Supplemental Materials

40S ribosomal subunits scan mRNA for the start codon by one-dimensional diffusion

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Supplemental Figures

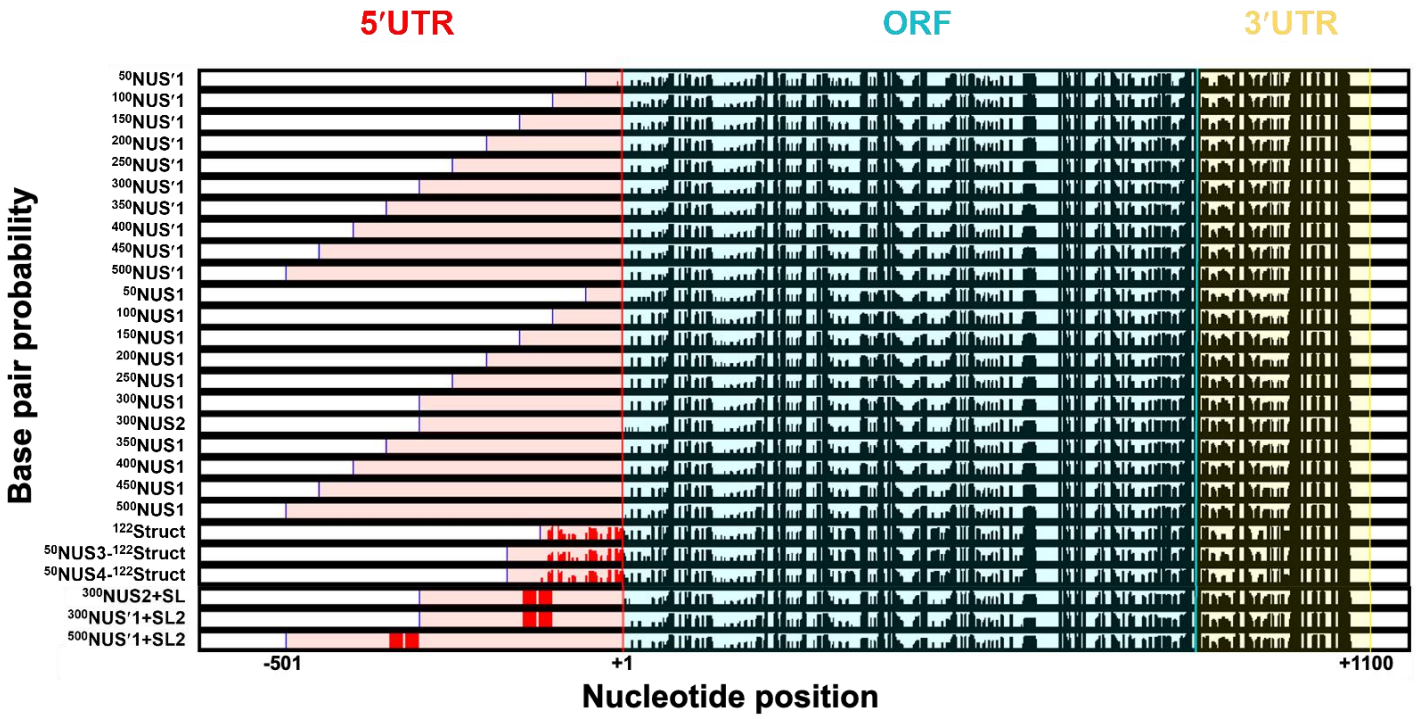

Figure S1. The nucleotide base pairing probability of GFP mRNA variants. The base pairing probabilities were estimated by a partition function calculation. The Y-axis is the probability of base pairing. The X-axis represents the nucleotide position in the mRNA. The 5' UTR, ORF and 3′ UTR are highlighted in red, cyan and yellow, respectively.

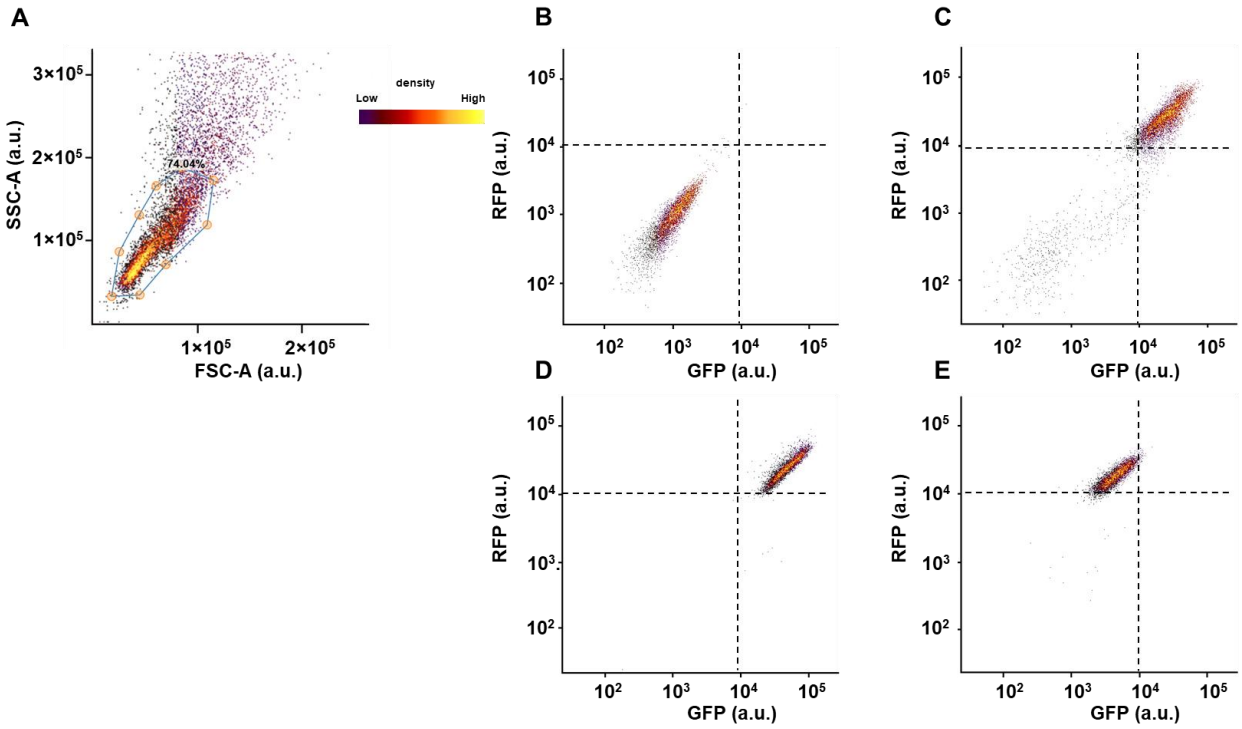

Figure S2. Representative flow cytometry scatter plots for NUS' RNA-ID constructs in which each cell is represented by a dot. (A) Initial forward / side scattering plot (area values) and gating scheme (enclosed area by light blue edges with orange vertices). The gated population was analyzed by GFP / RFP fluorescence emission scatter plots (B-E): (B) uninduced GO control cells, (C) GO control cells after induction by galactose, (D) 50NUS' cells after induction by galactose, and (E) 500NUS' cells after induction by galactose. Flowcytometry data files (FCS files) were analyzed, and illustrations were generated by Cytobank (https://community.cytobank.org).

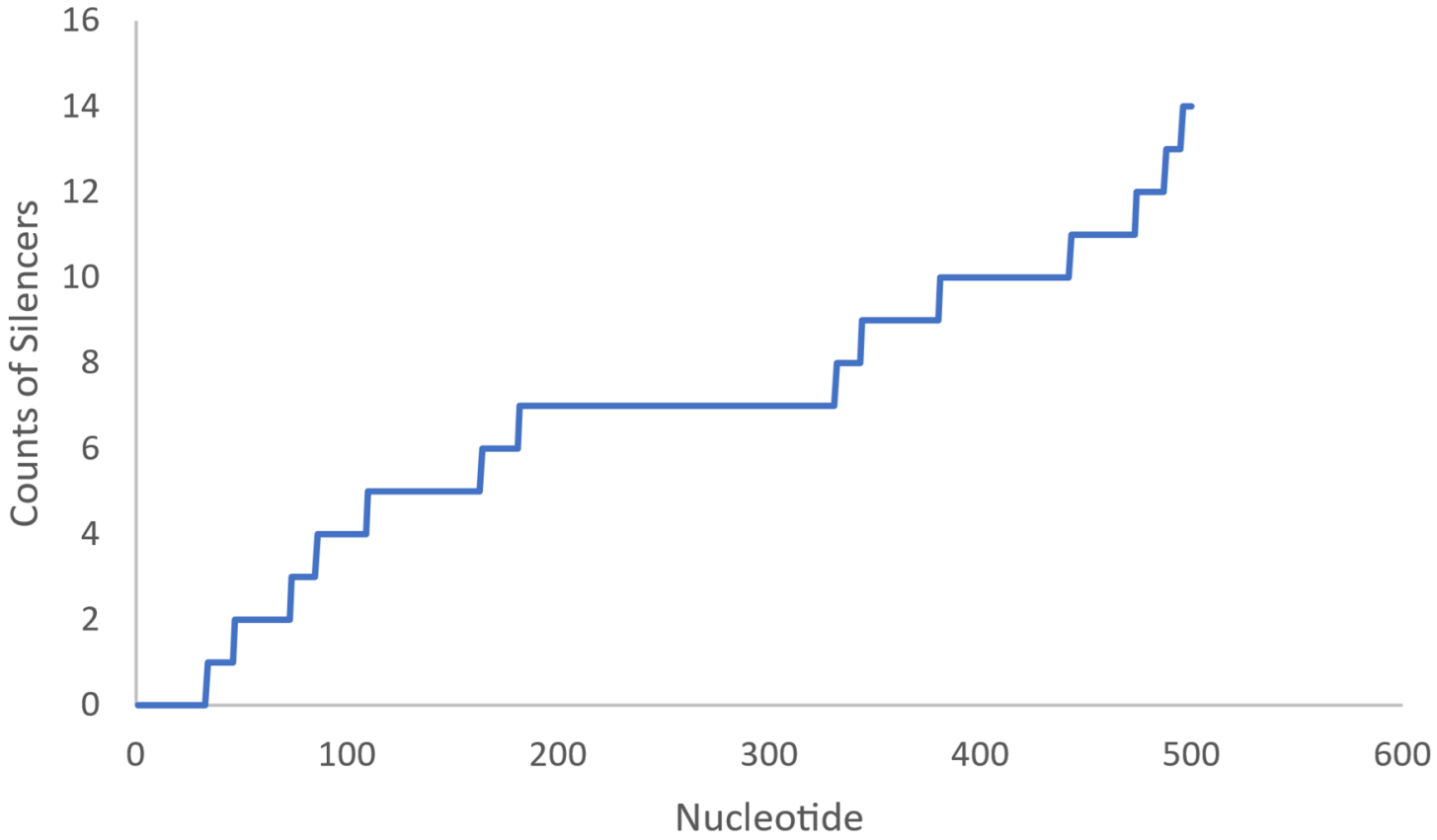

Figure S3. Cumulative number of UCC and ACCAC translation silencing sequences (y axis) in ^{500}NUS' 5' UTR (nucleotide position in the ^{500}NUS' 5' UTR, x axis).

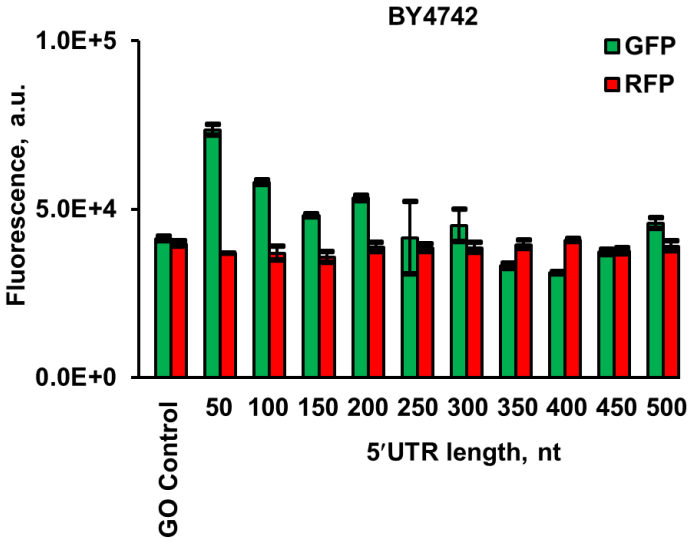

Figure S4. Mean GFP and RFP fluorescence measured in BY4742 yeast cells, which were transformed with different NUS1 5' UTR RNA-ID constructs as indicated.

