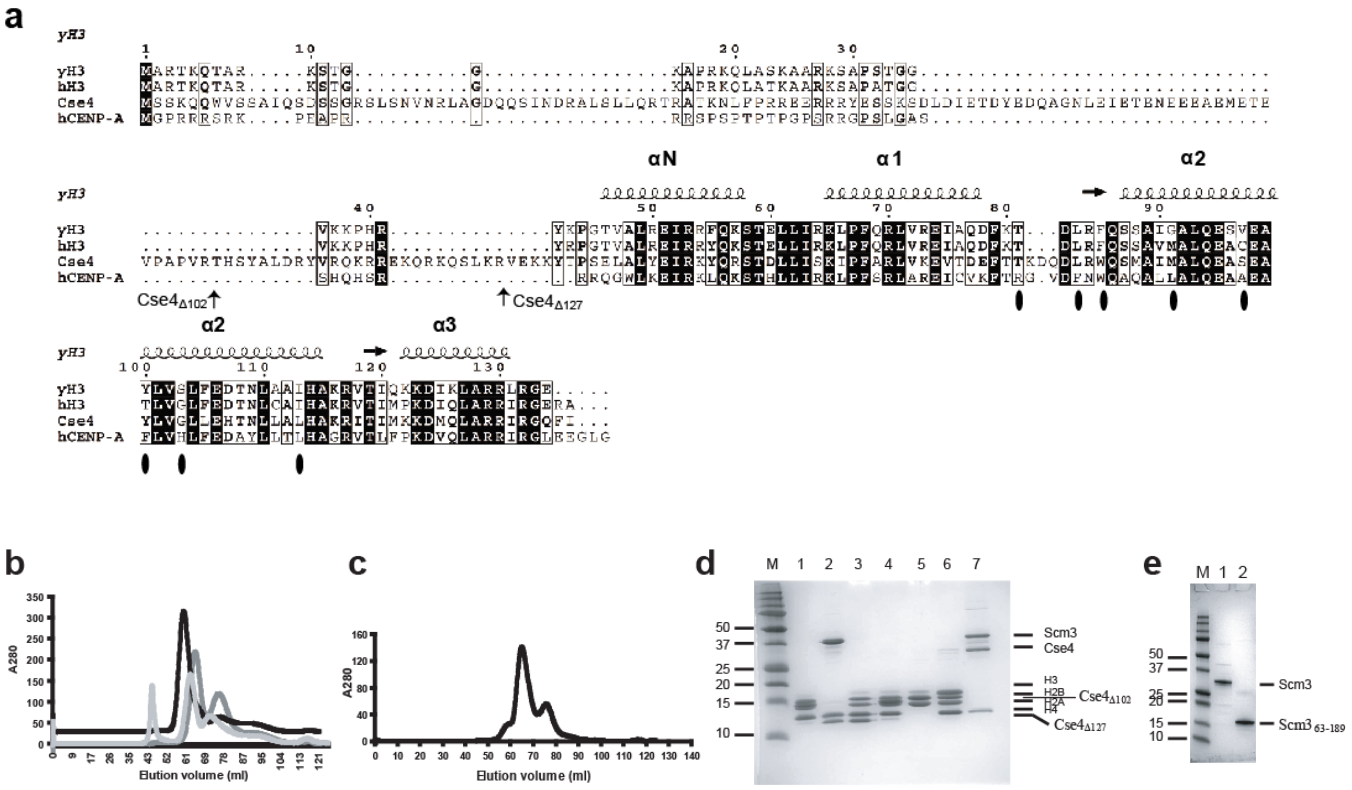
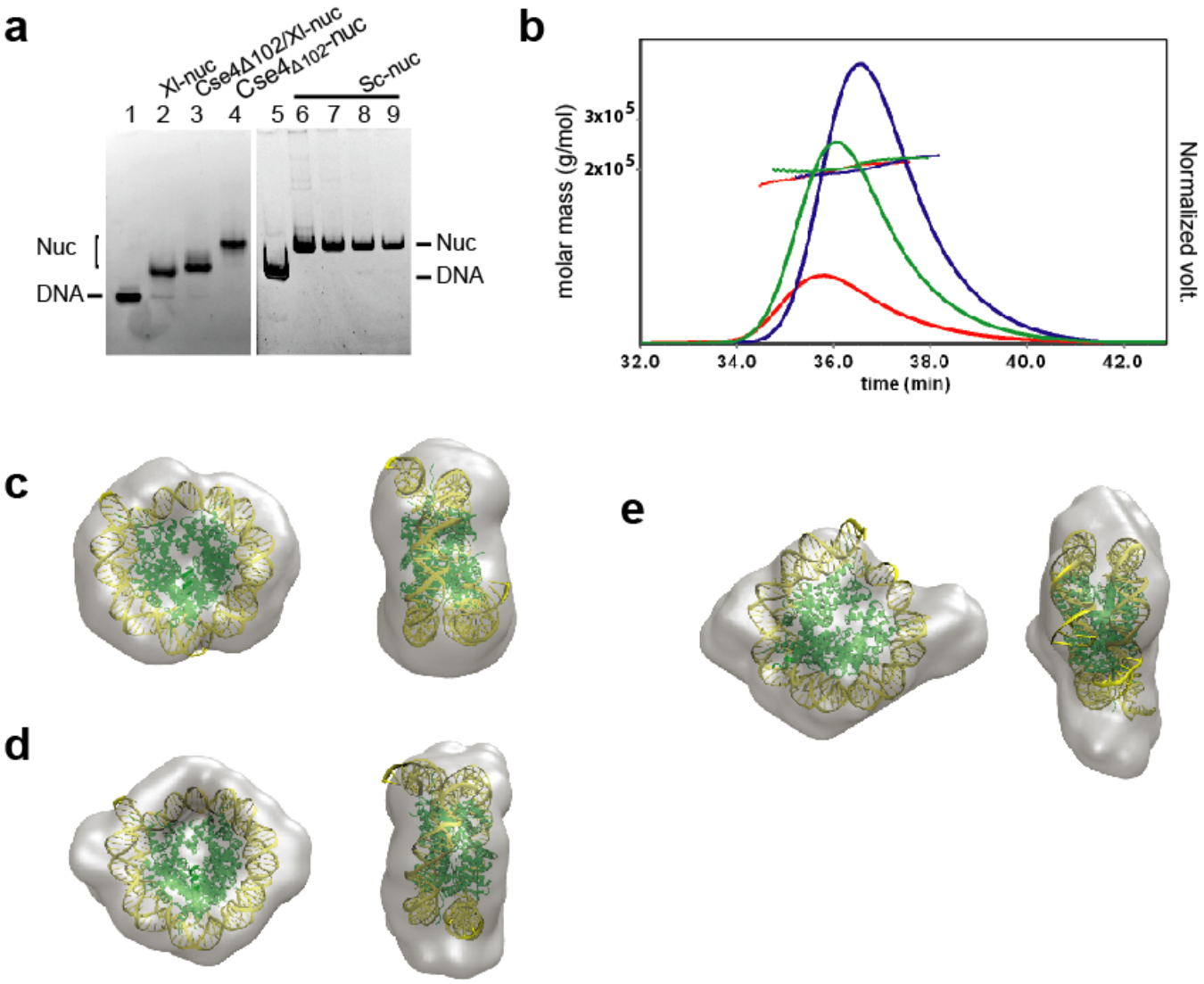
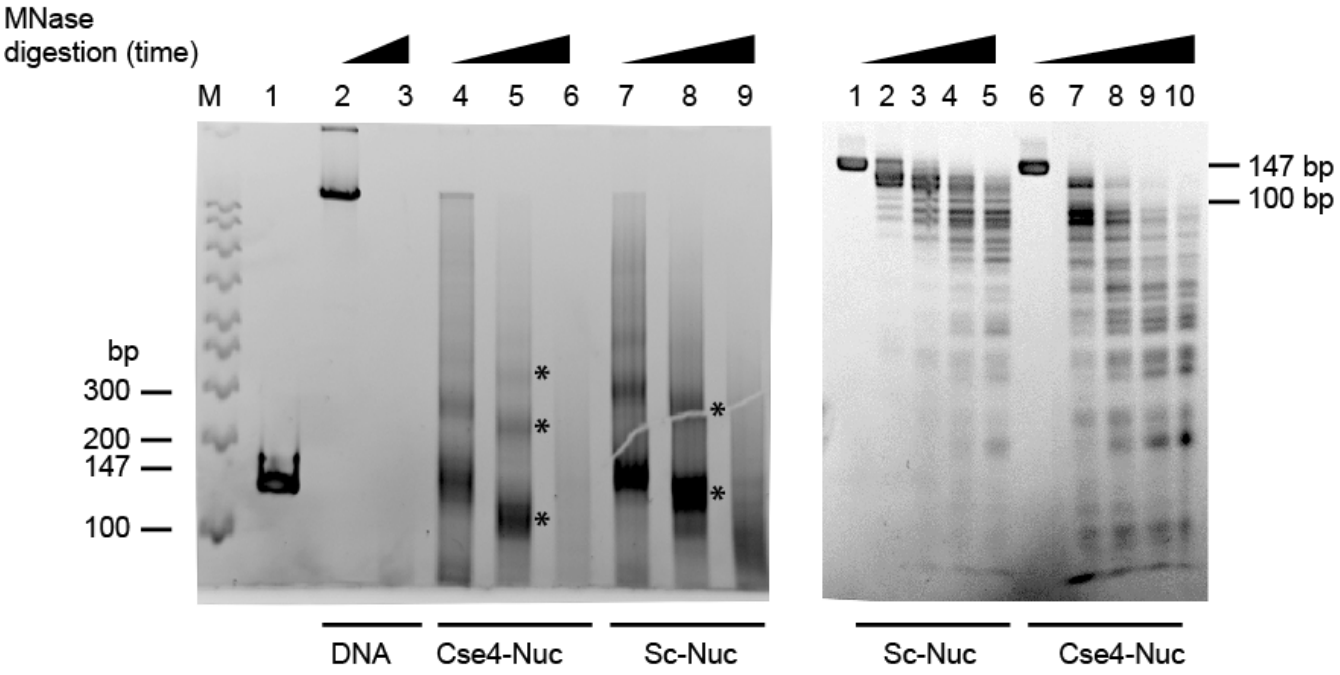
Supplementary Information



