

Supplemental Information

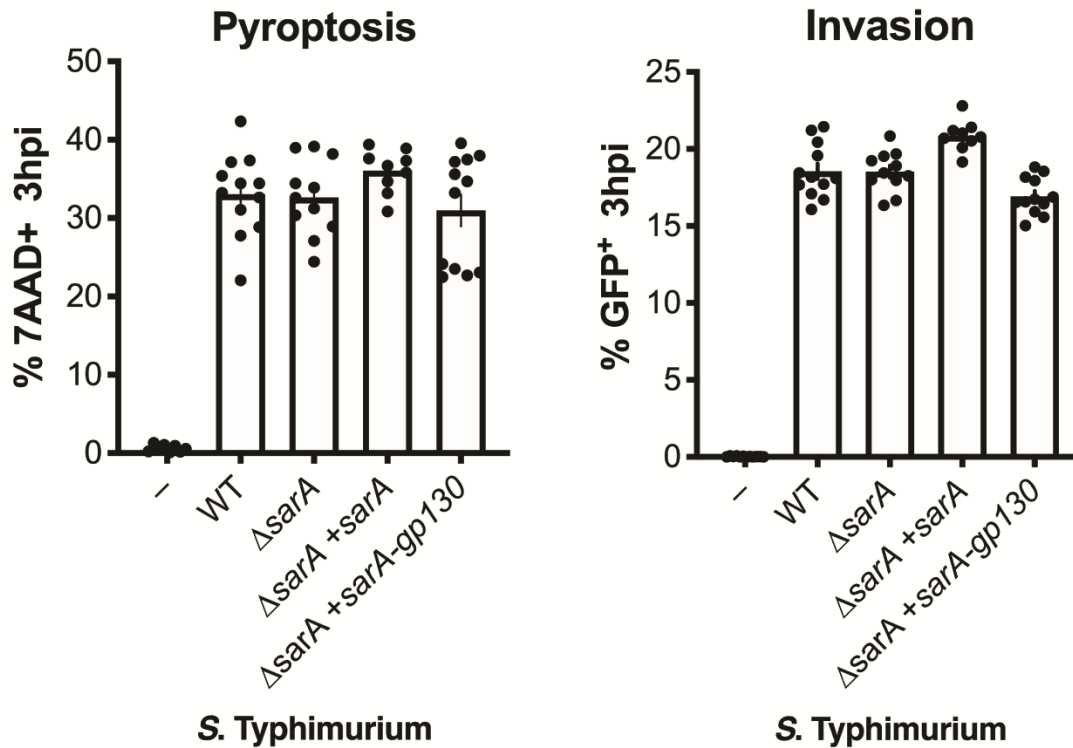


Figure S1. Complementation with endogenous chimera does not affect *Salmonella* invasion or host cell pyroptosis, Related to Figure 1E. Invasion and pyroptosis of GM19154 was measured 3 hpi after 70 min of IPTG treatment to induce GFP in infected cells and 5 min of 7AAD staining to mark dead cells. Percent GFP+ and 7AAD+ quantified by flow cytometry. Data from experiment in Figure 1E.

