Supplementary Tables and Figure Legends

Supplementary Tables

Table SI: Oligonucleotides

Oligonucleotide Name	Construct or Use	Sequence
QB832ADFP	Full length GLN4+ His6	AATTCCATCAACCTTAAA ATGGCTCACCATCACCATCACCAT ATGTCTTCTGTAGAAGAATTGACT
QB832ADRP	GLN4 to end	CTTCCAAACCACTCTTGGAAGTTGCGTCCTTCAA
QB1115ADFP	GLN4 187 – end	AATTCCATCAACCTTAAAATGATCAAGAAGAAGACCAAGAAT
QB1114ADFP	GLN4 211 – end	AATTCCATCAACCTTAAAATGTCTTCCGGTCCAAAGAGG
QB1034ADFP	GLN4 216 – end	AATTCCATCAACCTTAAAATGAGGACTATGTTCAATGAAGGTTTC C
QB1012ADFP	GLN4 1-187	AATTCCATCAACCTTAAAATGTCTTCTGTAGAAGAATTGACT
QB1012ADRP	GLN4 1-187	CTTCCAAACCACT GATTAAGTCTCTCTCATCCTT
HWI P239	gln4::Kan DNA	GAAGACATATATAAGAAACAAAAGGCAC
HWI P234	gln4::Kan DNA	GCCGTATATTGCTAAGGACACC
HWI P237	GLN4 -517	GGGTCCTGGTTCGAATCTGCAAATAAGCTTCCTAACGC
HWI P238	GLN4 +527	CTTGTTCGTGCTGTTTAAGAATCCCAGTGACAAGAATGACA
HWI P181	$tRNA^{Gln(CUG)}$	GGGTCCTGGTTCGAGAGCTTCTACTATAAACCTCACTC
HWI P182	$tRNA^{Gln(CUG)}$	CTTGTTCGTGCTGTTTACTATCAGGCTCTCAGAAGGC
HWI P235	$tRNA^{Gln(CUG)}$	TCTCCTGGATCCGGCTCGTATGTTGTGTGG
HWI P236	$tRNA^{Gln(CUG)}$	CCAGTGAATTCGAGCTCGGTAC
HWI P257	Purification of $tRNA^{Gln(CUG)}$	5`-Bio- TGGAGGTCCCACCCGGATTCGAACTGG 3`
HWI P237	JE1060AP and JE1062AP	GGGTCCTGGTTCGAATCTGCAAATAAGCTTCCTAACGC
HWI P238	JE1060Ap and JE1062AP	CTTGTTCGTGCTGTTTAAGAATCCCAGTGACAAGAATGACA
HWI P239	gln4-ΔKAN (-554)	GAAGACATATATAAGAAACAAAAGGCAC
HWI P234	gln4-ΔKAN (+586)	GCCGTATATTGCTAAGGACACC
HWI P40	Check gln4-ΔKAN (-790)	AGCAGCTGGAGCCACTAATGTTAG
P256	Check gln4-ΔKAN +811	CCAAATCCTAGCCCAAACTCTTCG
HWI P285	G-432 Xho1	CCTCCACTCGAG CCCTTCACGTTTCTGACAATAGTTCTG
HWI P288	G+288R Mlu1	CTCCAC ACGCGT CCATGTAGATTAACGTTATATTTTCCTTC

Table S2: Yeast Strains

Strain	Genotype	Source
BY4741	*MAT*a *his3Δ1 met15Δ0 leu2Δ0 ura3Δ0*	Open Biosystems
MEM 70	BY4741 *gln4-Δ::kan*^{R} [*CEN URA3 GLN4*]	This study
MEM 133	BY4741 *gln4-Δ::kan*^{R} *ade2*$^-$::*GLN4*::*MET15*	This study
MEM 141	BY4741 *gln4-Δ::kan*^{R} *ade2*$^-$::*gln4(211-809)*::*MET15*	This study
BCY123	*MAT*a, *pep4-3*::*HIS3*, *prb1*::*LEU2*, *bar1*::*HISG*, *lys2*::*GAL1/10-GAL4*, *can1*, *ade2*, *trp1*, *his3*, *ura3-52*, *leu2-3,112*	Mark Macbeth[23]
QB1012AD	BCY123 JE1012A [2 micron *URA3 GLN4*(1-187)-PT]	This study
EJG1117	*MAT*X *leu2 trp1ura3 prb1-112 pep4-1 his3Δ-pGAL10-GAL4*	Erin O'Shea
EJG1473	EJG117 *sam1Δ*::*NatR sam2Δ*::*KanR*	This study
MA337	EJ1473 JE1012A [2 micron *URA3* GLN4(1-187)-PT]	This study

23. Macbeth, M. R., Lingam, A. T. & Bass, B. L. (2004) Evidence for auto-inhibition by the N terminus of hADAR2 and activation by dsRNA binding. *RNA* **10**, 1563-1571.

