Supplementary Information

Supplementary Methods

RNA-seq and ChIP-seq datasets

RNA-seq and ChIP-seq datasets used in this manuscript are listed in Table S4.

Computational analysis

Alignment: RNA-seq data were aligned using tophat v2.0.5 and bowtie indices for the sacCer2 and ce10 genomes. RNA-seq tags were trimmed to 35bp before alignment, and tags mapping to rDNA loci were removed after alignment. Libraries were stranded and tophat was run using the parameter --library-type fr-firststrand to reflect this. Additionally, only the optimal alignment for each tag was recorded (-g 1), and importantly, data were not aligned using a reference transcriptome as a guide. ChIP-seq data were aligned using bowtie v0.12.7 and v0.12.5 in the case of the first and second replicate of H3K36me3 data respectively. ChIP-seq data were aligned with parameters -m 1 --best, to guarantee a single optimal alignment for each tag and to remove tags mapping optimally to multiple loci.

Track Creation and Data Averaging: Track visualizations were created for the UCSC Genome Browser in the following way. First, replicate data were individually converted from bowtie output to BED files using in-house scripts. BEDs were made into coverage maps using the BEDtools program genomeCoverageBed with the -bg option. The resulting bedGraphs were then adjusted to the number of billions of bases sequenced for each sample. The adjusted bedGraphs were then combined by unweighted average. ChIP-seq data were then normalized to H3 (subtraction) to correct for local sonication efficiency bias and estimate modification changes.

RNA-seq data were split according to tags on the plus or minus reference strand after conversion to BEDs. Resulting bedGraphs were converted into bigWigs using the UCSC tool bedGraphToBigWig.

Discovery of Cryptic Transcripts: RNA-seq tags in WT and *rph1*Δ time series were treated in the following way. All annotated SGD genes in the sacCer2 assembly were broken into 40 equal-sized units of one base-pair or more. For any given genetic background, time point, and replicate, an in-house script was employed to measure sense-strand tag density over each unit of each gene, resulting in a length-40 vector of RNA-seq enrichment for each known transcript. The first third of the transcript (subvector of size 13) was taken to be the 5' end, and the last third was the 3' end. Cryptic genes were defined as those satisfying three criteria: (a) that the 3':5' ratio in old cells exceed the 5th percentile value of all total-transcript enrichments in the old cells; (b) that the total old enrichment exceed that same cutoff; and (c) that the increase in cryptic enrichment (the ratio of ratios, 3':5' old : 3':5' young) exceed 1.5-fold. Genes were defined as cryptic in each of five replicate experiments, then the group of genes common to all five were taken to be "common cryptic genes" referred to as cryptic in the text.

Meta-Gene Plots: Meta-gene plots are made in the following way. For RNA-seq figures, sense-strand aligned tags are selected for each transcript. For each of the five short fragment replicate experiments, 40-unit vectors representing transcription from 5’ to 3’ were assessed and length-normalized, and a genome-wide average profile was constructed from these. Average curves were adjusted by subtracting the average 5’ gene half expression score from the entire profile.

Curves were then averaged over five replicates and the standard error was calculated at each unit of the vector and represented as shaded error bands.

Heatmaps: The tag count profile for each gene was averaged over all five RNA-seq experiments. The profiles were then adjusted by subtracting the average 5' gene half expression score. Negative profile scores were zeroed out (black) and finally the profiles were plotted in descending order (from top to bottom) by the strength of the 3' gene half expression score in the old population.

Boxplots: Boxplots are generated in R using the distribution of various qualities of cryptic genes vs an equal-sized set of randomly selected non-cryptic genes for (a) length and (b) transcription rate. Boxplots are generated for the WT-*rph1*Δ using the 3' half of the gene only (normalizing to the 5' half) and the WT- *rph1*Δ Old/Young using the same method. p-values are based on a one-sided Mann-Whitney test.

