Modularity of select riboswitch expression platform enables facile engineering of novel genetic regulatory devices

Supporting Information

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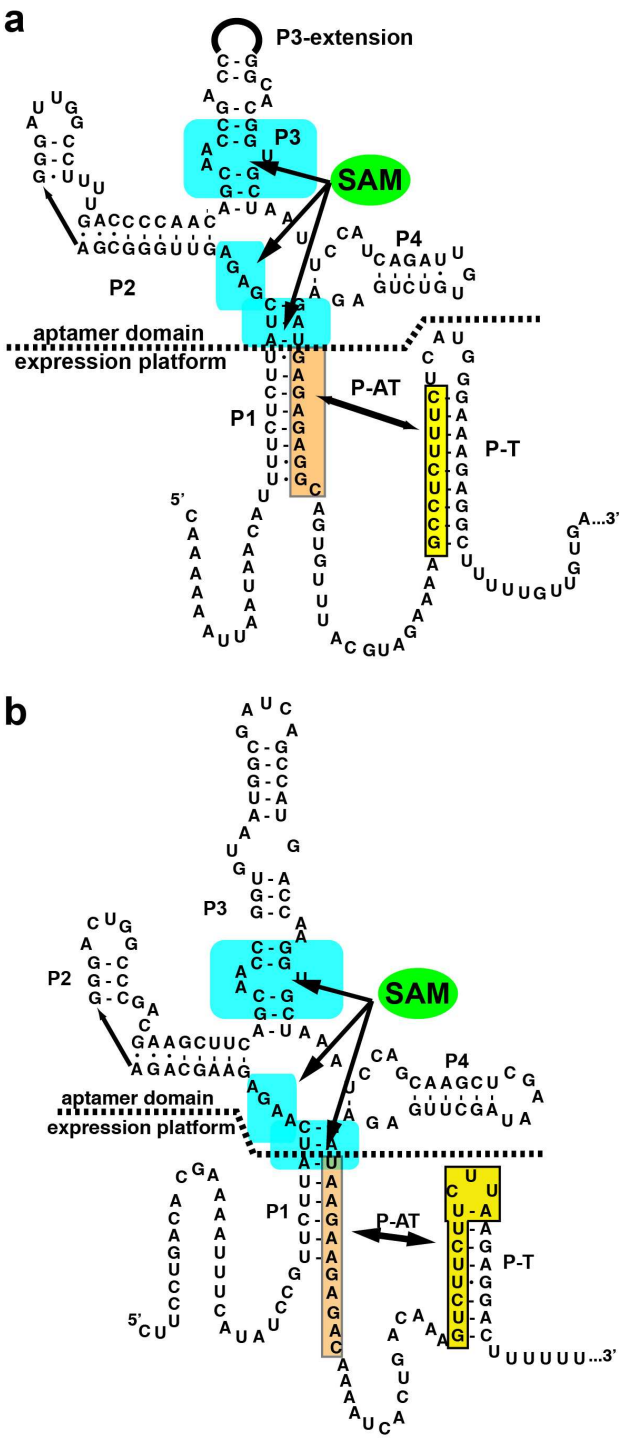
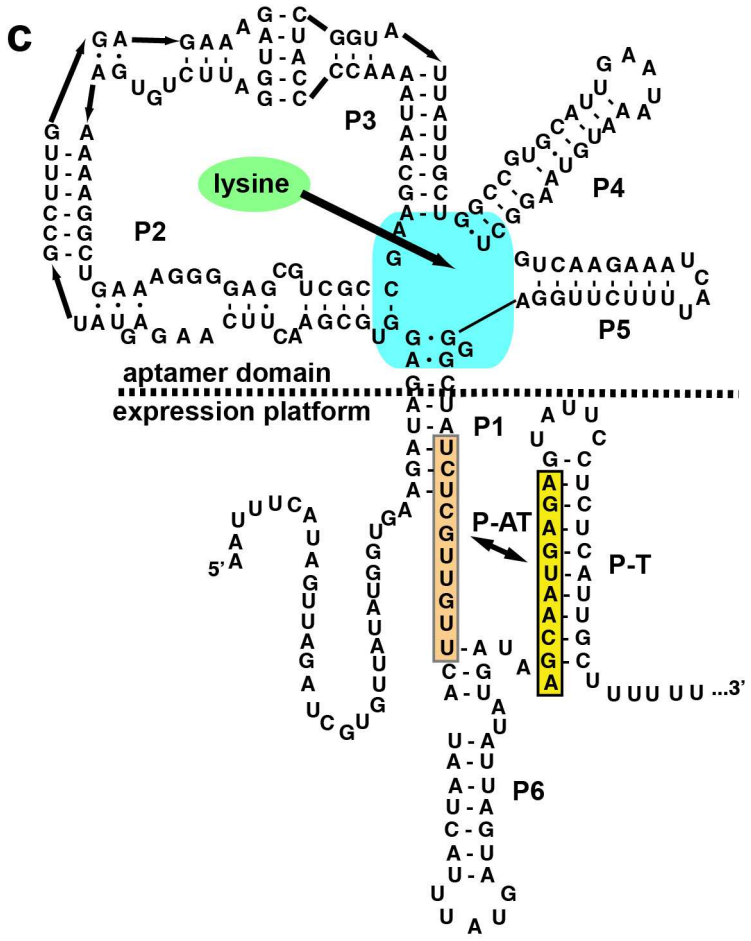
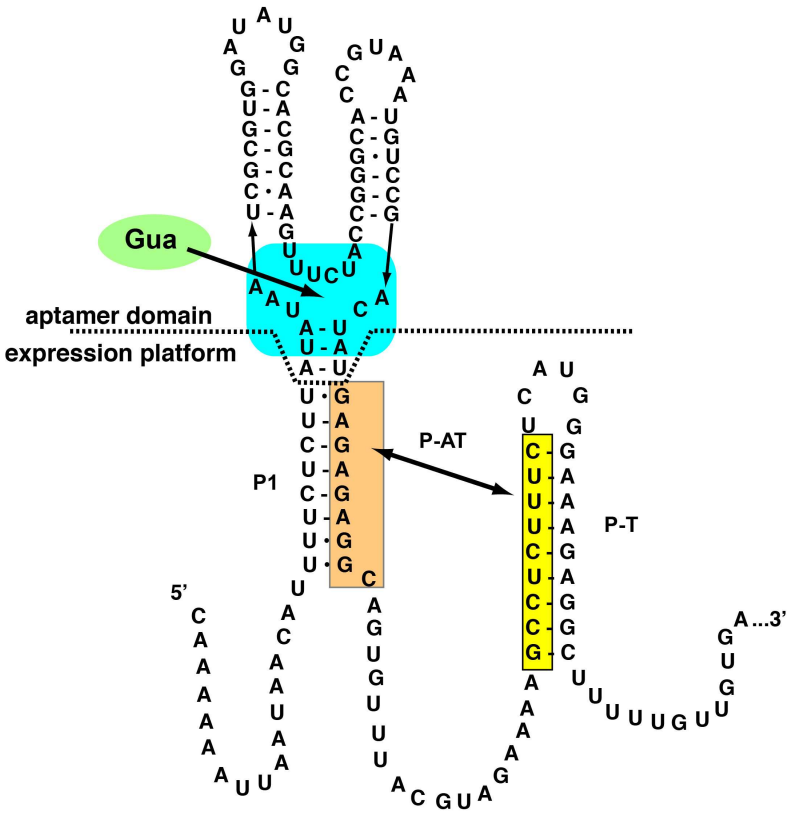
SUPPLEMENTARY FIGURES



