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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 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.

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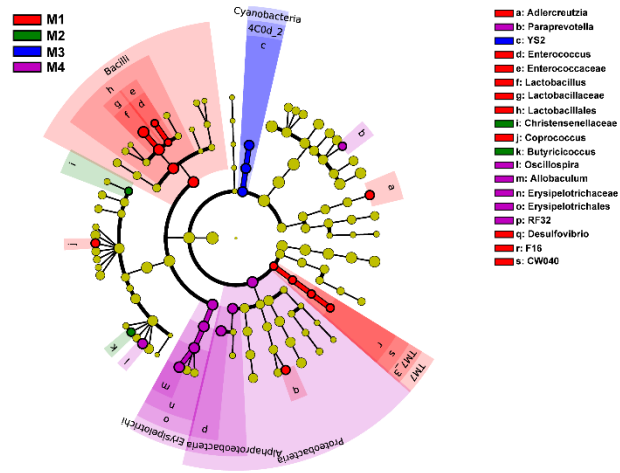
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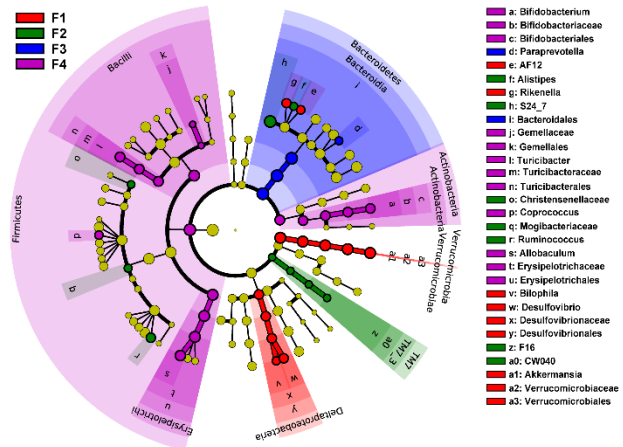
Table S12. Summary data for Fig. S5A and B.

