SUPPLEMENTARY MATERIAL

RNA helicase-mediated regulation of snoRNP dynamics on pre-ribosomes and rRNA 2'-*O*-methylation

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SUPPLEMENTARY FIGURES

Supplementary Figure S1. Generation of yeast strains lacking Dbp3 or expressing Nop56 at a reduced level. (A) Schematic view of the PCR-based approach to verify the identity of the yeast strains generated. **(B)** Genomic DNA extracted from the indicated yeast strains was used as a template for PCR reactions using the primer pairs indicated. PCR products were visualised using SafeView and UV light. **(C)** RNA extracted from wild type yeast or cells of the *NOP56DAmP* strain was separated by denaturing PAGE and subjected to northern blotting using probes hybridising to the indicated snoRNAs and the 5S rRNA was used as a loading control.

Supplementary Figure S2. RNase H analysis of Am876, Um898, Um2724 and Gm2619 in snoRNA deletion strains. (A) The normalized numbers of sequencing reads mapping to the 18S and 25S rRNAs was determined in the RMS datasets for wild type yeast and the *Δdbp3* strain. Relative rRNA levels from three biologically independent RMS experiments is shown as mean ± standard error and significance was determined using Student's t-test (*=p<0.05, **=p<0.01, ***=p<0.001 n.s.= non-significant). **(B-E)** RNA from wild type yeast or *Δsnr78-72*, *Δsnr40* or *Δsnr67-snr53* annealed to chimeric RNA-DNA oligonucleotides targeting the 25S-Am876, 25S-Um898, 25S-Um2724 or 25S-Gm2619 sites as appropriate was either treated with RNase H (RH, +) or left untreated (-). Samples were separated by denaturing agarose gel electrophoresis and northern blotting was performed using probes hybridising both

upstream and downstream of the modification sites. Specific cleavage products are indicated by arrow heads; Asterix indicates non-specific cleavage products.

Supplementary Figure S3. The levels of snoRNAs guiding Dbp3-dependent 2'-*O*-methylation are not affected by the absence of Dbp3. (A) The numbers of sequencing reads mapping to each snoRNA was determined in the RMS datasets for wild type yeast and the *Δdbp3* strain. Relative snoRNA levels from two biologically independent RMS experiments in shown as mean ± standard error. **(B)** RNA extracted from wild type yeast or cells of the *Δdbp3* strain was separated by denaturing PAGE and subjected to northern blotting using probes hybridising to the indicated snoRNAs and the 5S rRNA was used as a loading control.

Supplementary Figure S4. Structure and maturation of the U24 snoRNA. (A) RNA extracted from wild type yeast or cells of the *Δdbp3* strain was separated by denaturing PAGE and subjected to northern blotting using probes hybridising to the indicated snoRNAs and the 5S rRNA was used as a loading control. **(B and C)** RMS sequencing reads derived from wild type yeast and the *Δdbp3* strain were mapped to the annotated U24 sequence +/- 50 nt, and after normalisation for expression level, the numbers of reads mapping to each nucleotide were determined. Profiles for the 5' end (B) and 3' end (C) are shown. Nucleotides of box C (B) and box D (C) are underlined and the annotated 5' and 3' ends (58) are indicated. **(D)** Schematic view of the secondary structure of the pre-U24 snoRNA with key features indicated. Basepairing is shown according to (59).

Supplementary Figure S5. Levels of box H/ACA snoRNAs on pre-ribosomes in cells lacking Dbp3 compared to wild type. Wild type yeast and the *Δdbp3* strains were used to prepare cell extracts that were separated by sucrose density gradient centrifugation. Fractions containing either (pre-)ribosomal complexes or non-(pre-)ribosome associated proteins were pooled and RNA was extracted. Polyadenylation and reverse transcription were performed

and the level of each of the 75 yeast snoRNAs in each sample was determined by qPCR. The relative distribution of each box H/ACA snoRNA between (pre-)ribosome-bound and non-ribosome-associated fractions was calculated and differences in this ratio between the wild type and *Δdbp3* strains are shown graphically. Three independent experiments were performed and the data are presented as mean ± standard deviation. # indicates a snoRNA that is observed to accumulate on pre-ribosomes upon depletion of many other RNA helicases and ribosome AFs, and is therefore considered unspecific.

Supplementary Figure S6. Overview of rRNA 2'-*O*-methylations affected in *Δdbp3* mapped on the secondary structure of the 25S rRNA. 2'-*O*-methylations reduced in *Δdbp3* and the snoRNAs that guide them were highlighted on the secondary structure of the 25S rRNA [41] in red and the pre-rRNA nucleotides involved in basepairing interactions with the guiding snoRNAs are indicated in green. Extra snoRNA basepairing is shown in blue. The areas shown in Figure 6 as magnified views are indicated with boxes with labels corresponding to the individual panels.

