Supplemental material

Biedka et al., https://doi.org/10.1083/jcb.201711037

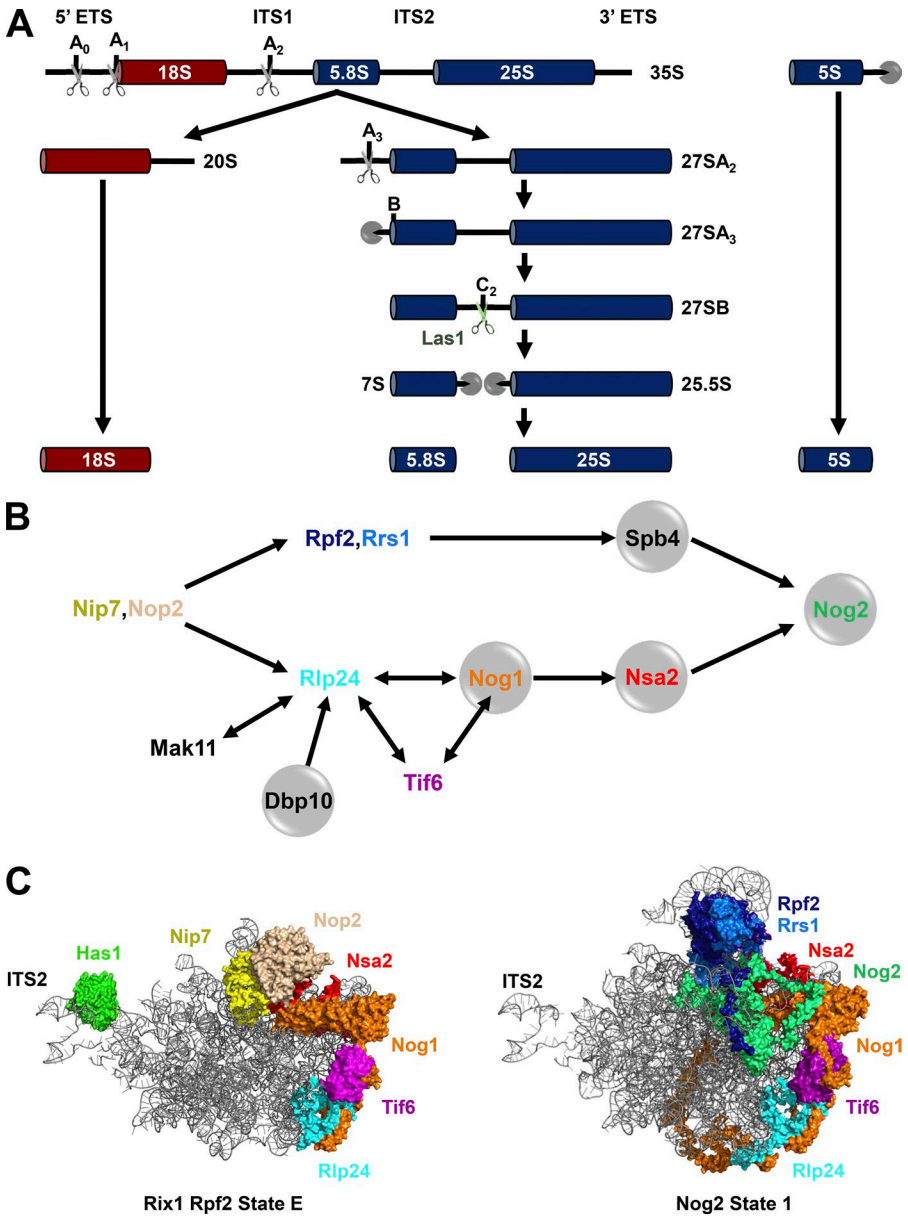

Figure S1. **The pre-rRNA processing pathway in *S. cerevisiae* and hierarchical assembly of AFs required for 27SB pre-rRNA processing. (A)** Endo- and exonucleolytic processing events are indicated. Large and small subunit rRNAs are colored blue and red, respectively. ETS, external transcribed spacer; ITS, internal transcribed spacer. **(B)** Hierarchical recruitment of B-factors, ending with recruitment of Nog2 by two parallel pathways. B-factors in gray circles were analyzed in this study. **(C)** The locations of B-factors in the Nsa1 state E and Nog2 state 1 particles. The structures shown are PDB IDs 6ELZ and 3JCT.

