Fig. S1, related to Fig. 1

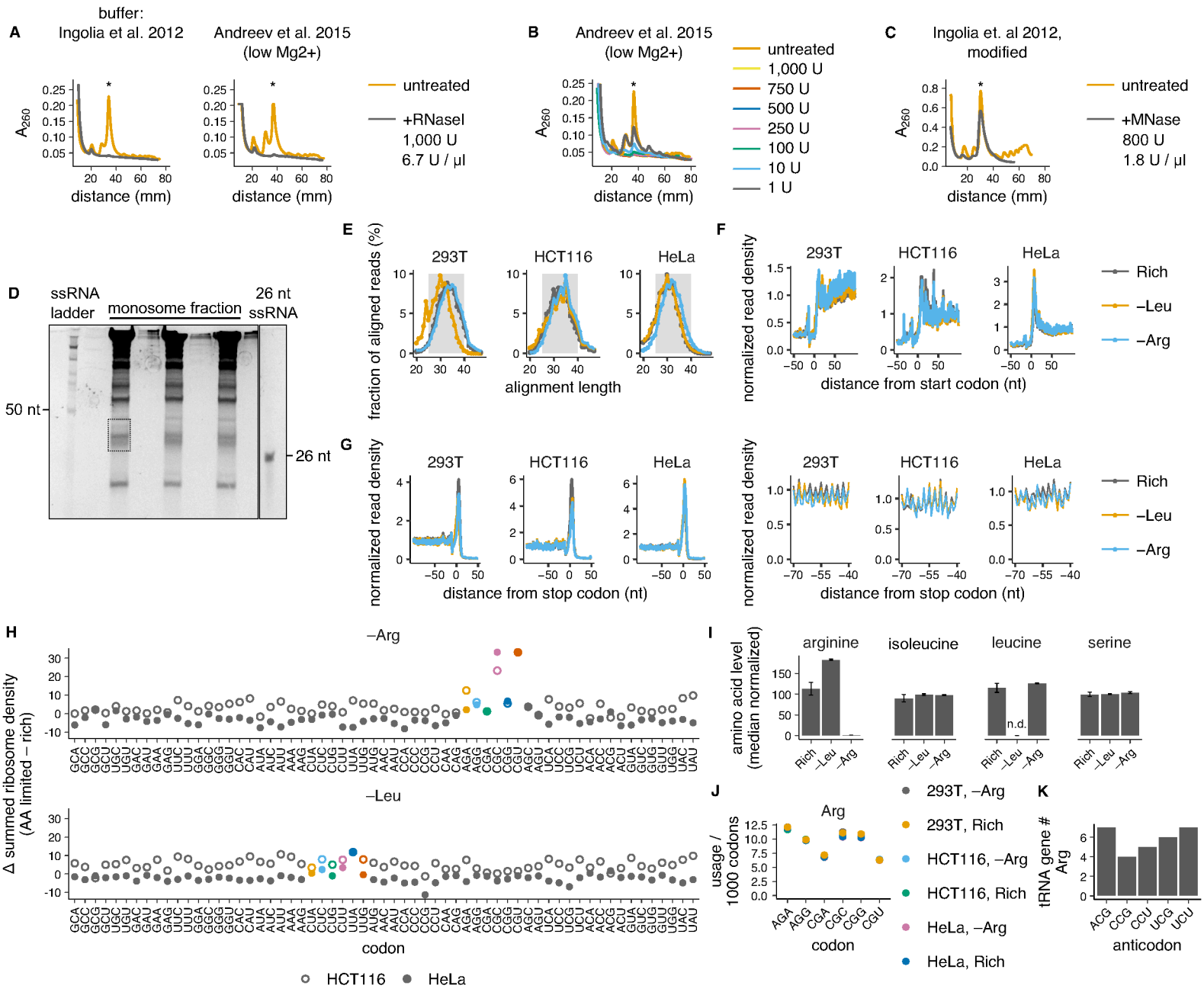


Figure S1

Codon-specific ribosome pausing emerges during limitation for arginine, but not leucine. (**A-C**) HEK293T cell polysome digestion into monosomes with varying amounts of RNaseI (B) in buffers with varying Mg2+ concentrations (A), or with MNase (C), was assessed by sucrose density gradient fractionation. (* = monosome fraction). (**D**) Representative size selection gel for RNA footprint extraction for library preparation. Box indicates the region excised. (**E-G**) Aligned read length distribution (E) and genome-wide read density profiles around annotated start (F) and stop codons (G) for data in Fig. 1A-C & S1H. After 3' end trimming, normalized read density is calculated as described in Fig. 1A & Methods. A region of the read density profile in G is magnified in a second (right) panel. (**H**) Overlaid summed changes in codon-specific ribosome density for HCT116 and HeLa cells following 3 hours of leucine or arginine limitation, calculated as described in Fig. 1A & Methods. Arg and Leu codons are colored according to the legend in Fig. 1. (**I**) Intracellular arginine, isoleucine, leucine, and serine levels in HEK293T cells following limitation for leucine or arginine for 3 hours or growth in rich medium. Error bars represent the standard