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Supplementary Information For

Total chemical synthesis of sumoylated histone H4 reveals negative biochemical crosstalk with histone ubiquitylation

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1. General Methods

2-Chlorotrityl resin (1.5 mmol/g substitution) was purchased from AnaSpec (Fremont, CA). Fmoc-Gly-Wang resin (0.32 mmol/g substitution) was purchased from EMD Millipore (Gibbstown, NJ). Fmoc-L-amino acids were purchased from Chem-Impex Int'l. (Wood Dale, CA), AGTC Bioproducts (Wilmington, MA), Creosalus (Louisville, KY), and Sigma-Aldrich (St. Louis, MO). Other chemical reagents were purchased from either Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich Chemical Company (St. Louis, MO). Plasmid mini-prep, PCR purification and gel extraction kits were purchased from QIAGEN (Valencia, CA). Reversed-phase HPLC (RP-HPLC) was performed on either a Varian (Palo Alto, CA) ProStar HPLC or an Agilent (Santa Clara, CA) 1260 Infinity II HPLC using an Agilent Poroshell 120 SB C18 analytical column (2.7 micron, 100 x 4.6 mm) at a flow rate of 1 mL/min, a Grace-Vydac (Deerfield, IL) semi-preparative C18, C4 column (5 micron, 150 x 4.6 mm) at a flow rate of 3.5 mL/min, or a Grace-Vydac preparative C18 column (10 micron, 250 x 22 mm) at a flow rate of 9 mL/min. Mobile phases were Buffer A containing 0.1% trifluoroacetic acid (TFA) in water, and Buffer B containing 90% acetonitrile and 0.1% TFA in water. Solid phase peptide synthesis was performed on a Liberty Blue Automated Microwave Peptide Synthesizer (CEM Corporation, Matthews, NC). Mass spectrometric analysis was conducted on a Bruker (Billerica, MA) Esquire, or a Thermo Scientific (Waltham, MA) LTQ Orbitrap ESI-MS.

2. Synthesis of H4(1-37)K12$_{Cys}$ (1)

H_2N-SGRGKGGKGLGK(Cys)GGAKRHRKVLRDNIQGITKPAIRRL-C(O)$NHNH_2$

The 2-chlorotrityl hydrazide resin for peptide synthesis was prepared by reacting 2-chlorotrityl chloride resin (1.5 mmol/g, 0.3 mmol) in a 10% (v/v) solution of hydrazine in DMF at 30 °C for 30 min.[1] The reaction was repeated once with fresh 10% (v/v) hydrazine solution. The resin was then treated with 10% (v/v) methanol in DMF for 10 min to cap any unreacted sites on the resin. In order reduce the loading capacity of resin the first amino acid, Leu, was coupled as mixture of Fmoc-Leu-OH and Boc-Leu-OH in a 1:2 molar ratio, respectively. The coupling reaction with Fmoc-Leu-OH (0.4 mmol), Boc-Leu-OH (0.8 mmol) *O*-(6-Chlorobenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HCTU, 1.1 mmol), and DIEA (2.4 mmol) proceeded for 60 min at 30 °C. Any unreacted hydrazine sites were then capped by treating the resin with

acetic anhydride (0.6 mmol), and DIEA (0.6 mmol) for 30 min at 25 °C and the reduced Fmoc-amino acid loading of the resin was confirmed by suspending the Fmoc-protected resin in 20% (v/v) piperidine in DMF to generate the dibenzofulvene-piperidine adduct that was quantified by absorbance at 304 nm (E. Next, each sequential amino acid was coupled in a 5-fold molar excess based on the reduced resin loading using a CEM microwave-assisted peptide synthesizer. Deprotection of the Fmoc- group was achieved by treating the resin with 5% (w/v) piperazine with 0.1 M HOBt in DMF for 3 min at 75 °C. Coupling reactions were undertaken for 10 min at 75 °C with a mixture of Fmoc-amino acid (0.5 mmol), DIC (0.49 mmol), Oxyma (0.49) and DIEA (0.98 mmol) in DMF. For Fmoc-Arg(Pbf)-OH, an additional coupling reaction was performed for 10 min at 75 °C. The Lys at position 12 was orthogonally protected with the 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene (ivDde) protecting group. The N-terminal amino acid, Ser was coupled as Boc-Ser-(O^{t}Bu)-OH. After the completion of linear amino acid couplings, the orthogonal deprotection of ivDde protecting group at K12 was performed by treating the resin-bound peptide with a solution of 5% (v/v) hydrazine in DMF for 5 min. The deprotection was repeated twice to ensure completion. Finally, the side-chain deprotected peptidyl resin was coupled with Boc-Cys(Trt)-OH (0.5 mmol) with DIC (0.49 mmol), Oxyma (0.49) and DIEA (0.98 mmol) in DMF to attach Cys at K12 as a ligation handle for SUMO-3 α-thioester ligation.

Peptide cleavage from the resin. The final resin was successively washed with DMF and DCM, then dried under vacuum. A cleavage cocktail consisting of TFA, triisopropylsilane and water, 95:2.5:2.5 (v/v), was added to the resin and the mixture shaken for 2 h at 25 °C. The resin was filtered, and the combined filtrate was added dropwise to a 10-fold volume of ice-cold diethyl ether and the resulting precipitate was centrifuged at 3000 rpm for 5 min at 25 °C. The clear solution was decanted and the precipitated crude peptide was dissolved in 50% (v/v) AcN in H_2O, filtered through a 0.45 μm filter and lyophilized to a dry powder. The dry peptide was dissolved in RP-HPLC Buffer A and then purified by C18 preparative RP-HPLC with a gradient of 0-73% B over 30 min. The isolated yield after lyophilization was 12% based on the initial resin loading. Calculated for **1**, M_{avg} 4,055.2 Da and observed 4,054.8 ± 0.6 Da.

3. Synthesis of H4(38-75)A38C$_{Acm}$ (2)

H_2N-C(Acm)RRGGVKRISGLIYEETRGVLKVFLENVIRDAVTYTEH-C(O)$NHNH_2$

The synthesis of the middle fragment of histone H4 on hydrazide resin followed the same protocol as described for fragment **1**. One key addition was the coupling of Cys(Acm) at the N-terminus using Fmoc-Cys(Acm)-OH (0.5 mmol) with DIC (0.49 mmol), Oxyma (0.49) and DIEA (0.98 mmol) in DMF. The isolated yield of purified peptide was 8% based on the initial resin loading. Calculated for **2**, M_{avg} 4,465.2 Da and observed 4,465.8 ± 0.6 Da.

4. Synthesis of H4(76-102)A76C (3)

H_2N-CKRKTVTAMDVVYALKRQGRTLYGFGG-CO_2H

Fmoc-Gly-Wang resin (0.32 mmol/gm) was allowed to swell for 10 min in excess DMF. Following resin deprotection with 20% piperidine in DMF (v/v), each amino acid was coupled in 5-fold molar excess based on resin loading using the CEM microwave peptide synthesizer. Deprotection of the Fmoc- group was achieved by treating resin with 5% (w/v) piperazine with 0.1 M HOBt in DMF for 3 min at 75 °C. Coupling reactions were undertaken for 10 min at 75 °C with a mixture of Fmoc-amino acid (0.5 mmol), DIC (0.49 mmol), Oxyma (0.49) and DIEA (0.98 mmol) in DMF. Cleavage and purification protocols were as mentioned for peptide **1**. The isolated yield of purified peptide was 14%. Calculated for **3**, M_{avg} 3,019.5 Da and observed 3,019.8 ± 0.5 Da.

5. Overexpression and purification of SUMO-3(2-91)C47S-MESNa

E. coli BL21(DE3) cells were transformed with the plasmid pTXB1-SUMO-3(2-91)C47S.[2] Cells were grown in 6 L Luria-Bertani medium supplemented with 100 μg/mL of Ampicillin at 37 °C with shaking at 250 rpm until OD_{600} ~0.6-0.8. Overexpression of the desired fusion protein was induced by the addition of 0.3 mM IPTG, and cells were grown for an additional 4 h at 25 ˚C with constant shaking. The cells were then harvested by centrifugation at 7,000xg for 15 min. The cell pellet was resuspended in a lysis buffer consisting of PBS, pH 7.2, containing 1 mM 2-Mercaptoethanesulfonic acid sodium salt (MESNa). Cells were lysed by sonication then centrifuged at 20,000xg for 15 min. The lysate supernatant was passed through a 0.45 μm filter then applied to a 30 mL chitin column pre-equilibrated with lysis buffer. Proteins were bound to the column over a period of 12 h at 4 °C. The column was then washed with 20 column volumes (CV) of lysis buffer followed by 2 CV of PBS at pH 7.75. The SUMO-3(2-91)C47S-MESNa α-thioester was generated from its intein-chitin-binding domain (CBD) fusion by incubation with 1.5 CV of PBS, pH 7.75, containing 100 mM MESNa for 72 h at 4 °C. The eluted α-thioester was

purified by C18 preparative RP-HPLC employing a gradient of 0-60% B over 60 min. Fractions containing the desired thioester were identified by ESI-MS. Calculated M_{avg} 10,444.7 Da and observed 10,443.8 ± 1.5 Da. The N-terminal Met of SUMO-3 is consistently processed in *E. coli* by the methionyl aminopeptidase enzyme, leading to the SUMO-3(2-91)C47S α-thioester product. Typical yields of the α-thioester were 4-5 mg/L of BL21(DE3) culture.