Gene Ontology: Functional characterization of cryptic genes was performed using DAVID. The Functional Annotation Clustering option was used to group similar categories. An enrichment score for each cluster was given as a mean of $-\log_{10}p$ of member GO categories with $p < 0.01$.

Sequence logos: A regular expression search for TATA[AT]A[AT][AG] in yeast and TATA[AT][AT]AG in worms revealed a number of internal TATA boxes in cryptic genes (start to stop codon only), which were then used as input to the online Weblogo tool. Similarly, a search for the initiator sequence moiety A (N5) NYA(A|T)NN N6 in yeast revealed a number of putative internal initiator sequences. These were judged to be true initiator sequences if the initial

N5 had four or more adenine and the trailing N6 matched the same criterion. Resulting sequences were also uploaded to Weblogo with default parameters. A permutation test was run to assess the statistical significance of these motifs. For each motif, the number of genes containing an internal motif was tabulated. Then 1,000 permutations were performed. In each iteration, an equal number of randomly selected non-cryptic genes was sampled and the number containing an internal TATA or initiator motif was assessed. These formed a background distribution; p-values reported are the fraction of permutations where the number of background genes with the internal motif exceeds the number of cryptic genes with internal motifs (Table S6).

Supplementary Figure Legends

Fig. S1 Replicability of aging screen

Pairwise correlation plots of four biological repeats (experiments C, D, E and F) of the aging screen with histone substitution mutant library. The relative enrichments of each mutant in the oldest population is plotted. The r values are indicated at the top.

Fig. S2 Performance of internal controls in the aging screen

Success of the aging screen was inferred from the enrichment/depletion profiles of *sir2*Δ, *SIR2-OE* and WT strains in the population. Six uniquely barcoded strains of each strain type (four for *SIR2-OE*) were included and their relative enrichments (RE) quantified in the aging time-course.

Fig. S3 Microarray versus next-generation sequencing readout in aging screen

Correlation plots of barcode enrichments in the aging screen as quantified by microarray (average of four biological replicates) and next-generation sequencing (one biological experiment). The r-value is indicated at the top.

Fig. S4 Deletion of Jhd1 has no effect on lifespan

Replicative lifespan assay with WT (blue) and *jhd1*Δ (purple). Mean lifespans are indicated in parentheses. Lifespan p-values are listed in Table S3.

Fig. S5 The rDNA locus does not play a role in lifespan extension of *rph1*Δ cells

(A) Real-time quantitative PCR was performed against the *RDN18, RDN58* and *NTS2* regions in the rDNA loci to detect ERC formation in WT and *rph1*Δ strains. Absolute quantities of PCR products were normalized to *ACT1* (one genomic copy in the cell). Finally, rDNA copies were calculated relative to the young of the same background. Error bars indicate standard error of the mean from two biological repeat experiments. **(B)** rDNA transcription from the *NTS2* locus was tested in the WT and *rph1*Δ young and old cells in two independent repeats of cell sorting.

Fig. S6 Deletion of Set2 or Rph1 does not affect telomeric silencing

Schematic showing the location (#1-5) of insertion of a *URA3* reporter at or near *TEL11L29* to assay silencing defects. WT, *set2*Δ and *rph1*Δ strains were assayed for growth on synthetic complete (SC) media, media lacking uracil (SC-ura) and media containing 5-fluoroorotic acid (SC+FOA). FOA sensitivity indicated a silencing defect.

Fig. S7 Comparison of long-fragment and short-fragment RNA-seq

(A) Comparative meta-gene plots of normalized tag counts over 244 cryptic genes in the yeast genome generated by two library preparation methods – traditional RNA-seq where the mRNA is fragmented to 200-300bp (long-fragment) and short fragment RNA-seq used in this study where the mRNA is fragmented to 40-50bp (short fragment). **(B)** Same data as in (A) except that the log ratio of tag counts in old over young were plotted. The plots are averaged over five short fragment replicates (E1-E5) and three long fragment replicates (E7-E9).

