Cell Reports, Volume *27*

Supplemental Information

Yeast Sirtuin Family Members Maintain Transcription Homeostasis to Ensure Genome Stability

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Figure S1

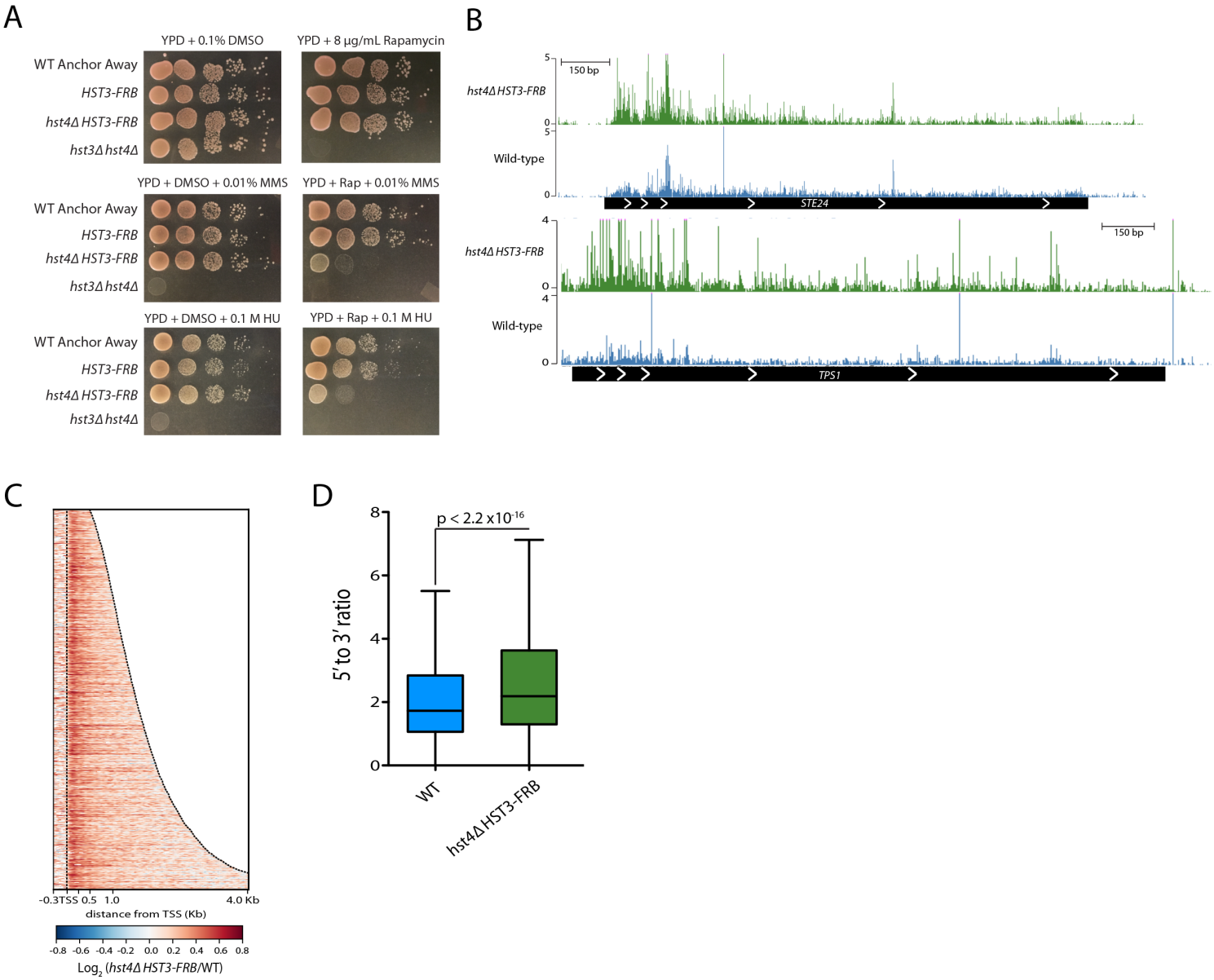


Figure S1. Hst3 and Hst4 maintain genome stability and regulate transcription, related to Figure 1. (A) WT anchor away, HST3-FRB, hst4Δ HST3-FRB, or hst3Δ hst4Δ strains were spotted (1/10 dilutions) on 2% glucose media containing either DMSO solvent or 8 µg/mL rapamycin (Rap), in the presence or absence of 0.01% Methyl methanesulfonate (MMS) or 0.1 M hydroxyurea (HU) and then grown for 3 days at 30˚C. Note that the hst3Δ hst4Δ strain does not harbor the tor1-1 allele, and therefore it does not grow on rapamycin media. **(B)** Representative genome browser views of S. pombe-normalized NET-seq reads for WT (blue) and hst4Δ HST3-FRB (green). **(C)** Heatmap displaying the $\log_2$ fold change between hst4Δ HST3-FRB and WT NET-seq reads from -0.3 kb from TSS to 4 kb downstream ordered by gene length. Black dotted lines represent TSS and TTS, respectively. **(D)** Box plot of the 5' to 3' ratios for WT and hst4Δ HST3-FRB calculated by summing the reads from TSS to +250 bp divided by the sum of the reads from -250 bp from TTS to TTS. p-value determined by Mann-Whitney U-test. Four biological replicates for WT and three biological replicates for hst4Δ HST3-FRB.

