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Supplementary Materials for

Rapamycin treatment during development extends life span and health span of male mice and *Daphnia magna*

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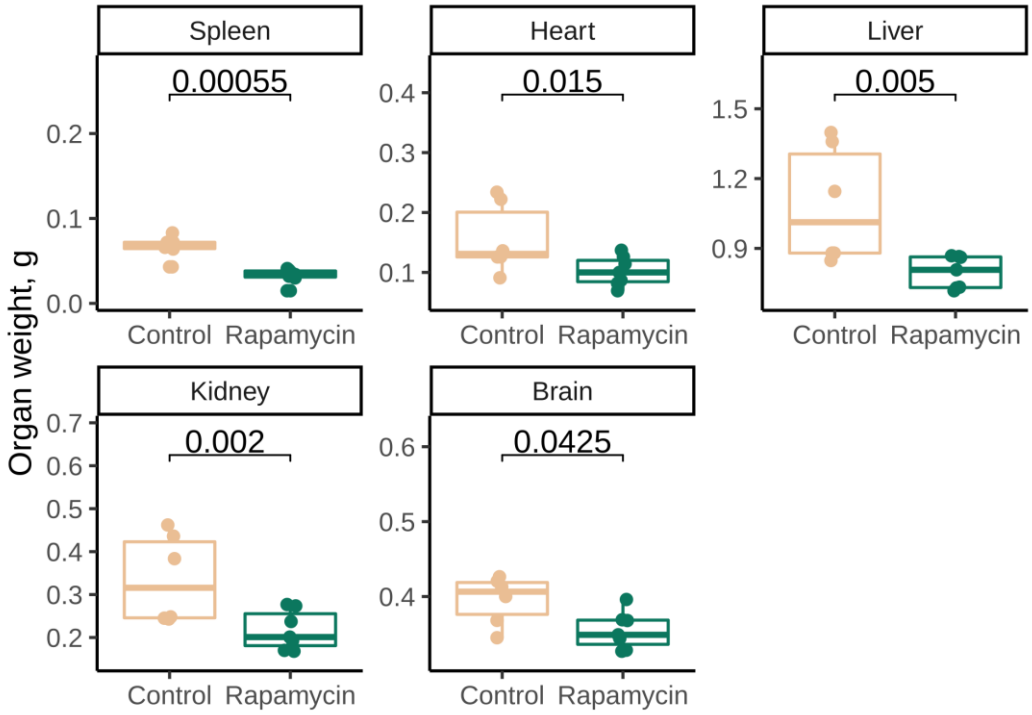
This PDF file includes:

Figs. S1 to S7
Legends for tables S1 to S5

Other Supplementary Material for this manuscript includes the following:

