SUPPLEMENTARY INFORMATION

Reversing Methanogenesis to Capture Methane for Liquid Biofuel Precursors

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Supplementary Table 1. Components in HS medium used to grow ANME-1 Mcr-producing *M. acetivorans* on methane and 0.1 mM or 10 mM $FeCl_3$.

Chemical	Final concentration (mM)	Amount in 5 mL (µmol)	Amount in 8 mL (µmol)
NaCl	400	2000	3200
$MgSO_4.6H_2O$	54	270	430
$NaHCO_3$	45	230	360
Methane	41	210	330
NH_4Cl	19	95	150
KCl	13	65	100
KH_2PO_4	5.0	25	40
Cysteine.HCl	3.2	16	26
$CaCl_2.2H_2O$	2.0	10	16
$Na_2S.9H_2O$	1.0	5.0	8.0
$MgSO_4.7H_2O$	0.10	0.50	0.80
$FeCl_3.6H_2O$	0.10 or 10	0.50 or 50	0.80 or 80
Nitrilotriacetic acid	0.079	0.40	0.63
$MnSO_4.H_2O$	0.030	0.15	0.24
$ZnCl_2$	0.0059	0.030	0.047
Pyridoxine hydrochloride	0.0049	0.025	0.039
Nicotinic acid	0.0041	0.021	0.033
Resazurin (sodium salt)	0.0040	0.020	0.032
p-Aminobenzoic acid	0.0037	0.019	0.030
Puromycin dihydrochloride	0.0037	0.019	0.030
$CoSO_4.7H_2O$	0.0036	0.018	0.029
$FeSO_4.7H_2O$	0.0036	0.018	0.029
Lipoic acid	0.0024	0.012	0.019
Boric acid	0.0016	0.0080	0.013
Thiamine hydrochloride	0.0015	0.0075	0.012
Riboflavin	0.0013	0.0065	0.010
Pantothenic acid (calcium salt)	0.0010	0.0050	0.0080
$Na_2MoO_4.2H_2O$	0.0010	0.0050	0.0080
$NiCl_2.6H_2O$	0.0010	0.0050	0.0080
Biotin	0.00082	0.0041	0.0066
Folic acid	0.00045	0.0023	0.0036
$CuSO_4.5H_2O$	0.00040	0.0020	0.0032
$KAl(SO_4)_2.12H_2O$	0.00021	0.0011	0.0017
Vitamin B_{12}.HCl	0.0000074	0.000037	0.000059

Supplementary Table 2. Differentially-expressed genes in *M. acetivorans*/pES1-MAT*mcr*3 grown on methane and 0.1 mM $FeCl_3$ compared to *M. acetivorans*/pES1-MAT*mcr*3 grown on methanol, as determined by RNA sequencing. Fold change is determined by the ratio of FPKM of three replicates grown on methane ($FPKM_{methane}$) to FPKM of three replicates grown on methanol ($FPKM_{methanol}$). A positive fold change indicates induced expression, whereas a negative fold change indicates repressed expression. Statistical significance after taking false discovery rate of 0.05 into account was determined at $q < 0.07$.

Gene	Gene product	$FPKM_{methane}$	$FPKM_{methanol}$	Fold change	Genome coordinates of mapped read
Induced					
MA1997	Hypothetical protein	9.2	0	∞	NC_003552.1: 2,473,596 → 2,473,934
MA0463	Ferredoxin	318.6	33.3	9.6	NC_003552.1:542,619 ← 542,799
Repressed					
MA3469	Hypothetical protein	16.3	3751.7	-229.1	NC_003552.1:4,280,293 ← 4,280,638
MA3300	Hypothetical protein	72.5	2104.0	-29.0	NC_003552.1: 4,075,459 → 4,075,671
novel transcript	Unannotated	15.4	433.1	-28.1	NC_003552.1:1,615,210 – 1,615,658
MA4384	Hypothetical protein	1.2	29.3	-25.1	NC_003552.1: 5,411,710 → 5,413,605
mcrA (Host)	Methyl coenzyme M reductase, subunit alpha	9.4	178.9	-18.6	NC_003552.1: 5,596,675 ← 5,598,387
mcrB (Host)	Methyl coenzyme M reductase, subunit beta	5.9	62.5	-10.7	NC_003552.1: 5,600,310 ← 5,601,614
mtaB1	Methanol-5-hydroxybenzimidazolylcobamide co-methyltransferase, isozyme 1	5.9	75.5	-12.8	NC_003552.1: 534,844 ← 536,229
mtaB2	Methanol-5-hydroxybenzimidazolylcobamide co-methyltransferase, isozyme 2	4.4	28.3	-6.4	NC_003552.1:5,420,051 → 5,421,437
novel transcript	Unannotated	0	322.5	-∞	NC_003552.1:1,440,944 – 1,441,203
novel transcript	Unannotated	0	116.7	-∞	NC_003552.1:63,866 – 64,037
atpC	V-type ATP synthase subunit C	0	24.7	-∞	NC_003552.1: 5,071,956 → 5,072,204

