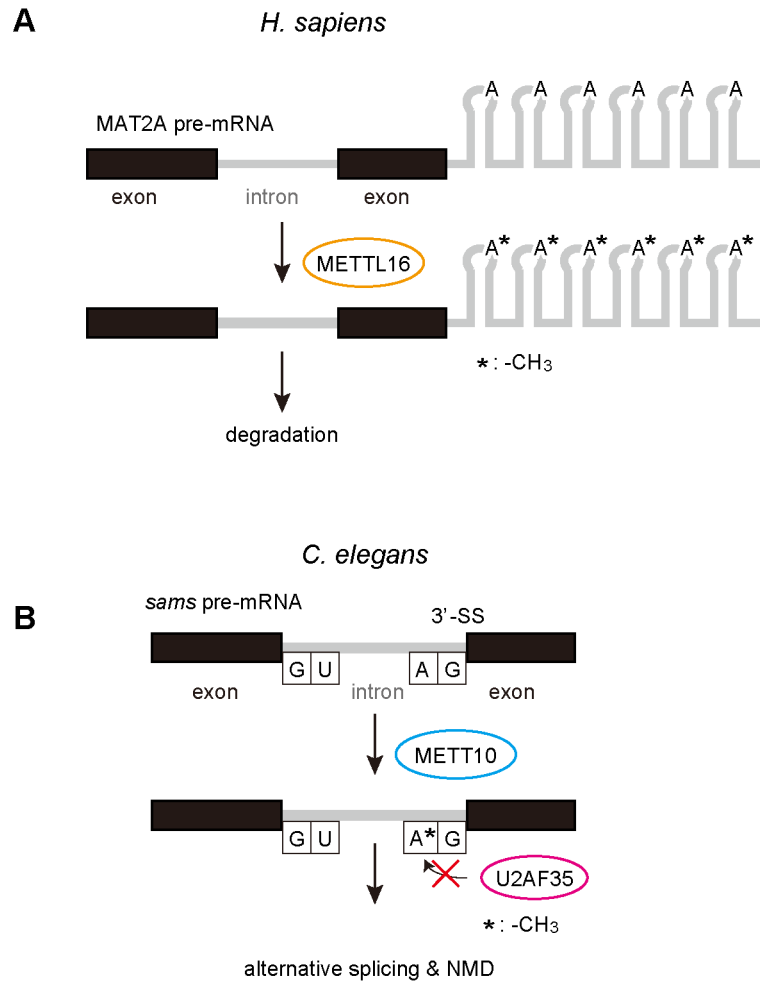


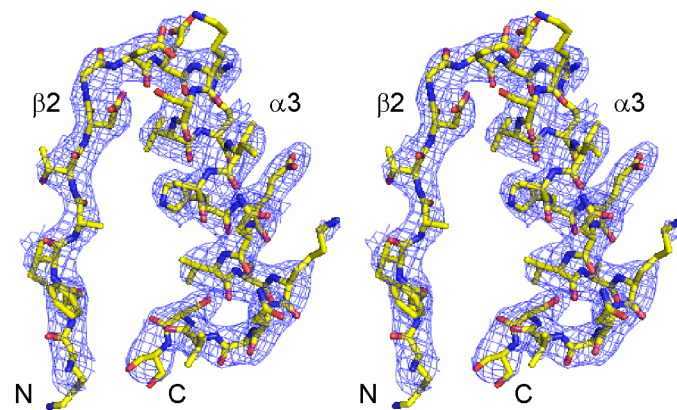
Supplementary Data

Structure of the *Caenorhabditis elegans* m⁶A methyltransferase METT10 that regulates SAM homeostasis

Jue Ju, Tomohiko Aoyama, Yuka Yashiro, Seisuke Yamashita, Hidehito Kuroyanagi,
Kozo Tomita

