Supplementary Data

De novo design of heat-repressible RNA thermosensors in *E. coli*

Allison Hoynes-O'Connor, Kristina Hinman, Lukas Kirchner and Tae Seok Moon

Supplementary Table 1. Parameters of thermosensors designed in this work (see Supplementary Figure 1 for their structures). "# RC" is the number of RNase E cleavage sites. "# Bulges" refers to the number of groups of unpaired nucleotides bulging out from the stem on either the side of the RNase E cleavage site (RC) or the anti-RNase E cleavage site (ARC). T_m and ΔG are estimated from Mfold (1) as described in the Materials and Methods.

Thermosensor	# RC	T_m (°C)	ΔG (kcal/mol)	Loop Size (nt)	# Bulges	
					RC	ARC
A1	2	27.5	-4.5	6	1	2
A2	2	27.5	-3.3	11	1	2
A3	2	27.5	-2.9	16	1	2
B1	2	29.5	-6.6	5	0	3
B2	2	29.5	-5.7	10	0	3
B3	2	29.5	-5.3	15	0	3
C1	2	29.2	-4.4	5	2	1
C2	2	29.2	-3.5	10	2	1
C3	2	29.2	-3.1	15	2	1
D1	1	37.8	-7.8	5	0	0
D2	1	37.8	-6.9	10	0	0
D3	1	37.8	-6.5	15	0	0
E1	1	32.4	-6.9	5	0	0
E2	1	32.4	-6.0	10	0	0
E3	1	32.4	-5.6	15	0	0
F1	1	37.8	-7.8	5	0	0
F2	1	37.8	-6.9	10	0	0
F3	1	37.8	-6.5	15	0	0
G1	2	34.0	-5.9	5	0	4
H1	2	36.5	-8.4	5	0	3
I1	2	35.9	-8.6	5	0	4
J1	2	25.6	-5.4	5	0	4
K1	2	25.6	-5.4	5	0	4
L1	2	25.6	-5.4	5	0	4

Supplementary Table 2. Plasmids used in this study.

Name	Parts
Controls	
pAH016	ColE1 ori; cm-R; Bba_J23104 – *gfp*
pAH021	ColE1 ori; cm-R; Bba_J23104 - No-ARC Control - *gfp*
pAH034	p15A ori; kan-R; Bba_J23105 – *rfp*
pAH048 (pTet-GFP)	ColE1 ori; cm-R; pTet – *gfp*
pAH049	ColE1 ori; cm-R; pTet - No-ARC Control – *gfp*
Thermosensors	
pAH050	ColE1 ori; cm-R; pTet - A1 Thermosensor - *gfp*
pAH051	ColE1 ori; cm-R; pTet - A2 Thermosensor - *gfp*
pAH052	ColE1 ori; cm-R; pTet - A3 Thermosensor - *gfp*
pAH053	ColE1 ori; cm-R; pTet - B1 Thermosensor - *gfp*
pAH054	ColE1 ori; cm-R; pTet - B2 Thermosensor - *gfp*
pAH055	ColE1 ori; cm-R; pTet - B3 Thermosensor - *gfp*
pAH056	ColE1 ori; cm-R; pTet - C1 Thermosensor - *gfp*
pAH057	ColE1 ori; cm-R; pTet - C2 Thermosensor - *gfp*
pAH058	ColE1 ori; cm-R; pTet - C3 Thermosensor - *gfp*
pAH059	ColE1 ori; cm-R; pTet - D1 Thermosensor - *gfp*
pAH060	ColE1 ori; cm-R; pTet - D2 Thermosensor - *gfp*
pAH061	ColE1 ori; cm-R; pTet - D3 Thermosensor - *gfp*
pAH062	ColE1 ori; cm-R; pTet - E1 Thermosensor - *gfp*
pAH063	ColE1 ori; cm-R; pTet - E2 Thermosensor - *gfp*
pAH064	ColE1 ori; cm-R; pTet - E3 Thermosensor - *gfp*
pAH065	ColE1 ori; cm-R; pTet - F1 Thermosensor - *gfp*
pAH066	ColE1 ori; cm-R; pTet - F2 Thermosensor - *gfp*
pAH067	ColE1 ori; cm-R; pTet - F3 Thermosensor - *gfp*
pAH068	ColE1 ori; cm-R; pTet - G1 Thermosensor - *gfp*
pAH069	ColE1 ori; cm-R; pTet - H1 Thermosensor - *gfp*
pAH070	ColE1 ori; cm-R; pTet - I1 Thermosensor - *gfp*
pAH071	ColE1 ori; cm-R; pTet - J1 Thermosensor - *gfp*
pAH072	ColE1 ori; cm-R; pTet - K1 Thermosensor - *gfp*
pAH073	ColE1 ori; cm-R; pTet - L1 Thermosensor - *gfp*
RNase E Rescue	
pAH045	p15A ori; kan-R; Bba_J23105 – *rfp;* Native promoters - *rne*
Genetic Circuits	
psicA-gfp (2)	ColE1 ori; cm-R; psicA - *gfp*
pTet-invF (3)	pSC101* ori; kan-R; pTet - *invF*
pBAD-sicA*(3)	p15A ori; amp-R; pBAD - *sicA**
pAH134	ColE1 ori; cm-R; psicA - D1 Thermosensor - *gfp*
pAH135	ColE1 ori; cm-R; psicA - E1 Thermosensor - *gfp*

pAH136	ColE1 ori; cm-R; psicA - E3 Thermosensor - *gfp*
pAH137	ColE1 ori; cm-R; psicA - F1 Thermosensor - *gfp*
pAH138	ColE1 ori; cm-R; psicA - F3 Thermosensor - *gfp*
pAH152	ColE1 ori; cm-R; psicA - B1 Thermosensor - *gfp*
pAH153	ColE1 ori; cm-R; psicA - C1 Thermosensor - *gfp*

Supplementary Table 3. *E. coli* strains used in this study.

Name	Host Strain	Plasmids
Controls		
DH10B	DH10B	None
BL21 Star (DE3)	BL21 Star (DE3)	None
Constitutive Positive Control	DH10B	pAH016
pTet Positive Control	DH10B	pAH048, pAH034
pTet No-ARC Control	DH10B	pAH049, pAH034
BL21 Star (DE3) pTet Positive Control	BL21 Star (DE3)	pAH048, pAH034
BL21 Star (DE3) No-ARC Control	BL21 Star (DE3)	pAH049, pAH034
RNase E Rescue pTet Positive Control	BL21 Star (DE3)	pAH048, pAH045
RNase E Rescue No-ARC Control	BL21 Star (DE3)	pAH049, pAH045
No Thermosensor Circuit Control	DH10B	pBAD-sicA*, pTet-invF, psicA-gfp
Thermosensors		
A1 Inducible	DH10B	pAH050, pAH034
A2 Inducible	DH10B	pAH051, pAH034
A3 Inducible	DH10B	pAH052, pAH034
B1 Inducible	DH10B	pAH053, pAH034
B2 Inducible	DH10B	pAH054, pAH034
B3 Inducible	DH10B	pAH055, pAH034
C1 Inducible	DH10B	pAH056, pAH034
C2 Inducible	DH10B	pAH057, pAH034
C3 Inducible	DH10B	pAH058, pAH034
D1 Inducible	DH10B	pAH059, pAH034
D2 Inducible	DH10B	pAH060, pAH034
D3 Inducible	DH10B	pAH061, pAH034
E1 Inducible	DH10B	pAH062, pAH034
E2 Inducible	DH10B	pAH063, pAH034
E3 Inducible	DH10B	pAH064, pAH034
F1 Inducible	DH10B	pAH065, pAH034
F2 Inducible	DH10B	pAH066, pAH034

