

SUPPLEMENTAL FIGURE AND TABLE LEGENDS

Figure S1. Supplemental Overview of Phenotype Data. Related to Figure 1.

(A) Histogram and dot plot of the animals with tissues collected at each time and diet, with the fraction on CD or HFD noted. Due to decreased lifespan in HFD, there is a disbalance in the CD/HFD ratio for the oldest timepoint at 24 months. (B) Significant Pearson lifespan correlations are observed between BXD strains in all three lifespan studies; 15 strains overlap in the left panel, 18 strains overlap in the studies portrayed in blue on the right panel and 16 strains for the red. (C) Proportion of variance explained by genotype, diet, age, or the interactions between these three variables, for key phenotypes in female mice, as calculated by ANOVA. (D) Lifespan calculations as a function of genotype for two strains with extreme differences in lifespan: BXD13 (very short lived) and BXD91 (very long lived). Significance in panels D and E are indicated by a Fleming-Harrington weighted log-rank test. (E) Kaplan–Meier curves for two strains with extreme effects of diet on lifespan. (F) Pearson correlation of measured lifespan by strain between CD and HFD fed cohorts in this study. 48 strains are shown, but with reciprocal B6D2F1 and D2B6F1s crosses are displayed separately although they diverge only for their mitochondrial genome (all are females, so the Y chromosome is irrelevant). (G–H) Body weight over time chart for two strains with extreme effects of diet on weight, showing the t-test result for the difference in body weight between CD and HFD using the weight for each animal taken between 470–530 days of age. (I) Serum alkaline phosphatase (ALPL) levels, segregated by different independent variables; t-test significance between groups is indicated.

Figure S2. Supplemental Patterns in Multifactorial, Multiomic Analysis. Related to Figure 2.

(A) Volcano plot showing the effect of age on mRNA and protein levels between old and young individuals. The number of genes which cross the t-test significance threshold below the nominal p -value of 0.05 or a Benjamini-Hochberg adjusted p -value of 0.05 are indicated. (B) Variation explained and F statistic for proteins and metabolites a function of the independent variables and their interactions. (C) Spearman correlation density plot of the relationship between diet (fold change) and age (correlation) on mRNA levels for all genes. The equivalent correlation for protein is not significant ($r = 0.01$, $p = 0.55$; not shown). (D) Histogram of the percentage of significant Pearson correlations for mRNA–protein pairs as a function of the variance of mRNA expression across the population. The color scale indicates each decile. (E)

Density plot of mRNA–protein Pearson correlations as a function of transcript abundance. (F) Spearman correlation plot between expression variance and abundance for mRNA. (G) Histogram of the percentage of significantly correlated mRNA–protein pairs as a function of the size of the protein complex to which the gene belongs, using CORUM annotation. (H) Empirical calculations of the false discovery rate of cis-eQTLs as a function of LOD score depending on if the gene is detected in only one dataset (“discovery”) or if it is the validation of expectations from independent cis-QTL data. (I) Left: Venn diagram of the overlap of cis-pQTLs for CD or HFD cohorts using a strict cutoff of $\text{LOD} \geq 4$. Middle: Slopegraph of LOD scores of all 165 genes with significant cis-pQTLs. Right: The same Venn diagram, but now using more flexible cutoffs. (J) cis-QTL consistency on a gene-level basis between mRNA and protein levels for just young individuals (left) and as a function across age for protein levels (right). The same patterns are observed here as for across-diet comparisons.

Figure S3. Supplemental Analysis of Aging Candidate Discovery & *C. elegans*. Related to Figure 3.

(A) Replicate study of the effect of *Ctsd* (*asp-4*) on lifespan in *C. elegans*. (B) (Left) *St7* has a negative Pearson correlation with *expected lifespan* of the individual in both CD ($n = 114$) and HFD ($n = 88$); (right) However, *St7* does not correlate with the *measured age* of the animal when it was harvested for expression analysis in either CD ($n = 161$) or HFD ($n = 129$). Note that not all individuals have “expected lifespan” measurements (i.e. indicating the diet and strain cohort to which the individual belonged had < 6 natural deaths). (C) The equivalent data as categorical comparisons, showing the t-test results and fold changes. (D) Repeat of *St7* (*st-7*) lifespan in *C. elegans*. Full details and significance tests for panels A and D are in Table S4.

