1   **Supplementary Material**

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3   **Aflatoxin B$_1$ exposure causes splenic pyroptosis via**

4   **disturbing gut microbiota-immune axis**

Huodai Chen [a], Lin Ye [a], Yurun Wang [a], Jiahong Chen [a], Jie Wang [a], Xueling Li [a],

Hongtao Lei *[a,b], Yunle Liu *[a,b].


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6   [a] Guangdong Provincial Key Laboratory of Food Quality and Safety / National-Local

7   Joint Engineering Research Center for Machining and Safety of Livestock and Poultry

8   Products, South China Agricultural University, Guangzhou 510642, China

9   [b] Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, 510642, China

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12   *Correspondence: Guangdong Provincial Key Laboratory of Food Quality and Safety,

13   South China Agricultural University, 483 Wushan Road, Tianhe District, Guangzhou

14   510642, China.

15   Yunle Liu, E-mail: liuyunle@scau.edu.cn.

16   Hongtao Lei, E-mail: hongtao@scau.edu.cn; Tel: +8620-8528 3925; Fax: +8620-8528

17   0270.
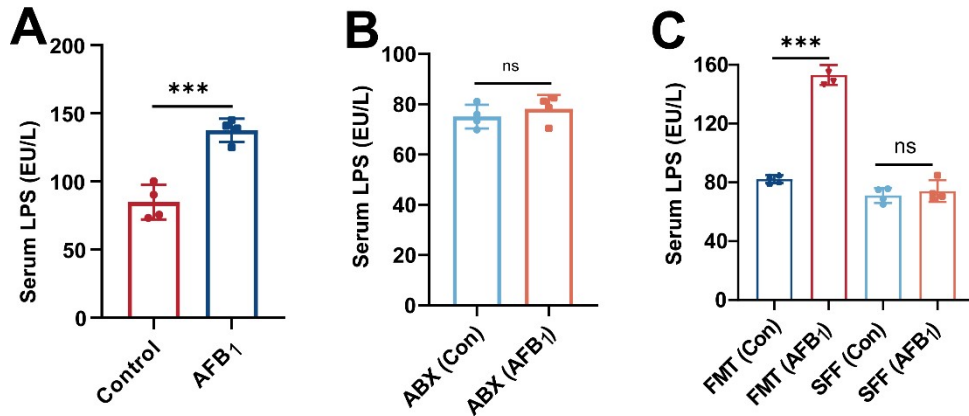
Supplementary figure 1. The levels of LPS in serum of mice. **(A)** The levels of LPS in normal groups ($n = 4$). **(B)** The levels of LPS in ABX groups ($n = 4$). **(C)** The levels of LPS in FMT groups and SFF groups ($n = 4$). Values are means ± SD. The statistical significance was determined using Student t-tests (A, B) and two-way ANOVA followed by post hoc test (C). The significance values are represented as follows: ns (not significant) for $p > 0.05$, * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.
