SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIALS AND METHODS

Construction of plasmids and yeast strains Strain YMP43 carrying chromosomal deletion of *GCN4* gene together with its 5´ UTR was constructed in the following steps. (i) Primers VM4 (it is a composite primer, the 5' part of which base pairs with the sequence corresponding to the extreme 5' UTR of the *GCN4* gene [far upstream of all RPEs] and the discontinuous 3' part of which base pairs with the sequence corresponding to the very beginning of the *GCN4::KanMx* cassette) and VM25 (base pairing with the very end of the *GCN4::KanMx* cassette) were used to PCR amplify a DNA fragment containing the *GCN4::KanMx* cassette using the genomic DNA isolated from the Euroscarf strain BY4741 *gcn4Δ* as the template. (ii) The purified PCR product was then used to transform H2880 to delete the *GCN4* gene and its entire 5' UTR and the resulting transformants were selected for the G418 resistance. The deletion of *GCN4* and its 5' UTR was verified by PCR. KanMX was then replaced with *HisG-URA-HisG* by transforming the BglII-EcoRI ~4.6 kb fragment from pAG25-*kanMX::HisG-URA-HisG* (pMP85). Uracil auxotrophy was regained by growing the cells on 5- FOA plates.

The strain YMP47 carrying a triple deletion of *GCN4* (includes its 5'-UTR), *TIF4631* and *TIF4632* genes was created in the following steps: (i) The strain YMP43 was transformed with the PCR amplified *tif4632::KanMX* DNA fragment using the primers MP224, MP227 and the genomic DNA isolated from the Euroscarf strain BY4741 *tif4632Δ* as the template. The resulting transformants were selected for the G418 resistance. The deletion of *TIF4632* was verified by PCR. K*anMX* was then replaced with *HisG-URA-HisG* as described above to generate YMP44. (ii) YMP44 was then transformed with pBAS2004 (*pTIF4632 URA3 CEN4*), subsequently with the PCR amplified *tif4631::KanMX* DNA fragment using the primers MP220, MP223 and the genomic DNA isolated from the Euroscarf strain BY4741 *tif4631Δ* as the template. The resulting transformants were selected for the G418 resistance. The deletion of *TIF4631* was verified by PCR.

The strain YMP61 carrying a triple deletion of *GCN4* (includes its 5'-UTR), *TIF1*, *TIF2* genes was created in the following steps: (i) The strain YMP43 was transformed with the PCR amplified *tif2::KanMX* DNA fragment using the primers MP156, MP157 and the genomic DNA isolated from the Euroscarf strain BY4741 *tif2Δ* as the template. The resulting transformants were selected for the G418 resistance. The deletion of *TIF2* was verified by PCR. *KanMX* was then replaced with *HisG-URA-HisG* as described above to generate YMP59. (ii) YMP59 was then transformed with p533 (*yEp195-TIF2-URA3*), subsequently with the PCR amplified *tif1::KanMX* DNA fragment using the primers MP153, MP154 and the genomic DNA isolated from the Euroscarf strain BY4741 *tif1Δ* as the template. The resulting transformants were selected for the G418 resistance. The deletion of *TIF1* was verified by PCR.

The strain YMP65 carrying a double deletion of *GCN4* (includes its 5'-UTR), *TIF45* genes was constructed by transforming the strain YMP43 first with pMP95 (*yEp195-TIF45-URA3*), subsequently with the PCR amplified *tif45::KanMX* cassette using the primers MP156, MP157 and the genomic DNA isolated from the Euroscarf strain BY4741 *tif45Δ* as the template. The resulting transformants were selected for the G418 resistance. The deletion of *TIF45* was verified by PCR.

To generate the yeast strains YMP52, YMP53, YMP55, YMP56, YMP57 and YMP91 the yeast strain YVM47 was first transformed with the plasmids pMP82, pMP83, pMP89, pMP90, pMP91, pMP124, respectively, and the uracil auxotrophy was regained by growing the cells that have evicted the original YEp-*TIF4632-URA* on SD plates containing 5-fluoroorotic acid.

To generate the yeast strains YMP63, YMP67 the yeast strains YVM61, YMP65 were first transformed with the plasmids pMP93, pMP97, respectively, and the uracil auxotrophy was regained by growing the cells that have evicted the original *URA* plasmids on SD plates containing 5-fluoroorotic acid.

The plasmids described below were produced with the help of PCR using the indicated primers and DNA templates: pMP82: MP1 / MP2 and pBAS2078; for pMP83: MP150 / MP2 and pBAS2078; for pMP91: MP294 / MP295 and pMP83; for pMP95: MP314 / MP315 and the genomic DNA of BY4741 *gcn4Δ;* for pMP124: MP307 / MP295 and pMP90. The resulting PCR products were digested with SalI & SacI (for pMP82), BamHI & SalI (for pMP83), BmgBI & NheI (for pMP91) and XmaI & XbaI (for pMP95), NheI & MscI (pMP124) ligated into equally digested p349 and p350, pMP90, respectively.

