$sRNA_{41}$ affects independently several ribosome binding sites within polycistronic mRNAs in *Methanosarcina mazei* Gö1

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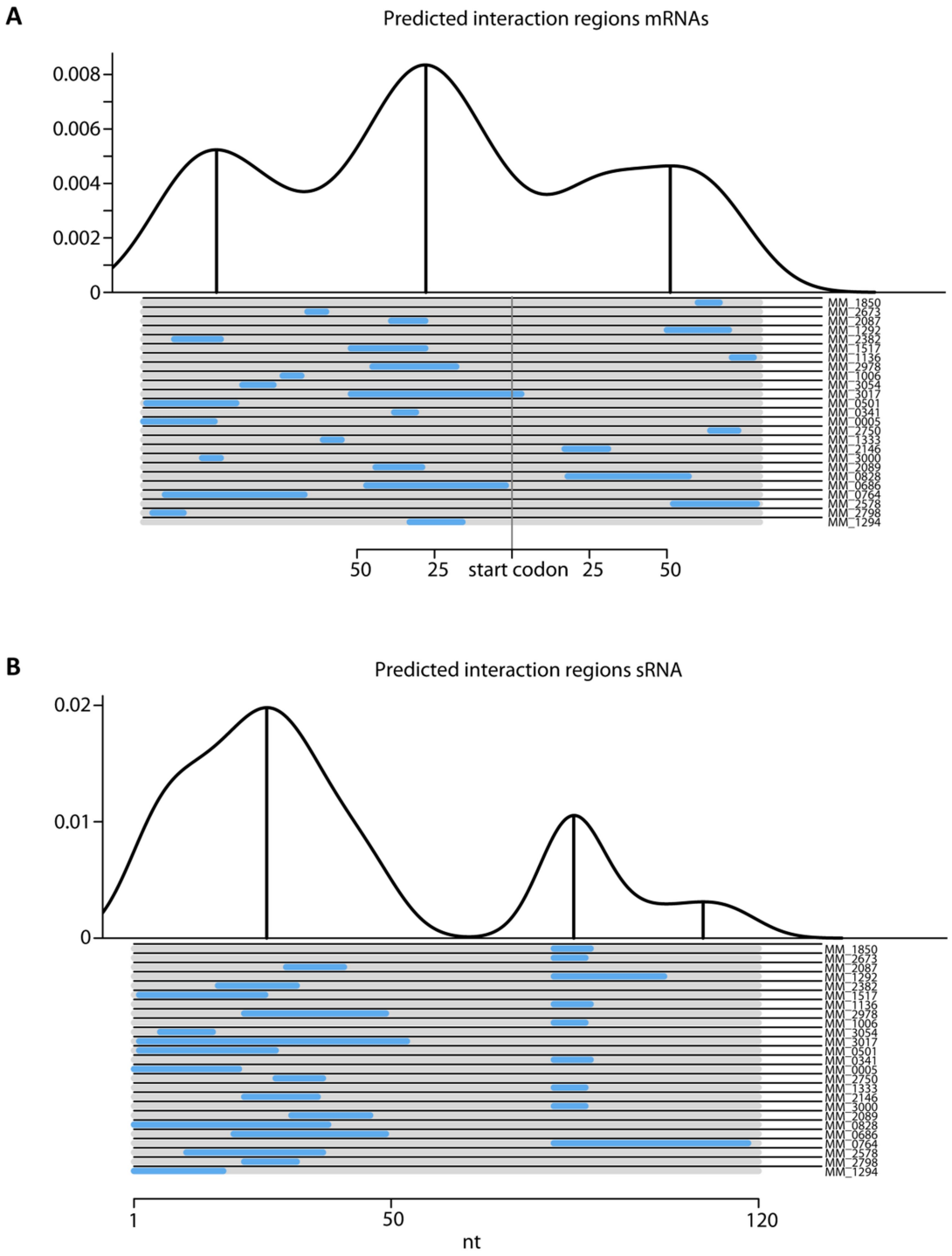
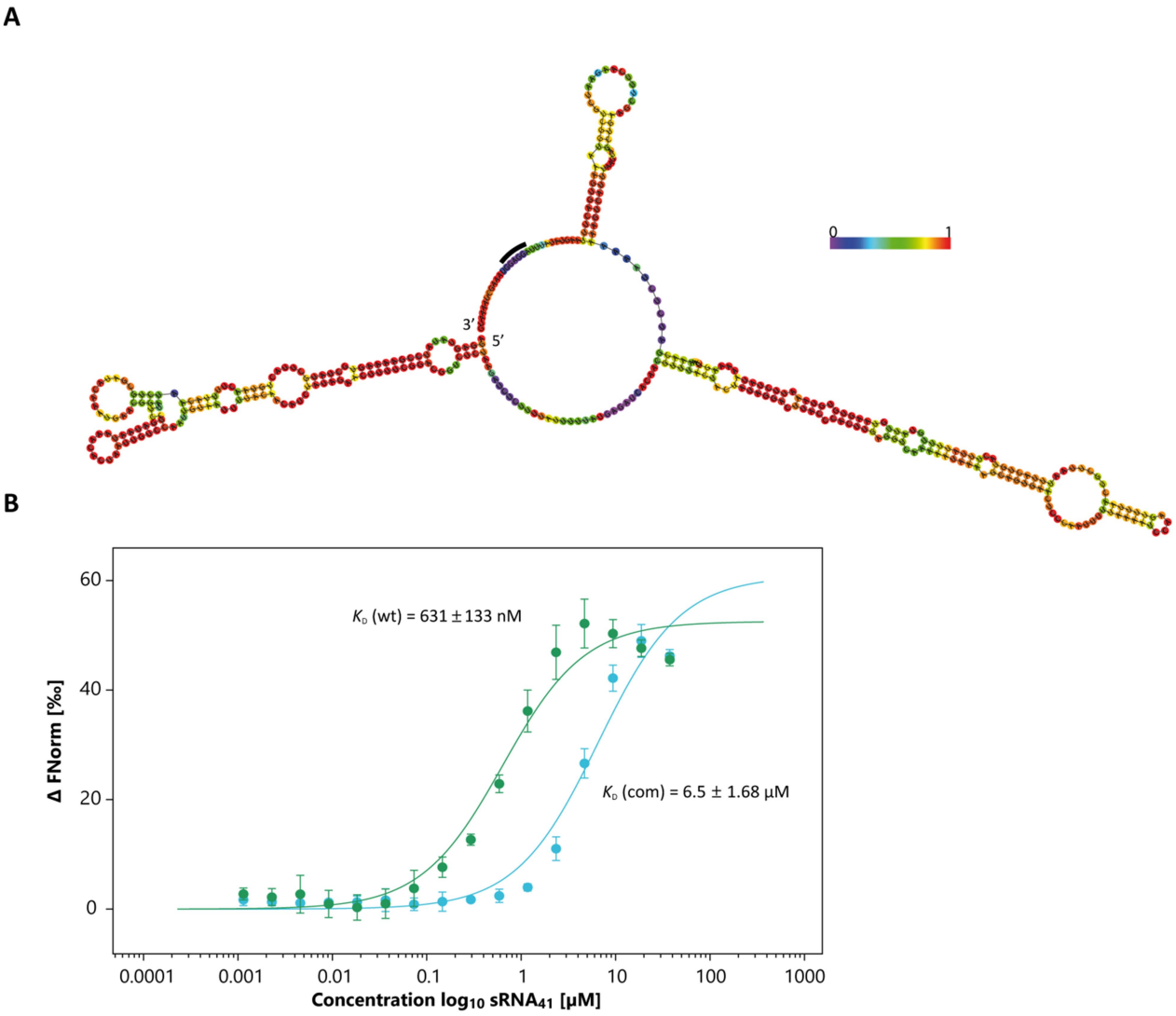
Supplemental material

