Global analysis of transcriptionally engaged yeast RNA polymerase III reveals extended tRNA transcripts

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Supplemental Material

Supplemental_Figure_S1

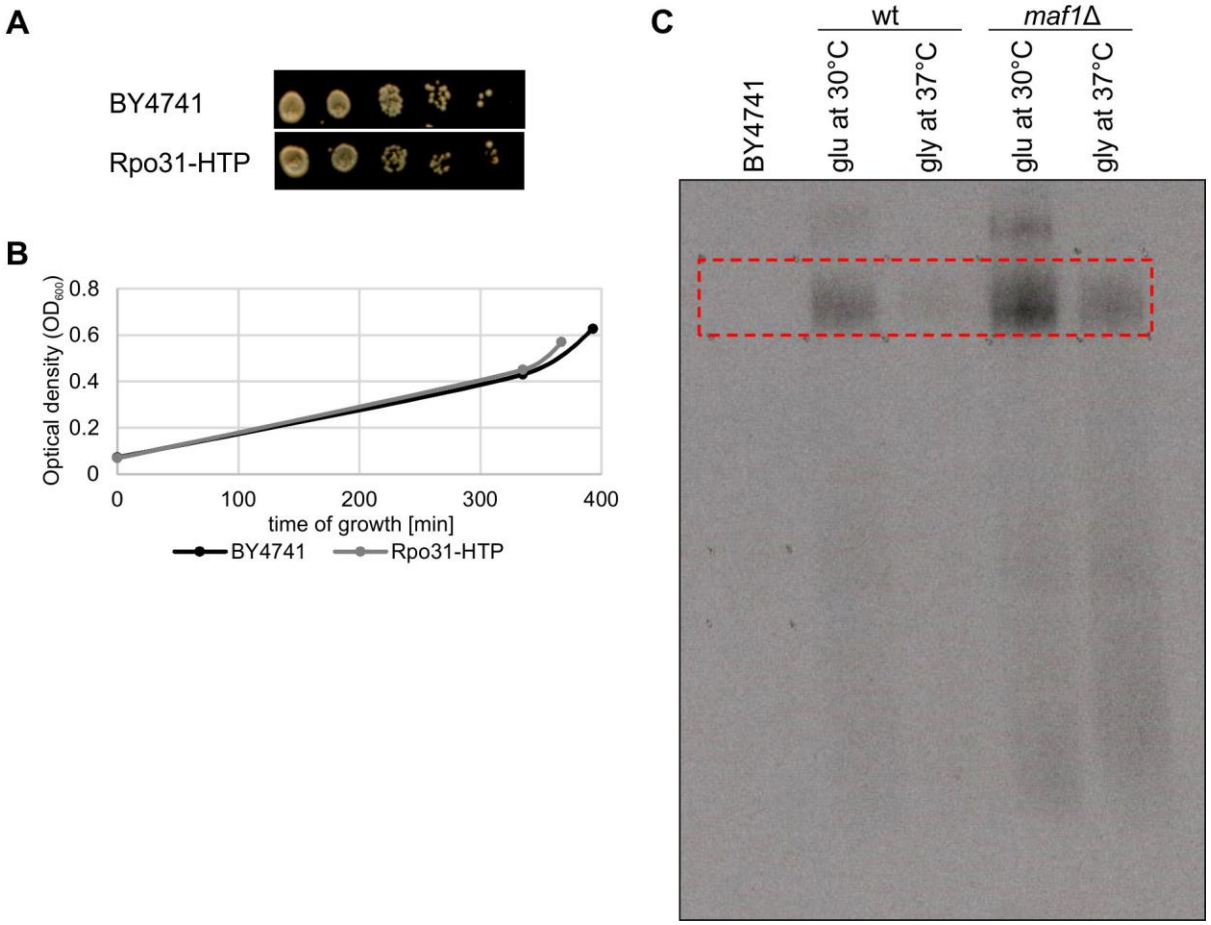


Figure S1.Strains growth and CRAC.

(A) Growth of wt (BY4741) and Rpo31-HTP strains. Overnight liquid cultures were diluted to OD_{600}=1.0 and serially 10-fold diluted, spotted on YPD plates and incubated at 30°C for two days.
(B) Growth curves of wt (BY4741) and Rpo31-HTP strains for CRAC culture.
(C) CRAC autoradiography. SDS-PAGE purification of the Rpo31-HTP protein, visualized by autoradiography of the cross-linked and radioactively labeled RNA. The red frame indicates the region excised for elution of RNA-protein complexes.

