SUPPLEMENTARY INFORMATION TO THE MANUSCRIPT ENTITLED:

Immature large ribosomal subunits containing the 7S pre-rRNA can engage in translation in *Saccharomyces cerevisiae*

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LEGENDS TO THE SUPLEMENTARY FIGURES

Figure S1. Maturation pathway of 5.8S and 25S rRNAs. The $27SA_2$ pre-rRNA, which is generated by either post-transcriptional cleavage of 35S pre-rRNA or co-transcriptional cleavage of nascent RNA polymerase I transcript (not shown), is processed by two alternative pathways. In the major one, about 85% of all $27SA_2$ pre-rRNA is cleaved at site A_3 and then exonucleolytically processed to site B_{1S} to yield $27SB_S$ pre-rRNA. The remaining 15% of $27SA_2$ pre-rRNA is directly processed at site B_{1L}, producing $27SB_L$ pre-rRNA. While processing at sites B_1 is completed, the 3' end of mature 25S rRNA is generated by trimming from site B_0 to site B_2. The subsequent processing of both 27SB species appears to be identical. Cleavage at site C_2 generates the 25.5S pre-rRNA and the two forms, long and short, of 7S pre-rRNA. Maturation of 25.5S pre-rRNA to 25S rRNA occurs by a two-step 5'-3' exonucleolytic trimming from site C_2. The 7S pre-rRNAs are converted to mature 5.8S rRNAs by a four-step 3'-5' exonucleolytic process. First, 7S pre-rRNAs are converted to 5.8S+30 pre-rRNAs, then, these precursors are trimmed to 6S pre-rRNAs. The 6S pre-rRNAs are converted to 5.8S+5 pre-rRNAs and these to mature 5.8S rRNAs. The enzymes involved in 7S pre-rRNA processing and their co-factors are shown. Note that, in wild-type cells, the pre-60S r-particles that are exported to the cytoplasm contain mature 25S and 5S rRNAs and either the long or the short form of 6S pre-rRNAs. For more details on yeast pre-rRNA processing, see [1].

Figure S2. The GTPase Lsg1 remains cytoplasmic in the *dob1-1* mutant. (**A**) Pre-60S r-particles containing 27S pre-rRNAs are not exported to the cytoplasm in the *dob1-1* mutant. The *dob1-1* (*mtr4*) strain was grown at 30 ºC in YPD medium to mid-log phase. Immunoprecipitation experiments were carried out using IgG-Sepharose and whole-cell extracts from untagged or TAP-tagged Nop7 and Lsg1 cells. RNA was extracted from the beads (lanes IP) or from an amount of total extract corresponding to 1/100 of that used for the immunoprecipitations (lanes T), separated on a 1.2% agarose-6% formaldehyde gel, transferred to a nylon membrane and subjected to northern hybridization with the probes indicated in parentheses to detect 27S pre-rRNAs and mature 25S rRNA. Probes are described in Figure 2 and Table S2. Signal intensities were measured by phoshorimager scanning; values (below each IP lane) refer to the percentage of each RNA recovered after purification. (**B**) Lsg1 is localized predominantly to the cytoplasm. Fluorescence microscopy was performed with exponentially growing cells expressing Lsg1-GFP from its cognate promoter in wild-type or *dob1-1* cells. Cells were stained with Hoechst for

localization of nuclei. The GFP and Hoechst signals were visualized by fluorescence microscopy. Arrows point to the position of the nuclei.

Figure S3. The *dob1-1* cells have no enlarged nucleoli. Wild-type (*MTR4*) and *dob1-1* (*mtr4*) cells were transformed with a plasmid that expressed Nop1-mRFP and S3-eGFP from their cognate promoters [2] (a gift from J. Bassler). Cells were grown at 30 ºC in SD-Leu. Cells were visualize under brightfield illumination (Visible). The subcellular localisation of the GFP-tagged ribosomal protein and the Nop1-mRFP nucleolar marker were analysed by fluorescence microscopy. The nucleoplasm was visualized by Hoechst 33342 staining. Arrows point to nucleolar fluorescence.