error of the mean from three technical replicate measurements. Intracellular leucine level was below the detection limit (n.d.) upon its limitation. (**J**) Usage frequencies for Arg codons in the transcriptome in HEK293T, HCT116, and HeLa cells following 3 hours of limitation for arginine or growth in rich medium. (**K**) Genomic copy number of Arg isoacceptor tRNAs (Chan and Lowe, 2016). (**L**) Arg and Leu codons matched with their cognate tRNA(s). Decoding by multiple tRNAs is indicated with a slash, I = inosine.

L

Codon	tRNA	Codon	tRNA
AGA	Arg_{UCU}	CUA	$Leu_{U/IAG}$
AGG	$Arg_{C/UCU}$	CUC	Leu_{IAG}
CGA	$Arg_{U/ICG}$	CUG	$Leu_{C/UAG}$
CGC	Arg_{ICG}	CUU	$Leu_{A/IAG}$
CGU	$Arg_{A/ICG}$	UUA	Leu_{UAA}
CGG	$Arg_{C/UCG}$	UUG	$Leu_{C/UAA}$

Fig. S2, related to Fig. 2

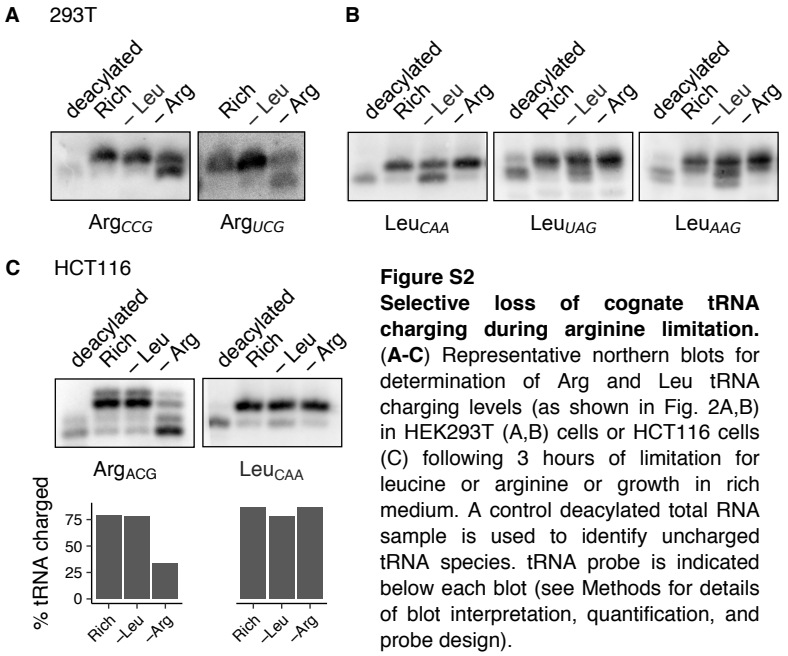


Figure S2
Selective loss of cognate tRNA charging during arginine limitation. (**A-C**) Representative northern blots for determination of Arg and Leu tRNA charging levels (as shown in Fig. 2A,B) in HEK293T (A,B) cells or HCT116 cells (C) following 3 hours of limitation for leucine or arginine or growth in rich medium. A control deacylated total RNA sample is used to identify uncharged tRNA species. tRNA probe is indicated below each blot (see Methods for details of blot interpretation, quantification, and probe design).

