Supporting Information

Regulation of Enteric *vapBC* Transcription: Induction by VapC Toxin Dimer-Breaking

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Plasmids Constructed

pKW71512. A DNA fragment containing *vapBC* from *S. enterica* LT2 was generated using primers VAPBC_LT2#1 and VAPBC_LT2#2. The PCR product was digested with BamHI and EcoRI and inserted in pNDM71, a mini-R1 cloning vector.

pKW512HB. Two PCR products containing either *vapB* or *vapC* from *S. enterica* LT2 was generated using primers H6B-down-EcoRI and VAPB_LT1#HIS62 or VAPC-1_LT2#12 and VAPC_LT2#HIS62, respectively. PCR product containing vapB was digested with *Eco*RI and BamHI and PCR product containing *vapC* was digested with BamHI and HindIII. The digests were then inserted into pMG25. pKW512HB produces VapB with a N-terminal tag of six histidines and VapC upon addition of IPTG.

pKW512HC. Two PCR products containing either *vapB* or *vapC* from *S. enterica* LT2 were generated using primers VAPB_LT2#HIS61 and VAPB_LT1#HIS62 or VAPC_LT2#HIS61 and VAPC_LT2#HIS62, respectively. The PCR product encoding *vapB* was digested with EcoRI and BamHI and the PCR product containing *vapC* was digested with BamHI and HindIII. The digests were then inserted into pMG25. pKW512HC produces VapC with an N-terminal tag of six histidines and VapB upon addition of IPTG.

pKW512TFZD7A. Two PCR products were generated using primers vapBC_down#EcoRI and D7A-up-N or vapBC-1LT2up#BamHI and H6C- down-D7A using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapBC-1LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254, a mini-R1 *lacZ* transcriptional

fusion vector. pKW512TFZD7A contains non-toxic *vapBC*^{D7A} in a transcriptional fusion to *lacZYA*.

pKW512TFZD7A-1. Two PCR products were generated using primers pOU254_CW and PBC-10_MUT_UP, or PBC-10_MUT_DOWN and pOU254_CCW using pKW512TFZD7A as template. Overlapping PCR products were then used as templates in a second round of PCR with primers pOU254_CW and pOU254_CCW. The resulting PCR product was digested with BamHI and EcoRI and inserted in pOU254. pKW512TFZD7A-1 encodes a non-toxic variant VapBC^{D7A} in a transcriptional fusion with *lacZYA* having an ATC to GAT mutation in the promoter.

pKW512TFZD7A-2. Two PCR products were generated using primers pOU254_CW and PBC-35_MUT_UP, or PBC-35_MUT_DOWN and pOU254_CCW using pKW512TFZD7A as template. Overlapping PCR products were then used as templates in a second round of PCR with primers pOU254_CW and pOU254_CCW. The resulting PCR product was digested with BamHI and EcoRI and inserted in pOU254. pKW512TFZD7A-2 encodes a non-toxic variant VapBC^{D7A} in a transcriptional fusion with *lacZYA* with GTA for TAC substitution in promoter

pKW512TFZD7A-1-2. Two PCR products were generated using primers pOU254_CW and PBC-10_MUT_UP, or PBC-10_MUT_DOWN and pOU254_CCW using pKW512TFZD7A-2 as template. Overlapping PCR products were then used as templates in a second round of PCR with primers pOU254_CW and pOU254_CCW. The resulting PCR product was digested with BamHI and EcoRI and inserted in pOU254. pKW512TFZD7A-1-2 encodes a

non-toxic variant VapBC^{D7A} in a transcriptional fusion with *lacZYA* with GTA for TAC and ATC for GAT substitution mutation in promoter

pKW254BC. A DNA fragment encoding *vapBC* from *S. enterica* LT2 was generated using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was digested with BamHI and EcoRI and inserted in pOU254. pKW254BC contains *vapBC* in a transcriptional fusion with *lacZYA.*

pKW254BCL40A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-L40A-rv or vapC-1_LT2up#BamHI and VAPC-L40A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The resulting PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCL40A encodes *vapBC*^{L40A} in a transcriptional fusion to *lacZYA.*

pKW254BCM41A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-M41A-rv or vapC-1_LT2up#BamHI and VAPC-M41A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCM41A encodes *vapBC*^{M41A} in a transcriptional fusion to *lacZYA.*

pKW254BCL43A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-L43A-rv or vapC-1_LT2up#BamHI and VAPC-L43A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR

using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCL43A encodes *vapBC*^{L43A} in a transcriptional fusion to *lacZYA.*

