

Table S1. Strains, plasmids, and primers used in this study

Name	Description	Source or reference
Strains		
<i>E. coli</i>		
DH5 α	Cloning strain	NEB
Rosetta (DE3)	Recombinant protein expression strain	NEB
pLacI		
<i>M. acetivorans</i>		
WWM73	$\Delta hpt::PmcrB-tetR-\phi C31-int-attP$	[1]
DJL60	WWM73 $\Delta iscSU2::pac-hpt$	This study
Plasmids		
pDL201	pET28a with <i>iscU2</i> cloned into <i>NdeI</i> & <i>XhoI</i> sites	This study
pDL202	pET28a with <i>iscS2</i> cloned into <i>NdeI</i> & <i>XhoI</i> sites	This study
pDL204	pET28a with <i>acnA</i> cloned into <i>NheI</i> & <i>XhoI</i> sites	This study
pJK301	Amp ^R vector for making markerless deletions in <i>Methanosarcina</i> spp.; contains <i>pac-hpt</i> flanked by Flp recombinase FRT recognition sites	[2]
pDL214	<i>iscSU2</i> knockout plasmid; pJK301 with regions upstream of <i>iscS2</i> cloned into <i>ApaI</i> & <i>HindIII</i> sites, downstream of <i>iscU2</i> cloned into <i>BamHI</i> & <i>SpeI</i> sites	This study
Primers		
IscSNdeF “P2 fwd”	ggtttgCATATGacaattgaaaacagaaccgtttac fwd primer with <i>NdeI</i> site to amplify <i>iscS2</i>	This study
IscSXhoR “P2 rev”	ggtggtCTCGAGTcaaagagctctgtattcctgaggg rev primer with <i>XhoI</i> site to amplify <i>iscS2</i>	This study
IscUNdeF “P1 fwd”	ggtggtCATATGgattacagcgttaaggtgttag fwd primer with <i>NdeI</i> site to amplify <i>iscU2</i>	This study
IscUXhoR “P1 rev”	ggtggtCTCGAGTcagtcccagggtcaagccc rev primer with <i>XhoI</i> site to amplify <i>iscU2</i>	This study
NheAconF	ggtggtGCTAGCatgagagaaggtctggacccc fwd primer with <i>NheI</i> site to amplify <i>acnA</i>	This study
AconXhoR	ggtggtCTCGAGTtattttttctaccgaatcccgc rev primer with <i>XhoI</i> site to amplify <i>acnA</i>	This study
ApausISCF	ggtggtGGCCCCcctgattccatttgaattcttc fwd primer with <i>ApaI</i> site to amplify <i>iscSU2</i> US region for insertion in pJK301	This study
HindusISCR	gttggtAAGCTTcgtagcagagtatccatgtaaacgg rev primer with <i>HindIII</i> site to amplify <i>iscSU2</i> US region for insertion in pJK301	This study
BamdsISCF	ggtggtGGATCCgggcttgagccctgggactgaatgagcc fwd primer with <i>BamHI</i> site to amplify <i>iscSU2</i> DS region for insertion in pJK301	This study
SpedISCR	ggtggtACTAGTgctgttcattgggtgtgaagctggatg	This study

	rev primer with <i>SpeI</i> site to amplify <i>iscSU2</i> DS region for insertion in pJK301	
Isc2USKOseqF “P3 fwd”	gatgtcaaacgggcgagaaacaagagg	This study
JK301SupR(new) “P3 rev”	tagtatattacgaatagggcg rev plasmid primer to confirm <i>iscSU2</i> knockout	This study
JK301SdownF “P4 fwd”	gctgctggtgaaagagacc fwd plasmid primer to confirm <i>iscSU2</i> knockout	This study
Isc2DSKOseqR “P4 rev”	ggaagagttcacggaagaattcccg rev genomic primer to confirm <i>iscSU2</i> knockout	This study

1. Guss, A. M., Rother, M., Zhang, J. K., Kulkarni, G. & Metcalf, W. W. (2008) New methods for tightly regulated gene expression and highly efficient chromosomal integration of cloned genes for *Methanosarcina* species, *Archaea*. **2**, 193-203.
2. Welander, P. V. & Metcalf, W. W. (2008) Mutagenesis of the C1 oxidation pathway in *Methanosarcina barkeri*: new insights into the Mtr/Mer bypass pathway, *J Bacteriol.* **190**, 1928-36.

Supplemental Figure Legends

Fig. S1. Arrangement of the gene clusters containing *iscS* and *iscU* in *E. coli* and *M. acetivorans* C2A. Other predicted gene functions: MA0806, conserved hypothetical protein; MA0809, DrsE superfamily protein; MA0810, SirA-like protein; MA0811, histidine triad protein; MA2714, homoserine O-acetyltransferase; MA2715, O-acetylhomoserine (thiol)-lyase; MA2716, quinolinate synthetase A; MA2719, helix-turn-helix XRE-family like protein; MA3262, LrgB superfamily protein; MA3263, LrgA superfamily protein.

