Turn-on DNA Damage Sensors for the Direct Detection of 8-Oxoguanine and Photoproducts in Native DNA

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Supplementary Information

Supplementary Methods

Cloning of DNA damage sensors. A plasmid encoding human oxoguanine glycosylase 1 (OGG1), pOTB7-hOGG1, was obtained from Open Biosystems (I.M.A.G.E. clone 3350168). Using standard techniques, the oxoguanine glycosylase 1 (OGG1) open reading frame was inserted into an existing EcoRV/NotI-digested pcDNA3.1(+) plasmid (Invitrogen) containing the C-terminal portion of luciferase (CLuciferase) followed by an 18 amino acid (AA) linker, yielding pcDNA3.1(+)-CLuciferase-18AA-OGG1. A K249Q mutation was introduced into the plasmid to create a catalytically inactive OGG1 mutant (Sequence S1). Mutagenesis was performed using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene) with designed mutagenic primers: K249Q top; 5'-CCTGGAGTGGGCACCCAGGTGGCTGACTGC and K249Q bottom; 5'-GCAGTCAGCCACCTGGGTGCCCACTCCAGG. To test the effect of linker length, a second construct was prepared with a 33 AA linker joining CLuciferase with OGG1(K249Q). This was achieved by PCR amplifying an existing 33 AA linker and ligating the insert in frame to the AgeI/EcoRV-digested pcDNA3.1(+) plasmid between CLuciferase and OGG1, yielding pcDNA3.1(+)-CLuciferase-33AA-OGG1 (Sequence S2). A final construct was generated by inserting the PCR-amplified OGG1(K249Q) gene in frame into a HindIII/BsiWI-digested pcDNA3.1/V5-His TOPO plasmid (Invitrogen) containing NLuciferase preceded by a 15 AA linker, generating pcDNA3.1-OGG1-15AA-NLuciferase (Sequence S3).

A plasmid encoding *Danio rerio* damaged-DNA binding protein 2 (DDB2) was purchased from Open Biosystems (I.M.A.G.E clone 7402966). The DDB2 gene was inserted into an EcoRV/NotI-digested vector that contained CLuciferase followed by an 18 AA linker, yielding pcDNA3.1(+)-CFluc-18AA-DDB2 (Sequence S4). A second construct was generated by inserting the gene encoding DDB2 into a HindIII/BsiWI-digested pcDNA3.1/V5-His TOPO plasmid containing NLuciferase preceded by a 15 AA linker, generating pcDNA3.1-DDB2-15AA-NLuciferase (Sequence S5).

Preparation of mRNA encoding the split-protein sensors. The following procedure applies to all split-protein sensors described in the manuscript. Genes encoding the split proteins were PCR amplified,

and the corresponding products served as templates for *in vitro* transcription using a T7 Ribomax RNA production kit (Promega) according to the manufacturer's suggestions. Generally, 3 μg of amplified DNA template was incubated at 37 °C for 3 h in the presence of 1× T7 transcription buffer, 7.5 mM rNTPs, and T7 enzyme mix. The transcribed RNA was purified over illustra ProbeQuant G-50 Micro Columns (GE Healthcare) and analyzed by agarose gel electrophoresis.

Monitoring MBD binding to damaged DNA. A methylation sensor was generated in which each half of split luciferase was attached to a methyl binding domain. The mRNA encoding the sensor was translated in a cell-free lysate system for generation of the corresponding proteins. A typical reaction was performed at 30 °C for 1.5 hours and contained 0.2 pmol of each mRNA transcript. Following translation, 1.25 μL of oxidized (30 μM $CuCl_2$ and 1 mM H_2O_2) or 2 hour UV-irradiated HeLa DNA (50 ng/μL) was added to 23.75 μL of the translation, and binding was allowed to occur for 1 hour at 4°C. Activity was monitored as a luminescent signal produced upon addition of luminescence reagent, where 20 μL of each translation was added to 80 μL of reagent. Luminescent readings were acquired 1 minute after mixing using a Turner TD-20e Luminometer with a 10 second integration time. Results are presented as the relative average of at least two independent DNA treatments. Within error, no change in luminescent signal was observed for the oxidized or UV-irradiated HeLa DNA as compared to the untreated equivalents (Figure S1).

