

Supplementary information for

Developmental changes of rRNA ribose methylations in the mouse

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The supplementary information contains:

Table S1

Table S2

Table S3

Supplementary Data 1

Supplementary Data 2

Figure S1

Figure S2

Figure S3

Figure S4

Figure S5

Supplementary references

Table S1. List of changes made to the curated mouse rDNA sequences based on reads obtained from RiboMeth-seq analyses. GenBank accession numbers for the original rDNA sequences are listed in the table and nucleotide numbers correspond to this. **Supplementary Data 1** contains a FASTA file with the corrected rDNA sequences.

SSU – GenBank accession: X00686.1

T inserted after T287	A inserted after G1228	G1561A	
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LSU – GenBank accession: NR_003279.1

C inserted after C251	C497Δ	C586Δ	C inserted after C665
A inserted after C829	T1354C	G inserted after G1887	T inserted after T1991
G2019C	C2020G	CGT inserted after G2322	G2365Δ
T2368C	G inserted after G2931	G2950Δ	G inserted after G2967
G3150Δ	T3151Δ	C3233T	C3235Δ
C3753Δ	G3754Δ	A3755G	G3756A

5.8S – GenBank accession: K01367.1

G23C			
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5S – GenBank accession: M31319.1

G38C	G43C	T69Δ	
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Table S2. List of oligonucleotides used in the study.

Northern blotting analysis (rRNA and small RNA detection)	
Purpose	Sequence
SNORD22 (mouse/human)	ATCCCTCAGACAGTTCCTTCTG
SNORD44 (mouse)	TCTTCAGGTAGTCAGTATTTGTTCCG
SNORD44 (human)	TTAGAGCTAATTAAGACCTTCATG
SNORD47 (mouse)	AACCTCAGAAATAAAATGGAACGGTTT
SNORD74 (mouse)	CAGTTGTCCCTACCAACATAGCCTTC
SNORD75 (mouse)	CAGAAATCCCTTCTGTCCACTACTCT
SNORD76 (mouse)	AGCTGTCAAGAGTAGCAAATATGA
SNORD78 (mouse/human)	TCAGACATTTGATCAACATCATTACA
SNORD79 (mouse)	TCAGAGAGATTCCCATCTGTTTAA
SNORD79 (human)	AGAGATTCCCATCTGCTTTAATTTT
SNORD80 (mouse/human)	AGTCTGCTGAACTATGTTATCATCAT
SNORD81 (mouse)	AGTAATCAGTGAGAGAGTTCAAG
SNORD126 (rat/mouse)	TGCTCAGCACATGTGTGTAATCA
7SK (mouse/human)	GTGTCTGGAGTCTTGGGAAGC
ITS1 – probe a (human)	TGGGTGTGCGGAGGGAAGC
ITS2 – probe b (human)	ACGCCGCCGGTCTGCGCTTA
SSU rRNA (human)	CCAGACAAATCGCTCCACCAACTAAG
LSU rRNA (human)	GCTCCCGTCCACTCTCGAC
Primer extension (detection of rRNA ribose methylations)	
LSU-Gm4593 (SNORD78)	GTCATAATCCCACAG
LSU-Am1310 (SNORD126)	TTCGGACGAGCCCTGA
CRISPR/Cas9-mediated deletion of the human SNORD78 gene	
sgRNA1	CCTTGAGCATGTTTGGGAACG
sgRNA2	TTTGTCTACATGCTCATTTT
PCR (screening for CRISPR/Cas9-mediated deletion at the human SNORD78 gene)	
Del_SNORD78_fd	CCTTGAGCATGTCTGGAACAGTG
Del_SNORD78_rv	CTTACCCAAGCAAGTCATCCATG
RT-qPCR and RT-PCR (mouse Gas5 detection)	
Gas5_ex1_fd	CGGAGCTGTGCGGCAT
Gas5_ex3_fd	TGTGGCAAAGGAGGATGAAGG
Gas5_ex6_fd	TGGAGTTTGAGGCTGGATAGAC
Gas5_ex3_rv	TCGTAAGCCTTCATCCTCCTTT
Gas5_ex6_rv	TTCAAAGTGTCTATCCAGCCTCA
Gas5_ex7_rv	GCAAGCCAGCCAAATGAACA
Gas5_ex8_rv	TGTTTGCAGTGCCTTCACTTG
GAPDH_fd	TGCACCACCAACTGCTTAG
GAPDH_rv	GGATGCAGGGATGATGTTT
RT-qPCR (human Gas5 detection)	
Gas5_fd	CTTCTGGGCTCAAGTGATCC
Gas5_rv	TTGTGCCATGAGACTCCATCAG
GAPDH_rv	GAGTCCTTCCACGATACCAAAG
GAPDH_fd	TGGGTGTGAACCATGAGAAGTA