pKW254BCI44A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-I44A-rv or vapC-1_LT2up#BamHI and VAPC-I44A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCI44A encodes *vapBC*^{I44A} in a transcriptional fusion to *lacZYA.*

pKW254BCY45A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-Y45A-rv or vapC-1_LT2up#BamHI and VAPC-Y45A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCY45A encodes *vapBC*^{Y45A} in a transcriptional fusion to *lacZYA.*

pKW254BCE48A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-E48A-rv or vapC-1_LT2up#BamHI and VAPC-E48A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCE48A encodes *vapBC*^{E48A} in a transcriptional fusion to *lacZYA.*

pKW254BCK49A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-K49A-rv or vapC-1_LT2up#BamHI and VAPC-K49A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCK49A encodes *vapBC*^{K49A} in a transcriptional fusion to *lacZYA.*

pKW254BCY72A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-Y72A-rv or vapC-1_LT2up#BamHI and VAPC-Y72A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCY72A encodes *vapBC*^{Y72A} in a transcriptional fusion to *lacZYA.*

pKW254BCA76S. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-A76S-rv or vapC-1_LT2up#BamHI and VAPC-A76S-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCA76S encodes *vapBC*^{A76S} in a transcriptional fusion to *lacZYA.*

pKW254BCA77S. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-A77S-rv or vapC-1_LT2up#BamHI and VAPC-A77S-f using S. *enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then

digested with EcoRI and BamHI and inserted into pOU254. pKW254BCA77S encodes *vapBC*^{A77S} in a transcriptional fusion to *lacZYA.*

pKW254BCI78A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-I78A-rv or vapC-1_LT2up#BamHI and VAPC-I78A-f using *S. enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCI78A encodes *vapBC*^{I78A} in a transcriptional fusion to *lacZYA.*

pKW254BCR89A. Two PCR products were generated using primers vapBC_down#EcoRI and VAPC-R89A-rv or vapC-1_LT2up#BamHI and VAPC-R89A-f using S. *enterica* LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers vapBC_down#EcoRI and vapC-1_LT2up#BamHI. The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCR89A encodes *vapBC*^{R89A} in a transcriptional fusion to *lacZYA.*

pKW512HBL43A. A PCR product containing *vapC* from *S. enterica* LT2 was generated using primers H6C-down-BamhI-KpnI and VAPC_LT2#HIS62 using pKW254BCL43A as template. The PCR product encoding *vapC*^{L43A} was digested with *Bam*HI and *Hind*III and inserted into BamHI and HindIII digested pKE512HB. pKW512HBL43A produces VapB with a N-terminal tag of six histidines and VapC^{L43A} upon addition of IPTG.

pKW512HBI44A. A PCR product containing *vapC* from *S. enterica* LT2 was generated using primers H6C-down-BamhI-KpnI and VAPC_LT2#HIS62 using pKW254BCI44A as

template. PCR product containing *vapC*^{I44A} was digested with *Bam*HI and *Hind*III and inserted into BamHI and HindIII digested pKE512HB. pKW512HBI44A produces VapB with a N-terminal tag of six histidines and VapC^{I44A} upon addition of IPTG.

pKW512HBY72A. A PCR product containing *vapC* from *S. enterica* LT2 was generated using primers H6C-down-BamhI-KpnI and VAPC_LT2#HIS62 using pKW254BCY72A as template. PCR product containing *vapC*^{Y72A} was digested with *Bam*HI and *Hind*III and inserted into BamHI and HindIII digested pKE512HB. pKW512HBY72A produces VapB with a N-terminal tag of six histidines and VapC^{Y72A} upon addition of IPTG.

pKW512HBA76S. A PCR product containing *vapC* from *S. enterica* LT2 was generated using primers H6C-down-BamhI-KpnI and VAPC_LT2#HIS62 using pKW254BCA76S as template. PCR product containing *vapC*^{A76S} was digested with *Bam*HI and *Hind*III and inserted into BamHI and HindIII digested pKE512HB. pKW512HBA76S produces VapB with a N-terminal tag of six histidines and VapC^{A76S} upon addition of IPTG.

Supporting Materials and Methods

β-galactosidase assays

Overnight cultures were diluted to an OD_{600} ~ 0.05 in LB supplemented with appropriate antibiotics and grown at 37°C to an OD_{600} of 0.5 and diluted to OD600 of 0.05. At OD600 of 0.4-0.5 samples of 500 µL were taken. Activity of the fusion was measured by monitoring β-galactosidase activity (Miller Units) in samples from cells carrying pOU254 derivatives using the chloroform protocol variant as described (41). Experiments were carried out in triplicates.