Additional File- Excel Document

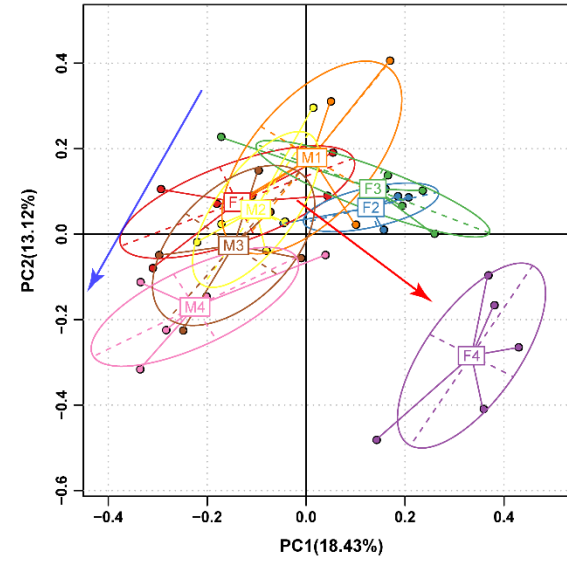
A



B



C



D

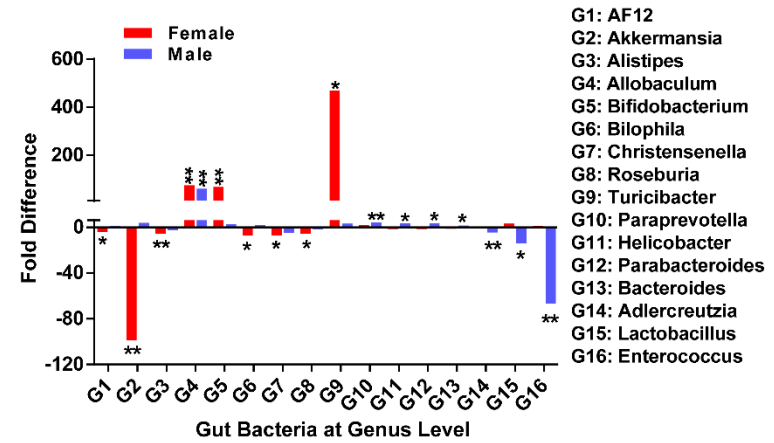


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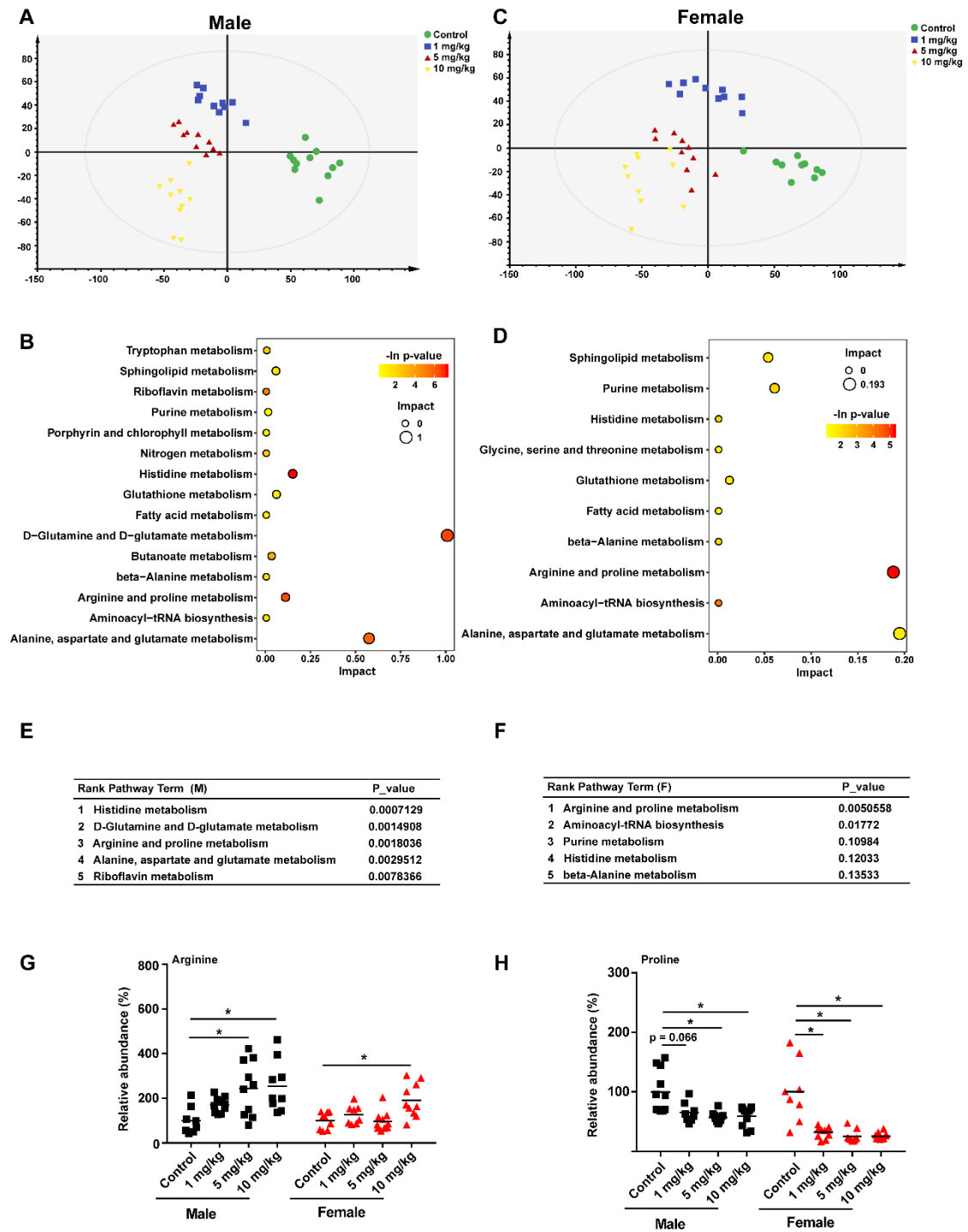


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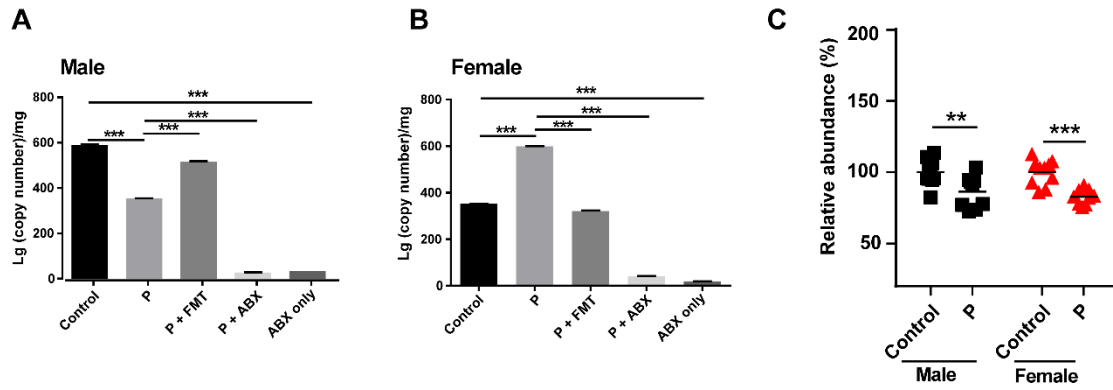


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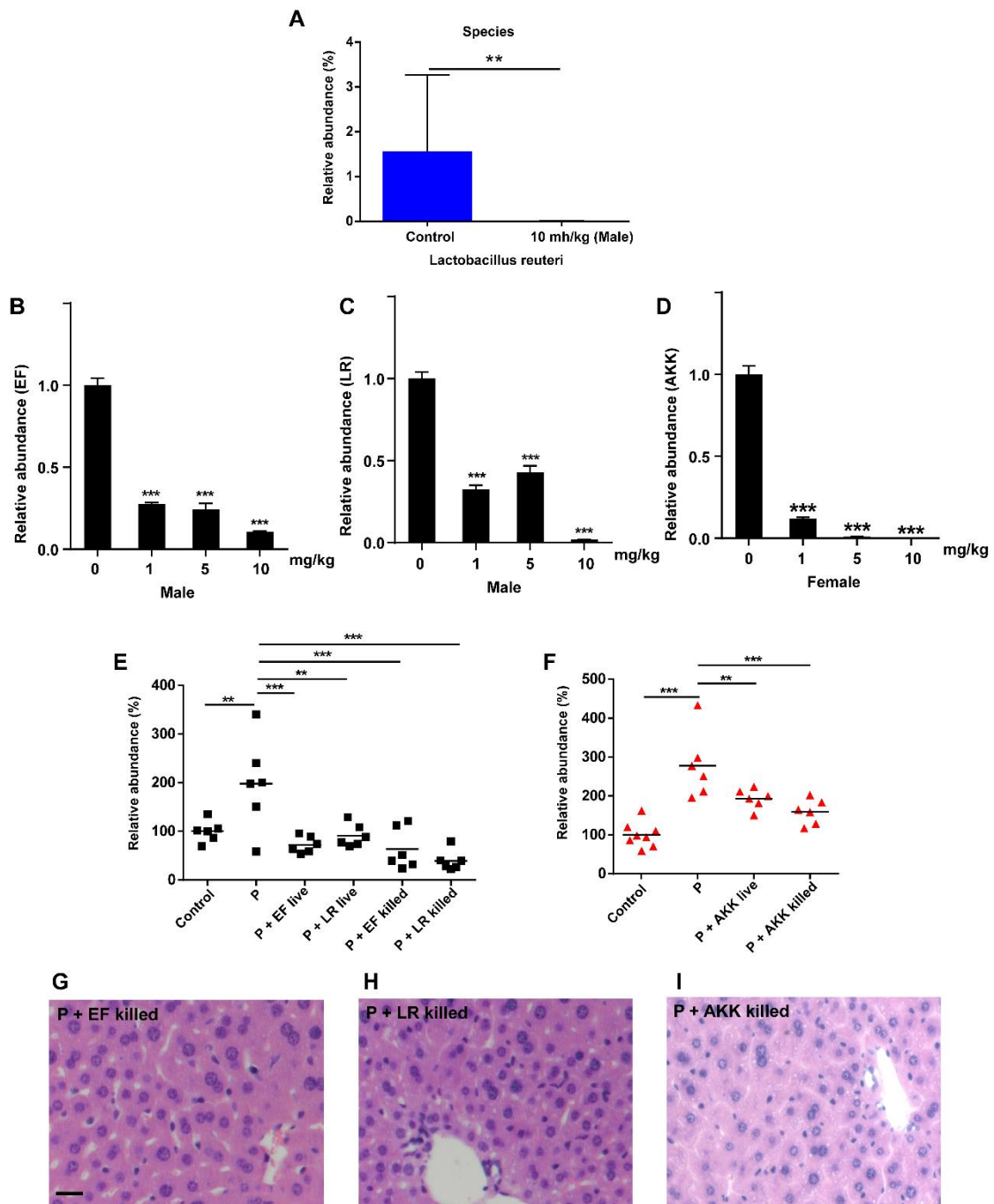