Figure S2

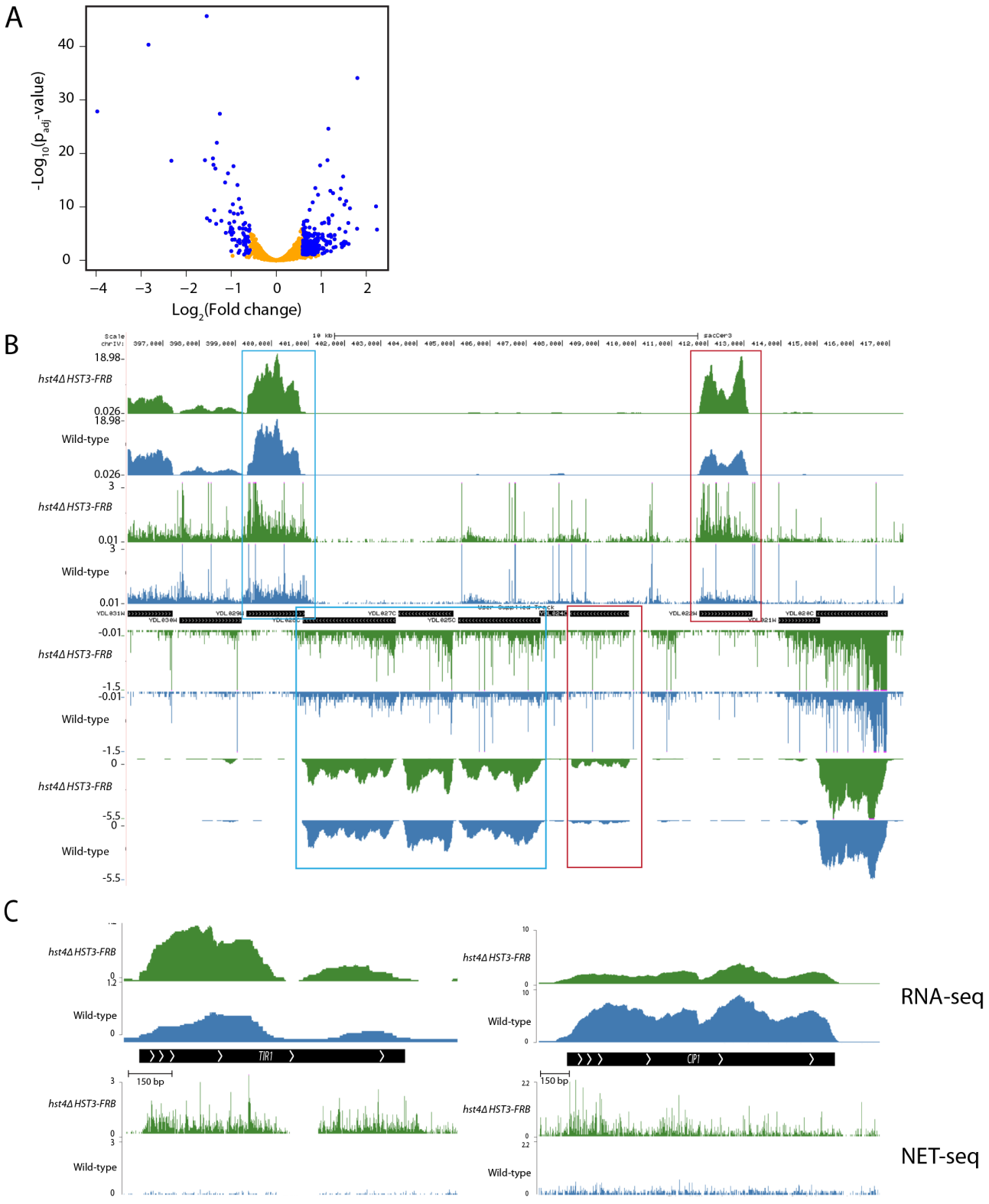


Figure S2. Steady-state mRNAs are minimally affected by loss of Hst3 and Hst4, related to Figure 3. (A) Volcano plot shows ORF transcripts that change significantly in the hst4Δ HST3-FRB mutant by RNA-seq. Significant genes are highlighted in blue (pAdj ≤ 0.1, Log_2 fold change ≥ 0.59 or ≤ -0.59). **(B)** Representative genome browser view comparing NET-seq and RNA-seq reads for WT (blue) and hst4Δ HST3-FRB (green). Red boxes highlight genes with increased NET-seq and RNA-seq reads in the hst4Δ HST3-FRB mutant. Blue boxes highlight genes with increased NET-seq reads in the hst4Δ HST3-FRB mutant, but not change by RNA-seq. **(C)** Zoomed-in genome browser view comparing NET-seq and RNA-seq reads for two representative genes for WT (blue) and hst4Δ HST3-FRB (green) mutnats. Left, nascent and steady-state RNA increased in hst4Δ HST3-FRB mutant for the TIR1 gene. Right, Nascent RNA increased in hst4Δ HST3-FRB mutant for the CIP1 gene, but decreased steady-state RNA.