The plasmids described below were produced with the help of fusion PCR using the indicated two to four pairs of primers and DNA templates: for pMP93: (i) MP2 / MP309 and p544, (ii) MP158 / MP308 and p544; for pMP97: (i) MP314 / MP318 and pMP95 (ii) MP315 / MP319 and pMP95; for pMP90 (i) MP307 / MP305 and pMP83 (ii) MP306 / MP303 and pMP83 (iii) MP304 / MP1 and pMP83; for pMP89 (i) MP296 / MP301 and pMP83, (ii) MP302 / MP299 and pMP83, (iii) MP300 / MP297 and pMP83 and (iv) MP298 / MP1 and pMP83. Thus obtained PCR products were used in a 1:1 ratio as templates for a third PCR amplification with primers MP2 / MP58 (for pMP93), MP314 / MP315 (for pMP97), MP1 / MP307 (for pMP90) and MP1 / MP296 (for pMP89). The resulting PCR products were cleaved with AgeI and SphI (for pMP93), XmaI & XbaI (for pMP97) and SacI & MscI (for pMP90), NheI & SacI (for pMP89) and ligated into equally digested p544, p349 and pMP83, respectively.

pMP85 was created by inserting the KpnI and SalI digested pMP42 into p471 digested with the same enzymes.

pMP98 was created by inserting the SpeI digested pMP80 into pMP96 digested with the same enzyme.

pMP122 and pMP123 were created by inserting the SalI & BstEII digested custom-made synthetic double-stranded DNA fragments (Invitrogen™ GeneArt™ Strings™ DNA fragments) into pMP29 digested with the same enzymes.

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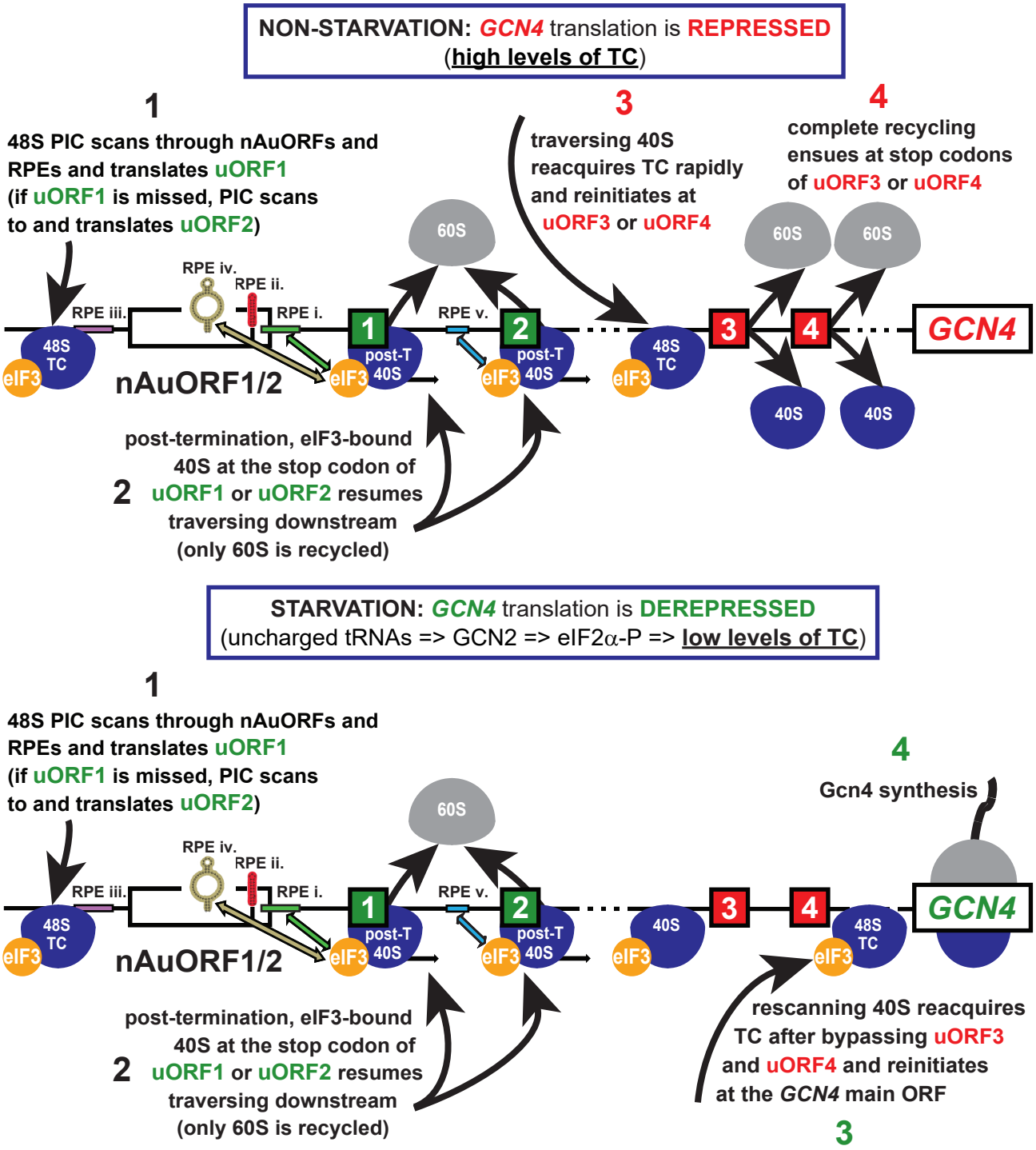
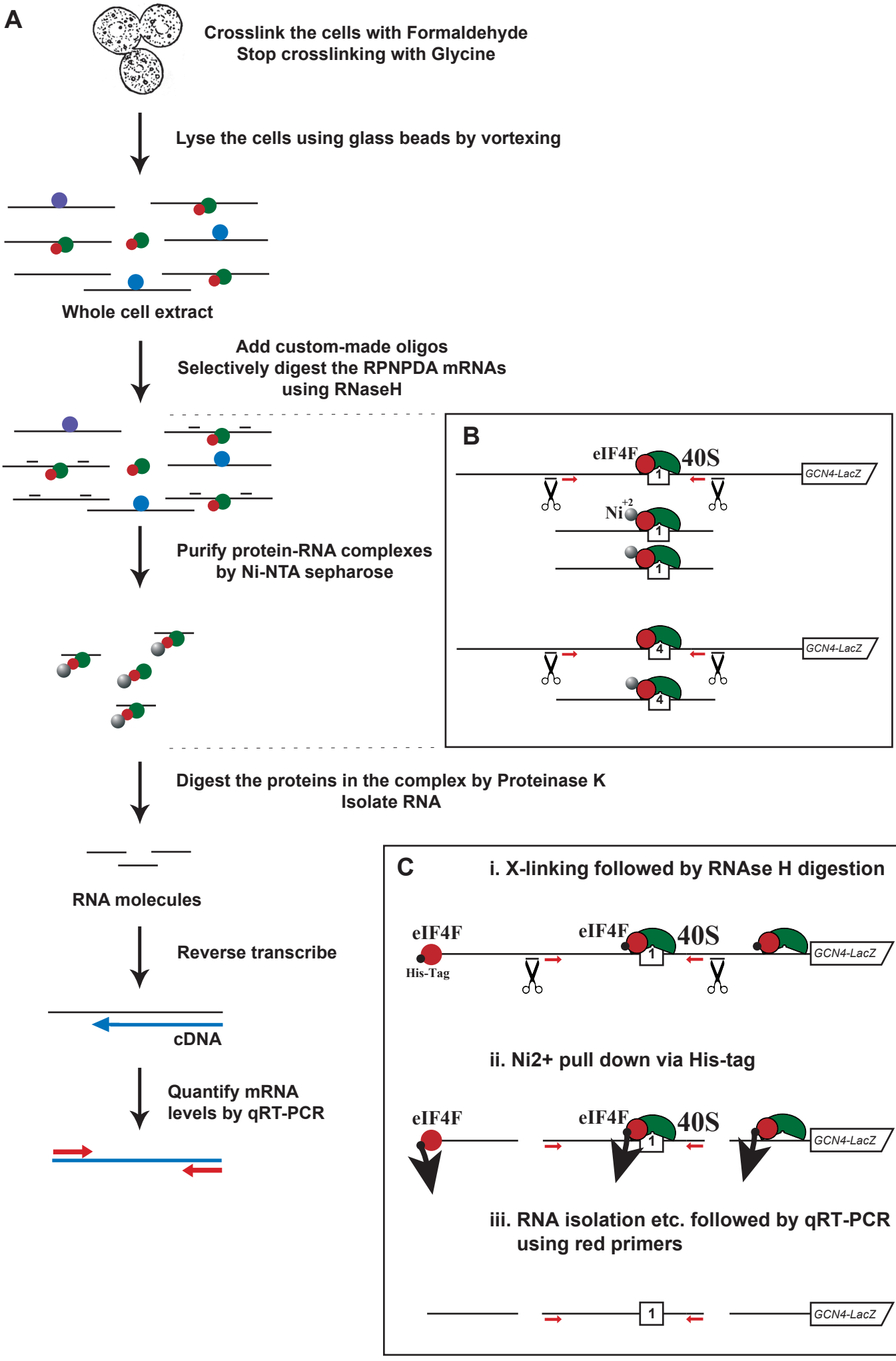
SUPPLEMENTARY FIGURES

