Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription.

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Supplementary Information

Supplementary Methods

RNA isolation

Total RNA was extracted from cells grown in YEA at 30°C using TRI Reagent® (Sigma Aldrich, T9424), following the manufacturer's instructions. Cells were disrupted at 4°C (2x20sec, 5 sec break in between) using Precellys24 (Peqlab).

Strand-specific RT-PCR

Levels of antisense (AS) transcripts were tested at selected loci using strand-specific reverse transcription followed by standard Taq-based PCR techniques (NEB M0267). Reverse transcription was performed on DNaseI-treated total RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, 4368814). Primers used are listed in Supplementary Table II (*, indicates primers used for the reverse transcription step). Controls lacking the reverse transcriptase enzyme (no RT) were included to exclude DNA contaminations. Quantification was performed with AIDA Image Data Analyzer v4.230.035. AS levels were normalized to actin and WT was set to 1.

Expression arrays

For expression array analyses ribosomal RNA (rRNA) was depleted using a mixture of eight rRNA-specific 5'-biotinylated probes (Sigma Aldrich, Supplementary Table III). 400 μL streptavidin magnetic beads (Hyglos, 311092) resuspended in 100 μL binding buffer (100 mM Tris-HCl pH 7.5, 1 mM EDTA, 2 M NaCl), 20 μg total RNA, 2 μL of 10 mM rRNA-specific probe-mix, and 2 μL RNase inhibitor (40 U, SUPERase-In, Ambion, AM2696) were incubated for 2 min at 65°C. The sample was cooled down and incubated for 5 min at 42°C, followed by a 25 min incubation with magnetic beads under constant shaking. The rRNA-depleted supernatant was collected using a magnet stand and subjected to ethanol precipitation. rRNA-depleted RNA was reverse transcribed using the SuperScript ®Indirect cDNA labeling system (Invitrogen, L101402), primed with random hexamers. Reference (WT) and test samples (mutant) were labeled with Cy3 and Cy5 (GE Healthcare, PA13105 and PA15100) and hybridized on high-resolution tiling microarrays (Agilent) consisting of forward- and reverse-DNA-strand-specific probes. Expression arrays were performed with at least two biological replicates, with the exception of *set2Δ*.

Scanning and initial processing of microarrays was performed using Agilent DNA Microarray Scanner and Agilent Scan Control software (version A.8.4.1.). Data extraction was performed using Agilent feature extraction software (version 10.7.3.1.). Significantly up-regulated AS transcripts were defined as a minimum of three consecutive probes (min. 240 nt) with 2-fold or higher up-

regulation of transcript levels in the mutant compared to WT. Significant changes in the S transcript levels were defined as a minimum 2-fold up- or down-regulation of the median ratio in a gene.

Antibodies

Antibodies used in western blots and CHIP-on-chip experiments included an anti-H3 antibody (Abcam, ab1791) [1], anti-H3K9/K14ac antibody (Diagenode, pAB-005-044) [2], and anti-actin antibody (Millipore, MAB1501R) [3]. Antibodies were used according to the manufacturer's instructions.

H3 and H3K9/K14ac western blots

Protein whole cell extracts (WCE) were performed using standard trichloroacetic acid (TCA) precipitation. Briefly, cells in stationary phase were treated with 20% TCA at 4°C and disrupted with glass beads using Precellys24 (Peqlab). Protein WCEs were isolated from glass beads with SDS loading buffer. WCEs were separated via 4-12% SDS-PAGE (Invitrogen, NP0323BOX) and transferred to nitrocellulose membranes (semi dry iBlot system, Invitrogen). Membranes were probed with either anti-H3- or anti-H3K9/K14ac-antibody. In addition, every blot was probed with an anti-actin antibody and stained with Ponceau S to compare WCE loading. Quantification of western blot signals was performed using AIDA Image Data Analyzer v4.230.035. Western blots were performed at least three times for each antibody in biological replicates. P-values were calculated using the student's t-test.

