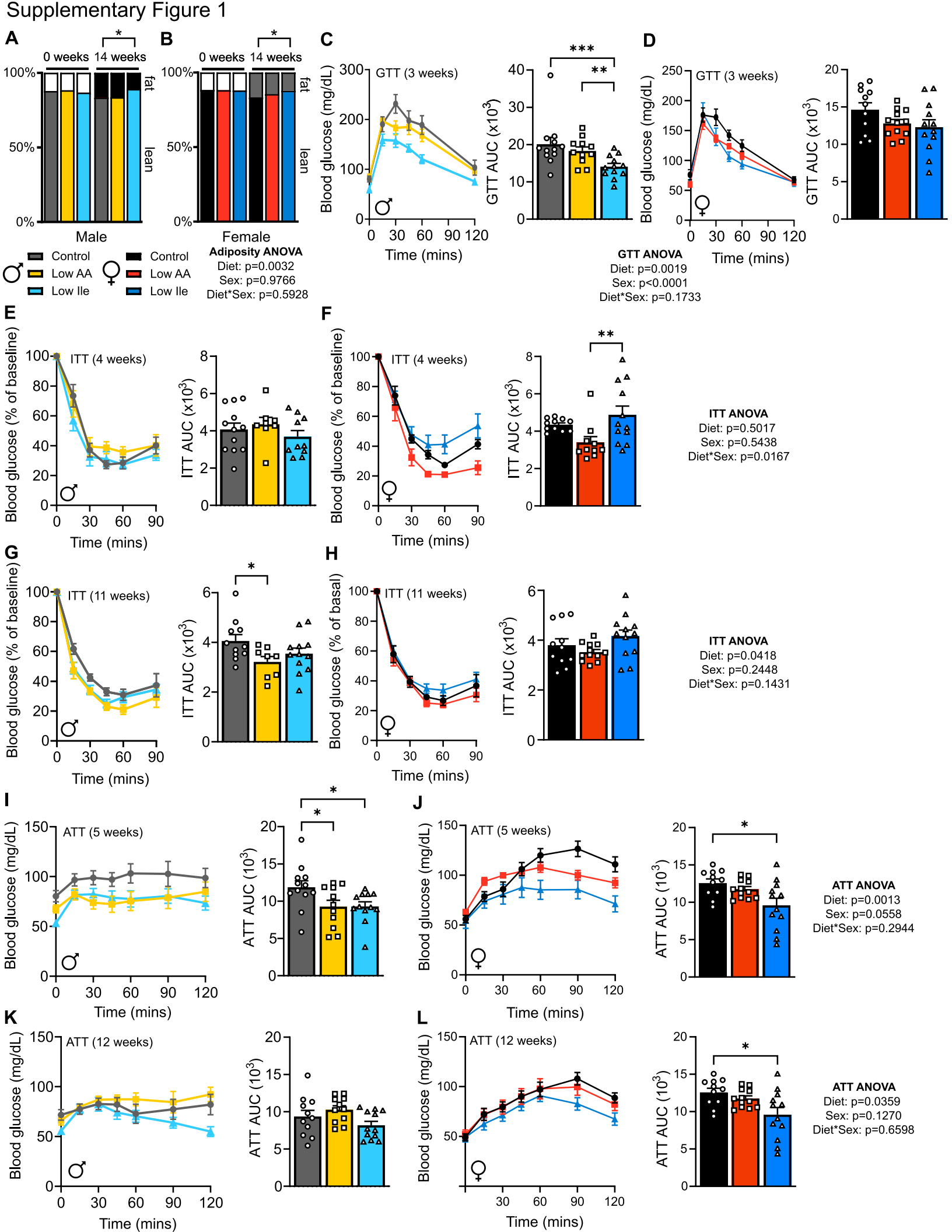
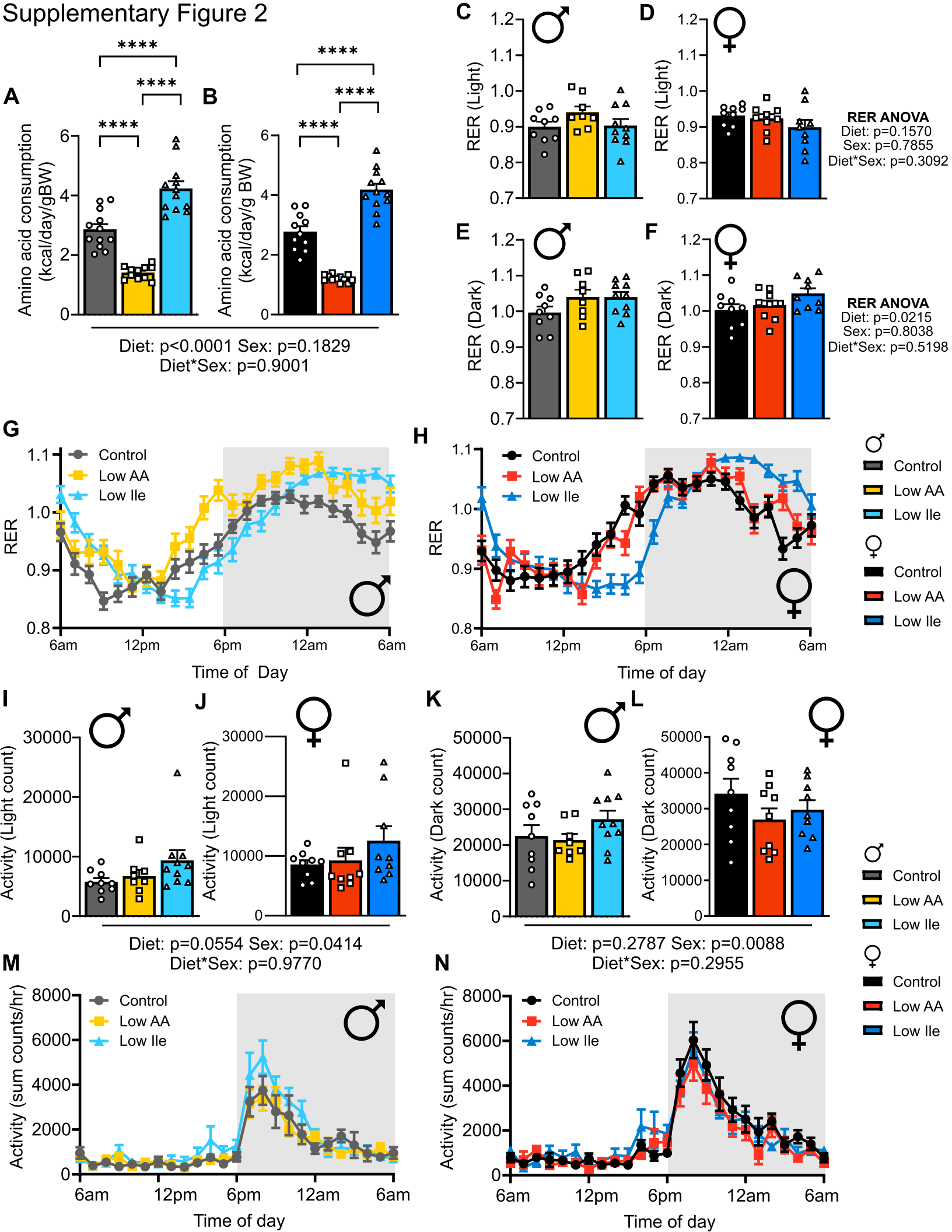
Supplementary Figure 1



