Legends to Supplementary Figures

Figure S1. Size distribution of MNase-seq libraries

The size distributions of aligned fragments (paired-end) of wild-type (WT), *hrp3Δ,* and *hrp3Δ* biological replicate were examined. The number of aligned reads (n) in each sample is shown.

.

Figure S2. The patterns of nucleosome positioning

(A) Heatmaps showing nucleosome patterns in wild-type, *hrp1Δ* and *mit1Δ*. The patterns of nucleosome positioning were profiled with a total of 1,718 non-overlapping regions ~ 2-kb of the TSSs. Yellow and blue indicate nucleosome-positioned and nucleosome-depleted regions, respectively. (B) A view of the nucleosome positions in genomic DNA, wild-type, *hrp1Δ*, *mit1Δ,* and *hrp3Δ*. The total reads in each sample were normalized with respect to the RPM. Nucleosome patterns on the *SPBC543.02C* and *pek1* genes are shown in red boxes. The y-axis indicates the nucleosome density.

Figure S3. Hrp1 and Mit1 do not affect the production of antisense transcripts

Loss of Hrp1 (A) or Mit1 (B) does not suppress antisense transcription at *zer1*$^{+}$. Strand-specific RT-qPCR of sense or antisense transcripts corresponding to *zer1*$^{+}$ was performed as described in Figure 3B. The graphs show the amount of transcripts relative to those of wild-type. The data represent the average of three independent experiments, and the error bars show the standard deviations.

Figure S4. Hrp3 is involved with transcriptional elongation

The 6-AU phenotype of cells lacking Hrp1 and Hrp3. Wild-type and the indicated mutant strains were grown on minimal medium with or without 6-AU (100 or 200 μg/ml). Cells were

serially diluted 5-fold and grown for 3 to 4 days. All strains were made by crossing with the 972 strain to select the *ura*$^{+}$ haploid; wild type (972), *hrp1Δ* (YS325), and *hrp3Δ* (YS251).

Figure S5. Hrp3 can be separated from Hrp1

(A) Fractionation scheme for separating Hrp3-TAP and Hrp1-3XFLAG using SP sepharose ion-exchange chromatography, and the obtained fractions. The indicated fractions were loaded and analyzed by Western blotting using α-FLAG and α–calmodulin-binding peptide (CBP) antibodies. (B) TAP purification of Hrp3 from peak fractions 40-44. Samples were resolved in 10% SDS PAGE gels and silver stained. Each band was identified using mass spectrometry.

Figure S6. The *hrp3*∆ mutation does not affect the localization of Clr6 HDAC complex

ChIP experiments were performed in either wild-type (no tag) or strains containing Sds3 (A) or Cph1 (B) with 13 Myc-epitope tags at the C-terminus. α-Myc antibody and the primer sets described in Figure 3C were used for ChIP assays. The occupancies of Sds3-Myc and Cph1-Myc were normalized with respect to the results obtained from an untagged control. The error bars in (A) and (B) represent the standard deviations from three independent repeats.

Figure S7. Hrp3 exhibits nucleosomal ATPase activity

ATPase activity assays were performed with FLAG-tagged Hrp3 and Hrp3^{K406A} purified from yeast cells. Free phosphate (Pi) from [r-^{32}P] ATP was separated on polyethyleneimine cellulose TLC plates. The relative ATPase activity was calculated as the ratio between the activity observed in the presence of the purified proteins and the basal activity observed in the presence of only LON (A) or dsDNA (B). The Hrp3^{K406A} protein was defective for ATPase activity. The (A) graph represents average values of three independent repeats but The (B) graph represents average values of two separate experiments.

Figure S8. Hrp3 modulates chromatin architecture

(A) Wild-type and *hrp3Δ* cells were grown in YES at 30°C and nuclei were digested with 500 U of MNase for 5, 15, 30, 45, and 60 min. The digested products were resolved on an ethidium-bromide-containing gel and visualized under UV illumination. The panels on the right show overlaid intensity traces of the 15 min MNase lanes (left panels) of wild-type and mutants, as analyzed using the Image J-software. (B) Nucleosomal DNA fragments of Hrp3-3XFLAG and *hrp3*^{K406A}-3X FLAG cells were prepared by digestion with 500 U of MNase for 5, 15, 30, 45, and 60 min, as described in (A).

Figure S9. Southern blot hybridization using probes specific for *sod2+*

Southern blot analysis was performed using wild-type, *hrp3*Δ (A), and Hrp3 3XFLAG, *hrp3*^{K406A}-3XFLAG cells (B) with probes corresponding to *sod2*$^{+}$. The DNA samples used in Supplementary Figure 7 (A) and (B) were analyzed by Southern blotting.

Figure S10. Hrp3 acts cooperatively with other heterochromatin factors to maintain *otr1R::ura4*$^{+}$ silencing

Serial dilution plating assays in the presence and absence of 5-fluoroorotic acid (FOA) were used to assess *otr1R::ura4*$^{+}$ expression in *hrp3*Δ*clr4*^{R320H} and *hrp3*Δ*alp13*Δ double mutants. Cells were spotted as serial 5-fold dilutions.

Figure S11. The chromatin remodeling activity of Hrp3 regulates *dg/dh* transcription

Strand-specific RT-qPCR of the *dg/dh* region was performed in Hrp3-3XFLAG and *hrp3*^{K406A}-3XFLAG strains, as described in Figure 5A. The graphs show the amount of transcripts relative to those of Hrp3-3XFLAG. The data represent the average of three independent experiments, and the error bars show the standard deviations between these experiments.

Figure S12. Genome-wide nucleosome patterns in wild-type and *hrp3Δ*

The top two tracks show nucleosome patterns of wild-type (wt, gray) and *hrp3Δ* (red) on chromosome II. The bottom track illustrates the relative level of nucleosome density between wild-type and *hrp3Δ,* using the log2 ratio (500-bp sliding window).

Figure S13. *hrp1Δ* doesn't affect cell-cycle dependent transcription of *dh* element

Strand-specific RT-PCR analysis of *dh* region in *cdc25-22 hrp1*Δ cells was performed as described in Figure 7B.

