

Supplementary Figures and Tables

FIGURE S1. Predicted structural features of *M. sedula* MCE. A) Multiple amino acid sequence alignment for MCE from *M. sedula*, *P. shermanii*, *T. tengcongensis*, and hypothetical protein of *Bacillus halodurans* C-125 (PDB: 3OA4). The conserved amino acid residues are shaded black, similar amino acids are boxed, and predicted residues for divalent cation binding are indicated by arrows. B) Predicted structure of *M. sedula* MMCE monomer, based on hypothetical protein BH1468 (PDB: [3OA4](#)) from *Bacillus halodurans* C-125, using the ModWeb online server (1, 2). C) The predicted metal binding site of *M. sedula* MCE, identified by comparison with that of *P. shermanii* and *T. tengcongensis* MCEs, metal binding residues were predicted to be H⁸, E⁵⁷, H⁸⁴, and E¹³⁴.

Figure S2. Alignment of amino acid sequences of MCM- α from archaea and bacteria, and other coenzyme B₁₂-dependent enzymes. The enzymes shown are as follows: MCM- α of *M. sedula* DSM 5348 (MCM α _Mse), MCM- α of *M. cuprina* Ar-4 (MCM α _Mcu), MCM- α of *S. solfataricus* P2 (MCM α _Sso), MCM- α of *S. tokodaii* str. 7 (MCM α _Sto), MCM- α of *S. acidocaldarius* DSM 639 (MCM β _Sac), MCM- α of *S. islandicus* M.14.25 (MCM α _Sis), MCM- α of *A. hospitalis* W1 (MCM α _Aho), MCM- α of *V. moutnovskia* 768-28 (MCM α _Vmo), MCM- α of *V. distributa* DSM 14429 (MCM α _Vdi), MCM- α of *A. saccharovorans* 345-15 (MCM α _Asa), MCM- α of *T. sibiricus* MM 739 (MCM α _Tsi), MCM- α of *T. barophilus* MP (MCM α _Tba), MCM- α of *P. furiosus* DSM 3638 (MCM α _Pfu), MCM- α of *P. sp.* NA2 (MCM α _Psp), MCM- α of *P. horikoshii* OT3 (MCM α _Pho), MCM- α of *T. gammatolerans* EJ3 (MCM α _Tga), MCM- α of *P. abyssi* GE5 (MCM α _Pab), MCM- α of *T. onnurineus* NA1 (MCM α _Ton), MCM- α of *C. sp.* Y-400-fl (MCM α _Csp), MCM- α of *C. aurantiacus* J-10-fl (MCM α _Cau), MCM- α of *C. aggregans* DSM 9485 (MCM α _Cag), N-terminal of MCM- α of *H. sapiens* (MCM α _Hsa), N-terminal of MCM- α of *M. musculus* (MCM α _Mmu), N-terminal of MCM- α of *P. shermanii* (MCM α _Psh), α -subunit of isobutyryl-CoA mutase of *S. cinnamomensis* (ICM α _Sci), α -subunit of glutamate mutase of *C. tetanomorphum* (GLM α _Cte).

Figure S3. A) Alignment of amino acid sequences of MCM- β from archaea and bacteria, and other coenzyme B₁₂-dependent enzymes. The enzymes shown are listed as follows: MCM- β of *M. sedula* DSM 5348 (MCM β _Mse), MCM- β of *A. pernix* K1 (MCM β _Ape), *V. moutnovskia* 768-28 (MCM β _Vmo), MCM- β of *A. saccharovorans* 345-15 (MCM β _Asa), MCM- β of *T. sibiricus* MM 739 (MCM β _Tsi), MCM- β of *C. sp.* Y-400-fl (MCM β _Csp), C-terminal of MCM- α of *H. sapiens* (MCM α _Hsa), C-terminal of MCM- α of *M. musculus* (MCM α _Mmu), C-terminal of MCM- α of *P. shermanii* (MCM α _Psh), β -subunit of isobutyryl-CoA mutase of *S. cinnamomensis* (ICM β _Sci), β -subunit of glutamate mutase of *C. tetanomorphum* (GLM β _Cte). The conserved amino acids are shaded black, and similar amino acids are boxed. The fingerprint “DXHXXG-SXL-GG” (where X is any amino acid) is indicated by an arrow. The residues H²¹-D¹⁹-K¹⁵ for the hydrogen-bonding network for coenzyme B₁₂ binding was indicated by asterisk. **B) Three dimensional model prediction for MCM- α (B) and MCM- β (C).** The three dimensional model of MCM- α and MCM- β were based, respectively, on MCM (PDB: [2XIJ](#), *Homo sapiens*) and MCM- β (PDB: [2YXB](#), *Aeropyrum Pernix*), using the ModWeb online server.

Figure S4. Relationship of 3-hydroxypropionate/4-hydroxybutyrate cycle with the metabolism of branched amino acids (valine, leucine, isoleucine, methionine) and propanoate to central carbon metabolism. Enzymes E1: ACC; E2: MCR; E3: MSR; E4: HPCS; E5: HPCD; E6: ACR; E7: MCE; E8: MCM; E9: SSR; E10: HBCS; E11: HBCD; E12: CCH; E13: ACK; E14: acetate kinase; E15: acetate-CoA ligase; E16: branched-chain-amino-acid transaminase; E17: 3-methyl-2-oxobutanoate dehydrogenase; E18: acyl-CoA dehydrogenase; E19: enoyl-CoA hydratase; E20: 3-hydroxyisobutyryl-CoA hydrolase; E21: 3-hydroxyisobutyrate dehydrogenase; E22: methylmalonate-semialdehyde dehydrogenase; E23: 3-hydroxyacyl-CoA dehydrogenase; E24: acetyl-CoA C-acyltransferase.