Figure S3

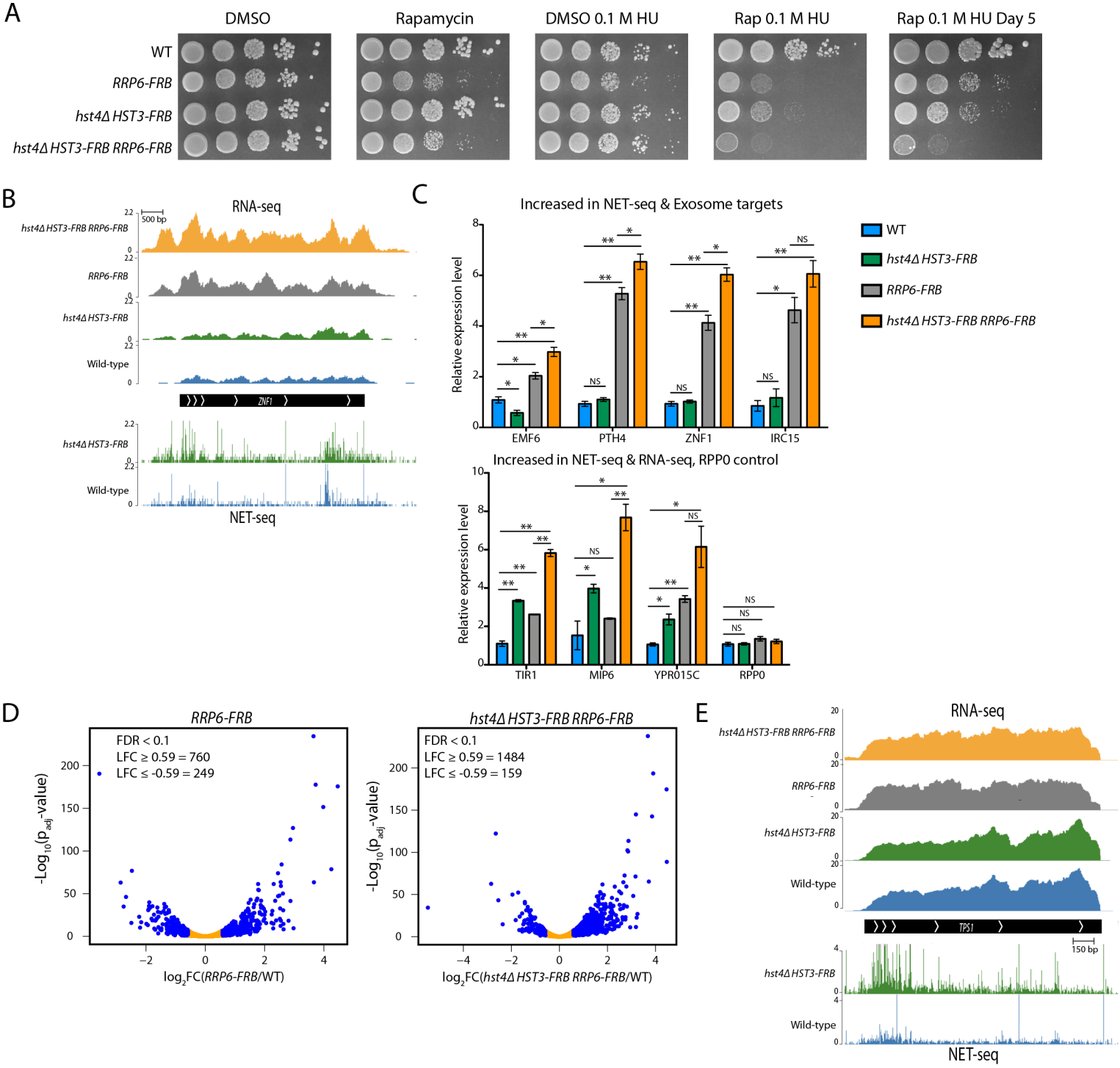
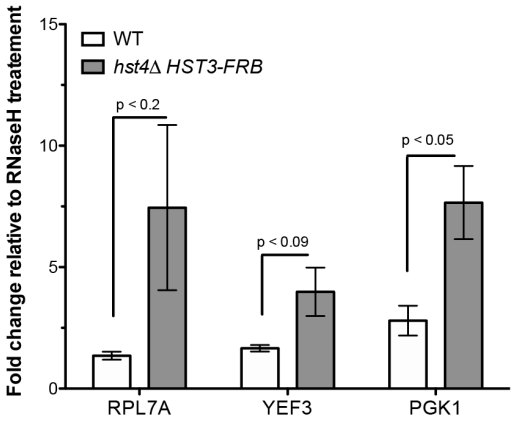


Figure S3. Nuclear exosome represses increased transcription in the hst4Δ HST3-FRB mutant, related to Figure 4. (A) WT anchor away, RRP6-FRB, hst4Δ HST3-FRB, or hst4Δ HST3-FRB RRP6-FRB strains were spotted (1/10 dilutions) on 2% glucose media containing either DMSO solvent or 8 µg/mL rapamycin (Rap), in the presence or absence of 0.1 M hydroxyurea (HU) and then grown for 3 days or 5 days at 30˚C. **(B)** Genome browser view of the ZNF1 gene that is regulated by Rrp6 displaying NET-seq data (bottom) for WT (blue) and hst4Δ HST3-FRB (green) as well as RNA-seq data for WT (blue), hst4Δ HST3-FRB (green), RRP6-FRB (gray), and hst4Δ HST3-FRB RRP6-FRB (orange). **(C)** RT-qPCR confirmation of exosome targets. Left, genes with increased transcription in the hst4Δ HST3-FRB mutant by NET-seq, but no change or decrease in RNA-seq. Increased in the RRP-FRB mutant and further increased in hst4Δ HST3-FRB RRP6-FRB mutant. Right, genes increased in both NET-seq and RNA-seq in the hst4Δ HST3-FRB mutant and further increased in the hst4Δ HST3-FRB RRP6-FRB mutant. RPP0 was also included as a control gene. P-values determined by student's t-test for 2 biological replicates. **(D)** Volcano plot shows ORF transcripts that change significantly in the RRP6 mutant (left) or the hst4Δ HST3-FRB RRP6-FRB (right) by RNA-seq. Significant genes are highlighted in blue (pAdj ≤ 0.1, Log2 fold change ≥ 0.59 or ≤ -0.59). **(E)** Representative genome browser view of a gene that is increased by NET-seq (bottom) in the hst4Δ HST3-FRB (green) compared to WT (blue), but does not change in RNA-seq (top) and is not an exosome target. WT (blue), hst4Δ HST3-FRB (green), RRP6-FRB (gray), and hst4Δ HST3-FRB RRP6-FRB (orange).