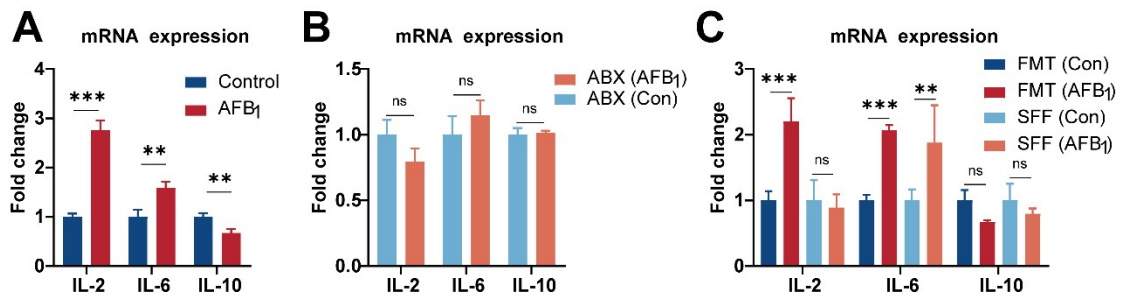
Supplementary figure 2. The effect of AFB_1 on cytokine expression levels in the spleen cells of mice. **(A)** Relative mRNA expression of IL-2, IL-6 and IL-10 in normal groups ($n = 3$). **(B)** Relative mRNA expression of IL-2, IL-6 and IL-10 in ABX groups ($n = 3$). **(C)** Relative mRNA expression of IL-2, IL-6 and IL-10 in FMT groups and SFF groups ($n = 3$). Values are means ± SD. The statistical significance was determined using Student t-tests (A, B) and two-way ANOVA followed by post hoc test (C). The significance values are represented as follows: ns (not significant) for $p > 0.05$, * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.
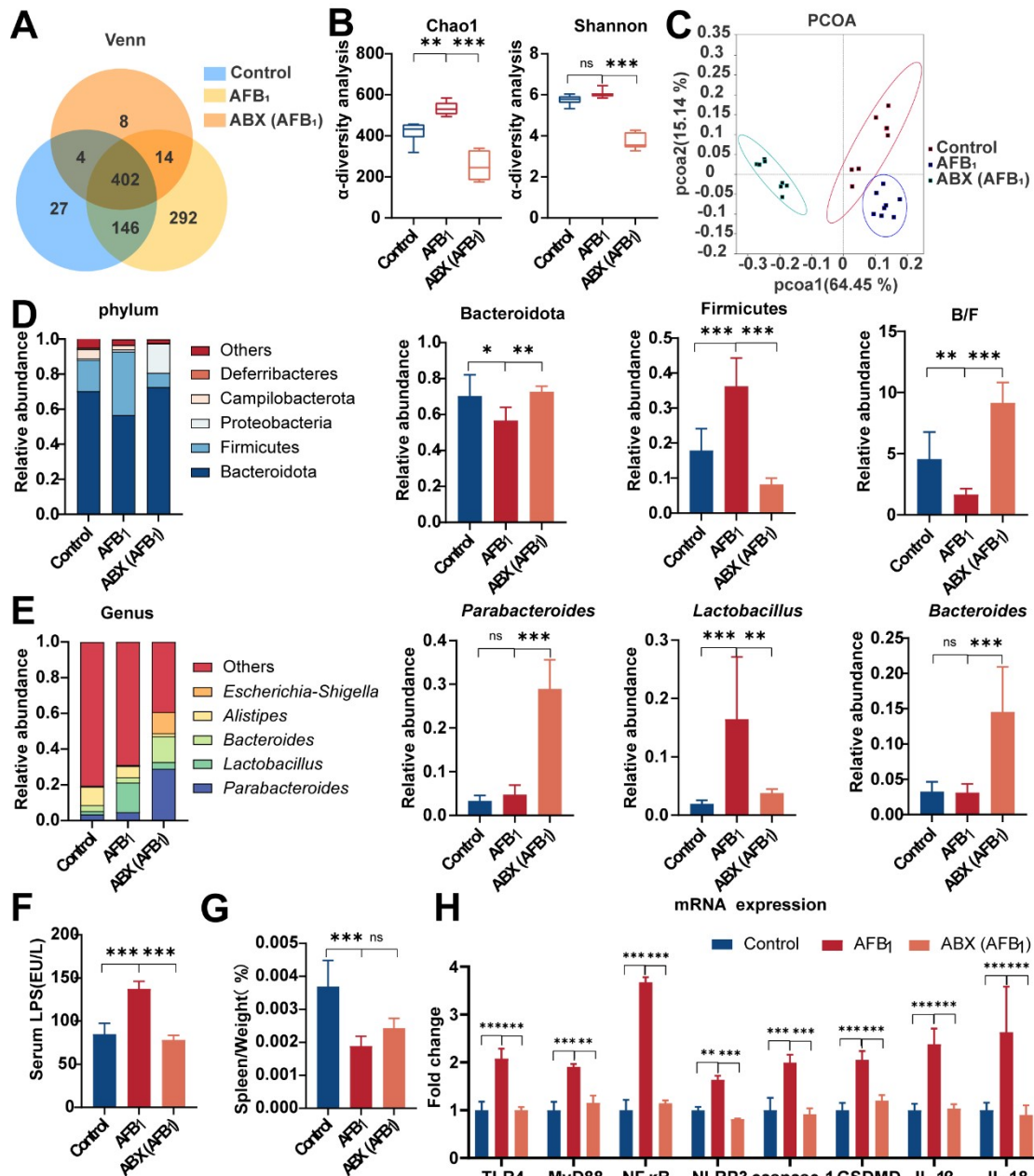
Supplemental figure 3. Antibiotic treatment reversed AFB₁-induced splenic pyroptosis mediated by disruption of gut microbiota. **(A)** OTU Venn diagram ($n = 7$). **(B)** Chao1 and Shannon indices in alpha diversity analysis ($n = 7$). **(C)** PCoA of weighted Unifrac distance matrix in inter-group distribution ($n = 8$). **(D)** The gut microbiota composition at the phylum level ($n = 7$). **(E)** The gut microbiota composition at the genus level ($n = 7$). **(F)** ELISA measurement of LPS levels in serum ($n = 5$). **(G)** Spleen index ($n = 6$). **(H)** Relative mRNA expression of TLR4, MyD88, NF-κB, NLRP3, caspase-1, GSDMD, IL-1β, and IL-18 ($n = 4$). Values are means ± SD. The statistical significance was determined using two-way ANOVA followed by post hoc