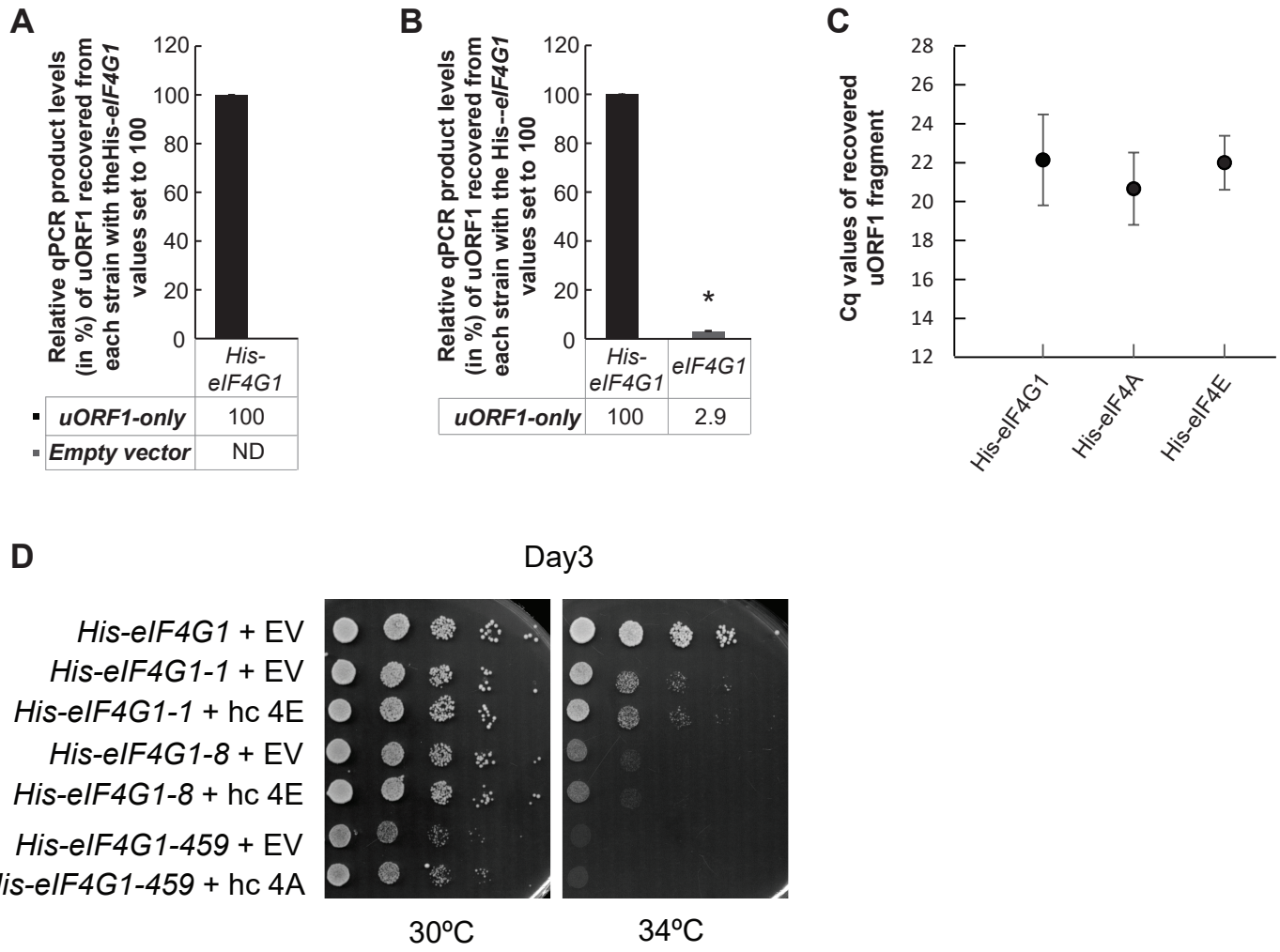

NON-STARVATION: GCN4 translation is REPRESSED
(high levels of TC)
1
48S PIC scans through nAuORFs and RPEs and translates uORF1 (if uORF1 is missed, PIC scans to and translates uORF2)
RPE iv.
RPE ii.
RPE iii.
RPE i.
RPE v.
48S TC
eIF3
nAuORF1/2
60S
1
post-T 40S
2
3
traversing 40S reacquires TC rapidly and reinitiates at uORF3 or uORF4
4
complete recycling ensues at stop codons of uORF3 or uORF4
40S
GCN4
2
post-termination, eIF3-bound 40S at the stop codon of uORF1 or uORF2 resumes traversing downstream (only 60S is recycled)
STARVATION: GCN4 translation is DEREPRESSED
(uncharged tRNAs => GCN2 => eIF2α-P => low levels of TC)
1
48S PIC scans through nAuORFs and RPEs and translates uORF1 (if uORF1 is missed, PIC scans to and translates uORF2)
4
Gcn4 synthesis
2
post-termination, eIF3-bound 40S at the stop codon of uORF1 or uORF2 resumes traversing downstream (only 60S is recycled)
rescanning 40S reacquires TC after bypassing uORF3 and uORF4 and reinitiates at the GCN4 main ORF
3

Supplementary Figure 1. Translational regulation of *GCN4*. The reinitiation (REI)-permissive uORF1 is translated under both, nutrient-replete and deplete conditions. After its translation, the 60S ribosomal subunit is released, whereas the 40S subunit remains bound to the *GCN4* mRNA – due to a specific interaction between REI-promoting elements (RPEs) of uORF1 and eIF3 (1,2) – to eventually resume traversing downstream. It cannot start "scanning" *per se* for the next AUG until it re-acquires an active eIF2 ternary complex (eIF2-TC), the levels of which are reduced under starvation/stress conditions. uORF2, functionally mimicking uORF1, was proposed to serve as its backup to capture 40Ses that 'leaky-scanned' past uORF1, thereby maximizing the REI potential as a fail-safe mechanism (3). In non-starved cells, where eIF2-TC levels are high, nearly all of the traversing 40Ses rebind the eIF2-TC before reaching the REI-non-permissive uORF3 or uORF4. Upon their translation, terminating ribosomes undergo full ribosomal recycling, thus preventing REI at the main CDS of *GCN4*. When eIF2-TC levels are low, a large proportion of the 40Ses will bypass uORFs 3 and 4 and – upon eventual acquisition of the eIF2-TC – reinitiate at the *GCN4* start codon. Hence, whereas the stress response shuts down general translation initiation, as eIF2-TCs are required for translation of most mRNAs, it at the same time stimulates *GCN4* translation to trigger stress adaptation programs (reviewed in (4,5)).


A
Crosslink the cells with Formaldehyde
Stop crosslinking with Glycine
Lyse the cells using glass beads by vortexing
Whole cell extract
Add custom-made oligos
Selectively digest the RPNPDA mRNAs
using RNaseH
Purify protein-RNA complexes
by Ni-NTA sepharose
Digest the proteins in the complex by Proteinase K
Isolate RNA
RNA molecules
Reverse transcribe
cDNA
Quantify mRNA
levels by qRT-PCR
B
eIF4F
40S
GCN4-LacZ
Ni+2
C
i. X-linking followed by RNAse H digestion
His-Tag
ii. Ni2+ pull down via His-tag
iii. RNA isolation etc. followed by qRT-PCR
using red primers

