SUPPORTING INFORMATION

Repair of hydantoin lesions and their amine adducts in DNA by base and nucleotide excision repair

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CONTENTS:

General methods and materials: DNA oligonucleotides containing standard phosphoramidites were purchased from Integrated DNA Technologies. The nonstandard oligonucleotides containing 8-oxo-7,8-dihydroguanine phosphoramidite (OG), and Fluorescein-dT Phosphoramidite (F) were synthesized at the University of Utah core facility utilizing phosphoramidite purchased from Glen research. Gh-, Sp-, and Sp-amine adduct containing oligonucleotides were synthesized from the corresponding OG-containing oligonucleotide (33, 59). All oligonucleotides were purified by HPLC using a Dionex 100 ion exchange column similarly to previously reported. [1-3] Within the context of this manuscript, the Sp1 and Sp2 nomenclature refers to the order in which the Sp diastereomers elute from a Dionax ion-exchange HPLC column, and do not denote specific stereochemistry at the C4 carbon in Sp. The two diastereomers of each Sp-amine adduct could not be separated via HPLC; therefore, each oligonucleotide containing Sp-amine adducts were used as a mixture of diastereomers. This was also true for the 33 base pair oligonucleotide used to construct the substrate for NER assays, where Sp-containing oligonucleotides are a mixture of Sp1 and Sp2. The 5' radiolabeling was performed using [γ-^{32}P] ATP (Perkin Elmer) with T4 polynucleotide kinase (New England Biolabs (NEB)). The 3'radiolabeling was prepared using [α- ^{32}P] cordycepin-5'triphosphate (Perkin Elmer) with terminal transferase (NEB). Labeled oligonucleotides were purified using G-50 spin columns (GE Healthcare). A Milli-Q PF system was used to purify distilled deionized water that was used to make all the buffers. Storage phosphor autoradiography was performed on a Typhoon Trio phosphorimager system. Data analysis was performed using ImageQuant software (version 5.2a) and the rate constants and dissociation constants were determined using GraFit 5.0 software. All other chemicals were purchased from Fisher Scientific, VWR, or Sigma.

Enzyme Purification and Preparation: Recombinant *Thermotoga maritima* UvrA and UvrB and *Bacillus caldotenax* UvrC were obtained as previously described. [4,5] Recombinant RNA edited NEIL1 (arginine at position 242) previously considered to be the wild type enzyme, and non-edited NEIL1 (lysine at position 242) and E3Q NEIL1 (edited) were purified and the active enzyme concentrations were determined as described previously.[2,3,6] The edited and non-edited NEIL1 enzyme concentrations listed represent active enzyme concentration. The glycosylase inactive variant E3Q and Nei glycosylase concentrations are listed as total protein. Fpg, E3Q Fpg, Nei, hOGG1 were purified and characterized as previously described. [2,7-9]

Hydroxyl Radical Footprinting with E3Q NEIL1: Fe•EDTA was used to generate hydroxyl radicals for footprinting experiments using the catalytically inactive E3Q NEIL1 variant. [7,10] Experiments were performed with the lesions; Gh, Sp, Sp-Lys, Sp-GlcN, and Sp-GPRPGP base paired to G and A. In the hydroxyl radical footprinting assays with Fe(II)•EDTA, either the lesion-containing or the complementary strand was 5'-end labeled with ^{32}P as described above, to facilitate determination of protected nucleotides on both strands of the duplex. Reaction conditions consisted of a 15 μL reaction volume containing 20 mM Tris-HCl pH 7.6, 10 mM EDTA, 1 μg/μL BSA, 0 nM - 2000 nM E3Q NEIL1 incubated at 37°C for 15 minutes, followed by the addition of 3 μL of 2 mM Fe(II), 3 μL of 2 mM EDTA and 3 μL of 10 mM Na ascorbate. The reaction was then incubated at 25°C for 30 minutes and loaded on a 15% acrylamine gel and run at 800 V for 4 hours.

