Supplement to:

The Cyclopropane Fatty Acid Synthase Mediates Antibiotic Resistance and Gastric Colonization of *Helicobacter pylori*

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Table S1. Oligonucleotide primers used in this study

Code	**Oligonucleotides**	**Oligonucleotides Sequence**
P1	aphAL-PstI	ATAAACTGCAGGCGAACCATTTGAGGTGATAG
P2	aphAR-BamHI	CGCGCGGATCCTTAAAACAATTCATCCAGTA
P3	CfaS-EcoRI-mut1	AATTGAATTCACTCTCACAGCTCACGCAA
P4	CfaS-PstI-mut2	TATACTGCAGTGTCTATTCCTTGAACTCT
P5	CfaS-BamHI-mut5	AATTGGATCCGATTTTAGCCCCTTCTTTCA
P6	CfaS-XbaI-mut6	AATATCTAGATTGTCCGTGCCTAATTCAGA
P7	HPCfaS-XhoI-L	TATACTCGAGTGTCCGCTTTAGAGATCG
P8	HPCfaS-PstI-R	GTGACTGCAGTTATTGGTAGATGTATTCTTTG
P9	pBS0203Cm04-KpnI-1	AATTGGTACCGGTTTAGAAGCGCAAAGT
P10	pBS0203Cm04-XhoI-2	GTCACTCGAGGCTGTTAATACTAATCCAA
P11	pBS0203Cm04-BamHI-3	AGATGGATCCAAGCCAATTCTTCCAATGA
P12	pBS0203Cm04-XbaI-4	AATTTCTAGAATGAGCCGGTGGTGATCA
P13	CatGC-PstI-L	CTGACTGCAGGATCCGCCATATTGTGTTGA
P14	CatGC-BamHI-R	ATATGGATCCCGCAGAACTGGTAGGTATGGA
P15	pHel3 HPcfa-XhoI-L	TATACTCGAGGGCCAATATGAGGGATGT
P16	pHel3 HPcfa-Kpnl-R	GCGCGGTACCTTATTGGTAGATGTATTCT
P17	HPCfaS-NdeI-L	ACGTGCATATGATTTCAAAATTTTTGCTCA
P18	HPCfaS-SalI-R	TCTTGTCGACTTATTGGTAGATGTATTCTT
P19	EcMtn-NdeI-L	GACGTCATATGAAAATCG GCATCATTG
P20	EcMtn-SalI-R	TCAAGTCGACTTAGCCATGTGCAAGTT
P21	BsLuxS-KpnI-L	ACGTAGGTACCATGCCTTCAGTAGAAAGT
P22	BsLuxS-SalI-R	TCTTGTCGACTTAGCCAAATACTTTTAGC

The underlined italic sequences are the introduced restriction sites.

Table S2. Fatty acid composition of *H. pylori* strain 26695 and its Δ*cfaS* mutant derivatives.

Fatty acid[a]	26695	HpS42 (Δ*cfaS*)	26995 +Dioc(50μM)[b]	HpS43 (Δ*cfaS:: cfaS*)
C14:0	36	17	18	33
C16:0	11	24	25	12
C18:0	12	25	26	13
C18:1	8	31	24	9
cycC19:0	28	ND	4	29

[a]The fatty acids listed are those positively assigned by gas chromatograpic data and with an abundance of greater that 1% in at least one sample. The data presented represent the mean of results from three independent experiments. cyc, cyclopropane ring; ND, not detected.

[b]Strain 26695 was cultured in the presence of 50 μM dioctylamine.

Table S3. Generation times of *H. pylori* strains

Strain	Generation time (h)[a]
26695	4.0±0.5
HpS42 (Δ*cfaS*)	6.7±0.8
26995+Dioc(10μM)[b]	5.0±0.3
26995+Dioc(50μM) [b]	6.6±0.2
HpS43 (Δ*cfaS:: cfaS*)	4.0±0.6
HpS48	3.9±0.5
HpS48+Dioc(50μM) [b]	6.4±1.0
HpS49	4.3±0.8
HpS49+Dioc(50μM) [b]	4.7±0.5

[a]The data presented represent the mean of results from three independent experiments.