Genome-wide chromatin-immunoprecipitation

ChIP experiments were performed as described previously [4] with the following modifications. Shearing of chromatin to an average fragment size of 650 bp was performed with a Bioruptor (Diagenode) at 4°C for 33 cycles (30sec on, 30sec off). Purified ChIP samples were amplified using the WGA2 kit (Sigma, 128K0755) followed by Bioprime Array CGH kit (Invitrogen, 45-0048) according the manufacturer's instructions. Genome-wide chromatin-Immunoprecipitation experiments were performed at least twice in biological replicates.

Isolation of mono-nucleosomal DNA and genome-wide nucleosome mapping

Isolation of mono-nucleosomal DNA was performed as described in Lantermann *et al*. [5] with the following modifications. Formaldehyde fixed and spheroplasted samples were evenly divided into ten samples. Genomic DNA of the mock control (reference) was sheared by sonication with the Bioruptor, for 25 cycles (30 sec on and 30 sec off, low power). Each of the additional nine samples was treated for 20 min at 37°C with increasing micrococcal nuclease (MNase) concentrations (2-32 U, Sigma, N5386), followed by an RNaseA (37°C, 2h) and proteinase K (65°C, overnight) treatment. Mono-nucleosomal DNA of the nine MNase-treated samples were agarose gel purified using a NucleoSpin kit with NTC buffer (Macherey Nagel, 740609.250) and pooled. Both the sheared reference sample and the pooled mono-nucleosomeal DNA were labeled using a Bioprime Array CGH kit (Invitrogen, 45-0048), and hybridized to 10 bp-resolution tiling microarrays following the manufacturer's instructions.

To generate the composite plots, we aligned the genes to the center of their first nucleosome, and plotted the geometric average of the relative nucleosome occupancy signals (Fig. 3B). We used an independent WT dataset to annotate the first and last nucleosomes at each gene, and applied this annotation to all data analyses. We decided to align genes to the center of their first nucleosome instead of the transcriptional start site (TSS), because the TSS is not always strictly positioned compared to the first nucleosome [6]. Aligning genes to their +1 nucleosome gives a sharper picture of the nucleosome organization at a genome-wide scale (Suppl. Fig. 2A). While composite plots summarize the average trend in the dataset, 2D-plots intuitively display the whole dataset, representing every gene as a horizontal line with points marking the position of the center of the nucleosomes (Fig. 3A and Fig. 4). Genes are aligned at the middle of their nucleosome array , and sorted vertically according to the length of their nucleosome array [7]. This representation doesn't use the nucleosome occupancy information and allows simpler comparison of genome-wide nucleosome position patterns between datasets.

For analyzing the nucleosome pattern of highly and lowly expressed genes, we used the mRNA copy number/cell dataset of Hiraoka *et al*. [8].

Microarray design

Expression arrays and ChIP-on-chip arrays (Design ID:036227): Agilent custom microarray design with 180K probes. The microarrays cover about 50% of the *S.pombe* genome (chr2, chr3:1-132300; 2320 genes) with 40bp resolution, and the remaining 50% with 250bp resolution. Probes alternate on the FW and Rev strand. Probe length is between 30-60 nt Tm optimized (75˚C). This array design allows us to monitor gene expression changes (sense, antisense and intergenic) in the entire genome, and to obtain a more detailed high resolution analysis of half of the *S.pombe* genome. Nucleosome mapping experiments (Design ID:036031): Agilent custom microarray design with 1M probes. Microarrays cover about 75% of the *S.pombe* genome (chr2, chr3, chr1:1-2500000; 3778 genes) with 10bp resolution, and the remaining 25% with 250bp resolution. Probes alternate on the FW and Rev strand. Probe length is between 30-60 nt Tm optimized (75˚C).

Supplemental Figure 1

Bulk H3 and and acetylated H3K9/K14 levels in WT and mutant strains.

(A) Whole cell extracts (WCE) were subjected to SDS-PAGE and H3 levels were monitored by Western blot for WT and the indicated mutant strains. Actin levels were determined and Ponceau S staining of the membranes was performed to quantify total protein levels in the WCEs. The relative H3 levels were quantified and normalized to either actin (upper chart, right panel) or total protein content in WCE (middle charts, right panel). In addition, actin levels were normalized to total protein content in WCEs (bottom chart, right panel). WT was set to 100%. Data represents the means of three independent experiments and error bars represent standard deviations.