Effect of hydrogen peroxide on 8-oxoguanine detection. To determine the effect of hydrogen peroxide concentration on DNA oxidation, 50 ng/μL HeLa DNA was treated with 0.5, 1, or 1.5 mM H_2O_2 in the presence of 15, 30, or 60 μM $CuCl_2$ for 30 min at RT, followed by quenching with 1 mM EDTA. Control reactions contained $CuCl_2$ in the absence of H_2O_2, followed by addition of 1 mM EDTA. The mRNAs encoding CLuciferase-OGG1 and MBD1-NLuciferase were translated in the Flexi Rabbit Reticulocyte Lysate System (Promega), consisting of 25 μL reactions prepared according to the manufacturer's instructions. A typical reaction was performed at 30 °C for 1.5 h and contained 0.2 pmol of each mRNA transcript. Following translation, 1.25 μL of 50 ng/μL HeLa DNA was added to 23.75 μL of the translation, and binding was allowed to occur for 1 h at 4 °C. Activity was monitored as a

luminescent signal produced upon addition of luminescence reagent, where 20 µL of each translation was added to 80 µL of reagent. Luminescent readings were acquired 1 min after mixing using a Turner TD-20e Luminometer with a 10 s integration time (Figure S2).

Effect of oxidation time on 8-oxoguanine detection. To assess the effect of oxidation time on induction of base damage, 50 ng/µL HeLa DNA was treated with 60 µM $CuCl_2$ and 1 mM H_2O_2 for 0.5, 1, 2, 3, 4, or 5 h, followed by quenching with 1 mM EDTA. Control reactions contained 60 µM $CuCl_2$ in the absence of H_2O_2, followed by addition of 1 mM EDTA. The HeLa DNA that was oxidized or non-oxidized for 0.5, 1, 2, or 3 h was analyzed using agarose gel electrophoresis (Figure S3). The mRNAs encoding CLuciferase-OGG1 and MBD1-NLuciferase were translated in a reticulocyte lysate system (Luceome Biotechnologies), consisting of 25 µL reactions with 0.2 pmol of each mRNA transcript. Following translation, 1.25 µL of 50 ng/µL HeLa DNA was added to 23.75 µL of the translation, and binding was allowed to occur for 1 h at 4 °C. Activity was monitored as a luminescent signal produced upon addition of luminescence reagent, where 20 µL of each translation was added to 80 µL of reagent. Luminescent readings were acquired 1 min after mixing using a Turner TD-20e Luminometer with a 10 s integration time (Figure S3).

Hydrolysis of HeLa DNA. HeLa DNA was exposed to oxidizing conditions consisting of treatment with 1 mM H_2O_2 in the presence of 60 µM $CuCl_2$ for 10 min at RT, followed by quenching with 1 mM EDTA, in a total volume of 5 mL. A non-oxidized sample was simultaneously prepared in the absence of H_2O_2. The samples were concentrated to ~ 1 mL using Amicon Ultra-15 centrifugal filters (Millipore) with a 3000 kDa molecular weight cut-off (MWCO). The concentrated samples were then dialyzed in 0.5-3 mL Slide-A-Lyzer dialysis cassettes (Pierce) with a 3500 MWCO against 850 mL hydrolysis buffer (10 mM ammonium acetate, pH 5.3) for 2 h at 4 °C. The samples were collected and further concentrated to ~ 40 µL using Amicon Ultra-0.5 centrifugal filters with a 3000 MWCO. Typical yields were > 1500 ng/µL DNA. The DNA (~ 60 µg) was heat denatured at 99 °C for 10 min followed by immediate cooling on ice. Nuclease P1 (Sigma) was added at 0.1 U/µg DNA in hydrolysis buffer and incubated at 50 °C for 1 h. Following hydrolysis, Antarctic Phosphatase (NEB) was added at 0.5 U/µg

DNA in phosphatase buffer (50 mM Bis-Tris-Propane-HCl, pH 6.0, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$), and incubated at 37 °C for 2 h. To isolate the digested nucleosides from the enzymes, each sample was applied to an Amicon Ultra-0.5 centrifugal filter with a 10000 MWCO. The total nucleoside concentrations of the oxidized and non-oxidized samples were determined by absorbance at 260 nm.