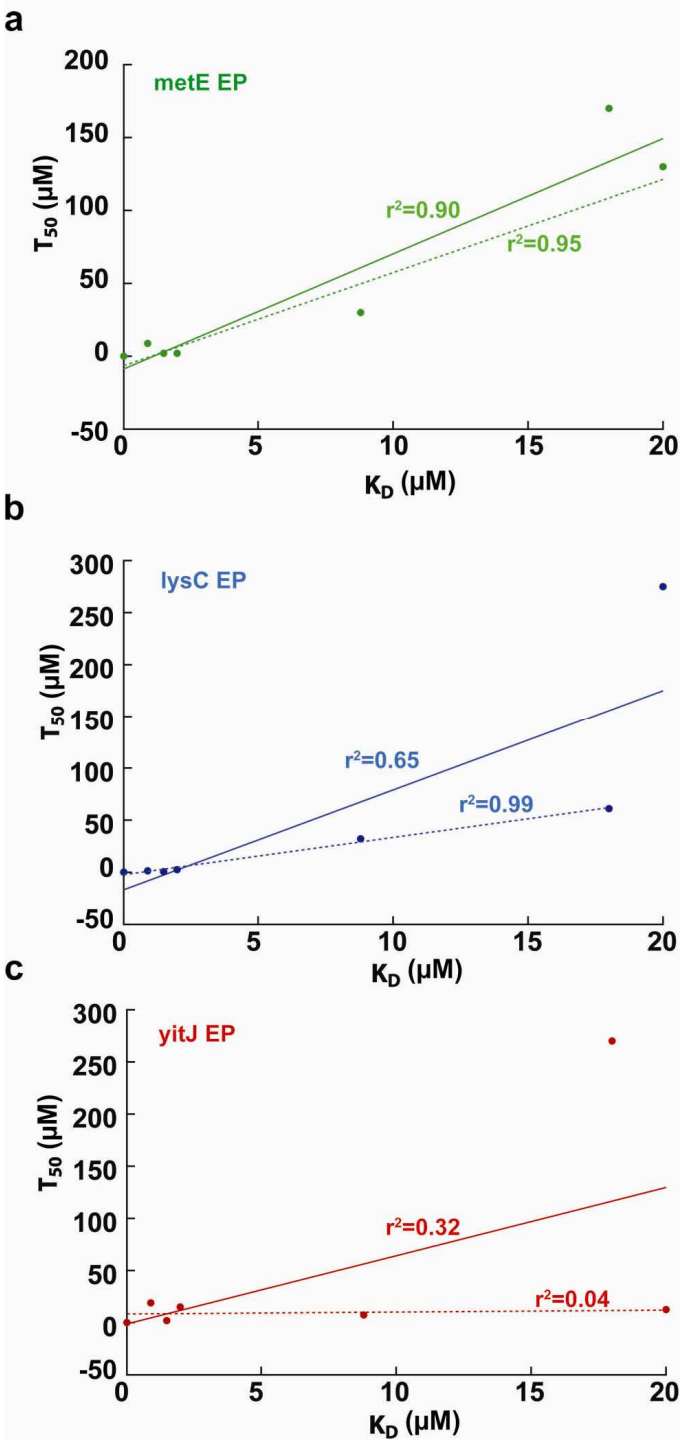
Supplementary Figure 1. Continued on the next page.



