CENP-A arrays are more condensed than canonical arrays at low ionic strength.

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Supporting Materials

CENP-A	Total Nucleosomes [N]	Average Nearest Neighbor Distance* [nm]
Tomo 1	2419	15.4±9.5
2	3301	15.7±9.7
3	2880	16.9±6.8
4	2656	19±6.5
ø	**2814**	**16.2±7**
H3.1		
Tomo 1	1984	19.9±7.6
2	2662	21.2±8.3
3	2039	19.9±9.5
4	1971	18.3±9.6
ø	**2164**	**19.8±5.9**

Table S1: Tomogram statistics of the analysis of nucleosome condensation (related to result section: 'Unfolded CENP-A arrays are more condensed than canonical arrays').* Average nearest neighbor distance of total nucleosomes of four tomograms (CENP-A: 11256, H3.1: 8656).

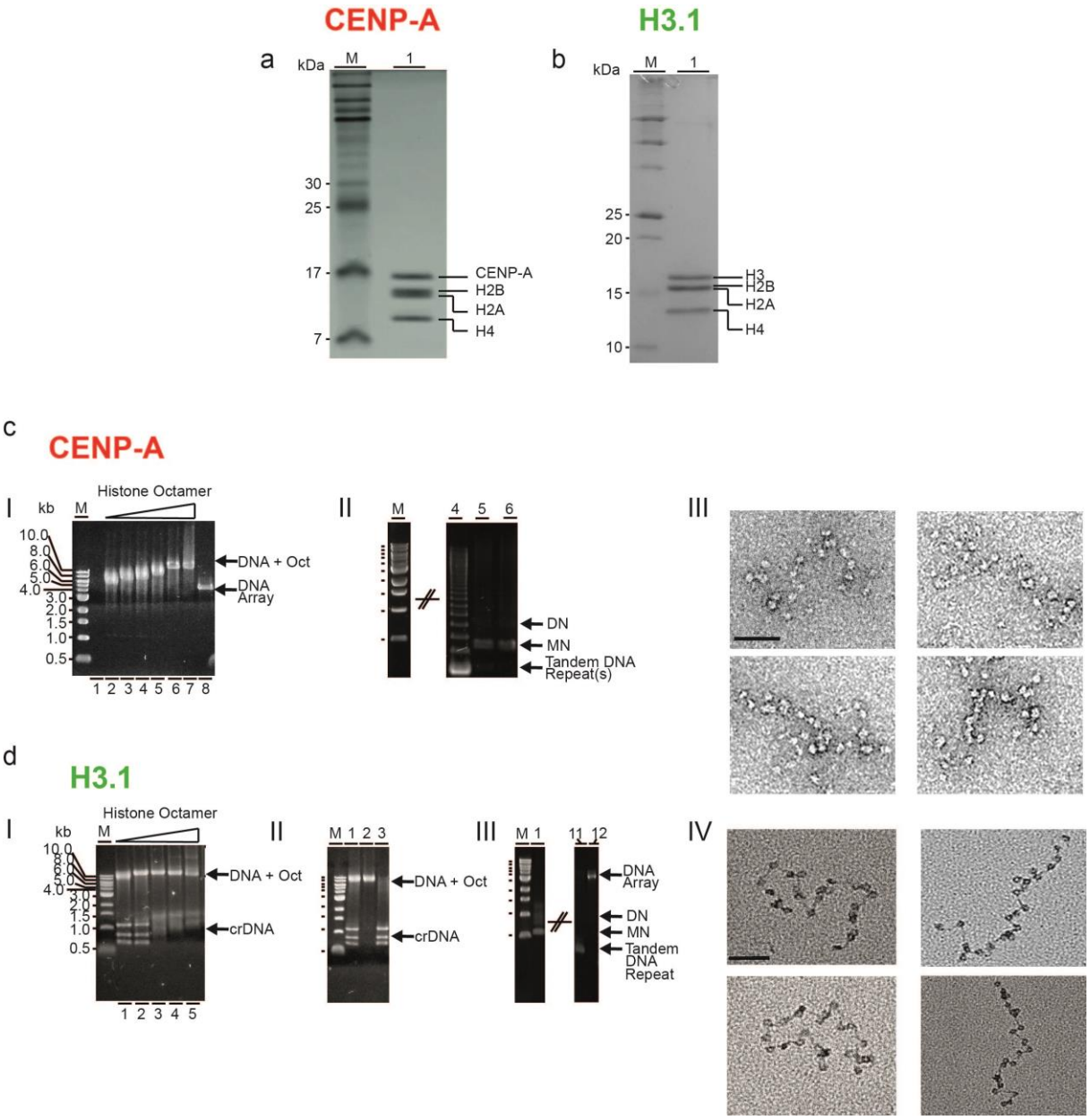

Figure S1: Nucleosome array reconstitution workflow (related to result section: 'Unfolded CENP-A arrays are more condensed than canonical arrays').

