

Supplemental information

***Caulobacter* lipid A is conditionally dispensable
in the absence of *fur* and in the presence
of anionic sphingolipids**

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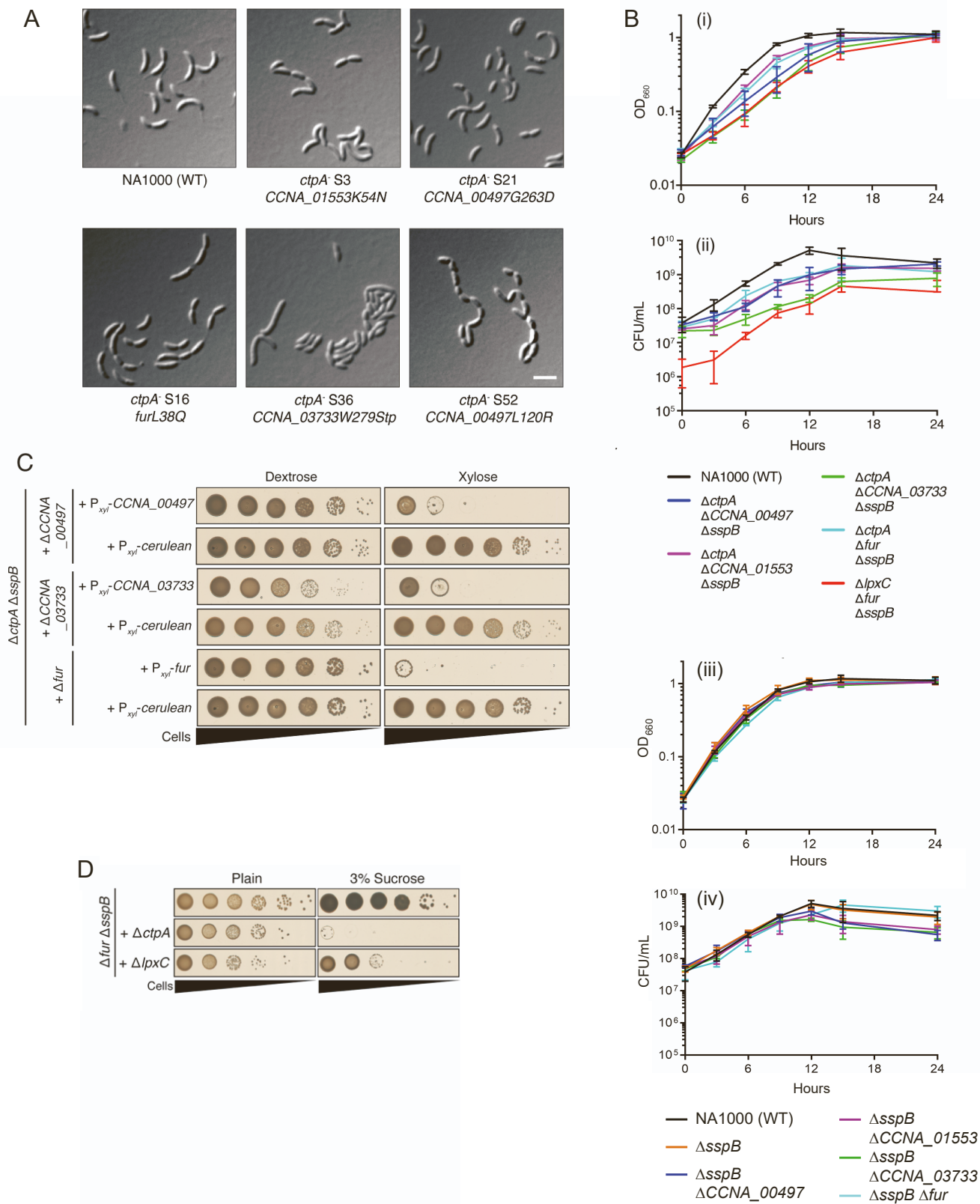


Figure S1, related to Fig. 1: Morphology, growth, and complementation of strains in which suppressor mutations permit the loss of *ctpA*. (A) Isolates from the $\Delta ctpA$ suppressor screen show variations in morphology. DIC images of selected suppressor isolates confirmed to have lost the *ctpA* covering plasmid. Putative suppressor mutations identified by whole-genome resequencing are indicated. Scale bar, 3 μm . (B) Growth curves of the indicated strains in PYE showing (i or iii) OD_{660} and (ii or iv) colony-forming units (CFU) per mL (mean \pm S.D., $N=3$). (C) Viability assays of $\Delta ctpA$ suppressor mutants, each harboring a vector for xylose-driven expression of the corresponding suppressor gene or the *cerulean* gene as a control. Kanamycin was included in media to retain expression vectors. (D) Viability of the indicated strains on PYE medium (Plain) or PYE medium + 3% sucrose.

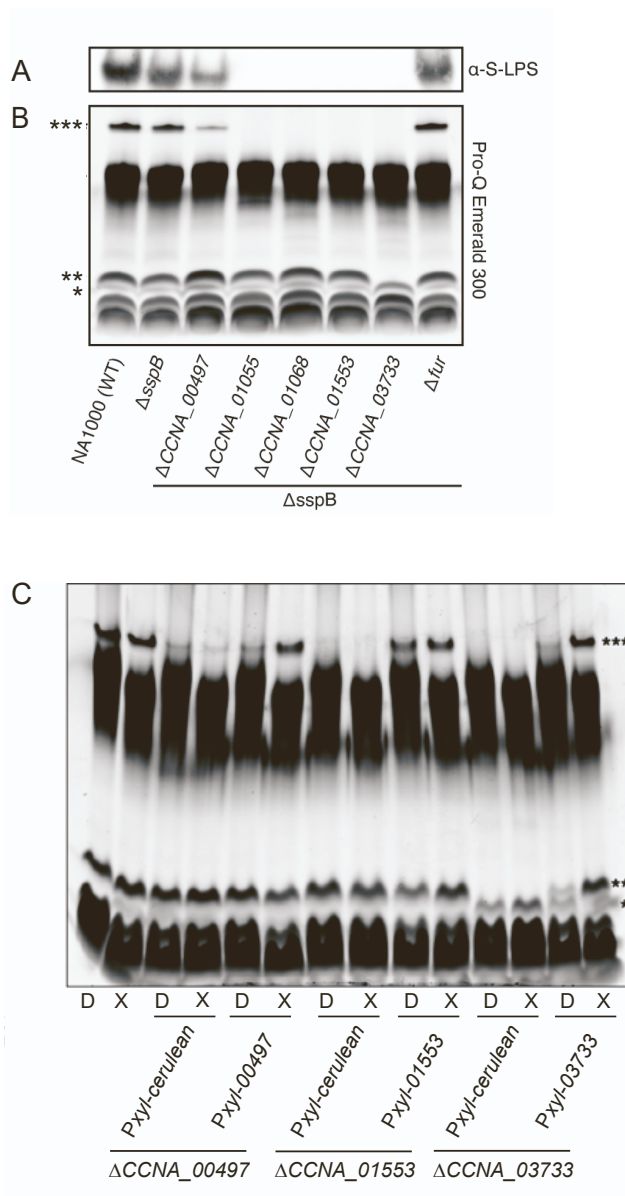


Figure S2, related to Fig.1: A subset of *ctpA* suppressor mutations impair or block S-LPS production. (A) α -S-LPS-probed immunoblot and (B) Pro-Q Emerald 300-stained gel of Proteinase K-treated whole-cell lysates. (C) Complementation of O-antigen biosynthesis using plasmid-borne genes driven by a xylose-inducible promoter. Pro-Q Emerald 300-stained polyacrylamide gel of Proteinase K-treated whole-cell lysates of strains grown in either PYED (D) or PYEX (X). Samples were normalized by OD_{660} . *** = S-LPS, ** = putative full-length lipid A+core, * = putative incomplete lipid A+core species in cells lacking *manC* activity (*CCNA_03733*).

