SUPPLEMENTARY DATA TO THE MANUSCRIPT ENTITLED

Ribosomal protein L35 is required for 27SB pre-rRNA processing in Saccharomyces cerevisiae

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SUPPLEMENTARY MATERIAL AND METHODS

Plasmid constructions

YCplac33-RPL35A and YCplac111-RPL35A were constructed as follows; a PCR was performed using yeast genomic DNA as a template and the oligonucleotides RPL35A-LEFT and RPL35A-RIGHT (for the sequence of the oligonucleotides, see Table S2), placed plus-minus 1 kb upstream and downstream from the start-stop codon of the RPL35A ORF, respectively. The ca. 2.4 kb PCR product was cloned into pGEM®-T (Promega), then restricted with BamHI and XbaI and cloned into YCplac33 and YCplac111 (1), which were also digested with the same enzymes. The resulting plasmids, whose correctness was confirmed by DNA sequencing, complemented the null rpl35A allele to the wild-type extent.

YCplac33-RPL35B and YCplac111-RPL35B were constructed as follows; a PCR was performed using yeast genomic DNA as a template and the oligonucleotides RPL35B-LEFT and RPL35B-RIGHT (Table S2), placed plus-minus 1 kb upstream and downstream from the start-stop codon of the RPL35B ORF, respectively. The ca. 2.6 kb PCR product was cloned into pGEM®-T, then restricted with EcoRI and cloned into YCplac33 and YCplac111, which were also digested with the same enzyme. The resulting plasmids, whose correctness was confirmed by DNA sequencing, complemented both the null rrpl35A and rpl35B allele to the wild-type extent (Figure S5).

To construct pAS24-RPL35A, a ca. 360 bp PCR product containing the L35A coding region was obtained by PCR using the RPL35AGAL-LEFT and RPL35AGAL-RIGHT primers (Table S2) and yeast genomic DNA as a template. This product was cloned into pGEM®-T, then restricted with SphI and SalI and cloned into pAS24 (2), which was also digested with the same enzyme. The resulting plasmid, whose correctness was confirmed by DNA sequencing, complemented the null rpl35A allele to the wild-type extent.
To generate YCplac111-RPL35B-GFP, a 1.8 kb PCR product containing the \textit{RPL35B} ORF lacking the termination codon and an additional 1 kb upstream the ORF was obtained by PCR using the oligonucleotides RPL35BpHAC-LEFT and RPL35BpHAC-RIGHT (Table S2) and yeast genomic DNA as a template. This product was cloned in pGEM\textregistered-T easy (Promega), then restricted with \textit{KpnI} and \textit{BamHI} and cloned into YCplac111-yGFP, which was also digested with the same enzymes. YCplac111-RPL35B-GFP complemented the null \textit{rpl35B} allele to the wild-type extent.

All plasmids used in this study are listed in Table S3.
Table S1. Yeast strains used in this study

| Strain | Relevant genotype | Reference |
|--------|-------------------|-----------|
| BY4743 | MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0 | (3) |
| BY4741 | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | (3) |
| BY4742 | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | (3) |
| Y23889 | As BY4743 but RPL35A::kanMX4/RPL35A | Euroscarf |
| Y23834 | As BY4743 but RPL35B::kanMX4/RPL35B | Euroscarf |
| RBY138 | MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 RPL35A::kanMX4 | This work |
| RBY139 | MATa his3Δ1 leu2Δ0 ura3Δ0 RPL35B::kanMX4 | This work |
| RBY168 | As RBY139 but MATα | This work |
| RBY175 | MATα his3Δ1 leu2Δ0 ura3Δ0 RPL35A::kanMX4 [pAS24-RPL35A (CEN, LEU2, GAL::RPL35A)] | This work |
| AJY1539 | MATa his3Δ1 leu2 met15Δ0 ura3 CRM1(T539C)-HA | (4) |
Table S2. Oligonucleotides used in this study

