Supporting Information

Hydrazide Mimics for Protein Lysine Acylation to Assess Nucleosome Dynamics and Deubiquitinase Action

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General

All commercially available reagents were used as purchased without further purification. Most chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Fairlawn, NJ) unless noted. Acetohydrazide was purchased from Sigma-Aldrich. NMR spectra were collected on a Bruker Avance III 500 MHz spectrometer. Chemical shifts are reported as δ in units of parts per million (ppm). Peptide and protein samples were spotted with MALDI matrix (α-cyano-4-hydroxycinnamic acid saturated in 50% aq. acetonitrile with 0.1% TFA), and spectra were acquired on a PerSeptive Biosystems Voyager-DE Pro. Electrospray ionization mass spectra (ESI-MS) were recorded on a UPLC-MS system consisting of Waters nanoACQUITY and Thermo Scientific TSQ mass spectrometer. The samples were eluted from either a Biobasic-C4 column (Thermo Scientific, Waltham, MA; column: 1×50 mm, 5 μm) or a monolithic column, ProSwift™ RP-4H (1×250 mm) using water (LCMS grade; solvent A) and acetonitrile (LCMS grade; solvent B) as eluent where both solvents A and B contained 0.01% formic acid as the phase modifier (100% solvent A to 70% solvent B, over a period of 25 min.) Occasionally, HPLC purified protein samples were introduced by direct infusion into ESI-MS using a syringe pump. Observed ESI-MS data are reported as average masses (M_{av}). The FRET measurements with nucleosomes were performed by exciting the labeled samples at 510 nm (donor excitation, Cy3) and collecting emission spectra from 530 to 750 nm; in addition, nucleosomes were further excited at 610 nm (acceptor excitation, Cy5) and emission

spectra from 630 to 750 nm were recorded using a Fluoromax-4 (Horiba Scientific, Kyoto, Japan) steady state spectrophotometer.

Synthesis of 2-hydroxy-2-methyl-propionohydrazide [CAS# 42596-46-3][1]

Ethyl 2-hydroxy-2-methyl-propionate (MW: 132.06 g/mol; d: 0.965 g/mL; 1 equiv.; 14.6 mmol; 2 mL) and anhydrous hydrazine (MW: 32.05 g/mol; d: 1.021 g/mL; 2 equiv.; 29.2 mmol; 916 µL) were added to a Biotage microwave reaction vial containing ethanol (3 mL) and the mixture was heated to 160 °C for 5 minutes by microwave irradiation. The reaction mixture was concentrated using a rotary evaporator and loaded onto a silica gel column and eluted with 3% ethanol in ethyl acetate. The desired fractions were pooled and concentrated to dryness leaving a white solid (1.51 g; 88%).

R_f = 0.21 (eluent: 2% EtOH/EtOAc).
^{1}H-NMR (500 MHz, CD_3OD): δ 1.25 (s, 6H, C$\underline{H}_3$), 3.19 (br s, 1H, exchangeable, N$\underline{H}$). ^{13}C-NMR (125 MHz, CD_3OD): δ 27.93 ($\underline{C}H_3$), 73.61 ($\underline{C}Me_2OH$), 178.29 ($\underline{C}$=O).
ESI-MS: *m/z* 119.56 (MH^+), 89.97 (M^+−28, base peak).

Installation of 2-hydroxyisobutyrl-lysine mimic on a model peptide

Reaction conditions for the three-step installation of acyl-lysine mimic was optimized using human H2B type-1C, C-terminal tail peptide 117-125 with K120C mutation which has the following sequence: Ac-AVT$\underline{C}$YTSSK-OH. To a 10 mM solution of the model peptide (2 mg by actual weight, stock solution was prepared by dissolving a lyophilized powder of the peptide in 60:40, acetonitrile/water) in 100 mM ammonium bicarbonate (pH 8.0) containting 25 mM tris(2-carboxyethyl)phosphine (TCEP), chloroacetaldehyde (50% w/v solution) was added to a final concentration of 10 mM, and the reaction mixture was stirred vigorously at room temperature for 30 minutes. The mixture was lyophilized overnight and the resulting solid was dissolved in minimum amount of 100 mM acetate buffer (NaOAc/AcOH, pH 5.0) and stirred at room temperature with 10 mM 2-Hydroxy-isobutyryl hydrazide for 18 hours. An aqueous solution of $NaBH_3CN$ (to a final concentration of 50 mM) was added and the reaction mixture was stirred for an additional 2 hours. The reaction mixture was then directly analyzed using ESI-MS (Supplementary Figure 1).

ESI-MS (-ve mode): m/z 1,144.31 $(M-H)^-$, 1,183.82 $(M+K)^-$

Production and purification of ubiquitin hydrazide (Ub-Hz)[2]

Our strategy for making Ub-Hz involved generating ubiquitin 2-mercaptoethanesulfonate ester (Ub-MESNa) first and treating that with hydrazine to produce Ub-Hz. Multiple protocols are described in the literature to generate Ub-Hz,[2] and we chose to employ intein mediated thioester formation followed by hydrazinolysis. Thus, frozen stock (−80 °C) of expression competent *Escherichia coli* Rosetta™(DE3)pLysS cells (EMD Millipore, Billerica, MA) transformed with the plasmid pTXB1-Ub (plasmid from New England Biolabs, Ipswitch, MA; wild type yeast ubiquitin 1–76 was inserted into pTXB1 upstream of *Mxe* intein/chitin binding domain) were plucked using a sterile pipette tip and inoculated a 20 mL lysogeny broth (LB) medium containing two antibiotics—carbenicillin (50 μg/mL) and chloramphenicol (35 μg/mL)—and incubated at 37 °C overnight (tubes placed on a rotisserie tube rotator housed in an incubator). This overnight starter culture was added to 1 L LB medium containing the two antibiotics at the concentration given above. The liter culture was incubated at 37 °C while shaking (220 rpm) until the OD_{600} reached 0.6–0.8 at which point isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM and the incubation was continued for 3 more hours. Cells were harvested by centrifugation at 5,000 rpm for 30 minutes (Beckman Coulter, Brea, CA; model: Avanti® J-E, rotor used: JA-10, temperature set to 4 °C). The cell pellet was resuspended in 30 mL of lysis buffer-A (20 mM HEPES, pH 6.5; 75 mM NaCl, 50 mM NaOAc [when all ingredients are mixed in dH_2O, pH becomes ~6.3, and the pH must be adjusted to 6.5 by adding 1 M NaOH dropwise]). The cells were lysed by passing through a french press (Thermo Electron Corporation, West Palm Beach, FL) three times at 16,000 psi and the cell debris was separated by centrifugation (20,000 rpm for 30 min, at 4 °C, rotor used: JA-20). The supernatants were filtered through a 0.22 μm filter and were loaded to a chitin column (5 mL bed volume/L culture) pre-equilibrated with lysis buffer-A (20 bed volumes) for 1 h at 4 °C. The column was washed with 20 bed volumes of lysis buffer-A and the column was incubated for 2 days at 37 °C with 7.5 mL cleavage buffer (lysis buffer-A with 100 mM MESNa (sodium 2-mercaptoethanesulfonate) and 5% (w/v) hydrazine acetate). The column was washed with lysis buffer-A (4 × 7.5 mL) and the eluate was collected. The cleavage protocol was repeated once more—incubation with cleavage buffer (7.5 mL) for 2 days at 37 °C and elution with lysis buffer-A. The pooled eluate containing ubiqutiyl hydrazide (Ub-Hz) was concentrated by ultrafiltration (Amicon Ultra-15, 3 kDa-cutoff, 4,000 rpm) to 2-3 mL volume. The concentrate was further washed with lysis buffer-A (3 × 11 mL) to ensure elimination of excess MESNa and hydrazine acetate. The crude sample of Ub-Hz was then purified by C18 semipreparative RP-HPLC (PROTO 300 C18, 4.6×250 mm, 5μ, 300 Å; Higgins Analytical, Mountain View, CA) while eluting with a gradient of

acetonitrile (20% to 80% over 25 min.; flow rate: 3.5 mL/min) and water with 0.05% trifluoroacetic acid (TFA) (Supplementary Figure 2A). Fractions corresponding to peaks with retention time (t_R) around 22 min were pooled from multiple injections after mass verification using ESI-MS (Supplementary Figure 2B, also Figure 1C shows mass deconvolution), and upon concentration and lyophilization Ub-Hz was obtained as white amorphous fluff (yield: 8-12 mg/liter culture). A 1 to 5 M stock solution of Ub-Hz may be prepared by dissolving the lyophilized material in neat DMSO.

