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Supplementary Materials

Lead exposure exacerbates liver injury in high-fat diet-fed mice by disrupting the gut microbiota and related metabolites

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13 **Table S1.** Target genes and primers for Real-time PCR in this study

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ZO-1	GATGTTTATGCGGACGGTGG	CATTGCTGTGCTCTTAGCGG
ZO-2	CGCGGGTTCCCACCG	TCCTCTTTTGGAATCCTTCTGC
Occludin	GCCCCTCTTTCCTTAGGCG	TCCCAAGATAAGCGAACCTGC
Claudin-1	CCCCCATCAATGCCAGGTATG	AGAGGTTGTTTTCCGGGGAC
Claudin-4	CTTCATCGGCAGCAACATCG	TACACATAGTTGCTGGCGGG
E-cadherin	AACCCAAGCACGTATCAGGG	GAGTGTTGGGGGCATCATCA
MUC1	CCCTATGAGGAGGTTTCGGC	GTGGGGTGACTTGCTCCTAC
MUC2	ACCTGGAAGGCCCAATCAAG	CTCAGCGTAGTTGGCACTCT
MUC4	ACCAGATGGCTCTGAACCTA	TGCATTGGCCTCCATTGTGA
TLR4	TCCCTGCATAGAGGTAGTTCC	TCAAGGGGTTGAAGCTCAGA
IL-6	GACAAAGCCAGAGTCCTTCAGA	TGTGACTCCAGCTTATCTCTCTTGG
IL-1β	TGCCACCTTTTGACAGTGATG	TGATGTGCTGCTGCGAGATT
TNF-α	GATCGGTCCCCAAAGGGATG	CCACTTGGTGGTGTGTGTGAGTG
MCP-1	CACTCACCTGCTGCTACTCA	GCTTGGTGACAAAAACTACAGC
NF-κB	ATGGCAGACGATGATCCCTAC	TGTTGACAGTGGTATTTCTGGTG
MYD88	CAGGAGATGATCCGGCAACT	CATGCGGCGACACCTTTTC
MLCK	CACTGTGGTCACAGGATGGG	TGCTTGCTCCTTGTTCGCAA
Collagen1	CGATGGATTCCCGTTCGAGT	GAGGCCTCGGTGGACATTAG
α-SMA	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
Fibronectin	GGCCACCATTACTGGTCTGG	GGAAGGGTAACCAGTTGGGG
FASN	GGCCCCTCTGTTAATTGGCT	CGCTTGTTGGTGGACACTTG
PPAR-γ	GGGGATGTCTCACAATGCCA	TGGTCATGAATCCTTGGCCC
ACC	TTGCCATGGGGATCCCTCTA	GCTGTTCCTCAGGCTCACAT
CD36	GACGTGGCAAAGAACAGCAG	CATGTCGCAATAGCTTGGCC
FABP1	TGAAGGCAATAGGTCTGCCC	CAGGGTGAACTCATTGCGGA
FABP4	TGTGTGATGCCTTTGTGGGA	TCCACCAGCTTGTCACCATC
AMPK-α1	GGGAAAGTGAAGGTGGGCAA	GATGTGAGGGTGCCTGAACA
ACOX	CTGGTGGGTGGTATGGTGTC	ATCATAGCGGCCGAGAACAG
CPT-1α	GCCCTCAAACAGATCTGCCT	CATGCGTTGGAAGTCTCCCT
CPT-1β	CATGTATCGCCGCAAACTGG	GTGTTCGGTGTTGAGGCCTA
CPT-2	CCCAAACCCAGTCGTGATGA	CCAGCCTTTAGAGCACTGCT
SREBP-1c	GACCCTACGAAGTGCACACA	GTGGCCTAGTCACAGGTTCC
SCD-1	GCCCACATGCTCCAAGAGAT	CTTTGACAGCCGGGTGTTTG
GPAT	AATAGGCCTCTGGAGGAGCTT	GCTTTGCTTACTGGTCCTGTATC
DGAT1	GAGGACGAGGTGCGAGAC	CAGACGATGGCACCTCAGAT
DGAT2	AACACGCCCAAGAAAGGTGG	GTAGTCTCGGAAGTAGCGCC
MTTP	CTCGCTAGAAGCCATCCTGG	CCCAATGGACAGCAGGATGT
APOB	AAGCACCTCCGAAAGTACGTG	CTCCAGCTCTACCTTACAGTTGA
SREBP2	GCAGCAACGGGACCATTCT	CCCCATGACTAAGTCCTTCAACT
HMGCR	AGCTTGCCCGAATTGTATGTG	TCTGTTGTGAACCATGTGACTTC
GPR41	TGTCCAATACTCTGCATCTGTG	CACGAGGAACACCAACAGGTA
GPR43	ATCACAGGAAACGGGAAGCC	CCAGTCTGGGGTCATTCTCC

