Supplemental Material

METTL7A (TMT1A) and METTL7B (TMT1B) are responsible for alkyl *S*-thiol methyl transferase activity in liver

Drake A. Russell, Marvin K. Chau, Yuanyuan Shi, Ian N. Levasseur, Benjamin J. Maldonato, Rheem A. Totah*

University of Washington

Department of Medicinal Chemistry

1959 NE Pacific Ave,

Box 357610

Seattle, WA 98195

Corresponding Author: Dr. Rheem A. Totah

Email: rtotah@uw.edu

Phone: 206-543-9481

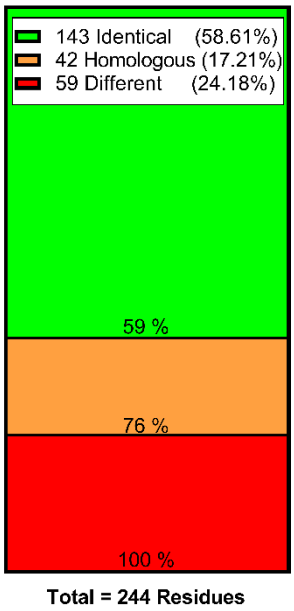
Liver ID	Sex
HL-102	Male
HL-111	Male
HL-112	Male
Hl-114	Male
HL-136	Male
HL-141	Male
HL-154	Male
HL-161	Male
HL-163	Male
HL-105	Female
HL-108	Female
HL-115	Female
HL-131	Female
HL-132	Female
HL-135	Female
HL-152	Female
HL-159	Female
HL-164	Female
HL-165	Female

Supplementary table 01. Table showing sex of individual liver donors.