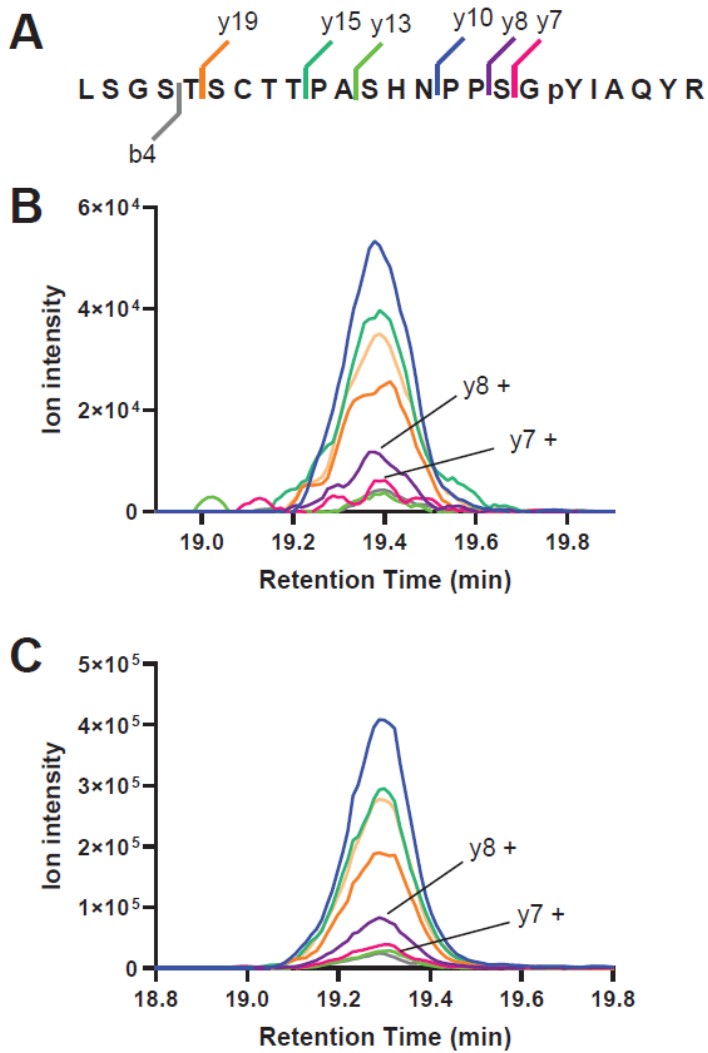


Figure S2. Targeted quantification of pTyr167 peptide by parallel reaction monitoring, Related to Figure 2C. (A) The endogenous pTyr167 peptide was identified on the basis of the labeled y- and b-ions, which are as follows: y10+, 1231.5507 m/z; y8+, 1037.4452 m/z; y7+, 950.4132 m/z; y19++, 1093.9699 m/z; y15++, 869.3909 m/z; y13++, 785.3459 m/z; y10++, 616.2790 m/z; b4+, 345.1769 m/z. B-C) Ion chromatograms of endogenous (light) pTyr167 peptide enriched from HeLa cells infected with (B) WT Salmonella or (C) SarA-overexpressing Salmonella. Ions are labeled as in (A). Phosphosite-localizing ions (y7 and y8) are labeled.

