

**Supplementary Figure 1. Structural comparison of FitAB and VapBC.** Left: The heterooctameric structure of *N. gonorrhoeae* FitAB with the FitB toxin shown in sea green and the FitA antitoxin in yellow (PDB 2H10<sup>17</sup>). The FitA antitoxins come together two-by-two to form two ribbon-helix-helix DNA binding domains (yellow, top and bottom). Right: The structure of hetero-octameric *S. flexneri* VapBC (this study) with the VapC toxin shown in blue and the VapB antitoxins in orange.

	α2		
	50	60	70
S. flexneri VapB (C-terminus)			
P18355 1PFU	AGETWDEWF	DGNSVSADFMDNR	EQPGMQERESF
	AGETWDEWF		
	AGETWDEWF		
	AGEIWDEWF		
E920B3JE920B	AGESWDEWF		
Q93GL5 Q93GL	AGESWDEWF		EQPSVQERESF
Deidroideidr	AGESWDSWF	- DGDSVSADFMNDR	EQPAVQERESF
	AGESWDSWF	- DGENVSADFMDIR	DQPAMQERESF
D4EA25 D4EA2	AGEGWDNWF	- EGENVSADEMISR	DOPPMOEREAF
Q/CPV2 VAPB	VGESWDSWF	- DGEGAS IDFMS IR	EQPAVQEREGE
Q5PL35 Q5PL3	VGESWDSWF	- DGEGASIDEMSIR	EQPAVQEREGF
A9N3K9 A9N3K	VGESWDSWF	- DGEGASTDFMSTR	EQLAVQEREGF
D4BHM7 D4BHM	AG E S WD S WF	- DGESVTSDFMAAR	EQPGDQDREGF
Q6X2S2 Q6X2S	AG E S WD S WF	- DGEGVTADFMAER	EQPADQERETF
F3GCP9 F3GCP	AG E MWN S WF	- DGESVSDDFMAER	EQPAEQQRESL
Q4ZY19 Q4ZY1	AG E MWN S WF	- DGESVSDDFMAER	GQPAEQQRESL
F3ITV9 F3ITV	AG E MWN S WF	- DGESVSDDFMAER	GQPAEQQRESL
Q888H8 Q888H	AG E MWN S WF	- DGESVSDDFMAER	EQPVEQLRESL
F3HT15 F3HT1	AG E S WN I WF	- EGENVSDDFMTAR	EQPADQQRESF
Q1I4A9 Q1I4A	AG E S WD S WF	- EGDDVSADFMASR	EQPADQEREGF
B1J5U8 B1J5	AG E S <mark>W</mark> D S WF	- DGEDASPDFMASR	DQPADQEREGF
C6CLI6 C6CLI	AG E S <mark>W</mark> D S WF	- DDAGVTPDFMSTR	EQPDEQIRDDF
Q6D393 Q6D39	AG E S WD S WF	- E E Q G V T P D F M I I R	EQPDDQIREDF
C6C2Z8 C6C2Z	AG E S WD S WF	- EEDGVTPDFMITR	EQPDDQIREDF
E3DCE6 E3DCE	VGESWDNWF	- DGENVTADFMDDR	EQPSEQRREHF
D2TCL7 D2TCL	VGESWDNWF	- DGENVTADFMDDR	EQPSEQRREHV
F5US91 F5US9	AG E S WE S WF	- DGEGVSADFMESR	EQPANQIREPL
B7J4Y7B7J4Y	AG E S WE S WF	- DGEGVSADFMESR	EQPAGQFREPL
E6QB21 E6QB2	AG E S <mark>W</mark> E G WF	- DGKGVSADFMESR	EQPADQHRECL
Q3JDY1 Q3JDY	AG D A <mark>W</mark> D V WF	- DGPAVSPDFMDDR	DQPDEQEREAF
F6DCU1 F6DCU	L E Q T WD D WF	- DAVGVSDDFMNER	AQEPDQLRESL
Q0I5P7 00I5P	VNQVWN SWF	Y D E H T I S D D F M T D R	EQPQMQERESF
F4HEX3 F4HEX	L N N V WD S WF	N D S N S V S D <mark>D F</mark> M L S <mark>R</mark>	EQPQIQEREEL

