

Supplementary Information

Modification of Adenosine196 by Mettl3 Methyltransferase in the 5'-External Transcribed Spacer of 47S Pre-rRNA Affects rRNA Maturation

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Received: 3 April 2020; Accepted: 21 April 2020; Published: date

Name	Sequences, 5'-3'
1	uuAuuuGGGuGAAGAcAAATsT
	UUuGUCUUcACCcAAAuAATsT
2	AcuucAGAcGAAuuAucAATsT
	UUGAuAAUUCGUCUGAAGUTsT
3	uGuuGAAAAAuuucGcucuTsT
	AGAGCGAAAUUUUUcAAcATsT
4	ccAAGGAAcAAuccAuuGuTsT
	AcAAUGGAUUGUUCCUUGGTsT
5	GGAAcAAuccAuuGuuGAATsT
	UUcAAcAAUGGAUUGUUCCTsT
6	uGAAcGGGuAGAuGAAAuuTsT
	AAUUUcAUCuACCCGUUcATsT
control	cuuAcGcuGAGuAcuucGATsT
	UCGAAGuACUcAGCGuAAGTsT

Supplementary Table S1. List of Mettl3 siRNA used in the study.

Upper case – ribonucleotide, lower case – 2'-O-methylribonucleotide, s –phosphothioate group.

Supplementary Table S2. List of primers used in the study.

Name	Forward, 5'-3'	Reverse, 5'-3'
47S (5'-ETS)	TCGCTGGAGAGGTTGGGCCT	TCGGACGCGCGAGAGAACAGCA

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A'	GTGTCAGGCGTTCTCGTCTCC	AGCGAGAAGGACGGTCCCGTT
A'-A0	GGTTTGCGCGAGCGTCGGCT	CAAACCGCCTCGAACCCCACAC
A0	GGCTCGTCGCCTACTGTG	CCAACCCGCTGGGAACG
A0-A1	GTGTTTTCCTGGTGGCCCGGC	GTTCGGTCCCAGGCGGGG
A1	GCCGCGCTCTACCTTACCTACCT	GCGAGCGACCAAAGGAACCATAACTG
18S	GGGGCCCGAAGCGTTTACTTTG	CAAGAATTTCACCTCTAGCGGCGC
5.8S	ACTCGGCTCGTGCGTC	GCGACGCTCAGACAGG
28S	AGAGGTAAACGGGTGGGGTC	GGGGTCGGGAGGAACGG
U8	ATCCTTACCTGTTCCTCCTCC	GGGTGTTGCAAGTCCTGA
UTP4	GTCCCTGTGACATCTAACAGCAGT	GCCTCCAGATATCAGCGCTGTT
UTP14	GCACCAAAACAGTGGGAAATGGG	TCTTCCTCACTCTCAGAGGCTACC
UTP24	GAATCTGTGCAGGGGGAAGCATATT	CTCTCCTCCTTTCCCAGGCATTGTA
MTREX	GTTTTGGTGCATCTCCTGTCTGC	GATGCCGTCAGGAAAACGTTTCTG
XRN2	CAGTGAACCTGAGCCAGAGGAT	GACTGCACAACTTTCCGACGGA
NCL	CCAGCCATCCAAAACTCTGT	TAACTATCCTTGCCCGAACG
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
β-Actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG
FBL	CCACACAAATACCGCATGCTCATC	GTCAATGCAGTTGGCCTTAATGGA
METTL3	CGGAACCAGCAAAGGAGCCAGCCAAG	GTAGTATTTAATAGCTCTAGGATC

Upper case – 2′-deoxynucleotide.

Supplementary Table S3. List of FISH probes used in the study.

FISH prob	e	Sequence, 5'-3'
47S		agacgagaacgccugacacgcacggcac-sCy3
	A'-A0	cgcuagagaaggcuuuucuc-sCy3
18S		accagacuugcccuccaaug-sCy3
Lower case — 2'-O-methylribonucleotide, sCy3—sulfo-Cyanine 3 dye.		

Supplementary Table S4. List of probes for T4 RNA ligation assay used in the study.