F3 Inducible	DH10B	pAH067, pAH034
G1 Inducible	DH10B	pAH068, pAH034
H1 Inducible	DH10B	pAH069, pAH034
I1 Inducible	DH10B	pAH070, pAH034
J1 Inducible	DH10B	pAH071, pAH034
K1 Inducible	DH10B	pAH072, pAH034
L1 Inducible	DH10B	pAH073, pAH034
RNase E Rescue		
BL21 Star (DE3) D1 Thermosensor	BL21 Star (DE3)	pAH059, pAH034
BL21 Star (DE3) D2 Thermosensor	BL21 Star (DE3)	pAH060, pAH034
BL21 Star (DE3) D3 Thermosensor	BL21 Star (DE3)	pAH061, pAH034
BL21 Star (DE3) E2 Thermosensor	BL21 Star (DE3)	pAH063, pAH034
BL21 Star (DE3) E3 Thermosensor	BL21 Star (DE3)	pAH064, pAH034
BL21 Star (DE3) F1 Thermosensor	BL21 Star (DE3)	pAH065, pAH034
BL21 Star (DE3) F2 Thermosensor	BL21 Star (DE3)	pAH066, pAH034
BL21 Star (DE3) F3 Thermosensor	BL21 Star (DE3)	pAH067, pAH034
RNase E Rescue D1 Thermosensor	BL21 Star (DE3)	pAH059, pAH045
RNase E Rescue D2 Thermosensor	BL21 Star (DE3)	pAH060, pAH045
RNase E Rescue D3 Thermosensor	BL21 Star (DE3)	pAH061, pAH045
RNase E Rescue E2 Thermosensor	BL21 Star (DE3)	pAH063, pAH045
RNase E Rescue E3 Thermosensor	BL21 Star (DE3)	pAH064, pAH045
RNase E Rescue F1 Thermosensor	BL21 Star (DE3)	pAH065, pAH045
RNase E Rescue F2 Thermosensor	BL21 Star (DE3)	pAH066, pAH045
RNase E Rescue F3 Thermosensor	BL21 Star (DE3)	pAH067, pAH045
Genetic Circuits		
D1 Thermosensor Circuit	DH10B	pBAD-sicA*, pTet-invF, pAH134
E1 Thermosensor Circuit	DH10B	pBAD-sicA*, pTet-invF, pAH135
E3 Thermosensor Circuit	DH10B	pBAD-sicA*, pTet-invF, pAH136
F1 Thermosensor Circuit	DH10B	pBAD-sicA*, pTet-invF, pAH137
F3 Thermosensor Circuit	DH10B	pBAD-sicA*, pTet-invF, pAH138
B1 Thermosensor Circuit	DH10B	pBAD-sicA*, pTet-invF, pAH152
C1 Thermosensor Circuit	DH10B	pBAD-sicA*, pTet-invF, pAH153

Supplementary Table 4. Genetic parts used in this study.

Part name	Type and source	DNA sequence
gfp	Gene (2)	atgagtaaaggagaagaacttttcactggagttgtcccaattcttgttgaattagatggtgatgttaatgggcac aaattttctgtcagtggagagggtgaaggtgatgcaacatacggaaaacttacccttaaatttatttgcactact ggaaaactacctgttccatggccaacacttgtcactactttgacttatggtgttcaatgcttttcaagatacccag atcatatgaaacggcatgactttttcaagagtgccatgcccgaaggttatgtacaggaaagaactatatttttca aagatgacgggaactataagacacgtgctgaagtcaagtttgaaggtgatacacttgttaatagaatcgagtta aaaggtattgattttaaagaagatggaaacattcttggacacaagttggaatacaactataactcacacaatgta tacatcatggcagacaaacaaaagaatggaatcaaagttaacttcaaaattagacacaacattgaagatggaa gcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtccttttaccagacaaccatt acctgtccacacaatctgccctttcgaaagatcccaacgaaaagagagaccacatggtccttcttgagtttgta acagctgctgggattacacatggcatggatgaactatacaaaaggcctgcagcaaacgacgaaaactacgc ttaagtagcttaa
rfp	Gene (4)	atggcgagtagcgaagacgttatcaaagagttcatgcgtttcaaagttcgtatggaaggttccgttaacggtca cgagttcgaaatcgaaggtgaaggtgaaggtcgtccgtacgaaggtacccagaccgctaaactgaaagtta ccaaaggtggtccgctgccgttcgcttgggacatcctgtccccgcagttccagtacggttccaaagcttacgtt aaacacccggctgacatcccggactacctgaaactgtccttcccggaaggtttcaaatgggaacgtgttatga acttcgaagacggtggtgttgttaccgttacccaggactcctccctgcaagacggtgagttcatctacaaagtt aaactgcgtggtaccaacttcccgtccgacggtccggttatgcagaaaaaaaccatgggttgggaagcttcc accgaacgtatgtacccggaagacggtgctctgaaaggtgaaatcaaaatgcgtctgaaactgaaagacgg tggtcactacgacgctgaagttaaaaccacctacatggctaaaaaaccggttcagctgccgggtgcttacaa aaccgacatcaaactggacatcacctcccacaacgaagactacaccatcgttgaacagtacgaacgtgctg aaggtcgtcactccaccggtgcttaa
rne	Gene *E. coli* MG1655 (5)	atgaaaagaatgttaatcaacgcaactcagcaggaagagttgcgcgttgcccttgtagatgggcagcgtctgt atgacctggatatcgaaagtccagggcacgagcagaaaaaggcaaacatctacaaaggtaaaatcacccg cattgaaccgagtctggaagctgcttttgttgattacggcgctgaacgtcacggtttcctcccactaaaagaaat tgcccgcgaatatttccctgctaactacagtgctcatggtcgtcccaacattaaagatgtgttgcgtgaaggtca ggaagtcattgttcagatcgataaagaagagcgcggcaacaaaggcgcggcattaaccacctttatcagtct ggcgggtagctatctggttctgatgccgaacaacccgcgcgcgggtggcatttctcgccgtatcgaaggcg acgaccgtaccgaattaaaagaagcactggcaagccttgaactgccggaaggcatggggcttatcgtgcgc accgctggcgtcggcaaatctgctgaggcgctgcaatgggatttaagcttccgtctgaaacactgggaagcc atcaaaaaagccgctgaaagccgcccggccccgttcctgattcatcaggagagcaacgtaatcgttcgcgc attccgcgattacttacgtcaggacatcggcgaaatccttatcgataacccgaaagtgctcgaactggcacgt cagcatatcgctgcattaggtcgcccggatttcagcagcaaaatcaaactgtacaccggcgagatcccgctg ttcagccactaccagatcgagtcacagatcgagtccgccttccagcgtgaagttcgtctgccgtctggtggttc cattgttatcgacagcaccgaagcgttaacggccatcgacatcaactccgcacgcgcgacccgcggcggc gatatcgaagaaaccgcgtttaacactaacctcgaagctgccgatgagattgctcgtcagctgcgcctgcgt gacctcggcggcctgattgttatcgacttcatcgacatgacgccagtacgccaccagcgtgcggtagaaaac cgtctgcgtgaagcggtgcgtcaggaccgtgcgcgtattcaaatcagccatatttctcgctttggcctgctgga aatgtcccgtcagcgcctgagcccatcactgggtgaatccagtcatcacgtttgtccgcgttgttctggtactg gcaccgtgcgtgacaacgaatcgctgtcgctctctattctgcgtctgatcgaagaagaagcgctgaaagaga acacccaggaagttcacgccattgttcctgtgccaatcgcttcttacctgctgaatgaaaaacgttctgcggta aatgccattgaaactcgtcaggacggtgtgcgctgtgtaattgtgccaaacgatcagatggaaaccccgcac taccacgtgctgcgcgtgcgtaaaggggaagaaaccccaaccttaagctacatgctgccgaagctgcatga agaagcgatggcgctgccgtctgaagaagagttcgctgaacgtaagcgtccggaacaacctgcgctggca acctttgccatgccggatgtgccgcctgcgccaacgccagctgaacctgccgcgcctgttgtagctccagca ccgaaagctgcaccggcaacaccagcagctcctgcacaacctgggctgttgagccgcttcttcggcgcact gaaagcgctgttcagcggtggtgaagaaaccaaaccgaccgagcaaccagcaccgaaagcagaagcga aaccggaacgtcaacaggatcgtcgcaagcctcgtcagaacaaccgccgtgaccgtaatgagcgccgcg acacccgtagtgaacgtactgaaggcagcgataatcgcgaagaaaaccgtcgtaatcgtcgccaggcaca gcagcagactgccgagacgcgtgagagccgtcagcaggctgaggtaacggaaaaagcgcgtaccgccg acgagcagcaagcgccgcgtcgtgaacgtagccgccgccgtaatgatgataaacgtcaggcgcaacaag aagcgaaggcgctgaatgttgaagagcaatctgttcaggaaaccgaacaggaagaacgtgtacgtccggtt cagccgcgtcgtaaacagcgtcagctcaatcagaaagtgcgttacgagcaaagcgtagccgaagaagcgg tagtcgcaccggtggttgaagaaactgtcgctgccgaaccaattgttcaggaagcgccagctccacgcaca gaactggtgaaagtcccgctgccagtcgtagcgcaaactgcaccagaacagcaagaagagaacaatgctg ataaccgtgacaacggtggcatgccgcgtcgttctcgccgctcgcctcgtcacctgcgcgtaagtggtcagc gtcgtcgtcgctatcgtgacgagcgttatccaacccagtcgccaatgccgttgaccgtagcgtgcgcgtctcc ggaactggcctctggcaaagtctggatccgctatccaattgtacgtccgcaagatgtacaggttgaagagca