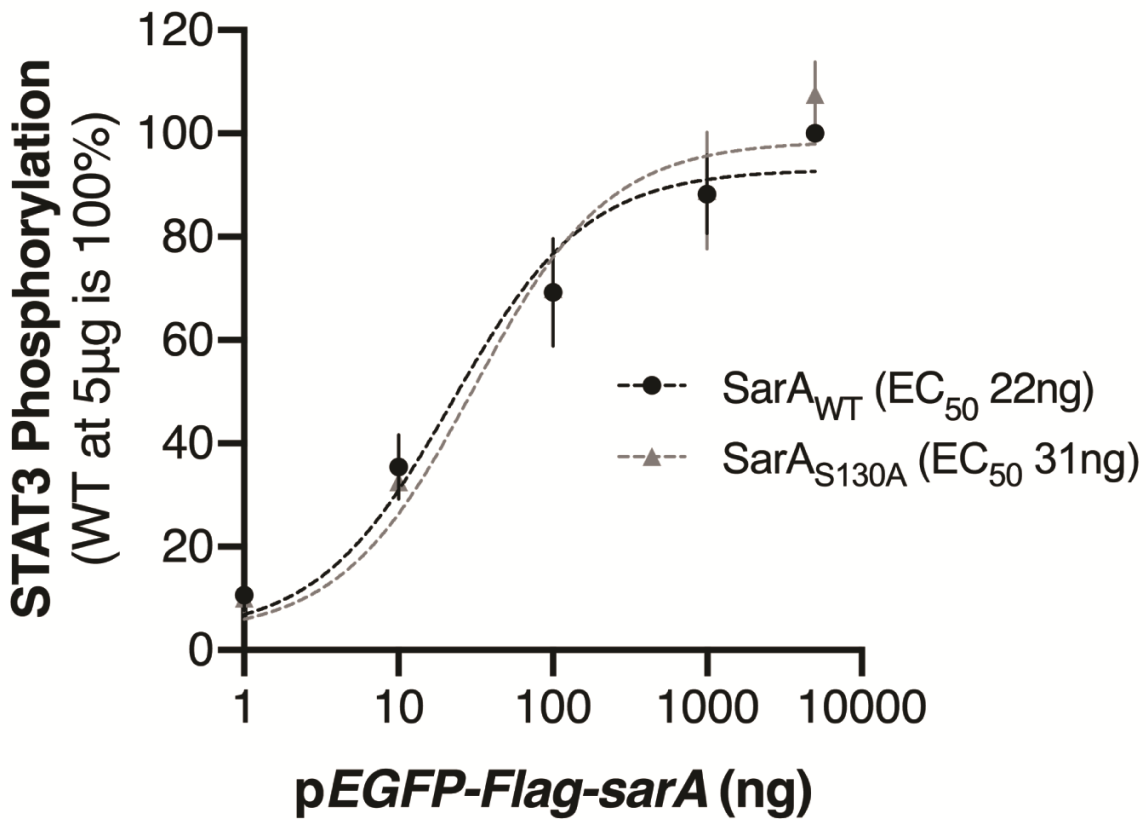


Figure S3. Overexpression of SarA S130A induces STAT3 phosphorylation with similar efficiency as wild-type SarA, Related to Figure 4M. HeLa cells transfected with increasing *pEGFP-Flag-sarA* (1ng to 5 µg) 24h before lysis and immunoblotting. Dots are means from four experiments with bars showing SEM. Wild-type SarA had an EC₅₀ of 22ng 95% CI [10,50] while the S130A mutation had a similar EC₅₀ of 30ng 95% CI [13,71].

Salmonella enterica Typhimurium (ATCC 14028s) Strains					
Strain	Genotype	Plasmid	Resistance	Derived from	Notes
DCK22	14028s	p67GFP	Amp	CS093	
DCK436	14028s <i>stm2585</i> ::Kan ^R	–	Kan	CS093	
DCK440	14028s Δ <i>stm2585</i>	–	–	DCK436	
DCK444	14028s Δ <i>stm2585</i>	p67GFP	Amp	DCK440	
DCK483	14028s	pWSK29	Amp	CS093	
DCK484	14028s	pWSK129	Kan	CS093	
DCK486	14028s Δ <i>stm2585</i>	pWSK129	Kan	DCK440	
DCK487a	14028s Δ <i>stm2585</i>	pWSK129- <i>stm2585</i>	Kan	DCK440	<i>stm2585</i> in plasmid is flanked by 786bp on 5' and 28bp on 3' from endogenous locus.
DCK487b	14028s Δ <i>stm2585</i>	pWSK129- <i>stm2585</i> , p67GFP	Amp, Kan	DCK444	
DCK499	14028s Δ <i>stm2585</i> -Kan ^R -3xFlag	–	Kan	CS093	
DCK698	14028s Δ <i>stm2585</i>	pWSK129- <i>stm2585</i> _{S130A}	Kan	DCK440	
DCK700	14028s Δ <i>stm2585</i>	pWSK129- <i>stm2585</i> _{Y167F}	Kan	DCK440	
DCK731	14028s Δ <i>stm2585</i>	pWSK129- <i>stm2585</i> _{S130A} , p67GFP	Amp, Kan	DCK444	
DCK733	14028s Δ <i>stm2585</i>	pWSK129- <i>stm2585</i> _{Y167F} , p67GFP	Amp, Kan	DCK444	
DCK830	14028s Δ <i>stm2585</i>	pUC57- <i>stm2585-gp130</i> , p67GFP	Amp, Kan	DCK444	<i>gp130</i> sequence in chimera is codon-optimized for <i>E. coli</i> expression.
DCK869	14028s Δ <i>stm2585</i>	pWSK129- <i>stm2585-gp130</i>	Kan	DCK440	

Table S2. Bacterial strains, Related to STAR Methods.