6. Synthesis of SUMO-3(2-92)C47S,G92A for Circular Dichroism studies

The purified SUMO-3(2-91)C47S-MESNa was dissolved in a buffer containing 6 M Gn•HCl, 200 mM NaPi, 100 mM L-Cysteine and 30 mM TCEP at pH 7.0. The reaction mixture was incubated at 25 °C for 4-6 h to allow L-Cys ligation. Reaction progress was followed by analytical C18 RP-HPLC with a gradient of 0-60% B over 30 min. After the reaction was considered to be complete, the reaction mixture was treated with 250 mM TCEP, 30 equivalents of VA-044 (0.08 mmol), and 10% (v/v) ^{t}BuSH and then incubated at 42 ^{o}C for 6 h in order to desulfurize the C-terminal Cys92 to Ala. The desulfurized product was purified by C18 analytical RP-HPLC with a gradient 0-60 % B over 30 min. Calculated M_{avg} for SUMO-3(2-92)C47S,G92A, 10,373.8 Da and observed 10,374.5 ± 1.4 Da.

7. Circular Dichroism studies of SUMO-3(2-92)C47S,G92A; SUMO-3(2-91)C47S-MESNa α-thioester and H4K12su.

Circular Dichroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter fitted with a Peltier temperature controller. Each pure protein was dissolved at a final concentration of 15 μM in 10 mM NaPi, 100 mM NaF, pH 7.4 at 20 °C in 1 mm quartz cuvettes and measurements were undertaken at a scanning speed of 100 nm/min over a range of 190-270 nm.

8. Expressed protein ligation of H4(1-37)K12$_{Cys}$ (1) with SUMO-3(2-91)C47S-MESNa to generate the sumoylated H4 tail peptide (4)

Purified peptide H4(1-37)K12$_{Cys}$-hydrazide (13 mg, 0.0032 mmol, 2 mM) was dissolved in ligation buffer containing 6 M Gn•HCl, 200 mM NaPi, 100 mM MPAA and 35 mM TCEP at pH 7.2. The dissolved peptide solution was added to SUMO-3(2-91)C47S-MESNa (28.2 mg, 0.0027 mmol, 1.6 mM), and the buffer pH was re-adjusted to 7.2 with dilute aqueous NaOH. The reaction mixture was incubated at 37 °C for 6-8 h to allow expressed protein ligation to proceed. Reaction

progress was followed by analytical C18 RP-HPLC with a gradient of 0-73% B over 30 min. After the ligation was considered to be complete, the reaction mixture was dialyzed against 500 mL of 6 M Gn•HCl, 200 mM NaPi buffer, pH 7.2, overnight to remove unreacted MPAA and TCEP. The dialyzed solution was treated with 10 equivalents of 20 mM aqueous $NaNO_2$ at -15 °C at pH ~3.0 for 20 min to undertake oxidation of the acyl hydrazide to an acyl azide intermediate. Subsequently, 50 equivalents of MPAA (100 mM) were added to the peptidyl azide *in situ* and the pH adjusted to 6.0. The displacement of azide by MPAA was followed by C18 RP-HPLC with a gradient of 20-60 %B over 30 min. The same gradient was used to purify the MPAA α-thioester product by C18 semipreparative RP-HPLC with an isolated yield of 40% over three steps. Calculated M_{avg} for MPAA α-thioester form of **4**, 14,359.4 Da and observed 14,359.9 ± 0.7 Da.

9. Native chemical ligation of H4(38-75)A38C$_{Acm}$ (2) and H4(76-102)A76C (3) to yield H4(38-102)A38C$_{Acm}$,A76C

The peptide H4(38-75)A38C$_{Acm}$ (**2**) (14.5 mg, 0.003 mmol, 2 mM) was dissolved in a buffer consisting of 6 M Gn•HCl, and 200 mM NaP_i at pH 3.0 and then treated with 10 equivalents of 20 mM aqueous $NaNO_2$ at -15 ºC for 20 min. Subsequently, 50 equivalents of MPAA (100 mM) were added to the peptidyl azide *in situ* and the pH adjusted to 6.0. The displacement of azide by MPAA was followed by C4 RP-HPLC with a gradient of 20-80 %B over 30 min. To the reaction mixture of H4(38-75)A38C$_{Acm}$ MPAA α-thioester a solution of H4(76-102)A76C (**3**) (11.6 mg, 0.0024 mmol) in 6 M Gn•HCl, 200 mM NaPi, and 35 mM TCEP at pH 7.2 was added and the pH of the ligation mixture was re-adjusted to 7.2 with dilute aqueous NaOH. The ligation mixture was incubated at 37ºC for 6 h and the reaction was followed by C4 analytical RP-HPLC with a gradient 20-80 % B over 30 min. After the ligation was considered complete, the product was purified using C4 preparative RP-HPLC with a gradient of 20-80% B over 60 min. The isolated yield of the ligation product was 35%. Calculated M_{avg} for **5** was 7,451.9 Da and observed 7,450.8 ± 1.1 Da.

10. Palladium-mediated Acetamidomethyl (Acm) protecting group removal from H4(38-102)A38C$_{Acm}$,A76C (5)

The ligation product H4(38-102)A38C$_{Acm}$,A76C (8.5 mg, 0.0011 mmol) with N-terminal Acm-protected Cys was dissolved in ligation buffer containing 6 M Gn•HCl, 200 mM NaPi, 100 mM

MPAA and 35 mM TCEP at pH 7.2, and incubated with 15 equivalents of $PdCl_2$ for 1 h at 37 °C.[3] The reaction mixture was quenched with 100 mM DTT and centrifuged at 13,000 rpm for 5 min to precipitate the Pd-complex, and the supernatant containing deprotected peptide **5** was purified by C4 preparative RP-HPLC with a gradient of 30-70% B over 60 min. This led to an isolated yield of 85%. Calculated M_{avg} for the Acm-deprotected form of **5**, 7,381.6 Da and observed 7,380.6 ± 1.2 Da.

11. Native chemical ligation of the sumoylated H4 tail peptide (4) with H4(38-102)A38C,A76C (5) to generate H4(1-102)K12SUMO-3(G92C),A38C,A76C and desulfurization to obtain H4K12su (6).

To a solution of the MPAA α-thioester form of peptide **4** (15.6 mg, 0.001 mmol, 2 mM) dissolved in ligation buffer containing 6 M Gn•HCl, 200 mM NaPi, 80 mM MPAA and 40 mM TECP at pH 7.2, the Acm-deprotected H4(38-102)A38C,A76C peptide **5** (7.2 mg, 0.0009 mmol) was added. The pH was re-adjusted to 7.2 and ligation allowed to proceed at 37 °C for 14 h. Reaction progress was followed by C4 analytical RP-HPLC with a gradient of 30-70% B over 30 min. After the ligation was considered complete, the reaction mixture was dialyzed against 500 mL of 6 M Gn•HCl, 200 mM NaPi buffer, pH 7.2, in a 3.5 kDa cut-off dialysis cassette in order to remove excess MPAA. The dialyzed reaction mixture was then treated with 250 mM TCEP, 90 equivalents of VA-044 (0.08 mmol), and 10% (v/v) ^{t}BuSH and then incubated at 42 °C for 6 h in order to desulfurize Cys38 and Cys76 and convert these to the native Ala found in wild-type H4. The reaction was analyzed by C4 analytical RP-HPLC with a gradient of 30-70% B over 30 min and purified by C4 semi-preparative RP-HPLC over a gradient of 30-70%B over 45 min. The isolated yield of pure H4K12sumo-3(C47S,G92A), or H4K12su, was 27%. Calculated M_{avg} for H4K12sumo-3(C47S,G92A), or H4K12su, 21,609.8 Da and observed 21,610.2 ± 1.3 Da.
For simplicity, hereon we refer to H4K12sumo-3(C47S,G92A) as H4K12su in the text below.

12. Octamer assembly

Individual histones H2A, H2B and H3 were dissolved at final concentrations of 4 mg/mL in an unfolding buffer consisting of 7 M Gn•HCl and 20 mM Tris, pH 7.5. Equimolar amounts of the three histones were combined with either wild-type H4 or synthetic H4K12su and the resulting mixture dialyzed into a refolding buffer consisting of 10 mM Tris, 2 M NaCl, 1 mM EDTA at pH

7.5. The self-assembled crude octamers were concentrated with a 10,000 MWCO Vivaspin concentrator and then purified by size-exclusion chromatography using a Superdex S-200 column attached to an AKTA FPLC pump. Fractions containing the desired octameric species were identified by 18% SDS-PAGE followed by coomassie staining, combined, and concentrated with a Vivaspin 500 concentrator prior to nucleosome assembly.

