Figure S1

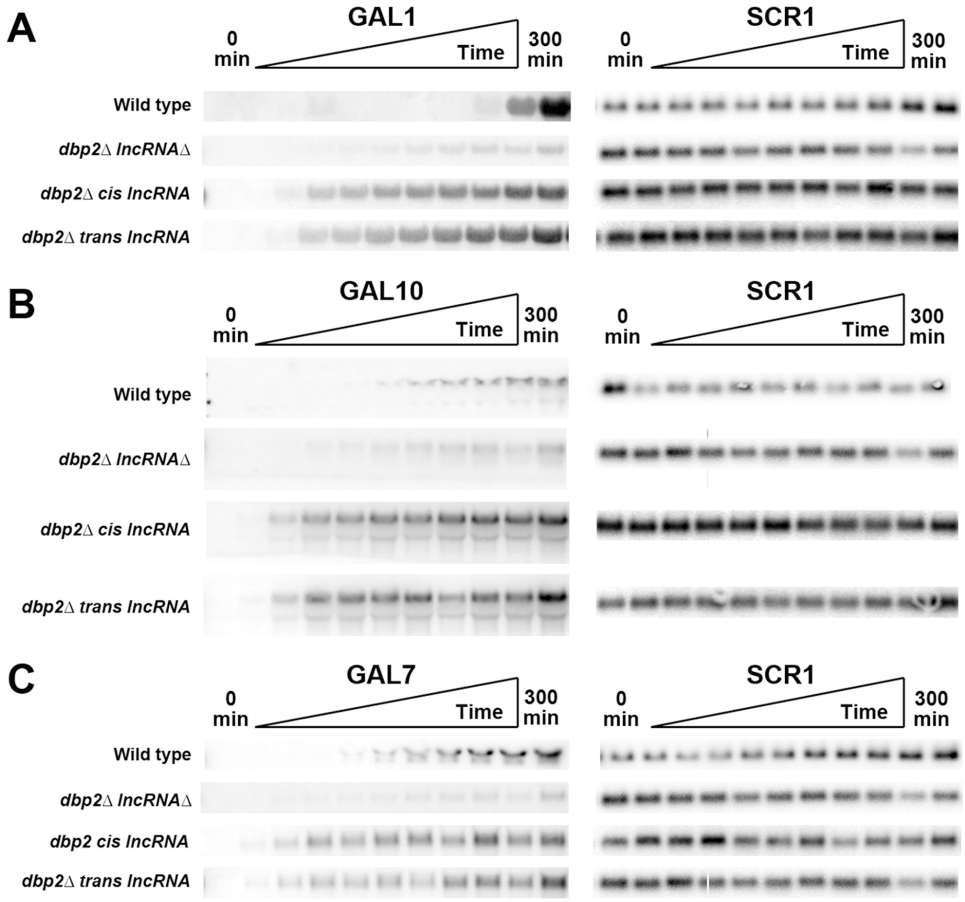

Figure S1, related to Figure 1. Representative Northern blots showing that the GAL lncRNAs function in *trans* to promote induction of the *GAL* cluster genes. (A-C) *GAL gene induction assay in wild type (cis-lncRNA), dbp2Δ cis-lncRNA, dbp2Δ lncRNAΔ, and dbp2Δ trans-lncRNAΔ cells*. Yeast cells were grown in transcriptionally repressive conditions (+glucose) before being shifted to the activated (+galactose) condition. RNA was isolated at 30-minute intervals over the course of 300 minutes. Representative Northern blots are shown for induction assays of *GAL1* (A), *GAL10* (B) and *GAL7* (C).