Supplementary Figure 1, continued. Sequence and secondary structure of (a) the *Bacillus subtilis metE* SAM-I, (b) *yitJ* SAM-I (b) the *lysC* lysine riboswitches used in this study. Nucleotides directly involved in ligand binding (green) are denoted by cyan rectangles. Sequence involved in formation of alternative secondary structures (P-T, intrinsic terminator; P-AT, antiterminator) are denoted with orange and yellow boxes. The boundary between the two domains as considered in this study to create chimeras is denoted by a dashed line.

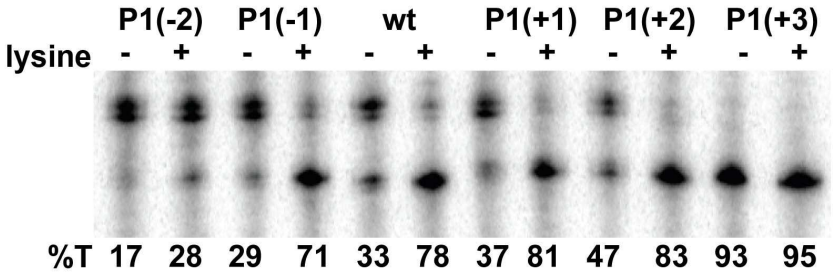


Supplementary Figure 2. Sequence and secondary structure of the chimera between the *B. subtilis xpt-pbuX* guanine riboswitch aptamer domain and the *B. subtilis metE* SAM-I riboswitch expression platform.

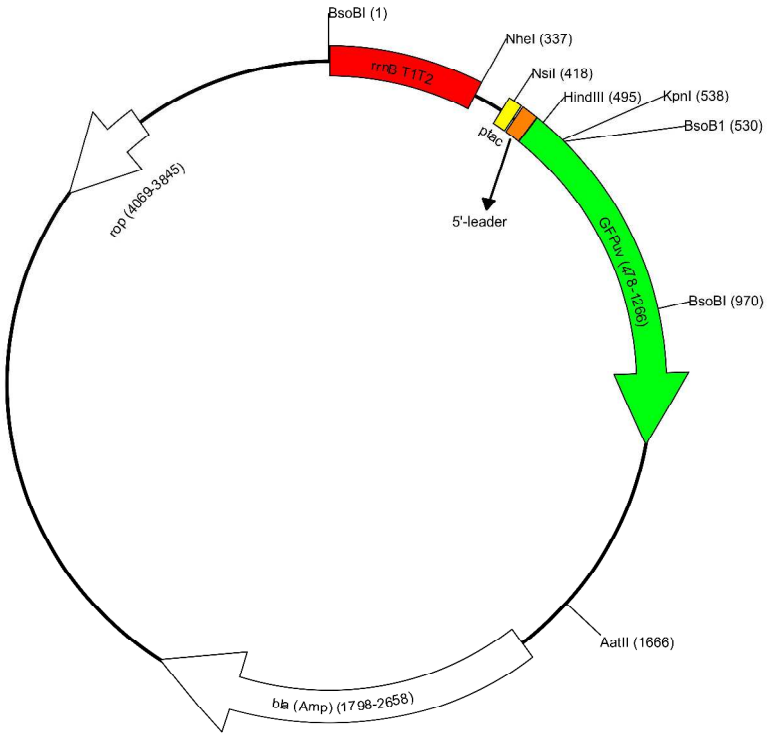


Supplementary Figure 3. Comparison of the T_{50} of each wild type or chimeric riboswitch determined by the in vitro transcription assay and the K_D of the aptamer domain as measured by ITC. The solid line represents the linear fit to all of the data whereas the dashed line is the fit to all the data except for the point that has the largest variance. While riboswitches containing the *metE* (a) and *lysC* (b) expression platforms show a strong correlation between T_{50} and K_D, that of *yitJ* is very weak (as judged from

