Supporting Information

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SI Methods

Nucleosomal Array Reconstitution and Analytical Ultracentrifugation. Nucleosomal arrays were reconstituted from purified components using salt dialysis (1). Briefly, human histones H3, H4, H2A, H2B were purified as monomers (2) and mixed to form $(H3/H4)_2$ tetramer and (H2A/H2B) dimer complexes (2, 3) while human $(CENP\text{-}A/H4)_2$ was purified from a bicistronic vector as a tetramer (Fig. S1*C*) (4). The 601 12×207 bp DNA template (5, 6) was purified by gel filtration (Sephacryl 1000) as described (7). Histone tetramers and dimers were combined with the DNA in 2 M NaCl and taken through stepwise dialysis against $1 \times$ TE (10 mM Tris pH 7.8, 0.25 mM EDTA) with 1 M NaCl, followed by $1 \times$ TE with 0.75 M NaCl, and completing dialysis against $1 \times$ TEN ($1 \times$ TE with 2.5 mM NaCl). Reconstituted nucleosomal arrays were characterized by sedimentation velocity in a Beckman Coulter XLA analytical ultracentrifuge. Boundaries were analyzed by the method of Demeler and van Holde (8) to obtain the integral distribution of sedimentation coefficients, G(s), plotted as boundary fraction against $S_{20,w}$. The extent of DNA template saturation with nucleosomes was determined from the sedimentation coefficient at boundary fraction of 0.5 (S_{ave}) as described (9). Sedimentation velocity experiments showed that both canonical and CENP-A nucleosomal arrays were stable in 1.25 mM $MgCl_2$ for at least 2.7 hours (the length of the longest H/DX time point; see below).

Electron Microscopy. Nucleosomal arrays were dialyzed into HEN buffer (10 mM Hepes, 0.25 mM EDTA, and 2.5 mM NaCl), fixed with 0.1% glutaraldehyde for 4 hr at 4 °C and dialyzed overnight against HEN. Samples were diluted as needed, applied to glow-discharged carbon-coated grids, and stained with 0.1% aqueous uranyl acetate. Grids were examined in a Tecnai 12 TEM operated at 100 kV in the tilted darkfield mode, and digital images were recorded using a TVIPS 2024×2024 CCD camera (10).

MNase Digestion. 2 μg of nucleosome arrays were digested with 0.2 or 2 units of MNase (Worthington) in the presence of 3 mM $CaCl_2$. Each reaction was quenched by addition of 10 μL of 0.5 M EDTA and Buffer PB (Qiagen), processed using a DNA purification kit (Qiagen) and subsequently analyzed by agarose gel electrophoresis.

Histone Fragmentation and MS. H/DX samples were individually melted at 0 °C and then injected (50 μL) and pumped through an immobilized pepsin (Sigma) column at initial flow rate of 50 μL/min for 90 s followed by 150 μL/min for another 90 s. Pepsin was immobilized by coupling to Poros 20 AL support (Applied Biosystems) and packed into column housings of 2 mm× 2 cm dimensions (IDEX). Protease-generated fragments were collected onto a C18 HPLC trap column (2.5×0.5 mm, LC Packings). Peptides were eluted into and through an analytical C18 HPLC column (0.3×75 mm, Agilent) by a linear 12–55% buffer B gradient at 6 μL/min (Buffer A: 0.1% formic acid; Buffer B: 0.1% formic acid, 99.9% acetonitrile). The effluent was electrosprayed into the mass spectrometer (LTQ Orbitrap XL, Thermo Fisher Scientific). The SEQUEST (Bioworks v3.3.1) software program (Thermo Fisher Scientific) was used to identify the likely sequence of the parent peptides using nondeuterated samples via tandem MS.

H/DX Data Processing and Validation. MATLAB based MS data analysis tool, ExMS, was used for data processing (11). Detailed information regarding the ExMS algorithm is described elsewhere (11). Briefly, the ExMS program searches raw MS data, identifies individual isotopic peaks/envelopes from a list of MS/MS peptides obtained from a SEQUEST search, as described above, and calculates centroid values of these envelopes. In the first step, the program is used to identify the isotopic envelope centroid and chromatographic elution time of each parental nondeuterated (ND) peptide. This information is subsequently used to identify deuterated peptides.