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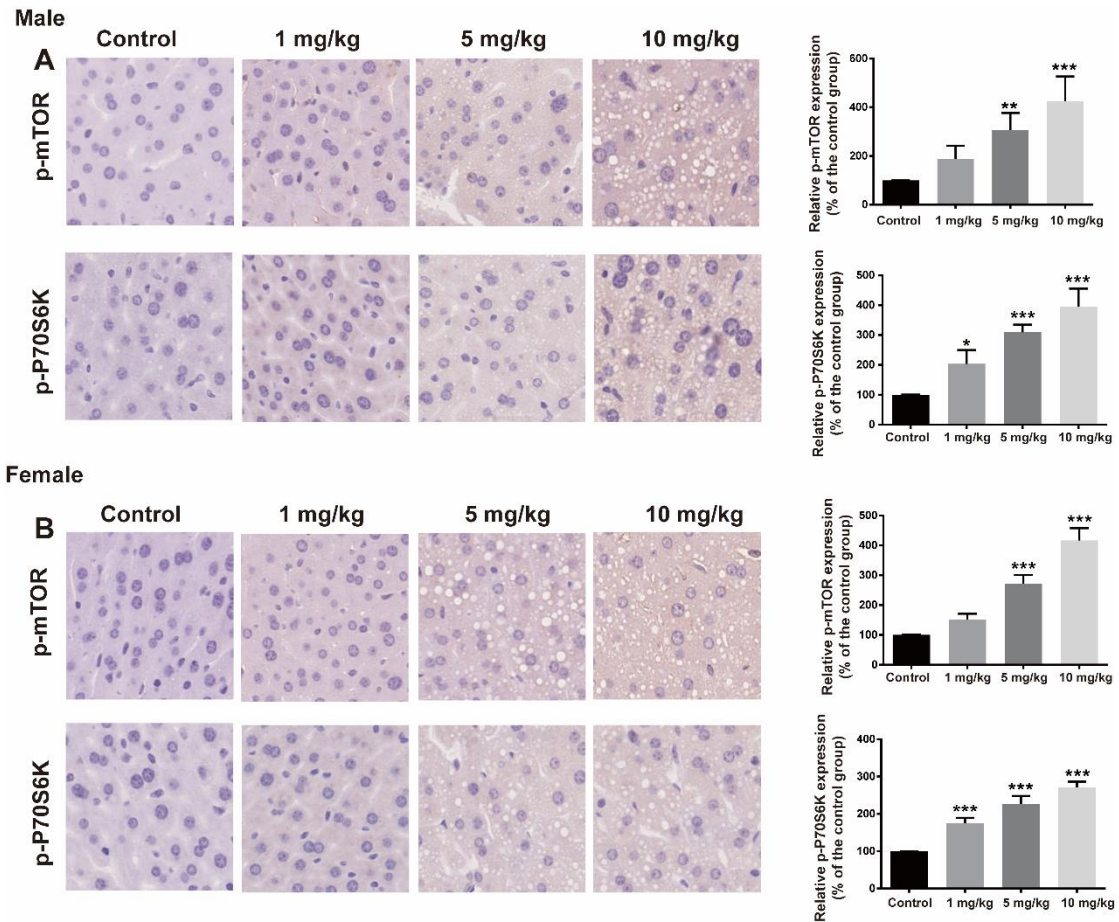


