Supplementary Information

CHD1 remodelers regulate nucleosome spacing *in vitro* and align nucleosomal arrays over gene coding regions in *S. pombe*

Julia Pointner[1#], Jenna Persson[2#], Punit Prasad[2#], Ulrika Norman[2], Annelie Strålfors[2], Olga Khorosjutina[2], Nils Krietenstein[1], J. Peter Svensson[2], Karl Ekwall[2]*, and Philipp Korber[1]*

[1]Adolf-Butenandt-Institut, University of Munich, Schillerstr. 44, 80336 Munich, Germany

[2]Karolinska Institutet, Department of Biosciences and Nutrition, Center for Biotechnology, Huddinge, Sweden

*Shared last authors. Address correspondence to: Karl.Ekwall@ki.se, pkorber@lmu.de

[#]These authors contributed equally to the work.

Supplementary Materials and Methods

Yeast strains and media, spotting assays, viability assays and microscopy

Standard fission yeast media and genetic techniques were used (Moreno et al., 1991). Liquid cultures were grown in YES media. Temperature sensitive strains were grown at the permissive temperature (25 °C) either for ~30 h or for ~24 h followed by 6 h at the nonpermissive temperature (34 °C) before harvesting. All other strains were grown at 30 °C.

Budding yeast strains were grown at 30 °C in YPD with 0.1 g/l adenine and 1g/l KH_2PO_4. As the *isw1::KANMX* deletion of the *S. cerevisiae* deletion strain collection (Euroscarf) used a wrongly annotated ATG start codon for *ISW1* and thereby also affects the upstream gene *RRT2* (J. Mellor, personal communication), we sequenced a PCR product using genomic DNA of strain YTT227 and the primers „Isw1 out rev“ (GCATTTAGTACATCTGATTCTTG) and „Ade2 ins rev“ (CCTCCTAATATACCAACTGTTCTAG) and confirmed that the *ADE2* marker cassette was inserted into the correctly annotated *ISW1* ORF.

Spotting assays: Solutions of 5 mM, 7.5 mM and 10 mM hydroxyurea (HU, Sigma, H8627) in water, or 100 µg/ml, 200 µg/ml and 300 µg/ml 6-azauracil (6-AU, Sigma, A1757) in 1 M ammonium hydroxide, or 0.003% and 0.006% w/v methanemethylsulfonate (MMS) in water were mixed into cooled, autoclaved YES media prior to pouring plates. Equal numbers of logarithmically growing cells were spotted in 4-fold (6-AU and HU) or 5-fold (MMS) serial dilutions 3-4 h after plates were poured.

Viability by Phloxin B staining: Cultures of Hu0303 (wt), Hu2262 (*snf21-36(ts)*), and Hu2314/5 (*snf31-36(ts) swr1Δ*) were grown to logarithmic phase at 25 °C or for 4 h at 34 °C following 24 h at 25°C in YES medium. Phloxin B fluorescent dye was then added to a final concentration of 15 mg/l and cultures were incubated for two more hours. Phloxin B was also added to a positive staining control culture of Hu0303 cells grown at 65 °C. Cells were then rinsed in PBS and the percent stained (dead) cells was quantified using a Zeiss Axioplan II microscope equipped with a Hamamatsu C4742-95 charge-coupled device camera. Hundred percent of control cells showed pink staining.

Viability by colony formation: Cultures of K240 (wt) or KYP176 (*snf21-36(ts)*) were grown at 25 °C for 24 h and afterwards for 10 h at 34 °C. Every two hours after shift to 34 °C, cells were counted in a Bürker counting chamber (Marienfeld) and 200 cells plated on YES plates in duplicates and incubated at 25 °C for four days. Colonies were counted and percentage of surviving cells was calculated with 200 colonies set as 100% survival.

Microscopy: cultures of K240 and KYP176 were grown as for the colony formation assay. 1-2 x 10^7 cells were harvested after 6 h at 34 °C, fixed in ethanol, rehydrated, spotted on slides, embedded in DAPI containing Vectashield mounting medium and visualized with an Axiovert 200M microsocope (EC Plan-Neofluar 63x/1.4 NA oil Ph2 (differential interference contrast [DIC]II) objective (Carl Zeiss, Inc.)). Images were processed with AxioVision software (Carl Zeiss, Inc.)

Tetrad analysis: Hu1294 and Hu2304 were crossed on a nitrogen-free plate. After 4 days, some of the cross was transferred to a YES plate. Asci were selected and spread on the plate using a SporePlay Dissection Microscope (Singer Instruments). Asci were allowed to dissintegrate at room temperature overnight, after which the individual spores were spread on the plate and allowed to grow for 5 days.

MNase indirect end labeling

locus	secondary cleavage	primers for probe generation
SPCC613.10	NdeI	SPCC613.10-F (TTCAAAGACTAAAGGAAGGGT) SPCC613.10-R (GTGGATTTGTTTAAAAGCTAAG)
SPCC4G3.08	PmlI	SPCC4G3.08-F (ACCTTGAGCCGATACCTGAG) SPCC4G3.08-R (TCTTCTTGAGACGCGCGTGT)
SPBC1685.14c	SpeI	SPBC1685.14c-F (GGACTGAGAATACTCCTAATGC) SPBC1685.14c-R

		(GATGAACAGTGCACAAAACAAAAGC)

Reverse transcription with actinomycin D

RNA from strains Hu303, Hu2239, Hu0574, Hu2303, and Hu2304 was treated as described in (Marvin et al., 2011) with slight modifications. The RNA was treated with Turbo DNaseI (Ambion #1907) according to manufacturer instructions. In two reactions per sample, cDNA was then synthesized from 9 µg total RNA using the SuperScript II Reverse Transcriptase Kit (Invitrogen). The RNA was incubated with 4.5 µg random hexamers in a volume of 124 µl at 70 °C for 10 min. After the addition of First Strand Buffer (final concentration 1x), DTT (final concentration 10 mM), dCTP, dGTP, dATP (each final concentration 0.25 mM), dTTP (0.2 mM), dUTP (0.05 mM) 2000 U Superscript Reverse Transcriptase, and Actinomycin D (final concentration 6.26 µg/mL), the 200 µl reaction was incubated at 42 °C for 1 h. Following deactivation of the reverse transcriptase at 70 °C for 15 min, the cDNA was incubated at 37 °C for 20 min with 3 µl of RNase Cocktail (Ambion #2286). Affymetrix GeneChip Sample Cleanup cDNA columns (Affymetrix #900371) were used to purify and concentrate the cDNA prior to fragmentation, biotinylation, and array hybridization by standard Affymetrix protocol (http://www.affymetrix.com).

Cloning and purification of recombinant S. pombe histones and His-Nap1

S. pombe histone genes were amplified from genomic DNA and cloned in pET-13 vector for overexpression in E. coli strain BL21 DE3 pLys (Stratagene), except histone H4 that was overexpressed in BL21 Star™ (DE3) (Invitrogen). Recombinant histones were purified and refolded into octamers as in (Luger et al., 1999). S. p*ombe* Nap1 was cloned in expression vector pET-M11 with 6X His tag at its N-terminus and was expressed in BL21(DE3) pLysS cells. The cells (1 l) were induced (λ_{600} = 0.6) with 1 mM IPTG for 3 h at 37^{o}C. Cells were lysed by sonication in ice-cold lysis buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 5 mM imidazole, 10 mM 2-mercaptoethanol and protease inhibitor cocktail (1 mM PMSF, 2 µM pepstatin, 0.6 µM leupeptin and 2 mM benzamidine)). Clarified extract was mixed with 2 ml of pre-equilibrated Ni-His-select beads (Sigma) and incubated for 1 h at 4 ^{o}C. Beads were washed with 7 column volumes (CV) of wash buffer containing 25 mM Tris-HCl, pH 8.0, 1 M NaCl, 5% glycerol, 10 mM 2-mercaptoethanol, protease inhibitor cocktail and 10 mM imidazole (0.01 WB; number indicates imidazole concentration in molar units), followed by two washes with 7 CV and 12 CV of 0.02 WB and 0.04 WB, respectively. Nap1 was eluted

with 0.25 WB. Fractions containing Nap1 were dialysed against buffer A-0.2 (10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 0.5 mM EDTA, 3% glycerol, 1 mM DTT and protease inhibitors, the number after hyphen indicates NaCl concentration in molar units). Dialysed Nap1 was passed through tandemly connected Hitrap SP sepharose and Hitrap Q sepharose columns (GE healthcare). Bound Nap1 was eluted from Hitrap Q sepharose with a linear gradient (15 CV) of buffer A-0.2 to A-1.2 where Nap1 was eluted at 400 mM NaCl concentration.

