Supporting Information

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SI Text

Materials. Unmodified ODNs used in this study were purchased from Integrated DNA Technologies. [γ-^{32}P]ATP was obtained from Amersham Biosciences. All enzymes unless specified were from New England Biolabs or Sigma–Aldrich. Shrimp alkaline phosphatase was obtained from Roche Diagnostics. [3,3,3-D_3]-DL-Alanine (D_3, >98%) was obtained from Cambridge Isotope Laboratories. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was purchased from TCI America.

M13mp7(L2) and wild-type *E. coli* strains were provided by Prof. John M. Essigmann, and polymerase-deficient AB1157 strains [Δ*pol B1*::spec (pol II-deficient), Δ*dinB* (pol IV-deficient), Δ*umuC*::*kan* (pol V-deficient), and Δ*umuC*::*kan* Δ*dinB* Δ*pol B1*::spec (triple knockout)] were generous gifts from Prof. Graham Walker (1). *E. coli* DNA polymerase IV was provided by Prof. Myron F. Goodman (2). Human polymerase κ was purchased from Enzymax.

Synthesis of [2,2,2-D_3]-N^2-(1-carboxyethyl)-2′-deoxyguanosine (D_3-N^2-CEdG). This compound was synthesized from 2-fluoro-2′-deoxyinosine and D_3-DL-alanine by using reported procedures (3). The resulting two diastereomers of D_3-N^2-CEdG were separated by HPLC and used as internal standards for the quantification of N^2-CEdG formed in cells, and the concentrations of the stock solutions of D_3-N^2-CEdG were determined by UV spectrophotometry using molar extinction coefficients of 13,600 liter/mol per centimeter at 260 nm (3).

Cell Culture and Methylglyoxal/D-Glucose treatment. WM-266-4 human melanoma cells (American Type Culture Collection, ATCC) were cultured at 37°C in 5% CO_2 atmosphere and in Eagle's minimum essential medium supplemented with 10% FBS (Invitrogen), 100 unit/ml penicillin, and 100 μg/ml streptomycin (ATCC). After growing to 80% confluence, cells were detached by trypsin-EDTA treatment and harvested by centrifugation to remove the medium. The cell pellets were subsequently washed twice with PBS and resuspended in 20 ml PBS (10^6 cells/ml) containing the desired concentrations of methylglyoxal (MG). The cells were incubated with MG at room temperature for 3.0 h with occasional shaking.

For D-glucose treatment, the cells were cultured in the same media containing certain concentrations of D-glucose. The media was discarded, and the cells were resuspended in fresh media containing the same concentration of glucose on day 3. The cells were harvested at the end of day 5. The nuclear DNA was isolated from cell lysates with phenol extraction and desalted by ethanol precipitation.

Enzymatic Digestion. Eight units of nuclease P1, 0.02 unit of calf spleen phosphodiesterase, and a 10-μl of solution carrying 300 mM sodium acetate (pH 5.0) and 10 mM zinc acetate were added to 90 μl of aqueous solution with 200 μg of genomic DNA, and the digestion was carried out at 37°C for 4 h. To the digestion mixture were then added 50 units of alkaline phosphatase, 0.2 unit of snake venom phosphodiesterase, and 10 μl of 0.5 M Tris·HCl buffer (pH 8.9). The digestion was continued at 37°C for 4 h, and the enzymes were removed by passing through a 10-kDa cutoff Centricon membrane (Millipore). The amount of nucleosides in the mixture was quantified by UV absorbance measurements, and to the mixture were then added 300 fmol of D_3-S-N^2-CEdG and D_3-R-N^2-CEdG. The resulting aliquots were subsequently subjected to HPLC enrichment and LC-MS/MS analysis.

HPLC Enrichment. The enrichment of DNA lesions from the digestion mixture of genomic DNA was performed on a Beckman Gold HPLC system (pump module 125, 32 Karat software version 3.0) with a UV detector (module 126) monitoring at 260 nm. A 4.6 × 250 mm Apollo reverse phase C18 column (5 μm in particle size, Alltech Associates) was used, and 10 mM ammonium formate buffer (pH 6.4, solvent A) and a mixture of 10 mM ammonium formate and acetonitrile (70/30, vol/vol, solvent B) were used as mobile phases. A gradient of 5 min of 0–10% B followed by 40 min of 10–35% was used, and the flow rate was 0.60 ml/min. We collected fractions in a wide retention time range (3–4 min) to ensure that the lesions were completely collected while the unmodified nucleosides were excluded as much as possible. The collected fractions were dried in a Speed-vac, reconstituted in 6 μl of H_2O, and 3-μl aliquot was injected for LC-MS/MS analysis.

LC-MS/MS Quantification of N^2-CEdG. Quantitative analysis of N^2-CEdG in the above DNA hydrolysates was performed by online capillary HPLC-ESI-MS/MS using an Agilent 1100 capillary HPLC pump (Agilent Technologies) interfaced with an LTQ linear ion-trap mass spectrometer (Thermo Fisher Scientific), which was set up for monitoring the fragmentation of the $[M + H]^+$ ions of N^2-CEdG and isotope-labeled internal standard D_3-N^2-CEdG. A 0.5 × 150 mm Zorbax SB-C18 column (5 μm in particle size, Agilent Technologies) was used for the separation of the DNA hydrolysates, and the flow rate was 6.0 μl/min. A 5-min gradient of 2–20% methanol in 0.1% formic acid, followed by a 35-min gradient of 20–45% methanol in 0.1% formic acid, was used for the separation. To eliminate the isobaric impurities present in MS/MS, we quantified N^2-CEdG using MS^3, where we monitored the further fragmentation of the $[M + H]^+$ ions of the nucleobase portions of N^2-CEdG and D_3-N^2-CEdG (i.e., the ions of *m/z* 224 and 227), respectively.

Preparation of ODN Substrates Containing an S-N^2-CEdG or R-N^2-CEdG. The 16- and 20-mer N^2-CEdG-containing ODNs (16 mer, 5′-GAAGACCAXCGACGCC-3′; 20 mer, 5′-ATGGCXCAC-TATGATCCTAG-3′, X = S/R-N^2-CEdG) were prepared as described (3).

***In Vitro* Replication Studies with Human Polymerase κ.** Primer extension assays were performed under standing-start conditions. The 20-mer lesion-containing template or undamaged template (20 nM) with dG in place of N^2-CEdG was annealed with a 5′-[^{32}P]-labeled 15-mer primer d(GCTAGGATCATAGTG) (10 nM), to which mixture was then added a mixture of all four dNTPs (200 μM) as well as the human DNA polymerase κ. The reaction was carried out at 37°C in a 20-μl solution containing 10 mM Tris·HCl (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM DTT for 60 min. The concentrations of the polymerase are indicated in Fig. S9. The reaction was terminated by adding 8 μl of gel-loading buffer containing 80% formamide, 10 mM EDTA (pH 8.0), 1 mg/ml xylene cyanol, and 1 mg/ml bromophenol blue. The products were resolved on 20% (acrylamide:bis-acrylamide, 29:1, wt/wt) cross-linked polyacrylamide gels containing 8 M urea. Gel-band intensities for the substrates and products were quantified by using a Typhoon 9410 Variable Mode Imager

(Amersham Biosciences) and ImageQuant version 5.2 (Amersham Biosciences).

The steady-state kinetic analyses were performed as described (4, 5). The primer-template duplex (10 nM) was incubated with human polymerase κ (5 ng) in the presence of an individual dNTP at various concentrations as indicated in Fig. S10. The reaction was carried out at room temperature with the same reaction buffer as described above. The dNTP concentration was optimized for different insertion reactions to allow for <20% primer extension (5). The products were resolved again by denaturing PAGE analysis. The observed rate of dNTP incorporation (V_{obs}) was plotted as a function of dNTP concentration, and the apparent K_m and V_{max} steady-state kinetic parameters for the incorporation of both the correct and incorrect nucleotides were determined by fitting the rate data with the Michaelis–Menten equation.