Fig. S8 Metagene profiles of cryptic genes in each RNA-seq dataset

Log ratios (Old/Young) of normalized RNA-seq tag counts in all RNA-seq replicates over bodies of genes that pass the defined cryptic gene filter (see Methods) in any one replicate.

Fig. S9 GO clustering analysis showing significantly enriched GO categories among cryptic genes

GO clustering analysis of the 244 cryptic genes using DAVID (http://david.abcc.ncifcrf.gov/). Enrichment scores are averages of $-\log_{10} p$ for each GO terms with $p < 10^{-3}$ in a given cluster.

Fig. S10 Deletion of subunits of the RNA degradation machineries does not produce cryptic transcripts

(A) Northern blot of mRNA isolated from yeast strains harboring no deletion (WT) or deletions in various components of RNA degradation machineries. Radiolabeled probes were designed against 5' end of *STE11*, 3' end of *STE11* and *ACT1*. RNA from *ste11*Δ strain was included as specificity control for hybridization. **(B)** Same as in (A) except mRNA was from yeast mutants known to form cryptic unstable transcripts.

Fig. S11 There is significant overlap of age-related cryptic transcripts with cryptic transcripts upregulated in transcription elongation mutants

(A-B) Four way Venn diagram showing the overlap of 244 age-related cryptic genes with cryptic genes in a *set2*Δ (Lieb strict (A) and Lieb lenient (B), *spt6* and *spt16* mutant strains. **(C)** Fold-enrichment of the Aging (age-related cryptic) genes in the other gene sets. p-values of the overlap (based on a hypergeometric test) are indicated.

Supplementary Tables

Table S1 – Yeast strains

Name	Description	Genotype
KS2	BY4741 (MATa)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
KS1	BY4742 (MATα)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
*rph1*Δ - a	*rph1*Δ in BY4741 (MATa)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rph1::KanMX4*
*rph1*Δ - α	*rph1*Δ in BY4742 (MATα)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rph1::KanMX4*
*jhd1*Δ - α	*jhd1*Δ in BY4742 (MATα)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 jhd1::KanMX4*
*set2*Δ - a	*set2*Δ in BY4741 (MATa)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set2::KanMX4*
*set2*Δ - α	*set2*Δ in BY4742 (MATα)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 set2::KanMX4*
pJD47_H3_wild-type	H3K36 (WT) - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-HHTS-HHFS*
Boeke-EMH-H3-142|K36R	H3K36R – JB*	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-hhts(K36R)-HHFS*
Boeke-EMH-H3-36|K36A	H3K36A - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-hhts(K36A)-HHFS*
Boeke-EMH-H3-158|K36Q	H3K36Q - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-hhts(K36Q)-HHFS*
Boeke-EMH-H3-158|K36E	H3K36E - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-hhts(K36E)-HHFS*
PSY40	*rph1*Δ in H3K36 - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-HHTS-HHFS rph1Δ::kanMX4*
PSY41	*rph1*Δ in H3K36R - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-hhts(K36R)-HHFS rph1Δ::kanMX4*
PSY42	*rph1*Δ in H3K36A - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-hhts(K36A)-HHFS rph1Δ::kanMX4*
PSY43	*rph1*Δ in H3K36Q - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-hhts(K36Q)-HHFS rph1Δ::kanMX4*
PSY44	*rph1*Δ in H3K36E - JB	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 can1::MFA1pr-HIS3 hht2-hhf2::URA3-hhts(K36E)-HHFS rph1Δ::kanMX4*
YBL619	Eaf3-chromodomainΔ/Rco1-TAP	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 eaf3-chd77-113Δ FLAG:Leu2 Rco1-TAP*
YBL634	Rco1-PHDΔ-TAP:HIS	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Rco1-PHDΔ-TAP:HIS*
YBL694	eaf3-chromodomainΔ/Rco1-PHDΔ-TAP:HIS (CR)	*MATα Rco1-PHDΔ-TAP:HIS/eaf3Δchromodomain*