Supplementary Figure S7. Pre-ribosome-association and overexpression of snR67. (A) Whole cell extracts prepared from wild type yeast and the *Δdbp3* strain were separated by sucrose density gradient centrifugation. RNA from individual fractions was separated by denaturing PAGE and analysed by northern blotting using probes to detect the snR67 snoRNA. The fractions containing "free" snoRNAs and pre-ribosome-associated snoRNAs are indicated. The upper panel is reproduced from Figure 6F. **(B)** RNA extracted from wild type yeast or *Δdbp3* cells transformed with either an empty pRS416 plasmid or a construct for overexpression of snR67 ($snR67_{OE}$) was separated by denaturing PAGE and subjected to northern blotting for the snR67 and snR64 snoRNAs. Mature 5.8S rRNA, visualised by methylene blue staining, served as a loading control.

Supplementary Figure S8. snoRNA and rRNA levels in wild type yeast and the pGAL$_S$-HA-Prp43 strain grown in glucose-containing media. (A-B) The numbers of sequencing reads mapping to each snoRNA (A), and the 18S and 25S rRNAs (B) was determined in the RMS datasets for wild type yeast and the pGAL$_S$-HA-Prp43 strain grown in glucose-containing media. Relative snoRNA levels from two biologically independent RMS experiments in shown as mean ± standard error.

SUPPLEMENTARY TABLES

Supplementary Table 1. DNA oligonucleotides used in this study. Primers used for qPCR analysis of snoRNA levels on pre-ribosomes are listed in [29].

Name	Sequence (5'-3')	Application
oMB1461	CGGTTTTAATTGTCCTA	Northern blot probe (ITS1)
oMB1468	TGAGAAGGAAATGACGCT	Northern blot probe (ITS2)
oMB2510	ATATTAGGATCCGCCATGACAAAGGAAGAAATCGCAG	Molecular cloning pMB312
oMB2511	ACGCGGTACCCTAATCGAAAGTAATTTTTTTTGGTTTC	Molecular cloning pMB312
oMB2594	CAGTTTGATATATAGGGGATTAAACGGATCCCCGGGTTAATTAA	Genomic deletion of *DBP3*
oMB2595	AACCCCTAATCGAAAGTAATTGAATTCGAGCTCGTTTAAAC	Genomic deletion of *DBP3*
oMB2625	TGATCAGTCGACCGTTATTTATTTT	Δ*dbp3* deletion strain verification (forward)
oMB2632	CTGCAGCGAGGAGCCGTAAT	Δ*dbp3* deletion strain verification (Kan MX6;TPB)
oMB3405	GAAGATAGACGAAATAGGAACAACAAACAGCTTATAAGCACCCAATAAGTGCGTTCGGATCCCCGGGTTAATTAA	Genomic deletion of *RRP6*
oMB3406	GAGGTCTTAAATGAAAATTACCATAATTTATAAATAAAAAAATACGCTTGTTTTACATAAGAATTCGAGCTCGTTTAAAC	Genomic deletion of *RRP6*
oMB3409	TGACAGAACCATTTCATGTTCAATA	Δ*rrp6* deletion strain verification (forward)
oMB3412	ATGTGAAGAAAAGAATTCCTGACAC	Δ*rrp6* deletion strain verification (reverse)
oMB3958	AAGTTCTCTAGTTGGGGAAATGACT	Δ*dbp3* deletion strain verification (reverse)
oMB5759	CGATAGTATAACCTTATAAACGCGCATAGAAAGATTAGGACTGCAAGAATAATAATGCGTACGCTGCAGGTCGAC	Genomic integration of pGALs promoter for *PRP43*
oMB5760	GGAATAGAGGTCTCAACTGGATCCGGGTGTTCGGACGAGAATCTTCTTTTGGAACCCATCGATGAATTCTCTGTCG	Genomic integration of pGALs promoter for *PRP43*
oMB6716	ATATATGGATCCGGTTATACTGCGTATTCGATTCTTTACTGG	Molecular cloning pMB1401
oMB6717	ATATATGCGGCCGCTGGTCCAATATTTGACGTTTCTAG	Molecular cloning pMB1401
oMB6720	GTTAATTACTTAGTATTAGACCAGGCAGACAGAATGTTGGAAAAAGG	Site-directed mutagenesis ($Dbp3_{E263Q}$)
oMB6721	CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAAGTAATTAAC	Site-directed mutagenesis ($Dbp3_{E263Q}$)
oMB1465	CTCCGCTTATTGATATGC	Northern blot probe 25S rRNA (RNase H)
oMB7263	GCCTGCTATGGTTCAGCGACG	Northern blot probe 25S rRNA (RNase H)
oMB6327	CTGTCTAGATGAACTAACACC	Northern blot probe 25S rRNA (RNase H)
oMB8611	AAAACTCGAGCTTCAACCTGTACGTGGATGG	Cloning snR67 for overexpression
oMB8612	AAAAGGTACCGAGGAGTGATAATGGCAATTAAGAC	Cloning snR67 for overexpression
oMB1537	GGTGATTAAACGACAGCATTGTCAAAGACTAGTCGA	Northern blot probe snR59
oMB1633	CAGTGTTTGTTGTTTGTAAAATCAG	Northern blot probe snR67
oMB1635	CAGTCATTTCAAAGATCCGCTTGG	Northern blot probe snR67

