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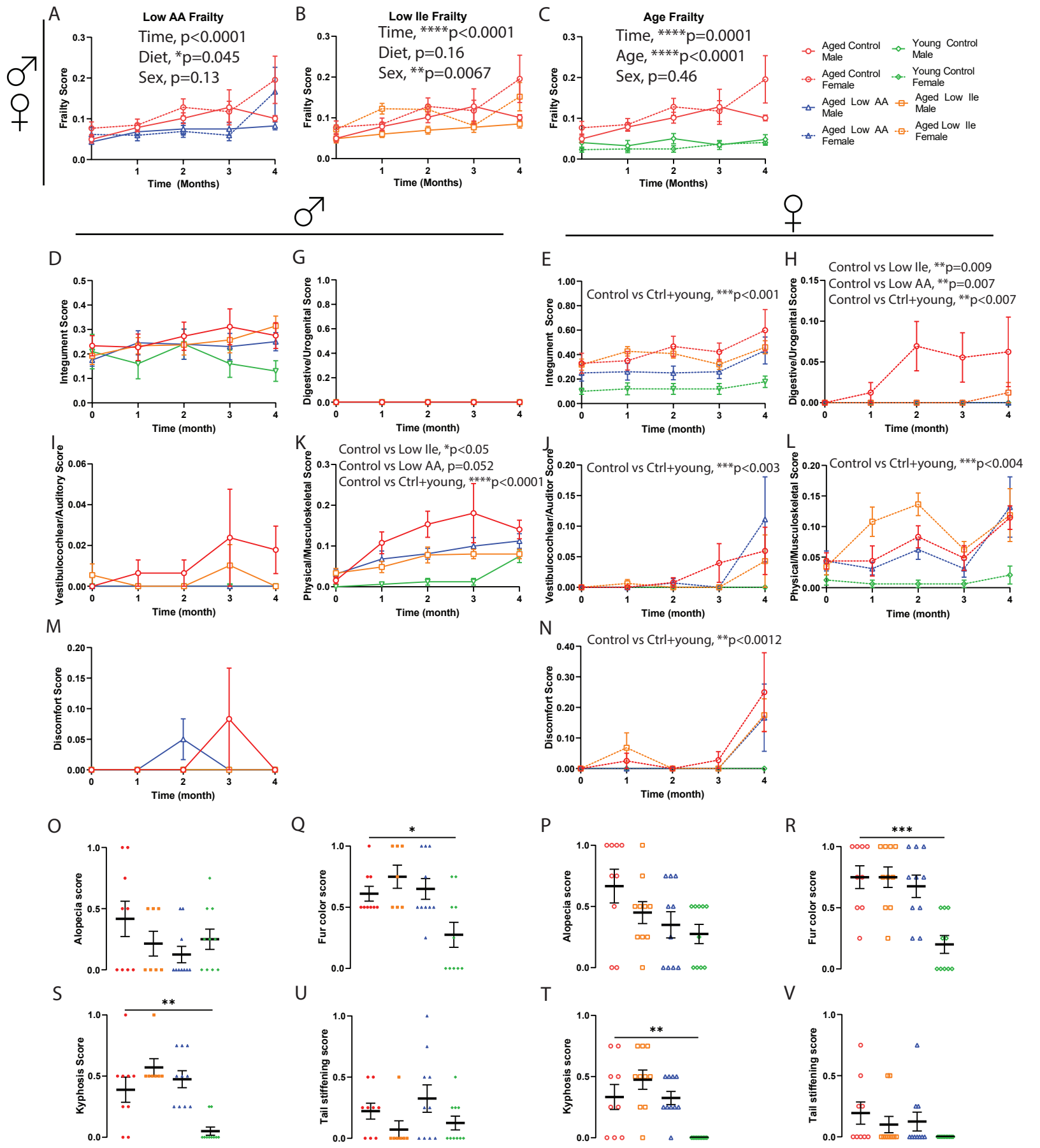
Supplemental Figure 1.



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Supplemental Figure 1. Frailty data 3-way ANOVA analysis and subcategories.

