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Supporting Online Material for

Combined Action of PHD and Chromo Domains Directs the Rpd3S HDAC to Transcribed Chromatin

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This PDF file includes:

Combinatorial action of the Rco1 PHD domain and the Eaf3 chromodomain targets the Rpd3S complex to deacetylate transcribed chromatin

Bing Li, Madelaine Gogol, Mike Carey, Daeyoup Lee, Chris Seidel and Jerry Workman

Supporting Online Material:

Material and Methods:

Plasmid constructs and yeast strains:

All *S. cerevisiae* strains (see Supplemental Table S1) are derived from either S288C or w303a background.

In order to engineer the mutations at genomic loci for the purpose of complex purification, a modified *delitto perfetto* (Storici and Resnick, 2006) strategy was employed. First, full-length *YNG2* and *RCO1* genes were cloned into the pCR-Blunt vector (Invitrogen) and then subjected to PCR-mediated mutagenesis. The resulting plasmids (pBL467, pBL468) contained the restriction enzyme sites of Age1 positioned at both sides of the targeted PHD domain. After Age1 digestion, the vector fragments were either self-ligated (to generate deletion construct-pBL472), or ligated with swapped inserts (to produce chimeric fusion plasmids pBL471 and pBL473). Next, knock-in yeast strains were constructed by transforming the PCR products that contain the CORE-I-SceI cassette and the homology regions corresponding to integration loci (Storici and Resnick, 2006). The URA+ and KAN+ colonies were selected, and confirmed by PCR analysis (YBL631 and YBL646). Finally, PCR products amplified from pBL467 and pBL468, which includes at least 100 bp of DNA flanking each side of PHD region, were denatured and transformed into YBL631 and YBL646 with carrier DNA (Storici and Resnick, 2006). The transformants were grown on YPD plates overnight before replication onto 5-FOA plates to screen for the pop-out of the integration cassette (URA). The colonies were then restreaked on G418 plates to confirm the loss of the KAN marker. The final

URA- and KAN- colonies were subjected to genomic sequencing. YBL694 was generated though a cross between YBL555 and YBL634, and YBL695 was from a cross between YBL555 and YBL677.

Protein purification

Recombinant Gst-Dot1, Gst-Set2, Gst-HYPB-SET and Gst-PHD were overexpressed in bacteria and purified through Glutathion-sepharoase (Amersham) according to manufacturer's instructions (Li et al., 2003). Rpd3S (Rco1-TAP), NuA4 (Epl1-TAP) and SAGA (Spt7-TAP) were purified through the standard tandem affinity purification (TAP) method (Li et al., 2003).

Nucleosome manipulation and EMSA assay

DNA templates used in the EMSA assays were PCR amplified from the pGEM-601R plasmid (Thastrom et al., 2004) using one 5' biontinylated primer (including a restriction enzyme linker) and one regular primer (to control the length of DNA). DNA was then gel purified prior to being reconstituted with recombinant *Xenopus* core histones via the serial dilution method (Li et al., 2005). Resulting mono-nucleosomes were then immobilized onto streptavidin-coated magnetic Dynal 280 beads in the final reconstitution buffer by overnight incubation at 4°C. Beads were washed twice in 1ml of wash buffer H (25mM HEPES.KOH pH7.6, 0.5mM EDTA pH8.0, 0.1mM EGTA pH8.0, 2.5mM MgCl2 , 10% Glyerol, 0.02% NP40, 1mM DTT, 0.1mg/ml BSA and 600mM KCl) after each step as illustrated in Figure1A. Histone modification reactions were performed in the presence or absence of 5μM cold Acetyl-CoA or SAM as described previously (Li et al., 2003) with one exception. 0.1mg/ml BSA was added in all reactions to stabilize the diluted nucleosomes. Methylation by rSet2 and rDot1 are of high efficiency. Based on the radioactivity incorporation, methylation should occur in more than 90% of the nucleosomal population, assuming they are in the di-methylated state. Acetylation reactions are also very robust, since they lead to slower migrating bands in the EMSA assay. Modified nucleosomes were finally released from magnetic beads by overnight incubation with EcoRV in 100ul of the digestion buffer D (10mM Tris.PH 7.9, 100mM NaCl, 2mM MgCl2, 5mM BME, 0.04% NP40, 0.1mg/ml BSA) at 37°C. The supernatant

