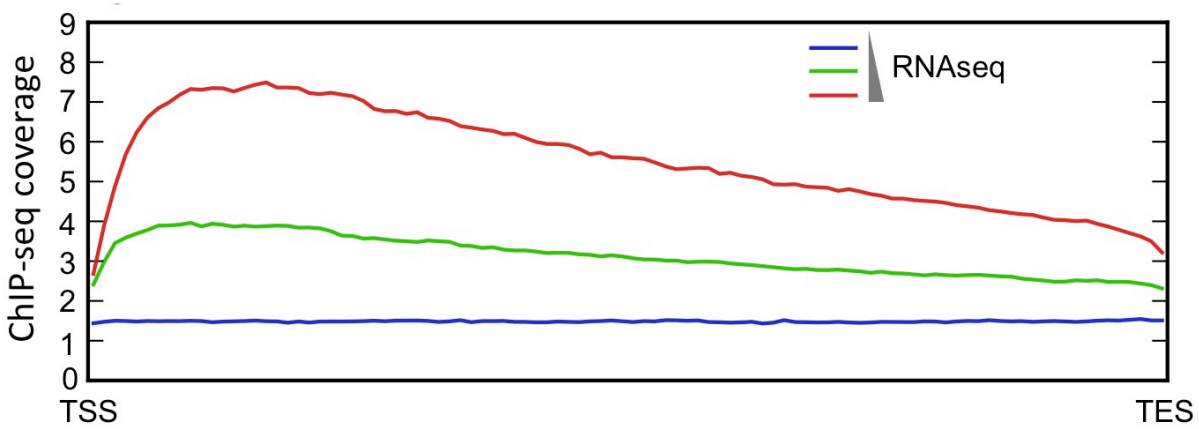
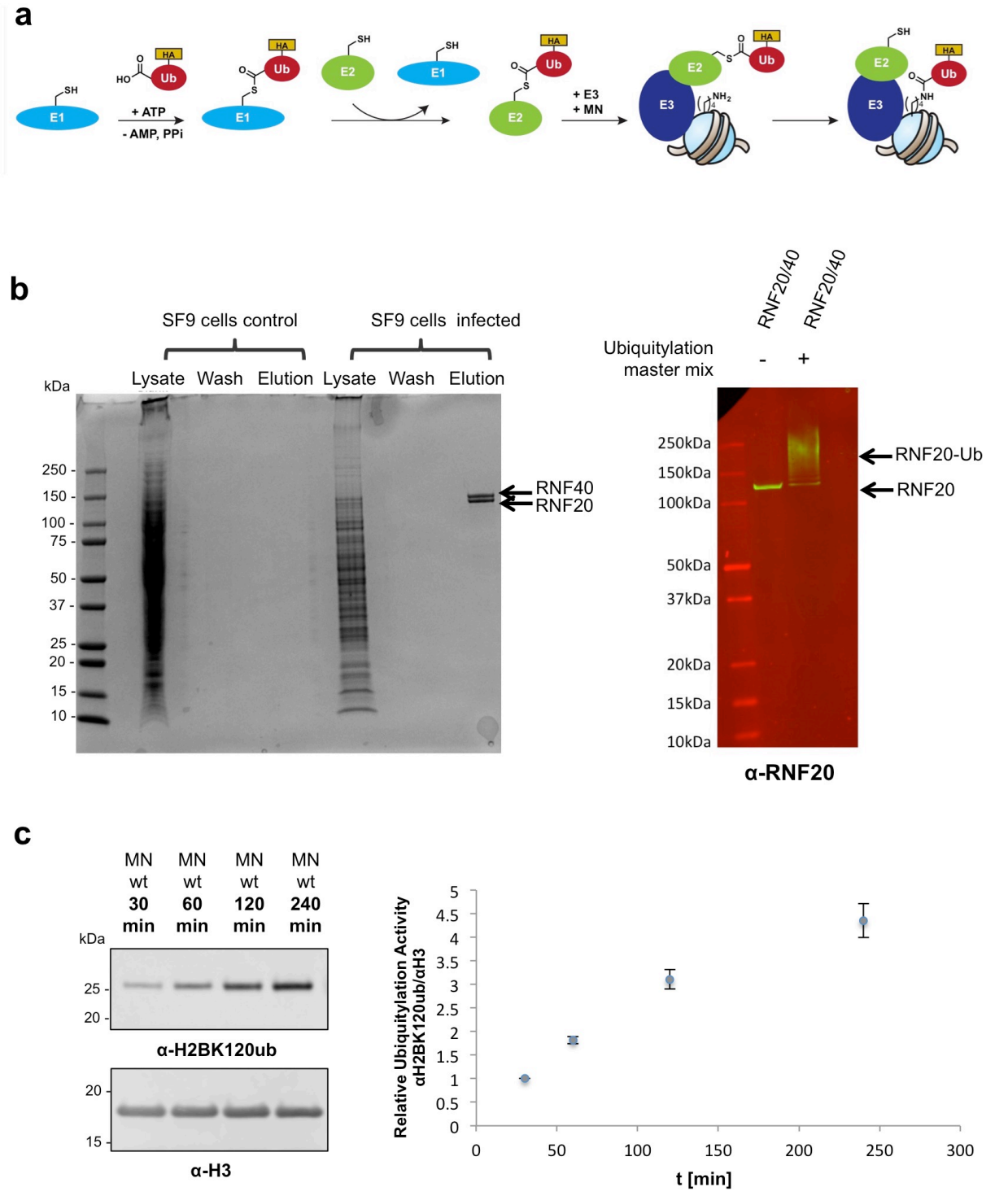
# Supplemental Information

## Functional Crosstalk between Histone H2B Ubiquitylation and H2A Modifications and Variants

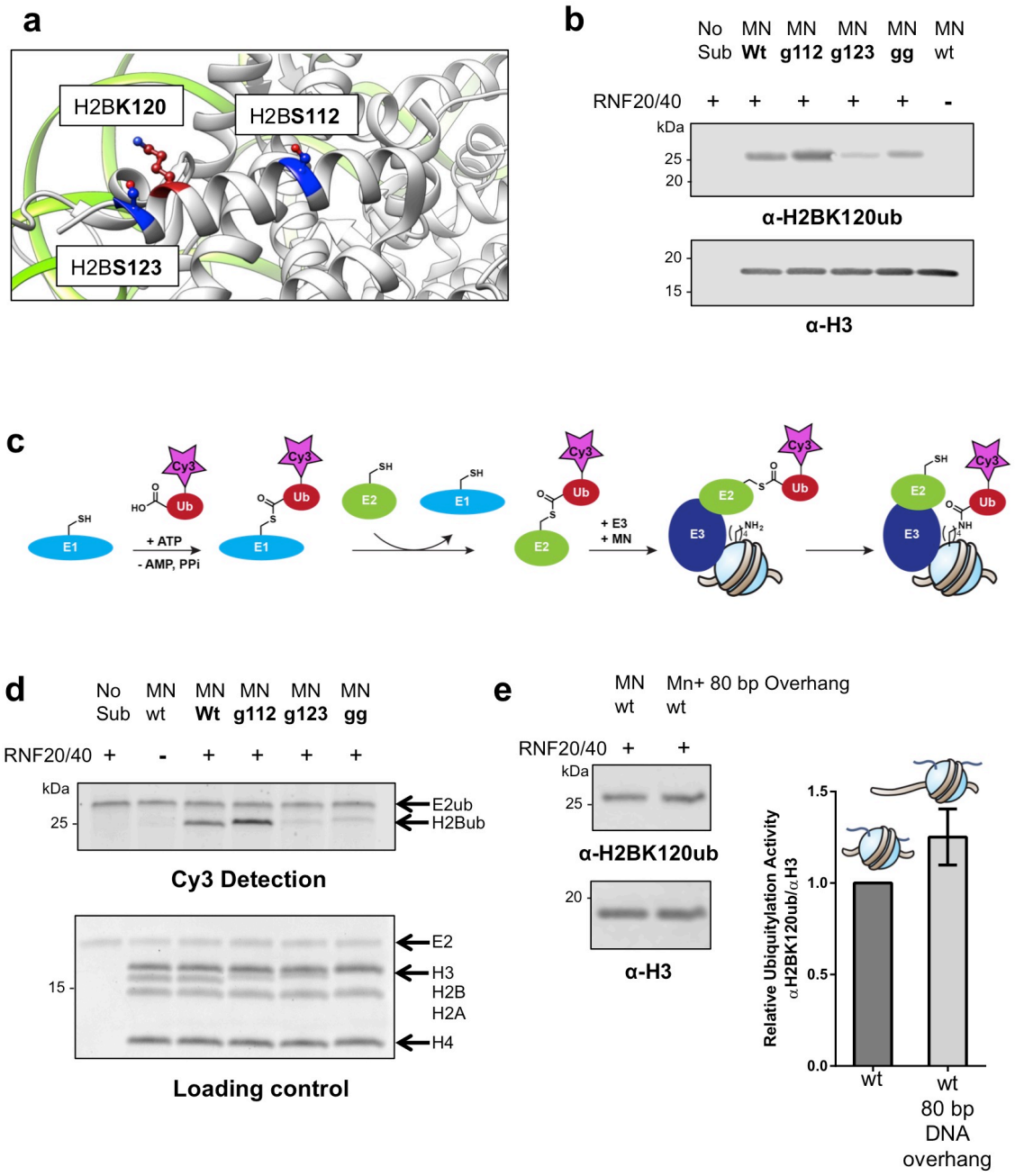
Wojcik et al.



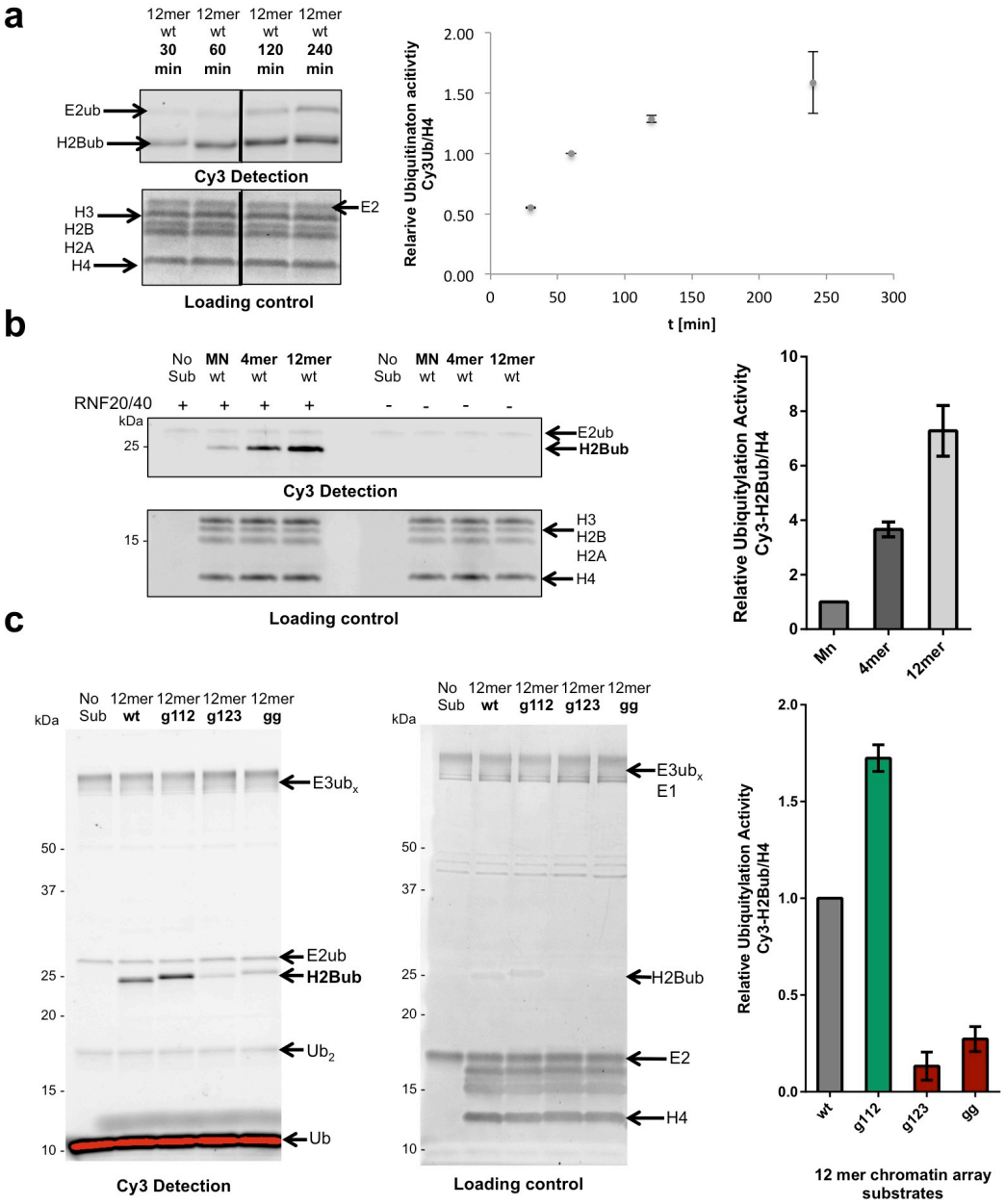

**Supplementary Figure 1**. **ChIP-seq profile of H2BK120ub in HeLa cells.** H2BK120ub is localized within gene bodies and is correlated with active transcription. H2BK120ub ChIP-seq coverage was scaled to a 1kb distance from the TSS to the TES. Genes were divided into three groups, based on their RNA-seq levels (quantified as reads per kilobase per million reads; RPKM). Data processed as described in Materials and Methods (data source listed in Supplementary Table 3).



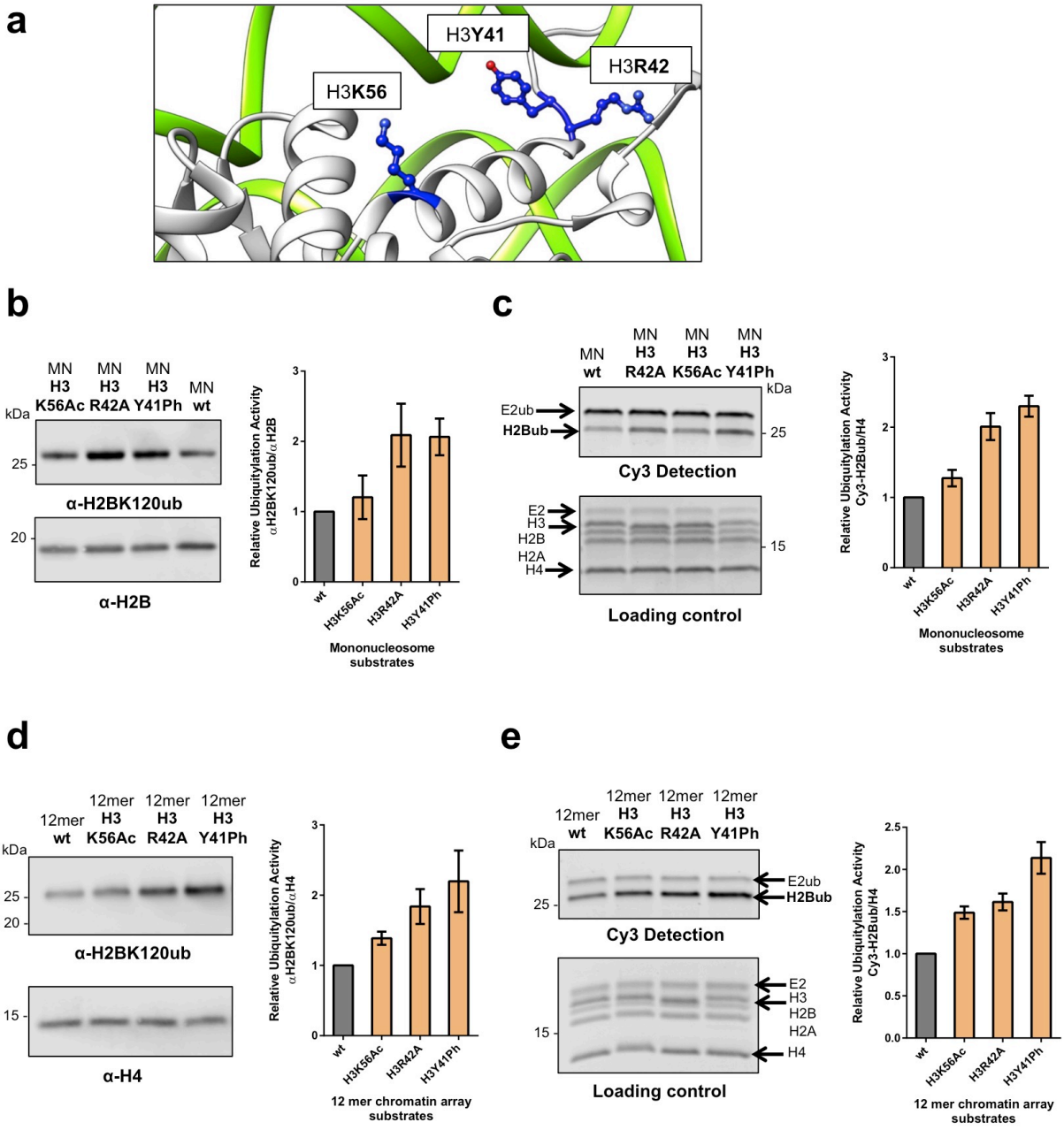

**Supplementary Figure 2.** ***In vitro*** **ubiquitylation of H2BK120.** (**a**) Schematic presentation of the RING-type ubiquitylation mechanism illustrated here using an HA-tagged ubiquitin as employed in the library-based screen. (**b**) Left: RNF20/40 expression and purification in SF9 cells analyzed by SDS-PAGE (stained with Coomassie Brilliant Blue). Right: α-RNF20 immunoblot with and without ubiquitylation master mix containing hE1 and UBE2A. Autoubiquitylation of functional RNF20/40 is observed in the presence of functional UBE2A. (**c**) *In vitro* ubiquitylation time course using MN substrates and native, unlabeled ubiquitin. Normalized H2BK120ub levels (αH2BK120ub/H3α) are plotted relative to the 30 min time point. Data are mean ± s.e.m. (n = 3). Full WB is shown in Supplementary Fig. 16a.