oMB6021	GATTCAGAAACTCTAGTTTG	Northern blot probe snR78
oMB6022	GAATAAACGTTCTAATCAC	Northern blot probe snR78
oMB6887	GATGGTGATAAGTTACGACAGC	Northern blot probe snR39
oMB1544	CGACAGCATCGTCAATGACTAGTCGAATATGTATTGG	Northern blot probe snR39
oMB1545	GGTGATAAGTTACGACAGC	Northern blot probe snR39
oMB6772	GGTATGTCTCATTCGGAACTCAAAG	Northern blot probe U24
oMB6773	GTGATAATTGGTATGTCTCATTCGG	Northern blot probe U24
oMB1541	GCTGCAAATTGCTACCTCTTTCA	Northern blot probe snR50
oMB1548	TGCTAGTCACTTTTTGGAATGCC	Northern blot probe snR39b
oMB6689	CTGAGTACTTGTGGCATCCATG	Northern blot probe snR40
oMB1928	TTCCAAAGGAATCATCG	Northern blot probe snR55
oMB1551	CATTTGATGAGACGTTTTCTTCA	Northern blot probe snR72
oMB6770	GGTTTATAGCATTGTCACTAAGGACG	Northern blot probe snR69
oMB6771	GCTGGGTTTATAGCATTGTCACTAAG	Northern blot probe snR69
oMB1546	GTCAGATACTGTGATAGTC	Northern blot probe U18
oMB1550	CTTCATTTCGATAGTATGTTCAATCAG	Northern blot probe snR60
oMB2213	GTCACAGGCGAAATATCATCAAAGTTAATC	Northern blot probe snR73
oMB6745	CCCGCTAAAGCATTGTCACTC	Northern blot probe snR76
oMB6888	CGTGCGTCTGATTATGGTCC	Northern blot probe snR63
oMB4421	CGGACGGGAAACGGTGCTTTCTGGTAGATATGG	Northern blot probe 5S rRNA
oMB1828	TCCTTTAGAGATGATAAAGACAACTTACAAGTACAGTTTTTTGTTGGTATCTCATCGGATCCCCGGGTTAATTAA	Genomic deletion of *SNR67-SNR53*
oMB7727	CTAATTCCAATACGAAGAGCCTAATTCTTTGATAGTTCCTTTTCTTTTCTAGTTTAGAGAATTCGAGCTCGTTTAAAC	Genomic deletion of *SNR67-SNR53*
oMB1845	ATCACTAACAGATGAAAAAGGTAGAATGGATAAAATACTTAAAGAATTTTATATGGAATTCGAGCTCGTTTAAAC	Genomic deletion of *SNR72-78*
oMB6001	GTTAGTTTTTCAGATATGTCTTTTGTCATTATCACAGGCCAGCTGAAGCTTCGTACGC	Genomic deletion of *SNR72-78*
oMB1832	TAGTACCTTAACACATGACGAAGATGAAAATTAAACATGAATTCAAGGAAAAATGCGGATCCCCGGGTTAATTAA	Genomic deletion of *SNR40*
oMB1848	TCATAATACAGTCACAGATGTGAGAGAAAAAAAAAAAAGAAAATAAAGGAAAATGGAATTCGAGCTCGTTTAAAC	Genomic deletion of *SNR40*
oMB7718	GCCCTAACTTTCCACCTCGC	Δ*snr67* deletion strain verification
oMB7719	CTAATTCCAATACGAAGAGC	Δ*snr67* deletion strain verification
oMB6006	CGTATCTTAGCATTACCCGC	Δ*snr72-snr78* deletion strain verification
oMB7721	CATCTAGTTCTTTGTCCAAAG	Δ*snr72-snr78* deletion strain verification
oMB7714	GAACACAACATTAAAAATGG	Δ*snr40* deletion strain verification
oMB7715	CTCCGAGCCATATCGGAAAG	Δ*snr40* deletion strain verification
oMB9191	GTAGATGAGGAGGTAATTGAAAAAAAGAAGGGTGAGAAGGAAGTGGAAG	Site-directed mutagenesis ($Dbp3_{\Delta 22-48}$)
oMB9192	CTTCCACTTCCTTCTCACCCTTCTTTTTTTCAATTACCTCCTCATCTAC	Site-directed mutagenesis ($Dbp3_{\Delta 22-48}$)
oMB9268	GTTGTCTGACATGGGTTCTTTAGTT	$NOP65_{DAmP}$ strain verification
oMB9269	GATAAACCAGCTGCAGAAGTGGAAG	$NOP65_{DAmP}$ strain verification

