SUPPLEMENTARY INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

FIG S1. Yeast rDNA and pre-rRNA processing pathway

A, rDNA unit and probes used in this work.

Three out of the four ribosomal RNAs, the 18S, 5.8S, and 25S, are encoded in a single long RNA Pol I transcript (35S). The fourth rRNA (5S) is synthesized independently by RNA Pol III (not represented). The coding sequences for the mature rRNAs are embedded in noncoding spacers, namely the 5'- and 3'-external transcribed spacers (5'- and 3'-ETS) and the internal transcribed spacers 1 and 2 (ITS1 and ITS2). The cleavage sites (A_0 to E) and oligonucleotides used in the northern-blot hybridizations are indicated.

B, Pre-rRNA processing pathway in budding yeast.

Full-length primary transcripts, 35S pre-rRNAs, are released by cotranscriptional cleavage by Rnt1 (*E. coli* RNase III) in the 3'-ETS at site B_0. Alternatively, nascent transcripts are cleaved cotranscriptionally in ITS1 at site A_2 (not represented). In fast-growing yeast cells, cotranscriptional cleavage in ITS1 occurs in up to 50-70% of cases.

The 35S RNA is initially cleaved at sites A_0, A_1, and A_2 by the SSU-processome, a large snoRNP, primarily organized around the box C+D snoRNA U3, and visualized on chromatin spreads as "terminal balls". The resulting 20S and $27SA_2$ pre-rRNAs are destined to the small and large subunits, respectively. The 20S pre-rRNA is exported to the cytoplasm, where it is converted to 18S rRNA, following 3'-end endonucleolytic cleavage at site D by Nob1. The $27SA_2$ pre-rRNA is matured following two alternative pathways. This results in the production of two forms (short

and long) of 5.8S rRNA, differing in size by about ~7/8 nucleotides at their 5'-ends. In the major pathway, representing ~80% of the molecules, $27SA_2$ is endonucleolytically cleaved at site A_3 by RNase MRP and digested to site B_{1S} by the 5'-3' exoRNases Rat1-Rai1 or Rrp17. The 5'-3' exoRNase Xrn1 can contribute to this step. In the minor pathway, corresponding to ~20% of cases, the $27SA_2$ is cleaved endonucleolytically at site B_{1L} by an unknown RNase. Both forms of 27SB pre-rRNA are cleaved at site C_2 within ITS2, generating the 7S pre-rRNA, precursors of the 5.8S rRNA, and 26S pre-rRNA, precursor of the 25S rRNA. The 7S pre-rRNA is digested to site E, corresponding to the 3'-end of 5.8S, by an extremely complex succession of reactions involving the core exosome, the nucleus-specific exosome subunit Rrp6, Ngl2, and the Rex exoRNases (see Fig 2). Discrete intermediates corresponding to 5.8S rRNA precursors extended at their 3'-ends by ~7/8 nucleotides (site E', 6S) are detected in wild-type cells. The final step in formation of the 3'-end of 5.8S rRNA occurs in the cytoplasm and is performed by Ngl2. The 26S pre-rRNA is digested to site C_1, the 5'-end of the 25S rRNA, by the Rat1-Rai1 complex or Rrp17. Xrn1 can also contribute to this step. Discrete intermediates corresponding to 25S rRNA precursors extended by ~7/8 (site C'_1) are detected in wild-type cells.

FIG S2 Yeast Las1 is required for large ribosomal RNA pre-rRNA processing

A, Pre-rRNA processing was analyzed by northern blotting. Total RNA was extracted from *las1-1* and *las1-DAmP* (*DAmP*) cells. *las1-1* cells were grown to mid log phase in YPD at 23°C and transferred to 37°C for up to 6 hours. *las1-DAmP* cells were grown to mid log phase in YPD at 30°C. An isogenic wild type was used as a control. Total RNA was separated on a denaturing agarose/formaldehyde gel, transferred to a nylon membrane and hybridized with a set of oligonucleotide probes specific to

mature and precursor rRNAs (see schematics to the right). Probes used are indicated in each panel and on the schematics. Fuji FLA7000 quantitations are summarized in cartoons (see Supplemental Table 2 for details).