A NA1000

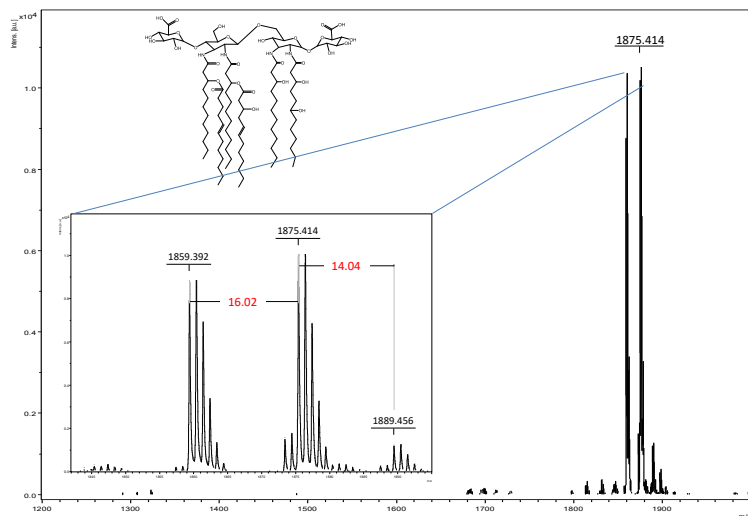
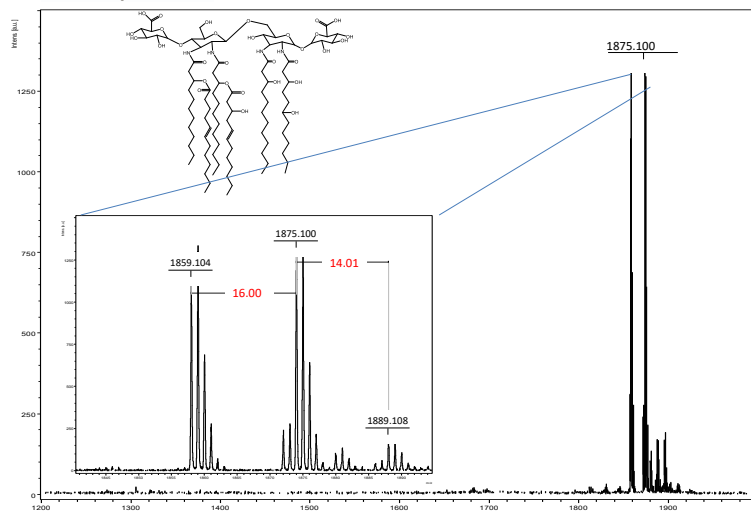
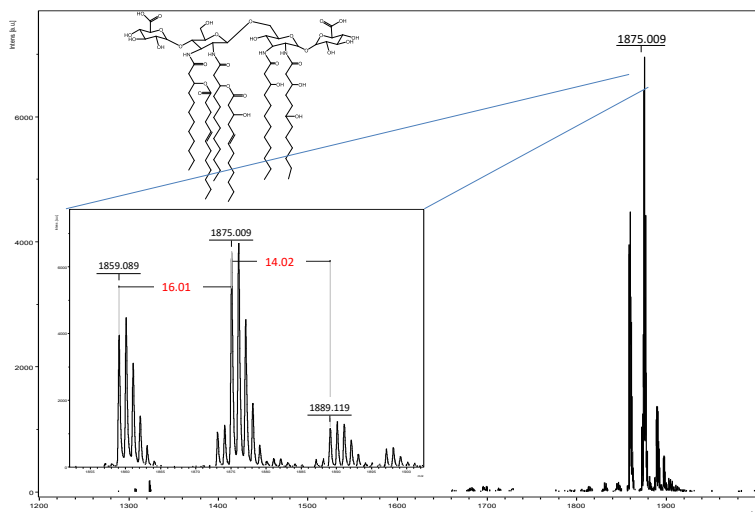
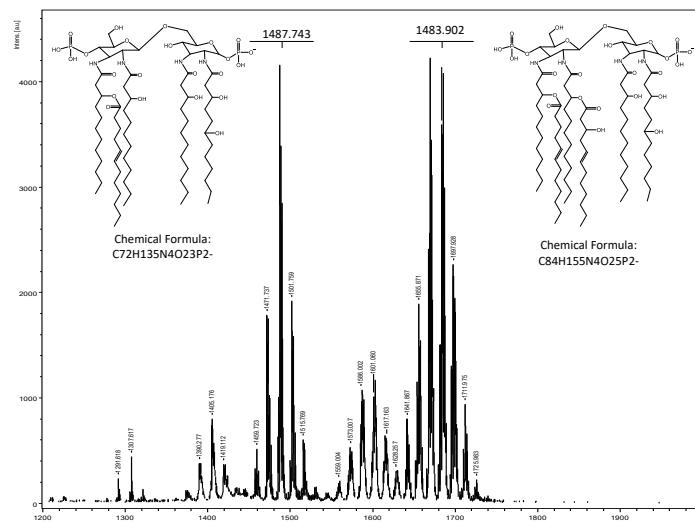
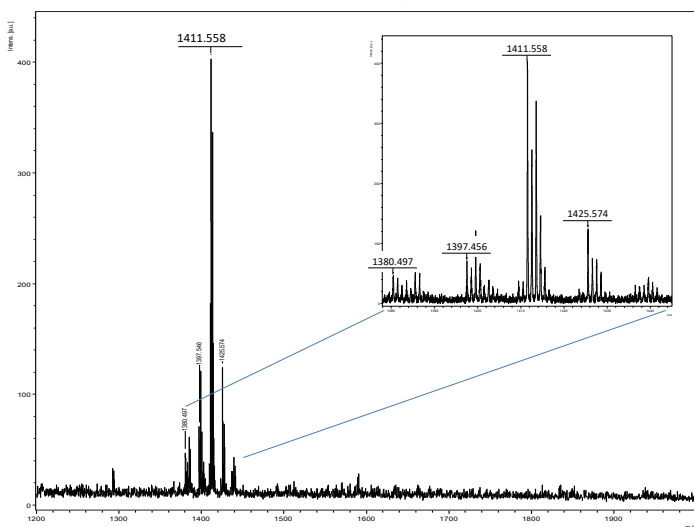
B Δ sspBC Δ fur Δ sspBD Δ ctpA Δ fur Δ sspBE Δ lpxC Δ fur Δ sspB

Figure S3, related to Figure 2: Δ ctpA and Δ lpxC strains with suppressor mutations lack wild-type lipid A. Tandem mass spectrometry (MS/MS)-derived structures of lipid A from the indicated strains. Lipid extraction and MS/MS analysis were performed as described in STAR Methods using the same protocols for all strains.

