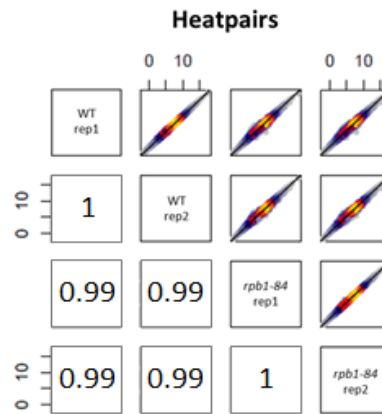
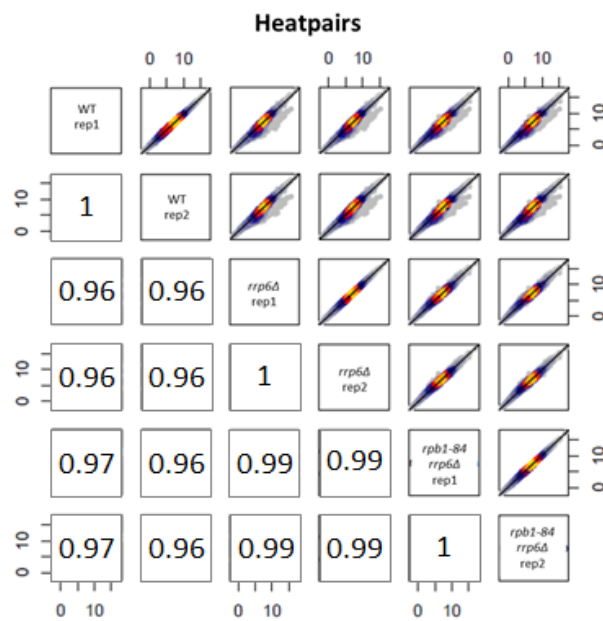
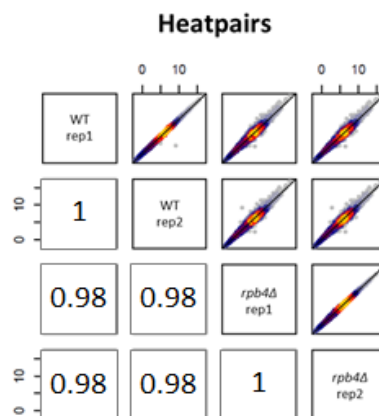
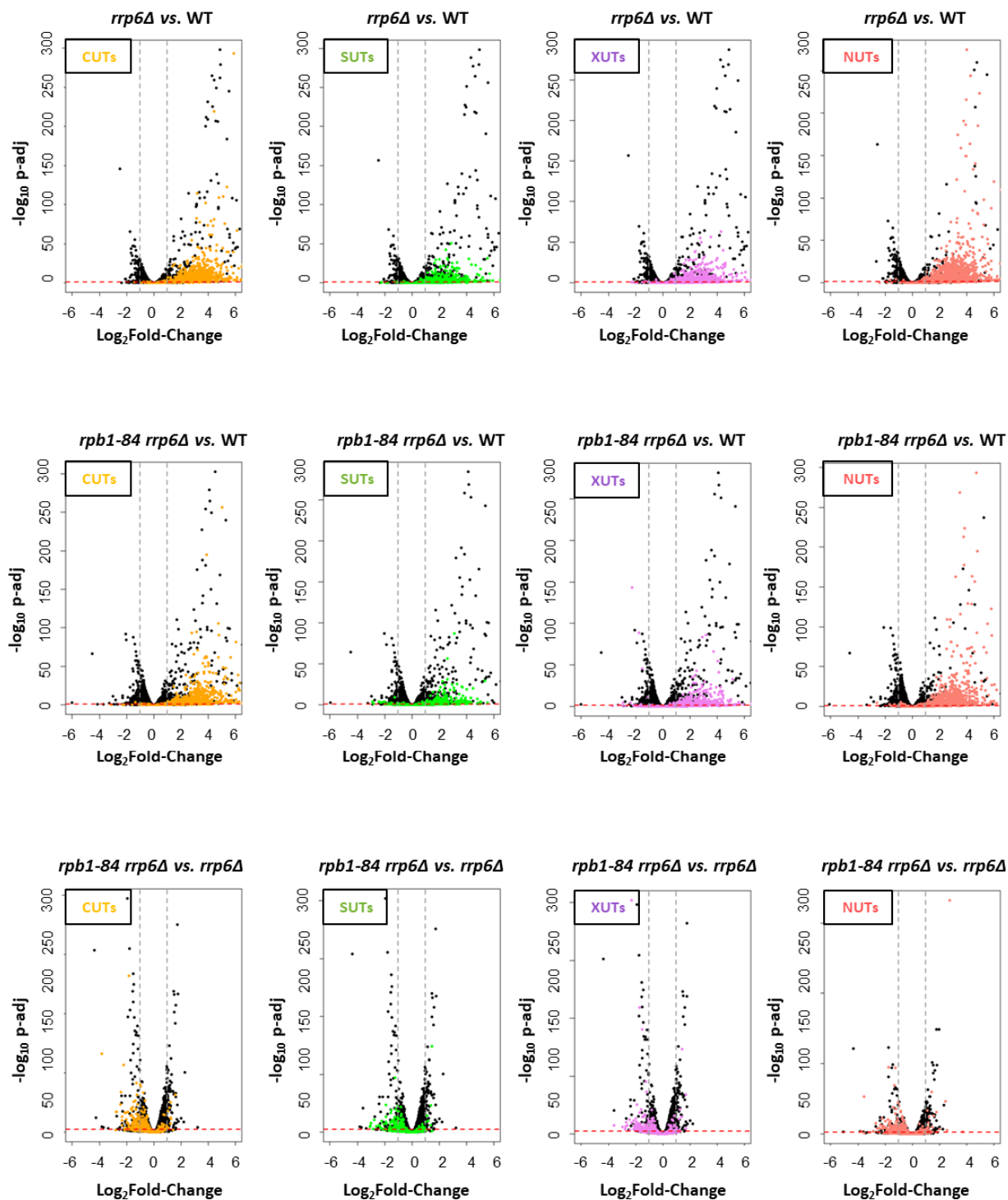


