Multivalent di-nucleosome recognition enables the Rpd3S histone deacetylase complex to tolerate decreased H3K36 methylation levels

Jae-Wan Huh [1,3,4], Jun Wu[1,4], Chul-hwan Lee[1], Miyong Yun[1], Daniel Gilada[1], Chad A. Brautigam[2] and Bing Li[1,*]

1. Department of Molecular Biology, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390
2. Department of Biochemistry, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390
3. Current address: Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul 138-736, Korea
4. These authors contributed equally

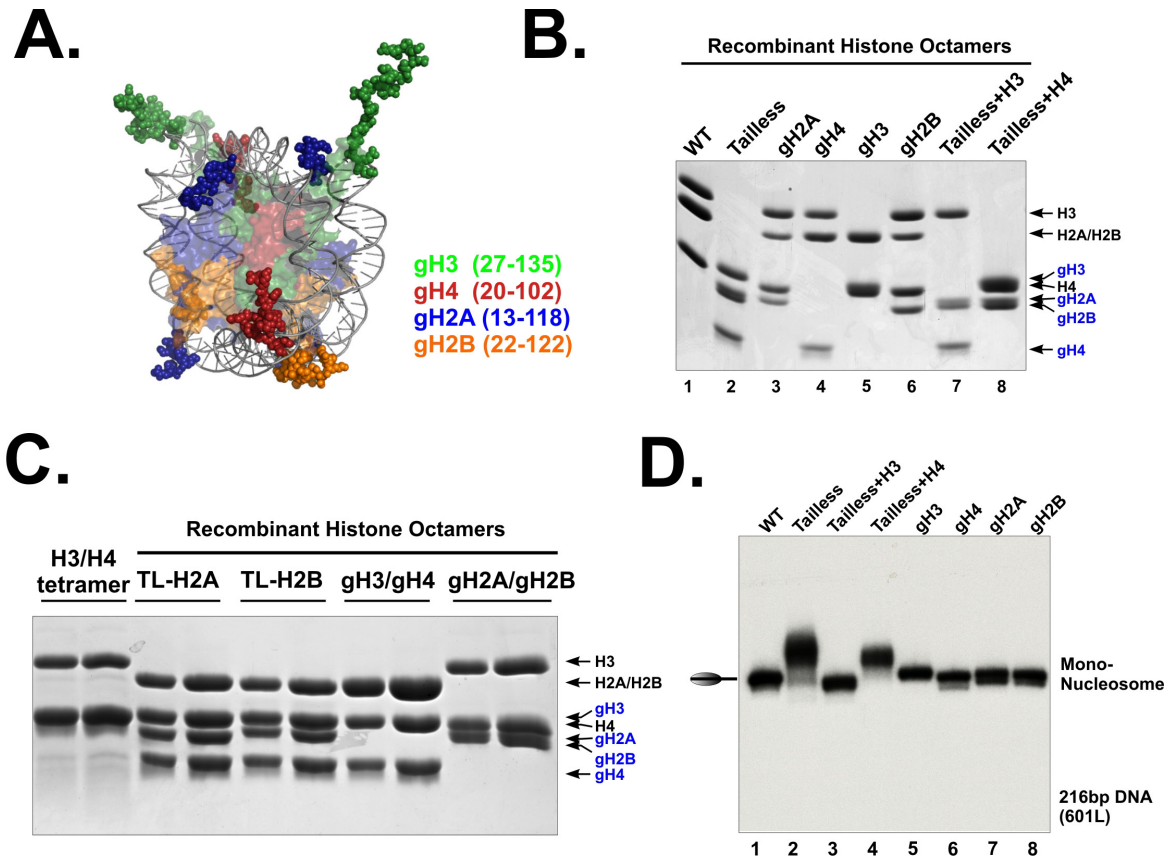
Supplementary information:

6 supplementary figures

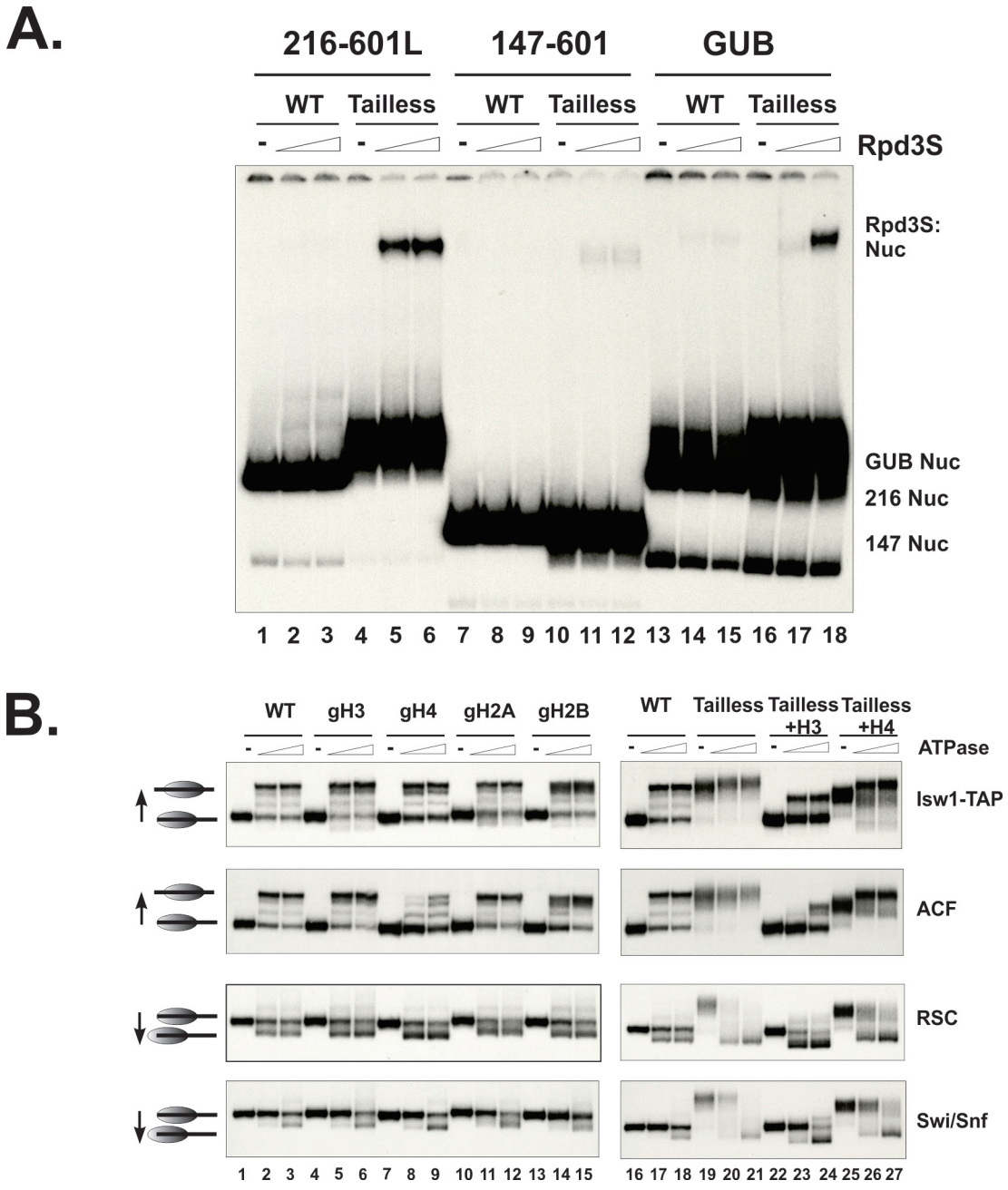
4 supplementary tables

Methods

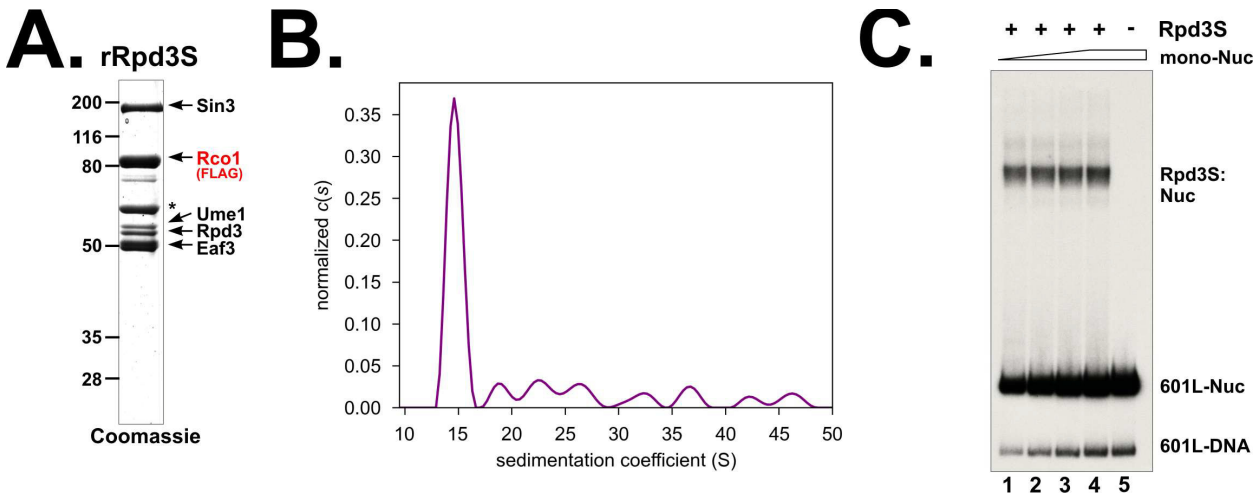
Supplementary Figures:



Supplementary Figure 1. Preparation of tailless nucleosomes.
(A) A structural view of nucleosomes (PDB 1KX5) rendered using the PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC. Truncated portions of each histone tail are highlighted, and the remaining residues are indicated on the right side. (B and C) Coomassie Blue staining of reconstituted recombinant tailless histone octamers. (D) Combinations of tailless histone octamers are reconstituted into mono-nucleosomes with a 216 bp DNA template containing a 601 sequence that is positioned at the 5'end (601L). The resulting nucleosomes are resolved on a 5% native PAGE gel.

