Identification of novel methyltransferases, Bmt5 and Bmt6, responsible for the m^{3}U methylations of 25S rRNA in *S.cerevisiae*

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Supplementary data:

Supplementary figures

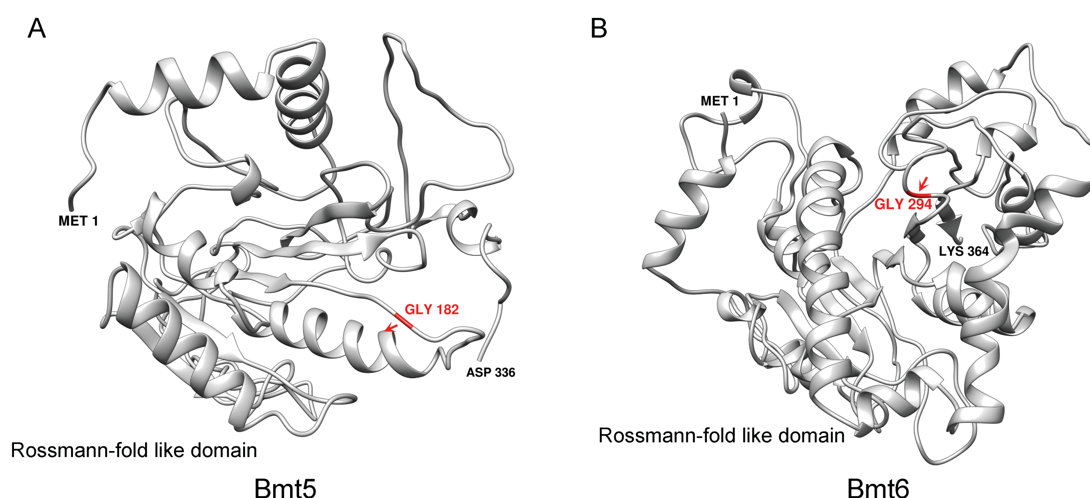


Figure S1. **Structural model of Bmt5 and Bmt6.** (A) Cartoon representing the 3D structure of Bmt5 and (B) Bmt6. The model was constructed using the protocol described by Kelley et.al (2009) (24). The 3D structure was constructed with sequence coverage of 100%, where all residues from Bmt5 and Bmt6 were modelled with 99.3% confidence by the single highest scoring template. This model further reinforced Bmt5 and Bmt6 to be SAM (S-adenosyl methionine) dependent methyltransferase belonging to Rossmann-fold superfamily. Highly conserved glycine residues, indispensible for the methylation reaction are labelled in red colour.