ELISA detection of 8-oxoguanosine. Quantification of 8-oxoguanine lesions in oxidized HeLa DNA was performed using an HT 8-oxo-dG ELISA kit (Trevigen) according to the manufacturer's instructions, where absorbance at 450 nm is inversely proportional to the 8-oxoguanosine concentration. Dilutions of the standard 8-oxoguanosine (10.8, 3.6, 1.2, and 0 ng/mL) were evaluated in the ELISA along with dilutions of the hydrolyzed oxidized and non-oxidized HeLa DNA (20, 10, and 5 μM). All samples were analyzed in duplicate. Prior to absorbance readings at 450 nm, samples were diluted as needed to obtain values < 1.0. The background absorbance from the wells lacking the ELISA secondary antibody was subtracted from the absorbance readings corresponding to the 8-oxoguanosine standards or HeLa hydrolysates. A standard linear curve was generated by plotting absorbance at 450 nm as a function of 8-oxoguanosine standard concentration (Figure S4). The three concentrations of oxidized and non-oxidized HeLa nucleosides were compared to the standard curve to yield values for the 8-oxoguanosine content in the hydrolysate (Figure S4). No absorbance above background was observed from any of the hydrolyzed non-oxidized HeLa DNA (data not shown). A value of 7.2 ng/mL (25 nM) 8-oxoguanosine was determined for the 20 μM hydrolyzed oxidized HeLa, indicating ~1200 8-oxoguanosines per 10^6 nucleosides.

DNA and protein sequences of DNA damage sensors

Sequence S1. CLuciferase-18AA-OGG1(K249Q). CLuciferase is red, the 18 amino acid linker is black, and OGG1 is blue. The K249Q mutation is colored orange.

```
atgggtacctccggttatgtaaacaatccggaagcgaccaacgccttgattgacaaggatggatggcta
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 H  S  G  D  I  A  Y  W  D  E  D  E  H  F  F  I  V  D  R  L  K  S  L
attaagtacaaaggctatcaggtggctcccgctgaattggaatccatcttgctccaacaccccaacatc
 I  K  Y  K  G  Y  Q  V  A  P  A  E  L  E  S  I  L  L  Q  H  P  N  I
ttcgacgcaggtgtcgcaggtcttcccgacgatgacgccggtgaacttcccgccgccgttgttgttttg
 F  D  A  G  V  A  G  L  P  D  D  D  A  G  E  L  P  A  A  V  V  V  L
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 E  H  G  K  T  M  T  E  K  E  I  V  D  Y  V  A  S  Q  V  T  T  A  K
aagttgcgcggaggagttgtgtttgtggacgaagtaccgaaaggtcttaccggaaaactcgacgcaaga
 K  L  R  G  G  V  V  F  V  D  E  V  P  K  G  L  T  G  K  L  D  A  R
aaaatcagagagatcctcataaaggccaagaagggcggaaagatcgccgtgaccggtggcggtgggggt
 K  I  R  E  I  L  I  K  A  K  K  G  G  K  I  A  V  T  G  G  G  G  G
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 P  A  L  W  A  S  I  P  C  P  R  S  E  L  R  L  D  L  V  L  P  S  G
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 T  P  D  E  L  E  A  V  R  K  Y  F  Q  L  D  V  T  L  A  Q  L  Y  H
cactggggttccgtggactcccacttccaagaggtggctcagaaattccaaggtgtgcgactgctgcga
 H  W  G  S  V  D  S  H  F  Q  E  V  A  Q  K  F  Q  G  V  R  L  L  R
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 Q  D  P  I  E  C  L  F  S  F  I  C  S  S  N  N  N  I  A  R  I  T  G
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 M  V  E  R  L  C  Q  A  F  G  P  R  L  I  Q  L  D  D  V  T  Y  H  G
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 F  P  S  L  Q  A  L  A  G  P  E  V  E  A  H  L  R  K  L  G  L  G  Y
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 F  S  A  D  L  R  Q  -
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Sequence S2. CLuciferase-33AA-OGG1(K249Q). CLuciferase is red, the 33 amino acid linker is black, and OGG1 is blue. The K249Q mutation is orange.

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H S G D I A Y W D E D E H F F I V D R L K S L
attaagtacaaaggctatcaggtggctcccgctgaattggaatccatcttgctccaacaccccaacatc
I K Y K G Y Q V A P A E L E S I L L Q H P N I
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F D A G V A G L P D D D A G E L P A A V V V L
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E H G K T M T E K E I V D Y V A S Q V T T A K
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K L R G G V V F V D E V P K G L T G K L D A R
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K I R E I L I K A K K G G K I A V T G G G G G
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S G G G G S G G G G S G G G G S G G G G S G G
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G S D I G S E G H R T L A S T P A L W A S I P
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C P R S E L R L D L V L P S G Q S F R W R E Q
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S P A H W S G V L A D Q V W T L T Q T E E Q L
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H C T V Y R G D K S Q A S R P T P D E L E A V
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R K Y F Q L D V T L A Q L Y H H W G S V D S H
ttccaagaggtggctcagaaattccaaggtgtgcgactgctgcgacaagaccccatcgaatgcctttc
F Q E V A Q K F Q G V R L L R Q D P I E C L F
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S F I C S S N N N I A R I T G M V E R L C Q A
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F G P R L I Q L D D V T Y H G F P S L Q A L A
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G P E V E A H L R K L G L G Y R A R Y V S A S
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E A H K A L C I L P G V G T Q V A D C I C L M
gccctagacaagccccaggctgtgcccgtggatgtccatatgtggcacattgcccaacgtgactacagc
A L D K P Q A V P V D V H M W H I A Q R D Y S
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W H P T T S Q A K G P S P Q T N K E L G N F F
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R S L W G P Y A G W A Q A V L F S A D L R Q -

Sequence S3. OGG1(K249Q)-15AA-NLuciferase. NLuciferase is red, the 15 amino acid linker is black, and OGG1 is blue. The K249Q mutation is orange.

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S E L R L D L V L P S G Q S F R W R E Q S P A
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H W S G V L A D Q V W T L T Q T E E Q L H C T
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V Y R G D K S Q A S R P T P D E L E A V R K Y
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F Q L D V T L A Q L Y H H W G S V D S H F Q E
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V A Q K F Q G V R L L R Q D P I E C L F S F I
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C S S N N N I A R I T G M V E R L C Q A F G P
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R L I Q L D D V T Y H G F P S L Q A L A G P E
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V E A H L R K L G L G Y R A R Y V S A S A R A
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I L E E Q G G L A W L Q Q L R E S S Y E E A H
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K A L C I L P G V G T **Q** V A D C I C L M A L D
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K P Q A V P V D V H M W H I A Q R D Y S W H P
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W G P Y A G W A Q A V L F S A D L R Q **G I P Y**
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A S R G G G S S G G G E D A K N I K K G P A P
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F Y P L E D G T A G E Q L H K A M K R Y A L V
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P G T I A F T D A H I E V D I T Y A E Y F E M
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E N S L Q F F M P V L G A L F I G V A V A P A
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S K K G L Q K I L N V Q K K L P I I Q K I I I
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G F N E Y D F V P E S F D R D K T I A L I M N
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S S G S T G L P K G V A L P H R T A C V R F S

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 T  L  F  S  F  F  A  K  S  T  L  I  D  K  Y  D  L  S  N  L  H  E  I
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 P  E  A  T  N  A  L  I  D  K  D  G  -
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Sequence S4. CLuciferase-18AA-DDB2. CLuciferase is red, the 18 amino acid linker is black, and DDB2 is green.

