Biophysical Journal, Volume *114*

Supplemental Information

Interactions of HP1 Bound to H3K9me3 Dinucleosome by Molecular Simulations and Biochemical Assays

Shuhei Watanabe, Yuichi Mishima, Masahiro Shimizu, Isao Suetake, and Shoji Takada

Supporting Figures for
Interactions of HP1 bound to H3K9m33 di-nucleosome by molecular simulations and biochemical assays

S. Watanabe, Y. Mishima, M. Shimizu, I. Suetake*, S. Takada*

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint first authors.

Supplementary Information

Expression vectors

For expressing HP1 α with GST and 3x Flag tag at N-terminus, cDNA corresponding to 3x repeated Flag tag was inserted between GST and HP1 α in pGEX6P-HP1α (Materials and Methods), in frame.

Purification of F- HP1 α

GST-Flag-tagged wild type and 3mut HPα α was expressed and purified with glutathione beads, as described in Materials and Methods. To obtain F-HP1α, purified GST-Flag-HP1α was treated with Turbo3C (Wako, Japan), and then further purified with HiTrapQ, as described in Materials and Methods.

Pulldown assay

F-HP1α (80pmol) was mixed to 10ul of anti-Flag M2 affinity gel (Sigma), and washed with binding buffer, and then the binding reaction was performed using di-nucleosomes with 25 bp DNA linker, as described in Materials and Methods. As the affinity beads contained proteins of which molecular weight is similar to histones, the reaction mixture was separated in 18% acrylamide gels and proteins were transferred onto nitro cellulose membrane and then histone H4 was detected by using primary antibodies (Abcam, ab10158) and HRP conjugated secondary antibodies (Thermo Fisher; #A27036). The bands were visualized with Western BLoT Quant HRP Substrate kit (Takara, Japan) and LAS1000 (Fuji Film, Japan). The density of band was quantified by using software, Image Gauge (Fuji Film, Japan).

Supplementary Figure

ATCGACGACA GTACTCGCCG CCCTGGAGAA TCCCGGTGCC GAGGCCGCTC

AATTGGTCGT AGACAGCTCT AGCACCGCTT AAACGCACGT ACGCGCTGTC

CCCCGCGTTT TAACCGCCAA GGGGATTACT CCCTAGTCTC CAGGCACGTG

TCAGATATAT ACATCCTGTG CACGTAAGAG TACTACTCGC CGCCCTGGAG

AATCCCGGTG CCGAGGCCGC TCAATTGGTC GTAGACAGCT CTAGCACCGC

TTAAACGCAC GTACGCGCTG TCCCCCGCGT TTTAACCGCC AAGGGGATTA

CTCCCTAGTC TCCAGGCACG TGTCAGATAT ATACATCCTG TGCACGTAAG

TACTCCGTCT TCGAT

Figure S1. The DNA sequence used for the reconstitution of di-nucleosomes. The expected positions of histone octamers are shown as underlines. The linker DNA between nucleosomes is 25 bp.

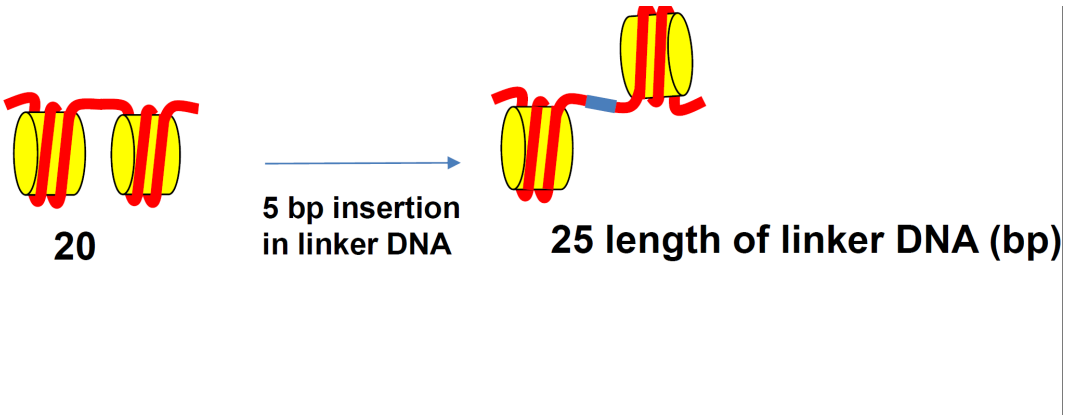


Figure S2. Schematic illustration of rotational positioning change of di-nucleosomes by 5 bp increment of linker DNA.

