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Supplementary Information for

Histone Tails Decrease N7-Methyl-2'-Deoxyguanosine Depurination and Yield DNA-Protein Crosslinks in Nucleosome Core Particles and Cells

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Supplementary text
Figs. S1 to S10
References for SI reference citations

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General Methods. Oligonucleotides used to generate 145 nt DNA used in NCPs were synthesized on an Applied Biosystems Incorporated 394 oligonucleotide synthesizer. Oligonucleotide synthesis reagents were from Glen Research. Oligonucleotides used for PCR amplification were purchased from Integrated DNA Technologies (IDT). T4 polynucleotide kinase (PNK), T4 DNA ligase, human alkyladenine DNA glycosylase (hAAG), formamidopyrimidine DNA glycosylase (Fpg), DNase I, and proteinase K were purchased from New England Biolabs (NEB). Sequenase was obtained from Affymetrix. Dimethyl sulfate (DMS), methyl methanesulfonate (MMS), and dGTP were purchased from Sigma. γ-^{32}P-ATP was from Perkin Elmer. Antibodies were purchased from Abcam. TMTsixplex™ isobaric label reagent was purchased from Thermo Fisher Scientific. Expression and purification of histone proteins, as well as refolding and purification of the histone octamers were performed using reported procedures (1). DNA containing the photolabile AP precursor was prepared as previously described (2). DNase I footprinting of NCPs was carried as previously described (3). MdGTP was characterized by MALDI-TOF MS and that of mutant histone proteins were determined by UPLC-MS. Radiolabeled oligonucleotide quantification was carried out using a Molecular Dynamics Phosphorimager 840 equipped with ImageQuant TL software. Chemiluminescence signals in western blotting experiments were detected by a Typhoon imager (GE Healthcare). All experiments were performed in clear siliconized tubes (Bio Plas Incorporated). Denaturing PAGE gels (10%) (40 x 32 x 0.04 cm) were run at room temperature under limiting power (50 W), and 10% SDS PAGE gels (20 x 16 x 0.1 cm) were run at 4°C under limiting power (7 W) until the bromophenol blue migrated to the bottom of the gel.

Synthesis of MdGTP. dGTP (100 μL, 10 μmol) was dried in a speed vacuum and the residue was resuspended in citrate buffer (pH 4.6, 500 mM, 100 μL) to a final concentration

of 0.1 M. DMS (6 μL) was added and the reaction mixture was vortexed at room temperature for 20 min. Additional DMS (6 μL) was added and the reaction was continued for another 70 min. The reaction mixture was diluted with 900 μL water, injected to a 1 mL Mono-Q column, and eluted at 4°C with HEPES buffer (pH 6.8, 50 mM) at 1 mL/min in a gradient of 0-500 mM NaCl over 60 min. MdGTP eluted at 12 min (100 mM NaCl), while unreacted dGTP eluted at 28 min (233 mM NaCl). The concentration of MdGTP was determined by UV-Vis. The λ_{max} increases from 252 nm to 258 nm following methylation, and the extinction coefficient at 258 nm (ε_{258}) of the methylated material is 8.9 $mmol^{-1}cm^{-1}$ (4). The typical yield of MdGTP within this scale is ~2 μmol (20%). The molecular weight was confirmed by MALDI-TOF MS using the negative reflectron mode and anthranilic acid/nicotinic acid (AA/NA, 1:1) as matrix (5). MdGTP ($C_{11}H_{17}N_5O_{13}P_3$): calculated 520.21, found 520.25 ($M\text{-}H^+$). MdGTP was stored in the elution buffer (50 mM HEPES, pH 6.8, 100 mM NaCl) at -80 °C.

Preparation of Oligonucleotides Containing MdG at 3′-termini. Chemically synthesized oligonucleotides (Fig. S1A) were enzymatically phosphorylated at their 5′-termini in a 50 μL reaction containing 4 nmol DNA, 1 x PNK buffer (70 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 5 mM DTT), 2 mM ATP and 50 units T4 PNK at 37 °C for 4 h. T4 PNK was inactivated by incubating at 65 °C for 30 min. The phosphorylated oligonucleotides were combined with the appropriate scaffolds (6 nmol) (Fig. S1A) in 1 x Sequenase buffer (40 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, 5 mM DTT and 50 mM NaCl) and hybridized by heating at 90 °C for 2 min, followed by slow cooling to room temperature. MdGTP (470 μM) and Sequenase (13 units) were added and the mixture (~150 μL) was incubated at room temperature for 2 h. The reaction mixture was mixed

with 30 μL 95% formamide and purified by a 20% denaturing PAGE gel (40 x 32 x 0.1 cm). The gel was run at 4 °C under limiting power (50 W) until the bromophenol blue (MdG_{123}, MdG_{158}, MdG_{89}) or xylene cyanol (MdG_{73}) migrated to the bottom of the gel. The product band was excised from the gel and the DNA was eluted overnight in 3 mL elution buffer (0.2 M NaCl and 1 mM EDTA) at 4 °C. The slurry was pelleted via a brief spin at 3000 g. The supernatant was filtered using a polyprep-column (BioRad) and the DNA was desalted using a 1 mL C18 Sep-pak desalting column. The eluted DNA (~2 nmol) was flash frozen in liquid nitrogen and dried by speed vacuum at low temperature, and finally resuspended in 20 μL water.