B, In *las1-DAmP* cells, pre-ribosomes are targeted by nucleolar surveillance. Total RNA extracted from cells expressing the *las1-DAmP* allele in the absence of functional surveillance (*trf5* deletion, lanes 4-5), and isogenic control strains, was analyzed as described in panel A. The blot was hybridized with oligonucleotide LD339. To the right, growth plate assay. Serial dilutions of the indicated strains grown at 30°C or 37°C.

FIG S3. *las1* mutants do not accumulate A_2-C_2, A_3-C_2, A_3-E rRNA fragments

Northern-blot analysis with oligonucleotide LD331 (left) or LD339 (right). Total RNA was extracted from the strain indicated and grown as described in Figs 2 and 7. The cartoon depicts the positions of the probes used. * denotes a long 3'-extended 5.8S rRNA precursor (see also Fig 7A).

FIG S4 Las1 interacts functionally with Rai1

Northern-blot analysis of low-molecular-weight RNAs. Total RNA extracted from the strains indicated. The membrane was hybridized with probe LD906.

FIG S5. Validation by RT-qPCR and western blotting of the RNAi-mediated depletions in HeLa cells.

A, Validation by RT-qPCR of the RNAi-mediated depletions. The residual level of each target mRNA was evaluated by RT-qPCR and normalized with respect to GAPDH. For all targets but ExoSC10, the mRNA level was reduced to ~10-20% of the wild-type level. For ExoSC10, the mRNA level was reduced by ~50%.

B, Validation by western blotting of RNAi-mediated Las1L depletion. The residual level of Las1L was tested on total protein by western blotting with a specific antibody. β-actin hybridization was used as a loading control. The level of Las1L protein fell below detection with both silencers used.

SUPPLEMENTAL MATERIALS AND METHODS

Yeast strains Strains were purchased from Euroscarf or OpenBiosys or created by transformation with suitable PCR cassettes and their integration into the chromosome by homologous recombination. The cassettes were generated with the DNA templates and oligonucleotides listed below. For the TAP-alone control strain, a TAP cassette was generated with oligonucleotides LD1936 and LD1937 and plasmid pDL091. It was integrated at the *BAT2* locus by homologous recombination. This resulted in expression of a stable full-length TAP polypeptide directly under the control of the constitutive *BAT2* promoter. *BAT2* is a metabolic gene encoding an enzyme involved in the last step of leucine synthesis. Genome-wide studies have determined that Bat2 is expressed at around 25 900 copies/cell (Ghaemmaghami et al, 2003), which is far more than most ribosome synthesis factors, making it a suitable control for unspecific interactions.

Characterizing the mutations in *las1-1*: genomic DNA was extracted from strain YDL2532 and the *LAS1* gene was PCR-amplified with oligonucleotides LD1953 and LD195, with LD1953 and LD1955, and with LD1953 and LD1956. Three individual PCR products were sequenced.

Strain name	PET Name	Genotype	Reference
YDL898	WT	*MAT a his3Δ2 leu2Δ1 met15Δ0 ura3Δ52 trp1Δ63*	Lafontaine & Tollervey, 1996
YDL793	WT	*MAT a his3Δ1 leu2Δ0 met15Δ0 URA3::CMV-tTA*	Mnaimneh et al, 2004
YDL1542	WT (BY4741) or YDL674	*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
YDL776	WT (BY4742)	*MAT α his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Euroscarf
YDL2596	WT	*MAT a leu2-3 112 his4-539 trp1 ura3 Δ 52 cup1::LEU2/PGK1pG/MFA2pG*	van Hoof et al, 2000
YDL2576	*ngl2Δ*	*Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0; YMR285c::kanMX4*	Euroscarf
YDL2597	*rex1Δ rex2Δ*	*MAT a leu2-3 112 lys2-201 his4-539 trp1 ura3-*	van Hoof et al, 2000

