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Supplemental Information

Endogenous rRNA Sequence Variation Can Regulate Stress Response Gene Expression and Phenotype

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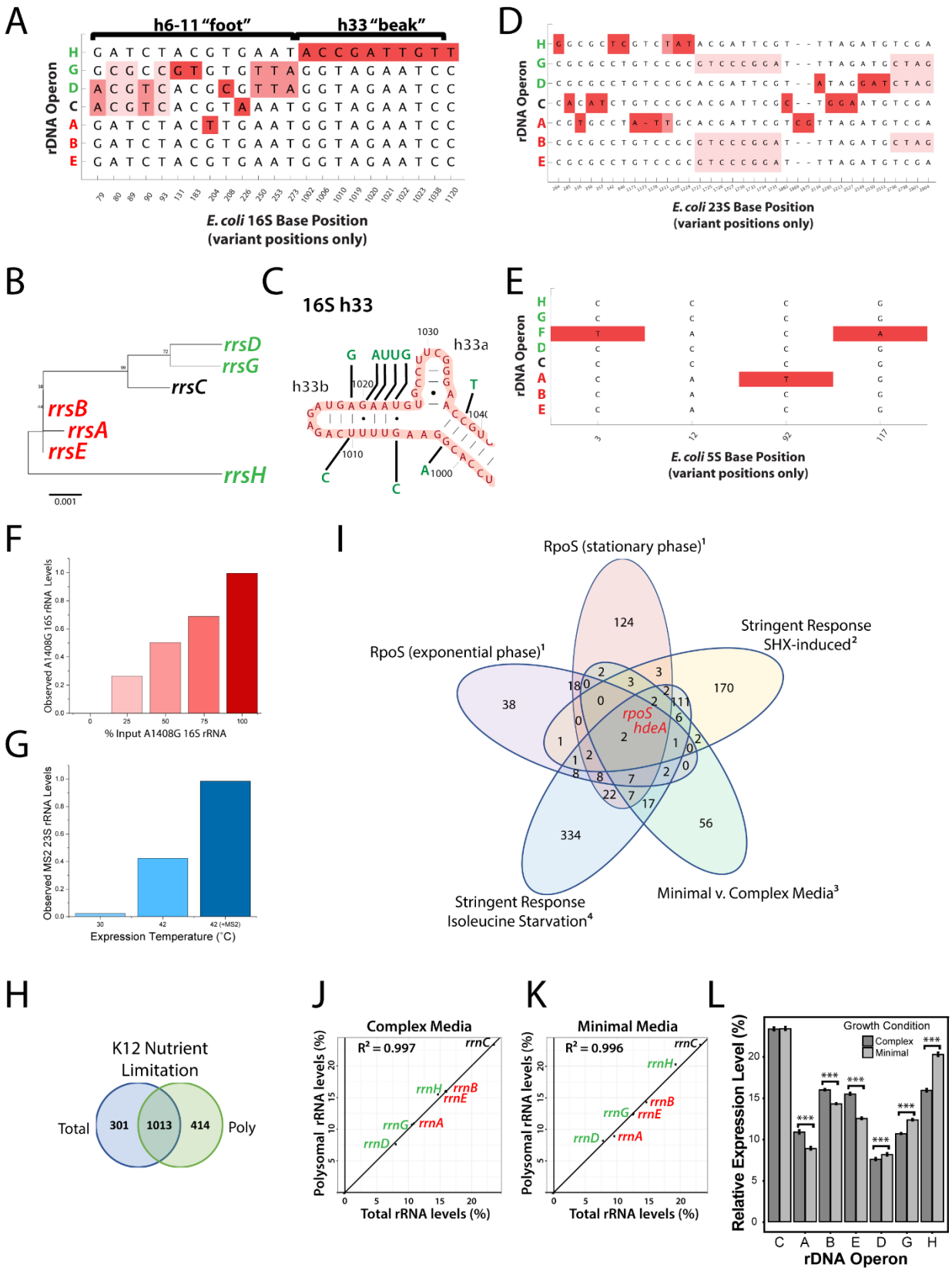
A
h6-11 "foot"
h33 "beak"
rDNA Operon
H
G
D
C
A
B
E
E. coli 16S Base Position
(variant positions only)
D
E. coli 23S Base Position
(variant positions only)
B
rrsD
rrsG
rrsC
rrsB
rrsA
rrsE
rrsH
0.001
C
16S h33
h33a
h33b
E
E. coli 5S Base Position
(variant positions only)
F
Observed A1408G 16S rRNA Levels
% Input A1408G 16S rRNA
G
Observed MS2 23S rRNA Levels
Expression Temperature (°C)
I
RpoS (stationary phase)[1]
RpoS (exponential phase)[1]
Stringent Response
SHX-induced[2]
Stringent Response
Isoleucine Starvation[4]
Minimal v. Complex Media[3]
rpoS
hdeA
124
170
38
334
56
H
K12 Nutrient
Limitation
Total
301
1013
414
Poly
J
Complex Media
R² = 0.997
Polysomal rRNA levels (%)
Total rRNA levels (%)
K
Minimal Media
R² = 0.996
L
Growth Condition
Complex
Minimal
Relative Expression Level (%)
C A B E D G H
rDNA Operon

