A conserved seed-pairing domain affords small RNA-mediated stress resistance in enterobacteria

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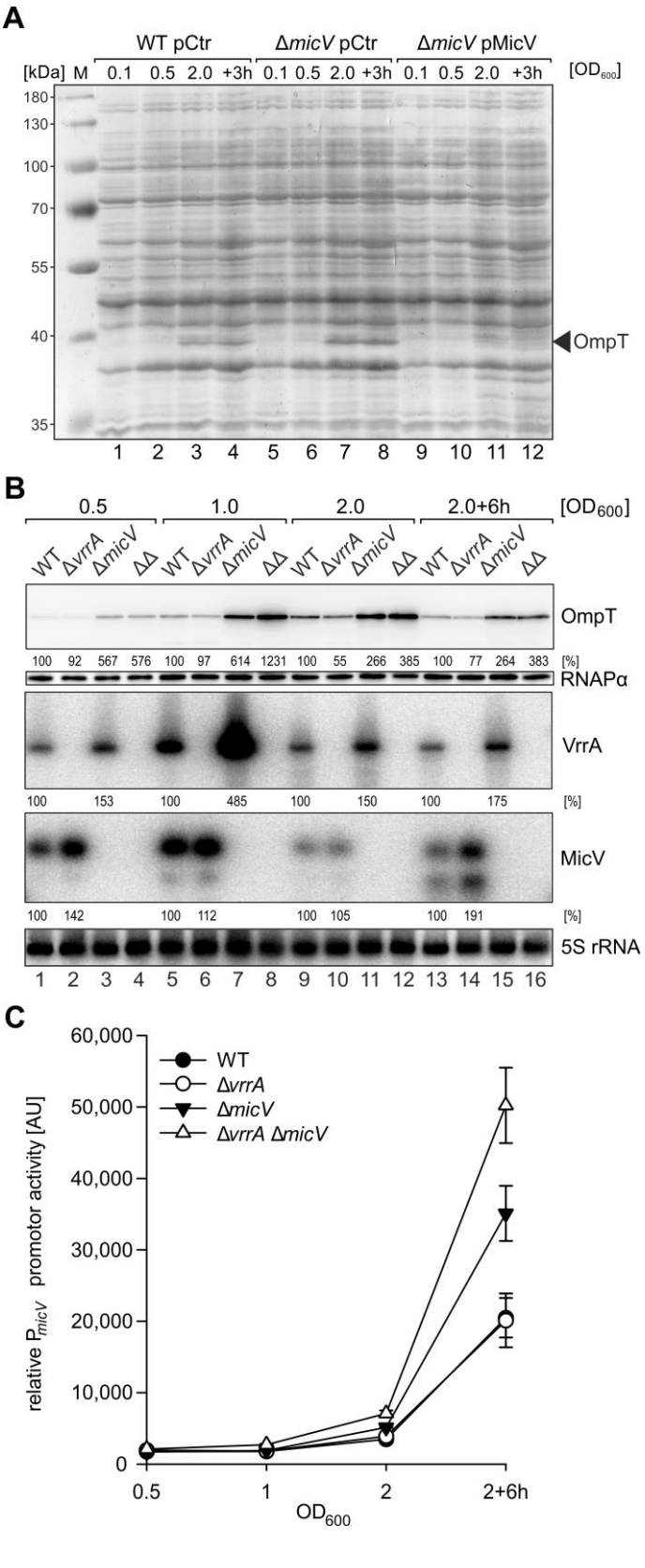
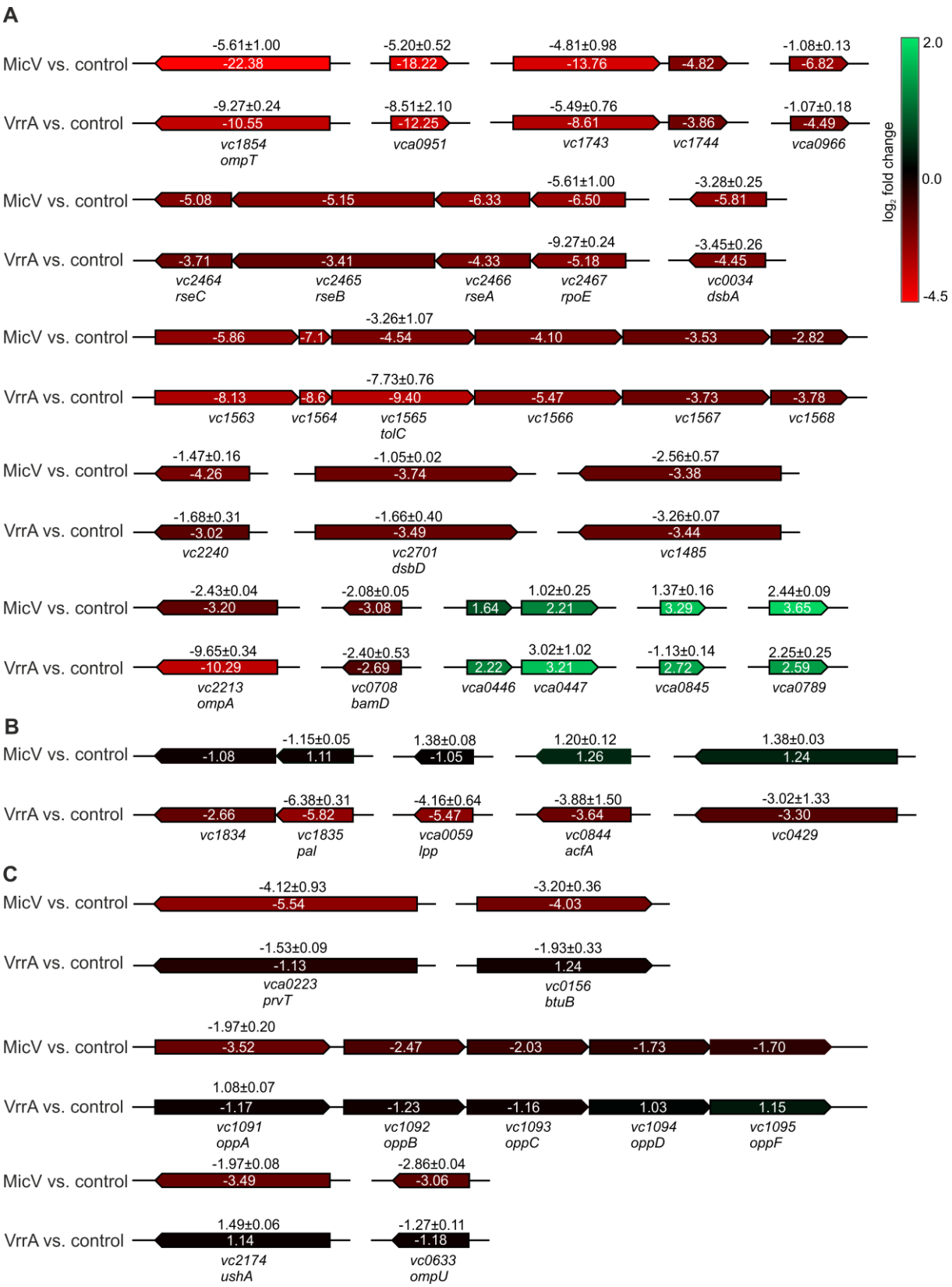

A
WT pCtr
ΔmicV pCtr
ΔmicV pMicV
[kDa] M 0.1 0.5 2.0 +3h 0.1 0.5 2.0 +3h 0.1 0.5 2.0 +3h [OD600]
180
130
100
70
55
40
35
OmpT
1 2 3 4 5 6 7 8 9 10 11 12
B
0.5
1.0
2.0
2.0+6h
[OD600]
WT ΔvrrA ΔmicV ΔΔ WT ΔvrrA ΔmicV ΔΔ WT ΔvrrA ΔmicV ΔΔ WT ΔvrrA ΔmicV ΔΔ
OmpT
100 92 567 576 100 97 614 1231 100 55 266 385 100 77 264 383 [%]
RNAPα
VrrA
100 153 100 485 100 150 100 175 [%]
MicV
100 142 100 112 100 105 100 191 [%]
5S rRNA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
C
relative PmicV promotor activity [AU]
60,000
50,000
40,000
30,000
20,000
10,000
0
WT
ΔvrrA
ΔmicV
ΔvrrA ΔmicV
0.5
1
2
2+6h
OD600