cdhB	Acetyl-CoA decarbonylase/synthase complex, subunit epsilon	0	13.7	-∞	NC_003552.1: 1,211,315 ← 1,211,827
fmdD	Formylmethanofuran dehydrogenase, subunit D	0	6.6	-∞	NC_003552.1: 367,095 → 367,484
groES	Co-chaperonin GroES	0	11.4	-∞	NC_003552.1: 741,925 → 742,203
MA0369	Hypothetical protein	0	6.4	-∞	NC_003552.1: 435,971 ← 436,561
MA0515	Hypothetical protein	0	19.5	-∞	NC_003552.1: 599,612 ← 600,484
MA0609	H/ACA RNA-protein complex component Gar1	0	9.5	-∞	NC_003552.1: 715,092 → 715,352
MA1027	Hypothetical protein	0	11.2	-∞	NC_003552.1: 1,228,492 → 1,228,737
MA1090	50S ribosomal protein L19	0	7.6	-∞	NC_003552.1: 1,295,017 → 1,295,472
MA1376	Phosphoribosylaminoimidazole carboxylase	0	8.6	-∞	NC_003552.1: 1,643,428 ← 1,643,823
MA1663	Hypothetical protein	0	89.3	-∞	NC_003552.1: 1,996,878 → 1,997,207
MA2558	Hypothetical protein	0	7.1	-∞	NC_003552.1: 3,183,587 ← 3,183,991
MA3479	Ferrous iron transport protein A	0	25.6	-∞	NC_003552.1: 4,305,144 ← 4,305,392
MA4194	Ferredoxin	0	92.5	-∞	NC_003552.1: 5,118,713 → 5,118,889
MA4277	50S ribosomal protein L12	0	151.1	-∞	NC_003552.1: 5,197,662 → 5,197,976
MA4508	Hypothetical protein	0	37.8	-∞	NC_003552.1: 5,555,059 → 5,555,268
MA4565	Hypothetical protein	0	690.5	-∞	NC_003552.1: 5,626,276 ← 5,626,488

