Supporting Information

A Nitrogen Mustard Induces Formation of DNA–Histone Cross-Links in Nucleosome Core Particles

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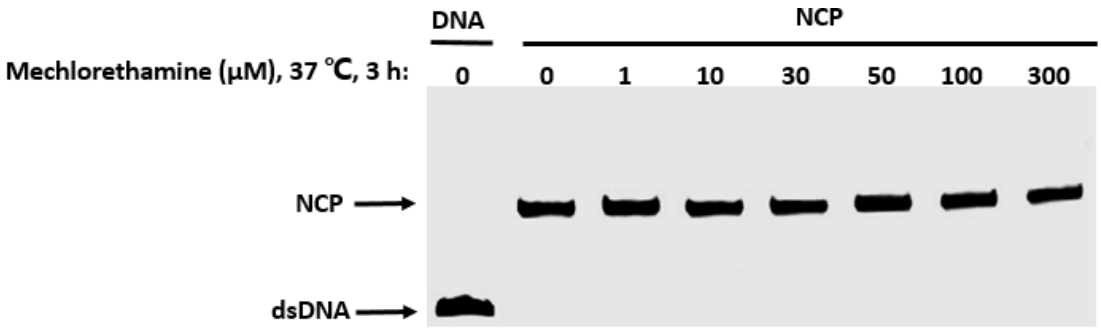


Figure S1. 5% native PAGE showing the stability of NCP after incubation with different concentrations of mechlorethamine.

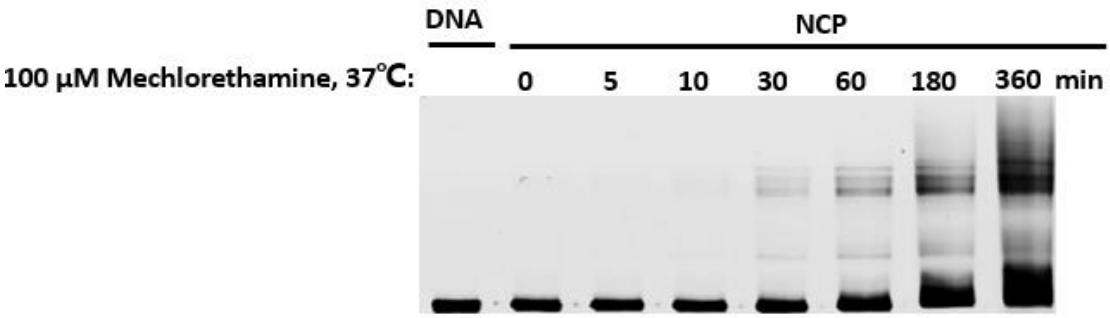
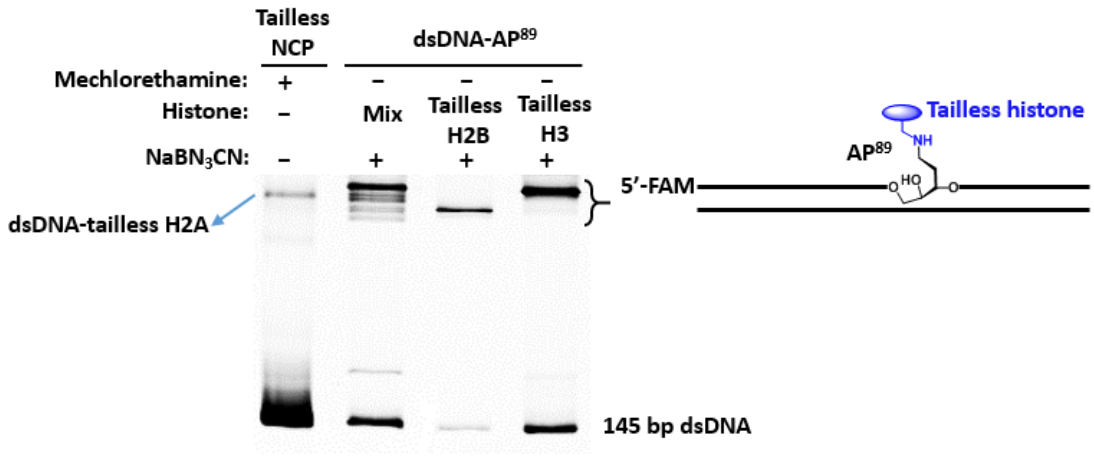


Figure S2. 12% SDS PAGE showing the time-dependent DPC formation in NCPs.