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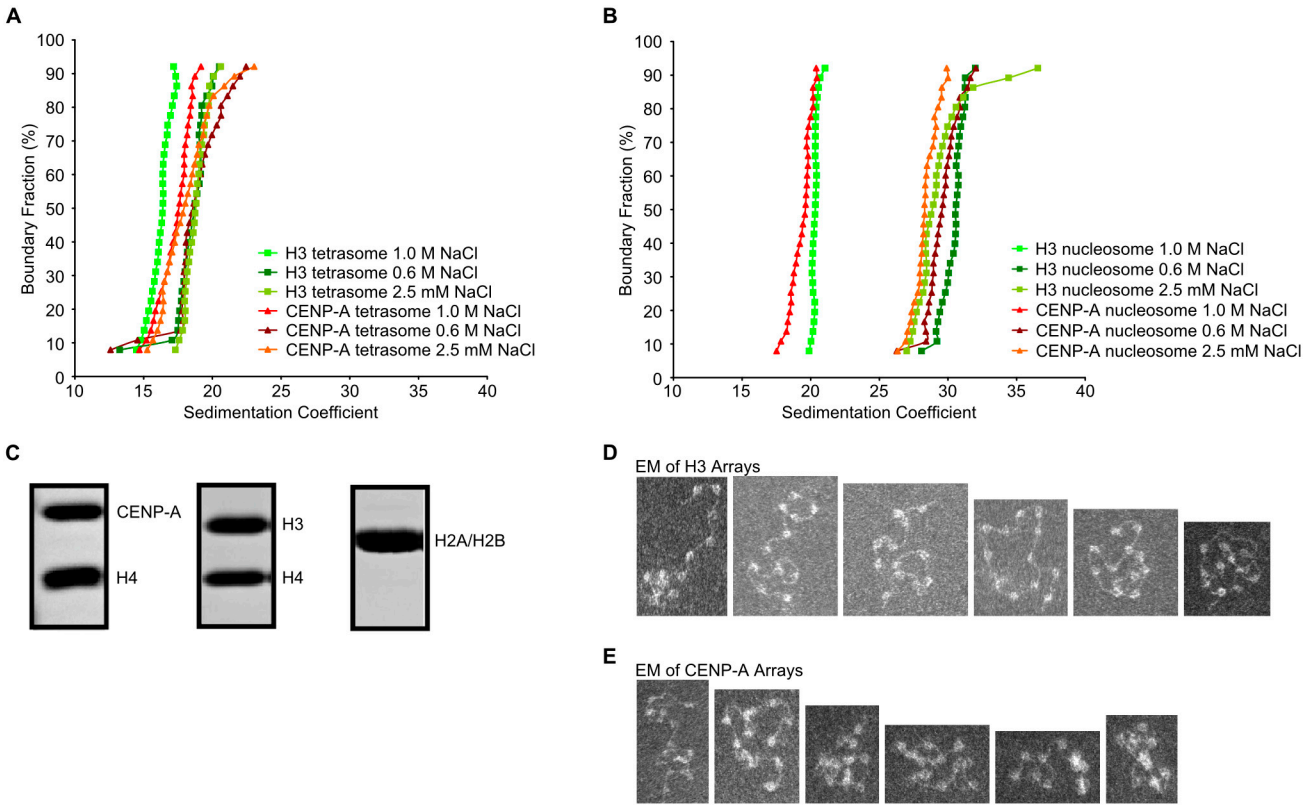

Fig. S1. Stepwise CENP-A nucleosome array assembly. (*A*) Sedimentation profiles of tetramer arrays at all NaCl concentrations as outlined in Fig. 1 *A* and *B*. (*B*) Sedimentation profiles of nucleosome arrays at all NaCl concentrations outlined in Fig. 1 *A* and *B*. (*C*) Coomassie-stained SDS/PAGE gels of purified histones used for array assembly. (*D*) Representative EM images of H3-containing nucleosome arrays. (*E*) Representative EM images of CENP-A containing nucleosome arrays.