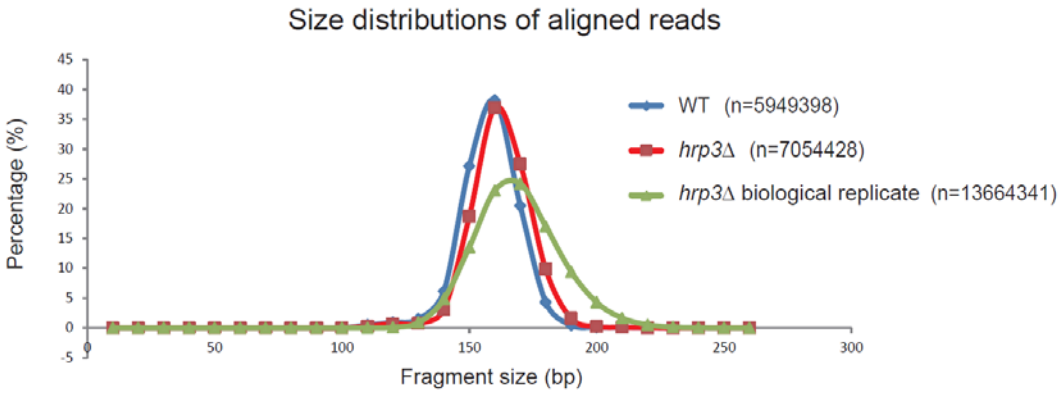

Size distributions of aligned reads
Percentage (%)
45
40
35
30
25
20
15
10
5
0
-5
0
50
100
150
200
250
300
Fragment size (bp)
WT (n=5949398)
hrp3Δ (n=7054428)
hrp3Δ biological replicate (n=13664341)

Figure S1

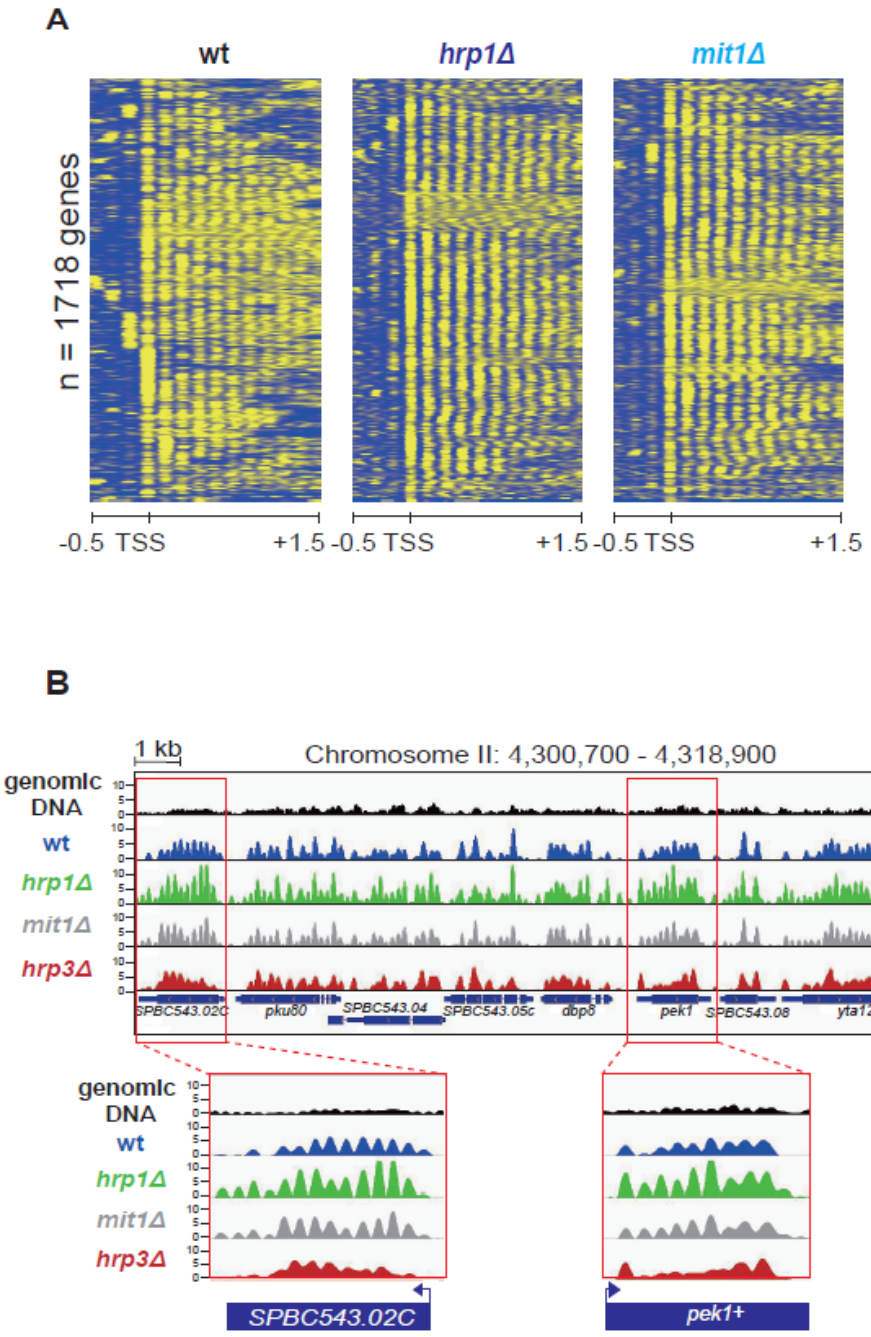

A
wt
hrp1Δ
mit1Δ
n = 1718 genes
-0.5 TSS
+1.5
-0.5 TSS
+1.5
-0.5 TSS
+1.5
B
1 kb
Chromosome II: 4,300,700 - 4,318,900
genomic DNA
wt
hrp1Δ
mit1Δ
hrp3Δ
SPBC543.02C
pku80
SPBC543.04
SPBC543.05c
dbp8
pek1
SPBC543.08
yta12
SPBC543.02C
pek1+