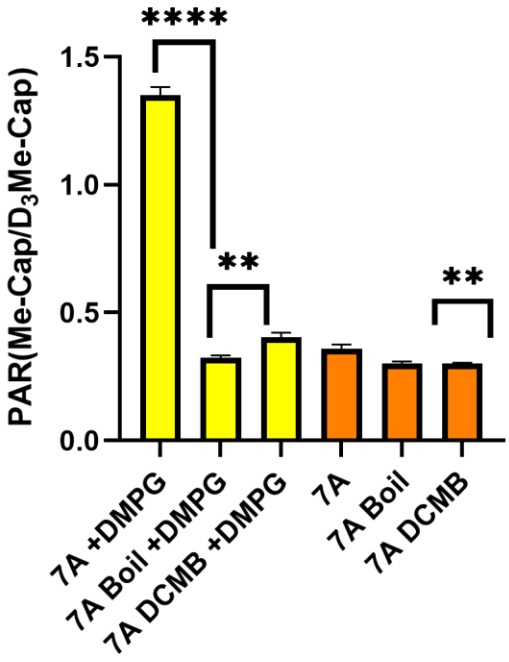
Peptide list	parent ions	fragmentations
sp|Q9H8H3|MET7A_HUMAN.VTC[CAM]IDPNPNFEK.+2y9.light	717.34	1073.53
sp|Q9H8H3|MET7A_HUMAN.VTC[CAM]IDPNPNFEK.+2y8.light	717.34	960.44
sp|Q9H8H3|MET7A_HUMAN.VTC[CAM]IDPNPNFEK.+2y7.light	717.34	845.42
sp|Q9H8H3|MET7A_HUMAN.VTC[CAM]IDPNPNFEK.+2y5.light	717.34	634.32
sp|Q9H8H3|MET7A_HUMAN.VTC[CAM]IDPNPNFEK.+2y9.heavy	721.35	1081.54
sp|Q9H8H3|MET7A_HUMAN.VTC[CAM]IDPNPNFEK.+2y8.heavy	721.35	968.46
sp|Q9H8H3|MET7A_HUMAN.VTC[CAM]IDPNPNFEK.+2y7.heavy	721.35	853.43
sp|Q9H8H3|MET7A_HUMAN.VTC[CAM]IDPNPNFEK.+2y5.heavy	721.35	642.33
MET7A.FYPPGC[CAM]R.+2y5.light	448.71	586.28
MET7A.FYPPGC[CAM]R.+2y4.light	448.71	489.22
MET7A.FYPPGC[CAM]R.+2y5+2.light	448.71	293.64
MET7A.FYPPGC[CAM]R.+2y4+2.light	448.71	245.12
MET7A.FYPPGC[CAM]R.+2y5.heavy	453.71	596.28
MET7A.FYPPGC[CAM]R.+2y4.heavy	453.71	499.23
MET7A.FYPPGC[CAM]R.+2y5+2.heavy	453.71	298.65
MET7A.FYPPGC[CAM]R.+2y4+2.heavy	453.71	250.12
sp|Q6UX53|MET7B_HUMAN.VTC[CAM]LDPNPHFEK.+3y7.light	486.23	868.43
sp|Q6UX53|MET7B_HUMAN.VTC[CAM]LDPNPHFEK.+3y11+2.light	486.23	679.31
sp|Q6UX53|MET7B_HUMAN.VTC[CAM]LDPNPHFEK.+3y10+2.light	486.23	628.79
sp|Q6UX53|MET7B_HUMAN.VTC[CAM]LDPNPHFEK.+3y7+2.light	486.23	434.72
sp|Q6UX53|MET7B_HUMAN.VTC[CAM]LDPNPHFEK.+3y7.heavy	488.91	876.45
sp|Q6UX53|MET7B_HUMAN.VTC[CAM]LDPNPHFEK.+3y11+2.heavy	488.91	683.32
sp|Q6UX53|MET7B_HUMAN.VTC[CAM]LDPNPHFEK.+3y10+2.heavy	488.91	632.8
sp|Q6UX53|MET7B_HUMAN.VTC[CAM]LDPNPHFEK.+3y7+2.heavy	488.91	438.73
MET7B.ELFSQIK.+2y6.light	432.74	735.44
MET7B.ELFSQIK.+2y5.light	432.74	622.36
MET7B.ELFSQIK.+2y4.light	432.74	475.29
MET7B.ELFSQIK.+2y6+2.light	432.74	368.22
MET7B.ELFSQIK.+2y6.heavy	436.75	743.45
MET7B.ELFSQIK.+2y5.heavy	436.75	630.37
MET7B.ELFSQIK.+2y4.heavy	436.75	483.3
MET7B.ELFSQIK.+2y6+2.heavy	436.75	372.23

Supplementary table 02. Table showing transitions for heavy labeled and unlabeled surrogate peptides for METTL7A (TMT1A) and METTL7B (TMT1B).

```
      #       1         10         20         30         40         50         60
METTL7A       MELTIFILRL AIYILTFPLY LLNFLGLWSW ICKKWFPYFL VRFTVIYNEQ MASKKRELFS
              M++ + +L+L  + +LT PL+ L+  LG W   +CK +FPY +    T   N + M SKKRELFS
METTL7B       MDILVPLLQL LVLLLTLPLH LMALLGCWQP LCKSYFPYLM AVLTPKSNRK MESKKRELFS
      #       61        70         80         90         100        110        120
METTL7A       NLQEFAGPSG KLSLLEVGCG TGANFKFYPP GCRVTCIDPN PNFEKFLIKS IAENRHLQFE
               ++   G SG K++LLE+GCG TGANF+FYPP GCRVTC+DPN P+FEKFL KS +AENRHLQ+E
METTL7B       QIKGLTGASG KVALLELGCG TGANFQFYPP GCRVTCLDPN PHFEKFLTKS MAENRHLQYE
      #       121       130        140        150        160        170        180
METTL7A       RFVVAAGENM HQVADGSVDV VVCTLVLCSV KNQERILREV CRVLRPGGAF YFMEHVAAEC
              RFVVA GE+M  Q+ADGS+DV VVCTLVLCSV ++  ++L+EV  RVLRPGG   +F EHVA
METTL7B       RFVVAPGEDM RQLADGSMDV VVCTLVLCSV QSPRKVLQEV RRVLRPGGVL FFWEHVAEPY
      #       181       190        200        210        220        230        240
METTL7A       STWNYFWQQV LDPAWHLLFD GCNLTRESWK ALERASFSKL KLQHIQAPLS WELVRPHIYG
               +W + WQQV  +P W  + D GC LTRE+WK  LE A FS++ +++    PL  W  V PHI G
METTL7B       GSWAFMWQQV FEPTWKHIGD GCCLTRETWK DLENAQFSEI QMERQPPPLK WLPVGPHIMG
      #          244
METTL7A       YAVK
               AVK
METTL7B       KAVK
```



