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 $\Delta dbp3$ 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 $\Delta snr78-72$, $\Delta snr40$ or $\Delta snr67$ -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'-Omethylation 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 $\Delta dbp3$ strain. Relative snoRNA levels from two biologically independent RMS experiments in shown as mean \pm standard error. (B) RNA extracted from wild type yeast or cells of the $\Delta dbp3$ 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 $\triangle dbp3$ 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 $\triangle dbp3$ 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 *△dbp3* 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 $\triangle dbp3$ 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 $\triangle dbp3$ mapped on the secondary structure of the 25S rRNA. 2'-O-methylations reduced in $\triangle dbp3$ 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 $\Delta dbp3$ 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 $\Delta dbp3$ 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 \pm 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].

ofMB1461 CGGTTTTAATTGTCCTA Northern blot probe (ITS1) oMB1468 TGAGAAGAAATGACGCT Northern blot probe (ITS2) oMB2510 ATATTAGGATCCGCCATGACAAAGGAAGAAATCGCAG Molecular cloning pMB312 oMB2511 ACGCGGTACCCTAATCGAAAGTAATTTTTTTGTTTC Molecular cloning pMB312 oMB2595 AACCCCTAATCGAAAGTAATTGAATTCGAGCTCCCGGGT Genomic deletion of <i>DBP3</i> aAC AACCCCTAATCGAAGGAAGTAATTGAATTCGAGCTCGTTTA Genomic deletion strain oMB2625 TGATCAGTCGACCGTTATTTATTTT Adbp3 deletion strain oMB2632 CTGCAGCGAGGAGCGTAAT Adbp3 deletion strain oMB3405 GAAGATAGACGAAATAGGAACAACAAACAGCTTATAA Genomic deletion of <i>RRP6</i> AATACCCATAACTGCGTTCGGATCCCCGGGTTAATTA Arpp6 deletion strain A CCACCAATAAGTGCGTTCAATAGAATTCCAGACTCGTTAT Arpp6 deletion strain AAC AAATACGCATTAATTAACGAATTCCAGACTCGATCGTTAT Arpp6 deletion strain A AAC AAATCGCATAATTAACGAAATGGAAATGACA Arpp6 deletion strain oMB3406 GAGGATCTAAATGAAATTACCATAATTTTAAAATAAAACGACTGTTAA	Name	Sequence (5'-3')	Application
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GACTGCAAGAATAATAATGCGTACGCTGCAGGTCGAC pGALs promoter for PRP43 oMB5760 GGAATAGAGGTCTCAACTGGATCCGGGTGTTCGGAC Genomic integration of pGALs promoter for PRP43 oMB6716 ATATATGGATCCGGTTATACTGCGTATTCGATTCTTTA Molecular cloning pMB1401 CTGG OMB6717 ATATATGGGCCGCTGGTCCAATATTTGACGTTTCTA Molecular cloning pMB1401 oMB6717 ATATATGCGGCCGCTGGTCCAATATTTGACGTTTCTA Molecular cloning pMB1401 G GTAATTACTTAGTATTAGACCAGGCAGACAGAATGTT Site-directed mutagenesis (Db93 _{E283Q}) OMB6721 CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAA oMB465 CTCCGCTTATTGATATGC Northern blot probe 25S rRNA (RNase H) Northern blot probe 25S oMB6327 CTGTCTAGATGAACTAACACC Northern blot probe 25S oMB8611 AAAACTCGAGGTTCAACCTGTACGTGGATGG Cloning snR67 for overexpression oMB8612 AAAAGGTACCGAGGAGTGATAATGGCAATTAAGAC Cloning snR67 for overexpression oMB1537 GGTGATTAAACGACCAGCATTGTCAAGAGCTAGTCGA Northern blot probe snR59 oMB1633 CAGTGTTTGTTGTTGTTGTAAAATCAG Northern blot probe snR67	oMB5759	CGATAGTATAACCTTATAAACGCGCATAGAAAGATTAG	Genomic integration of