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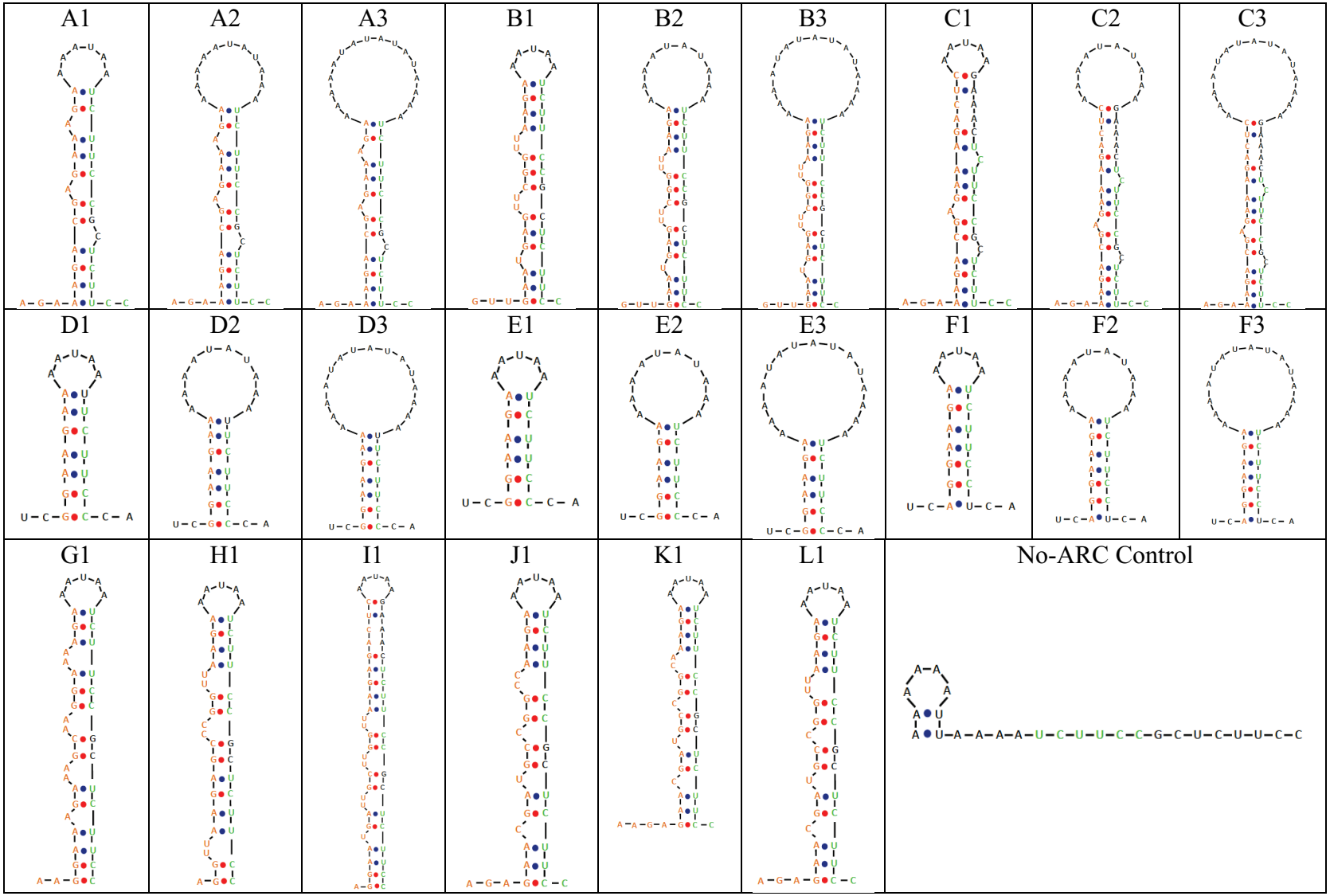
rne upstream region	Promoters and 5' UTR *E. coli* MG1655 (5)	acaggattcgcgccactcatttttctatgcttatatttacttttgcaccttattacttcactgcgtgatcactttattgat ggttattaaaccaatcaccagcaagaagtgaaaaaactgtgagtaagcgggtgataaatggtaaaagtcatct tgctataacaaggcttgcagtggaataatgaggccgtttccgtgtccatccttgttaaaacaagaaattttacgg aataacccattttgcccgaccgatcatccacgcagcaatggcgtaagacgtattgatctttcaggcagttagcg ggctgcgggttgcagtccttaccggtagatggaaatatttctggagagtaatacccagtctgtttctttgataatt gcgctgtttttccgcatgaaaaacgggcaaccgacactctgcgcctctttgagctgacgataaccgtgaggtt ggcgacgcgactagacacgaggccatcggttcacacccggaaaggcgttactttgcccgcagcttagtcgt caatgtaagaataatgagtaagttacg
invF	Gene (3)	atgtcattttctgaaagccgacacaatgaaaattgcctgattcaggaaggcgcgctgctttttgcgagcaggc cgttgtcgcaccagtatcaggagacctggtttttcgaccgttaaaaattgaagtactcagcaaattactggcatt tatcgatggcgcaggattagtggacacgacatatgctgaatccgataaatgggttttgctgagtcctgagtttc gcgctatttggcaagatcgtaaacgctgcgagtactggtttttgcagcaaattattacgccttctccggccttca ataaggtactggcgctgttacgaaaaagcgagagttactggttggttggctatttactcgctcagtcaaccagc ggcaacacgatgagaatgctgggagaagactatggcgtttcttataccatttttcgtcgtttgtgcagcagagc gttgggcggaaaagcgaagagtgaattacgaaactggcgtatggcgcaatcgctgctgaatagtgtagaag gccacgagaacatcacccaattagccgttaatcatggttactcatcgccttcacatttttctagtgagatcaaag agctgatcggcgtttcgccgcggaaattatcaaatattattcaattggcagacaaatga
*sicA**	Gene (3)	atggattatcaaaataatgtcagcgaagaacgtgttgcggaaatgatttgggatgccgttagtgaaggcgcca cgctaaaagacgttcatgggatccctcaagatatgatggacggtttatatgctcatgcttatgagttttataacca gggacgactggatgaagctgagacgttctttcgttacttatgcatttatgatttttacaatcccgattacaccatg ggactggcggcagtatgccaactgaaaaaacaatttcagaaagcatgtgacctttatgcagtagcgtttacgtt acttaaaaatgattatcgccccgttttttttaccgggcagtgtcaattattaatgcgtaaggcagcaaaagccag acagtgttttgaacttgtcaatgaacgtactgaagatgagtctctgcgggcaaaagcgttggtctatctggagg cgctaaaaacggcggagacagagcagcacagtgaacaagaaaaggaataa
psicA	Promoter (2)	ccacaagaaacgaggtacggcattgagccgcgtaaggcagtagcgatgtattcattgggcgtttttgaatgtt cactaaccaccgtcggggtttaataactgca
pTet	Promoter (3)	ttttcagcaggacgcactgacctccctatcagtgatagagattgacatccctatcagtgatagagatactgagc acatct
pBAD	Promoter (3)	agaaaccaattgtccatattgcatcagacattgccgtcactgcgtcttttactggctcttctcgctaaccaaaccg gtaaccccgcttattaaaagcattctgtaacaaagcgggaccaaagccatgacaaaaacgcgtaacaaaagt gtctataatcacggcagaaaagtccacattgattatttgcacggcgtcacactttgctatgccatagcatttttatc cataagattagcggatcctacctg
Bba_J23104	Promoter (http://parts.igem.org/Part:BBa_J23104)	ttgacagctagctcagtcctaggtattgtgctagc
Bba_J23105	Promoter (http://parts.igem.org/Part:BBa_J23105)	tttacggctagctcagtcctaggtactatgctagc

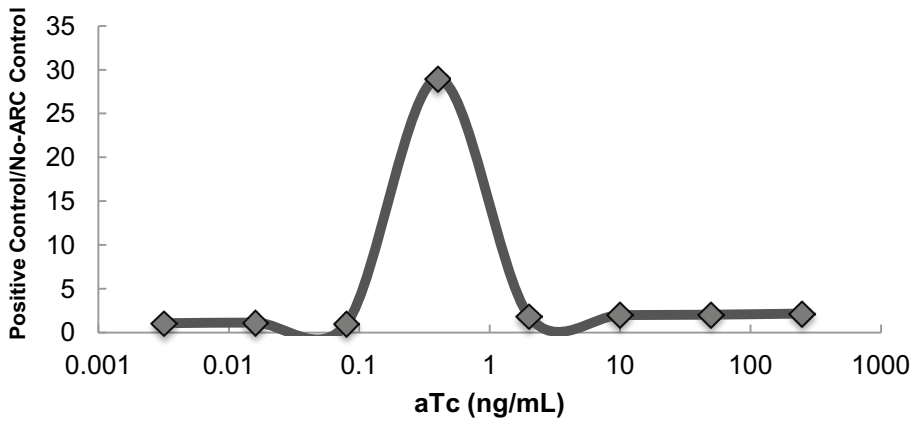
Supplementary Table 5. Primers for RT-qPCR.

