Supplementary Information

Switching fatty acid metabolism by an RNA-controlled feed forward loop

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This supplement contains:

www.pnas.org/cgi/doi/10.1073/pnas.1920753117

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Figure S1

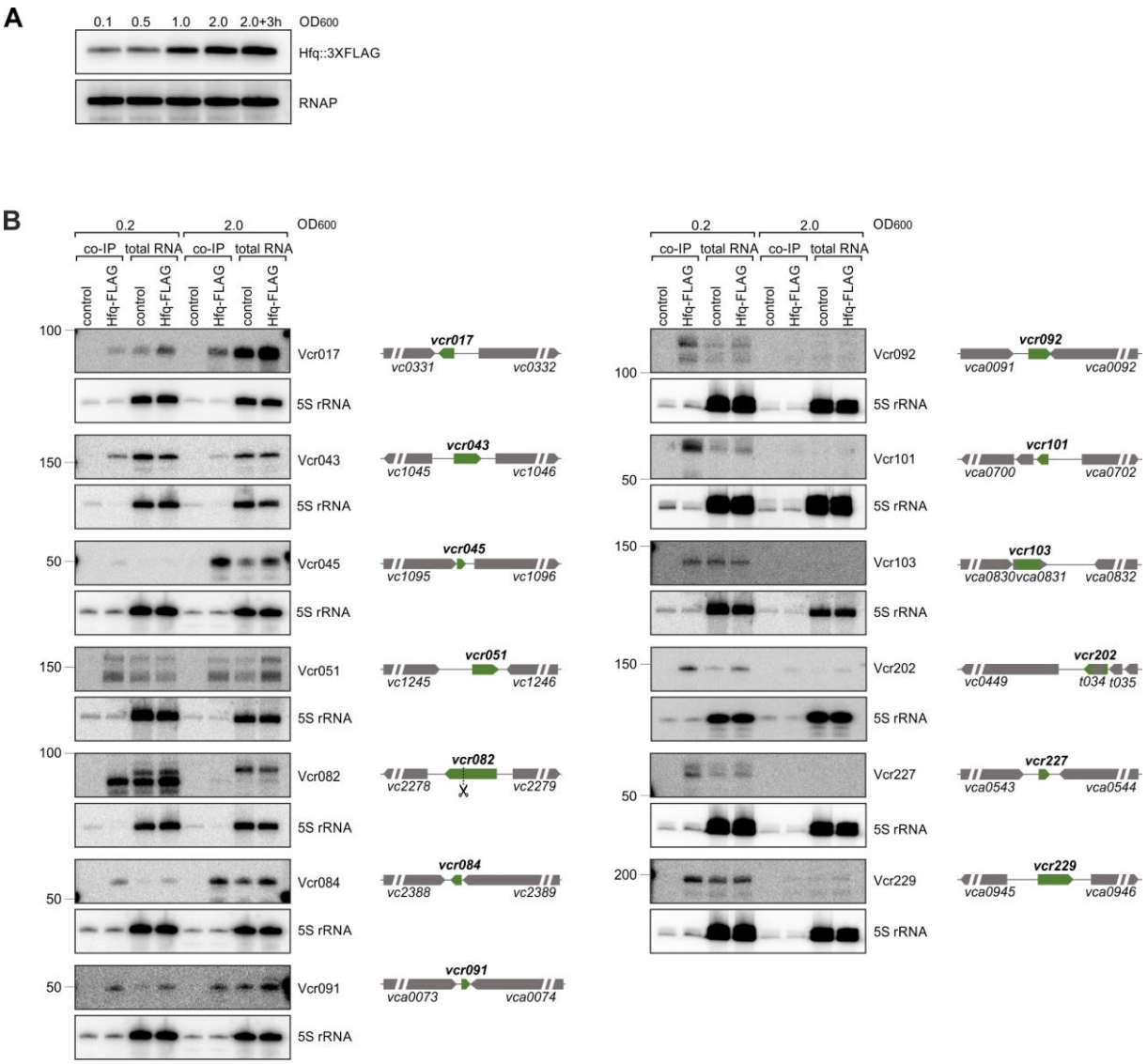


Figure S1: Expression of Hfq and further RIP-seq analysis

A) *V. cholerae* cells carrying a chromosomal 3XFLAG epitope at the *hfq* gene were cultivated in LB medium and protein samples were collected at the indicated OD_{600} readings. Production of Hfq was monitored by Western blot analysis. RNAP served as loading control. **B)** Co-IP and total RNA (lysate) fractions were obtained from *V. cholerae* wild-type and *hfq::3XFLAG* strains following growth in LB medium to low (OD_{600} of 0.2) and high cell densities (OD_{600} of 2.0). The RNA was loaded on Northern blots and probed for the indicated sRNAs. 5S rRNA served as loading control. The genomic locations of the sRNAs are shown to the right. Flanking genes are shown in gray, sRNAs are shown in green. Scissors indicate putative processing site.

Figure S2

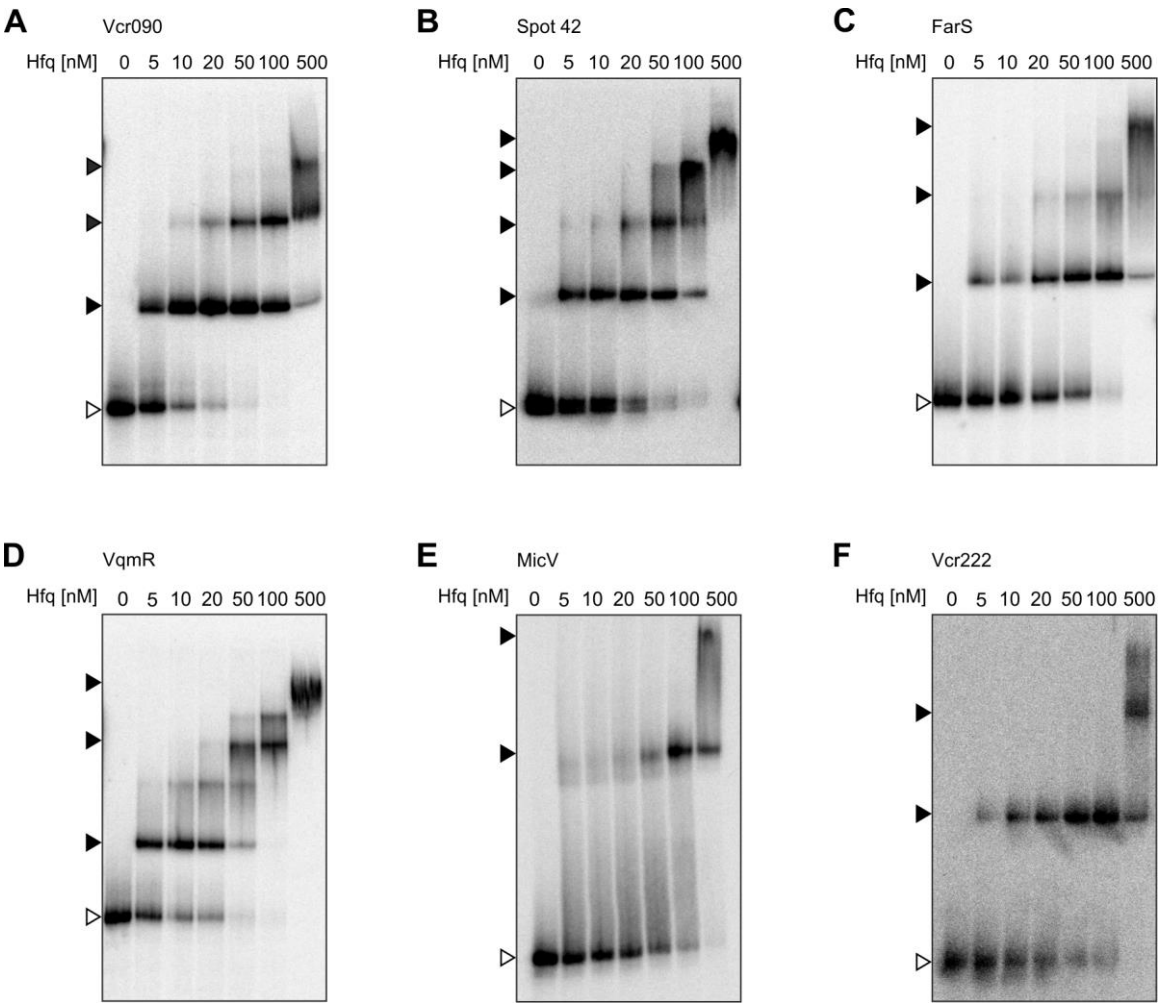


Figure S2: Hfq - sRNA binding experiments

A – F) Electrophoretic mobility shift assays (EMSAs) using *in vitro* synthesized, 5'end-labelled sRNAs (4nM; A: Vcr090, B: Spot 42, C: FarS, D: VqmR, E: MicV, F: Vcr222) and increasing concentrations of purified *V. cholerae* Hfq protein. Open triangles indicate free sRNAs, solid triangles indicate sRNA-Hfq complexes.