Figure S1 (Related to Figure 1). **Nutrient limitation alters the expression of rDNA operons in *E. coli*. (A)** Alignment highlighting all variant positions between 16S rRNA genes in *E. coli*. rDNA operons that are upregulated (green), and downregulated (red), on a relative basis in response to nutrient limitation are indicated on the vertical axis. The majority of variants in the 16S rRNA are located in either the h6-11 "foot" domain or the h33 "beak" domain. **(B)** Hierarchical clustering of *E. coli* 16S rRNA genes using the Neighbor-joining method (Saitou and Nei, 1987). Analysis was performed in MEGA6 (Tamura et al., 2013). **(C)** Secondary structure of h33 of the rrsB 16S rRNA according to (Stern et al., 1988). The position and identity of nucleotide variants in rrsH are indicated in green. **(D-E)** Alignment highlighting all variant positions between **(D)** 23S and **(E)** 5S rRNA genes in *E. coli*. rDNA operons that are upregulated (green), and downregulated (red), on a relative basis in response to nutrient limitation are indicated on the vertical axis. **(F)** RNA-Seq analysis of known mixtures of ribosomes with either wild-type or A1408G 16S rRNAs. The X-axis indicate the intended ratio, while the Y-axis indicates the observed ratio of A1408G to wild-type. **(G)** wild-type *E. coli* transformed with a heat-inducible rDNA expression plasmid encoding an MS2-tagged 23S rRNA (Wang et al., 2012). Consistent with previous literature (Youngman and Green, 2005) cells grown at 30 °C exhibit no plasmid induction, while cells grown at 42 °C exhibit strongly induced MS2-tagged rRNA expression (between 30 and 50%). MS2-tagged ribosomes can then be subsequently purified by affinity chromatography to approximately >85% purity, as previously reported (Youngman and Green, 2005). **(H)** Venn diagram showing the overlap between differentially expressed genes in total compared to polysomal RNA fractions of wild-type *E. coli* grown in complex or minimal media (this study). **(I)** Venn diagram showing the overlap between multiple independent studies of acute and chronic nutrient stress. Genes upregulated in all experiments are indicated in red. Differentially expressed gene sets obtained from [1](Dong and Schellhorn, 2009), [2](Durfee et al., 2008), [3](Tao et al., 1999), and [4](Traxler et al., 2008) as indicated. **(J,K)** Correlation between total RNA (X-axis) and polysomal RNA (Y-axis) levels of expressed rDNA in **(J)** complex and **(K)** minimal media. **(L)** The relative expression levels of individual rDNA operons in cells grown in complex or minimal media. Error bars represent the standard error of the mean and "***" indicates $p < 0.01$ across three biological replicates.