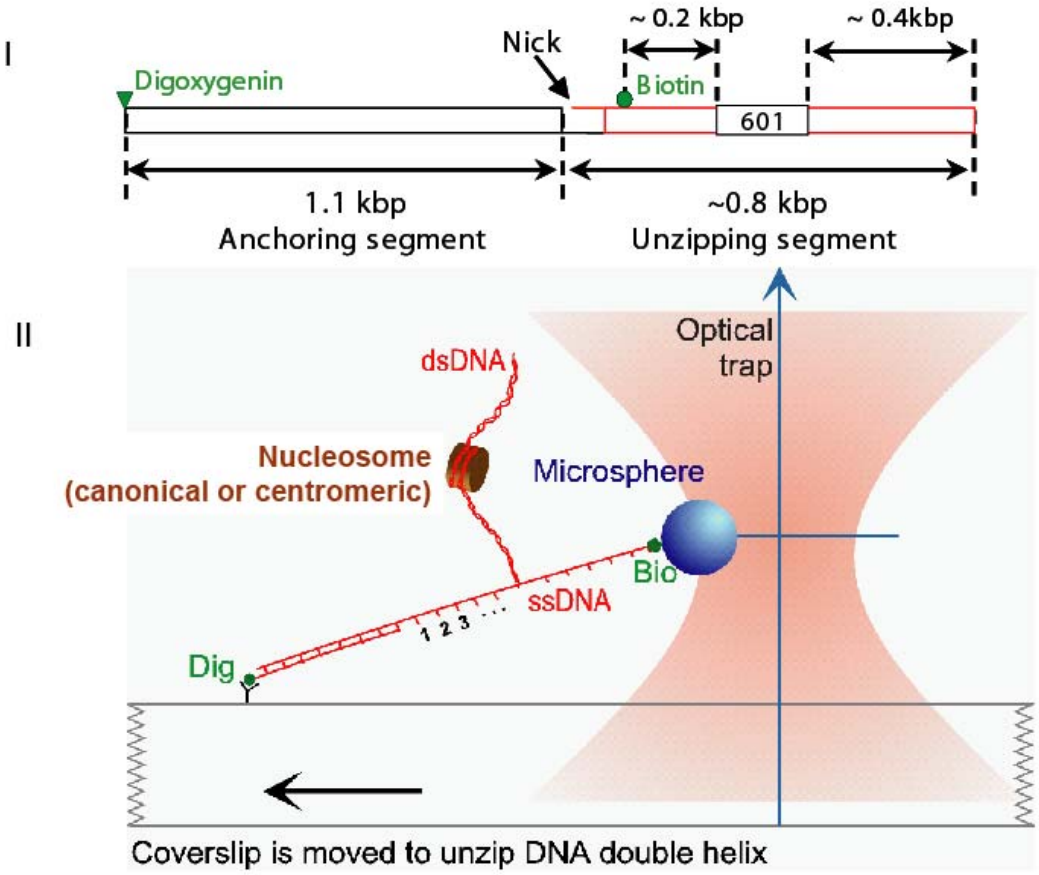
Supplementary Figure S1. **Sequence alignment and refolding of Cse4- and Sc-octamer**. **(a)** Sequence alignment of yeast H3, human H3, Cse4, and CENP-A. The sites of N-terminal truncations of Cse4 ($Cse4_{\Delta 102}$ and $Cse4_{\Delta 127}$) are shown. Amino acid residues proposed to be responsible for the unique features of the $(CENP\text{-}A/CENP\text{-}A)_2$ tetramer [5] are shown by black ellipsoids. **(b)** Histone octamers were refolded from H2A, H2B, H4 and H3 (or Cse4, $Cse4_{\Delta 102}$), and purified by gel filtration. Cse4-octamer (shown in black) elutes at 60 ml, $Cse4_{102}$-octamer (in dark grey) elutes at 65 ml and Sc-octamer (in light grey) elutes at 63 ml. **(c)** Refolding and purification of Cse4/H4/Scm3 complex, as in (b). The complex elutes at 65 ml. **(d)** SDS PAGE analysis of the final concentrated products. Lanes 1 and 4: $Cse4_{\Delta 102}$-octamer; lane 2: $Cse4_{\Delta 127}$ /H4/Scm3; lane 3: $Cse4_{\Delta 127}$-octamer; lane 5: Sc-H2A/H2B dimer; lane 6: Sc-octamer; lane 7: Cse4/H4/Scm3 complex. **(e)** SDS PAGE of refolded full length Scm3 (lane 1) and $Scm3_{63\text{-}189}$ (lane 2).



