Supplementary Data

Supplementary Figures

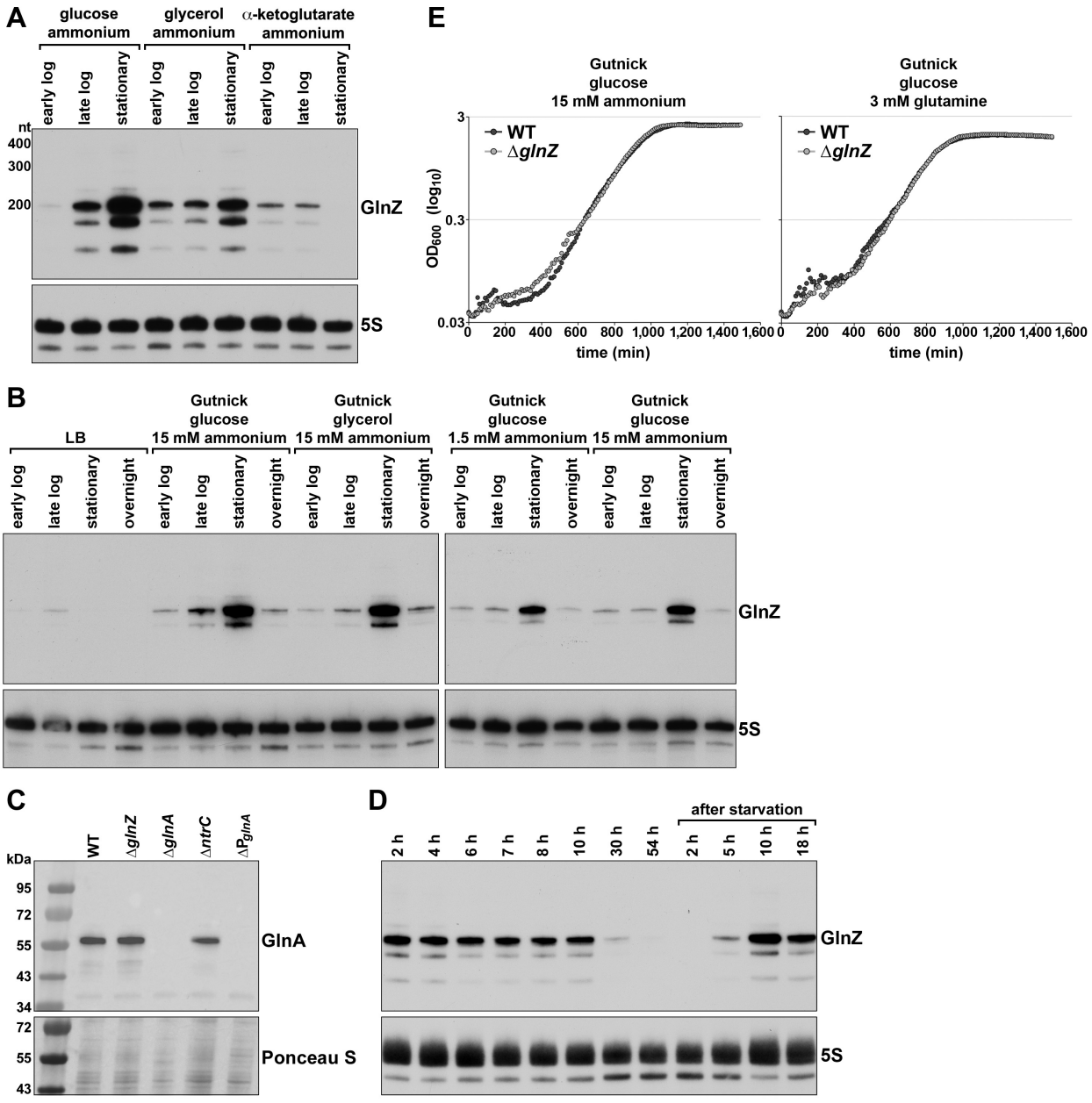


Figure S1. GlnZ expression across growth in different carbon and nitrogen sources. (**A**) Longer exposure of northern blot shown in Figure 1C. WT MG1655 was cultured to early logarithmic, late logarithmic or stationary phases in Gutnick medium with 0.4% glucose, glycerol or α-

ketoglutarate as the sole carbon source and 15 mM ammonium as the nitrogen source, and total RNA was isolated and analyzed as described in the Figure 1C legend. (**B**) Northern blot analysis of GlnZ levels with WT *crl*$^-$ MG1655 (GSO983) cultured to early logarithmic (OD_{600}~0.2), late logarithmic (OD_{600}~0.7), stationary phase (OD_{600}~3.5) or overnight (OD_{600}~3.5) in LB or Gutnick medium with 0.4% glucose or glycerol as the sole carbon source and 1.5 or 15 mM ammonium as the nitrogen source. Total RNA was isolated and subjected to northern blot analysis with labeled oligonucleotide probes to GlnZ and 5S. (**C**) Immunoblot analysis of glutamine synthetase levels. The indicated strains were grown to stationary phase (OD_{600} ~3.5) in Gutnick medium with 0.4% glucose and 15 mM ammonium and 1.5 mM glutamine (to allow growth of the Δ*glnA* and ΔP_{glnA} strains). Samples were normalized by OD_{600}, separated by SDS-PAGE, and analyzed using immunoblot analysis with an anti-glutamine synthetase antibody. The Ponceau S-stained membrane serves as the loading control. (**D**) Northern blot analysis of GlnZ levels with WT MG1655 subjected to long-term starvation. As indicated, samples were collected at 2 h (OD_{600} = 0.12), 4 h (OD_{600} = 0.56), 6 h (OD_{600} = 0.96), 7 h (OD_{600} = 0.98), 8 h (OD_{600} = 1.03), 10 h (OD_{600} = 1.13), 30 h (OD_{600} = 1.15), and 54 h (OD_{600} = 1.00) after subculturing into Gutnick medium with 0.4% glucose and 3.0 mM ammonium. Based on growth attenuation (1), cells become starved between 6 and 7 h. After 54 h, cells were rediluted to OD_{600} = 0.05 in Gutnick medium with 0.4% glucose and 3.0 mM ammonium and cells were collected at 2 h (OD_{600} = 0.045), 5 h (OD_{600} = 0.059), 10 h (OD_{600} = 0.69), and 18 h (OD_{600} = 1.19). Total RNA was isolated and subjected to northern blot analysis with labeled oligonucleotide probes to GlnZ and 5S. (**E**) Growth of WT MG1655 and Δ*glnZ* (GSO1153) strains after nitrogen limitation. Cells were cultured in Gutnick medium with 0.4% glucose and 3 mM ammonium to stationary phase (OD_{600}~1.5). Cultures were then incubated another 48 h before subculturing to an OD_{600} of 0.05 into Gutnick medium with 0.4% glucose and 15 mM ammonium or 3 mM glutamine. After

subculturing, OD_{600} was measured for 26 h. The average of four independent replicates is plotted.

