

FIGURE 1 - Supplement 1. Methotrexate increases the size of yeast cells. Cell size histograms of yeast cells (BY4742 strain background) treated with the indicated doses of methotrexate. Size (in fL) is on the x-axis, and the cell number on the y-axis.

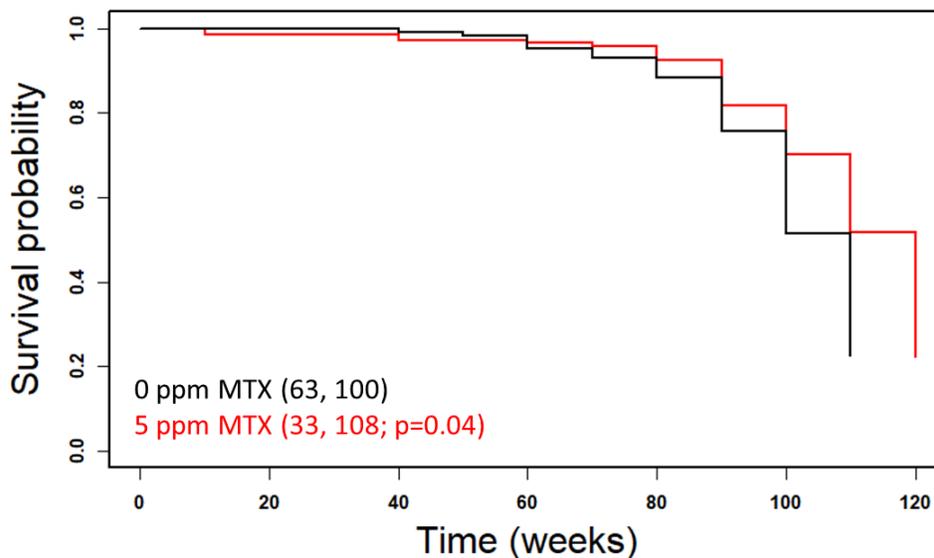


FIGURE 1 - Supplement 2. Survival curves of female Swiss mice on chronic, low-dose methotrexate (MTX). MTX was administered in the food at the indicated dose every other week, starting at 7 weeks of age. Raw data were from (Rustia and Shubik, 1973). Survival probability is on the y-axis, and time (in weeks) is on the x-axis. Mean lifespans and the number of animals assayed in each case are shown in parentheses. The indicated p value was based on the log-rank test.

