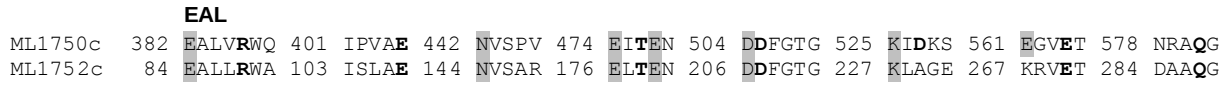
Supplemental data

Table S1. Plasmids used in this study

Plasmids	Description	Reference or source
pJN105	*P. aeruginosa* arabinose inducible expression vector, Gm^R, *araC-*P_{BAD}	(Newman & Fuqua, 1999)
pJN1120	pJN105 containing *tpbB* (PA1120)	(Hickman & Harwood, 2008)
pMRLB105	pJN105 containing *ml1419c*	This study
pMRLB108	pJN105 containing *ml1419c*$_{\Delta GGDEF}$	This study
pMRLB109	pET28a (Kan^R, IPTG inducible, *T7*) containing *ml1419c*	This study

Figure S1

(a)



(b)

Proteins	Active site residues						
ML1750c	E (382)	N (442)	E (474)	E (477)	D (504)	K (525)	E (561)
ML1752c	E (84)	N (144)	E (176)	E (179)	D (206)	K (227)	K (267)
MSMEG_2196	E (384)	N (444)	E (476)	E (479)	D (506)	K (527)	E (563)
Rv1354c	E (389)	N (449)	E (481)	E (484)	D (511)	K (532)	E (568)
Rv1357c	E (89)	N (149)	E (181)	E (184)	D (211)	K (232)	K (272)

Figure S1. Comparison of the active residues and positions in conserved EAL domain containing proteins from *M. leprae* TN, *M. smegmatis* mc^{2}155, and *M. tuberculosis* H37Rv. (a) Alignment of protein sequence containing EAL domains of *M. leprae* TN (ML1750c and ML1752c). The amino acids that contribute to PDE activity are highlighted with gray. The other known conserved amino acids surrounding the active site are in bold text (Romling *et al.*, 2013). (b) The conserved residues in proteins containing EAL domains. The position of each conserved residue is shown in parentheses. The conserved residues of the A-site in predicted EAL domains are present in all proteins as described as previously (Romling *et al.*, 2013). Interestingly, ML1752c from *M. leprae* TN and Rv1357c from *M. tuberculosis* H37Rv have K residues instead of E in the last position of predicted active sequence. However, Rv1357c has been shown to have PDE activity to convert cyclic di-GMP to pGpG in a previous study (Gupta *et al.*, 2010). Glutamic acid (E), Asparagine (N), Aspartic acid (D), Lysine (K).

