Supplementary Information

Ubiquitin Utilizes an Acidic Surface Patch to Alter Chromatin Structure

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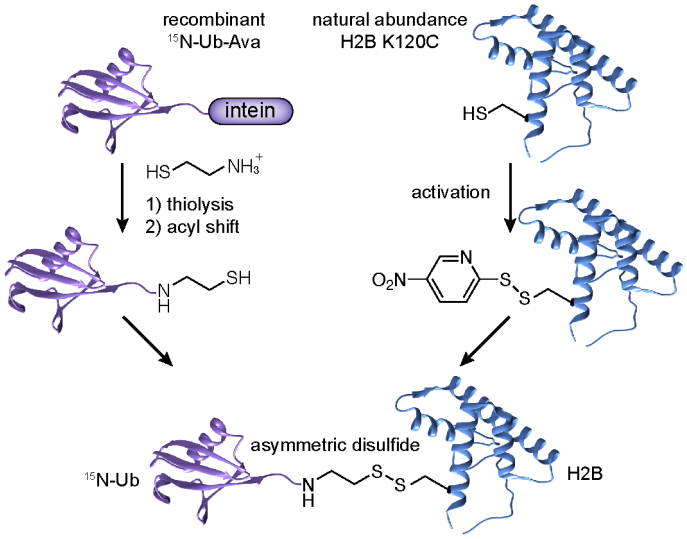
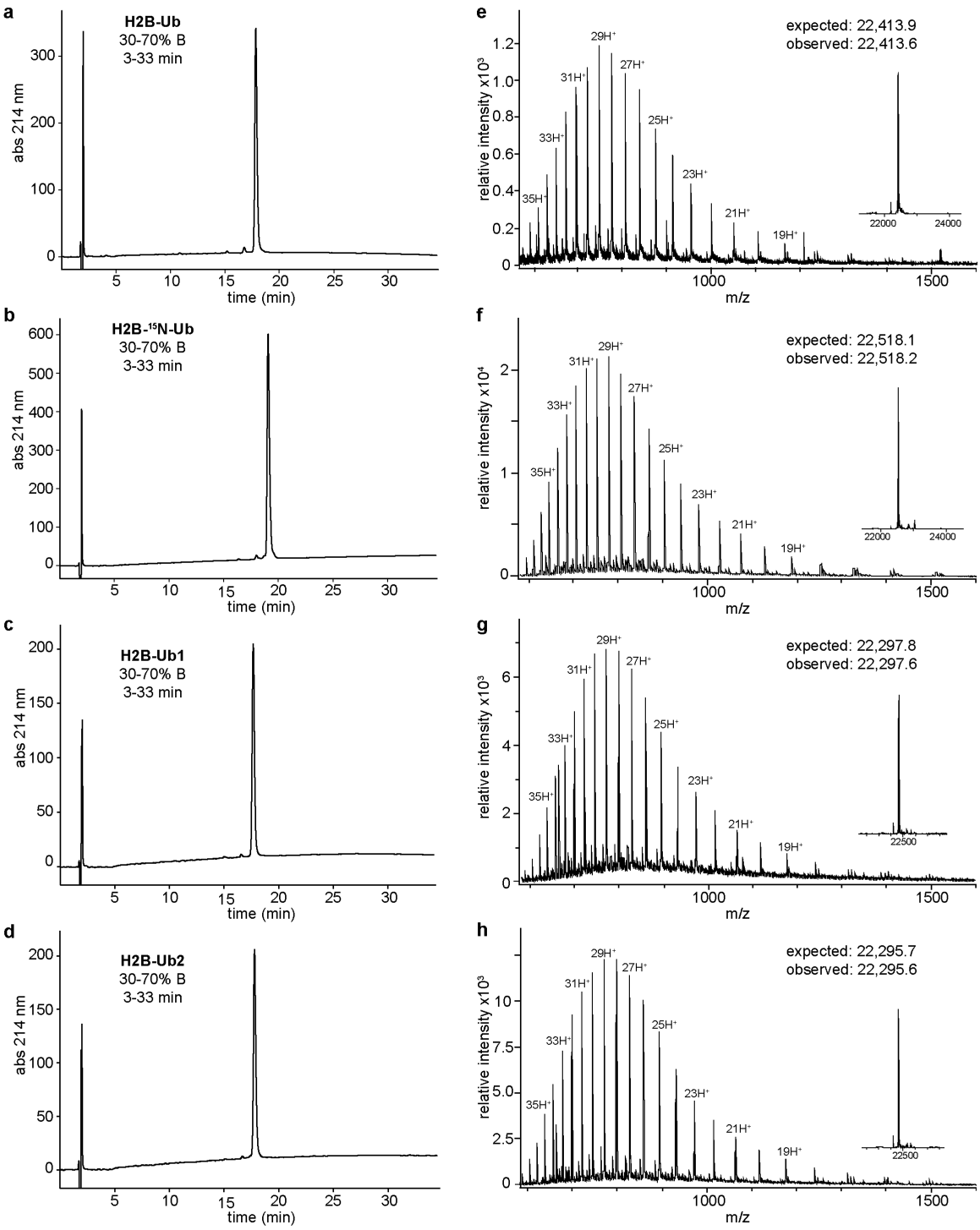
Supplementary Results

Supplementary Table 1: Resonance assignments for ubiquitin-aminoethanethiol in DMSO-d_6/D_2O/dichloroacetic acid-d_2 95/4.5/0.5%, pD = 5.0 adjusted with NaOD, 25 °C, 10 mM triphenylphosphine. Solution also contains buffer salts (TrisHCl, KCl, $MgCl_2$) that are left after lyophilization. See methods for more details.

