Supporting Information: Probing Interactions Between Histone Tails and Nucleosomal DNA via Product and Kinetic Analysis.

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General Methods. Oligonucleotides containing phenyl selenide or C4-AP precursors (3 and 2) were synthesized and purified as previously described.\textsuperscript{[1,2]} DNA containing 3 was subjected to additional purification by reversed-phase HPLC on a RP-C\textsubscript{18} column (VARIAN, Microsorb-MV 100-5 C\textsubscript{18} 250 4.6 mm). The peak of interest was collected using the following gradient conditions: 0-5 min 0-2\% B in A, 5-15 min 2-12\% B in A, 15-40 min 12-20\% B in A, 40-50 min 20-30\% B in A, 50-55 min 30-80\% B in A, 55-60 min 100\% A, at a flow rate 1.0 mL/min. [A: 0.05 M (Et\textsubscript{3}NH)OAc (pH 7.0)/MeCN 95:5; B: 0.05 M (Et\textsubscript{3}NH)OAc (pH 7.0)/MeCN 50:50]. Expression and purification of core histone
proteins, as well as refolding and purification of the histone octamer, were carried out as previously described. T4 polynucleotide kinase, T4 DNA ligase, and DNase I were purchased from New England Biolabs (NEB). Nuclease P1 (from Penicillium citrinum) was from Sigma and was dissolved in water (1 U/µL). Benzonase was purchased from Sigma. γ-32P-ATP was purchased from Perkin Elmer. C18-Sep-Pak cartridges were obtained from Waters. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics phosphorimager equipped with ImageQuant Version TL software. Salmon sperm DNA (10 mg/mL) was purchased from Invitrogen. All experiments with NCPs were conducted in clear siliconized tubes (Bio Plas Incorporated). Photolyses of oligonucleotides were carried out in a Rayonet photoreactor (RPR-100) equipped with 16 lamps with a maximum output at 350 nm. ESI-MS analysis was carried out on a LCQ-Deca Ion Trap. UPLC/MS system is an Acquity UPLC H-Class/Xevo G2 QTof from Waters. MALDI-TOF MS analysis was carried out on Bruker AutoFlexIII MALDI-TOF.

**Thermolysin digestion of isolated H4.** The preparation of NCPs containing 2 in large scale was described previously. After incubating NCP (~ 4 nmol) containing C4-AP89 and C4-AP234 at 37 °C for 8 h, 1 × benzonase buffer (50 mM Tris-HCl, pH 8.8, 10 mM MgCl2, 20 mM NaCl) was added, followed by the addition of 1 µL (250 units) of benzonase. The four histone proteins were separated by RP-HPLC as previously described. HPLC purified H4 (~ 1 nmol, 10 µg) and thermolysin (0.5 µg) were mixed in a 50 µL solution containing 50 mM NH4HCO3 and 1 mM CaCl2. The reaction mixture was incubated at 37 °C for 3 h, quenched with TFA to a final concentration of 1%. An aliquot (10 µL) was analyzed by UPLC-MS/MS. The column used was an ACQUITY UPLC HSS T3 Column, 100Å, 1.8 µm, 2.1 mm × 100 mm (From Waters). Buffer A is water, Buffer B is MeCN, Buffer C is 1% formic acid. Gradient: 0-1-36 min, B from 5%-5%-40%, keep C at 10% constantly. Column temperature: 35 °C, flow rate: 0.3 mL / min.
In-gel acetylation and trypsin digestion of H4. The histone H4 with modification was purified and subjected to in-gel acetylation followed by trypsin digestion as described previously. After drying the digested peptides in speedvac, the residue was dissolved in 0.1% TFA (30 µL). An aliquot (10 µL) was subjected to UPLC-MS/MS analysis under the same conditions as described above.

