Supporting Information

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SI Text

SI Materials and Methods. ***Synthesis of DNA Containing 5R,6S and 5S,6R Thymine Glycol.*** Thymidine glycol CE phosphoramidite was purchased from Glen Research and used per the manufacturers instructions with minor changes noted below. Oligonucleotide syntheses were performed on a Perseptive BioSystems Expedite 8909 using MILD amidites (Glen Research) and mild deprotection conditions. HPLC analyses were performed using a Beckman Coulter System Gold 125 HPLC with 168 PDA detector. HPLC purification was accomplished using a Beckman Coulter System Gold 126P HPLC with a 166 variable wavelength detector. MALDI-TOF mass spectra data were obtained on an ABI Voyager 4211 system using α-cyano-4-hydroxycinnamic acid as matrix and the system was calibrated against 10 and 4 kDa oligonucleotide standards from Genosys.

Thymine glycol oligonucleotide was synthesized using 10 mg of thymidine glycol CE phosphoramidite per coupling during a 1 μmole scale synthesis using ultramild CE phosphoramidites and ultramild deprotection reagents and conditions [Glen Research Catalog # GR10-1096-95 reagent instructions www.glenresearch.com/GlenReports/GR16-13.html (1)]. The coupling step for the thymine glycol monomer was performed using an offline coupling method extended to 45 min. The oligonucleotide was cleaved from the solid support under mild conditions in 30% ammonium hydroxide (1 mL) for 1 h at room temperature. The support column was washed with an additional 1 mL of 30% ammonium hydroxide and the sealed oligonucleotide solutions were allowed to sit at room temperature for 2 h to allow for complete deprotection of bases. The ammonium hydroxide solution was evaporated after addition of 20 uL triethylamine. DMT deprotection was accomplished on a Waters Sep-Pak column using standard conditions with aqueous 2% trifluoroacetic acid to remove the DMT group and 20%, followed by 50%, acetonitrile/water to remove the full length base deprotected thymine glycol modified oligonucleotide. The solvent was evaporated and the oligonucleotide further treated with a 200 uL mixture of TBAF/TEA- HF (5/1) solution overnight at 0–4 °C to remove the glycol TBDMS protecting groups. Water (2 mL) was added and the oligonucleotide solution was purified on a Pharmacia Nap 10 column using 0.1 M Sodium phosphate pH 7.4 as eluant to desalt the oligonucleotide and remove residual deprotection reagents. HPLC purification conditions used 0.1 M ammonium formate/1 mM EDTA (Buffer A) and acetonitrile (Buffer B). A gradient method was used as follows: Hold 0%B 5 min., 0–10%B gradient over 5 min., 10–14%B gradient over 15 min., hold 14%B 5 min., gradient 14–100%B over 2 min., hold 100% B 5 min. Two isomers were obtained with retention times of 11.7 min (major isomer 7 OD, cis-5R,6S) and 11.8 min (minor isomer, 5 OD, cis-5S,6R). Expected Mass Spec: 5001.9 m/z; Observed: 5001.2 m/z.

1. Iwai S (2000) Synthesis of thymine glycol containing oligonucleotides from a building block with the oxidized base. *Angew Chem Int Edit* 39:3874–3876.

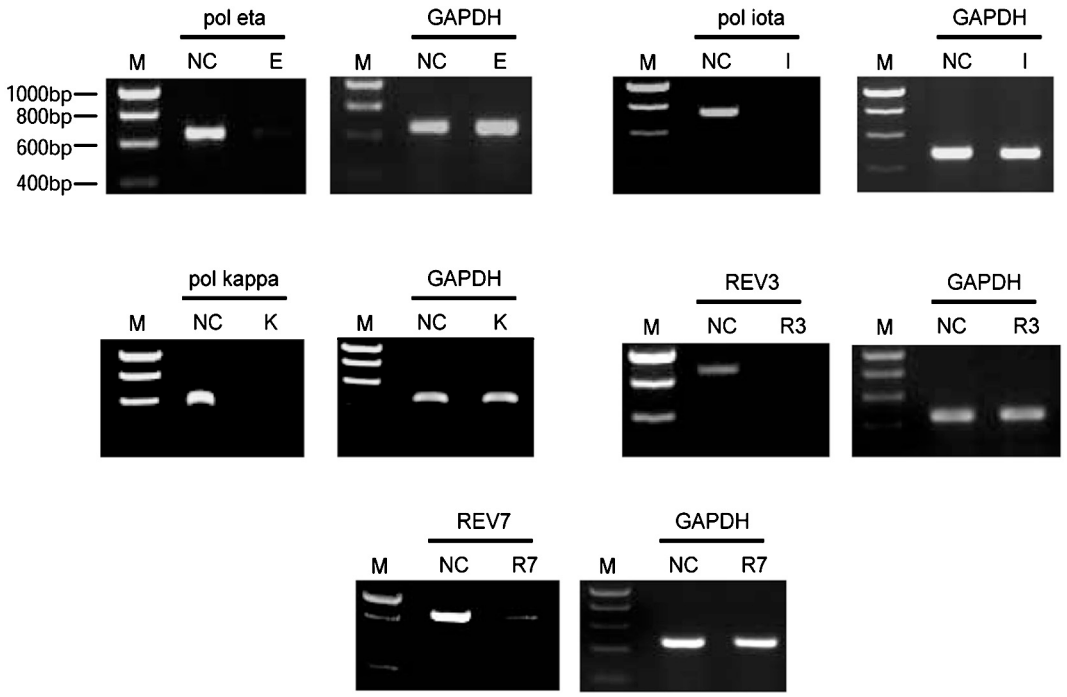

Fig. S1. RT-PCR analysis of the efficiency of siRNA inhibition of TLS Pols in normal human fibroblast cells. Cells were treated with 100 pmole of HPLC purified duplex siRNA. Total RNA was isolated 48 h after transfection with siRNA. RT-PCR was carried out to analyze the efficiency of siRNA inhibition on target gene mRNA expression. NC: negative control siRNA; E: human Polη siRNA; I, human Polι siRNA; K: human Polκ siRNA; R3: human Rev3 siRNA: R7: human Rev7 siRNA. For each analysis, the effects of that siRNA were examined on GAPDH mRNA expression.