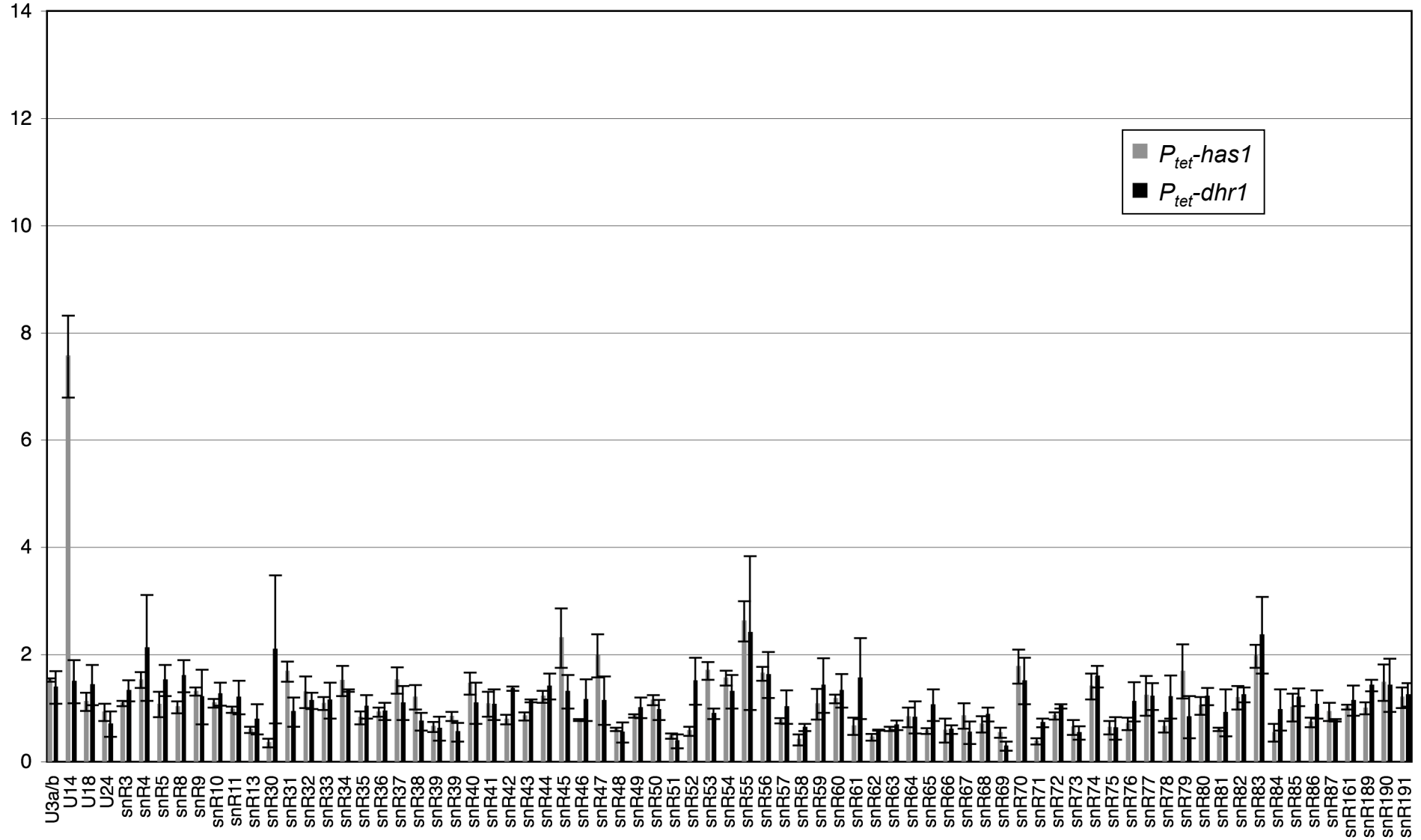