Figure S3

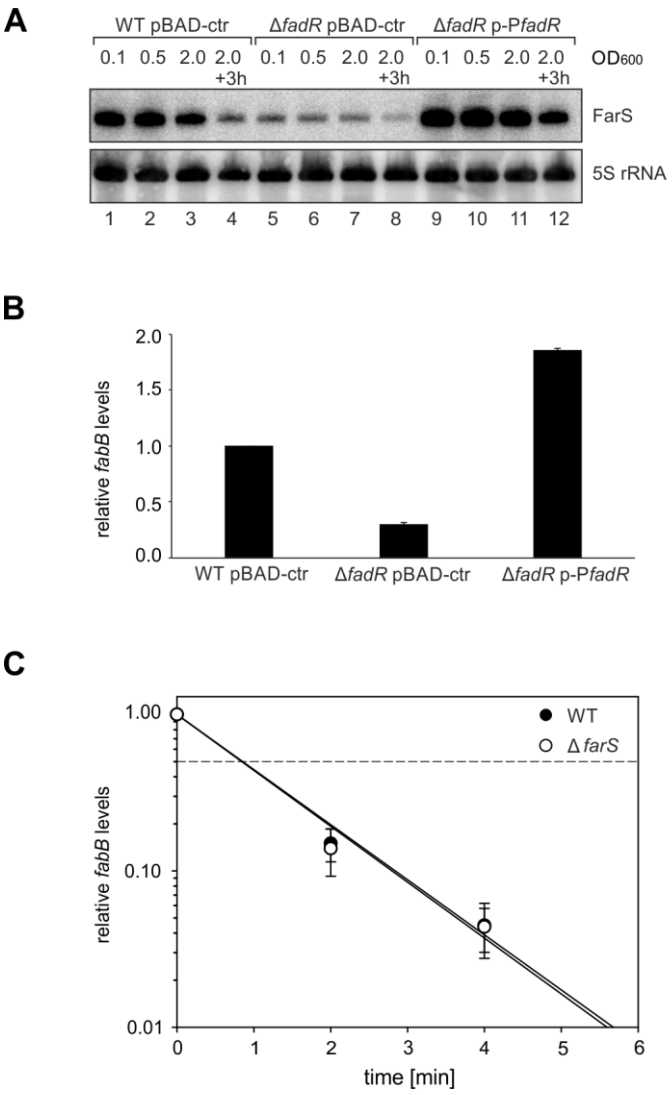


Figure S3: Expression of *fabB* and *farS* requires FadR

A) *V. cholerae* wild-type and Δ*fadR* cells harboring either a control plasmid (pBAD-ctr) or a plasmid containing the *fadR* gene with its native promotor (p-P*fadR*) were cultivated in M9 minimal medium. Total RNA samples were collected at different stages of growth and Northern blot analysis was performed to determine FarS levels. 5S rRNA served as loading control. **B)** *V. cholerae* wild-type and Δ*fadR* strains harboring the indicated plasmids were cultivated in LB medium to an OD_{600} of 1.0. RNA samples were collected and *fabB* mRNA levels were analyzed using qRT-PCR. Data are presented as mean ± SD. **C**) *V. cholerae* wild-type and Δ*farS* strains were grown in LB medium to an OD_{600} of 1.0. Cells were treated with rifampicin to terminate transcription. Total RNA samples were collected at the indicated time points and qRT-PCR was performed to monitor *fabB* transcript levels. Data are presented as mean ± SD.

Figure S4

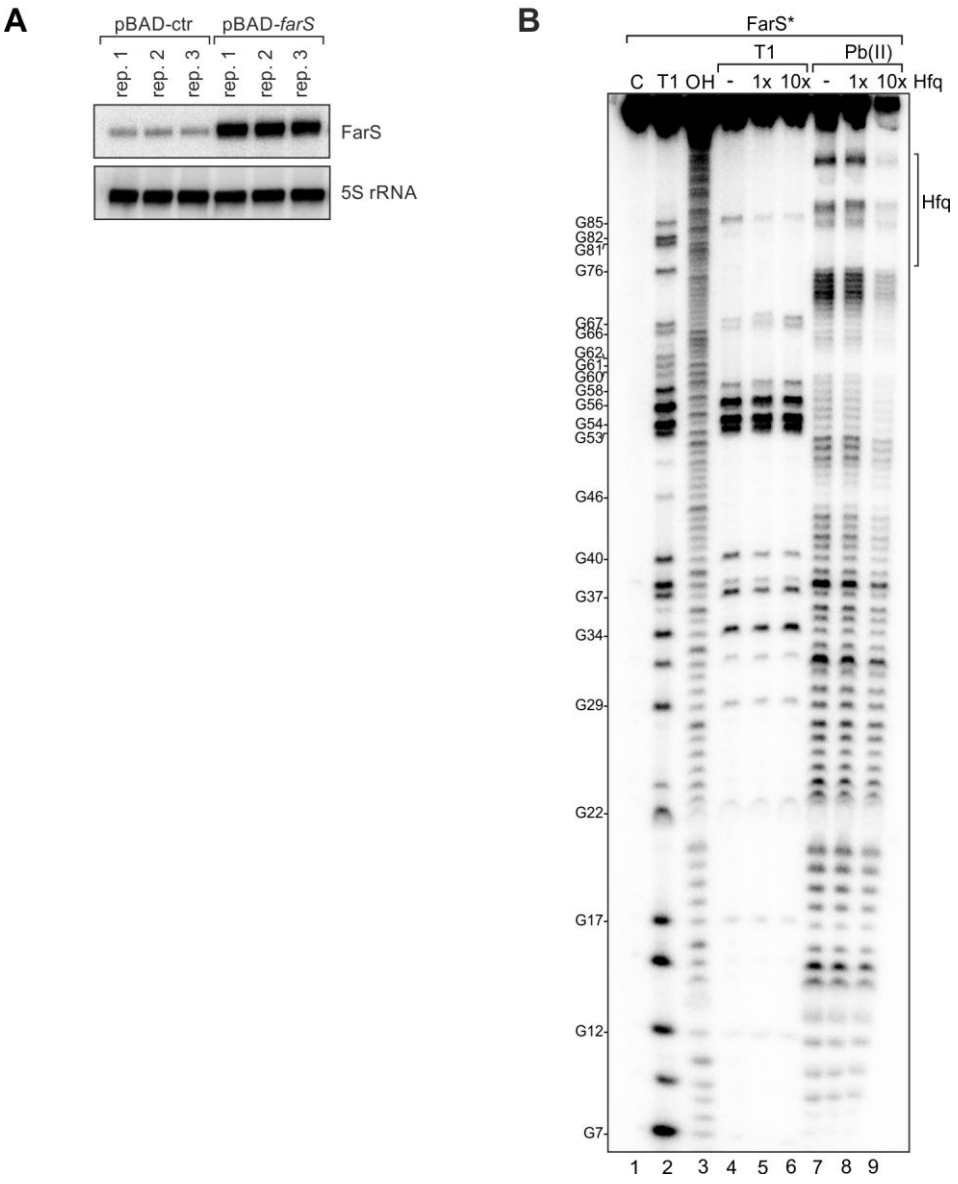
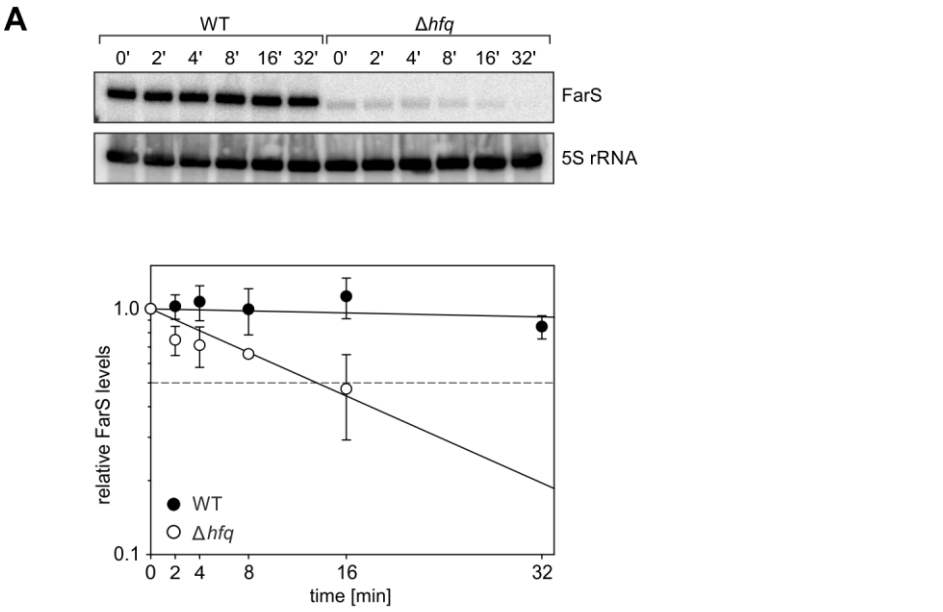
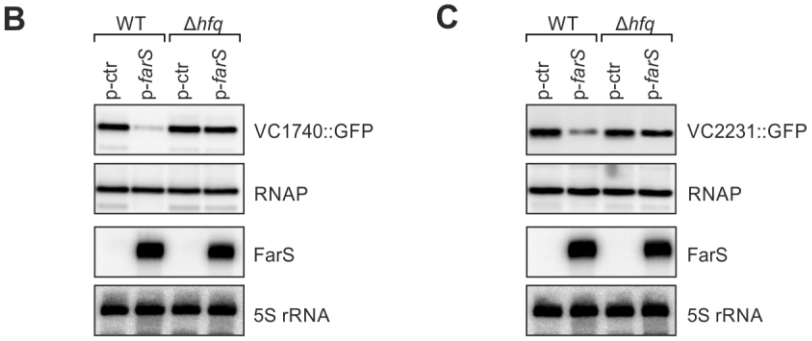