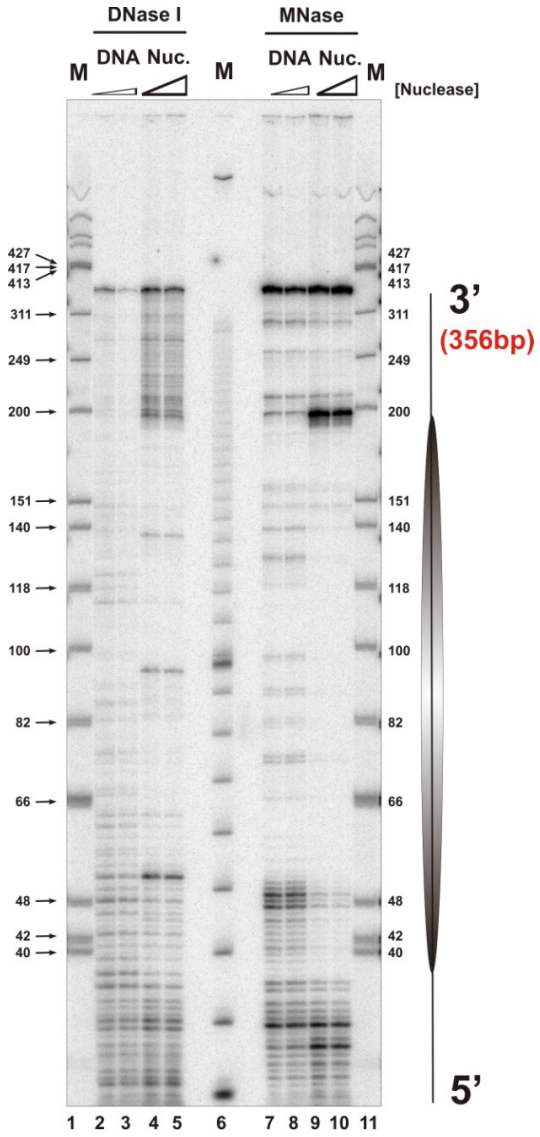


Supplementary Figure 2. Rpd3S preferentially binds to tailless nucleosomes.
(A).This experiment was carried out similarly to that shown in Figure 2, except that the 147 bp 601 DNA is amplified from pGEM-601R (Thastrom et al., 2004) and the GUB template was PCR amplified from pBL298 (pGUB ΔSH-A1)(Li et al., 2005). (B). Sliding of tailless nucleosomes mediated by chromatin remodeling complexes. The 216 bp 601L DNA templates were reconstituted with the indicated tailless histone octamers, and the resulting nucleosomes were gel-purified before being tested in sliding assays.

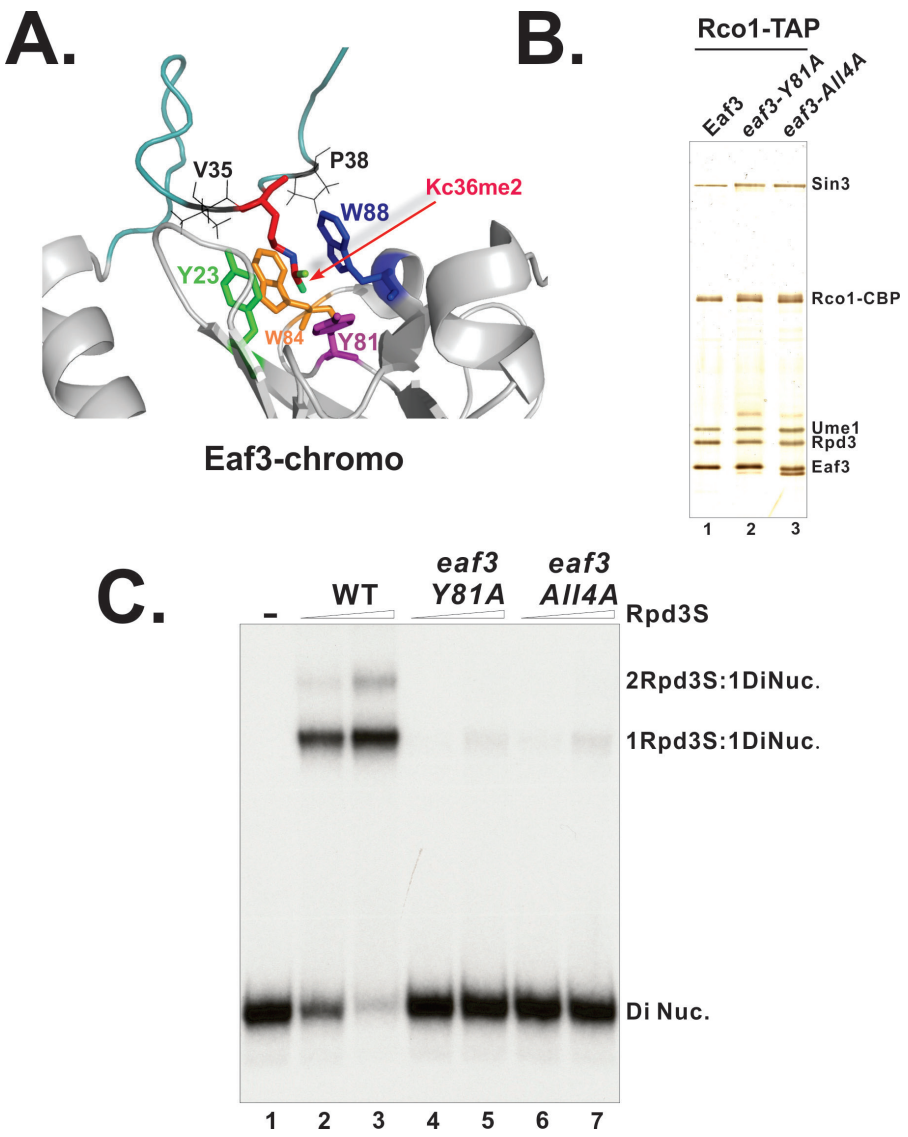


Supplementary Figure 3. Rpd3S exists as a monomer as determined by analytical ultracentrifugation sedimentation velocity analysis.

(A). Coomassie blue staining of purified recombinant Rpd3S complex which is used for AUC analysis. The asterisk represents a degraded form of Rco1-Flag. (B). Shown is the size distribution of Rpd3S normalized by the total signal (from analytical ultracentrifugation) observed sedimenting in the $s_{20,w}$-ranges displayed. (C) Rpd3S does not bind to two mono-nucleosomes at the same time. An increasing amount of mono-nucleosomes were incubated with fixed amount of Rpd3S.

