Supplementary Data

Supplementary Figures

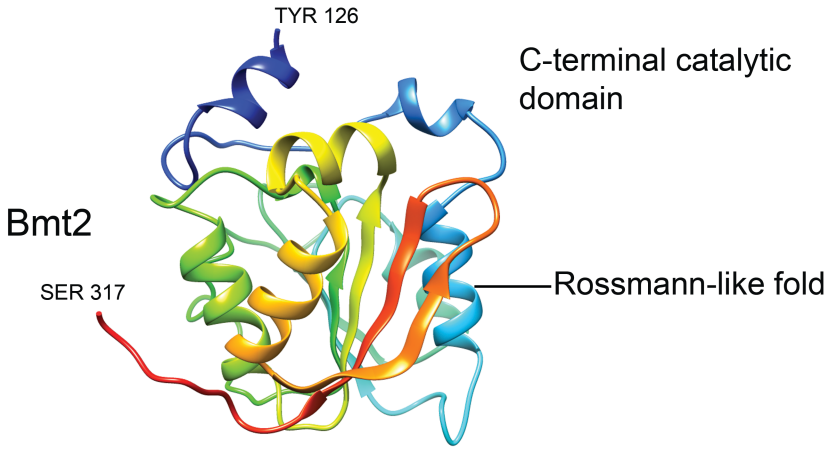

Figure S1. Structural model of Bmt2. Cartoon representing the 3D structure of the C- terminal catalytic domain of Bmt2. The model was constructed using the recent protocol described by Kelley et.al (2009). The 3D structure was constructed with sequence coverage of 52%, where 174 residues (52% of Bmt2 amino acid sequence) were modelled with 99.3% confidence by the single highest scoring template. This model further reinforced Bmt2 to be an Ado Met methyltransferase with the characteristic β sheets surrounded by helices belonging to Rossmann-fold superfamily.

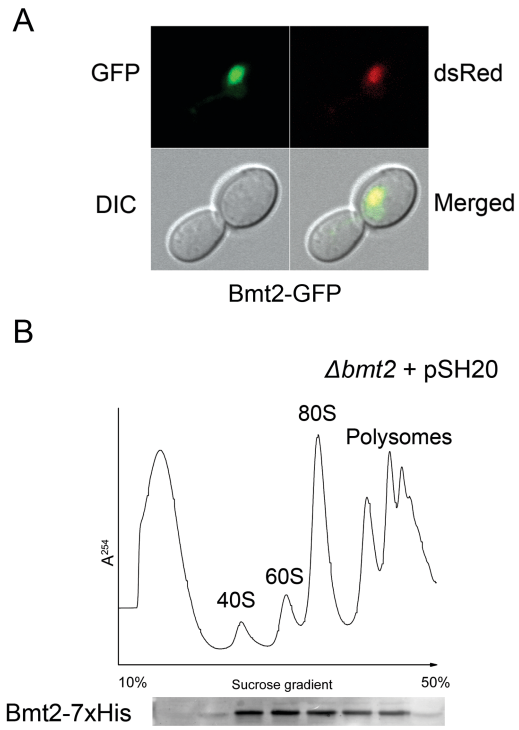

Figure S2. Cellular localization of the Bmt2 and its ribosomal association. (A) To test the nucleolar localization of Bmt2, pSH18 carrying Bmt2-GFP fusion constructs were transformed into strain ScNop56-mRFP and visualized with Leica TCS SP5. (B) To investigate any ribosomal or pre-ribosomal association of the Bmt2, plasmid pSH20 carrying heptahistidine tagged Bmt2 under a *TDH3* promoter, were transformed into *Δbmt2* mutant strain. The polysome profile was made from the transformed strain and the fractions corresponding to the cytoplasm, 40S, 60S, 80S, and polysomes were collected. The proteins from these fractions were precipitated and a western blot using anti-His antibodies was performed. The recombinant Bmt2 co-localized with the ribosomes.

