Supporting Information

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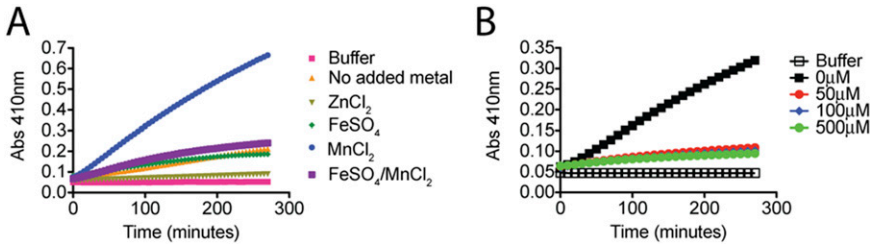

Fig. S1. Bis-PNPP hydrolysis activity of PgpH HD domain, related to Fig. 2. (*A*) Hydrolysis of Bis-pNPP to paranitrophenol was monitored at 410 nm in the presence of PgpH HD protein expressed with different metal ions. (*B*) Hydrolysis of Bis-pNPP by MBP-HD (Mn preparation) in the presence of increasing concentrations of c-di-AMP.

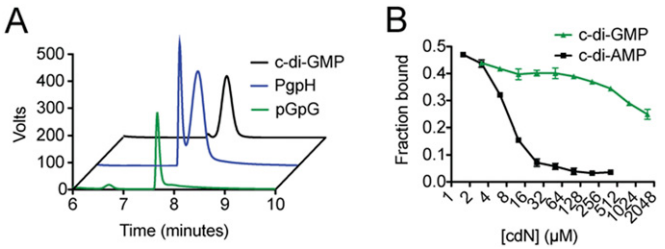

Fig. S2. c-di-GMP hydrolysis and interaction. (*A*) Hydrolysis of c-di-GMP into 5′-pGpG. Twenty micromolar MBP-HD (Mn preparation) was incubated with 100 μM c-di-GMP in storage buffer at room temperature for 22 h and analyzed with HPLC. (*B*) Competition titration DRCALA assays. Varying concentrations of unlabeled c-di-AMP or c-di-GMP were added to compete with bound ^{32}P-c-di-AMP.

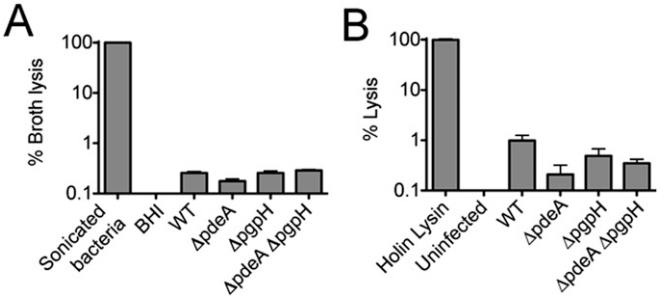

Fig. S3. Broth and intracellular lysis of *L. monocytogenes* strains, related to Fig. 6. (*A*) Lysis of the listed strains was quantified by monitoring the release of β-galactosidase into broth media (BHI). Lysis is reported based on the β-galactosidase enzymatic activity in sterile media (0% lysis) and sonicated bacteria (100% lysis). (*B*) Bone marrow-derived macrophages were infected with the indicated strains harboring a plasmid-encoding Luciferase driven by a eukaryotic-specific promoter. Six hours after infection, luminescence was measured in the infected host cells. DNA release is reported relative to uninfected (0%) and a holin lysin (100%) control strain.

Table S1. Metal incorporation by ICP-MS

	Metal: Protein stoichiometry*						
MBP-HD preparations	Mg	Mn	Fe	Co	Ni	Cu	Zn
WT no added metal	0.07	0.17	0.81	0.00	0.00	0.01	0.30
WT added Mn	0.05	1.08	0.51	0.00	0.00	0.00	0.63
WT added Fe	0.06	0.24	0.92	0.00	0.00	0.01	0.66
WT added Fe/Mn	0.06	0.87	0.72	0.00	0.01	0.01	0.39
H543A added Mn	0.02	0.60	0.22	0.00	0.00	0.00	0.15
D544A added Mn	0.04	0.00	0.02	0.00	0.00	0.00	0.28

*Average of two measurements.

Table S2. Summary of crystallographic information, related to Fig. 4

Structure	c-di-AMP complex (Fe peak)	c-di-AMP complex (native)	Free HD domain
Space group	$P6_1$	$P6_1$	$P6_1$
Cell dimensions			
a, Å	124.3	124.3	116.6
b, Å	124.3	124.3	116.6
c, Å	57.8	58.0	91.8
α, °	90	90	90
β, °	90	90	90
γ, °	120	120	120
Wavelength, Å	1.74	1.10	1.075
Resolution,* Å	25–2.7 (2.8–2.7)	25–2.1 (2.17–2.1)	25–2.4 (2.48–2.4)
No. of observations	51,226	120,944	105,385
R_{merge}, %	10.5 (33.8)	10.3 (40.8)	12.1 (40.6)
I/σI	11.2 (3.0)	12.9 (3.1)	10.7 (3.8)
Redundancy	3.7 (2.9)	4.0 (3.7)	3.8 (3.8)
No. of reflections	14,184	29,902	27,525
Completeness, %	98 (91)	99 (99)	99 (98)
R_{work}, %	—	16.3 (19.4)	18.5 (27.0)
R_{free},† %	—	20.2 (26.4)	23.1 (32.7)
rmsd			
Bond lengths, Å	—	0.010	0.009
Bond angles, °	—	1.2	1.1

*The numbers in parentheses are for the highest resolution shell.
†Five percent of the reflections were selected for free *R* calculation.

Table S3. Strains and plasmids used in this study

Strain number	Plasmid no.	Genotype	Source
L. monocytogenes:			
JW06		*L. monocytogenes* 10403S	
Derivatives of JW06:			
JW467	—	Δ*pdeA*	This study
JW221	—	Δ*pgpH*	—
JW468	—	Δ*pdeA* Δ*pgpH*	This study
JW469	—	$tRNA^{Arg}$::P_{spac}-*pgpH*$^+$ Δ*pgpH*	This study
JW470	—	$tRNA^{Arg}$::P_{spac}-*pdeA*$^+$ Δ*pdeA*	This study
JW471	—	$tRNA^{Arg}$::P_{spac}-*pgpH*$^+$ Δ*pdeA* Δ*pgpH*	This study
JW472	—	$tRNA^{Arg}$::P_{spac}-*pdeA*$^+$ Δ*pdeA* Δ*pgpH*	This study
E. coli:			
JW473	pTNH46	pMAL-c2x-PgpH HD (aa494-718) in BL21	This study
JW474	pTNH55	As pTNH46 but H543A	This study
JW475	pTNH56	As pTNH46 but D544A	This study