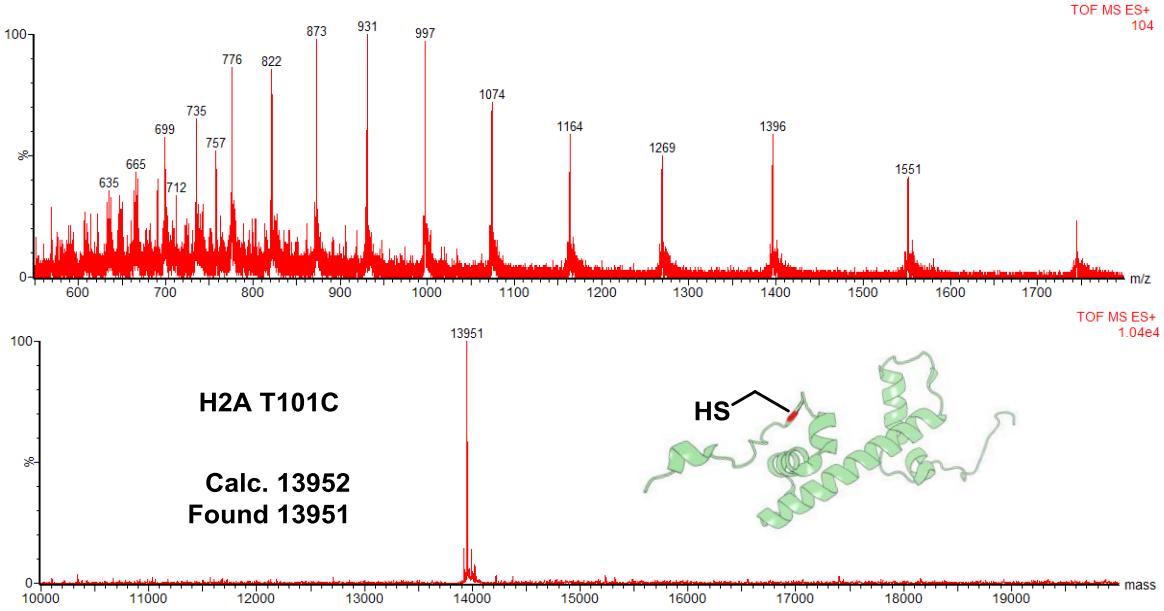
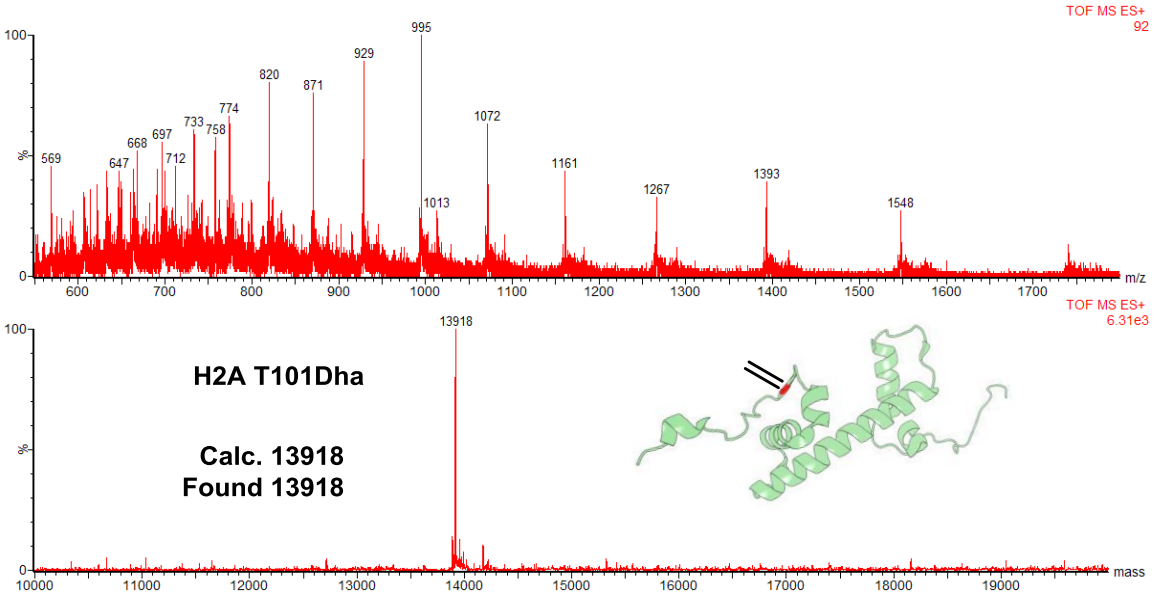
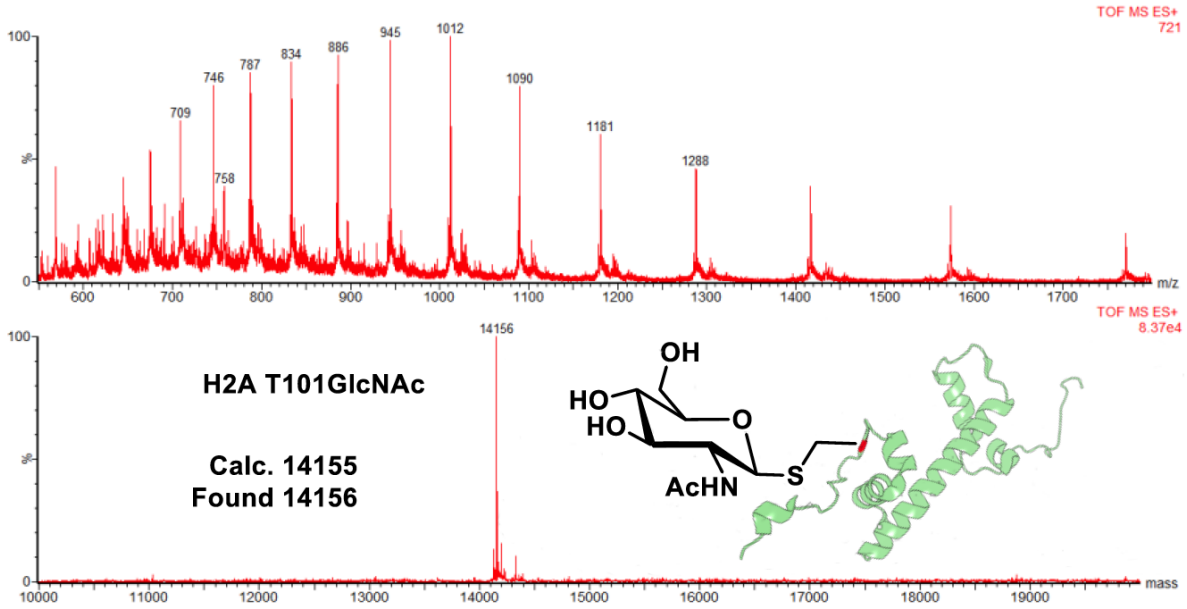