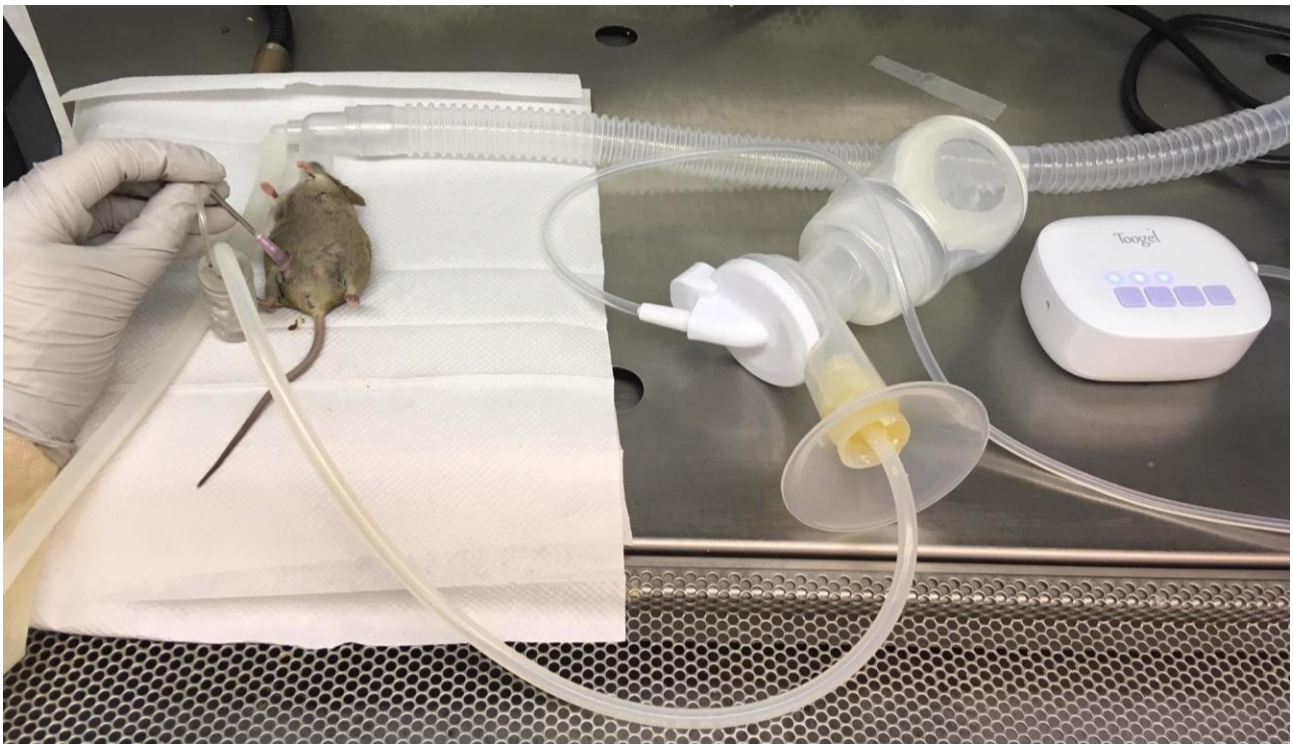
Tables S1 to S5

Supplementary Figure S1



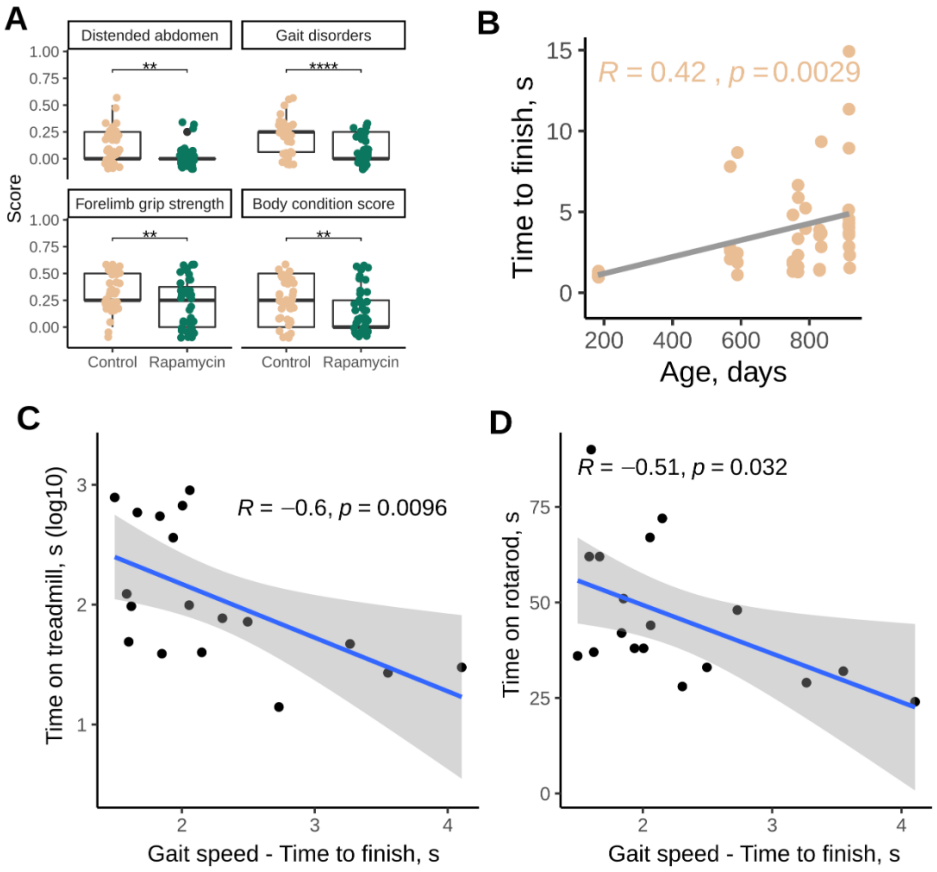
Supplementary Figure S1. Weight of organs of 51-day-old male and female UMHET3 mice subjected to rapamycin or control diets from birth to 45 days of age. P-values are calculated with ANCOVA with sex as a covariate and are FDR-adjusted for multiple testing.

