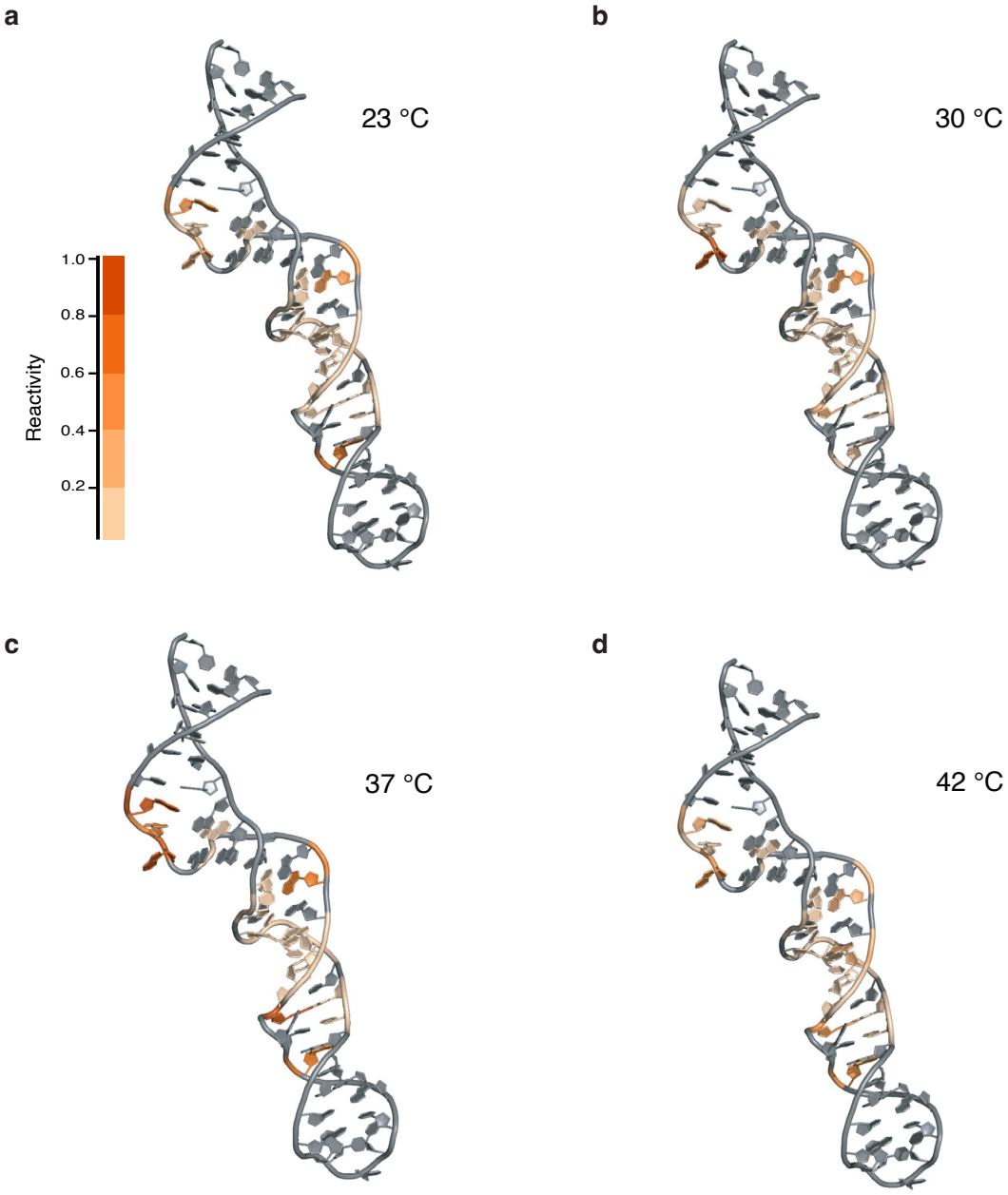
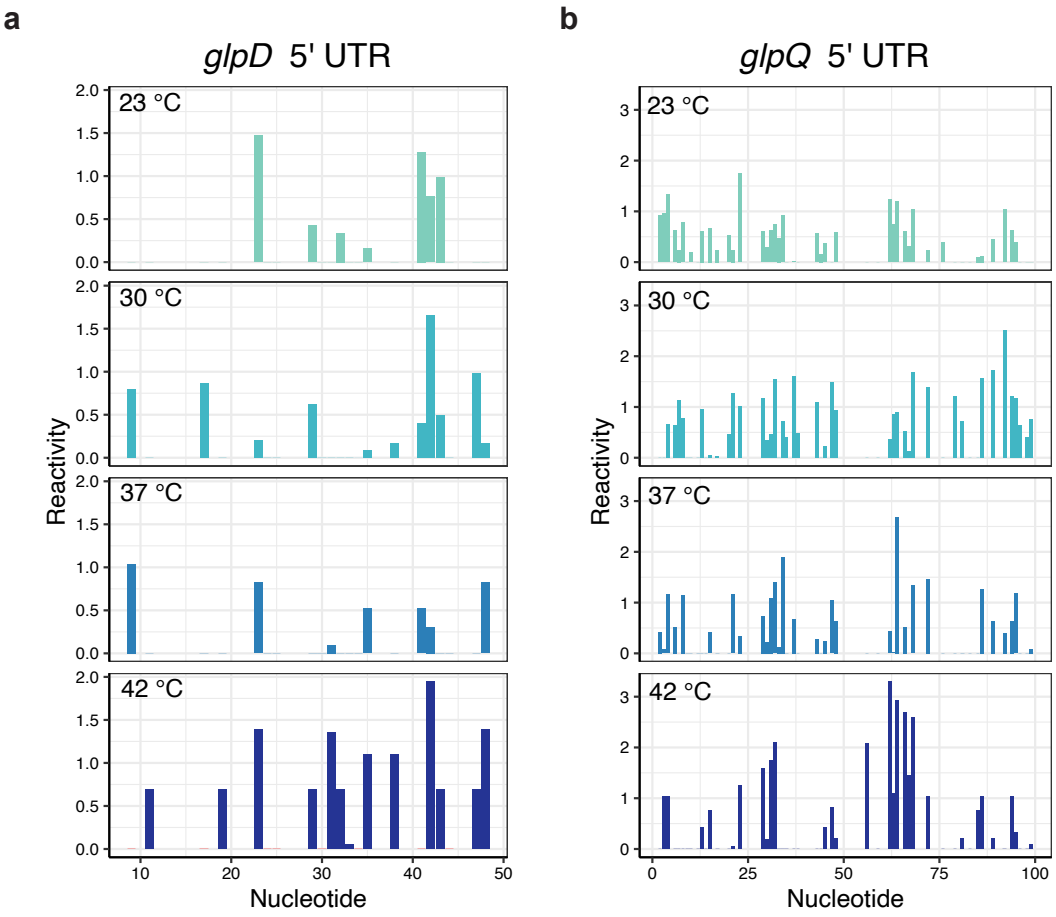
