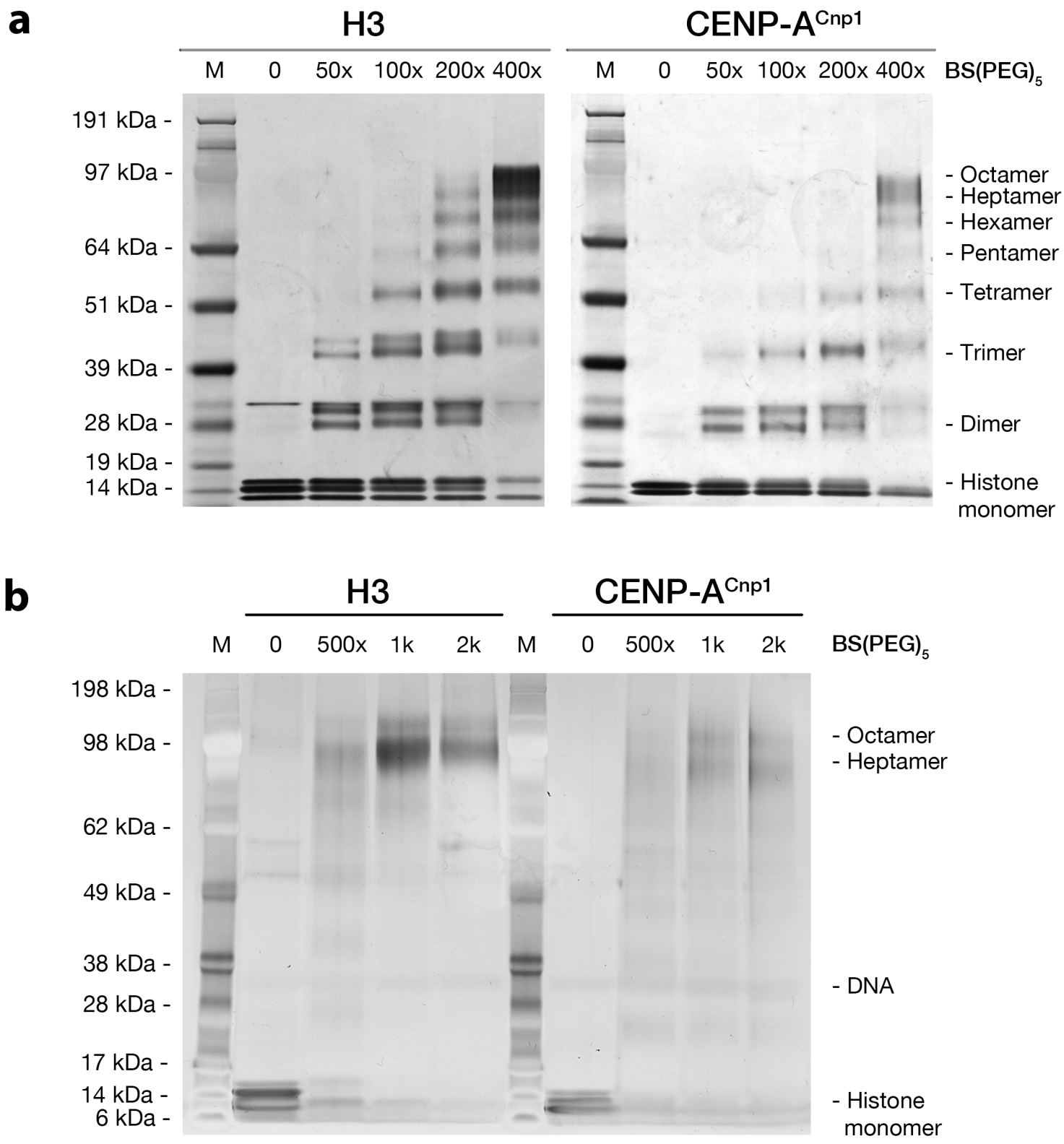
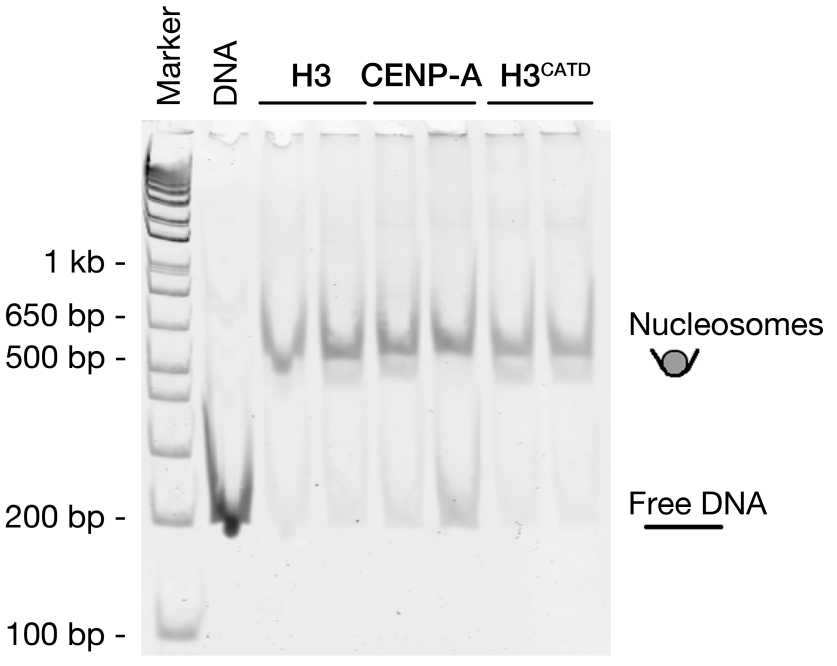
CENP-A confers a reduction in height on octameric nucleosomes