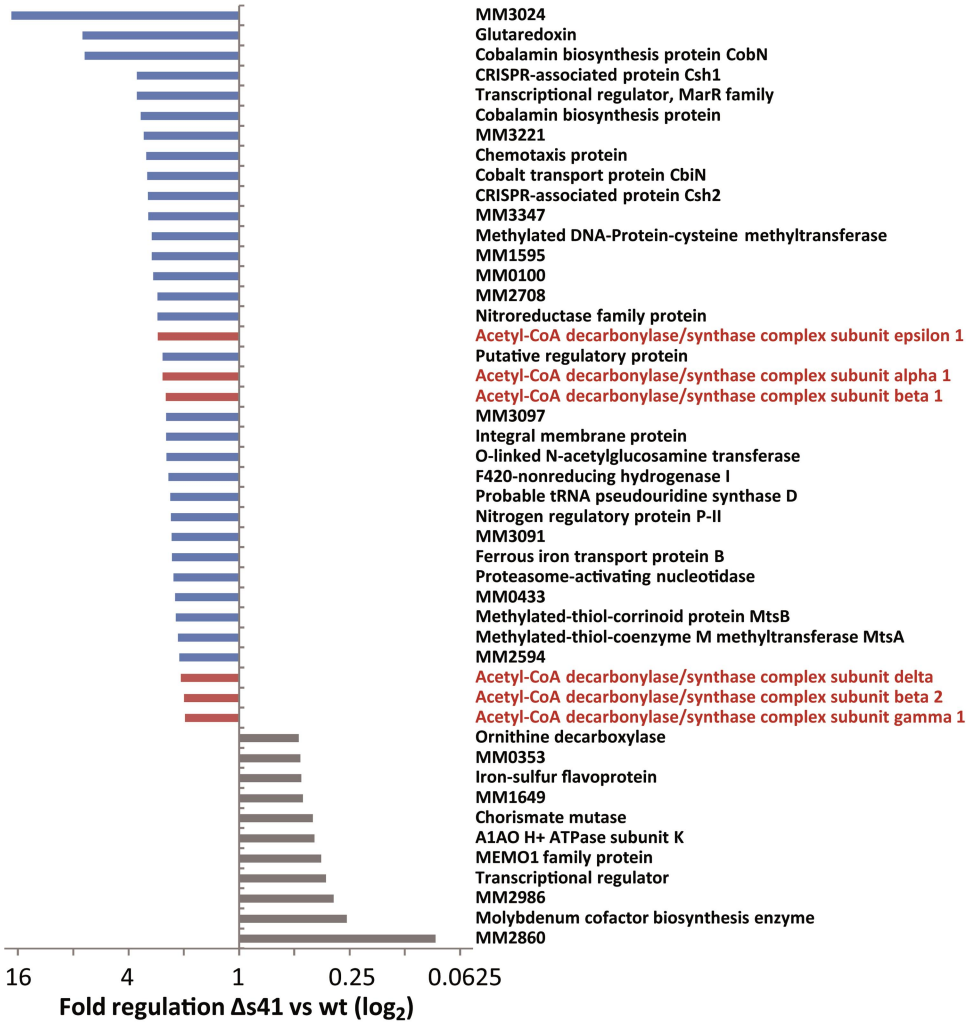
Supplementary figures S1-S5
Supplementary tables S1-S3



