Expanded View Figures

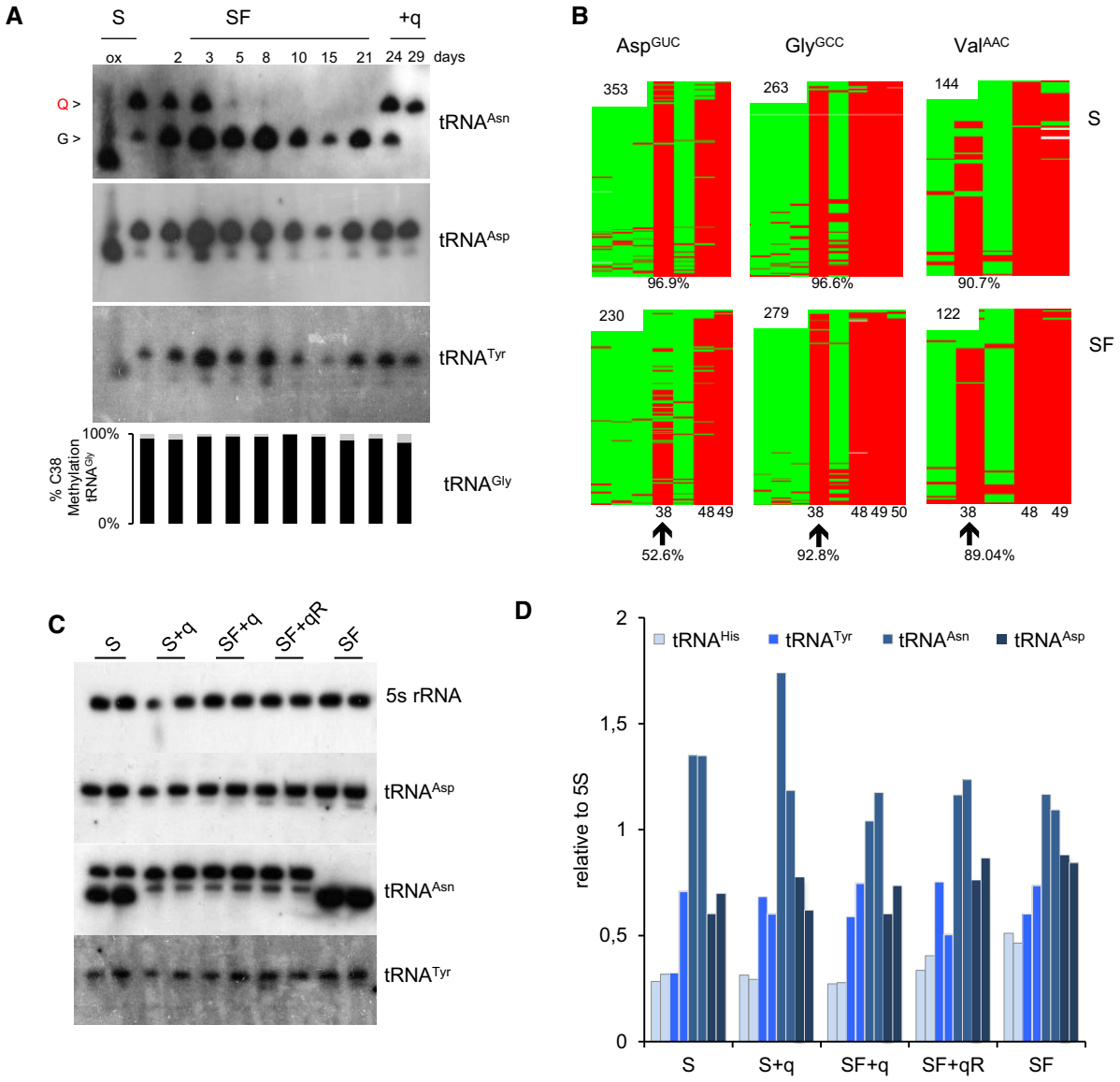

Figure EV1. $m^5C38\text{-}tRNA^{Asp}_{GUC}$ dependency on Q in HeLa cell culture and tRNA level quantifications.

A APB Northern blot using $tRNA^{Asn}$ probe shows that SF medium depletes Q-tRNA. No separate Q- and G-$tRNA^{Asp}$ and ^{Tyr} bands were detected. m^5C38 levels are measured by 454 bisulfite sequencing. A concomitant robust reduction in Q-$tRNA^{His}$ and $m^5C38\text{-}tRNA^{Asp}_{GUC}$ is observed in HeLa cells cultivated in SF medium. Both queuosinylation and methylation levels could be restored by the addition of queuine to the SF medium for 3 or 8 days after 21 days in SF medium. The differential migration is eliminated by oxidizing the ribose with periodate, producing a single faster migrating band (ox).