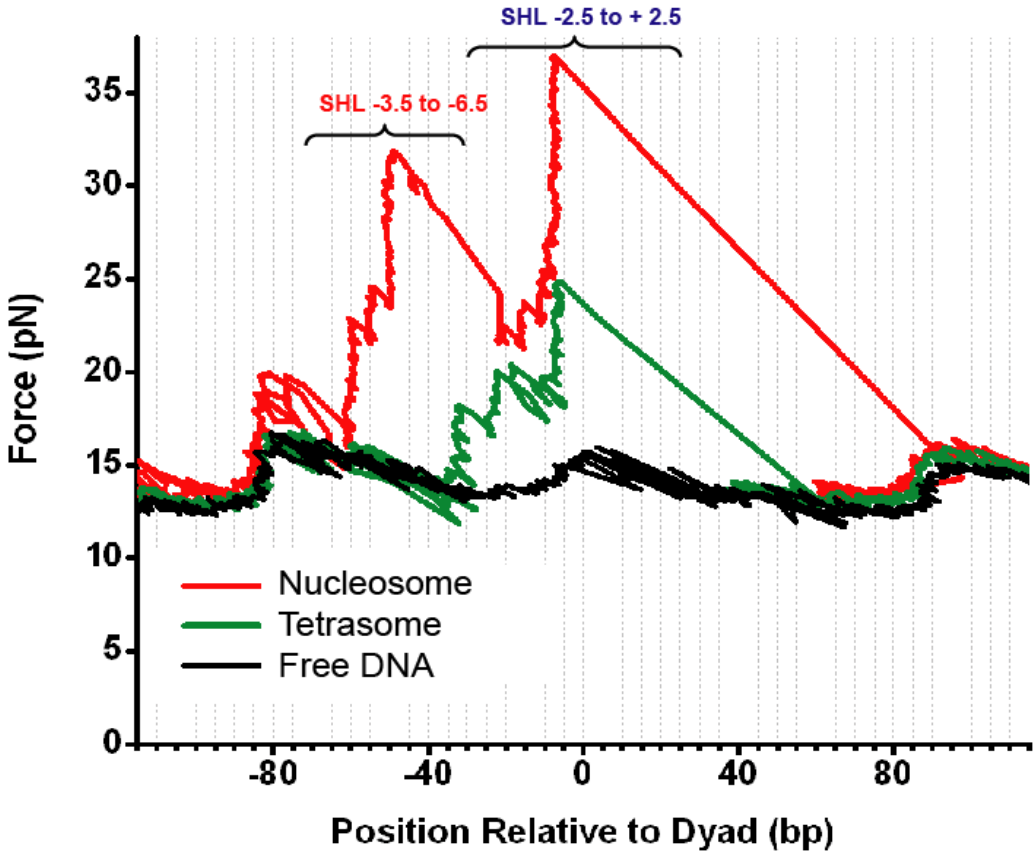
Supplementary Figure S2. Small angle X-ray scattering analysis demonstrates that Cse4 nucleosomes have an extended morphology. (a) Nucleosomes samples used for SAXS analysis, analyzed by 5% native PAGE and visualized by ethidium bromide staining. Lanes 1 and 5: DNA, lane 2: Xl-nucleosome, lane 3: Cse4$_{\Delta102}$/Xl-Nuc: nucleosome reconstituted with Cse4$_{\Delta102}$ and *Xenopus laevis* H2A, H2B and H4; lane 4: Cse4$_{\Delta102}$-nucleosome; lanes 6-9: Sc-nucleosomes, different concentrations loaded. **(b)** Monodispersity and molecular weight of the nucleosomes samples in (a) were assayed by SEC-MALS. X-axis shows the elution time from size-exclusion column; Left Y-axes: molecular mass in g/moles calculated from MALS; Right Y-axis: normalized volt. The molecular weight calculated from SEC-MALS were 206.3 (± 0.8%), 204.5 (±0.7%) and 198.3 (0.8%) kDa for Sc-nuc (green), Xl-nuc (blue) and Cse4$_{102}$-nucleosome (red), respectively**(c-e)** *Ab initio* envelopes calculated from the experimental scattering curves using DAMMIN [47]. Molecular envelopes were converted with Situs [49] and displayed with VMD [50].The nucleosome structural model (1kx5; reference 27) was fitted to the SAXS envelope with Colores. The location of the nucleosomal dyad axis has not been optimized, nucleosome models are shown for size comparison only. (**c**) Xl-nucleosome; (**d**) Sc-nucleosome; (**e**) Cse4$_{\Delta102}$-nucleosome. The envelopes are displayed in grey; the nucleosome crystal structure is shown in yellow (DNA) and green (histones).



