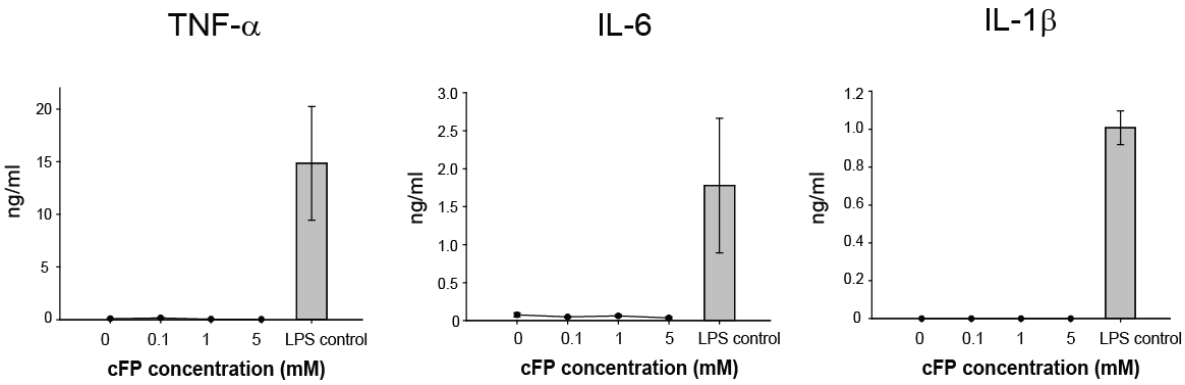

Figure S1. cFP does not induce pro-inflammatory cytokine expression in mouse macrophages.

(A) J774A.1 cells were treated with 100 ng/ml LPS or the indicated concentration of cFP for 24 h. The levels of TNF-α and IL-6 from the culture supernatants were measured by ELISA. Data represent mean +/- SD (n=3).

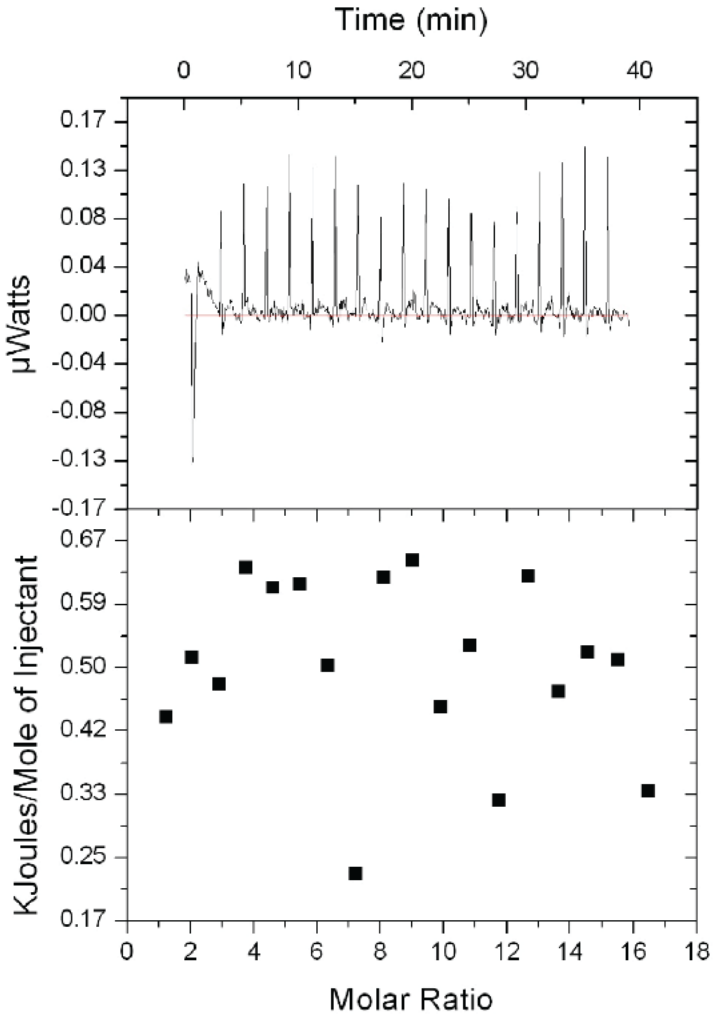

Figure S2. cFP does not bind to LPS.