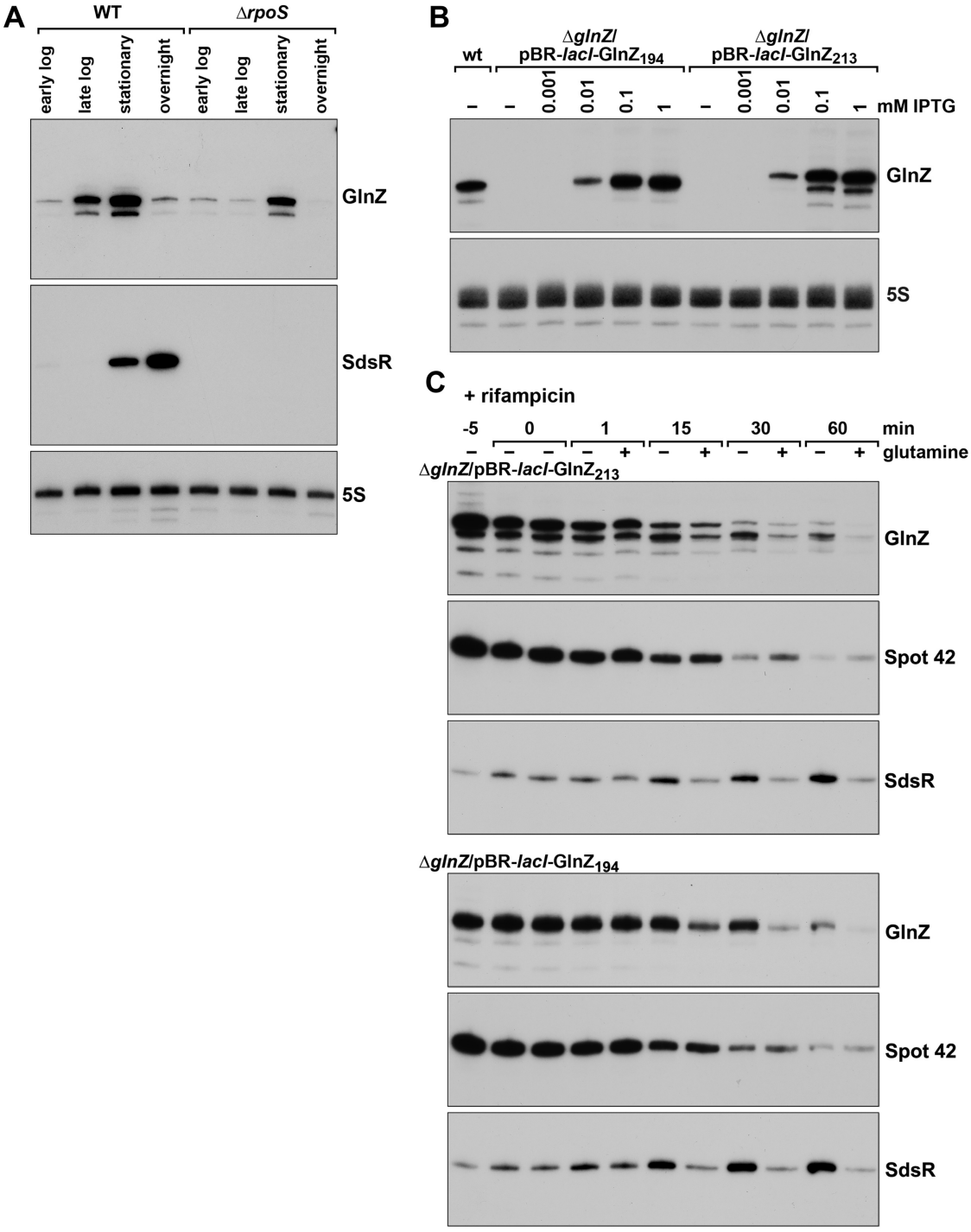


Figure S2. Effect of Δ*rpoS* and *glnZ* promoter on GlnZ levels. (**A**) The effect of Δ*rpoS* on GlnZ levels was evaluated by growing WT *crl*$^{-}$ MG1655 and Δ*rpoS* (GSO108) strains to early

logarithmic (OD_{600}~0.2), late logarithmic (OD_{600}~0.7), stationary phase (OD_{600}~3.5) or overnight (OD_{600}~3.5) in Gutnick medium with 0.4% glucose and 15 mM ammonium. Total RNA was collected and subjected to northern blot analysis as in Figure 1 with labeled oligonucleotide probes to GlnZ, SdsR and 5S. (**B**) WT MG1655 and Δ*glnZ* (GSO1153) cells harboring pBR-*lacI*-$GlnZ_{194}$ or pBR-*lacI*-$GlnZ_{213}$ were grown to stationary phase (OD_{600} ~ 3.5) in Gutnick medium with 0.4% glucose and 15 mM ammonium and induced with a range of IPTG concentrations. Samples were taken 1 h post-induction. Total RNA was collected and subjected to northern blot analysis as in Figure 1 with labeled oligonucleotide probes to GlnZ and 5S. (**C**) The Δ*glnZ* mutant (GSO1153) strain harboring pBR-*lacI*-$GlnZ_{213}$ or pBR-*lacI*-$GlnZ_{194}$ was cultured to late logarithmic phase and expression from the plasmids was induced with 100 μM IPTG for 1 h. The cultures were treated with rifampicin, then split 5 min post rifampicin addition with 15 mM glutamine spiked into one culture. Cells were collected at the times indicated, and total RNA isolated was subjected to northern blot analysis as in Figure 1 with labeled oligonucleotide probes to GlnZ, Spot 42 and SdsR. As we also observe here, SdsR was previously reported to be quite stable in cells treated with rifampicin (2).

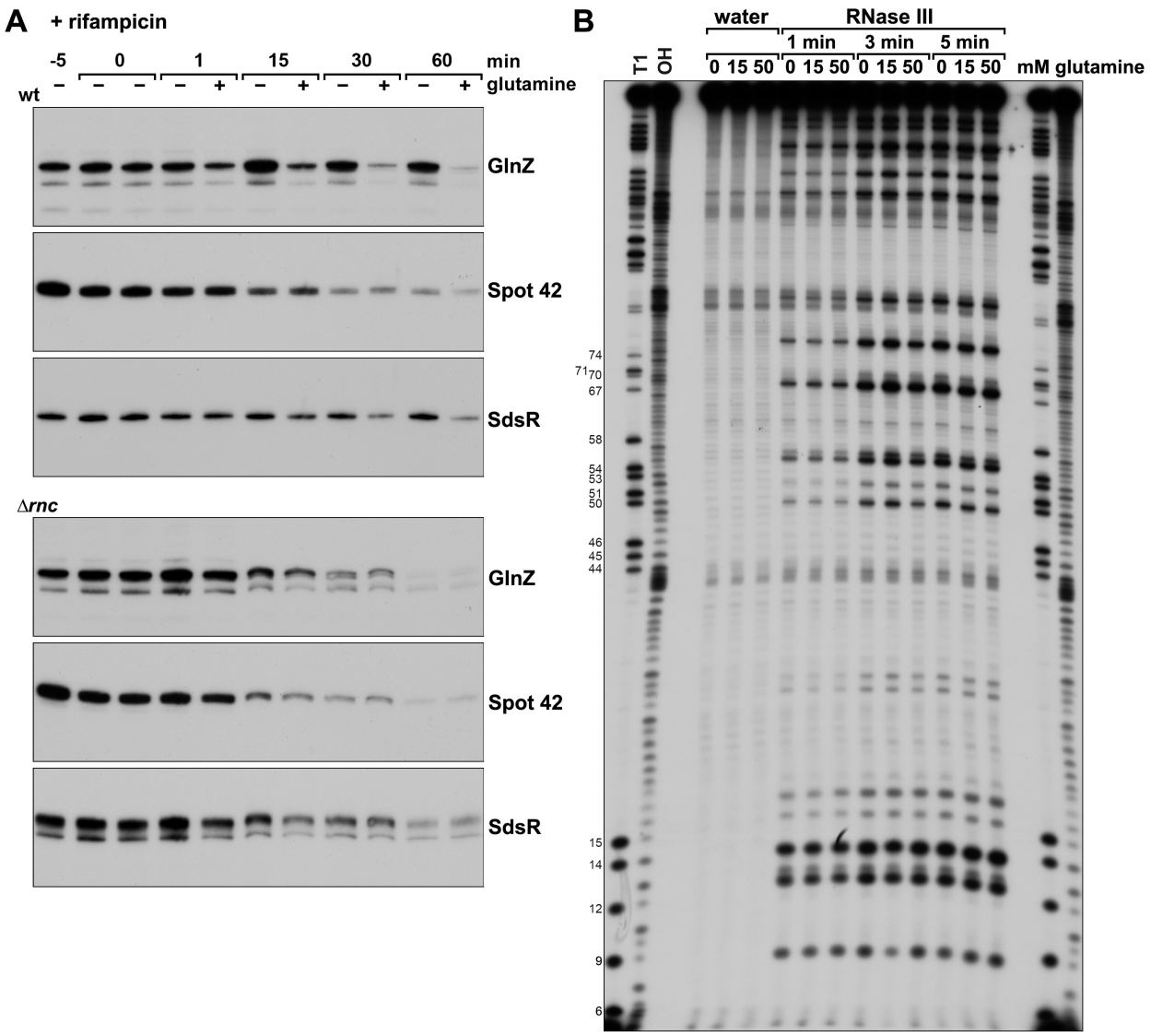
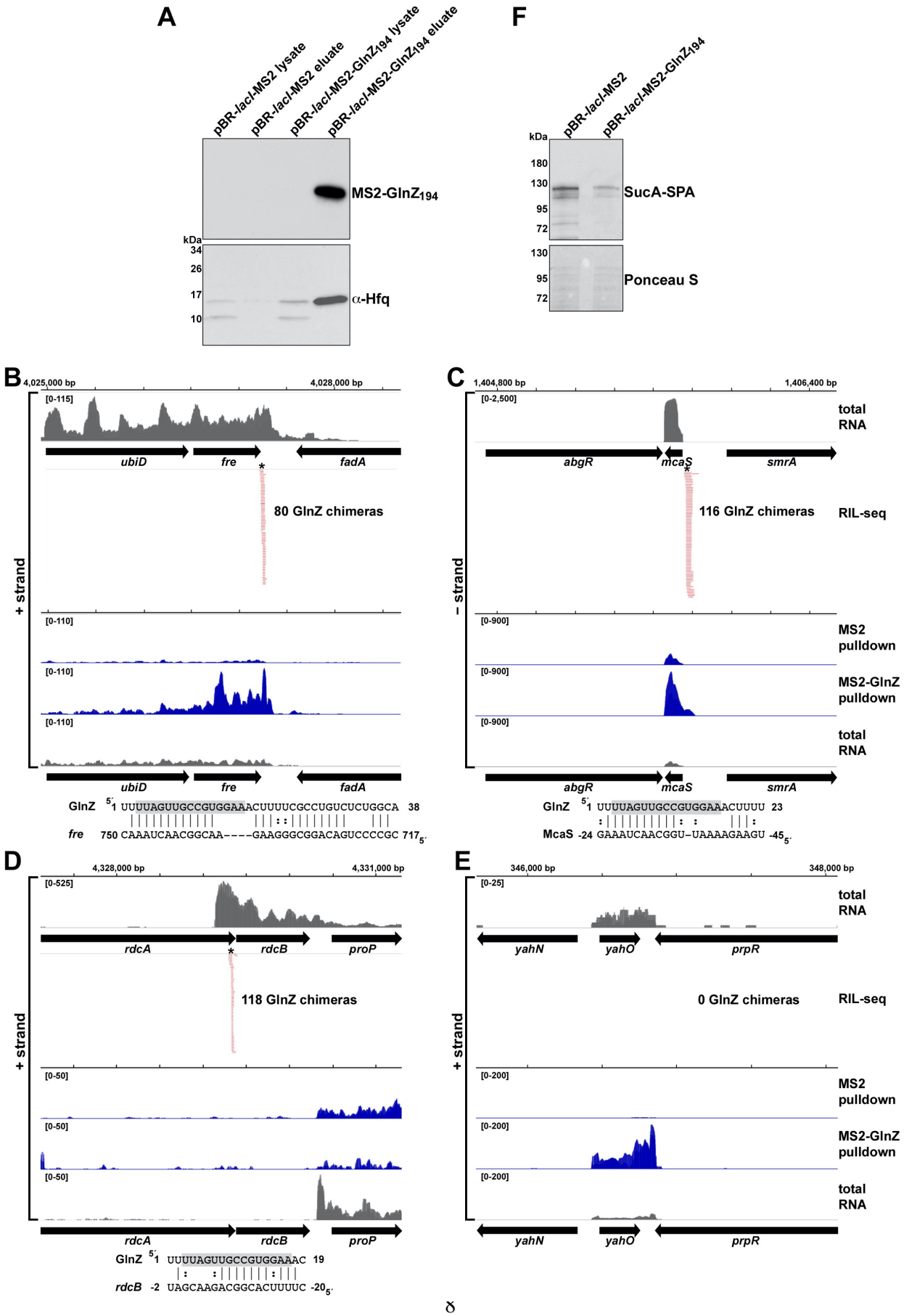