Quantitative PCR

Total RNA was extracted from cell samples using RNeasy mini kit (Qiagen). After elution, RNA was treated with DNase in an additional step to ensure that RNA was completely DNA free. Total RNA was converted to cDNA in 20 uL standard reactions with 0.32 µg of RNA and random primers using MultiScribe™ reverse transcriptase (AppliedBiosystems) according to manufactures instructions. The cDNA was diluted 100-fold and 6 µL used in 20 uL standard reactions containing 2uL of each primer and 10 µL 2x Sybr Green PCR mastermix (Qiagen). Primers used were q-vapB-f and q-vapB-rv for *vapB* mRNA and rpsA qPCR-f and rpsA qPCR-rv for internal reference gene (*rpsA*). The cDNA was amplified in a Rotor-Gene Q real-time PCR cycler (Qiagen) and the quantification performed using Rotor-Gene Q software by the $\Delta\Delta C_t$ relative quantification method (42). Standard curves for primer efficiencies were performed using 10, 100, 10^3 and 10^4 dilutions of cDNA and was taken into consideration in the C_t values obtained.

***In vitro* cross linking of purified VapC**

The presence of VapC dimer in solution was verified by *in vitro* cross linking with formaldehyde. 5 pmol Purified VapC or $VapC^{Y72A}$ variant was incubated with 0.7% formaldehyde in storage buffer (PBS + 20% glycerol) for 15 min at 37ºC. The cross linking reaction was terminated by addition of glycine to final concentration of 0.25M and Leammli buffer. The cross linked complexes were separated by SDS-PAGE and visualised by silver staining.

MW estimation of VapBC complex bound to DNA

The molecular weight (MW) of VapBC bound to DNA was estimated according to an EMSA-based method (43). 50 pmol of VapC was incubated with 50 pmol of VapB and 5 pmol DNA probe (containing *vapO1*) in binding buffer as described above. The binding

reactions were analyzed on a series of polyacrylamide gels (7%-10%) including four molecular weight markers (Lactal Albumin, 14.2kD, Carbonic Anhydrase, 29kD, Ovoalbumin, 44kD, Bovine Serum Albumin, 66kD). The gels were silver stained and the distance from the well to stained protein band was measured and divided by the bromophenol blue migration distance to give the relative mobility (R_f). The logarithm of the R_f values for protein complex and molecular weight marker was plotted against the gel acrylamide concentration and a best-line fit was obtained. The negative slopes of these fits were plotted against the molecular weights of the protein standards on a double-logarithmic scale and a best-line fit was obtained. The MW of the complex (VapBC bound to DNA) could then extrapolated according to the equation ($y = 0.0884x + 0.6332$) and the MW of the DNA (22.116 kD) was subtracted to give the MW of VapBC.

Western blot analysis

The stability of VapB after addition of chloramphenicol was determined by Western blot analysis. VapB was induced from pKW51, a low-copy-number plasmid, by addition of 1 mM IPTG for 10min to exponentially growing MG1655, KW10 (MG1655 Δ*lon*) and KW11 (MG1655Δ*clp*). Translation was inhibited by addition of 50 µg/mL choramphenicol and 1 mL samples were taken at time points indicated. Samples were rapidly harvested at 4ºC and cell pellet resuspend in 50 µL leammli buffer and boiled for 2 min. Proteins from 10 µL samples were separated by SDS-PAGE and electroblotted onto a PVDF transfer membrane (GE healthcare) and membrane blocked 1hr at ambient temperature in PBS-T (PBS with 0.1% Tween) with 5% milk powder. The membrane was briefly washed in PBS-T and then incubated for at least 1 hr at room temperature with VapB polyclonal antibodies (Eurogentec) in PBS-T and 2% milk powder. After 2 x 5 min washing steps in PBS-T was followed by incubation with HRP conjugated anti-rabbit IgG antibodies (Sigma) in PBS-T with 2% milk powder for 1 hr at room temperature. The membrane was then washed 2 x 5 min and 15 min

in PBS-T and the blot detected using ECL plus western blotting detection system (GE Healthcare).