Isothermal titration calorimetry was used to assess interactions between LPS and cFP. cFP (1 mM) was titrated into the solution of LPS (12 µM) at 37℃, and the enthalpy change was monitored. Data are representative of three independent experiments with similar results

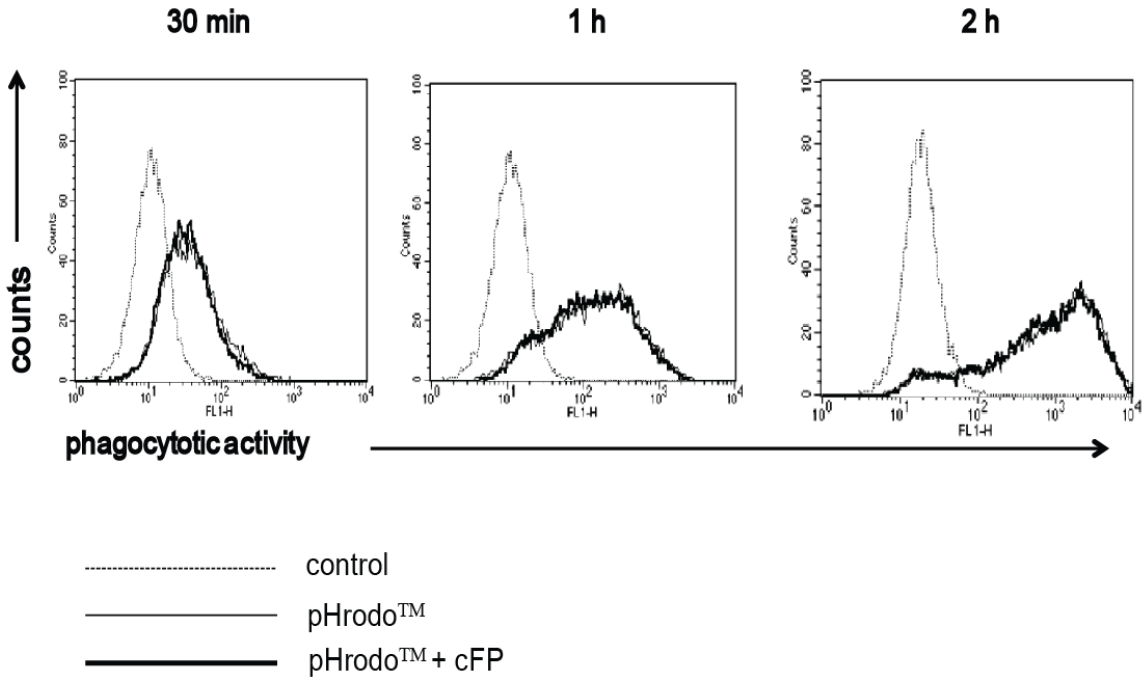

Figure S3. cFP does not affect phagocytosis of macrophages.

J774A.1 cells were treated with 100 μl pHrodo™ Green *E. coli* BioParticles® Conjugate in the presence or absence of 4 mM cFP for the indicated time, and the phagocytosis activity was detected by flow cytometry.

