SUPPLEMENTARY MATERIAL TO THE MANUSCRIPT ENTITLED

Saccharomyces cerevisiae ribosomal protein L26 is not essential for ribosome assembly and function

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Plasmid constructions

YCplac111-*RPL26A*; a PCR was performed using yeast genomic DNA as a template and the oligonucleotides RPL26A-LEFT and RPL26A-RIGHT (for the sequence of the oligonucleotides, see **Table S3**), placed plus-minus 1 kb upstream and downstream from the start-stop codon of the *RPL26A* ORF, respectively. The ca. 2.5 kb PCR product was cloned into pGEM®-T (Promega), then digested with *Eco*RI and cloned into YCplac111 (7), which was also digested with the same enzyme.

YCplac111-*RPL26B*; a PCR was performed using yeast genomic DNA as a template and the oligonucleotides RPL26B-LEFT and RPL26B-RIGHT (**Table S3**), placed plus-minus 1 kb upstream and downstream from the start-stop codon of the *RPL26B* ORF, respectively. The ca. 2.5 kb PCR product was cloned into pGEM®-T, then digested with *Sph*I *and Pst*I and cloned into YCplac111, which was also digested with the same enzymes.

YCplac111-*RPL26A-GFP*; a 1.8 kb PCR product containing the *RPL26A* ORF lacking the termination codon and an additional 1 kb upstream the ORF was obtained by PCR using the oligonucleotides RPL26A-LEFT and L26A-TAG (**Table S3**) and yeast genomic DNA as a template. This product was cloned into pGEM®-T Easy (Promega), then restricted with *Sma*I and *Bam*HI and cloned into YCplac111-yeGFP (gift from M. N. Hall), which was also digested with the same enzymes. YCplac111-*RPL26A-eGFP* complemented the *rpl26* null mutant to the wild-type extent.

YCplac111-*RPL26B-GFP*; a 1.8 kb PCR product containing the *RPL26B* ORF lacking the termination codon and an additional 1 kb upstream the ORF was obtained by PCR using the oligonucleotides RPL26B-LEFTpHTΔ and L26B-TAG (**Table S3**) and yeast genomic DNA as a template. This product was cloned in pGEM®-T Easy, then restricted with *Sal*I and *Bam*HI and cloned into YCplac111-yeGFP, which was also digested with the same enzymes. YCplac111-*RPL26B-GFP* complemented the *rpl26* null mutant to the wild-type extent.

The correctness of all resulting plasmids was confirmed by DNA sequencing.

Table S1. Yeast strains used in this study

Strain	Relevant genotype	Reference
BY4743	*MAT***a***/MAT*α *his3*Δ*1/his3*Δ*1 leu2*Δ*0/leu2*Δ*0 lys2*Δ*0/LYS2 met15*Δ*0/MET15 ura3*Δ*0/ura3*Δ*0*	(2)
BY4741	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*	(2)
Y25253	As BY4743 but *RPL26A*::kanMX4/*RPL26A*	Euroscarf
Y24664	As BY4743 but *RPL26B*::kanMX4/*RPL26B*	Euroscarf
RBY272	*MAT***a** *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 RPL26A*::kanMX4	This work
RBY273	*MAT*α *his3*Δ*1 leu2*Δ*0 ura3*Δ*0 met15*Δ*0 RPL26A*::kanMX4	This work
RBY274	*MAT***a** *his3*Δ*1 leu2*Δ*0 ura3*Δ*0 RPL26B*::kanMX4	This work
RBY275	*MAT*α *his3*Δ*1 leu2*Δ*0 ura3*Δ*0 met15*Δ*0 RPL26B*::kanMX4	This work
RBY276	*MAT***a** *his3*Δ*1 leu2*Δ*0 ura3*Δ*0 RPL26A*::kanMX4 *RPL26B*::kanMX4	This work
JWY6147	*MAT***a** *ura3-52 trp1-1 lys2-801 his3-*Δ*200 leu2-*Δ*1*	E. Jones
JWY9626	*MAT***a** *ura3-52 trp1-1 lys2-801 his3-*Δ*200 leu2-*Δ*1 TRP1::GAL::3xHA-RPL26A RPL26B:*::kanMX6	This work
JWY9634	*MAT***a** *ura3-52 trp1-1 lys2-801 his3-*Δ*200 leu2-*Δ*1 TRP1::GAL::3xHA-RPL26A RPL26B:*::kanMX6 *NOP7-TAP::URA3*	This work
JWY9663	*MAT***a** *ura3-52 trp1-1 lys2-801 his3-*Δ*200 leu2-*Δ*1 TRP1::GAL::3xHA-RPL26A RPL26B:*::kanMX6 *NOP7-TAP::URA3 RPL35B-13xMYC::HIS3*	This work
JWY9691	*MAT***a** *ura3-52 trp1-1 lys2-801 his3-*Δ*200 leu2-*Δ*1 TRP1::GAL::3xHA-RPL26A RPL26B:*::kanMX6 *NOP7-TAP::URA3 RPL37B-13xMYC::HIS3*	This work

