Supplemental Materials and Methods

Mass spectrometry of UTR::*cyaA'* fusions. Bacteria from overnight LB broth cultures were pelleted, washed with PBS, and flash frozen. Tryptic digests of cell lysates were analyzed at least two times by LC-MS/MS using an Orbitrap mass spectrometer. To determine if peptides matched UTR encoded residues, the *gtgA*, *cigR*, *gogB*, *sseL*, and *steD* UTRs were translated to KARLEP, HQKGNI, NNDSLV, SLYTEE, and VHEEVY, respectively. These motifs were appended to the CyaA' sequence and used as a reference for spectrum-to-peptide matching with MS-GFDB (1).

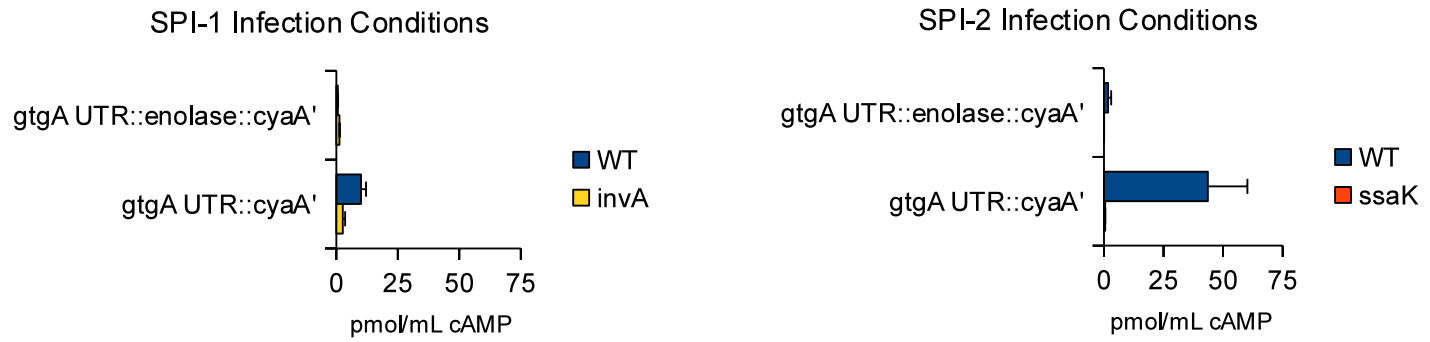
CyaA' Sequence:

MQGQQSHQAGYANAADRESGIPAAVLDGIKAVAKEKNATLMFRLVNPHSTSLIAEGVATKGLGV
HAKSSDWGLQAGYIPVNPNLSKLFGRAPEVIARADNDVNSSLAHGHTAVDLTLSKERLDYLRQA
GLVTGMADGVVASNHAGYEQFEFRVKETSDGRYAVQYRRKGGDDFEAVKVIGNAAGIPLTADID
MFAIMPHLSNFRDSARSSVTSGDSVTDYLARTRRAASEATGGLDRERIDLLWKIARAGARSAVG
TEARRQFRYDGDMNIGVITDFELEVRNALNRRAHAVGAQDVVQHGTEQNNPFPEADEKIFVVSA
TGESQMLTRGQLKEYIGQQRGEGYVFYENRAYGVAGKSLFDDGLGAAPGVPSGRSKFSPDVLET
VPASPGLRRPSLGAVERQDSGYDSLDG*

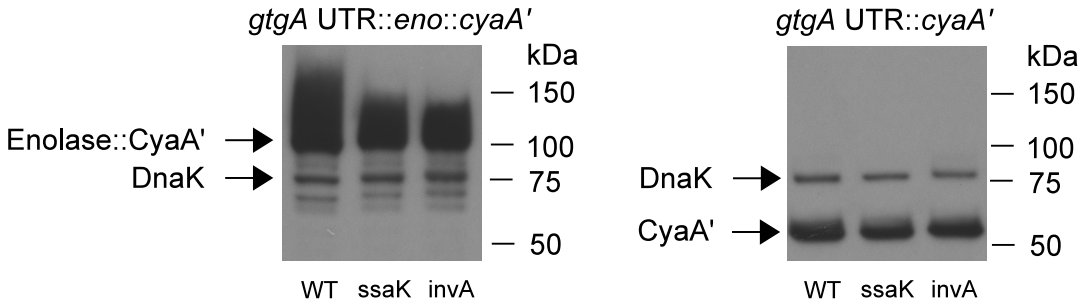
Because samples were derived from bacterial lysates, spectra were also searched against the *S.* Typhimurium 14028 proteome as well as a collection of contaminants commonly observed in MS experiments. Strict filters were used for peptide identifications. For peptides observed only once, we used an MS-GF spectral probability of $1E^{-10}$, PPM mass error < 2 and > -2, and a peptide false discovery rate (FDR) of 0. For peptides observed multiple times, we used a minimum MS-GF spectral probability of $2E^{-10}$, PPM mass error < 3 and > -3, and at least one observation with an FDR of 0.