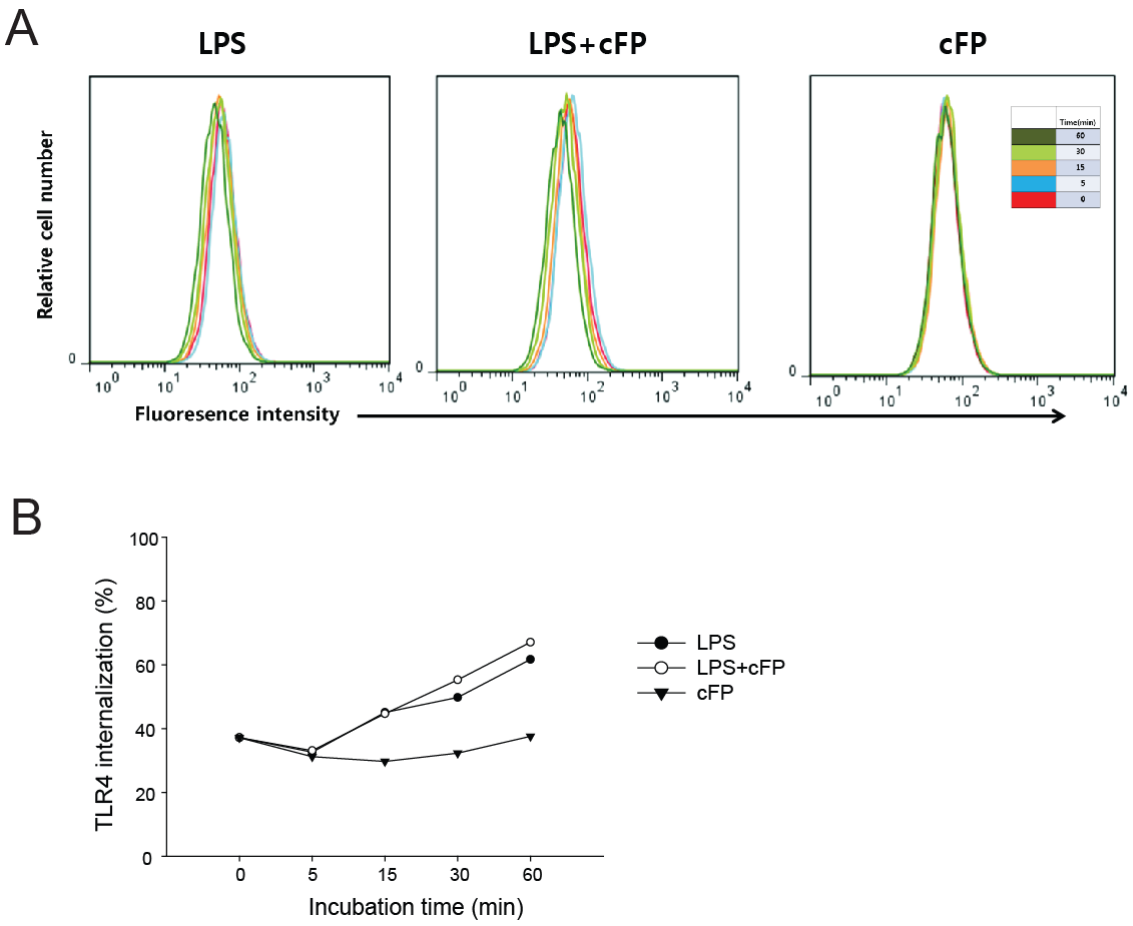

Figure S4. cFP does not affect TLR4 internalization induced by LPS.

(A) J774A.1 cells were treated with LPS in the presence or absence of 4 mM cFP for the indicated time. The TLR4 internalization ratio was measured using a TLR4-PE antibody by FACS analysis. Each experiment was performed in triplicate and repeated at least three times, which yielded similar results. (B) Plotting presentation of the data shown in (A).