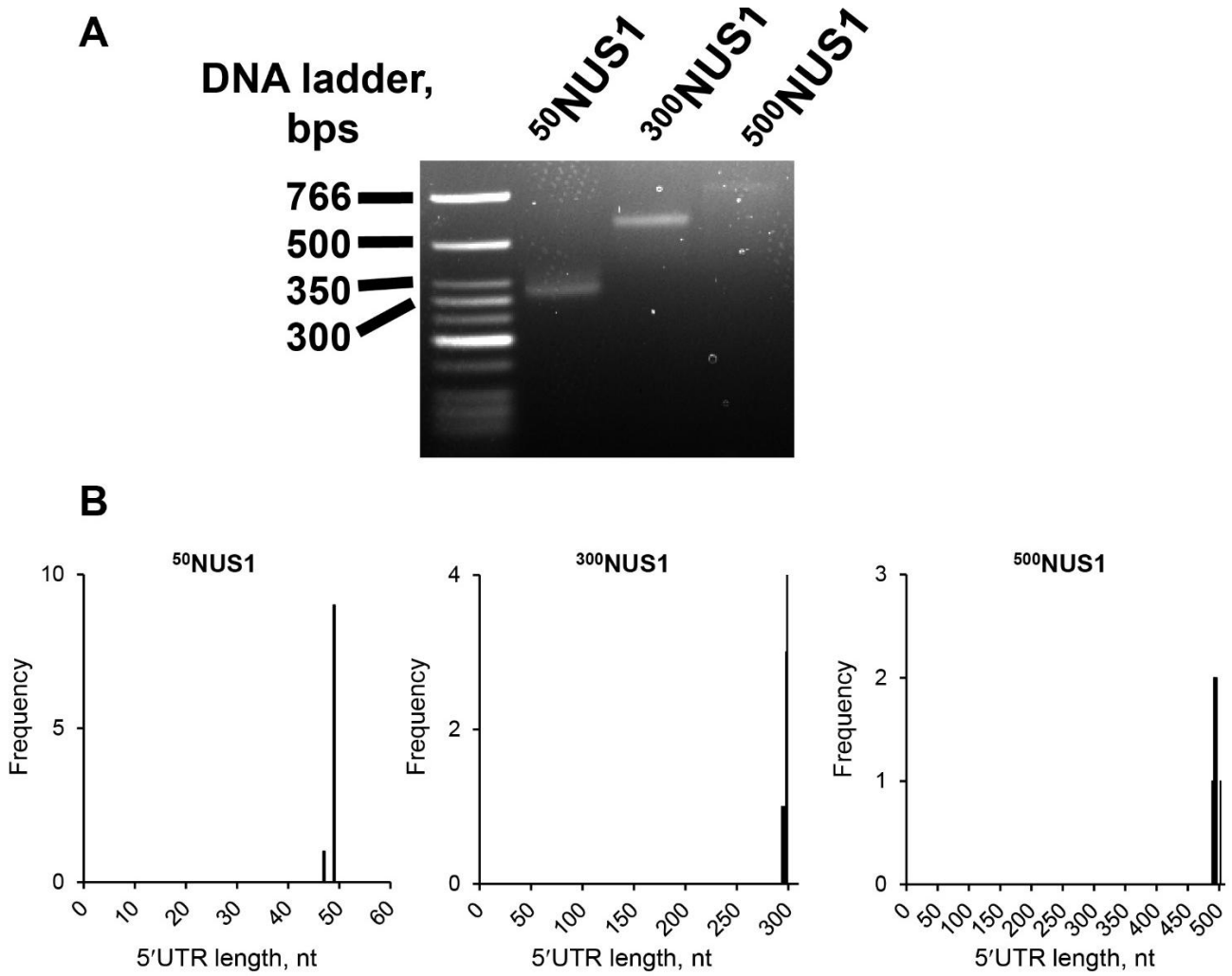

Figure S5. 5' RACE analysis of different NUS1 5' UTR RNA-ID variants. (A) The 5' end of NUS1 5' UTR RNA-ID was analyzed by RACE (Rapid Amplification of cDNA Ends) consisting of sequential dephosphorylation, de-capping, ligation to adaptor RNA (34 nts long) and reverse transcription of yeast total RNA. cDNAs were amplified using a forward primer (HIV-F1) which anneals to adaptor RNA and a reverse primer which anneals to GFP-ORF (5RACE-R2, Supplementary Table S7). PCR products were run on a 1% agarose gel. Expected lengths of ^{50}NUS1, ^{300}NUS1 and ^{500}NUS1 amplicons were of 345, 595 and 795 base pairs, respectively. (B) ^{50}NUS1, ^{300}NUS1 and ^{500}NUS1 RACE amplicons were cloned into a sequencing vector and then 10 clones for each construct were sequenced. 5' UTR length distributions were calculated using 5' RACE amplicon sequencing data. Average length of 5' UTR ± standard deviation in ^{50}NUS1, ^{300}NUS1, or ^{500}NUS1 constructs was 49±1, 298 ± 2 and 494 ± 4 nucleotides, respectively. When aligned with the original template, the average sequence identity of ^{50}NUS1, ^{300}NUS1, or ^{500}NUS1 5' RACE amplicons ± standard deviation was 98 ± 1, 99 ± 1, or 99 ± 1%, respectively.

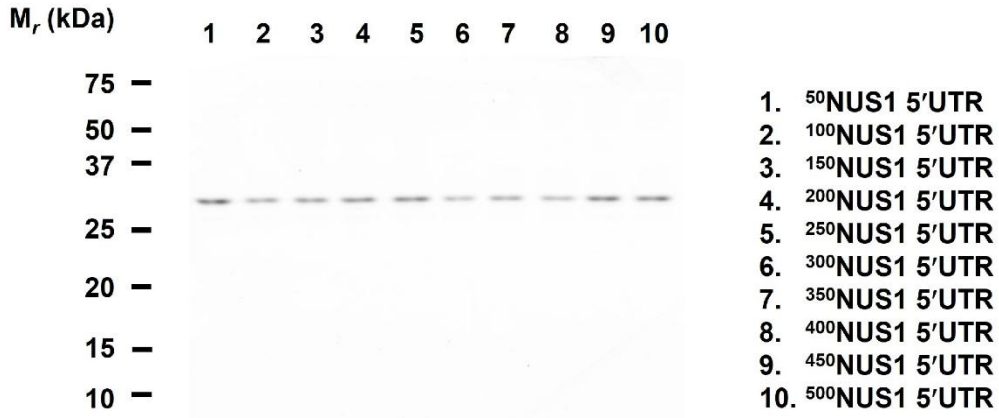

Figure S6. SDS-PAGE analysis of different NUS1 5' UTR RNA-ID variants. Lysates from yeast cells transformed with the NUS1 5' UTR RNA-ID variants were run on 15% SDS-PAGE gel and GFP fluorescence was detected with an Amersham Typhoon Biomolecule Imager (GE Healthcare, Chicago, IL) with settings of excitation wavelength = 488 nm, emission wavelength = 525 nm, PMT = 400V.

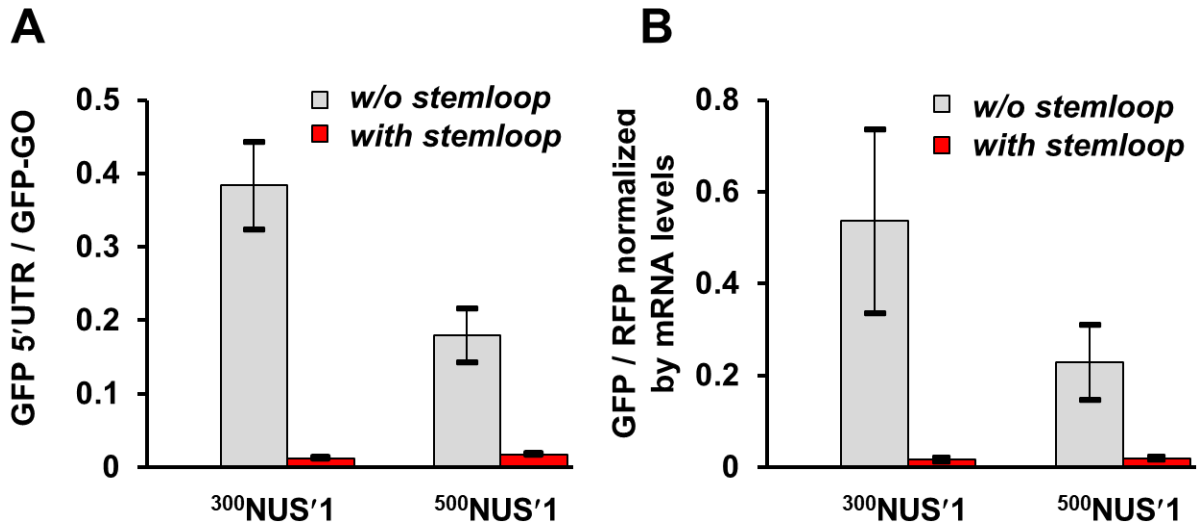

Figure S7. RNA secondary structure in the 5'UTR strongly inhibits translation of ^{300}NUS' and ^{500}NUS' GFP mRNAs. 54 nucleotides in the middle of ^{300}NUS' and ^{500}NUS' 5' UTR were replaced with a 44-nt long RNA stem-loop. (A) Mean GFP fluorescence was normalized to that in the GO RNA-ID reporter lacking both the 5' and 3' UTRs. (B) Mean GFP fluorescence was normalized to respective mRNA levels, which were determined by RT-qPCR.

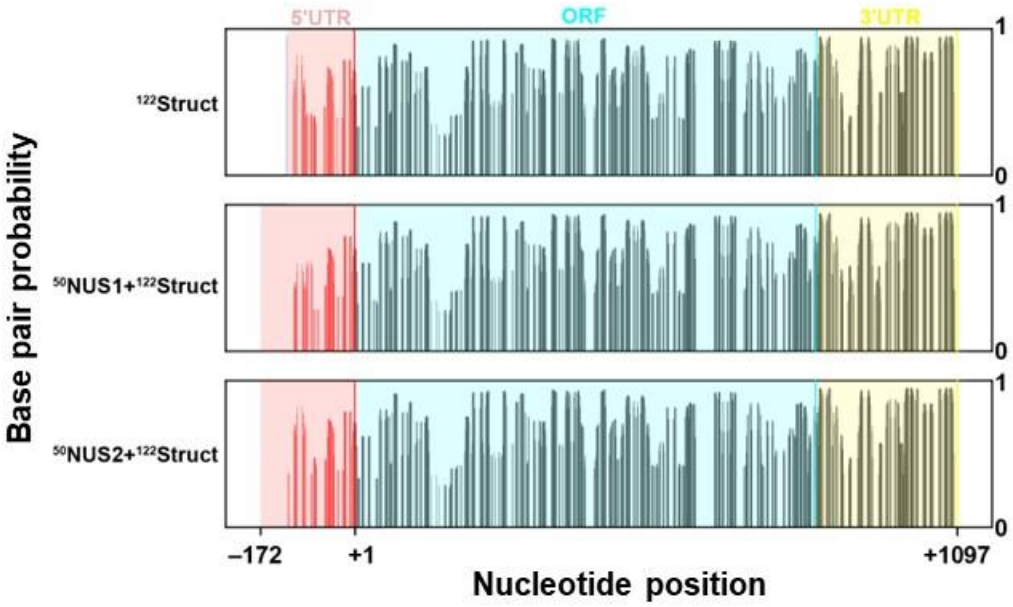

Figure S8. The nucleotide base pairing probability of ^{122}Struct, NUS3-^{122}Struc, NUS4-^{122}Struct GFP mRNA variants. The base pairing probabilities were estimated by a partition function calculation. The Y axis is the probability of base pairing. The X axis represents the nucleotide position in the mRNA. The 5′ UTR, ORF and 3′ UTR are highlighted in red, cyan and yellow, respectively.