The efficiency for nucleotide incorporation was determined by the ratio of V_{max}/K_m. The fidelity of nucleotide incorporation was then calculated by the frequency of misincorporation (f_{inc}) with the following equation:

$$f_{inc} = \frac{(V_{max}/K_m)_{incorrect}}{(V_{max}/K_m)_{correct}}$$

***In Vitro* Replication Studies with *E. coli* DNA Polymerase IV.** Primer extension assays and steady-state kinetic measurements with *E. coli* pol IV were performed under similar conditions as described above for human pol κ. For primer extension assays, a mixture of all four dNTPs (200 μM) as well as the *E. coli* pol IV (the concentrations of the polymerase are indicated in Fig. S7) were added to the same primer–template complex as described above. The reaction was carried out at 37°C in a 20-μl solution containing 20 mM Tris·HCl (pH 7.5), 8 mM $MgCl_2$, 5 mM DTT, 0.1 mM EDTA, 50 mM NaCl, 40 μg/ml BSA, and 4% glycerol for 60 min. The reaction was terminated by adding 8 μl of gel-loading buffer containing 80% formamide, 10 mM EDTA (pH 8.0), 1 mg/ml xylene cyanol, and 1 mg/ml bromophenol blue. The products were again resolved on 20% (acrylamide:bisacrylamide, 29:1, wt/wt) cross-linked polyacrylamide gels containing 8 M urea.

For steady-state kinetic measurements, the primer-template duplex (10 nM) was incubated with *E. coli* pol IV (20 ng) in the presence of an individual dNTP at various concentrations as indicated in the figures (Fig. S8). The reaction was carried out at 37°C for 10 min in the same reaction buffer as described above. The dNTP concentration was optimized for different insertion reactions to allow for less than 20% primer extension.

Construction of ss-M13 Genomes Harboring a Site-Specifically Inserted S-N^2-CEdG, R-N^2-CEdG or dG. The M13mp7(L2) viral genomes, either lesion-free or carrying a site-specifically inserted S- or R-N^2-CEdG, were prepared following the procedures described by Delaney and Essigmann (6, 7). Briefly, 20 pmol ss-M13mp7(L2) was digested with 40 units of EcoRI at 23°C for 8 h to linearize the vector. Two scaffolds, 5′-GGTCTTCCACTGAATCATGGTCATAGC-3′ and 5′-AAAACGACGGCCAGTGAATTGGCGTC-3′ (25 pmol), each spanning one end of the cleaved vector and the modified ODN insert, were annealed with the linearized vector. The 16-mer insert [d(GAAGACCAXCGACGCC), where "X" is dG, S-N^2-CEdG, or R-N^2-CEdG, 30 pmol] was 5′-phosphorylated in a 30-μl solution containing 1 × T4 polynucleotide kinase buffer, 1 mM ATP, 5 mM DTT, and 15 units of polynucleotide kinase at 37°C for 1 h. The 5′-phosphorylated 16-mer inserts were ligated by using T4 DNA ligase to the above vector in the presence of the two scaffolds at 16°C for 8 h. T4 DNA polymerase (22.5 units) was subsequently added and the resulting mixture was incubated at 37°C for 4 h to degrade the scaffolds. The solution was extracted once with phenol/chloroform/isoamyl alcohol (25:24:1, vol/vol), and the aqueous phase was passed through a Sephadex-G50 column (Amersham) to remove traces of phenol and salt. The constructed genomes were normalized against a lesion-free competitor genome, which was prepared by inserting a 19-mer unmodified ODN to the EcoRI-linearized genome, following the method described by Delanely *et al.* (7).

Transfection of *E. coli* Cells with ss-M13 Vectors Containing S-N^2-CEdG, R-N^2-CEdG or dG. Desalted genomes containing a lesion or unmodified dG (150 fmol) were mixed with the competitor genome at a ratio of 6:1 and transfected into the electrocompetent AB1157 *E. coli* cells. The M13 genome-carrying *E. coli* cells were grown in 3 ml of LB culture at 37°C for 6 h, after which the phage was recovered from the supernatant by centrifugation at 13,000 rpm for 5 min. The resulting phage was further amplified in SCS110 *E. coli* cells to increase the progeny/lesion-genome ratio (7). The phage recovered from the supernatant (700 μl) was passed through a QIAprep Spin M13 kit (Qiagen) to isolate the ss-M13 DNA.

Determination of the Bypass Efficiency Using Competitive Replication and Adduct Bypass (CRAB) Assay (7). PCR amplification of the region of interest in the resulting progeny genome was performed by using Phusion high-fidelity DNA polymerase. The primers were 5′-YCAG GGT TTT CCC AGT CAC GAC GTT GTA A-3′ and 5′-YCAG CTA TGA CCA TGA TTC AGT GGA AGA C-3′ (Y is an amino group), and the amplification cycle was 30, each consisting of 10 s at 98°C, 30 s at 62°C, 15 s at 72°C, with a final extension at 72°C for 5 min. The PCR products were purified by using QIAquick nucleotide removal kit (Qiagen).

For the bypass efficiency assay, a portion of the above PCR fragments was treated with 5 units of BbsI and 1 unit of shrimp alkaline phosphatase (Roche) in 10-μl NEB buffer 2 at 37°C for 4 h, followed by heating at 65°C for 20 min to deactivate the phosphatase. The above mixture was then treated in a 15-μl NEB buffer 2 with 5 mM DTT, ATP (50 pmol cold, premixed with 1.66 pmol [γ-^{32}P] ATP) and 10 units of polynucleotide kinase. The reaction was continued at 37°C for 1 h, followed by heating at 65°C for 20 min to deactivate the polynucleotide kinase. To the reaction mixture was subsequently added 10 units of Tsp509I, and the solution was incubated at 65°C for 1 h, followed by quenching with 15 μl formamide gel loading buffer containing xylene cyanol FF and bromophenol blue dyes. The mixture was loaded onto a 20% denaturing gel and products were quantified by phosphorimager analysis. After the restriction cleavages, the DNA fragment of interest from the full-length replication product was liberated as an 8mer ODN, d(p*XCGACGCC), where "X" designates the nucleobase present at the original lesion site after *in vivo* DNA replication, and "p*" represents the 5′-radiolabeled phosphate. On the other hand, the corresponding DNA fragment released from the competitor genome was an 11-mer ODN, d(p*GCTAGCTGCGG). The bypass efficiency was calculated using the following formula, %bypass = (lesion signal/competitor signal)/(non-lesion control signal/its competitor signal) (7).

Determination of Bypass Efficiency and Mutation Frequency Using LC-MS/MS. To examine the bypass efficiency using LC-MS, PCR products were treated with 45 units of BbsI and 20 units of shrimp alkaline phosphatase in 250 μl of NEB buffer 2 at 37°C for 4 h, followed by heating at 65°C for 20 min. To the resulting solution was then added 50 units of Tsp509I, and the reaction mixture was incubated at 65°C for 1 h followed by extraction once with phenol/chloroform/isoamyl alcohol (25:24:1, vol/vol), and the aqueous portion was dried with Speed-vac and desalted with HPLC using a 4.6 × 250-mm Apollo C18 column (5 μm in

particle size and 300 Å in pore size, Alltech Associate). The desalting was carried out by using H_2O as mobile phase A and acetonitrile as mobile phase B, where a gradient of 25 min 0% B, 1 min 0–50% B, and 25 min at 50% B was used. The flow rate was 0.8 ml/min.

After desalting, the ODN mixture was subjected to LC-MS/MS analysis. A 0.5 × 150 mm Zorbax SB-C18 column (5 μm in particle size, Agilent Technologies) was used for the separation, and the flow rate was 8.0 μl/min, which was delivered by using the Agilent 1100 capillary HPLC pump. A 5-min gradient of 0–20% methanol followed by a 35 min of 20–50% methanol in 400 mM HFIP buffer (pH was adjusted to 7.0 by the addition of triethylamine) was used for the separation (8). The effluent from the LC column was coupled directly to the LTQ linear ion trap mass spectrometer, which was set up for monitoring the fragmentation of the $[M\text{-}2H]^{2-}$ ions of the 8mer and 11-mer ODNs.

1. Jarosz DF, Beuning PJ, Cohen SE, Walker GC (2007) Y-family DNA polymerases in *Escherichia coli*. *Trends Microbiol* 15:70–77.
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6. Delaney JC, Essigmann JM (1999) Context-dependent mutagenesis by DNA lesions. *Chem Biol* 6:743–753.
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9. Gerlach VL, Feaver WJ, Fischhaber PL, Friedberg EC (2001) Purification and characterization of pol kappa, a DNA polymerase encoded by the human DINB1 gene. *J Biol Chem* 276:92–98.