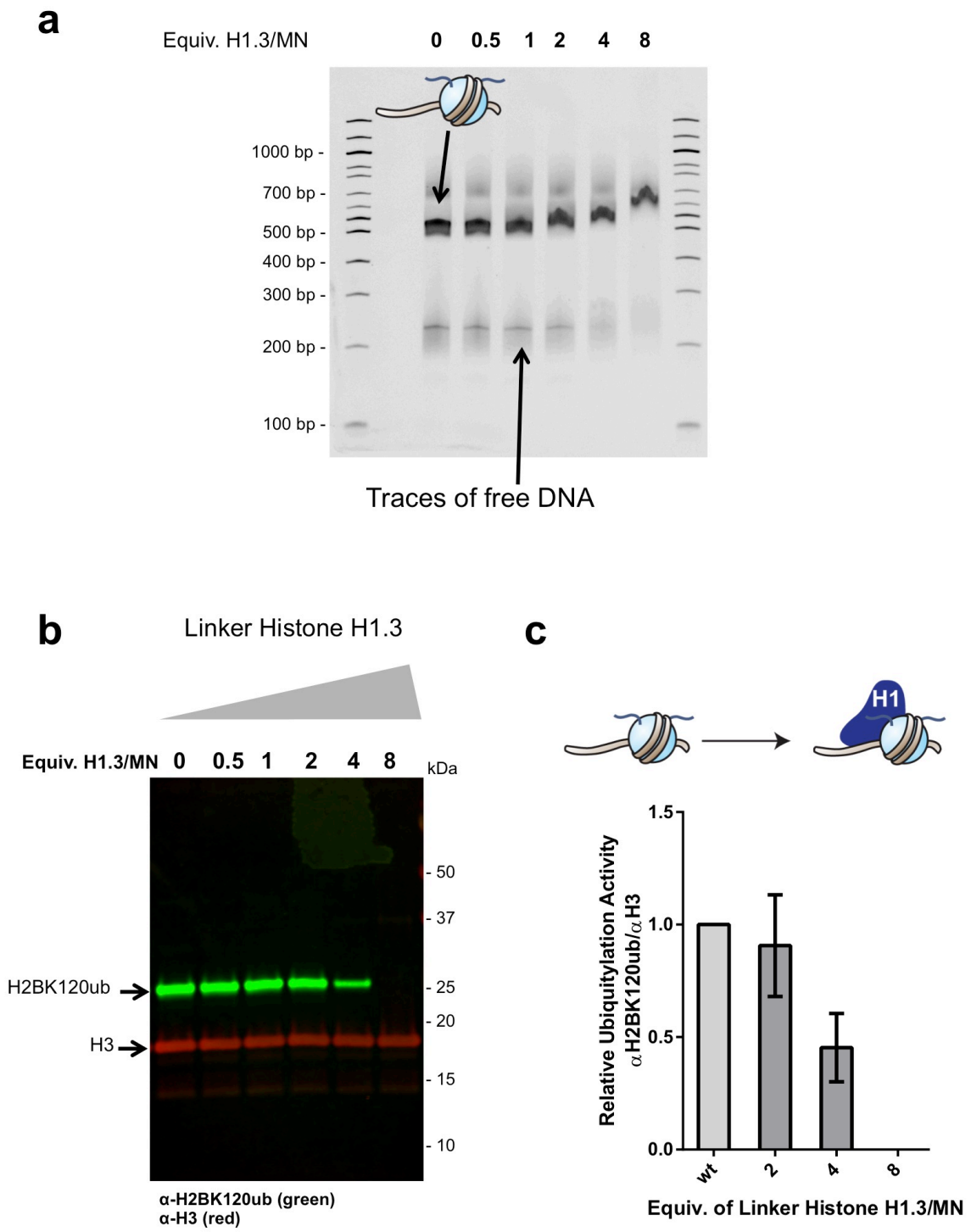

**Supplementary Figure 3. Crosstalk between H2BK120ub and GlcNAcylation at the C-terminal helix of histone H2B.** (**a**) Structure (PDB 1KX5) of the C-terminal helix of H2B within the nucleosome. GlcNAcylation sites are highlighted in blue and H2B K120 highlighted in red. Nucleosomal DNA is highlighted in green. (**b**) *In vitro* ubiquitylation of wt and GlcNAcylated mononucleosomes (MNs) (g112: H2BS112GlcNAc; g123: H2BS123GlcNAc; gg: H2BS112GlcNAc_S123GlcNAc) analyzed by western blotting. (**c**) Schematic presentation of the antibody-free Cy3-based *in vitro* ubiquitylation assay for a RING type E3 ligase. (**d**) *In vitro* ubiquitylation using Cy3-labeled ubiquitin and MNs as described in panel B. Cy3-readout by in-gel fluorescence and the corresponding protein stain used as loading control (SYPRO Ruby stain). (**e**) *In vitro* ubiquitylation of mononucleosomes with a minimal DNA overhang and mononucleosomes containing an asymmetrical 80 bp DNA overhang. Left: Representative western blot analysis after *in vitro* ubiquitylation. Right: Quantification of the immunoblotting data. Normalized H2BK120ub levels (αH2BK120ub/αH3) are plotted relative to wt-MNs. Data are mean ± s.e.m. (n = 3). Full gel images are presented in Supplementary Fig. 16b/c/d.



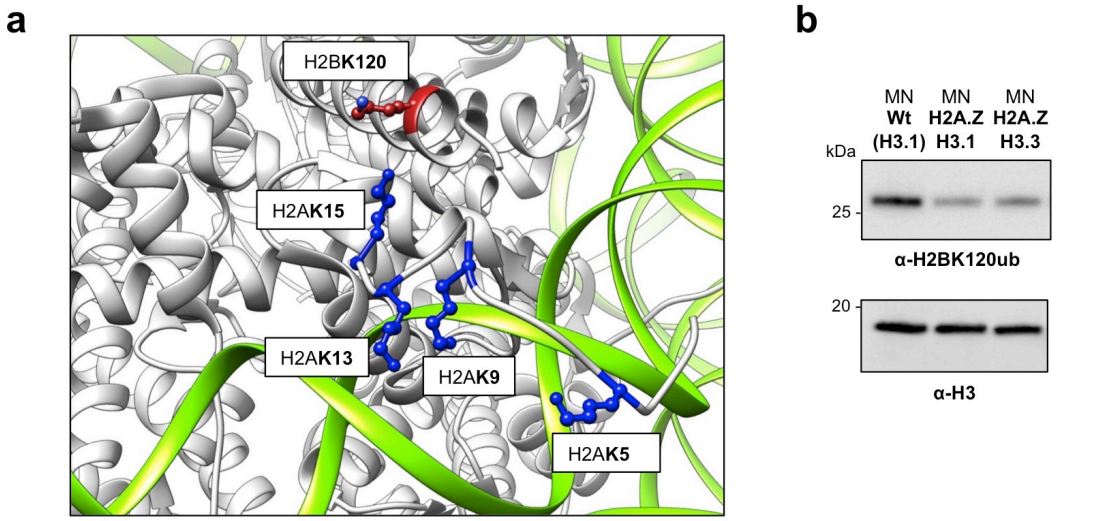

**Supplementary Figure 4.** ***In vitro*** **ubiquitylation of 12mer chromatin arrays.** (**a**) *In vitro* ubiquitylation time course using wt-12mer chromatin arrays and Cy3-labeled ubiquitin. Left: Representative SDS-PAGE after *in vitro* ubiquitylation (Cy3 fluorescence detection and SYPRO Ruby protein stain as loading control). The image was cropped for illustration purposes between lane 60 min and lane 120 min. Right: Quantification of the relative ubiquitylation levels by in-gel fluorescence. Normalized ubiquitylation levels (Cy3-H2Bub/H4) are plotted relative to the 60 min time point (n = 2). (**b**) *In vitro* ubiquitylation of mono- and oligonucleosomes. Left: Representative Cy3 readout and loading control. Right: Quantification of ubiquitylation activities (Cy3-H2Bub/H4) of wt mononucleosomes, 4mer and 12mer chromatin arrays. Ubiquitylation activities are normalized to MNs (n = 3). (**c**) *In vitro* ubiquitylation of wt- and GlcNAcylated-12mer chromatin arrays (g112: H2BS112GlcNAc; g123: H2BS123GlcNAc; gg: H2BS112GlcNAc_S123GlcNAc) using Cy3-labeled ubiquitin. Left: Representative Cy3 readout and loading control. Right: Quantification of ubiquitylation activities (Cy3-H2Bub/H4). Normalized ubiquitylation levels (Cy3-H2Bub/H4) are plotted relative to wt(n = 4). All data are mean ± s.e.m. Full gel images are given in Supplementary Fig. 17.




**Supplementary Figure 5. Crosstalk between H2BK120ub and modification/mutants at the DNA entry/exit site.** (**a**) Structure of the DNA entry/exit site of the nucleosome (PDB 1KX5). Positions H3Y41, H3R42A and H356 and their respective side chains are highlighted in blue. Nucleosomal DNA is highlighted in green. (**b,c**) *In vitro* ubiquitylation of wt- and modified MNs. (**b**) Left: Western blot analysis of *in vitro* ubiquitylation. Right: Quantification of immunoblot data (αH2BK120ub/αH2B) relative to wt (n = 3). (**c**) Left: In-gel fluorescence and protein stain (SYPRO Ruby) as loading control. Right: Quantification of ubiquitylation levels (Cy3-H2Bub/H4) relative to wt (n = 4). (**d,e**) *In vitro* ubiquitylation of wt- and modified 12mer chromatin arrays. (**d**) Left: Western blot analysis of *in vitro* ubiquitylation. Right: Quantification of immunoblot data (αH2BK120ub/αH4) relative to wt (n = 4). (**e**) Left: In-gel fluorescence and protein stain (SYPRO Ruby) as loading control. Right: Quantification of ubiquitylation levels (Cy3-H2Bub/H4) relative to wt (n = 3). All data are mean ± s.e.m. Full images are shown in Supplementary Fig. 18.




**Supplementary Figure 6. Addition of linker histone H1.3 inhibits ubiquitylation of H2BK120 *in vitro*.** (**a**) Native gel electrophoresis of MNs with a 80bp DNA overhang and different amounts of linker histone H1.3 (SYBR Gold nucleic acid stain). The gel shift indicates binding of linker histone H1.3. (**b,c**) *In vitro* ubiquitylation of MNs with a 80bp DNA overhang in presence of linker histone H1.3. (**b**) Western blot analysis of the *in vitro* ubiquitylation assay. (**c**) Quantification of immunoblot data (αH2BK120ub/αH3) relative to wt. Data are mean ± s.e.m. (n = 3). *In vitro* ubiquitylation was performed using standard conditions as described in the methods section (substrate concentration: 1µM of nucleosomal H2B corresponds to 0.5 µM mononucleosomes).




c

**Wt-H2A and H2A.Z**

**Human H2A type 2A**

```
        10         20         30         40         50         60
SGRGKQGGKA RAKAKSRSSR AGLQFPVGRV HRLLRKGNYA ERVGAGAPVY MAAVLEYLTA

        70         80         90         100        110        120
EILELAGNAA RDNKKTRIIP RHLQLAIRND EELNKLLGKV TIAQGGVLPN IQAVLLPKKT

ESHHKAKGK
```

**Human H2A.Z**

```
        10         20         30         40         50         60
AGGKAGKDSG KAKTKAVSRS QRAGLQFPVG RIHRHLKSRT TSHGRVGATA AVYSAAILEY

        70         80         90         100        110        120
LTAEVLELAG NASKDLKVKR ITPRHLQLAI RGDEELDSLI KATIAGGGVI PHIHKSLIGK

KGQQKTV
```

**H2A_K15 Mutants**

**Human H2A type 2A K15V**

```
        10         20         30         40         50         60
SGRGKQGGKA RAKAVSRSSR AGLQFPVGRV HRLLRKGNYA ERVGAGAPVY MAAVLEYLTA

        70         80         90         100        110        120
EILELAGNAA RDNKKTRIIP RHLQLAIRND EELNKLLGKV TIAQGGVLPN IQAVLLPKKT

ESHHKAKGK
```

**Human H2A type 2A K15Q**

```
        10         20         30         40         50         60
SGRGKQGGKA RAKAQSRSSR AGLQFPVGRV HRLLRKGNYA ERVGAGAPVY MAAVLEYLTA

        70         80         90         100        110        120
EILELAGNAA RDNKKTRIIP RHLQLAIRND EELNKLLGKV TIAQGGVLPN IQAVLLPKKT

ESHHKAKGK
```

**H2A_Tail Acetylation Mimic (poly Q, pQ)**

**Human H2A type 2A K5,9,13,15Q**

```
        10         20         30         40         50         60
SGRGQQGGQA RAQAQSRSSR AGLQFPVGRV HRLLRKGNYA ERVGAGAPVY MAAVLEYLTA

        70         80         90         100        110        120
EILELAGNAA RDNKKTRIIP RHLQLAIRND EELNKLLGKV TIAQGGVLPN IQAVLLPKKT

ESHHKAKGK
```

**Tail Swap (TS) chimeras**

**Human H2A type 2A tail swap (H2A_TS)**

```
        10         20         30         40         50         60
AGGKAGKDSG KAKTKAVSRS QRAGLQFPVG RVHRLLRKGN YAERVGAGAP VYMAAVLEYL

        70         80         90         100        110        120
TAEILELAGN AARDNKKTRI IPRHLQLAIR NDEELNKLLG KVTIAQGGVL PNIQAVLLPK

       130
KTESHHKAKG K
```

**Human H2A.Z tail swap (H2A.Z_TS)**

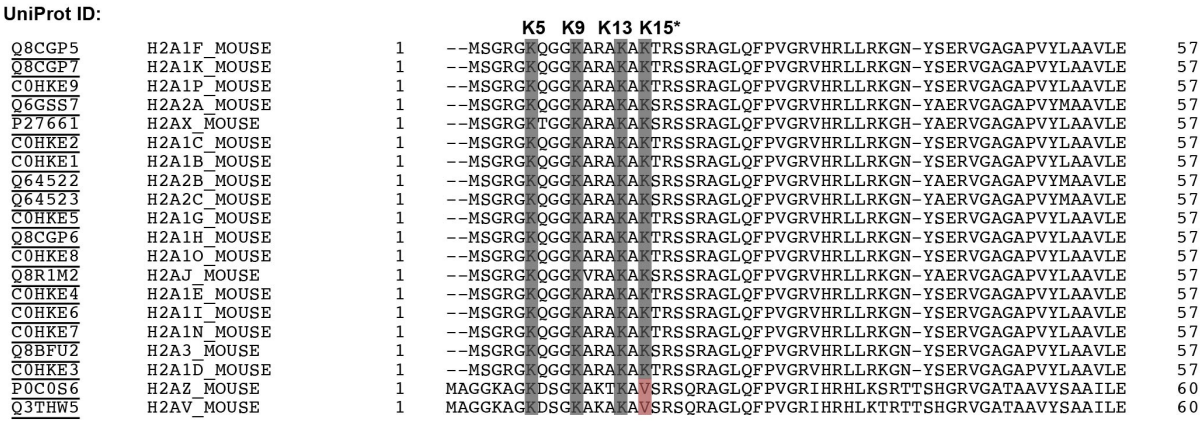
```
        10         20         30         40         50         60
SGRGKQGGKA RAKAKSRSSR AGLQFPVGRI HRHLKSRTTS HGRVGATAAV YSAAILEYLT

        70         80         90         100        110        120
AEVLELAGNA SKDLKVKRIT PRHLQLAIRG DEELDSLIKA TIAGGGVIPH IHKSLIGKKG

QQKTV
```

**Supplementary Figure 7. Crosstalk between H2BK120ub and the N-terminal tail of H2A.** (**a**) Structure of the H2A tail region within the nucleosome (PDB 1KX5). Positions H2AK15, H2AK13, H2AK9 and H2AK5 and the respective side chains are highlighted in blue. Nucleosomal DNA is highlighted in green. (**b**) *In vitro* ubiquitylation of MNs containing histone variant H2A.Z paired with H3.1 and H3.3. Western Blot analysis of *in vitro* ubiquitylation reactions. (**c**) Full amino acid sequences of H2A/H2A.Z mutants and chimeras employed in this study. Introduced changes to the canonical H2A/H2A.Z amino acid sequences are highlighted in red. Full WB is presented in Supplementary Fig. 19a.