Trypsin Digest and Mass Spectrometry

For Hrp1: Excised protein bands were destained twice with 100 µl of 100 mM NH_4HCO_3 / 50% acetonitrile (AcN) at 37 °C for 45 min, washed twice in 100 µl 100% AcN for 10 min and dried in Speedvac for 15 min at RT. Gel slices were reduced in 50 µl of 10 mM DTT at 56 °C for 45 min followed by alkylation with 50 µl of 55 mM iodoacetamide in dark for 30 min. Gel slices were washed twice in 100 µl of 40 mM NH_4HCO_3 for 10 min, dried and in-gel tryptic digestion was performed with 20 ng/µl of trypsin in 40 mM NH_4HCO_3 and incubated at 37 °C overnight. The supernatant was transferred to a new Eppendorf tube and 2 µl of 10% formic acid (FA) was added. To extract the peptides from the gel pieces, 50 µl of 50% AcN / 0.1% FA was added and the samples were put in ultrasonic bath for 5 min. Extraction was continued by adding 50 µl of 70% AcN / 0.1% FA to the gel pieces followed by ultrasonic bath for 5 min after which the supernatant was again transferred to the new Eppendorf tube. The last two extractions were performed with 50 µl of 100% AcN / 0.1% FA followed by ultrasonic bath for 5 min. After each extraction supernatant was transferred to the fresh eppendorf tube, eluates were combined and dried in Speedvac. For mass spectrometry analysis, the dried eluates were dissolved in 3% AcN / 0.1% FA. LC-MS was performed on a hybrid LTQ-Orbitrap Velos mass spectrometer (Thermo Fischer Scientific, San Jose, CA, USA). An Agilent HPLC 1200 system (Agilent Technologies, Santa Clara, CA, USA) was used for online reversed-phase nano-LC at a flow of 0.4 µl/min with solvent A (97% water, 3% ACN, 0.1% FA) and solvent B (5% water, 95% AcN, 0.1% FA). The fragments were separated on step gradient from 2% B up to 40% B in 45 min, followed by 100% B in 5 min. Samples 3 of 8 µl from each IPG fraction were bound on Zorbax 300SB-C18, 5 µm, 5 x 0.3 mm (Agilent Technologies, Santa Clara, CA, USA) and separated on a NTCC-360/100-5-153 C18 column (Nikkyo Technos Co., Tokyo, Japan) installed on to the nano electrospray ionisation (NSI) source of the Orbitrap Velos instrument. Acquisition proceeded in ~3.5 s scan cycles, starting by a single full scan MS at 30000 resolution (profile mode), followed by

two stages of data-dependent tandem MS (centroid mode): the top 5 ions from the full scan MS were selected firstly for collision induced dissociation (CID, at 35 % energy) with MS/MS detection in the ion trap, and finally for high energy collision dissociation (HCD, at 37.5% energy) with MS/MS detection in the Orbitrap. Precursors were isolated with a 2 m/z width and dynamic exclusion was used with 60 s duration. The MS/MS spectrum was searched using Proteome Discoverer 1.3 (Thermo Fischer Scientific, San Jose, CA, USA) against *S. pombe* protein sequence database. A precursor mass tolerance of 10 ppm, and product mass tolerances of 0.02 Da for HCD-FTMS and 0.8 Da for CID-ITMS were used. Further settings used were: trypsin with 1 missed cleavage; carbamidomethylation on cysteine as fixed modification and oxidation of methionine as variable modification

For Hrp3: In-gel digests were performed as described in standard protocols. Briefly, following the SDS-PAGE and washing of the excised gel slices proteins were reduced by adding 10 mM DTT (Sigma Aldrich) prior to alkylation with 55 mM iodoacetamide (Sigma Aldrich). After washing and shrinking of the gel pieces with 100% acetonitrile trypsin (Sequencing Grade Modified, Promega) was added and proteins were digested overnight in 40 mM ammoniumbicarbonate at 37°C. For protein identification samples were directly used for nano-ESI-LC-MS/MS. Each sample was first desalted on-line by a C18 microcolumn (300 µm i.d. x 5 mm, packed with C18 PepMap™, 5 µm, 100 Å by LC Packings). Peptides were then separated on a C18 reversed phase column via a linear acetonitrile gradient (UltiMate 3000 system (Dionex) and column (75 µm i.d. x 15 cm, packed with C18 PepMap™, 3 µm, 100 Å by LC Packings) before MS and MS/MS spectra were recorded on an Oribitrap mass spectrometer (Thermo Electron). The resulting spectra where analyzed via Mascot™ (Matrix Science) and Scaffold (Proteome Software) using the NCBInr Protein Database.

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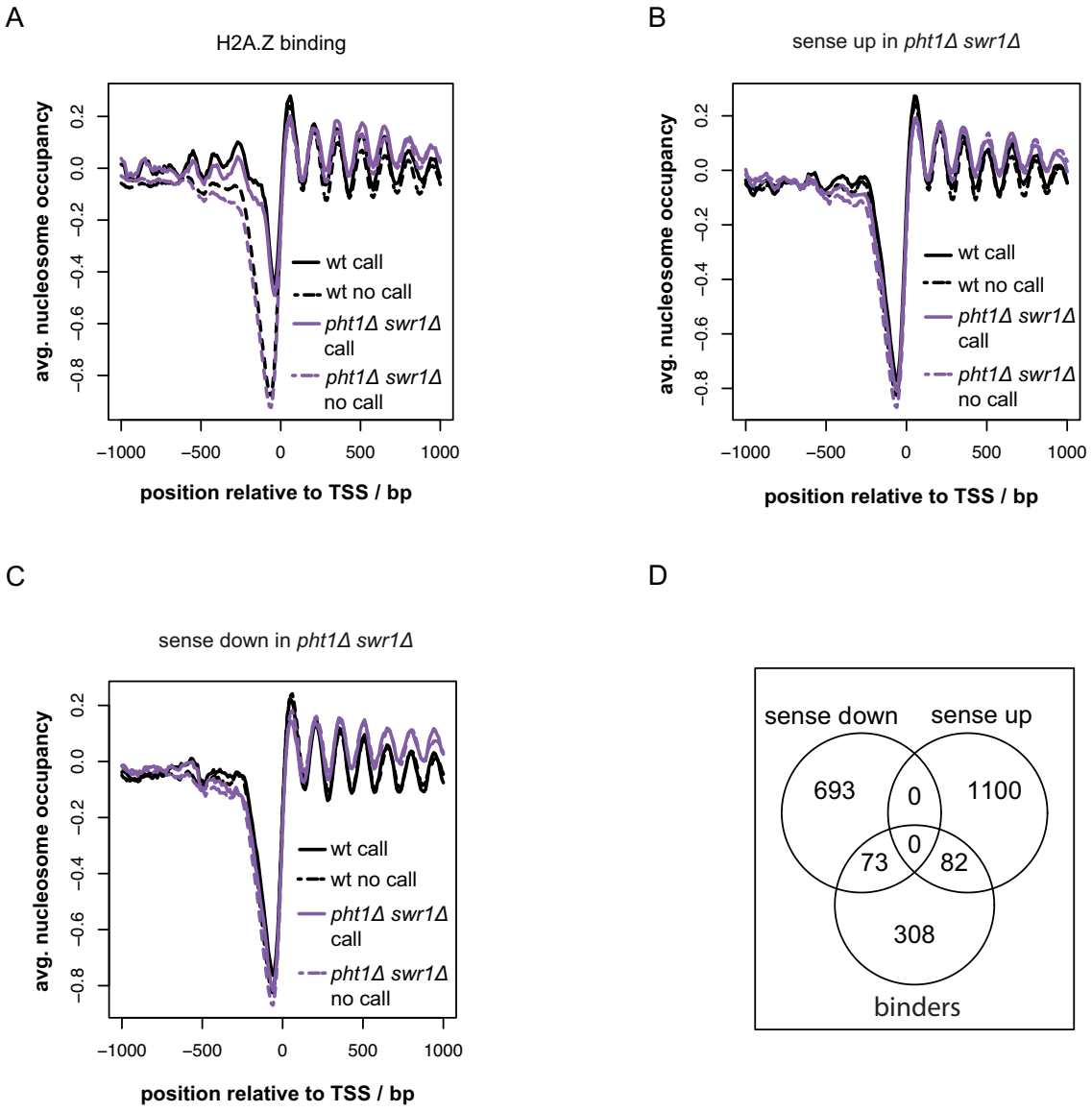
2. Marvin,M.C., Clauder-Munster,S., Walker,S.C., Sarkeshik,A., Yates,J.R., III, Steinmetz,L.M., and Engelke,D.R. (2011). Accumulation of noncoding RNA due to an RNase P defect in Saccharomyces cerevisiae. *RNA.,* **17,** 1441-1450.

