Supplemental Information for:

SPRINT: A Cas13a-based platform detection of small molecules

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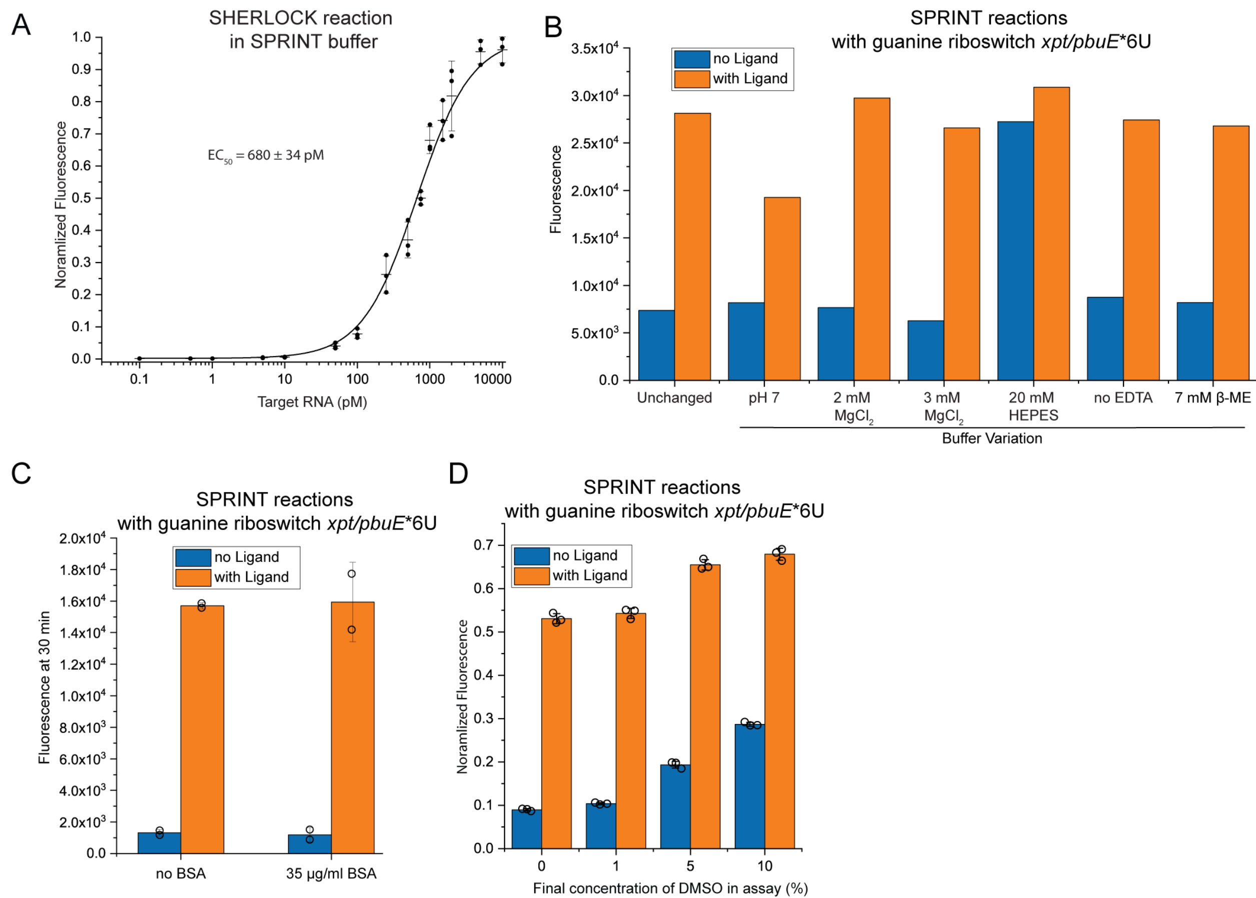


Figure S1: **Optimization of buffer conditions for SPRINT reactions**. All fluorescence measurements were background corrected, bars indicate mean value and error bars indicate s.d. from the mean. **(A)** SHERLOCK reactions were carried out in SPRINT buffer at varying concentrations of added target RNA. The measurements displayed were taken at 20 minutes reaction time. EC_{50} values were calculated from a biexponential model that was fit to the data; n = 3 (**B**) SPRINT reactions were carried out with the guanine riboswitch *xpt/pbuE**6U in unmodified SPRINT buffer. The riboswitch was induced with +/- 100 µM hypoxanthine. The other reactions were carried out in a SPRINT buffer with the indicated variation. In the “20 mM HEPES” condition, the Tris buffer was replaced by 20 mM HEPES, pH 8.0; n = 1. (**C**) SPRINT reactions were carried out with the guanine riboswitch *xpt/pbuE**6U in modified SPRINT buffer with or without BSA. The riboswitch was induced with +/- 100 µM hypoxanthine. Bars indicate mean value and error bars indicate s.d. from the mean. All fluorescence measurements are background subtracted; n = 3. (**D**) SPRINT reactions were carried out with the guanine riboswitch *xpt/pbuE**6U in modified SPRINT buffer at varying concentrations of dimethyl sulfoxide (DMSO). The riboswitch was induced with +/- 10 µM guanine; n = 3.

