Supporting Information

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SI Methods

Identification of genes encoding for putative c-di-GMP synthetases. Genes encoding for GGDEF-domain proteins in the *S.* Typhimurium LT2 genome (Genome Sequencing Center, Washington University) were identified using the SMART Architecture Analysis tool (http://smart.embl-heidelberg.de). Discernible domains of the corresponding proteins were confirmed with the Pfam database (http://pfam.sanger.ac.uk/search).

Analysis of GGDEF-Domain Protein-Expression Patterns. Strains were incubated under LB and atmospheric biofilm-formation conditions and on LB agar plates for different times and temperatures. In the Western blot assays, probing was carried out with anti-3xFlag antibodies at a concentration of 0.5 μg ml^{-1} for 60 min at room temperature. Alkaline phosphatase-conjugated secondary antibodies (goat anti-mouse; Sigma) diluted 1:2500 were used for 60 min at room temperature. Bound ligands were detected using the ECL™ Western blotting analysis system (GE Healthcare).

Construction of Strains Expressing a 3xFlag-Tagged GGDEF-Domain Protein. Twelve strains, derivatives of the clinical isolate *S.* Enteritidis 3934, each of which presented a 3xFlag epitope-encoding tail added to a gene encoding a GGDEF-domain protein, were constructed as described (1).

Construction of Strains Containing a Single 3xFlag-Tagged GGDEF-Domain Protein. Derivatives of the chloramphenicol-resistant mutant ΔXII containing a single GGDEF protein with an added 3xFlag epitope were constructed by transductions between the 12 strains expressing a 3xFlag-tagged GGDEF-domain protein and mutant ΔXII according to recommended protocols (2) with Phage P22 HT105/1 int-201 (3).

Construction of ΔXII. A new plasmid, pKO3blue, was constructed as a derivative of plasmid pKO3 (4) so that it contained the *lacZ* gene from *Bacillus stearothermophilus* under the constitutive promoter PclpB coming from plasmid pMAD (5). For that construction, a BamHI StuI fragment of plasmid pMAD was cloned into a linearized SmaI BamHI pKO3 plasmid. The pKO3blue shuttle vector was used to perform precise, markerless deletions of all of the genes encoding GGDEF-domain proteins present in the *S.* Enteritidis 3934 genome. First, a collection of single mutants in each gene encoding GGDEF protein (except for the *yeaJ* gene) was constructed. For that construction, DNA fragments corresponding to the upstream (fragment AB) and downstream (fragment CD) regions of the target genes were amplified with the primer pairs specified in Table S3, with chromosomal DNA from strain *S.* Enteritidis 3934 as a template. The PCR products were cloned in the pGEMt-easy vector (Promega), digested with NotI and XhoI enzymes in the case of AB fragments and XhoI and BglII enzymes in the case of CD fragments, and ligated in the same ligation mixture with the pKO3blue vector digested with NotI and BglII enzymes. The recombinant pKO3blue::AD vector was extracted from *E. coli* XL1 Blue and electroporated into strain *S.* Enteritidis 3934. Blue, chloramphenicol-resistant transformants were selected after 72 h of incubation at 28 °C in the presence of X-gal (BioLine). Homologous recombination and excision of the integrated plasmid was performed with a protocol described (4) and improved by the ability of blue and white screening of colonies that correlated with the presence and absence of the plasmid, respectively. Gene deletion was confirmed in white colonies by PCR using primers E and F flanking the targeted ORF, sequencing and digestion of the amplified EF fragment with XhoI enzyme. Second, mutant ΔXII was constructed in a sequential way (Fig. S3) by transduction between strains containing the pKO3blue::AD integrated plasmid and the corresponding recipient strain using Phage P22 HT105/1 int-201 (3). Excision of the plasmid to obtain the corresponding mutant was carried out as described previously. Disruption of the *yeaJ* gene was performed either by transduction between mutant ΔII and a *yeaJ* insertional mutant (6), leading to a kanamycin-resistant mutant ΔXII, or by the insertion of a chloramphenicol resistance gene (7, 8), leading to a chloramphenicol-resistant derivative of mutant ΔXII that was used in the construction of strains containing a single 3xFlag-tagged GGDEF-domain protein. Absence of all of the genes encoding GGDEF-domain proteins in the 2 derivatives of mutant ΔXII was confirmed by PCR using primers E and F flanking the targeted ORFs and Southern blot analysis.

Construction of a Collection of Strains Containing a Single GGDEF-Domain Protein. DNA fragments corresponding to the coding sequences of the *adr*A, *stm1987, yci*R, *yeg*E, *yfi*N, *yhd*A, *stm3388*, and *yhj*K genes were amplified with primer pairs A and D and chromosomal DNA from strain *S.* Enteritidis 3934 as a template. In the restoration of the *yeaJ* gene, primers 02H and 02D were used. Amplified fragments were sequenced and cloned into the pKO3blue plasmid that was electroporated into mutant ΔXII. Strains ΔXII+P_{4551}*adrA* and ΔXII+P_{4551}*hmsT* were constructed by amplifying the *adrA* and *hmsT* genes with primers 01G/XhoI3xflag and Hmst fw/Hmst rv, cloning into the pKO3blue::11AD plasmid, and electroporating into ΔXII. Strain ΔXII+*stm4551*+*adrA* was constructed by amplifying the *adrA* gene with primers 01G/XhoI3xflag, cloning into the pKO3blue::01AD plasmid, and electroporating into ΔXII+*stm4551*. Strains ΔXII+*stm4551* GGGSF, ΔXII+P_{4551}*adrA* GGGSF, and ΔXII+P_{4551}*hmsT* GGGSF were constructed by amplifying fragments containing the GGGSF motif with primers 11, 01, and HmsT GGGSF A to D, cloning into the pKO3blue plasmid, and electroporating ΔXII+*stm4551*, ΔXII+P_{4551}*adrA*, and ΔXII+P_{4551}*hmsT* strains. Integration and excision of the plasmid was used as described previously to obtain the corresponding restored strains. Strain ΔXII+*stm4551* was constructed from mutant ΔIX by deleting the *stm2503* gene with the use of the pKO3blue plasmid and mutating the *yfeA* gene by the insertion of a chloramphenicol resistance gene (7, 8). Strain ΔXII+*yfe*A was constructed from mutant ΔX by deleting the *stm2503* gene with the use of the pKO3blue plasmid.

Microarrays. Total RNA of the wild-type strain *S.* Enteritidis 3934 and ΔXII was isolated after 72 h of incubation in LB under biofilm-forming conditions. Two hundred μl of chilled stop mix (5% [vol/vol] phenol in ethanol) were added to 1 ml from cells grown in RNA-extraction conditions, kept in ice for 30 min, and then cells were harvested at 4 °C by centrifugation. Pellets were used for total RNA extraction using Promega's SV 96 Total RNA purification kit. Total RNA was quantified using the NanoDrop ND-1000 UV-Vis Spectrophotometer, and RNA quality was assessed on a Bioanalyzer 2100 (Agilent). When necessary, DNA was eliminated with Turbo DNase (Ambion). Three independent RNA samples were pooled and retrotranscribed using SuperScript III Reverse Transcriptase to obtain cDNA purified using Qiagen's QIAquick PCR purification kit.