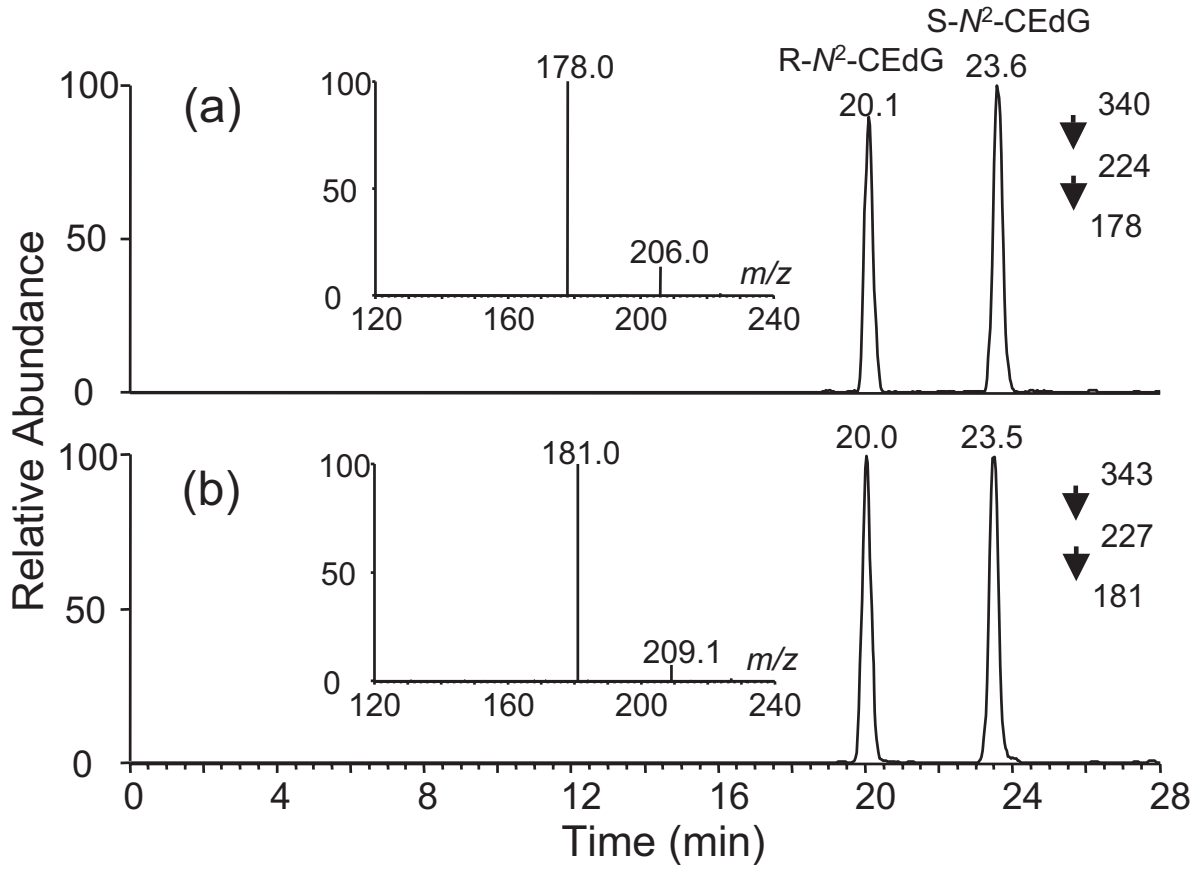

Fig. S1. SICs for the monitoring of the *m/z* 340.1 224 224.1 224 178.1 (*a*, for N^2-CEdG) and *m/z* 343.1 224 227.1 224 181.1 (*b*, for D_3-N^2-CEdG) transitions of the digestion mixtures of genomic DNA extracted from cells treated with 25 μM methylglyoxal; 300 fmol of D_3-N^2-CEdG internal standard was added before HPLC enrichment.