Primer extension analysis

To map the promoter *vapBC* from *S. enterica* LT2, primer extension analysis was performed on total RNA from KP1001 *S. enterica* LT2 or *E. coli* K-12 variants containing a plasmid encoding the *vapBC* operon. This was done in accordance with (32), however with modifications. The vapB-5#PE primer (4 pmol) which is specific to *vapB* in *vapBC* mRNA was labelled in a reaction with 4μL [γ-^{32}P]-ATP and T_4 polynucleotide kinase (NEB) for 30 min at 37ºC. Labelled primer (0.2 pmol) was hybridized with 10 μg of total RNA isolated from *S. enterica* LT2 or *E. coli* K-12 using RNase easy RNA extraction kit (Qiagen). Hybridization with primer was followed by reverse transcription using 0.1U of SuperScript II Reverse transcriptase (Invitrogen) in 1 x First Strand buffer, 10 mM DTT and 1 mM dNTP for 1 hr at 42ºC. Radiolabelled cDNA was separated in a 6% PAGE gel containing 8 M Urea and visualized by phosphor imaging.

Supporting Figures

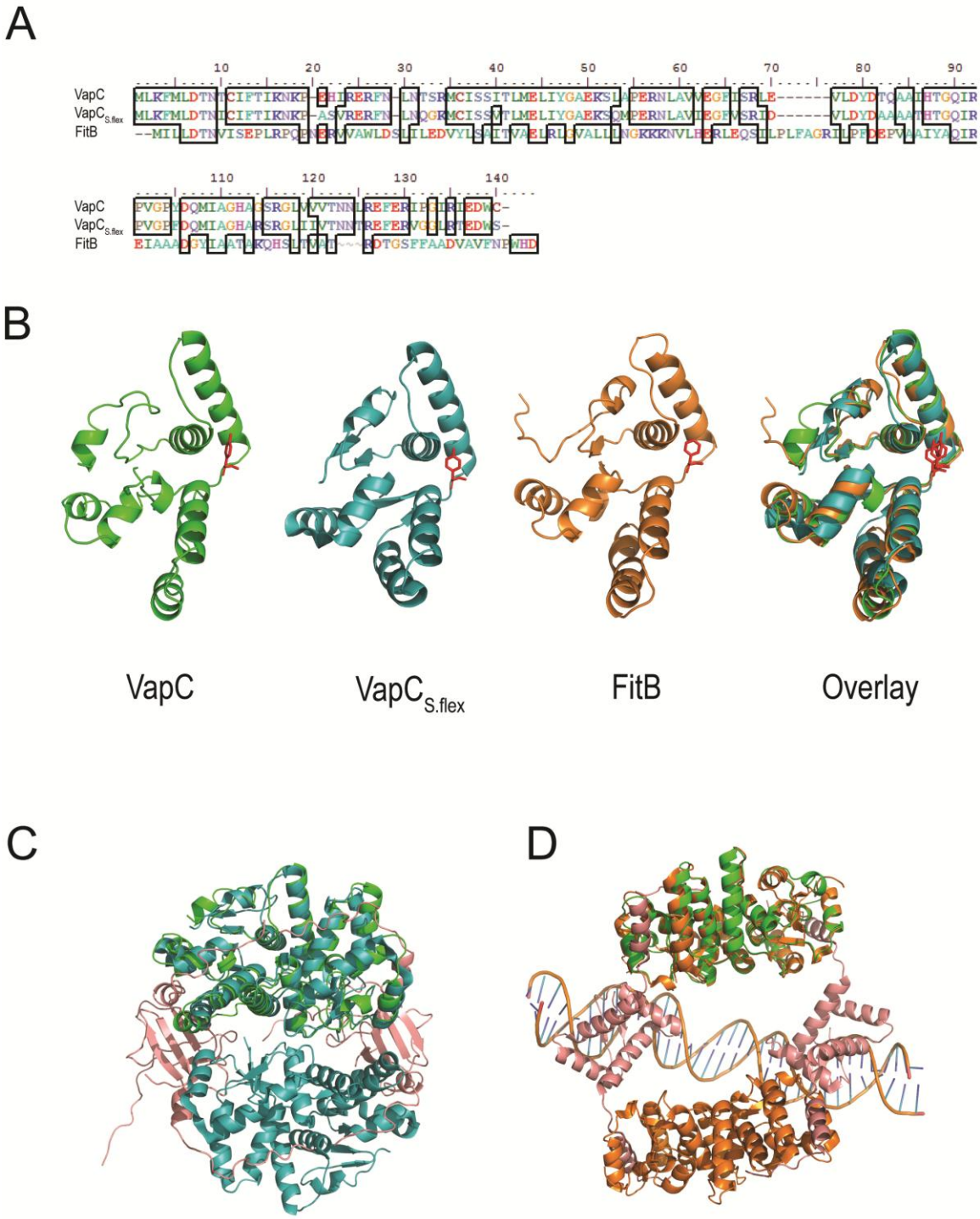
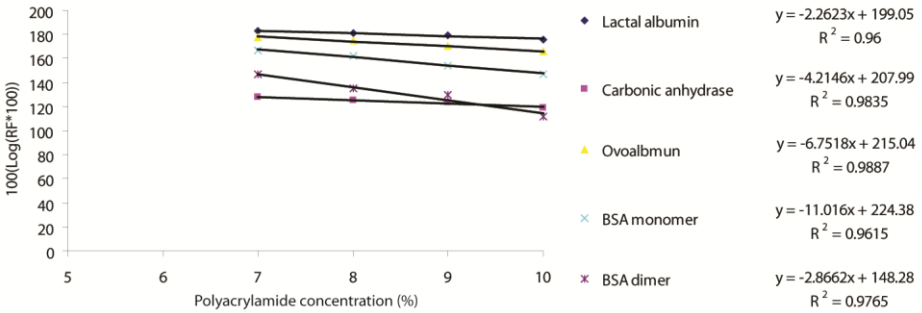