Supplementary Figure 1: A Low Ile diet alters body composition and improves glycemic control in male and female HET3 mice.

(A-B) Percentage body composition at the beginning and end of study in males (A) and females (B). (C) Male glucose tolerance test (GTT) after 3 weeks on diet and representative area under the curve (AUC). (D) Female GTT after 3 weeks on diet and representative area under the curve (AUC). (E) Male insulin tolerance test (ITT) after 4 weeks on diet and representative area under the curve (AUC). (F) Female ITT after 4 weeks on diet and representative area under the curve (AUC). (G) Male insulin tolerance test (ITT) after 11 weeks on diet and representative area under the curve (AUC). (H) Female ITT after 11 weeks on diet and representative area under the curve (AUC). (I) Male alanine tolerance test (ATT) after 5 weeks on diet and representative area under the curve (AUC). J) Female ATT after 5 weeks on diet and representative area under the curve (AUC). (K) Male alanine tolerance test (ATT) after 12 weeks on diet and representative area under the curve (AUC). (L) Female ATT after 12 weeks on diet and representative area under the curve (AUC). (A-L) n=8-12 mice/group. Two-way ANOVA between Sex and Diet groups with post-hoc Tukey test for pairwise comparisons, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. P-values for the overall effect of Sex, Diet and the interaction represent the significant p-values from the two-way ANOVA. Data are represented as mean ± SEM. Related to **Figure 1**.