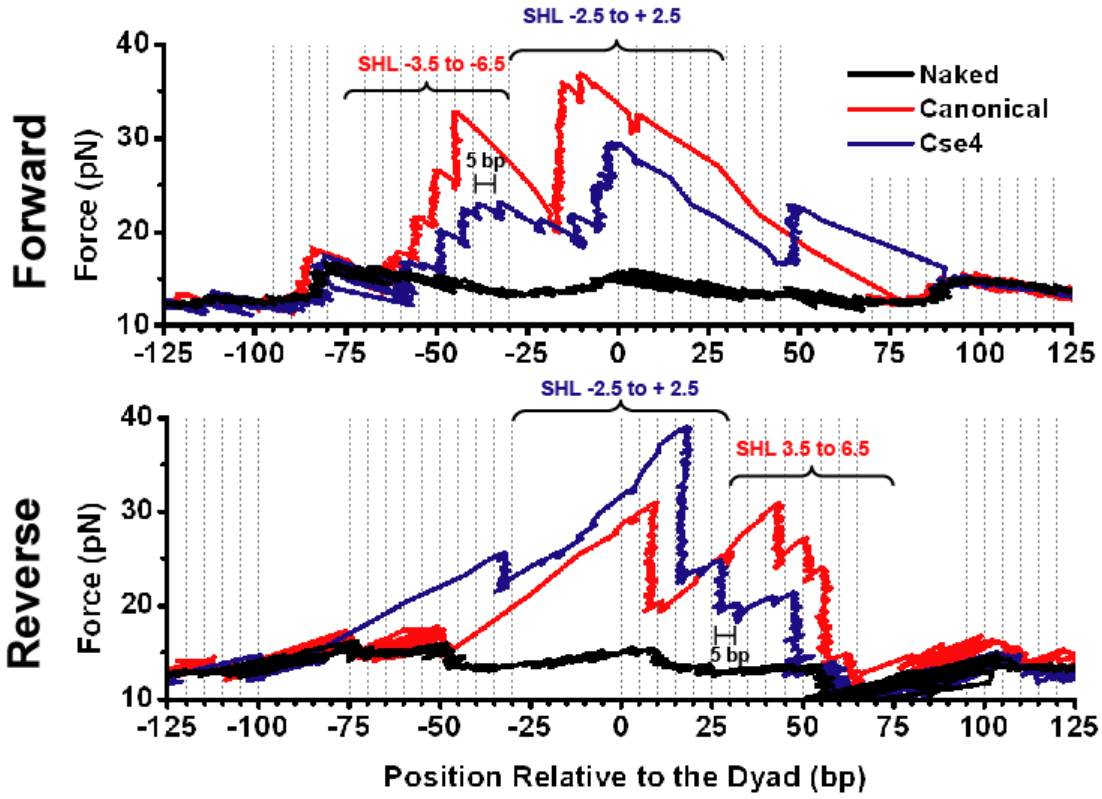
Supplementary Figure S3. The peripheral DNA regions are less well organized in Cse4-nucleosomes. MNase digestion mapping shows that Cse4 nucleosome organizes less than 147 bp DNA. Left panel: Nucleosomes were reconstituted onto linearized pBR322 plasmid DNA by yNap1, and digested by micrococcal nuclease (MNase). Extracted DNA was analyzed by 5% native PAGE. M: DNA marker; lane1: 147 bp DNA; lane 2: uncut DNA; lane 3: DNA digested for 60 seconds; lanes 4-6: Cse4 nucleosomal array digested for 30, 60 and 300 seconds; lanes 7-9: Sc-nucleosomal arrays digested similarly. Protected DNA bands after 60 seconds digestion are indicated with an asterisk. Right panel: Nucleosomes were reconstituted on 147 bp 601 DNA by salt dilution, and digested with MNase for 1, 2, 4 and 10 minutes (lanes 2-5 and 7-10). The DNA was extracted and analyzed on 6% native PAGE. Lanes 1 and 6: DNA from uncut nucleosome.



