Supporting Information

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SI Results

Construction and Characterization of a DNA Vaccine Vector Encoding an Arabinose-Dependent Regulatory Domain that Contributes to the Lysis Phenotype. Based on our previous success with development of a programmed regulated lysis system for protective antigen delivery (1–3), we used a similar strategy to achieve an improved regulated lysis phenotype. The *Salmonella* Typhimurium host strain, χ8806 (Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}:: TT *araC* P_{BAD} *murA*) (Table S1), which features an arabinose-dependent programmed lysis phenotype, was chosen as the starting strain. The Δ*araBAD1923* and Δ*araE25* mutations were added sequentially using suicide vectors (Table S1), resulting in vaccine strain χ8807. Three additional mutations, Δ(*gmd-fcl*)-26, Δ*relA1123*, and Δ*endA2311* (Table S1) were also included, resulting in recombinant attenuated *Salmonella* vaccine (RASV) strain χ8888 (Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ(*gmd-fcl*)-26 Δ*relA1123* Δ*endA2311*) (Table S1). We then introduced the Δ*sifA26* mutation, a defined in-frame deletion of the *sifA* gene, into strain χ8888 to generate strain χ9354. The presence of each mutation was verified by PCR. The presence of the Δ*asdA19* mutation in *Salmonella* was confirmed by the strain's dependence on DAP for growth. The presence of the ΔP_{murA7}::TT *araC* P_{BAD} *murA* mutation (Table S1) was verified by the strain's dependence on arabinose for growth.

We constructed a DNA vaccine vector pYA3650 (Fig. S1*A* and Table S1) that complemented the regulated delayed lysis strain described above. Then, pYA3650 was first introduced into the delayed regulated lysis host strains χ8854 and χ8888 (Table S1) to yield strains χ8854(pYA3650) and χ8888(pYA3650). Growth of strain χ8854(pYA3650) depended on arabinose (Fig. S1*B*). The plasmids were stably maintained for 50 or more generations when grown in the presence of arabinose and DAP. Colonization by strain χ8854(pYA3650) was evaluated in 8-wk-old female BALB/c mice orally inoculated with 10^9 CFU, a dose 50,000-times the LD_{50} of the wild-type parent strain, χ3761. The strain transiently colonized lymphoid tissues (Fig. S1*C*) and no bacteria were recovered by 11 d after inoculation; however, nonlysis attenuated strains will persist in lymphoid tissues much longer (Fig. S1*D*). No arabinose-independent *Salmonella* mutants were recovered at any time during this experiment. These results indicate that a wild-type *Salmonella* strain engineered with this programmed lysis system is attenuated and is efficiently cleared from the host following colonization of lymphoid tissues.

Regulated Programmed Lysis. The RASV strain χ8888(pYA3650) grew well in LB broth supplemented with 0.02% arabinose but began to die after 1 h of incubation in LB broth without arabinose. To evaluate cell lysis, release of the cytoplasmic enzyme β-galactosidase into culture supernatants was used as an indicator. The *atrB13*::Mud*J* allele, which directs constitutive expression of β-galactosidase, was transduced into strain χ8888, resulting in strain χ8933 (χ8888 harboring *atrB13*::Mud*J* mutation). We then introduced plasmid pYA3650 into χ8933 to yield χ8933 (pYA3650). The ratio of β-galactosidase activity in the supernatant (released β-galactosidase) or cell pellet (retained cell-associated β-galactosidase) versus total β-galactosidase activity (supernatant plus cell pellet) indicated the extent of cell lysis. Release of β-galactosidase by strain χ8933(pYA3650) occurred only in medium lacking arabinose (Fig. S7*A*). Conversely, the amount of cell-associated β-galactosidase decreased over time when χ8933 (pYA3650) was grown in medium without arabinose, but no decrease was seen in media containing arabinose (Fig. S7*B*). These results are consistent with our expectations for the arabinose-regulated cell lysis phenotype.

SI Materials and Methods

Bacterial Strains and Plasmids. The bacterial strains and plasmids used are listed in Table S1. *S.* Typhimurium strains with *asdA* and *murA* gene deletions were grown at 37 °C in LB broth or on LB agar (4) supplemented with 50 μg/mL DAP (5) and 0.2% arabinose. Transformants containing plasmids harboring the *araC* P_{BAD} *asdA murA* cassette were selected on LB agar plates containing 0.2% arabinose (1). LB agar containing 5% (wt/vol) sucrose and no sodium chloride, was used for *sacB* gene-based counter selection in allelic exchange experiments (6). When required, antibiotics were added to culture media at the following concentrations: chloramphenicol, 25 μg/mL; tetracycline, 12 μg/mL; and kanamycin, 50 μg/mL.

