Supplementary Data

for

Guanidine biosensors enable comparison of cellular turn-on kinetics of riboswitch-based biosensor and reporter

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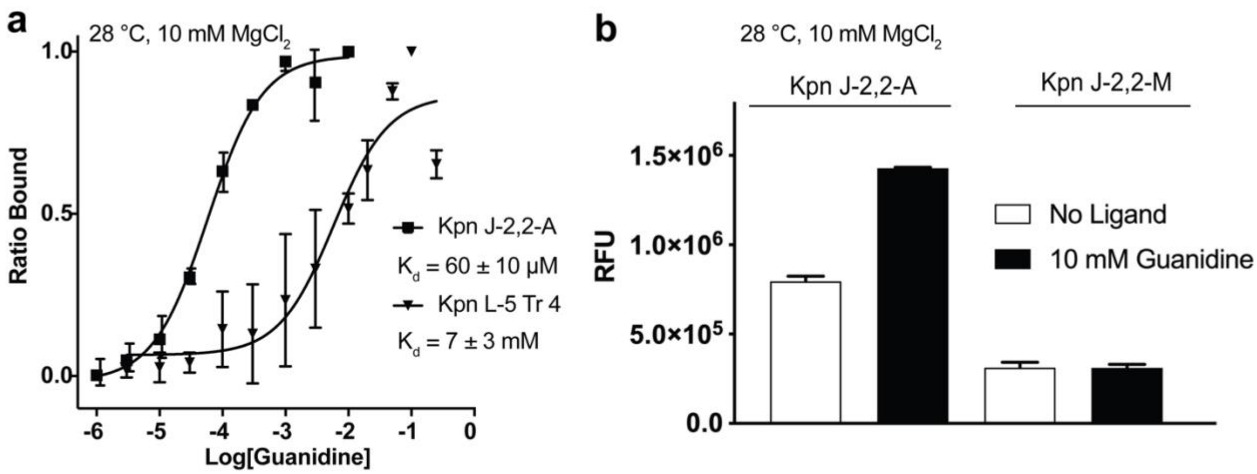

Figure S1. (a) Apparent dissociation constant (K_d) of representative junction (Kpn J-2,2-A) and linker (Kpn L-5 Tr4) biosensors towards guanidine. (b) Fluorescence of Kpn J-2,2-A and Kpn J-2,2-A M4 Mutant (J-2,2-M) in absence and presence of guanidine. Data shown are the average with standard error of the mean taken from at least two independent replicates.