[b]The strains were cultured in the presence of 10 μM or 50 μM dioctylamine.

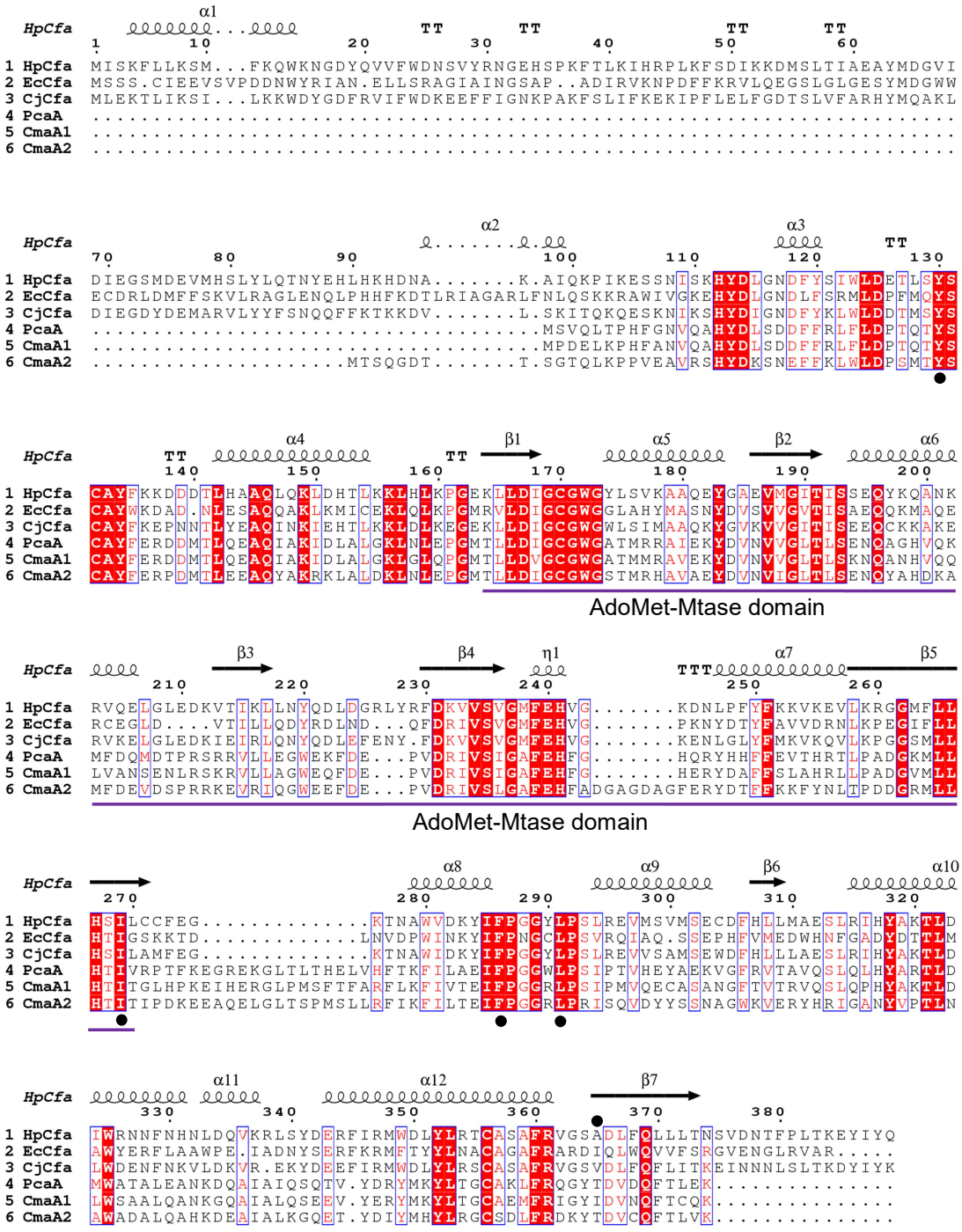

Fig S1. Multiple protein sequence alignments of CfaS homologues. The predicted protein sequence of HpCfaS (WP_000623798) is compared with the protein sequences of *E. coli* CfaS (WP_000098896), *Campylobacter jejuni* CfaS (WP_002892711), PcaA (WP_003402323), CmaA1 (WP_003900041) and CmaA2 (WP_003402621) from *M. tuberculosis*. The secondary structure diagrammed at the top is