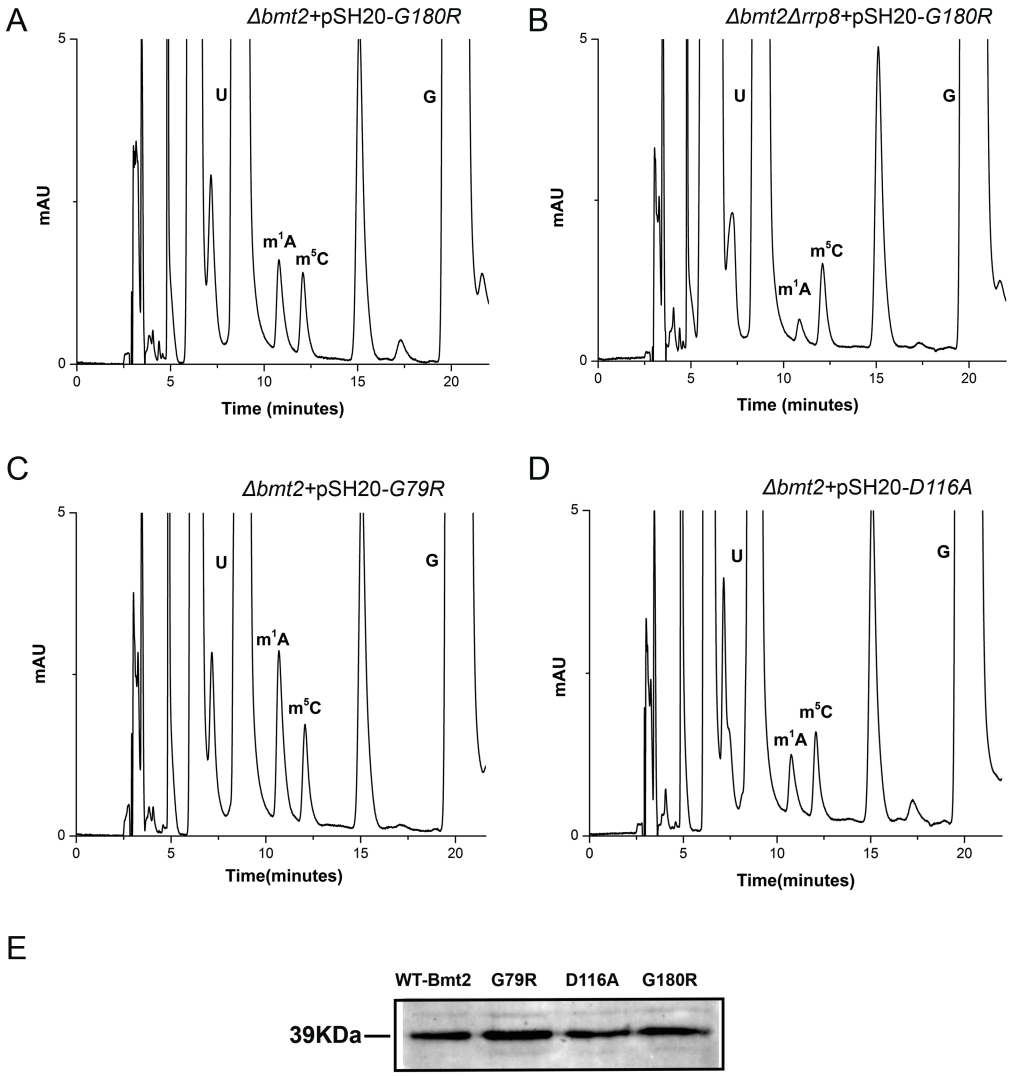

Figure S3. RP-HPLC analysis of the Bmt2-point mutants. The RP-HPLC chromatogram from the nucleosides derived from the 25S rRNA of *Δbmt2* strain carrying mutant bmt2 protein, where amino acid glycine180 was substituted with an arginine (*G180R*) (A). B) RP-HPLC chromatogram from the nucleosides derived from the 25S rRNA of *Δbmt2 Δrrp8* strain carrying mutant bmt2-*G180R* protein. C) RP-HPLC chromatogram from the nucleosides derived from the 25S rRNA of *Δbmt2* strain carrying mutant protein bmt2-*G79R* and (D) bmt2-*D116A*. The mutant bmt2 proteins were expressed from plasmids pSH20-*G180R*, pSH20-*G79R,* pSH20-*D116A.* The substitution of *G180R* and *D116A* influenced the catalytic function of Bmt2, as seen by loss of m^1A peak in the mutants. Interestingly, the bmt2-*G180R* possessed a slight residual activity as observed in *Δbmt2 Δrrp8* strain. E) Western blot analysis with the mutant proteins bmt2-*G79R*, bmt2-*D116A* and bmt2-*G180R* using the anti-His antibodies. The mutant proteins were expressed as C-terminally heptahistidine tagged protein from the respective plasmid.