		52 cup1::LEU2/PGK1pG/MFA2pG rex1Δ::TRP1 rex2Δ::LYS2	
YDL2598	*rex1Δ rex2Δ rex3Δ*	*MAT α leu2-3 112 lys2-201 his4-539 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG rex1Δ::TRP1 rex2Δ::LYS2 rex3Δ::TRP1*	van Hoof et al, 2000
YDL1608	*pMET3 ::RAT1 xrn1Δ*	*W303 1-a HIS3-pMET3-RAT1 xrn1::NAT*	El Hage et al, 2008
YDL2250	*rai1Δ*	*MAT a his3Δ1 leu2Δ0 met15Δ0 URA3::CMV-tTA rai1Δ::CgLEU2*	Lafontaine Lab
YDL1539	*rat1-1*	*MATα ura3Δ52 leu2Δ1 his3Δ200 rat1-1*	Tollervey Lab
YDL1788	*rrp6 Δ*	*MATa ade2 can1-100ura3Δ0 leu2Δ3,112 trp1Δ1, nab3::URA3Δ1 , rrp6:: CgLEU2*	Lepore and Lafontaine, 2011
YDL2532	*las1-1*	*MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 met15Δ0 can1Δ::LEU2-MFA1pr::His3 las1-1::URA3*	Ben-Aroya et al, 2008
YDL2533	*Las1-DAmP*	*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 las1:: KAN*	Open Biosystems
YDL2534	*Las1-TAP*	*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LAS1::TAP-HIS3MX6*	Euroscarf
YDL2492	*trf5Δ*	Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *trf5Δ::CgHIS3*	Lafontaine Lab

Strain name	**PET Name**	**Genotype**	**DNA template and oligonuleotides used to generate PCR casette**	**Host Strain**
YDL2618	*TAP-only*	MAT a his3-Δ1 leu2-Δ0 met15Δ0 + pBS1539::TAP-URA3-KI	pDL091 LD1936 and LD1937	YDL674
YDL2625	*Las1-GFP*	*MAT a ura3-52 trp1Δ63 leu2Δ1 hisΔ200* $GAL2^{+}$ *LAS1::GFP::KI-TRP1*	pDL0227 LD2023 and LD2024	YDL898
YDL2623	*pGAL1::3HA-las1*	*MAT α his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 His3MX6::pGAL1::3HA::LAS1*	pDL0438 LD1949 and LD1950	YDL776
YDL2637	*pGAL1::3HA-las1 rai1Δ*	*MAT α his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 His3MX6::pGAL1::3HA::LAS1 rai1Δ::CgLEU2*	pDL0093 LD1345 and LD1346	YDL2623
YDL2662	*Las1-DAmP trf5Δ*	*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 las1:: KAN trf5Δ::CgHIS3*	pDL0092 LD808 and LD809	YDL2533

Oligonucleotides used for the construction of yeast strains

LD1936	GAAATTTAAGGGAAAGCATCTCCACGAGTTTTAAGAACGATATGTCCATGGAAAAGAGAAG
LD1937	GTGGTAGTTATCTTAACTTTGGAGGCGTCTAGGGGTGCCAAGGTTACGACTCACTATAGGG
LD1949	CAAAAGTCATTTCTAGAAAGCAATGAAAAAAGGGCAATCTAGAATTCGAGCTCGTTTAAAC
LD1950	TCAGCAAAATCCCTCCATGGAACGATTCGTGGTGGTATCATGCACTGAGCAGCGTAATCTG
LD2023	CAAGGGTTCAAAATTGGAAACCCAAACCCTTTGGTGTCCTGCGTACGCTGCAGGTCGAC
LD2024	TAGTCTACAACGTATTTATAACGAGCATTGGAAACAGGTTAATCGATGAATTCGAGCTCG
LD1345	TGTAATATGGTGAAAGAATAGCGAAATATTAGACCAACATAGTGTATCCCACACAGGAAACAGCTATGACC
LD1346	TTTCGGATCATACAGGGTTTATCTCAATAATAAAGATACAAAATCGGTTCGGTTGTAAAACGACGGCCAGT
LD808	ATTTTTTATTTTTCAAATAAACAAACGAGGGCGGAGTTTATTGGGTCGTCCACAGGAAACAGCTATGACC
LD809	TGTGGTATTCTTGTATAAATAGTAAATAGTCTATAAGAGTCTATATTGTGGTTGTAAAACGACGGCCAGT

Silencer Select RNAs used in the RNAi depletions

scrambled control	As per Ambion
Human RPS11	AGUACAACCGCUUCGAGAAtt
Human Las1L # 1	GCUGCGCAUCUGUUCCAUUtt
Human Las1L # 2	GCGAAGAGGUGGAUUCUCAtt
Human Dom3Z # 1	GUACCAUGGAGAUGCCCGAtt
Human Dom3Z # 2	AGACCUUUCCUACCAUGAAtt
Human Dom3Z # 3	AGAGCACGGUUGUCCAGGAtt
Human Xrn2 # 1	GGAAAGUUGUGCAGUCGUAtt
Human Xrn2 # 2	GGAACUGAAUUCAUGGACAtt
Human Xrn2 # 3	GAACCGAACUUUACCAUUAtt
Human Eri1 # 1	CAGUCUAACUGGAAUUACUtt
Human Eri1 # 2	CCUUCCCUCAGGUACUAAAtt
Human Isg20L2 # 1	GAUACUCACAGGGAAGAUAtt
Human Isg20L2 # 2	GUUGCACUCUGAACCUUCAtt
Human Exosc10 # 1	GAGUAUGAUUUUUACCGAAtt
Human SkiV2L2 # 1	GCAUGGACUUUGCACCAAAtt