Figure S2

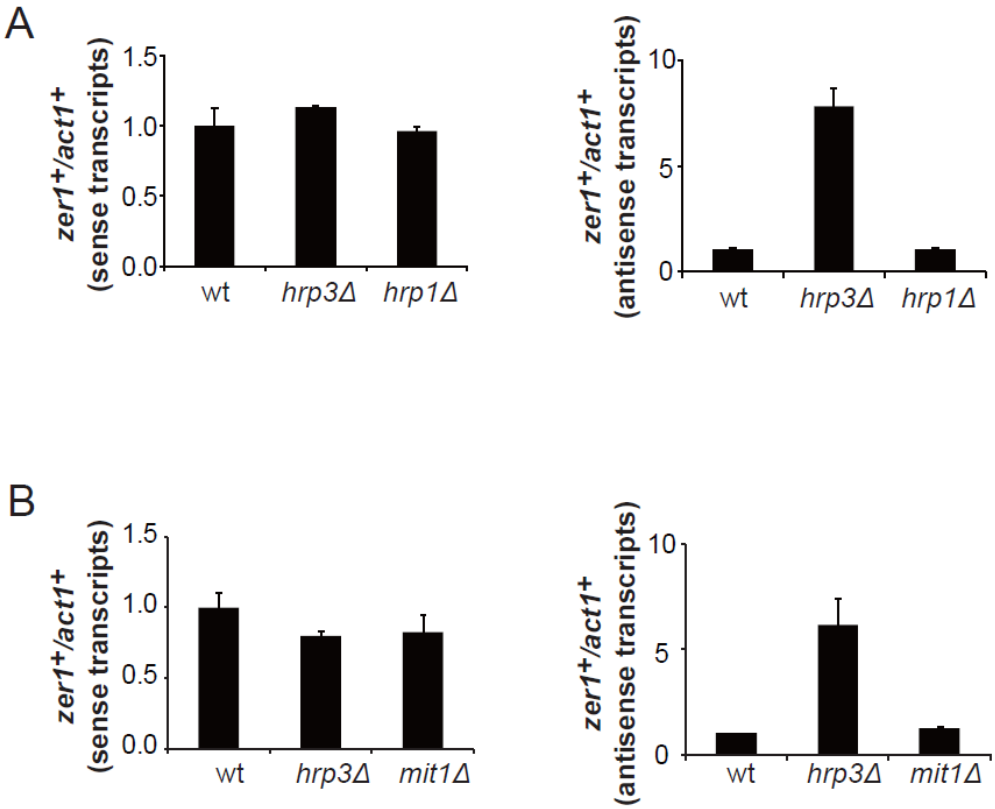

A
zer1+/act1+ (sense transcripts)
1.5
1.0
0.5
0.0
wt
hrp3Δ
hrp1Δ
zer1+/act1+ (antisense transcripts)
10
5
0
wt
hrp3Δ
hrp1Δ
B
zer1+/act1+ (sense transcripts)
1.5
1.0
0.5
0.0
wt
hrp3Δ
mit1Δ
zer1+/act1+ (antisense transcripts)
10
5
0
wt
hrp3Δ
mit1Δ

