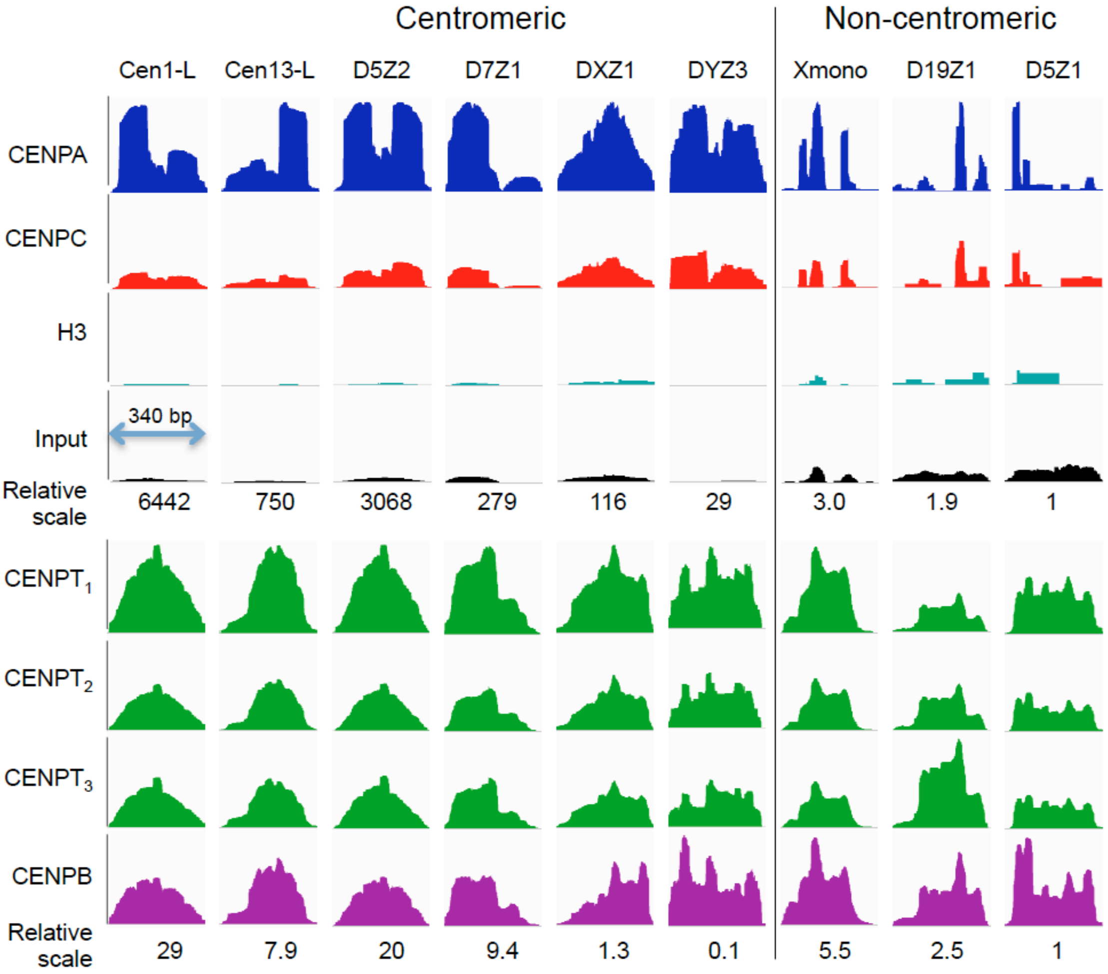
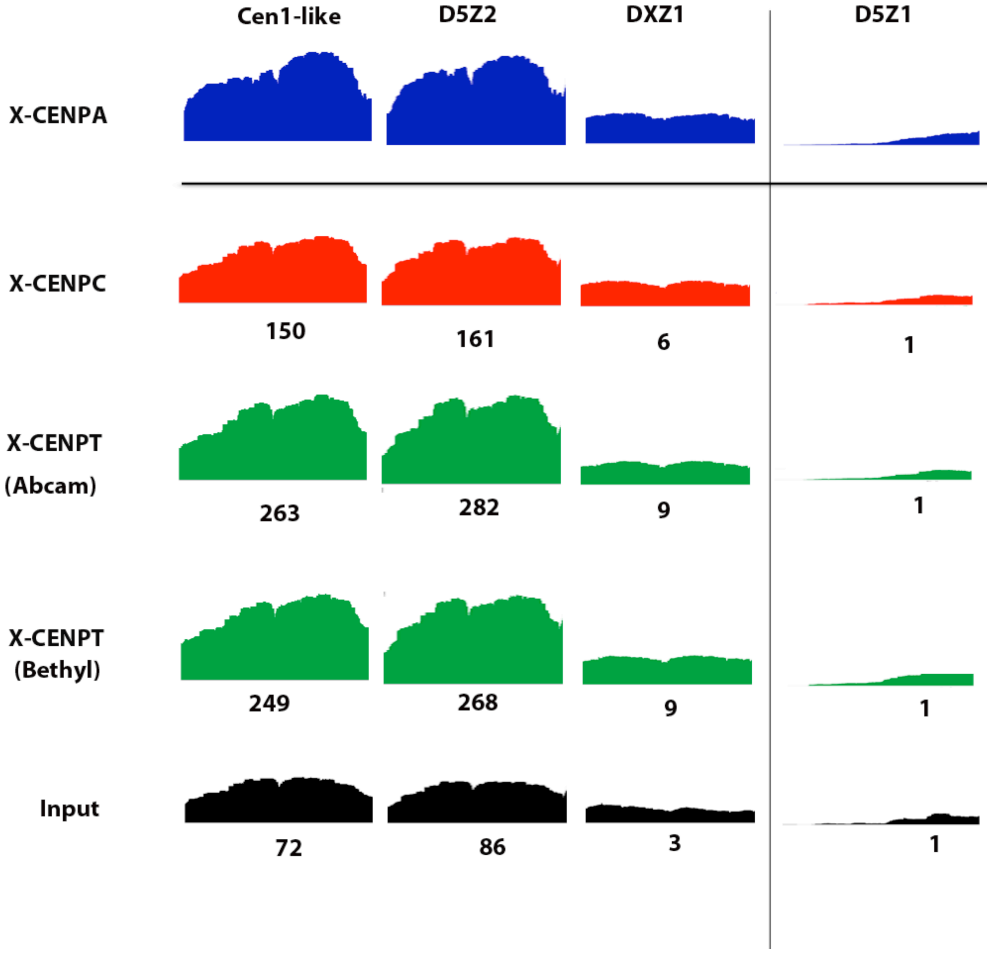