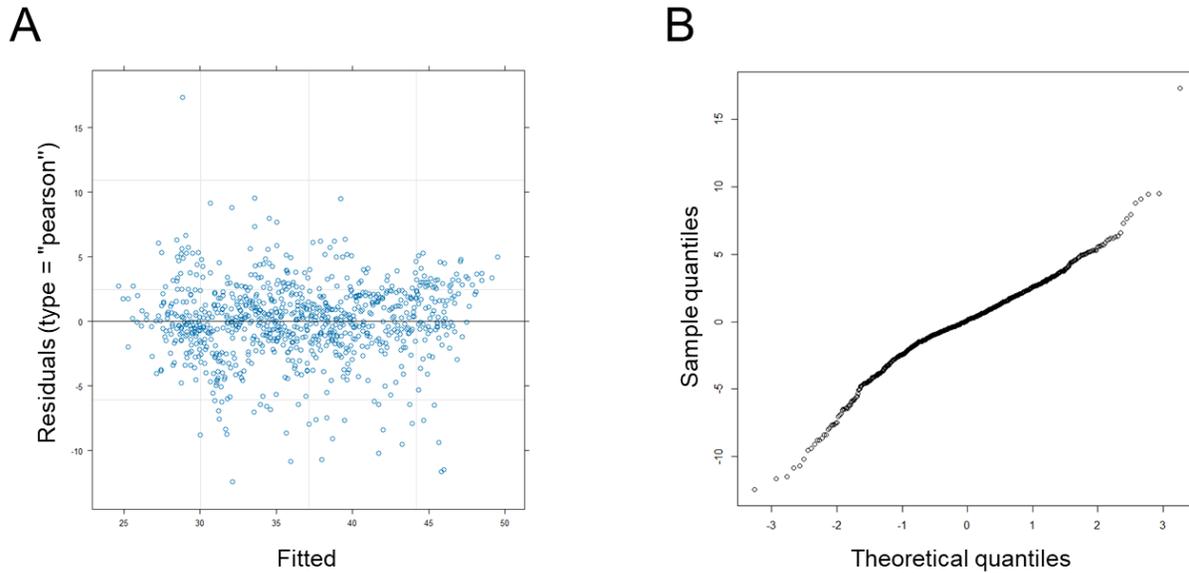


FIGURE 2 - Supplement 1. Evaluating assumptions of a linear regression model for longitudinal weight measurements. (A) Plot of the standardized residuals on the y-axis and the fitted values on the x-axis, from the data shown in Figure 2C. **(B)** Q-Q plot of the residuals, with the sample quantiles from the measurements in Figure 2C (y-axis), against the theoretical normally distributed ones (x-axis).

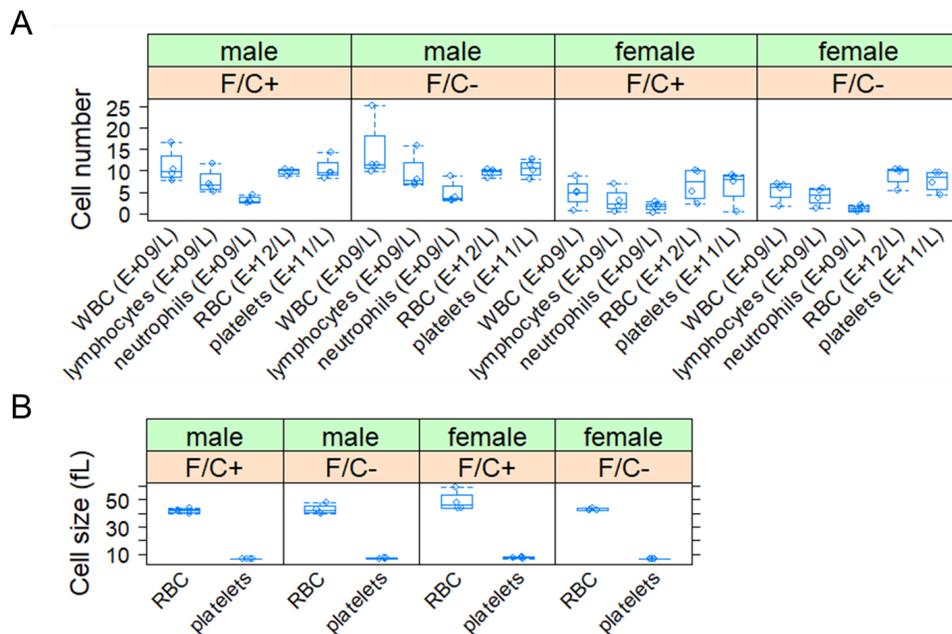


FIGURE 2 - Supplement 2. Number and size of blood cells from mice placed on dietary folate restriction late in life. (A) Blood cell numbers were measured at 108 weeks of age from animals of the indicated sex and diet group. **(B)** Cell size (y-axis; in fL) was measured from the same samples shown in (A) from animals of the indicated sex and diet group. The boxplots were drawn as in Figure 2.