Oligonucleotides used for northern-blot and primer extension analysis

LD331	CCAGTTACGAAAATTCTTG
LD339	GGCCAGCAATTTCAAGTTA
LD359	TTGTTACCTCTGGGCCC
LD366 (U3)	CCAAGTTGGATTCAGTGGCTC
LD471	CGGTTTTAATTGTCCTA
LD556 (5S)	CTACTCGGTCAGGCTC
LD871	CATGGCTTAATCTTTGAGAC
LD906	TGAGAAGGAAATGACGCT
LD1099	CTCCGCTTATTGATATGC
LD1290	ATCCCGGCCGCCTCCATCAC
LD1827	CCTCGCCCTCCGGGCTCCGTTAATGATC
LD1828	CTGCGAGGGAACCCCCAGCCGCGCA
LD1829	GCGCGACGGCGGACGACACCGCGGCGTC
LD1844	CGGAGGCCCAACCTCTCCGACGACAGGTCGCCAGAGGACAGCGTGTCAGC
LD2079	GGGGCGATTGATCGGCAAGCGACGCTC
LD2132	CAATGTGTCCTGCAATTCAC
LD2133 (7SL)	GCTCCGTTTCCGACCTGGGCC
LD915	GCGTTCTTGATCGATGC
LD1148	CTCCGCTTATTGATATGC
LD2293	GCCTCAGGCCGGCCAGACGAGAC

Oligonucleotides used for RT-qPCR analysis

LD714	Actin 1-F	CGTTCCAATTTACGCTGGTT
LD715	Actin 1-R	AGCGGTTTGCATTTCTTGTT
LD1818	GAPDH-F	TGCACCACCAACTGCTTAG
LD1819	GAPDH-R	GTTCAGCTCAGGGATGACC
LD1957	Las1-F	TTCCGTTCCATTGAAGAAGG
LD1958	Las1-R	GGGTTTGGGTTTCCAATTTT
LD2110	Eri1-F	TGCTGAAAGAGAGCAATTTTGC
LD2111	Eri1-R	CCAAGAACCATCTGTTAAAAGTGAG

LD2112	Isg20L2-F	GCTCATTCAGAGAATAAATGCTCC
LD2113	Isg20L2-R	GGTGTGGCATTCACCATG
LD2114	SkiV2L2-F	GTGATGTTACTATTAATCCTACGG
LD2115	SkiV2L2-R	CGATAATCTGTGTAAATAACATGAC
LD2116	Las1L-F	CCCCACATTTGAACAGTTGG
LD2117	Las1L-R	CCATCTGAGGATGTAGGTAGG
LD2118	Dom3Z-F	CCCGAATGTTGTTGCTGGC
LD2119	Dom3Z-R	CTGCCTCCCTCTAAAGCATTACT
LD2033	Xrn2-F	AGGTGGCTTTCTTCCTCCAG
LD2034	Xrn2-R	AGATCAGCATCTGCTCCACA
LD1979	Exosc10-F	AAGCCCAGAACATCATGGAG
LD1980	Exosc10-R	GTTTGCTCAGCTGCCTTCTT

Plasmids used in this study

pDL091	pBS1539	Kind gift from B Séraphin
pDL0227	pYM26	Euroscarf
pDL0438	pFA6a-His3MX6-pGAL1-3HA	Longtine et al, 1998
pDL0092	pBS1762	Kind gift from B Séraphin
pDL0093	pBS1763	Kind gift from B Séraphin

Protein extraction and western blotting Total protein was extracted from ~2x10^8 yeast cells resuspended in 200 µl lysis buffer (KOH pH7.5, 0.5 mM EDTA pH8.0, 100 mM NaCl, 1 mM DTT, 20% glycerol, 0.05% NP40, 2.5 mM PMSF (Sigma), mixed with an isovolume of 425-600-µm-diameter glass beads (Sigma). The cells were lysed by vortexing for 5x 30 sec, with 1 min resting time on ice. The lysate was cleared by centrifugation at 13 krpm and 4°C for 15 min, and the supernatants were assayed for protein content. Total protein was extracted from ~150 000 HeLa cells

inactivated by RNAi for 72h in TRI Reagent (AM9738, Ambion), according to the manufacturer's instructions.