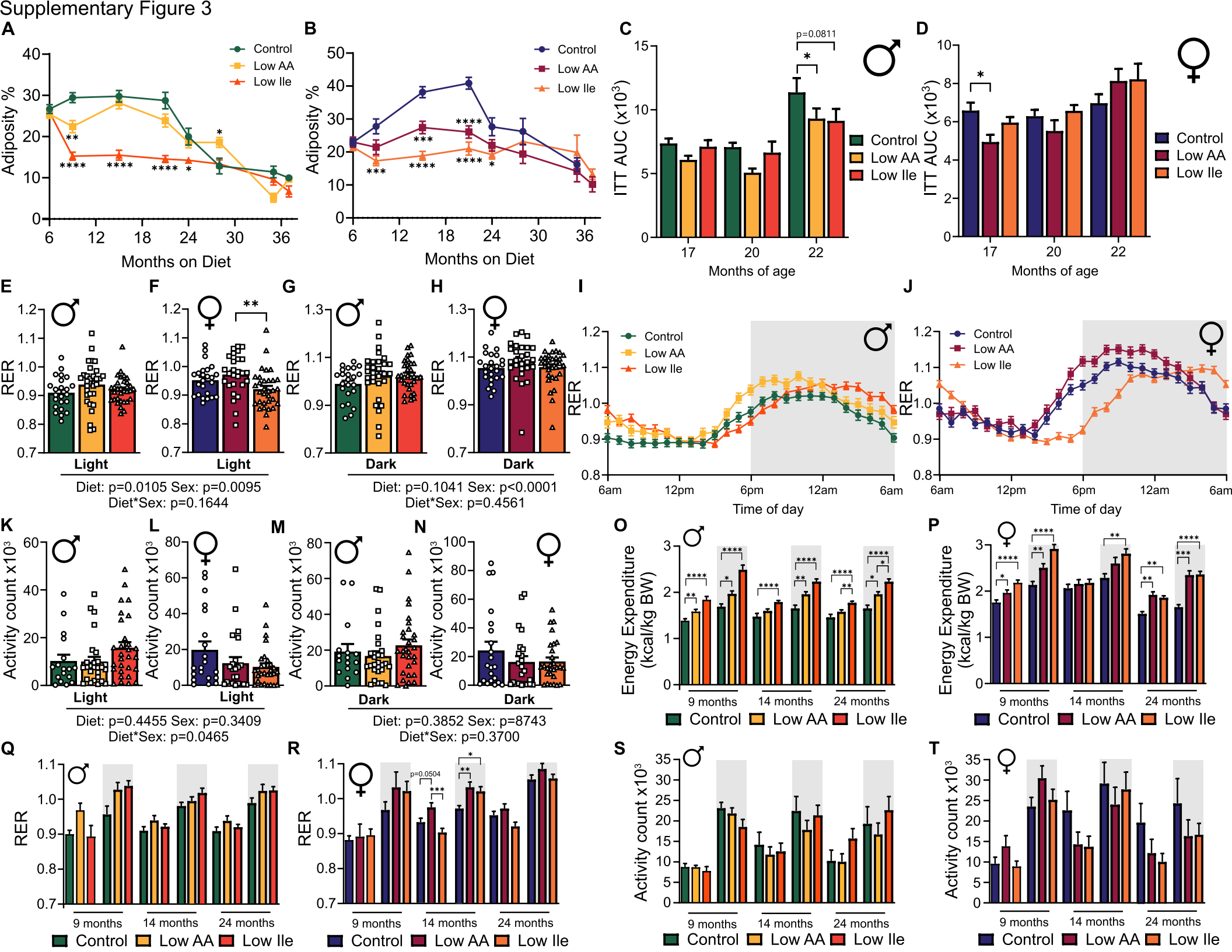
Supplementary Figure 2



Supplementary Figure 2: A Low Ile diet increases amino acid consumption, but not RER or activity levels in male and female HET3 mice.

(A-B) Food consumption was measured in home cages after 3 weeks on diet and amino acids intake was calculated for male (A) and (B) female mice (n=11-12 mice/group). (C-F) RER was measured using metabolic cages in the light (C-D) and dark (E-F) phases in male (C, E) and female (D, F) mice (n=8-10 mice/group). (G-H) RER of a 24 hour period in male (G) and female (H) mice (n=8-10 mice/group). (I-L) Spontaneous activity measured in the light (I) and dark (J) phases in male and female mice (n=8-10 mice/group). (M-N) Spontaneous activity over a 24-hour period in male (M) and female (N) mice (n=8-10 mice/group). (A-F, I-J) Two-way ANOVA for Diet and Sex with post-hoc Tukey multiple comparison test, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. P-values for the overall effect of Diet, Sex and the interaction represent the significant p-values from the two-way ANOVA. Data represented as mean ± SEM. Related to **Figure 2**.