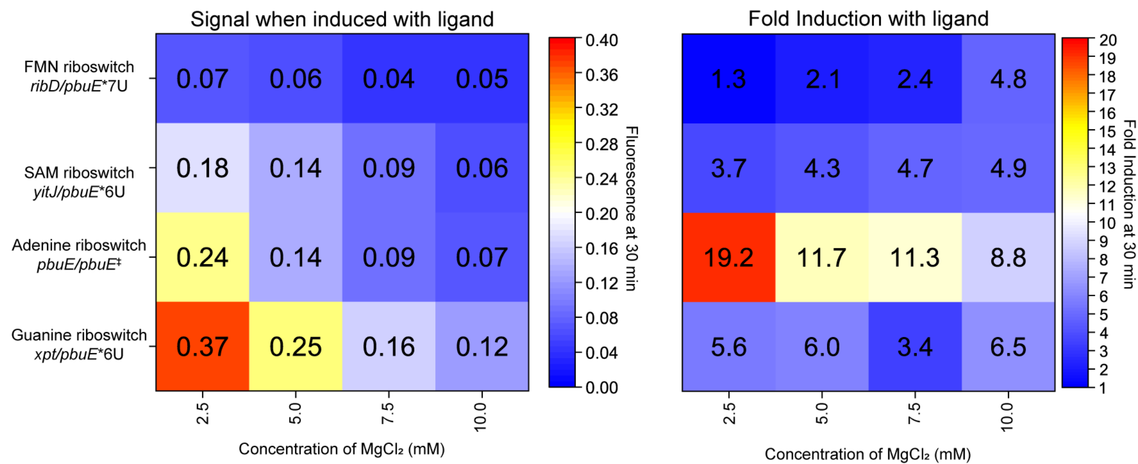
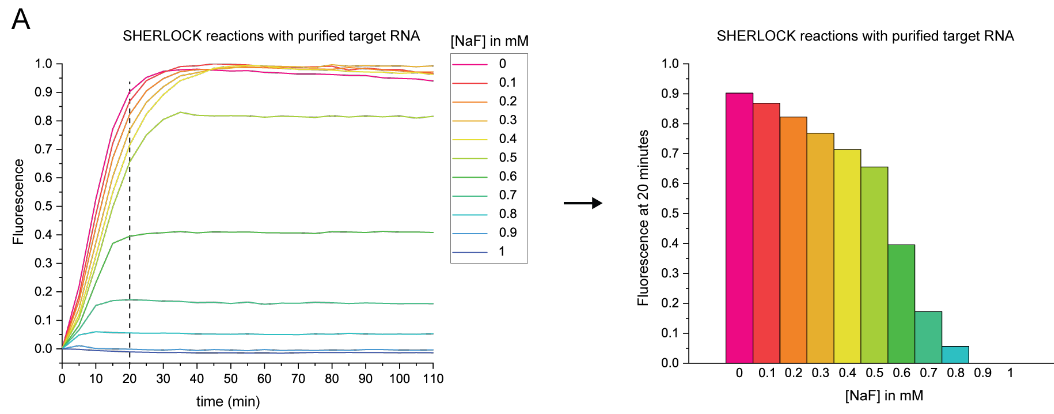
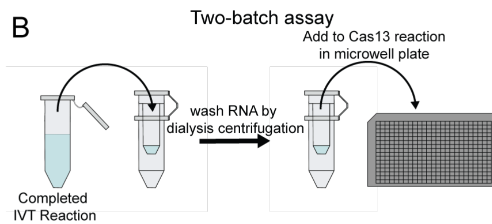
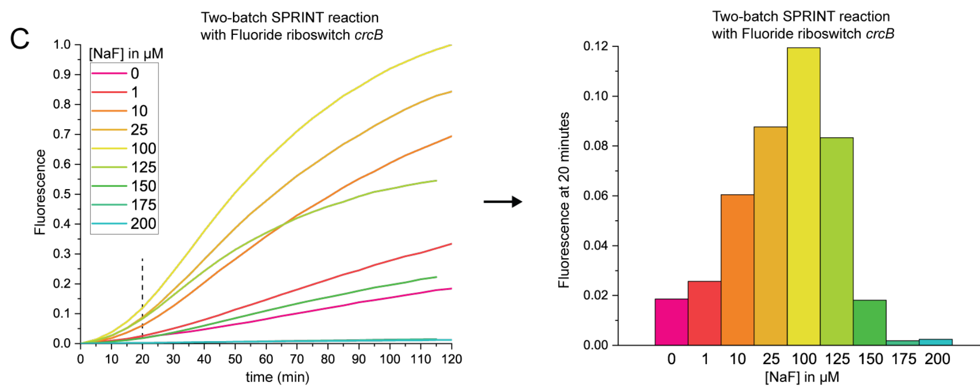
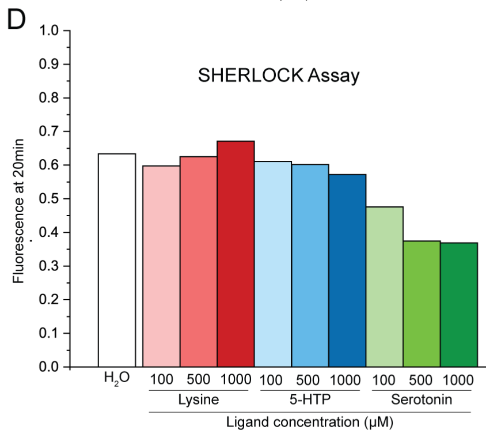