13. Mononucleosome (MN) assembly

Octamers and 147 bp of Widom *601* DNA were combined in 10 µL of the high-salt octamer refolding buffer consisting of 10 mM Tris, 2 M NaCl, 1 mM EDTA at pH 7.5 to a final concentration of 2 µM. After incubation at 37 ^{o}C for 15 min, 3.3 µL of dilution Buffer 1 (10 mM HEPES, 1 mM EDTA, 0.5 mM PMSF, pH 7.9) was added and the incubation temperature was reduced to 30 ^{o}C. Further additions of 6.7, 5.0, 3.6, 4.7, 6.7, 10, 30, and 20 µL of Buffer 1 were undertaken after every 15 min. A final addition of 100 µL of dilution Buffer 2 (10 mM Tris, 1 mM EDTA, 0.5 mM PMSF, 20% (v/v) Glycerol, pH 7.5) was undertaken and after an additional 15 min at 30 ^{o}C, the MNs were concentrated by Vivaspin 500 concentrator, and their composition verified by 5% Tris-Borate-EDTA (TBE) non-denaturing polyacrylamide gel electrophoresis followed by staining with SYBR Safe DNA stain.

14. Desumoylation of synthetic H4K12su by Sentrin-specific protease 2 (SENP2)

The recombinant human His_6-SENP2 (Sentrin-specific protease 2) catalytic domain cloned in pET28a (Addgene Plasmid 16357) was expressed in *E. coli* BL21(DE3) cells and purified using Ni-affinity chromatography using 20 mM Tris and 500 mM Imidazole buffer, pH 7.5, as the eluent.[4] The H4K12su octamer (1 µM) was incubated with His_6-SENP2 (4.5 µM) in desumoylation buffer consisting of 50 mM Tris-HCl, 1 mM DTT, 1 mM PMSF, 0.1 mM EDTA, 10% (v/v) glycerol, pH 7.5 at 4 ^{o}C for 6 hrs. The products were resolved by 18% SDS-PAGE followed by coomassie staining and anti-histone H4 immunoblotting.

15. Overexpression and purification of the full-length Rad6-Bre1 complex.

The full-length *Rad6* and *Bre1* genes were PCR amplified from *Saccharomyces cerevisiae* genomic DNA and sub-cloned into a modified pFastBac vector for co-expression in Sf9 insect

cells. Recombinant viruses were produced as per manufacturer instructions, and subsequently amplified in Sf9 monolayer cells to generate high-titer viruses for protein expression in HighFive monolayer cells. Cells were harvested 2-3 days post-infection and lysed by sonication in 40 mM HEPES buffer, pH 7.5, 350 mM NaCl, 5mM β-Mercaptoethanol. The lysate was cleared of insoluble matter by ultracentrifugation at 30,000*g* for 30 min and the protein complex purified by Glutathione-affinity chromatography (Thermo Fisher) using the GST-tag on Bre1. The protein complex was obtained by overnight on-column cleavage of the GST-tag by TEV protease at 4 °C. The eluted Rad6-Bre1 protein complex was further purified by anion-exchange chromatography on a 1 mL HiTrap Q-HP column (GE Healthcare) followed by size-exclusion chromatography with a Superdex 200 increase column (GE Healthcare) in a final buffer containing 40 mM HEPES, pH 7.5, 100 mM NaCl, and 1mM DTT. The pure proteins were mixed with 20% sterile glycerol and stored in frozen aliquots at -20 °C.

16. Nucleosome ubiquitylation assays with Rad6-Bre1.

For nucleosome ubiquitylation assays, 0.5 μM wild-type or sumoylated mononucleosomes, 100 nM UBE1 (E1 activating enzyme) , 10 μM Rad6 (E2 ligase)-Bre1 (H2BK120-specific E3 ligase) complex, 100 μM Ubiquitin, 3 mM ATP, and 100 μM DTT were mixed in a reaction volume of 10 μL buffered with 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 50 mM KCl, and 10 mM $MgCl_2$. Assays were undertaken for 90 min at 30 °C and stopped by the addition of 4 μL SDS-containing gel-loading buffer. Assay products were analyzed by 15% SDS-PAGE followed by immunoblotting with anti-H2B and anti-H2BK120ub-specific antibodies.

17. Rad6-Bre1 nucleosome binding assays

A fixed concentration of reconstituted wild-type or sumoylated nucleosomes (0.1 μM) was incubated with increasing concentrations (0 to 6 μM) of the Rad6-Bre1 complex in a nucleosome-binding buffer consisting of 50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM $MgCl_2$ at 4 ºC for 45 min. Bound/unbound nucleosomes were resolved by 5% TBE non-denaturing polyacrylamide gel electrophoresis followed by staining with SYBR Safe DNA stain.

18. Transient transfection of HEK293T cells with the HA-SUMO-3(ΔGG)-H4(Δ1-11) fusion, the HA-SUMO3(ΔGG)-H4 fusion, and the Myc_2-H4(Δ1-11) truncant.

HEK293T human embryonic kidney cells were cultured in T75 flasks containing Dulbecco's modified Eagle medium supplemented with 10% (v/v) fetal bovine serum. Cells were grown at 37 °C in an atmosphere of 5% CO_2 until ~50% confluency. Each T75 flask of cells was transiently transfected with 15 µg of pcDNA3.1-HA-SUMO3(ΔGG)-H4(Δ1-11), pcDNA3.1-HA-SUMO3(ΔGG)-H4, or pcDNA3.1-Myc_2-H4(Δ1-11) using Lipofectamine 3000 (Invitrogen). The growth media was changed once 24 h post-transfection and cells grown for an additional 24 h prior to cellular detachment by trypsination. Detached cells were gently collected by centrifugation at 3,000 rpm for 3 min. The resulting cell pellet was washed thrice with Dulbecco's phosphate-buffered saline (PBS) before being subjected to protein purification and subsequent ChIP assays.

19. Native chromatin immunoprecipitation (ChIP) of sumoylated or truncated H4(Δ1-11) nucleosomes.

HEK293T cells harvested 48 h post-transfection were resuspended in a modified STM-N/D buffer consisting of 250 mM Sucrose, 50 mM Tris-HCl, pH 7.4 at 4°C, 5 mM $MgCl_2$, 10 mM Iodoacetamide (IA), 20 mM N-Ethylmaleimide (NEM), 0.5 % (v/v) IGEPAL CA-630, 1x cOmplete protease inhibitor cocktail, 0.5% (w/v) Sodium deoxycholate in order to lyse cells and nuclei were isolated by centrifugation 2.5 kRCF for 5 min at 4 ºC. The precipitated nuclei were resuspended in Buffer 150 consisting of 20 mM Tris, pH 7.4 at 4°C, 0.15 M NaCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 0.1% (v/v) IGEPAL CA-630, 1 mM PMSF, 10 mM IA, 20 mM NEM, 1x cOmplete protease inhibitor cocktail, and 10% (v/v) glycerol followed by digestion with *Micrococcal nuclease* (MNase) diluted in MNase Buffer consisting of 50 mM Tris-HCl, pH 8.0 at 37 °C, 5 mM $CaCl_2$, 20 mM NEM, 10 mM IA, 0.1% (v/v) Triton X-100, and 1x cOmplete protease inhibitor cocktail. Cellular chromatin (400 µL volume) was digested with MNase for 1 hour at 37 °C with mixing every 30 minutes to ensure solution homogeneity. After 1 h, chromatin digestion was halted by the addition of 54 µL of 0.5 M metal-chelator, EGTA, and the digested chromatin mixture was passed through a 25-gauge needle several times in order to reduce solution viscosity. The digested chromatin was mixed with 450 µL of Buffer 500 consisting of 20 mM Tris-HCl, pH 7.4 at 4 °C, 0.5 M NaCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 0.1% (v/v) IGEPAL CA-630, 1 mM PMSF, 10 mM IA, 20 mM NEM, 1x cOmplete protease inhibitor cocktail, and 10% (v/v) glycerol. The

mixture of chromatin and Buffer 500 was supplemented with 5 M NaCl to adjust the final salt concentration back to 0.5 M NaCl, then centrifuged at 17.2 kRCF for 20 min at 4 °C to remove any insoluble material. After sample clarification, an aliquot of the soluble fraction was analyzed by non-denaturing agarose gel electrophoresis to ascertain the generation of mononucleosomes through the observation of low molecular weight ~150 bp DNA. The remaining majority of soluble fraction was mixed with anti-HA magnetic resin beads, pre-equilibrated in Buffer 500, for 2 h at 4 °C. The unbound fraction was subsequently removed by pipetting and the magnetic beads washed several times with Buffer 500. The HA-SUMO3(ΔGG)-H4(Δ1-11), HA-SUMO3(ΔGG)-H4, or Myc_2-H4(Δ1-11) were eluted by boiling the magnetic beads in SDS-containing gel-loading dye for no more than 3 minutes.

20. Western blotting to detect H2BK120ub and either HA-SUMO3(ΔGG)-H4(Δ1-11), HA-SUMO3(ΔGG)-H4, or Myc_2-H4(Δ1-11).

Immunoprecipitated histones were transferred to a PVDF membrane using 35 V for 2 h at 4 °C in a modified Towbin transfer-buffer consisting of 25 mM Tris-HCl, 192 mM Glycine, 4 mg/L SDS, and 10% (v/v) methanol. After protein transfer, the PVDF membrane was blocked with 5% (w/v) non-fat milk in PBS at 25 °C for 1 h prior to overnight incubation at 4 ºC with specific primary antibodies. Primary antibodies were diluted in PBST (PBS containing 0.05% (v/v) Tween-20), supplemented with 5% (w/v) non-fat milk powder. The next day, membranes were washed in PBST and then incubated for 1 h at 25 °C with the appropriate IR-dye-conjugated secondary antibody diluted in PBST supplemented with 5% (w/v) non-fat milk powder. The membrane was first washed with PBST and then PBS and subsequently scanned on a Li-COR Biosciences Odyssey IR scanner.