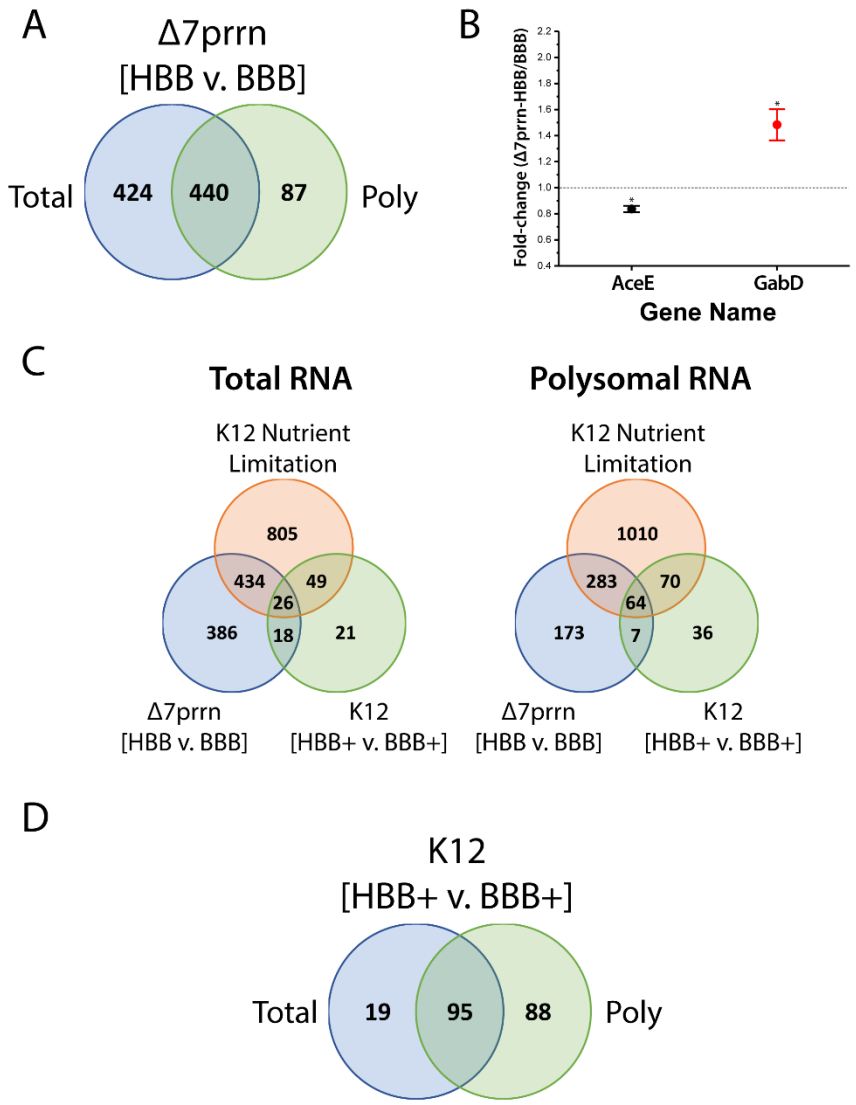

Figure S2 (Related to Figure 2). rrsH-bearing ribosomes alter gene expression at both the transcriptional (total RNA) and translational (polysomal RNA) levels. (A) Venn Diagram showing the overlap between differentially expressed genes in total compared to polysomal RNA fractions of Δ*7prrn* strains expressing either pKK3535-BBB or pKK3535-HBB plasmids and grown in minimal media. **(B)** qRT-PCR validation of RNA-Seq results from the Δ7prrn-HBB v. Δ7prrn-BBB experiment. Two genes, one that was downregulated (*aceE*) and one that was upregulated (*gabD*) by Δ7prrn-HBB, were selected for validation. "*" indicates $p < 0.05$ compared to Δ7prrn-BBB. Error bars indicate SEM. **(C)** Venn diagrams showing the number of overlapping differentially expressed genes between wild-type *E. coli* grown in complex or minimal media (this study), Δ*7prrn* strains expressing either pKK3535-BBB or pKK3535-HBB plasmids grown in minimal media, and wild-type *E. coli* K12 MG1655 expressing either pKK3535-BBB or pKK3535-HBB plasmids grown in minimal media. The left panel describes the total RNA fraction (transcriptional level), while the right panel describes the polysomal RNA fraction (translational level). **(D)** Venn Diagram showing the overlap between differentially expressed genes in total compared to polysomal RNA fractions of K-12 MG1655 strains expressing either pKK3535-BBB or pKK3535-HBB plasmids and grown in minimal media.