Figure S4

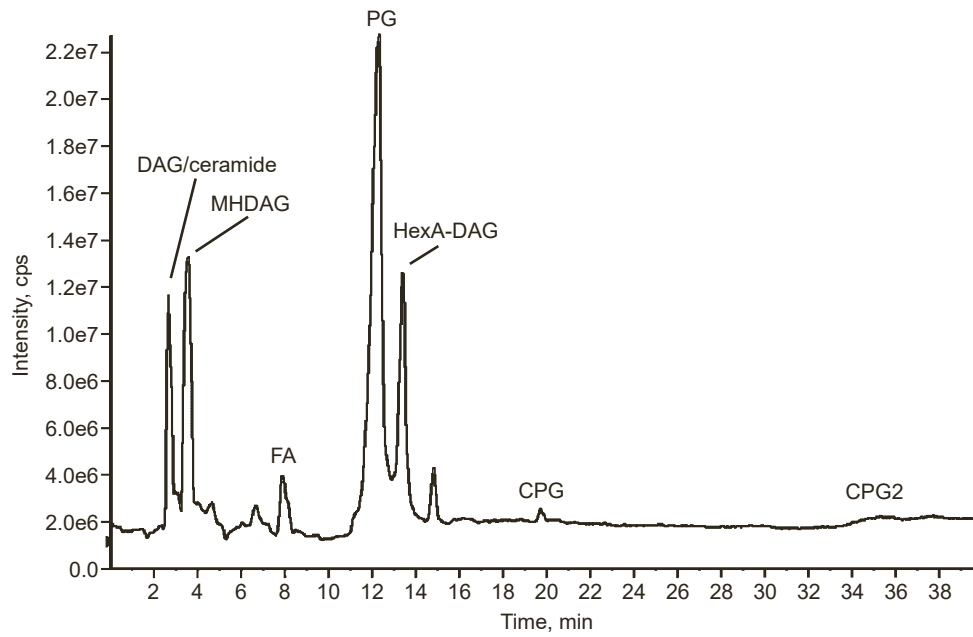


Figure S4, related to Figure 6: The total ion chromatogram of the *C. crescentus* lipidome shows the major lipid species present. DAG: diacylglycerol; MHDAG: mono-hexosyl diacylglycerol; FA: fatty acids; PG: phosphatidylglycerol; HexA-DAG: hexuronic acid-diacylglycerol; CPG: ceramide phosphoglycerate.

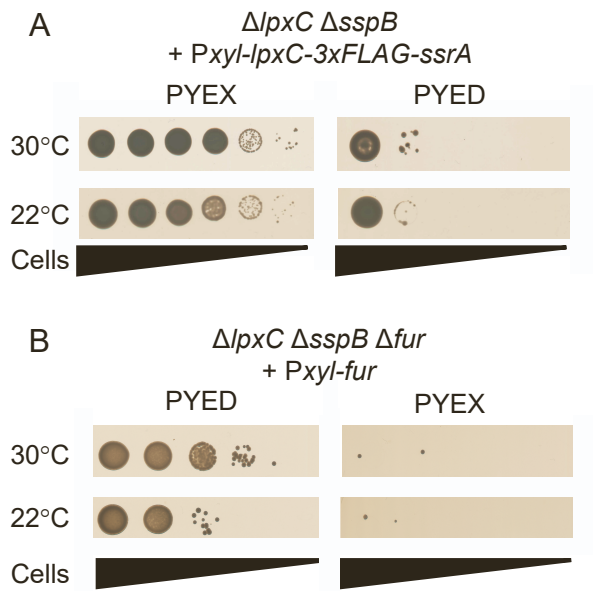


Figure S5, related to Figure 3. Slow growth at a reduced temperature in complex medium is insufficient for viability of $\Delta lpxC$ strains. (A) Viability of the LpxC depletion strain grown in inducing (PYEX) or depleting (PYED) conditions, at the indicated temperatures. (B) Viability of the stable $\Delta lpxC \Delta fur \Delta sspB$ strain harboring a P_{xyl}-fur plasmid, grown in noninducing (PYED) or inducing (PYEX) conditions, at the indicated temperatures. Cells were plated from 10-fold serially diluted suspensions normalized to OD₆₆₀ = 0.1. Plates were incubated for 3 days (30°C) or 6 days (22°C) and are representative of at least three independent trials. Plates in B included kanamycin to retain the *fur* expression vector.

Supplementary Tables

Table S1, related to Figure 1: Single-nucleotide polymorphisms and indels in *ΔctpA* suppressors^a

Strain	Sequence position ^b	Gene ^c	Annotation	Base Change	Amino Acid Substitution
S3	1668695	01553	Undecaprenyl-phosphate beta-N-acetyl-D-fucosaminophosphotransferase ^d	G>T	K54N
S3	2383967	02235	SGNH hydrolase family protein	G>A	G109D
S8	3492550	03316	UDP-N-acetylglucosamine 4,6-dehydratase/UDP-D-quinovosamine 4-dehydrogenase ^d	AG>ATG	E487Frameshift
S16	58496	00055	Ferric uptake regulation protein	A>T	L38Q
S21	515085	00497	Smooth LPS biosynthesis glycosyltransferase ^d	G>A	G263D
S21	1378399	01250	FecCD-family transporter protein	GCC>GC	A340Frameshift
S32	1157376	01056	Methyltransferase ^e	A>AG	S31Framshift
S32	2164248	02016	nuoMNADH-quinone oxidoreductase chain M	C>T	M7I
S32	2949436	02792	TonB-dependent outer membrane receptor	A>G	S516G
S36	3901011	03733	Mannose-1-phosphate guanylyltransferase ^d	C>T	W279Stop
S38	726779	00669	Glycosyltransferase family 99 protein WbsX ^d	CTG>CG	Q476Frameshift
S40	378822	00362	Zinc uptake regulation protein	A>T	C156S
S40	2868272	-	-	G>C	-
S40	3913883	03744	dTDP-glucose 4,6-dehydratase ^d	T>A	I269F
S43	1377138	01249	ABC-transporter substrate binding protein	C>T	A169V
S43	1668861	01553	Undecaprenyl-phosphate beta-N-acetyl-D-fucosaminophosphotransferase ^d	CC>GA	P110E
S43	2976030	02820	TadG-family protein	C>T	Silent
S44	727833	00669	Glycosyltransferase family 99 protein WbsX ^d	A>C	L125R
S47	537654	00524	Conserved hypothetical cytosolic protein	A>AG	L369Frameshift
S47	1173251	01068	Glycosyltransferase ^d	GCC>GC	R293Frameshift
S52	514656	00497	Smooth LPS biosynthesis glycosyltransferase ^d	T>G	L120R
S52	810384	00752	3-hydroxybutyryl-CoA dehydrogenase	T>A	Stop>Y
S52	3797776	-	-	T>C	-

