**Supplemental Information**

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# Defining the Pathway of Cytoplasmic Maturation of the 60S Ribosomal Subunit

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**Table S1. Strains Used in This study**

| Strain | Genotype | Source |
|---|---|---|
| W303 | *MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1* | (Santos and Ballesta, 1994) |
| W303' | *MATa ade2 his3Δ1 leu2Δ0 trp1 ura3Δ0* | Goyenechea and Warren, unpublished |
| W303-GP0 | *MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 URA3::$P_{GAL1}$-RPP0* | (Santos and Ballesta, 1994) |
| BSY28 | *MATα can1Δ::MFA1pr-HIS3 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sdo1ts* | Goyenechea and Warren, unpublished |
| BY4741 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0* | Open Biosystems |
| FWY111 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ afg2-18* (*drg1ts*) | (Pertschy et al., 2007) |
| Y5563 | *MATα can1Δ::MFA1pr-HIS3 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0* | (Menne et al., 2007) |
| AJY1539 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 CRM1(T539C-HA)* | (Hedges et al., 2005) |
| AJY1699 | *MATa his3Δ1 leu2Δ0 ura3Δ0 tif6Δ::KanMX with pAJ1194 ($P_{GAL}$::TIF6-myc URA3 CEN)* | This study |
| AJY1700 | *MATα his3Δ1 leu2Δ0 ura3Δ0 tif6Δ::KanMX with pAJ1194 ($P_{GAL}$::TIF6-myc URA3 CEN)* | This study |
| AJY1903 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 arx1::KanMX rei1Δ::KanMX* | (Hung and Johnson, 2006) |
| AJY1909 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ ARX1-GFP::HIS3MX* | (Hung and Johnson, 2006) |
| AJY1917 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rei1Δ::KanMX* | (Hung and Johnson, 2006) |
| AJY1948 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 ARX1-GFP::HIS3MX* | Hung, unpublished |
| AJY2467 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rlp24Δ::KanMX with pAJ898 (RLP24-HA URA3 CEN)* | This study |
| AJY2474 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 jjj1Δ::KanMX* | This study |
| AJY2547 | *MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 yvh1Δ::Nat$^r$* | (Lo et al., 2009) |

| | | |
|---|---|---|
| AJY2551 | *MAT*a *ura3Δ0 his3Δ1 leu2Δ0 lys2Δ0 mrt4Δ::KanMX* | (Lo et al., 2009) |
| AJY2553 | *MATa ura3Δ0 his3Δ1 leu2Δ0 lys2Δ0 yvh1Δ::NAT$^r$ mrt4Δ::KanMX* | (Lo et al., 2009) |
| AJY2909 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 TIF6-GFP::HIS3MX* | Research Genetics |
| AJY2976 | *MATa his3Δ1 leu2Δ0 ura3Δ0 yvh1Δ::KanMX* | This study |
| AJY2981 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ KanMX::P$_{GAL1}$-3xHA-EFL1* | This study |
| AJY3005 | *MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sdo1$^{ts}$ tif6Δ::KanMX with pAJ2451 (TIF6 LEU2 CEN)* | This study |
| AJY3006 | *MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sdo1$^{ts}$ tif6Δ::KanMX with pAJ2250 (TIF6(V192F) LEU2 CEN)* | This study |
| AJY3013 | *MATα his3Δ leu2Δ ura3Δ KanMX::P$_{GAL1}$-3xHA-EFL1 tif6Δ::KanMX with pAJ2451 (TIF6 LEU2 CEN)* | This study |
| AJY3014 | *MATα his3Δ leu2Δ ura3Δ KanMX::P$_{GAL1}$-3xHA-EFL1 tif6Δ::KanMX with pAJ2250 (TIF6(V192F) LEU2 CEN)* | This study |
| AJY3040 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MRT4-GFP::HIS3MX* | Open Biosystems |
| AJY3072 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 jjj1Δ::KanMX TIF6-GFP::HIS3MX* | This study |
| AJY3073 | *MATa his3Δ1 leu2Δ0 ura3Δ0 yvh1Δ::KanMX TIF6-GFP::HIS3MX* | This study |
| AJY3074 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rei1Δ::KanMX TIF6-GFP::HIS3MX* | This study |
| AJY3075 | *MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2Δ0 mrt4Δ::KanMX TIF6-GFP::HIS3MX* | This study |
| AJY3078 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ TIF6-GFP::HIS3MX* | This study |
| AJY3079 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ drg1ts TIF6-GFP::HIS3MX* | This study |
| AJY3080 | *MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 URA3::P$_{GAL1}$-RPP0 TIF6-GFP::HIS3MX* | This study |
| AJY3083 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ KanMX::P$_{GAL1}$-3xHA-EFL1 TIF6-GFP::HIS3MX* | This study |
| AJY3088 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ drg1$^{ts}$ ARX1-GFP::HIS3MX* | This study |
| AJY3089 | *MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 URA::P$_{GAL1}$-RPP0 ARX1-GFP::HIS3MX* | This study |
| AJY3086 | *MATα can1Δ::MFA1pr-HIS3 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sdo1ts ARX1-GFP::HIS3MX* | This study |
| AJY3093 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 arx1Δ::KanMX rei1Δ::KanMX TIF6-GFP::HIS3MX* | This study |
| AJY3090 | *MATα can1Δ::MFA1pr-HIS3 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 ARX1-GFP::HIS3MX* | This study |