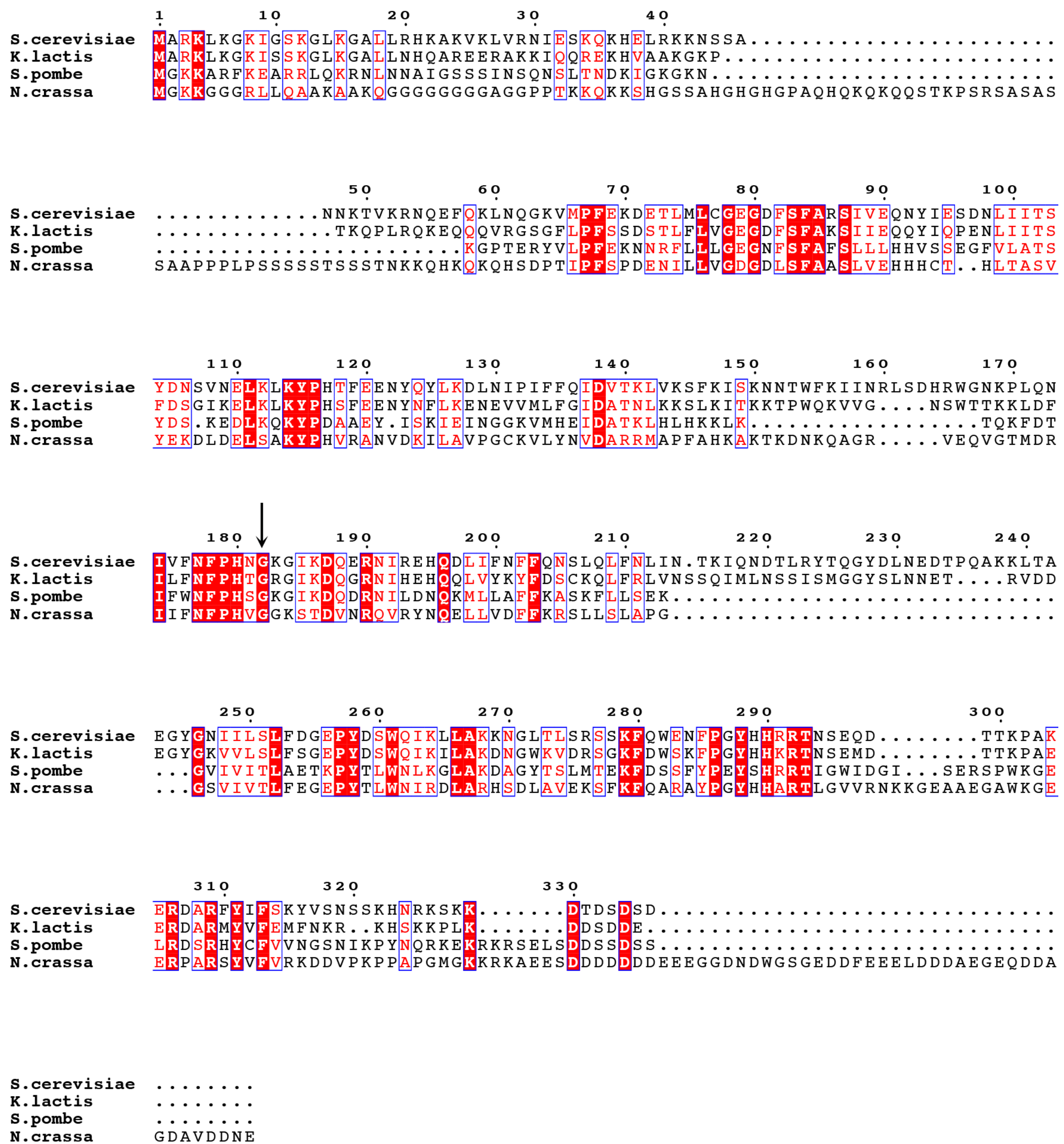


Figure S2. Bmt5 homology among different yeasts. The amino acid sequence of Bmt5 from *S. cerevisiae*, *K. lactis*, S.pombe and *N.crassa* were aligned using ClustalW2 (EMBL-EBI) and the alignment file was analyzed with ESPript 2.2. Bmt5 protein is highly conserved among these organisms.

