# Supplemental Materials

***Molecular Biology of the Cell***

Novačić *et al.*

**Table S1. *S. cerevisiae* strains**

| Strain ID | Genotype | Source |
|---|---|---|
| BMA41 wild type | *MATa ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1Δ can1-100* | (Baudin-Baillieu *et al.*, 1997) |
| BMA41 *rrp6Δ* | BMA41 with *rrp6::KanMX4* | (Mosrin-Huaman *et al.*, 2009) |
| BMA41 Psa1-Myc | BMA41 with *PSA1-Myc::hph* | This work |
| BMA41 *rrp6Δ* Psa1-Myc | BMA41 *rrp6* with *PSA1-Myc::hph* | This work |
| BMA41 Rrp6-Y361A | BMA41 with *rrp6Y361A* | (Stuparevic *et al.*, 2013) |
| BMA41 *rrp47Δ* | BMA41 with *rrp47::KanMX4* | (Stuparevic *et al.*, 2013) |
| BMA41 *trf4Δ* | BMA41 with *trf4::KanMX4* | (Stuparevic *et al.*, 2013) |
| BMA41 *trf5Δ* | BMA41 with *trf5::KanMX4* | (Stuparevic *et al.*, 2013) |
| BMA41 *mpp6Δ* | BMA41 with *mpp6::KanMX4* | (Stuparevic *et al.*, 2013) |
| BMA41 *air1Δ* | BMA41 with *air1::KanMX4* | (Stuparevic *et al.*, 2013) |
| BMA41 *air2Δ* | BMA41 with *air2::KanMX4* | (Stuparevic *et al.*, 2013) |
| BMA41 *air1Δ air2Δ* | *MATa ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1Δ can1-100 air1::HIS3 air2::KanMX4* | (Mosrin-Huaman *et al.*, 2009) |
| *DIS3* | *MATa ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100 dis3::KanMX4 [pBS3269-DIS3, LEU2]* | (Stuparevic *et al.*, 2013) |
| *dis3 endo*$^{-}$ | *MATa ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100 dis3::KanMX4 [pBS3278-dis3D171N, LEU2]* | (Stuparevic *et al.*, 2013) |
| *dis3 exo*$^{-}$ | *MATa ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100 dis3::KanMX4 [pBS3270-dis3D551N, LEU2]* | (Stuparevic *et al.*, 2013) |
| BY4741 wild type | *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | (Brachmann *et al.*, 1998) |
| BY4741 *rrp6Δ* | BY4741 with *rrp6::KanMX4* | This work |
| JHY222 wild type | *MATa/MATα HAP1/HAP1 MKT1(D30G)/MKT1(D30G) RME1(INS 308A)/RME1(INS 308A) TAO3(E1493Q)/TAO3(E1493Q)* | (Lardenois *et al.*, 2011) |
| JHY222 *rrp6Δ/rrp6Δ* | JHY222 with *rrp6::KanMX4/rrp6::KanMX4* | (Lardenois *et al.*, 2011) |

**Table S2. Primers**

| Primer ID | Sequence (5'→3') | Description |
|---|---|---|
| RRP6-Kan1 | GATAGACGAAATAGGAACAACAAACAGCTTATAA GCACCCAATAAGTGCGTT CCCGGCCAGCGACATGGAGGCCCAG | Deletion of *RRP6* |
| RRP6-Kan2 | GCCCTTGGTCCATTACTATCGCTAGATGATGGGT CGAATCTCCTTTTCCGAATCG ACAGCAGTATAGCGACCAGGCT | Deletion of *RRP6* |
| PSA1Ctag_fwd | TACCTCATAAGTCTATCTCCGATAATGTTCCAAAG GAAGCTATTATTATGCGTACGCTGCAGGTCGAC | C-terminal tagging of Psa1 |
| PSA1Ctag_rev | ACAGATGAGTGATATAGATTATATTCATTACAGTT CGTTTTCCTAACTCAATCGATGAATTCGAGCTCG | C-terminal tagging of Psa1 |
| PSA1_fwd | GATTGGCCCAGACGTGGTTA | RT-qPCR |
| PSA1_rev | ACTTCAACGTCGTCACCCAA | RT-qPCR |
| CUT488_fwd | GCTGGTGGTTTCTCGCTTTG | RT-qPCR |
| CUT488_rev | TGTGAAACTTCTAACAATGACGTG | RT-qPCR |
| PMA1_fwd | GGCTTCCATAACGAATTGAATTGGACCG | RT-qPCR |
| PMA1_rev | CAATCTAATCACGGTGTCGACGACGAAGAC | RT-qPCR |
| DPM1_fwd | TGGCCAGACCTTTGACCATC | RT-qPCR |
| DPM1_rev | CCTTGCGAGTTGATGTCCCT | RT-qPCR |
| ALG7_fwd | TTCAAATTGGTCCCCTGCCC | RT-qPCR |
| ALG7_rev | TGCTCTTTGGCGGTTCTTCT | RT-qPCR |
| PSA1prom_fwd | TGTTCTCACCTCTTTGACTTTGA | ChIP qPCR |
| PSA1prom_rev | GTTGTAGCTTGTCTTGTGCTGA | ChIP qPCR |
| TAF10prom_fwd | AGCTGCAGATTCAGCATTCA | ChIP qPCR |
| TAF10prom_rev | TCCGCATCGTAATCTTCCTCA | ChIP qPCR |