*Mus Musculus* (Mouse)



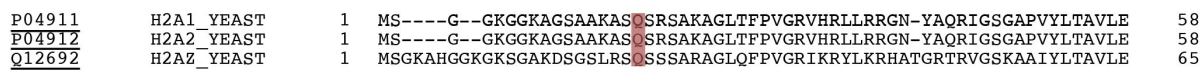

*Xenapus laevis* (African clawed frog)


*Gallus gallus* (Chicken)

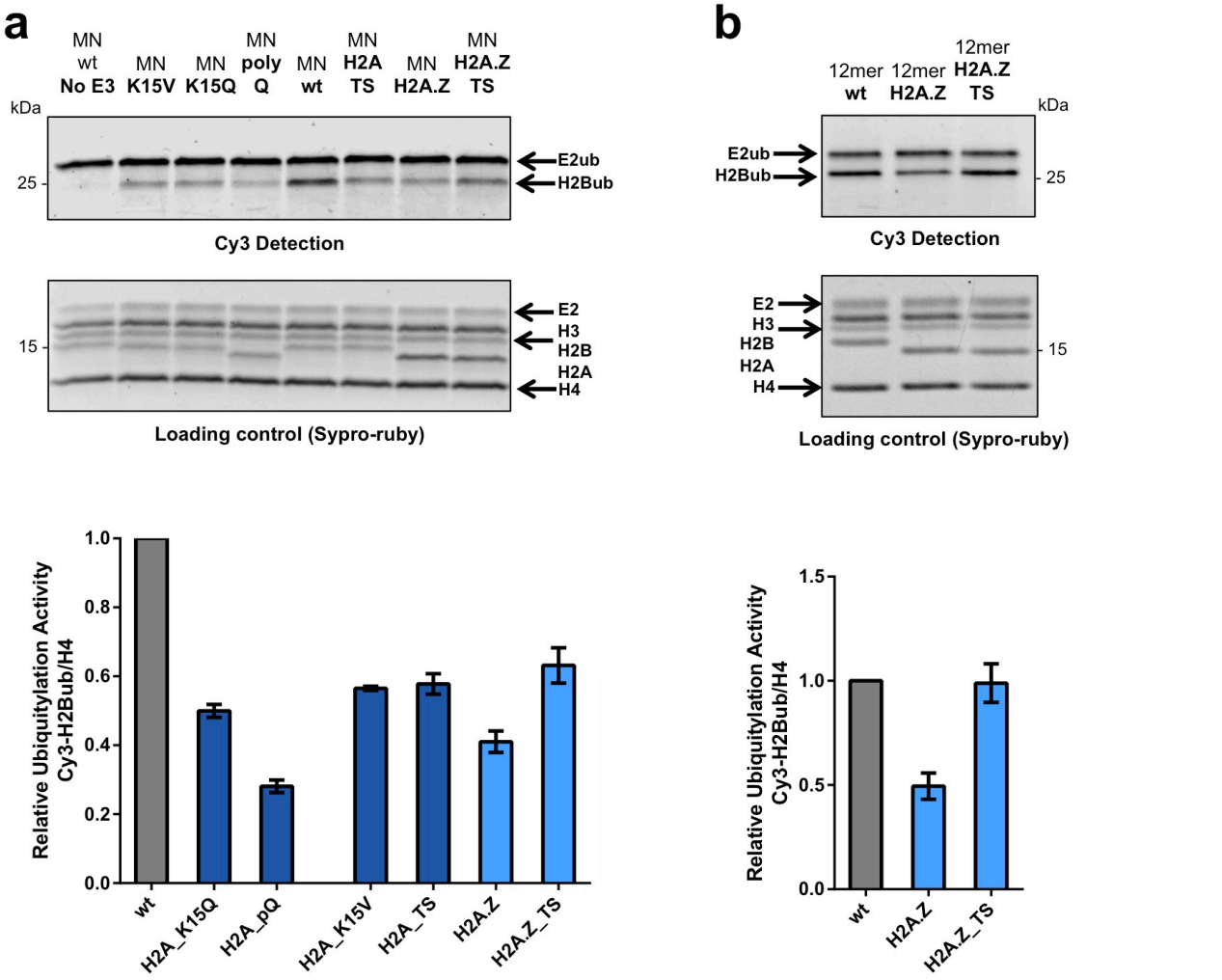

*Drosophila melanogaster* (Fruit fly)


*Caenorhabditis elegans* (Roundworm)

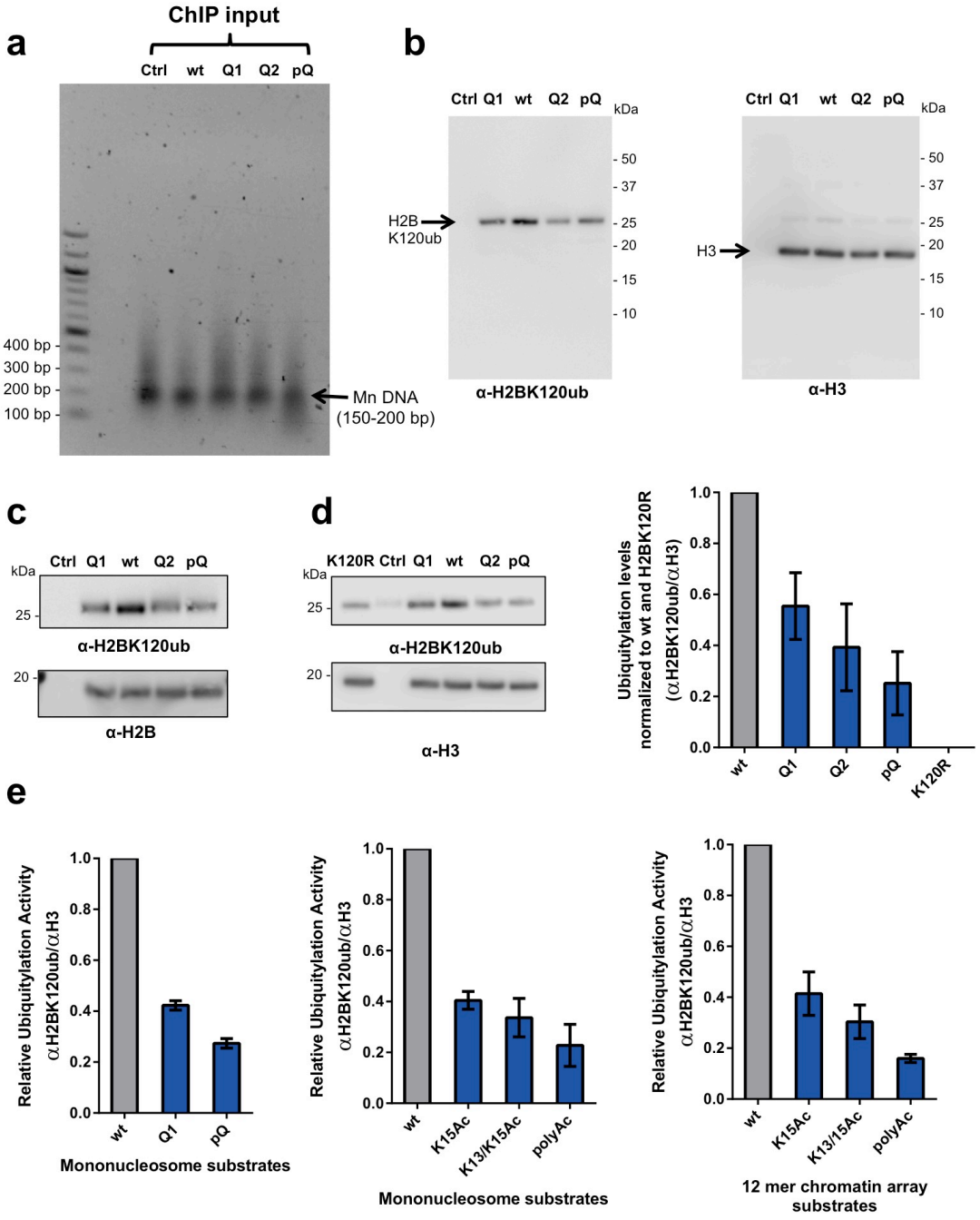

*Saccharomyces cerevisiae* (Baker's yeast)




**Supplementary Figure 8. Interplay between Lys-15 of H2A and Val-17 of H2A.Z (and H2A.V) is highly conserved in metazoans.** Amino acid sequence of canonical H2A and H2A variants were aligned using the UniProt database (H2A variants H2A.bbd and macroH2A are not shown in the alignment). H2AZL_Xena (UniProt ID P70094) encodes for histone H2A.Z-like variant found in Xenopus lavis.

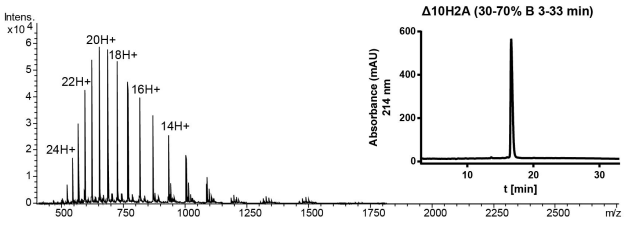


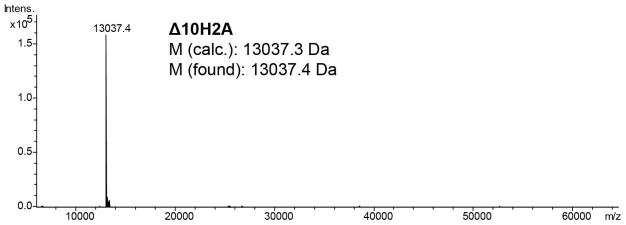

**Supplementary Figure 9. Crosstalk between H2BK120ub and the N-terminal tail of H2A analyzed by *in vitro* ubiquitylation using Cy3-Ub.** (**a**) *In vitro* ubiquitylation of mononucleosomes containing H2A/H2A.Z mutants using Cy3-Ub. Top: Cy3 readout and corresponding loading control (SYPRO Ruby stain). Bottom: Quantification of ubiquitylation activities (Cy3-H2Bub/H4) plotted relative to wt-MN (n = 4). (**b**) *In vitro* ubiquitylation of 12mer chromatin arrays containing H2A.Z and H2A.Z_TS measured by in-gel fluorescence. Top: Cy3 readout and corresponding loading control (SYPRO Ruby stain). Bottom: Quantification of ubiquitylation activities (Cy3-H2Bub/H4) plotted relative to wt-12mer chromatin arrays (n = 4). All data are mean ± s.e.m. Full gel images are shown in Supplementary Fig. 19b/c.




**Supplementary Figure 10. Regulation of H2BK120ub by the H2A N-terminal tail in HEK293T cells.** (**a**) Analysis of ChIP input (after MNase digestion and DNA isolation), showing that MNase digestion provided predominately MNs. (**b**) Full image of WB analysis, which was cropped for illustration purposes in Fig. 5a. (**c**) WB analysis of H2BK120ub levels after ChIP using H2B as loading control. (**d**) Left: Representative WB including a negative control: H2BK120R (K120R). Right: Quantification of H2BK120ub levels after ChIP in HEK 293T cells. Each data set of ubiquitylation levels (αH2BK120ub/αH3) was normalized to wt (100%) and H2BK120R (0%) followed by calculating the mean ± s.e.m. (n = 4). (**e**) Previously determined *in vitro* ubiquitylation activities for comparison: MN containing H2A K→ Q mutants (Fig. 3d), acetylated H2A (Fig. 3a) and 12mer chromatin arrays containing acetylated H2A (Fig. 3b). Full WBs are shown in Supplementary Fig. 20. Ctrl: Control (mock transfection); wt: H2A-FLAG; Q1: H2A_K15Q-FLAG; Q2: H2A_K13/15Q-FLAG; pQ: H2A_K5/9/13/15Q-FLAG).

Δ10H2A




**Δ10H2A**
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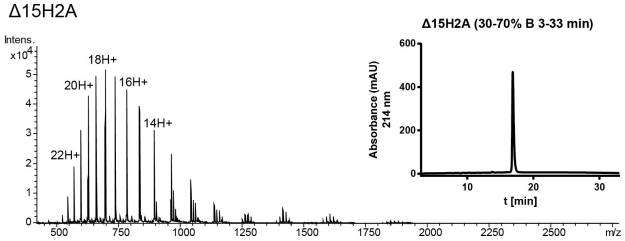
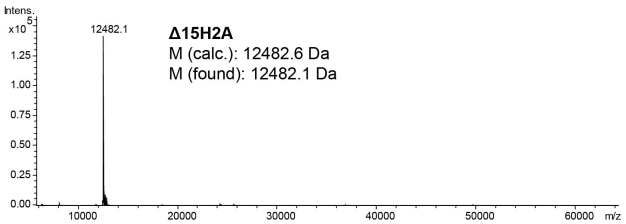
M (found): 13037.4 Da

Δ15H2A




**Δ15H2A**
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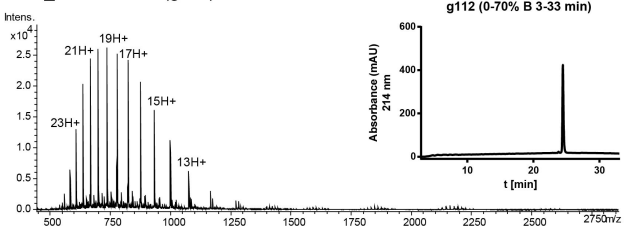
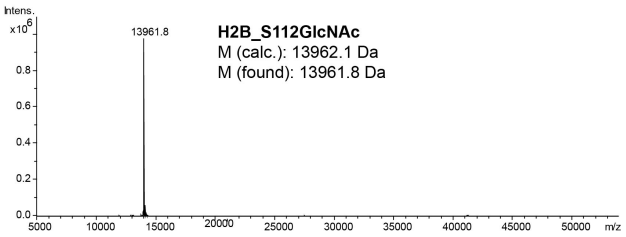
H2B_S112GlcNAc (g112)




**H2B_S112GlcNAc**
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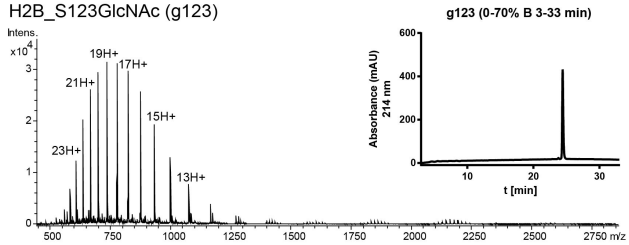
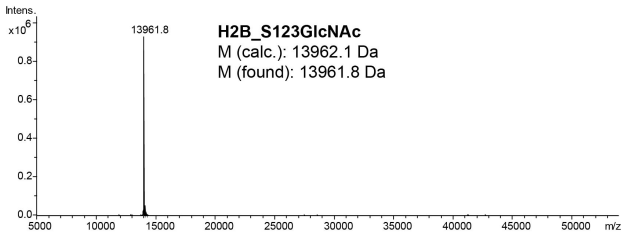
M (found): 13961.8 Da

