## SUPPLEMENTARY INFORMATION

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#### Extended Data Figure 2a





# 3 107 108 109 110 Free barco 601 DNA ded

#### Extended Data Figure 2b



#### Extended Data Figure 3b









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Extended Data Figure 8a





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#### Extended Data Figure 8b



Extended Data Figure 8c



#### Extended Data Figure 8d

Data for unmodified nucleosomes were calculated from the same gels as in Figure 4b.

#### 1 1 H2BK108ac H2BK108ac H2BK108ac nucleosomes nucleosomes nucleosomes replicate 1 replicate 2 replicate 3 Time (min.) 1 2.5 5 15 30 60 1 2.5 5 15 30 60 1 2.5 5 15 30 60

### H2BS112GlcNAc H2BS112GlcNAc H2BS112GlcNAc nucleosomes replicate 1 nucleosomes replicate 2 nucleosomes replicate 3 Time (min.) 1 2.5 5 15 30 60 1 2.5 5 15 30 60 1 2.5 5 15 30 60



#### Supplementary Figure 3a



#### Supplementary Figure 5a



#### Supplementary Figure 5b



#### Supplementary Figure 6b

See section of this figure corresponding to 'Extended Data Figure 2a' for uncropped gels of nucleosomes in Supplementary Figure 6b

Supplementary Figure 1 | Gel Source Data. Gel source data from relevant experiments performed in this study are labeled according to their corresponding figure. Pertinent information for interpretation is noted in red, and cropped areas

#### Supplementary Data Figure 3b







consistent with how they were displayed in figures are approximately enclosed in red boxes.





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Supplementary Figure 2 | Characterization of Histones by RP-HPLC/E-SI-MS. (Left) Analytical C-18 RP-HPLC chromatograms (insets; gradients used are individually noted) and corresponding ESI-MS spectra of indicated histones prepared in this study. Corresponding deconvoluted spectra are shown on the right. Lysine acetylation: Kac; lysine mono, di, and trimethylation: Kme, Kme2

and Kme3, respectively; arginine mono, asymmetric and symmetric demethylation: Rme, Rme2a and Rme2s, respectively; serine and tyrosine phosphorylation: Sph and Yph, respectively; serine modified with N-acetylglucosamine: SGIcNAc. TFA = trifluoroacetic acid adduct.



Supplementary Figure 3 | Nucleosome Thermal Mobility Shift Experiments. a, Nucleosomes analyzed directly after assembly by salt gradient dialysis. b, Nucleosomes from a re-analyzed after incubation for 1 hour at either 37 °C or 47 °C. Mobile nucleosomes will shift and coalesce in to the most thermodynamically favorable position along the DNA fragment used in assembly. Experimental replicates are displayed (n = 3). Unmod. = unmodified. All histones are unmodified unless otherwise specified. For gel source data, see Supplementary Fig. 1.

NH<sub>2</sub>-APPG(Nle)RLRSGRSTGAPLTRGS-amide



Supplementary Figure 4 | Characterization of LANA Peptides. Analytical C-18 RP-HPLC chromatograms (insets; gradients used are individually noted)



and corresponding ESI-MS spectra of indicated peptides are shown.

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Supplementary Figure 5 | Characterization of Recombinant BRG1 and CHD4 Chromatin Remodelers. a, Purified remodelers were run on a 4-20% Mini-PROTEAN® TGX™ gel (Bio-Rad) and run for 35 min at 180 V. Proteins were stained with Coomassie. Expected molecular weights: BRG1: 185 kDa,

а

CHD4: 218 kDa. **b**, BRG1 and CHD4 remodelers display ATP-dependent nucleosome remodeling activity as detected by a restriction enzyme accessibility assay. For gel source data, see Supplementary Fig. 1.

\_ ATP + + \_ 1 60 min. 1 60 BRG1 + ATP + 60 1 60 min. 1 CHD4

b



Supplementary Figure 6 | Assessment of Modeling Nucleosome Library Remodeling as a 1- vs. 2-phase Exponential Decay. a, Rates calculated for ACF remodeling against select nucleosome library members by fitting to a 1-phase or 2-phase exponential decay equation. b, Gel images are the same as in Extended Data Fig. 2a. c and d, Curves used to calculate rates as in a for a H2A+H2B+H3+H4KpolyAc modified nucleosome (c) and an unmodified nucleosome (d) (nucleosome 93 and 42 in Supplementary Table 1, respective-





f

ly). e, Mean rate values ( $k_{obs}$  min<sup>-1</sup>) calculated for ACF remodeling against all nucleosome library members fit to either a 1-phase or 2-phase exponential decay equation. Nucleosomes remodeled at a faster rate when fit to a 1-phase vs. a 2-phase exponential decay equation are highlighted in red and labeled. f, Same as in e, but without highlighted nucleosomes. For a, c, and d data are represented as the mean of experimental replicates ± s.e.m. (n = 3). For gel source data, see Supplementary Fig. 1.