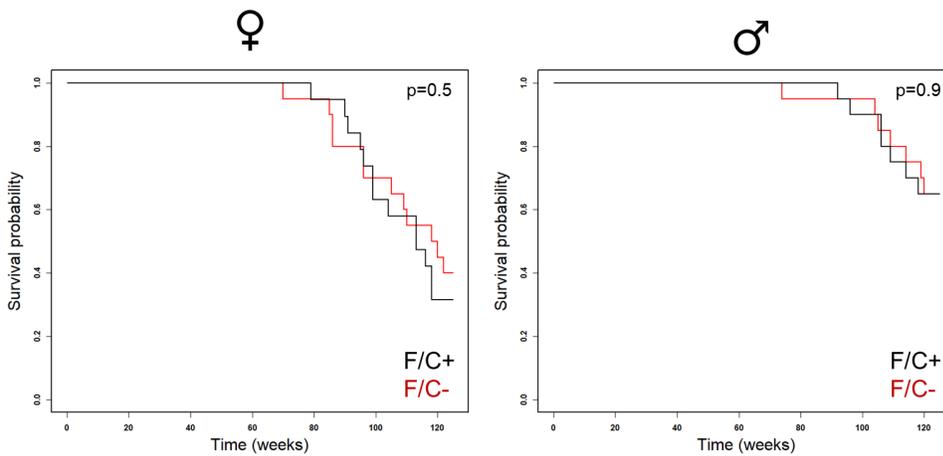


FIGURE 2 - Supplement 3. Survival curves of mice placed on dietary folate restriction late in life. Survival probability is on the y-axis, and time (in weeks) on the x-axis, from female (right panel) and male (left panel) animals of each diet test group. The indicated p value was based on the log-rank test.

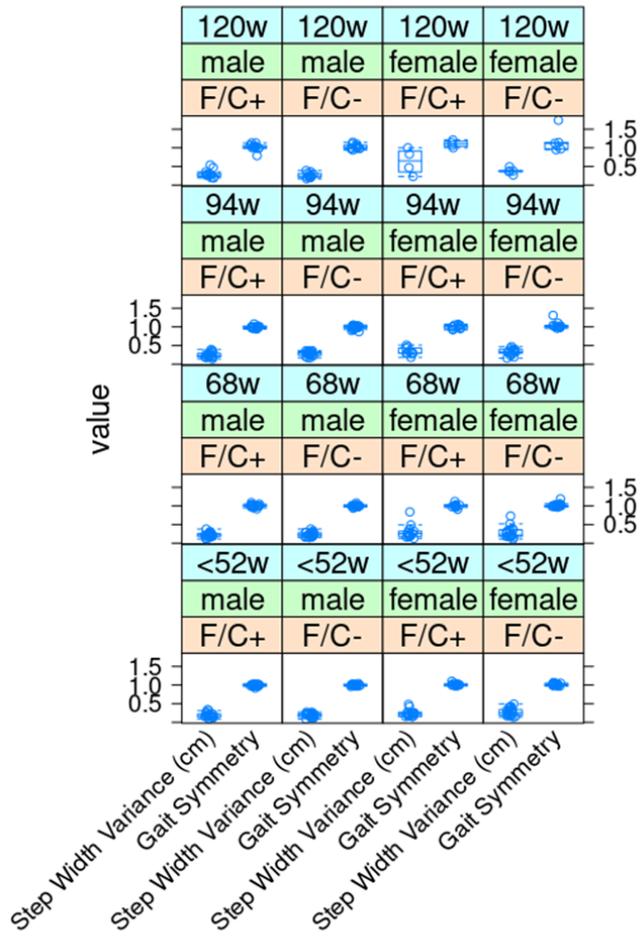


FIGURE 3 - Supplement 1. No significant gait changes in mice placed on dietary folate restriction late in life. As described in Materials and Methods, step width variance and gait symmetry values (shown on the y-axis) were measured with the Digigait system. As indicated, measurements were taken at the indicated times from female and male animals of each diet test group. The boxplots were drawn as in the previous figures.