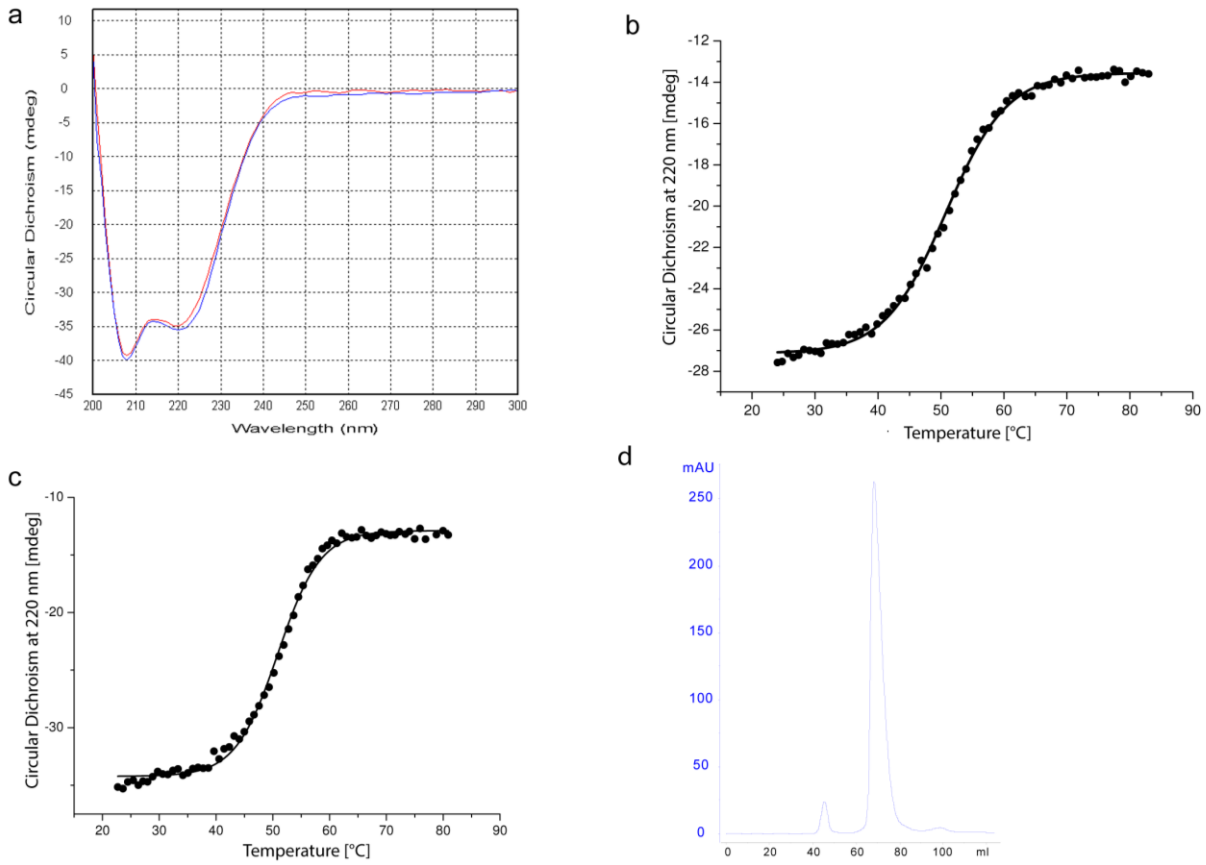

**Supplementary Figure 1.** LC-MS analysis of the reduced H2A T101C.



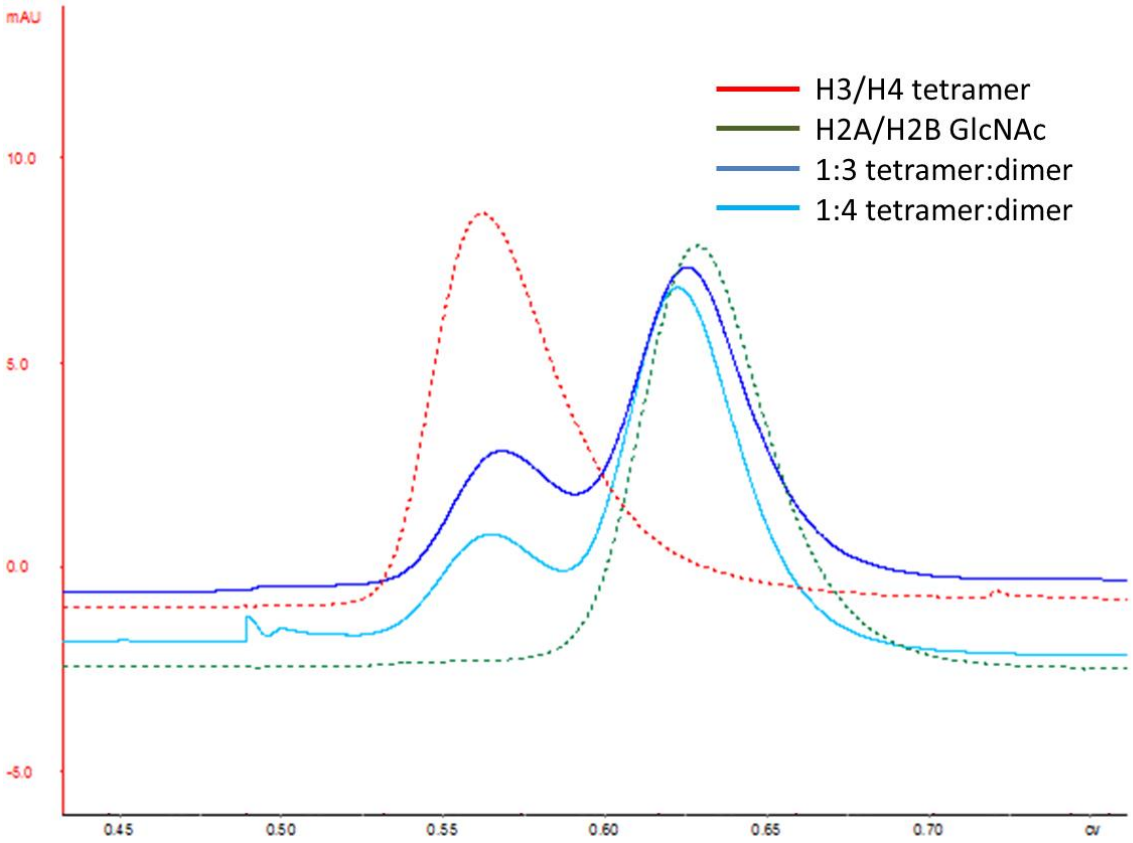

**Supplementary Figure 2.** LC-MS analysis of H2A T101Dha.



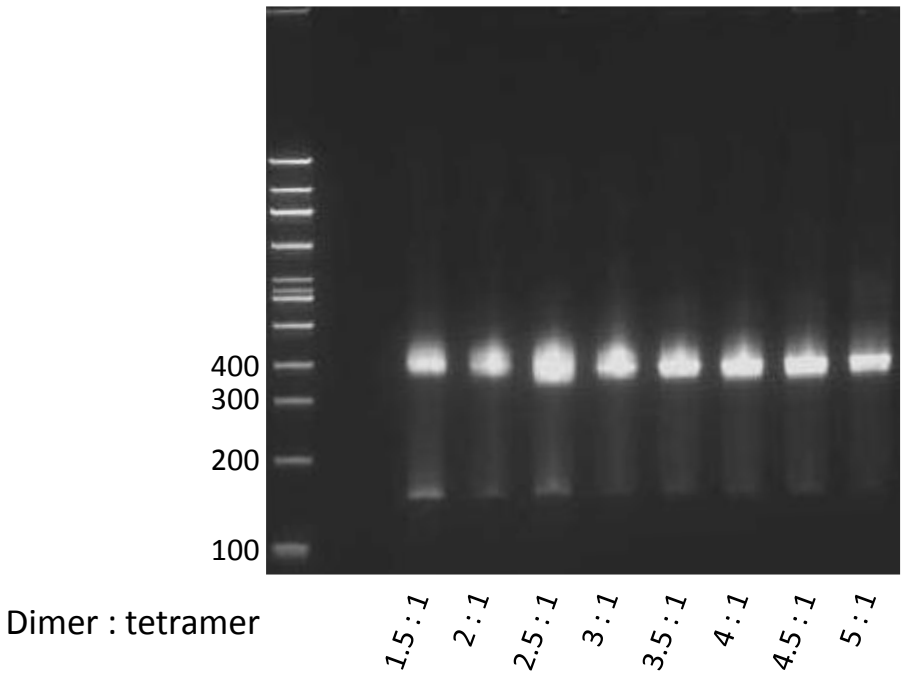

**Supplementary Figure 3.** LC-MS analysis of H2A T101GlcNAc.



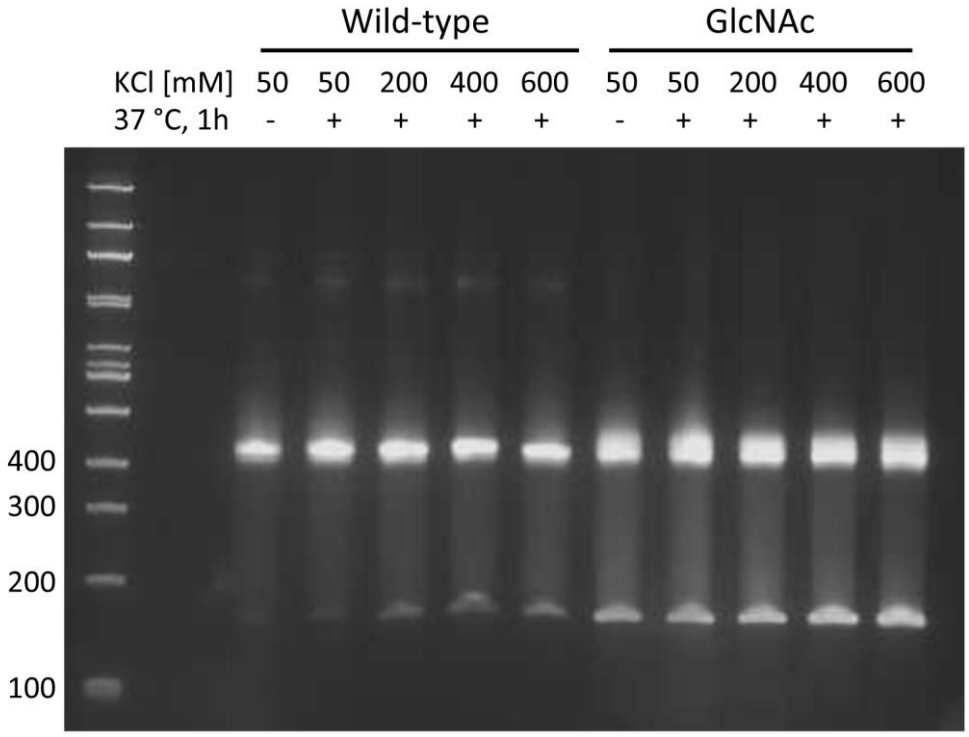

