Supporting Information

Oxidation of 8-oxo-7,8-dihydro-2'-deoxyguanosine leads to substantial DNA-histone cross-links within nucleosome core particles

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Contents:

(f601-F1) (FAM)-ATCGATGTATATATCTGACACGTGCCTGGA (30 nt)

(p601-F2) pGACTAGGGAGTAATCCCCTTGGCGGTTAAAACGCG (35 nt)

(p601-F3) pGGGGACAGCGCGTACGTGCGTTTGAGCGGTGCTAG (35 nt)

(p601-F3^{89}) pGGGGACAGCGCGTACGTGCGTTT X^{89}AGCGGTGCTAG (35 nt)

(p601-F3^{73}) pGGGGACAX^{73}CGCGTACGTGCGTTTGAGCGGTGCTAG (35 nt)

(p601-F4) pAGCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGGATTCTGAT (45 nt)

(p601-F4^{137}) pAGCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGX^{137}ATTCTGAT (45 nt)

(601-T1) CTCCCTAGTCTCCAGGCACG (20 nt)

(601-T2) CGCTGTCCCCCGCGTTTTAA (20 nt)

(601-T3) GTAGACAGCTCTAGCACCGC (20 nt)

(Zhou-R1) ATCAGAATCCCGGTGCCGAGGCCGCTCAATTGGTC (35 nt)

(pZhou-R2) pGTAGACAGCTCTAGCACCGCTCAAACGCAC (30 nt)

(pZhou-R3) pGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGG (35 nt)

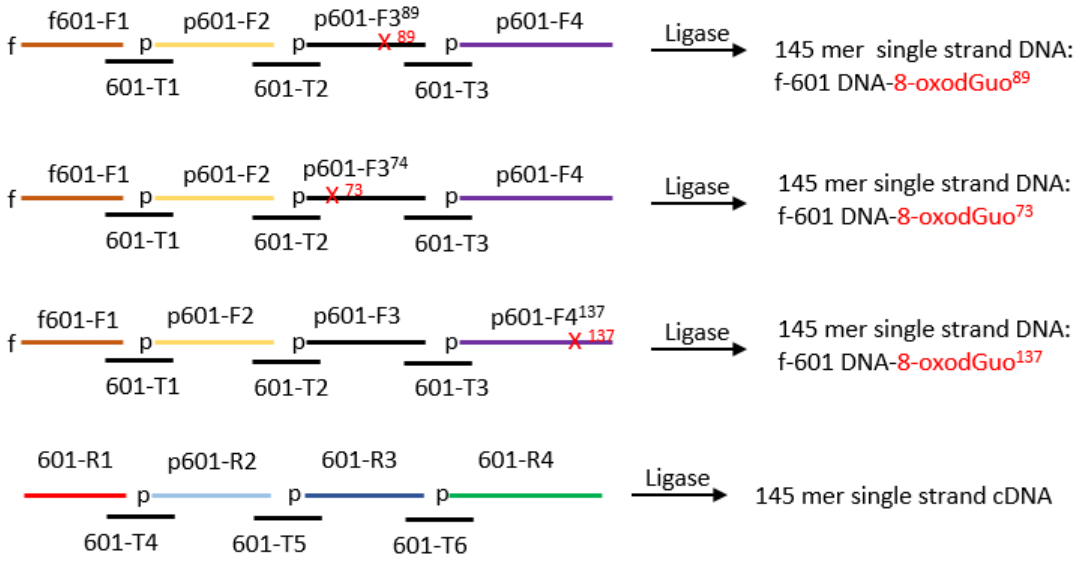
(pZhou-R4) pGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGAT (45 nt)

(601-T4) AGCTGTCTACGACCAATTGA (20 nt)

(601-T5) CAGCGCGTACGTGCGTTTGA (20 nt)

(601-T6) GGGAGTAATCCCCTTGGCGG (20 nt)

(601-T7) CGCTGAAACGCACGTACG CG (20 nt)



Sequence of 145 nt single-stranded 601 DNA:

5′(FAM)ATCGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTGGCGGTTAAAACGCGGGGGACAG^{73}GGCGTACG

TGCGTTTG^{89}AGCGGTGCTAGAGCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGG^{137}ATTCTGAT 3′