General procedure for the oxidation reaction of NCPs containing 3. The ligation of 145 mer 601 DNA containing 3 and the reconstitution of NCPs were carried out as described previously. However, the oligonucleotides and ligated strands were different (Figure S2). To the reconstituted NCP solution was added 5 mM NaIO4. Following incubation at 37 °C for 2 h, the samples were divided into two portions. One portion was treated with proteinase K (0.1 µg) for 5 min at room temperature and analyzed by 8% denaturing PAGE (40 × 32 × 0.04 cm). To the second portion was directly added 4 × SDS loading buffer (400 mM Tris•HCl, 400 mM DTT, 8% SDS, 40% glycerol) and analyzed by SDS PAGE (10% resolving acrylamide/bisacrylamide = 29:1, 5% stacking layer, 20 × 16 × 0.1 cm). The gel was run at 250 V until the bromophenol blue band migrated to the bottom.

General procedure for the kinetic studies. NCPs containing 5’-radiolabeled DNA duplex were mixed with 3 mM NaIO4 without additional buffer. Na2SO3 (30 mM) was added 5 min afterwards to react with excess NaIO4. An aliquot was withdrawn from the reaction and immediately put in -80 °C at each indicated time point. The aliquot was mixed with 4 × SDS loading buffer before being subjected to SDS PAGE analysis (10% resolving layer, acrylamide/bisacrylamide 29:1, 5% stacking layer, 20 × 16 × 0.1 cm).

LC-MS analysis for the reactions between phenyl-selenide-containing monomer (3) and protected amino acids. Typically, monomer 3 (1 mM) was mixed with protected amino acid 6a-f (50 mM) and 10 mM sodium phosphate buffer (pH 7.2), to which NaIO4 (5 mM) was added. If the removal of excess NaIO4 was required, Na2SO3 (50 mM) was added to the mixture 5 min after the
addition of NaIO₄. The resulting solution was incubated at 37 °C for overnight. The reaction was diluted by 5-fold prior to being subjected to UPLC-MS analysis. The column and the temperature of the column was the same as mentioned above. The separation condition is 0.1 % formic acid in water (solvent A) and acetonitrile (solvent B), 0.3 mL/min and using the following linear gradient: (time (min), % B) 5, 0; 18, 10; 25, 25; 30, 90; 35, 0. Mass spectra were acquired in positive ion mode with MSE using a capillary voltage of 3 kV, a sample cone voltage of 30 V and an extraction cone voltage of 4 V. The cone gas flow was set up to 30 L/h and desolvation gas flow was 800 L/h. Desolvation temperature and source temperature were set to 400 and 150 °C, respectively. The acquisition range was m/z 100-3000. The scan acquisition rate was 10 Hz. The LC-MS system was operated by the Mass Lynx software.

**DNase I footprinting of NCPs.** To the concentrated NCP solution (from 240 µL to ~ 40 µL by 10K Amicon concentration device) was added 2 µL of 10 × DNase I buffer (10 mM Tris•HCl, 2.5 mM MgCl₂, 0.5 mM CaCl₂, pH 7.6) and indicated amounts of DNase I (Figure S11-S12). The reaction was carried out for 5 min at room temperature and quenched by adding 1 µL of 0.5 M EDTA. Nucleoprotein gel electrophoresis (10 × 8 × 0.15 cm, 6% acrylamide/bisacrylamide, 59:1, 0.6 × TBE buffer) was used to purify NCP with cleaved DNA. The intact NCP band was excised from the gel and the DNA was eluted overnight in 500 µL of elution buffer (0.2 M NaCl and 1 mM EDTA) containing 0.1% SDS. The gel particle was filtered using a polyprep-column (BioRad) and the filtrate was mixed with 1 µg of salmon sperm DNA followed by ethanol precipitation. The obtained samples were analyzed by 8% denaturing PAGE (40 × 32 × 0.04 cm).

