Supporting Information.

Effect of Histone Lysine Methylation on DNA Lesion Reactivity in Nucleosome Core Particles

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Contents:

Nano LC-Orbitrap MS experimental procedure. Peptides were analyzed by on-line nanoflow LC-Orbitrap MS on a Dionex UltiMate 3000 RSLCnano system connected to a QExactive HF instrument (Thermo Fisher Scientific) via a Nanospray Flex ion source. Peptides were trapped on a C18P Pepmap100, 5 µm, 100 Å (5 mm x 300 µm internal diameter) column and separated on an Acclaim Pepmap RSLC C18, 2 µm, 100 Å analytical column (15 cm × 75 µm internal diameter; Thermo Fisher Scientific). Mobile phases consisted of 0.1% formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Chromatographic separation was achieved using the following gradient: 0-5 min, 5% B; 5-40 min, 5-55% B; 40-40.1 min 55-90% B; 40.1-45 min 90% B; 45-45.1 min 90-5% B; 45.1-60 min 5% B. The flow rate was set to 300 µL min^{-1}, the temperature of the sample tray was set to 4 °C and the injection volume was 1 µL.

The QExactive HF mass spectrometer was operated using positive ionization in full MS/dd-MS^2 mode. The spray voltage and capillary temperature were maintained at 1.8 kV and 250°C, respectively. The S-Lens RF level was set at 65. Full MS scans were acquired over the *m/z* 200−2000 range with a resolving power of 120,000 at *m/z* 200. The five most intense peaks were selected for fragmentation (MS^2). Detection for all MS^2 spectra was performed with a resolving power of 30,000 at *m/z* 200. The target value for MS scans was $3x10^6$ ions, and the target value for MS^2 scans was $1x10^5$ ions. HCD collision energy was 30 eV.

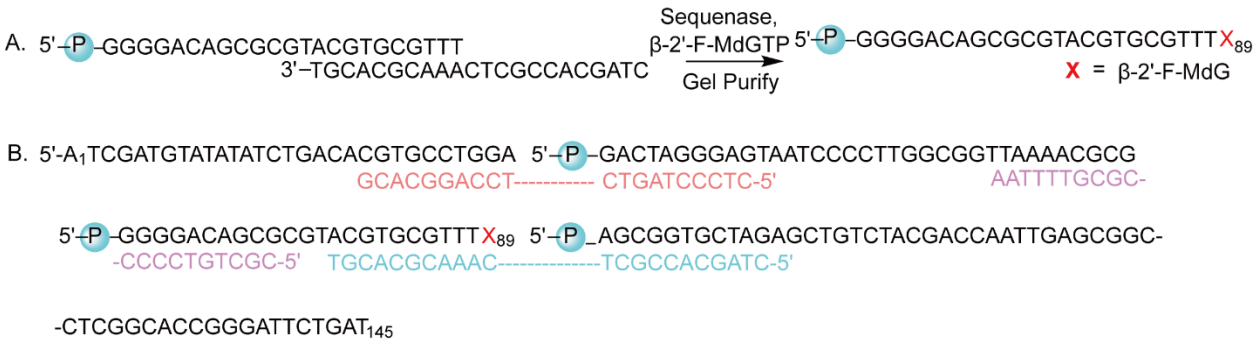


Figure S1: Preparation of 145-mer DNA containing β-2'-F-MdG_{89}. A. Incorporation of β-2'-F-MdG to position 89 by Sequenase. B. Enzymatic ligation to generate 145-mer 601 DNA containing β-2′-F-MdG_{89}. The ligated product was purified by denaturing PAGE.

