

Electronic Supplementary Information

Recognition of forcible curvature in circular DNA by human topoisomerase I

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Table S1. The sequences of DNA circles. All of the circular DNAs are only show in one strand from 5 end to 3 end. (84 bp cDNA and 84 bp linear DNA sequences consist of same composition)

Circle size (bp)	Sequence
84	ATCTTATCGAACAAGACCCGTGCAATGCTATCGACATCAAGGCCTATCGCTGGGA GTCAATGGGTTTCAGGATGCAGGTGAGGAT
300	CCATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATA TTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTT TTGCGGCATTTTGCCTTCCCTGTTTTTGGCTCACCCAGAAACGCTGGTGAAAGTAAA AGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAAC AGCGGTAAGTTAAGCTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATC GAAGGAGAGAAGAGCTGGAGCT
535	CCCAGTCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTACCAACCTAGGT ATCTAGAACC GGCTCGAGCCATAACTTCGTATAGCATAATTATAACGAAGTTATAT AAGCTGTCAAACATGAGAATTCTTGTATAGGTTAATGTCATGATAATAATGGTTTC TTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTA TTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATG CTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCT TATCCCTTTTTTGCGGCATTTCCTTCCCTGTTTTTGGCTCACCCAGAAACGCTGG TGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAAC TGGATCTCAACAGCGGTAAGTTAAGCTTTTTGCACAACATGGGGGATCATGTAAC TCGCCTTGATCGAAGGAGAGAAGAGCTGGAGCT
936	CATCCGCTCCCGGCGGATTTGTCTACTCAGGAGAGCGTTCACCGACAAACAAC AGATAAACGAAAGGCCAGTCTACCGACTGAGCCTTTCGTTTTATTTGATGCCT GGCAGTTCCTACTCTCGCGTTAACGCTAGCATGGATGTTTTCCAGTCACGACG TTGTAAAACGACGGCCAGTCTTAAGCTCGGGCCCAATAATGATTTTATTTTGAC TGATAGTGACCTGTTTCGTTGCAACAATTGATGAGCAATGCTTTTTTATAATGCCA ACTTTGTACAAAAAAGCAGGCTTGAAGGAATTCGGCAAGTCTTCCCACTTAGTG GATCCTCGTCGCAAAACCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAAGTCAG ACGTTGTAAAACGACGGCCAGTCCGTAATACGACTCACTTAAGGCCTTGACTAGA GGGTACCAACCTAGGTATCTAGAACC GGTCGAGCCATAACTTCGTATAGCATA ATTATACGAAGTTATATAAGCTGTCAAACATGAGAATTCTTGTATAGGTTAATGTC ATGATAATAATGGTTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCG GAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGAC AATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAA CATTTCCGTGTCGCCCTTATCCCTTTTTTGCGGCATTTCCTTCCCTGTTTTTGGCT CACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGA GTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGTTAAGCTTTTTGCACAACA TGGGGGATCATGTAACCTCGCCTTGATCGAAGGAGAGAAGAGCTGGAGCT

