Supplemental Figures

Preparation of a site-specific T=^{m}CG cis-syn cyclobutane dimer-containing template and its error-free bypass by yeast and human polymerase eta

[1]Qian Song[1], Shanen M. Sherrer[2], Zucai Suo[2], John-Stephen Taylor[1]

[1]Department of Chemistry, Washington University, St. Louis, MO 63130

[2]Department of Biochemistry, The Ohio State University, Columbus, OH 43210

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9-mer	5’-GCTCGTCAC-3’
9A-mer	5’-GCTCGTCAC**A**-3’
9AA-mer	5’-GCTCGTCAC**AA**-3’
9G-mer	5’-GCTCGTCAC**G**-3’
9GA-mer	5’-GCTCGTCAC**GA**-3’
AA-14-mer	5’-GCTCGTCAC**A**AT AC-3’
GA-14-mer	5’-GCTCGTCAC**G**AT AC-3’

T^{m}C-14-mer	5’ GTA**T^{m}C**GTGACGAGC 3’
T=^{m}C-14-mer	5’ GTA**T=^{m}C**GTGACGAGC 3’
TT-14-mer	5’ GTA**TT**GTGACGAGC 3’
T=T-14-mer	5’ GTA**T=T**GTGACGAGC 3’

FIGURE S1. **Oligodeoxynucleotides used in this study.**

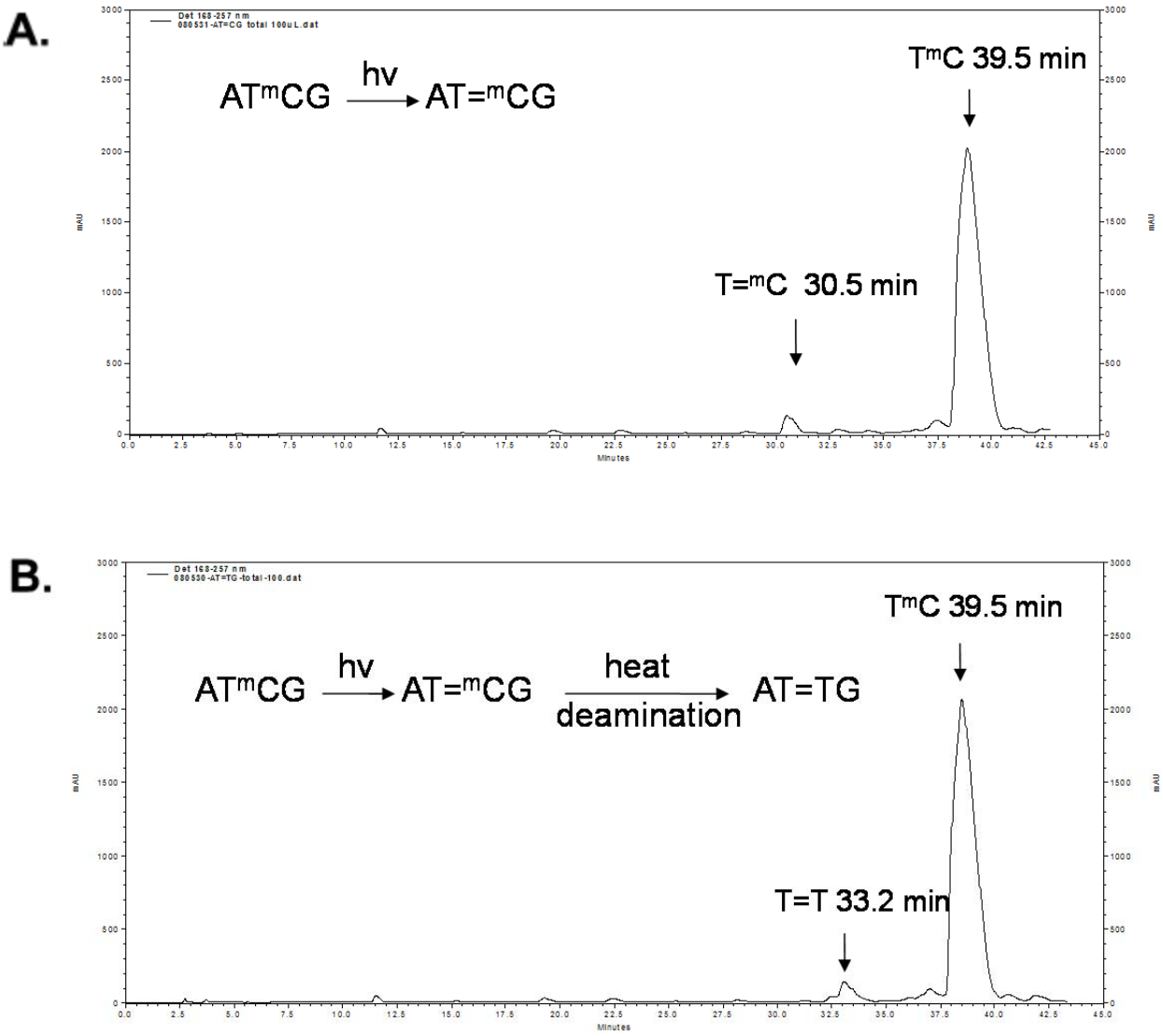

FIGURE S2. **Analysis of the UV irradiation products of T^{m}C-14-mer before and after deamination.** (A) HPLC trace of T^{m}C-14-mer after UV irradiation at 4°C for 1 hour at pH 8.5. (B) HPLC trace of T^{m}C-14-mer after UV irradiation at 4°C for 1 h followed by 3 h at 67°C and pH 6.5