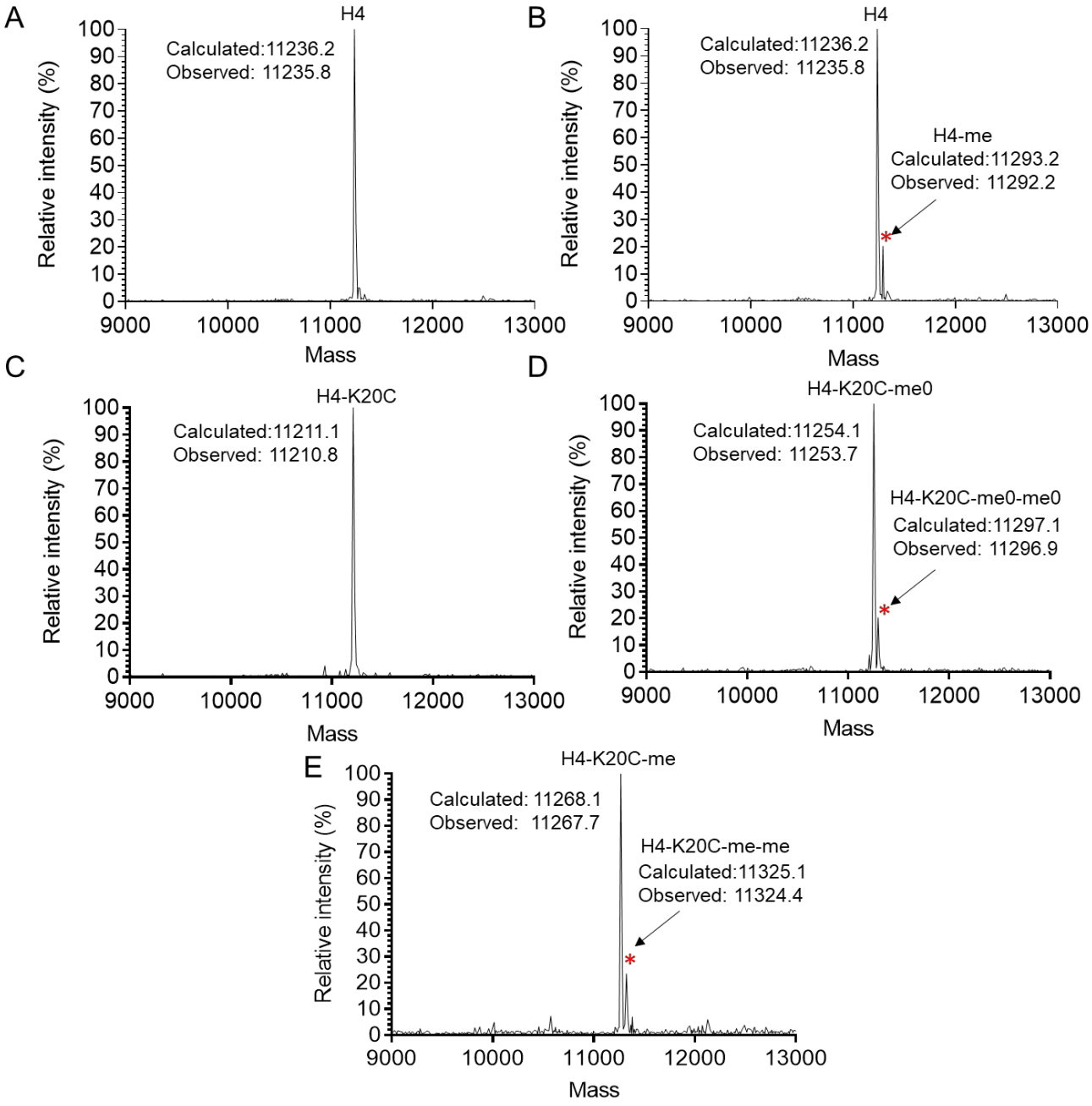
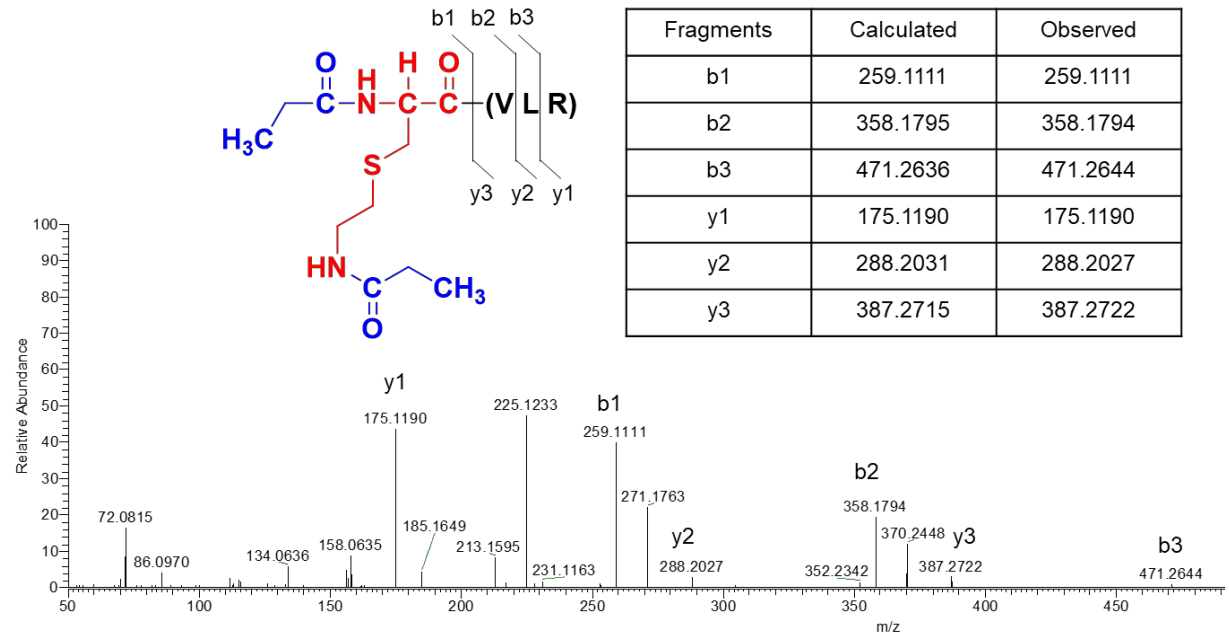