General DNA Procedures. DNA manipulations were carried out as described by Sambrook et al. (7). Oligonucleotide synthesis was done commercially. *Escherichia coli* and *Salmonella* were transformed by electroporation (Bio-Rad). We used suicide vector technology (8) to generate precise deletion/deletion-insertion mutations (9). Conjugational transfer of suicide vectors was performed using the suicide vector donor strain MGN617. PCR amplification was used to obtain DNA fragments for cloning and for verification of chromosomal deletion mutations. Nucleotide sequencing reactions were performed by the sequencing facility at Arizona State University, using ABI Prism fluorescent Big Dye terminators according to the instructions of the manufacturer (PE Biosystems).

Strain Characterization. Molecular genetic attributes of vaccine strains were confirmed by PCR with appropriate primers. LPS profiles of *Salmonella* strains were examined by previously described methods (10). Vaccine strains were compared with vector controls for stability of plasmid maintenance and arabinose-dependent growth (1).

Construction of the Regulated Programmed Lysis *S.* Typhimurium Vaccine Host-Vector System. The *S.* Typhimurium host strain, χ8806 (Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA*), which features arabinose-dependent programmed lysis phenotype, was chosen as a starting strain. The Δ*araBAD1923* and Δ*araE25* mutations were added sequentially using suicide vectors resulting in vaccine strain χ8807. Three additional mutations, Δ(*gmd-fcl*)-26, Δ*relA1123*, and Δ*endA2311* mutations were also added sequentially using suicide vectors resulting in vaccine strain χ8888 (Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ(*gmd-fcl*)-26 Δ*relA1123* Δ*endA2311*). Then we introduced the Δ*sifA26* mutation, a defined in-frame deletion of the *sifA* gene, into strain χ8888 by conjugation with suicide vector pYA3716 to generate strain χ9354. The presence of each mutation was verified by PCR. The presence of the Δ*asdA19* mutation in *Salmonella* was confirmed by its dependence on DAP for growth. The presence of the ΔP_{murA7}:: TT *araC* P_{BAD} *murA* mutation was verified by arabinose dependency for growth.

To construct a DNA vaccine vector, we first create a proper eukaryotic expression cassette in plasmid pVAX-1 by inserting a transcriptional terminator (*rrfG* TT: *aac tgc agt cta gat tat gcg aaa ggc cat cct gac gga tgg cct ttt tgt tta aac gga tcc gc*) at the HincII site so that expression in pUC origin (*ori*) domain does not affect the

transcriptional activities from P_{CMV} promoter. The resulting plasmid was designated pYA3587. Then, a DNA fragment including pUC *ori*, *rrfG* TT, and a eukaryotic expression cassette from plasmid pYA3587 was inserted into the lysis vector pYA3681 (1) to replace the original pBR *ori* and a prokaryotic expression cassette using primers pVAX-SmaI-SalI and pVAX-SmaI-CalI. This process resulted in DNA vaccine vector pYA3650. Then pYA3650 was introduced into the regulated delayed lysis host strain χ8888.

Examination of Cell Lysis in Vitro. Overnight cultures of strains grown in LB broth supplemented with 0.002% arabinose (0.002% arabinose was supplied to avoid accumulation of arabinose in bacteria to be able to detect cell lysis during the short incubation period) were diluted 1:400 into fresh prewarmed LB broth supplemented with or without 0.02% arabinose β-galactosidase activity in the supernatant and cell-pellet fractions were assayed at indicated time points as previously described (1, 11).

Colonization of Mice with the *Salmonella* DNA Vaccine Strains. All animal experiments were conducted as per protocols approved by the Arizona State University Institutional Animal Care and Use Committee. Mice were kept 1 wk after arrival to acclimate them to our animal facility before immunization. Each group of three inbred 7-wk-old female BALB/c mice (Charles Rivers Laboratories) were deprived of food and water for 4 h before oral administration of *Salmonella* vaccine strains. These strains were grown with aeration in LB broth supplemented with 0.2% arabinose to an optical density at 600 nm (OD_{600}) of 0.85 from a nonaerated static overnight culture. Next, 1.0×10^9 CFU of χ8888 (pYA3650) or other RASV strains in 20 μL of PBS containing 0.01% gelatin (BSG) was orally administered to mice at the back of the mouth with a pipette tip. Food and water were returned to mice 30 min later. Mice were killed at indicated times and their Peyer's patches, spleens, and livers were collected aseptically. Tissues were homogenized and plated on LB agar with 0.2% arabinose to evaluate colonization and persistence, and onto LB agar plates without arabinose to confirm arabinose-dependent growth.