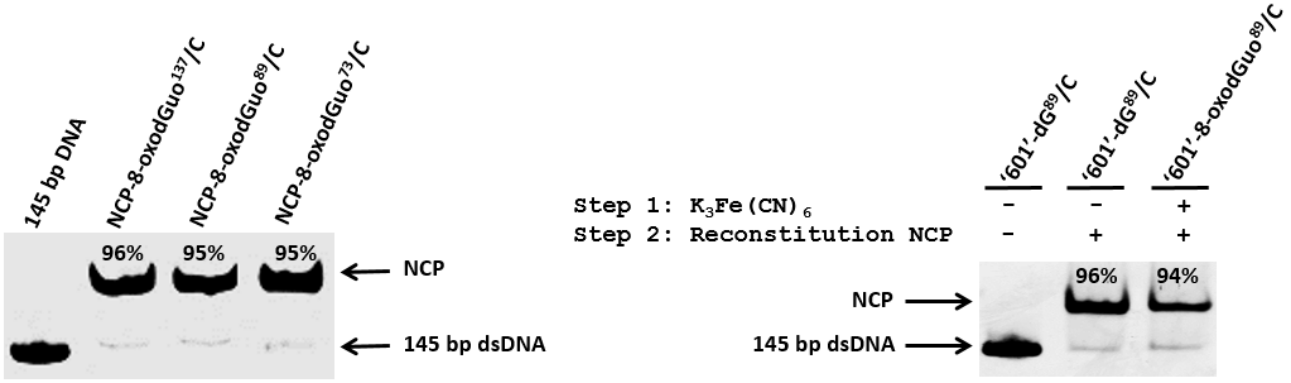
Sequence of 145 nt single-stranded 601 cDNA:

3′AGCTACATATATAGACTGTGCACGGACCTCTGATCCCTCATTAGGGGAACCGCCAATTTTGCGCCCCCTGTCGCGCATGCACGCAAAC

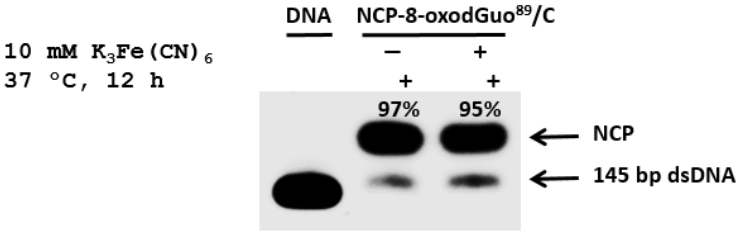
TCGCCACGATCTCGACAGATGCTGGTTAACTCGCCGGAGCCGTGGCCCTAAGACTA 5′ (cDNA)

Figure S1. Preparation of 145 nt singl-stranded 601 DNA by ligation. "X" denotes 8-oxodGuo. "f" denoate 5'-FAM labeling. "p" denotes 5'-phosphate group.

(S2A) Electrophoretic mobility shift assay showing the efficiency of NCP reconstitutions



(S2B) Electrophoretic mobility shift assay showing the stability of NCP upon 10 mM $K_3Fe(CN)_6$ treatment



(S2C) Electrophoretic mobility shift assay showing the stability of NCP upon heat treatment

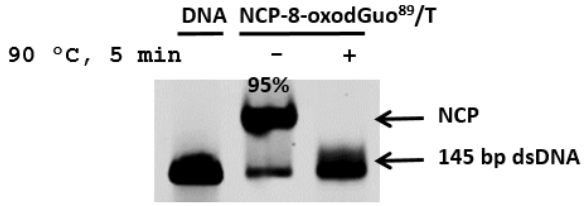


Figure S2. 5% (w/v) nondenaturing PAGE analysis of the formation and stability of NCPs.