**Determining the protein(s) involved in cross-linking with 4.** DNA duplexes containing 5′-³²P-3 were assembled in the same manner as previously described[^4] except the oligonucleotides and ligated duplexes were those depicted in Figure S4. To the NCP solution (typically 240 µL) was added 5 mM
NaIO₄. Following incubation for 4 h, the mixture was concentrated with Amicon Ultra 10K centrifugal filter to 50 µL. The resulting solution was diluted to 500 µL of 0.1% SDS in water followed by concentration again to 50 µL in the same Amicon tube. An aqueous solution of benzonase (2 U, 0.5 µL) and buffer are added to create a 100 µL solution containing 50 mM Tris•HCl pH 8.0, 2 mM MgCl₂, 20 mM NaCl. After incubation at 37 °C for 2 h, the reaction mixture was diluted with 1 × Antarctic phosphatase buffer (50 mM bis-Tris-Propane•HCl, pH 6.0, 1 mM MgCl₂, 0.1 mM ZnCl₂) to 500 µL and concentrated using Amicon Ultra 10K centrifugal filter to 50 µL. Repeat the dilution-concentration once more in the same Amicon tube. The final volume was adjusted to 100 µL, to which was added 2 U nuclease P1. Following 4 h incubation, 900 µL of cold acetone was added and the mixture was kept at -20 °C overnight. The proteins were pelleted by centrifugation (16,000 g) and washed with 500 µL of cold acetone. After drying, the proteins were analyzed by SDS-PAGE (18% resolving layer, acrylamide/bisacrylamide 29:1, 20 × 16 × 0.1 cm, 5% stacking layer). Samples analyzed by TAU PAGE were dissolved in 8 M urea solution containing 10 mM DTT and 5% acetic acid. TAU gels (15 %, acrylamide/bisacrylamide 59:1, 8 M urea, 5 % acetic acid, 0.37 % Triton X-100) were prepared and eletrophoresed as described previously. [6] Wild-type histone octamer were loaded as reference. Both SDS and TAU PAGE were first stained with Coomassie blue, and then subjected to phosphorimaging analysis.

**Preparation of the plasmids for expression of mutated histones.** The plasmids used for expression of mutated H4 were prepared by site-directed mutagenesis. The WT histone H4 (pET3a-H4-WT) plasmid was obtained as a gift from Professor Greg Bowman (JHU Biophysics). Plasmid for H4 K8, 12, 16, 20R was obtained from template of pET3a-H4-K5, 8, 12, 16, 20R, which was previously prepared. [7] Similarly, plasmid for H4 K8, 12, 16, 20R, H18A was prepared from template of pET3a-H4-K5, 8, 12, 16, 20R, H18A. The vector is pET3a and inset DNA sequences are listed below.
Sequence of pET3a-H4-K5, 8, 12, 16, 18R:

-CTTTAAGAAGGAGATACA TATG (start codon)

1
tct ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt cgt cgt cgt cac cgt
tct ggt cgt cgt gac aac atc cag ggt atc acc aag ccg gct atc cgt atg gct
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt ctc atg tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaa cgt atc ctc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaa cgt atc ctc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaa cgt atc ctc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaa cgt atc ctc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaa cgt atc ctc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaa cgt atc ctc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaa cgt atc ctc ggt atc tac gaa gaa acc cgc ggt gtt

Sequence of pET3a-H4-K5, 8, 12, 16, 18R, H18A:

-CTTTAAGAAGGAGATACA TATG (start codon)

1
tct ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt cgt cgt cgt cac cgt
tct ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt ggt cgt cgt cgt cgt cac cgt
tct ggt cgt ggt cgt ggt cgt ggt ggt ggt cgt cgt cgt cgt cgt cgt cgt cgt cgt cgt cgt cgt cgt cgt cgt

cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt
cgt gtt cgt ggt gtt aaaa cgt atc tcc ggt atc tac gaa gaa acc cgc ggt gtt

AAA CAA ACC GGT TAC TAC GAA GGT CAC
aaa cgt aaa acc gtt acc gct atg gac gtt gtt tac gct ctg aaa cgt cag ggt cgt