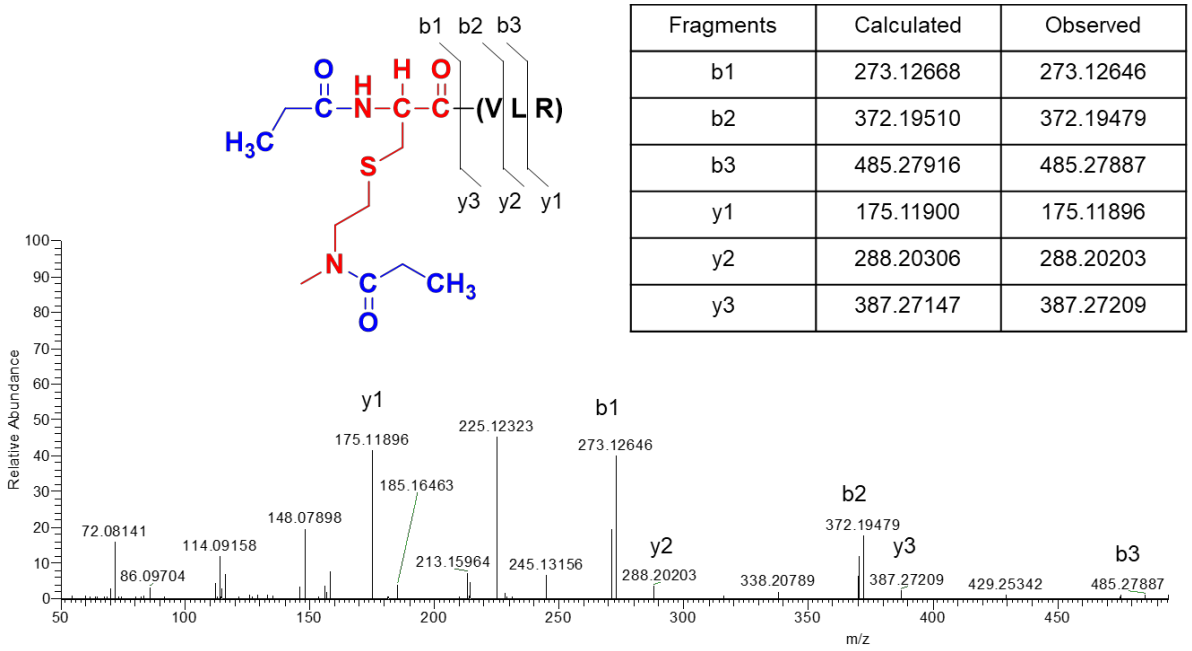