```
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```

Sequence S5. DDB2-15AA-NLuciferase. NLuciferase is red, the 15 amino acid linker is black, and DDB2 is green.

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Q S I H A Q L R Q C L Q E P F I R S L K S Y K
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 F H H G F G M F T T L G Y L I C G F R V V L M
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 Y R F E E E L F L R S L Q D Y K I Q S A L L V
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 I A S G G A P L S K E V G E A V A K R F H L P
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aatccggaagcgaccaacgccttgattgacaaggatggatga
 N P E A T N A L I D K D G -

Figures and Tables

Table S1. Sequences of methylated DNA target oligonucleotides. Zinc finger binding sites are in blue.

bp	Sequence
21	GCGTAmCGCGCCCACGCCACCG
	CGCATGmCGCGGGTGCGGTGGC
22	GCGTAmCGTCGCCCACGCCACCG
	CGCATGmCAGCGGGTGCGGTGGC
23	GCGTAmCGTACGCCCACGCCACCG
	CGCATGmCATGCGGGTGCGGTGGC
24	GCGTAmCGTAGCGCCCACGCCACCG
	CGCATGmCATCGCGGGTGCGGTGGC
31	GCGTAmCGTAGGACGATACGCCCACGCCACCG
	CGCATGmCATCCTGCTATGCGGGTGCGGTGGC

