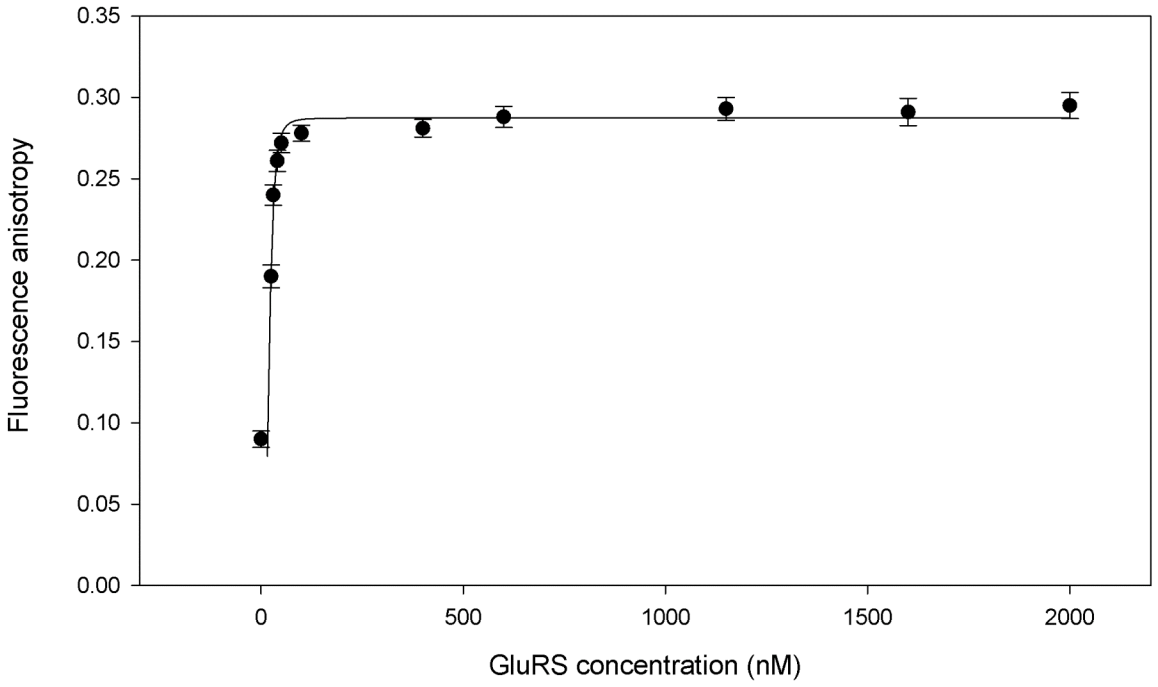
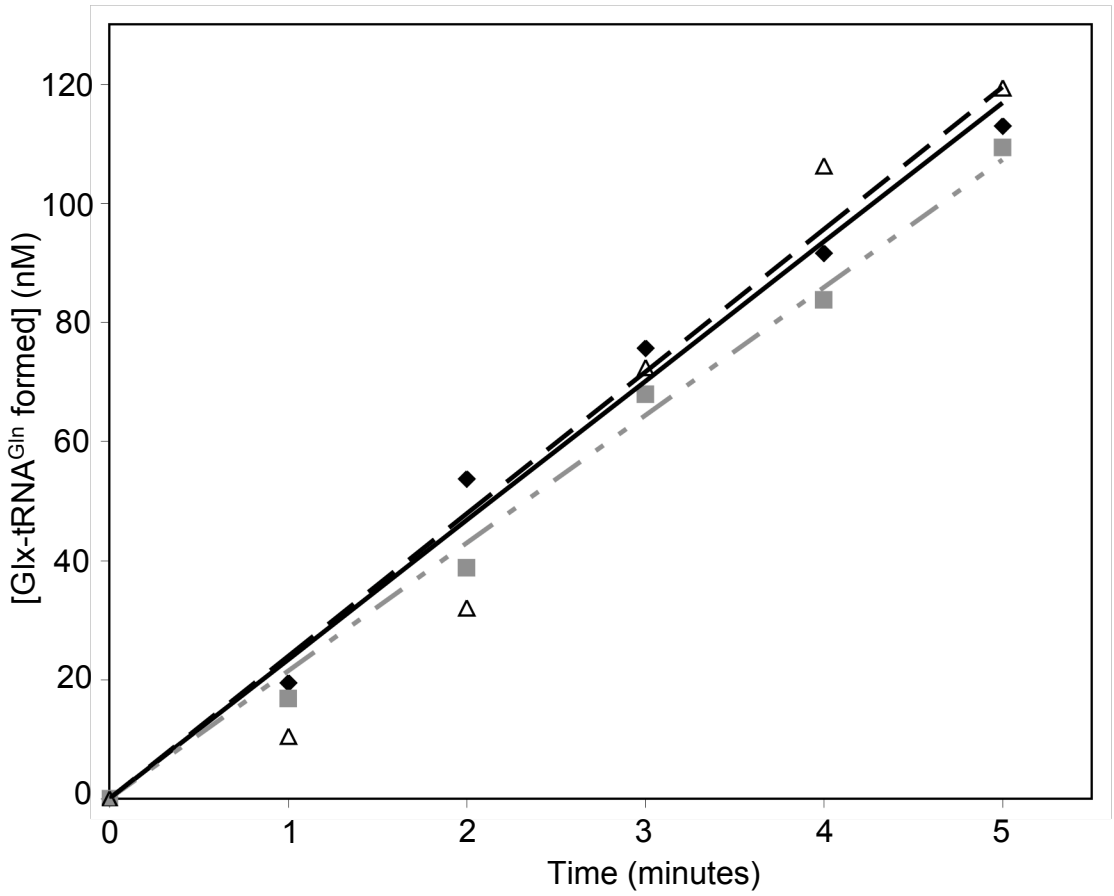
Supplemental Table 1: Deacylation of Glx-tRNA^{Gln} in the presence or absence of ND-GluRS and/or GatDE.