Preparation of 145 mer DNA Containing Site-specific MdG. All oligonucleotides, except those containing MdG which was already phosphorylated, and the 5'-terminal fragment (Fig. S1B), were treated with T4 PNK (50 units) in a 20 μL reaction containing 1.5 nmol DNA, 1 x T4 DNA ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP) at 37 °C for 4 h. T4 PNK was inactivated by incubating at 65 °C for 30 min. The phosphorylated oligonucleotides (except those containing MdG) were combined with the unphosphorylated oligonucleotide (1.5 nmol, Fig. S1B) and scaffolds (2 nmol, Fig. S1B). The mixture was heated at 95 °C for 2 min, followed by chilling on ice. The 5'-phosphorylated MdG containing oligonucleotide (1.5 nmol) was added and the mixture was incubated at room temperature for 2 h. T4 DNA ligase (3600 units) was added and the mixture (~150 μL) was incubated overnight at 16 °C. The reaction was phenol extracted (equal volume). The supernatant was mixed with 30 μL 95% formamide and purified by an 8% denaturing PAGE gel (20 x 16 x 0.1 cm). The gel was run at 4 °C under limiting power (10 W) until the xylene cyanol migrated to the bottom of the gel. The product

band was excised from the gel and the DNA was eluted overnight in 1 mL elution buffer (0.2 M NaCl and 1 mM EDTA) at 4 °C. The slurry was pelleted via a brief spin at 3000 g. The supernatant was filtered using a polyprep-column (BioRad) and the DNA was concentrated and buffer exchanged extensively with water using a 10K Amicon Ultra membrane at 4 °C. The DNA (~350 pmol) in H_2O was stored in -80 °C until use.

Characterization of the MdG Containing 601 DNA. The MdG containing 145 nt DNA was 5'-^{32}P labeled in a 25 μL reaction containing 20 pmol DNA, 1 x PNK buffer (70 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 5 mM DTT), 30 μCi γ-^{32}P-ATP and 15 units T4 PNK at room temperature for 2 h. Free γ-^{32}P-ATP was removed by G-50 sephadex column. A 10 μL reaction containing 25 pmol complementary DNA, 10 mM phosphate buffer, pH 7.2, and 100 mM NaCl was heated at 95 °C for 1 min and chilled on ice. The 5'-^{32}P labeled MdG containing DNA was then added and incubated at room temperature for 2 h to generate MdG containing 145 bp DNA. To determine the MdG content, DNA (14 fmol) was treated with hAAG (5 units) in a 10 μL reaction containing 20 mM Tris-HCl, pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$ and 0.1% Triton X-100 at 37 °C for 1 h. NaOH (1.2 μL, 1 M) was then added and the mixture was incubated at 37 °C for 30 min. The reaction mixture was then neutralized by HCl. To determine the AP content, DNA (14 fmol) was treated with 100 mM NaOH in a 10 μL reaction at 37 °C for 30 min. The reaction mixture was then neutralized by HCl. To determine the MeFapy•dG content, DNA (14 fmol) was treated with Fpg (5 units) in a 10 μL reaction containing 10 mM Bis-Tris-propane-HCl, pH 7.0, 10 mM $MgCl_2$, 1 mM DTT and 0.1 mg/ml BSA at 37 °C for 1 h. After each treatment, the DNA was ethanol precipitated, resuspended in 95% formamide and analyzed by 10% denaturing PAGE.

Generation of Mutant H2A and H2B Proteins. The DNA fragments of mutant H2A and H2B were amplified from pET3a-H2A or pET3a-H2B (6) by PCR using the following primers: H2A 1-15 Del.-Forward 5'-CGCGCTAAGCATATGACTCGCTCATCTCGGGCTGGG; H2A 1-15 Del.-Reverse 5'-GTTAGCAGCCGGATCCGATCACTTGCTCTTGGCCG; H2B 1-23 Del.-Forward 5'-CAGAAGAAACATATGAAAAAGCGCAGGAAGACAAGGAAG; H2B 1-31 Del.-Forward 5'-AGGAAGACACATATGGAGAGTTATGCCATTTACGTGTAC; H2B 1-23 Del.-K24,K25,K28,K31A-Forward 5' –AAGAAACATATGGCAGCACGCAGGGCAACAAGGGCA GAGAGTTATGCCATTTAC; H2B 1-23 Del.-R26,R27,R30,A-Forward 5'-AAGAAACATATGAAAAAGGCAGCAAAGACAGCAAAGGAGAGTTATGCCATT TAC; H2B-Reverse 5'-ATCCGGCAGGGATCCTCACTTGGCGCTGGTGTACTTG. The amplified DNA and pET3a plasmids were digested by NdeI and BamHI and the fragments were ligated using the Quick Ligation™ Kit (NEB) to generate the plasmids expressing the mutant H2A and H2B proteins. Expression and purification of the mutant histone proteins were performed using the reported procedures used for purification of the wild type histone proteins (1). The correct molecular weights were confirmed by UPLC-MS.

Reconstitution of Nucleosome Core Particles. Salmon sperm DNA (~20 µg, 31 nmol base pairs) and 5'-^{32}P labeled 145 bp DNA (4 pmol) were combined in a siliconized tube to a final volume of 20 µL containing 2 M NaCl and 0.1 mg/ml BSA. Various amounts of histone octamer (0.8-1.2 equivalent of total DNA) were added to the DNA sample. The mixture was incubated at 4 °C for 30 min before a series of dilutions at 4 °C using a buffer

containing 10 mM HEPES, pH 7.5, 1 mM EDTA and 0.1 mg/ml BSA. Dilution #: volume of buffer added in μL, incubation time in min-1: 24, 60; 2: 12, 60; 3: 12, 60; 4: 20, 30; 5: 20, 30; 6: 40, 30; 7: 100, 30; 8: 200, 30. After the final dilution (total volume ~448 μL), any precipitate was pelleted via a brief spin at 13,000 g at 4 °C. The solution was then transferred to a fresh siliconized tube. A small aliquot (~5 μL) was mixed with 3 μL 40% sucrose and analyzed by a 6% native PAGE gel (10 x 8 x 0.15 cm) to determine the reconstitution efficiency. The gel was run at 4 °C under limiting power (3 W) until the bromophenol blue migrated to the bottom of the gel. NCPs with reconstitution efficiency higher than 95% were immediately used for reactions.