Construction of Improved DNA Vaccine Vector Encoding WSN HA. Plasmid pPolI-Wsn, all kindly provided by Yoshihiro Kawaoka (University of Wisconsin, Madison, WI) was used as the template for construction of pYA4545 encoding WSN HA. Primers KpnI-WSN HA and XhoI-WSN HA were used to amplify the DNA sequence of WSN HA gene. A DNA fragment encoding the WSN HA antigen with Kozak sequence was inserted downstream of the CMV promoter of the improved DNA vaccine vector pYA4545 at the KpnI/XhoI site to obtain plasmid pYA4859. We then introduced plasmid pYA4859 into χ9354 to yield χ9354(pYA4859).

Cultured Cell Invasion and Survival Assay. The method of Galan and Curtiss (11) was adapted and modified as follows. Monolayers of INT-407 cells (human embryonic intestine; ATCC CCL 6) were seeded at 5×10^5 cells per well in a 24-well tissue culture plate in Eagle's MEM (EMEM) supplemented with 10% (vol/vol) FBS. The plates were incubated at 37 °C in a humidified, 5% (vol/vol) CO_2 incubator to obtain confluent monolayers. The cells were rinsed with EMEM-1% (vol/vol) FBS and inoculated with ~5×10^6 CFU of a bacterial suspension. To measure bacterial internalization, the tissue culture plates were centrifuged at $600 \times g$ for 5 min, and incubated for 2 h at 37 °C in a humidified, 5% (vol/vol) CO_2 incubator. Then, the infected monolayers were rinsed three times with MEM-1% (vol/vol) FBS, and incubated for an additional 3 h in MEM-1% (vol/vol) FBS containing 100 μg/mL of gentamicin. For the bacterial survival assay, the infected monolayers were incubated a further 18 h in MEM-1% (vol/vol) FBS containing 10 μg/mL of gentamicin. The epithelial cells lysed with a solution of 0.1% Triton X-100. The suspensions were serially diluted and the number of viable and internalized bacteria determined by counting the resultant colonies on LB agar plates. The reported values represent the mean counts ± SD derived from triplicate wells.

Examination of EGFP Synthesis Using Confocal Microscopy. Next, 5×10^4 cells of INT-407 and Vero (ATCC CCL-8), derived from the kidney of an African green monkey, were cultured on glass coverslips in the six-well plates for 18 h at 37 °C in a humidified, 5% (vol/vol) CO_2 incubator. The INT-407 and Vero cells were transfected with 1 μg of the DNA vaccine vectors pYA4272 (pYA3650 encoding EGFP) and pYA4685 (pYA4545 encoding EGFP) using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). Twenty hours after initiating the transfection, the cell monolayers were rinsed three times with PBS and stained with 5 μM TO-PRO-3 iodide nuclear stain (Molecular Probes). Then, cells were washed with PBS and fixed with 3.0% (wt/vol) paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Stained cells were mounted using Vectashield mounting medium (Vector Laboratories) and stored at 4 °C in darkness before analysis. Slides were observed using a confocal epifluoresecence microscope (Leica TCS SP2 Microsystems). Images were captured through the 20× objective and analyzed using Leica Lite software (Leica Microsystems).

Virus Strain Propagation, Purification, and Titration. rWSN virus was provided by Andrew Pekosz (The Johns Hopkins University, Baltimore, MD). This virus is a mouse-adapted strain created by reverse genetics and is lethal to mice in doses above 10^3 $TCID_{50}$ (12, 13). The virus was propagated and titrated in Madin–Darby canine kidney cells cultured in RPMI-1640 (Gibco) containing 2 μg/mL acetyl-trypsin (Sigma). The virus was passed through a 30% (wt/vol) sucrose cushion at $11,620 \times g$ for 3 h in a Surespin Sorvall 630 rotor using a WK ultra 90 centrifuge (Thermo Electron). The resulting pellet was resuspended in PBS pH 7.2 and centrifuged at $11,620 \times g$ for 1 h. The viral pellet was finally dissolved in 500 μL of PBS and kept frozen at −80 °C until used.