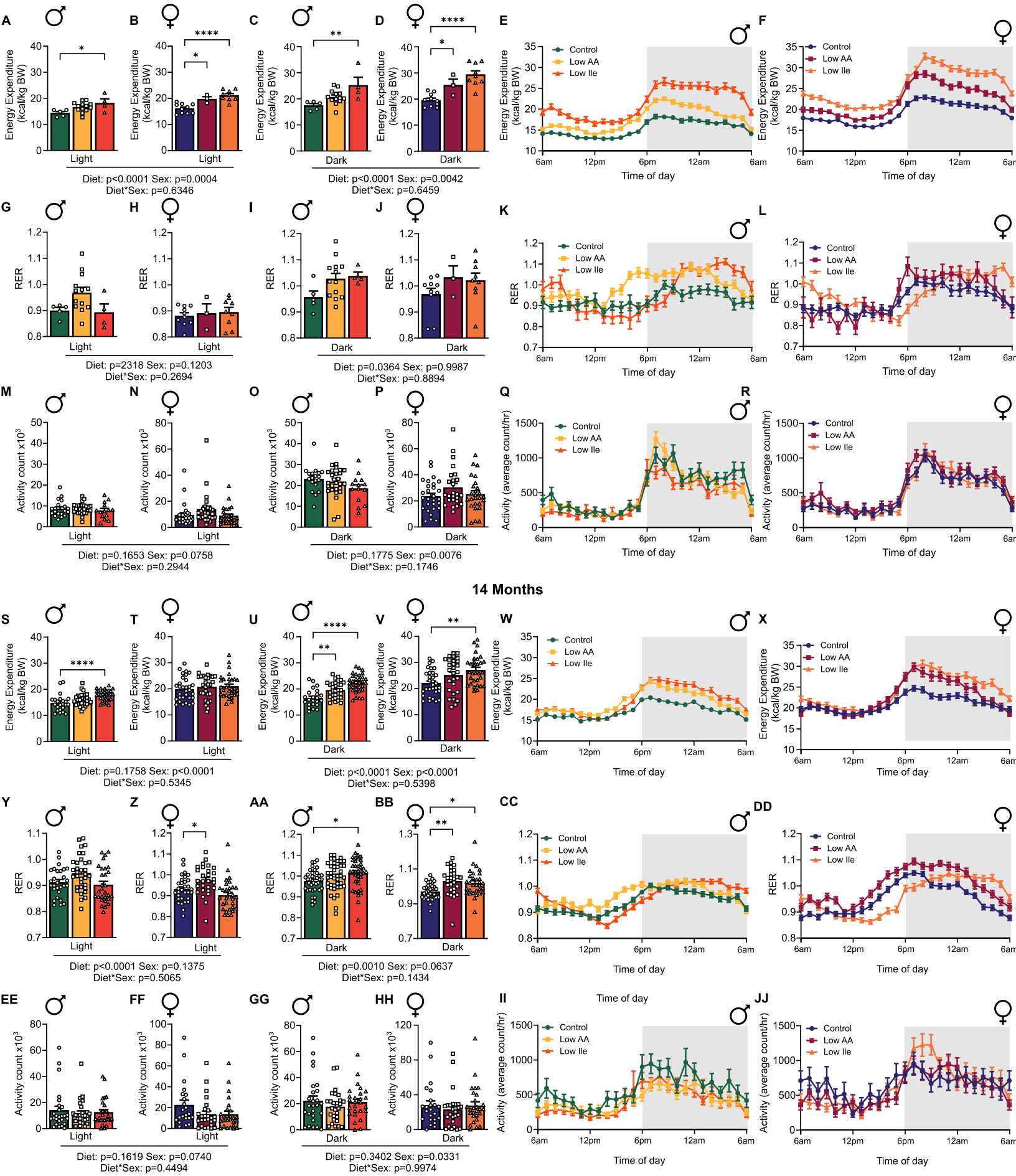
Supplementary Figure 3



Supplementary Figure 3: A low isoleucine diet improves insulin sensitivity in aged males only and increases energy expenditure but not in males and females longitudinally.

(A) Adiposity over time in males, n=47-51 mice/group. (B) Adiposity over time in females, n=51-53 mice/group. Mixed-effects model with Geisser-Greenhouse correction followed by Dunnett's multiple comparison correction test, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. (C) ITT AUC for male mice over the course of the experiment, n=12-17 mice/group. (D) ITT AUC for female mice over course of experiment, n=14-21 mice/group. (E-N) Metabolic chamber data from 24-month-old mice. (E-F) Male (E) and female (F) RER in the light phase. (G-I) Male (G) and female (H) RER in the dark phase. (I-J) RER over a 24-hour period for males (I) and females (J). (E-J) n=23-32 mice/group. (K-N) Male (K, M) and female (L, N) summed activity counts in the light (K-L) and (M-N) dark phases (K-N) n=16-30 mice/group. (O-T) Summary metabolic chamber data for mice analyzed at 9, 14, and 24 months of age.(O-P) Male (O) and female (P) energy expenditure between 9 and 24 months of age in the light (white) and dark (grey shaded) phases (n=14-33 mice/group). (Q-R) Male (Q) and female (R) RER between 9 and 24 months of age in the light (white) and dark (grey shaded) phases (n=14-33 mice/group). (S-T) Male (S) and female (T) longitudinal activity between 9 and 24 months of age in the light (white) and dark (grey shaded) phases (n=14-30 mice/group). (C-T) Two-way ANOVA for Diet and Age with post-hoc Tukey multiple comparison test, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Data represented as mean ± SEM. Related to **Figure 3**.