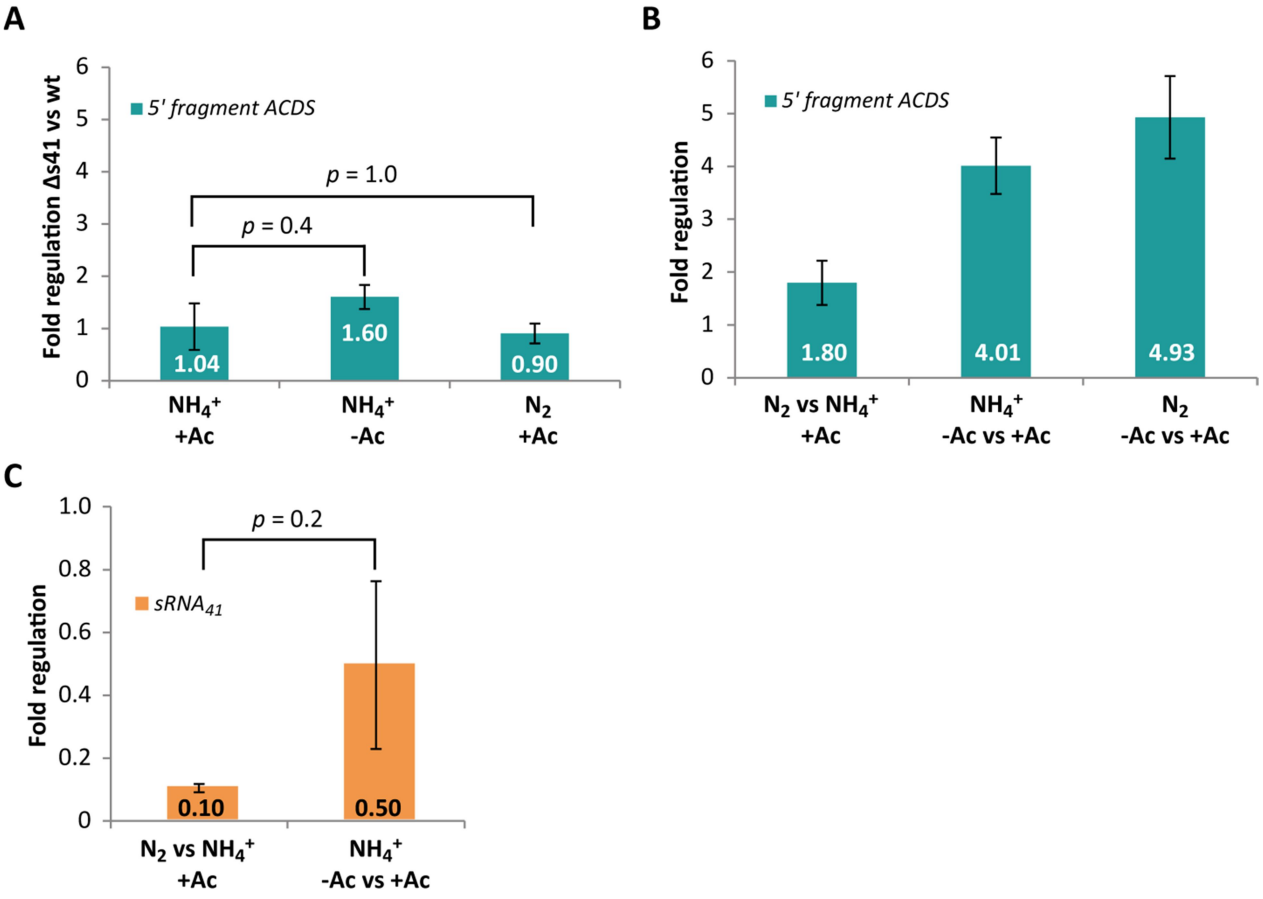
Supplementary Figure 1 Overview of the regions that play predominant roles in the statistically significant interactions between $sRNA_{41}$ and target mRNAs given by IntaRNA (Busch *et al.*, 2008). Interactions displayed for **A,** the target mRNAs and **B,** $sRNA_{41}$ sequence. The density plot in the top of each image is calculated from all predicted interactions with an IntaRNA p-value ≤ 0.01. The interactions pictured in the bottom are shown for the top 25 predicted targets (bottom-up). Blue bars showing interaction regions for each mRNA and the $sRNA_{41}$, respectively.