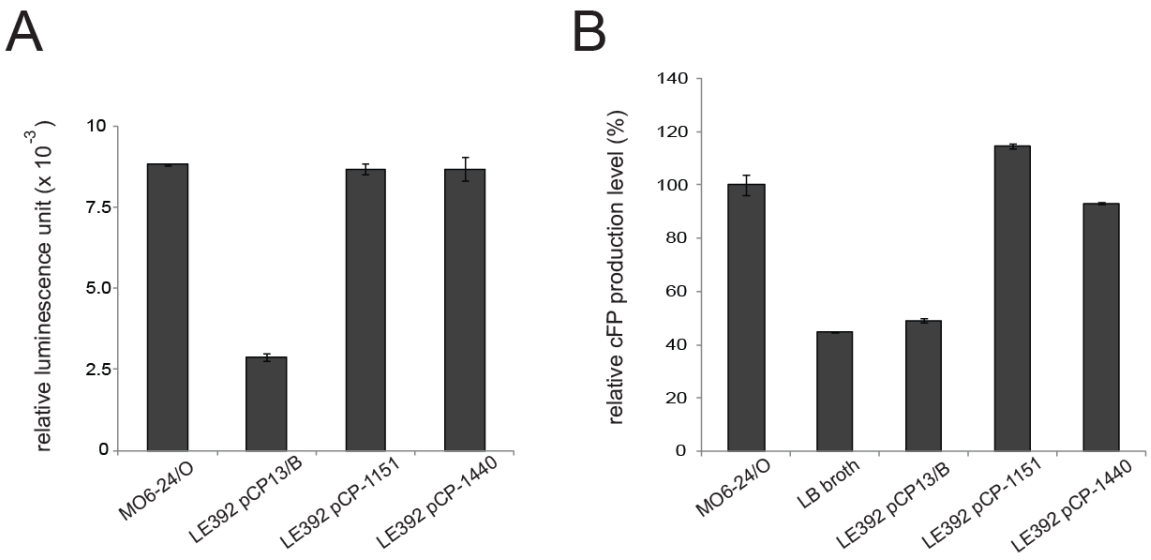

Figure S5. The cFP production in wild-type V. vulnificus, LE392 pCP-13/B, LE392 pCP-1151, and pCP-1440.

(A) Luciferase activity of wild-type *V. vulnificus* MO6-24/O, LE392 pCP-13/B, LE392 pCP-1151, and pCP-1440 was measured using the cFP bio-reporter MT102 pSB403 as described in the Materials and Methods. The relative luminescence units (RLU) represent the luminescence values normalized to cell density (OD_{600}). (B) Relative cFP production levels of wild-type *V. vulnificus* MO6-24/O, LB broth, LE392 pCP-13/B, LE392 pCP-1151, and pCP-1440. cFP production was measured using HPLC as described in the Materials and Methods. The data are the average values of three independent experiments, and error bars denote SDs.