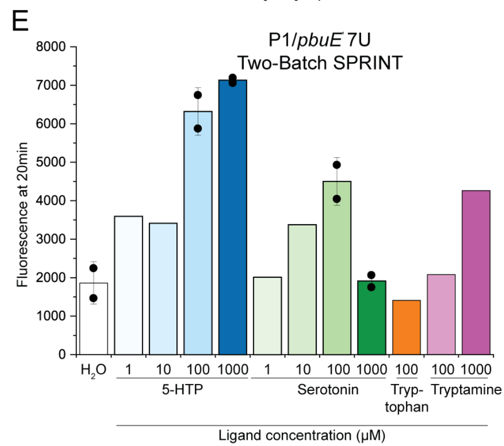
Figure S2: **Effect of $MgCl_2$ on riboswitch activation**. The FMN riboswitch was induced with +/- 100 µM FMN, the SAM riboswitch was induced with +/- 100 µM SAM, the adenine riboswitch was induced with 1 mM adenine, the guanine riboswitch was induced with +/- 100 µM guanine. Left panel shows the fluorescent signal after 30 minutes reaction time with ligand. Right panel shows the fold induction at 30 minutes reaction time. All fluorescence measurements are background subtracted and normalized to 125 nM fluorescein. Values indicate the mean; n = 3.


A
SHERLOCK reactions with purified target RNA
[NaF] in mM
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Fluorescence
time (min)
SHERLOCK reactions with purified target RNA
Fluorescence at 20 minutes
[NaF] in mM


B
Two-batch assay
Add to Cas13 reaction
in microwell plate
wash RNA by
dialysis centrifugation
Completed
IVT Reaction


C
Two-batch SPRINT reaction
with Fluoride riboswitch crcB
[NaF] in μM
0
1
10
25
100
125
150
175
200
Fluorescence
time (min)
Two-batch SPRINT reaction
with Fluoride riboswitch crcB
Fluorescence at 20 minutes
[NaF] in μM


D
SHERLOCK Assay
Fluorescence at 20min
H2O
100
500
1000
Lysine
5-HTP
Serotonin
Ligand concentration (μM)


E
P1/pbuE 7U
Two-Batch SPRINT
Fluorescence at 20min
H2O
5-HTP
Serotonin
Tryp-
tophan
Tryptamine
Ligand concentration (μM)

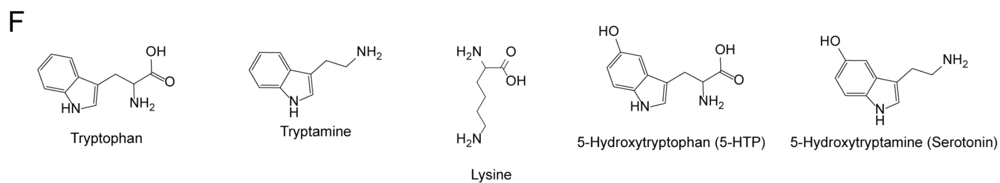
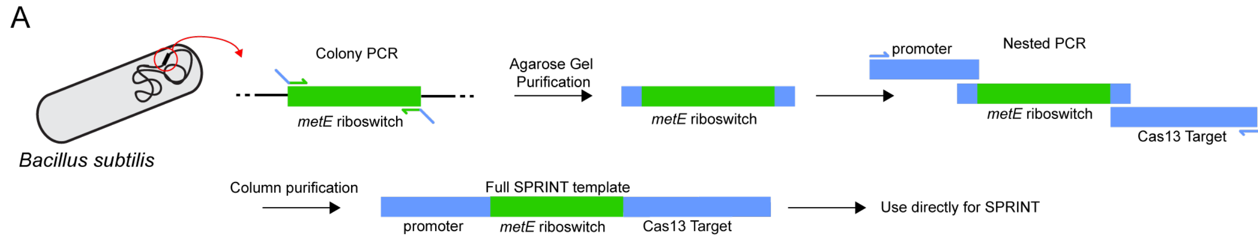