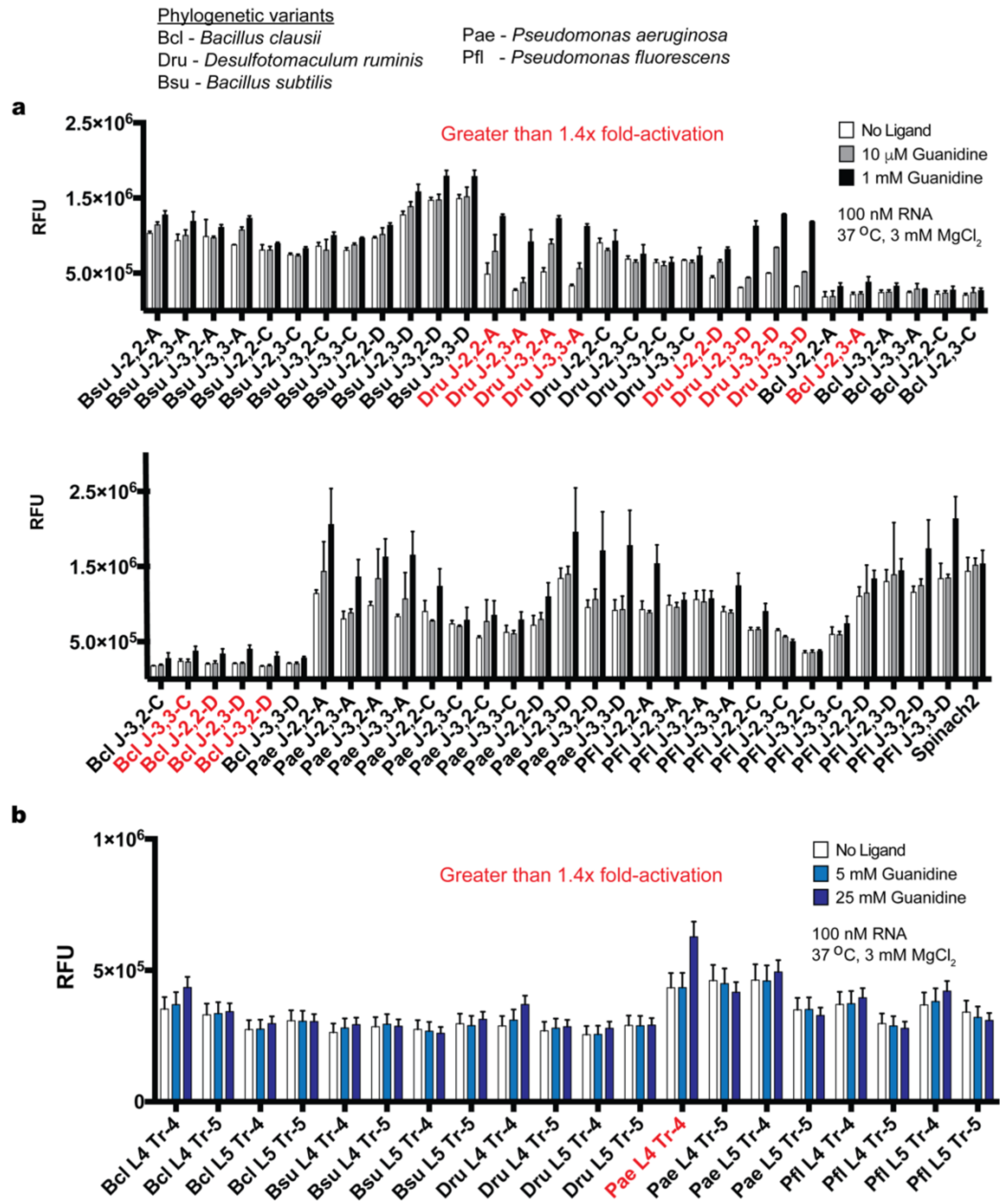

Figure S2. Screen of phylogenetic junction and linker biosensor libraries

Phylogenetic junction library consisting of 60 total biosensors from 5 riboswitch sequences with 13 constructs with greater than 1.4x fold activation (red). (b) Phylogenetic linker library consisting of 20 total biosensors from 5 riboswitch sequences with 1 construct with greater than 1.4x fold activation (red). Data shown are the average with standard error of the mean taken from three independent replicates

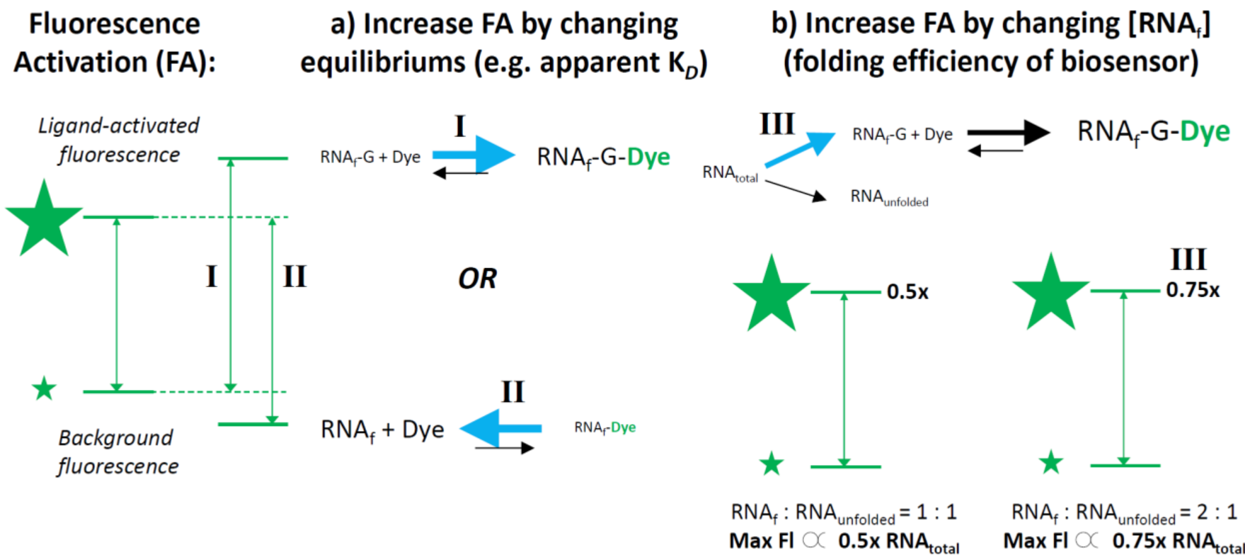

Figure S3. Three mechanisms that lead to increased fluorescence activation

(a) Fluorescence activation (FA) is the difference between background fluorescence (mainly due to dye binding to the RNA without ligand) and ligand-activated fluorescence (due to dye binding to the RNA-ligand complex). Observed FA can be increased either by increasing dye affinity to the RNA-ligand complex (**I**) or by decreasing dye affinity to the RNA alone (**II**). Our data indicate that Kpn and Dru biosensors have similar affinities, so their difference in FA is not due to these two mechanisms. (b) Instead, we expect that observed FA for Dru biosensors is increased due to higher folding efficiency (**III**). For RNA-based biosensors, the total RNA is partitioned into binding-competent folded states (RNA_f) and unfolded states ($RNA_{unfolded}$). Thus, the maximal fluorescence for a given biosensor construct is proportional to RNA_f and riboswitch sequences that fold better increase RNA_f and FA. As a note, background fluorescence does not change because it is independent of ligand binding.