Determination of AP and DPC Yields from MdG Containing NCPs. NCPs (140 μL, ~2 million cpm) were incubated at 37 °C. Aliquots (14 μL) were removed at appropriate times, immediately frozen in dry ice, and stored at -80 °C until the final time point. To determine the amounts of AP, half of the NCP samples were treated with 100 mM NaOH at 37 °C for 30 min, followed by neutralization with HCl and treatment with proteinase K (8 units) at room temperature for 30 min. The cleaved DNA products were analyzed by 10 % denaturing PAGE. To determine the amounts of DPCs, the other half of the NCP samples were analyzed by 10% SDS PAGE at 4 °C.

Analysis of the DNA Within Isolated DPCs from NCPs Containing AP or MdG. NCPs containing AP precursor at position 123 (~10,000 cpm) were photolyzed at 350 nm for 15 min and then incubated at 37 °C for the duration of the time course experiment. NCPs containing MdG_{89} or MdG_{123} (~4 million cpm) were incubated at 37 °C and aliquots were removed at the appropriate times. All samples were analyzed by 10% SDS PAGE at 4 °C. The DPC bands were excised from the gel and eluted at 4 °C for 12 h with 500 μL buffer

containing 10 mM HEPES, pH 7.5, 0.2 M NaCl and 0.1% SDS. The slurry was pelleted via a brief spin at 3000 g at 4 °C. The supernatant was filtered using a polyprep-column (BioRad) and the DPCs were concentrated and buffer exchanged extensively at 4 °C with 10 mM HEPES buffer (pH 7.5) using a 10K Amicon Ultra membrane. The isolated DPCs were treated with proteinase K (8 units) at room temperature for 5 min, and the DNA samples were analyzed by 10 % denaturing PAGE.

Determination of the Decomposition Rate of MdG DPCs. NCPs containing MdG_{123}were incubated at 37 °C for 60 h, followed by DPC isolation as described above. The isolated DPCs (~30,000 cpm) were incubated at 37 °C. Aliquots (~3000 cpm) were removed at the appropriate times and analyzed by 10% SDS PAGE at 4 °C.

DNA Analysis of the Decomposed MdG DPCs. MdG_{123} DPCs (~20,000 cpm) were decomposed by incubating at 37 °C for 24 h. The amounts of MdG, AP and MeFapy•dG in the released DNA were determined by the battery of chemical and/or enzymatic treatments described above to characterize the MdG containing DNA.

Determination of the Protein(s) in MdG_{123} DPCs by Western Blotting. NCPs were reconstituted by dialysis at 4°C (7) using histone octamer (660 pmol) and DNA consisting of both 5'-^{32}P labeled (1 pmol) and unlabeled (600 pmol) MdG_{123} containing DNA. The labeled DNA was present to facilitate determination of the reconstitution efficiency and DPC yield. The NCP samples were incubated at 37°C for 36 h. DPCs were purified by 10% SDS PAGE at 4 °C. The yield of DPCs (~4 pmol) was estimated by the counts of radiation. The isolated DPCs were decomposed by heating at 95 °C for 10 min. The released proteins were resolved by four different 20% SDS PAGE gels and detected by western blotting with individual anti-histone antibody. Due to different sensitivities of the

anti-histone antibodies, the following approximate amounts of DPCs were used to detect each histone: H2B, 0.4 pmol; H4, 0.8 pmol; H2A, 1 pmol; H3, 1 pmol. The proteins were transferred to nitrocellulose membranes and the membranes were blocked with 3% BSA at room temperature for 2 h. Each anti-histone antibody (anti-H2A, ab13923, 1:300; anti-H2B, ab1790, 1:3000; anti-H3, ab1791, 1:1000; anti-H4, ab10158, 1:1000) was added and incubated at room temperature for 2 h with gentle shaking. The secondary antibody (Goat Anti-Rabbit IgG H&L, ab6721, 1:20,000) was added and incubated for 2 h. Extensive washing was performed during each step. The signals were detected by chemiluminescence with a Typhoon imager.

Determination of AP and Overall DPC Yields in MMS-treated NCPs. NCPs containing native 601 DNA with both strands 5'-^{32}P labeled were reconstituted in the presence of salmon sperm DNA using the salt dilution, as described above. MMS was added to 0.8 mL NCPs (~8 million cpm) to a final concentration of 10 mM. The mixture was incubated at 37 °C for 1 h. The unreacted MMS was removed by extensive buffer change at 4 °C with a buffer containing 10 mM HEPES, pH 7.5, 0.1 mg/ml BSA and 90 mM NaCl using a 10 K Amicon Ultra membrane. To determine the extent of alkylation, 10 μL of the MMS treated NCPs (~0.1 million cpm) was phenol extracted (equal volume), ethanol precipitated and resuspended in 36 μL H_2O. The methylated DNA was then depurinated by heating at 90 °C for 30 min. The sample was cooled to room temperature, and NaOH was added to a final concentration of 100 mM. The mixture (~40 μL) was incubated at 37 °C for 30 min, at which time it was neutralized by HCl. The DNA was ethanol precipitated, resuspended in 95% formamide and analyzed by 10% denaturing PAGE. To determine the yields of AP in the MMS-treated DNA and NCPs, the precipitated DNA from the MMS treated NCPs

was resuspended in a buffer containing 10 mM HEPES, pH 7.5, 0.1 mg/ml BSA and 90 mM NaCl. MMS-treated DNA and NCPs (~1 million cpm) were incubated at 37 °C for 24 h. The yields of AP were determined as the amounts of cleaved DNA (DNA_{cl}) analyzed by 10% denaturing PAGE following alkaline cleavage. To determine the amounts of DPCs, the incubated NCP samples were analyzed by 10% SDS PAGE at 4 °C. The yields of AP and DPCs were normalized based on the extent of DNA alkylation.