Figure S4

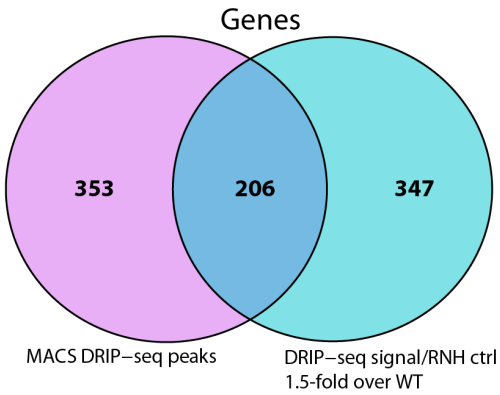
A



B

	Number of peaks
Total	667
Overlap genes	588
Overlap snRNA	30
Overlap ARS	22
Overlap transposons	78
Overlap tRNA	56

C



D

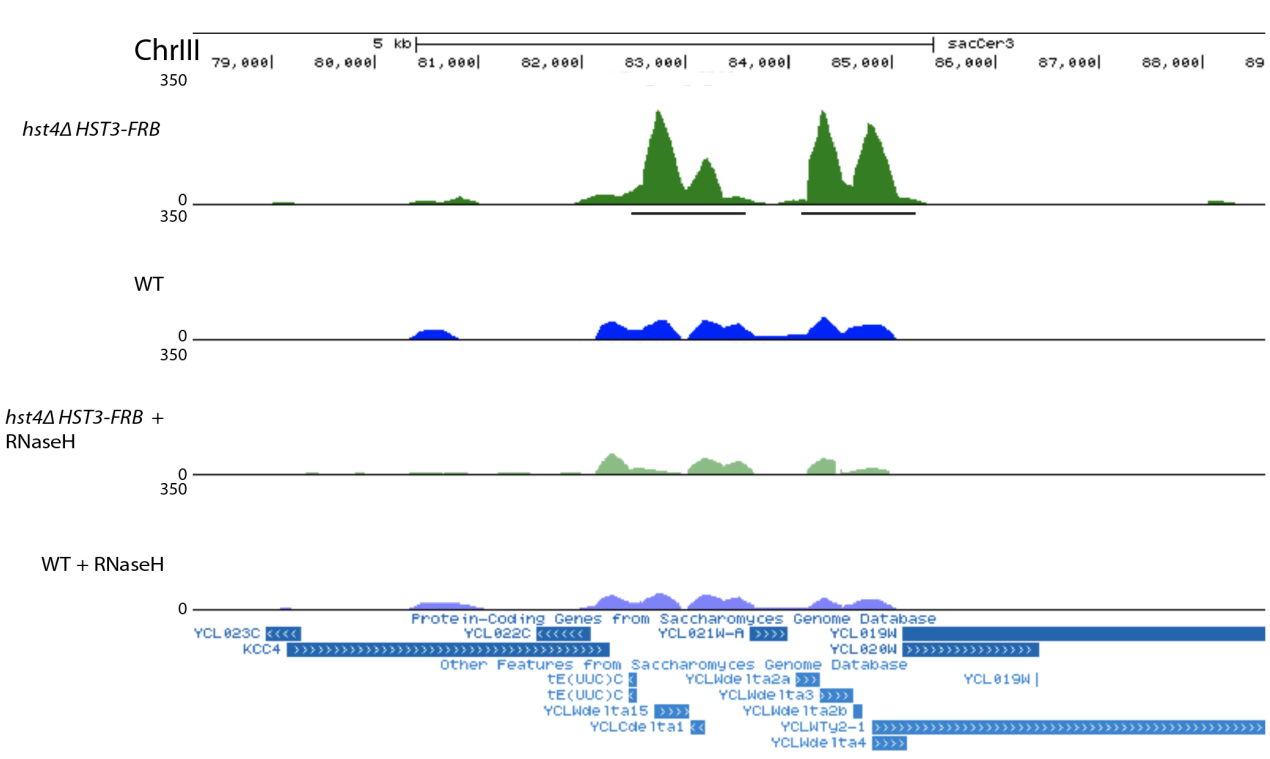
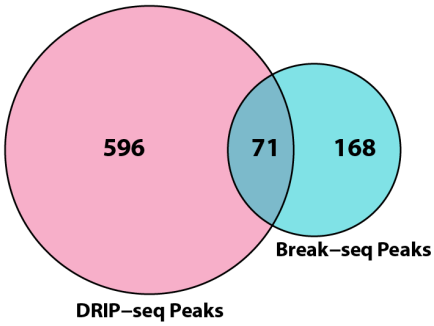