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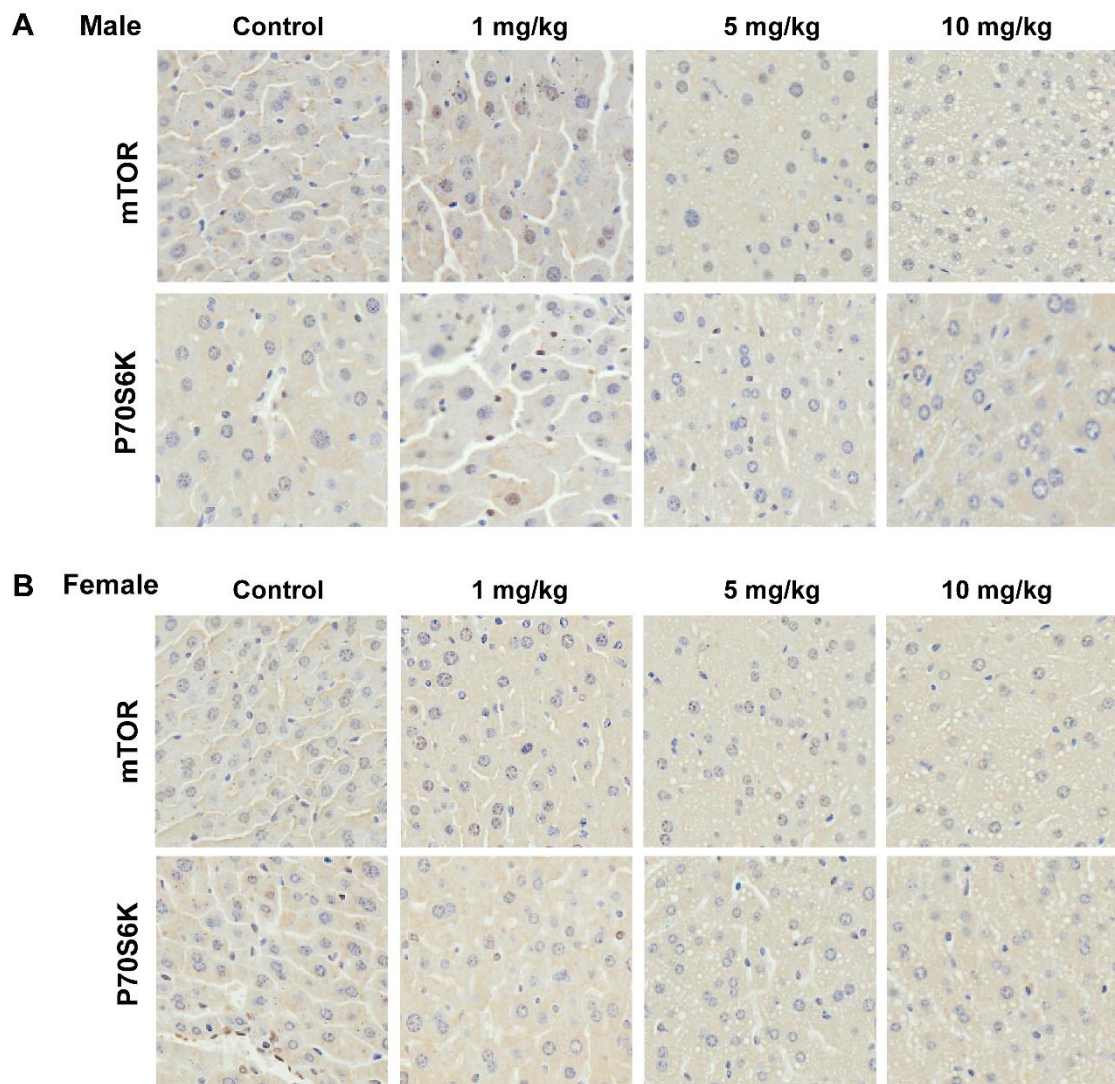


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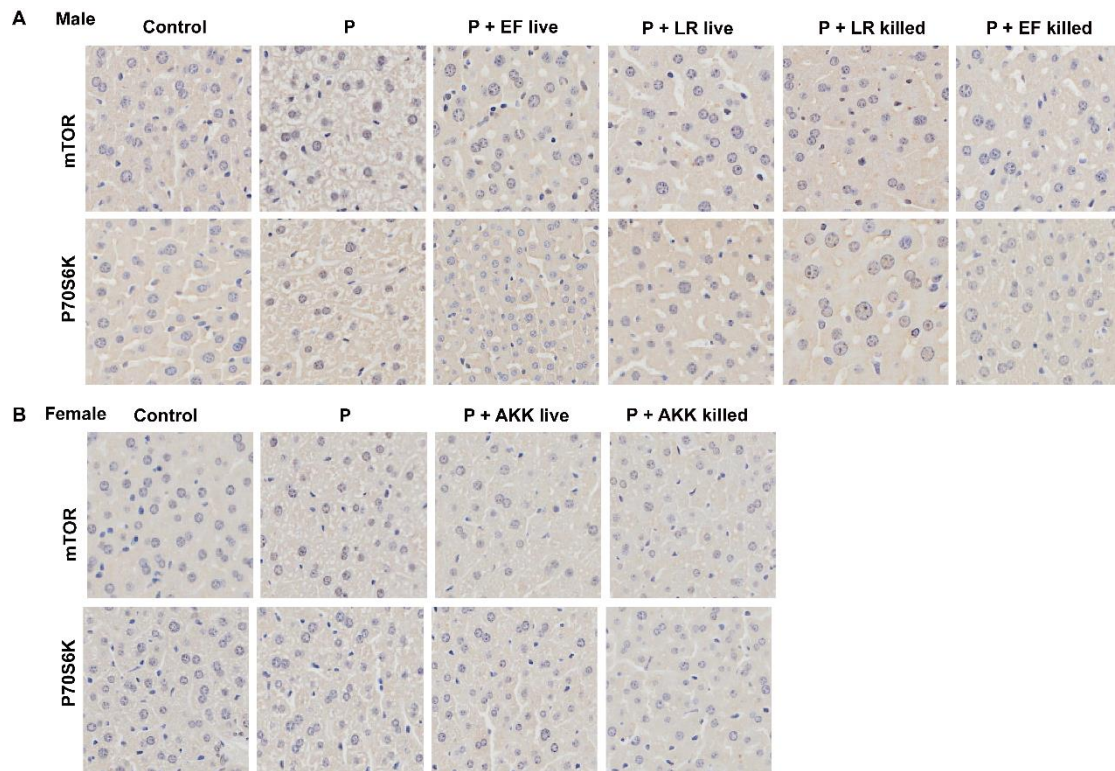


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Table S1 Hepatosomatic indices (%) of the mice liver treated with 2 mg/kg PFOS in pre-experiments.

