Supporting Information

Kong *et al.* 10.1073/pnas.0803801105

SI Materials and Methods

General DNA Procedures. DNA manipulations were carried out as described by Sambrook *et al.* (1). Oligonucleotide synthesis was done commercially. *Escherichia coli* and *Salmonella* were transformed by electroporation. We used suicide vector technology (2, 3) and P22-mediated transduction to generate precise deletion/deletion-insertion mutations (4). Conjugational transfer of suicide vectors was performed by using the suicide vector donor strain MGN617 (5). PCR amplification was used to obtain DNA fragments for cloning and for verification of chromosomal deletion mutations. Nucleotide sequencing reactions were performed by using ABI Prism fluorescent Big Dye terminators according to the instructions of the manufacturer (PE Biosystems).

Construction of Regulated Programmed Lysis *S.* Typhimurium Vaccine Host-Vector System. The ΔP_{murA7}::*araC* P_{BAD} *murA* deletion-insertion mutation was constructed by standard methods and introduced into wild-type strain χ3761 to yield χ8645. The Δ*asdA19*::*araC* P_{BAD} *c2* deletion-insertion mutation was introduced into χ8645 by P22HT *int* transduction from χ8290 (Δ*asdA19*::TT *araC* P_{BAD} *c2*). The Δ*endA2311*, Δ(*gmd-fcl*)-26 and Δ*relA1123* mutations were added sequentially, using suicide vectors (Table S1) resulting in vaccine strain χ8937. The presence of mutations and absence of suicide vector sequences were confirmed by PCR, using suitable primer sets (Table S2). The primers and steps used to construct plasmids are described as follows. The *E. coli* B/r *araC* P_{BAD} activator-promoter was derived from pBAD18 (6). The *E. coli* K-12 *araC* P_{BAD} activator-promoter was PCR amplified by using primers *araC-Nsi*I and E*araBAD*-EcoRI from strain χ289. The SD-GTG mutation in pYA3530 was introduced into the *asdA* gene by PCR. The ATG-*murA* gene was amplified by PCR from *E. coli* K-12 strain χ289 (*glnV42* λ^- $T3^r$), using primers E*murA*-EcoRI 5′ and E*murA*-EcoRI 3′, then the ATG start codon of the *murA* gene was changed to GTG by PCR. The P22 P_R promoter was derived from plasmid pMEG104. The P_{trc}-5ST1T2-P_{BR} *ori* fragment came from plasmid pYA3342. The fragment including in-frame fusion of the rPspA Rx1 (α-helical region of PspA from amino acid residue 3 to 257of mature $PspA_{Rx1}$) to the β-lactamase signal sequence was derived from pYA3634 (7). Expression of the rPspA Rx1 antigen was verified by SDS/PAGE and Western blot analysis with the anti-PspA monoclonal antibody Xi126 (8).

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4. Kang HY, Dozois CM, Tinge SA, Lee TH, Curtiss R, III (2002) Transduction-mediated transfer of unmarked deletion and point mutations through use of counterselectable suicide vectors. *J Bacteriol* 184:307–312.
5. Roland K, Curtiss R, III, Sizemore D (1999) Construction and evaluation of a cya crp Salmonella typhimurium strain expressing avian pathogenic Escherichia coli O78 LPS as a vaccine to prevent airsacculitis in chickens. *Avian Dis* 43:429–441.
6. Guzman LM, Belin D, Carson MJ, Beckwith J (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. *J Bacteriol* 177:4121–4130.
7. Curtiss R, III *et al.* (2007) in *Virulence Mechanisms of Bacterial Pathogens,* eds Brogden KA (ASM Press, Washington D.C), pp 297–313.
8. McDaniel LS, Scott G, Kearney JF, Briles DE (1984) Monoclonal antibodies against protease sensitive pneumococcal antigens can protect mice from fatal infection with Streptococcus pneumoniae. *J Exp Med* 160:386–397.