Production and purification of K→C mutant proteins used for hydrazide mimic installation (histones and ubiquitin)

We prepared H3-K56C and H3-K122C mutant proteins from *Xenopus laevis* histone 3.2 construct in pET3d vector additionally harboring a G102A mutation. This version of xlH3.2 has been previously employed for nucleosome core particle crystallography.[3,4] It should also be noted that the xlH3.2-G102A variant differs from canonical human histone H3.1 with respect to only two amino acids—namely, C96S and G102A. These C96S and C110A facilitate selective modification of the introduced Cys in the H3 forms used here. Refolded octamers also included the three other human core histones—H2A type 1-C, H2B type 1-H with D2E mutation, and H4.

We generated H3-K56C and H3-K122C mutant DNA constructs (plasmid: pET3d) using QuikChange site-directed mutagenesis kit (Agilent, Santa Clara, CA) by adopting a two-stage PCR protocol.[5] The primers used in the experiments are listed below.

H3-K56C: Forward—5'-ccgacgttaccagTGCtccactgagctgc-3' and Reverse—5'-gcagctcagtggaGCActggtaacgtcgg-3'

H3-K122C: Forward—5'-gtcaccatcatgcccTGCgacatccagttggc-3' and Reverse—5'-gccaactggatgtcGCAgggcatgatggtgac-3'

These mutant H3 DNA plasmids in a pET3d vector were transformed into Rosetta(DE3)pLysS cells (MilliporeSigma, Burlington, MA) and H3-K56C and H3-K122C proteins were expressed and purified according to the protocol described by Klinker et al.[6] Briefly, upon cell lysis and histone extraction under denaturing conditions (this protocol avoids inclusion body preparation), the histone extract was cleared by centrifugation (20 min, 20,000 rpm) and filtration through glass-fiber filter (0.45 µm, HPF Millex, MilliporeSigma). The pre-cleared cell extract was directly passed through a pre-equilibrated (with SAU 200 buffer: 40 mM NaOAc, pH 5.2, 6 M urea, 1 mM EDTA, 5 mM 2-mercaptoethanol, 10 mM lysine, supplemented with 200 mM NaCl) tandem anion-cation

exchange column—namely, HiTrap Q HP stacked on top of HiTrap SP HP and (both 5 mL, GE Healthcare, Pittsburgh, PA). After the extract had passed through the Q column, it was disconnected and histone was eluted from the SP column with a salt gradient up to 1 M NaCl. Purified fractions were pooled based on Coomassie-stained SDS-PAGE, dialyzed (3 kDa cutoff, 4 changes of 5 L, dH_2O with 0.01% TFA; note, the first two changes also included 1 mM 2-mercaptoethanol) and then lyophilized resulting in a powder that was stored at −80°C. The final yield of H3-K56C and H3-K122C proteins ranged from 25-40 mg per liter culture. Histone H2B mutants used in this work were of *Xenopus laevis* origin and were truncated versions (aa4–125, xlH2B-1.1). Preparation of H2B-K120C protein has been described previously.[4] After preparing a Rosetta(DE3)pLysS cells with the plasmid bearing an additional K116A mutation (QuikChange, primers used: Forward—5'-gtgtccgagggcaccgcggctgtcacctgttaca-3' and Reverse—5'-tgtaacaggtgacagccgcggtgccctcggacac-3') H2B-K116A,K120C protein was also produced and purified as described above. Ubiqutin-K48C was expressed and purified according to the method described by Raasi and Pickart.[7]

Immunoblotting

Histone H3 acetyl-K56 specific antibody was purchased from Abcam (Cambridge, MA). Pan 2-hydroxyisobutryllysine antibody was prepared as described previously.[8] Although we have used monoclonal anti-ubiquitin antibody VU-1 from LifeSensors (Malvern, PA) with great success, in the experiment leading to Figure 3C in the main text, we used the anti-ubiquitin antibody from Santa Cruz Biotechnology (Dallas, TX; mAb P4D1 clone).

For experiments with antibodies, 0.2–0.3 µg proteins were loaded per lane and after running 14% SDS-PAGE (gels run in parallel to serve as loading controls were stained with Colloidal Blue, #LC6025, from ThermoFisher Scientific, Waltham, MA) and transferring onto nitrocellulose or PVDF membranes, the membrane-blots were washed with 5–10 mL Milli-Q water for 2-3 min with mild agitation and blocked with 5–10 mL of TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.01% Tween-20) with 4% BSA for 1 h at room temperature with agitation. The blots were then agitated at 4 °C overnight with respective primary antibodies (titer: 1:500), rinsed three times (each 5 min durations with 5–10 mL TBST), and incubated at RT for 1–2 h with appropriate HRP-conjugated secondary antibodies (titer: 1:5,000) in 5–10 mL TBST with 4% BSA. Upon washing three times with TBST (5–10 mL, 5 min duration), the blots were visualized by chemiluminescence per the manufacturers' instructions (Pierce ECL Plus from Thermo Fisher).

Other proteins used in nucleosome dynamics experiments

Human histones: H2A type 1-C, hH2A-K119C (used for labeling with Cy5-maleimide, GE LifeSciences, #PA25031), hH2B-2DE type 1-H, hH3.1-C96S-G102A-C110A, hH4, hH4-V21C (used for labeling with Cy5-maleimide) were prepared as described previously.[9] The mutant histones required for fluorescent label attachment, hH2A-K119C[10] and hH4-V21C[11] have been reported. LexA repressor protein (binds to SOS boxes) that has been used previously in evaluating DNA accessibility in the entry/exit region, was expressed and purified according to the protocol of Little et al.[12]

Histone octamer refolding and purification

Each of the four core histones were combined at equal molar ratios, refolded and purified as previously described.[13,14] Octamers used in reconstitution of nucleosomes for entry/exit region studies (bearing modifications at H3-K56) were labeled with Cy5-maleimide (GE Healthcare, #PA25031, labeling was done at the heterodimer stage) on H2A-K119C. Octamers meant for the study of PTM effects at the dyad region of the nucleosomes were labeled with Cy5-maleimide on H4-V21C at the octamer stage.