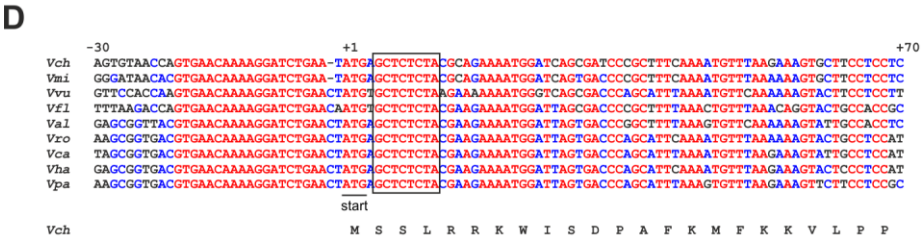
Figure S4: Pulse induction of FarS and structure probing experiments

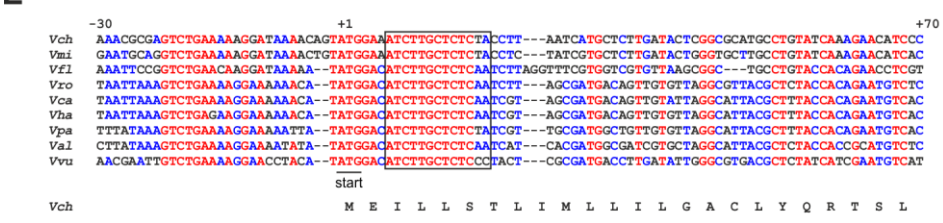
A) *V. cholerae* wild-type cells carrying the indicated plasmids were cultivated in LB medium to exponential phase (OD_{600} of 0.5) and induced with L-arabinose (0.2% final conc.) for 15 minutes. FarS levels were determined by Northern blot analysis and 5S rRNA was used as loading control. **B)** *In vitro* structure probing of 5′-end-labelled FarS sRNA (0.4 pmol) with RNase T1 (lanes 4 to 6) and lead(II) acetate (lanes 7 to 9) in the presence of 0.4 pmol (1x) or 4 pmol (10x) Hfq protein. RNase T1 and alkaline ladders of FarS were used to map the position of individual nucleotides. The positions of G residues are indicated relative to the transcriptional start site.

Figure S5


A
WT
Δhfq
0' 2' 4' 8' 16' 32' 0' 2' 4' 8' 16' 32'
FarS
5S rRNA
relative FarS levels
1.0
0.1
WT
Δhfq
0 2 4 8 16 32
time [min]


B
WT
Δhfq
p-ctr
p-farS
p-ctr
p-farS
VC1740::GFP
RNAP
FarS
5S rRNA
C
WT
Δhfq
p-ctr
p-farS
p-ctr
p-farS
VC2231::GFP
RNAP
FarS
5S rRNA


D
-30
+1
+70
Vch AGTGTAACCAGTGAACAAAAGGATCTGAA-TATGAGCTCTCTACGCAGAAAATGGATCAGCGATCCCGCTTTCAAAATGTTTAAGAAAGTGCTTCCTCCTC
Vmi GGGATAACACGTGAACAAAAGGATCTGAA-TATGAGCTCTCTACGCAGAAAATGGATCAGTGACCCCGCTTTCAAAATGTTTAAAAAAGTGCTTCCTCCTC
Vvu GTTCCACCAAGTGAACAAAAGGATCTGAACTATGTGCTCTCTAAGAAAAAAATGGGTCAGCGACCCAGCATTTAAAATGTTCAAAAAAGTACTTCCTCCTT
Vfl TTTAAGACCAGTGAACAAAAGGATCTGAACAATGTGCTCTCTACGAAGAAAATGGATTAGCGACCCCGCTTTTAAACTGTTTAAACAGGTACTGCCACCGC
Val GAGCGGTTACGTGAACAAAAGGATCTGAACTATGAGCTCTCTACGAAGAAAATGGATTAGTGACCCGGCTTTTAAAGTGTTCAAAAAAGTATTGCCACCTC
Vro AAGCGGTGACGTGAACAAAAGGATCTGAACTATGAGCTCTCTACGAAGAAAATGGATTAGTGACCCAGCATTCAAAATGTTTAAAAAAGTACTGCCTCCAT
Vca TAGCGGTGACGTGAACAAAAGGATCTGAACTATGAGCTCTCTACGAAGAAAATGGATTAGTGACCCAGCATTTAAAATGTTTAAGAAAGTATTGCCTCCAT
Vha GAGCGGTGACGTGAACAAAAGGATCTGAACTATGAGCTCTCTACGAAGAAAATGGATTAGTGACCCAGCATTCAAAATGTTTAAGAAAGTACTCCCTCCAT
Vpa AAGCGGTGACGTGAACAAAAGGATCTGAACTATGAGCTCTCTACGAAGAAAATGGATTAGTGACCCAGCATTTAAAGTGTTTAAGAAAGTTCTTCCTCCGC
start
Vch M S S L R R K W I S D P A F K M F K K V L P P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-30
+1
+70
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Vmi GAATGCAGGTCTGAAAAAGGATAAAACTGTATGGAAATCTTGCTCTCTACCTC---TATCGTGCTCTTGATACTGGGTGCTTGCCTGTATCAAAGAACATCAC
Vfl AAATTCCGGTCTGAACAAGGATAAAAA--TATGGACATCTTGCTCTCAATCTTAGGTTTCGTGGTCGTGTTAAGCGGC---TGCCTGTACACAGAACCTCGT
Vro TAATTAAAGTCTGAAAAGGAAAAAACA--TATGGACATCTTGCTCTCAATCTT---AGCGATGACAGTTGTGTTAGGCGTTACGCTCTACCACAGAATGTCTC
Vca TAATTAAAGTCTGAAAAGGAAAAAACA--TATGGACATCTTGCTCTCAATCGT---AGCGATGACAGTTGTATTAGGCATTACGCTTTACCACAGAATGTCAC
Vha TAATTAAAGTCTGAGAAGGAAAAAACA--TATGGACATCTTGCTCTCAATCGT---AGCGATGACAGTTGTGTTAGGCATTACGCTTTACCACAGAATGTCAC
Vpa TTTATAAAGTCTGAAAAGGAAAAATTA--TATGGACATCTTGCTCTCTATCGT---TGCGATGGCTGTTGTGTTAGGCATTACGCTTTACCACAGAATGTCAC
Val CTTATAAAGTCTGAAAAGGAAAATATA--TATGGACATCTTGCTCTCAATCAT---CACGATGGCGATCGTGCTAGGCATTACGCTCTACCACCGCATGTCTC
Vvu AACGAATTGTCTGAAAAGGAACCTACA--TATGGACATCTTGCTCTCCCTACT---CGCGATGACCTTGATATTGGGCGTGACGCTCTATCATCGAATGTCAT
start
Vch M E I L L S T L I M L L I L G A C L Y Q R T S L

