Supplemental Online Information

Supplemental Figure legends

Figure S1 (related to Figure 1). (A) Mutation of Fap7 produces the same phenotype as deletion of Fap7. (B) Biological repeat of Gal1::Fap7 samples grown for 16h in dextrose. (top) Absorbance profiles at 254 nm for 10-50% sucrose gradients; (middle) Western blots for assembly factors in each fraction; (bottom) Northern blots for rRNAs.

Figure S2 (related to Figure 3). Depletion of eIF5B accumulates Rio2 in 40S particles. (A) BY4741 wild type control; (B) eIF5B::KanMX6 cells. Absorbance profiles at 254 nm for 10-50% sucrose gradients (top), Western blots for assembly factors (middle) and Northern blots for rRNAs and precursors (bottom) are shown. (C) Biological repeat of the experiment in Figure S2B. (D) Biological repeat of the experiment in Figure 3.

Figure S3 (related to Figure 4). (A) Accumulation of 20S rRNA in lysates from strains used herein. (B) Depletion of Rli1 leads to degradation of pre-40S assembly factors.

Figure S4 (related to Figure 4). (A) Biological repeat of the experiment in Figure 4A. (B) Biological repeat of the experiment in Figure 4B.

Figure S5 (related to Figure 5). (A) Biological repeat of the experiment in Figure 5A. (B) Biological repeat of the experiment in Figure 5B.

Table S1: Yeast strains used in this study

Strain	Description	Genotype	Reference
YKK87	Rio2TAP	Rio2TAP::HIS3	Open Biosystems
YKK392	eIF5BΔ	Fun12::KanMX6	Open Biosystems
YKK395	Dom34Δ	Dom34::KanMX6	Open Biosystems
YKK216	Rio2TAP,Gal1::Fap7	Rio2TAP::HIS3, GalFap7::NatMX6	This work
YKK178	eIF5BΔ,Gal1::Fap7	Fun12::KanMX6, GalFap7::NatMX6	This work
YKK82	Gal1::Rli1TAP	Gal::Rli1TAP::KanMX6, HIS3	This work
YKK217	Gal1::Nob1, Rio2TAP	Rio2TAP::HIS3, GalNob1::KanMX6	This work
YKK398	Gal1::Nob1, Rio2TAP, Gal::Rli1	Rio2TAP::HIS3, GalNob1::KanMX6, GalRli1::NatMX6	This work

All strains have the BY4741 background provided by Open Biosystems.

Table S2: Oligonucleotides used in this study.

#	name	sequence
1	Fap7F2	GGAATAGTATATTTTGATTCTCTTCTCTTCCTGTTTTCCG AATTCGAGCTCGTTTAAAC
2	Fap7R2	TTCCTGTAACAATGATATTCGGTCCATACCGTCTTGTTC CATTTTGAGATCCGGGTTTT
3	Fap7up	CCACTTCGCTTCTGGATGTT
4	Fap7down	CGTCCCGTTTACTTCAATGC
5	Rli1F4	CTGTATTCTTAGATCTTCATTGTGATCTGTTTCAGCGCG A GAATTCGAGCTCGTTAAC
6	Rli1R2	TATCAGCGCTAACGATAGCGATACGACTGTTTTTATCAC TCATTTTGAGATCCGGGTTTT
7	Probe 5S	CTACTCGGTCAGGCTC
8	Probe tRNA^{Met}	TCGGTTTCGATCCGAGGACATCAGGGTTATGA
9	Probe b/20S	GCTCTCATGCTCTTGCC
10	Probe 25S	GCCCGTTCCCTTGGCTGTG
11	Probe 18S	CATGGCTTAATCTTTGAGAC
12	Probe U2	CAGATACTACACTTG