(a) 15% SDS-PAGE of CENP-A octamer. (b) 12% SDS-PAGE of canonical H3 octamer. (c1) CENP-A octamer titration series of 24x207bp-mer 601 DNA array. Lane 6 indicates total loading of the 24x207bp-mer 601 DNA array with CENP-A octamer. (c2) EcoRI digestion assay to assess the homogeneous loading of the DNA array (24x207bp-mer 601 DNA array contains behind each tandem repeat an EcoRI cleavage site). (c3) Negative stain EM of reconstituted CENP-A arrays. (d1) Same as in panel (c1) except of using canonical H3 octamer as titration molecule and carrier DNA (pUC19 cleavage side products during the processing of the 24x207bp-mer 601 DNA amplification) to prevent the array from being overloaded. (d2) $MgCl_2$ precipitation assay to remove the carrier DNA from the reconstituted H3 arrays. (d3) Same as in panel (c2). (d4) Positive stain EM of reconstituted canonical H3 arrays. Gelelectrophoretic analysis were carried out with native 0.7% agarose gels in 0.2x TBE Buffer and poststaining with ehtidium bromide. Scale bar is in all panels 50 nm. Discontinuity of gels (if lanes are not illustrated) is marked by double-crossed dashes. DNA markers are the same for each electrophoretic analysis and are indicated by horizontal bars.

Oct = Octamer, crDNA = carrier DNA, DN = Dinucleosomes, MN = Mononucleosomes, M = Marker, kb = kilobases.

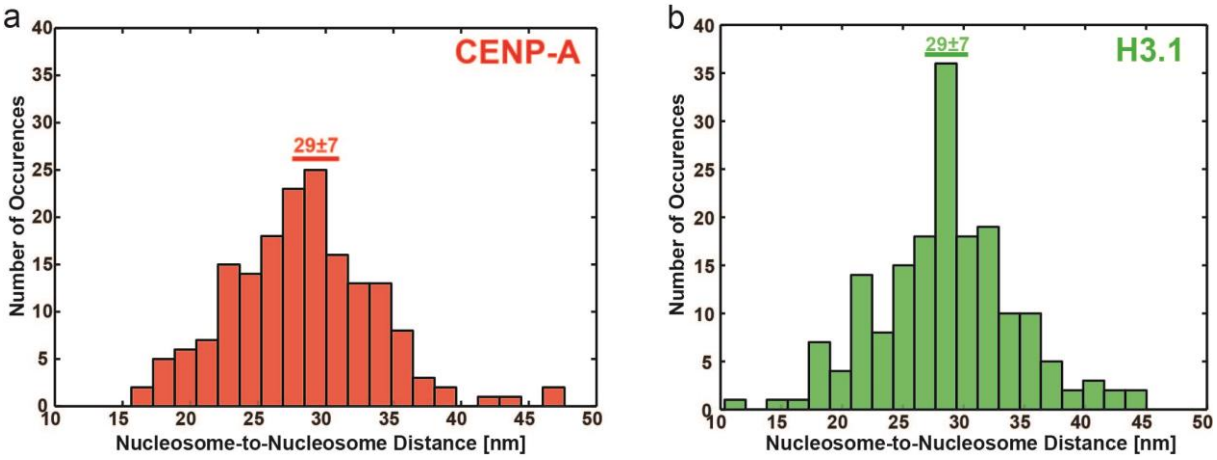

Figure S2: Histograms of nucleosome-to-nucleosome distance (NN1) (related to result section: 'Unfolded CENP-A arrays are more condensed than canonical arrays').

(a) Nucleosome-to-nucleosome distance of CENP-A trinucleosomes. (b) Nucleosome-to-nucleosome distance of canonical H3 trinucleosomes. Sample size is 175 for CENP-A and 176 for canonical H3 trinucleosomes (the same trinucleosome datasets that have been used for determining the omega angle).

