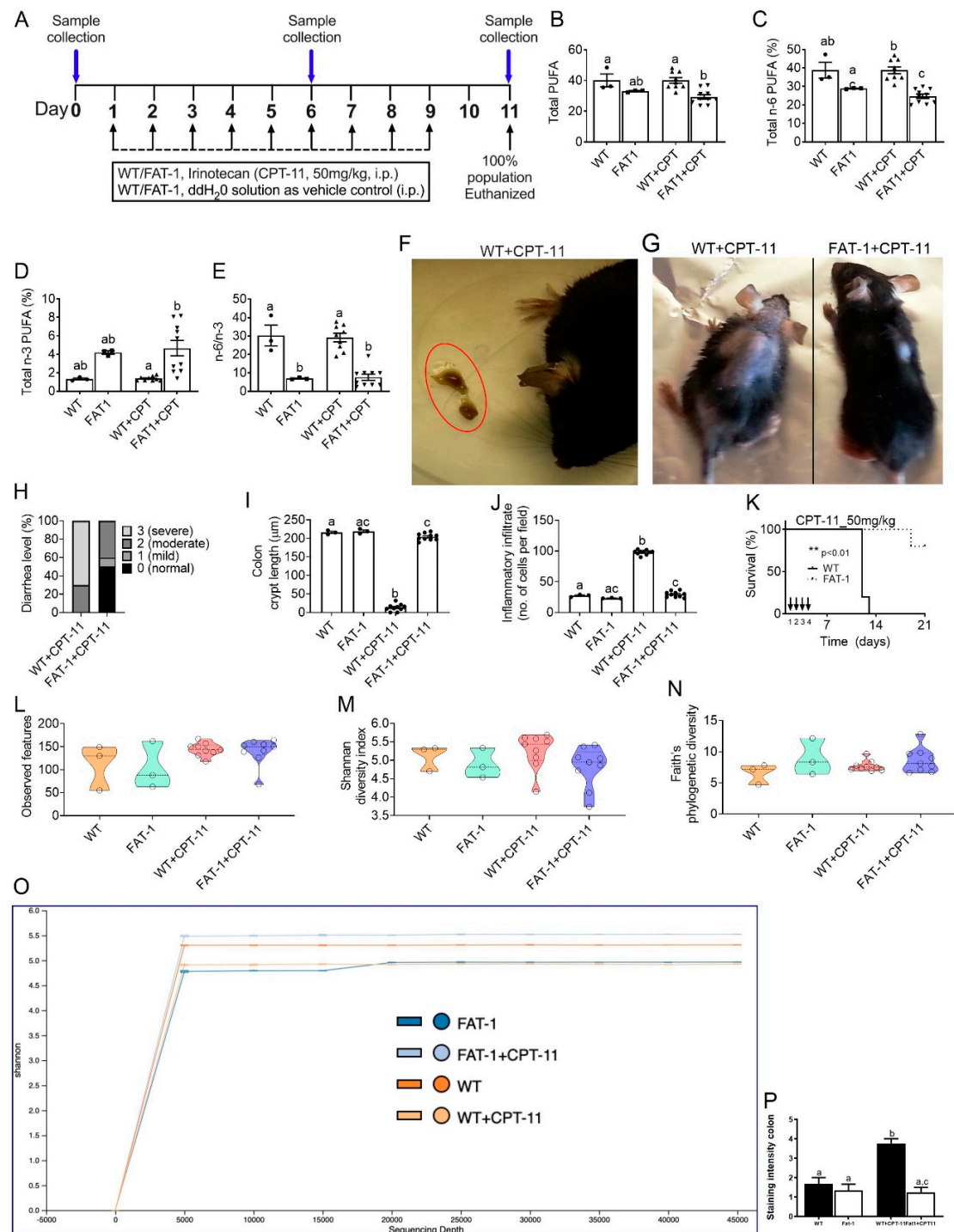


Supplementary Figures:

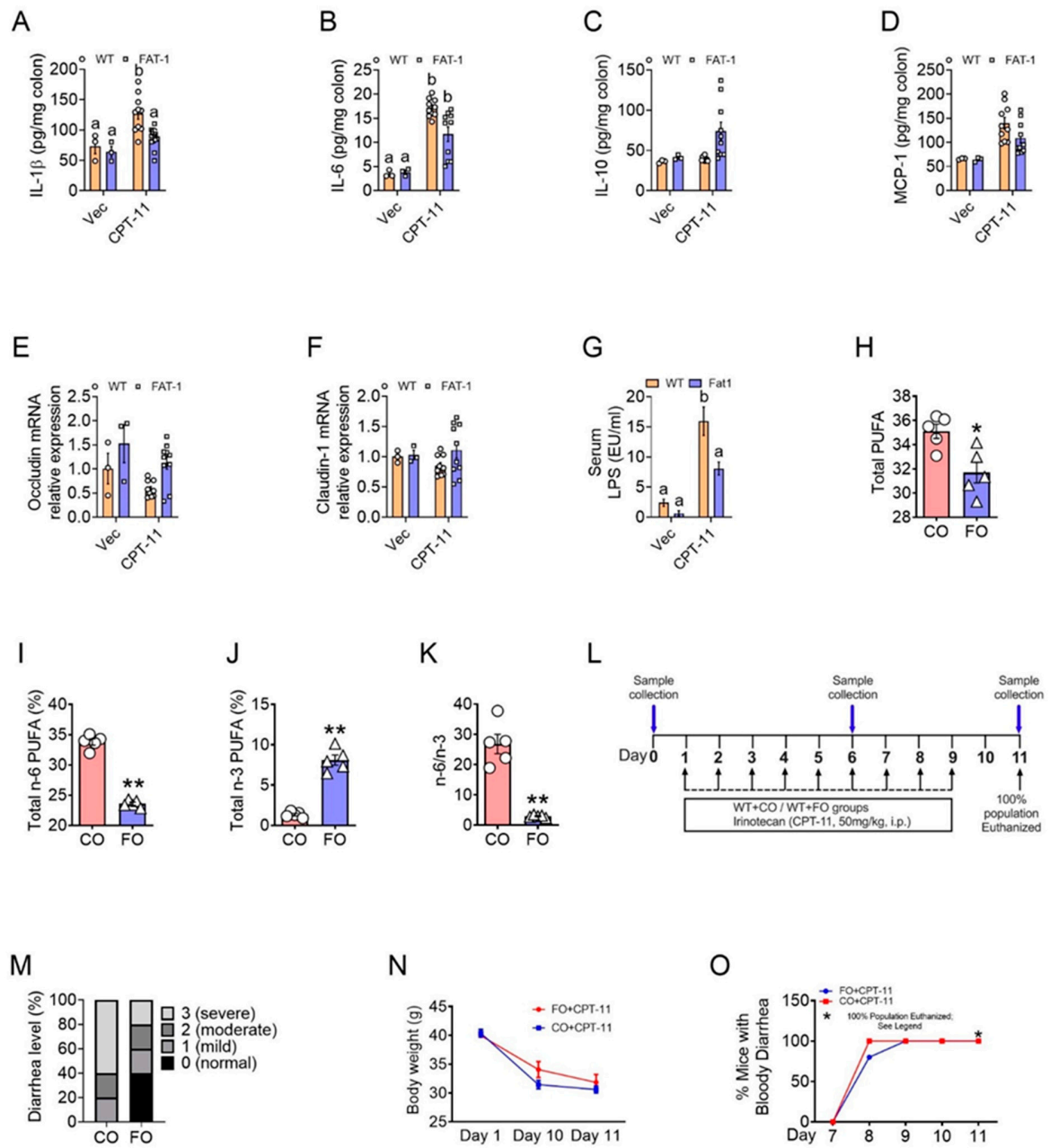
**Decreased Tissue Omega-6/Omega-3 Fatty Acid Ratio Prevents  
Chemotherapy-Induced Gastrointestinal Toxicity by Modulating  
Gut Microbiome**

