Supplemental Information for:

Rcs phosphorelay activation in cardiolipin-deficient *Escherichia coli* reduces biofilm formation

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Running title: Biofilm reduction in cardiolipin-deficient *E. coli*

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Supplemental Methods

Determination of* fim *invertible element orientation. We grew *E. coli* cells in 96 well microplates and used sonication to harvest biofilm cells as described above. We used an Epicentre Master Pure Complete DNA and RNA Purification kit (Madison, WI, USA) to extract genomic DNA from cells. Multiplex PCR was performed using New England Biolabs Q5 High-Fidelity DNA Polymerase (Ipswich, MA, USA), following the manufacturer's instructions. Primers were included to amplify off-oriented *fimAp* (INV and FIME), on-oriented *fimAp* (INV and FIMA), and *ftsZ* (EcFtsZ 1 and EcFtsZ 2; loading control) (1, 2).

Tables

Table S1. Strains of *E. coli* used in this study

Strain name	**Relevant genotype**	**Reference**
MG1655	Wild-type *E. coli*	Laboratory strain
PO10	MG1655 Δ*clsABC::FRT*	(3)
Δ*clsA*	MG1655 Δ*clsA::FRT*	This work
Δ*clsB*	MG1655 Δ*clsB::FRT*	This work
Δ*clsC*	MG1655 Δ*clsC::FRT*	This work
Δ*clsBC*	MG1655 Δ*clsBC::FRT-kan-FRT*	This work
Δ*clsAC*	MG1655 Δ*clsAC::FRT-kan-FRT*	This work
Δ*clsAB*	MG1655 Δ*clsAB::FRT-kan-FRT*	This work
Δ*flhD*	BW25113 Δ*flhD::FRT-kan-FRT*	(4)
Δ*cls* Δ*rcsA*	MG1655 Δ*clsABC::FRT* Δ*rcsA::FRT-cam-FRT*	This work
Δ*cls* Δ*rcsC*	MG1655 Δ*clsABC::FRT* Δ*rcsC::FRT-cam-FRT*	This work
Δ*cls* Δ*rcsF*	MG1655 Δ*clsABC::FRT* Δ*rcsF::FRT-cam-FRT*	This work
DH5α	F^- *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG* Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, *hsd*R17(r_K^- m_K^+), λ^-	Laboratory strain
5-alpha F' I^q	F′ *proA*$^+$*B*$^+$ *lacI*^{q} *Δ(lacZ)M15 zzf::Tn10* (Tet^R) / *fhuA2Δ(argF-lacZ)U169 phoA glnV44 Φ80Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	New England Biolabs

Table *S2.* Plasmids used in this study

Plasmid	**Relevant genotype**	**Reference**
pMP4641	TetR	(5)
pMP4658	TetR	(5)
pBad33	Low copy arabinose inducible expression plasmid	(6)
pBad33-*clsA*	clsA inserted between XbaI and SphI sites; CamR	This work
pBad33-*clsB*	clsB inserted between XbaI and HindIII sites; CamR	This work
pBad33-*ymdBC*	ymdB-clsC inserted between XbaI and HindIII sites; CamR	This work
pBad33-*clsA*(H224F H404F)	clsA with H224F and H404F substitutions	This work
pBad33-*clsB*(H113A)	clsB with H113A substitution	This work
pBad33-*clsB*(H290A)	clsB with H290A substitution	This work
pBad33-*ymdB-ymdC*(H130A)	clsC with H130A substitution, ymdB wild-type	This work
pBad33-*ymdB-ymdC*(H369A)	clsC with H369A substitution, ymdB wild-type	This work
pBad33-*phoA*	phoA inserted into X site; CamR	This work
pCP20	FLP recombinase expression plasmid; AmpR CamR; temperature sensitive	(7)
pKD3	AmpR CamR	(7)
pKD46	AmpR; temperature sensitive	(7)
pBad33-*ompA*-6XHis	CamR	This work

