## Supplementary Figures

**Figure S1**. *Conserved structures of the bacterial and human m$^1$A58 MTase.*

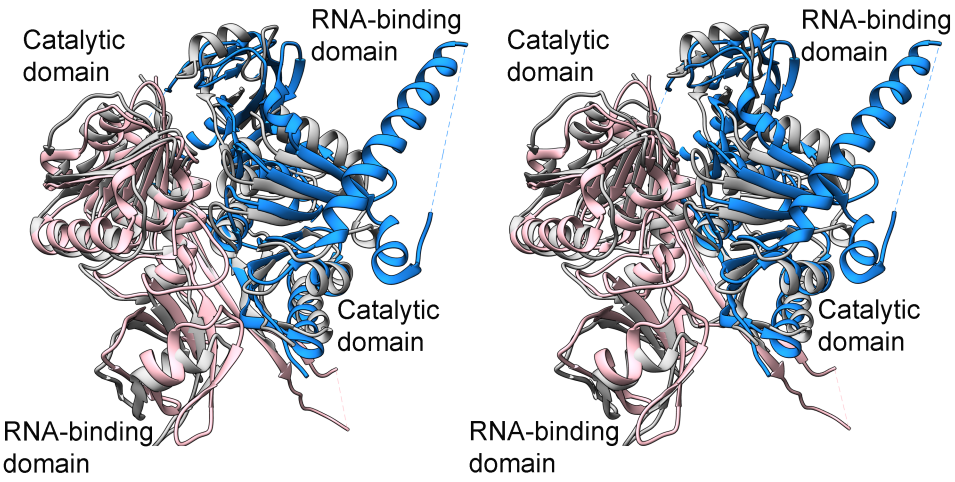


**Figure S1a.** Stereo cartoon rendition of the molecular replacement solution from a search with the *T. thermophilus* tight dimer (grey) superimposed on the refined human m$^1$A58 MTase heterodimer structure (Trm61 in pink and Trm6 in blue).

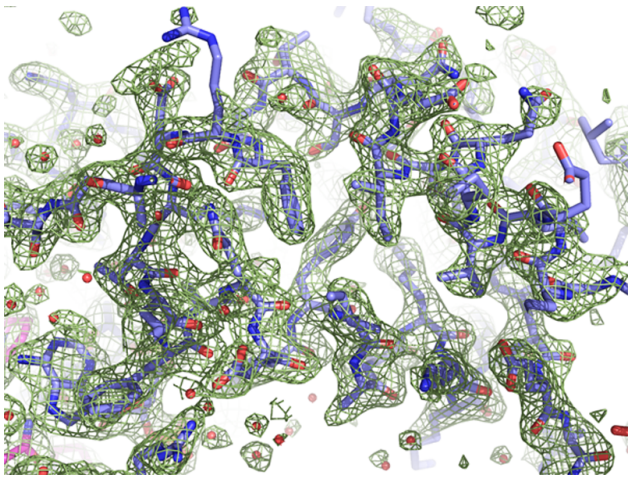

**Figure S1b.** Stick rendition of residues from the Trm6 83-residue insert, which were not present in the molecular replacement search model, overlaid with a composite omit map.