Figure S4. Supplemental Functional Network Analysis and False Discovery. Related to Figure 4.

(A) Spearman correlation network for the 75 OXPHOS genes measured at both the mRNA and protein level, showing minimal connectivity between the two layers (~3% of edges are across mRNA to protein). Network p -values are compared against 10,000 randomly selected gene sets of the same size from the same data. (B) Correlation density plots of all Spearman correlations for the ribosome, mitochondrial ribosome, beta oxidation, and TCA cycle as a function of mRNA level, protein level, or across the two. The correlations of random genes within mRNA/protein or across layer is shown

at the top-right. The average correlation of two mRNAs is similar to the average of two random proteins (+0.04 for mRNA, +0.03 for protein), but the standard deviation is different: ± 0.252 for mRNA, ± 0.12 for protein.

Figure S5. Supplemental Stability Inference and False Discovery. Related to Figure 5.

(A) A conceptual schematic for how to interpret prediction–stability plots. (B) The overlap of 100 negative control permutation networks using the mitochondrial translation gene set (top) and the PPAR signalling pathway gene set (bottom). The theoretical upper-bound false discovery in this area is 1 discovery per test in the green area regardless of gene set. Here, for this randomized gene set, the sum of the permutation tests discovers only 4 nodes in the “green” sections of the mRNA and 2 in the protein network, empirically indicating a false positive of approximately 0.03 per test averaged across all runs. Permuted false discovery in the white area is approximately 1 node per test. Note that the exact cutoff values change slightly depending on input. The reported p -values are for the effect of the stated independent variable (e.g. age) on the target pathway (e.g. mitochondrial translation) by t-test. (C) Spearman correlation plot of the cholesterol biosynthesis transcripts in CD and HFD conditions. (D) Pearson correlation plots between the mean cholesterol biosynthesis pathway expression and three candidates selected from different “quadrants” of the prediction–stability plots.

Figure S6. Supplemental Quality Control for Metabolomics. Related to Figure 2.

(A) Violin plot of correlations between all 629 back-to-back technical injection replicates in metabolomics Run 2 (mean $\rho = 0.99$), showing significant differences with the correlations of back-to-back injections of 629 unrelated samples (mean $\rho = 0.91$; $p = 1e-59$ t-test). (B) Comparison of the effect of *D2hgdh* allele on D-2-hydroxyglutarate levels. Animals with the C57BL/6 allele of *D2hgdh* had significantly higher D-2-hydroxyglutarate than those with the DBA/2 allele in both runs (t-test). (C) Comparison of three metabolites with large fold changes caused by HFD in our earlier 2016 BXD liver metabolome study (Williams et al., 2016), showing a general concordance in the effect of diet across study (t-test).

