Supplemental Materials

Molecular Biology of the Cell

Sung et al.

Figure S1. Characterization of Rpl26 used as a substrate in this study.

(A) *rpl26a*Δ*rpl26b*Δ mutants are viable. Cells of the indicated genotypes were spotted on YPD in 10-fold serial dilutions and incubated at 30°C for 2 days. (B) Polysome fraction is slightly decreased in *rpl26a*Δ*rpl26b*Δ mutants. WT and *rpl26a*Δ*rpl26b*Δ cells grown in YPD were treated with cycloheximide for 15 min before lysis to stabilize polysomes. The UV absorbance profiles of lysates fractionated by sucrose gradient are shown. Gray line: WT. Red line: *rpl26a*Δ*rpl26b*Δ. (C) Location of Rpl26 in 80S ribosome. The cartoons were generated with the UCSF Chimera program (Pettersen et al., 2004), using the atomic model for the crystal structure of the yeast 80S ribosome (PDB files 3U5D and 3U5E) (Ben-Shem et al., 2011). Left panel: entire 80S subunit with Rpl26 in blue. Right panel: Interactions between Rpl26 (blue) and rRNA species, with 25S, 5.8S, and 5S rRNAs colored in gray, yellow, and red, respectively.

Figure S2. Overexpressed Rpl26a does not accumulate to higher levels in *san1*Δ.

WT or *san1*Δ cells were induced with galactose for 1 hr to express Rpl26a-FLAG, and cell lysates were fractionated by SDS-PAGE. Immunoblotting was performed using an antibody against FLAG. Hexokinase was used as an internal control.

Figure S3. Newly synthesized endogenous ribosomal proteins aggregate in proteasome inhibitor-treated cells.

(A) Proteins aggregate in *pre9*Δ and cells treated with the proteasome inhibitor MG132. Cells of the indicated genotype were mock-treated or treated with 50 μM

bortezomib (btz) or 50 μM MG132 for 1 hr. Whole cell extract (WCE) was fractionated into soluble and pellet fractions, which were analyzed by SDS-PAGE followed by Coomassie Blue staining. (B) Scatter plots representing the ΔiBAQ of biological replicate C *vs*. A (left panel) and replicate C *vs.* B (right panel) for aggregated proteins in bortezomib (btz)-treated cells. Proteins are color-coded as indicated. Pearson's r-value is indicated on top of the plot. (C) The 20 ribosomal proteins that exhibited the largest increase in the pellet fraction upon proteasome inhibition. Values are the average difference of iBAQ between bortezomib (btz)-treated and non-treated samples with error bars indicating the standard error of the mean (SEM). Blue and red bars correspond to 60S and 40S proteins, respectively. (D) Violin plot representing the distribution of the ΔiBAQ for proteins of the large (60S) subunit (blue), small (40S) subunit (green) and non-ribosomal proteins (red).#

