Supplemental Material

The sRNA PinT Contributes to PhoP-mediated regulation of the SPI1 T3SS in *Salmonella enterica* serovar Typhimurium

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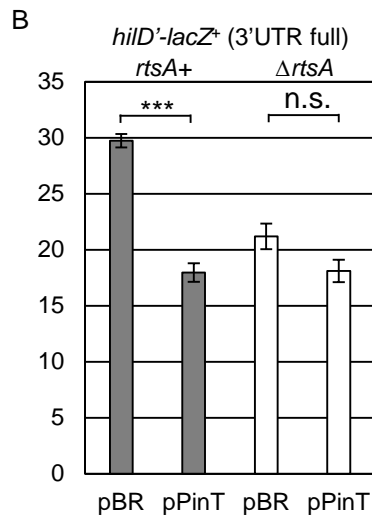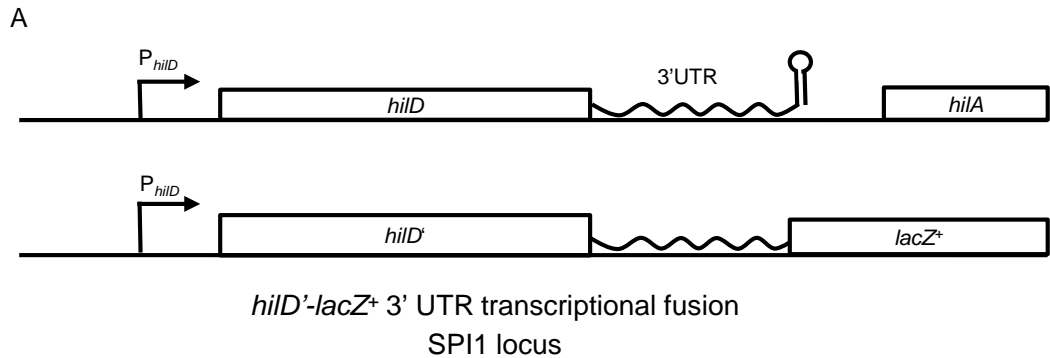#Corresponding Author. E-mail: slauch@illinois.edu

A

P$_{hilD}$

hilD

3'UTR

hilA

P$_{hilD}$

hilD'

lacZ$^+$

hilD'-lacZ$^+$ 3' UTR transcriptional fusion
SPI1 locus

B

hilD'-lacZ$^+$ (3'UTR full)

rtsA+   Δ*rtsA*

***   n.s.

pBR   pPinT   pBR   pPinT

Figure S1. PinT does not affect *hilD* expression through the 3' UTR.
A. Schematic representation of the *hilD'-lacZ$^+$* 3'UTR fusion. The *lacZ* gene is integrated just upstream of the hilD mRNA terminator. This is associated with a deletion that removes through *hilA.* B. β-galactosidase activity in *Salmonella* strains containing the *hilD'-'lacZ$^+$* transcriptional fusion in either *rtsA$^+$* (grey) or Δ*rtsA* (white) and either the empty vector or plasmids overexpressing PinT grown in SPI1 inducing conditions. β-galactosidase activity units are defined as (μmol of ONP formed min^{-1}) × 10^6/(OD$_{600}$ × ml of cell suspension) and are reported as mean ± standard deviation where n = 3. *P* values (unpaired *t* test) are indicated as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001. Strains used: JS2351 and JS2352, each with plasmid pBRplac or pPinT.
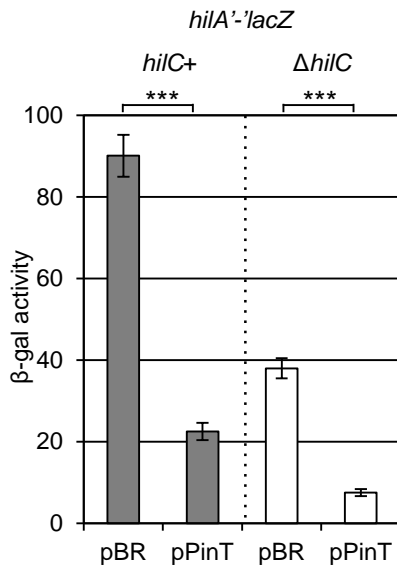
Figure S2. PinT represses *hilA* translation independent of HilC.
β-galactosidase activity in *Salmonella* strains containing the *hilA'-'lacZ* translational fusion in either *hilC*+ (grey) or Δ*hilC* (white) and either the empty vector or plasmids overexpressing PinT grown in SPI1 inducing conditions. β-galactosidase activities are reported as mean ± standard deviation where n = 3. *P* values (unpaired *t* test) are indicated as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001. Strains used: JS2333 and JS2353, each with plasmid pBRplac or pPinT.
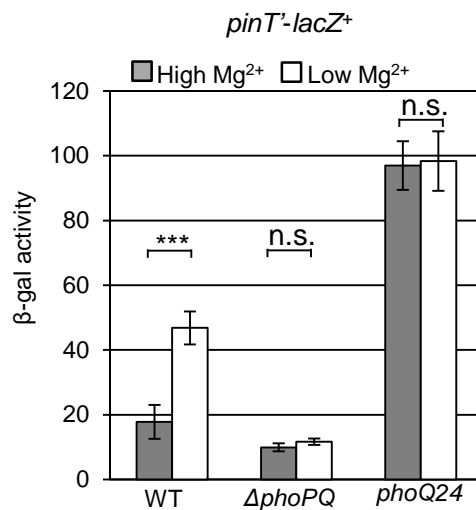
pinT'-lacZ+

