Supplementary Materials

1.1 Subacute toxicity experiment

Building upon the research methodology outlined by Ramesh et al.[1], C57BL/6J mice were assigned to seven experimental groups based on different EGCG doses (10, 20, 25, 50, 100, 200, and 400 mg kg-[1]), with three mice per group. The mice were treated with a 14-day continuous gavage of PBS containing EGCG. Clinical signs related to mortality were systematically assessed, and dynamic changes in body weight and food intake rate were closely monitored.

1.2 Establishment of the ARDS model

In accordance with established methods from previous literature[2], the ARDS models induced by intratracheal instillation, intraperitoneal injection, and nasal instillation of LPS were compared. The model construction details are as follows: For intratracheal instillation, LPS was administered at doses of 5 mg kg^{-1}, 10 mg kg^{-1}, and 20 mg kg^{-1}; for intraperitoneal injection, doses were 10 mg kg^{-1}, 20 mg kg^{-1}, and 40 mg kg^{-1}; and for nasal instillation, doses were 5 mg kg^{-1}, 10 mg kg^{-1}, and 20 mg kg^{-1}. Mice were sacrificed for sample collection 24 hours post-treatment. Survival curves were plotted, and pathological morphological changes were observed throughout the study, serving as criteria for confirming the successful establishment of the ALI model.

1.3 Lung wet/dry (W/D) ratio

The W/D ratio protocol described by Wang et al.[3] was followed. Twenty-four hours after treatment, the mice were sacrificed, and the entire lungs were excised for wet weight measurement. The lungs were then placed in an oven at 60°C for 72 hours to obtain the dry weight. Pulmonary edema was assessed by calculating the W/D ratio.

1.4 Inflammatory cell counting and protein content determination by the BCA method

Cell pellets obtained from the centrifuged BALF were collected. A Mindray hematology analyzer (Model: BC-7500) was used to accurately count the total number of white blood cells,

monocytes, and polymorphonuclear cells.

Following the approach of Wang et al.[3], a BCA kit (Cat. No. P0010) was employed to quantify the total protein in the supernatant and BALF. The protocol was strictly followed according to the manufacturer's instructions, with absorbance measured at 562 nm using a Bio-Tek Synergy H1 Multi-Mode Microplate Reader.

1.5 Fecal DNA determination

Based on the detailed methodology outlined by Guo et al.[4], a 200 mg portion of feces was weighed and placed into a 2 mL centrifuge tube. Reagent A from the fecal examination kit (Cat. No. D2700-50T) was added (1 mL), and the test tube was inverted to ensure thorough mixing. The tube was then incubated in a 70°C water bath for 20 minutes, with repeated inversions for proper mixing during incubation. Following incubation, the tube was centrifuged at 12,000 rpm for 5 minutes, and the supernatant was discarded. The precipitate was resuspended by adding 800 μL of reagent B, followed by the addition of 2 μL of RNase A. After mixing by tube inversion, the tube was left at room temperature for 15 minutes. Then, 20 μL of proteinase K was added, and the tube was inverted to ensure proper mixing before being incubated at 65°C for 30 minutes with multiple inversions. Centrifugation at 12,000 rpm for 2 minutes followed, and the supernatant was transferred to a fresh 2 mL centrifuge tube. Reagent C (800 μL) was added, and the mixture was inverted. It was then transferred to a DNA adsorption column. After standing for 2 minutes, centrifugation at 12,000 rpm for 1 minute was performed. The waste liquid in the collection tube was discarded, and the adsorption column was placed back into the collection tube. Washing solution I (600 μL) was added, and centrifugation at 12,000 rpm for 1 minute was performed. The waste liquid was discarded, and the adsorption column was placed back in the collection tube. Washing solution II (600 μL) was added, followed by centrifugation at 12,000 rpm for 1 minute. After discarding the waste liquid, the adsorption column was placed back into the collection tube and centrifuged at 12,000 rpm for 1 minute. The adsorption column was removed and air-dried at room temperature for 2 minutes before being transferred to a new centrifuge tube, and 60 μL of elution buffer was added. After standing for 5 minutes at room temperature, centrifugation at 12,000 rpm for 1 minute was performed. The liquid in the centrifuge tube represented the fecal microbial DNA solution. The DNA concentration was measured using a NanoDrop Eight nucleic acid micro-detector (Thermo Fisher).

1.6 Growth, preparation, and detection of AKK.

Capitalizing on the structured and clearly defined methodology proposed by Han et al.[5], Akkermansia muciniphila (A. muciniphila, strain number ATCC BAA-835 AKK) was anaerobically cultured at 37°C in chopped meat carbohydrate (CMC) broth medium. The formula for CMC medium per liter includes 30.0 g of Casitone (trypticase peptone), 10.0 g of Beef Extract, 5.0 g of Yeast Extract, 5.0 g of Dipotassium Phosphate, 0.5 g of L-Cysteine·HCl, 0.0005 g of Resazurin, 4.0 g of Glucose, 1.0 g of Cellobiose, 1.0 g of Maltose, and 1.0 g of Starch. To prepare the medium, 57.5 g of these substances were dissolved in 1 L of distilled or deionized water. The solution was heated to boiling and maintained for over 1 minute. The medium was then dispensed into test tubes, with an appropriate amount of chopped meat (beef particles, approximately one-third the height of the liquid) added. The tubes were autoclaved at 121°C for 30 minutes. After cooling to below 50°C, under aseptic conditions, one vial of hemin chloride (0.5 mg) and one vial of vitamin K1 (5 mg) were added per 100 mL of medium. After culturing A. muciniphila in an anaerobic chamber (AKD63s-03, Changzhou Pule Experiment System Co., Ltd.) for 72 hours, the culture was centrifuged at 8000 g for 10 minutes. The bacteria were washed twice with sterile PBS, and the concentration was adjusted to 4×10^8 colony-forming units per milliliter (cfu/mL) using a turbidity tube (Bikeman, product number: BC1114) for subsequent intragastric administration.