Supplementary Figure 2. Schematic representation of yeast *in vivo* RNA-protein Ni^{2+}-pull down (RaP-NiP) assay using formaldehyde crosslinking. (**A**) The basic scheme of the RaP-NiP is described in the form of a flowchart. Green and red balls represent 40S ribosomes and eIF4F complexes, respectively, grey balls stand for the Ni^{2+} beads, and purple and blue balls depict some non-specific RNA binding proteins. Exponentially growing yeast cells were crosslinked with 1% formaldehyde. Crosslinking was stopped by adding glycine and the fixed cells were lysed using glass beads by rigorous vortexing. Pre-cleared whole cell extract (WCE) containing RaP-NiP mRNAs in protein-RNA complexes were selectively digested with RNase H using sequence specific custom-made oligos. The resulting specific mRNA segments were purified with the help of a given His-tagged component of the yeast eIF4F complex, or its mutant variants, using the Ni-NTA sepharose beads. Thus isolated protein-RNA complexes were subsequently treated with Proteinase K, and the captured RNAs were further purified by hot phenol extraction, reverse transcribed and their amounts were then quantified by qRT-PCR. (**B**) The schematic boxed on the right-hand side illustrates typical amounts of RNAse H digested RNA segments of REI-permissive uORF1 and REI-non-permissive uORF4 from the *GCN4* mRNA leader co-purifying with His-tagged eIF4G or eIF4A, the typical ratio of which is ~2:1. (**C**) The schematic boxed at the bottom illustrates in detail that only the segment of interest spanning the particular uORF region and no upstream or downstream sequences is specifically analyzed in our assay. In other words, since we first cut with RNAse H to eliminate the upstream part of the reporter including the cap, as well as the downstream part following our segment of interest from our analysis, and only after that we carry out the pull down step, we retrieve and subsequently analyze with qRT-PCR only those mRNA segments that originated from ribosomes bound by our factor of interest and only at the site of our interest.

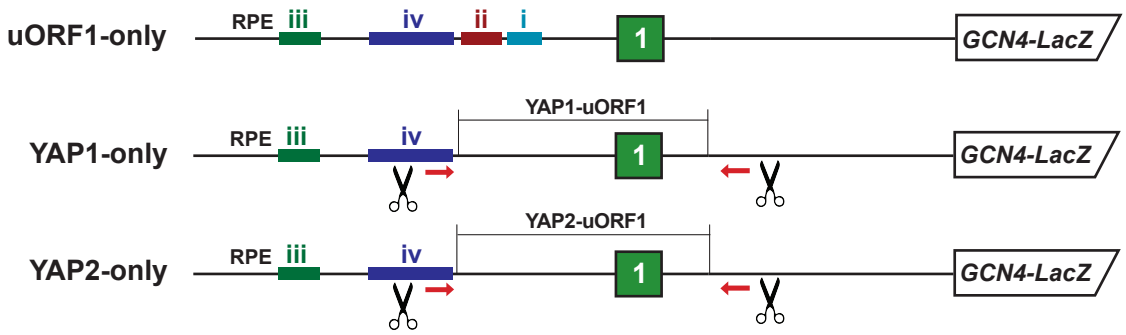
Supplementary Figure 3



Supplementary Figure 3. Cells expressing an empty vector or untagged eIF4G recover only background amounts of the uORF1 segment. (**A - B**) The yeast strains YMP53 (*gcn4Δ(incl. its 5' UTR), tif4631Δ, tif4632Δ* His-eIF4G1), YMP52 (*gcn4Δ(incl. its 5' UTR), tif4631Δ, tif4632Δ* eIF4G1) were introduced either with an empty vector or the uORF1-only RaP-NiP construct shown in Figure 1A and treated as described in Figure 1B. Relative qPCR product levels (in %) of the Y1 segment of uORF1 recovered from each strain were processed as described in Figure 1B with the values of His-eIF4G1 uORF1-only set to 100 % (asterisks indicate that $p<0.05$); ND-not determined. (**C**) The yeast strains YMP53, YMP63, YMP67 were introduced with the uORF1-only construct shown in Figure 1A and treated as described in Figure 1B. The quantification cycle (Cq) values of recovered uORF1 segment from each strain are displayed with standard deviations obtained from at least 3 independent experiments from three independent transformants (i.e. biological replicates). (**D**) Growth defects of individual eIF4G mutants are not suppressible by high copy expression of factors whose eIF4G-binding domains are not affected. The strains described in Figure 3C were transformed individually with YEplac112-based plasmids carrying either eIF4A or eIF4E alleles, or an empty plasmid, and the resulting transformants were spotted in five serial 10-fold dilutions on SD plates and incubated at 30°C or 34°C for 3 days.

