



### 1 Supplementary Data

### 2 <u>SEC-MALS</u>

- 3 The approximate molecular weight of MpMetRS was determined using size exclusion
- 4 chromatography coupled with multiangled light scattering [23,24]. The recombinant MpMetRS
- 5 M568A [1 mg/mL] or Mp $\Delta$ MetRS [2 mg/mL] was equilibrated in SEC-MALS buffer (50 mM Sodium
- 6 Phosphate•NaOH (pH 8.0), 250 mM NaCl and 5% glycerol). The protein was resolved using an
- 7 analytical size exclusion TSKgel column (7.8 mm × 30 cm, 8 μm particle size; Tosoh Bioscience) at a
- 8 flow rate of 0.5 mL/min over 35 minutes. Elution was monitored by UV<sub>280</sub> absorbance and molecular
- 9 mass determined by MALS (HELIOS II; Wyatt Technology) with linked refractive index determined
- 10 (Oprilab rEX; Wyatt Technology). Astra 6.1 software (Wyatt Technology) was used to analyze peaks
- 11 based on UV<sub>280</sub> and figures were generated using Prism Graphpad 8.0.
- 12 <u>PLP Occupancy</u>
- 13 Extraction of pyridoxal 5'-phosphate PLP was adapted from Wada, L. et al [21]. A PLP stock
- solution was made using 20 mg PLP dissolved in 10 mM Tris, pH 8.0 in a 100 mL volumetric flask.
- 15 A range of standards was generated using the PLP stock (0-75 nmol) in 10 mM Tris, pH 8.0 in 600
- 16 μL. The MpMetRS sample (50 nmol) was diluted in 10 mM Tris, pH 8.0 in 600 μL used directly after
- 17 purification and dialysis. To each sample, 70 μL of 5 M NaOH was added and the reaction was
- 18 placed at 70 °C for 10 minutes. To each sample 35 μL HCl [12 N] and 150 μL Tris, pH 8.0 [1 M] and
- 19 centrifuge for 5 minutes at 12k rpm. The resulting supernatants were placed into clear 96 well plates
- 20 and their absorbance measured at 415 nm wavelength.

## 21 Circular Dichroism and Thermal Stability

- 22 The MpMetRS samples were dialyzed twice in 1X PBS buffer (pH7.5) and the A280 was monitored on
- a Cary 50 UV-Vis spectrophotometer to asses protein concentration. In a 0.1 mm quartz cuvette the
- 24 0.1 mg/mL protein samples were run at 10 nm/min from 200-260 nm with three scans at 25 °C.
- 25 Samples were then tested for their thermal stability by increasing the temperature 2 C/min from 20-
- 26 80 °C. Samples were analyzed using a Jasco J-720 spectropolarimeter.
- 27 <u>Supplemental figures</u>

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**30** Figure S1. SEC-MALS of MpMetRS. A 1 mg/ml MpMetRS or 2 mg/mL Mp∆MetRS sample was passed through a HPLC

31 system and filtered through a Wyatt Dawn Helios-II and Optilab rEX to measure the light scattering and refractive index of

32 the protein. The absorbance at 280 nm was monitored by a Waters 2417 absorbance detector. SEC-MALS showed the

molecular weight at the peak reading was 253 kDa and monodisperse indicating the dimeric state of the MpMetRS protein

and 77 kDa and monodisperse for Mp $\Delta$ MetRS. The A<sub>280</sub> trace is represented as a solid green line, the differential refractive

35 index (dRI) is represented by a blue dashed line and the molecular weights are represented by red dots. Molecular weights

are plotted on the left y-axis while the UV and dRI trace were normalized to 1 and plotted on the right y-axis.