SUPPLEMENTARY TABLES AND FIGURES

TABLE S1. Primer sequences used for cloning *M. sedula* MCE (Msed_0639) and MCM (Msed_0638, Msed_2055)

| Gene | Orientation | Sequence |
|--------------|--------------------|---|
| <i>mce</i> | Forward | 5'-GACGACGACAAGATGAAGGGCAACAGTAAAGAAAG-3' |
| | Reverse | 5'-GAGGAGAAGCCCGGTAAACAACCTTCCACTTTTG-3' |
| <i>mcm-a</i> | Forward | 5'-CCCATTATACCTATTCTCTTCACTTTGCTCTCCTCTGCG-3' |
| | Reverse | 5'-TGCTCAATTGAGCGATTTTTTCATCCAAGGTCATCCTGG-3' |
| <i>mcm-β</i> | Forward | 5'-GATGGACTGGTGGTGTCTGGCTTACTTTGGTGTTAATATGC-3' |
| | Reverse | 5'-GCATATTAACACCAAAGTAAGCCGACACCACCAGTCCATC-3' |

TABLE S2. Enzymes of 3-hydroxypropionate/4-hydroxybutyrate cycle

| | | Identified in genome (Y/N) | | | | | | | | | | | | |
|--------------------------------------|--|----------------------------|------------------|------------------|-------------------|-------------------|------------------|------------------|------------------|------------------|-------------------|-------------------|------------------|------------------|
| | | ACC ¹ | MCR ¹ | MSR ¹ | HPCS ¹ | HPCD ¹ | ACR ¹ | MCE ¹ | MCM ¹ | SSR ¹ | HBCS ¹ | HBCD ¹ | CCH ¹ | ACK ¹ |
| Sulfolobaceae | <i>Metallosphaera sedula</i> DSM | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A ² | Y | Y | Y |
| | <i>Metallosphaera cuprina</i> Ar-4 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Acidianus hospitalis</i> W1 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Sulfolobus tokodaii</i> str. 7 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Sulfolobus acidocaldarius</i> DSM | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Sulfolobus islandicus</i> Y.G.57.14 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Sulfolobus islandicus</i> Y.N.15.51 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Sulfolobus islandicus</i> L.S.2.15 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Sulfolobus islandicus</i> L.D.8.5 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Sulfolobus islandicus</i> M.16.4 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| | <i>Sulfolobus solfataricus</i> P2 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y |
| <i>Sulfolobus islandicus</i> M.14.25 | Y | Y | Y | Y | Y | Y | Y | Y | Y | N/A | Y | Y | Y | |
| Thermoproteales | <i>Vulcanisaeta moutnovskia</i> 768-28 | N | N | N | Y | N | Y | N | Y | N | N/A | N | Y | Y |
| | <i>Vulcanisaeta distributa</i> DSM | N | N | N | Y | Y | Y | Y | Y | N | N/A | N | Y | Y |
| Acidilobales | <i>Acidilobus saccharovorans</i> 345- | N | N | N | Y | Y | Y | N | Y | N | N/A | N | Y | Y |
| Desulfurococcales | <i>Aeropyrum permix</i> K1 | N | N | N | N | Y | N | N | Y | N | N/A | N | Y | Y |
| | <i>Ignicoccus hospitalis</i> KIN4/I | N | Y | N | N | N | N | N | N | N | N/A | Y | Y | Y |
| Euryarchaeotes (Thermococcales) | <i>Thermococcus sibiricus</i> MM 739 | N | N | N | N | N | N | Y | Y | N | N/A | N | N | N |
| | <i>Thermococcus barophilus</i> MP | N | N | N | N | N | N | Y | Y | N | N/A | N | N | N |
| | <i>Pyrococcus furiosus</i> DSM 3638 | N | N | N | N | N | N | Y | Y | N | N/A | N | N | N |
| | <i>Pyrococcus</i> sp. NA2 | N | Y | N | N | N | N | Y | Y | N | N/A | N | N | N |
| | <i>Pyrococcus horikoshii</i> OT3 | N | N | N | N | N | N | Y | Y | N | N/A | N | N | N |

| | | | | | | | | | | | | | | |
|----------------|---|---|---|---|---|---|---|---|---|---|-----|---|---|---|
| | <i>Thermococcus gammatolerans</i> | N | N | N | Y | N | N | Y | Y | N | N/A | N | N | N |
| | <i>Pyrococcus abyssi</i> GE5 | N | N | N | N | N | N | Y | Y | N | N/A | N | N | N |
| | <i>Thermococcus onnurineus</i> NA1 | N | N | N | N | N | N | Y | Y | N | N/A | N | N | N |
| Chloroflexales | <i>Chloroflexus</i> sp. Y-400-fl | Y | Y | N | N | N | N | Y | Y | N | N/A | N | N | N |
| | <i>Chloroflexus aurantiacus</i> J-10-fl | Y | Y | N | N | N | N | Y | Y | N | N/A | N | N | N |
| | <i>Chloroflexus aggregans</i> DSM | Y | Y | N | N | N | N | Y | Y | N | N/A | N | N | N |

¹ACC(Msed_0147, 0148, 1375), MCR(Msed_0709) , MSR(Msed_1993) , HPCS(Msed_1456),HPCD(Msed_2001), ACR(Msed_1426) , MCE(Msed_0639), MCM(Msed_0638, Msed_2055), SSR(Msed_1424), HBCS, HBCD(Msed_1321), CCH(Msed_0399), ACK(Msed_0656).

²N/A: No information is available.