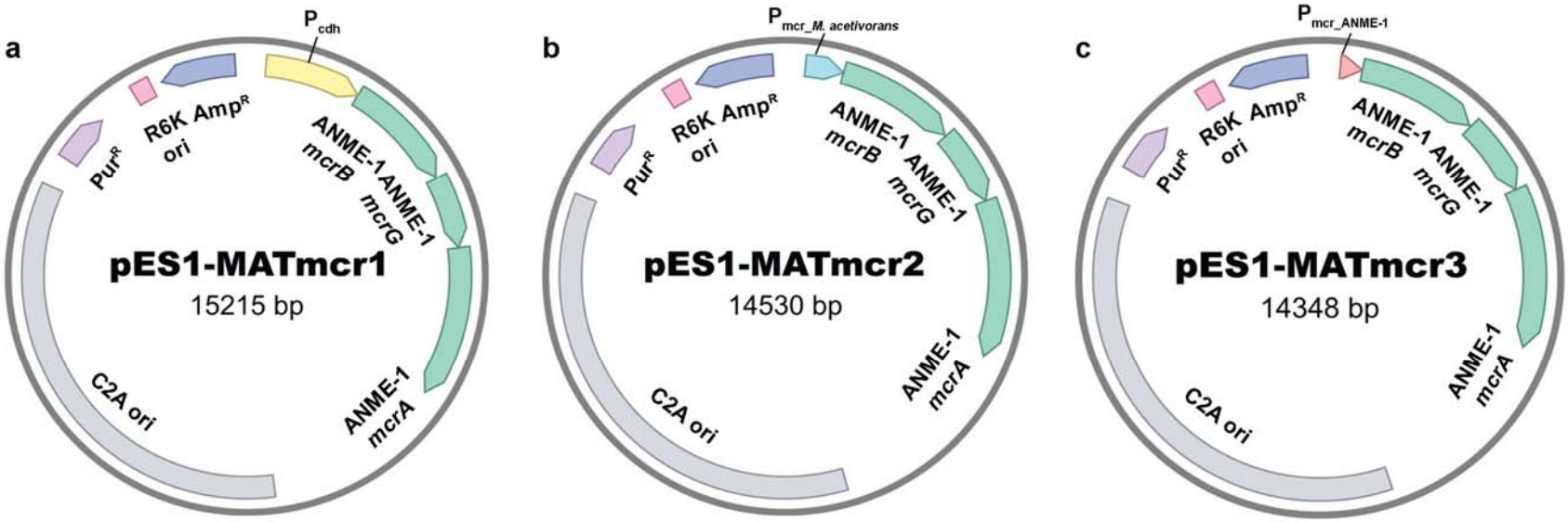
Supplementary Table 3. Strains and plasmids used in this study.

Strain/Plasmid	Description	Source
M. acetivorans C2A	Wildtype *M. acetivorans**	J. G. Ferry
E. coli DH5α-λ*pir*	F^- *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 hsdR17*(r_K^- m_K^+) λ*pir*	W. W. Metcalf
pES1	Amp^R, Pur^R, R6K *ori*, C2A *ori*, P_{cdh}	K. R. Sowers
pES1(Pmat)	Amp^R, Pur^R, R6K *ori*, C2A *ori*, $P_{mcr\_ANME\text{-}1}$	This study
pES1-MAT*mcr*1	Amp^R, Pur^R, R6K *ori*, C2A *ori*, P_{cdh}::$mcr_{ANME\text{-}1}$	This study
pES1-MAT*mcr*2	Amp^R, Pur^R, R6K *ori*, C2A *ori*, $P_{mcr\_M.\ acetivorans}$::$mcr_{ANME\text{-}1}$	This study
pES1-MAT*mcr*3	Amp^R, Pur^R, R6K *ori*, C2A *ori*, $P_{mcr\_ANME\text{-}1}$::$mcr_{ANME\text{-}1}$	This study
pES1-MAT*mcr*1-*flag*	Amp^R, Pur^R, R6K *ori*, C2A *ori*, P_{cdh}::$mcr_{ANME\text{-}1}$-*flag*	This study
pES1-MAT*mcr*2-*flag*	Amp^R, Pur^R, R6K *ori*, C2A *ori*, $P_{mcr\_M.\ acetivorans}$::$mcr_{ANME\text{-}1}$-*flag*	This study
pES1-MAT*mcr*3-*flag*	Amp^R, Pur^R, R6K *ori*, C2A *ori*, $P_{mcr\_ANME\text{-}1}$::$mcr_{ANME\text{-}1}$-*flag*	This study

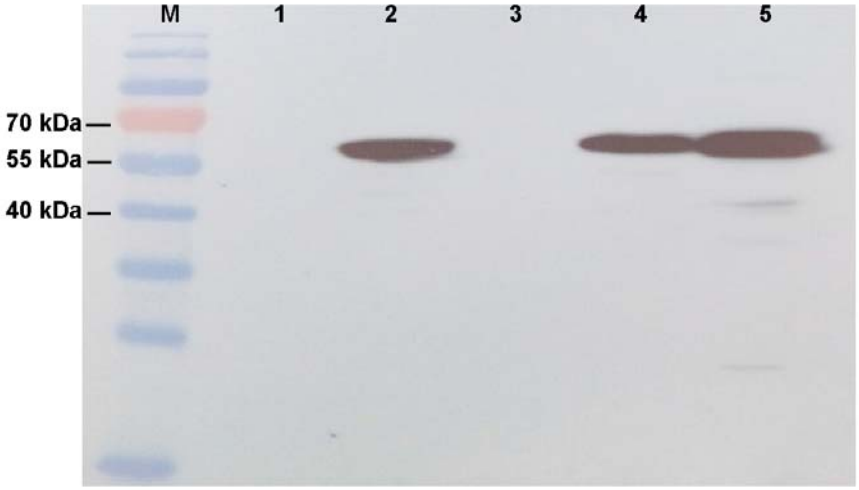
*In comparison to the reference genome of *M. acetivorans* (Genbank accession NC_003552.1), our wildtype *M. acetivorans* contains five amino acid substitutions, as revealed by genome sequencing: H313Q in MA2328 (hypothetical protein), A255T in MA1265 (DNA-directed RNA polymerase, beta subunit), A389T in MA0003 (sodium:proline symporter), F64L in MA2304 (phosphoserine aminotransferase), and W122R in MA2577 (hypothetical protein).