Fig. S3, related to Fig. 3

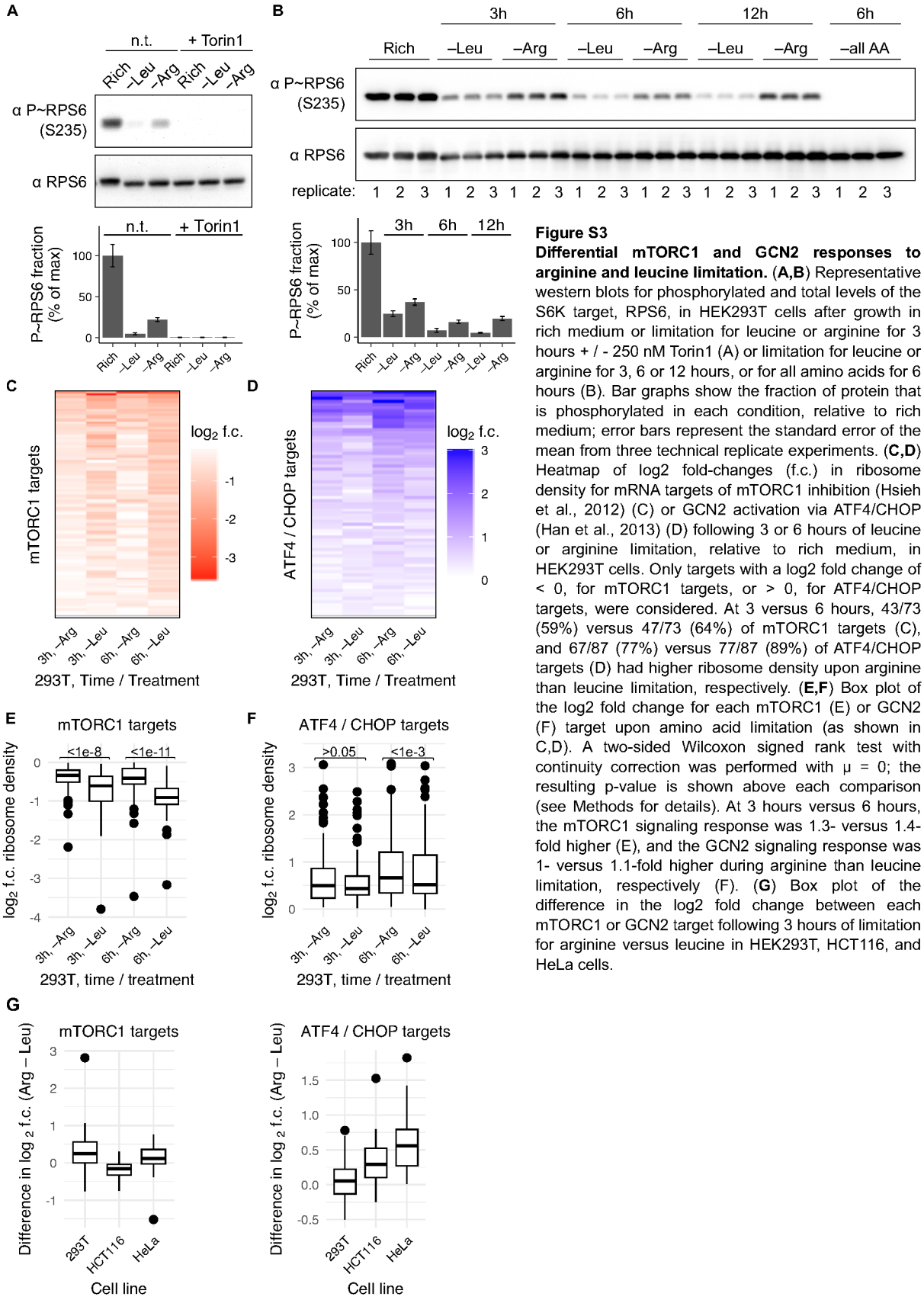