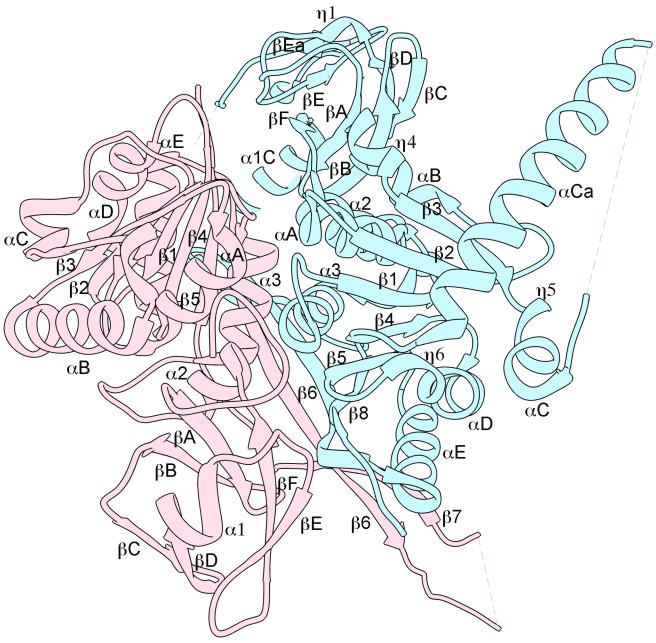


**Figure S1c**. Cartoon of the human m$^1$A58 MTase tight heterodimer. Trm61 is in pink and Trm6 is in blue. Both subunits have an N-terminal TRAM-like domain and a C-terminal domain with a class I MTase fold. Most of the 83-residue insert in the N-terminal domain of Trm6 is behind the clipping plane and so not visible in the figure. Secondary structure elements are labeled.

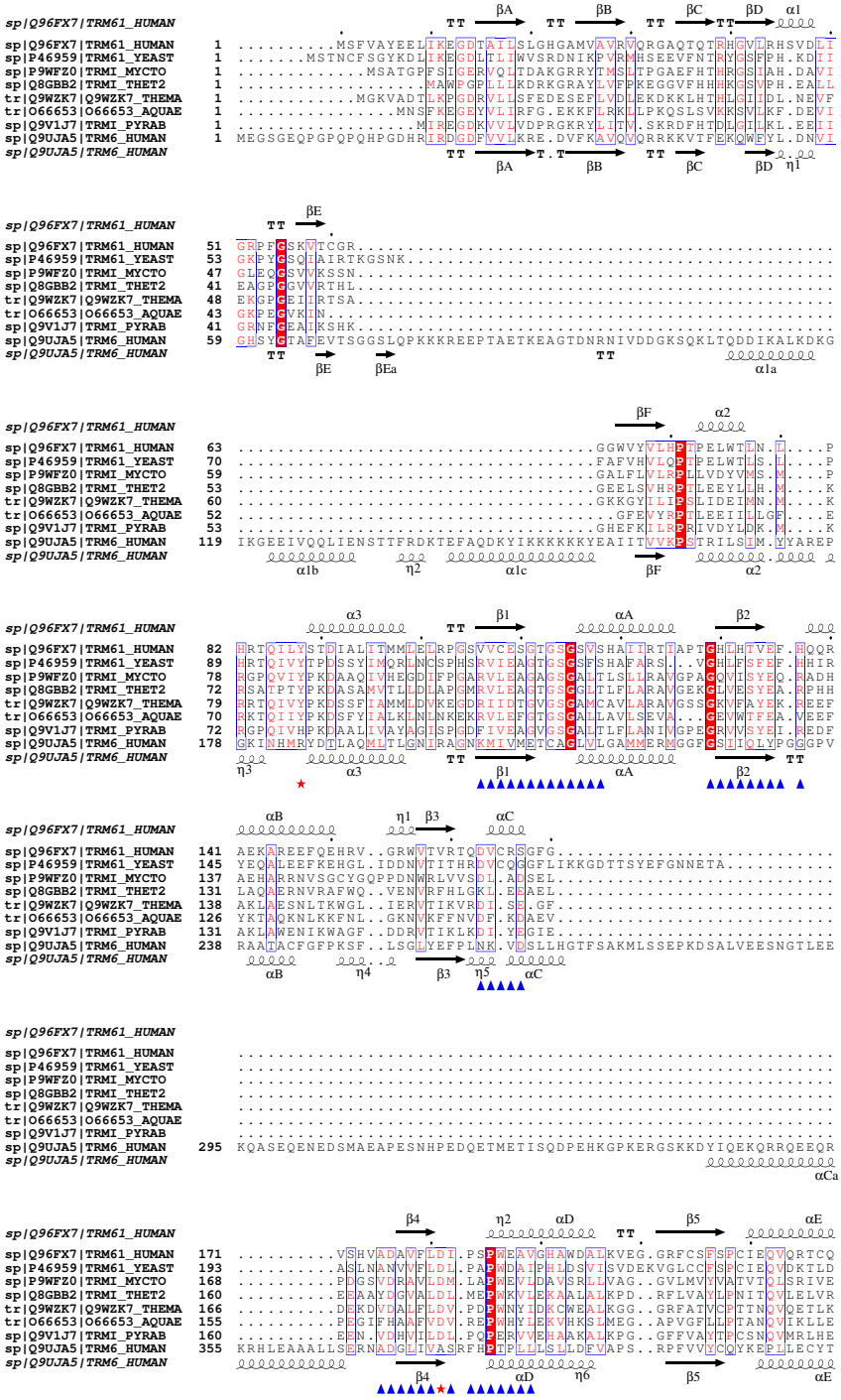
sp|Q96FX7|TRM61_HUMAN
sp|Q96FX7|TRM61_HUMAN 1
sp|P46959|TRM61_YEAST 1
sp|P9WFZ0|TRMI_MYCTO 1
sp|Q8GBB2|TRMI_THET2 1
tr|Q9WZK7|Q9WZK7_THEMA 1
tr|O66653|O66653_AQUAE 1
sp|Q9V1J7|TRMI_PYRAB 1
sp|Q9UJA5|TRM6_HUMAN 1
sp|Q9UJA5|TRM6_HUMAN
βA
βB
βC
βD
α1
η1
βE
βEa
α1a
βF
α2
α1b
η2
α1c
α3
β1
αA
β2
η3
αB
η1
β3
αC
η4
η5
αCa
β4
η6
β5
αE