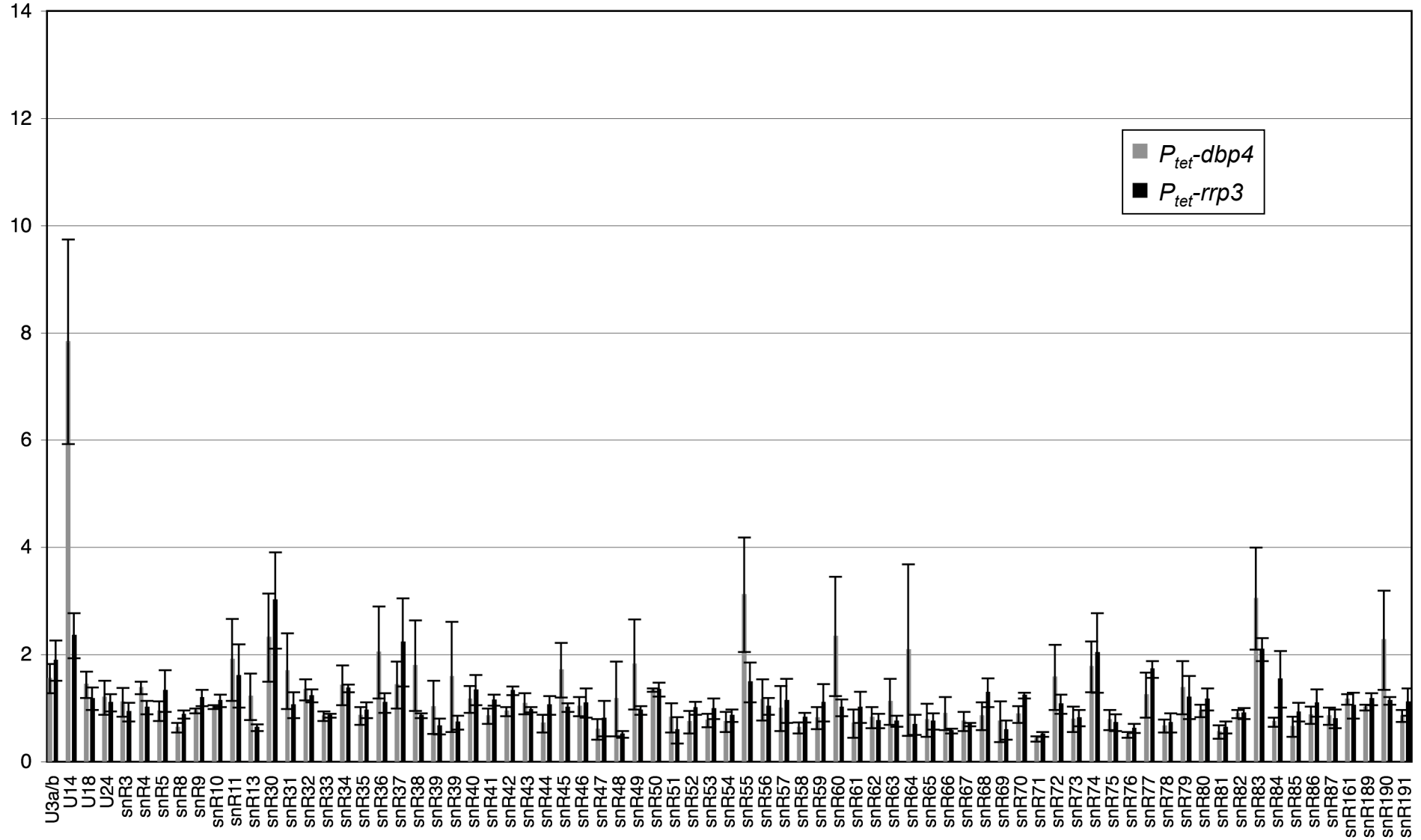
