SUPPLEMENTAL DATA

Supplemental Table S1. Yeast strains used in this study

Strain	Relevant genotype	Reference
W303	*MAT***a**/α *ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1*	(1)
W303-1A	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(1)
W303-1B	*MAT*α *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(1)
JDY919	As W303 but *rpl40a*::kanMX4/*RPL40A*	This study
JDY920	As W303-1A but *rpl40a*::kanMX4	This study
JDY921	As W303 but *rpl40b*::kanMX4/*RPL40B*	This study
JDY922	As W303-1A but *rpl40b*::kanMX4	This study
JDY923	As W303-1B but *rpl40b*::kanMX4	This study
JDY925	As W303-1A but *rpl40a*::kanMX4 *rpl40b*::kanMX4 [YCplac33-UB-HA-RPL40A]	This study [(a)]
JDY711	As W303-1A but *rpl35b*::kanMX4	This study
JDY906	As JDY925 but *NOP58*-GFP(S65T)::*TRP1*	This study [(b)]
JDY907	As JDY925 but *SSF1*-GFP(S65T)::natNT2	This study [(b)]
JDY908	As JDY925 but *NSA1*-GFP(S65T)::natNT2	This study [(b)]
JDY909	As JDY925 but *NOP7*-GFP(S65T)::natNT2	This study [(b)]
JDY910	As JDY925 but *ARX1*-GFP(S65T)::natNT2	This study [(b)]
JDY911	As JDY925 but *KRE35*-GFP(S65T)::natNT2	This study [(b)]
JDY912	As JDY925 but *RPP0*-GFP(S65T):: *HIS3MX6*	This study [(b)]

(a) This strain requires a plasmid-borne *RPL40A* or *RPL40B* allele to support growth. Depending on the experimental conditions and as indicated in the text, this plasmid might be different to YCplac33-UB-HA-RPL40A.

(b) Strains JDY906, JDY907, JDY908, JDY909, JDY910, JDY911 and JDY912 are segregants of the genetic cross between JDY925 and strains YMD24 (gift from M. Dosil), JDY850, JDY854, JDY851, JDY855, JDY853 and JDY861 (gift from M. Remacha), respectively. For details about the genotypes of all latter strains, see (2).

Supplemental Table S2. Oligonucleotides used in this study

Name	5'-3' Sequence
RPL40A-kanUP	TATTACCGCTTATTATCCCATAGTTGAGACGACCAAGATTCAAAC CGTACGCTGCAGGTCGAC
RPL40A-kanDOWN	AAAGTATACAGTAAATAAATGTATAGATTGATTGGGCGAAACAG AATCGATGAATTCGAGCTCG
RPL40A-UP	CTCATAGCTTCATGGCTCACATGC
RPL40A-DOWN	ACGTGCTACCGGAGGTAAGAAGTAAAGG
RPL40B-kanUP	CACTTTTTCCCGTTCAGCAAGAGGTAAAGCCACCAAAGGTTCAA ACGTACGCTGCAGGTCGAC
RPL40B-kanDOWN	GTGATGTATACAAACTTTGGATTTGTGGAGATCGTAATAAATCG AATCGATGAATTCGAGCTCG
RPL40B-UP	CGGATCCCCTAGTCAGCACAAGAG
RPL40B-DOWN	CGGATCCGGGCTGCTGTTCTACAAAG
GAL-RPL40A-UP	GCGTCGACATGCAAATGTATGCACCATATCC
GAL-RPL40A-DOWN	ACGTGCTACCGGAGGTAAGAAGTAAAGG
HA-RPL40A-P1	CTCATAGCTTCATGGCTCACATGC
HA-RPL40A-P2	AGGGGTACCCCGCATAGTCAGGAACATCGTATGGGTATTCAATG ATACCACCTCTC
HA-RPL40A-P3	TGACTATGCGGGGTACCCCTATGACGTCCCGGACTATGCAGGAT CCCCATCTTTGAAAGCTTTGG
HA-RPL40A-P4	ACGTGCTACCGGAGGTAAGAAGTAAAGG
RLP24GFP-UP	GGAGAACCACCTTTGTCAGG
RLP24GFP-DOWN	CGGATCCAAAAGCAATTTTCTTTGTATTTC
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

Supplemental Table S3. Plasmids used in this study

Name	Relevant information	Reference
YCplac111	CEN, *LEU2*	(3)
YCplac111-RPL40A	CEN, *LEU2,* Wild-type L40A	This study
YCplac111-RPL40B	CEN, *LEU2,* Wild-type L40B	This study
YCplac111-UB-HA-RPL40A	CEN, *LEU2,* N-terminal HA-tagged L40A	This study
YCplac33-RPL40A	CEN, *URA3,* Wild-type L40A	This study
YCplac33-RPL40A	CEN, *URA3,* Wild-type L40A	This study
YCplac33-UB-HA-RPL40A	CEN, *URA3,* N-terminal HA-tagged L40A	This study
pAS24-RPL40A	CEN, *LEU2, GAL1-10,* N-terminal 2x HA-tagged L40A	This study
pRS314-RFP-NOP1-RPL25-eGFP	CEN, *TRP1,* C-terminal RFP-tagged Nop1 and C-terminal GFP-tagged L25	(4)
pRS314-RFP-NOP1-RPS3-eGFP	CEN, *TRP1,* C-terminal RFP-tagged Nop1 and C-terminal GFP-tagged S3	(4)
pRS316-GAL-NMD3Δ100	CEN, *URA3, GAL1,* Dominant negative Nmd3Δ100 protein	(5)
pRS316-MEX67-GFP	CEN, *URA3,* GFP-tagged Mex67	(6)
YCplac33-MRT4-eGFP	CEN, *URA3,* GFP-tagged Mrt4	(7)
pAJ755	CEN, *URA3,* GFP-tagged Nmd3	(8)
YCplac33-RLP24-eGFP	CEN, *URA3,* GFP-tagged Rlp24	This study
pTIF6-GFP	CEN, *URA3,* GFP-tagged Tif6	(9)