Figure S5: Hfq is required for FarS-mediated target regulation

A) *V. cholerae* wild-type and Δ*hfq* strains were cultivated in LB medium to an OD_{600} of 1.5. Cells were treated with rifampicin and total RNA was collected at the indicated time points. Northern blot analysis was performed to monitor FarS levels. 5S rRNA was used as loading control. Data are presented as mean ± SD. **B and C)** *E. coli* wild-type and Δ*hfq* strains harboring a reporter plasmid for VC1740::GFP (A) or for VC2231::GFP (B) were co-transformed with plasmids p-ctr or p-*farS* and grown in LB medium to stationary phase (OD_{600} of 2.0). GFP levels were analyzed by Western blotting and FarS levels were determined by Northern blot analysis. RNAP and 5S rRNA served as loading controls for Western and Northern blots, respectively. **D and E)** Alignments of *fadE* sequences in different *Vibrio* species (D: *vc1740*, E: *vc2231*). The sequences were aligned using the Multalign algorithm (1). Numbers above the sequences indicate the distance to the first nucleotides of the *fadE* start codons (marked with +1). The start codons are underlined. Black boxes indicate sequences base-pairing to FarS. The corresponding amino acid sequences for *V. cholerae* are shown below, respectively. *Vch*, *Vibrio cholerae; Vmi, Vibrio mimicus; Vvu*, *Vibrio vulnificus; Vfl, Vibrio fluvialis; Val, Vibrio alginolyticus; Vro, Vibrio rotiferianus; Vca, Vibrio campbellii; Vha*, *Vibrio harveyi; Vpa*, *Vibrio parahaemolyticus.*

Figure S6

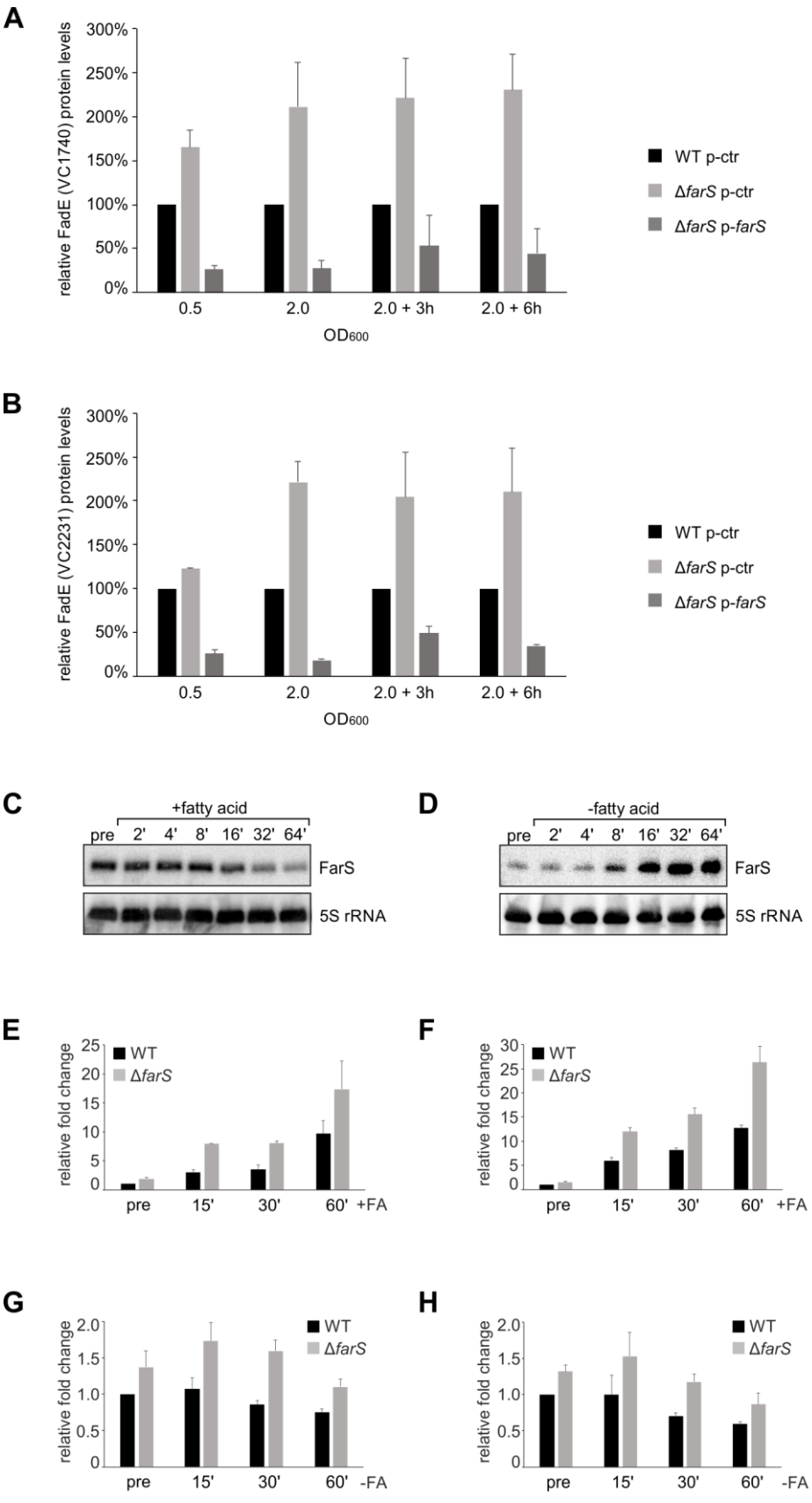
A
relative FadE (VC1740) protein levels
300%
250%
200%
150%
100%
50%
0%
0.5
2.0
2.0 + 3h
2.0 + 6h
OD600
WT p-ctr
ΔfarS p-ctr
ΔfarS p-farS
B
relative FadE (VC2231) protein levels
300%
250%
200%
150%
100%
50%
0%
0.5
2.0
2.0 + 3h
2.0 + 6h
OD600
WT p-ctr
ΔfarS p-ctr
ΔfarS p-farS
C
+fatty acid
pre 2' 4' 8' 16' 32' 64'
FarS
5S rRNA
D
-fatty acid
pre 2' 4' 8' 16' 32' 64'
FarS
5S rRNA
E
relative fold change
25
20
15
10
5
0
WT
ΔfarS
pre
15'
30'
60'
+FA
F
relative fold change
30
25
20
15
10
5
0
WT
ΔfarS
pre
15'
30'
60'
+FA
G
relative fold change
2.0
1.5
1.0
0.5
0
WT
ΔfarS
pre
15'
30'
60'
-FA
H
relative fold change
2.0
1.5
1.0
0.5
0
WT
ΔfarS
pre
15'
30'
60'
-FA