Maxam-Gilbert Sequencing: Modified Maxam-Gilbert G+A sequencing reaction was performed to localize the sites of UvrABC mediated oligonucleotide incision, as well as the nucleotides that are protected from hydroxyl radical cleavage by E3Q NEIL1.[10] The reactions consisted of the appropriately labeled DNA oligonucleotide in a solution with the final volume of 23 μL containing 17 nM unlabeled ssDNA, 0.11 M piperidine, 15% formic acid, and 0.18 mg/mL calf thymus DNA was incubated at 37˚C for 45 minutes. The reaction was quenched with the addition of 225 μL of a stop solution (0.3 M sodium acetate, pH 7, 0.1 mM EDTA, 0.03 mg/mL calf thymus DNA) The DNA strands were ethanol precipitated followed by the addition of 1.0 M piperidine. The mixture was incubated at 90˚C for 45 min. The sample was then lyophilized to dryness and denaturing loading dye was then added. Maxam-Gilbert C+T, and C only reactions were performed to confirm the sites of UvrABC mediated oligonucleotide incision. [10] A solution of 23 μL final volume containing 17 nM DNA, (1.5 M NaCl for C only reactions), 13 mM hydrazine, and 0.18 mg/mL calf thymus DNA was incubated at 20˚C for 7 minutes followed by ethanol precipitation. Samples were then electrophoresed on a 15% polyacrylamide gel for 3 hours at 800V. Gels were quantified using storage phosphor autoradiography.

REFERENCES FOR SUPPORTING INFORMATION:

(1) Schibel, A. E.; An, N.; Jin, Q.; Fleming, A. M.; Burrows, C. J.; White, H. S. *J Am Chem Soc* **2010**, *132*, 17992.

(2) Krishnamurthy, N.; Zhao, X.; Burrows, C. J.; David, S. S. *Biochemistry* **2008**, *47*, 7137.

(3) Zhao, X.; Muller, J. G.; Halasyam, M.; David, S. S.; Burrows, C. J. *Biochemistry* **2007**, *46*, 3734.

(4) Wang, H.; DellaVecchia, M. J.; Skorvaga, M.; Croteau, D. L.; Erie, D. A.; Van Houten, B. *Journal of Biological Chemistry* **2006**, *281*, 15227.

(5) Croteau, D. L.; DellaVecchia, M. J.; Wang, H.; Bienstock, R. J.; Melton, M. A.; Van Houten, B. *J Biol Chem* **2006**, *281*, 26370.

(6) Yeo, J.; Goodman, R. A.; Schirle, N. T.; David, S. S.; Beal, P. A. *Proc Natl Acad Sci U S A* **2010**, *107*, 20715.

(7) Krishnamurthy, N.; Muller, J. G.; Burrows, C. J.; David, S. S. *Biochemistry* **2007**, *46*, 9355.

(8) McKibbin, P. L.; Kobori, A.; Taniguchi, Y.; Kool, E. T.; David, S. S. *J Am Chem Soc* **2012**, *134*, 1653.

(9) Chu, A. M.; Fettinger, J. C.; David, S. S. *Bioorg Med Chem Lett* **2011**, *21*, 4969.

(10) Sambrook, J.; Russell, D. W. *Molecular Cloning, A Laboratory Manual*; 3 ed.; Cold Spring Harbor Laboratory Press: Plainview, NY, 2001; Vol. 1.

SI Table 1. Kinetic rate constants for edited NEIL1 with oxidized lesions determined under STO conditions.