	Control	2 mg/kg
Male	3.68 ± 0.20	4.91 ± 0.26
Female	4.97	5.56 ± 0.40

Note: PFOS: Perfluorooctane sulfonate. The pre-experiments were performed to select a dosage with moderate liver injury in mice for fecal microbiota transplantation and antibiotic treatment studies. Mice were exposed to either 2 or 5 mg/kg PFOS via daily oral gavage once daily for 14 days. Twenty-four hours after the last dose, the liver was collected, and the hepatosomatic index was calculated as the percentage of the ratio of liver weight (g) to body weight (g). While 5 mg/kg PFOS dosage induced severe liver injury in mice (Fig. 1B), 2 mg/kg dosage exposure (14 days) induced moderate liver injury and was selected for further study. n = 1-3 for both male and female mice. Significant differences were not calculated in the pre-experiments. The results just provided the reference for the selection of 2 mg/kg dosage in the formal experiments.

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

Fig 1B	Hepatosomatic index (%)	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	4.08 ± 0.34	4.82 ± 0.44	7.48 ± 0.47	9.49 ± 0.56
	Female	3.73 ± 0.21	4.22 ± 0.19	6.66 ± 0.40	9.70 ± 0.66

Fig 1C	ALT activity (U/L)	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	8.38 ± 4.94	8.08 ± 1.64	18.80 ± 7.76	43.15 ± 17.52
	Female	4.00 ± 1.84	5.48 ± 2.10	11.32 ± 3.72	36.68 ± 16.90

Fig 1D	AST activity (U/L)	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	21.55 ± 4.97	22.71 ± 6.08	21.54 ± 4.98	33.79 ± 6.40
	Female	24.39 ± 3.86	24.29 ± 3.23	31.21 ± 5.32	30.84 ± 5.22

Fig 1E	ALP activity (U/L)	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	21.85 ± 10.32	33.84 ± 6.71	32.20 ± 3.75	38.70 ± 11.73
	Female	21.92 ± 8.51	33.88 ± 6.73	36.13 ± 8.92	33.63 ± 8.73

Note: For ALT activity analysis, ALT reacted with alanine and α -ketoglutarate, and produced pyruvic acid. Pyruvic acid was then reacted with 2,4-dinitrophenylhydrazine (DNPH) to form phenylhydrazone, which could be dissolved in sodium hydroxide and then detected at 505nm. For AST activity analysis, AST catalyzed aspartate and α -ketoglutarate to form oxalacetate and glutamate. Oxalacetate could decarboxylate automatically to pyruvic acid, which could react with DNPH and then detected at 510nm. For ALP activity analysis, ALP catalyzed disodium phenyl phosphate to form phenol, which could react with 4-aminoantipyrine and potassium ferricyanide, and could be detected at around 520nm. Hepatosomatic index was calculated as the percentage of the ratio of liver weight (g) to body weight (g). n = 8-11. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. ALP: Alkaline phosphatase.

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

Fig 2B and D	Alpha diversity (observed OTUs)	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Male	395.20 ± 47.80	407.40 ± 17.05	375.20 ± 12.28	373.40 ± 40.01
	Female	348.20 ± 30.78	419.80 ± 30.46	395.00 ± 25.70	397.40 ± 27.52

	Male-relative abundance (%)			
Fig 2E	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
Lactobacillus	7.36	5.67	4.95	0.53
Oscillospira	1.78	1.12	0.82	2.20
Adlercreutzia	0.54	0.25	0.19	0.12
Desulfovibrio	0.39	0.13	0.063	0.22
Coprococcus	0.34	0.14	0.28	0.21
Butyricoccus	0.084	0.12	0.027	0.061
Allobaculum	0.074	0.67	1.09	4.43
Paraprevotella	0.028	0.027	0.052	0.11
Enterococcus	0.028	0.019	0.019	0.00042
Escherichia	0	0	0.00082	0.018

	Female-relative abundance (%)			
Fig 2F	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
Akkermansia	16.17	2.38	2.84	0.16

Prevotella	6.57	4.63	4.49	1.04
Desulfovibrio	1.13	0.38	0.075	0.16
Rikenella	0.63	0.30	0.10	0.14
AF12	0.29	0.24	0.12	0.078
Bilophila	0.17	0.075	0.028	0.023
Alistipes	0.13	0.13	0.085	0.024
Coprococcus	0.11	0.11	0.11	0.26
Allobaculum	0.093	1.14	0.42	6.95
Paraprevotella	0.039	0.040	0.10	0.063
Bifidobacterium	0.027	0.37	0.25	1.78
Turicibacter	0.0048	0.00081	0.00083	2.25

Note: n = 5.

Table S4 Summary data for Fig. 3I and J

Fig 3I	Relative abundance (%) - Arginine	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Male	100.00 ± 21.05	154.06 ± 74.34	295.43 ± 107.51	415.69 ± 220.94
	Female	100.00 ± 36.00	119.95 ± 61.66	190.05 ± 70.61	388.30 ± 190.44

Fig 3J	Relative abundance (%) - Proline	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Male	100.00 ± 14.12	107.72 ± 28.74	131.08 ± 9.79	123.41 ± 15.64
	Female	100.00 ± 28.09	114.24 ± 23.69	141.32 ± 11.17	128.96 ± 18.35

Note: n = 8-11.

Fig 3I- actual measured values	Ratio between intensities of arginine and the internal standard	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Male	0.069022591 ± 0.014526771	0.106339601 ± 0.051314282	0.203913873 ± 0.074203956	0.286920799 ± 0.152498646
	Female	0.130367623 ± 0.046930922	0.15637837 ± 0.080385322	0.247759899 ± 0.092056884	0.50621457 ± 0.248275028

Fig 3J- actual measured values	Ratio between intensities of proline and the internal	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg

	standard				
	Male	36.99470964 ± 5.221912944	39.84961111 ± 10.63174848	48.49275993 ± 3.621766676	45.65616264 ± 5.786170206
	Female	40.36369171 ± 11.33741718	46.11227368 ± 9.560844823	57.04218667 ± 4.506983375	52.05133427 ± 7.408030754