**Supplementary Figure 4** CD analysis of wildtype and GlcNAcylated H2A/H2B dimers. **a** Overlay of the CD spectra for wildtype (red) and GlcNAcylated (blue) H2A / H2B dimers. **b** Melting profile of GlcNAcylated H2A / H2B dimer measured by CD. Experiments were performed in triplicates. **c** Melting profile of wildtype H2A / H2B dimer measured by CD. Experiments were performed in triplicates. **d** UV trace of the size exclusion purification of reconstituted wildtype H3/H4 tetramers.



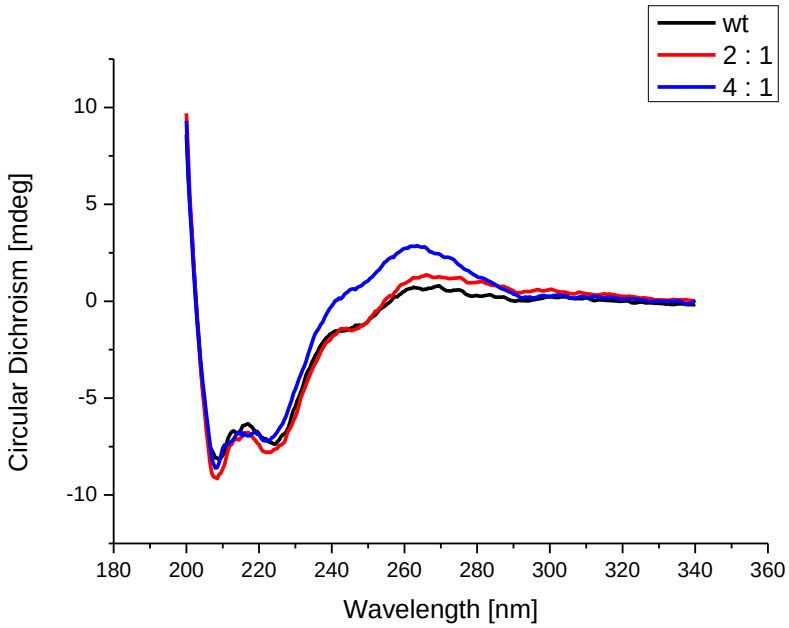

**Supplementary Figure 5**. Octamer refolding using different H3/H4 tetramer : H2A/H2B-GlcNAc dimer ratios. For reference, SEC traces for the H3/H4 tetramer (red) and the H2A/H2B-GlcNAc dimer (green) are shown.