| | | |
|---|---|---|
| AJY3098 | *MATa ura3Δ0 his3Δ1 leu2Δ0 lys2Δ0 yvh1Δ::NAT$^r$ mrt4Δ::KanMX TIF6-GFP::HIS3MX* | This study |
| AJY3100 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ MRT4-GFP::HIS3MX* | This study |
| AJY3101 | *MATa ade2 his3Δ leu2Δ trp1Δ ura3Δ afg2-18* (*drg1$^{ts}$*) *MRT4-GFP::HIS3MX* | This study |
| AJY3102 | *MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 URA3::P$_{GAL1}$-RPP0 MRT4-GFP::HIS3MX* | This study |
| AJY3110 | *MATα ade2-1 ura3 leu2 his3 yvh1Δ::NAT$^r$ URA3::P$_{GAL1}$-RPP0* | This study |
| AJY3118 | *MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rei1Δ::KanMX ARX1-GFP::HIS3MX* | This study |
| AJY3121 | *MAT his3Δ1 leu2Δ0 ura3Δ0 jjj1Δ::KanMX ARX1-GFP::HIS3MX* | This study |

**Table S2. Plasmids Used in This Study**

| Plasmids | Relevant markers | Source |
|---|---|---|
| pAJ538 | *NMD3-13xmyc LEU2* CEN | (Ho et al., 2000) |
| pAJ582 | *NMD3-GFP LEU2* CEN | (Hedges et al., 2005) |
| pAJ754 | *NMD3(AAA)-GFP LEU2* CEN | (Hedges et al., 2005) |
| pAJ758 | *NMD3(AAA)-GFP URA3* CEN | (Hedges et al., 2005) |
| pAJ898 | *RLP24-HA URA3* CEN | This study |
| pAJ901 | *LSG1-myc URA3* CEN | (Kallstrom et al., 2003) |
| pAJ903 | *LSG1-myc LEU2* CEN | (Kallstrom et al., 2003) |
| pAJ1003 | *TIF6-GFP LEU2* CEN | This study |
| pAJ1004 | *TIF6-GFP URA3* CEN | This study |
| pAJ1025 | *ARX1-GFP LEU2* CEN | This study |
| pAJ1015 | *ARX1-GFP URA3* CEN | (Hung and Johnson, 2006) |
| pAJ1018 | *REI1-myc URA3* CEN | (Hung and Johnson, 2006) |
| pAJ1028 | *REI1-myc LEU2* CEN | This study |
| pAJ1139 | *RLP24-HA HIS3* CEN | This study |
| pAJ1682 | *arx1-S347P URA3* CEN | This study |
| pAJ1875 | *RLP24 HIS3* CEN | This study |
| pAJ1895 | *RLP24ΔC-HA HIS3* CEN | This study |
| pAJ2064 | $P_{GAL}$*::RLP24 URA3* CEN | This study |
| pAJ2065 | $P_{GAL}$*::rlp24ΔC-HA URA3* CEN | This study |
| pAJ2074 | *NOG1-myc LEU2* CEN | This study |
| pAJ2075 | *DRG1-myc LEU2* CEN | This study |
| pAJ2239 | *DRG1-myc URA3* CEN | This study |
| pAJ2250 | *TIF6(V192F) LEU2* CEN | This study |
| pAJ2423 | *arx1-S347P-GFP URA3* CEN | This study |
| pAJ2425 | *ARX1 URA3* CEN | This study |
| pAJ2451 | *TIF6 LEU2* CEN | This study |
| pAJ2481 | $NLS_{SV40}$*-YVH1-GFP LEU2* CEN | (Lo et al., 2009) |