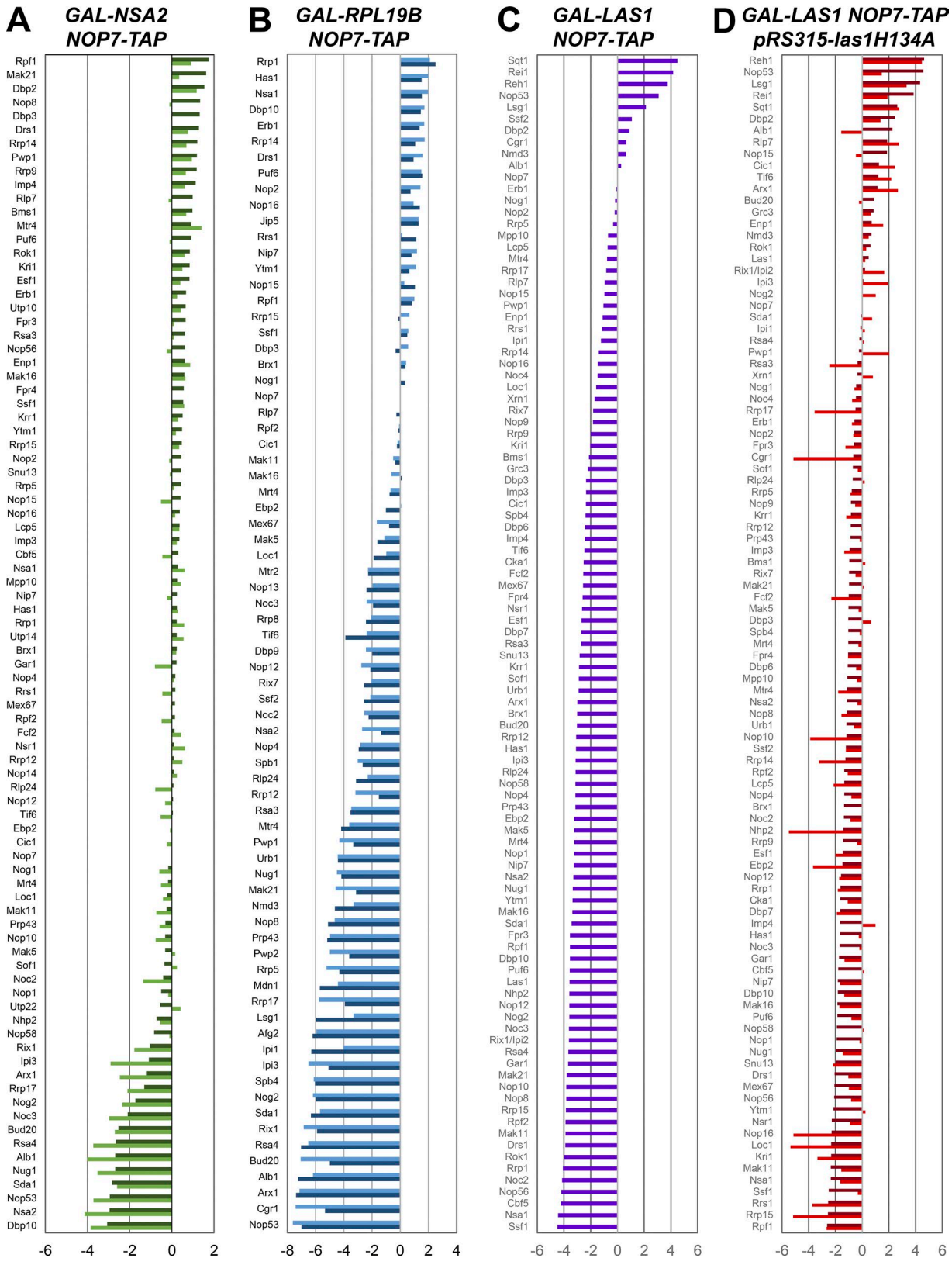

Figure S2. **Changes in pre-60S subunit protein composition caused by depletion of Nsa2, L19, or Las1 or by mutational inactivation of Las1.** Semi-quantitative mass spectrometry (iTRAQ) was used to quantify the relative changes in levels of 60S subunit AFs in the presence and absence of Nsa2 (A), L19 (B), or Las1 (C), or when WT Las1 is expressed or depleted and las1H134A is expressed (D). The ratios were normalized to levels of Nop7, and the fold change in log2 scale is shown. For depletion of Nsa2 and expression of las1H134A, two biological replicates are shown, and a single replicate is shown for depletion of Las1. For L19, both samples of yeast grown in glucose were compared with a single sample grown in galactose.

