**Supplementary Figures and Tables**

**Figure S1.- Characterization of cytoplasmic version of Rat1 protein (cRat1).** A) Protein expression levels of Xrn1 and Rat1-FLAG in wild-type (wt) and *xrn1Δ* cells transformed with the indicated cRat1 derivatives were analyzed by Western blotting using phosphoglycerate kinase (PGK) as a loading control. Note that a non-specific band just below the Xrn1 band is seen in all wt and *xrn1Δ* samples when using the Xrn1 antibody. B) mRNA half-life (HL) in minutes, determined as described in Materials and Methods, for two specific mRNAs (*RPL25* and *ACT1*) in the four strains. Note that the highly increased HL of both mRNAs in the *xrn1∆* strain (with regards to the wt) was fully complemented by the expression of cRat1-3xFLAG. C) The generation times (GT), cell volumes and global poly(A) mRNA stability were determined as described in Materials and Methods for the four strains and relativized to the values of the wt strain (transformed with the empty plasmid YCpLac33), which is taken as 1.00. Because of this the wt values lack standard deviation (SD). The experiments were repeated one to four times and the results were averaged. Actual values for the wt were 86±9 min of GT, 52±2 fL for cell volume and 38±3 min for poly(A) stability. Note that the relative global half-life of *xrn1Δ* strain with regards to the wild type one (4.81x), although higher than that observed for the other strains set described in Figure 1A, is similarly complemented by cRat1, . The Xrn1 deletion produces an increase in mRNA stability from 1 (reference value in wt) to 2.43 in *xrn1Δ* in the strain set shown in Figure 1. However, the global mRNA stability was much similar to wt in the cRat1 strain (1.38). Therefore, the addition of cRat1 changed the difference (regarding the wt) from 1.43 to 0.38, which represents 73.4% recovery. On the other hand, in the second strain set shown in this Supplementary Figure the global mRNA stability in *xrn1Δ* strain was 4.81x compared to wt and was compensated to 1.79 in the cRat1 strain. In this case the recovery was 79.4%, from 3.81 to 0.79. The small differences between the fold changes between strains in Figure 1 and these data might be due to the described strain differences or due to the presence of GFP tag in Dcp2 (see Table S1). D) Growth curves at 30°C for *xrn1Δ* cells transformed with the indicated Rat1 derivative proteins. This figure is complementary to Figure 1. E) Generation times (GT), cell volumes and global poly(A) mRNA stability were determined as described in the Materials and Methods for the six studied strains grown in SC-ura. Values were relativized to those of the wild-type (wt) strain (transformed with empty plasmid YCpLac33), which was taken as 1.00. As a result, the wt values lack standard deviation (SD) and statistical comparison. The two cRat1 samples (centromeric pBBM3, and multicopy pRScRAT1), however, statistically differ from their respective *xrn1Δ* ones at the p < 0.001 (\*\*\*), p < 0.05 (\*\*), p < 0.09 (\*) or non-significant (n.s.) level according to a two-tailed Student’s t-test. Similar complementation of the *xrn1Δ* phenotypes is observed in centromeric and multicopy cRat1 strains. Experiments were repeated 3 times and averaged. The actual values for the wt (YCpLac33) were 98±12 min for GT and 50.13±1.22 fL for cell volume. F) qRT-PCR quantification of endogenous *RAT1* and plasmid-expressed cRAT1 genes using specific primers which discriminate between both alleles. Values are relativized to the expression value of the endogenous *RAT1* in wt (YCpLac33) and *cRAT1* in cRat1 strain (*xrn1Δ*+pBBM3). Experiments were repeated 3 times and averaged. p < 0.05 (\*\*) or non-significant (n.s.) level according to a two-tailed Student’s t-test.

**Figure S2.- The cytoplasmic version of Rat1 protein (cRat1) partially restores the wild-type HT-5Pseq profile in a *xrn1∆* strain.** A) HT-5Pseq high-resolution plot around the start and stop codons showing the profiles of a different set of samples (those described in Supplementary Figure S1) from the strains set shown in Figure 2. B) Low-resolution average metagene plot panels of HT-5Pseq data, as shown in Figure 2C but divided into gene categories as marked. ALL (n= 6664), TATA (n= 1086), TATA-like (n= 4554), Ribosomal protein (RP) (n= 129) and Ribosome Biogenesis (RiBi) (n= 236). This figure is complementary to Figure 2A.

**Figure S3.- Heatmaps of HT-5Pseq and 5’Capseq data for all individual protein-coding genes aligned by their transcription start sites (TSS) and ordered by increasing 5’UTR length.** Corresponding summary average count metagene plots are shown on top of each heatmap. This figure is complementary to Figure 2F where the alignment of the same data was by the AUG codon.

**Figure S4.- Characterization of the only-cytoplasmic version of Rat1 protein with C- terminal domain of Xrn1 (cRat1-CtermXrn1).** A) Western blot analysis of protein expression levels in *xrn1Δ* cells transformed with FLAG-tagged cRat1 derivatives. B) Growth curves at 30°C of wild-type (wt) and *xrn1Δ* cells transformed with the indicated cRat1 derivatives. C) Histogram depicts FLAG protein expression levels normalized by PGK expression and measured by Western blot in wt and *xrn1Δ* cells transformed with the indicated cRat1 derivatives. This figure is complementary to Figure S1.

**Table S1.- List of yeast strains and plasmids used in this work.**

**Table S2. Gene set enrichment analysis (GSEA) of the changes in synthesis rates (SR) and mRNA stabilities (HL) in strains expressing cRat1.**