B Bisulfite sequencing maps from a biological replicate culture under S and SF for 3 weeks are depicted. Each row represents one sequence read and each column a cytosine residue. Green boxes represent unmethylated cytosine residues, and red boxes indicate methylated cytosine residues. Sequencing gaps are shown in white. Numbers in the maps indicate the number of reads. The position of specific cytosine residues and level of C38 methylation are indicated at the bottom.

C APB Northern blot analysis that complete Fig 2B with $tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{Tyr}$, and 5S rRNA as a loading control.

D Quantitative analysis of signals for tRNAs at the indicated culture conditions. tRNA signal intensities were normalized to 5S rRNA levels for each of the two replicates.

Data information: S (standard medium), SF (serum-free medium), q (queuine), Q (queuosine), and G (guanine).
Source data are available online for this figure.

Figure EV2. Translation speed in Q-free cells.

A Correlation between ribosome profiling dataset replicates. Spearman rho, 95% confidence interval for each pair of replicates.

B Changes in bulk codon occupancy in −Q cells compared to the relative rescue condition. All Q-decoded codons and Glu/Lys codons are highlighted with the indicated colors. Error bars: ± SE of the permutated ratios of bulk codon occupancy for the indicated conditions; (n = 4). For each distribution, the statistical significance calculated using a Kolmogorov–Smirnov test is shown. *P*-values (*t*-test, adjusted for multiple testing) relative for each codon occupancy are indicated by a color scale heatmap at the bottom.

C The heatmap shows codon occupancy correlations between replicates using Ward's maximum distance clustering method. Q-decoded codons are shown in green and Lys and Glu codons in orange. Note that hierarchical clustering grouped all Q-decoded codons together.

Data information: S (standard medium), SF (serum-free medium), q (queuine), Q (queuosine), and G (guanine).

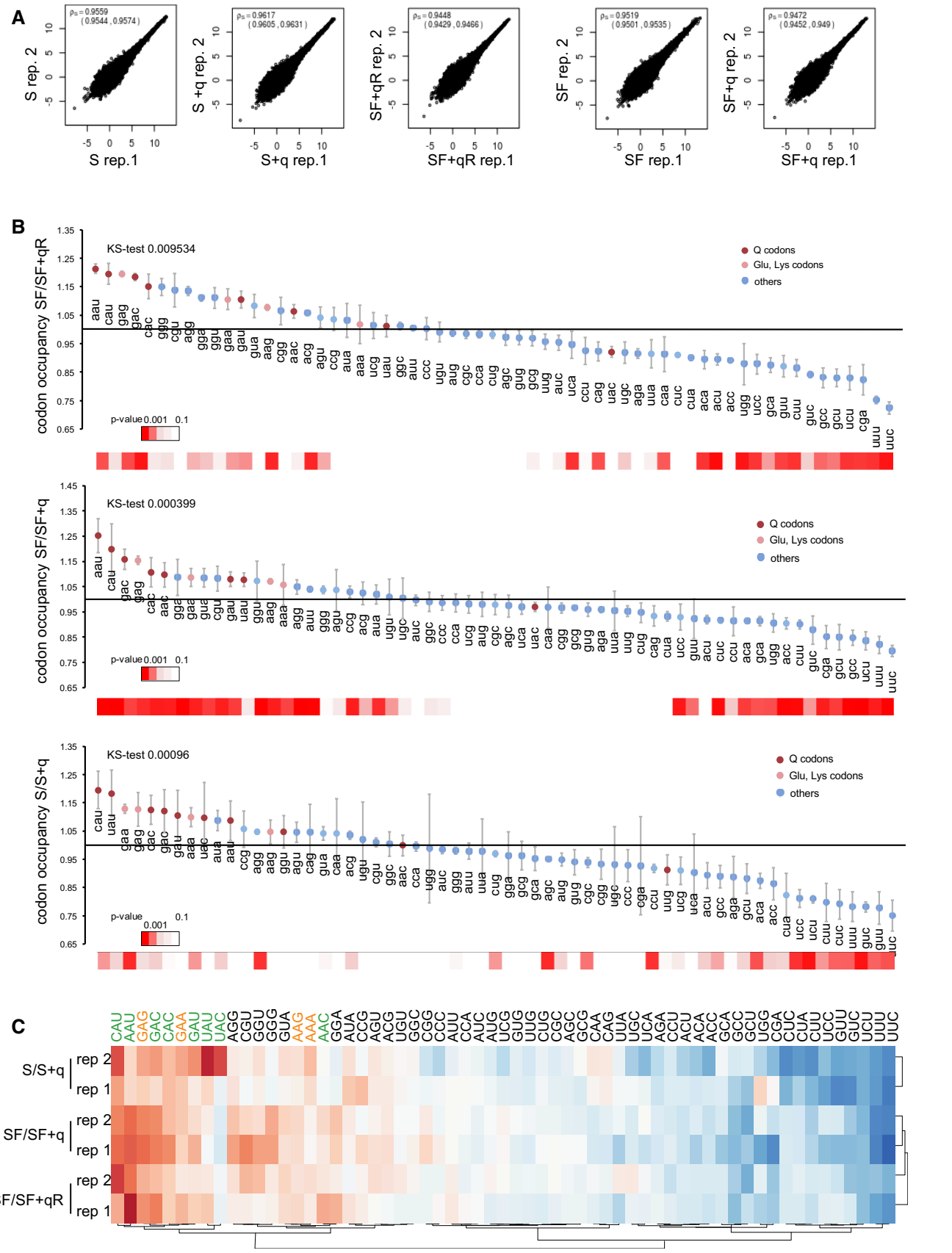
A
S rep. 2
S rep.1
S +q rep. 2
S+q rep.1
SF+qR rep. 2
SF+qR rep.1
SF rep. 2
SF rep.1
SF+q rep. 2
SF+q rep.1
B
codon occupancy SF/SF+qR
KS-test 0.009534
codon occupancy SF/SF+q
KS-test 0.000399
codon occupancy S/S+q
KS-test 0.00096
Q codons
Glu, Lys codons
others
p-value 0.001 0.1
C
S/S+q
SF/SF+q
SF/SF+qR
rep 2
rep 1