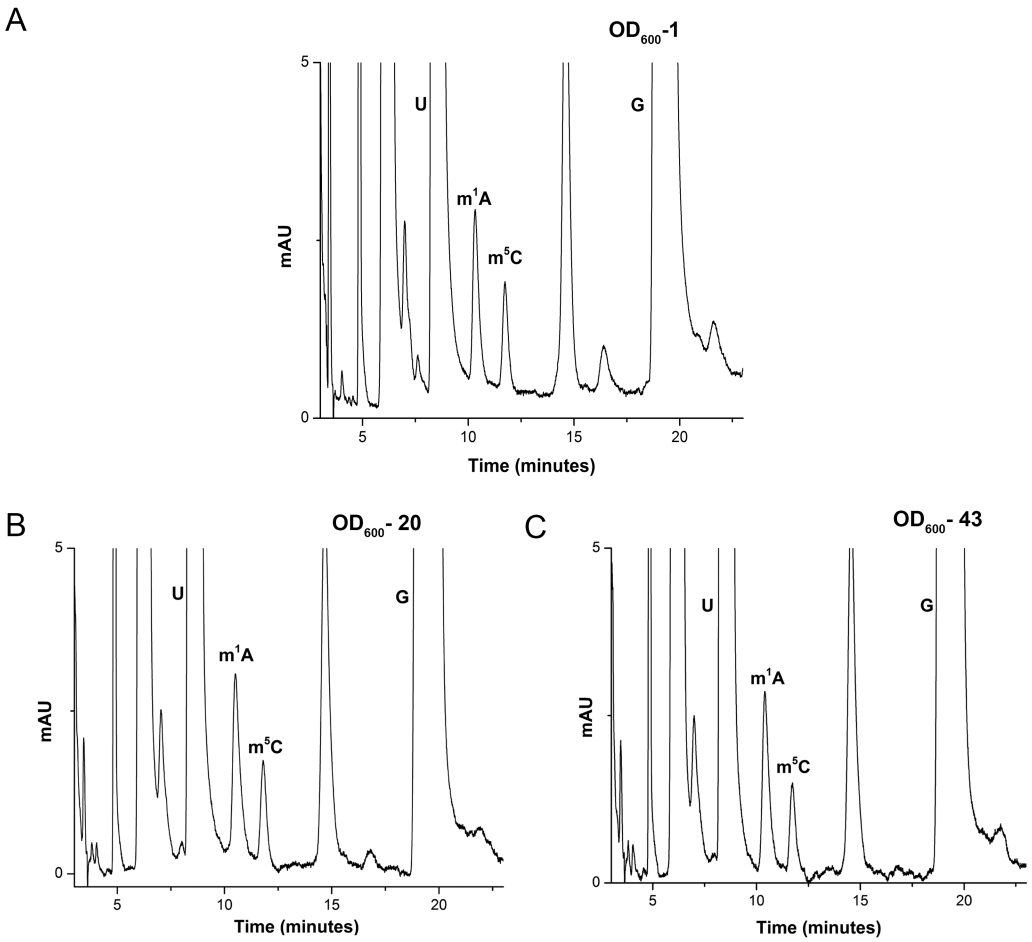

Figure S4. Investigation of m^1A modification of 25S rRNA at different growth phase. The RP-HPLC chromatogram from the 25S rRNA derived from the culture grown at different growth phase. A) RP-HPLC Chromatogram of the 25S rRNA from the yeast cell culture at early exponential phase (OD_{600} -1). B) RP-HPLC Chromatogram of the 25S rRNA from the yeast cell culture at the end of first growth phase (OD_{600} -20), where the glucose is completely depleted. C) RP-HPLC Chromatogram of the 25S rRNA from the yeast cell culture at stationary phase OD_{600} -43, where all carbon sources are depleted.

