Supporting Information

Krassovsky et al. 10.1073/pnas.1118898109

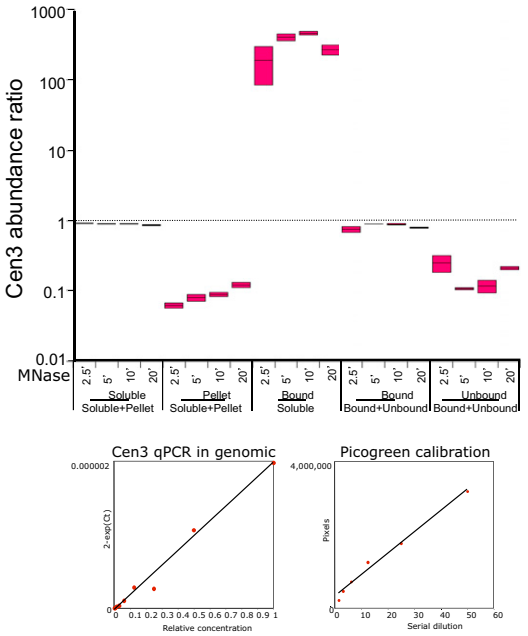

Fig. S1. Quantitative PCR (qPCR) measurement ratios showing relative abundance of Cen3 in chromatin fractions. PCR primers were designed to amplify the 125-bp Cen3 sequence (ChrIII:114383–114404 and 114487–114508) using a 46 °C annealing/extension temperature. Each qPCR measurement was divided by the total DNA level as determined by Picogreen fluorescence in the presence of RNase A. Ratios are calculated as ($qPCR_2$/$Picogreen_2$)/ ($qPCR_1$/$Picogreen_1$).

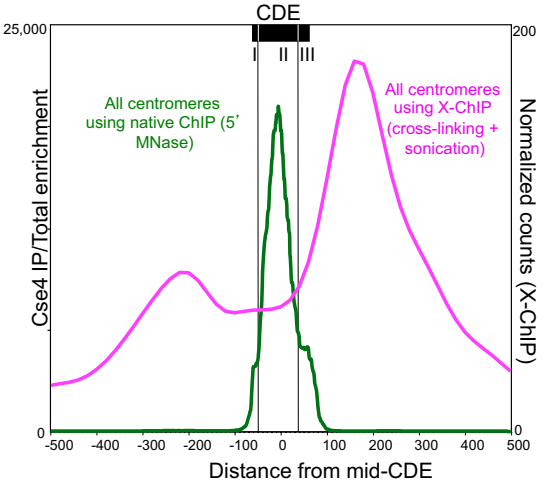

Fig. S2. Comparison between Native-ChIP and cross-linking and sonication before immunoprecipitation (X-ChIP) mapping profiles for all 16 aligned yeast centromeres. A normalized enrichment profile for all 16 yeast centromeres was computed from triplicate Cse4 X-ChIP data obtained from the Gene Expression Omnibus (Accession no. GSE13322) (1). These data are shown superimposed over the N-ChIP profiles from Fig. 2*B*. For X-ChIP data, plus and minus reads were offset by the maximum cross-correlation value for normalized counts around the mid-CDE (centromere DNA element) (130 bp), and the average of offset plus and minus reads within each 20-kb window was plotted.