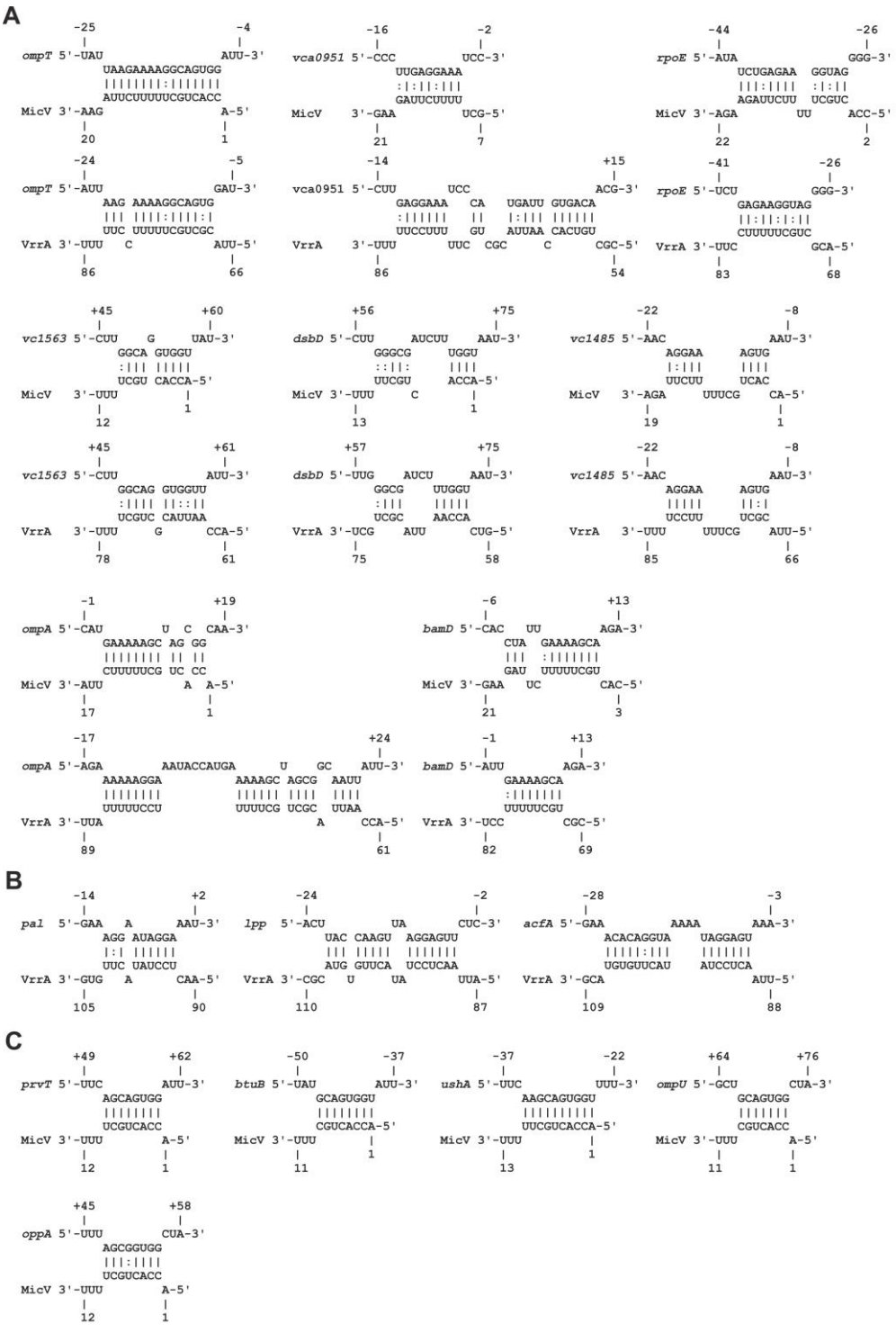
Figure Appendix S1: MicV controls OmpT production and OMP homeostasis

A) *V. cholerae* wild-type and Δ*micV* strains carrying empty vector control (pCtr) or *micV* expression plasmids (pMicV) were grown in LB. At the indicated time points, protein samples were collected and analyzed using SDS-PAGE and Coomassie staining. A molecular weight marker is provided on the left (M). Bands with different intensities were analyzed by mass spectrometry. **B)** *V. cholerae* wild-type, Δ*vrrA*, Δ*micV* or Δ*vrrA* Δ*micV* (ΔΔ) strains carrying the *ompT*::3XFLAG gene were grown in LB and at the indicated stages of growth, RNA and protein samples were collected. RNA samples were monitored for MicV and VrrA expression on Northern blots. Protein samples were investigated for OmpT::3xFLAG production using Western blot analysis. RNAPα served as a loading control for Western blots and 5S rRNA served as loading control for Northern blots. **C)** *V. cholerae* wild-type, Δ*vrrA*, Δ*micV* or Δ*vrrA* Δ*micV* strains carrying P*micV::mKate2* plasmids were cultivated in M9 minimal medium and at the indicated stages of growth, samples were collected and tested for mKate2 fluorescence. Data information: In (C), data are presented as mean ± SD, n = 3.


A
MicV vs. control
-5.61±1.00
-22.38
-5.20±0.52
-18.22
-4.81±0.98
-13.76
-4.82
-1.08±0.13
-6.82
VrrA vs. control
-9.27±0.24
-10.55
vc1854
ompT
-8.51±2.10
-12.25
vca0951
-5.49±0.76
-8.61
vc1743
-3.86
vc1744
-1.07±0.18
-4.49
vca0966
MicV vs. control
-5.08
-5.15
-6.33
-5.61±1.00
-6.50
-3.28±0.25
-5.81
VrrA vs. control
-3.71
vc2464
rseC
-3.41
vc2465
rseB
-4.33
vc2466
rseA
-9.27±0.24
-5.18
vc2467
rpoE
-3.45±0.26
-4.45
vc0034
dsbA
log2 fold change
2.0
0.0
-4.5
MicV vs. control
-3.26±1.07
-5.86
-7.1
-4.54
-4.10
-3.53
-2.82
VrrA vs. control
-7.73±0.76
-8.13
vc1563
-8.6
vc1564
-9.40
vc1565
tolC
-5.47
vc1566
-3.73
vc1567
-3.78
vc1568
MicV vs. control
-1.47±0.16
-4.26
-1.05±0.02
-3.74
-2.56±0.57
-3.38
VrrA vs. control
-1.68±0.31
-3.02
vc2240
-1.66±0.40
-3.49
vc2701
dsbD
-3.26±0.07
-3.44
vc1485
MicV vs. control
-2.43±0.04
-3.20
-2.08±0.05
-3.08
1.64
1.02±0.25
2.21
1.37±0.16
3.29
2.44±0.09
3.65
VrrA vs. control
-9.65±0.34
-10.29
vc2213
ompA
-2.40±0.53
-2.69
vc0708
bamD
2.22
vca0446
3.02±1.02
3.21
vca0447
-1.13±0.14
2.72
vca0845
2.25±0.25
2.59
vca0789
B
MicV vs. control
-1.08
-1.15±0.05
1.11
1.38±0.08
-1.05
1.20±0.12
1.26
1.38±0.03
1.24
VrrA vs. control
-2.66
vc1834
-6.38±0.31
-5.82
vc1835
pal
-4.16±0.64
-5.47
vca0059
lpp
-3.88±1.50
-3.64
vc0844
acfA
-3.02±1.33
-3.30
vc0429
C
MicV vs. control
-4.12±0.93
-5.54
-3.20±0.36
-4.03
VrrA vs. control
-1.53±0.09
-1.13
vca0223
prvT
-1.93±0.33
1.24
vc0156
btuB
MicV vs. control
-1.97±0.20
-3.52
-2.47
-2.03
-1.73
-1.70
VrrA vs. control
1.08±0.07
-1.17
vc1091
oppA
-1.23
vc1092
oppB
-1.16
vc1093
oppC
1.03
vc1094
oppD
1.15
vc1095
oppF
MicV vs. control
-1.97±0.08
-3.49
-2.86±0.04
-3.06
VrrA vs. control
1.49±0.06
1.14
vc2174
ushA
-1.27±0.11
-1.18
vc0633
ompU

Appendix Figure S2: MicV and VrrA mRNA target validation

A, B and C) *V. cholerae* Δ*vrrA* Δ*micV* strains carrying either an empty vector control (control), pBAD-micV (MicV) or pBAD-vrrA (VrrA) were grown in LB to OD_{600}=1.5. RNA samples were collected 10 min after induction with L-arabinose (0.2% final conc.) and analyzed for mRNA levels using RNA-seq or qRT-PCR. Targets determined by RNA-seq are depicted by arrows and are labelled with the fold change (white numbers). Arrows are colored according to the log_2 transformed fold change (right scalebar). For targets validated using qRT-PCR, the resulting fold-change is indicated above the tested mRNA target. The targets were grouped as follows: regulated by both sRNAs (A), regulated only by VrrA (B) or regulated only by MicV (C). Data information: In (A-C), qRT-PCR data are presented as mean ± SD, n = 2.