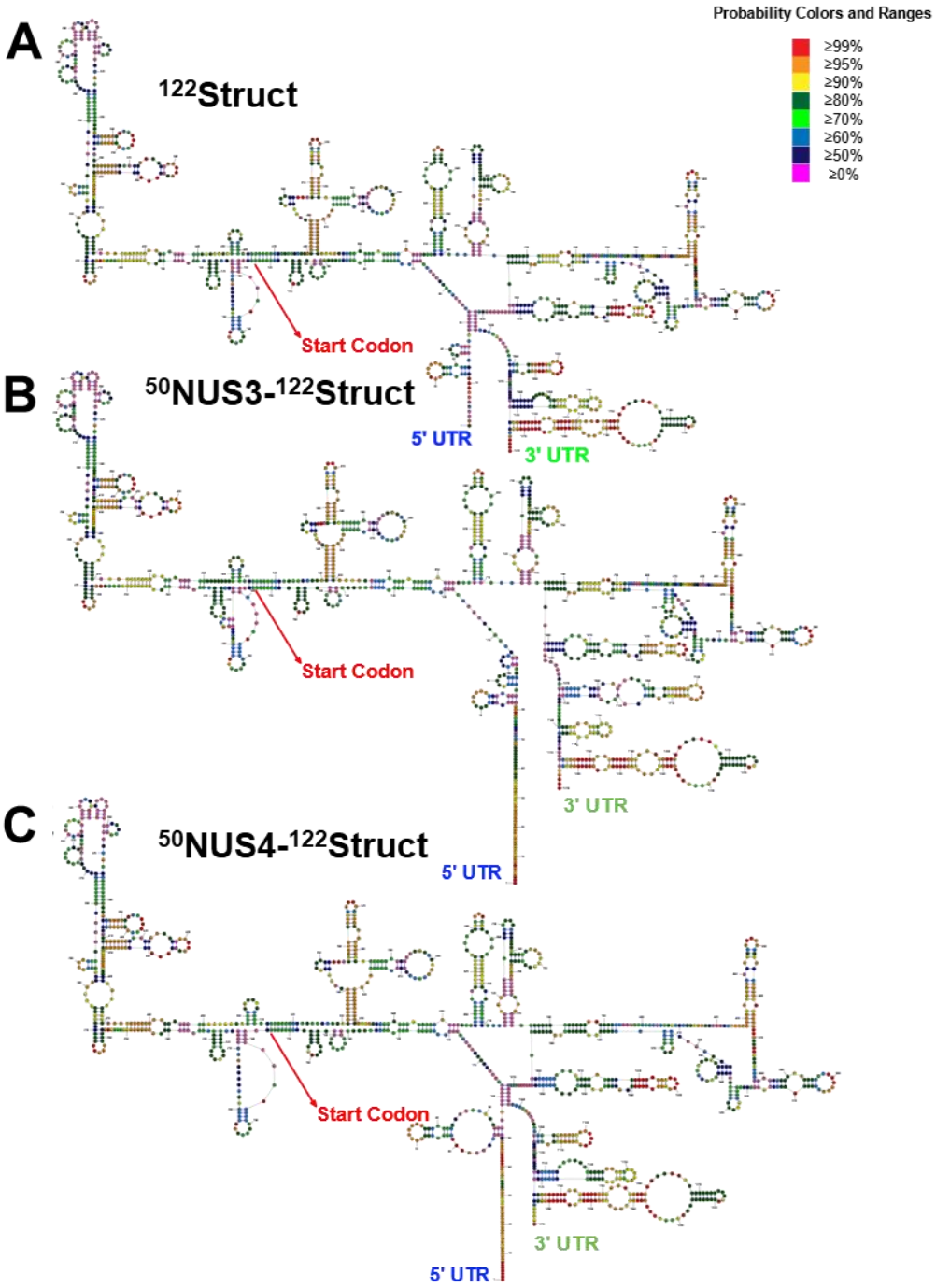

Figure S9. Secondary structures of ^{122}Struct, NUS3-^{122}Struc, NUS4-^{122}Struct GFP mRNA variants. The predicted lowest free energy structure of ^{122}Struct (A), NUS3-^{122}Struc (B), NUS4-^{122}Struct (C) GFP mRNAs. The structures were drawn with *StructureEditor* program from *RNAstructure* software package (https://rna.urmc.rochester.edu/RNAstructure.html). Base pair probabilities, predicted using a partition function, are indicated by the color key.

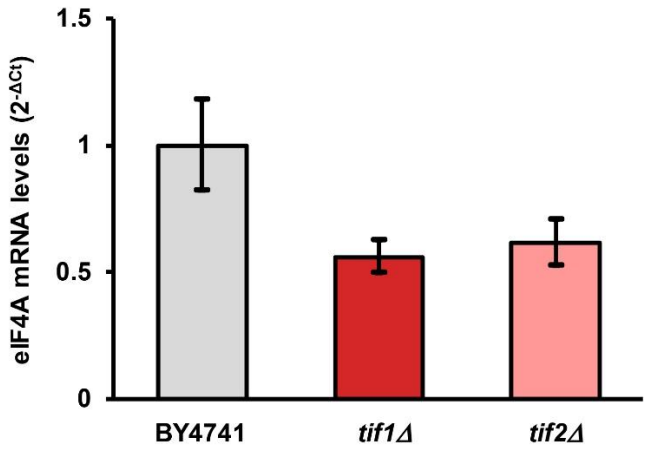

Figure S10. Changes in eIF4A mRNA levels in *tif1*Δ and *tif2*Δ strains relative to those in the wild-type (BY4147) strain, $2^{-\Delta Ct}$, measured by RT-qPCR.

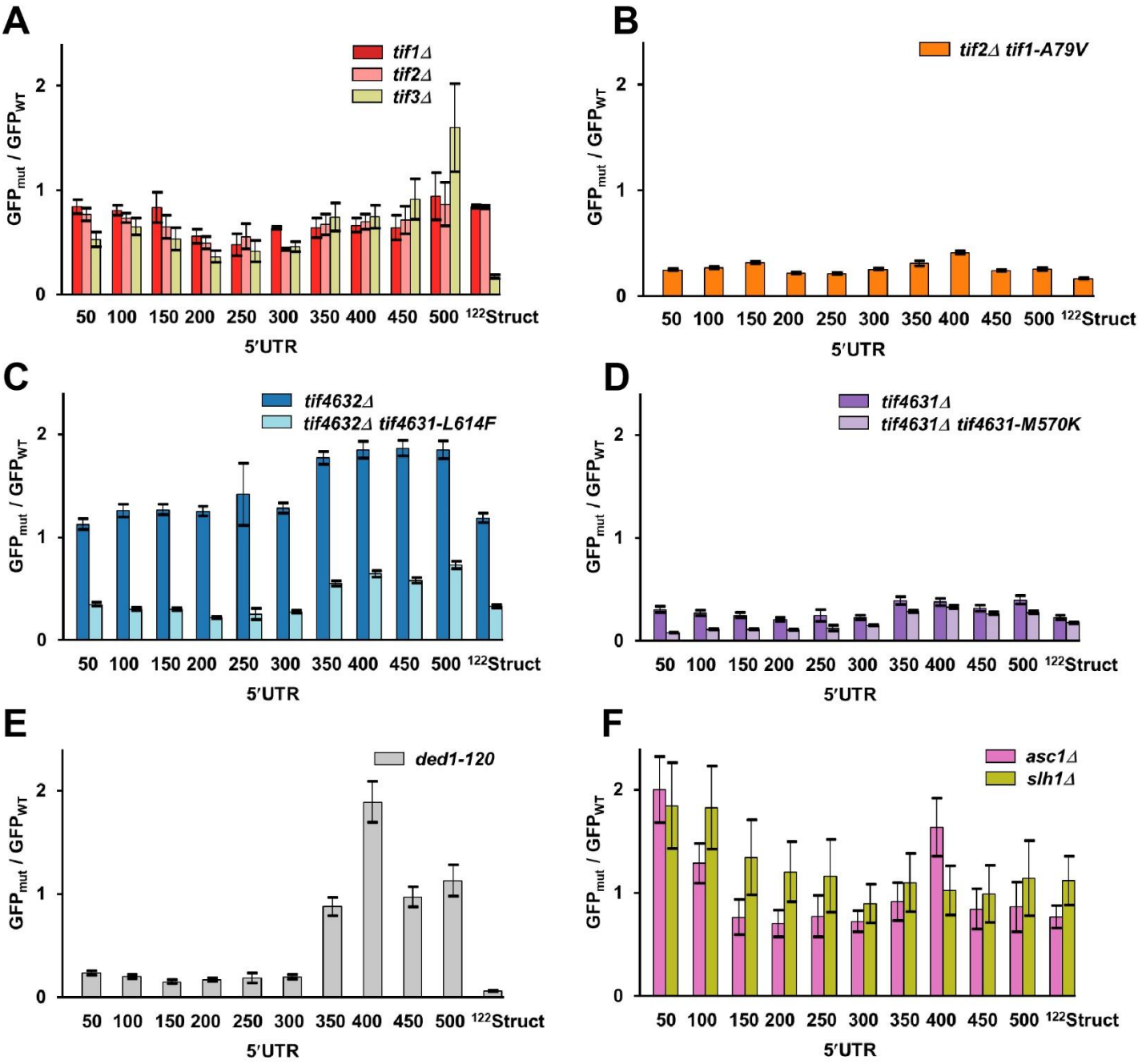

Figure S11. Mutational perturbations of initiation factors similarly reduce translation of GFP mRNAs with short and long unstructured NUS' 5' UTRs. NUS' 5' UTR RNA-ID variants were transformed in yeast strains bearing mutations/deletions of initiation factors as indicated. GFP fluorescence measured in mutant strains normalized to respective GFP fluorescence measured in wild-type strain. Error bars indicate standard deviations determined from three biological replicates. (A) eIF4A-encoding *tif1, tif2* or eIF4B-encoding *tif3* were deleted in BY4741 background as indicated. (B) Either wild-type (*TIF1*) or temperature sensitive (*tif1-A79V*) alleles of eIF4A-encoding *tif1* were expressed in the strain lacking both chromosomal copies of *tif1* and *tif2*. (C) An

amino acid substitution L614F, which reduces eIF4G binding to eIF4A, was introduced into *tif4631* in the background of the *tif4632* deletion to create temperature-sensitive *tif4632Δtif4631-L614F* strain. (D) An amino acid substitution M570K, which reduces eIF4G binding to eIF4A, was introduced into *tif4632* in the background of the *tif4631* deletion to create temperature-sensitive *tif4631Δtif4632-M570K* strain. (E) Amino acid substitutions G108D and G494D were introduced in Ded1-encoding *ded1* to create the *ded1-120* strain. (F) In *slh1Δ* or *asc1Δ* strains, helicase Slh1 and ribosomal protein Asc1, respectively.