H2B_S123GlcNAc (g123)




**H2B_S123GlcNAc**
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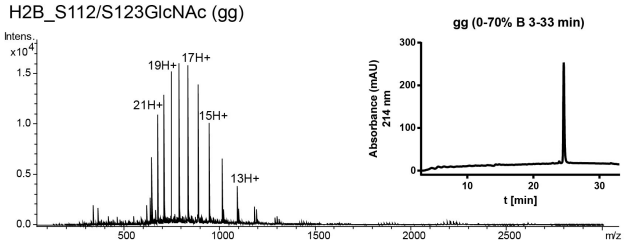
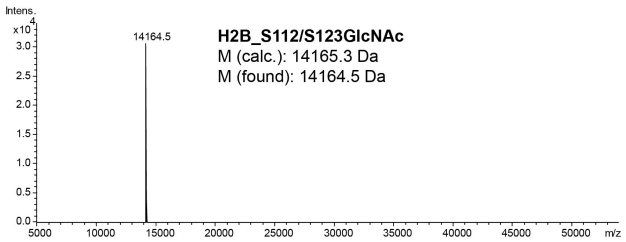
M (found): 13961.8 Da

H2B_S112/S123GlcNAc (gg)




**H2B_S112/S123GlcNAc**
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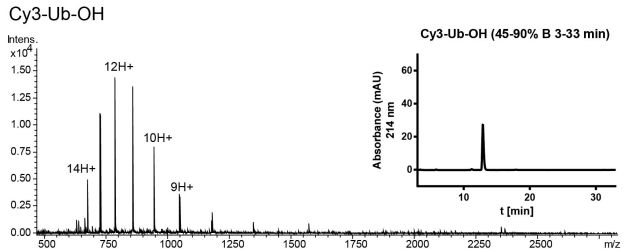
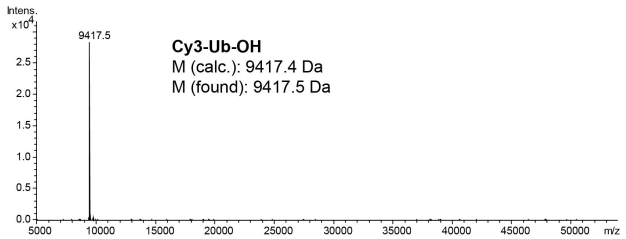
M (found): 14164.5 Da

Cy3-Ub-OH




**Cy3-Ub-OH**
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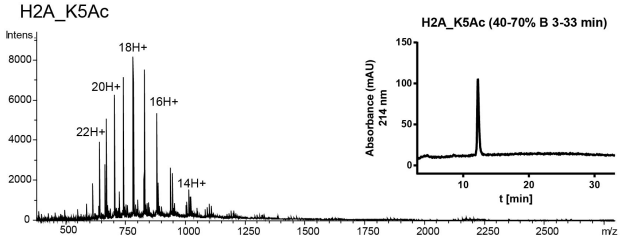
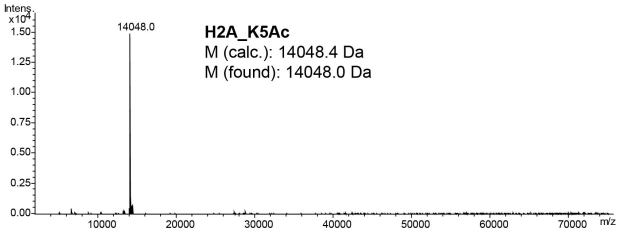
M (found): 9417.5 Da

H2A_K5Ac




**H2A_K5Ac**
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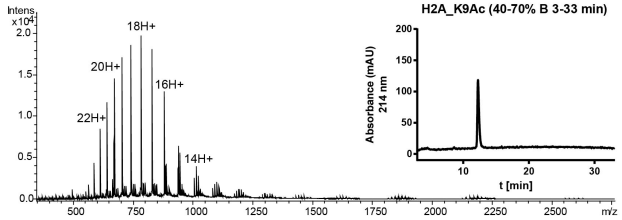
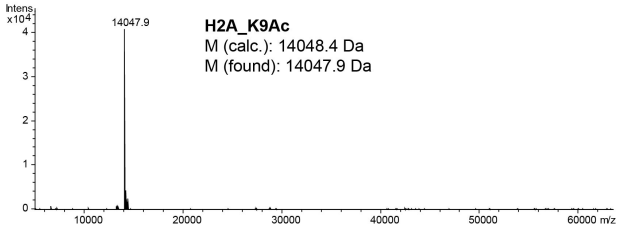
M (found): 14048.0 Da

H2A_K9Ac

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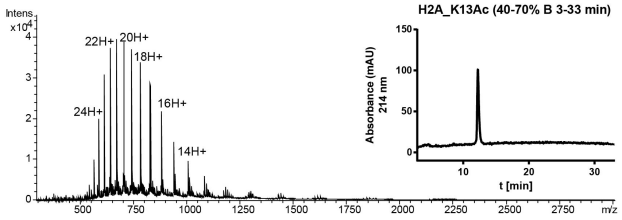
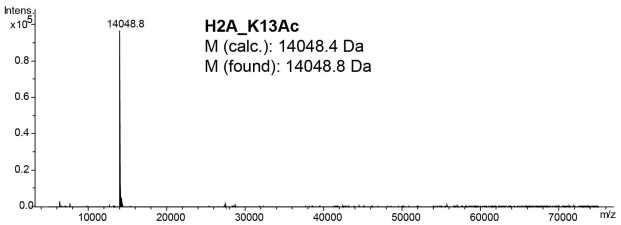
M (found): 14047.9 Da

H2A_K13Ac

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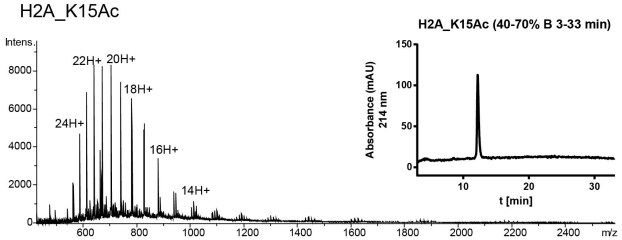
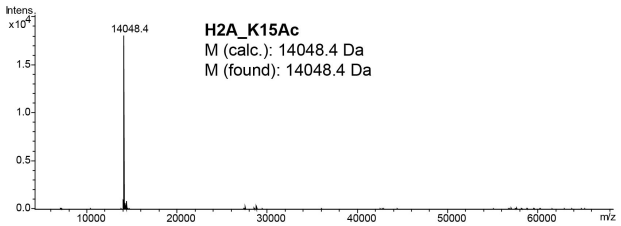
M (found): 14048.8 Da

H2A_K15Ac




**H2A_K15Ac**
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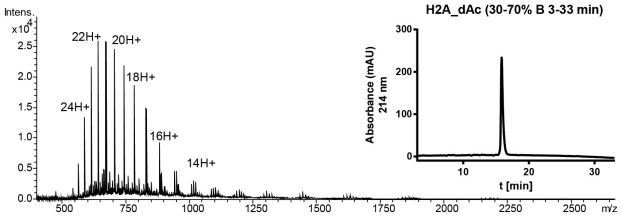
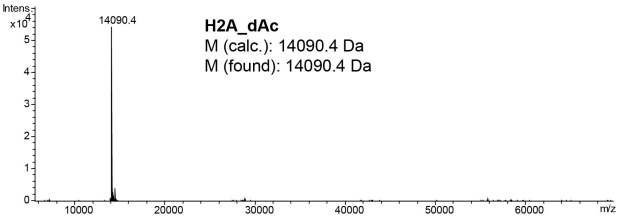
M (found): 14048.4 Da

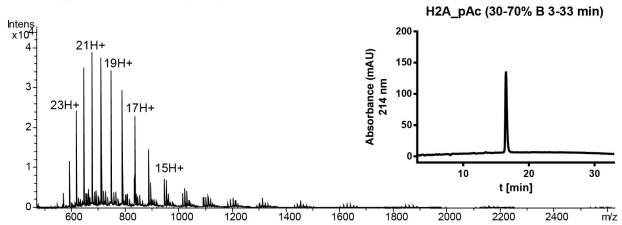
H2A_K13/15Ac (dAc)

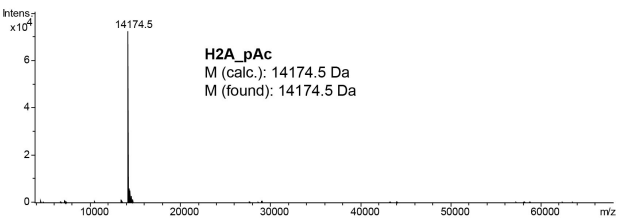



**H2A_dAc**
M (calc.): 14090.4 Da
M (found): 14090.4 Da

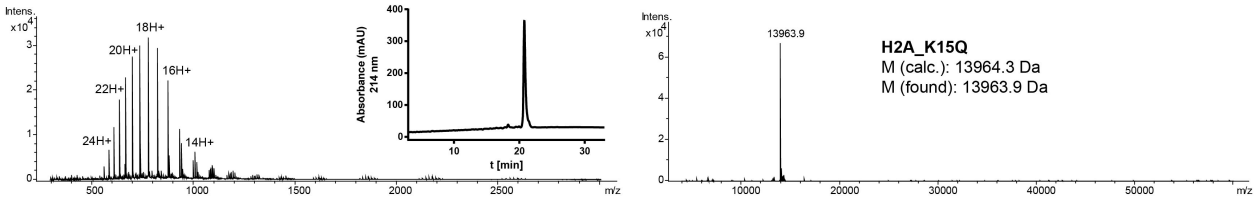
H2A_K5/9/13/15Ac (pAc)



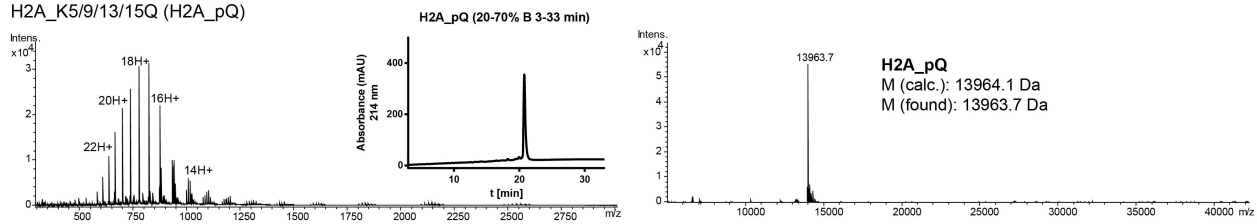

**H2A_pAc**
M (calc.): 14174.5 Da
M (found): 14174.5 Da

H2A_K15Q



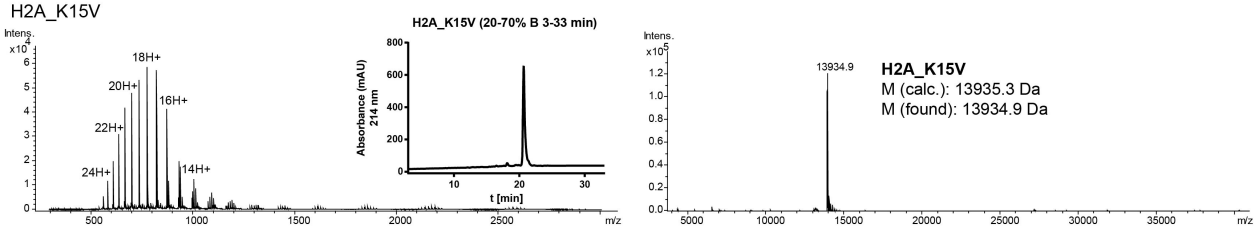

**H2A_K15Q**
M (calc.): 13964.3 Da
M (found): 13963.9 Da

H2A_K5/9/13/15Q (H2A_pQ)