Table *S*3. Primers

Name	**Sequence (5′→3′)**
INV[1]	GAGGTGATGTGAAATTAATTTAC
FIMA[1]	GATGCGGTACGAACCTGTCC
FIME[1]	GCAGGCGGTTTGTTACGGGG
EcFtsZ 1[2]	TAGCGGTATCACCAAAGGACT
EcFtsZ 2[2]	GTGATCAGAGAGTTCACATGCT

[1]Schwan *et al.* (1992), *J Bacteriol*

[2]Schwan *et al.* (2007), *J Bacteriol*

qPCR		
Gene	**Forward (5′→3′)**	**Reverse (5′→3′)**
clsA	ATATAATCGTCCTGAACGGCGGC	TGGTATGCAGTAACCCGCCTTCA
clsB	TAAAAAGTGGAACGCCAGCACG	AGTGACAAACTGAGCGGATCGAGA
clsC	CCGAAATGGCAGAGCATACGCTC	CGCTTGCTTGACAGTCATCCACG
cpsB	GTTGGCTCCTGGTCTTCATTA	CAGGCCAGATTCAGCATACA
cpsG	TGACGTGCTGGATATTGGTATG	GATTATGGCTGGCGGTAACT
degP	GCCTTCAGTGGTCAGCATTA	GGCAGAACGGAGAATCATCA
htrA	TATCGCGCTGATCCAAATCC	ACCAATCGCTACGGTGTAATC
mdtA	CAAGCAGGTTGATGTTGGTAAC	CCGGCAGGGTAAAGACTAAAT
acrD	TGACCTCGCTGGCATTTATC	CGAAATCATCCCGCCCATTA
pspA	CTGATGATCCAGGAGATGGAAG	TGTTCAATACGGCGAGTCAG
pspC	CTGGTGGTGCTGTCGATTT	AAGGTAGCTGCTCACCAAAG
idnT[3]	CTGTTTAGCGAAGAGGAGATGC	ACAAACGGCGGCGATAGC
gapA[4]	GTGATCCGGCTAACCTGAAA	GTCCTGGCCAGCATATTTGT

[3]Zhou *et al.* (2011), *BMC Mol Biol*

[4]Uchiyama *et al.* (2010), *FEMS Microbiol Lett*

Table *S4.* Minimum inhibitory concentration (MIC) of antimicrobial compounds in *E. coli* grown at 30°C in M9 minimal medium supplemented with amino acids. Values shown are the averages of 3 independent biological replicates and the standard error of the mean.

Compound	**MG1655**			**Δ*clsABC***		
Cecropin A[a]	12.5	±	0	4.1	±	1.1
Ampicillin[a]	16.0	±	2.7	13.3	±	2.7
Kanamycin[a]	16.0	±	5.3	10.7	±	2.7
EDTA[a]	64.0	±	10.7	16.0	±	0
Polymyxin B[a]	8.7	±	4.1	4.0	±	0
Cefuroxime[a]	32.0	±	0	8.0	±	4.0