Figure S1. Supplemental Overview of Phenotype Data

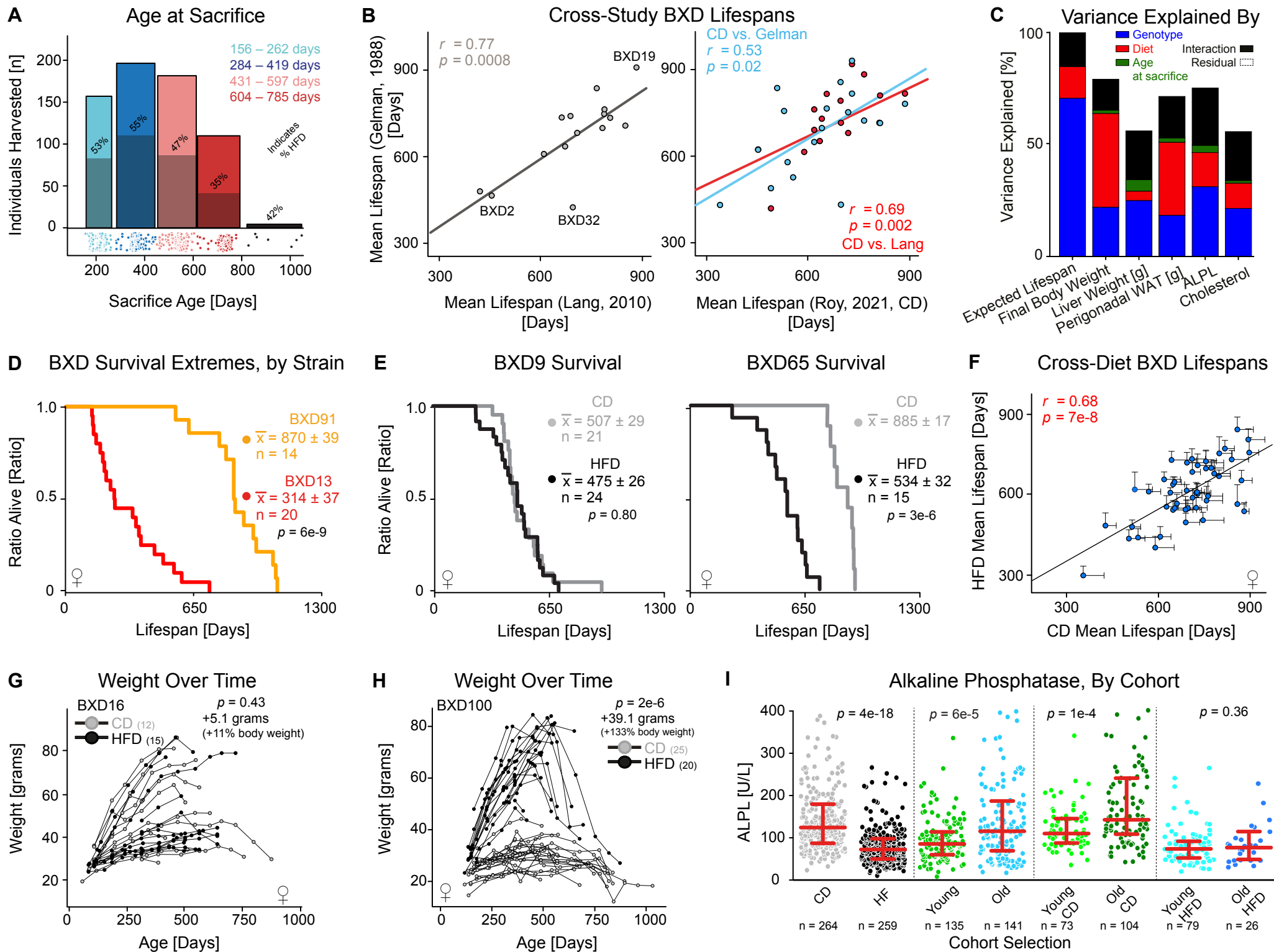


Figure S2. Supplemental Patterns in Multifactorial, Multiomic Analysis