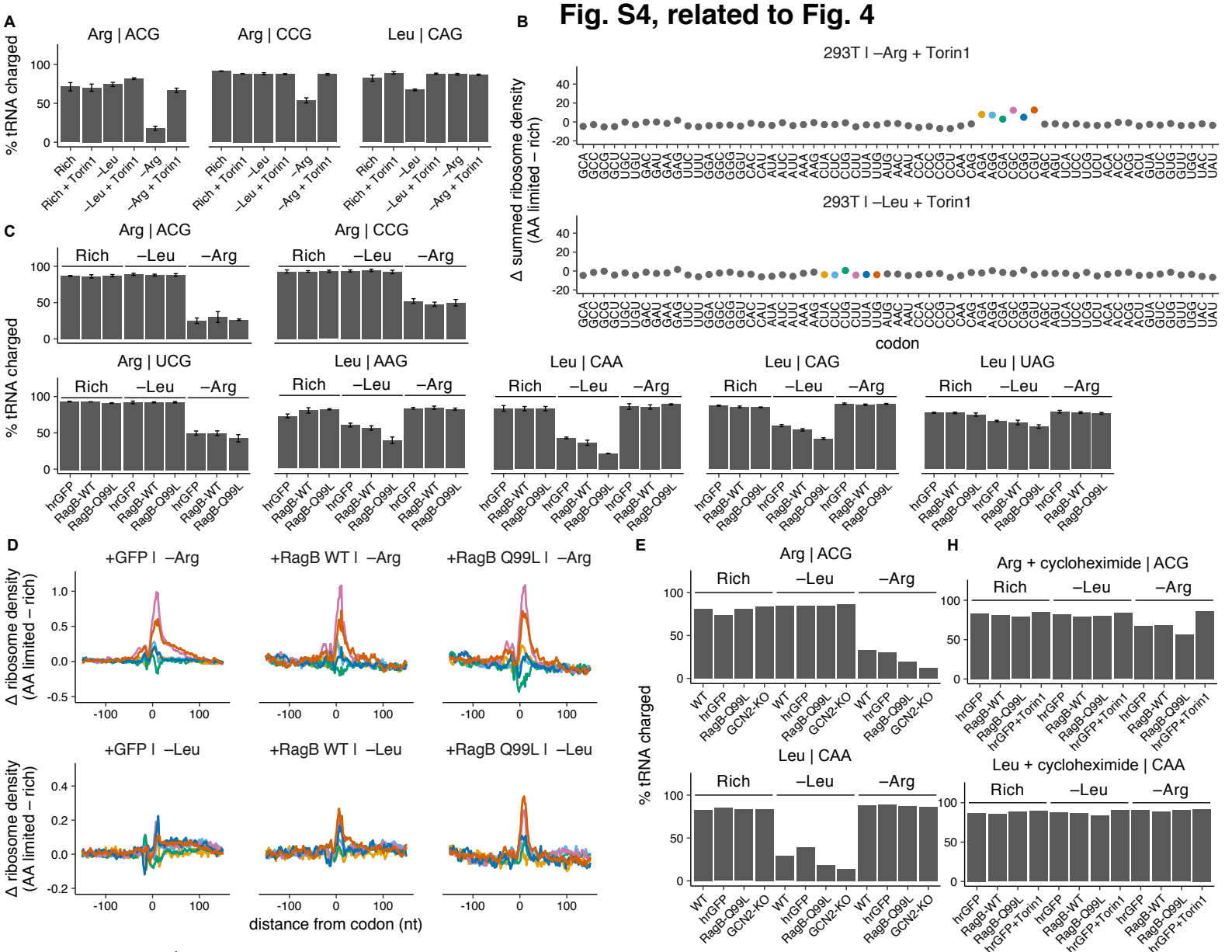
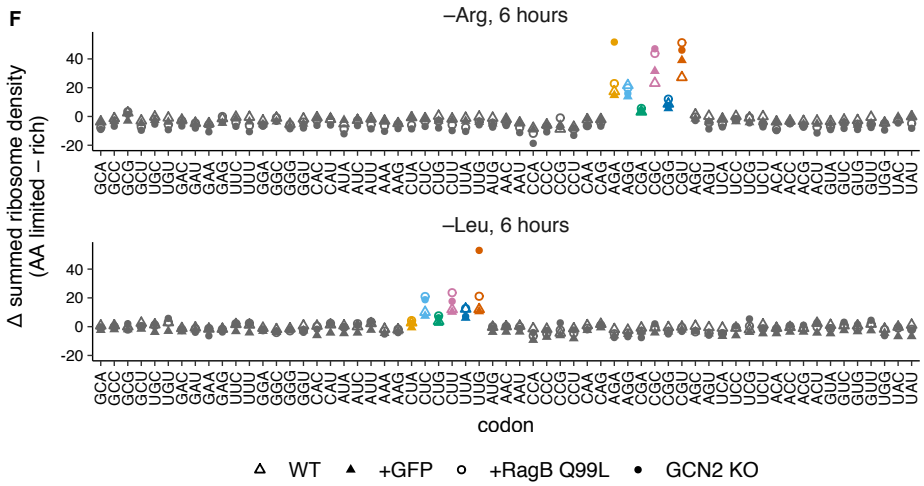