(A)



(B)

ProteinID	ProteinName	Coverage	# Unique Peptides	# Peptides	# AAs	MW [kDa]	calc. pI
A0A310TML1	CENP-T_C domain-containing protein	23.26	1	1	43	4.9	9.70
A0A1L8EWC7	Histone_H2A_C domain-containing protein	7.69	1	1	91	9.8	11.17
A0A310U679	VLIG-type G domain-containing protein	0.45	1	1	1546	178.1	6.29

(C)

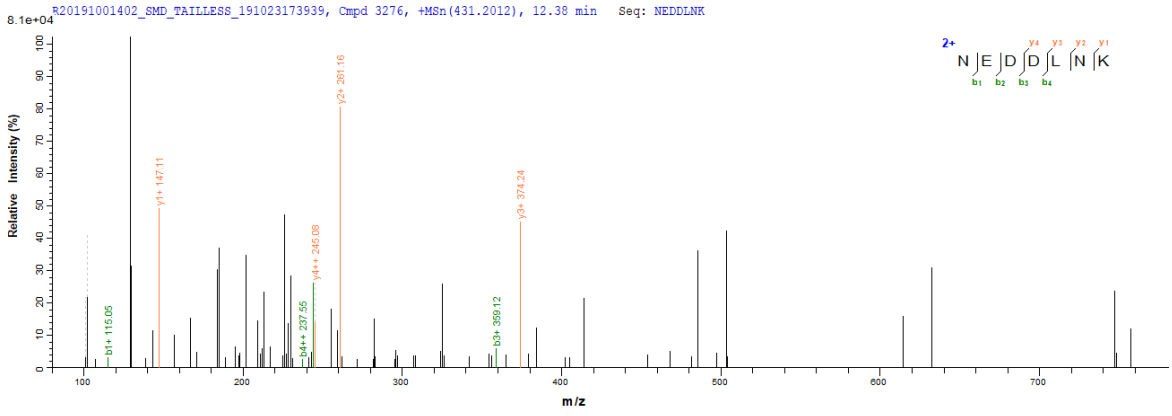
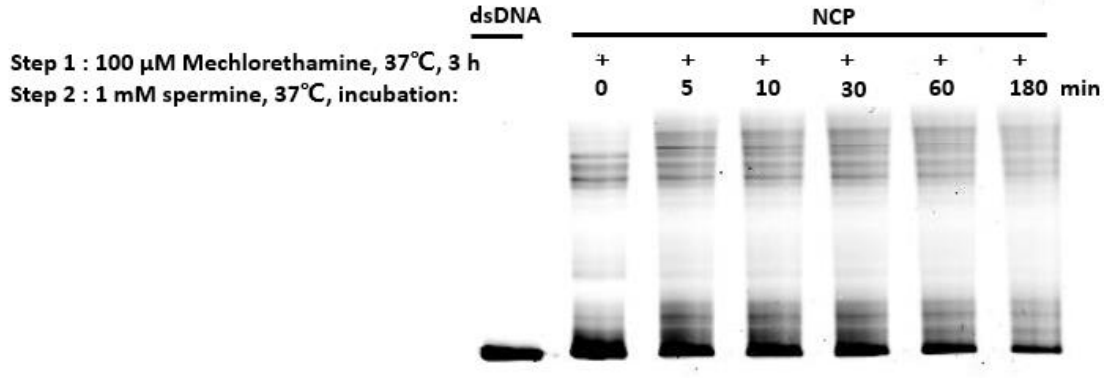


Figure S3. Identification of the DPC formed in tailless NCP. (A)12% SDS PAGE showing that the main DPC induced by mechlorethamine in tailless NCPs co-migrate with the AP^{89}-tailless H2A conjugate. Mix: mixture of H4-del 1-20, H3-del 1-37, H2A-del 1-15, H2B-del 1–31. The dsDNA molecule is 5'-FAM labeled and the gel was visualized with an Amersham Typhoon Gel and Blot Imaging System at excitation and emission wavelengths of 488 and 526 nm, respectively. The DPC migrated faster than dsDNA–H3 but slower than dsDNA–H2B.
(B) Proteins identified for the DPC formed in tailless NCP by MS. The DPC band formed in the

tailess NCP was excised and analyzed by trypsin digest-coupled LC-MS/MS. MS/MS spectra were searched using MASCOT engine (Matrix Science, London, UK; version 2.2) embedded into Proteome Discoverer 1.4 (Thermo Electron, San Jose, CA.) against Uniprot Xenopus laevis database (uniprot_Xenopus_laevis_57803_20191021.fasta) and the decoy database.
(C) The MS/MS spectrum of the unique peptide from the identified protein H2A.