TABLE S3. Comparisons of putative gene clusters containing MCE, MCM in microbial genomes

| Organism | | Genetic constitution | PF ¹ |
|-----------------|--|----------------------|-----------------|
| Sulfolobaceae | <i>Metallosphaera sedula</i> DSM 5348 | | AB ² |
| | <i>Metallosphaera cuprina</i> Ar-4 | | |
| | <i>Acidianus hospitalis</i> W1 | | |
| | <i>Sulfolobus tokodaii</i> str. 7 | | |
| | <i>Sulfolobus acidocaldarius</i> DSM 639 | | |
| | <i>Sulfolobus islandicus</i> | | |
| | <i>Sulfolobus islandicus</i> Y.N.15.51 | | |
| | <i>Sulfolobus islandicus</i> L.S.2.15 | | |
| | <i>Sulfolobus islandicus</i> L.D.8.5 | | |
| | <i>Sulfolobus islandicus</i> M.16.4 | | |
| | <i>Sulfolobus solfataricus</i> P2 | | |
| | <i>Sulfolobus islandicus</i> M.14.25 | | |
| Thermoproteales | <i>Vulcanisaeta moutnovskia</i> 768-28 | | B ² |
| | <i>Vulcanisaeta distributa</i> DSM 14429 | | |
| Acidilobales | <i>Acidilobus saccharovorans</i> 345-15 | | |

| | | | |
|---|---|--|-----------------|
| Desulfurococcales | <i>Aeropyrum pernix</i> K1 | | |
| Euryarchaeotes (Thermococcales) | <i>Thermococcus sibiricus</i> MM 739 | | |
| | <i>Thermococcus barophilus</i> MP | | |
| | <i>Pyrococcus furiosus</i> DSM 3638 | | |
| | <i>Pyrococcus</i> sp. NA20788 | | |
| | <i>Pyrococcus horikoshii</i> OT3 | | |
| | <i>Thermococcus gammatolerans</i> EJ3 | | |
| | <i>Pyrococcus abyssi</i> GE5 | | |
| | <i>Thermococcus onnurineus</i> NA1 | | |
| Chloroflexales | <i>Chloroflexus</i> sp. Y-400-fl | | BC ² |
| | <i>Chloroflexus aurantiacus</i> J-10-fl | | |
| | <i>Chloroflexus aggregans</i> DSM 9485 | | |
| | | | |
| ¹ PF: proposed function; | | | |
| ² A: Autotrophic carbon fixation through 3-hydroxypropionate/4-hydroxybutyrate cycle; B: Metabolism of branched amino acids (valine, isoleucine, methionine) and propanoate. C: Autotrophic carbon fixation through 3-Hydroxypropionate bicycle. | | | |

TABLE S4. Methylmalonyl-CoA epimerase and methylmalonyl-CoA mutase homologs

| Organism | | MCE (Msed_0639) | | MCM- α (Msed_0638) | | MCM- β (Msed_2055) | |
|------------------------------------|--|-----------------|--------------|---------------------------|--------------|--------------------------|--------------|
| | | Gene symbol | Identity (%) | Gene symbol | Identity (%) | Gene symbol | Identity (%) |
| Sulfolobaceae | <i>Metallosphaera sedula</i> DSM 5348 | Msed_0639 | 100 | Msed_0638 | 100 | Msed_2055 | 100 |
| | <i>Metallosphaera cuprina</i> Ar-4 | Mcup_1517 | 91 | Mcup_1516 | 93 | Mcup_0235 | 86 |
| | <i>Acidianus hospitalis</i> W1 | Ahos_2217 | 67 | Ahos_2216 | 83 | Ahos_0509 | 78 |
| | <i>Sulfolobus tokodaii</i> str. 7 | ST0554 | 74 | ST0552 | 82 | ST2096 | 76 |
| | <i>Sulfolobus acidocaldarius</i> DSM 639 | Saci_0923 | 64 | Saci_0924 | 79 | Saci_0062 | 74 |
| | <i>Sulfolobus islandicus</i> Y.G.57.14 | YG5714_0220 | 66 | YG5714_0219 | 79 | YG5714_0078 | 76 |
| | <i>Sulfolobus islandicus</i> Y.N.15.51 | YN1551_2823 | 66 | YN1551_2822 | 79 | YN1551_0076 | 76 |
| | <i>Sulfolobus islandicus</i> L.S.2.15 | LS215_0247 | 65 | LS215_0246 | 79 | LS215_0076 | 76 |
| | <i>Sulfolobus islandicus</i> L.D.8.5 | LD85_0221 | 66 | LD85_0220 | 78 | LD85_0076 | 76 |
| | <i>Sulfolobus islandicus</i> M.16.4 | M164_0235 | 66 | M164_0234 | 79 | M164_0076 | 76 |
| | <i>Sulfolobus solfataricus</i> P2 | SSO2425 | 64 | SSO2424 | 78 | SSO2266 | 79 |
| | <i>Sulfolobus islandicus</i> M.14.25 | M1425_0216 | 65 | M1425_0215 | 78 | M1425_0076 | 76 |
| Thermoproteales | <i>Vulcanisaeta moutnovskia</i> 768-28 | VMUT_0926 | 43 | VMUT_0924 | 61 | VMUT_0925 | 63 |
| | <i>Vulcanisaeta distributa</i> DSM 14429 | Vdis_0039 | 42 | Vdis_0037 | 61 | Vdis_0038 | 59 |
| Acidilobales | <i>Acidilobus saccharovorans</i> 345-15 | N/A | N/A | ASAC_1077 | 57 | ASAC_1078 | 61 |
| Desulfurococcales | <i>Aeropyrum pernix</i> K1 | N/A | N/A | APE_1687 | 57 | APE_1686 | 63 |
| | <i>Ignicoccus hospitalis</i> KIN4/I | N/A | N/A | N/A | N/A | N/A | N/A |
| Euryarchaeotes (Thermococcales) | <i>Thermococcus sibiricus</i> MM 739 | TSIB_1401 | 43 | TSIB_0812 | 61 | TSIB_1402 | 59 |
| | <i>Thermococcus barophilus</i> MP | TERMP_1057 | 45 | TERMP_1292 | 61 | TERMP_1059 | 58 |
| | <i>Pyrococcus furiosus</i> DSM 3638 | PF1948 | 46 | PF1477 | 62 | PF1946 | 60 |
| | <i>Pyrococcus</i> sp. NA2 | PNA2_0788 | 48 | PNA2_1880 | 62 | PNA2_0709 | 61 |