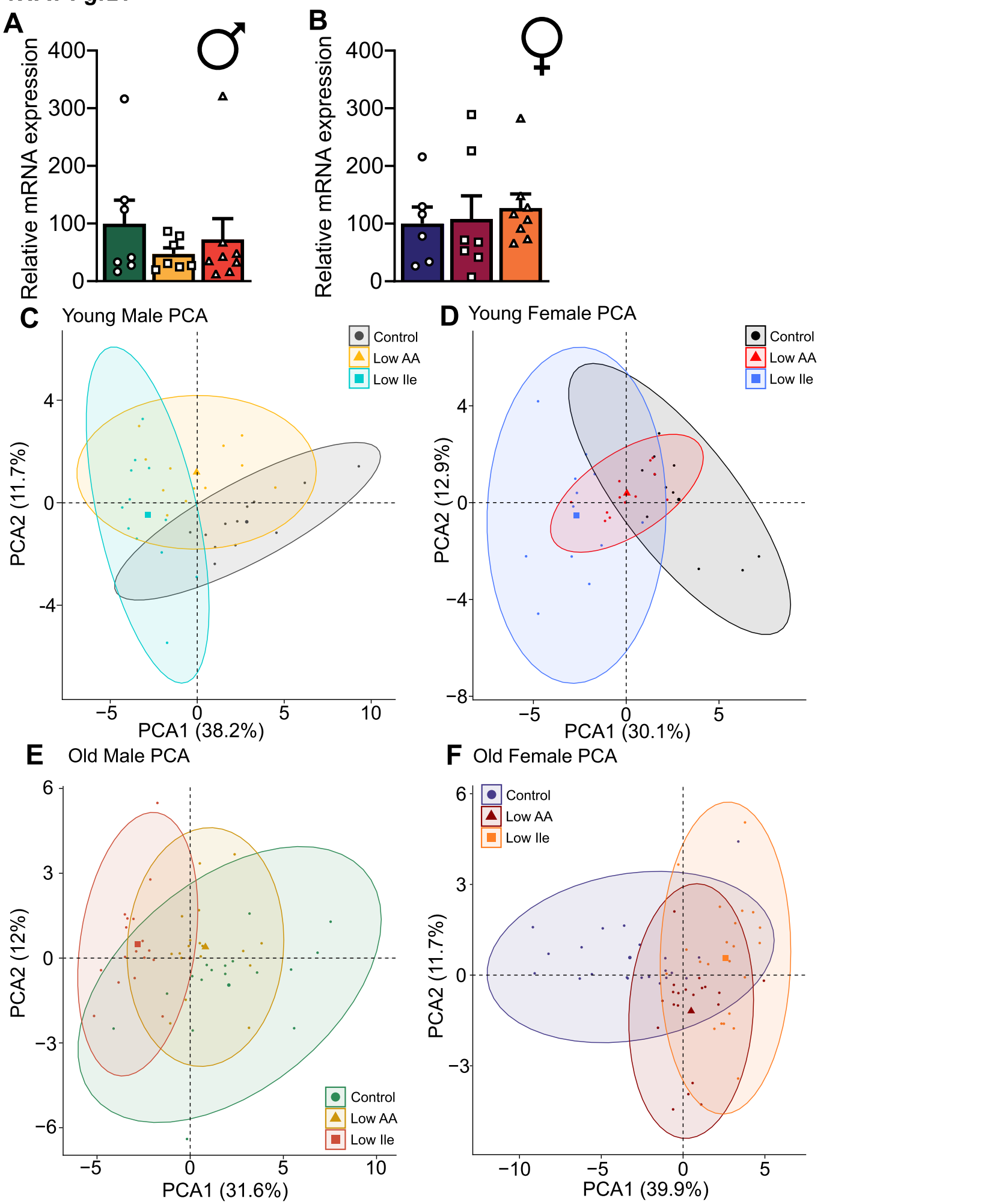
Supplementary Figure 4



Supplementary Figure 4: A low isoleucine diet increases energy expenditure but not RER or activity levels longitudinally in males and females.

(A-JJ) Overview of chamber data for male and female mice at 9 months (A-R) and 14 months (S-JJ) of age. (A-F) Energy expenditure at 9 months of age (male n=4-13 mice/group, female n=3-10 mice/group). (G-L) RER at 9 months of age (male n=4-13 mice/group, female n=3-10 mice/group). (M-R) Activity at 9 months of age (male n=14-28 mice/group, female n=25-28 mice/group). (S-X) Energy expenditure at 14 months of age (male n=28-33 mice/group, female n=28-31 mice/group). (Y-DD) RER at 14 months of age (male n=28-33 mice/group, female n=31-28 mice/group). (EE-JJ) Activity at 14 months of age (male n=26-27 mice/group, female n=23-27 mice/group).(A-JJ) Two-way ANOVA for Diet and Sex with post-hoc Tukey multiple comparison test, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Data represented as mean ± SEM. Related to **Figure 4**.