(B) Western blot analysis of the same WCEs using a polyclonal antibody recognizing both acetylated H3K9 and H3K14 (H3K9/K14ac). Ponceau staining of the Western blot membrane was performed and used to quantify total protein levels in the WCEs. The relative H3K9/K14ac levels were quantified and normalized to either actin, total protein content in WCE, or H3 level(charts, right panel). WT was set to 100%. Data represents the means of three independent experiments and error bars represent standard deviations.

Supplemental Figure 2

Hrp1 and Hrp3, have redundant functions in nucleosome organization in *S.pombe*.
(A) Composite plots of relative nucleosome occupancy for WT strain. Positions of the first and last nucleosomes were determined for each gene in an independent experiment with WT strain. 3778 genes were aligned either at their first nucleosome (left panel) or the transcriptional start site (TSS) and the average of their $\log_2$ nucleosome occupancy data (geometric mean) was plotted.

(B) Composite plots of relative nucleosome occupancy for the *hrp1Δ* mutant (blue line) and the *hrp1Δhrp3Δ* mutant (orange line) compared to WT (gray shading). Using the same annotation from the independent WT data set, 3778 genes were aligned at their first nucleosome and the average of their $\log_2$ nucleosome occupancy data (geometric mean) was plotted.

(C) Composite plots of relative nucleosome occupancy were generated, as in part (B), for the the *hrp3Δ* mutant (blue line) and the *hrp1Δhrp3Δ* mutant (orange line) compared to WT (gray shading).

Supplemental Figure 3

Regular organization of nucleosome arrays is unaffected in *mit1Δ*, *set2Δ*, and *alp13Δ* strains and is moderately affected in the *pob3Δ* strain.
Composite plots comparing relative nucleosome occupancy for WT (gray shading) and *hrp1Δhrp3Δ* mutant strains (orange line) to either (A) *mit1Δ,* (B) *pob3Δ*, (C) *set2Δ* and (D) *alp13Δ* strains (blue lines). Positions of the first and last nucleosomes were determined for each gene in an independent experiment with WT strain. 3778 genes were aligned at their first nucleosome and the average of their log2 nucleosome occupancy data (geometric average) was plotted.

Supplemental Table I: Yeast strains used in this study.

Supplemental Table II: Primers used for RT-PCR. Primers used for the reverse transcription step are marked with *. The subsequent PCR reaction was performed using the corresponding forward and reverse primers.

Supplemental Table III: Ribosomal RNA (rRNA) specific 5' biotinylated primers.