Determination of the Yields of DPC_{AP} and DPC_{MdG} in MMS-treated NCPs. NCPs containing native 601 DNA with one strand 5'-^{32}P labeled were reconstituted by salt dilution in the presence of salmon sperm DNA. MMS was added to 0.8 mL reconstituted NCPs (~8 million cpm) to a final concentration of 10 mM. The mixture was incubated at 37 °C for 1 h. The unreacted MMS was removed by extensive buffer change. The DNA alkylation efficiency was determined as described above. The MMS treated NCPs were incubated at 37 °C for 24 h. The DPCs were purified by 10% SDS PAGE at 4 °C. To determine the amounts of DPC_{AP}, an aliquot of the isolated DPCs (~50,000 cpm) was treated with 100 mM NaOH at 37 °C for 30 min, followed by neutralization and treatment with proteinase K (8 units) at room temperature for 5 min. The DNA was ethanol precipitated, resuspended in 95% formamide and analyzed by 10% denaturing PAGE. The amount of DNA_{cl} equals that of DPC_{AP}. To determine the amount of total DPCs in the labeled strand, an aliquot of the isolated DPCs (~50,000 cpm) was decomposed and depurinated by heating at 90 °C for 30 min. The sample was cooled to room temperature and NaOH was added to a final concentration of 100 mM. The mixture was then incubated at 37 °C for 30 min, followed by neutralization. The DNA was ethanol precipitated, resuspended in 95% formamide and analyzed by 10% denaturing PAGE. The amount of

DNA_{cl} equals the total amount of DPCs from the labeled strand. The yield of DPC_{MdG} was calculated by subtracting the amount of DPC_{AP} from the total DPCs in the labeled strand. The amounts of DPC_{AP} and DPC_{MdG} were normalized according to the alkylation efficiency of the corresponding DNA strand.

Distinguishing between DPCs from AP and MdG. AP_{123} DPCs and MdG_{123} DPCs were isolated from NCPs containing AP_{123} or MdG $_{123}$, respectively, as described above. DPCs (~8000 cpm) were treated with 0.1 M $NaBH_4$ at 4 °C for 1 h, followed by neutralizing with 0.1 M acetic acid. The DPCs were heated at 70 °C. Aliquots were removed and stored on dry ice until the final time point. The heated DPCs were analyzed by 10% SDS-PAGE at 4 °C.

Determining the DPC Formation in MMS Treated Chinese Hamster Lung Cells. Chinese hamster lung fibroblast V79 cells were plated at a density of 3 x 10^6 in 15 cm dishes containing Ham's F-12 modified essential Eagle's media (Life Technologies, Grand Island, NY) supplemented with 9% fetal bovine serum (Atlanta Biologics, Atlanta, GA) and cultured until ~90% confluence. Cells (in triplicate) were treated with MMS (0 or 25 mM) for 3 h at 37 °C in serum-free growth media. Following MMS treatment, cells were detached with trypsin and washed twice with cold PBS buffer. Cells were counted using a TC20^{TM} automated cell counter (Bio-Rad). The harvested cells (~2.1 x 10^7, ~90% live) from each dish were used for isolating genomic DNA containing DPCs following a reported protocol.(8). The DNA was resuspended in HEPES buffer (200 μL, 100 mM, pH 7.5) and the concentrations were determined by dG analysis. A yield of ~70-100 μg DNA was obtained.

Equal amounts of DNA (30 μg) isolated from cells without or with MMS treatment were either treated with 0.1 M $NaBH_4$ at 4 °C for 1 h, followed by quenching with 0.1 M acetic acid, or carried forward without $NaBH_4$ treatment. All samples were subsequently heated at 70 °C for 1 h to release proteins from DPCs. DNA and any stabilized DPCs were precipitated by adding 3 volume equivalents of cold ethanol, incubating on dry ice for 30 min, followed by centrifugation (14000 g) at 4 °C for 15 min. The supernatants were carefully removed, dried under vacuum and dissolved in HEPES buffer (500 μL, 100 mM, pH 8.0). The HEPES buffer was exchanged with ammonium bicarbonate buffer (25 mM, pH 8.0) using Amicon 3K filters and concentrated to ~200 μL. Trypsin (1 μg) was added directly to the filters and the mixtures were incubated at 37 °C overnight. The trypsin digested peptides were recovered by centrifugation (14000 g, 15 min) and concentrated to dryness in glass MS vials. The dried peptides were resuspended in HEPES buffer (35 μL, 100 mM, pH 8.0), mixed with acetonitrile (5 μL) and TMT 6-plex reagent (10 μL, 19.5 μg/μL). The labeling mixtures were incubated at room temperature for 2 h and quenched by adding 5% hydroxylamine (4 μL). The peptides from each condition (control, $NaBH_4$ control, MMS, MMS + $NaBH_4$) were labeled using a different TMT. The TMT-labeled peptides from all conditions were combined and desalted with C-18 columns. The desalted peptides were dried and resuspended in 0.1% formic acid (4 μL).