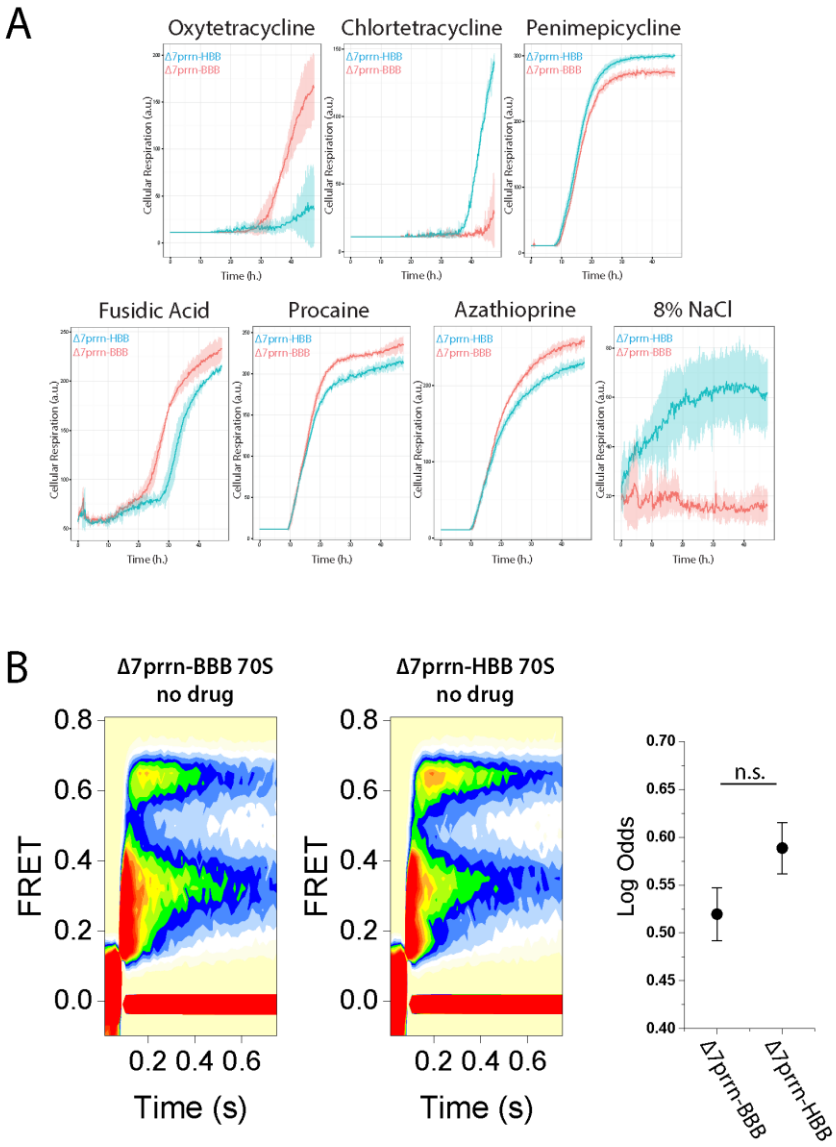

Figure S3 (Related to Figure 3). rrsH-bearing ribosomes alter cellular phenotype. (A) Growth phenotype analysis of *E. coli* strains Δ7prrn-HBB (blue) and Δ7prrn-BBB (red) using the BIOLOG phenotype microarray (Bochner et al., 2001). Error region indicates the standard deviation. All experiments were performed in triplicate. The top three panels are tetracycline derivatives; fusidic acid is a small-molecule inhibitor of elongation factor G (EF-G), the GTPase that catalyzes ribosome translocation (Wasserman et al., 2016); procaine is thought to affect membrane integrity related to osmotic stress (Guo and Gross, 2014); azathioprine is thought to be a purine biosynthesis inhibitor and is implicated in regulating cyclic di-GMP synthesis (Antoniani et al., 2013); 8% NaCl is an established osmotic stress (Solheim et al., 2014). **(B)** Post-synchronized, ensemble smFRET histograms of tRNA selection on (left) rrsB- and (middle) rrsH-bearing ribosomes (as indicated) in the absence of tetracycline. (Right) Log odds of tRNA accommodation (p=0.1). Error bars indicate standard error from the mean.