1. Lefrançois P, et al. (2009) Efficient yeast ChIP-Seq using multiplex short-read DNA sequencing. *BMC Genomics* 10:37.

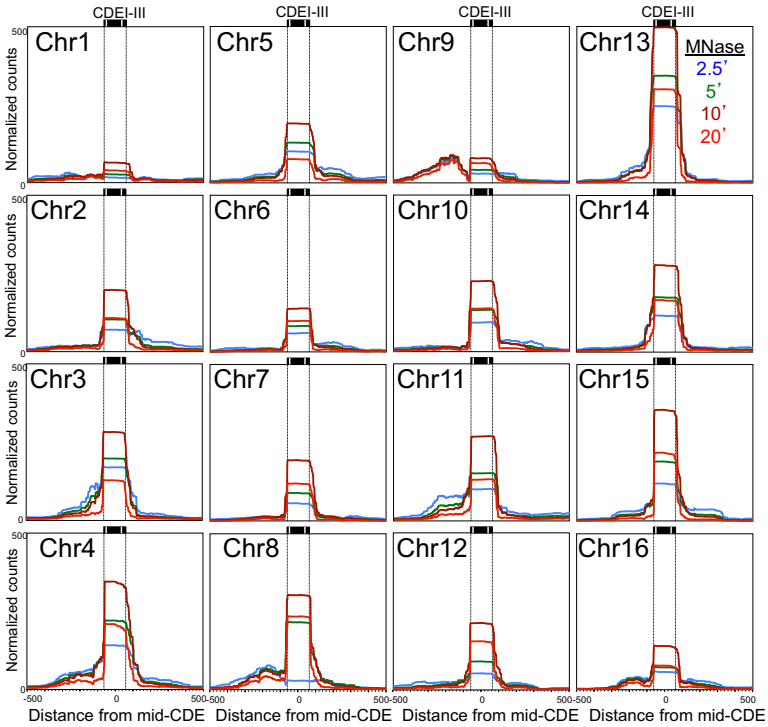

Fig. S3. Cse4-containing particles are precisely positioned over the CDE. Average Cse4 ChIP profiles for all centromeres and all mapped fragment sizes. The square signal over each CDE increases during digestion up to 10 min, then generally decreases, whereas the rounded signal to the left of Chr9 shows little if any change in signal, indicating the robustness of the profile regardless of the level of MNase digestion.

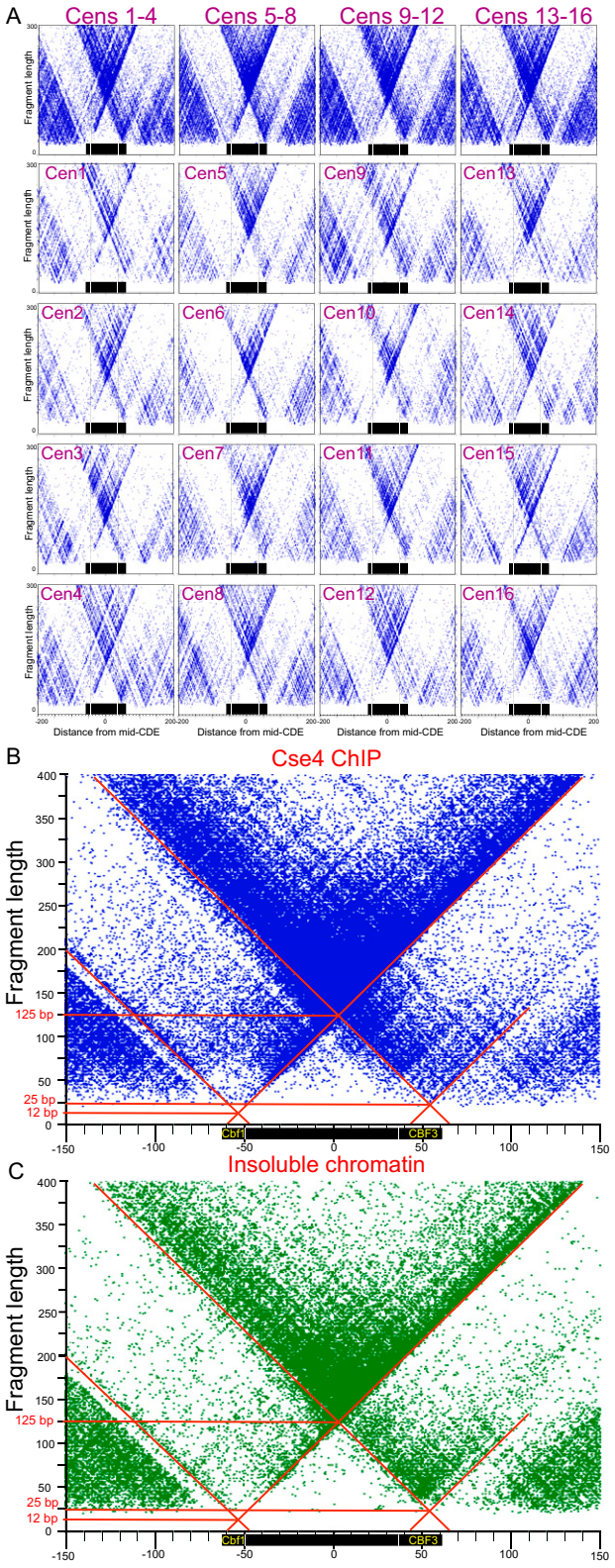

Fig. S4. V-plots of DNA from insoluble chromatin for individual centromeres. See the legend to Fig. 3 for details on interpretation of V-plots. (*A*) All 16 centromeres are shown either in groups of four or individually. (*B* and *C*) Direct comparison of Cse4 ChIP-seq (*A*) and pellet (*B*) from the same 20-min MNase sample, showing vertexes corresponding to ~10 bp for intersection of diagonals over CDEI, ~20 bp for intersection of the diagonals over CDEIII, and ~125 bp for intersection of the diagonals over the full CDE.