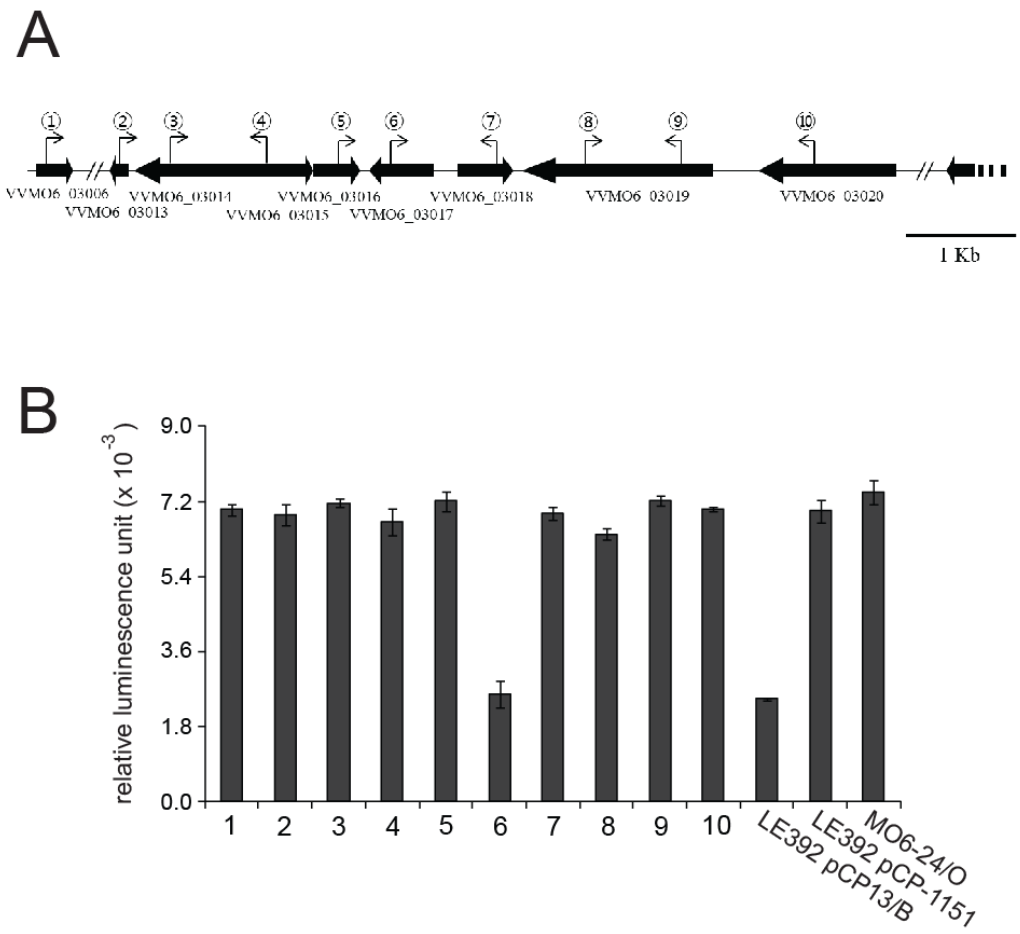

Figure S6. A transposon insertion mutant of VVMO6-03017 decreased cFP production. (A) The list of genes in the pCP-1151 clone and the position of the transposon insertion. The directions of the transposons are marked with arrows. (B) The luminescence of each transposon insertion mutant was measured using the cFP bio-reporter MT102 pSB403 as described in the Methods. Numbers 1 to 10 represent mutants with transposon insertions indicated in the gene illustration shown in (A). The relative luminescence units (RLU) represent the luminescence values normalized to cell density (OD_{600}). The data are the average values of three independent experiments, and error bars denote SDs.