| | | | | | | | |
|----------------|---|-------------|----|-------------|----|-------------|----|
| | <i>Pyrococcus horikoshii</i> OT3 | PH0272 | 48 | PH1306 | 61 | PH0275 | 61 |
| | <i>Thermococcus gammatolerans</i> EJ3 | TGAM_1929 | 46 | TGAM_1685 | 61 | TGAM_1931 | 55 |
| | <i>Pyrococcus abyssi</i> GE5 | PAB2438 | 48 | PAB1800 | 62 | PABmcmA2 | 61 |
| | <i>Thermococcus onnurineus</i> NA1 | TON_1074 | 45 | TON_1110 | 61 | TON_1076 | 63 |
| Chloroflexales | <i>Chloroflexus</i> sp. Y-400-fl | Chy400_3283 | 39 | Chy400_1995 | 53 | Chy400_0048 | 56 |
| | <i>Chloroflexus aurantiacus</i> J-10-fl | Caur_3037 | 39 | Caur_1844 | 53 | Caur_0042 | 56 |
| | <i>Chloroflexus aggregans</i> DSM 9485 | Cagg_0981 | 41 | Cagg_2545 | 52 | Cagg_0587 | 56 |

TABLE S5. Transcription analysis of gene cluster of MCE (Msed_0639), MCM (Msed_0638, Msed_2055) for *M. sedula* under autotrophic (AC) and heterotrophic (HT) conditions.

| | Annotation | Transcription level AC ¹ | Transcription level HC ² | Fold change ³ | Significance ⁴ (-log ₁₀ P value) |
|------------------|---|-------------------------------------|-------------------------------------|--------------------------|---|