GPR109A	TTTGTGTTCGGACTCCTGGG	AACAAGATGATGGCCAGGGG
β-actin	CTGGTCGTACCACAGGCATT	TGCTAGGAGCCAGAGCAGTA

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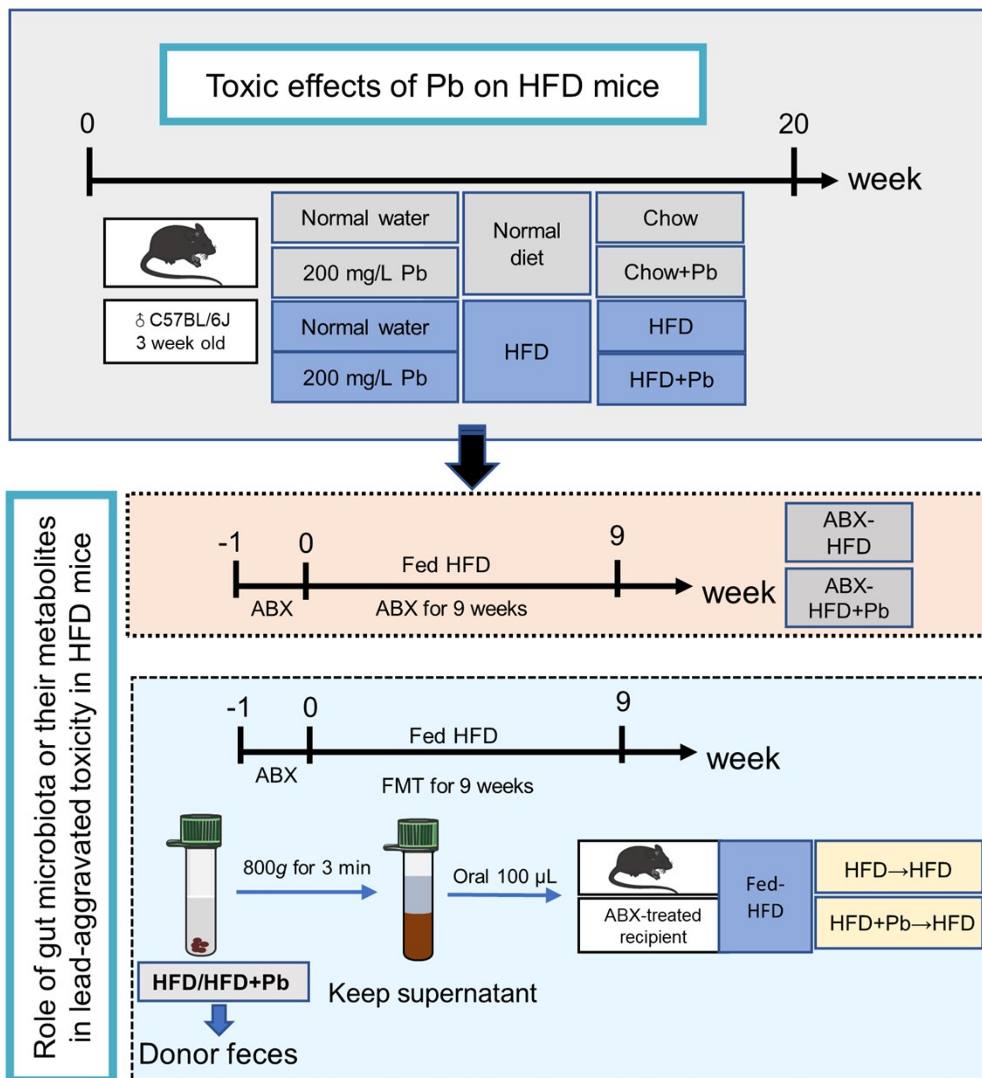