[a] Values in μg/mL

Supplemental Figures

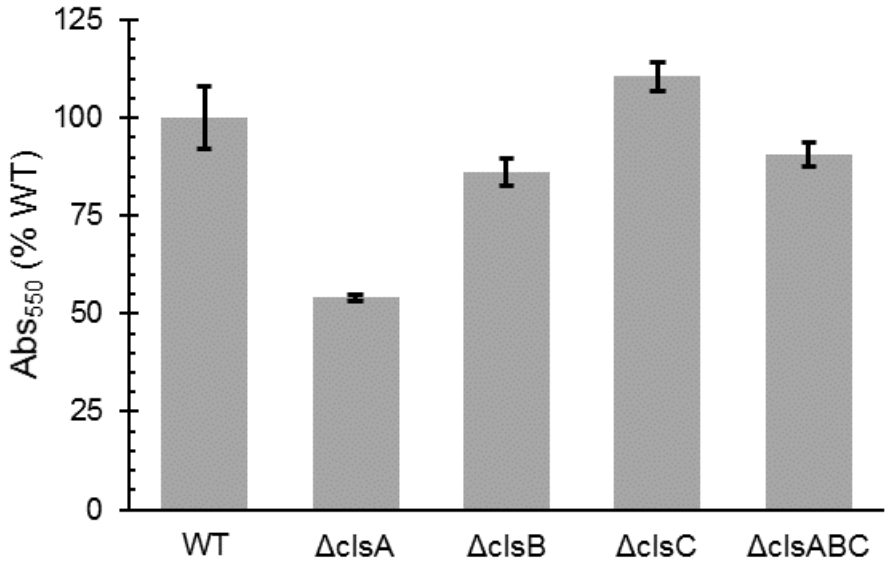

Figure S1. Surface attachment in rich nutrient medium. Cells were grown in lysogeny broth (LB) in microtiter plates for 24 h at 30°C without shaking. Adherent cells were stained with crystal violet (CV), and CV absorbance was measured at 550 nm. Error bars indicate standard error (N ≥6); differences between strains were not significant based on a Student's *t* test ($p > 0.05$), except WT vs. Δ*clsA* ($p < 0.0001$).