Figure S3. Effect of glutamine on RNase III cleavage in vivo and in vitro. (**A**) Wild type and Δ*rnc* strains (GSO1154) were cultured to stationary phase (OD_{600}~3.5) in Gutnick medium with 0.4% glucose and 15 mM ammonium and treated with rifampicin. Five min post rifampicin treatment, cultures were split, and 15 mM glutamine was spiked into one culture. Cells were collected at the times indicated and total RNA isolated was subjected to northern analysis as in Supplementary Figure SC. (**B**) RNase III in vitro cleavage assay was performed with $GlnZ_{194}$ transcribed in vitro from a T7 promoter and then radiolabeled. This RNA was pre-incubated with

either no glutamine, 15 mM or 50 mM glutamine for 1 h. Subsequently, water (as a control) or RNase III was added, and samples were incubated for 1, 3, or 5 min at 37˚C before stop solution was added. Samples were then resolved on a urea polyacrylamide gel, with T1 and OH ladders. Black dots denote strong cleavage by RNase III.


A
pBR-lacI-MS2 lysate
pBR-lacI-MS2 eluate
pBR-lacI-MS2-GlnZ194 lysate
pBR-lacI-MS2-GlnZ194 eluate
MS2-GlnZ194
kDa
34
26
17
10
α-Hfq
F
pBR-lacI-MS2
pBR-lacI-MS2-GlnZ194
kDa
180
130
95
72
SucA-SPA
Ponceau S
B
4,025,000 bp
4,028,000 bp
[0-115]
ubiD
fre
fadA
80 GlnZ chimeras
+ strand
[0-110]
[0-110]
[0-110]
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fre 750 CAAAUCAACGGCAA----GAAGGGCGGACAGUCCCCGC 717 5′
C
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1,406,400 bp
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mcaS
smrA
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RIL-seq
− strand
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MS2 pulldown
[0-900]
MS2-GlnZ pulldown
[0-900]
total RNA
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McaS -24 GAAAUCAACGGU-UAAAAGAAGU -45 5′
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4,331,000 bp
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rdcB
proP
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+ strand
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348,000 bp
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yahN
yahO
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MS2-GlnZ pulldown
[0-200]
total RNA

Figure S4. RNAs associated with $GlnZ_{194}$ identified by MAPS. (**A**) Northern blot and immunoblot assay after performing the MAPS co-purification protocol with lysates from Δ*glnZ* (GSO1153) overexpressing either MS2 or MS2-$GlnZ_{194}$. Lysate and eluate of indicated samples after 1 h overexpression with 1 mM IPTG were probed for GlnZ or Hfq. Browser images depict RIL-seq datasets (3) or MAPS data for GlnZ interactions with (**B**) *fre*, (**C**) McaS, (**D**) *rdcA,* and (**E**) *yahO*. Total RNA is depicted in grey, RIL-seq chimeras detected in M63 medium in red, and MAPS data for cultures grown in Gutnick medium with 0.4% glucose and 15 mM ammonium at stationary phase is depicted in blue for the pulldown with either MS2 or MS2-GlnZ overexpression. Predicted regions of interaction with GlnZ are depicted below each browser image with GlnZ coordinates given relative to +1 of $GlnZ_{194}$ and target coordinates given relative to the start codon A residue for the mRNAs and relative to +1 of McaS (4). The GlnZ seed region is highlighted in grey. (**F**) Immunoblot analysis of SucA-SPA levels. Δ*glnZ* (GSO1153) cells harboring pBR-*lacI*-MS2 or pBR-*lacI*-MS2-$GlnZ_{194}$ were grown to late logarithmic phase (OD_{600} ~1.0) in Gutnick medium with 0.4% glucose and 15 mM ammonium and plasmids were induced with 1 mM IPTG. Samples were normalized by OD_{600}, separated by SDS-PAGE, and analyzed using immunoblot analysis. The Ponceau S-stained membrane serves as the loading control.

***glnA* 3′** (- strand)

attgatgcgtacatcgctctgcgtcgcgaagaagatgaccgcgtgcgtatgactccgcatccggtagagtttgagct
GTACTACAGCGTCtaaGTG***T***TTTAGTTGCCGTGGAAACT***T***TCGCCTGTCTCTGGCAGGCCTGGGATCGGTGGCAAG
CACATCACGCCGGATGCGACGCAAATGCGTCTTATCCGGCCTACACGGTGATGATGTGGTAGGCCGGAGCAGGTGAG
TCGCTCTCCAACGTGAAGTTTGTCAGCTATCTGTAGCCCATCTCTGCATGGGCTTTTTT

***glnP* 5′** (- strand)

taaTAACGCTACACCTGTAAAACGCACTGGCAGTTCCCTCTCCCCTATGGGGAGAGGATTAGGGTGAGGGGCGCAAA
CCCGCTCCGGGGCCATTAATTACCCTGAATTTGATTATTTACACCACGGTAACAGGAACAACATatgCAGTTTGACT
GGAGTGCCATCTGGCCTGCCATTCCGC