21. Table of antibodies and cell lines used in this study.

No.	Catalog #	Supplier	Protein target	Dilution employed
1.	ab1790	Abcam	Histone H2B	0.1µg/mL (WB 1:10,000)
2.	39623	Active Motif	Histone H2BK120ub	1 µg/mL (WB 1:1,000)
3.	ab10158	Abcam	Histone H4	1 µg/mL (WB 1:1,000)
4.	ab24834	Abcam	Histone H3	1µg/mL (WB 1:1,000)
5.	ab1791	Abcam	Histone H3	1 µg/mL (WB 1:1,000)
6.	3724S	Cell Signaling Technologies	HA-tag	22 ng/mL (WB 1:1,000)
7.	2276S	Cell Signaling Technologies	Myc-tag	80 µg/mL (WB 1:1,000)
8.	926-32210	LI-COR	IR Dye 800CW Goat anti-Mouse	66.67 ng/µL (WB 1:15,000)
9.	926-68071	LI-COR	IR Dye 680RD Goat anti-Rabbit	66.67 ng/µL (WB 1:15,000)
10.	926-32351	LI-COR	IR Dye 800CW Goat anti-Mouse IgG2a	66.67 ng/µL (WB 1:15,000)
11.	CRL-3216	ATCC	HEK293T cell line used for studies of biochemical crosstalk	Lot number 70029111

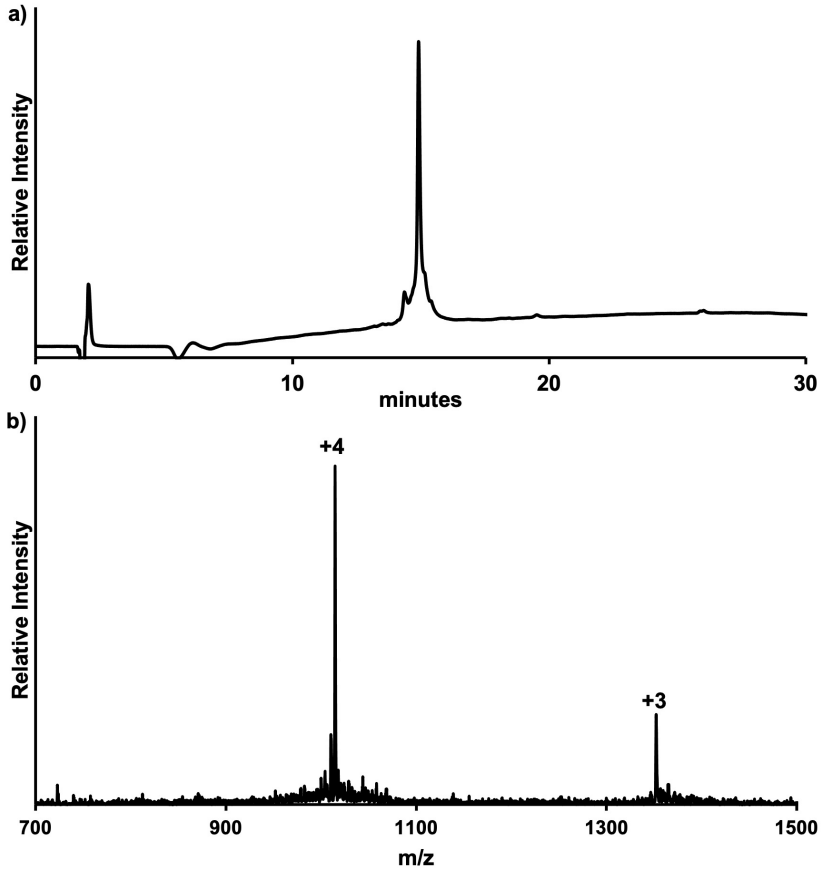

Figure S1. Synthesis of H4(1-37) K12$_{cys}$ (1). (a) C18 analytical RP-HPLC trace of pure **1** injected on a 0-73% B gradient over 30 min. (b) ESI-MS of pure **1**. Calculated M_{avg} 4,055.2 Da and observed 4,054.8 ± 0.6 Da.

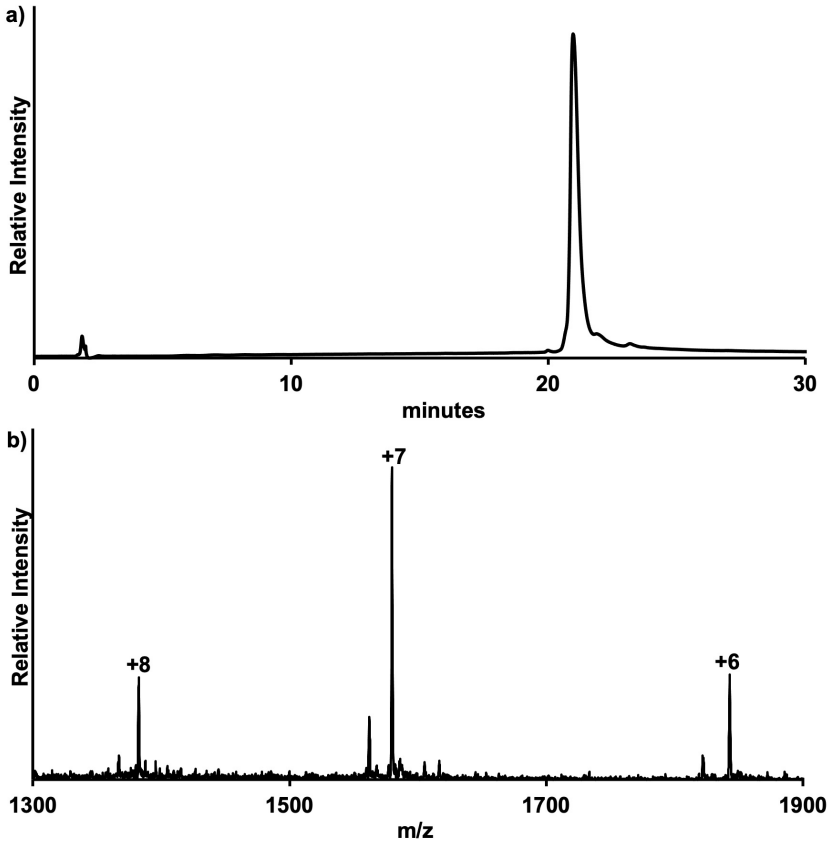

Figure S2. Intein-mediated generation of SUMO-3(2-91)C47S α-thioester. (a) C18 analytical RP-HPLC trace of pure SUMO-3(2-91)C47S α-thioester injected on a 0-60% B gradient over 30 min. (b) ESI-MS of pure SUMO-3(2-91)C47S α-thioester. Calculated M_{avg} 10,444.7 Da and observed 10,443.8 ± 1.5 Da.