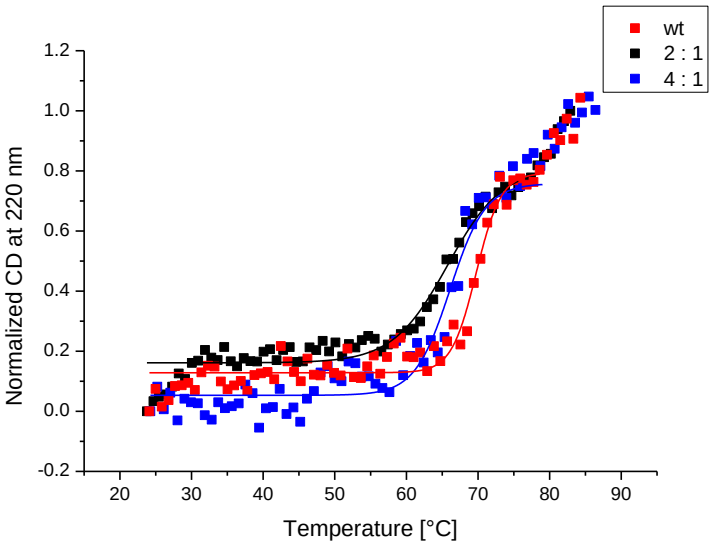

**Supplementary Figure 6.** Non denaturing PAGE analysis of nucleosome reconstitutions using different H3/H4 tetramer : H2A/H2B-GlcNAc dimer ratios.