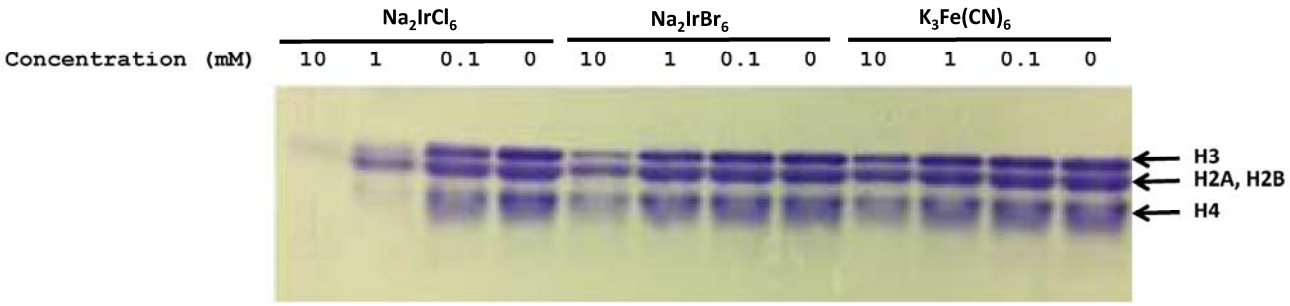
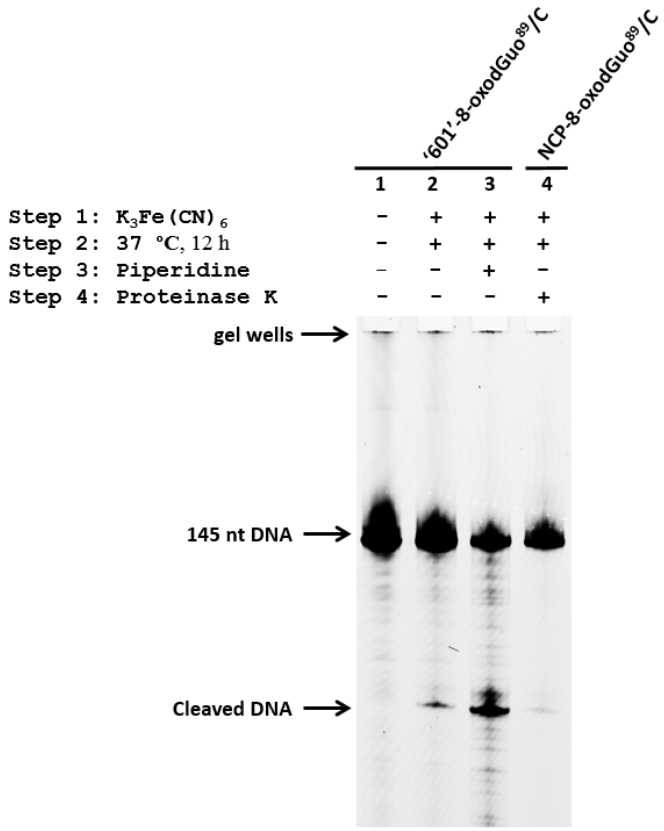
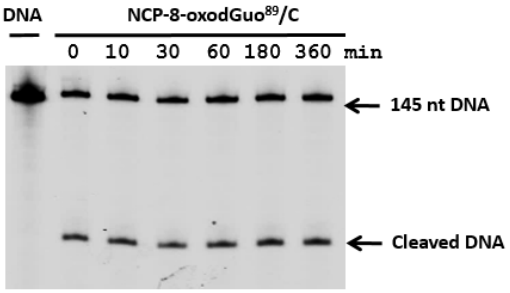


Figure S3. 10% (w/v) SDS PAGE analysis of histone octamers treated with different concentrations of Na_2IrCl_6, Na_2IrBr_6 and $K_3Fe(CN)_6$.

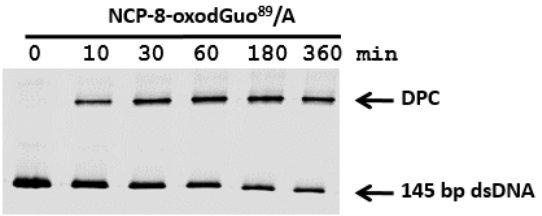
(S4A) 8% (w/v) denaturing PAGE analysis of reactions of NCP-8-oxodGuo^{89}/C and free DNA '601'-8-oxodGuo^{89}/C upon $K_3Fe(CN)_6$ oxidation



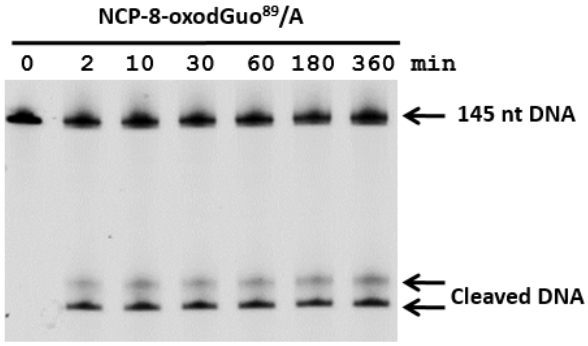
(S4B) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo^{89}/C upon $K_3Fe(CN)_6$ oxidation



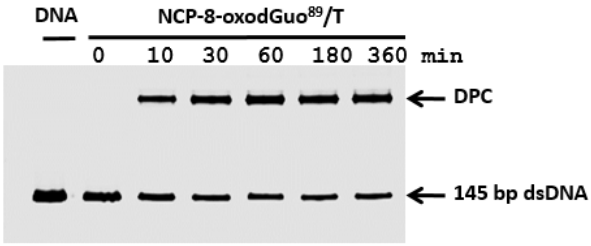
(S4C) 10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo^{89}/A upon $K_3Fe(CN)_6$ oxidation