Table S3. List of methylated sites and their relative position in the rRNA sequences of human and mouse, respectively. Methylated positions in human rRNA are according to the sequences in the snoRNA-LMBE-database (1). Mouse positions are according to the corrected sequences from this study (**Supplementary Data 1** and **Table S1**).

SSU

Human	Mouse	Human	Mouse	Human	Mouse	Human	Mouse	Human	Mouse
A27	A27	U354	U356	C517	C519	C797	C799	A1383	A1385
A99	A99	U428	U430	A576	A578	U799	U801	C1391	C1393
U116	U116	G436	G438	A590	A592	G867	G869	U1442	U1444
U121	U121	C462	C464	G601	G603	A1031	A1033	G1447	G1449
A159	A159	A468	A470	U627	U629	C1272	C1274	G1490	G1492
A166	A166	A484	A486	G644	G646	U1288	U1290	A1678	A1680
U172	U172	G509	G511	A668	A670	U1326	U1328	C1703	C1705
C174	C174	A512	A514	G683	G685	G1328	G1330	U1804	U1806

LSU

Human	Mouse	Human	Mouse	Human	Mouse	Human	Mouse	Human	Mouse
A389	A398	G2351	G2122	C3680	C3360	C3866	C3546	U4468	U4151
A391	A400	C2352	C2123	A3697	A3377	G3878	G3558	G4469	G4152
G1303	G1131	A2350	A2121	G3606	G3286	C3848	C3528	G4464	G4147
A1310	A1138*	A2388	A2159	A3703	A3383	U3904	U3584	A4493	A4176
A1313	A1141	U2402	U2173	G3723	G3403	G3923	G3603	C4506	C4189
C1327	C1155	C2409	C2180	A3739	A3419	G4020	G3695	A4541	A4224
G1509	G1336	G2411	G2182	A3764	A3444	C4032	C3707	A4560	A4243
A1511	A1338	A2774	A2545	G3771	G3451	G4166	G3849	G4588	G4271
A1521	A1348	C2791	C2562	C3787	C3467	U4197	U3880	U4590	U4273
G1612	G1439	A2802	A2573	U3797	U3477	G4198	G3881	G4593	G4276
G1747	G1563	C2811	C2582	A3804	A3484	U4276	U3959	G4607	G4290
A1858	A1674	U2824	U2595	A3809	A3489	G4340	G4023		
C1868	C1684	C2848	C2619	C3820	C3500	G4362	G4045		
C2338	C2109	G2863	G2634	A3846	A3526	C4426	C4109		

5.8S

Human	Mouse	Human	Mouse
U14	U14	G75	G75

*No evidence for methylation at LSU-A1310 in mouse, but the site is detected in human and rat.

Supplementary Data 1. Mouse rDNA sequence. A FASTA file with the mouse rDNA reference sequence used for mapping of sequencing reads is deposited at NCBI Gene Expression Omnibus (GEO) and accessible through GSE128947.