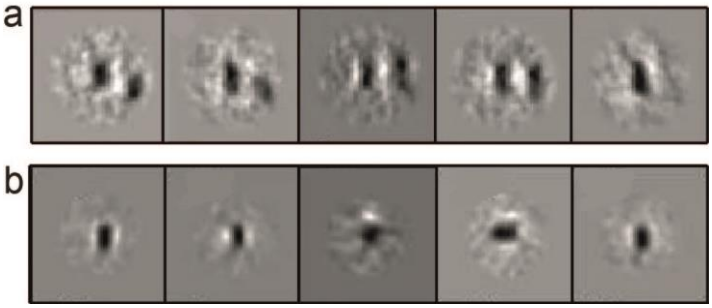

Figure S3: PCA class averages of CENP-A and canonical H3 nucleosomes (related to result section: 'Unfolded CENP-A arrays exhibit more juxtaposed nucleosomes').

(a) Gallery view of class averages of CENP-A nucleosomes upon classification of 1810 CENP-A nucleosomes. The first four class averages show the "stacking motif" and the last class average resembles mononucleosomes that do not reveal neighboring nucleosomes with distinct orientation to each other. (b) Gallery view of class averages of canonical H3 nucleosomes upon classification of 1286 canonical H3 nucleosomes. All five class averages resemble mononucleosomes with various orientations. Both panels represent five class averages out of hundred class averages in total.