Figure S2: ESI-MS of histone proteins determined by UPLC-MS. A. Wild type H4. B. Reaction product of wild type histone H4 with 2-chloroethyl methylammonium chloride. C. H4-K20C. D. H4-K20C-me0. E. H4-K20C-me. The asterisk (*) indicates the undesired alkylation possibly at lysine residues of histone H4.



Fragments	Calculated	Observed
b1	259.1111	259.1111
b2	358.1795	358.1794
b3	471.2636	471.2644
y1	175.1190	175.1190
y2	288.2031	288.2027
y3	387.2715	387.2722



Fragments	Calculated	Observed
b1	273.12668	273.12646
b2	372.19510	372.19479
b3	485.27916	485.27887
y1	175.11900	175.11896
y2	288.20306	288.20203
y3	387.27147	387.27209

Figure S3. Nano LC-Orbitrap MS of peptide fragments (a.a. 20-23) obtained by trypsin digestion of propionic anhydride treated H4-K20C-me0 and H4-K20C-me. (Note: Samples were treated again with propionic anhydride after trypsin digestion.)

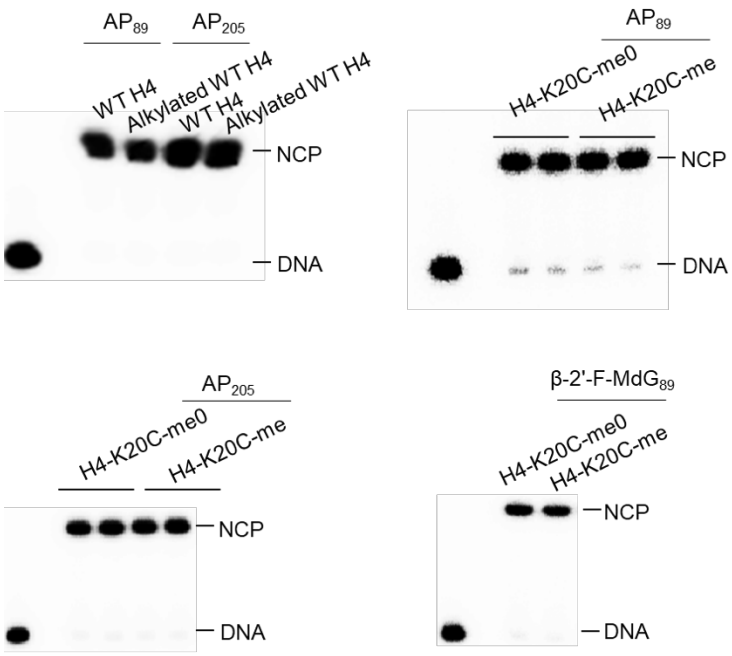


Figure S4: Native PAGE analysis of the reconstituted NCPs.