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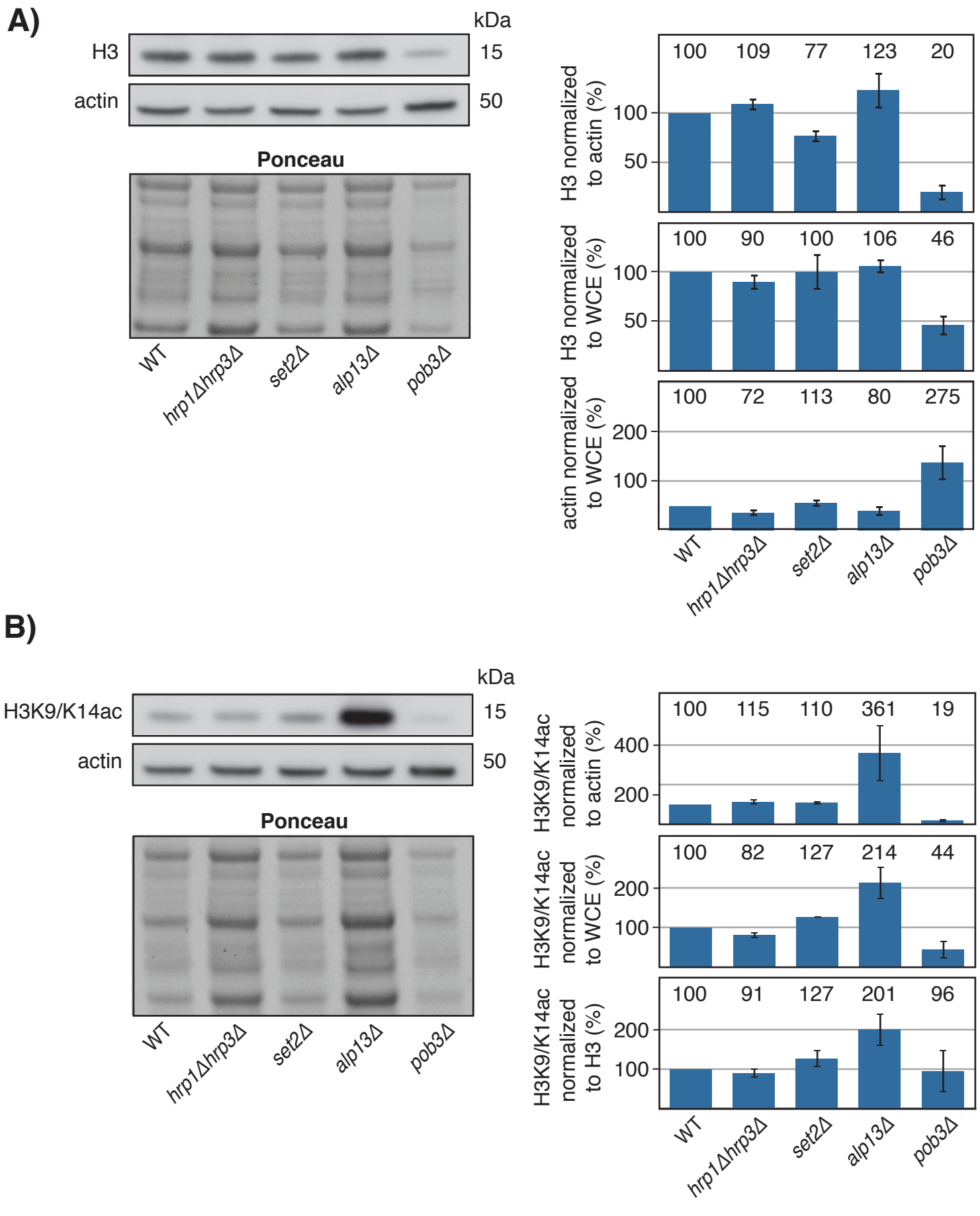
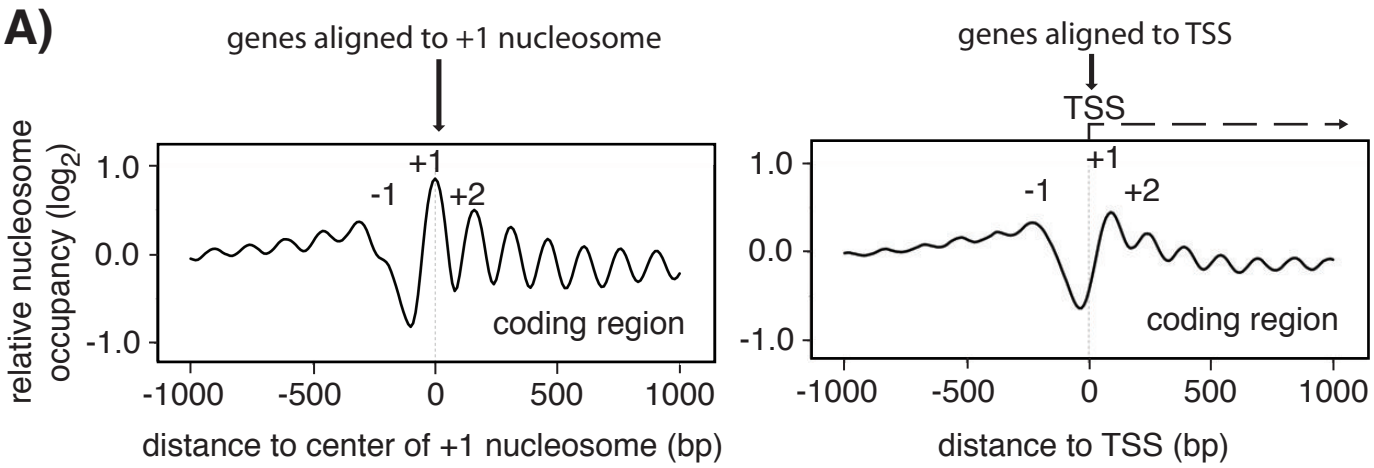