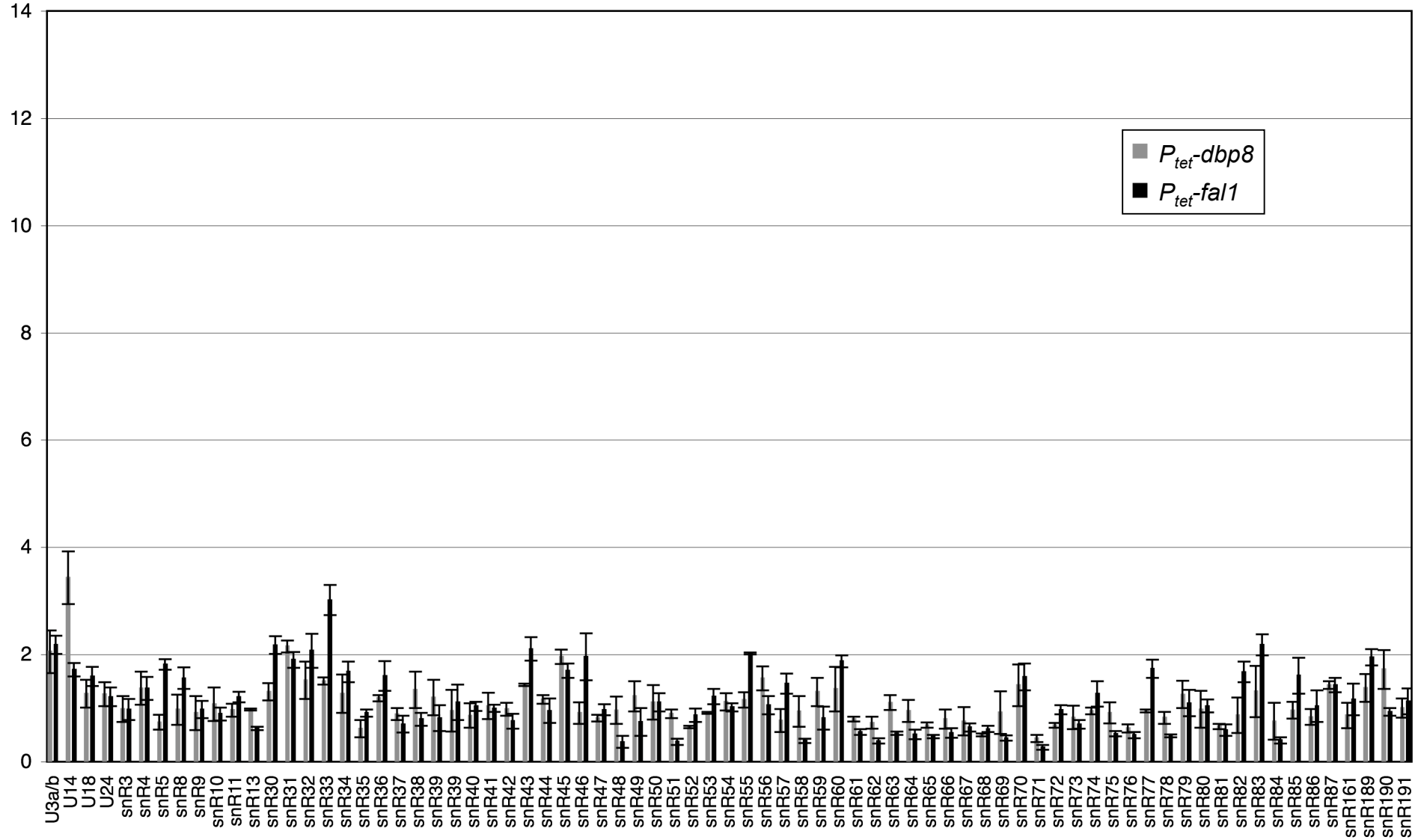