Matthew D. D. Miell, Colin J. Fuller, Annika Guse, Helena M. Barysz, Andrew Downes, Tom Owen-Hughes, Juri Rappsilber, Aaron F. Straight, and Robin C. Allshire

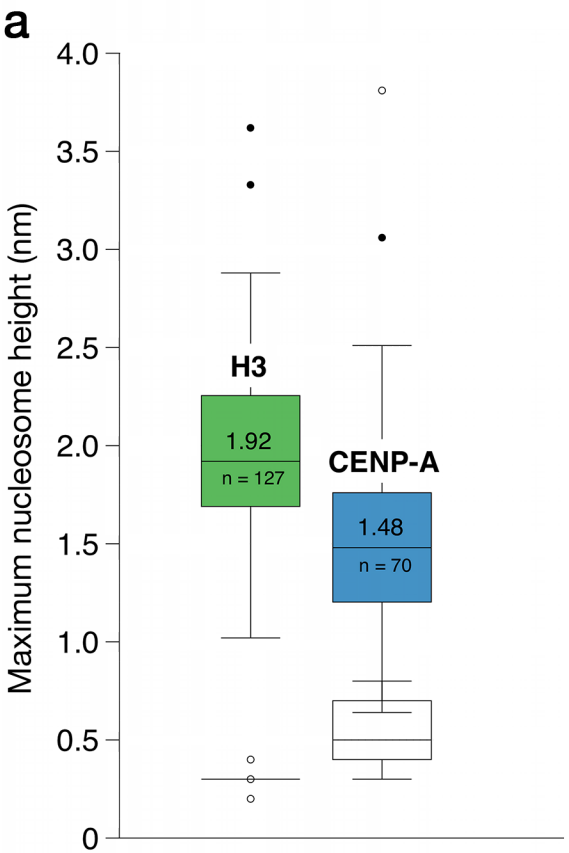
Supplementary information

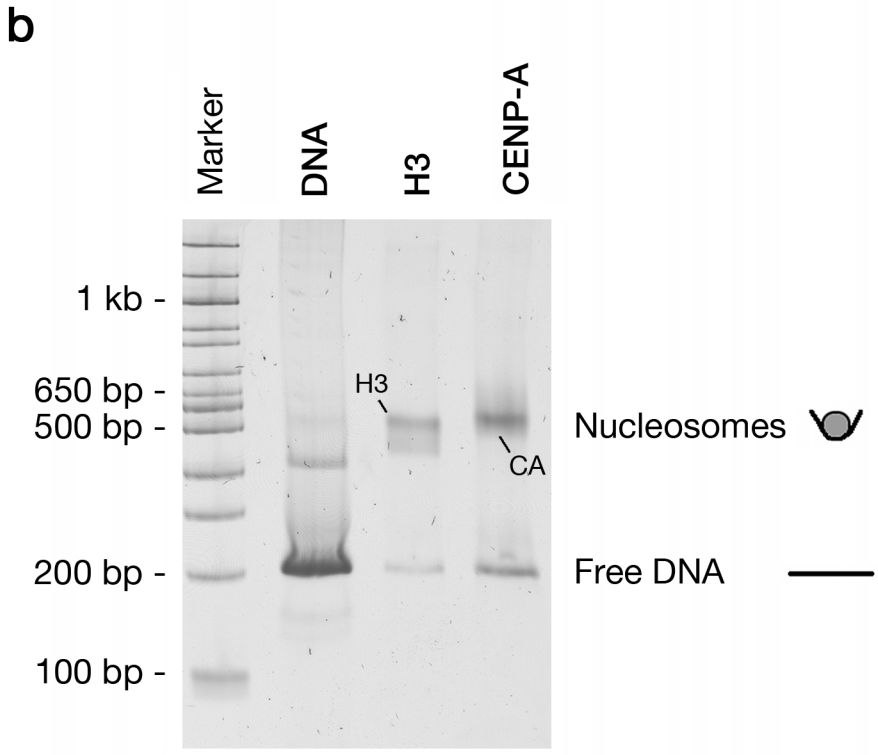


Supplementary Figure 1
***In vitro* assembled *S. pombe* H3 and CENP-A^{Cnp1} nucleosomes behave as octamers**
(a) Silver stained SDS-PAGE gel of recombinant *S. pombe* nucleosomes following fixation with BS(PEG)$_5$. Numbers above each lane show the molar excess of BS(PEG)$_5$ relative to the number of nucleosomes.
(b) Silver stained SDS-PAGE gel of recombinant *S. pombe* H3 and CENP-A^{Cnp1} nucleosome arrays following fixation with up to a five-fold increase in the concentration of BS(PEG)$_5$ required to cross-link octameric complexes.



Supplementary Figure 2
***In vitro* assembled CENP-A and H3^{CATD} nucleosomes migrate similarly to octameric H3 nucleosomes**
EtBr stained native PAGE gel of *in vitro* assembled human H3, CENP-A and chimeric H3^{CATD} nucleosome arrays, digested to mono-nucleosomes with AvaI.





