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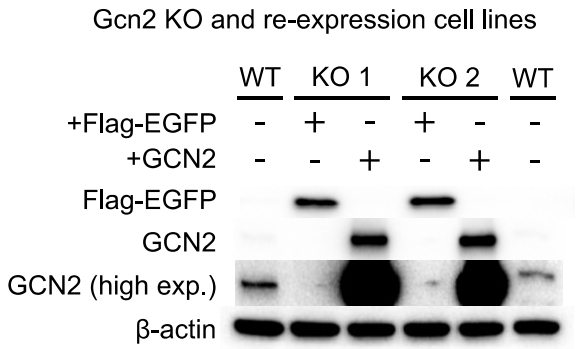
Supplemental information

GCN2 adapts protein synthesis to scavenging-dependent growth

Michel Nofal, Tim Wang, Lifeng Yang, Connor S.R. Jankowski, Sophia Hsin-Jung Li, Seunghun Han, Lance Parsons, Alexander N. Frese, Zemer Gitai, Tracy G. Anthony, Martin Wühr, David M. Sabatini, and Joshua D. Rabinowitz

A



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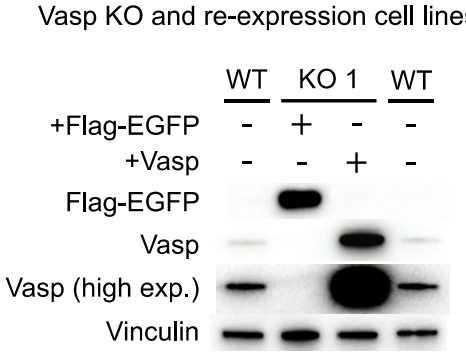
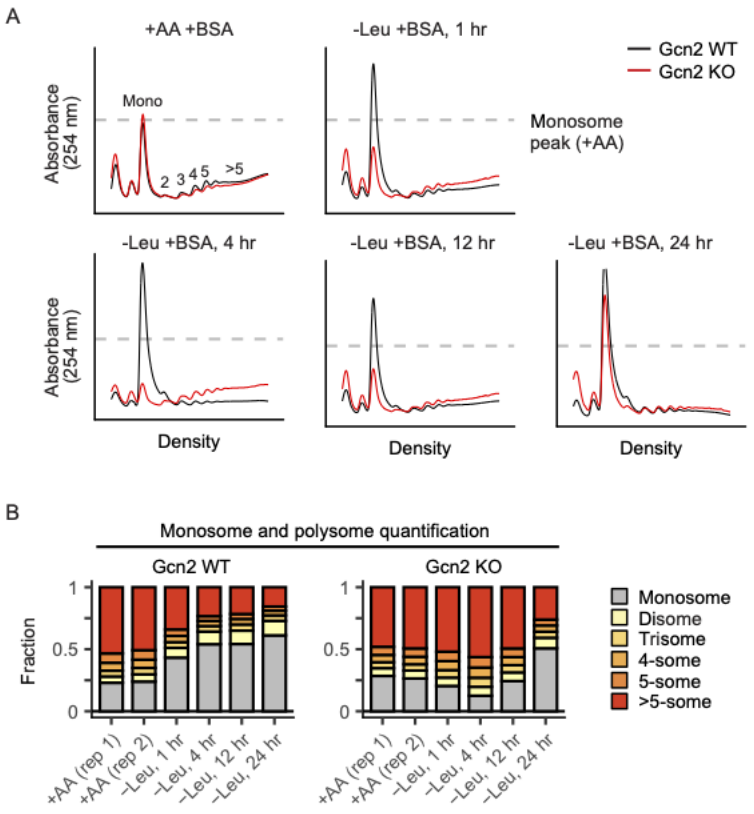
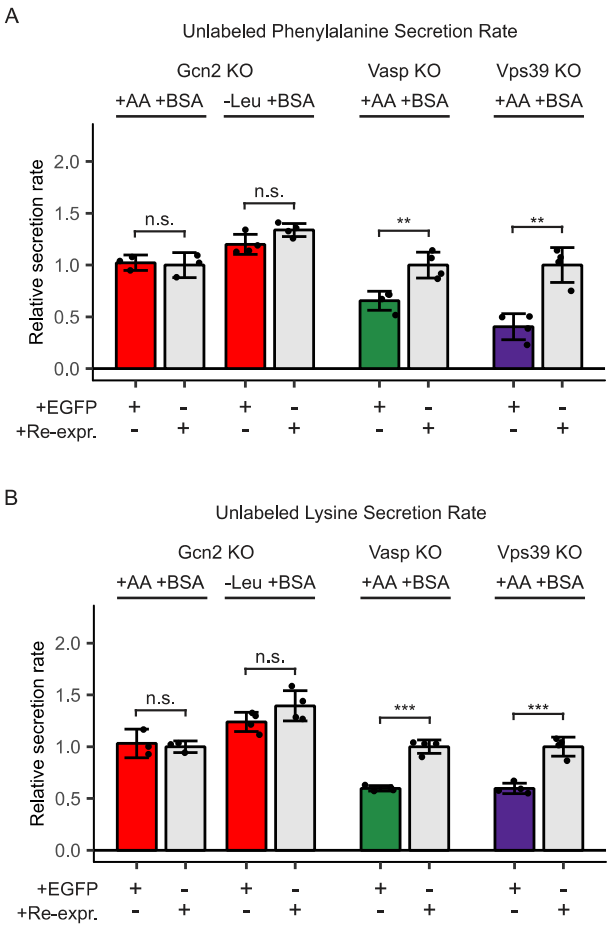