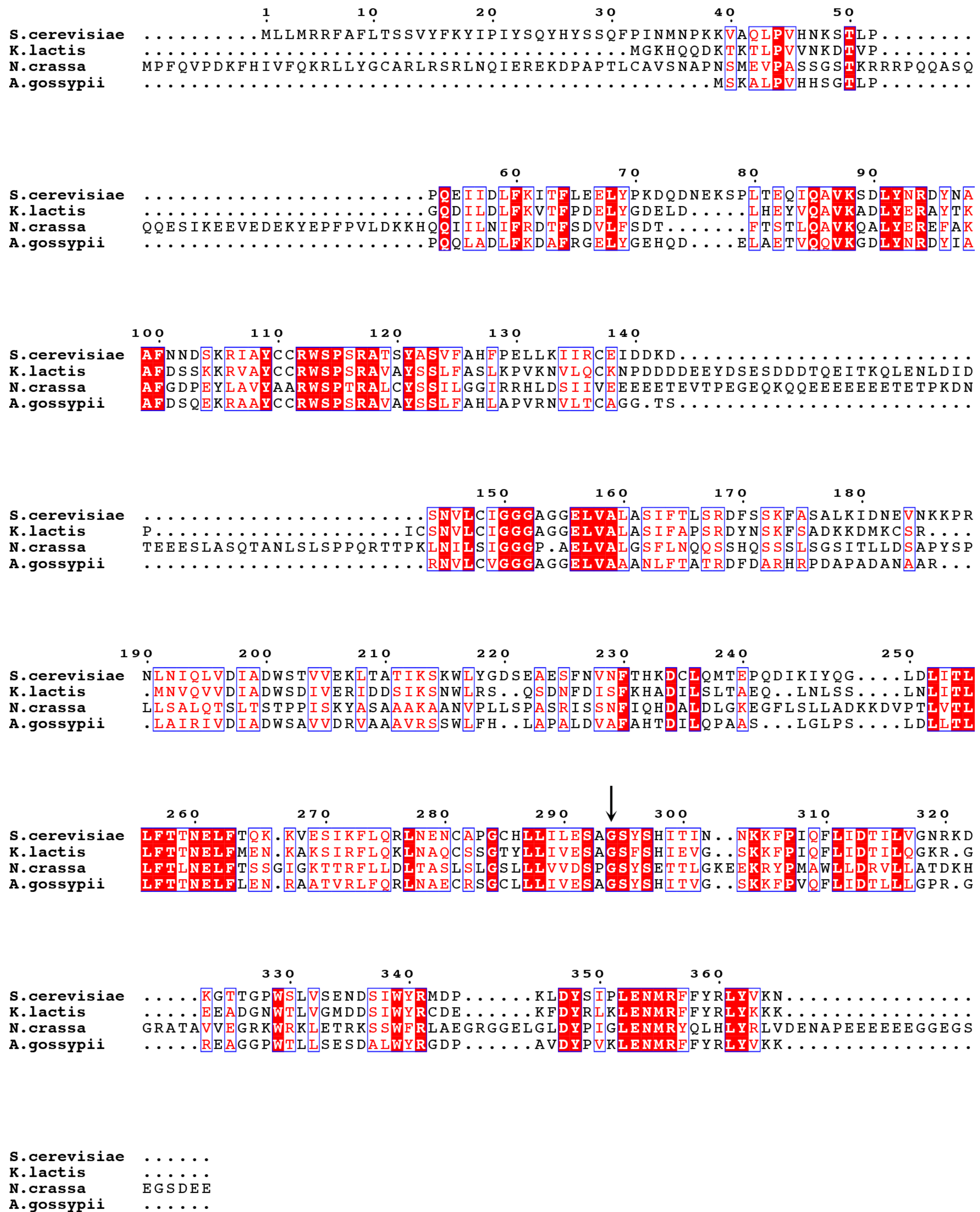


Figure S3. Bmt6 homology among different yeasts. The amino acid sequence of Bmt6 from *S. cerevisiae*, *K. lactis*, *N.crassa* and *A. gossypii* were aligned using ClustalW2 (EMBL-EBI) and the alignment file was analyzed with EsPript 2.2. Bmt6 protein is highly conserved among these organisms.