Figure S4. Pre-60S r-particles containing 7S pre-rRNAs are associated with polysomes in the *dob1-1* mutant. Wild-type (*MTR4*) and *dob1-1* (*mtr4*) cells were grown in YPD medium at 30 ºC. Cell extracts were prepared under polysome run-off conditions, by omission of cycloheximide, either in standard buffer (**A**) or in a buffer lacking $MgCl_2$ to dissociate 80S ribosomes into 40S and 60S r-subunits (**B**). Cell extracts were resolved in 7-50% sucrose gradients and fractionated. Total RNA was extracted from each fraction and analyzed by northern hybridization with probes that reveal 7S pre-rRNAs and mature 5.8S rRNAs. The position of free 40S and 60S r-subunits, 80S ribosomes and polysomes, obtained from the recorded A_{254} profiles, are indicated.

Figure S5. Association of pre-60S r-particles containing 7S pre-rRNAs with polysomes is not significantly enhanced upon TRAMP inactivation. The TRAMP component Trf4 was deleted in the indicated mutants. A second TRAMP component, Air1, was additionally deleted in the *rrp4-1* mutant background. All mutants were grown at 30 ºC in YPD medium to mid-log phase. Cell extracts were prepared and 10 A_{260} units of each extract were resolved in 7-50% sucrose gradients and fractionated. RNA was extracted from each fraction and analysed by northern blotting using probes f and e, which reveal 7S pre-rRNAs and mature 5.8S rRNAs, respectively. The position of free 40S and 60S r-subunits, 80S ribosomes and polysomes, obtained from the recorded A_{254} profiles, are indicated.

Figure S6. Functional sites of the *S. cerevisiae* ribosome. View of the X-ray structure of the yeast 80S ribosome. The approximate positions of the polypeptide exit tunnel (PET), the mRNA path (mRNA), the peptidyl transferase center (PTC), the GTPase-associated center (GAC) and the 3' end of 5.8S rRNA are highlighted. The 25S rRNA from

the large subunit is shown in grey, 5S rRNA in black, 5.8S rRNA in red and the 60S r-proteins in blue. The 18S rRNA from the small subunit is shown in pale green and the 40S r-protein in gold. The cartoon was generated with the UCSF Chimera program [3], using the crystal structure of the yeast 80S ribosome (PDB files 3U5F, 3U5G, 3U5H and 3U5I, [4]).

Table S1. Yeast strains used in this study

Strain	Relevant genotype	Reference
W303-1A	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	Ref. [5]
W303-1B	As W303-1A but *MAT*α	Ref. [5]
MS157-1A	As W303-1A but *dob1-1*	Ref. [6]
MS157-13B	As W303-1B but *dob1-1*	Ref. [6]
JDY4	As W303-1B but *rrp4::HIS3* [pRS415-rrp4-1]	Ref. [6]
R95-1-1	*MAT***a** *ade2-1 ade3 cep1*Δ *cry1 cyh2 his4-580 leu2 lys2 ura3 tyr1 SUP4-3* [pAP2-*CEP1/LEU2/ADE3*]	Ref. [7]
BM3-40A	As R95-1-1 but *csl4-1*	Ref. [7]
YTK301	*MAT*α *ura3-52 mtr3-1*	Ref. [8]
YAM587	As W303-1A but *rrp6*::kanMX4	Ref. [9]
BY4741	*MAT***a** *his3*Δ*0 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*	Euroscarf
Y1909	As BY4741 but *rrp47*::kanMX4	Euroscarf
Y871	As BY4741 but *ngl2*::kanMX4	Euroscarf
JDY1234	As W303-1A but *NOP7*-TAP::natNT2	This study [(a)]
JDY1235	As W303-1A but *ARX1*-TAP::natNT2	This study [(a)]
JDY1236	As W303-1A but *LSG1*-TAP::natNT2	This study [(a)]
Y3906	As W303-1B but *RPL24A*-TAP::HIS3MX4	This study [(b)]
JDY1238	As W303-1A but *dob1-1 NOP7*-TAP::natNT2	This study [(b)]
JDY1239	As W303-1B but *dob1-1 ARX1*-TAP::natNT2	This study [(b)]
JDY1240	As W303-1B but *dob1-1 LSG1*-TAP::natNT2	This study [(b)]
JDY1237	As W303-1A but *dob1-1 RPL24A*-TAP::HIS3MX4	This study [(b)]
JDY1241	As W303-1B but *rrp4::HIS3* [pRS415-rrp4-1] *NOP7*-TAP::natNT2	This study [(c)]
JDY1242	As W303-1B but *rrp4::HIS3* [pRS415-rrp4-1] *LSG1*-TAP::natNT2	This study [(c)]
Y6265	As BY4741 but *trf4*:: kanMX4	Euroscarf
Y196	As W303-1A but *trf4*::HIS3MX4	This study [(d)]
Y207	As W303-1A but *air1*::HIS3MX4	This study [(d)]
JDY1106	As R95-1-1 but *csl4-1 trf4*:: kanMX4	This study [(e)]
JDY1104	As YTK301 but *trf4*:: kanMX4	This study [(e)]
JDY1246	As W303-1B but *rrp4::HIS3* [pRS415-rrp4-1] *trf4*:: kanMX4	This study [(e)]
JDY1247	As W303-1B but *rrp4::HIS3* [pRS415-rrp4-1] *trf4*:: kanMX4 *air1*::HIS3MX4	This study [(f)]
JDY911	As W303-1A but *LSG1-GFP*::natNT2	Ref. [10]
JDY1248	As W303-1A but *dob1-1 LSG1-GFP*::natNT2	This study [(g)]