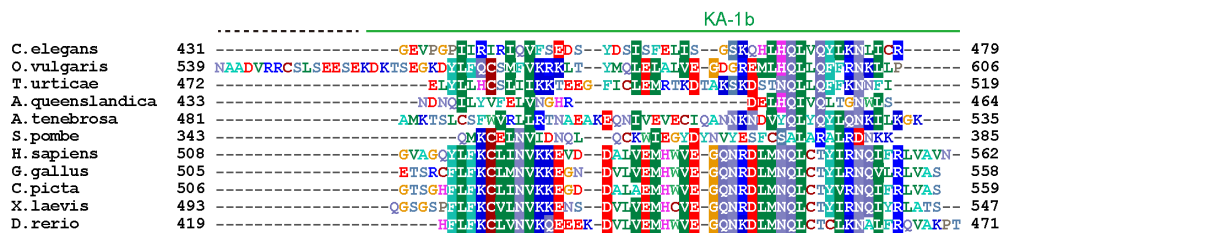
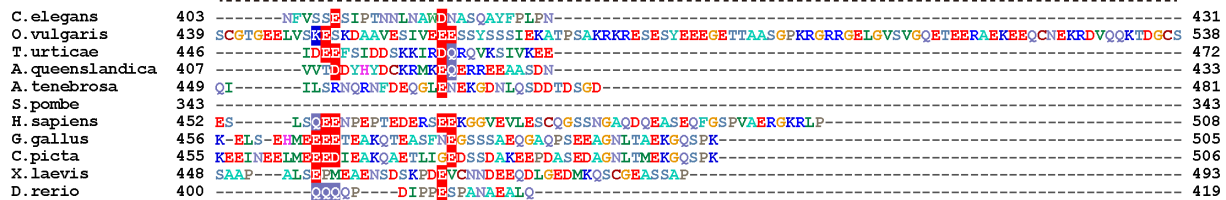
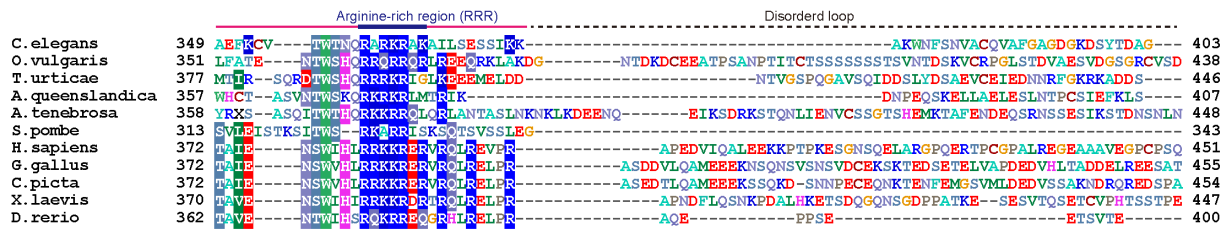
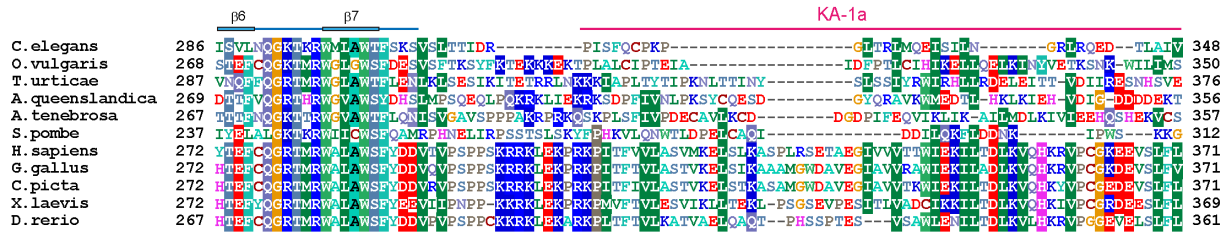
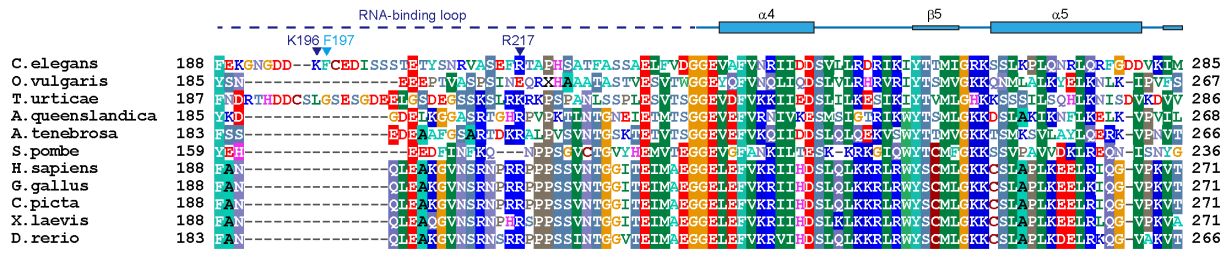
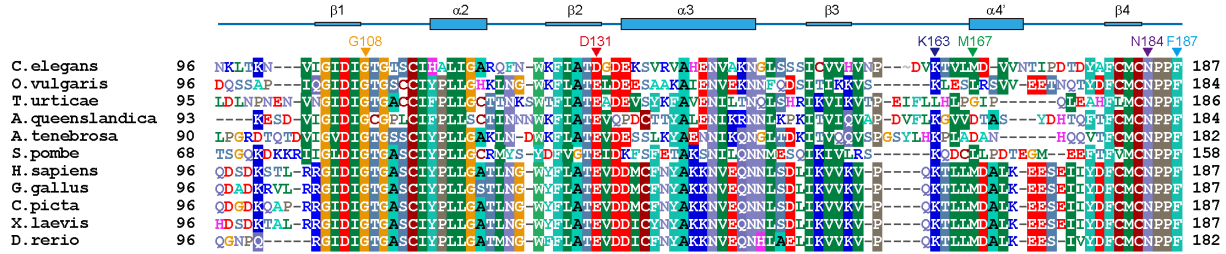
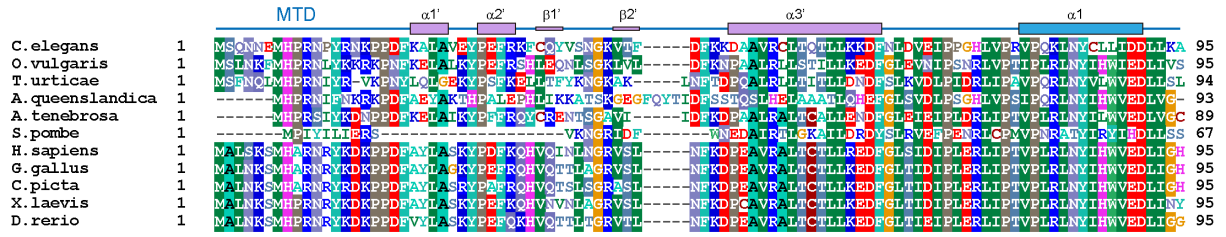


Supplementary Figure 1: Different mechanisms of SAM homeostasis between (A) *H. sapiens* and (B) *C. elegans*.