## Supplementary Figure 2. Multiple sequence alignment of homologous VapB C-termini.

Sequences with 54-95% identity to *S. flexneri* VapB are aligned and shown with their UniProt ID. Only the VapC-interacting region is shown corresponding to residues 43-75 of *S. flexneri* VapB. Conserved residues are marked in blue and residues directly involved in VapC inhibition in the structure presented here are shown with red letters in the first line. The secondary structure of VapB is shown at the top.

		β1	β2		α1	β3	β4
				20		30	40
S. flexneri VapB (N-terminus)	M	- E T T <mark>V</mark> F	LSNRSQAV	/ R L <mark>P</mark> K A V A		<b>/ E V</b> I A V <mark>G</mark>	R T - R I I T <mark>P A G</mark> E -
P18355 YPFU	M	- E T T <mark>V</mark> F	LSNRSQAV	/ R L <mark>P</mark> K A V A		/ E V I A V <mark>G</mark>	R T - R I I T <mark>P A G</mark> E -
Q7CPV2 VAPB	M	- <mark>H</mark> T T L F	F S N R T Q A V	/ R L <mark>P</mark> K S I S	F P E D V K H V	/ E I I A V <mark>G</mark>	R S - R I I T <mark>P V G</mark> E -
Q57120 VAPB2	M	- E A S <mark>V</mark> F	M T N R S Q A V	/ R L <mark>P</mark> A E V R	FSEEIKKL	S V R V S G	SD-RILSPLNQ-
Q05459 VAGC	M R	- <b>T</b> V S I F	K N <mark>G</mark> N N R A I	I R L <mark>P</mark> R D L D	F - E <mark>G</mark> V S E L	EIVREG	DS-IILR <mark>PVRP</mark> -
Q46558 VAPB	M K	- V A K F	T T <mark>GV</mark> R S Q A V	/ R	F - D - TKE 🛚	/ I I Q R F <mark>G</mark>	NG-ILLIPKNS-
E4QWH3 VAPB1	M	- L T K V F	Q S G N S Q A V	/ R I <mark>P</mark> M D F R	F D V D T \	/ E I F R K E	N <mark>G</mark> D V V L R <mark>P</mark> V S K -
Q4QNL8 VAPB1	M	- L T K V F	Q S G N S Q A V	/ R I <mark>P</mark> M D F R	F D V D T \	/ E I F R K E	N <mark>G</mark> D V V L R <mark>P</mark> V S K -
Q57534 VAPB1	M	- L T K V F	Q S G N S Q A V	/ R I <mark>P</mark> M D F R	F D V D T \	/ E I F R K E	N <mark>G</mark> DVVLR <mark>P</mark> VSK-
P08365 CHPS	M R	- I T - I K	R W <mark>G</mark> N <mark>S</mark> A <mark>G N</mark>	AV I <mark>P</mark> N I V M K E L N	L - Q <mark>P G</mark> Q S N	/	NQ - LILT <mark>P</mark> ISR -
P13975 PEMI	MH	- <mark>T</mark> T R L K	R V <mark>G G S</mark> V M L	T V P P A L L N A L S	L - <mark>G</mark> TD <u>N</u> E <b>N</b>	/ <mark>G M</mark> V I D N	GR-LIVEPYRR-
P0AE73 MAZE	M	- <mark>H</mark> S S V K	R W <mark>G</mark> N <mark>S P</mark> A V	/ R I <mark>P</mark> A T L MQ A L N	L - N I D D E N	/ K I D L V D	G K - L I I E <mark>P V</mark> R K E
P0AE72 MAZE	M	- <mark>H</mark> S S V K	R W <mark>G</mark> N <mark>S P</mark> A V	/ R I <mark>P</mark> A T L MQ A L N	L - N I D D E N	KIDLVD	G K - L I I E <mark>P V</mark> R <u>K</u> E
P39758 ABH_B	M K S	- I <mark>G</mark> VVR	K V D E L <mark>G</mark> R I	I V M <mark>P</mark> I E L R R A L <mark>D</mark>	I - A I K <mark>D</mark> S I	IEFFVD <mark>G</mark>	DK-IILKK <mark>Y</mark> K <mark>P</mark> -
P08874 ABRB	M F M K S	- <mark>TG</mark> IVR	K V D E L <mark>G</mark> R V	/ V I <mark>P</mark> I E L R R T L <mark>G</mark>	I - A <u>E K </u> D A L	EIYVDD	E <u>K</u> - I <u>I</u> L K K <mark>Y K P</mark> -
O07779 VPB27	MKA	- <mark>V</mark>	- V D A A <mark>G</mark> R I	I	L - Q <mark>P G</mark> S T <b>V</b>	/ E I S R Y <mark>G</mark>	A <mark>G</mark> - L <mark>H</mark> L I <mark>P TG</mark> R -
P65027 VPB40	MRT	- <mark>T</mark>	- I D V A <mark>G</mark> R L	_ V I <mark>P</mark> K R I R E R L <mark>G</mark>	L - R <mark>G</mark> N D Q N	/ EI TERD	GR-IEIEPAPT-
P65028 Y2626	MRT	- <u>T</u> <u>-</u> -	- I D V A <mark>G</mark> R L	_ V I <mark>P</mark> K R I R E R L <mark>G</mark>	L - R <mark>G </mark> N D Q \	/ E I TERD	GR-IEIEPAPT-
O29774 Y476	M <u>K</u> SCKAT	K I E A <mark>V</mark> L	TMDSKGQI	I L <mark>L P</mark> K E L R E R A <mark>G</mark>	L - K A <mark>G D</mark> R L	VAIA <mark>G</mark> -	. <u>.</u> . <u></u>
P81551 S7C3	M <mark>P</mark>	- V E E V V	' K V S R N Y Q <mark>V</mark>	/ T	V - K E <mark>G D</mark> L <b>\</b>	/ K V I <mark>Y</mark> D E	N <mark>G</mark> - <mark>G V V</mark> K I
P81552 S7C4	M A	- <mark>V</mark> E E I V	K V S R N Y Q <mark>V</mark>	/ T <mark>I P</mark> A K V R Q K <mark>F</mark> Q	I - K E <mark>G D</mark> L <b>\</b>	/ K V T F D E	S <mark>G</mark> - <mark>G V V</mark> K I