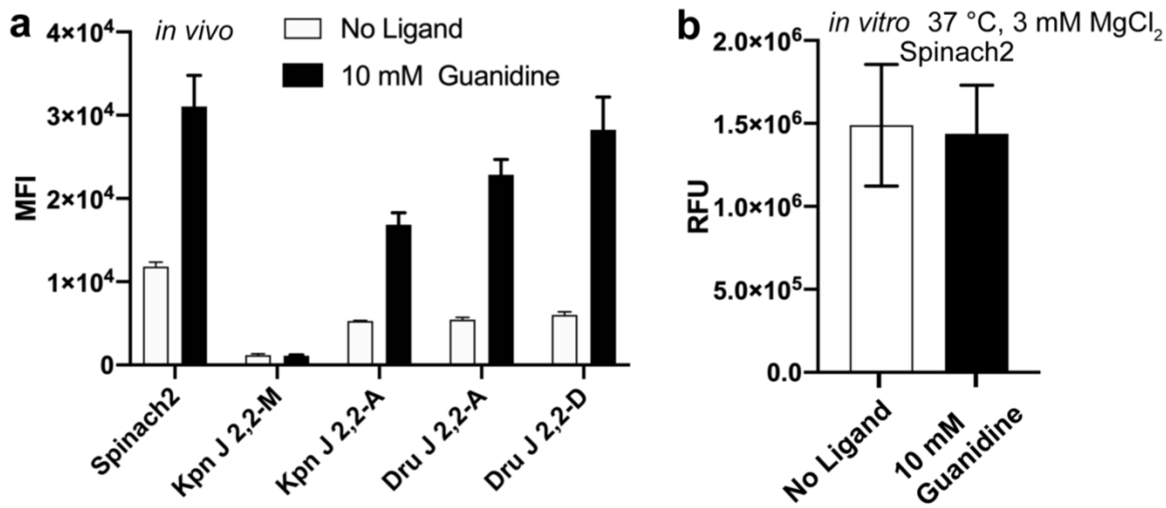

Figure S4. Non-specific fluorescence activation *in vivo* observed with 10 mM guanidine

(a) Live cell fluorescence measured by flow cytometry for *E. coli* BL21 (DE3) Star cells expressing RNA constructs in the absence or presence of 10 mM guanidine. (b) *In vitro* fluorescence for Spinach2 in the absence or presence of 10 mM guanidine.

At 10 mM of guanidine, we found that cells expressing Spinach2 also showed a significant increase in fluorescence (**Fig. S4a)**, even though Spinach2 RNA did not respond to 10 mM guanidine *in vitro* (**Fig. S4b**). One plausible explanation for this result is that guanidine at high concentration may have non-specific effects on the cell due to its chaotropic properties. We speculate that mild, non-lethal perturbation of the membrane itself, the membrane potential, or membrane proteins could increase permeability or decrease export of DFHBI-1T, leading to increased fluorescence. Under these conditions, cells expressing the biosensors still show greater fold activation than the background activation observed for Spinach2, which suggests that the biosensors still are binding and responding to guanidine. However, going forward, all subsequent experiments were performed with 500 μM of guanidine, which did not appear to have non-specific effects on cellular fluorescence.

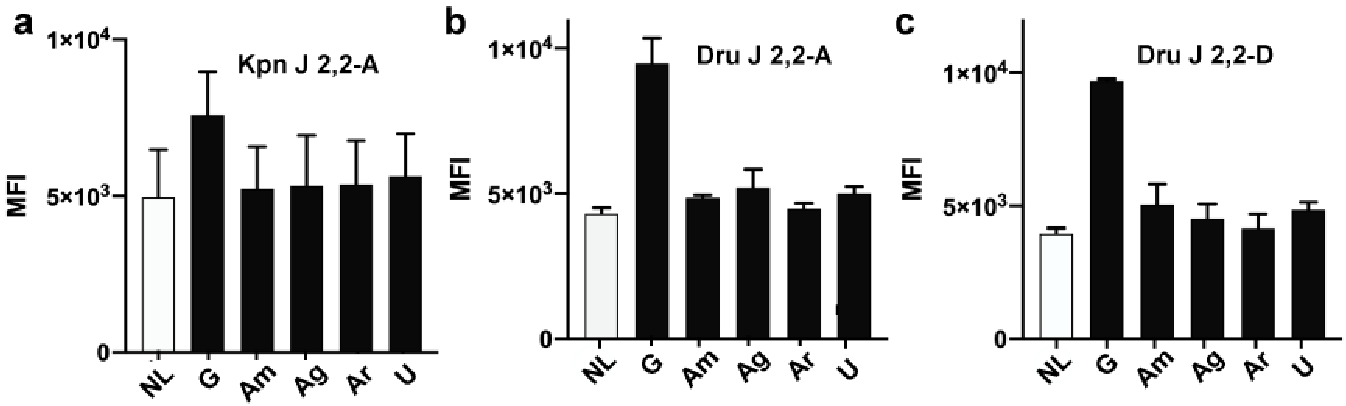

Figure S5. Ligand selectivity of guanidine biosensors *in vivo*

Live cell fluorescence measured by flow cytometry for *E. coli* BL21 (DE3) Star cells expressing (a) Kpn J 2,2-A, (b) Dru J 2,2-A, and (c) Dru J2,2-D biosensor RNA constructs in the absence or presence of 500 µM guanidine or structural analogs (see **Fig. 5**). Data shown are the average with standard deviation for three biological replicates.