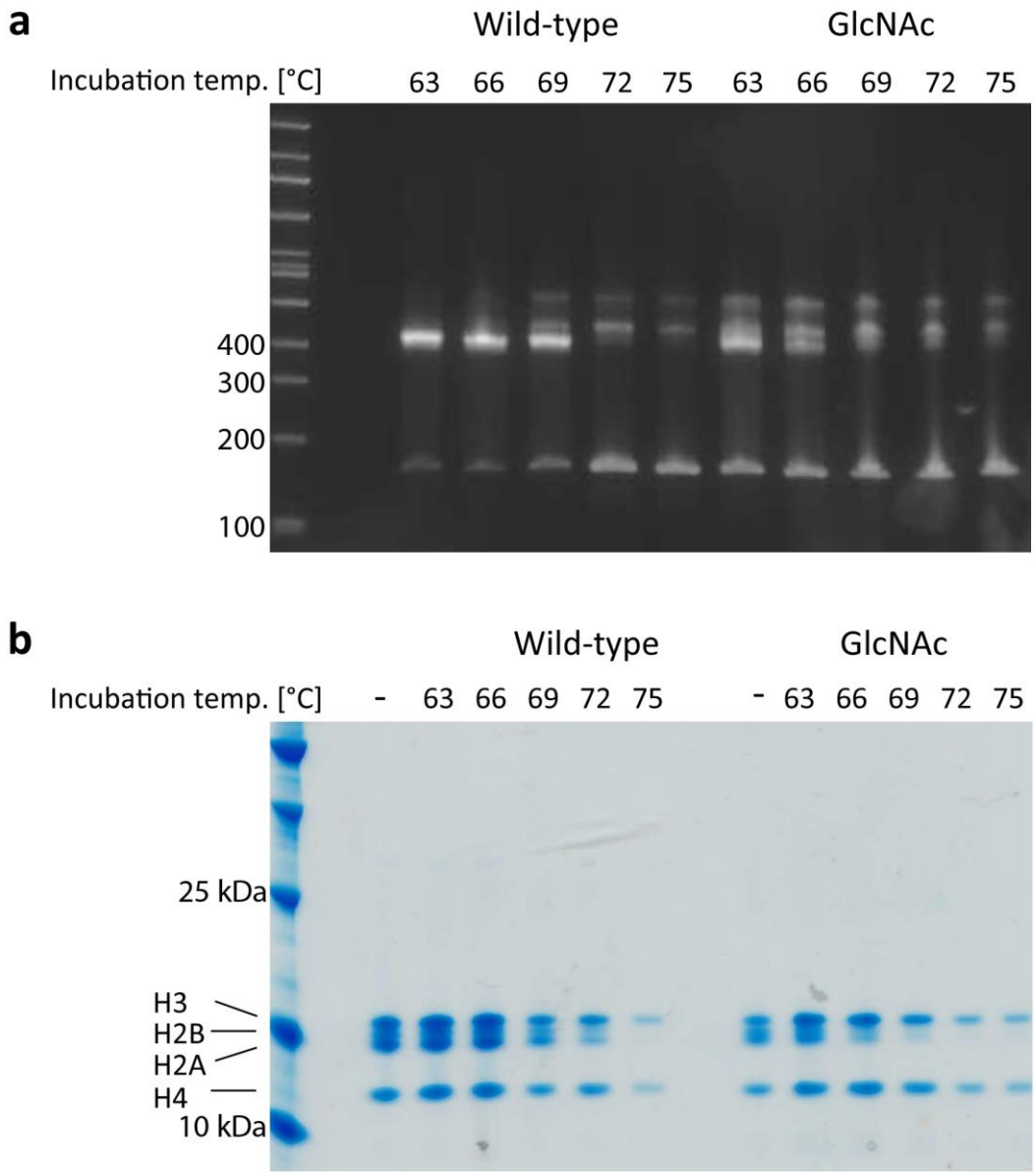

**Supplementary Figure 7.** Incubation of wt and GlcNAcylated nucleosomes for 1 h at 37°C with different concentrations of KCl.



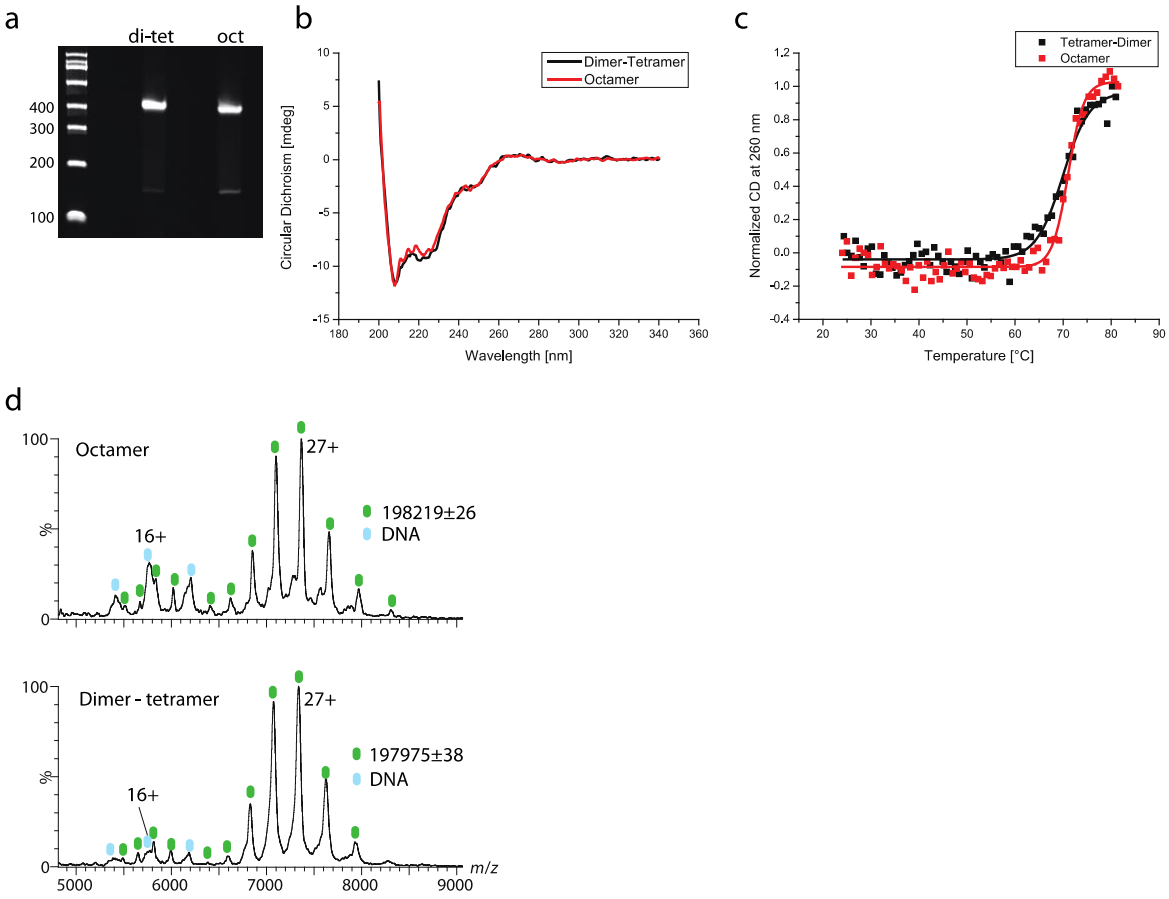

**Supplementary Figure 8.** CD analyis of wt nucleosomes (black) and nucleosomes reconstituted with different H2A/H2B-GlcNAc dimer : H3/H4 tetramer ratios (2:1, red; 4:1 blue).