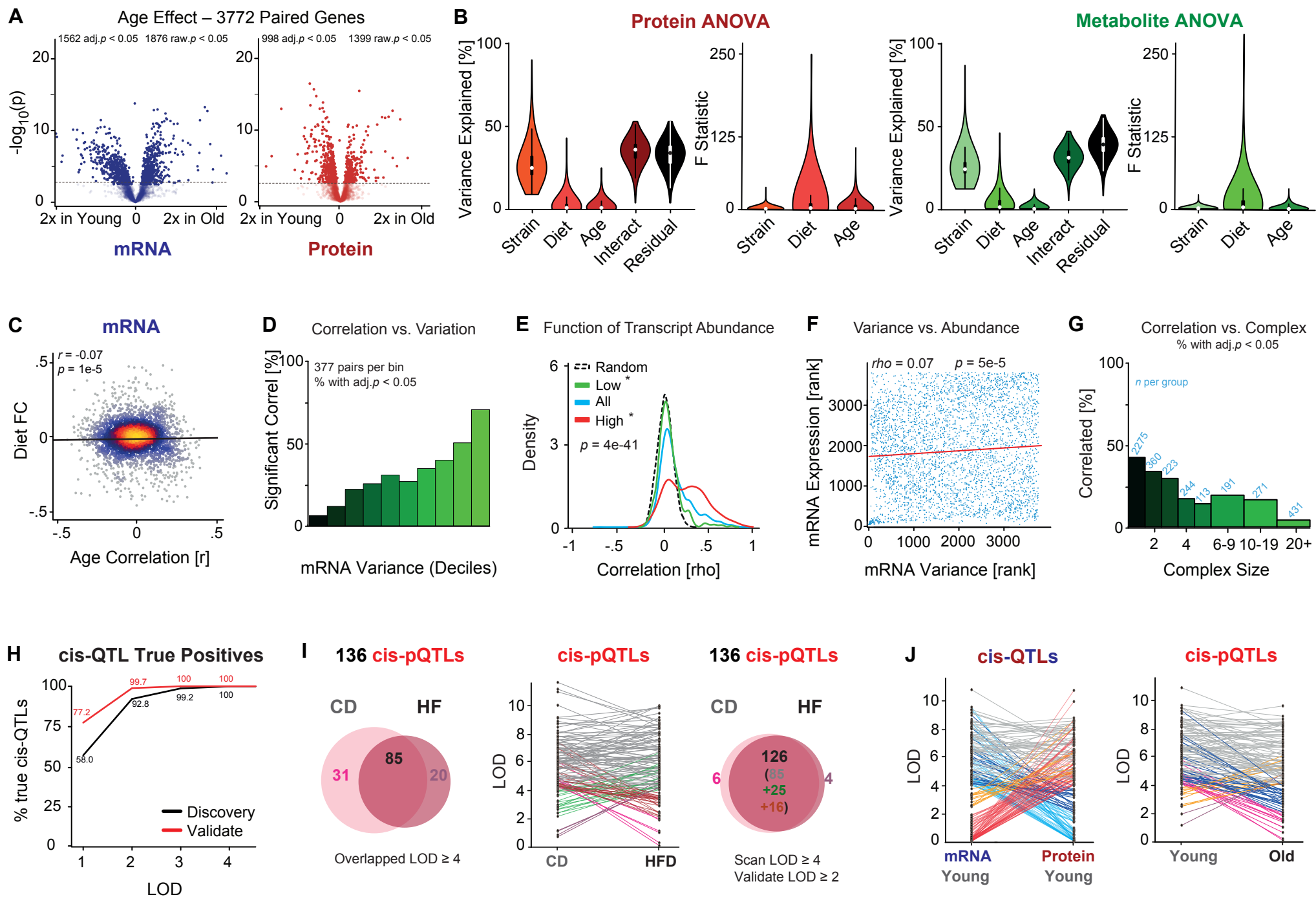


Figure S3. Supplemental Analysis of Aging Candidate Discovery & *C. elegans*