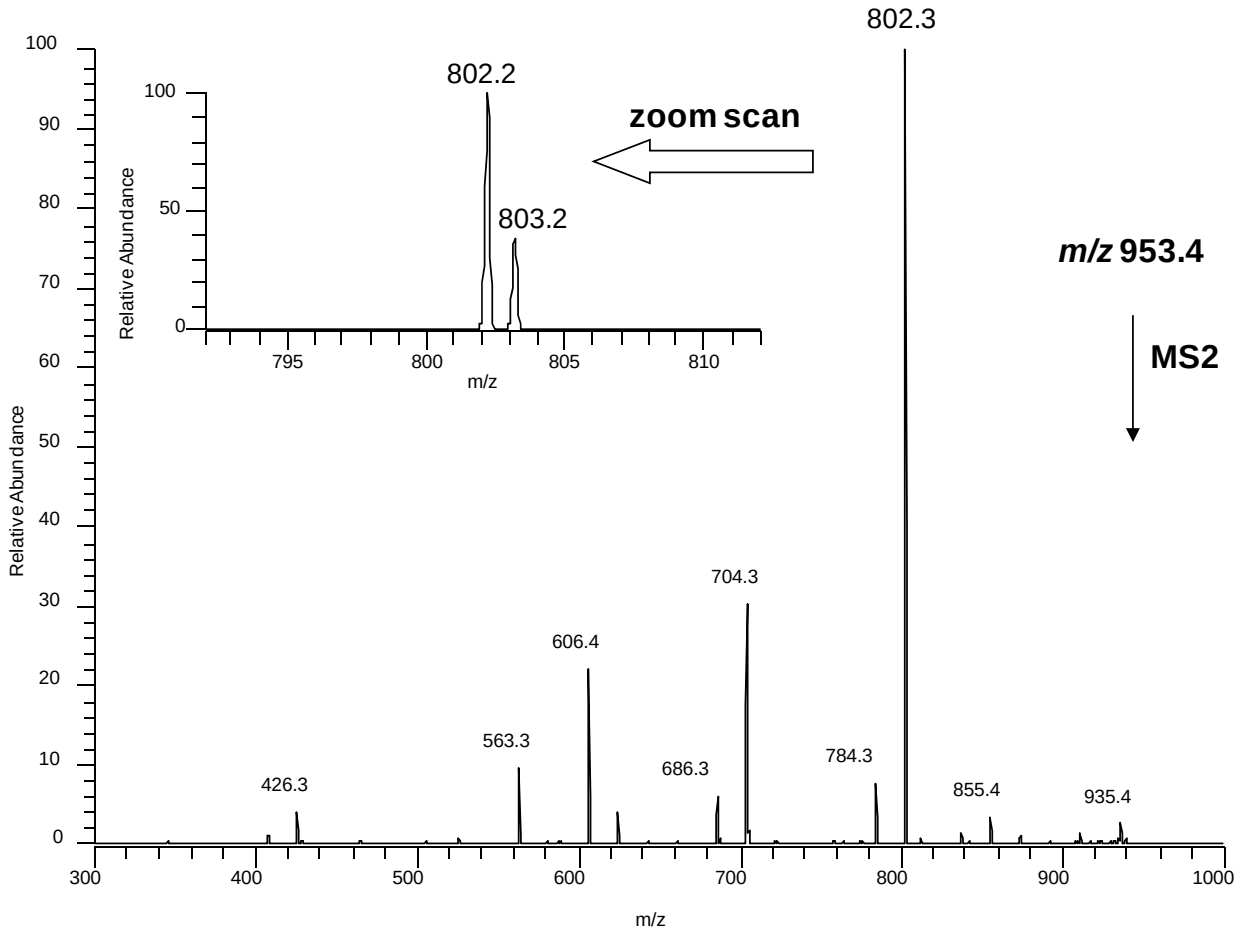

FIGURE S3. **Nuclease P1-coupled ESI-MS/MS analysis of T=^{m}C-14-mer.** (A) MS/MS of nuclease P1 digestion products of the HPLC peak corresponding to the cis-syn CPD of T^{m}C-14-mer (A1: parent ion *m*/*z*: 953, full MS; A2: zoom scan at *m*/*z*: 802). The 953 parent ion corresponds to [pT=^{m}CG-H]$^{-}$.