| Msed_0637 | Phosphoribosyltransferase | -0.31 | 0.24 | 0.68 | 1.58 |
| Msed_0638 | Methylmalonyl-CoA mutase-catalytic subunit (α) | 4.36 | 1.21 | 8.91 | 15.48 |
| Msed_0639 | Methylmalonyl-CoA epimerase | 3.16 | 1.00 | 4.47 | 6.54 |
| Msed_0640 | Heat shock protein Hsp20 | 2.74 | 0.88 | 3.63 | 7.13 |
| Msed_0641 | ZPR1-related zinc finger protein | 0.67 | 1.01 | 0.79 | 0.71 |
| Msed_2054 | Hypothetical protein | -0.26 | 1.37 | 0.32 | 5.26 |
| Msed_2055 | Methylmalonyl-CoA mutase-coenzyme B ₁₂ binding subunit (β) | 3.14 | 1.52 | 3.08 | 3.97 |
| Msed_2056 | LAO/AO transport system ATPase | 1.83 | -0.77 | 6.05 | 9.58 |
| Msed_2057 | Hypothetical protein | -0.05 | -1.88 | 3.57 | 5.64 |

¹ Least-squares mean (lsm) of normalized log₂-transformed transcription levels; AC: Autotrophic condition

² Least-squares mean (lsm) of normalized log₂-transformed transcription levels; HC: Heterotrophic condition

³ Fold change = 2^(AC-HC)

⁴ Bonferroni correction for this data set was 5.4

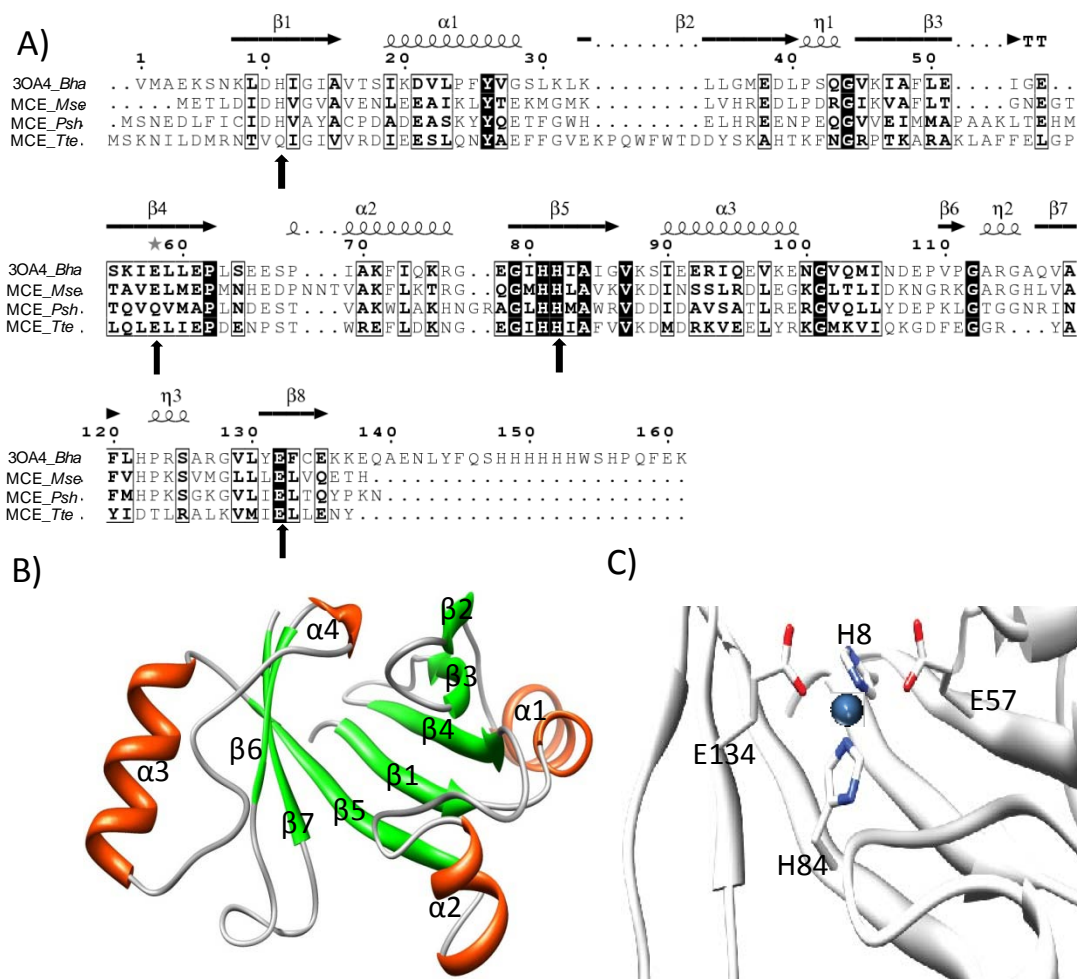
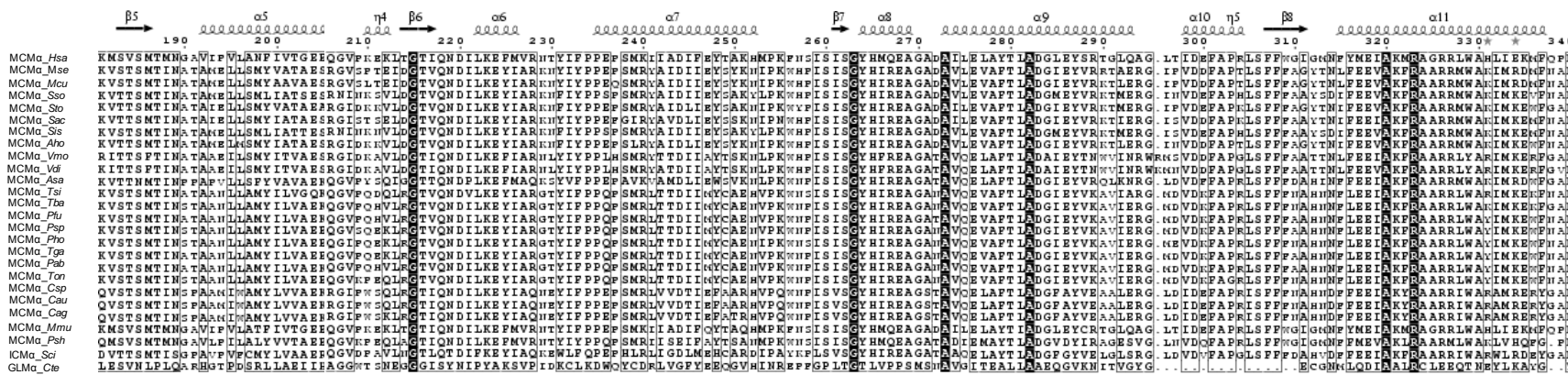