Figure S3

Differential mTORC1 and GCN2 responses to arginine and leucine limitation. (**A,B**) Representative western blots for phosphorylated and total levels of the S6K target, RPS6, in HEK293T cells after growth in rich medium or limitation for leucine or arginine for 3 hours + / - 250 nM Torin1 (A) or limitation for leucine or arginine for 3, 6 or 12 hours, or for all amino acids for 6 hours (B). Bar graphs show the fraction of protein that is phosphorylated in each condition, relative to rich medium; error bars represent the standard error of the mean from three technical replicate experiments. (**C,D**) Heatmap of log2 fold-changes (f.c.) in ribosome density for mRNA targets of mTORC1 inhibition (Hsieh et al., 2012) (C) or GCN2 activation via ATF4/CHOP (Han et al., 2013) (D) following 3 or 6 hours of leucine or arginine limitation, relative to rich medium, in HEK293T cells. Only targets with a log2 fold change of < 0, for mTORC1 targets, or > 0, for ATF4/CHOP targets, were considered. At 3 versus 6 hours, 43/73 (59%) versus 47/73 (64%) of mTORC1 targets (C), and 67/87 (77%) versus 77/87 (89%) of ATF4/CHOP targets (D) had higher ribosome density upon arginine than leucine limitation, respectively. (**E,F**) Box plot of the log2 fold change for each mTORC1 (E) or GCN2 (F) target upon amino acid limitation (as shown in C,D). A two-sided Wilcoxon signed rank test with continuity correction was performed with μ = 0; the resulting p-value is shown above each comparison (see Methods for details). At 3 hours versus 6 hours, the mTORC1 signaling response was 1.3- versus 1.4-fold higher (E), and the GCN2 signaling response was 1- versus 1.1-fold higher during arginine than leucine limitation, respectively (F). (**G**) Box plot of the difference in the log2 fold change between each mTORC1 or GCN2 target following 3 hours of limitation for arginine versus leucine in HEK293T, HCT116, and HeLa cells.

Fig. S4, related to Fig. 4





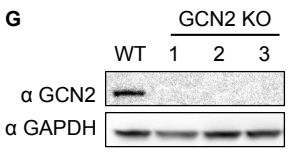


Figure S4

Signaling through the mTORC1 and GCN2 pathways regulates the magnitude of ribosome pausing during amino acid limitation. (**A**) tRNA charging levels for 2 Arg tRNAs and 1 Leu tRNA in HEK293T cells following 3 hours of leucine or arginine limitation or growth in rich medium, + / - 250 nM Torin1 (calculated as described in Methods). Error bars represent the standard error of the mean from three technical replicate experiments. (**B**) Summed changes in codon-specific ribosome density in the hrGFP cell line following 3 hours of limitation for leucine or arginine with 250 nM Torin1, relative to rich medium. Arg and Leu codons are colored according to the legend in D. (**C**) tRNA charging levels for 3 Arg and 4 Leu tRNAs in the hrGFP, RagB-WT, or RagB-Q99L cell lines following limitation for leucine or arginine for 3 hours or growth in rich medium (calculated as described in Methods). Error bars represent the standard error of the mean from three technical replicate experiments. (**D**) Changes in codon-specific ribosome density for the hrGFP, RagB-WT, and RagB-Q99L cell lines following limitation for leucine or arginine for 3 hours, relative to rich medium. (**E**) tRNA charging levels for 1 Arg and 1 Leu tRNA in the WT, hrGFP, RagB-Q99L, or GCN2 KO cell lines following limitation for leucine or arginine for 3 hours or growth in rich medium. (**F**) Overlaid summed changes in codon-specific ribosome density for the WT, hrGFP, RagB-Q99L, and GCN2 KO cell lines following 6 hours of leucine or arginine limitation, relative to rich medium. Arg and Leu codons are colored according to the legend in D. (**G**) Western blots for GCN2 and GAPDH proteins in HEK293T and 3 clonal replicate GCN2 KO cell lines. (**H**) tRNA charging levels for 1 Arg and 1 Leu tRNA in the hrGFP cell line + / - 250 nM Torin1, the RagB-WT, and the RagB-Q99L cell lines after exposure for <1 minute to 100 μg/mL cycloheximide in ice-cold PBS following limitation for leucine or arginine for 3 hours or growth in rich medium.