Nucleosome reconstitution and purification

Mononucleosomes used in this work were reconstituted using 147 bp Widom 601 DNA sequence and histone octamers with and without PTM mimics. The nucleosomes for the entry/exit region study contained 601-DNA with LexA binding site from bases 8–27 and Cy3 label on the first base (5'-end) and a protocol for its preparation and purification has been described previously.[15] The 601 Widom sequence used for the dyad region study was prepared as described before as well,[15] and in this case Cy3 label was placed on an internal thymine base (57th base from 5'-end, or −17 position from dyad) via hexylamino linker. Nucleosomes were reconstituted by salt double dialysis and purified by sucrose gradient centrifugation as described before.[16]

The nucleosomes containing H2B-K$_c$120ub and H2B-K116A,K$_c$120ub used in the UBP8/SAGA DUB module reaction were assembled with *Xenopus laevis* histones and they were prepared according to a previously described protocol.[4]

Site accessibility measurements

Nucleosomes (5 nM) bearing Cy3 (on 5'-end near the LexA target sequence) and Cy5 (appended to H2A-K119C on the octamer) FRET pairs and PTM mimics on H3-K56 were titrated against LexA (0 to 30 μM) as described previously[14] and the FRET efficiencies were determined by the (ratio)$_A$

method, performed in triplicate for each LexA concentration. LexA concentration of half-saturation ($S_{½}$) was determined by fitting the data to a noncooperative binding isotherm.

Salt titrations and kinetics to assess nucleosome disassembly

In order to assess the effect of PTM mimics in the dyad region of nucleosomes (mimics on H3-K122), we recorded in triplicate, the FRET efficiencies between the donor Cy3 (position −17 from dyad center of DNA, namely, on 5'-amino-modifier-C6 dT with Cy3-NHS ester) and the acceptor Cy5 (on H4-V21C, using Cy5-maleimide) pairs by inducing nucleosome disassembly with increasing salt concentrations (75, 500, 750, 875, 1000, 1125, 1250, 1375, 1500, 1750, and 2000 mM NaCl; at each point of measurement samples in the cuvette were allowed to stand for 3.5 min prior to the start of recording on the fluorometer) at a fixed concentration (5 nM in 0.5× TE buffer) of nucleosomes (without and with H3-K_c122- ac/hib/ub modifications). The salt concentration eliciting half-maximal FRET response was determined for each nucleosome by plotting normalized FRET efficiencies against salt concentrations. Next, acceptor response over a period of 30 min was recorded for each nucleosome (5 nM in 0.5× TE buffer) in triplicate, upon addition of 1,125 mM NaCl (EC50 average for unmod, H3-K_c122ac, -hib, and -ub nucleosomes in the previous salt titrations). The exponential decay data (only the linear range spanning up to 800 s was used) were processed as semi-log plots and the slopes represent the mean lifetime for these nucleosomes at 1,125 mM salt.

Production and Purification of UBP10.

The *ubp10* gene was amplified from *S. Cerevisiae* genomic DNA and cloned into a pET32a vector, which contains an ampicillin resistance gene. After a sequencing confirmation, the vector containing UBP10 DNA construct was transformed into Rosetta 2 (DE3) E. Coli cells. These bacteria were grown in LB suspension at 37 °C to an OD_{600} of 0.8, followed by induction of protein synthesis with 0.5 mM IPTG at 16 °C for 20 hours. Cells were harvested by centrifugation. Cell pellets containing Ubp10 were resuspended in 30 mL/L media of lysis buffer (25 mM HEPES pH7.5, 20 mM imidazole, 600 mM NaCl, 10 mM β-mercaptoethanol (βME), and 1 mM PMSF) and then lysed using a microfluidizer. The lysates were clarified by centrifugation for 30 min and 17,000×*g*. The supernatant containing Ubp10 was then loaded onto a 5 mL HisTrap column (GE Healthcare) equilibrated with lysis buffer and eluted with a linear gradient of 20 mM to 300 mM imidazole. Fractions containing Ubp10 were pooled and dialyzed at 4 °C overnight into TEV cleavage buffer (25 mM HEPES pH7.5, 20 mM imidazole, 600 mM NaCl, 10 mM βME) in the presence of 0.1 mg/mL Tobacco Etch Virus (TEV) protease to remove the affinity tag. The cleaved Ubp10 was then passed

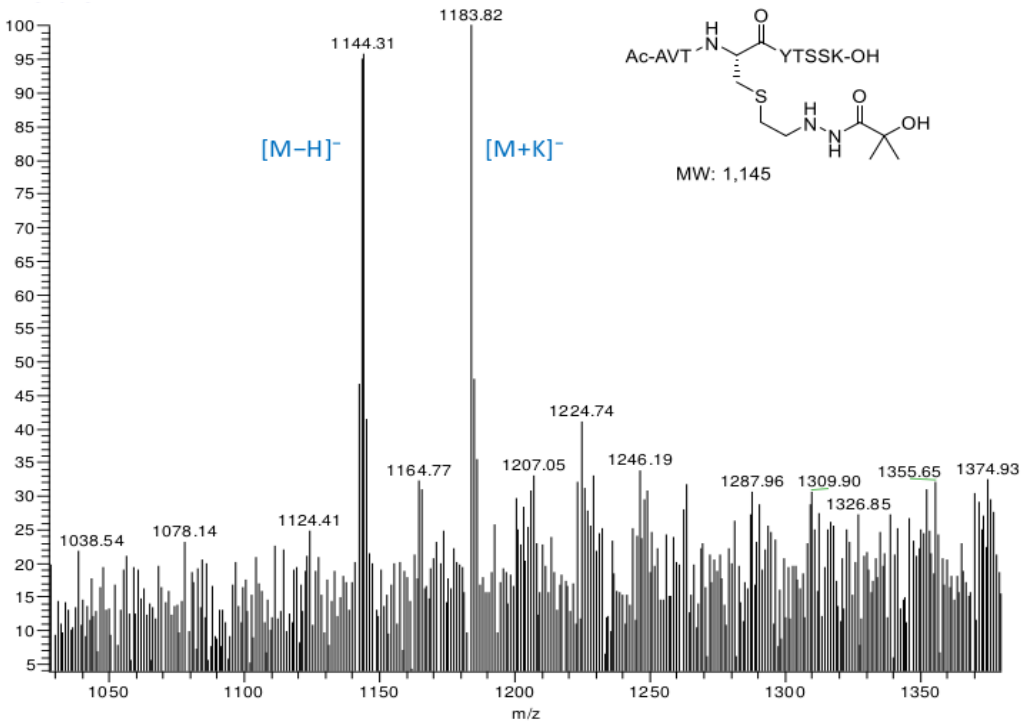
over the HisTrap column again, with the cleaved protein in the flow-through. Subsequently, we reduced the salt concentration of the flow-through to 100 mM by dilution with no-salt buffer, and the sample was further purified by SPHP column (GE Healthcare) equilibrated with ion exchange buffer (25 mM HEPES pH7.5, 50 mM NaCl, 10 mM βME). Ubp10 was then eluted with a gradient of 50 mM to 1M NaCl, concentrated to ~10 mg/mL, and loaded onto a Superdex 200 26/60 column (GE Healthcare) equilibrated with SEC buffer (25 mM HEPES pH7.5, 250 mM NaCl, 10 mM βME). Peak fractions containing purified Ubp10 were then pooled, concentrated to 10 mg/mL, flash frozen in liquid nitrogen and stored at −80 °C until further use.