**A****B****C**

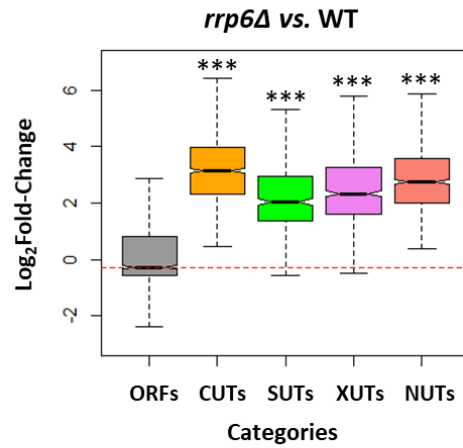
**Figure S1: Heatpairs showing a high correlation between every two biological replicates.** Similarity between samples is indicated by a numerical value, where 1 is the value of the greatest similarity, and scatterplots where the deviation of points from the trend line indicated variability between samples. Warmer colours denote the higher density of overlapping genes. Spearman rank correlation values are indicated. (A) Heatpairs of RNA-seq of the *rpb1-84* mutant and its isogenic wild-type (WT) strain. (B) Heatpairs of RNA-seq of the *rpb1-84 rrp6Δ* mutant, the *rrp6Δ* mutant and its isogenic WT strain. (C) Heatpairs of RNA-seq of the *rpb4Δ* mutant and its isogenic WT strain.