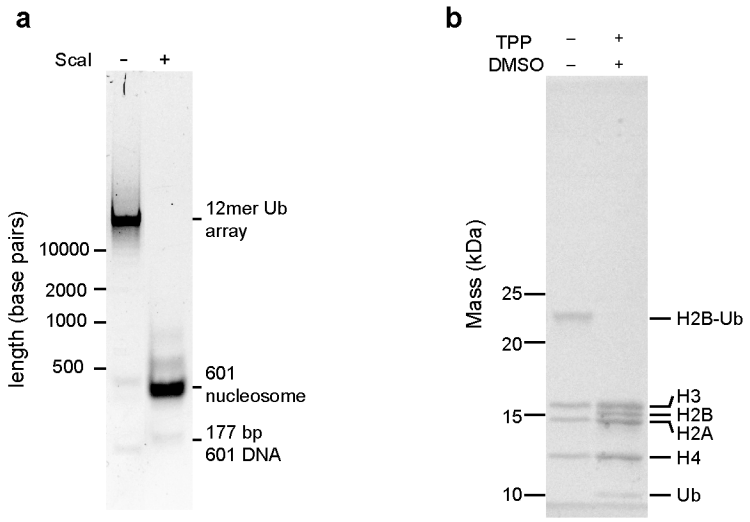
Residue	HN	N	Ca	Cb	Residue	HN	N	Ca	Cb
M1	-	-	55.34	34.94	**D39**	8.265	114.7	54.11	39.11
Q2	8.764	123.1	56.28	31.68	**Q40**	7.797	116.7	56.48	-
I3	8.031	117.2	60.74	40.78	**Q41**	-	-	-	31.22
F4	8.169	120.4	57.30	41.20	**R42**	8.004	118.2	56.13	32.74
V5	8.052	116.0	61.45	34.70	**L43**	7.998	119.7	51.62	44.12
K6	8.214	121.8	56.21	34.99	**I44**	7.749	116.8	61.16	40.30
T7	7.902	111.8	61.85	70.67	**F45**	8.078	120.3	57.74	40.83
L8	8.144	120.6	55.69	44.08	**A46**	8.096	121.4	52.47	21.83
T9	7.758	109.9	62.53	70.57	**G47**	8.086	105.2	46.04	-
G10	8.081	107.8	46.11	-	**K48**	8.023	117.7	56.43	35.02
K11	7.869	117.8	56.26	35.07	**Q49**	8.197	118.6	56.52	31.21
T12	8.096	114.1	62.64	70.40	**L50**	7.942	119.2	55.21	44.34
I13	7.869	118.2	60.86	40.80	**E51**	7.988	117.2	55.89	30.89
T14	7.948	116.0	62.39	70.45	**D52**	8.196	117.9	53.52	39.86
L15	7.955	120.9	54.93	44.63	**G53**	8.091	105.7	46.05	-
E16	8.111	118.4	55.82	30.82	**R54**	8.007	117.8	56.05	32.85
V17	7.656	114.7	60.98	34.73	**T55**	7.945	112.9	62.45	70.46
E18	8.196	121.3	53.77	30.02	**L56**	7.957	120.6	55.18	44.38
P19	-	-	63.33	32.98	**S57**	8.014	113.5	58.79	65.56
S20	8.134	113.0	59.37	65.35	**D58**	8.340	119.6	53.94	39.58
D21	8.285	119.1	53.61	39.50	**Y59**	7.806	115.8	58.53	40.38
T22	7.634	110.9	62.42	70.55	**N60**	8.189	117.9	53.87	40.69
I23	7.873	118.4	61.63	40.00	**I61**	7.780	116.7	61.39	-
E24	8.070	120.0	56.50	30.74	**Q62**	8.142	120.8	56.58	31.10
N25	8.119	118.5	54.05	40.40	**K63**	7.916	118.3	56.60	34.86
V26	7.889	116.7	63.34	33.60	**E64**	8.032	117.6	56.03	31.01
K27	8.092	119.7	57.59	34.20	**S65**	8.069	114.4	58.95	65.40
A28	7.941	120.5	53.05	21.16	**T66**	7.926	113.4	62.73	70.12
K29	7.905	116.9	57.27	34.45	**L67**	7.936	120.2	55.72	43.81
I30	7.820	116.9	62.32	39.75	**H68**	8.207	116.6	55.76	30.43
					L69	8.023	119.6	55.71	44.31
D32	-	-	53.99	39.57	**V70**	8.001	117.2	62.41	33.99
K33	7.829	117.2	56.83	34.80	**L71**	8.048	121.8	55.24	44.32
E34	7.951	116.5	56.14	30.93	**R72**	8.037	119.7	56.06	32.53
G35	8.003	105.9	45.60	-	**L73**	7.967	118.4	55.22	44.31
I36	7.936	118.3	58.34	40.02	**R74**	8.069	118.1	56.38	32.52
					G75	8.329	107.5	46.15	-
P38	-	-	64.12	32.81	**G76**	8.174	105.9	46.02	-



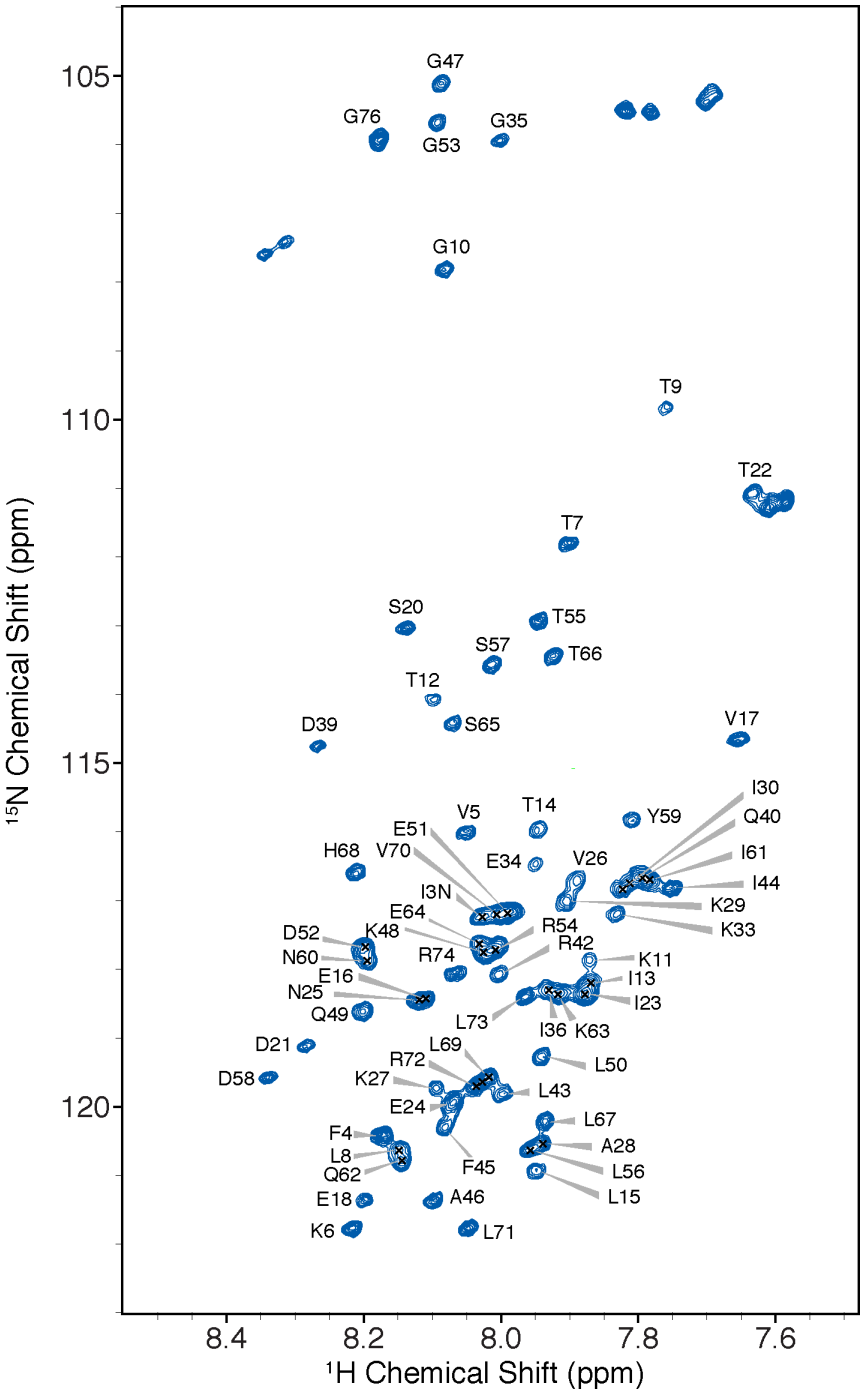
Supplementary Figure 1: Disulfide directed scheme to generate segmentally labeled H2B-^{15}N-Ub.