Human OTUB1 was expressed and purified as described previously.[17] DUB module used in deubiquitination experiments was prepared according to a previous report.[18]

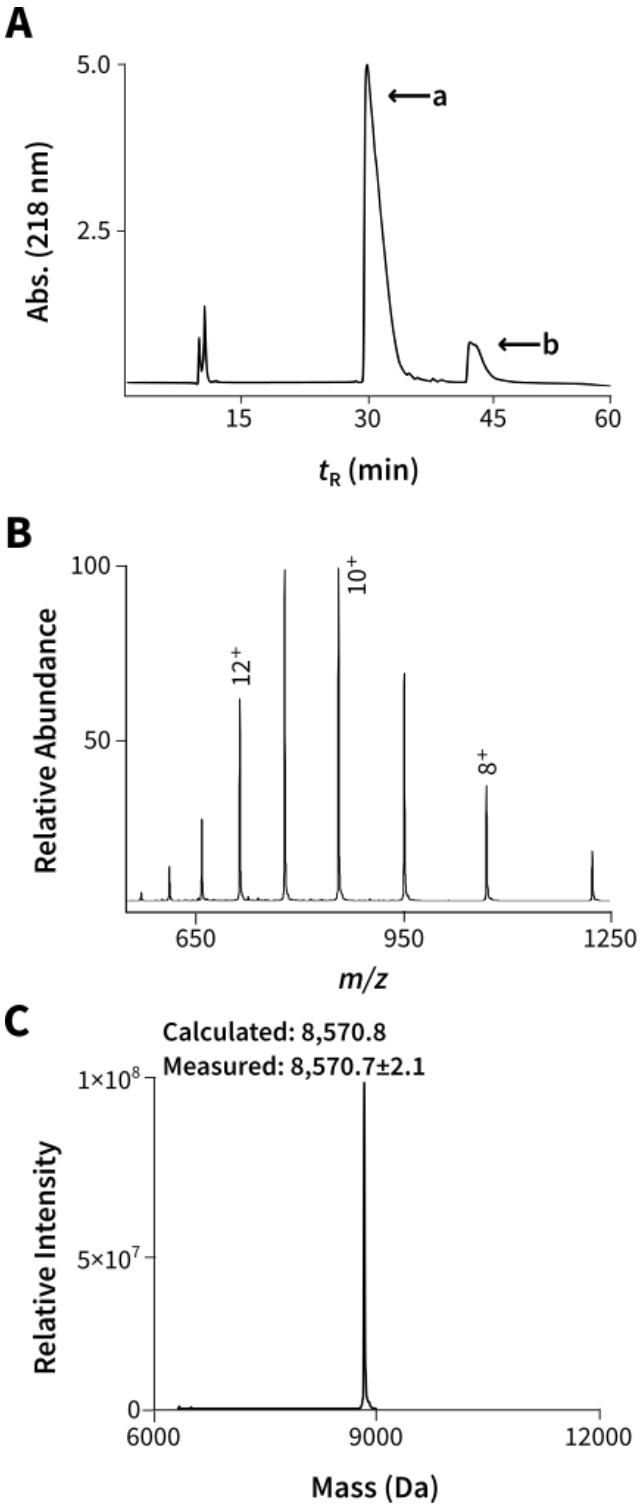
Deubiquitination assays

Deubiquitination assays were performed as described previously.[18]

