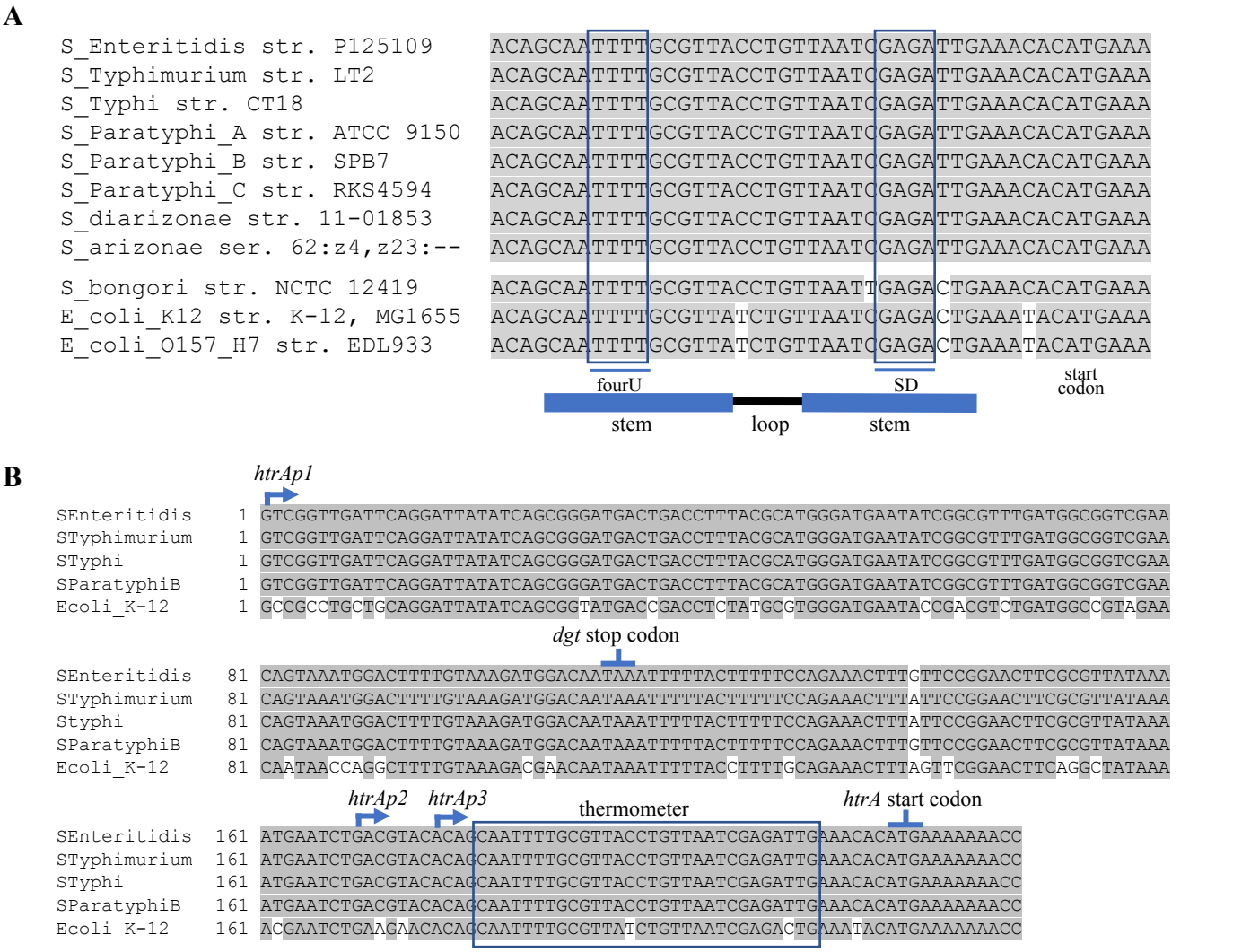
Supplemental Table 1. Plasmids and oligonucleotides used.

Plasmids:

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

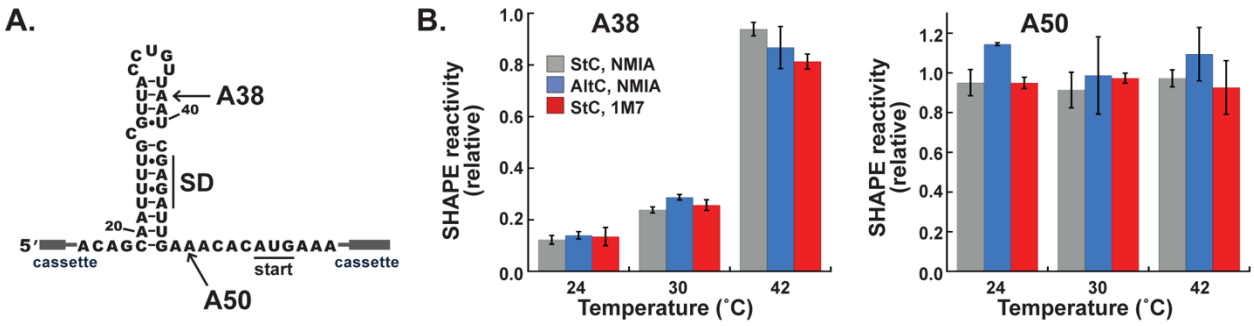
Primers and DNA oligonucleotides:

Name	Purpose	Sequence
222	5′ end of template for *htrA* RNA in SHAPE cassette, top strand	5′-TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTTGCGTTACCTGTTAATCGAGATTGAAAC
223	3′ end of template for *htrA* RNA in SHAPE cassette, bottom strand	5′-GAACCGGACCGAAGCCCGATTTGGCTTCGGCGAACC GAAGCTTTTTCATGTGTTTCAATCTCGATTAACAGG
224	5′ end of template for ΔC *htrA* 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 *htrA* RNA in alternate SHAPE cassette, bottom strand	5′-GAACCGGACCGAAGCCCGATTTGGATCCGGCGAACC GGATCGATTTCATGTGTTTCAATCTCGATTAACAGG
319	Forward primer for creation of U25C mutation in *bgaB* plasmid	5′- ACAGCAATTTCGCGTTACCTG
320	Reverse primer for creation of U25C mutation in *bgaB* plasmid	5′- GCTAGCGAGAAACAGTAG
323	5′ end of template for U25C *htrA* RNA in SHAPE cassette, top strand	5′- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTCGCGTTACCTGTTAATCGAGATTGAAAC
324	5′ end of template for AAAAA *htrA* RNA in SHAPE cassette, top strand	5′- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAAAAAAACGTTACCTGTTAATCGAGATTGAAAC
341	5′ end of template for U23C *htrA* RNA in SHAPE cassette, top strand	5′- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATCTTGCGTTACCTGTTAATCGAGATTGAAAC
342	5′ end of template for G28A *htrA* RNA in SHAPE cassette, top strand	5′- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTTGCATTACCTGTTAATCGAGATTGAAAC
348	5′ end of template for U40C *htrA* RNA in SHAPE cassette, top strand	5′- TTCTAATACGACTCACTATAGGCCTTCGGGCCAAAC AGCAATTTTGCGTTACCTGTTAACCGAGATTGAAAC
349	3′ end of template for U40C *htrA* RNA in SHAPE cassette, bottom strand	5′-GAACCGGACCGAAGCCCGATTTGGCTTCGGCGAACC GAAGCTTTTTCATGTGTTTCAATCTCGGTTAACAGG
275	gBlock dsDNA used for *htrAp1* RNA in SHAPE cassette	5′-GATGCCGGGAATTCTAATACGACTCACTATAGGCCTTCGGGCCAAGTCGGTTGATTCAGGA TTATATCAGCGGGATGACTGACCTTTACGCATGGGATGAATATCGGCGTTTGATGGCGGTCGA ACAGTAAATGGACTTTTGTAAAGATGGACAATAAATTTTTACTTTTTCCAGAAACTTTGTTCCG GAACTTCGCGTTATAAAATGAATCTGACGTACACAGCAATTTTGCGTTACCTGTTAATCGAGA TTGAAACACATGAAAAAGCTTCGGTTCGCCGAAGCCAAATCGGGCTTCGGTCCGGTTCGCGAG TTAACGGATCCTCTAGACGCG

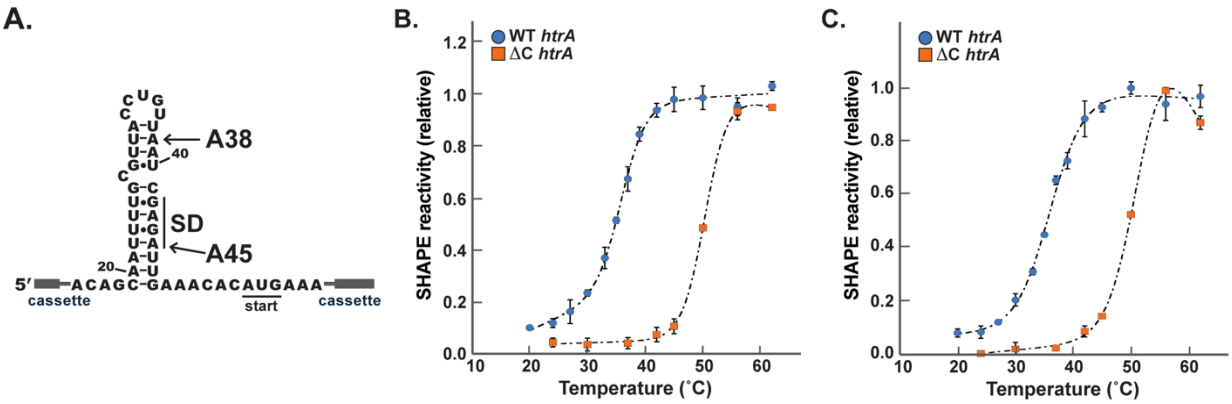


Supplemental Figure 1. The *htrA* thermometer and upstream sequence is conserved across *Salmonella enterica*, but differs in more diverse prokaryotes. A. Alignment of the *htrA* thermometer sequence from diverse strains of *S. enterica* with the corresponding sequence from *S. bongori* and *E. coli*. 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. doi:10.1128/mBio.00579-12.