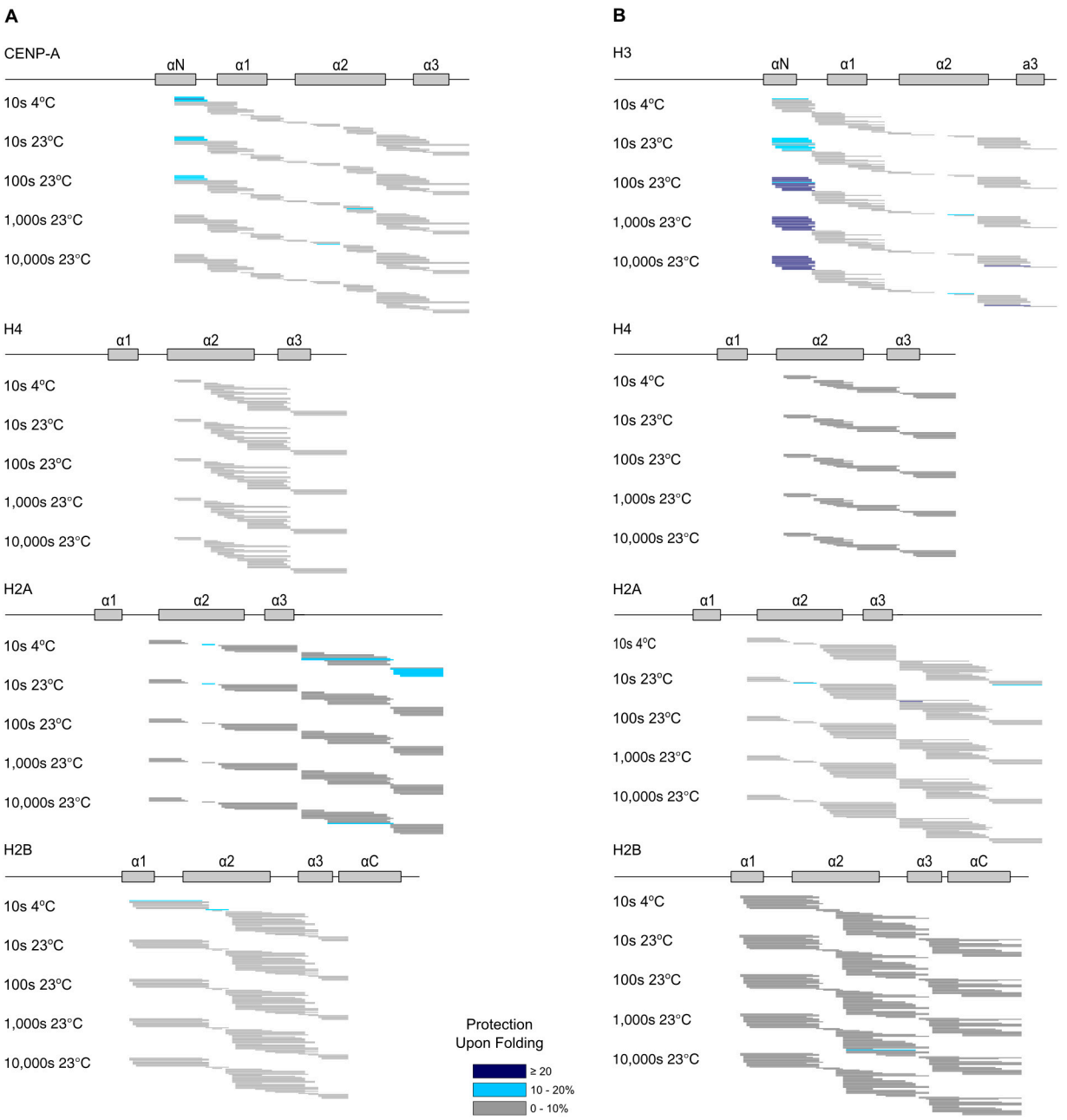

Fig. S2. Nucleosome array H/DX difference plots. (*A*) H/DX difference plots of CENP-A containing arrays at all time points. (*B*) H/DX difference plots of H3 containing arrays at all time points. Plots are displayed as in Fig. 2*D* and *E*.