FIGURE S1. Predicted structural features of *M. sedula* MCE. A) Multiple amino acid sequence alignment for MCE from *M. sedula*, *P. shermanii*, *T. tengcongensis*, and hypothetical protein of *Bacillus halodurans* C-125 (PDB: 3OA4). The conserved amino acid residues are shaded black, similar amino acids are boxed, and predicted residues for divalent cation binding are indicated by arrows. B) Predicted structure of *M. sedula* MMCE monomer, based on hypothetical protein BH1468 (PDB: [3OA4](#)) from *Bacillus halodurans* C-125, using the ModWeb online server (1, 2). C) The predicted metal binding site of *M. sedula* MCE, identified by comparison with that of *P. shermanii* and *T. tengcongensis* MCEs, metal binding residues were predicted to be H⁸, E⁵⁷, H⁸⁴, and E¹³⁴.



MCMa_Hsa LIQORLLHQQOPLRFENALAKKQLKGNHFDLILHHEPISISIKFLYSR...RDTIDLPEELPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Mse NVTFERVDEKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KGDVEKHYGPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Mcu NVTOBRVDEKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KGDVEKHYGPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Sso NDVDRIDKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KGDVEKHYGPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Sip EKLEIKYQDEKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KGSXAKIIGPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Sac DKLEIKYQDEKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KGDVLSLQPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Sis NDLEDRIDKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KGDVLSLQPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Aho NDLEDRIDKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KGDVLSLQPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_V60 DKIKERVDKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KDDVLDLQPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_V66 DKIKERVDKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KDDVLDLQPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Asa KRKESDIAKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KFDKXNELLQPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Tsi DEIKKTEDEKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KNDVLELQPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
 MCMa_Tba DEIKKTEDEKESKYLQPLKIKRKRRIKHFITPSSGELHNTLYTFLDLD...KNDVLELQPGVYRPFTRGYPYPTQTFEPRWTRIQYAGFQSSAEDDNRFRRLLEAGQTGSTAPDLPLQLGLDDEHDLAETVEGVVGVSMFHKRMDLVTHGDFLH
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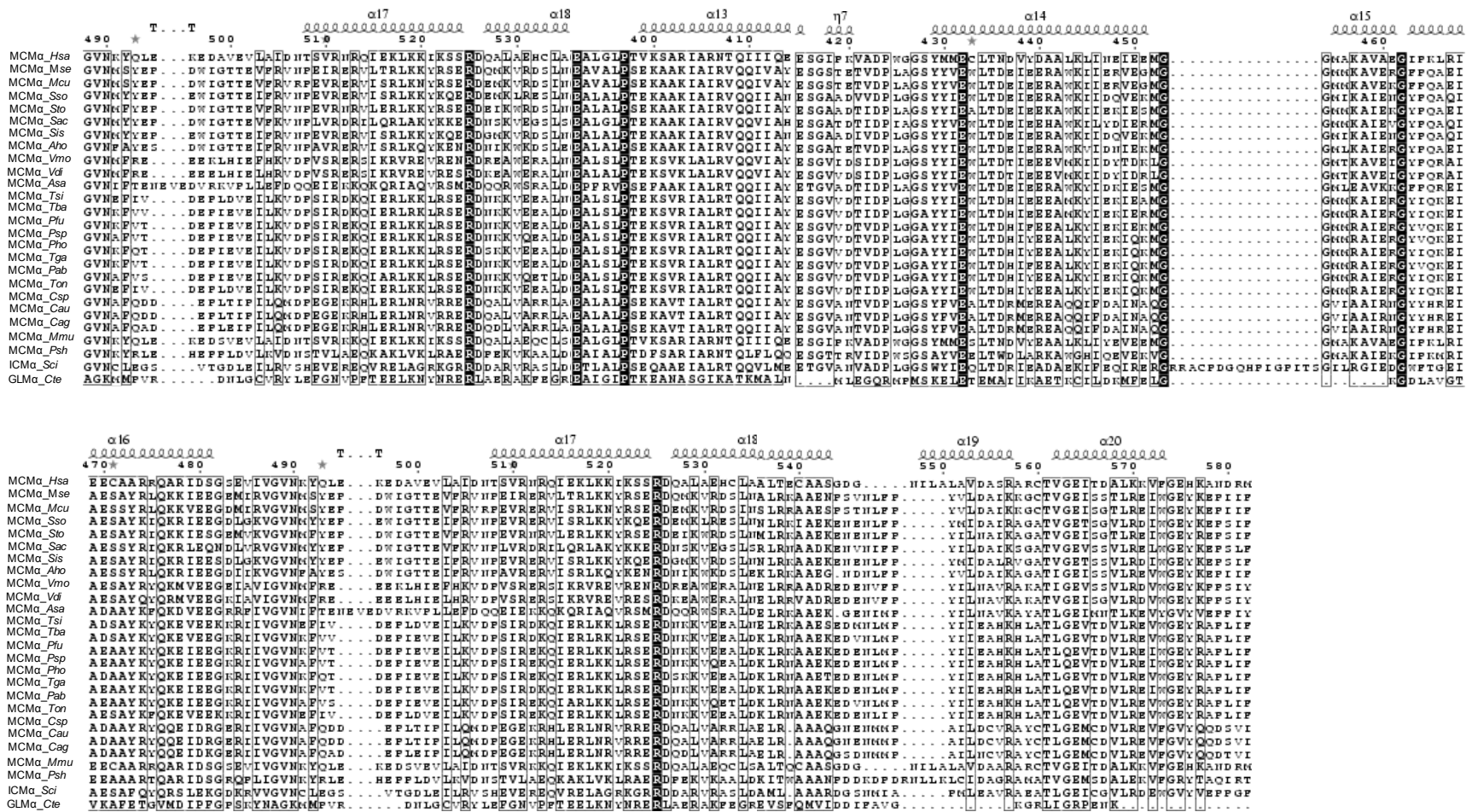


Figure S2. Alignment of amino acid sequences of MCM-α from archaea and bacteria and other coenzyme B₁₂-dependent enzymes. The enzymes shown are as follows: MCM-α of *M. sedula* DSM 5348 (MCMα_Mse), MCM-α of *M. cuprina* Ar-4 (MCMα_Mcu), MCM-α of *S. solfataricus* P2 (MCMα_Sso), MCM-α of *S. tokodaii* str. 7 (MCMα_Sto), MCM-α of *S. acidocaldarius* DSM 639 (MCMβ_Sac), MCM-α of *S. islandicus* M.14.25 (MCMα_Sis), MCM-α of *A. hospitalis* W1 (MCMα_Aho), MCM-α of *V. moutnovskia* 768-28 (MCMα_Vmo), MCM-α of *V. distributa* DSM 14429 (MCMα_Vdi), MCM-α of *A. saccharovorans* 345-15

(MCM α _Asa), MCM- α of *T. sibiricus* MM 739 (MCM α _Tsi), MCM- α of *T. barophilus* MP (MCM α _Tba), MCM- α of *P. furiosus* DSM 3638 (MCM α _Pfu), MCM- α of *P. sp.* NA2 (MCM α _Psp), MCM- α of *P. horikoshii* OT3 (MCM α _Pho), MCM- α of *T. gammatolerans* EJ3 (MCM α _Tga), MCM- α of *P. abyssi* GE5 (MCM α _Pab), MCM- α of *T. onnurineus* NA1 (MCM α _Ton), MCM- α of *C. sp.* Y-400-fl (MCM α _Csp), MCM- α of *C. aurantiacus* J-10-fl (MCM α _Cau), MCM- α of *C. aggregans* DSM 9485 (MCM α _Cag), N-terminal of MCM- α of *H. sapiens* (MCM α _Hsa), N-terminal of MCM- α of *M. musculus* (MCM α _Mmu), N-terminal of MCM- α of *P. shermanii* (MCM α _Psh), α -subunit of isobutyryl-CoA mutase of *S. cinnamomensis* (ICM α _Sci), α -subunit of glutamate mutase of *C. tetanomorphum* (GLM α _Cte).