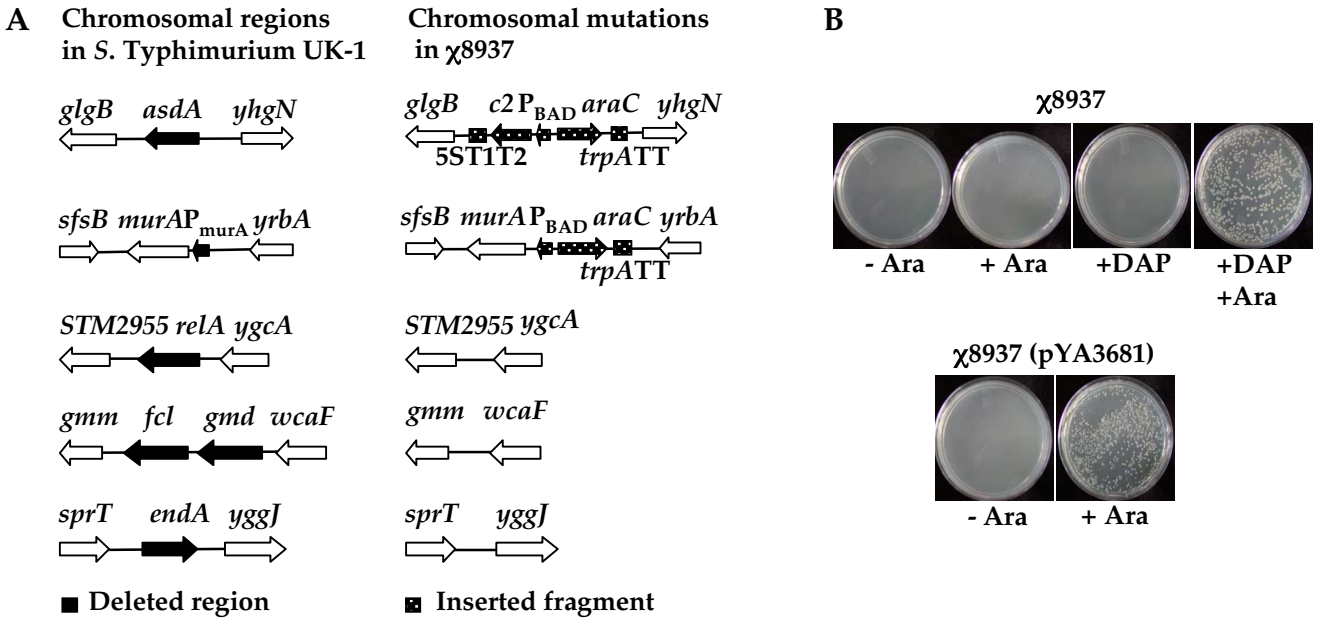

Fig. S1. Defined deletion mutations of strain χ8937 and nutritional requirements. (*A*) The defined deletion chromosomal mutations in wild-type *Salmonella* UK-1 and in strain χ8937. (*P*) promoter, TT: transcriptional terminator. (*B*) The growth of host strain χ8937 alone or strain χ8937 harboring pYA3681 on LB agar plates with or without supplementations.

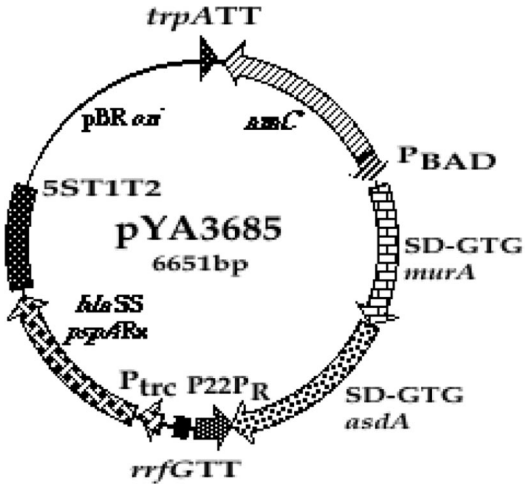

Fig. S2. Map of plasmid pYA3685. Plasmid sequences include the *trpA, rrfG*, and 5S ribosomal RNA transcriptional terminators; the P_{BAD}, P_{trc}, and P22 P_R promoters; the *araC* gene and start codon-modified *murA* and *asdA* genes; and the *bla-pspA* fusion protein.