[(a)] Strains JDY1234, JDY1235 and JDY1236 are meiotic segregants of the genetic cross between W303-1B and strains Y3852, Y3858 and Y3860, respectively, described in [11]; all these strains differ in the genes subjected to the integration of the C-terminal TAP-tag and have in common the genotype of strain Y4219 (As W303-1A but *rix7*::kanMX6 *ade3*::kanMX4 *NSA1*-GFP::HIS3MX6 [pHT4467Δ-RIX7]) [11].

[(b)] Strain Y3906 was generated by integration of a C-terminal TAP tag at the genomic locus, performed by a standard procedure [12].

[(c)] Strains JDY1241 and JDY1242 are meiotic segregants of the genetic cross between JDY4 and strains JDY1234 and 1236, respectively.

[(d)] Strains Y196 and Y207 were generated by deletion disruption of the *TRF4* and *AIR1* genes, respectively, with the cassette HIS3MX4, performed by a standard procedure [12].

[(e)] Strains JDY1106, JDY1104 and JDY1246 were obtained by deletion disruption of the *TRF4* gene in strains BM3-40A, YTK301 and JDY4, respectively. The *trf4*::kanMX4 cassette was amplified from genomic DNA of strain Y6265.

[(f)] Strain JDY1247 is a meiotic segregant of the genetic cross between JDY1246 and Y207.

[(g)] Strain JDY1248 is meiotic segregant of the genetic cross between MS157-13B and JDY911.

Table S2. Oligonucleotides used in this study

Name [a]	5'-3' Sequence
Probe e (5.8S)	TTTCGCTGCGTTCTTCATC
Probe f (E/C2)	GGCCAGCAATTTCAAGTTA
Probe g (C1/C2)	GAACATTGTTCGCCTAGA
Probe h (25S)	CTCCGCTTATTGATATGC
Probe 5S	GGTCACCCACTACACTACTCGG
FISH ITS1	ATGCTCTTGCCAAAACAAAAAAATCCATTTTCAAAATTATTAAATTTCT
FISH ITS2-1	AGGCCAGCAATTTCAAGTTAACTCCAAAGAGTATCACTC
FISH ITS2-2	AGAACATTGTTCGCCTAGACGCTCTCTTCTTATCGATAACGTTCCAATAC

[a] All primers used to delete genes, construct fusions or verify strains are not shown in this table and are available upon request.