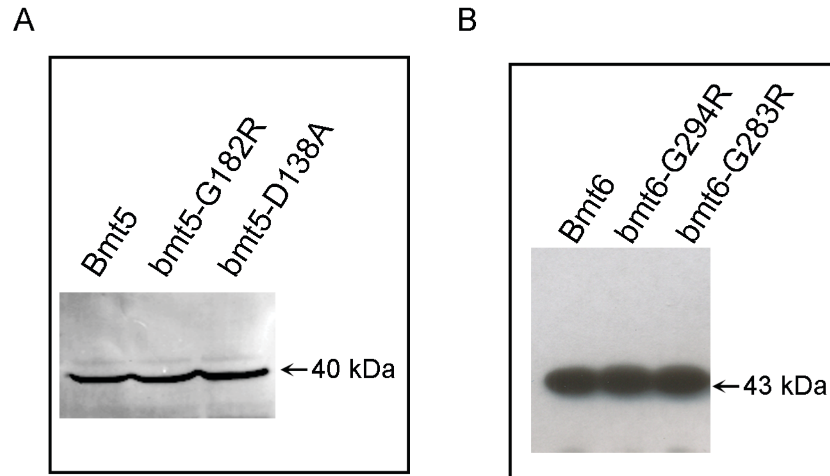


Figure S4. Western blot analysis of the Bmt6 and Bmt5 point mutants. The expression of both Bmt5 and Bmt6 along with the point mutants were analysed by Western blotting using anti-His antibodies as described in Materials and Methods. The blot shown in panel A was developed using Typhoon 9400 (GE Life sciences), whereas the blot in panel B was developed using X ray film. Apart from bmt5-*G182R* and bmt6-*G294R,* we also tested other point mutants of Bmt5 and Bmt6, bmt5-*D138A* and bmt6-*G283R* for expression analysis. As observed in the Western blot, point mutations did not influence the expression of respective mutants of both Bmt5 and Bmt6.

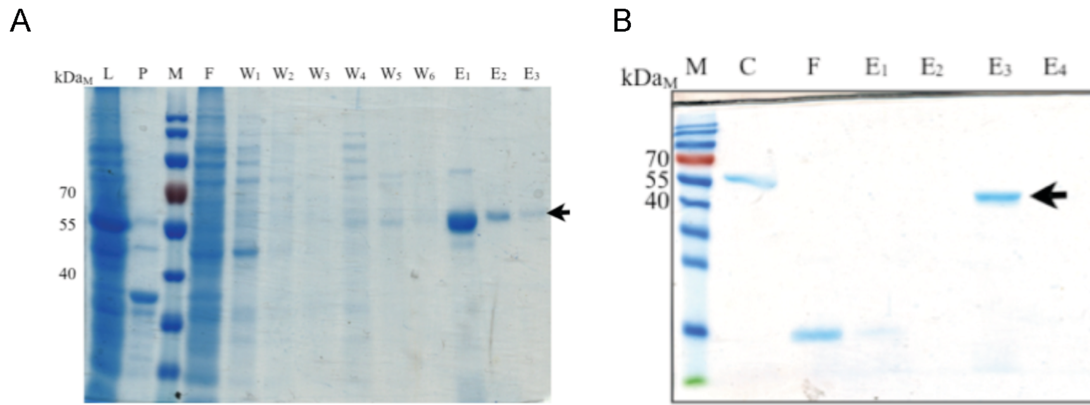


Figure S5. Purification of Bmt5. Protein samples were analyzed by SDS-PAGE. **(**A)**.** Purification of 6xHis-Smt3-Bmt5 (MW 53.4 kDa) over NiNTA IMAC. Gel lanes with different samples (L) Lysate, (P) Sediment, (M) Protein marker (7µl), (F) flow through, (W_1-W_3) washing fractions with wash buffer, (W_4-W_6) washing fraction with 10mM imidazole, (E_1, E_2) Elution with 100 mM imidazole and (E_3) Elution 500 mM imidazole were loaded on to 10% SDS-PAGE gel and was stained with Coomassie staining. (B) Bmt5 and 6xHis-Smt3 tag were separated by cation exchange chromatography as explained in Materials and Methods. The 6xHis-Smt3 tag of the 6xHis-Smt3-Bmt5-fusion protein was cleaved by Ulp1 enzyme. Bmt5 protein with molecular weight of 39.4 kDa was eluted with 1M NaCl (elution fraction E3), marked with a black arrow. The protein samples in different gel lanes are (M) Protein marker (7µl), (C) protein before cleavage, (F) primary flow through, (E_1) elution with PBS 400 mM NaCl, (E_2) elution with 800 mM NaCl, (E_3) elution with 1000 mM NaCl and (E_4) elution with 1200 mM NaCl.