was collected and supplemented with 25ul of 5X storage buffer (buffer D plus 50% glycerol) and kept at 4°C for no more than 2 months. All nucleosomes indicated either unmodified or modified are reconstituted using recombinant core histones. EMSA experiments were performed at 30°C in 15ul of EMSA reaction buffer (10mM HEPES pH7.8, 50mM KCl, 4mM MgCl2, 5mM DTT, 0.25mg/ml BSA, 5% Glycerol and 0.1mM PMSF). The total reactions were directly loaded onto a 3.5-5% native polyacrylamide gel (37.5:1) in 0.3 x TBE (In Figure 4B and Figure S11, 0.3x TBE plus 1% glycerol buffer was used). Electrophoresis was carried out at 4°C for 4-5 hours.

Chromatin Immunoprecipitation and ChIP-chip:

ChIP was performed essentially as described in two previous publications (Li and Reese, 2001; Strahl-Bolsinger et al., 1997). Immunoprecipitations were carried out using 1ul of anti-acetylated H4 antibody (Upstate), 1 ul of Rabbit IgG (Sigma) and 10 ul of anti-Eaf3 antibody (Abcam). The primer sets used in Figure4C and Figure S8 are *STE11* -432 and -157 for promoter; and *STE11* +904 and +1196 for 3' ORF as described in (Carrozza et al., 2005). Immunoprecipitated DNA was quantified by real time PCR using the DyNAmo™ qPCR Kits (NEB) on the Opticon® 2 Continuous Fluorescence Detection System (MJ Research).

DNA microarrays used for ChIP-chip are manufactured by Agilent technologies with the probe design from (Pokholok et al., 2005). Our amplification and labeling procedures are adopted from earlier publications (Iyer et al., 2001; Li et al., 2005). Briefly, in Round A amplification, a degenerate primer with an universal oligo tag at the 5' end are used in a two-round primer extension reaction catalyzed by T7 sequenase version 2.0 (USB); 1/6 of Round A products are then subjected to Round B amplification with ExTaq (Takara) using the universal primer. DNA is subsequently purified using a Qiagen PCR purification kit and chemically labeled through a platinum-based coordination compounds as previously described (Heetebrij et al., 2003). All microarray based experiments were carried out at least in duplicate. The final datasets are generated from the median values of all repetitions with Pearson correlation exceeding 0.85.

Microarray data analysis:

Final datasets from ChIP-chip experiments were pipelined into a modified average gene analysis based on the frame work originated by the Young laboratory (Pokholok et al., 2005). Basically, the coding region of each gene in the yeast genome was divided into 40 equally sized bins. The upstream and downstream intergenic regions for the gene were each divided into 20 equally sized bins. The microarray expression value for each probe was assigned to the closest bin (which ever bin midpoint was closest to the probe midpoint). Empty bin values were calculated using linear interpolation from the last and next non-zero expression values. In this way, an expression matrix was generated with 80 columns (corresponding to the intergenic and ORF bins) and a row for each gene in the yeast genome. Each column was then averaged throughout entire matrix to create the average expression values for each bin position.

Peptide binding assay:

The reactions were carried out in 300 µl binding buffer (50mM Tris pH7.5, 300mM NaCl and 0.1% NP-40) with 1µg biotinylated histone peptides (H3K4me set (Upstate) and H3K36me set (custom made by Sigma) (Carrozza et al., 2005)) and 1 µg of each GST fusion protein. The mixtures were incubated at 4°C for 5 hours before adding 30 µl (50% slurry) of pre-equilibrated streptavidin sepharose beads (Amersham). After another hour of incubation at 4°C, the beads were washed with 1 ml of binding buffer for three times at room temperature. Proteins bound to the beads were eventually eluted off by boiling in 1xSDS-PAGE loading buffer and western blotted using anti-GST antibody (Santa Cruz).

