Supplementary Materials

A *Salmonella* Typhi homolog of bacteriophage muramidases controls Typhoid toxin secretion

Hélène Hodak and Jorge E. Galán*
Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT06536

Materials and Methods

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Supplementary Table S2. All *S.* Typhi strains are derived from the clinical isolate ISP2825 (1). All in frame deletions or insertions into the *S.* Typhi chromosome were generated using the R6K-derived suicide vector pSB890 as previously described (2). Chromosomal DNA from *S.* Enteritidis (from the Roy Curtiss III strain collection) and *Yersinia enterocolitica* strain 8081 (3) (generously provided by Virginia Miller) was obtained using standard methods and used for PCR amplification of the *sty1889* homologs *sen1395* and *ye*1815, respectively. For complementation assays, relevant genes were expressed using the expression plasmid pSB3324. This plasmid is derived from the low copy vector pWSK129 (4) and was generated by exchanging the 0.28 kb *SacI-EcoRI* fragment containing the *lacZ* promoter for a 1.3 kb fragment containing *araC* and the arabinose-inducible promoter amplified from pBAD24 (5). This plasmid vector also allows the placing of a 3xFLAG epitope tag at the carboxy terminus of the protein of interest. Chimeras between different fragments of Sty1889 and Sen1395 were generated using overlapping PCRs following standard methods and were expressed using plasmid pSB3324. For bacteriolytic assays and overexpression purposes, all relevant DNA fragments were subcloned into pBAD24. Site directed mutagenesis was carried out by mismatched PCR using KOD Hot Start DNA polymerase (EMD Millipore).

Bacterial and eukaryotic cell growth conditions. *S.* Typhi strains were grown in L-broth (LB) on a rotating wheel at 37°C for approximately 16 h followed by a subculture in fresh LB. For infection assays, the subculture was done in LB containing 0.3 M NaCl (to stimulate expression of the SPI-1 type III secretion system). When appropriate 50 μg/mL Kanamycin, 100 μg/mL or 100 μg/ml Ampicillin were added. Henle-407 cells were grown at 37˚C and 5% CO_2 in DMEM medium supplemented with 10% bovine calf serum (BCS, HyClone).

Cultured epithelial cell infection assays. Overnight cultures of *S.* Typhi strains were diluted 1:33 in fresh LB medium containing 0.3 M NaCl and grown for 3 h to an OD600 nm of 0.9. Infections were carried out in HBSS medium for 1 h using a multiplicity of infection of 30. Cells were then incubated in DMEM containing 10% BCS and 100 μg/mL Gentamicin for 1 h followed by 10 μg/mL Gentamicin incubation for additional 20 h. For *S.* Typhi strains harboring pSB3324-derived plasmids, protein expression was induced with 0.0008% arabinose for 20 hs. The induction conditions were optimized to match plasmid-mediated expression levels of StsA after 22 hours of infection to those of chromosomally expressed StsA from its natural promoter, as assessed by Western blot.

Immunofluorescence microscopy. Twenty-two hours after infection, Henle-407 cells (grown on glass coverslips) were rinsed with phosphate buffered saline (PBS) and fixed 15 min at room temperature with 4% paraformaldehyde in PBS. Samples were incubated in 50 mM NH_4Cl in PBS for 10 min to quench free aldehydes and then blocked in 1% bovine serum albumin and 0.1% Triton X-100 in PBS (BT-PBS) for 20 min. Coverslips were incubated in BT-PBS for 30 min with primary anti-FLAG M2 mouse monoclonal antibody (Sigma) (1:10,000) and anti-*Salmonella* O poly A-I & Vi rabbit antiserum (Becton, Dickinson and Co.) (1:2,000), followed by 30 min incubation with 0.5 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) and secondary antibodies anti-mouse Alexa Fluor-488 and anti-rabbit Alexa Fluor-594 (Invitrogen) (1:2,000). Samples were visualized in an Eclipse TE2000-U (Nikon) microscope equipped with a CCD camera (MicroMAX RTE/CCD-1300Y; Princeton Instruments). Quantification of fluorescence intensity was carried out using ImageJ.

Bacteriolytic assays. *S.* Typhi Δ*ttsA* expressing various 3xFLAG tagged proteins from pBAD24-derived vectors were grown overnight in LB medium supplemented with 100 μg/mL Ampicillin. Overnight grown bacteria were diluted to an OD600 nm of 0.1 in fresh LB medium containing 0.2% arabinose. After 60 min of induction, 0.3% $CHCl_3$ was added to the cultures and lysis was monitored for additional 60 min by measuring their OD600 nm. Aliquots were collected immediately prior to adding $CHCl_3$ and boiled in Laemmli buffer for 5 min. The OD600 nm of the bacterial cultures was used to standardize the samples prior to western blot analysis using an anti-FLAG M2 monoclonal antibody 1:10,000 (Sigma) and a secondary anti-mouse infra red-800 nm antibody 1:16,000. To grow strains carrying pSB3324-derived vectors, the medium was supplemented with 50 μg/ml kanamycin. In this case, induction of expression was carried out by adding 0.3% arabinose and the assay carried out as described above. For holin assisted bacteriolysis, overnight cultures were diluted in fresh LB medium to an OD600 nm of 0.1 and further grown to an OD 600 nm of 0.5. At that time, 0.002% arabinose was added to the cultures in order to induce holin and/or endolysin expression and bacterial lysis was monitored for additional 280 min by measuring the OD600 nm of the bacterial cultures. Culture aliquots were collected 30 min following the induction with arabinose and protein expression analyzed as indicated above.

Detection of TtsA and CdtB during the course of infection. Henle-407 cells were infected as described above with a strain of *S.* Typhi encoding chromosomally-encoded FLAG-tagged TtsA and CdtB. At various times of infection, cells were lysed using 0.1% sodium deoxycholate and 10 μg/mL DNAse I in PBS and sample aliquots were plated on LB medium to determine c.f.u. The remaining samples were centrifuged at 10,000 g for 20 min at 4˚C. Bacterial pellets were resuspended in Laemmli buffer and boiled for 5 minutes. The presence of StsA and CdtB was determined by Western blot analysis. C.f.u. counts were used to load equivalent amounts of bacterial lysates for each time point.

Phylogenetic, genomic, and structural analyses. Phylogenetic trees were generated using ClustalW2 (6). Homologs of Sty1889 were identified using BLASTP. Only homologs with identity superior to 50% were retained for further analysis. To simplify the tree, only one representative of proteins exhibiting >95% identity to each other was included. For genomic analyses, the PHAge Search Tool (PHAST) web server (7) was used to search for the presence of phage genes in the vicinity of the Sty1889 homologs. Genomic context of endolysin coding genes shown in Supplementary Table S1 was manually obtained from the NCBI nucleotide data-

bases. Each locus containing an endolysin coding gene was analyzed for the presence of a holin as well as extracellular enzyme encoding genes using the NCBI nucleotide database in combination with manual *in silico* analyses. Modeling of the atomic structure of TtsA was done using (http://www.sbg.bio.ic.ac.uk/~phyre/) with the structure deposited as pdb2IS5 as a template.

References

1. Galan JE, Curtiss R, 3rd. Distribution of the invA, -B, -C, and -D genes of Salmonella typhimurium among other Salmonella serovars: invA mutants of Salmonella typhi are deficient for entry into mammalian cells. Infect Immun. 1991;59(9):2901-8. Epub 1991/09/01.
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3. Portnoy D, Moseley S, Falkow S. Characterization of plasmids and plasmid-associated determinants of Yersinia enterocolitica pathogenesis. Infect Immun. 1981; 31: 775–82.
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5. Guzman LM, Belin D, Carson MJ, Beckwith J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol. 1995;177:4121-30.
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7. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. PHAST: a fast phage search tool. Nucleic Acids Res. 2011;39:W347-52.
8. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. PHAST: a fast phage search tool. Nucleic Acids Res. 2011;39(Web Server issue):W347-52. Epub 2011/06/16.