Supplemental_Figure_S2

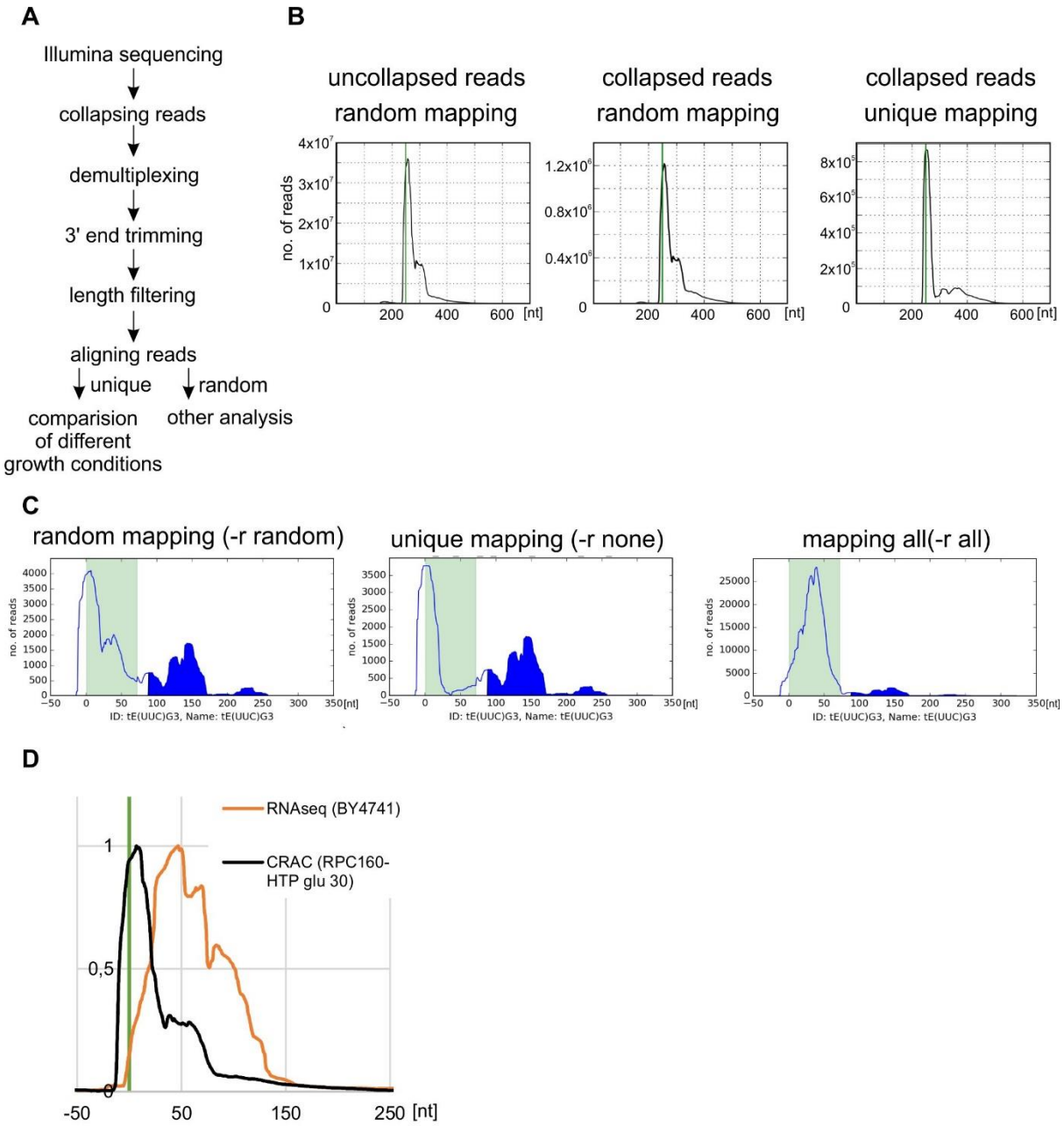


Figure S2. Data analysis.

(A) Overview of data analysis.

(B) Genome-wide profiles for all nuclear tRNA genes showing comparison between different processing schemes. Most analyses was performed on collapsed and randomly mapped reads.

(C) Hit distributions along the tE(UUC)G3 gene showing differences resulting from alternatives in read mapping. The x-axis shows distance (nt) from the 5' end of mature tRNA. Green background indicates exon position(s). Only comparisons between different datasets were performed using uniquely mapped reads.

(D) Comparison of genome-wide profiles for all nuclear tRNA genes obtained from CRAC and RNA-seq datasets. Validation showing that the predominant 5´ peak is not an

artificial pattern resulting from read mapping. Profile maximum was normalized to 1 for easier comparison. Both datasets use uncollapsed, randomly mapped reads.

Supplemental_Figure_S3

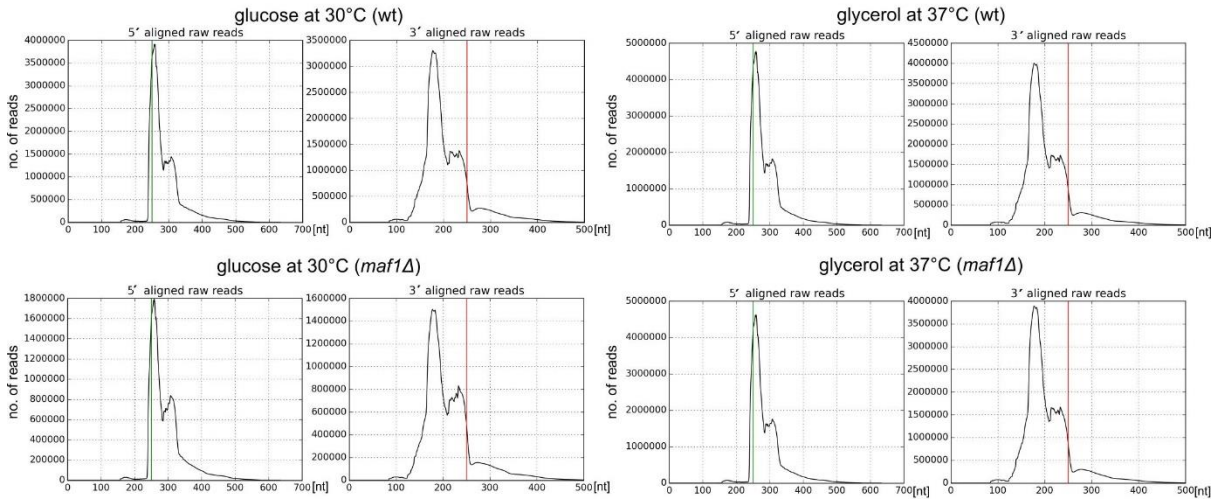


Figure S3. tRNA genome-wide profiles. Genome-wide profiles of all nuclear tRNA genes under tested growth conditions with high 5´ peak, aligned to 5´ end of mature tRNA. As default random mapping was applied.