Figure S1

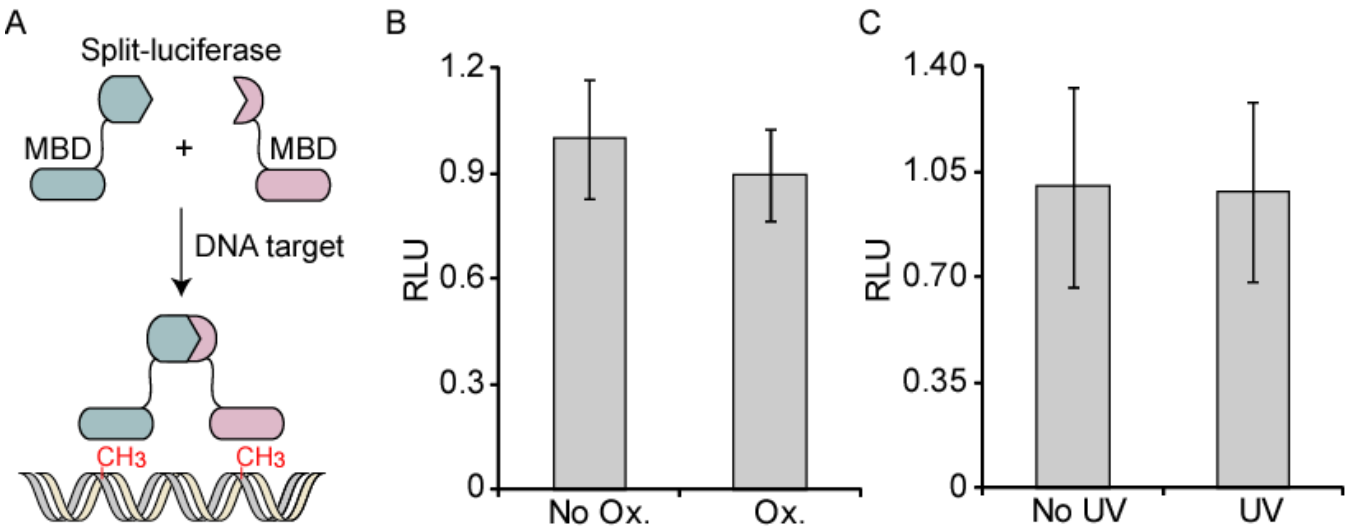


Figure S1. Methylation detection of damaged DNA. (A) Each half of split luciferase is attached to a methyl binding domain, thus luminescence reports on the total density of accessible methylated CpG sites. (B) MBD-based sensors were incubated with 50 ng of oxidized (Ox.) vs. non-oxidized (No Ox.) HeLa DNA, followed by luminescence readings. (C) MBD-based sensors were incubated with 50 ng HeLa DNA exposed to 2 hours UV or no UV, followed by luminescence readings. Results are presented as the luminescence readings of two independent trials, normalized to the maximum signal. It appears that neither the oxidation conditions nor the UV irradiation results in a detectable decrease in MBD binding to methylated CpG sites.