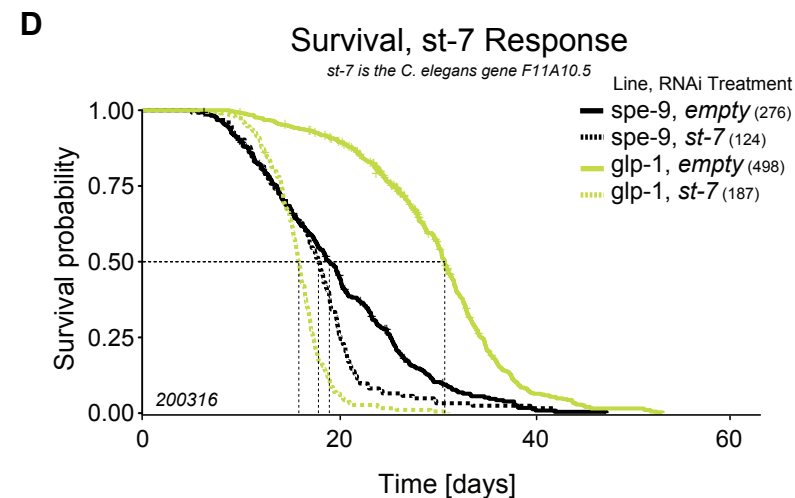
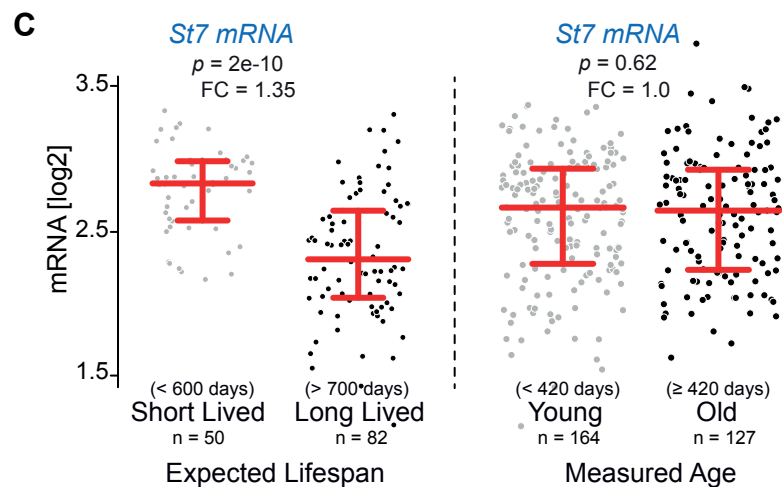
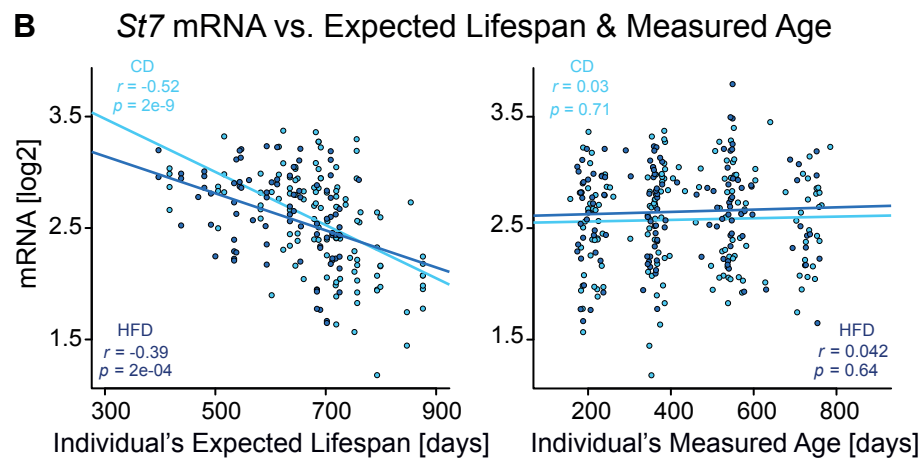
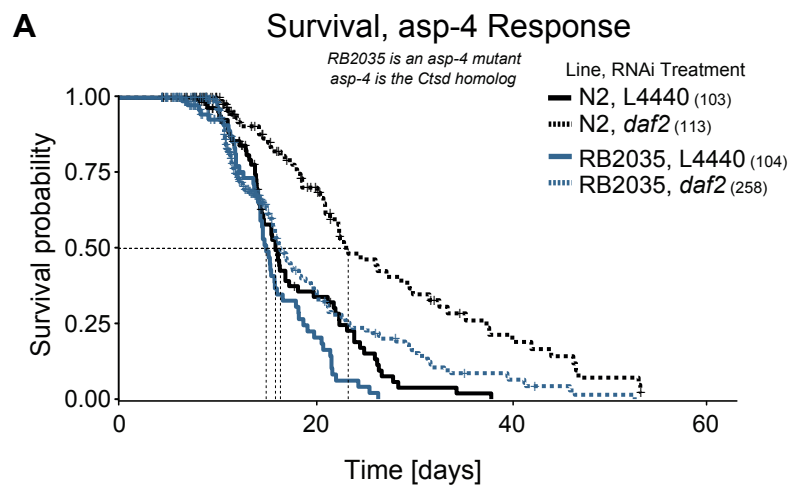