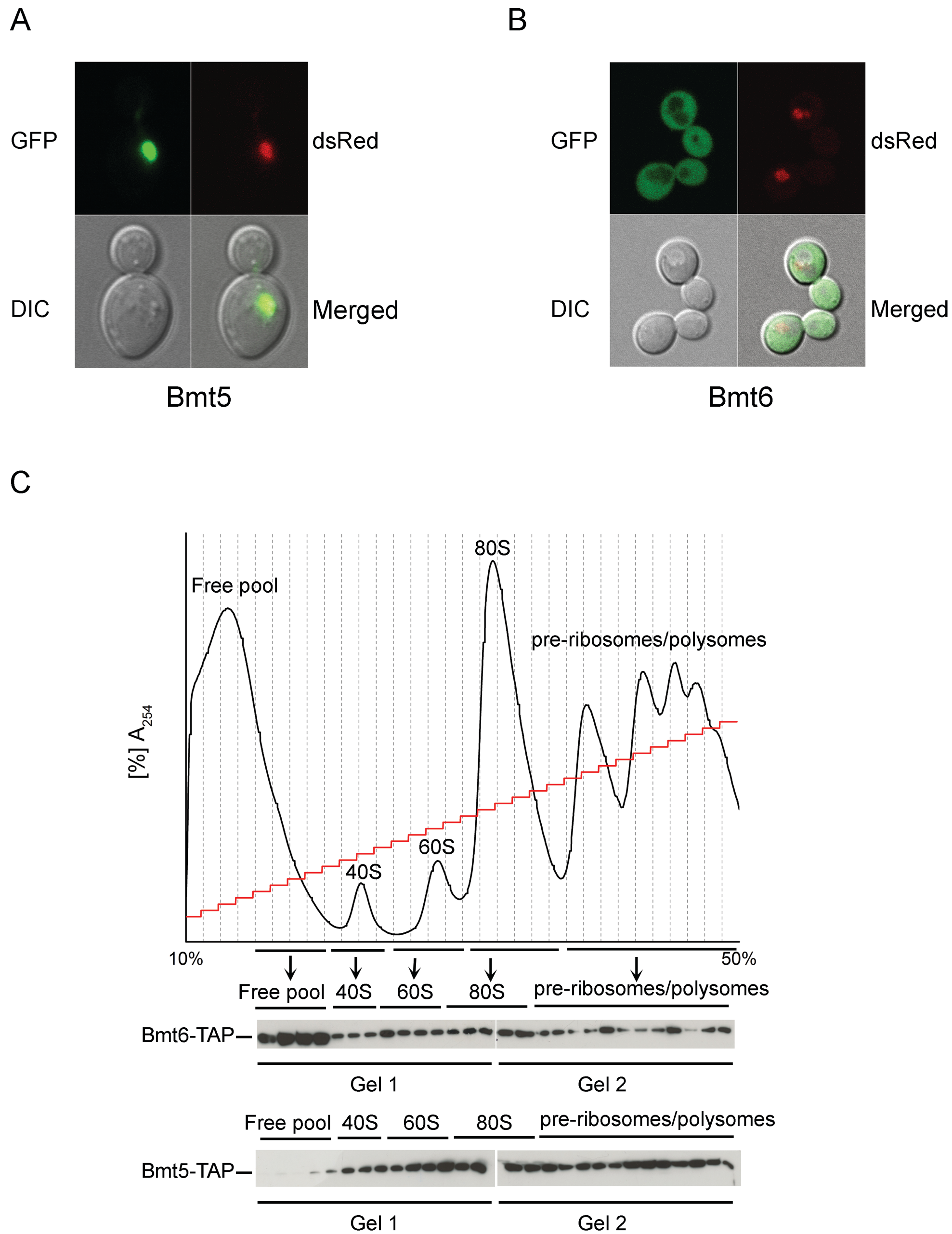
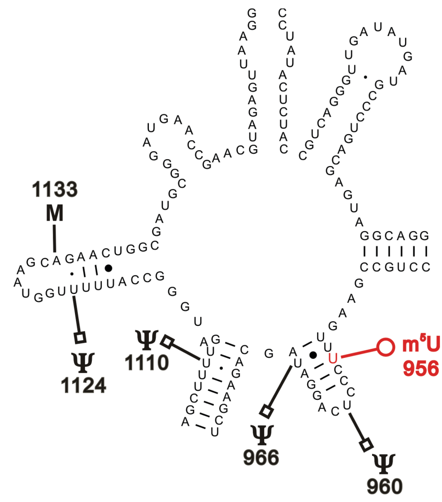