Supplementary Figure S2



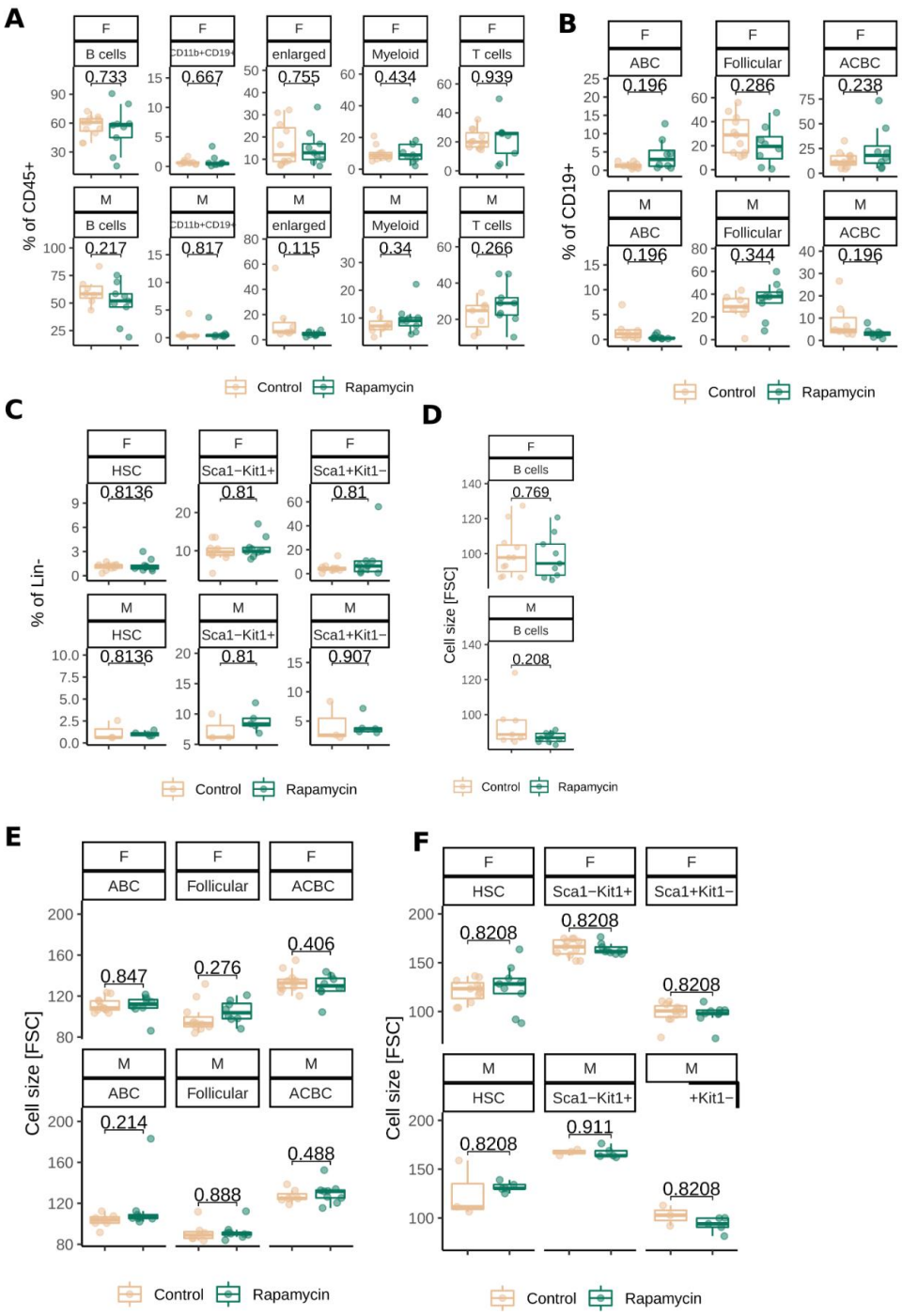
Supplementary Figure S2. Photo of the custom setup for milk collection from mice.