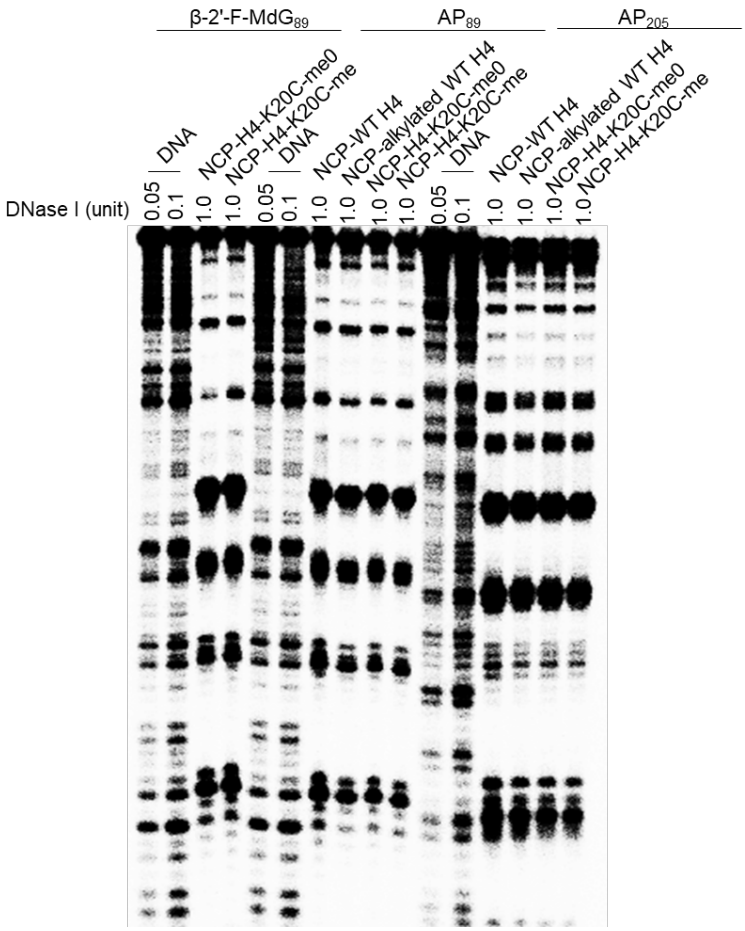


Figure S5: DNase I digestion of NCPs.