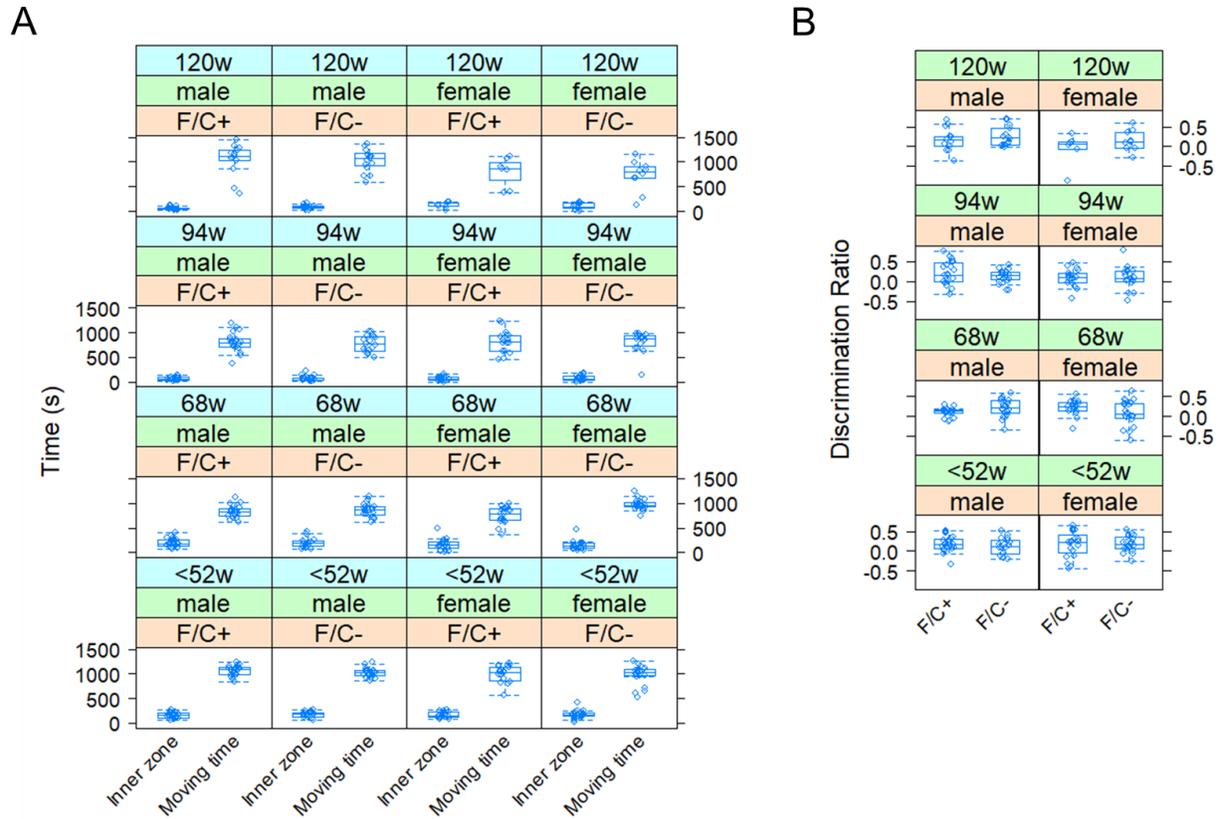


FIGURE 3 - Supplement 2. Open field (A) and novel object recognition (B) assays in mice placed on dietary folate restriction late in life. In (A), the inner zone and moving times during open field evaluation are on the y-axis. In (B), the discrimination ratio values, reflecting the ability of the mice to recognize a new object, are on the y-axis. Detailed descriptions of the assays are in Materials and Methods. The boxplots were drawn as in the previous figures.