Table S3: Plasmids used in this study

Plasmid	Description	Source/ Reference
BG2483	2 micron, *URA3* vector with P_{GAL1}, LIC cloning sites, ORF is fused to PT tag containing a recognition site for protease 3C, HA epitope, His6, ZZ domain of protein A	Malkowski[24]
JE1012	*GLN*4(1-187) in BG2483	This study
JE1115	*GLN4*(187-809) in BG2483	This study
JE1033	*GLN4* (212-809) in BG2483	This study
JE1034	*GLN4* (216-809) in BG2483	This study
JE1135	*GLN4*-G_{112}A V_{113}A G_{114}A in BG2483	This study
JE1136	*GLN4*-G_{112}P in BG2483	This study
JE1137	*GLN4*-W_{160}A in BG2483	This study
JE1140	*GLN4*-W_{160}F in BG2483	This study
PYEX4T	2 micron, *URA3 leu2D*	Martzen[25]
JE1135	tRNA Glu-CUC (AB209-11) in PYEX4T	This study
JE1060	GLN4 +/- 500 in AVA579[URA3 CEN]	This study
AVA579	URA3 CEN plasmid derived from YCPlac33 by insertion of LIC cloning site	

24. Malkowski, M. G. *et al.* (2007) Blocking S-adenosylmethionine synthesis in yeast allows selenomethionine incorporation and multiwavelength anomalous dispersion phasing. *Proc Natl Acad Sci U S A* **104**, 6678-6683.

25. Martzen, M. R. *et al.* (1999) A biochemical genomics approach for identifying genes by the activity of their products. *Science* **286**, 1153-1155..

Table S4: Data collection, phasing and refinement statistics for **MAD** (SeMet) structures

	Gln4 N-term		
Data collection			
Space group	$P12_11$		
Cell dimensions			
a, *b*, *c* (Å)	40.79 34.61 74.25		
α, β, γ (º)	90.00 97.61 90.00		
	Peak	*Inflection*	*Remote*
Wavelength	0.97937	0.97904	0.91162
Resolution (Å)	29.2-2.30 (2.42-2.30)		
R_{merge}	0.098(0.485)	0.098(0.467)	0.100(0.490)
R_{pim}	0.062(0.309)	0.062(0.297)	0.063(0.312)
$I/\sigma I$	12.9(3.4)	12.7(3.6)	13.0(3.4)
Completeness (%)	100.0(100.0)	100.0(100.0)	99.9(99.8)
Redundancy	6.5(6.6)	6.5(6.6)	6.5(6.6)
Refinement			
Resolution (Å)	29.2-2.30		
No. reflections	9385		
R_{work}/ R_{free}	0.182/0.211		
No. atoms			
Protein	1489		
Ligand/ion	0		
Water	82		
B-factors			
Protein	35.10		
Ligand/ion			
Water	42.36		
R.m.s deviations			
Bond lengths (Å)	0.007		
Bond angles (º)	1.035		
Ramachandran			
Favored	98.4%		
Allowed	1.6%		
Disallowed	0.0%		