S53	58379	00055	Ferric uptake regulation protein	G>T	S77Stop
S53	1778110	01656	Endonuclease/exonuclease/phosphatase family protein	A>C	L4R
S53	3900664	03733	Mannose-1-phosphate guanylyltransferase ^d	G>A	Q395Stop
S54	1377127	01249	ABC-transporter substrate binding protein	CGG>CG	G166Frameshift
S54	3492771	03316	UDP-N-acetylglucosamine 4,6-dehydratase/UDP-D-quinovosamine 4-dehydrogenase ^d	G>A	E561K
S57	1492703	01378	Protein-L-isoaspartate O-methyltransferase	G>GC	G59Frameshift
S57	2487074	02347	Phosphomannomutase/ phosphoglucomutase ^d	G>A	G266D
S111	295119	00283	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	A>T	L284Q
S111	1173965	01068	Glycosyltransferase ^d	TGC>T	R55Frameshift
S112	311705	00297	Two-component response regulator	C>T	W64Stop
S112	315570	00301	Phosphotransferase family protein	T>G	I319S
S112	2487307	02347	Phosphomannomutase/ phosphoglucomutase ^d	G>A	E344K
S112	3569520	03399	Flavin prenyltransferase UbiX	C>T	A159T

^aIn addition to the indicated mutations, each strain is also $\Delta ctpA \Delta sspB$.

^bSequence positions refer to the genome of *Caulobacter crescentus* NA1000 (NC_011916.1).

^cThe prefix for each gene number is *CCNA_*.

^dGenes known or predicted to be involved in O-antigen synthesis.

^eWe speculate that the frameshift in *CCNA_01056* affects the expression of the downstream gene *CCNA_01055*, annotated as a GT1 family glycosyltransferase.

Table S2, related to Figure 3: Single-nucleotide polymorphisms and indels identified in $\Delta lpxC$ suppressors^a

Strain	Sequence position ^b	Gene ^c	Annotation	Base Change	Amino Acid Substitution
S1	58446	00055	Ferric uptake regulation protein Fur	A>T	Y55N
	4000704	03835	3-oxoacyl-(Acyl-carrier-protein) synthase	C>T	G396S
S5	58436	00055	Ferric uptake regulation protein Fur	A>T	V58E

^aIn addition to the indicated mutations, each strain is also $\Delta lpxC \Delta 00497$.

^bSequence positions refer to the genome of *Caulobacter crescentus* NA1000 (NC_011916.1).

^cThe prefix for each gene number is *CCNA_*.

Table S3, related to Fig. 2: Fur or iron regulation of genes predicted to participate in lipid synthesis.

Gene ^a	Name ^b	Regulation by Fur and/or iron ^c
CCNA_00363	<i>pgpB</i>	None
CCNA_01382	<i>fadD</i>	None
CCNA_01431	<i>plsX</i>	None
CCNA_01432	<i>fabH</i>	None
CCNA_01746	<i>fabD</i>	None
CCNA_01747	<i>fabG</i>	None
CCNA_01750	<i>fabF</i>	None
CCNA_01932	<i>plsC</i>	None
CCNA_01960	<i>accB</i>	None
CCNA_01961	<i>accC</i>	Downregulated by iron limitation only (Leaden, <i>et al.</i> , 2018)
CCNA_01977	<i>plsB</i>	None
CCNA_01989	<i>fabZ</i>	None
CCNA_01995	<i>cdsA</i>	None
CCNA_02001	<i>pgpC</i>	None
CCNA_02040	<i>pgpC</i>	None
CCNA_02302	<i>plsC</i>	None
CCNA_03002	<i>pgsA</i>	None
CCNA_03090	<i>accA</i>	None
CCNA_03656	<i>accD</i>	Upregulated by iron limitation only (Leaden, <i>et al.</i> , 2018)
CCNA_03833	<i>fabI</i>	Downregulated by iron limitation only (Leaden, <i>et al.</i> , 2018)
CCNA_03835	<i>fabB</i>	None
CCNA_03836	<i>fabA</i>	None

^aGene numbers refer to the genome of *Caulobacter crescentus* NA1000 (NC_011916.1).

^bNames are derived from annotations in NCBI associated with NC_011916.1 or from BLAST or PSI-BLAST searches of the *Caulobacter crescentus* NA1000 genome initiated with the indicated *E. coli* amino acid sequence ((Altschul, 1997; Altschul *et al.*, 1990).

^cGene regulation was assessed by inspection of results (Leaden *et al.*, 2018; da Silva Neto *et al.*, 2013).

Table S4, related to Figure 5: Genes whose average fitness scores were lower in CHIR-090-treated cultures than in control cultures by >1.

Gene ^a	Average fitness PYE ^b	Average fitness PYE + 2 µg/ml CHIR-090 ^c	Net average fitness ^d	Annotation
CCNA_03609	-0.287	-3.176	-2.889	Outer membrane protein
CCNA_01222	-0.290	-2.953	-2.662	NADH-ubiquinone oxidoreductase (<i>cerR</i>)
CCNA_03983	-0.726	-3.246	-2.520	HicA-related toxin-antitoxin protein
CCNA_01060	0.175	-2.191	-2.365	Type I protein secretion ATP-binding protein, RsaD
CCNA_01638	0.122	-2.195	-2.317	Beta-lactamase family protein
CCNA_01162	-0.391	-2.668	-2.277	Beta-D-glucoside glucohydrolase
CCNA_01220	0.019	-2.222	-2.240	BioF-family ceramide biosynthesis protein CcbF (<i>spt</i>)
CCNA_01223	-0.354	-2.549	-2.195	Acyl-CoA synthetase (<i>acps</i>)
CCNA_03026	-1.443	-3.605	-2.162	Two-component response regulator, PetR
CCNA_01817	-0.016	-2.139	-2.123	Nitrogen assimilatory regulatory protein, NtrX
CCNA_01212	-0.470	-2.589	-2.120	dATP pyrophosphohydrolase (<i>bcerS</i>)
CCNA_01219	-0.383	-2.337	-1.955	Putative cytosolic protein (<i>cpgC</i>)
CCNA_01067	0.164	-1.730	-1.894	Type I secretion outer membrane protein RsaFa
CCNA_00252	-0.523	-2.345	-1.822	Multimodular transpeptidase-transglycosylase PbpX
CCNA_01217	-1.063	-2.808	-1.746	Phosphatidylglycerophosphate synthase (<i>cpgA</i>)
CCNA_02127	0.155	-1.521	-1.676	Bifunctional lysylphosphatidylglycerol flippase/synthetase MprF
CCNA_00525	-1.296	-2.941	-1.645	Prolipoprotein diacylglyceryl transferase
CCNA_03027	-0.219	-1.855	-1.636	Two-component sensor histidine kinase
CCNA_03321	0.202	-1.390	-1.593	VanZ superfamily protein
CCNA_01218	-0.894	-2.458	-1.564	Sphingosine kinase/diacylglycerol kinase-related protein (<i>cpgB</i>)
CCNA_03864	-0.087	-1.572	-1.485	DUF3576 domain-containing protein
CCNA_00001	-0.980	-2.436	-1.456	Pyruvate, phosphate dikinase regulatory protein
CCNA_01427	-0.619	-2.040	-1.421	Beta-barrel assembly machine protein BamE
CCNA_03876	0.509	-0.880	-1.389	Transcription termination factor rho
CCNA_00512	-0.689	-2.035	-1.345	GTP-binding protein, probable translation factor
CCNA_00050	-1.925	-3.242	-1.317	Apolipoprotein N-acyltransferase Lnt