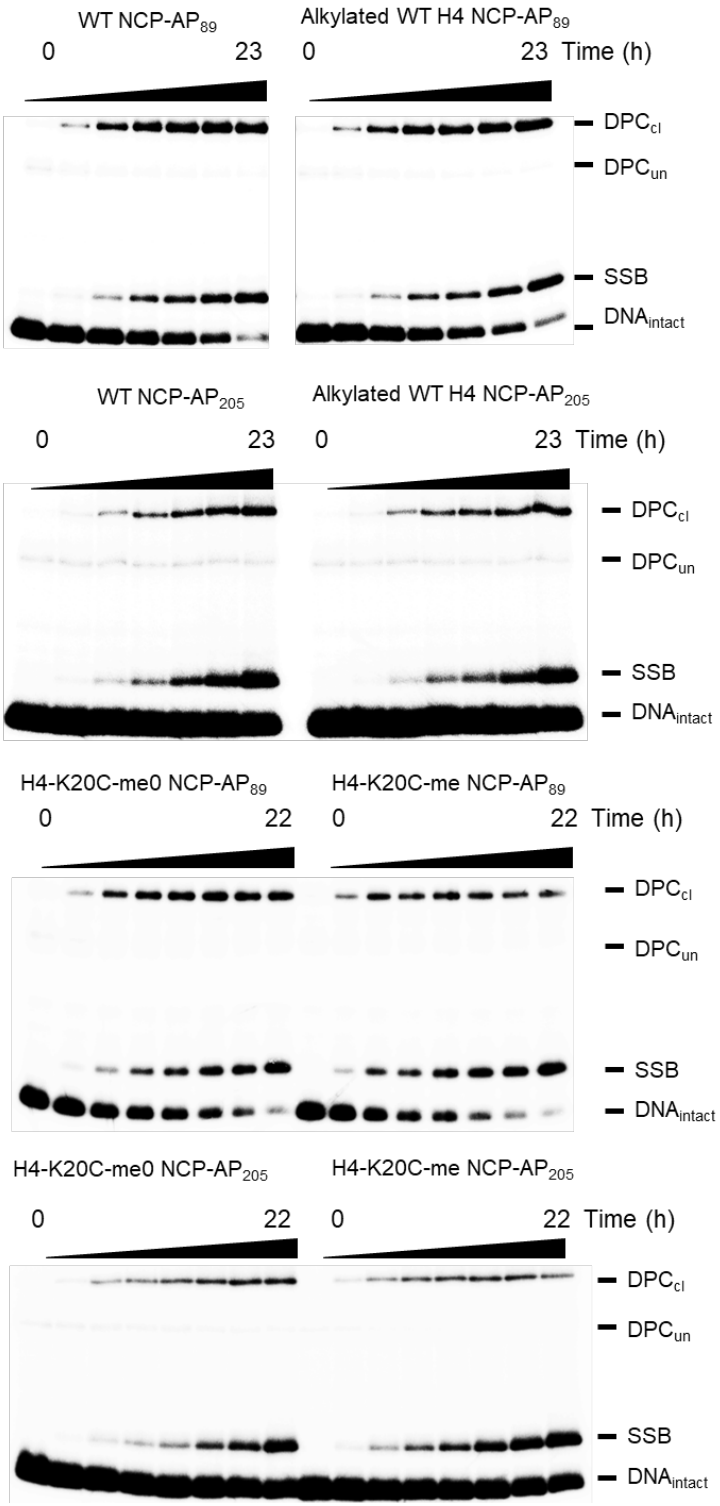


Figure S6: Representative 10% SDS-PAGE analysis of the products from NCPs containing AP in the absence of $NaBH_3CN$.

