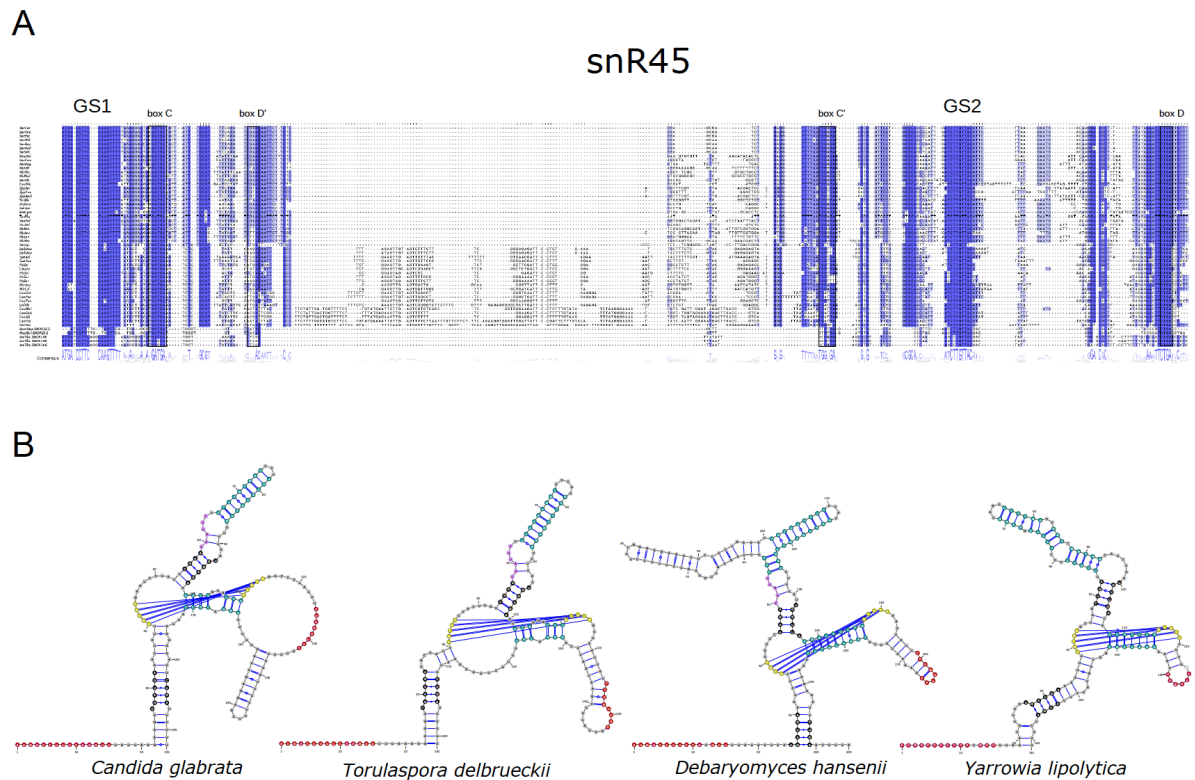
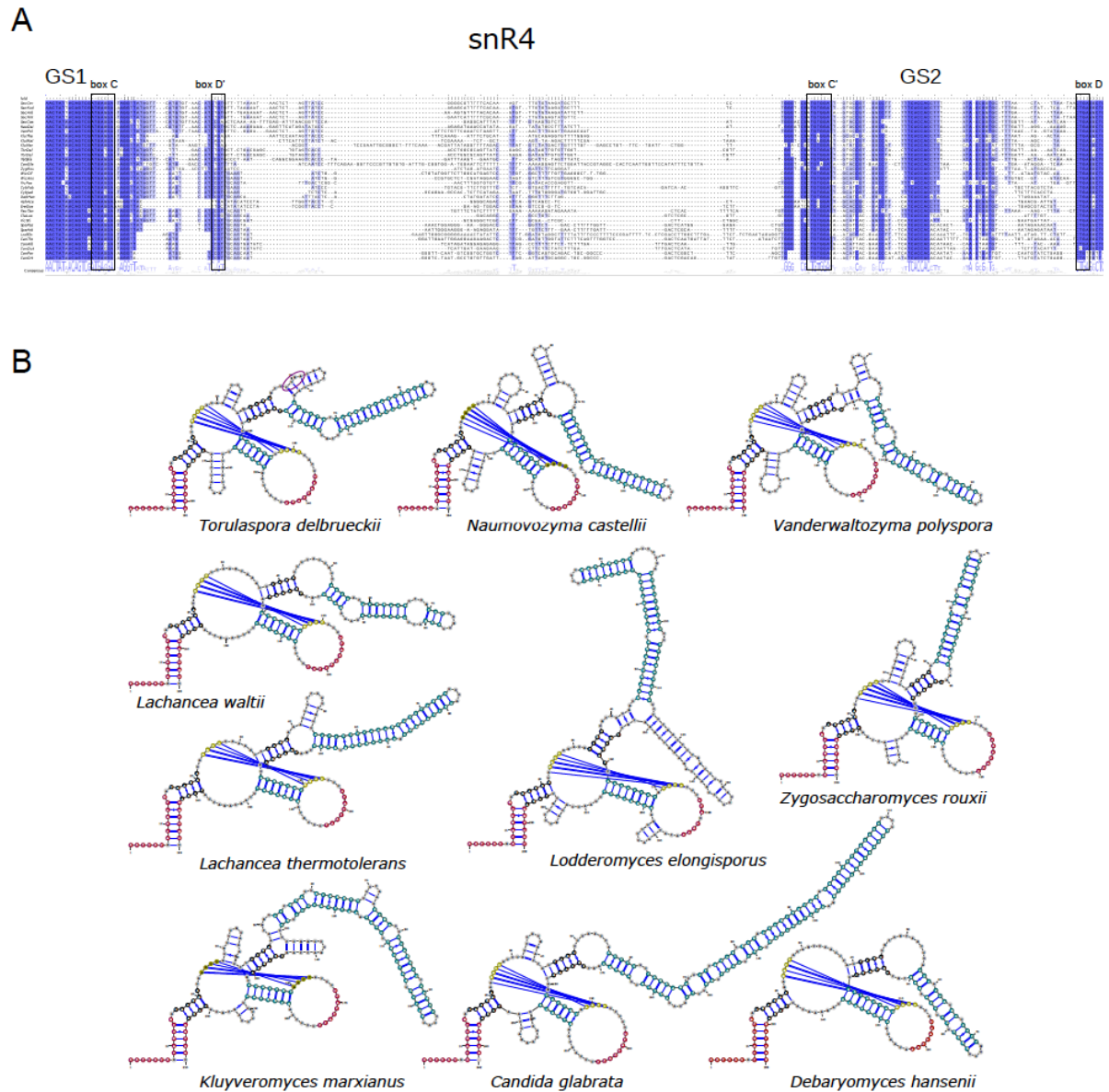