Supplementary Table 4. **Oligonucleotides used for cloning, sequencing, and verification of chromosomal and plasmid-based genes.** "f" indicates forward primer and "r" indicates reverse primer. All restriction enzyme sites used for cloning are underlined. Sequence for the FLAG tag is boxed. "*Phos*" indicates that the oligonucleotide is phosphorylated at the 5' end.

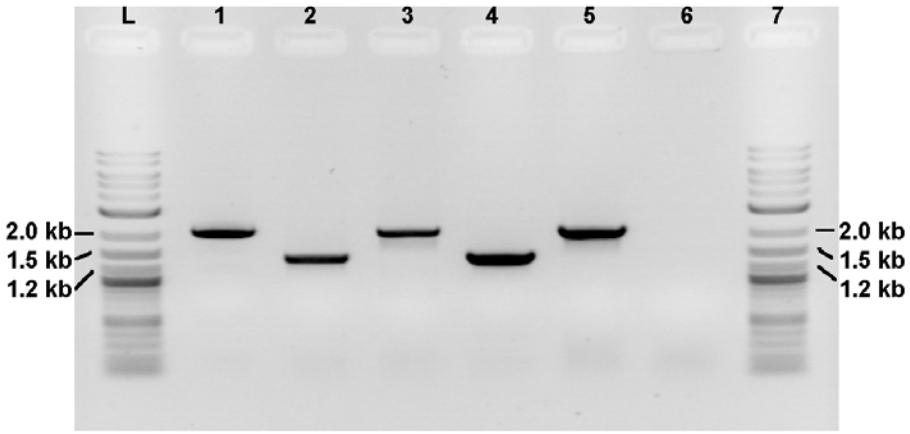
Primer name	Sequence (5'→3')
Cloning of pES1-MAT*mcr*1 and sequencing of ANME-1 *mcrBGA* genes	
veri-p-f	TAGTAGGTTGAGGCCGTTGAGC
pES1-f	CAATATGCCGCTTATCAGGATTAC
MATmcrB2-f	TATAGCCTGATAAAAGACAACG
MATmcrB3-f	ATCCATCCACCATTGGAAGAG
MATmcrB4-r	CAGGATGTCCATGTTGAGCTTG
pES1-r	CTTTGTTAGCAGCCGGATCTC
Cloning of pES1-MAT*mcr*2	
Pmcr-f2	GAATTCGGCTTCACGGTACCTTCTGCAGCACGGTATTATTAAACTTTCGTATTAAGAGG
Pmcr-r2	TTGCCTTTGTCGTCATATAAATCTATTTCATCTGCCATTTTAATTTCCTCCTTAATTTATTAAAATC
B6-r1	CAGTCATGCTAGCTTACAGTTTTGCACCGGGTTC
Cloning of pES1-MAT*mcr*3	
Pmat-f	GAATTCGGCTTCACGGTACCTTC
Pmat-r	TTGCCTTTGTCGTCATATAAATC
B6-r1	See above.
Cloning of pES1(Pmat)	
pES1(Matprom)-f	*Phos*-GCTAGCATGACTGGTGGACAGC
pES1(Matprom)-r	*Phos*-ATCTTCTTTTTCCTCCTTTTACTATTACCC
Cloning of pES1-MAT*mcr*1-*flag*	
pES1-f	See above.
B6-r-flag	AGTCATGCTAGCTTACTTGTCATCGTCGTCCTTGTAGTCCAGTTTTGCACCGGGTTCTC
Cloning of pES1-MAT*mcr*2-*flag*	
Pmcr-f2	See above.
B6-r-flag	See above.
Cloning and pES1-MAT*mcr*3-*flag*	
Pmat-f	See above.
B6-r-flag	See above.
16S rDNA amplification and sequencing	
ARCH109-F	AHDGCTCAGTAACACRT
ARCH934-R	GTGCTCCCCCGCCAATTCCT
27F	AGAGTTTGATCMTGGCTCAG
1492R	CGGTTACCTTGTTACGACTT
Plasmid-based ANME-1 *mcrA* amplification	
B4-f	ATACACTGGAAAAGGTCAGTA
pES1-r	See above.
Chromosomal *mcrCD* amplification	
mcrB-f	AACATCTGGCCTCATCGGTGAC
mcrG-r	CCCTGAGTTTCTCAAGTTTTCC