Supplemental Figures:

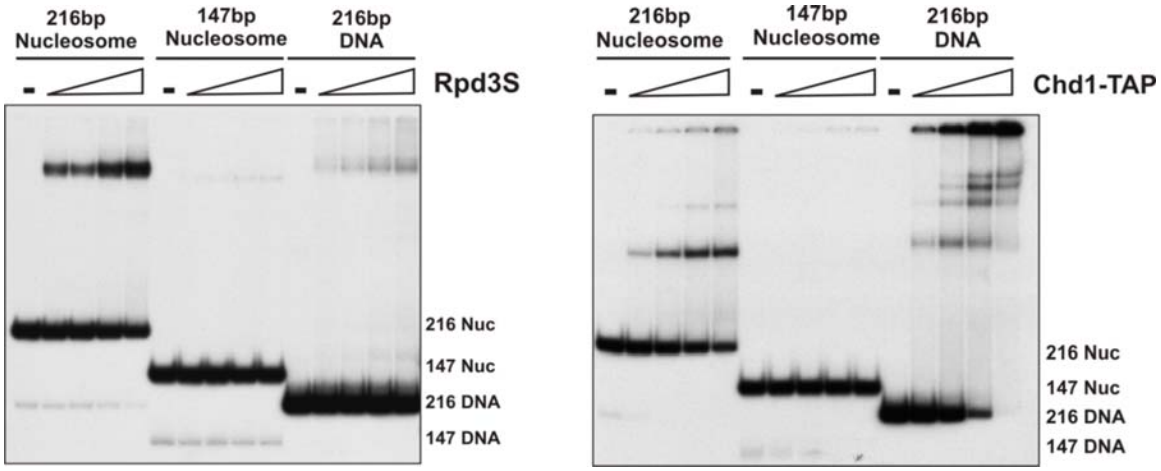


Figure S1. Linker DNA and histone contact are essential for Rpd3S nucleosome binding. The 147bp DNA is directly amplified from the 601 nucleosome position sequence (Thastrom et al., 2004) and the 216bp DNA is the 601 sequence plus a 69bp DNA linker at the 3' end. The end-labeled DNA fragments were reconstituted into mononucleosomes with HeLa short oligo nucleosomes (SON) using the octamer transfer method. The resulting nucleosomes are gel-purified before being subjected to EMSA assays. Rpd3S weakly binds to DNA (216bp-DNA) and nucleosomes without linker DNA, but has high affinity to the nucleosomes with a linker. Chd1-TAP is a representative of common chromatin-related complexes that tightly bind to DNA but can not bind to nucleosomes without the linker DNA.

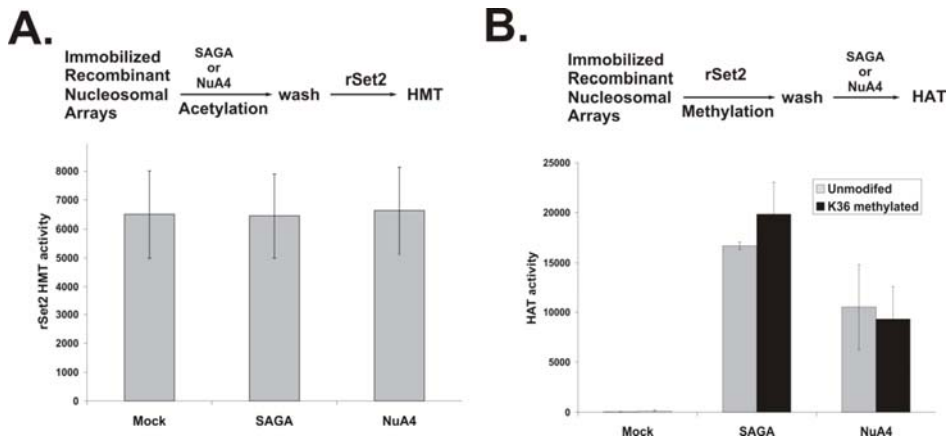


Figure S2. The interplay between acetylation by SAGA or NuA4 and methylation by rSet2 is minimal. Recombinant histone octamers were reconstituted into nucleosomal arrays using biotinylated 1.5 KB-pBluescript fragments (A). Histone methyltransferase activity was measured using the immobilized templates pre-acetylated by SAGA or NuA4. (B). HAT assays were performed on K36 nucleosomes methylated by rSet2.