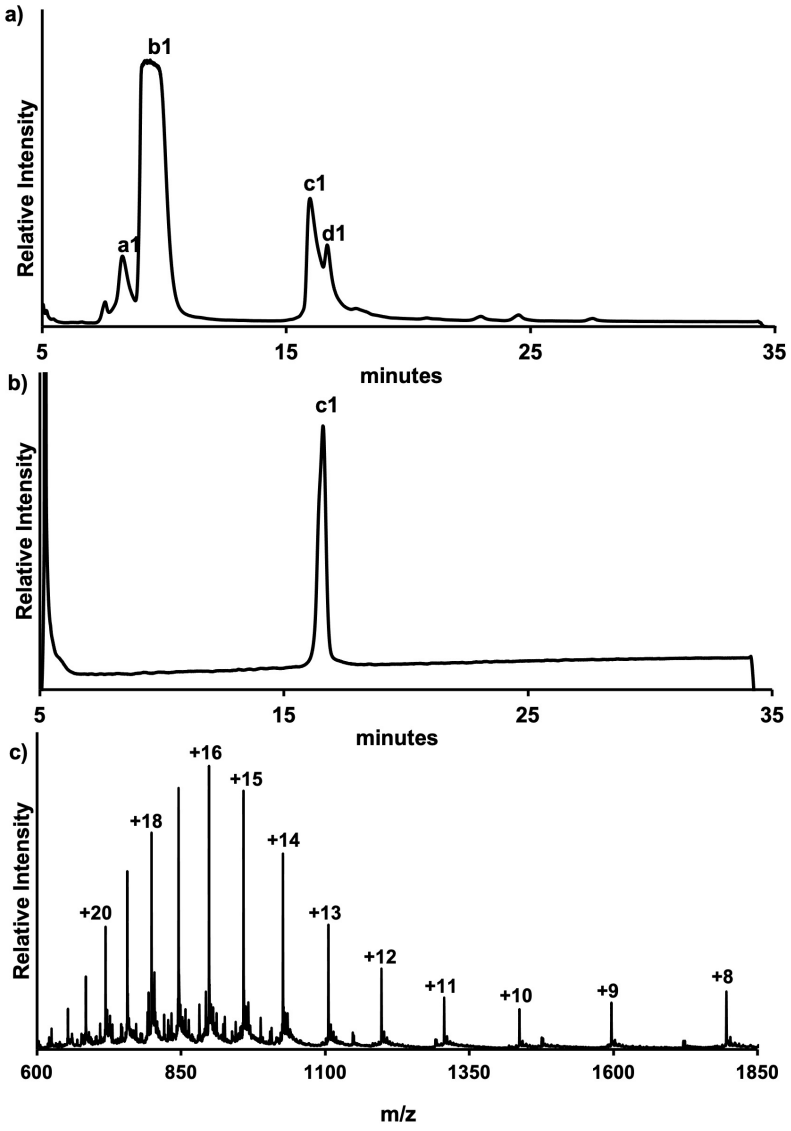

Figure S3. Ligation of H4(1-37)K12$_{cys}$ (1) with SUMO-3(2-91)C47S α-thioester. (a) C18 analytical RP-HPLC trace after 8 h of ligation injected on a 0-73% B gradient over 30 min. Peak **a1** corresponds to unreacted H4(1-37)K12$_{cys}$, peak **b1** is the excess MPAA in ligation buffer, peak **d1** is hydrolyzed SUMO-3(2-91)-CO_2H, and peak **c1** corresponds to the desired ligation product . (b) C18 RP-HPLC trace of the MPAA α-thioester form of the ligation product (**4**). (c) Calculated M_{avg} for MPAA α-thioester form **4**, 14,359.4 Da and observed 14,359.9 ± 0.7 Da.

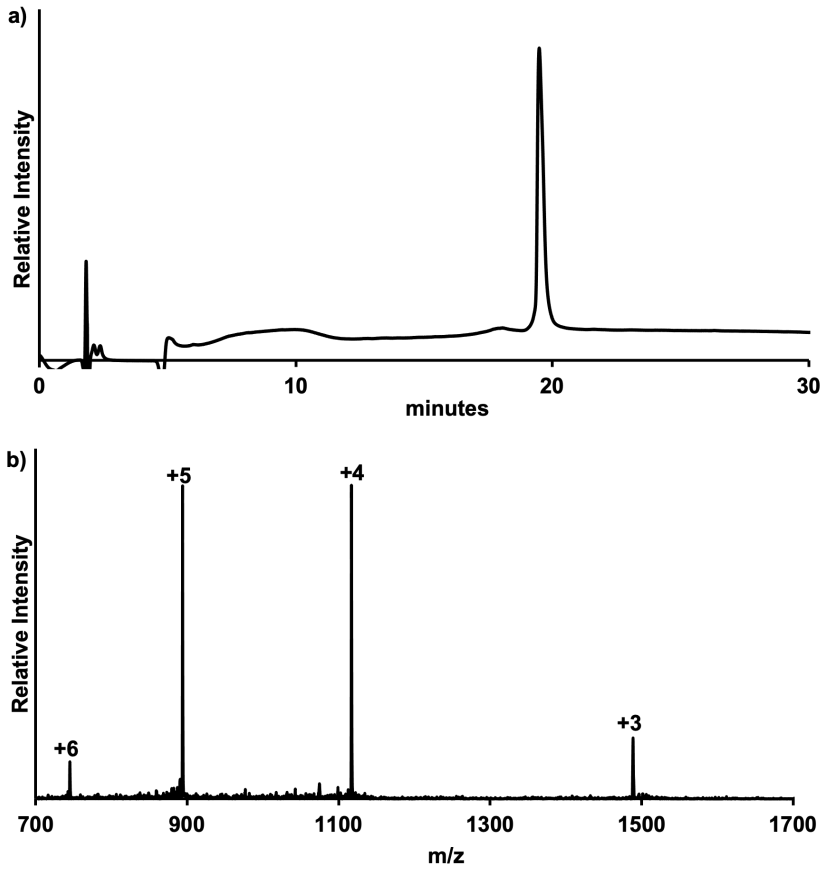

Figure S4. Synthesis of central fragment H4(38-75)A38C$_{Acm}$ (2). (a) C18 analytical RP-HPLC trace of pure **2** injected on a gradient of 20-80% B over 30 min. (b) ESI-MS of pure **2**. Calculated M_{avg} 4,465.2 Da and observed 4,465.8 ± 0.6 Da.

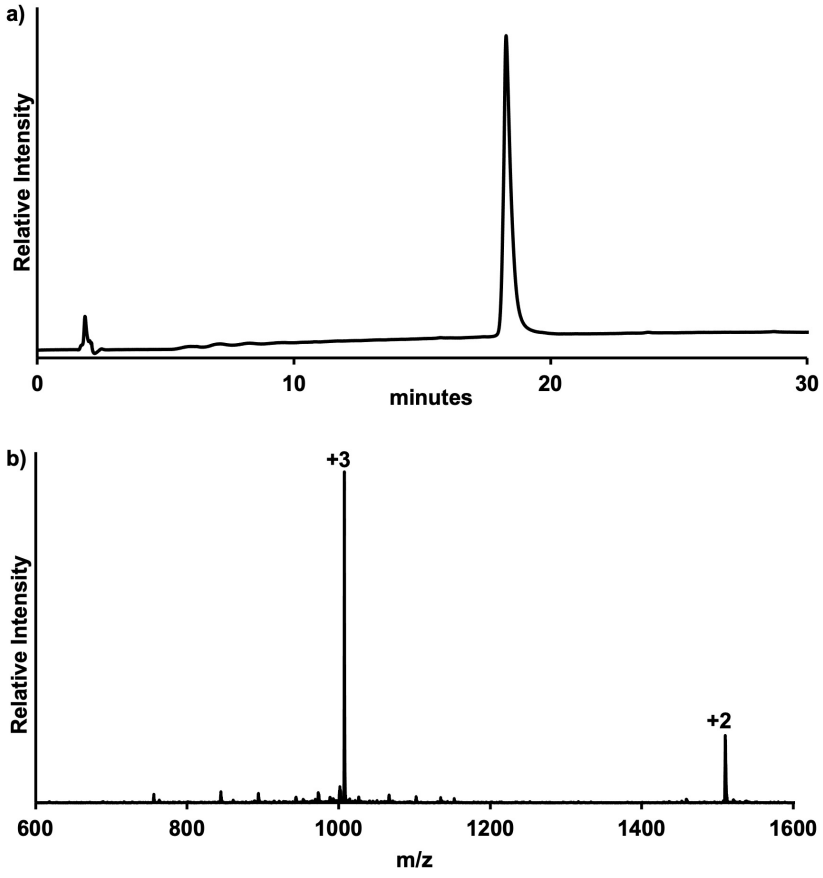

Figure S5. Synthesis of H4(76-102)A76C (3). (a) C18 analytical RP-HPLC trace of pure **3** injected on a gradient of 0-73% B over 30 min. (b) ESI-MS of pure **3**. Calculated for **3**, M_{avg} 3,019.5 Da and observed 3,019.8 ± 0.5 Da.