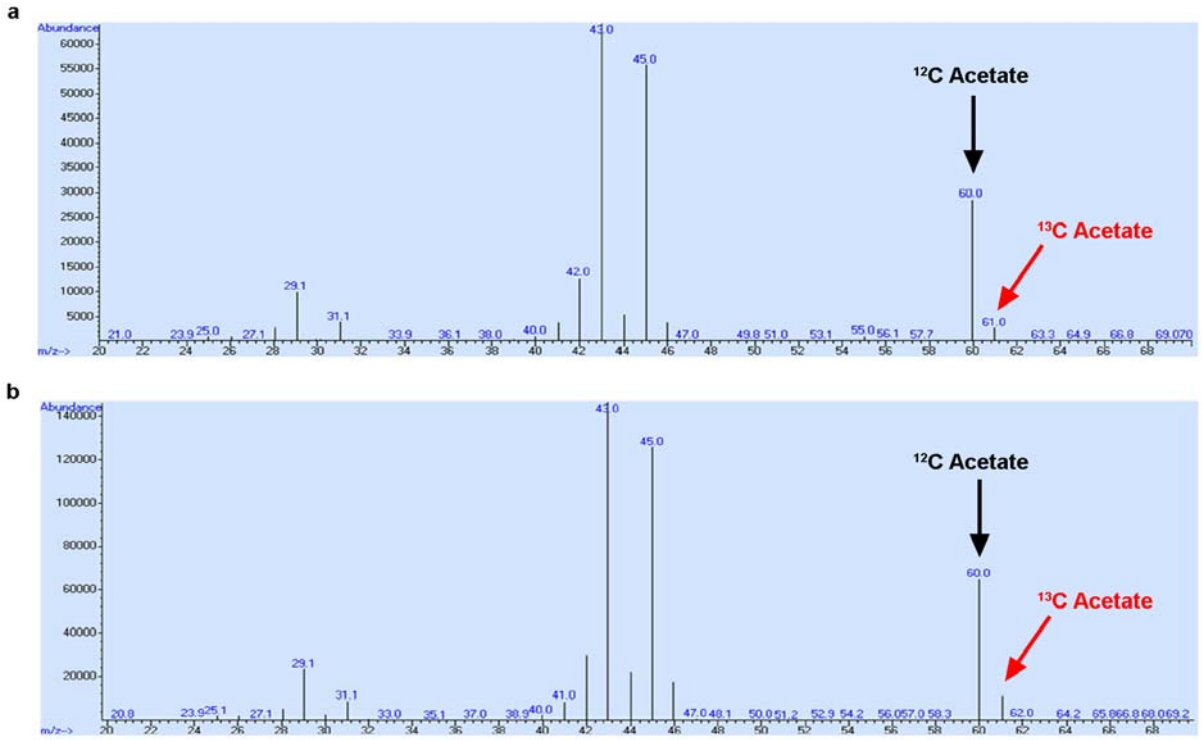
Supplementary Figure 1. Promoters used for expressing ANME-1 *mcrBGA*. Plasmid map of (a) pES1-MAT*mcr*1 with the CO dehydrogenase/acetyl-CoA synthase promoter (P_{cdh}) from *M. thermophila* for ANME-1 *mcrBGA*, (b) pES1-MAT*mcr*2 with the *mcr* promoter from *M. acetivorans* ($P_{mcr\_M.\ acetivorans}$) for ANME-1 *mcrBGA*, and (c) pES1-MAT*mcr*3 with the native *mcr* promoter from ANME-1 ($P_{mcr\_ANME\text{-}1}$) for ANME-1 *mcrBGA*. Similar plasmids were constructed with the sequence added for the FLAG tag to *mcrA* (see **Supplementary Table 4**).