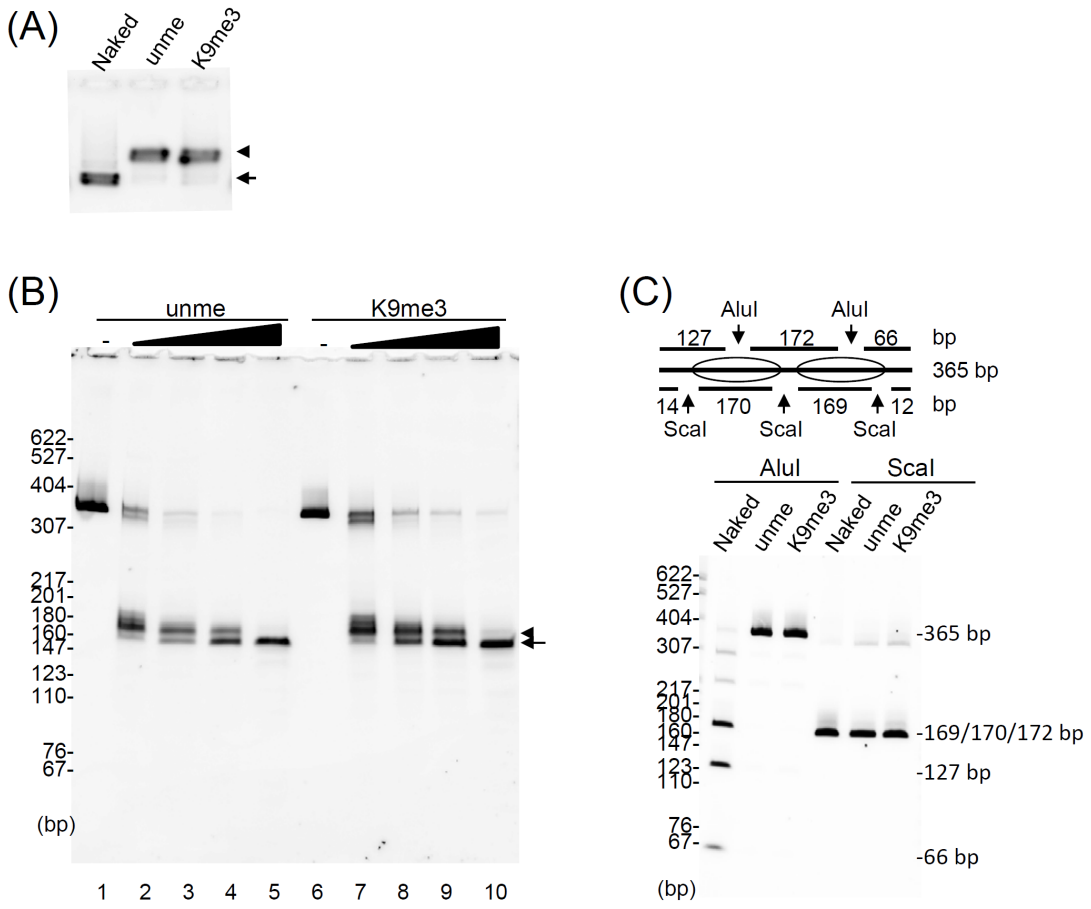


Figure S3. Properties of the reconstituted di-nucleosomes. A) Agarose gel electrophoresis of naked and reconstituted di-nucleosomes. Di-nucleosomes reconstituted with unmethylated H3 (unme) or H3K9me3 (K9me3) and naked DNA (naked) were electrophoresed in a 0.7% agarose gel. Reconstituted nucleosome and free DNA were indicated with arrowhead and arrow, respectively. B) MNase analysis for nucleosome assembly. Reconstituted di-nucleosomes were treated with MNase (2.6 U/ml for lanes 2 and 7; 8.8 U/ml for lanes 3 and 8; 26.5 U/ml for lanes 4 and 9; and 88.4 U/ml for lanes 5 and 10), or without MNase (lanes 1 and 6). DNA was separated in a 5% native polyacrylamide gel. Size markers (bp) are indicated at left of the gel. The positions of the 160 bp (arrowhead) and 146 bp (arrow) bands protected from MNase digestion are indicated. C) Restriction enzyme susceptibility of reconstituted di-nucleosomes. The expected positions of histone octamers (ellipses) and the length of digested DNA are shown (upper panel). ScaI sites are located at outside of the expected positions of nucleosomes, while AluI sites are at the region the core particles sit. Naked DNA or reconstituted were digested with either AluI or ScaI, and separated in a 5% native

polyacrylamide gel (lower panel).