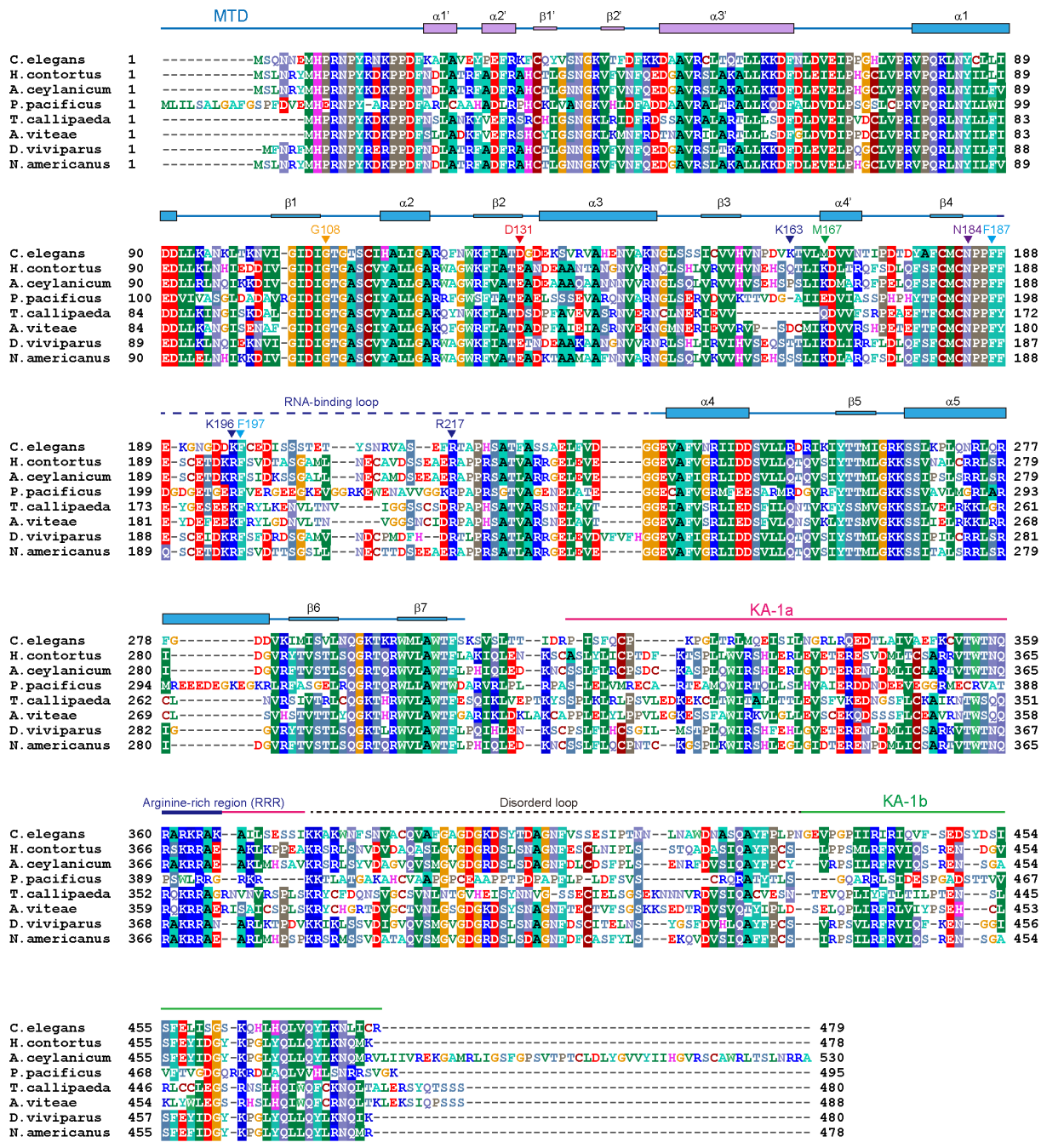
Supplementary Figure 2: 2Fo-Fc electron density map of CeMETT10-MTD.

2Fo-Fc electron density map contoured at 1.0 σ around the residues corresponding to $\beta 2$ and $\alpha 3$ of CeMETT10 (residues Lys126 - Ser151).



Supplementary Figure 3: Alignment of the amino acid sequences of *C. elegans* METT-10 homologs from other organisms.

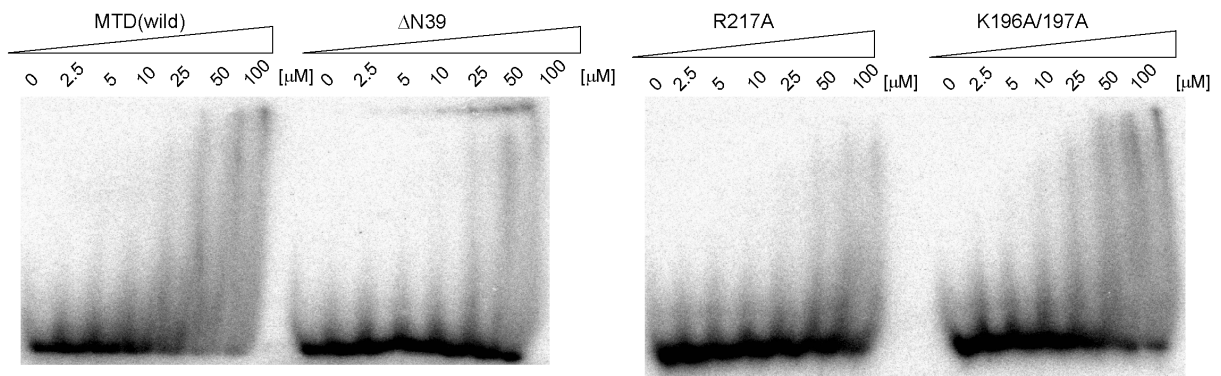
Invertebrates [*C. elegans* (MET16_CAEEL), *O. vulgaris* (A0A6P7TIF1_OCTVU), *T. urticae* (T1JVZ0_TETUR), *A. queenslandica* (A0A1X7U5N0_AMPQE), and *A. tenebosa* (A0A6P8ITS0_ACTTE),] fungus [*S. pombe* (MTL16_SCHPO)] and vertebrates [*H. sapiens* (MET16_HUMAN), *G. gallus* (MET16_CHICK), *C. picta* (A0A8C3F3B3_CHRPI), *X. laevis* (MET16_XENLA), and *D. rerio* (MET16_DANRE)].



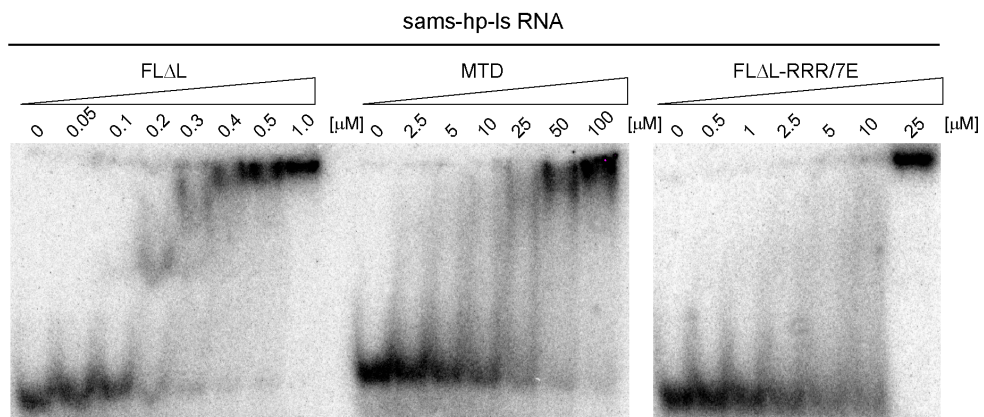
Supplementary Figure 4: Alignment of the amino acid sequences of *C. elegans* METT-10 homologs from other worms.