Figure S3

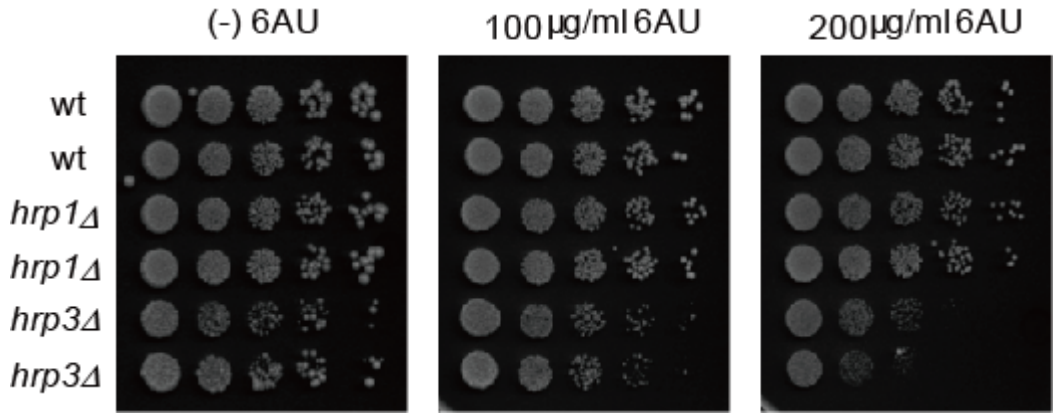


Figure S4

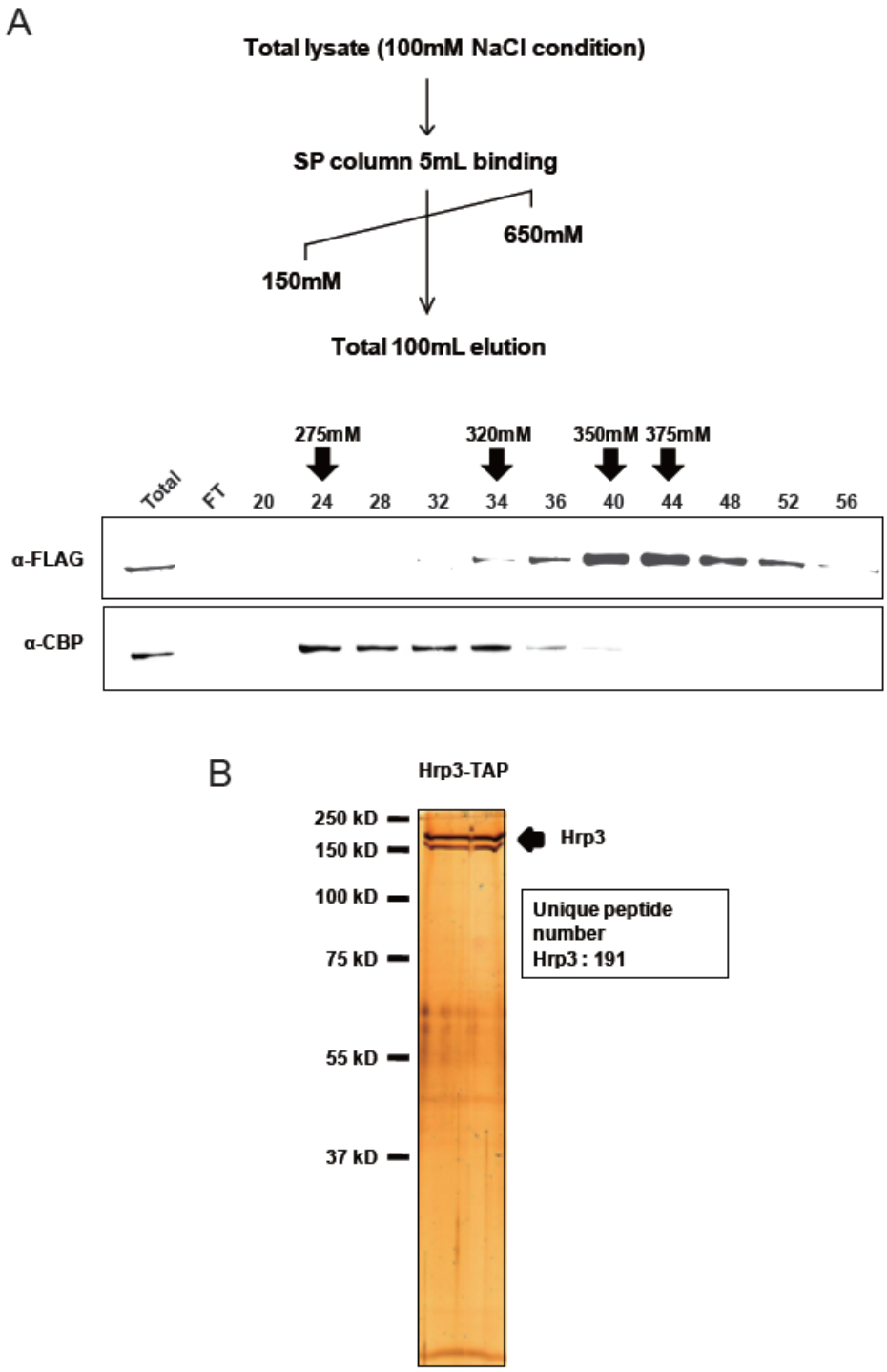

A
Total lysate (100mM NaCl condition)
SP column 5mL binding
650mM
150mM
Total 100mL elution
275mM
320mM
350mM
375mM
Total
FT
20
24
28
32
34
36
40
44
48
52
56
α-FLAG
α-CBP
B
Hrp3-TAP
250 kD
150 kD
100 kD
75 kD
55 kD
37 kD
Hrp3
Unique peptide number
Hrp3 : 191

Figure S5

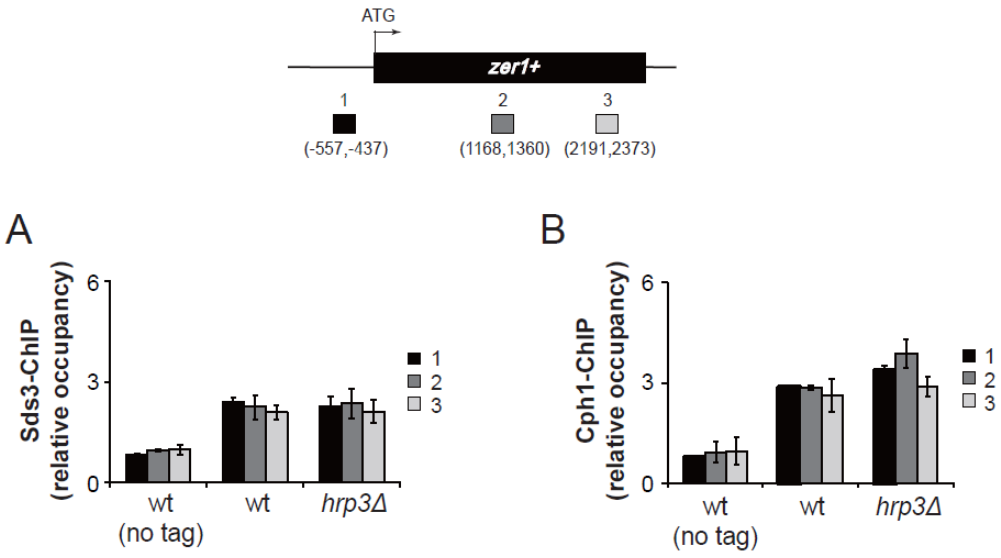

ATG
zer1+
1
2
3
(-557,-437)
(1168,1360)
(2191,2373)
A
Sds3-ChIP
(relative occupancy)
6
3
0
wt
(no tag)
wt
hrp3Δ
1
2
3
B
Cph1-ChIP
(relative occupancy)
6
3
0
wt
(no tag)
wt
hrp3Δ
1
2
3

Figure S6

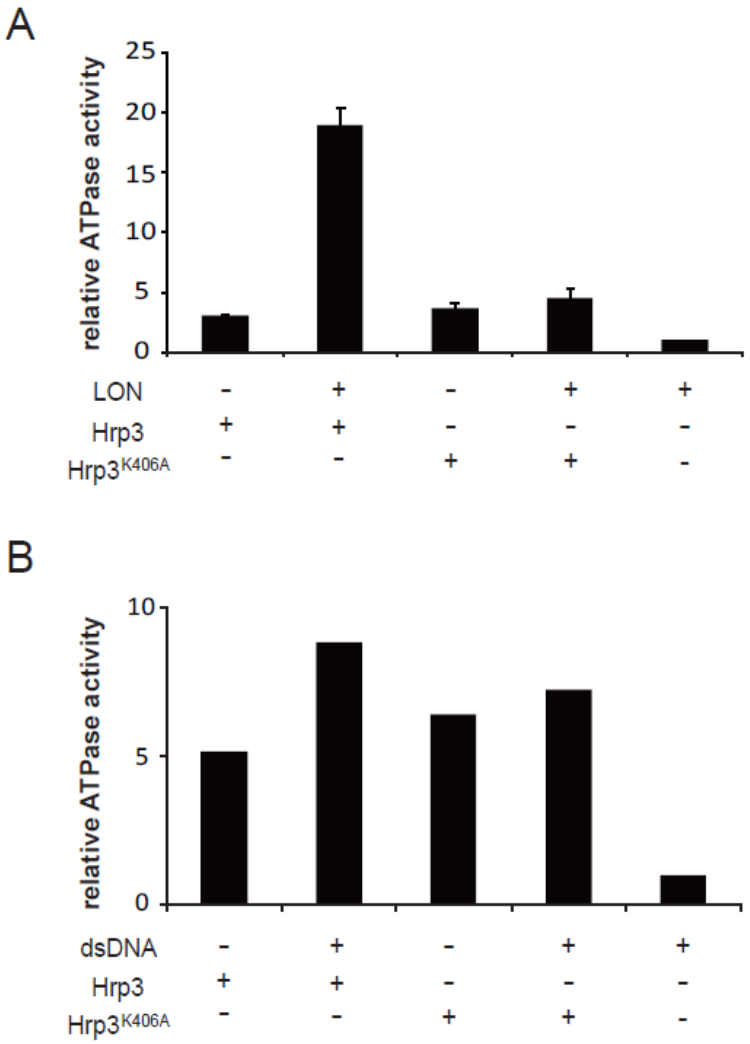
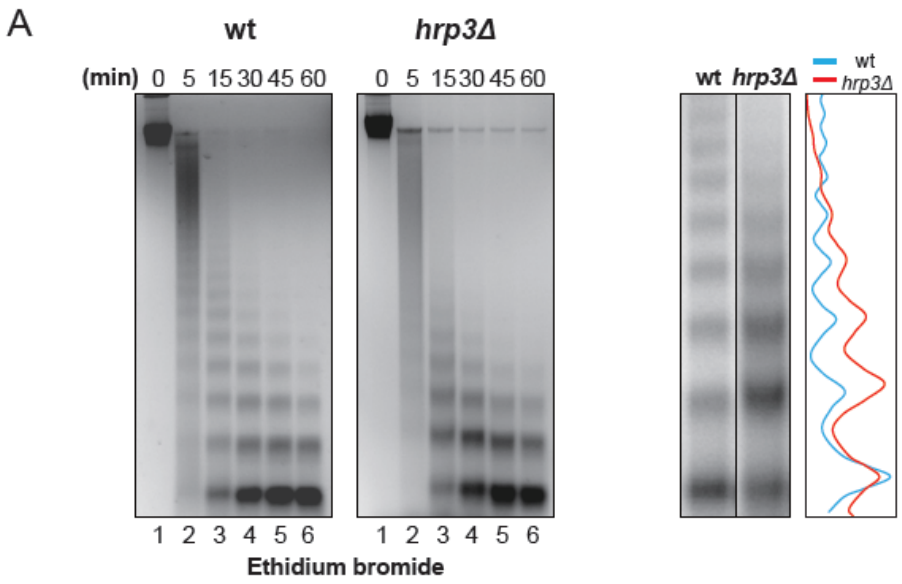