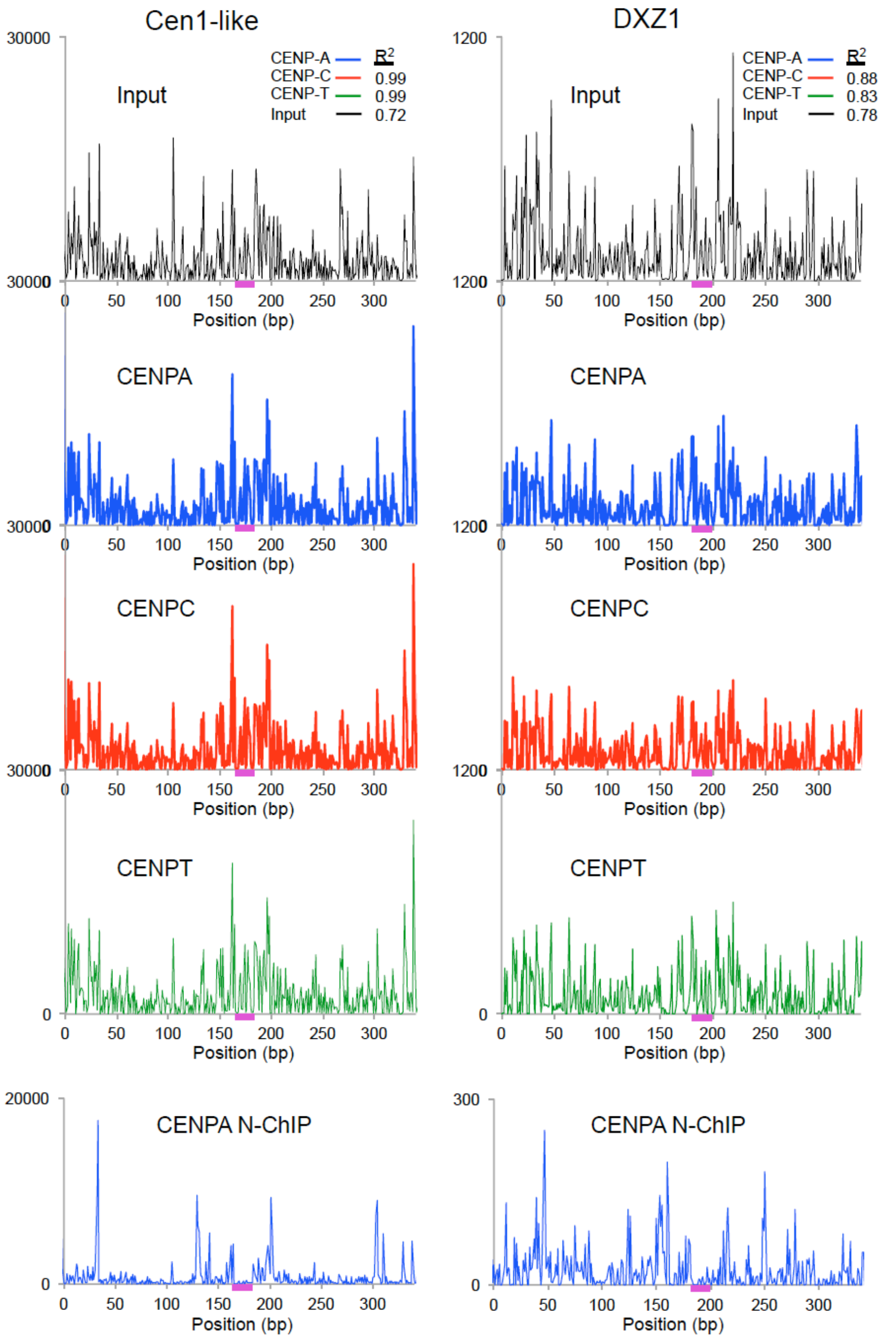
Supplemental information