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| TdPhpW                      |  |
|-----------------------------|--|
| BoPhnW                      | TILING PATTED TVKYAOVUSDICH PEEPEG-OTLEWISDET WILVADEKYYTSVLEGGS TAAVEATISSIVG-DEKTITINNGAYGER   |
| BmPhnW                      |  |
| StPhnW                      | VI.INFGEATTTDSVKYAOVVPDICPERKEEG-DIMOOTREDITSEVADWSHYTTVI.EGGSCTAADEAMICSVVPEDGELLVIDNEVVGER   |
| AmPhnW                      | ILINE CATTTENTYKHAOLVADIC DECODEG-ETMTEVSHALTEEVGNENEYSTILEGGSCTAAVESILSSVD-KEATVLVNNCAYCKE  |
| PbPhnW                      | ILINE CATTTOTY KYAOVYDDIC PREGEEG-DIMOFISTELTE FVADPEEYTAILEGS CTAVVESILSSVIG-EDTVLIINNEVYGR   |
| StPhnW                      | LLLTPCLTTSRTVKEAMLF-DSCTWDDDYNIGVVEOIROOLTALATASEGYTSVLLOGS SYAVEAVLGSALGPODKVLIVSNEAYGAR  |
| PaPhnW                      | ILLTPGPLTTSYRTRRAMMV-DWGSWDSDFN-ELTASYCORLLKTVGGEGSHTCVPLOGSCTFAVEAAIGTLVPRDGKVLVLINCAYGKR   |
| AvAGXT                      | LLLGPCTSNAHPAVLOAMNVSPVGHLDPAFL-ALMDEIOSLLRYVWOTENPLTIAVSGTCTAAMEATIANAVEPGDVVLIGVACYFGNR  |
| HsAGXT1                     | LLLGPGSSNLPPRIMAAGGLOMIGSMSKDMY-QIMDEIKEGIQYVFOTRNPLTLVISGSCHCALEAALVNVLEPGDSFLVGANCIWGOR  |
| ScAGXT1                     | TLLIPGETILSGAVOKALDVPSLGHTSPEFV-SIFORVLKNTRAVFKSAAASKSOPFVLAGSETLGWDIFASNFILSK-APNKNVLVVSTETFSDR   |
| SsSPAT                      | LLLHVGPTTIKEDVLVAGLENNVGFTSKEFV-EALAYSLKGLRYVMGASKNYQPLIIPGGCTSAMESVTSLL-KPNDKILVVSNCVFGDR   |
|                             |  |
|                             | 110 120 130 140 150 160 170 180 190 200  |
|                             |  |
| MpATD                       | LIDIAKRHQINQSLLQIK-QGESFDLKEVENLIKNKKAVFMVYMDTSCGILNPVKKVGELCKDYGCLFI <mark>VP</mark> AISGILNEEFNFDEYGVTAAVST  |
| TdPhnW                      | MAKIADIYKIPMDIFKSS-TYEPLDLQKLEAEFATKKYTHLACVYHETTTGLLNPLHIICPMAKKYGMVTI <mark>VD</mark> AVSAYCGMPMDLKSLGIDFMAST  |
| BoPhnW                      | MCKIANVYELDYIEYKSP-ATDKINLINLEQLISHSDEKIAHLAVVHCETTTGMLNPIKEIGDLCKKYEINLIVDAMSSFAAIPIDMKEMNIGYLAAS   |
| BmPhnW                      | MCKIAEIYGLNYLEYKGS-AEQAIDMNNLEIFIKKISTNVSYLALVHCETSTGLLNDIEKIGEFCAVKNIEMI <mark>VD</mark> AMSSYAAVPIDMQKMNISYLAAS  |
| StPhnW                      | MARMASVYKLNYEVFESS-PTGPVDLDVMEHTLKKGKFTHLAVVYHETTTGLLTDLPSIGEICKRLGIVTIVLAVSAYAGIPMDLEKLNVHFMAAS   |
| AmPhnW                      | ILKIATVYGLNVLEYRSP-LESAIDLTKLELLIKNAGVTISHLVIVHCETTTGLLNNIEAVGNICKKYNVAMIVPAMSSYGAIPIEMKRMNISYLAAS   |
| PbPhnW                      | MCQMAEVYGLKYIEYRSA-PDEALDLASLEAVIQNSTAKISHLAVVHNETTTGLLNDIGSIGQLCKKYHVYMIVPAVSSYGAIPIDMNVMNISYLAAS   |
| StPhnW                      | MVEMAGIMGIAHHAYDCG-EVARPDVQAIDAIINA-DPTISHIAMVHSETTTGMINPIDEVGALAHRYGKTYIVPAMSSFGGIPMDIAALHIDYLISS   |
| Paphnw                      | LAKICEVLQRPFSTLETE-ENVPTTAADVERLLAA-DPAISHVALIHCETSTGILNPLEATAKVVERHGKRLIVDANSSFGAIGIDARKVPFDALIAA   |
| AVAGXT                      | LVDMAGRYGADVRTISKP-WGQVFSLEELRTALENNRPATIALVHAETSTGARQPLEGVGELCREFGTLLIVDTVTSLGGVP1FLDGWGVDLAYSC   |
| HSAGXTI                     | AVDIGERIGARVHPMTRD-PGGHYTLGEVEEGLAQHKPVLLFLTHGESSTGVLQPLDGFGELCHRYKCLLLVDSVASLGGTPLLMDRQGIDILYSG   |
| ScAGXT1                     | FADCLRSYGAQVDVVRPLKIGESVPLELITEKLSQNSYGAVTVTHVDTSTAVLSDLKATSQAIKQTSPETFFVVPAVCSIGCEEFEFDEWGVDFALTA   |
| SSSPAT                      | WEQIFKRYPVNVKVLRPS-PGDYVKPGEVEEEVRKSEYKLVALTHVETSTGVREPVKDVINKIRKYVELIVUGVSSVGAEEVKAEEWNVDVYLTA  |
|                             | # 000 000 000 000 000 000 000 000  |
|                             | 210 220 230 240 250 260 270 280 290 300  |
| Manama                      |  |
| MADAN                       |  |
| RoPhnW                      | SNANLQAMA VGFVICUNAAELEKTADIPMKNIILALDIQIA-IPAKTHQTKPIPI VQTIMIALKQAVLETAQE-T-VQKIEKITACWNILVAA  |
