SUPPLEMENTARY MATERIAL

The Rio1p ATPase hinders premature entry into translation of late pre-40S pre-ribosomal particles

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SUPPLEMENTARY MATERIALS AND METHODS

Sucrose gradient fractionation of cells extracts

Fractionation experiments were performed as described in the Materials and Methods section of the main text with the following modifications. 0.25 mg of total extracts and a centrifugation speed of 39 000 rpm were used. 400 µl of each fraction were precipitated with 1600 µl of 25% trichloroacetic acid (TCA) in the presence of 2.4 µl of glycogen (20 µg/µl).

Western analysis

It was performed as described in the Materials and Methods section of the main text except that Nob1p was detected using peroxidase anti-peroxidase soluble complex antibody produced in rabbit (rabbit PAP, Sigma) diluted 2500 fold.

Reverse transcription and PCR amplification

5 µl of deionized water, 1 µl of random primers (Promega) and 5 µl of input extract (S10) or final tandem affinity purification E3 elutions obtained from the TetO7-RIO1, NOB1-FPZ or the TetO7-RIO1 strain were mixed and incubated during 1 min at 95°C. After cooling at 42°C, 1 µl (200 units) of reverse transcriptase SuperScript II (ThermoFischer Scientific) and 8 µl of RT mix (1 µl 10 mM dNTP (Promega), 4 µl 5 x first strand buffer, 2 µl 0.1 M DTT, 0.25 µl 40 units/µl RNasin (Promega)) were added. After 1 h 15 min at 42°C, the enzyme was inactivated by incubating 15 min at 70°C and the RNA was hydrolysed by addition of 2.3 µl 1M NaOH and incubation for 1 h at 55°C. The pH was then adjusted by addition of 2.3 µl 1 M HCl. PCR reactions were then performed using primers listed in Supplementary Table 1.

Supplementary Table 1. Oligonucleotides used.

Pgk1-RT-F	5’TGGTGGTATGGCTTTCACCT3’
Pgk1-RT-R	5’TGACAGTCTTGGTGTTGGCA3’
RpS14-RT-F	5’CCGCTAAGTGTAAGGAAGTCGGT3’
RpS14-RT-R	5’CCACCCTTCTTTCTGGTGGAGTCA3’
Actin-RT-F	5’TACGTTTCCATCCAAGCCGT3’
Actin-RT-R	5’GGAGAAAGAGTAACCACGTTCACT3’
U1-RT-F	5'TCAAACATGCGCTTCCAATA3'
U1-RT-R	5'CCAAAAATCCCCAAAGGAAT3'
U5-RT-F	5'GCGGAGGGAGGTCAACAT3'
U5-RT-R	5'AAAATATGGCAAGCCCACAG3'