H. contortus (A0A7I4YYI6_HAECO), *A. ceylanicum* (A0A016V3Y4_9BILA), *P. pacificus* (A0A2A6CIQ0_PRIPA), *T. callipaeda* (A0A0N5D327_THECL), *A. viteae* (A0A498S9S7_ACAVI), *D. viviparus* (A0A0D8YCA1_DICVI), and *N. americanus* (W2TKK9_NECAM). Residues K196/F197 and R217 are highlighted. The secondary structural elements of the CeMETT10-MTD are depicted in parentheses above the alignment. The C-terminal regions predicted to be KA-1 (KA-1a and KA-1b) by AlphaFold2 are also shown above the alignment. The region between KA-1a and KA-1b is predicted to be disordered.

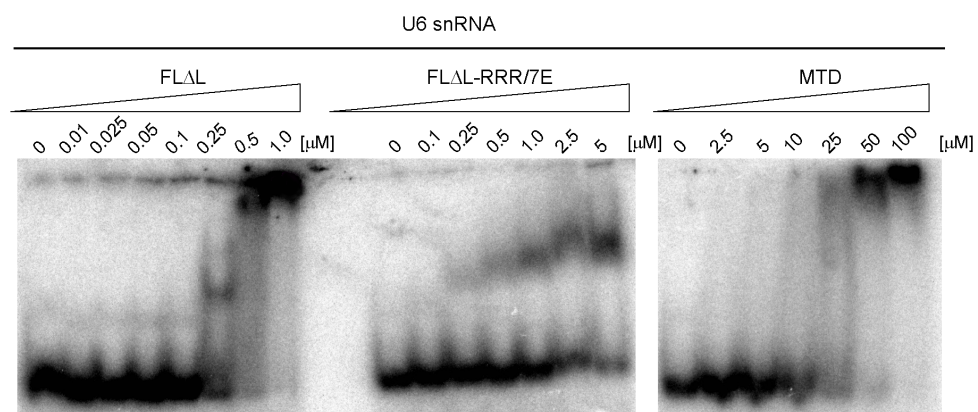
A



B

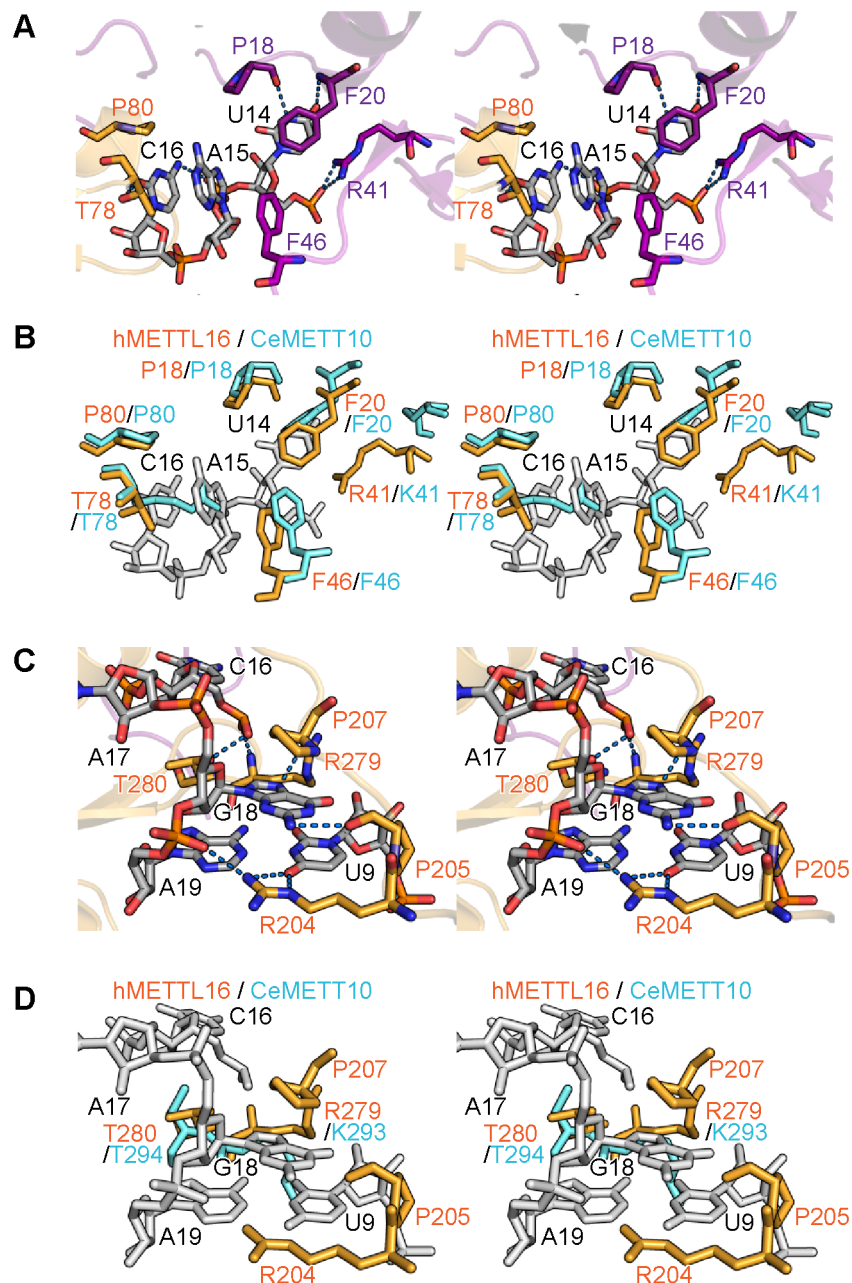


C



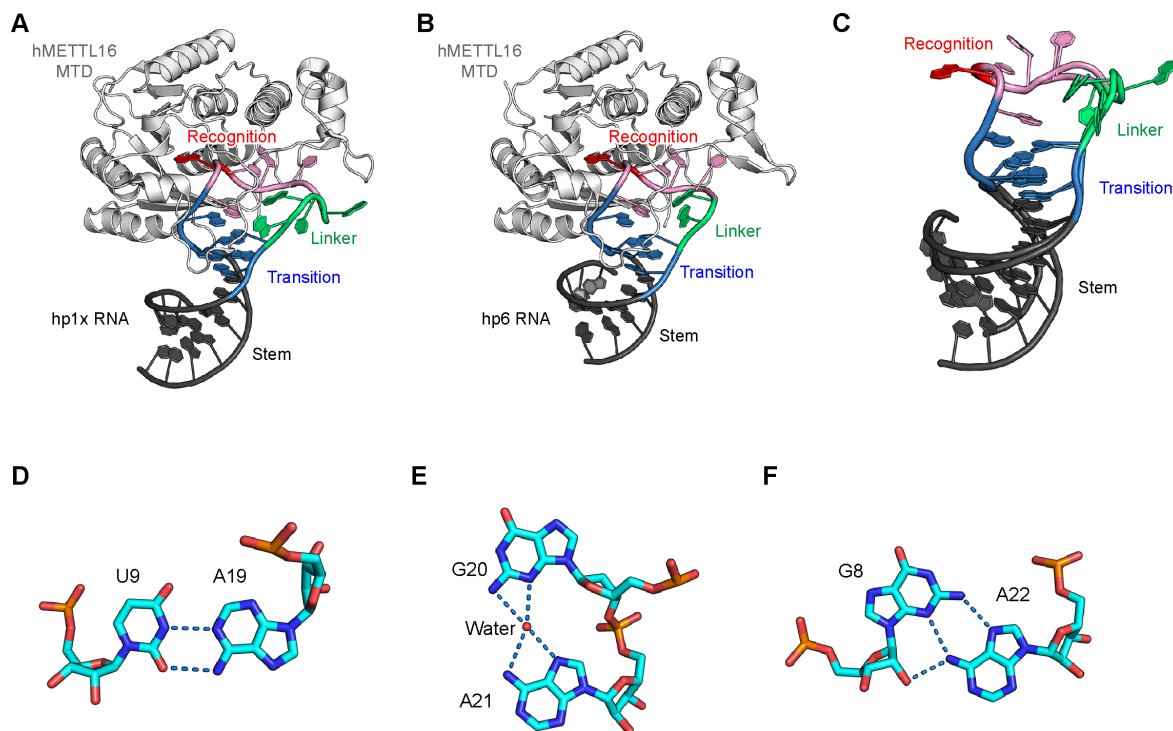
Supplementary Figure 5: Gel-shift assays

