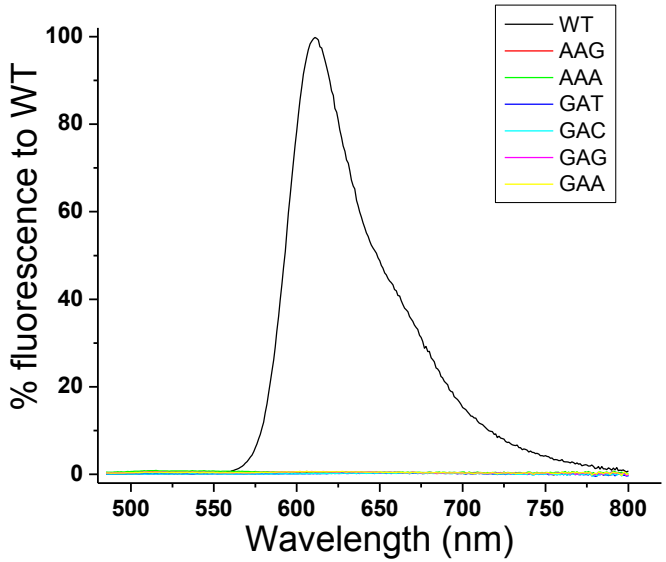

Figure S1. Emission spectra of purified WT and Met72→Lys/Asp/Glu mutant proteins from *E. coli*.