## REFERENCES


Baudin-Baillieu, A, Tollervey, D, Cullin, C, and Lacroute, F (1997). Functional analysis of Rrp7p, an essential yeast protein involved in pre-rRNA processing and ribosome assembly. Mol Cell Biol 17, 5023–5032.

Brachmann, CB, Davies, A, Cost, GJ, Caputo, E, Li, J, Hieter, P, and Boeke, JD (1998). Designer deletion strains derived from Saccharomyces cerevisiae S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115–132.

Lardenois, A, Liu, Y, Walther, T, Chalmel, F, Evrard, B, Granovskaia, M, Chu, A, Davis, RW, Steinmetz, LM, and Primig, M (2011). Execution of the meiotic noncoding RNA expression program and the onset of gametogenesis in yeast require the conserved exosome subunit Rrp6. Proc Natl Acad Sci U S A 108, 1058–1063.

Mosrin-Huaman, C, Honorine, R, and Rahmouni, AR (2009). Expression of bacterial Rho factor in yeast identifies new factors involved in the functional interplay between transcription and mRNP biogenesis. Mol Cell Biol 29, 4033–4044.

Stuparevic, I, Mosrin-Huaman, C, Hervouet-Coste, N, Remenaric, M, and Rahmouni, AR (2013). Cotranscriptional recruitment of RNA exosome cofactors Rrp47p and Mpp6p and two distinct Trf-Air-Mtr4 polyadenylation (TRAMP) complexes assists the exonuclease Rrp6p in the targeting and degradation of an aberrant messenger ribonucleoprotein particle (mRNP) in yeast. J Biol Chem 288, 31816–31829.

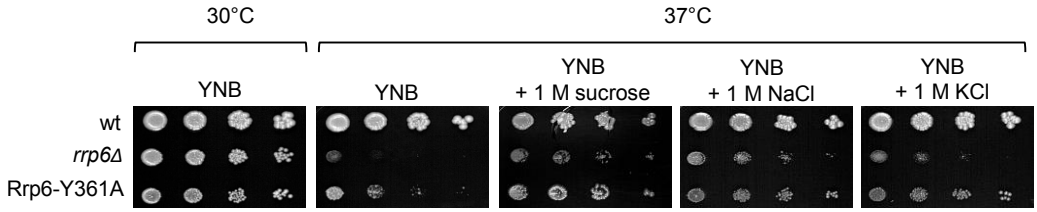


**Figure S1. Osmotic stabilization of *rrp6Δ* cells at high temperature happens regardless of osmotic stabilizer used.** 10-fold serial dilutions of BMA41 wild type (wt) and isogenic mutant strains cells were spotted on plates and were photographed after 3 days at indicated temperature.