(A) Gel-shifts of *sams-hp* by CeMETT10-MTD and its variants (Δ N39, R217A and K196A/F197A). (B) Gel-shifts of *sams-hp-ls* by CeMETT10-FL Δ L and its variants (MTD and FL Δ L-RRR/7E). (C) Gel-shifts of U6 snRNA by CeMETT10-FL Δ L and its variants (MTD and FL Δ L-RRR/7E).



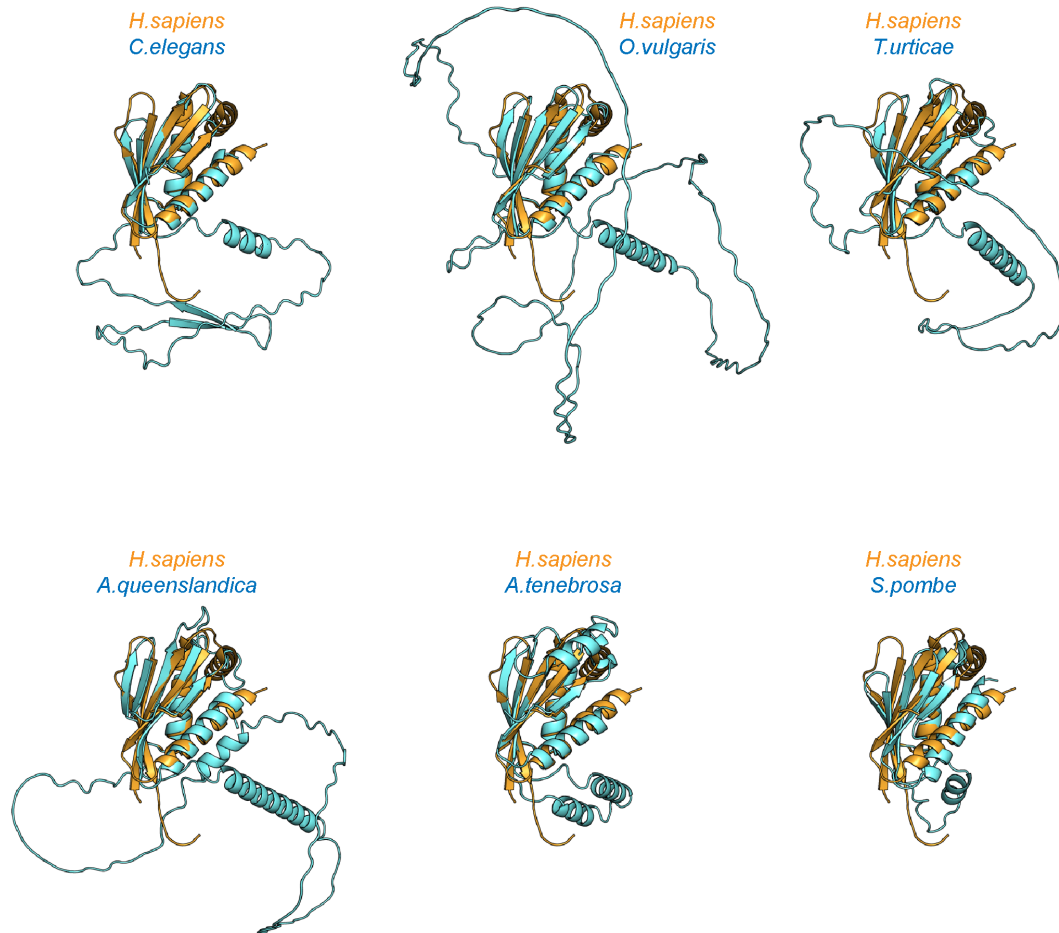
Supplementary Figure 6

(A) Interaction between hMETTL16 and MAT2A-hp (residues U14A15C16 are shown). (B) Superimposition of the structure of CeMETT10 (cyan) on the structure of hMETTL16 (orange) complexed with MAT2A-hp in (A). MAT2A-hp is colored gray. (C) Interaction between hMETTL16 and MAT2A-hp (residues A17G18A19 are shown). (D) Superimposition of the structure of CeMETT10 (cyan) on the structure of hMETTL16 (orange) complexed with MAT2A-hp in (C).