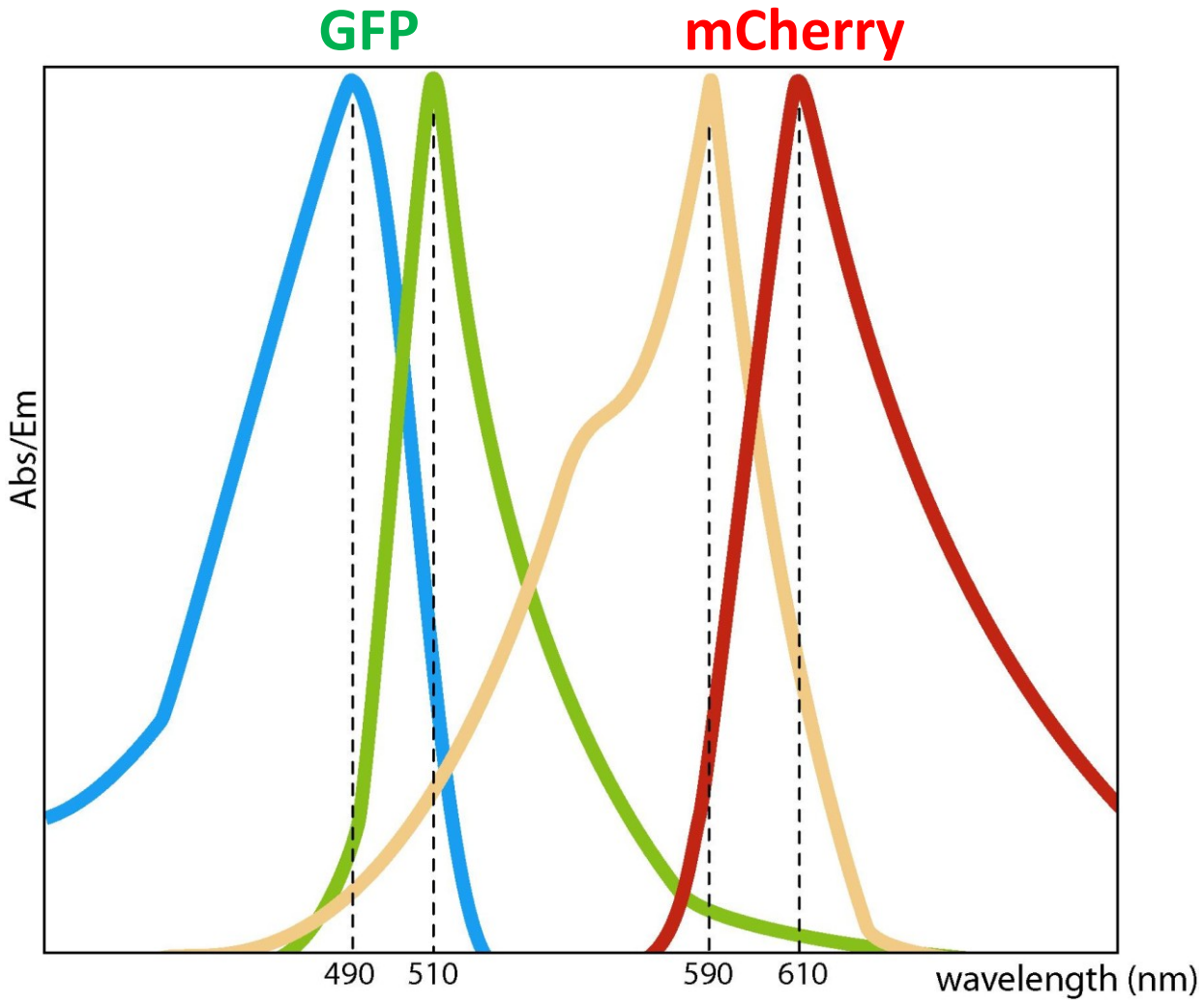

Figure S2. The excitation and emission spectra of GFP and mCherry have minimal overlap.

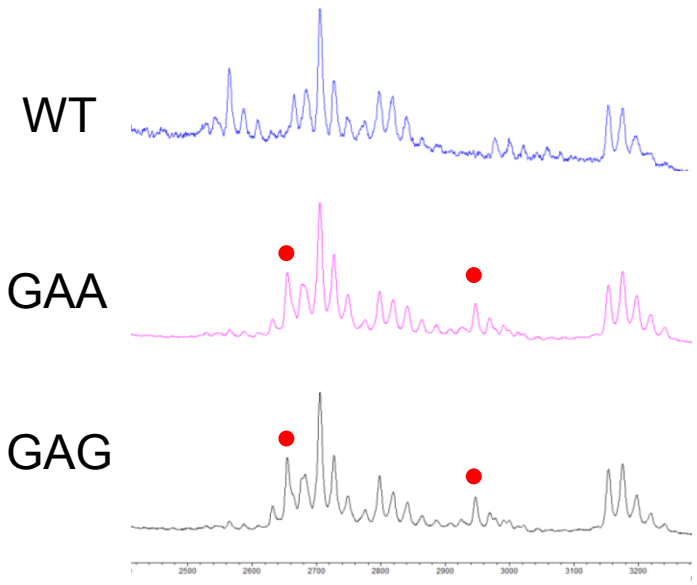

Figure S3. MALDI of Glu-C protease digested GFP-mCherry proteins isolated using GFP antibody. The mass region shown contains two additional peaks (marked by red dots) in the GAA/GAG constructs derived from the Glu residue at the site of mutation that is not incorporated into a fluorophore.