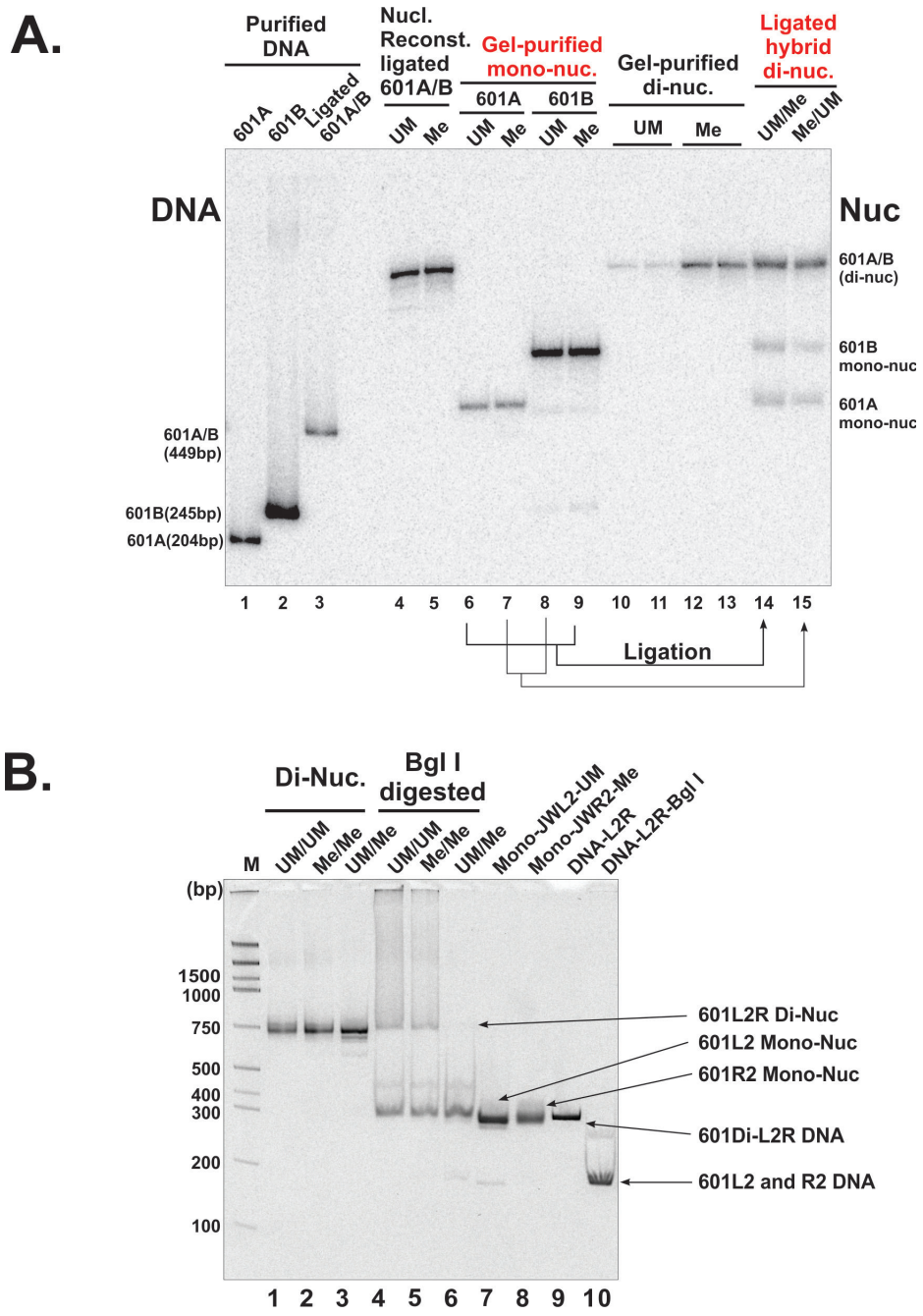


Supplementary Figure 4. Mapping the positions of 356 bp mono-nucleosomes.
By reconstituting the 356 bp DNA templates with a sub-saturating level of histone octamers, we detected a band on a native PAGE gel that migrates faster than the 356 bp di-nucleosomes prepared under standard conditions (Figure 4A, lane 13 vs. lane 16). To confirm that this band represents a mono-nucleosome species, we performed DNase I and MNase mapping of this nucleosomal population after gel purification. The results showed that only one nucleosome can be detected in this template, and its position is indicated on the right.



Supplementary Figure 5. Mutations within the chromodomain of Eaf3 compromise the binding of Rpd3S to di-nucleosome templates.

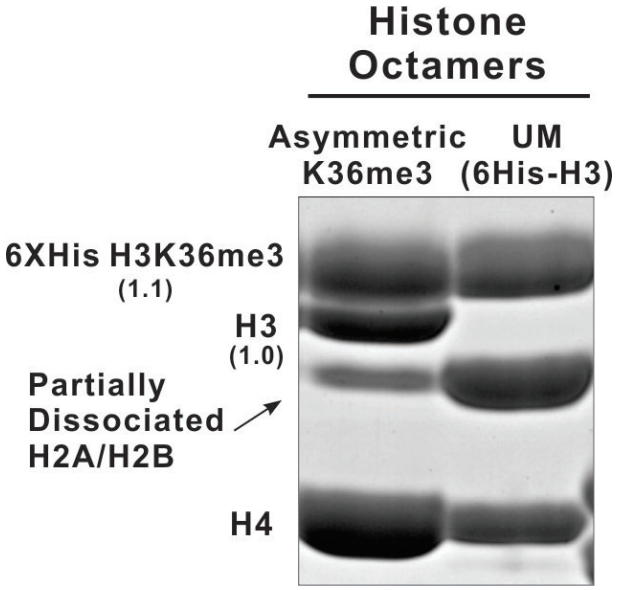
(A) A structural view of H3K36me2 binding to the chromo domain of Eaf3 (PDB 2K3Y) rendered using PyMOL. Four residues that form an aromatic cage for K36me2 (red) binding are labeled in different colored and highlighted. (B) Point mutations within the aromatic cage of CHD_{Eaf3} do not disrupt the complex integrity of Rpd3S. Wide type and mutant Rpd3S complexes were TAP-purified from yeast strains (YBL583, YBL805(a single mutation Y81A) and YBL806 (quadruple mutations of all four residues to alanine (All4A)) and visualized by silver staining. (C). Mutations within CHD_{Eaf3} compromise the binding of Rpd3S to di-nucleosome templates. Gel-mobility shift assays were carried out using wild type and mutated Rpd3S complexes, and H3K36 tri-methylated di-nucleosomes.



