Supplementary Information

Translation regulation by a Guanidine-II riboswitch is highly tunable in sensitivity, dynamic range, and apparent cooperativity

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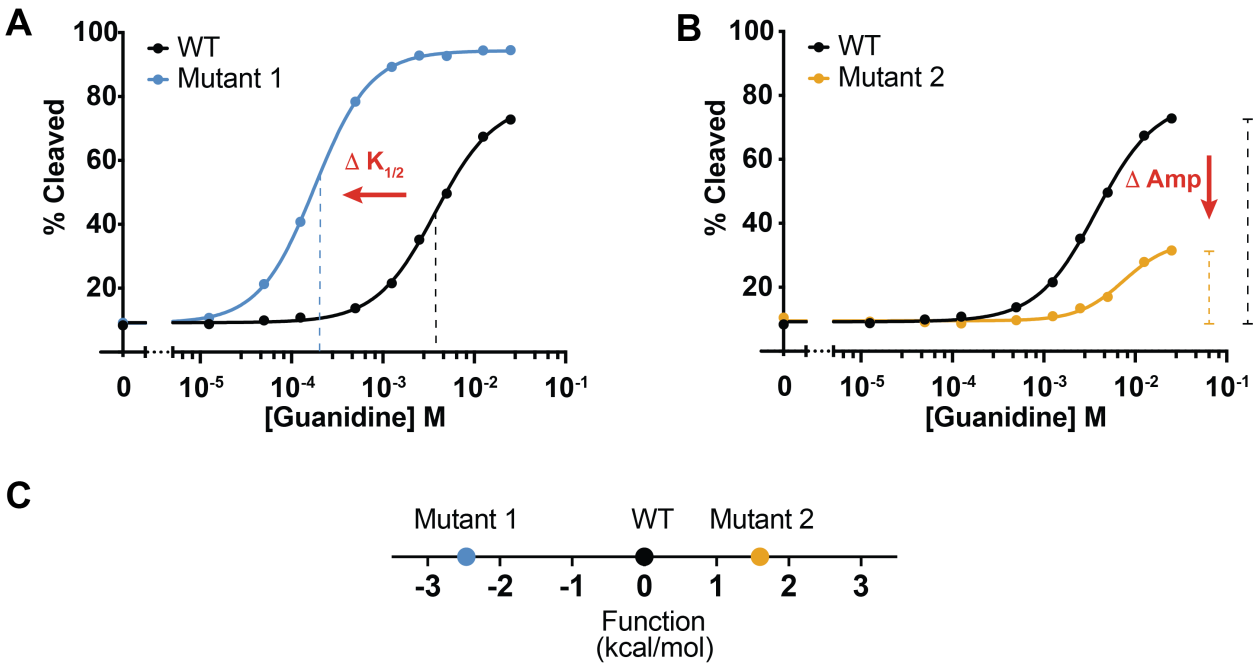
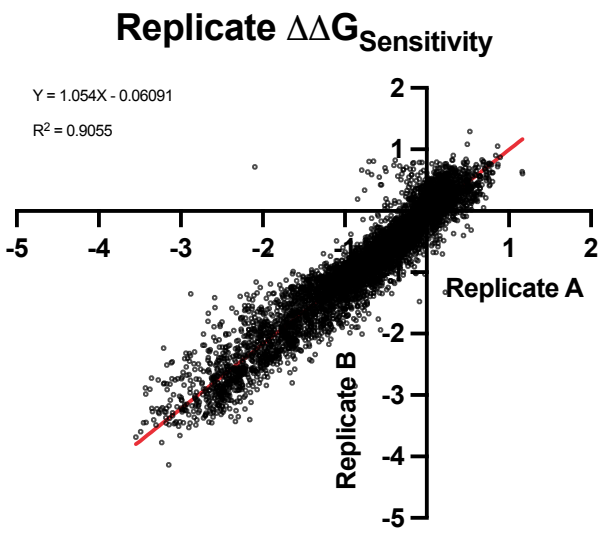
DNA sequence:

TAATACGACTCACTATA (T7 promoter) - GCGTTATCTCTTATCAGGCAGG (5' constant handle) - TTTCAGCCGGAAGCGGGACGACCCGTTTTCCCTCTTTCATTGCGCGGGGACGACCCTGCAGAGAGGCTG AAA (Mutated Region) - ATG (Start Codon) - TAG (Stop Codon) – TGGATCTATCTCTTGCTCGTA (CDS)