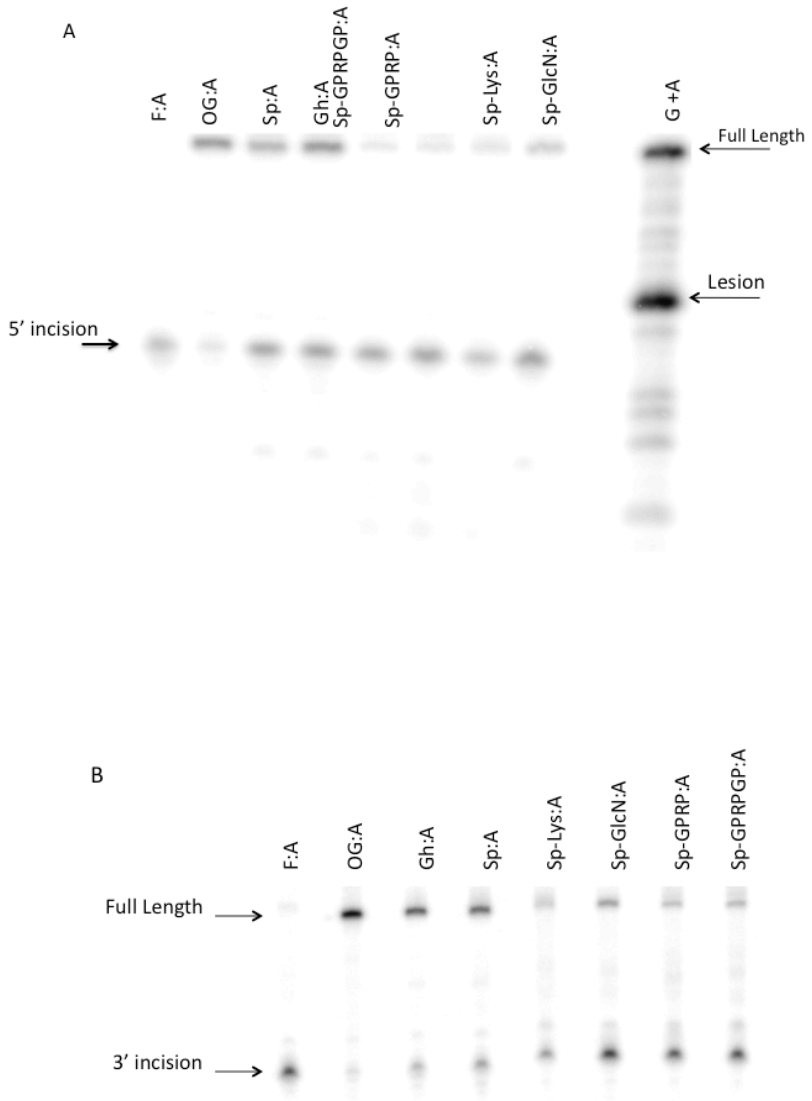
Base Pair	k_g' (min^{-1})	capacity	k_g" (min^{-1})	capacity
Sp1:A	320 ± 30		NA	
Sp1:C	>500		NA	
Sp1:G	>500		NA	
Sp1:T	>500		NA	
Sp2:A	47 ± 5		NA	
Sp2:C	>500		NA	
Sp2:G	>500		NA	
Sp2:T	>500		NA	
Gh:A	130 ± 10		NA	
Gh:C	>500	69 ± 2	6 ± 1	31 ± 2
Gh:G	>500	70 ± 1	1.3 ± 0.3	30 ± 1
Gh:T	>500	79 ± 1	4 ± 1	21 ± 1
Sp-Lys:A	370 ± 70	50 ± 2	7 ± 1	50 ± 2
Sp-Lys:C	>500	70 ± 2	11 ± 2	30 ± 2
Sp-Lys:G	>500	68 ± 2	12 ± 3	32 ± 2
Sp-Lys:T	>500	73 ± 4	18 ± 5	27 ± 4
Sp-GlcN:A	210 ± 90	41 ± 2	3 ± 1	59 ± 2
Sp-GlcN:C	>500	56 ± 4	14 ± 5	44 ± 4
Sp-GlcN:G	>500	43 ± 4	9 ± 1	57 ± 4
Sp-GlcN:T	>500	53 ± 6	20 ± 3	47 ± 6
Sp-GPRP:A	330 ± 80	36 ± 2	9 ± 2	64 ± 2
Sp-GPRP:C	360 ± 40	68 ± 4	13 ± 4	32 ± 4
Sp-GPRP:G	360 ± 70	67 ± 4	26 ± 7	33 ± 4
Sp-GPRP:T	380 ± 40	66 ± 7	30 ± 7	34 ± 7

Rate constants determined at 37°C under STO conditions with hydantoin lesions in a 30 base pair duplex. Reactions proceeded to >75% completion. Data was fit with either a single or a double exponential model generating k_g' and k_g". Capacity represents the % of each rate in the double rate fits. Errors reported are the standard deviations of the average of at least three independent trials. NA represents not applicable.