Figure S2

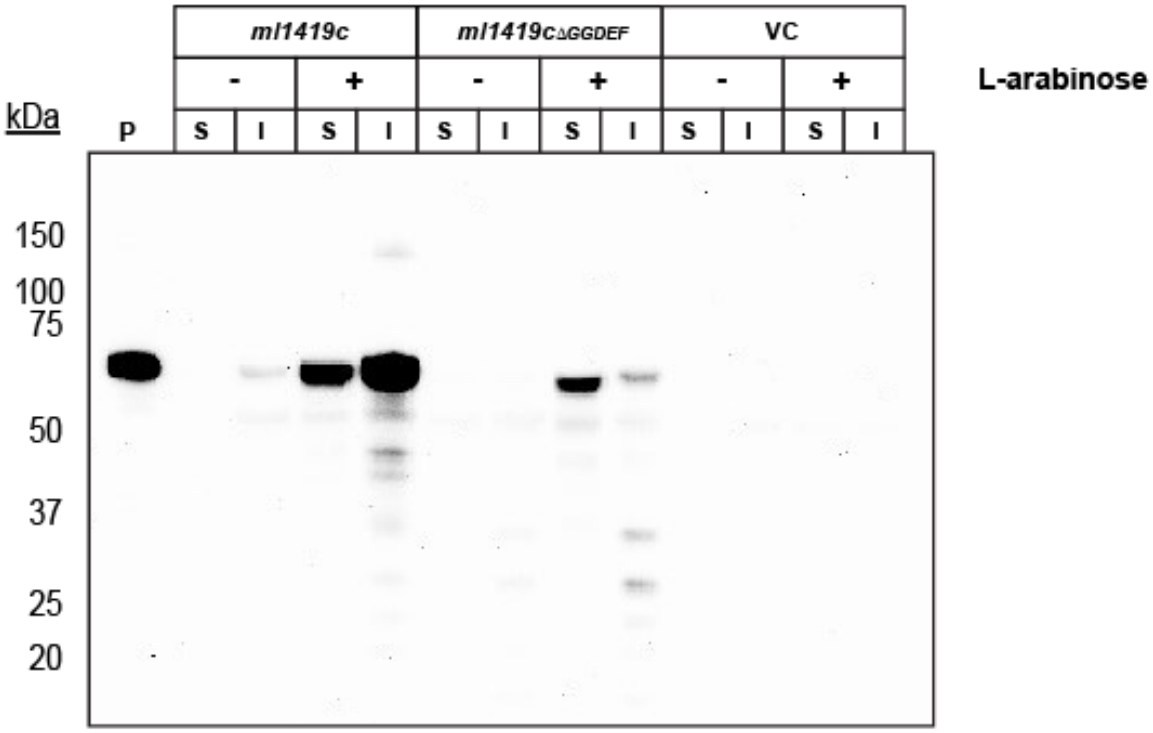


Figure S2. Expression of *ml1419c* from *M. leprae* in subcellular fractions from *P. aeruginosa* PAO1. Recombinant *P. aeruginosa* PAO1 containing *ml1419c*, *ml1419c*$_{\Delta GGDEF}$, and the vector control (VC) were grown in the presence (+) or absence (-) of 0.2% L-arabinose. Soluble (S) and insoluble (I) protein fractions (5 µg) from whole cell lysates of *P. aeruginosa* PAO1 containing *ml1419c*, *ml1419c*$_{\Delta GGDEF}$, and the vector control (VC) were analyzed by SDS-PAGE with Western blot. Purified recombinant ML1419c produced in *E. coli* was used as a positive control (lane P) (25 ng).

Figure S3

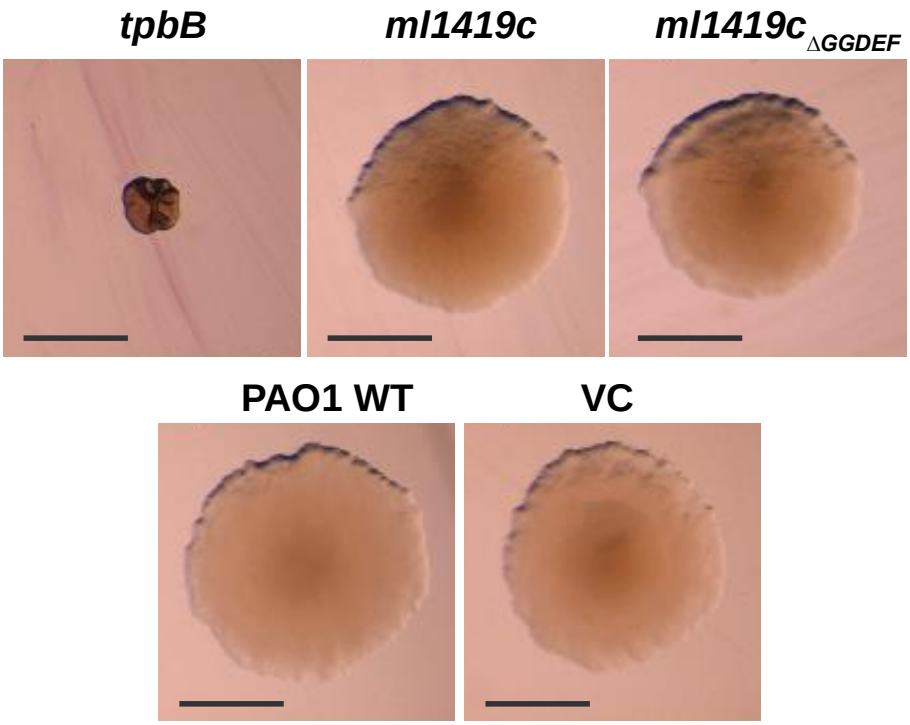


Figure S3. Colony morphology of *P. aeruginosa* PAO1 expressing *ml1419c* grown in the absence of arabinose. Recombinant *P. aeruginosa* PAO1 strains were grown without arabinose on VBMM agar containing Congo red, brilliant blue. Rugose colonies were observed in *P. aeruginosa* constitutively expressing *tpbB*. PAO1 wild type, *P. aeruginosa* containing arabinose inducible *ml1419c*, $ml1419c_{\Delta GGDEF}$, and the vector control (VC) form round colonies with smooth surfaces. Scale bar corresponds to 1 mm.