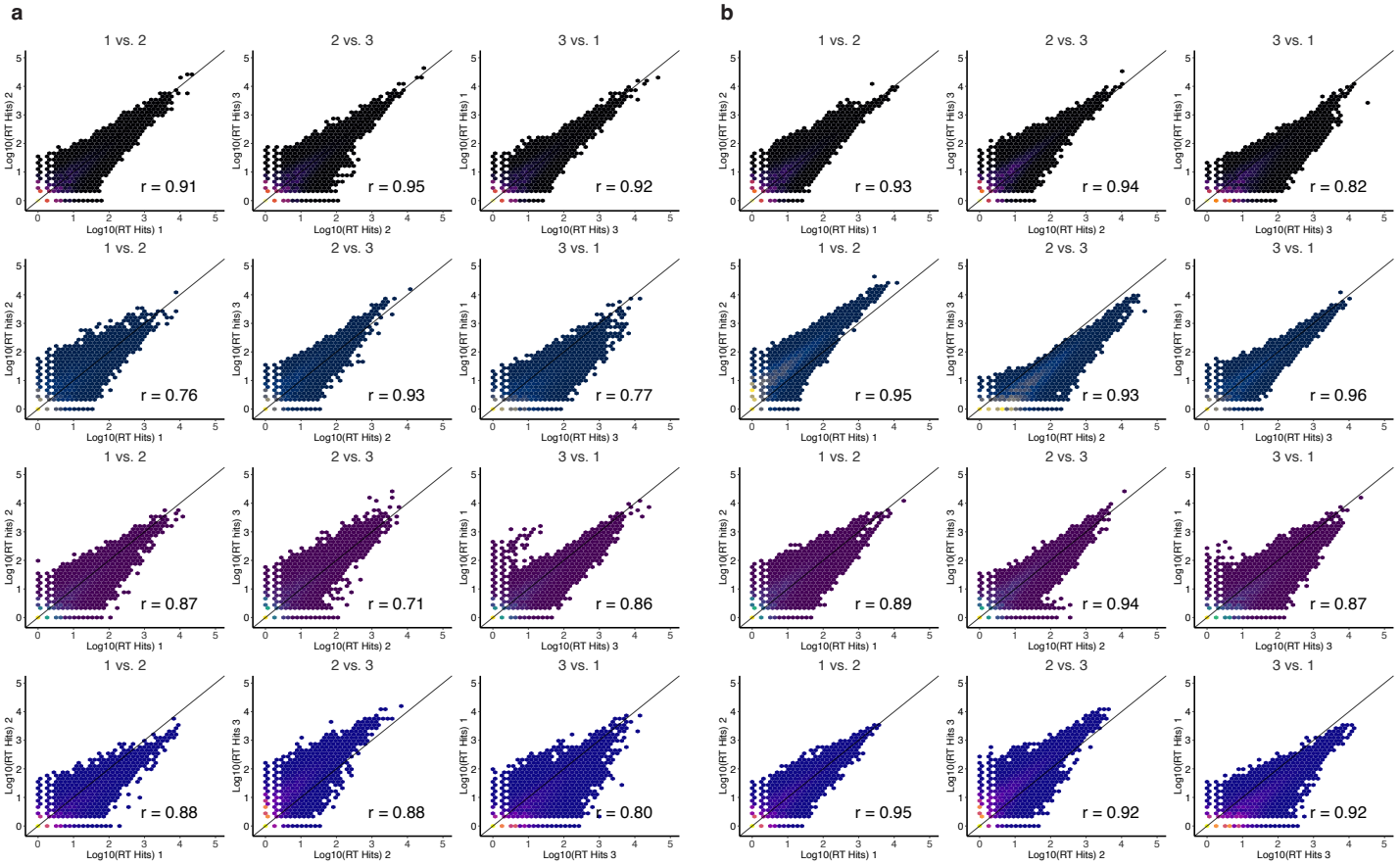