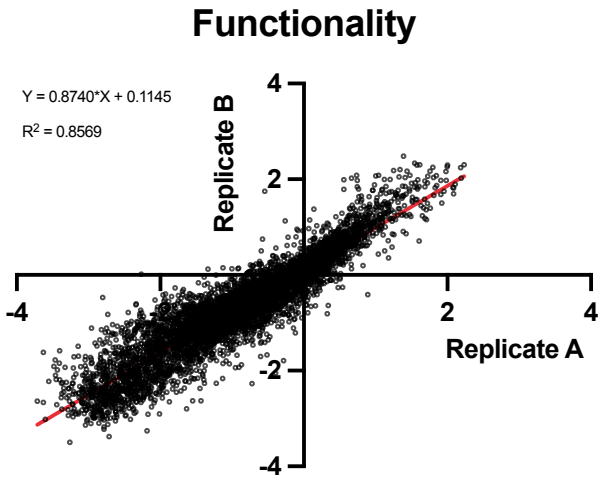
Sequencing coverage of mutants
All single mutants had an average of 1000 reads per concentration of guanidine tested. For double mutants, 98% of variants had over 50 reads per concentration.



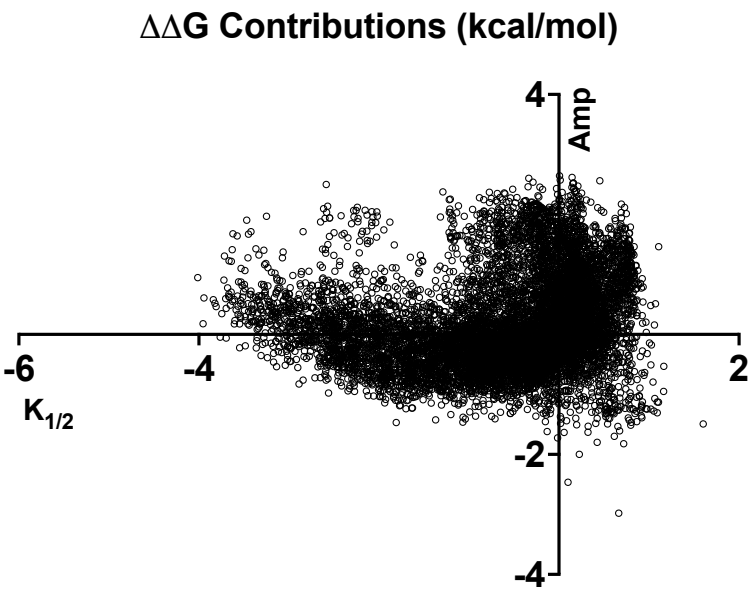
Supplemental Figure 1. Fit parameter description. A) Representative curve indicating shift in $K_{1/2}$ value B) Representative curve indicating shift in amplitude value C) Schematic depicting effect of shifting $K_{1/2}$ and amplitude on function parameter



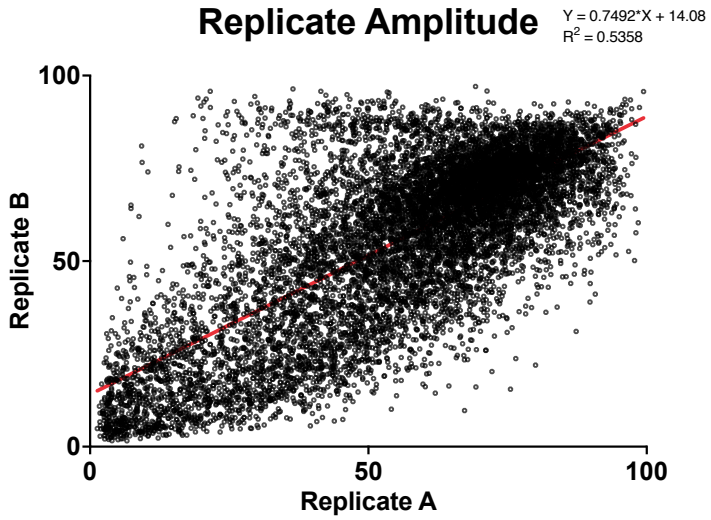
Supplemental Figure 2. Correlation of single and double mutant $\Delta\Delta G_{Sensitivity}$ between two replicates