3. Moreno,S., Klar,A., and Nurse,P. (1991). Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. *Methods Enzymol.,* **194,** 795-823.

Supplementary Figures

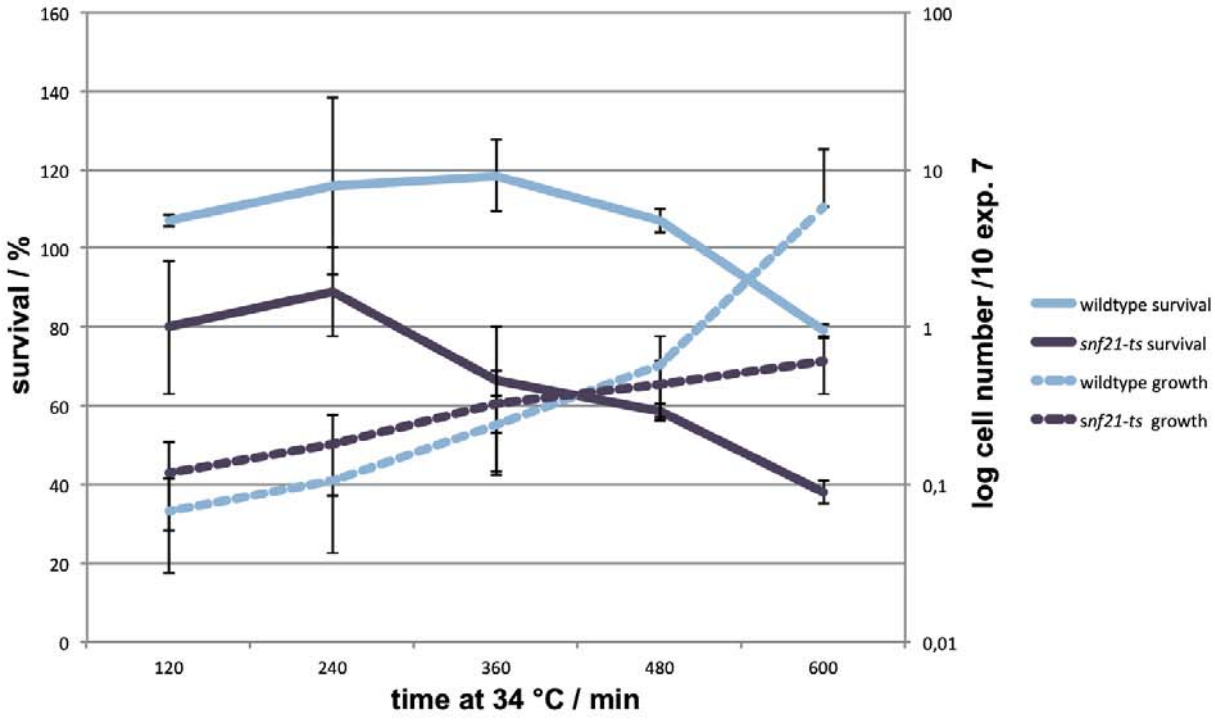
Suppl. Fig. 1



Supplementary Figure 1 H2A.Z related subgroups of genes do not show more pronounced defects in nucleosome positioning in *pht1Δ swr1Δ* cells. Same data as in Figure 1A, but (**A**) divided into H2A.Z-bound (384 calls) and -unbound (3629) loci, and (**B**) into genes with >1.5 fold upregulated sense transcription in *pht1Δ swr1Δ* cells (589 calls) and genes without significant changes (1463). (**C**) As (**B**) but for >1.5 fold downregulated sense calls (591). (**D**) Venn diagram showing the overlaps between the called loci from panels (**A**) to (**C**).

Suppl. Fig. 2

A



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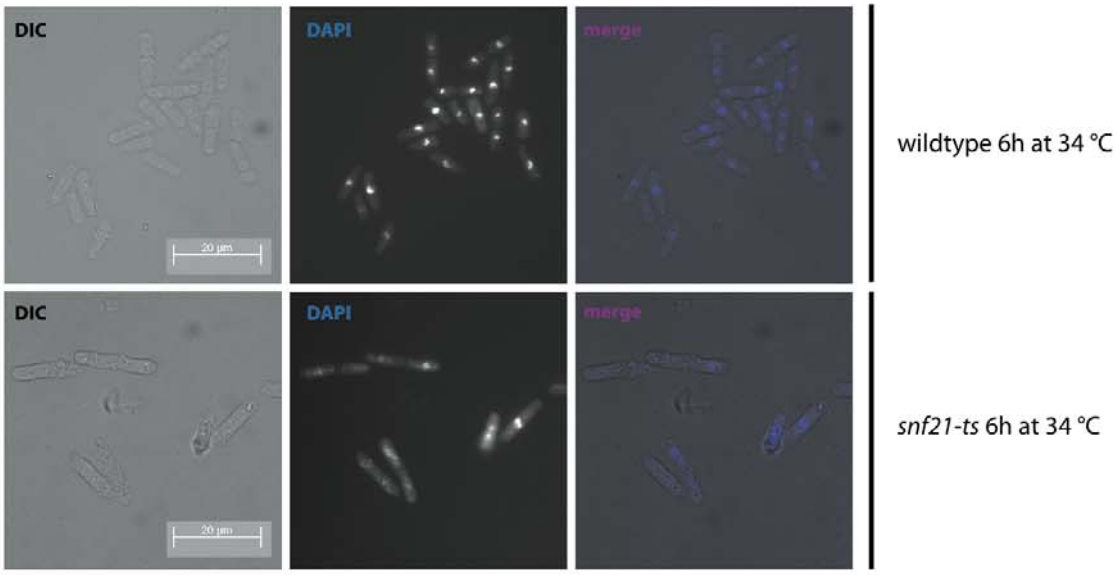
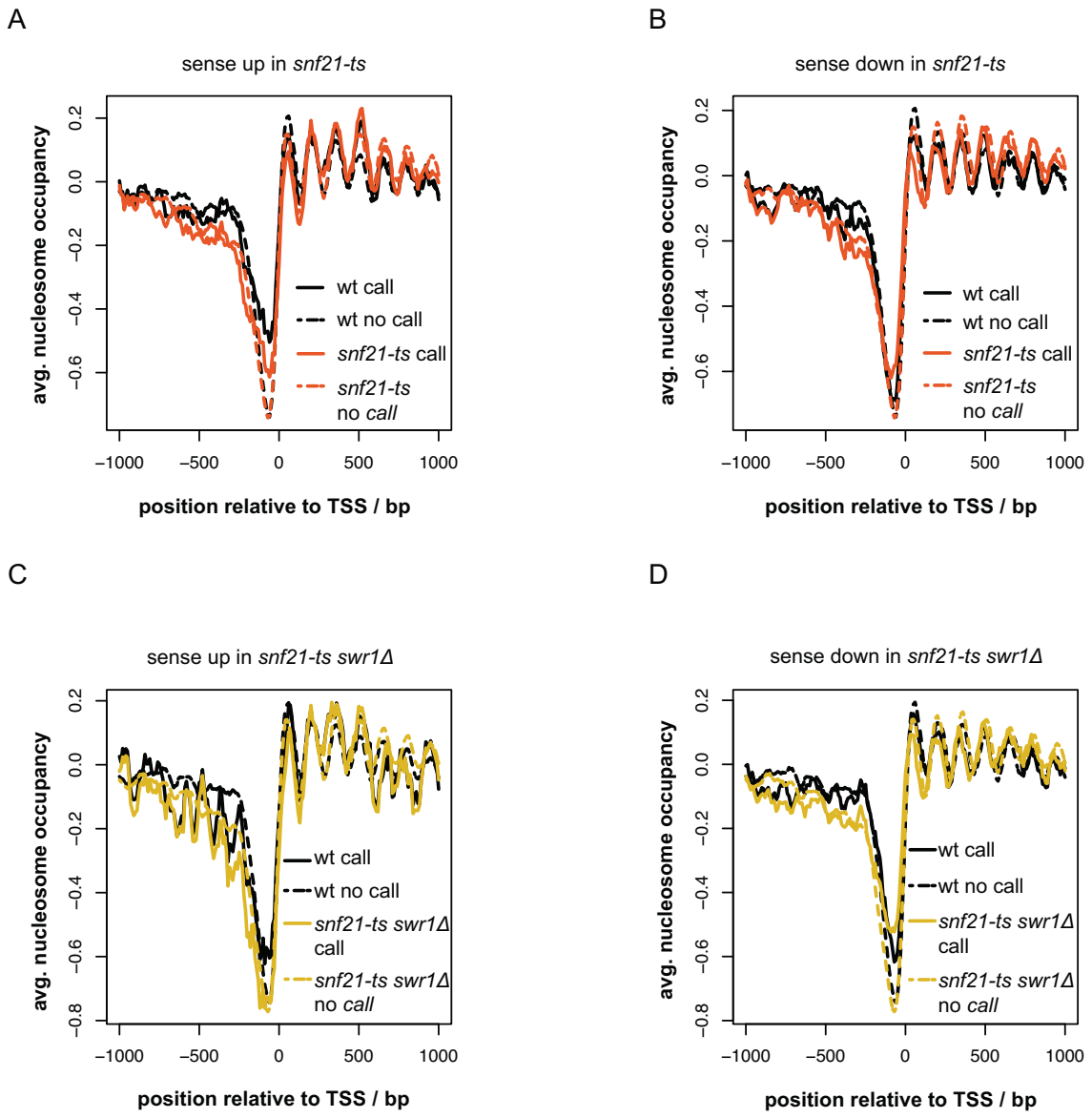