Figure S2

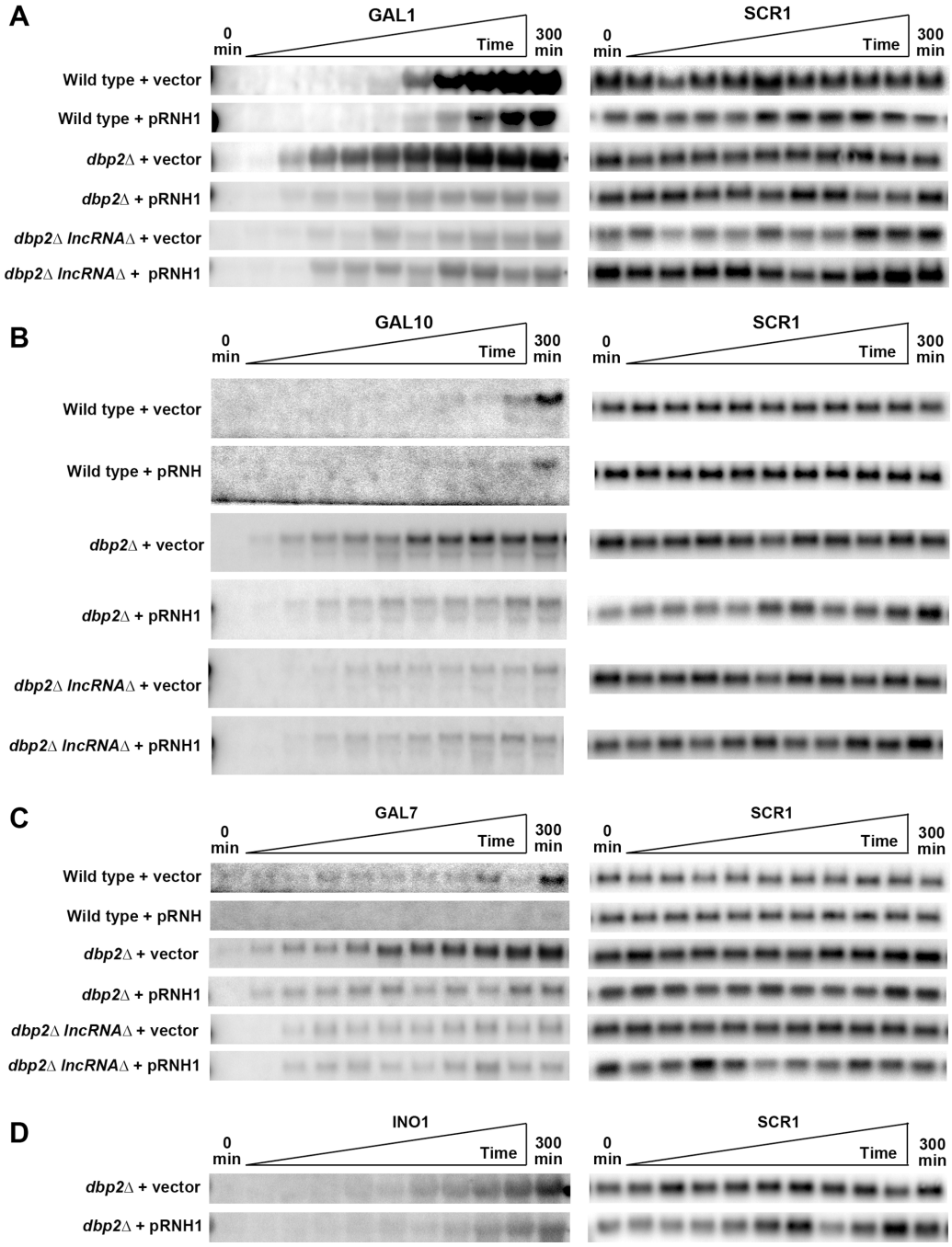

Figure S2, related to Figure 2. Representative Northern blots with ectopic expression of RNase H1. *(A-C) GAL gene induction assays of wild type, dbp2Δ and dbp2Δ lncRNAΔ cells harboring a plasmid encoding the human RNase H1 (pRNH1) gene or empty vector.* Strains were grown as in Figure S1, but in –LEU selective media for plasmid maintenance. Representative Northern blots for GAL1 (A), GAL10 (B) and GAL7 (C) are shown. *(D) INO1 gene induction assays of dbp2Δ cells with a plasmid encoding human RNase H1 or empty vector.* Strains were grown as above in –LEU selective media containing inositol before being shifted to –LEU media lacking inositol with time points taken over the course of 300 minutes.