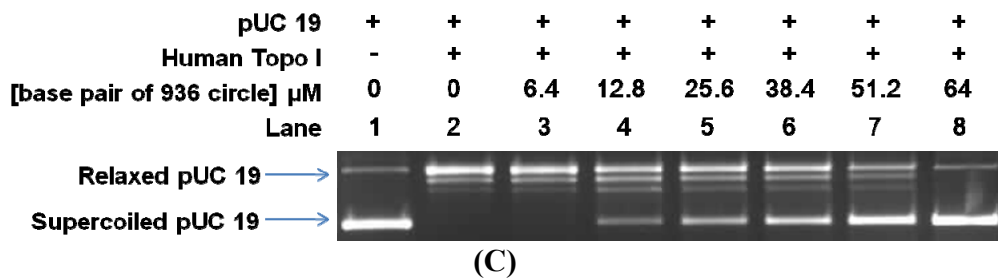
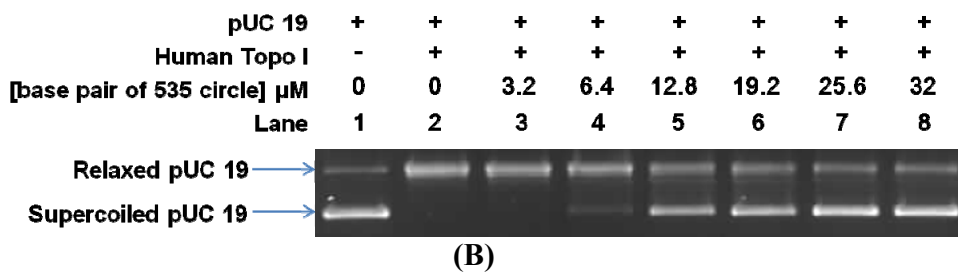
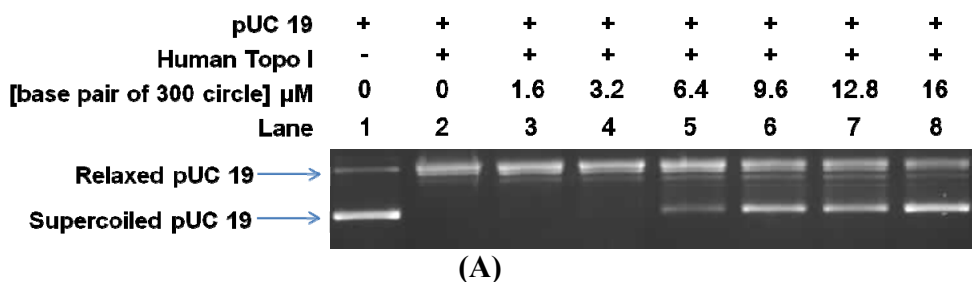


Figure S1. pUC 19 relaxation assay for the inhibitory effect of 300 bp cDNA (A), 535 bp cDNA (B), 936 bp cDNA (C) on hTopo I. Assay mixture containing 10 mM Tris-HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin (BSA), 0.1 mM spermidine, 5% glycerol, 250 ng pUC 19, 0.5 U of hTopo I, and each cDNA was prepared and further incubated at 37°C for 15 min before loading on agarose gel.