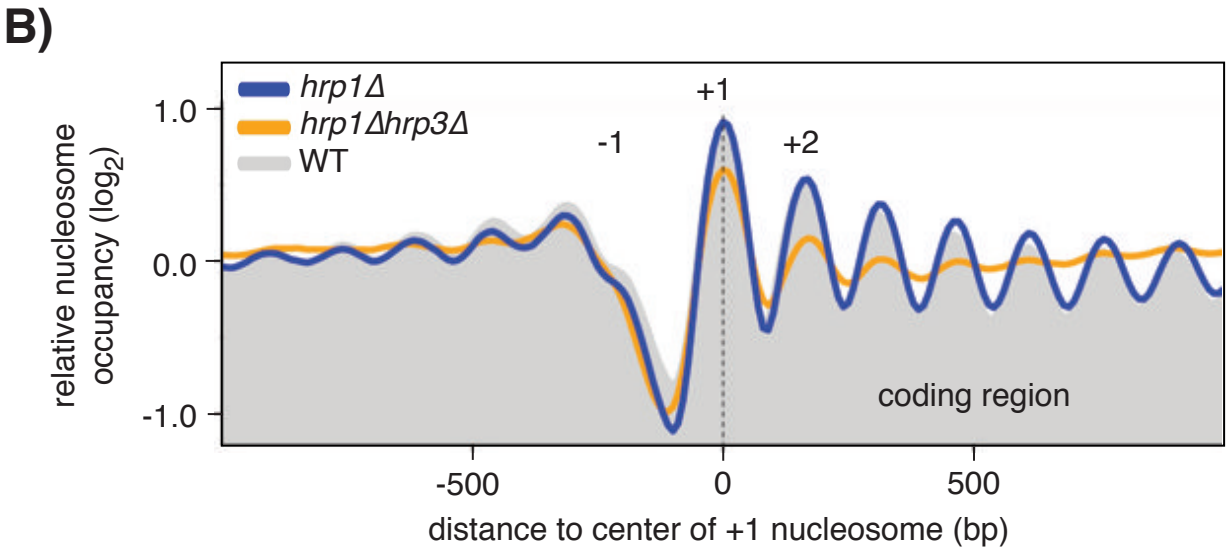
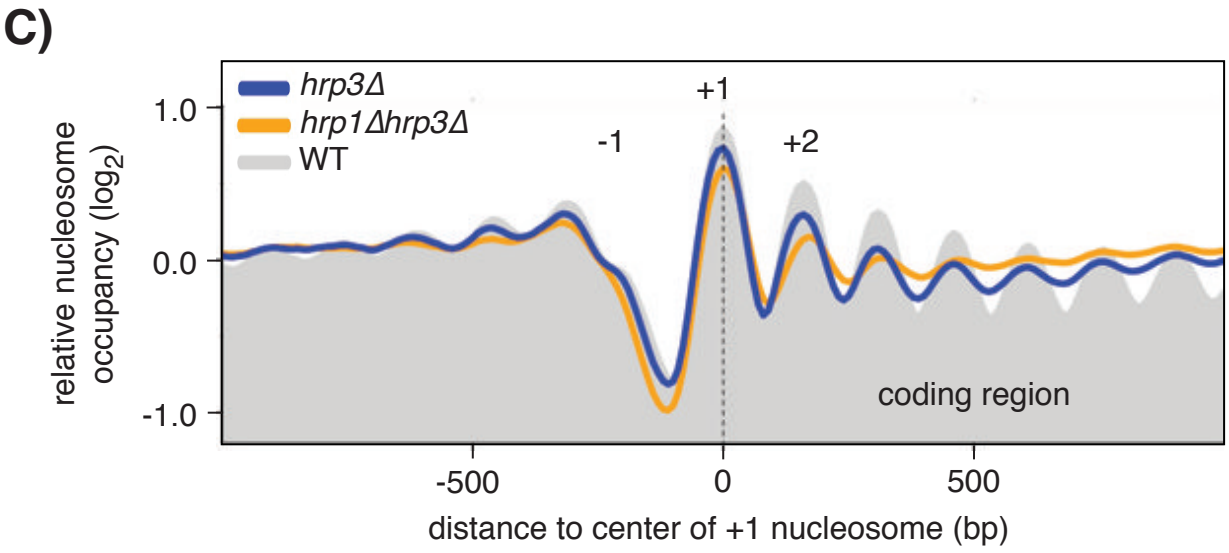
Supplemental Figure 1