Appendix Figure S3: Base-pairing interactions of MicV and VrrA with target mRNAs

A, B and C) Prediction of base-pairing interactions of the MicV and VrrA sRNAs with their respective target mRNAs. The numbers indicate the position relative to the AUG start codon (mRNA) or the transcriptional start site (sRNA). The targets were grouped as follows: regulated by both sRNAs (A), regulated only by VrrA (B) or regulated only by MicV (C).

Appendix Table S1: Global identification of σ^E-dependent promoters in *V. cholerae*

Potential promotor sites in *V. cholerae* predicted with MEME on a motif search based on σ^E-dependent promotor sites that are found in maximal distance of 50 nt to a TSS (see Appendix Supplementary Methods). Entries marked as "orphans" are TSS which are not associated with any gene, i.e. are not located 300 nt or less upstream or downstream of an ORF (see Papenfort *et al.,* 2015, Figure 1B).

NC_002505						
Motif hit start	**Motif hit end**	**Motif hit seq**	**TSS position**	**TSS strand**	**TSS locus_tag**	**TSS gene product**
13737	13764	GAAATTTAGAGAAAAAAGGAAAGTCTAA	13729	-	orphan	orphan
134057	134084	TAAACGTTCTTCACAACTGGTAATCAAT	134017	-	VC0140	hypothetical protein
139822	139849	TGAACCTGTTTAAGTGATGGTGGTCATA	139855	+	VC0150	RNA polymerase factor sigma-32
142040	142067	CAAATTTATCGGCACTGTAGGTGTCGAT	142104	+	VC0151	soluble pyridine nucleotide transhydrogenase
163858	163885	GGAACTAGAGCAAAAAATGTTTGCCAAA	163852	-	VC0165	hypothetical protein
255476	255503	CGAACTCTATAAAATTTAGCTGTTCTGA	255435	-	VC0249*,#	RfbL protein
282410	282437	CGAACTCAACAATGCCGGTTTTATCTGA	282451	+	VC0276	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase%2FIMP cyclohydrolase
369659	369686	TGAACTTCGCTTTGAGAAGATGGTCGAA	369692	+	VC0346	tRNA delta%282%29-isopentenylpyrophosphate transferase
477347	477374	TGAATTTTTCTGGAGTGAAGCGGTCTGA	477308	-	VC0446*	organic solvent tolerance protein
491005	491032	GGCACTTTATCGGTCCACTACAGTCGAA	490998	-	VC0461	hypothetical protein
522911	522938	TGAATTTATTGGTTTTGGTTTTATCTGT	522955	+	orphan	orphan
523404	523431	GGAAATAAACTTGACTTGGCTGATCATA	523444	+	VC0490	hypothetical protein
530444	530471	TGAACTTATAGATAGTTGAACGGCCTAA	530436	-	VC0496	hypothetical protein
573241	573268	TGAACCGTTTGGCGCTTTGGATGCCAAA	573275	+	VC0541	sulfate ABC transporter ATP-binding protein
580501	580528	GGCACCATTGAGAGTTTACGATGTCATA	580551	+	VC0548	carbon storage regulator
586889	586916	CGAACTGATTCACAAAAACAAGGTCATA	586922	+	VC0554	insulinase family protease%2Finsulinase family protease
598379	598406	GGAACCTTTCAGAATCACACTCGTCTAA	598413	+	VC0565	protease DegS
609707	609734	GGAACTTCCACAGATGAAAATCGTCGAA	609700	-	VC0580	hypothetical protein
743157	743184	GAACATAAACACTGATTTTGTGGTCAAA	743191	+	VC0694	hypothetical protein
757821	757848	CGAACTTTTTAAAAAACCGTGAGACTAA	757815	-	VC0708*,#	hypothetical protein
772170	772197	TGAACCTTATATGAAAATTTGTTTGCAA	772227	+	VC0719	DNA-binding response regulator PhoB
803818	803845	CGAACTTTGCCCCGGCTCTGCAATCTGA	803811	-	VC0751	co-chaperone HscB
867987	868014	CGCATTTTCCGGGTTAATTGCTGCCAAA	868043	+	VC0812	helicase-like protein
916669	916696	GCAACCAAACTCAAAATTCACAGTCTCA	916662	-	VC0851*	small protein A
929880	929907	TGAATTAAATCGCTTTCCTGTTGTCAGA	929861	-	orphan	orphan
1063364	1063391	GAACCTTGAATATGTTGAGTCGATCAAA	1063426	+	VC0997	glutaminyl-tRNA synthetase
1114450	1114477	TGAACTCCTTCGCATAATTTCTGTCCTA	1114444	-	VC1045	RNA polymerase sigma factor
1158499	1158526	TGAATTAATTTGCCATAAAAATGTCTTA	1158493	-	orphan	orphan
1169175	1169202	TCAAATTTTTTTTGTTATATTTGTCCAT	1169156	-	VC1098	acetate kinase
1301806	1301833	GGAACTCCATCGAAAACTCGAAGTCTGA	1301799	-	orphan	orphan
1374686	1374713	CGAACATTTTTTGAGTGGTCGTATCAGA	1374748	+	orphan	orphan
1469405	1469432	TCCACTTCTTCCTTATTATGTTATCTAT	1469364	-	VC1376	GGDEF family protein
1591531	1591558	GGAACTTTTGGAAGAATTGCTTGCCAAT	1591525	-	VC1486	ABC transporter ATPase
1591564	1591591	TGAACCAACCAACGATTTAGATATCGAA	1591525	-	VC1486	ABC transporter ATPase
1602295	1602322	GAAATGTTACTGAACAGGTGTTGTCCAA	1602328	+	VC1492	hypothetical protein
1617120	1617147	AAAACCTGTCGCTAATTTCAGTATCTGT	1617163	+	orphan	orphan
1675554	1675581	TGAACTTTCCTTATCATCCTTAGTCTGA	1675587	+	VC1563	pseudo

1744512	1744539	GGAACATCACGCCATTAATCGAATCGAA	1744545	+	VC1623	carboxynorspermidine decarboxylase
1856343	1856370	GGAACTTTTTGCGTGTCCAGTTGACTGA	1856377	+	VC1718	hypothetical protein
1878650	1878677	GGAACTCTTTGCCAAACGCCCAGTCTGA	1878684	+	orphan	***vrrA***
1903913	1903940	TGCACTAATCAGCATATTGTTTATCTGA	1903967	+	VC1764	hypothetical protein
1937499	1937526	CAAACTATTAGCTGTAGTGGTCAACTAA	1937568	+	VC1788	hypothetical protein
2049280	2049307	TAAACTTTCGTTAAAAAACGCGATCTAA	2049274	-	orphan	orphan
2068844	2068871	CGAACCTTTTGAAATTATGCGCATCCTA	2068808	-	VC1918	peptidyl-prolyl cis-trans isomerse D
2093516	2093543	CAAACGTTTGCCTGTGGATGTTATCAAA	2093565	+	VC1942	bifunctional 5%2C10-methylene-tetrahydrofolate dehydrogenase%2F 5%2C10-methylene-tetrahydrofolate cyclohydrolase
2111173	2111200	GAACAGTATGCGCAATTTGGTTGTCAGA	2111167	-	VC1957	hypothetical protein
2140363	2140390	GGAACTTGCGCAGCTACTTGGGGTCGAT	2140356	-	VC1987*,#	outer membrane lipoprotein Slp
2164623	2164650	TGACTTTATCGAGGATTATGGTGTCTGA	2164617	-	orphan	orphan
2196838	2196865	GCAACCAAAGCTGGAATTCACTGTCTGA	2196871	+	VC2040	hypothetical protein
2248788	2248815	GGAATTTCGCACCAAGATAGCGCTCTAA	2248822	+	VC2087	2-oxoglutarate dehydrogenase E1
2302696	2302723	TAAATCGATTGGCAAGTTATTGATCAAA	2302657	-	VC2149	hypothetical protein
2306486	2306513	GGAACCAGCACGCCCAATCGTTGCCCAA	2306480	-	VC2156*	lipoprotein
2524108	2524135	GGAACCCTGAGAGTATTCGCTTGTCAGA	2524101	-	VC2366	ribonuclease activity regulator protein RraA
2613708	2613735	TGAATTTTTAGCGCAATATCTGGTCTTA	2613701	-	VC2437	pseudo
2649846	2649873	TGAACTTTCTCGATAATGCCGAGTCTCT	2649839	-	VC2467*,#	RNA polymerase sigma factor RpoE
2654232	2654259	CACACTATTTTTGTTTAGGTTTTTCTAT	2654270	+	VC2473	hypothetical protein
2709569	2709596	GGAACCTTCACTGCTGGAGATTGCCAAA	2709603	+	VC2524	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase
2812169	2812196	TGAACCTTTTGCTTAGAGCTCTGTCTAT	2812162	-	VC2640	***micV***
2908373	2908400	GGAACTCATTGCCACATTGCCTCTCTAA	2908365	-	VC2734	general secretion pathway protein C