**FIG S1 : CRAC analysis revealed specific binding of Kre33 to leucine and serine tRNAs (A), 18S rRNA (B) and snoRNAs snR4 and snR45 (C).** **A)** Overview of CRAC-results for Kre33 binding to serine and leucine tRNA species. The left y-axis shows the total number of times each nucleotide within an RNA fragment was mapped to the RNA sequence (x-axis); the right y-axis shows the number of reads carrying a substitution (red) or deletion (orange). Around the acetylated cytosine (\*) a specific peak is observed in Kre33 cross-linked tRNAs. For other tRNA species or for the same species in control CRAC-experiments these substitution-peaks were not found. **B)** Read-alignments for Kre33 on the 18S rRNA show specific binding to the 5' domain (pink), around helices 34 (orange) and 45 (blue). Putative crosslink sites (yellow circles) are seen as a gap in these sequences. Acetylated residues C1280 and C1773 (purple) fall within cross-linked segments. Note that Kre33 binding could be incompatible with that of modifying snoRNPs snR44, snR49, snR51, snR55, snR40, and snR83/Ruf3. **C)** As (B) but then for Kre33 binding to snR4 and snR45. Models of snoRNAs interacting with target rRNA sequence (blue) with the acetylated cytosine residue (red) are as in Fig 4.

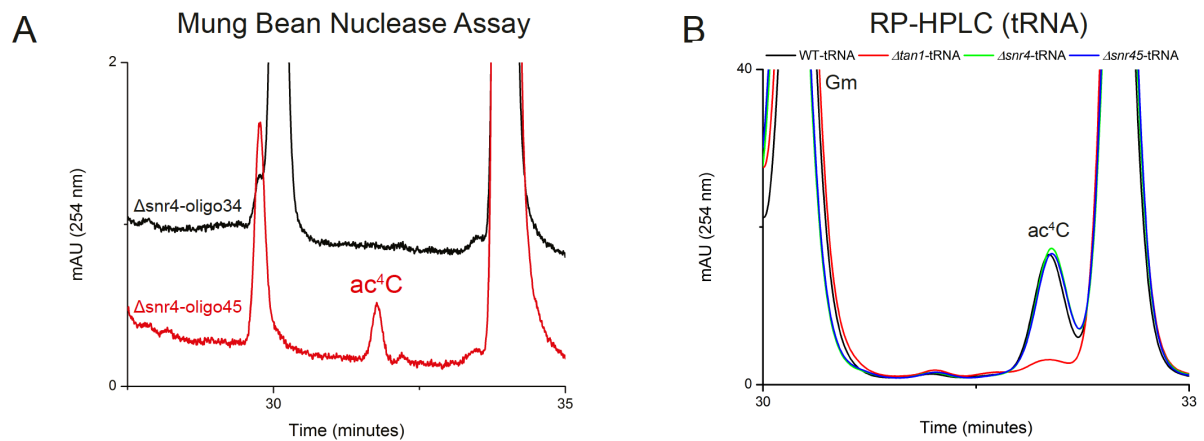


**FIG S2: Phylogenetic analysis of snR45**

**A)** Alignment of snoRNAs homologous to snR45. Sequences were retrieved and aligned as described previously [4]. The guide sequences (GS1 and GS2) and the C/D and C' motifs stand out due to their high level of conservation (deep blue shade). The non-canonical D' motif is quite variable and assigned based on 2D-modelling (B). Dot-bracket-notation shows the general 2D-structure (top-line). **B)** Secondary structure models of snR45 for the indicated yeast species. Shown are the conserved regions with guide sequences GS1 and GS2 (red), the C/D and C'/D' motifs (black), the pseudo-knot (olive) and helices with strong phylogenetic support (teal).