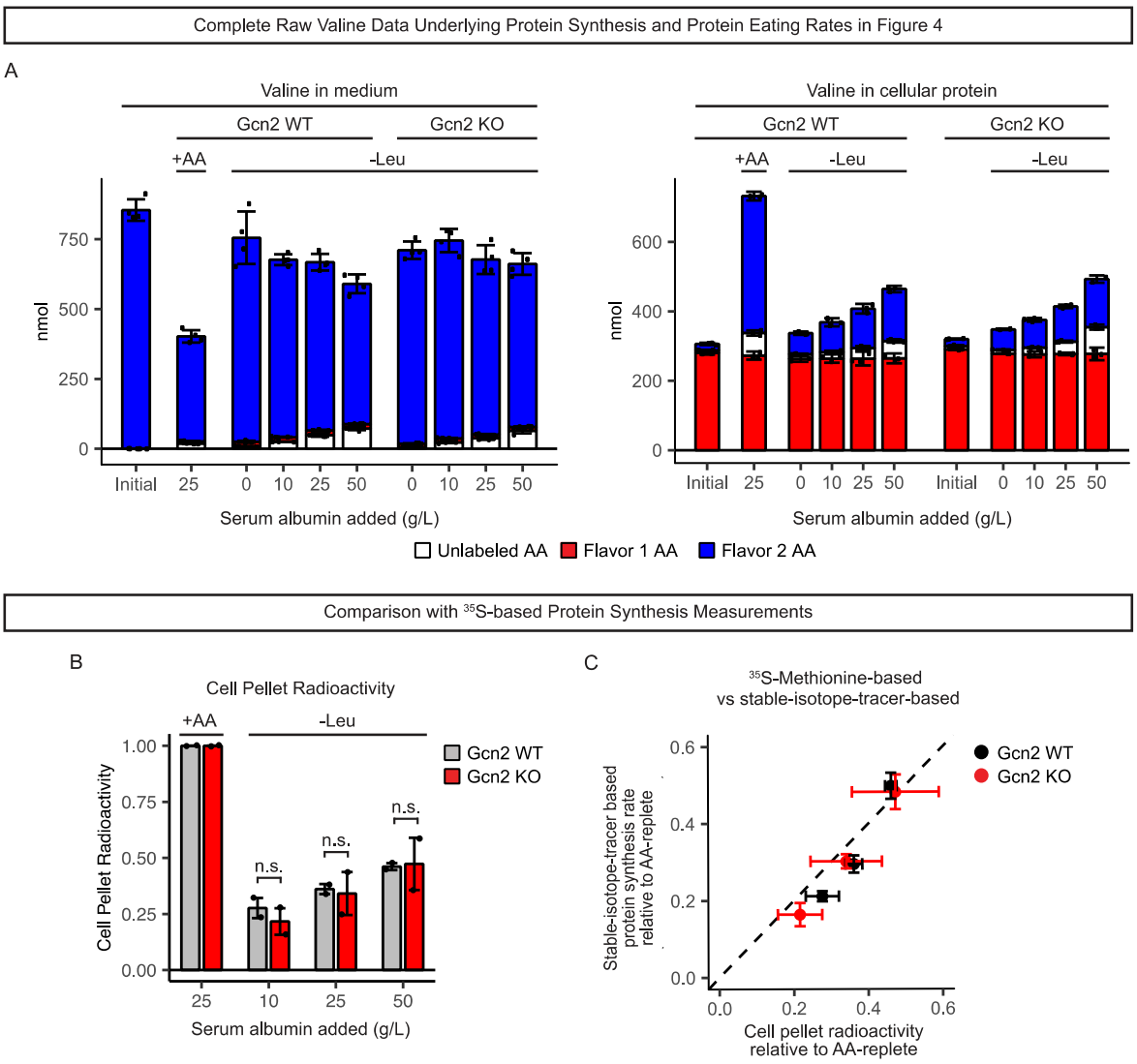
Supplementary Figure 1 – Western blots showing knockout and re-expression of *Gcn2* and *Vasp*. (A-B) Cells were cultured in standard DMEM. Protein was extracted and analyzed by Western Blot. (*Vps39* KO was validated by Sanger sequencing, as there is no reliable VPS39 antibody.)



