Table S1. Ribosome binding and depurination activity of mRTA and RTA mutants

	Ribosome binding	Depurination activity
mRTA	+++	+++
R193A/R235A	+	++
G212E	+++	+

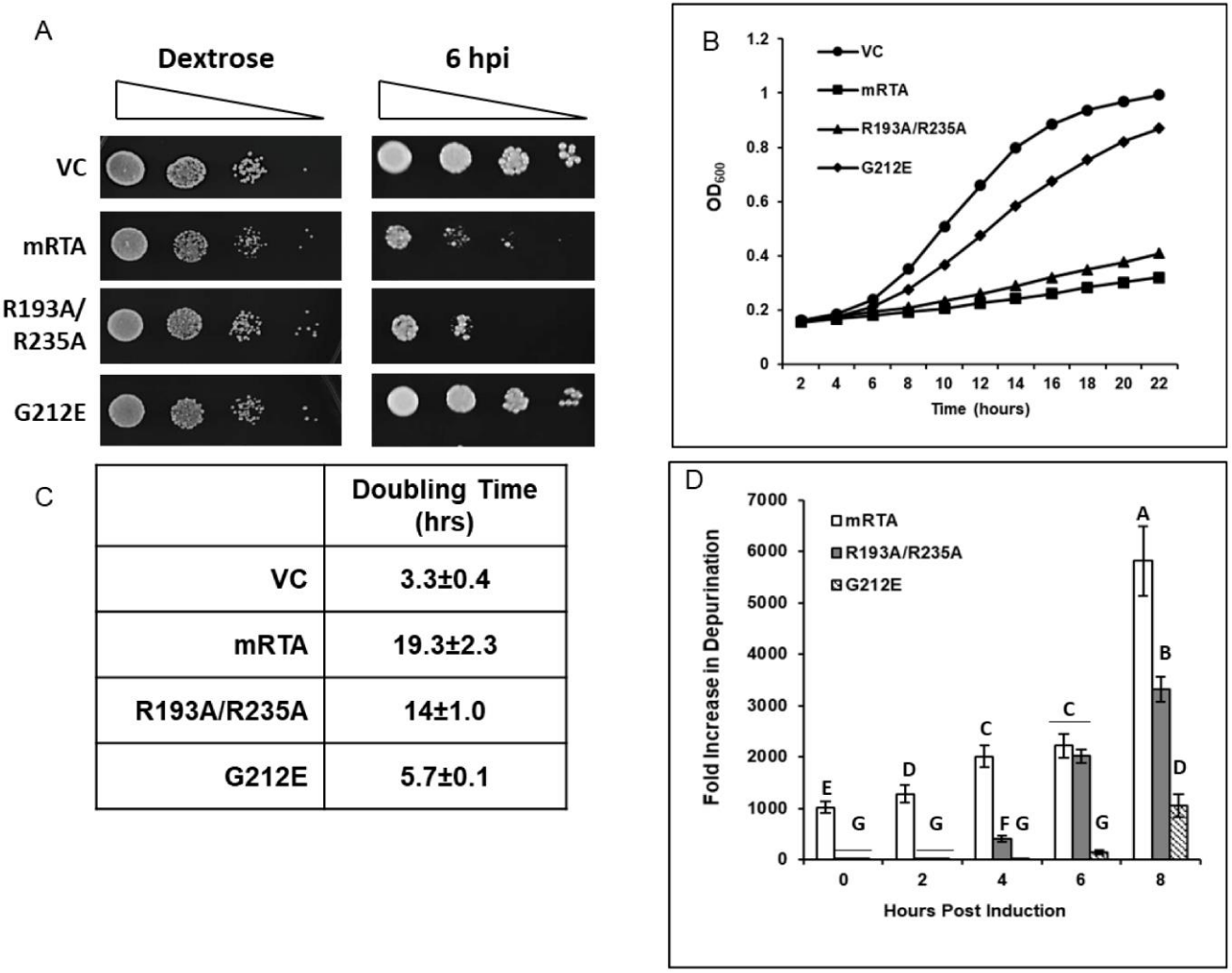

Fig. S1. **Cell viability and growth curve analysis of yeast expressing WT and mutant RTAs.** *A*, Yeast cells transformed with VC, WT or mutant RTA expression plasmids were grown in dextrose or galactose for 6 hours to induce RTA expression. A series of 10-fold dilutions were spotted on dextrose media and incubated at 30° C for 48 hours. *B*, Yeast from mid-log phase cultures were back diluted into galactose media to induce RTA expression and incubated at 30° C in a BioTek Synergy 4 plate reader. OD_{600} was measured every 2 hours. *C*, Average doubling time was calculated using the exponential fit of 3 growth curves. *D*, Ribosome