Half of the labeled peptide samples were used for nano-LC-MS/MS analysis (Thermo Orbitrap Fusion). The samples were loaded onto a pulled-tip fused silica column with a 100 μm innerdiameter packed in-house with 45 cm of 5 μm Luna-C18 resin (Phenomenex, Torrence, CA). 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) were used for eluting the samples from the LC. The raw data was

searched against the UniProt Chinese Hamster database with Thermo Scientific Proteome Discoverer 2.1 software. The database searches were carried out using the following settings in the Sequest HT algorithm: precursor ion mass tolerance, 10 ppm; Fragment mass tolerance 0.02 Da; variable modification, TMT-6plex, methylation (Lys and Arg), dimethylation (Lys and Arg), trimethylation (Lys and Arg), acetylation (Lys) and phosphorylation (Ser, Thr and Try); allowed number of mis-cleavages, 3.

Fig. S1. Oligonucleotides used in preparation of 145 nt DNA containing MdG at specific sites. (A) MdG was incorporated at the 3'-terminus of an oligonucleotide hybridized to a complementary template by Sequenase. (B) The gel purified MdG containing oligonucleotide was mixed with 3 or 4 other oligonucleotides and scaffolds (colored) to generate a 145 nt DNA containing MdG at position 73 (i), 89 (ii), 123 (iii) or 158 (iv) by enzymatic ligation.

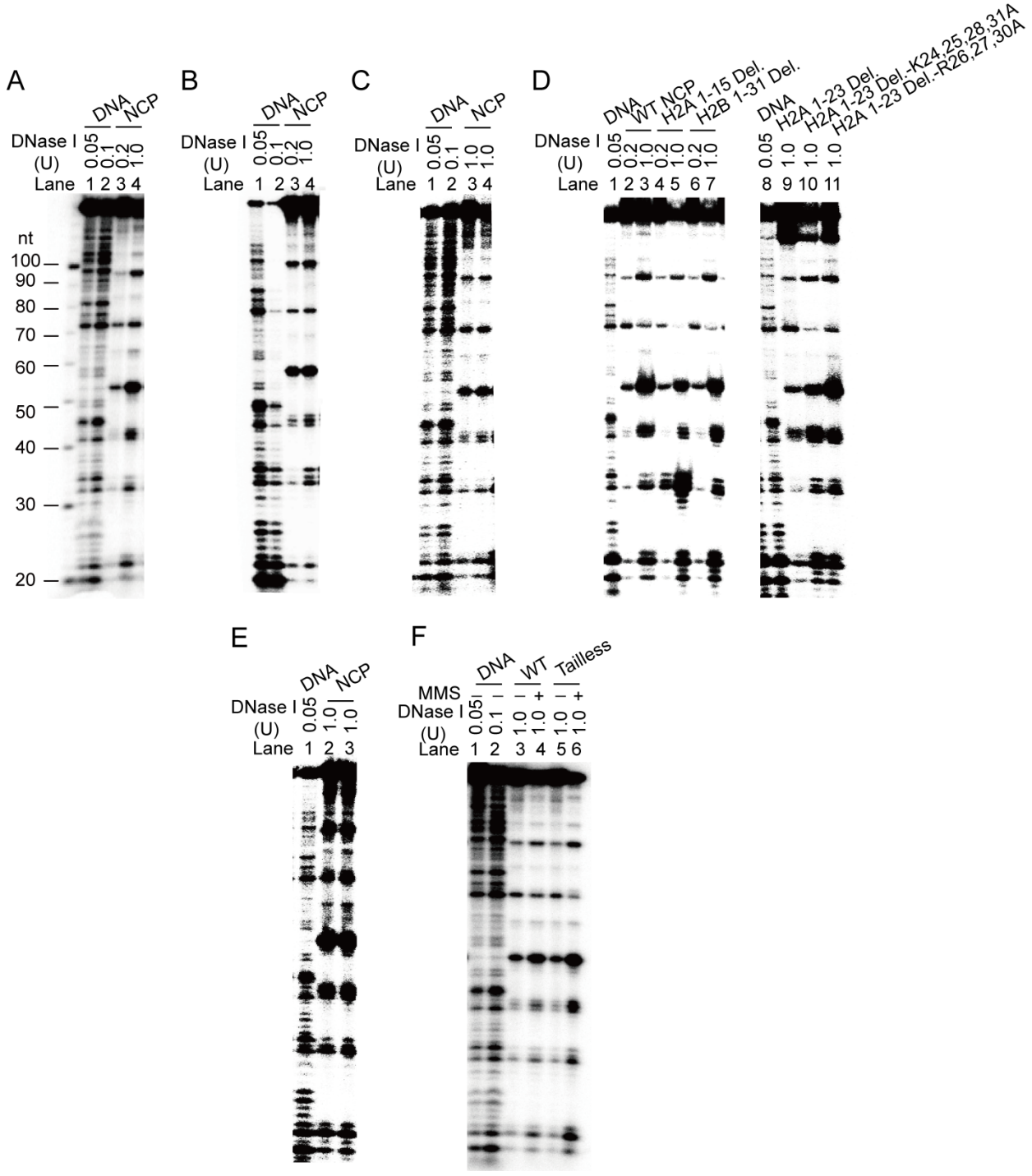

Fig. S2. DNase I footprinting of nucleosome core particles. Denaturing PAGE analysis of the DNase I digested NCPs containing MdG_{73} (A), MdG_{89} (B), MdG_{158} (C), MdG_{123} (D), AP_{123} (E), NCPs containing 601 DNA before and after MMS treatment (F). Free DNA and NCPs were digested at room temperature for 30 s and 5 min respectively with the indicated amounts of DNase I.