SUPPLEMENTAL REFERENCES

1. Thomas, B. J., and Rothstein, R. (1989) Elevated recombination rates in transcriptionally active DNA. *Cell* **56**, 619-630
2. García-Gómez, J. J., Lebaron, S., Froment, C., Monsarrat, B., Henry, Y., and de la Cruz, J. (2011) Dynamics of the putative RNA helicase Spb4 during ribosome assembly in Saccharomyces cerevisiae. *Mol Cell Biol* **31**, 4156-4164
3. Gietz, D., St. Jean, A., Woods, R. A., and Schiestl, R. H. (1992) Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res* **20**, 1425
4. Ulbrich, C., Diepholz, M., Bassler, J., Kressler, D., Pertschy, B., Galani, K., Bottcher, B., and Hurt, E. (2009) Mechanochemical removal of ribosome biogenesis factors from nascent 60S ribosomal subunits. *Cell* **138**, 911-922
5. Belk, J. P., He, F., and Jacobson, A. (1999) Overexpression of truncated Nmd3p inhibits protein synthesis in yeast. *RNA* **5**, 1055-1070
6. Segref, A., Sharma, K., Doye, V., Hellwig, A., Huber, J., Luhrmann, R., and Hurt, E. (1997) Mex67p, a novel factor for nuclear mRNA export, binds to both poly(A)+ RNA and nuclear pores. *EMBO J* **16**, 3256-3271
7. Rodríguez-Mateos, M., García-Gómez, J. J., Francisco-Velilla, R., Remacha, M., de la Cruz, J., and Ballesta, J. P. G. (2009) Role and dynamics of the ribosomal protein P0 and its related *trans*-acting factor Mrt4 during ribosome assembly in *Saccharomyces cerevisiae*. *Nucleic Acids Res* **37**, 7519-7532
8. Hedges, J., West, M., and Johnson, A. W. (2005) Release of the export adapter, Nmd3p, from the 60S ribosomal subunit requires Rpl10p and the cytoplasmic GTPase Lsg1p. *EMBO J* **24**, 567-579
9. Menne, T. F., Goyenechea, B., Sanchez-Puig, N., Wong, C. C., Tonkin, L. M., Ancliff, P. J., Brost, R. L., Costanzo, M., Boone, C., and Warren, A. J. (2007) The Shwachman-Bodian-Diamond syndrome protein mediates translational activation of ribosomes in yeast. *Nature Genet* **39**, 486-495
10. Kos, M., and Tollervey, D. (2010) Yeast pre-rRNA processing and modification occur cotranscriptionally. *Mol Cell* **37**, 809-820
11. Henras, A. K., Soudet, J., Gerus, M., Lebaron, S., Caizergues-Ferrer, M., Mougin, A., and Henry, Y. (2008) The post-transcriptional steps of eukaryotic ribosome biogenesis. *Cell Mol Life Sci* **65**, 2334-2359
12. Fromont-Racine, M., Senger, B., Saveanu, C., and Fasiolo, F. (2003) Ribosome assembly in eukaryotes. *Gene* **313**, 17-42
13. Venema, J., and Tollervey, D. (1999) Ribosome synthesis in *Saccharomyces cerevisiae*. *Annu Rev Genet* **33**, 261-311
14. Kressler, D., Hurt, E., and Bassler, J. (2010) Driving ribosome assembly. *Biochim Biophys Acta* **1803**, 673-683
15. Kressler, D., Linder, P., and de la Cruz, J. (1999) Protein *trans*-acting factors involved in ribosome biogenesis in *Saccharomyces cerevisiae*. *Mol Cell Biol* **19**, 7897-7912

SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE S1. Yeast pre-rRNA processing pathway. (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 (10) (not shown). For further description of the yeast pre-rRNA processing pathway, see (11-13).

SUPPLEMENTAL FIGURE S2. Model depicting the temporal assembly position of r-protein L40. The major intermediates of pre-ribosomal particles along the pathway of 60S ribosomal subunit maturation and their predominant pre-rRNA content are shown. These particles are termed, according to their position in the pathway, early 0 (E_0), early 1 (E_1), early 2 (E_2) and middle (M) 66S pre-ribosomal particles and late (L) and cytoplasmic (pre-60S) pre-60S r-particles. 90S and 43S pre-ribosomal particles are also indicated. The existence of these particles is inferred from the RNA and protein composition of purified complexes associated with selected ribosomal assembly factors. The relative abundance in the different pre-ribosomal particles of the assembly factors used as baits in Fig. 8 is shown. Herein, we propose that L40 binds to cytoplasmic pre-60S ribosomal particles; the assembly position of L1 and L3 is also shown. Note that assembly of L40 is required for the release of both Nmd3 and Rlp24 from cytoplasmic pre-60S ribosomal particles. Nucleolus, grey rectangle; pre-ribosomal particles and mature r-subunits, ovals; nuclear envelope, rods; L1, L3 and L40, blue are drawn as green and red structures, respectively. For further description of the ribosome assembly pathway, see (11,12,14,15).