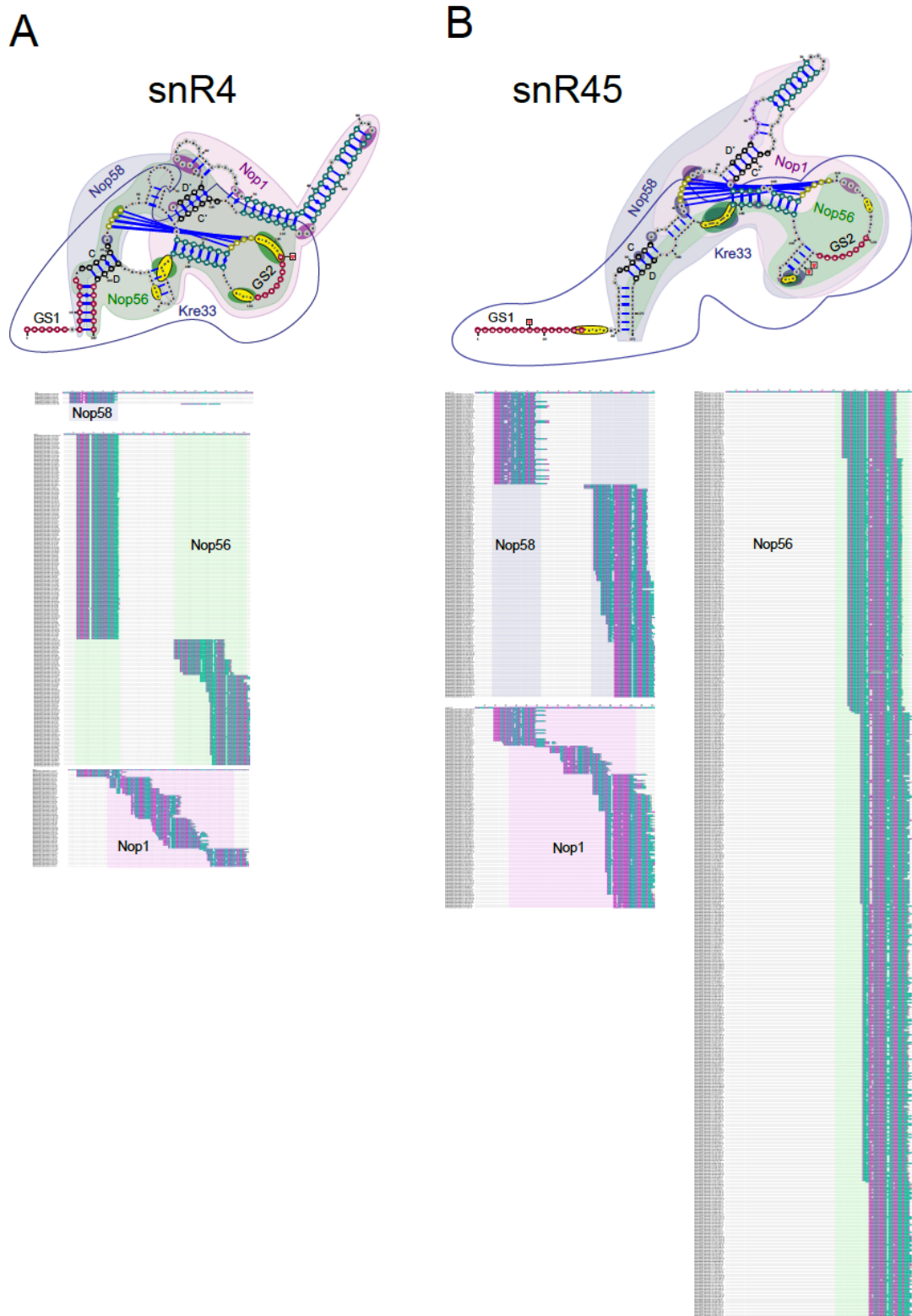


**FIG S3:** Phylogenetic analysis of snR4 with alignment (**A**) and 2D-models (**B**) as described for [S2 Fig](#). Note, *Torulaspora delbrueckii* snR45 could be modeled with a D' box using a canonical sequence (CUGA, red ellipse) instead of the CUGU found in *S. cerevisiae* and closely related species.