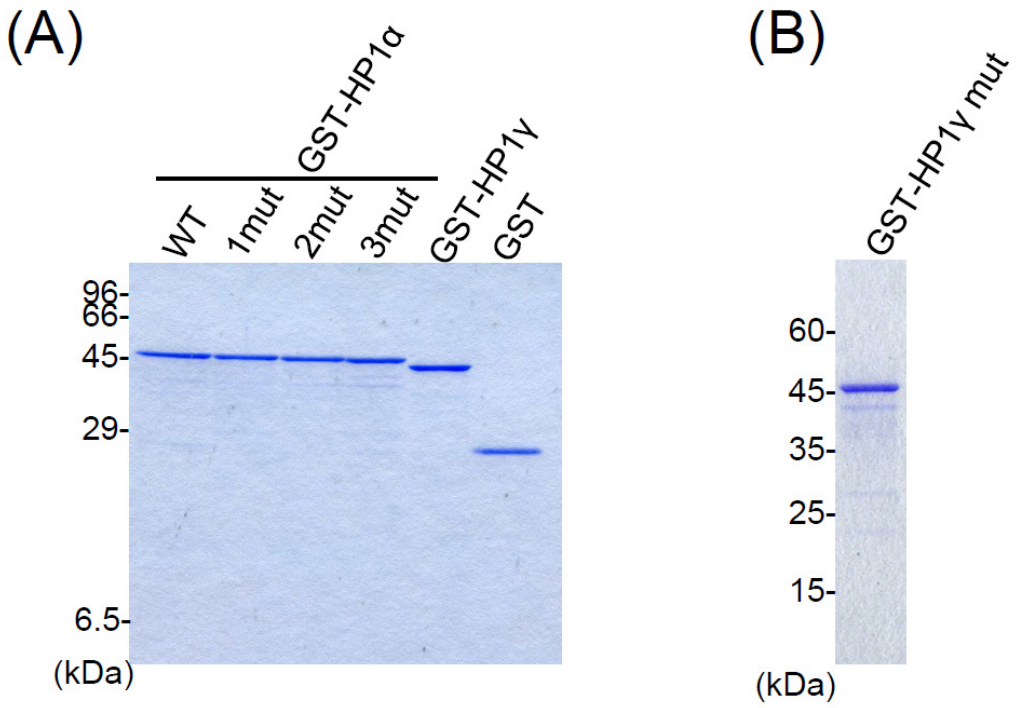
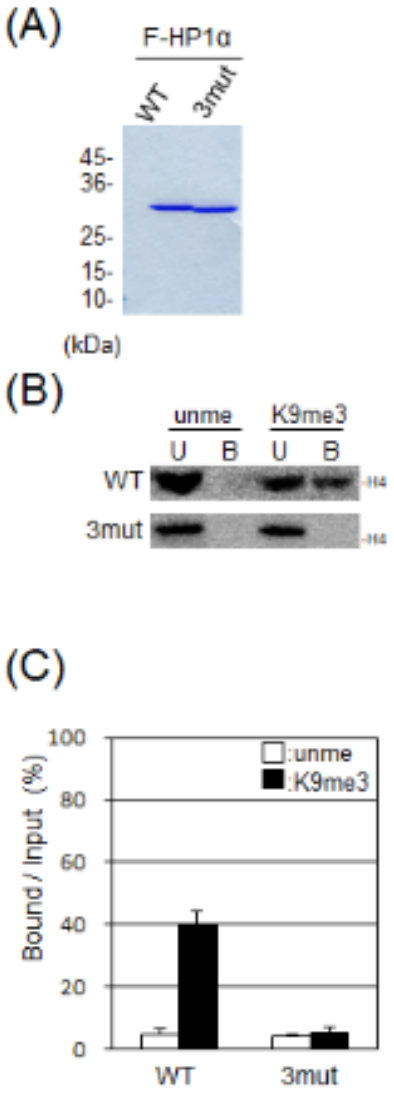


Figure S4. SDS-PAGE of purified HP1. A) One ug of proteins (GST-HP1α (wild type (wt), 1mut, 2mut and 3mut), GST-HP1γ, and GST) was separated in a 12% SDS-polyacrylamide gel, and stained with CBB. Size markers are indicated at the left of the gel. B) One μg of purified GST-HP1γ mutant (N68K, S69K, Q70Y, A72K, and G73M) was separated in a 5-20% gradient acrylamide gel , and stained with CBB. Size markers are indicated at the left of the gel.



