Name	Description	Reference
Strains		
S. Typhimurium		
∆spvD	12023 <i>AspvD</i>	(11)
∆spvD, pACYC (EV)	<i>∆spvD p</i> ACYC	This study
<i>∆spvD</i> , <i>p</i> ACYCspvD <sup>R161</sup>	<i>∆spvD p</i> ACYCspvD <sup>R161</sup>	(11)
<i>∆spvD</i> , <i>p</i> ACYCspvD <sup>R161/C73A</sup>	<i>∆spvD p</i> ACYCspvD <sup>R161/C73A</sup>	This study
<i>∆spvD</i> , <i>p</i> ACYCspvD <sup>G161</sup>	<i>∆spvD p</i> ACYCspvD <sup>G161</sup>	This study
⊿spvD, pACYCspvD <sup>G161/C73A</sup>	<i>∆spvD p</i> ACYCspvD <sup>G161/C73A</sup>	This study
∆spvD, pWSK29 (EV)	<i>∆spvD p</i> WSK29	This study
<i>∆spvD</i> , <i>p</i> spvD <sup>R161</sup> -2HA	<i>∆spvD p</i> WSK29spvD <sup>R161</sup> -2xHA	This study
<i>∆spvD</i> , <i>p</i> spvD <sup>R161/C73A</sup> -2xHA	<i>∆spvD p</i> WSK29spvD <sup>R161/C73A</sup> -2xHA	This study
⊿spvD, pspvD <sup>G161</sup> -2xHA	<i>∆spvD p</i> WSK29spvD <sup>G161</sup> -2xHA	This study
⊿spvD, pspvD <sup>G161/C73A</sup> -2xHA	<i>∆spvD p</i> WSK29spvD <sup>G161/C73A</sup> -2xHA	This study
E. coli		
DH5a	wt	NCTC
BL21(DE3)	wt	NCTC
PC2	wt	(29)
Plasmids		
pET22b	Protein expression vector used for expression and purification of SpvD and SpvC proteins	Merck Millipore
pHiSH	Protein expression vector with prescision protease cleavable His-tag for expressing proteins for crystallography	(28)
pACYC184	Rep <sub>p15A</sub> low copy number vector	(43)
pACYCspvD and mutant derivatives	pACYC184 with <i>spvD</i> cloned into <i>EcoRV</i> and <i>SalI</i> sites, Cm <sup>R</sup>	This study
pGEX-4T-2sseL	GST fused to sseL	(22)
<i>p</i> WSK29SpvD-2xHA and mutant derivatives	<i>p</i> WSK29 with spvD containing C-terminal 2xHA tag, Amp <sup>R</sup>	This study
pRK5-myc	Eukaryotic expression vector	Laboratory stock
<i>p</i> RK5-myc-SpvD and mutant derivatives	<i>p</i> RK5 with N-terminal myc-tagged SpvD cloned into <i>BamHI</i> and <i>EcoRI</i> sites, Amp <sup>R</sup>	This study
ptCMV-IκBα <sup>S32A/S36A</sup>	Positive NF-KB inhibition protein	Laboratory stock
ptCMV-GFP-SseK3	GFP-fused effector known to inhibit NF-kB in TNFα stimulated cells	Laboratory stock
M3psinrevkB-luc	<i>luc</i> gene under control of NF-кВ consensus promoter	Gift from Felix Randow
pRLTK	constitutively active Renilla luciferase	Gift from Felix Randow

Table S1. Strains and plasmids used in this study

Pseudomonas sp. CF149 Pseudomonas psychrophila	1	HVQAIRFTQROIEDNETHDINKIKQSLHIÄTDE-SELPEHLDIESLEQIASFTNFTYLHYSGNCOLL 70 HAOAIRFTQROIEDNETHDINKIKOSILHIINTD-SELPEHLDIESLEQIASFINKTYLHYSGNCOLL 70	6 6
Providencia burhodogranariea	1 MPISTININSPRONTRENNTGISDNIKLEESSNIVAIKPVNKATKLRKVSNNFLERIENNLNTNAINIG	EIHTVWLGNTKISNNEMTDSDFKDVIRIKDALCVKRKEKKLYPQTLRKNDLEKMAKYVNKPFIHYSGNCAIL 14	4
