SUPPLEMENTARY INFORMATION

Structural analysis of human 2′-*O*-ribose methyltransferases involved in mRNA cap structure formation

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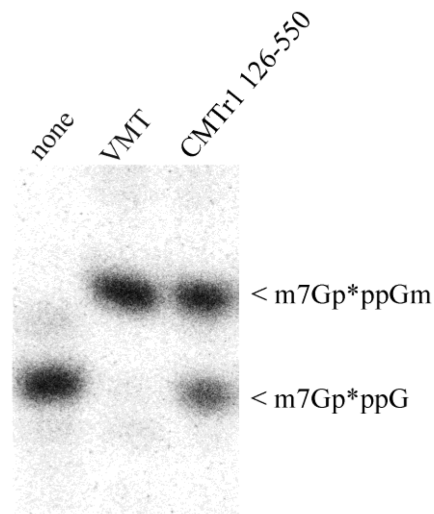
This Supplementary Information contains:

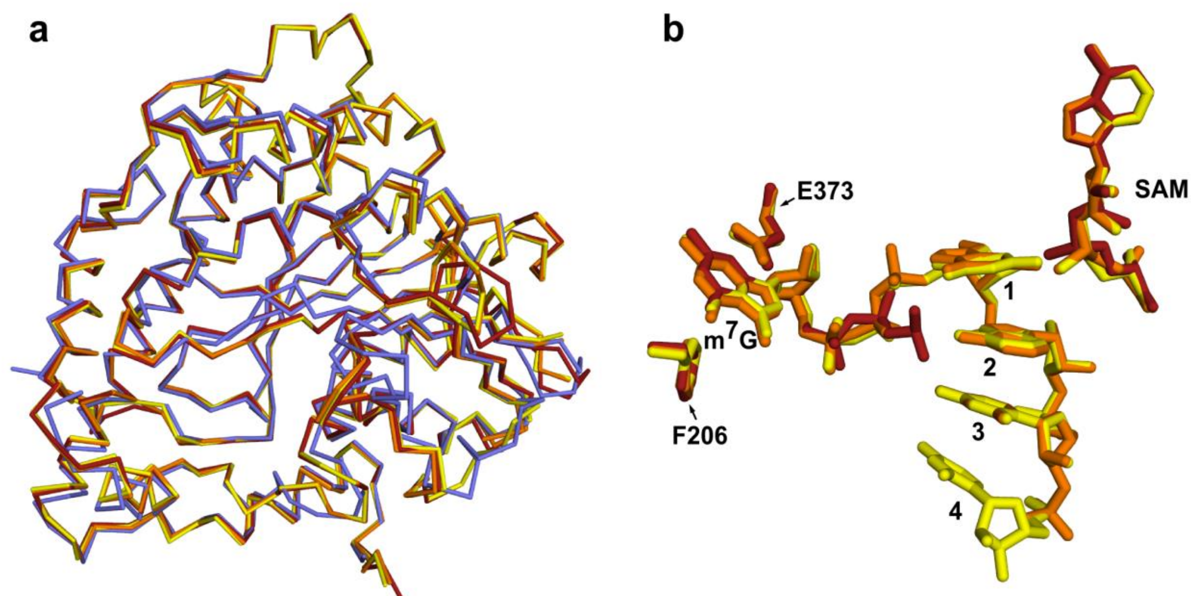
Supplementary Figures S1–S6

Supplementary Table S1

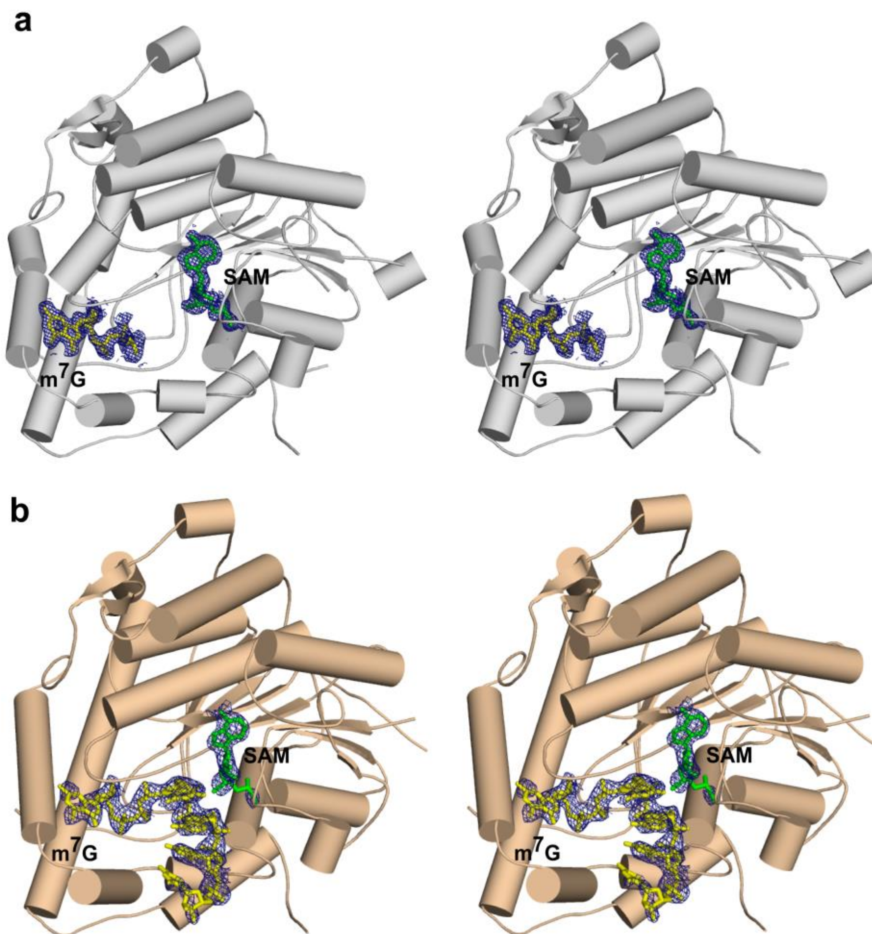
SUPPLEMENTARY FIGURES



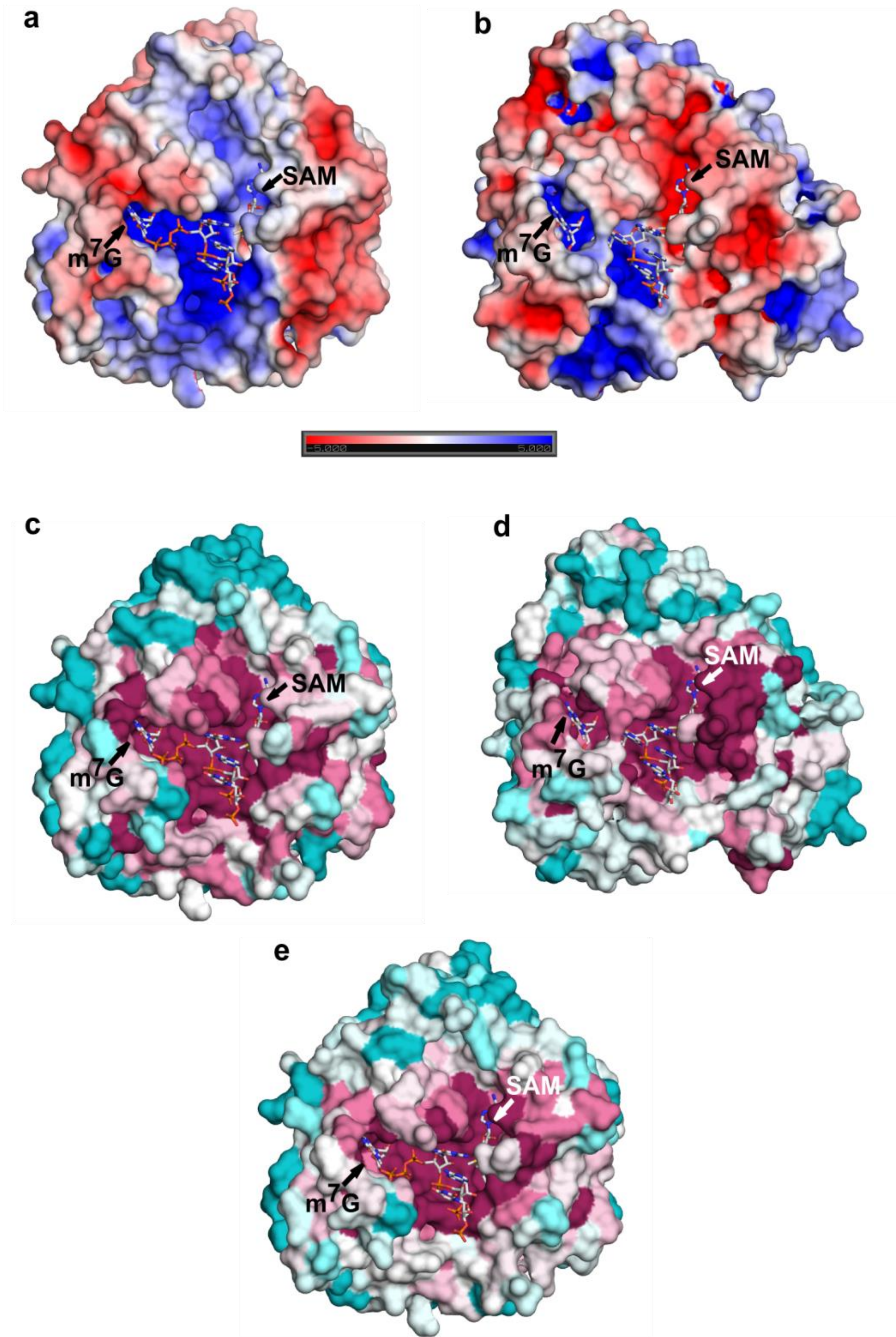
Supplementary Figure S1 | Methyltransferase activity of CMTr1$_{126\text{-}550}$. Reaction mixtures that contained RNA-GG molecules with a ^{32}P-labeled cap0 structure were incubated with enzymes indicated above the gel picture in the presence of SAM at 