Supplemental Figure 1

A

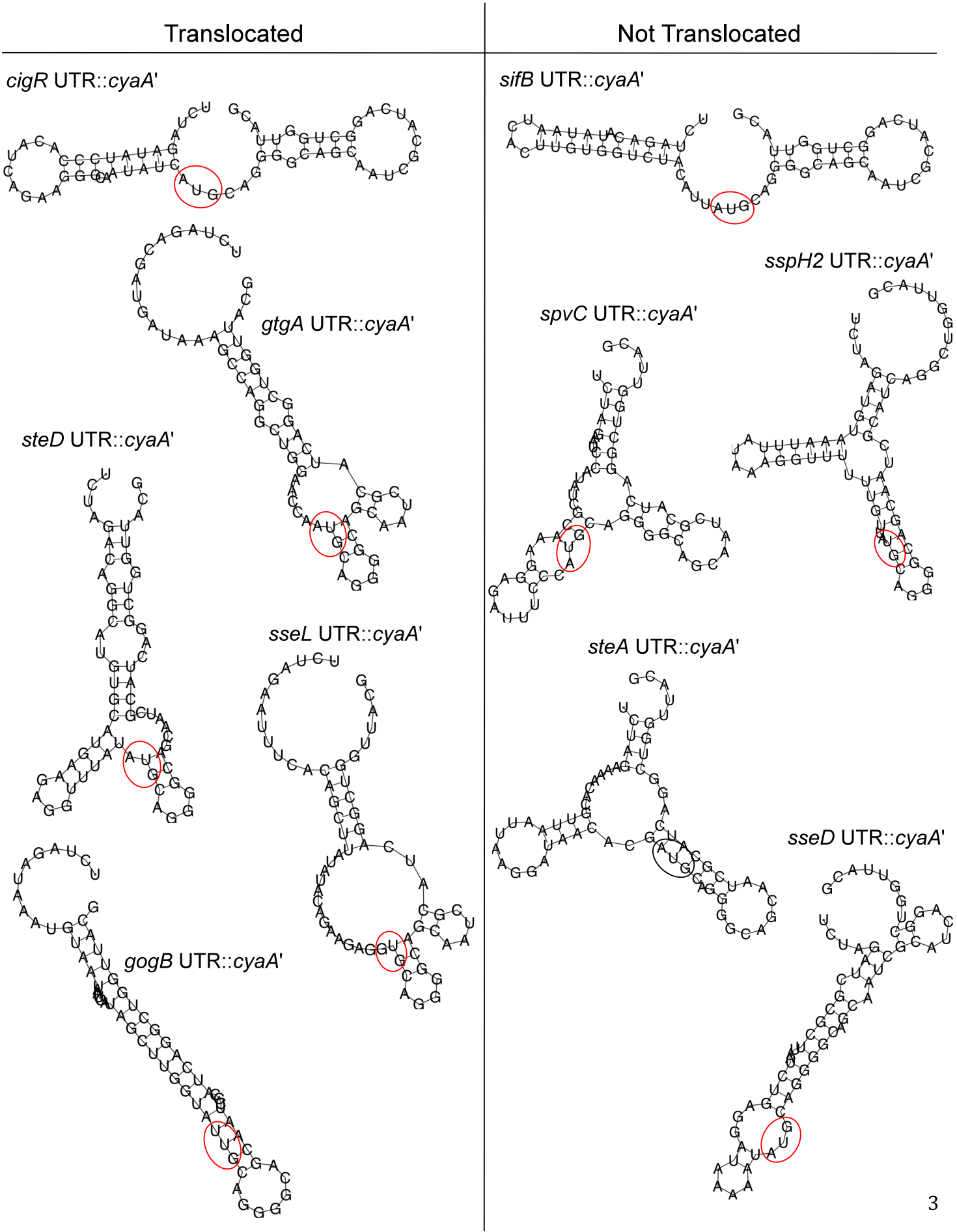


B



Supplemental Figure 1. General utility of the *gtgA* UTR as a T3S signal. (A) Translocation of enolase::CyaA'. The *gtgA* UTR was fused to enolase::*cyaA'* and tested for secretion into J774 macrophages. For comparison, the *gtgA* UTR::*cyaA'* fusion was tested in parallel. Bacteria were induced for SPI-1 and SPI-2 expression and used to infect J774 macrophages. Translocation was evaluated by cAMP ELISA. The *invA* and *ssaK* backgrounds are SPI-1 and SPI-2 functional mutants, respectively. (B) Construct expression. Bacteria from LB broth cultures were analyzed by Western blot using antibodies raised against CyaA' and DnaK to evaluate expression and loading, respectively. Approximately 10^5 bacteria were loaded per well.

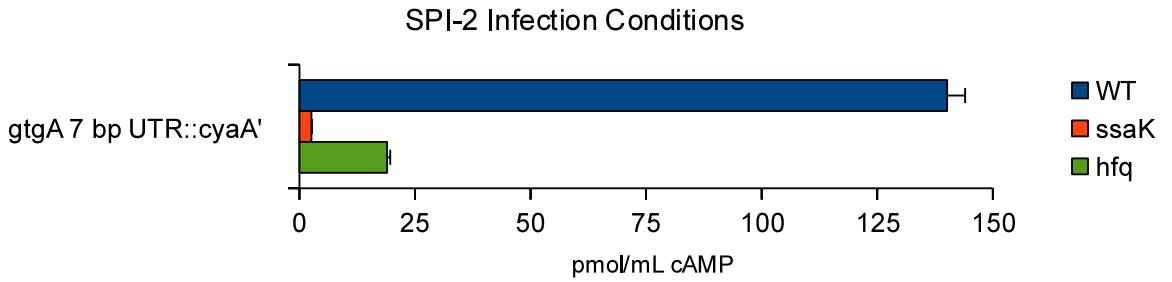
Supplemental Figure 2



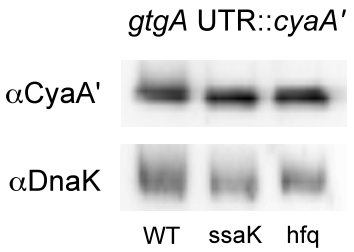
Supplemental Figure 2. RNAfold structural analysis. RNAfold structural prediction of UTR::*cyaA'* fusions. Sixty five bp nucleotides were chosen for analysis. Predicted start codons are circled. (Left) Fusions sufficient for translocation. (Right) Fusions insufficient for translocation.

Supplemental Figure 3

A



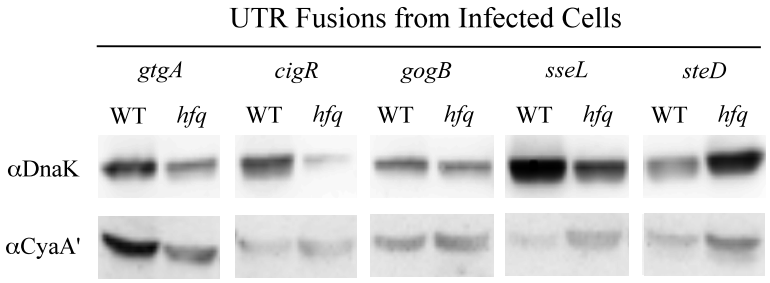
B



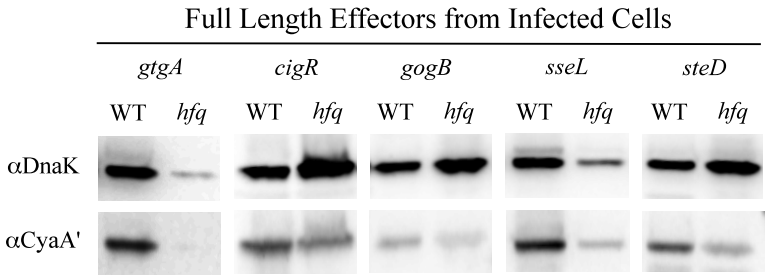
Supplemental Figure 3. Translocation of the gtgA 7 bp UTR by an *hfq* mutant. (A) Bacteria were induced for SPI-2 expression and used to infect J774 macrophages. Translocation was evaluated by cAMP ELISA. The *ssaK* background is a SPI-2 functional mutant. (B) Construct expression. Bacteria from LB broth cultures were analyzed by Western blot using antibodies raised against CyaA' and DnaK to evaluate expression and loading, respectively. Approximately 10^5 bacteria were loaded per well.

Supplemental Figure 4

A



B



Supplemental Figure 4. Protein expression from infected cells. (A) Expression of UTR fusions. J774 cells were infected with wild type and *hfq* mutant bacteria expressing various UTR::CyaA' fusions. Six to eight hours post-infection, lysates were analyzed by Western blot using anti CyaA' and DnaK antibodies. Representative Western blot shown. (B) Expression of intact effectors fused to CyaA'. Experiments performed as described above. Representative Western blots shown.

Supplemental Table 1. Strains and plasmids used in this study.

Category	Strain/Plasmid	Genotype/Description	Reference/Source
S. Typhimurium	14028	Wild type	ATCC
	MJW1301	14028 *ssaK*::*cat*	(2)
	MJW1835	14028 *invA*::*cat*	(2)
	GSN3001	14028 transformed with pGSN4001	This study
	GSN3002	MJW1301 transformed with pGSN4001	This study
	GSN3003	MJW1835 transformed with pGSN4001	This study
	GSN3004	14028 transformed with pGSN4002	This study
	GSN3005	MJW1301 transformed with pGSN4002	This study
	GSN3006	MJW1835 transformed with pGSN4002	This study
	GSN3007	14028 transformed with pGSN4003	This study
	GSN3008	MJW1301 transformed with pGSN4003	This study
	GSN3009	MJW1835 transformed with pGSN4003	This study
	GSN3010	14028 transformed with pGSN4004	This study
	GSN3011	MJW1301 transformed with pGSN4004	This study
	GSN3012	MJW1835 transformed with pGSN4004	This study
	GSN3013	14028 transformed with pGSN4005	This study
	GSN3014	MJW1301 transformed with pGSN4005	This study
	GSN3015	MJW1835 transformed with pGSN4005	This study
	GSN3016	14028 transformed with pGSN4006	This study
	GSN3017	MJW1301 transformed with pGSN4006	This study
	GSN3018	MJW1835 transformed with pGSN4006	This study
	GSN3019	14028 transformed with pGSN4007	This study
	GSN3020	MJW1301 transformed with pGSN4007	This study
	GSN3021	MJW1835 transformed with pGSN4007	This study
	GSN3022	14028 transformed with pGSN4008	This study
	GSN3023	MJW1301 transformed with pGSN408	This study
	GSN3024	MJW1835 transformed with pGSN408	This study
	GSN3025	14028 transformed with pGSN4009	This study
	GSN3026	MJW1301 transformed with pGSN4009	This study
	GSN3027	MJW1835 transformed with pGSN4009	This study
	GSN3028	14028 transformed with pGSN4010	This study
	GSN3029	MJW1301 transformed with pGSN4010	This study
	GSN3030	MJW1835 transformed with pGSN4010	This study
	GSN3031	14028 transformed with pGSN4011	This study
	GSN3032	MJW1301 transformed with pGSN4011	This study
	GSN3033	MJW1835 transformed with pGSN4011	This study
	GSN3034	14028 transformed with pGSN4012	This study
	GSN3035	MJW1301 transformed with pGSN4012	This study
	GSN3036	MJW1835 transformed with pGSN4012	This study
	GSN3037	14028 transformed with pGSN4013	This study
	GSN3038	MJW1301 transformed with pGSN4013	This study
	GSN3039	MJW1835 transformed with pGSN4013	This study