Primer	Sequence	Source
cysGF	ttgtcggcggtggtgatgtc	(6)
cysGR	atgcggtgaactgtggaataaacg	(6)
hcaTF	gctgctcggctttctcatcc	(6)
hcaTR	ccaaccacgctgaccaacc	(6)
idnTF	ctgtttagcgaagaggagatgc	(6)
idnTF	acaaacggcggcgatagc	(6)
gfpF	ctgtccacacaatctgccct	This Study
gfpR	gtttgctgcaggccttttgt	This Study

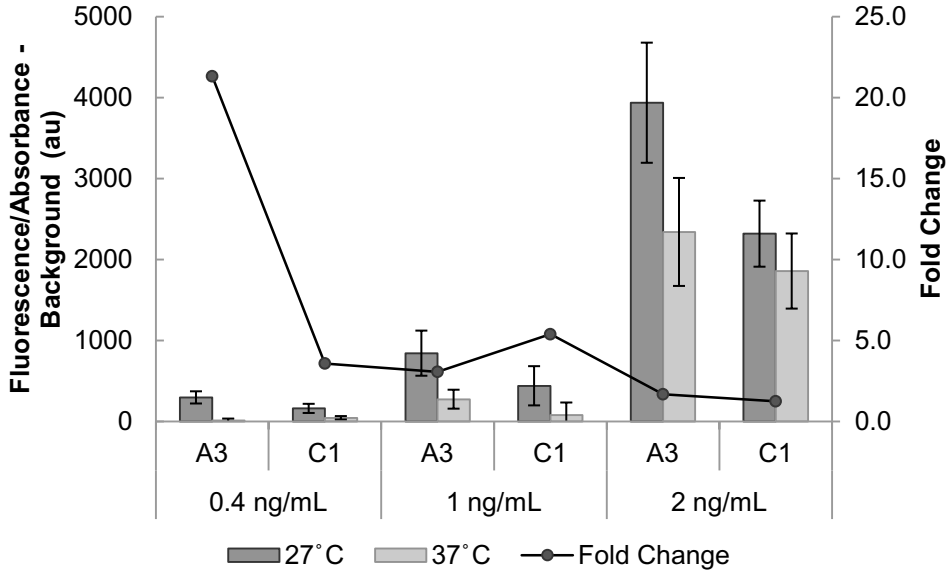


Supplementary Figure 1. Thermosensor structures predicted by Mfold. The structure includes the sequence ranging from the ARC to RC, but does not extend into the SD and coding sequence. The RC is in green and the ARC is in orange. When two RNase E cleavage sites are included in the structure, a GC spacer was placed between the two sequences, as has been observed in nature (7).