CCNA_00190	-0.624	-1.885	-1.261	Acyl-CoA hydrolase
CCNA_02386	-0.650	-1.875	-1.225	O-antigen ligase related enzyme
CCNA_00080	0.341	-0.862	-1.203	LexA-related transcriptional repressor
CCNA_00924	0.373	-0.813	-1.186	MarR/EmrR family transcriptional regulator
CCNA_00354	-0.212	-1.384	-1.172	NlpC/P60 CHAP domain amidase protein
CCNA_00027	0.109	-1.045	-1.154	2OG-Fe(II) oxygenase
CCNA_01324	-0.445	-1.588	-1.143	LSU ribosomal protein L30P
CCNA_01226	-1.084	-2.210	-1.126	OstA family protein
CCNA_01221	-0.499	-1.623	-1.124	acyl carrier protein (<i>acp</i>)
CCNA_00851	0.073	-1.030	-1.103	periplasmic multidrug efflux lipoprotein precursor
CCNA_03782	-1.055	-2.153	-1.098	cytochrome c biogenesis ATP-binding export protein CcmA
CCNA_03336	-3.118	-4.188	-1.071	Tol-Pal system periplasmic component YbgF
CCNA_03820	-1.783	-2.837	-1.055	LolA-family outer membrane lipoprotein carrier protein
CCNA_03260	0.243	-0.810	-1.054	queuosine biosynthesis protein QueD
CCNA_00850	0.065	-0.980	-1.045	cation/multidrug efflux pump acrB2
CCNA_02081	0.006	-1.013	-1.019	Sec-independent protein translocase protein tatB
CCNA_00735	0.361	-0.644	-1.005	beta-barrel assembly machine protein BamF

^aGene numbers refer to the genome of *Caulobacter crescentus* NA1000 (NC_011916.1).

^bAverage fitness in PYE was calculated from fitness values in set8IT011, set8IT023, and set8IT035.

^cAverage fitness in PYE + 2 µg/ml CHIR-090 was calculated from fitness values in set8IT012, set8IT024, and set8IT036.

^dNet average fitness = (Average fitness PYE + 2 µg/ml CHIR-090)-(Average fitness PYE).

Table S5, related to Figure 3: Growth rates in PYE medium at 22°C.

Strain	Doubling time +/- standard deviation (hours) ^a	
	PYE 30°C	PYE 22°C
NA1000	1.61 +/- 0.04	2.50 +/- 0.17
$\Delta sspB$	1.51 +/- 0.04	2.44 +/- 0.09
$\Delta fur \Delta sspB$	1.77 +/- 0.08	3.16 +/- 0.21

^aDoubling times were calculated from optical density measurements (660 nm) during exponential growth.

Table S6, related to STAR Methods: Strains used in this study.

Strain Number	Description ^a	NCBI BioSample Accession ^b or Note	Reference
KR4000	Wild-type <i>Caulobacter</i> NA1000		(Evinger and Agabian, 1977)
KR3180	NA1000 pJS14		This study
KR1499	Δ <i>sspB::aadA</i>		(Shapland et al., 2011)
KR3877	Δ <i>CCNA_00497::hyg</i>		This study
KR4198	Δ <i>CCNA_00497::hyg</i> pJS14		This study
KR3871	Δ <i>CCNA_01553::hyg</i>		This study
KR4197	Δ <i>CCNA_01553::hyg</i> pJS14		This study
KR4076	Δ <i>fur::hyg</i>		This study
KR4199	Δ <i>fur::hyg</i> pJS14		This study
KR3953	Δ <i>CCNA_00497::hyg</i> Δ <i>sspB::aadA</i>		This study
KR4115	Δ <i>CCNA_01055::hyg</i> Δ <i>sspB::aadA</i>		This study
KR4116	Δ <i>CCNA_01068::hyg</i> Δ <i>sspB::aadA</i>		This study
KR3954	Δ <i>CCNA_01553::hyg</i> Δ <i>sspB::aadA</i>		This study
KR3955	Δ <i>CCNA_03733::hyg</i> Δ <i>sspB::aadA</i>		This study
KR4077	Δ <i>fur::hyg</i> Δ <i>sspB::aadA</i>		This study
KR4153	Δ <i>sspB::aadA</i> pXCERN-2		This study
KR4154	Δ <i>CCNA_00497::hyg</i> Δ <i>sspB::aadA</i> pXCERN-2		This study
KR4155	Δ <i>CCNA_01553::hyg</i> Δ <i>sspB::aadA</i> pXCERN-2		This study
KR4156	Δ <i>CCNA_03733::hyg</i> Δ <i>sspB::aadA</i> pXCERN-2		This study
KR4157	Δ <i>fur::hyg</i> Δ <i>sspB::aadA</i> pXCERN-2		This study
KR4158	Δ <i>CCNA_00497::hyg</i> Δ <i>sspB::aadA</i> pZIK172		This study
KR4159	Δ <i>CCNA_01553::hyg</i> Δ <i>sspB::aadA</i> pZIK173		This study
KR4160	Δ <i>CCNA_03733::hyg</i> Δ <i>sspB::aadA</i> pZIK174		This study
KR4161	Δ <i>fur::hyg</i> Δ <i>sspB::aadA</i> pZIK175		This study
KR4269	Δ <i>fur::hyg</i> Δ <i>sspB::aadA</i> pZIK197		This study
KR3906	Δ <i>ctpA::tetAR</i> Δ <i>sspB::aadA</i> pAB6	Restock of KR2423 from Shapland 2011	(Shapland et al., 2011)
KR4111	Δ <i>ctpA::tetAR</i> Δ <i>sspB::aadA</i> Δ <i>CCNA_00497::hyg</i> pAB6	SAMN12568762	This study
KR4112	Δ <i>ctpA::tetAR</i> Δ <i>sspB::aadA</i> Δ <i>CCNA_01553::hyg</i> pAB6	SAMN12568763	This study
KR4092	Δ <i>ctpA::tetAR</i> Δ <i>sspB::aadA</i> Δ <i>CCNA_03733::hyg</i> pAB6	SAMN12568761	This study
KR4090	Δ <i>ctpA::tetAR</i> Δ <i>sspB::aadA</i> Δ <i>fur::hyg</i> pAB6	SAMN12568759	This study
KR4007	Δ <i>lpxC::tetAR</i> Δ <i>sspB::aadA</i> pZIK133		This study
KR4008	Δ <i>lpxC::tetAR</i> Δ <i>sspB::aadA</i> Δ <i>CCNA_00497::hyg</i> pZIK133		This study
KR4223	Δ <i>lpxC::tetAR</i> Δ <i>CCNA_00497::hyg</i> pZIK133		This study
KR4009	Δ <i>lpxC::tetAR</i> Δ <i>sspB::aadA</i> Δ <i>CCNA_01553::hyg</i> pZIK133		This study
KR4010	Δ <i>lpxC::tetAR</i> Δ <i>sspB::aadA</i> Δ <i>CCNA_03733::hyg</i> pZIK133		This study
KR4091	Δ <i>lpxC::tetAR</i> Δ <i>sspB::aadA</i> Δ <i>fur::hyg</i> pZIK133	SAMN12568760	This study