Supplementary Figures

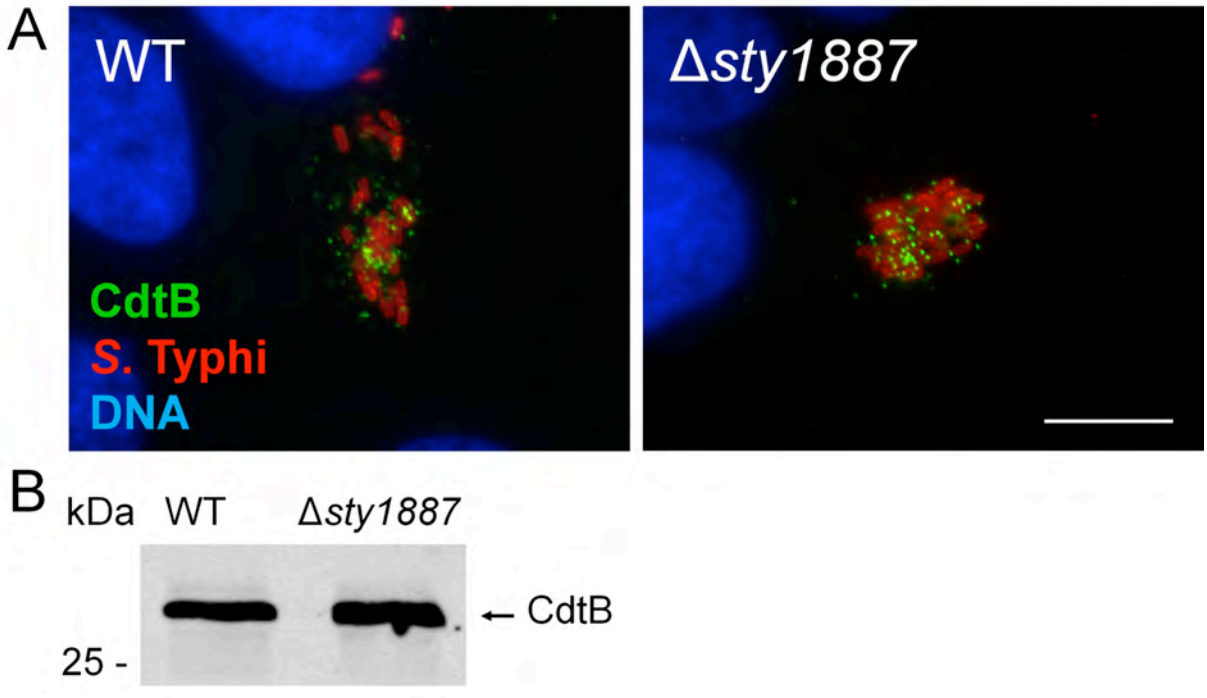

Figure S1 | Deletion of *sty1887* has no effect on typhoid toxin secretion. **(A)** Henle-407 cells were infected with *S.* Typhi expressing chromosomally encoded 3xFLAG epitope tagged *cdtB* or an isogenic Δ*sty1887* mutant. Twenty-two hours after infection cells were stained with an antibody directed to the FLAG epitope (green) (to visualize CdtB), a rabbit antibody directed to *S.* Typhi LPS (red), and DAPI for DNA detection (blue). The bar represents 10 μm. **(B)** Western blot analysis of the expression levels of CdtB in the strains used in panel (A).

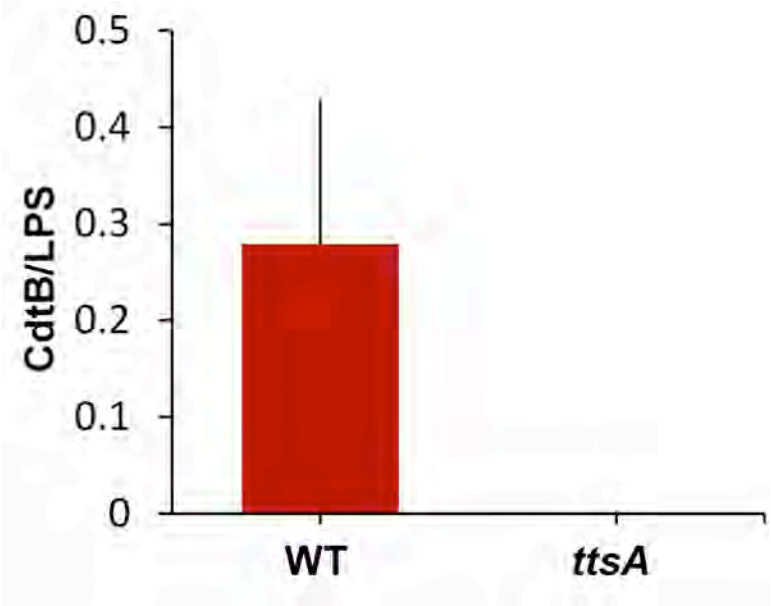

Figure S2 | Typhoid toxin secretion is abolished in Δ*ttsA* background. Henle-407 cells were infected with *S.* Typhi expressing chromosomally encoded 3xFLAG-epitope-tagged CdtB or a Δ*ttsA* isogenic mutant. Twenty-two hours after infection cells were stained with an antibody directed to the FLAG epitope (to visualize CdtB), and a rabbit antibody directed to *S.* Typhi LPS (to visualized bacteria). The fluorescence intensity associated with toxin detection was quantified as indicated in the Materials and Methods section. 52 and 50 images of host cells infected by the wild type or Δ*ttsA S.* Typhi, respectively, were acquired. The LPS signal was used to normalize the Typhoid toxin signal. The averaged ratios green/red and the corresponding standard deviations are shown (note: the signal in the *ttsA* mutant strain was too low to appear in the scale of the graph).

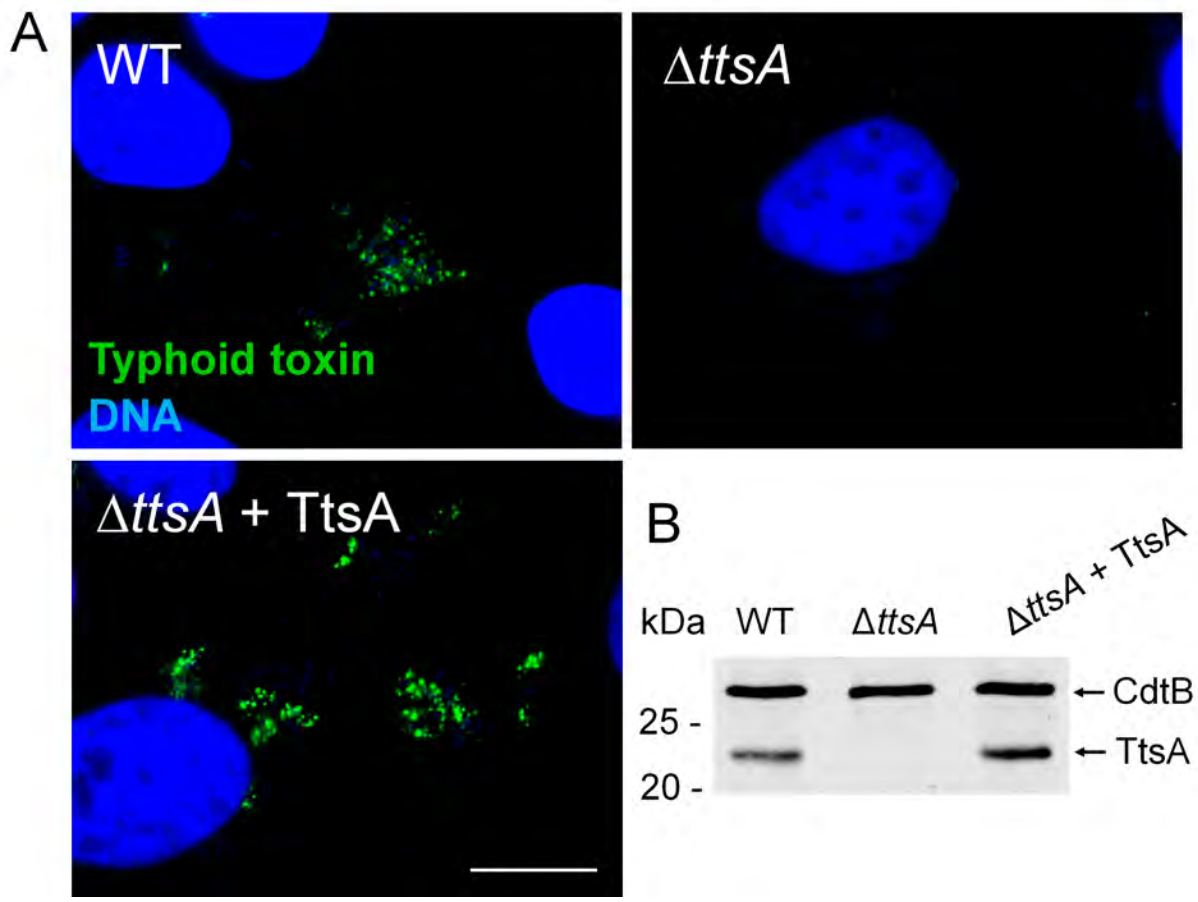

Figure S3 | Typhoid toxin secretion is abolished in Δ*ttsA* background. **(A)** Henle-407 cells were infected with *S.* Typhi, a Δ*ttsA* isogenic mutant, or the Δ*ttsA* complemented derivative. Twenty-two hours after infection cells were stained with a rabbit polyclonal antibody directed to the typhoid toxin (green), and DAPI for DNA detection (blue). The bar represents 10 μm. **(B)** Western blot analysis of the expression levels of the indicated proteins in the strains used in panel (A).