96                                102
acc ctg tac ggt ttc ggt ggt TAA (stop codon) AGA TCCGGCTGC-

**Preparation of plasmid for H4 K8, 12, 16, 20R (pET3a-H4-K8, 12, 16, 20R)**

Template: pET3a-H4-K5, 8, 12, 16, 20R

Forward primer: 5’–CATATGTCTGGTCGTGGTAAAAGGTGGTGGCTTGGTGTC–3’
Reverse primer: 5’-GACCCAGACCACGACCACCTTTACCACGACCAGACATATG-3’

**Preparation of plasmid for H4 K8, 12, 16, 20R, H18A (pET3a-H4-K8, 12, 16, 20R, H18A)**

Template: pET3a-H4-K5, 8, 12, 16, 20R, H18A

Forward primer: 5’–CATATGTCTGGTCGTGGTAAAAGGTGGTGGCTTGGTGTC–3’
Reverse primer: 5’-GACCCAGACCACGACCACCTTTACCACGACCAGACATATG-3’

[1] Sczepanski, J. T. et al. *J. Am. Chem. Soc*. 2008, 130, 9646.

[2] Peng, X. et al. *J. Am. Chem. Soc*. 2008, 130, 12890.

[3] Dyer, P. N. et al. *Methods Enzymol.* 2004, 375, 23.

[4] Zhou, C. et al. *J. Am. Chem. Soc*. 2013, 135, 5274.

[5] Sczepanski, J. T. et al. *Proc. Nat. Acad. Sci. USA* 2010, 107, 22475.

[6] Lennox, R. W. et al. *Methods Enzymol.*, 1989, 170, 532.

[7] Sczepanski, J. T. *Biochemistry*, 2013, 52, 2157
(S1) ATC GAT GTA TAT ATC TGA CAC GTG CCT GGA
(S2) GAC TAG GGA GTA ATC CCC TTG GCG GTT AAA ACG CG
(S3) GGG GAC AGC GCG TAC
(S4) GGG GAC A3,3C GCG TAC G
(S5) GTG CGT TT3,6 A GC GGT GCT AG
(S6) TG CGT TTA AGC GGT GCT AG
(S7) AGC TGT CTA CGA CCA ATT GAG CGG CCT CGG CAC CGG GAT TCT GAT
(S8) CTC CCT AGT CTC CAG GCA CG
(S9) GTA GAC AGC TCT AGC ACC GCT AAA ACG CAC GTA CGC GCT GTC CCC CGC GTT TT
(S10) GGG GAC AGC GCG TAC GTG CGT TTA AGC GGT GCT AG
(S11) AG CTG TCT ACG ACC AAT T3,13A GCG GCC T
(S12) CGG CAC CGG GAT TCT GAT
(S13) CGC TGT CCC CCG CGT TTT AA
(S14) GTA GAC AGC TCT AGC ACC GC
(S15) CGG TGC CGA GCC CGC T
(S16) ATC AGA ATC CCG GTG CCG AGG CCG CTC AAT TGG TC
(S17) GTA GAC AGC TCT AGC ACC GCT 3,22AA ACG CAC
(S18) GTA GAC AGC TCT AGC ACC GCT AAA ACG CA
(S19) GTA CGC GCT GTC CCC CGC GTT TT
(S20) CG TAC GCG 3,21TG TCC CCC GCG TTT TAA
(S21) CGG CCA AGG GGA TTA CTC CTT AGT CTC CAG GCA CGT GTC AGA TAT ATA CAT CGA T
(S22) AGC TGT CTA CGA CCA ATT GA
(S23) CGC GTA CGA AAA CGC G
(S24) CCC TTG GCG GTT AAA ACG CG
(S25) GTA GAC AGC TCT AGC ACC GCT AAA A
(S26) 3,205 CG CAC GTA CGG GCT GTC CCC CGC GTT TTA A
(S27) ATC AGA ATC CCG GTG CCG
(S28) AGG CCG CT\textsubscript{3,72} AAT TGG TC
(S29) GGG GAC AGC GCG TAC GTG CGT TTA
(S30) GGG GAC A
(S31) \textcolor{green}{3\textsubscript{8}}AG CGG TGC TAG
(S32) \textcolor{green}{3\textsubscript{174}}CG CGT ACG TGC GTT TAA GCG GTG CTA G
(S33) AGC TGT CTA CGA CCA ATT
(S34) \textcolor{green}{3\textsubscript{16}}AG CGG CCT CGG CAC CGG GAT TCT GAT
(S35) GTA GAC AGC TCT AGC ACC GCT
(S36) GTA GAC AGC TCT AGC ACC GCT AAA ACG CAC GTA CGC G
(S37) \textcolor{green}{3\textsubscript{202}}AA ACG CAC GTA CG
(S38) \textcolor{green}{3\textsubscript{213}}TG TCC CCC GCG TTT TAA
(S39) ATC AGA ATC CCG GTG CCG AGG CCG CT
(S40) \textcolor{green}{3\textsubscript{312}}AA TTG GTC