F
Tryptophan
Tryptamine
Lysine
5-Hydroxytryptophan (5-HTP)
5-Hydroxytryptamine (Serotonin)

Figure S3: **Effect of fluoride and serotonin on *E. coli* RNA polymerase and LwaCas13a**. (**A**) Cas13a in SHERLOCK reactions was activated with 10 nM of purified target RNA. All fluorescence measurements are background subtracted and normalized to 125 nM fluorescein; n = 1. (**B**) Workflow for two-batch SPRINT reactions. First, *in vitro* transcriptions are carried out. Then transcripts are washed three times with water. Next, washed transcription product is added to SHERLOCK reactions to quantify RNA. (**C**) SPRINT reactions with the fluoride riboswitch *crcB*; n = 1. (**D**) SHERLOCK reactions with addition of various small molecules; n = 1. (**E**) Ligands were added to the transcription step, washed out and the transcription product was added to a SHERLOCK reaction. Bars indicate mean value and error bars indicate s.d. from the mean; n = 1-2. (**F**) Molecular structures of tested ligands, drawn with ChemDraw 19.0. All fluorescence measurements were background subtracted and all except (E) were normalized to 125 nM fluorescein.



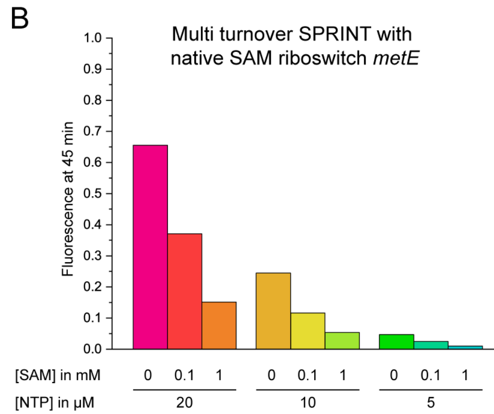


Figure S4: **SPRINT assays with the *metE* riboswitch**. (**A**) Workflow for preparing SPRINT template with a genomic riboswitch. First, the riboswitch is amplified by PCR from genome and overhangs are created. In a second PCR, the overhangs are used to attach a *tac* promoter at the 5'-end and the Cas13a target transcript at the 3'-end. After column purification of the PCR product, the DNA can be used for SPRINT assays. (**B**) Repression of the riboswitch was measured without heparin at different SAM concentrations and varied NTP concentrations; n = 1.