Supplemental Fig. 1. Mapping *in vivo* reactivity onto the *tyrS* T box riboswitch (PDB ID: 2KZL) from *B. subtilis* for **a** 23 °C, **b** 30 °C, **c** 37 °C, and **d** 42 °C.



Supplemental Fig. 2. Reactivity per nucleotide for genes in the *glp* regulon. **a** Reactivity changes per nucleotide for the *glpD* 5' UTR at 23, 30, 37, and 42 °C. **b** Reactivity changes per nucleotide for the *glpQ* 5' UTR at 23, 30, 37, and 42 °C.



Supplemental Fig. 3. Correlation of reverse transcription stops between replicates. **a** Comparison of three biological replicates from control samples for 23 °C (row 1), 30 °C (row 2), 37 °C (row 3), and 42 °C (row 4) growth temperatures. Pearson correlation coefficients (r) are shown. **b** Comparison of three biological replicates from DMS samples for 23 °C (row 1), 30 °C (row 2), 37 °C (row 3), and 42 °C (row 4) growth temperatures. Pearson correlation coefficients (r) are shown.

Supplemental Table S1. *B. subtilis* strains

Strain	Genotype[a]	Source
PLBS338	Prototroph	(Yakhnin 2004)
PLBS1103	PLBS338/*amyE::*P_{glpT}*-glpT'-'bgaB* translation fusion (-76 to +158) Cm^R	this study
PLBS1104	PLBS338/*amyE::*P_{glpF}*-glpF'-'bgaB* translation fusion (-92 to +150) Cm^R	this study
PLBS1110	PLBS338/*amyE::*P_{glpT} *-glpT'-'bgaB* translation fusion (-76 to +5, +86 to +158) Cm^R	this study
PLBS1111	PLBS338/*amyE::*P_{glpF}*-glpF'-'bgaB* translation fusion (-92 to +150, ΔT99, T102C) Cm^R	this study
PLBS1113	PLBS338/*amyE::*P_{glpT}*-glpT'-'bgaB* translation fusion (-76 to +5, +86 to +158, T87C, T89C, T90C) Cm^R	this study
PLBS1114	PLBS338/*amyE::*P_{glpT}*-glpT'-'bgaB* translation fusion (-76 to +158, T87C, T89C, T90C) Cm^R	this study

[a] Nucleotides in parentheses are relative to the start of transcription. ' implies truncation.

Yakhnin H, Zhang H, Yakhnin AV, Babitzke P. (2004). The *trp* RNA-binding attenuation protein of *Bacillus subtilis* regulates translation of the tryptophan transport gene *trpP* (*yhaG*) by blocking ribosome binding. *J. Bacteriol.* 186, 278-286.

