

SUPPORTING INFORMATION

for

Nucleosome Core Particles Lacking H2B or H3 Tails are Altered Structurally and have Differential Base Excision Repair Fingerprints

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Scheme S1. Widom 601 and Internal Standard Sequences.

Designation of the strands as I or J based on the crystal structure of Vasudevan, *et al.* for the Widom 601 NCP.¹ εA was distributed across all A positions in the I strand.

Lesion containing I strand:

5'-ATC AGA ATC CCG GTG CCG AGG CCG CTC AAT TGG TCG TAG ACA GCT CTA GCA CCG
CTT AAA CGC ACG TAC GCG CTG TCC CCC GCG TTT TAA CCG CCA AGG GGA TTA CTC
CCT AGT CTC CAG GCA CGT GTC AGA TAT ATA CAT CGA T- 3'

Complementary J strand:

5'-ATC GAT GTA TAT ATC TGA CAC GTG CCT GGA GAC TAG GGA GTA ATC CCC TTG GCG
GTT AAA ACG CGG GGG ACA GCG CGT ACG TGC GTT TAA GCG GTG CTA GAG CTG TCT
ACG ACC AAT TGA GCG GCC TCG GCA CCG GGA TTC TGA T- 3'

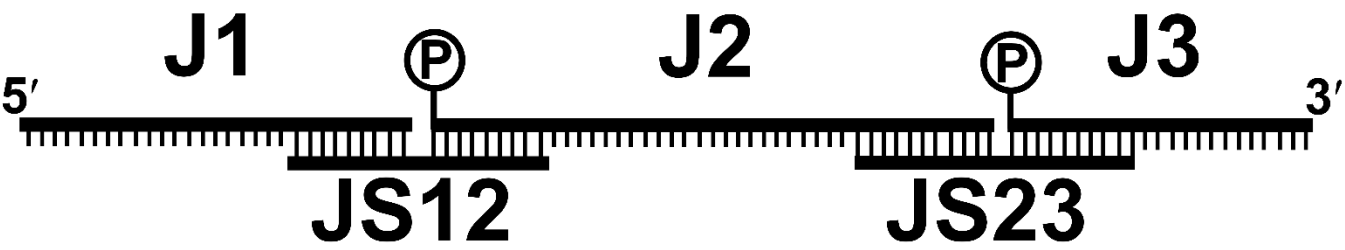
23 mer internal standard:

5'-ATC AGA ATC CCG GTG CCG AGG CC- 3'

92 mer internal standard:

5'-ATC AGA ATC CCG GTG CCG AGG CCG CTC AAT TGG TCG TAG ACA GCT CTA GCA CCG
CTT AAA CGC ACG TAC GCG CTG TCC CCC GCG TTT TAA CC- 3'

Scheme S2. Ligation Scheme for Complementary Strand Lacking εA.



Component	Length	Sequence (5' to 3')
J1	45mer	ATCGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAATC
J2	65mer	CCCTTGGCGGTTAAACGCGGGGGACAGCGCGTACGTGCGTTTAAGC GGTGCTAGAGCTGTCTAC
J3	35mer	GACCAATTGAGCGGCCTCGGCACCGGGATTCTGAT
JS12	30mer	TTTAACCGCCAAGGGGATTACTCCCTAGTC
JS23	32mer	GCCGCTCAATTGGTCGTAGACAGCTCTAGCAC