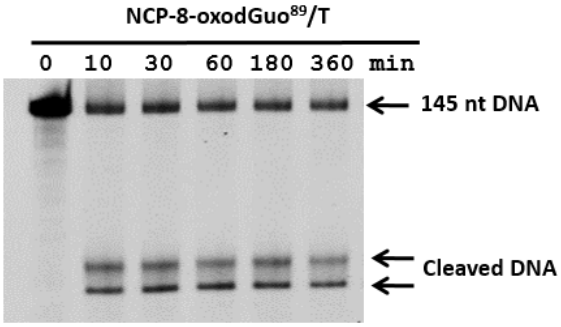
(S4D) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo^{89}/A upon $K_3Fe(CN)_6$ oxidation



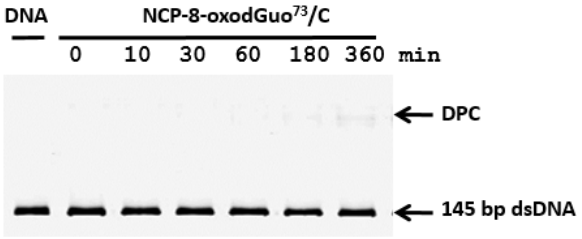
(S4E) 10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo^{89}/T upon $K_3Fe(CN)_6$ oxidation



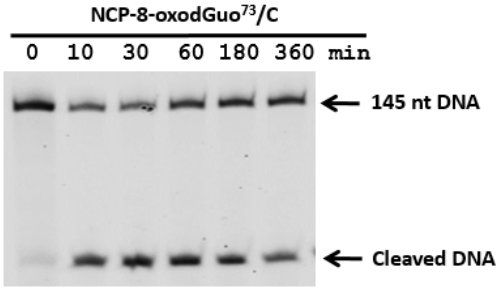
(S4F) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo^{89}/T upon $K_3Fe(CN)_6$ oxidation



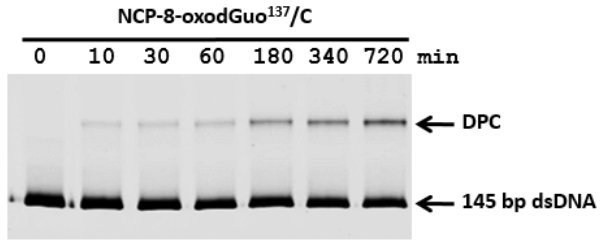
(S4G) 10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo^{73}/C upon $K_3Fe(CN)_6$ oxidation



(S4H) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo^{73}/C upon $K_3Fe(CN)_6$ oxidation



(S4I)10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo^{137}/C upon $K_3Fe(CN)_6$ oxidation



(S4J) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo^{137}/C upon $K_3Fe(CN)_6$ oxidation

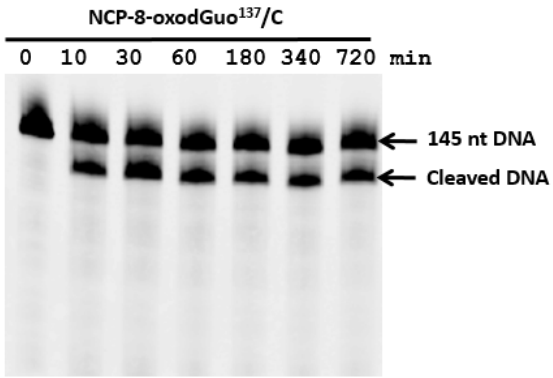


Figure S4. Representative gels of DPC formation and DNA scission in NCPs containing wide type histones.