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Table S2 – Statistical data for lifespan experiments validating the histone mutant screen (colored rows indicate significant p-values)

Histone	Mutation	Scr2 Log2	Scr2 LS	LS	LS N	LS WT	LS WT N	p-value	Change
H3	T80D	-2.250	Short-lived	16.39	80	23.84	80	7.94E-04	-31.3%
H3	K79E	-2.025	Short-lived	13.77	47	18.64	45	9.09E-03	-26.2%
H3	DEL9-16	-1.750	Short-lived	11.00	20	10.50	20	6.89E-01	4.8%
H4	R36K	-1.700	Short-lived	17.09	140	24.58	140	1.80E-09	-30.5%
H3	K9Q,K14Q,K18Q,K23Q	-1.700	Short-lived	21.51	140	21.57	140	8.37E-02	-0.3%
H3	DEL9-32	-1.625	Short-lived	11.21	19	12.10	20	7.84E-01	-7.4%
H3	DEL17-28	-1.625	Short-lived	11.55	20	12.10	20	8.98E-01	-4.5%
H4	N25D	-1.575	Short-lived	18.74	123	25.49	123	2.14E-05	-26.5%
H3	S86D	-1.525	Short-lived	19.95	124	23.29	125	1.15E-01	-14.3%
H3	K56Q	-1.500	Short-lived	6.68	59	27.18	60	1.14E-15	-75.4%
H4	R19E	-1.475	Short-lived	21.69	80	29.28	80	1.22E-05	-25.9%
H3	K14R	-1.475	Short-lived	23.57	120	22.68	120	3.89E-01	3.9%
H3	DEL5-24	-1.475	Short-lived	10.70	20	12.10	20	4.68E-01	-11.6%
H3	DEL13-20	-1.475	Short-lived	19.80	20	14.40	20	5.21E-03	37.5%
H3	L82A	-1.450	Short-lived	15.17	60	27.18	60	3.52E-08	-44.2%
H3	DEL21-36	-1.325	Short-lived	14.05	20	14.40	20	5.67E-01	-2.4%
H4	R3A	-1.250	Short-lived	22.45	145	24.43	143	1.01E-01	-8.1%
H4	K16R	-1.225	Short-lived	21.94	99	27.00	100	7.59E-04	-18.7%
H4	L22A	-1.175	Short-lived	21.03	80	29.28	80	1.70E-03	-28.2%
H3	K9A,K14A,K18A,K23A	-1.175	Short-lived	15.49	79	16.33	80	7.91E-01	-5.1%
H3	E73Q	-1.175	Short-lived	19.97	60	27.18	60	8.09E-04	-26.6%
H3	K14Q	-1.150	Short-lived	22.87	60	18.18	60	2.33E-02	25.8%
H3	DEL13-16	-1.125	Short-lived	18.85	20	14.40	20	2.32E-02	30.9%
H3	DEL17-20	-1.100	Short-lived	27.55	20	14.40	20	1.08E-06	91.3%
H4	G101A	-1.075	Short-lived	16.95	20	18.15	20	8.60E-01	-6.6%
H3	K36E	-1.075	Short-lived	17.49	140	28.48	140	3.59E-18	-38.6%
H3	DEL4-30	-1.050	Short-lived	6.33	15	12.10	20	2.17E-03	-47.7%
H3	DEL4-20	-1.025	Short-lived	14.70	20	14.40	20	7.65E-01	2.1%
H3	DEL17-24	-1.000	Normal	23.95	20	14.40	20	4.45E-05	66.3%
H3	DEL17-32	-1.000	Normal	10.25	20	17.90	20	7.98E-05	-42.7%
H3	DEL9-28	-0.950	Normal	6.94	18	12.10	20	3.35E-03	-42.6%
H3	DEL13-28	-0.875	Normal	7.88	16	12.10	20	2.72E-02	-34.9%
H3	DEL13-32	-0.850	Normal	8.35	20	17.90	20	1.06E-04	-53.4%
H3	K36R	-0.850	Normal	20.04	140	28.48	140	4.02E-12	-29.6%
H3	DEL1-24	-0.800	Normal	10.30	20	17.90	20	5.51E-04	-42.5%