Figure S2

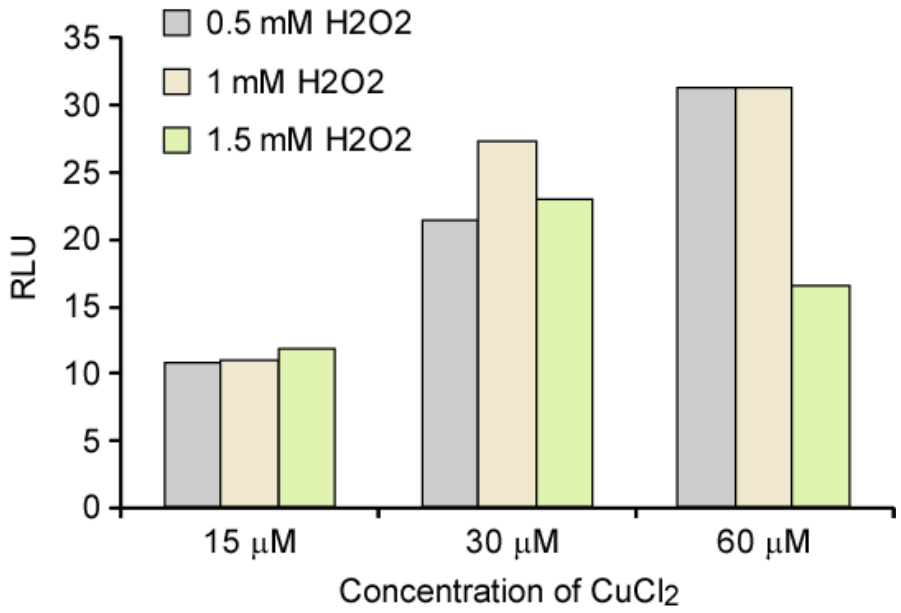


Figure S2. Effect of hydrogen peroxide concentration on DNA oxidation. HeLa DNA was treated for 30 min with various concentrations of H_2O_2 (0.5, 1, and 1.5 mM) using three different $CuCl_2$ concentrations (15, 30, and 60 μM). The CLuciferase-OGG1 and MBD1-NLuciferase sensor pair was incubated with 50 ng of each of the 9 oxidized HeLa targets or their non-oxidized equivalents. Results are presented as the signal in the presence of the oxidized target relative to the non-oxidized target. At a given concentration of $CuCl_2$, the concentration of H_2O_2 does not appear to have a significant effect, particularly for the 15 μM and 30 μM $CuCl_2$ samples. At 60 μM $CuCl_2$, a decrease in signal is observed at the highest concentration of H_2O_2 tested (1.5 mM).

Figure S3

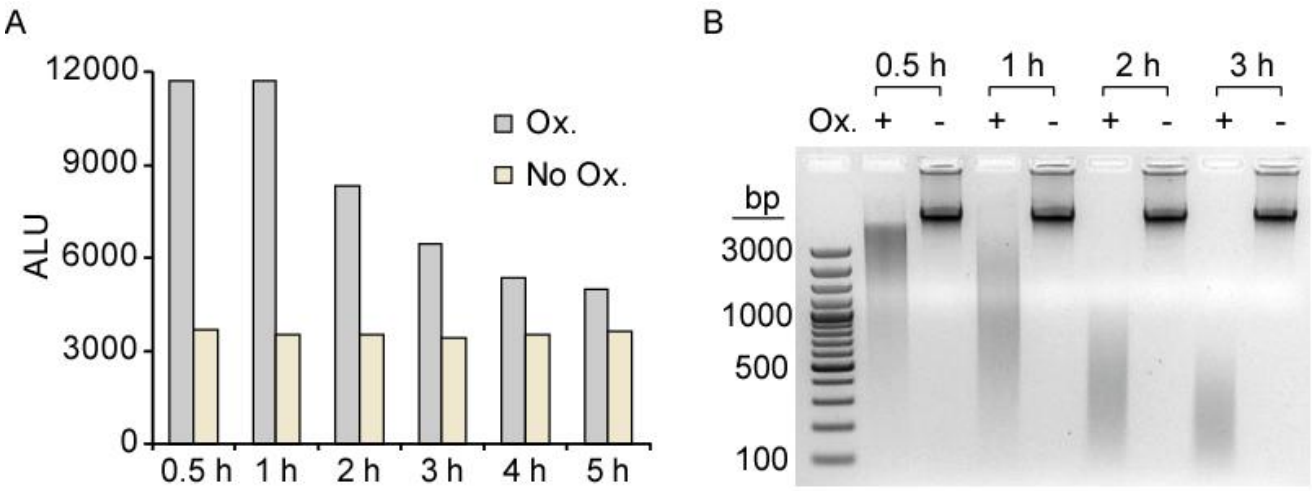


Figure S3. Effect of oxidation time on 8-oxoguanine sensor recognition. (A) HeLa DNA was exposed to 60 μM $CuCl_2$ and 1 mM H_2O_2 for various times (0.5, 1, 2, 3, 4, and 5 h). The CLuciferase-OGG1 and MBD1-NLuciferase sensor pair was incubated with 50 ng of each of the 6 oxidized HeLa targets or their non-oxidized equivalents. Results are presented as the luminescence readings obtained in arbitrary units (ALU). (B) The HeLa DNA was analyzed using agarose gel electrophoresis to determine the extent of target degradation. Only the first four time points are presented. Oxidation conditions ($CuCl_2$ + H_2O_2) are indicated with a plus sign (+), while non-oxidizing conditions ($CuCl_2$ only) are indicated with a minus sign (–). The degraded oxidized DNA observed in (B) appears to directly correlate with the decreased signal observed in (A), thus providing an explanation for a lack of sensor recognition at longer oxidation times.