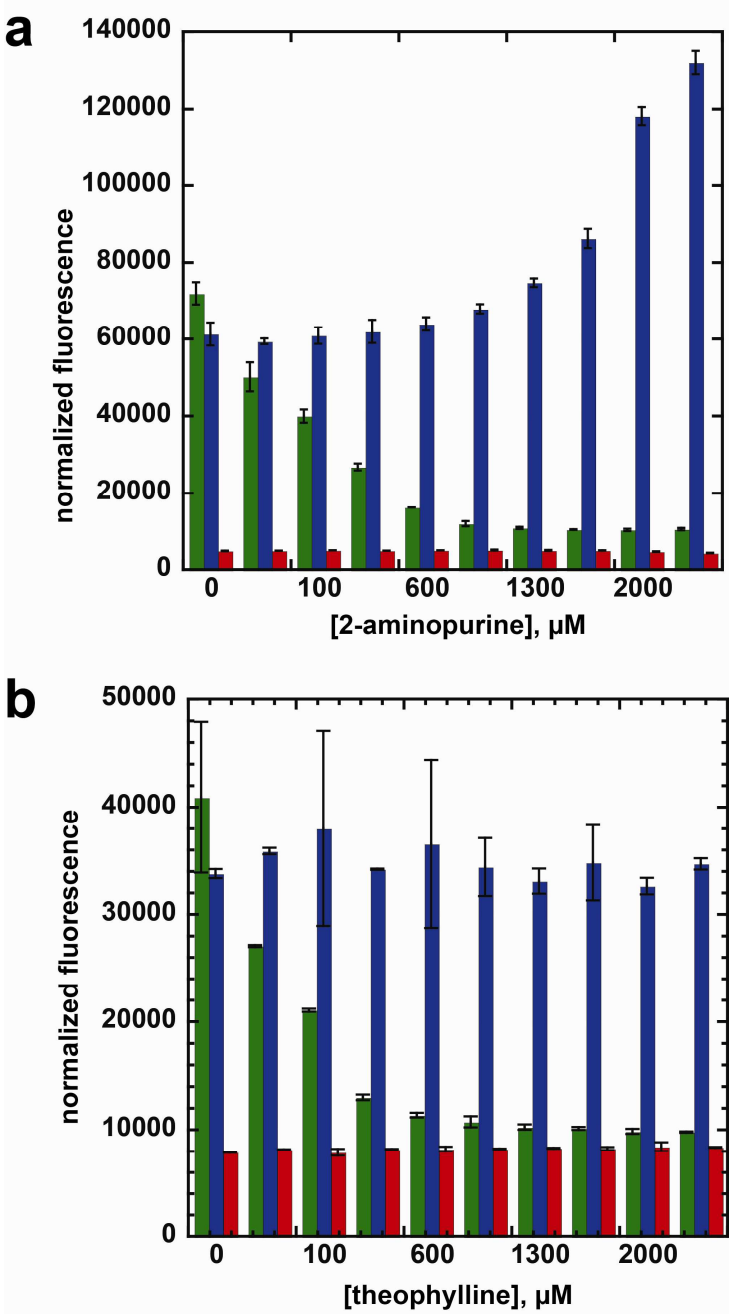
the r^2 of the fit).



Supplementary Figure 4. Lysine-dependent transcriptional termination of the *B. subtilis lysC* lysine riboswitch as a function of the length of the P1 helix. Point mutations were introduced into the 5'-side of the P1 helix to either disrupt Watson-Crick pairs (P1(-1), P1(-2)) or introduced additional pairs (P1(+1), P1(+2), P1(+3)) (see sequence in Supplementary Table 1). Transcription reactions were performed in the absence (-) or presence (+) of lysine. For each RNA, the percent termination in the absence and presence (1mM) of the ligand are given as the average of three independent experiments.



Supplementary Figure 5. Map of the parental riboswitch reporter plasmid (pRR1). The plasmid is derived from pBR322, from which the tetracycline resistance gene was removed and replaced with a gene encoding GFPuv (green) under control of a constitutively expressed *tac* promoter (yellow). The sequence immediately upstream of the *tac* promoter is an "insulator" sequence preceded by a strong *rrnB* T_1T_2 terminator (red). All riboswitch leader sequences (orange) were cloned between the *Nsi*I and *Hind*III sites. The sequence of the full plasmid is given in Supplementary Table 2.



Supplementary Figure 6. Raw normalized fluorescence data for graphs shown in Figure 5b, d. (a) Raw normalized data for titration of the *xpt*(C74U)/*metE* (green) and *xpt*(C74U,U51C)/*metE* (blue) reporter with 2-aminopurine in the defined growth medium. Red bars represent the background fluorescence as measured using the parental pBR322 plasmid with no GFPuv reporter. Note that while the U51C negative control displays a ~2-fold increase in total fluorescence at high 2-AP concentrations, this transition occurs at higher 2-AP concentrations that the repression of the reporter. (b) Raw normalized data for titration of the *theo*/*metE* (green) and *theo*(U24A)/*metE* (blue) reporters with theophylline in the defined growth medium. Red bars represent the pBR322 background control. Error bars represent the standard deviation of at least 3 independent measurements.