ELISA. IgG responses against HA or LPS in sera were determined by ELISA (14). Briefly, 96-well flat-bottom polystyrene microtiter plates (Dynatech Laboratories) were coated with 100 ng per well of inactivated purified WSN virus or LPS (Sigma) suspended in carbonate coating buffer (pH 9.5) and incubated at 4 °C overnight. Free binding sites were blocked with PBS-0.05% Tween 20 containing 3% (wt/vol) BSA for 2 h at room temperature. Sera were serially diluted from an initial dilution of 1:50. A 100-μL volume of diluted sample was added to duplicate wells and incubated for 1 h at room temperature. Plates were treated with biotinylated goat anti-mouse IgG (Southern Biotechnology). Wells were developed with streptavidin-alkaline phosphatase conjugate (Southern Biotechnology) followed by *p*-nitrophenyl phosphate (Sigma). Color development (absorbance) was recorded at 405 nm using an automated ELISA plate reader (SpectraMax; Molecular Devices). Endpoint titers were expressed as the reciprocal $\log_2$ values of the last positive sample dilution. Absorbance reading two-times higher than preimmune serum baseline values was considered positive.

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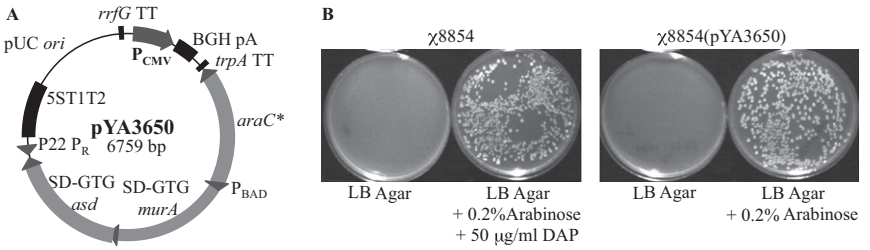

C

χ8854(pYA3650)				
Organ	3 days	7 days	11 days	15 days
Peyer's patch (CFU/PP)	3.3×10^3	2.5×10^3	0	0
Spleen (CFU/g)	3.3×10^1	3.3×10^1	0	0

D

χ8926 (Δ*sifA*)				
Organ	4 days	7 days	11 days	15 days
Peyer's patch (CFU/PP)	3.7×10^4	2.5×10^3	1.8×10^2	1.1×10^2
Spleen (CFU/g)	1.9×10^4	1.4×10^4	2.2×10^4	1.5×10^4

Fig. S1. In vitro and in vivo lysis of programmed lysis system in the absence of arabinose. (*A*) Map of plasmid pYA3650. Plasmid sequences include the *rrfG*, *trpA*, and 5S ribosomal RNA transcriptional terminators, the P_{BAD}, P22 P_R, and P_{CMV} promoters, the *araC* gene, and start codon-modified *murA* and *asdA* genes. (*B*) Growth of *Salmonella* strains χ8854 and χ8854(pYA3650) with and without arabinose on the indicated plates. (*C*) Colonization of mice with *S*. Typhimurium χ8854 (pYA3650) following oral inoculation with 10^9 CFU bacteria. (*D*) Colonization of mice with *S*. Typhimurium χ8926 following oral inoculation with 10^9 CFU bacteria.