SUPPLEMENTAL FIGURE S3. Complementation of the *rpl40a* and *rpl40b* null mutants by plasmid-borne *RPL40A* and *RPL40B*. Strains W303-1A (*Wild-type*), JDY920 (*rpl40a*Δ) and JDY922 (*rpl40b*Δ) were transformed with an empty YCplac111 plasmid, YCplac111-RPL40A or YCplac111-RPL40B. Transformants were grown in SD-Leu and diluted to an OD_{600} of 0.05. Cells were spotted in 10-fold serial dilutions onto SD-Leu plates and incubated at 30 ºC for 3 days. The doubling times in liquid SD-Leu are indicated in minutes. Values correspond to the mean and the standard deviation of five independent experiments.

SUPPLEMENTAL FIGURE S4. Polysome analysis of the complemented *rpl40a* and *rpl40b* null mutants. Strains JDY920 (*rpl40a*Δ) and JDY922 (*rpl40b*Δ), transformed with YCplac111-RPL40A or YCplac111-RPL40B (see Fig. S3), were grown in SD-Leu medium at 30 ºC to an OD_{600} of around 0.8. Whole cell extracts were prepared and 10 A_{260} of each extract were resolved in 7-50% sucrose

gradients. The A_{254} was continuously measured. Sedimentation is from left to right. The peaks of free 40S and 60S ribosomal subunits, 80S free couples/monosomes and polysomes are indicated.

SUPPLEMENTAL FIGURE S5. The HA-L40A fusion protein is partially functional. (A) W303-1A (*Wild-type*) and the *rpl40* null strain JDY925, transformed with either with YCplac111-RPL40A (*RPL40A*) or YCplac111-UB-HA-RPL40A (*HA-RPL40A*), were grown in liquid YPD medium. Ten-fold serial dilutions were spotted on YPD plates and incubated at 30 ºC for 3 days. The doubling times in liquid YPD are indicated in minutes. Values correspond to the mean and the standard deviation of five independent experiments. (B) Polysome profile analysis of cell extracts of the *rpl40* null strain transformed with plasmid YCplac111-UB-HA-RPL40A. For details, see legend to Fig. 1B. The peaks of free 40S and 60S ribosomal subunits, 80S free couples/monosomes and polysomes are indicated. (C) Fractions were collected from the above-shown gradient, then proteins were extracted from each fraction, and equal volumes were subjected to western blotting with antibodies against the HA tag or the ribosomal protein L35.

SUPPLEMENTAL FIGURE S6. Nmd3 and Rlp24 are not properly recycled back to the nucleus upon depletion of L40. *GAL::RPL40* cells expressing GFP-tagged versions of either Nmd3 or Rlp24 were grown at 30 ºC in SGal-Leu-Ura (Gal) and shifted for 18 h to SD-Leu-Ura (18 h Glc). Then, the subcellular localization of these GFP-tagged proteins was examined by fluorescence microscopy. DAPI was used to visualize the nucleoplasm. Arrows point to the nuclear fluorescence of the GFP-tagged proteins.

SUPPLEMENTAL FIGURE S7. Nmd3 and Rlp24 are retained on cytoplasmic 60S ribosomal particles upon depletion of L40. *GAL::RPL40* cells expressing GFP-tagged versions of either Nmd3 or Rlp24 were grown at 30 ºC in SGal-Leu-Ura (Gal) and shifted for 18 h to SD-Leu-Ura (18 h Glc). Cell extracts were prepared and 10 A_{260} of each extract were resolved in 7-50% sucrose gradients. Fractions were collected, then proteins were extracted from each fraction, and equal volumes were subjected to western blotting using anti-GFP antibodies to examine the position of Nmd3- or Rlp24-GFP. Antibodies against L1 ribosomal protein were used to monitor the position of 60S ribosomal subunits and polysomes. The peaks of free 40S and 60S ribosomal subunits, 80S and polysomes are indicated.

SUPPLEMENTAL FIGURE S8. Analysis of ribosomes from cells expressing the HA-L40A. Ribosomal particles (100 μg) from W303-1A (Wild-type) and JDY925 [YCplac111-UB-HA-RPL40A] (HA-RPL40A) cells were prepared (lanes -) and further purified by a 0.5 M NH_4Cl wash (lanes 0.5M) as described in "Experimental Procedures". Proteins were resolved by SDS-PAGE and analysed by Coomassie staining. Selected ribosomal proteins were detected by western blotting using specific antibodies against the HA-tag and the ribosomal proteins L1, L3, L5, L10, L12, L35 and P0.

