Supplemental Table 1. Plasmids and oligonucleotides used. Plasmids:

TTAACGGATCCTCTAGACGCG

Name	Purpose/modification	Source
pBAD2-bgaB-htrAp3	pBAD2-bgaB driven by htrAp3; Apr	Klinkert, et al. 2012
pBAD2-bgaB-htrAp3-∆C	pBAD2- <i>bgaB</i> driven by <i>htrAp3</i> -ΔC; Ap ^r	Klinkert, et al. 2012
pBAD2-bgaB-htrAp3-U25C	pBAD2-bgaB driven by htrAp3-U25C; Apr	This work
pUC19	Backbone for full-length <i>htrA</i> SHAPE template	New England Biolabs
htrAp1-cass1-pUC19	Production of full-length htrA SHAPE template	This work

Primers and DNA oligonucleotides:

Name	Purpose	Sequence
222	5' end of template for <i>htrA</i> RNA in SHAPE cassette, top strand	5 ⁻ -TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTTGCGTTACCTGTTAATCGAGATTGAAAC
223	3' end of template for <i>htrA</i> RNA in SHAPE cassette, bottom strand	5'-GAACCGGACCGAAGCCCGATTTGGCTTCGGCGAACC GAAGCTTTTTCATGTGTTTCAATCTCGATTAACAGG
224	5' end of template for ΔC <i>htrA</i> RNA in SHAPE cassette, top strand	5'-TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTTGGTTACCTGTTAATCGAGATTGAAAC
225	Reverse primer for SHAPE template	5'-GAACCGGACCGAAGCCCG
225-6FAM	SHAPE reaction primer, 5' 6-FAM label	5'-6-FAM-GAACCGGACCGAAGCCCG
225-VIC	SHAPE sequencing primer, 5' VIC label	5'-VIC-GAACCGGACCGAAGCCCG
226	Forward primer for SHAPE template	5'-TTCTAATACGACTCACTATAGGCCTTCGGG
272	3' end of template for <i>htrA</i> RNA in alternate SHAPE cassette, bottom strand	5'-GAACCGGACCGAAGCCCGATTTGGATCCGGCGAACC GGATCGATTTCATGTGTTTCAATCTCGATTAACAGG
319	Forward primer for creation of U25C mutation in <i>bgaB</i> plasmid	5'- ACAGCAATTTCGCGTTACCTG
320	Reverse primer for creation of U25C mutation in <i>bgaB</i> plasmid	5'- GCTAGCGAGAAACAGTAG
323	5' end of template for U25C <i>htrA</i> RNA in SHAPE cassette, top strand	5'- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTCGCGTTACCTGTTAATCGAGATTGAAAC
324	5' end of template for AAAAA <i>htrA</i> RNA in SHAPE cassette, top strand	5'- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAAAAAAACGTTACCTGTTAATCGAGATTGAAAC
341	5' end of template for U23C <i>htrA</i> RNA in SHAPE cassette, top strand	5'- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATCTTGCGTTACCTGTTAATCGAGATTGAAAC
342	5' end of template for G28A <i>htrA</i> RNA in SHAPE cassette, top strand	5'- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTTGCATTACCTGTTAATCGAGATTGAAAC
348	5' end of template for U40C <i>htrA</i> RNA in SHAPE cassette, top strand	5'- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTTGCGTTACCTGTTAACCGAGATTGAAAC
349	3' end of template for U40C <i>htrA</i> RNA in SHAPE cassette, bottom strand	5'-GAACCGGACCGAAGCCCGATTTGGCTTCGGCGAACC GAAGCTTTTTCATGTGTTTCAATCTCGGTTAACAGG
275	gBlock dsDNA used for <i>htrAp1</i> RNA in S 5'-GATGCCGGGAATTCTAATACG TTATATCAGCGGGATGACTGACC ACAGTAAATGGACTTTTGTAAAG GAACTTCGCGTTATAAAATGAAT TTGAAACACATGAAAAAAGCTTCG	SHAPE cassette ACTCACTATAGGCCTTCGGGCCAAGTCGGTTGATTCAGGA TTTACGCATGGGATGAATATCGGCGTTTGATGGCGGTCGA ATGGACAATAAATTTTTACTTTTTCCAGAAACTTTGTTCCG CTGACGTACACAGCAATTTTGCGTTACCTGTTAATCGAGA GTTCGCCGAAGCCAAATCGGGCTTCGGTCCGGTTCGCGAG