Supplementary Fig S5. The interaction between F-HP1α and di-nucleosomes

(A) Purified F-HP1α. Wild type F-HP1α (lane 1) and F-HP1α-3mut (lane 2) (1 μg) were separated with a 5-20% gradient acrylamide gel, and the bands were detected with CBB. Molecular weight markers are indicated at the left of the gel. An arrow indicates F-HP1α. (B) Interaction between F-HP1α (WT or 3mut) and di-nucleosomes. After the binding reaction using anti-Flag matrices, unbound (U) and bound (B) fractions are obtained. Histone H4 was detected by western blotting. A representative result is shown. Average +/- SD (intensity of bound H4/(that of bound + unbound H4) (%)) of the binding activity was calculated from three independent experiments, and shown in panel C.

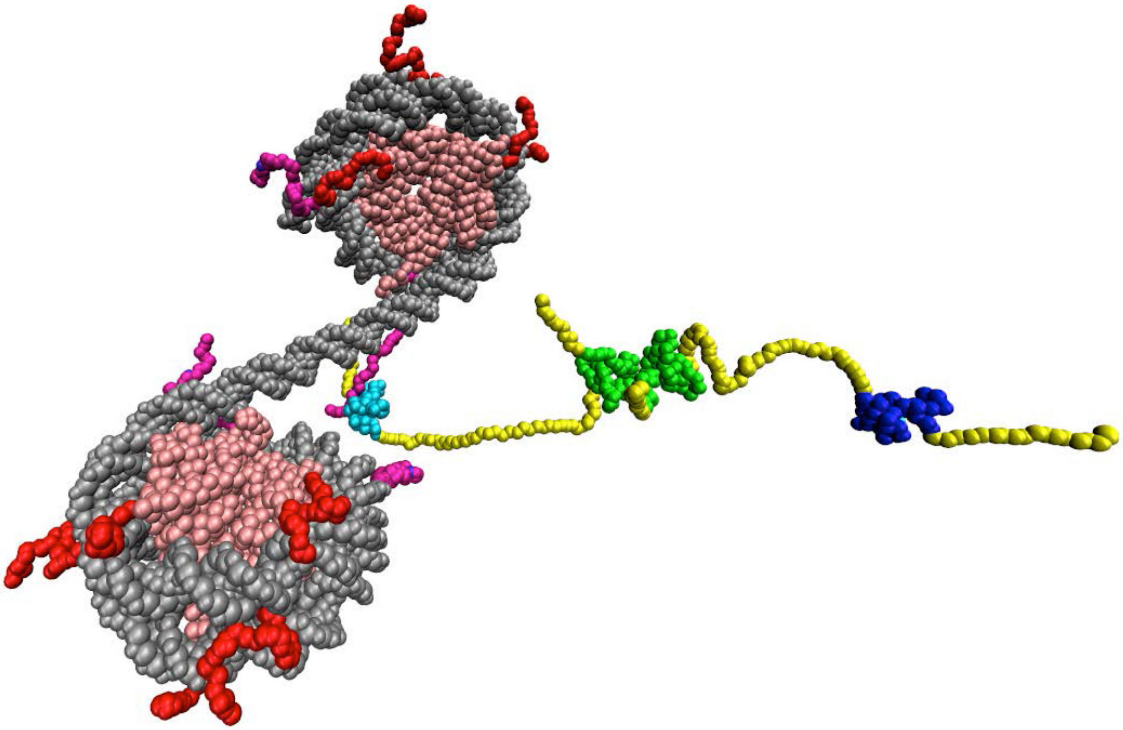


Figure S6. An example of initial structure for molecular simulations for "A prox bound". The color usage is the same as that of Fig.3.