| Name             | 5'-3' Sequence                                      |
|------------------|-----------------------------------------------------|
| RPL35A-LEFT      | TGTATGAAGGTTTGGATGGTTAAG                             |
| RPL35A-RIGHT     | GAACTAAAAGTGAAAGACCCACAAA                            |
| RPL35B-LEFT      | TAGATGGTTCCGGTAAGACC                                 |
| RPL35B-RIGHT     | TTGGCGAAATGGAAAGAGTC                                 |
| RPL35AGAL-LEFT   | GCGTCGACATGGTATGTGTGGATG                             |
| RPL35AGAL-RIGHT  | GCGCATGCATTGAACATTTGGCCAATA                           |
| RPL35BpHAC-LEFT  | CTAGAGGATCCCCCGGTACCAGCTC                            |
| RPL35BpHAC-RIGHT | CGCGGATCCAGCCTTGATAGCGTAC                            |
| Probe a (5'A0)   | GGTCTCTCTGCTGCCGG                                   |
| Probe b (18S)    | CATGGCTTAATCTTGAGAC                                 |
| Probe c (3-D/A2) | GACTCTCCATCTCTGGATCTGG                              |
| Probe d (A2/A3)  | TGTACCTCTGGGCCC                                     |
| Probe e (5.8S)   | TTTGCTGCTGTTCTTCATC                                 |
| Probe f (E/C2)   | GGCCAGCAATTTCAGTCA                                  |
| Probe g (C1/C2)  | GAACATGGTGCGCTAGA                                   |
| Probe h (25S)    | CTCCGCTTATTGGATATGC                                 |
| Probe 5S         | GGTCACCCACTACACTACTCGG                              |
Table S3. Plasmids used in this study

| Plasmid               | Features                                | Reference |
|-----------------------|-----------------------------------------|-----------|
| pAS24-RPL35A          | GAL-HA::RPL35A CEN LEU2                | This study|
| YCplac33              | CEN URA3                                | (1)       |
| YCplac111             | CEN LEU2                                | (1)       |
| YCplac33-RPL35A       | RPL35A CEN URA3                        | This study|
| YCplac111-RPL35A      | RPL35A CEN LEU2                        | This study|
| YCplac111-RPL35B      | RPL35B CEN LEU2                        | This study|
| YCplac-111-RPL35B-eGFP| RPL35B-eGFP CEN LEU2                  | This study|
| pRS315-RPL25-eGFP     | RPL25-eGFP CEN LEU2                   | (5)       |
| pRS316-RPL25-eGFP     | RPL25-eGFP CEN URA3                   | (5)       |
| pRS315-RPS2-eGFP      | RPS2-eGFP CEN LEU2                    | (6)       |
| pRS316-RPS2-eGFP      | RPS2-eGFP CEN URA3                    | (6)       |
| pUR34-DsRed-NLS       | DsRed-NLS CEN HIS3                     | (7)       |
| pRS413-DsRed-NOP1     | DsRed-NOP1 CEN HIS3                    | This study|
| pRS316-GAL-NMD3FL     | GAL::NMD3 CEN URA3                     | (8)       |
| pRS316-GAL-nmd3Δ100   | GAL::nmd3Δ100 CEN URA3                 | (8)       |
LEGENDS TO THE SUPPLEMENTARY FIGURES

Figure S1. Pre-rRNA processing in *S. cerevisiae*. (A) Structure and processing sites of the 35S pre-rRNA. This precursor contains the sequences for the mature 18S, 5.8S and 25S rRNAs that are separated by two internal transcribed spacer sequences, ITS1 and ITS2, and flanked by two external transcribed spacer sequences, 5' ETS and 3' ETS. The mature rRNA species are shown as bars and the transcribed spacer sequences as lines. The processing sites and the various probes used are indicated. (B) Pre-rRNA processing pathway. Cleavage and trimming reactions are indicated. Note that pre-rRNA processing can also occur co-transcriptionally at site A2. For reviews on pre-rRNA processing, see (9,10).

Figure S2. Model for the assembly position of ribosomal protein L35. (A) Current model for the formation, maturation and export of 66S pre-ribosomal particles in *S. cerevisiae*. A series of distinct particles are predicted to be intermediates during the synthesis of 60S r-subunits. These are termed, according to their position in the pathway, early 0 (E0), early 1 (E1), early 2 (E2) and middle (M) 66S pre-ribosomal particles and late (L) and cytoplasmic (pre-60S) pre-60S r-particles. All these particles are defined by the purification of complexes associated with TAP-tagged versions of selected r-subunit biogenesis factors. The predominant pre-rRNAs associated with the different particles are indicated. Nucleolus, grey rectangle; pre-ribosomal particles and mature r-subunits, ovals; nuclear envelope, rods; L35, red structure. For reviews see (11-13). Herein, we propose that L35 (at least L35B) binds to 5.8S rRNA in early E0 pre-60S r-particles. (B) The depletion of L35 leads to an inhibition of 27SA3 and 27SB pre-rRNA processing, thus, pre-60S r-particles containing these precursors accumulate (indicated by dark blue ovals with thick continuous lines). As a consequence, there is a clear reduction in medium, late and cytoplasmic pre-60S r-particles and a deficit in mature 60S r-subunits (indicated by light blue ovals with discontinuous lines). In addition, upon depletion of L35, there is a delay of 35S pre-rRNA processing, which causes a mild decrease in early E0 pre-60S r-particles (also indicated by light blue ovals with discontinuous lines).