Figure S1. Characterization of Rpl26 used as a substrate in this study

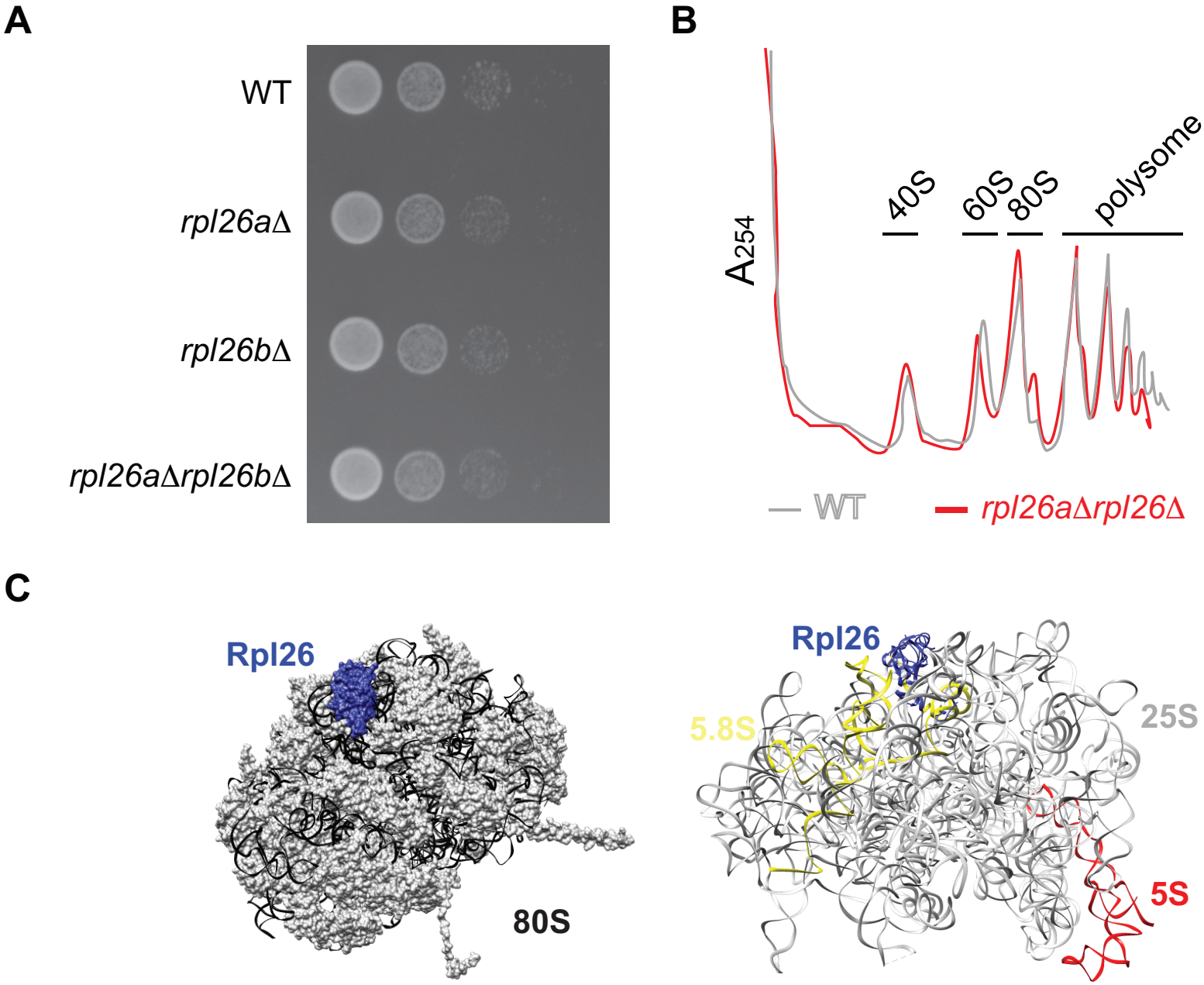


Figure S2. Overexpressed Rpl26a does not accumulate to higher levels in *san1*Δ

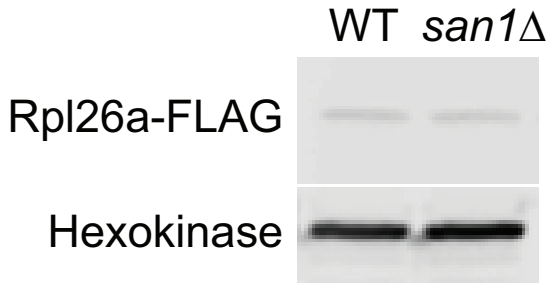
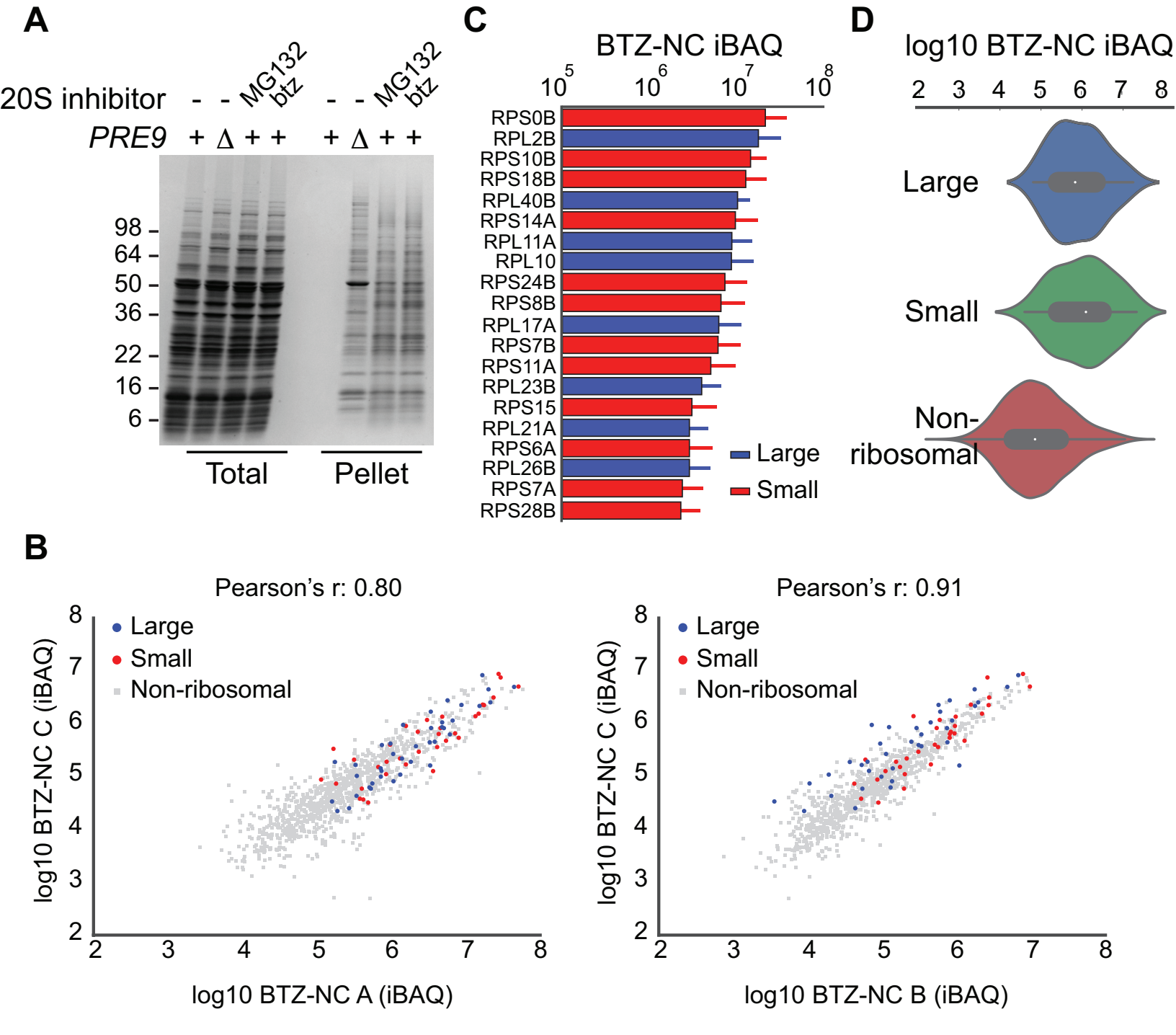


Figure S3. Newly synthesized endogenous ribosomal proteins aggregate in proteasome inhibitor-treated cells



Supplemental table 1. Yeast strains used in this study

RJD	Genotype	Source
1721	(BY4741) *MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	-
6427	BY4741 *pdr5*Δ*::KanMX4*	OBS*
6428	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-RPL13B-HHZ**	This study
6429	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-RPL26A-HHZ**	This study
6430	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-RPL34A-HHZ**	This study
6431	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-RPL36A-HHZ**	This study
6432	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-RPS17B-HHZ**	This study
6433	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-RPS18A-HHZ**	This study