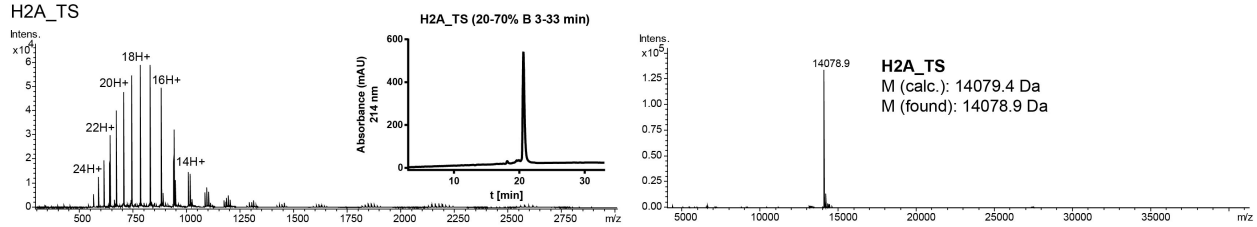

**H2A_pQ**
M (calc.): 13964.1 Da
M (found): 13963.7 Da

H2A_K15V



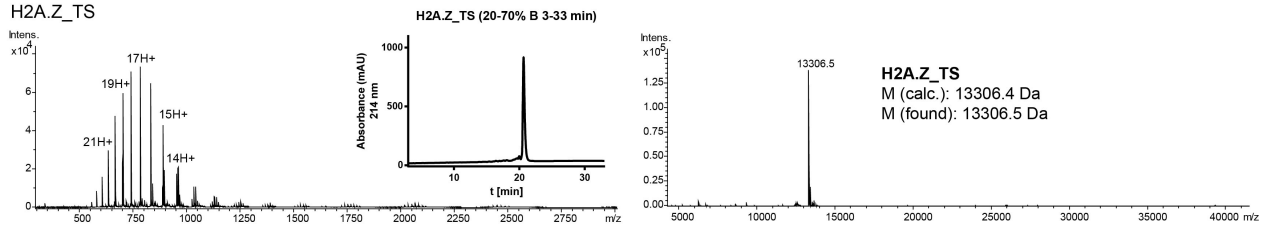

**H2A_K15V**
M (calc.): 13935.3 Da
M (found): 13934.9 Da

H2A_TS



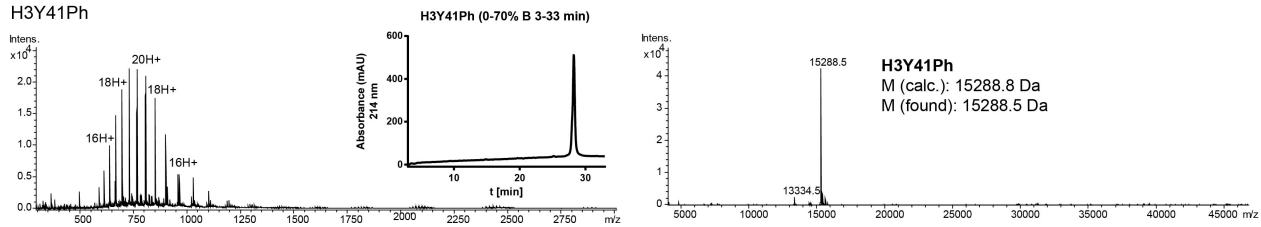

**H2A_TS**
M (calc.): 14079.4 Da
M (found): 14078.9 Da

H2A.Z_TS

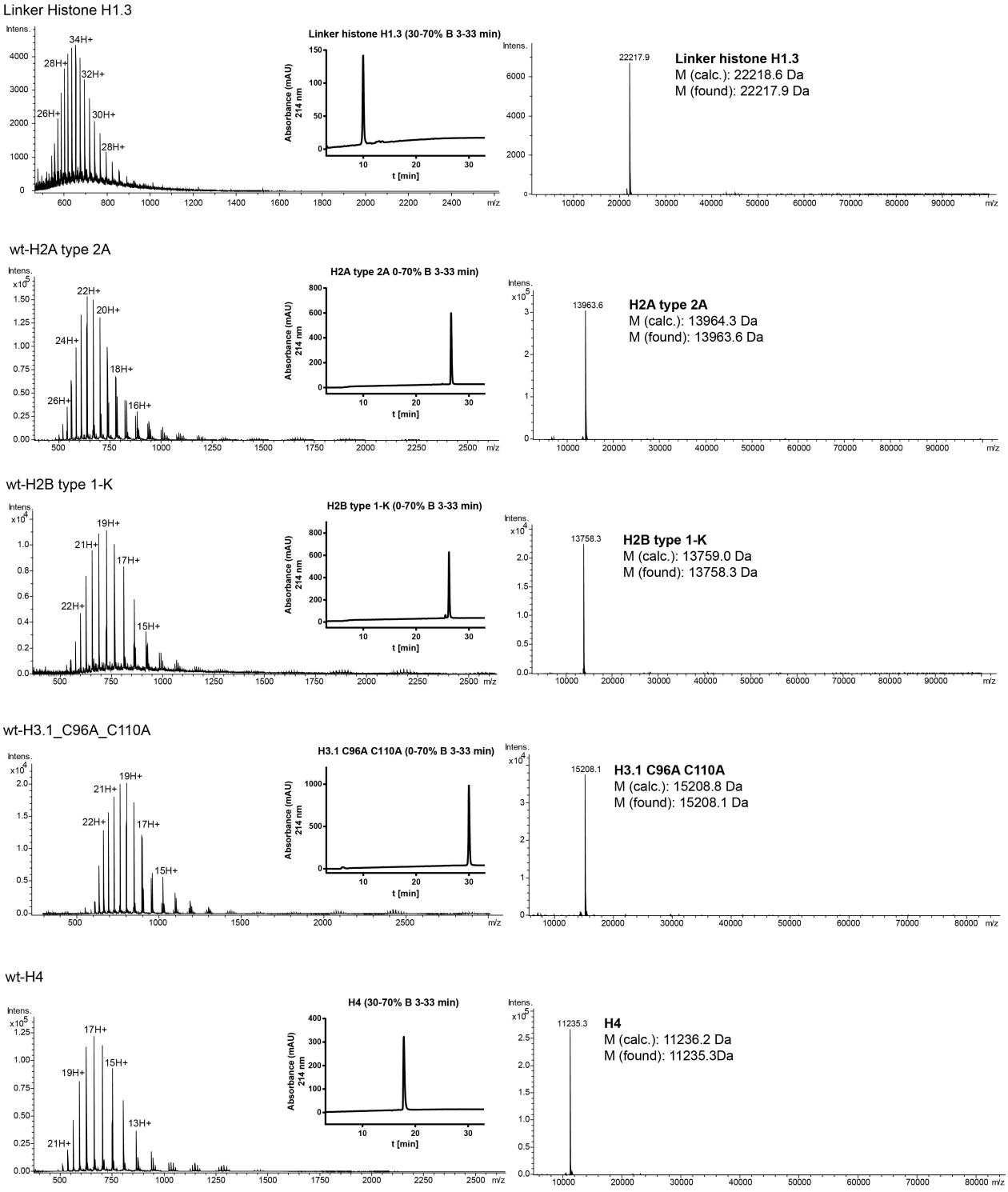



**H2A.Z_TS**
M (calc.): 13306.4 Da
M (found): 13306.5 Da

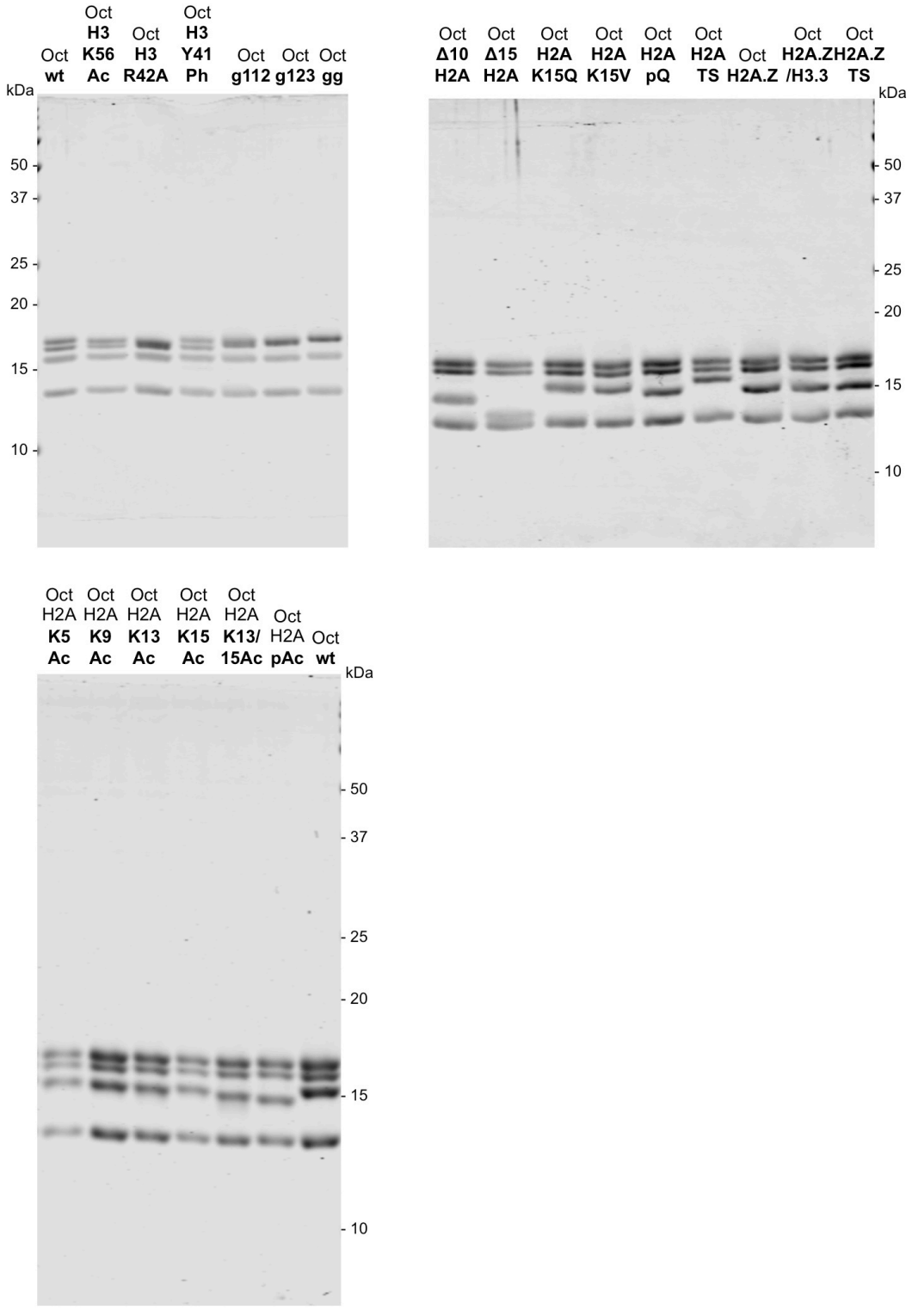
H3Y41Ph



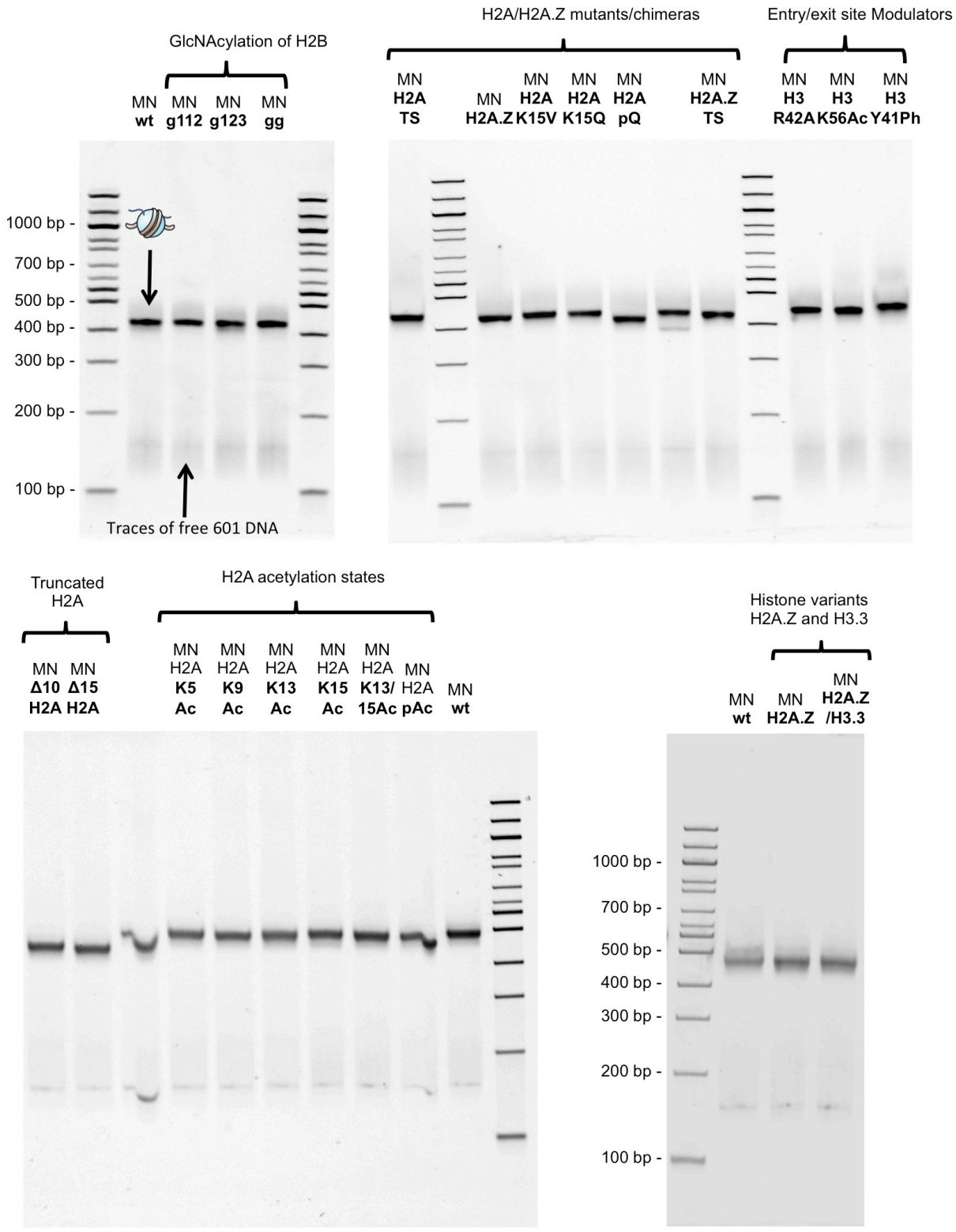

**H3Y41Ph**
M (calc.): 15288.8 Da
M (found): 15288.5 Da



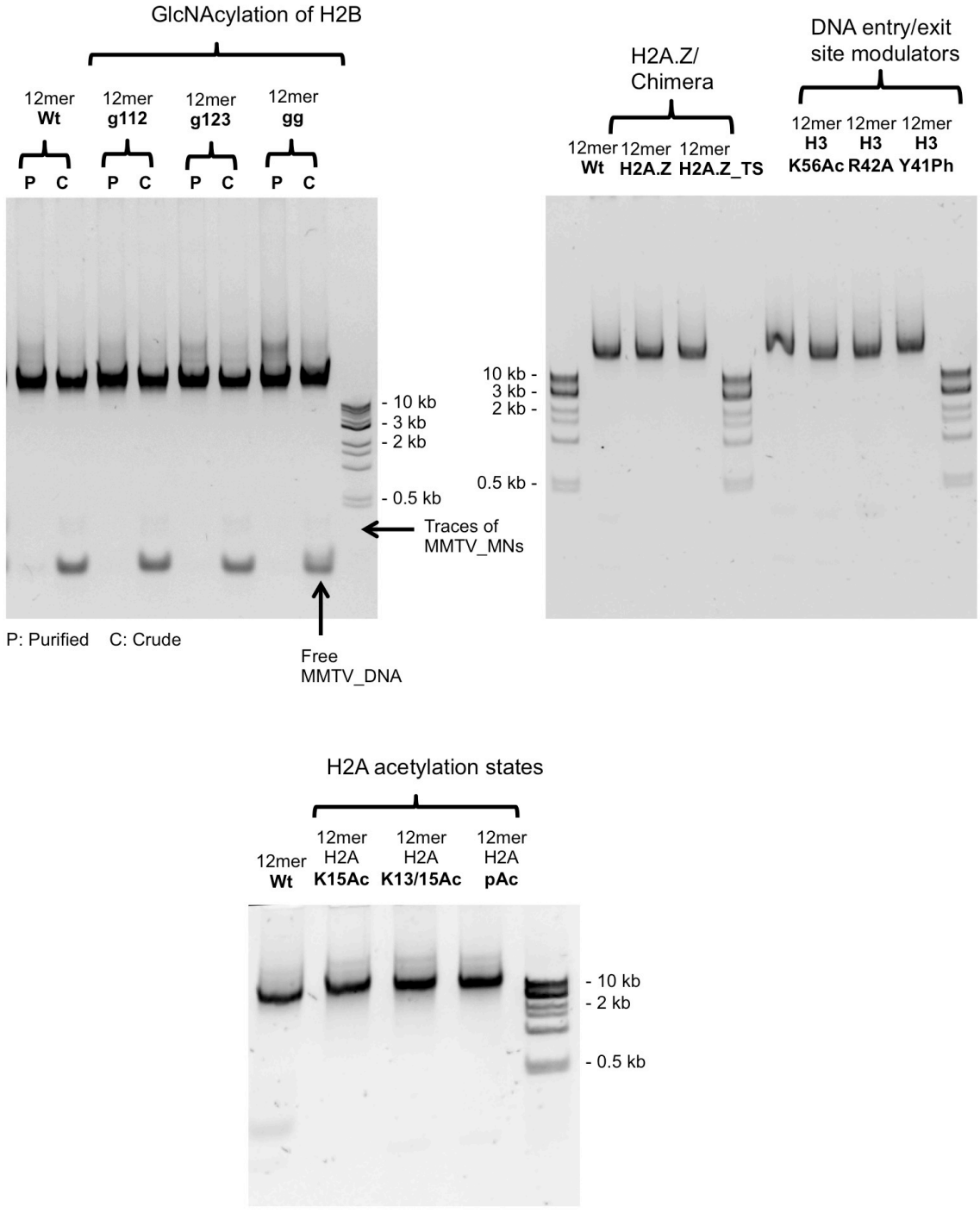

**Supplementary Figure 11. Characterization of recombinant and semisynthetic proteins used in this study.** Left: MS spectra of the indicated proteins and RP-HPLC chromatogram (solvent gradients are individually noted). Right: Deconvoluted MS-spectra and comparison of calculated and found MW. Serine modified with N-acetylglucosamine: SGlcNAc; Tail swap mutant: TS (see Supplementary Fig. 7c); Lysine acetylation: KAc; tyrosine phosphorylation: YPh.