*Highest resolution shell is shown in parenthesis

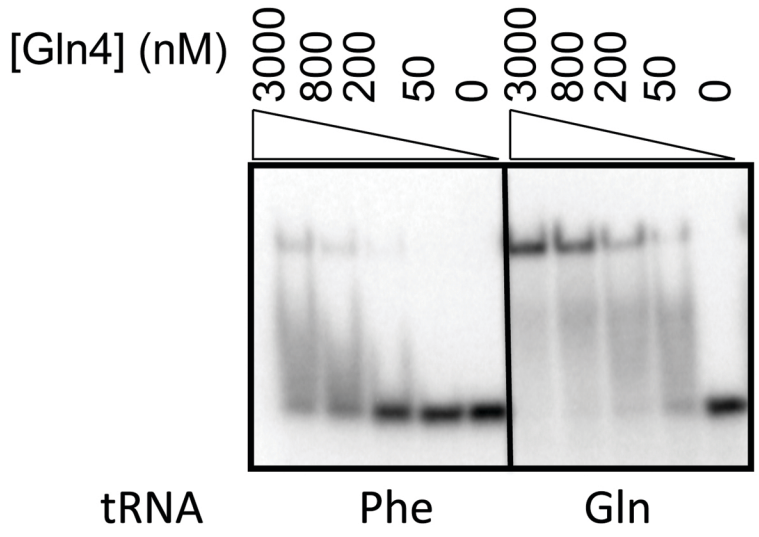

Figure S1. Gln4 protein binds more efficiently to native tRNA$^{Gln(CUG)}$ than to native tRNA^{Phe}.

