**Table S1. Grubbs test for outliers in RPF RPKM data at HC and LC.**

**Table S2. RNA RPKM, RPF RPKM, and translational efficiencies at HC and LC.**

**Table S3. Intragenic PS pattern correlation between samples.**

**Figure S1. Puromycin incorporation reveals translation under $C_i$ starvation.** The magnitude of each bar (left panel) shows the relative total cellular protein synthesis rate as inferred from content of puromycin in protein lysates. Measurements were performed in biological duplicates (A and B) at -5 min (HC), 2 h (HC) and 24 h (LC). "2 h B" is likely underestimated since the sample was contaminated with sedimented cells. Cells not treated with puromycin (-pur) and cells treated with puromycin and the translation inhibitor chloramphenicol (+pur, +Cm) were used as negative controls. A similar reduction in protein synthesis rate at 100 μg/mL chloramphenicol was reported by Xu Y et al. (2000, EMBO J 19:3349–57). The content of puromycin in protein lysates were analyzed by western blot probed with an anti-puromycin antibody (right panel). Dilutions of "-5 min A" were used to correlate background-subtracted lane intensities to the relative content of puromycin.

**Figure S2. Comparison and Pearson correlation of RPF RPKM under the different conditions.** Each point is one ORF passing filtration cutoffs. The overall distribution of RPF RPKM values in each condition is shown along the diagonal. Axes display log10-transformed values.

**Figure S3. Transcription level (mRNA) and protein synthesis rate (RPF; ribosome protected footprints) of the *slr0373-slr0374-slr0376* operon in HC* (-5 min sample) and LC (24 h sample) conditions.** Black vertical lines indicate ORF boundaries. Red vertical bars indicate RPM greater than 250. *HC sample at -5 min.

**Figure S4. Pause score (PS) distribution for every codon in each condition.** The box plots show the median PS surrounded by hinges indicating the upper and lower quartiles, and whiskers indicating the minimum and maximum PS. PS is displayed on an inverse hyperbolic sine transformed axis. Note that ATG includes start codons.

**Figure S5. Detailed view of the 5' UTR pause score (PS) profile in each condition.** Box plots (*ggplot2* function *geom_boxplot* at default settings) show the distribution of above-zero PS for each position in the 50 nt upstream and downstream of the start codon, with the median surrounded by

hinges indicating the first and third quartiles, and whiskers indicating the furthest values not further away than 1.5 times the inter-quartile range. Values outside that range were plotted individually as outliers. Bars indicate the number of ORFs having a PS of zero (light gray) or above zero (dark gray) at each position.

**Figure S6. Average ORF pause score (PS) at 5' and 3' ends with different filtering of ORFs.** Rows show increasing minimum distance to the nearest upstream (for the 5' end) or downstream (for the 3' end) ORF. ORFs with a neighbor closer than the indicated number of nucleotides were excluded. "All positions" means that no ORF vicinity or overlap filtering was performed. The number (n) of ORFs contributing to the average PS profile is shown in the upper right hand corner of each panel. Note that slr1474 was removed from the dataset altogether in order to avoid noise associated with the upstream non-coding sequence SGL_RS18415.

**Figure S7. Pause score (PS) plots for (A) *ssl1911* (*gifA*), (B) *slr1834* (*psaA*) and (C) *ssr0390* (*psaK1*).** Only shows the strand on which the ORF is present. Black vertical lines indicate ORF boundaries. Note that only the 5' end of the *psaA* ORF is shown.

**Figure S8. Correlation of 5' UTR, ORF, and 3' UTR RPF RPKM, for each condition, and correlation of 5' UTR, ORF, and 3' UTR fold change (FC) in RPF RPKM from HC* (-5 min) to LC (24 h).** We define the 5' UTR as the region encompassing positions -50 to -1 relative to the start codon, excluding positions within other ORFs. ORFs were divided into the categories Above average intergenic read density (AIRD) 5' UTR, Missing 5' UTR (the ORF has no direct upstream region that does not overlap with another ORF), and Other 5' UTR, or considered regardless of 5' UTR classification ("All 5' UTRs"). See Methods for more details. FC correlation was calculated only for genes with matching 5' UTR classification in HC* and LC. *HC sample at -5 min.

**Figure S9. ORF pause score (PS) patterns are highly correlated between two conditions if the RPF RPKM is high.** The histograms show the distribution of intragenic PS Pearson's r values for the ORFs. *The average RPF RPKM in the two samples being correlated is displayed as a colour-coded horizontal line at the exact Pearson's r value for each ORF.

**Figure S10. Sucrose density gradient profiles of MNase digested polysomes shows elevated levels of dimerized ribosomes (100S) at LC.** Graphs show the nucleic acid concentration along sucrose gradients of different samples. The area under each peak corresponds to the abundance of a ribosome subunit or a ribosome complex and was used to calculate the abundance ratios (inset

table).

**Figure S11. RPF read length distribution for each sample.** The shaded areas indicate reads that were used in the data analyses (read length ≥ 25 nt).

**Figure S12. Total read count per nucleotide at gene 5' and 3' ends for each sample.** These data did not have their nucleotide positions shifted and counts indicate the 3' ends of RPF reads. The red vertical lines indicate the positions of start and stop codons if a -12 nt shift is applied to the read counts.

**Text S1. Supplemental methods with details of ribosome profiling and RNAseq sample preparation.**