**Figure S1d**. Structure-based alignment of sequences of, in the following order, human Trm61, *Saccharomyces cerevisiae* Trm61, *Mycobacterium tuberculosis* TrmI, *T. Thermophilus* TrmI, *Thermatoga maritima* TrmI, *Aquifex aeolicus* TrmI, *Pyrococcus abyss*i TrmI, and human Trm6. Secondary structure elements for Trm61 and Trm6 are indicated above and below the sequences with a coil for helix and an arrow for β-strand, and are labeled as in (**c**). Invariant residues are shown with white letters against a red background. Blue triangles below the aligned sequences identify conserved sequence motifs that surround the cofactor-binding site in SAM-dependent methyltransferases. The red stars identify the Asp and Tyr that are important for catalysis in TrmI.

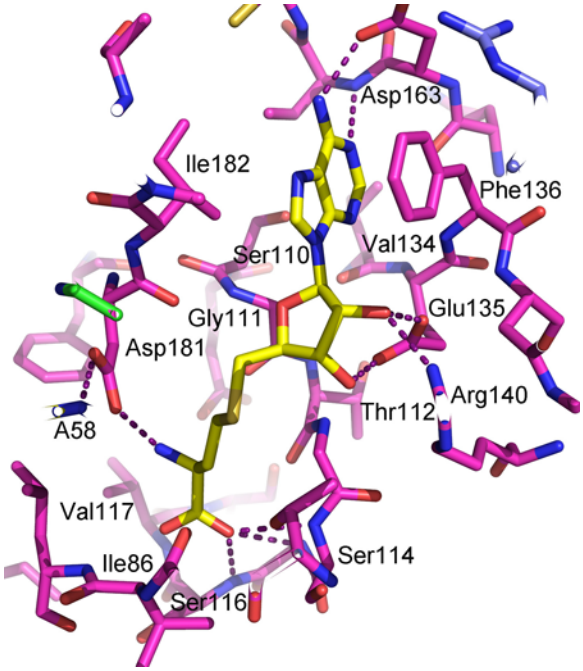


**Figure S2**. *Binding site for SAM in the Trm61 subunit*. SAH is shown in stick rendition with carbons colored yellow, nitrogen atoms blue and oxygen atoms red. Trm6 residues lining the binding site are shown with carbon atoms colored magenta. Hydrogen bonds between SAH and the protein are shown with dashed lines.