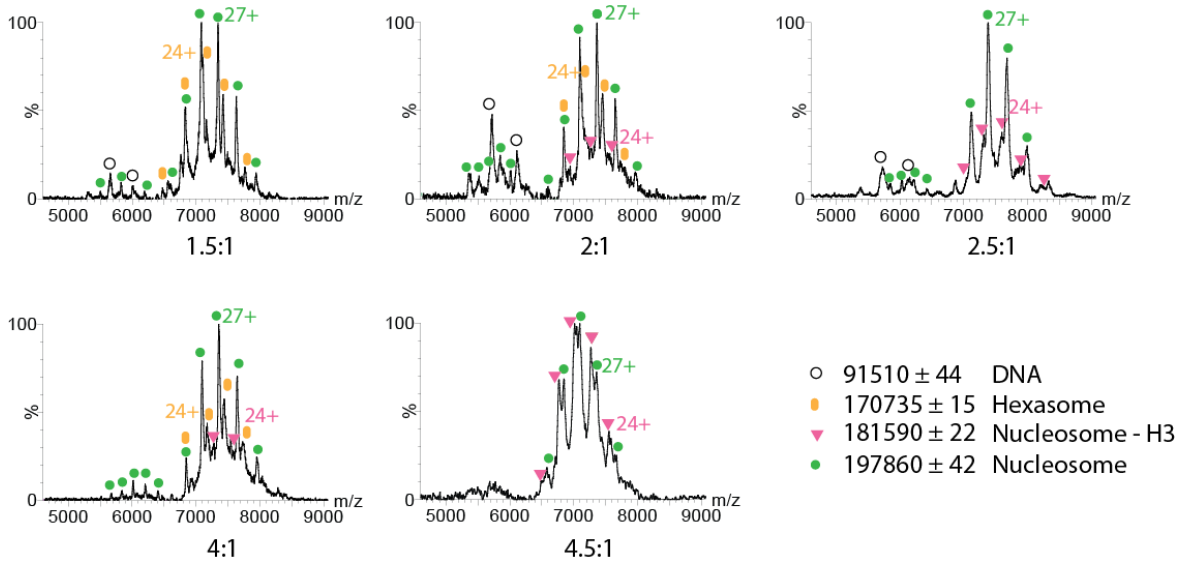

**Supplementary Figure 9.** Melting profile of wt and GlcNAcylated nucleosomes reconstituted with different dimer : tetramer ratios monitored by CD at 220 nm.




**Supplementary Figure 10.** Thermal denaturation of wt and GlcNAcylated nucleosomes. **a** Non-denaturing **'**Native' PAGE analysis of nucleosomes incubated at the indicated temperature for 30 min. **b** SDS-PAGE analysis of the supernatant of the thermal disassembly.




**Supplementary Figure 11.** Comparison of wt nucleosome reconstituted with wt octamers or wt H2A/B dimers and H3/4 tetramers. **a** PAGE analysis of differently reconstituted nucleosomes **b** Overlay of the CD spectra for nucleosome reconstituted with histone octamers (red) and histone di- and tetramers (black). **c** Melting profiles of nucleosome reconstituted from histone octamers (red) and histone di- and tetramers (black) measured by CD at 260 nm. The melting temperatures of the nucleosomes reconstituted from separate di- and tetramers displayed only a slight reduction in melting temperature (Tm 70.0 ± 0.4 °C; wt: 71.0 ± 0.2 °C). **d** Analysis of differently reconstituted nucleosomes using nESI-MS



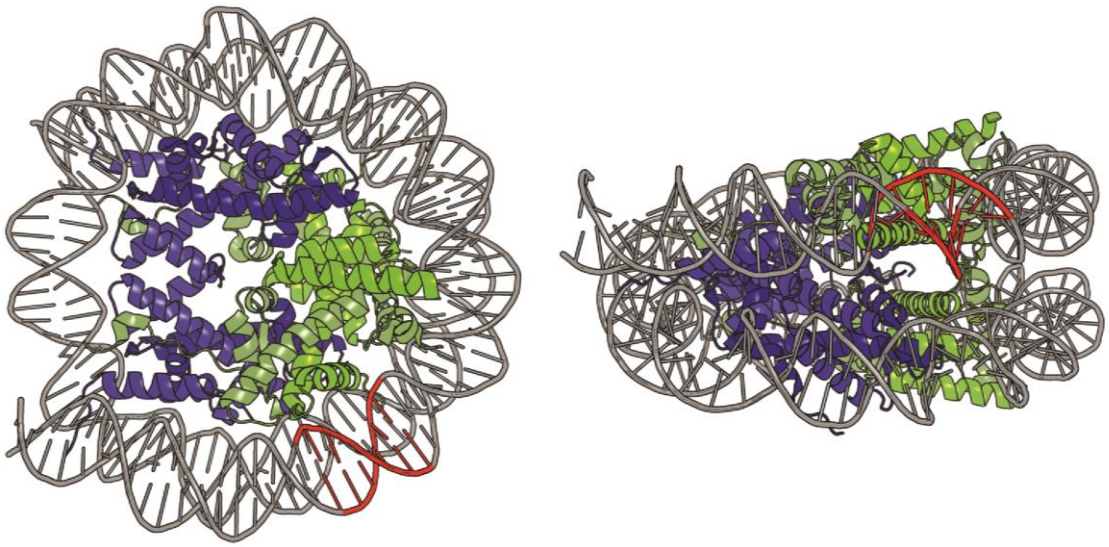

**Supplementary Figure 12.** Non denaturing MS analysis of nucleosomes reconstituted with different H3/H4 tetramer : H2A/H2B-GlcNAc dimer ratios. The dimer : tetramer ratio is given below the spectrum.