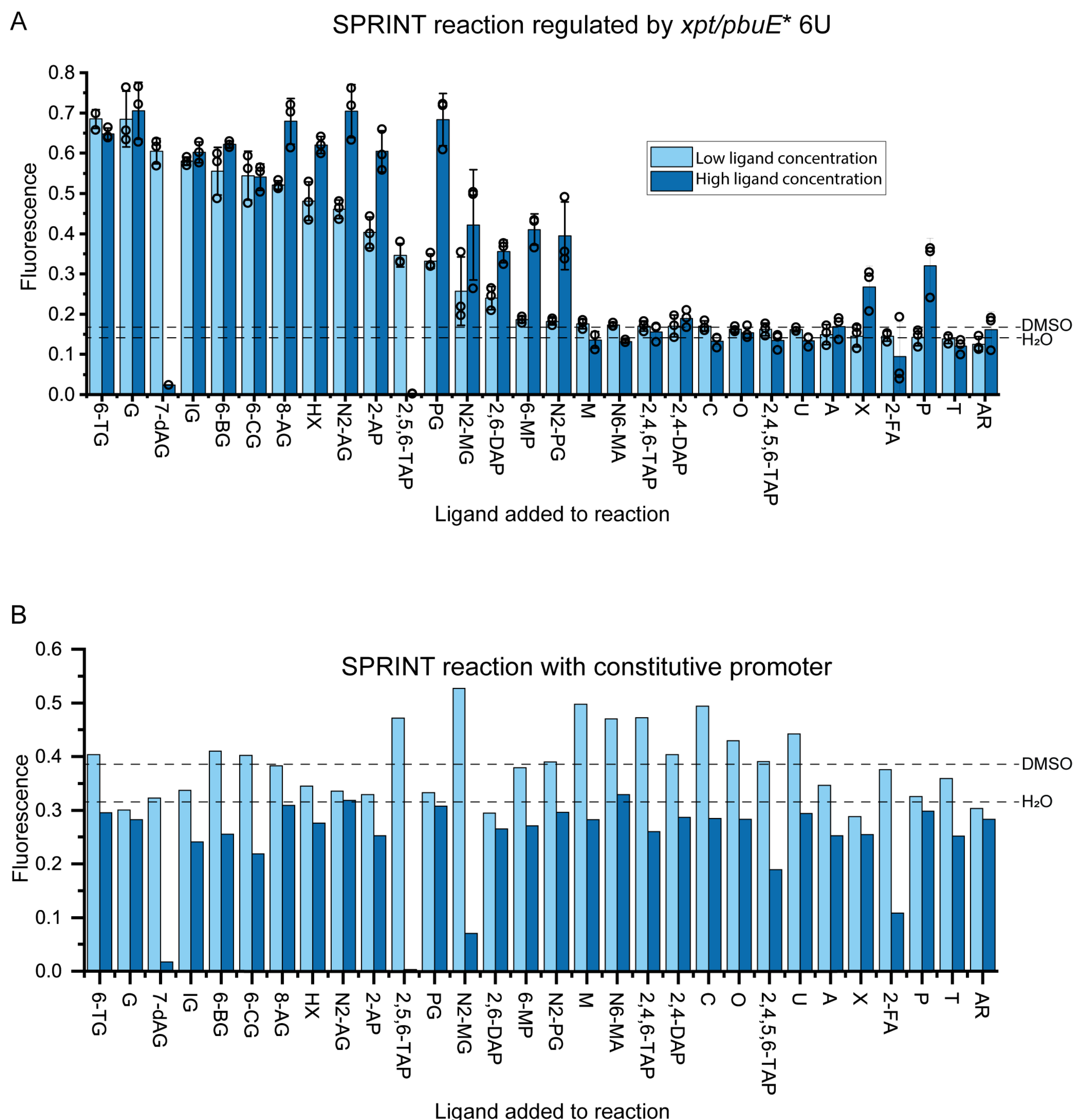


Figure S5: **Screen of 30 compounds for their effect on the guanine riboswitch *xpt/pbuE**6U**. Fluorescent signal of the SPRINT reactions was measured at 20 minutes reaction time, background was subtracted, and signal normalized to 125 nM fluorescein. Dashed lines indicate value of signal that was measured with just solvent. Full names of the abbreviated compounds and exact concentrations can be found in Table S3. (**A**) The guanine riboswitch *xpt/pbuE**6U regulated transcription in response to 30 different compounds. Bars indicate mean value and error bars indicate s.d. from the mean; n = 3. (**B**) The effect of the compounds on transcription from a constitutive promoter was measured. n = 1.

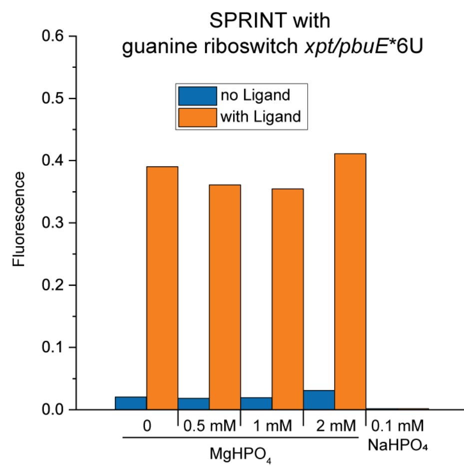


Figure S6: **Effect of phosphate salts on SPRINT reaction.** Addition of $MgHPO_4$ did not affect response of guanine riboswitch but $NaHPO_4$ inhibited the reaction entirely. Ligand was 100 µM guanine; n = 1.

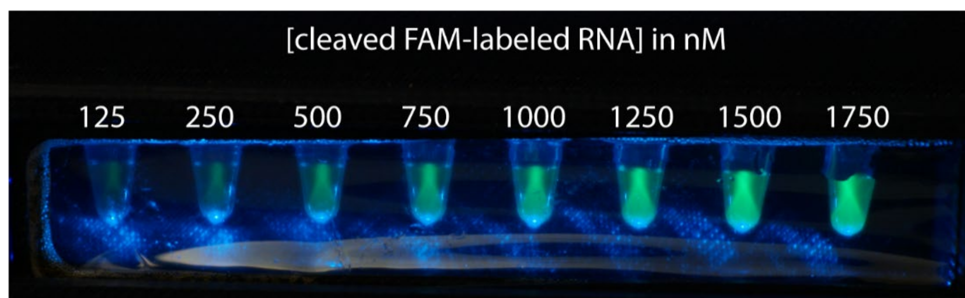


Figure S7: **Fluorescently labeled RNA oligonucleotides in the portable illuminator device**. Concentrations of fully cleaved RNA oligos were varied. All reactions were carried out in SPRINT buffer.