SUPPLEMENTARY REFERENCES

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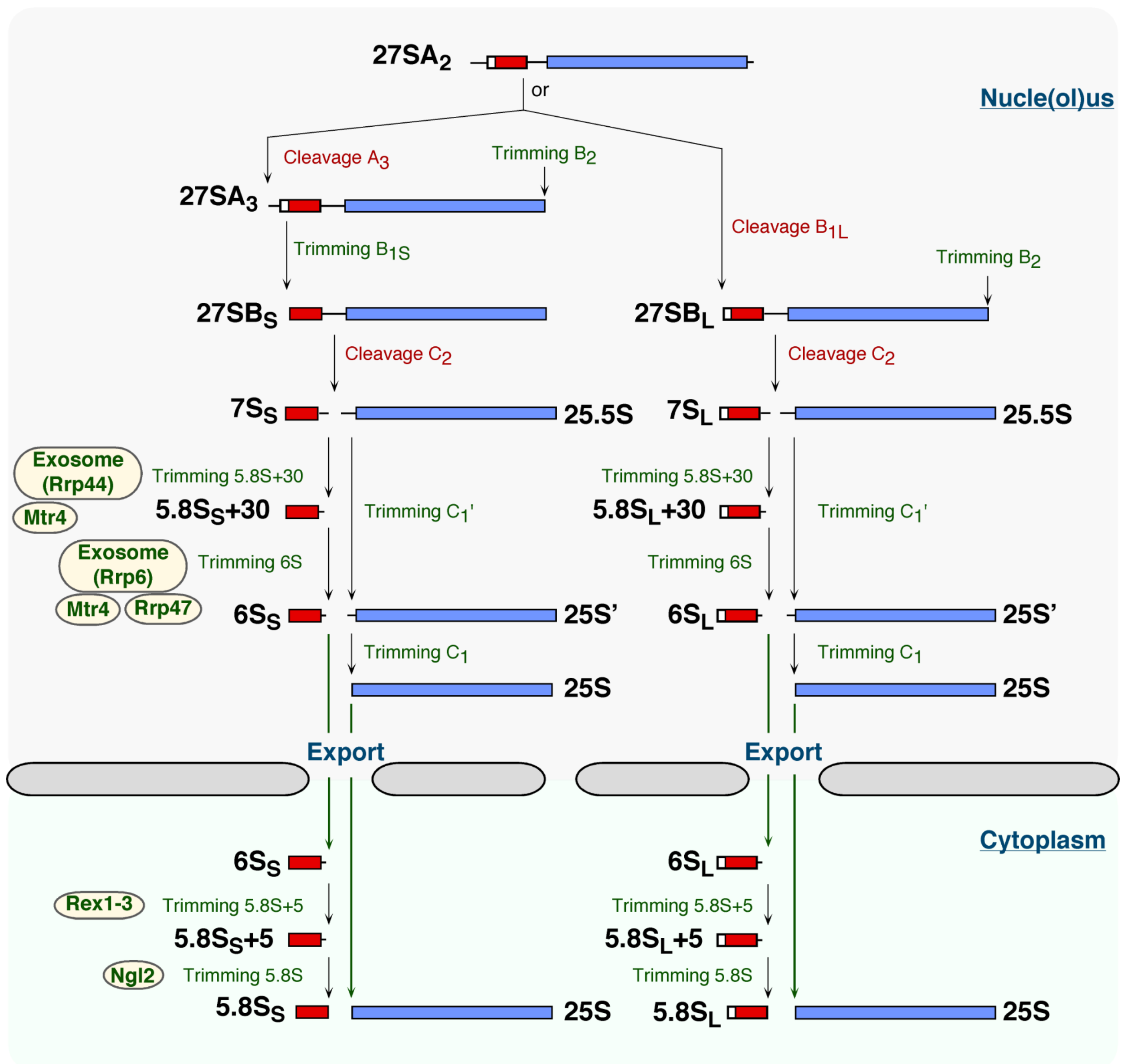
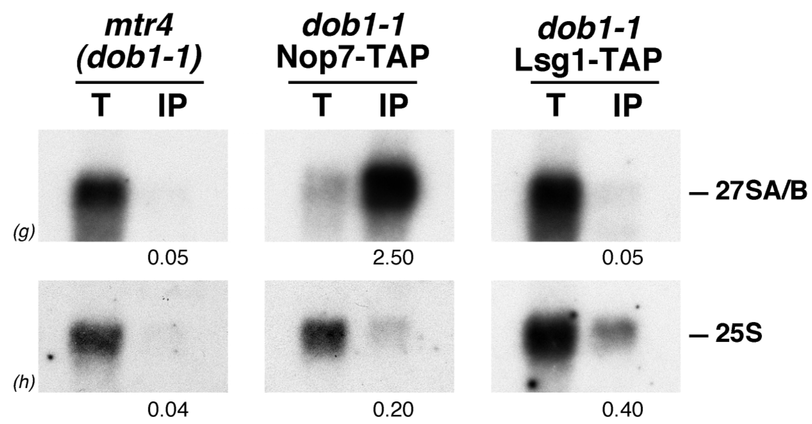