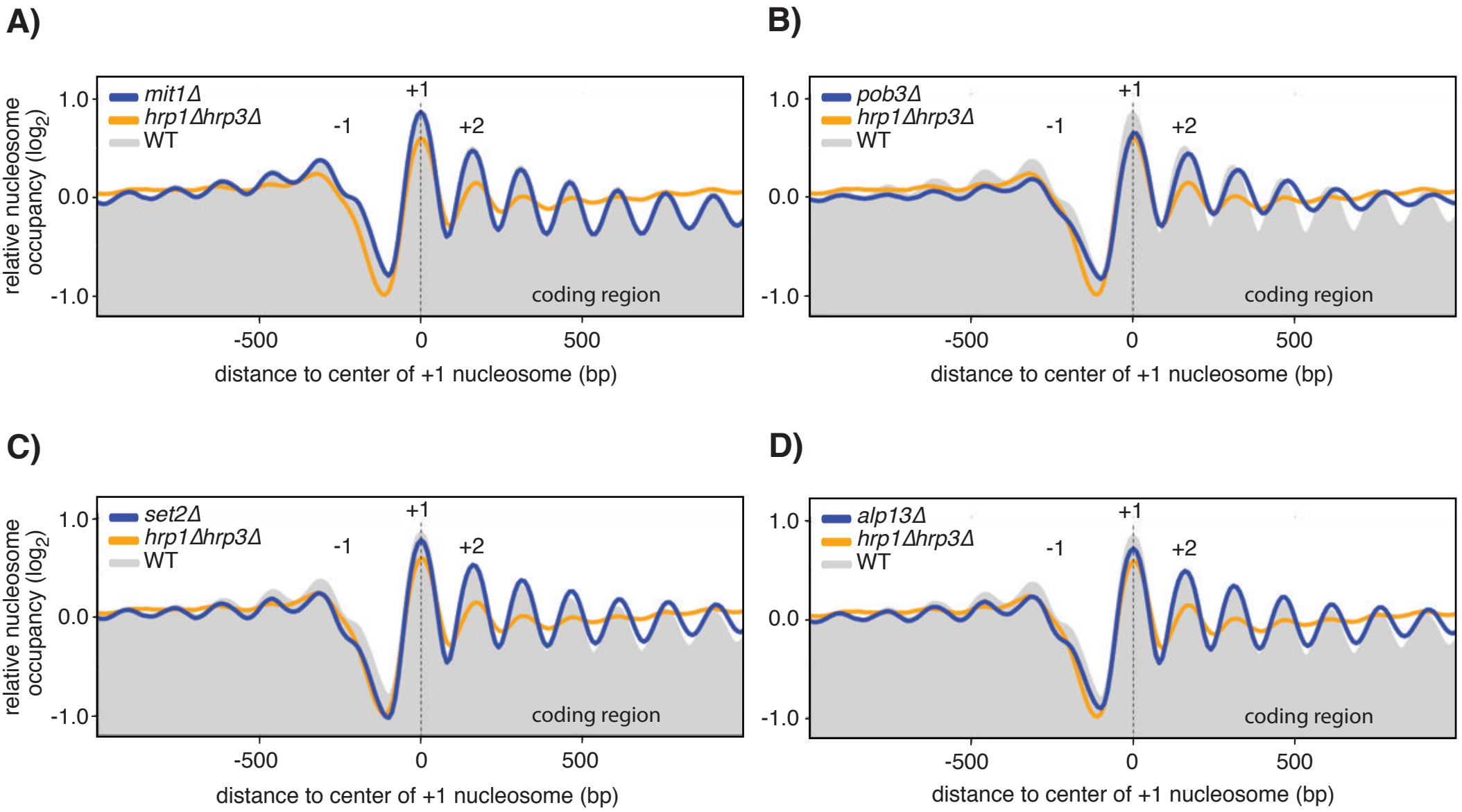
Supplemental Figure 2







Supplemental Figure 3



Supplemental Table I: Yeast strains used in this study.

Strain	Genotype	Origin	Used for
p20 (WT)	h+,his+, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-216	constructed in the lab	everything
p45 *(hrp1Δ)*	h+,his+, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-216, hrp1Δ::hphNT1	constructed in the lab	dot spots
p26 *(hrp1Δ)*	MatMsmt0, his2, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-210, hrp1Δ::hphNT1	constructed in the lab	nucleosome mapping, expression array
p46 *(hrp3Δ)*	h+,his+, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-210, hrp3Δ::NatNT2	constructed in the lab	dot spots
p27 *(hrp3Δ)*	MatMsmt0, his2, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-210, hrp3Δ::NatNT2	constructed in the lab	nucleosome mapping, expression array
p54 *(hrp1Δhrp3Δ)*	h+,his+, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-216, hrp1Δ::hphNT1, hrp3Δ::NatNT2	constructed in the lab	dot spots, expression array
p56 *(hrp1Δhrp3Δ)*	MatMsmt0, his2, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-210, hrp1Δ::hphNT1, hrp3Δ::NatNT2	constructed in the lab	ChIPs, nucleosome mapping
p65 *(set2Δ)*	h+,his+, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-216, set2Δ::KanMx	constructed in the lab	everything