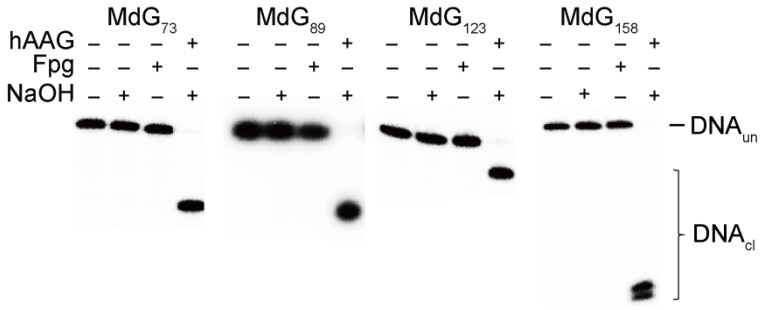

Fig. S3. Denaturing PAGE analysis of MdG-containing DNA following chemical and/or enzymatic treatments.

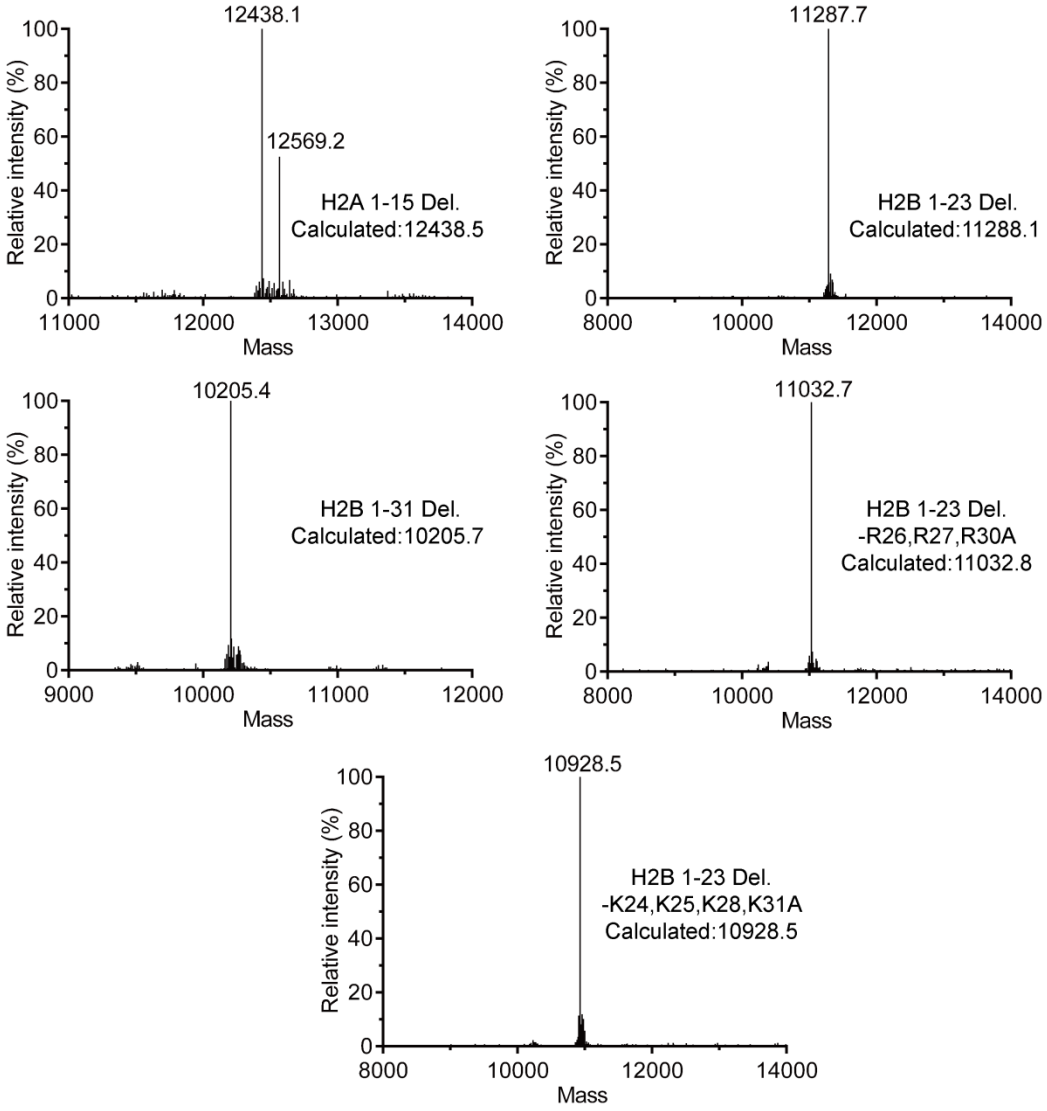

Fig. S4. Mass spectra of mutant H2A and H2B proteins. The peak with mass of 12569.2 in H2A 1-15 Del. corresponds to the protein with cleavage of the starting methionine residue during expression in *E. coli*.