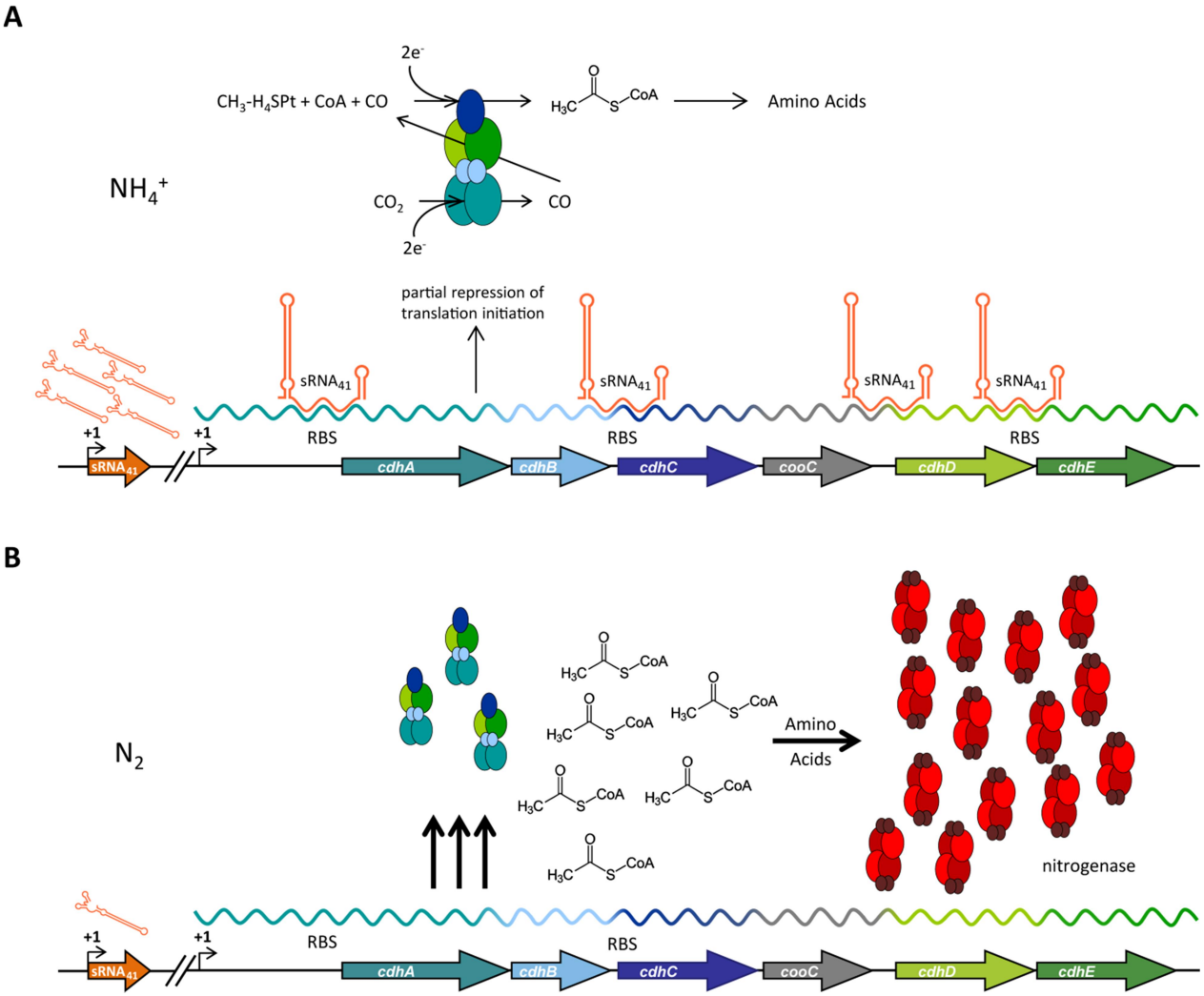
Supplementary Figure 2 A, Structure prediction of full length 5'UTR *MM2089* (400 nt) performed with RNAfold (Hofacker *et al.*, 1994). Color code indicates base pairing probability. Single stranded RBS is indicated by a black bar. **B,** MST experiment demonstrated interaction between a longer 5'UTR *MM2089* and $sRNA_{41}$. 6 µM of unlabeled 240 nt of 5'UTR *MM2089* was incubated together with the wt combination of 15 nM Cy3-labeled '5'UTR MM2089 and with increasing amounts of $sRNA_{41}$ (1.14 nM to 37.5 µM) (light blue graph, K_D (com)). Green graph demonstrates the wt '5'UTR MM2089 interaction without competitor 5'UTR *MM2089* (K_D (wt)).