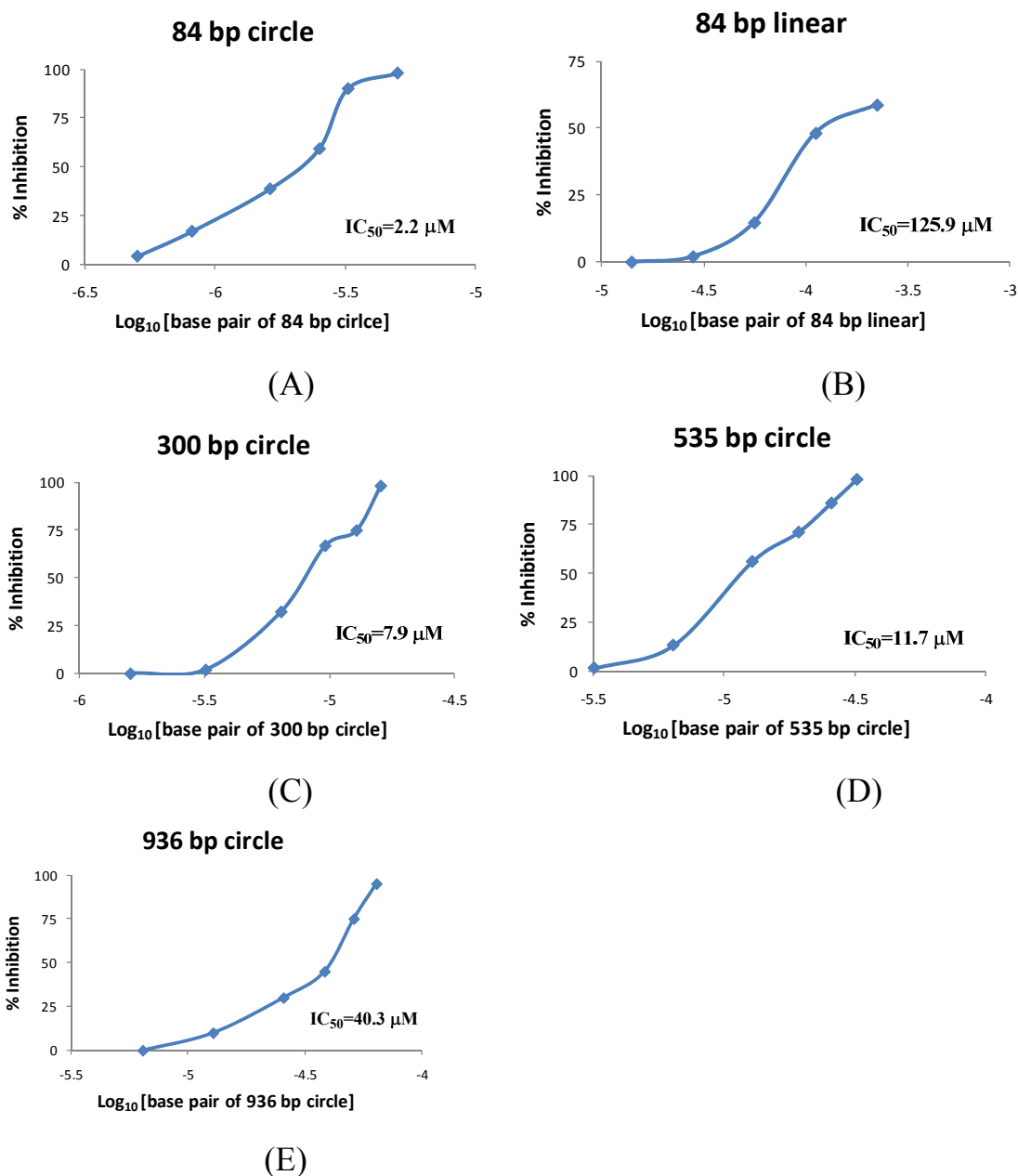


Figure S2. Correlations between base pairs concentration of 84 bp cDNA (A), 84 bp linear DNA (B), 300 bp cDNA (C), 535 bp cDNA (D), 936 bp cDNA (E) and the corresponding inhibitory effects on hTopo I. Percentages of pUC 19 relaxation were defined as the ratio of band density of relaxed DNA over the some of relaxed DNA plus supercoiled DNA [relaxed DNA/ (relaxed DNA + supercoil DNA)].¹ The DNA bands were quantified using Gel Documentation System (G:Box HR, Syngnene, Cambridge, UK) equipped with Gene Tools Software. The quantity of DNA sample was measured by NanoDrop 2000C (Thermo Fisher Scientific Inc, Wilmington, USA).

