Ribosomal protein eL24, involved in two intersubunit bridges, stimulates translation initiation and elongation

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SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURES

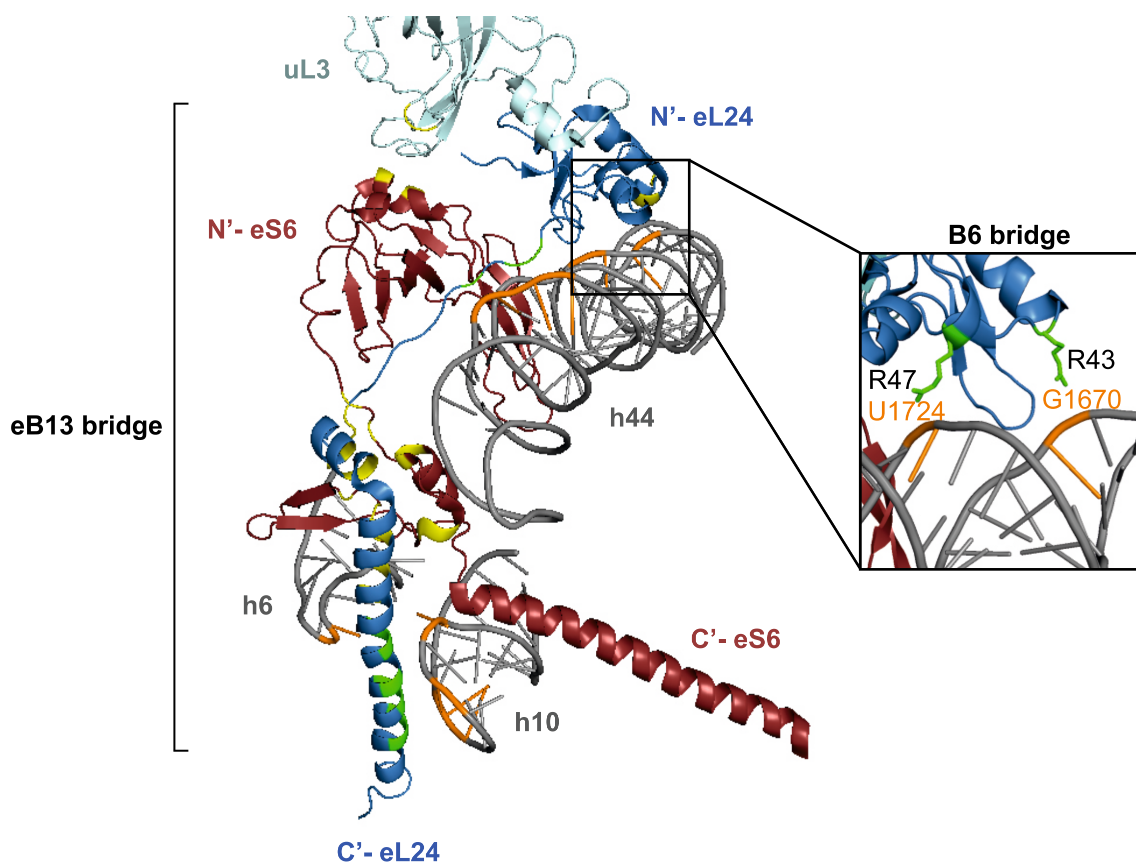
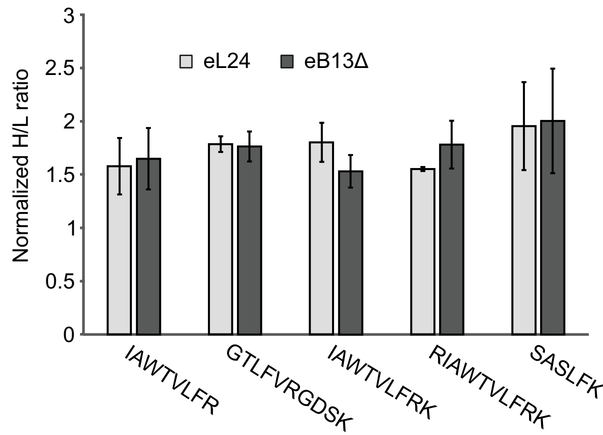


Figure S1. Intersubunit bridges eB13 and B6 in the post-translocational state of the *S. cerevisiae* ribosome. eL24 (blue), eS6 (red), uL3 (light cyan) and 18S rRNA h6, h10 and h44 (grey) are shown. Amino acid residues forming protein-protein and protein-rRNA type contacts are colored yellow and green, respectively. 18S rRNA nucleotides involved in protein-rRNA type contacts are colored orange. Ribosomal structures were generated by PyMol using coordinates from (1).