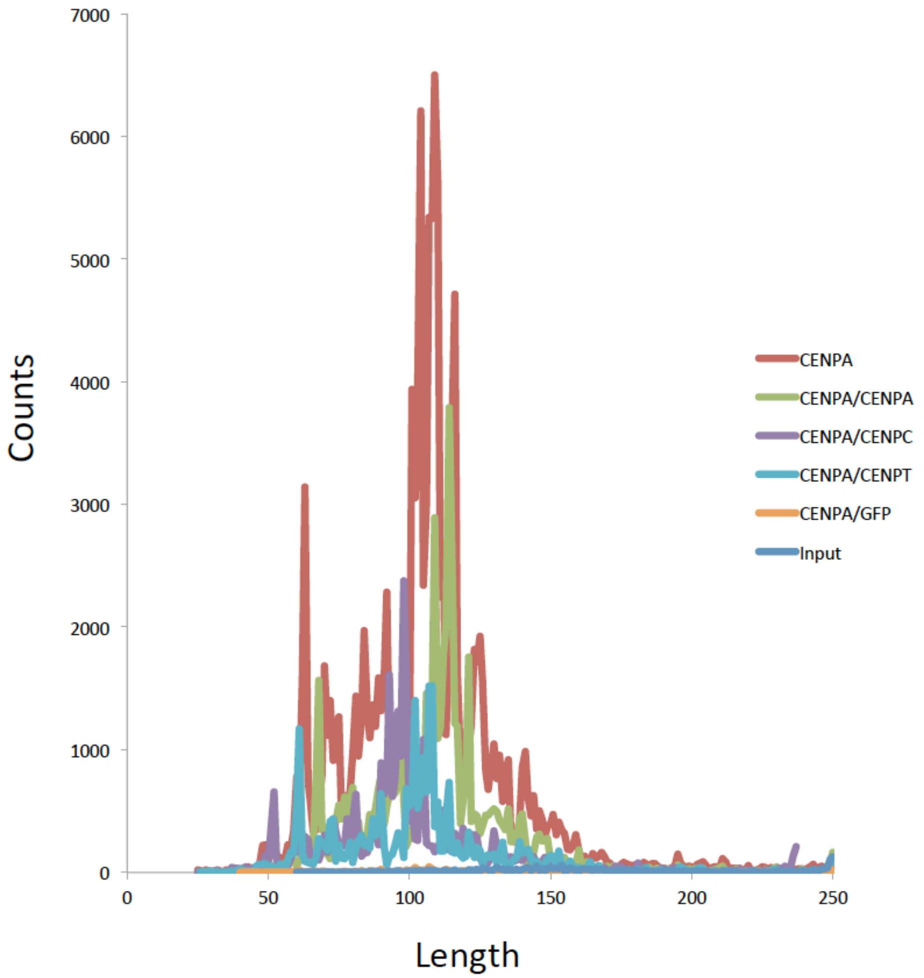
Supplemental Fig. S1. CENPT is not recovered under native conditions. N-ChIP-seq merged pairs for CENPA, CENPT, H3 and Input (top, from GEO GSE60951 (Henikoff et al. 2015) and paired-end reads for 3 biological replicates of CENPT (bottom) were mapped to the indicated 340-bp α-satellite dimers. Each column of four profiles is scaled to the maximum peak. The relative scale is the area of the CENPA, CENPC or H3 profile (top) or the CENPT$_1$, CENPT$_2$ or CENPT$_3$ profile (bottom) divided by the area of the D5Z1 profile, where the numbers reflect the product of the total sequence abundance times the enrichment.