	GSN3040	14028 transformed with pGSN4014	This study
	GSN3041	MJW1301 transformed with pGSN4014	This study
	GSN3042	MJW1835 transformed with pGSN4014	This study
	GSN3043	14028 transformed with pGSN4015	This study
	GSN3044	MJW1301 transformed with pGSN4015	This study
	GSN3045	MJW1835 transformed with pGSN4015	This study
	GSN3046	14028 transformed with pGSN4016	This study
	GSN3047	MJW1301 transformed with pGSN4016	This study
	GSN3048	MJW1835 transformed with pGSN4016	This study
	GSN3049	14028 transformed with pGSN4017	This study
	GSN3050	MJW1301 transformed with pGSN4017	This study
	GSN3051	MJW1835 transformed with pGSN4017	This study
	GSN3052	14028 transformed with pGSN4018	This study
	GSN3053	MJW1301 transformed with pGSN4018	This study
	GSN3054	MJW1835 transformed with pGSN4018	This study
	GSN3055	14028 transformed with pGSN4019	This study
	GSN3056	MJW1301 transformed with pGSN4019	This study
	GSN3057	MJW1835 transformed with pGSN4019	This study
	GSN3058	14028 transformed with pGSN4020	This study
	GSN3059	MJW1301 transformed with pGSN4020	This study
	GSN3060	MJW1835 transformed with pGSN4020	This study
	GSN3061	14028 transformed with pGSN4021	This study
	GSN3062	MJW1301 transformed with pGSN4021	This study
	GSN3063	MJW1835 transformed with pGSN4021	This study
	GSN3064	14028 transformed with pGSN4022	This study
	GSN3065	MJW1301 transformed with pGSN4022	This study
	GSN3066	MJW1835 transformed with pGSN4022	This study
	GSN3067	14028 transformed with pGSN4023	This study
	GSN3068	MJW1301 transformed with pGSN4023	This study
	GSN3069	MJW1835 transformed with pGSN4023	This study
	GSN3070	14028 transformed with pGSN4024	This study
	GSN3071	MJW1301 transformed with pGSN4024	This study
	GSN3072	MJW1835 transformed with pGSN4024	This study
	GSN3073	14028 transformed with pGSN4025	This study
	GSN3074	MJW1301 transformed with pGSN4025	This study
	GSN3075	MJW1835 transformed with pGSN4025	This study
	GSN3076	14028 transformed with pGSN4026	This study
	GSN3077	MJW1301 transformed with pGSN4026	This study
	GSN3078	MJW1835 transformed with pGSN4026	This study
	GSN3079	14028 transformed with pGSN4027	This study
	GSN3080	MJW1301 transformed with pGSN4027	This study
	GSN3081	MJW1835 transformed with pGSN4027	This study
	GSN3082	14028 transformed with pGSN4028	This study
	GSN3083	MJW1301 transformed with pGSN4028	This study
	GSN3084	MJW1835 transformed with pGSN4028	This study

	GSN3085	14028 transformed with pGSN4029	This study
	GSN3086	MJW1301 transformed with pGSN4029	This study
	GSN3087	MJW1835 transformed with pGSN4029	This study
	GSN3088	14028 transformed with pGSN4030	This study
	GSN3089	MJW1301 transformed with pGSN4030	This study
	GSN3090	MJW1835 transformed with pGSN4030	This study
	GSN3091	14028 transformed with pGSN4031	This study
	GSN3092	MJW1301 transformed with pGSN4031	This study
	GSN3093	MJW1835 transformed with pGSN4031	This study
	GSN3094	14028 transformed with pGSN4032	This study
	GSN3095	MJW1301 transformed with pGSN4032	This study
	GSN3096	MJW1835 transformed with pGSN4032	This study
	GSN3097	14028 transformed with pGSN4033	This study
	GSN3098	MJW1301 transformed with pGSN4033	This study
	GSN3099	MJW1835 transformed with pGSN4033	This study
	GSN3100	14028 transformed with pGSN4034	This study
	GSN3101	MJW1301 transformed with pGSN4034	This study
	GSN3102	MJW1835 transformed with pGSN4034	This study
	GSN3103	14028 transformed with pGSN4035	This study
	GSN3104	MJW1301 transformed with pGSN4035	This study
	GSN3105	MJW1835 transformed with pGSN4035	This study
	GSN3106	14028 transformed with pGSN4036	This study
	GSN3107	MJW1301 transformed with pGSN4036	This study
	GSN3108	MJW1835 transformed with pGSN4036	This study
	GSN3109	14028 transformed with pGSN4037	This study
	GSN3110	MJW1301 transformed with pGSN4037	This study
	GSN3111	MJW1835 transformed with pGSN4037	This study
	GSN3112	14028 transformed with pGSN4038	This study
	GSN3113	MJW1301 transformed with pGSN4038	This study
	GSN3114	MJW1835 transformed with pGSN4038	This study
	GSN3115	14028 transformed with pGSN4039	This study
	GSN3116	MJW1301 transformed with pGSN4039	This study
	GSN3117	MJW1835 transformed with pGSN4039	This study
	GSN3118	14028 transformed with pGSN4040	This study
	GSN3119	MJW1301 transformed with pGSN4040	This study
	GSN3120	MJW1835 transformed with pGSN4040	This study
	GSN3121	14028 transformed with pGSN4041	This study
	GSN3122	MJW1301 transformed with pGSN4041	This study
	GSN3123	MJW1835 transformed with pGSN4041	This study
	GSN3124	14028 transformed with pGSN4042	This study
	GSN3125	MJW1301 transformed with pGSN4042	This study
	GSN3126	MJW1835 transformed with pGSN4042	This study
	GSN3127	14028 transformed with pGSN4043	This study
	GSN3128	MJW1301 transformed with pGSN4043	This study
	GSN3129	MJW1835 transformed with pGSN4043	This study