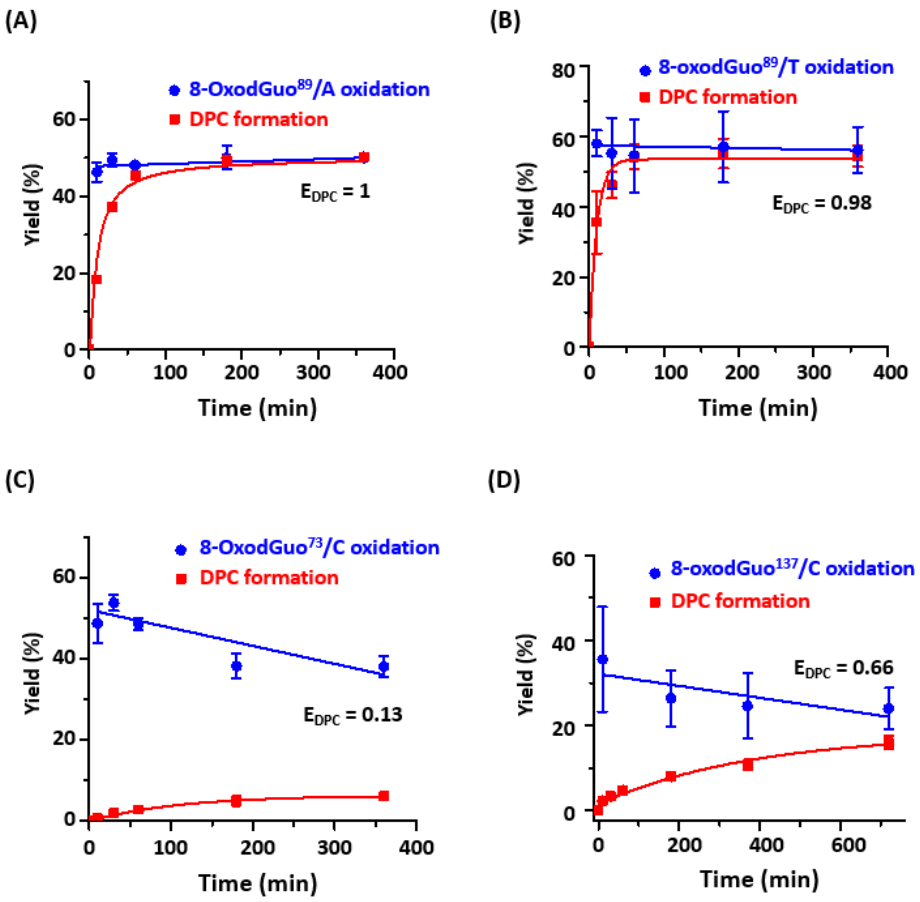
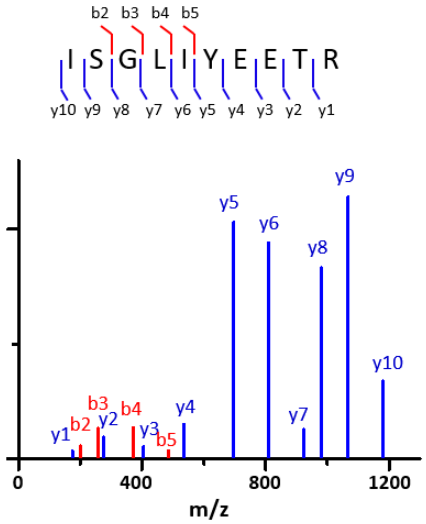


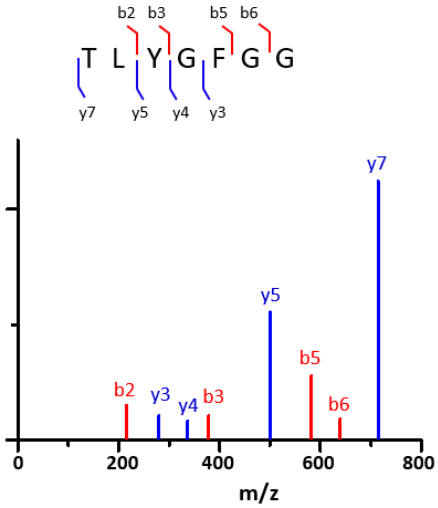
Figure S5. Kinetics of 8-oxodGuo oxidation and DPC formation in NCPs containing wide type histones. (A) NCP-8-oxodGuo^{89}/A; (B) NCP-8-oxodGuo^{89}/T; (C) NCP-8-oxodGuo^{73}/C; (D) NCP-8-oxodGuo^{137}/C.

(S6A) Identified fragment ISGLIYEETR of H4



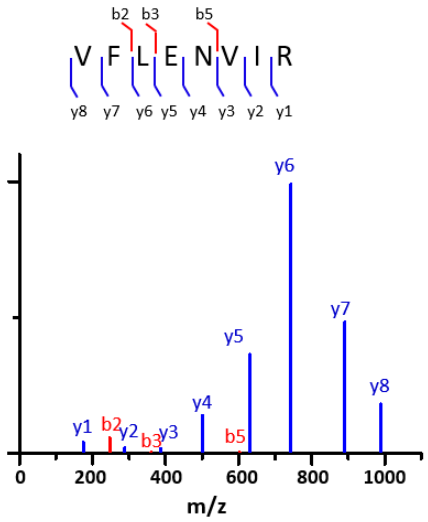
	Found (Da)	Cal. (Da)
y10	1180.621	1180.618
y9	1067.537	1067.534
y8	980.5052	980.5026
y7	923.4838	923.4808
y6	810.3997	810.3977
y5	697.3157	697.3138
y4	534.2524	534.2518
y3	405.2098	405.2102
y2	276.1672	276.168
y1	175.1195	175.1204
b5	484.3135	484.3131
b4	371.2294	371.2297
b3	258.1454	258.1460
b2	201.1239	201.1250