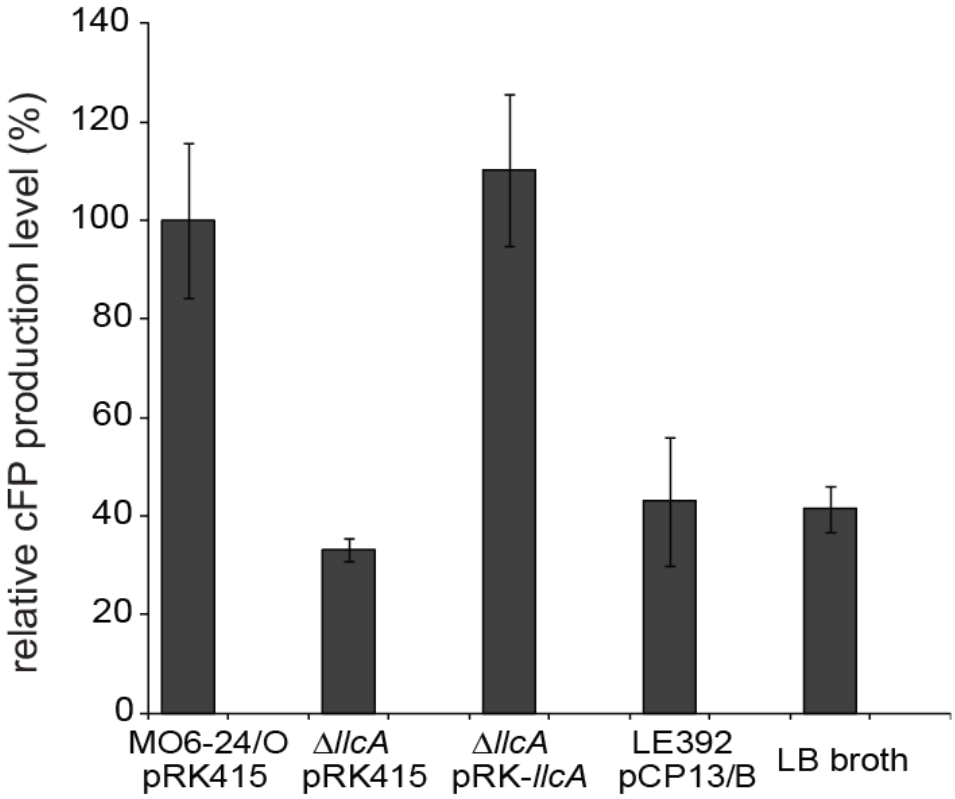

Figure S7. The *llcA* gene was related to cFP production.

Relative cFP production levels of *V. vulnificus* MO6-24/O (pRK415), Δ*llcA* (pRK415), and the complementation strain Δ*llcA* (pRK-*llcA*) were measured using HPLC as described in the Materials and Methods. The data are the average values of three independent experiments, and error bars denote SDs.

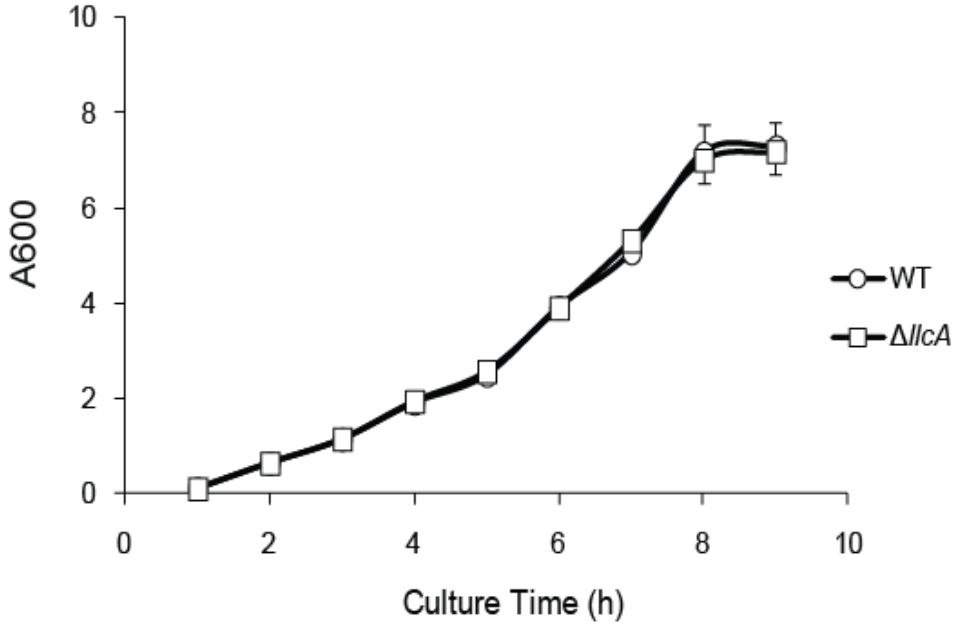

Figure S8. Δ*llcA* mutant has no defect in growth rate.

Overnight cultures of wild type (MO6-24/O) and Δ*llcA* mutant grown in LB were washed and diluted to the A_{600} (absorbance at 600 nm) value of 0.1 into fresh LB medium. Bacteria were grown at 30 ^{o}C for indicated times, and A_{600} was measured.