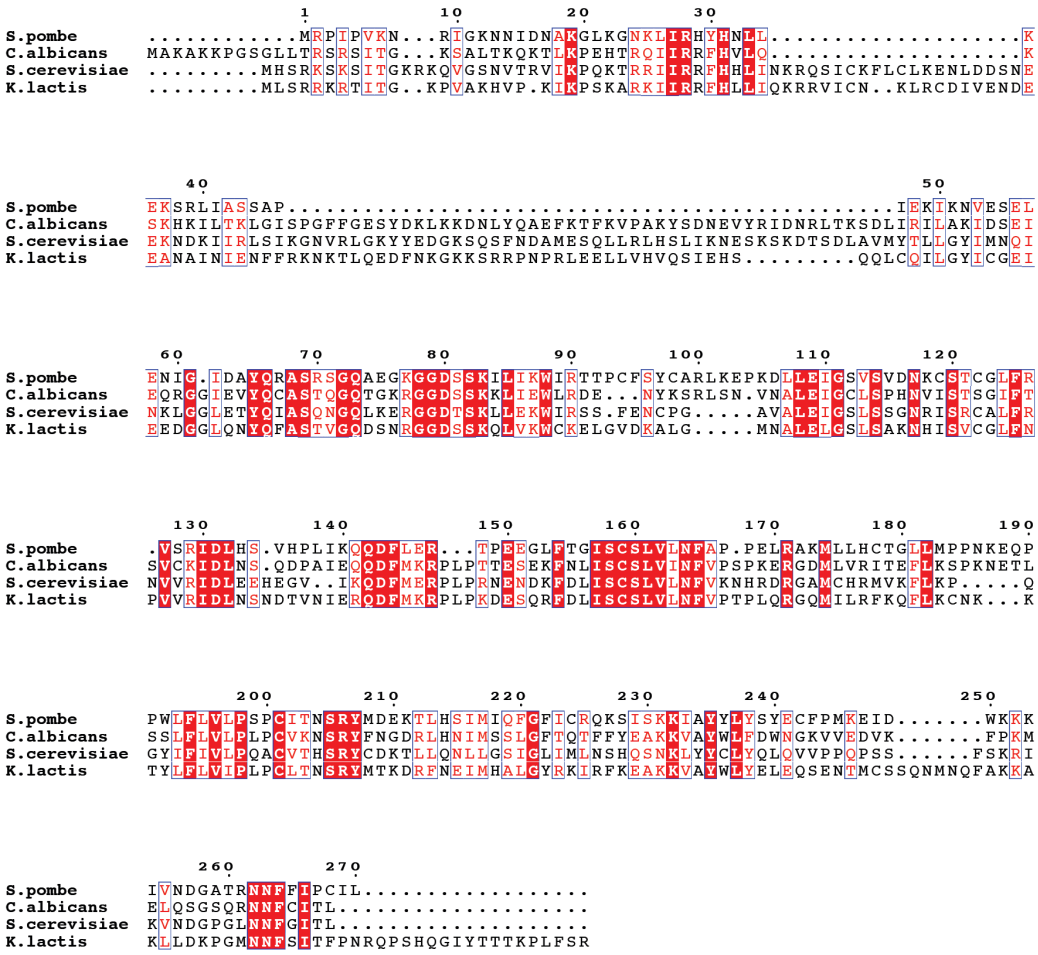

Figure S5. **Bmt2 homology among different yeasts**. The amino acid sequence of Bmt2 from *S. cerevisiae*, *K. lactis*, *C. albicans* and *S. pombe* were aligned using ClustalW2 (EMBL-EBI) and the alignment file was analyzed with EsPript 2.2. The Bmt2 protein is highly conserved among these organisms.