Figure S1. Structural alignment of VapC with FitAB. **A**) Protein sequence alignment of VapC (STM3033) from S. *enterica* LT2, VapC$_{S.flex}$ (MvpT) of *S. flexneri* 2a YSH6000 virulence plasmid pMYSH6000 and FitB from *N. gonorrhoea*. Identical residues are boxed.

B) The tertiary structure of VapC from S. *enterica* LT2 was predicted using PHYRE, Protein Homology/analogY Recognition Engine (34), http://www.sbg.bio.ic.ac.uk/~phyre/index.cgi). The predicted VapC (green) structure was aligned with the structure of *S. flexneri* $VapC_{S.flex}$ (cyan) (33) and *N. gonorrhoea* VapC homologue, FitB (2BSQ chain A in orange) (29) using PYMOL v1.3. Y72 in VapC and P78 in FitB is highlighted in red. **C**) Proposed VapC dimer (green) aligned with $VapC_{S.flex}$ dimer (cyan) in $vapBC_{S.flex}$ complex. VapB antitoxin dimers containing AbrB DNA binding domain are shown in salmon. The *S. flexneri* VapBC complex protein structure was acquired from the protein data bank PDB ID; 3TND. **D**) Proposed VapC dimer (green) aligned with FitB dimer (orange) in FitAB complex bound to DNA inverted repeat. FitA dimers containing RHH DNA binding domain, binding to DNA repeats are shown in salmon. The FitAB complex protein structure was obtained from the protein data bank PDB ID; 2BSQ.

A



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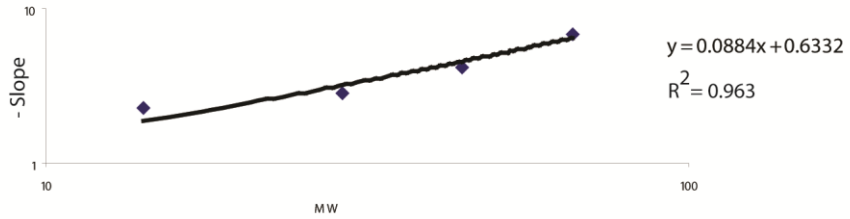

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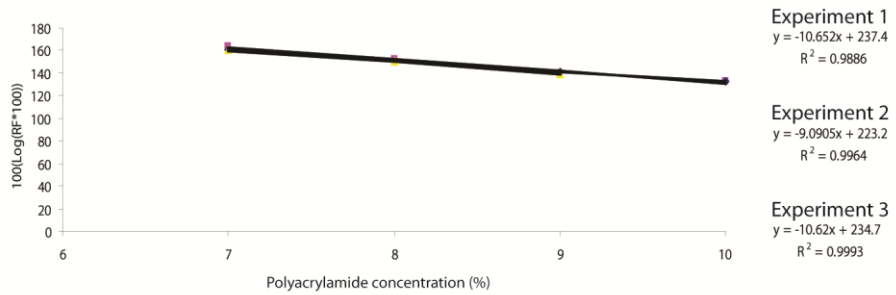

D

	-slope	MW (kD)	MW - DNA (kD)
Exp 1	10.652	119.8645	97.74854
Exp 2	9.0905	102.2005	80.08451
Exp 3	10.62	119.5025	97.38655