Figure S6. Cellular localisation and sedimentation profiles of Bmt5 and Bmt6. To test the cellular localization of Bmt5 and Bmt6, plasmids pSH22 (Bmt5-GFP) and pSH28 (Bmt6-GFP) were transformed into strain ScNop56-mRFP and visualized with Leica TCS SP5. (A) Cells carrying Bm5-GFP and (B) Bmt6-GFP in a strain where Sik1-mRFP is used as a reporter for the nucleolus. (C) Sedimentation pattern of TAP-tagged Bmt5 and Bmt6 on sucrose gradient (10% to 50%). Two sucrose gradients with the cell extract from Bmt5-TAP tagged strain and Bmt6-TAP tagged strain were made (only one from Bmt6-TAP shown here). Both Bmt5-TAP and Bmt6-TAP proteins were detected by Western blotting using PAP (peroxidase anti peroxidase) antibody.

A



B

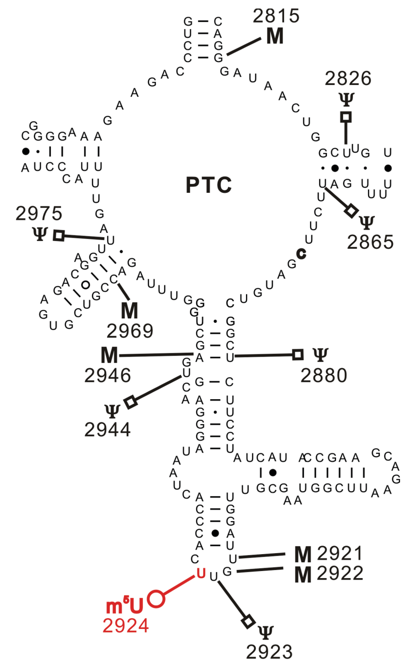


Figure S7. Secondary structure of yeast 25S rRNA. 25S rRNA has been predicted to contain two m^5U residues at position 956 in the helix 37 (A) and at position 2924 in the helix 92 (B). The files for secondary structure were taken from (http://www.rna.icmb.utexas.edu/).