Figure EV2.

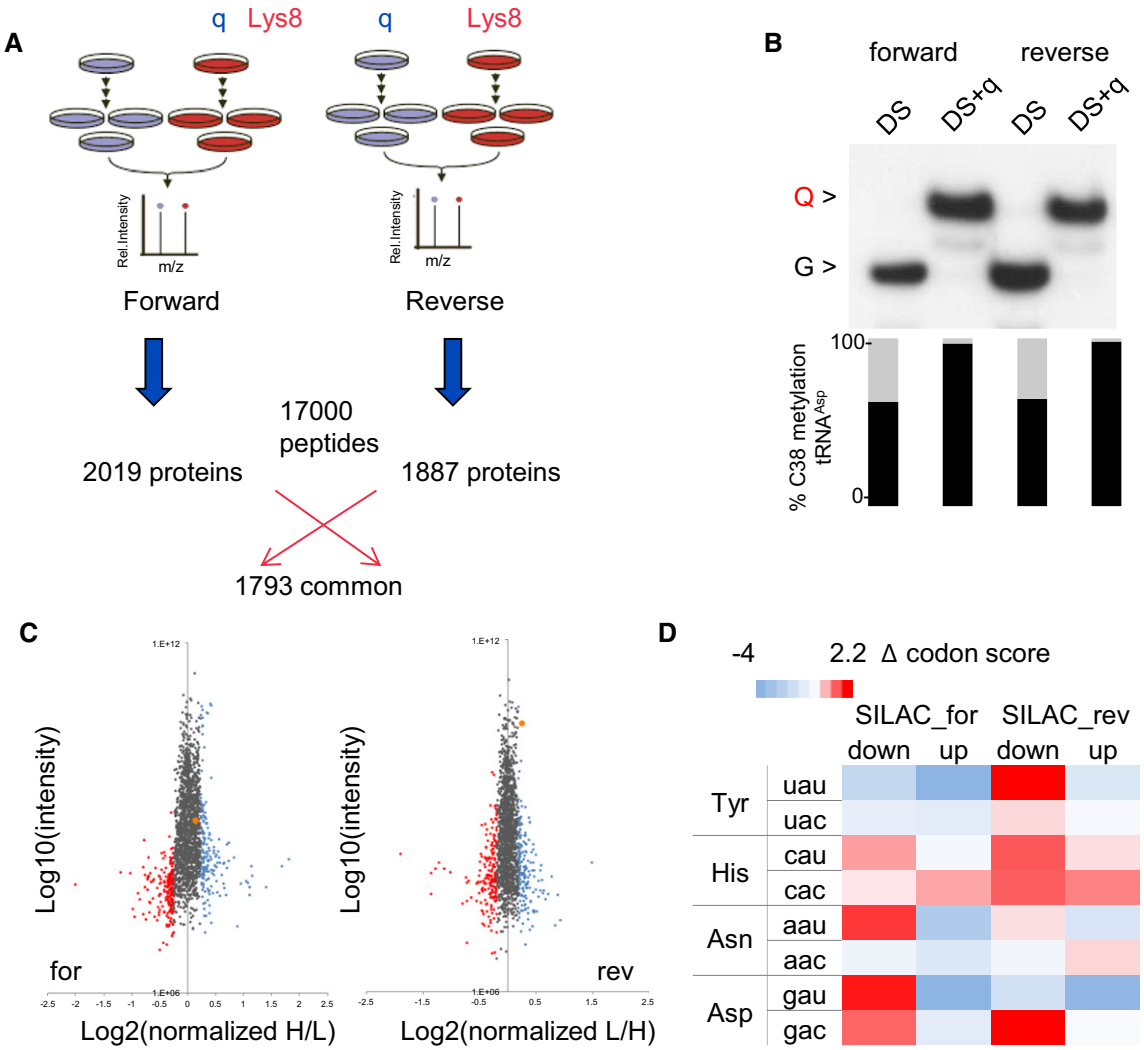

Figure EV3. SILAC.

A Labeling culture conditions and numbers of identified proteins.

B APB Northern blot using $tRNA^{His}$ probe and C38 methylation of $tRNA^{Asp}$ in SILAC labeled cells.

C SILAC analysis of deregulated proteins in the absence of Q. The top 10% of down-regulated proteins are indicated in red, the top 10% of up-regulated proteins are indicated in blue, HSPA5/BiP is indicated in orange.

D Increase or decrease in a codon frequency (‰) in up- or down-regulated proteins relative to the average frequency of that codon in unchanged proteins is displayed as heatmap according to the color scale.

Data information: DS (dialyzed medium), q (queuine), Q (queuosine), and G (guanine).
Source data are available online for this figure.

Figure EV4. Molecular functions affected by Q.

A Log2 fold changes in transcripts abundance and footprints at the indicated cell culture conditions. Pearson correlation coefficient (*r*) between footprints and mRNA abundance changes is shown.

B Volcano plot showing differentially translated mRNAs against adjusted *P*-values for SF compared with SF + qR. Red dots indicate $P_{adj} < 0.1$.

C Venn diagram showing the correlation of mRNA with high and low ribosome occupancy between the two rescue experiments (SF compared to SF + q and SF compared with SF + qR).

D Ingenuity molecular function analysis of differentially translated mRNAs. The top eight categories according to the ingenuity causal network *P*-value are shown.

E Transmission electron microscopy images showing cellular overview with increased cystic vacuoles of the ER upon Q depletion. Arrowheads point to rough endoplasmic reticulum while expansions are indicated by asterisk. N, nucleus. Scale bar 500 nm. Automated quantification of the size of all vacuoles is presented in violin plot between the indicated grown conditions (5 optical fields). Black lines show the medians; white lines represent individual data points; polygons represent the estimated density of the data; $*P < 0.05$ (*t*-test).

F Automated quantification of fluorescence intensity (AU: arbitrary units) relative to the immunofluorescence in Fig 6B. Error bars: ± SD (all cells in $n \geq 3$ optical fields); $*P < 0.05$ (*t*-test).

G Western blot showing increased eIF2α phosphorylation upon Q depletion. Actin and global eIF2 levels are used as loading controls. Quantification of the eIF2α phosphorylation signal normalized to β-actin. Each bar represents three independent biological replicates. Error bars: ± SD; $*P < 0.05$ (*t*-test).

Data information: S (standard medium), SF (serum-free medium), and q (queuine).
Source data are available online for this figure.