91.74 kD

Figure S2. Estimation of the Molecular weight (MW) of VapBC bound to *vapO1*. A) A graph showing the logarithm of the relative mobility (RF) of marker proteins; Lactal albumin (blue), Carbonic anhydrase (magenta), Ovoalbumin (Yellow), BSA monomer (teal) and BSA dimer (purple) plotted as a function of polyacrylamide concentration (%). The calculated

slopes of regression equations are shown on the right. **B**) The slopes of the marker protein regressions are plotted as a function of the molecular weight. The value of the regression equation of the plot is shown on the right. **C**) A graph showing the logarithm of the relative mobility of VapBC-DNA complex of three independent experiments. The calculated slopes of regression equations of the three experiments are shown on the right. **D**) Table with the molecular weight of VapBC-DNA complexes and VapBC (subtracted with mass of promoter DNA fragment 22.106 kD) extrapolated according to the equation $y = 0.0884x + 0.6332$. The average mass of three experiments is 91.74 ± 10.09 kD.

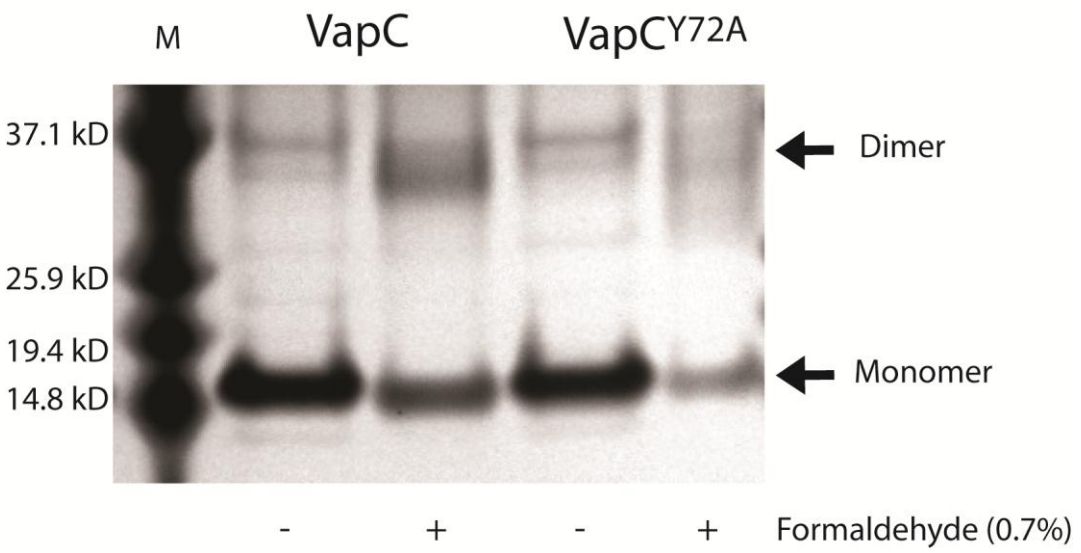

Figure S3. *In vitro* cross-linking of VapC shows mutant being deficient in dimerization. Five pmol purified VapC or VapC^{Y72A} were cross-linked by incubation with 0.7% formaldehyde or storage buffer for 15' at 37ºC. Cross-linked complexes were resolved by SDS-PAGE and subsequent silver staining. M is a molecular weight marker and position of monomer and dimer complexes are indicated with arrows.

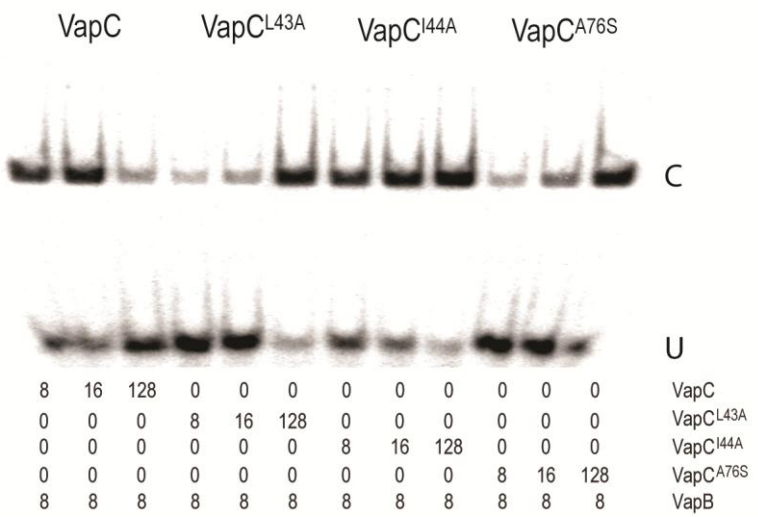

Figure S4 Effect of substitution mutations in VapC dimerization interface on conditional cooperativity. Electrophoretic mobility shift assay with purified VapC, $VapC^{L43A}$ $VapC^{I44A}$ $VapC^{A76S}$ and VapB with promoter DNA. The proteins were mixed with radiolabelled promoter DNA containing one binding site in concentrations shown (nM). Protein-DNA complexes were separated by 6% native PAGE and analysed by phosphorimaging. U and C indicate position of unbound and bound DNA, respectively.