Bovismorbificans	1MRVSGSASS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYVHYSGNCVLL 76	6
Enteritidis LA5	1MRVSGSASS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYVHYSGNCVLL 70	6
Enteritidis EC201200 Enteritidis PT4	1MRVSGSASS	DIISKINSKNINNDSNEVKRIKDALCIESKERILYPQNLSKDNLKQMARYVNNYYWHYSGNCVLL /{ DIISRINSKNINNNDSNEVKRIKDALCIESKERIIYPONI.SRDNI.KOMARYVNNTY <mark>V</mark> HYSGNCVLI. //	0 0
Enteritidis D7795	1SGSASS	DIISRINSKNINNDSNEVKRIKDALCIESKERILYPONLSRDNLKOMARYVNNTYVHYSGNCVLL 70	0
Enteritidis 2713/98	1MRVSGSAS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPONLSRDNLKOMARYVNNTYVHYSGNCVLL 76	6
Abortus equi	1MRVSGSASS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYUHYSGNCVLL 76	φ
Paratyphi C	1MRVSGSASS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYVHYSGNCVLL 76	φ
Typhimurium DT104	1MRVGGSAS	DIISRINSKNINNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYVHYSGNCVLL 70	0
Typhimurium 14028s	1MRVSGSASS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYVHYSGNCVLL 7 DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNIKQMARYVNNTVHYSGNCVII 72	6 1
ryphilmurrum 247333 Tyrchimurium 4/74		DITSETNSKNTNNNDSNEVKETKDALCIESKERTLYDDNLSEDNLSEDNLKOMARYNNUTYVHYSGNCVLL.76	ρια
TVDhimurium DT2	1SGSASS	DIISRINSKNINNDSNEVKRIKDALCIESKERILYPONLSRDNLKOMARYVNNTYVHYSGNCVLL 76	0
Typhimurium LT2	1MRVSGSASS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTY <mark>V</mark> HYSGNCVLL 7(	Q
Dublin	1MRVSGSASS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTY <mark>V</mark> HYSGNCVLL 7(	6
Choleraesuis RF-1	1MRVSGSASS	DIISRINSKNINNNDSN <mark>D</mark> VKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYVHYSGNCVLL 76	ø
Choleraesuis OU7025	1MRVSGSASS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYIHYSGNCVLL 76	φ
Pullorum	1MRVSGSÀSE	NIISRINSKNINNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYIHYSGNCVLL 76	Q
Gallinarum 1910/93	1MRVSGSASS	NIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYLHYSGNCVLL 76	9
Gallinarum 72/805	1MRVSGSAS	DIISRINSKNINNDSNEVKRIKDALCIESKERILYP <mark>K</mark> NLS <mark>L</mark> DNLKQMARYVNNTYIHYSGNCVLL 76	ø
Typhimurium ST313	1MRVSGSAS	DIISRINSKNINNNDSNEVKRIKDALCIESKERILYPQNLSRDNLKQMARYVNNTYUHYSGNCVLL 76	6
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Pseudomonas sp. CF149	77 SACUHYNIENNASIL <mark>RIENTSOPHIGLEGDVVDEV</mark> IFGKVL <mark>PS</mark> SY <mark>PLE</mark> SIDEVSAE <mark>VLR</mark> RY <mark>EVNGE-R</mark> S	I <mark>VSTY</mark> NYI <mark>IP</mark> LIGE <mark>SG</mark> ĤDENAVVVLDSQNKP <mark>A</mark> VQYLD <mark>A</mark> WKTSN <mark>TTPDSKALEAREPPSARETLRSEGSV</mark> 21	14
