SUPPLEMENTARY TABLE 1. Plasmids used in this study

Plasmid	Precursor	Description	Source
pC3286	p4437	integrating *URA3* P_{CUP1}*-UBI-R-tif51a-td* plasmid	This study
pC3287	YCplac111	sc *LEU2 TIF51A* plasmid	This study
pC3288	YCplac33	sc *URA3 TIF51A* plasmid	This study
pC3289	YEplac181	hc *LEU2 TIF51A* plasmid	This study
pC3290	pC3289	hc *LEU2* C-terminal FLAG-tagged *TIF51A* plasmid	This study
pC3291	pC3290	hc *LEU2* C-terminal FLAG-tagged *tif51a*^{K51R} plasmid	This study
pC3293	pC3292	sc *LEU2 tif51a-D63V* plasmid	This study
pC3294	pC3292	sc *LEU2 tif51a-S149P* plasmid	This study
pYDL-control			Dinman JD[1]
pYDL-LA			Dinman JD[1]
pYDL-Ty1			Dinman JD[1]
pYDL-LA0			Dinman JD[1]

Single copy (sc) or high copy (hc) number plasmid

[1] Harger, J. W. & Dinman, J. D. An *in vivo* dual-luciferase assay system for studying translational recoding in the yeast *Saccharomyces cerevisiae*. *RNA* **9**, 1019-1024 (2003).

SUPPLEMENTARY TABLE 2. *S. cerevisiae* strains used in this study

Strain	Precursor	Genotype	Source
H2557		*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ*	Hinnebusch AG
J691	H2557	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ tif51b::KANMX4*	This study
J692	J691	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ tif51b::NAT*	This study
J693	J692	*MATα/a trp1-63/trp1-63 ura3-52/ura3-52 leu2-3/leu2-3 leu2-112/leu2-112 GAL2^{+}/GAL2^{+} gcn2Δ/gcn2Δ tif51b::NAT/tif51b::NAT*	This study
J694	J693	*MATα/a trp1-63/trp1-63 ura3-52/ura3-52 leu2-3/leu2-3 leu2-112/leu2-112 GAL2^{+}/GAL2^{+} gcn2Δ/gcn2Δ tif51b::NAT/tif51b::NAT* pC3288[*TIF51A, URA3*]	This study
J695	J694	*MATα/a trp1-63/trp1-63 ura3-52/ura3-52 leu2-3/leu2-3 leu2-112/leu2-112 GAL2^{+}/GAL2^{+} gcn2Δ/gcn2Δ tif51b::NAT/tif51b::NAT tif51a::KANMX4/TIF51A* pC3288[*TIF51A, URA3*]	This study
J696	J695	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ tif51b::NAT tif51a::KANMX4* pC3288[*TIF51A, URA3*]	This study
J697	J696	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ tif51b::NAT tif51a::KANMX4* pC3287[*TIF51A, LEU2*]	This study
J698	J696	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ tif51b::NAT tif51a::KANMX4* pC3293[*tif51a-D63V, LEU2*]	This study
J699	J696	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ tif51b::NAT tif51a::KANMX4* pC3294[*tif51a-S149P, LEU2*]	This study
J700	H2557	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ ubr1::P_{GAL1}-myc-UBR1-TRP1*	This study
J701	J700	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ ubr1::P_{GAL1}-myc -UBR1-TRP1 P_{CUP1}-UBI-R-tif51a-td::URA3::tif51a*	This study