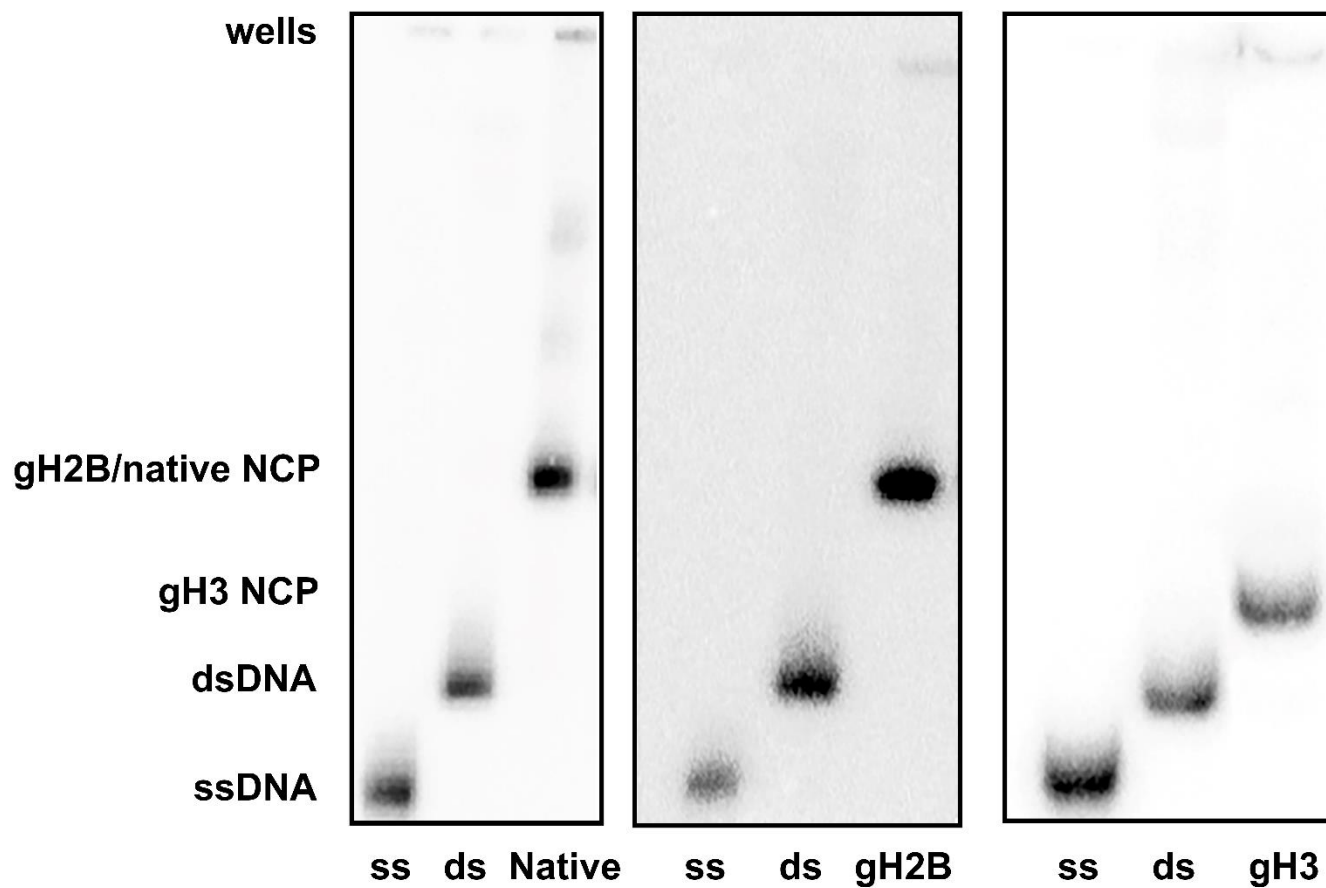


Figure S1. Native PAGE Analysis of ϵ A Containing NCPs. Representative native PAGE gels showing successful NCP formation as indicated by slower band migration in the NCP lane relative to the unincorporated duplex (ds) lane. Separate gels were run for the native, gH2B, and gH3 NCPs respectively. Only NCPs containing <5% dsDNA were used in these studies.