depurination in yeast expressing WT or mutant RTAs compared to VC was quantified by qRT-PCR using total RNA (375 ng) prepared from 1 OD of cells taken at 0, 2, 4, 6 and 8 hpi. The y-axis shows the average fold increase in ribosome depurination compared to VC with error bars representing the range of depurination from 2 biological replicates using 3 technical replicates for each. Means with different letters show significant differences according to the LSD test ($p<0.01$).

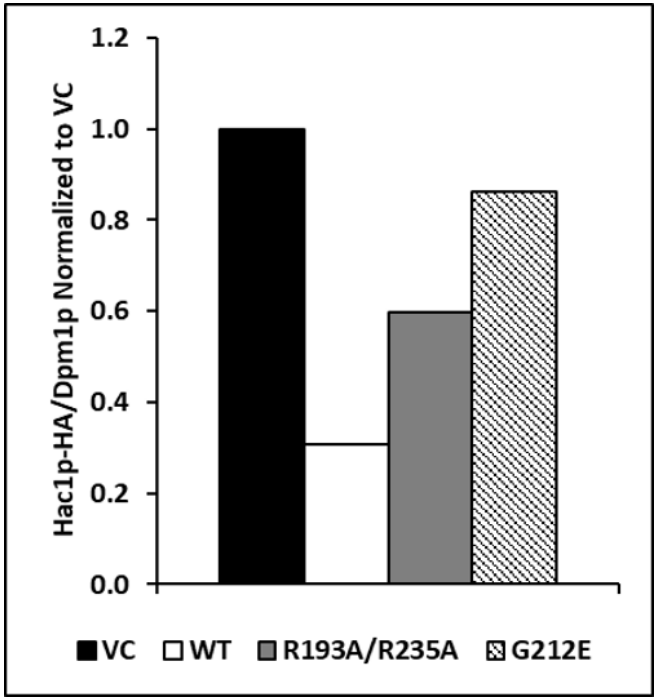

Fig. S2. **Expression of WT mRTA decreases the level of constitutively expressed HAC1-HA.** HAC1-HA protein was normalized to Dpm1p by densitometry using Li-COR analysis software in yeast carrying VC, WT or mutant RTA expression plasmids grown in dextrose and galactose. The y-axis shows the normalized signal intensity.