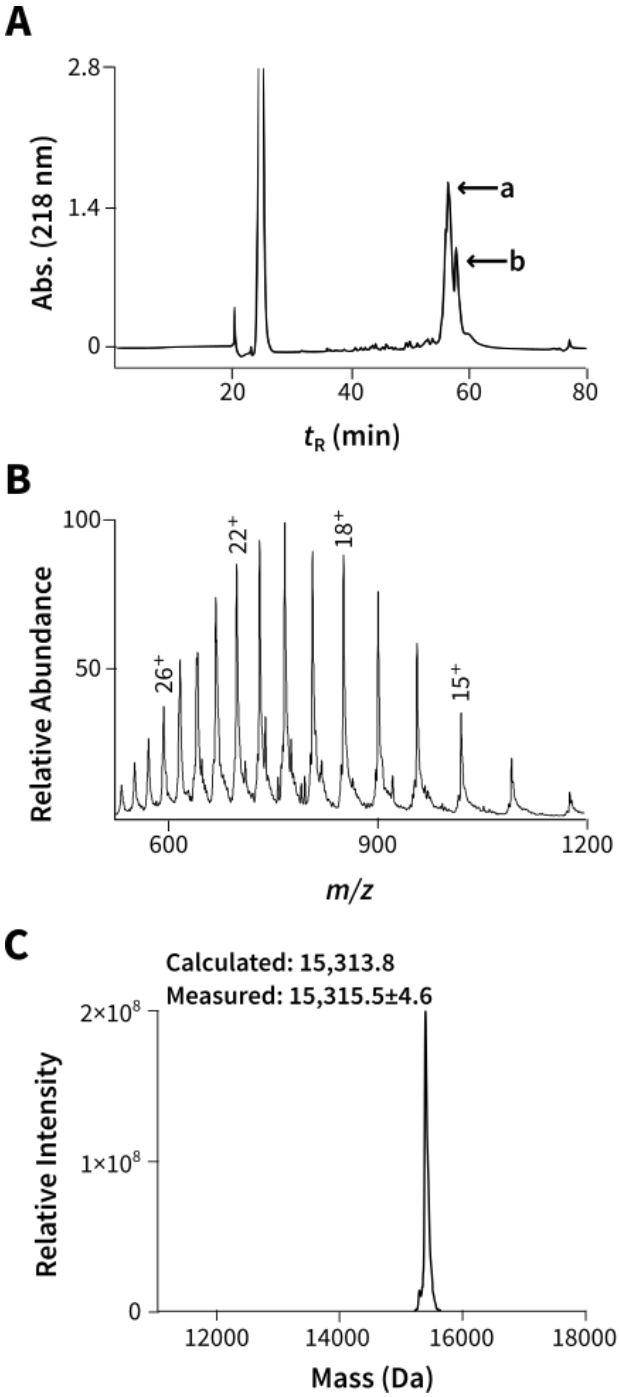
Supplementary figures 1 through 11



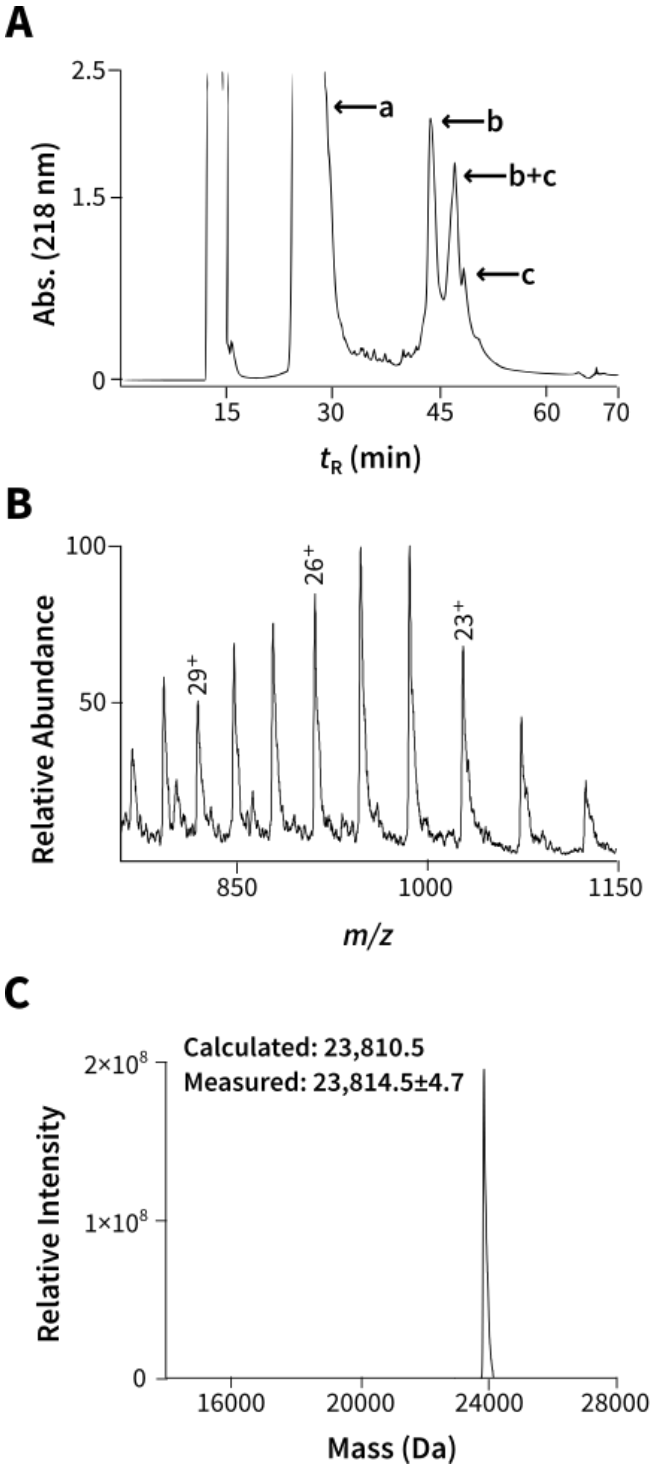
Supplementary Figure 1: ESI-MS (-ve mode) of the product from a model reaction with peptide xlH2B-118-126 sequence: Ac-AVT**C**YTSSK-OH; MW: 1,001).



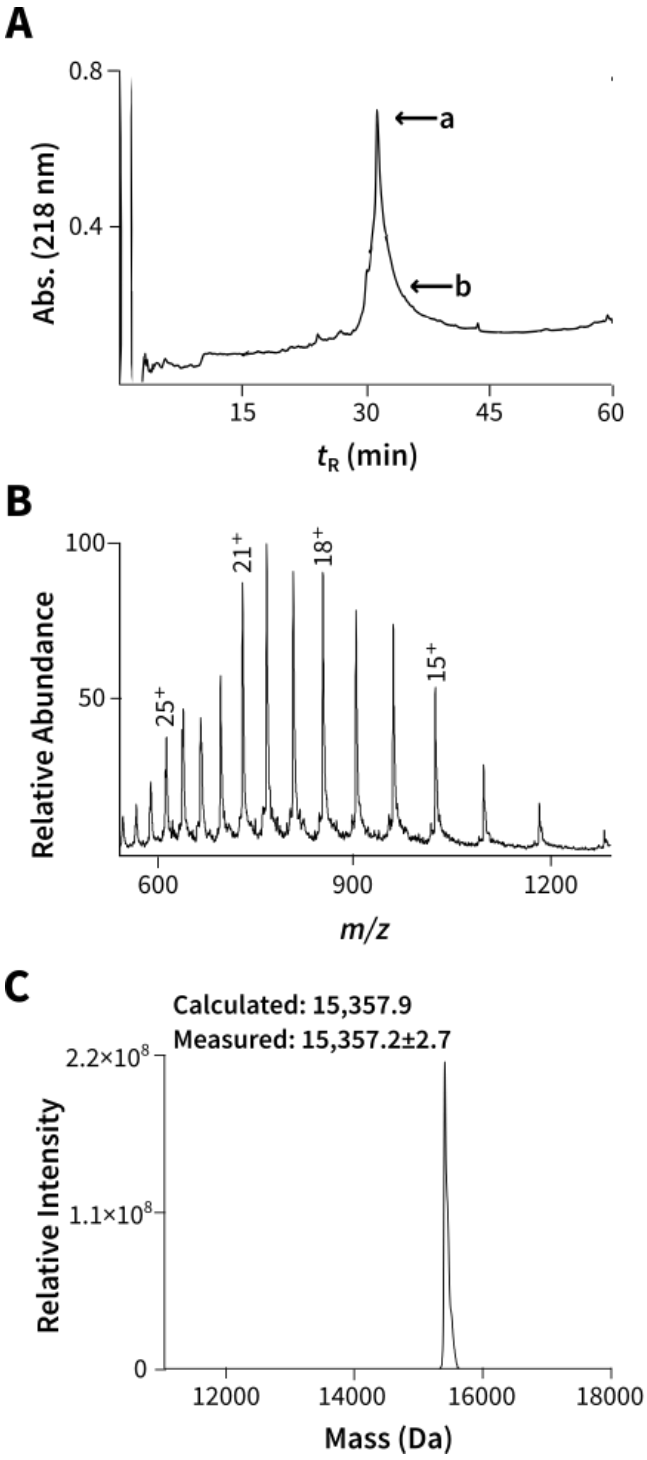
Supplementary Figure 2: Ubiquitin hydrazide (Ub-Hz). (A) HPLC purification of Ub-Hz. Peaks—'**a**' desired Ub-Hz, '**b**' unwanted impurity possibly due to intein-CBD construct. (B) ESI-MS of HPLC purified Ub-Hz. (C) Deconvoluted spectrum showing average mass.



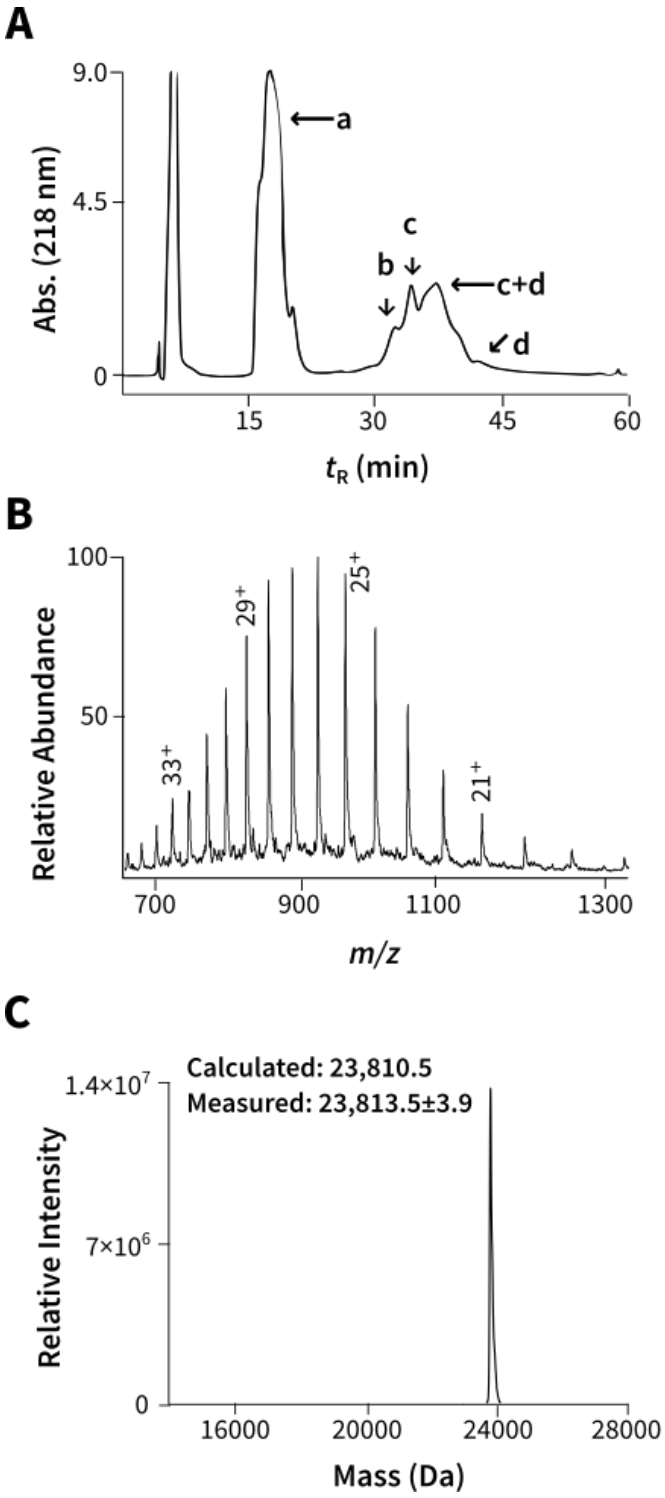
Supplementary Figure 3: H3-K_c56ac. (A) HPLC chromatogram of H3-K_c56ac purification. Peak '**a**' is the desired protein product, whereas, '**b**' turned out to be (upon ESI-MS analysis) the protein species resulting from alkylation with chloroacetaldehyde and reduction with cyanoborohydride, i.e. H3-K56C cysteine-sulfhydryl group transformed into -S-CH_2CH_2OH (1st step: chloroacetaldehyde alkyalation almost always goes to completion, whereas, the 2nd step of hydrazone formation is reversible. The 3rd step is also reliable and the unreacted/reverted aldehyde will be reduced to the corresponding alcohol). (B) ESI-MS characterization of H3-K_c56ac. (C) Deconvoluted spectrum showing the average mass.