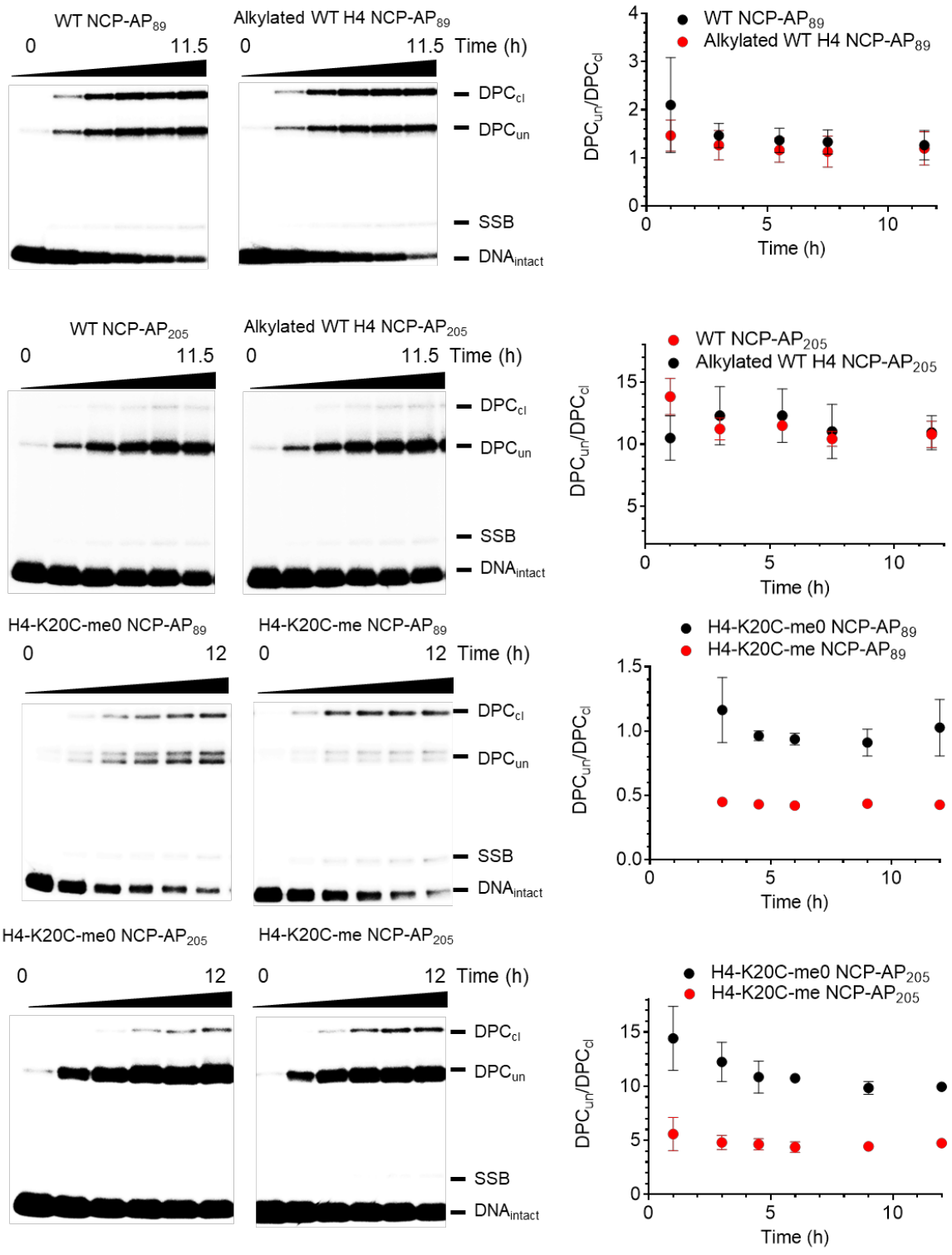


Figure S7: Representative 10% SDS-PAGE analysis of the products from NCPs containing AP in the presence of 30 mM $NaBH_3CN$.

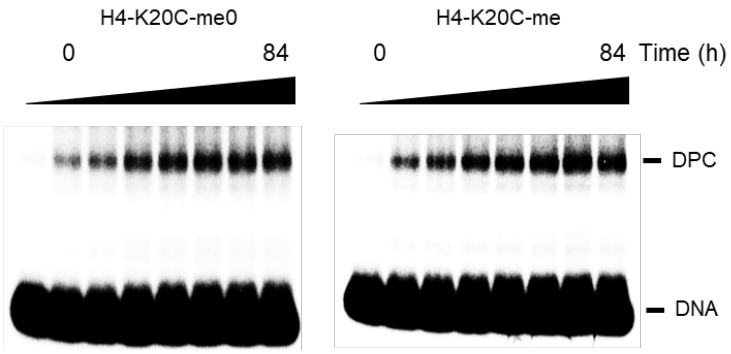


Figure S8: Representative 10% SDS-PAGE analysis of the DPCs from NCPs containing β-2'-F-MdG_{89}.

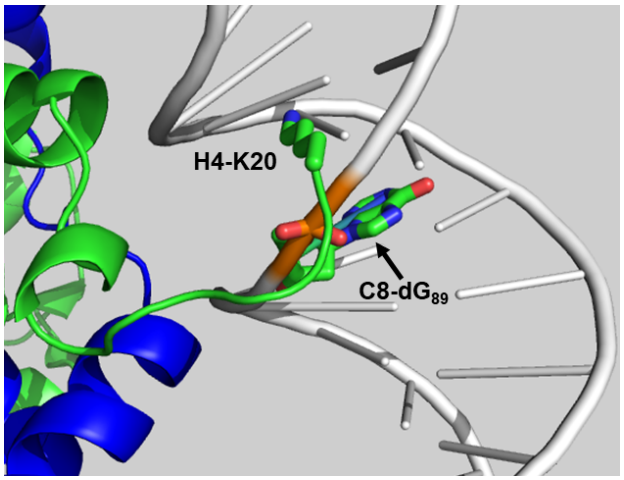


Figure S9: Proximity of C8-dG_{89} to H4-K20 in a NCP. PDB: 1aoi.