***sucA* 5′** (+ strand)

taaACCGTAGGCCTGATAAGACGCGCAAGCGTCGCATCAGGCAACCAGTGCCGGATGCGGCGTGAACGCCTTATCCG
GCCTACAAGTCATTACCCGTAGGCCTGATAAGCGCAGCGCATCAGGCGTAACAAAGAAATGCAGGAAATCTTTA***A***AA
ACTGCCCCTGACACTAAGACAGTTTTTA***A***AGGTTCCTTCGCGA***G***CCACTACGTAGACAAGAGCTCGCAAGTGAACCC
CGG*C*ACGCACATCACTGTGCGTGGTAGTATCCACGGCGAAGTAAGCATAAAAAAGATGCTT*AAGGG*ATCACGatgCA
GAACAGCGCTTTGAA

***aceE* 5′** (+ strand)

tagTGA***T***TTTTCTGGTAAAAATTATCCAGAAGATGTTGTAAATCAAGCGCATATAAAAGCGCGGCAACTAAACGTAG
AACCTGTCTTATTGAGCTTTCCGGCGAGAGTTCAATG*GG*ACAGGTTCCAGAAAACTCAACGTTATTAGATAGAT*AAG*
*GAA*TAACCCatgTCAGAACGTTTCCCAAATGACGTGGATCCGATCGAAA

***tmaR* (*yeeX*)** (- strand)

taaTTCCTGAACTTCAGAATCATCTTGCTGCTGCTTCGATTCAGCAAGGATAAAGGGTATGATAGT**G**AAAAGGGATA
AAAGCATTGTCATCTGCGGCAGCTATGAGTAATGTTGGCCCTAACGAATAGCGGTTGCTTAAACGAATCCGA***C***TCTC
ACATTATCAGGGGTATAAAAatgGAAACTACCAAGCCTTCATTCCAGGACGTACTGGAATTTGTTCGTCTGTTCCGT
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CTGAAAGCTATGGGCGAAATGAAAAACGGCGAAGCGAAGtaaTTCCCGTTTTATTCAATGAGGGTTGCCCGGCAACC
CTCATTG*C*TCATTGA

***fre* 3′** (+ strand)

tgaTGCGCGTTTGTTTTGCCCTATTTATCGATCCGAC*AGAGAA*AGCGCatgACAACCTTAAGCTGTAAAGTGACCTC
GGTAGAAGCTATCACGGATACCGTATATCGTGTCCGCATCGTGCCAGACGCGGCCTTTTCTTTTCGTGCTGGTCAGT
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McaS (- strand)

GATATGATAACCAGACCGGGTCGGTCCAACAACGTATTACCCAAATTTCCAGTAATAAGTTCCAAATATTGCCGATA
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GACGCGGATGCACTGCTGTGTGTACTGTAGAGTCTGGCGGATGTCGACAGACTCTATTTTttt

***rdcB* (*yjcZ*) regulator of diguanylate cyclase** (+ strand)

TT**A**CGGCAGGCATGAATACTTTTTTCGCTGAGTTCGCTTCATGTTTGACGGAATTACAGACGCGTTTACGCGAAAGT
CTGGCTCTGCGTCAACAAAATGAATCGGTGGTCAGGCTGATGCAGCAGCAATTGCAGCAGACTGTGATGACTCACGG
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***yahO* DUF1471 domain-containing protein** (+ strand)

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A*TAAG*AAGAAG**taa**TTCTGAATCCTATGTAAACATCTCCGATGCGTAAGTTTATCGGTGATCATCTATTGAAATTTA
TGCCGGATAAAGCGTTCGCGCTGCATTCGGCAGT*TCA*GCTTTTCAGCCGCCGCCAGAACGTCGTCCGGCTGATGCCT
AAATAATTCGCCGCTGCTGTTTTATCGCCATTAAATTTCTCCAGTGCCTGTTGTGGTGTC

Figure S5. Sequences of *glnZ* as well as *sucA*, *glnP*, *aceE, tmaR, fre,* McaS, *rdcB*, and *yahO* surrounding predicted regions of base pairing. Stop codons are in red font, start codons are in green font, possible stem defining terminators are in gray font and possible 3′ ends from (5) and ribosome binding sites are in italics. Transcription start sites from (6) are indicated in bold. Underlined nucleotides indicate positions of RNase III cleavage from (7,8). Sequences highlighted in blue correspond to possible regions of pairing with the GlnZ seed sequence and sequences in orange font are ARN sequences that could be Hfq binding sites. The sequence upstream of the start of $GlnZ_{213}$ is in lower case with predicted -10 and -35 sequences underlined.

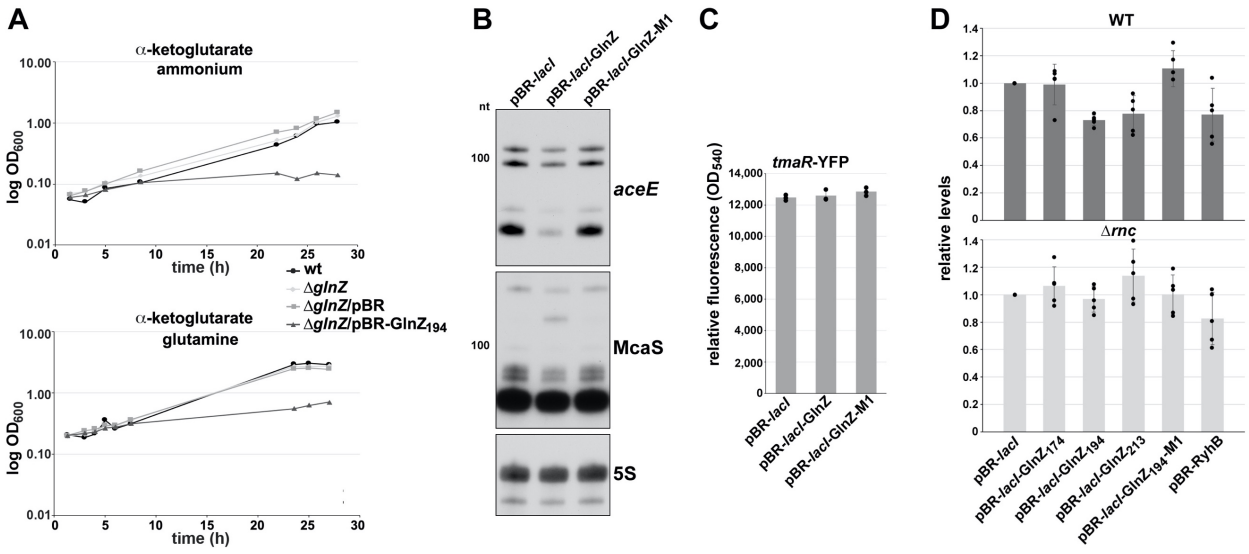
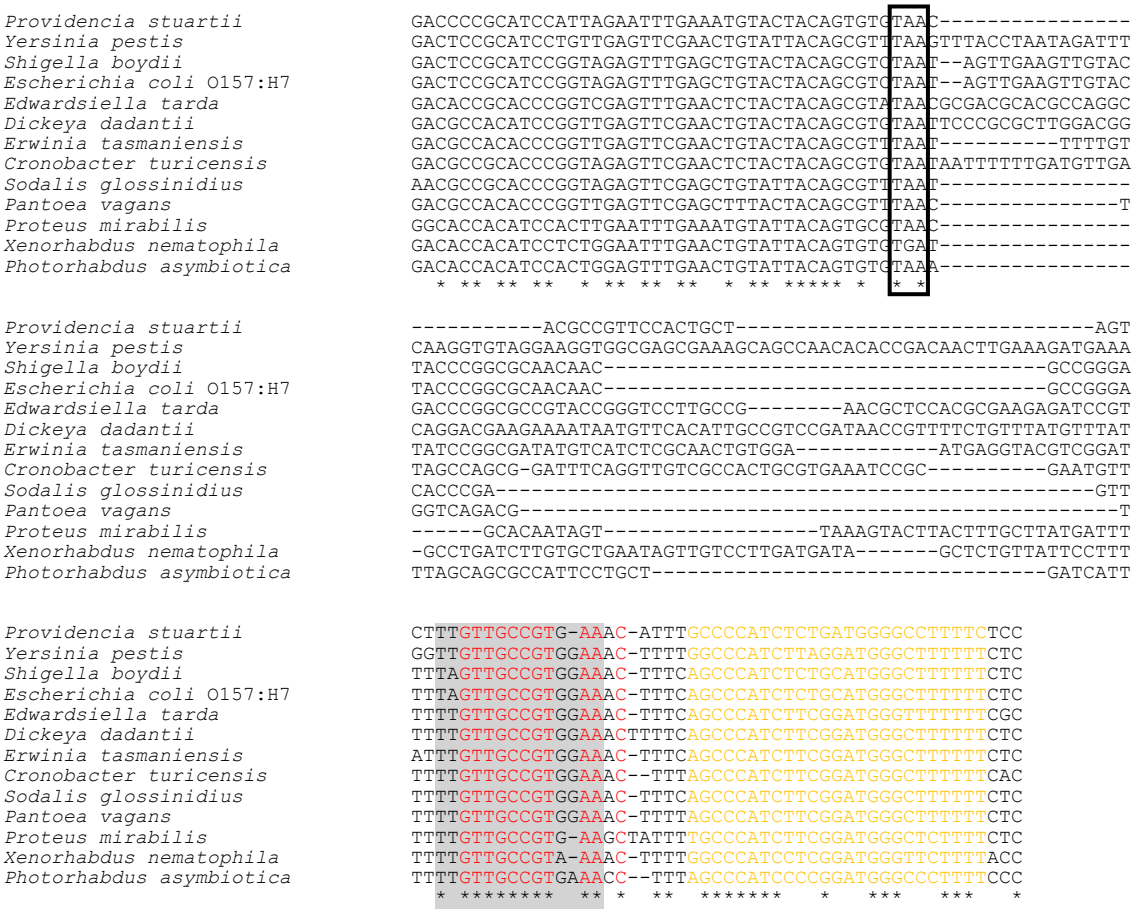