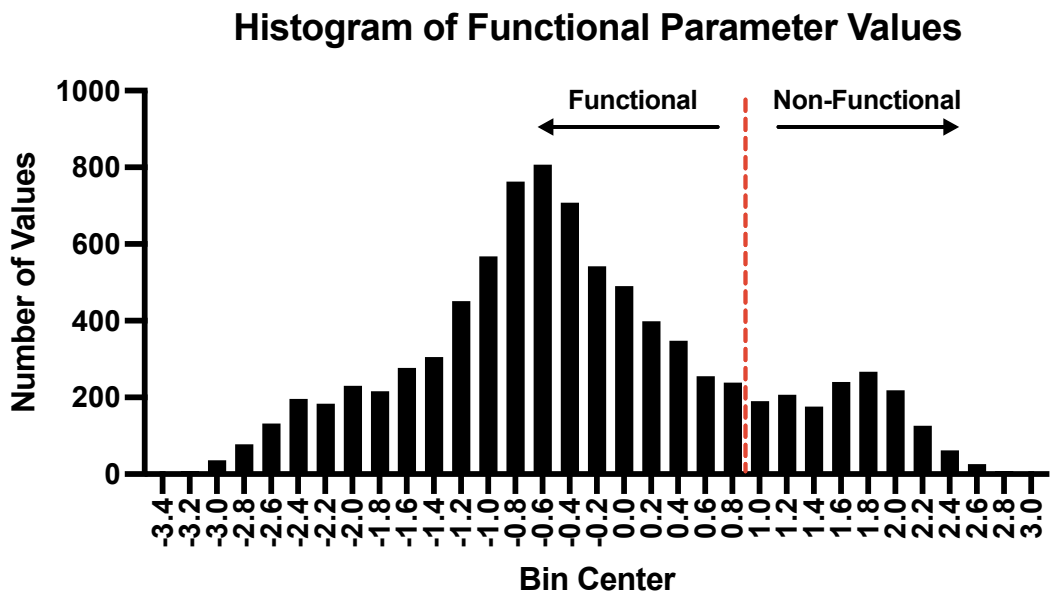
Supplemental Figure 3. Correlation of single and double mutant function parameter between two replicates



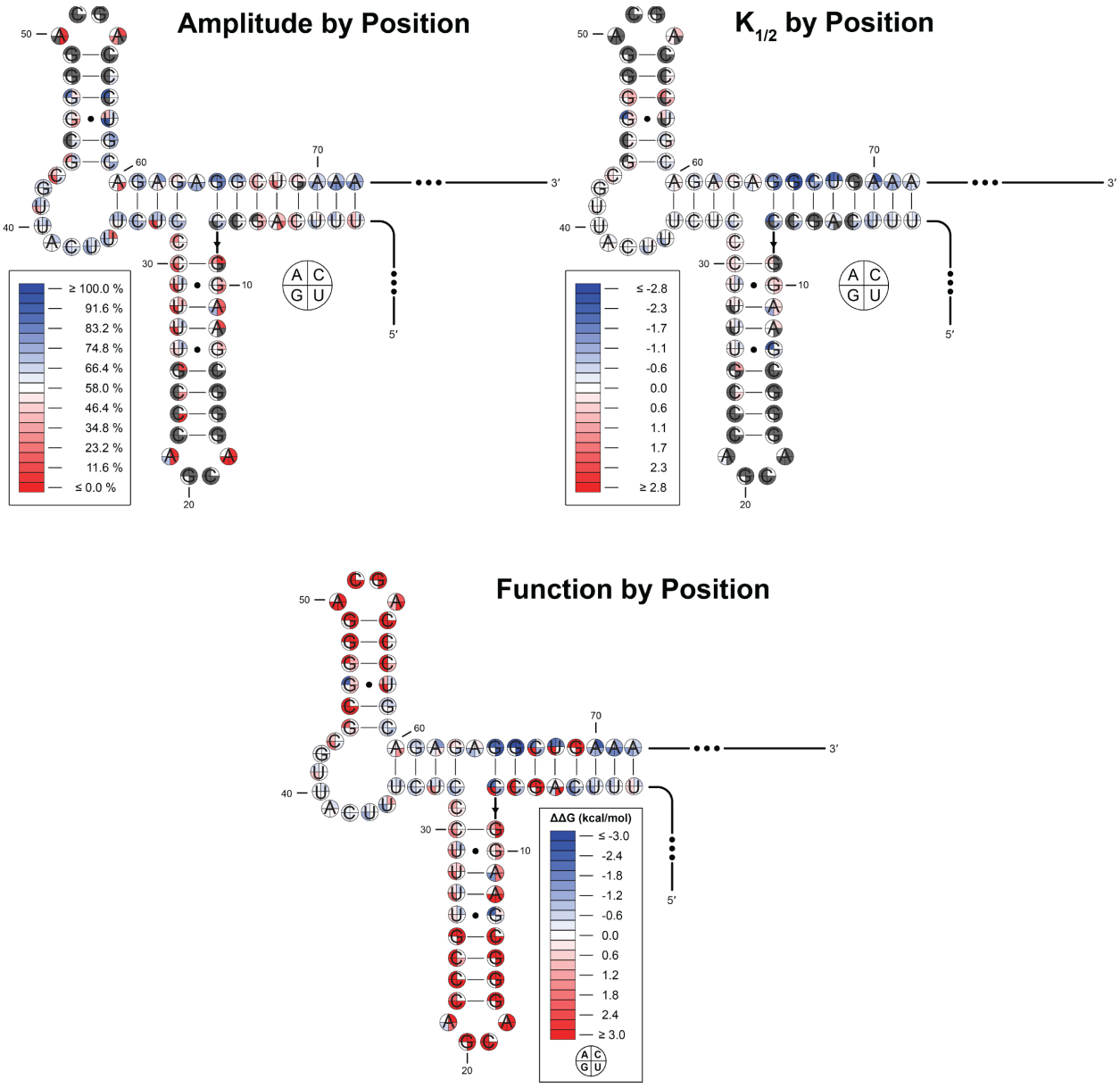
Supplemental Figure 4. Contributions of the sensitivity ($K_{1/2}$) and dynamic range (amplitude) to the function parameter.