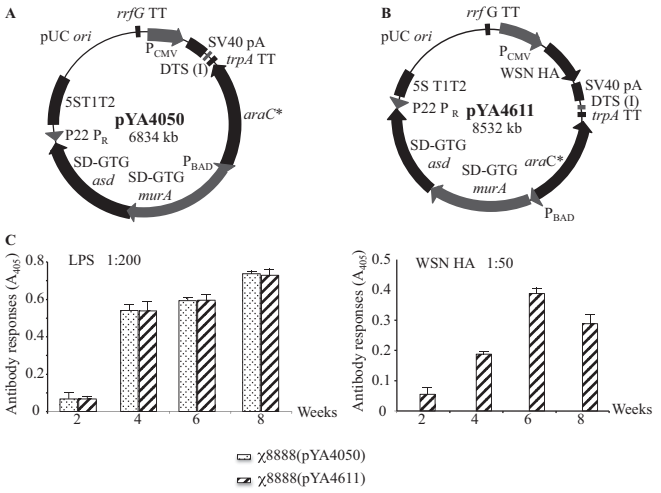

Fig. S2. Efficacy evaluation of DNA vaccine. (*A*) Map of plasmid pYA4050. Plasmid sequences of pYA4050 include the *rrfG*, *trpA*, and 5S ribosomal RNA transcriptional terminators, the P_{BAD}, P22 P_R, and P_{CMV} promoters, the *araC* gene, and start codon-modified *murA* and *asdA* genes, DNA nuclear targeting sequences (DTS) (I), and SV40 late poly(A). (*B*) Map of plasmid pYA4611. This plasmid was created by insertion of WSN HA into pYA4050. (*C*) Immune responses in mice after oral primary immunization and one boost dose with RASV strains χ8888(pYA4050) (vector control) and χ8888(pYA4611). Induction of IgG titers against purified *S*. Typhimurium LPS (*Left*) and inactivated influenza WSN virus (*Right*) detected by ELISA. Pooled serum samples ($n = 5$) from mice within a group were assayed and analyzed by ANOVA followed by Tukey's range test.

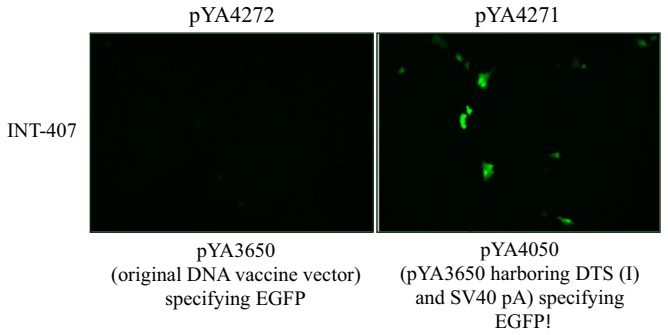

Fig. S3. Fluorescence imaging of EGFP (green signals) synthesis by DNA vaccine vector pYA3650 (original) (*Left*) and pYA4050 [improved by insertion of DTS (I) and SV40 late polyA into pYA3650] (*Right*) in INT-407 cells. One microgram plasmid DNA was transfected into INT-407 cell by using transfection reagent Lipofectamine, the fluorescence of EGFP was visualized using a microscope (Zeiss Axioskop 40, objective 10×) at 20 h posttransfection.

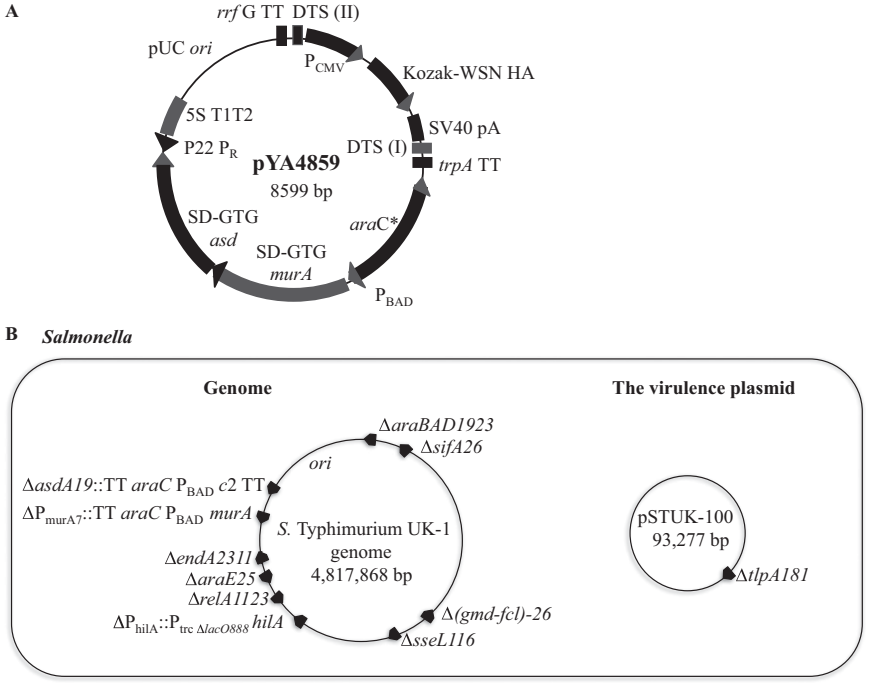

Fig. S4. Regulated delayed lysis system for WSN HA delivery by DNA vaccine. (*A*) Maps of plasmid pYA4859. Plasmid sequences of pYA4859 include the *rrfG*, *trpA*, and 5S ribosomal RNA transcriptional terminators, the P_{BAD}, P22 P_R, and P_{CMV} promoters, the *araC* gene, and start codon-modified *murA* and *asdA* genes, DTS (I), DTS (II), SV40 late poly(A), and WSN HA gene. (*B*) The defined deletion chromosomal mutations and locations in the genome of strain χ11218. P, promoter; TT, transcriptional terminator.