(S6B) Identified fragment TLYGFGG of H4



	Found (Da)	Cal. (Da)
y7	714.3463	714.3451
y5	500.2145	500.2147
y4	337.1512	337.153
y3	280.1297	280.1308
b6	639.3142	639.3127
b5	582.2928	582.2923
b3	378.2029	378.2033
b2	215.1396	215.1408

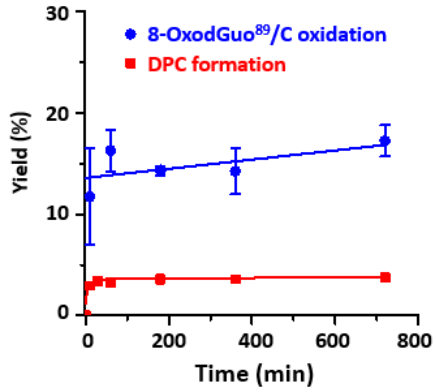
(S6C) Identified fragment VFLENVIR of H4



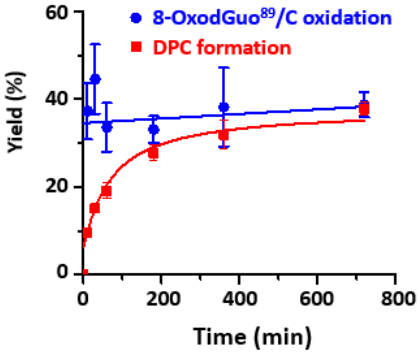
	Found (Da)	Cal.(Da)
y8	989.5784	989.5753
y7	890.5099	890.5081
y6	743.4415	743.4395
y5	630.3575	630.3562
y4	501.3149	501.3146
y3	387.272	387.2721
y2	288.2036	288.2042
y1	175.1195	175.1204
b5	603.3142	603.3133
b3	360.2287	360.2282
b2	247.1446	247.1459

Figure S6 Protein identification though MS/MS analysis of tryptic peptide fragments of DPC generated from NCP-8-oxodGuo[89]/C.