Supplementary Table 2. Plasmids used in this study

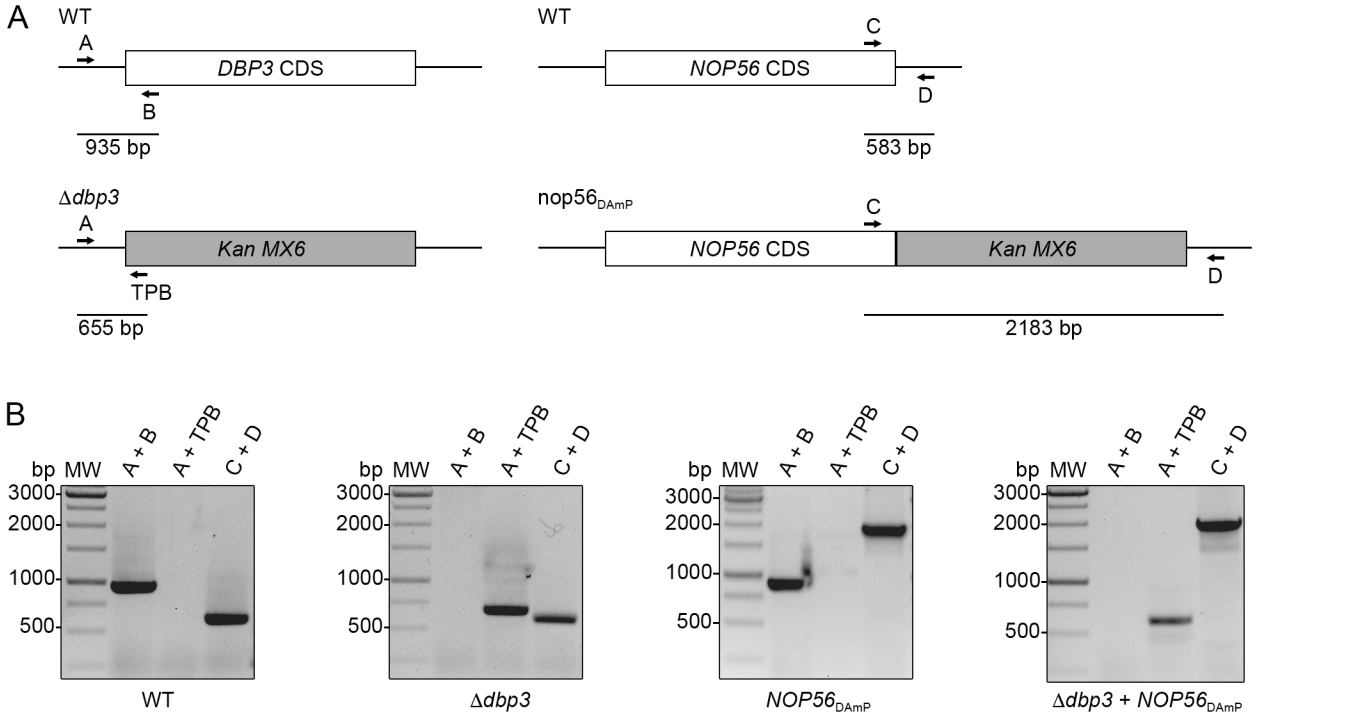
Name	Description	Application
pMB031	pRS415	Protein expression in yeast
pMB312	A21-Dbp3	Protein expression in *E. coli*
pMB1400	A21-Dbp3$_{E263Q}$	Protein expression in *E. coli*
pMB1401	pRS415-Dbp3+/-500	Yeast complementation
pMB1402	pRS415-Dbp3 $_{E263Q}$ +/-500	Yeast complementation
pMB1730	pRS416-*ACT*intron-pGAL$_1$	Yeast complementation
pMB1732	pRS416-*ACT*intron-pGAL$_1$-*SNR67*	Yeast complementation
pMB1822	pRS415-Dbp3$_{\Delta 22-48}$+-500bp	Yeast complementation