Supplementary Figure S3



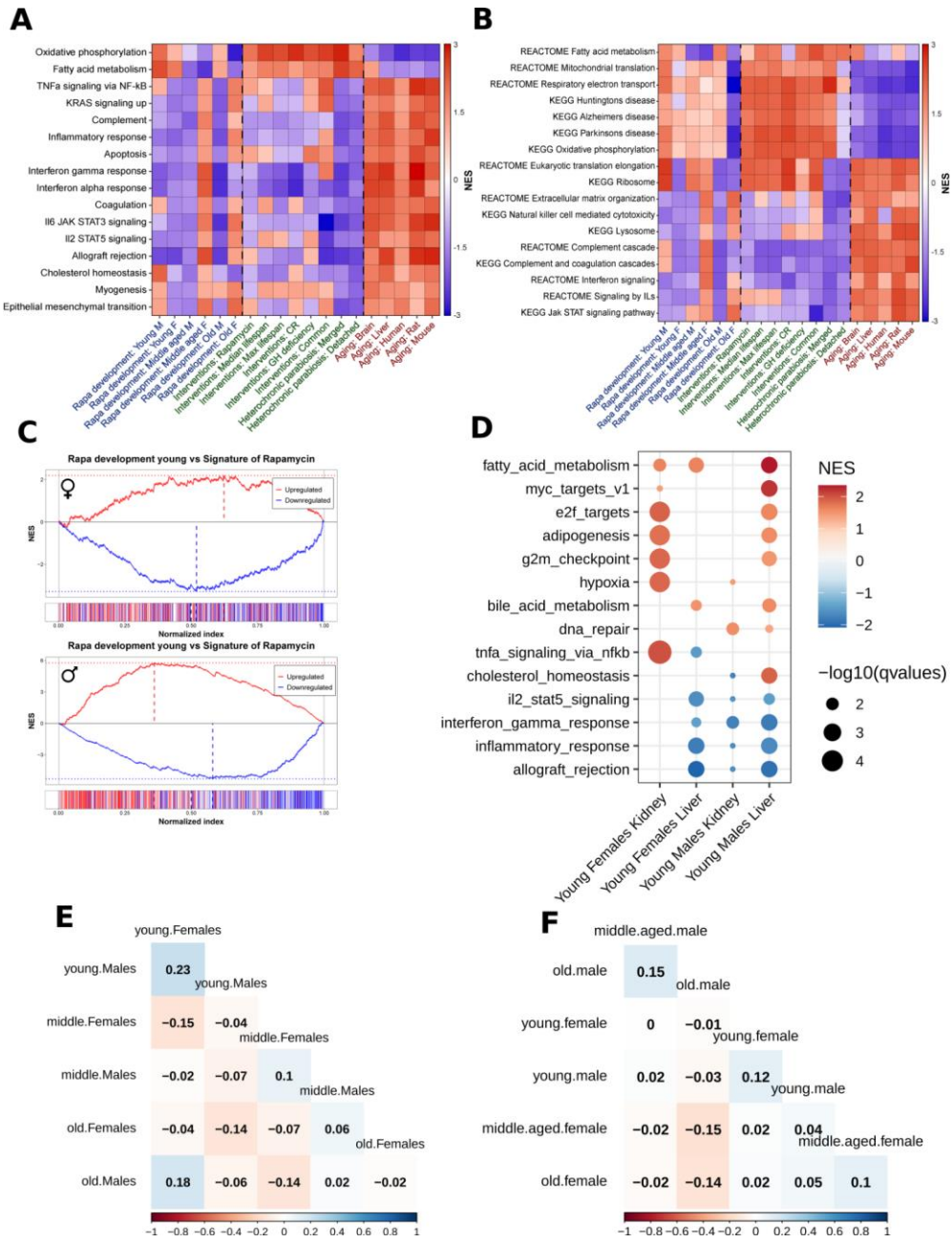
Supplementary Figure S3. Rapamycin treatment during development improved the health of aged mice based on gait speed and frailty index. (A) Frailty index features significantly affected by rapamycin treatment. P-value is calculated with repeated measures ANOVA test. (B) Correlation between median time to finish a run and age in UMHET3 mice. (C) Correlation between the time mice spent on treadmill or (D) rotarod and time to finish in the gate speed assay. Correlations were evaluated using Pearson's correlation coefficient.