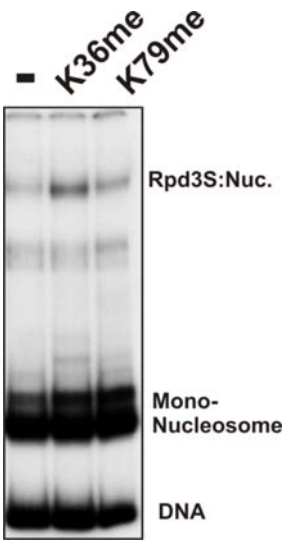


Figure S3. Rpd3S specifically recognizes K36 methylated but not K79 methylated nucleosomes. Mononucleosomes used in this assay were mock-methylated, methylated by rSet2 or rDot1, respectively.

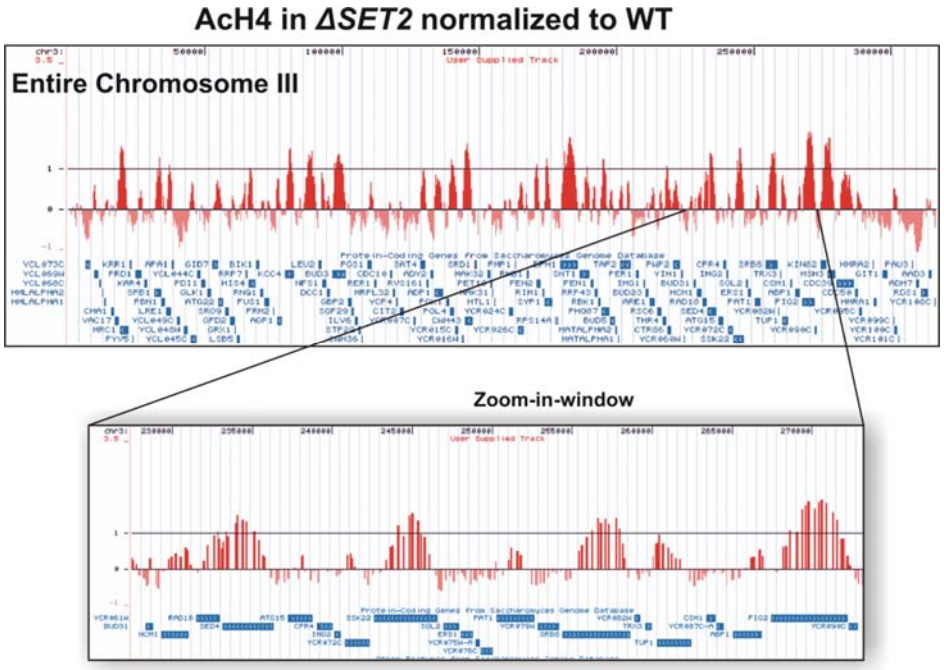


Figure S4. Chromosomal views of distribution pattern changes of acetylated histone H4 upon deletion of SET2. Genome wide localization study of acetylated histone H4 was carried out using WT and Δ*set2* yeast strains as described in Figure 4B. Log2 ratio of the enrichment of acetylated H4 in Δ*set2* over the enrichment of acetylated H4 (AcH4) in WT was plotted as a function of corresponding chromosome coordinates and displayed in the Genome Browser format (UCSC). The upper window displays the overall distribution profile across the entire chromosome III, with one region being detailed in the zoom-in window.