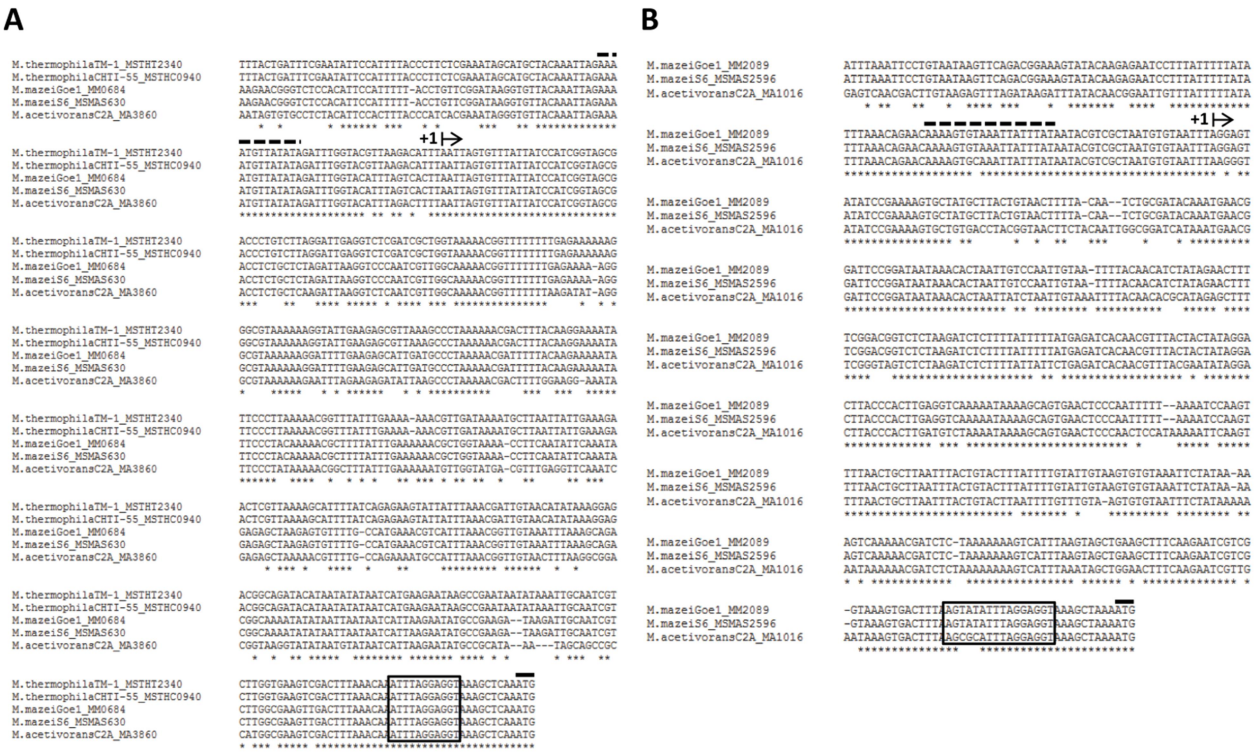
Supplementary Figure 3 Proteome analysis of a chromosomal *M. mazei* Gö1 $sRNA_{41}$ deletion mutant (Mm_Δs41) vs. wild type (Mm_wt) under N-sufficiency. Up and down-regulated proteins in the Mm_Δs41 mutant vs. Mm_wt with a minimum fold-change of 2 (blue and red bars) and 0.5 (grey bars). Protein levels were determined in exponential growth phase by LC-ESI MS/MS (see Experimental procedures). Diagram represents the second of two independent experiments. MM-numbers are given in case no function is assigned to the protein. Marked in red are the up regulated subunits of the ACDS complex.