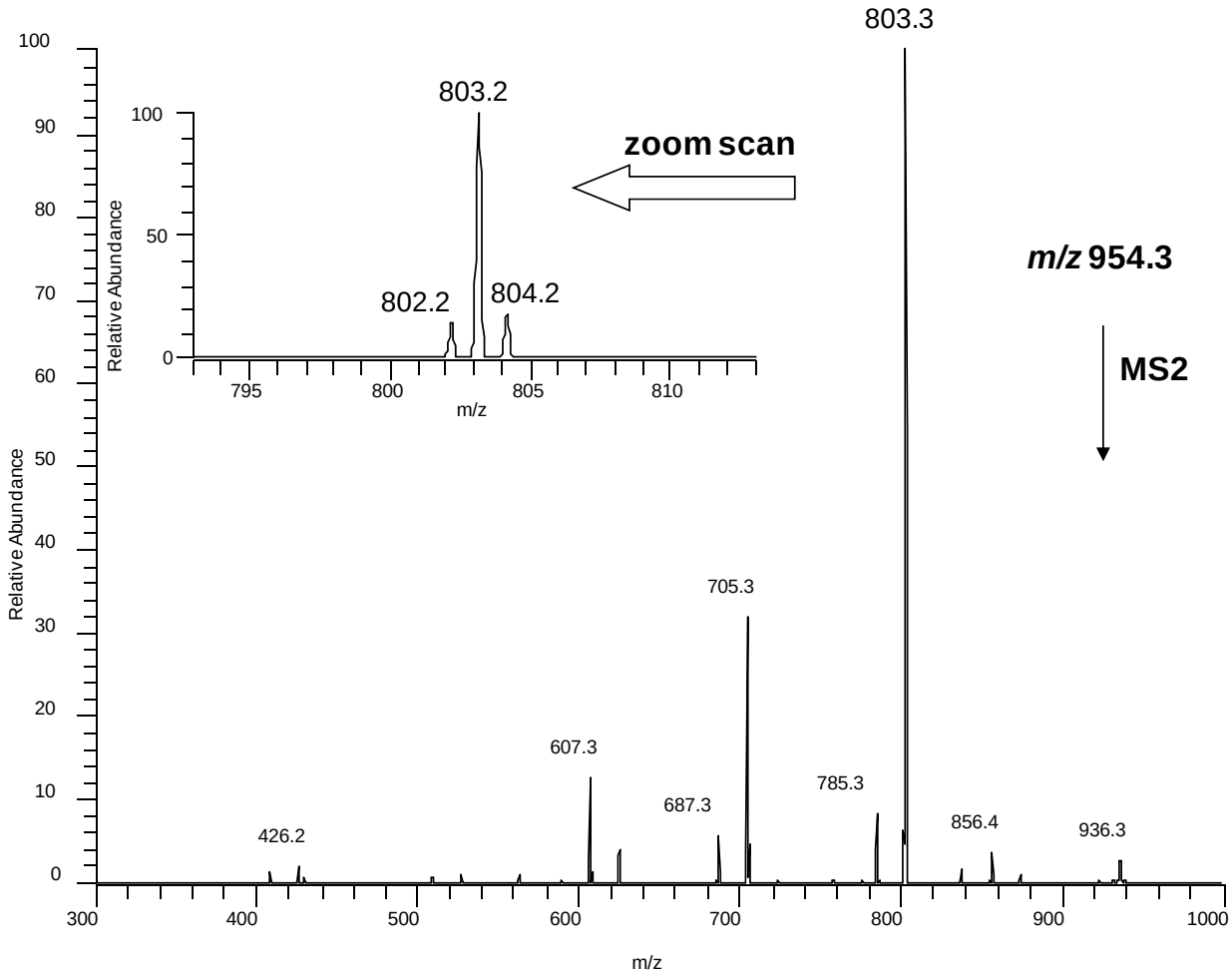

FIGURE S4. **Nuclease P1-coupled ESI-MS/MS analysis of the deamination products of T=^{m}C-14-mer.** Spectra of the nuclease P1 digested HPLC peak corresponding to the deaminated product of cis-syn CPD T^{m}C-14-mer (A1: parent ion *m*/*z*: 954, full MS; A2: zoom scan at *m*/*z*: 803). The 954 parent ion corresponds to $[pT=TG-H]^-$.