H3	DEL1-28	-0.775	Short-lived	7.15	34	14.47	60	1.43E-10	-50.6%
H4	DEL1-12	-0.700	Normal	12.05	20	9.95	20	1.86E-01	21.1%
H3	DEL13-24	-0.700	Normal	18.05	20	14.40	20	5.53E-02	25.3%
H3	K36Q	-0.550	Normal	19.16	140	28.48	140	7.11E-14	-32.7%
H3	DEL9-24	-0.500	Normal	13.70	40	14.47	60	2.72E-01	-5.3%
H3	K115A	-0.475	Normal	19.20	20	14.40	20	1.46E-02	33.3%
H3	K36A	-0.350	Normal	17.33	139	28.48	140	1.92E-18	-39.1%
H3	K122R	-0.325	Normal	14.20	20	14.40	20	7.14E-01	-1.4%
H4	DEL5-12	-0.300	Normal	13.89	19	15.85	20	6.77E-01	-12.3%
H3	DEL5-32	-0.200	Normal	5.88	17	10.50	20	5.00E-04	-44.0%
H3	K9R,K14R,K18R,K23R	-0.150	Normal	17.08	40	14.83	40	4.15E-02	15.2%
H3	T58D	-0.150	Normal	17.22	60	16.25	60	1.90E-01	6.0%
H3	K122A	-0.125	Normal	19.10	20	14.40	20	4.03E-03	32.6%
H3	R72K	-0.125	Normal	28.73	60	27.18	60	5.13E-01	5.7%
H4	K79Q	-0.025	Normal	14.35	20	17.90	20	4.20E-02	-19.8%
H4	DEL1-8	-0.025	Normal	17.55	20	15.85	20	1.95E-01	10.7%
H3	L60A	-0.025	Normal	13.80	40	14.47	60	2.36E-01	-4.6%
H4	I46A	0.050	Normal	8.94	17	9.95	20	4.13E-01	-10.1%
H3	L61A	0.050	Normal	15.00	60	15.72	60	8.67E-01	-4.6%
H3	T32D	0.100	Normal	15.30	20	14.40	20	3.63E-01	6.3%
H4	R39K	0.125	Normal	10.95	39	15.68	60	1.09E-05	-30.2%
H3	K4A	0.125	Normal	14.85	120	20.81	80	1.12E-06	-28.6%
H3	A1S	0.150	Normal	13.05	20	14.40	20	1.59E-01	-9.4%
H3	S135D	0.175	Normal	17.65	20	14.40	20	1.16E-02	22.6%
H4	R23A	0.200	Normal	13.41	39	15.68	60	7.77E-02	-14.5%
H3	S31D	0.250	Normal	16.90	20	14.40	20	6.69E-02	17.4%
H3	R72A	0.325	Long-lived	15.53	40	14.47	60	2.92E-01	7.3%
H4	K77A	0.325	Long-lived	22.65	20	17.90	20	8.28E-02	26.5%
H3	L70A	0.350	Long-lived	21.95	20	17.90	20	4.47E-02	22.6%
H3	E133Q	0.350	Long-lived	19.00	20	14.40	20	1.87E-02	31.9%
H4	Q27E	0.350	Long-lived	12.90	20	9.95	20	4.36E-02	29.6%
H3	R128A	0.375	Long-lived	20.55	20	14.40	20	5.35E-04	42.7%
H3	T3A	0.400	Long-lived	16.65	20	14.40	20	6.10E-02	15.6%
H4	G99A	0.400	Long-lived	14.07	58	18.32	60	2.79E-04	-23.2%
H3	G132A	0.675	Long-lived	14.95	20	14.40	20	6.34E-01	3.8%
H3	K64A	0.750	Long-lived	18.50	20	14.40	20	2.07E-01	28.5%
H3	A111S	0.750	Long-lived	18.60	20	14.40	20	1.19E-01	29.2%
H4	K31A	0.825	Long-lived	13.80	60	18.32	60	3.02E-02	-24.7%
H4	T30D	0.875	Long-lived	18.52	44	18.44	43	8.25E-01	0.4%
H3	K121Q	0.925	Long-lived	15.98	45	18.64	45	1.52E-01	-14.3%
H3	A110S	0.925	Long-lived	23.20	20	14.40	20	2.60E-05	61.1%