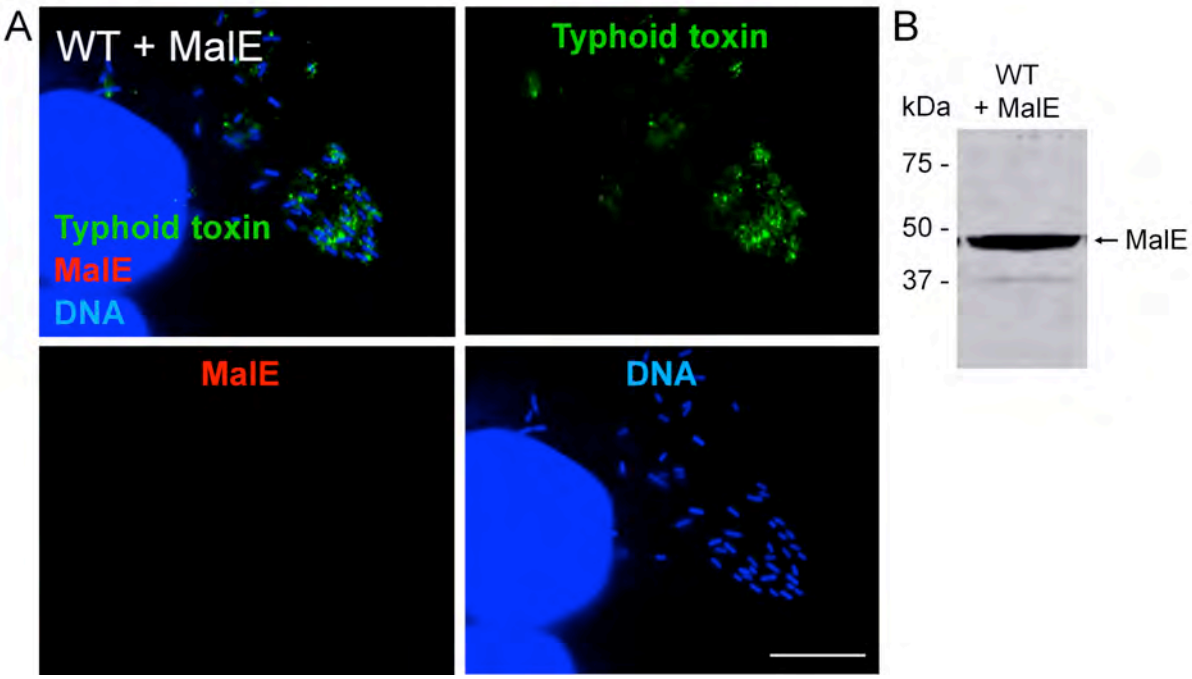

Figure S4 | Typhoid toxin secretion is specific. **(A)** Henle-407 cells were infected with wild type *S.* Typhi expressing from a low copy plasmid 3xFLAG tagged periplasmic protein MalE. Twenty-two hours after infection cells were stained with a rabbit polyclonal antibody directed to the typhoid toxin (green), a mouse monoclonal antibody directed to the FLAG epitope (red) (to detect MalE) and DAPI for DNA detection (blue). The bar represents 10 μm. **(B)** Western blot analysis was used to confirm the expression of MalE.

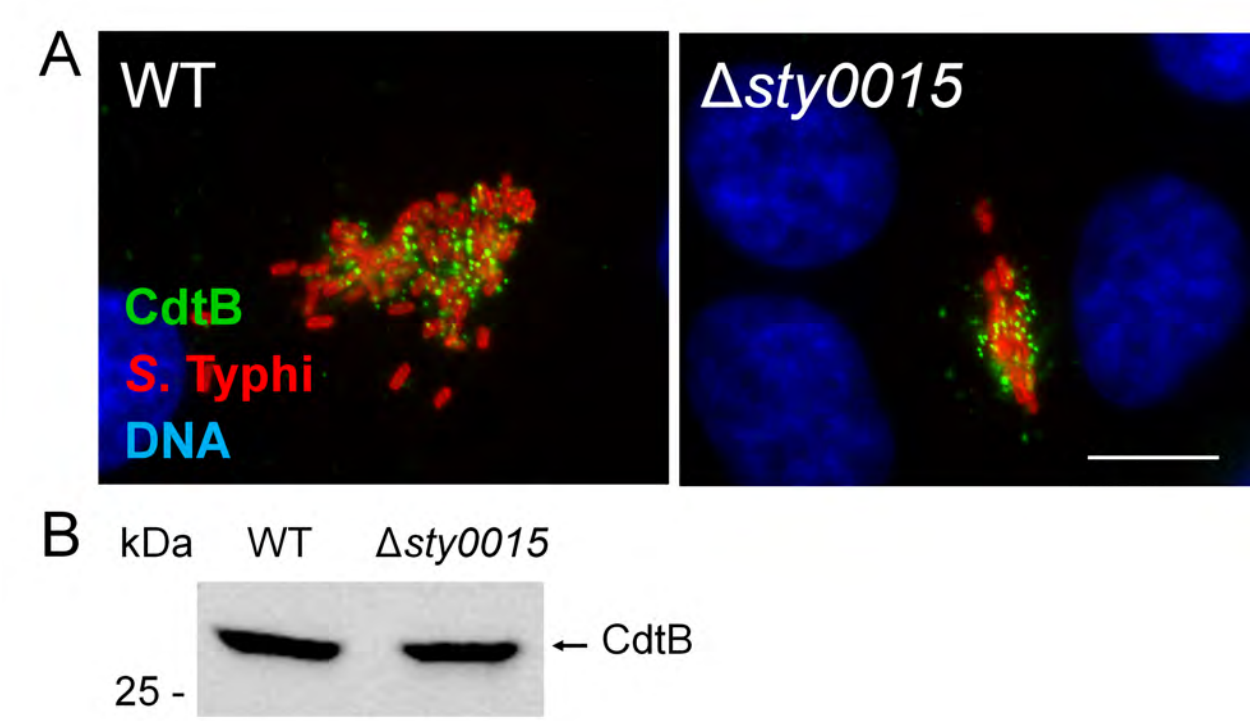

Figure S5 | The *S.* Typhi holin Sty0015 is not required for toxin secretion. **(A)** Henle-407 cells were infected with *S.* Typhi expressing chromosomally encoded 3xFLAG epitope tagged *cdtB* or an isogenic Δ*sty0015* mutant. Twenty-two hours after infection cells were stained with an antibody directed to the FLAG epitope (green) (to visualize CdtB), a rabbit antibody directed to *S.* Typhi LPS (red), and DAPI for DNA detection (blue). The bar represents 10 μm. **(B)** Western blot analysis of the expression levels of CdtB in the strains used in panel (A).