(A-C) Three-way mixed-effects analysis of the frailty data as separated by the indicated factors. At the beginning of the experiments n=10-13/group; p-values represent the overall effect of time, diet, and sex. **(D-N)** Subcategory averages of the frailty data. n=10-13/group; p-values represent result of the 2-way mixed-effects analysis. **(O-V)** Selected individual frailty categories, presented as the average of 3- and 4-month scores. At the beginning of the experiments n=10-13/group, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ANOVA followed by Dunnett's test. Data presented as mean ± SEM.

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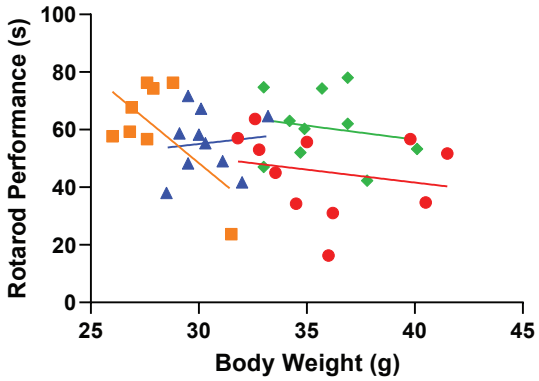
Supplemental Figure 2.

Aged Control Aged Low Ile Aged Low AA Young Control

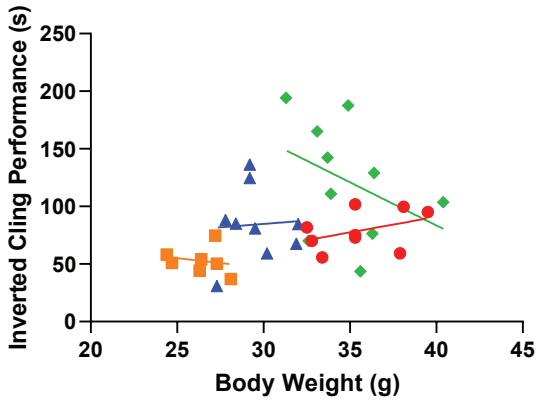
♂

A



Male group comparisons	Slope		Elevation	
Control vs	p-value	Significance	p-value	Significance
Low Ile	0.17	No	0.83	No
Low AA	0.62	No	0.47	No
Young Ctrl	0.97	No	0.02	Yes

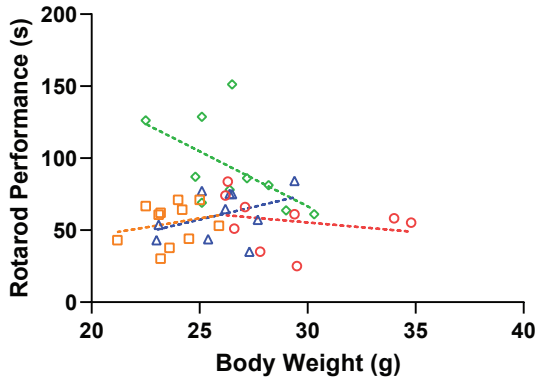
B



Male group comparisons	Slope		Elevation	
Control vs	p-value	Significance	p-value	Significance
Low Ile	0.40	No	0.66	No
Low AA	0.83	No	0.41	No
Young Ctrl	0.19	No	0.04	Yes

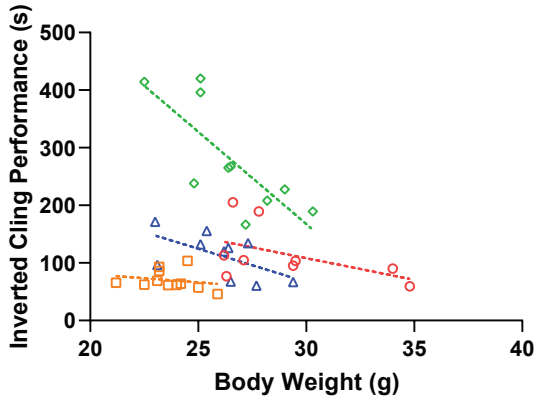
♀

C



Male group comparisons	Slope		Elevation	
Control vs	p-value	Significance	p-value	Significance
Low Ile	0.41	No	0.63	No
Low AA	0.19	No	0.64	No
Young Ctrl	0.17	No	0.04	Yes