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46 test (H). The significance values are represented as follows: ns (not significant) for $p >$
47 0.05, * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

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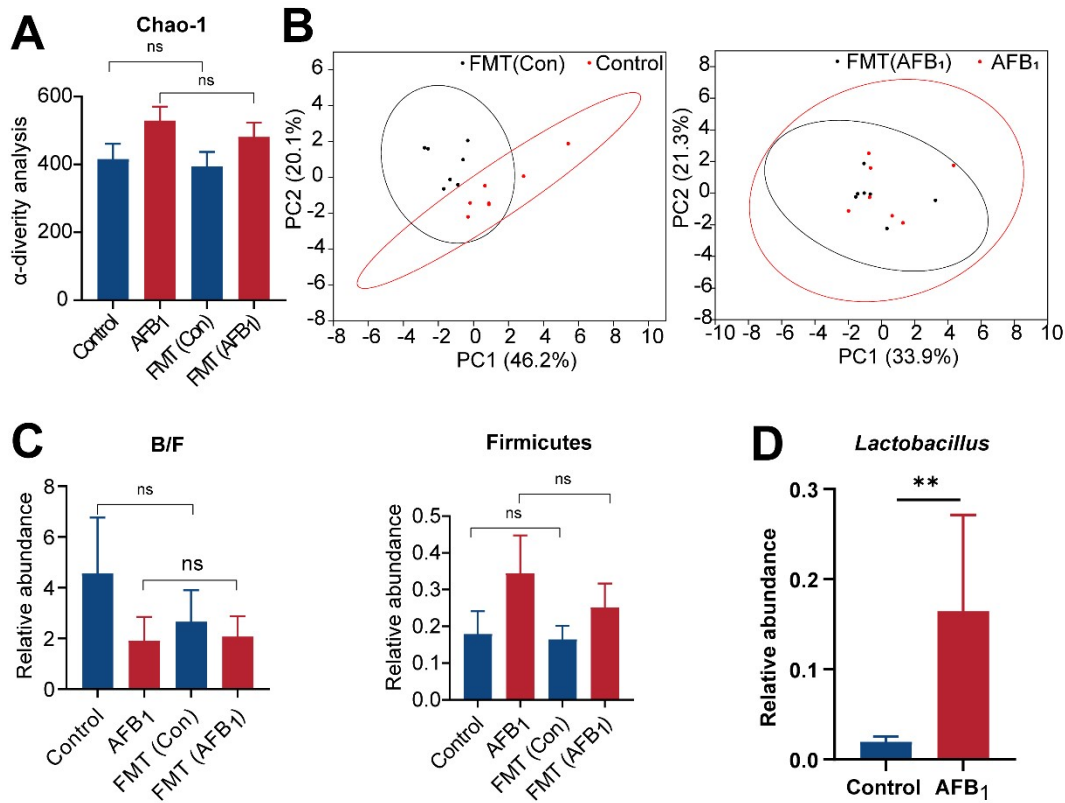
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50 **Supplementary figure 4.** Gut microbiota from the control group and AFB_1 group have
51 been colonized in the colon of recipient mice. **(A)** Chao1 index ($n = 7$). **(B)** Principal
52 component analysis (PCA) ($n = 7$)**. (C)** The ratio of Bacteroidota to Fimicutes at the
53 phylum level ($n = 7$). **(D)** The abundance of *lactobacillus*. Values are means ± SD. The
54 statistical significance was determined using two-way ANOVA followed by post hoc
55 test (A, C) and student t-tests (D). The significance values are represented as follows:
56 ns (not significant) for $p > 0.05$, * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