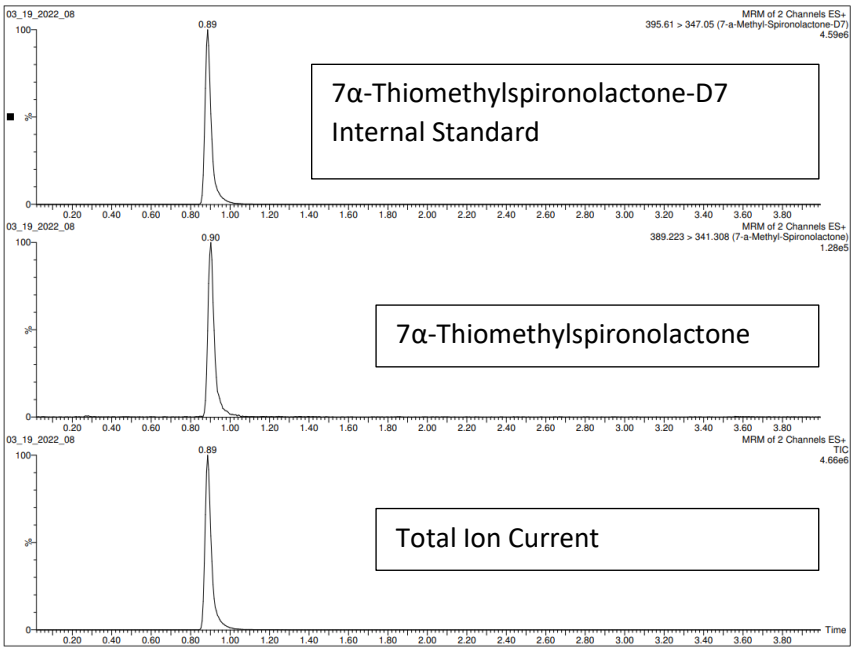
Supplementary Figure 01. Basic local alignment results for METTL7A (TMT1A) compared with METTL7B (TMT1B).