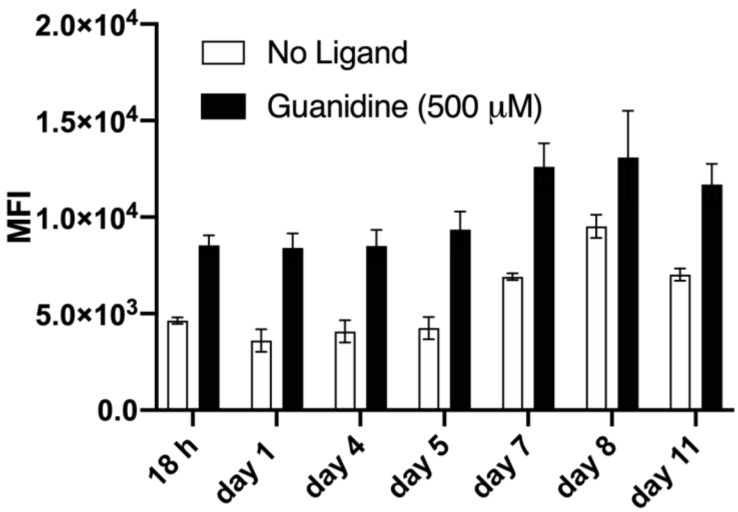

Figure S6. MFI values of the RBF biosensor Dru J 2,2-A in the presence and absence of guanidine after storage in NI media at 4 °C for number of days indicated. The data shown are the average with standard deviation of three biological replicates.

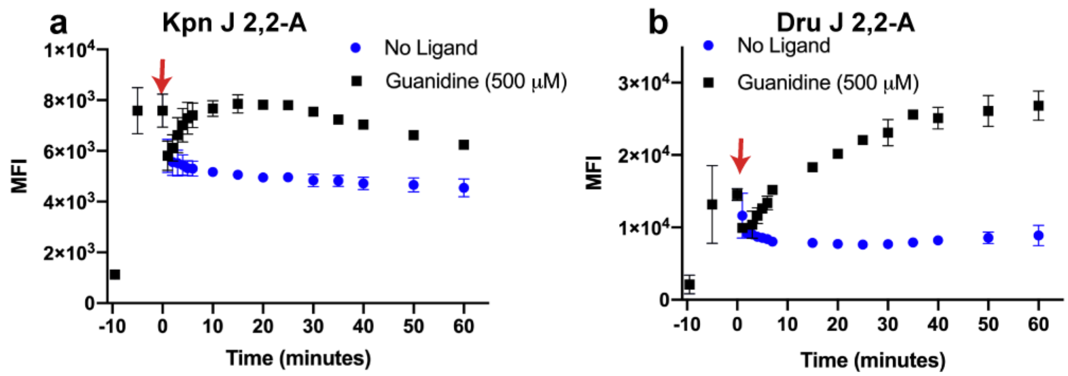

Figure S7. *In vivo* response kinetics of additional guanidine biosensors

Plot of average MFI values over time for cells expressing (a) Kpn J 2, 2-A or (b) Dru J 2, 2-D biosensors. Water or guanidine was added at time 0 (indicated by red arrow).