Supplementary Figure 7

(A) Structure of METTL16-MTD in complex with MAT2A-hp1x RNA (PDB: 6DU4) (1). (B) Structure of METTL16-MTD in complex with MAT2A-hp6 RNA (PDB:6DU5) (1). (C) Superimposition of the MAT2A- hp1x and MAT2A-hp6 RNAs (1) (D) Structures of the base pairs in the transition region of *MAT2A*-hp1 in a complex with hMETTL16-MTD. U9 and A19 form the reverse Watson-Crick base pair. (E) G20 and A21 are in the same plane and interact through water-mediated hydrogen bonds. (F) Hydrogen bonds between the sugar edge of G8 and the Hoogsteen edge of A22.



Supplementary Figure 8: Structural comparison of the C-terminal region of h METTL16 to those of other organisms.

Model structures of the C-terminal regions of human METTL16 homologs from invertebrates [*C. elegans* (MET16_CAEEL), *Octopus vulgaris* (A0A6P7TIF1_OCTVU), *Tetranychus urticae* (T1JVZ0_TETUR), *Actinia tenebrosa* (A0A6P8ITS0_ACTTE), and *Amphimedon queenslandica* (A0A1X7U5N0_AMPQE) and fungus [*Schizosaccharomyces pombe* (MTL16_SCHPO)] generated by AlphaFold2 (2,3). The C-terminal region of human METTL- 16, termed KA-1, is colored orange (PDB: 6M1U) (4).

```

hMETTL16_6gfnA 53 -----ALCTLLRREDPGL---SIDIPLERL 75
CeMETT10      53 -----CLTQTLLKKDFNL---DVEIIPPHL 75
hBCDIN3D_618uA 28 -----APFGNFPFH 42
hMePCE_6dcca 411 -----KFQYGNFYCK 420
hTRMT61A_5ccbA 64 -----WVYVLPHTPELWTLNLPH---RT 84
hNSUN6_5wwsA 133 PGIVSASQFMKAGDVISVYSDIKKCKKGAKEFDGTVKVLNGISELRSKEIFSGLPPELKGMGIRMTPEVYVLSPSFDSVLPRLYLF 217
mDNMT1_4da4A 1092 -----

```

```

                                D108 G110                                E133 V134
hMETTL16_6gfnA 76 IP-----TVPLRNYTHWSDLIGH---QDKSTLRRGTDICFGASCIIYFELGATLNG-----W-YF-LATEVDDMCFNIAKKNVEQNN-LSDLRKV 157
CeMETT10      76 VP-----RVPQRKNYCLLIDLLKANKLT---KNVIGHDIDEGTSCITHALIGARQFN-----W-KF-LARDGDEKSVRVAEENVAKNG-LSSSTCV 155
hBCDIN3D_618uA 43 YS---RFHPPEQRTR--LPPPELLRQLFPSPSENGPILGDDVGCN--SGDSDVLYKHFSLASREF-RL-LCCDIDPVLVVRABREC---P-FEDADTF 131
hMePCE_6dcca 421 YYGYRNPSCEDEGRVR--LKPESW-----FRGRDVIDLGCNV--GHDTLSLACKWG-----PSRM-VGLDIDSRILHSARONIRHYLSVFPNNVVF 555
hTRMT61A_5ccbA 85 QI---LYSTDIALITMME---LR---PGSVVCESGTG--SGSVSHAIRITA-----PTG-HL-HIVVEFHQQRARAKARBEQERH-VGRVAVV 159
hNSUN6_5wwsA 217 -L---QNLPSALVSBVLN--PQ---PGEKIIDICAA--EGSKTTHLALMHH---DQG-EV-LALDKIFNKVEKIKONALLG--LN-SIRA 289
mDNMT1_4da4A 1092 -----NSKTKNFEDPPNHARSPGNIKL---PKLRTIDVPSGC--GGLSSEGFHQAGI-----S-ETLWATEMWDPAQAQFRNNP-----GTIV 1189
                                Motif 1                                Motif 2

```

K-loop

```

                                T164
hMETTL16_6gfnA 158 VKVP--QKTLIM-----DAI--KEES--EIIIDFQMCNPE--F-----GEL---EF-----VKRIITHDSLQ--LKK 240
CeMETT10      156 VHVNDVKTVM-----DVV--NTIP--DIDAFQMCNPE--F-----GEV---AF-----VRIITDSDVL--LRD 253
hBCDIN3D_618uA 132 IITL--DFMNRTRK-----VIL--SSFLSQFGRSVVDIGFCMSI-----TMW---IHLNHGDHGLWBEIAHL--SS 188
hMePCE_6dcca 556 VTG---NRYVID-----RDDLVEAQTEPDVVLCSLTK-----WVHLNWDEEG--LKRMRRIYR--HLR 608
hTRMT61A_5ccbA 160 RQV---DVCRSG--FCV--S---HVADAVFDIE-----PWEVGLAWDALKVE 199
hNSUN6_5wwsA 290 FCF---DQKAVKLDVVEPFL--S---PESDRIITDAP--CSGMGQRPMACTWSVKEVASYQ--PL---QRKLFPAVQ--LLK 363
mDNMT1_4da4A 1190 FTE---DCNVLKLVMAGEVTNSLGEQLPQ--KGDVEMLCGGPE--C---QGFSGMNRNFSRTYSKFKN-----SL---VVSFTSYCDY--YR- 1262
D135(hBCDIN3D) Motif 3

```

```

hMETTL16_6gfnA 241 RLRWYSCML-GKK-----C-SIAPLKEPRIQG-V-EKV-T-----Y-TEFC-Q----- 277
CeMETT10      254 RIKLYTMTI-GRK-----S-SIKPLQNRQRFQDD-VKI-M-----I-SVLN-Q----- 291
hBCDIN3D_618uA 189 LCHYLVWEP-QPWKCYRAARLRKLGHDHDFHSLAIRGD-MENQIVQIHTQD-HG-METIC-----C-FGN----- 252
hMePCE_6dcca 609 PGGIILWLEP-QPW-----SSYGKRKRLTETIYKNIYRIQLKKEPFSSYITSDVGFSSY-E-----LVATPHNT----- 670
hTRMT61A_5ccbA 200 G-GRFCSSFS-PCI-----E-VQRTQQLAAR--CFSEL-S-----T-LEVL-PQVYV 240
hNSUN6_5wwsA 364 PEGVIVYSCTIT-----LAENDEQAVAWALKFPC-LQL-QPQEPQIGGEGMRGAGLSCBQLKQL-QRFD-P----- 426
mDNMT1_4da4A 1262 -PRELELEN-VRN-----FVSYRRSM-VIKRLTRCIVRM--S-YQC-T-----F-GVLQ-A----- 1304