Fig. S5, related to Fig. 5

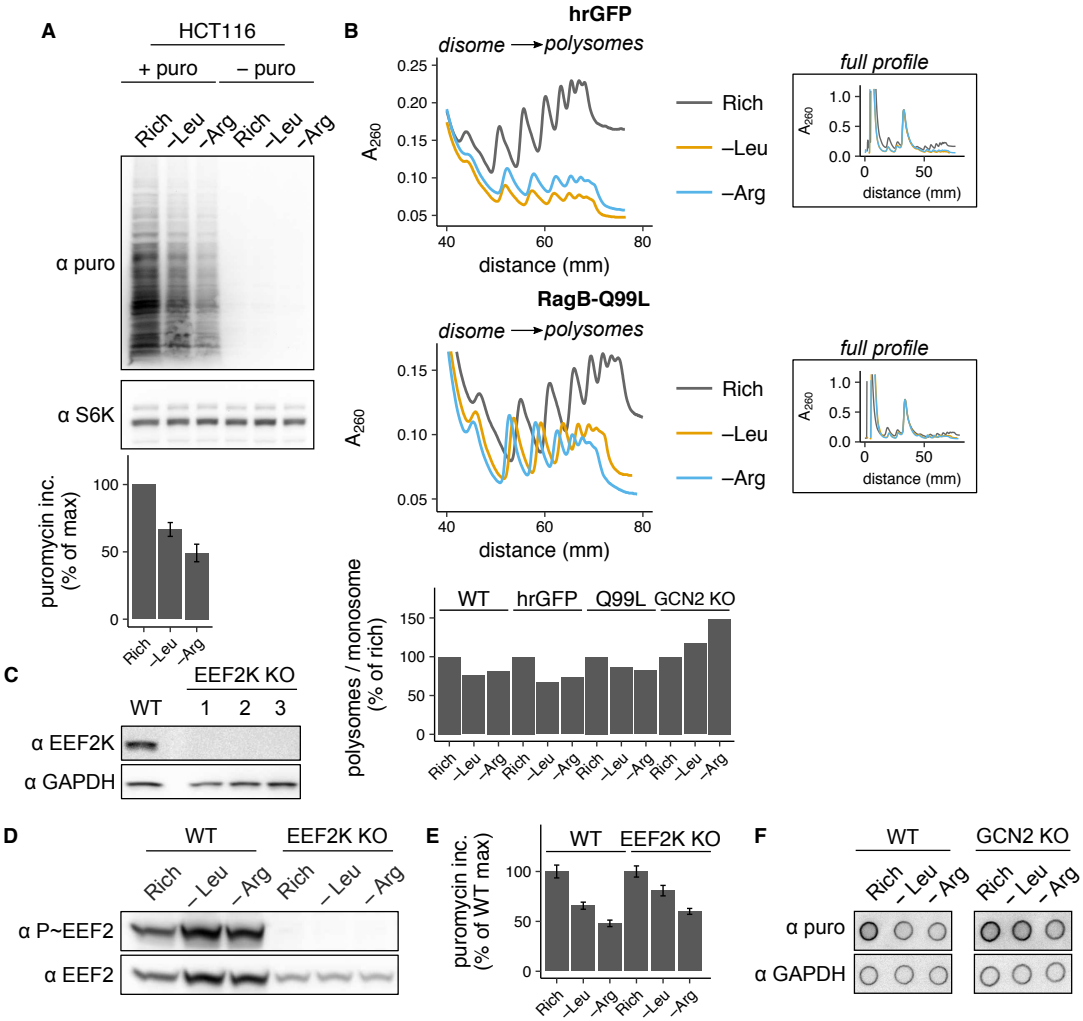


Figure S5

Ribosome pausing reduces global protein synthesis rate during amino acid limitation. (**A**) Representative western blots for puromycin and S6K in HCT116 cells after (+ puro) or without (– puro) a pulse of 10 μg/mL puromycin following 3 hours of leucine or arginine limitation, treatment with 250 nM Torin1, or growth in rich medium. Bar graph shows puromycin incorporation relative to rich medium (calculated as described in Methods); error bars represent the standard error of the mean from three technical replicate experiments. (**B**) Polysome profiles from the hrGFP and RagB-Q99L cell lines following 6 hours of leucine or arginine limitation or growth in rich medium. The main plot shows overlaid polysome profiles from the disome (2 ribosome) peak to the end of the polysomes, the inset plots show the entire profile. Bar graph shows the area in the polysome to monosome fraction, relative to that in rich medium (calculated as described in Methods). (**C**) Representative western blots for EEF2K and GAPDH in HEK293T and 3 clonal replicate EEF2K KO cell lines. (**D**) Western blots for phosphorylated and total EEF2 in WT and EEF2K KO cell lines following 3 hours of growth in rich medium, leucine limitation, or arginine limitation. (**E**) Puromycin incorporation in the WT (same data as Fig. 5D) or EEF2K KO cell lines following 3 hours of leucine or arginine limitation, relative to rich medium. Error bars represent the standard error of the mean for three technical replicate measurements. (**F**) Representative dot blots for puromycin and GAPDH in WT cells and the GCN2 KO cell line following 3 hours of leucine or arginine limitation or growth in rich medium.