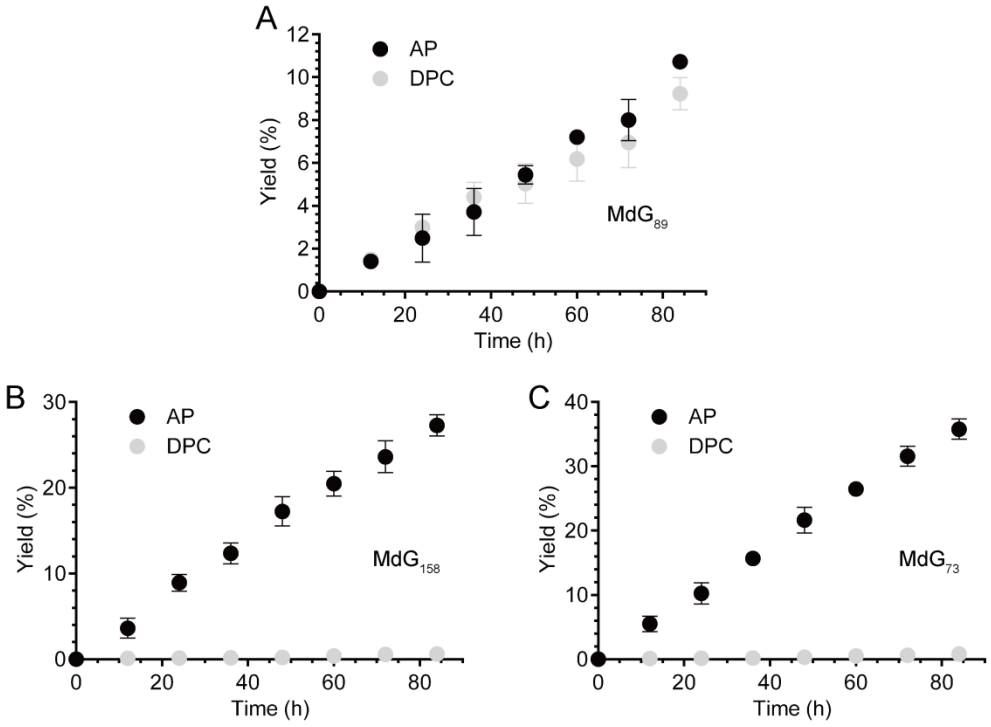

Fig. S5. Yields of AP and total DPCs from MdG_{89} (A), MdG_{158} (B) and MdG_{73} (C) within NCP as a function of time.

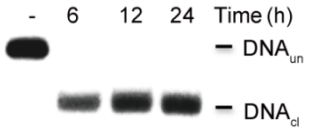

Fig. S6. Denaturing PAGE analysis of DNA in the DPCs isolated from AP_{123} NCP. The NCP was incubated at 37 °C as a function of time followed by DPC isolation.

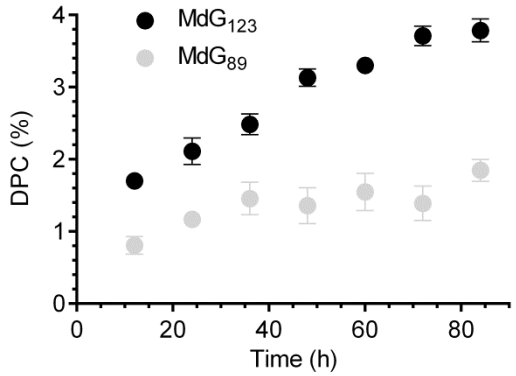

Fig. S7. The yields of MdG DPC at position 89 and 123 within NCPs as a function of time. The yields of MdG DPCs were determined as the amount of DPCs containing uncleaved DNA.

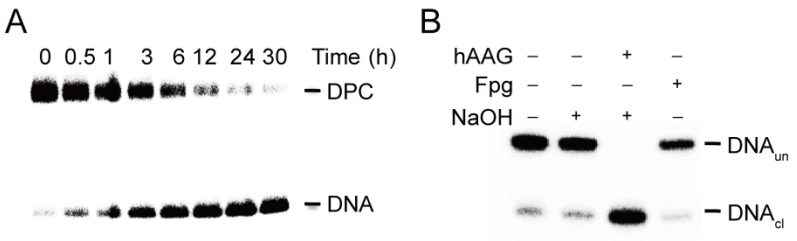

Fig. S8. MdG_{123} DPC formation is reversible. (A). SDS PAGE analysis of MdG_{123} DPC decomposition following incubating at 37 °C as a function of time. (B). Denaturing PAGE analysis of the DNA from the decomposed MdG_{123} DPC following chemical and/or enzymatic treatments. The MdG_{123} DPC was isolated from NCPs after incubating at 37 °C for 60 h. The isolated DPC was decomposed after incubating at 37 °C for 24 h.

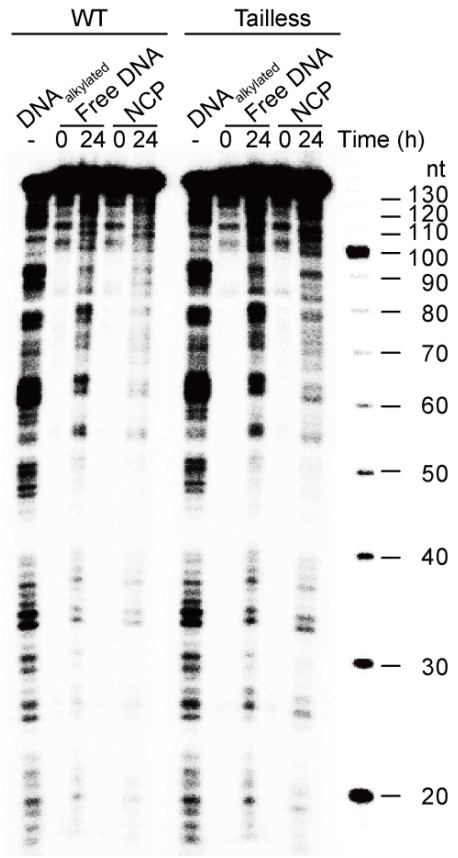

Fig. S9. DNA alkylation by MMS in NCPs. A representative 10% denaturing PAGE gel showing the DNA alkylation and determining the AP yields from the MMS (10 mM, 1 h, 37 °C) treated DNA and NCPs. The free DNA was prepared by isolation from the MMS treated NCPs in order to achieve the identical methylation efficiency and patterns. The free DNA and NCPs were incubated at 37 °C for 24 h and followed by alkaline cleavage. Both DNA strands were 5'-^{32}P labeled.