	GSN3130	14028 transformed with pGSN4044	This study
	GSN3131	MJW1301 transformed with pGSN4044	This study
	GSN3132	14028 transformed with pGSN4045	This study
	GSN3133	MJW1301 transformed with pGSN4045	This study
	GSN3134	14028 transformed with pGSN4046	This study
	GSN3135	MJW1301 transformed with pGSN4046	This study
	GSN3136	14028 transformed with pGSN4047	This study
	GSN3137	MJW1301 transformed with pGSN4047	This study
	GSN3138	14028 transformed with pGSN4048	This study
	GSN3139	MJW1301 transformed with pGSN4048	This study
	GSN3140	14028 transformed with pGSN4049	This study
	GSN3141	MJW1301 transformed with pGSN4049	This study
	GSN3142	14028 transformed with pGSN4050	This study
	GSN3143	MJW1301 transformed with pGSN4050	This study
	HY4261	14028 Δ*hfq*	(3, 4)
	GSN3144	HY4261 transformed with pGSN4004	This study
	GSN3145	HY4261 transformed with pGSN4022	This study
	GSN3146	HY4261 transformed with pGSN4023	This study
	GSN3147	HY4261 transformed with pGSN4040	This study
	GSN3148	HY4261 transformed with pGSN4043	This study
	GSN3149	14028 transformed with pGSN4051	This study
	GSN3150	MJW1301 transformed with pGSN4051	This study
	GSN3151	HY4261 transformed with pGSN4051	This study
	GSN3152	14028 transformed with pGSN4052	This study
	GSN3153	MJW1301 transformed with pGSN4052	This study
	GSN3154	MJW1835 transformed with pGSN4052	This study
	GSN3155	14028 transformed with pGSN4053	This study
	GSN3156	MJW1301 transformed with pGSN4053	This study
	GSN3157	HY4261 transformed with pGSN4053	This study
	GSN3057	14028 transformed with pGSN2014	(8)
	GSN3058	MJW1301 transformed with pGSN2014	(8)
	GSN3158	HY4261 transformed with pGSN2014	This study
	GSN3159	14028 transformed with pGSN4054	This study
	GSN3160	MJW1301 transformed with pGSN4054	This study
	GSN3161	HY4261 transformed with pGSN4054	This study
	GSN3162	14028 transformed with pGSN4055	This study
	GSN3163	MJW1301 transformed with pGSN4055	This study
	GSN3164	HY4261 transformed with pGSN4055	This study
	GSN3048	14028 transformed with pGSN2011	(8)
	GSN3049	MJW1301 transformed with pGSN2011	(8)
	GSN3165	HY4261 transformed with pGSN2011	This study
	GSN3166	HY4261 transformed with pGSN4046	This study
Plasmids	pMJW1753	CyaA' fusion plasmid	(2)
	pGSN4001	pMJW1753 R17 phage A UTR::*cyaA'*	This study
	pGSN4002	pMJW1753 *sipA* UTR::*cyaA'*	This study

	pGSN4003	pMJW1753 *steA* UTR::*cyaA'*	This study
	pGSN4004	pMJW1753 *gtgA* UTR::*cyaA'*	This study
	pGSN4005	pMJW1753 *sipB* UTR::*cyaA'*	This study
	pGSN4006	pMJW1753 *sipC* UTR::*cyaA'*	This study
	pGSN4007	pMJW1753 *sipD* UTR::*cyaA'*	This study
	pGSN4008	pMJW1753 *sopA* UTR::*cyaA'*	This study
	pGSN4009	pMJW1753 *sopB* UTR::*cyaA'*	This study
	pGSN4010	pMJW1753 *sopD* UTR::*cyaA'*	This study
	pGSN4011	pMJW1753 *sopE2* UTR::*cyaA'*	This study
	pGSN4012	pMJW1753 *avrA* UTR::*cyaA'*	This study
	pGSN4013	pMJW1753 *gtgE* UTR::*cyaA'*	This study
	pGSN4014	pMJW1753 *slrP* UTR::*cyaA'*	This study
	pGSN4015	pMJW1753 *sptP* UTR::*cyaA'*	This study
	pGSN4016	pMJW1753 *spvC* UTR::*cyaA'*	This study
	pGSN4017	pMJW1753 *spvD* UTR::*cyaA'*	This study
	pGSN4018	pMJW1753 *sseK1* UTR::*cyaA'*	This study
	pGSN4019	pMJW1753 *sspH1* UTR::*cyaA'*	This study
	pGSN4020	pMJW1753 *steB* UTR::*cyaA'*	This study
	pGSN4021	pMJW1753 *steE* UTR::*cyaA'*	This study
	pGSN4022	pMJW1753 *cigR* UTR::*cyaA'*	This study
	pGSN4023	pMJW1753 *gogB* UTR::*cyaA'*	This study
	pGSN4024	pMJW1753 *srfH* UTR::*cyaA'*	This study
	pGSN4025	pMJW1753 *pipB* UTR::*cyaA'*	This study
	pGSN4026	pMJW1753 *pipB2* UTR::*cyaA'*	This study
	pGSN4027	pMJW1753 *sifA* UTR::*cyaA'*	This study
	pGSN4028	pMJW1753 *sifB* UTR::*cyaA'*	This study
	pGSN4029	pMJW1753 *sopD2* UTR::*cyaA'*	This study
	pGSN4030	pMJW1753 *spiC* UTR::*cyaA'*	This study
	pGSN4031	pMJW1753 *spvB* UTR::*cyaA'*	This study
	pGSN4032	pMJW1753 *sseB* UTR::*cyaA'*	This study
	pGSN4033	pMJW1753 *sseC* UTR::*cyaA'*	This study
	pGSN4034	pMJW1753 *sseD* UTR::*cyaA'*	This study
	pGSN4035	pMJW1753 *sseF* UTR::*cyaA'*	This study
	pGSN4036	pMJW1753 *sseG* UTR::*cyaA'*	This study
	pGSN4037	pMJW1753 *sseJ* UTR::*cyaA'*	This study
	pGSN4038	pMJW1753 *sseK2* UTR::*cyaA'*	This study
	pGSN4039	pMJW1753 *sseK3* UTR::*cyaA'*	This study
	pGSN4040	pMJW1753 *sseL* UTR::*cyaA'*	This study
	pGSN4041	pMJW1753 *sspH2* UTR::*cyaA'*	This study
	pGSN4042	pMJW1753 *steC* UTR::*cyaA'*	This study
	pGSN4043	pMJW1753 *steD* UTR::*cyaA'*	This study
	pGSN4044	pMJW1753 *gtgA* 5 bp UTR::*cyaA'*	This study
	pGSN4045	pMJW1753 *gtgA* 6 bp UTR::*cyaA'*	This study
	pGSN4046	pMJW1753 *gtgA* 7 bp UTR::*cyaA'*	This study
	pGSN4047	pGSN4052 AGA → CGA point mutant	This study

	pGSN4048	pGSN4052 AGA → ATA point mutant	This study
	pGSN4049	pGSN4052 AGA → AGC point mutant	This study
	pGSN4050	pGSN4052 AGA → CTC point mutant	This study
	pGSN4051	pMJW1753 *spvD*$_{1\text{-}90}$::*cyaA'*	This study
	pGSN4052	pMJW1753 *gtgA* UTR::*enolase*::*cyaA'*	This study
	pGSN4053	pMJW1753 *gtgA*::*cyaA′*	This study
	pGSN2014	pMJW1753 *cigR*::*cyaA′*	(8)
	pGSN4055	pMJW1753 *gogB*::*cyaA′*	This study
	pGSN2014	pMJW1753 *sseL*::*cyaA′*	This study
	pGSN2011	pMJW1753 *steD*::*cyaA′*	(8)

Supplemental Table 2. Oligonucleotide primers used in this study.