H3	T3D	0.975	Long-lived	16.60	123	22.06	125	4.69E-04	-24.8%
H3	Q68A	1.000	Long-lived	10.37	60	27.18	60	1.12E-11	-61.9%
H4	DEL1-4	1.025	Long-lived	19.90	80	29.28	80	2.50E-07	-32.0%
H4	V81A	1.075	Long-lived	15.81	67	29.28	80	8.60E-14	-46.0%
H3	R63A	1.125	Long-lived	19.48	60	27.18	60	9.75E-04	-28.3%
H3	K42A	1.150	Long-lived	8.43	60	27.18	60	8.75E-14	-69.0%
H4	R55A	1.150	Long-lived	22.21	80	29.28	80	1.30E-04	-24.1%
H4	L62A	1.150	Long-lived	23.69	80	29.28	80	1.18E-03	-19.1%
H3	G44A	1.200	Long-lived	10.18	60	27.18	60	1.56E-12	-62.5%
H3	R69K	1.300	Long-lived	17.43	60	27.18	60	1.00E-05	-35.9%
H3	K18Q	1.350	Long-lived	19.57	60	27.18	60	6.79E-04	-28.0%
H4	E52R	1.375	Long-lived	6.47	79	29.28	80	1.17E-25	-77.9%
H4	D85A	1.400	Long-lived	14.14	111	25.49	123	1.63E-12	-44.5%
H4	L97A	1.425	Long-lived	11.99	80	29.28	80	2.21E-19	-59.1%
H3	K42Q	1.625	Long-lived	12.00	60	27.18	60	3.37E-10	-55.9%
H4	K44A	2.250	Long-lived	18.85	105	27.98	104	1.91E-07	-32.6%

Table S3 – Statistical data for replicative lifespan experiments in this study, excluding those validating histone mutant lifespan.

Figure	Test	Mating type	Test LS	Test cell #	Reference (Ref)	Ref LS	Ref cell #	p-value
2F	*rph1*Δ	Combined	35.71	85	WT	28.10	105	4.27E-06
		MATa	34.48	60	WT	30.22	60	0.0276
		MATα	38.64	25	WT	25.27	45	5.59E-05
S4	*jhd1*Δ	MATα	29.12	25	WT	32.02	45	0.458
2G	*set2*Δ	Combined	24.05	599	WT	28.15	239	1.10E-07
		MATa	23.13	360	WT	28.81	159	1.93E-08
		MATα	25.45	239	WT	26.86	80	0.175
2E, 2H	H3K36E	MATa	17.49	140	H3WT	28.48	140	3.59E-18
	H3K36E *rph1*Δ	MATa	19.31	140	H3K36E	17.49	140	0.151
	H3WT *rph1*Δ	MATa	31.3	120	H3WT	26.93	120	0.005
2E, 2I	H3K36R	MATa	22.42	100	H3WT	28.48	140	5.25E-06
	H3K36R *rph1*Δ	MATa	24.24	100	H3K36R	22.42	100	0.137
	H3WT *rph1*Δ	MATa	31.3	120	H3WT	26.93	120	0.005
2E, 2J	H3K36A	MATa	17.33	139	H3WT	28.48	140	1.92E-18
	H3K36A *rph1*Δ	MATa	20.96	139	H3K36A	17.33	139	0.0444
	H3WT *rph1*Δ	MATa	31.3	120	H3WT	26.93	120	0.005
2E, 2K	H3K36Q	MATa	19.16	140	H3WT	28.48	140	7.11E-14
	H3K36Q *rph1*Δ	MATa	20.37	138	H3K36Q	22.65	75	0.110
	H3WT *rph1*Δ	MATa	31.3	120	H3WT	26.93	120	0.005
5A	*eaf3-CHD*Δ	MATα	18.58	80	WT	26.86	80	1.52E-08
	*eaf3-CHD*Δ *rco1-PHD*Δ	MATα	20.11	80	eaf3-CHDΔ	18.58	80	0.162
5B	*rco1-PHD*Δ	MATa	24.26	80	WT	27.54	79	0.0366