KR4113	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_00497::hyg</i>	SAMN12568767	This study
KR4114	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_01553::hyg</i>	SAMN12568768	This study
KR4104	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_03733::hyg</i>	SAMN12568766	This study
KR4102	<i>ΔctpA::tetAR ΔsspB::aadA Δfur::hyg</i>	SAMN12568764	This study
KR4103	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg</i>	SAMN12568765	This study
KR4176	<i>ΔctpA::tetAR ΔsspB::aadA Δfur::hyg pZIK175</i>		This study
KR4270	<i>ΔctpA::tetAR ΔsspB::aadA Δfur::hyg pZIK197</i>		This study
KR4177	<i>ΔctpA::tetAR ΔsspB::aadA Δfur::hyg pXCERN-2</i>		This study
KR4178	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg pZIK175</i>		This study
KR4271	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg pZIK197</i>		This study
KR4179	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg pXCERN-2</i>		This study
KR4180	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_03733::hyg pZIK174</i>		This study
KR4181	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_03733::hyg pXCERN-2</i>		This study
KR4182	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_00497::hyg pZIK172</i>		This study
KR4183	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_00497::hyg pXCERN-2</i>		This study
KR4148	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg pZIK133</i> Plasmid re-introduced for complementation		This study
KR4149	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg pJS14</i>		This study
KR4150	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_03733::hyg pAB6</i> Plasmid re-introduced for complementation		This study
KR4151	<i>ΔctpA::tetAR ΔsspB::aadA ΔCCNA_03733::hyg pJS14</i>		This study
KR4264	NA1000 pZIK200		This study
KR4147	NA1000 pZIK171		This study
KR4170	NA1000 pZIK179		This study
KR4489	<i>ΔCCNA_01217</i>		This study
KR4549	<i>ΔCCNA_01217 vanA::01217::FLAG</i>	pKR438 integrated	This study
KR4450	<i>ΔCCNA_01218</i>		This study
KR4505	<i>ΔCCNA_01218 vanA::01218::FLAG</i>	pKR435 integrated	This study
KR4430	<i>ΔCCNA_01219</i>		This study
KR4501	<i>ΔCCNA_01219 vanA::01219::FLAG</i>	pKR436 integrated	This study
EK720	<i>ΔCCNA_01220</i> also known as KR4431		(Stankeviciute et al., 2019)
KR4530	<i>ΔCCNA_01220 vanA::01220::FLAG</i>	pKR437 integrated	This study
KR4517	<i>Δfur::hyg ΔsspB::aadA ΔCCNA_01217</i>		This study
KR4551	<i>Δfur::hyg ΔsspB::aadA ΔCCNA_01217 vanA::01217::FLAG</i>	pKR438 integrated	This study
KR4442	<i>Δfur::hyg ΔsspB::aadA ΔCCNA_01218</i>		This study
KR4503	<i>Δfur::hyg ΔsspB::aadA ΔCCNA_01218 vanA::01218::FLAG</i>	pKR435 integrated	This study
KR4438	<i>Δfur::hyg ΔsspB::aadA ΔCCNA_01219</i>		This study
KR4513	<i>Δfur::hyg ΔsspB::aadA ΔCCNA_01219 vanA::01219::FLAG</i>	pKR436 integrated	This study
KR4439	<i>Δfur::hyg ΔsspB::aadA ΔCCNA_01220</i>		This study
KR4532	<i>Δfur::hyg ΔsspB::aadA ΔCCNA_01220 vanA::01220::FLAG</i>	pKR437 integrated	This study
KR4509	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg ΔCCNA_01217</i> pZIK133		This study

KR4555	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg ΔCCNA_01217 vanA::01217::FLAG pZIK133</i>	pKR438 integrated	This study
KR4451	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg ΔCCNA_01218 pZIK133</i>		This study
KR4527	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg ΔCCNA_01218 vanA::01218::FLAG pZIK133</i>	pKR435 integrated	This study
KR4441	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg ΔCCNA_01220 pZIK133</i>		This study
KR4545	<i>ΔlpxC::tetAR ΔsspB::aadA Δfur::hyg ΔCCNA_01220 vanA::01220::FLAG pZIK133</i>	pKR437 integrated	This study
KR4205	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #3	SAMN11107060	This study
KR4206	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #8	SAMN11107061	This study
KR4207	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #16	SAMN11107062	This study
KR4208	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #21	SAMN11107063	This study
KR4209	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #32	SAMN11107064	This study
KR4210	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #36	SAMN11107065	This study
KR4211	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #38	SAMN11107066	This study
KR4212	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #40	SAMN11107067	This study
KR4213	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #43	SAMN11107068	This study
KR4214	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #44	SAMN11107069	This study
KR4215	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #47	SAMN11107070	This study
KR4216	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #52	SAMN11107071	This study
KR4217	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #53	SAMN11107072	This study
KR4218	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #54	SAMN11107073	This study
KR4219	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #57	SAMN11107074	This study
KR4220	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #111	SAMN11107075	This study
KR4221	<i>ΔctpA::tetAR ΔsspB::aadA</i> suppressor isolate #112	SAMN11107076	This study
KR4224	<i>ΔlpxC::tetAR ΔCCNA_00497::hyg</i> suppressor isolate #1	SAMN11107077	This study
KR4225	<i>ΔlpxC::tetAR ΔCCNA_00497::hyg</i> suppressor isolate #5	SAMN11107078	This study

^a For all strains designated “suppressor isolate,” additional genotype information is available in Table S1 or Table S2.