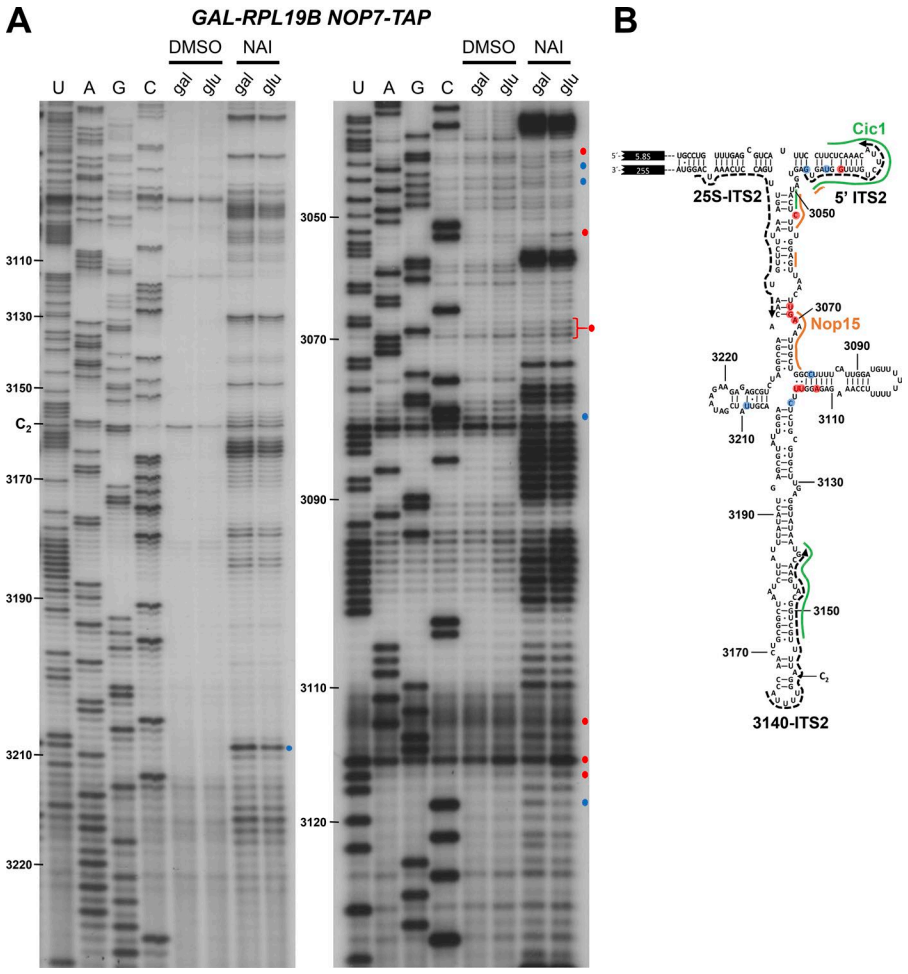

Figure S3. **The structure of ITS2 in preribosomes is largely unaffected by depletion of r-protein L19. (A)** In vivo structure probing of ITS2 (SHAPE) was conducted by treating cells with NAI and assaying modified nucleotides via primer extension with two oligonucleotides, one of which is complimentary to the 5′ end of 25S rRNA and the 3′ end of ITS2 and the other of which is complimentary to ITS2 near the C2 site. Cells were grown in galactose or grown in galactose and shifted to glucose for 16 h. Nucleotides with increased or decreased modification by NAI in the absence of L19 are indicated by red and blue circles, respectively. RNA extracted from cells treated with DMSO instead of NAI was used as a control. **(B)** Modified nucleotides are indicated on the predicted hairpin secondary structure of ITS2. The positions of the 25S-ITS2, 3140-ITS2, and 5′ ITS2 primers used for primer extension are indicated by black dashed lines. The 5′ ITS2 oligonucleotide products extend into 5.8S rRNA. Data from primer extension with the 5′ ITS2 primer are shown in Fig. 6. Green and orange lines represent the binding sites of Cic1 and Nop15, respectively, as determined by cross-linking and analysis of cDNAs (Granneman et al., 2011) and cryo-EM (Wu et al., 2016).