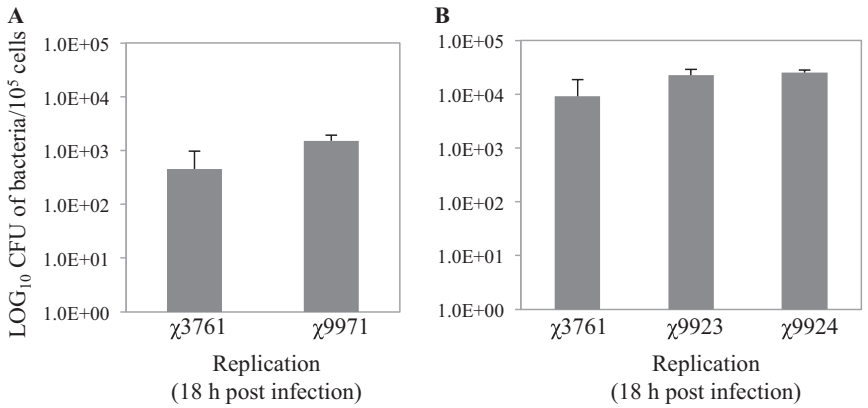

Fig. S5. Replication of *S*. Typhimurium χ3761 (wild-type), χ9923 (Δ*tlpA*), χ9924 (Δ*sseL*) (*B*), and χ9971 ($\Delta P_{hilA}::P_{trc\ \Delta lacO888}$ *hilA*) (*A*) in INT-407 cells. Values are the mean ± SD of three experiments with triplicate wells.

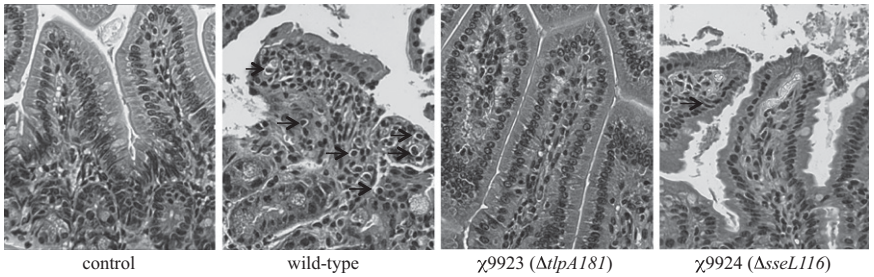

Fig. S6. Elimination of the synthesis of TlpA and SseL reduce apoptosis in the murine model of infection. Representative H&E images show apoptotic bodies (arrows) in the lower villus/crypt regions of ileum in control BALB/c mice or animals infected with wild-type *S*. Typhimurium UK-1 strain harboring the *tlpA* gene mutation or the *sseL* gene mutation. (Magnification: 40× objective.)

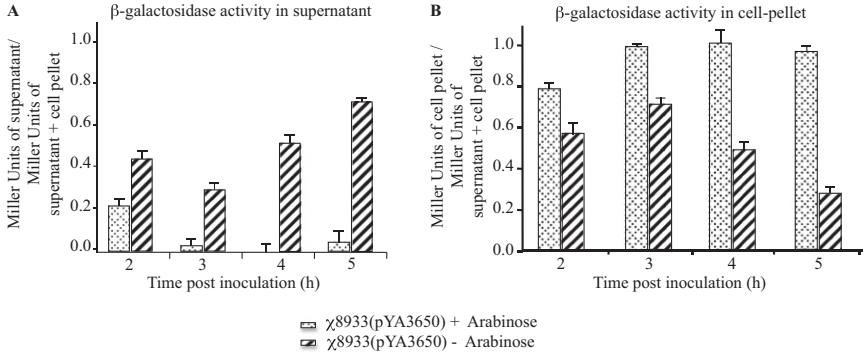

Fig. S7. In vitro demonstration of programmed lysis of *Salmonella* strain χ8888(pYA3650) in the absence of arabinose. (*A*) The ratio of released β-galactosidase versus total β-galactosidase when strain χ8888(pYA3650) with arabinose-regulated *asdA* and *murA* expression and constitutive *lacZ* expression was grown in LB broth with or without 0.02% arabinose. (*B*) The ratio of remaining β-galactosidase in nonlysed cells versus total β-galactosidase when strain χ8888 (pYA3650) was grown in LB broth with or without 0.02% arabinose.

Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source
S. Typhimurium UK-1		
χ3761	Wild-type	(1)
χ8806	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA*	Present study
χ8807	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25*	Present study
χ8854	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ*endA2311*	Present study
χ8888	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ*endA2311* Δ(*gmd-fcl*)*-26* Δ*relA1123*	(2)
χ8933	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ*endA2311* Δ(*gmd-fcl*)*-26* Δ*relA1123* Δ*atrB13*::MudJ	Present study
χ9354	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ*endA2311* Δ(*gmd-fcl*)*-26* Δ*relA1123* Δ*sifA26*	Present study
χ9923	Δ*tlpA181*	Present study
χ9924	Δ*sseL116*	Present study
χ9971	ΔP_{hilA}::$P_{trc\ \Delta lacO888}$ *hilA*	Present study
χ11214	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ*endA2311* Δ(*gmd-fcl*)*-26* Δ*relA1123* Δ*sifA26* ΔP_{hilA}::$P_{trc\ \Delta lacO888}$ *hilA*	Present study
χ11215	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ*endA2311* Δ(*gmd-fcl*)*-26* Δ*relA1123* Δ*sifA26* Δ*tlpA181* Δ*sseL116*	Present study
χ11218	Δ*asdA19*::TT *araC* P_{BAD} *c2* TT ΔP_{murA7}::TT *araC* P_{BAD} *murA* Δ*araBAD1923* Δ*araE25* Δ*endA2311* Δ(*gmd-fcl*)*-26* Δ*relA1123* Δ*sifA26* Δ*tlpA181* Δ*sseL116* ΔP_{hilA}::$P_{trc\ \Delta lacO888}$ *hilA*	Present study
E. coli K-12		
MGN-617	*thi-1 thr-1 leuB6 fhuA21 lacY1 glnV44 asdA4 recA1* RP4 2-Tc::Mu [*pir*]	(3)
χ289	*glnV42* λ^- $T3^r$	(4)
Recombinant plasmid		
pVAX-1	A 3.0-kb plasmid vector designed for use in the development of DNA vaccines	Invitrogen
pYA3587	pVAX-1 with *rrf*GTT at HincII site	Present study
pYA3650	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22 P_R anti-sense mRNA eukaryotic expression cassette	Present study
pYA3681	pBR *ori araC** P_{BAD} SD-GTG *asdA* SD-GTG *murA* P22 P_R anti-sense mRNA prokaryotic expression cassette	(5)
pYA3836	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22 P_R anti-sense mRNA eukaryotic expression cassette with DTS (I)	Present study
pYA4050	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22 P_R anti-sense mRNA eukaryotic expression cassette with DTS (I) and SV 40 polyA	Present study
pYA4271	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22P_R anti-sense mRNA eukaryotic expression cassette of EGFP with DTS (I) and SV 40 polyA	Present study
pYA4272	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22 P_R anti-sense mRNA eukaryotic expression cassette of EGFP	Present study
pYA4545	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22 P_R anti-sense mRNA eukaryotic expression cassette of with DTS (I), DTS (II) and SV 40 polyA	Present study
pYA4611	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22 P_R anti-sense mRNA eukaryotic expression cassette of WSN-HA with DTS (I) and SV 40 polyA	Present study
pYA4685	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22 P_R anti-sense mRNA eukaryotic expression cassette of EGFP with DTS (I), DTS (II) and SV 40 polyA	Present study
pYA4859	pUC *ori araC** P_{BAD} SD-GTG *murA* SD-GTG *asdA* P22 P_R anti-sense mRNA eukaryotic expression cassette of WSN-HA with DTS (I), DTS (II) and SV 40 polyA	Present study
Suicide vector		
pYA3484	Δ*araBAD1923*	(6)
pYA3485	Δ*araE25*	(6)
pYA3629	Δ(*gmd-fcl*)*-26*	(6)
pYA3652	Δ*endA2311*	(6)
pYA3679	Δ*relA1123*	(6)
pYA3716	Δ*sifA26*	(7)
pYA4620	Δ*tlpA181*	Present study
pYA4621	Δ*sseL116*	Present study
pYA4641	ΔP_{hilA}::$P_{trc\ \Delta lacO888}$ *hilA* *araC** P_{BAD} from χ289	Present study

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Table S2. Primers used in this study