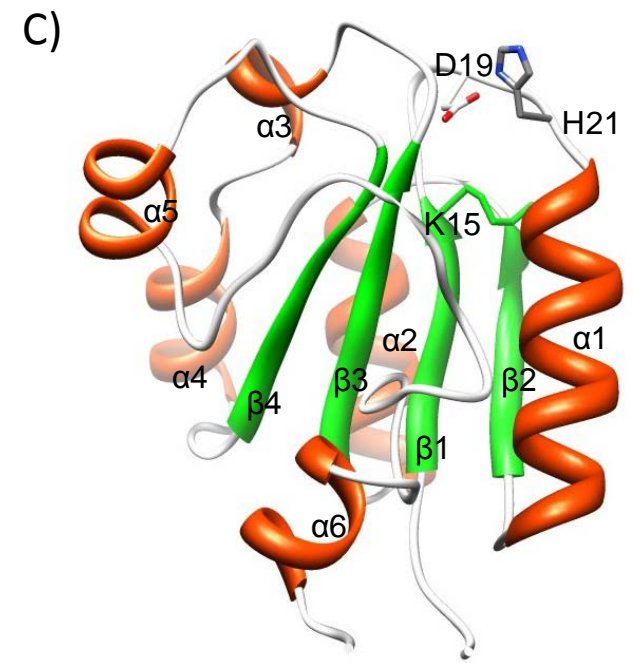
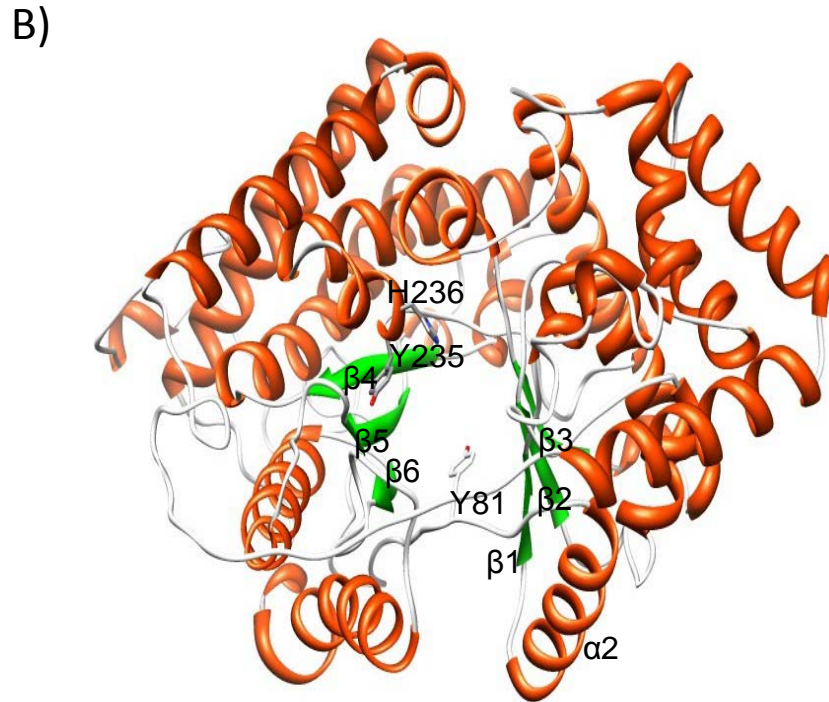
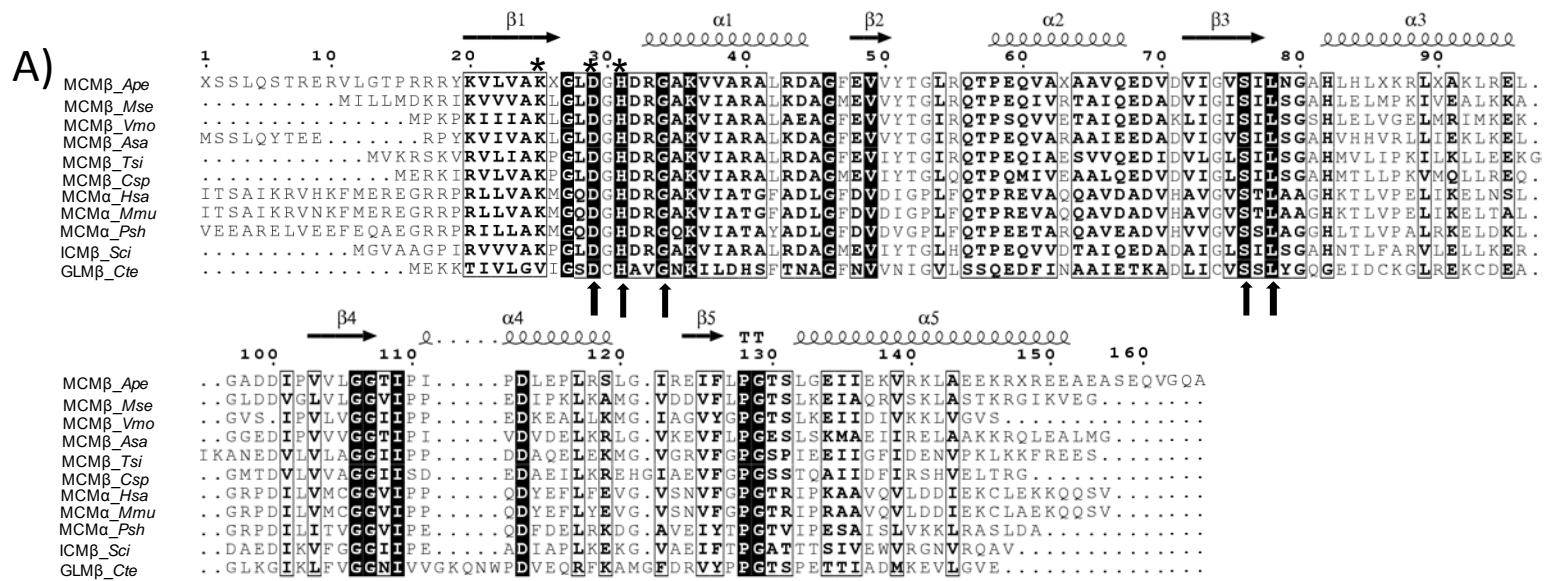