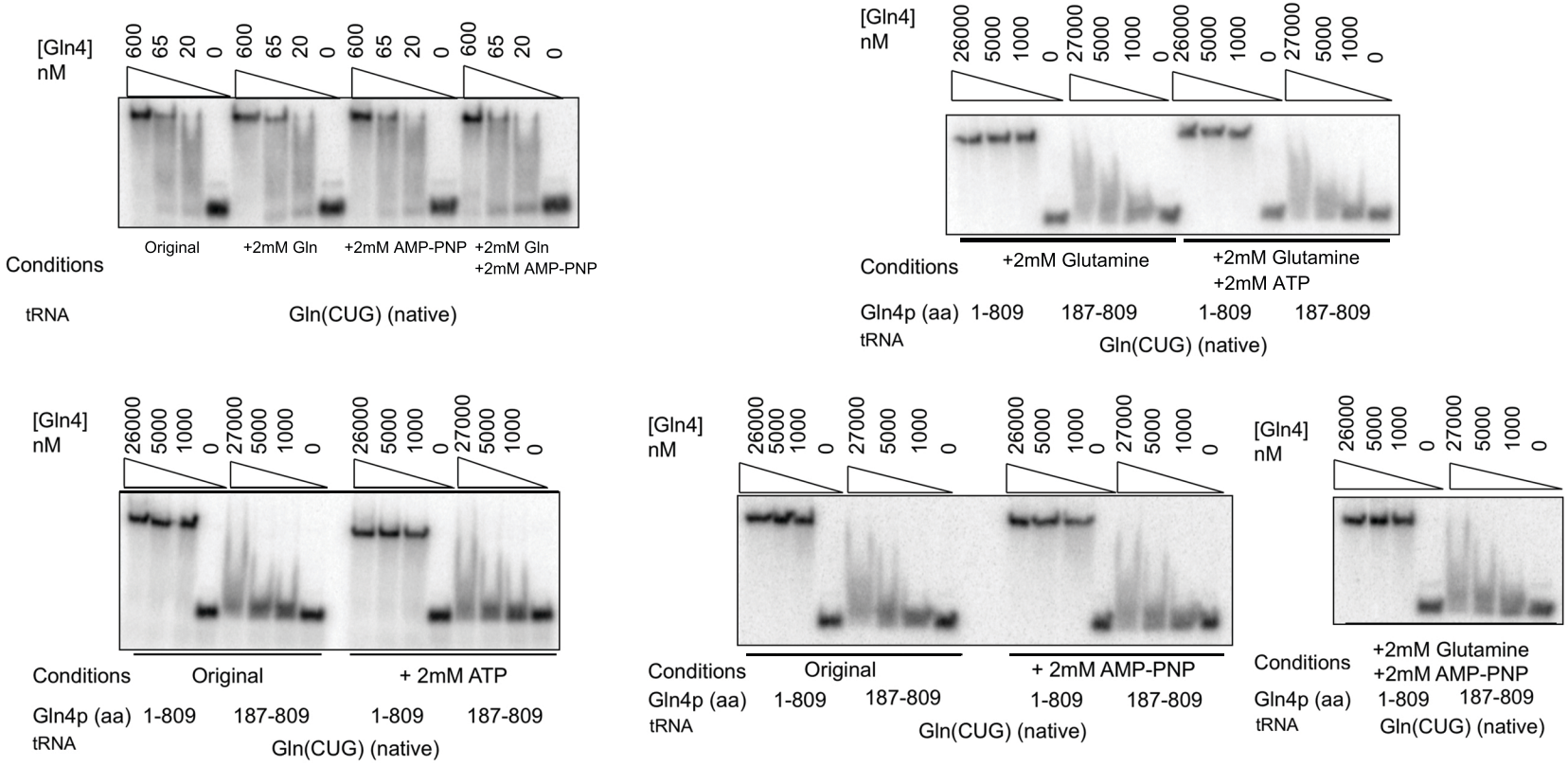

Figure S2. Effects of glutamine, ATP and AMP-PNP on binding of Gln4 and Gln4 (187-809) to tRNA$^{Gln(CUG)}$.

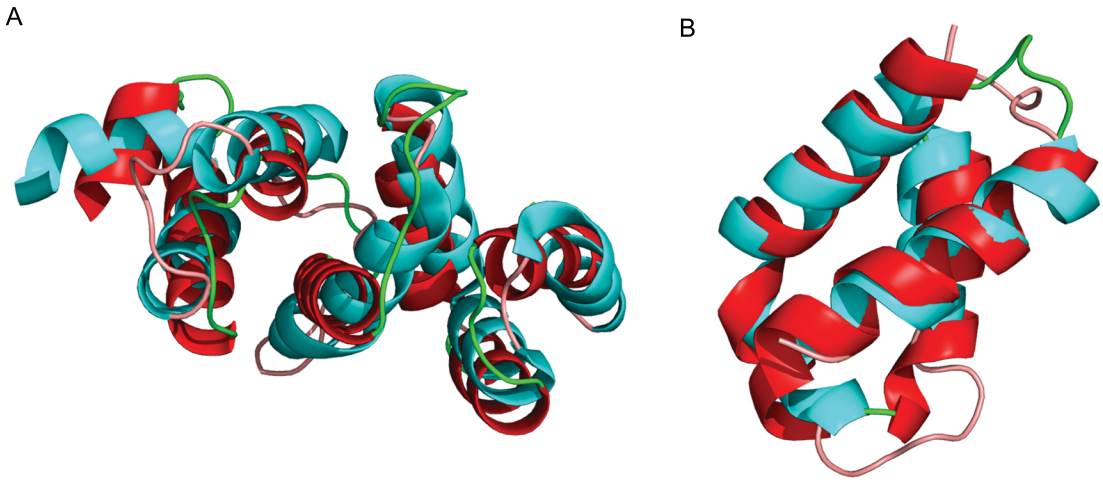

Figure S3. Structural alignment of helical and tail domains of Gln4 NTD and *Thermotoga maritima* GatB (PDB ID: 3AL0). A. The crystal structure of Gln4(2-110) (red) is superposed to the helical domain of *T. maritima* GatB(303-415) (cyan). B. The crystal structure of Gln4 (119-178) (red) is superposed on the tail domains of GatB (422-481) (cyan).

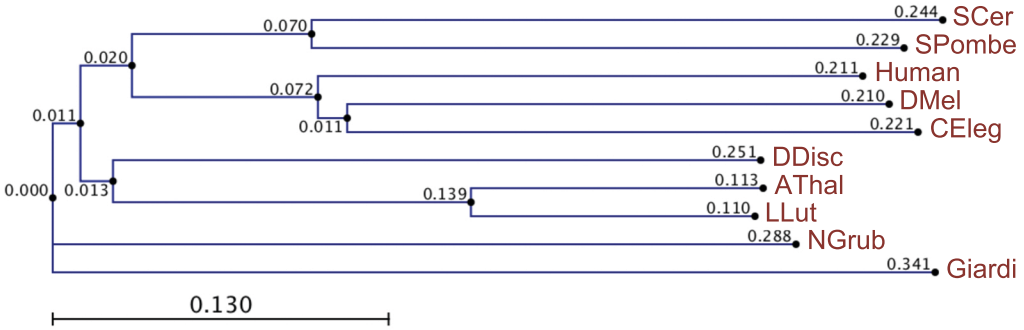

Figure S4. Phylogenetic tree indicating the relationships between the organisms from which GlnRS NTDs were compared in Fig 4B.

49. Corpet, F. (1988) Multiple sequence alignment with hierarchical clustering. *Nucl. Acids Res.*, **16** (22), 10881-10890.