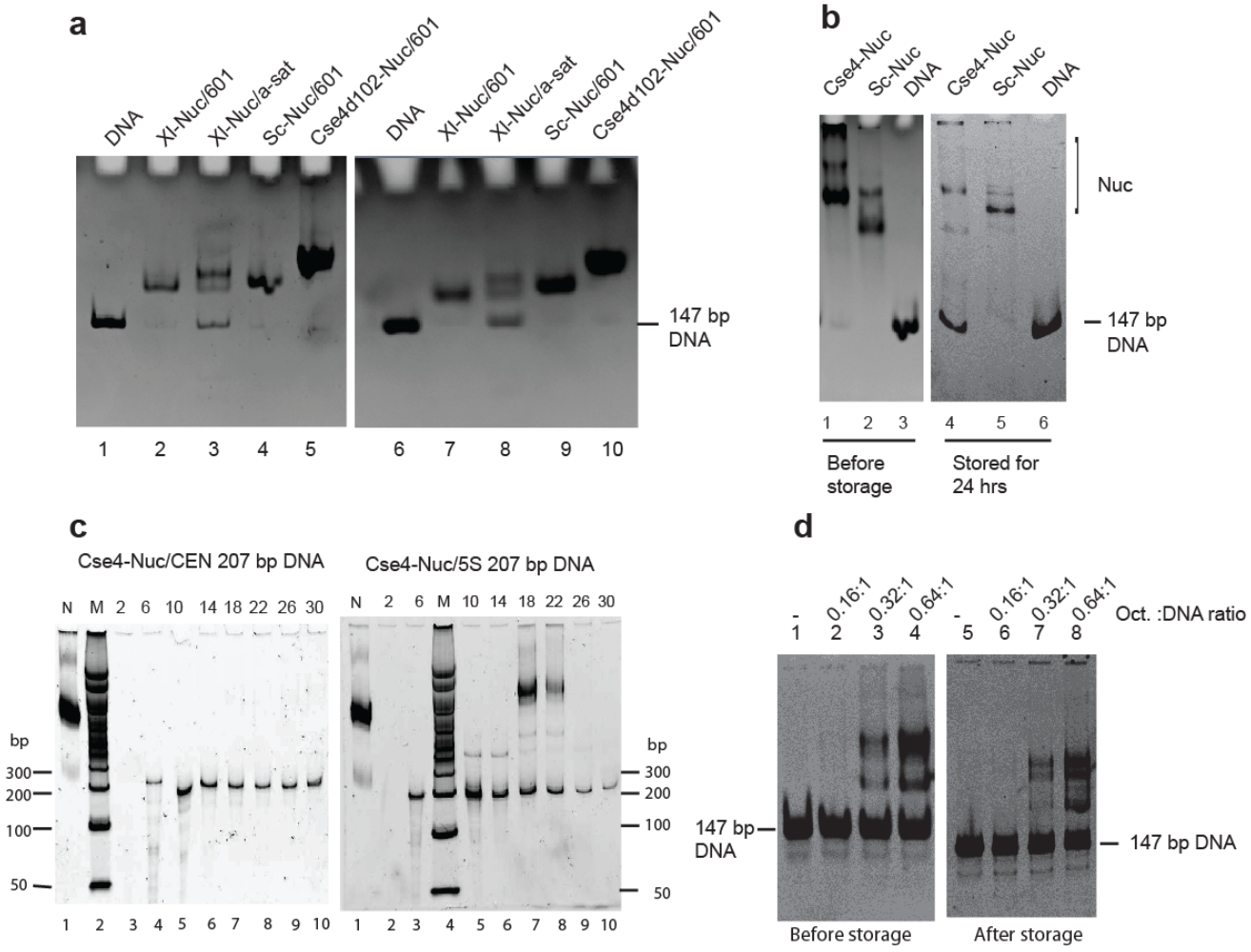
Supplementary Figure S4. The peripheral DNA regions are less well organized in Cse4-nucleosomes. Experimental configuration (adapted from [34]). I: The nucleosome unzipping template for single molecule experiments consist of two segments, separated by a nick in one DNA strand: (1) digoxygenin-labeled anchoring segment always remains double stranded and (2) biotin-labeled unzipping segment has its two DNA strands separated (unzipped) during experiments. The unzipping segment contained a single strong 601 nucleosome positioning sequence (601). II: The nucleosomal template was suspended between the glass coverslip surface and a microsphere via a digoxigenin-antidigoxigenin linkage at the coverslip and a biotin-streptavidin linkage at the microsphere. An optical trap was used to apply a force necessary to unzip through the DNA and nucleosome as the coverslip was moved away from the trapped microsphere.