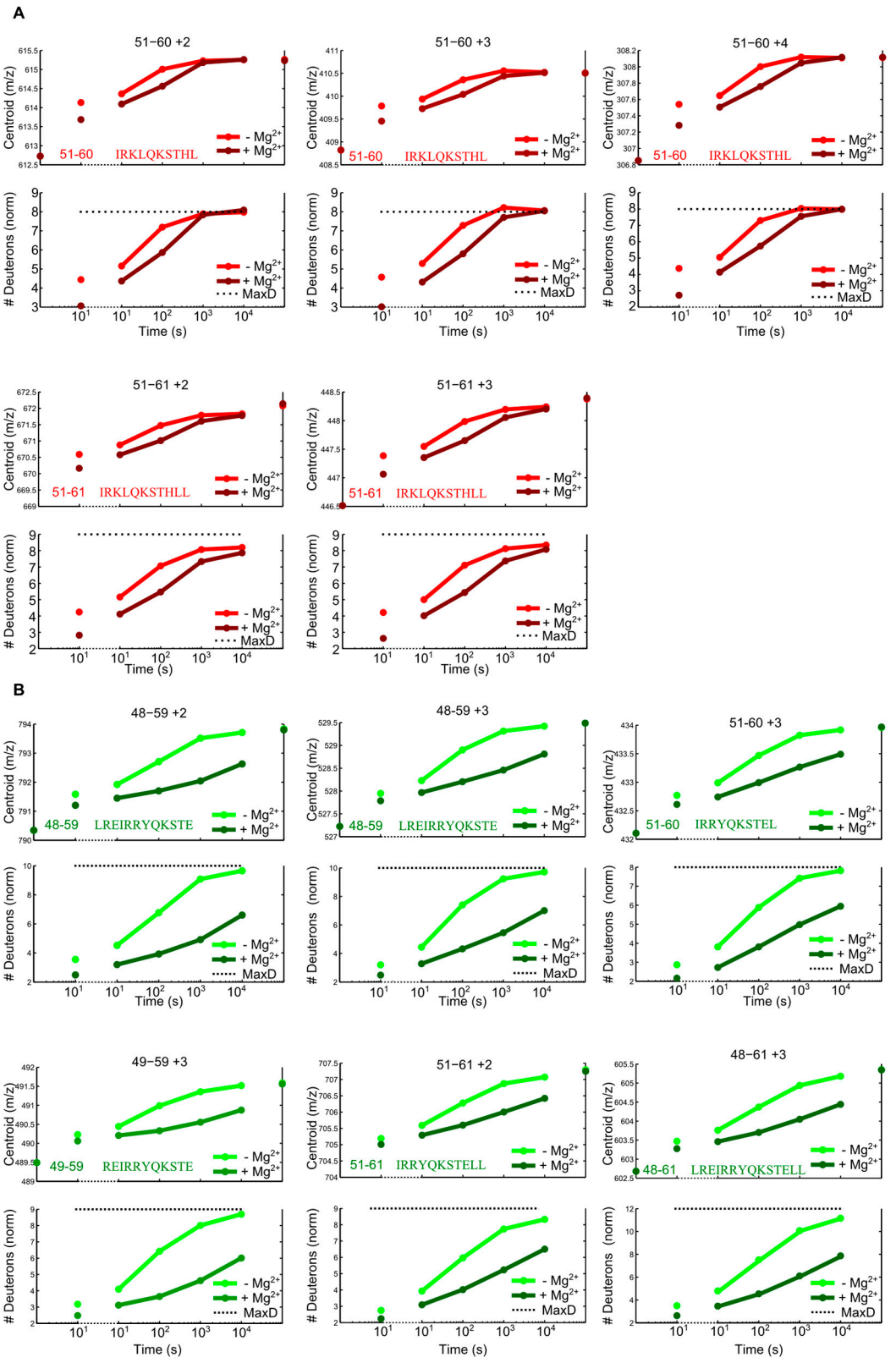

Fig. S3. Additional peptides from αN region. (*A*) Peptides from the αN region of CENP-A containing arrays. The a.a. coordinates are shown at the top of each centroid plot. (*B*) Peptides from the αN region of H3 containing arrays. Data points shown as separate points at 10 s correspond to 4 °C reactions while the rest of the time course corresponds to 23 °C reactions.