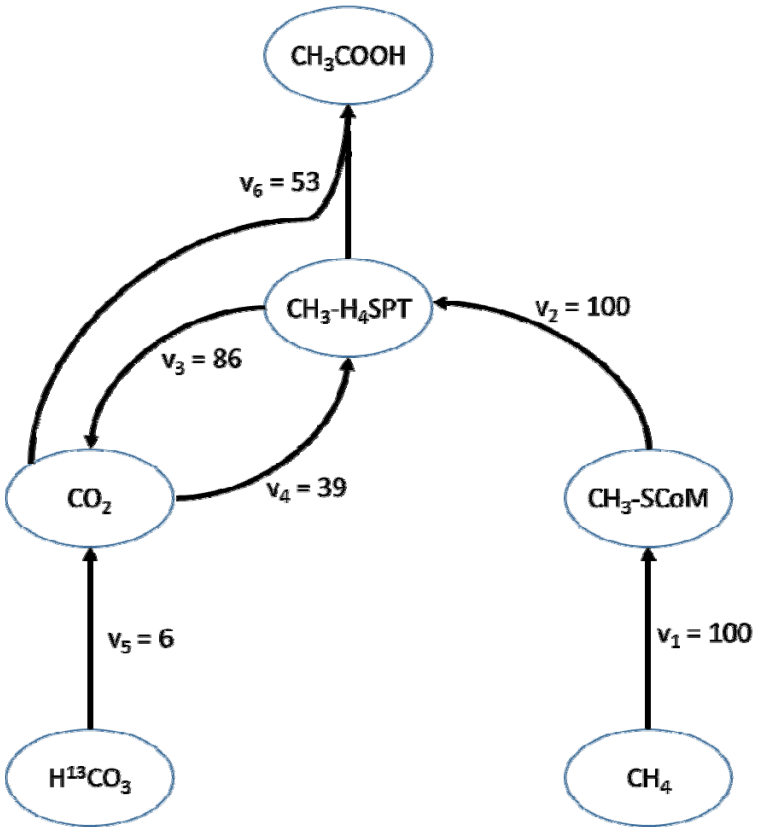
Supplementary Figure 2. Detection of ANME-1 McrA-FLAG in *M. acetivorans*/pES1-MAT*mcr*3 grown on methane after five days. Western blot shows ANME-1 McrA-FLAG (65 kDa) in *M. acetivorans*/pES1-MAT*mcr*1-*flag* (lane 2) and *M. acetivorans*/pES1-MAT*mcr*3-*flag* (lane 4) grown on methane and 0.1 mM $FeCl_3$ after five days (in high cell-density cultures). *E. coli* DH5α-λ*pir*/pES1-MAT*mcr*3 (lane 5) was used as the positive control. Lanes 1 and 3 correspond to methane-contacted *M. acetivorans* and *M. acetivorans*/pES1-MAT*mcr*2-*flag*, respectively.