Fig. S6, related to Fig. 6

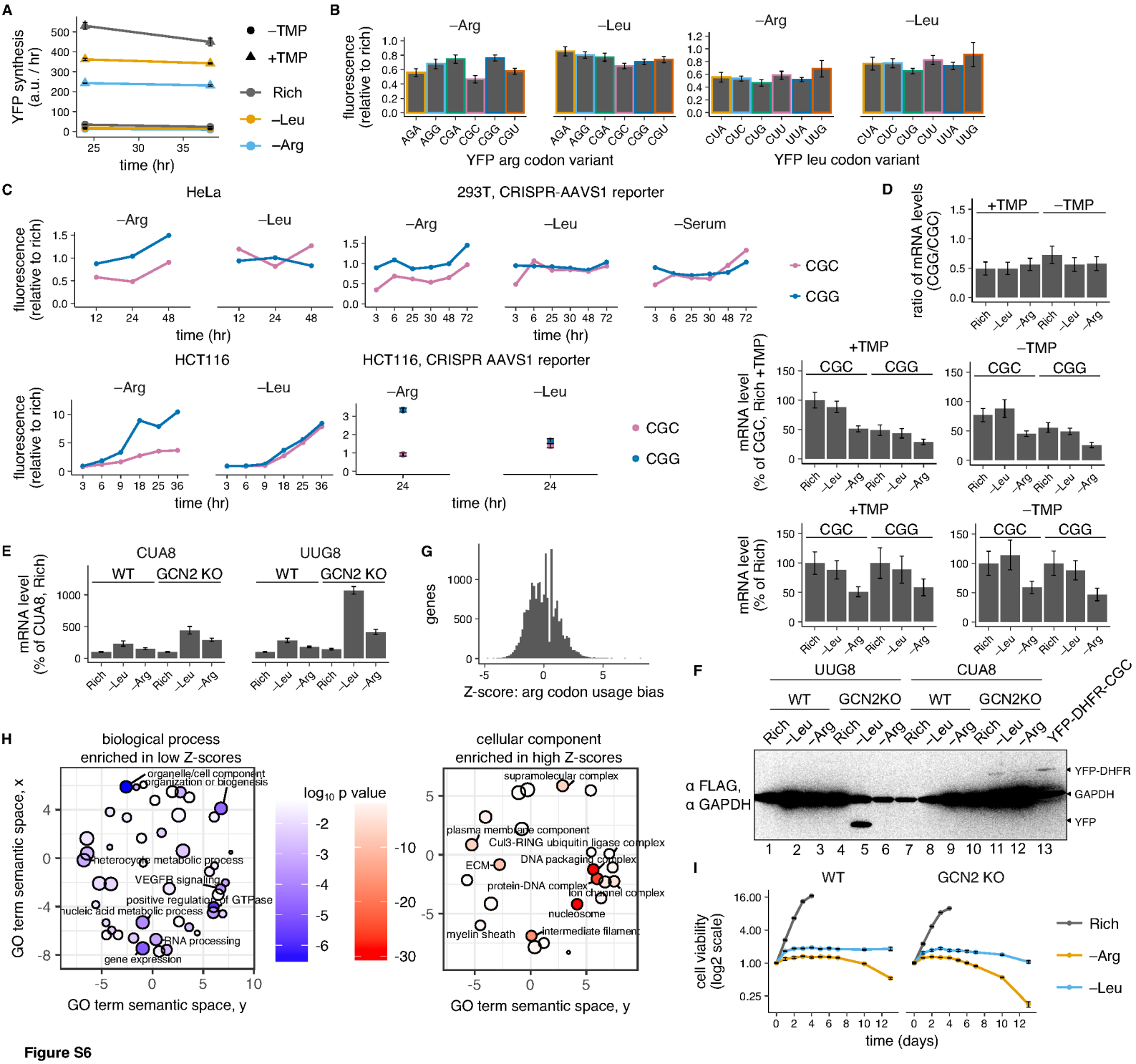


Figure S6

Ribosome pausing reduces protein expression from reporter mRNAs and induces premature termination of translation. (**A-C**) YFP codon variant reporter fluorescence measurements across multiple time points, cell lines, and reporter constructs (see Methods for details). Error bars represent the standard error of the mean from three technical replicate experiments. (**A**) Mean increase in YFP fluorescence per hour, + / - 10 μM TMP in HEK293T cells stably expressing the YFP-CGC reporter, following 24 or 38 hours of leucine or arginine limitation or growth in rich medium. (**B**) Mean YFP fluorescence in the HEK293T cells stably expressing the leucine or arginine YFP codon variant reporters following limitation for leucine or arginine with 10 μM trimethoprim for 24 hours, relative to rich medium +TMP. Arg and Leu codons are colored according to the legend in Fig. 6. (**C**) Mean YFP fluorescence in the HCT116, HeLa, and HEK293T cell lines stably expressing the YFP-CGC and -CGG reporters, following limitation for arginine, leucine or serum +TMP for 12, 24, or 48 hours, relative to rich medium +TMP. (**D**) YFP-CGC and -CGG CRISPR-AAVS1 reporter mRNA levels in HEK293T cells following 24 hours of limitation for leucine or arginine + / - TMP (calculated as described in Methods). From the top to bottom panel, the data is plotted 1) as the ratio of the YFP-CGG variant to the YFP-CGC variant for each condition, 2) normalized to the rich condition for the YFP-CGC reporter, 3) normalized to the rich condition