Supporting Tables

Table S1 Strains and plasmids used and constructed

Strain/Plasmid	Description	Reference
MG1655	*E. coli* K-12	Laboratory collection
KP1001	*S. enterica* LT2	Laboratory collection
TB28	MG1655 Δ*lacIZYA*	Laboratory collection
C41	BL21 derivative	Laboratory collection
KW10	Δ*lon*::*aphA*	This study
KW11	Δ*clp*::*aphA*	This study
pKW71512	pNDM71::*vapBC*	This study
pMG25	$p_{A1/O3/O4}$	Laboratory collection
pKW512HB	$p_{A1/O3/O4}$::H6-*vapB*-*vapC*	This study
pKW512HC	$p_{A1/O3/O4}$::*vapB*-H6-*vapC*	This study
pBAD33	p_{BAD}	(44)
pKW3353HC	p_{BAD}::SD_{opt}::$vapC^{D7A}$ -H6	(25)
pOU254	*lacZYA* transcrional fusion vector	Laboratory collection
pKW512TFZD7A	pOU254, $vapBC^{D7A}$::*lacZYA*	This study
pKW512TFZD7A-1	pKW512TFZD7A, mutation in IR of *vapO1*	This study
pKW512TFZD7A-2	pKW512TFZD7A, mutation in IR of *vapO2*	This study
pKW512TFZD7A-1-2	pKW512TFZD7A, mutation in IR of *vapO1* and *vapO2*	This study
pKW254BC	pOU254, *vapBC*::*lacZYA*	This study
pKW254BCL40A	pKW254BC, L40A substitution	This study
pKW254BCM41A	pKW254BC, M41A substitution	This study
pKW254BCL43A	pKW254BC, L43A substitution	This study
pKW254BCI44A	pKW254BC, I44A substitution	This study
pKW254BCY45A	pKW254BC, Y45A substitution	This study
pKW254BCE48A	pKW254BC, E48A substitution	This study
pKW254BCK49A	pKW254BC, K49A substitution	This study
pKW254BCY72A	pKW254BC, Y72A	This study

	substitution	
pKW254BCA76S	pKW254BC, A76S substitution	This study
pKW254BCA77S	pKW254BC, A77S substitution	This study
pKW254BCI78A	pKW254BC, I78A substitution	This study
pKW254BCR89A	pKW254BC, R89A substitution	This study
pKW512HBL43A	pKW512HB, with $vapC^{L43A}$	This study
pKW512HBI44A	pKW512HB, with $vapC^{I44A}$	This study
pKW512HBY72A	pKW512HB, with $vapC^{Y72A}$	This study
pKW512HBA76S	pKW512HB, with $vapC^{A76S}$	This study