Figure S3

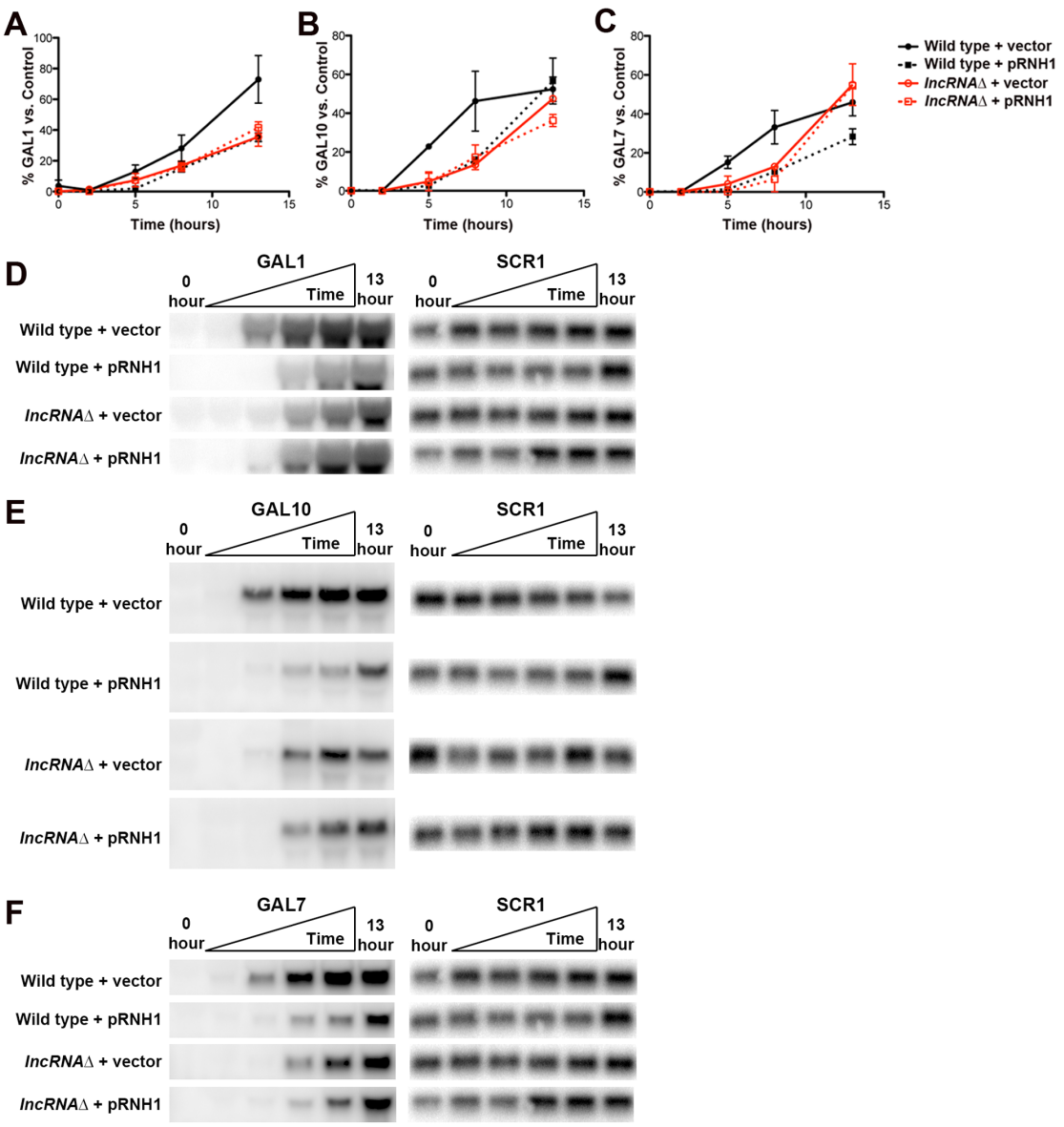

Figure S3, related to Figure 2. Ectopic expression of RNase H1 suppresses induction of *GAL* cluster genes in wild type cells. (A-F) *GAL gene induction assays of wild type and lncRNAΔ cells harboring either empty vector or a plasmid expressing human RNase HI*. Galactose induction assays were performed as described above with time points taken over the course of a 13 hour time period. *(A-C) Graphical representations of transcript levels during a GAL gene induction assay*. *GAL1* (A), *GAL10* (B) and *GAL7* (C) transcript levels were assayed by Northern blotting. Signal was quantified by densitometry. Results are presented relative to a fully induced wild type control, and represent 3 biological replicates. (D-F) Representative Northern blots for *GAL1* (D), *GAL10* (E) and *GAL7* (F) are shown.

Figure S4

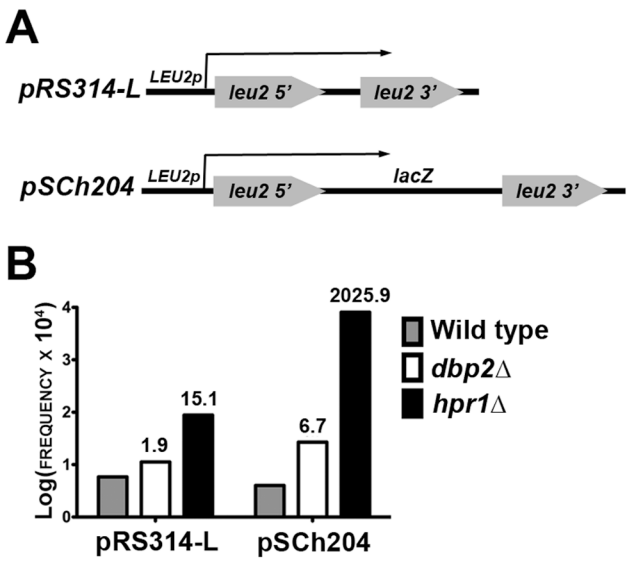

Figure S4, related to Figure 4. *DBP2*-deficient cells do not show hyper-recombination of a plasmid-encoded reporter gene. The recombination frequencies of the DNA direct repeat construct were determined for wild type, *dbp2*Δ and *hpr1*Δ cells. *(A) Schematic of reporter constructs used to determine recombination frequencies adapted from (Chavez and Aguilera, 1997; Prado et al., 1997).* DNA direct repeat constructs contain 5' and 3' regions of *LEU2* gene separated by a short intergenic region (pRS314-L) or the lacZ gene (pSCh204). Both vectors contain *TRP1* for selection after transformation. Constructs that undergo recombination will produce a functional *LEU2* transcript allowing for growth in SC-Leu media. *(B) Frequency of recombination in wild type, dbp2*Δ *and hpr1*Δ *cells and correlation with spacer length.* Recombination frequencies of the pRS314-L and pSCh204 reporters were measured in wild type, *dbp2*Δ and *hrp1*Δ strains. Strains were transformed with reporter constructs and plated on SC-Trp agar plates. Colonies were resuspended in 1xTE and dilutions were plated on SC-Leu and SC-Trp agar plates. Recombination was assayed by assessing growth on SC-Leu agar plates as a fraction of growth on SC–Trp agar plates. Strains lacking *HPR1* serve as a positive control for hyper recombination (Chavez and Aguilera, 1997).

Table S1. Plasmids used in this study. Related to Experimental Procedures.