Figure S1



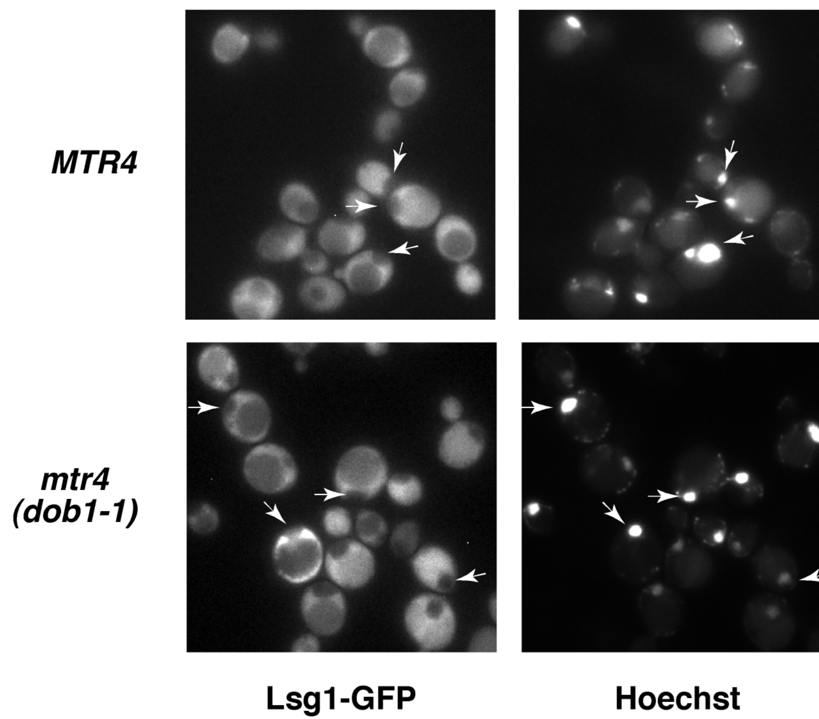
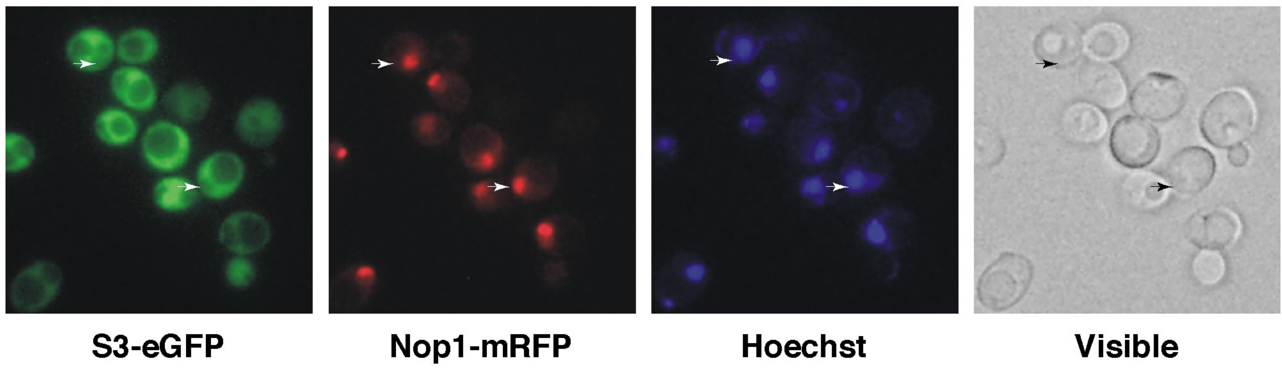