Figure S3. A) Alignment of amino acid sequences of MCM- β from archaea and bacteria, and other coenzyme B₁₂-dependent enzymes. The enzymes shown are listed as follows: MCM- β of *M. sedula* DSM 5348 (MCM β _Mse), MCM- β of *A. pernix* K1 (MCM β _Ape), *V. moutnovskia* 768-28 (MCM β _Vmo), MCM- β of *A. saccharovorans* 345-15 (MCM β _Asa), MCM- β of *T. sibiricus* MM 739 (MCM β _Tsi), MCM- β of *C. sp.* Y-400-fl (MCM β _Csp), C-terminal of MCM- α of *H. sapiens* (MCM α _Hsa), C-terminal of MCM- α of *M. musculus* (MCM α _Mmu), C-terminal of MCM- α of *P. shermanii* (MCM α _Psh), β -subunit of isobutyryl-CoA mutase of *S. cinnamomensis* (ICM β _Sci), β -subunit of glutamate mutase of *C. tetanomorphum* (GLM β _Cte). The conserved amino acids are shaded black, and similar amino acids are boxed. The fingerprint “DXHXXG-SXL-GG” (where X is any amino acid) is indicated by an arrow. The residues H²¹-D¹⁹-K¹⁵ for the hydrogen-bonding network for coenzyme B₁₂ binding was indicated by asterisk. **B) Three dimensional model prediction for MCM- α (B) and MCM- β (C).** The three dimensional model of MCM- α and MCM- β were based, respectively, on MCM (PDB: [2XIJ](#), *Homo sapiens*) and MCM- β (PDB: [2YXB](#), *Aeropyrum Pernix*), using the ModWeb online server.

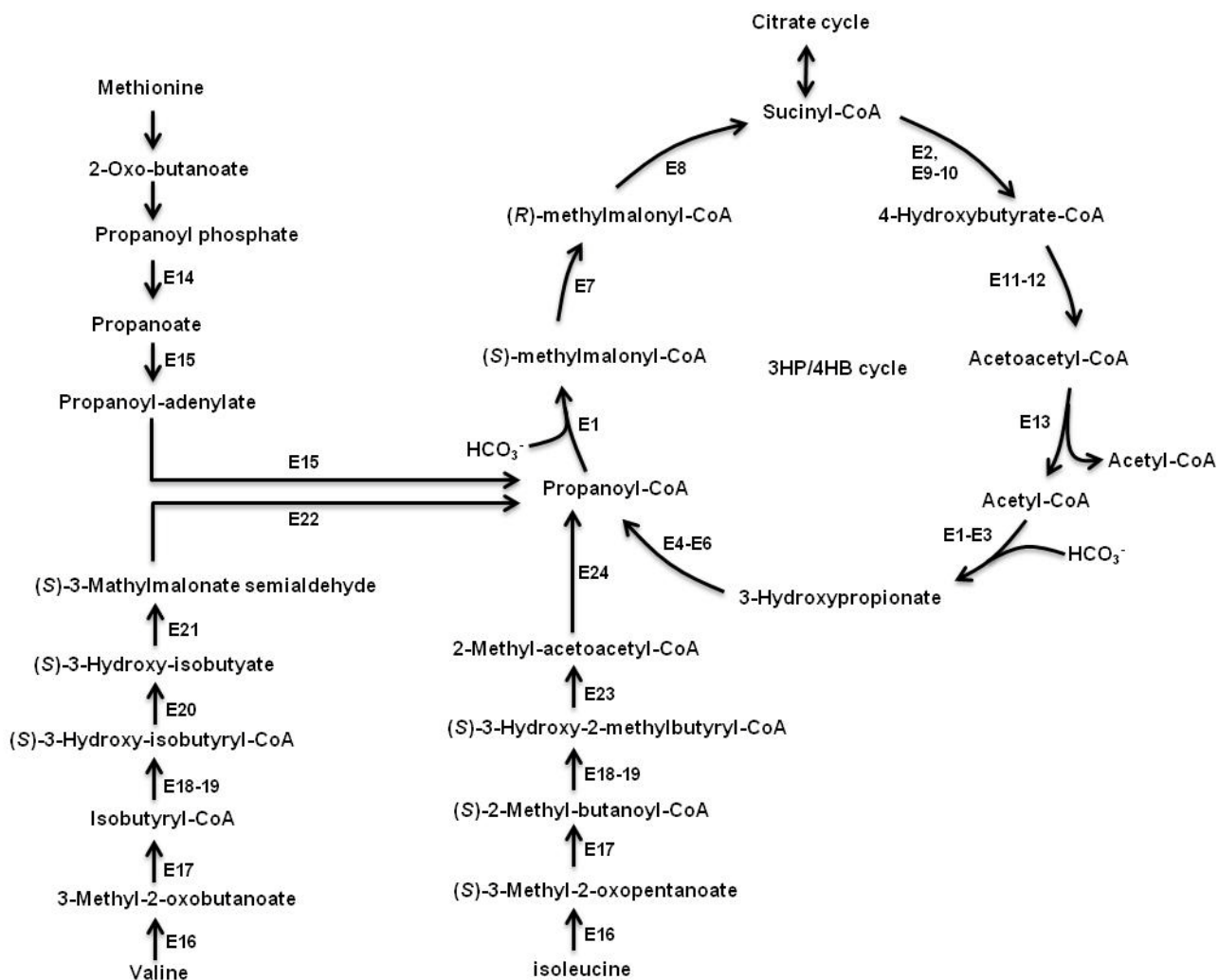


Figure S4. Relationship of 3-hydroxypropionate/4-hydroxybutyrate cycle with the metabolism of branched amino acids (valine, leucine, isoleucine, methionine) and propanoate to central carbon metabolism. Enzymes E1: ACC; E2: MCR; E3: MSR; E4: HPCS; E5: HPCD; E6: ACR; E7: MCE; E8: MCM; E9: SSR; E10: HBCS; E11: HBCD; E12: CCH; E13: ACK; E14: acetate kinase; E15: acetate-CoA ligase; E16: branched-chain-amino-acid transaminase; E17: 3-methyl-2-oxobutanoate dehydrogenase; E18: acyl-CoA dehydrogenase; E19: enoyl-CoA hydratase; E20: 3-hydroxyisobutyryl-CoA hydrolase; E21: 3-hydroxyisobutyrate dehydrogenase; E22: methylmalonate-semialdehyde dehydrogenase; E23: 3-hydroxyacyl-CoA dehydrogenase; E24: acetyl-CoA C-acyltransferase.

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