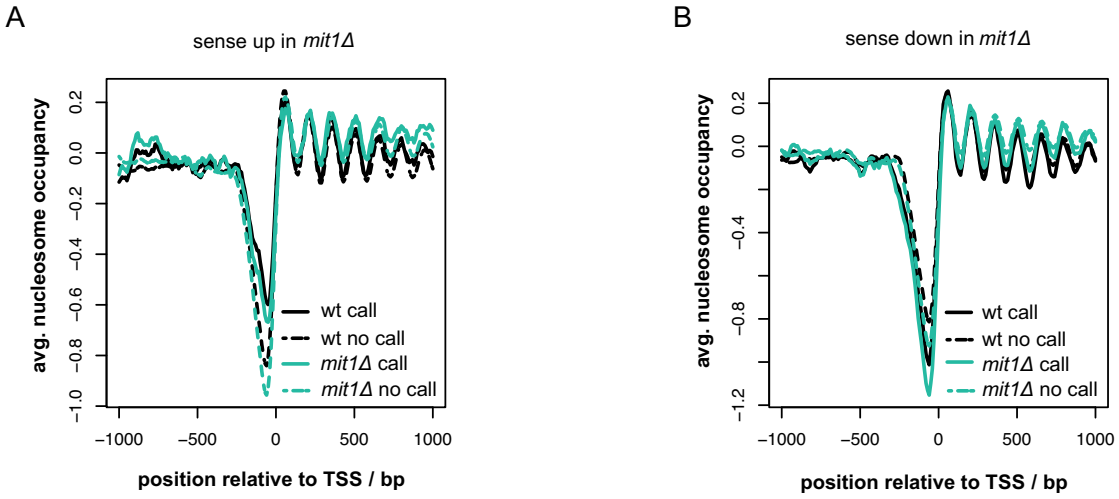
Supplementary Figure 2 Confirmation of an *snf21-ts* phenotype at 34 °C. (**A**) Growth by counting cell numbers and survival by colony formation after replating at 25 °C was monitored for wt (K240/Hu2261) and *snf21-ts* (KYP176/Hu2262) cells after shift to 34 °C. (**B**) Differential interference contrast (DIC) and fluorescence (DAPI stain) microscopy of wt and *snf21-ts* (as in (**A**)) under the indicated conditions.

Suppl. Fig. 3



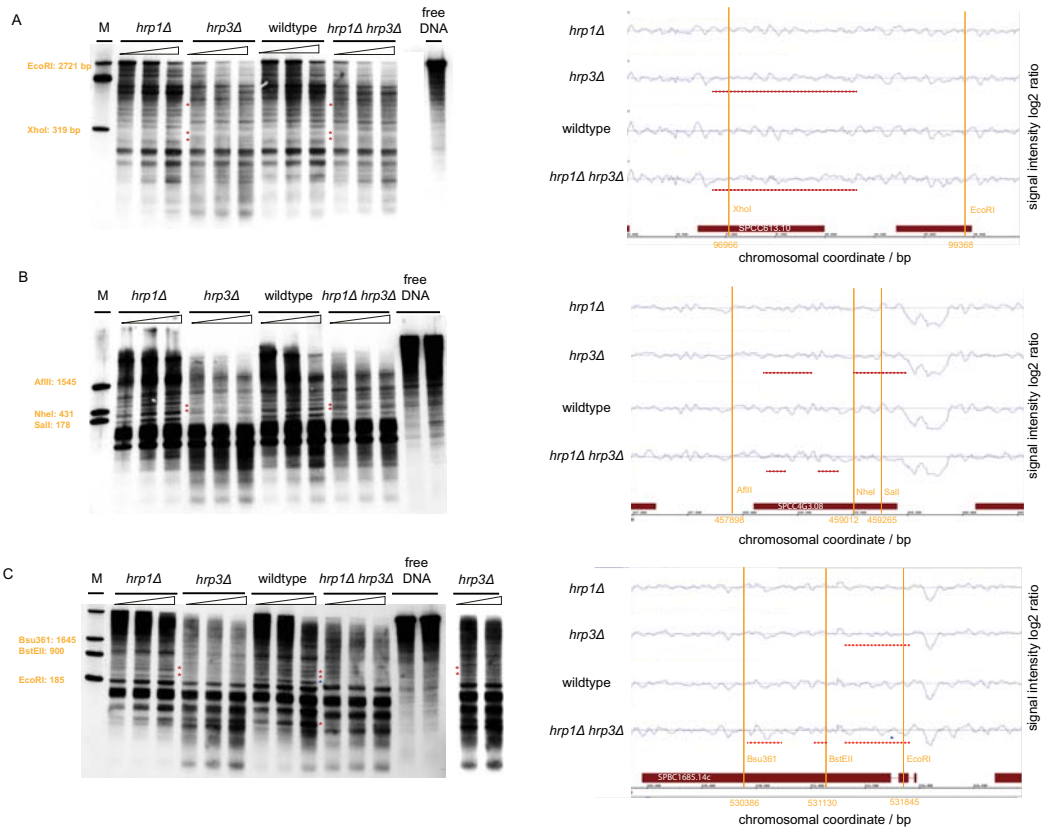
Supplementary Figure 3 *snf21-ts* and *snf21-ts swr1Δ* transcriptome responders do not have more pronounced defects in nucleosome positioning in the respective mutants. Same data as in Figure 1B, but divided into loci (**A**) >1.5 fold upregulated (138 calls) or no changes (1256), and (**B**) >1.5 fold downregulated (228 calls) or no changes (1256) in *snf21-ts* cells. Same data as in Figure 1C, but divided into loci (**C**) >1.5 fold upregulated (55 calls) or no changes (1425), and (**D**) >1.5 fold downregulated (175 calls) or no changes (1425) in *snf21-ts swr1Δ* cells.