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**Figure. S1. Decreased tissue n-6/n-3 ratio reduces CPT-11-induced gastrointestinal toxicities and guts microbiome alterations. (A)** Schematic diagram summarizing the timeline and experimental procedures of this study. WT and FAT-1 transgenic mice were arranged into

four groups, and mice from WT+CPT-11 and FAT-1+CPT-11 groups and WT and FAT-1 control groups were subjected to nine consecutive days of treatment with intraperitoneal injections (i.p) of 50 mg/kg CPT-11 and vehicle (double-distilled water) for CPT-11, respectively. Fecal samples were collected at the baseline before CPT-11 administration and at days 6 after CPT-11 treatment. Cecal contents, serum, and tissue samples were collected on days 11. **(B-E)** Parameters of colon-tissue fatty acids profile such as total polyunsaturated fatty acids (PUFA), total omega-6 (n-6) PUFA, total omega-3 (n-3) PUFA, and n-6/n-3 PUFA ratio. **(F)** Representative picture of bloody diarrhea (red circle) on day 6 in the WT+CPT-11 group. **(G)** Gross appearance of CPT-11 treated WT and FAT-1 mice at the time of euthanasia. **(H)** Differences in diarrhea severity between WT+CPT-11 and FAT-1+CPT-11 groups. **(I)** Crypt length measurements. **(J)** Inflammatory cells infiltrate. **(K)** Differences in the CPT-11–induced lethality in WT and FAT-1 mice treated with CPT-11 (50mg/kg). Kaplan-Meier survival curves and Log-rank (Mantel-Cox) test were used to graph the survival vs. time curves and calculate the P-value. **(L-N)** Violin plots showing the distribution, median (middle line), and quartiles (lines at the top and bottom) values of measures of gut microbiota  $\alpha$ -diversity. **(O)** QIIME2 created an alpha rarefaction plot (alpha diversity as a function of sampling depth). **(P)**. The staining intensity score was measured from the immunohistochemical analysis of colonic GUSB using the ImageJ software package. Data are shown as mean  $\pm$  standard error of the mean. Data with different superscript letters are significantly different ( $P < 0.05$ ) according to two-way ANOVA followed by Tukey's multiple comparisons test or Mann Whitney test (**L-N**).