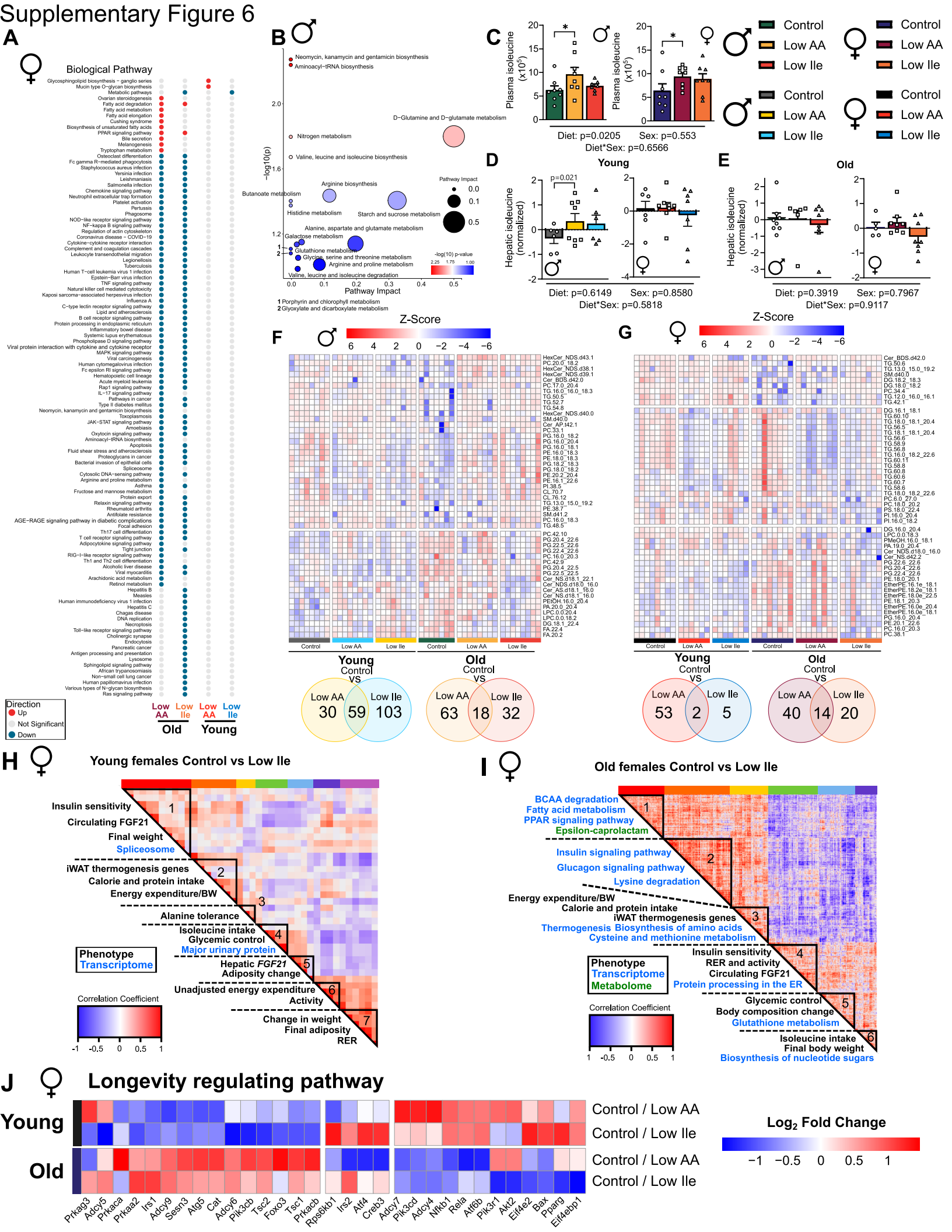
Supplementary Figure 5



Supplementary Figure 5: A low isoleucine diet does not alter iWAT *FGF21* expression in aged HET3 mice, but all measured phenotype changes promote global differentiation between groups in old and young mice.

(A-B) Relative expression of *Fgf21* in the iWAT of male (A) and female (B) mice fed the indicated diets (n=6-8 mice/group). Two-way ANOVA for Diet and Age with post-hoc Tukey multiple comparison test, **P*<0.05, ***P*<0.01, ****P*<0.001 and *****P*<0.0001. (C-F) Principal component analysis for 27 measured phenotypes in Young Male Mice. (C) Young Female Mice (D), Old Male Mice (E) and Old Female Mice. (n=11-22 mice/group). Data represented as mean ± SEM. Related to **Figure 5**.