that of HpCfaS. Identical residues are in white letters with red background, similar residues are in red letters with white background, varied residues are in black letters, and dots represent gaps. α: α-helix; β: β-sheet; T: β-turns/coils. The AdoMet-MTase domains are indicated by an underline. The solid circles denote the dioctylamine-interacting amino acid residues.

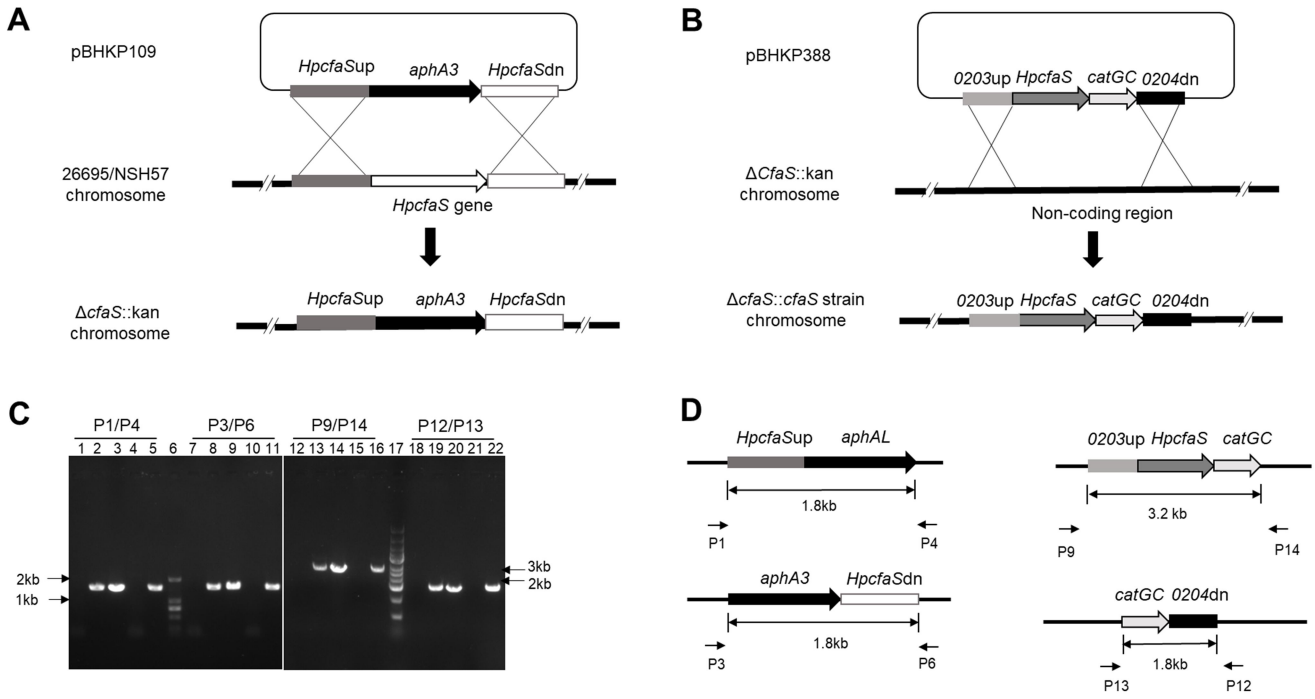

Fig S2. Construction and PCR confirmation of the Δ*cfaS* and Δ*cfaS* ::*cfaS* strains.