Name	Description	Source
pGAL1-GAL10-GAL7	pYGPM11I14	Open Biosystems
pSCR1	pYGPM29b01	Open Biosystems
pUG6	KanMx disruption cassette plasmid	(Guldener et al., 1996)
pAG32	HygB disruption cassette plasmid	Euroscarf (Goldstein and McCusker, 1999)
pUG6-GAL10-7	plasmid template for mutation of GAL4 binding sites	This study
pUG6-GAL10-7-Gal4-BS	Plasmid for integration of "trans-lncRNA" construct	This study
pRS306	URA3 integration template plasmid	(Sikorski and Hieter, 1989)
pRS304	TRP1 integration template plasmid	(Sikorski and Hieter, 1989)
pAG32	HygB disruption cassette plasmid	(Goldstein and McCusker, 1999)
p3xFLAG	p3xFLAG:KanMX	(Gelbart et al., 2001)
p425-GPD (vector)	pRS425-GPD	(Mumberg et al., 1995)
pRNH1	pRS425-GPD-RNH1	(Wahba et al., 2011)
pRS314-L	pRS314 containing two 598bp ClaI-EcoRI *LEU2* fragments repeated in direct orientation, separated by 31bp of polylinker	(Prado et al., 1997)
pSCh204	YCp plasmid based on the *TRP1* marker and containing the *LEU2*-LacZ construct	(Chavez and Aguilera, 1997)
pFA6a-FRB-GFP-KanMX6	FRB-GFP tag template for anchor away	Euroscarf (Haruki et al., 2008)

Table S2. Yeast strains used in this study. Related to Experimental Procedures.

Name	Genotype	Source
Wild type (FT4)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56*	(Houseley et al., 2008)
lncRNAΔ	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56 gal10::URA3::pMV12 (EcoRI/XhoI-Reb1 BSΔ with BS2 silent) dbp2::KanR*	(Houseley et al., 2008)
dbp2Δ (BTY219)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56*	(Cloutier et al., 2013)
dbp2Δ lncRNAΔ (BTY220)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56 gal10::URA3::pMV12 (EcoRI/XhoI-Reb1 BSΔ with BS2 silent) dbp2::KanR*	(Cloutier et al., 2013)
"trans-lncRNA" (BTY387)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56 gal10::URA3::pMV12 (EcoRI/XhoI-Reb1 BSΔ with BS2 silent) lys2::(GAL10-7 Gal4 BSΔ--KanR)*	This study
dbp2Δ "trans-lncRNA" (BTY388)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56 gal10::URA3::pMV12 (EcoRI/XhoI-Reb1 BSΔ with BS2 silent) dbp2::HygR lys2::(GAL10-7 Gal4 BSΔ-KanR)*	This study
Wild type (BY4741)	*Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
Wild type (W303)	*MATa ade2-1 his3-11,15 ura3-1 leu2-1 trp1-1 can1-100*	Rothstein
dbp2Δ (BTY110)	*dbp2::KanR ade2-1 his3-11,15 ura3-1 leu2-1 trp1-1 can1-100*	This study
hpr1Δ (BTY318)	*ade2-1 his3-11,15 ura3-1 leu2-1 trp1-1 can1-100 hpr1::KanR*	This study
lncRNAΔ TRP1 (BTY392)	*MATa, ura3-52, TRP1, his3-Δ200, leu2::PET56 gal10::URA3::pMV12 (EcoRI/XhoI-Reb1 BSΔ with BS2 silent) dbp2::KanR*	This study
HHY168	*MATalpha tor1-1 fpr1::NAT RPL13A-2xFKBP12*	Euroscarf (Haruki et al., 2008)
DBP2-AA (anchor away)	*MATalpha tor1-1 fpr1::NAT RPL13A-2×FKBP12::TRP1*	This study

(BTY369)	*DBP2-FRB-GFP-KanMX6*	
CYC8-3xFLAG (BTY340)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56 CYC8-3xFLAG*	This study
lncRNAΔ CYC8-3xFLAG (BTY341)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56 gal10::URA3::pMV12 (EcoRI/XhoI-Reb1 BSΔ with BS2 silent) CYC8-3xFLAG*	This study
dbp2Δ CYC8-3xFLAG (BTY347)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56 dbp2::HygR CYC8-3xFLAG*	This study
dbp2Δ lncRNAΔ CYC8-3xFLAG (BTY348)	*MATa, ura3-52, trp1-Δ63, his3-Δ200, leu2::PET56 gal10::URA3::pMV12 (EcoRI/XhoI-Reb1 BSΔ with BS2 silent) CYC8-3xFLAG DBP2::URA3*	This study

Table S3. Oligos for homologous recombination in this study. Related to Experimental Procedures.