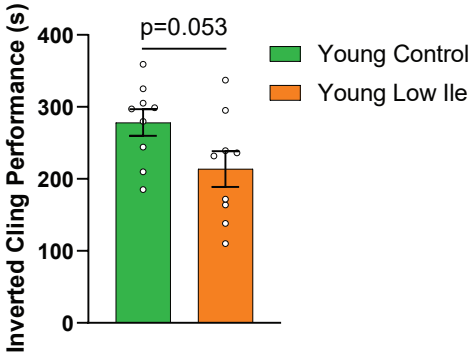
D



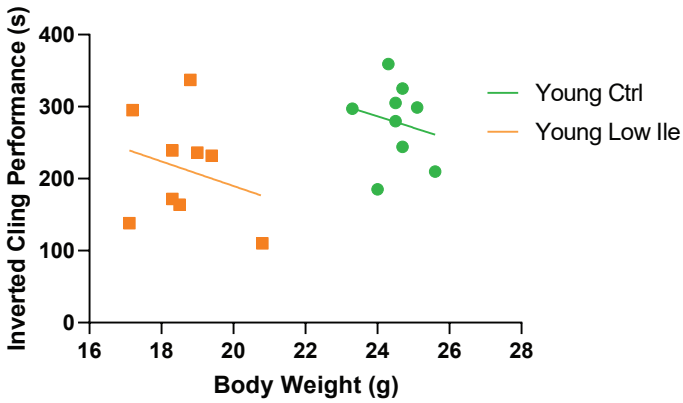
Male group comparisons	Slope		Elevation	
Control vs	p-value	Significance	p-value	Significance
Low Ile	0.61	No	0.002	Yes
Low AA	0.62	No	0.19	No
Young Ctrl	0.03	Yes	N/A	N/A

♂

E



F



Male group comparisons	Slope		Elevation	
Young Control vs	p-value	Significance	p-value	Significance
Young Low Ile	0.98	No	0.16	No

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Supplemental Figure 2. ANCOVA analysis of rotarod and inverted cling assay performance.

(A-D) Rotarod and inverted cling performance as a function of body weight (n=8-11/group, slopes and intercepts were calculated using ANCOVA). **(E-F)** Young 3-month old male mice were fed either a Control or a Low Ile diet for at least 1 month before inverted cling assay (E), and inverted cling performance as a function of body weight (n=9/group, *p<0.05, t-test (E); slopes and intercepts were calculated using ANCOVA (F)). Data presented as mean ± SEM.