Protein concentration was estimated with the Bio-Rad Protein Assay (REF 500.0006). Total protein extracts were resolved by 8-to-15% SDS-PAGE and transferred to either PVDF or hybond-C membranes (GE Healthcare). HA- and GFP-tagged proteins were detected respectively with an anti-HA antibody (MMS-101P, Covance) or an anti-GFP antibody (1814460, Roche). As a loading control in the yeast experiments, membranes were probed with anti-glucose-6-phosphate dehydrogenase (G6PDH, A9521, Sigma). For detection of human Las1L, a specific anti-Las1L antibody (AV34629, Sigma) was used at 1:500 for 2 h, followed by incubation with a donkey anti-rabbit IgG-HRP (C2609, Santa Cruz) at 1:1000 for 1 h at RT. As a loading control in the human experiments, an anti-β-actin antibody (AC-15 sc-69879, Santa Cruz) was used at 1:1000 for 1 h, followed by incubation with an anti-mouse-HRP (K1710, Santa Cruz) at 1:1000 for 1 h at RT.

Western blotting of sucrose gradient fractions: TAP-tagged proteins were detected with a rabbit peroxidase antiperoxidase antibody (PAP, Sigma) used at 1:1000 for 1 h at RT. Rps8 was detected with a specific antibody raised in rabbit and used at 1:1000 (a gift from Dr Giorgio Dieci), Rpl3 with a specific antibody raised in mouse, used at 1:2000 (a gift from Dr Jonathan Warner, Albert Einstein College, NYC), and Nog1 with an antibody raised in rabbit, used at 1:500 (a gift from Dr Micheline Fromont-Racine, Institut Pasteur, Paris).

Tandem affinity purification A single-step purification was performed as described previously by Oeffinger et al, 2007; Zhang et al, 2007. Briefly, yeast cells were collected, resuspended in resuspension buffer, frozen in liquid nitrogen, and lysed in solid phase by cryo-milling on a Planetary Mill (Retsch's) and stored at –80°C. The resuspension buffer consisted of: 1.2% PVP-40, 20 mM Hepes pH7.4, Sigma protease inhibitor cocktail 1:100, solution P 1:100 (2 mg pepstatin A, 90 mg PMSF, 5 ml ethanol) and 1M DTT 1:1000. The grindate was resuspended in RNP buffer (20 mM Hepes pH7.4, 110 mM KOAc, 0.5% TritonX100, 0.1% Tween-20, Ambion SuperRNAsin 1:5000, Sigma antifoam 1:5000, solution P 1:100, 150 mM NaCl) and incubated with rabbit-IgG-conjugated magnetic beads (Dynal) for 1 h at 4°C. The beads were collected with a magnet, washed four times, and eluted twice for 20 minutes at RT under denaturing conditions (500 mM NH_4OH/0.5 mM EDTA). The pooled eluates were separated on 4-12% NuPAGE Novex Bis-Tris precast gels (Invitrogen) and visualized by Coomassie blue staining. The material from duplicate experiments was analyzed by mass spectrometry (LC/MS-MS).

The Las1-TAP interactants were curated manually as follows: (1) the interactions found in two individual affinity purification experiments were combined, (2) only hits with more than 5 significant peptides were retained, (3) hits that were also identified in a TAP-alone control strain were discarded, (4) all hits found with a similar peptide number with an unrelated bait were discarded.