Suppl. Fig. 4



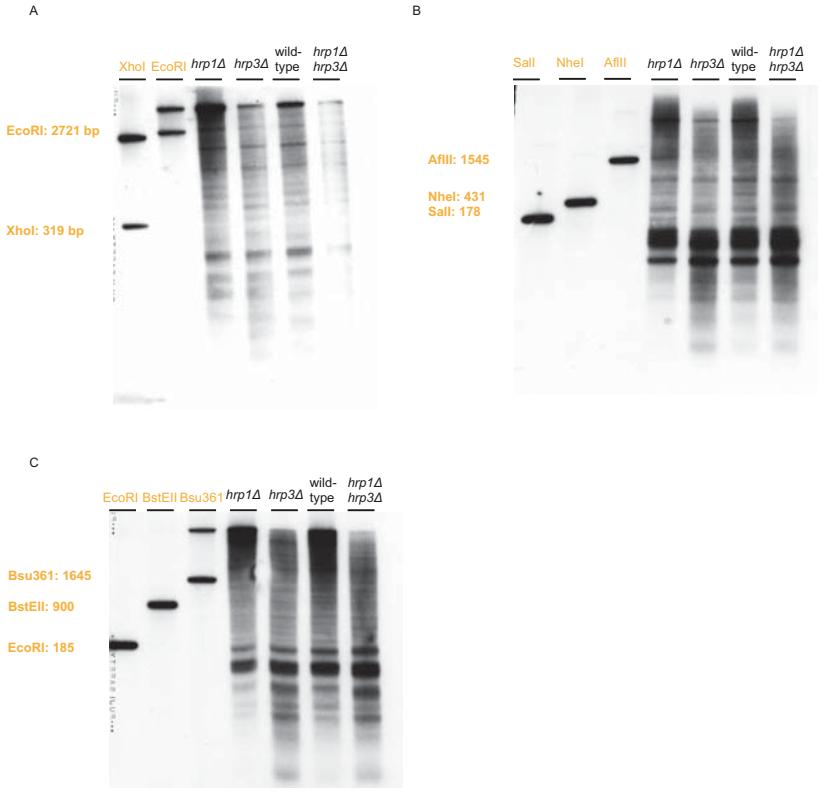
Supplementary Figure 4 *mit1Δ* transcriptome responders do not have more pronounced defects in nucleosome positioning in *mit1Δ* cells. Same data as in Figure 1D, but divided into loci (**A**) >1.5 fold upregulated (187 calls) or not (3826), and (**B**) >1.5 fold downregulated (309 calls) or not (3704) in *mit1Δ* cells.

Suppl. Fig. 5



Supplemantary Figure 5 Changed genic nucleosomal pattern in *hrp3Δ* and *hrp1Δ hrp3Δ* mutants relative to wt can also be seen at single loci. MNase indirect end labeling and corresponding nucleosome occupancy data by microarray hybridization are shown for three loci (**A**) SPCC613.10, (**B**) SPCC4G3.08 and (**C**) SPBC1685.14c. Sizes of marker fragments for indirect end labeling is given relative to the transcriptional start site. Wedges above the lanes indicate increasing MNase concentrations. All samples per locus were electrophoresed in the same gel, but an additional longer exposure is shown for *hrp3Δ* mutant in panel (**C**), two rightmost lanes. Red asterisks in indirect end labeling panels indicate pattern changes relative to wt. Blue asterisks show changed pattern revealed by both techniques at the same position. Red dashed horizontal lines in hybridization data panels point to changed nucleosome patterns relative to wt.

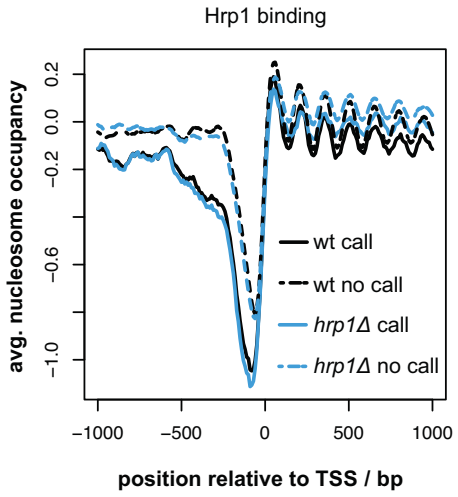
Suppl. Fig. 6



Supplemantary Figure 6 Mock MNase control for indirect end labeling of loci (**A**) SPCC613.10, (**B**) SPCC4G3.08 and (**C**) SPBC1685.14c. Samples were treated in the same way as for indirect end labeling in Suppl. Fig. 5 but without addition of MNase. Markers as in Suppl. Fig. 5.

Suppl. Fig. 7

A



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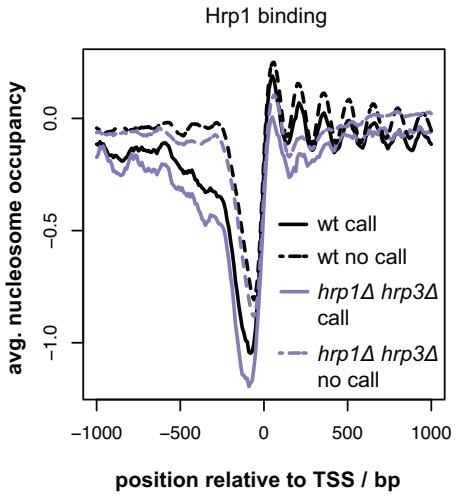