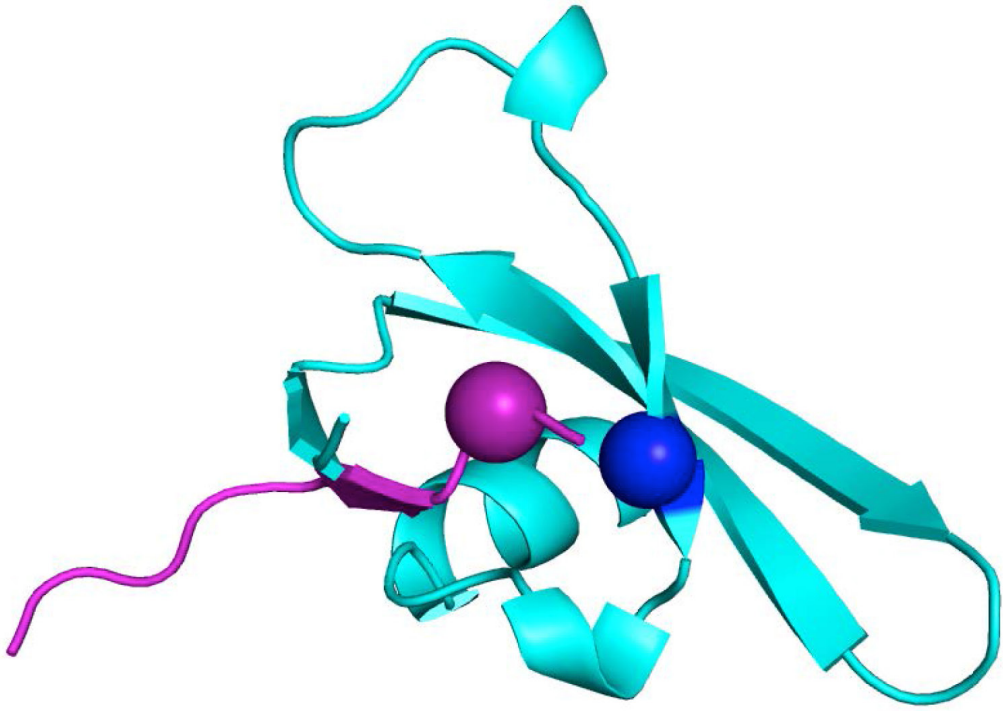


Figure S7. The crystal structure of the CD domain complex with a H3K9me3-containing peptide. The Cα positions of H3K9 and HP1 Glu52 are shown in purple and blue spheres. The distance between the two Cα atoms was 6.6Å. The structure is from 3FDT.

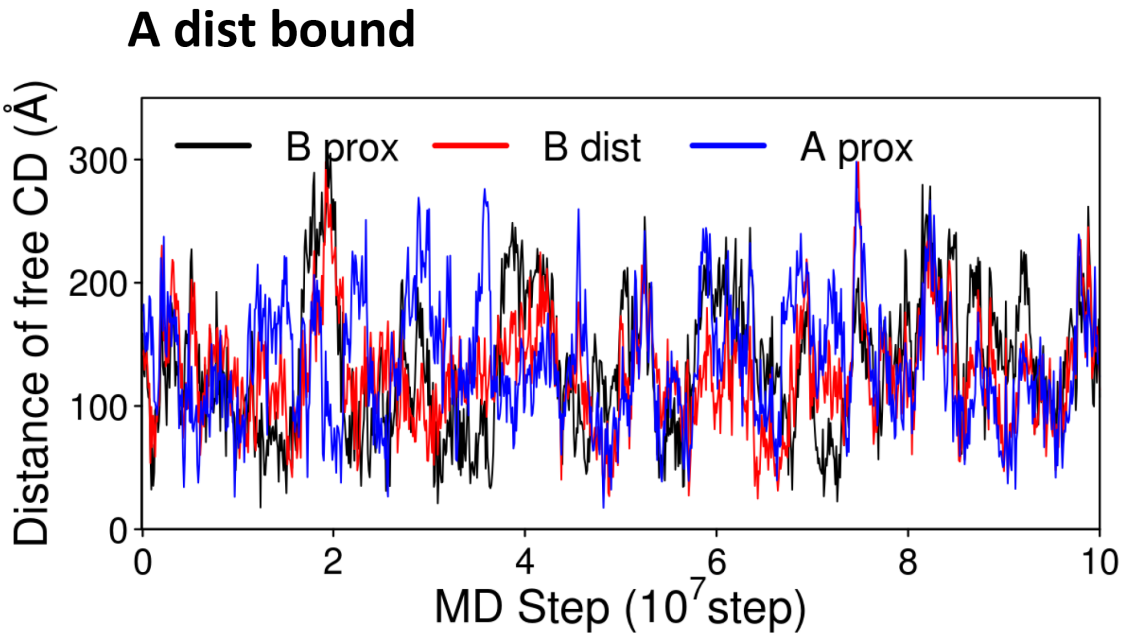


Figure S8. A representative trajectory of HP1α on nucleosomes where one CD is bound on the tri-methylated "A distal H3K9" and the other CD is free at the initial conformation. The other three H3K9s are assumed to be un-methylated. Time courses of the distances from the free CD to three H3K9s are plotted.