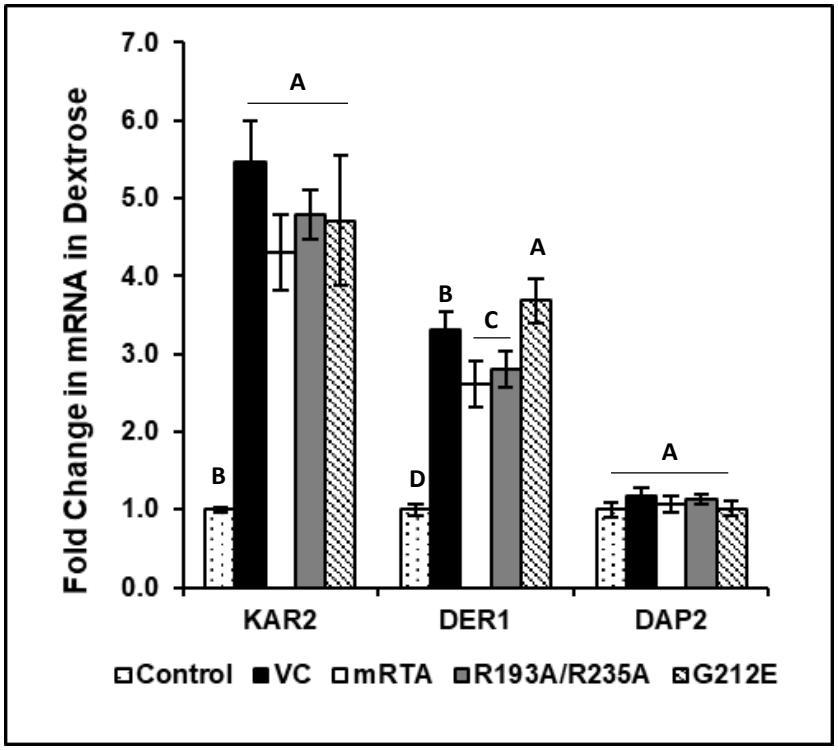

Fig. S3. **Expression of genes associated with the UPR is induced when HAC1-HA is provided *in trans*.** Fold increase in *KAR2*, *DER1* and *DAP2* mRNA in yeast carrying VC, WT or mutant RTA expression plasmids and p*HAC1*^{i}-HA was quantified by qRT-PCR using total RNA prepared from cells grown in dextrose. The y-axis shows the average fold increase in mRNA compared control yeast carrying the p*HAC1*^{u} plasmid representing the range of expression from 2 biological replicates using 3 technical replicates for each. Statistical analysis was conducted separately for each gene. Means with different letters show significant differences according to the LSD ($p<0.001$).

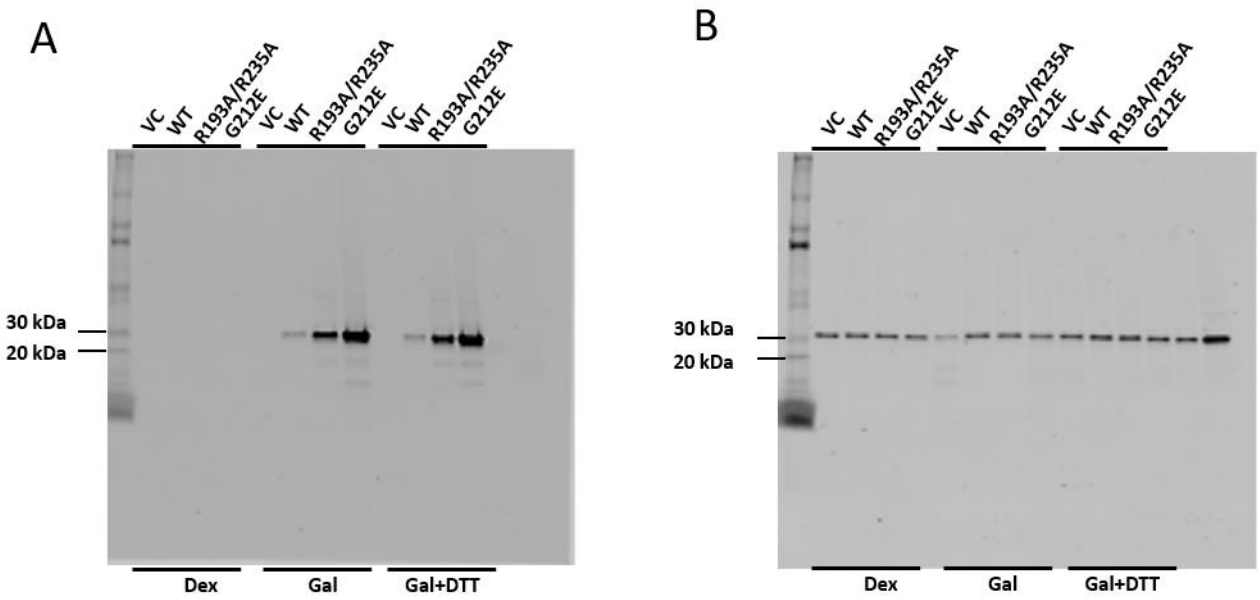

Fig. S4. **Uncropped western blots used in Fig. 1A to show expression of RTA and Dpm1p.** *A*, uncropped anti-RTA and *B*, uncropped anti-Dpm1p western blots.