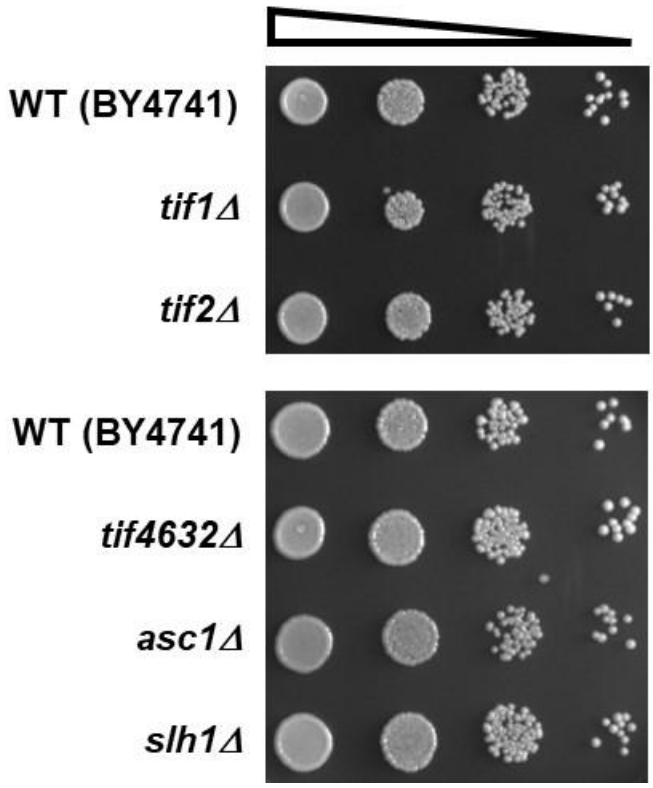

Figure S12. Deletion of *tif1, tif2, tif4632, asc1* or *slh1* does not affect cell growth on YPD plates. Strains were growth on YPD media for 48 hours at 30°C.

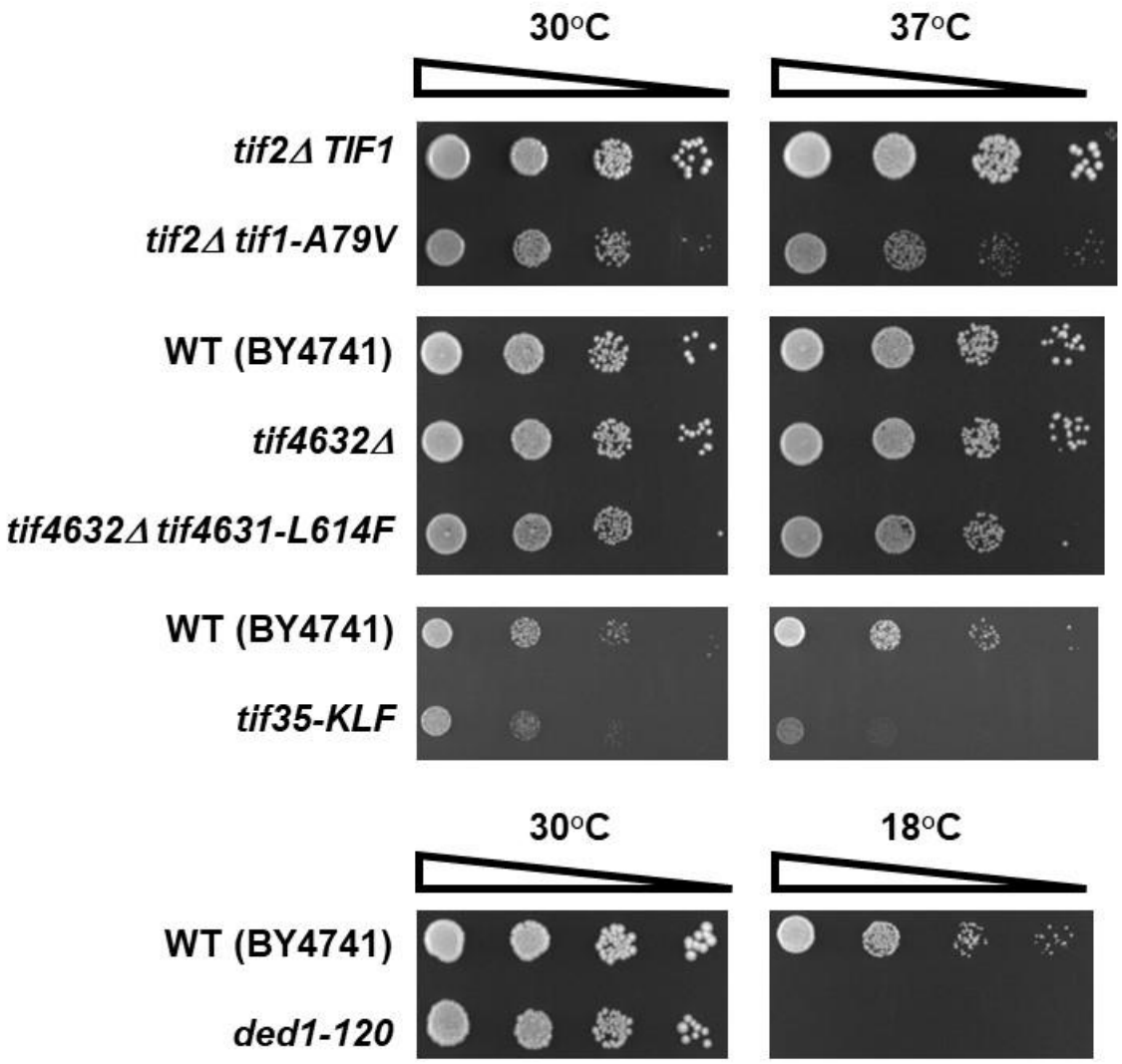

Figure S13. Temperature and cold-sensitive mutations in *tif1*, *tif4631*, *tif35* and *ded1* slowed yeast growth at non-permissive temperatures. Mutant strains were growth for 48 hours on YPD media at 30, 37 or 18°C as indicated.

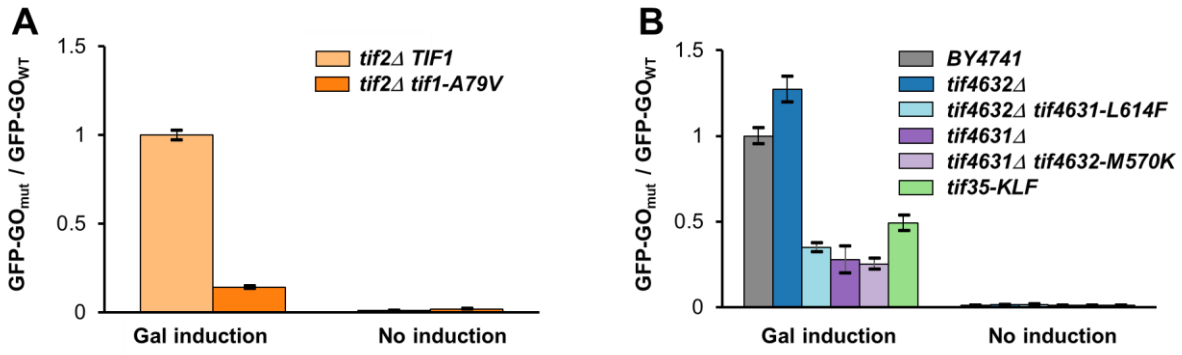

Figure S14. GFP is not synthesized in the absence of galactose. The GFP-GO RNA-ID construct was transformed in yeast strains bearing temperature sensitive mutations or deletions of initiation factors as indicated. GFP fluorescence measured in mutant strains normalized to the respective GFP fluorescence measured in wild-type (*tif2ΔTIF1* in panel (A) or BY4147 in panel (B)) strain. Galactose was added to induce the *Gal1,10* promoter concurrently to switching from 30 to 37°C. Error bars indicate standard deviations determined from three biological replicates.

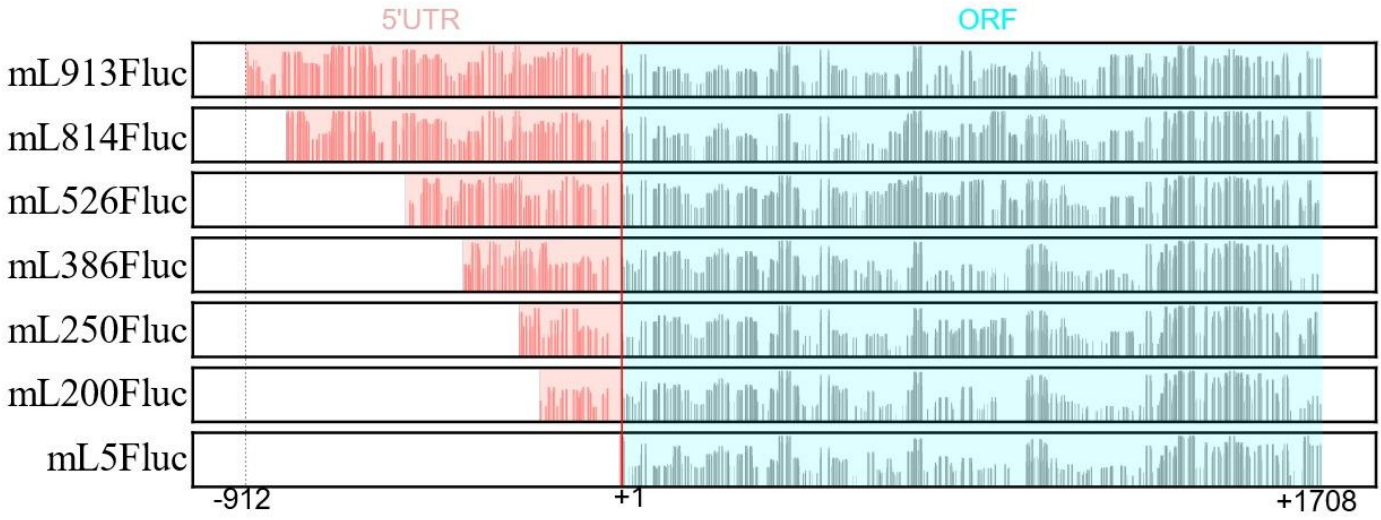

Figure S15. The nucleotide base pairing probability of reporter mRNAs used by Vassilenko et al. (Vassilenko et al. 2011). The base pairing probabilities were estimated with a partition function calculation. The Y axis is the probability of base pairing. The X axis represents the nucleotide position in the mRNA. The 5′ UTR and ORF are highlighted in red and cyan, respectively.

Supplementary Methods

Yeast genomic DNA (gDNA) purification.

Cultured yeast cells on YPD plate were collected and lysed by vortexing for 5 min in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2% Triton-X 100, 1% SDS, 100 mM NaCl with micro-glass beads. The supernatant was collected, and 8-fold excess volume of 100% ethanol was added and incubated for 1 hr at -20°C. After centrifuge at 1330 rpm for 10 min, the supernatant was removed. The precipitate was dissolved in 400 µl 10 mM Tris-HCl, pH 8.0, 1 mM EDTA with 1 µl RNase A (10 mg/mL) and incubated for 5 min at 23°C. The pure genomic DNA was collected by ethanol precipitation.

Cloning of RNA-ID plasmid containing desired sequences at 5′UTR of GFP-ORF.

The original RNA-ID plasmid contains *MET15* sequence for auxotrophic selection (Brule et al. 2016). Since we intended to use different yeast strains (BY4741 and BY4742) and BY4742 contains a native *MET15* gene, we modified the original RNA-ID plasmid by swapping the *MET15* sequence with the *URA3* sequence by Gibson assembly. The *MET15* eliminated RNA-ID DNA was amplified by PCR using the original RNA-ID plasmid as a template DNA and primers (*URA3*-Replace-Vec-F1 and *URA3*-Replace-Vec-R1). Insert *URA3* DNAs were amplified using S288C gDNA as a template DNA. At the same time, we eliminated the StuI site present in the native *URA3* gene with a synonymous mutation codon by doing two separate PCR reactions. Upstream of *URA3* gene was amplified using *URA3*-replace-ins-R1 and StuI-remove-R1 primers. Downstream of *URA3* gene was amplified using *URA3*-replace-ins-F1 and StuI-remove-F1 primers. The *MET15* eliminated linear RNA-ID DNA, upstream of *URA3*, and downstream of *URA3* were combined by Gibson assembly to obtain the RNA-ID-*URA3* plasmid. For further cloning we used this RNA-ID plasmid.