Bohnsack et al., suppl. Figure 1: Data were analysed and are presented as described for Figure 2



Bohnsack et al., suppl. Figure 1: Data were analysed and are presented as described for Figure 2



Bohnsack et al., suppl. Figure 1: Data were analysed and are presented as described for Figure 2

Bohnsack *et al*., supplementary Figure S2

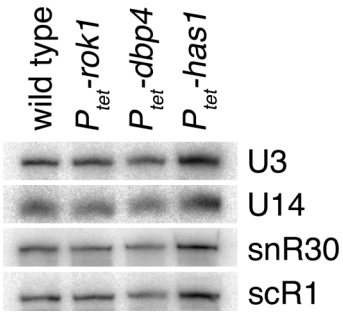


Figure S2: snoRNA expression levels are not affected by helicase depletion. RNA was extracted from soluble cell lysates and the levels of different snoRNAs and scR1 as loading control were analyzed for the wild type and cells after helicase depletion. The depletion of the helicases does not change the total cellular levels of the snoRNAs analyzed.

Bohnsack *et al*., supplementary Figure S3

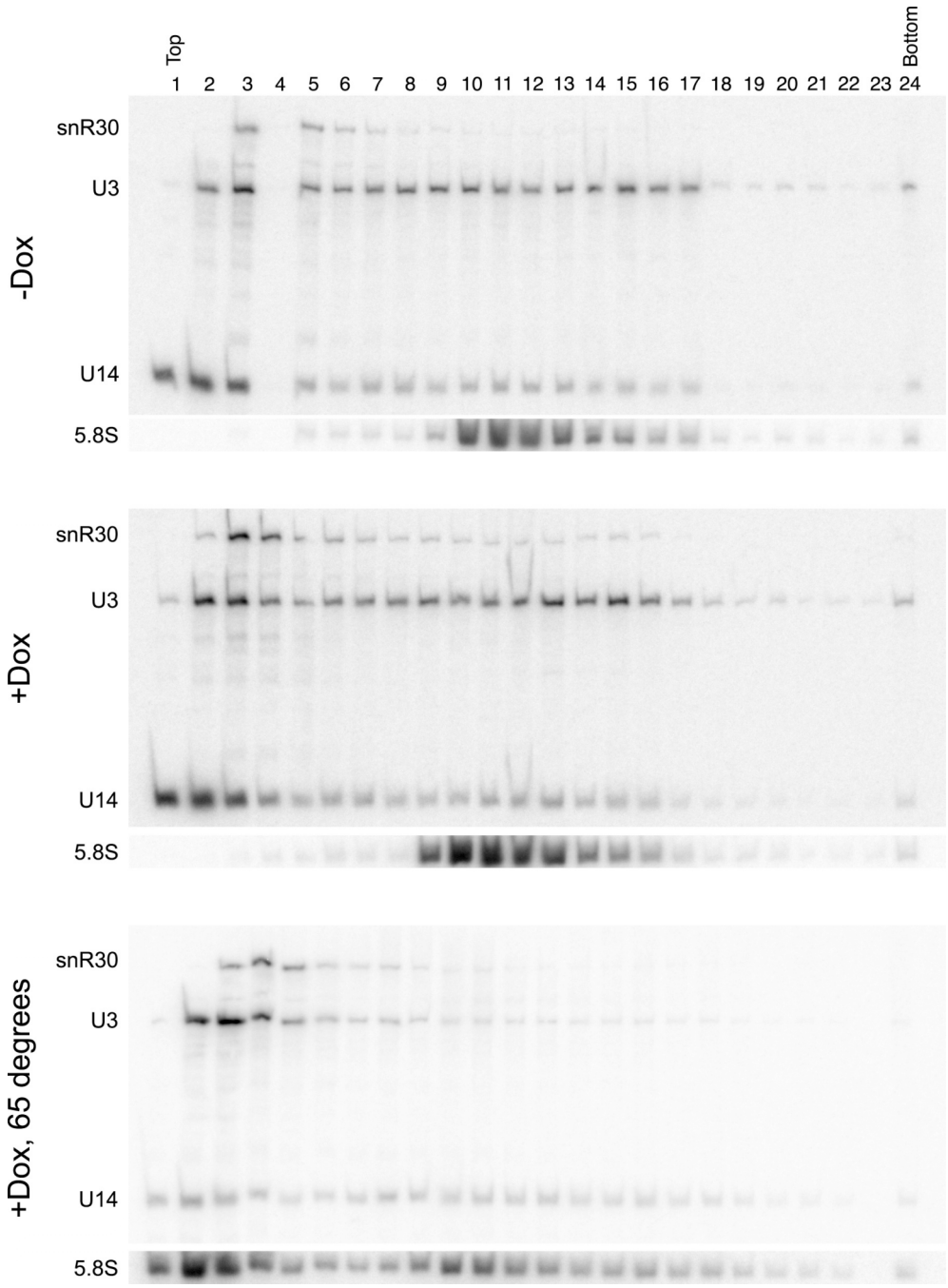


Figure S3: Depletion of Rok1 leads to increased basepairing of snR30 with ribosomal RNA. Cell lysates from the P_{tet}-*rok1* strain grown in the presence (+Dox) or absence of doxycycline (-Dox) were deproteinized by treatment with proteinase K, and loaded onto sucrose gradients. One sample (+Dox, 65 degrees) was heated to 65°C for disruption of base pairing, prior to loading of the gradient. The distributions of the indicated snoRNAs and 5.8S rRNA were detected by Northern blotting.