Table S2. Plasmids used in this study

Name	Relevant information	Reference
YCplac33	*CEN URA3*	(6)
YCplac111	*CEN LEU2*	(6)
YCplac-111-*RPL26A*	*RPL26A CEN LEU2*	This work
YCplac-111-*RPL26B*	*RPL26B CEN LEU2*	This work
YCplac111-yeGFP	C-terminal yeGFP *CEN LEU2*	M. Hall
YCplac-111-*RPL26A-eGFP*	*RPL26A-eGFP CEN LEU2*	This work
YCplac-111-*RPL26B-eGFP*	*RPL26B-eGFP CEN LEU2*	This work
pRS316-*RPL25-eGFP-NOP1-mRFP*	*RPL25-eGFP NOP1-mRFP CEN URA3*	(13)
pRS316-*RPS3-eGFP-NOP1-mRFP*	*RPS3-eGFP NOP1-mRFP CEN URA3*	(13)
pRS316-*GAL-NMD3FL*	*GAL::NMD3 CEN URA3*	(1)
pRS316-*GAL-NMD3Δ100*	*GAL::NMD3Δ100 CEN URA3*	(1)
p0-LacZ	*PGK1::lacZ 2μ TRP1*	(4, 5)
p1-LacZ	*PGK1::lacZ* (-1 frameshift) *2μ TRP1*	(4, 5)
p+1-LacZ	*PG1K::lacZ* (+1 frameshift) *2μ TRP1*	(4, 5)
pUK815	*PGK1::lacZ CEN URA3*	(12)
pUK817	*PGK1::lacZ* (premature TAA codon) *CEN URA3*	(12)
pUK818	*PGK1::lacZ* (premature TAG codon) *CEN URA3*	(12)
pUK819	*PGK1::lacZ* (premature TGA codon) *CEN URA3*	(12)
p367	*HIS4-lacZ* (ATG start codon) *URA3*	(3)
p391	*HIS4-lacZ* (TGG start codon) *URA3*	(3)
pBS1479	TAP tag fragment *CEN K. lactis-TRP1*	(11)
pFA6a-TRP1-PGAL1-3xHA	GAL promoter 3xHA tag *CEN K.lactis-TRP1*	(10)
pFA6a-kanMX6	kanMX6	(15)
pFA6a-HIS3MX6	HIS3MX6	(15)