Supplemental_Figure_S4

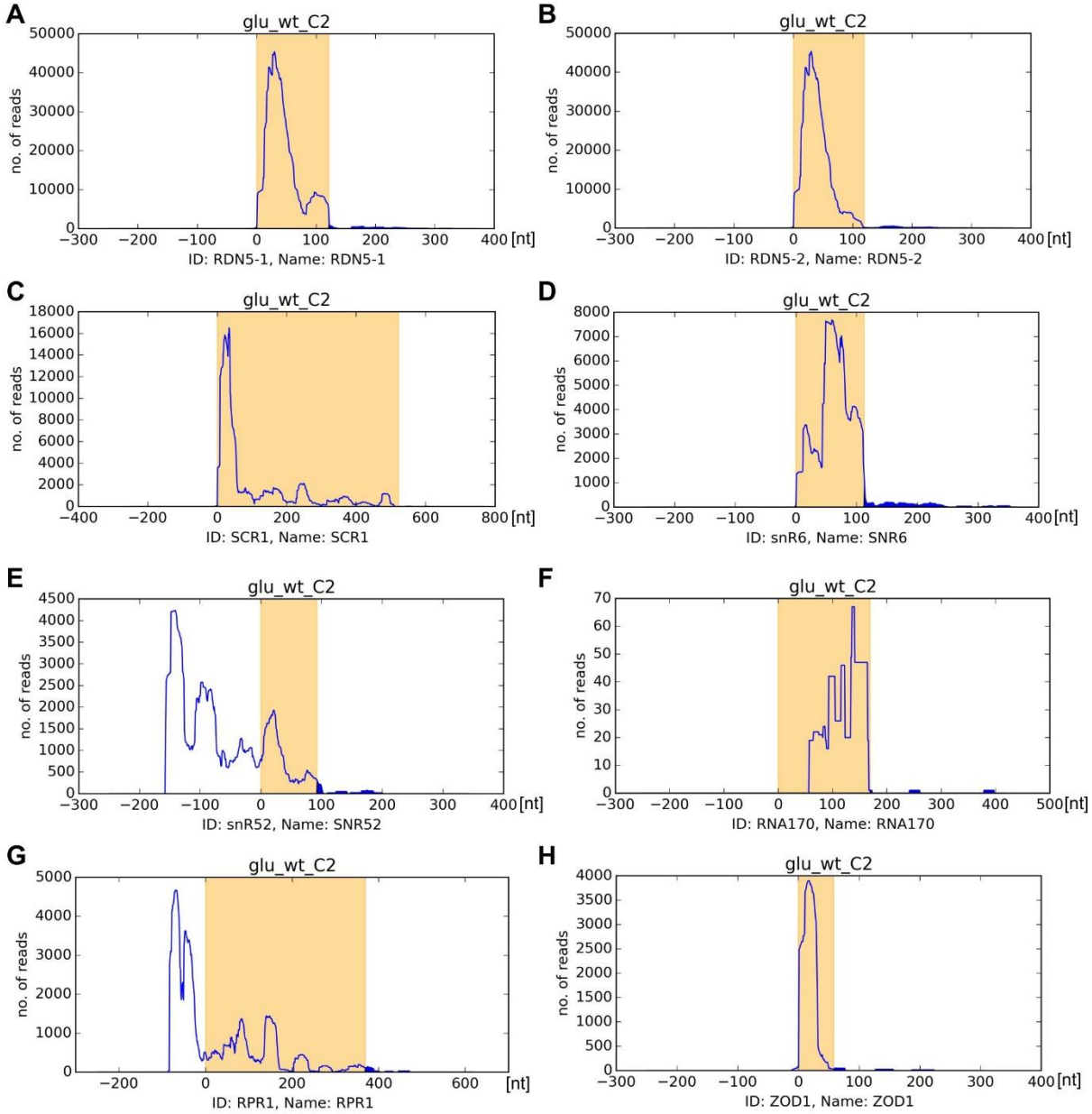


Figure S4. Hit densities across non-tRNA RNAPIII transcripts

Mature RNA regions are colored:

(A) RDN5-1
(B) RDN5-2
(C) SCR1
(D) SNR6
(E) SNR52
(F) RNA170
(G) RPR1
(H) *ZOD1*

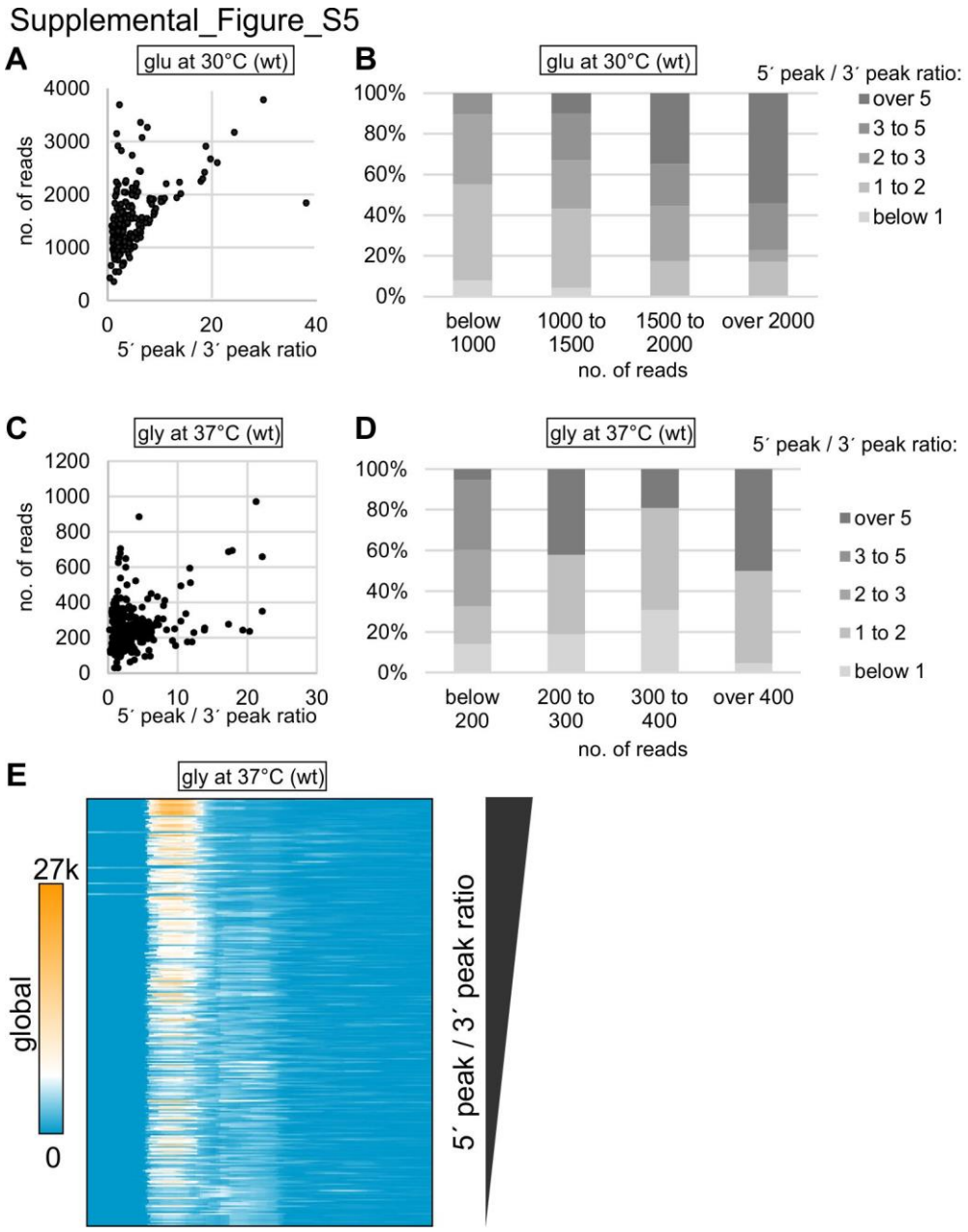


Figure S5. Predominant 5´ to 3´ peak ratios correlate with highly transcribed genes.

(A) Ratio of 5´ to 3´ peaks plotted against relative gene transcription (total number of reads mapped to a gene) is generally greater for highly expressed tRNA genes under permissive conditions

(B) 5´ to 3´ peak ratio distribution among tRNA genes grouped by transcription rate.

(C) Under restrictive growth conditions the 5´ to 3´ peak ratios are not well correlated with gene transcription rate.

(D) As (B) but RNAPIII activity is repressed.

(E) All nuclear tRNA genes were ordered according to 5´ to 3´ peak ratio. The color intensity shows number of reads in global scale of mapped reads, showing that under restrictive growth conditions 5´ to 3´ peak ratio is not well correlated with gene transcription rate.