**FIG S4: snR4 is involved in 18S rRNA acetylation at C1280.**

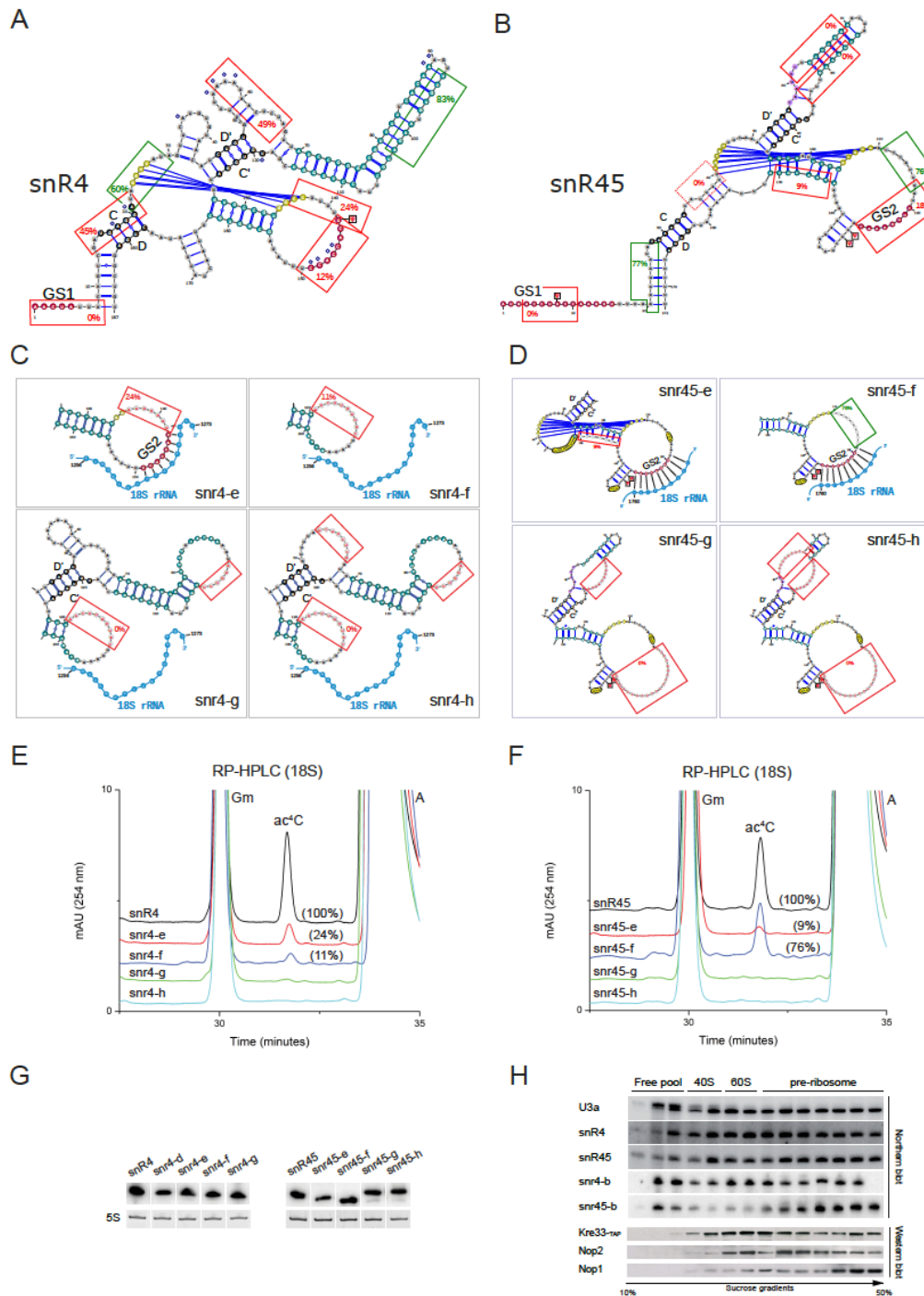
**A)** Overlaid RP-HPLC chromatograms of the nucleosides derived from fragments isolated using mung bean nuclease assay, containing ac<sup>4</sup>C1280 (oligo 34, black) and ac<sup>4</sup>C1773 (oligo 45, red) isolated from a strain lacking snR4 ( $\Delta$ snr4). Loss of snR4 leads to complete loss of ac<sup>4</sup>C1280 without affecting ac<sup>4</sup>C1773. **B)** Overlaid RP-HPLC chromatogram of tRNAs from strains lacking snR4 ( $\Delta$ snr4, green), snR45 ( $\Delta$ snr45, blue) and Tan1 ( $\Delta$ tan1, red) strain. Loss of snR4 and snR45 does not affect tRNA acetylation.



**FIG S5: Crosslinking of Nop56, Nop58 and Nop1 to snR4 and snR45**

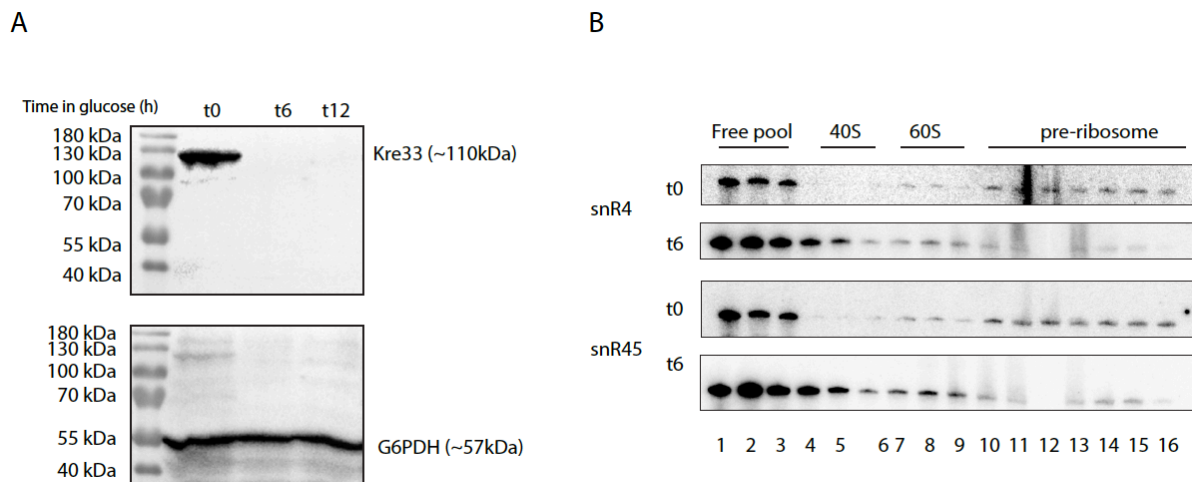
2-D structure models (top panel) based on phylogenetic comparison and DMS-modification for snR4 (**A**) and snR45 (**B**) showing Kre33 cross-linked sites (identified in the present study, yellow ovals), along with the binding regions (transparent) and cross-linking sites (opaque ovals) of Nop1 (purple), Nop56 (green), and Nop58 (blue) determined previously [4] and as colored squares overlaid on the alignments (bottom panel) of unique reads with the snoRNA sequences. For further details, see legend [Fig 4](#).