Genomic DNA of the wild-type strain was obtained from stationary growth cultures, using Qiagen's Genomic DNA and then was fragmented by sonication before array hybridization. Labeling of cDNA and gDNA with fluorescence molecules Alexa Fluor 647 or Alexa Fluor 555, respectively, was performed using exo-Klenow enzyme and the BioPrime Plus Array CGH Indirect Genomic Labeling System (Invitrogen). Labeled cDNA and gDNA were purified further, and the amount of incorporated labeling was estimated in a NanoDrop ND-1000 UV-Vis Spectrophotometer. Slides were prehybridized for 1 h at 42 °C with a solution containing 6× SSC, 0.5% SDS, and 1% BSA. cDNA and gDNA were mixed in a 3:1 ratio. This mixture was reduced to a 10-μl final volume, and 70 to 100 μl of the following solution was added: 50% formamide; 3X SSC, 1% SDS, 5X Denhart's solution, 5% dextran-sulfate. The solution then was incubated at 95 °C for 5 min. This mixture was added to the slide, and the hybridization continued overnight at 42 °C. After this time, the following washes were made, always in shaking conditions (230 rpm): 3 washes of 5 min each at 42 °C with 0.5X SSPE buffer (3.0 M Sodium Chloride, 0.2 M Sodium Hydrogen Phosphate, 0.02 M EDTA, pH 7.4) 0.1% Tween20 (Merck); 3 washes of 5 min each at 42 °C with 0.5X SSPE; and 2 final washes of 5 min each with 0.1X SSPE at 37 °C. Data acquisition, normalization, and statistical analysis of the microarray results have been described (9). We compared in duplicate 3 independent hybridizations of the wild-type strain vs. 3 independent hybridizations of ΔXII. Changes in expression were considered significant when the p-value was lower than 0.01. Only genes selected in both comparisons are included in Table S1.

Statistical Analysis. The statistical analysis was performed using GraphPad Prism version 5.00 for Windows. Gene expression (n = 10): According to the associated probability of the Kolmogorov-Smirnov normality test, 1-way ANOVA or the equivalent non-parametric Kruskal Wallis test was applied. When 1-way ANOVA was used, the variance heterogeneity detected through the Bartlett's test led us to choose Dunnett's post tests. Mann-Whitney U post tests with the Bonferroni correction followed the Kruskal-Wallis analysis. Survival curves (n = 10) were compared by the log-rank (Mantel-Cox) test. For the analysis of multiple survival curves, the Bonferroni-corrected threshold was computed for each individual comparison. Bacterial counts (n = 4 animals): Mann-Whitney U tests were used to analyze data corresponding to bacterial counts in organs. When multiple samples were compared, Kruskal-Wallis with Bonferroni-corrected Mann-Whitney U post tests were performed. In all cases α = 0.05 and 2-tail p-values are reported.

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2. Maloy SR, Stewart VJ, Taylor RK (1996) *Genetic Analysis of Pathogenic Bacteria: A Laboratory manual* (Cold Spring Harbor, N.Y.).
3. Schmieger H (1972) Phage P22-mutants with increased or decreased transduction abilities. *Mol Gen Genet* 119:75–88.
4. Link AJ, Phillips D, Church GM (1997) Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli*: Application to open reading frame characterization. *J Bacteriol* 179:6228–6237.
5. Arnaud M, Chastanet A, Débarbouillé M (2004) A new vector for efficient allelic replacement in naturally non transformable low GC% gram-positive bacteria. *Appl Environ Microbiol* 70:6887–6891.
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9. Mariscotti JF, Garcia-del Portillo F (2009) Genome expression analyses revealing the modulation of the *Salmonella* Rcs regulon by the attenuator IgaA. *J Bacteriol* 191:1855–1867.

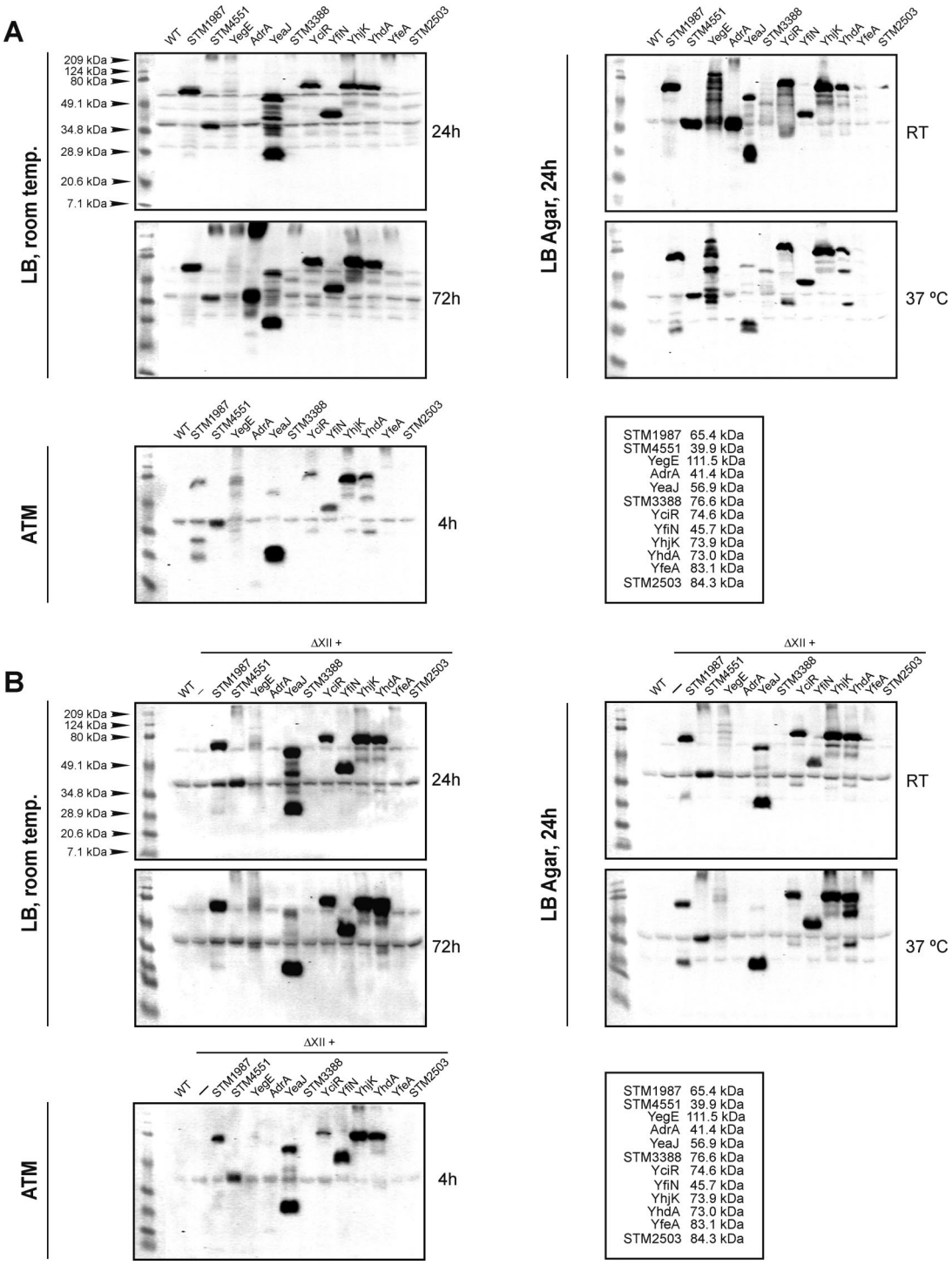

Fig. S1. Expression pattern of GGDEF-domain proteins. (*A*) Western blot of cellular extracts obtained from the wild-type strain and each of the 12 strains expressing a 3xFlag tagged GGDEF-domain protein. (*B*) Western blot experiments on cellular extracts of the wild-type strain, ΔXII, and a collection of 12 strains in which each GGDEF-domain protein tagged with a 3xFlag epitope was restored in the chromosome of ΔXII. During the biofilm formation process in LB conditions (after 24 h of incubation and after 72 h of incubation, when a visible biofilm has formed); after 24 h of incubation at room temperature (RT) or 37 °C on LB medium plates, and in ATM biofilm-forming conditions (after 4 h of incubation, when a biofilm has formed).