Supplementary Figure 4 Transcriptome analysis via qRT-PCR of the polycistronic mRNA of the operons encoding ACDS complexes and $sRNA_{41}$ in *M. mazei* wildtype (Mm_wt) and $sRNA_{41}$ chromosomal deletion mutant (Mm_Δs41). **A**, Regulation of the 5' fragment of polycistronic mRNA of ACDS (*MM2089* and *MM0686*) in comparison between Mm_Δs41 mutant and Mm_wt under different growth conditions. **B**, Regulation of 5' fragment of polycistronic mRNA of ACDS and **C**, $sRNA_{41}$ in Mm_wt comparing different nutrient compositions. NH_4^+, supplemented with ammonium; N_2, molecular nitrogen as sole nitrogen source; +Ac, supplemented with 11 mM sodium acetate trihydrate; -Ac, without acetate source. Fold regulation and respective standard deviations were calculated of three independent experiments. p-values in sections A and C were calculated with Mann-Whitney-Test showing no significant changes in expression levels ($p \geq 0.05$).



Supplementary Figure 5 Proposed model of the $sRNA_{41}$ post-transcriptional regulation. **A**, Regulation of $sRNA_{41}$ under NH_4^+. Translation of *cdh* genes is partially repressed by $sRNA_{41}$ binding to the translation start site (RBS). $sRNA_{41}$ is highly expressed under NH_4^+. ACDS complex catalyzes among others the synthesis of acetyl-CoA from CO_2, CH_3-H_4SPt and CoA. **B**, Under nitrogen fixing conditions (N_2) $sRNA_{41}$ is only slightly expressed causing a regular translation of ACDS. Thus more acetyl-CoA is generated for amino acid synthesis and therefore more nitrogen fixing enzymes (nitrogenase) are synthesized.



Supplementary Figure 6 Conservation of 5'UTRs of ACDS operons in different *Methanosarcina* species. **A,** Alignment of a short upstream region (115 nt) and the 5'UTR of the ACDS operon conserved in *Methanosarcina thermophila*, *M. mazei and M. acetivorans*. **B**, Alignment of a short upstream region the 5'UTR (115 nt) of the second ACDS operon, only present in *M. mazei* and *M. acetivorans*. Asterisks indicate conservation of DNA sequences, TSS are marked with an arrow and +1, potential consensus promoter sequences with dashed lines, TLS of both operons with continuous lines and verified interaction regions of $sRNA_{41}$ with the RBS in *M. mazei* are boxed. Alignments were performed with Clustal Omega (Sievers *et al.*, 2011).

Supplementary Table 1 Predicted interaction partners of $sRNA_{41}$. Prediction was performed using IntaRNA (Busch *et al.*, 2008) determining interaction ± 100 bp of translation start site.