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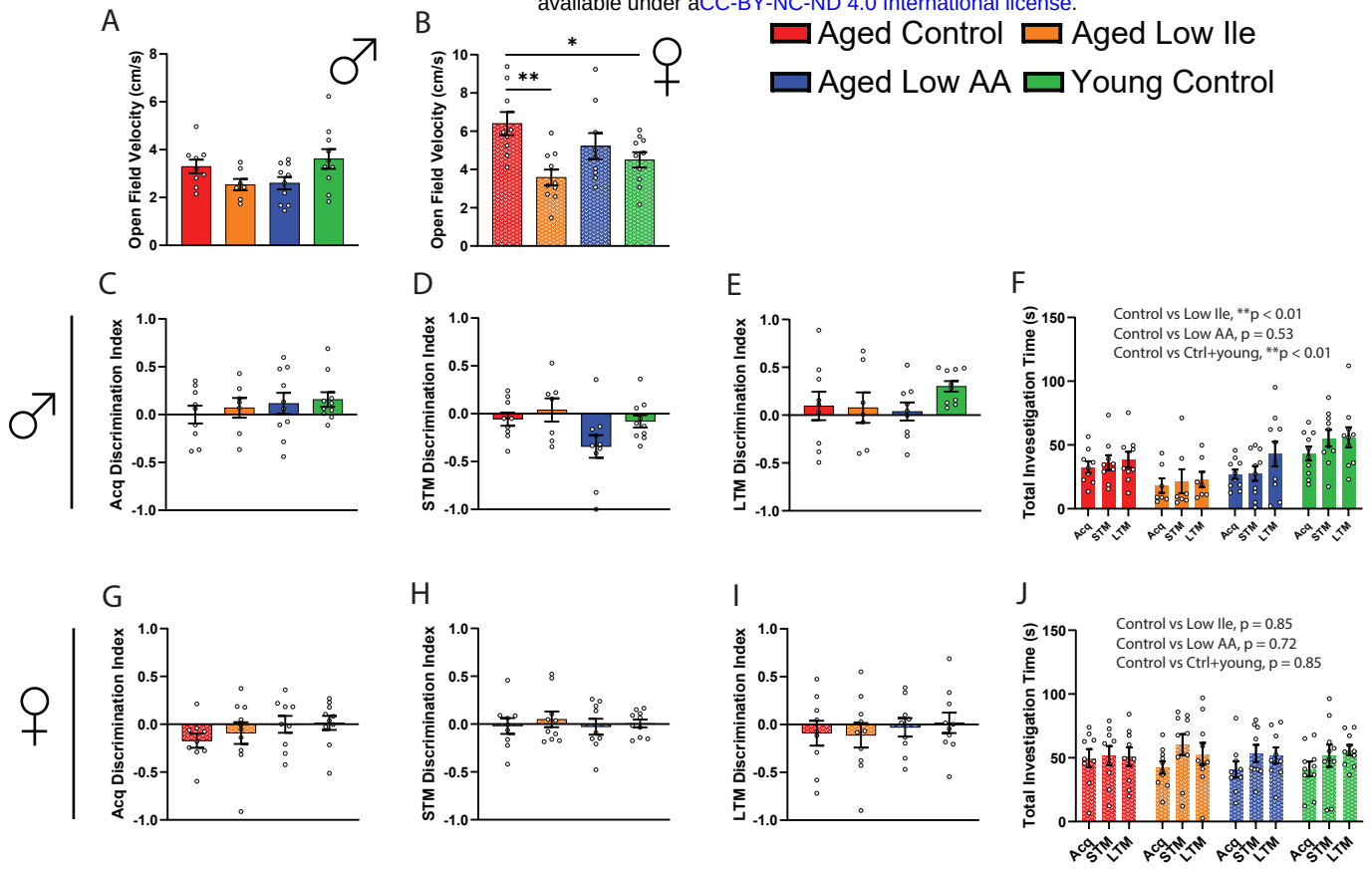
Supplemental Figure 3



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Supplemental Figure 3. Open field and novel object recognition test.

(A-B) Male (A) and female (B) mice in open field test. **(C-F)** Male mice novel object recognition test discrimination index in the acquisition phase (C), the short-term memory test (D), and the long-term memory test (E). (F) total investigation time in each trial. **(G-J)** Female mice novel object recognition test discrimination index in the acquisition phase (G), the short-term memory test (H), and the long-term memory test (I). (J) total investigation time in each trial. (A-E, G-I) n=7-10/group, *p<0.05, **p<0.01, ANOVA followed by Dunnett's test. (F, J) n=7-10/group, p-values represent the main effect of diet from the indicated 2-way ANOVA. Data represented as mean ± SEM.