Supplementary Table 3. Yeast strains used in this study

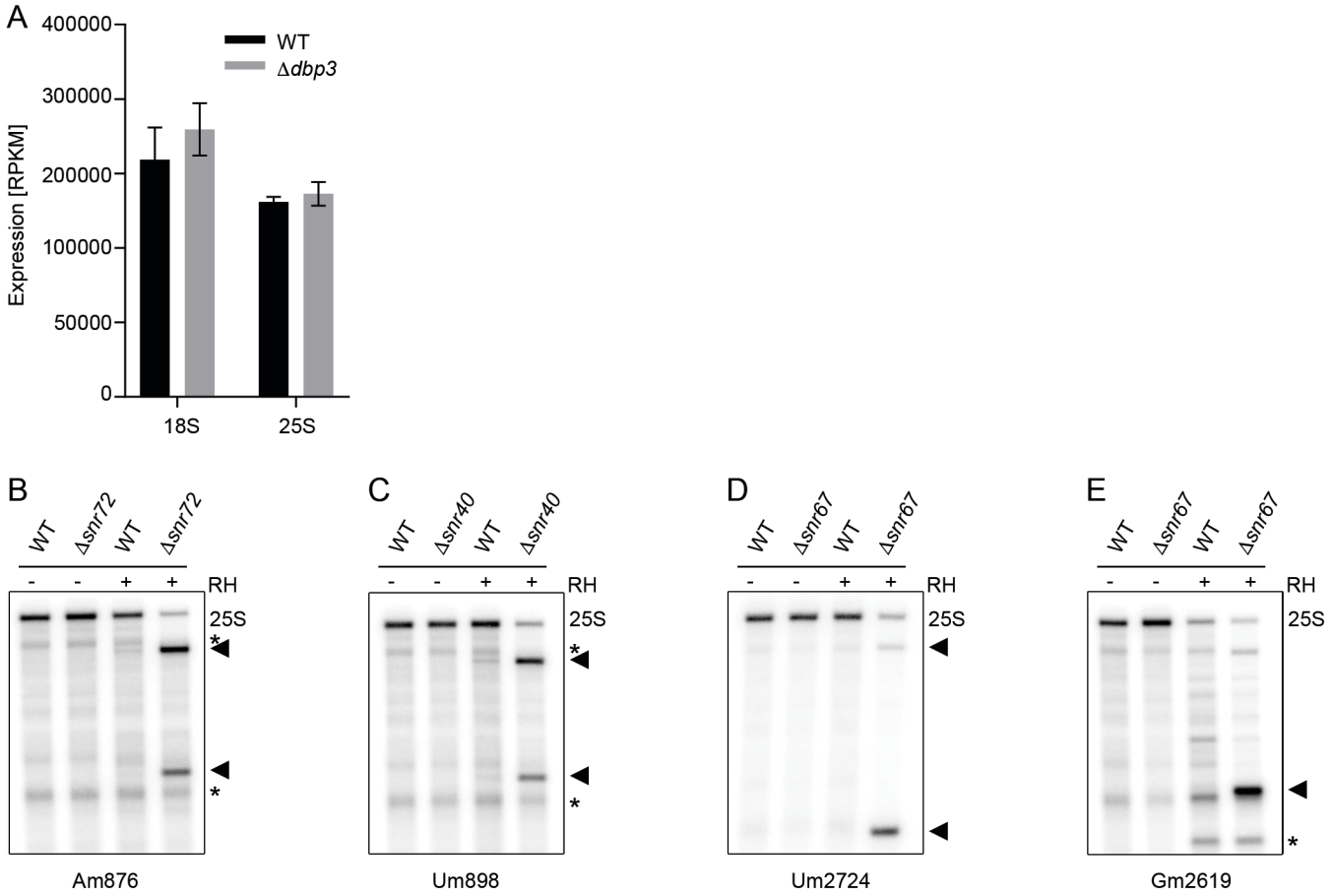
Name	Geneotype	Reference
YMB006/BY4741a	*MATa;his∆1;leu2∆0;met15∆0;ura∆0*	Euroscarf
YMB724	YMB006; *dbp3*::kanMX6	This study
YMB909	YMB006; rrp6::kanMX6	This study
YMB1088	YMB006; pGAL$_S$ prp43 natNT2	This study
YMB1487	YMB006; pMB031 (LEU2)	This study
YMB1538	YMB724; pMB1401 (LEU2)	This study
YMB1539	YMB724; pMB1402 (LEU2)	This study
YMB1540	YMB724; pMB031 (LEU2)	This study
YMB1566	YMB006; nop1-HTP (URA3)	This study
YMB1567	YMB724; nop1-HTP (HIS3)	This study
YMB1718	YMB006; *SNR78-SNR72*::natNT2	This study
YMB1720	YMB724; *SNR78-SNR72*::natNT2	This study
YMB1748	YMB006; *SNR40*::natNT2	This study
YMB1750	*NOP65*DAmP kanMX	Dharmacon
YMB1715	YMB006; *SNR67-SNR53*::natNT2	This study
YMB1903	YMB006; pMB1730 (URA3)	This study
YMB1905	YMB724; pMB1730 (URA3)	This study
YMB1895	YMB006; pMB1732 (URA3)	This study
YMB1899	YMB724; pMB1732 (URA3)	This study
YMB1963	YMB724; pMB1822 (LEU2)	This study
YMB1965	YMB1750; *dbp3*::kanMX6	This study

Supplementary Table 4. Chimeric oligonucleotides used for RNase H-based cleavage assays. mN indicates 2'-*O*-methylated RNA nucleotides and dN are DNA nucleotides.