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58 **Supplementary table 1**

59 Primer sequences used for RT-qPCR

Gene	Direction	Primer sequence (5′–3′)

TLR4	Forward primer	5'-TGA CCA CCC ATA TTG CCT ATA C-3'
TLR4	Reverse primer	5'-TGA TGG TGT GAG CAG GAG AG-3'
MyD88	Forward primer	5'-CGG CAA CTA GAA CAG ACA GAC TAT CG-3'
MyD88	Reverse primer	5'-TCT CAA TTA GCT CGC TGG CAA TGG-3'
NF-κB	Forward primer	5'-GAA CGA TAA CCT TTG CAG GC-3'
NF-κB	Reverse primer	5'-TTT CGA TTC CGC TAT GTG TG-3'
NLRP3	Forward primer	5'-ATT ACC CGC CCG AGA AAG G-3'
NLRP3	Reverse primer	5'-TCG CAG CAA AGA TCC ACA CAG-3'
Caspase-1	Forward primer	5'-ACA GCT CTG GAG ATG GTG A-3'
Caspase-1	Reverse primer	5'-GGT CCC ACA TAT TCC CTC CT-3'
GSDMD	Forward primer	5'-CCA TCG GCC TTT GAG AAA GTG-3'
GSDMD	Reverse primer	5'-ACA CAT GAA TAA CGG GGT TTC C-3'
IL-18	Forward primer	5'-GAC TCT TGC GTC AAC TTC AAG G-3'
IL-18	Reverse primer	5'-CAG GCT GTC TTT TGT CAA CGA-3'
IL-1β	Forward primer	5'-TCG CTC AGG GTC ACA AGA AA-3'
IL-1β	Reverse primer	5'-CAT CAG AGG CAA GGA GGA AAC-3'
GADPH	Forward primer	5'-AGG TCG GTG TGA ACG GAT TTG-3'
GADPH	Reverse primer	5'-GGG GTC GTT GAT GGC AAC A-3'

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61 **Supplementary table 2**

62 Histopathological scoring of spleen tissue from different groups

Degree Rating (1-10) / Group	Blurring at the Junction of Red and White Marrow	Scattering in Cell Distribution	Severity of Cell Congestion
Control	6	4	5
AFB$_1$	7	9	8
ABX (Con)	5	4	3
ABX (AFB$_1$)	5	5	4
FMT (Con)	5	4	3
FMT (AFB$_1$)	6	7	7
SFF (Con)	3	4	3
SFF (AFB$_1$)	6	8	7

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Supplementary table 3

ANOVA with an appropriate post hoc test for Figure 5B

Significance / Figure 5	main effects		interactions	post hoc test	
	FMT vs SFF	Con vs AFB$_1$		FMT(Con) vs FMT(AFB$_1$)	SFF(Con) vs SFF(AFB$_1$)
(B) Chao-1	F =7.895 P < 0.05	F = 2.634 P > 0.05	F = 0.316 P > 0.05	P > 0.05	P > 0.05
(B) Shannon	F = 3.652 P > 0.05	F = 18.442 P < 0.05	F = 1.989 P > 0.05	P < 0.05	P > 0.05