p69 *(alp13Δ)*	h+,his+, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-216, alp13Δ::NatNT2	constructed in the lab	everything, except expression array
p21 *(alp13Δ)*	MatMsmt0, his2, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-210, alp13Δ::KanMx	constructed in the lab	expression array
p550 *(pob3Δ)*	MatMsmt0, his2, leu1-32, ura4-DS/E, Otr::Ura4(SphI), ade6-216, pob3Δ::KanMX	constructed in the lab	everything
p440 *(mit1Δ)*	h+, his+, leu1-32, ura4-D18, ade6-210 or ade6-216, mit1Δ::KanMX	Bioneer mutation library	everything

Supplemental Table II: Primers used for RT-PCR.

	forward	reverse
SPBP35G2.10	CTCATACGACGCAACTAAG*	TAAACCACGCCAAGAAAC
SPBC1778.02	AGAGAAGTCTAAACGAATTGTG*	AATATCAGCAACCTCATTATCC
SPBC36B7.09	TGGCGGAAAAATCTGAACTC*	TGAAGGAGAATGCTTGCTTG
actin	TACTACCGCCGAACGTGAAATTG	AGGGAGGAAGATTGAGCAGCAGT*

Supplemental Table III: Ribosomal RNA (rRNA) specific 5' biotinylated primers.

	probe				
5S_1	TTAACTGCAGTGATCGGACGGGAAC	5S_depl_Sp_1			
5S_2	CAACCATAGTACTAACGAGGCCCTC	5S_depl_Sp_2			
5.8S_1	AATGACACTCAAACAGGCATGCC	5.8S_depl_1			
5.8S_2	tctgcaattcacattacgtatcgc	5.8S_depl_2			
18S_1	aactgatttaatgagccattcgcag	18S_depl_1			
18S_2	gatccttccgcaggttcacctacgg	18S_depl_2			
25/28S_1	gccaatccttatcccgaagttacgg	25/28S_depl_1			
25/28S_2	aaaactaacctgtctcacgacggtc	25/28S_depl_2			