NC_002506

Motif hit start	Motif hit end	Motif hit seq	TSS position	TSS strand	TSS locus_tag	TSS gene product
35140	35167	GGCACTTTCTGCTCCTGCATCAGTCAAA	35174	+	VCA0027	chitinase
67115	67142	CAAATTTTTTCCAGACAAATTTGTCACT	67182	+	VCA0061	DEAD%2FDEAH box helicase
92487	92514	TGAACAAGCTGTCATTCTCTCTATCAAA	92447	-	VCA0080	diguanylate cyclase
214685	214712	GAAACTCATTGACAAAACGAACATCAAA	214667	-	VCA0198	site-specific DNA-methyltransferase
357164	357191	CAAACTATTAGCTGTAGTGGTCAACTAA	357233	+	VCA0370	hypothetical protein
400386	400413	GGCAATTTAATGTCAAAAATTTATCAAA	400463	+	VCA0447	hemagglutinin associated protein
424133	424160	TAAACAAGAAGTCGATGAAGTTGTCGAA	424183	+	VCA0485	MazG domain-containing protein
510200	510227	GAAACTTCACATTGAATGAACTATCATA	510193	-	VCA0572	D-alanyl-alanine synthetase A
513961	513988	CAACCTTATATTGATAAAGGTGAACTAA	513940	-	VCA0575	LysR family transcriptional regulator
524874	524901	GAAACTCAAAGCCTATTTGAGAATCCAA	524866	-	VCA0588	peptide ABC transporter ATP-binding protein
921498	921525	TGCCATTTTCGCTGAAAAACTTGTCCAT	921452	-	VCA0974	methyl-accepting chemotaxis protein
929810	929837	GGAATCCAAAGCCATTTGCTTAGTCCAT	929863	+	VCA0981	hypothetical protein
949359	949386	TAAAATTTATCGATTGAAATTCATCAAA	949317	-	VCA0994	hypothetical protein
957852	957879	TAAACTAACCGCTGATAAACTACTCAGA	957911	+	VCA1004	hypothetical protein

*Listed as σ^E-dependent in *E. coli* K12, according to Ecocyc database (https://ecocyc.org/)

#Listed as σ^E-dependent in *E. coli* K12 (Rhodius *et al.*, 2006)

Appendix Table S2: Genes differentially regulated by either *micV* or *vrrA* pulse expression

Gene	Description[#]	Fold change* *micV* pulse	Fold change* *vrrA* pulse
ompT	outer membrane protein OmpT	-22.38	-10.55
vca0951	hypothetical protein	-18.22	-12.25
vc1743	hypothetical protein	-13.76	-8.61
vca0966	hypothetical protein	-6.82	-4.49
rpoE	RNA polymerase sigma factor RpoE	-6.50	-5.18
rseA	sigma-E factor negative regulatory protein RseA	-6.33	-4.33
vc1563	pseudogene	-5.86	-8.13
dsbA	thiol:disulfide interchange protein DsbA	-5.81	-4.45
rseB	sigma-E factor negative regulatory protein RseB	-5.15	-3.41
rseC	sigma-E factor negative regulatory protein RseC	-5.08	-3.71
vc1744	hypothetical protein	-4.82	-3.86
tolC	outer membrane protein TolC	-4.54	-9.40
vc2240	phenolic acid decarboxylase	-4.26	-3.02
vc1566	putative ABC transport system permease	-4.10	-5.47
dsbD	thiol:disulfide interchange protein DsbD	-3.74	-3.49
vc1567	putative ABC transport system permease	-3.53	-3.73
vc1485	hypothetical protein	-3.38	-3.44
ompA	outer membrane protein OmpA	-3.20	-10.29
bamD	outer membrane protein assembly factor BamD	-3.08	-2.69
vc1568	ABC transporter ATP-binding protein	-2.82	-3.78
vca0447	site-specific DNA-methyltransferase	2.21	3.21
vca0845	hypothetical protein	3.29	2.72
vca0789	putative membrane protein	3.65	2.59
pal	peptidoglycan-associated lipoprotein	1.11	-5.82
lpp	major outer membrane lipoprotein	-1.05	-5.47
acfA	accessory colonization factor AcfA	1.26	-3.64
vc0429	hypothetical protein	1.24	-3.30
prtV	immune inhibitor A, protease	-5.54	-1.31
btuB	vitamin B12 transporter	-4.03	1.24
oppA	oligopeptide transport substrate-bind. protein	-3.52	-1.17
ushA	5'-nucleotidase / UDP-sugar diphosphatase	-3.49	1.14
ompU	outer membrane protein OmpU	-3.06	-1.18

[#]Description is based on the annotation at KEGG (https://www.genome.jp/kegg)

*Fold change is based on transcriptomic analysis of pBAD-derived *micV* or *vrrA* expression using RNA-seq. Genes with a fold-change of at least 3.0-fold in either condition and a FDR adjusted p-value ≤ 1E-8 were considered to be differentially expressed.

Appendix Table S3: Bacterial strains used in this study

Strain	Relevant markers/ genotype	Reference/ source
V. cholerae		
KPS-0014	C6706 Wild-type	(Thelin & Taylor, 1996)
KPS-0054	C6706 Δ*hfq*	(Svenningsen et al., 2009)
KPS-0995	C6706 *hfq::hfq*-3XFlag	This study
KPVC-10072	C6706 Δ*vrrA*	This study
KPVC-10075	C6706 Δ*micV* Δ*vrrA*	This study
KPVC-10076	C6706 Δ*micV*	This study
KPVC-10122	C6706 *ompT::ompT-3xFLAG*	This study
KPVC-10124	C6706 Δ*micV ompT::ompT-3xFLAG*	This study
KPVC-10137	C6706 Δ*vrrA ompT::ompT-3xFLAG*	This study
KPVC-10139	C6706 Δ*micV* Δ*vrrA ompT::ompT-3xFLAG*	This study
KPVC-10814	C6706 Δ*vchM*	This study
KPVC-10822	C6706 Δ*vchM* Δ*rpoE*	This study
KPVC-10824	C6706 Δ*vchM* Δ*rpoE* Δ*vrrA*	This study
KPVC-10826	C6706 Δ*vchM* Δ*rpoE* Δ*micV*	This study
KPVC-10828	C6706 Δ*vchM* Δ*rpoE* Δ*vrrA* Δ*micV*	This study
KPVC-12139	C6706 Δ*ompA*	This study
KPVC-12143	C6706 Δ*vchM* Δ*rpoE* Δ*ompA*	This study
KPVC-12203	C6706 Δ*micV* Δ*vrrA ompA::ompA-3xFlag*	This study
KPVC-12647	C6706 *ompA::ompA* scr	This study
KPVC-12651	C6706 Δ*vchM* Δ*rpoE ompA::ompA* scr	This study
E. coli		
BW25113	*lacI*$^{+}$*rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR*514 Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$ *rph-1* Δ*(araB–D)567* Δ*(rhaD–B)568* Δ*lacZ4787*(::*rrnB-3*) *hsdR514 rph-1*	(Datsenko & Wanner, 2000)
TOP10	F- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*ΔM15 Δ*lacX*74 *nupG recA*1 *araD*139 Δ(*ara-leu*)7697 *galE*15 *galK*16 *rpsL*(Str^{R}) *endA1* λ^{-}	Invitrogen
S17λpir	Δ*lacU*169 (Φ*lacZ*ΔM15), *recA*1, *endA*1, *hsdR*17, *thi*-1, *gyrA*96, *relA*1, *λpir*	(Simon et al., 1983)
ECA101	*E.coli* BW25113 Δ*rpoE*	(Egler et al., 2005)
KPEC-52214	BW25113 *ompA::kan*^{R}	(Baba et al., 2006)
KPEC-52215	BW25113 *ompC::kan*^{R}	(Baba et al., 2006)