Name	Sequence
dbp2::URA3 F	CAACAACCTGTAACAGAATTAAGCACTATTAAGGCAAATTTAGAGCAAATATG TCAATTCATCATTTTTTTTTTATTC
dbp2::URA3 R	GCAGTCAACTTATATAATTATTATTAATAGAGATGAATGAATTGAATCACTTTG GGGTAATAACTGATATAATTAAATTG
dbp2::HygB	CAACAACCTGTAACAGAATTAAGCACTATTAAGGCAAATTTAGAGCAAATATG CAGCTGAAGCTTCGTACGC
dbp2::HygB	GCAGTCAACTTATATAATTATTATTAATAGAGATGAATGAATTGAATCACTTTG GCATAGGCGACTAGTGGATCT
hpr1::KanMX F	GATGGTAAGCAATAAATGGGTGC
hpr1::KanMX R	GCATCCTTTGCGCTACAACTTTATC
CYC8:3xFLAG F	TGTAGTAAGGCAAGTGGAAGAAGATGAAAACTACGACGACAGGGAACAAAAG CTGGAG
CYC8:3xFLAG R	GATTATAAATTAGTAGATTAATTTTTTGAATGCAAACTTTCTATAGGGCGAATT GGGT
TRP1 integration F	GCACACAAAGGCAGCTTGG
TRP1 integration R	CTACTCAGTAATAACCTATTTCTTAGC
LYS2 integration F	TCCTGGAATTTCAGTGAAAAACTGCTAATTATAGAGAGATATCACAGAGTTACT CACTACTGGGATCCGGCCGCGCAAC
LYS2 integration R	GGTTGAGCATTACGTATGATATGTCCATGTACAATAATTAAATATGAATTAGGA GAAAGAGGCGGCCGCCTATTAGTTAA

Table S4. Oligos for Northern Blot probe PCRs in this study. Related to Experimental Procedures.

Name	Sequence
GAL1 F	TTGGACGGTTCTTATGTCAC
GAL1 R	GAGACTCGTTCATCAAGGC
GAL10 F	GCATCACATTCCCTTCTATGAG
GAL10 R	ACGATTAGCATACCTGCCG
GAL7 F	CCTTGGTTAGGTCAACAGGAG
GAL7 R	AGTCGCATTCAAAGGAGCC
INO1 F	TGGGTTAGGTGGCAACAATGGC
INO1 R	GGGAACAAAAGTATTCTGCGGTG
SCR1 F	GGATACGTTGAGAATTCTGGCCGAGG
SCR1 R	AATGTGCGAGTAAATCCTGATGGCACC

Table S5. Oligos for strand-specific quantitative PCRs in this study. Related to Experimental Procedures.

Name	Sequence
ACT1 F	TGGATTCCGGTGATGGTGTT
ACT1 R	TCAAAATGGCGTGAGGTAGAGA
GAL10 lncRNA F	GAGGTCTTGACCAAGCATCACA
GAL10 lncRNA R	TTCCAGACCTTTTCGGTCACA
GAL10s lncRNA F	CGCTTCAGTGAAAAAATCATAAGG
GAL10s lncRNA R	GAGAGGCAAAGCAATTTAAGAATGT

Table S6. Primer/probe sets for ChIP and DRIP in this study. Related to Experimental Procedures.

Name	Sequence
GAL1 5' F	GAAGTTCGATTTGCCGTTGG
GAL1 5' R	AAGAGTGAGCAACATGGAGAC
GAL1 5' Probe	AGACCAGTCCGACACAGAAGGATCA
GAL1/10 P F	CTTTATTGTTCGGAGCAGTGC
GAL1/10 P R	GCTCATTGCTATATTGAAGTACGG
GAL1/10 P Probe	CGGTGAAGACGAGGACGCACG
GAL10 5' F	TGGTGCTGGATACATTGGTTC
GAL10 5' R	AGGGAATGTGATGCTTGGTC
GAL10 5' Probe	TGACTGTGTTGTTGCTGATAACCTGTCG
GAL10 3' F	GGTTTTGCAATTGAGCCTGG
GAL10 3' R	GCTGGCAAATCAGGAAAATCTG
GAL10 3' Probe	AAACGGTGAAACTTACGGGTCCAAGA
GAL7 P F	GCGCTCGGACAACTGTTG
GAL7 P R	TTTCCGACCTGCTTTTATATCTTTG
GAL7 P Probe	CCGTGATCCGAAGGACTGGCTATACA
GAL7 5' F	ATCATACAATGGAGCTGTGGG
GAL7 5' R	CTAGCCATTCCCATAGACGTTAC
GAL7 5' Probe	AAGCAGCCTCCTGTTGACCTAACC
KAP104 P F	CGGTGTCGTTATCGATGGAAA
KAP104 P R	CACTGCTCTTCTGCATAATTAATACTG
KAP104 P Probe	CCTCTTTTCGTAGTTTTCTGATCCGGGT

Table S7. Oligos for Chromosome Conformation Capture in this study. Related to Experimental Procedures.

Name	Sequence
GAL10 P1	CTTCACCGGTCGCGTTCC
GAL10 T1	GACCGAATTGTTTACAAGTTCTCTGTAC
GAL10 F	CCGTATGGTCATACGAAATACGC
GAL10 R	GCAACAAATTGTTTGGTATACCTAGC

Supplemental Table Legends

Table S8. Genes with R-loops in wild type cells and differentially expressed transcripts in *dbp2*Δ cells. Related to Figure 4.

Lists of genes that are both enriched for R-loops in wild type cells (Chan et al., 2014) and whose sense or antisense transcripts are differentially expressed in *dbp2*Δ (Beck et al., 2014). For the *DBP2*-dependent genes that are enriched in R-loops in wild type, 220 out of 322 (68.3%) of these genes were up-regulated in *dbp2*Δ, which is significantly more than the proportion of up-regulated genes (56.6%) in genes that lack R-loops (X-squared = 14.6, df = 1, $p = 1.31 \times 10^{-4}$). In contrast to the protein-coding sense transcripts, however, there was no difference in the proportion of up-regulated antisense RNAs for genes associated with R-loops (X-squared = 1.16, df = 1, p = 0.28)

Table S9. Functional annotation clustering analysis for the genes with R-loops in wild type cells and differentially expressed transcripts in *dbp2*Δ. Related to Figure 4.

