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 EcoRI 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 $\textit{vapBC}^{D7A}$ in a transcriptional fusion to $\textit{lacZYA}$.

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 $\textit{lacZYA}$ 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 $\textit{lacZYA}$ 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
\textit{vapBC}\textsuperscript{L43A} in a transcriptional fusion to \textit{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 \textit{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
\textit{vapBC}\textsuperscript{I44A} in a transcriptional fusion to \textit{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 \textit{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
\textit{vapBC}\textsuperscript{Y45A} in a transcriptional fusion to \textit{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 \textit{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
\textit{vapBC}\textsuperscript{E48A} in a transcriptional fusion to \textit{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 \( \text{vapBC}^{K49A} \) 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 \( \text{vapBC}^{Y72A} \) 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 \( \text{vapBC}^{A76S} \) 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 \(\text{vapBC}^{\text{A77S}}\) in a transcriptional fusion to \(\text{lacZYA}\).

pKW254BCI78A. Two PCR products were generated using primers \(\text{vapBC}\_\text{down}\#\text{EcoRI}\) and \(\text{VAPC}\_\text{I78A}\_\text{rv}\) or \(\text{vapC}\_1\_\text{LT2up}\#\text{BamHI}\) and \(\text{VAPC}\_\text{I78A}\_\text{f}\) using \(S.\ enterica\) LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers \(\text{vapBC}\_\text{down}\#\text{EcoRI}\) and \(\text{vapC}\_1\_\text{LT2up}\#\text{BamHI}\). The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCI78A encodes \(\text{vapBC}^{\text{I78A}}\) in a transcriptional fusion to \(\text{lacZYA}\).

pKW254BCR89A. Two PCR products were generated using primers \(\text{vapBC}\_\text{down}\#\text{EcoRI}\) and \(\text{VAPC}\_\text{R89A}\_\text{rv}\) or \(\text{vapC}\_1\_\text{LT2up}\#\text{BamHI}\) and \(\text{VAPC}\_\text{R89A}\_\text{f}\) using \(S.\ enterica\) LT2 as DNA template. The resulting products were then used as templates in a second round of PCR using primers \(\text{vapBC}\_\text{down}\#\text{EcoRI}\) and \(\text{vapC}\_1\_\text{LT2up}\#\text{BamHI}\). The PCR product was then digested with EcoRI and BamHI and inserted into pOU254. pKW254BCR89A encodes \(\text{vapBC}^{\text{R89A}}\) in a transcriptional fusion to \(\text{lacZYA}\).

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

pKW512HBI44A. A PCR product containing \(\text{vapC}\) from \(S.\ enterica\) LT2 was generated using primers \(\text{H6C-down-BamhI-KpnI}\) and \(\text{VAPC}\_\text{LT2}\#\text{HIS62}\) using pKW254BCI44A as
template. PCR product containing \text{vapC}^{444A} was digested with \textit{BamHI} and \textit{HindIII} and inserted into \text{BamHI} and \text{HindIII} digested \text{pKE512HB}. \text{pKW512HBI44A} produces \text{VapB} with a N-terminal tag of six histidines and \text{VapC}^{444A} upon addition of IPTG.

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

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

**Supporting Materials and Methods**

\textbf{β-galactosidase assays}

Overnight cultures were diluted to an OD$_{600} \sim 0.05$ 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 ∆∆Ct 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 Ct 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 37°C. 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 (Rf). The logarithm of the Rf 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 Δlon) and KW11 (MG1655Δclp). 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 4°C 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-32P]-ATP and T4 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](http://www.sbg.bio.ic.ac.uk/~phyre/index.cgi). The predicted VapC (green) structure was aligned with the structure of *S. flexneri* VapC<sub>S.flex</sub> (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<sub>S.flex</sub> dimer (cyan) in vapBC<sub>S.flex</sub> 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.
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 $91.74 \pm 10.09$ kD.
Figure S3. *In vitro* cross-linking of VapC shows mutant being deficient in dimerization.