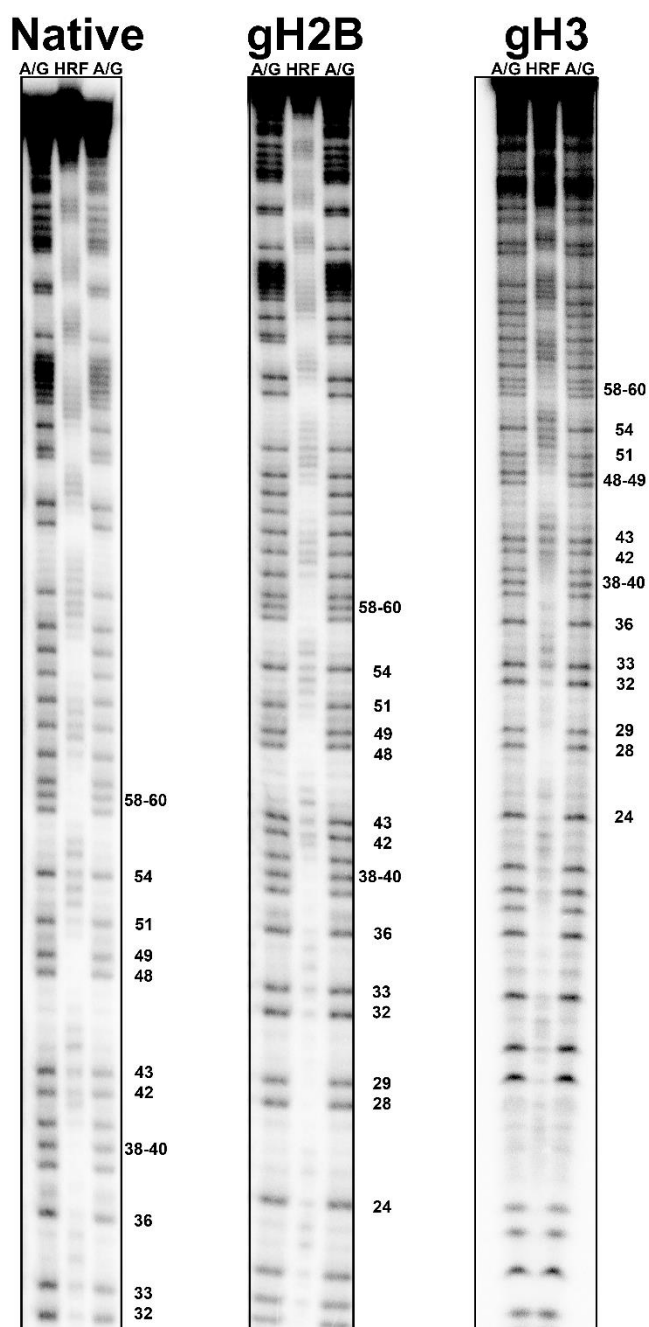


Figure S2. Hydroxyl Radical Footprinting of NCPs. Representative HRF denaturing PAGE gels from which rotational orientation of nucleobases was assigned. The oscillatory pattern of bands in the HRF lanes reflects the rotational position of DNA in the NCP. Quantitation of band intensity is provided in Figure 3. Lane A/G is the Maxam-Gilbert sequencing reaction (A+G) use for sequence alignment.