Figure S4

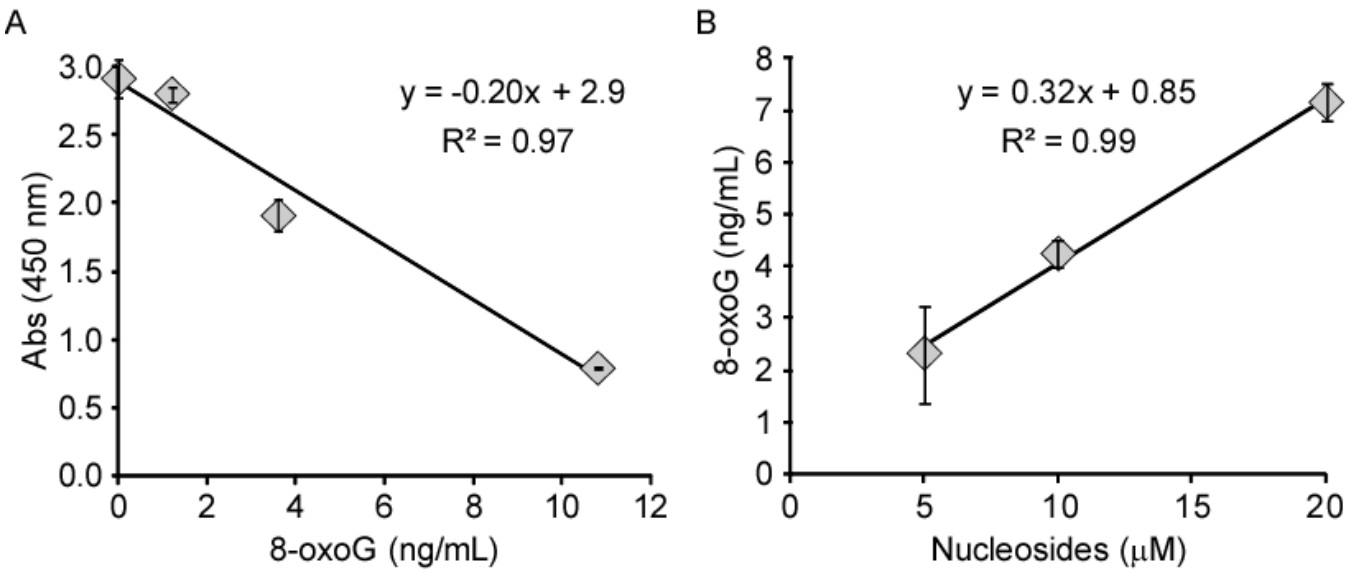
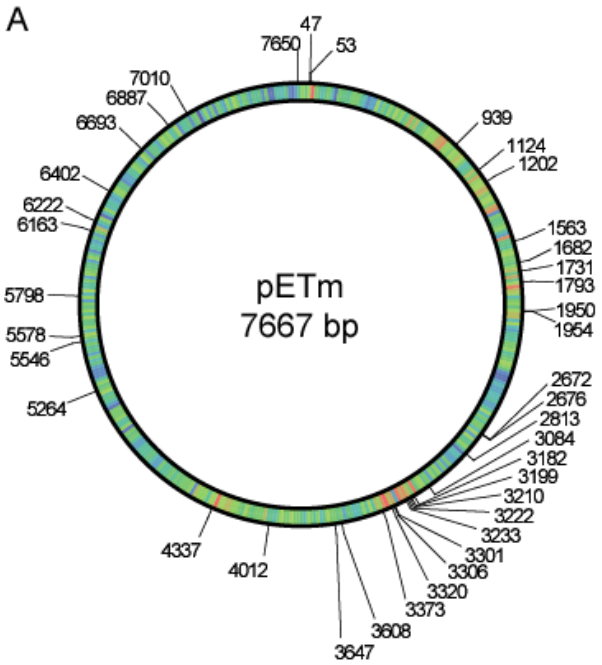


Figure S4. ELISA evaluation of 8-oxoguanosine. (A) A standard curve for quantifying 8-oxoguanosine nucleosides was prepared using a commercially available ELISA kit along with 8-oxoguanosine standard samples at 10.8, 3.6, 1.2, and 0 ng/mL. (B) Oxidized HeLa DNA was hydrolyzed to generate nucleosides, and three different concentrations (20, 10, and 5 μM) were evaluated in the 8-oxoguanosine ELISA. Using the standard curve, the ELISA output was extrapolated to provide an estimate of the 8-oxoguanosine content of each sample. The results for the oxidized HeLa hydrolysate are the average of results acquired from two independent trials. The non-oxidized HeLa hydrolysate did not provide any measurable signal in the ELISA assay.

Figure S5



B

Expected products (bp)
927
886
718
640
365
361
325
291
282
271
235
220

Figure S5. MseI digestion of the methylated plasmid target. (A) The sites of MseI cleavage (5'-TTAA) of the methylated plasmid, pETm, are indicated. (B) A list of the expected size of MseI cleavage products greater than 200 base pairs (bp) is presented.