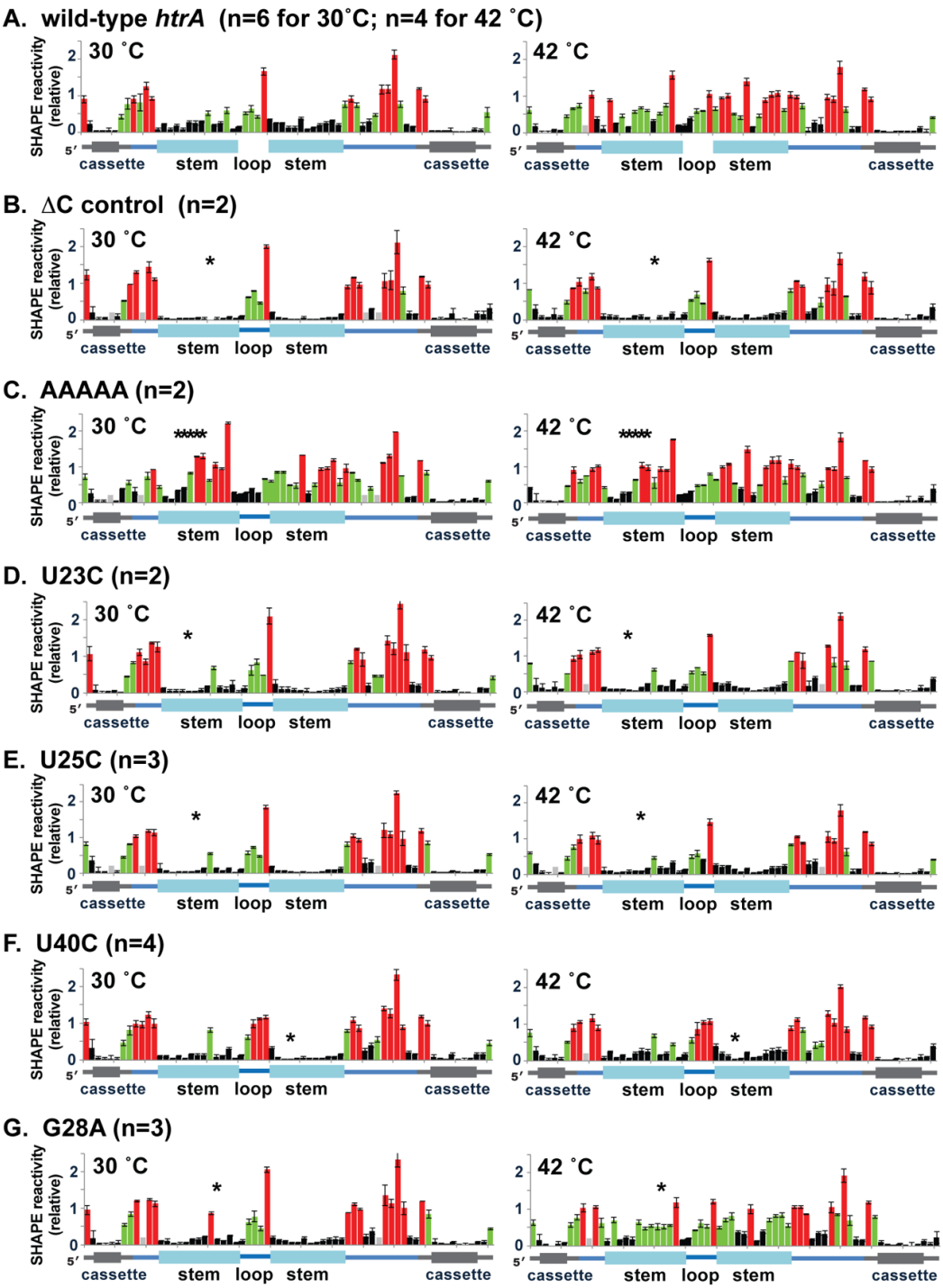
B. Alignment of the extended region upstream of the *htrA* coding region of several *S. enterica* strains with that of *E. coli*. Transcription start sites are noted, with the two upstream start sites in the coding region of the gene *dgt* (stop codon marked). The *S.* Enteritidis sequence was used for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