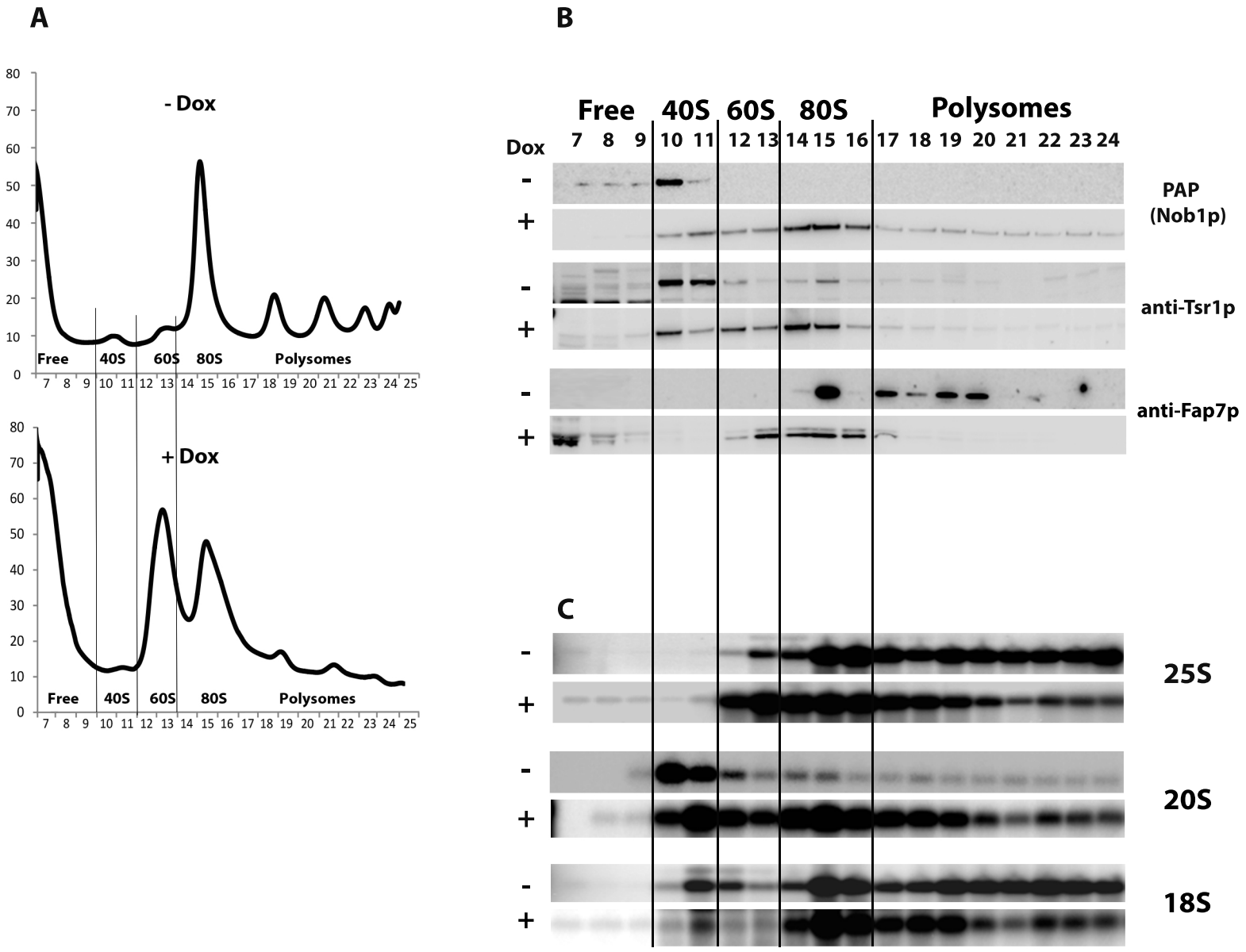
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Sucrose gradient fractionation of total extracts from a *TetO7-RIO1, NOB1-FPZ* strain grown in YPD or in YPD plus doxycycline for 16 h to repress *RIO1* expression. Same legend as that of main Figure 1, except that Nob1p was detected using rabbit PAP.

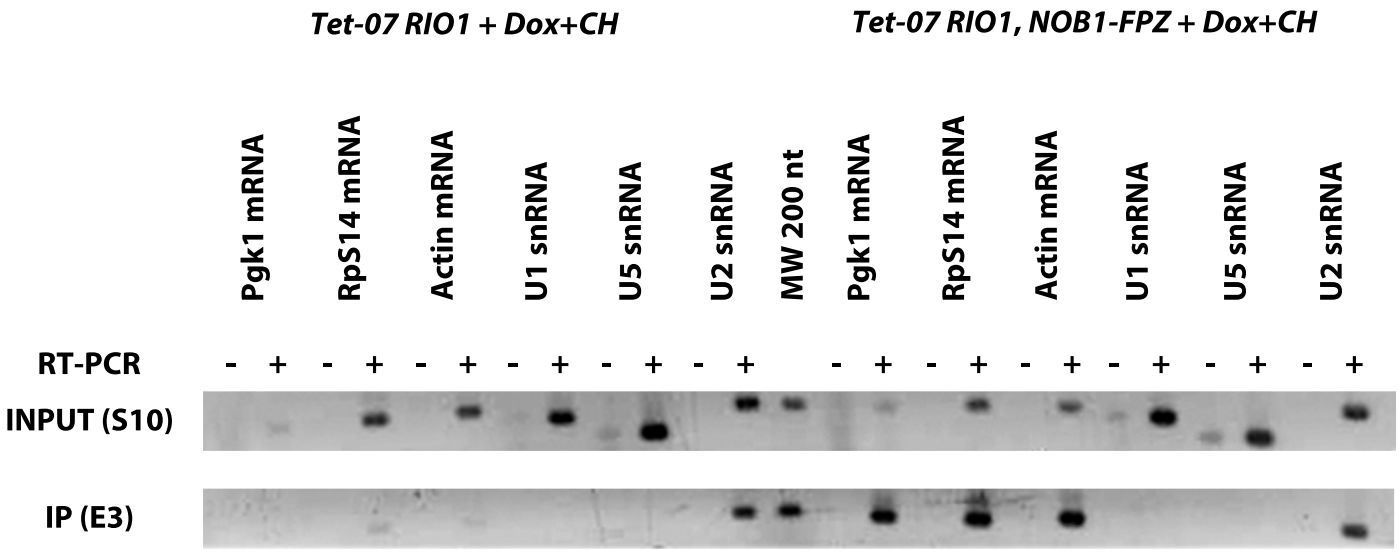
Supplementary Figure S2. Nob1p-containing pre-40S particles associate with mRNAs when Rio1p is depleted. Tandem affinity purifications were performed from the *TetO7-RIO1, NOB1-FPZ* or the *TetO7-RIO1* strain (as a control) both grown in the presence doxycycline to repress *RIO1* expression. Cycloheximide was added shortly before cell harvest and during extract preparation. RNAs from aliquots of input extracts (S10) and the final E3 elutions (see Figure 3 legend for details) were reverse transcribed and the cDNAs of the PGK1, RPS14, ACT1 mRNAs, or the U1 and U5 snRNAs were PCR amplified (+). Genomic DNA contamination was evaluated by direct PCR amplification without reverse transcription (-).