**Figure S1**

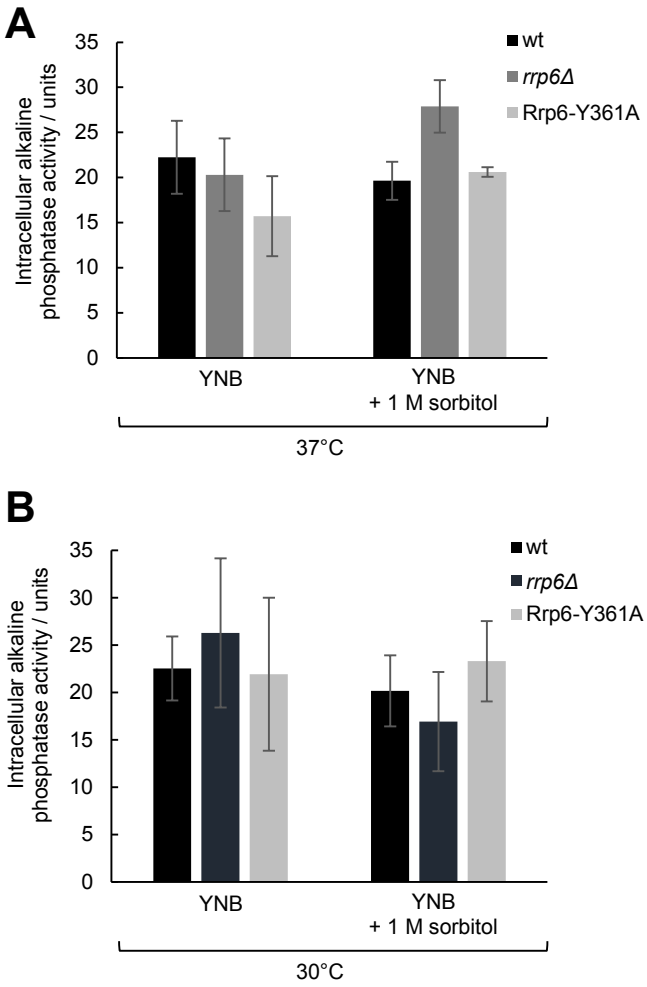


**Figure S2. Alkaline phosphatase is not differentially expressed in *rrp6Δ* or Rrp6-Y361A mutant.** BMA41 wild type (wt) and isogenic mutant strains were grown in YNB medium for 3 days at 37°C (A) or 30°C (B) and intracellular alkaline phosphatase activity was measured. Measurements were performed in duplicate, and reported values represent the means and standard deviations of three independent experiments (n=3).

**Figure S2**

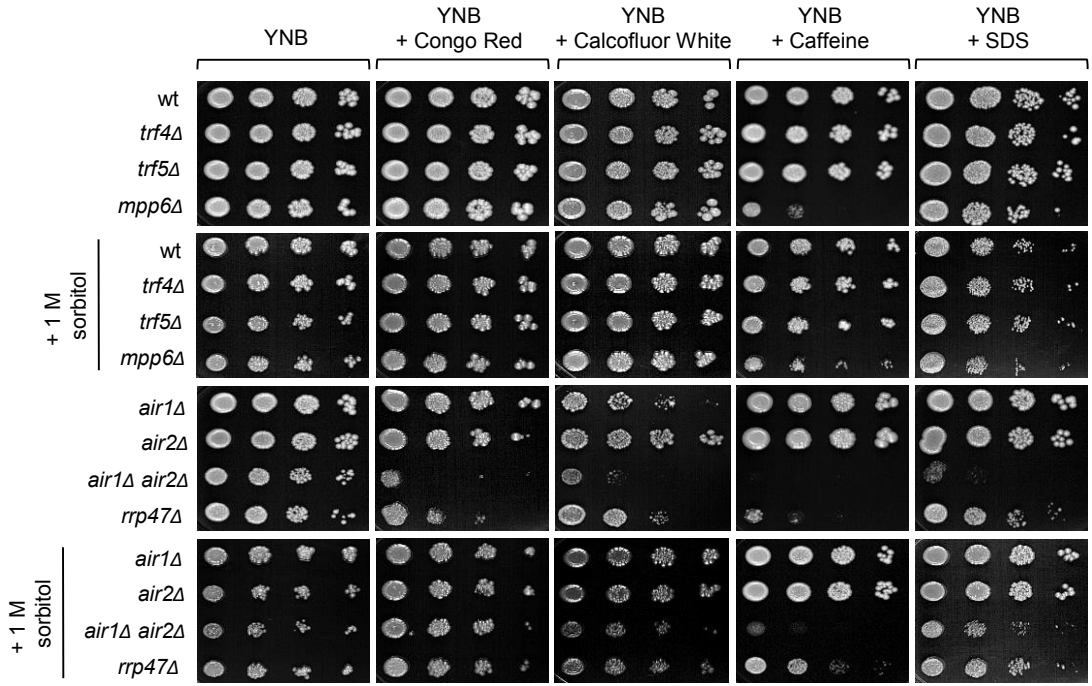


**Figure S3. Temperature-sensitive mutants in RNA exosome cofactors are hypersensitive to cell wall stressors.** Strains are described in Figure 2. 10-fold serial dilutions of cells were spotted on plates and were photographed after 3 days at 30°C. Concentrations of compounds used: Congo Red 10 µg/ml, Calcofluor White 20 µg/ml, Caffeine 6 mM, SDS 0,0075%.