Supplemental Table S2. Plasmids

Plasmid	Characteristics[a]	Source
pDL	contains *bgaB* Ap^R Cm^R	BGSC[b]
pTZ19R	cloning vector Ap^R	Thermo Fisher
pYH379	N-terminal portion of *bgaB* (*bgaB'*) cloned into pTZ19R Ap^R	this study
pYH380	P_{glpF}-*glpF' bgaB*' (-92 to +150) cloned into pYH379 Ap^R	this study
pYH381	P_{glpT}-*glpT' bgaB*' (-76 to +158) cloned into pYH379 Ap^R	this study
pYH382	P_{glpT}-*glpT'-'bgaB*' (-76 to +158), derivative of pYH381 Ap^R	this study
pYH383	P_{glpF}-*glpF'-'bgaB*' (-92 to +150), derivative pYH380 Ap^R	this study
pYH384	P_{glpT}-*glpT'-'bgaB* translation fusion (-76 to +158), derivative of pDL Ap^R Cm^R	this study
pYH385	P_{glpF}-*glpF'-'bgaB* translation fusion (-92 to +150), derivative of pDL Ap^R Cm^R	this study
pYH396	P_{glpF}-*glpF'-'bgaB*' (-92 to +150, ΔT98, T101C), derivative pYH383 Ap^R	this study
pYH398	P_{glpT}-*glpT'-'bgaB*' (-76 to +5, +86 to +158), derivative of pYH382 Ap^R	this study
pYH402	P_{glpT}-*glpT'-'bgaB* translation fusion (-76 to +5, +86 to +158), derivative of pDL Ap^R Cm^R	this study
pYH403	P_{glpF}-*glpF'-'bgaB* translation fusion (-92 to +150, ΔT99, T102C), derivative of pDL Ap^R Cm^R	this study
pYH406	P_{glpT}-*glpT'-'bgaB*' (-76 to +5, +86 to +158, T87C, T89C, T90C), derivative of pYH398 Ap^R	this study
pYH407	P_{glpT}-*glpT'-'bgaB* translation fusion (-76 to +5, +86 to +158, T87C, T89C, T90C), derivative of pDL Ap^R Cm^R	this study
pYH409	P_{glpT}-*glpT'-'bgaB*' (-76 to +158, T87C, T89C, T90C), derivative of pYH382 Ap^R	this study
pYH410	P_{glpT}-*glpT'-'bgaB* translation fusion (-76 to +158, T87C, T89C, T90C), derivative of pDL Ap^R Cm^R	this study

[a] Nucleotides in parentheses are relative to the start of transcription. ' implies truncations

[b] Bacillus Genetic Stock Center, Columbus, OH.

Supplemental Table S3. Oligonucleotides

Oligonucleotide	Sequence	Use
glpT fwd	GGGGATCCTACGTAGGTACCGGATAATATTCACTTCCCTAAGGC	P_{glpT}-*glpT'-'bgaB*
glpT rev	CGTTTAATTATAAATTAAGTTAAAATTTAGGTACCTAATAATTATCCCCCTTTGCTG	P_{glpT}-*glpT'-'bgaB*
glpF fwd	GGGGATCCTACGTAGGTACCCACAACATCTAATACCAAATTGTGG	P_{glpF}-*glpF'-'bgaB*
glpF rev	TTCGTTTAATTATAAATTAAGTTAAAATTTAGGTACCAGCACATTCCTCCTAAAGTCACG	P_{glpF}-*glpF'-'bgaB*
bgaB fwd	ATGAATGTGTTATCCTCAATTTGTTACGGAG	P_{glpT}-*glpT'-'bgaB,* P_{glpF}-*glpF'-'bgaB*
glpF TLN rev	AGCACATTCCTCCTAAAGTCACG	P_{glpF}-*glpF'-'bgaB*
glpT TLN rev	TAATAATTATCCCCCTTTGCTGTTTGTG	P_{glpT}-*glpT'-'bgaB*
glpF mut fwd	GTTGTGGTTTTTTTATTCCTCCTTCTCTATCATGC	P_{glpF}-*glpF'-'bgaB* (ΔT99, T102C)
glpF mut rev	GCATGATAGAGAAGGAGGAATAAAAAAACCACAAC	P_{glpF}-*glpF'-'bgaB* (ΔT99 , T102C)
glpT mut-1 fwd	CCGGACTATGATAATTTAAGAAAGCGCT	P_{glpT}-*glpT'-'bgaB* (-76 to +5, +86 to +158)
glpT mut-1 rev	GCCTCCTTTCCCGAAAAACAAAAAG	P_{glpT}-*glpT'-'bgaB* (-76 to +5, +86 to +158)
glpT mut-2 fwd	GACAAGGGGTGTTTTCTGGCCCCCTTTGTTTTTCGGGAAAGG	P_{glpT}-*glpT'-'bgaB* (T87C, T89C, T90C)
glpT mut-2 rev	CCTTTCCCGAAAAACAAAGGGGGCCAGAAAACACCCCTTGTC	P_{glpT}-*glpT'-'bgaB* (T87C, T89C, T90C)
glpT mut-3 fwd	GATAATTTAAGAAAGCGCCCCCTTTGTTTTTCGGGAAAGG	P_{glpT}-*glpT'-'bgaB* (-76 to +5, +86 to +158, T87C, T89C, T90C)
glpT mut-3 rev	CCTTTCCCGAAAAACAAAGGGGGCGCTTTCTTAAATTATC	P_{glpT}-*glpT'-'bgaB* (-76 to +5, +86 to +158, T87C, T89C, T90C)