6434	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-RPS24A-HHZ**	This study
6435	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-RPS24B-HHZ**	This study
6436	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-HOG1-HHZ**	This study
6437	BY4741 *pdr5*Δ*::KanMX4*, p*GAL1-HHT2-HHZ**	This study
6438	BY4741 *rpl26a*Δ*::KanMX4*	OBS*
6439	BY4741 *rpl26b*Δ*::KanMX4*	OBS*
6440	BY4741 *rpl26a*Δ*::KanMX4 rpl26b*Δ*::KlLEU2*	This study
6441	BY4741,pESC(HIS3)-P_{GAL10}-*RPL26A-GFP*	This study
6442	BY4741 *rpl26a*Δ*::KanMX4 rpl26b*Δ*::KlLEU2*,pESC(HIS3)-P_{GAL10}-*RPL26A-GFP*	This study
6443	BY4741,pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6444	BY4741 *rpl26a*Δ*::KanMX4 rpl26b*Δ*::KlLEU2*,pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6449	BY4741 *RPL26A-GFP::KanMX4 RPL26B-GFP::KlURA3*,pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6450	BY4741 *rpl26a*Δ*::KanMX4 RPL26B-GFP::KlURA3*,pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6451	BY4741 *rpl26b*Δ*::KanMX4 RPL26A-GFP::KlURA3*,pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6452	BY4741 *pdr5*Δ*::KanMX4,* pESC(HIS3)	This study