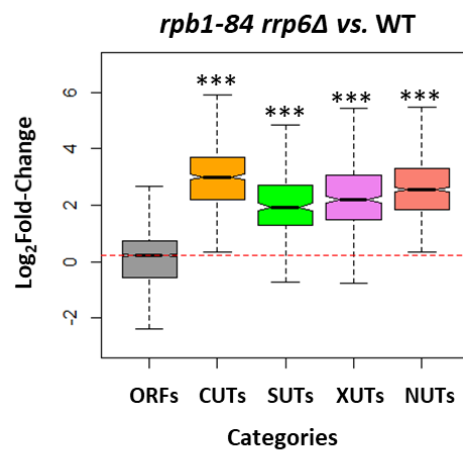
**Figure S2**

**Figure S2: ORFs and ncRNAs analysis from the RNA-seq of the wild-type strain, the *rrp6Δ* mutant and the *rpb1-84 rrp6Δ* mutant strains.** Volcano plots generated to identify the differentially expressed transcripts in the *rrp6Δ* mutant vs. the wild type (WT; upper panel), the *rpb1-84 rrp6Δ* double mutant vs. the WT strain (middle panel) and the *rpb1-84 rrp6Δ* double mutant vs. the *rrp6Δ* mutant strain (bottom panel) ( $n=2$ , DESeq2) [55]. A specific cut-off was applied to define the altered accumulation transcripts consisting in  $\text{Log}_2 \text{Fold-Change}$  higher than  $\pm 1$  (dashed grey lines) and  $p\text{-adj} < 0.1$  (dashed red line). Specific classes of ncRNAs are coloured: CUTs (orange), SUTs (green), XUTs (violet) and NUTs (salmon). ORFs are coloured in black.

A



B



C

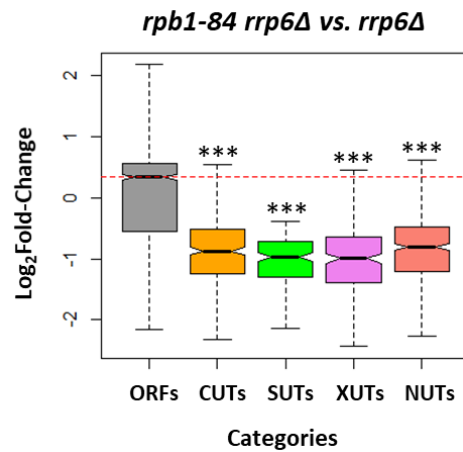
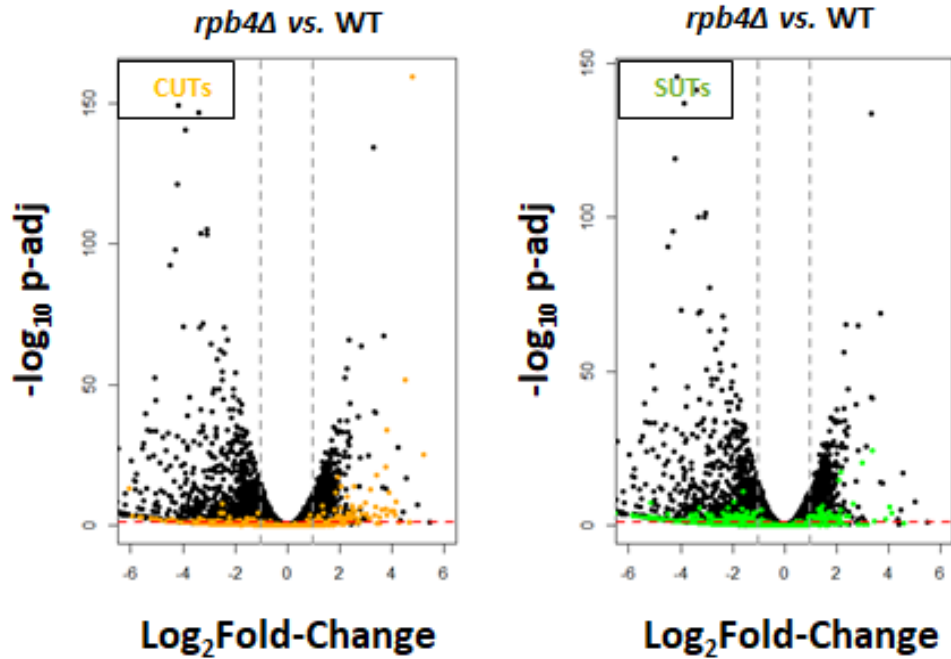


Figure S3

**Figure S3: RNA-seq of the wild-type strain, the *rrp6Δ* mutant and the *rpb1-84 rrp6Δ* mutant strains.** Box-plots showing the distribution of ORFs, CUTs, SUTs, XUTs and NUTs in the *rrp6Δ* mutant vs. the wild type (WT) (A), the *rpb1-84 rrp6Δ* mutant vs. the WT strain (B) and the *rpb1-84 rrp6Δ* mutant vs. the *rrp6Δ* mutant strain (C). The indicated p-values were obtained during two-sided Wilcoxon rank-sum test. \* Wilcoxon-test p-value < 10<sup>-3</sup>; \*\* p-value < 10<sup>-5</sup>; \*\*\* p-value < 10<sup>-9</sup>. Each group of analysed transcripts was compared to the 'ORFs' group.