Figure S6. Growth assays and GlnZ regulation of *aceE*, McaS, *tmaR*-YFP and SucA-SPA. (**A**) WT and Δ*glnZ* (GSO1153) strains harboring the plasmids indicated were subcultured to an OD_{600} ~0.05 in Gutnick medium with 0.4% α-ketoglutarate as the carbon source and either 15 mM ammonium or 15 mM glutamine as the nitrogen source. Plasmids with induced with 1 mM IPTG. Cultures were incubated for 28 h and OD_{600} was measured at the times indicated. The average of three independent replicates is plotted. (**B**) GlnZ downregulation of *aceE* and McaS transcripts. The Δ*glnZ* (GSO1153) strain harboring the indicated plasmids was grown in Gutnick medium with 0.4% glucose and 15 mM ammonium to stationary phase (OD_{600} ~3.5). Plasmids were induced for 1 h with 1 mM IPTG. Total RNA was collected and subjected to northern blot analysis with labeled oligonucleotide probes to *aceE*, McaS, and 5S. For *aceE* and 5S, this figure shows the first three lanes of the northern presented in Figure 7D. The same blot was sequentially probed for McaS shown here. (**C**) $GlnZ_{194}$ does not regulate a *tmaR*-YFP fusion. The indicated strains were grown for 3 h in LB medium with 1 mM IPTG to induce WT and mutant GlnZ expression. Relative fluorescence units were determined by measuring OD_{540} for

each sample and normalizing by OD_{600}. Each bar corresponds to the average of three independent replicates with individual data points shown as dots. Error bars represent one standard deviation. (**D**) Quantification of immunoblot shown in Figure 6A together with four independent repeats of the same experiment. Each bar corresponds to the average of the five independent replicates relative to the pBR-*lacI* vector control with individual data points shown as dots. Error bars represent one standard deviation.

A

Class I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Escherichia coli O157:H7
Edwardsiella tarda
Dickeya dadantii
Erwinia tasmaniensis
Cronobacter turicensis
Sodalis glossinidius
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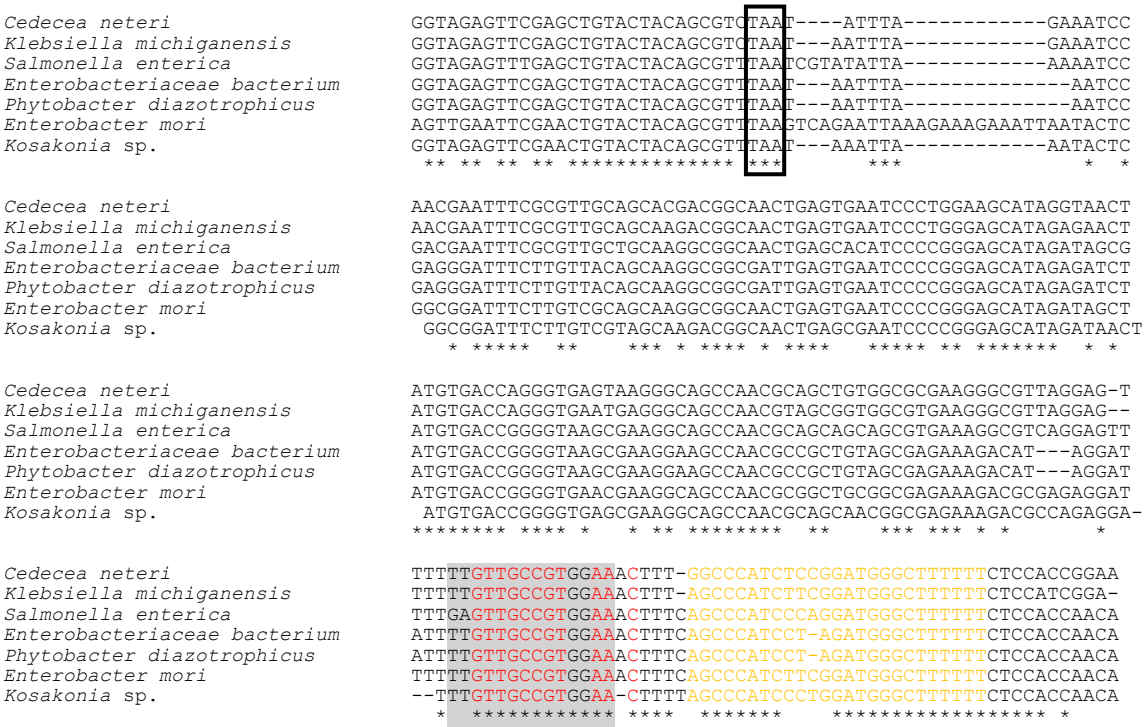
Xenorhabdus nematophila
Photorhabdus asymbiotica

Class II



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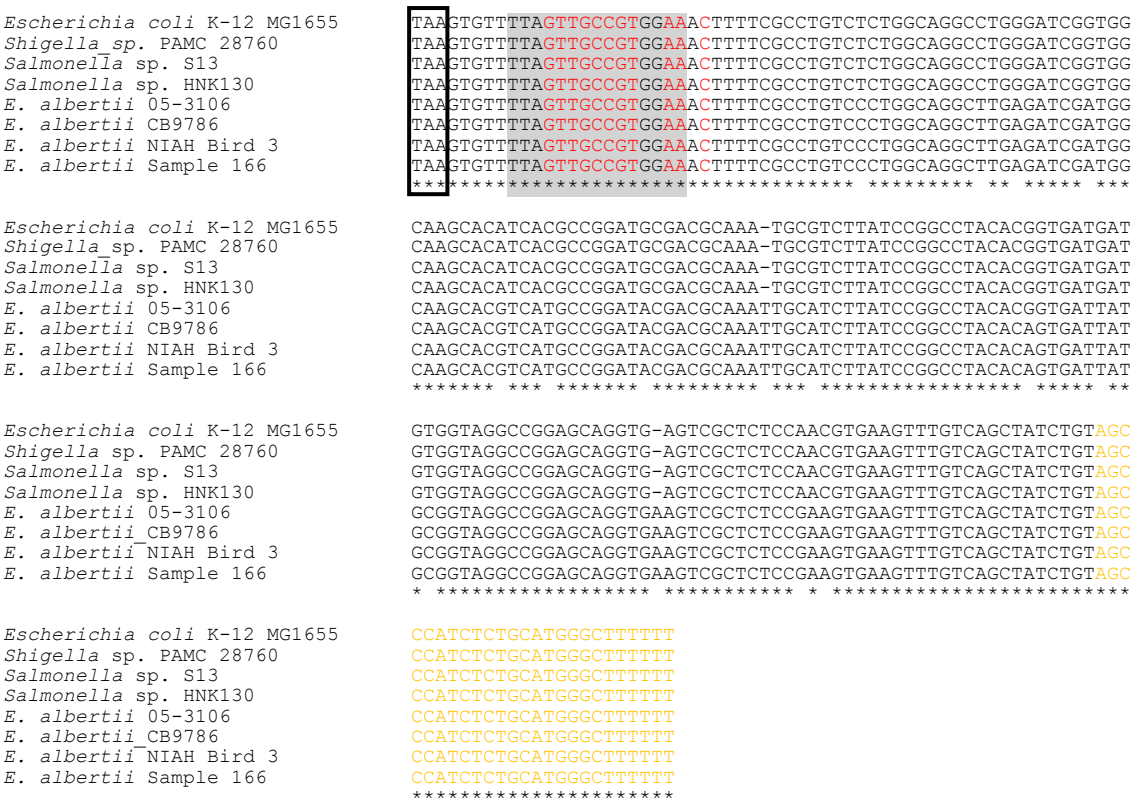