(A)



(B)

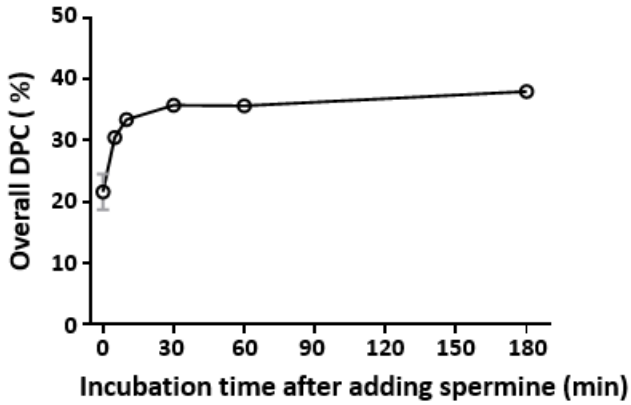


Figure S4. Stability of mechlorethamine-induced DPC in the presence of 1 mM spermine. (A) 12% SDS PAGE showing the time-dependent DPC stability in the presence of 1 mM spermine. NCPs were first incubated with mechlorethamine at 37 °C for 3 h to allow DPC formation, then spermine (final 1 mM) was added followed by incubation at 37 °C. The variation of DPC yields is shown in (B).

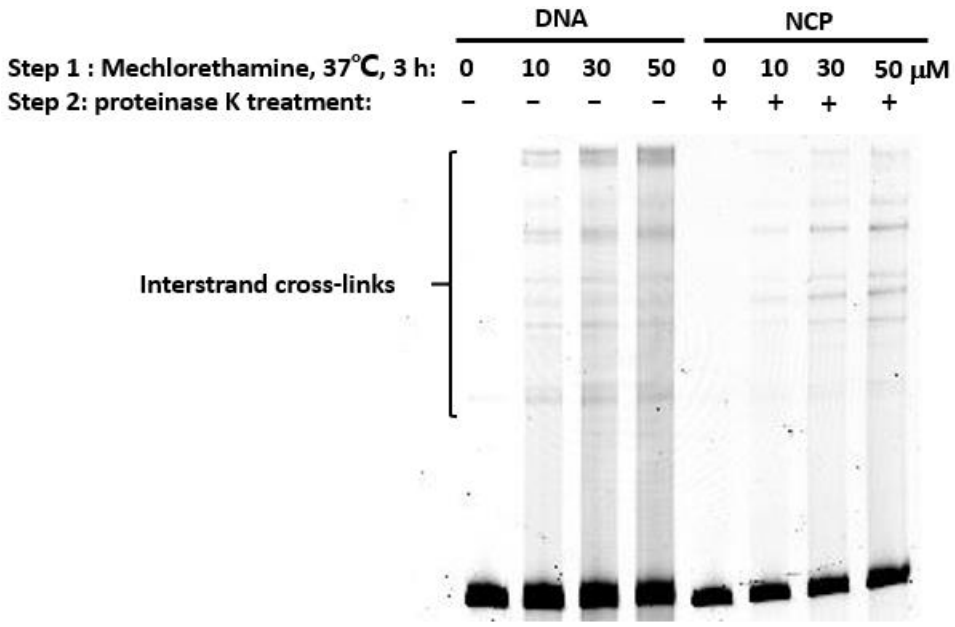


Figure S5. 8% denaturing PAGE showing the interstrand cross-link formation in dsDNA and NCPs upon treatment with different concentrations of mechlorethamine at 37 °C for 3 h.