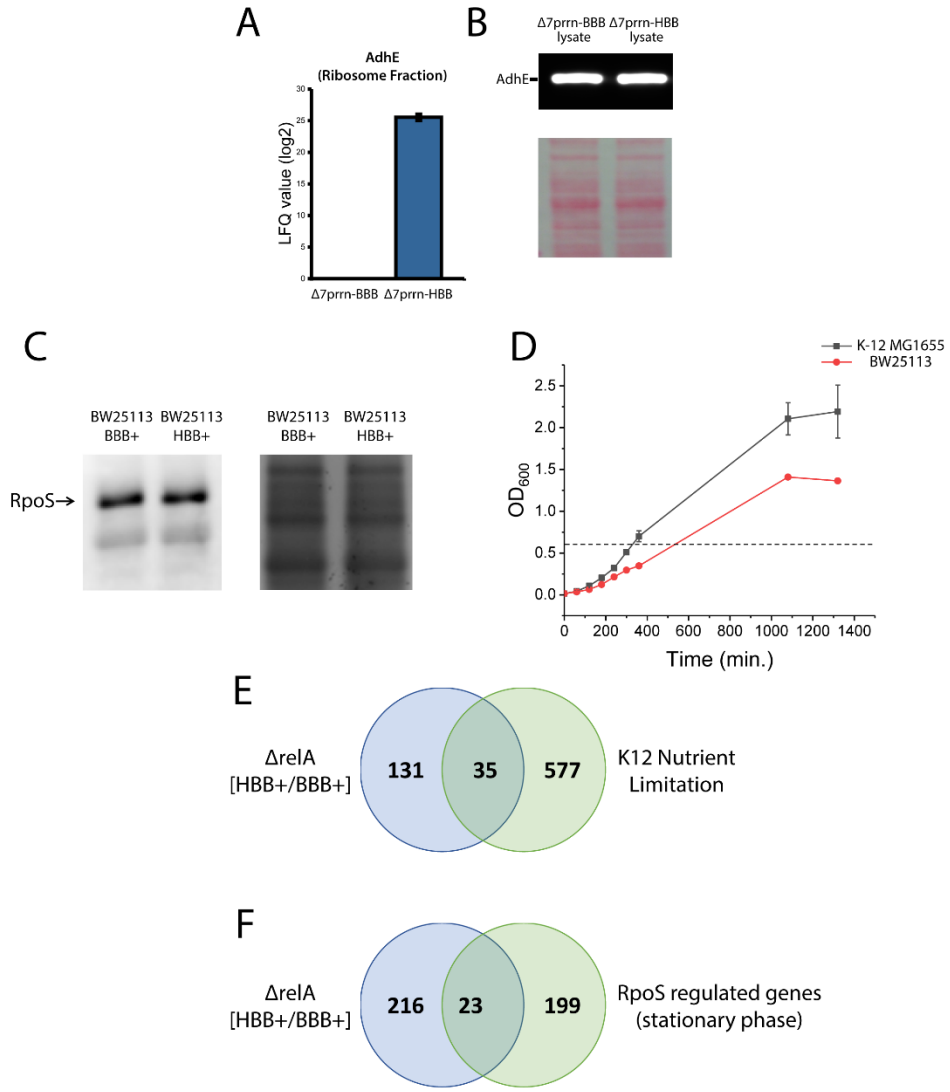

Figure S4 (Related to Figure 4). Mass spectrometry quantification of AdhE protein levels in cells expressing rrsB- and rrsH-bearing ribosomes, characterization of BW25113-BBB+/HBB+ RpoS levels and growth in minimal media, and overlap between rrsH-regulated genes in the *ΔrelA* background and stress response programs. (A) Quantification of AdhE data obtained from quantitative mass spectrometry investigations performed on high salt-washed TC-70S ribosomes (n = 3, **Methods**). Error bars indicate standard deviation. **(B)** Western blot for the 96 kDa AdhE protein from cell lysate. Ponceau stain of the same membrane, located beneath the blot, is included as a loading control. **(C)** Western blot for RpoS in BW25113 strains expressing either pKK3535-BBB or -HBB and harvested at OD_{600} = 0.6 in minimal media. **(D)** Growth curves of *E. coli* strains K-12 MG1655 and BW25113 