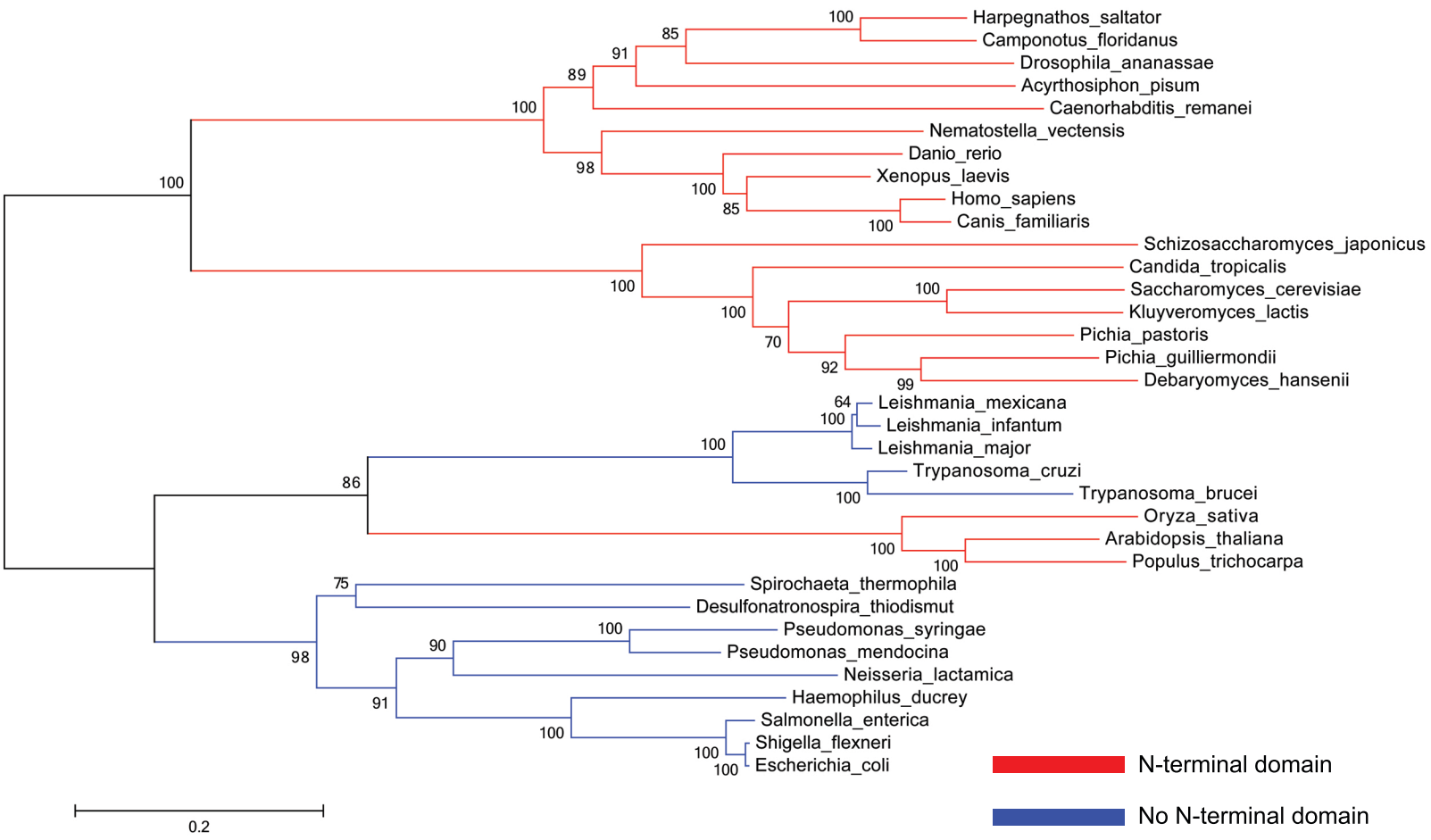

Figure S5. Phylgenetic distribution of GlnRs with and without N-terminal appended domains. Eukaryotic and bacterial GlnRS sequences were aligned using ClustalW and a phylogenetic tree was constructed using MEGA5, with 200 bootstraps carried out to test statistical relevance. In addition to bacteria, a set of Euglenozoa protists lack the appended domain.

54. Tamurka, K. *et. al.* (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution.* doi: 10.1093/molbev/msr121.