For/Rev	5'-Sequence-3'	Description
Forward	CTGATCTAGAAGGAGGTTTGACCTATGCAGCTGGTAC	pGSN4001
Reverse	GTACCAGCTGCATAGGTCAAACCTCCTTCTAGATCAG	
Forward	CTGATCTAGAAGGATAACAGAAGAGGATATTAATAATGC AGCTGGTAC	pGSN4002
Reverse	GTACCAGCTGCATTATTAATATCCTCTTCTGTTATCCTTCT AGATCAG	
Forward	CTGATCTAGATGAGCAAAATTTGAAGGAGTAGGATATGC AGCTGGTAC	pGSN4003
Reverse	GTACCAGCTGCATATCCTACTCCTTCAAATTTTGCTCATCT AGATCAG	
Forward	CTGATCTAGACGATGATAAAGCCAGGCTGGAACCAATGC AGCTGGTAC	pGSN4004
Reverse	GTACCAGCTGCATTGGTTCCAGCCTGGCTTTATCATCGTC TAGATCAG	
Forward	CTGATCTAGAACAGTGAACAAGAAAAGGAATAATTATGC AGCTGGTAC	pGSN4005
Reverse	GTACCAGCTGCATAATTATTCCTTTTCTTGTTCACTGTTCT AGATCAG	
Forward	CTGATCTAGAACTGCCAAAATAAAGGGAGAAAAATATGC AGCTGGTAC	pGSN4006
Reverse	GTACCAGCTGCATATTTTTCTCCCTTTATTTTGGCAGTTCT AGATCAG	
Forward	CTGATCTAGACTCCTGATGGCGAACTGGGGATATTATGCA GCTGGTAC	pGSN4007
Reverse	GTACCAGCTGCATAATATCCCCAGTTCGCCATCAGGAGTC TAGATCAG	
Forward	CTGATCTAGAATAATGATGTTGATAAGGAATTCTAATGCA GCTGGTAC	pGSN4008
Reverse	GTACCAGCTGCATTAGAATTCCTTATCAACATCATTATTC TAGATCAG	
Forward	CTGATCTAGACCTATTCAGGAATATTAAAAACGCTATGCA GCTGGTAC	pGSN4009
Reverse	GTACCAGCTGCATAGCGTTTTTAATATTCCTGAATAGGTC TAGATCAG	
Forward	CTGATCTAGATAATATAAATTTGAAGGAAAATATTATGC AGCTGGTAC	pGSN4010
Reverse	GTACCAGCTGCATAATATTTTCCTTCAAATTTATATTATCT AGATCAG	
Forward	CTGATCTAGAATAGTTATCTAAAAGGAGAACTACCGTGC AGCTGGTAC	pGSN4011
Reverse	GTACCAGCTGCACGGTAGTTCTCCTTTTAGATAACTATTC TAGATCAG	
Forward	CTGATCTAGAATATATTAAATCTGAAAAGTTAAAGATGC AGCTGGTAC	pGSN4012
Reverse	GTACCAGCTGCATCTTTAACTTTTCAGATTTAATATATTCT AGATCAG	
Forward	CTGATCTAGAAAAATTACTATTCGGCGAGTATATTATGCA GCTGGTAC	pGSN4013
Reverse	GTACCAGCTGCATAATATACTCGCCGAATAGTAATTTTTC TAGATCAG	

Forward	CTGATCTAGATACGTTCAGATCAGGTAGGGAAAATATGC AGCTGGTAC	pGSN4014
Reverse	GTACCAGCTGCATATTTTCCCTACCTGATCTGAACGTATC TAGATCAG	
Forward	CTGATCTAGAGCGCTCAAAAACATACTGCAGGAATATGC AGCTGGTAC	pGSN4015
Reverse	GTACCAGCTGCATATTCCTGCAGTATGTTTTTGAGCGCTC TAGATCAG	
Forward	CTGATCTAGAATCCATATCGCAAAGGAGATTTCCCATGCA GCTGGTAC	pGSN4016
Reverse	GTACCAGCTGCATGGGAAATCTCCTTTGCGATATGGATTC TAGATCAG	
Forward	CTGATCTAGATGCATTTTATTGAGGTAGTGTAACTATGCA GCTGGTAC	pGSN4017
Reverse	GTACCAGCTGCATAGTTACACTACCTCAATAAAATGCATC TAGATCAG	
Forward	CTGATCTAGAAGTAAGTATGGAGCATTTAATTGTTATGCA GCTGGTAC	pGSN4018
Reverse	GTACCAGCTGCATAACAATTAAATGCTCCATACTTACTTC TAGATCAG	
Forward	CTGATCTAGATGTGCTGTAAATTAGGCAGTGGAATATGC AGCTGGTAC	pGSN4019
Reverse	GTACCAGCTGCATATTCCACTGCCTAATTTACAGCACATC TAGATCAG	
Forward	CTGATCTAGATGAAATCAATCTCAGGTAATAATCCATGCA GCTGGTAC	pGSN4020
Reverse	GTACCAGCTGCATGGATTATTACCTGAGATTGATTTCATC TAGATCAG	
Forward	CTGATCTAGATGTAAAAGGGTCTCCTCTTGTTGTGATGCA GCTGGTAC	pGSN4021
Reverse	GTACCAGCTGCATCACAACAAGAGGAGACCCTTTTACAT CTAGATCAG	
Forward	CTGATCTAGATATCCCACATCAGAAGGGCAATATCATGC AGCTGGTAC	pGSN4022
Reverse	GTACCAGCTGCATGATATTGCCCTTCTGATGTGGGATATC TAGATCAG	
Forward	CTGATCTAGATAAATGTAATAATGATAGCTTGGTATTGCA GCTGGTAC	pGSN4023
Reverse	GTACCAGCTGCAATACCAAGCTATCATTATTACATTTATC TAGATCAG	
Forward	CTGATCTAGATGTAAATTTATAAAGGTTTTTTGTTATGCA GCTGGTAC	pGSN4024
Reverse	GTACCAGCTGCATAACAAAAAACCTTTATAAATTTACATC TAGATCAG	
Forward	CTGATCTAGACTCACTTCCATAAGAAGGAATCAAAATGC AGCTGGTAC	pGSN4025
Reverse	GTACCAGCTGCATTTTGATTCCTTCTTATGGAAGTGAGTC TAGATCAG	
Forward	CTGATCTAGATGTTGCTGTCTCTGGGAGAAAATATATGCA GCTGGTAC	pGSN4026
Reverse	GTACCAGCTGCATATATTTTCTCCCAGAGACAGCAACATC TAGATCAG	
Forward	CTGATCTAGATTACTCCAGTATAAGTGAGATTAATATGCA GCTGGTAC	pGSN4027

Reverse	GTACCAGCTGCATATTAATCTCACTTATACTGGAGTAATC TAGATCAG	
Forward	CTGATCTAGACATATAATCACTTGTGGTCTACATTATGCA GCTGGTAC	pGSN4028
Reverse	GTACCAGCTGCATAATGTAGACCACAAGTGATTATATGTC TAGATCAG	
Forward	CTGATCTAGAGCGGTAAATAATCAAGGGAGTTATTATGC AGCTGGTAC	pGSN4029
Reverse	GTACCAGCTGCATAATAACTCCCTTGATTATTTACCGCTC TAGATCAG	
Forward	CTGATCTAGAGCGAATTTGATAGAAACTCCCATTTATGCA GCTGGTAC	pGSN4030
Reverse	GTACCAGCTGCATAAATGGGAGTTTCTATCAAATTCGCTC TAGATCAG	
Forward	CTGATCTAGAGACGGCCAGTTTCAGGAGATAGTGTATGC AGCTGGTAC	pGSN4031
Reverse	GTACCAGCTGCATACACTATCTCCTGAAACTGGCCGTCTC TAGATCAG	
Forward	CTGATCTAGAAGGAAAAACAAAAAGGTAAAGCATAATGC AGCTGGTAC	pGSN4032
Reverse	GTACCAGCTGCATTATGCTTTACCTTTTTGTTTTTCCTTCT AGATCAG	
Forward	CTGATCTAGAAGCAACTTTCTGACAGGAGCTAAAAATGC AGCTGGTAC	pGSN4033
Reverse	GTACCAGCTGCATTTTTAGCTCCTGTCAGAAAGTTGCTTC TAGATCAG	
Forward	CTGATCTAGATCGCGCTTAATCTGAGGATAAAAATATGC AGCTGGTAC	pGSN4034
Reverse	GTACCAGCTGCATATTTTTATCCTCAGATTAAGCGCGATC TAGATCAG	
Forward	CTGATCTAGATATTGCTTAAATAACAGAACGAAATATGC AGCTGGTAC	pGSN4035
Reverse	GTACCAGCTGCATATTTCGTTCTGTTATTTAAGCAATATC TAGATCAG	
Forward	CTGATCTAGACTGATCATACATCTCGGGGAGAACCATGC AGCTGGTAC	pGSN4036
Reverse	GTACCAGCTGCATGGTTCTCCCCGAGATGTATGATCAGTC TAGATCAG	
Forward	CTGATCTAGATGTTTAATAAAGTAAGGAGGACACTATGC AGCTGGTAC	pGSN4037
Reverse	GTACCAGCTGCATAGTGTCCTCCTTACTTTATTAAACATC TAGATCAG	
Forward	CTGATCTAGAACTGATAATTTAAGCGTGTAAAAATATGC AGCTGGTAC	pGSN4038
Reverse	GTACCAGCTGCATATTTTTACACGCTTAAATTATCAGTTC TAGATCAG	
Forward	CTGATCTAGAATATTAATAGCGTAAGGGTTGAAAAATGC AGCTGGTAC	pGSN4039
Reverse	GTACCAGCTGCATTTTTCAACCCTTACGCTATTAATATTCT AGATCAG	
Forward	CTGATCTAGAATTTCACAGCTTATATACAGAAGAGGTGC AGCTGGTAC	pGSN4040
Reverse	GTACCAGCTGCACCTCTTCTGTATATAAGCTGTGAAATTC TAGATCAG	