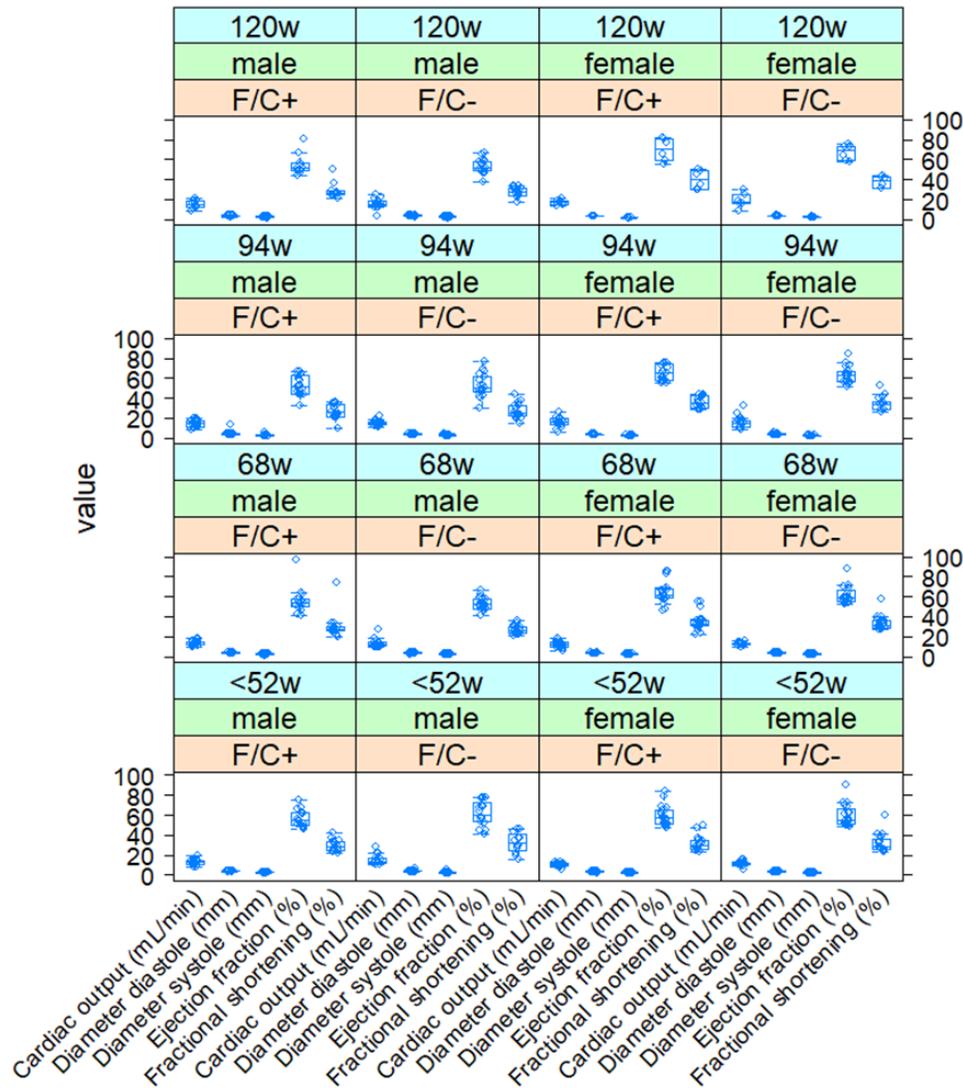


FIGURE 3 - Supplement 3. Normal cardiac function of mice placed on dietary folate restriction late in life. Several parameters of cardiac function (x-axis) were measured by echocardiography as described in Materials and Methods, and the corresponding values are on the y-axis. Measurements were taken at the indicated times from female and male animals of each diet test group, as indicated. The boxplots were drawn as in the previous figures.

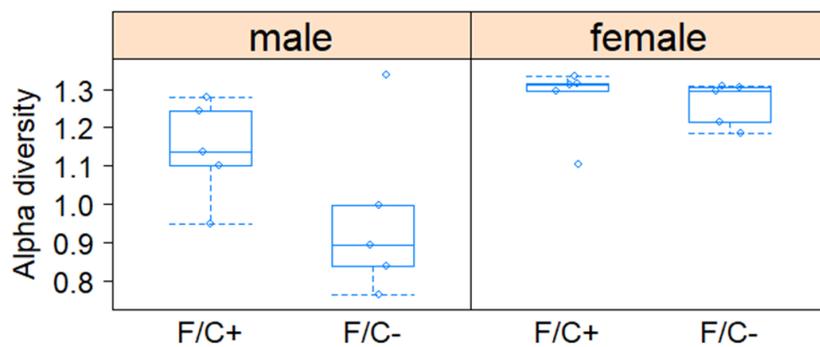


FIGURE 5 - Supplement 1. Species diversity within the gut microbiome of mice placed on dietary folate restriction late in life. Shannon's diversity index (y-axis) is shown for each sex and diet as a metric of the alpha diversity of the fecal microbiome sampled and sequenced at 90 weeks of age, from 5 mice in each test group. The boxplots were drawn as in the previous figures.