Position	L probe, 5'-3'	R probe, 5'-3'
A5	p-CAGCTCTATGGGCAGTCGGTGAT	CCATCTCATCCCTGCGTGTC AGAGGACAGCGTgu
A80	p-CACCGGTAGGCCATCTATGGGCAGTCGGTGAT	CCATCTCATCCCTGCGTGTC CGGCTAGCCGGgu
A196	p-CAGAGACCCGGACTCTATGGGCAGTCGGTGAT	CCATCTCATCCCTGCGTGTC GCCGCCCCGGgu
A2577	p-CCAATGCAAAAACTCTATGGGCAGTCGGTGAT	CCATCTCATCCCTGCGTGTCCTTAACTCAAAgu
A2488	p-ATATTTAAGGCTCTATGGGCAGTCGGTGAT	CCATCTCATCCCTGCGTGTCAACTAAGCTAcu

Upper case – 2'-deoxynucleotide, lower case – ribonucleotide, p – phosphate group.



Figure S1. A. Determination of the Mettl3 siRNA efficacy by RT-qPCR (* p < 0.05, ** p < 0.01). 1–6 are the numbers of siRNA from Suppl. table 1. B. Determination the efficacy of the Mettl3 protein inhibition by Western-blot. C. Schematic representation of the human pre-rRNA processing with the cleavage sites.



Figure S2. A. Gel electrophoresis of PCR analysis (18, 20 and 24 cycles of PCR) of T4 RNA ligase assay for the known m6A and A positions in Malat1 RNA. B. Control of the quality of the cell fractions separation by RT-qPCR snoRNAs and mRNA Gapdh measurement. RT-qPCR analysis of the A' (C.) and A0 (D.) cleavage efficacy in cells with Mettl3 KD using siRNA-3 and siRNA-4 (* p < 0.05).

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Figure S3. A. FISH analysis of mature rRNAs and U2 snRNA in control and Mettl3 KD cells. DNA is stained with DAPI, rRNA with Cy3-labeled probes. B. Polysome profiling for control and Mettl3 KD cells.



Figure S4. Analysis of Mettl3-dependent m6A residues (highlighted in yellow) in UTP14 and nucleolin mRNA by MeT-DB V2.0 browser 31. .

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RNA45SN5 at chr22_KI270733v1_random:122273-135645 - (NR_046235)

Human	
Chimp	
CHIMP	gtccgaccgtcccgactccc-ggtgctagcccgggtctgggtctctgg-acca-agg
Gorilla	<u>gtccgagcgtcccgact</u> ccc-ggtgcc <u>gggtccgggt</u> <u>ctctg</u> -acccacccgggg
Gibbon	gtccgagcgtcccgactcccccc-ggtgccggcccgggtccgggtctctgacccacct-ggg
Rhesus	atccgagcgtcccagctccc-ggtgccgccttgggtccgggtctctgacc
Crab-eating macaque	atccgagcgtcccagctccc-ggtgccgccttgggtccgggtctc <mark>tg</mark> acccacct-ggg
Pig-tailed macaque	atgcgagcgtcctgactccc-ggtgccgccttgggtccgggtctctgacccacct-ggg
Sooty mangabey	atccgagcgtcctgactcct-ggtgccgccttggttccgggtctctgacccaccc-ggg
Drill	atccgagcgtcctgactcct-ggtgccgccttggttccgggtctc <mark>tg</mark> acccaccc-ggg
Marmoset	tctatctgagtgtcctggctccctggtgctgcccgtagcccaggtctcctctgacccaccc-ttg
Dog	ggactccaggtggcccgggtcgc-ggtgtcgccctccggtctccggcacccgagg-gag
Tarsier	

RNA45SN5 at chrUn_GL000220v1:105424-118780 - (NR_046235)

Human	ggtgccggcccgggtccgggtctc <mark>tgacc</mark> cacccggggg
Chimp	ggtgctagcccgggtctgggtctc <mark>tgacc</mark> caccaagggg
Gibbon	ggtgccggcccgggtccgggtctc <mark>tgacc</mark> cacctggggg
Rhesus	ggtgccgccttgggtccaggtctc <mark>tgacc</mark> catctggggg
Crab-eating macaque	ggtgccgccttgggtccgggtctc <mark>tgacc</mark> cacctggggg
Baboon	ggtgccaccttgggtccaggtctccaacccacctggggg
Marmoset	ggtgctgcccgtagcccaggtctcctctctgacccacccttgtg
Dog	ggtgtcgccctccggtctccggcacccgagggaggg
Proboscis monkey	

Figure S5. Multiple sequence alignment of 5'-ETS parts with highlighted m6A nucleotide (yellow) and DRACH sequence (green) in different species.



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