The ^{50}NUS 5′UTR ~ ^{500}NUS 5′UTR containing RNA-ID-plasmid construction was done as follows. We first constructed the RNA-ID plasmid carrying the ^{500}NUS 5′UTR. The ^{500}NUS 5′ UTR DNA fragment containing the 15 bp 3' end of *Gal1,10* promoter at the 5' end and 31 bp 5' end of tagged GFP-ORF sequence at the 3' end was produced by PCR reaction combining 3 synthesized DNA oligo nucleotides with a size of 200 bp in the presence of a forward primer (NR-5UTR-F1) and reverse primers (500NR-5UTR-R1 and 500NR-5UTR-R2). Also, the upstream flanking DNA fragment and downstream flanking DNA fragment were produced by

PCR. Then the upstream DNA, ^{500}NUS 5′UTR DNA, and downstream DNA were combined by Gibson assembly. Assembled DNA was further amplified by PCR using SpeI-F1 and SalI-R1 primers, agarose-gel purified, and digested by SpeI/SalI (^{500}NUS 5′UTR insert DNA). The original RNA-ID plasmid was also digested by SpeI/SalI and the vector part of DNA was agarose-gel purified (RNA-ID vector). Finally, the complete ^{500}NUS 5′UTR-RNA-ID plasmid was generated by ligation of the ^{500}NUS 5′ UTR insert DNA and RNA-ID vector. The obtained plasmid was verified by Sanger sequencing (Genewiz-Azenta). Then we generated the rest of the plasmid (^{50}NUS 5′UTR-RNA-ID ~ ^{450}NUS 5′UTR-RNA-ID) as follows. Upstream and downstream insert DNA fragments were produced by PCR using the ^{500}NUS 5′UTR-RNA-ID plasmid as a template DNA and the primer sets (SpeI-F1/50NR-5UTR-R1 ~ 450NR-5UTR-R1 for upstream DNA and 50NR-5UTR-F1 ~ 450NR-5UTR-F1/SalI-R1 for upstream DNA). These upstream and downstream DNAs were combined by Gibson assembly, digested by SpeI/SalI, and ligated with the SpeI/SalI digested vector part of RNA-ID DNA. The obtained plasmid was verified by Sanger sequencing (Genewiz-Azenta).

The ^{300}NUS-SL 5′UTR containing the RNA-ID-plasmid construction was done as follows. Using the ^{300}NUS RNA-ID plasmid as a template DNA, we performed PCR to amplify an upstream fragment of insert DNA fragment with primers (SpeI-F1, 50SL2-300NR-5UTR-DNA1-R1, and 50SL2-300NR-5UTR-DNA1-R2), and a downstream fragment of insert DNA fragment with primers (Sal1-R1, 50SL2-300NR-5UTR-DNA1-F1, and 50SL2-300NR-5UTR-DNA1-F2). The upstream of insert DNA fragment was digested by SpeI/AflII and the downstream of insert DNA fragment was digested by SalI/AflII. Finally, the digested upstream of insert DNA fragment, the downstream of insert DNA fragment, and the RNAID vector DNA (see above) were ligated to create the ^{300}NUS-SL 5′UTR RNA-ID plasmid. The obtained plasmid was verified by Sanger sequencing (Genewiz-Azenta).

The ^{50}NUS3-^{122}Struct 5′UTR and ^{50}NUS4-^{122}Struct containing the RNA-ID-plasmid construction was done as follows. Upstream and downstream insert DNA fragments were produced by PCR using the ^{122}Struct 5′UTR-RNA-ID plasmid as a template DNA. For producing ^{50}NUS3-^{122}Struct 5′UTR insert DNAs, SpeI-F1 and RNAID-Vec-5'-350-R1 were used to produce the upstream part of the insert DNA. Also, RNAID-Vec-5'-GAPDH-50-F1, GAPDH-50-F2, and SalI-R1 were used to produce the downstream part of insert DNA. For producing ^{50}NUS4-^{122}Struct 5′UTR insert DNAs, SpeI-F1 and GAPDH-50RE-R2 were used to produce the

upstream part of the insert DNA. Also, GAPDH-50RE-F1, GAPDH-50RE-F2, and SalI-R1 were used to produce the downstream part of insert DNA. Then, these upstream and downstream DNAs were combined by Gibson assembly, digested by SpeI/SalI, and ligated with the SpeI/SalI-digested vector part of RNA-ID DNA. The obtained plasmid was verified by Sanger sequencing (Genewiz-Azenta).

Cloning of plasmid for gene mutation.

At first, DNA fragments for Gibson assembly were produced by PCR using the primer-DNA template pairs as listed in **Supplementary Tables 2-12**. Next, the appropriate DNA segment sets and corresponding plasmid vector were combined by Gibson assembly to generate the complete plasmid. The obtained plasmid was verified by Sanger sequencing (Genewiz-Azenta). Purified plasmids were digested by restriction enzymes as indicated in **Supplementary Table 13** and DNA fragments other than the carrier vector part were agarose-gel purified.

SDS-PAGE analysis with fluorescence detection.

Cells were lysed in 1.5 mL tubes containing 200 µL of 50 mM HEPES, pH 7.5, 1 mM EDTA, 0.5% Triton X-100, 1 mM DTT, 10% glycerol, 1 M NaCl, Thermo Scientific Pierce Protease Inhibitor Mini Tablets, EDTA-free (1 tablet in 50 mL, Fisher Scientific), and 1 mM PMSF (Vorvis et al. 2008). The solution was mixed with 300 mg acid-washed Glass Beads (Sigma-Aldrich, St. Louis, MO) and vortexed for 10 min at 4°C. After centrifuging at 13,300 rpm for 10 min, the supernatant was collected. ~20 Abs_{260} of lysate were mixed with equal volume of 2X Laemmli buffer containing beta-mercaptoethanol and loaded onto a 15% SDS-PAGE gel. After running at 150V for 100 min, the gel was visualized by Amersham Typhoon Biomolecule Imager (GE Healthcare, Chicago, IL) with excitation at 488 nm, emission filter set to 525 nm and PMT set to 400V.

RT-qPCR.

RT-qPCR was performed according to the method described previously (Lai et al. 2022) using primers provided in **Supplementary Table 14**. Yeast total RNA was prepared by using formamide-EDTA as described elsewhere (Shedlovskiy et al. 2017). cDNA was synthetized using Superscript II Reverse Transcriptase (Invitrogen). cDNA was amplified using Fast SYBR Green Master Mix (Applied Biosystems), detected using the 7500 Fast Real-Time PCR system, and analyzed with the 7500 Software v2.3 (Applied Biosystems).

To validate TIF1 and TIF2 gene transcription, we used TFC1, TAF10, and ALG9 as reference genes (Teste et al. 2009). The primer pair used to quantify TIF1 or TIF2 transcripts was tif1-2-semiqPCR-F1/ tif1-2-qPCR-R1 and reference primer pairs were TFC1-F1/TFC-R1, TAF10-F1/TAF10-R1, and ALG9-F1/ALG10-R1. Primer pairs used to quantify GFP transcript and reference RFP transcripts were GFP-qPCR-F4/GFP-qPCR-R4 and RFP-qPCR-F5/RFP-qPCR-R5, respectively.

5′RACE.

Approximately 10 µg of yeast total RNA from ^{50}NUS, ^{300}NUS, or ^{500}NUS RNA-ID DNA transformed BY4741 cells were first treated with Calf Intestinal Alkaline Phosphatase (NEB) to dephosphorylate RNAs without cap. Then the cap on the mRNA was removed by mRNA Decapping Enzyme (NEB), leaving a phosphate bound at 5' end. Adaptor RNA was synthesized by IDT. The RNA sequence is 5′-UUCUUCUGAAGAUAAAGCAACAACAACAAGGCAA -3′. Ligation of the adaptor RNA to the samples was performed using T4 RNA ligase I (NEB). Reverse transcription was performed by the method (see RT-qPCR section) and each target cDNA was amplified using a forward primer (HIV-F1) that anneals to the adaptor RNA and a reverse primer that anneals to the GFP-ORF (5RACE-R2, **Supplementary Table 14**). Since some transcription starts not exactly at the expected 5' end of the 5' UTR, we cloned the 5'RACE amplicon into an pSP64 plasmid to validate sequence by Sanger sequencing. In order to clone the 5'RACE amplicon into pSP64 using SacI and HindIII site, we created a SacI site at the 5' end of the 5'RACE amplicon by PCR using a primer set (NR-Lig-SacI-F2-2, NR-Lig-SacI-F3, and 5RACE-R2). There is a HindIII site ~50bp downstream from the 3' end of 5' UTR. After PCR, SacI/HindIII restriction digestion, each purified DNA was ligated with the SacI/HindIII digested pSP64 plasmid. We collected ~10 ligated plasmid clones for each ^{50}NUS, ^{300}NUS, or ^{500}NUS RNA-ID sample and Sanger sequencing was performed at Genewize-Azenta using a pSP64 specific primer (M13R-Hiro).

Spot assay.

A small volume (5 ml) Yeast cells were cultured in YPD medium overnight at 30°C, adjusted concentration at OD_{600}~0.2, and cultured 4 hrs in YPD medium at indicated temperature. Cells were appropriately diluted in

YPD and 5 µl of each dilution was spotted on YPD solid Medium. After the indicated time (1 – 4 days) plates were photographed with a Molecular Imager Gel Doc XR+ system (Bio-Rad, Hercules, CA).

Supplementary Table 1. 5' UTR sequences of GFP mRNA in RNA-ID constructs.

5' UTR	Sequence (5' to 3')	Linguistic complexity†
GO	GCUAGC	0.600
^{50}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAAA	0.0278
^{100}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAAAAA	0.00494
^{150}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAACAAACAACAACAAAACACAAAACACACACAACACACACACAACA CAACAAAAA	0.00226
^{200}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAACAAACAACAACAAAACACAAAACACACACAACACACACACAACA CAACAACAAAAAACACAUACUAAACACAACACAAAAAACACACAACAC ACAACACAAAA	0.00160
^{250}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAACAAACAACAACAAAACACAAAACACACACAACACACACACAACA CAACAACAAAAAACACAUACUAAACACAACACAAAAAACACACAACAC ACAACACACAACAACAAACCCCCACACCCCCCCCCCUACAAAAACACA AAAAACACACAAA	0.00182
^{300}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAACAAACAACAACAAAACACAAAACACACACAACACACACACAACA CAACAACAAAAAACACAUACUAAACACAACACAAAAAACACACAACAC ACAACACACAACAACAAACCCCCACACCCCCCCCCCUACAAAAACACA AAAAACACACACACUACACACAUCAUUACAACAAUCACAACCUCACAC AACCCUAUACCAAAA	0.00216
^{350}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAACAAACAACAACAAAACACAAAACACACACAACACACACACAACA CAACAACAAAAAACACAUACUAAACACAACACAAAAAACACACAACAC ACAACACACAACAACAAACCCCCACACCCCCCCCCCUACAAAAACACA AAAAACACACACACUACACACAUCAUUACAACAAUCACAACCUCACAC AACCCUAUACCAAAACAAAAAACCCCUACACAAAAAACACACACACAA CUACACUAAAUCUCAAA	0.00221
^{400}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAACAAACAACAACAAAACACAAAACACACACAACACACACACAACA CAACAACAAAAAACACAUACUAAACACAACACAAAAAACACACAACAC ACAACACACAACAACAAACCCCCACACCCCCCCCCCUACAAAAACACA AAAAACACACACACUACACACAUCAUUACAACAAUCACAACCUCACAC AACCCUAUACCAAAACAAAAAACCCCUACACAAAAAACACACACACAA CUACACUAAAUCUCACAAACAACAACACAAAACACAACCUACACACAC AAAACACAACCCCUCAAAA	0.00182
^{450}NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAACAAACAACAACAAAACACAAAACACACACAACACACACACAACA CAACAACAAAAAACACAUACUAAACACAACACAAAAAACACACAACAC ACAACACACAACAACAAACCCCCACACCCCCCCCCCUACAAAAACACA	0.00186

