III binding assays**

Topo III protein was purified according to a previously published procedure (39). oligonucleotides were labeled at the 5' end with [g-32P]-ATP using T4 Polynucleotide Kinase (New England Biolabs^{R}). Electrophoretic mobility shift assays using Topo III were performed in 10 ml of the following solution: 50 mM HEPES (pH 7.9), 100 mM NaCl, 0.1mM EDTA, 4% w/v sucrose, 2% v/v glycerol, 0.1 mg/ml BSA, 0.02% w/v bromophenol blue, 20 nM labeled oligonucleotide and Topo III (50 nM). For binding inhibition the indicated concentrations of 360A were added to the reaction mixture in a volume of 1 ml. The reaction mixture was incubated at room temperature for 30 min. Each individual mixture was separated immediately by electrophoresis on 1% agarose gels in 0.5X Tris-Borate-EDTA buffer. The gels were run at 80 V for 45 min, dried on Whatman DE81 paper, and visualized by a phosphorimager (Typhoon 9210, Amersham). Data was analyzed using ImageQuant software (Amersham).

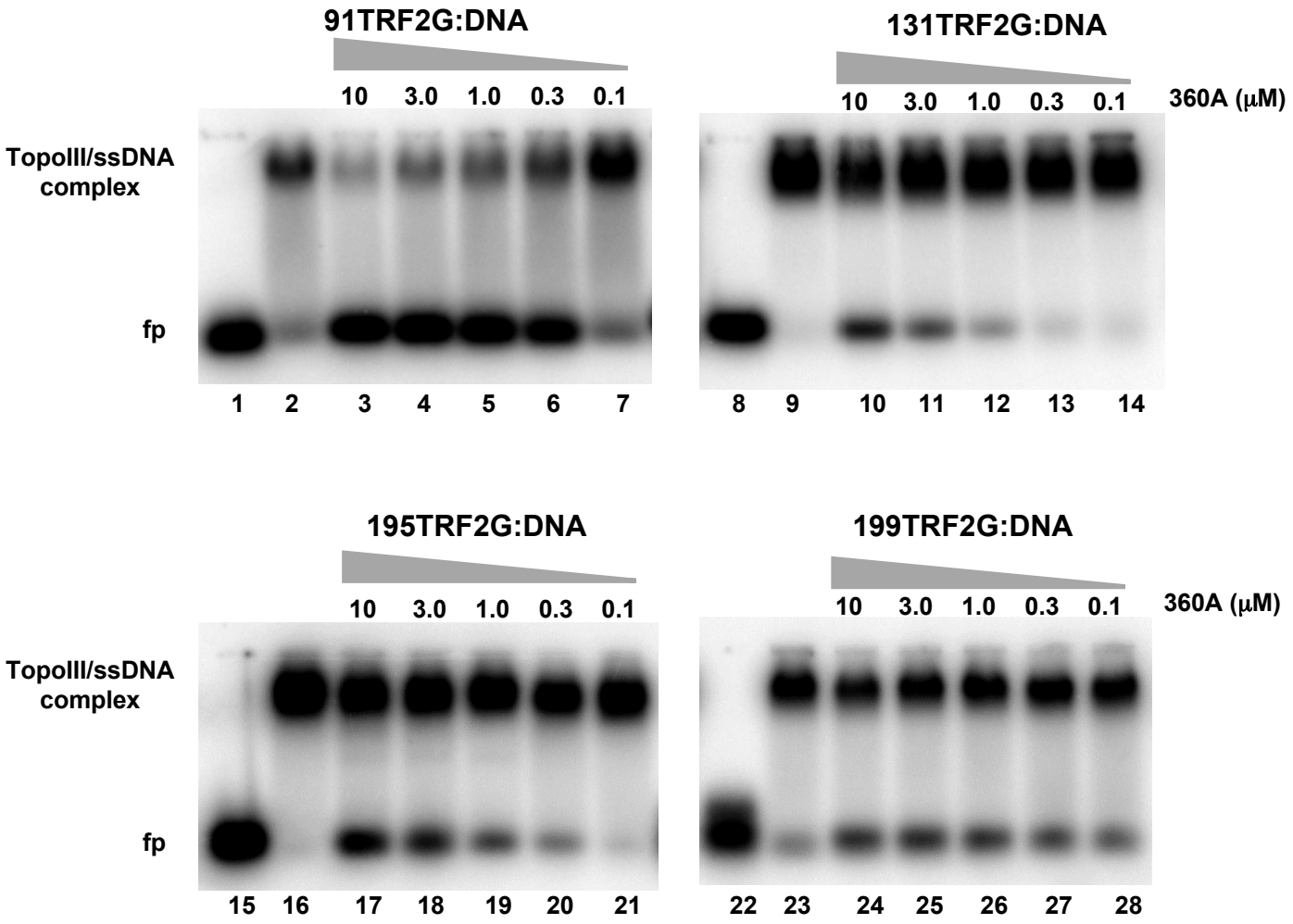

91TRF2G:DNA
10 3.0 1.0 0.3 0.1
131TRF2G:DNA
10 3.0 1.0 0.3 0.1
360A (μM)
TopoIII/ssDNA complex
fp
1 2 3 4 5 6 7
8 9 10 11 12 13 14
195TRF2G:DNA
10 3.0 1.0 0.3 0.1
199TRF2G:DNA
10 3.0 1.0 0.3 0.1
360A (μM)
TopoIII/ssDNA complex
fp
15 16 17 18 19 20 21
22 23 24 25 26 27 28