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

Fig 4B and C	Hepatosomatic index (%)	Control	P	P + ABX	P + FMT
	Male	4.62 ± 0.18	6.28 ± 0.52	5.26 ± 0.24	5.73 ± 0.29
	Female	3.97 ± 0.25	5.49 ± 0.32	4.60 ± 0.13	5.05 ± 0.23

Fig 4F	Relative abundance (%) - Hepatic arginine	Control	P	P + ABX	P + FMT	ABX
	Male	100.00 ± 30.14	205.17 ± 46.04	91.56 ± 49.16	128.26 ± 24.88	82.46 ± 24.48
	Female	100.00 ± 12.68	154.02 ± 39.79	89.89 ± 42.69	89.54 ± 28.37	93.80 ± 21.97

Fig 4G	Relative abundance (%) - Fecal arginine	Control	P	P + ABX	P + FMT	ABX
	Male	100.00 ± 49.39	306.93 ± 99.77	836.66 ± 166.74	155.48 ± 81.23	781.14 ± 238.42
	Female	100.00 ± 37.23	281.09 ± 125.74	1494.88 ± 486.59	97.35 ± 26.17	1552.38 ± 274.43

Note: n = 8-10. P: Perfluorooctane sulfonate. ABX: antibiotic treatment, FMT: fecal microbiota transplantation.

Fig 4F- actual measured values	Hepatic arginine- Ratio between intensities of arginine and the internal standard	Control	P	P + ABX	P + FMT	ABX
	Male	0.026399301 ± 0.007956411	0.054163324 ± 0.012153047	0.024171273 ± 0.012978209	0.033860725 ± 0.006568348	0.021767998 ± 0.006461411
	Female	0.034635245 ± 0.00439002	0.053346242 ± 0.013782627	0.031013437 ± 0.009824434	0.031134341 ± 0.014784152	0.032486282 ± 0.007608523

Fig 4G- actual measured values	Fecal arginine- Ratio between intensities of arginine and the internal standard	Control	P	P + ABX	P + FMT	ABX
	Male	0.020302134 ± 0.010026691	0.062314043 ± 0.020255893	0.169859093 ± 0.033851616	0.031565557 ± 0.01649069	0.158587759 ± 0.048405023
	Female	0.013207867 ± 0.004917805	0.037126112 ± 0.016607629	0.197442412 ± 0.06426833	0.012857852 ± 0.0034566	0.205035731 ± 0.036246714

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

Fig 5A	Relative abundance (%)	Control	10 mg/kg
	Lactobacillales (A1)	7.40 ± 7.62	0.55 ± 0.73
	Lactobacillaceae (A2)	7.36 ± 7.62	0.53 ± 0.75
	Lactobacillus (A3)	7.36 ± 7.62	0.53 ± 0.75
	Enterococcaceae (A4)	0.028 ± 0.017	0.00042 ± 0.00093
	Enterococcus (A5)	0.028 ± 0.017	0.00042 ± 0.00093
	Verrucomicrobiaceae (A6)	16.17 ± 9.17	0.16 ± 0.36
	Akkermansia (A7)	16.17 ± 9.17	0.16 ± 0.36
	Akkermansia muciniphila (A8)	16.17 ± 9.17	0.16 ± 0.36

Fig 5C	Hepatosomatic index (%)	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	3.96 ± 0.27	6.67 ± 0.30	4.84 ± 0.39	4.86 ± 0.24	4.88 ± 0.20	4.67 ± 0.21

Fig 5C	Hepatosomatic index (%)	Control	P	P + AKK live	P + AKK killed
	Female	4.04 ± 0.28	5.41 ± 0.18	4.80 ± 0.11	5.16 ± 0.19

Fig 5F	Relative abundance (%)	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	100.00 ± 18.12	404.92 ± 157.97	152.44 ± 33.85	195.11 ± 97.30	136.56 ± 20.17	206.63 ± 47.24

Fig 5G	Relative abundance (%)	Control	P	P + AKK live	P + AKK killed
	Female	100.00 ± 29.67	149.15 ± 20.42	110.96 ± 24.46	121.37 ± 20.09

Note: n = 8-10. P: Perfluorooctane sulfonate. LR: *L. Reuteri*, EF: *E. faecalis*, AKK: *Akk.*

Muciniphila.

Fig 5F- actual measured values	Hepatic arginine- Ratio between intensities of arginine and the internal standard	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	0.04188 415 ± 0.00759 0716	0.1695 96159 ± 0.0661 63923	0.06384 8729 ± 0.01417 5764	0.08172 1403 ± 0.04075 1893	0.05719 8707 ± 0.00844 6963	0.08654 5754 ± 0.01978 5022

Fig 5G- actual measured values	Hepatic arginine- Ratio between intensities of arginine and the internal standard	Control	P	P + AKK live	P + AKK killed
	Female	0.038559357 ± 0.011439739	0.057509426 ± 0.007874974	0.042786684 ± 0.00943053	0.04679821 ± 0.007746512

Table S7 Summary data for Fig. 6A and B

Fig 6A	Relative p-mTOR expression (% of the control group)	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	100.00 ± 1.07	239.64 ± 21.84	123.68 ± 1.55	124.05 ± 4.88	172.27 ± 4.26	176.82 ± 5.12

Fig 6A	Relative p-P70S6K expression (% of the control group)	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	100.00 ± 0.31	326.92 ± 31.38	198.05 ± 28.56	197.47 ± 16.34	222.61 ± 21.98	226.96 ± 22.41

Fig 6B	Relative p-mTOR expression (% of the control group)	Control	P	P + AKK live	P + AKK killed
	Female	100.00 ± 0.91	249.44 ± 28.32	123.91 ± 4.25	180.99 ± 18.28

Fig 6B	Relative p-P70S6K expression (% of the control group)	Control	P	P + AKK live	P + AKK killed
	Female	100.00 ± 0.82	213.75 ± 20.50	130.65 ± 20.02	152.38 ± 11.79

Note: n = 3. P: Perfluorooctane sulfonate. mTOR: Mammalian target of rapamycin. LR:

L. Reuteri, EF: *E. faecalis*, AKK: *Akk. Muciniphila*.

Fig 6A- actual measured values	p-mTOR expression	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	0.997751198 ± 0.010707932	2.391033313 ± 0.217955541	1.234032321 ± 0.01550843	1.237753771 ± 0.048645592	1.71881657 ± 0.042503314	1.764185853 ± 0.051114333

Fig 6A- actual measured values	p-P70S6K expression	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	0.995091306 ± 0.003120881	3.253136886 ± 0.312304404	1.970737914 ± 0.284245689	1.964975523 ± 0.162589574	2.215194316 ± 0.218710354	2.25849388 ± 0.222985102

Fig 6B- actual measured values	p-mTOR expression	Control	P	P + AKK live	P + AKK killed
	Female	1.010456515 ± 0.009181529	2.520502667 ± 0.286170398	1.252010919 ± 0.042899717	1.82885823 ± 0.184691985

Fig 6B- actual measured values	p-P70S6K expression	Control	P	P + AKK live	P + AKK killed
	Female	1.00208246 ± 0.00817589	2.141938952 ± 0.205395652	1.309249198 ± 0.200579989	1.526959658 ± 0.118174553

Table S8 Summary data for Fig. S1D

Figure S1D	Fold difference (10 mg/kg vs. Control)	Male	Female
	G1	1.12	-3.76
	G2	3.82	-98.77
	G3	-2.90	-5.49
	G4	59.59	74.50
	G5	2.38	66.98
	G6	1.77	-7.14
	G7	-4.87	-6.84
	G8	-1.64	-5.64
	G9	3.10	468.85
	G10	4.05	1.61
	G11	3.52	-1.47
	G12	3.34	-1.55
	G13	1.56	-1.29
	G14	-4.69	-1.11
	G15	-14.01	3.04
	G16	-66.78	1.02

Note: “-” indicated that PFOS-treated mice had significantly lower bacterial abundances. PFOS: Perfluorooctane sulfonate. G: genus.

Figure S1D actual measured values of 0 mg/kg and 10 mg/kg groups	Relative abundance (%)	Male-0 mg/kg	Male-10 mg/kg	Female-0 mg/kg	Female- 10 mg/kg
	G1	0.30429 ± 0.348464	0.341986 ± 0.4803 53	0.29158 ± 0.221665	0.077568 ± 0.063663
	G2	4.27556 ± 3.77576	16.352922 ± 9.732615	16.169607 ± 9.174985	0.163716 ± 0.362672
	G3	0.127315 ± 0.158873	0.043854 ± 0.020356	0.131338 ± 0.089567	0.023942 ± 0.017259
	G4	0.07426 ± 0.052115	4.425235 ± 3.079619	0.093278 ± 0.059322	6.94967 ± 5.182951
	G5	0.191328 ± 0.215489	0.45615 ± 0.480968	0.026645 ± 0.027047	1.784651 ± 1.69673
	G6	0.081512 ± 0.080588	0.144675 ± 0.107254	0.167438 ± 0.108175	0.023454 ± 0.023696
	G7	0.005804 ± 0.00633	0.001193 ± 0.001089	0.002792 ± 0.002258	0.000408 ± 0.000912
	G8	0.00455 ± 0.009122	0.002767 ± 0.006187	0.009157 ± 0.001707	0.001624 ± 0.002657
	G9	0.042713 ± 0.073657	0.132492 ± 0.154155	0.004795 ± 0.008554	2.248125 ± 0.606816
	G10	0.028285 ± 0.01106	0.114646 ± 0.057087	0.038963 ± 0.021969	0.062824 ± 0.059242
	G11	0.862783 ± 0.550485	3.032973 ± 1.726229	3.447228 ± 3.984886	2.349771 ± 1.799486
	G12	0.402318 ± 0.105421	1.34258 ± 0.82091	0.406054 ± 0.235654	0.262308 ± 0.288879
	G13	9.179983 ± 3.417691	14.35912 ± 3.244313	8.636494 ± 2.015126	6.672421 ± 3.61246
	G14	0.541383 ± 0.309034	0.115538 ± 0.089943	0.291208 ± 0.070067	0.261937 ± 0.119354

	G15	7.359306 ± 7.618082	0.525287 ± 0.751838	3.322033 ± 4.010776	10.092741 ± 5.497853
	G16	0.027782 ± 0.016822	0.000416 ± 0.000931	0.000408 ± 0.000912	0.000418 ± 0.000934

Table S9 Summary data for Fig. S2G and H

Fig S2G	Relative abundance (%) - Arginine	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	100.00 ± 60.82	170.28 ± 34.35	244.21 ± 123.25	254.48 ± 113.32
	Female	100.00 ± 37.13	126.90 ± 42.36	97.11 ± 44.04	191.07 ± 75.79

Fig S2H	Relative abundance (%) - Proline	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	100.00 ± 35.41	65.28 ± 16.69	57.19 ± 8.52	59.12 ± 17.02
	Female	100 ± 51.88	32.01 ± 9.39	25.35 ± 10.52	25.73 ± 5.91

Note: n = 8-11.

Fig S2G- actual measured values	Ratio between intensities of arginine and the internal standard	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	1.211215453 ± 0.736633621	2.062487118 ± 0.416132151	2.957845173 ± 1.492843595	3.082258268 ± 1.372553037
	Female	1.754760941 ± 0.651594727	2.226656727 ± 0.743235857	1.703961571 ± 0.772802769	3.352825443 ± 1.329872745

Fig S2H- actual measured values	Ratio between intensities of proline and the internal standard	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	23.82965523 ± 8.438010129	15.55518102 ± 3.976586516	13.62813482 ± 2.031109962	14.08885321 ± 4.055395441
	Female	57.97630525 ± 30.07537214	18.55993259 ± 5.443139737	14.69526487 ± 6.096841524	14.91801937 ± 3.425789784

Table S10 Summary data for Fig. S3

Fig S3A and B	Lg (copy number)/mg	Control	P	P + FMT	P + ABX	ABX only
	Male	589.52 ± 2.65	353.03 ± 1.16	515.28 ± 2.94	27.82 ± 0.29	32.92 ± 0.21
	Female	351.35 ± 1.72	598.75 ± 1.57	320.74 ± 2.72	41.25 ± 0.57	18.76 ± 0.13

Note: n = 4. P: Perfluorooctane sulfonate. ABX: antibiotic treatment. FMT: fecal microbiota transplantation.