Forward	CTGATCTAGATGTAAATTTATAAAGGTTTTTTGTTATGCA GCTGGTAC	pGSN4041
Reverse	GTACCAGCTGCATAACAAAAAACCTTTATAAATTTACATC TAGATCAG	
Forward	CTGATCTAGAAATAAATTTTCAGAGGATGAGACATATGC AGCTGGTAC	pGSN4042
Reverse	GTACCAGCTGCATATGTCTCATCCTCTGAAAATTTATTTC TAGATCAG	
Forward	CTGATCTAGACAGGCATGTGCATGAAGAGGTTTATATGC AGCTGGTAC	pGSN4043
Reverse	GTACCAGCTGCATATAAACCTCTTCATGCACATGCCTGTC TAGATCAG	
Forward	CTGATCTAGAAACCAATGCAGCTGGTAC	pGSN4044
Reverse	GTACCAGCTGCATTGGTTTCTAGATCAG	
Forward	CTGATCTAGAGAACCAATGCAGCTGGTAC	pGSN4045
Reverse	GTACCAGCTGCATTGGTTCTCTAGATCAG	
Forward	CTGATCTAGAGGAACCAATGCAGCTGGTAC	pGSN4046
Reverse	GTACCAGCTGCATTGGTTCCTCTAGATCAG	
Forward	CACCGCGGTGGCGGCCGCTCTCGAGGAACCAATGCAGGG GCAGCA	pGSN4047
Reverse	TGCTGCCCCTGCATTGGTTCCTCGAGAGCGGCCGCCACCG CGGTG	
Forward	CACCGCGGTGGCGGCCGCTCTATAGGAACCAATGCAGGG GCAGCA	pGSN4048
Reverse	TGCTGCCCCTGCATTGGTTCCTATAGAGCGGCCGCCACCG CGGTG	
Forward	CACCGCGGTGGCGGCCGCTCTAGCGGAACCAATGCAGGG GCAGCA	pGSN4049
Reverse	TGCTGCCCCTGCATTGGTTCCGCTAGAGCGGCCGCCACCG CGGTG	
Forward	CACCGCGGTGGCGGCCGCTCTCTCGGAACCAATGCAGGG GCAGCA	pGSN4050
Reverse	TGCTGCCCCTGCATTGGTTCCGAGAGAGCGGCCGCCACC GCGGTG	
Forward	TAATACGACTCACTATAGGGAGAAAAAAAAAAAAAAGC CCGGATAGCTCAGTCGGTAGAGCAGCGGCCTCGACCAGA ATCATGCAAGTGCGTAAGATAGTCGCGGGTCGAGGCCGC GTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAA AAAAAAAAAATCTAGATGCATTTTATTGAGGTAGTGTAA CTATGCAGGGGCAGCAATCGC	pGSN4051
Reverse	TAATACGACTCACTATAGGGAGAAAAAAAAAAAAAAGC CCGGATAGCTCAGTCGGTAGAGCAGCGGCCTCGACCAGA ATCATGCAAGTGCGTAAGATAGTCGCGGGTCGAGGCCGC GTCCAGGGTTCAAGTCCCTGTTCGGGCGCCACTGCAGAA AAAAAAAAAATCTAGACGATGATAAAGCCAGGCTGGAA CCAATGCAGGGGCAGCAATCGC	
Forward	TAATACGACTCACTATAGGGAGA	pGSN4052
Reverse	GCGATTGCTGCCCCTG	
Forward	CCGATAAAACCGGCACGAT	tRSA::*spvD* UTR aptamer
Forward	TGACGTTGGTGAAGGTTTCG	tRSA::*gtgA* UTR aptamer
Forward	ATCCCCGCAGCCGTACTC	tRSA primers

Reverse	TCAATGTGGCGTTTTTTTCCT	
Forward	CTGATCTAGATGCATTTTATTGAGGTAGTGTAACTATGAG	*gyrB* qRT-PCR
Reverse	GTACCAGCTGCTTGACTTCATTTGAATCATTATTA	
Forward	CTGATCTAGACGATGATAAAGCCAGGCTGGAACCAATGT CCAAAATCGTTAAAGTCATC	*cyaA'* qRT-PCR
Reverse	GTACCCCGGGCGCCTGGCCTTTGATCTC	
Forward	CTGATCTAGATAATAAAAAGGATGTGTAACTCATC	pGSN4053
Reverse	GTACCAGCTGATTACTAAATTCGTAGGCGATTCTTGG	
Forward	CTGATCTAGATAAATGTAATAATGATAGCTTGGTATTGAC ATA	pGSN4054
Reverse	GTACCAGCTGACGATTTCTATTTTTAGGCTTATATTTATCC	
Forward	CTGATCTAGAATTTCACAGCTTATATACAGAAGA	pGSN4055
Reverse	GTACGATATCCTGGAGACTGTATTCATATATTTG	

Supplemental Table 3. Screen for RNA Leader Sequences Sufficient for CyaA' Secretion.

Infection Conditions	UTR	*S.* Typhimurium Background	cAMP (pmol/mL)	Standard Deviation	Secreted
SPI-1	R17 phage A	WT	0.69	0.03	No
		invA::*cat*	0.73	0.28	
	sipA	WT	1.753	0.316	No
		invA::*cat*	1.75	0.84	
	sipB	WT	1.411	0.109	No
		invA::*cat*	1.656	0.045	
	sipC	WT	1.392	0.753	No
		invA::*cat*	4.288	0.284	
	sipD	WT	0.855	0.368	No
		invA::*cat*	1.436	0.058	
	sopA	WT	0.700	0.241	No
		invA::*cat*	2.187	0.241	
	sopB	WT	1.259	0.078	No
		invA::*cat*	2.214	0.485	
	sopD	WT	3.358	0.134	No
		invA::*cat*	9.203	0.508	
	sopE2	WT	0.998	0.139	No
		invA::*cat*	1.760	0.613	
	avrA	WT	1.857	0.194	No
		invA::*cat*	2.711	0.323	
	gtgE	WT	1.086	0.336	No
		invA::*cat*	1.261	0.085	
	slrP	WT	1.461	0.442	No
		invA::*cat*	2.365	0.052	
	sptP	WT	1.144	0.139	No
		invA::*cat*	2.391	0.493	
	spvC	WT	4.536	2.343	No
		invA::*cat*	15.260	0.303	
	spvD	WT	0.775	0.234	No
		invA::*cat*	1.514	0.080	
	sseK1	WT	1.082	0.481	No
		invA::*cat*	1.429	0.135	
	sspH1	WT	0.542	0.195	No
		invA::*cat*	1.525	0.003	
	steA	WT	2.233	0.62	No
		invA::*cat*	2.767	0.09	
	steB	WT	0.911	0.192	No
		invA::*cat*	1.637	0.101	
	steE	WT	1.755	0.043	No
		invA::*cat*	3.117	0.309	
	cigR	WT	0.930	0.320	No
		invA::*cat*	1.937	0.086	
	gogB	WT	2.014	0.178	No
		invA::*cat*	2.632	0.250	
	gtgA	WT	14.290	1.607	Yes
		invA::*cat*	5.313	0.714	
	pipB	WT	0.564	0.100	No
		invA::*cat*	1.286	0.116	