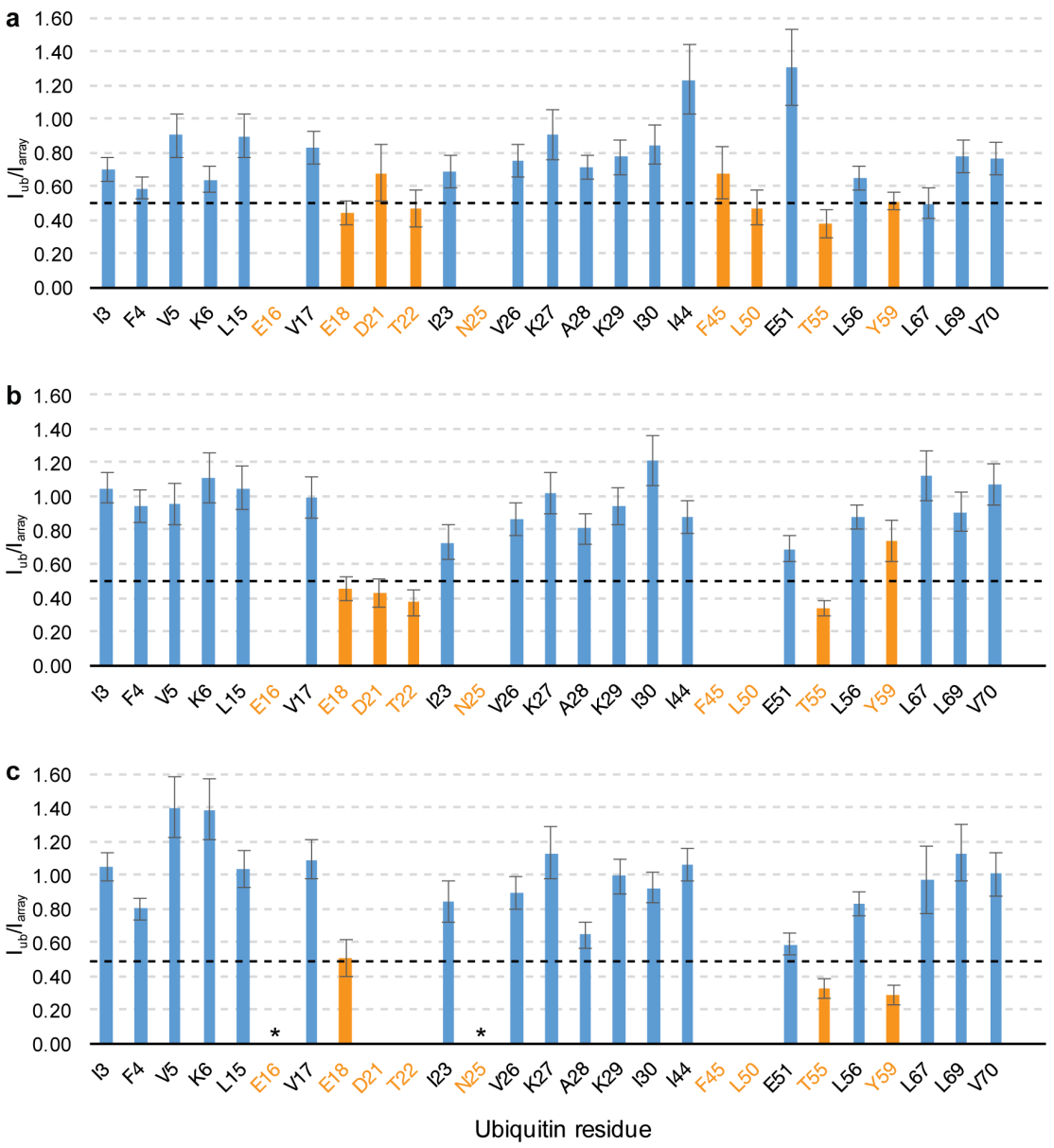
Supplementary Figure 2: Characterization of H2B-Ub prepared using an asymmetric disulfide approach. **(a-d)** Analytical C18 reverse-phase chromatograms of purified H2B-Ub, H2B-^{15}N-Ub, H2B-Ub1 and H2B-Ub2 respectively. **(e-h)** ESI-MS of purified proteins.



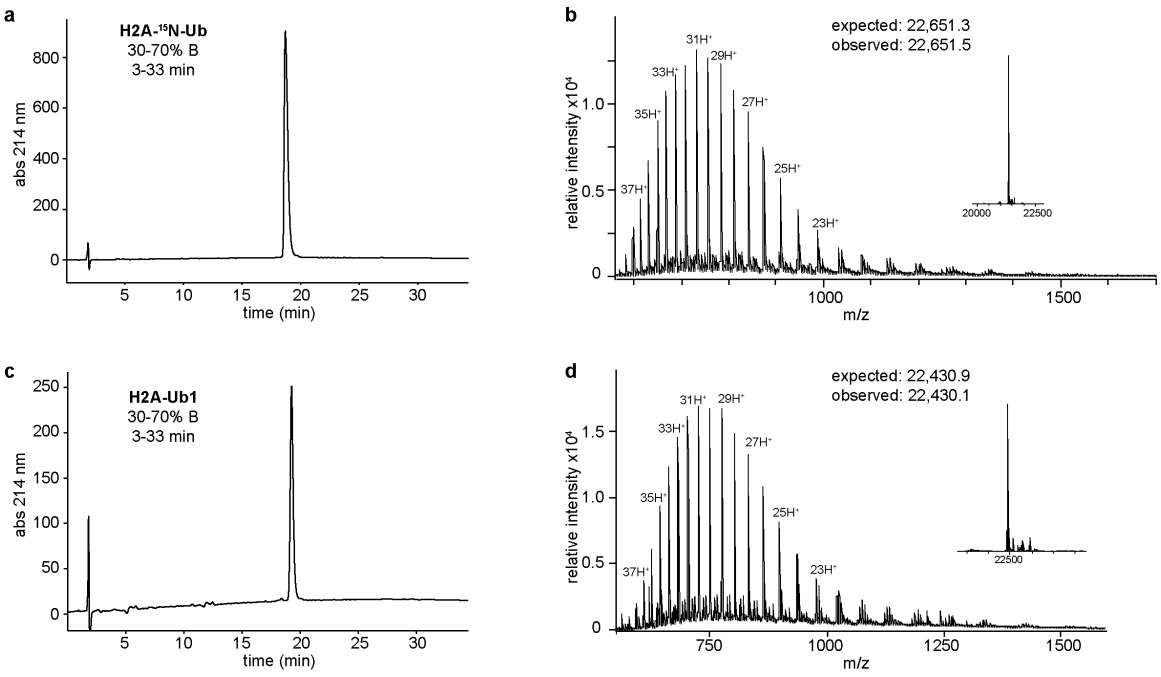
Supplementary Figure 3: Analysis of 12-mer nucleosome arrays containing H2B-^{15}N-Ub. **(a)** Native gel of the arrays (Lane 1). The arrays can be digested to mononucleosomes using the restriction enzyme ScaI to assess the saturation of all twelve 601 DNA nucleosome positioning sites (Lane 2). Minimal amounts of free 177 bp 601 DNA are indicative of well-formed arrays. **(b)** SDS-PAGE of the arrays. After H/D exchange (Lane 1), the arrays were lyophilized and transferred to a solution containing dimethyl sulfoxide (DMSO) and triphenylphosphine (TPP) to record NMR spectra (Lane 2). 10 mM TPP successfully reduced the asymmetric disulfide between H2B and Ub at the low pD and aprotic conditions required for the experiment.