**FIG S6: Physical interaction of snR4 and snR45 with 18S rRNA is indispensable for 18S rRNA acetylation**

2D models of snR4 (A) and snR45 (B) along with zoomed-in views (C and D) for mutated regions. The dotted red box in (B) indicate a mutation that interfered with stable expression of the snoRNA. See for further details the legend for Fig 5, F) RP-HPLC chromatograms of 18S rRNA isolated from a strain carrying a double deletion for *SNR4* and *SNR45* expressing the indicated mutant versions of snR4 (E) and snR45 (F). (G) Northern blot developed with snoRNA-specific probes showing stable expression of snr4 and snr45 mutants. 5S rRNA was used as loading control. (H) Sedimentation profile of snR4 (snr4-b) and snR45 (snr45-b) with an altered GS2 (cf. Fig 5) to test their association with ribosomes/pre-ribosomes; northern analysis in (H), snoRNA-specific probes and western-blot were probed with anti-TAP, anti-Nop1, and anti-Nop2 antibodies to detect Kre33-TAP, Nop1 and Nop2, respectively.



**FIG S7: Sedimentation profiles of snR4 and snR45 upon Kre33 depletion**

Kre33 was depleted using a strain containing HA tagged Kre33 under galactose promoter (pGAL1::3HA-kre33) that was grown in YPGSR (yeast extract, peptone, galactose–sucrose–raffinose, 2% w/v each) to mid-log phase, washed in pre-warmed water and transferred to YPD for up to 12 h. We compared t0 (time point at which cells were transferred to YPD) and t6 (6hours after transfer to YPD). **A**) Western blot showing that glucose mediated depletion of Kre33 (top panel) was extremely effective and after 6 hours Kre33 was depleted to a level beyond the Western blot detection limit. The blot was developed using mouse anti-HA (SIGMA) and goat anti-mouse-HRP (Santa Cruz Biotechnology) antibodies. Glucose 6 phosphate dehydrogenase (G6PDH) was used as a loading control (lower panel). The blot was developed using rabbit anti G6PDH and donkey-anti rabbit (Santa Cruz Biotechnology) antibodies. **B**) Sedimentation profiles of snR4 and snR45 at t0 and t6.

S1 Table. Hybrids containing snoRNA sequences

Read No. ‡	Sequence of hybrids between 18S rRNA and snoRNA	ΔG*	5' Source	from	to	3' Source (motif)	from	to
130876-1_2	TAGTGAAACTGCGAATGGCTCCACG <b>GTGATGA</b> AAGACTGGT	-3	RDN37-1	80	99	U14 / snR128 (C)	2	21
-12350-2_5	AGTGAAACTGCGAATGGCTCAA <b>CATTCGCAGTTTC</b> CACG <b>G</b>	-20.6	RDN37-2	80	100	U14 / snR128	27	46
63756-1_6	TTGGCCTTTT <b>TCACCACC</b> TTTAT <b>TTTTGTG</b> <b>GGTGGTGG</b> TGC	-14.5	snR4	133	154	RDN37-1	1257	1274
97648-1_3	TAAGGAAGGCAGCAGGCGCGCTACT <b>ATGATGA</b> ATGACATTA	-1.8	RDN37-1	416	435	snR52 (C)	1	20
99477-1_3	TGGAGGGCAAGTCTGGTGCCAGCAAAAAGCTGT <b>GCACCAG</b> T	-18.1	RDN37-1	547	570	snR40	14	31
133303-1_2	<b>TGG</b> TGCATGGCCGTTCTTGTA <b>ATGACGA</b> GAAAAAAGCTG	-7.4	RDN37-1	1269	1289	snR40 (C)	1	22
301878-1_1	<b>TGGTGG</b> TGCATGGCCGTTCTTGTA <b>ATGACGA</b> GAAAAAAGC	-3.2	RDN37-1	1266	1286	snR40	1	20
77693-1_4	CACAT <b>CATGCATCACCATCT</b> <b>TTTTGTG</b> <b>GGTGGTGG</b> TGCATG	-21.2	snR55	76	94	RDN37-1	1257	1277
-18530-1_2	GAT <b>CATGCATCACCATCTGA</b> TTAAGGATTGACAGATTGAG	-6.3	snR55	78	98	RDN37-2	1225	1243
125983-1_2	<b>GATTTTGTG</b> <b>GGTGGTGG</b> TGCATGATCCG <b>GCGA</b> TGATTCTT	-6.8	RDN37-1	1255	1277	snR55 (D')	38	55
251846-1_1	GG <b>ATTTGTG</b> <b>GGTGGTGG</b> TGCAAAT <b>CATGCATCACCATCTGA</b>	-19.9	RDN37-1	1258	1275	snR55 (D)	78	96
62794-1_6	<b>GTTTTGTG</b> <b>GGTGGTGG</b> TGCATAGCAT <b>CATGCATCACCATCT</b>	-23.2	RDN37-1	1257	1279	snR55	77	94
310610-1_1	<b>TTTTGTG</b> <b>GGTGGTGG</b> TGCATTGGAAT <b>ATGTGCC</b> ATGGATT	-10.3	RDN37-1	1257	1276	snR55 (C')	55	75
301495-1_1	<b>TGGGTGGTGG</b> TGCATGGCCGTT <b>C</b> ATGGATTACAT <b>CATGCAT</b>	-8.9	RDN37-1	1262	1284	snR55	68	86
92915-1_3	CCGCGTAATTCCAGCTCCAATAAAAACAAA <b>AGCTGGAATT</b>	-13	RDN37-1	572	594	snR77	20	38
99442-1_3	TGCGGTAATTCCAGCTCCAATAAAA <b>AGCTGGAATTACTG</b> <b>GCT</b>	-20.6	RDN37-1	574	594	snR77	26	45
183610-1_1	CAGTAATTCCAGCTCCAATAAAA <b>AGCTGGAATTACTG</b> <b>GCTGA</b>	-17.2	RDN37-1	577	594	snR77 (D')	26	47
32255-1_15	CGGTAATTCCAGCTCCAATAAAA <b>AGCTGGAATTACTG</b> <b>GCTGA</b>	-20.4	RDN37-1	575	594	snR77 (D')	26	47
202069-1_1	CGGTAATTCCAGCTCCAATAAAA <b>AGCTGGAATTACTG</b> <b>GCTG</b>	-20.4	RDN37-1	575	594	snR77	26	46
202068-1_1	CGGTAATTCCAGCTCCAATAAAA <b>AGCTGGAATTACTG</b> <b>GC</b>	-20.4	RDN37-1	575	594	snR77	26	44
125360-1_2	GATGATCAGATACCGTCGTAGT <b>TTTA</b> <b>ATGATGA</b> TAGCATGA	-7.1	RDN37-1	994	1020	snR79 (C)	1	18
	<b>Sequence of hybrids between 25S rRNA and snoRNA</b>							
-10468-1_11	<b>CTA</b> TTACAGTCG <b>ATGAGGA</b> TGATCGTCGAATTTGGGTATA	-9.8	snR4	3	22	RDN37-2 (snR40/snR60)	3438	3457