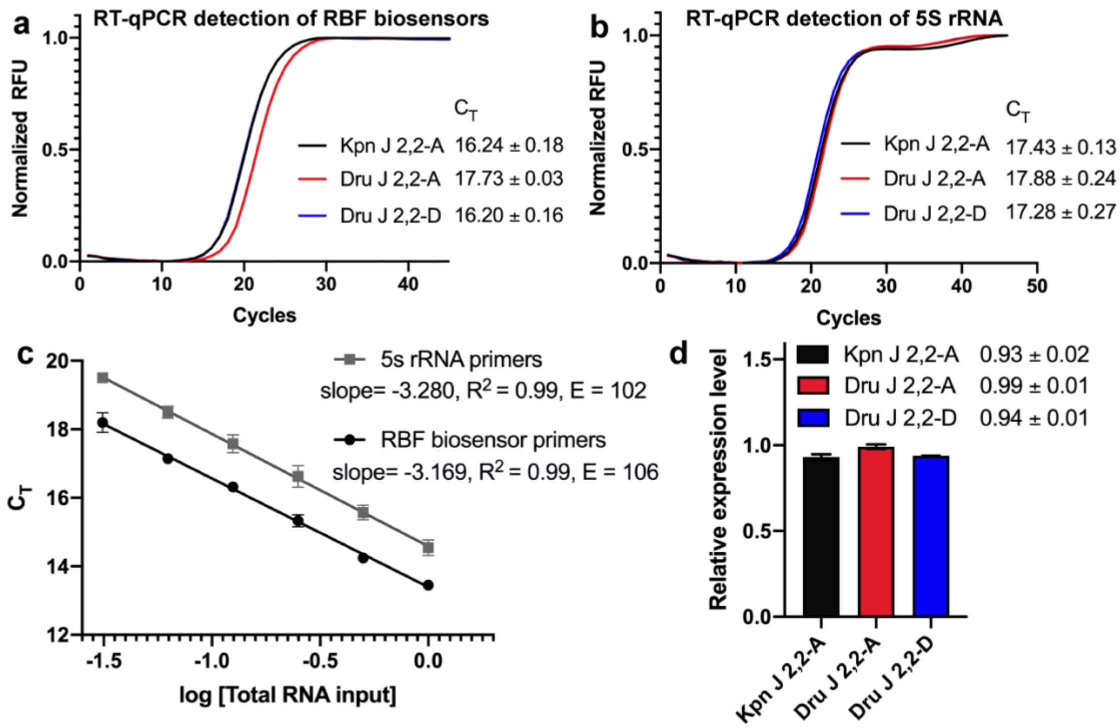

Figure S8. Quantification of relative *in vivo* expression levels of the guanidine biosensors

(a) qRT-PCR plot of the biosensors obtained for the amplification with 1:8000 dilution of the total RNA sample using the biosensor-specific primers. (b) qRT-PCR plot of the biosensors obtained for the amplification with 1:8000 dilution of the total RNA sample using the 5s rRNA-specific primers. A portion of the 5s rRNA was amplified as endogenous control to compare the amounts of RNA used in each amplicon.(c) Real-Time PCR Standard Curve with the samples of serial dilution (1: 1000 to 1: 32000 dilution) representing ~100% PCR efficiency for both sets of primers, biosensors-specific primers and 5s rRNA-specific primers. PCR efficiency for each set of primer was calculated by using the equation: E = $(10^{\wedge(-1/slope)} - 1)*100$ (d) Relative in vivo expression level of each biosensor. All reactions were performed in triplicate. For experimental details see method section.

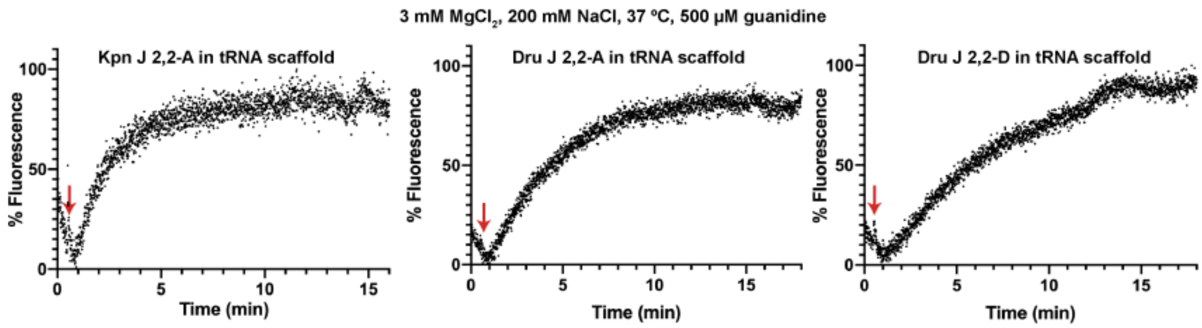

Figure S9. *In vitro* response kinetics of guanidine biosensors in tRNA scaffold.

In vitro response kinetics of guanidine biosensors in tRNA scaffold after injection of guanidine by an automated injector on a SpectraMax i3x plate reader (Molecular Devices). The final concentration of guanidine is 500 µM. The red arrow indicates when guanidine was injected. Data shown are the average of 3 independent replicates and error bars are not shown for clarity.

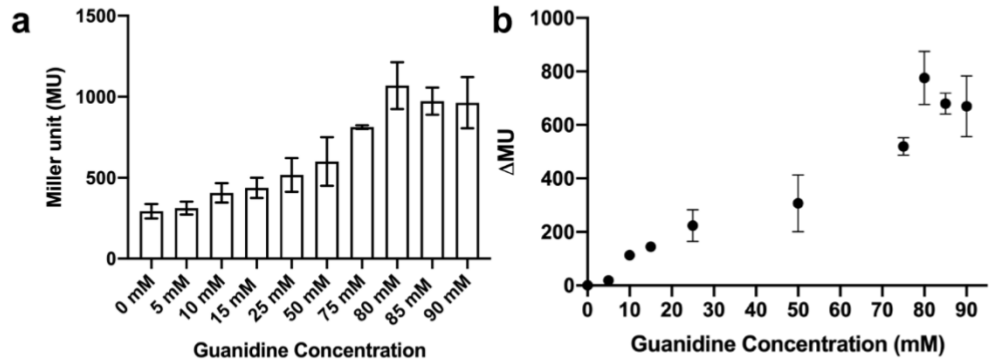

Figure S10. In vivo guanidine reporter response in presence of the increasing concentration of guanidine. (a) Plot of Miller Unit of the cells expressing the Kpn-WT lacZ reporter in the presence of increasing concentration of guanidine. (b) Plot of ΔMU (MU at a particular guanidine concentration – MU in the absence of guanidine) with increasing concentration of guanidine.