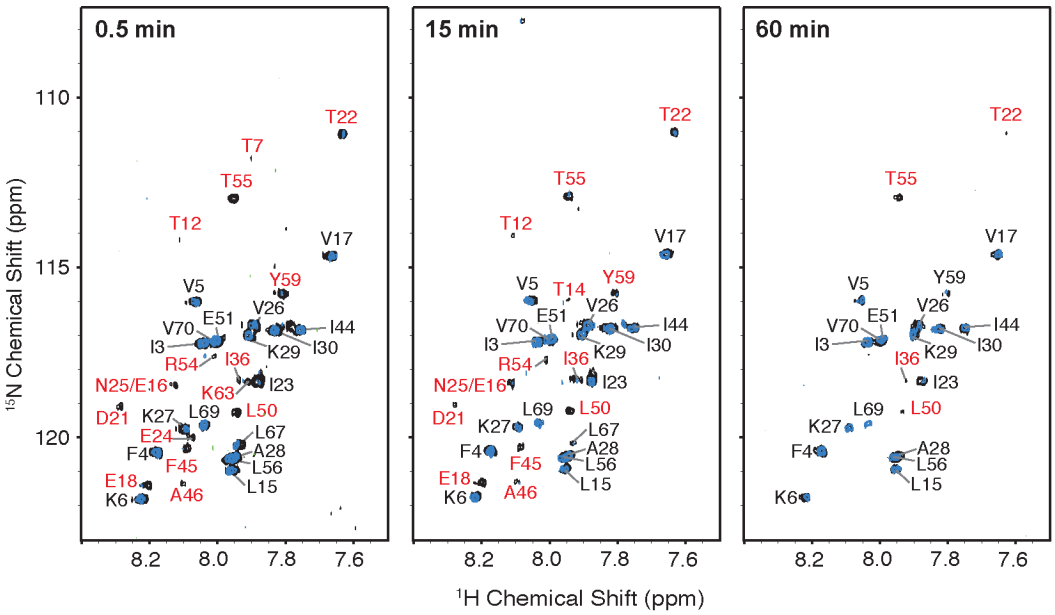
Supplementary Figure 4: ^{1}H-^{15}N HSQC spectrum of ^{15}N,^{13}C-Ub-SH in DMSO-d_6/H_2O/dichloroacetic acid-d_2 95:4.5:0.5%, pD adjusted to 5.2 with 4% NaOD. The sample was treated identically to the array samples, namely ^{15}N-Ub-SH was initially dissolved in protonated exchange buffer containing 1 mM $MgCl_2$, 10 mM Tris, 10 mM KCl. The sample was then lyophilized, and subsequently re-dissolved in the DMSO-based solvent for NMR data acquisition. Therefore, the final sample contains salts and buffer components, which influence the chemical shifts of the cross-peaks. Assignments were based on a set of 3D HNCA, HNCACB and HNCO experiments acquired with the same sample.