Fig. S2. Amino acid sequence alignment of *M. acetivorans* IscS1-3 with IscS from *Escherichia coli* and *Archaeoglobus fulgidus*. The active site residues in *E. coli* IscS are red and boxed. The PLP binding residues in *E. coli* IscS are green. Ec: *E. coli*; Af: *A. fulgidus*.

Fig. S3. Amino acid sequence alignment of *M. acetivorans* IscU1-3 with IscU from *Escherichia coli* and *Archaeoglobus fulgidus*. The Fe-S cluster binding residues in *E. coli* IscU are red, and the conserved aspartate and histidine are green. The HscA-interacting region is boxed. Ec: *E. coli*; Af: *A. fulgidus*.

Fig. S4. Characterization of recombinant *M. acetivorans* aconitase (AcnA). A) SDS-PAGE analysis of purified recombinant AcnA. B) UV-visible spectrum of purified AcnA after in vitro reconstitution with iron and sulfur.

FIGURE S1

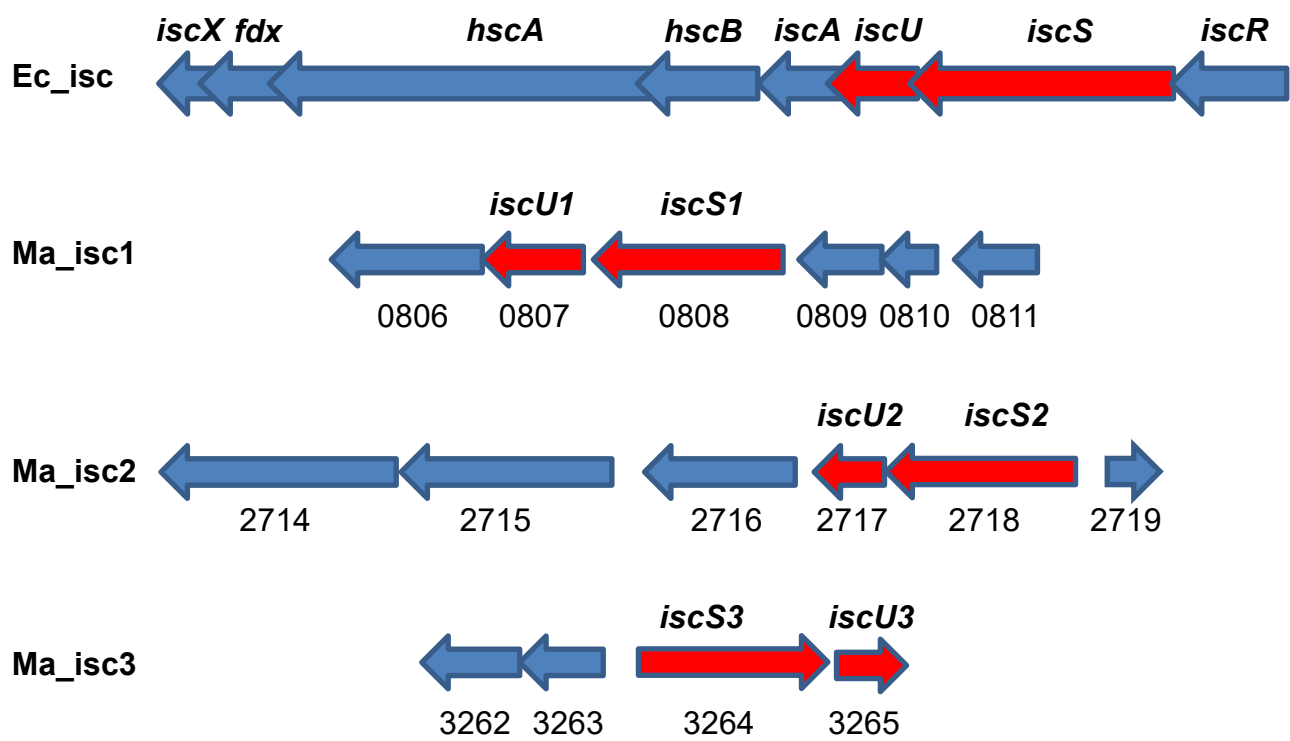


FIGURE S2

IscS1	-----MIYL
IscS2	-----MTIENRTVYM
IscS3	MLSSRLFSQDYFPQIHSHLRYFHTFMMQTERFQKAHNKFISSKLLFSLGVLMGETHLIYM
AfIscS	-----MAYF
EcIscS	-----MKLPIYL
	* :
IscS1	DNAACTRLDERVFEAMKPYFFDT-YAVATSEFGYSMGIDAKEGLENSREGIASGLGAAP-
IscS2	DNSATTPVRKEVVEEMLPYLTEN--FGNPSS-IYELGKISKHAVENARKRVADAIGAE-
IscS3	DHAATTFTKPEVIEAMLPLKEH--FGNPSS-LYSIGREGKEAVETSRKKLAKALGAAQP
AfIscS	DYTSAKPVDERVLEAMLPMTES--FGNPSS-VHSYGFKAREAVQEAREKVAKLVNGGG-
EcIscS	DYSATTPVDPRVAEKMMQFMTMDGTFGNPASRSHRFGWQAEAAVDIARNQIADLVGADP-
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IscS1	EEIVFTSGDTESSNMALKGVAWALREKKKGKHIIISKIEDFPVLNTAKTLQKQGFVDVTFLD
IscS2	NEIYFTSGGTESDNWTVKGVAFAN-KNRGKHIIITSSIEHHAVLHACAWLEGQGFEVITYLP
IscS3	EEIYFTSGGTESDNWAIKGTAFSR-QKKGKHIIITPIEHHAVLYPCEYLETQGFEVITYLP
AfIscS	GTVVFTSGATEANNLAIIGYAMRN-ARKGKHILVSAVEHMSVINPAKFLQKQGFVEVEYIP
EcIscS	REIVFTSGATESDNLAIKGAANFY-QKKGKHIIITSKTEHKAVLDTCRQLEREGFEVITYLA
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IscS1	VDAEGFADLEELKKAITKETILVSIQHSNQEIGTAQDLKAISEICEEKDVLVLTATHSF