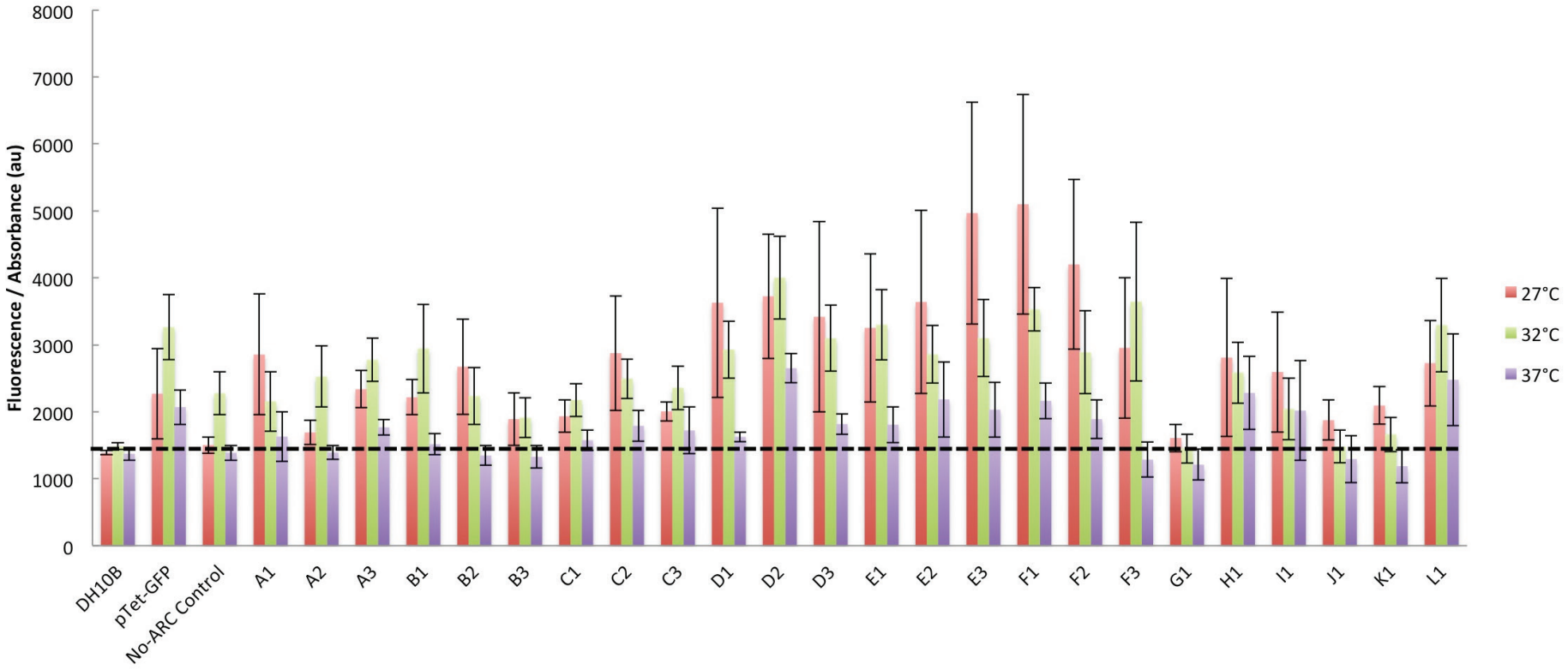
A



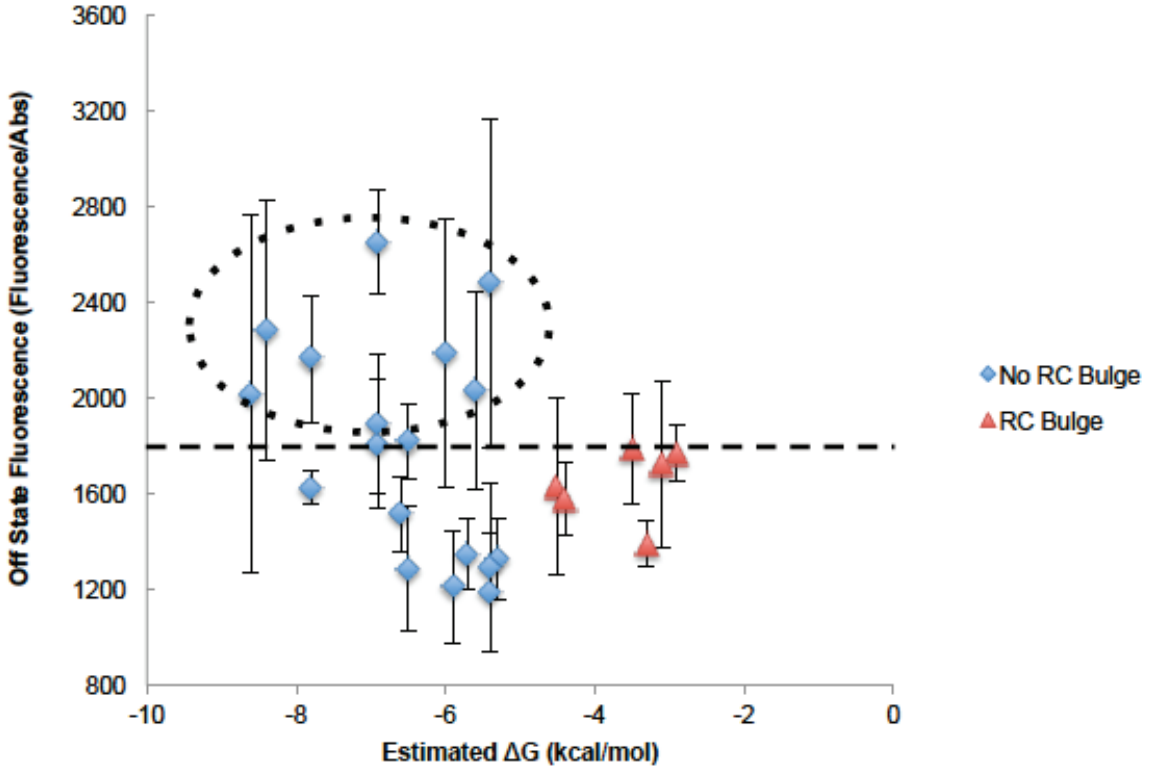
B



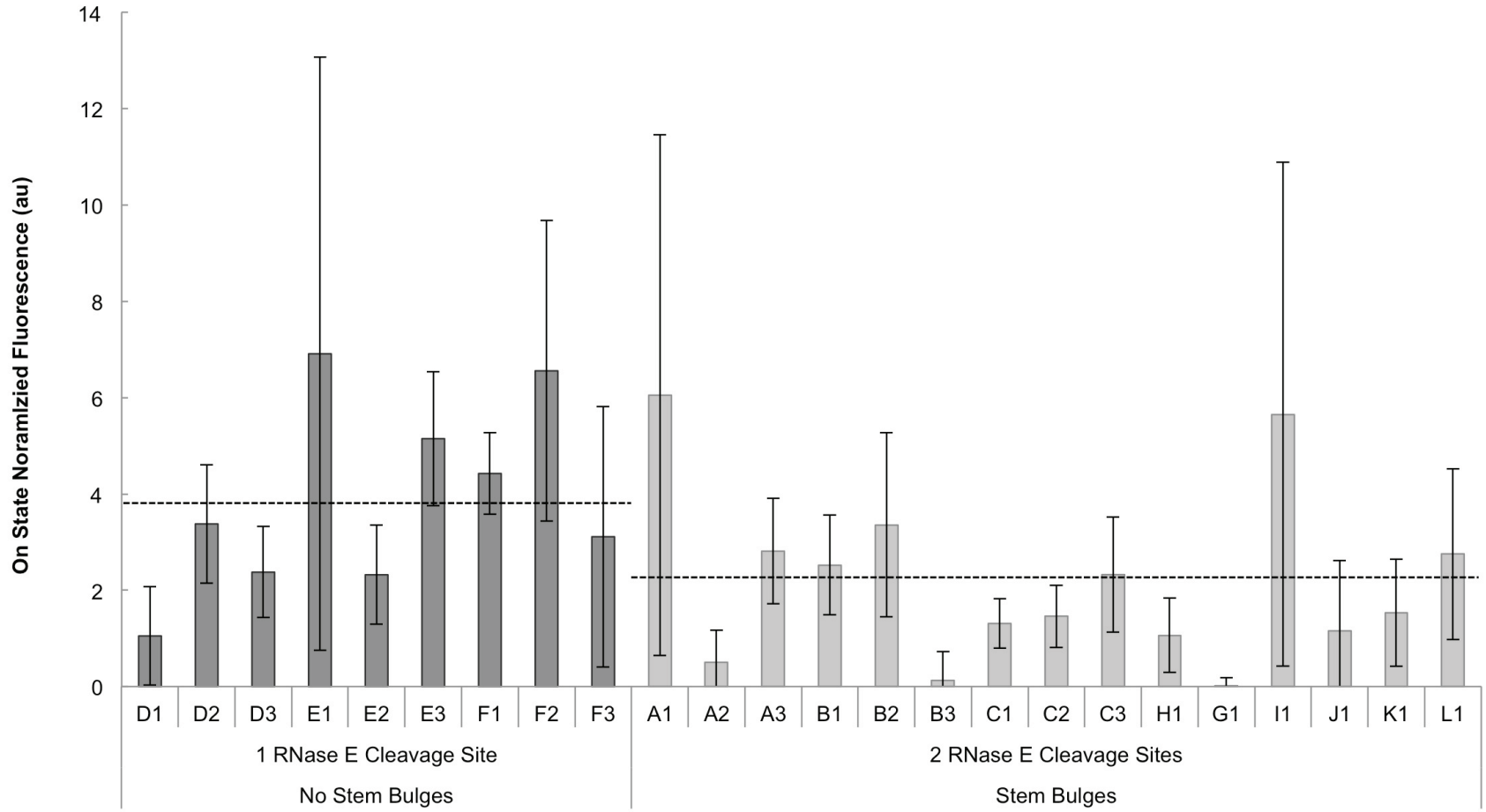
Supplementary Figure 2. Transcription level optimization. Optimization of transcript level is necessary to ensure that the thermosensors will function correctly within the cell. An aTc level of 1 ng/mL was used in all experiments unless otherwise indicated. (A) Ratio of the fluorescence output of positive control (pTet-GFP) to that of No-ARC control at 37°C at 0.0032, 0.016, 0.08, 0.4, 2, 10, 50, and 250 ng/mL aTc. The line is added as a guide to the eye. Thermosensors were under the control of pTet, which allowed for control of the transcript level within the cell. A transcript level that is too low will be undetectable, and a transcript level that is too high will overwhelm the capacity of RNase E. (B) A3 and C1 thermosensors were tested at 0.4 ng/mL, 1 ng/mL, and 2 ng/mL aTc to identify the optimum aTc level for thermosensor response. Per cell fluorescence is shown by dividing fluorescence by absorbance, and subtracting the autofluorescence levels, divided by absorbance, of background cells (DH10B). GFP expression is compared at 0.4 ng/mL, 1 ng/mL, and 2 ng/mL, at both 27°C and 37°C. Fold change between 27°C and 37°C is shown by the circles, and the line is added as a guide to the eye. At 0.4 ng/mL, high fold changes are likely due to extremely low fluorescence levels. These fluorescence levels are too low to be clearly distinguished from the background. At 2 ng/mL, fluorescence levels were high, but fold changes decreased. At 1 ng/mL, fluorescence levels were high enough that they could be clearly distinguished from the background, and fold changes were sufficiently high. Data is the average of three biological replicates. Error bars represent standard error of the mean (s.e.m.).



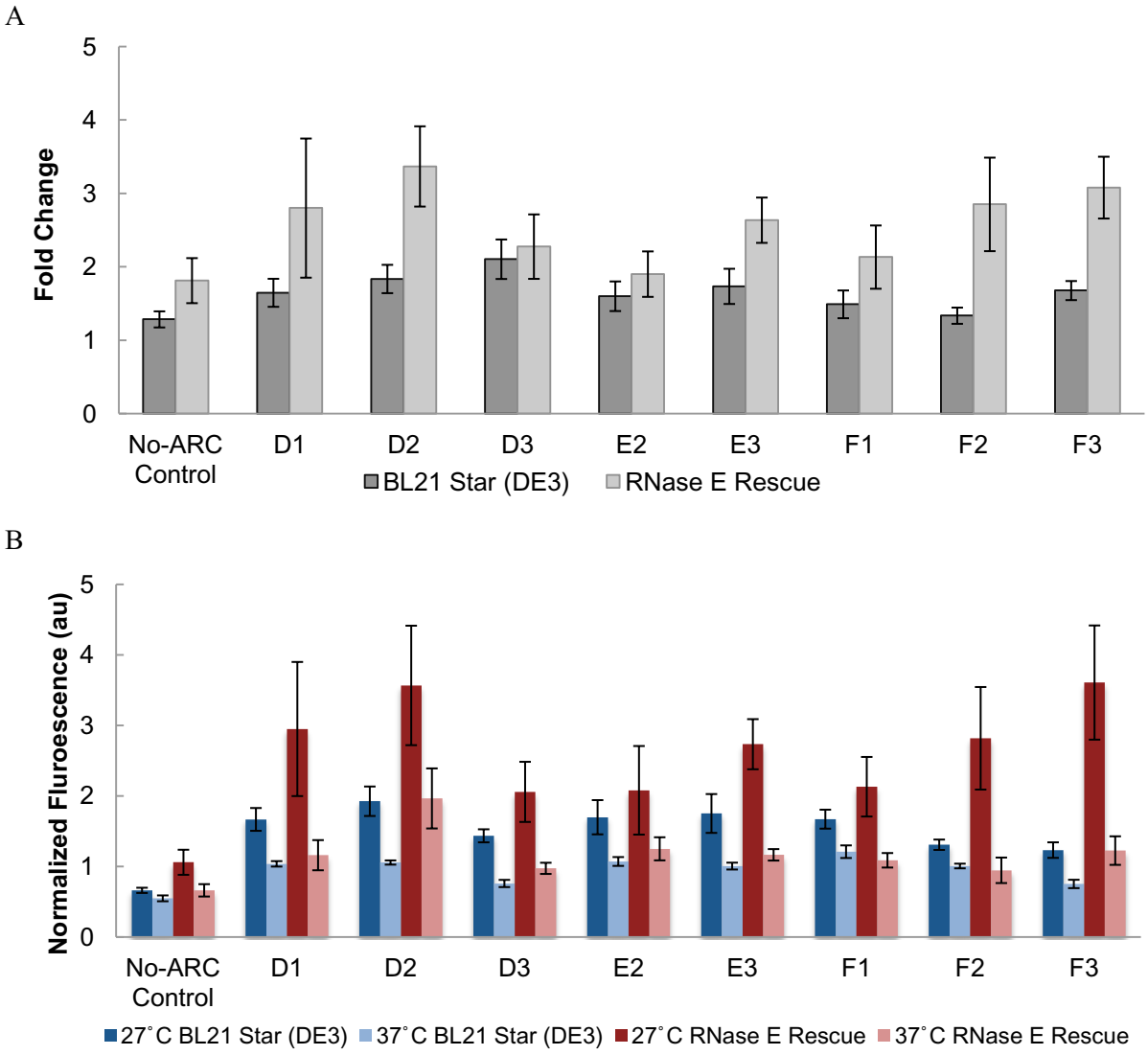
Supplementary Figure 3. Thermosensor response in *E. coli* DH10B. Fluorescence is shown for all 24 thermosensors, as well as a variety of controls, at 27°C, 32°C, and 37°C. Fluorescence is divided by absorbance to give an approximate "per cell" expression level. The dashed line represents the autofluorescence level (divided by absorbance) measured in DH10B. Though in some cases expression appears to be higher at 32°C than at 27°C, slightly higher expression in the positive control (pTet-GFP) at 32°C can account for this trend. Normalized values show that for the majority of thermosensors, expression is highest at 27°C, and the expression level at 32°C is between expression levels at 27°C and 37°C. Data is the average of six biological replicates, over two different days. Error bars represent standard error of the mean (s.e.m.).