Supplementary Data 2. RiboMeth-seq scores and statistics. An excel sheet with raw read-end data, calculated RiboMeth-seq scores, and information on 2'-O-Me sites that have been subjected to barcode correction (2) is deposited at NCBI Gene Expression Omnibus (GEO) and accessible through GSE128947.

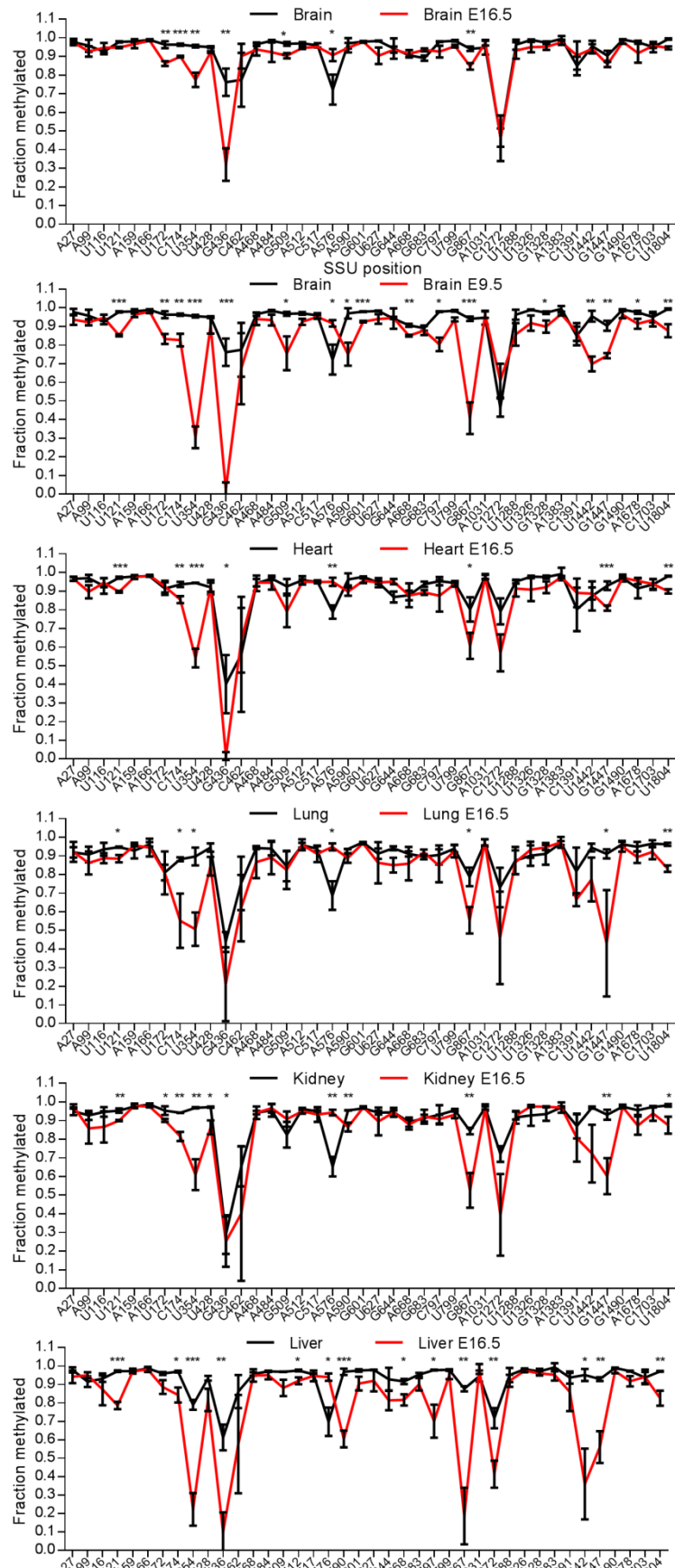


Figure S1. Stoichiometry of methylation at all 2'-O-Me sites in SSU rRNA in whole cell RNA isolated from adult tissues and developmental stages. Brain E9.5 corresponds to the whole head from stage E9.5.