Figure S6: Effect of FarS and fatty acids on FadE production

A and B) *V. cholerae* wild-type and Δ*farS* strains carrying a chromosomal 3XFLAG epitope either at the *vc1740* (A) or at the *vc2231* (B) gene and harboring the indicated plasmids were cultivated in M9 minimal medium. FadE::3XFLAG protein production (A: VC1740::3XFLAG, B: VC2231::3XFLAG) was analyzed by Western blotting (see Figs. 5A-B). Bar graphs show quantification of Western blots obtained from three independent biological replicates. FadE levels in the wild-type strains were set to 100%. Data are presented as mean ± SD. **C)** *V. cholerae* wild-type cells were grown in M9 minimal medium and fatty acids (sodium oleate, 0.005% final conc.) were added when cells reached an OD_{600} of 1.0. RNA samples were collected at the indicated time points before and after treatment. Northern blot analysis was performed to determine FarS levels. 5S rRNA was used as loading control. **D)** *V. cholerae* wild-type cells were cultivated in M9 minimal medium supplemented with external fatty acids (sodium oleate, 0.005% final conc.). When reaching an OD_{600} of 1.0, cells were washed and transferred into fresh M9 minimal medium lacking fatty acids. RNA samples were collected at the indicated time points. The Northern blot was probed for FarS and 5S rRNA served as loading control. **E and F)** *V. cholerae* wild-type and Δ*farS* strains carrying a chromosomal 3XFLAG epitope either at the *vc1740* (E) or at the *vc2231* (F) gene were cultivated in M9 minimal medium. When cells reached stationary phase (OD_{600} of 2.0), fatty acids (+FA, sodium oleate, 0.005% final conc.) were added and FadE expression patterns were analyzed on Western blots (see Figs. 6B-C). Bar graphs show quantification of Western blots obtained from three independent biological replicates. Expression in the wild-type strain before treatment (pre) was set to 1. Data are presented as mean ± SD. **G and H)** *V. cholerae* wild-type and Δ*farS* strains carrying a chromosomal 3XFLAG epitope either at the *vc1740* (G) or at the *vc2231* (H) gene were cultivated in M9 minimal medium containing sodium oleate (0.005% final conc.). When reaching stationary phase, cells were washed and resuspended in M9 minimal medium lacking fatty acids (-FA) and FadE levels were monitored on Western blots (see Figs. 6D-E). Bar graphs show quantification of Western blots obtained from three independent biological replicates. Expression in the wild-type strain before treatment (pre) was set to 1. Data are presented as mean ± SD.

Supplementary Materials and Methods

Plasmid construction

All plasmids used in this study are listed in Table S5, and all DNA oligonucleotides in Table S6. The plasmid pMH029 was constructed by amplifying the *hfq* gene (*vc0347*) from *V. cholerae* (KPS-0014) genomic DNA (gDNA) with KPO-2292 and KPO-2293 and inserting it into the linearized pTYB11 plasmid (NEB, KPO-2294/2295), using Gibson assembly (GA). To generate the sRNA expression plasmids pJR5 and pJR6, the *farS* gene was PCR amplified from gDNA, using primer sets KPO-2450/2452 and KPO-2451/2452, respectively. The fragments were fused to linearized pBAD1K (pMD004) or pEVS143 plasmid backbones (KPO-0196/1397 or KPO-0092/1397) via GA. pJR6 served as template to insert a single point mutation in the *farS* gene using site-directed mutagenesis and oligonucleotides KPO-3026/3027, yielding plasmid pJR14. The plasmid pMH034 was obtained by linearizing pMD004 with KPO-1792 and KPO-1397, and inserting the fragment amplified from gDNA with oligonucleotides KPO-2453 and KPO-2452, using GA. The *fabB* promotor truncation plasmids pJR8, pJR9 and pJR10 were cloned in the same way as pMH034 using the oligonucleotide combinations KPO-2454/2452, KPO-2455/2452 and KPO-2456/2452, respectively, for insert amplification. pMH034 served as template to construct pJR34 via site-directed mutagenesis using the oligonucleotides KPO-3963 and KPO-3964. To generate pJR22 by GA, the *fabB-farS* fragment was amplified from gDNA with KPO-3771 and KPO-2452 and pMD004 was linearized with pBAD-ATGrev and KPO-1397. To construct plasmid pJR12, the *farS* flanking regions were amplified with primer sets KPO-1278/2458 and KPO-2459/1281 respectively, and subsequently cloned via GA into the pKAS32 plasmid backbone, linearized with KPO-0267 and KPO-0268. pMH043 was obtained by linearizing pMH001 with KPO-1792 and KPO-1423 and inserting the fragment amplified from gDNA with oligonucleotides KPO-2764 and KPO-2765 via GA. GFP fusions were cloned as described previously (2). Briefly, *vc2231* (pMH037) and *vc1741/40* (pMH042) inserts for translational reporters were PCR amplified with the oligonucleotide sets KPO-2797/2798 and KPO-2546/2923 and introduced via GA into linearized pXG10 (KPO-1702/1703) and pXG30 (KPO-2662/1703) backbones, respectively. Single point mutations in the *vc2231* and *vc1740* genes were implemented by PCR using KPO-3030/3031 and KPO-3028/3029, resulting in plasmids pJR16 and pMH051, respectively. The plasmids pJR20 and pJR21 were constructed by GA using pKAS32 backbone that was linearized with KPO-0267 and KPO-0268. The insert fragments for pJR20 were amplified from KPS-0014 gDNA (KPO-3080/3081 and KPO-3084/3019) and KPS-0995 gDNA (KPO-3082/3083) that carries the 3XFLAG coding sequence. For pJR21, primer pairs KPO-3075/3076 (KPS-0014), KPO-3079/3015 (KPS-0014) and KPO-3077/3078 (KPS-0995) were used for insert amplification.

Strain construction

All strains used in this study are listed in Table S4. *V. cholerae* C6706 was used as wild-type strain throughout the study. KPVC-11255 was constructed using natural transformation as described previously (3). Briefly, the flanking regions of *fadR* were amplified from *V. cholerae* gDNA with KPO-2766/2767 and KPO-2768/2769 and the FRT-flanked kanamycin cassette was amplified with KPO-1771/1772 from pBR-FRT-KAN-FRT (3). The three fragments were fused and amplified using KPO-2766/2769. Mutant cells were selected on kanamycin plates and confirmed by PCR using KPO-2698/1820. All other *V. cholerae* mutants were generated using the pKAS32 suicide vector (4) and established cloning strategies (5). Briefly, pKAS32-plasmids (pJR12, pMD003, pJR20 and pJR21) were conjugated into *V. cholerae* and cells were selected for ampicillin resistance. Polymyxin B was used to specifically inhibit *E. coli* growth. Single colonies were transferred to fresh plates and selected for streptomycin resistance. Mutants were confirmed by PCR and sequencing. KFS-01032 was established by P1 *vir* transduction of the Δ*hfq::KanR* allele from the KEIO collection (6) using standard protocols.