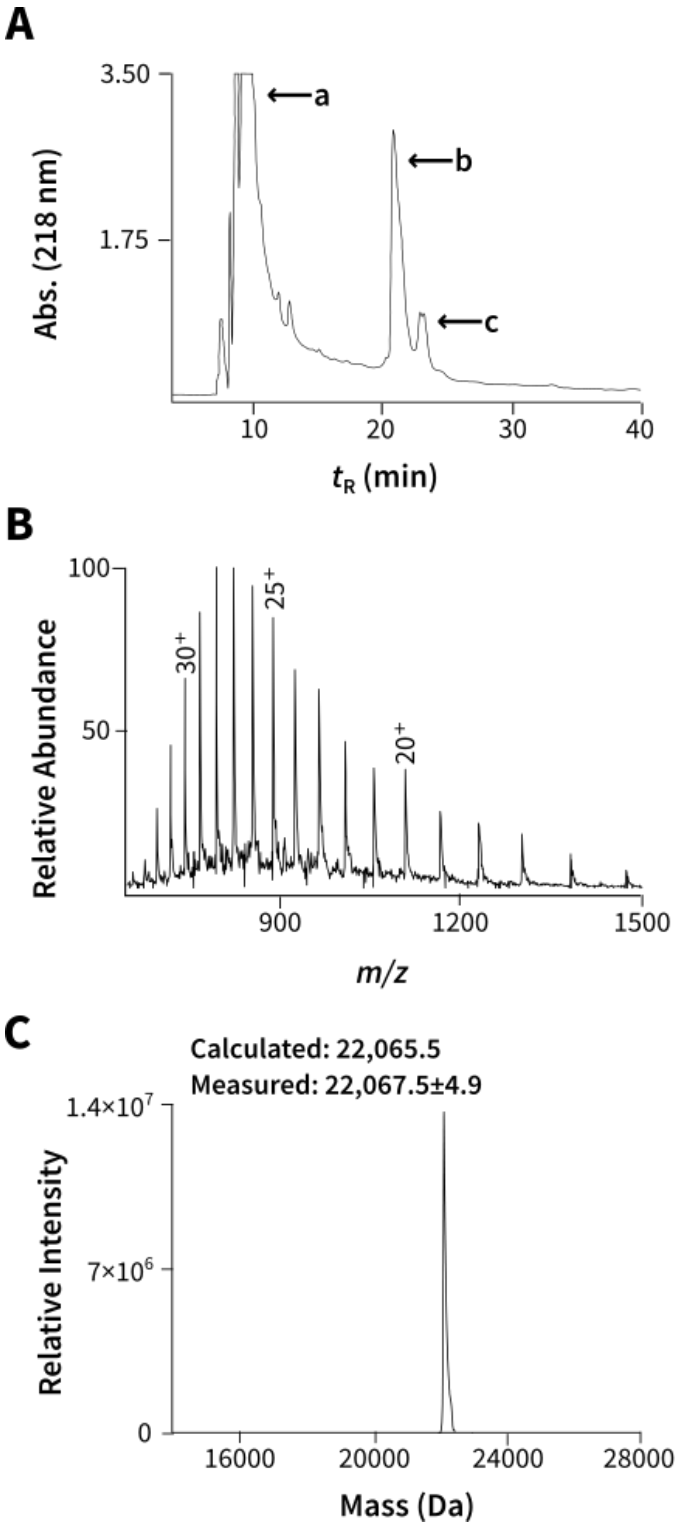
Supplementary Figure 4: H3-K_c56ub. (A) HPLC chromatogram of H3-K_c56ub. Peaks labelled: '**a**' – unreacted ubiquitin hydrazide; '**b**' – desired H3-K_c56ub; '**b+c**' – mixture of desired H3-K_c56ub and alkylated (Cys-S-CH_2CH_2OH) histone; '**c**' – mostly alkylated histone. (B) ESI-MS of purified H3-K_c56ub. (C) Mass deconvolution showing M_{av}.