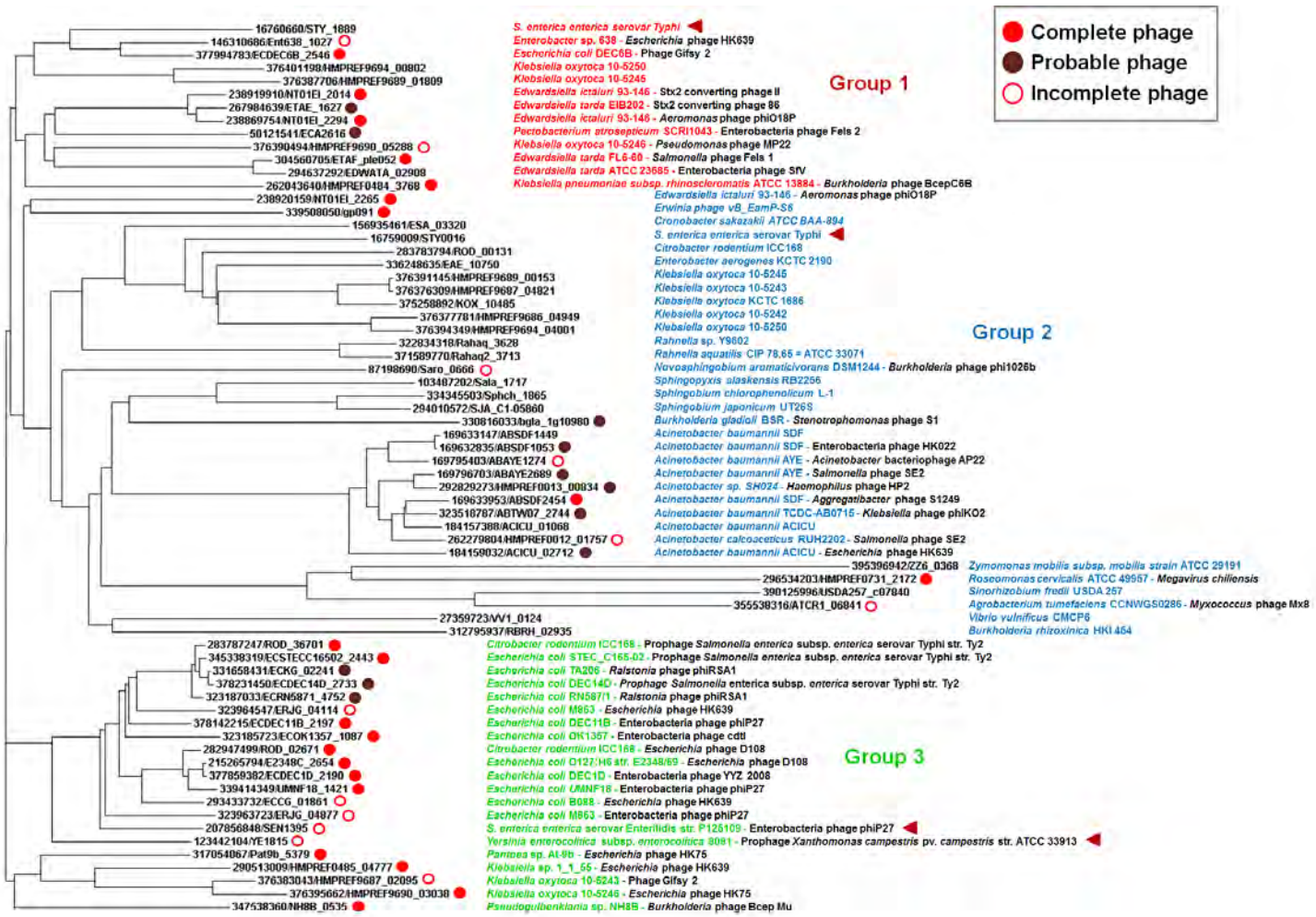

Figure S6 | Phylogenetic representation of TtsA homologs. The phylogenetic tree was generated using ClustalW2. TtsA homologs were identified using NCBI and coliBASE BLASTP. Homologs with identity superior to 50% are represented. In addition, ZliS (*ZZ*6_0368) from *Zymomonas mobilis* and its close homologs encoded by *Roseomonas cervicalis*, *Sinorhizobium fredii*, and *Agrobacterium tumefaciens* were also added to the tree. Homologs are depicted in three major groups 1, 2 and 3 (depicted in red, blue and green, respectively) based on their similarity. The PHAge Search Tool (PHAST) web server was used to analyze the loci of all the TtsA homologs represented in the tree to identify possible prophages. The red, plum and empty red dots indicate TtsA homologs located in complete, probably complete, or incomplete phage loci, respectively, as indicated by the PHAST tool. The likely identity of each phage is also indicated. The proteins investigated in this study are indicated with red arrows.

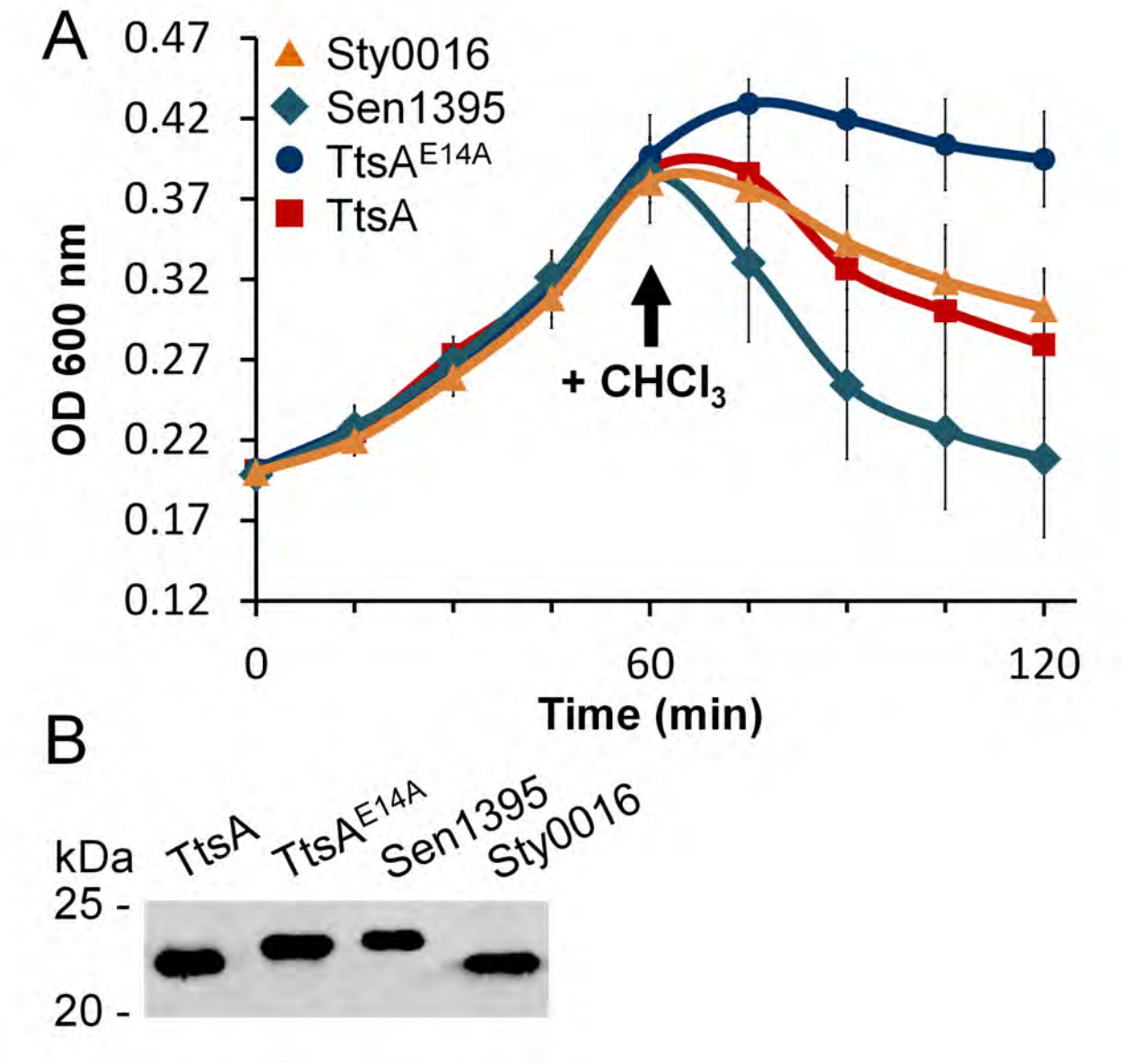

Figure S7 | Bacteriolytic activity of TtsA's homologs. **(A)** 3xFLAG-tagged TtsA, TtsA^{E14A}, Sty0016 or Sen1395 were overexpressed in Δ *ttsA S.* Typhi from an arabinose-inducible promoter. Overnight cultures were diluted in fresh medium containing 0.2% arabinose and grown for 60 minutes. Lysis was induced by adding 0.3% chloroform ($CHCl_3$) and monitored by measuring the OD600 nm of the bacterial cultures. The graph shows the average and standard deviations of 6 independent assays. **(B)** Western blot analysis of the expression levels of the different muramidases assayed in panel (A).