^bBioSample Accession numbers refer to genome resequencing data for the indicated strains in the Sequence Read Archive at <https://www.ncbi.nlm.nih.gov/sra>, under BioProject ID PRJNA526705

Table S7, related to STAR Methods: Plasmids used in this study.

Name	Description	Reference
pJS14	Broad host range cloning vector; high copy; chlor ^R ; pBBR1MCS derivative with unique EcoRI site	(J. Skerker, unpublished)
pNPTS138	kan ^R ; sacB-containing integration vector	(M.R. Alley, unpublished)
pXCERN-2	For integration at P _{xyiX} ; encodes xylose-inducible <i>cerulean</i> that can be exchanged for gene of interest; kan ^R	(Thanbichler et al., 2007)
pMCS-2	For integration at locus specified by insert sequence; kan ^R	(Thanbichler et al., 2007)
pAB6	pJS14-P _{xyiX} - <i>ctpA</i> -3xFLAG- <i>ssrA</i>	(Shapland et al., 2011)
pZIK133	pJS14-P _{xyiX} - <i>lpxC</i> -3xFLAG- <i>ssrA</i>	This study
pZIK172	pXCERN-2-P _{xyiX} - <i>CCNA_00497</i>	This study
pZIK173	pXCERN-2-P _{xyiX} - <i>CCNA_01553</i>	This study
pZIK174	pXCERN-2-P _{xyiX} - <i>CCNA_03733</i>	This study
pZIK175	pXCERN-2-P _{xyiX} - <i>CCNA_00055 (fur)</i>	This study
pZIK78	pNPTS138- <i>CCNA_00497::hyg</i> ; for replacing <i>CCNA_00497</i> with hygromycin resistance cassette	This study
pZIK82	pNPTS138- <i>CCNA_01055::hyg</i> ; for replacing <i>CCNA_01055</i> with hygromycin resistance cassette	This study
pZIK81	pNPTS138- <i>CCNA_01068::hyg</i> ; for replacing <i>CCNA_01068</i> with hygromycin resistance cassette	This study
pZIK73	pNPTS138- <i>CCNA_01553::hyg</i> ; for replacing <i>CCNA_01553</i> with hygromycin resistance cassette	This study
pZIK80	pNPTS138- <i>CCNA_03733::hyg</i> ; for replacing <i>CCNA_03733</i> with hygromycin resistance cassette	This study
pZIK161	pNPTS138- <i>CCNA_00055::hyg</i> ; for replacing <i>fur</i> with hygromycin resistance cassette	This study
pZIK134	pNPTS138- <i>CCNA_02064::tetAR</i> ; for replacing <i>lpxC</i> with tetracycline resistance cassette	This study
pHP45Ω-hyg	For isolating <i>hyg</i> fragment; hyg ^R ; amp ^R	(Blondelet-Rouault et al., 1997)
pKOC3	Contains <i>tetAR</i> flanked by EcoRI sites; amp ^R ; tet ^R	(Skerker et al., 2005)
pVMCS-4	For amplification of <i>aacC1</i> ; gent ^R	(Thanbichler et al., 2007)
pMCS-4	For single integration to disrupt genomic loci; gent ^R	(Thanbichler et al., 2007)
pVGFPC-2	Complementation vector for expressing target genes from <i>vanA</i> locus; kan ^R	(Thanbichler et al., 2007)

pVGFPC-4	Complementation vector for expressing target genes from <i>vanA</i> locus; gent ^R	(Thanbichler et al., 2007)
pVCHYC-5	Complementation vector for expressing target genes from <i>vanA</i> locus; tet ^R	(Thanbichler et al., 2007)
pFLGC-1	Vector for adding FLAG tag to the C-terminus of open reading frames; spec ^R	(Thanbichler et al., 2007)
pGS74	pNPTS138-based plasmid for markerless deletion of <i>CCNA_01217</i>	This study
pEK406	pVCHYC-5-based complementing vector, <i>vanA::CCNA_01217-FLAG</i>	This study
pKR438	pVGFPC-4-based complementing vector, <i>vanA::CCNA_01217-FLAG</i>	This study
pKR429	pNPTS138-based plasmid for markerless deletion of <i>CCNA_01218</i>	This study
pKR432	<i>CCNA_01218::FLAG</i> in pFLGC-1	This study
pKR435	pVGFPC-2-based complementation vector, <i>vanA::CCNA_01218-FLAG</i>	This study
pGS76	pNPTS138-based plasmid for markerless deletion of <i>CCNA_01219</i>	This study
pKR433	<i>CCNA_01219::FLAG</i> in pFLGC-1	This study
pKR436	pVGFPC-2-based complementation vector, <i>vanA::CCNA_01219-FLAG</i>	This study
pEK722	pNPTS138-based plasmid for markerless deletion of <i>CCNA_01220</i>	(Stankeviciute et al., 2019)
pKR434	<i>CCNA_01220::FLAG</i> in pFLGC-1	This study
pKR437	pVGFPC-2-based complementation vector, <i>vanA::CCNA-01220-FLAG</i>	This study
pKR438	pVGFPC-4-based complementation vector, <i>vanA::CCNA-01217-FLAG</i>	This study

Table S8, related to STAR Methods: Primers used in this study.

Name	Sequence (5'-3')
pJS14-PxylX	AGAACTAGTGGATCCTCACATGGTCTCGAA
PxylX-lpxC R	ACCCGAAGCCGACACGGCGTCGTCTCCCA
PxylX-lpxC F	TGGGGAGACGACGCCGTGTCCGGCTTCGGGT
lpxC-3xFLAG R	GTAGTCCATGGATCCAACCGCTTCTGCAAG
lpxC-3xFLAG F	CTTGCAGAAGCGGTTGGATCCATGGACTAC
ssrA-pJS14	CTTGATATCGAATTCTCACGCAGCGACGGC
pVCERN-2 00497 F	ACGCATATGAACAGCATTCTCCCG
pVCERN-2 00497 R	CCGGAGCTCCTAGATCGGCCGGCC
pVCERN-2 01553 F	ACGCATATGAAGCGTATGTTTGAT
pVCERN-2 01553 R	CCGGAGCTCCTAACGGGTGACGCC
pVCERN-2 03733 F	ACGCATATGGCTGCGATCTATCCG
pVCERN-2 03733 R	CCGGAGCTCTCAACGCGGCTTCGT
Pvan-fur	GAGGAAACGCATATGGATCGACTCGAAAAG
fur-pVCERN	AATTCTCCGGAGCTCTTACTCCTCCAGCGG
3xFLAG-F	cgccggcgatccatggactacaaag
3xFLAG-JS14	CTTGATATCGAATTCTCACTTGTCATCGTCATC
00497::hyg UpF	CTCACTAGTAGGACGACGCCATA
00497::hyg UpR	ATCCCCGGGGGCAAGGGTCGAGAC
00497::hyg DownF	ATCCCCGGGCGCCCGCTGCTGTGG
00497::hyg DownR	AGCGAATTCACCGAGGATTTGGTT
01055::hyg UpF	CTCACTAGTGCTGGCGCTGGAAGA
01055::hyg UpR	ACCGGATCCTTGAGCGCCATGGGC
01055::hyg DownF	ACCGGATCCGATGGACGAGCGCAG
01055::hyg DownR	AGCGAATTCTACGATGACGAGTCG
01068::hyg UpF	CTCACTAGTGCGCGAGGACACCGT
01068::hyg UpR	ACCGGATCCGACGCCTGGGTGGCG
01068::hyg DownF	ACCGGATCCCAGGCGGCGCCATAT
01068::hyg DownR	AGCGAATTCTTGACCTGCTTGAGC
01553 UpF	AAAACCTAGTATCGAGCAGGGCGTC
01553 UpR	GGCCCCGGGATCAAACATACGCTT
01553 DownF	ATCCCCGGGCGCGGTGCTGACCGCA
01553 DownR	AAAGAATTCTATGCCGCCAAGCT
03733::hyg UpF	CTCACTAGTGACGCTGGCCCTTGT
03733::hyg UpR	ACCGGATCCGAAGGCGTGATCGAG