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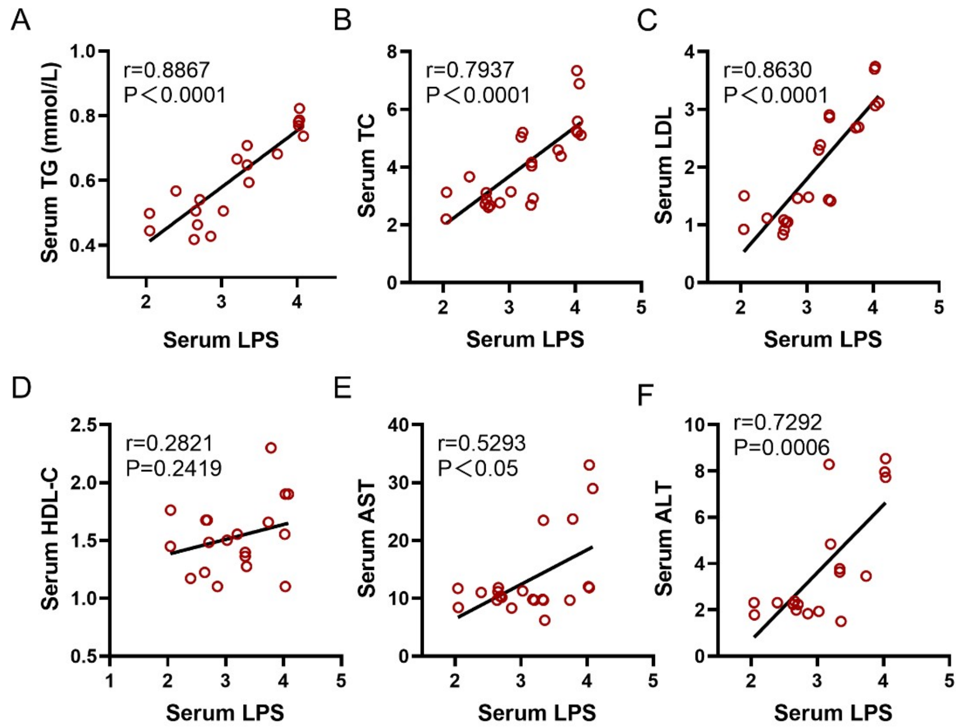
16 **Fig. S1** Protocol design of the whole experiment. HFD, high fat diet; Pb, lead; FMT, fecal

17 microbiota transplantation; ABX, antibiotic cocktail



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19 **Fig. S2** Pearson correlation analysis of serum LPS and related biochemical indices. Data are

20 expressed as mean ± SD. A value of $p < 0.05$ was considered to be significant.

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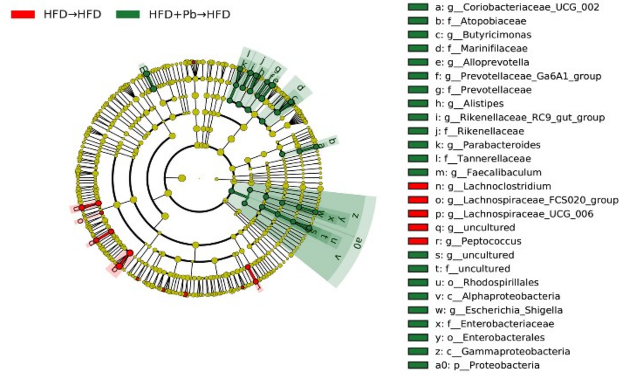

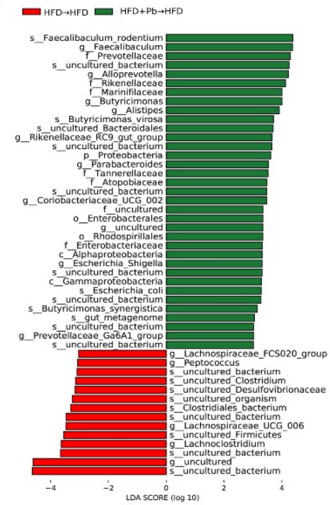

Fig. S3 LEfSe analysis of cladogram (A) and LDA distribution histograms (B) (LDA score log10 >3).

26 **SUPPLEMENTARY MATERIALS AND METHODS**

27 **1. Verification of the success of pseudo germ-free mice model with antibiotics**

28 **1.1 Methods**

29 **1.1.1 Animals and treatments**

30 C57BL/6 mice (n=10) were purchased from Shanghai Slack Experimental Animal Co., Ltd
31 (Shanghai) and placed in a specific pathogen-free environment with an environmental temperature
32 of 23±2°C, relative humidity of 50±10%, and a light/dark cycle of 12 hours. After 1 week of
33 acclimation, all mice were divided into non-antibiotic treated group (NAB, n=5) and antibiotic
34 treated group (AB, n=5). The AB group was allowed to drink antibiotics for 7 days, while the
35 NAB group was given conventional treatment. Mice in the NAB and AB groups were humanely
36 sacrificed to verify the effect of antibiotics on the degree of intestinal disturbance.

37 In detail, mice were fed freely for 7 days with 1 mg/mL ampicillin (Beijing Solarbio Science
38 & Technology Co. Ltd., China), cefoperazone sodium salt (Shanghai Yuanye Bio-Technology Co.,
39 Ltd., China), and clindamycin hydrochloride (Beijing Solarbio Science & Technology Co., Ltd.,
40 China) (1-3).

41 **1.1.2 Sample collection**

42 Mice were sacrificed after overnight fasting. The contents of mouse jejunum, ileum and
43 colon were collected, and the contents were placed in 0.9% sterile saline at a ratio of 1:9 for
44 colony counting.