Supplementary Figure 4

A



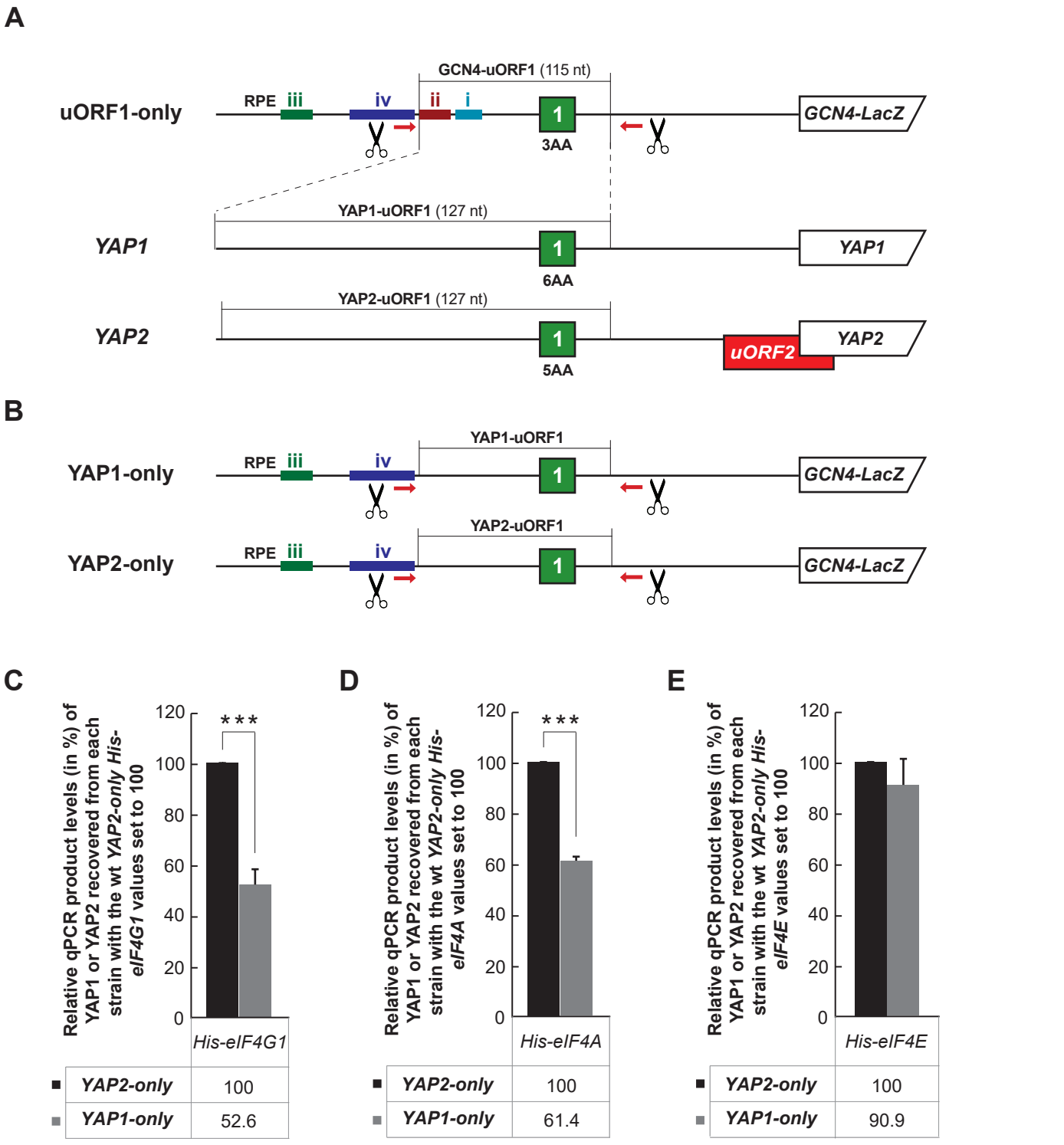
B

	His-eIF4G1			
	30ºC		34ºC	
uORF1-only	**542 ± 43**	**100%**	**277 ± 18**	**100%**
YAP1-only	**1850 ± 48**	**341%**	**1983 ± 71**	**365%**
YAP2-only	**662 ± 3.4**	**122%**	**635 ± 26**	**229%**

Units of β-galactosidase activity

Supplementary Figure 4. uORF1 of *YAP1* and *YAP2* promote reinitiation. (**A**) Schematics showing the wt uORF1-only (pMP29), and *YAP1*- and *YAP2*-only RaP-NiP constructs (for details, please, see Supplementary Figure 5A and B). (**B**) The *GCN4-lacZ* constructs from panel A were introduced individually into the strain YMP53 (*gcn4Δ tif4631Δ tif4632Δ* His-eIF4G1). The resulting transformants were pre-cultured in minimal media overnight, diluted to OD_{600} ~ 0.35, grown for an additional 6 hr and the β-galactosidase activities were measure and analyzed as described in Figure 4B (*p<0.05)**.**

Supplementary Figure 5



Supplementary Figure 5. eIF4F complex is retained on post-termination ribosomes on REI-permissive uORF1 of *YAP1 and 2* mRNA leaders. (**A**) Schematics showing the design of *YAP1*- and *YAP2*-only RaP-NiP constructs. *YAP1*- or *YAP2*-only constructs were constructed by swapping the *YAP1*- or *YAP2*-uORF1 fragment with the *GCN4*-uORF1 fragment in the context of the uORF1-only construct. The RNase H cutting sites (indicated by scissors) and the RT-PCR primer binding sites (indicated by red arrows) were preserved. (**B**) Schematics showing the resulting *YAP1*- and *YAP2*-only RaP-NiP constructs. (**C**) The YMP53 (*gcn4Δ tif4631Δ tif4632Δ* His-eIF4G1) strain was introduced with the *YAP1*- or *YAP2-only* RaP-NiP constructs shown in panel A and treated as described in panel B of Figure 1. Relative qPCR product levels (in %) of the *YAP1*-uORF1 or *YAP2*-uORF1 segments recovered from the His-eIF4G1 expressing strain were processed as described in panel B of Figure 1 with the values of His-eIF4G1 YAP2-only set to 100 % (***p<0.0005). (**D**) The YMP63 (*gcn4Δ tif1Δ tif2Δ* His-eIF4A) strain was introduced with the *YAP1*- or *YAP2*-only RaP-NiP constructs shown in panel A and treated as described in panel B. Relative qPCR product levels (in %) of the *YAP1*-uORF1 or *YAP2*-uORF1 segments recovered from the His-eIF4A expressing strain were processed as described in panel B with the values of His-eIF4A *YAP2*-only set to 100 % (***p<0.0005). (**E**) The YMP67 (*gcn4Δ cdc33/tif45Δ* His-eIF4E) strain was introduced with the *YAP1*- or *YAP2*-only RaP-NiP constructs shown in panel A and treated as described in panel B. Relative qPCR product levels (in %) of the *YAP1*-uORF1 or *YAP2*-uORF1 segments recovered from His-eIF4E expressing strains were processed as described in panel B with the values of His-eIF4E *YAP2*-only set to 100 %

SUPPLEMENTARY TABLES

Supplementary Table 1. Yeast strains used in this study.