FIGURE S1

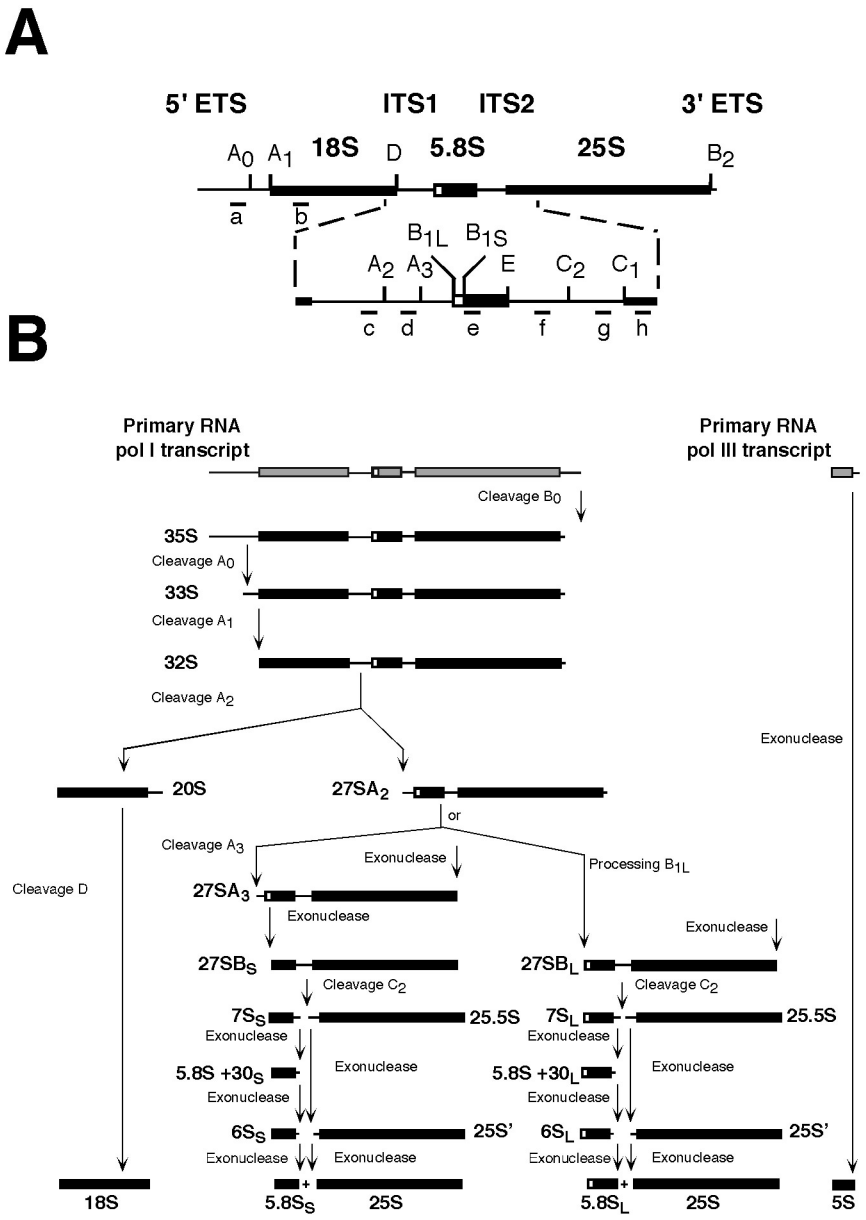
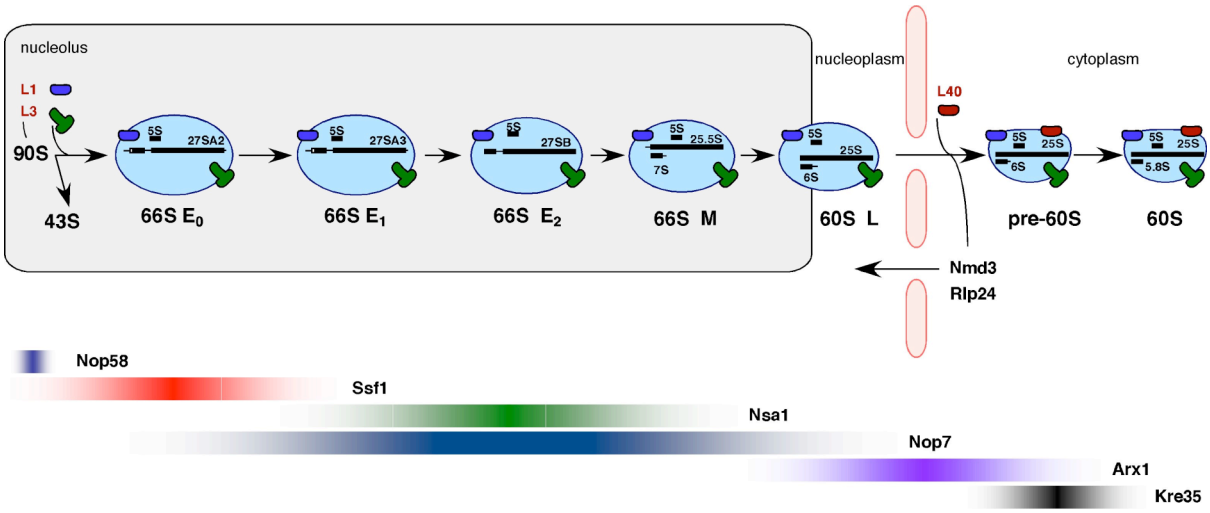

A
5' ETS
ITS1
ITS2
3' ETS
A0 A1 18S D 5.8S 25S B2
a b
B1L B1S
A2 A3 E C2 C1
c d e f g h
B
Primary RNA pol I transcript
Primary RNA pol III transcript
Cleavage B0
35S
Cleavage A0
33S
Cleavage A1
32S
Cleavage A2
Exonuclease
20S
27SA2
or
Cleavage A3
Exonuclease
Processing B1L
Cleavage D
27SA3
Exonuclease
Exonuclease
27SBS
27SBL
Cleavage C2
Cleavage C2
7SS
25.5S
7SL
25.5S
Exonuclease
Exonuclease
Exonuclease
5.8S +30S
Exonuclease
Exonuclease
5.8S +30L
Exonuclease
Exonuclease
6SS
25S'
6SL
25S'
Exonuclease
Exonuclease
Exonuclease
Exonuclease
18S
5.8SS
25S
5.8SL
25S
5S