Figure S2

MTR4



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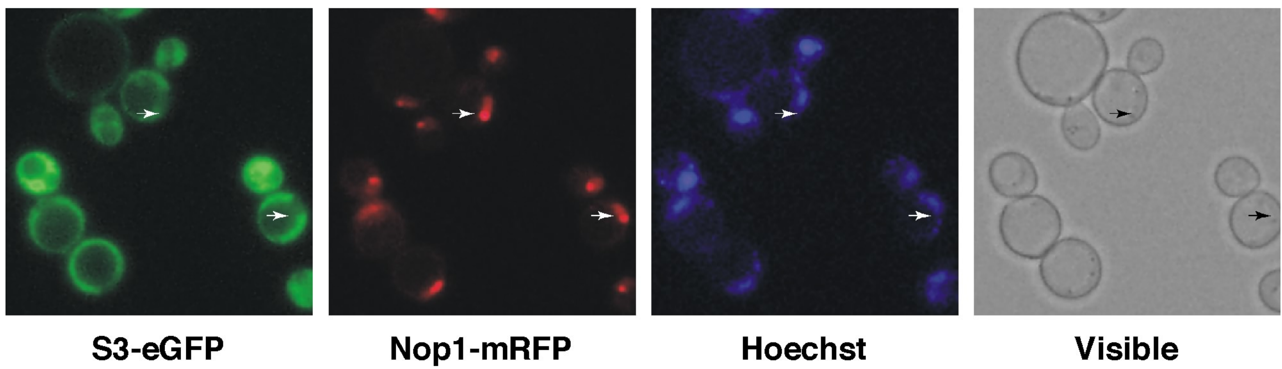
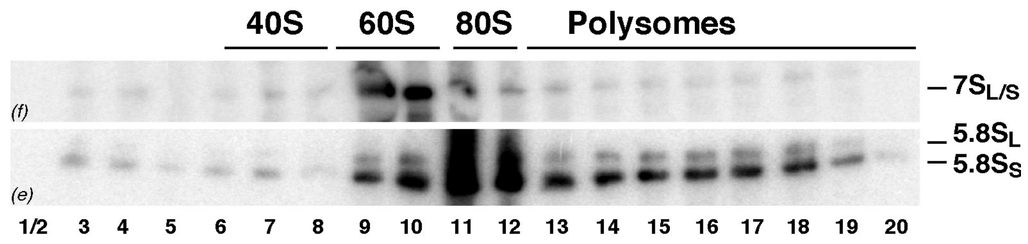


Figure S3

A

MTR4



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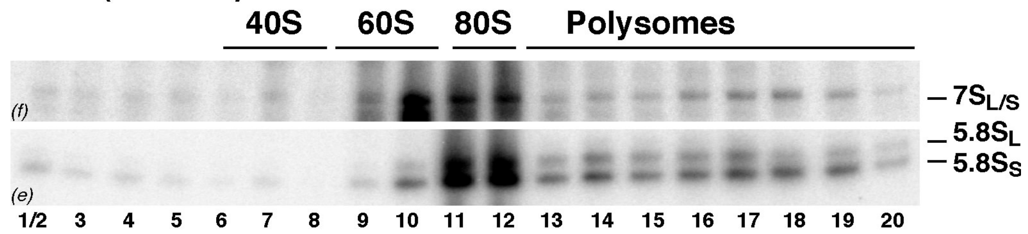
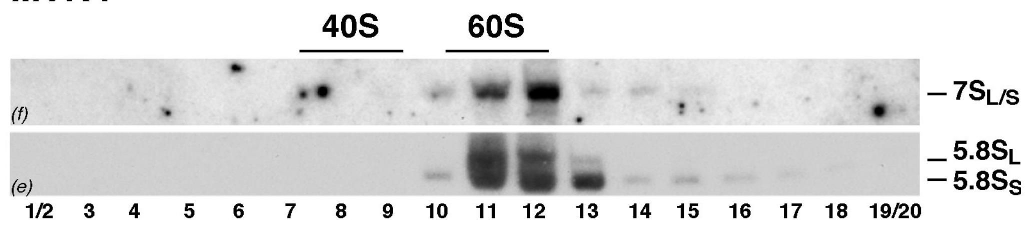