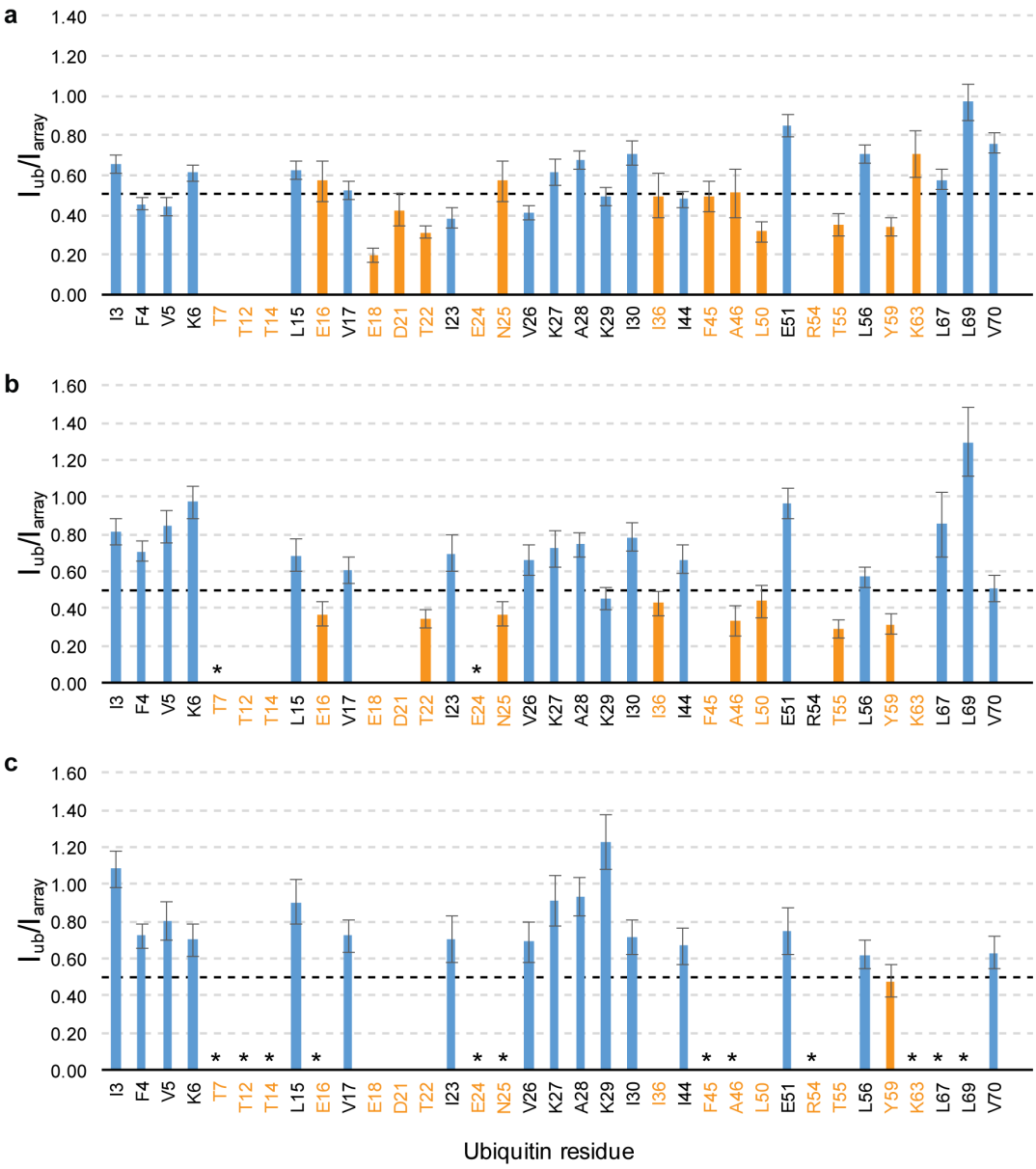
Supplementary Figure 5: Ratio of the cross-peak intensities of the ubiquitin control and array samples for H2B-Ub as presented in **Fig. 1c**. **(a)** 0.5 min time point, **(b)** 15 min time point, **(c)** 60 min time point. Residues displaying low ratios (< 0.5 average for the three time points) were assigned as array specific and labeled in orange. The black dashed line indicates a ratio of 0.5. I_{ub} is the intensity of the corresponding peak in the HSQC spectrum of the ubiquitin control sample, I_{array} is the intensity of the cross-peak in the HSQC of the array sample. For quantifying the cross-peaks, the intensity cutoff was $4 \times \sigma_{noise}$ where σ_{noise} is the average noise level of each spectrum. Error bar = $\sqrt{\left(\sigma_{array}/I_{array}\right)^2 + \left(\sigma_{ub}/I_{ub}\right)^2}$, where σ_{array} and σ_{ub} are the noise levels of each array and ubiquitin control spectrum. The asterisks denote I_{array} and I_{ub} both equal to zero.



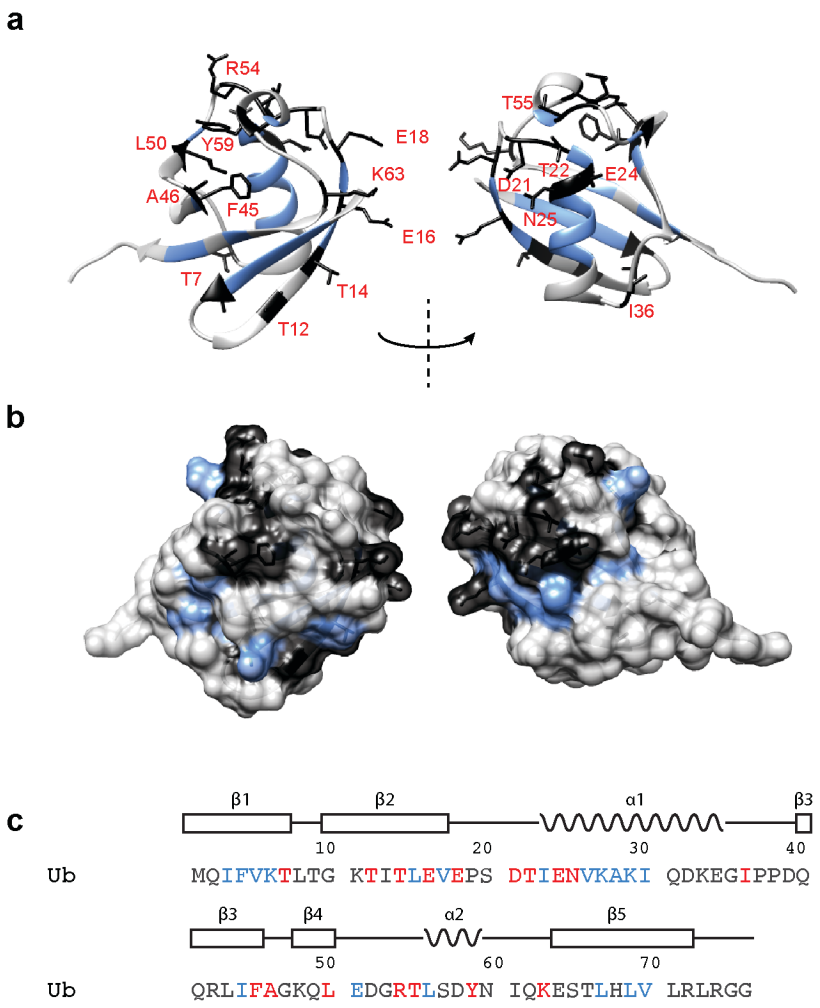
Supplementary Figure 6: Characterization of H2A-Ub prepared using an asymmetric disulfide approach. **(a)** and **(c)** Analytical C18 reverse-phase chromatograms of purified H2A-^{15}N-Ub, and H2A-Ub1 respectively. **(b)** and **(d)** ESI-MS of purified proteins.



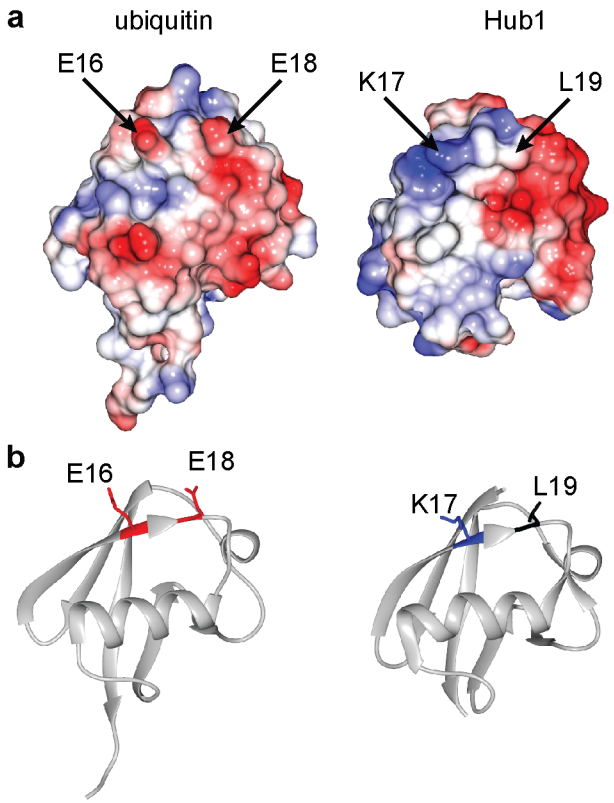
Supplementary Figure 7: ^{1}H-^{15}N HSQC spectra of nucleosome arrays incorporating H2A-^{15}N-Ub incubated in deuterated buffer for 0.5, 15 and 60 min. Cross-peaks from experiments performed with H2A-Ub arrays are depicted in black, while cross-peaks from control Ub experiments are shown in blue. Resonance assignments for array-specific cross-peaks are labeled in red. Contour levels were set to $5 \times \sigma_{noise}$ where σ_{noise} is the average noise level of each spectrum.