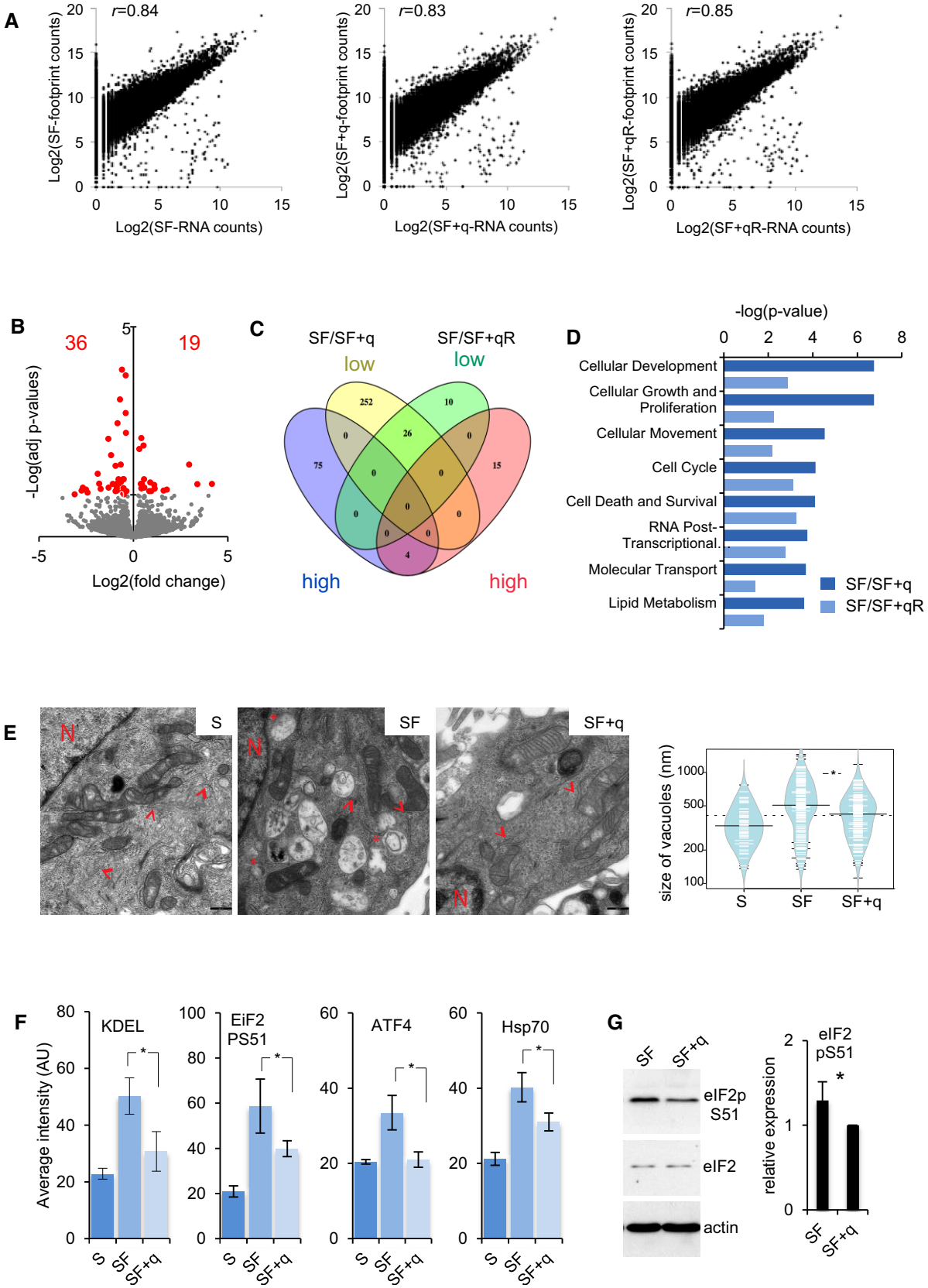

Figure EV4.

Figure EV5. Q-dependent phenotypes in Q-deficient mice.

A Mouse feeding timing schedule.

B APB Northern blot using $tRNA^{His}$ probe indicates that liver and brain axenic mouse tissues show physiological levels of Q-tRNA and that q-free diet depletes Q-tRNA. Q-tRNA level is obtained by quantification of Q and G signals and is presented on the right graph.

C Liver C38 methylation of $tRNA^{Asp}$ and Q-tRNA percentage obtained by quantification of the Q and G signals in Fig 7A. Error bars: ± SD; (n = 3).

D APB Northern blot using $tRNA^{His}$ probe shows that q-free diet depletes Q-tRNA. m^5C38 state measured by 454 bisulfite sequencing in the same RNA. Both queuosinylation and methylation levels could be slightly restored by the addition of q to the diet for 8 days. Q-tRNA percentage is obtained by quantification of the Q and G signals.