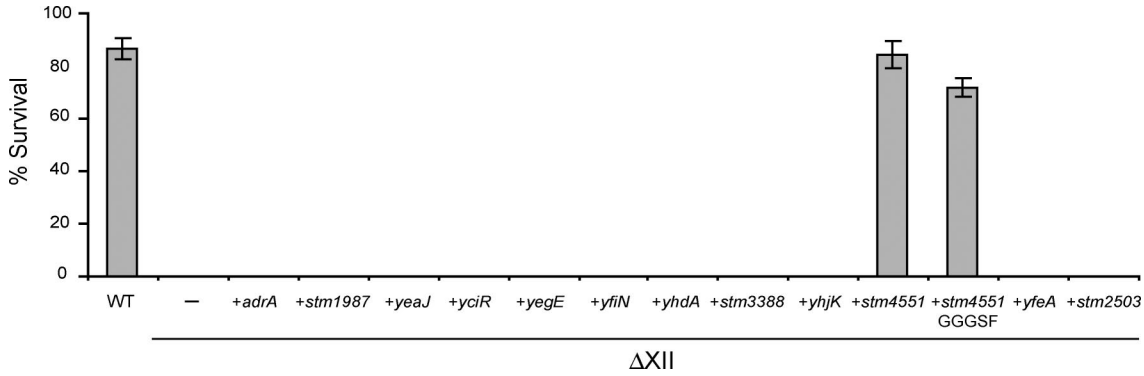

Fig. S2. Differences in resistance to desiccation. Survival of the wild-type strain, ΔXII, a collection of ΔXII derivative strains containing a single GGDEF-domain protein, and ΔXII+*stm4551* GGGSF after desiccation. The surviving bacteria were enumerated by viable plate counts, and their numbers (log cfus) were compared with those of initial inocula, which defined 100% survival. Bars represent the median, and error bars represent the interquartile range ($n = 3$).

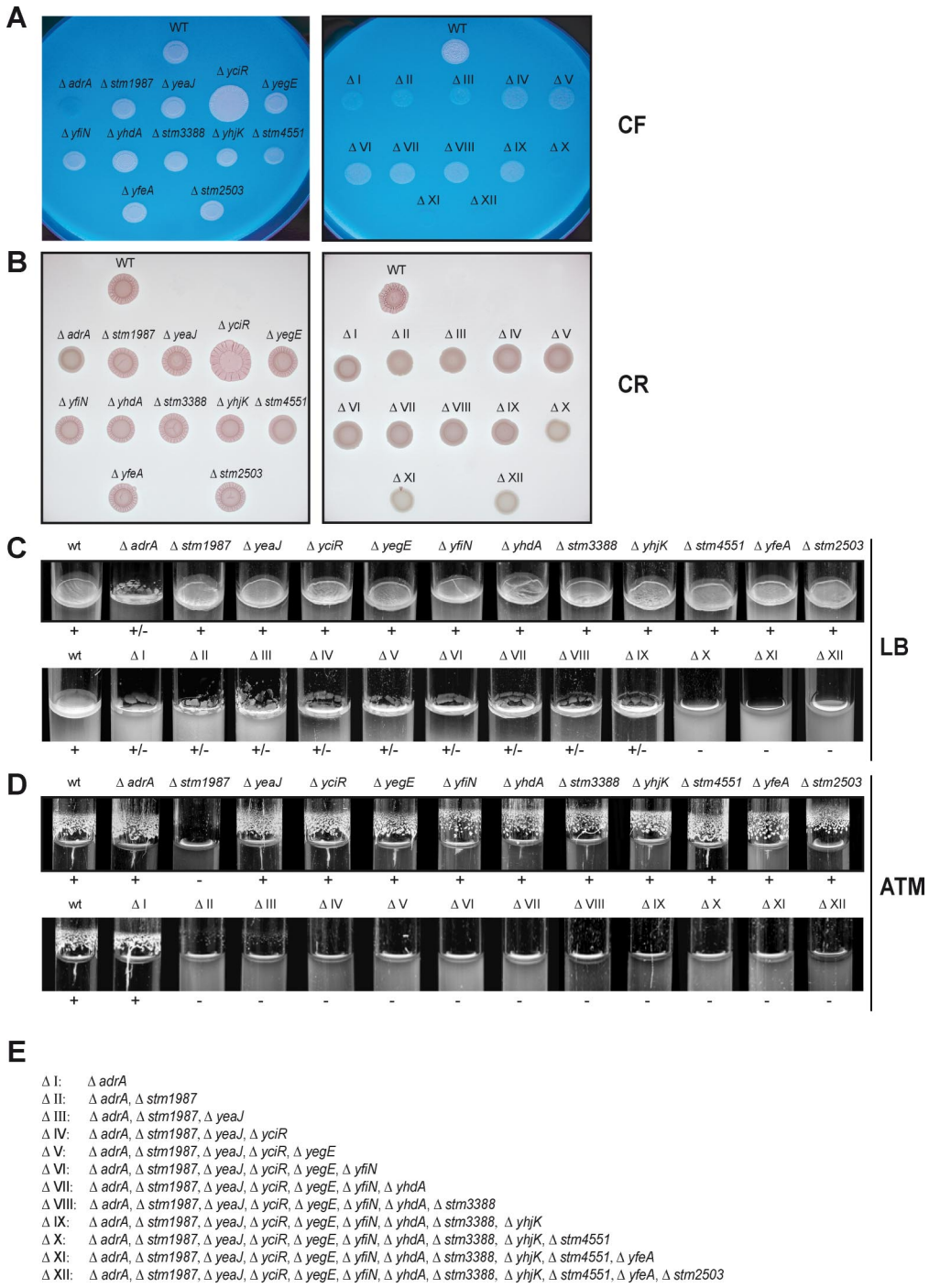

Fig. S3. Phenotypic analysis of simple GGDEF mutants vs. sequential mutants obtained during the process of construction of ΔXII. (*A*) Cellulose production on calcofluor (CF) plates. (*B*) Cellulose and fimbriae production on Congo red (CR) agar plates. (*C*) Biofilm formation capacity in LB media conditions and (*D*) in ATM conditions. (*E*) Heading of genotype from partial mutants.

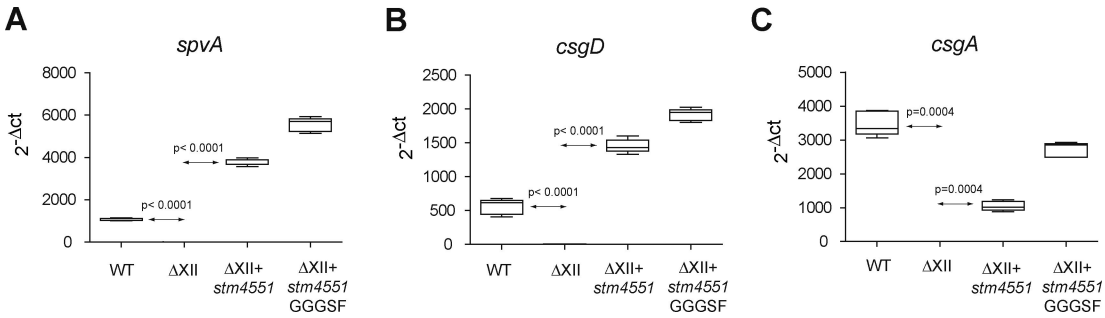

Fig. S4. RT-PCR experiments to correlate mRNA levels with analyzed phenotypes. mRNA levels were determined for (*A*) *spvA,* (*B*) *csgD*, and (*C*) *csgA* genes in the wild-type, ΔXII, ΔXII+*stm4551*, and ΔXII+*stm4551* GGGSF strains.