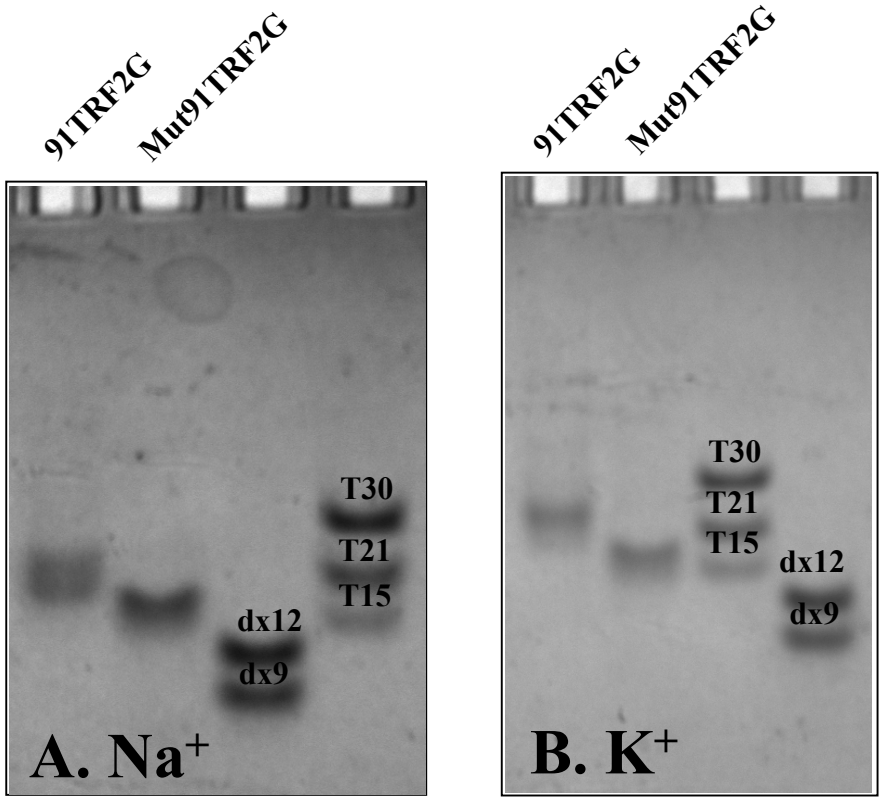
Figure S1

Support Information Figure S1

In a previous work we have shown that purified recombinant Topo III preferentially interacts with single-stranded DNA (39). In the presence of G-rich sequences having the potential to adopt G4 structures, Topo III binding is strongly inhibited by G4 stabilization using specific G4 ligands such as telomestatin or 360A.
As shown in figure S1, and as expected, the binding of Topo III to the different TRF2G oligonucleotides was not affected by theoretical ability of these sequences to adopt G4 structures *in vitro* (figure S1, compare lines 2, 9, 16 and 23). The addition of increasing concentration of 360A (0.1 to 10 µM) strongly impairs the binding of Topo III to 91TRF2G:DNA with an IC_{50} < to 0.3 µM (Figure S1, lanes 3-7). In contrast, 360A treatment partially inhibits the binding of Topo III to 131TRF2G:DNA, 195TRF2G:DNA and 199TRF2G:DNA oligonucleotides, even when we used a 1000-fold excess of G4 ligand relative to ssDNA, IC_{50}>10 mM (Figure S1 lanes 10, 17 and 24).

Figure S2 : **: Non denaturing gel electrophoresis**.

Oligonucleotides at 30µM. Samples were incubated in 10mM TRIS/HCl pH 7.5 buffer with 100mM NaCl (**A**) or KCl (**B**). The gel was prepared at 12% acrylamide supplemented with 20mM of the corresponding salt and run at 20°C. The gel was revealed by UV-Shadow



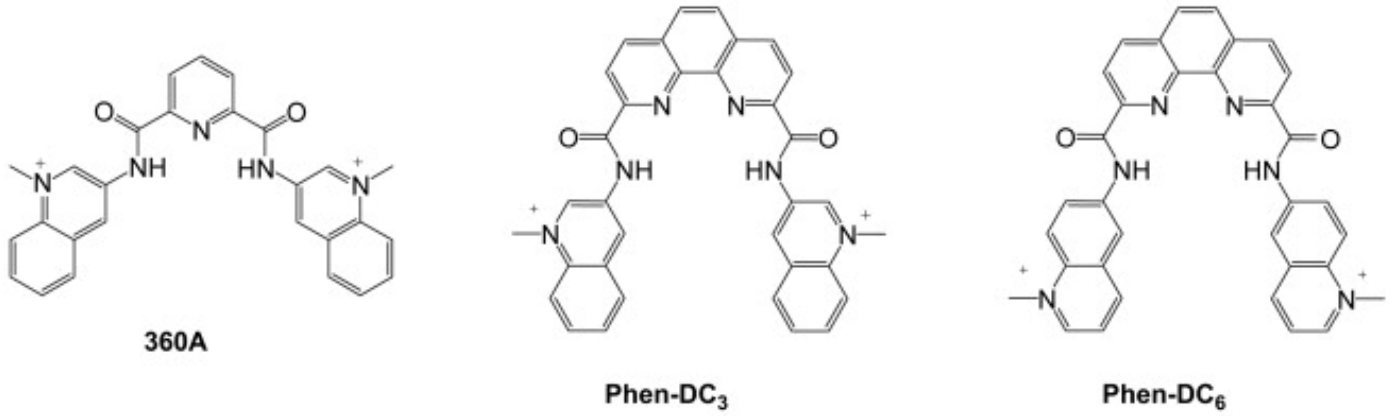


Figure S3: Molecular structure of G4 ligands used in this work