IscS2	VDRYGMVSPEELKNAIRDDTILISIMLANNEIGTIQPVEEIGKISRENSIYFHTDAVQAI
IscS3	VDEYGLVNPAEVEASIKKDTVLSVMYANNEIGTIEPILEIGKIAREHGIPFHTDAVQVI
AfIscS	VGKYGEVDVSFIDQKLRRDDTILVSVQHANNEIGTIQPVEEISEVLG-KAALHIDATASV
EcIscS	PQRNGIIDLKELEAAMRDDTILVSIHVNNNEIGVVQDIAAIGEMCRARGIYHV DATQSV
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IscS1	TRLPLNVKDLP--VDLVTMSAHTIHGPRGIGALCIRKD--TPIVKFMDGGGFQEFNLRAGV
IscS2	GHVPIDVKKMN--VDLLSLSGHKGFGGPKGCGALYIRKG--TKIEAFLHGGAQERKRRAGT
IscS3	GKVPLDLQREHKDVDMLSLSSHKFYGPKGIGALYIREG--TEIDNYMHGGAQERGKRAGT
AfIscS	GQIEVDVEKIG--ADMLTISSNDIYGPKGVGALWIRKE--AKLQPVILGGGQENGLRSGS
EcIscS	GKLPIDLSQLK--VDLMSFSGHKIYGPKGIGALYVRRKPRVRIEAQMHHGGGHERGMRSGT
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IscS1	ENIPGAVGFATAVKLVTEENRQLAAMRDR--VIERALSEIPEVTLNGSREKRLPQNANL
IscS2	ENVPSIVGLGKAIGLATGEMEETNKPLLEMRRERLIKGLLQIPKTHLNGHPTERLANNVNV
IscS3	ENVVGIVGMGKAIELATANIEAHNEKLSKMRARLMAGILEIPYCRLNGHPEKRLPGNLFNF
AfIscS	ENVPSIVGFGKAAEITAMEWREEAERLRRLRDRIIDNVLKIEESYLNHPEKRLPNNVNV
EcIscS	LPVHQIVGMGEAYRIAKEEMATEMERLRGLRNRLWNGIKDIEEVYLNGLDLEHGAPNILNV
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IscS1	TFHYVEGESVTLHMDMRGFAVSTGSACFSRSLEASHVIRGIGGDHERAHGSVRFTFGRYN
IscS2	TFEYIEGESLLLLLNAGIFASTGSACNSTSLEPSHVLTAACVPHEIVHGSRLSLGRMN
IscS3	SFEYIEGESLLLLMLDQMGICSTGSACSSGSSEPSHVLRAIGLAPKTAQGTLRLTLGDAN
AfIscS	RFSYIEGESIVLSLDMAGIQASTGSACSSKTLPQSHVLMACGLKHEEAHGTLTLLTLGRYN
EcIscS	SFNHYVEGESLIMALKDLAVSS--GSACTSASLEPSYVLRALGLNDELAHSSIRFSLGRFT
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IscS1	RMEDADAAIDAMSEIVARLREISPLAKK-----
IscS2	TLEDVDRVLEVLPEIVQKLRNMSPLTPQEYRAL-----
IscS3	SEEDIDYVLEVLPEVVGKLRAISPFYKPESKCEK-----
AfIscS	TDDEDVDRLLLEVLPGVIERLRMSPLYRR-----
EcIscS	TEEEIDYTIELVRKSIGRLRDLSPLEWEMYKQGVDLNSIEWAHH
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FIGURE S3



FIGURE S4