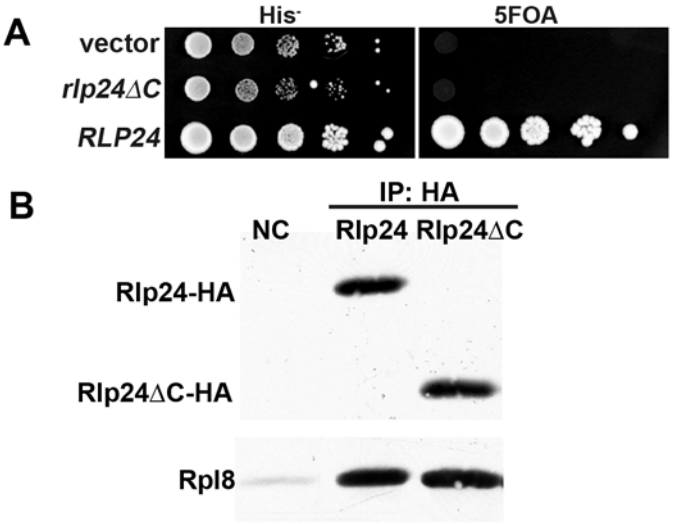


Figure S1, Lo et al

**Figure S1. Rlp24ΔC does not complement *rlp24*Δ but retains 60S subunit binding.** (A) AJY2467 (*rlp24*Δ with plasmid pAJ898 (*RLP24-HA URA3*)) was transformed with vector, *rlp24*Δ*C-HA* (pAJ1895) or *RLP24* (pAJ1875). Ten-fold serial dilutions were plated on His drop out and on 5FOA media to select against the wild-type *RLP24 URA3* vector. (B) Rlp24-HA (pAJ1139) or rlp24ΔC-HA (pAJ1895) was expressed in BY4741, extracts were prepared and immunoprecipitated. Western blotting was done with anti-HA and anti-Rpl8 antibodies.

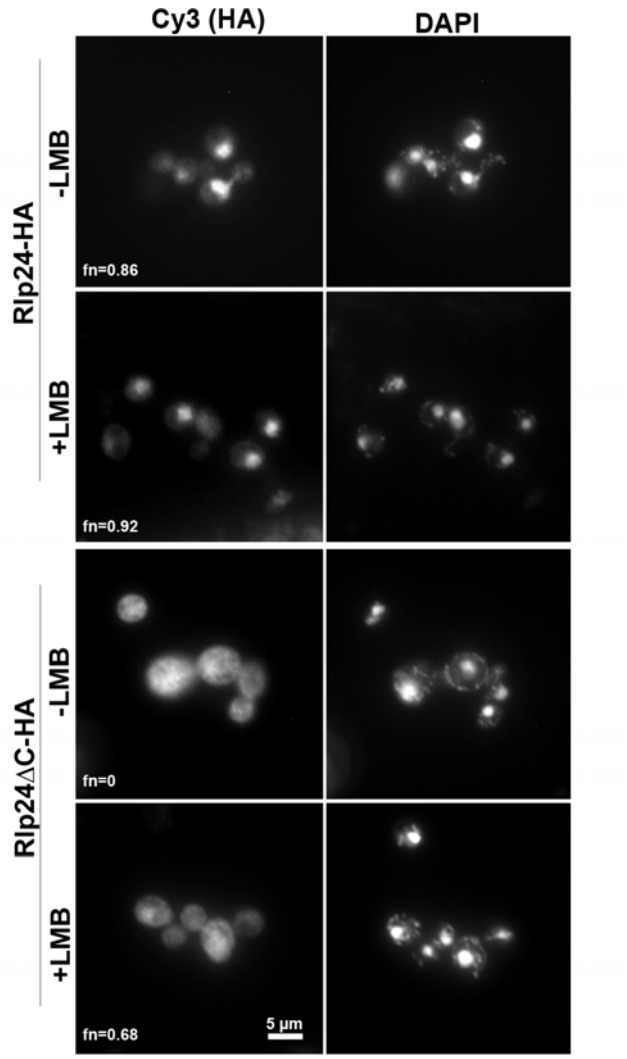


**Figure S2, Lo et al**

**Figure S2. Rlp24ΔC can recycle to the nucleus.** The leptomycin B-sensitive strain AJY1539 containing Rlp24-HA (pAJ1139) or Rlp24ΔC-HA (pAJ1895) was treated with 0.1µg/ml LMB as indicated for 30 minutes, fixed with 3.7% formaldehyde and prepared for indirect immunofluorescence. Cy3 was used to visualize Rlp24 or Rlp24ΔC and DAPI for DNA.

## Supplemental Experimental Procedures

### Strains and Plasmids

AJY2474, AJY2467, AJY2956, and AJY2957 were obtained by transforming the indicated plasmids into the appropriate heterozygous diploid deletion strain (Research Genetics) followed by sporulation. The GAL1 promoter and 3xHA tag was amplified from pFA6A pGAL-3HA::KanMX (Longtine et al., 1998) and integrated into W303 to generate AJY2981. AJY3005 and AJY3006 were derived from crossing BSY28 and AJY1699 (tif6Δ pTIF6), and AJY3013 and AJY3014 were from crossing AJY2981 with AJY1700 (tif6Δ pTIF6).