Bohnsack *et al*., supplementary Methods

Quantitative PCR and data analysis

The preparation of RNA isolated from pools of gradient fractions was performed as shown in Figure 1A and essentially as described in Ro et al. (2006). After polyadenylation using *E. coli* poly(A) polymerase (New England Biolabs), an oligo(dT) linker was annealed and reverse transcribed using Superscript III (Invitrogen), followed by RNA digestion with RNase A and RNase H (New England Biolabs). qPCR was performed using the general reverse primer and snoRNA specific forward primers (see supplementary Tables S2). Data from depletion strains were normalized to the YMB279 sample processed in parallel as "wild type" and qPCRs were performed as described (Houseley et al., 2007). The values of the depletion samples were normalized to the "wild type" and ratios of pre-ribosomal versus unbound levels for each snoRNA were calculated using the formula $2^{-(Ct(WT) - Ct(mutant)\ pool1)}/2^{-(Ct(WT) - Ct(mutant)\ pool2)}$ from approximately 30,000 qPCR reactions. Samples were found to have at least 10^9-fold higher signal than controls processed without reverse transcriptase. Original qPCR data will be supplied upon request.

References:

Houseley J, Kotovic K, El Hage A, Tollervey D (2007) Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. *EMBO J* **26**: 4996-5006.

Ro S, Park C, Jin J, Sanders KM, Yan W (2006) A PCR-based method for the detection and quantification of small RNAs. *Biochem Biophys Res Commun* **351**: 756–763.

Bohnsack *et al*., supplementary Tables

Yeast strains used in this study

Strain	Genotype	Reference
BY4741	MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0	Brachmann et al., 1998
YMK119	MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; tTA::lys2; tetR'-URA3 K.L::leu2	Kos and Tollervey, in prep
YMB137	YMK119 with P_{tet}-*3HA-rrp3* (NatMX6)	This study
YMB138	YMK119 with P_{tet}-*3HA-dhr2* (NatMX6)	This study
YMB144	YMK119 with P_{tet}-*3HA-dhr1* (NatMX6)	This study
YMB146	YMK119 with P_{tet}-*3HA-rok1* (NatMX6)	This study
YMB152	YMK119 with P_{tet}-*3HA-dbp8* (NatMX6)	This study
YMB155	YMK119 with P_{tet}-*3HA-dbp4* (NatMX6)	This study
YMB322	YMK119 with P_{GAL}-*3HA-fal1* (KanMX9)	This study
YMB323	YMK119 with P_{tet}-*3HA-has1* (NatMX6)	This study
YMB374	YMK119 with P_{tet}-*3HA-rok1* (NatMX6); P_{tet}-*3HA-dhr1* (HygMX6)	This study
YMB348	YMB146 with pRS415	This study
YMB350	YMB146 with pRS415-Rok1-WT	This study
YMB351	YMB146 with pRS415-Rok1-ΔSAT	This study
YMB352	YMB146 with pRS415-Rok1-K172L	This study

Plasmids used in this study

Plasmid	Description
pRS415	Centromeric yeast shuttle vector with LEU2 as auxotrophic marker (Stratagene)
pRS415-Rok1-WT	pRS415 with wild type *ROK1* under native promotor
pRS415-Rok1-K172L	K172L mutation in Q-domain
pRS415-Rok1-ΔSAT	SAT domain in Rok1 mutated to AAA (S312A-T314A)

Oligonucleotides for qantitative PCR

U3a/b	GGTACAAATGGCAGTCTGAC
U14	TTCTTTAGAGACCTTCCTAGG
U18	TGACAAAAGAGATGTGGTTGAC
U24	GAGACATACCAATTATCACCAAG
snR3	GTTTTGATTAGCTGAATGAGAC
snR4	CCTTTATAGCGGTGCTTTAAC
snR5	ATTGGTTCGCTCTAGGTGTAC
snR8	ATCGGTACTGCGCGAGTGAG
snR9	ACCTATCATTAGTCCTTCAGAC
snR10	TCTGTCGTCTGTTTTTTAGCAG
snR11	AAGAAAGTGAGTGGATCTTCCC
snR13	GTGTGGAAAAACTCAAGCTAC
snR30	CATTTGGGTAAAACCATACTG
snR31	TAAACACCTGATACAGTTGGTC
snR32	GAAATGAGATATTGGGAATCAG
snR33	AATTGATATAGAAGTGTGTGGAC
snR34	TGTCTCAAACGAGGCGATAG
snR35	CAAGGGCTGGTAGGACAGAC
snR36	GCTATTTTTATCTCACGGTATC
snR37	CCTAAGCGACTCTTCTTCATG
snR38	CTGAATGGGTAATAATAGGTAACC
snR39	GCTGTCGTAACTTATCACCATC
snR39b	ATGTTGTCAACTTAAATTACACC
snR40	AAGTTTCAGCCTTGTATGAG
snR41	CCTTTTTCGTTAAGTTTCAG
snR42	GTTAAGCGACCCATGAAATG
snR43	AATCTCTGGGTTGTTTAGATG
snR44	TCCATTACCGTTTACTTTTCC
snR45	GCAACCCATTGATCTTGTTAC
snR46	ATAAAGTTGTGCTATTTCCATG
snR47	ACAATAGCTTTTTAACACTG