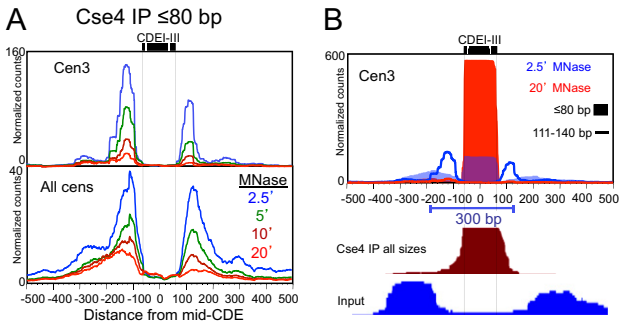

Fig. S5. The CDE is closely flanked by subnucleosomal particles and phased nucleosomes. (*A*) Mapped paired-end read counts of ≤80-bp fragments show enrichment after Cse4 ChIP and high sensitivity to MNase digestion on both sides of the centromere after ChIP. (*B*) Mapped paired-end reads for Cse4 ChIP at two MNase concentrations show subnucleosomal particles flanking the Cen3 CDE, where a square peak indicates nearly perfect protection of the 111- to 140-bp centromeric DNA segments. The ≤80-bp fragments are lost from the immunoprecipitated material with concomitant enrichment of 110- to 140-bp fragments during MNase digestion. Below are densities for Cse4 immunoprecipitation (brown) and >140-bp soluble chromatin fragments lined up to illustrate the relative locations of the different MNase-protected particles after 20-min digestion.

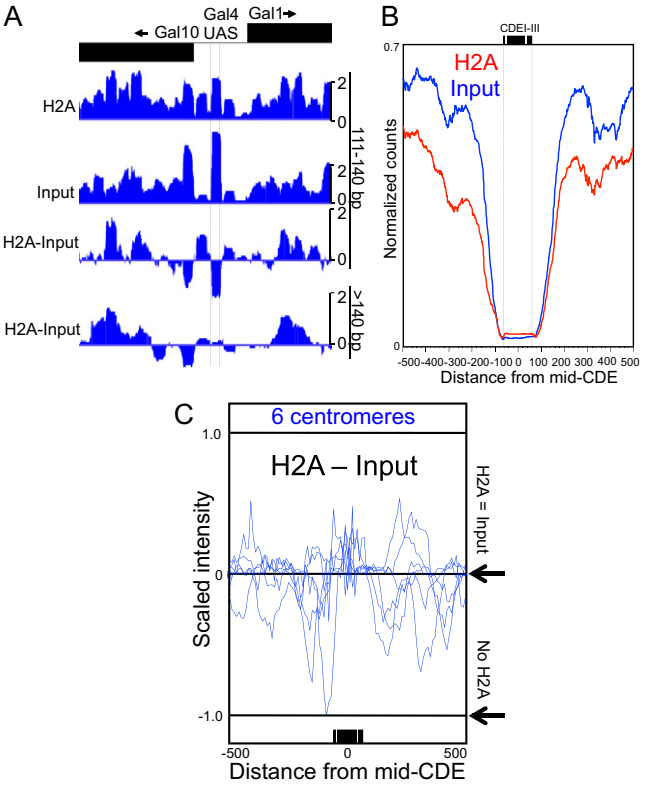

Fig. S6. Depletion of H2A at the H2A.Z-enriched nucleosome over the Gal4 UAS. (*A*) Positive control for the experiment shown in Fig. 5*A*, showing the 2.5-min MNase-digestion profile for total chromatin at the Gal4 region, where the well-positioned nucleosome over the Gal4 UAS was reported to wrap only ∼135 bp, and to be depleted for H2A and enriched for H2A.Z, in contrast to flanking nucleosomes which showed the opposite enrichment (1). Note that there is high occupancy of input for this nucleosome but lower occupancy of H2A relative to the genome as a whole (H2A − Input < 0) and to flanking nucleosomes. (*B*) Occupancies of Input and H2A ChIP showing the depletion of H2A in nucleosomes flanking the CDE for all 16 yeast centromeres, probably because these nucleosomes are relatively enriched for H2A.Z. (*C*) X-ChIP data showing that the Cxe4 nucleosome contains H2A. Data are converted from the sum of scaled intensities from "AA" and "AZ" (total H2A-containing) nucleosomes (2). See the legend to Fig. 6 for details.