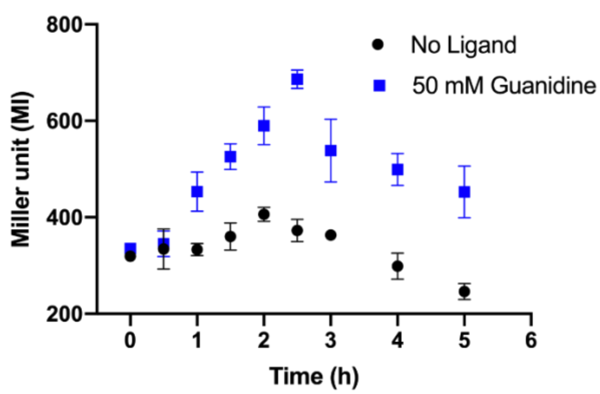

Figure S11. ***In vivo*** **response kinetics of guanidine reporter**

Plot of average MU values over time for cells expressing the Kpn-WT lacZ reporter in the presence or absence of 50 mM guanidine.

Table S1. Junction biosensor sequences. Bold sequences indicate Spinach2 sequence, which flanks the guanidine-I riboswitch sequence used. Orange indicates the artificial transducer stem or adenosine spacers for junction biosensors. Underlined residues indicate the adenosine spacers which are not part of the stem. Red residue represents G to C mutation.

Name	Sequence (5' to 3')
Kpn J-1,1-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-1,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-1,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-2,1-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-2,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-2,2-M (G to C)	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGGCUAGcGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-2,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-3,1-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-3,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-3,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J 1,1-B	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
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Kpn J-2,1-B	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**

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Kpn J-2,3-B	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucaaGCUGGCUAGGGUUCCGGUUCACC GCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCC GGGAGaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-3,1-B	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucaaaGCUGGCUAGGGUUCCGGUUCAC CGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCC CGGGAGagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-3,2-B	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucaaaGCUGGCUAGGGUUCCGGUUCAC CGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn J-3,3-B	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucaaaGCUGGCUAGGGUUCCGGUUCAC CGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
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Bsu J-3,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaCUCUUCAAGUUUUCUAGGGUU CCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAU GCACACGGAGGGAAAAAAGCCCGGGAGaaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACU AGUUACAUC**
Bsu J-2,2-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaCUCUUCAAGUUUUCUAGGGUU CCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAU

	GCACACGGAGGGAAAAAAGCCCGGGAGaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu J-2,3-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaCUCUUCAAGUUUUCUAGGGUUCCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAUGCACACGGAGGGAAAAAAGCCCGGGAGaaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu J-3,2-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaaCUCUUCAAGUUUUCUAGGGUUCCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAUGCACACGGAGGGAAAAAAGCCCGGGAGaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu J-3,3-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaaCUCUUCAAGUUUUCUAGGGUUCCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAUGCACACGGAGGGAAAAAAGCCCGGGAGaaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu J-2,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaCUCUUCAAGUUUUCUAGGGUUCCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAUGCACACGGAGGGAAAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu J-2,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaCUCUUCAAGUUUUCUAGGGUUCCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAU**G**CACACGGAGGGAAAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu J-3,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaCUCUUCAAGUUUUCUAGGGUUCCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAU**G**CACACGGAGGGAAAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu J-3,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaCUCUUCAAGUUUUCUAGGGUUCCGCAUGUCAAUUGACAUGGACUGGUCCGAGAGAAAACACAUACGCGUAAAUAGAAGCGCGUAUGCACACGGAGGGAAAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-2,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGUUUUCUAGGGUUCCGCAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-2,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGUUUUCUAGGGUUCCGCAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-3,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGUUUUCUAGGGUUCCGCAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-3,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGUUUUCUAGGGUUCCGCAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-2,2-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaGUUUUCUAGGGUUCCGCAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-2,3-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaGUUUUCUAGGGUUCCGCAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCC**C**GGGAGaaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-3,2-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaaGUUUUCUAGGGUUCCGCAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAG**CC**CGGGAGaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-3,3-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaaGUUUUCUAGGGUUCCGCAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**