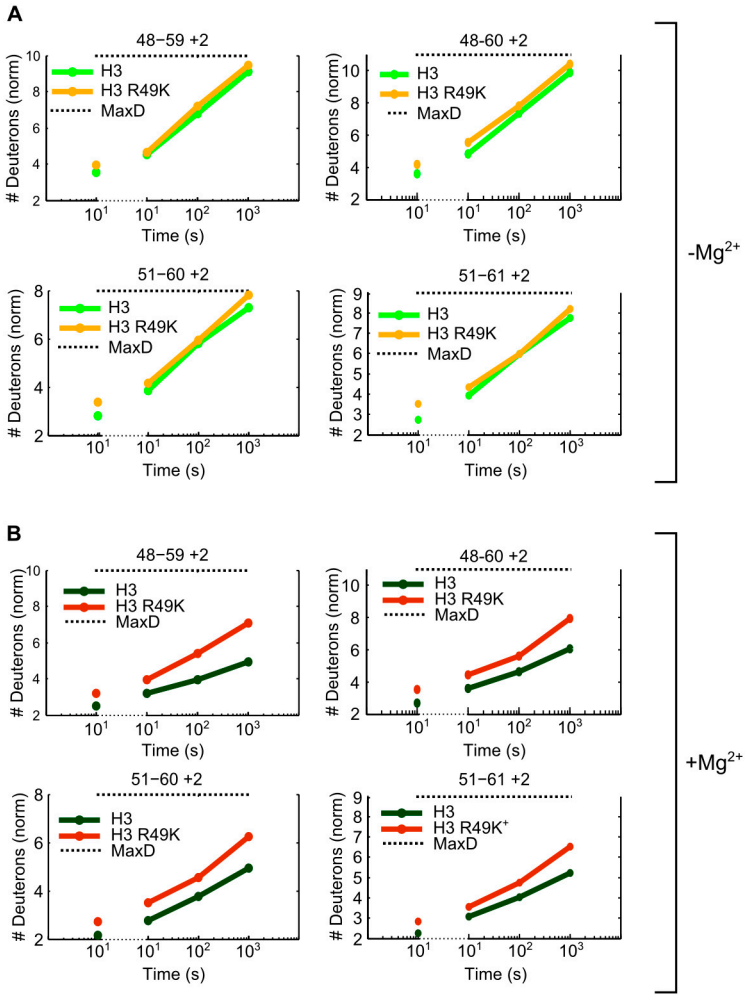

Fig. S4. Additional peptides from H3 R49K containing arrays. Peptides from the αN region of H3- and H3 R49K- containing arrays are shown. The a.a. coordinates are shown at the top of each centroid plot. Data points shown as separate points at 10 s correspond to 4 °C reactions while the rest of the time course corresponds to 23 °C reactions. (*A*) Unfolded arrays ($-Mg^{2+}$). (*B*) Folded arrays ($+Mg^{2+}$).