1. Floer M, et al. (2010) A RSC/nucleosome complex determines chromatin architecture and facilitates activator binding. *Cell* 141:407–418.
2. Luk E, et al. (2010) Stepwise histone replacement by SWR1 requires dual activation with histone H2A.Z and canonical nucleosome. *Cell* 143:725–736.

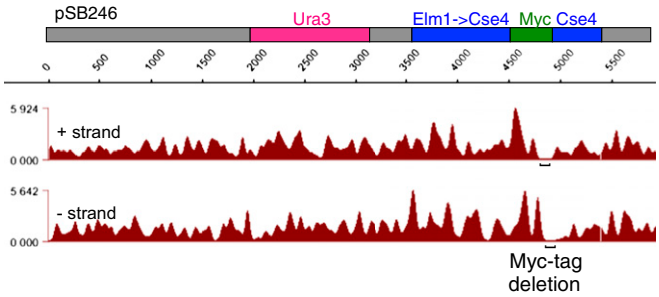

Fig. S7. Multiple copies of CSE4-Myc in strain SBY8796. The plasmid pSB246 was searched using the first one-million single-end 36-bp reads either not mapped to the yeast genome (364,160) or mapped uniquely (635,840). The 1,736 hits with an exact match for 36 bases (834+ and 902−) are displayed, using the left end of the mapped segment in all cases. Copy number estimate based on read density: (1,736 plasmid reads per 6 kb per plasmid)/(635,840 genomic reads per 12,000 kb per genome) = 5.5 copies per plasmid. This value was confirmed by average URA3 and CSE4 normalized peak heights for the whole dataset (~120/20 = 6). The map of pSB246 shows *Escherichia coli* sequence in gray, Ura3 sequence in magenta, Cse4 and upstream Elm1 sequence in blue, and 11 tandem Myc sites in green. Sequence assembly revealed that three of the 11 Myc sites have been deleted from all copies (bracketed region).

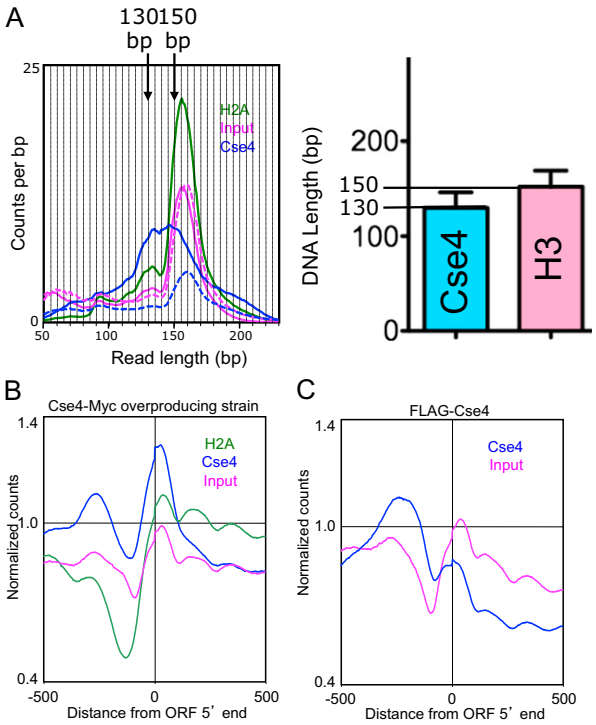

Fig. S8. Overproduced Cse4 nucleosomes deposit at "hot" nucleosome positions. (*A*) Length distributions of mapped fragments genome-wide, showing a comparison with lengths of MNase-protected fragments extracted from purified nucleosomes assembled in vitro and measured on agarose gels (1). The results from two separate experiments are shown, where little difference is seen in the degree of MNase digestion based on the slight offset in input peak position. (*B*) To confirm that hot nucleosomes are generally enriched for Cse4 nucleosomes relative to H2A nucleosomes around promoters (Fig. 7*B*), we aligned all yeast genes at their 5′ ORF ends and plotted the total number of normalized counts at each base pair. Strong enrichment is seen for overproduced Cse4-Myc and depletion of FLAG-H2A relative to Input from the −1 to +1 nucleosome positions. (*C*) In a single-copy control strain, Cse4 enrichment is seen over highly active promoter regions, as previously reported (2, 3).