Figure S4. Increased R-loop abundance in the absence of Hst3 and Hst4, related to Figure 5. (A) DRIP-qPCR showing RNase-H normalized R-loop abundance in WT (white) and hst4Δ HST3-FRB (gray) for 3 biological replicates. Error bars represent standard error of the mean. P-values determined by student's t-test. **(B)** Distribution of DRIP-seq peaks determined by MACS for various genomic loci. **(C)** Venn diagram showing the overlap of DRIP-seq peaks at genes identified by MACS or by the method using an RNaseH control. **(D)** Representative genome browser view of normalized DRIP-seq reads in WT (blue) and hst4Δ HST3-FRB (green) cells at tRNA and transposon loci.

Figure S5

A



B

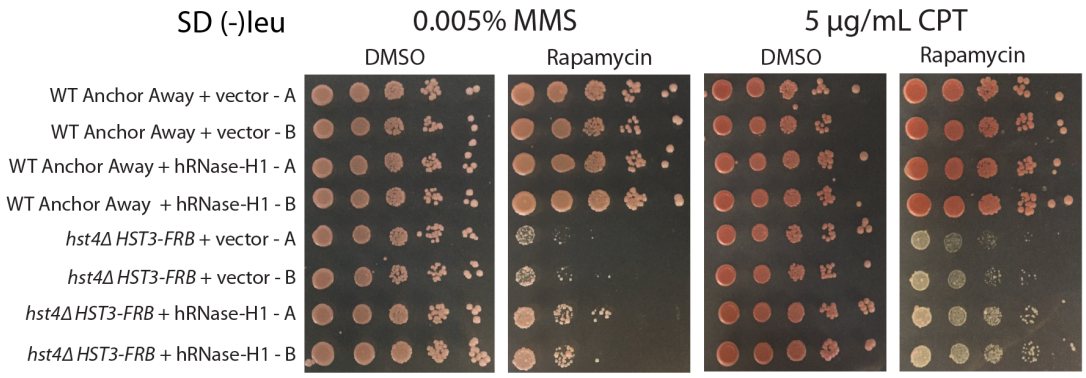


Figure S5. R-loop formation and genomic stability, related to Figure 6. (A) Venn diagram showing the overlap between Break-seq peaks and DRIP-seq peaks. **(B)** WT anchor away or hst4Δ HST3-FRB strains transformed with empty vector or a vector overexpressing human RNase-H1. Strains were spotted (1/10 dilutions) on 2% glucose media containing either DMSO solvent or 8 µg/mL rapamycin in the presence of 0.005% MMS or 5 µg/mL CPT and then grown for 3 days at 30˚C. Two transformants were spotted for each strain.

Table S3.