Fig S3C	Relative abundance (%)	Control	P
	Male	100.00 ± 9.18	86.37 ± 11.17
	Female	100.00 ± 8.89	82.85 ± 5.20

Note: n = 8-10. P: Perfluorooctane sulfonate.

Fig S3C-actual measured values	Ratio between intensities of proline and the internal standard	Control	P
	Male	114.5966445 ± 10.52197604	98.98168281 ± 12.79722434
	Female	117.4671085 ± 10.44220445	97.31715834 ± 6.103782578

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

Fig S4A	Relative abundance (%)	Control	10 mg/kg
	Lactobacillus reuteri	1.56 ± 1.71	0.0085 ± 0.0090

Note: n = 5.

Fig S4B	Relative abundance (EF)	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Male	1.00 ± 0.043	0.28 ± 0.0072	0.24 ± 0.035	0.10 ± 0.0052

Note: n = 3-5. EF: *E. faecalis*.

Fig S4C	Relative abundance (LR)	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Male	1.00 ± 0.040	0.32 ± 0.027	0.43 ± 0.041	0.019 ± 0.00080

Note: n = 3-5. LR: *L. Reuteri*.

Fig S4D	Relative abundance (AKK)	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Female	1.00 ± 0.052	0.12 ± 0.0085	0.010 ± 0.00061	0.00030 ± 4.37E-05

Note: n = 3-5. AKK: *Akk. Muciniphila*.

Fig S4E	Relative abundance (%)	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	100.00 ± 21.89	198.22 ± 93.53	72.67 ± 16.91	91.29 ± 23.27	63.52 ± 42.49	39.65 ± 20.84

Note: n = 6-8. P: Perfluorooctane sulfonate. LR: *L. Reuteri*. EF: *E. faecalis*. AKK: *Akk. Muciniphila*.

Fig	Relative	Control	P	P + AKK live	P + AKK killed
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S4F	abundance (%)				
	Female	100.00 ± 32.04	277.87 ± 85.24	193.25 ± 25.49	159.19 ± 32.23

Note: n = 6-8. P: Perfluorooctane sulfonate. LR: *L. Reuteri*. EF: *E. faecalis*. AKK: *Akk.*

Muciniphila.

Fig S4B- actual measured values	EF	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Male	7.96589E-05 ± 3.39725E-06	2.2058E-05 ± 5.74743E-07	1.94342E-05 ± 2.77624E-06	8.34189E-06 ± 4.11855E-07

Fig S4C- actual measured values	LR	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Male	0.000494826 ± 1.98934E-05	0.000159906 ± 1.33858E-05	0.000211423 ± 2.03989E-05	9.19014E-06 ± 3.97562E-07

Fig S4D- actual measured values	AKK	0 mg/kg	1 mg/kg	5 mg/kg	10 mg/kg
	Female	0.000652189 ± 3.38438E-05	7.83075E-05 ± 5.55637E-06	6.75527E-06 ± 3.95532E-07	1.96013E-07 ± 2.84723E-08

Fig S4E- actual measured values	Ratio between intensities of arginine and the internal standard	Control	P	P + EF live	P + LR live	P + EF killed	P + LR killed
	Male	0.173506279 ± 0.037986799	0.343928222 ± 0.162279954	0.126080295 ± 0.029336935	0.158386817 ± 0.040379263	0.110212726 ± 0.073728943	0.068789159 ± 0.036155296

Fig S4F- actual measured values	Ratio between intensities of arginine and the internal standard	Control	P	P + AKK live	P + AKK killed
	Female	0.011482495 ± 0.003679216	0.031906282 ± 0.009787179	0.022190001 ± 0.002927457	0.018279539 ± 0.003700917

Table S12 Summary data for Fig. S5A and B

Fig S5A	Relative p-mTOR expression (% of the control group)	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	100.00 ± 0.48	187.18 ± 54.89	305.20 ± 71.71	424.92 ± 101.96

Fig S5A	Relative p-P70S6K expression (% of the control group)	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	100.00 ± 0.47	203.61 ± 46.13	309.31 ± 25.57	395.08 ± 60.40

Fig S5B	Relative p-mTOR expression (% of the control group)	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Female	100.00 ± 0.090	150.93 ± 20.23	271.69 ± 28.98	416.56 ± 41.05

Fig S5B	Relative p-P70S6K expression	Control	1 mg/kg	5 mg/kg	10 mg/kg
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	(% of the control group)				
	Female	100.00 ± 0.0093	175.31 ± 13.98	226.64 ± 21.34	270.67 ± 15.56

Note: n = 3. mTOR: Mammalian target of rapamycin.

Fig S5A- actual measured values	p-mTOR expression	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	1.001612839 ± 0.004779506	1.874868532 ± 0.549764679	3.056934629 ± 0.718282689	4.256026061 ± 1.02126754

Fig S5A- actual measured values	p-P70S6K expression	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Male	1.002069406 ± 0.004700499	2.040293808 ± 0.46226399	3.099482023 ± 0.256180497	3.958930823 ± 0.605201532

Fig S5B- actual measured values	p-mTOR expression	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Female	1.003176673 ± 0.000906625	1.514046054 ± 0.202963647	2.725537528 ± 0.29071039	4.178845234 ± 0.411796059

Fig S5B- actual measured values	p-P70S6K expression	Control	1 mg/kg	5 mg/kg	10 mg/kg
	Female	1.002140753 ± 9.32231E-05	1.756848545 ± 0.140086363	2.27129019 ± 0.213876842	2.712478252 ± 0.155962589