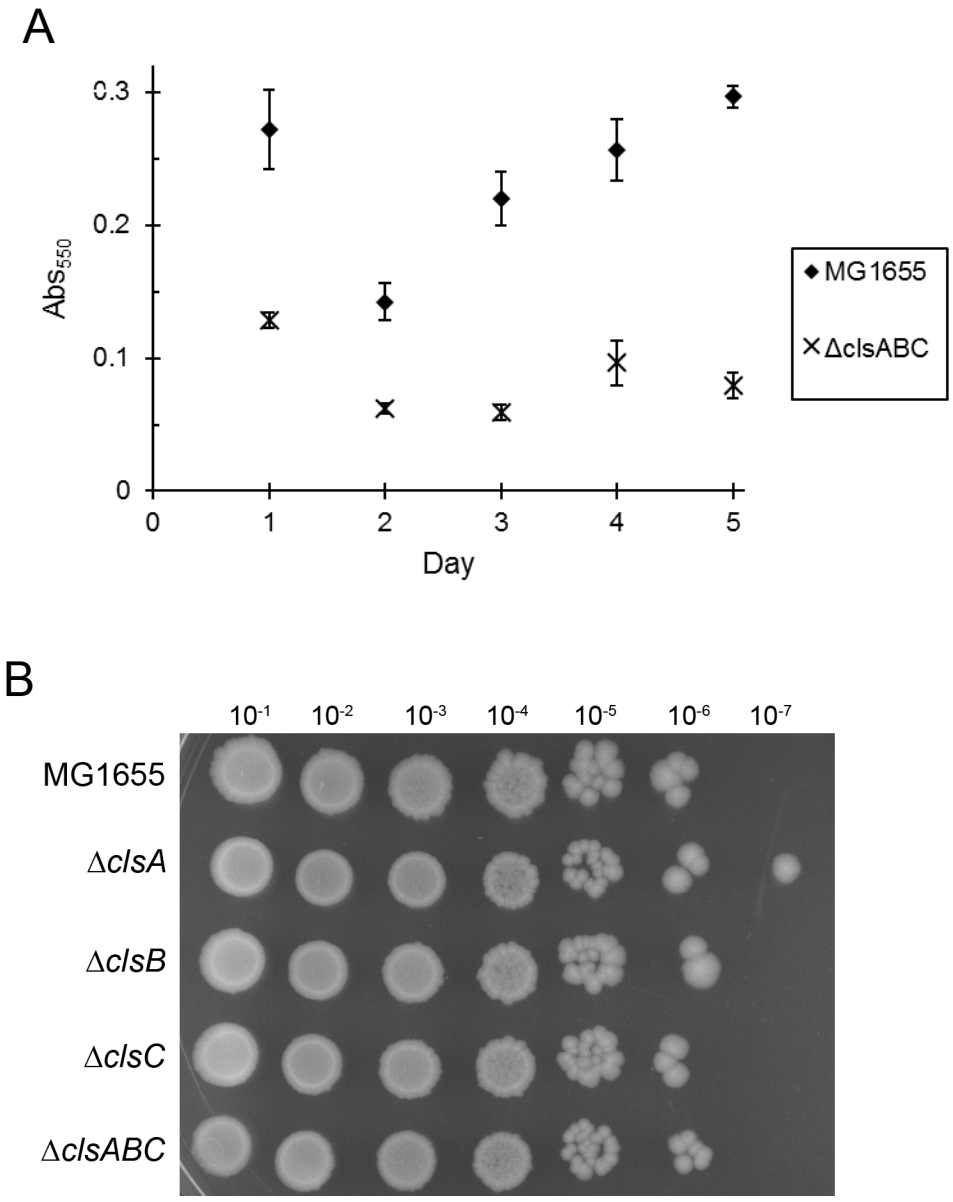

Figure S2. Surface attachment over multiple days. Cells were grown in microtiter plates for 24 h at 30°C without shaking. (A) Adherent cells were stained with crystal violet (CV), and CV absorbance was measured at 550 nm. Error bars indicate standard error ($N \geq 6$). (B) Colony forming units were determined for 5 day-old cultures.

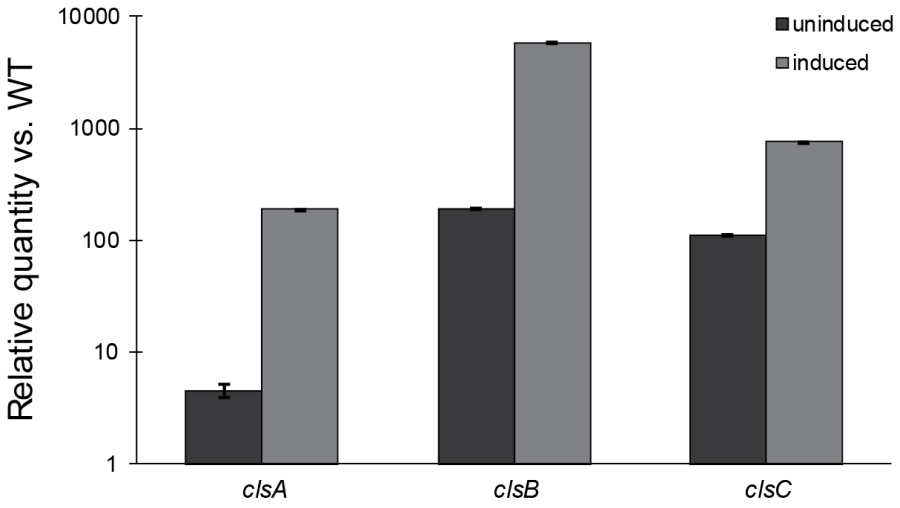

Figure S3. Induction level of *cls* transcripts. We extracted RNA from 24 h old cultures grown with (induced) or without (uninduced) 0.2% arabinose to stimulate protein expression. All strains lacked a chromosomal copy of one *cls* gene, which was replaced with a vector copy of the gene in the arabinose-inducible plasmid pBad33 (e.g. Δ*clsA*/pBad33-clsA).