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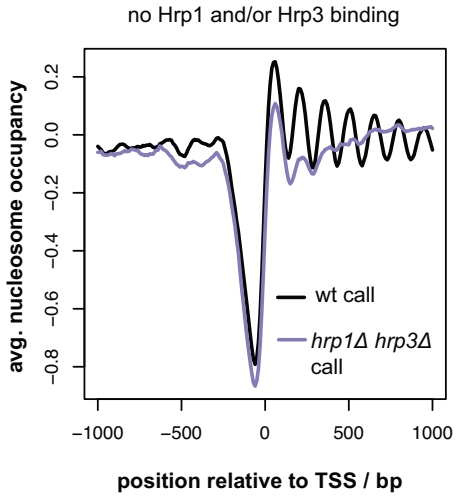
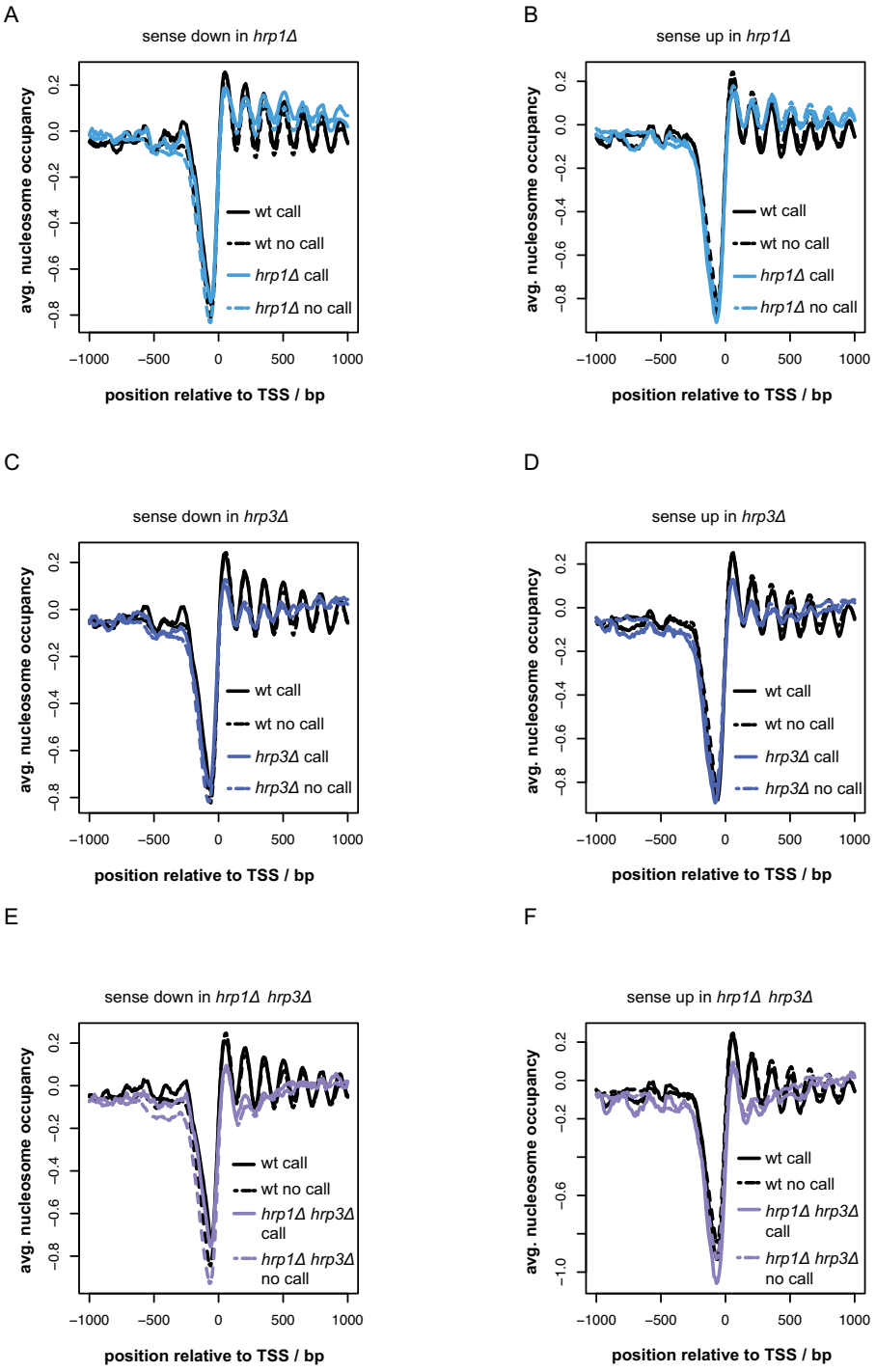
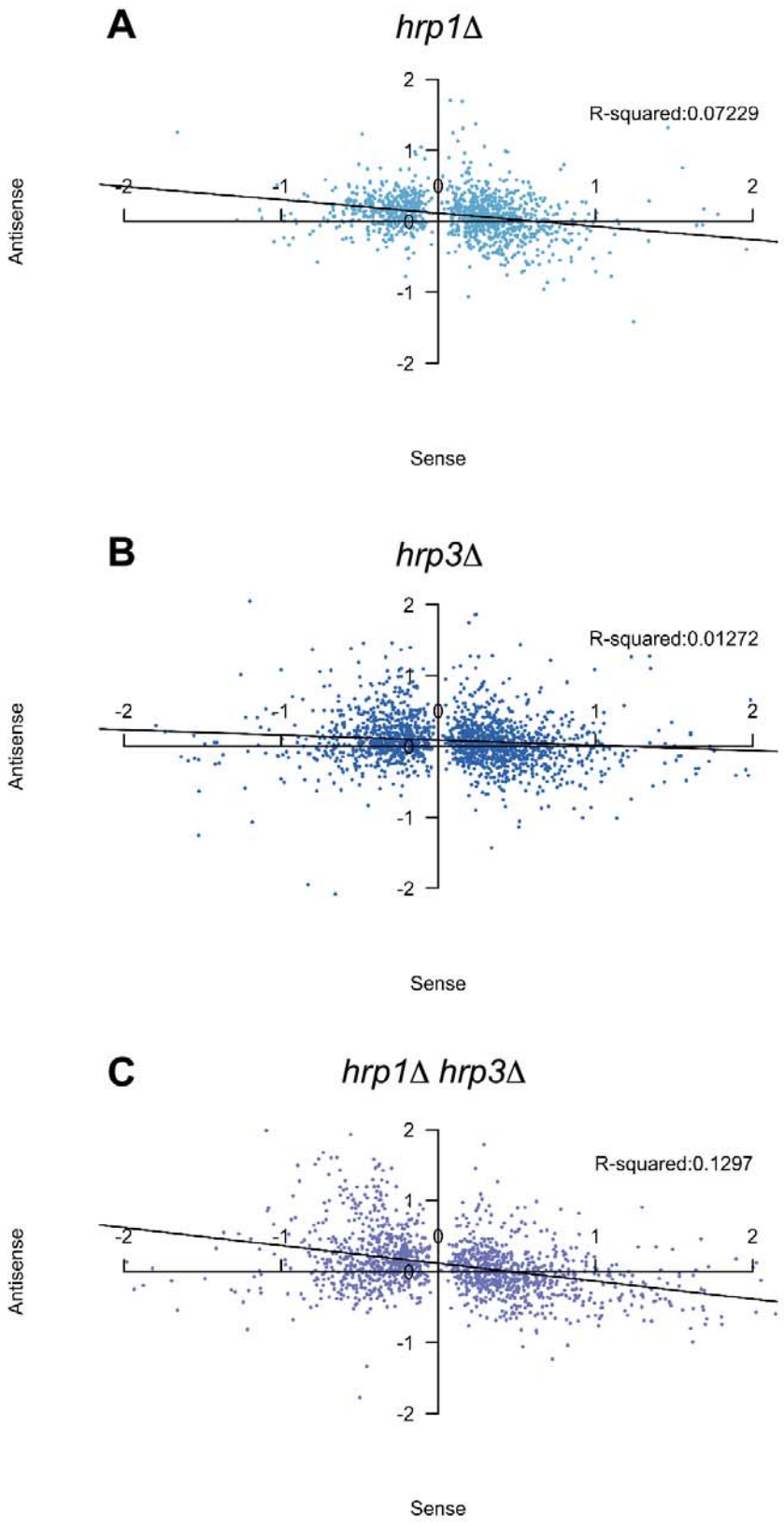


Supplementary Figure 7 Hrp1 binding targets do not really show more pronounced defects in nucleosome positioning in *hrp1Δ* and *hrp1Δ hrp3Δ* cells. (**A**) Same data as in Figure 3A, but divided into Hrp1-bound (428 calls) and -unbound (3585) loci. (**B**) Same data as in Figure 3C, but divided into subgroups as in panel (**A**). (**C**) Same data as in Figure 3C, but only loci are shown which are bound by neither Hrp1 nor Hrp3 (3443).



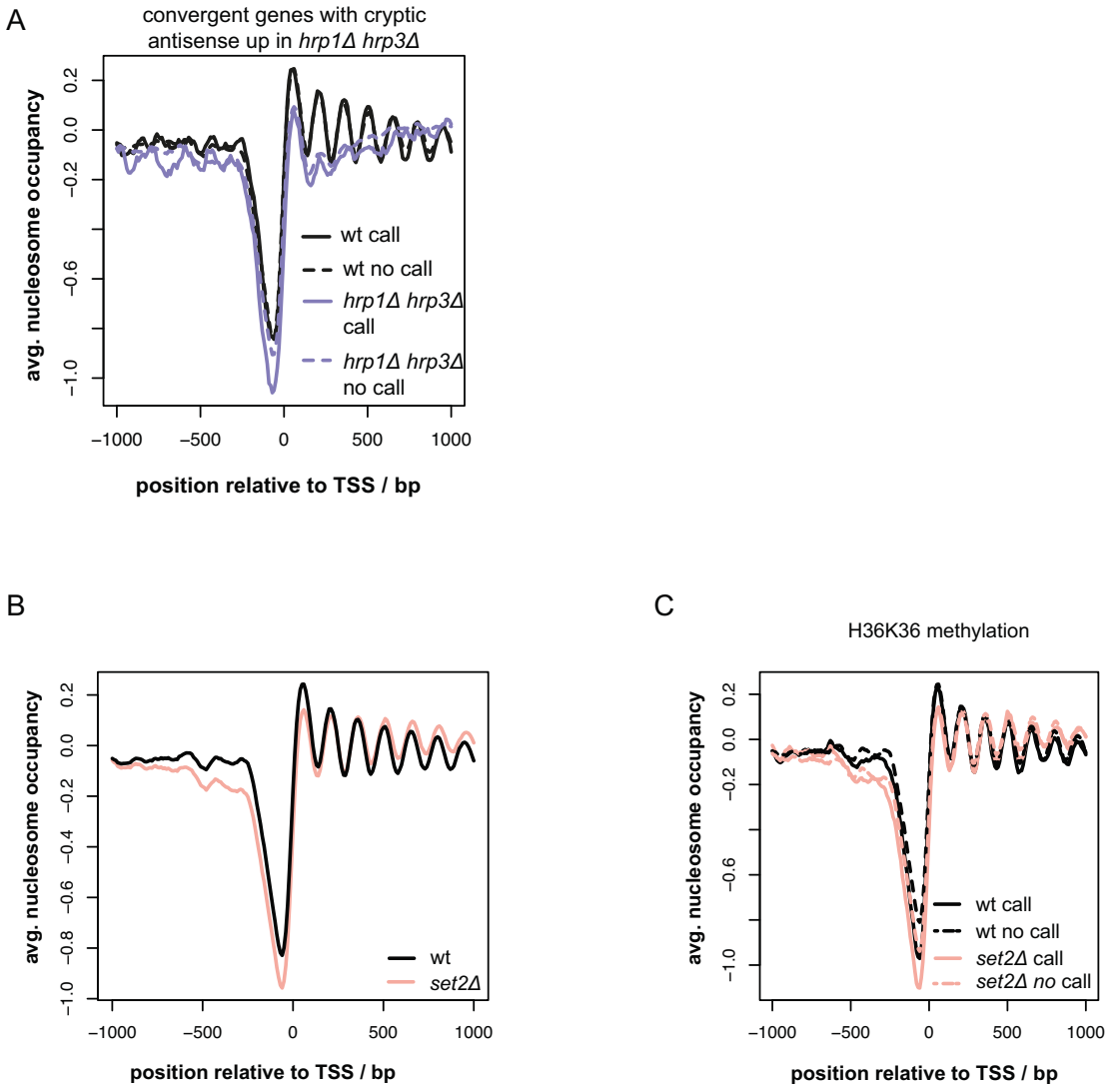
Supplementary Figure 8 Nucleosome occupancy patterns of *hrp* mutant responders. Same data as in Figure 3A, B and C, respectively, but divided into loci downregulated in (**A**) *hrp1Δ* cells (342 calls) or not changed (2835), (**C**) *hrp3Δ* cells (507 calls) or not changed (2481), and (**D**) *hrp1Δ hrp3Δ* cells (450 calls) or not changed (2570); correspondingly for the loci upregulated in (**B**) *hrp1Δ* cells (374 calls) or not changed (2835), (**D**) *hrp3Δ* cells (515 calls) or not changed (2481), and (**F**) *hrp1Δ hrp3Δ* cells (341 calls) or not changed (2570).