6453	BY4741 *pdr5Δ::KanMX4,* pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6454	BY4741 *pre9Δ::KlLEU2*	This study
6455	BY4741 *pre9Δ::KlLEU2*, pESC(HIS)-P_{GAL10}-*RPL26A-GFP*	This study
6456	BY4741 *pre9Δ::KlLEU2*, pESC(HIS3)	This study
6457	BY4741 *pdr5Δ::KanMX4*, pESC(HIS3)-P_{GAL10}-*RPL26A-GFP*	This study
6459	BY4741 *NOP56-RFP::KlURA3*, pESC(HIS3)-P_{GAL10}-*RPL26A-GFP*	This study
6461	BY4741 *NOP56-RFP::KlURA3 pre9Δ::KlLEU2*, pESC(HIS3)-P_{GAL10}-*RPL26A-GFP*	This study
6462	BY4741 *pre9Δ::KlLEU2,* pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6464	BY4741 *rpl26aΔ::KanMX4 rpl26bΔ::KlLEU2 pre9Δ::KlURA3*,pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6465	BY4741 *RPL26A-GFP::HIS3MX6,* pESC(URA)	This study
6466	BY4741 *RPL26A-GFP::HIS3MX6,* p*GAL1-RPL26A-HHZ**	This study
6467	BY4741 *rpl26aΔ::KanMX4 rpl26bΔ::KlLEU2,* p*GAL1-RPL26A-HHZ**	This study
6615	BY4741 *atg7Δ::KanMX4*, pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study
6616	BY4741 *pep4Δ::KanMX4*, pESC(HIS3)-P_{GAL10}-*RPL26A-FLAG*	This study

OBS* : OpenBiosystems, yeast knockout collection

HHZ* : 6×His-HA-Protein A (ZZ domain)

Supplemental table 2. Plasmids used in this study

RDB	Plasmid	Source
3112	pGAL1-RPL26A-HHZ*	Open Biosystems
3113	pGAL1-RPL34A-HHZ*	Open Biosystems
3114	pGAL1-RPL36A-HHZ*	Open Biosystems
3115	pGAL1-RPL13B-HHZ*	Open Biosystems
3116	pGAL1-RPS18A-HHZ*	Open Biosystems
3117	pGAL1-RPS24A-HHZ*	Open Biosystems
3118	pGAL1-RPS24B-HHZ*	Open Biosystems
3119	pGAL1-RPS17B-HHZ*	Open Biosystems
3120	pGAL1-HOG1-HHZ*	Open Biosystems
3121	pGAL1-HHT2-HHZ*	Open Biosystems
3122	pESC (HIS)	Open Biosystems
3123	pESC (URA)	Open Biosystems
3124	pESC(HIS)-P_{GAL10}-RPL26A-FLAG	This study
3125	pESC(HIS)-P_{GAL10}-RPL26A-GFP	This study
3145	pKlLEU2	EUROSCARF (pUG73)
3146	pFA6a-GFP-KlURA3	(Sung et al., 2008)

HHZ* : 6×His-HA-Protein A (ZZ domain)

Sung, M.K., Ha, C.W., and Huh, W.K. (2008). A vector system for efficient and economical switching of C-terminal epitope tags in Saccharomyces cerevisiae. Yeast *25*, 301-311.