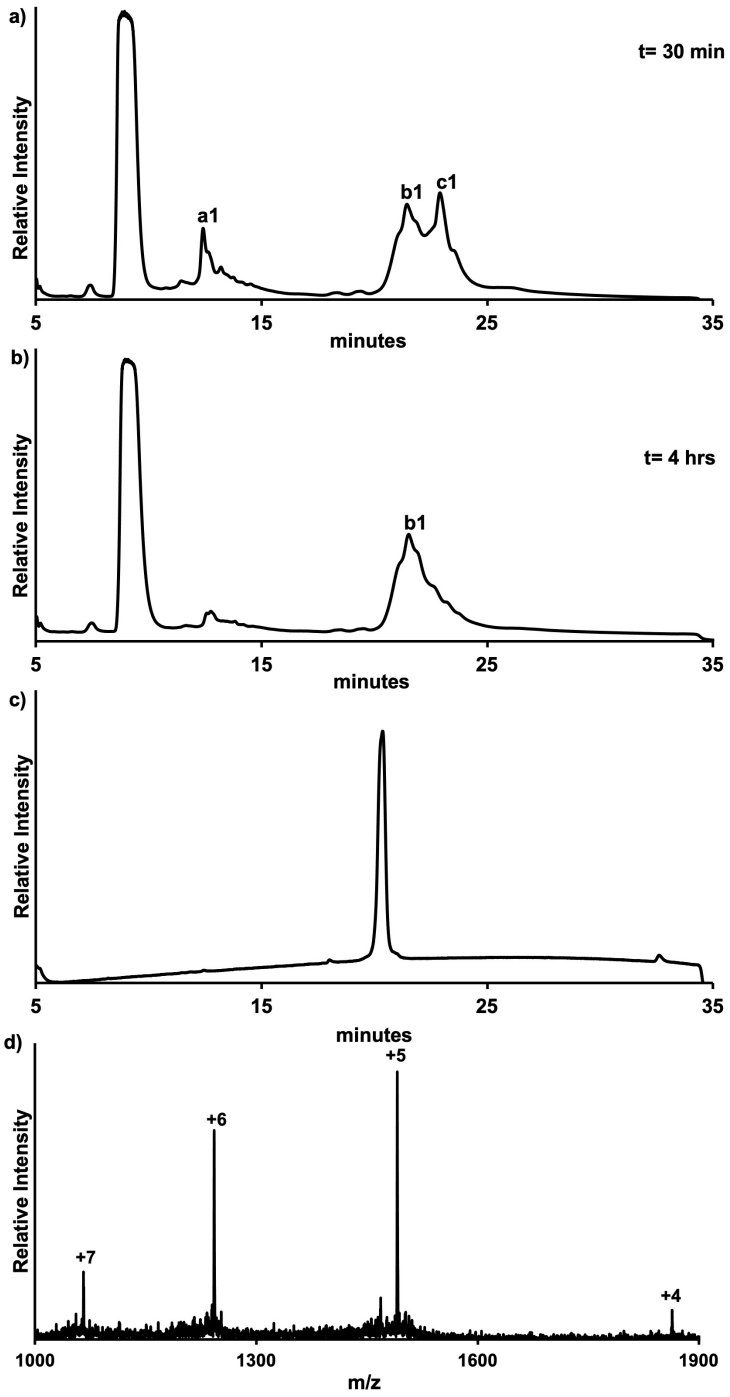

Figure S6. Ligation of H4(38-75)A38C$_{Acm}$ (2) and H4(76-102)A76C (3). C4 analytical RP-HPLC trace after (a) 30 min, and (b) 4 h of ligation. Peak **a1** corresponds to unreacted H4(76-102)A76C, peak **b1** contains the desired product, and peak **c1** corresponds to unreacted H4(38-75)A38C$_{Acm}$-MPAA α-thioester. (c) C4 analytical HPLC trace of the purified ligation product on a 20-80% B gradient over 30 min. (d) ESI-MS of purified H4(38-102)A38C$_{Acm}$,A76C. Calculated M_{avg} for H4(38-102)A38C$_{Acm}$,A76C was 7,451.9 Da and observed 7,450.8 ± 1.1 Da.

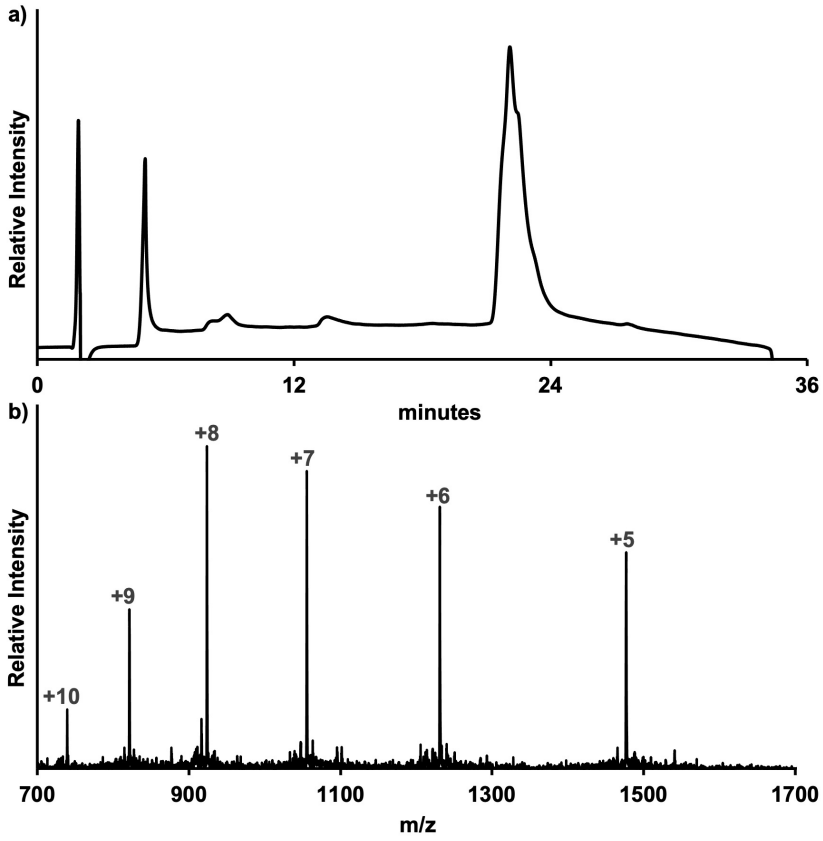

Figure S7. Acm-deprotected H4(38-102)A38C,A76C peptide (5). (a) C4 analytical HPLC trace of pure **5** injected on a gradient of 30-70% B over 30 min. (b) and ESI-MS of purified **5**. Calculated M_{avg} 7,381.6 Da and observed 7,380.6 ± 1.2 Da.

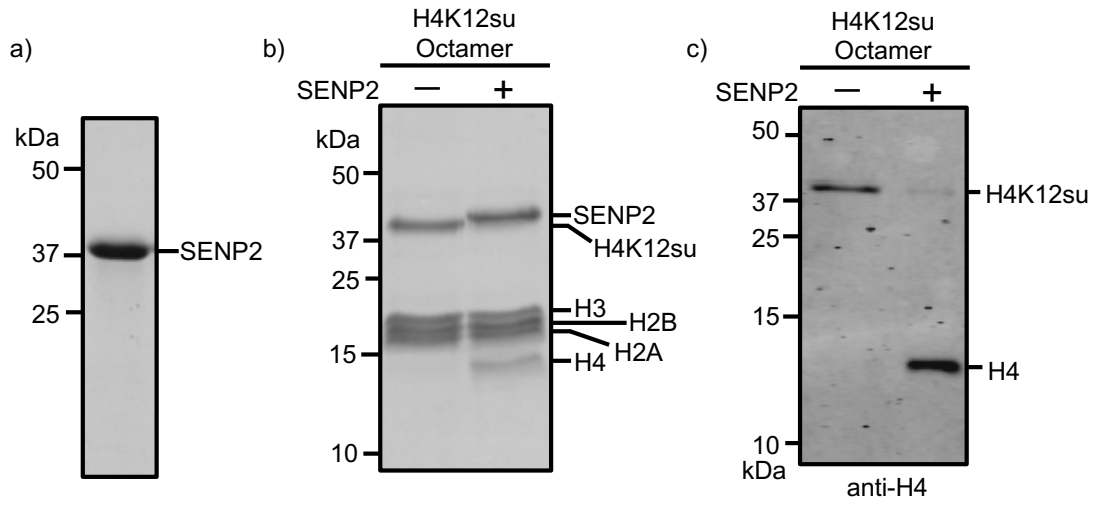

Figure S8. SENP2-mediated desumoylation of H4K12su. (a) Coomassie-stained 15% SDS-PAGE of pure SENP2 catalytic domain. (b) 18% SDS- PAGE of H4K12su desumoylation by the SENP2 catalytic domain. (c) Western blot with anti-H4 antibody to detect H4K12su desumoylation by the SENP2 catalytic domain.

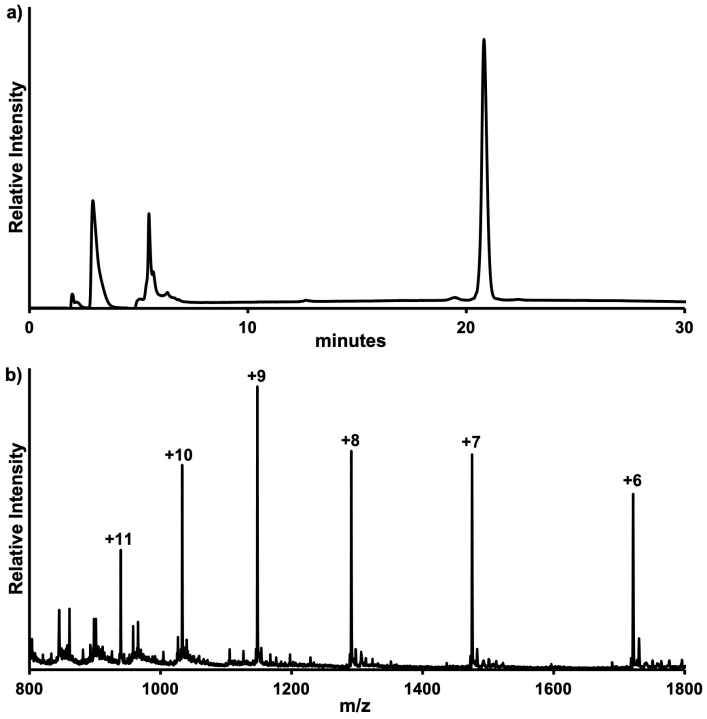

Figure S9. Synthesis of SUMO-3(2-92)C47S,G92A. (a) C18 analytical RP-HPLC trace of pure SUMO-3(2-92)C47S,G92A injected on a 0-60% B gradient over 30 min. (b) ESI-MS of pure SUMO-3(2-92)C47S,G92A. Calculated M_{avg} 10,373.8 Da and observed 10,374.5 ± 1.4 Da.