```

```

hMETTL16_6gfnA 277 -----G--R-----T-MRWALAMSEYD----- 291
CeMETT10      291 -----G--K-----T-KRRLAMTFESKS-VSL----- 309
hBCDIN3D_618uA 252 -----S--K-----TSW-DRSLLPRA----- 264
hMePCE_6dcca 670 -----S--K-----GFQREVILFHKARSP----- 687
hTRMT61A_5ccbA 241 RTVSLPPPDLGTGDTSPFRSGTFMKEAV--G-----H-TGYLTFATKTPG----- 289
hNSUN6_5wwsA 427 -----S-----AVPLPDTDMDSLREARRDMLRLANKDSI--GEIYAKFVKC----- 466
mDNMT1_4da4A 1304 -----GQYGVQAQT-----R-RRATHLAAAP-G-EKLPPLPEPLHVFAPRACQLSVV 1347

```

Supplementary Figure 9: Alignment of the amino acid sequences of the Rossmann-fold methyltransferases. Human METTL-16 (hMETTL16), *C. elegans* METT10, human MePCE (hMePCE) (5), human BCDIN3D (hBCDIN3D)(6,7), human TRMT61A (hTRMT61A) (8), human NSUN6 (hNSUN6)(9) and mouse DNMT1 (mDNMT1)(10). Motifs 1, 2 and 3 are involved in the recognition of SAM.

Supplementary Table 1: Nucleotide sequences of synthetic genes

<p><i>C. elegans</i> METT-10 gene</p> <p>5'- ATGAGTCAGAACAACGAAATGCATCCTCGTAATCCTTACCGCAACAAACCGCCGGATTT CAAAGCGTTAGCTGTGGAATATCCGGAATTTTCGCAAATTCTGTCAGTATGTGTCTAACGG AAAAGTTACCTTCGACTTCAAAAAAGACGCCGCGGTTTCGTTGTCTAACACAAACCTTGC TGAAGAAAGATTTCAATCTGGATGTCGAGATTCCGCCTGGTCATCTGGTCCCAAGGGTTC CTCAGAAACTGAACTATTGCCTGCTGATTGATGATCTGCTGAAAGCCAACAAACTCACC AAGAATGTGATCGGCATTGATATAGGTACCGGCACTTCCTGCATTCATGCACTGATTGGG GCTCGTCAGTTCAACTGGAAGTTCATCGCTACAGATGGCGACGAAAAAGCGTGCGAG TTGCACACGAGAATGTGCTAAAAACGGCTTGTCTTCTAGCATTGCGTTGTGCACGTTA ATCCGGATGTGAAGACGGTGCTGATGGATGTGGTCAATACGATTCCAGACACTGACTATG CGTTTTGCATGTGCAATCCGCCGTTTTTTGAGAAAGGTAATGGCGATGACAAATTTTTCG AGGATATCTCAAGTTCGACAGAAACCTACAGCAACCGTGTAGCCTCCGAATTTTCGGACT GCACCGCATTCTGCGACCTTTGCGTCGAGTGGCGAACTGTTTGTGATGGTGGAGAAGT GGCGTTCGTGAACCGCATTATCGATGACTCCGTGTTGTTACGCGATCGAATTAAGATCTA CACCACGATGATTGGCCGCAAATCATCGCTCAAACCCTTGCAGAACCGCTTACAGCGCT TTGGTGATGACGTAAAAATCATGATCTCAGTCTTGAACCAAGGCAAAACGAAACGTTGG ATGCTGGCGTGGACCTTTTCCAAAAGCGTAAGTCTGACGACCATTGATCGTCCCATCTCG TTTCAGTGTCCAAAACCCGGCTTAACTCGGCTGATGCAGGAAATTAGCATCTTAAACGG ACGTCTGCGTCAGGAAGACACACTGGCCATTGTGGCGGAGTTCAAGTGTGTAACGTGG ACCAATCAACGTGCCAGAAAACGCGCAAAAGCCATTCTTAGCGAAAGCTCAATCAAAA AGGCCAAGTGGAACCTTAGCAACGTGGCATGTCAGGTTGCATTTGGTGCTGGTGTATGGG AAAGACTCCTATACCGATGCAGGGAACCTCGTTAGTTCGGAATCTATTCGACGAATAAT CTGAATGCCTGGGACAATGCGTCACAAGCGTATTTCCCGCTTCCAAATGGCGAAGTTCC GGGTCCGATCATTTCGCATCCGTATACAGGTATTTTCGGAAGATAGCTACGACAGCATCAG TTTTGAGCTTATTTCCGGCAGCAAACAACATCTCCACCAACTCGTCCAGTACCTGAAAA ACCTTATCTGCCGCTGA -3'</p>
<p>sams hp-ls gene for <i>in vitro</i> transcription</p> <p>5'- ATCGGAATTCTAATACGACTCACTATAGGCAACAATATGTTTCTTTACCTGTTACAACGGT TACTTTGATTACAGAAACGGTGACTAAAACGGGTATGATCATGTTGTGCCCCATACGCGT CATCCAAGCTTATCG -3'</p>
<p><i>C. elegans</i> U6 snRNA gene for <i>in vitro</i> transcription</p> <p>5'- ATCGGAATTCTAATACGACTCACTATAGTTCTTCCGAGAACATATACTAAAATTGGAACAA TACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCAAATTCGTGAAGCGTTC CAAATTTTTTCATACGCGTCATCCAAGCTTATCG -3'</p>