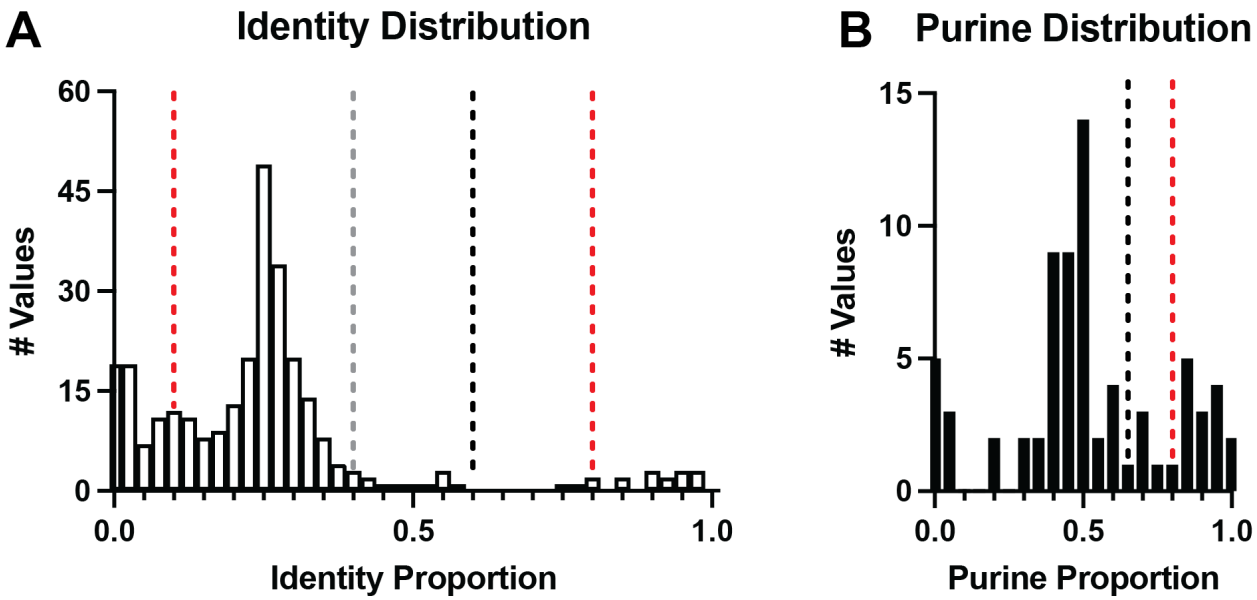
Supplemental Figure 5. Correlation of single and double mutant amplitude fit data between two replicates.