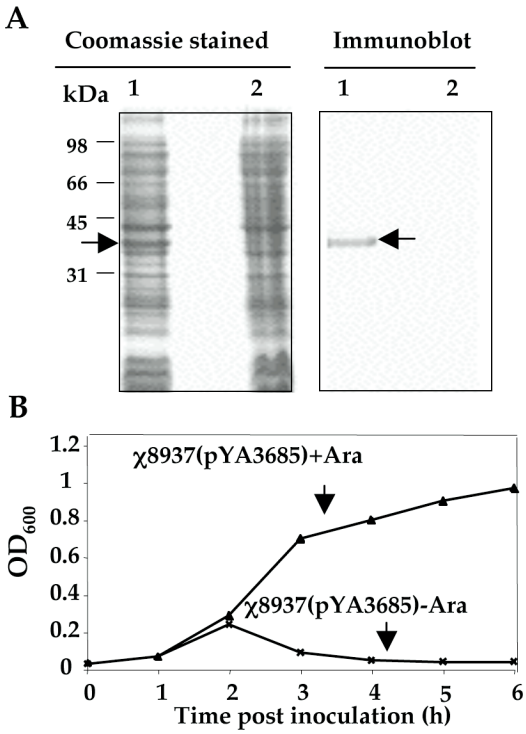

Fig. S3. Expression of PspA Rx1 and arabinose-dependent growth of χ8937(pYA3685). (*A*) The expression of rPspA Rx1 in the programmed lysis *S.* Typhimurium strain χ8937(pYA3685) grown in LB broth with 0.02% arabinose at 37°C. Aliquots of mid-log phase cultures were subjected to SDS/PAGE or immunoblot analysis. The immunoblot was probed with anti-PspA antibody. PspA proteins are indicated by arrows. Lanes 1 and 2, protein from χ8937(pYA3685) and χ8937(pYA3681), respectively. (*B*) Growth curves of PspA-expressing strain χ8937(pYA3685) in LB broth with or without the addition of 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
χ8276	Δ*asdA16*	2
χ8290	Δ*asdA19*::TT *araC* P_{BAD} *c2*	This study
χ8645	ΔP_{murA7}::*araC* P_{BAD} *murA*	This study
χ8831	Δ(*gmd-fcl*)*-26*	This study
χ8844	Δ*endA2311*	This study
χ8882	Δ*relA1123*	This study
χ8937	Δ*asdA19*::*araC* P_{BAD} *c2* ΔP_{murA7}::*araC* P_{BAD} *murA* Δ(*gmd-fcl*)*-26* Δ*relA1123* Δ*endA2311*	This study
χ9379	χ3761 Δ*atrB13*::MudJ	This study
χ9380	χ8937 Δ*atrB13*::MudJ	This study
E. coli K-12		
χ289	*glnV42* λ^- $T3^r$	3
MGN-617	*thi-1 thr-1 leuB6 fhuA21 lacY1 glnV44 asdA4 recA1* RP4 2-Tc::Mu [*pir*]	4
Suicide vectors		
pMEG-443	Δ*asdA16*	Megan Health, Inc.
pMEG-611	Δ*asdA19*::TT *araC* P_{BAD} *c2*	Megan Health, Inc.
pMEG-902	ΔP_{murA7}::*araC* P_{BAD} *murA*	Megan Health, Inc.
pYA3629	Δ(*gmd-fcl*)*-26*	This study
pYA3652	Δ*endA2311*	This study
pYA3679	Δ*relA1123*	This study
Plasmids		
pYA3450	p15A *ori araC* P_{BAD} SD-ATG *asdA*	This study
pYA3530	p15A *ori araC* P_{BAD} SD-GTG *asdA*	This study
pYA3681	pBR *ori araC** P_{BAD} SD-GTG *asdA* SD-GTG *murA* P22 P_R anti-sense mRNA	This study
pYA3685	pBR *ori araC** P_{BAD} SD-GTG *asdA* SD-GTG *murA* P22 P_R anti-sense mRNA, rPspA Rx1 *araC** P_{BAD} from χ289	This study