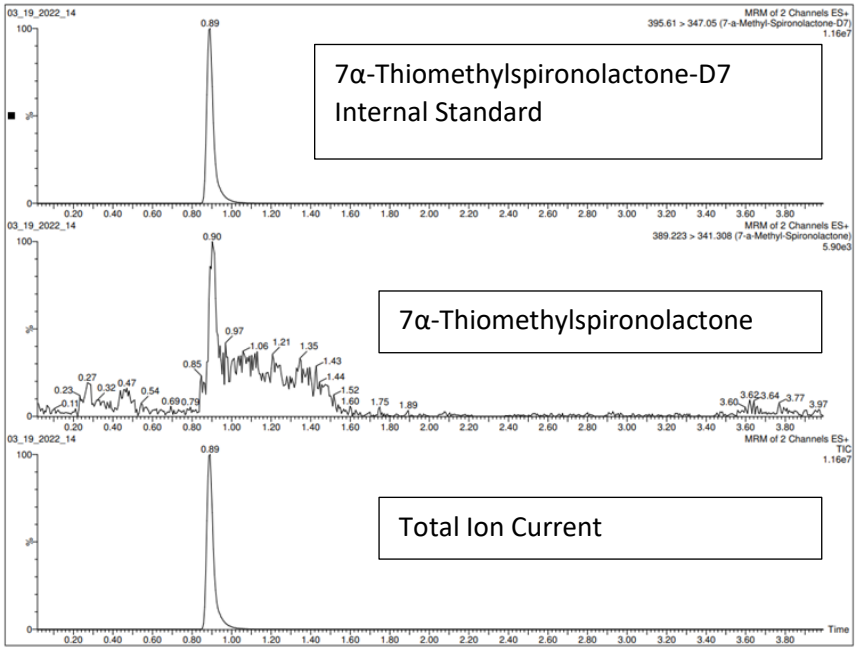
Supplementary Figure 02. Activity assay monitoring the formation of S-methyl captopril +/- DMPG by recombinant purified *N*-GST-METTL7A (*N*-GST-TMT1A). All data (n=3) are presented as the mean ±s.d.

For this captopril methylation assay, samples were incubated for 45 min +/- DMPG, the final DCMB concentration was 100 µM, and all other parameters and analysis techniques were performed similar to the captopril assay that is described in the in vitro 7α-thiospironolactone and captopril methylation methods section.