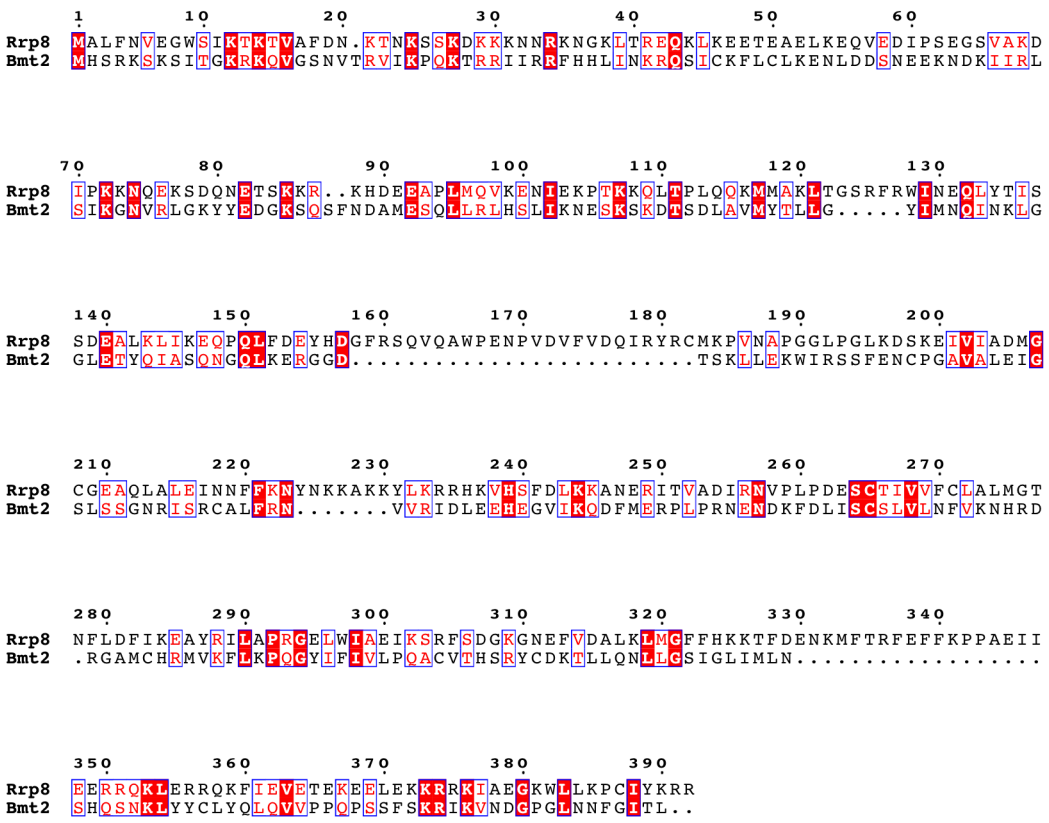

Figure. S6. Amino acid sequence alignment of Bmt2 and Rrp8. The complete amino acid sequence of Bmt2 and Rrp8 from S. *cerevisiae* was aligned using ClustalW2 (EMBL-EBI) and the alignment files was analyzed with EsPript 2.2. The Bmt2 protein and Rrp8 share significant homology. Both Rrp8 and Bmt2 are the class I methyltransferases responsible for m^1A modification of 25S rRNA at position 645 and 2142, respectively.

Supplementary Tables

Table S1: Yeast Strains

Strain	Genotype	Origin
Y10000	BY4742; MATα; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0	EUROSCARF
Y13280	BY4742; MATα; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; YBR141c::up-kanMX4-down	EUROSCARF
Y04018	BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YDR083w::up-kanMX4-down	EUROSCARF
ScNop56-mRFP	MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0 Sik1-RFP-KANMX6 (in BY4742)	Huh et al. Nature, 2003
CEN.PK920-1C	MATa ura3-52 trp1-289 leu2-3, 112 his3Δ1 ΔΔrdn::pNOY455 [HIS3] + pRDN-hyg1(pNOY290) [URA3, leu2D]	Meyer et al., 2011
BY.PK1022-6D	MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ybr141c::up-kanMX4-down rrp8::up-kanMX4-down	This study (from crossing Y13280 x Y04018)
CEN.PK1167-1C	MATa ura3-52 trp1-289 leu2-3,112 his3Δ1 ΔΔrdn::pNOY455 [HIS3] + pPK622 (A2142C)	This study (CEN.PK920-1C after transformation with pPK622 and plasmid loss of pRDN-hyg1 after 5-FOA selection
CEN.PK1168-1C	MATa ura3-52 trp1-289 leu2-3,112 his3Δ1 ΔΔrdn::pNOY455 [HIS3] + pPK623 (A2142T)	This study (CEN.PK920-1C after transformation with pPK622 and plasmid loss of pRDN-hyg1 after 5-FOA selection
CEN.PK1169-1C	MATa ura3-52 trp1-289 leu2-3,112 his3Δ1 ΔΔrdn::pNOY455 [HIS3] + pPK624 (A2142G)	This study (CEN.PK920-1C after transformation with pPK622 and plasmid loss of pRDN-hyg1 after 5-FOA selection)