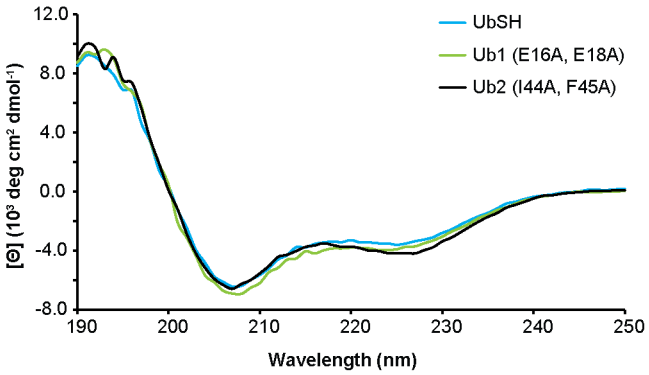
Supplementary Figure 8: Ratio of the cross-peak intensities of the ubiquitin control and array samples for H2A-Ub as presented in **Supplementary Fig. 7**. **(a)** 0.5 min time point, **(b)** 15 min time point, **(c)** 60 min time point. Residues displaying low ratios (< 0.5 average for the three time points) were assigned as array specific and labeled in orange. The black dashed line indicates a ratio of 0.5. I_{ub} is the intensity of the corresponding peak in the HSQC spectrum of the ubiquitin control sample, I_{array} is the intensity of the cross-peak in the HSQC of the array sample. For quantifying the cross-peaks, the intensity cutoff was $4 \times \sigma_{noise}$ where σ_{noise} is the average noise level of each spectrum. Error bar $= \sqrt{\left(\sigma_{array}/I_{array}\right)^2 + \left(\sigma_{ub}/I_{ub}\right)^2}$, where σ_{array} and σ_{ub} are the noise levels of each array and ubiquitin control spectrum. The asterisks denote I_{array} and I_{ub} both equal to zero (except for L69 where only I_{array} and is zero).



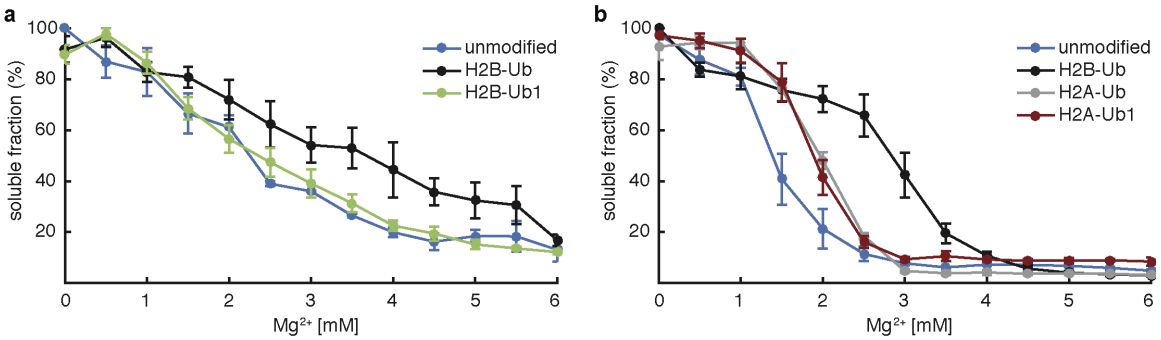
Supplementary Figure 9: Ubiquitin residues protected from backbone H/D exchange in nucleosome arrays incorporating H2A-Ub. **(a)** Residues unique for the array sample are shown in black, while residues common for both the array and ubiquitin only control samples are depicted in blue. The structure represents the ubiquitin fold (PDB ID: 1UBQ). **(b)** Surface representation of the residues depicted in (a). **(c)** Ubiquitin sequence and secondary structure representation. Residues labeled in red are uniquely protected from H/D exchange for the array samples, while the residues labeled in blue appear in both array and control samples. **(a-c)** Residues colored in grey undergo rapid H/D exchange and are not detectable in the NMR spectra.



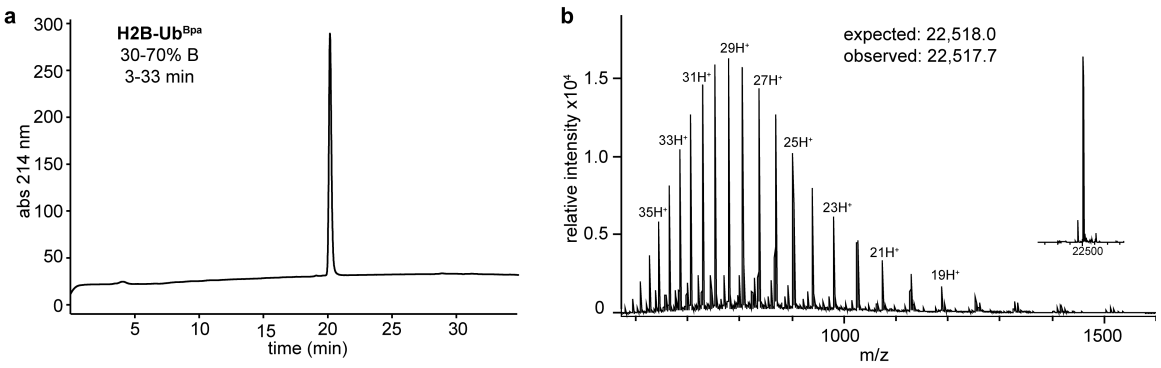
Supplementary Figure 10: Comparison of the electrostatic properties of ubiquitin and Hub1. **(a)** Coulombic surface potential of ubiquitin (PDB ID: 1UBQ) and yeast Hub1 (PDB ID: 1M94). The location of the acidic patch on ubiquitin and the corresponding surface features on Hub1 are denoted by arrows. **(b)** Ribbon representation of the ubiquitin and Hub1 structural folds.