Supplemental_Figure_S6

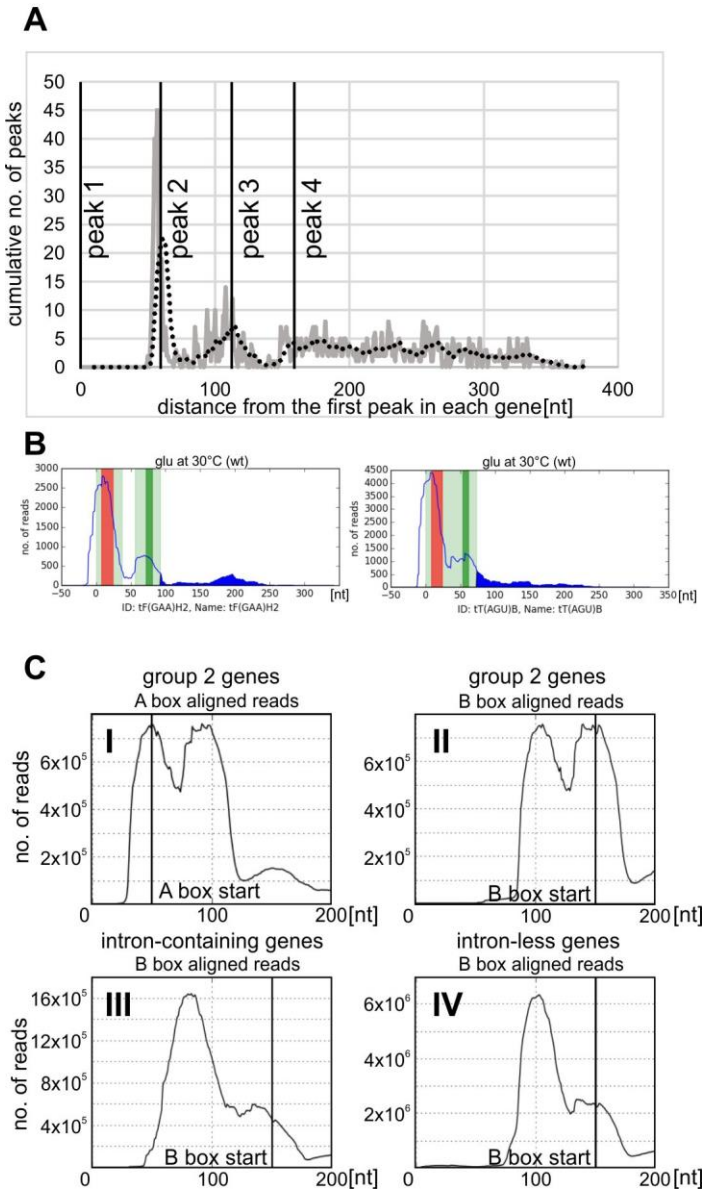


Figure S6. Possible explanations of RNAPIII density patterns

(A) Genome-wide distribution of peaks along tRNA genes. Each peak for each tRNA gene was counted as 1 and the sum of peaks was plotted starting from the first peak. Raw plot (grey line) and trend line (black dotted line) are presented. Positions of the first four peaks are marked.

(B) Hit distributions along individual genes (tF(GAA)H2 and tT(AGU)B) with annotated A box (red background) and B box (dark green background). Light green background indicates exon position(s). Filled area represents RNAPIII read-through.

(C) Genome-wide profile for group 2 tRNAs (50 genes with 5´ to 3´ peak ratio <1.5) (panel I and II) and for intron-containing and intron-less tRNA genes (panels III and IV). Vertical lines show start sites of A and B box sequences.

Supplemental_Figure_S7

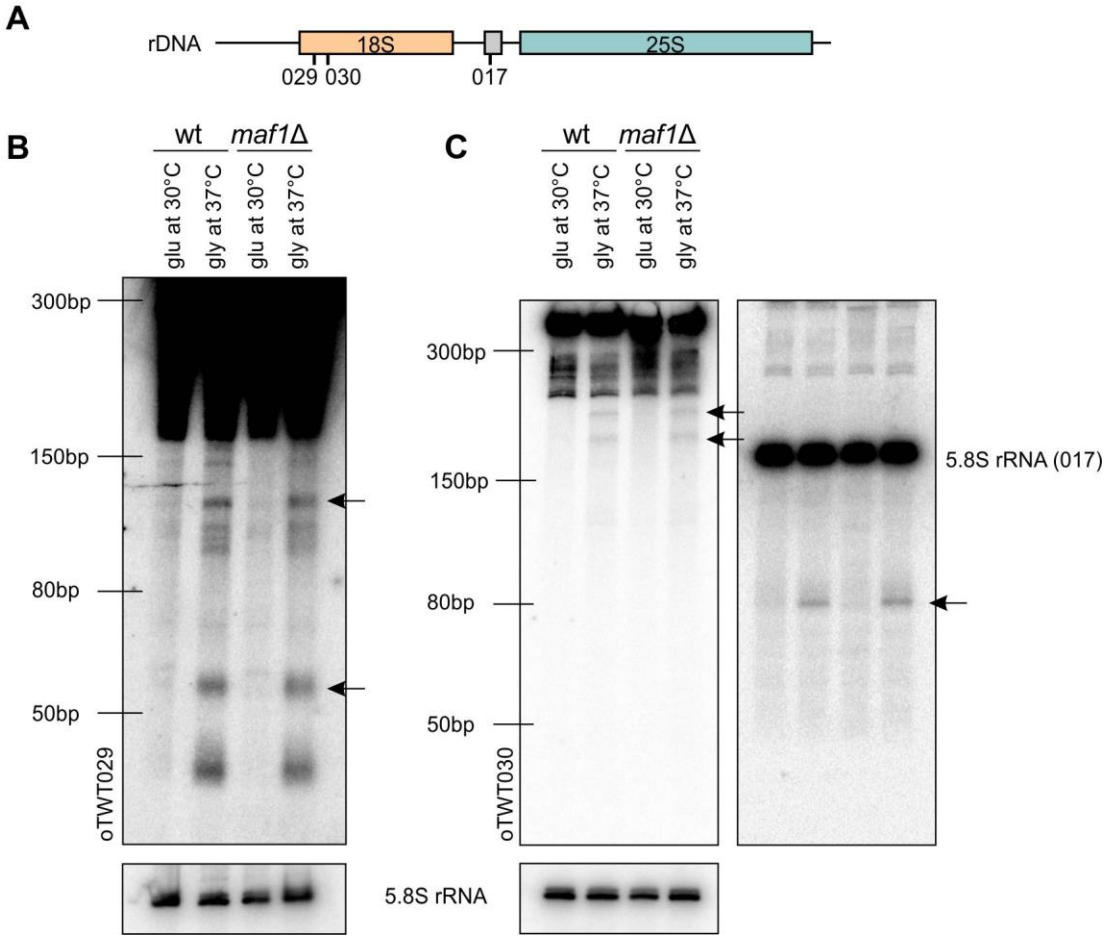
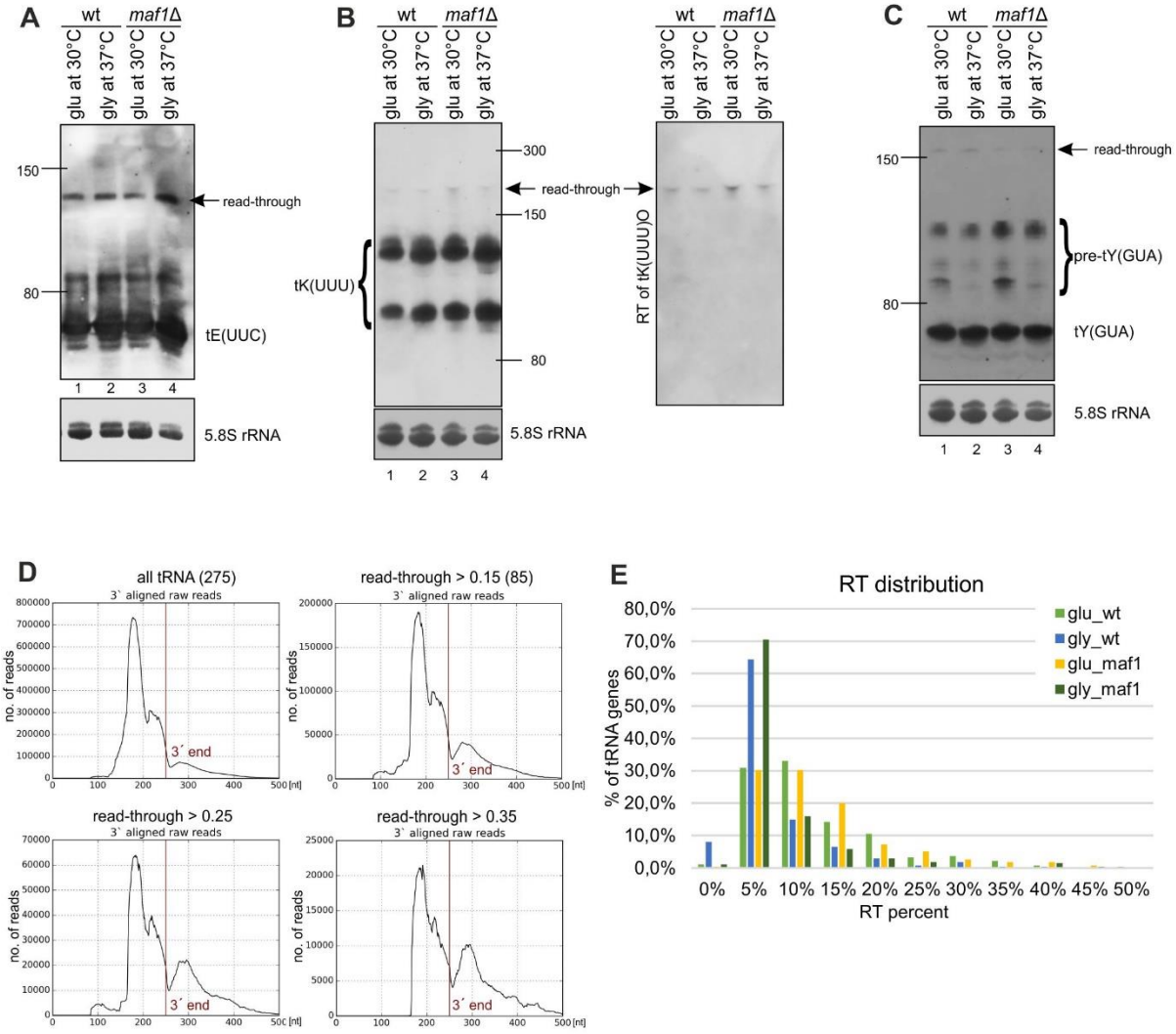