Pseudomonas psychrophila	77 SACVHYNIENNASILRTENTSOPHIGLEGDVVDEVIFGKVLPSSYPLESIDEVSAEVLRRYENNGE-RS	I <mark>VSTY</mark> NYIIPLIGESGHDFNAVVVLDSQNKPAVQYLDAWKTSNTTPDSKALEARFPESARFTLRSFGRV 21 TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	14
Providencia burnodogranariea Bowismorhificans	143 SACLHINIH <mark>I</mark> KQUI <mark>I</mark> SANNITASETVGL <mark>ESIL</mark> VUANIFG <mark>VALAGANANA</mark> II-ISUU <mark>UVANEVLK</mark> KI <mark>NLNNEULE</mark> 77 SACIHYNTHHRODTISSKNTASPTVCLDSATVDKITFGHEINOSYCINSTDEVEKETINRYDTKRE-SS	IISAENIILK <sup>N</sup> IGEC <mark>H</mark> HDENAV <mark>IIKENANEK</mark> UVQEIDAMAIE <mark>NNEENNEKHESSSGEEVVRAVDEKHD</mark> 20 TISAENVIVVDIIGECGHDENAVVICEVDKKPVVOETDSWFTSNIIDSLOFIKKHESSSGEEVVRAVDEKHD 21	2 V Π C
Euvismonutricans Enteritidis 1.25	77 SACHULUINIINII SSKNTASFIYGIDSALVDKIIFGULUNGSTCHWSIDEVEKETISNIIIIINII 77 SACHUYNTHHRODII.SSKNTASPIYGIDSALVDKIIFGHRINOSYCINSIDEVEKETISNII	TISAENTIVEIIGEOGUDE NAVVICEIDANKEIVEIDEN MALABARAN DE SAGELANNIE SUGGEFIVIATDEAUD 21 TISAENVIVDTIGEOGUDE NAVVICEYDKK PVVOETDSWKTSNIILSIGGODE FAKRESSSGEFEVVRAVDEKED 21	2 4
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ELCOLLOLALS FIL	2. Construction and the second state of the second state of the second seco	LL ORDINEL MELTODOCOLLENAY Y TOELDUKE VYEE DOWNLOWLE DETECTED FUNCTION DOUL TIESADNYING FUNCTOORDUNATION VERENVINGERENGENERDEN DETECTED VERUEGESEGED VAN VERUEU 	1 -
Enteritidia 2713/98	77 SACTHYNTHHRODTI.SSKNTASPTVCLINSATVDKTTFGHEIMOSYCINSTDEVEKETLNRYDTKRESS	TISAENVIADITIGECCCHDENAVVICEVDKKPVUCETDSWETSNILDSLOEDIUUESSSGEFVVRAVDEKHD 21	2
Abortus admi		TISSANDATION CONTRACTOR DEPENDENT OF A CONTRACTOR DEPENDENT OF A CONTRACTOR DEPENDENT OF A CONTRACTOR DEPENDENT	1 -
Paratvohi C	77 SACLHYNTHHRODTLSSKNTASPTVGLDSATVDKITFGHELMOSYCLMSTDEVEKETLNRYDIKRE-SS	TISAENYIVPIIGECRHDFNAVVICEYDKKPYVOFIDSWKTSNILPSLOEIKKHFSSSGEFYVRAYDFKHD 21	19
Tvohimurium DT104	77 SACLHYNTHHROITSSKNTASPTVGLDSATVDKIIFGHELNOSYCLNSIDEVEKEILNRYDIKRE-SS	IISAENYIAPIIGECEHDENAVVICEYDKKPYVOEIDSWKTSNILPSLOEIKKHESSSGEEYVRAYDEKHD 21	191
Tvphimurium 14028s	77 SACLHYNIHHRODILSSKNTASPTVGLDSAIVDKIIFGHELNOSYCLNSIDEVEKEILNRYDIKRE-SS	IISAENYIAPIIGECRHDFNAVVICEYDKKPYVOFIDSWKTSNILPSLOEIKKHFSSSGEFYVRAYDEKHD 21	191
Tvphimurium SL1344	77 SACLHYNIHHRODILSSKNTASPTVGLDSAIVDKIIFGHELNOSYCLNSIDEVEKEILNRYDIKRE-SS	IISAENYIAPIIGECRHDENAVVICEYDKKPYVOFIDSWKTSNILPSLOEIKKHFSSSGEFYVRAYDEKHD 21	191
Typhimurium 4/74	77 SACLHYNIHHRQDILSSKNTASPTVGLDSAIVDKIIFGHELNQSYCLNSIDEVEKEILNRYDIKRE-SS	IISAENYI <mark>A</mark> PIIGEC <mark>R</mark> HDFNAVVICEYDKKPYVQFIDSWKTSNILPSLQEIKKHFSSSGEFYVRAYDEKHD 21	16