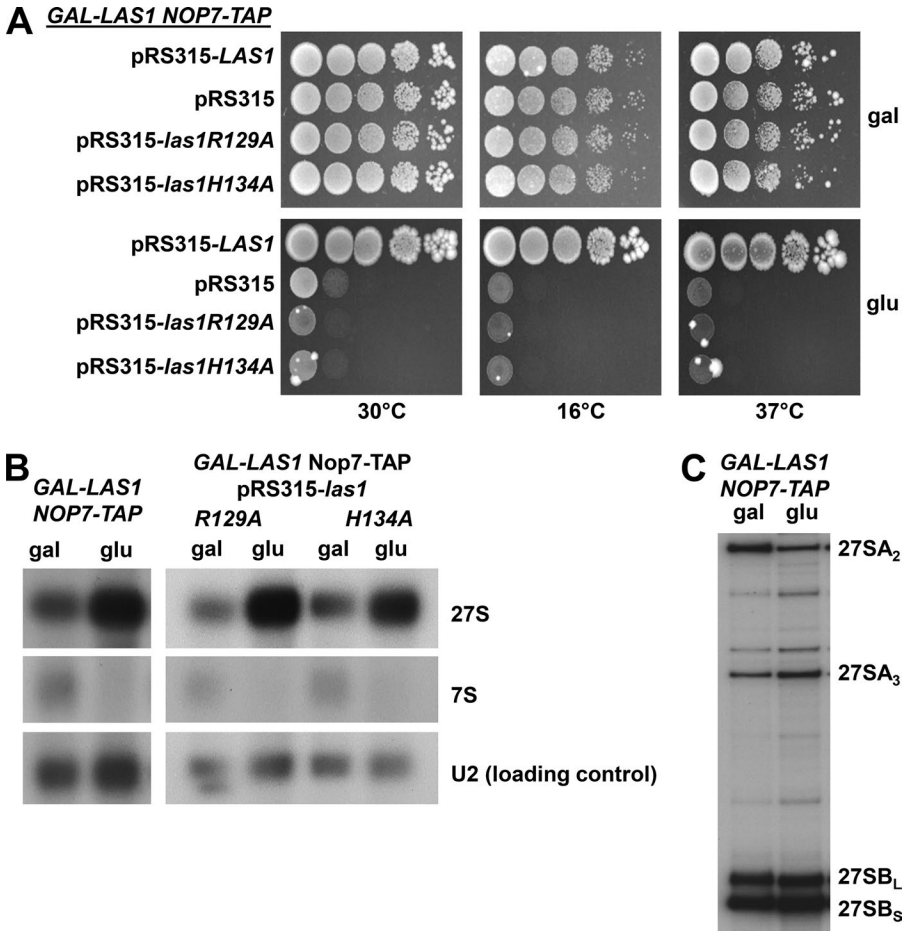

Figure S4. **Depletion or mutational inactivation of Las1 is lethal and blocks formation of 7S pre-rRNA. (A)** Serial dilutions (1:10,000) of *GAL-LAS1 NOP7-TAP* yeast containing positive (pRS315-*LAS1*) or negative (pRS315) control vectors or expressing las1 mutant proteins that lack endonucleolytic activity were spotted on C-leu solid media and incubated at 30°C, 16°C, or 37°C. **(B and C)** Total RNA was extracted from cells grown in galactose or grown in galactose and shifted to glucose and steady-state levels of pre-rRNAs were analyzed by Northern blotting (B) and primer extension (C).