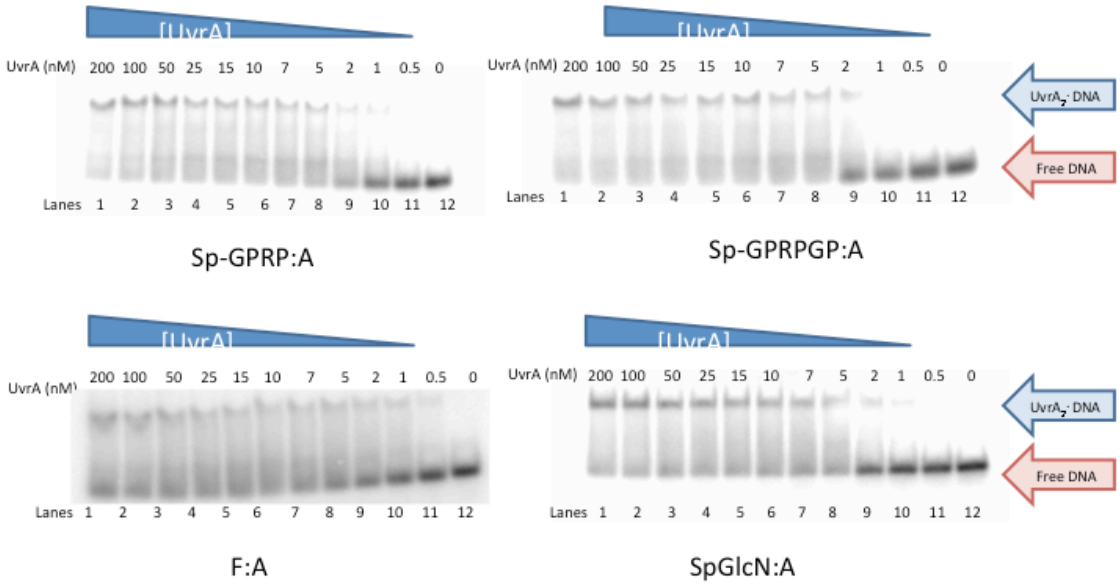
SI Table 2. Kinetic rate constants for non-edited NEIL1 with oxidized lesions determined under STO conditions.

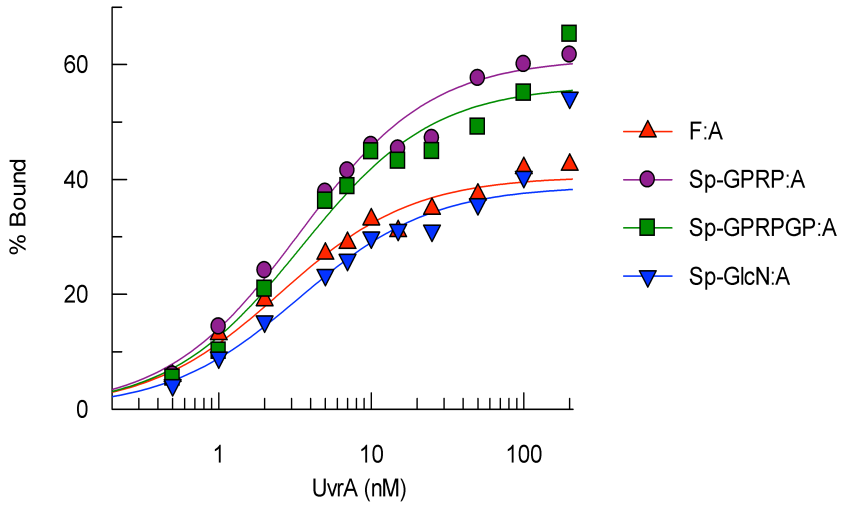
Base Pair	k_g' (min^{-1})	capacity	k_g" (min^{-1})	capacity
Gh:G	>500	74 ± 2	3± 2	26 ± 2
Gh:T	>500	74 ± 6	2.8 ± 0.3	26 ± 6
Sp-Lys:A	340 ± 70	49 ± 3	6 ± 2	51 ± 3
Sp-Lys:C	>500	62 ± 1	7 ± 2	38 ± 1
Sp-Lys:G	>500	60 ± 3	10 ± 2	40 ± 3
Sp-Lys:T	>500	55 ± 11	14 ± 12	45 ± 11
Sp-GlcN:A	200 ± 100	40 ± 4	2 ± 1	60 ± 4
Sp-GlcN:C	300 ± 200	51 ± 3	5 ± 2	49 ± 3
Sp-GlcN:G	400 ± 100	46 ± 2	4 ± 1	54 ± 2
Sp-GlcN:T	>500	50 ± 2	8 ± 2	50 ± 2
Sp-GPRP:A	230 ± 90	24 ± 4	6 ± 1	76 ± 4
Sp-GPRP:C	360 ± 40	55 ± 3	7 ± 1	45 ± 3
Sp-GPRP:G	210 ± 40	60 ± 4	12 ± 4	40 ± 4
Sp-GPRP:T	310 ± 50	62 ± 3	12 ± 2	38 ± 3