Figure S7. RNAPIII CRAC reveals new RNAPIII transcripts.

(A) Schematic representation of the rDNA transcription unit with positions of northern probes marked.
(B) Northern blot showing short RNA fragments arising from rDNA repeats, visualized using specific probe (oTWT029), and the 5.8S rRNA loading control.
(C) Northern using probe oTWT030 and longer exposure of 017 probe recognizing an 80 nt fragment.

Supplemental_Figure_S8



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gene name	gene ID	RT	last 10 nt of tRNA gene + 50 nt after
tE(UUC)E2	tE(UUC)E2	56,1%	CGTATCGGAGTACTTTTTTGACATCATACAGTGTATGTATGGGGTTAGGAGCCACCTTCC
SOE1	tE(UUC)G1	47,4%	CGTATCGGAGTTTTTTGTCGTAAGTTGAGAAAGTTTTTTCCAGTTAAGTTATTATGACGC
tE(UUC)E1	tE(UUC)E1	39,3%	CGTATCGGAGTACTTTTTTGATGGCTACAATCATAATTTGACATACCTCATATGAATATG
tE(UUC)G3	tE(UUC)G3	32,5%	CGTATCGGAGTGTTCTTTTTCTTACCTCTTGAAAAAGAGGTAGTAAAGTCAATATAAATT
tE(UUC)P	tE(UUC)P	29,3%	CGTATCGGAGTTTTCTTTTTGCTTCCTTCCGCCCTTCCAAGTTGTTGCATAATCTTATCT
tE(UUC)I	tE(UUC)I	17,7%	CGTATCGGAGTATGTTTATTTTATTTTTTTCATATCCGCTCGAATATTGCCTTCGTTTAA
tE(UUC)E3	tE(UUC)E3	15,6%	CGTATCGGAGTATTCGTTATATTTTTTCAATCGCTTACGATCAAAAGCTTTGAAAACATT
tE(UUC)K	tE(UUC)K	13,5%	CGTATCGGAGTTTTCATTATTATTTTTAATCTAGCAGTACTAGCGTGATGTTAATGTTAC
tE(UUC)L	tE(UUC)L	10,8%	CGTATCGGAGATATATTTAATATTTTTTTTGATTTGACTTCAAATGAGAACTATTTTTTT
tE(UUC)M	tE(UUC)M	10,7%	CGTAGCGGAGTTTTCTTTTTGAAGAAATTTGAAAATTTTTTTCTTCACGTCGACCAAGGT
tE(UUC)B	tE(UUC)B	10,7%	CGTATCGGAGTTATTTTTTGTTTCTATACCATTATTTTTCTTGAATGTTGCATTATGATA
tE(UUC)G2	tE(UUC)G2	9,5%	CGTATCGGAGTATTTTTTTGAAAGTTTTTTTTCACGTTAGTTCCGTGAAGAAAATTTTTT
tE(UUC)J	tE(UUC)J	9,5%	CGTATCGGAGTATTTTTTATGTGATTAGCTAAATAGTTATTTTTTGAAATAGTTTGTAGT
tE(UUC)C	tE(UUC)C	8,1%	CGTATCGGAGTTTTTTTTTTCTGCTATTACAATTAAAGATTTTTTCAACTATATCTGAGA