Dru J-2,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGUUUUCUAGGGUUCCGCGAUAA AAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCG GGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-2,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGUUUUCUAGGGUUCCGCGAUAA AAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCC**G** GGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-3,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaGUUUUCUAGGGUUCCGCGAUA AAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGC**CC** **GGGAG**aagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru J-3,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaGUUUUCUAGGGUUCCGCGAUA AAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCC**C** GGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-2,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-2,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-3,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-3,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-2,2-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-2,3-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-3,2-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaaGCCGACUAGGGUUCCGACUC GCUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGC CCGGGAGaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-3,3-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaaGCCGACUAGGGUUCCGACUC GCUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGC CCGGGAGaaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-2,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-2,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-3,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae J-3,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaGCCGACUAGGGUUCCGACUCG CUCGCGAGUGGCUGGUCCGAGAGUUGGCGACCUCCAGUGAGGUUACACGGCGGGAUAAAAGCC CGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-2,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGACUAGGGUUCCGGCUCG CUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAA **GCCCGGGAG**aagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**

Pfl J-2,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-3,2-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-3,3-A	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-2,2-C	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cuucucgaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaacgaaagaag**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-2,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-3,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-3,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-2,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-2,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-3,2-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl J-3,3-D	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaaGCUGACUAGGGUUCCGGCUCGCUAAGGCGAGUGGCUGGUCCGAGAGUCGGCGACCUCCAGUUGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**

Table S2. **Linker biosensor sequences**. Bold sequences indicate the Spinach2 sequence, which flanks the guanidine-I riboswitch sequence used. Orange indicates the poly-adenosine linker length for linker biosensors.

Name	Sequence (5' to 3')
Kpn L-3	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uccgagagcuggcgaccucggcgagguuacacggcggga uaaaagcccgggagAAAgcuggcuaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uccgagagcuggcgaccucggcgagguuacacggcggga uaaaagcccgggagAAAAgcuggcuaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uccgagagcuggcgaccucggcgagguuacacggcggga uaaaagcccgggagAAAAAgcuggcuaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-6	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uccgagagcuggcgaccucggcgagguuacacggcggga uaaaagcccgggagAAAAAAgcuggcuaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-4 Tr 1	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCC**uccgagagcuggcgaccucggcgagguuacacggcggga uaaaagcccgggagAAAAgcuggcuaggg**UGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-4 Tr 2	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCC**uccgagagcuggcgaccucggcgagguuacacggcggga uaaaagcccgggagAAAAgcuggcuaggg**GUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-4 Tr 3	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagcuggcgaccucggcgagguuacacggcgggau aaaagcccgggagAAAAgcuggcuaggg**UUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-4 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagcuggcgaccucggcgagguuacacggcgggau aaaagcccgggagAAAAgcuggcuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-4 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagcuggcgaccucggcgagguuacacggcgggau aaaagcccgggagAAAAgcuggcuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-4 Tr 6	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGU**uccgagagcuggcgaccucggcgagguuacacggcgggaua aagcccgggagAAAAgcuggcuaggg**UAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-4 Tr 7	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGG**uccgagagcuggcgaccucggcgagguuacacggcgggauaa agcccgggagAAAAgcuggcuaggg**AGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-5 Tr 1	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCC**uccgagagcuggcgaccucggcgagguuacacggcggga uaaaagcccgggagAAAAAgcuggcuaggg**UGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-5 Tr 2	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCC**uccgagagcuggcgaccucggcgagguuacacggcggga uaaaagcccgggagAAAAAgcuggcuaggg**GUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-5 Tr 3	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagcuggcgaccucggcgagguuacacggcgggau aaaagcccgggagAAAAAgcuggcuaggg**UUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-5 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagcuggcgaccucggcgagguuacacggcgggau aaaagcccgggagAAAAAgcuggcuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**

Kpn L-5 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagcuggcgaccucggcgagguuacacggcgggauaaaagcccgggagAAAAAgcuggcuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-5 Tr 6	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGU**uccgagagcuggcgaccucggcgagguuacacggcgggauaaaagcccgggagAAAAAgcuggcuaggg**UAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Kpn L-5 Tr 7	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGU**uccgagagcuggcgaccucggcgagguuacacggcgggauaaaagcccgggagAAAAAgcuggcuaggg**AGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bcl L-4 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uccgagaggugcacacggcgucugccgugacacggagggauaaaagcccgggagAAAAugcgccuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bcl L-4 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uccgagaggugcacacggcgucugccgugacacggagggauaaaagcccgggagAAAAugcgccuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bcl L-5 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uccgagaggugcacacggcgucugccgugacacggagggauaaaagcccgggagAAAAAugcgccuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bcl L-5 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uccgagaggugcacacggcgucugccgugacacggagggauaaaagcccgggagAAAAAugcgccuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu L-4 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagaaaacacauacgcguaaauagaagcgcguaugcacacggagggaaaaaagcccgggagAAAAguuuuc**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu L-4 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagaaaacacauacgcguaaauagaagcgcguaugcacacggagggaaaaaagcccgggagAAAAguuuuc**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu L-5 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagaaaacacauacgcguaaauagaagcgcguaugcacacggagggaaaaaagcccgggagAAAAAguuuuc**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Bsu L-5 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagaaaacacauacgcguaaauagaagcgcguaugcacacggagggaaaaaagcccgggagAAAAAguuuuc**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru L-4 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccaagagaaaacacacagccuagcugugacacggagggacaaaagcccgggagAAAAguuuucuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru L-4 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccaagagaaaacacacagccuagcugugacacggagggacaaaagcccgggagAAAAguuuucuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru L-5 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccaagagaaaacacacagccuagcugugacacggagggacaaaagcccgggagAAAAAguuuucuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Dru L-5 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccaagagaaaacacacagccuagcugugacacggagggacaaaagcccgggagAAAAAguuuucuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae L-4 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagaguuggcgaccuccagugagguuacacggcgggauaaaagcccgggagAAAAgccgacuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae L-4 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagaguuggcgaccuccagugagguuacacggcgggauaaaagcccgggagAAAAgccgacuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**