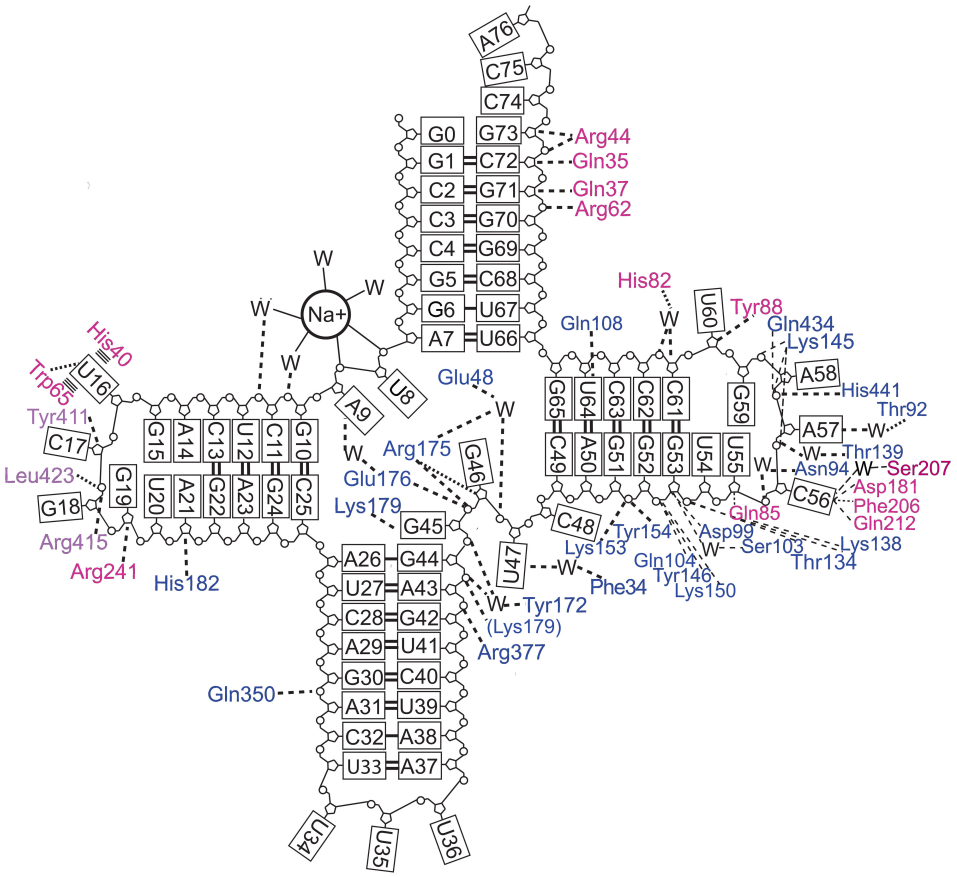


**Figure S3**. *Diagram of protein - $tRNA_3^{Lys}$ interactions*. Dashed lines indicate hydrogen bonds with protein side chains and dotted lines indicate hydrogen bonds with the protein backbone. Three parallel lines indicate stacking interactions. Double lines between tRNA bases indicate Watson-Crick base pairing while single lines indicate non-Watson-Crick base pairing. Residue names are color-coded according to the subunit they are part of, using the color scheme from Fig. 2.