Table S3. Oligonucleotides used in this study

Name	5'-3' Sequence
L26A-LEFT	AGTCGCCGAGTTTTGTCTTC
L26A-RIGHT	GCTGACCTGACAAGTCGTTG
L26B-LEFT	CGGAGTAAAGACCTAGGCAAA
L26B-RIGHT	AAACAACTAGTGACAAGACGGAAG
L26A-TAG	CGGGATCCTTCCAACTTACC
L26B-TAG	GCGTCGACTTCCAATTTACC
RPL26B-LEFTpHTΔ	CGGGATCCCGCGGAGTAAAGACCTAGGCAAA
RPL26A-RIGHTpHTΔ	CGGGATCCCGGCTGACCTGACAAGTCGTTG
RPL26A-LEFTpHTΔ	CGGGATCCCGAGTCGCCGAGTTTTGTCTTC
L26 Probe	ACGTTTCCTCTGACAGAAGAAAGG
RPL35AGAL-LEFT	GCGTCGACATGGTATGTTTGAGATG
RPL35AGAL-RIGHT	GCGCATGCATTGAACTTTGCCAATA
ADH1 UP	GCACGGTGACTGGCCATT
ADH1 LOW	AGACTCTGTAACCCATAGCCTTGG
Probe a (5'A_0)	GGTCTCTCTGCTGCCGG
Probe b (18S)	CATGGCTTAATCTTTGAGAC
Probe c (3-D/A_2)	GACTCTCCATCTCTTGTCTTCTTG
Probe d (A_2/A_3)	TGTTACCTCTGGGCCC
Probe e (5.8S)	TTTCGCTGCGTTCTTCATC
Probe f (E/C_2)	GGCCAGCAATTTCAAGTTA
Probe g (C_1/C_2)	GAACATTGTTCGCCTAGA
Probe h (25S)	CTCCGCTTATTGATATGC
Probe 5S	GGTCACCCACTACACTACTCGG
5.8S_3'END	AAATGACGCTCAAACAGGCATGC
25S_421	CCATGTCTGATCAAATGCCC
25S_330	CTTGTTCGCTATCGGTCTCTC
25S_233	CTTTACAAAGAACCGCACTCC
25S_117	GCACCGAAGGTACCAGATTTC

Table S4. Quantification of pre- and mature rRNAs in the *rpl26* null strain

Pre- and mature rRNAs	Relative value[a]
35S	2.2 ± 0.7
32S	2.4 ± 0.9
$27SA_2$	1.5 ± 0.5
27SB	1.6 ± 0.5
25S	1.1 ± 0.4
20S	0.9 ± 0.4
18S	1.2 ± 0.6

[a] Pre- and mature rRNAs were detected by Northern blot hybridisation as shown in Fig. 7A. Signal intensities were measured by phosphoimager scanning. Values were normalised to those obtained for the wild-type control strain, arbitrarily set at 1.0. The mean and the standard error were calculated from three independent experiments.

Table S5. Summary of the translation phenotypes of the *rpl26* null mutant

		Strain	
		Wild-type	*rpl26* null mutant
Assays			
Drug sensitivity[a]			
	Anisomycin	No	No
	AZC	No	No
	Cycloheximide	No	No
	Hygromycin B	No	No
	Paramomycin	No	No
	Neomycin	No	No
Killer maintenance[b]		K^+	K^+
Programmed frameshifting[c]			
	-1 PRF	0.08 ± 0.03	0.08 ± 0.02
	+1 PRF	0.15 ± 0.03	0.15 ± 0.06
Readthrough activity[d]			
	UAA	0.04 ± 0.01	0.03 ± 0.01
	UAG	0.02 ± 0.01	0.02 ± 0.01
	UGA	0.16 ± 0.04	0.17 ± 0.06
Start codon selection[e]		0.08 ± 0.02	0.08 ± 0.02

[a]Effective concentrations of each drug are described in Materials and Methods; No: no effect.

[b]Killer phenotype was evaluated as described in Materials and Methods; K^+: stable killer phenotype.

[c]PFR efficiencies were determined *in vivo* using LacZ reporters as described in Materials and Methods. The values correspond to the ratio of β-galactosidase activity produced by cells harbouring either p-1-LacZ or p+1-LacZ divided by the β-galactosidase activity produced by cell harbouring p0-LacZ. The mean and the standard error were calculated from at least three independent transformants of each plasmid in triplicate.

[d]Readthrough activities were determined *in vivo* using LacZ reporters as described in Materials and Methods.The values correspond to the ratio of β-galactosidase activity produced by cells harbouring either pUK817 (UAA), pUKC818 (UAG) or pUK819 (UGA) divided by the β-galactosidase activity produced by cell harbouring pUKC815 (no premature stop codon). The mean and the standard error were calculated from at least three independent transformants of each plasmid in triplicate.

[e]Start codon selection stringency was determined *in vivo* using LacZ reporters as described in Materials and Methods. The values correspond to the ratio of β-galactosidase activity produced by cells harbouring p391 (*HIS4*^{UUG}*-lacZ)* divided by the β-galactosidase activity produced by cell harbouring p387 (*HIS4*^{AUG}*-lacZ).* The mean and the standard error were calculated from at least three independent transformants of each plasmid in triplicate.