Figure S1. Oligonucleotides synthesized for preparing 145 nt single stranded 601 DNA.
Figure S2. Preparation of 145mer ssDNA by ligation.

Figure S3. Sequence of 601 DNA and position of 3.
Figure S4. Preparation of internally radiolabeled strands.
Figure S5. Representative SDS and TAU gel image for identifying cross-linked proteins with 4 in NCPs. (A) Example SDS PAGE (18%) of histone proteins following the 5'-$^{32}$P-3$_{89}$ transfer assay. Lanes 1, 3, 5 are histone octamers with WT H4 (lane 1), H4 H18A (lane 3) and H4 K5, 8, 12, 16, 20R (lane 5) stained by coomassie blue; lanes 2, 4, 6 are the autoradiograms of histone octamers comprising WT H4 (lane 2), H4 H18A (lane 4) and H4 K5, 8, 12, 16, 20R (lane 6) following DPC formation with the 5'-$^{32}$P-3$_{89}$ and subsequent DNA digestion. (B) Sample TAU PAGE (20%) of histone proteins following 5'-$^{32}$P-3$_{205}$ transfer assay. Lane 1 is commassie blue stained WT histone octamer. Lane 2 is the autoradiogram of the histone octamers composed of WT histone proteins following DPC formation and subsequent DNA digestion.
Figure S6. Kinetic study of 489 in NCPs containing (A) H4 K16A variant; (B) H4 K20A variant; (C) H4 K16,20A variant; (D) H4 K5, 8, 12A variant; (E) H4 H18A variant; (F) H4 K5, 8, 12, 16, 20R variant; (G) H4 K8, 12, 16, 20R variant; (H) H4 K5, 8, 12, 16, 20R/H18A variant; (I) H4 K8, 12, 16, 20R/H18A.
Figure S7. Structures of protected amino acids used in the monomer study: lysine (6a), histidine (6b), cysteine (6c), tyrosine (6d), alanine (6e), aspartic acid (6f), serine (6g), tryptophan (6h) and arginine (6i).