Figure S4. Supplemental Functional Network Analysis and False Discovery

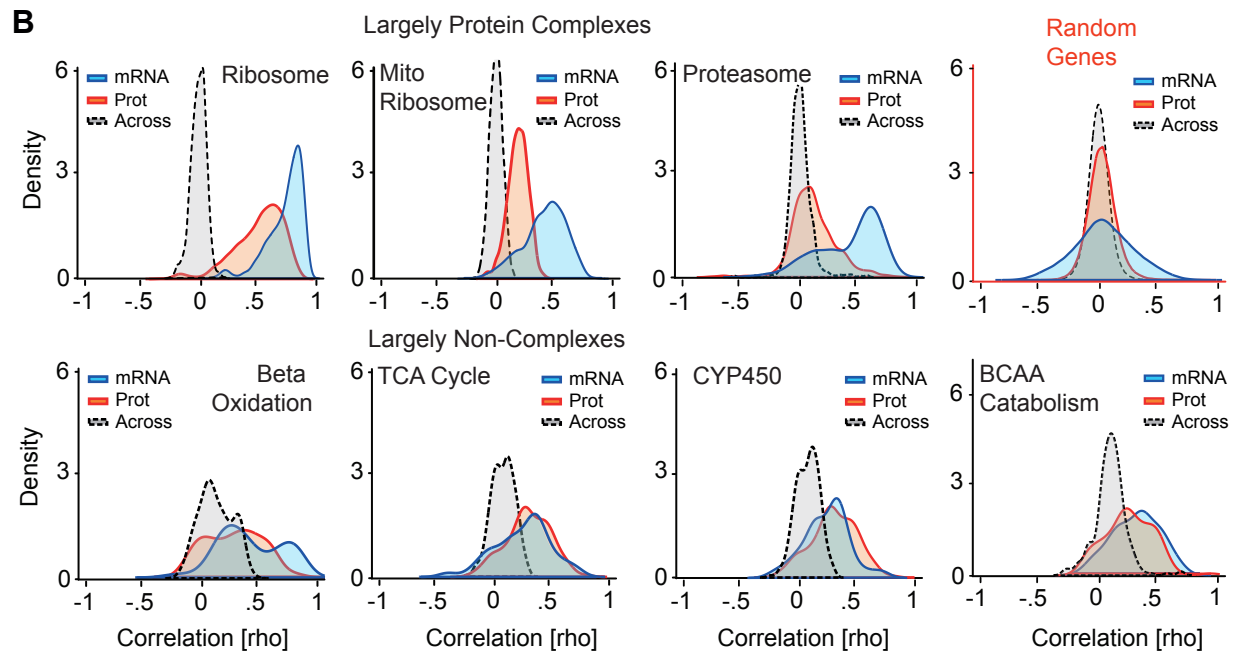
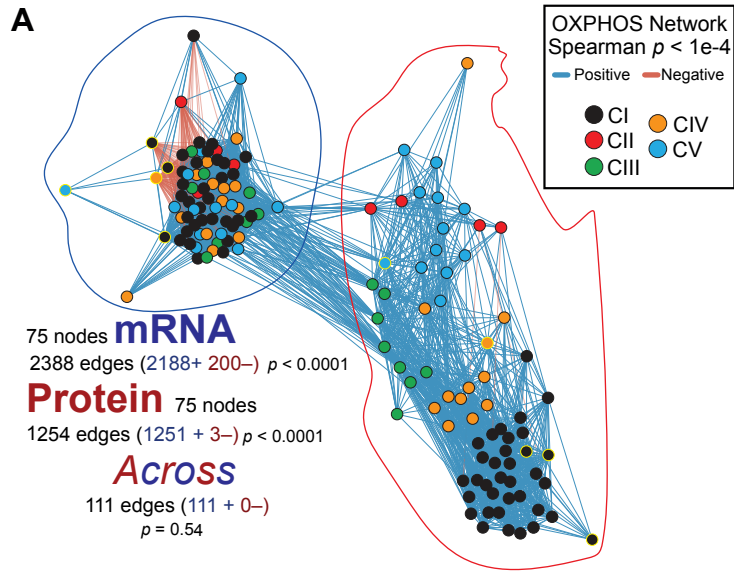