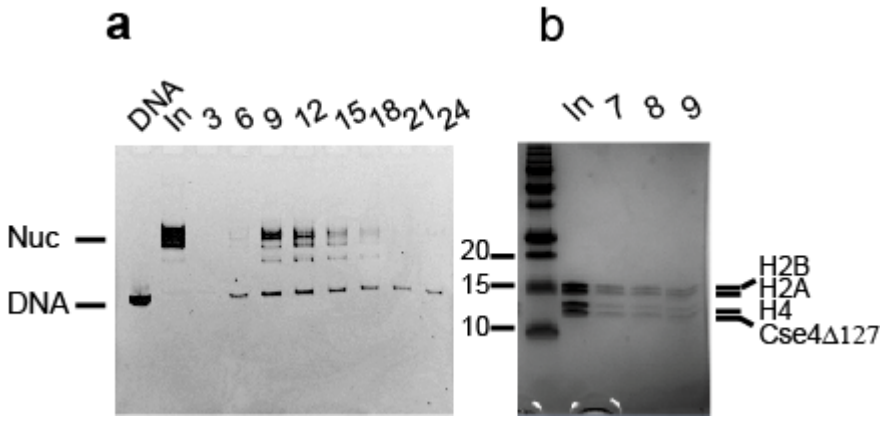
Supplementary Figure S5. The peripheral DNA regions are less well organized in Cse4-nucleosomes. Comparison of force signatures for a nucleosome and a tetrasome. Unzipping signatures of nucleosomes (red) and tetrasomes (green) assembled from recombinant *Xenopus laevis* histones. Nucleosome signatures exhibit two regions of interactions which correlate well with locations along the DNA that are expected to interact with H3/H4 tetramer ("dyad region") and H2A/B dimer ("off-dyad region"). Tetrasome signatures exhibit only a single region of interactions, which substantially overlaps the dyad region identified in the nucleosome signature and also correlates well with expected H3/H4 tetramer interactions. This signature is consistent with our previous results obtained on tetrasomes assembled with HeLa histones [34].



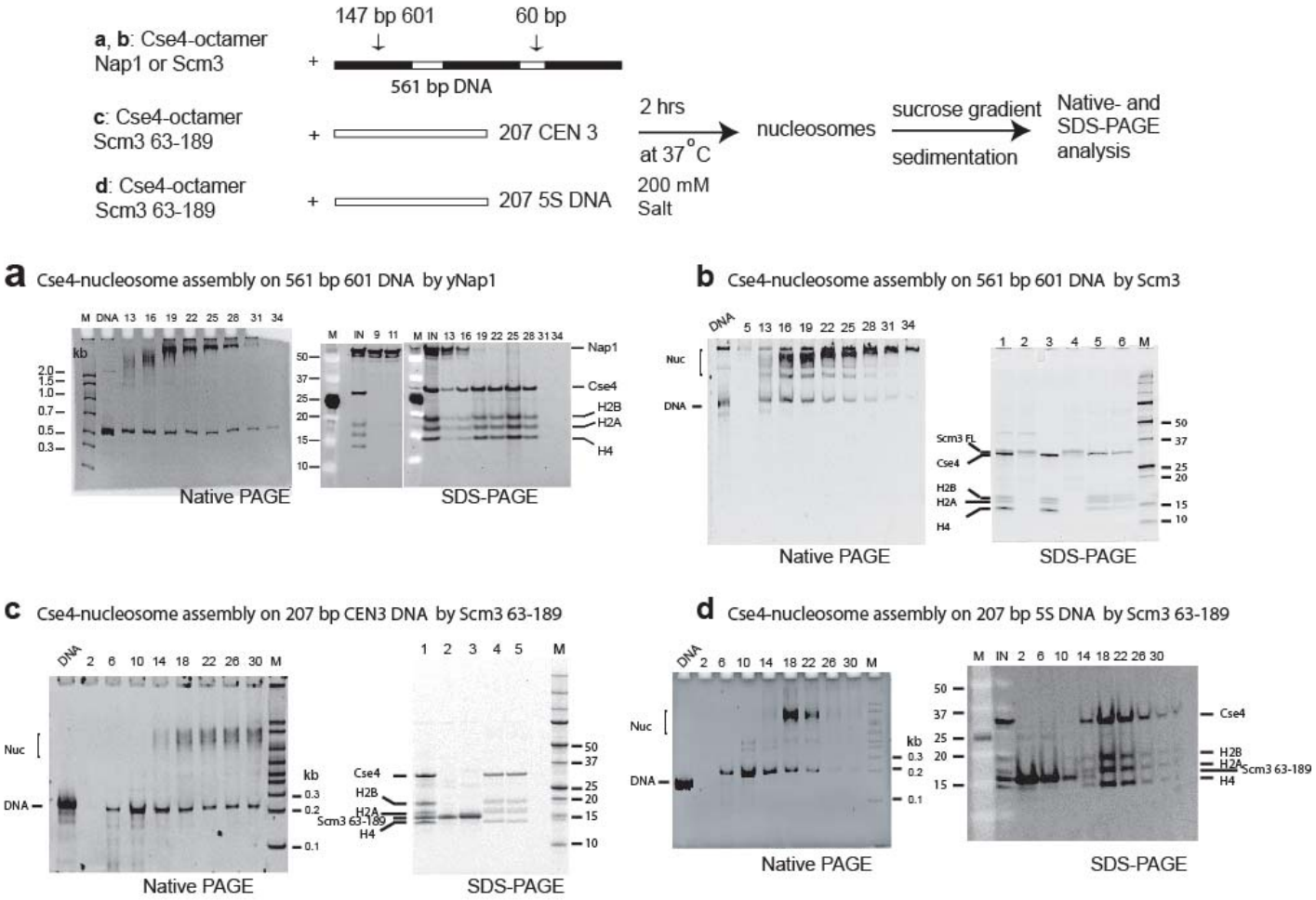
Supplementary Figure S6. The peripheral DNA regions are less well organized in Cse4-nucleosomes Comparison of force signatures for Sc-nucleosome and Cse4-nucleosome. Single representative traces are shown for forward (upper panel) and reverse (lower panel) unzipping of a Sc-nucleosome (red) and Cse4-nucleosome (blue). The baseline unzipping signature of naked DNA (black) is also shown. Data obtained under the same conditions as in Figure 3. Regions are defined as in Figure 3. Three distinct regions of interaction were observed for both nucleosomes. The distinct 5 bp periodicity within each region was also apparent, as described previously for HeLa nucleosomes [34]. This suggests that, overall, the structure of histone-DNA interactions are conserved between these two types of nucleosomes. However, for a centromeric nucleosome, the outer regions are disrupted at lower forces compared to that of Sc-nucleosome, indicating that its outer turn DNA is more weakly bound.