A
relative ATPase activity
25
20
15
10
5
0
LON - + - + +
Hrp3 + + - - -
Hrp3K406A - - + + -
B
relative ATPase activity
10
5
0
dsDNA - + - + +
Hrp3 + + - - -
Hrp3K406A - - + + -

Figure S7


A
wt
hrp3Δ
(min) 0 5 15 30 45 60
0 5 15 30 45 60
wt hrp3Δ
wt
hrp3Δ
1 2 3 4 5 6
1 2 3 4 5 6
Ethidium bromide

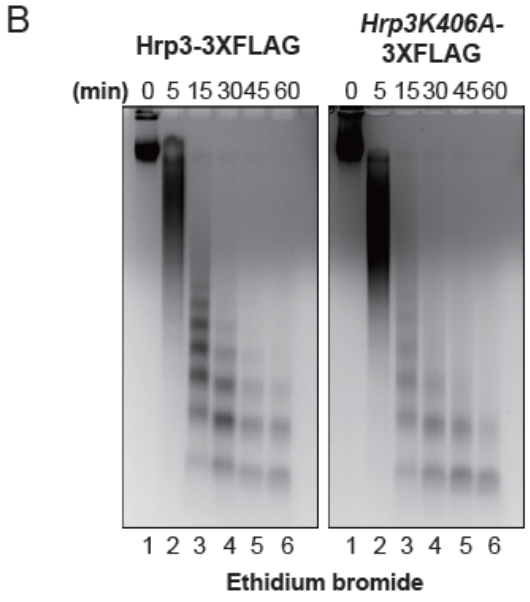

B
Hrp3-3XFLAG
Hrp3K406A-3XFLAG
(min) 0 5 15 30 45 60
0 5 15 30 45 60
1 2 3 4 5 6
1 2 3 4 5 6
Ethidium bromide

Figure S8

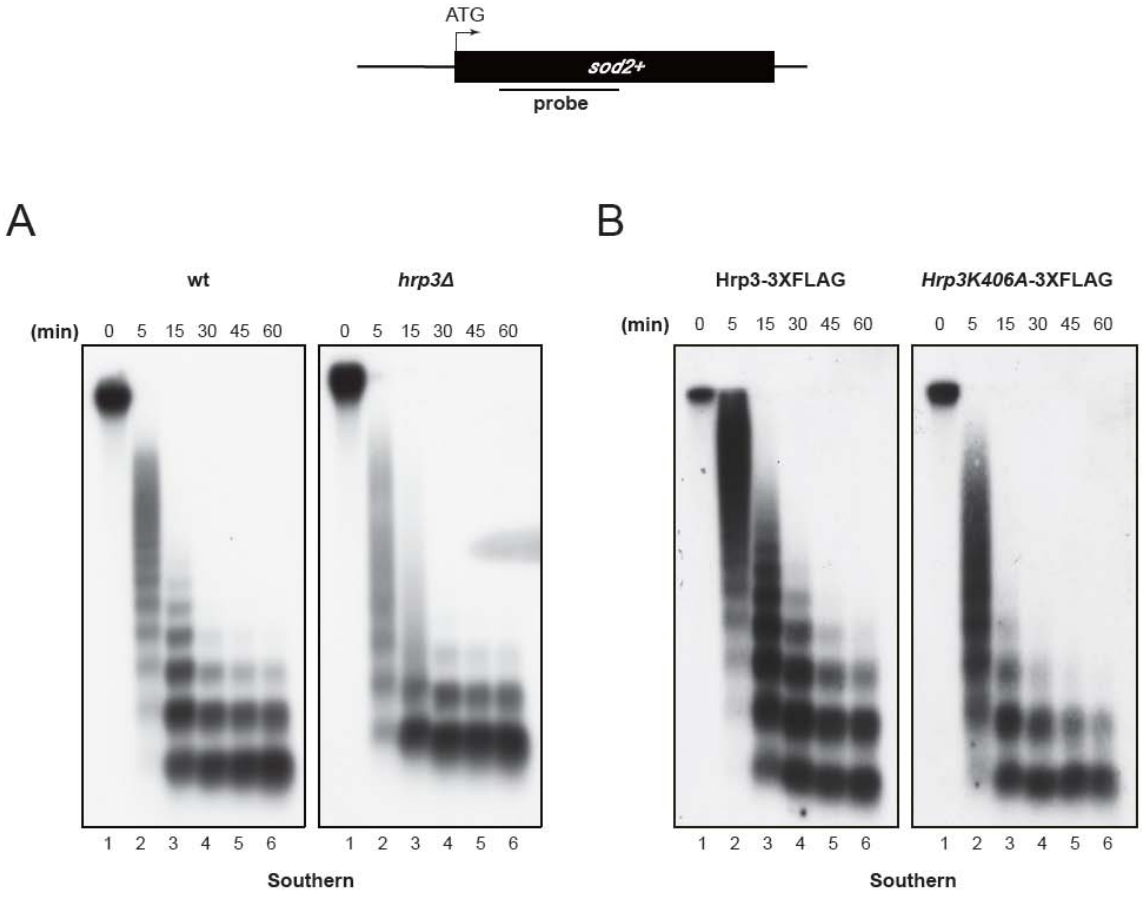

ATG
sod2+
probe
A
wt
hrp3Δ
(min) 0 5 15 30 45 60 0 5 15 30 45 60
1 2 3 4 5 6 1 2 3 4 5 6
Southern
B
Hrp3-3XFLAG
Hrp3K406A-3XFLAG
(min) 0 5 15 30 45 60 0 5 15 30 45 60
1 2 3 4 5 6 1 2 3 4 5 6
Southern