Complete results of the functional annotation clustering analysis using DAVID Functional Annotation Tool for the genes listed in Table S8. Genes common between Chan et al., 2014 and this study were used as the background gene input.

Table S10. Comparison between genes with increased R-loop formation in *rnh1*Δ *rnh201*Δ, *hpr1*Δ *or sen1-1* and genes whose sense or antisense transcripts are differentially expressed in *dbp2*Δ. Related to Figure 4.

Results for two-sided Fisher's exact tests and relevant gene lists are included.

Supplemental Experimental Procedures

Plasmids and Cloning

Plasmids were constructed by standard genetic techniques and are listed in Table S1. The pUG6-GAL10-7 plasmid was synthetized by Genescript and contains the *GAL10-7* genomic region (Chr II sites according to SGD) in the pUG6 vector. The pUG6-*GAL10-7-Gal4-BS* plasmid was constructed by PCR-based mutation of Gal4 binding sites in pUG6-*GAL10-7*.

Yeast Strain Construction

Yeast strains were constructed using classical yeast genetics and are listed in Table S2. Oligos used for generation of PCR templates for homologous recombination are listed in Table S3. The *dbp2*Δ "*trans-lncRNA*" strain was constructed by integration of PCR from pUG6-*GAL10-7-Gal4-BS* into the *LYS2* locus of the *lncRNA*Δ strain. *DBP2* was then deleted by homologous recombination using pAG32 as a template. Expression of the GAL lncRNAs from the *trans*-lncRNA construct was verified by qRT-PCR whereas loss of *GAL10* and *GAL7* expression was confirmed by integration of the "*trans-lncRNA*" construct into a *gal10*Δ or *gal7*Δ strain, respectively (data not shown). For the assay to determine recombination frequencies, the *hpr1*Δ in the W303 background was constructed using the BY4741 *hpr1::KanR* as a template. The Dbp2-Anchor away (*DBP2-AA*) strain was constructed by PCR-mediated integration of the *FRB-GFP-KanMX6* cassette at the 3' end of the endogenous *DBP2* locus in the HHY168 strain containing the *RPL13A-2xFKBP12:TRP1* construct. Addition of rapamycin induces dimerization of FRB with FKB12 and transport of nuclear proteins to the cytoplasm during ribosome subunit export (Haruki et al., 2008). For competition assays, *TRP1* was integrated into the *trp1-*Δ*63* locus in the *lncRNA*Δ strain to serve as a selective marker.

Yeast Growth

Unless otherwise noted, all yeast cultures were grown at 30˚C in YP+ glucose (YP+2%D) media. Transcriptional induction assays of the *GAL* genes were conducted as described previously following a shift of exponentially growing cells from YP+2%D to YP+2%Gal (Cloutier et al., 2013). Strains containing p*RNH1* or pRS415-GPD empty vector were grown in synthetic complete-leucine (SC-Leu+2%D) media and shifted to SC-LEU+2%Gal for *GAL* gene induction. *INO1* inductions were performed by growing strains in SC-Leu media containing inositol to an OD_{600} of 0.4. Cells were then shifted to SC-Leu media lacking inositol for 5 hours, with samples taken at 30-minute intervals.

Northern Blotting

Northern blotting was performed as described previously (Cloutier et al., 2013). Plasmids used as PCR templates for Northern blotting probes are listed in Table S2 whereas oligos are listed in Table S4. Probes were generated from PCR templates and labeled with ^{32}P-dCTP using the Decaprime II kit according to manufacturer's instructions (Invitrogen). Northern blots were quantified by densitometry using Imagequant TL v7.0 (GE). Transcript levels were determined as the percentage of a fully induced wild type *GAL* control RNA as follows: (*GAL* Transcript Signal/*SCR1* signal)÷(*GAL* Control/*SCR1* Control)×100%, whereby *GAL* control represents total RNA from a wild-type culture following a 300-min induction from derepressive (+raffinose) conditions. For INO1 induction assays, RNA from wild type cells shifted to media lacking inositol for 5 hours was used for normalization. The control RNA is used for normalization of all Northern blots as previously described (Cloutier et al., 2013).

Strand-specific Reverse Transcription (ssRT) qPCR

Strand-specific qPCR was performed as described previously (Beck et al., 2014). Strains were grown as described above. Total RNA was extracted using a standard hot phenol protocol. RNA was treated with TurboDNase I (Ambion) according to manufacturer's instructions. cDNA was prepared with a Quantitect RT-PCR kit (Qiagen) according to manufacturer's instructions using 2 ug of RNA per reaction. Primers for cDNA preparation were removed using a PCR Clean-Up kit (Qiagen) according to manufacturer's instructions and eluting in 20 µL water. Quantitative PCR was performed in a BioRad CFX-96 PCR machine, and expression was determined using BioRad CFX Manager 3.1 software. *ACT1* was used as an internal control. Oligos used for cDNA preparation and qPCR reactions are listed in Table S5. Results are presented as the mean of 3 biological replicates with the SEM.