	S Enteritio	dis	str. 1	P125109	9	ACAGC	AATTTT	GCGTT	ACCTGT	TAATC	'GAGA'	TTGAAA	CACATGA	AA
	S Typhimur:	ium	str. 3	LT2		ACAGC	AATTTT	GCGTT	ACCTGT	TAATC	'GAGA'	TTGAAA	CACATGA	AA
	S Typhi st	r. (CT18			ACAGC	AATTTT	GCGTT	ACCTGT	ТААТС	GAGA'	TTGAAA	CACATGA	AA
	S Paratyph:	i A	str. i	ATCC 91	L50	ACAGC	AATTTT	GCGTTA	ACCTGT	TAATC	'GAGA'	TTGAAA	CACATGA	AA
	S Paratyph	i B	str. 3	SPB7		ACAGC	ΔρͲͲͲͲ	GCGTT	ACCTGT	ТААТС	'GAGA'	TTGAAA	CACATGA	AA
	S Paratyph	 i C	str 1	RKS4594	1	ACAGC	ልልጥጥጥጥ	GCGTT		 	GAGA		CATGA	ΔΔ
	S_diarizon:		r + r = 1	1 - 01853	2	ACACC	. <u>.</u>	CCCTT			GAGA			7 7 7 7
						ACAGO								<u>7</u> 7
	s_arizonae	sei	2. 62:	24,223		ACAGU	AATTTTT	GCGTTA	ACCIGI	TAATC	GAGA	ITGAAA	LACATGA	AA
	S bongori s	str.	NCTC	12419		ACAGC	AATTTT	GCGTT	ACCTGT	TAATI	GAGA	CTGAAA	CACATGA	AA
	E coli K12	stı	с. к-12	2, MG10	655	ACAGC	AATTTT	GCGTT	ATCTGT	TAATC	GAGA	CTGAAA	TACATGA	AA
	E coli 015	7 н7	/ str.	EDL933	3	ACAGC	AATTTT	GCGTT	ATCTGT	TAATC	GAGA	CTGAAA	TACATGA	AA
		_								-			start	
							fourU		_		SD		codon	
							stem	1	loop	5	stem			
			htrAp1											
	ORatavitidia	1			י א רח ה א ר		CC3 003 0			CCC3 00	7 7 M 7 M 7		23 mccoccm	0011
	STyphimurium	1	GICGGII	IGATICAGO IGATTCAGO	3AIIA. 3ATTA:	TATCAGCG	GGATGAC	TGACCII	TACGCAT	GGGAIG GGGATG	AAIAIC	GGCGIII GGCGTTT	SATGGCGGT	CGAA
	STyphi	1	GTCGGTT	GATTCAG	3011111. 3077701	TATCAGCG	GGATGAC	TGACCTT	TACGCAT	GGGATG	AATATC	CGCCGTTT	GATGGCGGT	CGAA
	SParatvphiB	1	GTCGGTT	GATTCAG	3ATTA'	TATCAGCG	GGATGAC'	TGACCTT	TACGCAT	GGGATG	AATATC	CGGCGTTT	GATGGCGGT	CGAA
	Ecoli K-12	1	GCCGCCI	GCTGCAG	GATTA	TATCAGCG	GTATGAC	CGACCTC	TATGCGT	GGGATG	AATACC	GACGTCT	GATGGCCGT	AGAA
	SEnteritidis	81	CAGTAAA	ATGGACTTT	TGTA	AAGATGGA	CAATAAA	TTTTTAC	TTTTTCC	AGAAAC	TTTGTI	CCGGAAC	ITCGCGTTA	TAAA
	STyphimurium	81	CAGTAAA	ATGGACTTI	TGTA	AAGATGGA	CAATAAA	TTTTTAC	TTTTTTCC	AGAAAC	TTTATT	CCGGAAC	ITCGCGTTA	TAAA
	Styphi	81	CAGTAAA	A'I'GGAC'I''I''	l'TGTA	AAGA'I'GGA	CAA'I'AAA'		TTTTTCC	AGAAAC	TTTATT	'CCGGAAC'	I'TCGCGTTA	'I'AAA
	SParatyphiB	81	CAGTAAA	ATGGACTTI	L'IGTAA	AAGA'I'GGA				AGAAAC	TTTGTT	CCGGAAC'	PTCGCGTTA	'I'AAA
	ECOIL_K-12	81	CAATAAC	CAGGCTTT	L'IGTAA	AAGACGAA	CAATAAA	ITTTTTAC	CITILGC	AGAAAC	TTTAGI	TCGGAAC	ITCAGGCTA	TAAA
				htrAp2	htrAp.	3	thermon	neter		htr2	4 start c	odon		
	SEnteritidis	161	ATGAATC	CTGACGTAC	CACAG	CAATTTTG	CGTTACC	IGTTAAT	CGAGATT	GAAACA	CATGAA	AAAAACC		
	STyphimurium	161	ATGAATC	CTGACGTAC	CACAG	CAATTTTG	CGTTACC	IGTTAAT	CGAGATT	GAAACA	CATGAA	AAAAACC		
	STyphi	161	ATGAATC	CTGACGTAC	CACAG	CAATTTTG	CGTTACC	IGTTAAT	CGAGATT	GAAACA	CATGAA	AAAAACC		
	SParatyphiB	161	ATGAATC	CTGACGTAC	CACAG	CAATTTTG	CGTTACC	IGTTAAT	CGAGATT	GAAACA	CATGAA	AAAAACC		
	Deal: V 10	161	ACCAATC		TACAC	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	COMMANC		CCACACT	<u> </u>	CATCA7	77777CC		