in minimal media. Error bars represent the standard deviation of three biological replicates and the dotted line demarcates cell density at OD_{600} = 0.6. **(E,F)** Venn diagrams showing the number of overlapping differentially expressed genes between Δ*relA E. coli* expressing either pKK3535-BBB or pKK3535-HBB plasmids grown in minimal media and **(E)** wild-type *E. coli* K-12 MG1655 grown in complex or minimal media (this study) and **(F)** previously identified RpoS-regulated genes (Dong and Schellhorn, 2009).

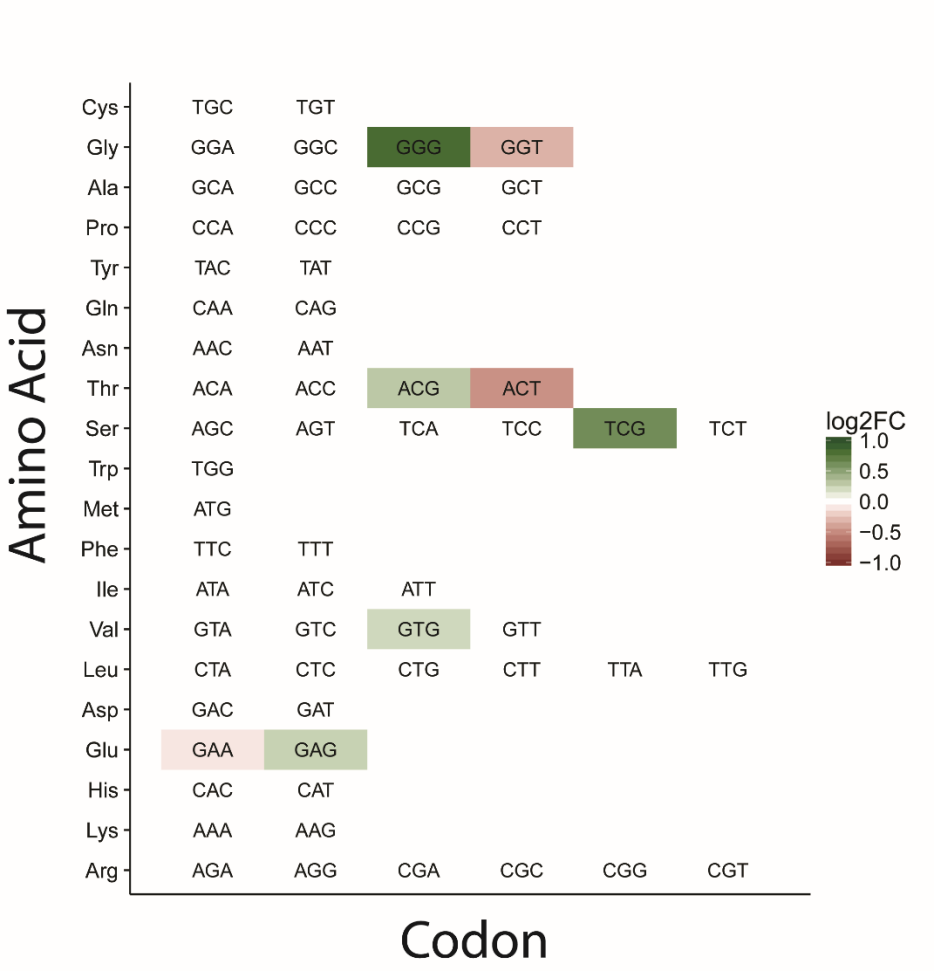

Figure S5 (Related to Figure 5). Codon usage analysis of differentially translated genes between *E. coli* strains Δ7prrn-HBB and Δ7prrn-BBB. Green-colored codons are enriched within upregulated genes in Δ7prrn-HBB compared to Δ7prrn-BBB, while red-colored codons are enriched within the downregulated genes in Δ7prrn-HBB compared to Δ7prrn-BBB **(Methods)**.