E The upper panels show liver sections from indicated mice stained with hematoxylin and eosin, whereas the lower panels show an overview of the immunostaining with antibodies against KDEL. Scale bar 50 μm. Automated quantification of KDEL intensity. Error bars: ± SD; $*P < 0.05$ (t-test); $n \geq 3$ optical fields; for three mice for condition.

Data information: h holoxenic mice, con mice were fed a conventional sterilized food, −q mice were fed a q-free synthetic diet for 60 days, and +q were mice fed a synthetic diet supplemented with 40 nM queuine for 60 days.
Source data are available online for this figure.

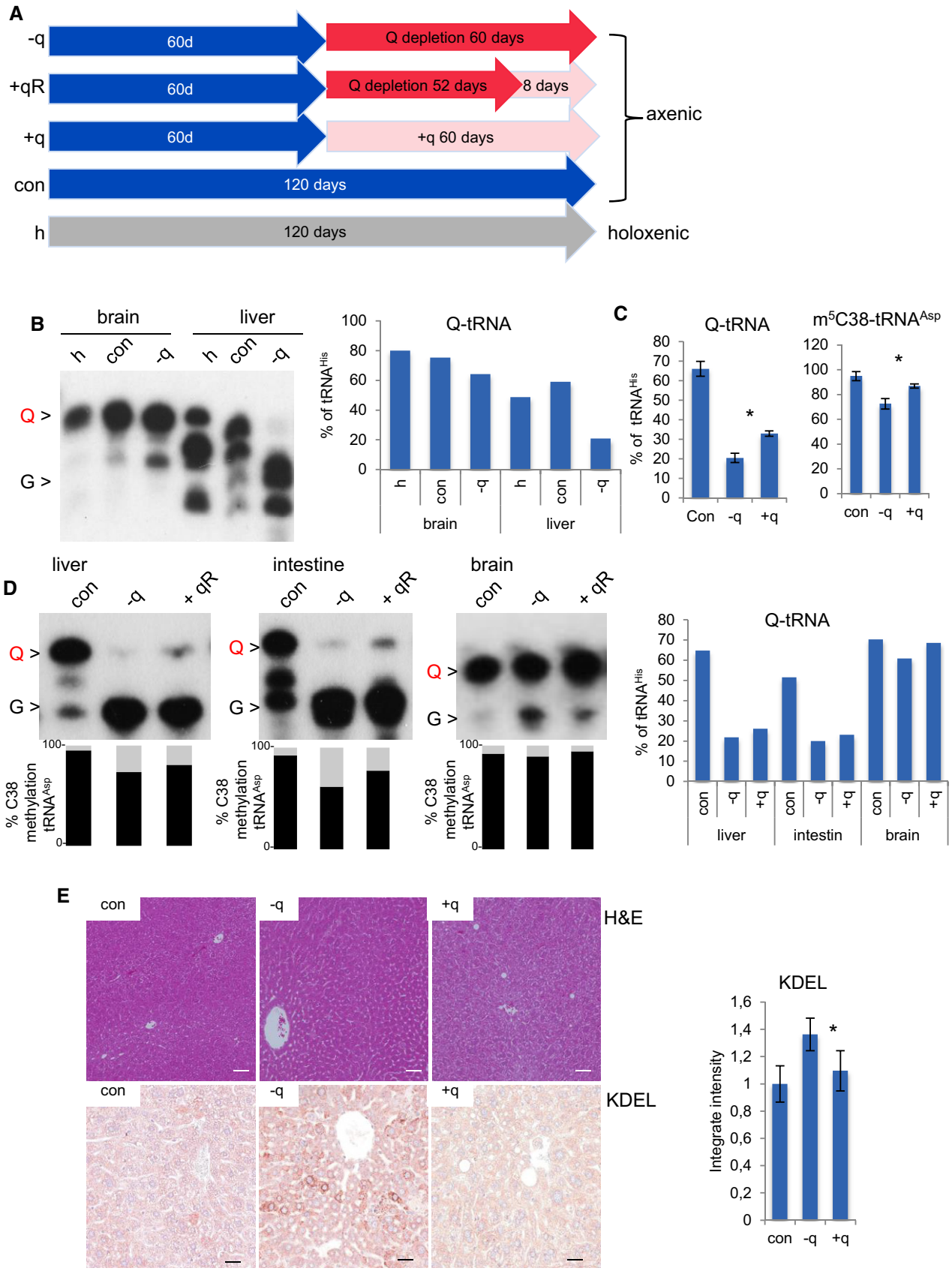

Figure EV5.