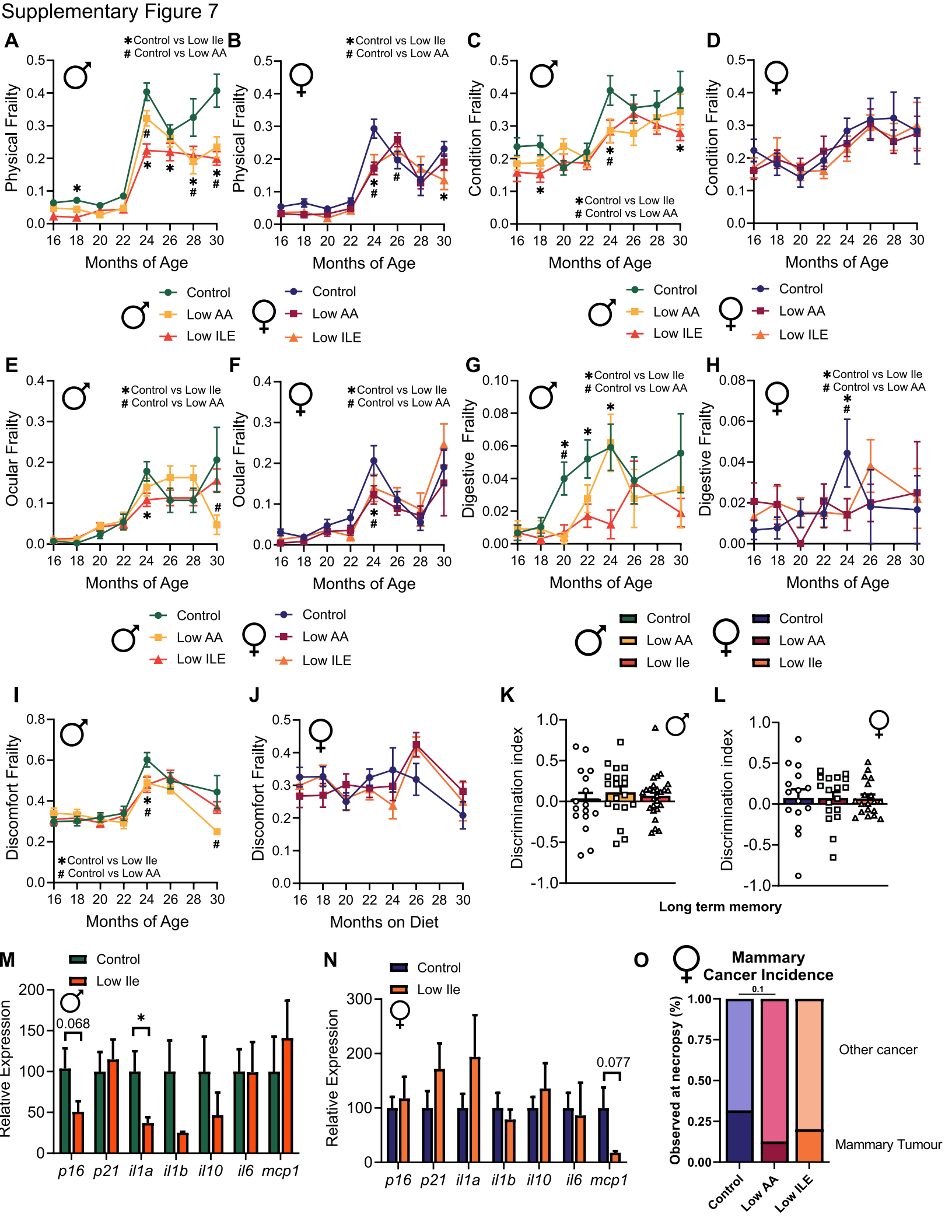
Supplementary Figure 6



Supplementary Figure 6: A low isoleucine diet induces molecular changes in male and female mice.

(A) Enriched KEGG pathways from transcriptional analysis of female mice (n=4-8 mice/group). (B) Enriched pathways from metabolomics analysis of Control-fed and Low Ile-fed 24-month-old male mice (n=6-8 mice/group). (C) Plasma isoleucine levels in old male and female mice. n=7-8 mice/group; significance between contrasts (Control vs Low AA and Control vs Low Ile) analyzed using Empirical Bayes Moderated Linear model, *=$p<0.05$. Two-way ANOVA for Diet and Sex. (D) Hepatic isoleucine levels in young male and female mice. n=6-8 mice/group; significance between contrasts (Control vs Low AA and Control vs Low Ile) analyzed using Empirical Bayes Moderated Linear model. Two-way ANOVA for Diet and Sex. (E) Hepatic isoleucine levels in old male and female mice. n=6-8 mice/group; significance between contrasts (Control vs Low AA and Control vs Low Ile) analyzed using Empirical Bayes Moderated Linear model. Two-way ANOVA for Diet and Sex. (F) Hierarchical clustering of the top 50 most significantly altered lipids between Old Control Males vs Low Ile, n=7-8 mice/group. Venn diagram show overlap of significantly altered lipids between Low AA and Low Ile fed mice vs Control fed for each age/diet (unadjusted $p<0.05$). (G) Hierarchical clustering of top 50 most significantly altered lipids between Old Control Females vs Low Ile, n=6-8 mice/group. Venn diagram show overlap of significantly altered lipids between Low AA and Low Ile fed mice vs Control fed for each age/diet (unadjusted $p<0.05$). (H) Spearman's rank order correlation matrix of phenotypic, transcriptomic, metabolomic and lipidomic changes across young female Control vs Low Ile mice. Hierarchical clustering identified 7 mega-clusters (outlined in black; Table S). N=6-8 mice/group. (I) Spearman's rank order correlation matrix of phenotypic, transcriptomic, metabolomic and lipidomic changes across old female Control vs Low Ile mice. Hierarchical clustering identified 6 mega-clusters (outlined in black; Table S). N=6-8 mice/group. (J) Log_2 fold changes of genes from KEGG Longevity regulating pathway that were significantly changed across old and young female mice on Low AA or Low Ile diets vs Control fed mice, n=4-8 mice/group. Related to **Figure 6** and **Supplementary Tables 3-5**.