Supplementary Figure S7. Cse4-nucleosomes assembled on centromeric DNA are unstable. (**a**). Nucleosomes were reconstituted on 147 bp 601 or α-satellite (a-sat) DNA by salt deposition and analyzed on 5% native PAGE. Lane 1-5: before storage; 6-10: same samples after storage for a week at 4° C. The type of DNA and octamer used were indicated above the gel. (**b**). Cse4-nucleosomes were reconstituted on 147 bp CEN3 DNA by salt deposition and analyzed on 5% native PAGE before (lane 1-3) and after storage at 4°C for 24 hrs (lane 4-6). Note that the data in lane 1-3 is the same as the data in Figure 1a (lane 7-9). The storage resulted in disassembly of the nucleosome band and in the enrichment of the free DNA. (**c**). Cse4-nucleosomes were assembled on 207 bp CEN3 and 5S DNA, and fractionated by sucrose gradient sedimentation. The same samples in Supplementary Figures S9c and d were analyzed on 5% native PAGE after 24 hrs storage at 4°C. Left panel: Cse4-nucleosomes assembled on 207 bp CEN3 were disassembled after 24 hrs; Right panel: Cse4-nucleosomes on 207 bp 5S DNA were stable. M- DNA marker; N- Nucleosome assembled on 207 bp 601 DNA used as loading control. (**d**). Cse4-nucleosomes were reconstituted on 147 bp DNA (from pBR322 plasmid, see Supplementary Table S1 for the DNA sequence) and the samples were analyzed on 5% native PAGE before and after storage at 4°C for 24 hrs.



Supplementary Figure S8. Cse4-nucleosomes remain intact during sucrose gradient fractionation. Sucrose density gradient fractionation of $Cse4_{\Delta 127}$-nucleosomes reconstituted on 601 DNA (207 bp) by a salt dilution protocol. **(a)** Intermittent fractions were analyzed on 5% native PAGE as in Figure 5c (top panel). **(b)** Fraction samples were analyzed by SDS-PAGE as indicated in Figure 5c (bottom panel).