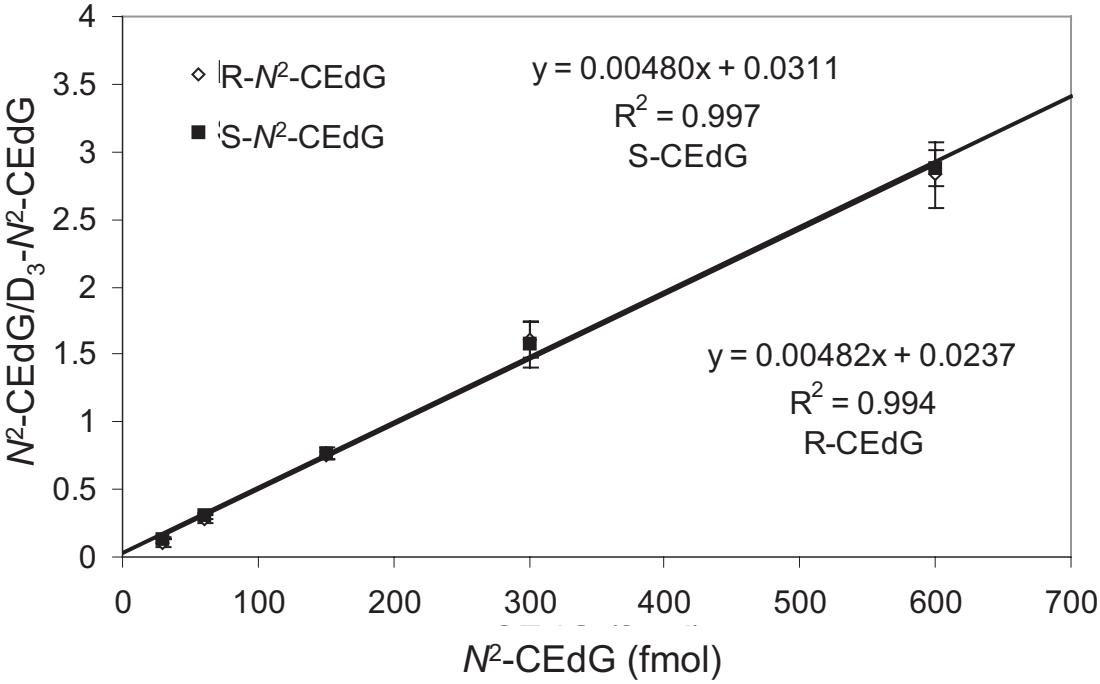

Fig. S2. Calibration curves for quantification of S-N^2-CEdG and R-N^2-CEdG

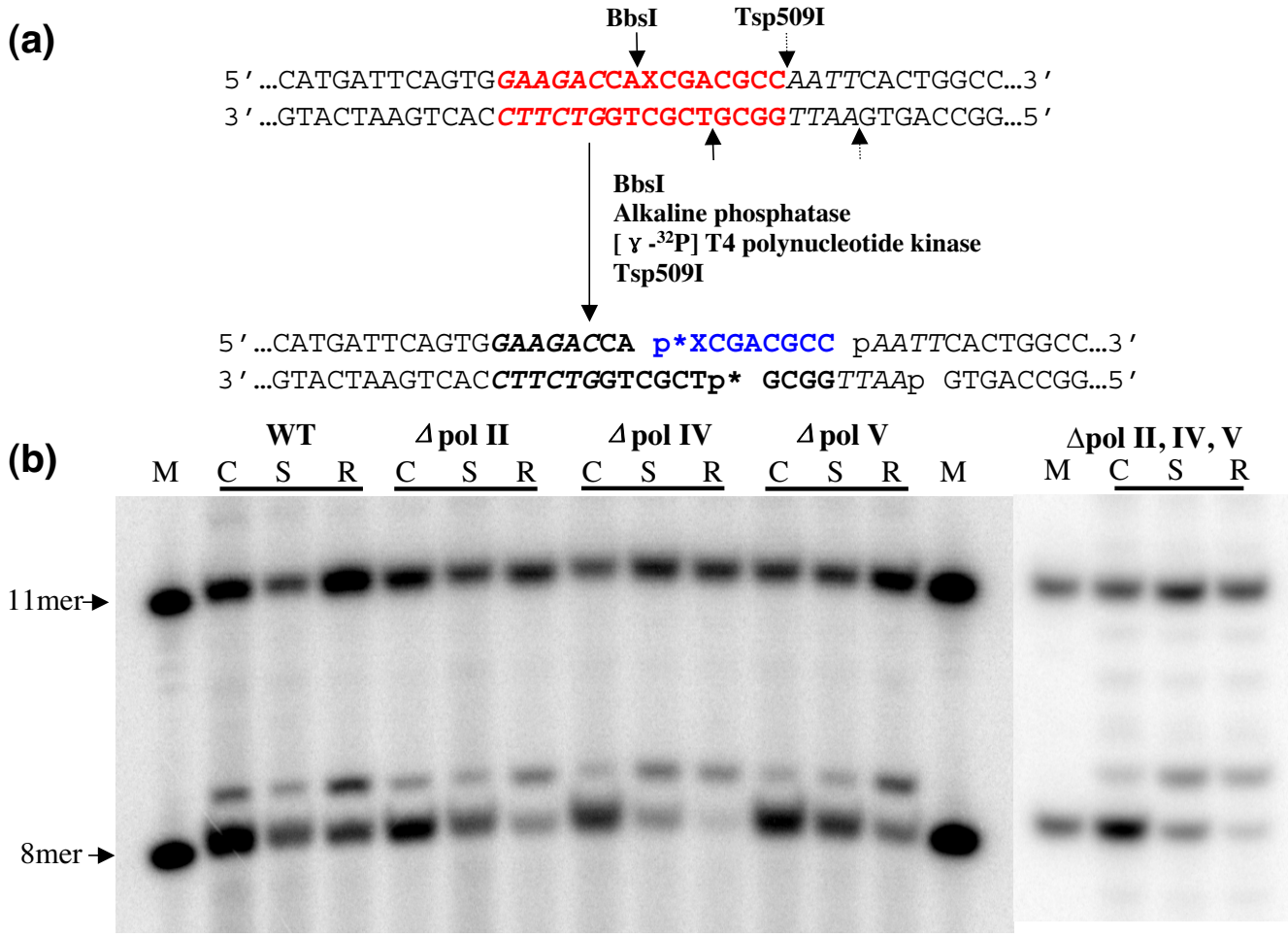

Fig. S3. Measurement of the bypass efficiencies of the two diastereomers of N^2-CEdG *in vivo* by the CRAB assay. (*a*) Sample processing (''p*'' represents ^{32}P-labeled phosphate group); (*b*) gel image showing the 11 and 8 mer released from the PCR products of the progeny resulting from the replication of competitor genome and the control or lesion-carrying genome. ''M'' represents markers which include the unmutated 8-mer sequence from the control genome and authentic 11-mer sequence from the competitor genome. ''C'', ''S,'' and ''R'' represent control as well as S-N^2-CEdG- and R-N^2-CEdG-bearing genomes, respectively. The band above the 8 mer is most likely a nonspecific digestion product from the competitor genome, because, in each lane, the intensity for this band is proportional to the band intensity of the 11 mer.

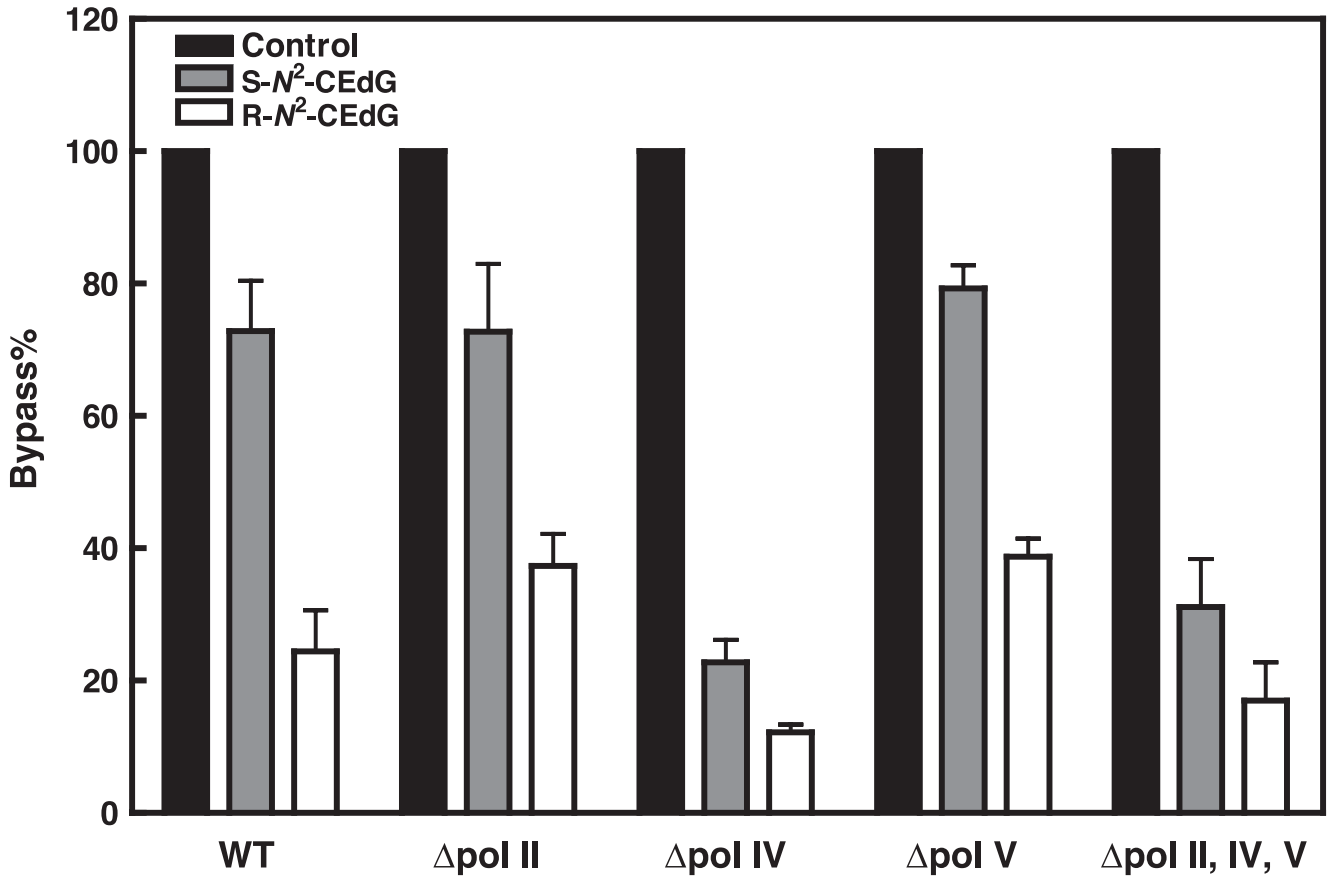

Fig. S4. *In vivo* bypass efficiencies of S-N^2-CEdG and R-N^2-CEdG in wild-type, pol II-, pol IV-, pol V-deficient, and triple knockout AB1157 *E. coli* cells determined by LC-MS/MS assay. Black, gray, and white column represent control DNA, S-N^2-CEdG and R-N^2-CEdG, respectively. The data represent the means and standard deviations of results from three independent transformation and LC-MS/MS experiments.