Suppl. Fig. 9



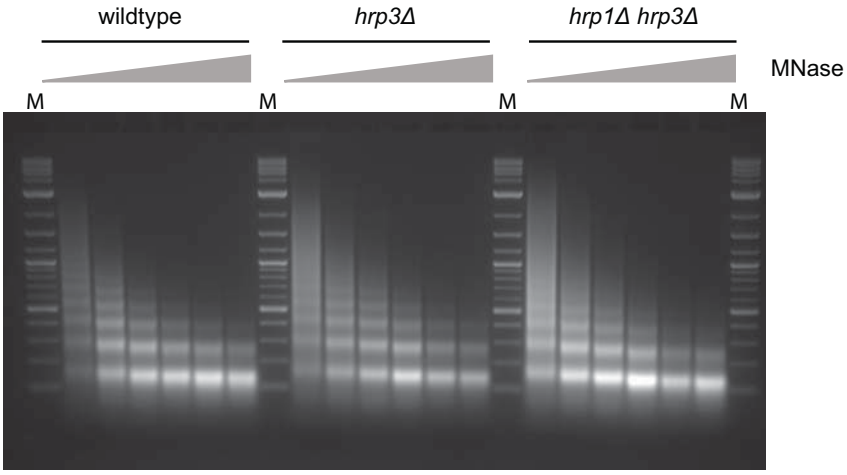
Supplementary Figure 9 Changes in cryptic antisense transcription do not correlate well with changes of sense transcription at the same locus. Scatter plot of sense and cryptic antisense expression changes vs. wt for (**A**) *hrp1Δ*, (**B**) *hrp3Δ*, and (**C**) *hrp1Δ hrp3Δ* cells.

Suppl. Fig. 10



Supplementary Figure 10 Impaired genic arrays did not correlate with convergent gene orientation and were not detected in *set2Δ* cells. Same data as in Figure 3C, but divided into subgroups of (**A**) convergent genes with upregulated cryptic antisense transcription (424 calls) or genes without transcriptional changes (2570). (**B**) TSS-aligned nucleosome occupancy profiles for 4013 genes in wt (Hu303, average of five biological replicates) and *set2Δ* (Hu1582, average of two biological replicates) at 30 °C. (**C**) Same data as in panel (**B**), but divided into loci with H3K36Me mark (469 calls) or not (3544).

A



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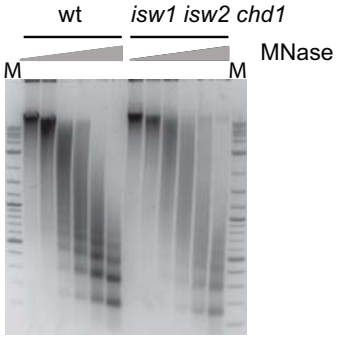

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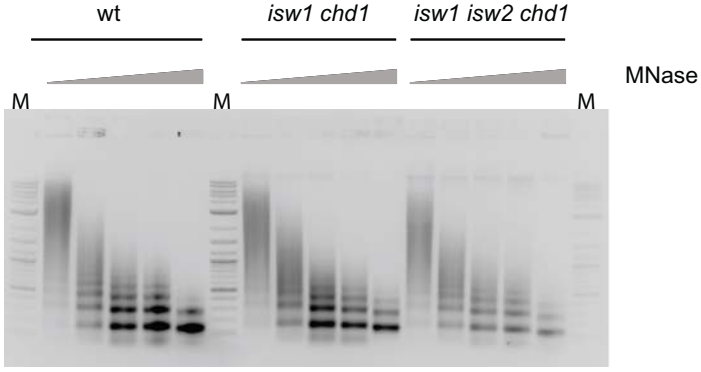
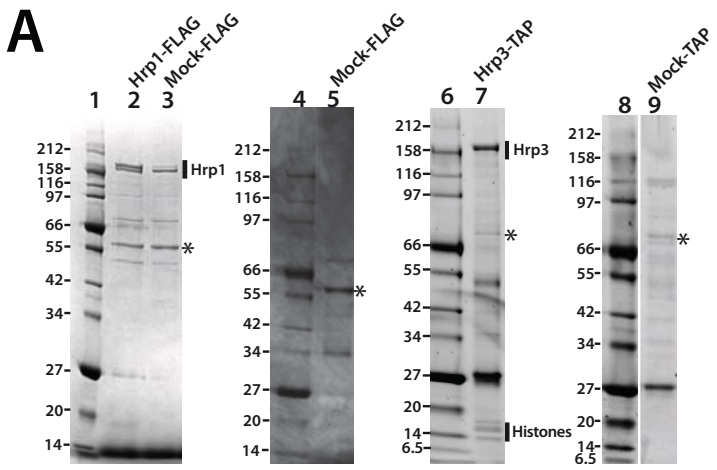
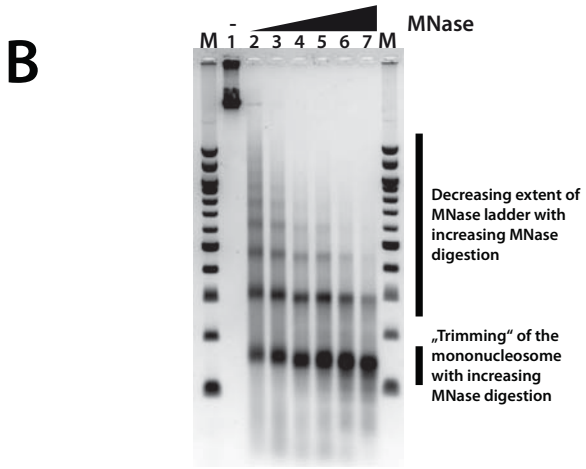


Supplementary Figure 11 Fission and budding yeast mutants with impaired genic arrays still show substantial bulk MNase ladders. MNase ladder assay for (**A**) wt (Hu303), *hrp3Δ* (Hu0575/EJY321) and *hrp1Δ hrp3Δ* (Hu2303), (**B**) wt (BMA64) and *isw1 isw2 chd1* (MP28/MP280), and (**C**) wt (W1588-4C), *isw1 chd1* (YTT223), and *isw1 isw2 chd1* (YTT227). One representative example of two biological replicates for each strain is shown. Twice the amount of material was loaded for Hu2303 as lower signal intensity was expected in analogy to respective *S. cerevisiae* mutants. However, this resulted in almost twice as strong signal arguing against increased MNase sensitivity in this mutant. Wedges on top of the lanes denote increasing MNase concentrations of 0.38; 0.96; 1.5; 3.1; 4.6; 6.2 U/ml for panel (A), 0.025; 0.05; 0.09; 0.19; 0.38, 0.75 U/ml for panel (B), and 0.05; 0.09; 0.19; 0.38; 0.75 U/ml for (C). Marker lanes "M" contain 2log-ladder (New England Biolabs).