B

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Yersinia pestis                  GGTTGTTGCCGTGGAAAC
Salmonella enterica              TTGAGTTGCCGTGGAAAC
Shigella boydii                  TTTAGTTGCCGTGGAAAC
Escherichia coli O157:H7         TTTAGTTGCCGTGGAAAC
Escherichia coli K-12 MG1655     TTTAGTTGCCGTGGAAAC
Shigella sp. PAMC 28760          TTTAGTTGCCGTGGAAAC
Salmonella sp.                   TTTAGTTGCCGTGGAAAC
Kosakonia sp.                    TTTTGTTGCCGTGGAA-C
Edwardsiella tarda               TTTTGTTGCCGTGGAAAC
Dickeya dadantii                 TTTTGTTGCCGTGGAAAC
Cronobacter turicensis           TTTTGTTGCCGTGGAAAC
Sodalis glossinidius             TTTTGTTGCCGTGGAAAC
Pantoea vagans                   TTTTGTTGCCGTGGAAAC
Cedecea neteri                   TTTTGTTGCCGTGGAAAC
Klebsiella michiganensis         TTTTGTTGCCGTGGAAAC
Enterobacteriaceae bacterium     TTTTGTTGCCGTGGAAAC
Phytobacter diazotrophicus       TTTTGTTGCCGTGGAAAC
Enterobacter mori                TTTTGTTGCCGTGGAAAC
Erwinia tasmaniensis             ATTTGTTGCCGTGGAAAC
                                   ********  ** *
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Figure S7. Conservation of GlnZ seed sequence and terminator despite overall lack of conservation. (**A**) Sequence alignment of the 3′UTR of *glnA* from various Gammaproteobacteria containing class I, class II, or class III GlnZ homologs. The *glnA* stop codon is indicated with a box. Nucleotides conserved in all sequences are indicated with an asterisk. The GlnZ seed sequence predicted by RIL-seq data is highlighted in grey, and the seed residues conserved across all species used in our analysis are in red font. The predicted terminator stem-loops are in orange font. (**B**) Sequence alignment of seed sequence from Gammaproteobacteria. All alignments were generated using MUSCLE (9).

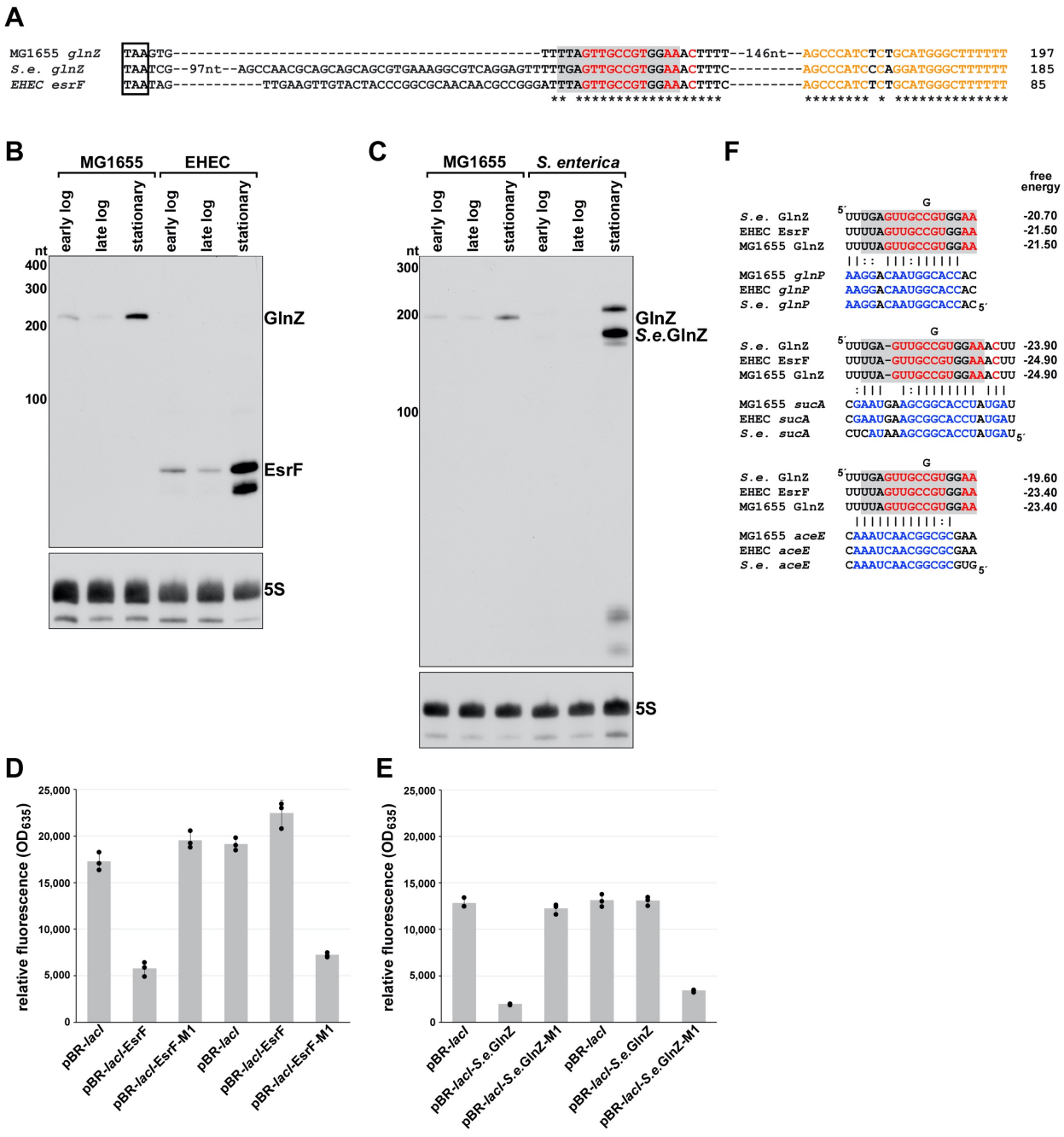

Figure S8. Conservation of GlnZ expression, and function. (**A**) Sequence alignment of the GlnZ seed sequence region (seed sequence predicted by RIL-seq data highlighted in grey, and residues conserved across all species used in our analysis in red font) and terminator stem-loop (conserved residues in orange font) for the 3′ UTRs of *glnA* from *E. coli* MG1655, EHEC (NC_002695.2) and *Salmonella enterica* (NC_003197.2). Nucleotides conserved in all three

sequences are indicated with an asterisk. Positions are given relative to the *glnA* stop codon. Alignment was generated using MUSCLE (9). (**B**) Northern blot analysis of GlnZ levels with *E. coli* MG1655 or EHEC cultured to early logarithmic, late logarithmic, or stationary phases in Gutnick medium with 0.4% glucose and 15 mM ammonium. RNA was isolated from samples and subjected to northern analysis as described in Figure 1. (**C**) Northern blot analysis of GlnZ levels with *E. coli* MG1655 or *S. enterica* LT2 cultured to early logarithmic, late logarithmic, or stationary phases in Gutnick medium with 0.4% glucose and 15 mM ammonium. RNA was isolated from samples and subjected to northern analysis as described in Figure 1. Regulation by (**D**) EsrF-M1 and (**E**) *S.e*.GlnZ-M1 is restored for the *glnP-M1-mCherry* fusion. The indicated strains were grown for 3 h in Gutnick medium with 0.4% glucose and 15 mM ammonium and 1 mM IPTG to induce WT and mutant EsrF and *S.e*.GlnZ expression and either 0.2% arabinose (*glnP-mCherry*) or 2% arabinose (*glnP-M1-mCherry*) to induce the mCherry fusion. Relative fluorescence units were determined by measuring OD_{635} for each sample and normalizing by OD_{600}. Each bar corresponds to the average of three independent replicates with individual data points shown as dots. Error bars represent one standard deviation. (**F**) Conservation of the region of base pairing with GlnZ targets *glnP*, *sucA*, and *aceE*. The sequences of the base pairing regions for the targets are shown with the GlnZ seed sequence predicted by RIL-seq data highlighted in grey, the GlnZ residues conserved across all species used in our analysis in red font, and the conserved target nucleotides involved in base pairing in blue font. The M1 mutant sequence is indicated above the alignments. The interaction between GlnZ and its targets in *E. coli* is indicated with lines.