Figure S5. Supplemental Stability Inference and False Discovery

A Interpreting Stability Plots:

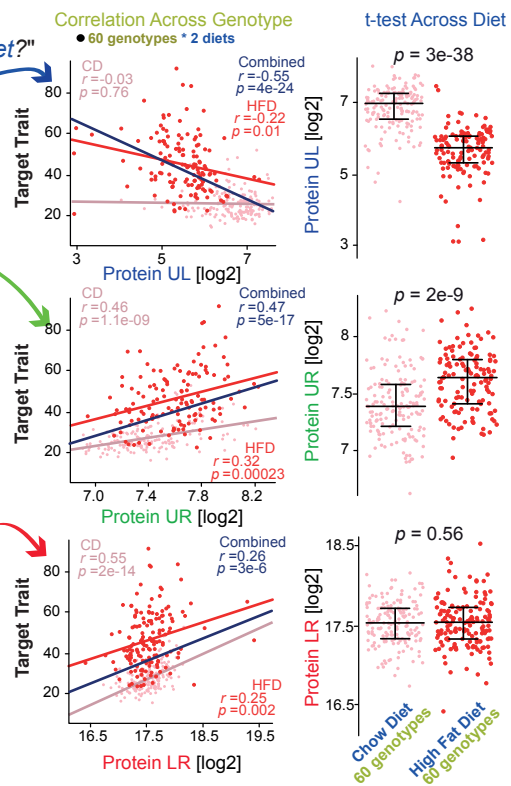
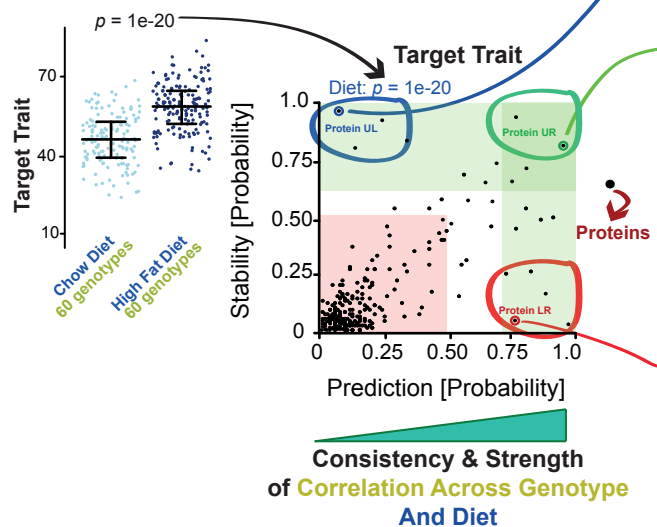
"Which **proteins** change a **target trait** across strains according to **diet**?"