Figure S3. The PhoPQ two-component system activates the expression of PinT.
β-galactosidase activity in *Salmonella* strains containing the *pinT'-lacZ+* transcriptional fusion in either wild type or the indicated mutant background. The strains were grown in either PhoPQ-noninducing (10 mM Mg^{2+}) or -inducing conditions (10 µM Mg^{2+}) in N-minimal medium. β-galactosidase activities are reported as mean ± standard deviation where n = 3. *P* values (unpaired *t* test) are indicated as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001. Strains used: JS2355, JS2357 and JS2356.
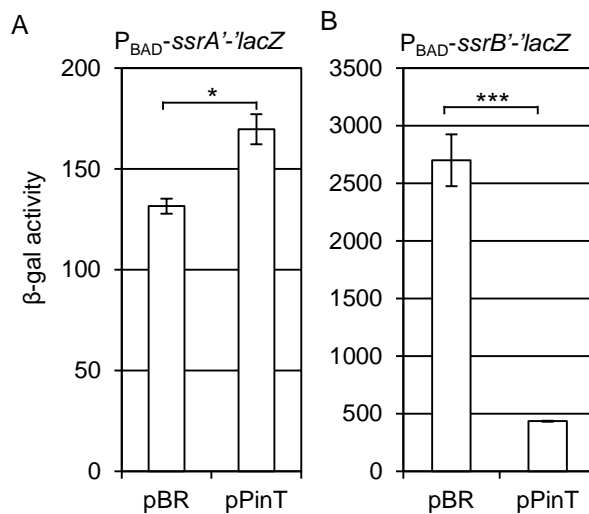
Figure S4. PinT directly regulates the expression of SPI2 T3SS through repressing *ssrB* translation.
β-galactosidase activity in *E. coli* strains containing (A) P_{BAD}-*ssrA'-'lacZ* or (B) P_{BAD}-*ssrB'-'lacZ* translational fusion and plasmids overexpressing PinT grown in the presence of 100 µM IPTG and 0.001% arabinose to induce the sRNA expression and the fusion *lacZ* protein expression, respectively. β-galactosidase activities are reported as mean ± standard deviation where n = 3. *P* values (unpaired *t* test) are indicated as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001. Strains used: JMS6508 and JMS6509, each with plasmid pBRplac or pPinT.

Table S1. Conservation of genes in the tRNA-PheR island in representative *Salmonella*.

Serovar/ Strain	% identity[a] to indicated *S*. Typhimurium 14028 gene						
	PhoN STM14_5193	RtsA STM14_5188	RtsB STM14_5187	RtsC STM14_5186	RtsD STM14_5185	PinT	STM14_5184
Typhimurium LT2	100%	100%	100%	100%	100%	100%	100%
Enteritidis BAA-708	98%	99%	99%	Pseudogene	Pseudogene	100%	100%
Gallinarum 9184	98%	99%	97%	Pseudogene	Pseudogene	---	99%
Typhi TY2	95%	100%	100%	Pseudogene	In frame insertion (61%)	100%	99%
Bongori SA19983605	---	77%	94%	---	---	---	---

[a]Identity is denoted at the amino acid level for ORFS and at the nucleotide level for PinT.

Table S2. Bacterial strains and plasmids used in this study.