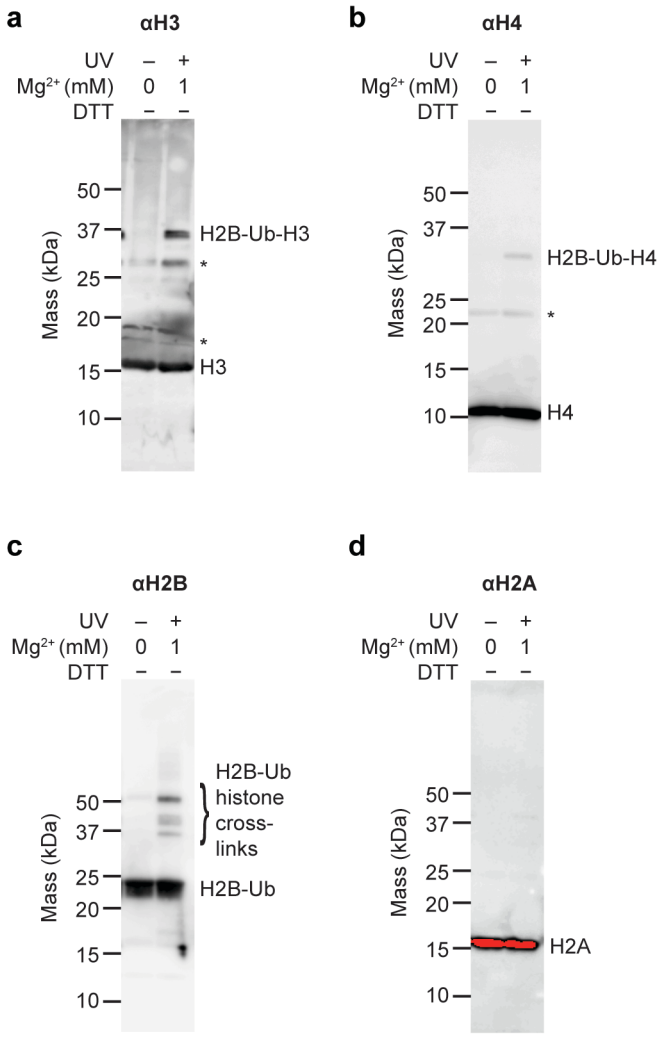
Supplementary Figure 11: Circular dichroism spectra of ubiquitin and ubiquitin mutants. Spectra were collected at 20 ºC at 20 μM protein in 10 mM potassium phosphate (pH 7.3), 100 mM potassium fluoride and 0.5 mM DTT. UbSH – ubiquitin with aminoethanethiol linker.



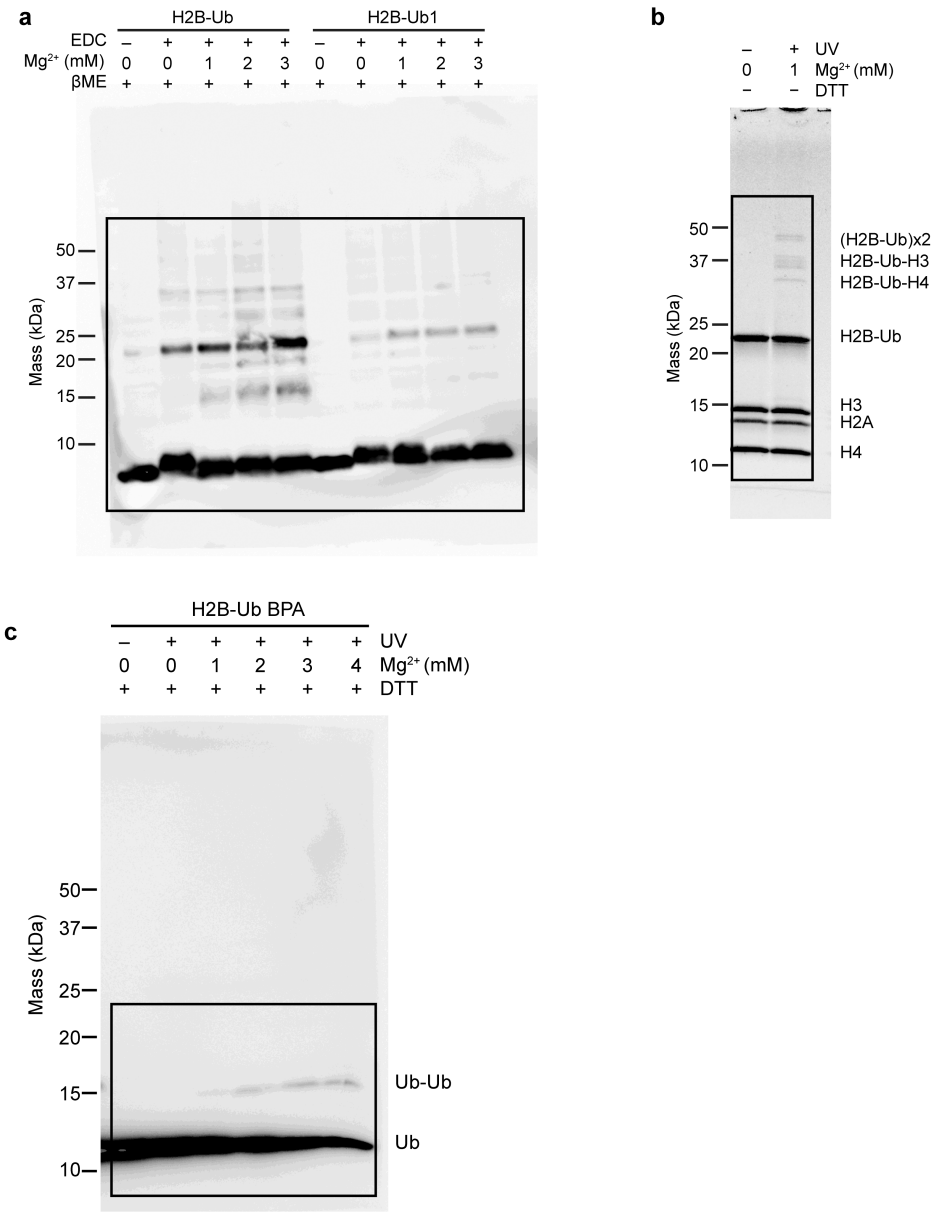
Supplementary Figure 12: Oligomerization of nucleosome arrays. **(a)** Oligomerization of H2B-Ub arrays in the presence of 100 mM KCl, 10 mM Tris, pH 7.8. **(b)** Oligomerization of H2A-Ub and H2A-Ub1 arrays as compared to unmodified and H2B-Ub arrays using the same conditions as in **Fig. 3** (10 mM KCl, 10 mM Tris, pH 7.8). Ub1 – ubiquitin with E16A, E18A substitutions. For **(a)** and **(b)**, error bars, s.e.m. (n = 3).