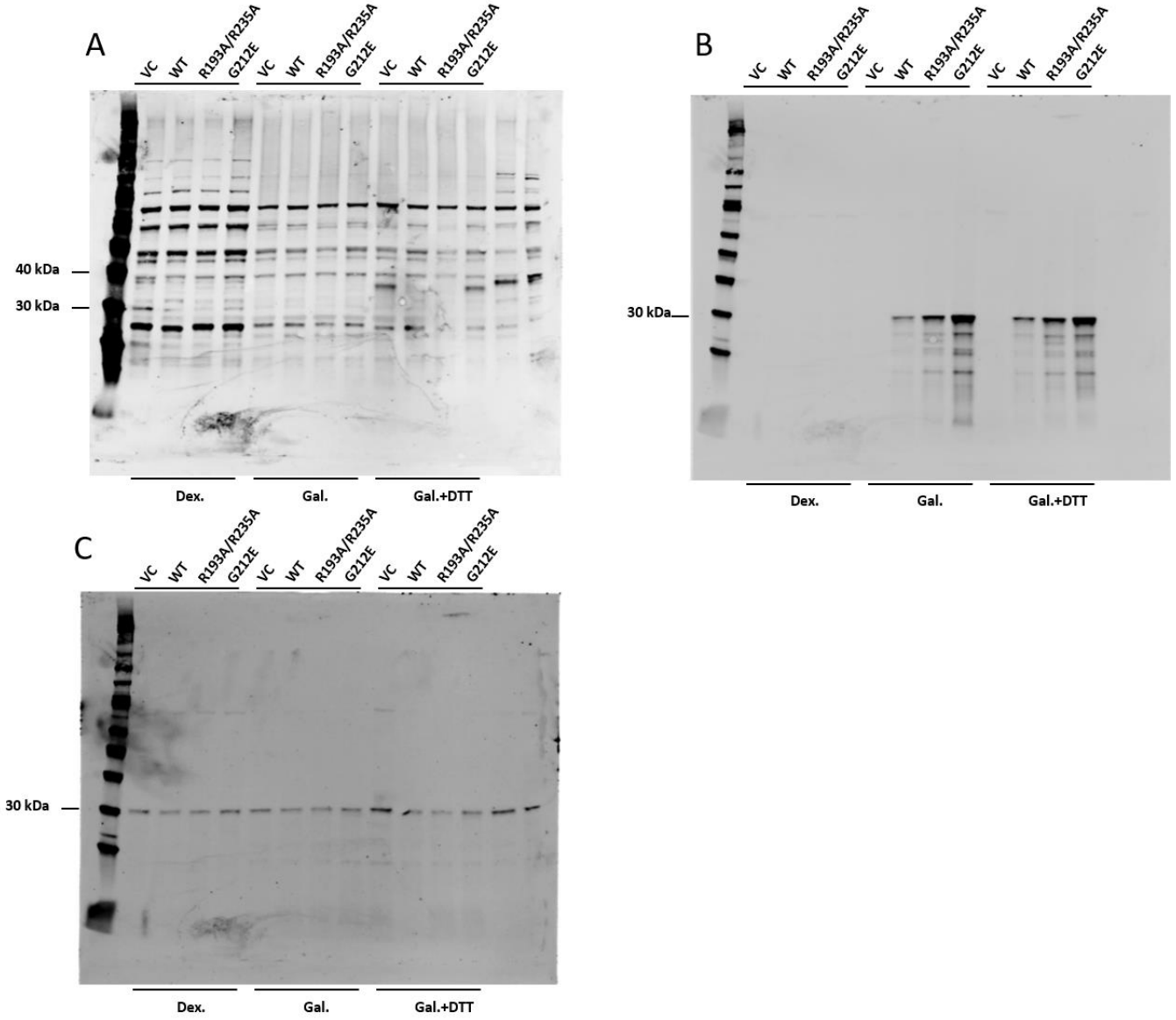

Fig. S5. **Uncropped western blots used in Fig. 3D to show expression of HAC1, RTA and Dpm1p.** *A*, uncropped anti-HAC1, *B*, anti-RTA, and *C*, anti-Dpm1p western blots.