oMB5760GGAATAGAGGTCTCAACTGGATCCGGGTGTTCGGAC GAGAATCTTCTTTTGGAACCCATCGATGAATTCTCTGT CGGenomic integration of pGALs promoter for PRP43oMB6716ATATATGGGATCCGGTTATACTGCGTATTCGATTCTTA CTGGMolecular cloning pMB1401oMB6717ATATATGCGGCCGCTGGTCCAATATTTGACGTTTCTA GMolecular cloning pMB1401oMB6720GTTAATTACTTAGTATTAGACCAGGCAGACAGAATGTT GGAAAAAGGSite-directed mutagenesis (Dbg3E263Q)oMB6721CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAA GTAATTAACSite-directed mutagenesis (Dbg3E263Q)oMB7263GCCTGCTATGGTTCAGCGACG CTCCGCTTATGGTTCAGCGACGNorthern blot probe 25S rRNA (RNase H)oMB6327CTGTCTAGATGAACTAACACC CTGTCTAGATGAACTAACACCNorthern blot probe 25S rRNA (RNase H)oMB8611AAAAGGTACCGAGGATGACAGTGGATGG OMB8612Cloning snR67 for overexpressionoMB1537GGTGATTAAACGACAGCATTGTCAAAGACTAAGAC OMB1633CAGTGTTTGTTGTTGTTGTTGTAAAAATCAG OMB1635Northern blot probe snR67 oMorthern blot probe snR67		GACTGCAAGAATAATAATGCGTACGCTGCAGGTCGAC	pGALs promoter for PRP43
GAGAATCTTCTTTTGGAACCCATCGATGAATTCTCTGT CGpGALs promoter for PRP43oMB6716ATATATGGATCCGGTTATACTGCGTATTCGATTCTTTA CTGGMolecular cloning pMB1401oMB6717ATATATGCGGCCGCTGGTCCAATATTTGACGTTTCTA GMolecular cloning pMB1401oMB6720GTTAATTACTTAGTATTAGACCAGGCAGACAGAATGTT GGAAAAAGGSite-directed (Dby3E283Q)oMB6721CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAA GTAATTAACSite-directed (Dby3E283Q)oMB1465CTCCGCTTATTGATATGCNorthern Northern blot probe 25S rRNA (RNase H)oMB6327CTGTCTAGATGAACTAACACC MOB8611Northern blot probe 25S rRNA (RNase H)oMB8612AAAAGGTACCGAGGAGTGATAATGGCAATTAAGAC OWB8612Cloning snR67 for overexpressionoMB1537GGTGATTAAACGACAGCATTGTCAAGGCAATTAAGAC OMB1633Northern blot probe snR67 of oVerexpression	oMB5760	GGAATAGAGGTCTCAACTGGATCCGGGTGTTCGGAC	Genomic integration of
oMB6716ATATATGGATCCGGTTATACTGCGTATTCGATTCTTTA CTGGMolecular cloning pMB1401oMB6717ATATATGCGGCCGCTGGTCCAATATTTGACGTTTCTA GMolecular cloning pMB1401oMB6720GTTAATTACTTAGTATTAGACCAGGCAGACAGAATGTT GGAAAAAGGSite-directed (Dbp3E2630)oMB6721CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAA GTAATTAACSite-directed (Dbp3E2630)oMB1465CTCCGCTTATTGATATGCNorthern Northern blot probeoMB7263GCCTGCTATGGTTCAGCGACG GCCTGCTAGGTGCAACACCNorthern RNA (RNase H)oMB6327CTGTCTAGATGAACTAACACCNorthern RNA (RNase H)oMB8611AAAACTCGAGCTTCAACCTGTACGTGGATGG OMB8612Cloning SnR67 AAAAGGTACCGAGGAGTGATAATGGCAATTAAGACoMB1537GGTGATTAAACGACAGCATTGTCAAGCAACTAACACCCloning SnR67 OMB1633oMB1633CAGTGTTTGTTGTTGTTGTTGTTAAAATCAGNorthern blot probe snR67 OMB1635oMB1635CAGTCATTCAAAGATCCGCTTGGNorthern blot probe snR67 OMB1635		GAGAATCTTCTTTTGGAACCCATCGATGAATTCTCTGT CG	pGALs promoter for PRP43