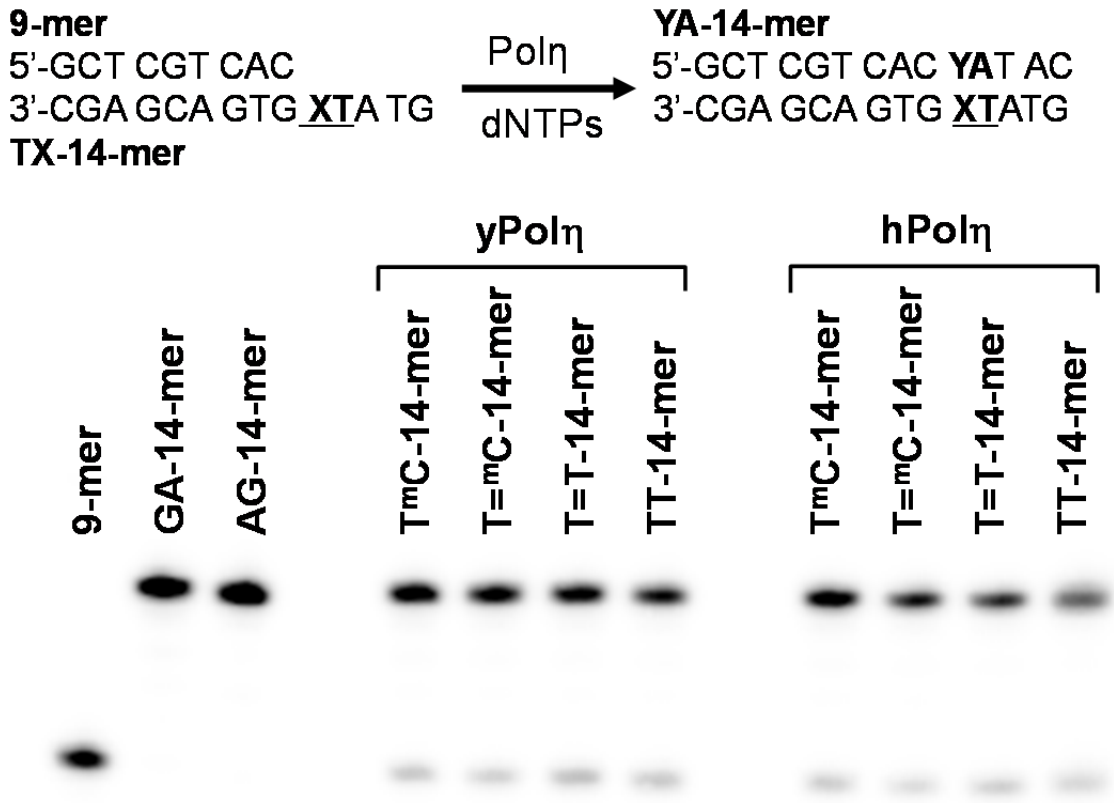

FIGURE S5. **Multiple hit full length primer-extension experiment.** The 9-mer primer/14-templates were incubated with Polη and 100 μM of each dNTP until complete extension was achieved. Denaturing electrophoresis gel (20% TBE PAGE) showing production of full-length primer extension products under the reaction conditions.