1 MKVEIDSFSG AKIYPGRGTL FVRGDSKIFR FQNSKSASLF KQRKNPRRIA WTVLFRKHHK KGITE 65

GTL FVRGDSK
SASLF K
RIA WTVLFRK
IA WTVLFRK
IA WTVLFR

Figure S2. "Heavy"/"light" ratios of peptides originated from the N-terminal domain of eL24 in ribosomes of eB13Δ and control cells. The average (mean ± SD) ratios across all biological replicates are plotted. Sequence of the N-terminal domain of eL24 is presented in black letters. Identified peptides are shown in red letters.

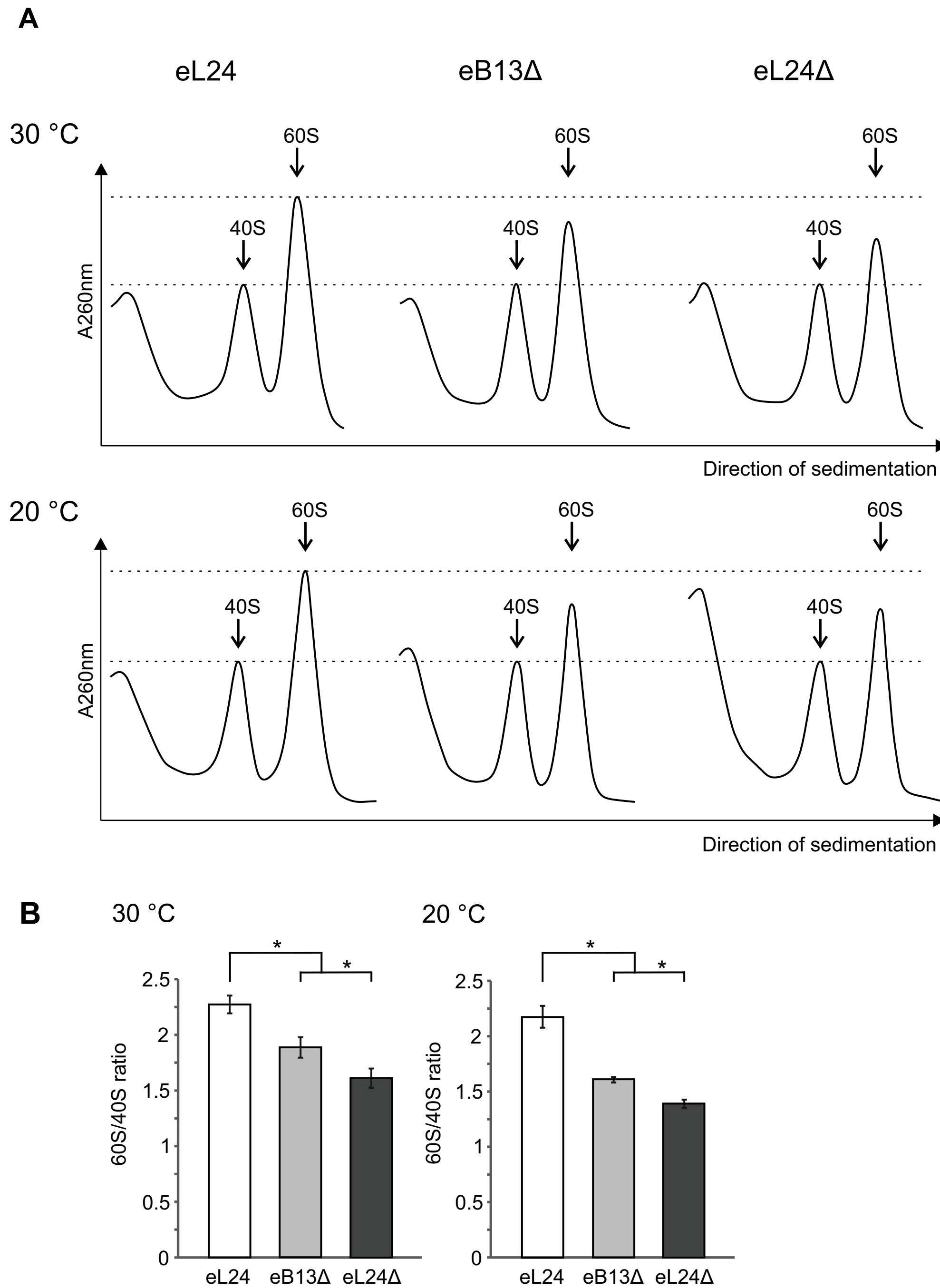


Figure S3. Relative amounts of 60S and 40S subunits in extracts of eL24 mutants.
(A) Extracts of *rpl24AΔrpl24BΔ* strains carrying either eL24 wild-type (eL24) or mutant (eB13Δ) allele and *rpl24AΔrpl24BΔ* strain (eL24Δ, TYSC488) were analyzed by sedimentation in sucrose density gradient. Cells were grown in rich medium at 30 °C or 20 °C to mid-exponential phase. The whole cell extracts were prepared in presence of low concentration of Mg^{2+} and subjected to sedimentation analysis in 7%-47% sucrose gradients. Gradients were visualized at 260 nm (A260 nm). Sedimentation is from left to right. The peaks of 60S and 40S subunits are indicated.

(B) 60S/40S ratios of cell extracts analyzed in (A). Areas under 60S and 40S peaks were quantified by ImageJ and 60S/40S ratios were calculated. The averages (mean ± SD) of at least three biological replicates are plotted. Statistical significance was determined by the unpaired two sample Student's t test (*, $p < 0.01$).