45 **1.1.3 Colony count**

46 The intestinal contents were mixed with sterile saline (0.9%) according to the ratio of 1:9. Take
47 0.1 mL of the mixture and continue to mix with 0.9 mL of sterile saline. And continue this procedure

48 by preparing a 10-fold dilution of the sample. An exact 100 μL sample of each concentration was
49 taken and applied to nutrient agar medium. Incubate at 37°C for 24h, and record the colony number
50 and bacterial amount (4). Bacterial count (CFU/mL) = number × 10^n × 10 (n, dilution ratio).

51 **1.1.4 Statistical analysis**

52 All values were expressed as mean ± standard deviation (SD). The independent sample *t* test
53 was used to compare the differences between the two groups. $p < 0.05$ was considered statistical
54 significance.

55 **1.2 Results**

56 **1.2.1 The result of colony count**

57 The results in Table S1 show that the number of colonies in the jejunum, ileum and colon of
58 the AB group was significantly lower than that of the NAB group after 24 h of incubation. The
59 results indicate that the antibiotic-treated pseudo-sterile mouse model is successful and could be
60 used for fecal microbial transplantation experiments.

61 **Table S2.** Effect of antibiotic treatment on bacterial colonies of jejunum, ileum, and colon of mice.

bacterial colonies	NAB (CFU/mL)	AB (CFU/mL)
jejunum	$2.75\times10^6\pm9.57\times10^5$	$3.83\times10^5\pm2.53\times10^{5}$ **
ileum	$2.87\times10^7\pm4.04\times10^6$	$4.67\times10^6\pm5.8\times10^{5}$ ***
colon	$2.93\times10^{10}\pm5.13\times10^9$	$3.47\times10^8\pm1.00\times10^{8}$ ***

62 Data are expressed as mean ± SD. AB *vs* NAB, **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001.

63 **2 Biochemical analyses**

64 Serum insulin, diamine oxidase (DAO) and lipopolysaccharide (LPS) were detected by mouse
65 ELISA kit (Quanzhou Kainodibio Co., Ltd., China). Serum content of total cholesterol (TC),
66 triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein
67 cholesterol (HDL-C) were determined using commercial kits (Jiancheng Bio Co., Nanjing, China).

68 Serum alanine transaminase (ALT), and glutamic oxaloacetic transaminase (AST) were measured
69 by an automated blood biochemistry analyzer (C8000, Abbott Laboratories, Abbott Park, IL, USA).

70 **3 Gut microbiota analysis**

71 The intestinal contents (n=3-5/group) were used for gut microbiota assays. Briefly, DNA was
72 extracted from the samples, and the purity of the DNA was tested by 1% agarose gel electrophoresis.
73 The 16S V3-V4 region was amplified; the target band size was detected by 2% agarose gel
74 electrophoresis and purified by recovery with magnetic beads. Libraries were sequenced on the
75 NovaSeq250 platform. Alpha diversity was described by richness (Chao1 and Observed_species)
76 and diversity (Shannon and Simpson). Beta diversity was expressed using a weighted uniform
77 method based on PCoA. The composition of microorganisms was analyzed at the phylum and genus
78 level. Microbial biomarkers between different groups were analyzed using LEfSe. Regarding the
79 analysis of gut microbiota results, among the four groups of Chow, Chow+Pb, HFD, and HFD+Pb,
80 the Chow group was used as the control group, the FMT experiment used the HFD→HFD group as
81 the control group, and the ABX experiment used the ABX-HFD group as the control group. Alpha
82 diversity and species composition were statistically analyzed by One-way ANOVA with Tukey post
83 hoc test (more than two groups) and independent samples *t*-test (two groups). Correlations between
84 gut microbiota and physiological indicators were analyzed using Spearman's correlation.

85 **4 SCFA analysis**

86 SCFA content in feces was measured according to the previous method (5). First, 0.1 g of feces
87 was weighed to which 1 mL 50% phosphoric acid was added. The mixture was ground for 5 min
88 and centrifuged at 21000 × g at 4 ℃ for 10 min to obtain supernatant. After diluting 0.01g of
89 supernatant with 1.8 mL of 50% phosphoric acid solution, the mixture vortexed for 30s and

90 centrifuged for another 30s. Added to 20 μL of the diluted supernatant was 10 μL 200 mM 3-

91 NPH·HCl, 10 μL 200mM EDC·HCl, 80 μL 50% phosphoric acid, 50 μL 7% Pyridine, and 1 μL

92 isotope internal standard solution. This mixture was vortexed for 1min, centrifuged for 1 min,

93 derivatized at 40°C for 30 min and centrifuged at 18000 × g for 1 min at 4 °C. Twenty μL of the

94 derivatized reaction solution was added to 280 μL of 50% phosphoric acid solution (containing

95 0.1% formic acid), vortex mixed for 30 s, and centrifuged at 18000 × g for 10 min at 4°C. The

96 resulting supernatant was placed in an injection bottle for LC-MS/MS analysis.

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