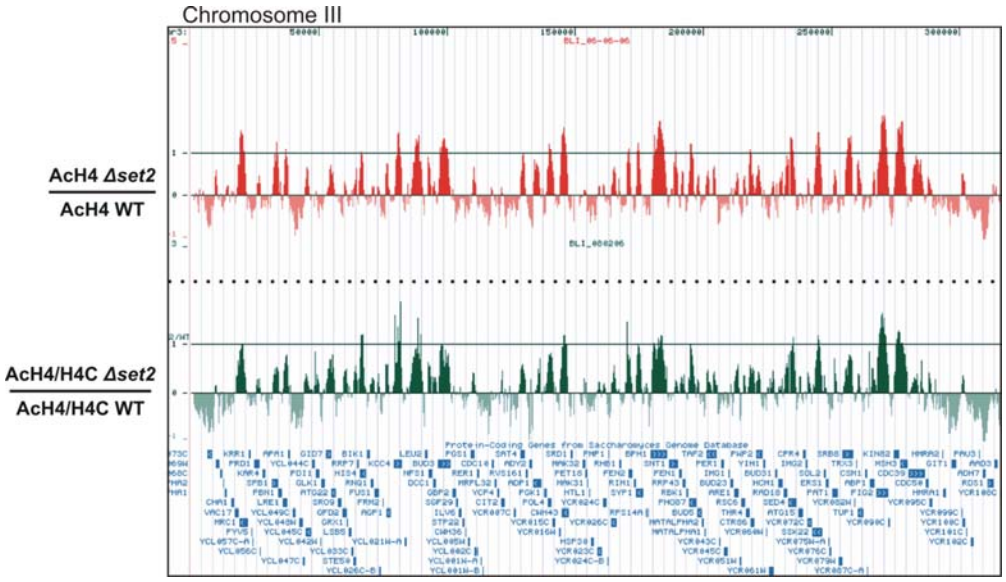
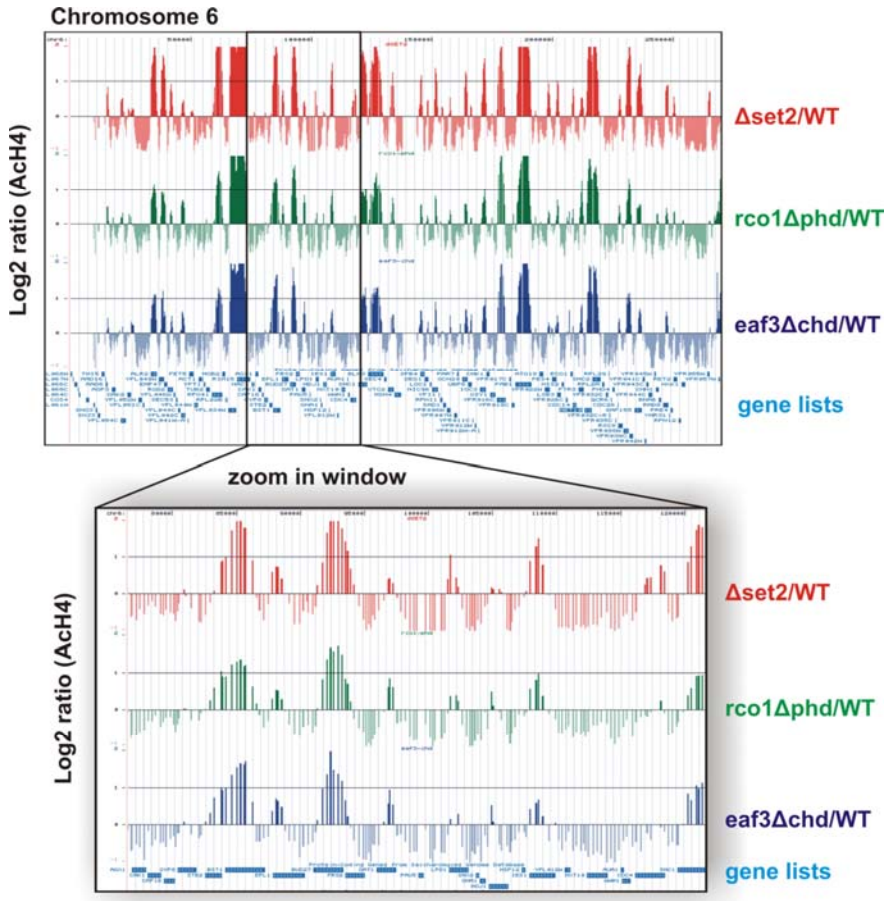