Supplementary Figure S3. Relative density profiles of total RPFs from Rio1p-expressing cells (+ Rio1) or Rio1p-depleted cells (- Rio1) or immuno-purified RPFs from Rio1p-depleted cells (two separate experiments termed IP1 and IP2), mapped onto specific ORFs.

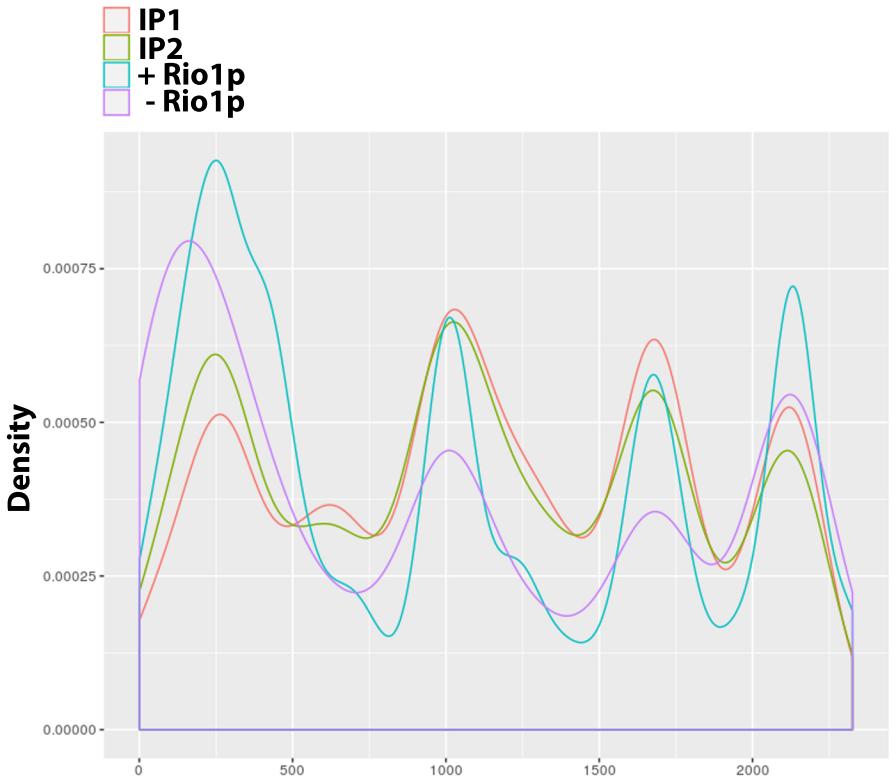
