### **Supporting Information for**

#### METTL21C methylates Alanine tRNA synthetase at lysine 943 in muscle tissue

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#### **Supplementary Figure Legends**

# Supplementary Figure 1. Mass spectrometry analysis of AARS1 as a methylated substrate of METTL21C.

Representative tandem mass spectra identifying of the *in vitro* methylation of indicated fractions using deuterated SAM as a methyl donor, proteins were in-gel digested by trypsin. Spectra are representative of experiments independently performed three times with similar results. (A-B) MS/MS of the unmodified or monomethylated AARS1 peptide (935aa- 943aa) with the indicated *m/z*. *m/z* for b and y ions observed in spectra were indicated in blue and red, respectively. (C-E) MS/MS of the mono, di and trimethylated AARS1 peptide (935aa- 962aa) with the indicated m/z. Digested peptides analyzed by Fusion Mass spectrometer.

#### Supplementary Figure 2. Mass spectrometry analysis of methylated recombinant AARS1.

Representative tandem mass spectra of (A) unmodified, (B) mono and (C) di-methylated GST-AARS1 (935aa- 943aa) peptide after *in vitro* methylation of AARS1 by METTL21C using deuterated SAM as a methyl donor. Proteins were in-gel digested by GluC. m/z for b and y ions observed in spectra are indicated in blue and red, respectively.

#### Supplementary Figure 3. Sequence alignment of METTL21 family.

Protein sequence alignment of the METTL21 family of enzymes. METTL21A, B, C and D sequences alignments were performed with the MUSCLE algorithm using Jalview software. Conserved catalytic residues between the METTL21 family members is indicated with arrows. The colors in the figure indicating the default color scheme used for alignments in ClustalX [38, 39]. Blue- Hydrophobic, red-positive charged, magenta- negative charge, green- polar, pink- cystines, orange- glycines, yellow- prolines, cyan- aromatic.

# Supplementary Figure 4. Mass spectrometry analysis of GFP-AARS1 methylation in METTL21C expressing cells.

Representative LC-MS/MS analysis of GFP-AARS1 after IP from 293T transfected cells, proteins were ingel digested by Glu-C. (A-D) Representative tandem mass spectra analysis of the (A) unmodified, (B) mono-methylated (C) di-methylated (D) tri-methylated peptide of AARS1 with the indicated m/z. m/z for b and y ions observed in spectra were indicated in blue and red, respectively. Digested peptides analyzed by Elite Mass spectrometer.

# Supplementary Figure 5: Mass spectrometry analysis of AARS1 methylation in METTL21C expressing cells.

Representative tandem mass spectra analysis of AARS1 after IP from 293T cells using anti-AARS1 antibody, proteins were in-gel digested by Glu-C. (A-D) Representative tandem mass spectra analysis of the (A) unmodified, (B) monomethylated (C) demethylated (D) trimethylated peptide of AARS1. m/z for b and y ions observed in spectra were indicated in blue and red, respectively. Digested peptides analyzed by Elite Mass spectrometer. (E) i- Chromatograms for quantification of GFP-AARS1 from Flag-METTL21C transfected cells (see Supplementary Figure 4), ii- table represents the peptide quantification. (F) i- Chromatograms for quantification Flag-METTL21C transfected cells, ii- table represents the peptide quantification.



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### Zoabi et al., Supplementary Figure 2

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## Zoabi et al., Supplementary Figure 3

Α.