Supplementary Figure 6. Generation of hybrid di-nucleosomal templates.
(A). As illustrated in Figure 5A, hybrid di-nucleosomes were created by ligating two mono-nucleosomes (either unmodified or methylated) through non-palindromic restriction sites. Lanes 1 and 2, gel-purified 601A (digested by DraIII/BglI) and 601B (digested by BglI/PflMI) fragments before nucleosome reconstitution. These two templates were reconstituted with either unmodified (UM) or K36me3 (me) histone octamers, and the resulting nucleosomes were gel-purified (lanes 6-9). Mono-nucleosomes in lanes 6 and 9 were ligated and the ligation mixtures were directly loaded in lane 14. Likewise, lanes 7 and 8 were paired and the ligated products

were run in lane 15. As a control, we also ligated 601A and 601B DNA together to form a 449 bp di-nucleosome DNA template (lane 3). Using the ligated DNA, we performed standard di-nucleosome reconstitution (lanes 4 and 5), and the resulting nucleosomes served as a marker to identify correct products from nucleosome ligation reactions. Lanes 10-13 contain gel-purified di-nucleosomes that consist of two identical mono-nucleosomes.

(B). Preparation of di-nucleosomal templates for histone deacetylase assay. To form hybrid di-nucleosomes, we ligated unmodified mono-nucleosomes (lane7) with methylated mono-nucleosomes (lane8) through a non-palindromic restriction site (Bgl I). The resulting ligated di-nucleosomes were purified through native preparative PAGE. To confirm that two nucleosomes within di-nucleosome templates are each properly positioned, purified di-nucleosomes (lane1-3) were digested with Bgl I and then loaded in lane 4-6. The migration of digested mono-nucleosomes (601JWL2 and 601JWR2) indicates that all three di-nucleosomes are positioned as expected.



Supplementary Figure 7. Preparation of asymmetric methylated histone octamers.
Purified histone octamers are resolved on a 16% SDS-PAGE and stained by Coomassie blue. Wild-type octamers containing His-tagged H3 were loaded on the right as a control. The intensity of methylated H3 (6His H3K36me3) and wild-type H3 within asymmetric histone octamers are indicated in the parentheses, demonstrating an expected the ratio of 1.1 to 1.

Supplementary Tables:

Supplemental Table1. The list of chromatin templates used in this study

Chromatin templates	Description	Number of Nucleosomes	Product size (bp)	PCR based			RE digested		Figure Numbers
				Plasmid templates	Primers[a]		Vectors	Enzymes	
					1	2			
ChrT01	216-601L	Mono	216	pBL386	772	773			1A-C; 2A-F; 4A; S2
ChrT02	216-601C	Mono	227	pBL386	1041	1042			1A
ChrT03	222-601L	Mono	222	pBL630	772	1111			3C, D; 4C
ChrT04	196-Di	Di	462	pBL634	1111	1112			3A, B; 4C; S5
ChrT05	153-Tri or 153-3	Tri	565	pBL675	1111	1112			4E
ChrT06	196-Tri or 196-3	Tri	712	pBL674	1111	1112			4E
ChrT07	187-601L	Mono	187	pBL386	772	1042			4A
ChrT09	266-601L	Mono	266	pBL386	772	1126			4A
ChrT10	316-601L	Mono	316	pBL386	772	1127			4A
ChrT11	356-601C	Mono or Di	356	pBL386	1041	1127			4A; S4
ChrT12	153-Di	Di	364	pBL635	1111	1112			4B
ChrT13	Ligated ChrT19 and ChrT20	Di	452						5B, C
ChrT14	216EV-601L	Mono	222				pBL645	EcoR V	6B, C
ChrT17	147-601	Mono	147	pBL386	772	801			S2
ChrT18	GUB	Mono	216	pBL298	196	197			S2
ChrT19	601-L1 or 601A	Mono	204				pBL754	Dra III / Bgl I	S5A
ChrT20	601-R2 or 601B	Mono	248				pBL756	Bgl I / PflM I	S5A
ChrT23	Di-L2R	Di	353				pBL837	DraIII/PflMI	6D, E , F
ChrT24	601-JWL2	Mono	178				pBL897	Dra III / Bgl I	S5B
ChrT25	601-JWR2	Mono	175				pBL903	Bgl I / PflM I	S5B
ChrT26	Ligated ChrT24 and ChrT25	Di	353						6F

a. the serial number for each primer (see Supplemental Table 3)