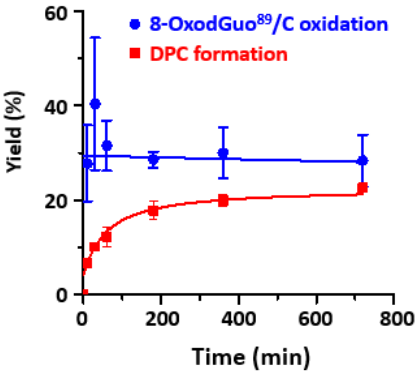
(A) $Del_{1\text{-}20}$



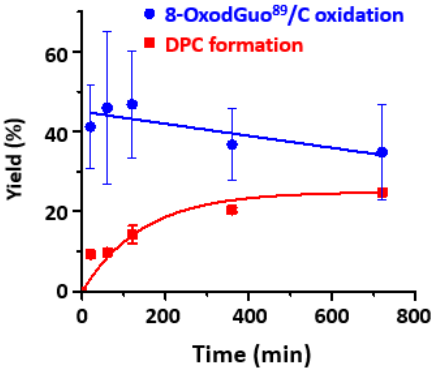
(B) K5,8,12,16,20R



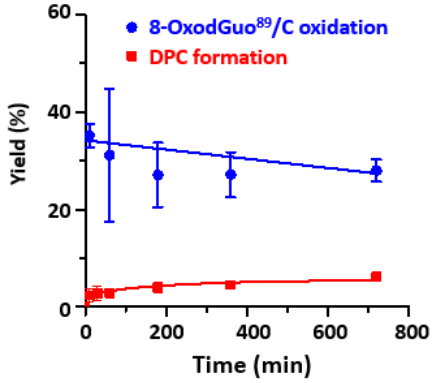
(C) S1A- K5,8,12,16,20R



(D) S1A- K5,8,12,16,20R-H18A



(E) CapN



(F) CapN- K5,8,12,16,20R

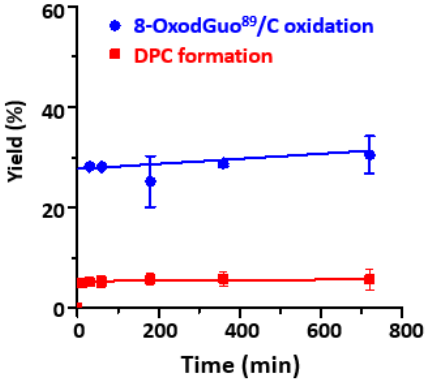
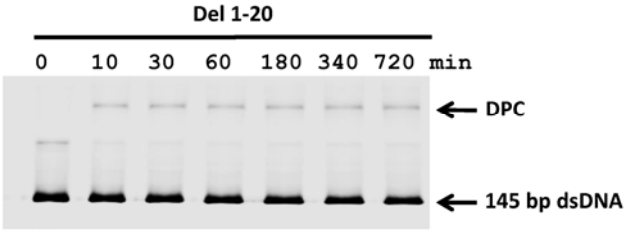
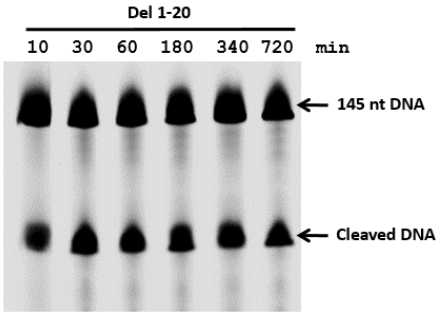


Figure S7. Kinetics of 8-oxodGuo oxidation and DPC formation in NCP-8-oxodGuo^{89}/C containing mutated histone H4.

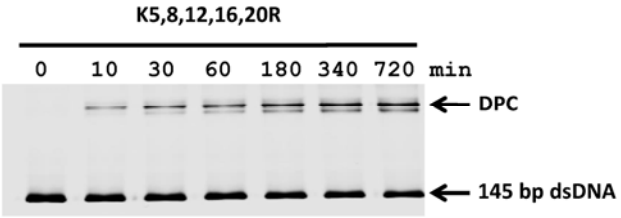
(S8A) 10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo[89]/C containing H4-Del$_{1\text{-}20}$ mutant



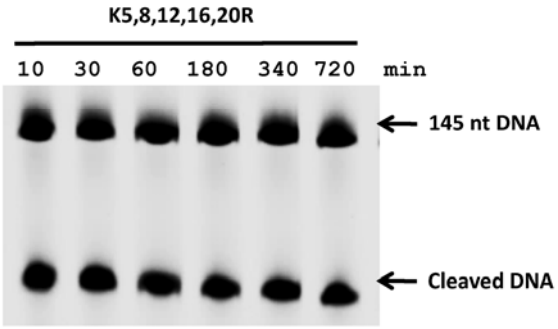
(S8B) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo[89]/C containing H4-Del$_{1\text{-}20}$ mutant



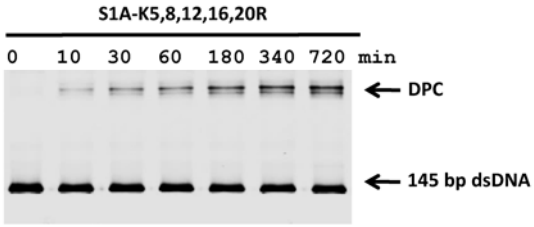
(S8C) 10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo[89]/C containing H4-K5,8,12,16,20R mutant



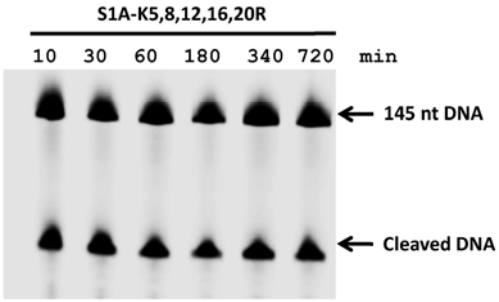
(S8D) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo[89]/C containing H4-K5,8,12,16,20R mutant



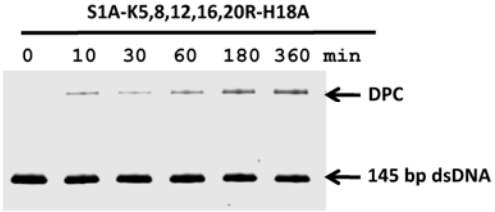
(S8E) 10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo[89]/C containing H4-S1A-K5,8,12,16,20R mutant



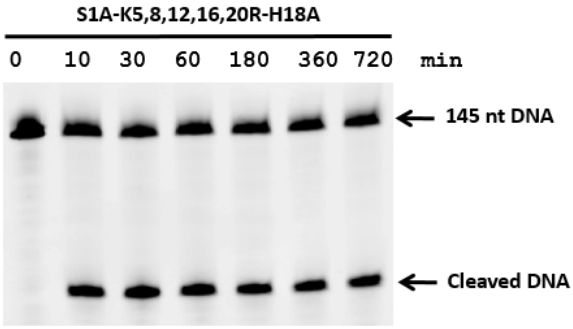
(S8F) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo[89]/C containing H4-S1A-K5,8,12,16,20R mutant