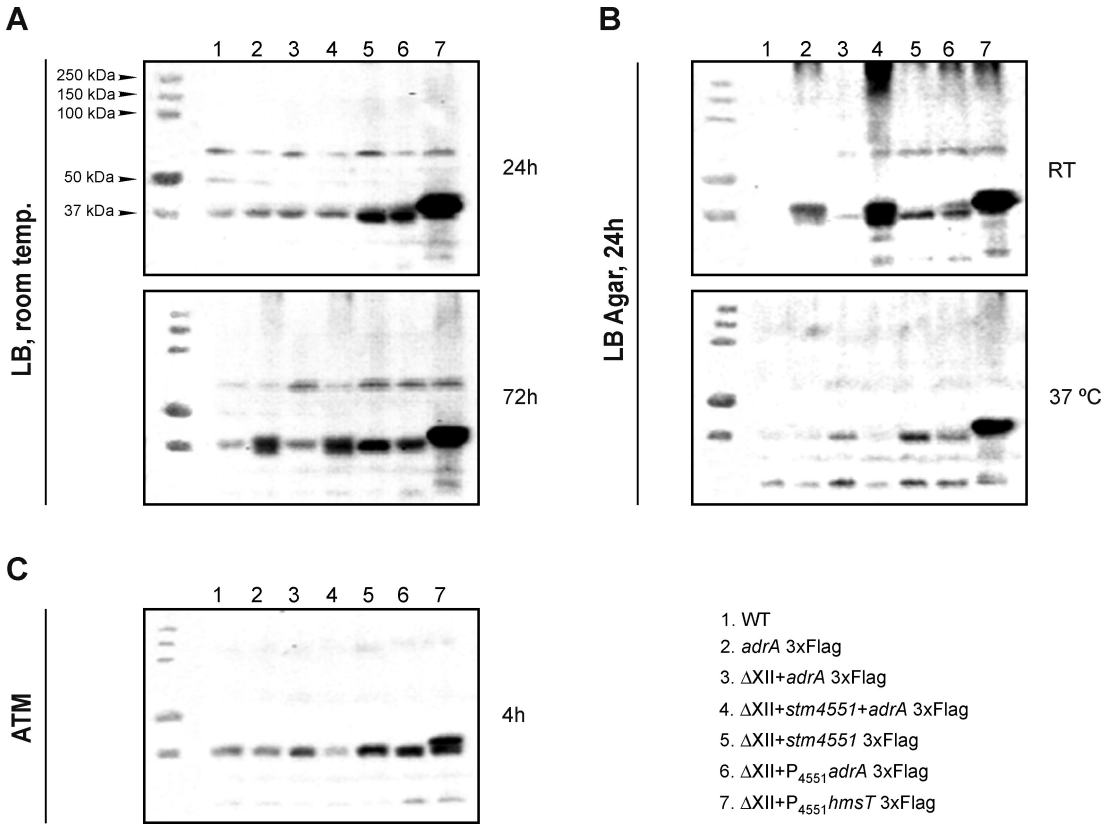

Fig. S5. Expression assays showing that AdrA and HmsT are expressed under the *stm4551* promoter and that induction of AdrA depends on the presence of Stm4551. Western blot experiments on cellular extracts of the wild-type strain (1), the wild-type strain expressing a 3xFlag-tagged AdrA protein (2), ΔXII restored with a 3xFlag-tagged AdrA protein (3), or STM4551 protein (5), ΔXII double restored with STM4551 and a 3xFlag-tagged AdrA protein (4), and ΔXII restored with a 3xFlag-tagged AdrA protein (6) or HmsT protein (7) under the *stm4551* promoter. The expected molecular weights of AdrA, STM4551, and HmsT are 41.4kDa, 39.9 kDa, and 44.3 kDa, respectively during the biofilm-formation process in LB conditions (after 24 h of incubation and after 72 h of incubation when a visible biofilm has formed) (*A*), after 24 h of incubation at room temperature or 37 °C on LB medium plates (*B*), and in ATM biofilm-forming conditions (after 4 h of incubation when a biofilm has been formed) (*C*).

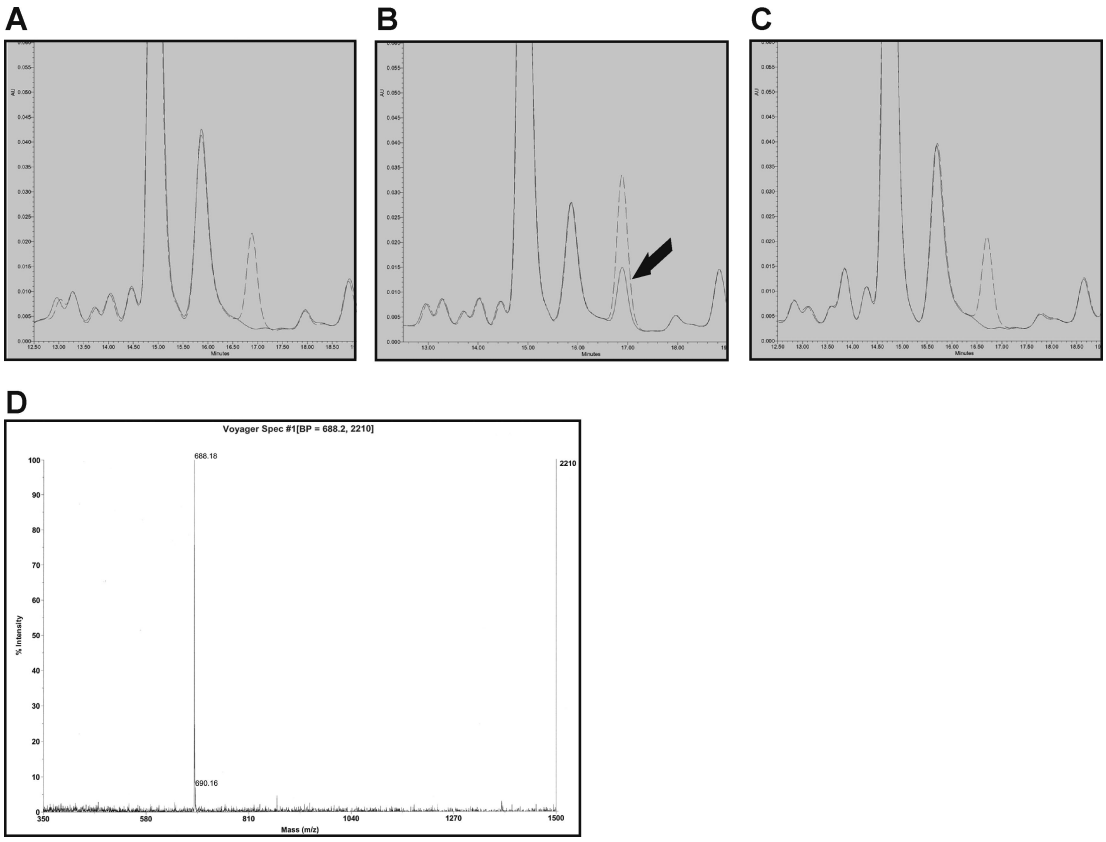

Fig. S6. STM4551 presents DGC activity that is dependent on a catalytic active site. Representative chromatograms showing detection of c-di-GMP by HPLC analysis of nucleotide extracts (equivalent to 10 mg wet weight) from cells grown on LB without NaCl agar plates for 16 h at 28 °C of (*A*) an *adrA* mutant complemented with the low-copy plasmid pBR328; (*B*) an *adrA* mutant complemented with pBR328::*stm4551*; and (*C*) an *adrA* mutant complemented with pBR328::*stm4551* GGGSF. Addition of synthetic c-di-GMP was used to identify the c-di-GMP peak and quantification (*dashed lines*). The arrow indicates the position of the c-di-GMP peak. The area of the c-di-GMP peak was used to estimate the amount of c-di-GMP in a sample referred to wet cell weight. (*D*) Identification of c-di-GMP from relevant HPLC fractions by MALDI-TOF MS analysis. The fraction covering the c-di-GMP area present in *B* and the fraction from a positive control (namely an *adrA* mutant complemented with the arabinose-inducible plasmid pBAD30::*adrA*) were collected and analyzed. A major ion was detected at *m/z* of 688.18 $[M–H]^-$.