37 ºC for 90 min. Vaccinia virus cap1 MTase VMT was used as a positive control. After the reaction, RNA molecules were purified by phenol/chloroform extraction and ethanol precipitation, followed by digestion with nuclease P1. The digestion products were resolved on 21% polyacrylamide/8M urea gel and visualized by autoradiography. Asterisks indicate the positions of ^{32}P-labeled phosphates.



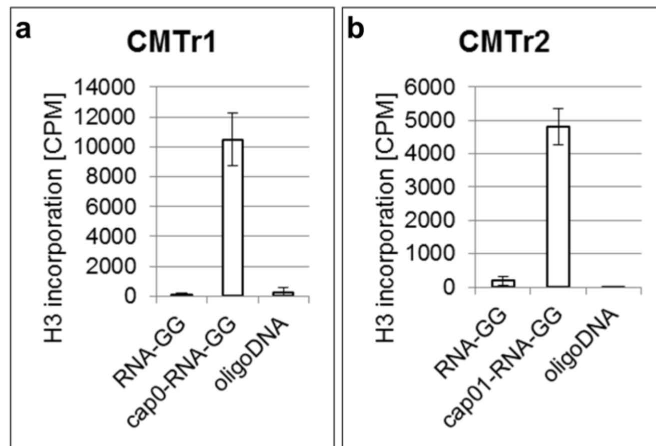
Supplementary Figure S2 | Structural comparison of all determined CMTr1$_{126\text{-}550}$ variants and their ligands. (**a**) Ribbon representation of superimposition of all crystallized CMTr1$_{126\text{-}550}$ variants. Both particles of CMTr1$_{126\text{-}550}$ crystallized with m^{7}GpppGAUC and SAM (structure [iii]), and CMTr1$_{126\text{-}550}$ in complex with the cap analog and SAM (structure [ii]) are nearly identical. The structure of the unliganded enzyme (structure [i]) is slightly different, especially in the region of loops involved in substrate binding. (**b**) Superimposition of the ligands present in both copies of the complex in structure (iii) (particle A is colored orange and particle B is colored yellow) and structure (ii) (colored red). The conformation of the 5′-5′ triphosphate bridge structure is significantly different with the cap analog, although the F206 and E373 stacking residues have the same orientation in all of the CMTr1$_{126\text{-}550}$ crystal structures.