Pae L-5 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagaguuggcgaccuccagugagguuacacggcggga uaaaagcccgggagAAAAAgccgacuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pae L-5 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagaguuggcgaccuccagugagguuacacggcggga uaaaagcccgggagAAAAAgccgacuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl L-4 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagucggcgaccuccaguugagguuacacggcggg auaaaagcccgggagAAAAgcugacuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl L-4 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagucggcgaccuccaguugagguuacacggcggg auaaaagcccgggagAAAAgcugacuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl L-5 Tr 4	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagucggcgaccuccaguugagguuacacggcggg auaaaagcccgggagAAAAAgcugacuaggg**UGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**
Pfl L-5 Tr 5	**GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUC**uccgagagucggcgaccuccaguugagguuacacggcggg auaaaagcccgggagAAAAAgcugacuaggg**GAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC**

Table S3. RNA-based fluorescent biosensor sequences. *Underlined italics* indicate tRNA scaffold which flanks the guanidine biosensors. *Italics* represent T7 terminator sequence. Bold sequences indicate Spinach2 sequence, which flanks the guanidine-I riboswitch sequences used. Orange indicates the artificial transducer stem or adenosine spacers for junction biosensors. Underlined orange residues indicate the adenosine spacers which are not part of the stem. Red residue represents G to C mutation.

Name	Sequence (5' to 3')
Spinach2	GGG*GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCG* **GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**GUAGGCUGCUUCGGCAGCCUAC**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC** *CGGCCGCGGGUCCAGGGUUCAAGUCCCUGUUCGGGCGCCAUAGCAUAACCCCUUGGGGCCUCUAAACGGGUCUUGAGGGGUUUUUUG*
Kpn J-2,2-A	GGG*GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCG* **GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC** *CGGCCGCGGGUCCAGGGUUCAAGUCCCUGUUCGGGCGCCAUAGCAUAACCCCUUGGGGCCUCUAAACGGGUCUUGAGGGGUUUUUUG*
Kpn J-2,2-M (G to C)	GGG*GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCG* **GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGGCUAGcGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC** *CGGCCGCGGGUCCAGGGUUCAAGUCCCUGUUCGGGCGCCAUAGCAUAACCCCUUGGGGCCUCUAAACGGGUCUUGAGGGGUUUUUUG*
Dru J-2,2-A	GGG*GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCG* **GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGUUUUCUAGGGUUCCGCGAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC** *CGGCCGCGGGUCCAGGGUUCAAGUCCCUGUUCGGGCGCCAUAGCAUAACCCCUUGGGGCCUCUAAACGGGUCUUGAGGGGUUUUUUG*
Dru J-2,2-D	GGG*GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCG* **GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGUUUUCUAGGGUUCCGCGAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC** *CGGCCGCGGGUCCAGGGUUCAAGUCCCUGUUCGGGCGCCAUAGCAUAACCCCUUGGGGCCUCUAAACGGGUCUUGAGGGGUUUUUUG*

Table S4. RNA-based fluorescent biosensor sequences used for in vitro kinetic study.

Name	Sequence (5' to 3')
Kpn J-2,2-A	GGG*GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCG* **GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGCUGGCUAGGGUUCCGGUUCACCGCGGUGAACGUCUGGUCCGAGAGCUGGCGACCUCGGCGAGGUUACACGGCGGGAUAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC** *CGGCCGCGGGUCCAGGGUUCAAGUCCCUGUUCGGGCGCCA*
Dru J-2,2-A	GGG*GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCG* **GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**cucuucaaGUUUUCUAGGGUUCCGCGAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaagaaggg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC** *CGGCCGCGGGUCCAGGGUUCAAGUCCCUGUUCGGGCGCCA*
Dru J-2,2-D	GGG*GCCCGGAUAGCUCAGUCGGUAGAGCAGCGGCCG* **GAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCA**uacuucaaGUUUUCUAGGGUUCCGCGAUAAAAUUAUCGGACUGGUCCAAGAGAAAACACACAGCCUAGCUGUGACACGGAGGGACAAAAGCCCGGGAGaagaagg**UUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC** *CGGCCGCGGGUCCAGGGUUCAAGUCCCUGUUCGGGCGCCA*