Rate constants determined at 37°C under STO conditions with hydantoin lesions in a 30 base pair duplex. Reactions proceeded to >75% completion. Data was fit with either a single or a double exponential model generating k_g' and k_g". Capacity represents the % of each rate in the double rate fits. Errors reported are the standard deviations of the average of at least three independent trials. NA represents not applicable.

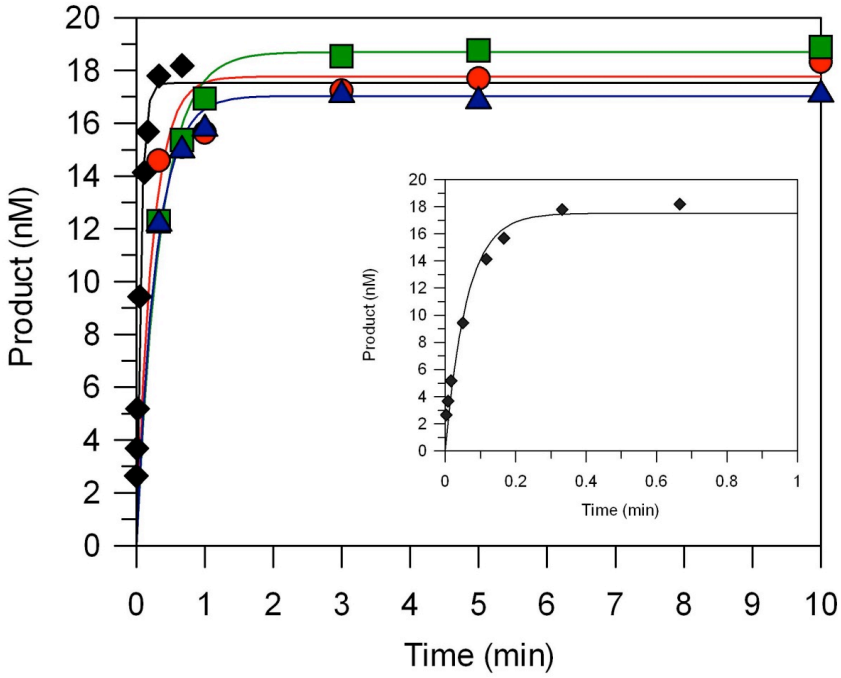


SI Figure 1. UvrABC Incision sites are the same with all lesions. Cleavage pattern of UvrABC reactions with 5'-labeled (A) or 3'-labeled (B) ^{32}P- labeled F, OG, Gh, Sp-containing 50 bp duplex, and Sp-amine adduct-containing 51 bp duplex. Four hour reaction time point incubated at 55˚C 2 nM DNA duplex with 20 nM UvrA, 100 nM UvrB, and 50 nM UvrC. Sites were the same for all duplexes. The Maxam-Gilbert sequencing G+ A reaction is shown (labeled G +A in Panel A) to illustrate cleavage site relative to the lesion.