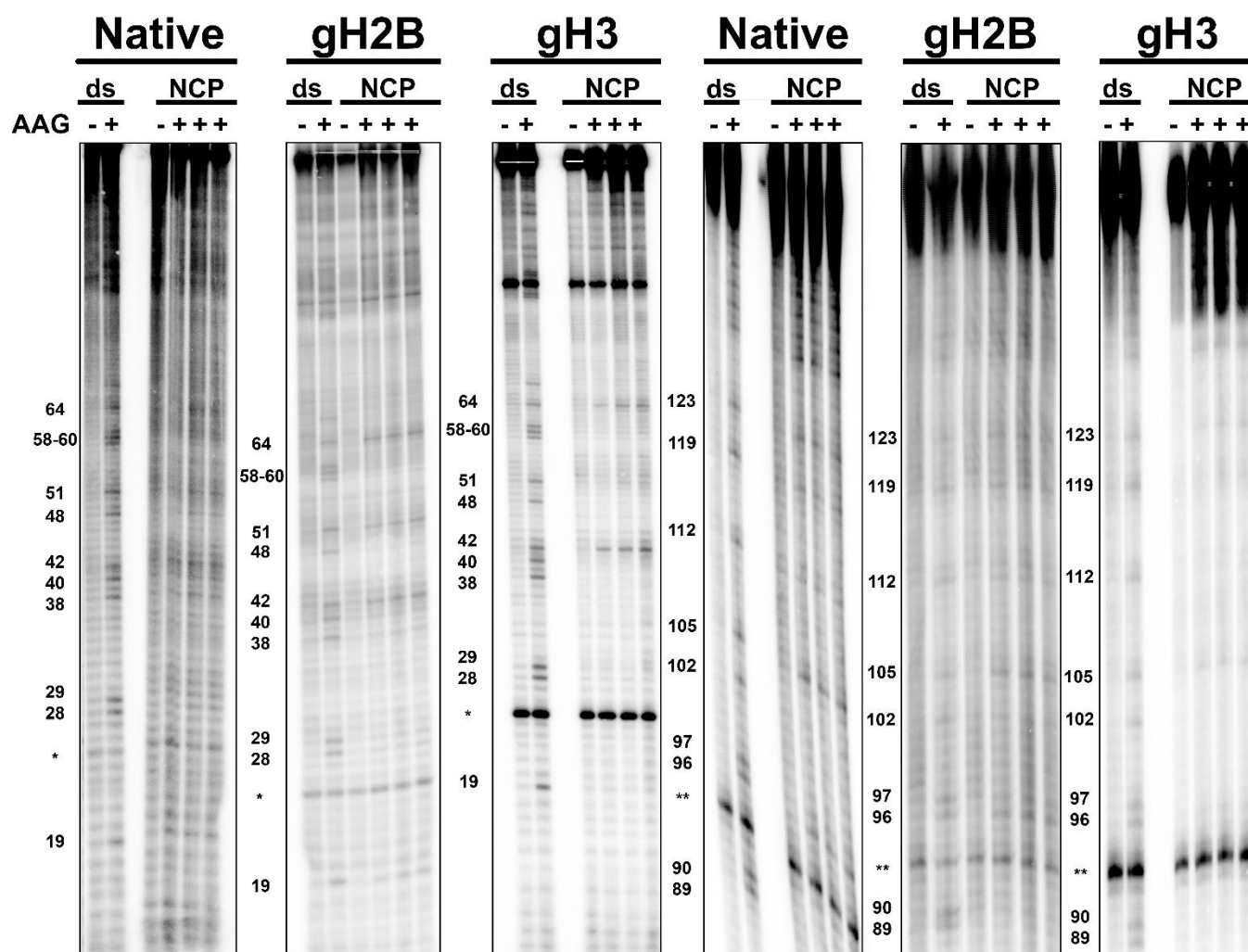


Figure S3. Denaturing PAGE analysis of AAG activity on NCPs and Duplex. Evaluation of AAG activity on NCPs and Duplex containing globally substituted ϵ A lesions. All samples were incubated with 2000 nM AAG and 50 nM of either ds or NCP substrate for 1 h at 37 °C and then strand breaks were catalyzed by incubation with 0.5 M NaOH at 90 °C for 3 min to visualize successful ϵ A excision. Each data set consists of the following lanes: a no enzyme duplex sample, duplex treated with AAG, a no enzyme NCP sample, and three replicates of AAG treated NCPs. The internal standards are indicated by the asterisks on the gel with a single asterisk and double asterisk corresponding to the 23 mer and 92 mer standards, respectively. When necessary, the PAGE images were aligned using SAFA software to correct for any slant in the gels.

Table S1. HRF reactivity used to assign rotational orientation of ϵ A lesions. These values are derived from band intensities quantitated in gels provided in Figure S2.

ϵA Position	Rotational Orientation	Native NCP	gH2B NCP	gH3 NCP
19	IN	0.30	0.23	0.44
28	IN	0.11	0.06	0.05
29	IN	0.14	0.00	0.05
38	IN	0.09	0.28	0.10
40	MID	0.25	0.19	0.34
42	OUT	0.69	0.51	0.75
48	IN	0.08	0.01	0.00
51	MID	0.35	0.43	0.44
58	IN	0.24	0.24	0.19
59	IN	0.20	0.40	0.13
60	IN	0.18	0.47	0.23
64	OUT	0.78	0.64	0.77
89	IN	0.04	0.28	0.44
90	IN	0.01	0.24	0.36
96	OUT	0.80	0.57	0.77
97	OUT	0.92	0.72	0.79
102	MID	0.64	0.59	0.24
105	MID	0.49	0.65	0.65
112	MID	0.43	0.14	0.52
119	IN	0.35	0.32	N/A
123	OUT	0.80	0.45	N/A

SUPPLEMENTARY REFERENCES

1. Vasudevan, D., Chua, E. Y. D., Davey, C. A. (2010) Crystal structures of nucleosome core particles containing the '601' strong positioning sequence. *J. Mol. Biol.* 403 (1), 1-10. DOI: 10.1016/j.jmb.2010.08.039