Table S2 Oligonucleotides used in the study

Oligo name	Sequence
VAPBC_LT2#1	CCCCCGGATCCGACAAGGATGCCCTGCGGAG
VAPBC_LT2#2	CCCCCGAATTCGAATCCGGGGATAACATCTGA
H6B-down-EcoRI	CCCCCGAATTCAAAATAAGGAGGAAAAAAAAATGCATCACCATCACCATCACCACACAACACTTTTTTTTAG
VAPB_LT1#HIS62	CCCCCGGATCCCAGCATCAAAATCCTTCCCG
VAPC-1_LT2#12	CCCCCGGTACCGGATCCAAAATAAGGAGGAAAAAAAAATGCTGAAATTCATGCTTGATAC
VAPC_LT2#HIS62	CCCCCAAGCTTGCAGAAATTAGCACCAGTCTTC
VAPB_LT2#HIS61	CCCCCGAATTCAAAATAAGGAGGAAAAAAAAATGCACACAACACTTTTTTTTAG
VAPC_LT2#HIS61	CCCCCGGATCCAAAATAAGGAGGAAAAAAAAATGCATCACCATCACCATCACCTGAAATTCATGCTTGATACCAA
vapBC_down#EcoRI	CCCCGAATTCGACAAGGATGCCCTGCGGAG
vapC-1_LT2up#BamHI	CCCCGGATCCATGCAGAAATTAGCACCAGTCTTC
H6C- down-D7A	CATGCTTGCGACCAATACCTGTATTTTC
D7A-up-N	GTATTGGTCGCAAGCATGAATTTCAG
pOU254_CW	TAGGGGTTCCGCGCA
pOU254_CCW	TGTGGGATTAACTGC
PBC-10_MUT_DOWN	ATATACGCGTTGTATGATCAGAAAGGAGGCAGTTATGCAC
PBC-10_MUT_UP	CTGCCTCCTTTCTGATCATACAACGCGTATATCCATTATGAG
PBC-35_MUT_DOWN	GGATTATTTTTTCCTTACTATCTCTTTGACATATACATCGCCTC
PBC-35_MUT_UP	ATATGTCAAAGAGATAGTAAGGAAAAAATAATCCAGTCACATGACG
VAPC-	CAGCTCCATCACCGCGATGGAGCTGATTTACGGTGC

L40A-f	
VAPC-L40A-rv	GTAAATCAGCTCCATCGCGGTGATGGAGCTGATACAC
VapC-M41A-f	CAGCTCCATCACCTTAGCGGAGCTGATTTACGGTGCTG
VapC-M41A-rv	CACCGTAAATCAGCTCCGCTAAGGTGATGGAGCTGATAC
VapC-L43A-f	CATCACCTTAATGGAGGCGATTTACGGTGCTGAAAAAAG
VapC-L43A-rv	TTTCAGCACCGTAAATCGCCTCCATTAAGGTGATGGAGC
VAPC-I44A-f	CCTTAATGGAGCTGGCGTACGGTGCTGAAAAAAGCCTG
VAPC-I44A-rv	CTTTTTTCAGCACCGTACGCCAGCTCCATTAAGGTGATGG
VAPC-Y45A-f	CTTAATGGAGCTGATTGCGGGTGCTGAAAAAAGCCTGGC
VAPC-Y45A-rv	GCTTTTTTCAGCACCCGCAATCAGCTCCATTAAGGTGATG
VapC-E48A-f	CTGATTTACGGTGCTGCGAAAAGCCTGGCGCCGGAGCG
VapC-E48A-rv	CGGCGCCAGGCTTTTCGCAGCACCGTAAATCAGCTCC
VapC-K49A-f	GATTTACGGTGCTGAAGCGAGCCTGGCGCCGGAGCGTAATC
VapC-K49A-rv	CTCCGGCGCCAGGCTCGCTTCAGCACCGTAAATCAGCTC
VapC-F72A-f	GCCTTGAGGTTTTGGATGCGGATACACAGGCAGCGATAC
VapC-F72A-rv	TCGCTGCCTGTGTATCCGCATCCAAAACCTCAAGGCGG
VAPC-A76S-f	GGATTACGATACACAGTCGGCGATACATACCGGTCAAATC
VAPC-A76S-rv	GACCGGTATGTATCGCCGACTGTGTATCGTAATCCAAAAC
VAPC-A77S-f	TTACGATACACAGGCATCGATACATACCGGTCAAATCCG
VAPC-A77S-rv	TTTGACCGGTATGTATCGATGCCTGTGTATCGTAATCC
VAPC-I78A-f	GATACACAGGCAGCGGCGCATACCGGTCAAATCCGTGC
VAPC-I78A-rv	GATTTGACCGGTATGCGCCGCTGCCTGTGTATCGTAATC

VapC-R89A-f	CCGTGCCGAACTGGCCGCGAAGGGAACACCTGTCGGGCC
VapC-R89A-rv	GACAGGTGTTCCCTTCGCGGCCAGTTCGGCACGGATTTG
vapBC_EMSA_down	CAGAAGCCATGGGCACATATG
vapBC_EMSA_up	GATTTCGACATGTTTCACATCC
VapBCbinding#small-down	CTCATAATGGATATACGCGTTGTATATCCAGAAAGG
VapBCbinding#small-up	CCTTTCTGGATATACAACGCGTATATCCATTATGAG
q-vapB-f	GCAGTCAGATTGCCTAAATCG
q-vapB-rv	AAATCAGTACTGGCGCCTTC
rpsA qPCR-f	CTGGGTCTGAAACAGTGCAA
rpsA qPCR-r	TCCAAGCCGATGAAGATACC