FIGURE S2


nucleolus
nucleoplasm
cytoplasm
L1
L3
90S
43S
5S
27SA2
66S E0
27SA3
66S E1
27SB
66S E2
25.5S
7S
66S M
25S
6S
60S L
L40
pre-60S
5.8S
60S
Nmd3
Rlp24

Nop58
Ssf1
Nsa1
Nop7
Arx1
Kre35

FIGURE S3

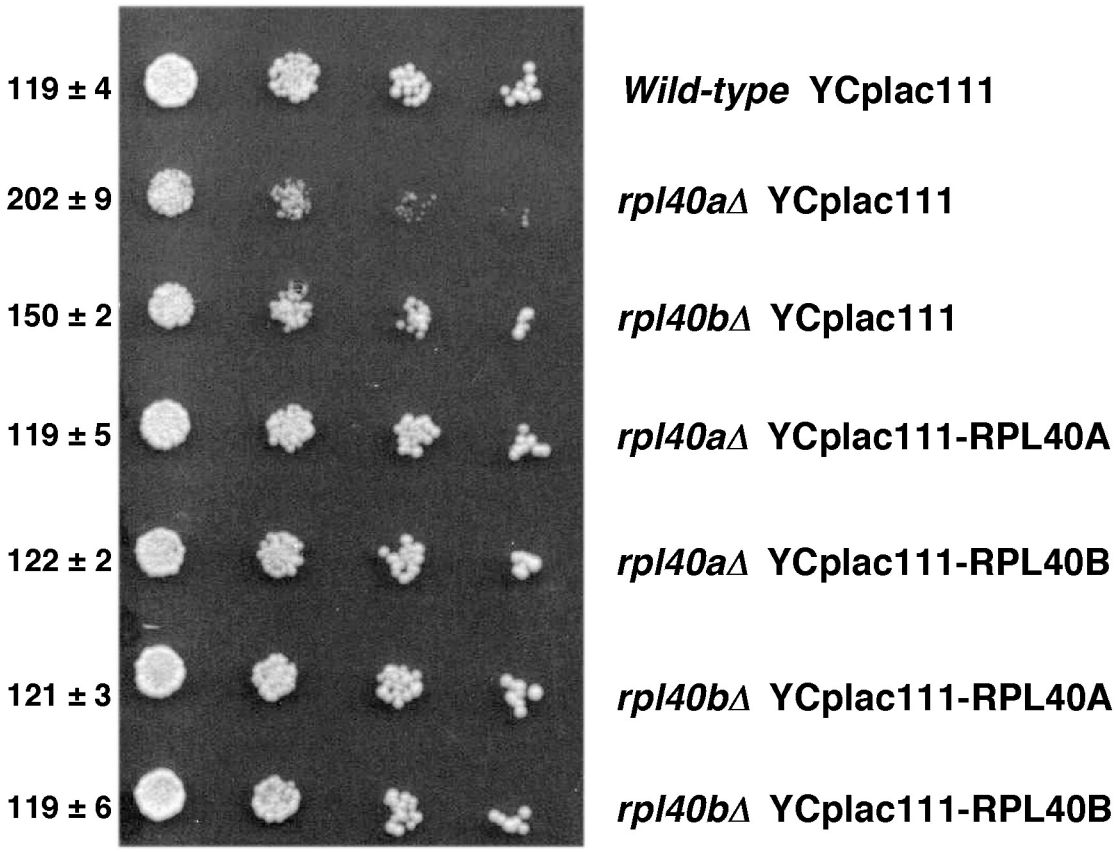

119 ± 4
Wild-type YCplac111
202 ± 9
rpl40aΔ YCplac111
150 ± 2
rpl40bΔ YCplac111
119 ± 5
rpl40aΔ YCplac111-RPL40A
122 ± 2
rpl40aΔ YCplac111-RPL40B
121 ± 3
rpl40bΔ YCplac111-RPL40A
119 ± 6
rpl40bΔ YCplac111-RPL40B