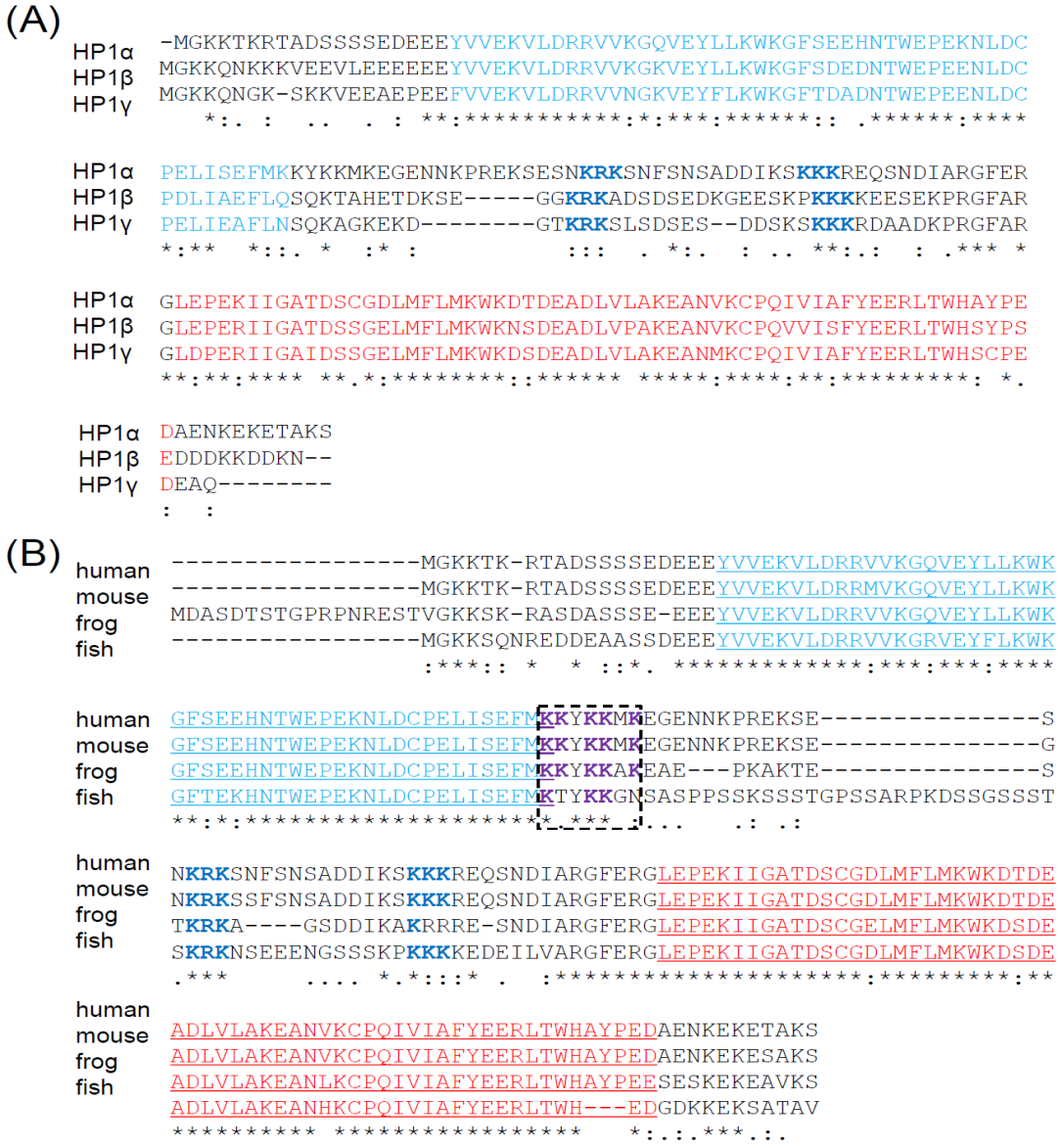


Figure S9. Alignment of HP1 sequences. Human HP1 isoforms (A) and HP1α from several species (B) were aligned using ClustalW (http://www.genome.jp/tools/clustalw). Regions corresponding to CD and CSD were indicated with light blue and red letters, respectively. Conserved two basic amino acids patches (KRK and KKK) in HR are shown in blue bold letters. Lysine residues close to CD are highly conserved among species (purple letters) B) The accession number of human HP1α, β, γ, mouse HP1α, frog HP1α, and fish HP1α are NM_012117.1, NM_006807, AB030905, NP_001070257, NP_001080863, and AAI29318, respectively. Identical amino acid is indicated as *, while homologous amino acid is as . or :.