Flow cytometry analyses Cells-cycle progression was analyzed by determining of the DNA content by propidium iodide (PI) labelling followed by flow cytometry analysis. About $8x10^6$ yeast cells from an exponentially growing culture were fixed with 70% ethanol for 15 min at RT, collected by centrifugation, and resuspended in

200 mM TrisCl pH 7.8. The cells were treated overnight at 37°C with 10 µg/ml RNase A (Sigma), collected by centrifugation, and resuspended in buffer A (200 mM TrisCl pH 7.5, 200 mM NaCl, 78 mM MgCl2). Aggregated cells were separated by means of a series of three 10-s sonication pulses (on a Vibra Cell TM sonicator, Sonics & Materials Inc. with power setting 2 out of 10 and 50% output pulse) with an incubation step in iced water. Cells were collected by centrifugation and resuspended in buffer B (180 mM Tris-Cl pH 7.5, 190 mM NaCl, 70 mM MgCl2) supplemented with 55 µg/ml of propidium iodide (Sigma). The DNA was stained for 1 h at RT. 100 µl cell suspension was added to 900 µl of 200 mM TrisCl pH 7.8 and 3x45 000 cells from each sample were analyzed with a CyAn ADP (Dako) flow cytometer. The data were acquired with the Summit 4.3 software (Dako). To quantify the proportion of cells in each phase of the cell cycle, the data were quantified with the Dean/Jett/Fox model in the cell cycle module of the Flowjo 8.8.6 software (Tree Star, Ashland, Oregon).

Sucrose gradient sedimentation analysis Separation by sucrose gradient was performed essentially as described previously in Gerus et al, 2010 with minor modifications. About $2x10^9$ yeast cells growing exponentially were collected by centrifugation, rinsed in buffer K (20 mM TrisHCl pH7.4, 50 mM KCl, 5 mM $MgCl_2$), and collected again by centrifugation. Cell pellets were resuspended with approximately 1 volume of ice-cold buffer K, supplemented with 1 mM DTT, 1x Complete EDTA-free protease inhibitor cocktail (Roche), 0.1 U/µl RNasin (Promega). About 500 µl of ice-cold glass beads (diameter 425-600 µm, Sigma) were added to the cells. These were broken by twelve rounds of vigorous shaking for 30 s three times, with a 30-s incubation on ice after each shaking. Extracts were clarified

by three successive centrifugations at 16 krpm and 4°C for 10 min, and quantified by OD_{260} reading. About 30 A_{260} units were loaded onto 10-50% sucrose gradients prepared in buffer K. Salt condition was as follows: 20 mM TrisHCl pH7.4, 50 mM KCl and 5 mM $MgCl_2$. Gradients were prepared in Beckman ultra-clear centrifugation tubes (REF344059, 14 x 89 mm). Five 2.2 ml layers of saccharose solution at decreasing concentration (50%, 40%, 30%, 20%, and 10% saccharose) were added one by one, with an incubation of at least 20 min at -80°C in between. Unless otherwise stated, a 50% saccharose stock solution was prepared in a 1x buffer containing 50 mM Tris-HCl pH 7.4, 12 mM $MgCl_2$, 625 mM NH_4Cl and 1 mM DTT. Gradients were centrifuged for 165 min at 39 krpm and 4°C in an Optima L-100XP Ultracentrifuge (Beckman-Coulter) using the SW41Ti rotor. After centrifugation, 24 fractions of 500 µl each were collected from the top of each gradient, by use of a Foxy Jr. fraction collector (Teledyne ISCO). The absorbance at 254 nm was measured during collection with a UA-6 cell (Teledyne ISCO). Proteins were precipitated with TCA from the first half of each fraction; the second half of each fraction was used to extract RNA by a phenol/chloroform protocol. Proteins were loaded onto a precast gel (Criterion XT, Biorad) and transferred to a PVDF (GE Healthcare) membrane; RNAs were separated on a denaturing agarose gel. The same protocol was used for polysome profiles, except that exponentially growing cells were treated with 50 µg/ml cycloheximide (Sigma) added directly to the culture medium.

Fluorescence microscopy To amplify the weak GFP signal observed in cells expressing a functional Las1-GFP construct from the endogenous *LAS1* promoter, standard immunofluorescence with an anti-GFP antibody was used (Pringle et al, 1991). Cells were spheroplasted and incubated overnight at 4°C with a mouse

monoclonal anti-GFP antibody at 1:500 (Roche) and then for 1 hour at RT with a goat anti-mouse IgG coupled to Alexa 488 at 1:1000 (Molecular Probes, A-11001). Yeast cells were observed with a Zeiss Axio Imager Z1 microscope equipped with a 100x objective (N.A. of 1.46) and standard filter sets. Images were captured with a Zeiss HRm CCD camera and the native Axiovision software (v4.5) and transferred to Photoshop and Illustrator (CS3, Adobe).

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