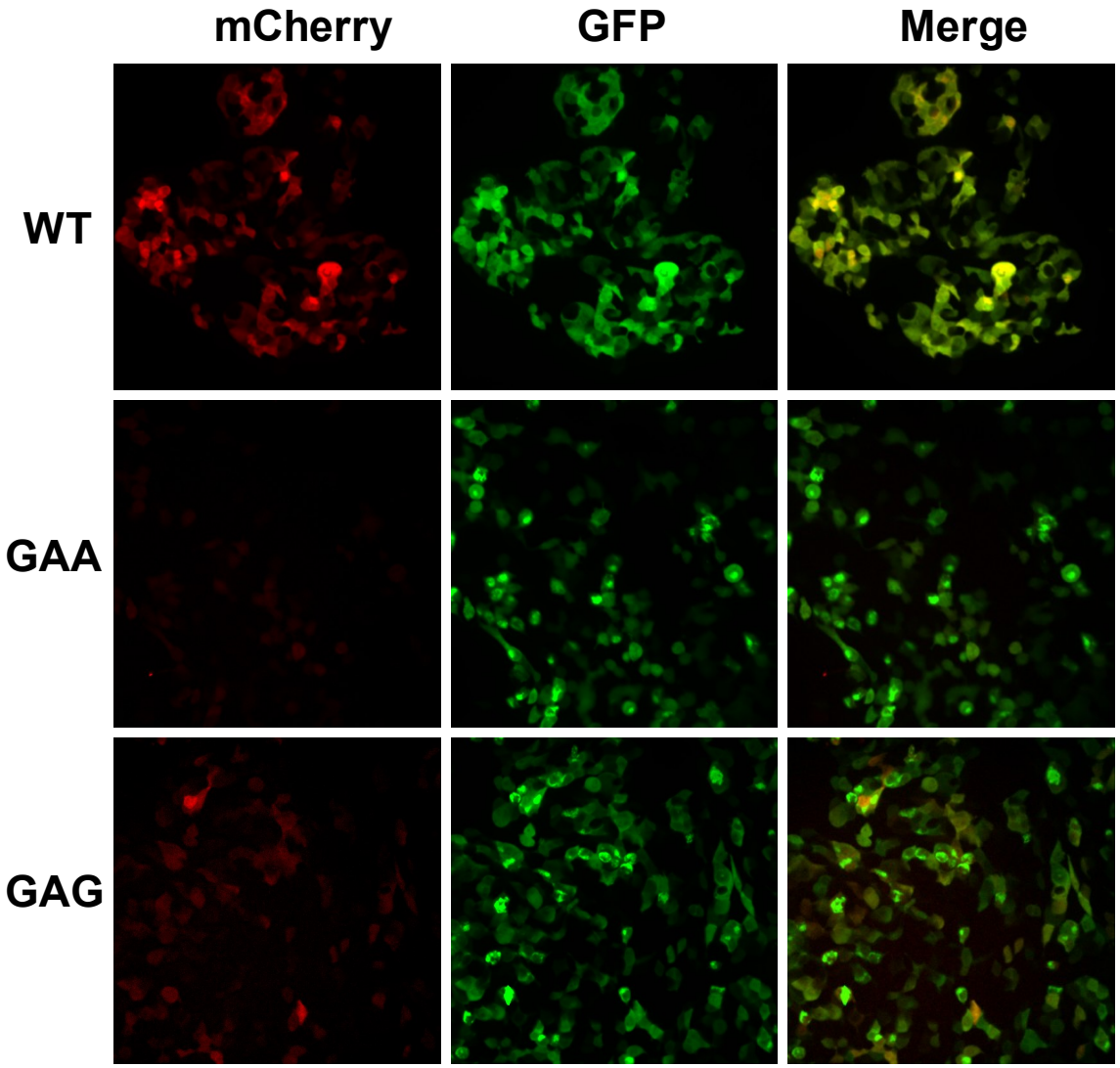

Figure S4. Fluorescence microscopy images of the cell lines.