FIGURE S4

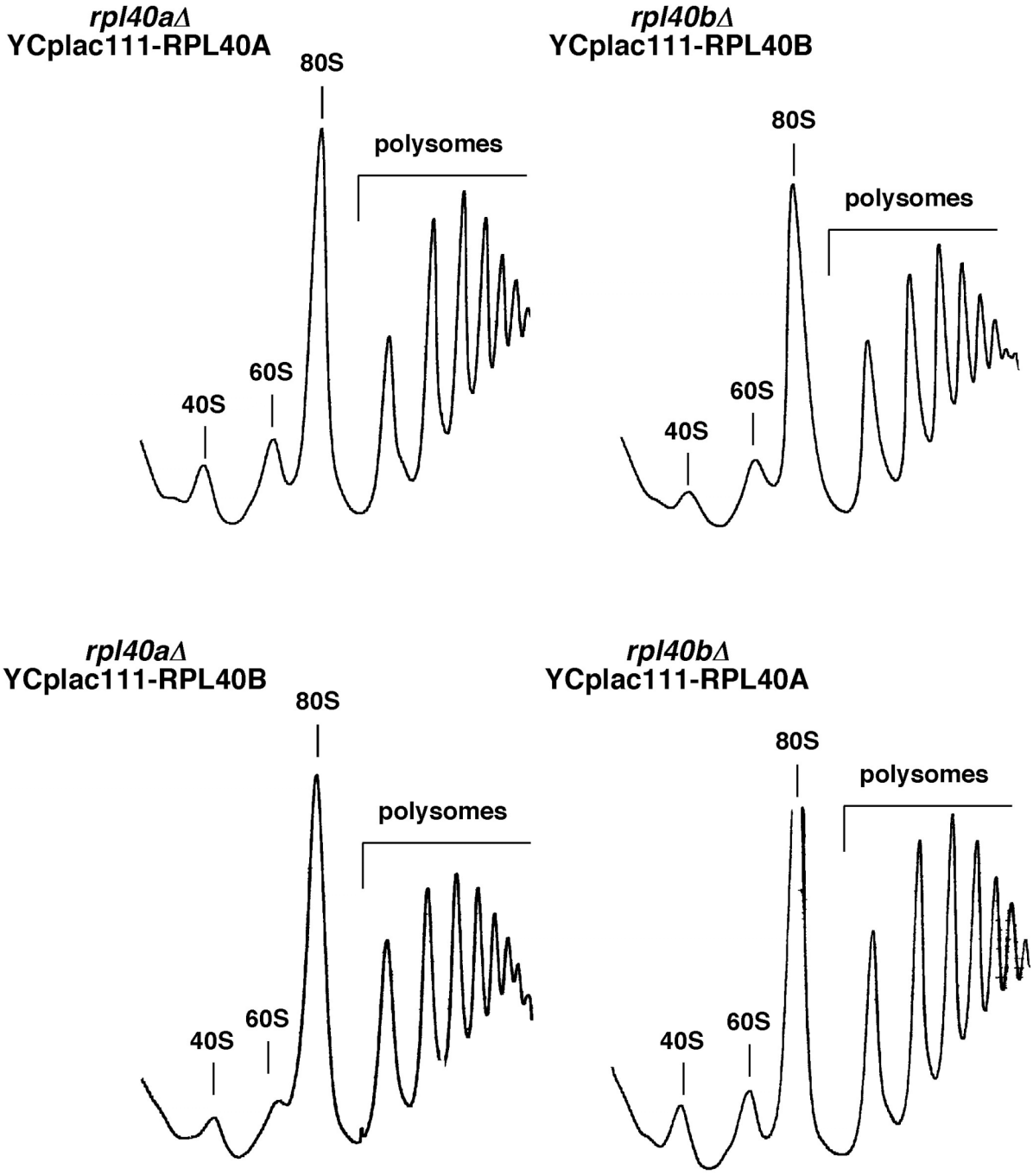

rpl40aΔ
YCplac111-RPL40A
80S
polysomes
60S
40S
rpl40bΔ
YCplac111-RPL40B
80S
polysomes
60S
40S
rpl40aΔ
YCplac111-RPL40B
80S
polysomes
60S
40S
rpl40bΔ
YCplac111-RPL40A
80S
polysomes
60S
40S