Figure S5. An alternative way to normalize genome wide histone distribution changes upon deletion of SET2. Chromatin immunoprecipitation assays were performed using either an antibody against acetylated H4 (Upstate) or an antibody recognizing unmodified H4 c-term tail (Abcam) in WT and Δ*set2* strains. Immunoprecipitated DNA and input DNA from each combination were subjected to competitive hybridization on high resolution tiling arrays. Log2 Ratio of immunoprecipitated DNA over Input (or total chromatin) was defined as the enrichment value for each antibody. Since we were primarily interested in the distribution changes between WT and mutants, rather than the distribution pattern in any given condition, the Log2 ratios of enrichment values of Δ*set2* over WT were used in all analyses shown in Figure 5 and the upper panel of this figure. Given the background problem raised by Pokholok and colleagues in their recent paper (Pokholok et al., 2005), we performed the suggested control experiments. In this experiment, the distribution of AcH4 from each strain was normalized to the histone H4 density (H4C) as measured by ChIP-chip using the antibody against unmodified histone H4 c-terminal region (H4C from Abcam). The Log2 ratios of normalized AcH4 in Δ*set2* over normalized AcH4 in WT were then displayed in the genome browser format (the lower panel). These two analyses gave rise to almost identical results. For consistency and economical considerations, we chose to use the ratio of enrichment values as the indication of distribution changes.



FigureS6 PHD_{Rco1} and CHD_{Eaf3} are required for regulating global acetylation at ORFs
Wild type yeast and strains bearing *eaf3*Δchd (YBL619), *rco1*Δphd (YBL632) or *set2Δ* mutations were subjected to ChIP-chip analysis using anti-acetylated H4 antibody. The immunoprecipitated (IP) DNA and total chromatin (input) were labeled with florescent dyes and hybridized on high resolution tiling arrays manufactured by Agilent technologies. The enrichment values were first calculated using the log2 ratio of IP versus input. Subsequently, the enrichment of AcH4 from each mutant was normalized to WT and displayed in the *Genome Browser* format (UCSC). Chromosomal views show distribution changes of acetylated histone H4 upon each indicated mutation.

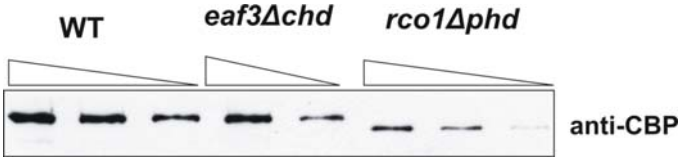
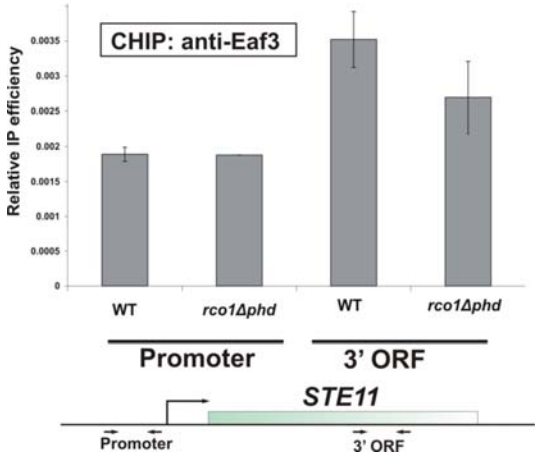


Figure S7. Quantification of Rpd3S complexes Rpd3S complexes (WT, *eaf3*Δchd and *rco1*Δphd) were purified using the standard TAP method (Li et al., 2003) and separated on a SDS-PAGE gel followed by western blotting using antibody against the Calmodulin binding protein (CBP) peptide. The intensity of each band was then quantified using *ImageQuant* (GE Science) software.



FigureS8. The PHD domain of Rco1 is important for targeting Rpd3S complex to the STE11 coding region. Formaldehyde cross-linked whole cell extracts from wild-type (BY4741) and *rco1Δphd* (YBL632) were subjected to immuoprecipitation using 10 µl of anti-Eaf3 antibody. Purified DNA was quantified by real-time PCR using indicated primer sets.