03733::hyg DownF	ACCGGATCCGCCGCCACACAGGAT
03733::hyg DownR	AGCGAATTCATGCTCAAGGACCTC
fur UpF	CTCACTAGTAAGAGGGTGACCTCG
fur UpR	ACCGGATCCGAGCTCTACGGGATG
fur DownF	ACCGGATCCTTCGATACAGGCCTT
fur DownR	AGCGAATTCTATATGCAGGCCTTC
lpxC KO F	CTTCGAAACGGCCGATGATG
lpxC KO R	GATCAACAACGCCGATGACG
lpxC UpF	CTCACTAGTTCAGATAGGCTTCGA
lpxC UpR	CATGAATTCCTCAATAACGCCGTG
lpxC DownF	CTTGAATTCCGTGTGCTGAAAATA
lpxC DownR	GACGCATGCTGGCCGCAAGCCGCG
aacC1 EcoRI F	CGCGAATTCgaattgacataagcc
aacC1 EcoRI R	GGGAATTCgaattggccgcgcg
ctpA KO F	GAAGAAGCGCGGGATCAAGA
ctpA KO R	GTTGCCATGCTTGATGTGCA
EK897	AAGCTTGGCGCCAGCCGG
EK898	GAATTCGCTAGCTTCGGC
EK1047	gctggcgccaagcttAGGCGCTCGATCTGATCTTG
EK1048	caggaagatCGGCCCTTTTTGAACTTCAC
EK1049	aagggccgATCTTCCTGGCCCTCTTC
EK1050	cgaagctagcgaattcCTTGATCGTCGGGTTCTC
EK1055	gctggcgccaagcttGGCTTCTTCCACAACCTTG
EK1056	accttgagGAGGACCCCGGACATATC
EK1057	gggtcctcCTCAAGGTCTGGAGAGAG
EK1058	cgaagctagcgaattcCGTCATCTGTCGTCCCTAC
01218 up_fwd	attgaagccggctggcgccaTGGCACGGCCATTTCCGGC
01218 up_rev	tgagcccaaaGCCGTCGATCACCAGCAAG
01218 down_fwd	gatcgacggcTTTGGGCTCAAGCCGTTCCG
01218 down_rev	cgtcacggccgaagctagcgGCCGTTCCGACAAGCCGCG
EK1357	tactcatATGAGTAGTGAAGTTCAAAAAGGGCCG
EK1358	tactgctagcTTActgtcatgcatcctttagtcTTTCGCCAGCCAGGACTGG
EK S216	ACCCCGTCTGATAAAGGCTTC
EK S217	GCGAGACCGTGATCGACT
EK S238	GTAGGCAGGGTCCGACAGT
EK S239	ACCGCAAAGTTGTGGAAGAA

EK S240	ATGACCTTCCTCGACACG
EK S241	GAGTCGATCACGGTCTCG
EK S242	AGGGCTTCTTCTTTGGCATT
EK S243	ACTTCATCGTCGGCACCTT
RecUni-1	ATGCCGTTTGTGATGGCTTCCATGTCG
RecVan-2	CAGCCTTGGCCACGGTTTCGGTACC
Nde-01218	TATATTCATATGCTTCGTCTGCACGCCATCC
01218-Mlu	ATTATACGCGTTCCGACCAGGAACCGCAAGGC
Nde-01219	TATATTCATATGAGCCGCCTGCGCGGCCTC
01219-Mlu	TAATAACGCGTTGCGGCTTGCCGCCGCTCTC
Nde-01220	TATATTCATATGGGGCTATTTGATAAGCACCTGGCC
01220-Mlu	AATAACGCGTGGCGCGGGCGCGCTTGAG
01217-FLAG F	CCACGATGCGAGGAAACGCATATGAGTAGTGAAGTTCAAAAAG
01217-FLAG R	AATTAAGGCGCCTGCAGGCAGCTAGCTCACTTGTCATC
01218-FLAG F	CCACGATGCGAGGAAACGCATATGCTTCGTCTGCACG
01218-FLAG R	AATTAAGGCGCCTGCAGGCAGCTAGCTTACTTGTCATCGTC
01219-FLAG F	CCACGATGCGAGGAAACGCATATGAGCCGCCTGCGCGG
01219-FLAG R	AATTAAGGCGCCTGCAGGCAGCTAGCTTACTTGTCATCGTCATCCTTGTAG
01220-FLAG F	CCACGATGCGAGGAAACGCATATGGGGCTATTTGATAAG
01220-FLAG R	AATTAAGGCGCCTGCAGGCAGCTAGCTTACTTGTCATC

The following primer pairs were used for qRT-PCR analyses of the indicated genes.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>CCNA_03142 rpoD</i>	CTCTATGCGATCAACAAGCG	ATAGGCCTTGAGGAACTCGC
<i>CCNA_03372 bfd</i>	GTCTGCAACTGTAACGGCATC	CAGCCCTTGTGACGGAAG
<i>CCNA_00793 sgt1</i>	AGCCTTTGTCAGGACCAGAA	CACCACCTCGTCGAGAATTT
<i>CCNA_00792 sgt2</i>	CCACGAGCTGTTTCGTCATC	TGTAGTAGTTGGCGTCAAACG
<i>CCNA_01217 cpgA</i>	GTCACCTTCATCGGCTTTGT	CGAGGAAGGTCATGATCCAG
<i>CCNA_01218 cpgB</i>	GAGAGAACCCGACGATCAAG	CAGGTTGGTGGCCTTCAC
<i>CCNA_01219 cpgC</i>	CCGGGGTCCTCGACTACTAC	CCGTGCTCAAACCTCGTGATA
<i>CCNA_02064 lpxC</i>	TTGTTGATCACCGTGCCGAG	CCGACATGGGGATCGTCTTT