**Figure S4:** CUTs and SUTs analysis from the RNA-seq of the *rpb4Δ* mutant and its isogenic wild-type strain. Volcano plot generated to identify the differentially expressed transcripts in the *rpb4Δ* mutant vs. the wild-type (WT) strain (n=2, DESeq2) [55]. A specific cutoff was applied to define the altered accumulation transcripts consisting in  $\text{Log}_2$  Fold-Change of the *rpb4Δ* mutant vs. the WT higher than  $\pm 1$  (dashed grey lines) and  $p\text{-adj} < 0.1$  (dashed red line). Black dots show mRNA transcripts, whereas specific classes of ncRNAs are coloured: CUTs (orange) and SUTs (green).

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

Strain	Genotype	Relevant information	Origin
BY4741	<i>MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0</i>		Euroscarf
Gr21-2d	<i>MATα his3Δ200 leu2-3,112 trp1Δ63 ura3-52</i> <i>rpb1-Δ187::HIS3 + pFL44-RPB1 (2μm URA3 RPB1)</i>		P. Thuriaux
Nrd1-TAP	<i>MATa ade2 arg4 leu2-3,112 trp1-289 ura3-52</i> <i>nrd1::TAP::URA3</i>		[89]
Y01285	<i>MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0 rpb4</i> <i>Δ::kanMX4</i>		Euroscarf
Y01777	<i>MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0</i> <i>rrp6Δ::KanMX4</i>		Euroscarf
YFN116	<i>MATα his3-Δ200 leu2-3,112 trp1-Δ63 ura3-52</i> <i>rpb1-Δ187::HIS3 + pYEB220 (2μm LEU2 RPB1)</i>		[50]
YFN104	<i>MATα his3-Δ200 leu2-3,112 trp1-Δ63 ura3-52</i> <i>rpb1-Δ187::HIS3 + pYEB220-84 (2μm LEU2 rpb1-84)</i>		[50]
YFN161	<i>MATa his3-Δ1 leu2-Δ0 lys2-Δ0 ura3-Δ0</i> <i>rtr1Δ::KanMX4</i>		[42]
YFN166	<i>MATa his3-Δ1 leu2-3,112 trp1-Δ63 ura3-Δ0</i> <i>rpb1- Δ187:: HIS3 + pFL44-RPB1 (2μm URA3 RPB1) rtr1Δ::KanMX4</i>	YFN161 X Gr21-2d	This work
YFN241	<i>MATα his3-Δ200 leu2-3,112 trp1-Δ63 ura3-52</i> <i>rpb1-Δ187:: KanMX4 + pYEB220 (2μm LEU2 RPB1)</i>		[52]
YFN243	<i>MATα his3-Δ200 leu2-3,112 trp1-Δ63 ura3-52</i> <i>rpb1-Δ187:: KanMX4 + pYEB220-84 (2μm LEU2 rpb1-84)</i>		[52]
YFN471	<i>MATa his3-Δ1 leu2-3,112 trp1-Δ63 ura3-Δ0</i> <i>rpb1- Δ187:: TRP1 + pFL44-RPB1 (2μm URA3 RPB1) rtr1Δ::KanMX4</i>	<i>his3::TRP1</i> marker disruption conversion into YFN166 strain using pHT6 plasmid [90]	This work
YFN518	<i>MATα his3-Δ200 leu2-3,112 trp1-Δ63 ura3-52</i> <i>rpb1-Δ187::HIS3 + pYEB220 (2μm LEU2</i>		[65]