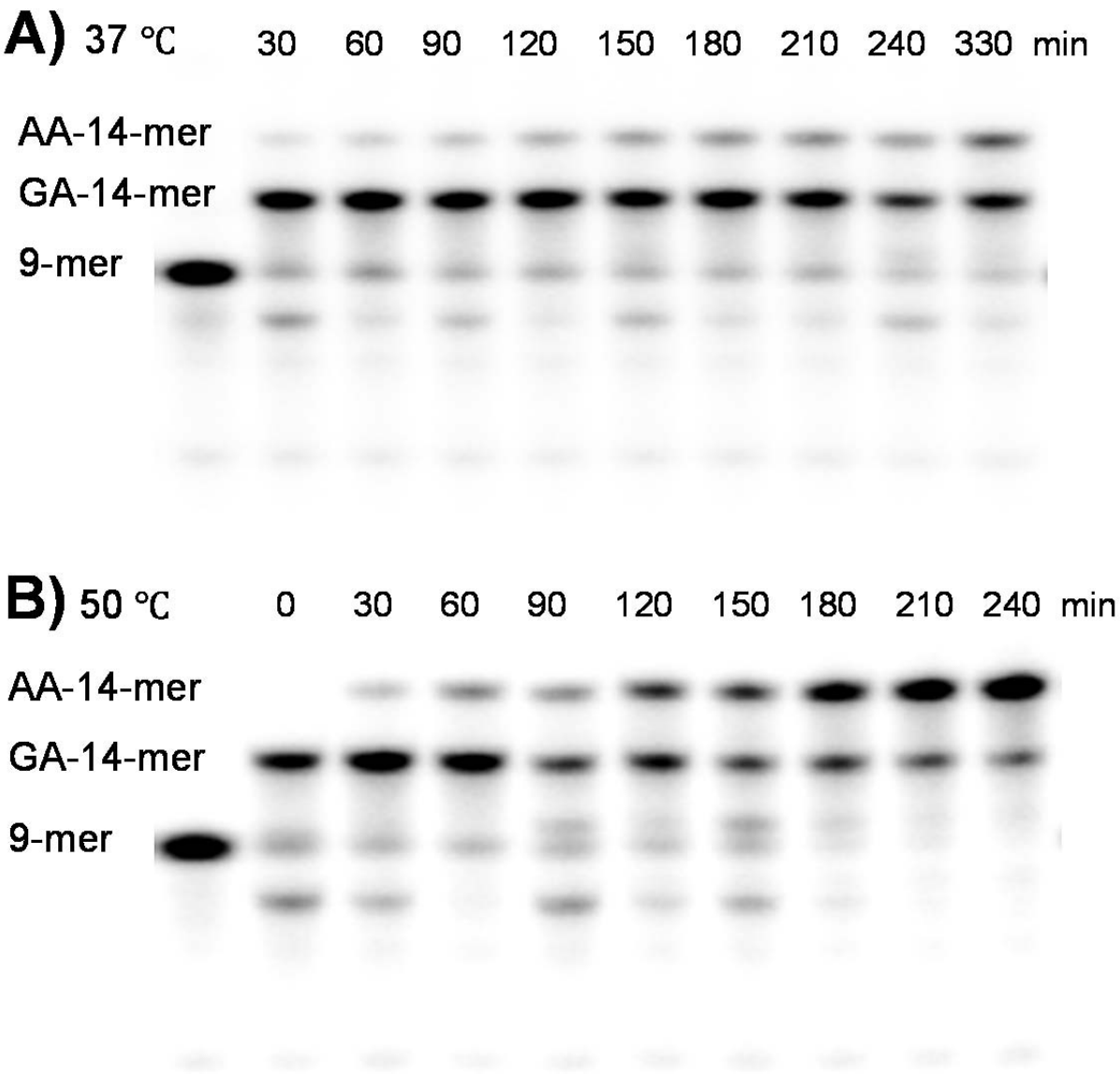

FIGURE S6. **Temperature dependence of ^{m}C deamination in T=^{m}C-14-mer CPD at pH 7.5.** Multiple-hit nucleotide insertion competition assay carried out with 100 μM of each dNTP at 37°C and 50 °C and electrophoresed on a 25% polyacrylamide, pH 3.5 citrate gel.