Supplemental Table 2. The list of plasmids used in this study

Plasmids	Backbone	Plasmid description	Source
pBL298	pBend	*pGUB ΔSH-A1*	Workman JL
pBL386	pGEM-T	*pGEM-3Z/601 reverse*	Widom J
pBL455	pET3a	*Xenopus gH3 (27-135)*	Luger K
pBL456	pET3a	*Xenopus gH4*	Luger K
pBL457	pET3a	*Xenopus gH2A*	Luger K
pBL458	pET3a	*Xenopus gH2B*	Luger K
pBL624	pCR-Blunt	*pCR-147bp-601L*	This study
pBL630	pBluescript	*pBS-1X 601-196L*	This study
pBL631	pBluescript	*pBS-1X 601-153C*	This study
pBL634	pBluescript	*pBS-2X 601-196L*	This study
pBL635	pBluescript	*pBS-2X 601-153C*	This study
pBL645	pBluescript	*pBS-216L-16X*	This study
pBL670	pET21a	*pET21-gH3K36C-6His*	This study
pBL674	pBluescript	*pBS-3X 601-196L*	This study
pBL675	pBluescript	*pBS-3X 601-153C*	This study
pBL749	pCR-Blunt	*pCR-B-601Di-L*	This study
pBL750	pCR-Blunt	*pCR-B-601Di-R*	This study
pBL752	pBluescript	*pBS-Di601-448bp 1X*	This study
pBL754	pCR-Blunt	*pCR-B-601Di-L1 (Dra III/Bgl I)*	This study
pBL756	pCR-Blunt	*pCR-B-601Di-R2(Bgl I/PflM I)*	This study
pBL760	pET21a	*pET21a-6His-xH3*	This study
pBL761	pET21a	*pET21a-6His-xH3K36C*	This study
pBL762	pET21a	*pET21a-gH3K36C*	This study
pBL808	pBluescript	*pBS-2bglIM-linker4*	This study
pBL818	pBL808	*pBs-2bglI&DraIIIM*	This study
pBL819	pBL818	*pBs-2bglI&DraIIIM-linker (KBg2-EV-DraIII-PflMI-EV-Bam)*	This study
pBL837	pBL819	*pBS-2bglI&DraIIIM-linker L2R-15*	This study
pBL897	pBL818	*pBs-2bglI&DraIIIM-linker-L2X10*	This study
pBL903	pBL818	*pBs-2bglI&DraIIIM-linker-R2X8*	This study

Supplemental Table 3. The list of primers used in this study

Primers	Sequence
P196	GATCCTCTAGACGGAGGACA
P197	GATCCCTCGATTCCATGG
P772	acaggatgtatatatctgacacgtgcctgg
P773	TGACCAAGGAAAGCATGATTCTTCACAC
P801	CTGGAGAATCCCGGTGCCGAG
P1004	tctGGTACCCGGGAGATCTGATATCacaggatgtatatatctgacacg
P1005	tctGGATCCGATATCTGACCAAGGAAAGCATGATTCTTCACAC
P1006	tctGGATCCGATATCCTTCACACCGAGTTCATCCCT
P1007	tctGGATCCGATATCCTGGAGAATCCCGGTGCCGAG
P1041	tatccgactggcaccggca
P1042	GAGTTCATCCCTTATGTGA
P1111	gctctagaactagtggatcc
P1112	GGTACCCGGGCGGGAGATCT
P1126	TGACACTATAGAATACTCAAGCT
P1127	AATTTCACACAGGAAACAGCT
P1188	tctGCCATTCCGGCGGACCCTATACGCGGCCGCCCT
P1189	tctGCCggaatGGCtatccgactggcaccggcaaggt
P1190	tctGGATCCGATATCTCTTCACACCGAGTTCATCCCT
P1191	GGTATGGTATTCTAGACACTACGTGccggcaaggtcgctgttcaataca
P1193	TCTGCAGCCAATAGTTGGTCTTCACACCGAGTTCATCCCT
P1369	ctctCCCGAGCACTACGTGcgctgttcaatacatgcacagga
P1371	ctctCTCGGGGCCATTCCGGCCGCCCTGGAGAATCCCGGTGCCGA
P1377	ctctCCCGAGGCCggaatGGCacaggatgtatatatctgaca
P1367	GATCCGATATCCACTACGTGAAACCAATAGTTGGGATATCAGATCTCCCGGGTAC
P1368	CCGGGAGATCTGATATCCCAACTATTGGTTTCACGTAGTGGATATCG
P1411	ctctCTCGGGCCAACTATTGGCCCTATACGCGGCCGCCCTGGA

Supplemental Table 4. Yeast strains used in this study

Name	Parental Strain	*Genotype*	Source
YBL202	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Rsc2-TAP::HIS3MX6*	Open Biosystems
YBL218	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 isw1-TAP::HIS3MX6*	Open Biosystems
YBL220	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 chd1-TAP::HIS3MX6*	Open Biosystems
YBL291	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Epl1-TAP::HIS3MX6*	Open Biosystems
YBL489	JLW553	*MAT**a**, ura3-1 lys2Δ::hisG trp1-1 his3-11,15 leu2-3,112 can1-100* ***SNF6TAP:KlTRP1, SWI2HA:KANMX6***	Workman J.
YBL583	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Rco1-TAP::HIS3MX6***	Open Biosystems
YBL584	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Ada2-TAP::HIS3MX6***	Open Biosystems
YBL619	YBL555	*MAT**alpha** his3Δ1 leu2Δ0 lys2Δ 0 ura3Δ0* ***eaf3-chd77-113*****Δ Flag:Leu2** ***Rco1-TAP::HIS***	*(Li et al., 2007)*
YBL634	YBL632	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Rco1-ΔPhD-TAP:HIS***	*(Li et al., 2007)*
YBL805	YBL742	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Eaf3-CD1-Y81A RCO1-C-TAP::HIS***	This study
YBL806	YBL745	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Eaf3-CD1-Y23A/Y81A/W84A/W88A RCO1-C-TAP::HIS***	This study

Methods:

Plasmid constructs

Vectors that contain different numbers of repeating sequences for nucleosome reconstitution were generated using two strategies:

(1) *Step-wise propagation of repeating sequences using a KpnI/BglI/BamHI cassette (Dyer et al., 2004)*. The basic units of each repeat were amplified from the pBL386 (pGEM-601R) plasmid (Thastrom et al., 2004) using specific primer sets listed in Supplemental Table S4. For instance: *for the 196L fragment* (a 196 bp DNA with the 601 positioning sequence being at the lateral position), primers P1004 and P1006 were used; whereas P1004 and P1007 were paired to amplify *the 153C fragment* (a 153 bp DNA with 601 locating at the center). Restriction-digested PCR products were then inserted into pBluescript vector at the BamHI/KpnI site to form pBL630 and pBL631, which contain one copy of each 601-containing fragment. Multiple copies of these single units were then built round-by-round using the KpnI/BglI/BamHI cloning strategy (Dyer et al., 2004) to make pBL634, pBL635, pBL674 and pBL675. pBL645, pBL674, pBL675 and pBL811 were also generated in similar fashion.

(2) Tandem ligation to generate multiple repeats of non-palindromic DNA fragments.

To create DNA fragments for the nucleosome ligation experiments (Figure 5A and Supplementary Figure 6A), a 204 bp DNA fragment (601A) and a 245 bp DNA (601B) were generated by PCR from pBL386 using primer set P1191/P1188 and P1189/P1193, respectively. Both PCR products were cloned into pCR-Blunt vectors to create pBL754 and pBL756. To engineer a vector free of the Bgl I site, pBluescript vector was subjected to multiple rounds of PCR mutagenesis to remove two internal Bgl I sites, and a non-palindromic AvaI (CCCGAG) linker was subsequently inserted between two original AvaI sites to generate pBL808. We further removed a DraIII site from pBL808 to generate pBL818. An adaptor DNA was made by annealing primer P1367/P1368 and inserted into pBL818 to form pBL819. To prepare a large quantity of ligatable DNA for HADC assay substratues, the 601JWL2 and 601JWR2 sequences were amplified from pBL386 using primers P1369/P1371 and P1377/P1411, all of which contain a compatible non-palindromic AvaI site at the 5'ends. Purified PCR products were digested by AvaI and ligated into AvaI-digested pBL818 to generate pBL897 (10 copies of 601JWL2) and pBL903 (8 copies of 601JWR2). DraIII/BglI fragment from pBL897 and BglI/PflMI fragment

from pBL903 were mixed together and ligated into pBL819 to form pBL837, which contains one copy of Di-L2R (ChrT23) that can be released by DraIII/PflMI double digestion.

Nucleosome preparation and chromatin-based biochemical assays

1. Preparation of recombinant histone octamers:

Recombinant *Xenopus* histones were individually purified and reconstituted into histone octamers using a previous protocol (Li et al., 2007). Histone H3K36 methylated histones were prepared using the Methyl-Lysine Analog (MLA) approach (Li et al., 2009; Simon et al., 2007). The completion of the chemical reaction was confirmed by mass-spectrometry analysis and/or western blotting.

Preparation of asymmetrically modified histone octamers (within a histone octamer, one histone H3 is methylated at K36 and the other one is unmethylated) was based on a published method (Li and Shogren-Knaak, 2008). Briefly, pBL761 was transformed into BL21(DE3) codon plus cells to over-express HIS tagged Xenopus H3K36C mutant (6His-xH3K36C). Recombinant histones were purified under the standard denaturing condition, and subjected to the chemical modification (MLA) as described above to make 6His-xH3K36me3. We then mixed 130.5nmol of wild-type histone H3, 14.5nmol of 6His-xH3K36me3, and 145nmol of each xH4, xH2A and xH2B. Histone octamers were isolated using a Superdex 200 gel-filtration column. After octamer-containing fractions were pooled, 150µl of pre-washed TALON affinity resin (Clontech) was added into the mixture and incubated at 4°C for 3hrs. Resins were then washed with 4 ml of AM-Ni-wash buffer (10mM Tris PH7.5, 2M NaCl and 20mM Imidazole) and eluted with 1ml of AM-Ni-elution buffer (10mM Tris PH7.5, 2M NaCl and 250mM Imidazole). The eluent was equilibrated in 10mM Tris PH7.5, 2M NaCl and 5mM βME, and concentrated. For some unknown reason, H2A/H2B dimer was partially dissociated from asymmetric octamer (Supplementary Figure 7) when TALON resin was used. To compensate for this loss, a proper amount of H2A/H2B dimer was added along with asymmetric octamers to ensure normal nucleosome formation.

2. Chromatin templates:

All DNA templates used for nucleosome reconstitution were listed in ***Supplemental table 1***, and sorted by the Chromatin Template (ChrT) number.

1) Small-scale preparation of radio-labeled probes:

DNA templates used in Figure 2-5 were end-labeled as previously described (Li et al., 2007). Di-nucleosome and tri-nucleosome DNA probes (ChrT04, ChrT05, ChT06 and ChrT12) were PCR amplified from corresponding vectors (pBL634, pBL635, pBL674 or pBL675) using the same primer set- P1111/P1112

2) Large-scale DNA template preparation:

Mono-nucleosome templates (ChrT14: 216EV-601L) were isolated using a PEG precipitation based method (Dyer et al., 2004) from 10mg EcoRV-digested pBL645 plasmid. Di-nucleosome templates (ChrT23: Di-L2R) were purified through a preparative 3.5% native PAGE electrophoresis (Prep Cell 491, BioRad) starting from 5 mg DraIII/PflMI-digested pBL837. To prepare mono-nucleosome templates bearing non-palindromic ends (ChrT24, ChrT25) for nucleosome ligation experiments, 5mgs of each pBL897 and pBL903 were digested with DraIII/BglI or BglI/PflMI, respectively, and the intended DNA fragments were purified through a preparative 4% native PAGE electrophoresis.