METTL21C/1-264	1 MDVCLSSAQQ <mark>PG</mark> RR <mark>G</mark> EGLSS <mark>PGG</mark> WLEAEKK <mark>G</mark> APQKDSTGGVLEESNK	IEPS 51
METTL21A/1-218	1MALVPYEET	TEFG 13
METTL21B/1-226	1PESES-ESVFPR	-EVG 21
METTL21D/1-229	1T	LE <mark>S</mark> - 7
METTL21C/1-264	52 LHSLQKFVPTDYASYTQEHYRFAGKEIVIQE SIESYGAVVWPGA	MALC 99
METTL21A/1-218	14 L QKF - HKPLATFSF ANHTIQIRODW - RHLGVAAVVWDAA	IVLS 54
METTL21B/1-226	22 L F ADS - YSEKSQFCF CGHVLTITONFGSRLGVAARVWDAA	L <mark>S</mark> LC 64
METTL21D/1-229	8 SL - EDPLRSFVRVLEKRDGTVLRL - QQY - SSGGVGCVVWDAA	IVLS 50
METTL21C/1-264	100 QYLEEHAEELNFQDAKILEIGAGPGLVSIVASILGAQVTATDL	<mark>P</mark> DVL 146
METTL21A/1-218	55 TYLEMGAVELRGRSAVELGAGTGLVGIVAALLGAHVTITDR	KVAL 99
METTL21B/1-226	65 NYFESQNVDFRGKKVIELGAGTGIVGILAALQGGDVTITDL	PLAL 109
METTL21D/1-229	51 KYLETPEFSGDGAHALSRRSVLELGSGTGAVGLMAATLGADVVVTDL	EELQ 101
METTL21C/1-264	147 GNLQYNLLKNTLQCTAHLPEVKELVWGEDLDKNFPKSAFYYDYVLAS	DVVY 197
METTL21A/1-218	100 EFLKSNVQANLPPHIQTKTVVKELTWGQNLGSFSPGEFDLILGA	DIIY 147
METTL21B/1-226	110 EQIQGNVQANVPAGGQAQVRALSWGIDHHVF-PANYDLVLGA	DIVY 154
METTL21D/1-229	102 DLLKMNINMNKHL-VTGSVQAKVLKWGEEIEGF-PSPPDFILMA	DCIY 147
METTL21C/1-264	198 HHYFLDKLLTTMVYLSQPGTVLLWANKFRFSTDYEFLDKFKQ	VFD <mark>T</mark> 243
METTL21A/1-218	148 LEETFTDLLQTLEHLCSNHSVILLACRIRYERDNNFLAMLER	QFTV 193
METTL21B/1-226	155 LEPTFPLLLGTLQHLCRPHGTIYLASKMRKEHGTESFFQHLLPQ	HFQL 202
METTL21D/1-229	148 YEESLEDLLKTLKDLSGEETCLLCCYEORTMGKNDELEKKYEELLQL	DEDE 198
METTL21C/1-264	244 TLLAEYPESSVKLFKGILKWD	264
METTL21A/1-218	194 RKVHYDPEKDVHI.YEAQKRNQKEDL	218
METTL21B/1-226	203 ELAQRDEDENVNI.YRARHREPRPA.	226
METTL21D/1-229	199 EKIPLEKHDEEYRSEDIHIIYIRKKKSKFPS.	229

Zoabi et al., Supplementary Figure 4



### Zoabi et al., Supplementary Figure 5









	VSAQATGK*NVGCLQE					
GLU-C digestion	AAR	AARS-GFP/ Flag-METTL21C-WT				
Peptide sequence	charge	m/z	Intensity	%		
unmodified	2	781.386	4487467	36.0336		
Mono-methylation	2	788.393	1540197	12.3675		
Dimethylation	2	795.401	1056462	8.48321		
Tri-methylation	2	802.409	5369444	43.1157		
Total			12453570			







ii.

	GKGGGKDVSAQATG <mark>K</mark> *NVGCLQE				
GLU-C digestion	AARS/Flag-METTL21C				
Peptide sequence	charge	m/z	Intensity	%	
unmodified	2	721.03	20972021	62.4581	
Monomethylation	2	725.7	3604660	10.7353	
Dimethylation	2	731.04	2001643	5.96122	
Trimethylation	2	735.38	6999401	20.8454	
Total			33577725		