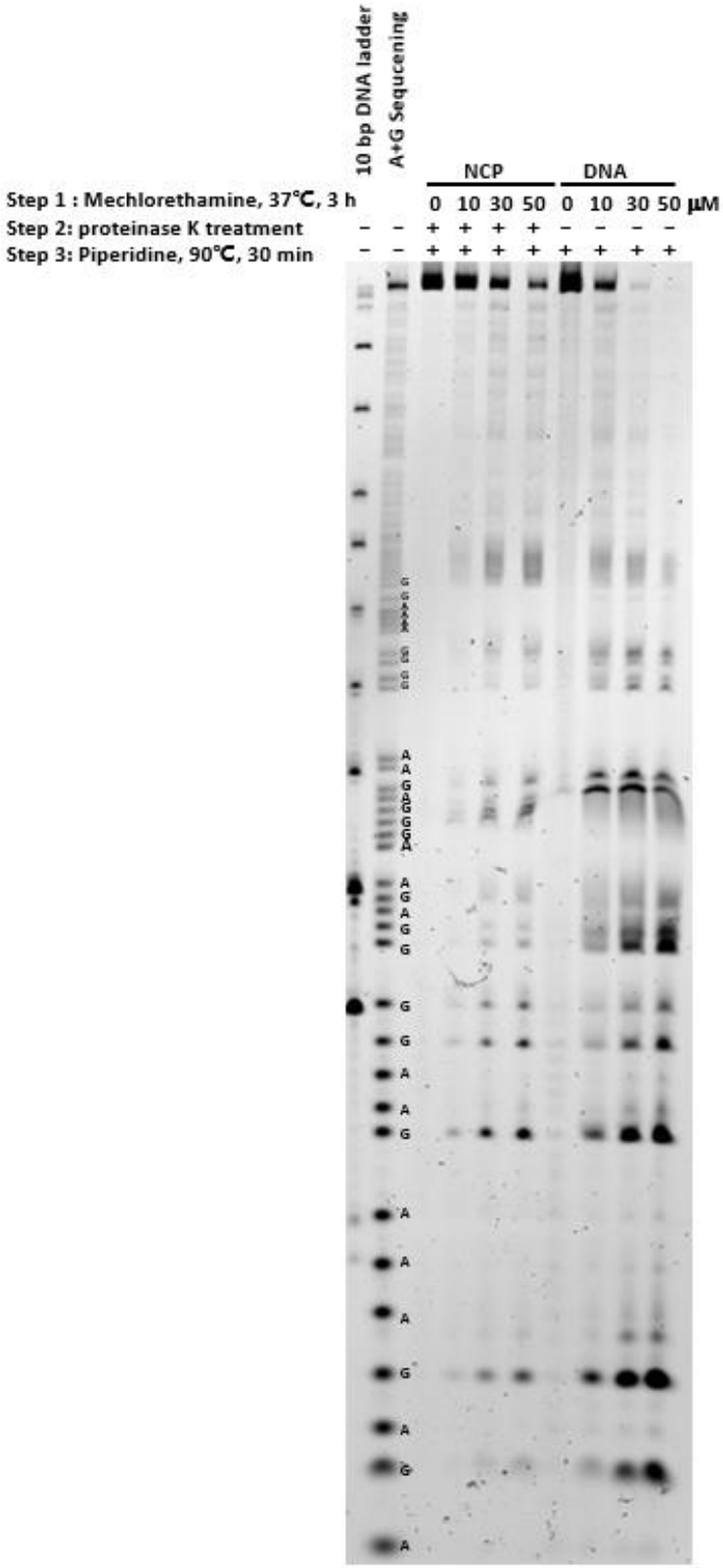


Figure S6. 8% denaturing PAGE showing the overall DNA damage in dsDNA and NCPs upon treatment with different concentrations of mechlorethamine at 37 °C for 3 h.