c

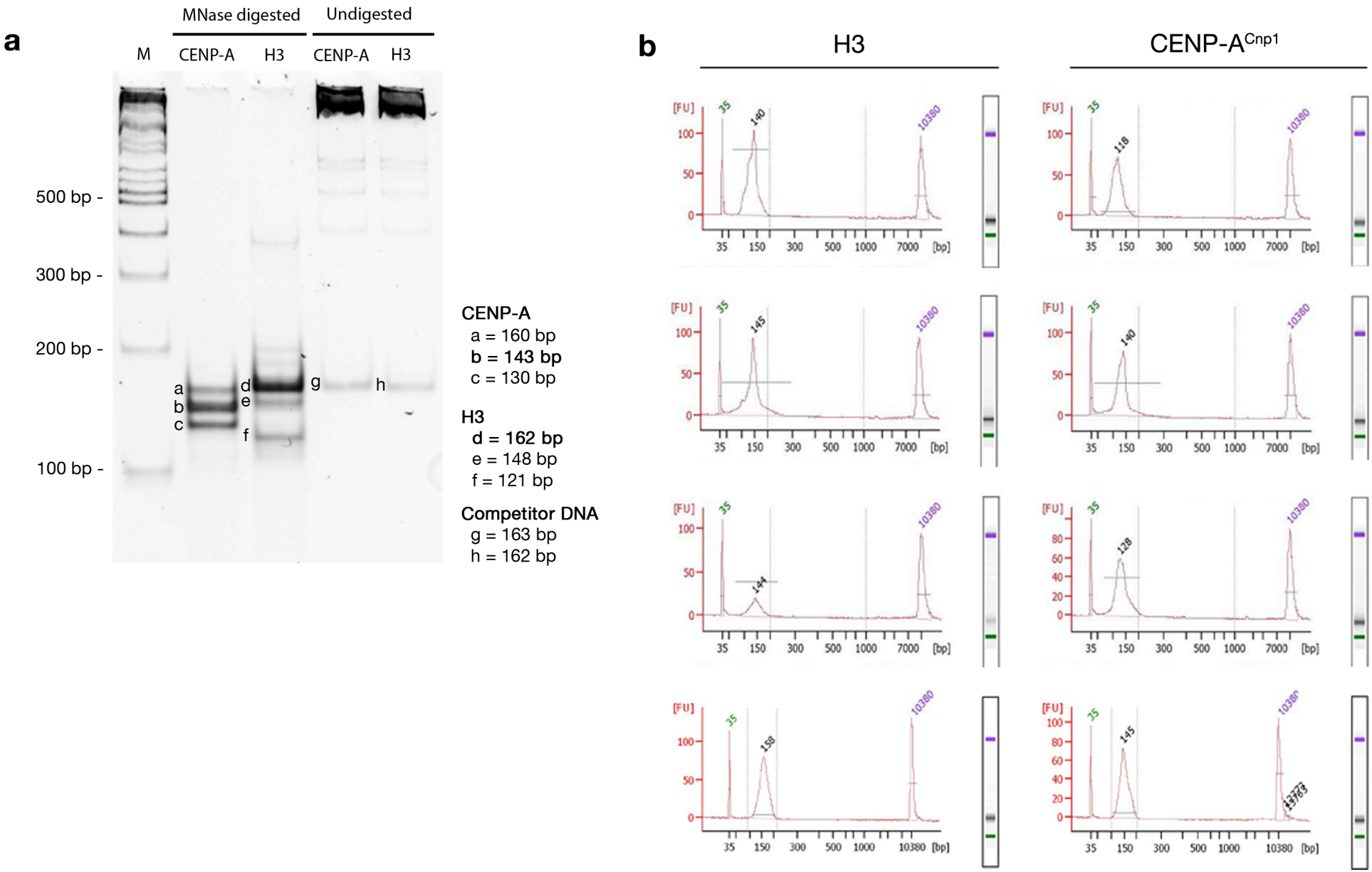
H3

Description	Peptides
Histone H2A	9
Histone H2B	10
Histone H3	15
Histone H4	18

CENP-A

Description	Peptides
Histone H2A	3
Histone H2B	7
CENP-A	7
Histone H4	18

Supplementary Figure 3
Mass spectrometry analysis of recombinant nuclesomes
a) Distributions of peak nucleosome heights measured using AFM for a singe batch of *in vitro* assembled recombinant human H3 and CENP-A nucleosomes.
b) Sybr Green stained native PAGE gel of the same nucleosome arrays inmaged in a). Arrays were Ava1-digested to release mono-nucleosomes from the arrays prior to native PAGE analysis. This is the same gel shown in Fig. 1b. Labelled nucleosome bands were cut from the gel for analysis by mass spectrometry.
c) Protein components (contaminants subtracted) of the labelled nucleosome bands in a) identified by mass spectrometry.



Supplementary Figure 4

***In vitro* assembled human and *S. pombe* CENP-A nucleosomes protect less DNA than H3 nucleosomes**

a) SyBr gold stained native PAGE gel of recombinant CENP-A or H3 nucleosome arrays following digestion with micrococcal nuclease (MNase) and subsequent phenol:chloroform extraction of the DNA. The length of DNA in each of the labelled bands was calculated by comparison with the migration of the five smallest bands (100-500 bp) of the ladder. Bands g and h represent the 160 bp competitor DNA included to allow proper reconsitution of nucleosomes on the arrays.
b) Arrays of recombinant *S. pombe* H3 and CENP-A^{Cnp1} nucleosomes were digested with MNase, phenol/chloroform extracted, and run on a high sensitivity Bioanalyser DNA chip to determine the protected length of DNA. Data are shown here for 4 replicas for each nucleosome type.