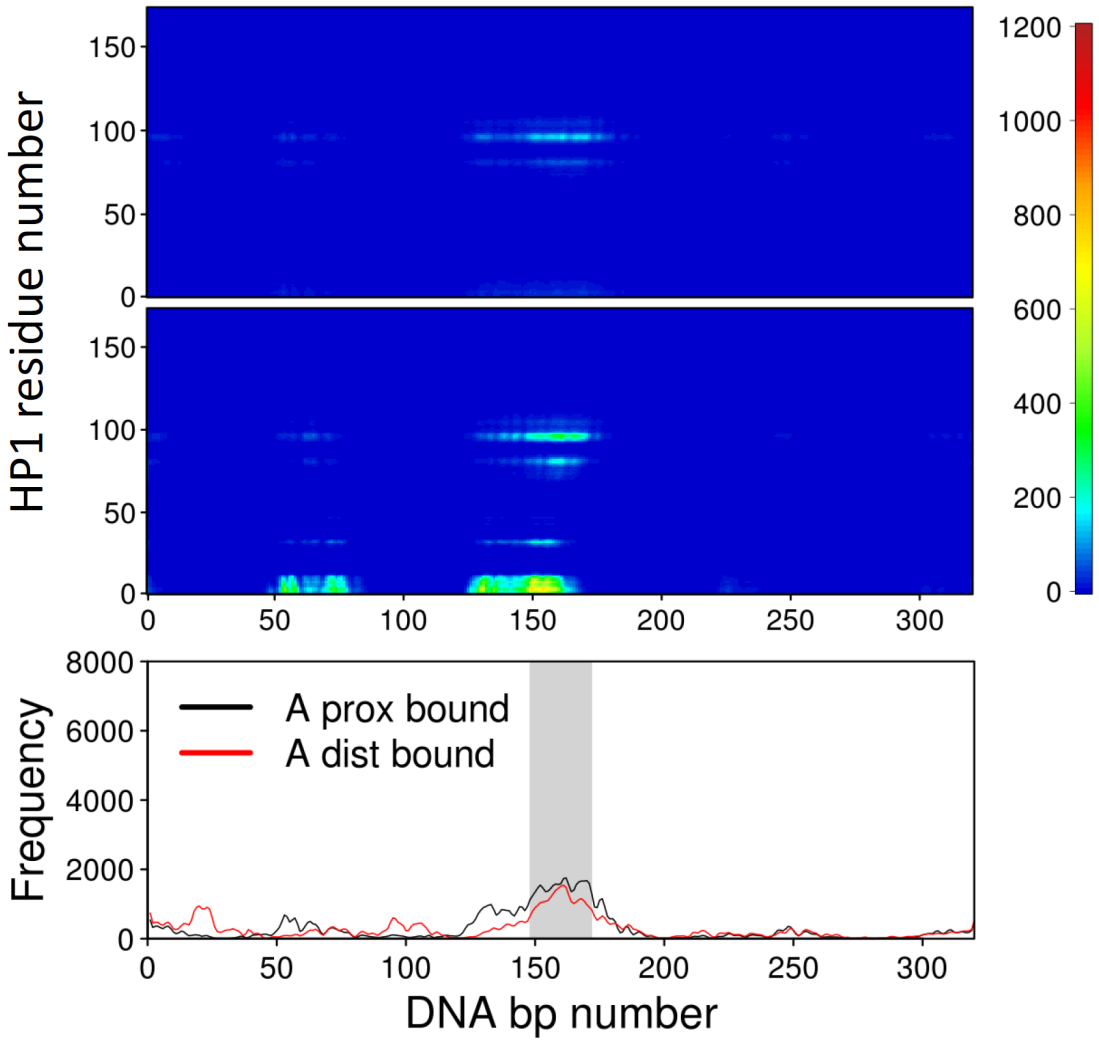


Figure S10. HP1γ-DNA interactions in molecular simulations. A) The horizontal axis indicates DNA base pair index of di-nucleosome; 1-147; nucleosome A, 148-172; linker DNA, 173-319. The vertical axis means residues in the HP1γ dimer; the bottom half for the bound HP1γ and the top half for the free HP1γ. Contact frequencies are represented by colors (see the right bar for the color definition). B) Frequency of HP1γ binding to every nucleotides in DNA.