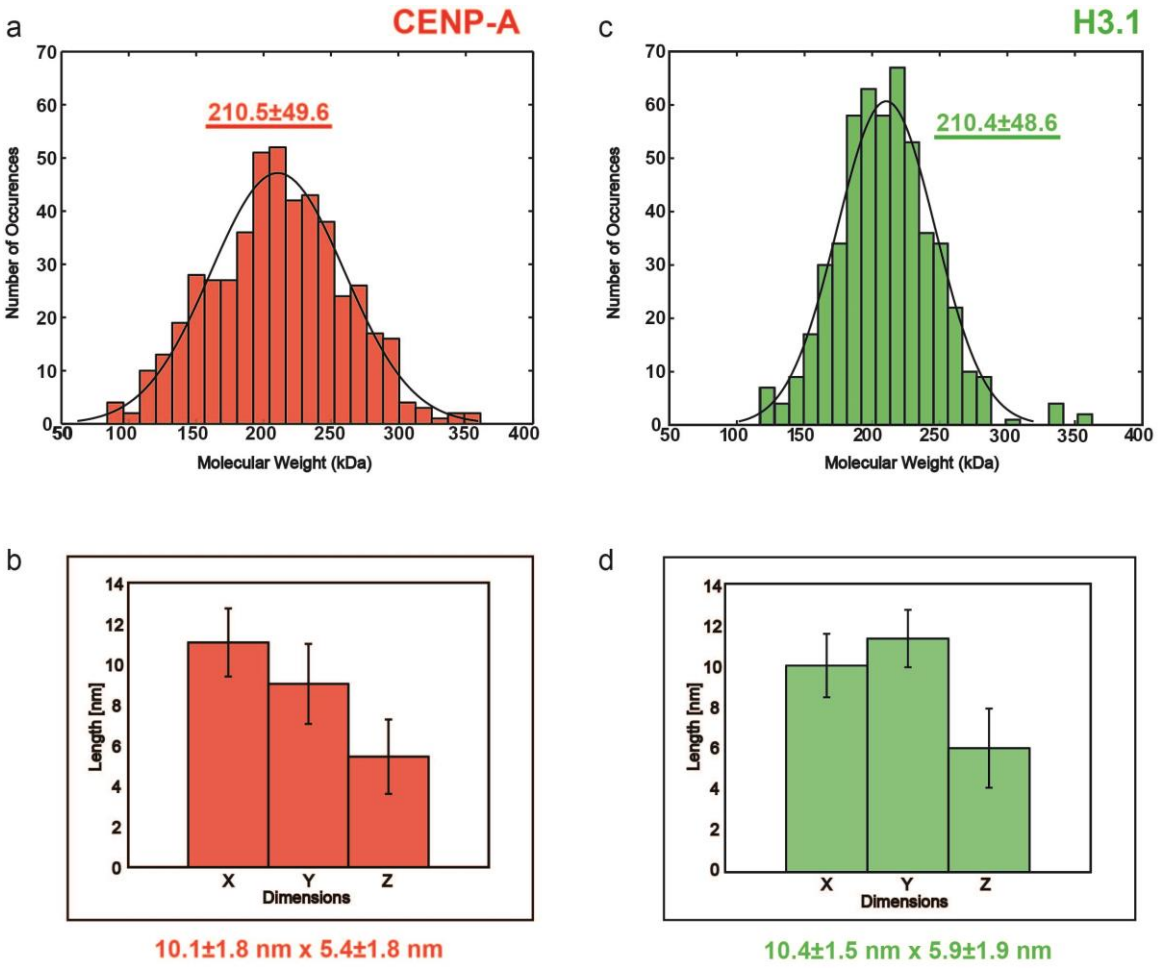

Figure S4: Dimensions and molecular weight distribution of CENP-A and canonical H3 nucleosomes (related to result section: 'Both CENP-A and H3 arrays have a higher ratio of crossed vs. open DNA entry/exit sites').

(a) Histogram of the molecular weight distribution of segmented CENP-A and (c) canonical H3 nucleosomes with a Gaussian distribution curve superimposed in black. (b) Bar chart of the measured dimensions of segmented CENP-A and (d) canonical H3 nucleosomes. The average diameter (x,y-value) and height (z-value) are given below the bar chart. The CENP-A dataset (Fig 1a, Table 1 *Tomo3*) corresponds to 487 and H3 dataset (Fig 1b, Table 1 *Tomo4*) to 518 nucleosomes. The broadening of the molecular weight and size distribution in comparison to canonical H3 nucleosomes is attributed to the compactness effect of CENP-A nucleosomes since technically the nucleosomes are measured in a spherical subvolume which corresponds to ~2 times the standard dimensions (10x5 nm) of a nucleosome so that neighboring nucleosomes with a N+2 distance (NN2, Fig 1b, lower inset) are probably also detected.

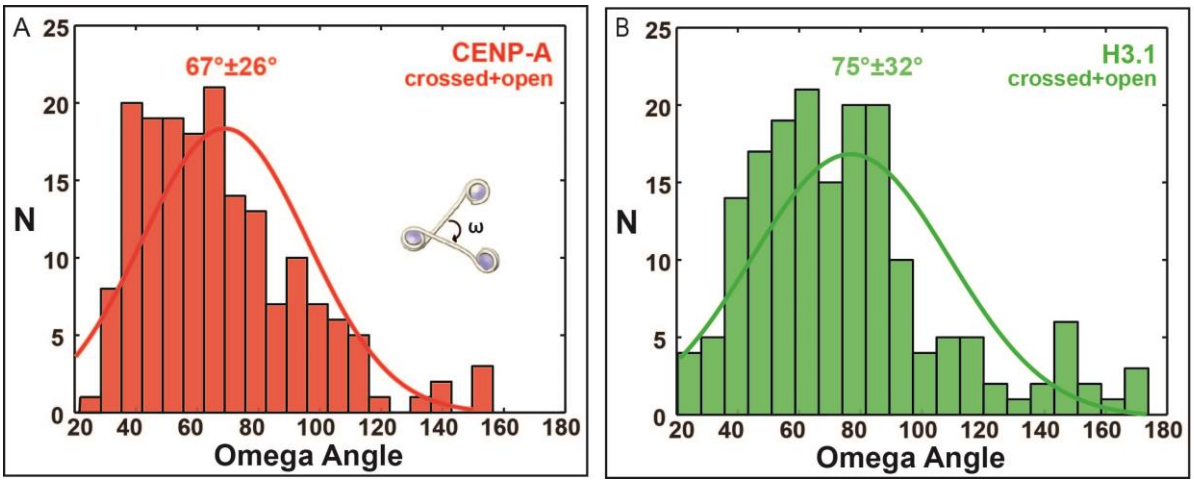

Figure S5: Omega angle distribution among CENP-A and canonical H3 nucleosomes with crossed and open DNA entry/exit site (related to result section: 'The DNA entry/exit angle is 8 degrees narrower in CENP-A vs. H3 arrays').

(a) Histogram of the omega angle distribution for the crossed/open DNA entry/exit site of CENP-A nucleosomes. (b) As in (a) but for the canonical H3 nucleosomes. When both datasets for crossed/open DNA entry/exit site are pooled, the mean omega angle is 75° (SD 32°) for canonical H3 and 67° (SD 26°) for CENP-A nucleosomes. The mean value and standard deviation is depicted above the histogram peak in all panels. In each panel the curve fitting is superimposed in its corresponding colour.