Appendix Table S4: Plasmids used in this study

Plasmid trivial name	Plasmid stock name-	Relevant fragment	Comment	Origin, marker	Reference
pBAD1K-Ctr	pMD004		Control plasmid	p15A, Kan^R	Papenfort lab plasmid collection
pBAD1K-*micV*	pNP016	P_{BAD}-*micV*	*micV* expression plasmid	p15A, Kan^R	This study
pBAD1K-rpoE	pNP018	P_{BAD}-*rpoE*	*rpoE* expression plasmid	p15A, Kan^R	This study
pBAD1K-vrrA	pNP022	P_{BAD}-*vrrA*	*vrrA* expression plasmid	p15A, Kan^R	This study
pBAD5A	pKP8-35	empty	Control plasmid	pBR322, Amp^R	(Papenfort et al., 2006)
pBAD5A-*rpoE* (*E.c.)*	pKP142-2	*rpoE* (*E.coli*)	*rpoE* expression plasmid	pBR322, Amp^R	(Papenfort et al., 2010)
pBAD5A-*rpoE* (*V.c.*)	pRH011	*rpoE* (*V.cholerae*)	*rpoE* expression plasmid	pBR322, Amp^R	This study
pCMW-1C	pCtr		Promotorless plasmid for transcriptional reporters	p15A, Cm^R	(Herzog et al., 2019)
pCMW-1C-*mKATE2*	pYH-010	*mKATE2*	Promoterless plasmid for transcriptional reporters	p15A, Cm^R	(Herzog et al., 2019)
pCMW-1C-P*micV*::*mKate2*	pNP074	P*micV*::*mKATE2*	Transcriptional reporter P*micV::mKATE2*	p15A, Cm^R	This study
pCMW-1C-P*vrrA*::*mKate2*	pNP075	P*vrrA*::*mKATE2*	Transcriptional reporter P*vrrA::mKATE2*	p15A, Cm^R	This study
pCMW-1K	pCtr		Control plasmid	p15A, Kan^R	Papenfort lab plasmid collection
pCMW-1K-P*micV*::*gfp*	pNP017	P*micV::gfp*	Transcriptional reporter P*micV::gfp*	p15A, Kan^R	This study
pEVS143-1K		P*tac* promoter	Constitutive over-expression plasmid	p15A, Kan^R	(Dunn et al., 2006)
pEVS143-1C		P*tac* promoter	Constitutive over-expression plasmid	p15A, Cm^R	Papenfort lab plasmid collection
pEVS-*micV*	pNP002	*micV*	*micV* expression plasmid	p15A, kan^R	This study
pEVS-*micV* M1	pRG001	*micV* M1	*micV* M1 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*rybB*	pMD030	*rybB*	*rybB* expression plasmid	p15A, Cm^R	This study
pEVS-P_L-*rybB*	pMD251	*rybB*	*rybB* expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*rybB*Δ9	pNP088	*rybB*Δ9	*rybB*Δ9 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.01	pMD241	*sRNA* var.1	*sRNA* var.1 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.02	pMD242	*sRNA* var.2	*sRNA* var.2 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.03	pMD243	*sRNA* var.3	*sRNA* var.3 expression plasmid	p15A, Kan^R	This study

pEVS-P_L-*sRNA* var.04	pMD244	*sRNA* var.4	*sRNA* var.4 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.05	pMD245	*sRNA* var.5	*sRNA* var.5 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.06	pMD246	*sRNA* var.6	*sRNA* var.6 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.07	pMD247	*sRNA* var.7	*sRNA* var.7 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.08	pMD248	*sRNA* var.8	*sRNA* var.8 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.09	pMD249	*sRNA* var.9	*sRNA* var.9 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.10	pMD250	*sRNA* var.10	*sRNA* var.10 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.11	pMD251	*sRNA* var.11	*sRNA* var.11 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.12	pMD252	*sRNA* var.12	*sRNA* var.12 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.13	pMD253	*sRNA* var.13	*sRNA* var.13 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.14	pMD254	*sRNA* var.14	*sRNA* var.14 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* var.15	pMD255	*sRNA* var.15	*sRNA* var.15 expression plasmid	p15A, Kan^R	This study
pEVS-P_L-*sRNA* library		*sRNA* 9nt variants	*sRNA* library expression plasmid	p15A, Cm^R	This study
pEVS-*rybB*	pRH013	*rybB*	*rybB* expression plasmid	p15A, Kan^R	This study
pEVS-*vrrA*	pRH001	*vrrA*	*vrrA* expression plasmid	p15A, kan^R	This study
pEVS-*vrrA* M1	pRG002	*vrrA* M1	*vrrA* M1 expression plasmid	p15A, Kan^R	This study
pEVS-*vrrA* M2	pRG004	*vrrA* M2	*vrrA* M2 expression plasmid	p15A, Kan^R	This study
pKAS32	pKAS32		suicide plasmid for allelic exchange	R6K, Amp^R	(Skorupski & Taylor, 1996)
pKAS32-*hfq*::3xFLAG	pKP431	*hfq:3xFLAG:*	*hfq::3xFLAG* allelic replacement	R6K, Amp^R	This study
pKAS32-*ompA::3xFLAG*	pNP089	*ompA::3xFLAG*	*ompA::3xFLAG* allelic replacement	R6K, Amp^R	This study
pKAS32-*ompA*	pNP090	*ompA*	*ompA* region	R6K, Amp^R	This study
pKAS32-*ompA* scr	pNP091	*ompA* scr	*ompA* scr allelic replacement	R6K, Amp^R	This study
pKAS32-*ompT::3xFLAG*	pNP021	*ompT::3xFLAG*	*ompT::3xFLAG* allelic replacement	R6K, Amp^R	This study
pKAS32-Δ*micV*	pNP024	up-/downstream flanks of *micV*	suicide plasmid for *micV* knock-out	R6K, Amp^R	This study
pKAS32-Δ*ompA*	pEE001	up-/downstream flanks of *ompA*	suicide plasmid for *ompA* knock-out	R6K, Amp^R	This study

pKAS32-Δ*rpoE*	pNP023	up-/downstream flanks of *rpoE*	suicide plasmid for *rpoE* knock-out	R6K, Amp^R	This study
pKAS32-Δ*vchM*	pNP076	up-/downstream flanks of *vchM*	suicide plasmid for *vchM* knock-out	R6K, Amp^R	This study
pKAS32-Δ*vrrA*	pNP026	up-/downstream flanks of *vrrA*	suicide plasmid for *vrrA* knock-out	R6K, Amp^R	This study
P_L-*rybB*	pFM1-1	P_L-*rybB*	*rybB* expression plasmid	ColE1, Amp^R	(Bouvier et al., 2008)
pXG10-1C	pXG10-1C	'*lacZ::gfp*	template plasmid for translational reporters	pSC101*, Cm^R	Papenfort lab plasmid collection
pXG10-1C-*acfA::gfp*	pNP059	*acfA::gfp*	Translational reporter *acfA::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*bamD::gfp*	pNP082	*bamD::gfp*	Translational reporter *bamD::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*btuB::gfp*	pNP029	*btuB::gfp*	Translational reporter *btuB::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*dsbD::gfp*	pNP079	*dsbD::gfp*	Translational reporter *dsbD::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*lpp* M2	pNP087	*lpp* M2**::gfp*	Translational reporter *lpp* M2**::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*lpp::gfp*	pNP086	*lpp::gfp*	Translational reporter *lpp::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*ompA::gfp*	pNP081	*ompA::gfp*	Translational reporter *ompA::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*ompT* M1	pRG009	*ompT* M1**::gfp*	Translational reporter *ompT* M1**::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*ompT::gfp*	pKP465	*ompT::gfp*	Translational reporter *ompT::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*ompU::gfp*	pNP085	*ompU::gfp*	Translational reporter *ompU::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*oppA::gfp*	pNP084	*oppA::gfp*	Translational reporter *oppA::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*pal::gfp*	pNP062	*pal::gfp*	Translational reporter *pal::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*prvT::gfp*	pNP083	*prvT::gfp*	Translational reporter *prvT::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*rpoE::gfp*	pNP044	*rpoE::gfp*	Translational reporter *rpoE::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*ushA* M1	pRG008	*ushA* M1**::gfp*	Translational reporter *ushA* M1**::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*ushA::gfp*	pNP054	*ushA::gfp*	Translational reporter *ushA::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*vc1485::gfp*	pNP080	*vc1485::gfp*	Translational reporter *vc1485::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*vc1563::gfp*	pNP078	*vc1563::gfp*	Translational reporter *vc1563::gfp*	pSC101*, Cm^R	This study
pXG10-1C-*vca0951::gfp*	pNP077	*vca0951::gfp*	Translational reporter *vca0951::gfp*	pSC101*, Cm^R	This study
pXG10-SF	pXG10SF	'*lacZ::gfp*	template plasmid for translational reporters	PSC101*, Cm^R	(Corcoran et al., 2012)