	$t_{1/2}$ (minutes)	
	Glu-tRNA^{Gln}	Gln-tRNA^{Gln}
No enzyme	3.3 ± 0.2	3.1 ± 0.1
ND-GluRS	1.7 ± <0.1	1.9 ± 0.1
GatDE	4.6 ± 0.5	2.3 ± 0.1
ND-GluRS + GatDE	3.5 ± 0.2	1.6 ± 0.1

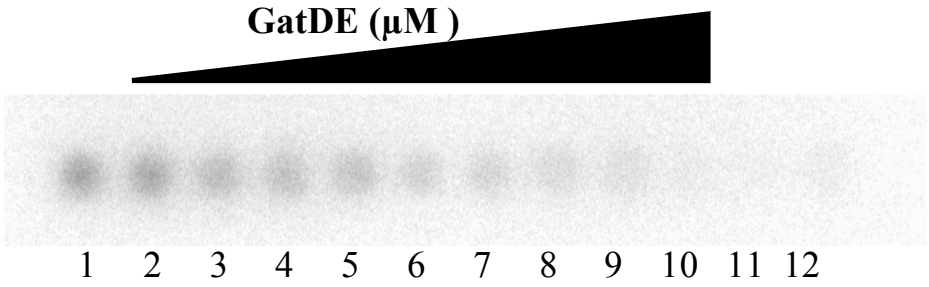
Glx-tRNA^{Gln} (200 nM) indicated incubated at 65 °C with or without ND-GluRS (2 μM) and/or GatDE (2 μM) and aliquots quenched in 100 mM sodium citrate, pH 4.5, with 0.2 mg/mL of nuclease P1 as described in Materials and Methods.



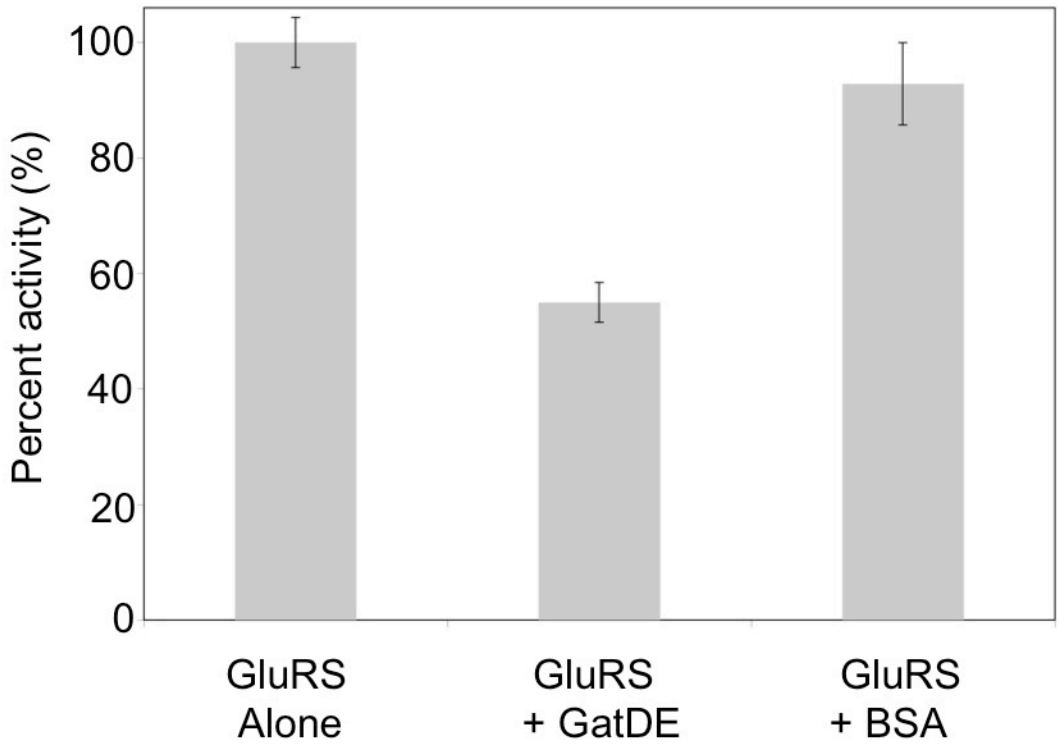
Supplemental Figure 1 AF-labeled GatDE (20 nM) incubated with increasing concentrations of ND-GluRS (up to 2 μM). Change in fluorescence anisotropy monitored as described in Materials and Methods.



Supplemental Figure 2 tRNA^{Gln} (10 μM) aminoacylated as described in the Material and Methods by ND-GluRS (100 nM) alone (△, black dashed line) or with either GatDE (2 μM)(◆, solid black line) or BSA (2 μM)(■, grey dashed line).



Supplemental Figure 3 [^{32}P] tRNA^{Glu} (5 nM) incubated at room temperature for 25 min with ND-GluRS (60 nM) (1), ND-GluRS (60 nM) and increasing concentrations of GatDE (7.8 nM to 2 µM) (2-10), GatDE (2 µM) (11), or no enzyme (12) as described in the Materials and Methods. Samples were spotted on a nitrocellulose membrane and visualized as described in the Materials and Methods.



Supplemental Figure 4 $tRNA^{Glu}$ (80 nM) glutamylated by ND-GluRS (50 nM) alone or in the presence of either GatDE (1.8 μM) or BSA (1.8 μM) as described in the Materials and Methods. Activity relative to the GluRS alone. Errors bars represent standard deviations.