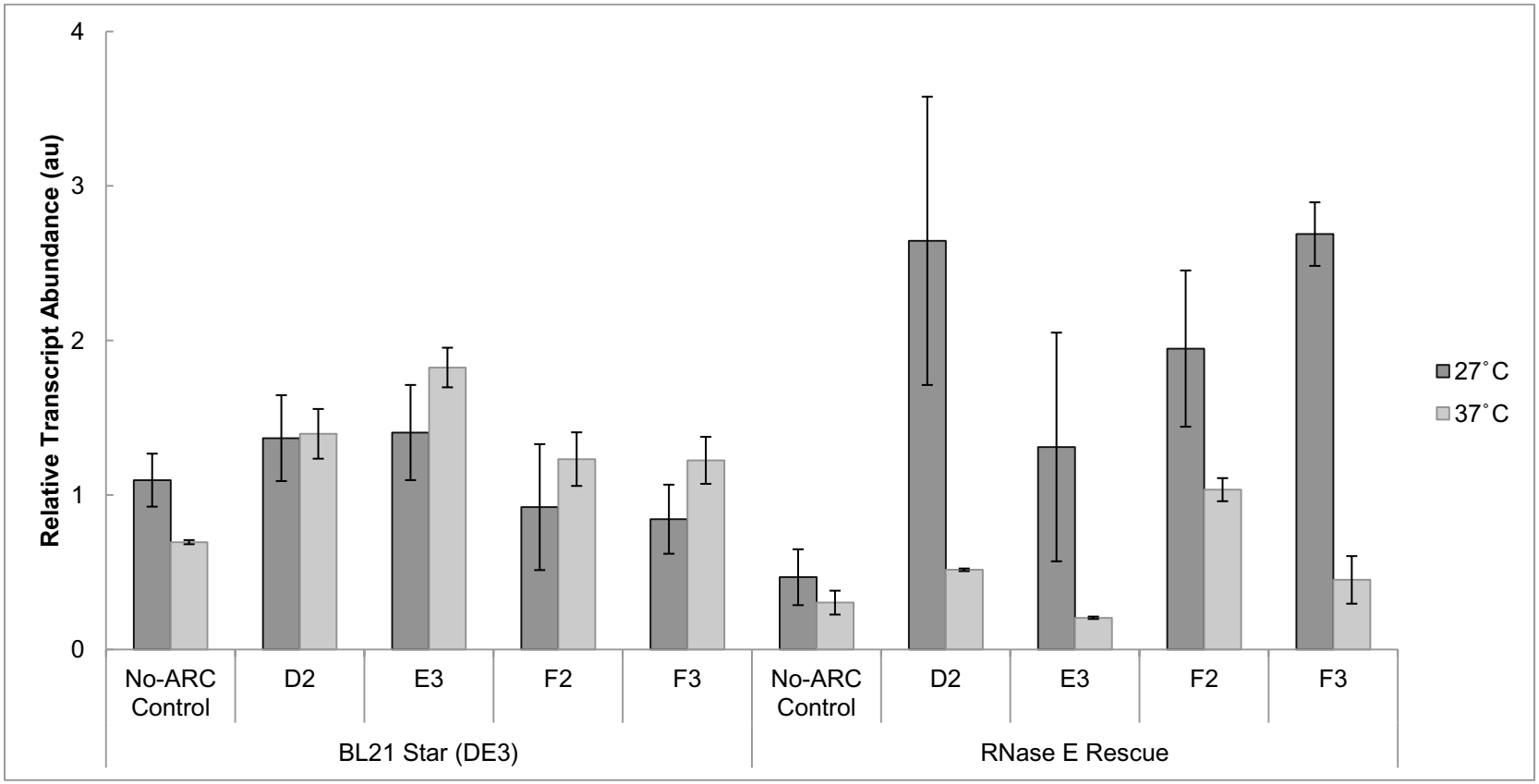
Supplementary Figure 4. Correlation between "off" state fluorescnce and estimated ΔG. “Off” state fluorescence is a measure of leakiness. The horizontal dashed line shows the threshold fluorescence, determined by DH10B autofluorescene divided by absorbance. Thermosensors with an “off” state fluorescence that exceeds the threshold are considered to be leaky. The threshold fluorescence is equal to one standard deviation above the average autofluorescence (devided by absorbance), as measured in the white cells (DH10B). The dotted circle indicates points representing the leaky thermosensors, which are all above the dashed line. Data is the average of six biological replicates, over two different days. Error bars represent standard error of the mean (s.e.m.).



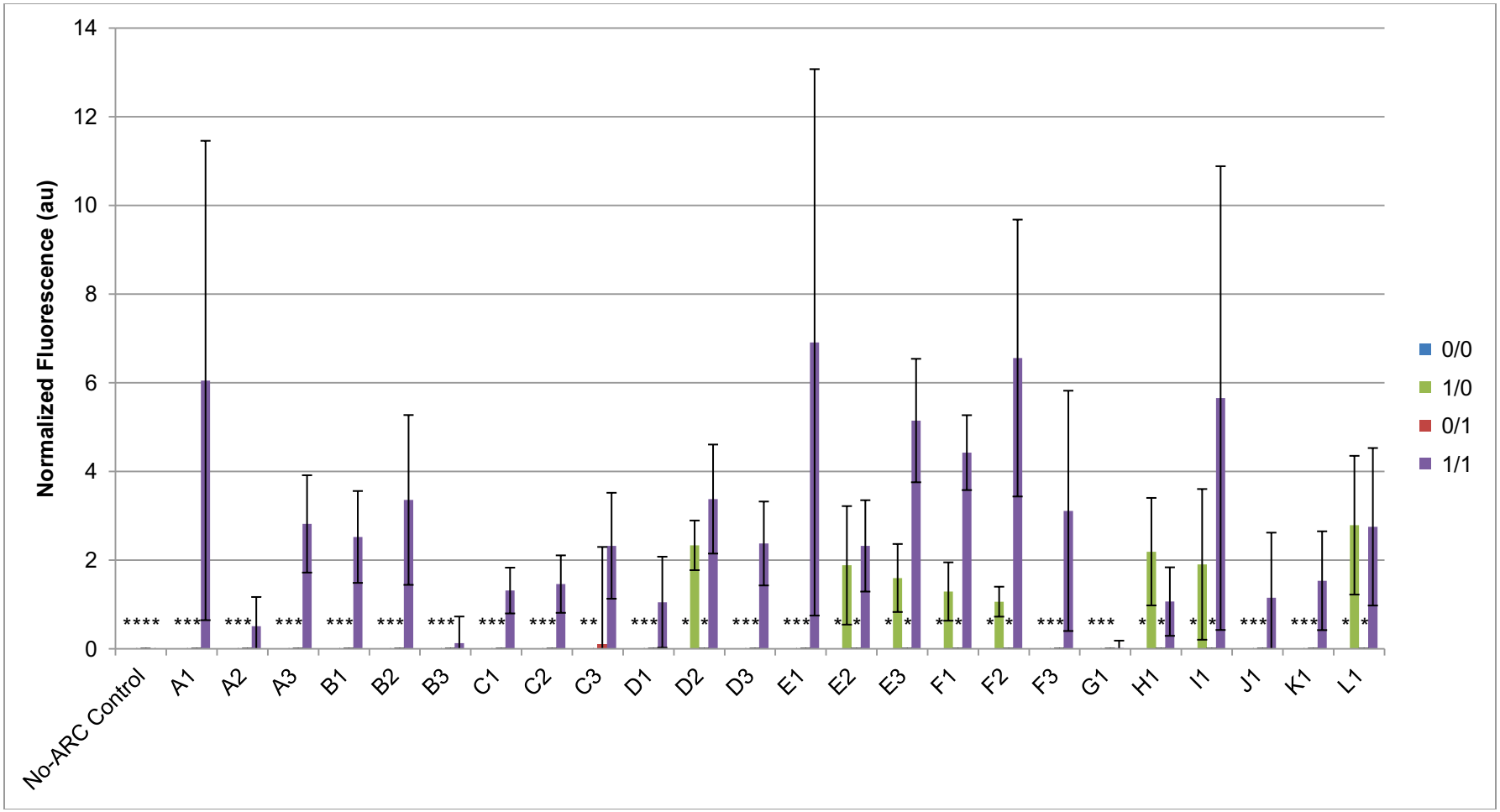
Supplementary Figure 5. "On" state of thermosensors with 1 RC and no stem bulges, or 2 RCs and stem bulges. The dashed lines show the average "on" state for each group. Data is the average of six biological replicates, over two different days. Error bars represent standard error of the mean (s.e.m.).