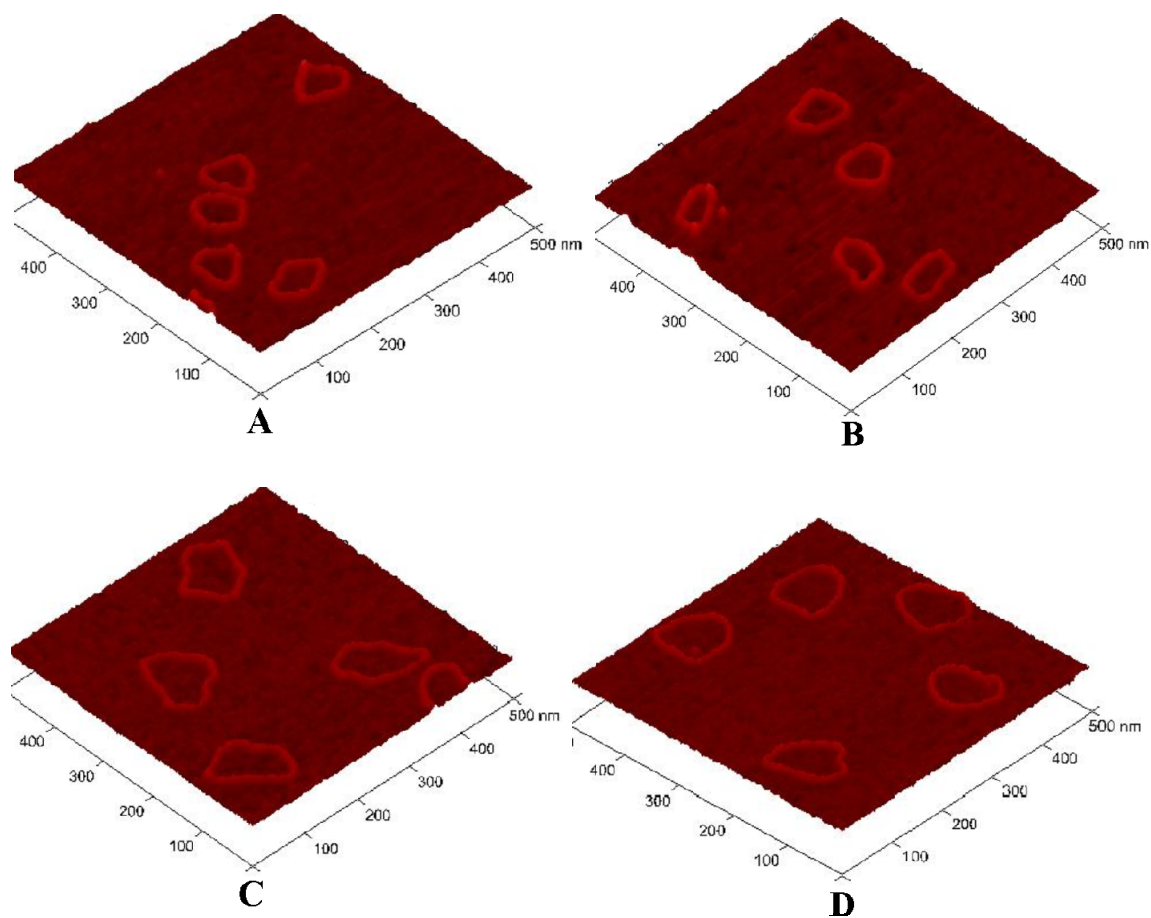


Figure S3. Three dimensions AFM plots of 535 bp circle and 936 bp circle in presence (A, C) and absence (B, D) hTopo I respectively. 400 nm x 400 nm scans.



Figure S4. EMSA analysis of hTopo I and 84 bp linear DNA interaction. Lane 1, 84 bp linear DNA alone; Lane 2, 84 bp linear DNA and 10 U hTopo I are incubate at 30 °C for 15 min; The gel electrophoresis was run at 4 °C, 100 V.

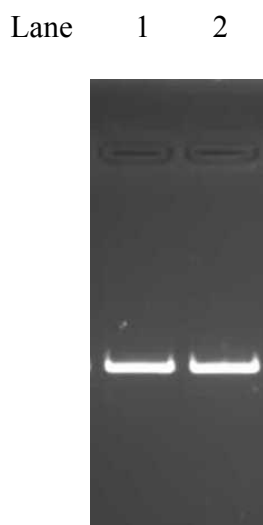


Figure S5. EMSA analysis of hTopo I and pUC 19 interaction. Lane 1, pUC 19 alone; Lane 2, pUC 19 and 10 U hTopo I are incubate at 30 °C for 15 min; The 0.8% agarose gel was run at 4 °C, 50 V.

Materials and Methods:

Reagents: Plasmid DNA pUC19, T4 DNA ligase, Nuclease BAL-31 and SacI were purchased from New England Biolabs (Ipswich, MA). Proteinase K was purchased from Fermentas (Singapore). Human Topo I was obtained from TopoGEN (Columbus, OH). Duplex linear DNA precursors were provided by Generay Biotech (Shanghai, China), single strand oligonucleotide were provided by Sigma-Aldrich (Singapore).