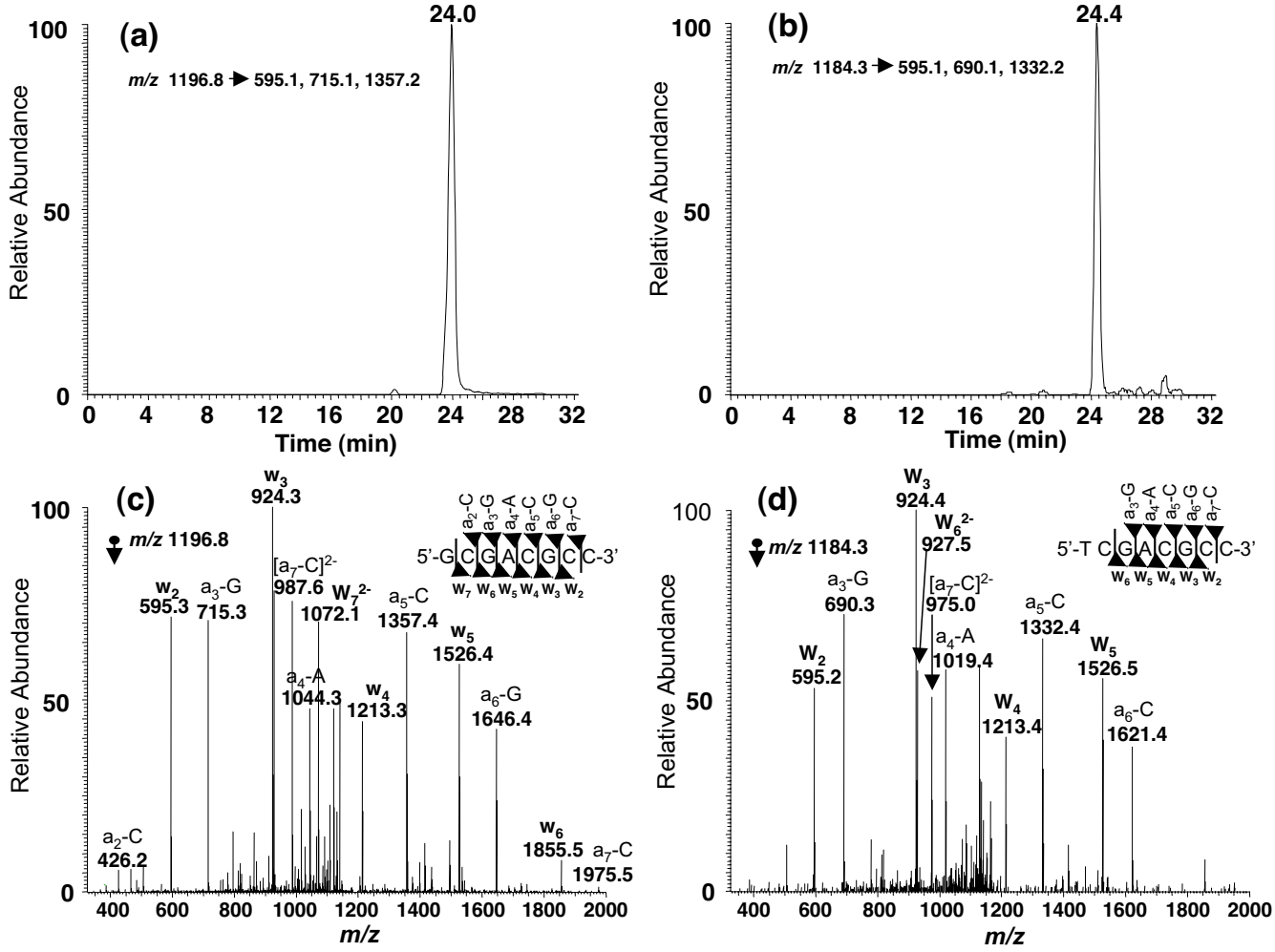

Fig. S5. LC-MS/MS for monitoring the restriction fragments of interest without mutation or with a G → T mutation at the original N^2-CEdG site [i.e., d(GCGACGCC) (8 mer, G) and d(TCGACGCC) (8 mer, T)]. Shown in *a* and *b* are the selected-ion chromatograms (SICs) for the formation of indicated fragment ions of these two ODNs, and illustrated in *c* and *d* are the MS/MS of the $[M–2H]^{2-}$ ions (*m/z* 1,196.9 and 1,184.3) of these two ODNs.

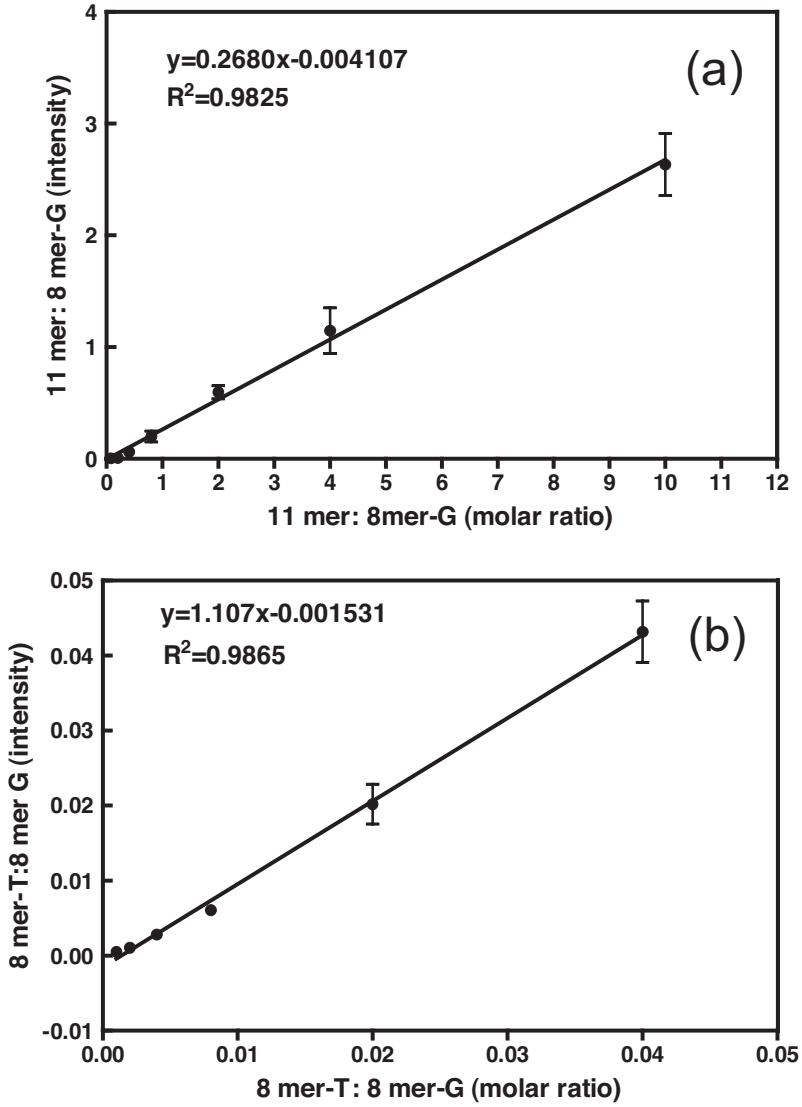

Fig. S6. Calibration curves for the quantification of 11-mer [i.e., d(GCTAGCTGCGG)] (*a*) and 8-mer-T [i.e., d(TCGACGCC)] (*b*). 2.5 pmol of 8-mer-G [i.e., d(GCGACGCC)] was mixed with 11-mer and 8-mer-T at different ratios. The normalized peak area ratios of 11-mer and 8-mer-T over 8-mer-G in the selected-ion chromatograph (SIC) were plotted against the molar ratio for these ODNs to give the calibration curves. The fragment ions, w_2, [a_3-Base] and [a_5-Base] ions were selected for the SIC monitoring of 8-mer ODN while the fragment ions, w_3, [a_4-base], and [a_5-Base] ions were selected for SIC monitoring of 11-mer ODN. The conditions for analysis are identical for the replication mixture and the calibration curves. The data represent the results from three independent measurements.