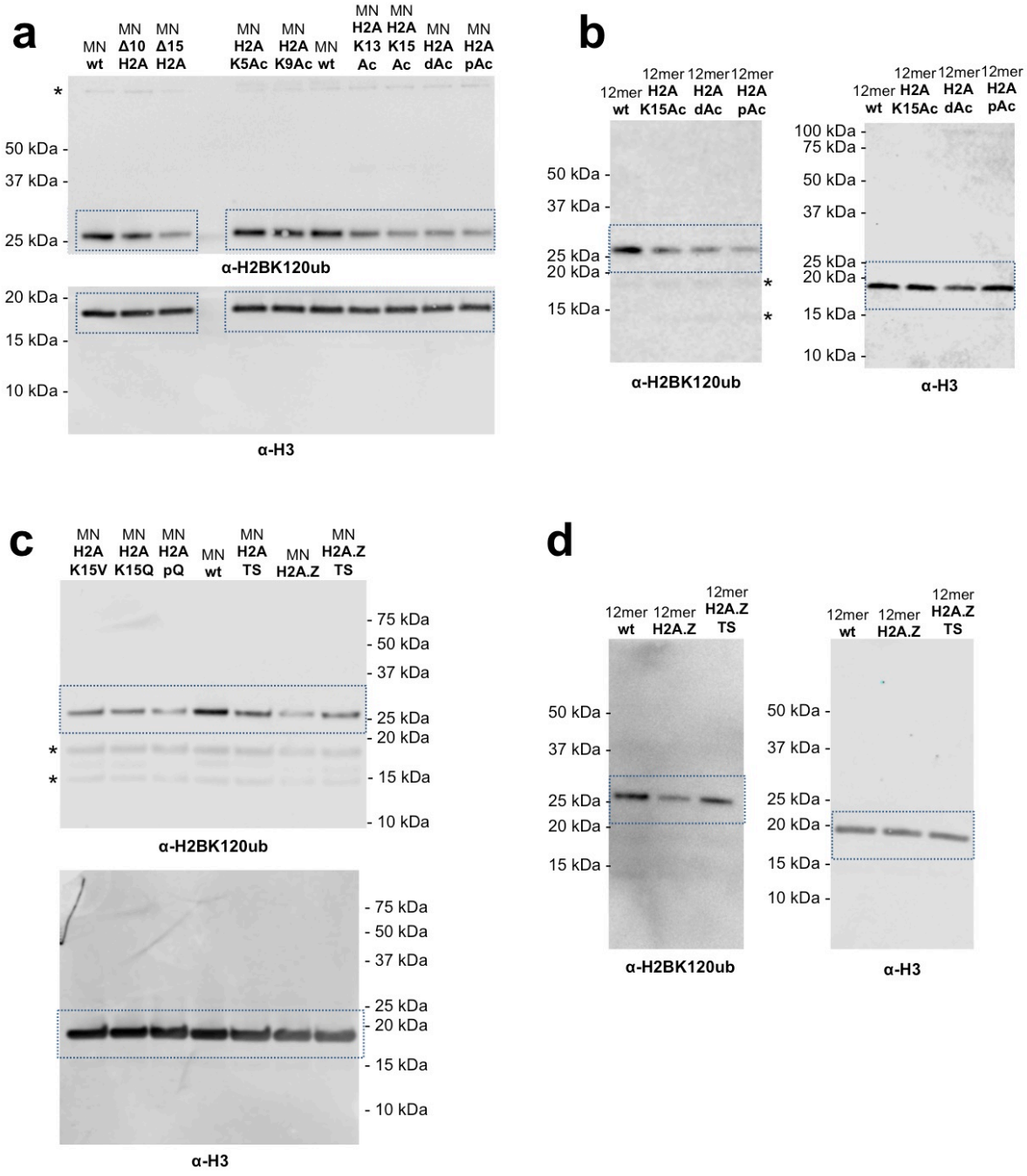

**Supplementary Figure 12. Characterization of histone octamers.** Individual histone octamers were analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue.



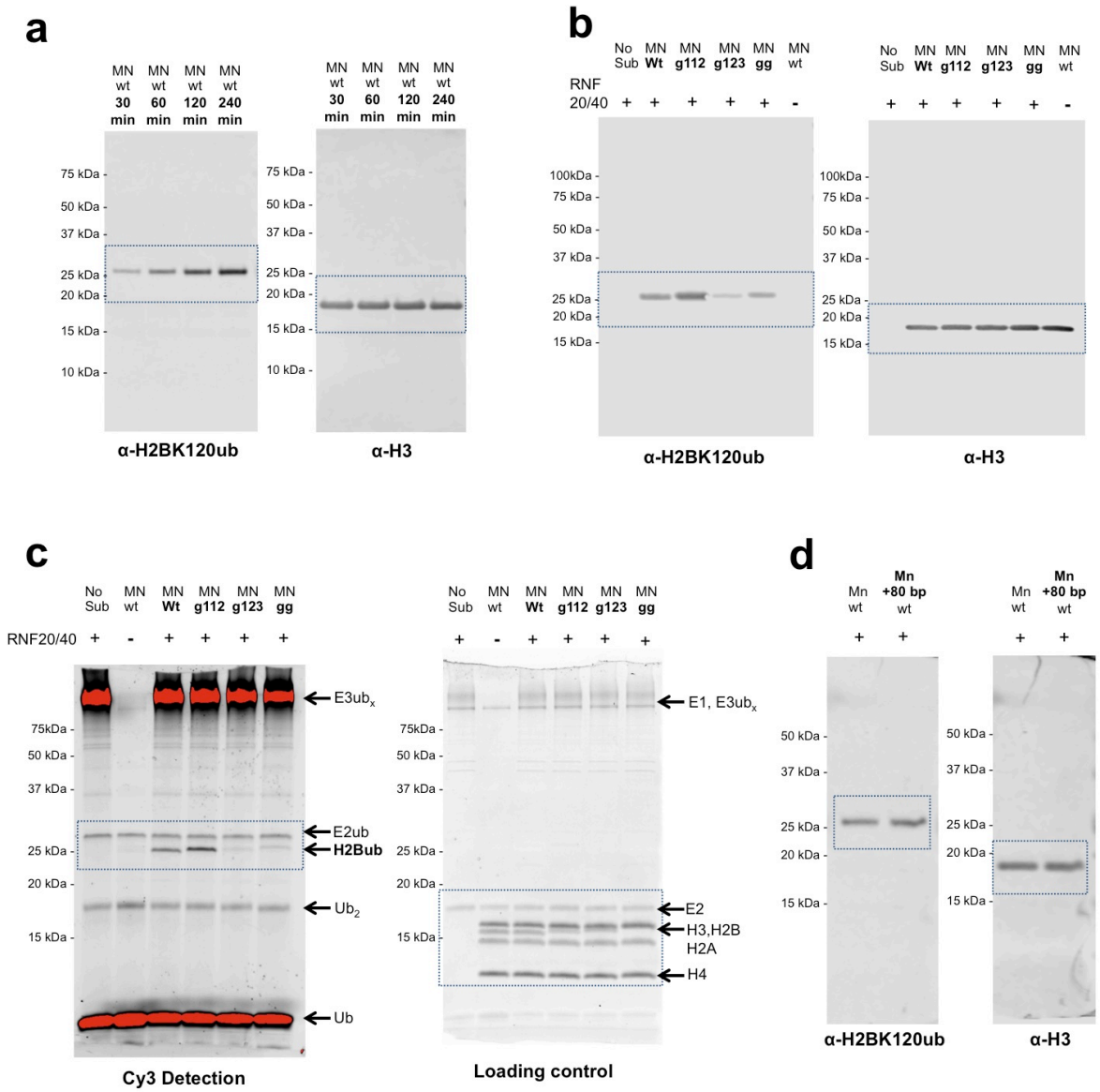

**Supplementary Figure 13. Characterization of mononucleosome substrates.** Individual MNs were analyzed by native gel electrophoresis and stained with SYBR Gold nucleic acid stain.



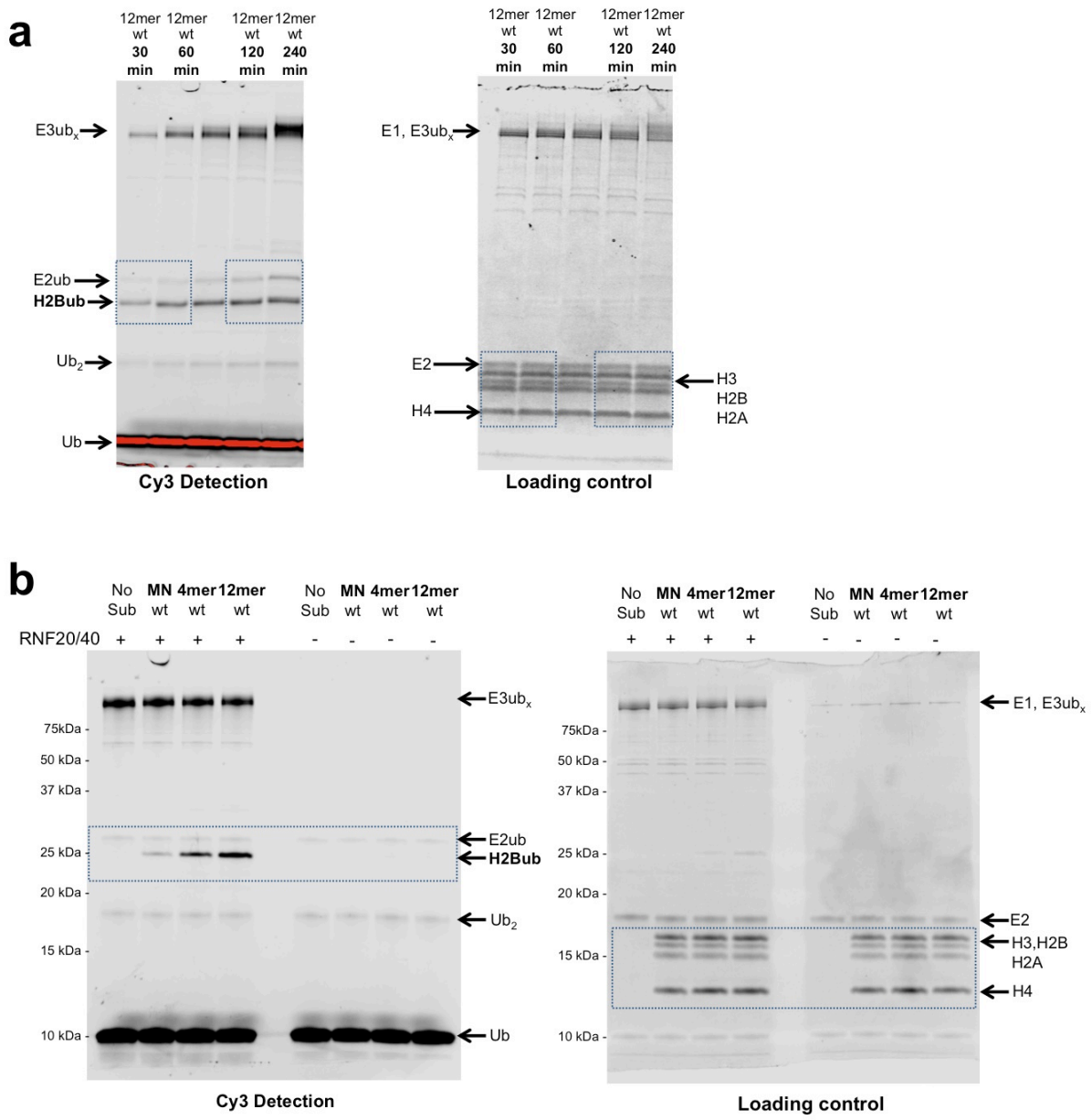

**Supplementary Figure 14. Characterization of 12mer chromatin array substrates.** Individual 12mer chromatin arrays were analyzed by native gel electrophoresis (APAGE gel) and stained with SYBR Gold nucleic acid stain. Crude assemblies and purified samples are shown for wt-, g112,- g123 -and gg-12mer chromatin arrays.



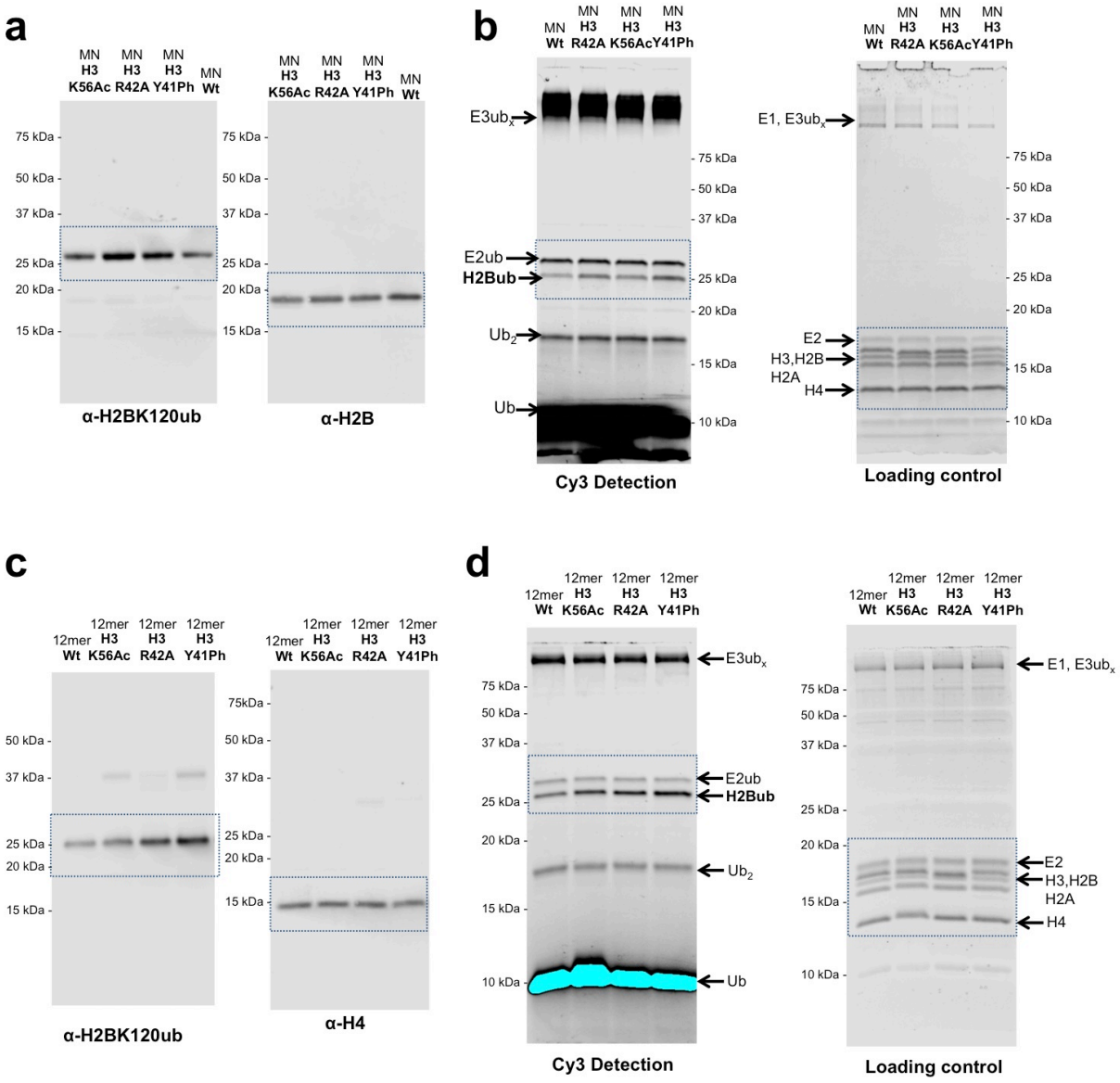

**Supplementary Figure 15. Full images for data presented in Fig. 3.** Blots were cropped for illustration purposes. (**a**) Full image for WB analysis presented in Fig. 3a. WB was physically cut before antibody incubation. (**b**) Full image for WB analysis presented in Fig. 3b. (**c**) Full image for WB analysis presented in Fig. 3d. The asterisks mark non-specific antibody binding. (**d**) Full image for WB analysis presented in Fig. 3e.