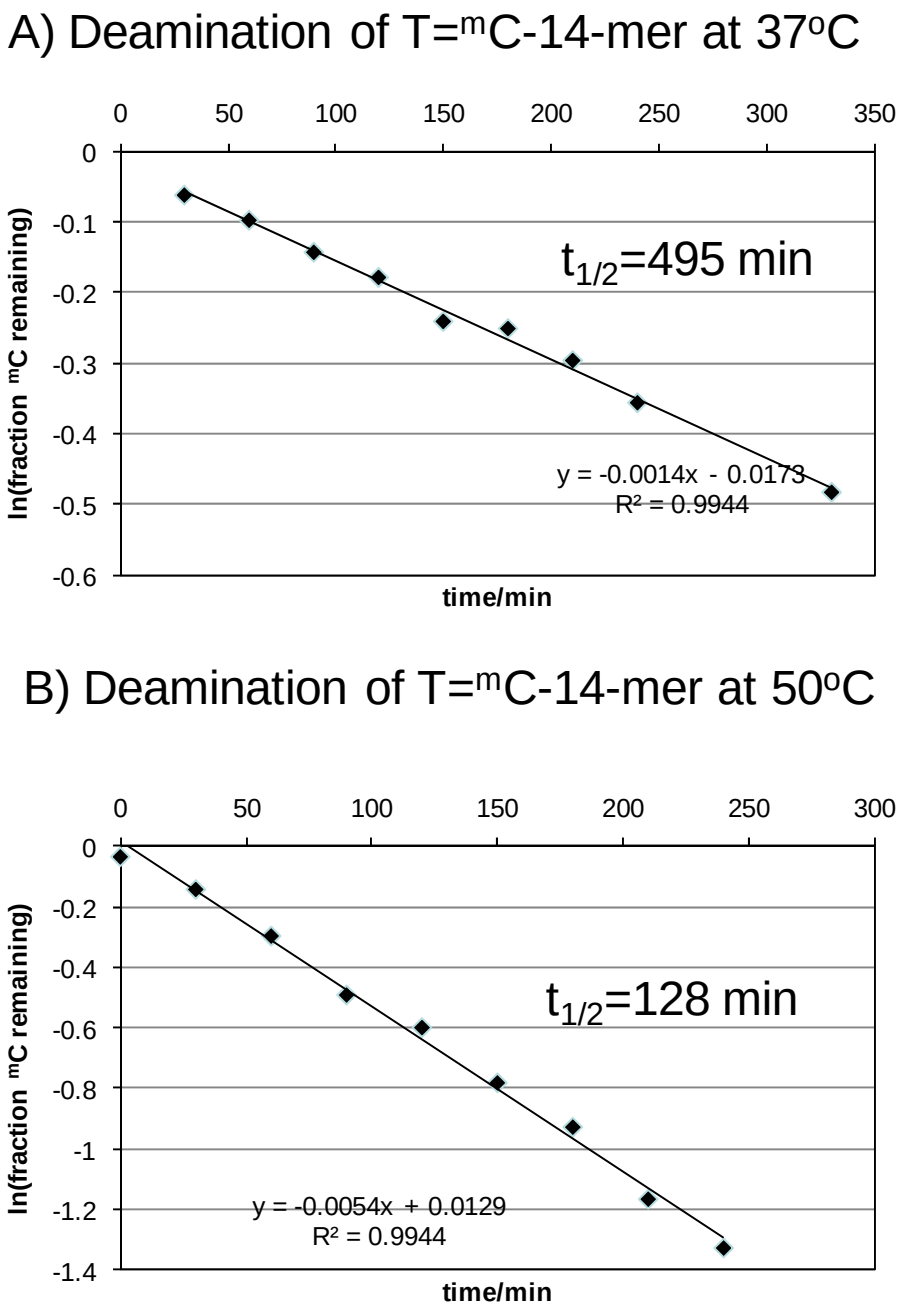

FIGURE S7. **Deamination rate constant determination.** Least squares fit to a plot of the natural log of the fraction of ^{m}C remaining in the T^{m}CG-14-mer CPD versus deamination time at two different temperatures. The fraction of ^{m}C remaining equals the fraction of G inserted, G/(G + A), opposite the ^{m}C of the T=^{m}C14-mer by yPolη.