Supplementary Tables

Table S1. Strains used in study

GSO Name	Strain Name	Relevent Genotype	Source
GSO983	MG1655	*Escherichia coli* wild-type strain (*crl*-)	Lab stock
GSO982	MG1655	*Escherichia coli wild-type strain* (*crl*+)	Lab stock
	NM400	MG1655 mini-λ cm^R ts	Gottesman Lab
	NM500	MG1655 mini-λ tet^R ts	Gottesman Lab
	PM1205	PM1203 *lacI'::* P_{BAD}*-cat-sacB:lacZ,* miniλ tet^R	(10)
	NRD1166	MG1655 Zeo T1 T2 P_{lac}-*ccdB-kan*-mCherry at *lac* locus, mini-λ:tet	Gottesman Lab
GSO108	MG1655 Δ*rpoS*	MG1655 Δ*rpoS*::*tet*	(11)
	JW3839 Δ*glnG*	Δ*glnG::kan* (=Δ*ntrC::kan*)	Keio collection
GSO1151	MG1655 Δ*glnG*	Δ*glnG::kan*	This study
GSO1152	MG1655 ΔP_{glnA}	ΔP_{glnA}*::kan*	This study
GSO1153	MG1655 Δ*glnZ*	Δ*glnZ::kan*	This study
GSO1154	MG1655 Δ*rnc*	Δ*rnc::cam*	Lab stock
GSO1155	MG1655 Δ*rnc*Δ*glnZ*	Δ*rnc::cam* Δ*glnZ::kan*	This study
GSO1156	PM1205 P_{glnA}*-lacZ*	P_{glnA}*:lacZ*	This study
GSO1157	PM1205 P_{glnZ}*-lacZ*	P_{glnZ}*:lacZ*	This study
GSO1158	NRD1166 *glnP*-mCherry	*glnP*-mCherry	This study
GSO1159	NRD1166 *glnP*-M1-mCherry	*glnP*-M1-mCherry	This study
	DY330 *sucA-SPA*	W3110 Δ*lacU169 gal490 λCI857* Δ*(cro-bioA) sucA:SPA::kan*	(12)
GSO1160	NM500 Δ*sucA::cat-sacB*	Δ*sucA::cat-sacB*	This study
GSO1161	MG1655 *sucA*-SPA	*sucA-SPA::kan*	This study
GSO1162	MG1655 *sucA*-M1-SPA	*sucA-M1-SPA::kan*	This study
GSO1163	MG1655 Δ*rnc sucA*-SPA	Δ*rnc::cam sucA-SPA::kan*	This study
	SX1989 *yeeX-YFP*	*yeeX793-YFP::cat*	(13)
GSO1164	MG1655 *tmaR-YFP*	*yeeX793-YFP::cat*	This study
	Escherichia coli EHEC O157:H7 EDL933		ATCC
	Salmonella enterica subs. e*nterica* LT2		Lab stock
GSO1165	NEB5α + pBR-lacI-$GlnZ_{213}$	pBR-lacI-$GlnZ_{213}$	This study
GSO1166	NEB5α + pBR-lacI-$GlnZ_{194}$	pBR-lacI-$GlnZ_{194}$	This study
GSO1167	NEB5α + pBR-lacI-$GlnZ_{194}$-M1	pBR-lacI-$GlnZ_{194}$-M1	This study
GSO1168	NEB5α + pBR-lacI-$GlnZ_{174}$	pBR-lacI-$GlnZ_{174}$	This study
GSO1169	NEB5α + pBR-lacI-MS2-$GlnZ_{194}$	pBR-lacI-MS2-$GlnZ_{194}$	This study
GSO1170	NEB5α + pBR-lacI-EsrF	pBR-lacI-EsrF	This study
GSO1171	NEB5α + pBR-lacI-EsrF-M1	pBR-lacI-EsrF-M1	This study
GSO1172	NEB5α + pBR-lacI-S.e.GlnZ	pBR-lacI-S.e.GlnZ	This study
GSO1173	NEB5α + pBR-lacI-S.e.GlnZ-M1	pBR-lacI-S.e.GlnZ-M1	This study
GSO1174	NEB5α + pBR-$GlnZ_{194}$	pBR-$GlnZ_{194}$	This study

GSO1175	NEB5α + pBR-GlnZ$_{194}$-M1	pBR-GlnZ$_{194}$-M1	This study
GSO1176	NEB5α + pUC19-SucA-SPA	pUC19-SucA-SPA	This study
GSO1177	NEB5α + pUC19-SucA-M1-SPA	pUC19-SucA-M1-SPA	This study

Table S2. Plasmids used in study

Plasmid Name	Plasmid Description	Source
pBR	pBR322 carrying an inducible PlacO-1 promoter. AmpR KanR	(14)
pBR-*lacI*	pNM46, pBR carrying the *lacI* gene (amp^R)	N. Majdalani
pBR-*lacI*-GlnZ$_{213}$	pBR-*lacI* carrying 213 nt variant of GlnZ	This study
pBR-*lacI*-GlnZ$_{194}$	pBR-*lacI* carrying 194 nt variant of GlnZ	This study
pBR-*lacI*-GlnZ$_{194}$-M1	pBR-*lacI* carrying 194 nt variant of GlnZ with M1 mutation	This study
pBR-*lacI*-GlnZ$_{174}$	pBR-*lacI* carrying 174 nt variant of GlnZ	This study
pBR-*lacI*-MS2	pBR-*lacI* carrying the MS2 tag	N. Thongdee
pBR-*lacI*-MS2-GlnZ$_{194}$	pBR-*lacI* carrying 194 nt variant of GlnZ	This study
pBR-*lacI*-EsrF	pBR-*lacI* carrying EsrF	This study
pBR-*lacI*-EsrF-M1	pBR-*lacI* carrying EsrF with seed sequence mutation	This study
pBR-*lacI*-*S.e.*GlnZ	pBR-*lacI* carrying *S. enterica* GlnZ	This study
pBR-*lacI*-*S.e.*GlnZ-M1	pBR-*lacI* carrying *S. enterica* GlnZ with M1 mutation	This study
pBR-GlnZ$_{194}$	pBR carrying 194 nt variant of GlnZ	This study
pBR-GlnZ$_{194}$-M1	pBR carrying 194 nt variant of GlnZ with M1 mutation	This study
pBR-RyhB	pBR carrying sRNA *ryhB*	(15)
pUC19	amp^R	New England Biolabs
pUC19-SucA-SPA	pUC19 carrying *sucA-SPA::kan* including the 5′ UTR of SucA	This study
pUC19-SucA-M1-SPA	pUC19 carrying *sucA-SPA::kan* including the 5′ UTR of SucA with M1 mutation	This study