	<i>RPB1) RPB4::TAP::TRP1</i>		
YFN519	<i>MAT<math>\alpha</math> his3-<math>\Delta</math>200 leu2-3,112 trp1-<math>\Delta</math>63 ura3-52 rpb1-<math>\Delta</math>187::HIS3 + pYEB220-84 (2<math>\mu</math>m LEU2 rpb1-84) RPB4::TAP::TRP1</i>		[65]
YFN542	<i>MAT<math>\alpha</math> his3-<math>\Delta</math>200 leu2-3,112 trp1-<math>\Delta</math>63 ura3-52 rpb1-<math>\Delta</math>187::HIS3 + pFL44-RPB1 (2<math>\mu</math>m URA3 RPB1) rrp6<math>\Delta</math>::KanMX4</i>	Chromosomal integration of rrp6 $\Delta$ ::KanMX4 allele in strain Gr21-2d	This work
YFN543	<i>MAT<math>\alpha</math> his3-<math>\Delta</math>200 leu2-3,112 trp1-<math>\Delta</math>63 ura3-52 rpb1-<math>\Delta</math>187::HIS3 + pYEB220 (2<math>\mu</math>m LEU RPB1) rrp6<math>\Delta</math>::KanMX4</i>	Plasmid shuffling in YFN542	This work
YFN545	<i>MAT<math>\alpha</math> his3-<math>\Delta</math>200 leu2-3,112 trp1-<math>\Delta</math>63 ura3-52 rpb1-<math>\Delta</math>187::HIS3 + pYEB220-84 (2<math>\mu</math>m LEU rpb1-84) rrp6<math>\Delta</math>::KanMX4</i>	Plasmid shuffling in YFN542	This work
YFN668	<i>MAT<math>\alpha</math> ade2 leu2-3,112 ura3-52 trp1-289 rpb1- D187::kanMX4 + pYEB220 (2<math>\mu</math>m LEU) nrd1::TAP::URA</i>	YFN241 X Nrd1-TAP	This work
YFN669	<i>MAT<math>\alpha</math> ade2 leu2-3,112 ura3-52 trp1-289 rpb1- D187::kanMX4 + pYEB220-84 (2<math>\mu</math>m LEU2 RPB1) nrd1::TAP::URA</i>	YFN243 X Nrd1-TAP	This work

**Table S2. Plasmids used**

Name		Origin
pYEB220	ORI (2μm) <i>LEU2</i>	[50]
pYEB220-84	ORI (2μm) <i>LEU2</i>	[50]
pCM185	ORI (CEN) <i>TRP1</i>	[91]
pCM185- <i>RPB6</i>	ORI (CEN) <i>TRP1</i>	[50]
pM647 [pRS423-SSU72]	ORI (2μm) <i>HIS3</i>	[92]
pHT6 <i>HIS3</i> → <i>TRP1</i> converter	KanR; <i>TRP1</i>	[90]

\* CEN: centromeric vector; 2μm: multicopy vector

**Table S3. Oligonucleotides used**

<i>18S-501</i>	CATGGCCGTTCTTAGTTGGT
<i>18S-301</i>	ATTGCCTCAAACCTCCATCG
<i>CUT474-501</i>	CGTGCGCTTGAGATTCAGTT
<i>CUT474-301</i>	AGCAAACAAGAGTCTCGGTTGA
<i>CUT638-501</i>	TTCAAGGACTACGAAACCACAT
<i>CUT638-301</i>	CCGGACGTCTATATCGCTAA
<i>CUT701-501</i>	CACTGAATGGGCCCTGAGG
<i>CUT701-301</i>	CAGGCCGAGCTTTGATTGTG
IntergChrV-F (intergenic region)	TGTTCCCTTTAAGAGGTGATGGTGAT
IntergChrV-R (intergenic region)	GTGCGCAGTACTTGTGAAAACC
<i>LYS20-501</i>	ATCACCGGGTTCTGTGCATT
<i>LYS20-301</i>	GCGTTCCAGCCAGTTAGTCT
<i>PIR1-501</i>	CTGTTGCTCCAGTCTCCCAA
<i>PIR1-301</i>	TGCCATTAGTAATTGGAGCTGGA
<i>RRP6-501</i>	GCATAAGCGACAGAAAACACT
<i>RRP6-301</i>	AACACGCACAATATTCGAGC
<i>SUT228-501</i>	AACGGGATGATAGGCGCA TT
<i>SUT228-301</i>	CTCTTACGCTAGCTTCCCGG
<i>SUT240-501</i>	ACCCAAAACGCCGATCTAGG
<i>SUT240-301</i>	CTT TTGATAGCCAGCCCATCT
<i>snR5-501</i>	GCTTAAGACATCACGCCTCC
<i>snR5-301</i>	ACACCTAGAGCGAACCAATGA
<i>snR13-501</i>	GGCATCTCAAATCGTCTCTATATC
<i>snR13-301</i>	GAGTTTTTCCACACCGTTACTGAT
<i>snR82-501</i>	TGGCTCTTCAACACATTTCAACA
<i>snR82-301</i>	AAAAC TCCCGGTAACCAGGC

The primers used were as follows (all 5'-3')