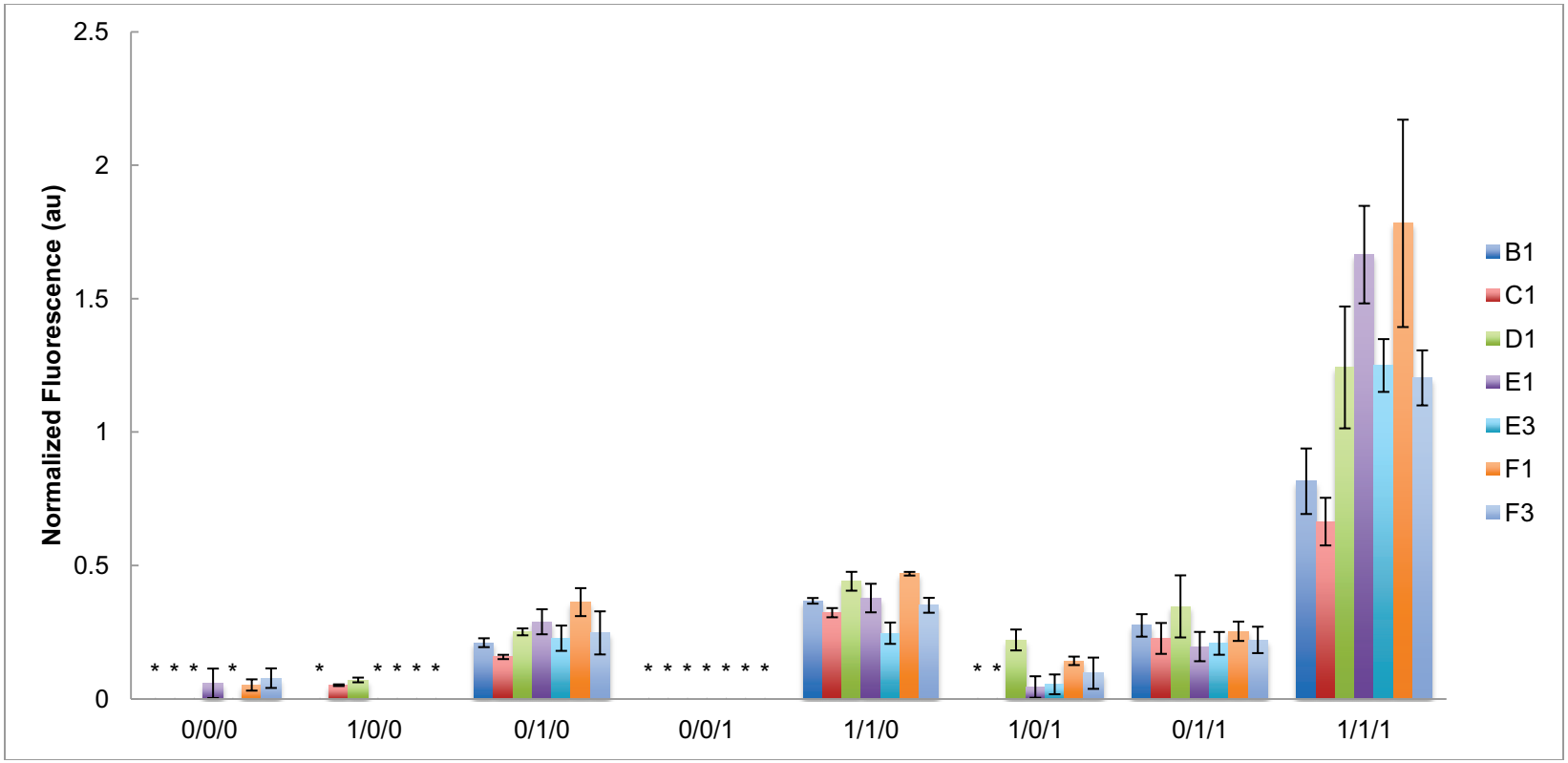
Supplementary Figure 6. Fluorescence results for thermosensors in the BL21 Star (DE3) and RNase E Rescue Strains. (A) Fold change (27°C fluorescence / 37°C fluorescence) of selected thermosensors in BL21 Star (DE3) stain and RNase E Rescue strain. The D2, E3, F2, and F3 thermosensors demonstrate a significant increase in the 27°C/37°C expression ratio from the BL21 Star (DE3) strain to the RNase E rescue strain ($P<0.05$; two-tailed, unpaired, Student's t-test). P-values are as follows: No-ARC control = 0.13, D1 = 0.25, D2 = 0.02, D3 = 0.74, E2 = 0.42, E3 = 0.03, F1 = 0.19, F2 = 0.04, and F3 = 0.01. (B) Normalized fluorescence of selected thermosensors at 27°C and 37°C in BL21 Star (DE3) stain and RNase E Rescue strain. Fluorescence was normalized to pTet-GFP output under each condition in each strain. Raw fluorescence values were generally higher in the BL21 Star (DE3) strain than in the RNase E Rescue strain, although normalized values do not represent this trend due to differences in pTet-GFP output. Data is the average of 14 biological replicates, over a total of three different days. Error bars represent standard error of the mean (s.e.m.).



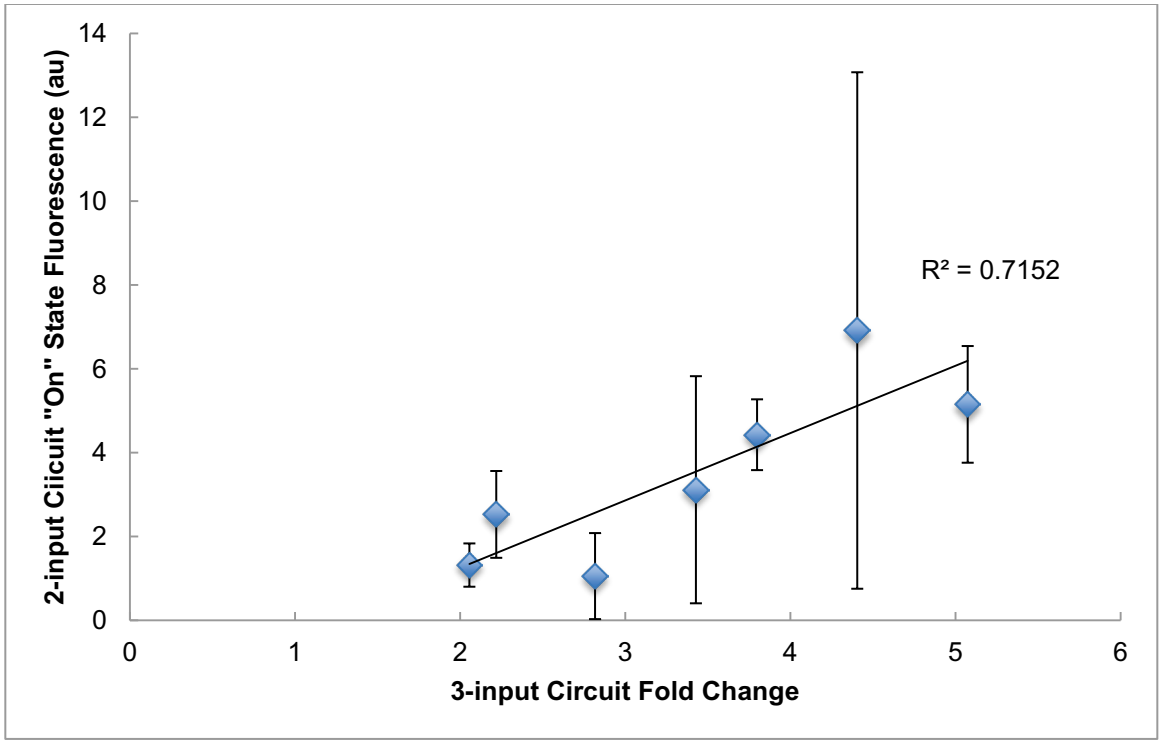
Supplementary Figure 7. Relative transcript abundance of selected thermosensors at 27°C and 37°C in BL21 Star (DE3) stain and RNase E Rescue strain based on RT-qPCR data. Transcript abundances do not display major changes in response to temperature in the BL21 Star (DE3) strain, but show higher transcript abundance at 27°C than at 37°C in the RNase E Rescue strain. Data was normalized to the positive control (pTet-gfp) in that strain and at that temperature, and corrections were applied (log transformation, mean centering, and autoscaling) in accordance with MIQE guidelines (8,9). The data shown is from two biological and two technical replicates. Error bars represent standard error of the mean (s.e.m.).