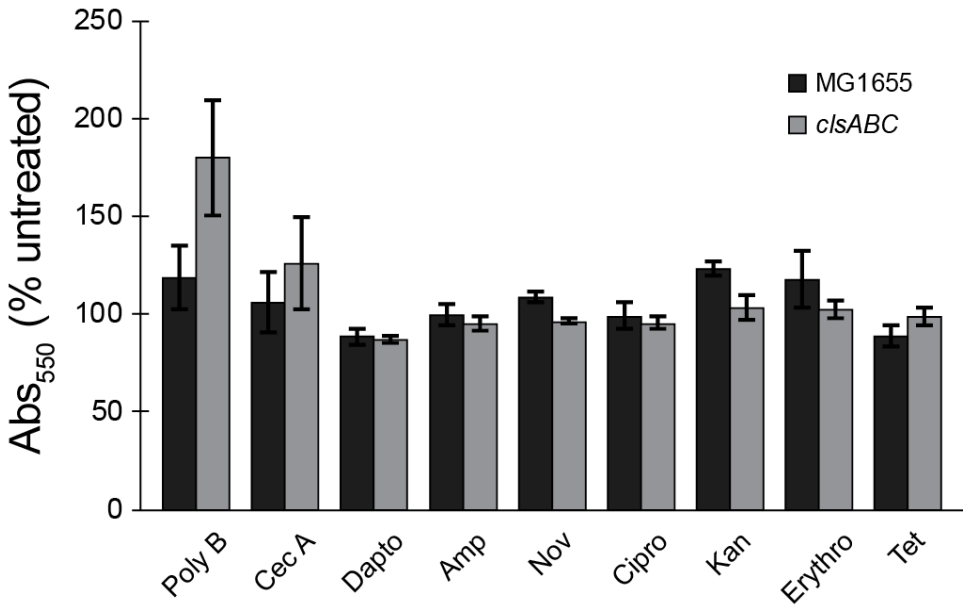

Figure *S*4. Effect of treatment with antimicrobials on biofilm formation in Δ*clsABC* cells. Cells were grown in M9 minimal medium in microtiter plates for 24 h at 37°C without shaking, then treated with antimicrobial compounds for 24 h as described in the Materials and Methods. Adherent cells were stained with CV, and CV absorbance was measured at 550 nm. Abs values were normalized to those obtained from untreated controls. Error bars indicate standard error (N ≥ 16); (Poly B, polymyxin B; Cec A, cecropin A; Dapto, daptomycin; Amp, ampicillin; Nov, novobiocin; Cipro, ciprofloxacin; Kan, kanamycin; Erythro, erythromycin; Tet, tetracycline).

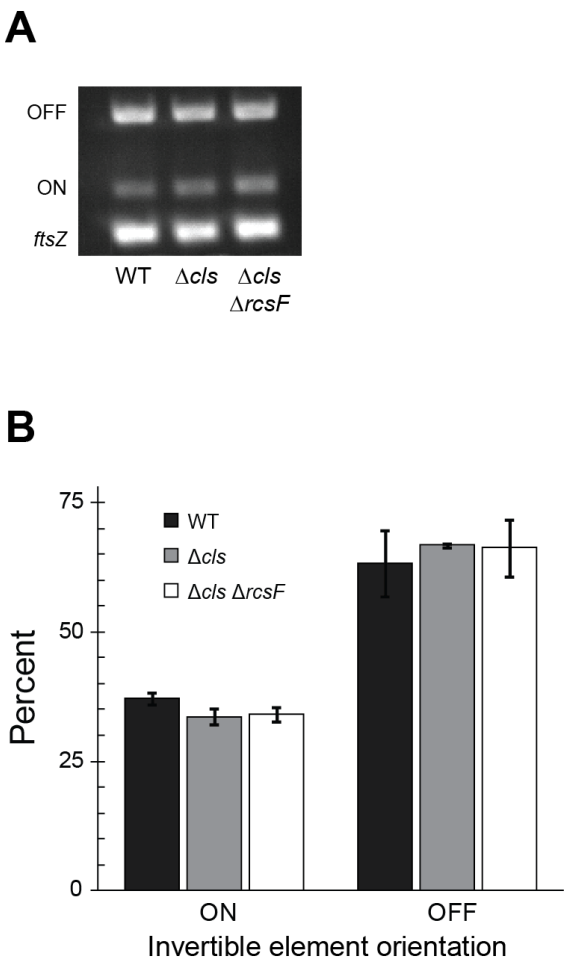

Figure S5. Fimbriation of CL deficient cells. Genomic DNA was extracted from *E. coli* biofilms as described in the Materials and Methods and PCR was used to determine the orientation of the FimA invertible element. (A) INV and FIMA primers amplify phase-on oriented DNA (450 bp product), INV and FIME primers amplify phase-off oriented DNA (750 bp product), and EcFtsZ1 and 2 primers amplify a 302 bp segment of the *ftsZ* gene. (B) ImageJ was used to determine the intensity of DNA bands, which were normalized to the *ftsZ* loading control. Error bars indicate standard error (N = 3); differences between strains were not significant based on a Student's *t* test ($p > 0.05$).

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