Appendix Table S5: DNA oligonucleotides used in this study

ID	Sequence (5'→3'); P denotes a monophosphate	Description
KPO-0012	GCAGATCGAACTGGAAGCT	pKP431
KPO-0019	CGAGATTATCGATCTTATTCA	pKP431
KPO-0066	TATCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCCTCTTCAGACTTC TCTGCTGG	pKP431
KPO-0067	GATCATGATATCGACTACAAAGATGACGATAAATAGTTCTTTGCACAATTATT TAAGGAG	pKP431
KPO-0092	CCACACATTATACGAGCCGA	pNP002, pRH001/013
KPO-0148	GTTTTTGGTACCATCCCAATGATCCACAAAGA	pKP431
KPO-0149	GTTTTTCCTAGGAAACAGTCTCTACCGCTTGG	pKP431
KPO-0196	GGAGAAACAGTAGAGAGTTGCG	pNP016/018/022
KPO-0236	GGGCGTACACAAGTATAGGAGT	VrrA oligoprobe
KPO-0243	TTCGTTTCACTTCTGAGTTCGG	5S rRNA oligoprobe
KPO-0267	TAATAGGCCTAGGATGCATATG	pNP026/076/089, pEE01
KPO-0268	CGTTAACAACCGGTACCTCTA	pNP026/076/089, pEE01
KPO-0282	CACTGACACCCTCATCAGTG	pMD241-255
KPO-0640	P-TTTTACCGCGACACCGTGGC	pNP16
KPO-0820	GGCCTTCTTAGAGTCTTCTAAGAA	MicV oligoprobe
KPO-0999	P-ACCACTGCTTTTTCTTAGAAGAC	pNP002/016
KPO-1000	GTTTTTTCTAGAGGATTAGAACCCGAATTAAACT	pNP002
KPO-1023	GTTTTTTCTAGAGGATCCGGTGATTGATTGAG	pRH001/013, pNP002
KPO-1064	GTTTTTTATGCATGAATCTAATGGCGGTGGTG	pKP465
KPO-1065	GTTTTTTGCTAGCAGCTGCGTTTACAGAGCCT	pKP465
KPO-1082	P-GTGATTGACAGAGCTTTGAGA	pRH001
KPO-1083	GTTTTTTCTAGATCGCCAATGAACCGACTTG	pRH001
KPO-1180	GCTTATTTGGAGATGTTTGAGC	pNP024
KPO-1181	AGAGCTCTAAGCAAAAGGTTCAT	pNP024
KPO-1182	AACCTTTTGCTTAGAGCTCTTGCGTAGCAGAAAGTTTAATTCG	pNP024
KPO-1183	ACCAAATCCCGCTGCTGCAT	pNP024
KPO-1184	GTTTTTGGTACCCTAGCGCGTTTAAACACCTCA	pNP024
KPO-1185	GTTTTTCCTAGGAGATCAAGGACGCATTGCCG	pNP024
KPO-1186	TCAATCGTAAAAGGCTCGACAC	pNP023
KPO-1187	GAAGGTAGGGGAATAACAATATTCCGTAATGACTATGGTGAATAG	pNP023
KPO-1188	ATTGTTATTCCCCTACCTTCTC	pNP023
KPO-1189	TTATCTTCAGTGATCAAATCCAGC	pNP023
KPO-1214	GTTTTTGGTACCTTCATCACCACGGCGGATC	pNP023
KPO-1215	GTTTTTCCTAGGTTATCTTGCAAGGACGTCTGC	pNP023
KPO-1235	GTTTTTTGTCGACTGCTCTTCAGCAAGCTCAAGC	pNP017/074
KPO-1236	GTTTTTTGCATGCGTGGTACAGTAATAGACAGAG	pNP017/074
KPO-1237	GTTTTTGGTACCGGATTAGAACCCGAATTAAACT	pNP016
KPO-1324	AGAGGTACCGGTTGTTAACGGATAATGGTGCAGCTTGGTG	pNP026
KPO-1325	ATGAACCGACTTGAACTATTCAGACTGGGCGTTTG	pNP026
KPO-1326	GTTCAAGTCGGTTCATTGG	pNP026
KPO-1327	TATGCATCCTAGGCCTATTAGGTGTAGATAAAGCAAGTTTC	pNP026
KPO-1397	GATCCGGTGATTGATTGAGC	pNP018/022
KPO-1398	CGCAACTCTCTACTGTTTCTCCTAGGGGAATAACAATAGGAGTG	pNP018
KPO-1399	GCTCAATCAATCACCGGATCACCATAGTCATTACGGAATTTGC	pNP018

KPO-1409	TCGTATAATGTGTGGGCCACTGCTTTTCTTTGATGTC	pRH013
KPO-1410	ACCGGATCCTCTAGAGGTTGAGAGGGTTGCAGGG	pRH013
KPO-1417	TAGAGGTACCGGTTGTTAACGCAAAGAGTTGGAAAACCACCTTC	pNP021
KPO-1418	CCAGTAGATACGAGCACCGA	pNP021
KPO-1419	GATCTCGAACACGTTTATTGAG	pNP021
KPO-1420	CATATGCATCCTAGGCCTATTAGAAGAGCGCTCTCGATTTC	pNP021
KPO-1421	TCGGTGCTCGTATCTACTGGGACTACAAAGACCATGACGGTG	pNP021
KPO-1422	CTCAATAAACGTGTTCGAGATCTTACTATTTATCGTCATCTTTGTAGTC	pNP021
KPO-1423	TCTAGATTAAATCAGAACGCAGAAG	pRH011
KPO-1424	GGAGAAACAGTAGAGAGTTGC	pRH011
KPO-1425	GCAACTCTCTACTGTTTCTCCTAGGGGAATAACAATAGGAGTG	pRH011
KPO-1426	GCGTTCTGATTTAATCTAGAACCATAGTCATTACGGAATTTGC	pRH011
KPO-1478	CGCAACTCTCTACTGTTTCTCCGTGATTGACAGAGCTTTGAGA	pNP022
KPO-1479	GCTCAATCAATCACCGGATCTCGCCAATGAACCGACTTG	pNP022
KPO-1491	CTTTCGTCTTCACCTCGAGAATTGTGAGCGGATAACAATTGAC	synthetic sRNA library
KPO-1492	GATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCG	synthetic sRNA library
KPO-1505	GTTTTTTTAATACGACTCACTATAGGGAGGGCACTGCGAGTGCTAATAGAG	*ompT* riboprobe
KPO-1506	GGTGACCAAACAAAGAGTTGG	*ompT* riboprobe
KPO-1525	GCGGCCCTCTCACTTCC	pMD241-255
KPO-1529	GGAAGTGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAG	pMD241-255
KPO-1660	GTTTTTATGCATATGACCTATACCGTCCGC	pMD241-255
KPO-1681	GTTTTTATGCATGTTATGCAGTGGTATTGCAC	pNP029
KPO-1682	GTTTTTGCTAGCGGTAAGCAGCGATGCTAGA	pNP029
KPO-1683	GTTTTTATGCATAAGTTTTATCCGCACTCCAAG	pNP054
KPO-1684	GTTTTTGCTAGCAATGGCTGCACTGAGGAC	pNP054
KPO-1702	ATGCATGTGCTCAGTATCTCTATC	pNP081/084/085
KPO-1703	GCTAGCGGATCCGCTGG	pNP081/084/085
KPO-1704	GAGATACTGAGCACATGCATACGAAAATGGCTGAGCCATC	pNP085
KPO-1712	GAGATACTGAGCACATGCATATGATTGCTAATGTGTGCCGCA	pNP084
KPO-1716	GAGATACTGAGCACATGCATGCGGTGAAACCAAGCGTTTAAC	pNP062
KPO-1717	GAGCCAGCGGATCCGCTAGCTGGTAGCGCAATCAGCAGAC	pNP062
KPO-1718	GAGATACTGAGCACATGCATAATAAAATGTGAAACACAGGTAAAAATAG	pNP059
KPO-1719	GAGCCAGCGGATCCGCTAGCCGGTGCTGCATTTGCTGATAAAG	pNP059
KPO-1826	GAGCCAGCGGATCCGCTAGCAGCAAAAAGTAACGTCGCTGAA	pNP081
KPO-1831	GAGATACTGAGCACATGCATGACAAAAAGGTGATCTGGCTC	pNP081
KPO-1840	GTTTTTATGCATGCTCATGCAAGTAGTGGTGTC	pNP044
KPO-1841	GTTTTTGCTAGCCTGAACTCGCTCAATCAACAC	pNP044
KPO-1846	GTGTGTATGGAAGGCCCTAATC	*ushA* qRT-PCR
KPO-1847	CACTCGTAAGCTTGAACAATGTAAG	*ushA* qRT-PCR
KPO-1850	TGCCGGAGAGAAAGACAAATC	*oppA* qRT-PCR
KPO-1851	ACCCATCATCATCACGAAGTAAG	*oppA* qRT-PCR
KPO-1852	CTGAGCAAGAACTGAAAGAACAAG	*pal* qRT-PCR
KPO-1853	AGCTAGCATTGCTTCGTAGTC	*pal* qRT-PCR
KPO-2193	AGAGGTACCGGTTGTTAACGCACTGTCTGATGAACTGATCTTC	pNP076
KPO-2194	TGAAGCATGTAAAAAGGGAGTTAACTGTATCACCATACTACCTC	pNP076
KPO-2195	CTCCCTTTTTACATGCTTCACAG	pNP076
KPO-2196	TATGCATCCTAGGCCTATTACGATCTTTGCGTTGATATTCAGG	pNP076
KPO-2297	GTCTGATGCACTACACGATTCT	*ompT* qRT-PCR
KPO-2298	GCTAGCTCTTGCTTTGCATTATC	*ompT* qRT-PCR