1. Curtiss R, III *et al.* (1991) in *Colonization Control of Human Bacterial Enteropathogens in Poultry*, eds Bailey JHS, Cox NA, Stern NJ, Meinersmann RJ (Academic, New York), pp 169–198.
2. Kang HY, Srinivasan J, Curtiss R, III (2002) Immune responses to recombinant pneumococcal PspA antigen delivered by live attenuated Salmonella enterica serovar Typhimurium vaccine. *Infect Immun* 70:1739–1749.
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Table S2. Primers used in this study

Primer name	Sequence
Construction of plasmid pYA3681	
GTG *asd*	
GTG *asd 5′*	cag gaa aaa aac gct gtg aaa aat gtt gg
GTG *asd 3′*	gtc ctt ttt ttg cga cac ttt tta caa cc
araC P_{BAD} GTG *asd*	
*araC-Sma*I	cga ccc ggg atc gat ctg tgc ggt att tca cac cg
*asd-Sma*I	gca ccc ggg tcg aca gat cct tgg cgg cga gaa ag
*araC** P_{BAD} (from χ289)	
E*araC-Nsi*I	cca atg cat aat gtg cct gtc aaa tgg
E*araBAD-Eco*RI	cgg aat tcg cta gcc caa aaa aac g
murA	
E*murA-Eco*RI 5′	cgg aat tct gag aac aaa cta aat gg
E*murA-Eco*RI 3′	cgg aat tct tat tcg cct ttc aca cgc
GTG *murA*	
EMGTGRV-*Nco*I	cat gcc atg gag ctc ggt acc cgg gga t
EMGTG-*Nco*I-*Eco*RI	cat gcc atg gaa ttc tga gaa caa act aag tgg ata aat ttc gtg ttc ag
P_{trc}-pBR *ori* cassette	
P_{trc}-*Pme*I	agc ttt gtt taa acg gat ctt ccg gaa gac ctt cca ttc
*Xba*I-pBR	gct cta gac tgt cag acc aag ttt act cat a
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
Construction of plasmid pYA3685	
*Nco*I-bla-PspA	cat gcc atg ggt att caa cat ttc cgt gtc gcc ctt att c
*Sma*I-TAA-PspA	tcc ccc ggg cta tta ttc tac att att gtt ttc t
Construction of suicide vectors	
Δ*relA1123*	
relA C-*Sph*I	aca tgc atg ccc aga tat ttt cca gat ctt cac
relA C-*Eco*RI	cgg aat tca ccc cag aca gta atc atg tag cgg
relA N-*Eco*RI	cgg aat tca agg gac cag gcc tac cga ag
relA N-*Bam*HI	cgg gat ccg agg gcg ttc cgg cgc tgg tag aa
Δ(*gmd-fcl*)-26	
*wcaF-Xba*I	gct cta gat cct caa ata gtc ccg tta gg
*wcaF-Sma*I	tcc ccc ggg caa aat att gta tcg ctg g
*gmm-Sph*I	gcacgc atg ctc agg cag gcg taa atc gct ct
*gmm-Xba*I	cct cta gac aat gtt ttt acg tca gga aga tt
Δ*endA2311*	
*endA*N-*Bam*HI	cgg gat ccg cta cga aat ccg cct caa c
*endA*N-*Hin*dIII	ccc aag ctt agc aaa acg agc ccg caa cg
*endA*C-*Hin*dIII	ccc aag ctt cct aca cta gcg gga ttc ttg
*endA*C-*Sph*I	aca tgc atg ccg cag cgc tca gag