1. Kingston IJ, Yung JS, Singleton MR (2011) Biophysical characterization of the centromere-specific nucleosome from budding yeast. *J Biol Chem* 286:4021−4026.
2. Lefrançois P, et al. (2009) Efficient yeast ChIP-Seq using multiplex short-read DNA sequencing. *BMC Genomics* 10:37.
3. Camahort R, et al. (2009) Cse4 is part of an octameric nucleosome in budding yeast. *Mol Cell* 35:794−805.

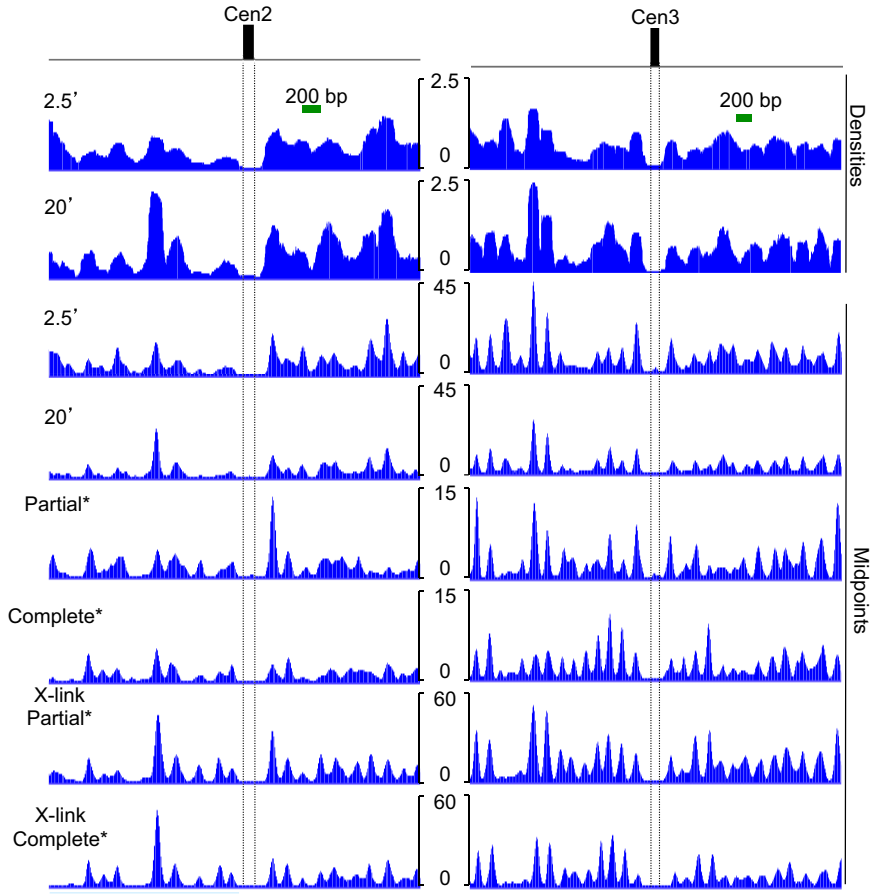

Fig. S9. Centromeric DNA is depleted from both native and cross-linked chromatin. Regions around Cen2 and Cen3 are shown. For midpoint landscapes from single-end reads, an offset of 75 bp was added to each plus end and subtracted from each minus end. A Gaussian kernel density function with bandwidth = 10 and window = 5 was applied to both single-end and paired-end reads.

Table S1. Yeast strains used in this study

Strain*	Genotype
SBY8796	MATa leu2-3,112 his3-11,15 trp1-1 lys- RAD5 hta2-htb2::NAT HTA1-L3FLAG-kanMX cse4ΔKAN ura3-1:CSE4-myc12:URA
SBY5146	MATa leu2-3,112 his3-11:pCUP1-GFP12-LacI12:HIS3 trp1-1:256lacO:TRP1 can1-100 ade2-1 Δlys2 Δbar1 cse4ΔKan ura3-1:pCse4-3xFLAG-CSE4 + 500 bp
SBY2688	MATa leu2-3,112 his3-11,15 trp1-1 LYS2 RAD5 hta2-htb2::NAT HTA1-L3FLAG-kanMX ura3-1:CSE4-myc12:URA

*All strains were derived from W303.