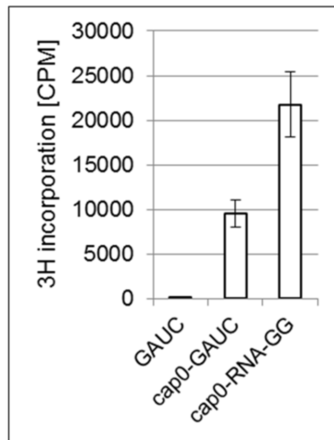
Supplementary Figure S3 | Stereo view of ligand orientation in structures of CMTr1 catalytic domain obtained by co-crystallization with (**a**) m^7GpppG and SAM and (**b**) m^7GpppGAUC and SAM. Simulated annealing omit maps (σ-cutoff = 3σ) for the ligands are shown as blue mesh.


a
SAM
m7G
b
SAM
m7G
-5.000
5.000
c
SAM
m7G
d
SAM
m7G
e
SAM
m7G
Color-coding scheme
1
2
3
4
5
6
7
8
9
Variable
Average
Conserved

Supplementary Figure S4 | Electrostatic potential and conservation of the active site in CMTr1 and CMTr2 catalytic domains. The crystallographic model of $CMTr1_{126\text{-}550}$ (**a,c,e**) and the predicted structure of $CMTr2_{1\text{-}423}$ (**b,d**) are shown in surface representation. (**a,b**) Mapping of the electrostatic potential (±5 kT/e, red – negative; blue – positive) reveals that the RNA-binding grooves in both CMTr1 and CMTr2 structures exhibit similar distribution of positive charge, while their cofactor-binding sites are charged differently: SAM-binding site in CMTr1 is charged positively (**a**), while the SAM-binding site in CMTr2 is charged negatively (**b**). Mapping of residues conserved in either MTR1 (**c**) or MTR2 (**d**) families onto the respective structures reveals large patches of conservation (colored in purple) in and around the cofactor- and RNA-binding sites, while the rest of the protein surface exhibits little or no conservation (light pink or green). (**e**) Residues conserved between MTR1 and MTR2 families are concentrated in the region that in the $CMTr1_{126\text{-}550}$ complex structure interacts with SAM and the substrate tetranucleotide.



Supplementary Figure S5 | No internal methylation by CMTr1 or CMTr2. *In vitro*-transcribed RNA-GG molecules without a cap structure, with a cap0 (**a**) or cap01 (**b**) structure, or a DNA oligonucleotide were incubated with CMTr1 (**a**) or CMTr2 (**b**) in the presence of [^{3}H-methyl]-SAM. After 90 min incubation, the enzyme was heat-denatured, and the samples were loaded on DEAE cellulose. The membrane was washed, and the amount of ^{3}H-methyl group incorporation into the substrates bound to the membrane was measured. The signal obtained for the sample without the substrate was subtracted from the signals of the remainder of the samples. The analyses were performed in triplicate. Average results with standard deviations are shown.