Supplementary Figure S1



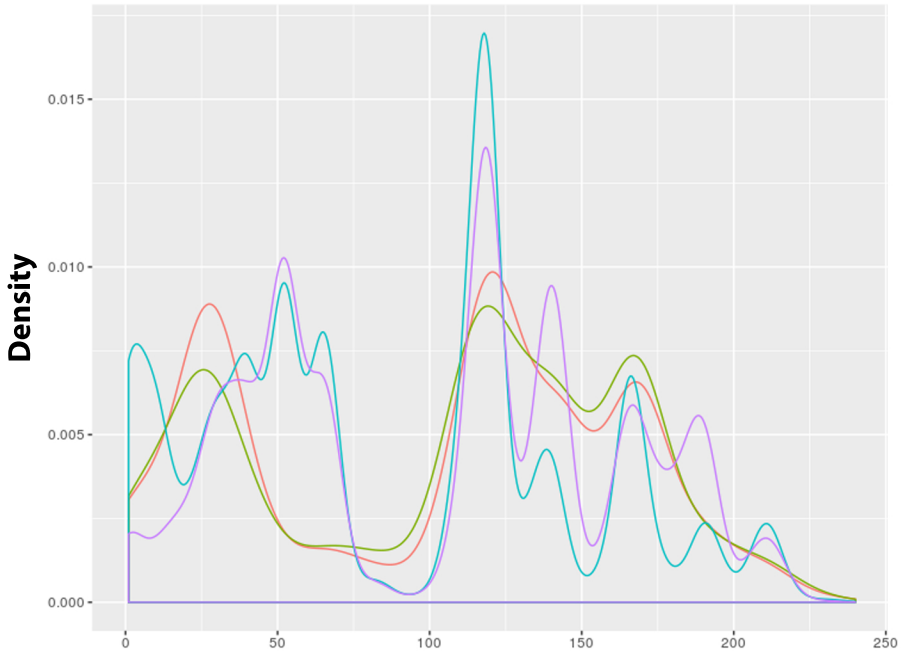
Supplementary Figure S2



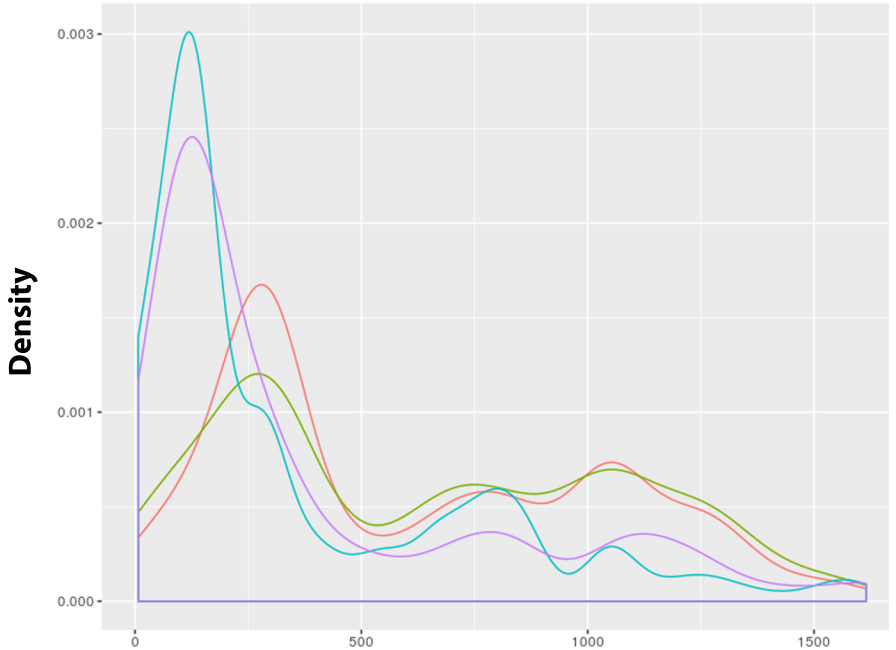
Supplementary Figure S3



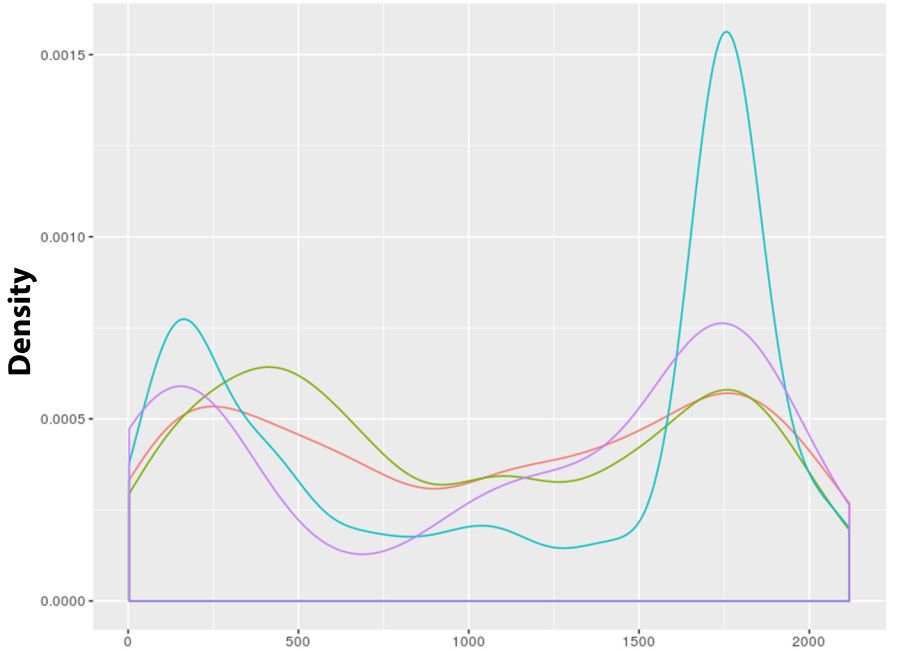
Distance of ribosome from start codon on the YLR304C mRNA