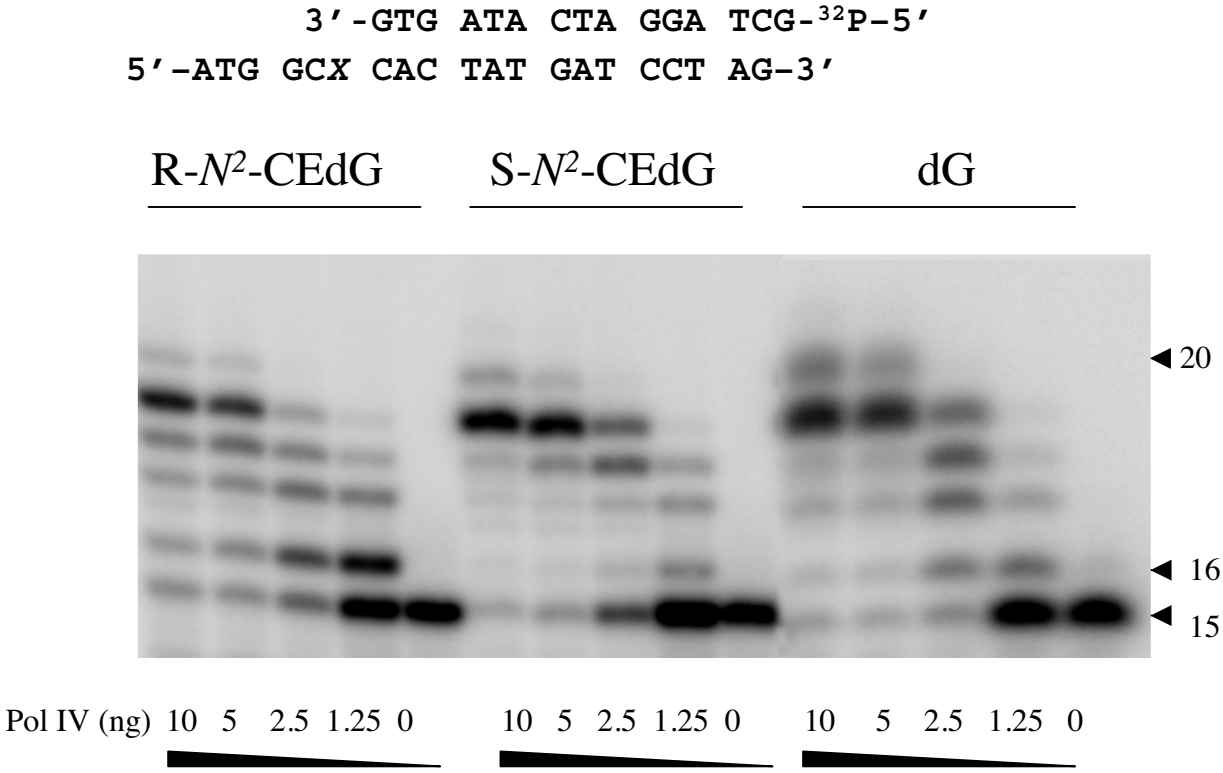

Fig. S7. *In vitro* replication studies of N^2-CEdG-bearing and control undamaged substrates with *E. coli* DNA polymerase IV (''X'' represents S-N^2-CEdG, R-N^2-CEdG or unmodified dG). The primer extension was carried out at 37°C in the presence of all four dNTPs at a concentration of 200 μM each for 60 min, and the amounts of pol IV were indicated. A 5′-[^{32}P]-labeled d(GCT AGG ATC ATA GTG) was used as the primer.

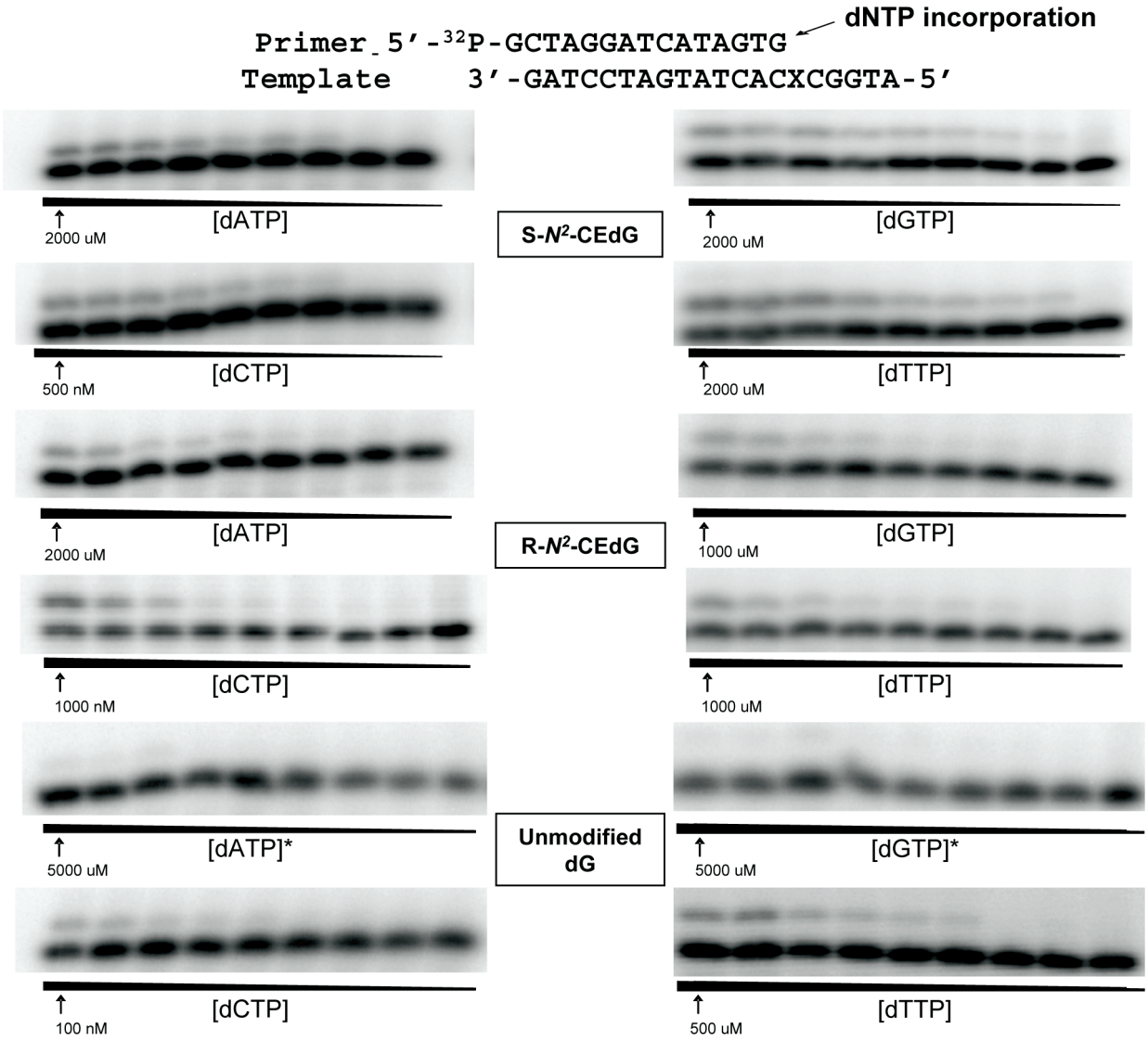

Fig. S8. Steady-state kinetic measurements for the *E. coli* pol IV-mediated incorporation of dAMP, dGMP, dCMP and dTMP opposite the S-N^2-CEdG, R-N^2-CEdG or undamaged dG in a 20-mer template ODN. *E. coli* DNA pol IV (20 ng) was incubated with primer-template complex at 37°C for 10 min for each reaction. The highest dNTP concentration is shown, and the concentration ratio of dNTP between adjacent lanes was 0.5–0.6. ''*'': The incorporation of dATP and dGTP opposite undamaged dG is barely detectable even at extraordinarily high dNTP concentration.

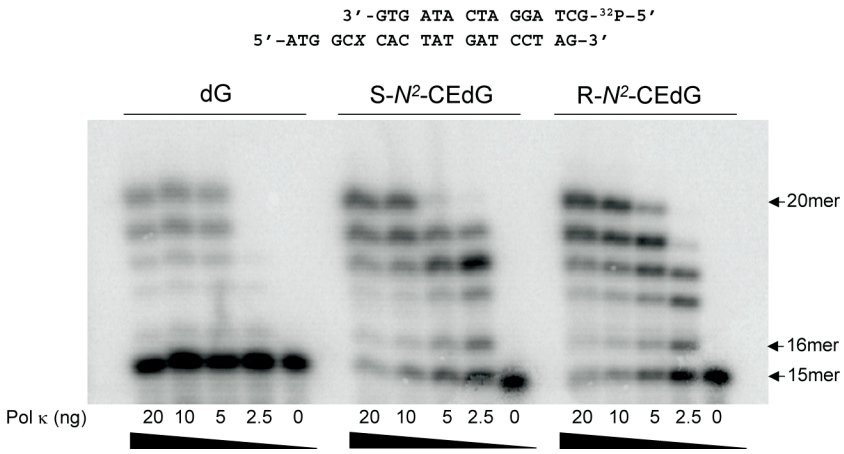

Fig. S9. *In vitro* primer extension assays of N^2-CEdG-bearing and control undamaged substrates with human polymerase κ (''X'' represents S-N^2-CEdG, R-N^2-CEdG or unmodified dG). The primer extension was carried out at 37°C in the presence of all four dNTPs at a concentration of 200 μM each for 60 min, and the amounts of human pol κ were indicated. A 5′-[^{32}P]-labeled d(GCT AGG ATC ATA GTG) was used as the primer.