Figure S9

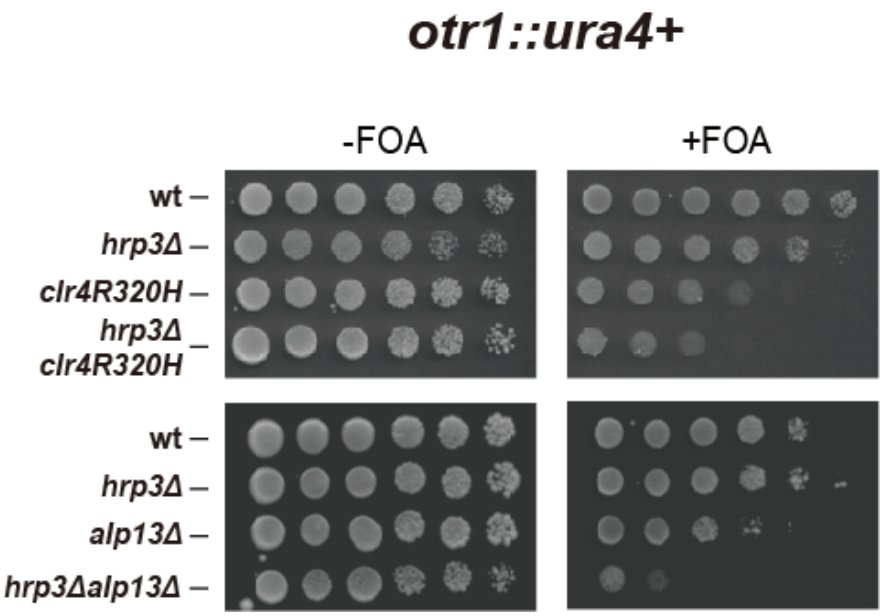

otr1::ura4+
-FOA
+FOA
wt
hrp3Δ
clr4R320H
hrp3Δ clr4R320H
wt
hrp3Δ
alp13Δ
hrp3Δalp13Δ

Figure S10

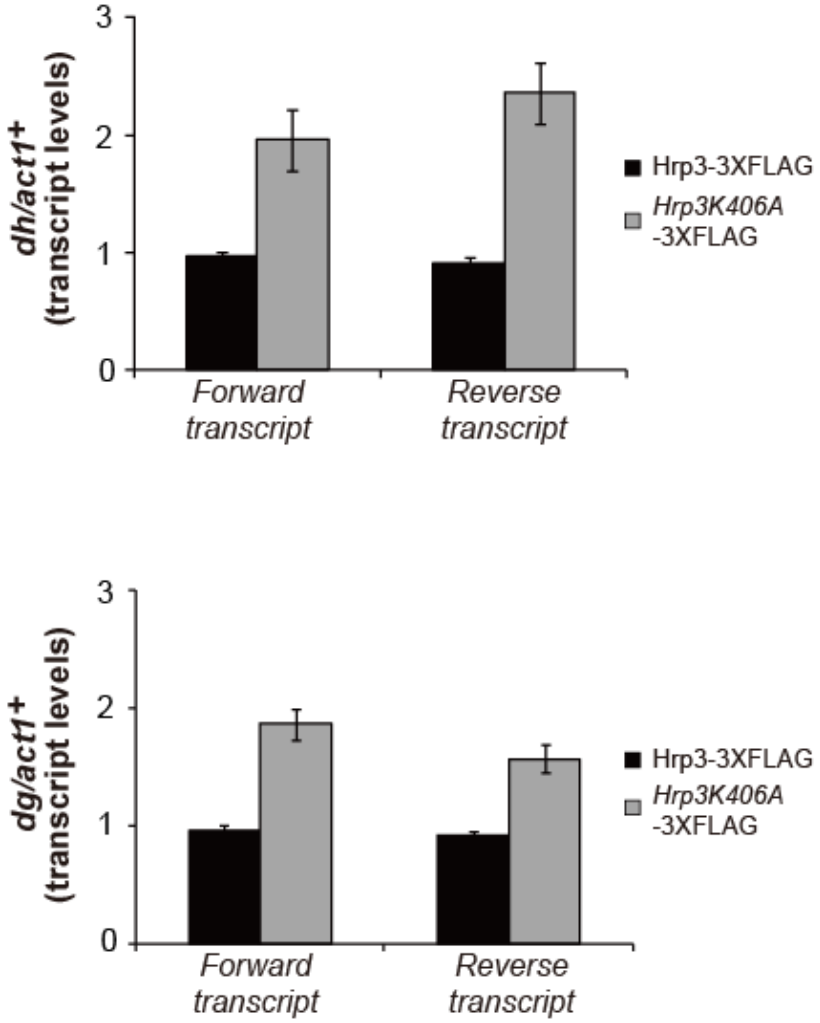

dh/act1+
(transcript levels)
3
2
1
0
Forward
transcript
Reverse
transcript
Hrp3-3XFLAG
Hrp3K406A
-3XFLAG
dg/act1+
(transcript levels)
3
2
1
0
Forward
transcript
Reverse
transcript
Hrp3-3XFLAG
Hrp3K406A
-3XFLAG