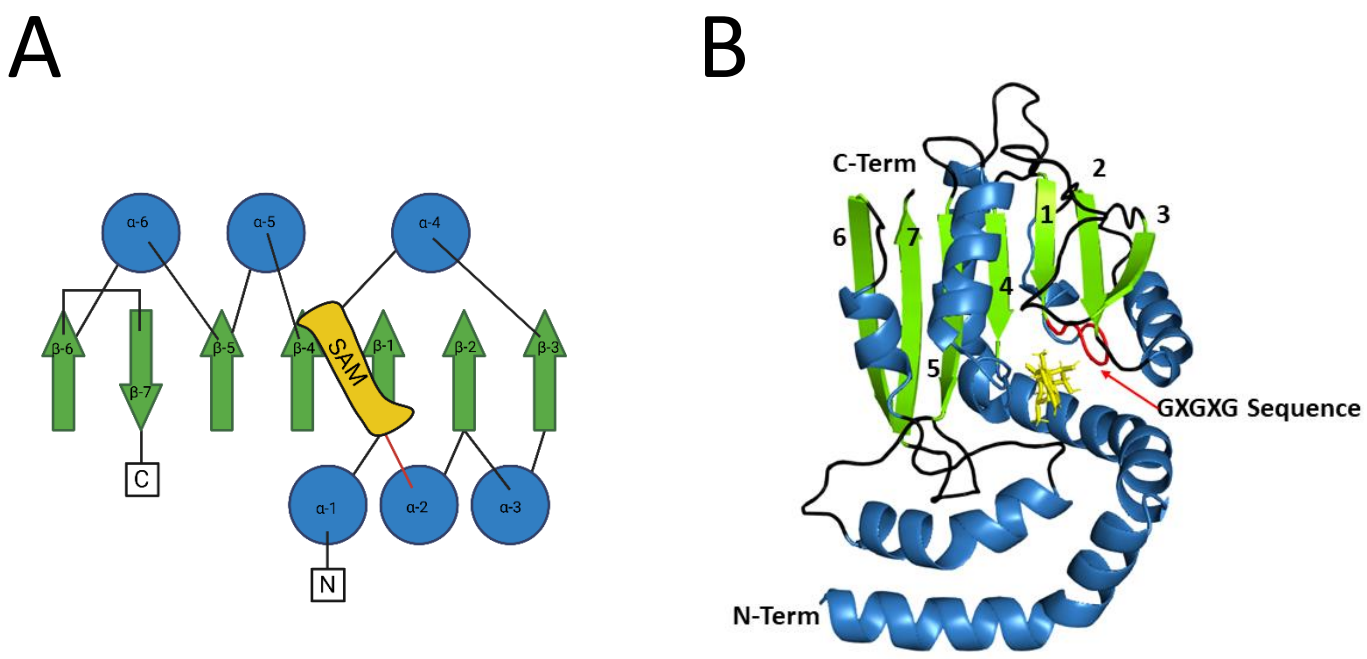
A



B

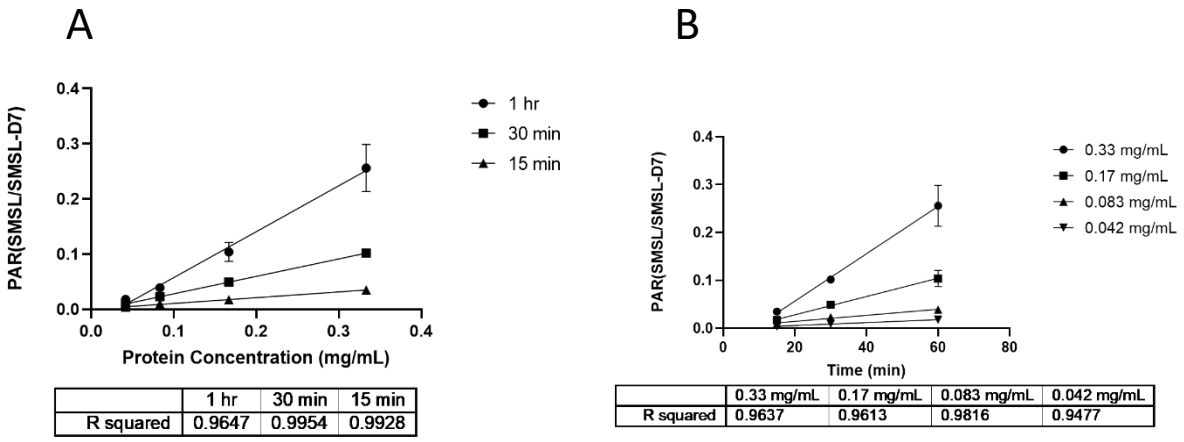


Supplementary Figure 03. Representative chromatogram traces from a TSL methylation activity assay with recombinant purified active (A) and boiled (B) *N*-GST-METTL7A (*N*-GST-TMT1A).



Supplementary Figure 04. Cartoon representation of class I methyltransferases (A) as described by Schubert et al., 2003. Alpha helices (blue) are labeled α-1 through α-6 beta sheets (green) are labeled β-1 through β-7. The SAM binding site is marked in yellow, and the GXGXG sequence location is marked by a red line. An AlphaFold produced homology model of METTL7A (TMT1A) (B) with beta sheets numbered in order along protein sequence and colored green. Alpha helices are colored blue, the GXGXG sequence is colored red, and SAM (yellow) is oriented in the SAM binding domain. The order and number of alpha helices and beta sheets along the AlphaFold-produced homology model is identical to what was described by Schubert et al., 2003.

The homology model for METTL7A was downloaded from The AlphaFold Protein Structure Database https://alphafold.ebi.ac.uk/files/AF-Q9H8H3-F1-model_v2.pdb. The homology model was colored using PyMOL Molecular Graphics System. Pymol renders secondary structures, which allowed us to distinguish segments as alpha helices or beta sheets. SAM was docked into the METTL7A homology model with The University of Hamburg's Center for Bioinformatics protein-ligand docking software, JAMDA.