LEGENDS TO THE SUPLEMENTARY 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 spacers as lines. The processing sites and the location of various probes used in this study are indicated. Probes are listed in Table S3. (**B**) Pre-rRNA processing pathway. Cleavage and trimming reactions are indicated. Note that pre-rRNA processing also occurs co-transcriptionally at site A_2 (9) (not shown). For more details on yeast pre-rRNA processing, see (8, 14).

Figure S2. L26 is conserved in the three domains of life. Comparison of the amino acid sequences of eukaryotic L26A and L26B from *S. cerevisiae*, eubacterial L24 from *E. coli* and archaeal L29 from *Haloarcula marismortui.* Proteins were aligned using ClustalW2 at EMBL-EBI (www.ebi.ac.uk/Tools/clustalw2/).

Figure S3. L26 is not essential for growth. The strains JWY6147 (Wild-type) and JWY9626 (*GAL::RPL26*), which is a *rpl26bΔ* strain that harbours a *HA-RPL26A* allele under the control of a *GAL* promoter, were grown in liquid YPGal to an OD_{610} of 0.5. Serial dilutions were spotted on YPGal (Galactose) and YPD (Glucose) plates and incubated for several days at the indicated temperatures.

Figure S4. Depletion of L26 partially perturbs production of 60S ribosomal subunits. Polysome profiles are shown for the *GAL::RPL26* strain described in Fig. S3. Cells were grown to mid log phase in YPGal (Galactose) and shifted for 17 h to YPD (Glucose). Total extracts were prepared and ten A_{260} units of each sample were resolved on 7%-47% sucrose gradients. The A_{254} was continuously monitored. Sedimentation is from left to right. The peaks of free 40S and 60S r-subunits, 80S free couples/monosomes and polysomes are indicated. Half-mer polysomes are labelled by arrows.

Figure S5. L26 depletion leads to a mild pre-rRNA processing defect. Total RNA was extracted from the *GAL::RPL26* strain grown at 30°C in YPGal (Gal) or shifted for 17 h to YPD (Glc). Primer extension was performed with probe f within ITS2 (Table S3). This

probe allows the detection of $27SA_2$, $27SA_3$ and $27SB_{L/S}$ and $7S_{L/S}$ pre-rRNAs as the stops at processing sites A_2, A_3, B_{1L} and B_{1S}, respectively.

Figure S6. Analysis of ribosomes from wild-type and L26-deficient cells. Ribosomal particles were enriched from wild-type and *rpl26* null (*rpl26aΔ rpl26bΔ*) cells as described in Materials and Methods. Then, they were analysed by SDS-PAGE and the presence of selected r-proteins in the preparations was analysed by western blotting using specific antibodies.

Figure S7. *In vivo* chemical probing of the yeast rRNA domain I. (**A**) Secondary structure of yeast 25S/5.8S rRNA domain I showing the location of the primers used for *in vivo* DMS chemical probing. (**B**) Wild-type and *rpl26* null (*rpl26aΔ rpl26bΔ*) cells were treated with DMS. Total RNA was extracted from unmodified (-DMS) and modified (+DMS) yeast cells and analysed by primer extension. Probes, between parentheses, are listed in Table S3. U, A, G, C represent dideoxy sequencing lanes done with the same primers. Stop controls indicate the efficiency of the quenching reaction after DMS treatment. Black and green dots denote nucleotides with stronger and weaker reactivity to DMS, respectively, in the *rpl26* null strain relative to that in the wild-type strain. For a three-dimensional view of the location within the ribosome of these nucleotides, see Fig. 10C.