T7 transcription and 5' end labelling of RNA

DNA templates carrying a T7 promoter for *in vitro* synthesis of RNA were prepared by PCR using the oligonucleotides listed in Table S6. Template DNA (200 ng) was *in vitro* transcribed using the AmpliScribe T7-Flash transcription kit (Epicentre) following the manufacturer's recommendations. RNA size and integrity were verified on denaturing polyacrylamide gels. 5' end labelling was performed as described previously (7). Briefly, RNA (20 pmol) was dephosphorylated using 10 units of calf alkaline phosphatase (NEB), followed by P:C:I extraction and ethanol precipitation of RNA. Dephosphorylated RNA was incubated with [^{32}P]-γATP (20 µCi) and 1 unit of polynucleotide kinase (NEB) for 1 h at 37°C. Unincorporated nucleotides were removed using Microspin G-50 columns (GE Healtcare). Labelled RNA was loaded on a 6% / 7 M urea gel, cut from the gel, eluted overnight at 4°C with RNA elution buffer (0.1 M sodium acetate, 0.1% SDS, 10 mM EDTA), and recovered by P:C:I extraction.

Purification of Hfq

The Hfq protein was expressed from the pTYB11 expression vector (NEB) in *E. coli* ER2566 Δ*hfq* cells and purified following the Impact Kit (NEB) protocol. Briefly, cells were grown to OD_{600} of 0.5 and induced with IPTG (0.5 mM final conc.) for 15 h at 20°C. Cells were harvested, resuspended in column buffer (20 mM Tris-HCl [pH 8.5], 500 mM NaCl, 1 mM EDTA) and lysed by sonication. Cleared lysates were loaded on a column containing the chitin binding domain. After 40 h of incubation at room temperature, on column cleavage was induced using cleavage buffer (20 mM Tris-HCl [pH 8.5], 500 mM NaCl, 50 mM DTT, 1mM EDTA). Protein purification

was verified by SDS-PAGE analysis. The Hfq protein was concentrated and buffer was exchanged to storage buffer (25 mM Tris-HCl [pH 7.6], 150 mM NaCl, 0.5 mM EDTA) using 5 kDA MWCO Vivaspin columns (GE Healthcare).

Electrophoretic Mobility Shift Assays (EMSA)

To analyze complex formation between sRNAs and Hfq *in vitro*, gel shift assays were performed following previously established protocols (8). Briefly, 5' end-labelled RNA (4 pmol) was supplemented with 1x structure buffer (0.01 M Tris-HCl [pH 7], 0.1 M KCl, 0.01 M $MgCl_2$) and 1 µg yeast RNA and incubated with increasing concentrations of purified Hfq or Hfq dilution buffer (1x structure buffer, 1% [vol/vol] glycerol, 0.1% [vol/vol] Triton X-100) at 37°C for 15 min. Prior to loading, reactions were mixed with native loading buffer (50% glycerol, 0.5x TBE, 0.2% [wt/vol] bromphenol blue) and separated by native PAGE. Signals were visualized on a Typhoon Phosphorimager (Amersham).

Quantitative real-time PCR (qRT-PCR)

Quantitative real-time PCR was performed as described previously (9). Briefly, RNA was extracted using the SV total RNA Isolation System (Promega) and *fabB* transcript levels were measured using the Luna Universal One-Step RT-qPCR Kit (NEB) and the MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad). Oligonucleotides used for qRT-PCR are listed in Table S6.

Transcript stability experiments

In order to analyze RNA stability, cells were treated with rifampicin (250 µg/ml final conc.) at the designated ODs to terminate transcription. RNA samples were collected at the indicated time points and transcript levels were determined either by Northern blot analysis or by qRT-PCR.

RNA structure probing

RNA structure probing was carried out as described previously (10) with few modifications. In brief, 0.4 pmol 5′ end-labelled FarS sRNA was denatured, quickly chilled on ice and mixed with 0.4 pmol or 4 pmol of purified *V. cholerae* Hfq protein or an equal volume of Hfq dilution buffer in the presence of 1x structure buffer and 1 µg yeast RNA. Samples were incubated at 37°C for 15 min, and treated with RNase T1 (0.1 U; Ambion, #AM2283) for 2.5 min or with lead(II) acetate (5 mM final conc.; Sigma, #316512) for 1.5 min. Reactions were stopped by the addition of 2 vol. stop/precipitation buffer (1 M guanidinium thiocyanate, 0.167% N-lauryl-sarcosine, 10 mM DTT, 83% 2-propanol). RNA was precipitated for 2 h at -20°C, and collected

by centrifugation (30 min, 4°C, 13.000 rpm). Samples were dissolved in GLII loading buffer, and separated on 10% polyacrylamide sequencing gels.

Table S1: Statistics of obtained and mapped cDNA reads for co-IP libraries

strain	condition	replicate	number of reads (million)	reads mapped on chr I (million)	reads mapped on chr II (million)	mapped reads in total
untagged control	0.2	I	11.0	10.4	0.3	97.6%
untagged control	0.2	II	13.3	12.5	0.4	97.8%
Hfq::3XFLAG	0.2	I	13.5	12.5	0.7	97.9%
Hfq::3XFLAG	0.2	II	12.3	10.8	1.2	97.6%
untagged control	2.0	I	39.1	36.9	0.9	96.7%
untagged control	2.0	II	12.1	11.7	0.2	98.0%
Hfq::3XFLAG	2.0	I	7.9	6.8	0.8	96.7%
Hfq::3XFLAG	2.0	II	14.5	12.6	1.6	97.5%

Table S3: Overview of new sRNA candidates

name	start	stop	orientation	size (nt)	enriched in Hfq co-IP
chromosome 1					
Vcr200	218.072	218.336	sense	265	yes
Vcr201	455.266	455.354	sense	89	yes
Vcr202	481.285	481.138	antisense	148	yes
Vcr203	606.829	606.883	sense	55	yes
Vcr204	677.937	678.072	sense	136	yes
Vcr205	714.030	714.121	sense	92	no
Vcr206	944.382	944.313	antisense	70	no
Vcr207	1.106.591	1.106.734	sense	144	yes
Vcr208	1.531.755	1.531.675	antisense	81	yes
Vcr209	1.578.023	1.578.082	sense	60	yes
Vcr210	1.582.874	1.582.933	sense	60	yes
Vcr211	1.861.483	1.861.570	sense	88	no
Vcr212	2.000.800	2.001.130	sense	331	yes
Vcr213	2.059.854	2.060.038	sense	185	yes
Vcr214	2.376.028	2.376.142	sense	115	yes
Vcr215	2.396.723	2.396.633	antisense	91	yes
Vcr216	2.518.934	2.518.785	antisense	150	yes
Vcr217	2.537.176	2.537.226	sense	51	yes
Vcr218	2.558.990	2.558.878	antisense	113	yes
Vcr219	2.639.102	2.639.035	antisense	68	yes
Vcr220	2.653.872	2.654.007	sense	136	yes
Vcr221	2.669.966	2.670.113	sense	148	no
Vcr222	2.783.908	2.783.840	antisense	69	yes
Vcr223	2.855.213	2.855.093	antisense	121	no
chromosome 2					
Vcr224	14.695	14.764	sense	70	yes
Vcr225	42.166	42.215	sense	50	yes
Vcr226	334.397	334.515	sense	119	no
Vcr227	479.958	480.023	sense	66	yes
Vcr228	787.266	787.431	sense	166	yes
Vcr229	897.527	897.726	sense	200	yes
Vcr230	937.994	938.066	sense	73	yes

Table S4: Bacterial strains used in this study

Strain	Relevant markers / genotype	Reference / Source
V. cholerae		
KPS-0014	C6706 wild-type	(11)
KPS-0054	C6706 Δ*hfq*	(12)
KPS-0995	C6706 *hfq::3Xflag*	(13)
KPVC-11063	C6706 Δ*farS*	This study
KPVC-11255	C6706 Δ*fadR::kan*	This study
KPVC-11437	C6706 Δ*vc2231*/Δ*vc1740*	This study
KPVC-11488	C6706 Δ*farS vc2231*::*3Xflag*	This study
KPVC-11492	C6706 *vc2231*::*3Xflag*	This study
KPVC-11525	C6706 *vc1740*::*3Xflag*	This study
KPVC-11526	C6706 Δ*farS vc1740*::*3Xflag*	This study
KPVC-11527	C6706 *rne*-3071 Δ*farS*	This study
E. coli		
Top10	*F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(StrR) endA1 λ-*	Invitrogen
S17λpir	*ΔlacU169 (ΦlacZΔM15), recA1, endA1, hsdR17, thi-1, gyrA96, relA1, λpir*	(14)
ER2566	*fhuA2 lacZ::T7 gene1 [Ion] ompT gal sulA11 R(mcr-73::miniTn10--TetS)2 [dcm] R(zgb-210::Tn10--TetS) endA1 Δ(mcrC-mrr) 114::IS10*	New England Biolabs
KFS-01032	ER2566 Δ*hfq::kan*	This study
KPEC-50812	MC4100 Δ*hfq*	(15)