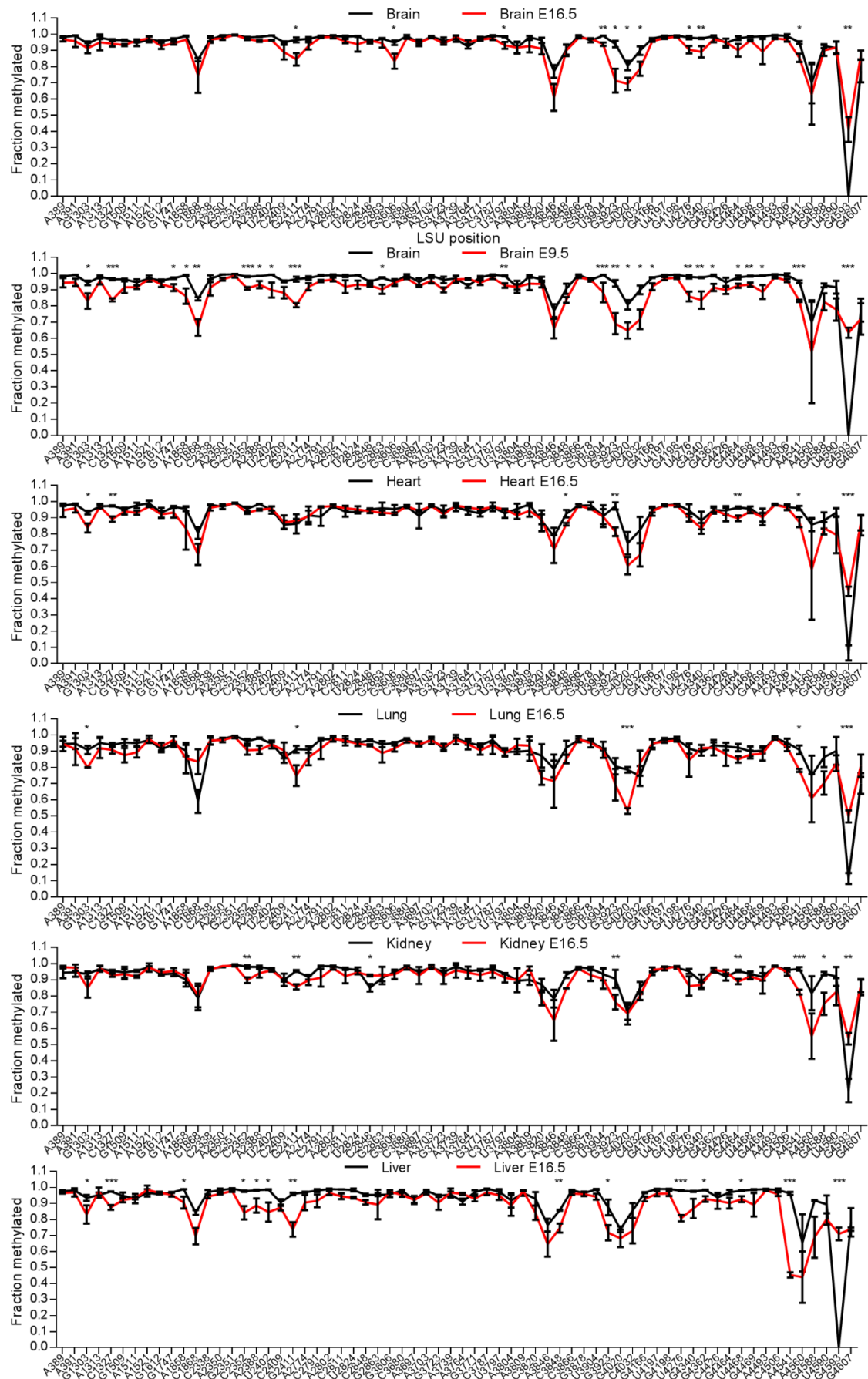


Figure S2. Stoichiometry of methylation at all 2'-O-Me sites in LSU rRNA in whole cell RNA isolated from adult tissues and developmental stages. Brain E9.5 corresponds to the whole head from stage E9.5.