Table S4 – RNA-seq and ChIP-seq datasets

Name of dataset	**Library Prep**	**Technical/Biological repeat**	**Samples**
E1	Short fragment RNA-seq	biological	WT
E2	Short fragment RNA-seq	biological	WT
E3	Short fragment RNA-seq	biological	WT and *rph1*Δ
E4	Short fragment RNA-seq	technical of E3	WT and *rph1*Δ
E5	Short fragment RNA-seq	biological	WT and *rph1*Δ
E6	Short fragment RNA-seq	biological	Day 1 non-FuDR, Day 1 FuDR, Day 8 FuDR and Day 12 FuDR
E7	Long fragment RNA-seq	biological	WT and *rph1*Δ
E8	Long fragment RNA-seq	biological	WT and *rph1*Δ
E9	Long fragment RNA-seq	biological	WT and *rph1*Δ
F1	ChIP-seq	biological	WT and *rph1*Δ
F2	ChIP-seq	biological	WT and *rph1*Δ

Table S5 – alignment and reproducibility parameters for high throughput sequencing datasets

A. Table of alignment parameters

Name of dataset	**Genetic background/treatment**	**Sort/timepoint**	**Total tags**	**Aligned tags**	**Percent aligned**	**Coverage**
E1	WT	Young	83775228	29749097	35.5	122.3
	WT	Old	87814164	25038374	28.5	102.9
E2	WT	Young	110931589	37070368	33.4	152.4
	WT	Old	114830029	36067983	31.4	148.3
E3	WT	Young	37162759	18004733	48.5	51.8
	WT	Old	27606218	16241864	58.8	46.7
	WT	Older	36790452	23061602	62.7	66.4
	*rph1*Δ	Young	37240522	20751442	55.7	59.7
	*rph1*Δ	Old	38579992	21952411	56.9	63.2
	*rph1*Δ	Older	43842136	27613288	63	79.5
E4	WT	Young	24605070	11921046	48.5	34.3
	WT	Old	26579917	17754303	66.8	51.1
	WT	Older	26728331	12762137	47.8	36.7
	*rph1*Δ	Young	26665617	14256359	53.5	41
	*rph1*Δ	Old	22632434	13185863	58.3	37.9
	*rph1*Δ	Older	31026649	21162082	68.2	60.9
E5	WT	Young	88043317	57786335	65.6	237.6
	WT	Old	74513438	42310819	56.8	173.9
	WT	Older	89871748	51406697	57.2	211.3
	*rph1*Δ	Young	67124411	46509057	69.3	191.2
	*rph1*Δ	Old	67217390	44879970	66.8	184.5
	*rph1*Δ	Older	104503620	65379134	62.6	268.8
E6	No FuDR	Day 1	128081626	94282933	73.6	70.5
	FuDR	Day 1	104362651	77954619	74.7	58.3