J702	J701	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ ubr1:: P_{GAL1}-myc -UBR1-TRP1 P_{CUP1}-UBI-R-tif51a-td::URA3::tif51a tif51b::KANMX4*	This study
J703	J702	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ ubr1:: P_{GAL1}-myc-UBR1-TRP1 P_{CUP1}-UBI-R-tif51a-td::URA3::tif51a tif51b::KANMX4* pC3290[*TIF51A-FLAG, LEU2*]	This study
J704	J702	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ ubr1:: P_{GAL1}-myc-UBR1-TRP1 P_{CUP1}-UBI-R-tif51a-td::URA3::tif51a tif51b::KANMX4* pC3291[*tif51a^{K51R}-FLAG, LEU2*]	This study
J713	J700	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ ubr1::P_{GAL1}-myc-UBR1-TRP1* YCplac33	This study
J714	J713	*MATα trp1-63 ura3-52 leu2-3 leu2-112 GAL2+ gcn2Δ ubr1::P_{GAL1}-myc-UBR1-TRP1 tif51b::KANMX4* YCplac33	This study
YAJ2		*MATa trp1Δ ura3-52 leu2-3,-112 gcn2Δ::hisG P_{GAL}-myc-UBR1::TRP1::ubr1*	Hinnebusch AG[1]
YAJ22		*MATa trp1Δ ura3-52 leu2-3,-112 gcn2Δ::hisG P_{GAL}-myc-UBR1::TRP1::ubr1 P_{CUP1}-UB I-R-HA- tif32 ^{td}::URA3::tif32*	Hinnebusch AG[1]
SL797-2C		*MATa met8-1 leu2-1 aro7-1 trp1-1 his5-2 lys2-1 ura3-52 sup35-4*	Liebman SW[2]

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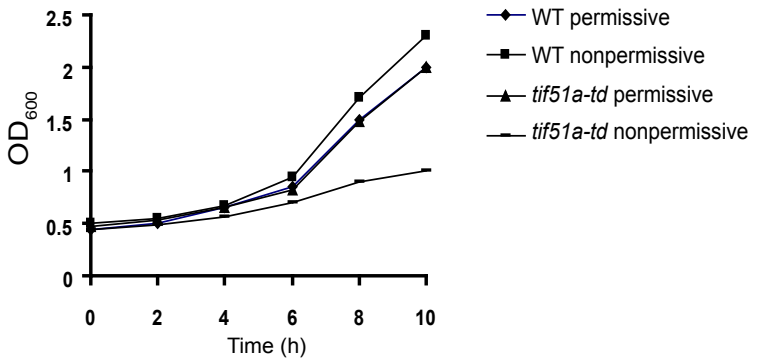
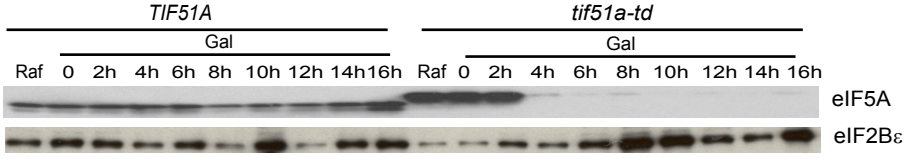
[1] Jivotovskaya, A. V., Valasek, L., Hinnebusch, A. G. & Nielsen, K. H. Eukaryotic translation initiation factor 3 (eIF3) and eIF2 can promote mRNA binding to 40S subunits independently of eIF4G in yeast. *Mol Cell Biol* **26**, 1355-1372 (2006).

[2] All-Robyn, J. A., Kelley-Geraghty, D., Griffin, E., Brown, N. & Liebman, S. W. Isolation of omnipotent suppressors in an [*eta*$^{+}$] yeast strain. *Genetics* **124**, 505-514 (1990).