oMB6717ATATATGCGGCCGCTGGTCCAATATTTGACGTTTCTA GMolecular cloning pMB1401oMB6720GTTAATTACTTAGTATTAGACCAGGCAGACAGAATGTT GGAAAAAGGSite-directed (Db3E263Q)mutagenesis (Db3E263Q)oMB6721CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAA GTAATTAACSite-directed (Db3E263Q)mutagenesis (Db3E263Q)oMB1465CTCCGCTTATTGATATGCNorthern ProbeNorthern Probe25S rRNA (RNase H)oMB6327CTGTCTAGATGAACTAACACCNorthern ProbeNorthern Probe25S rRNA (RNase H)oMB6611AAAACTCGAGCTTCAACCTGTACGTGGATGGCloning overexpressionsnR67 or overexpressionoMB1537GGTGATTAAACGACAGCATTGTCAAAGACTAAGACCloning overexpressionsnR67 or overexpressionoMB1633CAGTGTTTGTTGTTGTTAAAATCAGNorthern blot probe snR67oMB1635CAGTCATTTCAAAGATCCGCTTGGNorthern blot probe snR67	oMB6716	ATATATGGATCCGGTTATACTGCGTATTCGATTCTTA CTGG	Molecular cloning pMB1401
oMB6720GTTAATTACTTAGTATTAGACCAGGCAGACAGAATGTT GGAAAAAGGSite-directed (Dbp3E263Q)mutagenesis (Dbp3E263Q)oMB6721CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAA GTAATTAACSite-directed (Dbp3E263Q)mutagenesis (Dbp3E263Q)oMB1465CTCCGCTTATTGATATGC NorthernNorthern blot probe25S rRNA (RNase H)oMB6327CTGTCTAGATGAACTAACACC RNA (RNase H)Northern blot probe25S rRNA (RNase H)oMB8611AAAACTCGAGCTTCAACCTGTACGTGGATGG NorthernCloning overexpressionsnR67 for overexpressionoMB8612AAAAGGTACCGAGGAGTGATAATGGCAATTAAGAC OMB1537GGTGATTAAACGACAGCATTGTCAAAGACTAGTCGA Northern blot probe snR59Northern blot probe snR67 oMB1633Northern blot probe snR67	oMB6717	ATATATGCGGCCGCTGGTCCAATATTTGACGTTTCTA G	Molecular cloning pMB1401
oMB6721CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAA GTAATTAACSite-directed (Dbp3E263Q)oMB1465CTCCGCTTATTGATATGCNorthern RNA (RNase H)oMB7263GCCTGCTATGGTTCAGCGACGNorthern 	oMB6720	GTTAATTACTTAGTATTAGACCAGGCAGACAGAATGTT GGAAAAAGG	Site-directed mutagenesis (Dbp3 _{E263Q})
oMB1465CTCCGCTTATTGATATGCNorthern blot probe25S rRNA (RNase H)oMB7263GCCTGCTATGGTTCAGCGACGNorthern blot probe25S rRNA (RNase H)oMB6327CTGTCTAGATGAACTAACACCNorthern blot probe25S rRNA (RNase H)oMB8611AAAACTCGAGCTTCAACCTGTACGTGGATGGCloning overexpressionsnR67 overexpressionoMB8612AAAAGGTACCGAGGAGTGATAATGGCAATTAAGACCloning overexpressionsnR67 	oMB6721	CCTTTTTCCAACATTCTGTCTGCCTGGTCTAATACTAA GTAATTAAC	Site-directed mutagenesis (Dbp3 _{E2630})
oMB7263 GCCTGCTATGGTTCAGCGACG Northern blot probe 25S oMB6327 CTGTCTAGATGAACTAACACC Northern blot probe 25S oMB8611 AAAACTCGAGCTTCAACCTGTACGTGGATGG Cloning snR67 for oMB8612 AAAAGGTACCGAGGAGTGATAATGGCAATTAAGAC Cloning snR67 for oMB1537 GGTGATTAAACGACAGCATTGTCAAAGACTAGTCGA Northern blot probe snR59 oMB1633 CAGTGTTTGTTGTTGTTGTAAAATCAG Northern blot probe snR67	oMB1465	CTCCGCTTATTGATATGC	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 CAGTGTTTGTTGTTGTTAAAATCAG Northern blot probe snR67 oMB1635 CAGTCATTTCAAAGATCCGCTTGG Northern blot probe snR67	oMB7263	GCCTGCTATGGTTCAGCGACG	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 CAGTGTTTGTTGTTGTTGTAAAATCAG Northern blot probe snR67 oMB1635 CAGTCATTTCAAAGATCCGCTTGG Northern blot probe snR67	oMB6327	CTGTCTAGATGAACTAACACC	Northern blot probe 25S rRNA (RNase H)