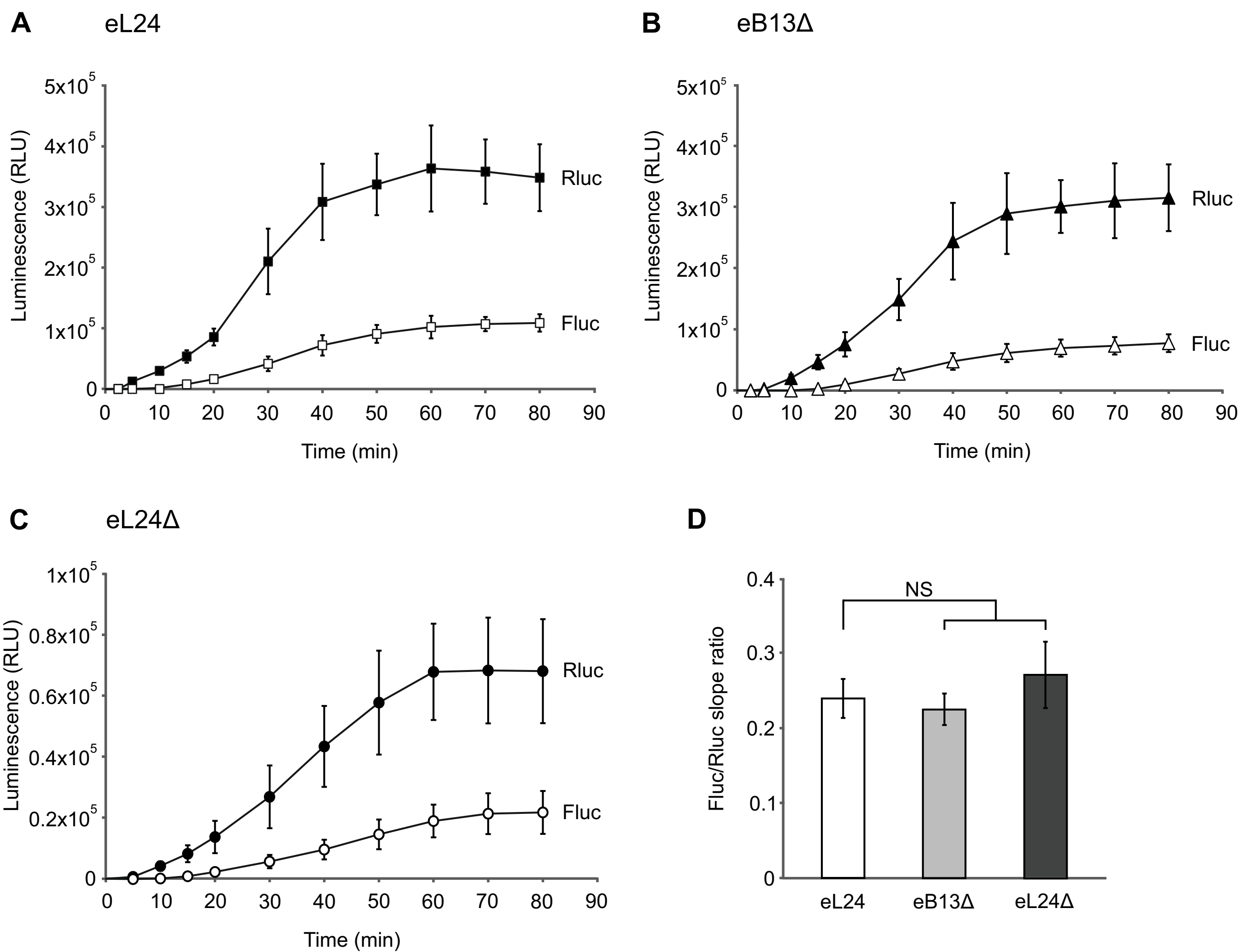


Figure S4. Synthesis of fusion Renilla-Firefly luciferase in yeast cell-free translation extracts prepared from eL24 control cells (A), eB13Δ cells (B) or eL24Δ cells (C). *In vitro* translation was carried out at 25 °C in 30 µl starting volume using 500 ng of mRNA as a template. For each strain at least three independent extracts were analyzed, each extract was analyzed by at least two independent reactions. The average (mean ± SD) relative light units (RLU) of all replicates are plotted.

(D) Ratios between slopes of Firefly and Renilla luciferase activity curves from (A, B, C). The average ratios (mean ± SD) of all replicates are plotted. Statistical significance was determined by the unpaired two sample Student's t test (NS, not significant).

SUPPLEMENTARY TABLES

Table S1. Yeast strains used in this study.

Strain	Strain name	Genotype	Source
TYSC309	WT	*MAT**a** ura3-52 leu2Δ1 his3Δ200 trp1Δ36 Δarg4 Δlys1*	Lab collection
TYSC310		*MAT*α *ura3-52 leu2Δ1 his3Δ200 trp1Δ36 Δarg4 Δlys1*	Lab collection
TYSC448		*MAT**a** ura3-52 leu2Δ1 his3Δ200 trp1Δ36 Δarg4 Δlys1 Δrpl24A::hphMX6*	This study