226958-1_1	GACGAATAAGGCGTCCTTG <b>TAC</b> ATTC <b>TTAAGAATGACAAG</b>	-3.5	<u>RDN37-1</u>	5731	5749	snR45	125	145
226959-1_1	GACGAATAAGGCGTCCTTG <b>TAC</b> ATTC <b>TTAAGAATGACAAGG</b>	-5.8	<u>RDN37-1</u>	5731	5749	snR45	125	146
114554-1_2	ACTGTTGGGACCCGAAAGATGATTTA <b>GTGATG</b> A <b>TA</b> CTGCCG	-2	<u>RDN37-1</u>	3344	3363	snR39B (C)	1	20
92889-1_3	CCGAGCTTGACTCTAGTTT <b>GA</b> CTCAA <b>ATGATGA</b> AATACCAA	-5.6	<u>RDN37-1</u>	4963	4982	snR66 (C)	2	20
285819-1_1	TATAGGGGCGAAAGACTACTATCGAA <b>ATGAAGA</b> TAAAAATT	-4	<u>RDN37-1</u>	3454	3471	snR60 (C')	46	70
117007-1_2	CAAGTGGGAGCTTCGGCGCCACTATCGAA <b>ATGAAGA</b> TAAAA	-5.7	<u>RDN37-1</u>	5012	5031	snR60 (C')	47	67
	<b>Sequence of other hybrids with snoRNA</b>							
97363-1_3	TAAAGGGTTGCGCTTCTTTCTGCGCAACCCA <b>TTGATCTTG</b>	-18.4	<u>TEF1/2</u>	1286	1304	snR45	100	123
<b>legend</b>	target <b>guide</b> <b>box C, D, C', or D'</b> Kre33- <i>Xlink</i> site <b>snR4-target</b>							

‡ Reads are from the main data set (data-set II) and from a library with ~5-fold less coverage (-*dataset I*)