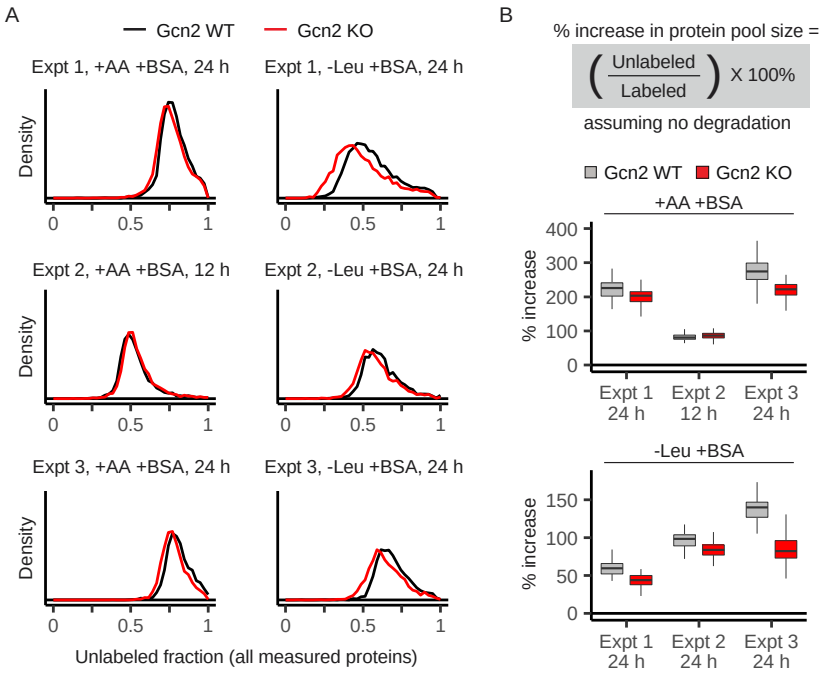
Supplementary Figure 2 - GCN2 knockout cells initially overload mRNAs with ribosomes, but the number of translating ribosomes normalizes after 24 hours. (A) Polysome profiles of *Gcn2* WT and *Gcn2* KO cells switched to either amino acid-replete medium or leucine-free medium, each supplemented with 50 g/L bovine serum albumin (BSA), for the indicated times. Cell lysates were loaded onto 10-50% sucrose gradients and spun at 35,000 g for 2.5 h at 4°C before measurement. Profiles were normalized such that the area under each curve was equal. (B) For each ribosome configuration (monosome, disome, etc), the area under the curve was calculated, and the fraction of each species was plotted.