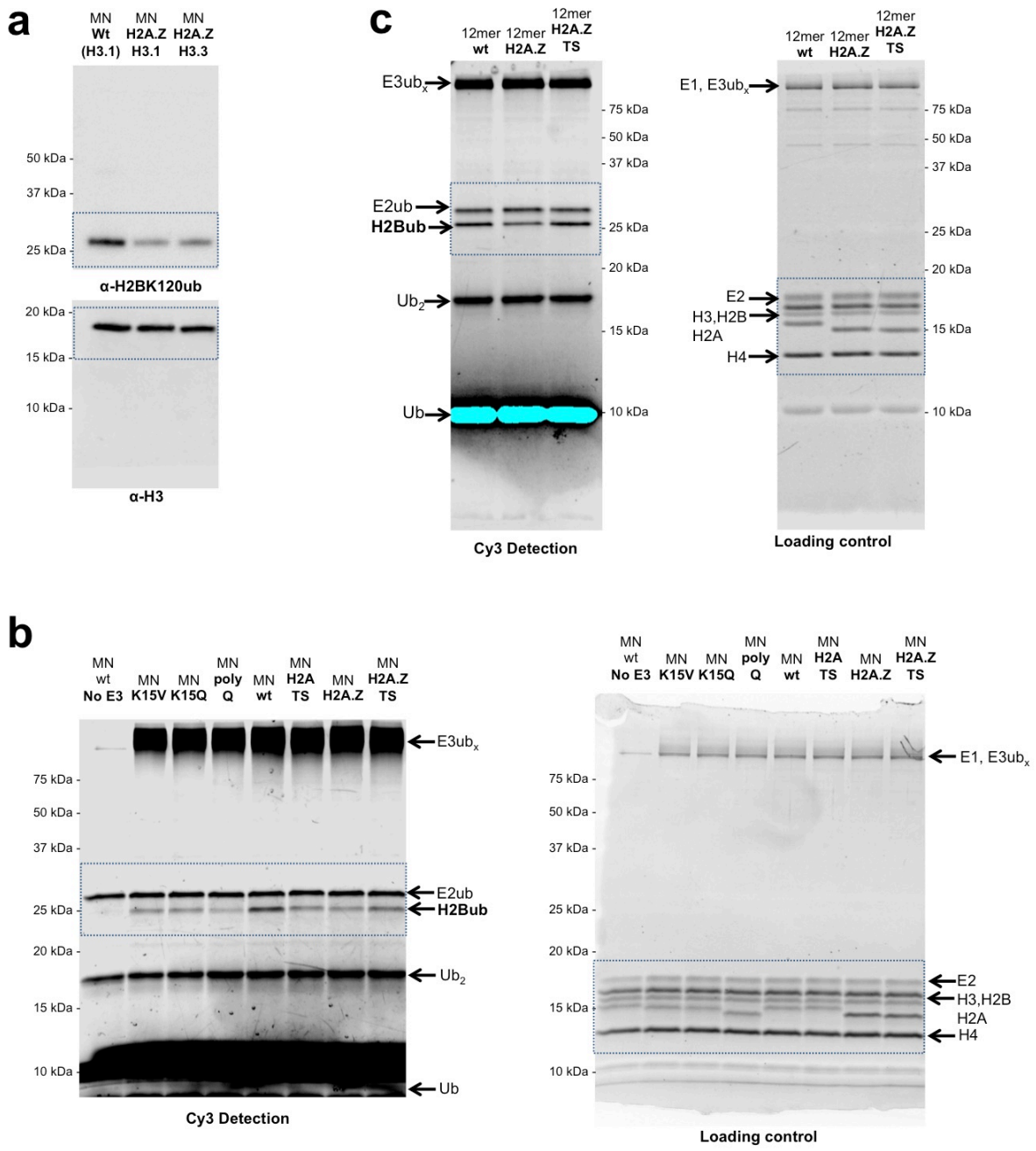

**Supplementary Figure 16. Full images for data presented in Supplementary Fig 2/3.** Images were cropped for illustration purposes. (**a**) Full image for WB analysis presented in Supplementary Fig. 2c. (**b**) Full image for WB analysis presented in Supplementary Fig. 3b. (**c**) Full image of Cy3 readout and loading control (SYPRO Ruby protein stain) presented in Supplementary Fig. 3d. (**d**) Full image for WB analysis presented in Supplementary Fig. 3e.



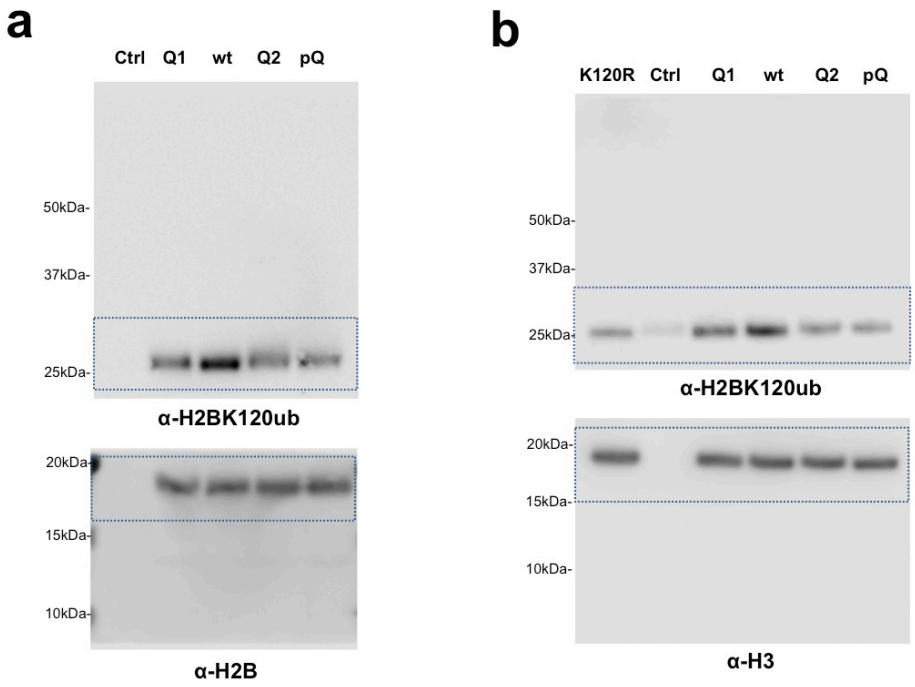

**Supplementary Figure 17. Full images for data presented in Supplementary Fig. 4.** Images were cropped for illustration purposes. (**a**) Full image of Cy3 readout and loading control (SYPRO Ruby protein stain) presented in Supplementary Fig. 4a. (**b**) Full image of Cy3 readout and loading control (SYPRO Ruby protein stain) presented in Supplementary Fig. 4b.




**Supplementary Figure 18. Full images for data presented in Supplementary Fig. 5.** Images were cropped for illustration purposes. (**a**) Full image for WB analysis presented in Supplementary Fig. 5b. (**b**) Full image of Cy3 readout and loading control (SYPRO Ruby protein stain) presented in Supplementary Fig. 5c. (**c**) Full image for WB analysis presented in Supplementary Fig. 5d. (**d**) Full image of Cy3 readout and loading control (SYPRO Ruby protein stain) presented in Supplementary Fig. 5e.




**Supplementary Figure 19. Full images for data presented in Supplementary Fig. 7/9.** Images were cropped for illustration purposes. (**a**) Full image for WB analysis presented in Supplementary Fig. 7b. WB was physically cut before antibody incubation. (**b**) Full image of Cy3 readout and loading control (SYPRO Ruby protein stain) presented in Supplementary Fig. 9a. (**c**) Full image of Cy3 readout and loading control (SYPRO Ruby protein stain) presented in Supplementary Fig. 9b.




**Supplementary Figure 20. Full images for data presented in Supplementary Fig. 10.** Images were cropped for illustration purposes. (**a**) Full image for WB analysis presented in Supplementary Fig. 10c. WB was physically cut before antibody incubation. (**b**) Full image for WB analysis presented in Supplementary Fig. 10d. WB was physically cut before antibody incubation.

**Supplementary Table 1. Library-based screen for *de novo* ubiquitylation activity.** Substrates are ranked by *de novo* ubiquitylation activity from least active to most active. DNA reads were normalized to library input and the average signal intensities of the six wt-controls. Data are mean of $\log_2$(fold change) values ± s.e.m. (n = 3). Individual nucleosomes are color coded as in Fig. 2. Gray: Wt-controls; Red: Negative controls (pre-modified H2BK120); Light red: Free DNA; Dark blue: H2A N-terminal tail acetylation (single modification or combinations of K5Ac, K9Ac, K13Ac, K15Ac); Light blue: Nucleosomes containing histone variant H2A.Z; Orange: Entry/exit site modulators; Green: H2BS112GlcNAc. Rounded figures are displayed, the calculation of the mean and the s.e.m. was carried out with exact values.

| | Barcode | Histone H2A | Histone H2B | Histone H3 | Histone H4 | Modified | log2(fold change) IP1 | log2(fold change) IP2 | log2(fold change) IP3 | log2(fold change) Average | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MN BC#116 | CACTGT | H2AK5,9,13,15ac | H2B WT | H3 WT | H4 WT | H2A | -1.06 | -0.60 | -0.76 | **-0.81** | 0.13 |
| MN BC#108 | ACGCAG | H2A WT | H2BK120ac | H3 WT | H4 WT | H2B | -1.04 | -0.50 | -0.73 | **-0.76** | 0.16 |
| MN BC#122 | CTGCTA | H2AK5,9,13,15ac | H2BK5,11,12,16,20ac | H3K9,14,18,23,27ac | H4 WT | H2A, H2B, H3 | -0.43 | -0.57 | -1.09 | **-0.70** | 0.20 |
| MN BC#32 | ACATGT | H2A WT | H2BK120ub | H3K9me3 | H4 WT | H2B, H3 | -1.17 | -0.47 | -0.41 | **-0.68** | 0.24 |
| MN BC#135 | ACGACT | H2A | H2BK120ub | H3 WT | H4R3me2a | H2B, H4 | -1.08 | -0.40 | -0.56 | **-0.68** | 0.21 |
| MN BC#31 | CACAGT | H2A WT | H2BK120ub | H3K4me3 | H4 WT | H2B, H3 | -1.13 | -0.35 | -0.52 | **-0.66** | 0.24 |
| MN BC#33 | CGTCTG | H2A WT | H2BK120ub | H3K27me3 | H4 WT | H2B, H3 | -1.09 | -0.45 | -0.42 | **-0.65** | 0.22 |
| MN BC#34 | CTCTCG | H2A WT | H2BK120ub | H3K9,14,18,23,27ac | H4 WT | H2B, H3 | -0.67 | -0.19 | -0.67 | **-0.51** | 0.16 |
| MN BC#119 | ACGTCG | H2A.Z | H2B WT | H3 WT | H4 WT | H2A | -0.72 | -0.41 | -0.40 | **-0.51** | 0.10 |
| MN BC#120 | CGAGAT | H2A.Z | H2B WT | H3.3 | H4 WT | H2A, H3 | -0.68 | -0.37 | -0.44 | **-0.50** | 0.09 |
| MN BC#130 | CTCTAT | H2AK15ac | H2B WT | H3 WT | H4 WT | H2A | -0.73 | -0.42 | -0.34 | **-0.50** | 0.12 |
| MN BC#547 | ACGTCT | x | x | x | x | free DNA no PST | -0.71 | -0.07 | -0.45 | **-0.41** | 0.19 |
| MN BC#40 | CTCAGT | H2A WT | H2B WT | H3K9,14,18,23,27ac | H4K5,8,12,16,20ac | H3, H4 | 0.13 | -0.42 | -0.87 | **-0.39** | 0.29 |
| MN BC#121 | ATCACG | H2AK5,9,13,15ac | H2BK5,11,12,16,20ac | H3K9,14,18,23,27ac | H4K5,8,12,16,20ac | H2A, H2B, H3, H4 | -0.13 | -0.36 | -0.65 | **-0.38** | 0.15 |
| MN BC#129 | GCGCTG | H2AK13ac | H2B WT | H3 WT | H4 WT | H2A | -0.35 | -0.28 | -0.23 | **-0.29** | 0.03 |
| MN BC#21 | CATGAT | H2A WT | H2B WT | H3K9me3 | H4K5,8,12,16,20ac | H3, H4 | -0.16 | -0.32 | -0.28 | **-0.25** | 0.05 |
| MN BC#136 | AGCTCG | H2AK119ub | H2B WT | H3 WT | H4R3me2a | H2A, H4 | -0.33 | -0.15 | -0.27 | **-0.25** | 0.05 |
| MN BC#18 | AGCGTA | H2A WT | H2B WT | H3K9me3 | H4K12ac | H3, H4 | -0.15 | -0.26 | -0.24 | **-0.22** | 0.03 |
| MN BC#30 | AGAGCA | H2A WT | H2BK120ub | H3 WT | H4 WT | H2B | -0.29 | 0.00 | -0.35 | **-0.21** | 0.11 |
| MN BC#3 | CAGTAT | H2A WT | H2B WT | H3 WT | H4K8ac | H4 | -0.22 | -0.20 | -0.20 | **-0.21** | 0.01 |
| MN BC#14 | GTATCT | H2A WT | H2B WT | H3K4me3 | H4K5,8,12,16,20ac | H3, H4 | -0.08 | -0.28 | -0.27 | **-0.21** | 0.07 |
| MN BC#109 | AGCATA | H2A WT | H2BK125ac | H3 WT | H4 WT | H2B | -0.24 | -0.08 | -0.21 | **-0.17** | 0.05 |
| MN BC#96 | GCAGCA | H2A WT | H2B WT | H3K79ac | H4 WT | H3 | -0.22 | -0.12 | -0.16 | **-0.17** | 0.03 |
| MN BC#5 | ACGTGA | H2A WT | H2B WT | H3 WT | H4K16ac | H4 | -0.15 | -0.17 | -0.16 | **-0.16** | 0.01 |
| MN BC#6 | CTCACG | H2A WT | H2B WT | H3 WT | H4K20ac | H4 | -0.17 | -0.20 | -0.09 | **-0.16** | 0.03 |
| MN BC#28 | AGCACG | H2A WT | H2B WT | H3K27me3 | H4K5,8,12,16,20ac | H3, H4 | -0.05 | -0.16 | -0.25 | **-0.15** | 0.06 |
| MN BC#16 | ATGTGT | H2A WT | H2B WT | H3K9me3 | H4K5ac | H3, H4 | 0.01 | -0.24 | -0.22 | **-0.15** | 0.08 |
| MN BC#111 | GTATAT | H2AK5ac | H2B WT | H3 WT | H4 WT | H2A | -0.13 | -0.12 | -0.18 | **-0.14** | 0.02 |
| MN BC#20 | CTGCGA | H2A WT | H2B WT | H3K9me3 | H4K20ac | H3, H4 | -0.09 | -0.12 | -0.17 | **-0.13** | 0.02 |
| MN BC#17 | GCTCGA | H2A WT | H2B WT | H3K9me3 | H4K8ac | H3, H4 | -0.09 | -0.13 | -0.12 | **-0.11** | 0.01 |
| MN BC#36 | CTATAG | H2AK119ub | H2B WT | H3 WT | H4 WT | H2A | -0.22 | -0.07 | -0.05 | **-0.11** | 0.06 |
| MN BC#10 | CATACA | H2A WT | H2B WT | H3K4me3 | H4K8ac | H3, H4 | -0.14 | -0.04 | -0.14 | **-0.11** | 0.03 |
| MN BC#2 | ACGACG | H2A WT | H2B WT | H3 WT | H4K5ac | H4 | -0.02 | -0.12 | -0.18 | **-0.11** | 0.05 |
| MN BC#106 | CGATCG | H2A WT | H2BK108ac | H3 WT | H4 WT | H2B | -0.10 | -0.05 | -0.15 | **-0.10** | 0.03 |
| MN BC#112 | GCTGAG | H2AK9ac | H2B WT | H3 WT | H4 WT | H2A | -0.12 | -0.05 | -0.14 | **-0.10** | 0.03 |
| MN BC#12 | CAGAGA | H2A WT | H2B WT | H3K4me3 | H4K16ac | H3, H4 | -0.02 | -0.05 | -0.24 | **-0.10** | 0.07 |
| MN BC#64 | CACACA | H2A WT | H2B WT | H3 WT | H4R17A,R19A | H4 | -0.10 | -0.04 | -0.13 | **-0.09** | 0.03 |
| MN BC#19 | ATACAG | H2A WT | H2B WT | H3K9me3 | H4K16ac | H3, H4 | -0.02 | -0.12 | -0.14 | **-0.09** | 0.04 |
| MN BC#98 | AGATCA | H2A WT | H2B WT | H3K122ac | H4 WT | H3 | -0.15 | -0.06 | -0.06 | **-0.09** | 0.03 |
| MN BC#75 | CGCGCG | H2A WT | H2B WT | H3 WT | H4K91ac | H4 | -0.09 | -0.02 | -0.17 | **-0.09** | 0.04 |
| MN BC#127 | ACTGCT | H2A WT | H2B WT | H3T118H | H4R17A,R19A | H3, H4 | -0.04 | -0.09 | -0.12 | **-0.08** | 0.02 |
| MN BC#15 | GAGCAG | H2A WT | H2B WT | H3K9me3 | H4 WT | H3 | -0.09 | -0.09 | -0.03 | **-0.07** | 0.02 |
| MN BC#7 | GTGACA | H2A WT | H2B WT | H3 WT | H4K5,8,12,16,20ac | H4 | 0.05 | -0.11 | -0.15 | **-0.07** | 0.06 |
| MN BC#43 | GTAGCT | H2A WT | H2B WT | H3K18ac | H4 WT | H3 | 0.02 | -0.01 | -0.18 | **-0.06** | 0.06 |
| MN BC#23 | GCTCAG | H2A WT | H2B WT | H3K27me3 | H4K5ac | H3, H4 | 0.00 | -0.10 | -0.05 | **-0.05** | 0.03 |
| MN BC#82 | GATGCT | H2A WT | H2B WT | H3.3K27M | H4 WT | H3 | 0.07 | 0.03 | -0.25 | **-0.05** | 0.10 |
| MN BC#9 | AGACTA | H2A WT | H2B WT | H3K4me3 | H4K5ac | H3, H4 | -0.03 | -0.09 | -0.01 | **-0.05** | 0.02 |
| MN BC#4 | ACAGTA | H2A WT | H2B WT | H3 WT | H4K12ac | H4 | -0.11 | -0.05 | 0.02 | **-0.04** | 0.04 |
| MN BC#11 | GCACAG | H2A WT | H2B WT | H3K4me3 | H4K12ac | H3, H4 | -0.04 | -0.11 | 0.02 | **-0.04** | 0.04 |
| MN BC#1 | CGAGAG | x | x | x | x | free DNA +PST | -0.04 | 0.34 | -0.42 | **-0.04** | 0.22 |
| MN BC#8 | GCGACA | H2A WT | H2B WT | H3K4me3 | H4 WT | H3 | -0.01 | -0.05 | -0.06 | **-0.04** | 0.02 |
| MN BC#26 | GTATGA | H2A WT | H2B WT | H3K27me3 | H4K16ac | H3, H4 | 0.04 | -0.02 | -0.12 | **-0.04** | 0.05 |
| MN BC#342 | CGCGCA | H2A WT | H2B WT | H3 WT | H4 WT | ALL WT | -0.03 | 0.01 | -0.08 | **-0.03** | 0.02 |
| MN BC#24 | CATCTA | H2A WT | H2B WT | H3K27me3 | H4K8ac | H3, H4 | 0.05 | 0.00 | -0.15 | **-0.03** | 0.06 |
| MN BC#63 | CGACGT | H2A WT | H2B WT | H3 WT | H4 WT | ALL WT | -0.03 | -0.02 | -0.04 | **-0.03** | 0.01 |
| MN BC#344 | GCAGTG | H2A WT | H2B WT | H3 WT | H4 WT | ALL WT | -0.04 | 0.02 | -0.04 | **-0.02** | 0.02 |
| MN BC#48 | CACTGA | H2A WT | H2B WT | H3K18acK23ac | H4 WT | H3 | 0.07 | 0.01 | -0.14 | **-0.02** | 0.06 |
| MN BC#128 | ACACTA | H2A WT | H2B WT | H3 WT | H4 WT | CpG | -0.01 | -0.03 | -0.02 | **-0.02** | 0.01 |
| MN BC#44 | CAGTCT | H2A WT | H2B WT | H3K23ac | H4 WT | H3 | 0.05 | 0.07 | -0.16 | **-0.02** | 0.07 |
| MN BC#139 | ACGTAG | H2A WT | H2B WT | H3K18cr | H4 WT | H3 | 0.13 | 0.00 | -0.18 | **-0.02** | 0.09 |
| MN BC#105 | ATCGCA | H2A WT | H2BK5,11,12,16,20ac | H3 WT | H4 WT | H2B | 0.02 | 0.03 | -0.09 | **-0.01** | 0.04 |
| MN BC#89 | GTCTCA | H2A WT | H2B WT | H3S10ph | H4 WT | H3 | -0.02 | 0.08 | -0.09 | **-0.01** | 0.05 |
| MN BC#137 | ATGTAT | H2A WT | H2B WT | H3K9cr | H4 WT | H3 | 0.07 | 0.07 | -0.16 | **0.00** | 0.08 |
| MN BC#138 | GACGCG | H2A WT | H2B WT | H3K14cr | H4 WT | H3 | 0.01 | -0.02 | 0.00 | **0.00** | 0.01 |
| MN BC#13 | ATATCA | H2A WT | H2B WT | H3K4me3 | H4K20ac | H3, H4 | 0.02 | -0.04 | 0.04 | **0.01** | 0.02 |
| MN BC#351 | ATACTG | H2A WT | H2B WT | H3 WT | H4 WT | ALL WT | 0.01 | 0.02 | -0.01 | **0.01** | 0.01 |
| MN BC#107 | GTACGA | H2A WT | H2BK116ac | H3 WT | H4 WT | H2B | 0.08 | 0.11 | -0.17 | **0.01** | 0.09 |
| MN BC#132 | GTACTA | H2A WT | H2B WT | H3K4me1 | H4 WT | H3 | 0.08 | 0.05 | -0.10 | **0.01** | 0.05 |
| MN BC#45 | GTGAGT | H2A WT | H2B WT | H3K27ac | H4 WT | H3 | 0.14 | 0.01 | -0.09 | **0.02** | 0.07 |
| MN BC#42 | CTGTCG | H2A WT | H2B WT | H3K14ac | H4 WT | H3 | 0.05 | 0.08 | -0.06 | **0.02** | 0.04 |
| MN BC#125 | CGTAGA | H2A WT | H2B WT | H3T118H | H4R45A | H3, H4 | 0.10 | 0.07 | -0.11 | **0.02** | 0.07 |
| MN BC#41 | CGACGA | H2A WT | H2B WT | H3K9ac | H4 WT | H3 | 0.08 | 0.07 | -0.08 | **0.02** | 0.05 |
| MN BC#104 | AGTGAT | H2A WT | H2BK20ac | H3 WT | H4 WT | H2B | 0.06 | 0.09 | -0.08 | **0.02** | 0.05 |
| MN BC#352 | ATATAT | H2A WT | H2B WT | H3 WT | H4 WT | ALL WT | 0.03 | -0.04 | 0.09 | **0.03** | 0.04 |
| MN BC#67 | GACTAG | H2A WT | H2B WT | H3 WT | H4R3me2a | H4 | 0.12 | 0.09 | -0.09 | **0.04** | 0.07 |
| MN BC#133 | ATGCGA | H2A WT | H2B WT | H3K4me2 | H4 WT | H3 | 0.07 | 0.01 | 0.05 | **0.04** | 0.02 |
| MN BC#27 | ATCGTG | H2A WT | H2B WT | H3K27me3 | H4K20ac | H3, H4 | 0.05 | 0.06 | 0.03 | **0.05** | 0.01 |
| MN BC#350 | ACATCA | H2A WT | H2B WT | H3 WT | H4 WT | ALL WT | 0.06 | 0.02 | 0.07 | **0.05** | 0.02 |
| MN BC#100 | ATGTCA | H2A WT | H2BK11ac | H3 WT | H4 WT | H2B | 0.08 | 0.02 | 0.05 | **0.05** | 0.02 |