3. Nucleosome preparation:

1-Reconstitution using radio-labeled probes: Nucleosome reconstitutions were carried out using a modified salt-dilution method (Owen-Hughes et al., 1999). Briefly, 1 pmol of gel-purified radio-labeled probe DNA (mono-, di- or tri-nucleosomal templates) was mixed with 2μg cold plasmid DNA, approximately 3 μg recombinant histone octamers (individually determined through titration), 100ng of BSA and 2 μl of 5M NaCl. The final volume was brought to 10 μl with H_2O and serial salt dilution steps were followed as described previously (Li et al., 2009). The resulting nucleosomes were gel-purified and stored at 4°C (Yun et al., 2012).

2-Large-scale nucleosome preparation: The optimal ratios of histone octamers and any given DNA were determined using a similar salt-dilution method as described above, except that 1μg of cold DNA template was tested in each condition without any competitor plasmid DNA. 300μg of histone octamers were mixed with a proper amount of template DNA in a 1.25ml system in 2M NaCl. The mixtures were stepwise dialyzed against 300ml of each TEB (10mM Tris.HCl pH7.5, 1mM EDTA and 1mM β-ME) containing 2M, 1.2M, 1M, 0.8M, 0.6M NaCl for 2hrs at

30°C, and finally switched to 500ml TEB for 2 hrs at 4°C. All nucleosomes were then purified through preparative electrophoresis (Dyer et al., 2004).

3-Preparation of hybrid di-nucleosomes: To prepare radio-labeled hybrid di-nucleosomes for EMSA assays (Figure 5A), 100 µg of pBL754 (601A) and 100 µg of pBL756 (601B) plasmids were digested with DraIII/BglI or BglI/PflMI, respectively. The desired mono-nucleosome fragments were purified from a 2% agarose gel. Then, 1.5 µg of each (601A and 601B) fragment was end-labeled in a 40 µl PNK exchange reaction by adding 8 µl of 5X PNK exchange buffer (250 mM imidazole pH 6.4, 350 µM ADP, 60 mM $MgCl_2$, 5 mM βME, 1 µl of γ-ATP and 3 µl of PNK from NEB). Reactions were carried out at 37°C for 1 hour followed by 55°C heat inactivation for 5 min. DNA was purified through a Qiagen PCR purification spin column. For each template, radioactivity incorporation (cpm/µl) was measured by a liquid scintillation counter, and DNA concentration (ng/µl) was determined by electrophoresis with standard DNA markers. The ratios of cpm over ng were then used to calculate and track the amount of DNA in the subsequent steps. Mono-nucleosome reconstitutions of 601A and 601B templates were carried out separately as described above. Nucleosomes were gel-purified and then mixed in pairs at equimolar amounts (shown in Supplementary Figure 6A). These mixtures were concentrated to a final volume of 45 µl using 30KD Amicon ultra 0.5 concentrators (Millipore). Then, 5 µl of 10X T4 ligase buffer and 10 units of T4 DNA ligase (NEB) were added. The ligation reactions were incubated at 16°C for 3 hrs before directly loading on a 4.5% native PAGE gel for purification.

To prepare a large quantity of hybrid di-nucleosomes for HDAC assays, 200µg of purified 601JWL2 (ChrT24) mono-nucleosomes and 220µg of purified 601JWR2 (ChrT25) mono-nucleosomes were mixed in a 300µl reaction containing 30µl 10X T4 ligase buffer, 3µl of 100mM ATP. 15µl of T4 ligase was then added and reactions were incubated at 16°C for 12 hrs. The resulting di-nucleosomes were finally purified through a preparative 3.5% native PAGE purification and concentrated. The quality of each nucleosome was confirmed as shown in Supplementary Figure 6B.

4. Nucleosome mapping by DNaseI and MNase

^{32}P-end-labled nucleosomes (~10,000cpm) were incubated with different amounts of DNase I and MNase (Worthington) at room temperature for 5 mins in a 15ul reaction system. The reactions were stopped by adding 120µl of STOP buffer (0.3MNaAC, 5mM EDTA, 0.1%SDS and 40µg/ml linear Acrylamide (Ambion)) and incubating at 55°C for 15mis. Control and Digested DNA was then purified and resolved on a 8% denaturing (urea) poly-acrylamide gel (Li and Reese, 2001).

Analytical Ultracentrifugation. The Rpd3S complex was prepared for centrifugation by dialyzing it extensively against a buffer containing 20 mM Tris pH 7.5, 50 mM NaCl, 0.02% IPEGAL, 0.17 mM EDTA, and 0.17 mM β-mercaptoethanol. The protein was placed in the sample sector a charcoal-filled Epon dual-sectored centerpiece that had been sandwiched between two sapphire windows and placed in a centerpiece housing. The dialysate was placed in the reference sector. The housing was positioned in an An50-Ti rotor (Beckman-Coulter) and placed in the centrifuge. This assembly was allowed to incubate overnight at 4 deg. C under vacuum. Afterwards, centrifugation was performed by rotor acceleration to 42,000 rpm. The data were analyzed using the c(s) distribution (Schuck (2000), Biophys. J., vol. 78, 1606) with systematic noise subtraction (Schuck & Demeler (1999), Biophys. J., vol, 76, 2288). Values for partial-specific volume, buffer density, and buffer viscosity were estimated using SEDNTERP (T.M. Laue, B.D. Shah, T.M. Ridgeway and S.L. Pelletier, Analyticall Ultracentrifugation in Biochemistry and Polymer Science, Royal Society of Chemistry (Edited by S. Harding and A. Rowe), 1992, pp. 90-125.)

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