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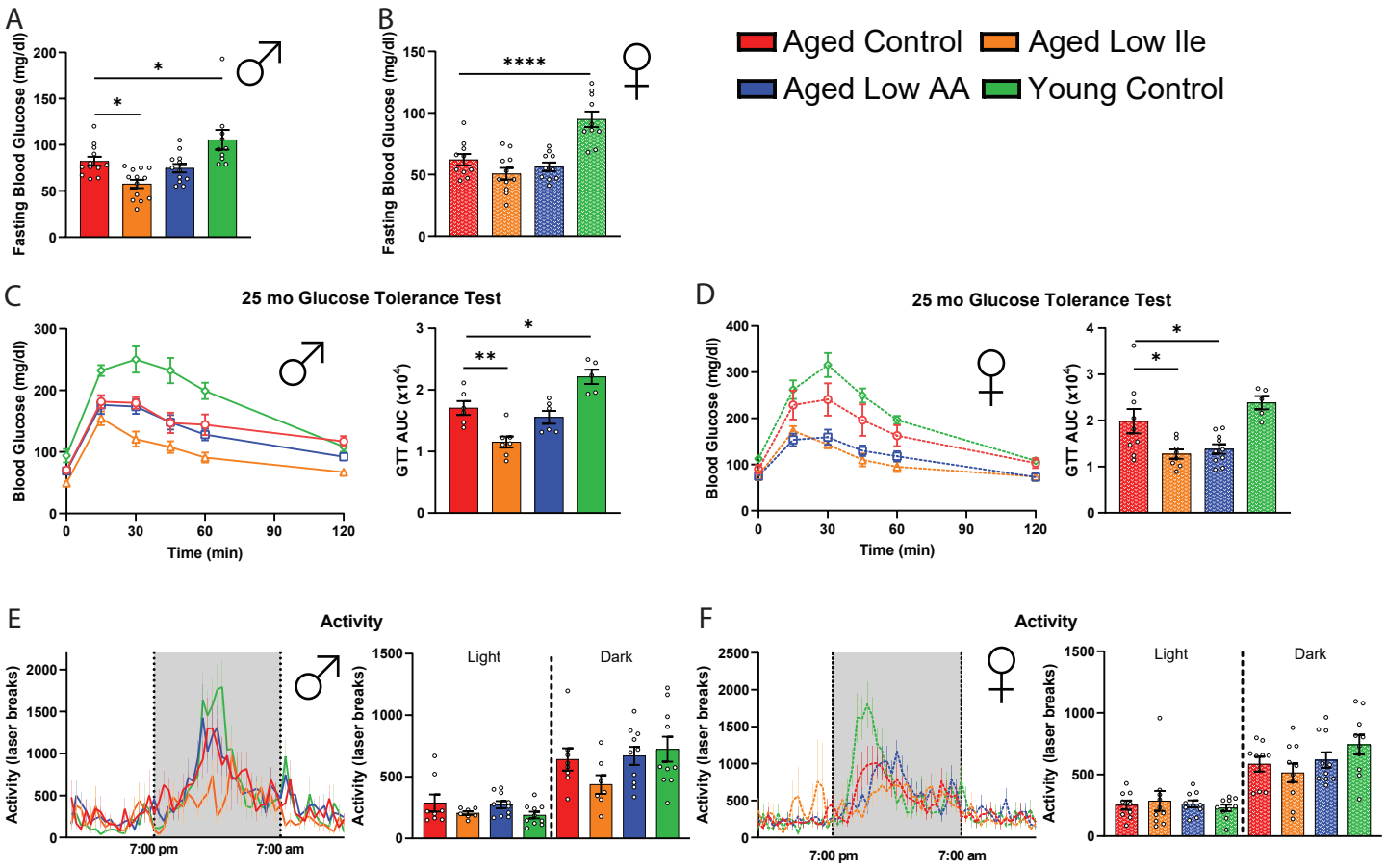
Supplemental Figure 4.



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Supplemental Figure 4. Effects of late-life Low AA and Low Ile diets on glycemic control and activity.

(A-B) Fasting blood glucose of 21-month-old male (A) and female (B) mice after 3 weeks on the indicated diets. n=10-13/group. **(C-D)** Glucose tolerance of 25-month old male (C) and female (D) mice after 3 weeks on the indicated diets. n=5-10/group. **(E-F)** Spontaneous activity of male (E) and female (F) mice during the metabolic chambers experiments shown in Fig. 3. n=7-10/group. (A-F) *p<0.05, **p<0.01, ****p<0.0001, ANOVA followed by Dunnett's test. Data represented as mean ± SEM.