**Supplementary Figure 13**: Quantitative proteomics identifies preferential interacting partners for H2A T101 GlcNAc nucleosome (bait) over wild-type nucleosome (control) among n=720 identified proteins identified in two independent biological replicates. Significant interactors identified by Label free quantification (LFQ) on Significance B analysis with a Benjamini-Hochberg FDR of 1 % are shown in blue in a scatter plot. Top 20 significant interactors among them are denoted in red with their gene name. Scatter plot: x axis, logarithmized ratio of average LFQ intensities of protein groups in the bait over control pulldown experiment from two independent replicates; y axis, log10-transformed summed up intensity of protein groups.

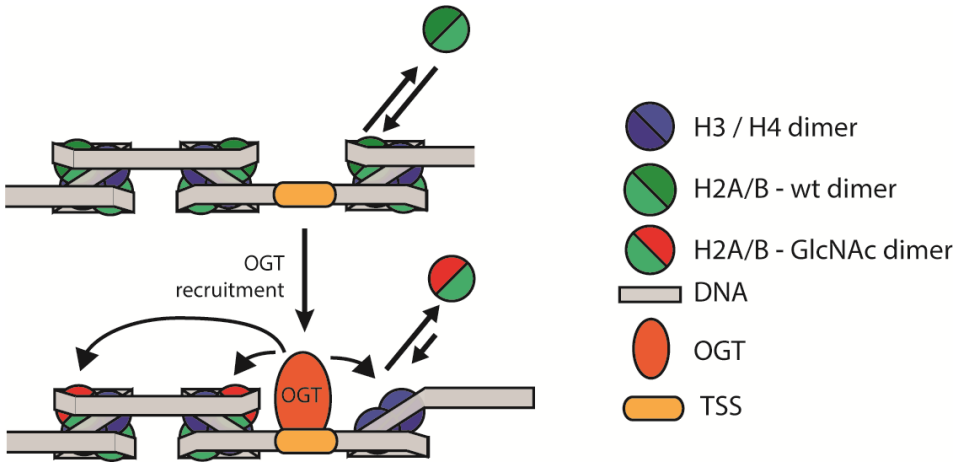
**a**

ATCGGATCTTACATGCACAG GATGTATATATCTGA**CACGT**
GCCTGGAGACTAGGGAGTAA TCCCCTTGGCGGTTAAAACG
CGGGGGACAGCGAGTACGTG CGTTTAAGCGGTGCTAGAGC
TGTCTACGACCAATTGAGCG GCCTCGGCACCGGGATTCTC
CAGGGCGGCCGCGTAGTACT GAAUbioUbio

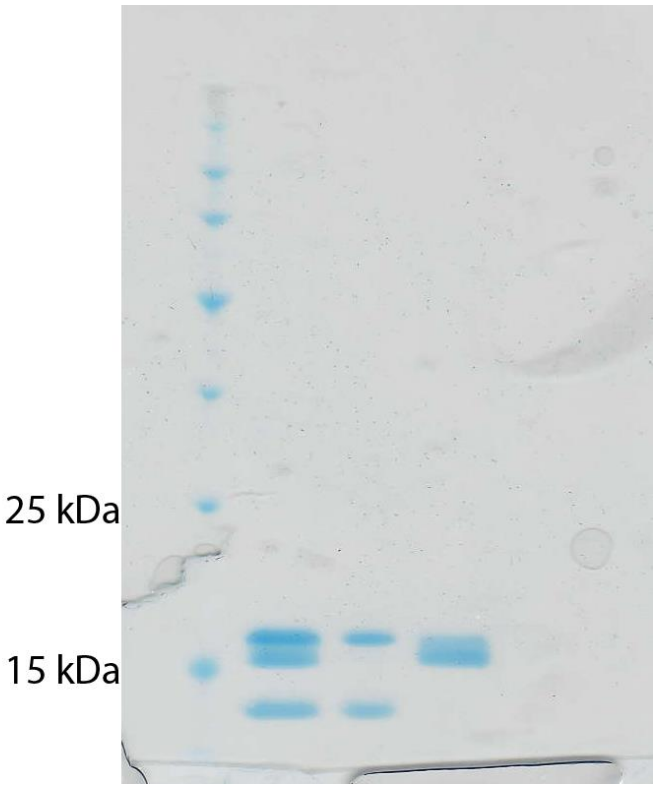
**b**



**Supplementary Figure 14.** Location of the E-Box sequence in the 601 DNA used for nucleosome reconstitution. **a** Sequence of the used 601 DNA with the E-Box sequence marked in red. **b** Location of the E-Box (red) in the 601 DNA in the crystal structure of the nucleosome (pdb: 3LZ0).




**Supplementary Figure 15.** A speculative additional role for OGT recruitment in the suggested mechanism of OGT (see also Figure 3 in main text). Localization of OGT is not due to direct DNA binding, but due to association with other chromatin regulating complexes.




**Supplementary Figure 16**. Uncropped image used in Fig. 1f