Figure S11

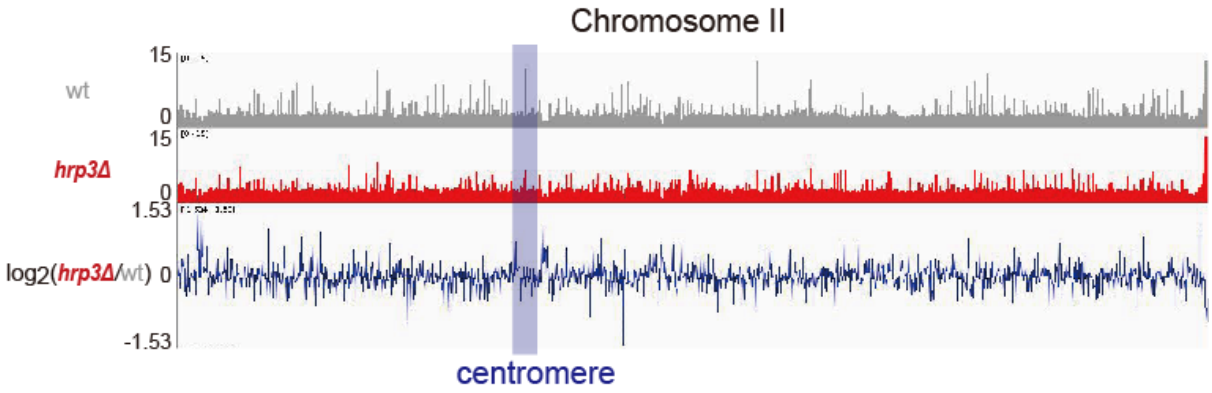

Chromosome II
15
wt
0
15
hrp3Δ
0
1.53
log2(hrp3Δ/wt) 0
-1.53
centromere

Figure S12

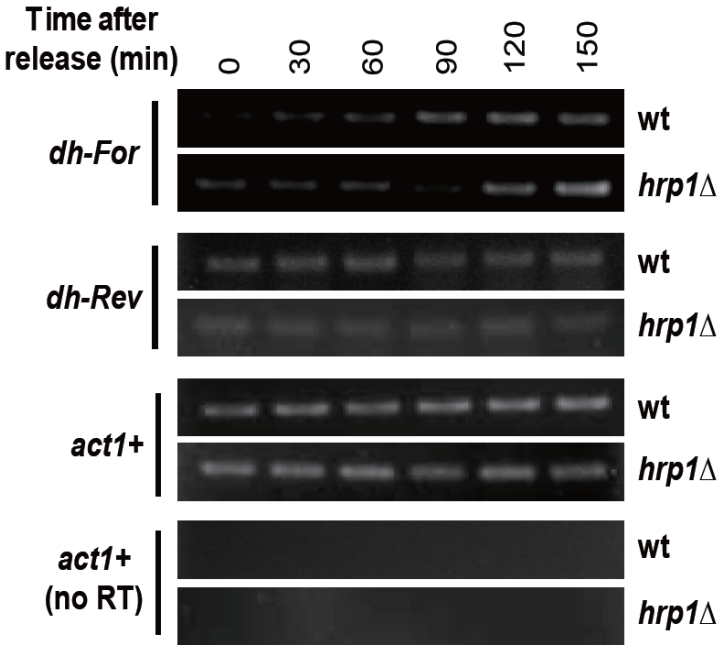

Time after release (min)
0
30
60
90
120
150
dh-For
wt
hrp1Δ
dh-Rev
wt
hrp1Δ
act1+
wt
hrp1Δ
act1+ (no RT)
wt
hrp1Δ

Figure S13

Table S1. Yeast strains used in this study

Strain		Genotype	Source
YS13	*h*$^-$	*h*$^-$ *ade6-M210 leu1-32 ura4-D18*	This study
YS14	*h*$^+$	*h*$^+$ *ade6-M210 leu1-32 ura4-D18*	This study
YS27	*h*$^+$	*hrp1Δ::kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
YS28	*h*$^+$	*hrp3Δ::hphMX6 ade6-M216 leu1-32 ura4-D18*	This study
YS39	*h*$^+$	*clr4Δ::kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS136	*h*$^-$	*Hrp1-3xFLAG::kanMX6 hrp3-3HA-TAP::kanMX6 ade6-210 leu1-32 ura4-D18*	This study
YS176	*h*$^+$	*Hrp3-3XFLAG:: kanMX6 ade6-M210 leu1-32 ura4-*	This study
YS229	*h*$^-$	*set2Δ:: kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS338	*h*$^+$	*hrp3Δ::hphMX6 set2Δ::ura4+ ade6-M216 leu1-32 ura4-D18*	This study
YS342	*h*$^+$	*alp13Δ::kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS374	*h*$^-$	*hrp3Δ::hphMX6 alp13Δ:: kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS487	*h*$^-$	*Hrp3^{K406A}-3XFLAG::kan ade6-M210 leu1-32 ura4-D18*	This study
YS543	*h*$^-$	*Cph1-13Myc::KanMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS547	*h*$^-$	*Sds3-13Myc::KanMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS569	*h*$^-$	*Cph1-13Myc::KanMX6 hrp3Δ::hphMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS639	*h*$^+$	*Sds3-13Myc::KanMX6 hrp3Δ::hphMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS675	*h*$^+$	*clr3Δ::kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
YS685	*h*$^-$	*hrp3Δ::hphMX6 clr3Δ:: kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
HG1089	*h*$^-$	*swi6Δ::kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
HG1139	*h*$^-$	*mit1Δ::kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
972	*h*$^-$		This study
YS325	*h*$^+$	*hrp1ΔkanMX6 ura4*$^+$	This study
YS251	*h*$^+$	*hrp3ΔhphMX6 ura4*$^+$	This study
YS867	*h*$^-$	*otr1::ura4 ade6-210 leu1-32 his3-D1 ura4DS/E*	This study
YS915	*h*$^-$	*otr1::ura4 hrp3 Δ::hygMX6 ade6-210 leu1-32 ura4DS/E*	This study
SPG1292 from Dr.Grewal	*h*$^-$	*otr1::ura4 clr4R320H ade6-210 leu1-32 ura4DS/E*	This study

YS854	h^+	*otr::ura4 hrp3Δ:: hygMX6 clr4R320H ade6-210 leu1-32 ura4DS/E*	This study
YS820	h^+	*otr1::ura4 ade6-210 ura4-D18 leu1-32 his3?*	This study
YS825	*?*	*otr1::ura4 hrp3Δ::hygMX6 ade6-? Leu1-32 ura4-D18*	This study
YS785	h^-	*otr1::ura4 alp13Δ::hygMX6 ade6-210 ura4-D18 leu1-32 his3-D1*	This study
YS869	h^-	*otr1::ura4 hrp3 Δ::hygMX6 alp13Δ::kanMX6 ade6-? ura4-D18 leu1-32 his3-?*	This study
DM151	h^-	*cdc25-22 ura4-D18*	This study
YS615		*cdc25-22 hrp3Δ::hphMX6 ade6-M216 leu1-32 ura4-D18*	This study
YS808		*cdc25-22 hrp1Δ::kanMX6 ade6-M216 leu1-32 ura4-D18*	This study