Figure S3. L35 is conserved throughout evolution. Comparison of the amino acid sequences of eukaryotic L35 from *Canis familiaris*, *Homo sapiens* and *S. cerevisiae*, eubacterial L29 from *Escherichia coli* and archaeal L29 from *Haloarcula marismortui*. Proteins were aligned using ClustalW2 at EMBL-EBI (www.ebi.ac.uk/Tools/clustalw2/). Note that the C-terminal extension of the eukaryotic L35 proteins is not present in the prokaryotic L29 proteins.

Figure S4. L35 is located close to L25 surrounding the exit tunnel of the nascent polypeptides. Three-dimensional model of the *S. cerevisiae* 60S r-subunit seen from the exit side (T) of the
nascent polypeptide tunnel (right) or rotated 180° in the y-axis (left). The r-proteins surrounding the tunnel exit are coloured in gold except L35 in red. To orientate the 60S r-subunit, 5S rRNA (yellow) and r-proteins L1 and L12 (green) are also highlighted. The rest of ribosomal proteins are coloured in grey and the rRNA in blue. The cartoons were generated with the UCSF Chimera programme (www.cgl.ucsf.edu/chimera/) using the yeast cryo-EM-based 60S ribosomal subunit mapped onto the *H. marismortui* X-ray structure (PDB code 1S1I; (14)).

**Figure S5. Functional analysis of the L35-eGFP construct.** (A) RBY138 (*Δrpl35A*), RBY139 (*Δrpl35B*) and BY4741 (*Wild-type*) were transformed with YCplac111 or the complementing YCplac111-RPL35A, YCplac111-RPL35B or YCplac111-RPL35B-eGFP, grown in SD-Leu and diluted to an OD₆₀₀ of 0.05. A 10-fold series of dilutions was performed for each strain and 5 µl drops were plated on SD-Leu plates. Plates were incubated at 30°C for 4 days. (B) The above strains were grown in SD-Leu at 30°C and harvested at an OD₆₀₀ of 0.8, cell extracts were prepared and 10 A₂₆₀ of each extract were resolved in 7-50% sucrose gradients. The A₂₅₄ was continuously measured. Sedimentation is from left to right. The peaks of free 40S and 60S r-subunits, 80S free couples/monosomes and polysomes are indicated. Half-mers are labeled by arrows.

**Figure S6. L35 binds to 5.8S rRNA.** (A) Detailed view of yeast L35 in the context of its binding site in the 60S r-subunit. Only rRNA residues situated at or closer than 10 Å from L35 are shown (bases and phosphate backbone). Note that only residues A2 to E60 of the yeast L35 structure could be modelled (surface and ribbons upper panel; only ribbon bottom panel). Base numbering follows the rRNA sequence deposited for the yeast cryo-EM-based 60S r-subunit fitted onto the *H. marismortui* X-ray structure, which for all residues numbered is indeed the 23S rRNA from *H. marismortui* (PDB code 1S1I; (14)). (B) Detail view of human L35 in the context of its binding site. As above, only rRNA residues situated at or closer than 10 Å from L35 are shown (only phosphate backbone). Note that only residues from I4 to T64 of the human L35 structure could be modelled (surface and ribbons upper panel; only ribbon bottom panel). Base numbering follows the rRNA sequence deposited for the model of canine cryo-EM-based 60S r-subunit, which for all residues numbered correspond to either 25S rRNA or 5.8S human rRNAs (PDB file 2ZKR; (15)). (C) Secondary structure of 23S rRNA domain I from *H. marismortui* as represented in (16). Blue spheres indicate rRNA residues situated closer than 5 Å from yeast L35. Note that these residues are equivalent to specific 5.8S rRNA residues in eukaryotes. (D) Secondary structure of the mammalian 25S/5.8S rRNA domain I as represented in (15). Blue spheres indicate rRNA residues situated closer than 5 Å from mammalian L35.
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Figure S1

Supplementary data, Babiano and de la Cruz
Figure S2
**Figure S3**
Figure S4
Figure S5
Figure S6