DNA-RNA Immunoprecipitation (DRIP)

DRIP was performed as previously described (El Hage et al., 2010) with the following modifications. Cells were crosslinked using 1% formaldehyde for 15 minutes at room temperature with shaking. After crosslinking, cells

were pelleted and washed twice with cold wash buffer (50 mM HEPES•KOH, 140 mM NaCl, and 1 mM EDTA) and frozen in liquid nitrogen. Cells were then lysed cryogenically using a Retsch Oscillating Mill MM400. Cell lysates were resuspended in cold Lysis Buffer (50 mM HEPES•KOH, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM PMSF and 1× protease inhibitor (complete, ETDA-free, Roche), and sheared by sonication in a Covaris E210 sonicator to a size of ~250-500 bp. Lysate was then cleared by centrifugation, diluted 1:5 in 0.1M sodium bicarbonate + 1% SDS, and then incubated overnight at 65˚C to reverse cross-linking. The DNA was then phenol/chloroform extracted twice without vortexing, precipitated with ethanol, 0.3M NaOAc, and 0.02 mg/mL glycogen (Thermo Scientific) and resuspended in nuclease-free water. Protein G Dynabeads (Invitrogen) were prebound overnight with 2.66 µg S9.6 antibody (Boguslawski et al., 1986; Hu et al., 2006). 50 ug gDNA was incubated with RNase H (NEB) or buffer for two hours at 37˚C in a total reaction volume of 50 µL. Reaction mixtures were then diluted in 500 uL Lysis buffer + 10mM EDTA + 0.1% Sodium deoxycholate and added to S9.6 antibody-coated beads. Beads were then incubated for two hours at 4˚C with rocking before being washed, eluted and precipitated as for ChIP (Cloutier et al., 2013). Precipitated elutions were resuspended in 30 uL of 1xTE and 1 uL was used for each qPCR reaction using Taqman 2x Master Mix (Life Technologies) and Primetime primer/probe assays (Table S5). Enrichment from samples was calculated as the percent of input consisting of 1 µl of 0.05 µg/µL genomic DNA. Results are displayed as the fold change above the no-antibody background control and are an average of five independent biological replicates with two technical repeats per sample with SEM.

Anchor-Away DRIP

For Anchor-away experiments, the Dbp2-Anchor-away strain (*DBP2-AA*) was grown to an OD of 0.4. Cultures were then divided and rapamycin dissolved in DMSO was added to a final concentration in the cultures of 10 µg/mL to half the cultures while an equal amount of DMSO was added to the other half. Cultures were then incubated at 30˚C with samples removed at the indicated time points and fixed with formaldehyde for DRIP. Cells were visualized by microscopy both before and after treatment with rapamycin at the indicated time points to confirm export of Dbp2-FRB-GFP from the nucleus. Cells were visualized on an Olympus BX51 fluorescent microscope. Images were captured with a Hamamatsu Orca R2 camera and MetaMorph software (Molecular Devices, Sunnyvale, CA).

Determination of Recombination Frequencies

Wild type W303, *dbp2*Δ W303, and *hpr1*Δ W303 strains were transformed with DNA direct repeat constructs pRS314-L or pSCh204 to assay the recombination frequencies as described previously (Chavez and Aguilera, 1997; Prado et al., 1997). The W303 strain background was used because of the Trp- phenotype. Reporters used to determine recombination frequencies consist of a *LEU2* open reading frame separated by either a short intergenic region (pRS314-L) or the *LacZ* gene (pSCh2014). Recombination between the direct repeats leads to formation of an intact *LEU2,* allowing growth on SC-Leu media. Both vectors contain the *TRP1* gene for selection after transformation. Strains were transformed with each vector, plated on SC-Trp and grown at the permissive temperature of 25˚C for wild type and *hpr1*Δ and 35˚C for *dbp2*Δ. Six independent colonies were selected from the transformation plates for each strain, resuspended in 1xTE and plated on both SC-Trp and SC-Leu to assay total growth and recombination events, respectively. The recombination frequency was calculated by dividing the number of recombinant cells by viable cells. The median of all the frequencies from 6 original colonies was used as the recombination frequency.

RNA-seq and Bioinformatics

RNA-Seq data was previously deposited in the Gene Expression Omnibus (GSE58097, (Beck et al., 2014)), and the alignments from this earlier analysis were used in this study. Aligned reads were imported into R using Rsamtools (Gentleman et al., 2004; Morgan M; RCoreTeam, 2014). The first mapped read in a pair (flags = 73, 83, 89, 99) were retained, and a mapping quality cutoff of mapq ≥ 40 was applied. Reads overlapping rDNA regions were excluded from the analysis. The gene annotation for *S. cerevisiae* was retrieved from Ensembl (R64-1-1). To resolve sense (mRNA expression) and antisense transcription, reads and gene annotations were partitioned into plus and minus strand lists. To determine the number of reads for RNA transcripts, plus strand reads were counted over plus strand ORF regions and minus strand reads were counted over minus strand ORF regions using GenomicRanges from Bioconductor (Lawrence et al., 2013). The two tables were combined for analysis of differential gene expression. To resolve antisense transcription, plus strand reads were counted over minus strand ORF regions and minus strand reads were counted over plus strand ORF regions. EdgeR (McCarthy et al., 2012; Robinson et al., 2010; Robinson and Smyth, 2007, 2008) was used to determine differential sense and antisense