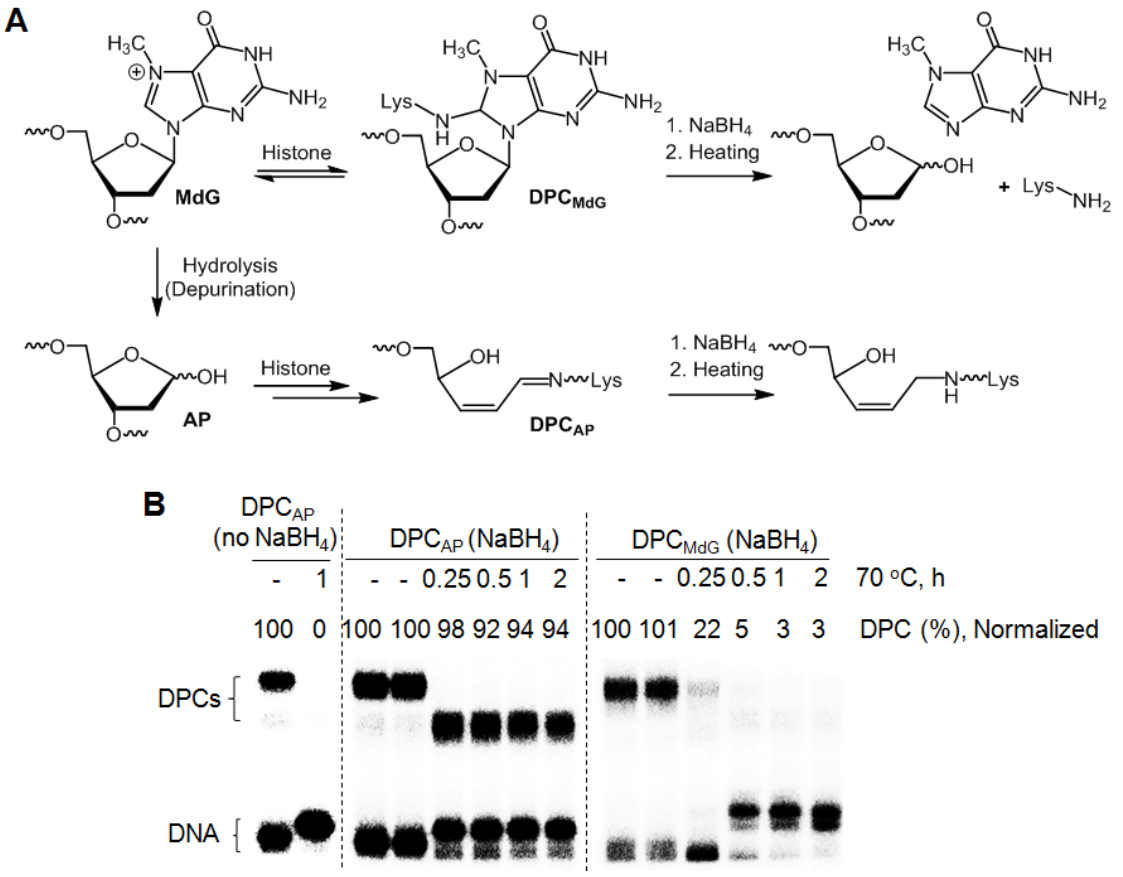

Fig. S10. Distinguishing DPC_{MdG} from DPC_{AP}. A. A scheme showing the different thermal stabilities of DPC_{MdG} and DPC_{AP} after $NaBH_4$ treatment. B. A 10% SDS-PAGE gel showing the thermal stability of DPC_{MdG} and DPC_{AP} isolated from NCPs containing MdG_{123} or AP_{123} following $NaBH_4$ treatment.

References

1. Dyer PN, Edayathumangalam RS, White CL, Bao Y, Chakravarthy S, Muthurajan UM, Luger K (2004) Reconstitution of nucleosome core particles from recombinant histones and DNA. *Methods Enzymol.*, 375:23-44.
2. Zhou C, Sczepanski JT, Greenberg MM (2012) Mechanistic studies on histone catalyzed cleavage of apyrimidinic/apurinic sites in nucleosome core particles. *J. Am. Chem. Soc.*, 134:16734-16741.
3. Weng L, Zhou C, Greenberg MM (2015) Probing interactions between lysine residues in histone tails and nucleosomal DNA via product and kinetic analysis. *ACS Chem. Biol.*, 10:622-630.
4. Hendler SS, Fuerer E, Srinivasan PR (1970) Synthesis and chemical properties of monomers and polymers containing 7-methylguanine and an investigation of their substrate or template properties for bacterial deoxyribonucleic acid or ribonucleic acid polymerases. *Biochemistry*, 9:4141-4153.
5. van Kampen JJ, Fraaij PL, Hira V, van Rossum AM, Hartwig NG, de Groot R, Luider TM (2004) A new method for analysis of azt-triphosphate and nucleotide-triphosphates. *Biochem. and Biophys. Comm.*, 315:151-159.
6. Sczepanski JT, Wong RS, McKnight JN, Bowman GD, Greenberg MM (2010) Rapid DNA-protein cross-linking and strand scission by an abasic site in a nucleosome core particle. *Proc. Natl. Acad. Sci. USA*, 107:22475-22480.
7. Weng L, Greenberg MM (2015) Rapid histone-catalyzed DNA lesion excision and accompanying protein modification in nucleosomes and nucleosome core particles. *J. Am. Chem. Soc.*, 137:11022-11031.
8. Groehler At, Villalta PW, Campbell C, Tretyakova N (2016) Covalent DNA-protein cross-linking by phosphoramide mustard and nornitrogen mustard in human cells. *Chem. Res. Toxicol.*, 29:190-202.