TYSC455		*MAT*α *ura3-52 leu2Δ1 his3Δ200 trp1Δ36 Δarg4 Δlys1Δrpl24B::hphMX6*	This study
TYSC488	eL24Δ	*MAT**a** ura3-52 leu2Δ1 his3Δ200 trp1Δ36 Δarg4 Δlys1 Δrpl24A::hphMX6 Δrpl24B::hphMX6*	This study

Table S2. Plasmids used in this study.

Plasmid	Description	Source, reference
pRS314	*TRP1 / CEN*	(2)
pRS314-RPL24	*RPL24A / TRP1 / CEN*	This study
pRS314-$rpl24_{1\text{-}111}$	$rpl24_{1\text{-}111}$ */ TRP1 / CEN*	This study
pRS314-$rpl24_{1\text{-}80}$	$rpl24_{1\text{-}80}$ */ TRP1 / CEN*	This study
pRS314-$rpl24_{1\text{-}65}$	$rpl24A_{1\text{-}65}$ */ TRP1 / CEN*	This study
pRS314-rpl24(R43A,R47A)	*rpl24A(R43A,R47A) / TRP1 / CEN*	This study
pRS314-$rpl24_{1\text{-}65}$*(R43,R47A)*	$rpl24A_{1\text{-}65}$*(R43A,R47A) / TRP1 / CEN*	This study
pUC18-Fluc	*pT7/PGK1 5'UTR/Fluc / poly*$(A)_{30}$	This study
pUC18-Rluc-Fluc	*pT7/PGK1 5'UTR/Rluc-Fluc/poly*$(A)_{30}$	This study

SUPPLEMENTARY REFERENCES

1. Ben-Shem, A., Garreau de Loubresse, N., Melnikov, S., Jenner, L., Yusupova, G. and Yusupov, M. (2011) The structure of the eukaryotic ribosome at 3.0 A resolution. *Science*, **334**, 1524-1529.
2. Sikorski, R. S. & Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* **122**, 19-27.