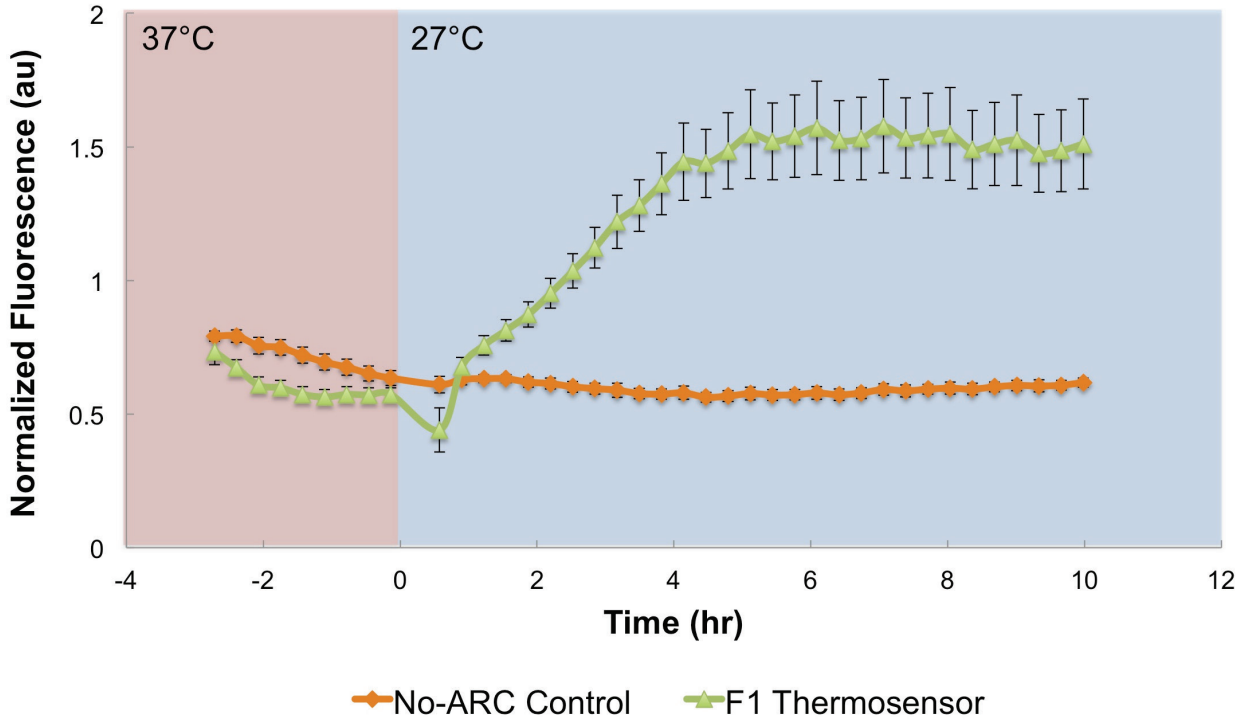
Supplementary Figure 8. Two-input composite circuits with all 24 thermosensors. Conditions are reported as "aTc/Temperature". For temperature, "0" = 37°C and "1" = 27°C. aTc was used at a concentration of 1 ng/mL. Data is the average of six biological replicates, over two separate days. The asterisk (*) indicates that the GFP/Abs value was within one standard deviation of the background DH10B GFP/Abs value (Materials and Methods). Error bars represent standard error of the mean (s.e.m.).



Supplementary Figure 9. Three-input composite circuits with the B1, C1, D1, E1, E3, F1 and F3 thermosensors. Conditions are reported as "Ara/aTc/Temp". For temperature, "0" = 37°C and "1" = 27°C. aTc was used at a concentration of 2 ng/mL, and arabinose (Ara) was used at a concentration of 0.32 μM. Fold changes were calculated by dividing normalized expression in the [111] condition by that of the [110] condition, which was the leakiest condition in all cases. Fold changes are as follows: B1 = 2.2; C1 = 2.1; D1 = 2.8; E1 = 4.4; E3 = 5.1; F1 = 3.8; F3 = 3.4. P-values were found with a one-tailed, unpaired Student's t-test, which compared expression in the [111] condition to expression in the [110] condition. P-values are as follows: B1 = 0.03; C1 = 0.03; D1 = 0.03; E1 = 0.01; E3 = 0.002; F1 = 0.04; F3 = 0.005. The change in expression was found to be significant ($p<0.05$) for all seven circuits. Data is the average of three biological replicates, over two separate days. The asterisk (*) indicates that the GFP/Abs value was within one standard deviation of the background DH10B GFP/Abs value (Materials and Methods). Error bars represent standard error of the mean (s.e.m.).



Supplementary Figure 10. Correlation of 2-input circuit "on" state with 3-input circuit fold change. Results are shown for the B1, C1, D1, E1, E3, F1, and F3 thermosensors. Fold changes were calculated by dividing normalized expression in the [111] condition by expression in the [110] condition, which was the leakiest condition in all cases (see Supplementary Figure 9). Error bars represent standard error of the mean (s.e.m.).



Supplementary Figure 11. Time response of the RNA thermosensor F1 is shown. Cells were grown at 37°C for four hours, then the temperature was reduced to 27°C. The time at which the temperature change occurs is set as t=0. Upon exposure to a colder temperature, fluorescence for the F1 thermosensor increases relative to the fluorescence of the No-ARC control. Fluorescence is normalized to the positive control (pTet-GFP). These fluorescence values cannot be directly compared to the data shown in Figure 2 because the experimental conditions were different (e.g. temporal separation of chemical and temperature induction; see the Methods below). Data shown is the average of eight biological replicates, and error bars represent standard error of the mean (s.e.m.).

Supplementary Methods

RNA Extraction

RNA isolation was performed using TRIzol Reagent (Life Technologies), according to manufacturer's instructions with the following modifications. After resuspension with 1 mL of TRIzol, cells were incubated at 95°C for 5 minutes, then incubated on ice for 5 minutes before phase separation. After the addition of chloroform, tubes were vigorously shaken and incubated at room temperature for 10-15 minutes prior to centrifugation. After the addition of isopropanol to the aqueous phase following chloroform extraction, samples were incubated at room temperature for 10 minutes and centrifuged at 12,000g for 30 minutes at 4°C. The RNA wash was performed with ice cold ethanol, and after air-drying the RNA pellet, the RNA was resuspended in DPEC-treated water. Concentrations and purities (A260/A280) were measured with a Nanodrop 2000 UV-Vis Spectrophotometer. Purities (A_{260}/A_{280} values) ranged from 1.80 to 2.05, with an average value of 1.94.

DNase Treatment

DNA was removed using the DNA-free Kit (Life Technologies) according to manufacturer's instructions. To check for DNA contamination, PCR was completed with RT-qPCR primers (50 nM), and DNase-treated RNA samples as the template. GoTaq DNA Polymerase (Promega Corporation) was used according to manufacturer's instructions. PCR reaction volumes were 50 μL, and were held at 95°C for 2 minutes, then underwent 40 cycles of 45 seconds at 95°C, 45 seconds at 60°C, and 20 seconds at 72°C, followed by 5 minutes at 72°C. No bands were detected when visualized in a gel, indicating that DNA had been completely removed from the sample. Concentrations and purities (A_{260}/A_{280}) were measured. Purities (A_{260}/A_{280} values) ranged from 1.67 to 2.02, with an average value of 1.91.

Reverse Transcription

The DNase-treated RNA samples were converted to cDNA libraries using the AffinityScript QPCR cDNA synthesis Kit (Agilent Technologies). To generate the cDNA

library, 10 μL of 2X cDNA synthesis master mix, 3 μL random primers, 1 μL AffinityScript RT/RNase block enzyme mixture, and 6 μL of RNA (0.8 – 2.5 μg of RNA, depending on concentration) were combined for a total reaction volume of 20 μL. Samples were cycled at 25°C for 5 minutes, 42°C for 15 minutes, and 95°C for 5 minutes. The concentrations of the cDNA libraries ranged from 1400 – 3200 ng/mL. Samples were stored at -20°C for up to 2 days.

RT-qPCR Primer Optimization and Efficiency

Primer concentration was optimized by performing PCR with a gradient of primer concentrations and identifying the concentration at which no primer dimers or non-specific binding occurred. PCR for this optimization step was performed using 0.5 μL of GoTaq polymerase and either plasmid DNA (containing GFP), gDNA (containing the reference genes), or water (no-template control) as the template. PCR reaction volumes were 50 μL, and were held at 95°C for 2 minutes, then underwent 40 cycles of 45 seconds at 95°C, 45 seconds at 60°C, and 20 seconds at 72°C, followed by 5 minutes at 72°C. Optimal primer concentration was found to be 50 nM. No bands were observed in the no-template control.

Calibration curves were generated for each set of primers to determine primer efficiency in RT-qPCR (>90% for each primer set) and ensure that cDNA concentration was within the linear range for each gene target. RT-qPCR conditions are described in the Methods. 6 μg of cDNA was used for both *idnT* and *hcaT*, 12 μg of cDNA was used for *cysG*, and 400 ng of cDNA was used for *gfp*. The concentrations of cDNA were different because reference genes expressed from the genome have a much lower copy number than *gfp*, which is expressed from a high-copy plasmid.

Characterization of Response Time

To determine the speed with which the thermosensor responds to a change in temperature, cells were prepared as described in the Methods. After resuspension in 1 ng/mL aTc-containing M9 minimal media with 4 g/L glucose, cells were cultured at 37°C and 250 rpm in the plate reader, with absorbance and fluorescence measurements taken

every 15 minutes. After 4 hours, the temperature was decreased to 27°C, and measurements were continued until stationary phase was reached.

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