To make pAJ1875 *RLP24* was amplified with primers AJO585 (GGCGTCGACTACGTTGATTCAAATGGC) and AJO613 (GCGCGACGTCACATCTCTAACTCCTAAG). The fragment was digested with SmaI and SalI and ligated into the same sites of pRS413. To make pAJ1895, PCR was carried out with primers AJO582 (GCGACTCGAGTGATATCTATCGCTTTTCTAGGA) and AJO1005 (GAATTAATTAATTTAGCCAACTTTCTGGC). The fragment was digested with SalI and PacI and ligated into the same sites of pAJ1139. pAJ2064 (*GAL:RLP24-HA*) and pAJ2065 (*GAL:rlp24*Δ*C-HA*) were made with the same primers but different templates. AJO1064 (GCGGAATTCATGAGAATTTATCAATGCCA) and AJO1037 (GCTACGGCTAGAGCTCTGGAGCTTTTGAATC) were used to amplify rlp24ΔC from pAJ1139 or RLP24 from pAJ1895. The PCR products were digested with EcoRI and PacI and ligated into pAJ1810. pAJ2074 (*NOG1-myc*) was made by PCR amplification using AJO1097 (CGTGAGCTCCTCTGGCTGTCTTGCAGATT) and AJO1098 (ACGTTAATTAAACGGAAATCTGTCTTACCGAC), and ligating the SstI

and PacI cut fragment into the same sites of pAJ1026. To make pAJ2075 (*DRG1-myc*), *DRG1* was amplified with AJO1099 (CGTCGGCCGAGTGGGCCCGTGGTTTATCA) and AJO1100 (ACGTTAATTAACGAAGATGAACCGCTTCTTAG). The PCR fragment was digested with EagI and PacI and ligated into the same sites of pAJ1026.

**HeLa Cell Work**

**Plasmid Construction**

DUSP12 was amplified from a full length cDNA clone (MGC: 10337, IMAGE: 3958403, OpenBiosystems) by using primers DUSP12-F1 (AGGGAGACCCAAGCTTATGTTGGAGGCTCCGGGC) and DUSP12-R1 ( CGTTACTAGTGGATCCCCTATTTTTCCTGTTTGTGATCCCAA), and inserted into pcDNA3-EGFP between HindIII and BamHI to generate pcDNA3-DUSP12-EGFP.

**Cell Culture and Transfection**

HeLa cells were cultured in glass bottom 6-well plates containing DMEM with 10% fetal calf serum and maintained at 37ºC with 5% $CO_2$. At about 30% confluency, HeLa cells were transfected with 10nM siRNA specific to either P0 or DUSP12 (ON-TARGETplus SMARTpool from Thermo Fisher Scientific) by using RNAiMAX (Invitrogen) for 48 hrs. ON-TARGETplus Non-targeting Pool (Thermo Fisher Scientific) was used as negative control for siRNA transfection. Knockdown efficiency was monitored by Western blotting using mouse anti-P0 (Abnova) or chicken anti-DUSP12 antibody (USBiological). GAPDH, as a loading control for western blot, was probed with mouse anti-GAPDH (Santa Cruz Biotechnology). Goat anti-mouse or goat anti-chicken HRP

conjugated secondary antibodies (Santa Cruz Biotechnology) were used for Western blotting. For plasmid transfection, FuGENE HD transfection reagent (Roche) was used following the manufacturer's instructions; after 12 hrs, HeLa cells were fixed with 4% formaldehyde for 15 min and nuclei were stained with Hoechst 33342 (Invitrogen).

**Immunofluorescence and Microscopy**

HeLa cells were fixed with 4% formaldehyde in PBS for 15 min at room temperature followed by permeabilization with cold methanol for 10 min at -20ºC. Cells were blocked with blotting grade 1% BSA (Promega) for 1 hr at room temperature and then incubated with mouse anti-MRTO4 (Santa Cruz Biotechnology) and rabbit anti-eIF6 (Cell Signaling) antibodies in blocking buffer at 4ºC overnight. After washing three times with PBS, cells were incubated with goat anti-mouse IgG Texas Red conjugated (Santa Cruz Biotechnology) and goat anti-rabbit IgG Alexa Fluor 488 conjugated (Invitrogen) secondary antibodies for 1 hr at room temperature. Cell nuclei were stained with Hoechst 33342 (Invitrogen). Fluorescence was visualized on an inverted microscope (Nikon Eclipse TE2000-E) fitted with a Plan Apo 60x/0.95 objective and a digital camera (Cascade II 512; Photometrics) controlled with the NIS Elements software (AR 3.0). Images were prepared using Photoshop.

## Supplemental References


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