Table S1. Genes differentially expressed in ΔXII

Protein Function	Name	Number	Fold Activation
Genes induced in ΔXII strain vs. WT			
Metabolism			
Energy production and conversion			
Putative cytochrome oxidase subunit		STM1792	+ 4.97
ATP synthase subunit C	*atpG*	STM3866	+ 3.48
Cytochrome d terminal oxidase polypeptide	*cydA*	STM0740	+ 3.40
subunit			
Flavodoxin	*fldB*	STM3045	+ 3.54
ATP synthase subunit E	*nuoE*	STM2325	+ 3.96
NADH dehydrogenase subunit I	*nuoI*	STM2321	+ 3.12
NADH dehydrogenase subunit J	*nuoJ*	STM2320	+ 3.07
Phosphoenolpyruvate carboxykinase	*pckA*	STM3500	+ 3.78
Dihydrolipoamide acetyltransferase	*sucB*	STM0737	+ 4.74
Succinyl-CoA synthetase subunit beta	*sucC*	STM0738	+ 3.74
Hypothetical protein	*ygiR*	STM3168	+ 3.40
Coenzyme transport and metabolism			
5–10-methylene-tetrahydrofolate	*folD*	STM0542	+ 3.69
Pyrimidine deaminase/reductase	*ribD*	STM0416	+ 3.22
Nucleotide transport and metabolism			
Nucleoside diphosphate kinase	*ndk*	STM2526	+ 3.57
Cellular processes and signaling			
Cell motility			
Flagellar basal body rod modification protein	*flgD*	STM1176	+ 3.32
Cell-proximal portion of basal-body rod	*flgF*	STM1178	+ 3.41
Flagellin-specific chaperone FliS	*fliS*	STM1961	+ 3.89
Cell wall/membrane/envelope biogenesis			
UDP-N-acetylmuramyl pentapeptide synthase	*murF*	STM0124	+ 3.59
Putative outer membrane porin precursor	*nmpC*	STM1572	+ 4.42
Putative glycosyl transferase	*wcaC*	STM2113	+ 4.00
Posttranslational modification, protein turnover, chaperones			
Periplasmic heme-dependent peroxidase	*ccmE*	STM3815	+ 8.93
Putative hydrogenase formation protein	*hypC*	STM2856	+ 3.28
Intracellular trafficking, secretion, and vesicular transport			
Preprotein translocase SecY	*secY*	STM3420	+ 3.83
Drug/analog resistance			
Polymyxin resistance protein B	*pmrD*	STM2304	+ 5.27
Information storage and processing			
Transcription			
Transcriptional regulator	*tyrR*	STM1683	+ 2.86
Translation, ribosomal structure and biogenesis			
50S ribosomal protein L2	*rplB*	STM3437	+ 3.89
50S ribosomal protein L3	*rplC*	STM3440	+ 5.07
50S ribosomal protein L4	*rplD*	STM3439	+ 4.68
50S ribosomal protein L5	*rplE*	STM3428	+ 5.62
50S ribosomal protein L14	*rplN*	STM3430	+ 5.31
50S ribosomal protein L15	*rplO*	STM3421	+ 3.98
50S ribosomal protein L22	*rplV*	STM3435	+ 3.94
50S ribosomal protein L23	*rplW*	STM3438	+ 3.76
50S ribosomal protein L24	*rplX*	STM3429	+ 4.64
50S ribosomal protein L30	*rpmD*	STM3422	+ 4.55
30S ribosomal protein S3	*rpsC*	STM3434	+ 4.25
30S ribosomal protein S4	*rpsD*	STM3416	+ 4.59
30S ribosomal protein S5	*rpsE*	STM3423	+ 5.57
30S ribosomal protein S6	*rpsF*	STM4391	+ 4.91
30S ribosomal protein S8	*rpsH*	STM3426	+ 5.83
30S ribosomal protein S11	*rpsK*	STM3417	+ 5.08
30S ribosomal protein S13	*rpsM*	STM3418	+ 4.46
30S ribosomal subunit protein S14	*rpsN*	STM3427-S	+ 5.99
30S ribosomal protein S18	*rpsR*	STM4393	+ 4.97
23S ribosomal RNA	*rrlA*	STM3991	+ 5.41
Threonyl-tRNA synthetase	*thrS*	STM1333	+ 4.17
RNA			
RNA-binding protein Hfq	*hfq*	STM4361	+ 4.41

Protein Function	Name	Number	Fold Activation
Regulatory RNA	*csrB*	STM2966	+ 5.78
Virulence plasmid pSLT			
Putative carbonic anhydrase		PSLT046	+ 3.53
Poorly characterized			
Translocation machinery component	*sipD*	STM2883	+ 4.64
Putative outer membrane protein	*ycbK*	STM0996	+ 3.71
Genes repressed in ΔXII strain vs WT[a]			
Metabolism			
Carbohydrate transport and metabolism			
Putative mannitol dehydrogenase		STM3083	−2.95
Cellular processes and signaling			
Cell wall/membrane/envelope biogenesis			
Putative nucleoside-diphosphate-sugar epimerase		STM2914	−5.21
Lipoprotein	*nlpD*	STM2925	−6.05
Glucosyltransferase	*rfaG*	STM3722	−3.71
Putative mechanosensitive channel	*yggB*	STM3067	−3.83
Intracellular trafficking, secretion, and vesicular transport			
Zinc-resistance associated protein	*zraP*	STM4172	−3.43
Information storage and processing			
Transcription			
Putative transcriptional regulator	*csgD*	STM1142	−4.84
Fimbriae			
Major curlin subunit precursor	*csgA*	STM1144	−8.04
Minor curlin subunit precursor	*csgB*	STM1143	−9.67
Putative curli production protein precursor	*csgC*	STM1145	−4.29
Curli production assembly/transport component	*csgF*	STM1140	−5.04
RNA			
Met tRNA	*metY*	STM3289	−3.91
Virulence plasmid pSLT			
Outer membrane protein	*spvA*	PSLT040	−3.56
Poorly characterized			
Putative inner membrane protein		STM3021	−3.60
Putative cytoplasmic protein		STM4103	−3.37
Putative inner membrane protein		STM4552	−3.50
Putative inner membrane protein		STM3774	−4.38
Hyperosmotically inducible periplasmic protein	*osmY*	STM4561	−3.82
Translocation machinery component	*sseC*	STM1400	−4.46
Putative periplasmic protein	*yahO*	STM0366	−4.03
Hypothetical protein	*ybgS*	STM0759	−4.30
Putative inner membrane protein	*ychH*	STM1782	−4.11
Putative cytoplasmic protein	*yciE*	STM1730	−5.96
Putative cytoplasmic protein	*yciF*	STM1729	−5,79
Putative cytoplasmic protein	*yciG*	STM1728	−7,32
Putative cytoplasmic protein	*yjbJ*	STM4240	−4.79
Putative cytoplasmic protein	*ymdF*	STM1121	−3.64

[a]All genes encoding GGDEF-domain proteins were found to be significantly repressed in the ΔXII strain. These data have been omitted from the table.