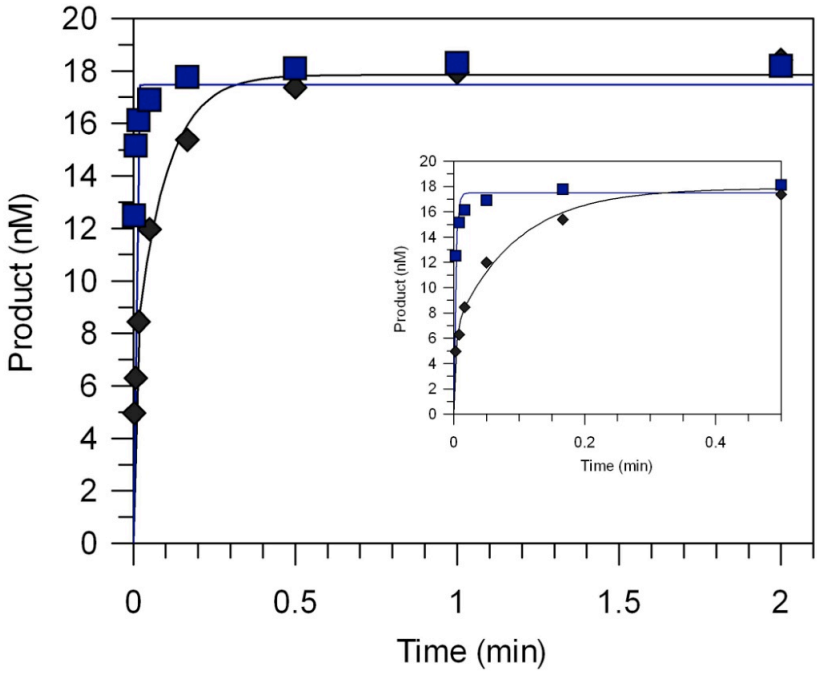




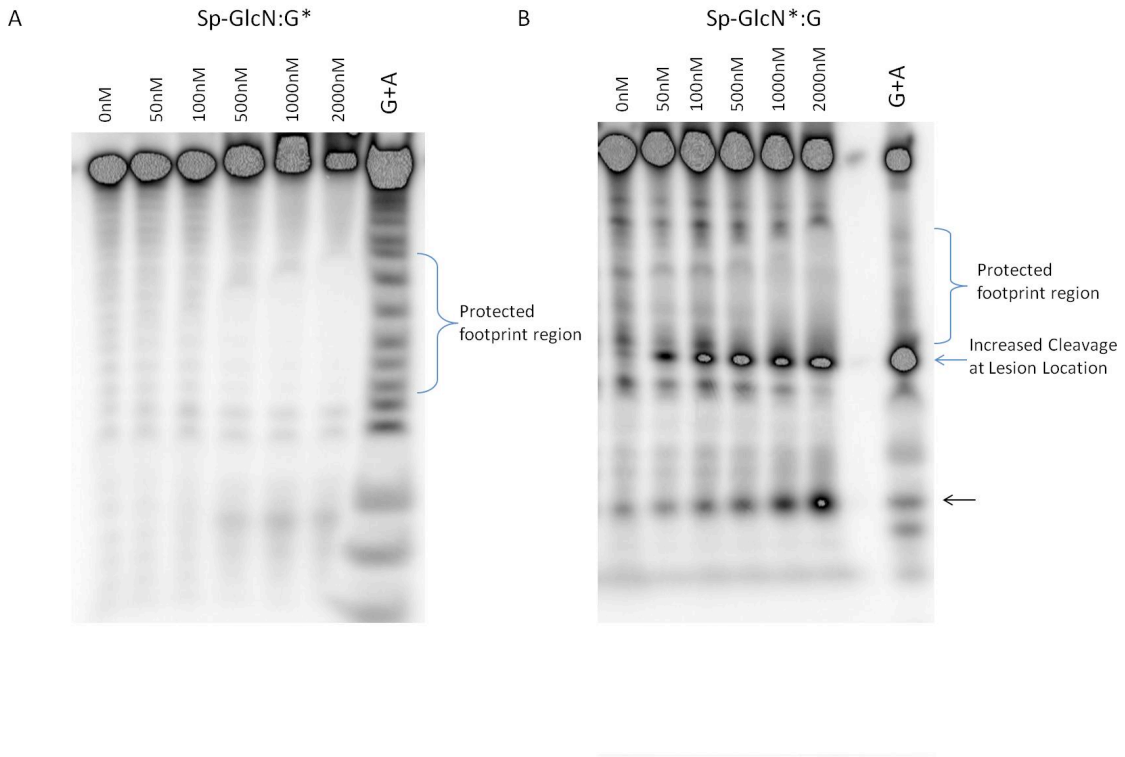
SI Figure 2. UvrA binding to Sp-amine adduct-containing DNA. **(A)** Representative storage phosphor autoradiograms of detect specific binding of UvrA to several Sp-amine adducts and F-containing duplex using EMSA. **(B)** Plot of the percent bound DNA determined from quantitation of the autoradiograms shown in panel A. The line represents the fitting of the data to a one-site binding isotherm for determination of relevant K_d.