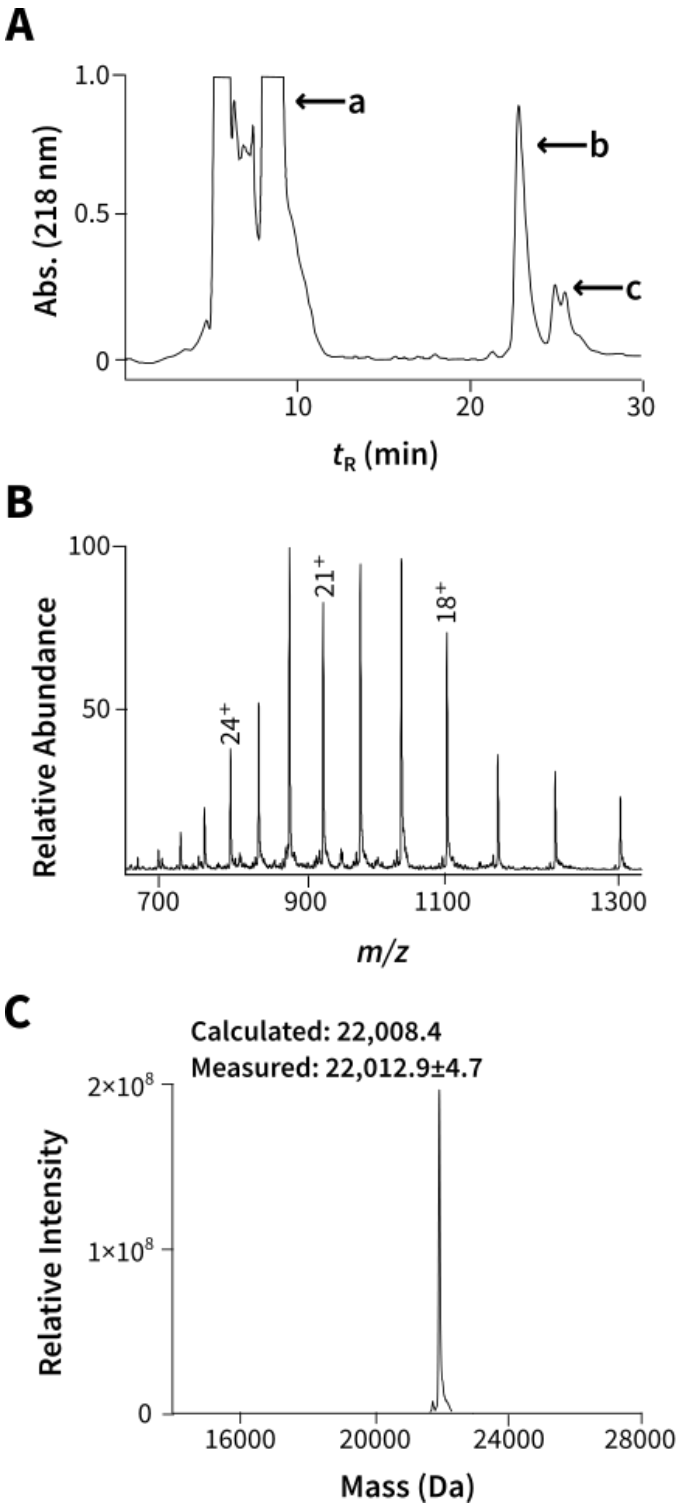
Supplementary Figure 5: H3-K_c122hib. (A) HPLC of H3-K_c122hib. Peaks—'**a**', H3-K_c122hib; '**b**', mostly alkylated H3. (B) ESI-MS of purified H3-K_c122hib. (C) Deconvolution of the ESI-MS of H3-K_c122hib.



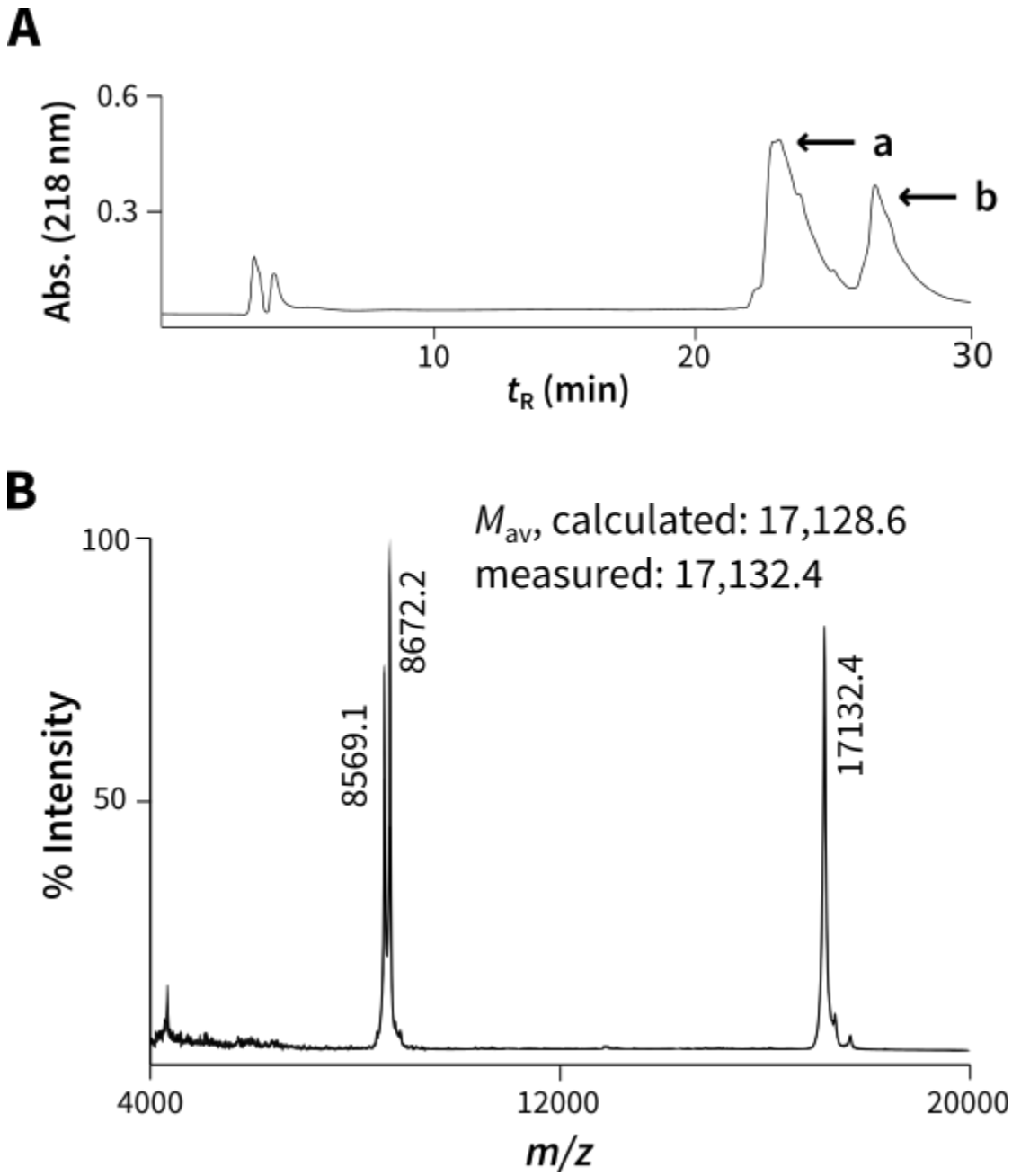
Supplementary Figure 6: H3-K_c122ub. (A) HPLC of H3-K_c122ub. Peaks—'**a**', unreacted ubiquitin hydrazide; '**b**', impure fraction of H3-K_c122ub; '**c**', fraction enriched in H3-K_c122ub; '**c+d**', mixture of H3-K_c122ub and alkylated H3; '**d**', mostly alkylated H3. (B) ESI-MS of purified H3-K_c122ub. (C) Mass deconvolution (M_{av} is shwon).