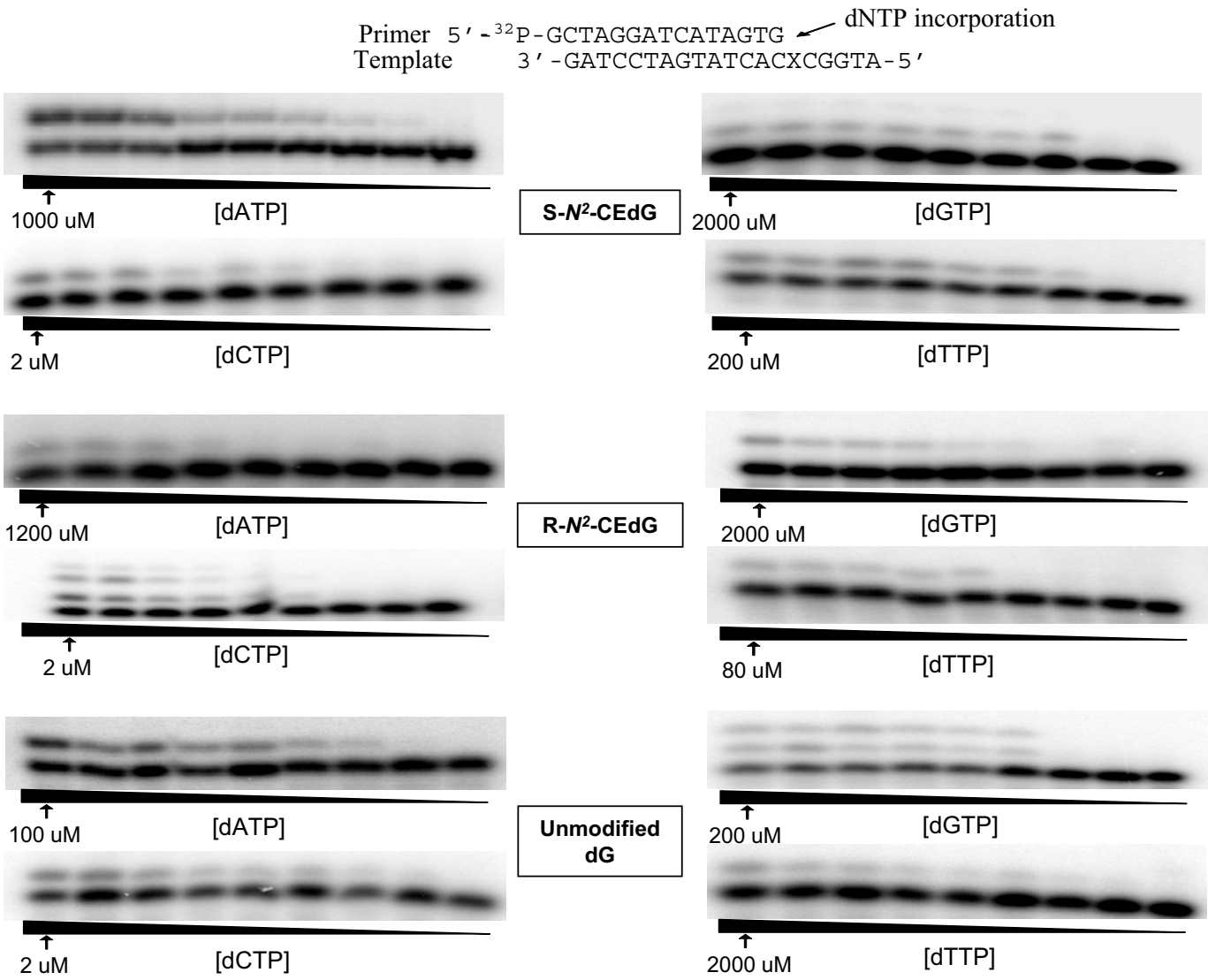

Fig. S10. Steady-state kinetic measurements for incorporation of dAMP, dGMP, dCMP, and dTMP opposite the S-N^2-CEdG, R-N^2-CEdG or undamaged dG on the 20-mer ODNs. Human pol κ (5 ng) was incubated with primer-template complex at room temperature for 10 min for each reaction. The highest dNTP concentration is shown, and the concentration ratio of dNTP between adjacent lanes was 0.5–0.6.

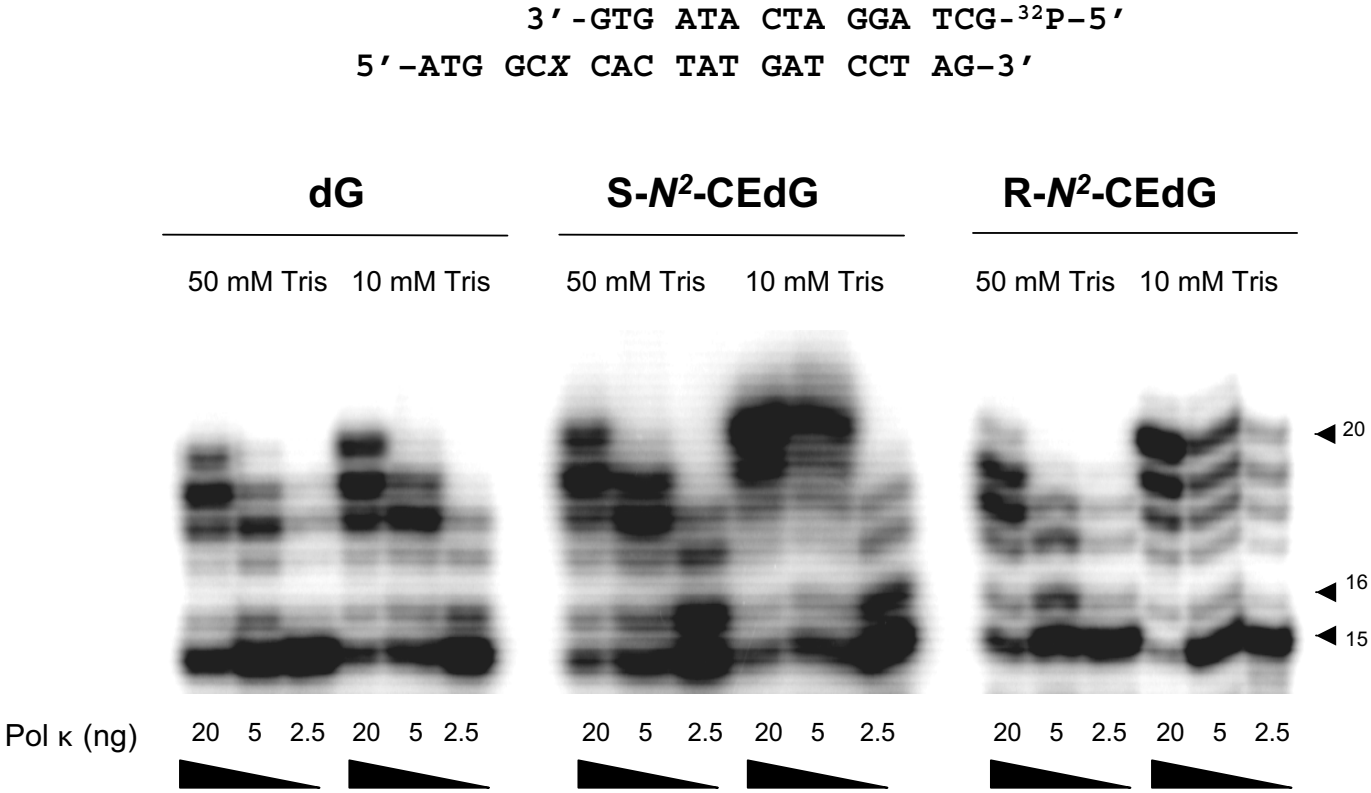

Fig. S11. Comparison of primer extension of N^2-CEdG-bearing and control dG-containing substrates with human pol κ (''X'' represents S-N^2-CEdG, R-N^2-CEdG or an unmodified dG) in a buffer containing 10 mM Tris·HCl (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM DTT [labeled as ''10 mM Tris''] or 50 mM Tris·HCl (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM DTT [labeled as ''50 mM Tris'']. The primer extension was carried out at 37°C in the presence of all four dNTPs at a concentration of 200 μM each for 60 min, and the amounts of pol κ are indicated. A 5′-[^{32}P]-labeled d(GCTAGGATCATAGTG) was used as the primer.