Plasmid	Resistance	In <i>E. coli</i> Strain	Notes
pEGFP-C1	Kan	DCK53	
pEGFP-Flag- <i>stm2585</i>	Kan	DCK528	<i>stm2585</i> (native sequence) in pEGFP-C1 with N-terminal Flag tag
pEGFP-Flag- <i>stm2585</i> _{Y167F}	Kan	DCK728	
pEGFP-Flag- <i>stm2585</i> _{S130A}	Kan	DCK736	
pEGFP-Flag- <i>stm2585</i> _{L139*}	Kan	DCK786	Removes the GBS sequence from <i>stm2585</i>
pcDNA3	Amp	DCK77	
pcDNA3.1-Flag- <i>stm2585</i>	Amp	DCK542, DCK796	From GenScript. <i>stm2585</i> is codon-optimized for mammalian expression
pcDNA3.1-Flag- <i>stm2585</i> _{Y167F}	Amp	DCK798	
pcDNA3.1-Flag- <i>stm2585</i> _{L139*}	Amp	DCK799	
pcDNA3.1-Flag- <i>stm2585-gp130</i>	Amp	DCK801	Human gp130 sequence, not codon-optimized
pcDNA3.1-Flag- <i>gp130dimer</i>	Amp	DCK824	Cytosolic domain of gp130 with C-C linker and Leu zipper for dimerization
pcDNA3.1-Flag- <i>gp130dimer-stm2585</i>	Amp	DCK825	<i>stm2585</i> insert (140-179) is codon-optimized for mammalian expression
pWSK129	Kan	DCK827	
pWSK129- <i>stm2585</i>	Kan	DCK809	<i>stm2585</i> is flanked by 786bp on 5' and 28bp on 3' from endogenous locus
pWSK129- <i>stm2585</i> _{Y167F}	Kan	DCK629	
pWSK129- <i>stm2585</i> _{S130A}	Kan	DCK628a	
pWSK129- <i>stm2585-gp130</i>	Kan	DCK852	
pWSK29	Amp	DCK826	
pUC57- <i>stm2585-gp130</i>	Kan	DCK828	From GenScript. Human gp130 insert is codon-optimized for <i>E. coli</i> expression.

Table S3. Plasmids, Related to STAR Methods.

Designation	Name	Purpose	Sequence
DK395	<i>stm2585_T388G_Fwd</i>	S130A in native <i>sarA</i>	5'-actaaagagtctttatcattaccagttGccccggatgcttta-3'
DK396	<i>stm2585_T388G_Rvr</i>	S130A in native <i>sarA</i>	5'-taaagcatccggggCaactggtaatgataaagactctttagt-3'
DK399	<i>stm2585_A500T_Fwd</i>	Y167F in native <i>sarA</i>	5'-aaccaccgtccggtttTtatcgctcaatacagg-3'
DK400	<i>stm2585_A500T_Rvr</i>	Y167F in native <i>sarA</i>	5'-cctgtattgagcgataAaaccggacggtgggtt-3'
DK580	<i>EGFP-Flag-stm2585_139*_Fwd</i>	Remove GBS from native <i>sarA</i>	5'-ccggatgctttaaccacaaaactattataagtgcacggtacc-3'
DK581	<i>EGFP-Flag-stm2585_139*_Rvr</i>	Remove GBS from native <i>sarA</i>	5'-ggtaccgtcgacttataatagttttgggttaaagcatccgg-3'
DK587	<i>EGFP-Flag-stm2585_Δ1-100_Fwd</i>	N-term truncation of native <i>sarA</i>	5'-gacgatgacgacaagctcgagtaccagccc-3'
DK588	<i>EGFP-Flag-stm2585_Δ1-100_Fwd</i>	N-term truncation of native <i>sarA</i>	5'-gggctggactcgagcttgtcgtcatcgtc-3'
DK652	<i>Flag-stm2585-gp130_139*_Fwd</i>	Remove gp130 from chimera	5'-cactgacacagaagctgctgtaagaattctgcagatatcc-3'
DK653	<i>Flag-stm2585-gp130_139*_Rvr</i>	Remove gp130 from chimera	5'-ggatatctgcagaattcttacagcagcttctgtgctcagtg-3'
DK670	<i>Flag-stm2585_ΔFlag_Fwd</i>	Untag codon-opt <i>sarA</i>	5'-agacaaatcgcatcatggtggcggatccgagc-3'
DK671	<i>Flag-stm2585_ΔFlag_Rvr</i>	Untag codon-opt <i>sarA</i>	5'-gctcggatccgccaccatgatgctgatttgtct-3'

Table S4. Primers, Related to STAR Methods.