	pipB2	WT	1.927	0.030	No
		invA::*cat*	2.878	0.374	
	sifA	WT	1.660	0.400	No
		invA::*cat*	1.355	0.326	
	sifB	WT	0.743	0.452	No
		invA::*cat*	1.214	0.142	
	sopD2	WT	1.004	0.279	No
		invA::*cat*	1.615	0.469	
	spiC	WT	0.922	0.285	No
		invA::*cat*	1.659	0.336	
	spvB	WT	1.469	0.037	No
		invA::*cat*	1.653	0.045	
	srfH	WT	0.952	0.11	No
		invA::*cat*	1.090	0.06	
	sseB	WT	1.289	0.576	No
		invA::*cat*	1.333	0.195	
	sseC	WT	1.710	0.388	No
		invA::*cat*	2.397	0.105	
	sseD	WT	1.935	0.603	No
		invA::*cat*	2.117	0.221	
	sseF	WT	0.905	0.078	No
		invA::*cat*	1.472	0.692	
	sseG	WT	0.988	0.061	No
		invA::*cat*	1.176	0.093	
	sseJ	WT	7.000	0.800	No
		invA::*cat*	5.548	0.810	
	sseK2	WT	1.448	0.374	No
		invA::*cat*	1.458	0.242	
	sseK3	WT	1.021	0.070	No
		invA::*cat*	1.490	0.058	
	sseL	WT	1.796	0.036	No
		invA::*cat*	1.376	0.023	
	sspH2	WT	1.634	0.181	No
		invA::*cat*	1.021	1.059	
	steC	WT	2.685	0.253	No
		invA::*cat*	2.946	0.164	
	steD	WT	4.579	0.123	No
		invA::*cat*	2.518	0.292	
SPI-2	R17 phage A	WT	0.807	0.128	No
		ssaK::*cat*	0.244	0.062	
	sipA	WT	0.435	0.087	No
		ssaK::*cat*	0.042	0.002	
	sipB	WT	1.559	0.671	No
		ssaK::*cat*	0.319	0.062	
	sipC	WT	1.022	0.150	No
		ssaK::*cat*	0.211	0.104	
	sipD	WT	0.634	0.450	No
		ssaK::*cat*	0.391	0.142	
	sopA	WT	0.574	0.080	No
		ssaK::*cat*	0.220	0.020	
	sopB	WT	1.110	0.132	No
		ssaK::*cat*	0.467	0.120	
	sopD	WT	6.336	0.431	No

		ssaK::*cat*	3.607	0.477	
	sopE2	WT	1.074	0.140	No
		ssaK::*cat*	0.286	0.038	
	avrA	WT	3.765	0.449	No
		ssaK::*cat*	0.920	0.035	
	gtgE	WT	1.445	0.080	No
		ssaK::*cat*	0.236	0.061	
	slrP	WT	6.179	2.845	No
		ssaK::*cat*	0.895	0.142	
	sptP	WT	4.512	1.069	No
		ssaK::*cat*	1.803	0.057	
	spvC	WT	4.692	0.129	No
		ssaK::*cat*	0.570	0.044	
	spvD	WT	1.103	0.233	No
		ssaK::*cat*	0.705	0.269	
	sseK1	WT	3.921	0.036	No
		ssaK::*cat*	0.251	0.000	
	sspH1	WT	0.531	0.136	No
		ssaK::*cat*	0.299	0.047	
	steA	WT	0.105	0.08	No
		ssaK::*cat*	0.063	0.02	
	steB	WT	1.475	0.539	No
		ssaK::*cat*	1.307	0.106	
	steE	WT	10.664	2.294	Weak
		ssaK::*cat*	0.362	0.027	
	cigR	WT	46.045	4.245	Yes
		ssaK::*cat*	2.805	2.805	
	gogB	WT	24.293	2.151	Yes
		ssaK::*cat*	0.435	0.156	
	gtgA	WT	136.521	4.082	Yes
		ssaK::*cat*	2.256	0.614	
	pipB	WT	0.243	0.048	No
		ssaK::*cat*	0.582	0.021	
	pipB2	WT	3.015	1.106	No
		ssaK::*cat*	0.191	0.195	
	sifA	WT	6.489	1.837	No
		ssaK::*cat*	1.116	0.157	
	sifB	WT	0.128	0.007	No
		ssaK::*cat*	0.101	0.045	
	sopD2	WT	1.107	0.072	No
		ssaK::*cat*	0.734	0.162	
	spiC	WT	0.412	0.104	No
		ssaK::*cat*	0.340	0.004	
	spvB	WT	1.481	0.206	No
		ssaK::*cat*	1.357	0.533	
	srfH	WT	0.985	0.165	No
		ssaK::*cat*	0.239	0.187	
	sseB	WT	1.376	0.270	No
		ssaK::*cat*	1.952	0.422	
	sseC	WT	1.978	0.816	No
		ssaK::*cat*	0.220	0.052	
	sseD	WT	1.680	0.285	No
		ssaK::*cat*	0.800	0.144	

	sseF	WT	0.124	0.060	No
		ssaK::*cat*	0.056	0.002	
	sseG	WT	0.522	0.337	No
		ssaK::*cat*	0.228	0.082	
	sseJ	WT	0.632	0.105	No
		ssaK::*cat*	0.220	0.056	
	sseK2	WT	1.129	0.131	No
		ssaK::*cat*	0.582	0.082	
	sseK3	WT	1.307	0.552	No
		ssaK::*cat*	0.882	0.222	
	sseL	WT	45.097	4.337	Yes
		ssaK::*cat*	0.562	0.039	
	sspH2	WT	1.681	0.087	No
		ssaK::*cat*	0.778	0.040	
	steC	WT	3.103	1.585	No
		ssaK::*cat*	2.202	0.106	
	steD	WT	23.119	0.610	Yes
		ssaK::*cat*	1.023	0.310	

Supplemental Table 4. Description of *Salmonella* effector proteins relevant to this work.

Effector	T3S Utilized	Genetic Islands	Homologues	Activity	Host cell target(s)	Infection Phenotypes	Refs.
GtgA (STM1026)	SPI-2	Gifsy-2	*S.* Typhimurium: GogA (STM2614) PipA (STM1087)			Required for full virulence	(5, 6)
CigR (STM3762)	SPI-2	SPI-3				Required for full virulence	(6, 7)
GogB (STM2584)	SPI-2	Gifsy-1	N-terminus: *Yersinia* YopM, *Shigella* IpaH$_{7.8}$/$_{9.8}$ *Salmonella* SspH1/2, SlrP C-terminus: *Yersinia* YP2634/Y1471 rabbit EPEC OrfL, EHEC 0157:H7 Z1829				(8, 9)
SseL (STM2287)	SPI-2			Cysteine protease with deubiquitinase activity	IκBα	Impairs IκBα ubiquitination and degradation but not phosphorylation, inhibits macrophage apoptosis, downregulates inflammatory responses	(10, 11)
SteD (STM2139)	SPI-2					Required for full virulence	(6)

Supplemental Table 5. LC-MS/MS Identification of CyaA' peptides.