**Figure S3**

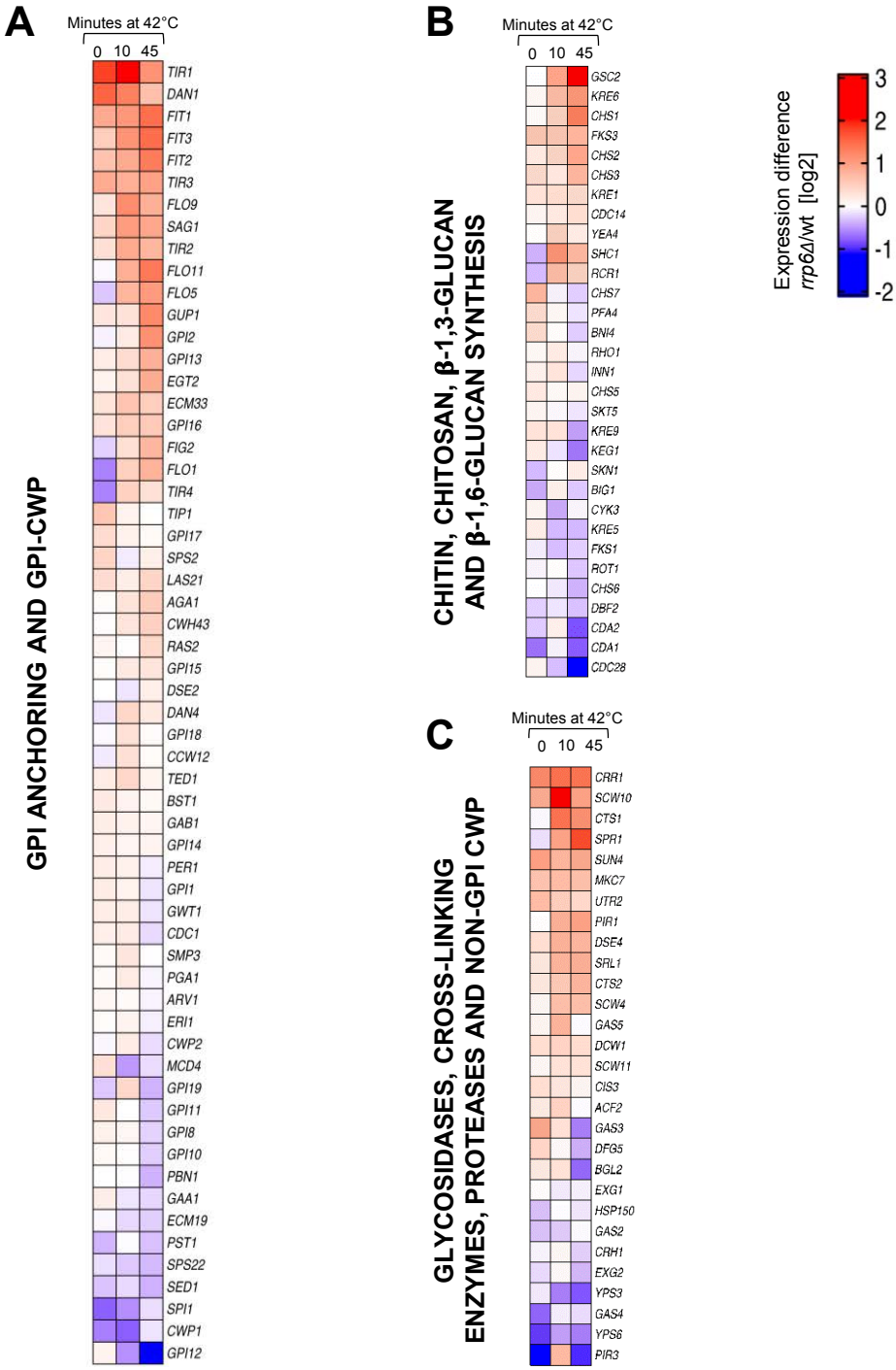


**Figure S4. Expression profiles of mRNAs encoding cell wall-related proteins in cells lacking Rrp6 during heat shock.** RNA-seq heat map with data from (Wang et al. 2020) showing the expression differences of mRNAs as *rrp6Δ*/wt mRNA ratio on a log2 scale, for genes subdivided into cell wall-related gene categories adapted from (Orlean 2012): (A) GPI anchoring and GPI-CWP (cell wall proteins), (B) Chitin, chitosan, **β**-1,3 