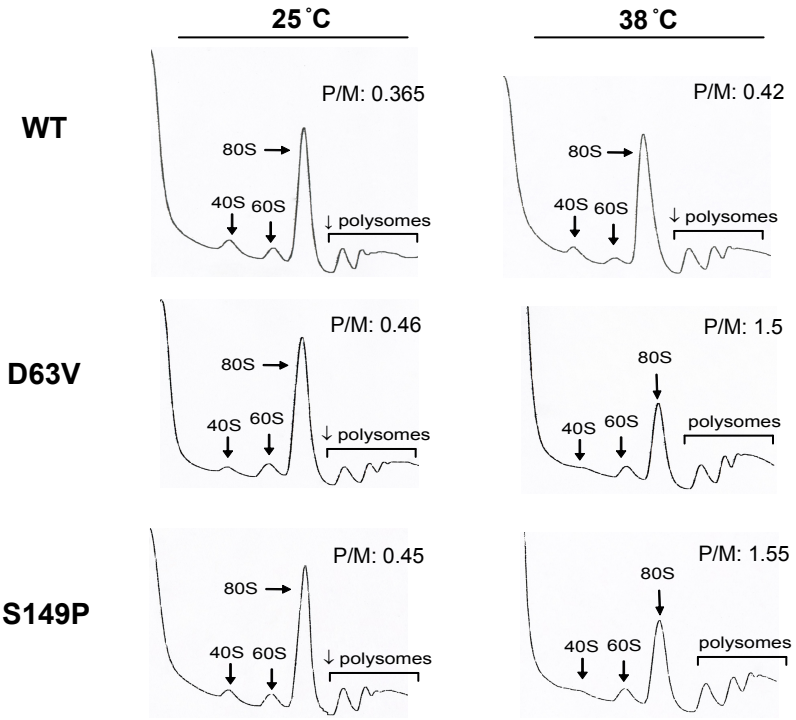
a



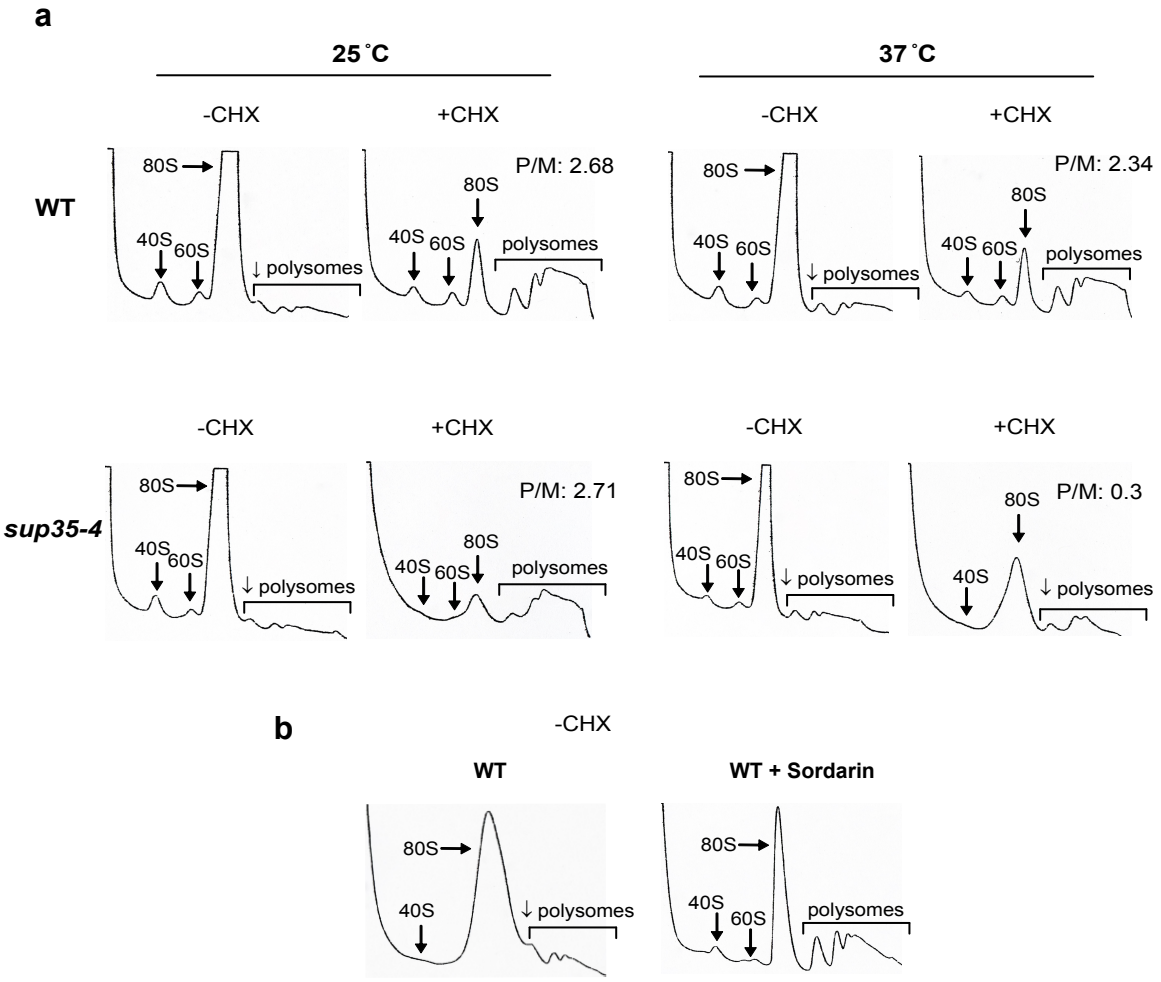
b



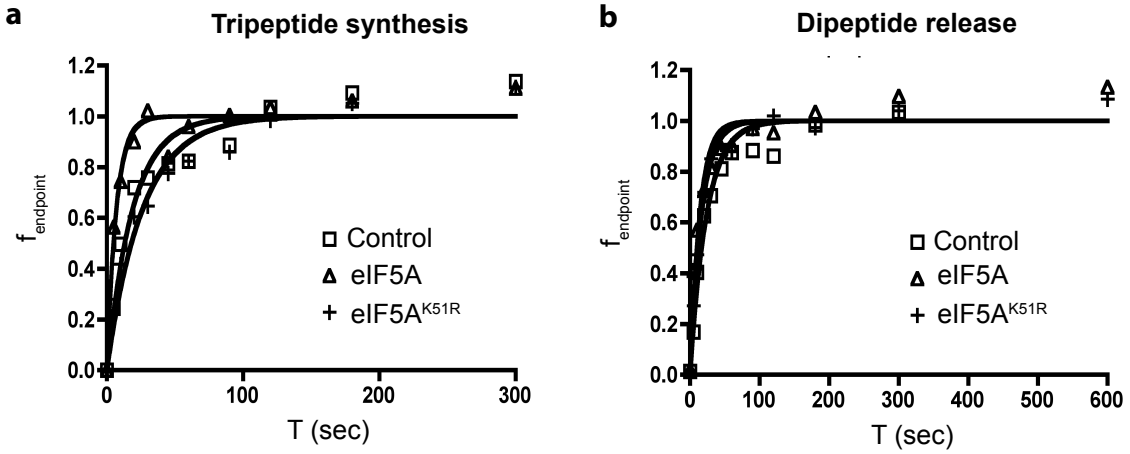
Supplementary Figure 1: Impaired growth and rapid depletion of eIF5A in *tif51a-td* strain under non-permissive conditions. a, Isogenic WT (J714) and *tif51a-td* mutant (J702) strains were grown in SC medium containing 2% raffinose as a carbon source and 100 μM copper sulphate (SC_{Raf} + Cu^{2+}; permissive condition) to OD_{600} ~ 1.0, washed at room temperature to remove Cu^{2+}, split in halves, and incubated under permissive or non-permissive conditions (SC_{Gal}; SC containing 2% galactose lacking copper). **b,** Western analysis of eIF5A. Strains described for panel **b** were cultured under the same conditions as for panel **a**, and WCEs were prepared and subjected to western analysis using antibodies against the indicated proteins. Immune complexes were visualized by chemiluminescence.