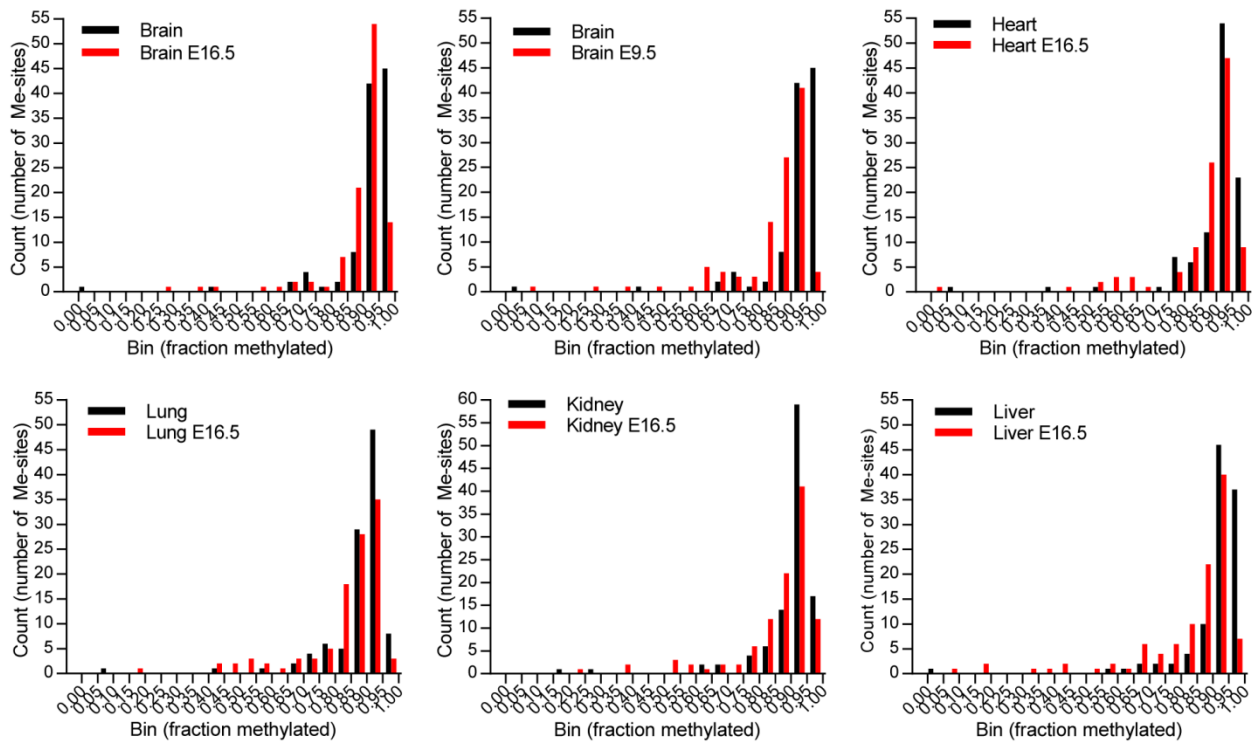


Figure S3. Bin diagrams of fraction methylated for all 2'-O-Me sites in 28S, 18S, and 5.8S rRNA from all adult and developmental tissues. Brain E9.5 corresponds to the whole head from stage E9.5.