Supplementary Figure S4



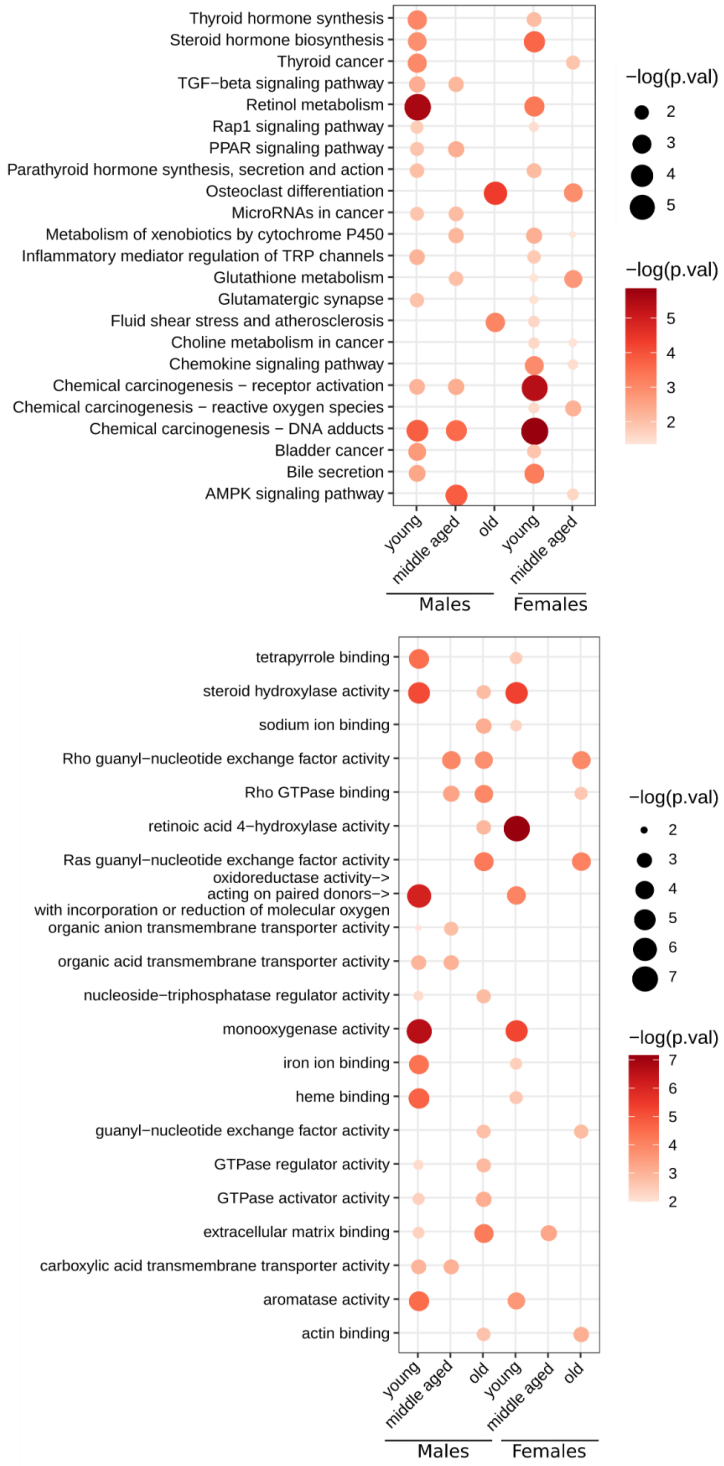
Supplementary Figure S4. Immune cell composition and cell sizes of 21-29-day-old UMHET3 mice. P-values are calculated with repeated measures ANOVA test.