А

B

Supplemental Figure 1. The *htrA* thermometer and upstream sequence is conserved across *Salmonella enterica*, but differs in more diverse prokaryotes. A. Alignment of the *htrA* thermometer sequence from diverse strains of *S. enterica* with the corresponding sequence from *S. bongori* and *E. coli*. The thermometer sequence is absolutely conserved in the *S. enterica* strains. Sequence differences in *S. bongori* and *E. coli* would be predicted to destabilize the hairpin significantly, as they disrupt a base pair observed in the *S. enterica htrA* hairpin. Sequences were retrieved from GenBank. *S. enterica* subsp. *enterica* serovars include: *S.* Enteritidis str. P125109 (NC_011294.1), *S.* Typhimurium str. LT2 (NC_003197.2), *S.* Typhi str. CT18 (NC_003198.1), *S.* Paratyphi A str. ATCC 9150 (NC_006511.1), *S.* Paratyphi B str. SPB7 (CP000886.1), and *S.* Paratyphi C str. RKS4594 (NC_012125.1). Other *S. enterica* subspecies include: *S. enterica* subsp. *diarizonae* str. 11-01853 (CP011289.1) and *S. enterica* subsp. *arizonae* serovar 62:z4,z23:-- (CP000880.1). The *S. bongori* sequence comes from NCTC 12419, culture collection SGSC SARC11 (FR877557.1), while the *E. coli* sequences come from str. K-12 MG1655 (U00096.3) and O157:H7 str. EDL933 (AE005174.2). Strains for the alignment were chosen based on the phylogenetic trees from Desai PT, Porwollik S, Long F, Cheng P, Wollam A, Clifton S, Weinstock GM, McClelland M. 2013. Evolutionary genomics of the *Salmonella enterica* subspecies. mBio 4(2):e00579-12.

B. Alignment of the extended region upstream of the *htrA* coding region of several *S. enterica* strains with that of *E. coli*. Transcription start sites are noted, with the two upstream start sites in the coding region of the gene *dgt* (stop codon marked). The *S.* Enteritidis sequence was used for the extended sequence in Figure 5.



Supplemental Figure 2. **Trends in reactivity with respect to temperature are independent of the structural cassette or of the SHAPE reagent used. A.** Position of the nucleotides A38 and A50, shown on the secondary structure of the *htrA* thermometer. **B**. SHAPE results for A38 and A50. A38 is part of the upper stem of the thermometer hairpin, and it is thus predicted to have increased reactivity at elevated temperatures. A50 is predicted to be unpaired at all temperatures. The standard cassette (StC) is the sequence used throughout the rest of the work. Alternate cassette (AltC, shown in blue) used a different sequence in the first hairpin of the 3' end of the structure cassette. Experiments testing the effect of using the SHAPE reagent 1M7 are shown in red. Error bars represent the standard deviation of two to six experiments.



Supplemental Figure 3. Representative SHAPE melting curves, with error shown. A. Position of the nucleotides A38 and A45, shown on the secondary structure of the *htrA* thermometer. **B., C.** SHAPE melting curves for A38 (**B**) and A45 (**C**) for the wild-type thermometer (blue circles) and for the Δ C mutant (orange squares). Error bars represent the standard deviation of two to six experiments.



Supplemental Figure 4. SHAPE data for the *htrA* stem mutants at 30 °C and 42 °C. Average values for the wild-type *htrA* thermometer and the Δ C control are shown in panels A and B, respectively, for comparison, for comparison with results from the AAAAA mutant (C), U23C (D), U25C (E), U40C (F), and G28A (G). Positions of mutation(s) have been indicated with an asterisk (for the Δ C control, the asterisk marks the site of the missing nucleotide). Coloring as in Figure 2. Positions for which SHAPE values could not be determined due to high background signal are shown in grey. Values are the average of several independent experiments, as marked, with standard deviation indicated with error bars. Negative values are set to zero.