Salmonella[a] or *E. coli* strain	Genotype	Deletion endpoint[b]	Source or reference
Salmonella			
14028	Wild type		ATCC[c]
PM1205	MG1655 *mal::lacI^q, ΔaraBAD araC^+, lacI'::P_{BAD}-cat-sacB:lacZ, miniλtet^R*		(1)
JS2333	ϕ(*hilA'-'lacZ)hyb116*	3040173 - 3038966	
JS892	ϕ(*hilD'-'lacZ)hyb139*		(2)
JS2334	ϕ(*rtsA'-'lacZ)hyb6*	4573742 - 4574496	
JS248	*ΔrtsA5*	4561755–4560884	(3)
JS2117	*rne131::Cm*		(4)
JS2335	ϕ(*hilA'-'lacZ)hyb116 rne131::Cm*		
JS2336	ϕ(*rtsA'-'lacZ)hyb6 rne131::Cm*		
JS2337	ϕ(*hilA'-'lacZ)hyb116 ΔrtsA5*		
JS2338	ϕ(*hilA'-'lacZ)hyb116 ΔrtsA5 rne131::Cm*		
JS325	ϕ(*rtsB'-lacZ^+)6*		(3)
JS2339	ϕ(*rtsB'-lacZ^+)6 rne131::Cm*		
JS542	*phoQ24*		(5)
JS2192	*phoQ24 ycfD612::Cm*		(6)
JS2340	*ΔpinT::tet*	4572735 - 4572805	
JS2341	ϕ(*hilA'-'lacZ)hyb116 ΔpinT::tet*		
JS2342	ϕ(*hilA'-'lacZ)hyb116 phoQ24 ycfD612::Cm*		
JS2343	ϕ(*hilA'-'lacZ)hyb116 ΔpinT::tet phoQ24 ycfD612::Cm*		

JS2344	φ(*rtsA'-'lacZ)hyb6 ΔpinT::tet*		
JS2345	φ(*rtsA'-'lacZ)hyb6 phoQ24 ycfD612::Cm*		
JS2346	φ(*rtsA'-'lacZ)hyb6 ΔpinT::tet phoQ24 ycfD612::Cm*		
TH4054	LT2 *flhC5456*::MudJ		(7)
JS2347	14028 *flhC5456*::MudJ		
JS2348	*Δcrp101::Cm*	3629505 - 3630136	
JS2349	14028 *flhC5456::MudJ Δcrp::Cm*		
JS696	φ(*fliZ'-lacZ+)8042*	1759979 - 1759907	(8)
JS746	*fliZ8041::tet*	2044136 - 2044684	(8)
JS2350	φ(*hilA'-'lacZ)hyb116 fliZ8041::tet*		
JS2351	φ(*hilD3'UTR-'lacZ+)116*	3039273 - 3041759	
JS2352	φ(*hilD-'lacZ+)3'UTR ΔrtsA5*		
JS252	*ΔhilC113::Cm*	3012135 - 3012976	(3)
JS2353	φ(*hilA'-'lacZ)hyb116 ΔhilC113::Cm*		
JS2354	*ΔpinT2::Cm*	4572745 - 4572805	
JS2355	φ(*pinT2'-lacZ+*)		
JS1068	*ΔphoPQ::cm*	1317242 - 1319310	(9)
JS2356	φ(*pinT'-lacZ+) phoQ24 ycfD612::Cm*		
JS2357	φ(*pinT'-lacZ+) ΔphoPQ::cm*		
JS749	*attλ::pDX1::hilA'-lacZ+*		(8)
JS2358	*attλ::pDX1::hilA'-lacZ+ ΔpinT::Cm*		
JS2359	*attλ::pDX1::hilA'-lacZ+ Δspi1-2916::FRT*		