| BOFILIW                     | SIGNING STATES TAR TRANSFORMED TEMPERSON TO DO THE STATES AND A STAT |
| S+DbpW                      |  |
| AmPhpW                      | SNANLQGRAGVAFVICURESLEATSTPRISTILLALDQVF FEATKYRFFFFVQTLALKQALLETVVE-T-VEATARTIACWELLMUQ<br>SNANLQGRAGVAFVILARESLEATSTPRISTILLALDQVF FEATKYRFFFFVQTVATVAT VALLETVVE-TFVQHQVGCQVATIVG   |
| DhDhaW                      |  |
| PDPIIIW<br>C+DhpW           | SIN I GENERAL VEFVI ANNAQUETI I C  |
| PaPhpW                      |  |
|                             |  |
| HebGYT1                     | SCALINADDETSLISTEDIKATKKWSSD-KTWADESEVIJIKWILDIKUMSA INGEL KVIMIATIVISIJESILAIJASIGO GLENSKUMPHDEAAVIHGD   |
| ScAGXT1                     | SO A LGAPACEST SI CSSFEMDYALNDSKNGHVHGVFSSLRRWTPTMENYEAGGAYFATPLVOLINSLDVALKETLEE-G-LHKRWDLHREMSDWFKDS   |
| SSSPAT                      | SOKALGSAACLGLILLSPKALSTLDSON-STAGYYLDLRNWLPVMRGAEEGKAAYFANPLVHVILOLAEAFRLIEKE-G-TENRIKRHTMVASATRAG   |
| DIDINI                      |  |
|                             | " 310 320 330 340 350 360 370 380 390 400  |
|                             |  |
| MpATD                       | LITLGFRHIIEN-E-NSRSNWVLVME-TPNIIKANELR-AYLYAMK-NILIECEIADSS-NRIVELAISAAHDIEDVTQLIEAIKEYIDL   |
| TdPhnW                      | IKKLGLKMLVKE-E-HQ-SHFITAIL-EPETPKYSFEALH-DFAA-EH-SFTIYPCKLGNI-DTFRIANIGDIQPEEMRRFTVKLKEYMNG  |
| BoPhnW                      | <mark>ieklgl</mark> thlvhk-n-hh-skiitsii-epnypnydfy <mark>0mh</mark> -dyfa-sk-gftiyp <mark>c</mark> kldklgtf <mark>rlanig</mark> nitykdmeQfidlldlyikg  |
| BmPhnW                      | ITQLGLTYLVPE-T-HH-SKIITSIL-EPSDKKYNFDIMH-DFFY-KQ-GFTIYP <mark>C</mark> KIDKLETF <mark>RIA</mark> NIGDITYRDIERFLHLLEQY <mark>L</mark> NG  |
| StPhnW                      | <mark>IARLGLE</mark> ALVPR-E-YQ-SHL <mark>ITAI</mark> V-EPSRPGYSFEDLH-AYAR-KR-GFTIYP <mark>G</mark> KLSNAQTF <mark>RIANIG</mark> DIQPEEMQRFVEILGEYILS  |
| AmPhnW                      | <mark>IKALGL</mark> THLIDE-A-NH-SRIITS <mark>I</mark> V-EPPHKGYDFNKLH-DFLY-ER- <mark>GFTIYP</mark> CKIDNLNTF <mark>R</mark> VANIGDITYKDMECFLTLLDQYLN-  |
| PbPhnW                      | ITQLGLTHLVKK-E-NH-SRIITAII-EPNTPSYDFEEMH-DYFS-RC-GYTIYPCKLDAKNTF <mark>R</mark> VANIGDITHKDMELFVSLLKQYLIS  |
| StPhnW                      | MRALGFNTLLDD-E-LH-SPIITAFY-SPEDPQYRFSEFY-RRLK-EQ-GFVIYP <mark>G</mark> KVSQSDCF <mark>R</mark> IGNIGEVYAADITALLTAIRTAMYW   |
| PaPhnW                      | MARLGFRSFLPA-E-IQ-APIIVTFH-APRDPRYRFADFY-QRVR-EK-GFILYP <mark>C</mark> KLTQV-ETF <mark>P</mark> VGCIGHVDAAEMRQAVAAIGEALRE  |
| AvAGXT                      | LEDIGLSLHVEK-E-YR-LPTLTTVR-IPDGVDGKAVARQLLN-EH-NIEVGG <mark>C</mark> LGELA-GKVW <mark>E</mark> VGLMGFNSRKESVDQLIPALEQVL  |
| HsAGXT1                     | LQALGLQLFVKD-PALR-LPTVTTVA-VPAGYDWRDIV-SYVI-DHFDIEIMG <mark>C</mark> LGPST-GKVL <mark>RIGLLGCNATRE</mark> NVDRVTEALRAALQH  |
| ScAGXT1                     | LVNGLQLTSVSR-YPS-N-MS-AHGLTAVY-VADPPDVI-AFLK-SH-GVVIAGCIHKDIGPKYICTACNKNLPYMKNCFDLIKLALQR  |
| SsSPAT                      | LEALGLEIVARRPE-SY-SNTVTGVILKVADPQKVL-AGTV-NE-GVEFAPGVHPAFKYF <mark>RI</mark> GHMGWVTPNDAIIAISVIERTLRK  |
|                             |  |
|                             | 410 420  |
|                             | <u></u>  |
| MpATD                       | KITSKG-EV  |
| TdPhnW                      |  |
| BoPhnW                      | LQGGEEY-FI-S   |
| BmPhnW                      |  |
| SCPNNW                      | LDESEENL-KCE-  |
| AmPhnW                      | G  |
| PoPhnW                      | 1SYYNS-RRENNGNSKT  |
| StPhnW                      |  |
|                             | T  |
| PaPhnW                      | TK<br>LEVLEI   |
| PaPhnW<br>AvAGXT            | TK<br>LEVLERI<br>RRR   |
| PaPhnW<br>AvAGXT<br>HsAGXT1 | TK<br>LEVLE  |