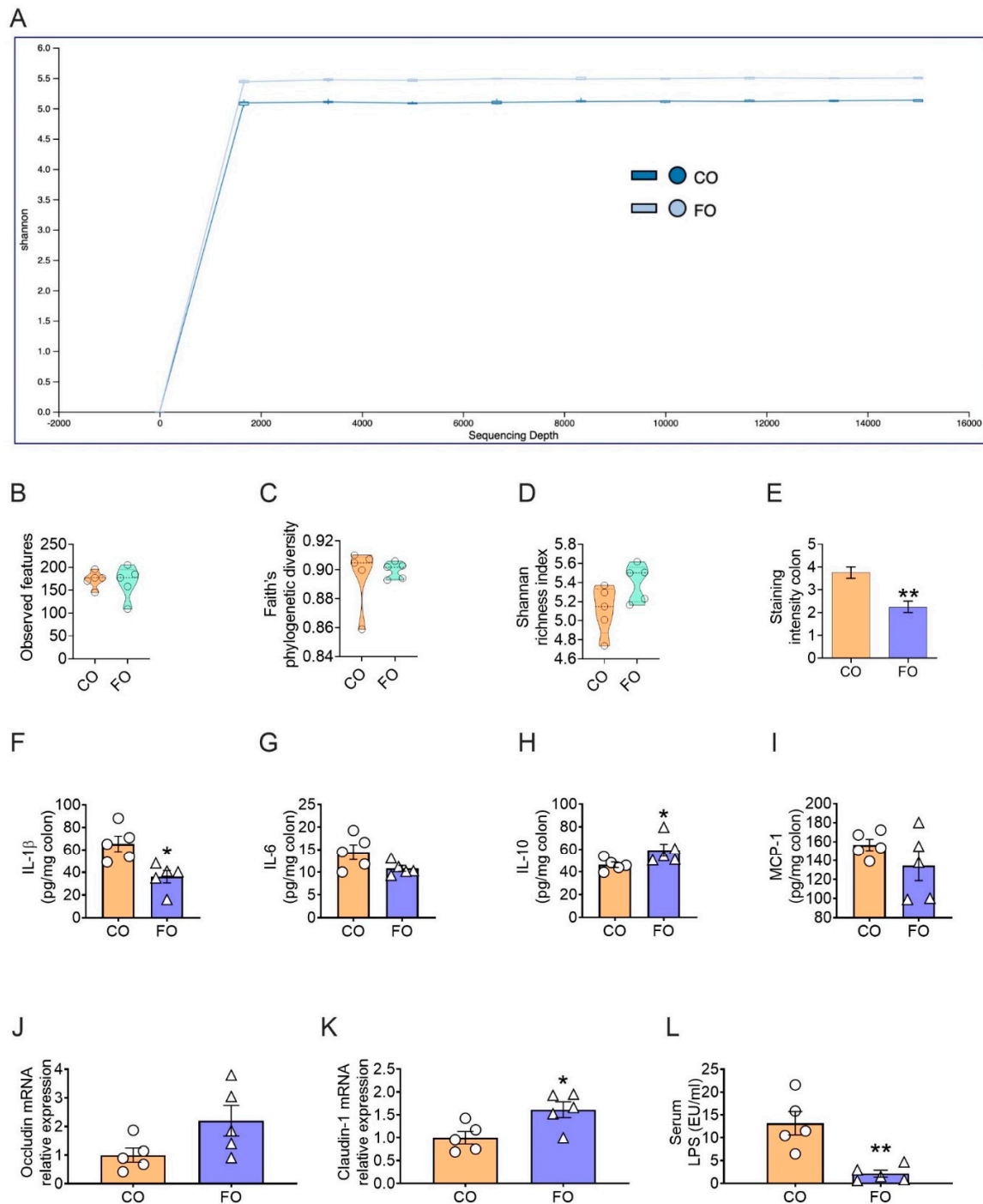


**Figure. S2. Decreased n-6/n-3 ratio reduces CPT-11-induced gut-barrier dysfunction. (A-D)**

Colonic tissue levels of Interleukin (IL)-1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-10, and monocyte chemoattractant protein-1 (MCP-1). (E-F) Differences in the occludin and claudin-1 tight-junction markers

relative gene expression (G) Serum lipopolysaccharides (LPS) levels (endotoxin units/ml). (H-K)

Parameters of colon-tissue fatty acids profile such as total polyunsaturated fatty acids (PUFA), total omega-6 (n-6) PUFA, total omega-3 (n-3) PUFA, and n-6/n-3 PUFA ratio. (**L**) Schematic diagram summarizing the timeline and experimental procedures of this study. (**M**) Differences in diarrhea severity between Corn Oil (CO)+CPT-11 and Fish Oil (FO)+CPT-11 groups. A10 days of either corn oil (CO) or fish oil (FO) supplementation to wild-type (WT) male mice followed by CPT-11 (50mg/kg; intraperitoneal route) administration for 11 days. CPT-11-induced gut toxicities such as body weight changes (**N**) and bloody diarrhea (**O**) between CO+CPT-11 and FO+CPT-11 groups. Data are shown as mean  $\pm$  standard error of the mean. According to one-way ANOVA, data with different superscript letters are significantly different ( $P < 0.05$ ), followed by Tukey's multiple comparisons test or Kruskal-Wallis tests followed by Dunn's multiple comparisons test when the homogeneity of variance was not met with one-way ANOVA. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , nonparametric Mann Whitney test. The scale bar for images in C and D panels are 3000  $\mu\text{m}$ .



**Figure. S3. Dietary n-3 PUFA supplementation reduces CPT-11-induced alterations in the gut microbiome. (A)** Alpha rarefaction plot (alpha diversity as a function of sampling depth)

was created by QIIME2. (**B-D**) Violin plot with lines at the median (dashed lines) and quartiles (complete lines) showing the differences in the  $\alpha$ -diversity measures. (**E**) The staining intensity score was measured from the immunohistochemical analysis of colonic GUSB using the ImageJ software package. (**F-I**) Colonic tissue levels of Interleukin (IL)-1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-10, and monocyte chemoattractant protein-1 (MCP-1). (**J-K**) Differences in the occludin and claudin-1 tight-junction markers relative gene expression (**L**) Serum lipopolysaccharides (LPS) levels (endotoxin units/ml). Data are shown as mean  $\pm$  standard error of the mean. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, nonparametric Mann Whitney test.