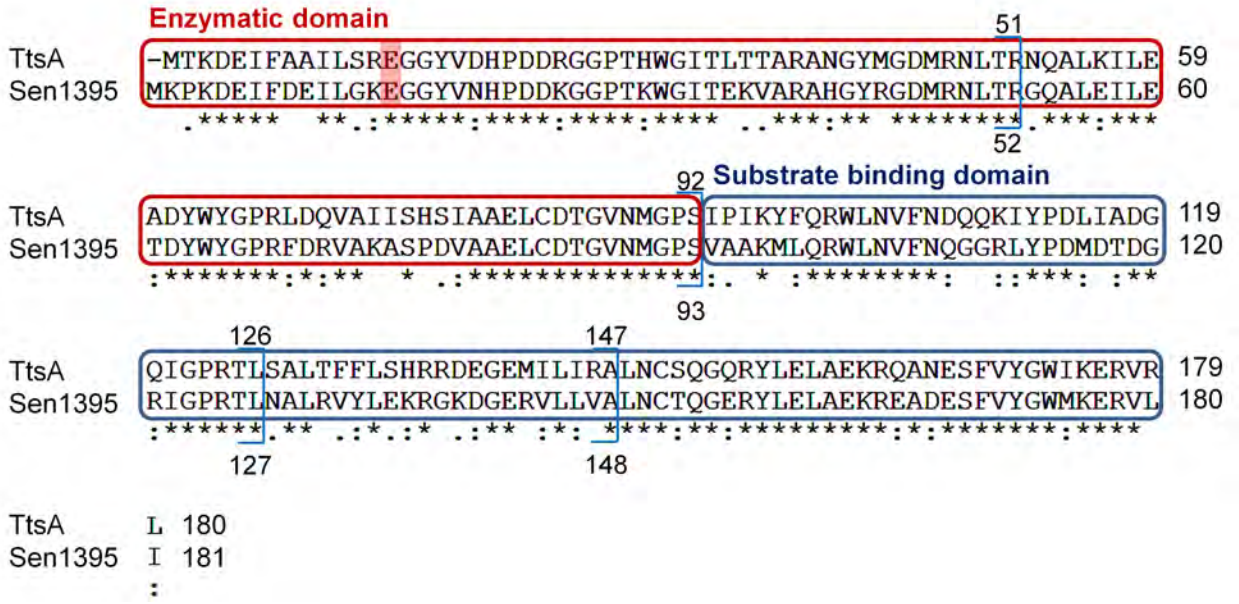

Figure S8 | Schematic representation of the chimeras analyzed in this study that are shown in Fig. 4**.** The alignment between TtsA and Sen1395 was generated using ClustalW2. The enzymatic and peptidoglycan-binding domains are indicated. Blue brackets indicate the junction region used to construct the chimeras. The residue number indicated above and below the bracket corresponds to TtsA and Sen1395 sequence, respectively.

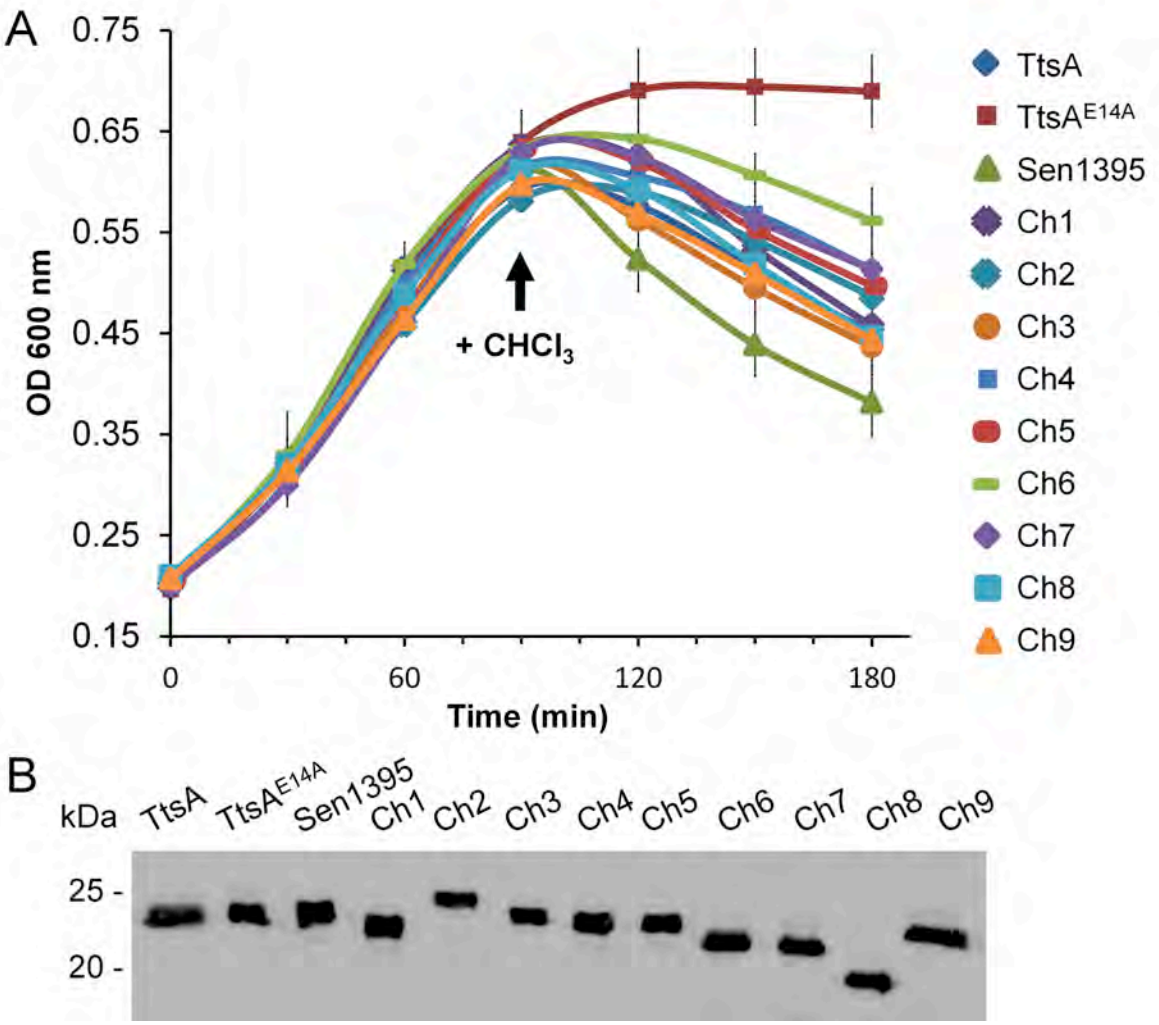

Figure S9 | Bacteriolytic activity of TtsA-Sen1395 chimeras. **(A)** TtsA, TtsA E14A, Sen1395 and the various TtsA-Sen1395 chimeras were expressed from an arabinose-inducible promoter. Overnight cultures were diluted in fresh medium containing 0.3% arabinose and grown for 180 minutes. Lysis was induced by adding 0.3% chloroform (CHCl3) and monitored by measuring the OD600 nm of the bacterial cultures. The graph shows the average and standard deviations of 4 independent assays. **(B)** Western blot analysis of the expression levels of the different muramidases assayed in panel (A).