Strains		
Name	***Genotype***	**Source**
CY1827	*MATa tor1-1 fpr1::loxP-LEU2-loxP RPL13A-2×FKBP12::loxP (HHY221) bar1Δ::HISG*	Euroscarf (Haruki et al., 2008)
CY2389	*MATa tor1-1 fpr1::loxP-LEU2-loxP RPL13A-2×FKBP12::loxP (HHY221)bar1Δ::HISG HST3-FRB:kanMX6*	This study
CY2390	*MATa tor1-1 fpr1::loxP-LEU2-loxP RPL13A-2×FKBP12::loxP (HHY221) bar1Δ::HISG HST3-FRB:kanMX6 hst4Δ::HPH*	This study
CY2028	W303 *MATa bar1Δ::his5+hst3Δ::NatMX hst4Δ::KanMX*	P. Kaufman
CY2394	*MATa tor1-1 fpr1::loxP-LEU2-loxP RPL13A-2×FKBP12::loxP (HHY221) bar1Δ::HISG RPB3-FLAG:NAT*	This study
CY2395	*MATa tor1-1 fpr1::loxP-LEU2-loxP RPL13A-2×FKBP12::loxP (HHY221)bar1Δ::HISG HST3-FRB:kanMX6 hst4Δ::HPH RPB3-FLAG:NAT*	This study
CY2474	*MATa tor1-1 fpr1::loxP-LEU2-loxP RPL13A-2×FKBP12::loxP (HHY221)bar1Δ::HISG RRP6-FRB:HisMX6 RPB3-FLAG:NAT*	This study
CY1885	*MATα tor1-1 fpr1::NAT RPL13A-2X FKBP12::TRP1 (HHY168)*	Euroscarf (Haruki et al., 2008)
CY2393	*MATα tor1-1 fpr1::NAT RPL13A-2X FKBP12::TRP1 (HHY168) HST3-FRB:kanMX6 hst4Δ::HPH*	This study
JY741/FLAG-Rpb3	h- flag-rpb3 ade6-M216 ura4-D18 leu1	NBRP (Kimura et al., 2001)
Plasmids		
Name	**Description**	**Source**
CP1458	pR5425-GPD	(Mumberg et al., 1995) From Elizabeth Tran
CP1459	pR5425-GPD-human RNase H1	(Wahba et al., 2011) From Elizabeth Tran

Table S4.

Primers		
Target	**Direction**	**Sequence**
RT-qPCR		
EMF6	Forward	5' AAGTGGTTGTGGCGGTAAAG
	Reverse	5' CCACGCACAATCCAACTAAA
PTH4	Forward	5' AACGGGCTTGCCCTTAAATC
	Reverse	5' TCACTTCCTGAGGAATCCAAGC
ZNF1	Forward	5' TTGTCTAAACCGCTGCTACG
	Reverse	5' TCAAAGTTTCCTCGCGATGG
IRC15	Forward	5' TCGGCGGCTATTTAACATGC
	Reverse	5' CGTAAACTGCCGCAAAATCC
OLE1	Forward	5' TTAACCGCTTTCGTCATTCC
	Reverse	5' GGGGTTCTTCTGTCATCGAA
PRM7	Forward	5' AATTCCTTCACGACCACCAC
	Reverse	5' TCAATGTTTGCGCTGCCTTC
CIP1	Forward	5' ACCGATCCAATCCCCTTATC
	Reverse	5' AGAATTTGTGCGGGCTAAGA
TIR1	Forward	5' ATTCGCTGCTATTGCTGCTT
	Reverse	5' GTCAGTGGCGGAAGCTAAAG
MIP6	Forward	5' TAGCTACGCCGATGAAACCT
	Reverse	5' GCTGCGCAGATTTTCTTCTT
YPR015C	Forward	5' ACACGACCAAAAGGGAAGTG
	Reverse	5' AAGGTGCGTAATGGTAAGCG
RPP0	Forward	5' CGGTAACAAGGTCGGTCAAT
	Reverse	5' AGCGGAAACGAAGTGAGAAA
PDC1	Forward	5' GGTTGGGACCACCTATCCTT
	Reverse	5' TTGTGGAGCATCGAAGACTG
DRIP-qPCR		
RPL7A	Forward	5' GATCTGCCTTGTATTGTCTTCG
	Reverse	5' TCAGCGGCCATTGTGATCTT
YEF3	Forward	5' GATTGCCGGTGGTAAGAAGA
	Reverse	5' CGTAAGCATCACCCAATTCC
PGK1	Forward	5' GGACAAGCGTGTCTTCATCA
	Reverse	5' CGTTTCTTTCACCGTTTGGT