Strain	Genotype	Source or reference
BY4741 *gcn4Δ*	*MATa gcn4::KanMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
BY4741 *tif4631Δ*	*MATa tif4631::KanMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
BY4741 *tif4632Δ*	*MATa tif4632::KanMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
BY4741 *tif1Δ*	*MATa tif1::KanMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
BY4741 *tif2Δ*	*MATa tif2::KanMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
H2880[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ*	(6)
H2881[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn2Δ*	(6)
YMP43[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3*	this study
YMP47[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif4632::KanMX ::hisG-URA3-HisG, ura3, tif4631::KanMX* (YCp-*TIF4632-URA*)	this study
YMP52[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif4632::KanMX ::hisG-URA3-HisG, ura3, tif4631::KanMX* (YEp-*TIF4631-LEU*)	this study
YMP53[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif4632::KanMX::hisG-URA3-HisG, ura3, tif4631::KanMX* (YEp-*His-TIF4631-LEU*)	this study
YMP55[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif4632::KanMX::hisG-URA3-HisG, ura3, tif4631::KanMX* (YEp-*His-tif4631-1-LEU*)	this study
YMP56[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif4632::KanMX ::hisG-URA3-HisG, ura3, tif4631::KanMX* (YEp-*His-tif4631-8-LEU*)	this study

YMP91[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif4632::KanMX ::hisG-URA3-HisG, ura3, tif4631::KanMX* (YEp-*His-tif4631-8*-LEU*)	this study
YMP57[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif4632::KanMX ::hisG-URA3-HisG, ura3, tif4631::KanMX* (YEp-*His-tif4631-459-LEU*)	this study
YMP61[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif2::KanMX ::hisG-URA3-HisG, ura3, tif1::KanMX* (YEp-*TIF2-URA*)	this study
YMP63[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif2::KanMX::hisG-URA3-HisG, ura3, tif1::KanMX* (YEp-*His-TIF2-LEU*)	this study
YMP65[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif45::KanMX* (YEp-*TIF45-URA*)	this study
YMP67[a]	*MATa, leu2-3, -112 ura3-52 trp1Δ gcn4 (incl. 5' UTR) ::KanMX::hisG-URA3-HisG, ura3, tif45::KanMX* (YEp-*His-TIF45-LEU*)	this study

[a] Isogenic strains

Supplementary Table 2. Plasmids used in this study.

Plasmid	Description	Source or reference
p180 (*YCp50–GCN4–lacZ*)	low copy *URA3* vector containing WT *GCN4* leader fused with *lacZ*	(7)
p349 (*YEplac181-LEU2*)	high copy *LEU2* empty vector	(8)
p533 (*YEplac195-TIF2-URA3*)	High-copy *URA3* vector containing *TIF2* ORF	(9)
p544 (*YEplac181-TIF2-LEU2)*	High-copy *LEU2* vector containing *TIF2* ORF	(10)
p350 (*YEplac195-URA3*)	high copy *URA3* empty vector	(8)
p467 (uORF1-extended)	low copy *URA3* vector containing uORF1 (GC) extends into GCN4 sequence	Grant et. al. (1994) MCB 14: 2616-28
pMP29 (uORF1-only)	low copy *URA3* vector containing uORF1 only with its genuine 5' and 3' flanking sequences	(1)
pMP30 (uORF4-only)	low copy *URA3* vector containing uORF4 only with its genuine 5' and 3' flanking sequences	(1)
pMP32 (uORF1-SUB40)	low copy *URA3* vector containing uORF1 only; the sequences -48 to -40 upstream of uORF1 were substituted by complementary sequences	(1)
pMP33 (uORF1-CAAII)	low copy *URA3* vector containing uORF1 only; the -143 to -122 upstream sequences were substituted by a stretch of CAA triplets of the identical length to the original sequence	(1)
pMP34 (uORF2-only)	low copy *URA3* vector containing uORF2 only with its genuine 5' and 3' flanking sequences	(1)
pMP35 (uORF4_2-only)	low copy *URA3* vector containing uORF4 only with its genuine 5' and 3' flanking sequences	(1)
pMP53 (uORF3-only)	low copy *URA3* vector containing uORF3 only with its genuine 5' and 3' flanking sequences	(1)

pBAS2078	Low copy *TRP2* vector containing *TIF4631* ORF with *TIF4632* 5' & 3'-UTRs	(11)
pBAS2004	Low copy *URA3* vector containing *TIF4632* ORF with *TIF4632* 5' & 3'-UTRs	(11)
pMP82 (*YEp181-TIF4631-LEU*)	High-copy *LEU2* vector containing *TIF4631* ORF	this study
pMP83 (*YEp181-His-TIF4631-LEU*)	High-copy *LEU2* vector containing His-*TIF4631* ORF	this study
pMP89 (*YEp181-His-TIF4631-1*)	High-copy *LEU2* vector containing His-*tif4631-1* ORF	this study
pMP90 (*YEp181-His-TIF4631-8*)	High-copy *LEU2* vector containing His- *tif4631-8* ORF	this study
pMP91 (*YEp181-His-TIF4631-459*)	High-copy *LEU2* vector containing His-*tif4631-459* ORF	this study
pMP85 (pAG25-*kanMX::HisG-URA-HisG*)	High-copy vector containing *kanMX::HisG-URA-HisG* disruption cassette	this study
pMP93 (*YEp181-His-TIF2*)	High-copy *LEU2* vector containing His-*TIF2* ORF	this study
pMP98 (*YEp181- tif45::KanMX*)	High-copy *LEU2* vector containing *tif45::KanMX* disruption cassette	this study
pMP95 (*YEp181-TIF45*)	High-copy *URA3* vector containing *TIF45* ORF	this study
pMP97 (*YEp181-His-TIF45*)	High-copy *LEU2* vector containing His-*TIF45* ORF	this study
pMP121 (Ycp22-*GCN4*)	low copy TRP2 vector containing WT GCN4 leader	this study
pMP122 (Ycp50-*YAP1-GCN4*)	low copy *URA3* vector containing uORF1 of *YAP1* with its genuine 5' and 3' flanking sequences	this study
pMP123 (Ycp50-*YAP2-GCN4*)	low copy *URA3* vector containing uORF1 of *YAP2* with its genuine 5' and 3' flanking sequences	this study
pMP124 (*YEp181-His-TIF4631-8**)	High-copy *LEU2* vector containing His- *tif4631-8** ORF	this study

Supplementary Table S3. Oligonucleotides used in this study.