KPO-2311	GACCACTCGTTTTTCTTAGAAGACTCTAAGAAGG	pRG001
KPO-2312	TCTAAGAAAAACGAGTGGTCCCACACATTATACG	pRG001
KPO-2313	CAATTACGCTCGTTTTTCCTTTTTATTAACTCCTATAG	pRG002
KPO-2314	AGGAAAAACGAGCGTAATTGGTGACAGCG	pRG002
KPO-2315	CTATAGAAGTGTACGCCCAAAGCCAGATTG	pRG004
KPO-2316	CTTTGGGCGTACACTTCTATAGGAGTTAATAAAAAG	pRG004
KPO-2378	GGTAACCCAGAAACTACCACTG	*recA* qRT-PCR
KPO-2379	CACCACTTCTTCGCCTTCTT	*recA* qRT-PCR
KPO-2418	CAACGAGTGGTTTTCATCAGTTCAAAGGTATGAC	pRG008
KPO-2419	GAACTGATGAAAACCACTCGTTGAAATGTCGTTG	pRG008
KPO-2426	CCATATTAAGAAAAGCGAGTGGATTAAC	pRG009
KPO-2427	CACTCGCTTTTCTTAATATGGGAATTCC	pRG009
KPO-2503	GTTTTTGCATGCCAAGCGATTAACATCACATTTTCTCG	pNP075
KPO-2504	GTTTTTGTCGACGTCTATTCAGACTGGGCGTTTG	pNP075
KPO-3328	GATGCGGTTGATTGGCTTAAA	*vca0447* qRT-PCR
KPO-3329	CCGTGTAGTCGTACCTATTTGTC	*vca0447* qRT-PCR
KPO-3330	TTCAGGGTAAGGTGGCTTTG	*vca0845* qRT-PCR
KPO-3331	GCGAGCAGCAGACTAAAGAT	*vca0845* qRT-PCR
KPO-3332	GACCGCCTATGTCTTGATGTT	*vca0789* qRT-PCR
KPO-3333	GTGTAGAGCCGATCAAGGTATT	*vca0789* qRT-PCR
KPO-3334	CAACAACGCATGCCCAATAC	*vc1743* qRT-PCR
KPO-3335	GGAGCCATTCGAGCATTTCTA	*vc1743* qRT-PCR
KPO-3336	CCAAGCAAAGATCTGACCAAAG	*vca0966* qRT-PCR
KPO-3337	CGCGTATTTCTTCACGCTTATG	*vca0966* qRT-PCR
KPO-3338	GAAGCCATTCTTGGTGCTAAC	*vca0951* qRT-PCR
KPO-3339	TCTCGTTCATAAGTGCCAGAG	*vca0951* qRT-PCR
KPO-3340	GTTTTTATGCATGTTTTTTTGAACTTTCCTTATCATCC	pNP078
KPO-3341	GTTTTTGCTAGCTGAAGACTCAGGGGTATAAGTG	pNP078
KPO-3346	GTTTTTATGCATTACTATCACCGGTAAATGATTAATC	pNP079
KPO-3347	GTTTTTGCTAGCGTTATTGCCAGCGTTATTACCAA	pNP079
KPO-3348	GTTTTTATGCATGAACTTGAAGCTCTTCCGCAA	pNP080
KPO-3349	GTTTTTGCTAGCCATCACTTGGTAGAGTGCCG	pNP080
KPO-3350	GTTTTTATGCATACATCAAAAACATCCCTTGAGGAA	pNP077
KPO-3351	GTTTTTGCTAGCGTTAGCACCAAGAATGGCTTC	pNP077
KPO-3360	GTTTTTATGCATACTAGTATGGAAAAATACGCCGAC	pNP082
KPO-3361	GTTTTTGCTAGCGCAACCAAATAACAGGGATAACG	pNP082
KPO-3362	GTTTTTATGCATAAATACTTTACATATGGATATGTACTATG	pNP083
KPO-3363	GTTTTTGCTAGCGCCTAAATCAATGGGTGTTTGAG	pNP083
KPO-3364	GTTTTTATGCATGTCCATATTTTAATTTTCGATAAGTATAG	pNP086
KPO-3365	GTTTTTGCTAGCTGCAGTGGTAGCTTCATCAGG	pNP086
KPO-3418	CCAGCGGATCCGCTAGCAACATTCGCTGCAAAAGAGGTG	pNP084
KPO-3419	CCAGCGGATCCGCTAGCAGCGTAAGCGCCAGTAGC	pNP085
KPO-3420	AGCGTCTTCCGTACTTCTACT	*lpp* qRT-PCR
KPO-3421	GCTGACCTGAGTGCTGATTT	*lpp* qRT-PCR
KPO-3422	CAGTGTCACCGAAAGTGTAGAT	*ompU* qRT-PCR
KPO-3423	CTGTTGACGCAATGGGTAATG	*ompU* qRT-PCR
KPO-3424	CTGAACTGGATGACTGGCTTAC	*vc1565* qRT-PCR
KPO-3425	CCACTTTGACTCTGCTGCTTAG	*vc1565* qRT-PCR
KPO-3426	CCAATTCGCTGCCTTTGATTAC	*prvT* qRT-PCR

KPO-3427	CCTGTGTACTGGGTGTCATATTC	*prvT* qRT-PCR
KPO-3428	AAACGGCGCACCATAGAA	*dsbA* qRT-PCR
KPO-3429	CGTAAGCCACCGAAAGATGA	*dsbA* qRT-PCR
KPO-3464	CCTCGTAACTCAAGCCATCAA	*rpoE* qRT-PCR
KPO-3465	ATCGAACCCGGAGAACATTAC	*rpoE* qRT-PCR
KPO-3466	CATCACGGTACGCTTCCATAA	*vc2240* qRT-PCR
KPO-3467	GCATGGTGCCATTTACTTTCC	*vc2240* qRT-PCR
KPO-3468	GTACTCATTGACCGAAGGTGAG	*dsbD* qRT-PCR
KPO-3469	AGGCAGCGGAACAGATAAAG	*dsbD* qRT-PCR
KPO-3470	TTCCATGCACGGGTATATAAGG	*vc1485* qRT-PCR
KPO-3471	GACGTGACAACGTATCGTAGAA	*vc1485* qRT-PCR
KPO-3472	CTGGAATTCAGGGATCACTAGC	*ompA* qRT-PCR
KPO-3473	CAGCTAAAGGTCTAGGCGAAAG	*ompA* qRT-PCR
KPO-3474	GTGCTGACCTTCACCTTCTT	*vc0429* qRT-PCR
KPO-3475	GCTCGACAATCTGCTCTAACT	*vc0429* qRT-PCR
KPO-3478	CGAAGCACAACCTCAAGAAAC	*btuB* qRT-PCR
KPO-3479	TCGATATCTTGGCGGGTAATG	*btuB* qRT-PCR
KPO-3480	CTCCAGCGTTACCCAAACA	*bamD* qRT-PCR
KPO-3481	AGAAATCTGCGGTCGCTAAA	*bamD* qRT-PCR
KPO-3484	TTTAGGCTAACAGCGTCACTT	*acfA* qRT-PCR
KPO-3485	GCAAATGCAGCACCGTATATT	*acfA* qRT-PCR
KPO-3562	CCTTCTTAAGGAGTTCTCTATGAAC	pNP087
KPO-3563	CTTAAGAAGGTAAGTCGGTGTTATTG	pNP087
KPO-4040	AGAGGTACCGGTTGTTAACGCACTGCTAAACCATGACTCAAG	pEE001
KPO-4041	CATCAAGATTCAATCTACAAAGGC	pEE001
KPO-4042	TTGTAGATTGAATCTTGATGAGCCTTTCGGTTATTATTTTGTCAC	pEE001
KPO-4043	TATGCATCCTAGGCCTATTAGTGCAATGATCTTGGGTGATG	pEE001
KPO-4110	AGAGGTACCGGTTGTTAACGGGAAATACCATGAAAAAGCTAGC	pNP089
KPO-4111	TTCAGTAACTTGGTACTGGAATTC	pNP089
KPO-4112	TCCAGTACCAAGTTACTGAAGACTACAAAGACCATGACGGTG	pNP089
KPO-4113	ATCTTGATGATTACCGTAAATTACTATTTATCGTCATCTTTGTAGTC	pNP089
KPO-4114	TTTACGGTAATCATCAAGATTCAATC	pNP089
KPO-4115	TATGCATCCTAGGCCTATTAAACCATGACTCAAGTCCATGC	pNP089
KPO-4308	GTGCTCAGTATCTTGTTATCCGCTC	pNP088
KPO-4309	GATAACAAGATACTGAGCACTTTCTTTGATGTCCCCATTTTGTGGAG	pNP088
KPO-4356	GCTCCACAAAATGGGGAC	rybB-scaffold oligoprobe
KPO-4962	AGAGGTACCGGTTGTTAACGCACTCGATTTTTGTTATCACCAG	pNP090
KPO-4963	TATGCATCCTAGGCCTATTAGTGCAATGATCTTGGGTGATG	pNP090
KPO-5122	ATTATAGCAGCAAATTTCTTCATGGTATTTCCTTTTTTCTTTATG	pNP091
KPO-5123	GAAGAAATTGGCTGCTATAATTTCAGCGACGTTACTTTTTGC	pNP091