Table S1. **Yeast strains used in this study**

Strain	Genotype	Source
JWY6147	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1*	E. Jones (Carnegie Mellon University, Pittsburgh, PA)
JWY7793	*MATa his3-1 leu2-0 ura3-0 met15D0/MET15D0 lys2-0 rpl19a::HIS3MX6 rpl19b::kanMX4 pGAL-RPL19B*	Pöll et al., 2009
JWY8188	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 spb4::GAL-HA3-SPB4 TRP1*	Talkish et al., 2012
JWY8437	*MATa his3-1 leu2-0 ura3-0 met15D0/MET15D0 lys2-0 rpl19a::HIS3MX6 rpl19b::kanMX4 nop7::NOP7-TAP URA3 pGAL-RPL19B*	This study
JWY8649	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 nog1::GAL-HA3-NOG1 TRP1*	Talkish et al., 2012
JWY8706	*MATα trp1 ade2-1 his3-11 dbp10::kanMX4 nop7::NOP7-TAP TRP1 pAS24-GAL-DBP10 LEU2*	Talkish et al., 2012
JWY8809	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 nog2::NOG2-TAP HIS3*	Ghaemmaghami et al., 2003
JWY8814	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 nsa1::NSA1-TAP HIS3*	Ghaemmaghami et al., 2003
JWY8854	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 nmd3::NMD3-TAP HIS3*	Ghaemmaghami et al., 2003
JWY10689	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 nog2::GAL-HA3-NOG2 TRP1*	This study
JWY10691	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 nog1::GAL-HA3-NOG1 TRP1 rpl26b::RPL26B-HA3 HIS3*	This study
JWY10692	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 nog1::GAL-HA3-NOG1 TRP1 rpl35b::RPL35B-HA3 HIS3*	This study
JWY10693	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 nog1::GAL-HA3-NOG1 TRP1 rpl37b::RPL37B-HA3 HIS3*	This study
JWY10695	*MATα trp1 ade2-1 his3-11 dbp10::kanMX4 nop7::NOP7-TAP TRP1 pAS24-GAL-DBP10 LEU2 rpl26b::RPL26B-HA3 HIS3*	This study
JWY10696	*MATα trp1 ade2-1 his3-11 dbp10::kanMX4 nop7::NOP7-TAP TRP1 pAS24-GAL-DBP10 LEU2 rpl35b::RPL35B-HA3 HIS3*	This study
JWY10697	*MATα trp1 ade2-1 his3-11 dbp10::kanMX4 nop7::NOP7-TAP TRP1 pAS24-GAL-DBP10 LEU2 rpl37b::RPL37B-HA3 HIS3*	This study
JWY10698	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 spb4::GAL-HA3-SPB4 TRP1 rpl26b::RPL26B-HA3 HIS3*	This study
JWY10700	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 spb4::GAL-HA3-SPB4 TRP1 rpl35b::RPL35B-HA3 HIS3*	This study
JWY10702	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 spb4::GAL-HA3-SPB4 TRP1 rpl37b::RPL37B-HA3 HIS3*	This study
JWY10778	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 nog2::GAL-HA3-NOG2 TRP1 rpl26b::RPL26B-HA3 HIS3*	This study
JWY10779	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 nog2::GAL-HA3-NOG2 TRP2 rpl35b::RPL35B-HA3 HIS3*	This study
JWY10780	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 nog2::GAL-HA3-NOG2 TRP3 rpl37b::RPL37B-HA3 HIS3*	This study
JWY11373	*MATa his3-1 leu2-0 ura3-0 met15D0/MET15D0 lys2-0 rpl19a::HIS3MX6 rpl19b::kanMX4 pGAL-RPL19B pRS316-RFP-NOP1-RPL25-GFP*	This study
JWY11409	*MATa ura3-52 trp1-289 ade2 leu2-3,-112 arg4 nop7::NOP7-TAP URA3 nsa2::GAL-NSA2 kanMX6*	This study
JWY11432	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 las1::GAL-HA3-LAS1 TRP1*	This study
JWY11438	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 las1::GAL-HA3-LAS1 TRP1 pRS315-las1R129A*	This study
JWY11440	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-TAP URA3 las1::GAL-HA3-LAS1 TRP1 pRS315-las1H134A*	This study
JWY11477	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 las1::GAL-LAS1 kanMX6*	This study
JWY11488	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nop7::NOP7-GFP TRP1 las1::GAL-LAS1 kanMX6*	This study
JWY11490	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 cic1::CIC1-GFP TRP1 las1::GAL-LAS1 kanMX6*	This study
JWY11492	*MATa ura3-52 trp1-1 lys2-801 his3-Δ200 leu2-Δ1 nmd3::NMD3-TAP TRP1 las1::GAL-LAS1 kanMX6*	This study
JWY11505	*MATa ura3-52 trp1-289 ade2 leu2-3,-112 arg4 nsa2::GAL-NSA2 kanMX6*	Lebreton et al., 2006

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