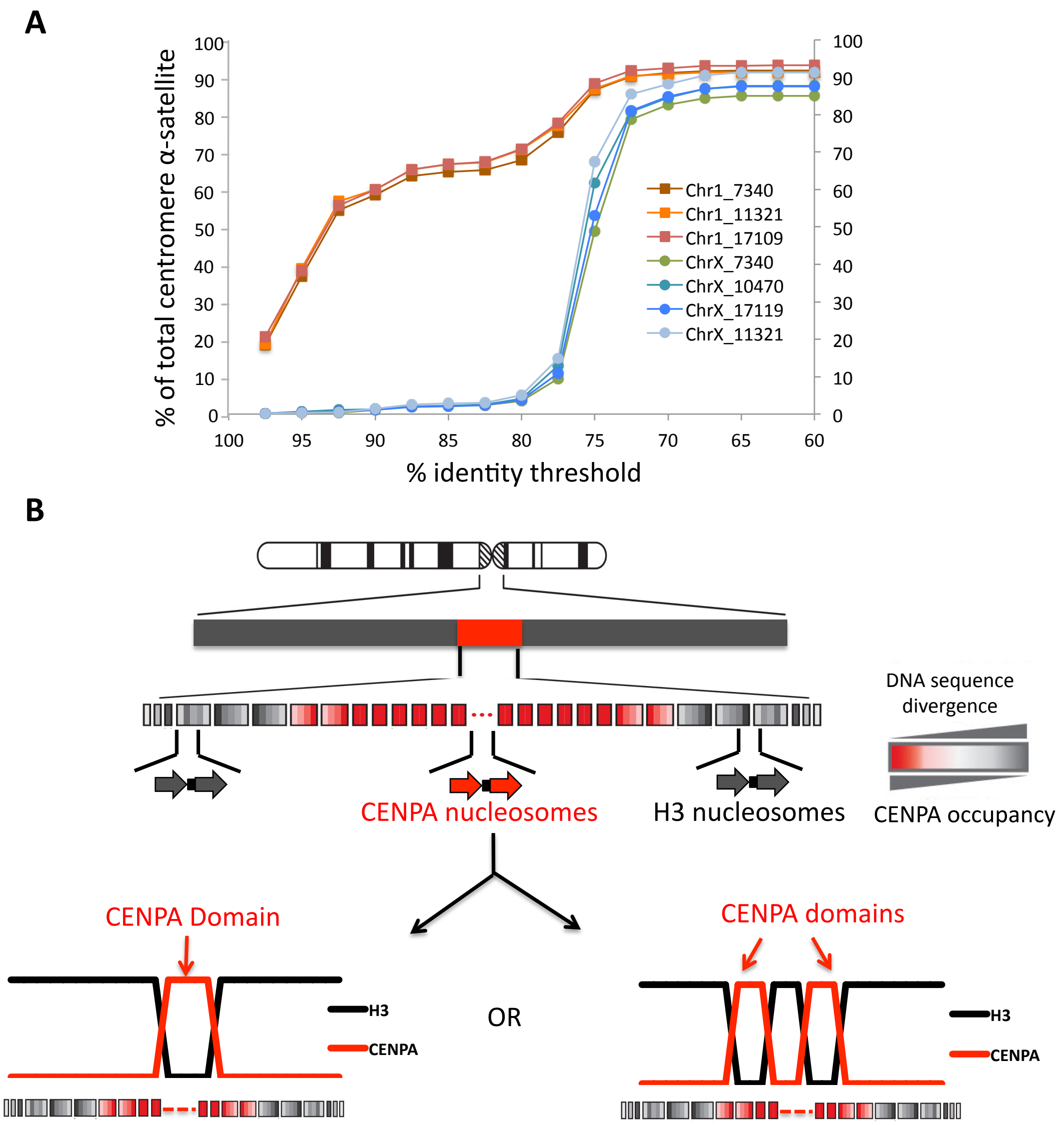
Supplemental Fig. S2. CENPA, CENP- C and CENPT are cross-linked to same α-satellites. The X-ChIP profiles of CENPT with two different antibodies (Abcam and Bethyl) on centromeric α-satellites are highly similar to each other and to those of CENPA and CENPC.