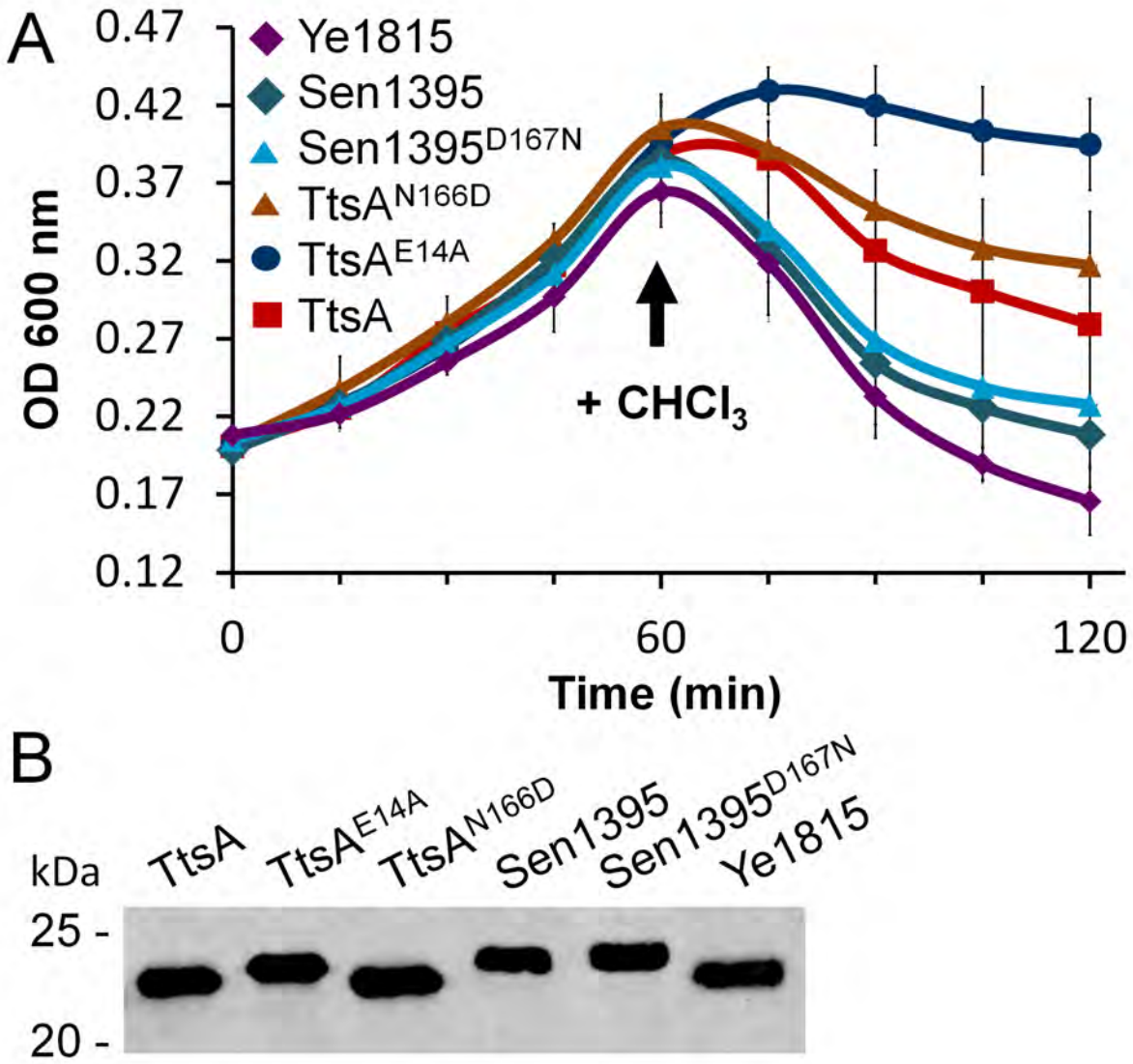

Figure S10 | Bacteriolytic ability of $TtsA^{N166D}$ and $Sen1395^{D167N}$. **(A)** 3xFLAG-tagged TtsA, $TtsA^{E14A}$, $TtsA^{N166D}$, Sen1395, $Sen1395^{D167N}$ or Ye1815 were overexpressed in Δ*ttsA S.* Typhi from an arabinose-inducible promoter. Overnight cultures were diluted in fresh medium containing 0.2% arabinose and grown for 60 minutes. Lysis was induced by adding 0.3% chloroform ($CHCl_3$) and monitored by measuring the OD600 nm of the bacterial cultures. The graph shows the average and standard deviations of 9 independent assays. **(B)** Western blot analysis of the expression levels of the different muramidases assayed in panel (A) immediately before addition of $CHCl_3$.

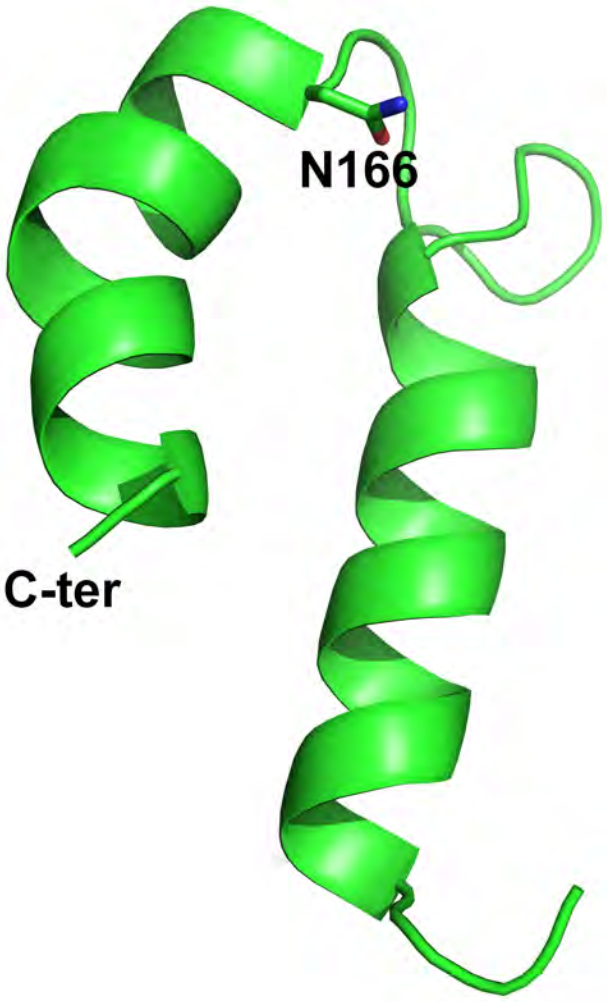

Figure S11 | Atomic model of the carboxy terminus of TtsA showing the position of the functionally critical residue N166.