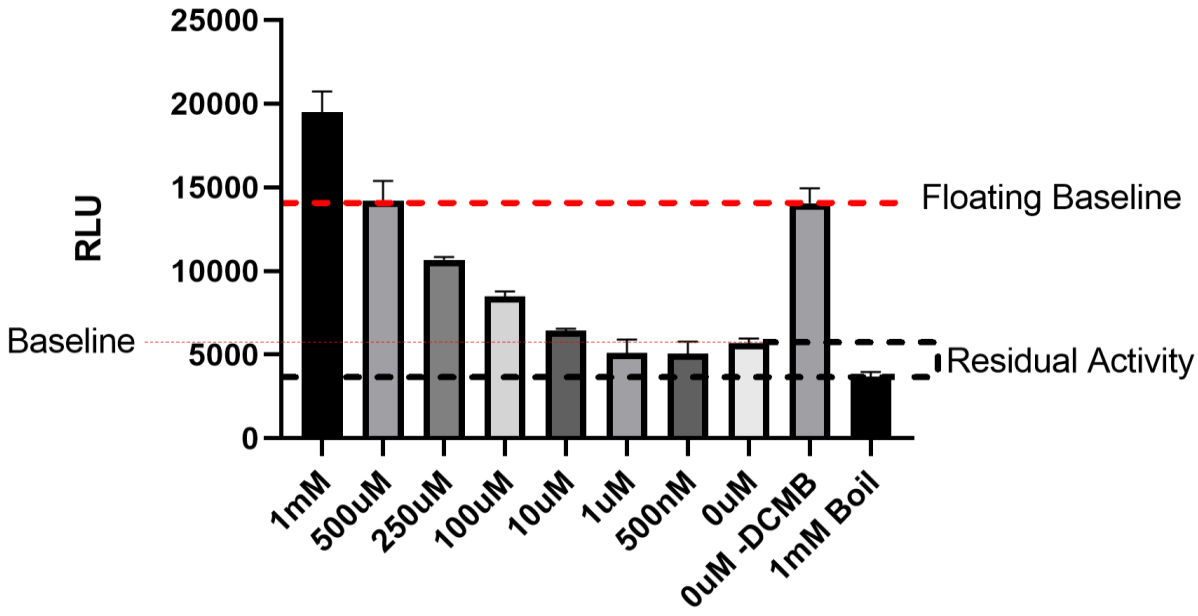


	1 hr	30 min	15 min
R squared	0.9647	0.9954	0.9928

	0.33 mg/mL	0.17 mg/mL	0.083 mg/mL	0.042 mg/mL
R squared	0.9637	0.9613	0.9816	0.9477

Supplementary Figure 05. The methylation of TSL at various concentrations of purified recombinant *N*-GST-METTL7A (*N*-GST-TMT1A) over the course of a 1 hr, 30 min, or 15 min incubation plotted with respect to protein concentration (A) or time increments (B).

Purified recombinant METTL7A was diluted to 0.125. 0.25, 0.5, and 1 mg/mL and then further diluted the purified protein into reaction buffer containing DMPG as described in the methods section; in vitro 7α-thio spironolactone and captopril methylation using recombinant METTL7A. TSL was deposited onto a 96-well plate as previously described, and the diluted protein was then added to the appropriate wells. The final protein concentrations were 0.042, 0.083, 0.17, and 0.33 mg/mL. The final TSL concentration was 100 µL. SAM was added in staggered time increments to induce the reaction. The final SAM concentration was 100 µM. The final incubation times for the various protein concentrations were 1 h, 30 min, and 15 min. The reaction was quenched, and the samples were analyzed by LC-MS/MS as was described in the method section; in vitro 7α-thio spironolactone and captopril methylation using recombinant purified METTL7A.



Supplementary Figure 06. The relative luminescence unit response from purified recombinant METTL7A incubated with various concentrations of 4-chlorothiophenol. The reaction was quenched by the addition of DCMB to a final concentration of 100 µM and all treatments were analyzed using Promega's MTaseGlo Assay. For one set of replicates, no substrate was included in the incubation and no DCMB was added to quench the reaction prior to adding the MTaseGlo development reagents. The difference between the response from this set of replicates and a set also incubated without substrate but with the addition of DCMB represents the activity that can be attributed to the methylation of a compound present in the MTaseGlo development reagents by METTL7A. There is still a response above boil in no-substrate treated replicates, but this response is low enough to identify the methylation of substrates during the activity assay.