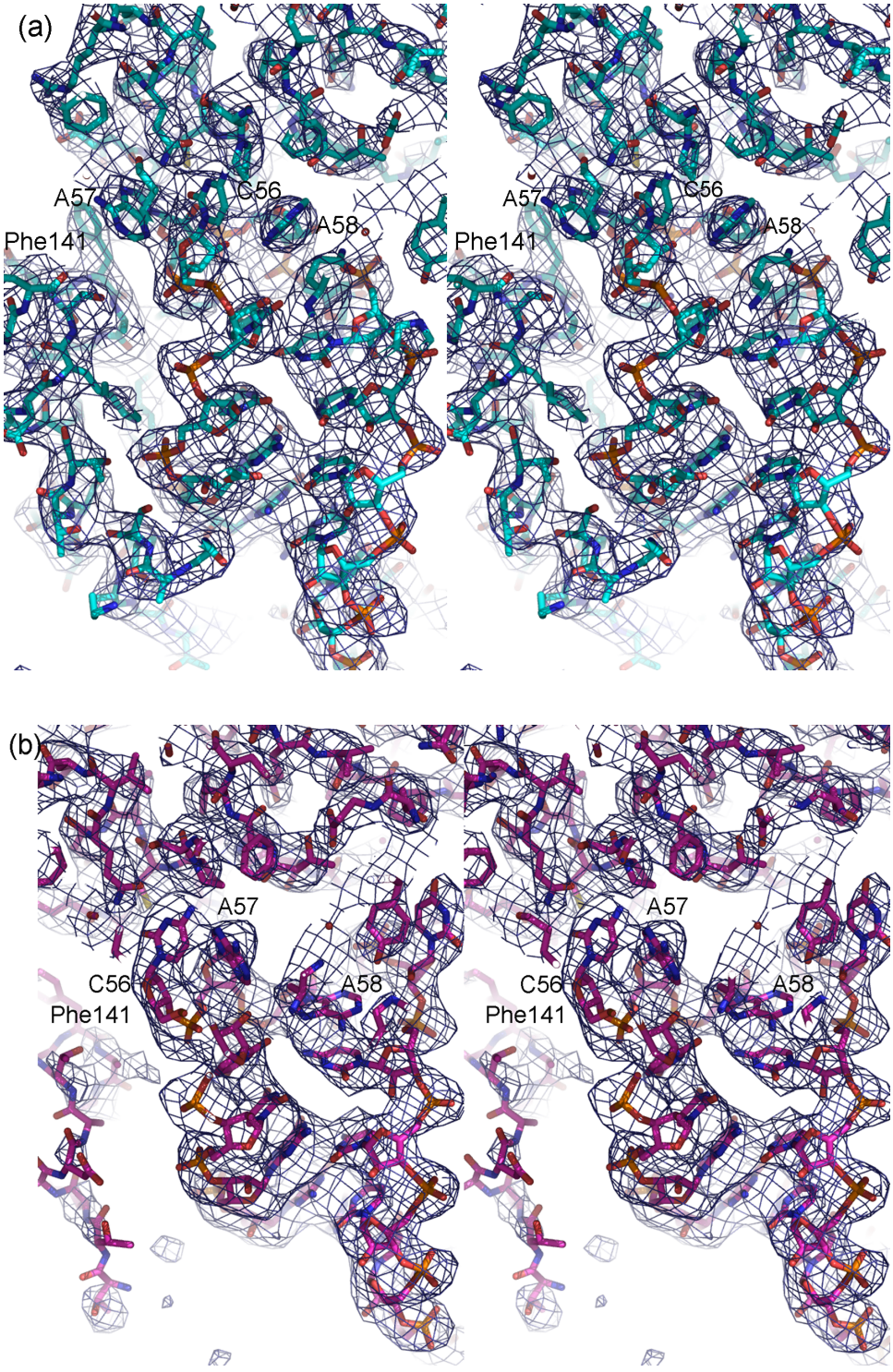


**Figure S4**. *Electron density at 3.6Å resolution for the $P2_12_12_1$ crystal form*. 2mFo-DFc density overlaid on portions of the TΨC-stem loops and Trm6 inserts from the a) productive heterodimer and b) nonproductive heterodimer in the $P2_12_12_1$ crystal structure

of the $m^1$A58 MTase-SAH-$tRNA_3^{Lys}$ complex. The regions shown and the views are approximately the same as in Fig. 6a.