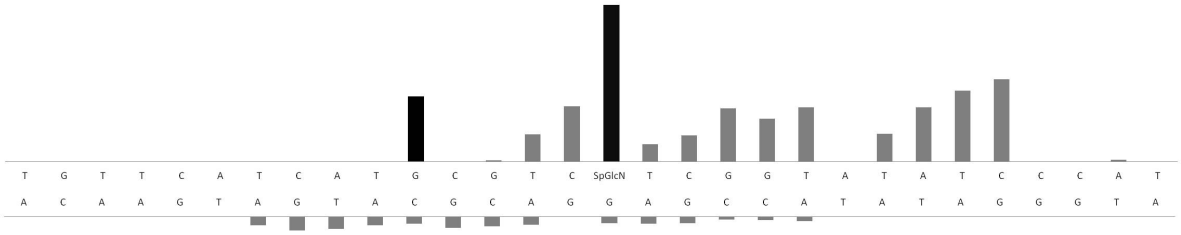
SI Figure 3. Sp-amine adduct removal by Fpg. Representative plot of extent of Fpg removal of OG and Sp-amine hydantoin lesions as a function of time under STO conditions from 30 bp duplex containing OG:C (♦), Sp-GlcN:C(•), Sp-GPRPGP:A (■), Sp-Lys:A(▲) fit to a single-exponential rate equation. Inset shows region of OG:C (♦) fit expanded to 1 minute time scale. All experiments were performed using 200 nM Fpg and 20 nM duplex DNA at 37˚C. Rate constants (min^{-1}) for the lesion removal determined from several experiments are as follows: Sp-Lys, $k_g = 3.0 \pm 0.4$; Sp-GlcN, $k_g = 4.4 \pm 0.2$; Sp-GPRPGP, $k_g = 2.9 \pm 0.1$. The rates for Fpg catalyzed removal of OG was similar to that previously reported in a different 30 bp sequence OG ($k_g = 14 \pm 1$ min^{-1}) and is similar in magnitude to that for removal of Sp ($k_g = 21 \pm 1$ min^{-1}) and Gh ($k_g = 21 \pm 1$ min^{-1}). [7,8]



SI Figure 4. Sp-amine lesion removal by NEIL1. Representative plot of the extent of edited NEIL1 catalyzed removal of the hydantoin lesions as a function of time under STO conditions using a 30 bp duplex containing Sp1:A(■) fit to a single-exponential rate equation and Sp-GPRP:A (◆) fit to a double-exponential rate model. Inset region expanded to 0.5 min time scale. All experiments were performed using 200 nM edited NEIL1 and 20 nM duplex DNA at 37 ˚C.



SI Figure 5. Footprinting experiments of Sp-GlcN with E3Q NEIL1. Storage phosphor autoradiogram of Fe•EDTA footprinting experiments performed with E3Q NEIL1 and Sp-GlcN:G containing DNA. (A) Guanine containing strand of the Sp-GlcN:G duplex 5'-^{32}P-labeled. (B) Sp-GlcN hydantoin lesion containing strand of the Sp-GlcN:G duplex 5'-^{32}P-labeled. Black arrow corresponds to G11 of lesion containing oligonucleotide which was also found to be hyper-reactive upon NEIL1 binding, which is consistent with dramatic distortion of the DNA duplex upon lesion recognition. Note that E3Q NEIL1 protection of the G-containing strand is modest compared to that of the lesion containing strand (see SI Figure 6).



SI Figure 6. Relative protection/hyperreactivity of specific nucleotides from hydroxyl radical footprinting experiments with E3Q NEIL1 and Sp-GlcN:G duplex. The histogram was generated by quantitation of the intensities of bands in the storage phosphor autoradiogram in the presence versus absence of E3Q NEIL1. The grey bars indicated the extent of protection at given nucleotides while the black bar indicates the extent of additional reactivity at the lesion site and G11 upon protein binding. The nucleotide with the maximum level of protection from hydroxyl radical cleavage is denoted by the highest bar. The absolute intensity of the cleavage at the Sp-GlcN nucleotide in the presence of 500 nM E3Q NEIL1 is approximately 2.4-fold increased relative to the control with no enzyme.