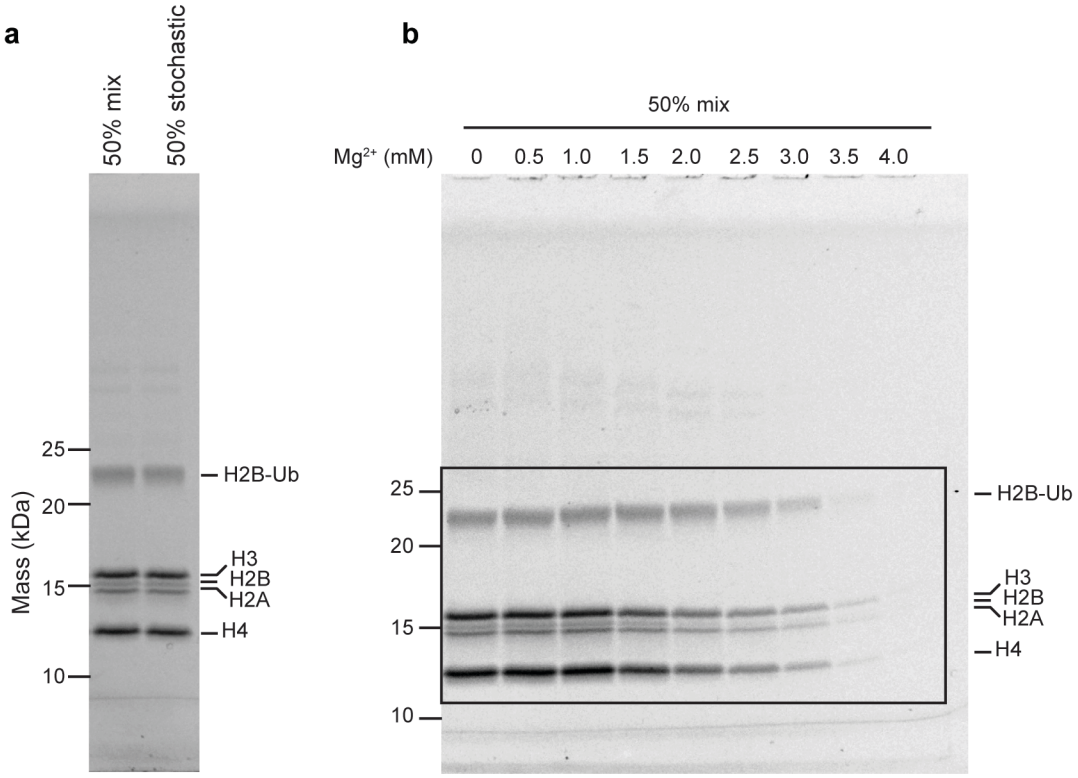
Supplementary Figure 13: Characterization of H2B-Ub^{Bpa} prepared using an asymmetric disulfide approach. **(a)** Analytical C18 reverse-phase chromatogram of purified H2B-Ub^{Bpa}. **(b)** ESI-MS of the purified protein.



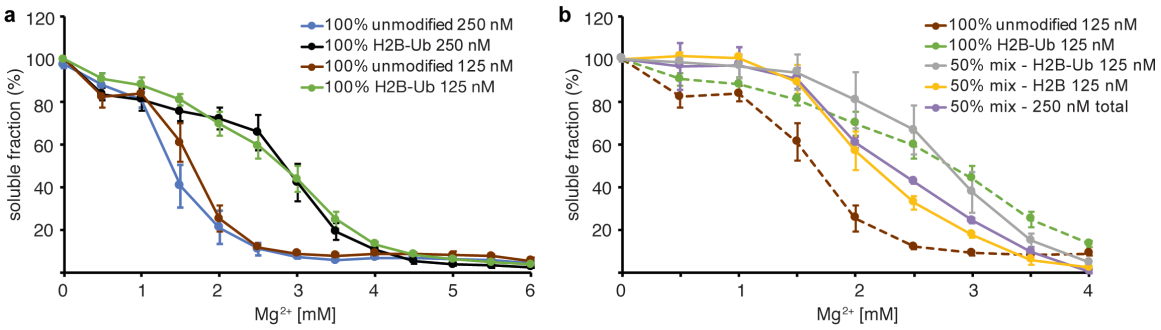
Supplementary Figure 14: Cross-linking of H2B-Ub^{Bpa} arrays analyzed by Western blotting. **(a)** Analysis of H2B-Ub^{Bpa} cross-links to H3 (αH3, Abcam ab1791, 1:10,000 dilution). **(b)** Analysis of H2B-Ub^{Bpa} cross-links to H4 (αH4, Active Motif 39270, 1:500 dilution). **(c)** Analysis of cross-links containing H2B (αH2B, Abcam ab1790, 1:8,000 dilution). **(d)** Analysis of H2B-Ub^{Bpa} cross-links to H2A (αH2A, Active Motif 39112, 1:1000 dilution). The asterisks denote bands due to non-specific antibody binding. All lanes contain 15 pmoles of 601 sites, and UV irradiation was performed for 5 min in the presence of 1 mM Mg^{2+}. Analysis was performed in non-reducing conditions.



Supplementary Figure 15: Full images for the data presented in **Fig. 4. (a)** EDC-based cross-linking of nucleosome arrays containing H2B-Ub and H2B-Ub1. Cross-linking was performed in the presence of increasing concentrations of Mg^{2+}, and all samples were subsequently reduced with β-mercaptoethanol to detach ubiquitin and ubiquitin cross-linked species from H2B. Analysis was performed by SDS-PAGE followed by western blotting with an antibody against ubiquitin (αUb, Biolegend/Covance P4D1/P4G7 #838701 1:1000 dilution). **(b)** UV-induced cross-linking of H2B-Ub^{Bpa} arrays in the presence of 1 mM Mg^{2+} (SYPRO® Ruby protein gel stain). The cross-linked bands were resolved by SDS-PAGE under non-reducing conditions. **(c)** UV-induced cross-linking of H2B-Ub^{Bpa} arrays in the presence of increasing concentrations of Mg^{2+}. Samples were reduced with 100 mM dithiothreitol, separated by SDS-PAGE, and analyzed by western blotting against ubiquitin (αUb, Abcam ab8134, 1:1000 dilution).



Supplementary Figure 16: Analysis of the mixed and stochastic array samples. **(a)** SDS-PAGE of 50% stochastic and 50% mixed arrays depicting equivalent amounts of H2B-Ub and H2B. **(b)** Full image of the SDS-PAGE analysis of mixed arrays presented in **Fig. 5c**.



Supplementary Figure 17: Concentration dependence of precipitation experiments. **(a)** Comparison of array concentrations equivalent to 250 nM and 125 nM 601 sites, respectively. The 250 nM data are the same as in **Fig. 3d** and **Fig. 5b,d**. **(b)** Quantitative analysis of mixed arrays (**Fig. 5**) containing 50% H2B-Ub and 50% unmodified arrays with equivalent concentrations of 100% H2B-Ub and 100% unmodified samples. Error bars, s.e.m. (n = 3).