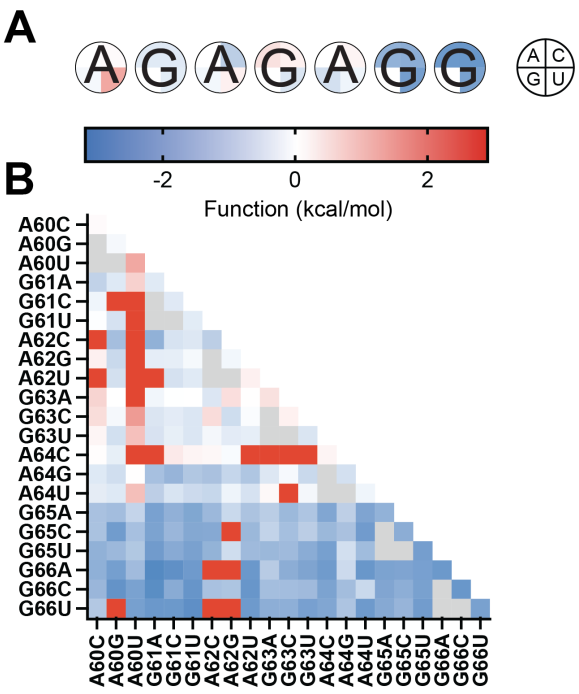
Supplemental Figure 6. Distribution of functional parameter values. Red line denotes the 1 standard deviation above the mean cutoff for eliminating "non-functional" mutants.



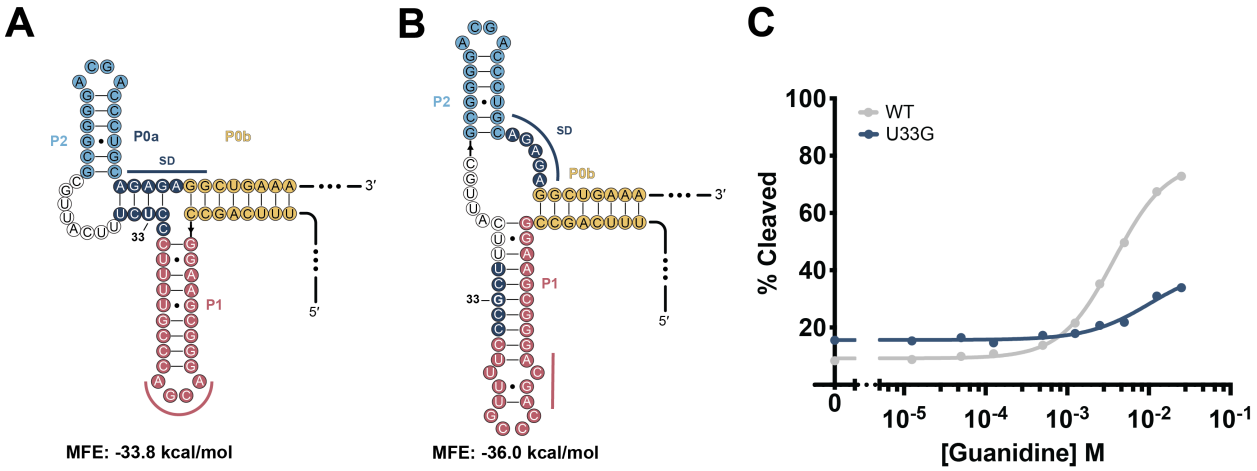
Supplemental Figure 7. Single mutant heatmaps of amplitude, $K_{1/2}$, and the function parameter.



Supplemental Figure 8. Cutoffs of nucleotide proportion for the biochemical consensus. A) Individual identity proportion distribution. >0.8 is red, >0.6 is black, >0.4 is grey. B) Purine identity distribution. >0.8 is red, >0.65 is black



Supplemental Figure 9. Functionality of variants in the SD of the Pae *Gdm-II riboswitch A) Single mutant function B) Double mutant heat map of SD variants*



Supplemental Figure 10. Functional deficiency of U33G. A) Modeling the WT MFE OFF state B) Modeling the U33G MFE OFF state C) Representative RelE cleavage guanidine response profiles of the WT (grey) and U33G (gold) mutant.