Supplementary Figure S9. Cse4-nucleosomes assembled by Scm3 are composed of all four histone proteins but do not contain Scm3. The schematic of the experimental strategy is show on the top. **(a)** Cse4-nucleosomes were reconstituted on 561 bp DNA (containing three 147 bp 601 sequences connected by 60 bp linker DNA) using Nap1 as assembly factor. **Left panel**: The assembled chromatin was purified by sucrose gradient sedimentation and the fractions were run on 5% native PAGE and visualized by ethidium bromide staining. The fractions analyzed (13-34; top to bottom of the sucrose gradient) and the control DNA are indicated. **Right panel**: Samples (in left panel and fractions 9 and 11)) were analyzed by SDS-PAGE and visualized by SYPRO Ruby (Invitrogen) staining. IN: input; M: protein molecular weight standard (kDa).**(b)** Cse4-nucleosomes was reconstituted on the same DNA as in (**a**) using Scm3 as a chaperone. **Left panel**: As described in (a) with the addition of fraction 5. **Right panel**: Samples (in left panel) were analyzed by SDS-PAGE and visualized by SYPRO Ruby staining. Lane 1: Input (Scm3 + Cse4-octamer); lane 2:Scm3; lane 3: Cse4-octamer; lane 4: fractions 1-5 combined; lane 5: fractions 13-21; lane 6: fractions 22-30; M- protein molecular weight standard. **(c)** Cse4-nucleosomes were reconstituted on 207 CEN3 DNA using $Scm3_{63-189}$ as nucleosome assembly factor. **Left panel**: As described in (a). The fractions analyzed are shown on top of the gel. M- DNA size marker. **(Right panel)** SDS-PAGE analysis of the sucrose gradient fractions of Cse4-nucleosome assembled on 207bp CEN3 DNA from (Left panel). Lane 1: input-$Scm3_{63-189}$/Cse4-octamer; lane 2: input- $Scm3_{63-189}$; lane 3: fractions 1-5 combined; lane 4: fractions 14-24 combined; lane 5: fractions 25-34 combined; M: protein molecular weight standard and the sizes in kDa are indicated. **(d)** Similar to (**c**) except the assembly was done on 207 bp 5S DNA fragment. IN- input materials for sucrose gradient fractionation.

Supplementary Table S1. DNA sequences used in nucleosomes reconstitution.

Type of DNA	Sequences	Remarks
147 bp α-satellite DNA	ATCAATATCCACCTGCAGATACTACCAAAAGTGTATTTGGAAACTGCTCCATC AAAAGGCATGTTCAGCTGGATTCCAGCTGAACATGCCTTTTGATGGAGCAGT TTCCAAATACACTTTTGGTAGTATCTGCAGGTGGATATTGAT	
146 bp 5S DNA	ATCGAGCCCTATGCTGCTTGACTTCGGTGATCGGACGAGAACCGGTATATTC AGCATGGTATGGTCGTAGGCTCTTGCTTGATGAAAGTTAAGCTATTTAAAGG GTCAGGGATGTTATGACGTCATCGGCTTATAAATCCCTGGAT	
147 bp CEN3 DNA	AAATAGTACAAATAAGTCACATGATGATATTTGATTTTATTATATTTTTAAAAAA AGTAAAAAATAAAAAGTAGTTTATTTTTAAAAAATAAAATTTAAAATATTAGtgtattt gatttccgaaagttaaaaaAGAAATAGTAAGAA	CDEI- underlined; CDEII-shaded; CDEIII- small font
147 bp DNA PCR amplified from pBR322 plasmid	TCCAGCAGCCGCACGCGGCGCATCTCGGGCAGCGTTGGGTCCTGG CCACGGGTGCGCATGATCGTGCTCCTGTCGTTGAGGACCCGGCTAG GCTGGCGGGGTTGCCTTACTGGTTAGCAGAATGAATCACCGATACG CGAGCGAACG	
207 bp CEN3 DNA	AAATCCACAGAAAGCTATTCATTGAAAAAATAGTACAAATAAGTCACATGATG ATATTTGATTTTATTATATTTTTAAAAAAAGTAAAAAATAAAAAGTAGTTTATTT TTAAAAAATAAAATTTAAAATATTAGtgtatttgatttccgaaagttaaaaaAGAAATAGTAA GAAATATATATTTCATTGAATGGATATATGAAACGT	CDEI- underlined; CDEII-shaded; CDEIII- small font
147 bp 601 DNA	ACTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAG CACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGG GATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGAT	
207 bp 601 is shaded	ATCTAATACTAGGACCCTATACGCGGCCGCACTGGAGAATCCCGGTGCCGA GGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACG CGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGG CACGTGTCAGATATATACATCGATTGCATGTGGATCCGAATTCATATTAATGA T	147 bp 601 is shaded

Supplementary Table S1 continued…

Type of DNA	Sequences	Remarks
Trimeric 601 DNA	ATCGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAG CACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGG GATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGATTGCATGT GGATCCGAATTCATATTAATCATATCTAATACTAGGACCCTATACGCGGCCG CATCGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTA GCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGG GGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGATTGCATG TGGATCCGAATTCATATTAATCATATCTAATACTAGGACCCTATACGCGGCCG CATCGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTA GCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGG GGATTACTCCCTAGTCTCCAGGCACGT GTCAGATATATACATCGAT	three 147 bp 601 is shaded dark
DNA template for single molecule DNA unzipping experiments	TTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAAC AATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTATTTAGGTG ACACTATAGAATACTCAAGCTTGCATGCCTGCAGGTCCGGGACCTAATGACC AAGGAAAGCATGATTCTTCACACCGAGTTCATCCCTTATGTGATGGACCCTA TACGCGGCCGCCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGT AGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTT AACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACA TCCTGTGCATGTATTGAACAGCGACCTTGCCGGTGCCAGTCGGATAGTGTTC CGAGCTCCCACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGCCCTAT AGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGG AAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGC CAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTT GCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCA TCTGTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCT GATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCC	147 bp 601 is shaded

Supplementary Table S2: Summary of the SAXS parameters for Cse4-containing and canonical nucleosomes.

Nucleosome	**Rg (Guinier) (Å)**	**Rg (Gnom) (Å)**	**Dmax (Å)**
Xl-Nuc	40.7 (0.1)	41.8	120
Sc-Nuc	42.0 (0.3)	42.9	135
$Cse4_{\Delta 102}$-Nuc	46.0 (0.1)	46.7	165