Supplementary Table 1: Sequences of riboswitches and chimeras used in this study.

Name	Sequence[1]
***lysC* expression platform chimeras**	
lysC wild type	AATTTCATAGTTAGATCGTGTTATATGGTGAAGATAGAGGTGCGAACT TCAAGAGTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTGAA AGGGGAGCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGCTG GCCGTGCATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTGGA GGGCTATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCA ATGAGAGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCT TTCTCCTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
lysC (P1-3)	AATTTCATAGTTAGATCGTGTTATATGGTGATCTTAGAGGTGCGAACT TCAAGAGTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTGAA AGGGGAGCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGCTG GCCGTGCATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTGGA GGGCTATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCA ATGAGAGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCT TTCTCCTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
lysC (P1-2)	AATTTCATAGTTAGATCGTGTTATATGGTGATCATAGAGGTGCGAACT TCAAGAGTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTGAA AGGGGAGCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGCTG GCCGTGCATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTGGA GGGCTATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCA ATGAGAGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCT TTCTCCTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
lysC (P1-1)	AATTTCATAGTTAGATCGTGTTATATGGTGATGATAGAGGTGCGAACT TCAAGAGTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTGAA AGGGGAGCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGCTG GCCGTGCATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTGGA GGGCTATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCA ATGAGAGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCT TTCTCCTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
lysC (P1+1)	AATTTCATAGTTAGATCGTGTTATATGGTGGAGATAGAGGTGCGAACT TCAAGAGTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTGAA AGGGGAGCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGCTG GCCGTGCATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTGGA GGGCTATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCA ATGAGAGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCT TTCTCCTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
lysC (P1+2)	AATTTCATAGTTAGATCGTGTTATATGGTCGAGATAGAGGTGCGAACT TCAAGAGTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTGAA AGGGGAGCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGCTG GCCGTGCATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTGGA GGGCTATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCA ATGAGAGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCT TTCTCCTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
lysC (P1+3)	AATTTCATAGTTAGATCGTGTTATATGGACGAGATAGAGGTGCGAACT TCAAGAGTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTGAA AGGGGAGCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGCTG GCCGTGCATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTGGA GGGCTATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCA ATGAGAGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCT TTCTCCTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG

metE/lysC	AATTTCATAGTTAGATCGTGTTATATGGTGAAGATAATCGAGAGTTGG GCGAGGGATTGGCCTTTTGACCCCAACAGCAACCGACCGTAATACCAT TGTGAAATGGGGCGCACTGCTTTTCGCGCCGAGACTGATGTCTCATAA GGCACGGTGCTAATTCCATCAGATTGTGTCTGAGAGATTATCTCGTTG TTCATAATCATTTATGATGATTAATTGATAAGCAATGAGAGTATTCCT CTCATTGCTTTTTTTATTGTGGACAAAGCGCTCTTTCTCCTCACCCGC ACGAACCAAAATGTAAAGGGTGGTAATACATG
yitJ/lysC	AATTTCATAGTTAGATCGTGTTATATGGTGAAGATATCAAGAGAAGCA GAGGGACTGGCCCGACGAAGCTTCAGCAACCGGTGTAATGGCGATCAG CCATGACCAAGGTGCTAAATCCAGCAAGCTCGAACAGCTTGGAAGATA TCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCAATGAGA GTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCTTTCTCC TCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
xpt/lysC	AATTTCATAGTTAGATCGTGTTATATGGTGAAGATAATATAATCGCGT GGATATGGCACGCAAGTTTCTACCGGGCACCGTAAATGTCCGACTATT ATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCAATGAG AGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCTTTCTC CTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
xpt(C74U)/lysC	AATTTCATAGTTAGATCGTGTTATATGGTGAAGATAATATAATCGCGT GGATATGGCACGCAAGTTTCTACCGGGCACCGTAAATGTCCGATTATT ATCTCGTTGTTCATAATCATTTATGATGATTAATTGATAAGCAATGAG AGTATTCCTCTCATTGCTTTTTTTATTGTGGACAAAGCGCTCTTTCTC CTCACCCGCACGAACCAAAATGTAAAGGGTGGTAATACATG
ribD/lysC	AATTTCATAGTTAGATCGTGTTATATGGTGAAGATATCGGGGCAGGGT GGAAATCCCGACCGGCGGTAGTAAAGCACATTTGCTTTAGAGCCCGTG ACCCGTGTGCATAAGCACGCGGTGGATTCAGTTTAAGCTGAAGCCGAC AGTGAAAGTCTGGATGGGAGATATCTCGTTGTTCATAATCATTTATGA TGATTAATTGATAAGCAATGAGAGTATTCCTCTCATTGCTTTTTTTAT TGTGGACAAAGCGCTCTTTCTCCTCACCCGCACGAACCAAAATGTAAA GGGTGGTAATACATG
theo/lysC	AATTTCATAGTTAGATCGTGTTATATGGTGAAGATAATACCAGCTTCG AAAGAAGCCCTTGGCAGTATCTCGTTGTTCATAATCATTTATGATGAT TAATTGATAAGCAATGAGAGTATTCCTCTCATTGCTTTTTTTATTGTG GACAAAGCGCTCTTTCTCCTCACCCGCACGAACCAAAATGTAAAGGGT GGTAATACATG
***yitJ* expression platform chimeras**	
yitJ wild type	CTTCCTGACACGAAAATTTCATATCCGTTCTTATCAAGAGAAGCAGAG GGACTGGCCCGACGAAGCTTCAGCAACCGGTGTAATGGCGATCAGCCA TGACCAAGGTGCTAAATCCAGCAAGCTCGAACAGCTTGGAAGATAAGA AGAGACAAAATCACTGACAAAGTCTTCTTCTTAAGAGGACTTTTTTA TTTCTCTTTTTTCCTTGCTGATGTGAATAAAGGAGGCAGACAATG
metE/yitJ	CTTCCTGACACGAAAATTTCATATCCGTTCTTAATCGAGAGTTGGGCG AGGGATTGGCCTTTTGACCCCAACAGCAACCGACCGTAATACCATTGT GAAATGGGGCGCACTGCTTTTCGCGCCGAGACTGATGTCTCATAAGGC ACGGTGCTAATTCCATCAGATTGTGTCTGAGAGATTAAGAAGAGACAA AATCACTGACAAAGTCTTCTTCTTAAGAGGACTTTTTTTATTTCTCTT TTTTCCTTGCTGATGTGAATAAAGGAGGCAGACAATG
xpt/yitJ	CTTCCTGACACGAAAATTTCATATCCGTTCTTAATATAATCGCGTGGA TATGGCACGCAAGTTTCTACCGGGCACCGTAAATGTCCGACTATTAAG AAGAGACAAAATCACTGACAAAGTCTTCTTCTTAAGAGGACTTTTTTT ATTTCTCTTTTTTCCTTGCTGATGTGAATAAAGGAGGCAGACAATG
xpt(C74U)/yitJ	CTTCCTGACACGAAAATTTCATATCCGTTCTTAATATAATCGCGTGGA TATGGCACGCAAGTTTCTACCGGGCACCGTAAATGTCCGATTATTAAG AAGAGACAAAATCACTGACAAAGTCTTCTTCTTAAGAGGACTTTTTTT ATTTCTCTTTTTTCCTTGCTGATGTGAATAAAGGAGGCAGACAATG