Supplementary Figure S6 | CMTr1 methylates capped oligoribonucleotide substrate. 5´-phosphorylated tetraribonucleotide GAUC with phosphate group or cap0 structure on 5′ end or *in vitro* transcribed RNA-GG molecule with cap0 structure was incubated with CMTr1 in the presence of [^{3}H-methyl]-SAM. After 90 min incubation, the enzyme was heat-denatured, and the samples were loaded on DEAE cellulose. The membrane was washed, and the amount of ^{3}H-methyl group incorporation into the substrates bound to the membrane was measured. The signal obtained for the sample without the substrate was subtracted from the signals of the remainder of the samples. The analyses were performed in triplicate. Average results with standard deviations are shown.

SUPPLEMENTARY TABLE

Supplementary Table S1 | Primers used in this study

Primers for site directed mutagenesis of CMTr1 and CMTr2	
hMTr1-K203A_f	5′-CAGTGTGCGAGCGTGTTTGATG-3′
hMTr1-R203A_r	5′-CAACACACTGTGAAGCAGC-3′
hMTr1-R228A_f	5′-GCGGGAGTCTTCTTTCTAAACAGGGC-3′
hMTr1-R228A_r	5′-GATCATCTCATAGGGATTGGCC-3′
hMTr2-K74A_f	5′-CCTAAATGAAGTAGCAAACCTACTGAGTGATAAGAAACTG-3′
hMTr2-K74A_r	5′-GAGTTCTTCAAATCAAGAAATGCATTAAGTTC-3′
hMTr2-L77A_f	5′- AAAACCTAGCGAGTGATAAGAAACTGGATGAGTG-3′
hMTr2-S78A_f	5′- AAAACCTACTGGCTGATAAGAAACTGGATGAGTG-3′
hMTr2-77/78A_r	5′- TTACTTCATTTAGGGAGTTCTTCAAATCAAG -3′
hMTr2-W85A_f	5′- GGATGAGGCGCATGAGCACACTGC-3′
hMTr2-W86A_f	5′- GGATGAGTGGGCTGAGCACACTGC-3′
hMTr2-85/86A_r	5′- AGTTTCTTATCACTCAGTAGGTT-3′
hMTr2-T89A_f	5′-ATGAGCACGCTGCTTTCACTAATAAAG-3′
hMTr2-T89A_r	5′-GCCACTCATCCAGTTTCTTATC-3′
hMTr2-Q113A_f	5′-CTTTGTACTGCAGCATGGTGTAAGTTCCATGAG-3′
hMTr2-Q113A_r	5′-TTCAGCATTCACAGATTTTCTAACATGAG-3′
hMTr2-H142A_f	5′-TCTCTAGCCCTTTGTGAAGCTCCAGGAG-3′
hMTr2-E145A_f	5′-TCTCTACACCTTTGTGCAGCTCCAGGAG-3′
hMTr2-142/145.r	5′-ATTCAGTTTTCCATTCTGAAAAGCTTCC-3′
hMTr2-K307A_f	5′-GCTACTAGCGCGGCAGGAAACTCCGAAG-3′
hMTr2-K307A_f	5′-AGGTTTGAAAACATGGACTTGGTC-3′
Primers for creating deletion variants of CMTr1 and CMTr2	
hMTr1_1-550_f	5′-CCTCCGACCCTAAATCG-3′
hMTr1_1-550_r	5′-CTATTAAGAAGGAGCCACACG-3′
hMTr2_1-430_f	5′-ACAAAATGGTTTGGGCAG-3′
hMTr2_1-430_r	5′-CTATTAATTTGTACTACAACCAATACTAGAT-3′
hMTr1_550-835_f	5′-TCTTCCTCCGACCCTAAATC-3′
hMTr2_430-770_f	5′-AATACAAAATGGTTTGGGCAGAGG-3′
hMTr1/2_550-835/430-770_r	5′- CATGGCGGCCGCAAG-3′
hMTr1_126-550_f	5′-AGAGGCTTGGGTCTGACAC-3′
hMTr1_126-550_r	5′-GGCGCCCTGAAAATAAAGATTC-3′