	AAAAACACACACACUACACACAUCAUUACAACAAUCACAACCUCACAC AACCCUAUACCAAAACAAAAAACCCCUACACAAAAAACACACACACAA CUACACUAAAUCUCACAAACAACAACACAAAACACAACCUACACACAC AAAACACAACCCCCUCACUAUUAUACACACACAAAAAAUACUAAACAAA AACAAAAAAUAAAAAACAAAA	
[500]NUS1	CACCAAAAAACACACACACAACAUAAAACACAAAAACAAAACAACACC UAAACAACACACACACACACACACACACACACACACACACACAAAACUAC ACAACAAACAACAACAAAACACAAAACACACACAACACACACACAACA CAACAACAAAAAACACAUACUAAACACAACACAAAAAACACACAACAC ACAACACACAACAACAAACCCCCACACCCCCCCCCCUACAAAAACACA AAAAACACACACACUACACACAUCAUUACAACAAUCACAACCUCACAC AACCCUAUACCAAAACAAAAAACCCCUACACAAAAAACACACACACAA CUACACUAAAUCUCACAAACAACAACACAAAACACAACCUACACACAC AAAACACAACCCCCUCACUAUUAUACACACACAAAAAAUACUAAACAAA AACAAAAAAUAAAAAACACACAAAAACCCCCCUACUAAAACAAAAACA AAAAUAAAUAAAAAAUACCCAAA	0.00168
[300]NUS2	ACACACUACACACACCAAACCCAUAAUCACAACACACACAAUUAAAUA CCAACUCUAAACACAAAAACUAUCAUUACACACACCAAAUUUCUCAUA CUACUACUCACUAUUACACAAACACCUCAUUUAUAACAACAACAUUC AUUUCUACAAAACACUCAUCAAACCUUACACAUACAACCCCAAUUAC CUUUUUACCAAUCACAAUACACAAAACCUACUAAACCCCCCAACCCU ACAACAUCUCAUACACACACAAAUACAAACAACACACUUAAACAACAA CAACCCCUAAAACCUAAA	0.0150
[300]NUS2 +SL	ACACACUACACACACCAAACCCAUAAUCACAACACACACAAUUAAAUA CCAACUCUAAACACAAAAACUAUCAUUACACACACCAAAUUUCUCAUA CUACUACUCACUAUUACACAAACACCUCAUUUAUAACAACAACAUUC AUUUCUACGAGCUUGCUUGGGUGAGGGGGCUUAAGCCCUUUCGCC UAGGUGGGCAAAAUCACAAUACACAAAACCUACUAAACCCCCCAACC CUACAACAUCUCAUACACACACAAAUACAAACAACACACUUAAACAAC AACAACCCCUAAAACCUAAA	0.144
[122]Struct	GCUAGCCCCCCGGUUUCUAUAAAUUGAGCCCGCAGCCUCCCGCUUC GCUCUCUGCUCCUCCUGUUCGACAGUCAGCCGCAUCUUCUUUUGC GUCGCCAGCCGAGCCACAUCGCUCAGACACC	0.441
[50]NUS3 - [122]Struct	CAAUAAAAACCUCCCUAAAACCCCAAUACUCCCUACUAAUAAAACCC CCCGCUAGCCCCCCGGUUUCUAUAAAUUGAGCCCGCAGCCUCCCGC UUCGCUCUCUGCUCCUCCUGUUCGACAGUCAGCCGCAUCUUCUUUU GCGUCGCCAGCCGAGCCACAUCGCUCAGACACC	0.373
[50]NUS4 - [122]Struct	CACCCCCUAAAUAAUCACAACCACACUCACAUUUCCUACCCCCCCCA UAAGCUAGCCCCCCGGUUUCUAUAAAUUGAGCCCGCAGCCUCCCGC UUCGCUCUCUGCUCCUCCUGUUCGACAGUCAGCCGCAUCUUCUUUU GCGUCGCCAGCCGAGCCACAUCGCUCAGACACC	0.452
[50]NUS′	CCCAACACUUACCCCCCCUACUCCCCAAAACCCCAUCCAAUACUCCC AAC	0.112
[100]NUS′	AACUACCCCAACAAAAACAAACACUACCCCCCAAUCUCUAUCCCCCA CCCCCCAACACUUACCCCCCCUACUCCCCAAAACCCCAUCCAAUACU CCCAACAAA	0.0351
[150]NUS′	CCCUAAAACAAAACCCCCCAACCAACAAUCCUCAACCUACCAAAACAA ACAACUACCCCAACAAAAACAAACACUACCCCCCAAUCUCUAUCCCC CACCCCCCAACACUUACCCCCCCUACUCCCCAAAACCCCAUCCAAUA CUCCCAACAAA	0.0265
[200]NUS′	ACCCCCAACCAACAAAACACAUAUAACACUCCCUCACAAAUUCCCCC ACACCCUAAAACAAAACCCCCCAACCAACAAUCCUCAACCUACCAAAA CAAACAACUACCCCAACAAAAACAAACACUACCCCCCAAUCUCUAUC	0.0262

	CCCCACCCCCCAACACUUACCCCCCCUACUCCCCAAAACCCCAUCCA AUACUCCCAACAAA	
^{250}NUS′	AACCCAACAACAACAAACAAAAACUCACAACCAAAUCUCACACACAAC CAACCCCCAACCAACAAAACACAUAUAACACUCCCUCACAAAUUCCC CCACACCCUAAAACAAAACCCCCCAACCAACAAUCCUCAACCUACCA AAACAAACAACUACCCCAACAAAAACAAACACUACCCCCCAAUCUCUA UCCCCCACCCCCCCAACACUUACCCCCCCCUACUCCCCCAAAACCCCAUC CAAUACUCCCAACAAA	0.0178
^{300}NUS′	ACAUACCCCACACUCAACCCAUACCUAAAACCCCCACUCAACUCAAA ACCAACCCAACAACAACAAACAAAAACUCACAACCAAAUCUCACACAC AACCAACCCCCAACCAACAAAACACAUAUAACACUCCCUCACAAAUUC CCCCACACCCUAAAACAAAACCCCCCAACCAACAAUCCUCAACCUAC CAAAACAAACAACUACCCCAACAAAAACAAACACUACCCCCCAAUCUC UAUCCCCCACCCCCCCAACACUUACCCCCCCCUACUCCCCCAAAACCCCA UCCAAUACUCCCAACAAA	0.00861
^{350}NUS′	CAAUAAAAACCUCCCUAAAACCCCAAUACUCCCUACUAAUAAAACCC CCCACAUACCCCCACACUCAACCCAUACCUAAAACCCCCACUCAACUC AAAACCAACCCAACAACAACAAACAAAAACUCACAACCAAAUCUCACA CACAACCAACCCCCAACCAACAAAACACAUAUAACACUCCCUCACAAA UUCCCCCACACCCUAAAACAAAACCCCCCAACCAACAAUCCUCAACC UACCAAAACAAACAACUACCCCAACAAAAACAAACACUACCCCCCAAU CUCUAUCCCCCCACCCCCCCAACACUUACCCCCCCCUACUCCCCCAAAACC CCCAUCCAAUACUCCCAACAAA	0.00750
^{400}NUS′	ACAAUACUCCUACAACACAAACAAAAUAAAACACUCACACAAAACAAC UACAAUAAAAACCUCCCUAAAACCCCAAUACUCCCUACUAAUAAAACC CCCCACAUACCCCCACACUCAACCCAUACCUAAAACCCCCCACUCAACU CAAAACCAACCCAACAACAACAAACAAAAACUCACAACCAAAUCUCAC ACACAACCAACCCCCCAACCAACAAAACACAUAUAACACUCCCUCACAA AUUCCCCCCACACCCUAAAACAAAACCCCCCCAACCAACAAUCCUCAAC CUACCAAAACAAACAACUACCCCAACAAAAACAAACACUACCCCCCCAA UCUCUAUCCCCCCACCCCCCCAACACUUACCCCCCCCUACUCCCCCAAAAC CCCAUCCAAUACUCCCAACAAA	0.00652
^{450}NUS′	CCCACACUAACUACUCACAACCACACUCACAUUUCCUACCCCACACA CACACAAUACUCCUACAACACAAACAAAAUAAAACACUCACACAAAAC AACUACAAUAAAAACCUCCCUAAAACCCCAAUACUCCCUACUAAUAAA ACCCCCCCACAUACCCCCACACUCAACCCAUACCUAAAACCCCCCACUCA ACUCAAAACCAACCCAACAACAACAAACAAAAACUCACAACCAAAUCU CACACACAACCAACCCCCCAACCAACAAAACACAUAUAACACUCCCUCA CAAAUUCCCCCCACACCCUAAAACAAAACCCCCCCAACCAACAAUCCUC AACCUACCAAAACAAACAACUACCCCAACAAAAACAAACACUACCCCC CAAUCUCUAUCCCCCCACCCCCCCAACACUUACCCCCCCCUACUCCCCAA AACCCCAUCCAAUACUCCCAACAAA	0.00645
^{500}NUS′	ACAACUAAAACCCACCAAUAACUAAAACAACCACAACAUAUCACUCCU ACCCCACACUAACUACUCACAACCACACUCACAUUUCCUACCCCACA CACACACAAUACUCCUACAACACAAACAAAAUAAAACACUCACACAAA ACAACUACAAUAAAAACCUCCCUAAAACCCCAAUACUCCCUACUAAUA AAACCCCCCACAUACCCCACACUCAACCCAUACCUAAAACCCCCACU CAACUCAAAACCAACCCAACAACAACAAACAAAAACUCACAACCAAAU CUCACACACAACCAACCCCCAACCAACAAAACACAUAUAACACUCCC UCACAAAUUCCCCCACACCCUAAAACAAAACCCCCCAACCAACAAUC CUCAACCUACCAAAACAAACAACUACCCCAACAAAAACAAACACUACC CCCCAAUCUCUAUCCCCCACCCCCCAACACUUACCCCCCCCUACUCCC CAAAACCCCAUCCAAUACUCCCAACAAA	0.00596

^{300}NUS′ +SL	ACAUACCCCACACUCAACCCAUACCUAAAACCCCCACUCAACUCAAA ACCAACCCAACAACAACAAACAAAAACUCACAACCAAAUCUCACACAC AACCAACCCCCAACCAACAAAACACAUAUAACACUCCCUCACAAAUUC CCCCACAAGACGCUUGCUUGUCACCGCUCCUUAAGGAGUGGUGGCA GGUAGGCGAAAAACUACCCCAACAAAAACAAACACUACCCCCCAAUC UCUAUCCCCCACCCCCCAACACUUACCCCCCCUACUCCCCAAAACCC CAUCCAAUACUCCCAACAAA	0.110
^{500}NUS′ +SL	ACAACUAAAACCCACCAAUAACUAAAACAACCACAACAUAUCACUCCU ACCCCACACUAACUACUCACAACCACACUCACAUUUCCUACCCCACA CACACACAAUACUCCUACAACACAAACAAAAUAAAACACUCACACAAA ACAACUAAGACGCUUGCUUGUCACCGCUCCUUAAGGAGUGGUGGCA GGUAGGCGAAAACAUACCCCACACUCAACCCAUACCUAAAACCCCCA CUCAACUCAAAACCAACCCAACAACAACAAACAAAAACUCACAACCAA AUCUCACACACAACCAACCCCCAACCAACAAAACACAUAUAACACUCC CUCACAAAUUCCCCCACACCCUAAAACAAAACCCCCCAACCAACAAU CCUCAACCUACCAAAACAAACAACUACCCCAACAAAAACAAACACUAC CCCCCAAUCUCUAUCCCCCACCCCCCAACACUUACCCCCCCUACUCC CCAAAACCCCAUCCAAUACUCCCAACAAA	0.0662

†Linguistic complexity(Trifonov 1990; Gabrielian and Bolshoy 1999) is defined as:

$$C = \prod_{k=1}^{w} U_k$$

where U_k is the fraction of possible sequences observed for a *k*-mer and *w* is the maximum *k*-mer size. U_k is:

$$U_k = \min[4^k, L - k + 1]$$

where *L* is the sequence length. *w* is adopted based on sequence length with $w=3$ for $L<18$, $w=4$ for $18\leq L<67$, $w=5$ for $67\leq L<260$, $w=6$ for $260\leq L<1029$, and $w=7$ for $L\geq 1029$.

Supplementary Table 2. Primers (DNA oligo nucleotides) used for PCR to generate RNA-ID plasmids with various 5′UTR insert DNAs.