Figure S4

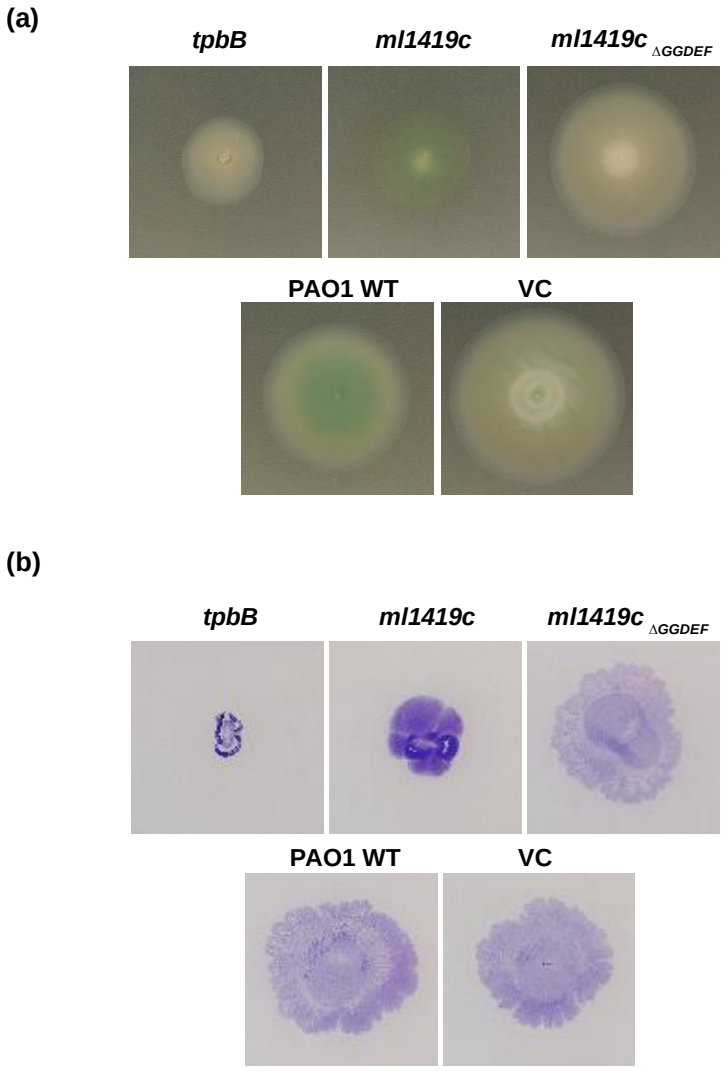


Figure S4. Suppression of *P. aeruginosa* motility expressing *ml1419c.* (a) Swimming and (b) twitching motility were suppressed in *P. aeruginosa* expressing *tpbB* and *ml1419c* as compared to the wild-type PAO1 strain and vector control (VC) in the presence of 0.2% L-arabinose. Twitching and swimming motility were restored in *P. aeruginosa* expressing mutated *ml1419c* (*ml1419c*$_{\Delta GGDEF}$).