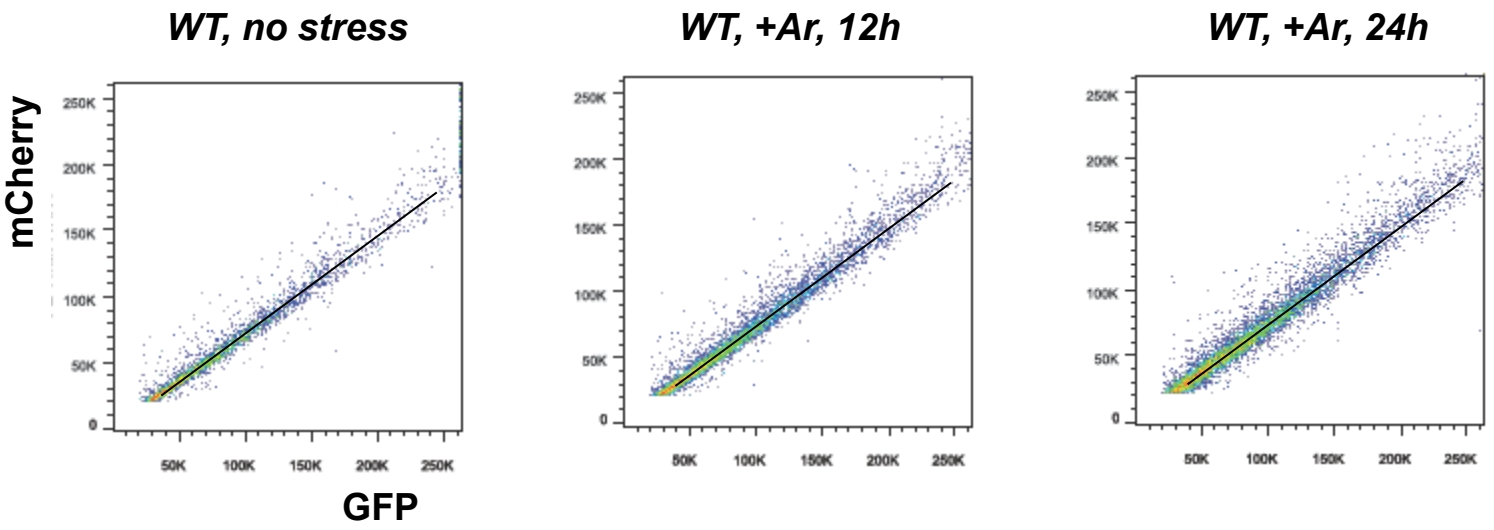

Figure S5. GFP and mCherry fluorescence have the same slope under no stress and stress conditions.

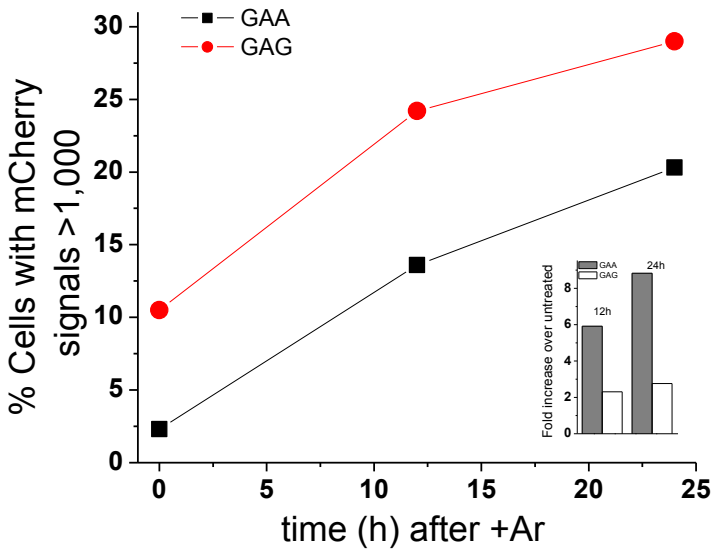

Figure S6. mCherry fluorescence increase is much higher when counting cells with fluorescence signal of >1,000. For comparison, some WT mcherry cells can have fluorescence signal of >100,000. Inset shows the fold change of samples ± arsenite.

A

Anticodon		Copy number	score
CUC	1	1	71.5
	2	7	76.61
UUC	1	1	54.07
	2	2	72.23
	3	1	75.96
	4	2	76.26
	5	2	76.46

B

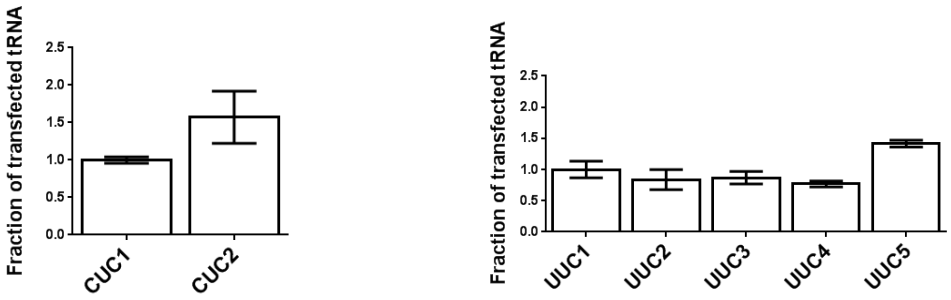

Figure S7. Human $tRNA^{Glu}$ isodecoders. (A) Anticodon, nomenclature used in this work, gene copy number in the human genome, and tRNAScan score. The higher the score, the more likely it folds into the standard tRNA structure. (B) Comparison of *in vivo* stability of $tRNA^{Glu}$ isodecoder transcripts. *In vitro* transcribed tRNAs were labeled with [α-^{32}P]ATP at A76 and transfected into GAA/GAG mutant stable cells for 36h. Total RNAs were extracted and loaded on 10% polyacrylamide urea gel. The gel was exposed to a phosphorimager screen and quantified.