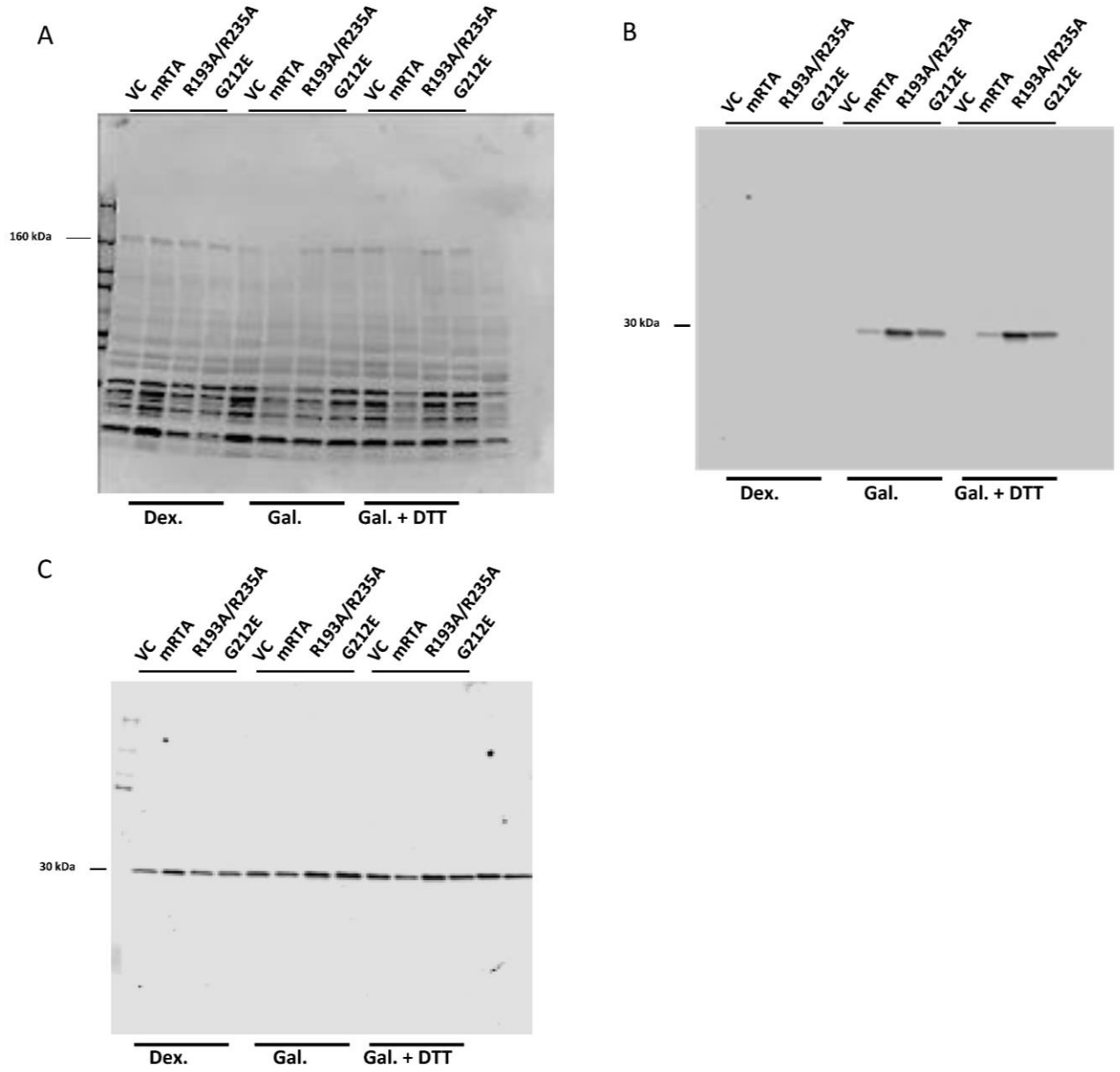

Fig. S6. **Uncropped western blots used in Fig. 4F to show expression of Ire1p-GFP, RTA and Dpm1p.** *A*, uncropped anti-GFP, *B*, anti-RTA, and *C*, anti-Dpm1p western blots.