Table S2. Strains and plasmids used in this study

Strains	Relevant Characteristics	Reference or Source
Salmonella Enteritidis		
3934	Wild-type clinical isolate	(Solano et al. 2002)
adrA-Flag	3934 *adrA*::3xFlag Km^R	This study
stm1987-Flag	3934 *stm1987*::3xFlag Km^R	This study
yeaJ-Flag	3934 *yeaJ*::3xFlag Km^R	This study
yciR-Flag	3934 *yciR*::3xFlag Km^R	This study
yegE-Flag	3934 *yegE*::3xFlag Km^R	This study
yfiN-Flag	3934 *yfiN*::3xFlag Km^R	This study
yhdA-Flag	3934 *yhdA*::3xFlag Km^R	This study
stm3388-Flag	3934 *stm3388*::3xFlag Km^R	This study
yhjK-Flag	3934 *yhjK*::3xFlag Km^R	This study
stm4551-Flag	3934 *stm4551*::3xFlag Km^R	This study
yfeA-Flag	3934 *yfeA*::3xFlag Km^R	This study
stm2503-Flag	3934 *stm2503*::3xFlag Km^R	This study
Δ*adrA*	3934 Δ*adrA*	This study
Δ*stm1987*	3934 Δ*stm1987*	This study
Δ*yeaJ*-Km	3934 Δ*yeaJ*::Km^R	(Garcia et al., 2004)
Δ*yciR*	3934 Δ*yciR*	This study
Δ*yegE*	3934 Δ*yegE*	This study
Δ*yfiN*	3934 Δ*yfiN*	This study
Δ*yhdA*	3934 Δ*yhdA*	This study
Δ*stm3388*	3934 Δ*stm3388*	This study
Δ*yhjK*	3934 Δ*yhjK*	This study
Δ*stm4551*	3934 Δ*stm4551*	This study
Δ*yfeA*	3934 Δ*yfeA*	This study
Δ*stm2503*	3934 Δ*stm2503*	This study
ΔI	3934 Δ*adrA*	This study
ΔII	3934 Δ*adrA* Δ*stm1987*	This study
ΔIII	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R	This study
ΔIV	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR*	This study
ΔV	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE*	This study
ΔVI	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN*	This study
ΔVII	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA*	This study
ΔVIII	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388*	This study
ΔIX	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK*	This study
ΔX	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551*	This study
ΔXI	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA*	This study
ΔXII::Km	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII::Clo	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*adrA*-Flag	3934 *adrA*:: 3xFlag-Km^R Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*stm1987*-Flag	3934 Δ*adrA stm1987*:: 3xFlag-Km^R Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yeaJ*-Flag	3934 Δ*adrA* Δ*stm1987 yeaJ*:: 3xFlag-Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yciR*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R *yciR*::3xFlag-Km^R Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yegE*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR yegE*::3xFlag-Km^R Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yfiN*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE yfiN*::3xFlag-Km^R Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yhdA*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN yhdA*::3xFlag-Km^R Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*stm3388*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA stm3388*::3xFlag-Km^R Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yhjK*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388 yhjK*::3xFlag-Km^R Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study

Strains	Relevant Characteristics	Reference or Source
ΔXII+*stm4551*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK stm4551*::3xFlag-Km^R Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yfeA*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551 yfeA*::3xFlag-Km^R Δ*stm2503*	This study
ΔXII+*stm2503*-Flag	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA stm2503*::3xFlag-Km^R	This study
ΔXII+*adrA*	3934 Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*stm1987*	3934 Δ*adrA* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yeaJ*	3934 Δ*adrA* Δ*stm1987* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yciR*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yegE*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yfiN*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yhdA*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*stm3388* Δ*yhjK* Δ*stm4551* ΔyfeA Δ*stm2503*	This study
ΔXII+*stm3388*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*yhjK* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*yhjK*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*stm4551* Δ*yfeA* Δ*stm2503*	This study
ΔXII+*stm4551* (02 Km^R, 06 Clo^R)	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*yfeA*::Clo^R Δ*stm2503*	This study
ΔXII+*stm4551* (02 Clo^R, 06 Tet^R)	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*yfeA*::Tet^R Δ*stm2503*	This study
ΔXII+*stm4551* (02 Km^R, 06 Tet^R)	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*yfeA*::Tet^R Δ*stm2503*	This study
ΔXII+*yfeA*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*stm2503*	This study
ΔXII+*stm2503*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*stm4551* Δ*yfeA*	This study
ΔXII+*stm4551* GGGSF	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*yfeA*::Tet^R Δ*stm2503* and *stm4551* containing E267G and E268S amino acid substitutions	This study
ΔXII+*stm4551* GxxE	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*yfeA*::Tet^R Δ*stm2503* and *stm4551* containing R256G and D259E amino acid substitutions	This study
ΔXII+*stm4551* Δ*bcsA*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Clo^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*yfeA*::Tet^R Δ*stm2503* Δ*bcsA*::*MudJ*	This study
ΔXII + P$_{4551}$*hmsT*	ΔXII::Km P*stm4551*::*hmsT*	This study
ΔXII + P$_{4551}$*hmsT* GGGSF	ΔXII::Km P*stm4551*::*hmsT* containing D290G and E291S amino acid substitutions	This study
ΔXII + P$_{4551}$*adrA* Δ*bcsA*	ΔXII::Km P*stm4551*::*adrA*::3xFlag Δ*bcsA*::*MudJ*	This study
ΔXII + P$_{4551}$*hmsT* Δ*bcsA*	ΔXII::Km P*stm4551*::*hmsT* Δ*bcsA*::*MudJ*	This study
ΔXII + P$_{4551}$*hmsT*-Flag	ΔXII::Km P*stm4551*::*hmsT*::3xFlag	This study
ΔXII + P$_{4551}$*adrA*-Flag	ΔXII::Km P*stm4551*::*adrA*::3xFlag	This study
ΔXII + P$_{4551}$*adrA* GGGSF	ΔXII::Km P*stm4551*::*adrA*::3xFlag from *S.* Enteritidis 3934 fusion containing D290G and E291S amino acid substitutions	This study
ΔXII+*stm4551*+*adrA*	3934 *adrA*::3xFlag Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*yfeA*::Tet^R Δ*stm2503*	This study
ΔXII+*stm4551* Δ*spvA*	3934 Δ*adrA* Δ*stm1987* Δ*yeaJ*::Km^R Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*stm3388* Δ*yhjK* Δ*yfeA*:: Clo^R Δ*stm2503* Δ*spvA*::Tet^R	This study
bcsA-MudJ	3934 *bcsA*::MudJ (Km^R)	(Solano et al. 2002)
Salmonella Typhimurium		
TT3699 *ara*651::Tn10	Used as template for the amplification of the Tetracycline resistance cassette.	Gift from G. Casadesús
SV4406 *rcsB*::Mud*Q*	Used as template for Chloramphenicol cassette resistance amplification	Gift from F. García del Portillo
Escherichia coli		

Strains	Relevant Characteristics	Reference or Source
MC4100 *ybeW*::Km	Used as template for the amplification of the Kanamycin resistance cassette.	Gift from J. M. Ghigo
XL1 Blue	*recA*1 *endA*1 *gyrA*96 *thi*-1 *hsd*R17 *supE*44 *relA*1 *lac*[F′*proAB lacI*^{q}ZΔ*M15* Tn10(Tet^{r})]	Stratagene
Yersinia pestis KIM	Wild-type isolate	Gift from I. Moriyón
Plasmids		
pKOBEGA	Amp^{R}, vector for recombination experiments	(Chaveroche et al., 2000)
pSUB11	Used as template for the amplification of the 3xFlag Kanamycin resistance cassette.	(Uzzau et al., 2001)
pKO3blue	Clo^{R}, derivative of pKO3 carrying the pMAD *lacZ* gene under control of the PclpB promoter. Vector used for deletion and insertion of genes.	This study
pWJB30	pBAD30::*adrA*	(Zogaj et al., 2001)
pBR328::*stm4551*	pBR328 containing *stm4551* from *S.* Enteritidis 3934	This study
pBR328::*stm4551* GGGSF	pBR328::*stm4551* containing E267G and E268S amino acid substitutions	This study