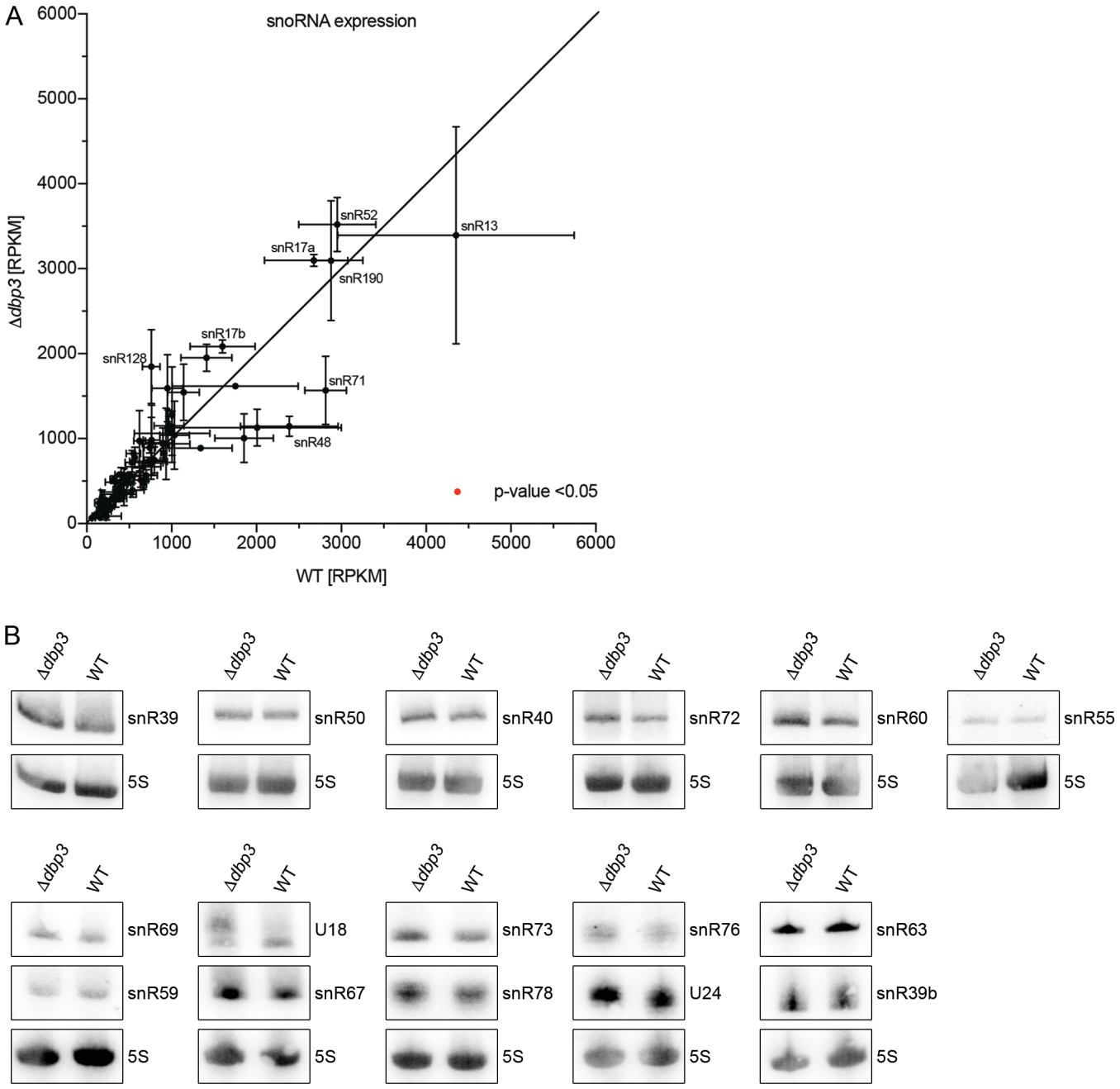
Name/Target modification	Sequence (5'-3')
25S-Am876	mAmCmGdTdCdAdGmAmAmCmCmGmCmUmAmCmGmAmG
25S-Um898	mCmCmAdAdAdTdTmCmGmAmCmGmAmUmCmGmAmUmUmU
25S-Gm2619	mGmCmCdCdCdAdGmCmCmAmAmAmCmUmCmCmCmCmAmC
25S-Um2724	mUmGmAdAdAdAdTmCmAmAmAmAmUmCmAmAmGmGmG



Aquino et al., Supplementary Figure S1



Aquino et al., Supplementary Figure S2



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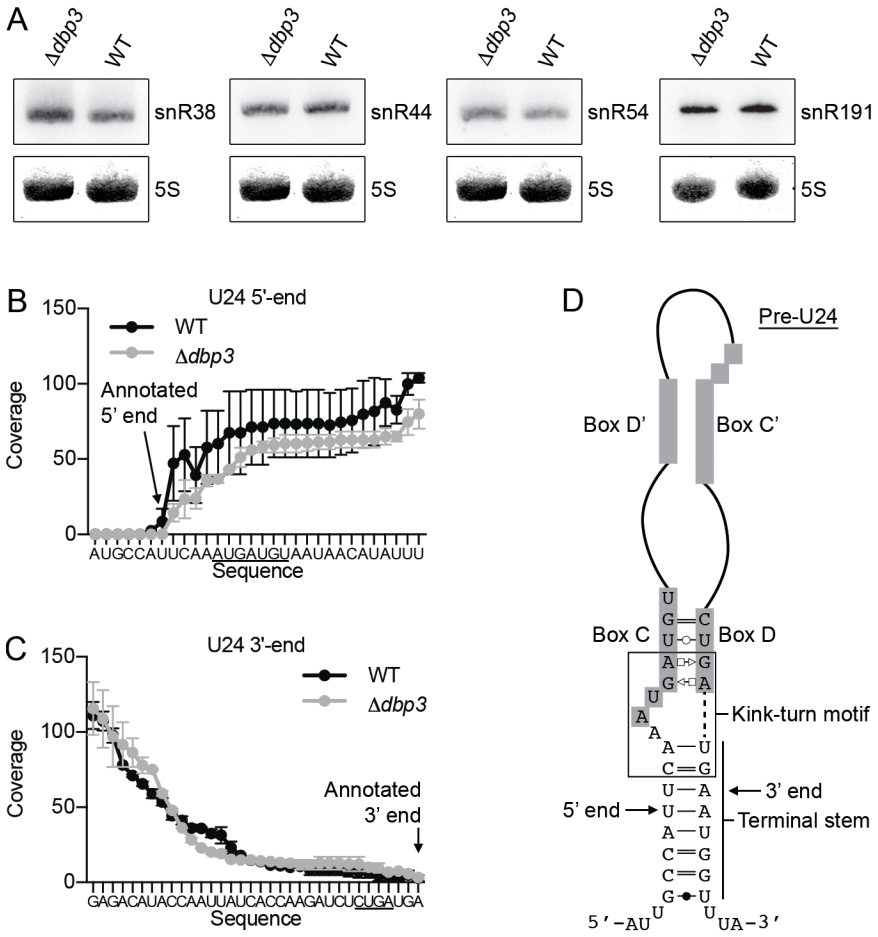