Supplementary Table 2: Oligo-nucleotide sequences

Primer name	Sequence
METT10_Forward	5'- CTGCAGCATATGAGTCAGAACAACGAAATGCATCCTC - 3'
METT10-FL_Reverse	5'- GACCTCGAGGCGGCAGATAAGGTTTTTCAGG- 3'
METT10-FL-ΔL_For	5'- GGCGAAGTTCCGGGTCCGATCATTG -3'
METT10-FL-ΔL_Rev	5'- TGCCACGTTGCTAAAGTTCCACTTG -3'
METT10-FLΔL- RRR/7E Forward	5'- GAAGAAGAAGAAGCCATTCTTAGCGAAAGCTC -3'
METT10-FLΔL- RRR/7E Reverse	5'- TTCTTCTTCTTGATTGGTCCACGTTACACAC -3'
METT10-MTD_1-314_Rev	5'- TATCTCGAGACGATCAATGGTCGTCGACTTACG -3'
METT10-MTD_ΔN-40- 314 Forward	5'- GGAAAAGTTACCTTCGACTTC -3'
METT10-MTD_ΔN-40- 314 Reverse	5'- CATATGTATATCTCCTTCTTAAAGT -3'
METT10-MTD_G108C_For	5'- TGCACCGGCACTTCCTGCATTCATG -3'
METT10-MTD_G108C_Rev	5'- TATATCAATGCCGATCACATTCTTGG -3'
METT10-MTD_D131A_For	5'- GCGGGCGACGAAAAAAGCGTGCGAG -3'
METT10-MTD_D131A_Rev	5'- TGTAGCGATGAACTTCCAGTTGAAC -3'
METT10-MTD_K163A_For	5'- GCGACGGTGCTGATGGATGTG -3'
METT10-MTD_K163A_Rev	5'- CACATCCGGATTAACGTGCACAAC -3'
METT10-MTD_M167A_For	5'- GCGGATGTGGTCAATACGATTC -3'
METT10-MTD_M167A_Rev	5'- CAGCACCGTCTTCACATCCG -3'
METT10-MTD_N184A_For	5'- GCGCCGCCGTTTTTTGAGAAAGGTAATG -3'
METT10-MTD_N184A_Rev	5'- GCACATGCAAAACGCATAGTCAGTGTCTG -3'
METT10-MTD_F187A_For	5'- GCGTTTGAGAAAGGTAATGGCGATGAC -3'
METT10-MTD_F187A_Rev	5'- CGGCGGATTGCACATGCAAAACGCATAG -3'
METT10- MTD_KF196/197AA_For	5'- GCGGCGTGCGAGGATATCTCAAGTTC -3'
METT10- MTD_KF196/197AA_Rev	5'- GTCATCGCCATTACCTTTCTCAAAAACGG -3'
METT10-MTD_R217A_For	5'- GCGACTGCACCGCATTCTGCGACCT -3'
METT10-MTD_R217A_Rev	5'- AAATTCGGAGGCTACACGGTTGCTGTAG -3'

Supplementary Table 3: Nucleotide sequences of RNA substrates.

RNA	Sequence
sams-hp	5'- GGGUUACUUUGAUUACAGAAACGGUGACCC -3'
Δ stem	5'- GGGUCUUUGAUUACAGAAACGGUGACCC -3'
R1	5'- GGGUUACUUUGAUCACAGAAACGGUGACCC -3'
R2	5'- GGGUUACUUUGAUUAUAGAAACGGUGACCC -3'
L1	5'- GGGUUACUUUCAUACAGAAACGGUGACCC -3'
L2	5'- GGGUUACUUUGUUUACAGAAACGGUGACCC -3'
T1	5'- GGGUUACUUAGAUUACAGAAACGGUGACCC -3'
T2	5'- GGGUUACUGUGAUUACAGAAACGGUGACCC -3'
T3	5'- GGGUUACUUUGAUUACAGAUUCGGUGACCC -3'
T4	5'- GGGUUACUUUGAUUACAGAUUGGGUGACCC -3'
sams hp-ls	5'- GGCAACAAUAUGUUUCUUUACCUGUUACAACGGUUACUUUGA UUACAGAAACGGUGACUAAAACGGGUAUGAUCAUGUUGUGCC C -3'
U6 snRNA	5'- GUUCUCCGAGAACAUAUACUAAAAUUGGAACAAUACAGAGA AGAUUAGCAUGGCCCCUGCGCAAGGAUGACACGCAAUUCGU GAAGCGUUCCAAUUUUUU -3'

References

1. Doxtader, K.A., Wang, P., Scarborough, A.M., Seo, D., Conrad, N.K. and Nam, Y. (2018) Structural Basis for Regulation of METTL16, an S-Adenosylmethionine Homeostasis Factor. *Mol Cell*, **71**, 1001-1011 e1004.
2. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A. *et al.* (2021) Highly accurate protein structure prediction with AlphaFold. *Nature*, **596**, 583-589.
3. Varadi, M., Anyango, S., Deshpande, M., Nair, S., Natassia, C., Yordanova, G., Yuan, D., Stroe, O., Wood, G., Laydon, A. *et al.* (2021) AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Res.*, **50**, D439-D444.
4. Aoyama, T., Yamashita, S. and Tomita, K. (2020) Mechanistic insights into m6A modification of U6 snRNA by human METTL16. *Nucleic Acids Res*, **48**, 5157-5168.
5. Yang, Y., Eichhorn, C.D., Wang, Y., Cascio, D. and Feigon, J. (2019) Structural basis of 7SK RNA 5'- γ -phosphate methylation and retention by MePCE. *Nat Chem Biol*, **15**, 132-140.
6. Martinez, A., Yamashita, S., Nagaïke, T., Sakaguchi, Y., Suzuki, T. and Tomita, K. (2017) Human BCDIN3D monomethylates cytoplasmic histidine transfer RNA. *Nucleic Acids Res.*, **45**, 5423-5436.
7. Liu, Y., Martinez, A., Yamashita, S. and Tomita, K. (2020) Crystal structure of human cytoplasmic tRNA^{His}-specific 5'-monomethylphosphate capping enzyme. *Nucleic Acids Res.*, **48**, 1572-1582.
8. Finer-Moore, J., Czudnochowski, N., O'Connell, J.D., 3rd, Wang, A.L. and Stroud, R.M. (2015) Crystal Structure of the Human tRNA m(1)A58 Methyltransferase-tRNA(3)(Lys) Complex: Refolding of Substrate tRNA Allows Access to the Methylation Target. *J Mol Biol*, **427**, 3862-3876.
9. Liu, R.J., Long, T., Li, J., Li, H. and Wang, E.D. (2017) Structural basis for substrate binding and catalytic mechanism of a human RNA:m5C methyltransferase NSun6. *Nucleic Acids Res*, **45**, 6684-6697.
10. Song, J., Teplova, M., Ishibe-Murakami, S. and Patel, D.J. (2012) Structure-based mechanistic insights into DNMT1-mediated maintenance DNA methylation. *Science*, **335**, 709-712.