Typhimurium DT2	77 SACLHYNIHHRQDILSSKNTASPTVGLDSAIVDKIIFGHELNQSYCLNSIDEVEKEILNRYDIKRE-SS	IISAENYI <mark>A</mark> PIIGEC <mark>R</mark> HDFNAVVICEYDKKPYVQFIDSWKTSNILPSLQEIKKHFSSSGEFYVRAYDEKHD 21	16
Typhimurium LT2	77 SACLHYNIHHRQDILSSKNTASPTVGLDSAIVDKIIFGHELNQSYCLNSIDEVEKEILNRYDIKRE-SS	IISAENYI <mark>A</mark> PIIGEC <mark>R</mark> HDFNAVVICEYDKKPYVQFIDSWKTSNILPSLQEIKKHFSSSGEFYVRAYDEKHD 21	16
Dublin	77 SACLHYNIHHRQDILSSKNTASPTVGLDSAIVDKIIFGHELNQSYCLNSIDEVEKEILNRYDIKRE-SS	IISAENYI <mark>A</mark> PIIGEC <mark>R</mark> HDFNAVVICEYDKKPYVQFIDSWKTSNILPSLQEIKKHFSSSGEFYVRAYDEKHD 21	16
Choleraesuis RF-1	77 SACLHYNIHHRQDILSSKNTASPTVGLDSAIVDKIIFGHELNQSYCLNSIEEVEKEILNRYDIKRE-SS	IISAENYI <mark>A</mark> PIIGEC <mark>R</mark> HDFNAVVICEYDKKPYVQFIDSWKTSNILPSLQEIKKHFSSSGEFYVRAYDEKHD 21	16
Choleraesuis 0U7025	77 SACLHYNIHHRQDILSSKNTASPTVGLDS <mark>T</mark> IVDKIIFGHELNQSYCLNSIDEVEKEILNRYDIKRE-SS	IISAENYIAPIIGEC <mark>R</mark> HDFNAVVICEYDKKPYVQFIDSWKTSNILPSLQEIKKHFSSSGEFYVRAYDEKHD 21	Ξ.
Pullorum	71 SACLHYNIHHRQDILSSKNTASPTVGLDSALVDKIIFGHELNQSYCLNSIDEVEKELLNRYDIKRE-SS 22 SACHYMMINIALISSKNTASPTVGLDSALVDKIIFGHELNQSYCLNSIDEVEKELLNRYDIKRE-SS	IIISAENYIAPIIGECRHDENAVVICEYDKKPYVQFIDSWKTSNILPSLQEIKKHFSSSGEFYVRAYDEKHD 21 TICAAPUVIAADIIGEORDINAWIITEEVERENDUIDEITEEMEENTI DEI GETRUUDEGGGEEEVUN DUIDEGG	- H
Gallinarum 72/805	71 SACLHINTHHRQUILSSKNTASFIVGLDSALVDALIFGHELNQSICLNSLDEVEKETINEVEKETSS 77 SACTHVNTHHPODTISSKNTASEPTVCIDSALVDATIFGHELNQSICLNSLDEVEKETINPODTKPE-SC	IISAENTIA <sup>R</sup> IIGEURHDENAVVIUEIDKKFIVQEIDSMKISNILFSLQEIKKHESSSGEEIVR <mark>E</mark> IDEKHD 21 TISAENVI <mark>A</mark> DTIGEURHDENAVVIUFYDKKDVVOEIDSMFISNILDSIOFIKKHESSSGEEEVVDAVDEKHD 21	2 4
TVDhimurium ST313	77 SACHTINITHINKELTANIN AND AND AND AND AND AND AND AND AND AN	IISAENYI <mark>a</mark> piigeckhdenavviceydkkpyvofidsmktsnildslobikk <mark>tlliikgilcogi</mark> 21	4 日
17	2		

Figure S1. Amino acid alignment of SpvD sequences.

The alignment was prepared using BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The protein BLAST search was performed with BLASTP 2.2.29+ software (BLOSUM62 matrix; E threshold= 1.0) using *S*. Typhimurium 14028s SpvD input sequence (http://www.uniprot.org/uniprot/A0A0F6AW50). Sequences lacking a significant portion of the protein or the catalytic triad components were omitted. Polymorphisms are highlighted in yellow and catalytic triad positions are marked with red asterisks.



**Figure S2**. Comparison of  $SpvD^{4CS/A154/R161}$  and  $SpvD^{4CS/A154/G161/C73A}$  structures. Catalytic triad residues are represented with red sticks. Additional loop regions that were mapped in  $SpvD^{4CS/A154/G161/C73A}$  structure (right) are coloured in blue.