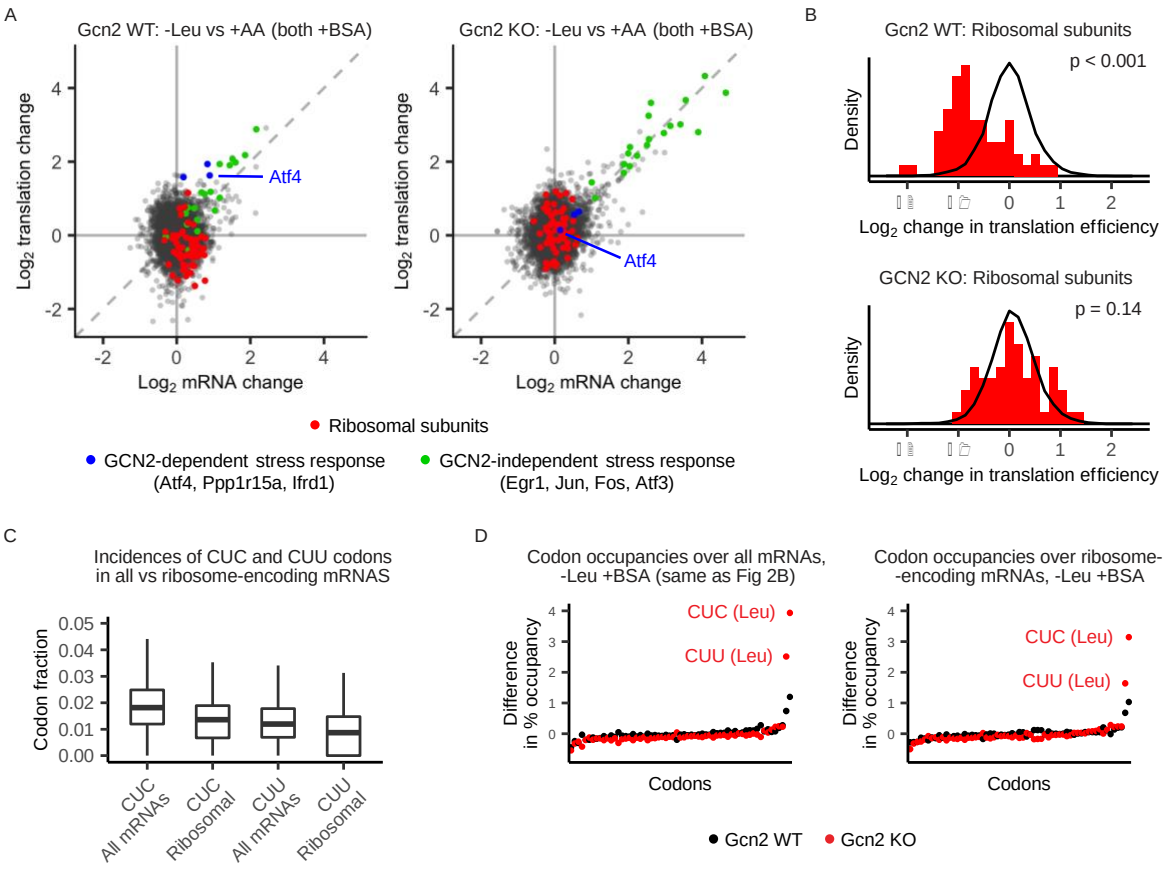
Supplementary Figure 3 – GCN2 is not required for protein scavenging: phenylalanine and lysine secretion data. Unlabeled phenylalanine (A) and lysine (B) secretion over 24 h in *Gcn2*, *Vasp*, and *Vps39* knockout cells expressing either EGFP or the corresponding sgRNA-resistant human cDNA. For each cell line, rates were normalized to the amino acid-replete condition with re-expression. All media were supplemented with 50 g/L bovine serum albumin (BSA). Error bars represent 95% confidence intervals (n=3). *p < 0.05, **p < 0.01, ***p < 0.001.