Five pmol purified VapC or VapC<sup>Y72A</sup> were cross-linked by incubation with 0.7% formaldehyde or storage buffer for 15’ at 37°C. 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 ΔlacIZYA | Laboratory collection |
| C41           | BL21 derivative | Laboratory collection |
| KW10          | Δlon::aphA | This study |
| KW11          | Δclp::aphA | This study |
| pKW71512      | pNDM71::vapBC | This study |
| pMG25         | pA1/O3/O4 | Laboratory collection |
| pKW512HB      | pA1/O3/O4::H6-vapB-vapC | This study |
| pKW512HC      | pA1/O3/O4::vapB-H6-vapC | This study |
| pBAD33        | pBAD | (44) |
| pKW3353HC     | pBAD::SDopt::vapC<sup>D7A</sup>-H6 | (25) |
| pOU254        | lacZYA transcrional fusion vector | Laboratory collection |
| pKW512TFZD7A  | pOU254, vapBC<sup>D7A</sup>::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 |
| pKW254BCA76S | pKW254BC, A76S | This study |
| pKW254BCA77S | pKW254BC, A77S | This study |
| pKW254BCI78A | pKW254BC, I78A | This study |
| pKW254BCR89A | pKW254BC, R89A | This study |
| pKW512HBL43A | pKW512HB, with $vapC^{L43A}$ | This study |
| pKW512HBI44A | pKW512HB, with $vapC^{L44A}$ | 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_LT 2#1 | CCCCCGGATCCGACAAAGGATGCCCTGCCAGAG |
| VAPBC_LT 2#2 | CCCCCGAATTCGATCCGGAGAATAACATCTGA |
| H6B-down-EcoRI | CCCCCGAATTCAAAAATAAGGAGGAAAAAATGCATCACTACCATCACCACACTTTTTCAG |
| VAPB_LT1# HIS62 | CCCCCGGATCCCGACAAAGGATGCCCTGCCAGAG |
| VAPC-1_LT2#12 | CCCCCGGGATCCGACAAAATAAGGAGGAAAAAATGCTGAATTCATGCTGATAAC |
| VAPC_LT2# HIS62 | CCCCCCAATTCAAAAATAAGGAGGAAAAAATGCACACACACACCTTTTTCAG |
| VAPB_LT2# HIS61 | CCCCCGAATTCGACAAAGGATGCCCTGCCAGAG |
| VAPC_LT2# HIS61 | CCCCCGAATTCGACAAAGGATGCCCTGCCAGAG |
| vapBC_down#EcoRI | CCCCCGAATTCGACAAAGGATGCCCTGCCAGAG |
| vapC-1_LT2up#Ba mHI | CCCCCGAATTCGACAAAGGATGCCCTGCCAGAG |
| H6C- down-D7A | CATGCTTGCGACCAATACCTGTATTTTC |
| D7A-up-N | GTATTTGGTGCAAGCATGAATTTTCAG |
| pOU254_C W | TAGGGGTTCCGCGCA |
| pOU254_CC W | TGTGGGATTAACCTGC |
| PBC-10_MUT_D OWN | ATATACGCGTTGTAGTACGAAAGGAGGCCAGTTATGCAC |
| PBC-10_MUT_UP | CTGCCTCCTTCTGATCATAAAACGCGTATATCCATTATGAG |
| PBC-35_MUT_D OWN | GGATTATTTTTTCTTACTATCTCTTTTGACATATACATCGCCTC |
| PBC-35_MUT_UP | ATATGTCAAAAGAGATAGTAAAGGAAAAAAATAATCCAGTCATGACG |
| VAPC- | CAGCTCCATCCCGCGATGGAGCTGATTTACCGGTGC |
| L40A-f | GTAATCAGCTCCATCGCGGTGATGGAGCTGATACAC |
| VapC-L40A-rv | CAGCTCCATCACCTTAGCGGAGCTGATTTACGGTGCTG |
| VapC-M41A-f | CACCGTAATCAGCTCCGCTAAGGTGATGGAGCTGATACAC |
| VapC-M41A-rv | CATCACCTTAATGGAGGCGATTTACGGTGCTGAAAAAG |
| VapC-L43A-f | TTTTACGGACCCTTAATGCCTCCATTAAGGTGATGAGC |
| VapC-L43A-rv | CCTTAATGGAAGCTCCGTACGGTGCTGAAAAAGCCTG |
| VAPC-I44A-f | CTTTTTTCAGCAACCAGATGACCCAGCTCCATTAAGGTGATGG |
| VAPC-I44A-rv | CTTTTTTCAGCAACCAGATGACCCAGCTCCATTAAGGTGATGG |
| VAPC-Y45A-f | CTTTAATGGAAGCTCCGTACGGTGCTGAAAAAGCCTG |
| VAPC-Y45A-rv | CTTTTTTCAGCAACCAGATGACCCAGCTCCATTAAGGTGATGG |
| VapC-E48A-f | CTGATTACCGGTGCTGCGAA |
| VapC-E48A-rv | CTGATTACCGGTGCTGCGAA |
| VapC-K49A-f | GATTTACGGTGCTGAAGCGATCAGCGAGCGGAGCGTAATC |
| VapC-K49A-rv | GATTTACGGTGCTGAAGCGATCAGCGAGCGGAGCGTAATC |
| VapC-F72A-f | GCCTTGAGGTTTTGGATGCGGAAATCAGCTCCATTAAGGTGATGAGC |
| VapC-F72A-rv | GCCTTGAGGTTTTGGATGCGGAAATCAGCTCCATTAAGGTGATGAGC |
| VAPC-A76S-f | GGATTACGATACACAGGCAGCGGATACACATACCGGTCAAATC |
| VAPC-A76S-rv | GGATTACGATACACAGGCAGCGGATACACATACCGGTCAAATC |
| VAPC-A77S-f | GACCGGTATGTATCGCGACTGTGTATCGTAATCCAAAC |
| VAPC-A77S-rv | GACCGGTATGTATCGCGACTGTGTATCGTAATCCAAAC |
| VAPC-I78A-f | GATTTGACCGGTATGCGCGGTGCTGCTGCTGCTGATACAC |
| VAPC-I78A-rv | GATTTGACCGGTATGCGCGGTGCTGCTGCTGCTGATACAC |
| VapC-R89A-f       | CCGTGCCGAACTGGCCGCAGAAGGAACACCTGTCGGGCC |
|-------------------|-----------------------------------------|
| VapC-R89A-rv      | GACAGGTGTTCCTTCGCGCCAGTTCCGCAAGGATTTC   |
| vapBC_EMS A_down  | CAGAAAGCCATGGGCACCATATG                  |
| vapBC_EMS A_up    | GATTTCGACATGTTTCACATCC                   |
| VapBCbinding#small-down | CTCATAATGGATATACGCGTTGTATATCCAGAAAGG |
| VapBCbinding#small-up  | CTTTTCTGGATATAACGCCTATATCCATTATGAG      |
| q-vapB-f          | GCAGTCAGATTGCCTAAATCG                    |
| q-vapB-rv         | AAATCAGTACTGGGCGCCTTC                    |
| rpsA qPCR-f       | CTGGGTCTGAAACAGTGCAA                     |
| rpsA qPCR-r       | TCCAAGCCGATGAAGATACC                     |