Table S3. Oligonucleotides used in this study

Oligonucleotide	Sequence (5′ to 3′)
Tagging of genes encoding for GGDEF proteins	
yciR 3FLAG.Fw	TGAACGCTGGTACAAACGTTATCAGACGAAAAAAATGCGTgactacaaagaccatgacgg[a]
yciR 3Flag 2.Rv	TGCCTGTCTGCACAGGCTGTTCTCCCTATTATCGTCGCCAcatatgaatatcctccttag[a]
yedQ 3FLAG.Fw	AAGGGCTAAACCGGGAGGGCGTACATTTGCGTAACGACGAcatatgaatatcctccttag[a]
stm1987 3Flag 2.Rv	GCCAGAACGAAGGGCCGGATGGCTGGCGCGAAGGAATGGAcatatgaatatcctccttag[a]
yegE 3FLAG.Fw	GCTGGATTTGTTACTGAATACCAGCTATTTCGCTATCCATgactacaaagaccatgacgg[a]
yegE 3Flag 2.Rv	CGCGCTACTGCATATCTGGTATACCCACGGCTGGCAACCGcatatgaatatcctccttag[a]
stm3388 3FLAG.Fw	CAATATATTCACAAGGTCAATATATAATATCAACTCAAAAgactacaaagaccatgacgg[a]
stm3388 3Flag 2.Rv	AATTAATGCCAAATGAACAACAGGCGGAAGAAGCCTGAGAcatatgaatatcctccttag[a]
yfiN 3FLAG.Fw	GTATCAGGCTAAACACCGGCGTGCGGAGCGCTCGCTAAACgactacaaagaccatgacgg[a]
yfiN 3Flag 2.Rv	GTGGTCTCAACGCTGAGTCAGAAACGGCCAGGCCCGTTCCcatatgaatatcctccttag[a]
adrA 3FLAG.Fw	AGCAAAGAATGCCGGACGTAACCGCACCGAAGTGGCGGCAgactacaaagaccatgacgg[a]
adrA 3Flag 2.Rv	AATCAGAGGCGCTCAGTAAATCCTGAAGCCCGGCTGGACGcatatgaatatcctccttag[a]
yeaJ 3FLAG.Fw	GCTCTATCTGAACAAACAACAAAAACAACATCGTTCATCAgactacaaagaccatgacgg[a]
yeaJ 3Flag 2.Rv	TTCTCACTCCTGGTGCAATATGAACATTTACTGCGAAGCAcatatgaatatcctccttag[a]
stm4551 3FLAG.Fw	ACGTAACCACATTCTGGTCAGCGACGACATGCGCGCCCTAgactacaaagaccatgacgg[a]
stm4551 3Flag 2.Rv	GGTTAACGCTGTTGGCGTAGCACGATTACGCCAACAGGATcatatgaatatcctccttag[a]
yhdA 3FLAG.Fw	TGACACAAACGTGAAAAAATATTCGCAAAGATACTCGGTTgactacaaagaccatgacgg[a]
yhdA 3Flag 2.Rv	AGCGCGCGTTATTCTACGTGAAAACAAATTAAACGGCAGGcatatgaatatcctccttag[a]
yfeA 3FLAG.Fw	GCAGGGATATCTGATTGGCCGCCCGCGCCCGTTAGGCAAAgactacaaagaccatgacgg[a]
yfeA 3Flag 2.Rv	TGCAGAAACCGGGGAGCTATCCCCGGTTTTTTTATGCCGCcatatgaatatcctccttag[a]
yhjK 3FLAG.Fw	AGAACGGTATCTGTCGCACGAAAATCCTGATTACAAAAGTgactacaaagaccatgacgg[a]
yhjK 3Flag 2.Rv	GTTAAAAAGTTTGAGCTGGCTCGCACAAGCGCGACCTTTTcatatgaatatcctccttag[a]
stm2503 3FLAG.Fw	ATATTTAATTGGCGAGCCGCAGCTATTATCTGAGATTCAGgactacaaagaccatgacgg[a]
stm2503 3Flag 3.Rv	CAATATTGAGGAAGAAAGCTCGCCGGAGATCGCCGCGTAAATTGTGAGCCAGACGCCGCGCCGCGGCGTCTGTCTcatatgaatatcctccttag[a]
01-G[e]	CTCGAGATGTTCCCAAAAATAATGAATG
XhoI3xflag	CTCGAGTTACTATTTATCGTCGTCATC
*hmsT*x3xf Fw	gcggccgcAGGCTCTGGTACGGATTTC
*hmsT*x3xf Rv	ctcgagttactatttatcgtcgtcatctttgtagtcgatatcatgatctttataatcaccgtcatggtctttgtagtcAGGGGAAGACTGTAC-ATTTG[b]
Deletion and restoration experiments	
01-A[e]	GCGGCCGCTGCCAGTGTAACTGTGGA
01-B[e]	CTCGAGACAATTTTCCCAAATTATAGAA
01-C[e]	CTCGAGGCCGGGCTTCAGGATTT
01-D[e]	AGATCTCTGGGACACGACCGTAA
01-E[e]	CACAGTTGTTATAACGTTAC
01-F[e]	CCTGAACAAAGACTCGCT
yeaJ-Km Fw	TTTCGGCTTTATCGCAACGTCACACGCGAAAATATTAGCGATGCCATGACCGGGCTTTACAAAGCCACGTTGTGTCTCAA[c]
yeaJ-Km Rv	GGGCTGCATATTATAAATCCCGGCAGAAAAATGGACTGTCTTATCCGGTGCGATAATTTGGCGCTGAGGTCTGCCTCGTG[c]
02 Clo-Fw[e]	atgaatttgcatcataaagcgctcaggcactttatctcggcaagcgtcatcgttttgacagtgtaggctggagctgcttc[d]
02 Clo-Rv[e]	ctatgatgaacgatgttgtttttgttgtttgttcagatagagctgcgcatcggaagcctgcatatgaatatcctccttag[d]
02-E[e]	AGCATATTCGCGATCAGG
02-F[e]	CGTTGTGTCGGTATTGCT
02-H[e]	GCGGCCGCATGAATTTGCATCATAAAGCG
02-D[e]	GGCGATGCGCAGATAGT
03-A[e]	GCGGCCGCGATATCACCCAACAAATG
03-B[e]	CTCGAGCATCCCATTTAAGCGCCA
03-C[e]	CTCGAGCGATAATAGGGAGAACAGC
03-D[e]	AGATCTCAGATACGCCGGTAATTTT
03-E[e]	TGGACCTCTCCTATCCG
03-F[e]	TGCTGCTGCCATTTTCAAT
04-A[e]	GCGGCCGCGGAATTGTCGTACACGGT
04-B[e]	CTCGAGAACTTCTGGTTATTGATACA
04-C[e]	CTCCAGCCTTCGCGCCAGCCATC
04-D[e]	AGATCTCTCACAACGAAATCCGCC
04-E[e]	AGCGTAGCGTCCTGGC
04-F[e]	CCAACTGGACGTTCATTG
05-A[e]	GCGGCCGCACCGGTAATTCAATCGCC
05-B[e]	CTCGAGTCTATCCGAATCGCCGG
05-C[e]	CTCGAGCCAGCCGTGGGTATACC
05-D[e]	AGATCTGTTTGAACAGGGCGTGC
05-E[e]	ATCTCTTCACGCAAACGC
05-F[e]	CGCGTCTGTTTGATCTTG
06-A[e]	GCGGCCGCCGTCATCCGTTCCTTGAA