Table S1. Bacterial strains and plasmids used in this study

Strains or plasmids	Genotypes	Sources
Strains		
E. coli		
DH5α	λ^- φ80d*lacZ* Δ*M15* Δ(*lacZYA-argF*)*U169 recA1 endA1 hsdR17* (r_K^- m_K^-) *supE44 thi-1 gyrA relA1*	Our collection
LE392	*glnV44 supF58* (*lacY1* or Δ*lacZY*) *glaK2 metB1 trpR55 hsdR514*(r_k^- m_k^+)	Promega
S17-1	[C600::RP4-2 (Tc::Mu)(Km::Tn*7*) *thi pro hsdRM*$^+$ *recA*, Tp^r	[1]
S17-1 λ *pir*	S17-1 with λ*pir* lysogen	[1]
V. vulnificus		
MO6-24/O	Pathogenic clinical isolate	[2]
Δ*llcA*	Derivative of MO6-24/O with a deletion in *llcA*	This study
Plasmids		
pCP13/B	Broad host range cosmid vector, Tc^r	[3]
pCP-1151	pCP 13/B vector containing the partial genome of *V. vulnificus*	This study
pGEM-T Easy	TA-cloning vector, *lacZ*, f1 origin, Ap^r	Promega
pGEM-llcKO	pGEM-T Easy vector containing the deletion in *llcA*	This study
pRK415	IncP *ori*, broad-host-range vector; *oriT* of RP4, Tc^r	[4]
pRK-*llcA*	pRK415 with *V. vulnificus llcA*	This study
pDM4	Suicide vector for allelic exchange, *sacB*, Cm^r	[5]
pDM4-llcKO	pDM4 with a *V. vulnificus llc* deletion	This study

* Numbers indicate nucleotide positions relative to the translational start site

Abbreviations: Tp, trimethroprim; Tc, tetracycline; Ap, ampicillin; Cm, chloramphenicol

Source references

1. Simon R, Priefer, U., Pühler, A. (1983) A broad host range mobilization system for in vivo genetic

engineering: transposon mutagenesis in gram negative bacteria. Nature Biotechnology 1: 784-791.

2. Reddy GP, Hayat U, Abeygunawardana C, Fox C, Wright AC, et al. (1992) Purification and determination of the structure of capsular polysaccharide of Vibrio vulnificus M06-24. Journal of bacteriology 174: 2620-2630.
3. Darzins A, Chakrabarty AM (1984) Cloning of genes controlling alginate biosynthesis from a mucoid cystic fibrosis isolate of Pseudomonas aeruginosa. Journal of bacteriology 159: 9-18.
4. Keen NT, Tamaki S, Kobayashi D, Trollinger D (1988) Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. Gene 70: 191-197.
5. Milton DL, O'Toole R, Horstedt P, Wolf-Watz H (1996) Flagellin A is essential for the virulence of Vibrio anguillarum. Journal of bacteriology 178: 1310-1319.

Table S2. Primers used for real time-PCR

Genes	Sequences (5'→3')	
Il6	F: TAGTCCTTCCTACCCCAATTTCC	R: TTGGTCCTTAGCCACTCCTTC
Tnfa	F: CCCTCACACTCAGATCATCTTCT	R: GCTACGACGTGGGCTACAG
Inos	F: GTTCTCAGCCCAACAATACAAGA	R: GTGGACGGGTCGATGTCAC
Rantes	F: GCTGCTTTGCCTACCTCTCC	R: TCGAGTGACAAACACGACTGC
Ip10	F: CCAAGTGCTGCCGTCATTTTC	R: GGCTCGCAGGGATGATTTCAA
Mcp1	F: TTAAAAACCTGGATCGGAACCAA	R: GCATTAGCTTCAGATTTACGGGT
Hprt	F: GTTGGATACAGGCCAGACTTTGTTG	R: GAGGGTAGGCTGGCCTATAGGCT

F: Forward direction, R: Reverse direction

Table S3. Primers used for cloning and construction of *llc* mutant

Purpose/ Name	Nucleotide sequence (5′ to 3′)[a]
Construction of a *llc* in-frame deletion mutant	
llcKO_F1	GTTACGCAACTTTTCATCTA
llcKO_B1	GAACCTTGGGGCGGTAGCAACA
llcKO_F2	GAGAAACCAAGGCACTGCCT
llcKO_B2	CTTCGGCCCCAACCCTTGCA
Cloning of *llc*	
llc_comF	GACGGCCAGTGAATTCCTGCGTTTTATGGTTTTTCTC
llc_comB	GCTTGCATGCCTGCAGTGATTAAATAGCTCGTGTTCTGGTGT

[a] Nucleotides modified for the generation of restriction sites are underlined.