Figure S8. Structures of amino acid adducts from monomer reactions with 4: lysine adduct (7a), histidine adducts (7b and 7c), cysteine adduct (7d).
Figure S9. Chromatograms of reacting 4 with (A) protected lysine (6a); (B) protected histidine (6b); (C) protected cysteine (6c).
Figure S10. Representative chromatogram for purifying oligonucleotides containing 3 (oligonucleotide S31).
Figure S11. DNase I footprinting of NCPs containing 3_{89} and 3_{205}. Lane 1: DNA 10-bp ladder; lane 2: A + G sequencing on DNA duplex containing 3_{89}; lane 3-4: DNase I digested free 601 DNA containing 3_{89} and 3_{205}; lane 5: DNase I digested NCPs composed of WT histone octamers with 3_{205}; lane 6: DNase I digested NCPs composed of WT histone octamers with 3_{89}; lane 7-12: DNase I digested NCPs composed of histone H4 H18A variant (lane 7-8) or H4 K5, 8, 12, 16, 20R (lane 9-10) or H4 K5, 8, 12, 16, 20R/H18A (lane 11-12) with 3_{89}; lane 13: A + G sequencing on DNA duplex containing 3_{205}. DNase I was added to each sample in the following amounts: 0.1 unit for lanes 7, 9, 11; 0.2 unit for lanes 5, 6, 8, 10, 12.
Figure S12. DNase I footprinting of NCPs containing 3_{89} and 3_{202}. Lane 1-2: DNase I digested free 601 DNA containing 3_{89} and 3_{202}; lane 3: DNA 10-bp ladder; lane 4-9: DNase I digested NCPs composed of WT histone proteins (lane 4-5) or histone H4 K16, 20A variant (lane 6-7) or histone H4 K5, 8, 12A variant (lane 8-9) with 3_{202}; lane 10-15: DNase I digested NCPs composed of histone H4 K16, 20A variant (lane 10-11) or histone H4 K5, 8, 12A variant (lane 12-13) or histone H4 del 1-20 (lane 14-15) with 3_{89}; DNase I was added to each sample in the following amounts: 0.1 unit for lanes 4, 6, 8, 10, 12, 14; 0.2 unit for lanes 5, 7, 9, 11, 13, 15.
Figure S13. (A) DNase I footprinting of NCPs containing 3₁₁₇ (lanes 4-5), 3₁₁₂ (lanes 6-7) and 3₂₁₈ (lanes 8-9). Lane 1: DNA 10-bp ladder; lane 2: A + G sequencing on DNA duplex containing 3₁₁₇; lane 3: DNase I digested free 601 DNA containing 3₁₁₇; DNase I was added to each sample in the following amounts: 0.1 unit for lanes 4, 6, 8; 0.2 unit for lanes 5, 7, 9. (B) DNase I footprinting of NCPs containing 3₇₃ (lanes 4-5) and 3₇₃⁺ (lanes 6-7, * indicates T₃A sequence); lane 1: DNA 10-bp ladder; lane 2: A + G sequencing on DNA duplex containing 3₇₃; lane 3: DNase I digested free 601 DNA containing 3₇₃; DNase I was added to each sample in the following amounts: 0.1 unit for lanes 4, 6; 0.2 unit for lanes 5, 7.
Figure S14. ESI-MS spectra of modified oligonucleotides.
(834) GAG CGG CCT CGG CAC CGG GAT TCT GAT
Mass Calc. [M-H]: 8448.4
**Figure S15.** MALDI-TOF mass spectra of modified oligonucleotides (the laser of MALDI-TOF instrument can cleave the phenyl selenide group, resulting in a peak with 156 Da less than the expected molecular weight).

(S4) GGG GAC A373C GCG TAC G
Mass Calc: [M+H]+: 5102.2

(S5) GTG CGT TT389 AGC GGT GCT AG
Mass Calc [M+H]+: 6350.0
(S11) AG CTG TCT ACG ACC AAT T3_{119} A GCG GCC T
Mass Calc [M+H]^+: 8391.4

(S17) GTA GAC AGC TCT AGC ACC GCT 3_{202} AA ACG CAC
Mass Calc [M+H]^+: 9290.0
(S20) CG TAC GCG 3_{18} TG TCC CCC GCG TTT TAA
Mass Calc [M+H]^+: 8044.1

(S28) AGG CCG CT_{172} AAT TGG TC
Mass Calc [M+H]^+: 5356.4
**(S32)** 3.CG CGT ACG TGC GTT TAA GCG GTG CTA G
Mass Calc [M+H]: 8806.6

**(S37)** 3.AA ACG CAC GTA CG
Mass Calc [M+H]: 4411.8
(S38) $3_{218}$TG TCC CCC GCG TTT TAA
Mass Calc [M+H]$^+$: 5571.6

(S40) $3_{172}$AA TTG GTC
Mass Calc [M+H]$^+$: 2883.8
Figure S16. ESI mass spectra of histone H4 K8, 12, 16, 20R and K8, 12, 16, 20R/H18A variants.