Purified recombinant METTL7A was incubated with various concentrations of 4-chlorothiophenol for 30 minutes. The final concentration of SAM was 100 µM. All reaction conditions and analyses using the MTaseGlo Assay were identical to what was described in the substrate screening methods section. For one set of replicates, no substrate was included in the incubation and no DCMB was added to quench the reaction prior to adding the MTaseGlo development reagents.

A.

METTL7B siRNA Sequence:

5’ CCUUCAUGUGGCAGCAAGUdTdT 3’ / 5’ ACUUGCUGCCACAUGAAGGdTdT 3’

B. METTL7A mRNA coding region sequence:

```
atctgttttttccccttctgagcaatggagcttaccatctttatcctgagactggccatt
 I  C  F  F  P  F  -  A  M  E  L  T  I  F  I  L  R  L  A  I
tacatcctgacatttcccttgtacctgctgaactttctgggcttgtggagctggatatgc
 Y  I  L  T  F  P  L  Y  L  L  N  F  L  G  L  W  S  W  I  C
aaaaaatggttcccctacttcttggtgaggttcactgtgatatacaacgaacagatggca
 K  K  W  F  P  Y  F  L  V  R  F  T  V  I  Y  N  E  Q  M  A
agcaagaagcgggagctcttcagtaacctgcaggagtttgcgggcccctccgggaaactc
 S  K  K  R  E  L  F  S  N  L  Q  E  F  A  G  P  S  G  K  L
tccctgctggaagtgggctgtggcacgggggccaacttcaagttctacccacctgggtgc
 S  L  L  E  V  G  C  G  T  G  A  N  F  K  F  Y  P  P  G  C
agggtgacctgtattgaccccaaccccaactttgagaagtttttgatcaagagcattgca
 R  V  T  C  I  D  P  N  P  N  F  E  K  F  L  I  K  S  I  A
gagaaccgacacctgcagtttgagcgctttgtggtagctgccggggagaacatgcaccag
 E  N  R  H  L  Q  F  E  R  F  V  V  A  A  G  E  N  M  H  Q
gtggctgatggctctgtggatgtggtggtctgcaccctggtgctgtgctctgtgaagaac
 V  A  D  G  S  V  D  V  V  V  C  T  L  V  L  C  S  V  K  N
caggagcggattctccgcgaggtgtgcagagtgctgagaccgggaggggctttctatttc
 Q  E  R  I  L  R  E  V  C  R  V  L  R  P  G  G  A  F  Y  F
atggagcatgtggcagctgagtgttcgacttggaattacttctggcaacaagtcctggat
 M  E  H  V  A  A  E  C  S  T  W  N  Y  F  W  Q  Q  V  L  D
cctgcctggcaccttctgtttgatgggtgcaacctgaccagagagagctggaaggccctg
 P  A  W  H  L  L  F  D  G  C  N  L  T  R  E  S  W  K  A  L
gagcgggccagcttctctaagctgaagctgcagcacatccaggccccactgtcctgggag
 E  R  A  S  F  S  K  L  K  L  Q  H  I  Q  A  P  L  S  W  E
ttggtgcgccctcatatctatggatatgctgtgaaatagtgtgagctggcagttaagagc
 L  V  R  P  H  I  Y  G  Y  A  V  K  -  C  E  L  A  V  K  S
```

Supplementary Figure 07. METTL7B (TMT1B) siRNA sequence with region complementary to METTL7A (TMT1A) coding region of NCBI Reference Sequence: NM_014033.4 highlighted (A). METTL7A (TMT1A) coding region of NCBI Reference Sequence: NM_014033.4 with region complementary to METTL7B (TMT1B) siRNA sequence highlighted (B).