Table S2: Plasmids

Plasmid	Description	Origin
pPK468	High copy number plasmid carrying *URA3* marker, 2µ ori, amp	P. Kötter
pSH18	A derivative pUG35 plasmid carrying Bmt2-GFP fusion proteins	This study
pSH20	A derivative pPK468 plasmid carrying Bmt2-7xHis fusion protein	This study
pSH20-*G180R*	A derivative pSH20 plasmid carrying bmt2-*G180R*-7xHis fusion protein	This study
pSH20-*G79R*	A derivative pSH20 plasmid carrying bmt2-*G79R*-7xHis fusion protein	This study
pSH20-*D116A*	A derivative pSH20 plasmid carrying bmt2-*D116A*-7xHis fusion protein	This study
pAV164	High copy number plasmid carrying wild type rDNA with the *TRP1* marker and *leu2d* gene	Chernoff et al., 1994 (1)
pPK622	A derivative of pAV164 plasmid carrying mutant A2142C 25SrDNA	This study
pPK623	A derivative of pAV164 plasmid carrying mutant A2142T 25SrDNA	This study
pPK624	A derivative of pAV164 plasmid carrying mutant A2142C 25SrDNA	This study

Table S3: Oligonucleotides

Oligonucleotides	Sequence
pSH18 FP	TACATAGATACAATTCTATTACCCCCATCCATACTCTAGAATGCATTCAAGAAAGTCGAAG
pSH18 RP	TTGGGACAACACCAGTGAATAATTCTTCACCTTTAGACATGAGGGTAATACCAAAATTGTTC
pSH20 FP	ACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACG ATGCATTCAAGAAAGTCGAAG
pSH20 RP	TATAAAAAGAAAATTTATTTAAATGCAAGATTTAAAGTAGTTAGTGATGGTGATGGTGATGGTGGAGGG TAATACCAAAATTGTTC
25S-A2142	GCACTGGGCAGAAATCACATTGCG
oligo-645	CACTCGCATAGACGTTAGACTCCTTGGTCCGTGTTTCAAGACGGGCGG
oligo-2142	CTGACCATCGCAATGCTATGTTTTAATTAGACAGTCAGATTCCCCTTG
25S-Mut3	TCTGACTGTCTAATTBAAACATAGCATTGCG
25S-Mut4	CAATGCTATGTTTVAATTAGACAGTCAGATTCC
ITS1 (D-A2)	GATTGCTCGAATGCCCAAAG
ITS2 (C1-C2)	CGCCTAGACGCTCTCTTCTTA
bmt2-G180R FP	GGAGCAGTGGCATTAGAAATTCGTTCATTGAGCTCCGGAAATC
bmt2-G180R-RP	GATTTCCGGAGCTCAATGAACGAATTTCTAATGCCACTGCTCC
bmt2-G79R-RP	ATCAGGTTAAGTATTAAAGGTAATGTAAGACTGCGCAAGTACTATGAAGACGGCAAATCG
bmt2-G79R-RP	TTTGCCGTCTTCATAGTACTTGCGCAGTCTTACATTACCTTTAATACTTAACCTGATAATTTTG
bmt2-D116A-RP	CATTAATTAAAAATGAATCCAAATCAAAGGATACTTCTGCCTTGGCTGTGATGTACACATTACTTGGTTAC
bmt2-D116A-RP	ACCAAGTAATGTGTACATCACAGCCAAGGCAGAAGTATCCTTTGATTTGGATTCATTTTTAATTAATGAGTG

References

1. Chernoff,Y.O., Vincent,A. and Liebman,S.W. (1994) Mutations in eukaryotic 18S ribosomal RNA affect translational fidelity and resistance to aminoglycoside antibiotics. *The EMBO Journal*, **13**, 906–913.