for each YFP variant separately. Error bars represent the standard error of the mean for three technical replicate experiments. (**E**) CUA8 and UUG8 premature termination YFP reporter mRNA levels in the WT and GCN2 KO cell lines following 48 hours of limitation for leucine or arginine –TMP, relative to rich medium –TMP (see Methods section for details of calculation). Error bars represent the standard error of the mean for three technical replicate experiments. (**F**) Overexpressed blot image from Fig. 6I, for FLAG and GAPDH in the WT or GCN2 KO cell lines stably expressing the UUG8 or CUA8 reporters after growth in rich medium or 48 hours of leucine or arginine limitation. (**G**) Distribution of Z-scores reflecting pause-site arginine codon usage bias in coding sequences (see Methods section for details of calculation). (**H**) Biological process (BP) or cellular component (CC) gene ontology (GO) categories enriched in genes with bias against (left plot) or in favor of (right plot) usage of pause-site codons to encode arginine; visualized using REVIGO (Supek et al., 2011). Each bubble represents a significantly enriched GO term; color represents $\log_{10}$ of the false-discovery rate adjusted p-value, and size scales with the number of genes for a term. (**I**) Cell viability in the HEK293T or GCN2 KO cell lines following 1 to 13 days of leucine or arginine limitation, or growth in rich medium, relative to day 0. Error bars represent the standard error of the mean from five technical replicate measurements. Both cell lines reached confluency in rich medium after 4 days.