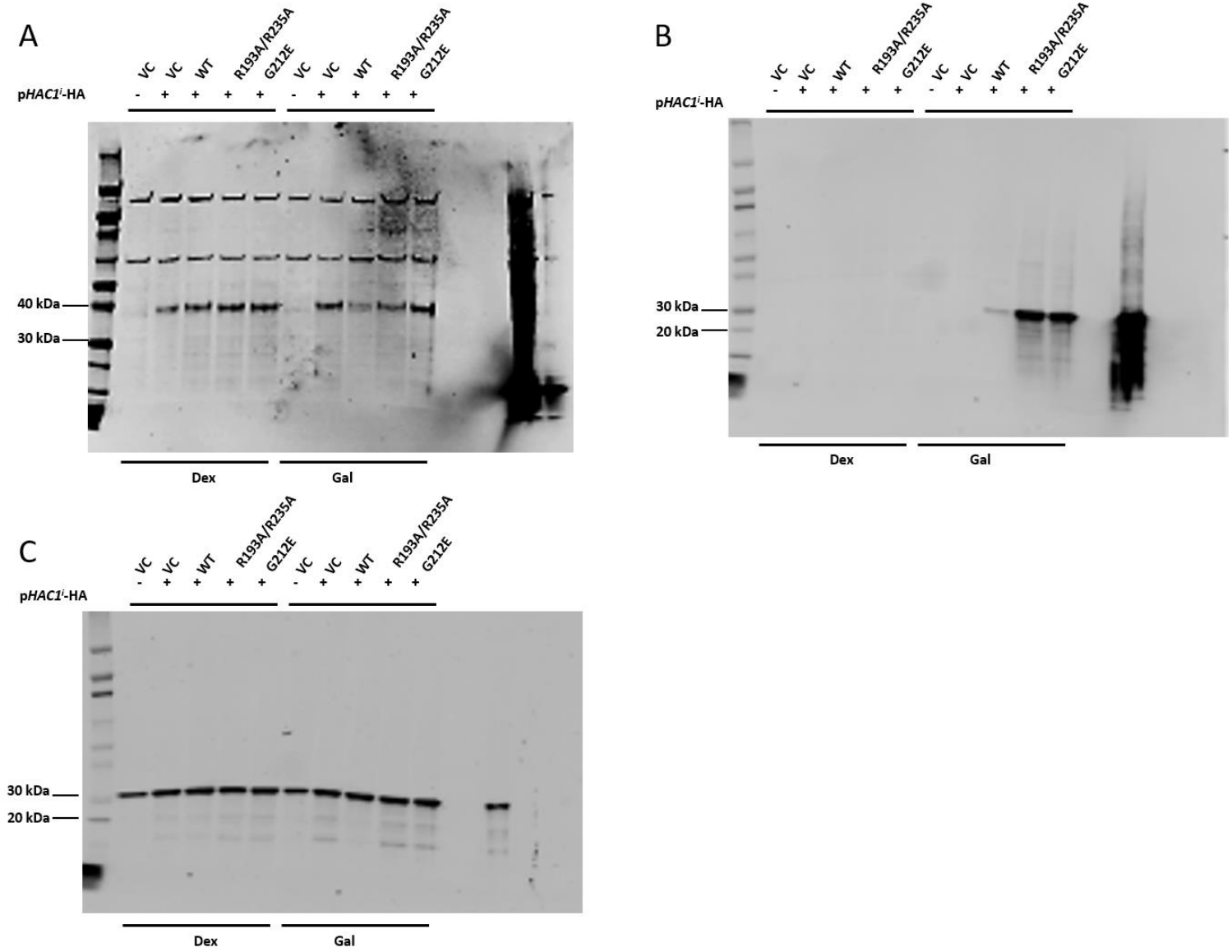

Fig. S7. **Uncropped western blots used in Fig. 6D to show expression of HAC1, RTA and Dpm1p.** *A*, uncropped anti-HA, *B*, anti-RTA, and *C*, anti-Dpm1p western blots.