oMB8612 AAAAGGTACCGAGGAGTGATAATGGCAATTAAGAC Cloning snR67 for overexpression oMB1537 GGTGATTAAACGACAGCATTGTCAAAGACTAGTCGA Northern blot probe snR59 oMB1633 CAGTGTTTGTTGTTGTTGTAAAATCAG Northern blot probe snR67 oMB1635 CAGTCATTTCAAAGATCCGCTTGG Northern blot probe snR67	oMB8611	AAAACTCGAGCTTCAACCTGTACGTGGATGG	Cloning snR67 for overexpression
oMB1537 GGTGATTAAACGACAGCATTGTCAAAGACTAGTCGA Northern blot probe snR59 oMB1633 CAGTGTTTGTTGTTGTTGTAAAATCAG Northern blot probe snR67 oMB1635 CAGTCATTTCAAAGATCCGCTTGG Northern blot probe snR67	oMB8612	AAAAGGTACCGAGGAGTGATAATGGCAATTAAGAC	Cloning snR67 for overexpression
oMB1633 CAGTGTTTGTTGTTGTTGTAAAATCAG Northern blot probe snR67 oMB1635 CAGTCATTTCAAAGATCCGCTTGG Northern blot probe snR67	oMB1537	GGTGATTAAACGACAGCATTGTCAAAGACTAGTCGA	Northern blot probe snR59
oMB1635 CAGTCATTTCAAAGATCCGCTTGG Northern blot probe snR67	oMB1633	CAGTGTTTGTTGTTGTAAAATCAG	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	TCCTTTAGAGATGATAAAGACAACTTACAAGTACAGTT	Genomic deletion of SNR67-	
	TTTTGTTGGTATCTCATCGGATCCCCGGGTTAATTAA	SNR53	
oMB7727	CTAATTCCAATACGAAGAGCCTAATTCTTTGATAGTTC	Genomic deletion of SNR67-	
		SNR53	
oMB1845	ATCACTAACAGATGAAAAAGGTAGAATGGATAAAATAC	Genomic deletion of SNR72-	
	TTAAAGAATTTTATATGGAATTCGAGCTCGTTTAAAC	78	
oMB6001	GTTAGTTTTTCAGATATGTCTTTTGTCATTATCACAGG	Genomic deletion of SNR72-	
oMB1832		78 Genomic deletion of SNR40	
010101002	GAATTCAAGGAAAAATGCGGATCCCCGGGTTAATTAA		
oMB1848	TCATAATACAGTCACAGATGTGAGAGAAAAAAAAAAAAA	Genomic deletion of SNR40	
-MD7710			
		$\Delta snr67$ deletion strain verification	
oMB7719	CTAATTCCAATACGAAGAGC	$\Delta snr67$ deletion strain	
oMP6006		verification	
		verification	
oMB7721	CATCTAGTTCTTTGTCCAAAG	<i>∆snr</i> 72- <i>snr</i> 78 deletion strain	
oMP7714		verification	
010107714	GACACACATTAAAATGG	Ashr40 deletion strain	
oMB7715	CTCCGAGCCATATCGGAAAG	$\Delta snr40$ deletion strain	
		verification	
oMB9191	GTAGATGAGGAGGTAATTGAAAAAAAGAAGGGTGAGA	Site-directed mutagenesis	
oMR0102		(UDD)3 <u>A22-48</u>) Site-directed mutaconocia	
		(Dbp3 _{A22-48})	
oMB9268	GTTGTCTGACATGGGTTCTTTAGTT	NOP65 _{DAmP} strain	
		verification	
oMB9269	GATAAACCAGCTGCAGAAGTGGAAG	NOP65 _{DAmP} strain	
		venilcation	

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-ACTintron-pGAL1	Yeast complementation
pMB1732	pRS416-ACTintron-pGAL1-SNR67	Yeast complementation
pMB1822	pRS415-Dbp3∆22-48+-500bp	Yeast complementation

Supplementary Table 2. Plasmids used in this study

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; <i>dbp3</i> ::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	NOP65DAmP 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; <i>dbp3</i> ::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	mUmGmAdAdAdAdTmCmAmAmAmAmUmCmAmAmGmGmGmG



Aquino et al., Supplementary Figure S1



Aquino et al., Supplementary Figure S2



Aquino et al., Supplementary Figure S3



Aquino et al., Supplementary Figure S4



Aquino et al., Supplementary Figure S5



Aquino et al., Supplementary Figure S6





Aquino et al., Supplementary Figure S7



Aquino et al., Supplementary Figure S8