expression. Genes with a low number of overlapping reads across all samples (< 10 for sense, < 5 for antisense) were removed. A cutoff of FDR ≤ 0.01 was used to determine differential expression. Lists of differentially expressed sense and antisense transcripts overlapping the ORF regions in *dbp2*Δ were compared to a list of ORFs enriched for RNA-DNA hybrids in wild type (Chan et al., 2014). Two-sided Fisher's exact tests and proportion tests were conducted using R. Venn diagrams were generated using the online Venn diagram Generator of Bioinformatics and Research Computing at Whitehead Institute for Biomedical Research (http://jura.wi.mit.edu/bioc/tools/venn.php).

Functional annotation clustering of biological processes was conducted using DAVID (Huang da et al., 2009a, b) to reveal overrepresented GO term clusters for ORFs that are both enriched in RNA-DNA hybrids in wild type and whose overlapped sense and antisense transcripts are differentially expressed in *dbp2*Δ. Functional clusters with enrichment scores ≥ 1.3 (equivalent to a p-value ≤ 0.05) are shown in Figure 4. Complete results from the functional annotation clustering are shown in Table S9.

Chromosome Conformation Capture (3C)

Wild type, *lncRNA*Δ*, dbp2*Δ and *dbp2*Δ *lncRNA*Δ cells were grown to mid-log phase at 30˚C in YP+2%D. Equal numbers of cells were collected at 0, 90 and 180 minutes following a shift to YP+2%Gal. 3C was performed as described previously (El Kaderi et al., 2012). The primers used for 3C PCR are listed in Table S6. P1T1 PCR represents the gene-looping signal, whereas the FR PCR product is the loading control. P1T1 PCR signal was normalized to the control PCR and plotted as shown in Figure 5.

Chromatin Immunoprecipitation (ChIP)

ChIP was performed as described previously (Cloutier et al., 2013), with the following modifications. For Cyc8–3×FLAG ChIP, chromatin from ~1.4×10^8 cells was immunoprecipitated with 1 µL of FLAG M2 monoclonal antibody (F3165, Sigma) and 12 µL of Protein G Dynabeads (30 mg/mL, Invitrogen) at 4°C for 2 h. Immunoprecipitated DNA was isolated, and quantitative PCR was performed using Bio-Rad CFX96 Real-time system using PrimeTime Assay primers purchased from IDT (Table S6). All ChIP experiments were performed with three biological replicates and three technical repeats. Error bars represent the SEM of three biological replicates.

Western Blotting

Wild type, *CYC8-FLAG*, *lncRNA*Δ *CYC8-FLAG*, *dbp2*Δ *CYC8-FLAG* and *dbp2*Δ*lncRNA*Δ *CYC8-FLAG* cells were grown to mid-log phase at 30˚C in YP+2%D. Whole cell lysate was prepared and western blotting was conducted as previously described (Cloutier et al., 2013).. FLAG-tagged Cyc8, G6PDH, and Pgk1 were detected by rabbit anti-3×FLAG (F7425, Sigma), rabbit anti-G6PDH (A9521, Sigma) and monoclonal mouse anti-yeast Pgk1 (459250, Invitrogen), respectively.

Growth Curves and Competition assay

Wild type and *lncRNA*Δ cells were grown to early log phase in SC+2%D media. Cells were then washed with SC media with no glucose before being resuspended in either SC+2%D or SC+2%Gal media to an OD600nm of 0.05. Cell density was then assessed over 30 hours using a Beckman-Coulter DU700 spectrophotometer. Growth curves were generated in GraphPad Prism and doubling times determined by fitting an exponential growth curve to the 0-10 hour points for growth in glucose and 0-24 hour points for growth in galactose. For cells expressing RNase H1 we used the points from 0-36 hours, and 0-28 hour for cells with empty vector. Lag times were determined separately using the Baranyi model using the Excel Add-In DMFit version 2.1 (http://www.combase.cc/tools/; (Baranyi and Roberts, 1994)). Results represent the mean of six biological replicates with the SEM. Wild type and *lncRNA*Δ *TRP1* cells were grown to early log phase in SC+2%D media. Equal amounts of wild type and *lncRNA*Δ *TRP1* cells were determined by by both optical density and cell counting and then combined in the same flask, washed with SC media, and resuspended to a final OD600nm of 0.1 in SC+2%Gal media. Samples were taken at intervals over the course of 30 hours and approximately 500 cells according to optical density were plated on YP+2%D and –TRP+D plates. The percentage of *lncRNA*Δ *TRP1* cells was determined as the number of Trp+ colony forming units (cfu's) over the total cfu's (YPD) and wild type as the number of Trp- colonies over the total cfu's. For *trans-lncRNA* competition assays, *lncRNA*Δ and *trans-lncRNA* cells were treated as above, before being selected on YPD and YPD-G418 plates to obtain total cell count and *trans-lncRNA* cell count respectively. Results were plotted in GraphPad Prism and represent three biological replicates with three technical replicates.

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