H4 K8, 12, 16, 20R, cal. [M+H]^+ = 11349.2

H4 K8, 12, 16, 20R/H18A, cal. [M+H]^+ = 11283.1
Figure S17. LC-MS/MS spectra of peptide fragments following thermolysin digestion of histone H4. A. Fragmentation pattern of peptide containing 1 at Lys16 (K16). B. Fragmentation pattern of peptide containing 1 at Lys12 (K12).
Table S1. Calculated and observed fragmentation patterns for peptides in Figures 2, 3, and Figure S17.

|                  | Cal.     | Found    |
|------------------|----------|----------|
| **Figure 2A.**   |          |          |
| Fragment 20-23   |          |          |
| (K20 mod.)       |          |          |
| y1               | 175.1195 | 175.1200 |
| y2               | 288.2036 | 288.2056 |
| y3               | 387.2720 | 387.2746 |
| a2*              | 278.1869 | 278.1870 |
| y4*              | 593.3775 | 593.3762 |
| y2-NH3           | 271.1770 | 271.1766 |

| **Figure 2B.**   |          |          |
| Fragment 4-17    |          |          |
| (K16 mod.)       |          |          |
| b2*              | 228.1348 | 228.1384 |
| b3*              | 285.1563 | 285.1574 |
| y1               | 175.1195 | 175.1212 |
| y4*              | 509.2836 | 509.2855 |
| y5*              | 566.3051 | 566.3029 |
| y6*              | 736.4106 | 736.4048 |
| y7*              | 793.4321 | 793.4268 |
| y9*              | 963.5376 | 963.5257 |
| y12*             | 1247.6862| 1247.6775|
| y14*             | 1474.8131| 1474.8136|

| **Figure 2C.**   |          |          |
| Fragment 4-17    |          |          |
| (K12 mod.)       |          |          |
| b8*              | 739.4103 | 739.4103 |
| y1               | 175.1195 | 175.1222 |
| y5*              | 530.3051 | 530.3022 |
| y14*             | 1474.8131| 1474.8159|
Figure 3. Fragment 1-9 (K8 mod.)

|   | Cal.   | Found   |
|---|--------|---------|
| b5 | 486.2788 | 486.2782 |
| b7 | 600.3218 | 600.3230 |
| a7 | 572.3268 | 572.3288 |
| a8* | 778.4324 | 778.4362 |
| y9* | 881.4594 | 881.4558 |

Figure S17A. Fragment 10-20 (K16 mod.)

|   | Cal.   | Found   |
|---|--------|---------|
| y3 | 440.2734 | 440.2713 |
| y7* | 930.5386 | 930.5446 |
| y8* | 987.5601 | 987.5624 |
| y10* | 1172.6765 | 1172.6753 |
| y10-H2O* | 1154.6659 | 1154.6577 |
| y11-H2O* | 1267.7500 | 1267.7426 |
| y11* | 1285.7605 | 1285.7614 |
| y7-NH3* | 913.5121 | 913.5135 |

Figure S17B. Fragment 10-20 (K12 mod.)

|   | Cal.   | Found   |
|---|--------|---------|
| y3 | 440.2734 | 440.2760 |
| y8 | 909.5495 | 909.5525 |
| y10* | 1172.6765 | 1172.6761 |
| y11* | 1285.7605 | 1285.7588 |
| y8-NH3 | 892.5229 | 892.5279 |