Primer name	Sequence (5' to 3')
500-5UTR-200nt-F1	TCAAATGTAATAAAACACCAAAAAACACACACACAACATAAAACAC AAAAACAAAACAACACCTAAACAACACACACACACACACACACACA CACACACACACAAAACTACACAACAAACAACAACAAAACACAAAAC ACACACAACACACACACAACACAACAACAAAAAACACATACTAAAC ACAACACAAAAAACAC
500-5UTR-200nt-F2	CAAAAAACACATACTAAACACAACACAAAAAACACACAACACACAA CACACAACAACAAACCCCCACACCCCCCCCCCTACAAAAACACAA AAAACACACACACTACACACATCATTACAACAATCACAACCTCACA CAACCCTATACCAAAACAAAAAACCCCTACACAAAAAACACACACA CAACTACACTAAATCTC
500-5UTR-200nt-F3	ACACAAAAAACACACACACAACTACACTAAATCTCACAAACAACAA CACAAAACACAACCTACACACACAAAACACAACCCCTCACTATTAT ACACACACAAAAAATACTAAACAAAAACAAAAAATAAAAAACACACA AAAACCCCCCTACTAAAACAAAAACAAAAATAAATAAAAAATACCCA AAATGTCTACTGAA
NR-5UTR-F1	TCAAATGTAATAAAACACCAAAAAACACAC
500NR-5UTR-F1	ACACAAAAAACACACACACAACTAC
500NR-5UTR-F2	CAAAAAACACATACTAAACACAACAC
500NR-5UTR-R1	CTTCTAACTTCAGTAGACATTTTGGGTATTTTTTATTTATTTTTG
500NR-5UTR-R2	CGTTATTTCTTCTAACTTCAGTAGACATTTTGGG
50NR-5UTR-F1	CAAAACAACACCTAAAAATGTCTACTGAAGTTAGAAGAAATAACG
50NR-5UTR-R1	CTAACTTCAGTAGACATTTTTAGGTGTTGTTTTGTTTTTGTGTTTTAT G
100NR-5UTR-F1	ACACACACAAAACTACACAAAAAATGTCTACTGAAGTTAGAAGAAA TAAC
100NR-5UTR-R1	TAACTTCAGTAGACATTTTTTGTGTAGTTTTGTGTGTGTGTG
150NR-5UTR-F1	CACAACAAAAAATGTCTACTGAAGTTAGAAGAAATAACG
150NR-5UTR-R1	TTCAGTAGACATTTTTTGTTGTGTTGTGTGTGTGTTG
150NR-5UTR-R2	CGTTATTTCTTCTAACTTCAGTAGACATTTTTTGTTGTGTTG
200NR-5UTR-F1	CACACAACACAAAAATGTCTACTGAAGTTAGAAGAAATAAC
200NR-5UTR-R1	GTAGACATTTTTGTGTTGTGTGTTGTGTGTTTTTTG
200NR-5UTR-R2	GTTATTTCTTCTAACTTCAGTAGACATTTTTGTGTTGTGTG
250NR-5UTR-F1	AAAAACACAAAAAACACACAAAATGTCTACTGAAGTTAGAAGAAAT AAC
250NR-5UTR-R1	TAACTTCAGTAGACATTTTGTGTGTTTTTTGTGTTTTTGTAGG
300NR-5UTR-F1	CACAACCCTATACCAAAAATGTCTACTGAAGTTAGAAGAAATAAC
300NR-5UTR-R1	TAACTTCAGTAGACATTTTTGGTATAGGGTTGTGTGAGG
350NR-5UTR-F1	ACACAACTACACTAAATCTCAAAATGTCTACTGAAGTTAGAAGAAA TAAC
350NR-5UTR-R1	TTCTAACTTCAGTAGACATTTTGAGATTTAGTGTAGTTGTGTGTG
400NR-5UTR-F1	ACAACCCCTCAAAAATGTCTACTGAAGTTAGAAGAAATAAC
400NR-5UTR-R1	TTCAGTAGACATTTTTGAGGGGTTGTGTTTTGTG
400NR-5UTR-R2	CGTTATTTCTTCTAACTTCAGTAGACATTTTTGAGGGG
450NR-5UTR-F1	AAAAACAAAAAATAAAAAACAAAAATGTCTACTGAAGTTAGAAGAAA TAAC
450NR-5UTR-R1	TTCAGTAGACATTTTTGTTTTTTATTTTTTGTTTTTGTTTAGTATTTTT TG
SpeI-F1	CCTATTTAAATGGTTGATGGATTACTAGTGG
SalI-R1	GGACAGATTGTGTCGACAGGTAATGG

URA3-Replace-Ins-F1	GGTTTATACATAATTTTACAACTCATTACGCACACTTAGTTTTGCTGGCCGCATCTTC
URA3-Replace-Vec-R1	GAAGATGCGGCCAGCAAAACTAAGTGTGCGTAATGAGTTGTAAAATTATGTATAAACC
URA3-Replace-Vec-F1	CACGTTCCTTATATGTAGCTTTCGACATTGTATGGATGGGGGTAATAGAATTGTATC
URA3-Replace-Ins-R1	GATACAATTCTATTACCCCCATCCATACAATGTCGAAAGCTACATATAAGGAACGTG
StuI-remove-F1	GTAACAAAGGAACCTAGAGGTCTTTTGATGTTAGCAG
StuI-remove-R1	CTGCTAACATCAAAAGACCTCTAGGTTCCTTTGTTAC
50SL2-300NR-5UTR-DNA1-R1	AGCCCCCTCACCCAAGCAAGCTCGTAGAAATGAATGTTGTTGTTATAAATGAGGTGTTTG
50SL2-300NR-5UTR-DNA1-R2	TTTCGCATCTTAAGCCCCCTCACC
50SL2-300NR-DNA2 -F1	CGAAACTTAAGCCCTTTCGCCTAGGTGGGCAAAATCACAATACACAAAACCTACTAAACC
50SL2-300NR-DNA2-F2	ATGCGAAACTTAAGCCCTTTCGCC
RNAID-Vec-5'-350-R1	TTGGGGTTTTAGGGAGGTTTTTATTGTTTTATTACATTTGAATAAGAAGTAATACAAACC
RNAID-Vec-5'-GAPDH-50 -F1	AAAACCCCAATACTCCCTACTAATAAAACCCCCCGCTAGCCCCCCGGTTTCTATAAATTG
GAPDH-50-F2	AAAACCCCAATACTCCCTACTAATAAAACCC
GAPDH-50RE-R2	GGGTGTTTTATTACATTTGAATAAGAAGTAATACAAACCGAAAATG
GAPDH-50RE-F1	ACAACCACACTCACATTTCCTACCCCCCCCCATAAGCTAGCCCCCCGGTTTCTATAAATTG
GAPDH-50RE-F2	TTATTCAAATGTAATAAAACACCCCCTAAATAATCACAACCACACTCACATTTCCTACCC

Supplementary Table 3. Primers used to generate gene deletion strain

Primer name	**Sequence (5' to 3')**	**Amplicon used for**
tif1-5UTR-F1	GTAGAGTAAACTTCCACGCACATATTAGG	tif1 deletion
tif1-3UTR-R1	CTCAACCATCCCAAACCTCGAG	tif1 deletion
tif2-5UTR-F1	GCCTGATACCTGATGCCATCCC	tif2 deletion
tif2-3UTR-R1	CAGCTAGTTACTCTTCACACACAAAG	tif2 deletion
tif3-5UTR-F1	GATTAGTACCAGATTGAGCTCAGC	tif3 deletion
tif3-3UTR-R1	TGCTGCAGTAACCTTCTTTGAAGG	tif3 deletion
tif4632-5UTR-F1	CTGTCGCCAAGCTGTTCAAG	tif4632 deletion
tif4632-3UTR-R1	CCAGAATACGTAAGCGTTCTGGATTC	tif4632 deletion
Tif-1-2-semiqPCR-F2	CACCATCATGAAGGAATTCAGAAGTGG	tif1 or tif2 deletion confirmation
Tif-1-2-semiqPCR-R2	GGATGGCAATTCTTCAATTTGAGTGGAG	tif1 or tif2 deletion confirmation
tif3-F1	ATGGCTCCACCAAAGAAAACC	tif3 deletion confirmation
tif3-R2	CTATTTCTTACCAACAACTTCCCAATTGTC	tif3 deletion confirmation
tif4632-F1	CCACACCCGCAGCAAGCC	tif4632 deletion confirmation

tif4632-R2	TAATCACTGTCCCCATCGTTATTCATTAA TG	tif4632 deletion confirmation

Supplementary Table 4. Primers used to generate mutant strains

No.	Primer name	Sequence (5' to 3')
1	Vec-DED1-site-F1	CAAGCTTGTCGACGGAGCTCGTAATGCATCATT CTATACGTGTCATTCTG
2	Vec-DED1-site-R1	CAGAATGACACGTATAGAATGATGCATTACGAG CTCCGTCGACAAGCTTG
3	DED1-TGA-3UTR-F1	GTTCAAACAACTCTTCTTGGTGGTGATTTCAGA CAAACTAGGGTGAGGATTC
4	DED1-TGA-3UTR-R1	GAATCCTCACCCTAGTTTGTCTGAAATCACCAC CAAGAAGAGTTGTTTGAAC
5	DED1-3UTR-KanMX-F1	CCTGTATATTCGTTTTTGAATATACTTTGTTCCC AGCTTGCCTCGTCCCC
6	DED1-3UTR-KanMX-R1	GGGGACGAGGCAAGCTGGGAACAAAGTATATT CAAAAACGAATATACAGG
7	KanMX500-vec-F1	GGTGCGACAATCTATCGATTGTATGGCTAGCCA TATGTATATCTCCTTCTTAAAG
8	KanMX500-vec-R1	CTTTAAGAAGGAGATATACATATGGCTAGCCAT ACAATCGATAGATTGTCGCACC
9	Vec-KanMX-up-F1	CAAGCTTGTCGACGGAGCTCAGCTTGCCTCGT CCCC
10	Vec-KanMX-up-R1	GGGGACGAGGCAAGCTGAGCTCCGTCGACAAG CTTG
11	KanMX-3UTR500-F1	CCGCCATCCAGTGTCGATTTCAGACAAACTAGG GTGAGGATTC
12	KanMX-3UTR500-R1	GAATCCTCACCCTAGTTTGTCTGAAATCGACAC TGGATGGCGG
13	3UTR500-vec-F1	GTATTCATTTCCTTCCTACATTTTCTCTCCGCTA GCCATATGTATATCTCCTTCTTAAAG
14	3UTR500-vec-R1	CTTTAAGAAGGAGATATACATATGGCTAGCGGA GAGAAAATGTAGGAAGGAAATGAATAC
15	Vec-TIF4631-site-F1	CTTGGGCTGCAGGTCGACGACTCGATAACGAC GTGAGAAACG
16	Vec-TIF4631-site-R1	CGTTTCTCACGTCGTTATCGAGTCGTCGACCTG CAGCCCAAG
17	TIF4631-5UTR-ORF-F1	CAAGGTAAGAGGACAACTGTAATTACCTATTAC AATAATGACAGACGAAACTGCTCACCC
18	TIF4631-5UTR-ORF-R1	GGGTGAGCAGTTTCGTCTGTCATTATTGTAATA GGTAATTACAGTTGTCCTCTTACCTTG
19	TIF4631-TAA-3UTR-F1	GGCTCCTCCTCCAAAGGAAGAACCAGCTGCAC CAACTTCTACCG
20	TIF4631-TAA-3UTR-R1	CGGTAGAAGTTGGTGCAGCTGGTTCTTCCTTTG GAGGAGGAGCC
21	TIF4631-3UTR-HgrB500-F1	CTTCGCCAGTCTTCGTTTACGGACATGGAGGCC CAG
22	TIF4631-3UTR-HgrB500-R1	CTGGGCCTCCATGTCCGTAAACGAAGACTGGC GAAG
23	HgrB500-vec-F2	GTGGATATGTCCTGCGGGTAAATGGATCCCCG GGCGAGCTCC