Table S2. List of primers used in this study

name	description	strand	sequence
zer1$^+$	#1-ChIP	Fw	CTGCAACGTCTGTCCAGTTATC
zer1$^+$	#1-ChIP	Rv	GTCGTGTAAAGTATGCAGAACGG
zer1$^+$	#2-ChIP	Fw	AGGATACTATTAGCTCGTGCCG
zer1$^+$	#2-ChIP	Rv	CCACCCGTGATACATTACCTTG
zer1$^+$	#3-ChIP	Fw	GCGAGAGTTGTGTTTGATAGGG
zer1$^+$	#3-ChIP	Rv	AGGTTCAAGCCATATAGCCTCC
sod2$^+$	#1-ChIP	Fw	GCCCACCTCAAATGTGCTTA
sod2$^+$	#1-ChIP	Rv	TGGTTTATACTTGAGGACCGGG
sod2$^+$	#2-ChIP	Fw	GTTAAGCCATCGAGGAATGCAG
sod2$^+$	#2-ChIP	Rv	TAAAGGTATCGCTAGCGGAAGG
sod2$^+$	#3-ChIP	Fw	CTGTTGAAGGCTTGCCTGTTTG
sod2$^+$	#3-ChIP	Rv	CCCTATTGGTCCGAAATGTCCA
JPO986	For forward transcript & ChIP	Rv	GATACTGATAATATTGAGATCCACAGCAC
JPO987	For reverse transcript & ChIP	Fw	GCGATGCCAAACAACAATATTG
GTO226	For forward transcript & ChIP	Rv	CGTCTTGTAGCTGCATGTGAA
GTO223	For reverse transcript & PCR	Fw	GAAAACACATCGTTGTCTTCAGAG
dh	RT-qPCR/PCR & ChIP	Fw	GCAAAACACACGGACATAGTAT
act1$^+$	For forward transcript	Rv	CAATTTCACGTTCGGCGGTAG
act1$^+$	For reverse transcript	Fw	GAAGTACCCCATTGAGCACGG
act1$^+$	RT-PCR(gel running)	Fw	GAAGTACCCCATTGAGCACGG
act1$^+$	RT-PCR(gel running)	Rv	CAATTTCACGTTCGGCGGTAG
act1$^+$	RT-qPCR	Fw	CCACTATGTATCCCGGTATTGC
act1$^+$	RT-qPCR	Rv	GAATGGATCCACCAATCCAGAC
zer1$^+$	For forward transcript	Rv	AGCTTGTGCTAACTTGATACC
zer1$^+$	For reverse transcript	Fw	GTTTGCTACTGCTCCATATGA
zer1$^+$	RT-PCR/qPCR check	Fw	AGGACTAAACAGCCGAGACGTGAA
zer1$^+$	RT-PCR/qPCR check	Rv	ACTTTGTCACGAGCAGCTCCAA
sod2$^+$	For forward transcript	Rv	AAATCGGAATCGGACTTGGT
sod2$^+$	For reverse transcript	Fw	GGATGTAATGATGGAATGGCGG
sod2$^+$	RT-qPCR, forward RT check	Fwd	GGAAAGAAGCCCTTTTCGTT
sod2$^+$	RT-qPCR, forward RT check	Rv	AAATCGGAATCGGACTTGGT
sod2$^+$	RT-qPCR, reverse RT check	Fwd	GTTAAGCCATCGAGGAATGCAG
sod2$^+$	RT-qPCR, reverse RT check	Rv	TAAAGGTATCGCTAGCGGAAGG
snoR58	Northern control		GATGAAATTCAGAAGTCTAGCATC
IK8	siRNA Northern*(dh and dg repeats)*		ATTCCTTTCTGAACCTCTCTGTTAT
IK9	siRNA Northern*(dh and dg repeats)*		TTTGATGCCCATGTTCATTCCACTTG
IK10	siRNA Northern*(dh and dg repeats)*		GGGAGTACATCATTCCTACTTCGATA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

6-AU sensitivity assay

Media containing 6-AU were prepared by supplementing SD-Ura medium with 100 ug/ml and 200 ug/ml 6-azauracil (Sigma). Five microliters of 5-fold serial dilutions (in water) of each culture were spotted on SD-Ura plates with or without 6-AU, and the plates were incubated at 30°C for 3 to 4 days.

Biochemical experiments for separating Hrp1 and Hrp3

Fifteen liters of yeast cell cultures expressing TAP-tagged Hrp3 and 3X FLAG-tagged Hrp1 (YS136) were grown in YES medium at 30°C to an optical density of ~ 1 at 600 nm. The cell pellet was resuspended in PDB 100 buffer (5 mM HEPES pH 7.4, 0.5% Triton X-100, 10% glycerol, 1 mM EDTA, and proteinase inhibitors) and disrupted in a bead beater (Biospec). The resulting crude whole-cell extracts were clarified by ultracentrifugation and directly applied to SP sepharose resin (GE Healthcare). After a 100 mM salt wash, proteins were eluted with a salt gradient from 150 mM to 650 mM. Each eluted fraction was subjected to Western blot analysis with α-FLAG or α-CBP antibodies. The purified complexes were resolved on a 10% SDS-PAGE gel and visualized with silver staining. Eluted proteins were subjected to mass spectrometry.

Silencing assay

The utilized FOA medium consisted of minimal medium supplemented with uracil (50 mg/L) and 1 g/L of 5-fluoroorotic acid (FOA; Biosynth). Cells were spotted on plates with or without 5-FOA, and the plates were incubated at 30ºC for 4 to 5 days.

Nucleosomal ATPase assay

ATPase assays were performed with FLAG-tagged Hrp3 and Hrp3^{K406A} purified from yeast cells under following condition: 50uM cold ATP, 1 uCi of [r-^{32}P] ATP, 5mg BSA. Where indicated, the reactions were supplemented with 100ng of pBS-KS(+) and LON, long oligo-nucleosomes (gift from Jerry Workman) and were incubated at 30°C for 1hr. Unhydrolyzed ATP and free phosphate were separated by thin-layer chromatography on PEI-cellulose plates (J.T.Baker, USA). Spots were visualized by exposure to a phosporimager cassette and quantified by densitometric analysis.