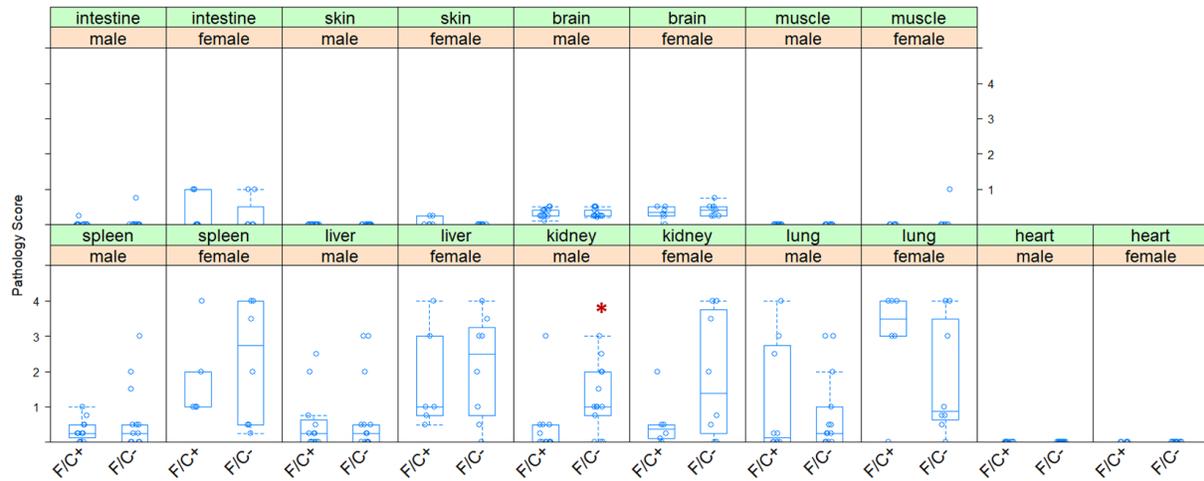


FIGURE 6 - Supplement 1. Disease load of mice placed on dietary folate restriction late in life. The pathology of the indicated tissues, collected at 120 weeks of age, was scored on a 0-4 scale (with 4 reflecting the highest degree of pathological changes; see Materials and Methods) and shown on the y-axis. The different diets are on the x-axis. Differences in kidney abnormalities in male mice were significant (indicated with a red asterisk; $p=0.0165$, based on the Wilcoxon rank sum test). The boxplots were drawn as in the previous figures.