Least active substrates shown in Figure 2B

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MN BC#99 | CGAGTA | H2A WT | H2BK5ac | H3 WT | H4 WT | H2B | 0.13 | 0.15 | -0.12 | **0.05** | 0.09 |
| MN BC#84 | GTGCTA | H2A WT | H2B WT | H3K36me3 | H4 WT | H3 | 0.09 | 0.07 | 0.01 | **0.05** | 0.02 |
| MN BC#22 | AGCTGT | H2A WT | H2B WT | H3K27me3 | H4 WT | H3 | 0.07 | 0.06 | 0.03 | **0.06** | 0.01 |
| MN BC#74 | GCGTGA | H2A WT | H2B WT | H3 WT | H4K79ac | H4 | 0.13 | 0.08 | -0.02 | **0.06** | 0.04 |
| MN BC#25 | CTCTCA | H2A WT | H2B WT | H3K27me3 | H4K12ac | H3, H4 | 0.11 | 0.09 | 0.01 | **0.07** | 0.03 |
| MN BC#97 | AGATAG | H2A WT | H2B WT | H3K115ac | H4 WT | H3 | 0.19 | 0.08 | -0.06 | **0.07** | 0.07 |
| MN BC#87 | GCACGA | H2A WT | H2B WT | H3R2me | H4 WT | H3 | 0.11 | 0.11 | 0.02 | **0.08** | 0.03 |
| MN BC#140 | CTATGT | H2A WT | H2B WT | H3K27cr | H4 WT | H3 | 0.28 | 0.01 | -0.05 | **0.08** | 0.10 |
| MN BC#102 | CGTCGA | H2A WT | H2BK15ac | H3 WT | H4 WT | H2B | 0.13 | 0.13 | -0.02 | **0.08** | 0.05 |
| MN BC#113 | GAGTCA | H2AK118ac | H2B WT | H3 WT | H4 WT | H2A | 0.21 | 0.06 | -0.02 | **0.08** | 0.07 |
| MN BC#29 | CGCGAT | H2A WT | H2B WT | H3K9,14,18,23,27ac | H4 WT | H3 | 0.35 | 0.11 | -0.19 | **0.09** | 0.16 |
| MN BC#73 | CGCACG | H2A WT | H2B WT | H3 WT | H4K77ac | H4 | 0.15 | 0.14 | 0.00 | **0.10** | 0.05 |
| MN BC#91 | CACACG | H2A WT | H2B WT | H3K4ac | H4 WT | H3 | 0.23 | 0.15 | -0.07 | **0.10** | 0.09 |
| MN BC#101 | CTCGTG | H2A WT | H2BK12ac | H3 WT | H4 WT | H2B | 0.14 | 0.14 | 0.02 | **0.10** | 0.04 |
| MN BC#78 | AGCTAG | H2A WT | H2B WT | H3.3 | H4 WT | H3 | 0.15 | 0.11 | 0.07 | **0.11** | 0.02 |
| MN BC#81 | CTCGAT | H2A WT | H2B WT | H3.3G34W | H4 WT | H3 | 0.13 | 0.18 | 0.05 | **0.12** | 0.04 |
| MN BC#80 | ATCTAT | H2A WT | H2B WT | H3.3G34R | H4 WT | H3 | 0.12 | 0.13 | 0.11 | **0.12** | 0.01 |
| MN BC#79 | ATGACT | H2A WT | H2B WT | H3.3G34V | H4 WT | H3 | 0.14 | 0.13 | 0.12 | **0.13** | 0.00 |
| MN BC#118 | ACAGAG | H2A.X | H2B WT | H3 WT | H4 WT | H2A | 0.27 | 0.15 | -0.02 | **0.14** | 0.08 |
| MN BC#86 | GTGCAT | H2A WT | H2B WT | H3K4me3K27me3 | H4 WT | H3 | 0.35 | 0.16 | 0.04 | **0.18** | 0.09 |
| MN BC#77 | ACTAGA | H2A WT | H2B WT | H3T118H | H4 WT | H3 | 0.23 | 0.29 | 0.04 | **0.19** | 0.07 |
| MN BC#65 | ATATAG | H2A WT | H2B WT | H3 WT | H4R45A | H4 | 0.27 | 0.16 | 0.18 | **0.20** | 0.03 |
| MN BC#88 | CGTATA | H2A WT | H2B WT | H3R2me2a | H4 WT | H3 | 0.22 | 0.20 | 0.21 | **0.21** | 0.01 |
| MN BC#95 | AGCATG | H2A WT | H2B WT | H3K64ac | H4 WT | H3 | 0.28 | 0.23 | 0.16 | **0.22** | 0.03 |
| MN BC#92 | CACTCG | H2A WT | H2B WT | H3K36ac | H4 WT | H3 | 0.34 | 0.28 | 0.10 | **0.24** | 0.07 |
| MN BC#103 | GCAGTA | H2A WT | H2BK16ac | H3 WT | H4 WT | H2B | 0.35 | 0.25 | 0.15 | **0.25** | 0.06 |
| MN BC#90 | GAGTCT | H2A WT | H2B WT | H3R42me2a | H4 WT | H3 | 0.33 | 0.22 | 0.21 | **0.25** | 0.04 |
| MN BC#93 | GCGAGA | H2A WT | H2B WT | H3K37ac | H4 WT | H3 | 0.44 | 0.36 | 0.13 | **0.31** | 0.09 |
| MN BC#68 | ACATGA | H2A WT | H2B WT | H3 WT | H4R3me2s | H4 | 0.42 | 0.35 | 0.22 | **0.33** | 0.06 |
| MN BC#134 | AGATGT | H2A | H2BS112GlcNAc | H3 WT | H4 | H2B | 0.51 | 0.32 | 0.38 | **0.40** | 0.06 |
| MN BC#83 | AGTAGA | H2A WT | H2B WT | H3.3K36M | H4 WT | H3 | 0.51 | 0.42 | 0.31 | **0.41** | 0.06 |
| MN BC#66 | AGCTCT | H2A WT | H2B WT | H3 WT | H4R3me | H4 | 0.47 | 0.49 | 0.34 | **0.44** | 0.05 |
| MN BC#94 | GTCTAG | H2A WT | H2B WT | H3K56ac | H4 WT | H3 | 0.62 | 0.45 | 0.27 | **0.45** | 0.10 |
| MN BC#126 | ATCATG | H2A WT | H2B WT | H3R42A | H4R17A,R19A | H3, H4 | 0.64 | 0.47 | 0.48 | **0.53** | 0.05 |
| MN BC#76 | CAGCTG | H2A WT | H2B WT | H3R42A | H4 WT | H3 | 0.80 | 0.56 | 0.53 | **0.63** | 0.08 |
| MN BC#124 | GTCTAT | H2A WT | H2B WT | H3R42A | H4R45A | H3, H4 | 0.88 | 0.58 | 0.53 | **0.66** | 0.11 |
| MN BC#85 | ATATGA | H2A WT | H2B WT | H3Y41ph | H4 WT | H3 | 1.41 | 1.09 | 1.05 | **1.18** | 0.12 |

Most active substrates shown in Figure 2B

**Supplementary Table 2. List of antibodies used in this study.**

| Epitope | Vendor (product #) | Antibody dilution | Application |
|---|---|---|---|
| Anti-H3 | Abcam (Ab1791) | 1:10000 in TBS-T | Loading control |
| Anti-H2B | Abcam (Ab1790) | 1:10000 in TBS-T | Loading control |
| Anti-H4 | Abcam (Ab31830) | 1:2000 in TBS-T | Loading control |
| Anti-H2BK120ub | Medimabs (MM0029) | 1:1000 in TBS-T | *In vitro* ubiquitylation levels |
| Anti-H2BK120ub | CST (Ubiquityl-Histone H2B XP® Rabbit mAb #5546) | 1:1000 in TBS-T containing 5% BSA | *In vitro* ubiquitylation levels and cellular ubiquitylation levels |
| Anti-HA | Covance (anti-HA.11 Clone 16B12, MMS-101R) | See method section | *In vitro* ChIP of the MN-Library |
| Anti-FLAG | Sigma Aldrich (M2 clone, F3165) | See method section | ChIP of cellular MNs |
| Anti-RNF20 | Abcam (Ab32629) | 1:1000 in TBS-T | Autoubiquitylation of RNF20 |
| Anti-Rabbit | Bio-Rad HRP conjugate (170-6515) | 1:10000 in TBS-T | Visualization of prim. antibody |
| Anti-Mouse | Bio-Rad HRP conjugate (170-6516) | 1:10000 in TBS-T | Visualization of prim. antibody |
| Anti-Rabbit | LI-COR (IRDye@800CW 926-32211) | 1:15000 in TBS-T | Visualization of prim. antibody |
| Anti-Rabbit | LI-COR (IRDye@680LT 877-11081) | 1:15000 in TBS-T | Visualization of prim. antibody |
| Anti-Mouse | LI-COR (IRDye@800CW 827-08364) | 1:15000 in TBS-T | Visualization of prim. antibody |

**Supplementary Table 3. Summary of ChIP-seq and RNA-seq datasets used in this study.**

| Dataset | Cell type | Data source | Laboratory responsible for data generation | Accession number |
|---|---|---|---|---|
| H2A.Z ChIP-seq | HeLa-S3 | ENCODE consortium | Bradley Bernstein, Broad Institute | ENCFF532VFI |
| H3K79me2 ChIP-seq | HeLa-S3 | ENCODE consortium | Bradley Bernstein, Broad Institute | ENCFF432DSJ |
| H3K4me3 ChIP-seq | HeLa-S3 | ENCODE consortium | Bradley Bernstein, Broad Institute | ENCFF699TXY |
| Strand-specific, rRNA-depleted, poly(A)+ RNA-seq | HeLa-S3 | ENCODE consortium | Thomas Gingeras, CSHL | ENCFF000FNX, ENCFF000FNY |
| Strand-specific, rRNA-depleted, poly(A)+ RNA-seq | HeLa-S3 | ENCODE consortium | Thomas Gingeras, CSHL | ENCFF084ARU |
| MNase-seq | HeLa-S3 | European Nucleotide Archive | Giuseppe Macino, Sapienza University of Rome | ERS345758 |
| H2BK120ub ChIP-seq | HeLa | NCBI Gene Expression Omnibus | Didier Devys, Institut de Génétique et de Biologie Moléculaire et Cellulaire | GSM1277116 |