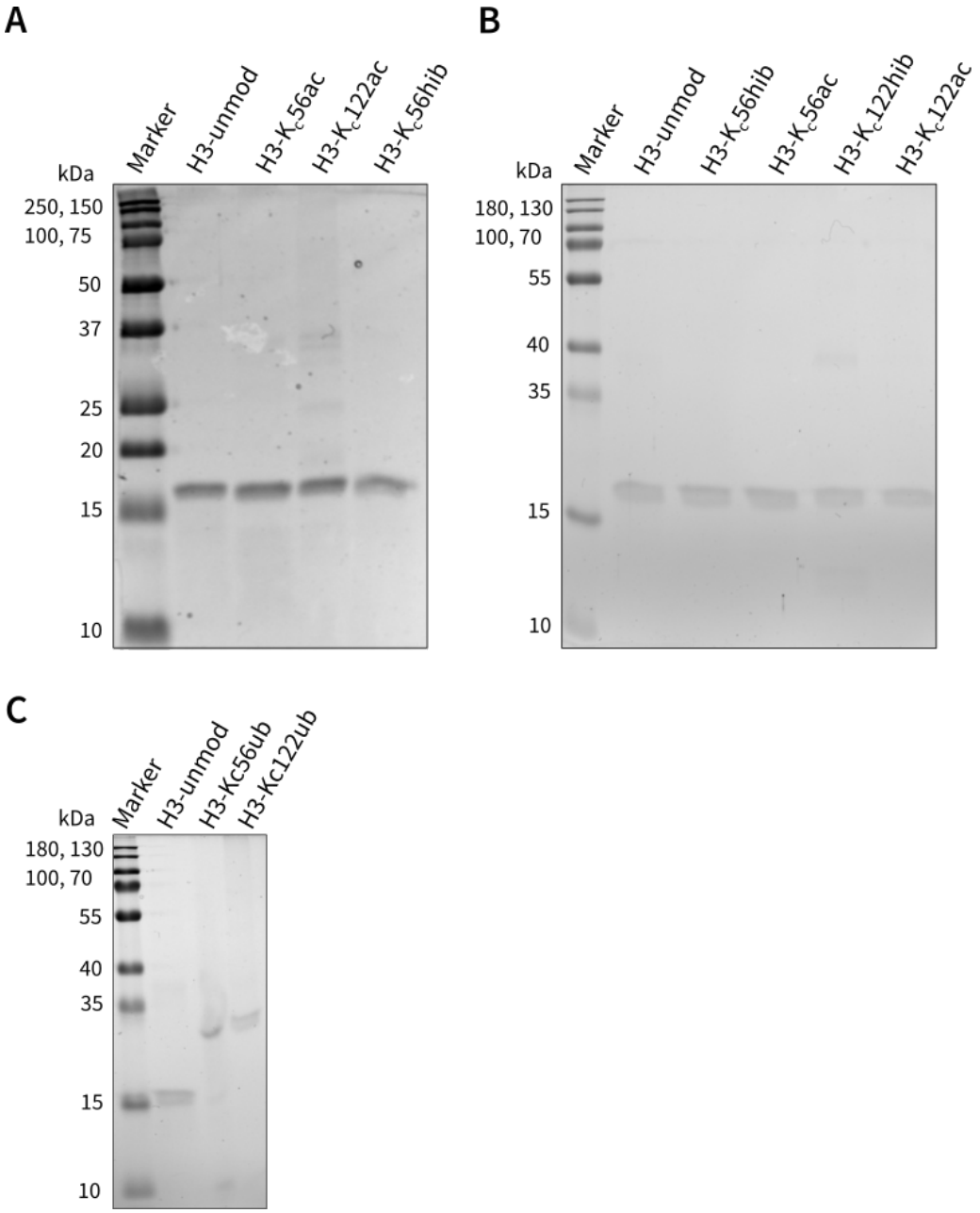
Supplementary Figure 7: H2B-K_c120ub. (A) HPLC purification. Peaks—'**a**' unreacted Ub-Hz; '**b**' desired, H2B-K_c120ub; '**c**' alkylated H2B-K120C (bears 2-hydroxyethyl group on Cys-sidechain). (B) ESI-MS of purified H2B-K_c120ub. (C) Deconvolution of the above ESI-MS.



Supplementary Figure 8: H2B-K116A,K_c120ub. (A) HPLC purification of H2B-K116A,K_c120ub. Peaks—'**a**' unreacted Ub-Hz; '**b**' desired, H2B-K116A,K_c120ub; '**c**' alkylated H2B-K116A,K120C. (B) ESI-MS of purified H2B-K116A,K_c120ub. (C) Mass deconvolution.

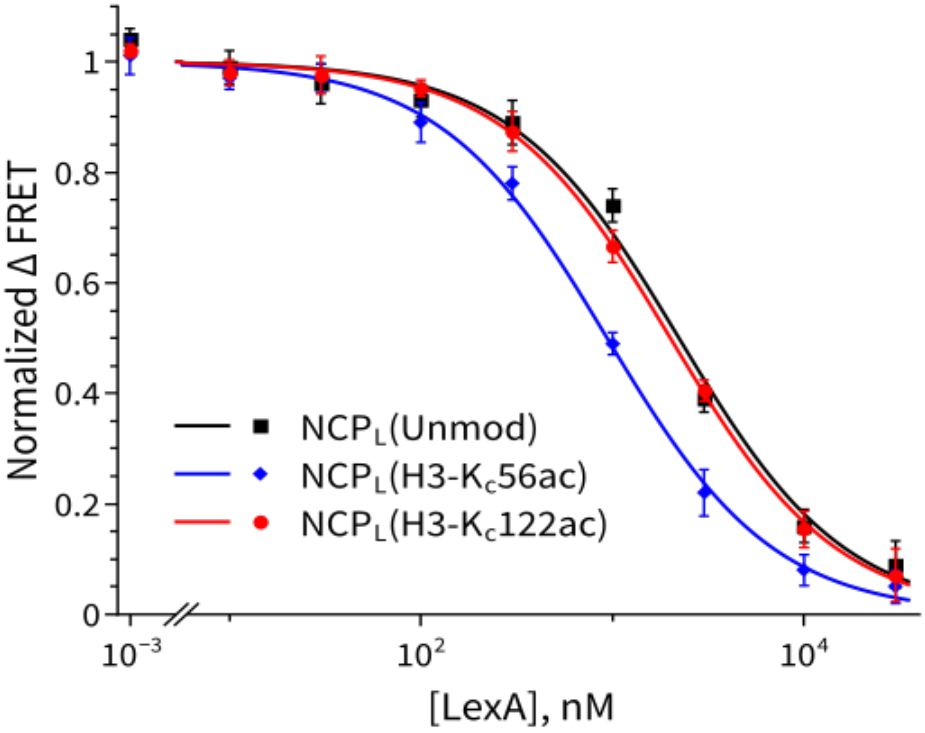


Supplementary Figure 9: K_c48-diUb. (A) HPLC purification of K_c48-diUb. Peaks—'**a**' unreacted Ub-Hz; '**b**' desired, K_c48-diUb. (B) MALD-TOF spectrum of purified K_c48-diUb.



Supplementary Figure 10: Full gels corresponding to Colloidal Blue visualization of loading controls depicted in Figure 3 A, B and C, respectively, in the main text.

A



B

Nucleosome	S½ (nM)	Relative DNA Accessibility
NCP_L(unmod)	2,219.7 ± 96.9	1.0 ± 0.0
NCP_L(H3-K_c56ac)	936.2 ± 64.4	2.4 ± 0.2
NCP_L(H3-K_c122ac)	2,001.7 ± 95.0	1.1 ± 0.1

Supplementary Figure 11: (A) LexA—nucleosome (NCP) binding isotherms comprising a negative control. Note that the NCP_L(H3-K_c122ac) which serves as a negative control is distinct from NCP(H3-K_c122ac) which was reconstituted to study nucleosome dynamics in the dyad region. Like the other NCPs used in this LexA binding isotherms, NCP_L(H3-K_c122ac) comprises LexA target sequence (bp 8–27), Cy3 on the first base of dsDNA, and Cy5 on H2A-K119C. LexA binding curves demonstrate that acyl-Lys mimic in the dyad region [NCP_L(H3-K_c122ac)] has a minimal impact on LexA binding, despite having gone through the three-step chemical process of PTM mimic installation. (B) Tabulation of S½ values and comparison of site accessibilities. The S½ value for NCP_L(H3-K_c122ac) is very close to that of NCP_L(unmod), whereas the same acyl-Lys mimic in the entry/exit region of nucleosome [NCP_L(H3-K_c56ac)] lowers the S½ by 2-fold.

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