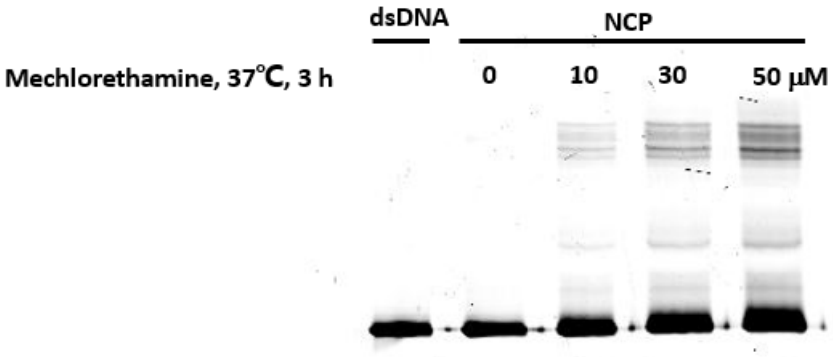
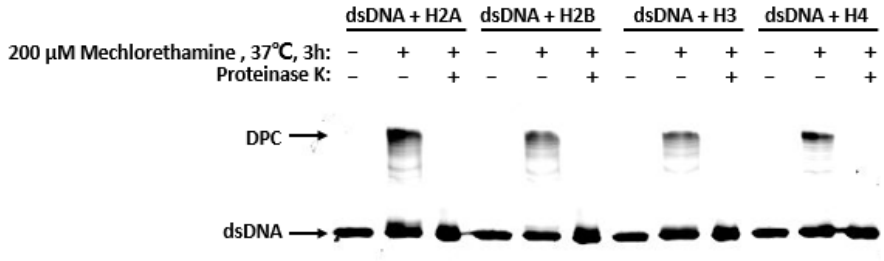


Figure S7. 12% SDS PAGE showing the DPC formation in NCPs upon treatment with different concentrations of mechlorethamine at 37 °C for 3 h.

(A)



(B)

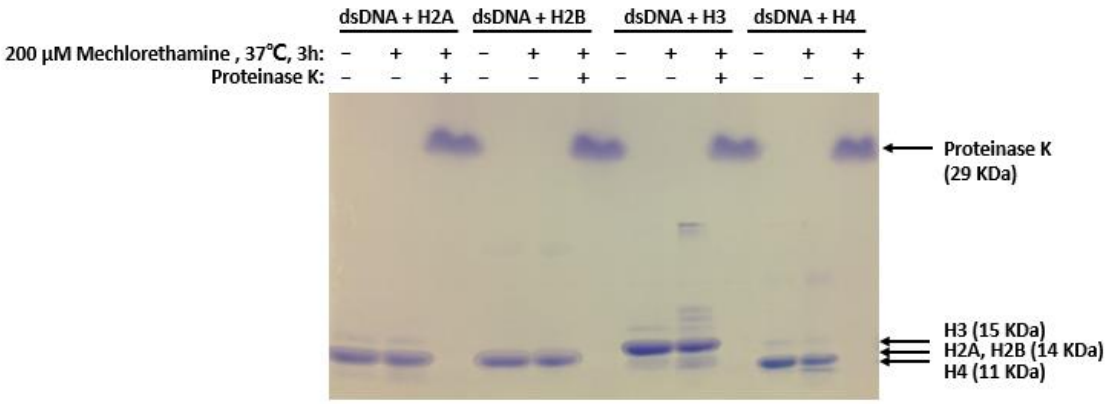


Figure S8. 12% SDS PAGE showing the reactions between dsDNA and free histones in the presence of mechlorethamine. Reaction conditions: 145 bp '601' dsDNA (0.2 μM) and histone (0.4 μM) were mixed in HEPES buffer (10 mM, pH 7.5), total volume 50 μL. Mechlorethamine (final 200 μM) was added and the mixture was incubated at 37 °C for 3 h. The reaction was split into two portions. One was directly analysed by 12% SDS PAGE. The another one was treated with proteinase K (10 μg) for 5 min prior to the 12% SDS PAGE analysis. The gel was visualized by fluorescence (A) and then stained with coomassie blue (B).

Sequence of 145 nt single-stranded 601 DNA:

5’(FAM)ATCGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTGGCG
GTTAAAACGCGGGGGACAGGGCGTACGTGCGTTTA^{89}AGCGGTGCTAGAGCTGTCTACG
ACCAATTGAGCGGCCTCGGCACCGGG^{137}ATTCTGAT 3’

Sequence of 145 nt single-stranded 601 cDNA:

3’TAGCTACATATATAGACTGTGCACGGACCTCTGATCCCTCATTAGGGGAACCGCCAATT
TTGCGCCCCCTGTCGCGCATGCACGCAAACTCGCCACGATCTCGACAGATGCTGGTTAA
CTCGCCGGAGCCGTGGCCCTAAGACTA 5’ (cDNA)

Figure S9. Sequence of the 5’-FAM labelled ‘601’ dsDNA.