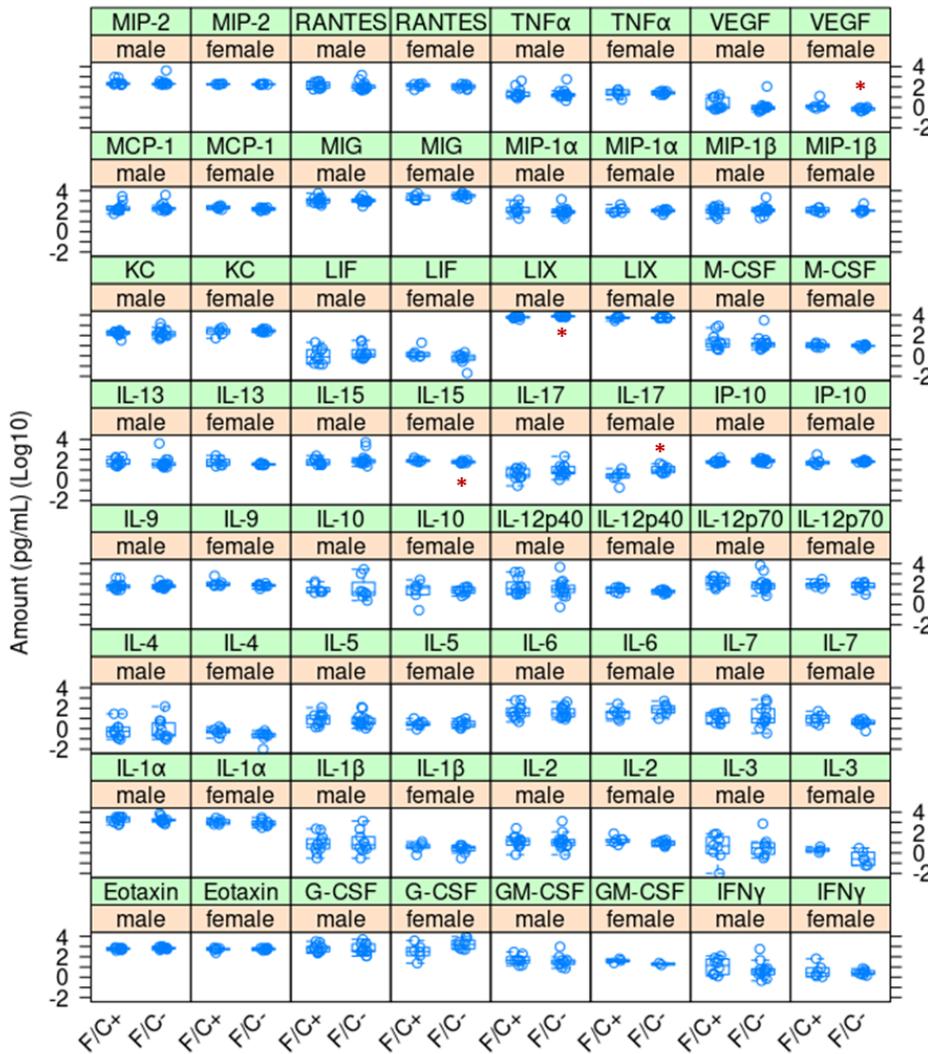


FIGURE 6 - Supplement 2. Cytokine levels of mice placed on dietary folate restriction late in life. Serum cytokine levels were measured at 120 weeks of age using a mouse multiplex cytokine assay service by Eve Technologies (see Materials and Methods). The measured amounts (in pg/mL; Log10-transformed) are on the y-axis. The different diets are on the x-axis for each cytokine, as indicated. Significant differences in the measured values within a sex and diet group are indicated with a red asterisk ($p < 0.05$, based on the Wilcoxon rank sum test). The boxplots were drawn as in the previous figures.