ORF ID (Deppenmeier *et al.*, 2002)	**Gene / protein designation**	**Interaction energy [kcal/mol]**
MM_1294	hypothetical protein	-17.33
MM_2798	hypothetical protein	-14.68
MM_2578	iron (II) transport protein A	-14.25
MM_0764	hypothetical protein	-13.49
MM_0686	acetyl-CoA decarbonylase/synthase complex	-13.32
MM_0828	hypothetical protein	-13.04
MM_2089	acetyl-CoA decarbonylase/synthase complex	-12.94
MM_3000	DNA repair helicase	-12.22
MM_2146	50S ribosomal protein L15	-12.18
MM_1333	zinc ABC transporter, zinc binding protein	-12.10
MM_2750	hypothetical protein	-11.97
MM_0005	peptide ABC transporter permease	-11.96
MM_0341	amidophosphoribosyl-transferase	-11.93
MM_0501	3-demethylubiquinone-9 3-methyltransferase	-11.63
MM_3017	ABC transporter, permease	-11.62
MM_3054	hypothetical protein	-11.59
MM_1006	proteasome-activating nucleotidase	-11.57
MM_2978	hypothetical protein	-11.55
MM_1136	hypothetical protein	-11.53
MM_1517	cysteine desulfurase nifS	-11.51
MM_2382	hypothetical protein	-11.49
MM_1292	archaeosine tRNA-ribosyltransferase	-11.46
MM_2087	acetyl-CoA decarbonylase/synthase complex	-11.20
MM_2673	hypothetical protein	-11.14
MM_1850	hypothetical protein (RNase P)	-11.11
MM_2106	glycosyltransferase	-11.08
MM_2145	50S ribosomal protein L30	-11.01

Supplementary Table S2 (Excel sheet) List of proteins quantified by LC-ESI MS/MS in Mm_Δs41 vs wt as forward and reverse experiment (see Experimental procedures).

Supplementary Table S3 Primer sequences and descriptions used in this study

Designation	Sequence (5'->3')	Comment
666-1	CAGGTACCGGATAAGGTTGGAAA	Construction of a chromosomal $sRNA_{41}$ deletion mutant
666-2	CGGAATTCTTGAGTTCACCTGC	Construction of a chromosomal $sRNA_{41}$ deletion mutant
666-3	AGGAATTCACTCCCTGAAGGAAAA	Construction of a chromosomal $sRNA_{41}$ deletion mutant
666-4	GAGAAAACGCTTGAGATCTAAG	Construction of a chromosomal $sRNA_{41}$ deletion mutant
sRNA666 for	CTCGAGAGCATAATCAGGGATCT	Construction of a $sRNA_{41}$ overexpression mutant
sRNA666 rev	CTCGAGTGTGTATAAATTGACGCG	Construction of a $sRNA_{41}$ overexpression mutant
sRNA41 T7 for	TAATACGACTCACTATAGGGGTCTAGCGAACAGACG	Construction of T7-$sRNA_{41}$
sRNA41 NheI rev	GTGCTAGCGTGACTCCCTTGCACCTGAAAC	Construction of T7-$sRNA_{41}$
sRNA41del18-46fo	GTACAAACTTGCAATGCTGGAATTCCC	Construction of 22 nt deletion in $sRNA_{41}$ by site-directed mutagenesis
sRNA41del18-46re	CATTTTTACGTCTGTTCGCTAGACATATG	Construction of 22 nt deletion in $sRNA_{41}$ by site-directed mutagenesis
s41 EMSA for	TTGCAATGCTGGAATTCCCCA	Construction of complementary mutations in $sRNA_{41}$ by site-directed mutagenesis
s41 EMSA rev	GTTTGTACAATATAAAATGGAGGCGTTTCCC	Construction of complementary mutations in $sRNA_{41}$ by site-directed mutagenesis
MM2089 T7 for	TAATACGACTCACTATAGGGGACTTACCCACTTGAGGTC	Construction of T7-5'UTR MM2089
MM2089 rev NheI	CTCCCGCTAGCTAATTTGCTCATTTTAGC	Construction of T7-5'UTR MM2089
2089-2088 for	CTGGTGGAAGTTTATCAGGAC	Amplification intergenic region *MM2089-MM2088*
2089-2088 rev	GATTATGGTGTCGTAGTTGCC	Amplification intergenic region *MM2089-MM2088*
2088-2087 for	GGCAACTACGACACCATAATC	Amplification intergenic region *MM2088-MM2087*
2088-2087 rev	CTGGCAGTAGTTGATGAAGTC	Amplification intergenic region *MM2088-MM2087*
2087-2086 for	CAGCTGAAGAAGAGGAAGAAG	Amplification intergenic region *MM2087-MM2086*
2087-2086 rev	CGGACTCCTTATTCATATCGG	Amplification intergenic region *MM2087-MM2086*
2086-2085 for	CCTCTCTTTGAAATACCCGAC	Amplification intergenic region *MM2086-MM2085*
2086-2085 rev	GAGATCAATCTCAATGTCCCC	Amplification intergenic region *MM2086-MM2085*
2085-2084 for	CAAACATGGAGCGTATCAGAC	Amplification intergenic region *MM2085-MM2084*
2085-2084 rev	CTGTCTAGTTCTGCCAGTTTC	Amplification intergenic region *MM2085-MM2084*
MM1621 for.rt	TAGGAGGTTTTCTCGGAAGCG	qRT-PCR
MM1621 rev.rt	AAGCGTATCTCCATCAAGCCC	qRT-PCR
MM2181 for.rt	GCCTCCATGAGAAGAATGCTC	qRT-PCR
MM2181 rev.rt	CTTCAAGGTCTCCAACTCCTG	qRT-PCR
MM0464_for	TATGTCATCCACGTCAGACGC	qRT-PCR
MM0464_rev	TCGTATCGGCCTTTTGCCTTG	qRT-PCR
MM0686_for	CTTGGTCTCGAACTTGTCAGG	qRT-PCR
MM0686_rev	GTTGATGAAGTCGTGGACACG	qRT-PCR
MM1602_for	TTCTTGACCTGAAGGGCAGTG	qRT-PCR
MM1602_rev	TCCGGTACCCGGTAACAATAG	qRT-PCR
MM2087_for	CTTGGTCTCGAACTTGTCAGG	qRT-PCR
MM2087_rev	GTTGATGAAGTCGTGGACACG	qRT-PCR
MM2089_for	GTGCTGTTACTGCACATACGG	qRT-PCR
MM2089_rev	CCGAGGTTGAGAGGTAACTCT	qRT-PCR