Independent Variable 1: **Diet**

Independent Variable 2: **Genotype**

Primary Dependent Variable: **Target Trait**

Unknown Mediating Variables: **Proteins**



B Sum of Results from 100 Random Permutations

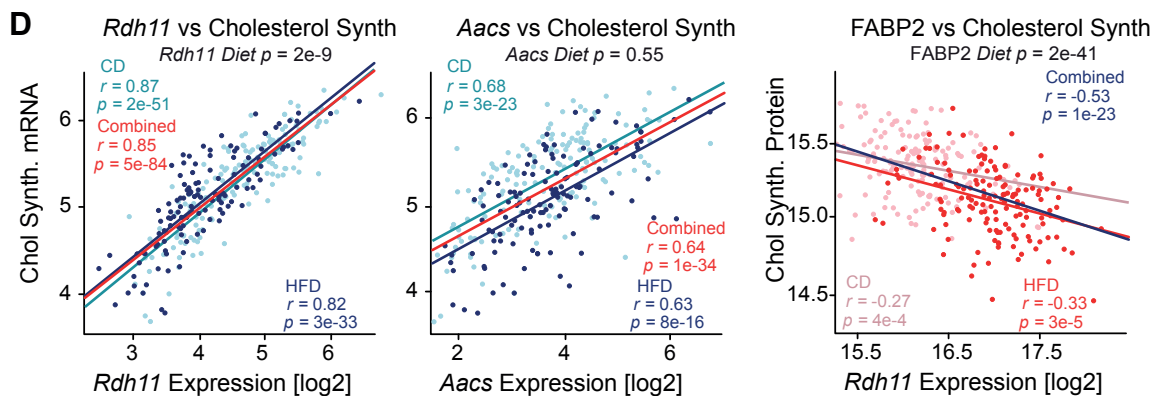
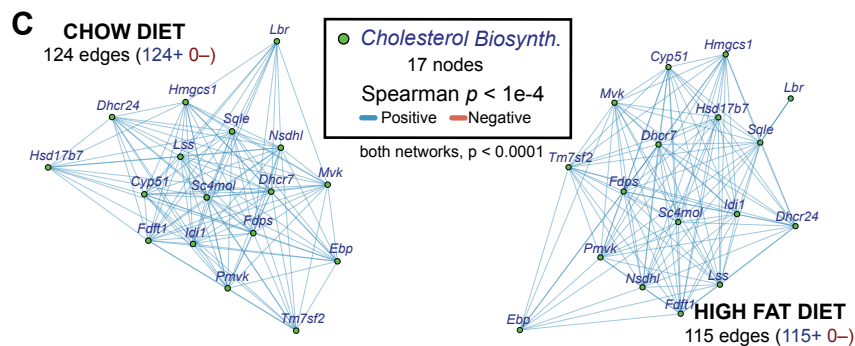
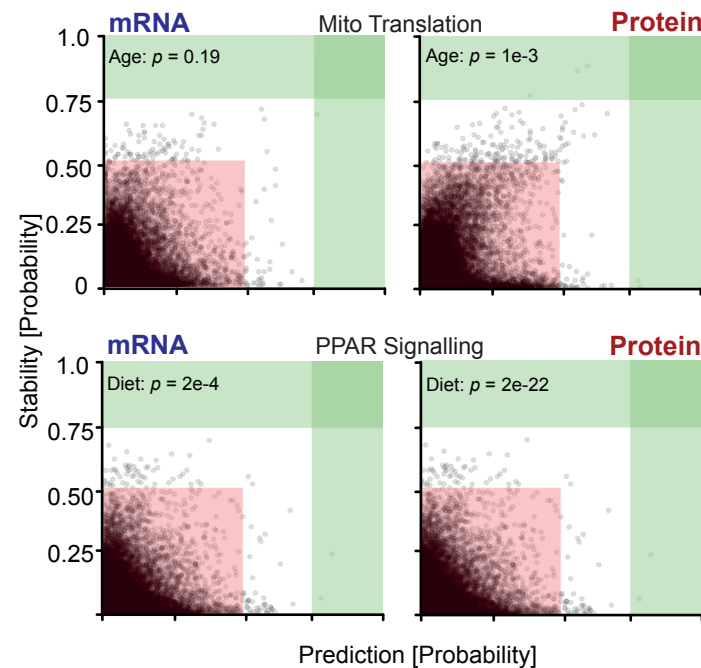


Figure S6. Supplemental Metabolomics Quality Control