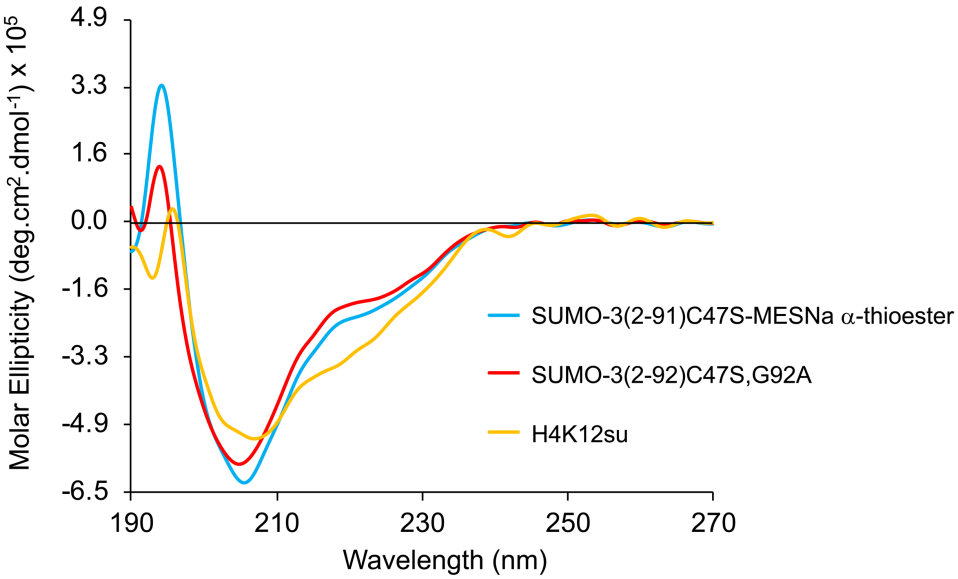

Figure S10. Circular Dichroism spectra of SUMO-3(2-92)C47S,G92A; SUMO-3(2-91)C47S-MESNa α-thioester and H4K12su. The maxima at ~195 nm and minima at ~208 nm are characteristic of the folded SUMO-3(2-91)C47S-MESNa α-thioester.[2]

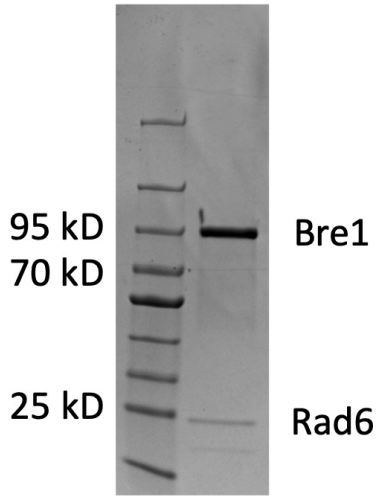

Figure S11. Purification of the Rad6-Bre1 complex. Coomassie-stained 10% SDS- PAGE of the pure Rad6 and Bre1 proteins.

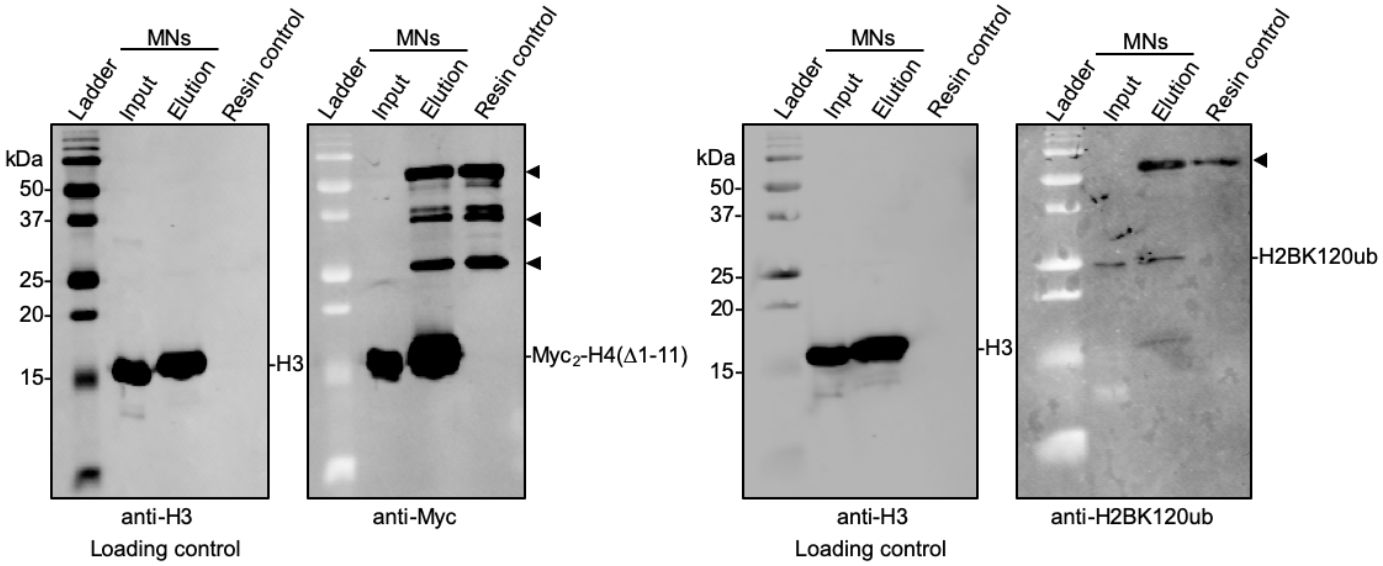

Figure S12. Myc-tag-mediated immunoprecipitation of mononucleosomes containing H4(Δ1-11). Input = input mononucleosomes (MNs) obtained by *Micrococcal nuclease* digestion of cellular chromatin. Elution = eluate from anti-Myc resin. Resin control = Anti-Myc resin prior to incubation with nucleosomes. Myc_2-H4(Δ1-11) = double Myc-tagged truncated H4(12-102) protein missing tail residues 1-11. An H3-specific antibody was used as a loading control for the total amount of MNs in input and elution lanes. A filled triangle indicates antibody-chains released from the resin under denaturing conditions.

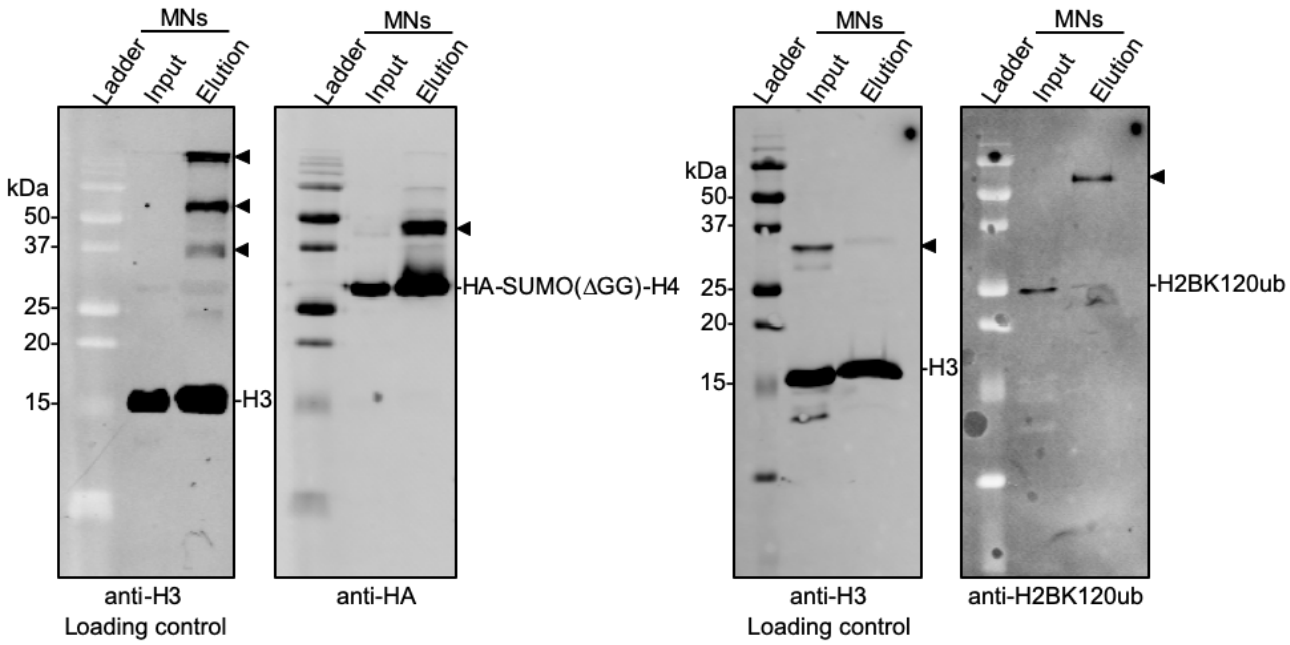

Figure S13. HA-tag-mediated immunoprecipitation of mononucleosomes containing SUMO-3(ΔGG)-H4. Input = input mononucleosomes (MNs) obtained by *Micrococcal nuclease* digestion of cellular chromatin. Elution = eluate from anti-HA resin. HA-SUMO(ΔGG)-H4 = HA-tagged protease-resistant SUMO-3 misssing its C-terminal Gly91 and Gly92 residues, fused to the N-terminus of full-length histone H4. An H3-specific antibody was used as a loading control for the total amount of MNs in input and elution lanes. A filled triangle indicates non-histone species cross-reactive to the secondary antibody used for imaging western blots.