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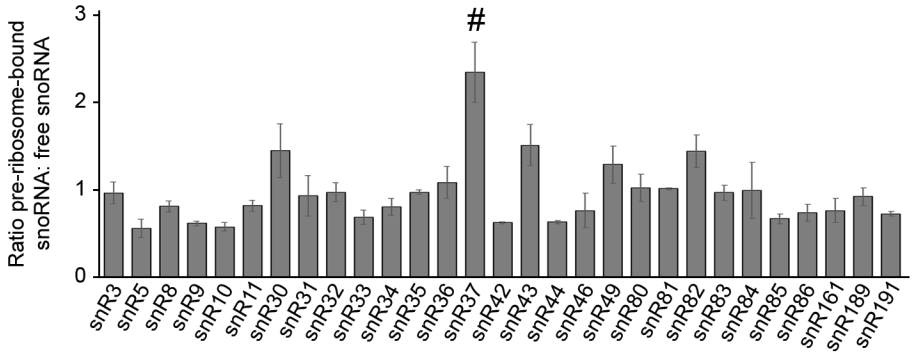

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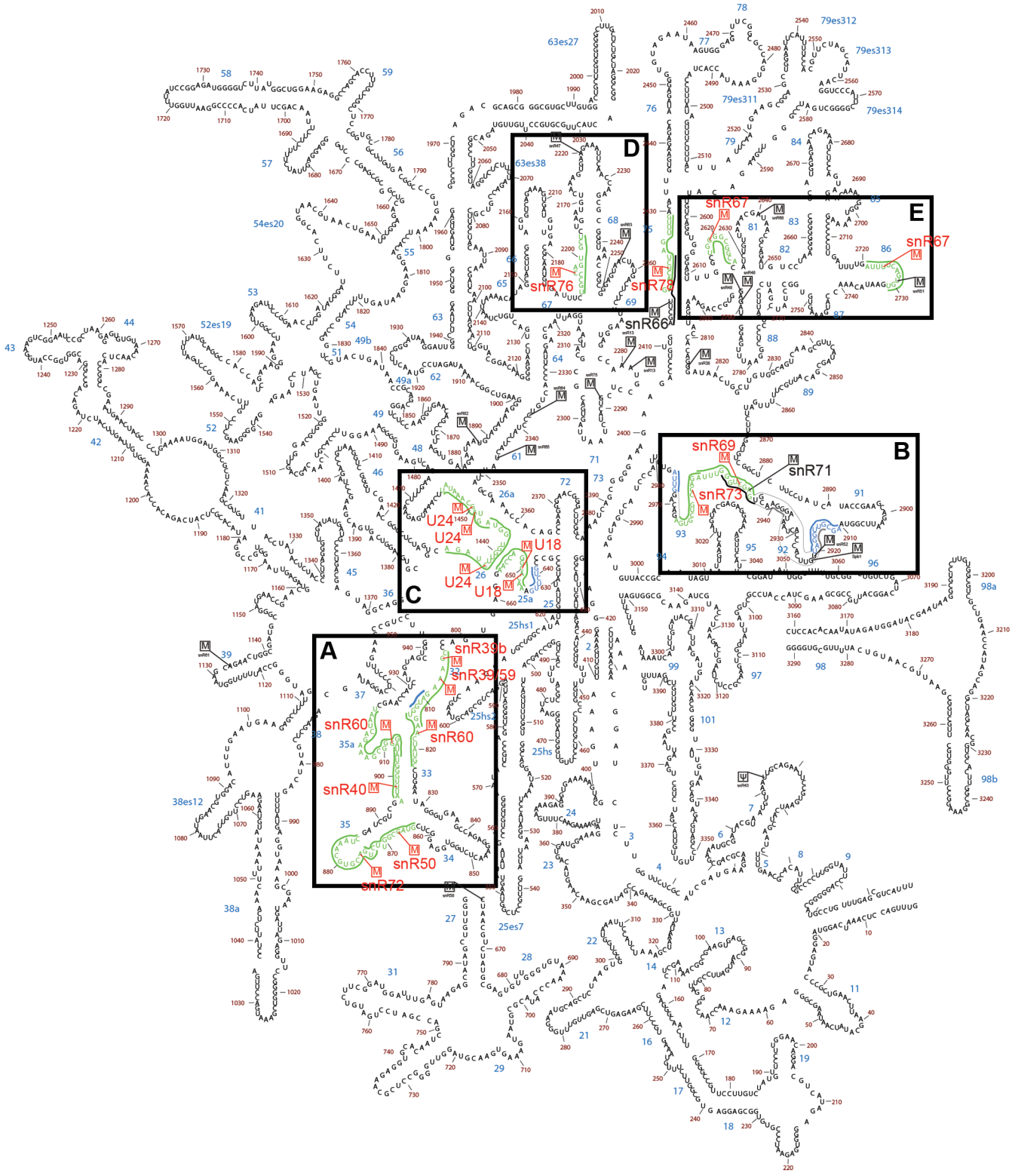