Figure 2B

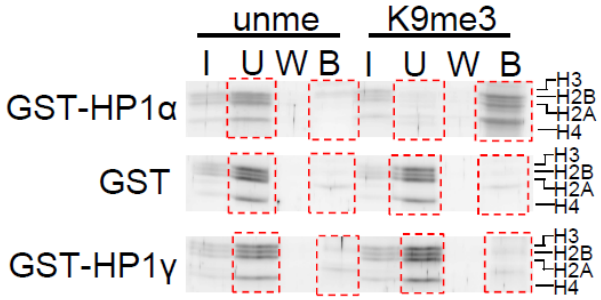


Figure 6B

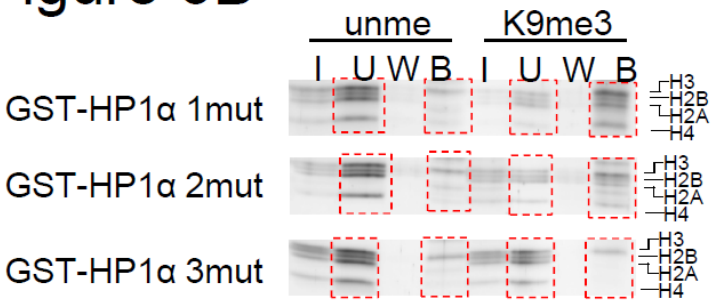


Figure 6E

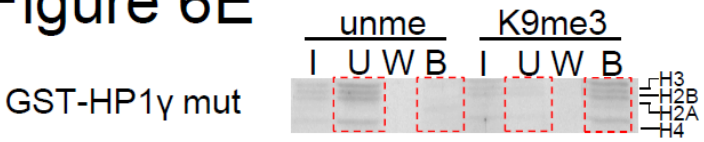


Figure S11. Whole gel images for the main figures. The regions excised are shown as red boxes with broken lines. In panels for Figures 2B, 6B, and 6E, the input (one-fourth of each) (I), unbound (U), wash (W), and bound (B) fractions were electrophoresed and visualized. For the binding experiments, unmethylated H3 (unme) or H3K9me3 (K9me3) di-nucleosomes were used. The positions of GST, GST-HP1α, GST-HP1γ, H2A, H2B, H3 and H4 are indicated in each corresponding images.

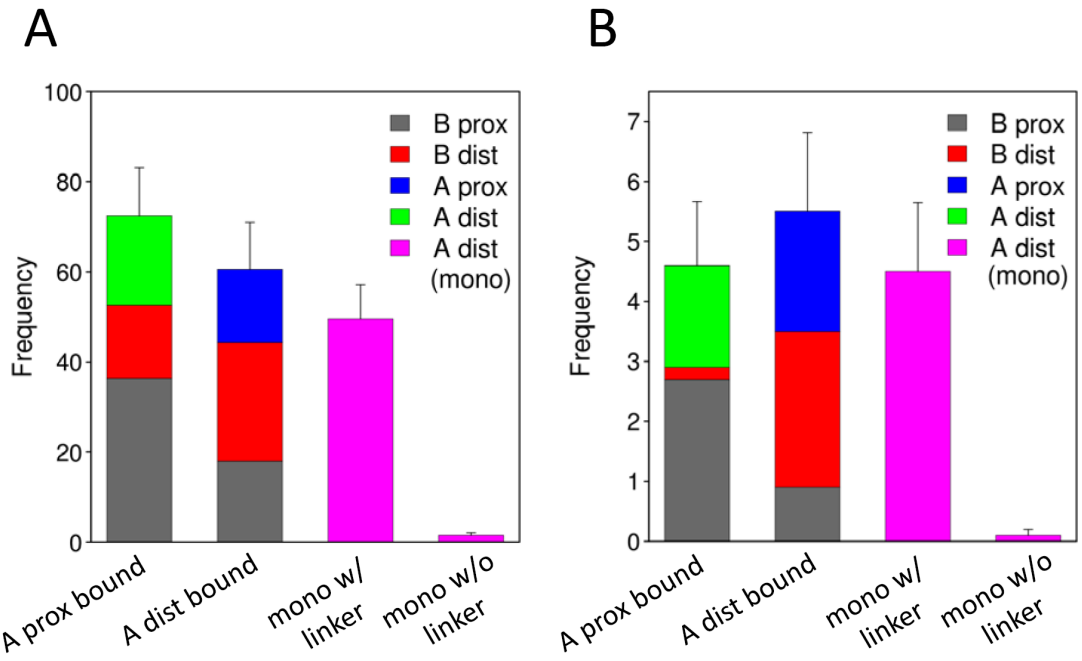


Figure S12. Results of HP1α-di-nucleosome molecular simulations where one CD is bound on the tri-methylated H3K while the other CD is free. This is similar plot to Fig.4 with the different cutoffs. The number of occurrence where the distance was below (A) 6.6 x 3 Å and (B) 6.6 x 2 Å are depicted.

Supporting Movies

Movie S1. A coarse-grained molecular simulation movie that corresponds to Fig. 3.

Movie S2. A fully-atomistic molecular simulation movie that corresponds to Fig. 9.