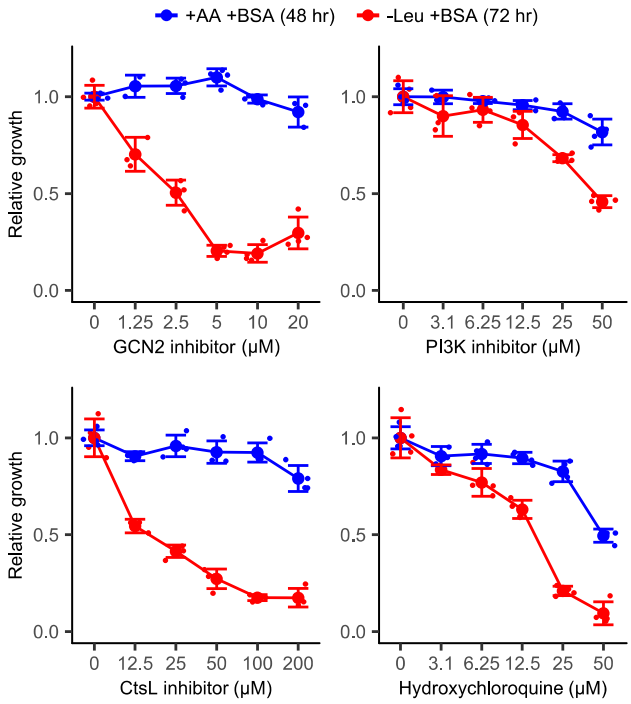
Supplementary Figure 4 – Complete unprocessed valine data and comparison of stable isotope tracer-based measurements with ^{35}S-methionine-based protein synthesis measurements. (A) Complete unprocessed valine data underlying the protein synthesis and protein scavenging rates in 4C-E. (B) *Gcn2* WT and *Gcn2* KO cells were cultured in leucine-free medium supplemented with the indicated amounts of serum albumin in the presence of 5 µCi ^{35}S methionine for 24 h. Monolayers were then washed three times with PBS, trypsinized, and resuspended in standard DMEM. Cell pellets were washed once with PBS, then resuspended in 200 µL PBS. 100 µL was transferred to scintillation fluid. Scintillation counts are reported relative to counts in AA-replete medium. Control samples to which 5 µCi ^{35}S methionine was added for only 10 min prior to cell pellet harvesting were used to estimate background due to unincorporated ^{35}S methionine. Background radioactivity was subtracted all other samples before normalization. (C) Comparison of stable isotope-based measurements with radioactivity-based measurements of protein synthesis. All measurements are reported relative to protein synthesis in amino acid-replete medium.



Supplementary Figure 5 – Protein labeling shows that GCN2 does not slow protein synthesis in KRPC-A cells cultured in amino acid-deficient medium. (A) Three separate proteomics experiments showing unlabeled fractions of all measured proteins in *Gcn2* WT and *Gcn2* KO cells grown in amino acid-replete or leucine-free medium supplemented with 50 g/L bovine serum albumin (BSA) for the indicated times. (B) Unlabeled fractions were converted into percent increases in protein pool size using the highlighted equation, and percent increases for 50 stable proteins (proteins with minimal degradation) are shown in boxplots (STAR Methods).



Supplementary Figure 6 – GCN2 suppresses the translation of ribosomal proteins. (A) Gcn2 WT and KO cells were switched to amino acid-replete medium or leucine-free medium, each supplemented with 50 g/L albumin (BSA). After one hour, cells were lysed in the presence of 100 ug/mL cycloheximide, and traditional RNA sequencing libraries as well as ribosome profiling libraries were prepared and analyzed by deep sequencing. Log-ratios of transcript levels in leucine-free medium versus amino acid-replete medium are plotted on the x axes; log-ratios of ribosome footprints are plotted on the y axes. (B) Differences in translation efficiencies (difference between translation change and mRNA change) in leucine-free medium versus amino acid-replete medium of all measured ribosomal subunits (red bars) and all measured transcripts (black lines). p-values were calculated using two-tailed t tests. (C) CUC and CUU codons, the two leucine codons at which stalling occurs in KRPC-A cells (Figure 2B), are not present at higher frequencies in ribosomal protein-encoding transcripts than in the average transcript. The lower and upper hinges correspond to the 25th and 75th percentiles, and the whiskers extend from the hinge to the most extreme values or, at most, 1.5X the interquartile range. (D) Comparison of codon occupancies in all mRNAs and ribosome-encoding mRNAs. The codon occupancy profiles look very similar; thus, the results in (A) and (B) cannot be explained by more stalling on this subset of transcripts.



Supplementary Figure 7 – Small molecule targeting of GCN2 and cathepsin L in *K-Ras*^{G12D} MEFs. *K-Ras*^{G12D} MEFs were cultured in amino acid-replete and leucine-free media in varying concentrations of GCN2iB (GCN2 inhibitor); GDC0941 (PI3-kinase class 1A inhibitor); cathepsin L inhibitor; and hydroxychloroquine. All media were supplemented with 50 g/L bovine serum albumin (BSA). Error bars represent 95% confidence intervals (n=3).