Oligonucleotide	**Sequence (5' to 3')**
VM4	TCGGCTCGCTGTCTTACCTTTTAAAATCTTCTACTTCTTGACAGTACTTATCTTCTTATATAATAGATATCG TACGCTGCAGGTCGAC
VM25	TTTAAAGTTTCATTCCAGCATTAGC
MP1	GTTTTCCCAGTCACGACGTT
MP2	AACAGCTATGACCATGATTACGC
MP7	GGGAAATTTTTATTGGCGAGTAAAC
MP79	GTGGCTGGTGAGTTGTATAATTCGC
MP8	GCTCACTCATCTACTTCGCAATC
MP46	TGGATAATTTGACAGAAAGGTAACC
MP145	GCCTTCTACGTTTCCATCCA
MP146	GGCCAAATCGATTCTCAAAA
MP150	CTG TGG ATC CAT GGT GAT GGT GGT GGT GAT GAT GCA TAT GTG CCT ACA ATT GAT CTA TTG
MP153	ATA CGC AGC CCT GAC ATT TGT AG
MP154	GA AAT ATA TGT TAC GTT ATC AAG ATA GCC TCA C

MP155	ATA TCA TCG AAC TTG TAG ACA ACC TTG TC
MP156	AGC AGA TCC CAA TAC ACA TAG TAG G
MP157	GGC AAA ATA ATA TCA TAT AGG GGG TCA AG
MP158	GAG AAA GTA CCG GTC TTA CCA GTA C
MP159	GTA CTG GTA AGA CCG GTA CTT TCT C
MP220	TTA AAG CTT CTT ACT TTA CTC CTT CTT GC
MP221	GAT TAG CAG GGG CAC TAC CAG
MP222	ATG AGG AAG AGG AGA GAC AAC GTC
MP223	TTA GAC TTT CTA CCA ACA TCC TTG TAT CC
MP224	CTA CGG CGA CTT CAT ATT TTT AGG C
MP225	CAT CTG AGG AGC ATA GTA GTT AGC TGG
MP226	CAA TTA CGT CAG AAA AAG AAC AGT CAG AG
MP227	TGA TGA AAG ATC AGG TCT AGT TAA TGC AC
MP294	GAA CAC GTC AAA TAT ACA TAT GGC CCA ACT TTC GCT GCT CAA TTT AAG G
MP295	GGG GCT AGC TTC TTT TCA GTC

MP296	GAA GCT AGC CCC TGA CGG AAA G
MP297	GAA TAA TAT TGT CAG AAA TAC CGA ACA AAC
MP298	GTT TGT TCG GTA TTT CTG ACA ATA TTA TTC
MP299	GTC TCA AAT TGA CCA CCA ACA GTA TTT AAC
MP300	GTT AAA TAC TGT TGG TGG TCA ATT TGA GAC
MP301	GAA AAT CTG TTC TAT ACC AGC CTT TAA CG
MP302	CGT TAA AGG CTG GTA TAG AAC AGA TTT TC
MP303	GAA AAT CTG TTC TAT ATC AGC CTT TAA CG
MP304	CGT TAA AGG CTG ATA TAG AAC AGA TTT TC
MP305	CTT TGA TCT TCT TCT TGA TGA AGT TCT TG
MP306	CAT CAA GAA GAA GAT CAA AGA GAA TGA ATG ACG ACT CTA GAT CTA ATA GAT C
MP307	TCG TGG CCA TGA CTT TAT TAA TAC CTC AG
MP308	CTA ATA AAT AGT AAT TGC AAT ATG CAT CAT CAC CAC CAC CAT CAC CAT GGA TCT GAA GGT ATT ACT G
MP309	CAT ATT GCA ATT ACT ATT TAT TAG TTT CTT GTA C
MP312	AGG AGT TTT ACG AAA AAT AAA AGC ATT TTT GTC TGA AAA CTA GTG AAA GGA AGA AAA ATG GAT GTC CAC GAG GTC TC

MP313	ATT ATA TAT ATA ATT TTG ATT AAA ATA ACA ATT ATC TTA AGA AAA ATT CAG ACT ATC TTA CGG TGT CGG TCT CGT AG
MP314	CCG CTC TAG AAG TTT GGA TAC TGG TAT TGT GAC TC
MP315	GCA GCC CGG GAC GAT TGA AGA CGA ACT AAT CGA AG
MP316	AGA CGG TAC CAT GTC CGT TGA AGA AGT TAG CAA G
MP317	CTA CGT CGA CTT ACA AGG TGA TTG ATG GTT GAG G
MP318	CTT GCT AAC TTC TTC AAC GGA TCC ATG GTG ATG GTG GTG GTG ATG ATG CAT TTT TCT TCC TTT CAC TAG
MP319	TCC GTT GAA GAA GTT AGC AAG AAG
MP320	AAAACTAGTGATGTCCACGAGGTCTCTCAC
MP321	CCTAACTAGTCGGTGTCGGTCTCGTAGC
MP322	CCCTCAACCATCAATCACCTTG