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SUPLEMENTARY FIGURES

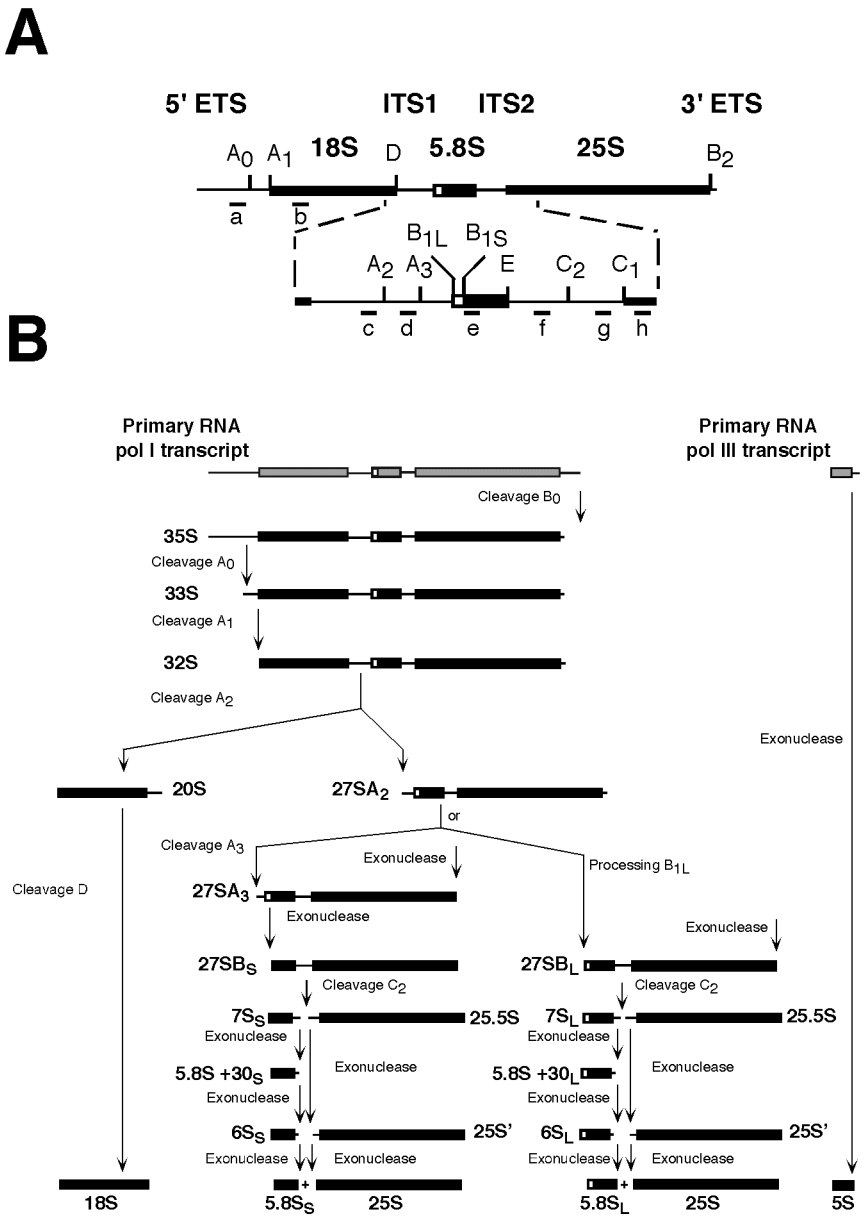

Figure S1

```
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H. marismortui L24  -------MSKQPDKQRKSQRRAPLHERHKQVRATLSADLREEYGQRNVRVNAGDTVEVLR 53
E. coli L24         ------MAAKIRRDDEVIVLTGKDKGKRGKVKNVLSSGKVIVEGIN-LVKKHQKPVPALN 53
                           :.   . .     .    ::  :   **       * . :  .  . * .:.

