

**Table S1.** Strains and plasmids used in this study

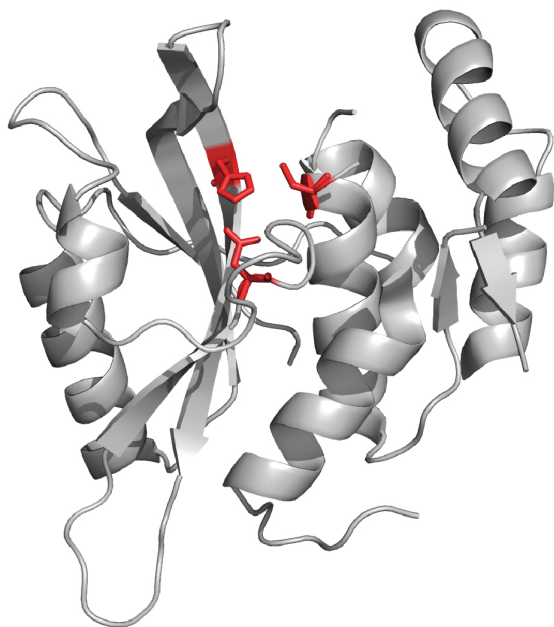
Name	Description	Reference
<b>Strains</b>		
<i>S. Typhimurium</i>		
$\Delta spvD$	12023 $\Delta spvD$	(11)
$\Delta spvD$ , pACYC ( <i>EV</i> )	$\Delta spvD$ pACYC	This study
$\Delta spvD$ , pACYCspvD <sup>R161</sup>	$\Delta spvD$ pACYCspvD <sup>R161</sup>	(11)
$\Delta spvD$ , pACYCspvD <sup>R161/C73A</sup>	$\Delta spvD$ pACYCspvD <sup>R161/C73A</sup>	This study
$\Delta spvD$ , pACYCspvD <sup>G161</sup>	$\Delta spvD$ pACYCspvD <sup>G161</sup>	This study
$\Delta spvD$ , pACYCspvD <sup>G161/C73A</sup>	$\Delta spvD$ pACYCspvD <sup>G161/C73A</sup>	This study
$\Delta spvD$ , pWSK29 ( <i>EV</i> )	$\Delta spvD$ pWSK29	This study
$\Delta spvD$ , pspvD <sup>R161</sup> -2HA	$\Delta spvD$ pWSK29spvD <sup>R161</sup> -2xHA	This study
$\Delta spvD$ , pspvD <sup>R161/C73A</sup> -2xHA	$\Delta spvD$ pWSK29spvD <sup>R161/C73A</sup> -2xHA	This study
$\Delta spvD$ , pspvD <sup>G161</sup> -2xHA	$\Delta spvD$ pWSK29spvD <sup>G161</sup> -2xHA	This study
$\Delta spvD$ , pspvD <sup>G161/C73A</sup> -2xHA	$\Delta spvD$ pWSK29spvD <sup>G161/C73A</sup> -2xHA	This study
<i>E. coli</i>		
DH5 $\alpha$	wt	NCTC
BL21(DE3)	wt	NCTC
PC2	wt	(29)
<b>Plasmids</b>		
pET22b	Protein expression vector used for expression and purification of SpvD and SpvC proteins	Merck Millipore
pHiSH	Protein expression vector with precision 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>Sall</i> sites, Cm <sup>R</sup>	This study
pGEX-4T-2sseL	GST fused to sseL	(22)
pWSK29SpvD-2xHA and mutant derivatives	pWSK29 with <i>spvD</i> containing C-terminal 2xHA tag, Amp <sup>R</sup>	This study
pRK5-myc	Eukaryotic expression vector	Laboratory stock
pRK5-myc-SpvD and mutant derivatives	pRK5 with N-terminal myc-tagged SpvD cloned into <i>BamHI</i> and <i>EcoRI</i> sites, Amp <sup>R</sup>	This study
ptCMV-IkB $\alpha$ <sup>S32A/S36A</sup>	Positive NF- $\kappa$ B inhibition protein	Laboratory stock
ptCMV-GFP-SseK3	GFP-fused effector known to inhibit NF- $\kappa$ B in TNF $\alpha$ stimulated cells	Laboratory stock
M <sub>3</sub> psinrev $\kappa$ B- <i>luc</i>	<i>luc</i> gene under control of NF- $\kappa$ B consensus promoter	Gift from Felix Randow
pRLTK	constitutively active Renilla luciferase	Gift from Felix Randow



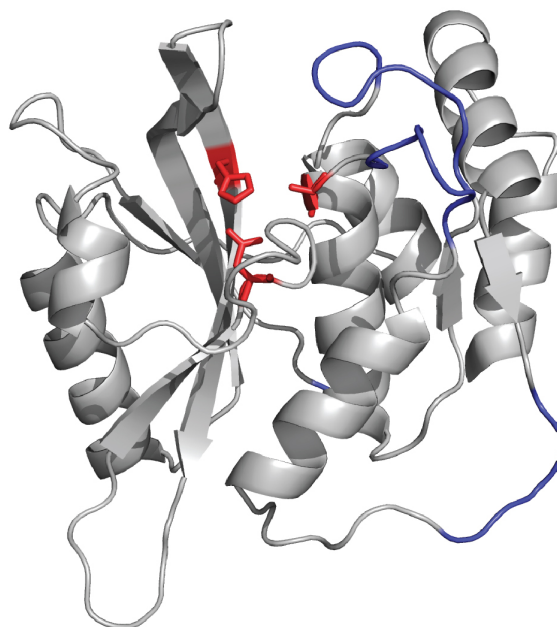
**Figure S1.** Amino acid alignment of SpvD sequences.

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.

SpvD<sup>4CS/R161</sup>



SpvD<sup>4CS/G161/C73A</sup>



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