Table S3. Oligonucleotides used in study

Name	Purpose	Sequence
AK280	Northern probe against GlnZ	ATGGGCTACAGATAGCTGACAAACTTCACG
LW030	Northern probe against 5S rRNA	CGGCGCTACGGCGTTTCACTTCTG
MR023	Northern probe against Spot 42	GGTCTGAAAGATAGAACATCTTACCTCTGT
LW066	Northern probe against SdsR	GTATTCGGTCCAGGGAAATGGCTCTTGGG
LW055	Oligo for AceE primer extension analysis	GATCGGATCCACGTCATTTGGG
LW138	Northern probe against EsrF and *S.e.*GlnZ	GCTGAAAGTTTCCACGGCAACTAAATCCCG
LW178	Northern probe against AceE 5'UTR	CTCTCGCCGGAAAGCTCAATAAGACAGGTTCTACGTTTAGTTGCCGCGC
LW251	Northern probe against McaS	TCCGCGTCTTAAATCCGGCATTGTCTCCTCTGCGCCGGT
LW043	Forward primer to amplify pBR-*lacI* plasmid for Gibson cloning	GAATTCTCATGTTTGACAG
LW044	Reverse primer to amplify pBR-*lacI* plasmid for Gibson cloning	GACGTCAGTATCTTGTTATC
LW045	Forward primer to amplify $GlnZ_{213}$ for Gibson cloning into pBR-*lacI*	GATAACAAGATACTGACGTCGTACTACAGCGTCTAAGTG
LW046	Reverse primer to amplify $GlnZ_{174}$/$GlnZ_{194}$/$GlnZ_{213}$ for Gibson cloning into pBR-*lacI*	GCTGTCAAACATGAGAATTCAAAAAAGCCCATGCAGAG
LW047	Forward primer to amplify $GlnZ_{194}$ for Gibson cloning into pBR-*lacI*	GATAACAAGATACTGACGTCTTTTAGTTGCCGTGGAAAC
LW060	Forward primer to amplify $GlnZ_{174}$ for Gibson cloning into pBR-*lacI*	GATAACAAGATACTGACGTCTTTCGCCTGTCTCTGGCAG
LW127	Forward QuikChange primer to make seed sequence mutant in pBR-*lacI*-$GlnZ_{194}$	AGGCGAAAAGTTTCCACCGCAACTAAAAGACGTCA
LW128	Reverse QuikChange primer to make seed sequence mutant in pBR-*lacI*-$GlnZ_{194}$	TGACGTCTTTTAGTTGCGGTGGAAACTTTTCGCCT
LW113	Forward primer to amplify pBR-*lacI*-MS2 plasmid for Gibson cloning	AACCATTATTATCATGACATTAACCTATAAAAATAG
LW114	Reverse primer to amplify pBR-*lacI*-MS2 plasmid for Gibson cloning	CAGACCCTGATGGTGTCTG
LW115	Forward primer to amplify $GlnZ_{194}$ for Gibson cloning into pBR-lacI-MS2	GCAGACACCATCAGGGTCTGTTTTAGTTGCCGTGGAAAC
LW116	Reverse primer to amplify $GlnZ_{194}$ for Gibson cloning into pBR-lacI-MS2	ATGTCATGATAATAATGGTTAAAAAAGCCCATGCAGAG
LW132	Forward primer to amplify EsrF for Gibson cloning into pBR-*lacI*	GATAACAAGATACTGACGTCTAGTTGAAGTTGTACTACCC
LW133	Reverse primer to amplify EsrF for Gibson cloning into pBR-*lacI*	GCTGTCAAACATGAGAATTCAAAAAAGCCCATGCAGAG
LW139	Forward QuikChange primer to make seed sequence mutant in pBR-*lacI*-EsrF	GGCTGAAAGTTTCCACCGCAACTAAATCCCGGC
LW140	Reverse QuikChange primer to make seed sequence mutant in pBR-*lacI*-EsrF	GCCGGGATTTAGTTGCGGTGGAAACTTTCAGCC
LW147	Forward primer to amplify *S.e.*GlnZ for Gibson cloning into pBR-*lacI*	GATAACAAGATACTGACGTCTCGTATATTAAAAATCCGAC

LW148	Reverse primer to amplify *S.e.*GlnZ for Gibson cloning into pBR-*lacI*	GCTGTCAAACATGAGAATTCAAAAAAGCCCATCC
AK305	Forward primer to clone $GlnZ_{213}$ into pBR	GACGTCGTACTACAGCGTCTAAGTGTTTTAGTTG
AK306	Forward primer to clone $GlnZ_{194}$ into pBR	GACGTCTTTTAGTTGCCGTGGAAACTTTTCG
AK307	Forward primer to clone $GlnZ_{174}$ into pBR	GACGTCTTTCGCCTGTCTCTGGCAG
AK308	Reverse primer to clone GlnZ isoforms into pBR	GAATTCAAAAAAGCCCATGCAGAGATGG
AK379	Forward primer to clone $GlnZ_{194}$-M1 into pBR	GACGTCTTTTACTTCGGCTCCAAACTTTTCGCCTGTCTCTGG
AK407	Forward primer to make a chromosomal deletion of the *glnA* promoter	ACTTTAACTCTCCTGGATTGGTCATGGTCGTCGTGGTAACGAAATCTGCAG TGTAGGCTGGAGCTGCTTC
AK415	Reverse primer to make a chromosomal deletion of the *glnA* promoter	TTACGCAATTTTTCGATCACAACTTTGCCTCAGGCATTAGAAATAGCGCGA TGGGAATTAGCCATGGTCC
AK318	Forward primer to delete *glnZ* from the MG1655 chromosome	TGCGTATGACTCCGCATCCGGTAGAGTTTGAGCTGTACTACAGCGTCTAAT GACTCTGCTAATACTGTTACGAGTGGCTGCTTACAGCGGTACTCTGCTACC GCTTTTTTGTGTAGGCTGGAGCTGCTTC
AK320	Reverse primer to delete *glnZ* from the MG1655 chromosome	AACGTGAAGTTTGTCAGCTATCTGTAGCCCATCTCTGCATGGGCTTTTTTG TGTAGGCTGGAGCTGCTTC
LW069	Forward primer to clone GlnA promoter into PM1205	CGAAGCGGCATGCATTTACGTTGACACCATCGAATGGCGCCCATTGAAGCA CTATATTGGTGC
LW070	Reverse primer to clone GlnA promoter into PM1205	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACAGCGGACATAC TTTAACTCTCC
LW025	Forward primer to clone GlnZ promoter into PM1205	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCATGAATTCTG TTTCCTGTGTGAAATTGTTATCCGCTCACAATTAGCTCAAACTCTACCGGA TGCG
LW068	Reverse primer to clone GlnZ promoter into PM1205	CGAAGCGGCATGCATTTACGTTGACACCATCGAATGGCGCGACCTGCCGCC AGAAGAAGC
LW189	Forward primer to replace SucA with *cat-sacB* in the chromosome	AGCGCAGCGCATCAGGCGTAACAAAGAAATGCAGGAAATCAATGAGACGTT GATCGGCACGTAAG
LW202	Reverse primer to replace SucA with *cat-sacB* in the chromosome	GGGACCAGAATATCTACGCTACTCATTGTGTATCCTTTATGTAACAGATGA ACAGCATGTAACACC
LW234	Forward primer to amplify pUC19 for Gibson cloning	ATCCGCTTACAGACAAGC
LW235	Reverse primer to amplify pUC19 for Gibson cloning	AGCTGTTTCCTGTGTGAAATTG
LW236	Forward primer to clone SucA-SPA into pUC19	ATTTCACACAGGAAACAGCTAGCGCAGCGCATCAGGCGTAACAAAGAAATG C
LW237	Reverse primer to clone SucA-SPA into pUC19	CAGCTTGTCTGTAAGCGGATGGGACCAGAATATCTACGCTACTCATTGTGT ATC
LW240	Forward QuikChange primer to make compensatory mutant in pUC19-SucA-SPA	TTTTATGCTTACTTCGCGGTGGATACTACCACGCA
LW241	Reverse QuikChange primer to make compensatory mutant in pUC19-SucA-SPA	TGCGTGGTAGTATCCACCGCGAAGTAAGCATAAAA
LW242	Forward primer to make SucA-SPA/SucA-M1-SPA on the chromosome	AGCGCAGCGCATCAGGCGTAAC
LW243	Reverse primer to make SucA-SPA/SucA-M1-SPA on the chromosome	GGGACCAGAATATCTACGCTACTCATTGTG

LW117	Forward primer to make *glnP*-mCherry on the chromosome of NRD1166	ATACTATGCCGATATACTATGCCGATGATTAATTGTCAACGCGCTTCAGCC ATACTTTTCATACTC
LW118	Reverse primer to make *glnP*-mCherry on the chromosome of NRD1166	CCTTGATGATGGCCATGTTATCCTCCTCGCCCTTGCTCACAAACTGCATAT GTTGTTCCTGTTACCG
LW137	Reverse primer to make *glnP*-M1-mCherry on the chromosome of NRD1166	CCTTGATGATGGCCATGTTATCCTCCTCGCCCTTGCTCACAAACTGCATAT GTTGTTCCTGTTA

Table S4. Summary of GlnZ targets based on RIL-seq experiments. Summary of sRNAs targets based on RIL-seq experiments. RIL-seq datasets from experiments done in six different conditions (3,16) were analyzed in order to predict GlnZ targets. Table is sorted according to the number of conditions in which a target was found. Red font indicates GlnZ was the first RNA in the chimera, for all others GlnZ was the second RNA. Targets also found in the top 50 candidates from the MAPS dataset are highlighted in grey.

Table S5. Summary of GlnZ targets based on MAPS experiments. MS2 affinity purification followed by RNA-seq was performed with stationary phase cultures grown in Gutnick medium with 0.4% glucose and 15 mM ammonium in duplicate. Differential expression analysis was carried out using DESeq2. Table is sorted by most significant adjusted p-value (padj). lfcSE corresponds to the standard error of the $\log_2$-fold change. Targets also found in the top 50 of the RIL-seq dataset are highlighted in grey.

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