Primer name	Sequence
Construction of plasmid pYA3650	
Synthetic *rrfG* TT	
rrfG TT	aac tgc agt cta gat tat gcg aaa ggc cat cct gac gga tgg cct ttt tgt tta aac gga tcc gc
Insertion of pUC *ori- rrfG* TT-P_{CMV}-BGH polyA cassette	
pVAX-SmaI-SalI	gca ccc ggg tcg aca gat cct tggcggcgagaaag
pVAX-SmaI-CalI	cgacccgggatcgatctgtgcggtatttcacaccg
Construction of plasmid pYA3836	
Insertion of DTS (I) fragment	
SphI-SV40 second	aca tgc atg caa cca gct gtg gaa tgt gtg tca gtt agg gtg tgg aaa gtc ccc agg ctc ccc agc agg ca
PflMI-*araC*-SV40	cgc cat ctg gtg gcg ggt tta acg ccg att gag gcc a
Construction of plasmid pYA4050	
Insertion of SV40 late polyA fragment	
XhoI-SV40 polyA	ccg ctc gag aat gct tcg agc aga cat gat aag ata cat tga tga gtt tgg aca
SphI-SV40 polyA	aca tgc atg ccc cgg atc gat cct tat cgg att tta cca cat ttg tag agg ttt tac ttg c
Construction of plasmids of pYA4272 and pYA4685	
Insertion of EGFP fragment	
KpnI-EGFP	ggg gta cca gga gcc gcc acc atg gtg agc aag ggc gag gag ctg
XhoI-EGFP	ccg ctc gag tta ctt gta cag ctc gtc cat gc
Construction of plasmid pYA4545	
DTS (II) cassette	
DTS (II)	ggg gac ttt ccg ggg act ttc ctc ccc acg cgg ggg act ttc cgc cac ggg cgg gga ctt tcc ggg gac ttt cc
Insertion of DTS (II) fragment	
PmlI-DTS up-1	ccg gca cgt gct aaa act tca ttt tta att taa aag g
DTS up-2	gcc cgt ggc gga aag tcc ccc gcg tgg gga gga aag tcc ccg gaa agt ccc cta atg acc ccg taa ttg att act att ggg gac ttt ccg cca cgg gcg ggg act ttc cgg gga ctt tcc gtt cat agc cca tat atg gag ttc cg
KpnI-DTS down-2	cgg ggt acc aag ctt aag ttt aaa cgc ta
Construction of plasmids of pYA4611 and pYA4859	
Insertion of WSN HA gene	
KpnI-WSN HA	ggg gta cca gga gcc gcc acc atg gct aag gca aaa cta ctg gtc ctg t
XhoI-WSN HA	ccg ctc gag tca gat gca tat tct gca ctg c
Construction of suicide vector	
pYA4620 (Δ*tlpA181*)	
HindIII-*tlpA* up-1	ccc aag ctt gcc ctg cct gtc gaa aag atg agc g
tlpA up-2	gag ctc ggt acc agc ctg cag gca tgc ctg tca gtc tcc gca taa agg ggt a
tlpA down-1	gca tgc ctg cag gct ggt acc gag ctc tga tga caa ttc aga cgc ctg tga t
EcoRI-*tlpA* down-2	cgg gaa ttc ctg tca taa cgg cgt cag tga cgg c
pYA4621 (Δ*sseL116*)	
HindIII-*sseL* up-1	ccc aag ctt ctg cga aca ccc cat tac gct act g
sseL up-2	gag ctc ggt acc agc ctg cag gca tgc ctc ttc tgt ata taa gct gtg aaa t
sseL down-1	gca tgc ctg cag gct ggt acc gag ctc tgg tga gta ctt aga gcc tat ccc a
EcoRI-*sseL* down-2	cgg gaa ttc ttg aag cgt gta ttt atc gta ttc g
pYA4641 (ΔP_{hilA}::$P_{trc\ \Delta lacO888}$ *hilA*)	
HindIII-$P_{trc\ 888}$ up-1	ccc aag ctt cat ttt ttg tat ctg tca ctt aag t
$P_{trc\ 888}$ up-2	gag ctc ggt acc cgg cct gca ggc atg cac aca tta tac gag ccg gat gat taa ttg tca aca gct cat ttc aga atg ata tcg tct ttt ctt tac tcc
$P_{trc\ 888}$ down-1	gca tgc ctg cag gcc ggg tac cga gct cca cac agg aaa cag acc atg cca cat ttt aat cct gtt cct gta t
BamHI-$P_{trc\ 888}$ down-2	cgc gga tcc cag ttc ttc gta atg gtc acc ggc a