Supplementary Figure 7



Supplementary Figure 7: A low isoleucine diet reduces frailty in male and female mice at specific timepoints but does not impact memory or senescence signaling.

(A-J) Disseminated frailty scores over time for male and female mice (n varies by month; max n=44-49 mice/group). (A-B) Physical frailty, (C-D) Body condition frailty, (E-F) Ocular frailty, (G-H) Digestive frailty, (I-J) Mouse discomfort. (A-J) Two-way ANOVA for Diet and Time with post-hoc Tukey multiple comparison test, **P*<0.05 Control vs Low Ile fed, #*P*<0.05 Control vs Low AA. (K-L) Novel object recognition of long-term memory in male (K) and female (L) mice (n=15-26). One-way ANOVA for Diet with post-hoc Dunnet's multiple comparison test. (M-N) Hepatic expression levels of senescence genes in 24-month-old male (M) and female (N) mice. n=5-8 mice/group; Unpaired t-tests, **P*<0.05, ***P*<0.01, ****P*<0.001 and *****P*<0.0001. Two-sided chi-squared test, **P*<0.05. (O) Prevalence of mammary cancer at necropsy in female mice (n=19-24 mice/group). Two-sided chi-squared test, **P*<0.05. Data represented as mean ± SEM. Related to **Figure 7**.

Supplemental Tables

Supplementary Table 1: Diet Composition.

Diet composition and calorie content for diets used in this study. Related to STAR METHODS Experimental Model and Subject Details.

Supplementary Table 2: The relationship between isoleucine intake and phenotypic changes is dependent on sex and age.

R values for correlations from **Figure 5A**. Related to **Figure 5**.

Supplementary Table 3: Changes in the hepatic transcriptome on Low AA and Low Ile diets are dependent on age and sex.

(A) Hepatic Log^2 fold-changes and related p-values for comparisons of interest from transcriptomic analysis in males. **(B)** Significant changes at each omics level (adjusted p-value<0.01 for transcriptomics, p-value=0.05 for metabolomics and lipidomics). **(C)** Hepatic Log_2 fold-changes and related p-values for comparisons of interest from transcriptomic analysis in females. **(D)** KEGG enriched pathways for significant genes found in **Tables S3A and S3C**. Related to **Figure 6** and **Supplementary Figure 6**.

Supplementary Table 4: Changes in the hepatic and circulating metabolome are dependent of age, diet and sex.

(A) Hepatic Log^2 fold-changes and related p-values for comparisons of interest from metabolomic analysis in males. **(B)** KEGG enriched pathways for metabolites found in **Tables S4A and S4C**. **(C)** Hepatic Log_2 fold-changes and related p-values for comparisons of interest from metabolomic analysis in females. **(D)** Plasma Log_2 fold-changes and related p-values for comparisons of interest from amino acid metabolomics analysis in 24 month old males and females. Related to **Figure 6** and **Supplementary Figure 6**.

Supplementary Table 5: Changes in the hepatic lipidome on Low AA and Low Ile diets are dependent on age and sex.

(A) Hepatic Log^2 fold-changes and related p-values for comparisons of interest from lipidomic analysis in males. **(B)** Hepatic Log_2 fold-changes and related p-values for comparisons of interest from lipidomic analysis in females. **(C)** LION Lipid Ontology enrichment for pathways of significant lipids (p<0.05) for each of the age/sex/diet groups. **(D)** Concatenated data for coexpression

network for Figure 6F and G, Supplementary Figures 6E and F. Related to **Figure 6** and **Supplementary Figure 6**.

Supplementary Table 6: A low isoleucine diet increases median lifespan in both sexes.

(A) Lifespan statistics for **Figure 7Q-T**. **(B)** Number of mice for each study cohort. Related to STAR METHODS Experimental Model and Subject Details. **(C)** Lifespan of all, including censored, mice. Related to **Figure 7**.

Supplementary Table 7: Primer sequences.

Primer sequences for each gene measured by qPCR. Related to STAR METHODS Quantitative PCR.