Table S5: Plasmids used in this study

Plasmid trivial name	Plasmid stock name	Relevant fragment	Comment	Origin, marker	Reference
p-ctr	pCMW-1		control plasmid	P15A, Kan^R	Papenfort plasmid collection
pBAD1K-ctr	pMD004		control plasmid	P15A, Kan^R	Papenfort plasmid collection
pBAD1C-ctr	pMH001		control plasmid	P15A, Cm^R	Papenfort plasmid collection
pEVS143	pEVS143	P*tac* promotor	constitutive overexpression plasmid	P15A, Kan^R	Papenfort plasmid collection
pKAS32	pKAS32		suicide plasmid for allelic exchange	R6K, Amp^R	(4)
pXG10-*gfp*	pXG10-*gfp*	*lacZ'::gfp*	template plasmid for translational reporters	pSC101*, Cm^R	(2)
pXG30-*gfp*	pXG30-*gfp*	*flag::lacZ'::gfp*	template plasmid for translational reporters	pSC101*, Cm^R	(2)
pTYB11-*hfq*	pMH029	*hfq (vc0347)*	intein fusion vector for Hfq protein purification	pBR322, Amp^R	This study
pBAD-*farS*	pJR5	*farS*	*farS* expression plasmid	P15A, Kan^R	This study
p-*farS*	pJR6	*farS*	*farS* expression plasmid	P15A, Kan^R	This study
p-P*fabB-farS*	pMH034	P*fabB-farS*	*fabB-farS* expression plasmid	P15A, Kan^R	This study
p-P*fabB-farS* -300 bp	pJR8	*fabB-farS*	*fabB-farS* promotor truncation plasmid	P15A, Kan^R	This study
p-P*fabB-farS* -600 bp	pJR9	*fabB-farS*	*fabB-farS* promotor truncation plasmid	P15A, Kan^R	This study
p-P*fabB-farS* -900 bp	pJR10	*fabB-farS*	*fabB-farS* promotor truncation plasmid	P15A, Kan^R	This study
pKAS32-Δ*farS*	pJR12	up/downstream flanks of *farS*	suicide plasmid for *farS* knock-out	R6K, Amp^R	This study
p-P*fadR*	pMH043	P*fadR*	*fadR* expression plasmid	P15A, Cm^R	This study
p-P*fabB-farS* > GGG	pJR34	P*fabB-farS*	mutated *rne* site (TTT1-3GGG)	P15A, Kan^R	This study
pKAS-*rne*-3071	pMD003	*rne*-3071	suicide plasmid for *rne* C202T base mutation	R6K, Amp^R	Papenfort plasmid collection
pBAD-*fabB-farS*	pJR22	*fabB-farS*	*fabB-farS* expression plasmid	P15A, Kan^R	This study
p-*farS**	pJR14	*farS**	*farS** (G54C) expression plasmid	P15A, Kan^R	This study
pXG10-*vc2231*	pMH037	*vc2231::gfp*	translational reporter for *vc2231*	pSC101*, Cm^R	This study
pXG10-*vc2231**	pJR16	*vc2231*::gfp*	translational reporter for *vc2231** (C17G)	pSC101*, Cm^R	This study
pXG30-*1741/40*	pMH042	*flag::vc1741 vc1740::gfp*	translational reporter for *vc1741* and *1740*	pSC101*, Cm^R	This study
pXG30-*vc1741/40**	pMH051	*flag::vc1741 vc1740*::gfp*	translational reporter for *vc1741* and *1740** (C10G)	pSC101*, Cm^R	This study
pKAS32-*vc2231*::3Xflag*	pJR20	*vc2231::3Xflag*	*vc2231*::3Xflag* allelic replacement	R6K, Amp^R	This study
pKAS32-*vc1740*::3Xflag*	pJR21	*vc1740::3Xflag*	*vc1740*::3Xflag* allelic replacement	R6K, Amp^R	This study

Table S6: DNA oligonucleotides used in this study

Name	Sequence 5' to 3'	Description
KPO-0009	CTACGGCGTTTCACTTCTGAGTTC	*E.c.* 5S oligoprobe
KPO-0063	CGTCTATAAGTGTGAACAATGGTG	Qrr4 oligoprobe
KPO-0092	CCACACATTATACGAGCCGA	plasmid construction
KPO-0196	GGAGAAACAGTAGAGAGTTGCG	plasmid construction
KPO-0216	AGTCGAGGACTCAGTTTATGATTA	Vcr017 oligoprobe
KPO-0243	TTCGTTTCACTTCTGAGTTCGG	*V.ch.* 5S oligoprobe
KPO-0267	TAATAGGCCTAGGATGCATATG	plasmid construction
KPO-0268	CGTTAACAACCGGTACCTCTA	plasmid construction
KPO-0331	GAGCCAATCTACAATTCATCAGA	Vcr090 oligoprobe
KPO-0452	ATCTTGTCGACGTGTAGAAGAGGTT	VqmR oligoprobe
KPO-0513	GTTTTTTTTAATACGACTCACTATAGCAGAGCATGAGTTGCATGAC	VqmR T7 transcription
KPO-0514	AAAAAAAGCCAGCCTGAAGACG	VqmR T7 transcription
KPO-0820	GGCCTTCTTAGAGTCTTCTAAGAA	MicV oligoprobe
KPO-0821	AGGTTGTCAGAGAGGCCTTGA	Vcr084 oligoprobe
KPO-0822	GCCAGGTGAATAATGCGCTTG	Vcr092 oligoprobe
KPO-0842	GTAAAGCAATTAACTTACGCCAATTG	Vcr043 oligoprobe
KPO-0845	TTGGCCCGTCACAGGCTGAA	Vcr045 oligoprobe
KPO-0873	CTCTCCATGGGACAGAGTCT	FarS oligoprobe
KPO-1278	TAGAGGTACCGGTTGTTAACGCACCGGTATGGGTATTATTTCG	plasmid construction
KPO-1281	CATATGCATCCTAGGCCTATTAGTTGGCTCATCACATACCTC	plasmid construction
KPO-1397	GATCCGGTGATTGATTGAGC	plasmid construction
KPO-1423	TCTAGATTAAATCAGAACGCAGAAG	plasmid construction
KPO-1702	ATGCATGTGCTCAGTATCTCTATC	plasmid construction
KPO-1703	GCTAGCGGATCCGCTGG	plasmid construction
KPO-1771	TAATGTCGGAGTAGGCTGGAGCTGCTTCGAAGTTCC	strain construction
KPO-1772	CTTCCAGAGACATATGAATATCCTCCTTAGTTCCTATTC	strain construction
KPO-1792	CAGTGCGCCTTTTTATAGTC	plasmid construction
KPO-1820	ATGGATACTTTCTCGGCAG	strain construction
KPO-2002	GTTTACCATCGCTTATAGTTATA	Vcr091 oligoprobe
KPO-2010	TAAAGCTTTCAACCTGTGACG	Vcr222 oligoprobe
KPO-2075	GTGTCTATGGCACAACTTTTAA	Vcr202 oligoprobe
KPO-2077	CCGCGAAAAGTAGGTTGTTTC	Vcr229 oligoprobe
KPO-2155	GGTATCTAAATTCTTTCGATACG	Vcr227 oligoprobe
KPO-2178	GTTTTTTTAATACGACTCACTATAGGGAGGGTGAATCATATCGACCAAATTTG	Vcr082 riboprobe
KPO-2179	GTCTGCAATGTTCTGGAACC	Vcr082 riboprobe
KPO-2292	CCCAGGTTGTTGTACAGAACATGGCTAAGGGGCAATCTCTA	plasmid construction
KPO-2293	CGGATCCCCTTCCTGCAGTTACTCTTCAGACTTCTCTGC	plasmid construction
KPO-2294	GTTCTGTACAACAACCTGGG	plasmid construction
KPO-2295	CTGCAGGAAGGGGATCCG	plasmid construction
KPO-2378	GGTAACCCAGAAACTACCACTG	*recA* qRT-PCR
KPO-2379	CACCACTTCTTCGCCTTCTT	*recA* qRT-PCR
KPO-2450	CGCAACTCTCTACTGTTTCTCCTTTCCAGAACAGATTAGTTTCGC	plasmid construction
KPO-2451	TCGGCTCGTATAATGTGTGGTTTCCAGAACAGATTAGTTTCGC	plasmid construction
KPO-2452	GCTCAATCAATCACCGGATCCAATCAAAGTTGCAGGCATTG	plasmid construction
KPO-2453	GACTATAAAAAGGCGCACTGCAGACCATTGACGTTAGAGAAA	plasmid construction
KPO-2454	GACTATAAAAAGGCGCACTGCTAAAAGCTGGCAAGTCAGG	plasmid construction
KPO-2455	GACTATAAAAAGGCGCACTGGTGGGTCCTTACATGGTG	plasmid construction
KPO-2456	GACTATAAAAAGGCGCACTGGGTTTCGTGATCTCTGGCG	plasmid construction
KPO-2458	CTAGGCCGCCGGGCAAACTGTGTTGGATCTGGTGCG	plasmid construction
KPO-2459	TTTGCCCGGCGGCCTAG	plasmid construction
KPO-2546	CCAGCGGATCCGCTAGCCACTTTCTTAAACATTTTGAAAGC	plasmid construction
KPO-2549	AAAAATACCCGACGACCTAGG	FarS T7 transcription
KPO-2650	CCCTCTTAGGAAAAATTGTCAC	Vcr101 riboprobe
KPO-2651	GTTTTTTTAATACGACTCACTATAGGGAGGCACCATAAAAAAAGCCCCG	Vcr101 riboprobe
KPO-2662	TTTATCGTCGTCATCTTTGTAG	plasmid construction
KPO-2698	GTTTCGCAGCTACCACTGG	strain construction