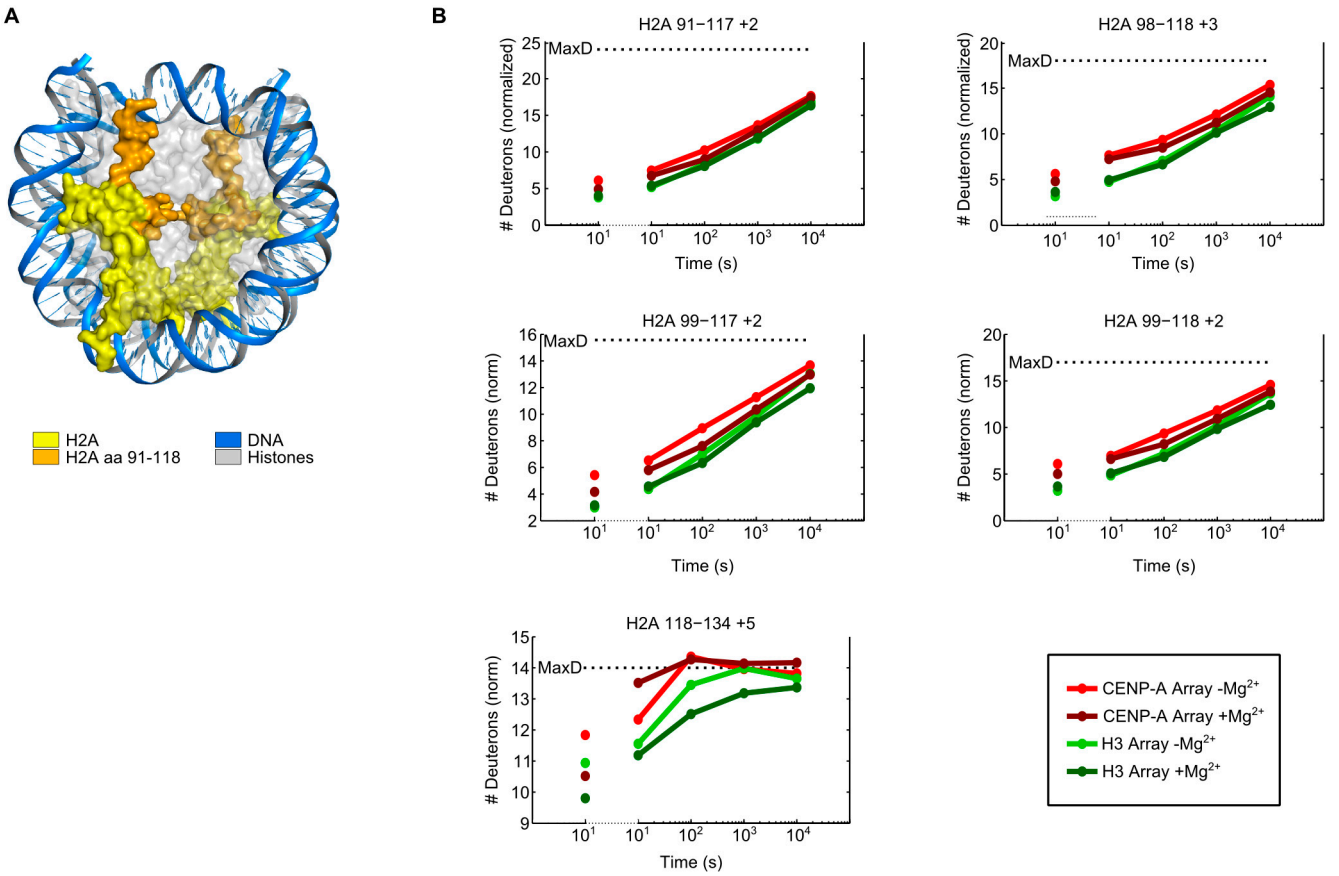

Fig. S5. Additional peptides from H2A C-terminal region. (*A*) Crystal structure of the human nucleosome particle containing histone H3 (PDB ID code 2CV5) (12) is shown to highlight the location of histone H2A (yellow) and the region of H2A where increased protection is observed in H3-containing arrays (91–134; a.a. 91–118 are in dark yellow; a.a. 118–134 are not visible in the structure). (*B*) Representative peptides spanning H2A region aa 91–134. Data points shown as separate points at 10 s correspond to 4 °C reactions while the rest of the time course corresponds to 23 °C reactions.