Supplementary Figure S5



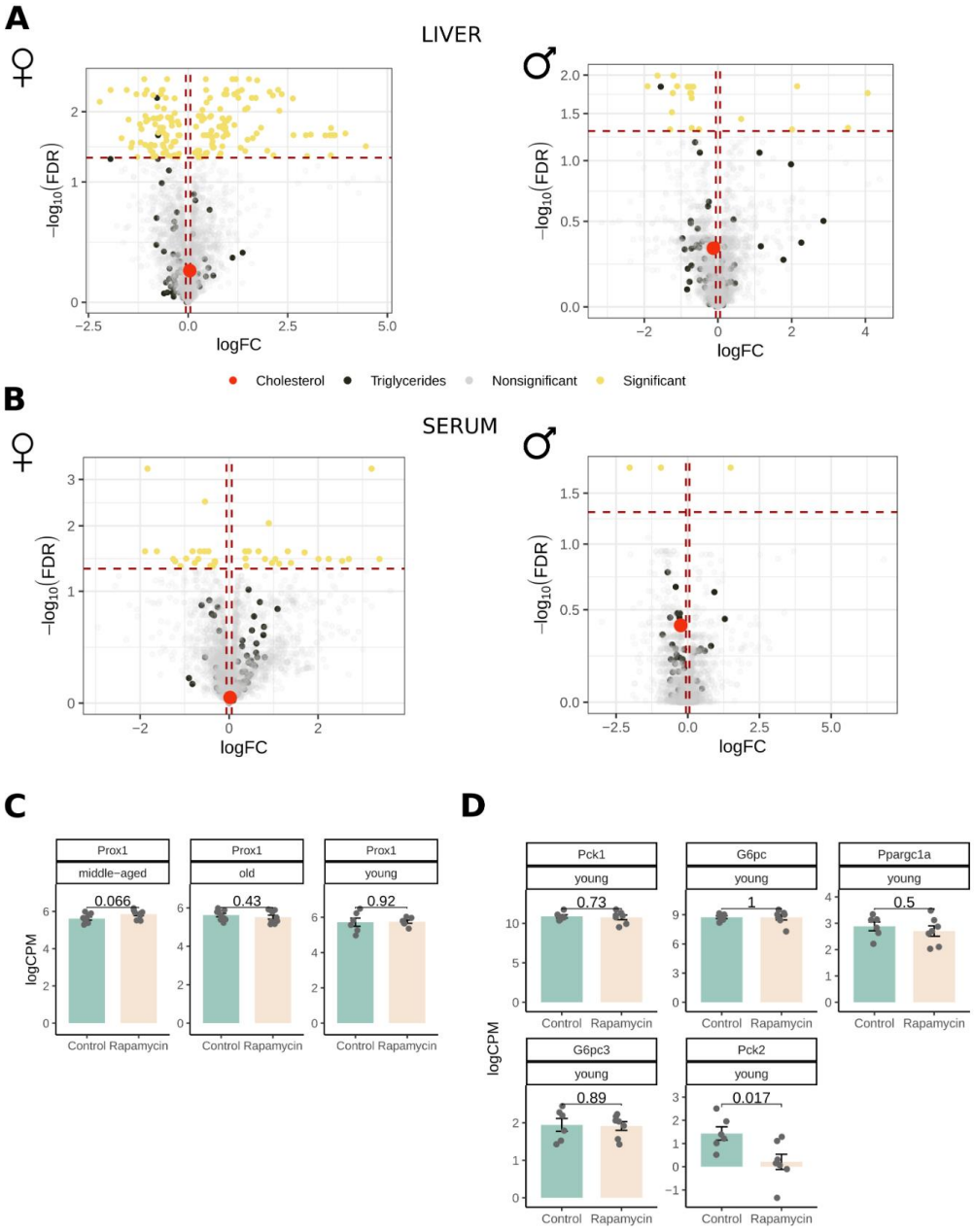
Supplementary Figure S5. Rapamycin treatment during development remodels the epigenome of liver and kidney. (A) Number of differentially expressed genes (DEGs, defined as FDR-adjusted p < 0.1). (B) and (C) Functional enrichment analyses of gene expression signatures and EL rapamycin treatment across age groups and sexes. Only functions significantly associated with at least one signature (q-value < 0.1) are shown. Cells are colored based on the normalized enrichment score (NES). (D) Gene set enrichment plots of young males and females against the signature of rapamycin. (E) Correlation matrix between ranks for gene expression and (F) CpG sites. Numbers displayed are spearman correlation coefficients.

Supplementary Figure S6



Supplementary Figure S6. KEGG and GO enrichment of genes affected in both gene expression and DNA methylation in treated animals compared to controls. Such genes from treated old females were not enriched in any of KEGG terms.

Supplementary Figure S7



Supplementary Figure S7. Rapamycin treatment during development has limited impact on liver and serum lipidome and on expression of genes involved in gluconeogenesis. (A) Volcano plots for lipids changing their levels in response to EL rapamycin treatment in the liver or (B) serum. Each dot is a lipid. Triglycerides are shown as black dots, and cholesterol is shown as a red dot. Horizontal red line is a cut-off for significant changes (FDR<0.05). Yellow dots are statistically significant changes. (C) LogCPM of Prox1 gene measured by RNA-seq in the liver. P-values are calculated with a two-sided Student t-test. (D) LogCPM of genes from gluconeogenesis pathways measured by RNA-seq in the liver. P-values are calculated with a two-sided Student t-test.

Supplementary Table 1. End of life pathologies of mice treated with rapamycin during development and controls.
Supplementary Table 2. Differentially methylated CpG sites in the livers of mice treated with rapamycin during development compared to controls.
Supplementary Table 3. DEGs in the liver of mice treated with rapamycin during development compared to controls.
Supplementary Table 4. DEGs in the kidney of mice treated with rapamycin during development compared to controls.
Supplementary Table 5. Differentially present lipids in 20-22 months old mice treated with rapamycin during development compared to controls.