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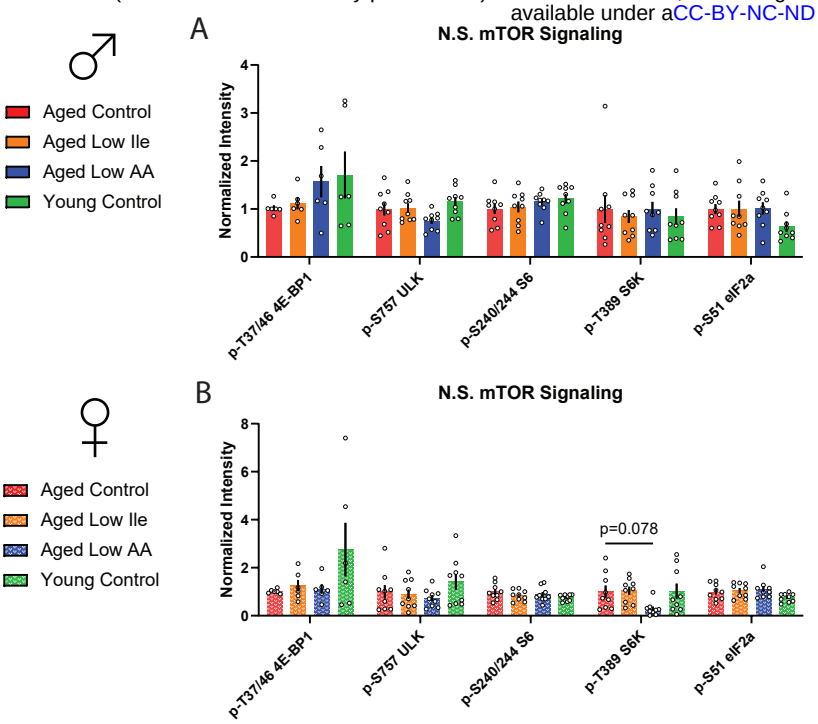
Supplemental Fig. 5



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Supplemental Figure 5. Non-significantly altered aging rate indicators in the aged mice liver.

(A) Proteins not significantly altered by either diet or age in the livers of male mice. **(B)** Proteins not significantly altered by either diet or age in the livers of female mice. n=6-9/group, *p<0.05, ANOVA followed by Dunnett's test. Data presented as mean ± SEM.

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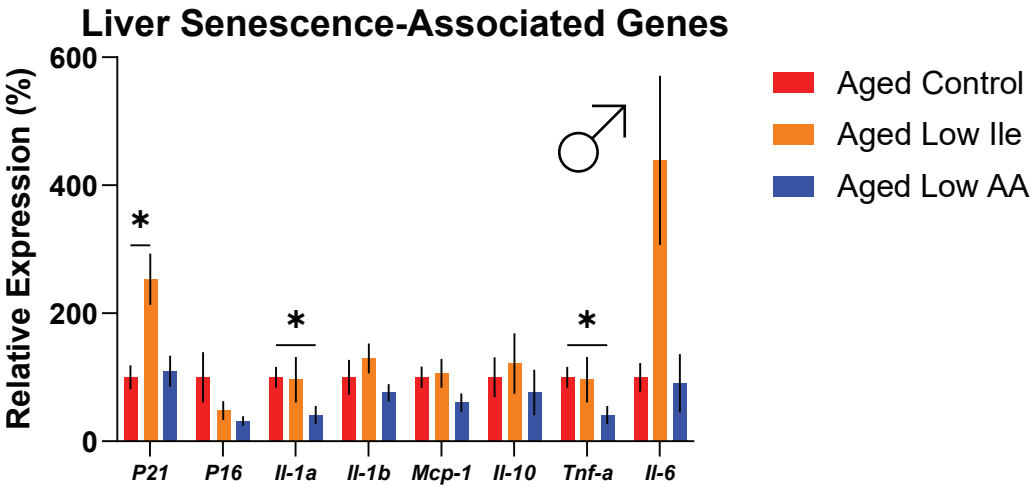
Supplemental Figure 6.



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Supplemental Figure 6. Expression analysis of senescence markers in the aged male liver.

Expression of the indicated genes in the livers of 20-month-old mice on the indicated diets for 4 months was determined by qPCR. n=5-8/group, *p<0.05, ANOVA followed by Dunnett's test. Data presented as mean ± SEM.