Distance of ribosome from start codon on the YHR021C mRNA

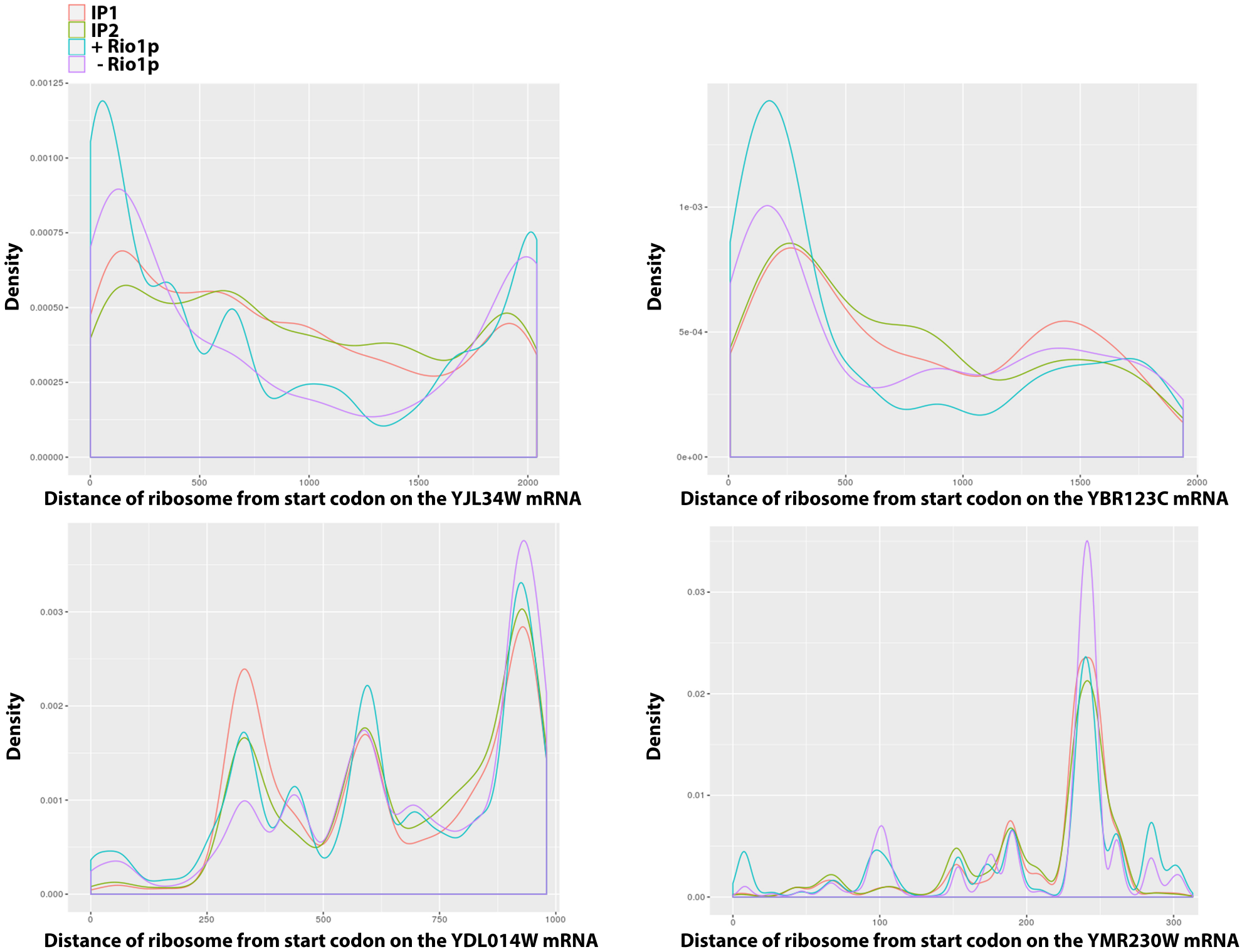


Distance of ribosome from start codon on the YMR011W mRNA

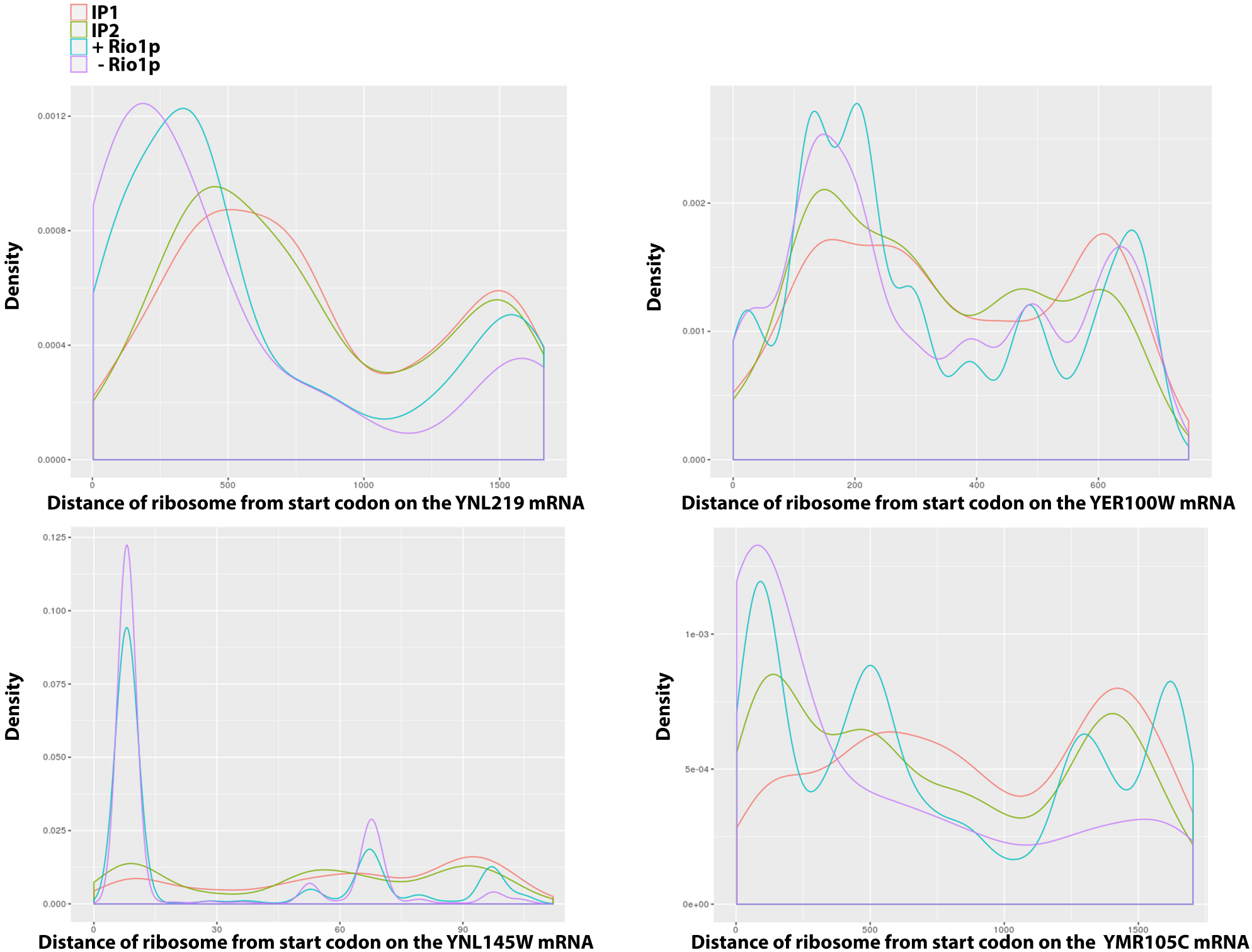


Distance of ribosome from start codon on the YFR015C mRNA

Supplementary Figure S3 continued



Supplementary Figure S3 continued



Supplementary Figure S3 continued