MM2427_for	ATGATGGCAGATCTTGCAGCC	qRT-PCR
MM2427_rev	GGAATCCTTCCGGTTTCTTCC	qRT-PCR
MM2860_for	TCACGGACAGATTGTTGCAGG	qRT-PCR
MM2860_rev	CCCGGTCAACTATTGTGTCTG	qRT-PCR
sRNA41 NheI for 2	ACATGCTAGCGTCTAGCGAAC	qRT-PCR
sRNA41 EcoRV rev	AGCCGGATATCTTGTGACTCC	qRT-PCR

Supplementary Table 4 Strains and Plasmids used and constructed in this study

Strain	**Description**	**Reference**
E. coli DH5α	General cloning strain	(Hanahan, 1983)
E. coli λpir JM109	General cloning strain	(Miller & Mekalanos, 1988)
E. coli BL21-pRIL	Protein expression strain, pRIL plasmid for expression of rare tRNAs codons for arginine, isoleucine and leucine	Stratagene, La Jolla, CA, USA
M. mazei 3A	Potential cell wall mutant, referenced as wildtype	(Ehlers *et al.*, 2005)
M. mazei pWM321	*M. mazei* 3A containing plasmid pWM321	(Metcalf *et al.*, 1997)
M. mazei Δ$sRNA_{41}$	*M. mazei* 3A chromosomal $sRNA_{41}$ deletion mutant, $sRNA_{41}$::pac	This study
M. mazei $OPsRNA_{41}$	*M. mazei* 3A $sRNA_{41}$ overexpression mutant containing plasmid pRS820	This study
Plasmid	**Description**	**Reference**
pWM321	*E.coli/M. mazei* shuttle vector, oriR6K, pC2A replicon, *pac*, *bla*	(Metcalf *et al*., 1997)
pRS207	pBlueskript SK+ containing *pac*-casette	(Ehlers *et al*., 2005)
pRS820	pWM321 containing $sRNA_{41}$	This study
pRS821	pBlueskript SK+ containing up- and downstream flanking regions of $sRNA_{41}$ and the *pac*-cassette replacing $sRNA_{41}$	This study
pRS913	pCRII-TOPO containing $sRNA_{41}$ under T7-promoter for *in vitro* transcription	This study
pRS1070	pCRII-TOPO containing *MM2087*	This study
pRS1071	pET-28a(+) containing *MM2087* for heterologous gene expression	This study
pRS1126	pCRII-TOPO containing $sRNA_{41}$ Δ18-46 under T7-promoter for *in vitro* transcription	This study
pRS1128	pCRII-TOPO containing 5'UTR of *MM2089* under T7-promoter for *in vitro* transcription	This study
pRS1274	pCRII-TOPO containing mutated $sRNA_{41}$ under T7-promoter for *in vitro* transcription	This study

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