S. cerevisiae L26A  GSKKGQEGKISSVYRLKFAVQVDKVTKEKVNGASVPINLHPSKLVITKLHLDKDRKALIQ 120
S. cerevisiae L26B  GSKKGQEGKISSVYRLKFAVQVDKVTKEKVNGASVPINLHPSKLVITKLHLDKDRKALIQ 120
H. marismortui L24  GDFAGEEGEVINVDLDKAVIHVEDVTLEKTDGEEVPRPLDTSNVRVTDLDLEDEKREARL 113
E. coli L24         -----QPG---GIVEKEAAIQVSNVAIFNAATGKADR---------VGFRFEDGKKVRFF 96
                         : *   .:   : .::*..*:  :.   ..           . : ::. ::

S. cerevisiae L26A  RKGGKLE- 127
S. cerevisiae L26B  RKGGKLE- 127
H. marismortui L24  ESEDDSA- 120
E. coli L24         KSNSETIK 104
                    .. ..
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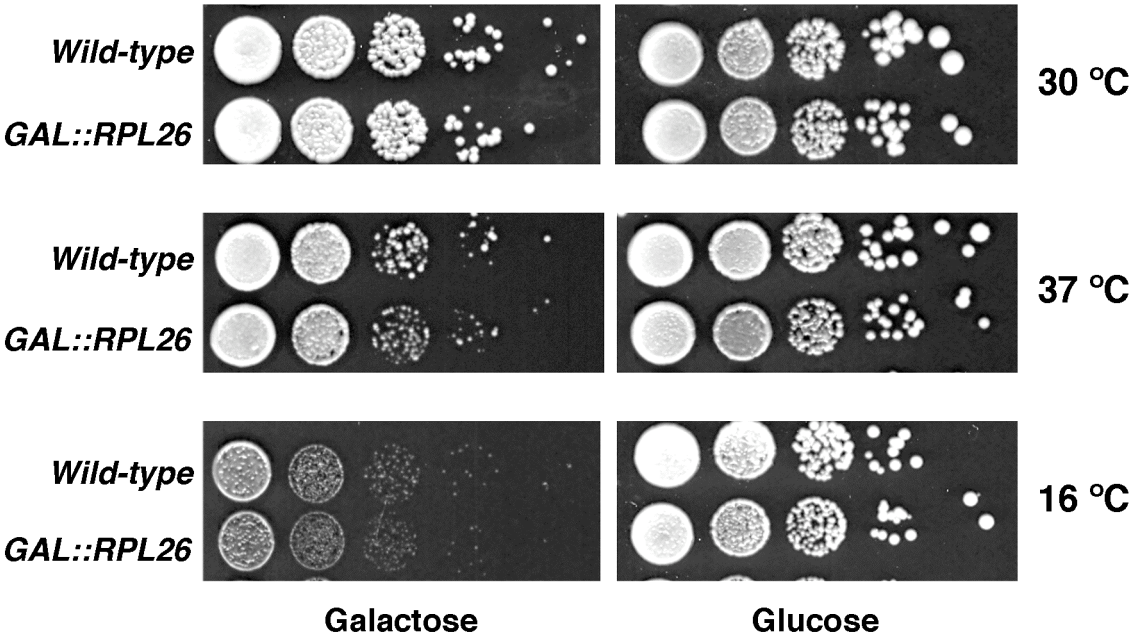
Figure S2


Wild-type
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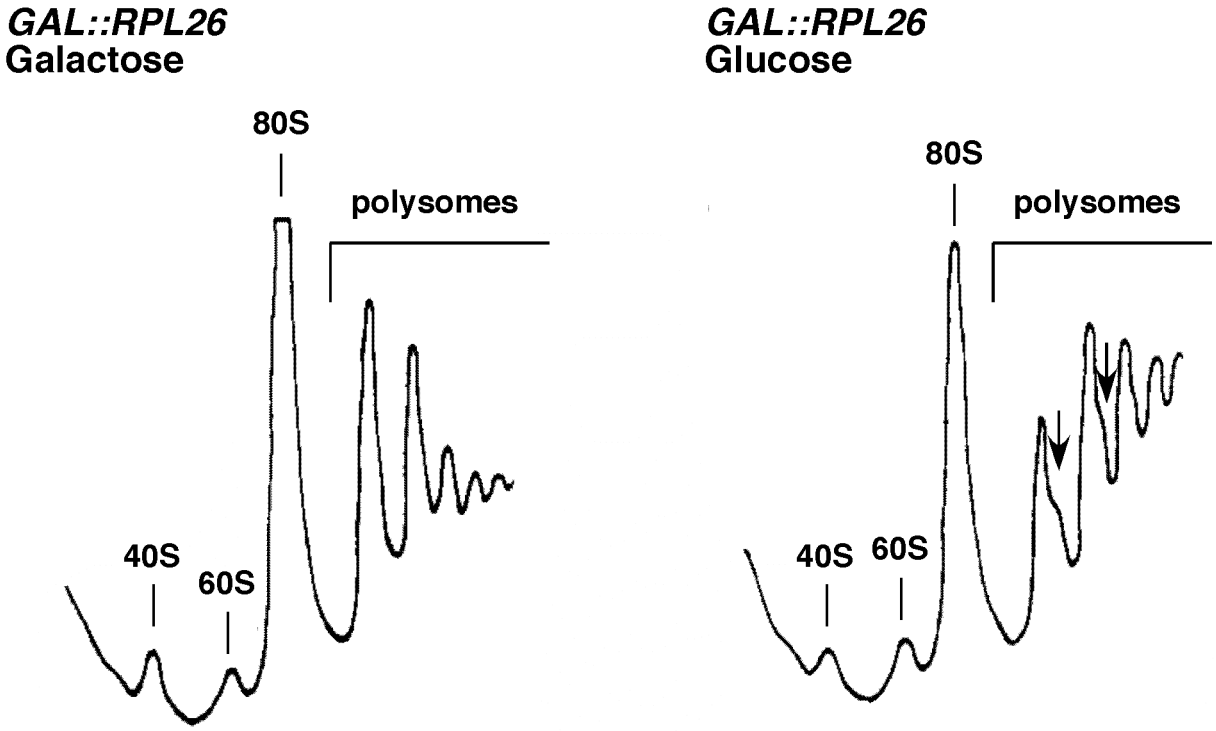
16 °C