Figure S6

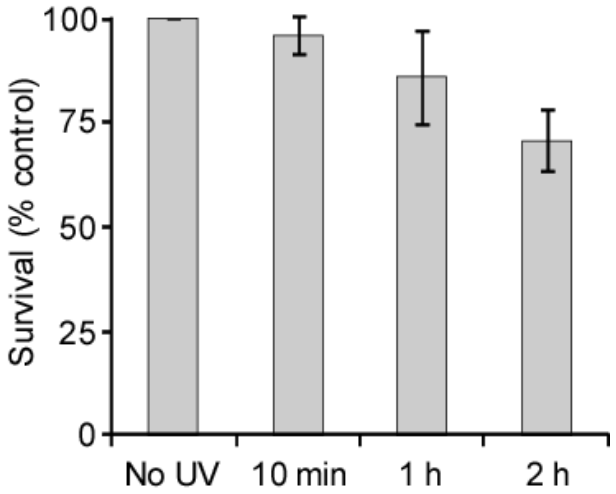


Figure S6. Cell survival following UV irradiation. HeLa cells were irradiated with UVC light with a peak output at 254 nm for 10 min, 1 h, or 2 h, followed by assessment of cell viability using the methylthiazolyl tetrazolium (MTT) assay. Results are presented as percent survival relative to the No UV control. The 10 min exposure time does not appear to adversely affect cell viability.

Figure S7

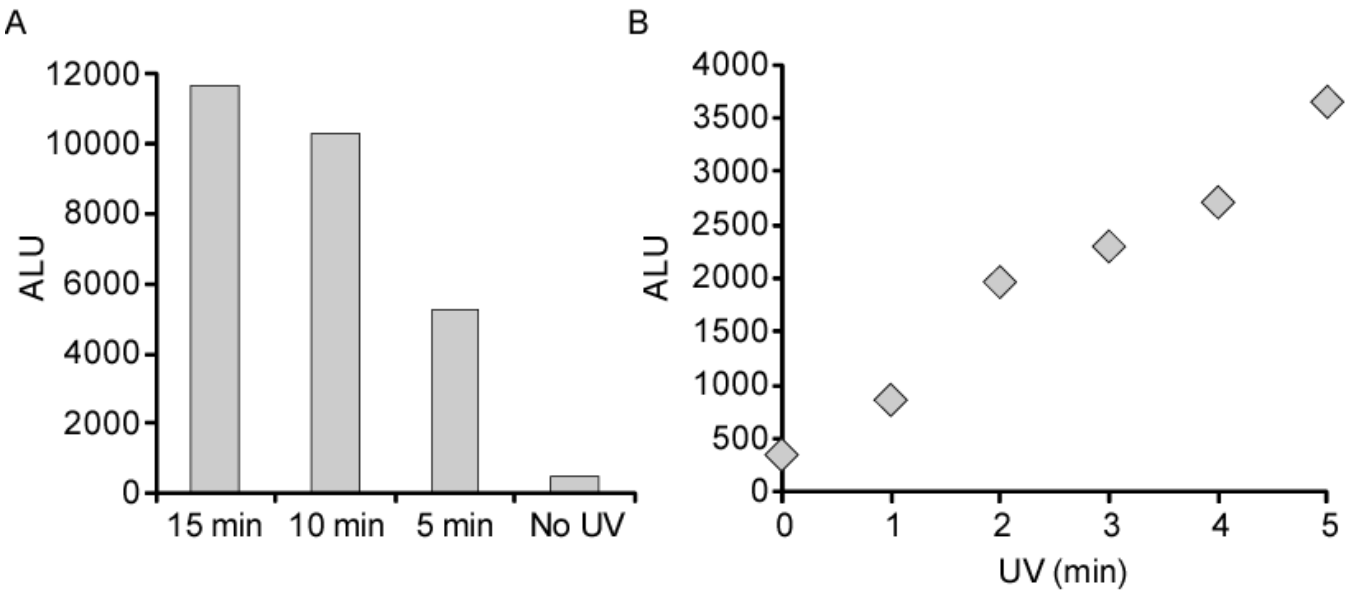


Figure S7. UV damage detection as a function of UV exposure time. HeLa cells were irradiated with UVC light with a peak output at 254 nm for the indicated times, followed by isolation of genomic DNA. The CLuciferase-DDB2 and MBD1-NLuciferase sensor pair was incubated with 50 ng of DNA from cells exposed to UV light or No UV. Results are presented as the luminescence readings obtained in arbitrary units (ALU).