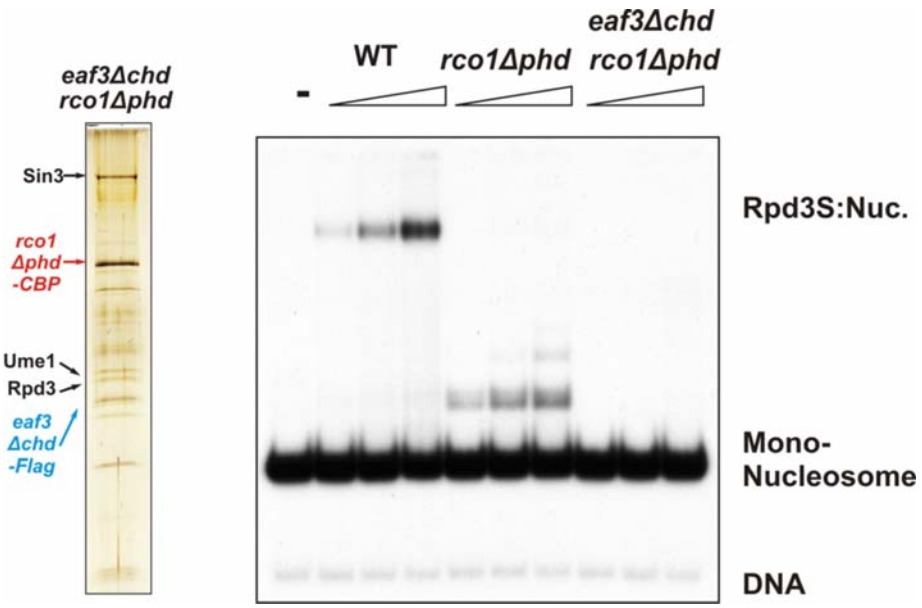


Figure S9. Deletion of PHD_{Rco1} and CHD_{Eaf3} results in the disruption of Rpd3S integrity and its binding to nucleosomes. The mutant Rpd3S was purified through Rco1-TAP from YBL694. (A) Silver staining (B) EMSA assay were performed using mono-nucleosomes reconstituted via octamer transfer from HeLa oligonucleosomes.

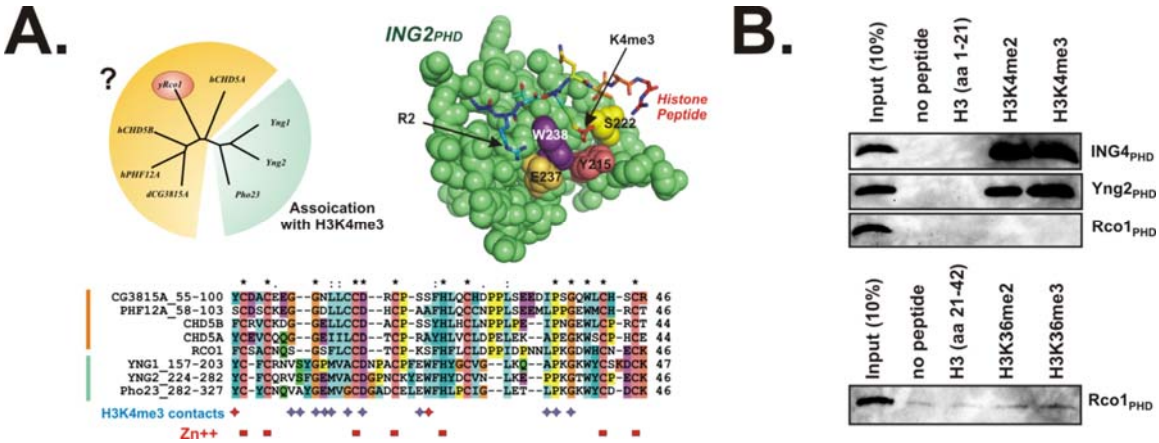


Figure S10. The PHD domain of Rco1 is structurally different from the ING family PHD domain and it does not bind to tri-methylated H3K4 peptides

(A). Upper left panel. Phylogenic tree analysis of the PHD domains of three ING family members (shaded in green) and the PHD domains of yeast Rco1 and its homologs in higher organisms using *PHYLIP (PHYLogeny Inference Package)* software *(*University of Washington). The crystal structure of the ING2 PHD domain binding to K4 trimethylated peptides (PDB#: 2G6Q) was rendered using *PyMOL* software (DeLano Scientific LLC.) The Four residues in ING-PHD domains that are important for contact with H3K4me3 peptides are highlighted. Sequence alignment of above PHD domains (Rco1 and its homologues) was performed using the *Clustal W* software. (B) Peptide pull down assay was performed to test the binding of Gst-Ing4$_{PHD}$, Gst-Yng2$_{PHD}$ and Gst-Rco1$_{PHD}$ to indicated histone H3 C-terminal peptides.