snR48	ATGTTAGGATGTGAAGTTTAAGTAC
snR49	AAGATTTATCTCTTTTGTCCATC
snR50	CTTTACAGAACCGCTACACTG
snR51	TAGATTGGTCTCTTTAACGAAGG
snR52	GACATTAGCGTGAACAATCTC
snR53	TGATTAAAATTGTTGTTTACGC
snR54	CGATCTTGTAGAGAACTTTTACTC
snR55	CACAATCGTCTTTTTTTTATCC
snR56	ACACAGACCTGTACTGAACTTTTC
snR57	ATTTTTCTGAGGAAGTATATGC
snR58	CTCCTATGGAAGAGAAACTC
snR59	AATCACCATCTTTCGGCTGAC
snR60	CAACTGATTGAACATACTATCG
snR61	AAGATAACCAATTTTACCAAAG
snR62	ATGGAAGATATACGACTATCAAC
snR63	GAGTCTTTTAATGTGATGAGTGG
snR64	TAAAGCCCAGTTTTTAGTAGAG
snR65	AACTTTATGATTACAGTGTTTTC
snR66	GAGATTGCTTTTTTATTACTGAC
snR67	GATTTTACAAACAACAAACACTG
snR68	CGAGGAAATTGACTCTTAACAG
snR69	AAAGGGAGAAGATTTTTTTGTC
snR70	TGATTGGTCACAAGACATCTG
snR71	ATTTCCTATCCAACATTCATC
snR72	GAGAACATCAATGAAGAAAACG
snR73	TGTGACAGGGCGTGGTACTG
snR74	GAAACAAATTACTCAAATAGACAAG
snR75	AACTATTAAAATTACCATTCATGC
snR76	TCCTTTCAAATGAGTGACAATG
snR77	GAGTATATGTTGATACGTTTTTGC
snR78	AGTTTCTGAATCTTTTGTGATTAG
snR79	CAAGACTACAACGGTATCTG
snR80	GGTTATATTAGTCCATTTCATAGC
snR81	ACGCTTTTTCACATCTTCTTG

snR82	TATAGTTTGATAGTAGATGGGCG
snR83	CCCGATTTGTATTTTATTTTTC
snR84	AGGAACATGACTCAAAGAGACAC
snR85	TACCATATAGAGGTGTCAAGTACAC
snR86	AGAATGTAGTTTCATACCCG
snR87	TGTTCTATATGGGTGATTAGCG
snR161	TCAGGCTGTATTTCATAACACTAC
snR189	ACTTTCAAGTACTTCACACG
snR190	GAAAAGATGTTGCTTCTGTGAC
snR191	TATGTTCGTAGTAAAGATCCTCAC
general reverse prim.	CGAATTCTAGAGCTCGAGGCAGG

Oligonucleotide used for reverse transcription

Oligo(dT) linker	CGAATTCTAGAGCTCGAGGCAGGCGACATGGCTGGCTAGTTA AGCTTGGTACCGAGCTCGGATCCACTAGTCCTTTTTTTTTTTTTT TTTTTTTTTTTTVN

Oligonucleotides used as probes for Northern blotting

U3	UUAUGGGACUUGUU
U14	TCACTCAGACATCCTAGG
snR30	CTAAGTTAAACTCGTCAACG
5.8S	GCGTTGTTCATCGATGC
scR1	ATCCCGGCCGCCTCCATCAC