Figure S8. Analysis of transcriptional read-through of RNAPIII

(A) Northern blotting reveals extended pre-tRNAs transcribed from tE(UUC) genes. Extended forms are of low abundance relative to mature tE(UUC). 5.8S rRNA is the loading control.

(B) Northern with probes specific for tK(UUU): 5´tK(UUU) and tK(UUU)O_RT (recognizing a sequence 40 nt downstream from gene tK(UUU)O).
(C) Northern with probe to tY(GUA).
(D) Cumulative plots of tRNA genes aligned to 3´-ends of mature tRNAs. Plots were generated for all tRNA genes and grouped by RT levels.
(E) Histogram showing distribution of RT under different growth conditions.
(F) Table presenting relationship between RT level and 3´-extension sequence. T4 and longer T tracts in genomic sequence are marked in bold.

Supplemental_Figure_S9

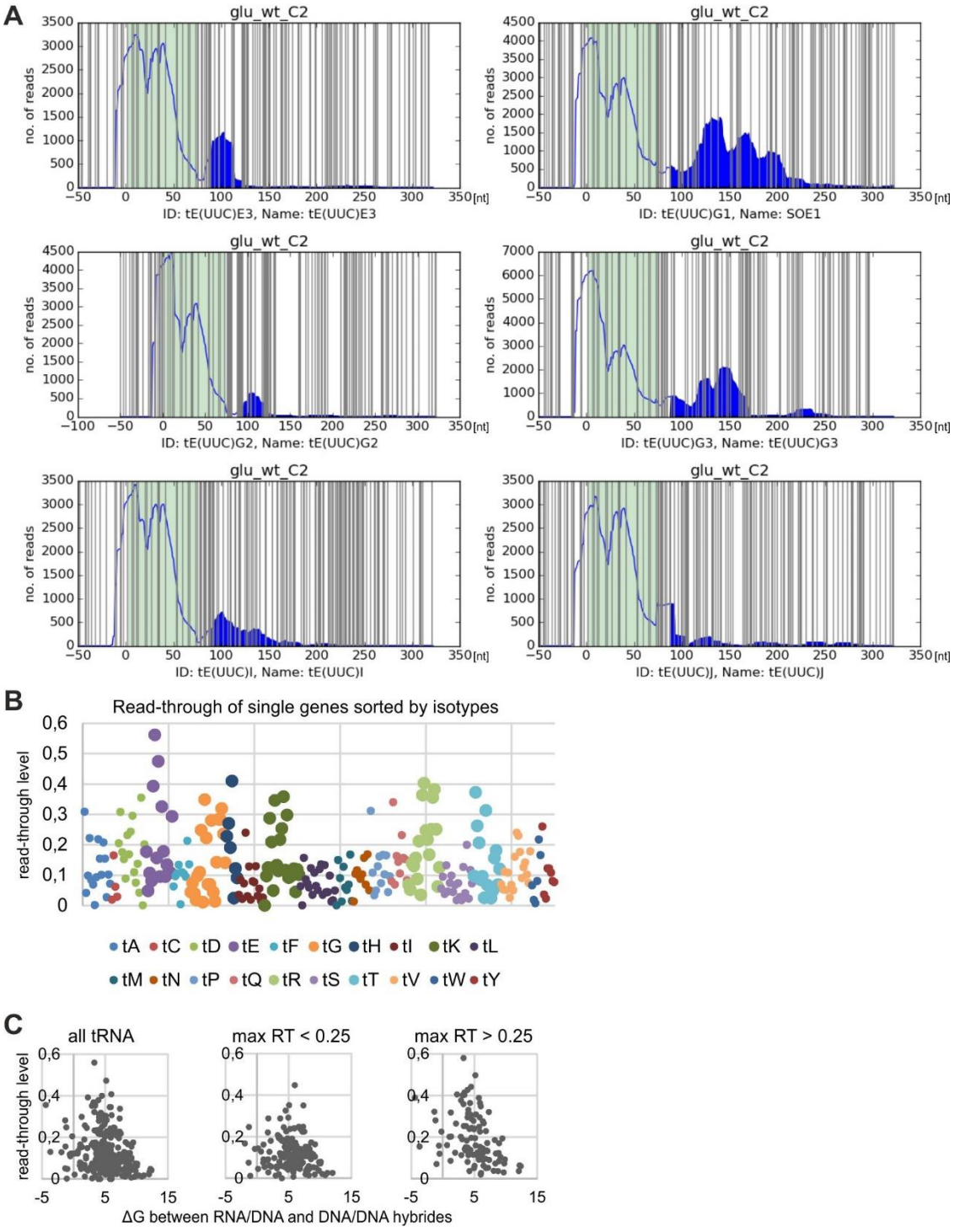

Figure S9. Reasons of transcriptional read-through of RNAPIII

(A) Hit distributions along selected genes encoding glutamic acid tRNA (tE(UUC)E3, *SOE1*, tE(UUC)G2, tE(UUC)G3, tE(UUC)I, tE(UUC)J) with all U nucleotides marked (grey lines). This shows a correlation between U-richness and RNAPIII transcription/termination. Green background indicates exon position(s). Filled area represents RNAPIII read-through (RT).