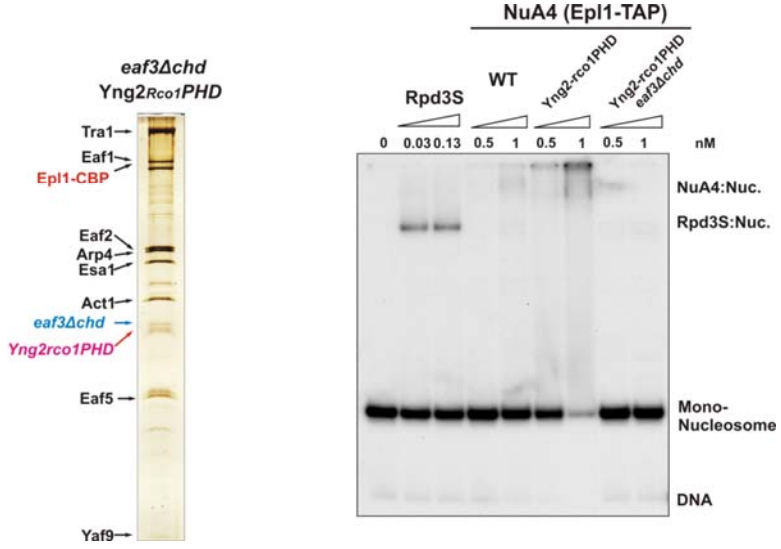


Figure S11. Combination of CHD$_{Eaf3}$ and PHD$_{Rco1}$ cause an artificially engineered NuA4 complex to have high affinity for nucleosomes.

Supplemental Table 1. Yeast strains used in this study

Name	Parental Strain	Genotype	Source
YBL250	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Δset2::KAN***	Open Biosystems
YBL555	YJW677	*MAT***alpha** *his3Δ1 leu2Δ0 lys2Δ 0 ura3Δ0* ***eaf3-chd77-113*Δ Flag:Leu2**	Carrozza M
YBL583	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Rco1-TAP::HIS3MX6***	Open Biosystems
YBL609	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***ΔJHD1:KAN***	Open Biosystems
YBL619	YBL555	*MAT***alpha** *his3Δ1 leu2Δ0 lys2Δ 0 ura3Δ0* ***eaf3-chd77-113*Δ Flag:Leu2**	This study
YBL631	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Rco1-PhD-I-core(URA+KAN+)***	This study
YBL632	YBL631	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Rco1-ΔPhD***	This study
YBL634	YBL632	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Rco1-ΔPhD-TAP:HIS***	This study
YBL646	**YBL291**	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Epl1-TAP:HIS YNG2-PhD-I-core KAN+URA+***	This study
YBL648	**YBL631**	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Rco1-yng2PhD***	This study
YBL649	**YBL648**	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Rco1-yng2PhD-TAP:HIS***	This study
YBL659	**YBL646**	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Yng2Δphd Epl1-TAP***	This study
YBL677	YBL646	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* ***Yng2-rco1PHD Epl1-TAP***	This study
YBL694	**YBL675**	*MAT***alpha** *his3Δ1 leu2Δ0 lys2Δ 0 ura3Δ0* ***Rco1-ΔPhD-TAP:HIS/ eaf3-chd77-113Δ Flag:Leu2***	This study
YBL695	**YBL676**	*MAT***alpha** *his3Δ1 leu2Δ0 lys2Δ 0 ura3Δ0* ***Yng2-rco1PHD Epl1-TAP/ eaf3-chd77-113Δ Flag:Leu2***	This study

Supplemental Table2. Plasmids used in this study

Plasmid	Backbone	Plasmid Description	Source
pBL382	pGEX5X1	*GST-HYPB 915-1211 SET*	Chen Zhu
pBL440	pGEX-6P	Gst-ING4 (184-249)	Gozani
pBL443	pGEX-6P	Gst-YNG2 (202-282)	Gozani
pBL467	pCR-Blunt	pCR-YNG2-Age1-PHD	This study
pBL468	pCR-Blunt	pCR-RCO1-Age1-PHD	This study
pBL471	pCR-Blunt	pCR-RCO1-yng2PHD(202-282)	This study
pBL472	pCR-Blunt	pCR-YNG2-Age1-minus-PHD	This study
pBL476	pCR-Blunt	pCR-YNG2-rco1PHD(241-329)	This study

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