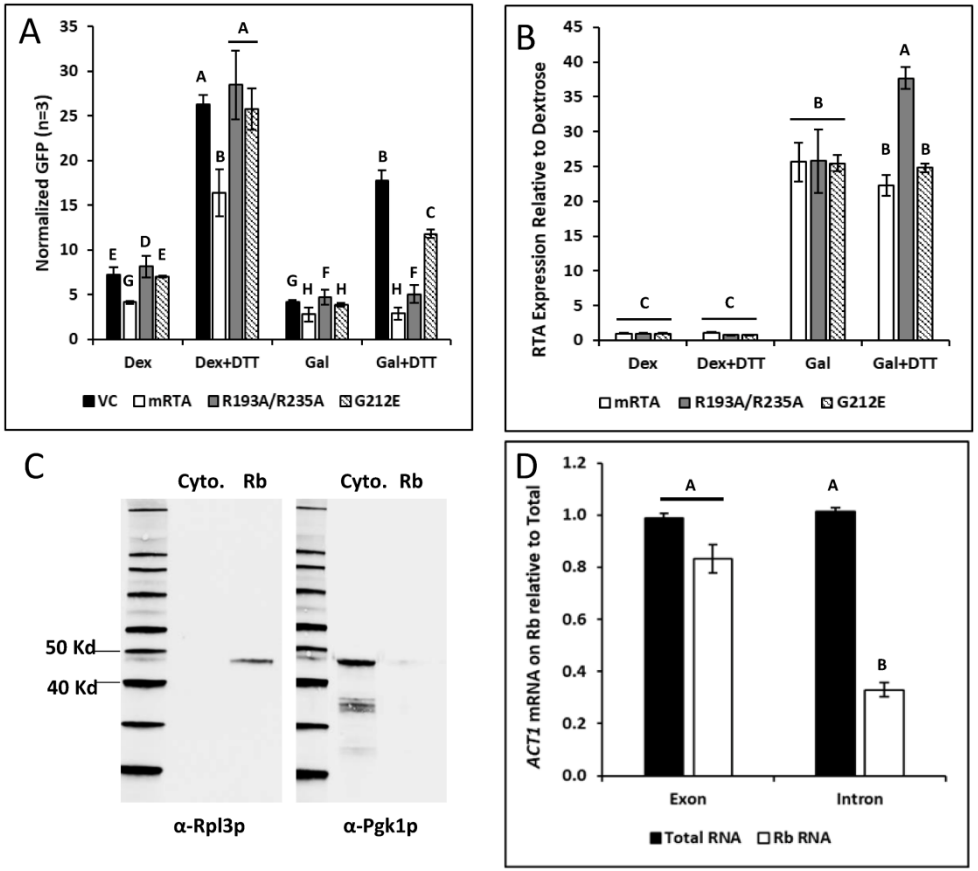

Fig. S8. **Analysis of ribosomes from yeast.** *A*, GFP fluorescence from a UPRE-GFP reporter measured by flow cytometry in yeast carrying VC, WT and mutant RTA expression plasmids grown in dextrose in the absence (Dex) or presence ER stress (Dex+DTT) and with RTA expression in the absence (Gal) or presence (Gal+DTT) of ER stress. The y-axis shows the GFP signal normalized to yeast lacking the UPRE-GFP reporter from 3 biological replicates along with the standard error (n=3). Statistical analysis was done using the means from all groups. Means with different alphabetical letters show significant differences according to the LSD test ($p<0.01$). *B*, RTA expression from yeast carrying VC, WT or mutant RTA plasmids grown in dextrose (Dex) or galactose (Gal) in the absence or presence of ER stress compared to growth in dextrose. The y-axis shows mean fold change in RTA expression with standard error representing the range of expression from 3 biological replicates using 3 technical replicates for each. Statistical analysis was done using the means from all groups. Means with different letters show significant differences according to the LSD test ($p<0.01$). *C*, Protein lysates from equal cell volumes of cytoplasmic and ribosomal fractions were subjected to western blot analysis with monoclonal antibodies against ribosomal protein L3 (Rpl3p) and cytoplasmic protein Pgk1p. *D*, The abundance of *ACT1* mRNA (exon) and pre-mRNA (intron) sequences associated with purified ribosomes relative to total RNA representing the range of expression from 3 biological replicates using 3 technical replicates for each. Means with different alphabetical letters show significant differences according to the LSD test ($p<0.001$).