24	HgrB500-vec-R2	GGAGCTCGCCCGGGGATCCATTTACCCGCAGGACATATCCAC
25	Vec-HgrB_sall-up-F1	CTTGGGCTGCAGGTCGACGACATGGAGGCCCAG
26	Vec-HgrB_sall-up-R1	CTGGGCCTCCATGTCGTCGACCTGCAGCCCAAG
27	HgrB-TIF4631-3UTR500-F1	GAATGCTGGTCGCTATACTGCAGCTGCACCAACTTCTACCG
28	HgrB-TIF4631-3UTR500-R1	CGGTAGAAGTTGGTGCAGCTGCAGTATAGCGACCAGCATTC
29	TIF4631-3UTR500-BamHI-vec-F1	CAGAGGCCACGAAAGGAAAGGGGATCCCCGGGCGAGCTCC
30	TIF4631-3UTR500- BamHI-vec-R1	GGAGCTCGCCCGGGGATCCCCTTTCCTTTCGTGGCCTCTG
31	Tif34Mut-Vec-ORF-F1	GGGCTGCAGGTCGACTCTAGACAAATTATGGGATGTGTCAAACGG
32	Tif34Mut-Vec-ORF-R1	CCGTTTGACACATCCCATAATTTGTCTAGAGTCGACCTGCAGCCC
33	Tif34Mut-Mut-F2	GGAATTCATTATTCTTGGTGGTGGTCGAGAGGCCAAGG
34	Tif34Mut-Mut-R2	GGTGGTGACATCCTTGGCCTCTCGACCACC
35	Tif34Mut-3UTR1-HgrB500-F1	CCCTTCGAGGTAATCTTCCGGTGGACATGGAGGCCCAG
36	Tif34Mut-3UTR1-HgrB500-R1	CTGGGCCTCCATGTCCACCGGAAGATTACCTCGAAGGG
37	pSP64-XbaI-HgrB-F1	GGGCTGCAGGTCGACTCTAGAGACATGGAGGCCCAG
38	pSP64-XbaI -HgrB-R1	CTGGGCCTCCATGTCTCTAGAGTCGACCTGCAGCCC
39	HgrB-Tif34-3UTR500-F1	GAATGCTGGTCGCTATACTGAGATCTTCCCTGTATGCAGTTCC
40	HgrB-Tif34-3UTR500-R1	GGAACTGCATACAGGGAAGATCTCAGTATAGCGACCAGCATTC
41	Tif34-3UTR500-pSP64-BamHI-F1	GAGGTTGTGTTATATAAGACAACGCTCGGATCCCCGGGCGAGCTCC
42	Tif34-3UTR500-pSP64-BamHI-R1	GGAGCTCGCCCGGGGATCCGAGCGTTGTCTTATATAACACAACCTC
43	Tif35Mut-Vec-ORF-F1	GGGCTGCAGGTCGACTCTAGACATACAAGATTGAAGACGGTGTCAAG
44	Tif35Mut-Vec-ORF-R1	CTTGACACCGTCTTCAATCTTGTATGTCTAGAGTCGACCTGCAGCCC
45	Tif35Mut-Mut1-F1	CGTGATGATATGTGTACTTTGGCGATTATGCAAGTTAATG
46	Tif35Mut-Mut1-R1	CATTTTCATTAACTTGCATAATCGCCAAAGTACACATATC
47	Tif35Mut-Mut2-F1	CAAGAGGTGCCGCCGCTGTTACCTTTTCGAG
48	Tif35Mut-Mut2-R1	CTCGAAAAGGTAACAGCGGCGGCACCTCTTG
49	Tif35Mut-3UTR1-HgrB500-F1	CAAAGCAAGGAAAAGCTCAGAGGACATGGAGGCCCAG
50	Tif35Mut-3UTR1-HgrB500-R1	CTGGGCCTCCATGTCCTCTGAGCTTTTCCTTGCTTTG
51	HgrB-Tif35-3UTR500-F1	GAATGCTGGTCGCTATACTGGCTGTCGTGAATATGTCAAATACCG

52	HgrB-Tif35-3UTR500-R1	CGGTATTTGACATATTCACGACAGCCAGTATAG CGACCAGCATTC
53	Tif35-3UTR500-pSP64-BamHI-F1	CTCGTTAAAGGAACTGTTGGTAGAATACCGGAT CCCCGGGCGAGCTCC
54	Tif35-3UTR500-pSP64-BamHI-R1	GGAGCTCGCCCGGGGATCCGGTATTCTACCAA CAGTTCCTTTAACGAG

Supplementary Tables 5-11. Primers/template DNA combination for the PCR reaction that were used to produce fragment DNAs for Gibson assembly. Primer sequences are shown in Supplementary Table 3.

Table 5. Gibson assembly fragments used to generate pET24B-ded1-120-KanMX500.

DNA fragment to produce	Forward primer No.	Reverse primer No.	Template DNA
5′UTR-ORF	1	4	gDNA from H5114-NSY5
3′UTR	3	6	gDNA from H5114-NSY5
5′end of hphMX	5	8	gDNA from H5120-NSY20
Carrier vector	7	2	plasmid (pET24b)

Supplementary Table 6. Gibson assembly fragments used to generate pET24B-KanMX-ded1-120-3UTR500.

DNA fragment to produce	Forward primer No.	Reverse primer No.	Template DNA
hphMX	9	12	gDNA from H5120-NSY20
5′end of 3′UTR	11	14	gDNA from H5114-NSY5
Carrier vector	13	10	plasmid (pET24b)

Supplementary Table 7. Gibson assembly fragments used to generate pSP64-tif4631-L614F-HgrB500.

DNA fragment to produce	Forward primer No.	Reverse primer No.	Template DNA
5′UTR	15	18	gDNA from BY4741
ORF	17	20	plasmid (B5608-pEP245)
3′UTR	19	22	gDNA from BY4741
5′end of hphMX	21	24	gDNA from H5120-NSY20
Carrier vector	23	16	plasmid (pSP64)

Supplementary Table 8. Gibson assembly fragments used to generate pSP64-tif4631-HgrB-3UTR500.

DNA fragment to produce	Forward primer No.	Reverse primer No.	Template DNA
hphMX	25	28	gDNA from H5120-NSY20
5′end of 3′UTR	27	30	gDNA from BY4741
Carrier vector	29	26	plasmid (pSP64)

Supplementary Table 9. Gibson assembly fragments used to generate pSP64-tif34-Q258R-HgrB500.

DNA fragment to produce	Forward primer No.	Reverse primer No.	Template DNA
5′UTR-ORF1	31	34	gDNA from BY4741
ORF2-3′UTR	33	36	gDNA from BY4741
5′end of HgrB	35	24	gDNA from H5120-NSY20
Carrier vector	23	32	plasmid (pSP64)

Supplementary Table 10. Gibson assembly fragments used to generate pSP64-tif34-HgrB-3UTR500.

DNA fragment to produce	Forward primer No.	Reverse primer No.	Template DNA
hphMX	37	40	gDNA from H5120-NSY20
5′end of 3′UTR	39	42	gDNA from BY4741
Carrier vector	41	38	plasmid (pSP64)

Supplementary Table 11. Gibson assembly fragments used to generate pSP64-tif35-KLF-HgrB500.

DNA fragment to produce	Forward primer No.	Reverse primer No.	Template DNA
ORF1	43	46	gDNA from BY4741
ORF2	45	48	gDNA from BY4741
ORF3-3′UTR	47	50	gDNA from BY4741
5′end of HgrB	49	24	gDNA from H5120-NSY20
Carrier vector	23	44	plasmid (pSP64)

Supplementary Table 12. Gibson assembly fragments used to generate pSP64-tif35-HgrB-3UTR500.

DNA fragment to produce	Forward primer No.	Reverse primer No.	Template DNA
hphMX	37	52	gDNA from H5120-NSY20
5′end of 3′UTR	51	54	gDNA from BY4741
Carrier vector	53	38	plasmid (pSP64)

Supplementary Table 13. Plasmids used for transformation to generate yeast mutant strains.

Mutant strain to generate	Plasmid	Restriction enzymes	Yeast strain to transform
ded1-120	pET24B-ded1-120-KanMX500 and pET24B-KanMX-ded1-120-3UTR500	SacI/NdeI	BY4741
tif4632 deletion + tif4631-L614F	pSP64-tif4631-L614F-HgrB500 and pSP64-tif4631-HgrB-3UTR500	SalI/BamH1	Δtif4632
tif34-Q258R	pSP64-tif34-Q258R-HgrB500 and pSP64-tif34-HgrB-3UTR500	XbaI/BamH1	BY4741
tif35-KLF	pSP64-tif35-KLF-HgrB500 and pSP64-tif35-HgrB-3UTR500	XbaI/BamH1	BY4741

Plasmids were digested by restriction enzymes indicated and linear DNAs were agarose-gel purified and used for transformation.

Supplementary Table 14. Primers used for RT-qPCR and 5′RACE

Primer name	Sequence (5' to 3')
tif1-2-semiqPCR-F1	CGAAGAACCATCTGCCATTCAACAACG
tif1-2-qPCR-R1	CCGGTCTTACCAGTACCAGATTGAGC
TFC-F1	GCTGGCACTCATATCTTATCGTTTCACAATGG
TFC-R1	GAACCTGCTGTCAATACCGCCTGGAG
TAF10-F1	ATATTCCAGGATCAGGTCTTCCGTAGC
TAF10-R1	GTAGTCTTCTCATTCTGTTGATGTTGTTGTTG
ALG9-F1	CACGGATAGTGGCTTTGGTGAACAATTAC
ALG9-R1	TATGATTATCTGGCAGCAGGAAAGAACTTGGG
GFP-qPCR-F4	ATGGCCCTGTCCTTTTACCAGA
GFP-qPCR-R4	AAGGACCATGTGGTCACG
RFP-qPCR-F5	TAGCAGTTTGAGTACCTTCATATGG
RFP-qPCR-R5	GTTCATATGGAAGGTTCAGTTAATGG
HIV-F1	CTTCTGAAGATAAAGCAACAACAACAAG
5RACE-R2	CTCTCCACGGACAGAAAATTTGTG
NR-Lig-SacI-F2-2	TTAACTATGAGCTCCTTCTGAAGATAAAGCAACAACAACAAG
NR-Lig-SacI-F3	TTAACTATGAGCTCCTTCTGAAGATAAAG
M13R-Hiro	CACACAGGAAACAGCTATGACATG

References:

Brule CE, Dean KM, Grayhack EJ. 2016. RNA-ID, a Powerful Tool for Identifying and Characterizing Regulatory Sequences. *Methods Enzymol* **572**: 237-253.

Gabrielian A, Bolshoy A. 1999. Sequence complexity and DNA curvature. *Comput Chem* **23**: 263-274.

Lai WC, Zhu M, Belinite M, Ballard G, Mathews DH, Ermolenko DN. 2022. Intrinsically Unstructured Sequences in the mRNA 3' UTR Reduce the Ability of Poly(A) Tail to Enhance Translation. *J Mol Biol* **434**: 167877.

Shedlovskiy D, Shcherbik N, Pestov DG. 2017. One-step hot formamide extraction of RNA from Saccharomyces cerevisiae. *RNA Biol* **14**: 1722-1726.

Teste MA, Duquenne M, Francois JM, Parrou JL. 2009. Validation of reference genes for quantitative expression analysis by real-time RT-PCR in Saccharomyces cerevisiae. *BMC Mol Biol* **10**: 99.

Trifonov EN. 1990. Making sense of the human genome. In *Structure and methods*, Vol 1 (ed. RH Sarma, MH Sarma), pp. 69-77. Adenine Press.

Vassilenko KS, Alekhina OM, Dmitriev SE, Shatsky IN, Spirin AS. 2011. Unidirectional constant rate motion of the ribosomal scanning particle during eukaryotic translation initiation. *Nucleic Acids Res* **39**: 5555-5567.

Vorvis C, Markus SM, Lee WL. 2008. Photoactivatable GFP tagging cassettes for protein-tracking studies in the budding yeast Saccharomyces cerevisiae. *Yeast* **25**: 651-659.