KPO-2764	GACTATAAAAAGGCGCACTGGATAGTGTGAGCTGTGTCC	plasmid construction
KPO-2765	CTGCGTTCTGATTTAATCTAGATTAGCAATCGTCTTCAGTAAAATTG	plasmid construction
KPO-2766	GGCAATAACGATACTCAAGTTC	strain construction
KPO-2767	TCCAGCCTACTCCGACATTATCTAGCACTGTTCGTTTTCGTTA	strain construction
KPO-2768	TATTCATATGTCTCTGGAAGCCACTAGTTGGTGTACGTCG	strain construction
KPO-2769	GCTATCGAAAGGAGAACTTTGG	strain construction
KPO-2797	GAGATACTGAGCACATGCAT ATCCGAACCCGCGCGCTT	plasmid construction
KPO-2798	GAGCCAGCGGATCCGCTAGTTGATACAGGCATGCGCCG	plasmid construction
KPO-2923	CTACAAAGATGACGACGATAAATCGTTAAGCGAATTGCGCCC	plasmid construction
KPO-3015	CATATGCATCCTAGGCCTATTAGAGCGGCATCACAGGAATC	plasmid construction
KPO-3019	CATATGCATCCTAGGCCTATTACTCCAATCTACAACTCACGAC	plasmid construction
KPO-3026	GTCCCATGCAGAGCGGGATAGGATCCTT	plasmid construction
KPO-3027	CTCTGCATGGGACAGAGTCTGCGTCTG	plasmid construction
KPO-3028	GAGCTCTGTACGCAGAAAATGGATCAGCG	plasmid construction
KPO-3029	CATTTTCTGCGTACAGAGCTCATATTCAG	plasmid construction
KPO-3030	CTTGCTCTGTACCTTAATCATGCTCTTG	plasmid construction
KPO-3031	GATTAAGGTACAGAGCAAGATTTCCATAC	plasmid construction
KPO-3075	TAGAGGTACCGGTTGTTAACGGATGGACCAATGAACTATCTGG	plasmid construction
KPO-3076	TGCCACACTGTTTAACTTAGG	plasmid construction
KPO-3077	CCTAAGTTAAACAGTGTGGCAGACTACAAAGACCATGACGG	plasmid construction
KPO-3078	CCTCGATACTCTATTTTATTTGTTATTACTATTTATCGTCATCTTTGTAG	plasmid construction
KPO-3079	TAACAAATAAAATAGAGTATCGAGG	plasmid construction
KPO-3080	TAGAGGTACCGGTTGTTAACGGTAAAGGCATCTGTTTAGGCC	plasmid construction
KPO-3081	AGCCACTTCAGCTTTACGTTG	plasmid construction
KPO-3082	CAACGTAAAGCTGAAGTGGCTGACTACAAAGACCATGACGG	plasmid construction
KPO-3083	GAATACTGCTCAATGTGGAACTTATTACTATTTATCGTCATCTTTGTAG	plasmid construction
KPO-3084	TAAGTTCCACATTGAGCAGTATTC	plasmid construction
KPO-3139	GGACAGAGTCTGCGTCTG	oligoprobe for mutated FarS (G54C)
KPO-3387	GTAACGCGGTTGAGCTTAT	*fabB* qRT-PCR
KPO-3388	CATGGTTTGTGACCAGTAGAG	*fabB* qRT-PCR
KPO-3726	CAGCCTAATCCAATAACGTGAAAC	Spot 42 oligoprobe
KPO-3771	GCTAACAGGAGGAATTAACCATGAAACGAGTCGTCATCAC	plasmid construction
KPO-3794	GACCCTTTCCTTTGTTGCTC	Vcr103 oligoprobe
KPO-3751	ACCTGATTCCATCCCGAA	5S qRT-PCR
KPO-3752	TGGCGATGTTCTACTCTCA	5S qRT-PCR
KPO-3963	CAACACAGGGGCCAGAACAGATTAGTTTCGC	plasmid construction
KPO-3964	CTGTTCTGGCCCCTGTGTTGGATCTGGTG	plasmid construction
KPO-4131	AAGAAAAAAGCCCTAAACCTAGTAC	MicV T7 transcription
KPO-4154	GTTTTTTTTAATACGACTCACTATAGACCACTGCTTTTTCTTAGAAGAC	MicV T7 transcription
KPO-4249	GTTTTTTTTAATACGACTCACTATAGGTTTCCAGAACAGATTAGTTTCGC	FarS T7 transcription
KPO-5083	GTTTTTTTTAATACGACTCACTATAGGAATAGACAACCTTTTGTCCT	Vcr090 T7 transcription
KPO-5084	AAAAAAAGAGCGAGCTATTTAAAC	Vcr090 T7 transcription
KPO-5085	GTTTTTTTTAATACGACTCACTATAGGACTCTAATCATAATTTATTTTGTGC	Vcr222 T7 transcription
KPO-5086	AGCTTTCAACCTGTGACGAA	Vcr222 T7 transcription
KPO-5087	GTTTTTTTTAATACGACTCACTATAGGCGTAGGGTACAGAGGTAAG	Spot 42 T7 transcription
KPO-5088	AATAAAAAACGCCCCAGTCAAA	Spot 42 T7 transcription
KPO-5415	CAACGGGAGAGAAAACGGTT	VSsrna24 oligoprobe
KPO-5468	CTTGTTAGGCTCATCACTCTTC	Vcr051 riboprobe
KPO-5469	GTTTTTTTTTTTAATACGACTCACTATAGGGAGGCAGTTCAGCACAAACTCAATAC	Vcr051 riboprobe
pBAD-ATGrev	GGTTAATTCCTCCTGTTAGC	plasmid construction

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