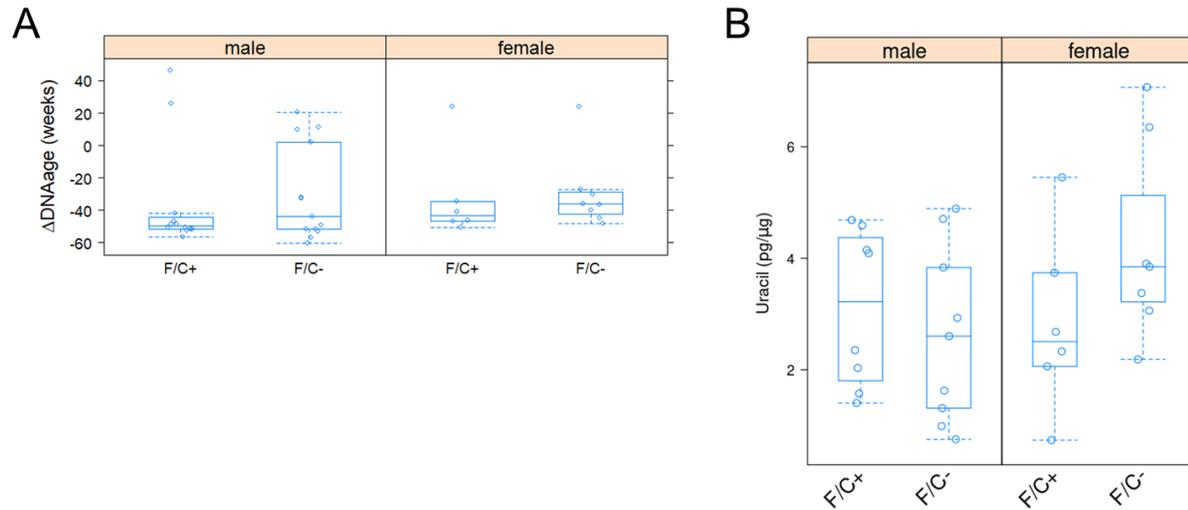


FIGURE 6 - Supplement 3. No significant changes in methylation status or uracil misincorporation in the genome of mice placed on dietary folate restriction late in life. (A) DNA methylation at multiple sites in the genome was measured with the epigenetic clock assay by ZymoResearch (see Materials and Methods) from liver samples at 120 weeks of age. Based on the measured against the predicted changes, a biological age estimate was compared to the actual age (Δ DNAage; shown on the y-axis). The different diets are on the x-axis. The boxplots were drawn as in the previous figures. **(B)** Uracil levels in the DNA were measured as described in Materials and Methods from liver tissue collected at 120 weeks of age (y-axis; in pg/ μ g). The different diets are on the x-axis. The boxplots were drawn as in the previous figures.

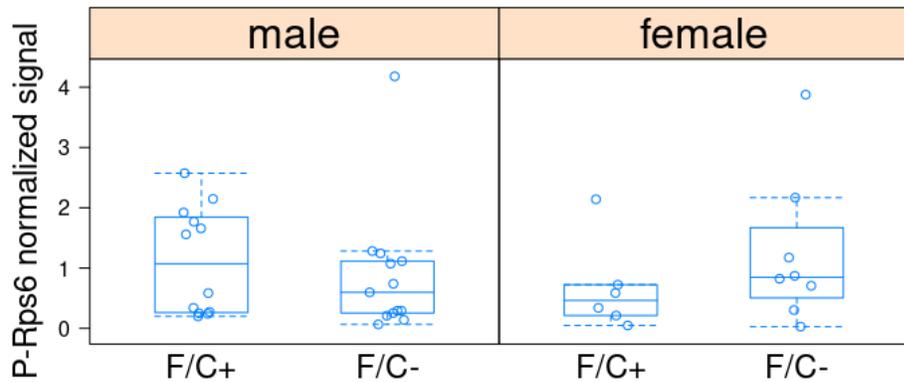


FIGURE 7 - Supplement 1. RPS6 phosphorylation levels in liver tissue are not significantly affected by dietary folate restriction late in life. Phosphorylated RPS6 (P-RPS6) levels (y-axis) were measured with a phosphospecific antibody and normalized against the signal from an antibody against RPS6 (detecting the total amount), as described in Materials and Methods. The different diets are on the x-axis. The boxplots were drawn as in the previous figures.

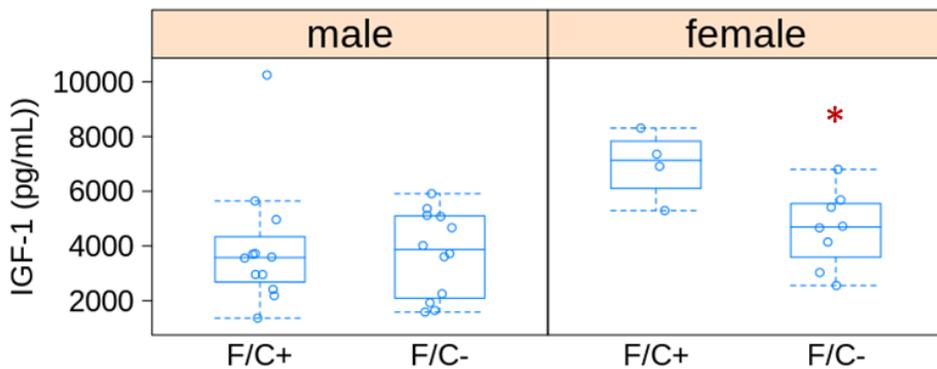


FIGURE 7 - Supplement 2. Lower IGF-1 levels in the serum of female mice placed on dietary folate restriction late in life. IGF-1 levels (in pg/mL; shown on the y-axis) were measured with a commercial mouse IGF-1 ELISA Kit (see Key Resources Table). The different diets are on the x-axis. The boxplots were drawn as in the previous figures. In female mice, the difference was statistically significant, indicated with a red asterisk ($p=0.028$, based on the Wilcoxon rank sum test).