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Supplementary table 4

ANOVA with an appropriate post hoc test for Figure 6

Significance / Figure 6	main effects		interactions	post hoc test	
	FMT vs SFF	Con vs AFB$_1$		FMT(Con) vs FMT(AFB$_1$)	SFF(Con) vs SFF(AFB1)
(A) Spleen/Weight (%)	F = 0.409 P > 0.05	F = 63.603 P < 0.05	F = 24.054 P < 0.05	P < 0.05	P > 0.05
(B) Histopathological score	F = 0.125 P > 0.05	F = 45.125 P < 0.05	F = 1.125 P > 0.05	P < 0.05	P < 0.05
(C) CD4/CD8	F = 4.549 P > 0.05	F = 42.314 P < 0.05	F = 0.292 P > 0.05	P < 0.05	P < 0.05
(D) TLR4 mRNA expression	F = 70.170 P < 0.05	F = 1.376E3 P < 0.05	F = 70.170 P < 0.05	P < 0.05	P < 0.05
(D) MyD88 mRNA expression	F = 26.483 P < 0.05	F = 379.401 P < 0.05	F = 26.483 P < 0.05	P < 0.05	P < 0.05

(D) NF-κB mRNA expression	F = 0.158 $P > 0.05$	F = 618.503 $P < 0.05$	F = 0.158 $P > 0.05$	$P < 0.05$	$P < 0.05$
(D) NLRP3 mRNA expression	F = 1.744 $P > 0.05$	F = 56.618 $P < 0.05$	F = 1.744 $P > 0.05$	$P < 0.05$	$P < 0.05$
(D) Caspase-1 mRNA expression	F = 44.079 $P < 0.05$	F = 155.801 $P < 0.05$	F = 44.079 $P < 0.05$	$P < 0.05$	$P < 0.05$
(D) GSDMD mRNA expression	F = 6.546 $P < 0.05$	F = 47.164 $P < 0.05$	F = 6.546 $P < 0.05$	$P < 0.05$	$P < 0.05$
(D) IL-1β mRNA expression	F = 0.041 $P > 0.05$	F = 91.684 $P < 0.05$	F = 0.041 $P > 0.05$	$P < 0.05$	$P < 0.05$
(D) IL-18 mRNA expression	F = 12.963 $P < 0.05$	F = 62.128 $P < 0.05$	F = 13.013 $P < 0.05$	$P < 0.05$	$P < 0.05$
(E) TLR4 Protein expression	F = 63.408 $P < 0.05$	F = 227.933 $P < 0.05$	F = 63.408 $P < 0.05$	$P < 0.05$	$P < 0.05$
(E) NF-κB Protein expression	F = 3.660 $P > 0.05$	F = 40.392 $P < 0.05$	F = 3.660 $P > 0.05$	$P < 0.05$	$P < 0.05$
(E) P-NF-κB Protein expression	F = 93.262 $P < 0.05$	F = 254.823 $P < 0.05$	F = 93.262 $P < 0.05$	$P < 0.05$	$P < 0.05$
(E) NLRP3 Protein expression	F = 12.491 $P < 0.05$	F = 90.092 $P < 0.05$	F = 12.491 $P < 0.05$	$P < 0.05$	$P < 0.05$
(E) Caspase-1 Protein expression	F = 11.663 $P < 0.05$	F = 21.402 $P < 0.05$	F = 11.663 $P < 0.05$	$P < 0.05$	$P < 0.05$
(E) Pro-IL-1β Protein expression	F = 2.724 $P > 0.05$	F = 28.929 $P < 0.05$	F = 2.724 $P > 0.05$	$P < 0.05$	$P < 0.05$
(E) IL-1β Protein expression	F = 4.956 $P > 0.05$	F = 18.003 $P < 0.05$	F = 4.956 $P > 0.05$	$P < 0.05$	$P > 0.05$
(E) Pro-IL-18 Protein expression	F = 7.609 $P < 0.05$	F = 69.655 $P < 0.05$	F = 7.609 $P < 0.05$	$P < 0.05$	$P < 0.05$
(E) IL-18 Protein expression	F = 10.074 $P < 0.05$	F = 99.479 $P < 0.05$	F = 10.074 $P < 0.05$	$P < 0.05$	$P < 0.05$

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