Table S1: Strains and plasmids used in the present study

Strain/ Plasmid	Genotype	Origin
Y02674	BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YLR063w::kanMX4	EUROSCARF
Y01487	BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YIL096C:: up-kanMX4	EUROSCARF
BY.PK1134-18B	BY4742; MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; YLR063W:: up-kanMX4-down YIL096C:: up-URA3-down	This study
ScNop56-mRFP	MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0 Sik1-RFP-KANMX6 (in BY4742)	(26)
pSH22	A derivative pUG35 plasmid carrying Bmt5-GFP fusion proteins	This study
pSH24	A derivative pPK468 plasmid carrying Bmt5-7xHis fusion protein	This study
pSH25	A derivative of pPK591 plasmid carrying mutant Bmt6-6xHis-Smt3 under T7 promoter	This study
pSH26	A derivative of pPK591 plasmid carrying mutant Bmt5-6xHis-Smt3 under T7 promoter	This study
pSH28	A derivative pUG36 plasmid carrying Bmt6-GFP fusion proteins	This study
pSH29	A derivative pPK468 plasmid carrying Bmt6-7xHis fusion protein	This study
pSH24a	A derivative pPK468 plasmid carrying bmt5-*G182R*-7xHis fusion protein	This study
pSH29a	A derivative pPK468 plasmid carrying bmt6-*G294R*-7xHis fusion protein	This study
pSH24h	A derivative pPK447 plasmid carrying Bmt5-7xHis fusion protein	This study
pSH29h	A derivative pPK447 plasmid carrying Bmt6-7xHis fusion protein	This study
pPK468	A multicopy plasmid carrying Ura3 as a marker for the expression of ORFs under *TDH3* promoter in yeast.	Peter Kötter (unpublished)
pPK447	A multicopy plasmid carrying His3 as a marker for the expression of ORFs under *TDH3* promoter in yeast.	Peter Kötter (unpublished)
pPK591	Plasmid for the heterologous expression in *E.coli*. The proteins are expressed as N-term. 6xHis-Smt3-fusion protein.	Peter Kötter (unpublished)

Table S2: Oligonucleotides used in the present study

Oligonucleotides	Sequence
pSH22 FP	TACATAGATACAATTCTATTACCCCCATCCATACTCTAGAATGGCCAGGAAATTGAAGGG
pSH22 RP	TTGGGACAACACCAGTGAATAATTCTTCACCTTTAGACATGTCTGAATCAGAATCTGTGTC
pSH24 FP	ACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACGATGGCCAGGAAATTGAAGGG
pSH24 RP	CTATAAAAAGAAAATTTATTTAAATGCAAGATTTAAAGTAGTTAGTGATGGTGATGGTGATGGTGGTCTGAATCAGAATCTGTG
pSH25 FP	GAAGATAACGATATCATTGAGGCCCATCGTGAACAGATTGGTGGTATGTTGCTTATGAGACGCTTTGC
pSH25 RP	AGCAGCCAACTCAGCTTCCTTTCGGGCTTTGTTAGCAGCCGGATCTTAGTTCTTGACATAAAGACG
pSH26 FP	GAAGATAACGATATCATTGAGGCCCATCGTGAACAGATTGGTGGTATGGCCAGGAAATTGAAGGG
pSH26 RP	AGCAGCCAACTCAGCTTCCTTTCGGGCTTTGTTAGCAGCCGGATCTTAGTCTGAATCAGAATCTGTG
pSH28 FP	TGCTGCTGGTATTACCCATGGTATGGATGAATTGTACAAAATGTTGCTTATGAGACGCTTTGC
pSH28 RP	GAGGGCGTGAATGTAAGCGTGACATAACTAATTACATGACTTAGTTCTTGACATAAAGACGG
pSH29 FP	ACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACGATGCACCATCACCATCACCATCACTTGCTTATGAGACGCTTTGC
pSH29 RP	TATAAAAAGAAAATTTATTTAAATGCAAGATTTAAAGTAG TTAGTTCTTGACATAAAGAC
pSH24a FP	GTTTTCAATTTTCCCCATAATAGAAAAGGTATTAAGGATCAAGAAA
pSH24a RP	TTTCTTGATCCTTAATACCTTTTCTATTATGGGGAAAATTGAAAAC
pSH29a FP	TCTCATACTAGAGAGTGCACGTAGCTATTCTCACATTACC
pSH29a RP	GGTAATGTGAGAATAGCTACGTGCACTCTCTAGTATGAGA
Oligo-2634	CTTAGGACATCTGCGTTATCGTTTAACAGATGTGCCGCCCCAGCC
Oligo-2843	GAATCAAAAAGCAATGTCGCTATGAACGCTTGACTGCCACAAGCCAG
d	CATGGCTTAATCTTTGAGAC
e	CGGTTTTAATTGTCCTA
f	GATTGCTCGAATGCCCAAAG
i	CGCCTAGACGCTCTCTTCTTA
j	CTCCGCTTATTGATATGC