glucan and **β**-1,6 glucan synthesis and (C) Glycosidases, cross-linking enzymes, proteases and non-GPI CWP.

**Figure S4**

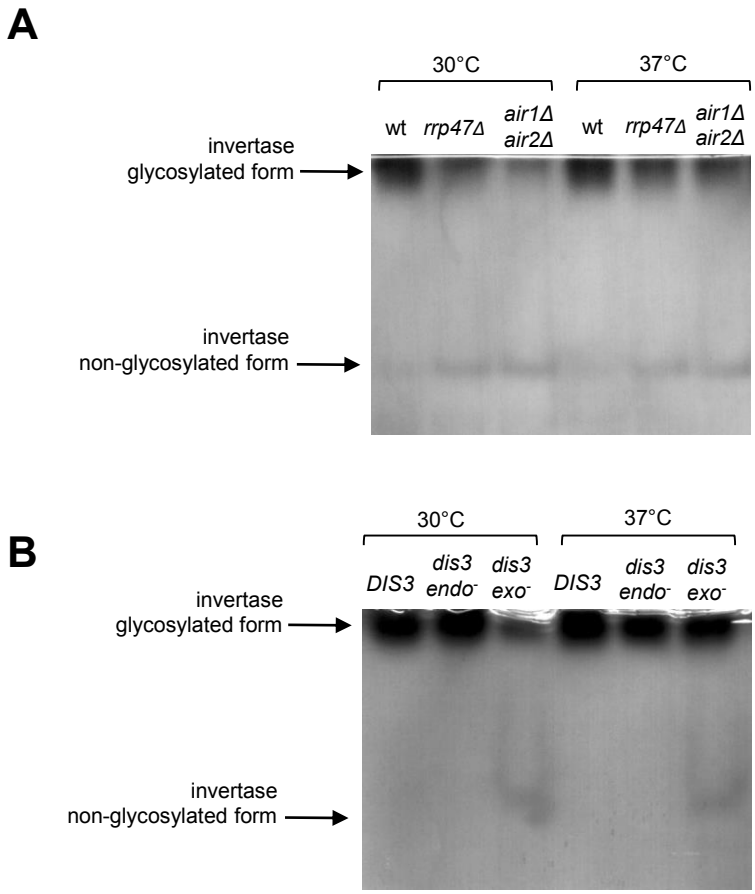


**Figure S5. Protein glycosylation is affected in RNA exosome mutants.** Strains are described in Figure 2. Activity staining of invertase from periplasmic extracts of *rrp47Δ* and *air1Δair2Δ* cells (A), as well as *dis3 exo*$^-$ cells (B) contain an additional non-glycosylated form of periplasmic invertase.