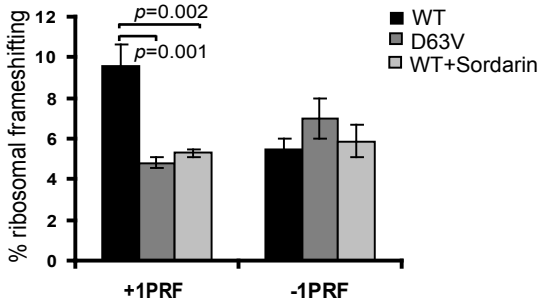
Supplementary Figure 2: Retention of polysomes in *tif51a-D63V* and *tif51a-S149P* mutant strains at restrictive temperatures. Isogenic WT (J697), *tif51a-D63V* (D63V; J698), and *tif51a-S149P* (S149P; J699) strains were grown to mid-log phase at 25 ˚C in SC medium, pelleted, and diluted to A_{600} of ~0.5 with either 25 ˚C or pre-warmed 38 ˚C media. Cultures were then incubated for 2 h at 25 or 38 ˚C, respectively, and WCEs were prepared and separated on 4.5% to 45% sucrose gradients by centrifugation at 39,000 rpm for 2.5 h. Gradients were fractionated while scanning at 254 nm to visualize the indicated ribosomal species. Polysome to monosome (P/M) ratios were calculated by comparing the areas under the 80S and polysome peaks. ↓polysomes indicates reduced amount of polysomes.



Supplementary Figure 3: Polysome analysis in termination factor eRF3 (*sup35-4*) mutant strain and in sordarin-treated WT strain. a, The *sup35-4* temperature-sensitive mutant strain SL797-2C transformed with either an empty vector (*sup35-4*) or a *SUP35* plasmid (WT) was grown to mid-log phase at 25 ˚C in YEPD medium, pelleted, and diluted to A_{600} of ~0.5 with either 25 ˚C or pre-warmed 37 ˚C media. Cultures were then incubated for 2 h at 25 or 37 ˚C and cells were either untreated or treated with 50 μg/ml CHX for 5 min before harvesting. WCE preparation, sucrose gradient analysis, and P/M ratio calculation were performed as described for supplementary Figure 2. **b,** The WT strain J714 was grown in SC_{Gal}-Cu^{2+} for 14 h with the final 5h in the absence or presence of 2 μg/ml of sordarin. WCE preparation, sucrose gradient analysis, and P/M ratio calculation were performed as described for supplementary Figure 2. ↓polysomes indicates reduced amount of polysomes.



Supplementary Figure 4: Representative fits from *in vitro* elongation and termination experiments. Reconstituted elongation and termination assays were carried out in triplicate as described in the text and Methods. As previously mentioned, single-exponential curves were fitted to the data to obtain rate constants and endpoints. Representative curves are shown for (**a)** an elongation assay and (**b**) a release assay. $f_{endpoint}$ is the ratio of peptide at any given data point to the endpoint value generated in the curve fitting process. Note that the time (x) axis in (**b**) is twice as long as in (**a**).



Supplementary Figure 5: eEF2 inhibitor sordarin and *tif51a-D63V* mutation decrease +1 ribosomal frameshifting at 38 ˚C. Dual-luciferase reporter plasmids containing firefly (F) and *Renilla* (R) luciferase coding regions separated by the +1 programmed ribosomal frameshift (PRF) signal from the yeast *Ty1* retrotransposon or the -1 PRF signal from the yeast L-A virus[1], or the relevant 0-frame control were introduced into WT and *tif51a-D63V* mutant strains. Where indicated, cultures contained 200 ng/ml sordarin. PRF efficiencies (%) were calculated by dividing the ratio of F to R luciferase obtained with the reporter versus the 0-frame control plasmid. Results are the average of at least three independent experiments; *P*-values for -1 PRF were 0.08 (WT vs. D63V) and 0.54 (WT +/- sordarin).

[1] Harger, J. W. & Dinman, J. D. An *in vivo* dual-luciferase assay system for studying translational recoding in the yeast *Saccharomyces cerevisiae*. *RNA* **9**, 1019-1024 (2003).