Tables

Table S1: DNA sequences used in this study. Sequences for promoters are marked yellow, riboswitches green, crRNA cyan, Cas13a target site in magenta, aTF operator sequences red. Aptamer sequences were highlighted in **bold**.

Description	**Sequence** (5' to 3')
pUC plasmid containing the sequence for a SPRINT template with the *xpt/pbuE**6U riboswitch	Sequence can be accessed under https://benchling.com/s/seq-UVhTBzr2J14rEuLO8FPc
pUC plasmid containing the sequence for a SPRINT template with the operator sequence for smtB (zinc aTF)	Sequence can be accessed under https://benchling.com/s/seq-F2XaV8atiR8Jqz1PhtWq
SPRINT template constant 5' region with tac promoter for *E. coli* RNAP	GCTAGCCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTGACAGGCATGCATAAGGCTCGTATAATATATTCa
SPRINT template constant 5' region with T7 promoter	CTAAGGATGATTTCTGGAATTCTAAAGATCTTAATACGACTCACTATAGGGA
SPRINT template constant 3' region with sequence for Target RNA (ssRNA1)	GGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATGGATTACTTGGTAGAACAGCAATCTACTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAG
DNA template for transcription of crRNA against ssRNA1	GGCGGCGAATTCTAATACGACTCACTATAGGGGAAGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACCTACCAAGTAATCCATATTTCTAGAGGATC
Full length SPRINT template	
none (constitutive tac promoter)	GCTAGCCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTGACAGGCATGCATAAGGCTCGTATAATATATTCGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATGGATTACTTGgtAGAACAGCAATCTACTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAG

xpt/*pbuE** 6U (guanine riboswitch)	GCTAGCCACAGCTAACACCACGTCGTCCCTATCTGCTGCC CTAGGTCTATGAGTGGTTGCTGGATAACTTGACAGGCATG CATAAGGCTCGTATAATATATTCaAATTAAATAGCTATTATC ACGATTTT**TATAATCGCGTGGATATGGCACGCAAGTTTCT** **ACCGGGCACCGTAAATGTCCGACTA**AAAATCCTGATTAC AAAATTTGTTTATGACATTTTTGTAATCAGGATTTTTTATT TATCAAAACATTTAAGTAAAGGAGTTTGTTATGTTTTTTTTG GCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGA AATATGGATTACTTGGgtAGAACAGCAATCTACTCGACCTGC AGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTC CTGTGTTTATCCGCTCACAATTCCACACAACATACGAGCC GGAAGCATAAAG
ribD/*pbuE** 7U (FMN riboswitch)	GCTAGCCACAGCTAACACCACGTCGTCCCTATCTGCTGCC CTAGGTCTATGAGTGGTTGCTGGATAACTTGACAGGCATG CATAAGGCTCGTATAATATATTCaAATTAAATAGCTATTATC ACGATTTTT**CGGGGCAGGGTGGAAATCCCGACCGGCGG** **TAGTAAAGCACATTTGCTTTAGAGCCCGTGACCCGTGTG** **CATAAGCACGCGGTGGATTCAGTTTAAGCTGAAGCCGAC** **AGTGAAAGTCTGGATGGGAG**AAAAATCCTGATTACAAAAT TTGTTTATGACATTTTTGTAATCAGGATTTTTTATTTATC AAAACATTTAAGTAAAGGAGTTTGTTATGTTTTTTTTGGCC AGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATA TGGATTACTTGGgtAGAACAGCAATCTACTCGACCTGCAGGC ATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGT GTTTATCCGCTCACAATTCCACACAACATACGAGCCGGAA GCATAAAG
yitJ/*pbuE** 6U (SAM riboswitch)	GCTAGCCACAGCTAACACCACGTCGTCCCTATCTGCTGCC CTAGGTCTATGAGTGGTTGCTGGATAACTTGACAGGCATG CATAAGGCTCGTATAATATATTCaAATTAAATAGCTATTATC ACGATTTT**ATCAAGAGAAGCAGAGGGACTGGCCCGACG** **AAGCTTCAGCAACCGGTGTAATGGCGATCAGCCATGACC** **AAGGTGCTAAATCCAGCAAGCTCGAACAGCTTGGAAGAT** AAAATCCTGATTACAAAATTTGTTTATGACATTTTTGTAAT CAGGATTTTTTATTTATCAAAACATTTAAGTAAAGGAGTTT GTTATGTTTTTTTTGGCCAGTGAATTCGAGCTCGGTACCC GGGGATCCTCTAGAAATATGGATTACTTGGgtAGAACAGCAA TCTACTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATG GTCATAGCTGTTTCCTGTGTTTATCCGCTCACAATTCCACA CAACATACGAGCCGGAAGCATAAAG
pbuE/*pbuE*[‡] (adenine riboswitch)	GCTAGCCACAGCTAACACCACGTCGTCCCTATCTGCTGCC CTAGGTCTATGAGTGGTTGCTGGATAACTTGACAGGCATG CATAAGGCTCGTATAATATATTCaCACTTG**TATAACCTCAA** **TAATATGGTTTGAGGGTGTCTACCAGGAACCGTAAAATC** **CTGATTA**CAAGCCGTTTTTCGGCTTGTAATCAGGATTTTT TTTGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTC TAGAAATATGGATTACTTGGgtAGAACAGCAATCTACTCGAC

	CTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTG TTTCCTGTGTTTATCCGCTCACAATTCCACACAACATACGA GCCGGAAGCATAAAG
crcB (fluoride riboswitch)	GCTAGCCACAGCTAACACCACGTCGTCCCTATCTGCTGCC CTAGGTCTATGAGTGGTTGCTGGATAACTTGACAGGCATG CATAAGGCTCGTATAATATATTCTAGGCGATGGAGTTCGC CATAAACGCTGCTTAGCTAATGACTCCTACCAGTATCACTA CTGGTAGGAGTCTATTTTTTTGGCCAGTGAATTCGAGCTC GGTACCCGGGGATCCTCTAGAAATATGGATTACTTGGTAG AACAGCAATCTACTCGACCTGCAGGCATGCAAGCTTGGC GTAATCATGGTCATAGCTGTTTCCTGTGTTTATCCGCTCAC AATTCCACACAACATACGAGCCGGAAGCATAAAG
metE (SAM riboswitch)	GCTAGCCACAGCTAACACCACGTCGTCCCTATCTGCTGCC CTAGGTCTATGAGTGGTTGCTGGATAACTTGACAGGCATG CATAAGGCTCGTATAATATATTCCAAAAAATTAATAACATTT TCTCTTATCGAGAGTTGGGCGAGGGATTGGCCTTTTGACC CCAACAGCAACCGACCGTAATACCATTGTGAAATGGGGC GCACTGCTTTTCGCGCCGAGACTGATGTCTCATAAGGCAC GGTGCTAATTCCATCAGATTGTGTCTGAGAGATGAGAGAG GCAGTGTTTTACGTAGAAAAGCCTCTTTCTCTCATGGGAA AGAGGCTTTTTGGCCAGTGAATTCGAGCTCGGTACCCGG GGATCCTCTAGAAATATGGATTACTTGGTAGAACAGCAAT CTACTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATG GTCATAGCTGTTTCCTGTGTTTATCCGCTCACAATTCCACA CAACATACGAGCCGGAAGCATAAAG
none (constitutive T7 promoter)	CTAAGGATGATTTCTGGAATTCTAAAGATCTTAATACGACT CACTATAGGGGCCAGTGAATTCGAGCTCGGTACCCGGGG ATCCTCTAGAAATATGGATTACTTGGTAGAACAGCAATCTA CTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTC ATAGCTGTTTCCTGTGTTTATCCGCTCACAATTCCACACAA CATACGAGCCGGAAGCATAAAG
Zinc aTF smtB	CTAAGGATGATTTCTGGAATTCTAAAGATCTTAATACGACT CACTATAGGGACACATGAACAGTTATTCAGATAGGCCAGT GAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATG GATTACTTGGTAGAACAGCAATCTACTCGACCTGCAGGCA TGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTG TTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAG CATAAAG
tetracycline aTF tetR	CTAAGGATGATTTCTGGAATTCTAAAGATCTTAATACGACT CACTATAGGGATCCCTATCAGTGATAGAGAGGCCAGTGAA TTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATGGATT ACTTGGTAGAACAGCAATCTACTCGACCTGCAGGCATGCA AGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTTTAT CCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAA AG