FIGURE S5

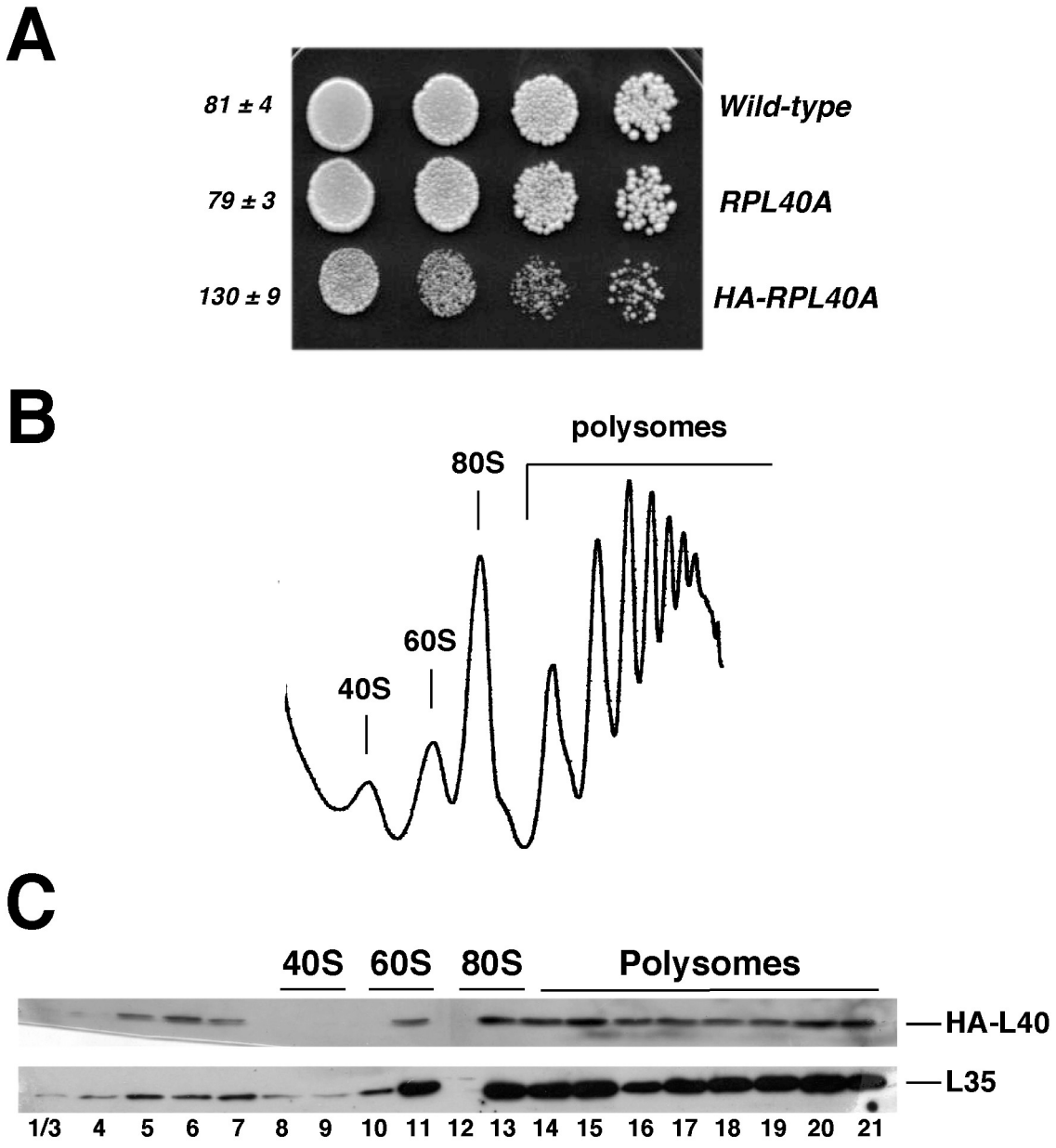

A
81 ± 4
79 ± 3
130 ± 9
Wild-type
RPL40A
HA-RPL40A
B
polysomes
80S
60S
40S
C
40S
60S
80S
Polysomes
HA-L40
L35
1/3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