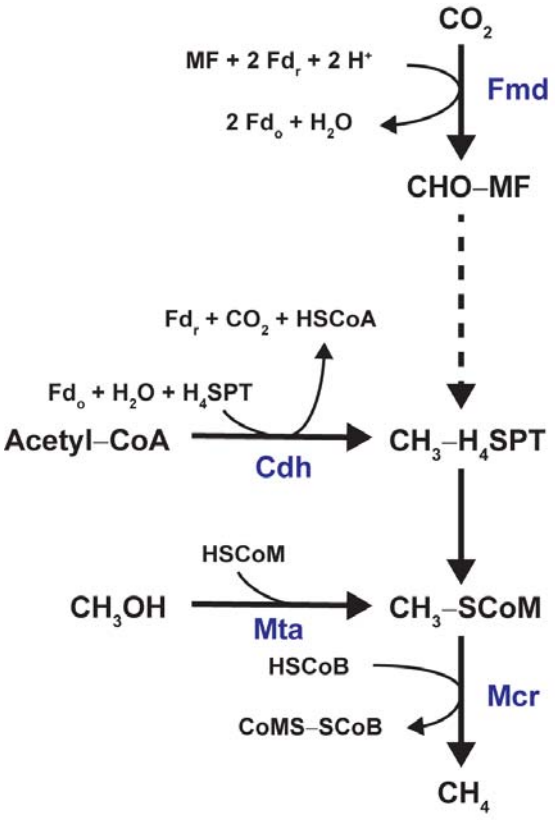
Supplementary Figure 3. Detection of ANME-1 *mcrA* after 30 days of growth on methane. PCR amplification of plasmid-based ANME-1 *mcrA* (2 kb, lanes 1, 3, and 5) and chromosomal-based *mcrCD* (1.3 kb, lanes 2, 4, and 6). DNA template for lanes 1 and 2: *M. acetivorans*/pES1-MAT*mcr*1 after 30 days (long-term) growth on methane and 0.1 mM $FeCl_3$. DNA template for lanes 3 and 4: freshly transformed *M. acetivorans*/pES1-MAT*mcr*1 growing on methanol. DNA template for lanes 5 and 6: *E. coli* DH5α-λ*pir*/pES1-MAT*mcr*1.



Supplementary Figure 4. Extracellular production of acetate from $H^{13}CO_3$ in ANME-1 Mcr-producing *M. acetivorans* via GC/MS. GC/MS spectra of culture supernatants used to identify acetate from $H^{13}CO_3$. Unlabeled acetate (^{12}C Acetate, black arrows) has a molecular mass of 60, whereas ^{13}C-labeled acetate (^{13}C Acetate, red arrows) has a molecular mass of 61. (a) *M. acetivorans* harboring empty plasmid pES1(Pmat) grown on ^{13}C-labeled bicarbonate for 10 days. (b) *M. acetivorans* harboring pES1-MAT*mcr*3 grown on ^{13}C-labeled bicarbonate for 10 days. Note that the y-axes differ for panels (a) and (b).



Supplementary Figure 5. Flux through the various reactions in the methanogenesis pathway of *M. acetivorans* estimated by ^{13}C-metabolic flux analysis using ^{13}C-labeled bicarbonate ($H^{13}CO_3$) as the input tracer. Methane uptake (v1) fixed at 100 mmol/gdw-h was used as the basis. The reduction of CO_2 in reaction v6 results in the incorporation of $H^{13}CO_3$ into the carbonyl carbon of acetate. The reverse flux through the methylotrophic pathway (v4) results in the incorporation of ^{13}C-bicarbonate into the methyl group of acetate, so both positions of acetate are labeled by $H^{13}CO_3$. Complete oxidation of methane to CO_2 via the methylotrophic pathway (v3) produces an unlabeled CO_2 pool resulting in a large unlabeled fraction of acetate.



Supplementary Figure 6. Simplified methanogenesis pathway from CO_2 and CH_3OH of *M. acetivorans*. Enzymes in blue are multienzyme complexes, and one or two gene(s) encoding their subunit is repressed upon growth on methane (see **Supplementary Table 2** for details). Solid arrows denote reaction catalyzed by the enzymes. Dashed arrows denote shortened steps of catalytic transformations. Enzymes: Fmd, formyl-methanofuran dehydrogenase; Cdh, CO dehydrogenase/acetyl-CoA synthase; Mta, methanol-specific methyltransferase; and Mcr, methyl-coenzyme M reductase of *M. acetivorans* (not cloned ANME-1 Mcr). Metabolites: MF, methanofuran; Fd_r, reduced ferredoxin; Fd_o, oxidized ferredoxin; CHO-MF, formyl methanofuran; H_4SPT, 5,6,7,8-tetrahydrosarcinapterin; CH_3-H_4SPT, methyl-H_4SPT; CH_3-SCoM, methyl-coenzyme M; HSCoM, coenzyme M; HSCoB, coenzyme B; and CoMS-SCoB, heterodisulfide of coenzyme M and coenzyme B.