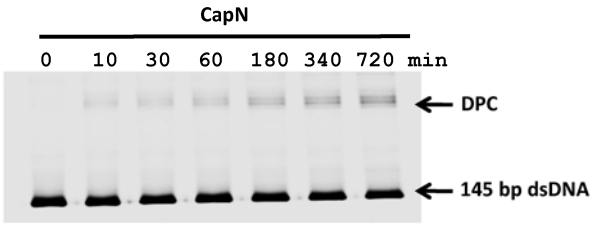
(S8G) 10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo[89]/C containing H4-S1A-K5,8,12,16,20R-H18A mutant



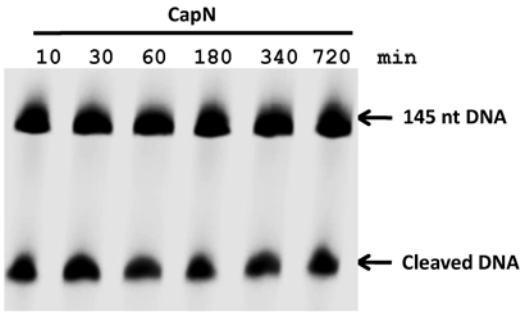
(S8H) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo[89]/C containing H4-S1A-K5,8,12,16,20R-H18A mutant



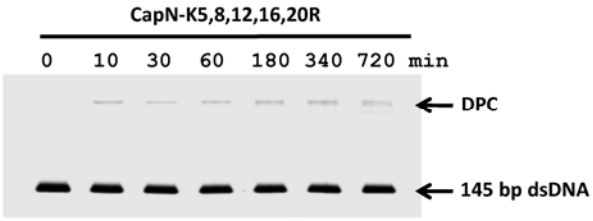
(S8I) 10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo[89]/C containing H4-CapN mutant



(S8J) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo[89]/C containing H4-CapN mutant



(S8K)10% (w/v) SDS PAGE analysis of DPC formation in NCP-8-oxodGuo[89]/C containing H4-CapN- K5,8,12,16,20R mutant



(S8L) 8% (w/v) denaturing PAGE analysis of DNA scission in NCP-8-oxodGuo[89]/C containing H4-CapN- K5,8,12,16,20R mutant

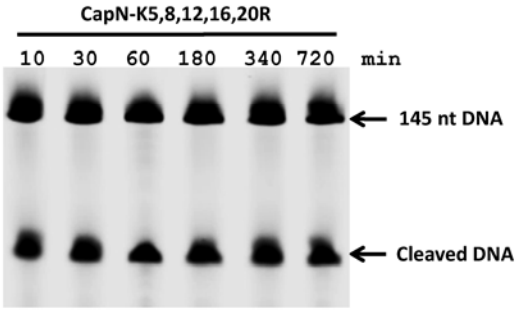
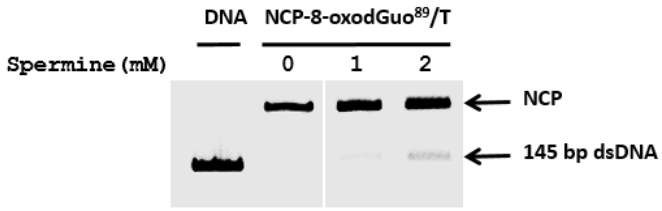


Figure S8. Representative gels of DPC formation and DNA scission in NCP-8-oxodGuo[89]/C containing mutated histone H4.

(A) 5% (w/v) nondenaturing PAGE analysis of the stability of NCP upon spermine treatment



(B) 10% (w/v) SDS PAGE analysis of DPC formation in the presence of spermine

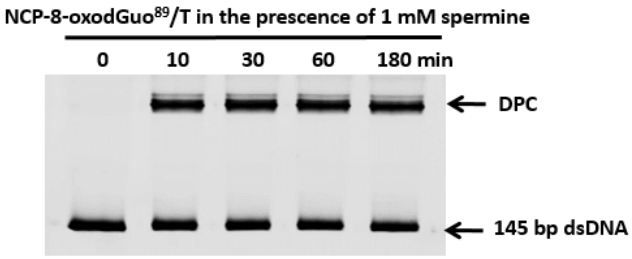


Figure S9. Representative gels of DPC formation in NCP-8-oxodGuo^{89}/T in the presence of 1 mM spermine.