lysC/yitJ	CTTCCTGACACGAAAATTTCATATCCGTTCTTAAGATAGAGGTGCGAA CTTCAAGAGTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTG AAAGGGGAGCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGC TGGCCGTGCATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTG GAGGGCTATCTTAAGAAGAGACAAAATCACTGACAAAGTCTTCTTCTT AAGAGGACTTTTTTTATTTCTCTTTTTTCCTTGCTGATGTGAATAAAG GAGGCAGACAATG
ribD/yitJ	CTTCCTGACACGAAAATTTCATATCCGTTCTTATCGGGGCAGGGTGGA AATCCCGACCGGCGGTAGTAAAGCACATTTGCTTTAGAGCCCGTGACC CGTGTGCATAAGCACGCGGTGGATTCAGTTTAAGCTGAAGCCGACAGT GAAAGTCTGGATGGGAGATAAGAAGAGACAAAATCACTGACAAAGTCT TCTTCTTAAGAGGACTTTTTTTATTTCTCTTTTTTCCTTGCTGATGTG AATAAAGGAGGCAGACAATG
theo/yitJ	CTTCCTGACACGAAAATTTCATATCCGTTCTTAATACCAGCTTCGAAA GAAGCCCTTGGCAGTAAGAAGAGACAAAATCACTGACAAAGTCTTCTT CTTAAGAGGACTTTTTTTATTTCTCTTTTTTCCTTGCTGATGTGAATA AAGGAGGCAGACAATG
***metE* expression platform chimeras**	
metE wild type[2]	CAAAAAATTAATAACATTTTCTCTTATCGAGAGTTGGGCGAGGGATTG GCCTTTTGACCCCAACAGCAACCGACCGTAATACCATTGTGAAATGGG GCGCACTGCTTTTCGCGCCGAGACTGATGTCTCATAAGGCACGGTGCT AATTCCATCAGATTGTGTCTGAGAGATGAGAGAGGCAGTGTTTTACGT AGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAAA ACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATAT AAAGGAGGAGAAACAATG
metE (P1-2)	CAAAAAATTAATAACATGGTCTCTTATCGAGAGTTGGGCGAGGGATTG GCCTTTTGACCCCAACAGCAACCGACCGTAATACCATTGTGAAATGGG GCGCACTGCTTTTCGCGCCGAGACTGATGTCTCATAAGGCACGGTGCT AATTCCATCAGATTGTGTCTGAGAGATGAGAGAGGCAGTGTTTTACGT AGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAAA ACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATAT AAAGGAGGAGAAACAATG
metE (P1-1)	CAAAAAATTAATAACATGTTCTCTTATCGAGAGTTGGGCGAGGGATTG GCCTTTTGACCCCAACAGCAACCGACCGTAATACCATTGTGAAATGGG GCGCACTGCTTTTCGCGCCGAGACTGATGTCTCATAAGGCACGGTGCT AATTCCATCAGATTGTGTCTGAGAGATGAGAGAGGCAGTGTTTTACGT AGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAAA ACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATAT AAAGGAGGAGAAACAATG
metE (P1+1)	CAAAAAATTAATAACAGTTTCTCTTATCGAGAGTTGGGCGAGGGATTG GCCTTTTGACCCCAACAGCAACCGACCGTAATACCATTGTGAAATGGG GCGCACTGCTTTTCGCGCCGAGACTGATGTCTCATAAGGCACGGTGCT AATTCCATCAGATTGTGTCTGAGAGATGAGAGAGGCAGTGTTTTACGT AGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAAA ACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATAT AAAGGAGGAGAAACAATG
metE (P1+2)	CAAAAAATTAATAACTGTTTCTCTTATCGAGAGTTGGGCGAGGGATTG GCCTTTTGACCCCAACAGCAACCGACCGTAATACCATTGTGAAATGGG GCGCACTGCTTTTCGCGCCGAGACTGATGTCTCATAAGGCACGGTGCT AATTCCATCAGATTGTGTCTGAGAGATGAGAGAGGCAGTGTTTTACGT AGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAAA ACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATAT AAAGGAGGAGAAACAATG

yitJ/metE	CAAAAAATTAATAACATTTTCTCTTTCAAGAGAAGCAGAGGGACTGGC CCGACGAAGCTTCAGCAACCGGTGTAATGGCGATCAGCCATGACCAAG GTGCTAAATCCAGCAAGCTCGAACAGCTTGGAAGAGAGAGAGGCAGTG TTTTACGTAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTT GTGAGAAAACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTT TTACATATAAAGGAGGAGAAACAATG
xpt/metE	CAAAAAATTAATAACATTTTCTCTTATATAATCGCGTGGATATGGCAC GCAAGTTTCTACCGGGCACCGTAAATGTCCGACTATGAGAGAGGCAGT GTTTTACGTAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGT TGTGAGAAAACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGT TTTACATATAAAGGAGGAGAAACAATG
xpt/metE (P1-2)	CAAAAAATTAATAACATGGTCTCTTATATAATCGCGTGGATATGGCAC GCAAGTTTCTACCGGGCACCGTAAATGTCCGACTATGAGAGAGGCAGT GTTTTACGTAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGT TGTGAGAAAACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGT TTTACATATAAAGGAGGAGAAACAATG
xpt/metE (P1-1)	CAAAAAATTAATAACATGTTCTCTTATATAATCGCGTGGATATGGCAC GCAAGTTTCTACCGGGCACCGTAAATGTCCGACTATGAGAGAGGCAGT GTTTTACGTAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGT TGTGAGAAAACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGT TTTACATATAAAGGAGGAGAAACAATG
xpt/metE (P1+1)	CAAAAAATTAATAACAGTTTCTCTTATATAATCGCGTGGATATGGCAC GCAAGTTTCTACCGGGCACCGTAAATGTCCGACTATGAGAGAGGCAGT GTTTTACGTAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGT TGTGAGAAAACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGT TTTACATATAAAGGAGGAGAAACAATG
xpt/metE (P1+2)	CAAAAAATTAATAACTGTTTCTCTTATATAATCGCGTGGATATGGCAC GCAAGTTTCTACCGGGCACCGTAAATGTCCGACTATGAGAGAGGCAGT GTTTTACGTAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGT TGTGAGAAAACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGT TTTACATATAAAGGAGGAGAAACAATG
xpt(C74U)/metE	CAAAAAATTAATAACATTTTCTCTTATATAATCGCGTGGATATGGCAC GCAAGTTTCTACCGGGCACCGTAAATGTCCGATTATGAGAGAGGCAGT GTTTTACGTAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGT TGTGAGAAAACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGT TTTACATATAAAGGAGGAGAAACAATG
lysC/metE	CAAAAAATTAATAACATTTTCTCTTAGATAGAGGTGCGAACTTCAAGA GTATGCCTTTGGAGAAAGATGGATTCTGTGAAAAAGGCTGAAAGGGGA GCGTCGCCGAAGCAAATAAAACCCCATCGGTATTATTTGCTGGCCGTG CATTGAATAAATGTAAGGCTGTCAAGAAATCATTTTCTTGGAGGGCTA TCTGAGAGAGGCAGTGTTTTACGTAGAAAAGCCTCTTTCTCTCATGGG AAAGAGGCTTTTTGTTGTGAGAAAACCTCTTAGCAGCCTGTATCCGCG GGTGAAAGAGAGTGTTTTACATATAAAGGAGGAGAAACAATG
ribD/metE	CAAAAAATTAATAACATTTTCTCTTTCGGGGCAGGGTGGAAATCCCGA CCGGCGGTAGTAAAGCACATTTGCTTTAGAGCCCGTGACCCGTGTGCA TAAGCACGCGGTGGATTCAGTTTAAGCTGAAGCCGACAGTGAAAGTCT GGATGGGAGAGAGAGAGGCAGTGTTTTACGTAGAAAAGCCTCTTTCTC TCATGGGAAAGAGGCTTTTTGTTGTGAGAAAACCTCTTAGCAGCCTGT ATCCGCGGGTGAAAGAGAGTGTTTTACATATAAAGGAGGAGAAACAAT G
theo/metE	CAAAAAATTAATAACATTTTCTCTTATACCAGCTTCGAAAGAAGCCCT TGGCAGGAGAGAGGCAGTGTTTTACGTAGAAAAGCCTCTTTCTCTCAT GGGAAAGAGGCTTTTTGTTGTGAGAAAACCTCTTAGCAGCCTGTATCC GCGGGTGAAAGAGAGTGTTTTACATATAAAGGAGGAGAAACAATG