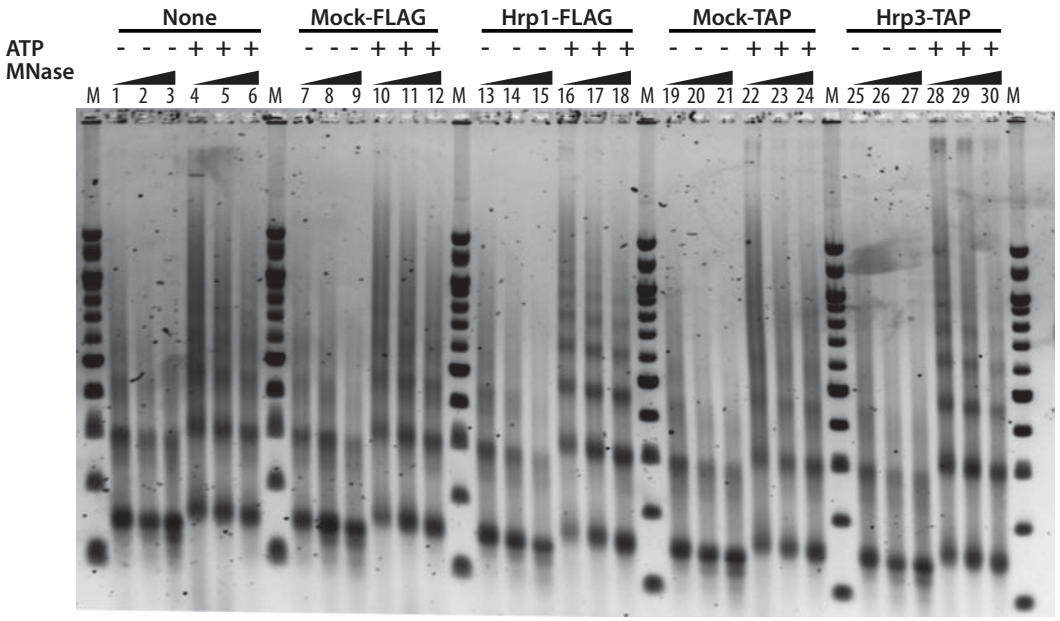
Hrp1-FLAG		Mock-FLAG (Hu0807)	
Protein	ΣCoverage	Protein	ΣCoverage
Hrp1	31.46	Hrp1	24.11
Hrp1	30.37	Hrp1	36.34
Ssa2	39.41	Ssa2	45.13
RBP	32.77	RBP	47.99
RBP	21.99	RBP	27.70
RBP	7.19	MUG14	5.06

Ssa2: heat shock protein; RBP: RNA binding protein; MUG14: Meiotically upregulated gene 14



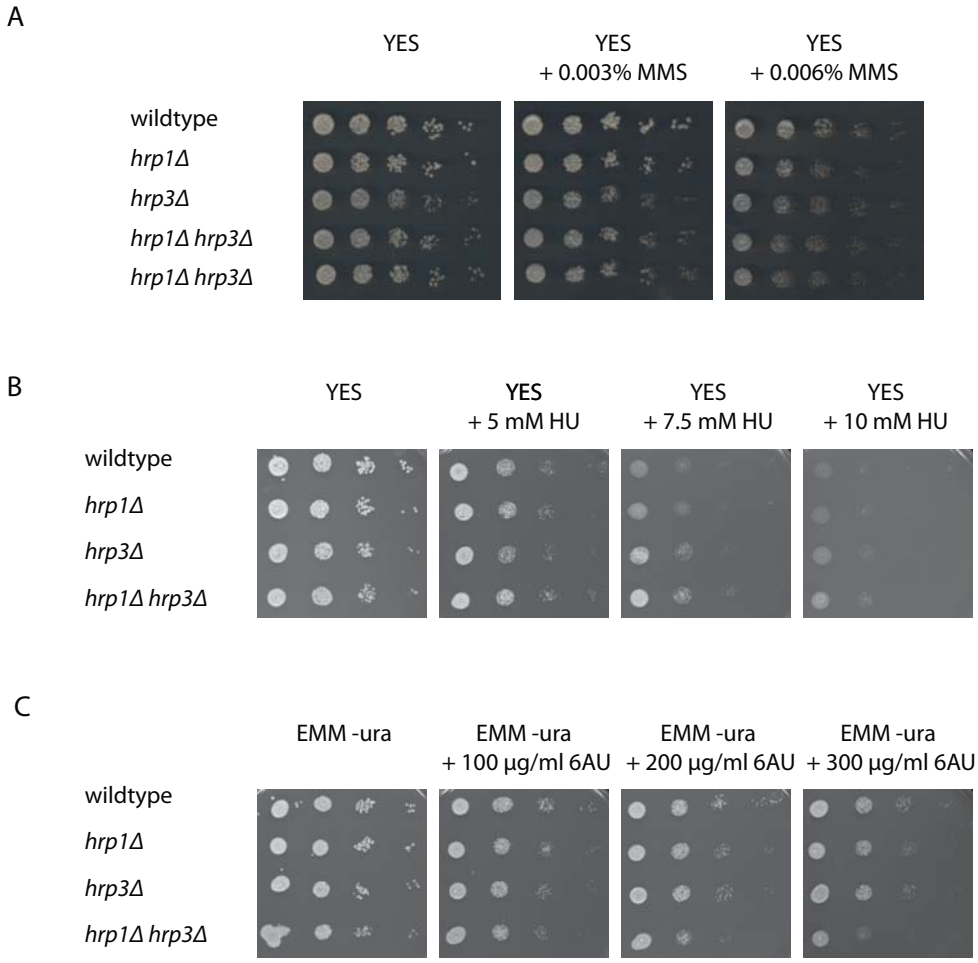
Supplementary Figure 12 Purification of Hrp1 and Hrp3 and titration of MNase digestion. (**A**) Affinity purified Hrp1-FLAG (lane 2) and Hrp3-TAP (lane 7) were analysed on 4-12% Bis-tris denaturing PAGE along with respective mock purifications from untagged strains (lanes 3 (Hu0807), 5 and 9 (both Hu2304)), and protein markers (lanes 1, 4, 6 and 8). The most prominent proteins in the preparations loaded in lanes 2 and 3 as detected by mass spectrometry are given in the table. Interestingly, Hrp1 binds non-specifically to FLAG M2-agarose beads (lane 3). Therefore mock-FLAG purification from Hu2304 (*hrp1Δ hrp3Δ*) was used for the control assay in Suppl. Fig. 13. For mass spectrometry analysis of Hrp3-TAP and respective mock preparation see Supplementary Table I. Asterisks next to lanes 3, 5, 7 and 9 denote the non-specifically co-purifying protein bands that were quantified in order to use equivalent amounts of mock and true purifications in the control assays in Suppl. Fig. 13. Samples in lane 8 and 9 were electrophoresed together in the same gel, but cut out and placed next to each other in PhotoshopCS5. (**B**) Sample as in Figure 6A, lanes 10-12, was digested with a wider range of MNase concentrations (0; 2; 4; 8; 16; 32 and 64 U/ml) and analyzed in a 1.7% agarose gel. Marker lanes „M" as in Figures 5 and 6.

Suppl. Fig. 13



Supplementary Figure 13 Hrp1 and Hrp3 but not mock preparations show ATP-dependent nucleosome spacing activity. Same experiment as in Figure 5 but also including equivalent amounts of mock-FLAG and mock-TAP preparations (see Suppl. Fig. 12A).

Suppl. Fig. 14



Supplementary Figure 14 Drug sensitivity assays for *hrp* mutants. The sensitivity for (**A**) methylmethanesulfonate (MMS), (**B**) hydroxyurea (HU), and (**C**) 6-azauracil (6-AU) was measured by spotting assay for the indicated strains (Hu303, Hu2239, Hu0807, Hu2303) and conditions.