\* predicted folding energy (kcal/mol) of the hybrid

## S2 Table. Strains, plasmids and oligonucleotides

Name	Description	Source
CEN.PK1303-5A	MAT a, ura3-52;his3-Δ1; leu2-3,112; trp1-289; MAL2-8 <sup>c</sup> , SUC2, snr45(1,172)::loxP-kanMX4-loxP	This study
CEN.PK1351-5B	MAT a, ura3-52;his3-Δ1; leu2-3,112; trp1-289; MAL2-8 <sup>c</sup> , SUC2, snr4(-50,186)::loxP-kanMX4-loxP	This study
CEN.WA4-9C	MAT α, ura3-52;his3-Δ1; leu2-3,112; trp1-289; MAL2-8 <sup>c</sup> , SUC2, snr4(-50,186)::loxP-kanMX4-loxP; snr45(1,172)::loxP-kanMX4-loxP	This study
BY4741	MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0	Euroscarf
SC1403	MATa; ura3-52; leu2-3,112; KRE33::TAP-KIURA3	Euroscarf
SC1541	MATa; ura3-52; leu2-3,112; NOP58::TAP-KIURA3	Euroscarf
SC1110	MATa; ura3-52; leu2-3,112; GAR1::TAP-KIURA3	Euroscarf
BY.WA5-2B	MAT α, ura3Δ0; leu2Δ0; his3Δ1; KRE33::kanMX4 + pSH35	(Sharma et al., 2015)
BY.WA6-1B	MAT α, ura3Δ0; leu2Δ0; his3Δ1; KRE33::kanMX4 + pSH35a	(Sharma et al., 2015)
BY.WA8-1B	MAT α, ura3Δ0; leu2Δ0; his3Δ1; KRE33::kanMX4 + pSH35c	(Sharma et al., 2015)
BY.WA9-1C	MAT A, ura3Δ0; leu2Δ0; his3Δ1; KRE33::kanMX4 + pSH35d	(Sharma et al., 2015)
pJN23	A derivative pRS426 plasmid carrying <i>SNR4</i>	This study
pJN23a	A derivative pRS426 plasmid carrying snr4(1,8)::TTGATTAT	This study
pJN23b	A derivative pRS426 plasmid carrying snr4(142,150)::GGTGGTGGG	This study
pJN23c	A derivative pRS426 plasmid carrying snr4(93,102)::GGTTGATTCT	This study
pJN23d	A derivative pRS426 plasmid carrying snr4(56,66)::TTAATGAGGT	This study
pJN23e	A derivative pRS426 plasmid carrying snr4(136,142)::AAGGGGG	This study
pJN23f	A derivative pRS426 plasmid carrying snr4(130,150)::ATCTTGTTAC	This study
pJN23g	A derivative pRS426 plasmid carrying snr4(93,102)::AGGTTT; (130,150)::ATCTTGTTAC	This study
pJN23h	A derivative pRS426 plasmid carrying snr4(56,66)::TCTTCTCG; (93,102)::AGGTTT; (130-150)::ATCTTGTTAC	This study
pJN28	A derivative pRS426 plasmid carrying <i>SNR45</i>	This study
pJN28a	A derivative pRS426 plasmid carrying snr45(3,10)::GACCTCC	This study
pJN28b	A derivative pRS426 plasmid carrying snr45(77,84)::GTAACCAG	This study
pJN28c	A derivative pRS426 plasmid carrying snr45(61,68)::AGGACGGC	This study
pJN28d	A derivative pRS426 plasmid carrying snr45(117,127)::TTGGAACAATG	This study
pJN28e	A derivative pRS426 plasmid carrying snr45(144,150)::Δ	This study
pJN28f	A derivative pRS426 plasmid carrying snr45(110,118)::Δ	This study
pJN28g	A derivative pRS426 plasmid carrying snr45(79,84)::TTGTGTAAGA; (118,127)::CCGTGGCCTTTTTCACCACCT	This study
pJN28h	A derivative pRS426 plasmid carrying snr45(61,68)::AAATAACTCTA; (79,84)::TTGTGTAAGA; (118,127)::CCGTGGCCTTTTTCACCACCT	This study
pSH35	A derivative pPK468 plasmid carrying Kre33-7xHis	(Sharma et al., 2015)
pSH35a	A derivative pPK468 plasmid carrying kre33-K289A	(Sharma et al., 2015)
pSH35c	A derivative pPK468 plasmid carrying kre33-H545A	(Sharma et al., 2015)
pSH35d	A derivative pPK468 plasmid carrying kre33-R637A	(Sharma et al., 2015)
<b>Name</b>	<b>Sequence 5' → 3'</b>	
pJN23-A1	GAATTGGGTACCGGGCCCCCCTCGAGGTCGACGGTATCGATAAGAAAGCTTTGTAATATG	
pJN23-A2	GAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCGGTATAATAGAAGAATTTAG	

pJN28-A1	GAATTGGGTACCGGGCCCCCCTCGAGGTGACGGTATCGATAGATTAA CTTGATAATGGAGTTG	
pJN28-A2	GAGCTCCACCGCGGTGGCGGCCGCTCTAGAAGTGTGGATCGAGACCAA TCGACAGTCTTC	
snR4-nb	CTAGAGTTATTTTAAAACAC	
snR45-nb	GCGCAGGAACCGCTATCTCC	
snR4-Mod- probe	TATTAATAGTTAAAGCACCG	
snR45-Mod- probe	ATTTTATAAAAAGCGTCCTTG	
pJN23a-sp-FP	CATTTTTTTATTTCAAATCCCCATCTTGATAATCAGTCGATGAGGATAGGT TATAG	
pJN23a-sp-RP	CTATAACCTATCCTCATCGACTGATTATCAAGATGGGGATTTTGAATAAA AAAATG	
pJN23b-sp-F2	GATTGTGCCGTGGCCTTTTGGTGGTGGTTATAGCGGTGCTTTAACTATT AATAACTG	
pJN23b-sp-R2	ATTAATAGTTAAAGCACCGCTATAACCCACCACCAAAGGCCACGGCACA ATCCACATC	
snR4-cF	GGCGTTTTTCACAAAGTGTTGATTCTTGCTTTTCTGGGTCGATGTGG	
snR4-cR	CGACCCAGGAAAGCAAGAATCAACCACTTTGTGAAAAACGCCCC	
pJN23d-sp-F2	GTGTAACATCTGTGTTTTATTTAATGAGGTGTTATCCGGGGCGTTTTTCAC AAAG	
pJN23d-sp-R2	GTGAAAAACGCCCGGATAACACCTCATTAATAAAACACAGATGTTACAC ATG	
snR4-eF	GGGTCGATGTGGATTGTGCCGTGGAAGGGGGCACCCACCTTTATAGCGGT GCTTTAAC	
snR4-eR	GCACCGCTATAAAGGTGGTGCCCCCTTCCACGGCACAATCCACATCGAC CCAGGAAAGC	
snR4-fF	GGATTGTGATCTTGTTACTTATAGCGGTGCTTTAACTATTAATAAC	
snR4-fR	CGCTATAAGTAACAAGATCACAATCCACATCGACCCAGG	
snR4-gF2	CACAAAGTAGGTTTTGCTTTCTGGGTCGATGTG	
snR4-gR2	GCAAAACCTACTTTGTGAAAAACGCCCG	
pJN23h-sp-F3	CATCTGTGTTTTATCTTCTCGTTATCCGGGGCGTTTTTCACAAAGTAGGT TTTGC	
pJN23h-sp-R3	CCCGGATAACCGAGAAGATAAAACACAGATGTTACACATGAACTATAACC	
pJN28a-sp-FP	GGTTTCATTTAAAGAAACCAATCTGGAAGGAAGTTTTTAAAAGAATACGAT G	
pJN28a-sp-RP	CGTATTCTTTTAAAACCTCCTTCCAGATTGGTTTCTTTAAATGAAACCTCC	
pJN28c-sp-F2	GCGTTTCAAATCGAACAATAGGACGGCGAGCGATCTGAGGTTTTAATGGA G	
pJN28c-sp-R2	CATTA AACCTCAGATCGCTCGCCGTCCTATTGTTGATTTGAAACGCAAA TAATATC	
pJN28e-sp-F2	CTTGTTACATTCTTAAGAATGACATTTTATAAAATTCTGATTCTTTTTCTTT C	
pJN28e-sp-R2	GAAAGAAAAAGAATCAGAATTTTATAAAATGTCATTCTTAAGAATGTAACA AGATC	
snR45-1	CTTGGAAGGTCATTGGTTTCTTTAAATGAAACCTCCAAAGAAAATCTAACG CGTCTTGGTAAAATATATGTACCGAGTGTTCGCTTTTTATACC	
snR45-2	GGAGGTTTCATTTAAAGAAACCAATGACCTTCCAAGTTTTTAAAGAATAC GATGATATTATTTGCGTTTCAAATCGAACAATTCTTCTCGGAGCGATCTGA GG	
snR45-2b	GGAGGTTTCATTTAAAGAAACCAATGACCTTCCAAGTTTTTAAAGAATAC GATGATATTATTTGCGTTTCAAATCGAACAATTCTTCTCGGAGCGATC	

snR45-3b	GTCCTTGTCAATTCTTAAGAATGTAACAAGATCAATGGGTTGCGCAGGAAC CGCTATCTCCACTTACTGGTTACGATCGCTCCGAGAAGAATTGTTTCGATTTG AAACGC	
snR45-4b	CCCATTGATCTTGTACATTCTTAAGAATGACAAGGACGCTTTTATAAAATT CTGATTCTTTTTCTTTTCATTCTTTTGTGTTTTGACTTGGGAG	
snR45-3d	CCTTGTCAATTCTTAAGAATCATTGTTCCAAAATGGGTTGCGCAGGAACCG CTATCTCCATTAACCTCAGATCGCTCCGAGAAGAATTGTTTCGATTTG	
snR45-4d	GCAACCCATTTTGGAAACAATGATTCTTAAGAATGACAAGGACGCTTTTATA AAATTCTGATTCTTTTTCTTTTCATTCTTTTGTGTTTTGACTTGGG	
snR45-3f	GCGTCCTTGTCAATTCTTAAGAATGTAACAAGATGCGCAGGAACCGCTATC TCCATTAACCTCAGATCGCTCCGAGAAGAATTGTTTCGATTTG	
snR45-4f	GGTTCTGCGCATCTTGTACATTCTTAAGAATGACAAGGACGCTTTTATA AAATTCTGATTCTTTTTCTTTTCATTCTTTTGTGTTTTGACTTGGG	
snR45-3g	GAATAGGTGGTGAAAAAGGCCACGGCAATGGGTTGCGCAGGAACCGCTA TCTCCATTATCTTACACAACAGATCGCTCCGAGAAGAATTGTTTCGATTTG	
snR45-2h	CATTAAAGAAACCAATGACCTTCCAAGTTTTTAAAGAATACGATGATATT ATTTGCGTTTTCAAATCGAACAATAAATAACTCTAGAGCGATCTGTTG	
snR45-3h	GAATAGGTGGTGAAAAAGGCCACGGCAATGGGTTGCGCAGGAACCGCTA TCTCCATTATCTTACACAACAGATCGCTCTAGAGTTATTTATTGTTTCG	
snR45-4h	CCCATTGCCGTGGCCTTTTTCCACCACCTATTCTTAAGAATGACAAGGACG CTTTTATAAAATTCTGATTCTTTTTCTTTTCATTCTTTTGTGTTTTGAC	
snR45-5	CTAGTCTACAATTTAAGAACATATAAAAAGCTACTTGTGTACAGATGAGAT GACTACTCCAAGTCAAAAACAACAAAAGAATGAAAGAAAAAAG	
probe 33	CGCTGCTCACCAATGG	
probe 20	TGAGAAGGAAATGACGCT	
probe 9	CCCACCTATTCCTCTTGC	
probe 5	GATTGCTCGAATGCCCAAAG	
probe 4	CGGTTTTAATTGCCTA	
PE_40_25S	TATCCTGAGGGAAACTTCGG	
PE_34_18S	TAAGGTCTCGTTCGTTATCGC	