Preparations of 84 bp circular DNA: 84 bp circle were prepared according to the process describe in previous literature.² The substrates were mixed in the reaction buffer (60 mM Tris-HCl, pH 7.6, 25 mM NaCl, 13 mM MgCl₂, 10 mM DTT, 1mM ATP, 25 mg/ml BSA), heated to 95°C for 2min and then chilled immediately on ice for 5min. The mixture was brought to 16°C and incubated with 10 U of T4 DNA ligase overnight. After the ligation steps, the DNA products were treated by BAL-31 at 30°C to digest the remaining single and double-stranded linear DNA. The circles were purified subsequently by the PCR Purification Kit (Qiagen). The circles did not contain chemically synthesized oligonucleotides to assure the consistency of DNA quality in the human Topo I relaxation reactions.

Preparations of 300 bp, 535 bp and 930 bp circular DNA: these circles were prepared according to the process describe in previous literature.² The substrates were mixed in the reaction buffer (60 mM Tris-HCl, pH 7.6, 25 mM NaCl, 13 mM MgCl₂, 10 mM DTT, 1mM ATP, 25 mg/ml BSA) and incubated with 10 U of T4 DNA ligase overnight at 16°C. After

the ligation steps, the DNA products were treated by BAL-31 at 30°C to digest the remaining double-stranded linear DNA. The obtained circular DNA products were analyzed by agarose gel electrophoresis (1% or 2%), then cut gel and further purified by Gel Extraction Kit (Qiagen). The circles did not contain chemically synthesized oligonucleotides to assure the consistency of DNA quality in the human Topo I relaxation reactions.

Reactions of human Topo I with pUC 19 and Circular DNA: A mixture containing 10 mM Tris-HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin (BSA), 0.1 mM spermidine, 5% glycerol, 250 ng pUC 19, 0.5 U of human Topo I, and each DNA circle was prepared respectively and further incubated at 37 °C for 15 min. After incubation, the product was analysis by 1% agarose gel electrophoresis.

AFM sample preparation: Immobilization of DNA samples on micas were carried out following the previously reported procedures³⁻⁶. AFM images were obtained in Tapping ModeTM on a MultimodeTM AFM (Veeco, Santa Barbara, CA) in connection with a Nanoscope VTM controller. Antimony (n) doped Si cantilevers with nominal spring constants between 20 and 80 N/m were selected. Scan frequency was 1.9 Hz per line and the modulation amplitude was in a nanometer range⁷⁻⁸. All DNA sample determinations were carried out in air at room temperature. A buffer contains 40 mM HEPES, 10 mM MgCl₂ and pH 7 was deposited on freshly cleaved mica for 5 min. DNA circle was incubated with 10 U human Topo I at 30°C for 15 min in the binding buffer [10 mM

Tris/HCl (pH 7.5), 3 mM CaCl₂, 50 mM NaCl, 0.1 M sucrose and 5% glycerol]. After the pre-prepared mica dried with a gentle N₂ flow, a 20 µl solution contain DNA sample, 10 U human Topo I and binding buffer was deposited on it incubated for 5min, washed with dd H₂O, dried with a gentle N₂ flow and then dried under vacuum.

Electrophoretic mobility shift assay (EMSA): EMSA was prepared according to the process describe in previous literature.⁹⁻¹⁰ A labeling 84 bp circle was incubated with 10 U human Topo I at 30°C for 15 min in the binding buffer [10 mM Tris/HCl (pH 7.5), 3 mM CaCl₂, 50 mM NaCl, 0.1 M sucrose and 5% glycerol]. After incubation, mixtures followed by treatment with 1µl proteinase K for 1 hour at 37°C. The reaction mixtures were electrophoresed in 8% non-denaturing polyacrylamide gel at 4 °C in 0.25×TBE buffer and DNA bands were detected by phosphorImage (GE Healthcare).

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