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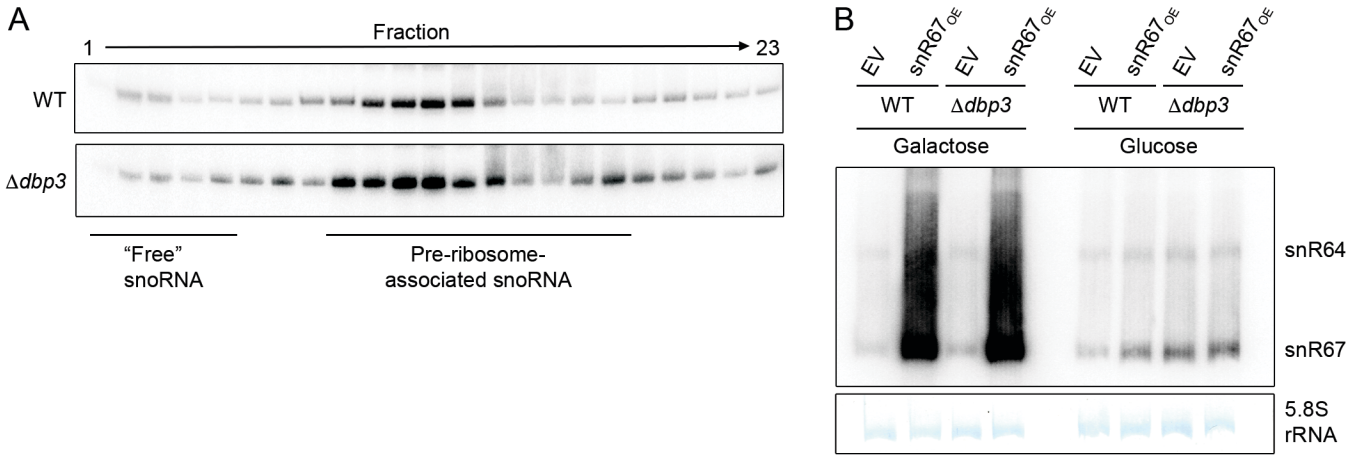

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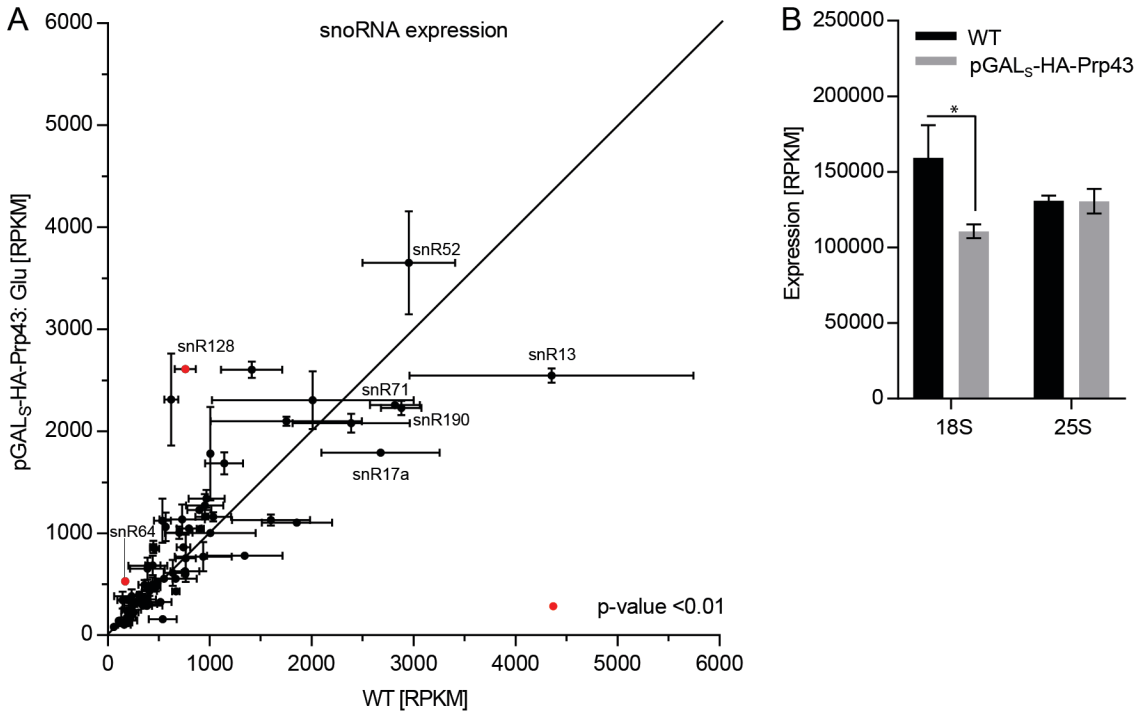

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