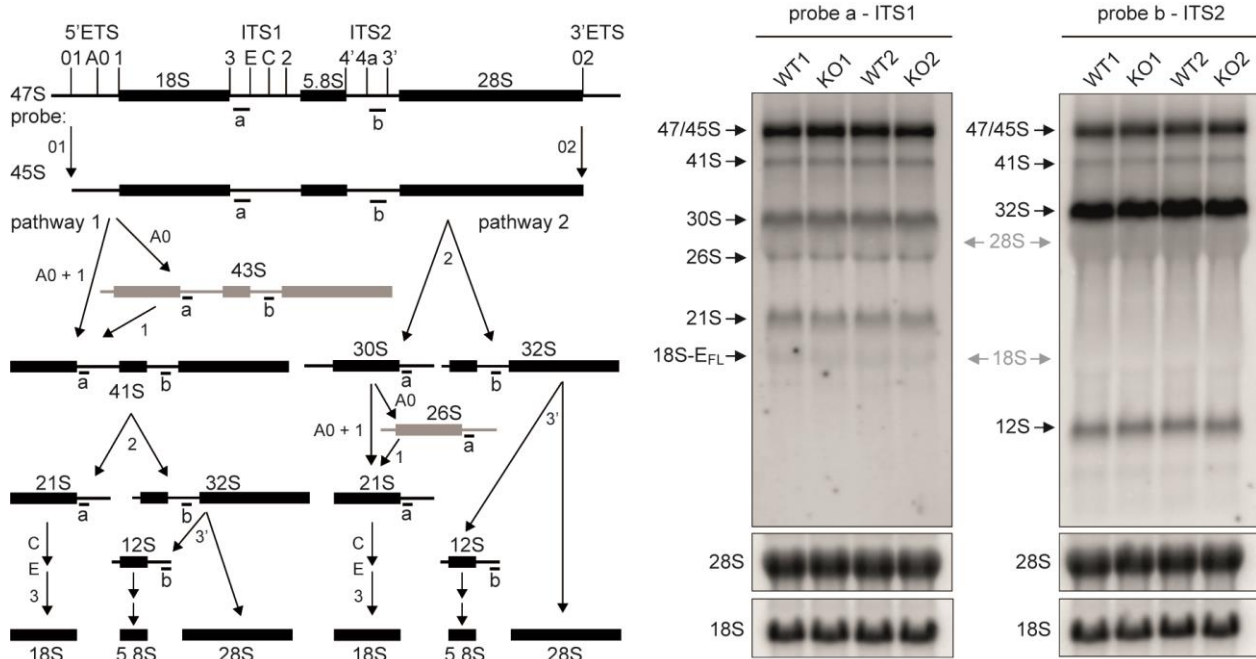


Figure S4. Pre-rRNA processing in WT HEK293T and SNORD78 knockout cells. To the left is shown an illustration of the human pre-rRNA transcript with annotated processing sites and a simplified diagram of the two main processing pathways, and with short lived intermediates in grey. The hybridization sites of probes a and b targeting ITS1 and ITS2, respectively, are indicated on the processing map. The right part of the figure shows northern blotting analyses of parallel gel runs of whole cell RNA samples from two WT HEK293T and two SNORD78 knockout cell cultures hybridized with either probe a or probe b. Black arrows indicate processing intermediates, while grey arrows indicate the migration of 28S and 18S rRNA inferred from re-hybridizing the membranes with probes targeting these rRNA species.



Figure S5. Sequences of SNORD78 in WT and SNORD78 knockout cell lines. PCR products from the KO1 cell line was cloned and 5 individual clones were sequenced. All gave the same sequence with an insertion of 98 nucleotides between the C box and the antisense element upstream of the D box. 3 individual clones of PCR products from the KO2 cell line showed the same sequence with a 42 nucleotide deletion spanning the C box. The antisense element base pairing with rRNA guiding methylation at LSU-G4953 is shown in bold italic.

Supplementary references

1. Lestrade L, Weber MJ. snoRNA-LBME-db, a comprehensive database of human H/ACA and C/D box snoRNAs. *Nucleic acids research*. 2006;34(Database issue):D158-62.
2. Krogh N, Kongsbak-Wismann M, Geisler C, Nielsen H. Substoichiometric ribose methylations in spliceosomal snRNAs. *Organic & Biomolecular Chemistry*. 2017;15(42):8872-6.