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**39** Figure S2. Sequence alignment of MpMetRS and other Class V aminotransferases. Aminotransferase domain (ATD), 2-

40 Aminoethylphosphonate-pyruvate aminotransferase (AEPT), Alanine-glyoxalate aminotransferase (AGXT), Serine-pyruvate

41 aminotransferase (SPAT); M. penetrans ATD, T. denticola AEPT, B. obstructivus AEPT, B. megaterium AEPT, S. thermophilla

42 AEPT, A. macyae AEPT, P. bacterium AEPT, S. typhimurium AEPT, P. aeruginosa AEPT, A. variabilis AGXT, H. sapiens AGXT1,

- S. cerevisiae AGXT, S. solfataricus SPAT; Catalytically important residues are denoted by #. All sequences were truncated to
- the first region of homology due to several being fusion proteins. Multiple sequence alignment was generated using T-coffee
- MSA service [25].



Figure S3. SDS-PAGE of purified MpMetRSs. MpMetRS samples were separated on a 10% SDS-PAGE followed by Coomassie Blue staining. The expected size of full-length MpMetRS is 126.4 kDa and 61.4 kDa for Mp∆MetRS.

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Figure S4. Circular dichroism of MpMetRS alanine variants. Circular dichroism spectroscopy was performed in 1X PBS (pH 7.5) scanning from 200-260 nm at 25 °C; M568A is in black, K386A in blue, D616A in red and W1005A in green.

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# 58 Table S1. MpMetRS structural properties.

|                   | <u>M568A</u>   | <u>K386A</u>      | <u>D616A</u>     | <u>W1005A</u>    |
|-------------------|----------------|-------------------|------------------|------------------|
| PLP Occupancy     | $72 \pm 9.1$ % | $66.2 \pm 11.2$ % | $63.5 \pm 6.6$ % | $63.8 \pm 6.9$ % |
| Thermal Stability | 54.0           | 55.7              | 52.3             | 57.8             |

59

## 60 Table S2. MpMetRS tRNA<sup>Met</sup> in vitro primers.

|                            | Forward Primer                      | Reverse Primer             |
|----------------------------|-------------------------------------|----------------------------|
| Mp-<br>tRNA <sup>Met</sup> | AATTCCTGCAG <b>TAATACGACTCACTAT</b> | mUmGGTGACAGAGGAGAGATTCGAAC |
|                            | AGGCAGAGTATCTCAGTGGTTAGAGA          | TCTCGACACCTCGGGTATGAGCCGAG |
|                            | A <u>CTCGGCTCATACCCGAGG</u>         |                            |

61 \*T7 promoter in bold and overlapping regions are underlined.

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