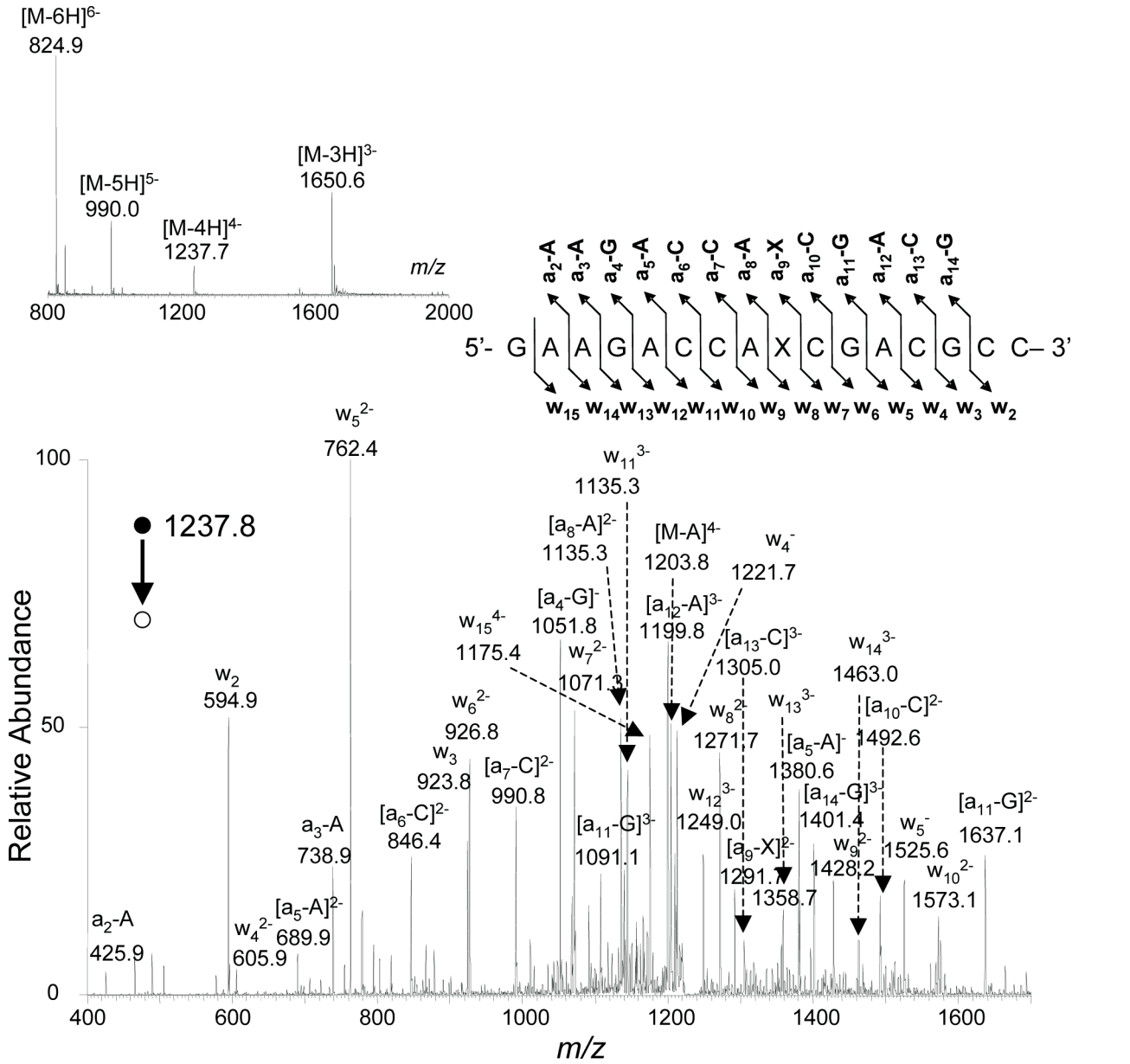

Fig. S12. The product-ion spectrum of the [M–4H]^4–ion (*m/z* 1237.8) of d(GAAGACCAXCGACGCC) (X = S-*N*^{2}-CEdG). Negative-ion ESI-MS and a scheme outlining the fragments found in MS/MS are shown above the MS/MS.

Table S1. Steady-state kinetic parameters for nucleotide incorporation opposite the two diastereomers of N^2-CEdG and unmodified dG by human DNA polymerase κ (K_m and V_{max} are average values based on three independent measurements)

dNTP	V_{max}, nM min^{-1}	K_m, nM	V_{max}/K_m, min^{-1}*	f_{inc}
S-N^2-CEdG-containing substrate				
dATP	0.24 ± 0.004	(1.21 ± 0.10) × 10^5	1.98 × 10^{-6}	6.39 × 10^{-3}
dGTP	0.08 ± 0.01	(3.31 ± 0.32) × 10^5	2.41 × 10^{-7}	7.77 × 10^{-4}
dCTP	0.25 ± 0.03	(8.06 ± 0.95) × 10^2	3.10 × 10^{-4}	1.00
dTTP	0.30 ± 0.02	(3.46 ± 0.55) × 10^4	8.67 × 10^{-6}	2.80 × 10^{-2}
R-N^2-CEdG-containing substrate				
dATP	0.28 ± 0.02	(9.83 ± 0.20) × 10^5	2.84 × 10^{-7}	1.49 × 10^{-3}
dGTP	0.10 ± 0.01	(5.28 ± 1.05) × 10^5	1.89 × 10^{-7}	9.90 × 10^{-4}
dCTP	0.91 ± 0.09	(4.77 ± 0.39) × 10^3	1.91 × 10^{-4}	1.00
dTTP	0.14 ± 0.02	(2.53 ± 0.43) × 10^4	5.53 × 10^{-6}	2.90 × 10^{-2}
dG-containing substrate				
dATP	0.26 ± 0.002	(7.48 ± 1.17) × 10^4	3.48 × 10^{-6}	6.4 × 10^{-2}
dGTP	0.30 ± 0.01	(1.84 ± 0.26) × 10^5	1.63 × 10^{-6}	3.0 × 10^{-2}
dCTP	0.27 ± 0.01	(5.00 ± 0.54) × 10^3	5.40 × 10^{-5}	1.00
dTTP	0.16 ± 0.06	(7.75 ± 0.02) × 10^5	2.06 × 10^{-7}	3.81 × 10^{-3}

*The V_{max}/K_m values for unmodified substrates were somewhat lower than reported previously, which might be attributed to the use of different preparations of the polymerases, different primer-template sequences, and/or different polymerase reaction buffers. In this respect, Gerlach *et al.* (9) optimized the conditions for pol κ polymerase activity, which showed that the polymerase was most active in the pH range of 6.5–7.5. Although in 50-mM Tris buffer, the pol κ activity was insensitive to NaCl concentration up to 50 mM, a metal cofactor, Mg^{2+} or Mn^{2+} (with the former being preferred), is required for the activity. Our reaction conditions are close to the optimal conditions except that we used 10 mM Tris, whereas 50 mM Tris was used in the previous study . To assess whether the lower concentration of Tris used in the present study can compromise the efficiency of nucleotide incorporation, we carried out the primer extension assays employing the buffer as described in *Materials and Methods* or under the same buffer, except that it contained 50 mM Tris. It turned out that the polymerase activity was slightly higher in 10- than in 50-mM Tris buffer (Fig. S11). Thus, the lower efficiency is unlikely due to the different buffer conditions used in the current and previous studies; rather, it might be attributed to the different preparations of pol κ or different primer-template sequence.