Table S2: Oligonucleotides used in this study

Name of oligonucleotide	**Sequence** (5' to 3')
InsulatorOligo_fwd	GCTAGCCACAGCTAACACCACGTC
ssDNA1_rev	CTTTATGCTTCCGGCTCGTATGTTGTG
CreateSeq_fwd	CTAAGGATGATTTCTGGAATTC
pbuEBB_fwd	ATTTATCAAAACATTTAAGTAAAGGAGTTTG
pbuEBB_rev	AATAGCTATTTAATTTGAATATATTATACGAGCC
CreateSeq_rev	CGCTTCTGCGTTCTG
RiboswitchBB_fwd	GGCCAGTGAATTCGAGCTCG
e.coliProm_rev	GAATATATTATACGAGCCTTATGCATG
CreateBBnogRNA2	GAAGCTTGGGCCCGAACAAAAAC
HA_rev	AGATCTTTAGAATTCCAGAAATCATC
crRNA1_rev	GATCCTCTAGAAATATGGATTACTTGGTAGG
5' gen	GGCGGCGAATTCTAATACGACTCACTATAG
metE_fwd	GCATAAGGCTCGTATAATATATTCCAAAAAATTAATAACATT TTCTCTTATCGAGAGTTG
metE_rev	CTCATGGGAAAGAGGCTTTTTGGCCAGTGAATTCGAG

Table S3: Chemical compounds screened against *xpt*/*pbuE**6U. Limited solubility of some compounds required the use of lower concentrations.

Full Name	Abbreviation	Solvent	Low concentration	High concentration	Published K_D values in µM (Refs.)
6-Thioguanine (2-amino-6-thiopurine)	6-TG	140 mM NaOH	10 µM	1 mM	1 (1)
Guanine	G	DMSO	10 µM	1 mM	0.004 ± 0.003 (1, 2)
7-deazaguanine	7-dAG	DMSO	10 µM	1 mM	0.0495 ± 0.013 (2)
Isobutyrylguanine	IG	DMSO	10 µM	1 mM	
6-bromoguanine (2-amino-6-bromopurine)	6-BG	DMSO	10 µM	1 mM	2.1 ± 0.6 (3)
6-chloroguanine	6-CG	140 mM NaOH	10 µM	1 mM	0.89 ± 0.06 (3)
8-aminoguanine	8-AG	Water	10 µM	1 mM	
hypoxanthine	HX	DMSO	10 µM	1 mM	0.759 ± 0.066 (2)
N2-acetylguanine	N2-AG	DMSO	1 µM	100 µM	
2-aminopurine	2-AP	water	10 µM	1 mM	4.4 ± 1.2 (3)
2,5,6-Triaminopyrimidin-4(3H)-one	2,5,6-TAP	DMSO	1 µM	100 µM	
N2-phenoxyacetylguanine	PG	DMSO	1 µM	100 µM	
N2-methylguanine	N2-MG	280 mM NaOH	10 µM	1 mM	0.1 (1)
2,6-diaminopurine	2,6-DAP	water	10 µM	1 mM	4.1 ± 0.6 (3)
6-mercaptopurine	6-MP	DMSO	10 µM	1 mM	
N2-pivaloylguanine	N2-PG	DMSO	10 µM	1 mM	
Melamine	M	Water	10 µM	1 mM	
N6-methyladenine	N6-MA	DMSO	10 µM	1 mM	
2,4,6-triaminopyrimidine	2,4,6-TAP	Water	10 µM	1 mM	
2,4-diaminopyrimidine	2,4-DAP	Water	10 µM	1 mM	
Cytosine	C	Water	10 µM	1 mM	
Orotate	O	DMSO	10 µM	1 mM	
2,4,5,6-tetraaminopyrimidine	2,4,5,6-TAP	Water	10 µM	1 mM	
Uracil	U	Water	10 µM	1 mM	
Adenine	A	water	10 µM	1 mM	>300 (1, 3)
Xanthine	X	water	10 µM	1 mM	39 ± 4.2 (3)
2-fluoroadenine	2-FA	DMSO	10 µM	1 mM	
Purine	P	Water	10 µM	1 mM	
Theophylline	T	Water	10 µM	1 mM	
Adenosine	AR	Water	10 µM	1 mM	

Supplementary References

1. Mandal,M., Boese,B., Barrick,J.E., Winkler,W.C. and Breaker,R.R. (2003) Riboswitches Control Fundamental Biochemical Pathways in Bacillus subtilis and Other Bacteria. *Cell*, **113**, 577–586.

2. Gilbert,S.D., Stoddard,C.D., Wise,S.J. and Batey,R.T. (2006) Thermodynamic and Kinetic Characterization of Ligand Binding to the Purine Riboswitch Aptamer Domain. *Journal of Molecular Biology*, **359**, 754–768.

3. Gilbert,S.D., Reyes,F.E., Edwards,A.L. and Batey,R.T. (2009) Adaptive Ligand Binding by the Purine Riboswitch in the Recognition of Guanine and Adenine Analogs. *Structure*, **17**, 857–868.