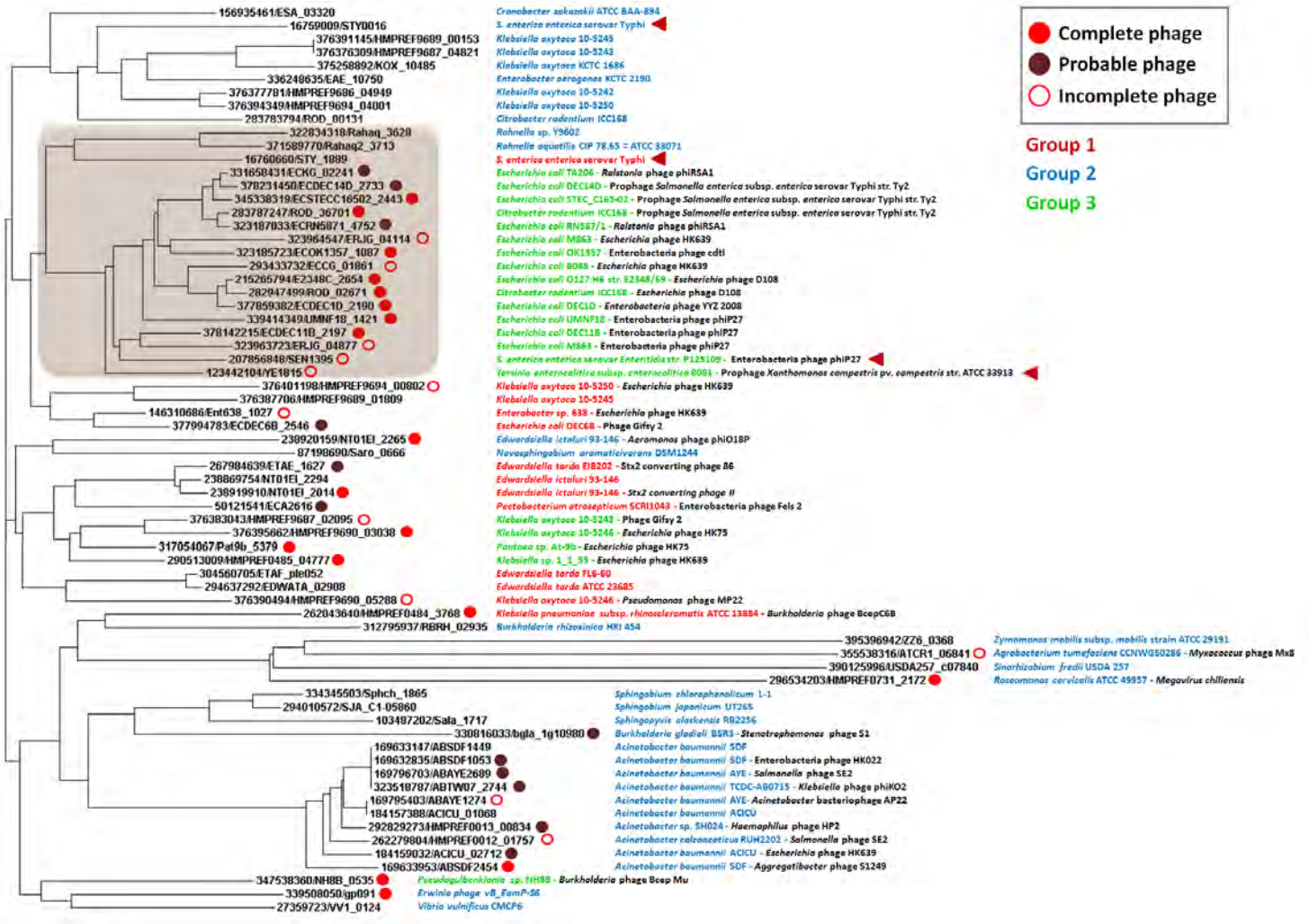

Figure S12 | Phylogenetic analysis of the C-terminus of the TtsA homologs. The phylogenetic tree was generated with the 36 C-terminal residues of the TtsA homologs using ClustalW2. TtsA homologs represented in this tree are the same as those represented in Supplementary Figure S6. The TtsA homologous proteins with the potential to complement typhoid toxin secretion identified in this study are highlighted in grey. PHAge Search Tool (PHAST) web server was used to analyze the loci of all the homologs represented in the tree and to identify possible prophages (8). The red, plum and empty red dots indicate TtsA homologs located in complete, probably complete or incomplete phage loci, respectively, as indicated by the PHAST tool. The likely identity of each phage is indicated. The proteins analyzed in this study are designated with red arrows. The color code for the groups 1, 2 and 3 determined in Supplementary Figure S6 was maintained in this figure.

Supplementary Table S1: Genomic contexts of TtsA-like endolysin coding genes.

Organism	Endolysin	Holin	Phage	Extracellular enzyme/toxin
S. enterica enterica serovar Typhi	STY1889	ND	ND	Typhoid toxin: STY1886 (cdtB), STY1890 (pltA), STY1891 (pltB)
Enterobacter sp. 638	Ent638_1027	yes* (between Ent638_1027 and Ent638_1028)	Escherichia phage HK639	
Escherichia coli DEC6B	ECDEC6B_2546	ECDEC6B_2547	Gifsy 2	
Klebsiella oxytoca 10-5250	HMPREF9694_00802	yes* (between HMPREF9694_00802 and HMPREF9694_00803)	Escherichia phage HK639	colicin* (between HMPREF9694_00801 and HMPREF9694_00802); HMPREF9694_00801 colicin/pyosin immunity protein
Klebsiella oxytoca 10-5245	HMPREF9689_01809	HMPREF9689_01808	Klebsiella_phage_phiKO2	
Edwardsiella ictaluri 93-146	NT01EI_2014	NT01EI_2015	Stx2_converting_phage_II	
Edwardsiella tarda EIB202	ETAE_1627	ETAE_1626	Stx2_converting_phage_86	
Edwardsiella ictaluri 93-146	NT01EI_2294	NT01EI_2295	Aeromonas_phage_phiO18P	
Pectobacterium atrosepticum SCRI1043	ECA2616	ECA2617	Enterobacteria_phage_Fels_2	
Klebsiella oxytoca 10-5246	HMPREF9690_05288	yes* (between HMPREF9690_05288 and HMPREF9690_05287)	Pseudomonas_phage_MP22	
Edwardsiella tarda FL6-60 plasmid pFL6-60	ETAF_ple052	ETAF_ple051	Fels 1	
Edwardsiella tarda ATCC 23685	EDWATA_02908	EDWATA_02909	Enterobacteria_phage_SfV	
Klebsiella pneumoniae subsp. rhinoscleromatis ATCC 13884	HMPREF0484_3768	HMPREF0484_3769	Burkholderia_phage_BcepC6B	
Edwardsiella ictaluri 93-146	NT01EI_2265	NT01EI_2264	Aeromonas_phage_phiO18P	
Erwinia phage vB_EamP-S6	gp091	gp092	Erwinia phage vB_EamP-S6	
Cronobacter sakazakii ATCC BAA-894	ESA_03320	ESA_03321	ND	ESA_03317 chitinase
S. enterica enterica serovar Typhi	STY0016	STY0015	ND	STY0018 chitinase
Citrobacter rodentium ICC168	ROD_00131	ROD_00122	ND	ROD_00151 chitinase
Enterobacter aerogenes KCTC 2190	EAE_10750	EAE_10745	ND	EAE_10760 chitinase
Klebsiella oxytoca 10-5243	HMPREF9689_00153	HMPREF9689_00152	ND	HMPREF9689_00155 chitinase
Klebsiella oxytoca 10-5243	HMPREF9687_04821	HMPREF9687_04822	ND	HMPREF9687_04819 chitinase
Klebsiella oxytoca KCTC 1686	KOX_1485	KOX_1480	ND	KOX_1495 chitinase
Klebsiella oxytoca 10-5242	HMPREF9686_04949	HMPREF9686_04948	ND	HMPREF9686_04952 chitinase
Klebsiella oxytoca 10-5250	HMPREF9694_04001	HMPREF9694_04000	ND	HMPREF9694_04003 chitinase
Rahnella sp. Y9602	Rahaq_3628	Rahaq_3626	ND	Rahaq_3630 chitinase
Rahnella aquatilis CIP 78.65 = ATCC 33071	Rahaq2_3713	ND	ND	Rahaq2_3715 chitinase
Novosphingobium aromaticivorans DSM1244	Saro_0666	Saro_0667; Saro_0668	Burkholderia_phage_phi1026b	
Sphingopyxis alaskensis RB2256	Sala_1717	Sala_1718	ND	
Sphingobium chlorophenolicum L-1	Sphch_1865	Sphch_1866	ND	
Sphingobium japonicum UT26S	SJA_C1-05860	SJA_C1-05850	ND	
Burkholderia gladioli BSR	bgla_1g10980	ND	Stenotrophomonas_phage_S1	
Burkholderia rhizoxinica HKI 454	RBRH_02935	ND	ND	
Vibrio vulnificus CMCP6	VV1_0124	ND	ND	
Acinetobacter baumannii SDF	ABSDF1449	ND	ND	
Acinetobacter baumannii SDF	ABSDF1053	ABSDF1052	Enterobacteria_phage_HK022	
Acinetobacter baumannii AYE	ABAYE1274	ABAYE1273	Acinetobacter bacteriophage AP22	
Acinetobacter baumannii AYE	ABAYE2689	ABAYE2690	Salmonella phage SE2	
Acinetobacter sp. SH024	HMPREF0013_00834	HMPREF0013_00835	Haemophilus_phage_HP2	
Acinetobacter baumannii SDF	ABSDF2454	ABSDF2455	Aggregatibacter phage S1249	
Acinetobacter baumannii TCDC-AB0715	ABTW07_2744	ABTW07_2745	Klebsiella_phage_phiKO2	
Acinetobacter baumannii ACICU	ACICU_01068	ACICU_01067	ND	
Acinetobacter calcoaceticus RUH2202	HMPREF0012_01757	HMPREF0012_01759; HMPREF0012_01758	Salmonella phage SE2	
Acinetobacter baumannii ACICU	ACICU_02712	ACICU_02714; ACICU_02713	Escherichia phage HK639	
Zymomonas mobilis subsp. mobilis strain ATCC 29191	ZZ6_0368	ZZ6_0369	ND	ZZ6_0875 levansucrase; ZZ6_0874 invertase
Sinorhizobium fredii USDA 257	USDA257_c07840	ND	ND	
Agrobacterium tumefaciens CCNWGS0286	ATCR1_06841	yes* (between ATCR1_06841 and ATCR1_06851)	Myxococcus_phage_Mx8	
Roseomonas cervicalis ATCC 49957	HMPREF0731_2172	HMPREF0731_2174	Megavirus chiliensis	
Citrobacter rodentium ICC168	ROD_36701	ROD_36711	Prophage Salmonella enterica subsp. enterica serovar Typhi str. Ty2	
Escherichia coli STEC_C165-02	ECSTECC16502_2443	ECSTECC16502_2442	Prophage Salmonella enterica subsp. enterica serovar Typhi str. Ty2	
Escherichia coli TA206	ECKG_02241	ECKG_02240	Ralstonia_phage_phiRSA1	
Escherichia coli DEC14D	ECDEC14D_2733	ECDEC14D_2732	Prophage Salmonella enterica subsp. enterica serovar Typhi str. Ty2	
Escherichia coli RN587/1	ECRN5871_4752	ECRN5871_4751	Ralstonia_phage_phiRSA1	
Escherichia coli M863	ERJG_04114	ERJG_04112; ERJG_04113	Escherichia phage HK639	ERJG_04110 ptxB; ERJG_04110 ptxA; ERJG_04115 heat-labile enterotoxin I; ERJG_04116 heat-labile enterotoxin II
Escherichia coli DEC11B	ECDEC11B_2197	ECDEC11B_2199; ECDEC11B_2198	Enterobacteria_phage_phiP27	
Escherichia coli OK1357	ECOK1357_1087	ECOK1357_1088	Enterobacteria_phage_cdtI	
Citrobacter rodentium ICC168	ROD_02671	ROD_02691; ROD_02681	Escherichia_phage_D108	
Escherichia coli O127:H6 str. E2348/69	E2348C_2654	E2348C_2652; E2348C_2653	Escherichia_phage_D108	
Escherichia coli DEC1D	ECDEC1D_2190	ECDEC1D_2188; ECDEC1D_2189	Enterobacteria_phage_YYZ_2008	
Escherichia coli UMNF18	UMNF18_1421	UMNF18_1419; UMNF18_1420	Enterobacteria_phage_phiP27	
Escherichia coli B088	ECCG_01861	ECCG_01859; ECCG_01860	Escherichia phage HK639	ECCG_01862 heat labile enterotoxin IIB; ECCG_01863 heat labile enterotoxin IIA (between ECCG_01862 and ECCG_01863)
Escherichia coli M863	ERJG_04877	ERJG_04878	Enterobacteria_phage_phiP27	
S. enterica enterica serovar Enteritidis str. P125109	SEN1395	SEN1394	Enterobacteria_phage_phiP27	
Yersinia enterocolitica enterocolitica 8081	YE1815	YE1814	Prophage Xanthomonas campestris pv. campestris str. ATCC 33913	
Pantoea sp. At-9b	Pat9b_5379	Pat9b_5378; Pat9b_5380	Escherichia phage HK75	
Klebsiella sp. 1_1_55	HMPREF0485_04777	HMPREF0485_04776; HMPREF0485_04778	Escherichia phage HK639	
Klebsiella oxytoca 10-5243	HMPREF9687_02095	HMPREF9687_02096; HMPREF9687_02094	Gifsy 2	
Klebsiella oxytoca 10-5246	HMPREF9690_03038	HMPREF9690_03039; HMPREF9690_03037	Escherichia phage HK75	
Pseudogulbenkiania sp. NH8B	NH8B_0535	ND	Burkholderia phage Bcep Mu	