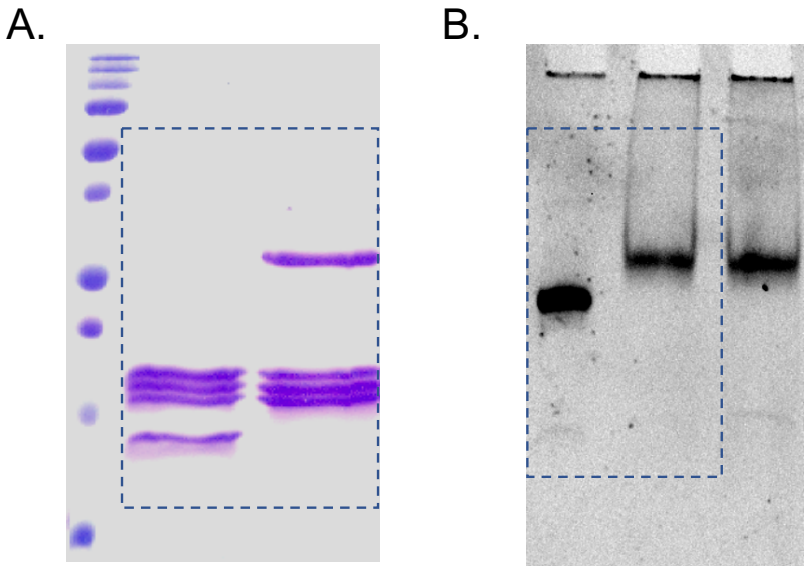

Figure S14. Uncropped gels for (A) octamer and (B) nucleosome assembly. Boxes indicate the area presented in the main text Figures 1C-D.

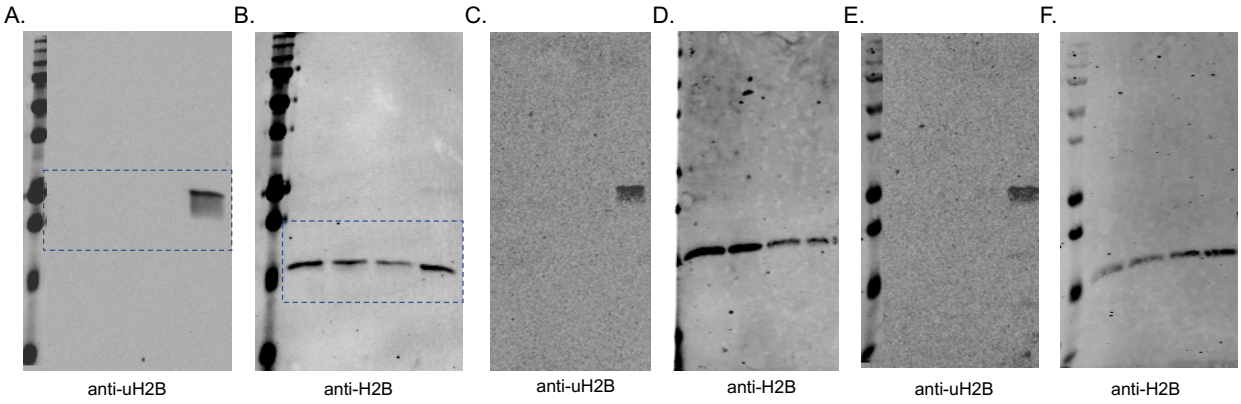

Figure S15. Uncropped blots for (A,C,E) anti-uH2B western and (B,D,F) anti-H2B western (n = 3). Boxes indicate the areas presented in the main text Figure 1E.

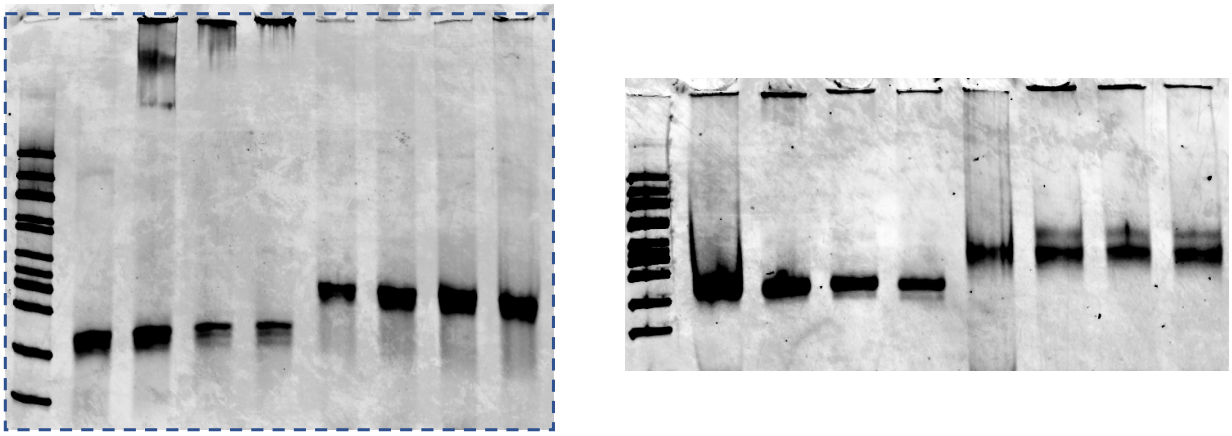

Figure S16. Uncropped gels for Rad6-Bre1 mononucleosome binding. (n = 2). A box indicates the area shown in Figure 2.

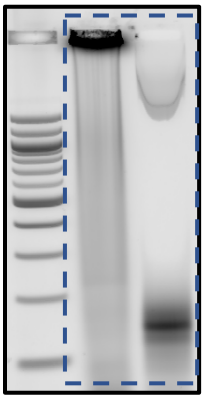

Figure S17. Uncropped gel for *Micrococcal nuclease* digestion of cellular chromatin. Box indicates the area shown in Figure 3A.

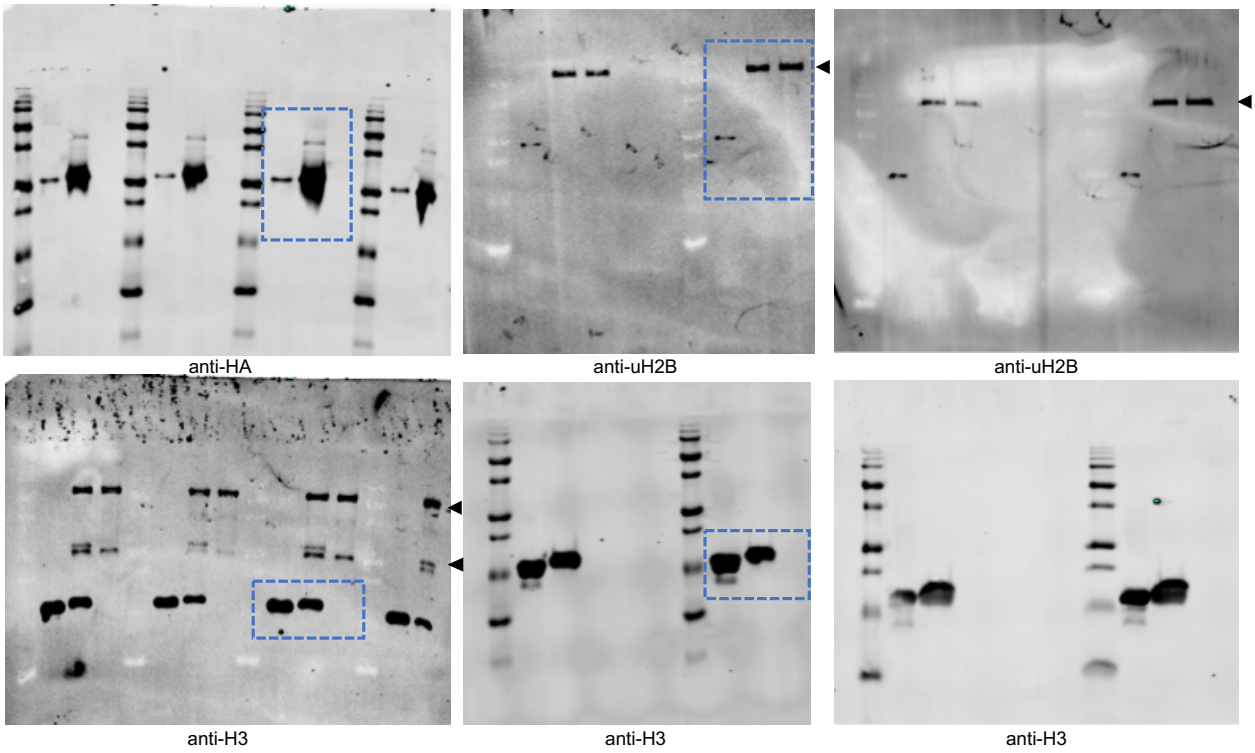

Figure S18. Uncropped western blots with anti-HA, anti-uH2B and anti-H3 antibodies (n=4). Boxes indicate the areas shown in Figure 3B. A filled triangle indicates antibody chains released from the resin under denaturing conditions.

References

1. J.-S. Zheng, S. Tang, Y.-K. Qi, Z.-P. Wang and L. Liu, *Nat. Protocols*, 2013, **8**, 2483-2495.
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