**Figure S5**

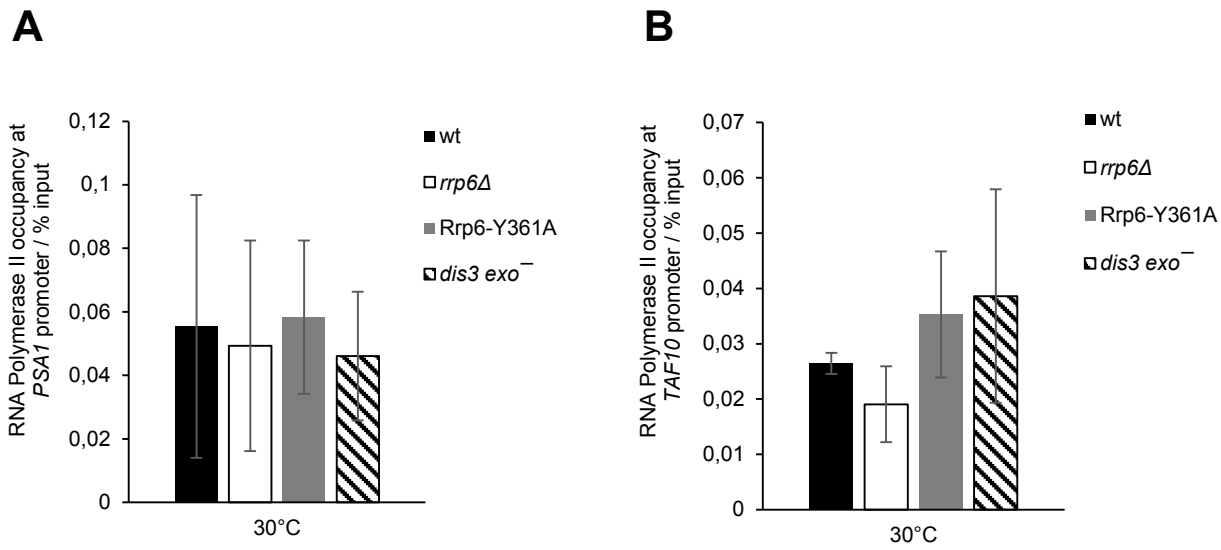


**Figure S6. There is no difference in recruitment of RNA Polymerase II to *PSA1* or *TAF10* gene promoters in RNA exosome mutants compared to wild type cells at physiological temperature.** Strains are described in Figures 1 and 2. Quantification was performed by ChIP of RNA polymerase II using specific antibodies 8WG16. Immunoprecipitated samples (output) were normalized to input following quantification by qPCR. Reported values represent the means and range of two independent experiments (n=2).

**Figure S6**

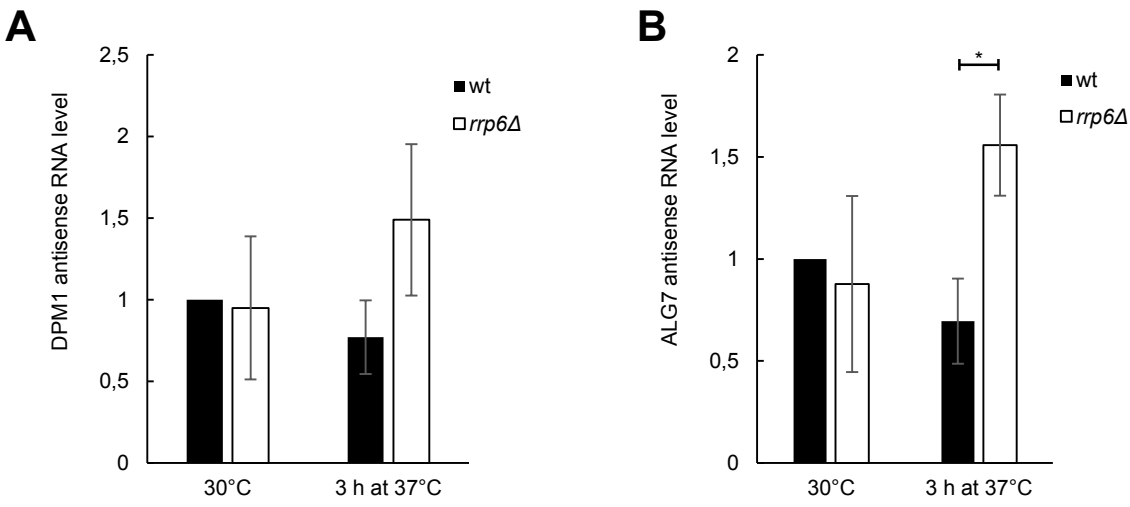


**Figure S7. DPM1 and ALG7 antisense RNAs accumulate in cells lacking Rrp6 at high temperature.** RT-qPCR values for DPM1 (A) and ALG7 (B) antisense RNAs for BMA41 wild type (wt) and isogenic *rrp6Δ* mutant strain are normalized to PMA1 mRNA and expressed relative to transcript abundance in wild type cells at 30°C, which is set as 1. Reported values represent the means and standard deviations of three independent experiments (n=3). Indicated differences show the significant differences using an unpaired student T-test. One (*) asterisk denotes a p-value lower or equal to 0.05.

**Figure S7**