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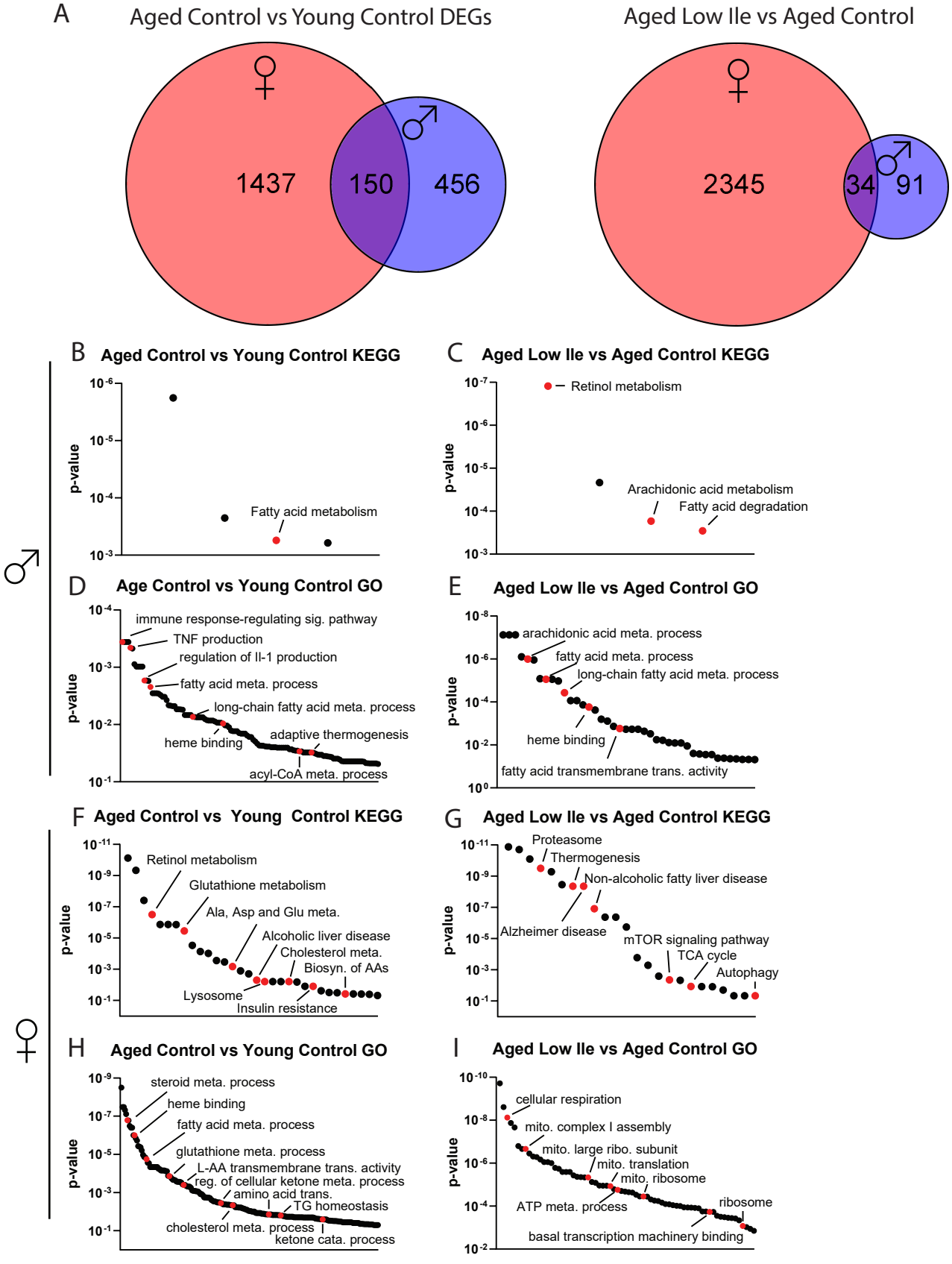
Supplemental Figure 7.



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Supplemental Figure 7. Venn diagram and enrichment analysis of differentially expressed hepatic genes.

(A) Venn diagram showing the number of overlapping and non-overlapping DEGs between male and females. **(B-C)** Significantly enriched KEGG pathways by age (B) and diet (C) in male mice. **(D-E)** Significantly enriched GO terms by age (D) and diet (E) in male mice. **(F-G)** Significantly enriched KEGG pathways by age (F) and diet (G) in female mice. **(H-I)** Significantly enriched GO terms by age (H) and diet (I) in female mice. Transcriptomic analysis, n=5-6/group.

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Supplemental Data Table. Spreadsheet containing significantly altered heart lipid species, top 50 liver DEG, significant liver KEGG and GO pathways.