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Supplemental Material

The Role of Fecal Microbiota in Liver Toxicity Induced by Perfluorooctane Sulfonate in Male and Female Mice

Lilong Jiang, Yanjun Hong, Pingting Xiao, Xiaoxiao Wang, Jinghui Zhang, Ehu Liu, Huijun Li, and Zongwei Cai

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Figure S1. The effects of PFOS on the fecal microbiota community structures in male and female mice. Taxonomic cladogram of bacterial 16S rDNA sequences in the feces of control and PFOS-exposed male (A) and female (B) mice. (C) PCA plot based on bacterial 16S rDNA gene sequence abundance in the feces. (D) Sex-specific bacterial genera alteration in the feces of control and 10 mg/kg PFOS-exposed male and female mice. The bacterial abundances were calculated from the data of 16S sequencing. The fold difference illustrates the ratio between the average bacterial abundances of 10 mg/kg PFOS exposure group and the average bacterial abundances of control group (n = 5). Colored notes in cladogram show the important microbiota in the indicated groups, and the yellow notes represent the microbiota that do not show any importance in groups. PFOS: Perfluorooctane sulfonate. PCA: Principal component analysis. M: male mice, M1-M4: control, 1 mg/kg, 5 mg/kg and 10 mg/kg. F: female, F1-F4: control, 1 mg/kg, 5 mg/kg and 10 mg/kg. G: Genus. Summary data can be found in Table S8. *$p < 0.05$, **$p < 0.01$ compared with the control group.

Figure S2. The effects of PFOS on the metabolic profiles of fecal samples of male and female mice. (A) PLS-DA score plots for discriminating the fecal metabolome in male mice. (B) Pathway analysis of differential metabolites in male mice. (C) PLS-DA score plots for discriminating the fecal metabolome in female mice. (D) Pathway analysis of differential metabolites in female mice. (E) Rank pathway term analysis of differential metabolites in male mice. (F) Rank pathway term analysis of differential metabolites in female mice. (G) Comparison of arginine levels in feces of male and female mice. (H) Comparison of proline levels in feces of male and female mice. The relative abundance was calculated by the ratio between the level of metabolite concentration in PFOS-exposed group and the average level of control group. PFOS: Perfluorooctane sulfonate. PLS-DA: Partial least squares discrimination analysis. Summary data can be found in Table S9 and Excel Tables S5 and S6. Statistical significance was analyzed by one-way ANOVA among multiple groups. n = 8-11. *$p < 0.05$ compared with the control group.

Figure S3. The effects of ABX and FMT on the relative abundance of proline in the mice liver with PFOS exposure. (A) Bacterial DNA contents in indicated male mice groups. n = 4. (B) Bacterial DNA contents in indicated female mice groups. n = 4. (C) The relative abundance of proline in the liver of indicated male and female mice groups. n = 8-10. The relative abundance was calculated by the ratio between the level of metabolite concentration in exposure group and the average level of control group. P: PFOS, Perfluorooctane sulfonate. ABX: antibiotic treatment. FMT: fecal microbiota transplantation. Summary data can be found in Table S10. Statistical significance was analyzed by one-way ANOVA. **p < 0.01, ***p < 0.001 compared with the indicated group. Results were presented as the mean ± SD.

Figure S4. The effects of bacterial administration on PFOS-induced liver injury. (A) Comparison proportion of *L. Reuteri* levels in feces detected by sequencing analysis. n = 5. (B-D) Relative abundance of specific live bacteria in the indicated groups measured by qPCR. n = 3-5. (E) The relative abundance of arginine in feces of male mice. n = 6-8. (F) The relative abundance of arginine in feces of female mice. n = 6-8. (G-H) Histopathology of liver tissues in male mice treated with killed EF (G) and LR (H). (I) Histopathology of liver tissues in female mice treated with killed AKK. The relative abundance was calculated by the ratio between the level of metabolite concentration in exposure group and the average level of control group. P: PFOS, Perfluorooctane sulfonate. LR: *L. Reuteri*. EF: *E. faecalis*. AKK: *Akk. Muciniphila*. scale bar = 25 μm. Summary data can be found in Table S11. Statistical significance was analyzed by one-way ANOVA. **p < 0.01, ***p < 0.001 compared with the indicated group. Results were presented as the mean ± SD.

Figure S5. The effects of PFOS on the expressions of mTOR and P70S6K. (A) Expression of phosphorylated mTOR and P70S6K in fixed liver tissues of male mice treated with or without PFOS. (B) Expression of phosphorylated mTOR and P70S6K in fixed liver tissues of female mice treated with or without PFOS. The relative intensity represents the ratio between the expression level of phosphorylated protein (p-mTOR and p-P70S6K) and the total protein expression level (mTOR and P70S6K). PFOS: Perfluorooctane sulfonate. mTOR: Mammalian target of rapamycin. Summary data can be found in Table S12. Statistical significance was analyzed by one-way ANOVA. n=3. *p < 0.05, **p < 0.01, ***p < 0.001 compared with the control group. Results were presented as the mean ± SD.

Figure S6. The effects of PFOS on the expressions of mTOR and P70S6K. (A) Expressions of mTOR and P70S6K in fixed liver tissues of male mice treated with or without PFOS. (B) Expressions of mTOR and P70S6K in fixed liver tissues of female mice treated with or without PFOS. PFOS: Perfluorooctane sulfonate. mTOR: Mammalian target of rapamycin.

Figure S7. The effects of PFOS on the expressions of mTOR and P70S6K. (A) Expressions of mTOR and P70S6K in fixed liver tissues of male mice in the indicated groups. (B) Expressions of mTOR and P70S6K in fixed liver tissues of female mice in the indicated groups. P: PFOS, Perfluorooctane sulfonate. LR: *L. Reuteri*. EF: *E. faecalis*. AKK: *Akk. Muciniphila*. mTOR: Mammalian target of rapamycin.

Table S1. Hepatosomatic indices (%) of the mice liver treated with 2 mg/kg PFOS in pre-experiments.

Table S2. Summary data for Fig. 1B, C, D and E.

Table S3. Summary data for Fig. 2B, D, E and F.

Table S4. Summary data for Fig. 3I and J.

Table S5. Summary data for Fig. 4B, C, F and G.

Table S6. Summary data for Fig. 5A, C, F and G.

Table S7. Summary data for Fig. 6A and B.

Table S8. Summary data for Fig. S1D.

Table S9. Summary data for Fig. S2G and H.

Table S10. Summary data for Fig. S3.

Table S11. Summary data for Fig. S4A, B, C, D, E and F.

Table S12. Summary data for Fig. S5A and B.

Additional File- Excel Document