FIGURE S6

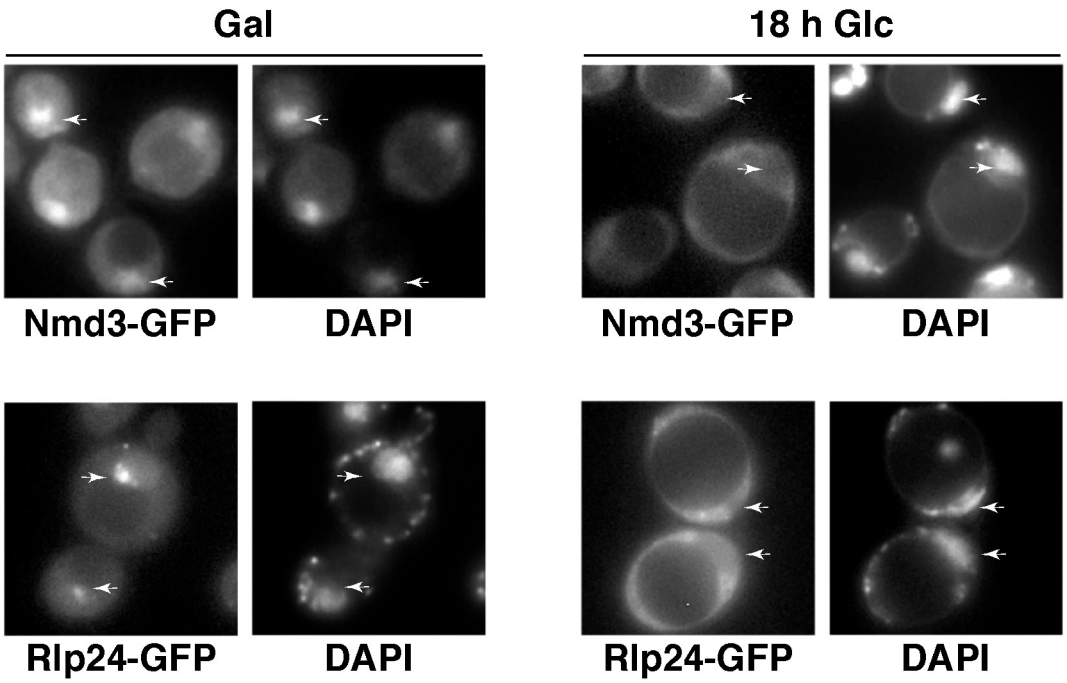

Gal
18 h Glc
Nmd3-GFP
DAPI
Nmd3-GFP
DAPI
Rlp24-GFP
DAPI
Rlp24-GFP
DAPI

FIGURE S7

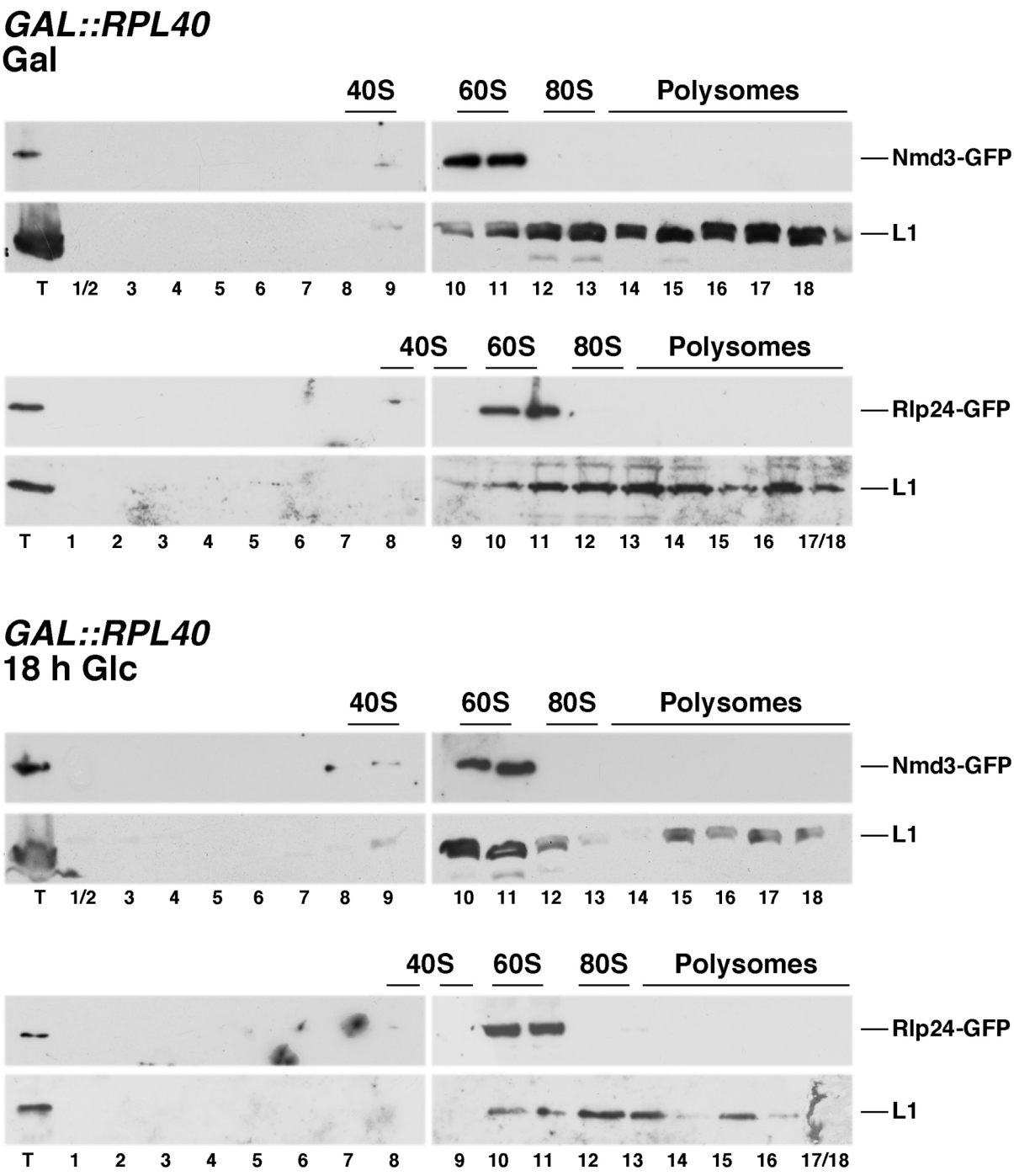
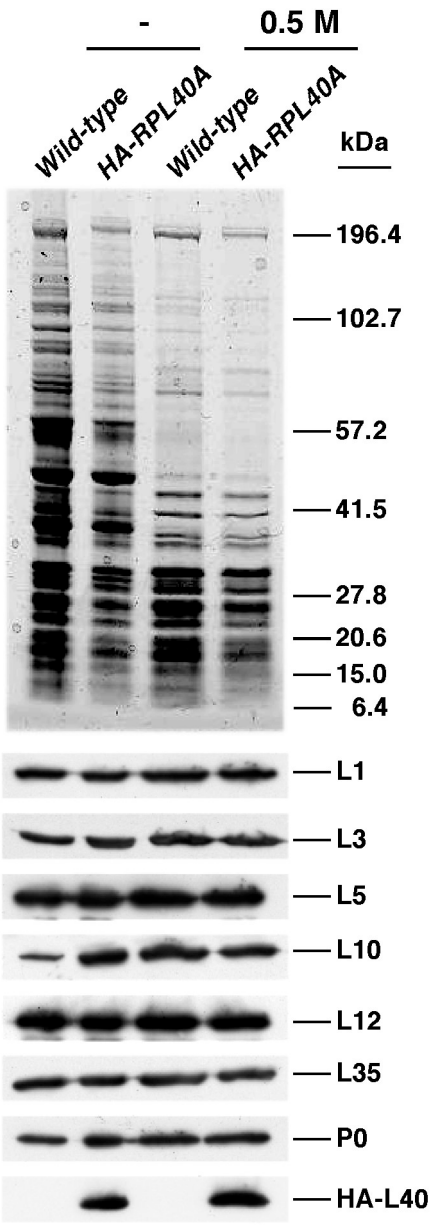

GAL::RPL40
Gal
40S
60S
80S
Polysomes
Nmd3-GFP
L1
T 1/2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
40S
60S
80S
Polysomes
Rlp24-GFP
L1
T 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17/18
GAL::RPL40
18 h Glc
40S
60S
80S
Polysomes
Nmd3-GFP
L1
T 1/2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
40S
60S
80S
Polysomes
Rlp24-GFP
L1
T 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17/18

FIGURE S8


-
0.5 M
Wild-type
HA-RPL40A
Wild-type
HA-RPL40A
kDa
196.4
102.7
57.2
41.5
27.8
20.6
15.0
6.4
L1
L3
L5
L10
L12
L35
P0
HA-L40