(B) High level transcriptional RT is characteristic for a subset of tRNA isotypes. On the plot RT values are ordered according to isotype.

(C) Correlation between transcriptional RT and ΔG between predicted stability of DNA/DNA versus RNA/DNA duplexes, over 20 nt 3´ to the ends of tRNA gene. Panels show correlation for all tRNA genes (left panel), isotypes with low RT (middle) and isotypes where RT of at least two genes exceeds 25% (right panel).

Yeast strains used in this study

All yeast analyses were performed in strains derived from (BY4741, *MAT*a; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*).

Yeast strains used in this study:

ID	Name	genotype	source
yTWT050	C160-HTP	a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RPC160-HTP::URA3MX	this study
yTWT057	C160-HTP maf1Δ::KanMX	a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RPC160-HTP::URA3MX maf1Δ::KanMX4	this study
118	Nab2-HTP	a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 NAB2-HTP::URA3MX	Tuck and Tollervey 2013
CDF21	Rrp6-HTP	MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0 ; RRP6-HTP-URA3	Schneider et 2012
CDF32	Δrrp44 shuffle strain	MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; rrp44Δ::kanMX6; [pRS316/RRP44-ssz]	Schneider et 2012
CDF33	Rrp44-HTP	MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0 RRP44::Kan + pRS315/RRP44-HTP	Schneider et 2012
CDF34	Rrp44exo-HTP	MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0 RRP44::Kan + pRS315/Rrp44-D551N-HTP	Schneider et 2012
CDF36	Mtr4-HTP	MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0 ; MTR4-HTP-URA3	this study

Supplemental Methods

Northern blotting

Yeast strains were grown in SC-trp medium containing 2% glucose at 30°C to exponential growth phase, then transferred to SC-trp medium containing 2% glycerol, incubated for 2 h at 37°C. Then RNA was isolated as described previously (Tollervey and Mattaj 1987; Foretek et al. 2016). Quantity and purity of RNA were analyzed using a NanoDrop 1000 (Thermo). To identify read-through products for individual tRNAs, 5µg of the total RNA and 2µl low range ssRNA ladder (NEB) were resolved by 10% PAGE with 8 M urea. Gels were stained with ethidium bromide for visualization of RNA ladder. Then RNA was transferred onto a Hybond-N+ membrane (Amersham) by electroblotting in 0.5× TBE and crosslinked by UV radiation. For tRNA detection a nonradioactive northern method was used (Wu et al. 2013). For some probes, the temperature of hybridization and the washing protocol were modified to increase specificity of the signal. Blots were developed using photographic films for higher resolution (CL-XPosure Film from Thermo Scientific Pierce). New RNAPIII transcripts were detected using radioisotope northern method.

Oligonucleotides used for Northern hybridization:

Oligo name	**Sequence**	**Labeling method**
His(GUG)	5’-ACTAACCACTATACTAAGA-3’	DIG
tH(GUG)G2_RT	5’-CTTCTCTTGGTCGGGTTTGG-3’	DIG
tE(UUC)	5’-AAAGCGTGATGTGATAGCCG-3’	DIG
5’tK(UUU)	5’-CAAGGATGAGTTCTTC-3’	DIG
tK(UUU)RT	5’-AGAGGATGGTACCCGAATAG-3’	DIG
tTYR(GUA)all	5’-CGAGTCGAACGCCCGAT-3’	DIG
5.8S rRNA	5’-GCGTTGTTCATCGATGC-3’	DIG
tY(GUA) SRM15	5’-GCGAGTCGAACGCCCGATCTCAAGATTTACAGT CTTGCGCCTTAAACCAACTTGGCTACC-3’	DIG
017 – 5.8 rRNA	5’-GCGTTGTTCATCGATGC-3’	Radioisotope
320 - tRNALys2-intron	5’-ATCCTTGCTTAAGCAAATGCGC-3’	Radioisotope
oTWT002	5’-CGGACAATTGATTAAACTATG-3’	Radioisotope
oTWT008	5’-TTTCTGTTCTCCATGAGTCC-3’	Radioisotope
oTWT029	5’-GCTCTAGAATTACCACAGTT-3’	Radioisotope
oTWT030	5’-CATCGAAAGTTGATAGGGC-3’	Radioisotope

RNA-seq

Yeast strains were grown at 30°C to OD600 0.5 in minimal media and RNAs were extracted using a standard acidic hot phenol method. RNA-seq libraries were prepared according to the protocol suggested by NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England Biolabs) and NEBNext Multiplex oligos for Illumina. 10 µg of RNA were used as input and treated with Ribominus Transcription Isolation Kit (AMBION) to reduce ribosomal RNA. The remaining RNA was used for the construction of both strands of cDNA. Adaptors were ligated and DNA fragments were amplified. Libraries were size-fractionated on agarose gels, run on a Bioanalyzer for quality control and sequenced using Illumina HiSeq with 50bp single-end reads (Edinburgh Genomics).