(A) Recombination events required to generate the Δ*cfaS* mutant strain using the *aphAL* cassette to displace *HpcfaS* gene in the wild type strain by natural transformation. (B) Recombination events required to generate the Δ*cfaS*::*cfaS* strain using chromosomal complementation system by natural transformation. (C) and (D). PCR confirmation of the mutant and complementation strains. As shown in D, expected product size using the primer pair P1/P4 and P3/P6 for mutant confirmation is ~1.8 kb, while the expected product size using the primer pair P9/P14 and P13/P12 for complementation confirmation is ~3.2 kb and 1.8 kb, respectively. From left to right, the temples (lane1-5 and lane7-11) used in the PCR reactions are the genomic DNAs of the strain 26695, 26695 Δ*cfaS*, Hp159 Δ*cfaS*, Hp159 and the plasmid DNA of pBHKP203, respectively, while the temples (lane12-16 and lane 18-22) are the genomic DNAs of the strain 26695 Δ*cfaS*, 26695 Δ*cfaS* ::*cfaS*, Hp159 Δ*cfaS* ::*cfaS*, Hp159 Δ*cfaS* and the plasmid DNA of pBHKP388, respectively.

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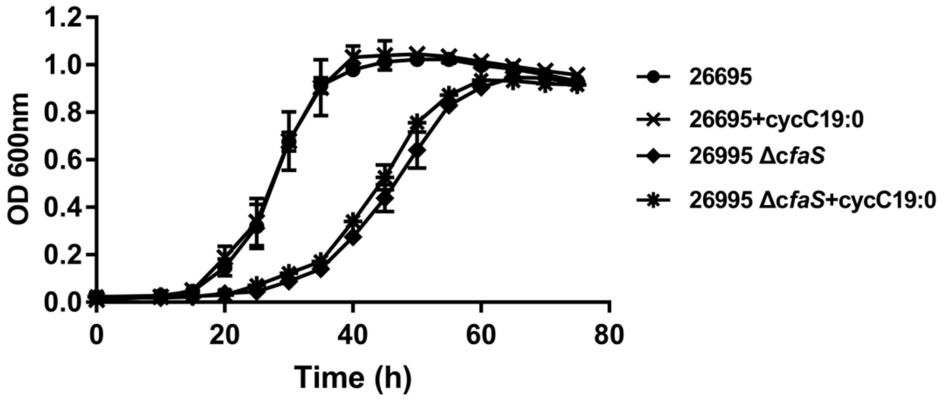

Fig S3. Growth curves of the *H. pylori* 26695 and Δ*cfaS* strains on BHI broths containing FCS supplemented with or without cycC19:0 (0.01%) at 37℃. At the indicated time points, data represented as OD_{600} measurements. The data are the means with standard error bars from three independent determinations.