APPENDIX SUPPLEMENTARY MATERIALS AND METHODS

Plasmid construction

The plasmids used in this study are listed in Table S4, used DNA oligonucleotides are listed in Table S5. The plasmids pNP074, pNP075 and pNP017 were obtained by amplification of the promotor regions of *micV* and *vrrA* from KPS-0014 chromosomal DNA, using the oligonucleotides KPO-1235/1236 and KPO-2503/2504, respectively. The promotor inserts were digested using SphI and SalI restriction enzymes and ligated into an equally treated pYH010 backbone (pNP074, pNP075) or a pCMW-1K backbone (pNP017). The inserts for the sRNA expression plasmids pRH001, pRH013 and pNP002 were obtained by amplification with KPO-1082/1083, KPO-1409/1410 or KPO-0999/1000, respectively. Fragments were introduced into linearized pEVS plasmid backbones (KPO-0092/1023) using XbaI restriction (pRH001, pNP002) and ligation, or Gibson assembly (pRH013). The plasmid pRH011 was generated via Gibson assembly using linearized pBAD5A backbone (KPO-1423/1424) and a KPO-1425/1426 amplified *rpoE* insert, obtained from KPS-0014 chromosomal DNA. The *micV, vrrA and rpoE* fragments were PCR amplified from KPS-0014 chromosomal DNA using the primer pairs KPO-0999/1237, KPO-1398/1399 or KPO-1478/1479, respectively. KpnI restriction of the *micV* fragment and a KPO-0196/0640 linearized pBAD5K backbone, yielded pNP016. Gibson assembly of the *vrrA* and *rpoE* fragments with KPO-0196/1397 linearized pBAD5K backbone yielded pNP022 and pNP018, respectively. Plasmid pKP431 was cloned by PCR amplification of the *hfq* flanking regions with KPO-0012/0066 and KPO-0019/0067, thereby introducing the 3xFLAG tag with primer overhangs. The resulting fragments were fused via overlap PCR using KPO-0148/0149, and introduced into pKAS32 backbone using KpnI and AvrII restriction sites. The plasmids pNP023 and pNP024 were constructed by amplification of insert fragments using KPO-1186/1187 and KPO-1188/1189, or KPO-1180/1181 and KPO-1182/1183, respectively. The inserts were fused using overlap PCR with the oligonucleotides KPO-1214/125 (pNP023) or KPO-1184/1185 (pNP024), digested with KpnI and AvrII and ligated into an equally digested pKAS32 backbone. The plasmids pNP026, pNP076, pEE001, pNP021 and pNP089 were constructed by Gibson assembly, using a KPO-0267/0268 linearized pKAS32 backbone. The insert fragments were PCR amplified from KPS-0014 chromosomal DNA using the following oligonucleotides: pNP021 (KPO-1324/1325 and KPO-1326/1327), pNP076 (KPO-2193/2194 and KPO-2195/2196), pEE001 (KPO-4040/4041, KPO-4042/4043), pNP021 (KPO-1417/1418, KPO-1419/1420 and KPO-1421/1422 amplified from KPS-0995 chromosomal DNA), pNP089 (KPO-4110/4111, KPO-4114/4115 and KPO-4112/4113 amplified from KPS-0995 chromosomal DNA). GFP fusions were cloned as described previously (Corcoran et al., 2012) and employing previously determined transcriptional start site annotations (Papenfort et al., 2015). Briefly, *acfA* (pNP059), *bamD* (pNP082), *btuB* (pNP029), *dsbD* (pNP079), *lpp* (pNP086), *ompA* (pNP081), *ompT* (pKP465),

ompU (pNP085), *oppA* (pNP084), *pal* (pNP062), *prvT* (pNP083), *rpoE* (pNP044), *ushA* (pNP054), *vc1485* (pNP080), *vc1563* (pNP078), *vca0951* (pNP077) inserts for translational reporters were PCR amplified using the primers indicated in Table S6 and introduced into pXG10-1C backbones using NheI, NsiI restriction sites or Gibson assembly. The pMD030 plasmid was constructed by restriction digest of pFM1-1 with XbaI and XhoI, yielding the P_L-*rybB* fragment and insertion into an equally treated pEVS backbone. pNP088 was obtained by site-directed mutagenesis PCR using KPO-4308/4309 and the parental plasmid pMD251 as a template. pMD241-255 plasmids were obtained by sequencing plasmids derived from EtOH resistant colonies. The Cm^R resistance cassettes were replaced with Kan^R cassettes using linearization with KPO-0282/1529 and amplification of the KanR cassette from the pCMW-1K plasmid using KPO-1160/1525. The plasmid pNP089 was generated by amplification of the insert from KPS-0014 chromosomal DNA using KPO-4962/4963 and Gibson assembly with KPO-0267/0268 linearized pKAS32 backbone. Quickchange PCR using pNP090 as template, and KPO-5122/5123 yielded pNP091. Mutations for compensatory base pair exchanges were introduced using the oligonucleotides listed in Table S5, and the respective parental plasmids as a template.

V. cholerae strain construction

A complete list of strains used in this study is provided in Table S3. *V. cholerae* C6706 was used as the wild-type strain in this study. *V. cholerae* mutant strains were generated as described previously (Papenfort et al., 2017). RK2/RP4-based conjugal transfer was used to introduce plasmids into *V. cholerae* from *E. coli* S17λpir plasmid donor strains. Subsequently, transconjugants were selected using appropriate antibiotics, and polymyxin B to specifically inhibit *E. coli* growth.

Identification of σ^E-dependent promoters in *V. cholerae*

To detect σ^E-dependent promoters in *V. cholerae*, the promotor sequence of 60 σ^E-dependent genes of *E. coli* (Mutalik et al., 2009) were used to construct a motif with MEME (Bailey et al., 2015). The motif was searched with FIMO (Bailey et al., 2015) in the genome sequence of *V. cholerae* (Accession NC_002505, NC_002506) accepting only hits with a p-value below 0.0001. The 626 motif matching sites in *V. cholerae* were filtered by proximity to transcription start site (Papenfort *et al.,* 2015) and only those with a maximal distance of 50 nt were reported in Table S1. A Unix shell script that represents the analyses has been deposited at Zenodo (https://doi.org/10.5281/zenodo.2543422)

In silico analyses

Genomic loci encoding *micV* in various *Vibrio* strains were analyzed for gene synteny using SyntTax (Fig. EV1A) (Oberto, 2013). The following strains were used for analysis: *V.ch*., *Vibrio cholerae* (NCBI:txid243277); *V.vu.*, *Vibrio vulnificus* (NCBI:txid914127); *V.co., Vibrio coralliilyticus* (NCBI:txid1384040); *V.tu*., *Vibrio tubiashii* (NCBI:txid1051646); *V.ha*., *Vibrio harveyi* (ATCC:33843); *V.an*., *Vibrio anguillarum* (NCBI:txid882102); *V.al*., *Vibrio alginolyticus* (NCBI:txid1219076). To generate the alignment of *micV* sequences (Fig. 1A), the following strains were used: Vch, *Vibrio cholerae* (NCBI:txid243277); Vfu, *Vibrio furnissii* (NCBI:txid903510); Vvu, *Vibrio vulnificus* (NCBI:txid216895); Van, *Vibrio anguillarum* (NCBI:txid882102); Vsp, *Vibrio splendidus* (NCBI:txid575788); Vex, *Vibrio* sp. Ex25 (NCBI:txid150340); Vpa, *Vibrio parahaemolyticus* (NCBI:txid223926); Vej, *Vibrio* sp. EJY3 (NCBI:txid1116375); Asa, *Aliivibrio salmonicida* (NCBI:txid316275); Afi, *Aliivibrio fischeri* (NCBI:txid312309). To generate the alignment of *vrrA* sequences (Fig. EV2A), the following strains were used: Vch, *Vibrio cholerae* (NCBI:txid243277); Vco, *Vibrio coralliilyticus* (NCBI:txid1384040); Vvu, *Vibrio vulnificus* (NCBI:txid672); Val, *Vibrio alginolyticus* (NCBI:txid1219076); Vsp, *Vibrio splendidus* (NCBI:txid575788).

APPENDIX SUPPLEMENTARY REFERENCES

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