	FuDR	Day 8	113350994	84867363	74.9	63.5
	FuDR	Day 12	103086819	79592292	77.2	59.5
E7	WT	Young	17981520	26810371	74.6	220.4
	WT	Old	16186073	22852969	70.6	187.9
	*rph1*Δ	Young	21186508	31410180	74.1	258.2
	*rph1*Δ	Old	15189759	20880878	68.7	171.7
E8	WT	Young	25280561	31426014	62.2	258.4
	WT	Old	20527733	20378949	49.6	167.6
	*rph1*Δ	Young	23787692	27402759	57.6	225.3
	*rph1*Δ	Old	19824118	18789072	47.4	154.5
E9	WT	Young	27997043	31879961	56.9	262.1
	WT	Old	25837586	28756961	55.7	236.4
	*rph1*Δ	Young	24535409	28761163	58.6	236.5
	*rph1*Δ	Old	26618078	25569726	48	210.2
F1	WT – input	Young	18873898	15379199	81.5	63.2
	WT – input	Old	25465014	11246820	44.2	46.2
	WT – H3K36me3	Young	16314160	14636461	89.7	60.2
	WT – H3K36me3	Old	14052366	6059872	43.1	24.9
	WT – H3	Young	15688854	13097761	83.5	53.8
	WT – H3	Old	22786162	9058257	39.8	37.2
	*rph1*Δ – input	Young	11830255	9521043	80.5	39.1
	*rph1*Δ – input	Old	6632095	2791428	42.1	11.5
	*rph1*Δ – H3K36me3	Young	9732730	8771822	90.1	36.1
	*rph1*Δ – H3K36me3	Old	8381044	3712409	44.3	15.3
	*rph1*Δ – H3	Young	7518831	6249703	83.1	25.7
	*rph1*Δ – H3	Old	8502975	3373504	39.7	13.9
F2	WT – input	Young	107866519	80240258	74.4	494.8

	WT – input	Old	53169531	22547738	42.4	139
	WT – H3K36me3	Young	55239956	45028512	81.5	277.7
	WT – H3K36me3	Old	52886977	21096496	39.9	130.1
	WT – H3	Young	46785809	35595219	76.1	219.5
	WT – H3	Old	51338271	21097609	41.1	130.1
	*rph1*Δ – input	Young	61591394	47253860	76.7	252.2
	*rph1*Δ – input	Old	48396395	21158802	43.7	166.6
	*rph1*Δ – H3K36me3	Young	73002519	58406281	80	360.2
	*rph1*Δ – H3K36me3	Old	55972074	25563972	45.7	157.6
	*rph1*Δ – H3	Young	58278447	40902143	70.2	291.4
	*rph1*Δ – H3	Old	58583365	27013429	46.1	130.5

B. Table of reproducibility parameters

Pearson correlation table for RNA-seq tags over 3' half of cryptic genes in WT Young

	E1	**E2**	**E3**	**E4**	**E5**
E1	1	0.98	0.81	0.78	0.81
E2	0.98	1	0.89	0.87	0.89
E3	0.81	0.89	1	1	0.97
E4	0.78	0.87	1	1	0.98
E5	0.81	0.89	0.97	0.98	1

Pearson correlation table for RNA-seq tags over 3' half of cryptic genes in WT Old

	E1	**E2**	**E3**	**E4**	**E5**
E1	1	0.99	0.89	0.83	0.81
E2	0.99	1	0.9	0.86	0.77
E3	0.89	0.9	1	0.96	0.8
E4	0.83	0.86	0.96	1	0.72
E5	0.81	0.77	0.8	0.72	1

Table S6 – TATA and Initiator motif density

	Number of cryptic genes	Number of annotated cryptic genes	Number of motifs in cryptic genes	Number of cryptic genes with motif	Motif density (number of motifs per Kb of cryptic gene sequence)	Motifs per gene (number of motifs per cryptic gene)	Background = non-cryptic genes			
							p-values for number of motifs	p-values for number of genes with motif	p-values for motif density	p-values of motifs per gene
Yeast TATA	244	244	140	107	0.19	0.57	0.007	<0.001	0.91	0.006
Yeast Initiator	244	244	410	171	0.56	1.7	<0.001	<0.001	0.282	<0.001
Worm TATA	443	414	145	110	0.35	0.07	<0.001	<0.001	0.158	<0.001