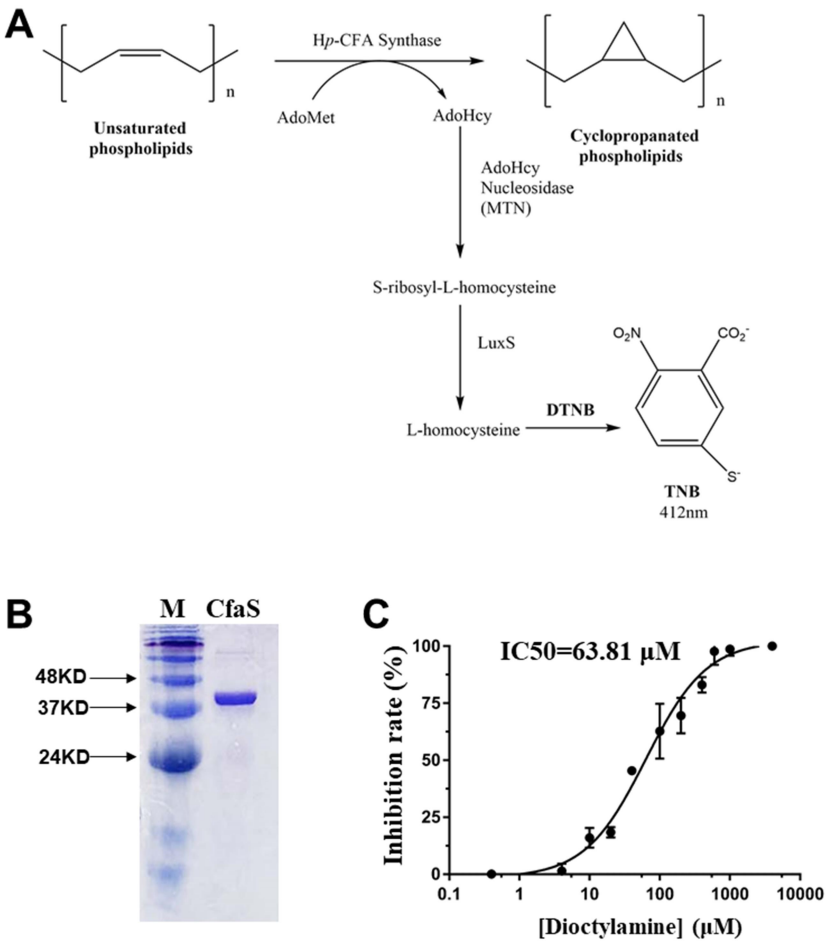

Fig S4. Dioctylamine inhibits HpCfaS methyltransferase activity. (A) Coupled Assay for HpCfaS Methyltransferase Activity. In this method, SAH, the product of cyclopropane mycolic acid synthases, is converted to homocysteine by the highly efficient SahH which can react readily with Ellman's reagent (DTNB) resulting in absorbance at 412 nm. (B) SDS-PAGE analysis of the purified HpCfaS. The apparent molecular weight of His-tagged RpfB is about 46 kDa. (C) Dose-response curve of dioctylamine inhibition of HpCfaS as measured in a coupled colorimetric assay. Values are plotted for percent inhibition relative to DMSO control. Concentration of HpCfaS used was 0.5 μM.

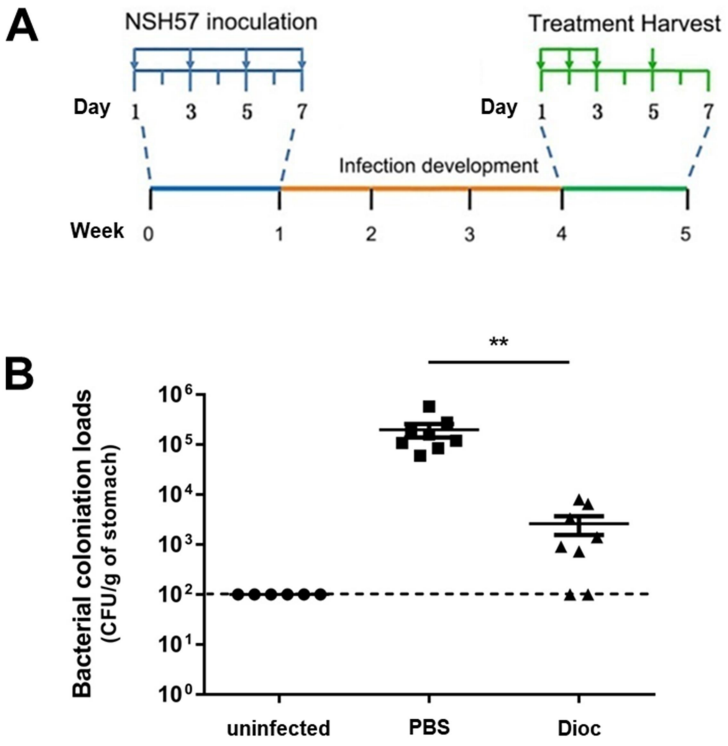

Fig S5. Anti-*H. pylori* efficacy *in vivo*. (A) The study protocol including *H. pylori* inoculation and infection development in C57BL/6 mice, followed by the treatments. (B) Quantification of bacterial burden in the stomach of *H. pylori*-infected mice treated with PBS or Dioctylamine. Error bars represent the SD derived from 6 mice (uninfected), 8 mice (PBS) or 8 mice (Dioc) per group. If no colonies were present, calculations were made using the limit of detection (100 CFU/g stomach). **$P < 0.01$.