Supplemental Fig. S3. Fragment ends from X-ChIP (top four panels) and native bottom panel) datasets were mapped to the Cen1-like consensus and DXZ1 sequences. The CENPB box region is indicated by the magenta box.



Supplemental Fig. S4. Fragment length distribution of Sequential ChIP datasets on Cen1-like α-satellites.



Supplemental Fig. S5. A) Only a fraction of α-satellites on chromosome 1 map with high identity to Cen1-like dimer. BLASTn of Cen1-like 340-bp dimeric consensus sequence against the α-satellite subset (identified using a Hidden Markov Model filter trained on α-satellite) of Chromosome 1 flow sorted sequence libraries showed that ~20% of the α-satellites on Chromosome 1 is ≥97.5% identical to the Cen1-like consensus, gradually increasing to ~65% when the stringency is relaxed to ≥80% identical. In contrast, only ~4% of the X-chromosome α-satellite is ≥80% identical to the Cen1-like 340-bp consensus sequence (Sivakanthan Kasinathan, personal communication). **B) A model for CENPA and H3 occupancies on a typical human centromere.**

Supplemental Table 1. Primers used for qPCR in this study.

Primer	Sequence
S1-F	AGGGAATGTCTTCCCATAAAAACT
S1R	GTCTACCTTTTATTTGAATTCCCG
S2F	TTTTTGTGCAATTGGCAAATGGAG
S2R	AGACTGTTTCCTCACTGCTCT
S3F	CAGCAGCATCCTCAGAAACTT
S3R	CTCCACTTGCAAATTCCACA
5SDNA-F1	CCGGACCCCAAAGGCGCACGCTGG
5SDNA-R1	TGGCTGGCGTCTGTGGCACCCGCT

Supplemental References

Henikoff JG, Thakur J, Kasinathan S, Henikoff S. 2015. A unique chromatin complex occupies young alpha-satellite arrays of human centromeres. *Science advances* **1**: e14000234.