UTR	CyaA' Peptide Identified	MS-GF Spectral Probability	PPM Mass Error	*P* Value	Peptide False Discovery Rate
gtgA	SSDWGLQAGYIPVNPNLSK	3.66E-18	-0.298	1.09E-11	0
	SSDWGLQAGYIPVNPNLSK	5.47E-18	-0.478	1.62E-11	0
	VIGNAAGIPLTADIDMFAIMPHLSNFR	1.51E-11	-1.185	4.5E-05	0
	VIGNAAGIPLTADIDMFAIMPHLSNFR	6.45E-10	-1.101	1.92E-03	0
	VIGNAAGIPLTADIDMFAIMPHLSNFR	7.42E-10	-1.439	2.20E-03	0.0011
	LVNPHSTSLIAEGVATK	2.55E-13	-2.602	7.56E-07	0
	LVNPHSTSLIAEGVATK	5.95E-13	-1.125	1.77E-06	0
	QAGLVTGMADGVVASNHAGYEQFEFR	2.04E-22	-0.709	6.07E-16	0
	RAASEATGGLDR	7.16E-12	-1.929	2.12E-05	0
	SSVTSGDSVTDYLAR	1.64E-19	-1.411	4.88E-13	0
	SSVTSGDSVTDYLAR	9.35E-19	-1.490	2.77E-12	0
	SSVTSGDSVTDYLAR	9.66E-12	-2.352	2.87E-05	0
cigR	VIGNAAGIPLTADIDMFAIMPHLSNFR	8.82E-12	-1.863	2.62E-05	0
gogB	VIGNAAGIPLTADIDMFAIMPHLSNFR	5.58E-13	-0.254	1.66E-06	0
	VIGNAAGIPLTADIDMFAIMPHLSNFR	3.82E-12	-0.593	1.14E-05	0
	LVNPHSTSLIAEGVATK	1.27E-13	-0.352	3.77E-07	0
	LVNPHSTSLIAEGVATK	1.15E-10	-0.633	3.40E-04	0
	RAASEATGGLDR	7.91E-13	1.015	2.34E-06	0
	SSVTSGDSVTDYLAR	1.44E-15	0.000	4.28E-09	0
sseL	VIGNAAGIPLTADIDMFAIMPHLSNFR	9.54E-11	-0.931	2.84E-04	0
	VIGNAAGIPLTADIDMFAIMPHLSNFR	1.30E-10	0.423	3.85E-04	0
	LVNPHSTSLIAEGVATK	1.25E-13	-1.195	3.70E-07	0
steD	SSDWGLQAGYIPVNPNLSK	1.12E-16	1.134	3.30E-10	0
	VIGNAAGIPLTADIDMFAIMPHLSNFR	3.66E-11	-0.593	1.09E-04	0
	VIGNAAGIPLTADIDMFAIMPHLSNFR	1.08E-10	0.169	3.20E-04	0.0003
	LVNPHSTSLIAEGVATK	1.31E-11	-0.774	3.88E-05	0
	SSVTSGDSVTDYLAR	1.85E-10	-1.960	5.48E-04	0
	SSVTSGDSVTDYLAR	7.30E-09	-2.431	2.14E-02	0.0149

Supplemental Table 6. LC-MS/MS Identification of RNA binding proteins.

14028 Locus	LT2 Locus	Gene	Mass (kDa)	Protein Description	Unique Peptides	Total Spectral Counts	*gtgA* RNA	*spvD* RNA
STM14_5242	STM4361	*hfq*	11.13	RNA-binding protein Hfq	3	4	4	
STM14_4121	STM3418	*rpsM*	13.16	30S ribosomal protein S13	2	4	2	2
STM14_4128	STM3425	*rplF*	18.86	50S ribosomal protein L6	2	2	2	
STM14_4142	STM3439	*rplD*	22.09	50S ribosomal protein L4	3	3	3	
STM14_4143	STM3440	*rplC*	22.25	50S ribosomal protein L3	2	6	6	
STM14_4119	STM3416	*rpsD*	23.49	30S ribosomal protein S4	4	7	7	
STM14_4987	STM4150	*rplA*	24.73	50S ribosomal protein L1	3	5	5	
STM14_4137	STM3434	*rpsC*	25.98	30S ribosomal protein S3	2	2	2	
STM14_0257	STM0216	*rpsB*	26.76	30S ribosomal protein S2	2	2	2	
STM14_4052	STM3359	*mdh*	32.48	malate dehydrogenase	2	3	2	1
STM14_2153	STM1780	*prsA*	34.22	ribose-phosphate pyrophosphokinase	4	5	4	1
STM14_4118	STM3415	*rpoA*	36.51	DNA-directed RNA polymerase α	5	12	6	6
STM14_3531	STM2928	*truD*	39.33	tRNA pseudouridine synthase D	2	2	2	
STM14_4149	STM4146	*tuf*	43.28	elongation factor Tu	6	14	9	5
STM14_0856	STM0737	*sucB*	43.86	dihydrolipoamide acetyltransferase	2	2	2	
STM14_3557	STM2952	*eno*	45.6	phosphopyruvate hydratase	3	3		3
STM14_0185	STM0154	*lpdA*	50.64	dihydrolipoamide dehydrogenase	2	2		2
STM14_4663	STM3867	*atpA*	55.11	F0F1 ATP synthase subunit alpha	3	6	1	5
STM14_5207	STM4330	*groEL*	57.29	chaperonin GroEL	11	17	1	16
STM14_1110	STM0981	*rpsA*	61.17	30S ribosomal protein S1	3	5	1	4
STM14_4440	STM3682	*selB*	68.65	selenocysteinyl-tRNA translation factor	4	5		5
STM14_0190	STM0158	*acnB*	93.53	aconitate hydratase 2/2-methylisocitrate dehydratase	3	11	4	7

Supplemental References

1. **Kim, S., N. Mischerikow, N. Bandeira, J. D. Navarro, L. Wich, S. Mohammed, A. J. R. Heck, and P. A. Pevzner**. 2010. The generating function of CID, ETD, and CID/ETD pairs of tandem mass spectra: applications to database search. Molecular & Cellular Proteomics **9**:2840-2852.
2. **Geddes, K., M. Worley, G. Niemann, and F. Heffron**. 2005. Identification of new secreted effectors in Salmonella enterica serovar Typhimurium. Infect Immun **73**:6260-6271.
3. **Ansong, C., H. Yoon, S. Porwollik, H. Mottaz-Brewer, B. O. Petritis, N. Jaitly, J. N. Adkins, M. McClelland, F. Heffron, and R. D. Smith**. 2009. Global systems-level analysis of Hfq and SmpB deletion mutants in Salmonella: implications for virulence and global protein translation. PLoS One **4**:e4809.
4. **Vogel, J., and B. F. Luisi**. 2011. Hfq and its constellation of RNA. Nat Rev Microbiol **9**:578-589.
5. **Smith, J. N., and B. M. Ahmer**. 2003. Detection of other microbial species by Salmonella: expression of the SdiA regulon. J Bacteriol **185**:1357-1366.
6. **Niemann, G. S., R. N. Brown, J. K. Gustin, A. Stufkens, A. S. Shaikh-Kidwai, J. Li, J. E. McDermott, H. M. Brewer, A. Schepmoes, R. D. Smith, J. N. Adkins, and F. Heffron**. 2011. Discovery of novel secreted virulence factors from Salmonella enterica serovar Typhimurium by proteomic analysis of culture supernatants. Infect Immun **79**:33-43.
7. **Blanc-Potard, A. B., F. Solomon, J. Kayser, and E. A. Groisman**. 1999. The SPI-3 pathogenicity island of Salmonella enterica. J Bacteriol **181**:998-1004.

8. **Coombes, B. K., M. E. Wickham, N. F. Brown, S. Lemire, L. Bossi, W. W. Hsiao, F. S. Brinkman, and B. B. Finlay**. 2005. Genetic and molecular analysis of GogB, a phage-encoded type III-secreted substrate in Salmonella enterica serovar typhimurium with autonomous expression from its associated phage. J Mol Biol **348**:817-830.

9. **Ramsden, A. E., D. W. Holden, and L. J. Mota**. 2007. Membrane dynamics and spatial distribution of Salmonella-containing vacuoles. Trends Microbiol **15**:516-524.

10. **Le Negrate, G., B. Faustin, K. Welsh, M. Loeffler, M. Krajewska, P. Hasegawa, S. Mukherjee, K. Orth, S. Krajewski, A. Godzik, D. G. Guiney, and J. C. Reed**. 2008. Salmonella secreted factor L deubiquitinase of Salmonella typhimurium inhibits NF-kappaB, suppresses IkappaBalpha ubiquitination and modulates innate immune responses. J Immunol **180**:5045-5056.

11. **Rytkonen, A., J. Poh, J. Garmendia, C. Boyle, A. Thompson, M. Liu, P. Freemont, J. C. Hinton, and D. W. Holden**. 2007. SseL, a Salmonella deubiquitinase required for macrophage killing and virulence. Proc Natl Acad Sci U S A **104**:3502-3507.