Strain/Plasmid	Relevant Features		Reference
JS2360	*attλ::pDX1::hilA'-lacZ⁺ Δspi1-2916::FRT ΔpinT::Cm*		
E. coli			
JMS6503	PM1205 *lacI'::P$_{BAD}$-hilC'-'lacZ*		(4)
JMS6504	PM1205 *lacI'::P$_{BAD}$-rtsA'-'lacZ*		(4)
JMS6505	PM1205 *lacI'::P$_{BAD}$-hilA'-'lacZ*		(4)
JMS6506	PM1205 *lacI'::P$_{BAD}$-hilAmt1'-'lacZ*		
JMS6507	PM1205 *lacI'::P$_{BAD}$-rtsAmt2'-'lacZ*		
JMS6508	PM1205 *lacI'::P$_{BAD}$-ssrA'-'lacZ*		
JMS6509	PM1205 *lacI'::P$_{BAD}$-ssrB'-'lacZ*		
Plasmid	**Relevant Features**		**Reference**
pBRplac	Amp^R, p*lac* promoter based expression vector		(10)
pPinT	AatII-EcoRI pinT(*Salmonella*) containing fragment cloned into pBRplac		
pPinT-mt1	G7C, G8C, A9T, T10A, T11A, A12T site directed mutation in pPinT		
pPinT-mt2	G19T, G20T, T21A, G22C, T23A site directed mutation in pPinT		

a All *Salmonella* strains are isogenic derivatives of *S. enterica* serovar Typhimurium strain 14028.

b Numbers indicate the base pairs that are deleted (inclusive) as defined in the *S. enterica* serovar Typhimurium 14028 genome sequence (National Center for Biotechnology Information).

c ATCC, American Type Culture Collection.

Table S3. Primers used in this study.

Name	Primer Sequence
F-AatII-PinT	5'GATCGACGTC AGTAACGGATTACTTTGTGGTGTAG3'
R-EcoRI-PinT	5'GATCGAATTC TGTTAATTATTACAGAGAGAGTTAATTTAT3'
mt1-PinT-F	5'GACGTCAGTAACCCTAATCTTTGTGGTGTAGCGTAACGGTAATTGTCCTCC3'
mt1-PinT-R	5'GGAGGACAATTACCGTTACGCTACACCACAAAGATTAGGGTTACTGACGTC3'
mt2-PinT-F	5'GACGTCAGTAACGGATTACTTTGTTTACAAGCGTAACGGTAATTGTCCTC3'
mt2-PinT-R	5'GAGGACAATTACCGTTACGCTTGTAAACAAAGTAATCCGTTACTGACGTC3'
F-hilA'-'lacZ E. coli	5'ACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATAAAACTAATCTCTATTGCAATGAGG3'
R-hilAmt1'-'lacZ E. coli	5'GTTTTCCCAGTCACGACGTTGTAAAACGACCGATACAGGAACACCTAATAAATGTGGCAT3'
F-rtsA'-'lacZ E. coli	5'TTTTATCGCAACTCTCTACTGTTTCTCCATAGAAATGCAATATAAAATAGCATTTTCCAT3'
R-rtsAmt2'-'lacZ E. coli	5'CCCAGTCACGACGTTGTAAAACGACGACAGGTGAGGGATTAAATACTTTTAGCATGATAATTTCCTTTTATTACCACAGC3'
KO-pinT-TC-F	5'CATTGTTGGGGATATTTATGTTTTACTTACCTCAGTAACGGATCCCTCTTGGGTTATCA3'
KO-pinT-TC-R	5'TGTTAATTATTACAGAGAGAGTTAATTTATAAAAAAAAGCTAGGTGGGTACGTTGGAGCC3'
KO-crp-F	5'ATGGTGCTTGGCAAACCGCAAACAGACCCGACTCTTGAATGGTTCTGTAGGCTGGAGCTG3'
KO-crp-R	5'TTAACGGGTGCCGTAGACGACGATGGTCTTGCC CATATGAATATCCTC3'
KO-hilD3-F	5'TGCCTTATTCACAGCGTAAGAATTCGTCCAGATGACACTATCTCCTGTAGGCTGGAGCTG3'
KO-hilD3-R	5'CAACCAGATTACGATGATAAAAAAATAATGCATATCTCCTCTCTCCATATGAATATCCTC3'
KO-pinT-F	5'TTCATTGTTGGGGATATTTATGTTTTACTTACCTCAGTAACGGATTGTAGGCTGGAGCTG3'
KO-pinT-R	5'TTGTCTGTTAATTATTACAGAGAGAGTTAATTTATAAAAAAAAGCCATATGAATATCCTC3'
R-ssrB'-'lacZ E. coli	5'TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACTTTAAAATGAGGCCAGGGTA3'
F-ssrB'-'lacZ E. coli	5'ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATGGTATGCTATGTCATAGACA3'
R-ssrA'-'lacZ E. coli	5'TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCCAAATAATTATTGTTGTTA3'
F-ssrA'-'lacZ E. coli	5'ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACATCGCCATCTTATTAAAA3'

Reference

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