tet/metE	CAAAAAATTAATAACATTTTCTCTTGGGAGAGGTGAAGAATACGACCA CCTAGGTAGAAATACCTAAAACATACCCGAGAGAGGCAGTGTTTTACG TAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAA AACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATA TAAAGGAGGAGAAACAATG
tet/metE (P1-1)	CAAAAAATTAATAACATTTACTCTTGGGAGAGGTGAAGAATACGACCA CCTAGGTAGAAATACCTAAAACATACCCGAGAGAGGCAGTGTTTTACG TAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAA AACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATA TAAAGGAGGAGAAACAATG
tet/metE (P1-2)	CAAAAAATTAATAACATTTAGTCTTGGGAGAGGTGAAGAATACGACCA CCTAGGTAGAAATACCTAAAACATACCCGAGAGAGGCAGTGTTTTACG TAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAA AACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATA TAAAGGAGGAGAAACAATG
tet/metE (P1-3)	CAAAAAATTAATAACATTTAGGCTTGGGAGAGGTGAAGAATACGACCA CCTAGGTAGAAATACCTAAAACATACCCGAGAGAGGCAGTGTTTTACG TAGAAAAGCCTCTTTCTCTCATGGGAAAGAGGCTTTTTGTTGTGAGAA AACCTCTTAGCAGCCTGTATCCGCGGGTGAAAGAGAGTGTTTTACATA TAAAGGAGGAGAAACAATG

[1]The coloring scheme for the sequences is as follows: cyan, expression platform; green, aptamer domain; red, ATG start codon of first coding region; yellow, mutation introduced to a wild type sequence.
[2]The “wildtype” *metE* sequence used in this paper starts at the +11 nucleotide of the predicted start site of the *metE* transcript.

Supplementary Table 2: Complete sequence of pRR1

CTCGGGTTGCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGACCCCATGCCGAACTCAGAAGTGAA
ACGCCGTAGCGCCGATGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAA
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TCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGAT
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CAACTTGTCTGGTGTCAAAAATAATAGGCCTACTAGTCGGCCGTACGGGCCCTTTCGTCTCGCGC
GTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTG
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Supplementary Table 3: Defined medium used in this study.

Trace metals solution:

HCl	10% v/v
$FeCl_3$-$6H_2O$	10 mg/mL
$ZnCl_2$-$4H_2O$	2 mg/mL
$CoCl_2$-$6H_2O$	2 mg/mL
Na_2MoO_4-$2H_2O$	2 mg/mL
$CaCl_2$-$2H_2O$	1 mg/mL
$CuCl_2$	1 mg/mL
$MnCl_2$	1 mg/mL
H_3BO_3	0.5 mg/mL

CSB supplement:

Glucose	20% (w/v)
0.5 M disodium citrate	67 mg/mL
Trace metals solution	10% v/v
$MgSO_4$	3.3 mg/ml
Thiamine	0.33 mg/mL

CSB salts:

NaH_2PO_4	37 mM
K_2HPO_4	66 mM
$(NH_4)_2SO_4$	15 mM

CSB Media:

CSB salts	500 ml
CSB supplement	15 ml
10% Casamino acids (sterile)	5 ml