Figure S5

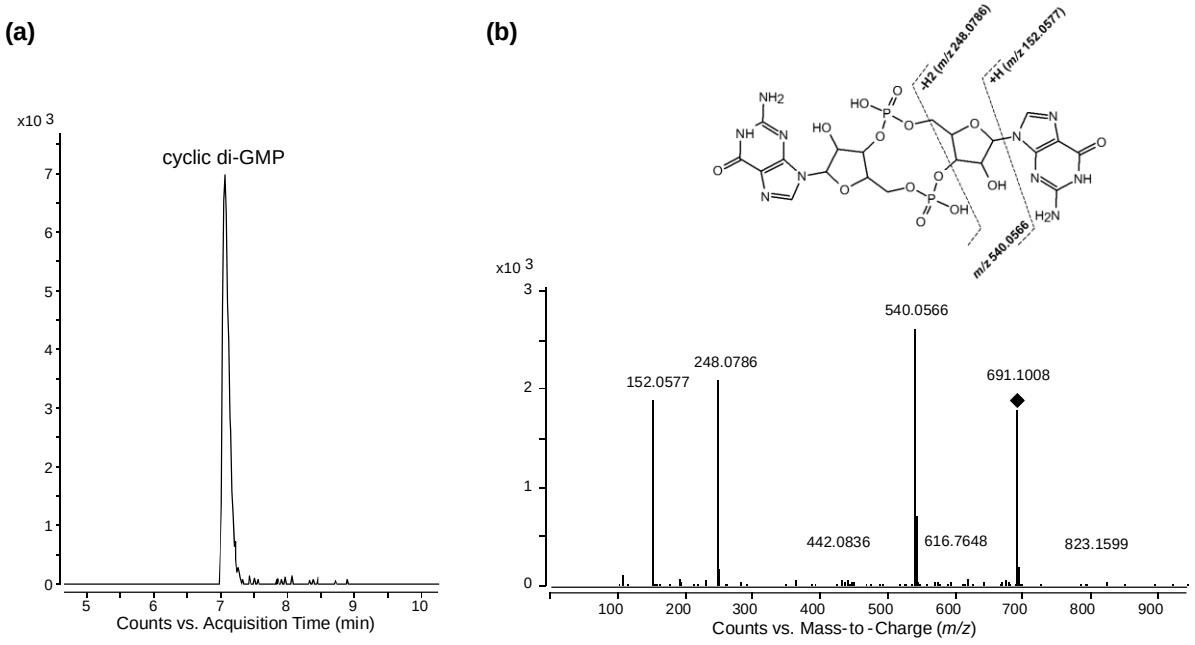


Figure S5. Detection of cyclic di-GMP standard by LC-MS. Cyclic di-GMP standard was separated and detected by LC-MS. (a) The extracted ion chromatogram (EIC) of cyclic di-GMP (*m/z* 691.102) revealed a retention time of 7.061 min. (b) The cyclic di-GMP peak was confirmed by tandem mass spectrometry (LC-MS/MS) with the transition ions of *m/z* 152.0577, 248.0786 and 540.0566. The *m/z* 248.0786 fragment ion is diagnostic for cyclic di-GMP.

Figure S6

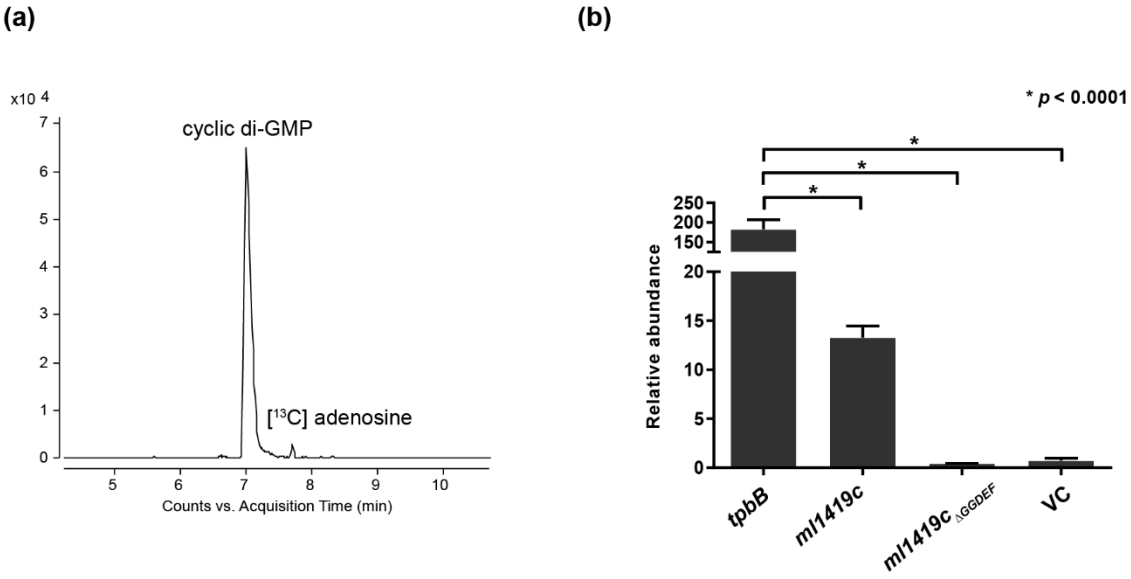


Figure S6. Cyclic di-GMP detection by LC-MS and relative quantification of cyclic di-GMP in *P. aeruginosa* recombinant strains. (a) The EIC of cyclic di-GMP detected in the extracts from *P. aeruginosa* expressing *tpbB*. (b) The relative quantification of cyclic di-GMP in *P. aeruginosa* expressing *tpbB* and other recombinant genes.

References

Gupta, K., Kumar, P. & Chatterji, D. (2010). Identification, activity and disulfide connectivity of c-di-GMP regulating proteins in *Mycobacterium tuberculosis*. *PLoS One* **5,** (11): e15072. doi:10.1371/journal.pone.0015072.

Hickman, J. W. & Harwood, C. S. (2008). Identification of FleQ from *Pseudomonas aeruginosa* as a c-di-GMP-responsive transcription factor. *Mol Microbiol* **69,** 376-89.

Newman, J. R. & Fuqua, C. (1999). Broad-host-range expression vectors that carry the L-arabinose-inducible *Escherichia coli araBAD* promoter and the *araC* regulator. *Gene* **227,** 197-203.

Romling, U., Galperin, M. Y. & Gomelsky, M. (2013). Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. *Microbiol Mol Biol Rev* **77,** 1-52.