Galactose
Glucose

Figure S3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60S

Figure S4

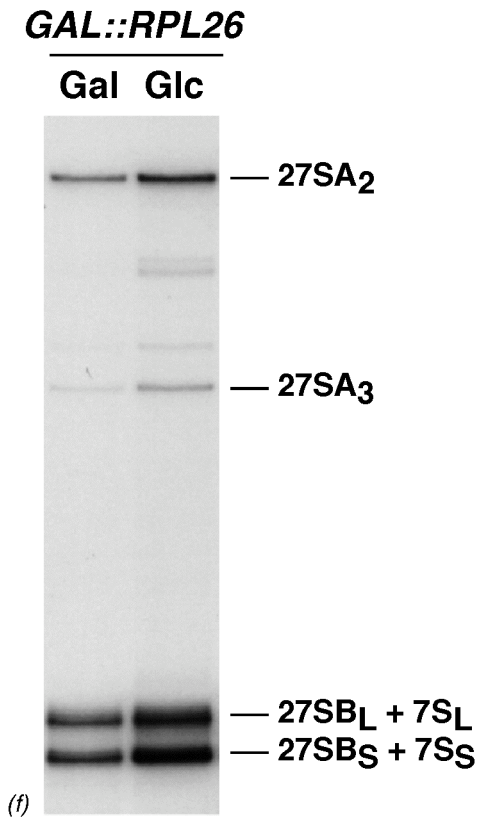


Figure S5

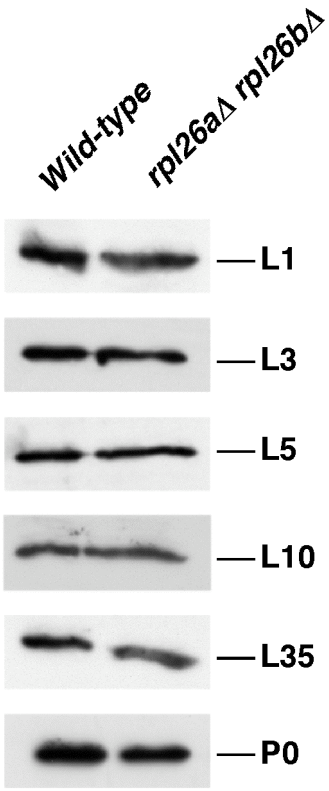
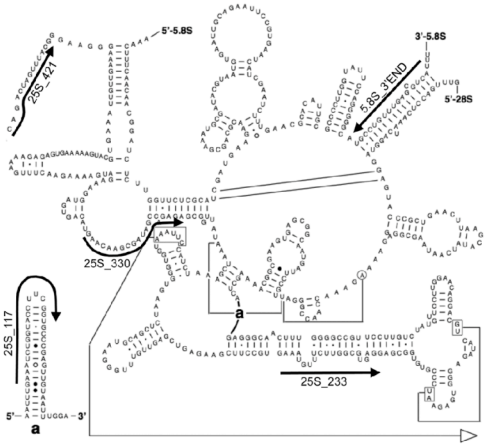


Figure S6

A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a
5'
3'

B

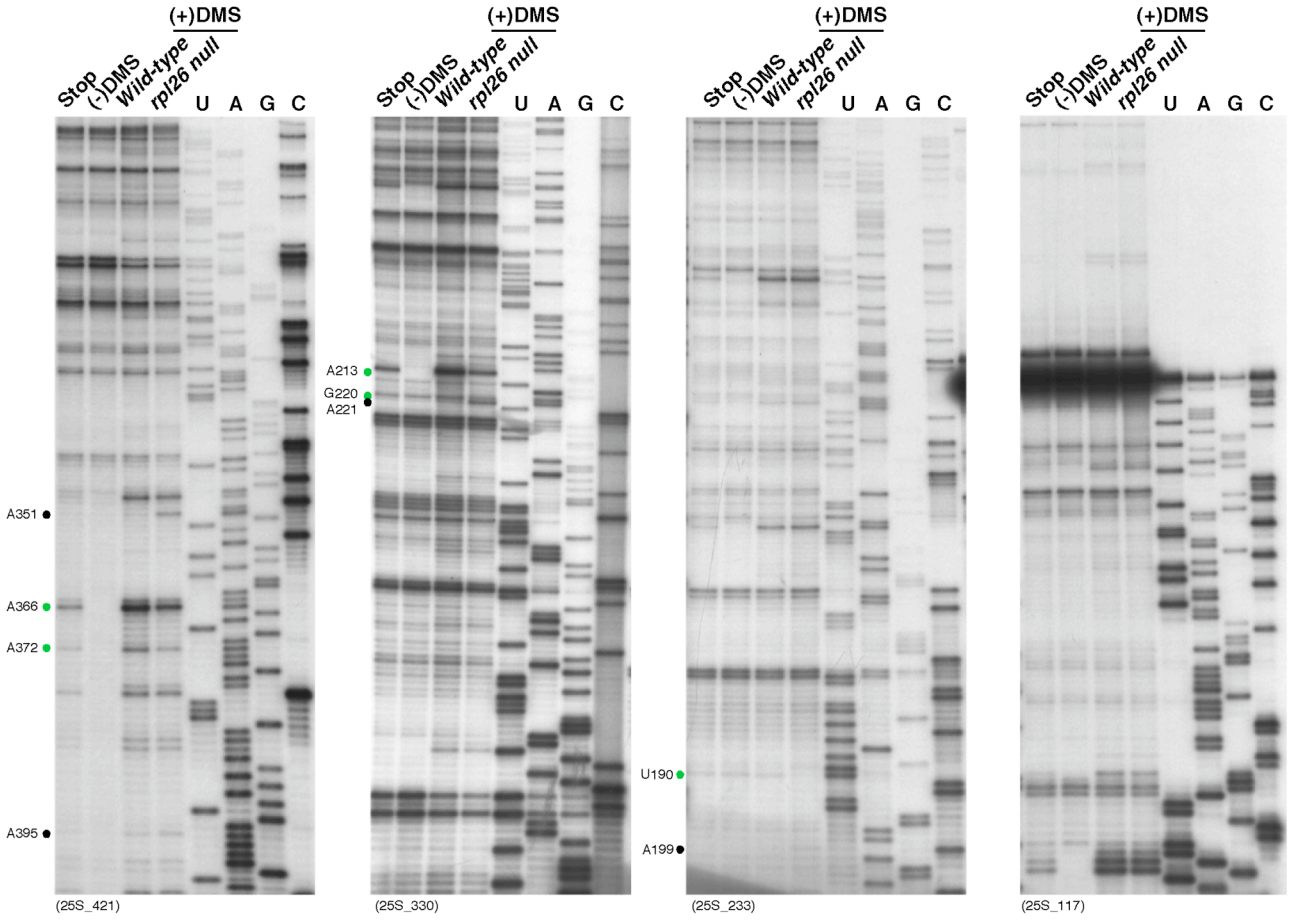
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Figure S7