Oligonucleotide	Sequence (5′ to 3′)
06-B[e]	CTCGAGAATGCTCACATCATTATATAAG
06-C[e]	CTCGAGTAAAAAAACCGGGGATAGCTCCCCGGTTTC
06-D[e]	AGATCTGATCGCGGCGCTGAACGC
06-E[e]	CCAGGTTTGGGCGATGT
06-F[e]	TATGTCGTTGATGCCAGC
06 Clo-Fw[e]	ATGCCGGATAAGTGTAACGTATTAAAAAATATAAAAATATTCTTACTGGCCTTCTGCCTGGTGTAGGCTGGAGCTGCTTC[d]
06 Clo-Rv[e]	TTATTTGCCTAACGGGCGCGGGCGGCCAATCAGATATCCCTGCAAACTGTGTACGCCAAGCATATGAATATCCTCCTTAG[d]
06 Tet-Fw[e]	ATGCCGGATAAGTGTAACGTATTAAAAAATATAAAAATATTCTTACTGGCCTTCTGCCTGCGCTGTTAATCACTTTACTT[f]
06 Tet-Rv[e]	TTATTTGCCTAACGGGCGCGGGCGGCCAATCAGATATCCCTGCAAACTGTGTACGCCAAGGGTTATCAAGAGGGTCATTA[f]
07-A[e]	GCGGCCGCGACGATATGGCAAAATAATG
07-B[e]	CTCGAGGATTCCGTGCAAGCATTAA
07-C[e]	CTCGAGGGCCTGGCCGTTTCTG
07-D[e]	AGATCTAGCAACTTGAACAAGAGCA
07-E[e]	GATTATTTTTTCTCCGCACA
07-F[e]	CTTAGAAGACCTGAACTTC
08-A[e]	GCGGCCGCCACAGCATGGCGGTAAAA
08-B[e]	CTCGAGGTTAACTCCGACGGTTATA
08-C[e]	CTCGAGGTTTAATTTGTTTTCACGTAG
08-D[e]	AGATCTGATATTGCCCGGCGTAC
08-E[e]	GATAGCCCAGCTTATGCA
08-F[e]	CGCATAAAGCTGTTGCTG
09-A[e]	GCGGCCGCAGTTTCACCACAGGCGC
09-B[e]	CTCGAGTTAGCTCATTGGTTATGCAG
09-C[e]	CTCGAGGCTTCTTCCGCCTGTTG
09-D[e]	AGATCTTTGAGAATAAAACGCAGTTG
09-E[e]	CGCGTACGTTATCTGATG
09-F[e]	TTGAAACCGCTATTGGCG
10-A[e]	GCGGCCGCTATAGCCCGCAGGAATAC
10-B[e]	CTCGAGAATTGTTAACGAGCGGCTG
10-C[e]	CTCGAGTCGGCGTTGTGCGAGC
10-D[e]	AGACTCATCGAGCGTTGCCGGAT
10-E[e]	AGGTGATTAACGAGAATAAC
10-F[e]	CAATCACATTGAAAATGAGC
11-A[e]	GCGGCCGCGTAAGATAACTGTGCGAAG
11-B[e]	CTCGAGTTGTCGTTATTTATCGGTGA
11-C[e]	CTCGAGTTGGCGTAATCGTGCTAC
11-D[e]	AGATCTTCCTGATGCACATCAAGC
11-E[e]	AAGGTGGCGGAATTGGTA
11-F[e]	CCGGTATTGCTCCAGATA
12-A[e]	gcggccgctaacagcttaacgttgtcc
12-B[e]	ctcgagtcagcagaaccccccaa
12-C[e]	ctcgagacgccgcggcgcggcgt
12-D[e]	agatctcagcttgaagcgttgctt
12-E[e]	TCCCGCGGTTGCTCTTT
12-F[e]	aacaggccagacgcgtg
Hmst.Fw	CTCGAGATGCAGAGTAAATTGAATATG
Hmst.Rv	CTCGAGTCAAGGGGAAGACTGTAC
spvA Tet-Fw[e]	GATATTGTCCGTCAGACCCGTAAACAGTTTATTAACGCCAATATGTCATGGCCGGGCTCGCTGTTAATCACTTTACTT[f]
spvA Tet-Rv[e]	GTAATCGCTAACTGTCGGGCAAAGGTATTCAGTGCTTCAAATGGCGTATAGTCGGCGGTTGGTTATCAAGAGGGTCATTA[f]
Primers to generate the inactive GGGSF motif	
11-A GGGSF[e]	gcggccgcgcctgtggctgaacggta
11-B GGGSF[e]	ggatcccccgccaaaacggcaga [g]
11-C GGGSF[e]	ggatcctttctggtgttgctgacc [g]
11-D GGGSF[e]	agatcttcatagggcgcgcatgt
01-A GGGSF[e]	gcggccgcTTCGCCTGGGTAAGTTAC
01-B GGGSF[e]	ggatccGCCGCCAAAGCGCCC[g]
01-C GGGSF[e]	ggatccTTTGCGGTGATTATGTGCG[g]
01-D GGGSF[e]	agatctTCATGCCGCCACTTCGG
HmsT-A GGGSF[e]	gcggccgcGTTGCGTCGCACTGATAA
HmsT -B GGGSF[e]	ggatccACCACCATAACGCACCAC[g]
HmsT -C GGGSF[e]	ggatccTTTCTCGTACTCCTAACAC[g]
HmsT -D GGGSF[e]	agatctTCAAGGGGAAGACTGTAC
Primers to generate the inactive GxxE motif	
11-A GGGSF[e]	gcggccgcgcctgtggctgaacggta
11-B GxxE[e]	ACCGGATCCCACGGCGTCCCGAATTG

Oligonucleotide	Sequence (5′ to 3′)
11-C GxxE[e]	ACCGGATCCCGCGAAGTCGTCTGCCGTTTTGG
11-D GGGSF[e]	agatcttcatagggcgcgcatgt
Primers to generate the probes for Southern analysis	
adrA int. Fw	cggctattcactgtcgg
adrA int. Rv	tagcgttatctgtaattgac
yeaJ int. Fw	acaccggtgctggaaca
yeaJ int. Rv	acgaataatacgctccgg
yciR int. Fw	accggcctgcccaatc
yciR int. Rv	gccgcgccaggtaattt
stm1987 II Fw	GATGACCAAAGCGATCG
stm1987 III Rv	AGTGACAGCCAGTCTAC
yegE VI Fw	TGACTCTATCGGAGAAGC
yegE V Rv	AACAGGCCAAACTCATCG
yfeA int. Fw	ccactctggcgacgct
yfeA int. Rv	cctgtcagcaccagcaa
yfiN III Fw	TTCCATTGCCGGTATCAC
yfiN III Rv	ATACAGCTGCGAAATGCC
yhdA int. Fw	gttagccggtagcgcat
yhdA int. Rv	tttatcggtgcgctggc
STM3388 IV Fw	TTATTTGACGCCCGGCTT
STM3388 V Rv	TTTTCCGTCCAGCGGATA
yhjK int. Fw	ttaatgaacgactgcccat
yhjK int. Rv	cggcagcgtgtggatc
stm4551 III Fw	GCCCATCATATGACCGTA
stm4551 II Rv	CTCTCGTTTTCCCCCTTT
stm2503 int. Fw	tatcagcgttatatgccct
stm2503 int. Rv	atagctcacgccgacag
Primers for RT-PCR	
gyrB.rt.Fw	cggtagtcaacgctctgtc
gyrB.rt.Rv	ggccagaaacgtaccatcgt
csgD.rt.Fw	gcaggataatttaagccgca
csgD.rt.Rv	taatccgctgaccacgtgttc
csgA.rt.Fw	caaacgatgcccgtaaatc
csgA.rt.Rv	tttagcgttccactggtcga
spvA.rt.Fw	agccggacaacagtaccgc
spvA.rt.Rv	ccgcaatcaactgttccacc

[a]Priming sequences designed to anneal to the beginning of the 3xFlag-coding sequence (Fw) and to the Km^R cassette (Rv) underlined.
[b]Complete 3xFlag-coding sequence underlined.
[c]Priming sequence for the Km resistance cassette underlined.
[d]Priming sequence for the Clo resistance cassette underlined.
[e]Equivalences of gene names and the internal code used to name primers utilized for pKO3blue experiments: 01 (*adrA*); 02 (*yeaJ*); 03 (*yciR*); 04 (*stm1987*); 05 (*yegE*); 06 (*yfeA*); 07 (*yfiN*); 08 (*yhdA*); 09 (*stm3388*); 10 (*yhjK*); 11 (*stm4551*); 12 (*stm2503*).
[f]Priming sequence for the Tet resistance cassette underlined.
[g]*BamH*I site underlined