**Supplementary Figure 3. Multiple sequence alignment of the VapB N-terminus with AbrB-like domains.** Multiple sequence alignment of 22 proteins containing an AbrB-like DNA-binding domain showing conservation of several aliphatic residues (light blue), which tether the domain together. Acidic residues are shown on purple, hydrophobic (including His) on sea green, Ser/Thr on dark green, proline on light green, and glycine on orange background (Clustal colour scheme). The secondary structure of VapB is shown at the top.



Supplementary Figure 4. Affinity measurements of the VapBC-DNA interaction. (a) Electrophoretic mobility shift assay (EMSA) with increasing concentrations of *S. flexneri* VapBC and constant amount of <sup>32</sup>P-labelled control DNA (lanes 1-6) or DNA I (lanes 7-14), see the Figure 5 legend for further details. DNA fragments were incubated with increasing concentrations of VapBC ( $0.2 - 2.5 \text{ ng/}\mu\text{L}$ ) and DNA-protein complexes separated by 6% native PAGE. ! indicates unbound DNA and arrows shifted DNA-protein complexes. (b) Electrophoretic mobility shift assay (EMSA) with purified VapBC and <sup>32</sup>P-labelled DNA II (lanes 1-6) or DNA III (lanes 7-14). DNA fragments were incubated with increasing concentrations of VapBC ( $0.5 - 4 \text{ ng/}\mu\text{L}$ ) and visualised as in (a).