B

MTR4



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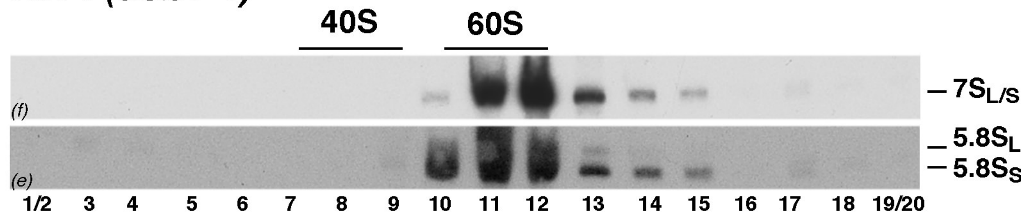

Figure S4

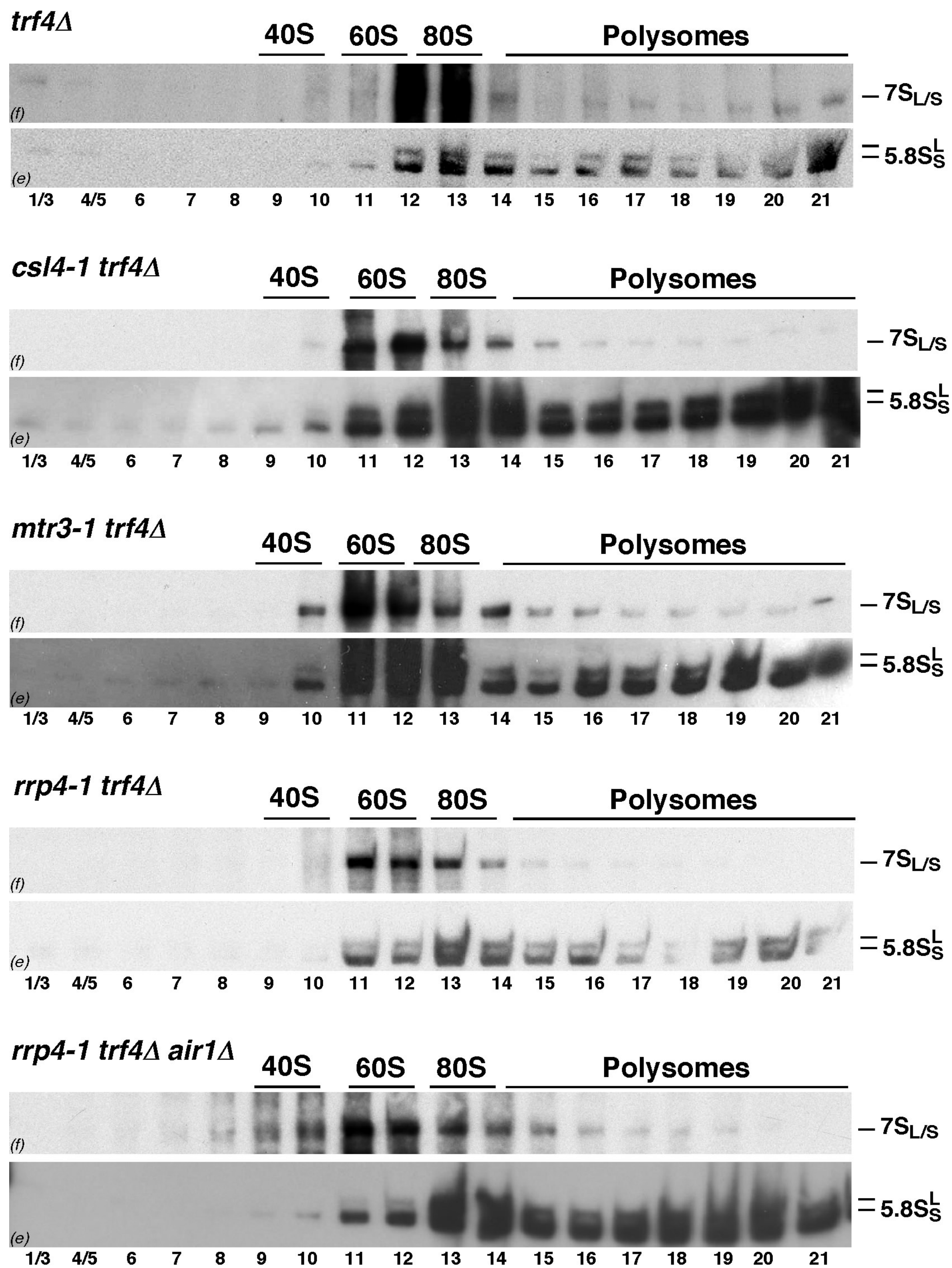

Figure S5

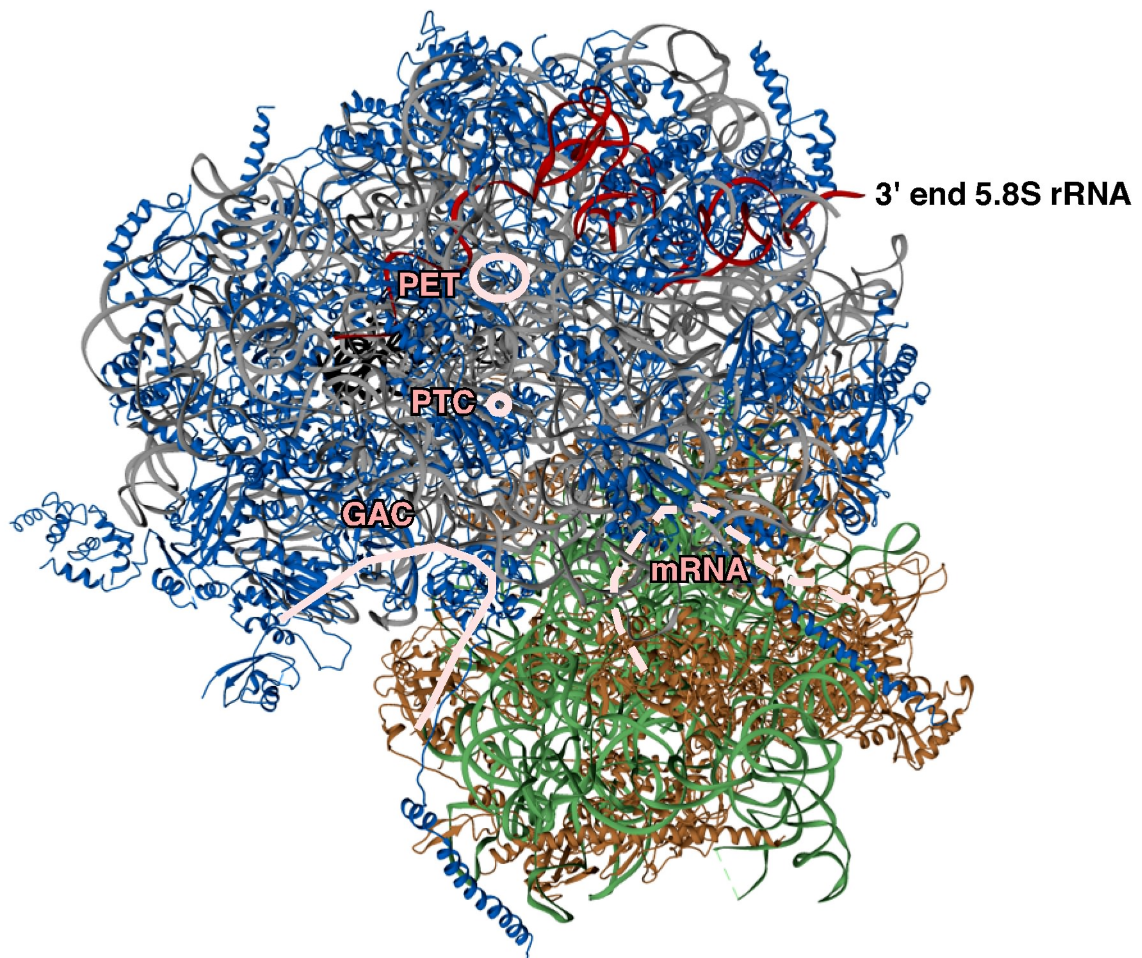

Figure S6