ND: not detected
*: identified in this study

Table S2: Bacterial strains and plasmids used in this study

Strains	Description	Reference
S. Typhi ISP2825	Reference strain	1
SB1944	*S*. Typhi *cdtB*^{3xFLAG}	2
SB2644	*S*. Typhi *cdtB*^{3xFLAG} Δ*ttsA*	This study
SB2349	*S*. Typhi Δ*STY1887*	This study
SB1844	*S*. Typhi Δ*ttsA*	This study
SB2142	*S*. Typhi *cdtB*^{3xFLAG} *ttsA*^{3xFLAG}	This study
SB2143	*S*. Typhi *cdtB*^{3xFLAG} Δ*STY0015*	This study

Plasmids	Description	Reference
pSB3324	pWSK129 derived, low copy, P_{ara}, allows C-terminal 3xFLAG tagging; Kan^{R}	This study
pSB3335	pSB3324 *ttsA*^{3xFLAG}	This study
pSB3950	pSB3324 *ttsA*$^{E14A\ 3xFLAG}$	This study
pSB4027	pSB3324 *SEN1395*^{3xFLAG}	This study
pSB4031	pSB3324 *STY0016*^{3xFLAG}	This study
pSB4512	pSB3324 *YE1815*^{3xFLAG}	This study
pSB4372	pSB3324 *SEN1395*$^{D167N\ 3xFLAG}$	This study
pSB4719	pSB3324 *STY0015 STY0016*^{3xFLAG}	This study
pSB4720	pSB3324 *STY0015 ttsA*^{3xFLAG}	This study
pSB4721	pSB3324 *STY0015 ttsA*$^{E14A\ 3xFLAG}$	This study
pSB4352	pSB3324 *ttsA*$^{Q154E\ 3xFLAG}$	This study
pSB4353	pSB3324 *ttsA*$^{Q164E\ 3xFLAG}$	This study
pSB4354	pSB3324 *ttsA*$^{N166D\ 3xFLAG}$	This study
pSB4355	pSB3324 *ttsA*$^{I174M\ 3xFLAG}$	This study
pSB4356	pSB3324 *ttsA*$^{R179L\ 3xFLAG}$	This study
pSB4201	pSB3324 *SEN1395*$^{[1-127]}$*ttsA*$^{[127-181]\ 3xFLAG}$	This study
pSB4202	pSB3324 *SEN1395*$^{[1-93]}$*ttsA*$^{[93-181]\ 3xFLAG}$	This study
pSB4203	pSB3324 *SEN1395*$^{[1-52]}$*ttsA*$^{[52-181]\ 3xFLAG}$	This study
pSB4204	pSB3324 *ttsA*$^{[1-126]}$*SEN1395*$^{[128-181]\ 3xFLAG}$	This study
pSB4205	pSB3324 *ttsA*$^{[1-92]}$*SEN1395*$^{[94-181]\ 3xFLAG}$	This study
pSB4206	pSB3324 *ttsA*$^{[1-51]}$*SEN1395*$^{[53-180]\ 3xFLAG}$	This study
pSB4207	pSB3324 *ttsA*$^{[1-147]}$*SEN1395*$^{[149-180]\ 3xFLAG}$	This study
pSB4210	pSB3324 *SEN1395*$^{[1-127]}$*ttsA*$^{[127-147]}$*SEN1395*$^{[149-181]\ 3xFLAG}$	This study
pSB4211	pSB3324 *SEN1395*$^{[1-148]}$*ttsA*$^{[148-180]\ 3xFLAG}$	This study
pSB3833	pSB3324 *sty4425*^{3xFlag}	This study
pSB3832	pBAD24 *ttsA*^{3xFLAG}	This study
pSB3957	pBAD24 *ttsA*$^{E14A\ 3xFLAG}$	This study
pSB4368	pBAD24 *ttsA*$^{N166D\ 3xFLAG}$	This study
pSB4380	pBAD24 *STY0016*^{3xFLAG}	This study
pSB4369	pBAD24 *SEN1395*^{3xFLAG}	This study
pSB4379	pBAD24 *SEN1395*$^{D167N\ 3xFLAG}$	This study
pSB4518	pBAD24 *YE181*^{3xFLAG}	This study
pSB890	R6K origin; can be counterselected for with sucrose; Tet^{R}	3
pSB3567	pSB890 for C-terminal 3xFLAG tagging of *ttsA* in chromosome of *S*. Typhi	This study
pSB3252	pSB890 for replacement of cdtB3xFLAG by phoA3xFLAG in SB1944	This study
pBAD24	Used for arabinose-inducible overexpression in *S*. Typhi Δ*ttsA*; Amp^{R}	4

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