The detection of AKK in mouse feces was conducted following the protocols in previous studies. Mouse feces were collected and centrifuged at 600 g for 5 minutes, and the supernatant was collected. AKK nucleic acid was detected using the AKK nucleic acid detection kit (product number: BNCC 372727). The detection steps were as follows: A 25-μL reaction mixture was prepared, comprising 15 μL of the centrifuged supernatant, 1 μL of the template, and 9 μL of DEPC-treated water. The mixture was thoroughly mixed and briefly centrifuged. The reaction conditions were as follows: an initial step at 95°C for 1 minute, followed by 40 cycles of 95°C for 10 seconds and 54°C for 30 seconds. The detection was carried out using the ABI 7500 PrepStation (Applied Biosystems; Thermo Fisher Scientific, Inc.). Using a mouse positive control as an internal reference, the relative levels of AKK were quantitatively analyzed by the $2^{-\Delta\Delta Ct}$ method.

1.7 Quantitative analysis method for SCFAs profile based on gas chromatography-mass spectrometry (GC-MS)

Utilizing the meticulously designed and clearly defined methodology proposed by Hildebrand et al.[6], 20 mg of fecal samples, 20 mg of lung tissue samples, or 100 μL of serum samples were accurately weighed or measured. The samples were dissolved, homogenized, and then centrifuged at 13,000 × g for 15 minutes. Next, 200 μL of the supernatant was taken, diluted 1:1, and centrifuged again at 13,000 × g for 5 minutes at 4°C. Finally, 120 μL of the supernatant was transferred to a sample vial. GC-MS detection was performed using an Agilent 7890B gas chromatography system with a DB-FATWAX UI column (30 m × 0.25 mm, 0.25 μm, part number G3903-63008). The inlet temperature was set at 250°C with a split ratio of 20:1, and helium was used as the carrier gas at a flow rate of 40 cm/s at 80°C. The oven temperature was initially set to 80°C and held for 1 minute, followed by an increase to 200°C at a rate of 10°C/min. A 1 μL injection volume was used, and mass spectrometry conditions were set with a flow rate of 1 mL/min. After completing the operations, Qualitative Navigator B.08.00 software was employed to perform a quantitative analysis of the short-chain fatty acid components.

Supplementary Table

Table. S.1 Primer sequences of RT-PCR test.

Name	Species	Forward primer (5′-3′)	Reverse primer (5′-3′)
IL-1β	Mouse	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
IL-6	Mouse	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
TNF-α	Mouse	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC
Occludin	Mouse	GCGAGGAGCTGGAGGAGGAC	CGTCGTCTAGTTCTGCCTGTAAGC
Zo-1	Mouse	AACCCGAAACTGATGCTGTGGATAG	CGCCCTTGGAATGTATGTGGAGAG
GAPDH	Mouse	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG

Table. S.2 primary and secondary antibodies.

Antibody Name	Catalog Number	Company Name	Location
ZO-1 Rabbit Polyclonal Antibody	PTG-21773-1-AP-50ul	proteintech	Shanghai, China

Occludin Polyclonal antibody	13409-1-AP-50ul	proteintech	Shanghai, China
phospho-STAT1 antibody	AF3300	Affinity Biosciences	Jiangsu, China
phospho-STAT2 antibody	AF3653	Affinity Biosciences	Jiangsu, China
STAT3 antibody	AF6294	Affinity Biosciences	Jiangsu, China
phospho-STAT3 antibody	AF3293	Affinity Biosciences	Jiangsu, China
phospho-STAT4 antibody	AF3441	Affinity Biosciences	Jiangsu, China
phospho-STAT5 antibody	AF3304	Affinity Biosciences	Jiangsu, China
JAK1 antibody	AF5012	Affinity Biosciences	Jiangsu, China
phospho-JAK1 antibody	AF2012	Affinity Biosciences	Jiangsu, China
JAK2 antibody	AF6022	Affinity Biosciences	Jiangsu, China
phospho-JAK2 antibody	32901T	Cell Signaling Technology	Danvers, Massachusetts
tubulin	T0023-1	Affinity Biosciences	Jiangsu, China
NF-kB p65 Antibody-C-terminal	AF5006-50ul	Affinity Biosciences	Jiangsu, China
Phospho-NF-kB p65 (Ser536) Antibody	AF2006	Affinity Biosciences	Jiangsu, China
Phospho-c-Jun (Ser73) Antibody	AF3095	Affinity Biosciences	Jiangsu, China
c-Jun Ab	AF6090	Affinity Biosciences	Jiangsu, China
ERK1/2 Ab	AF0155-50ul	Affinity Biosciences	Jiangsu, China
P-ERK1/2(Thr202/Tyr204) Ab	AF1015-50ul	Affinity Biosciences	Jiangsu, China
Phospho-p38 MAPK (Thr180/Tyr182) Antibody	AF4001-50ul	Affinity Biosciences	Jiangsu, China
p38 MAPK Antibody	AF6456	Affinity Biosciences	Jiangsu, China
Phospho-PI3K p85 (Tyr458)[Tyr467]/p55 (Tyr199) Antibody	AF3242	Affinity Biosciences	Jiangsu, China
PI3K p85 alpha Antibody	AF6241	Affinity Biosciences	Jiangsu, China
pan-AKT1/2/3 Antibody	AF6261-50ul	Affinity Biosciences	Jiangsu, China
Phospho-Akt(Ser473) Ab	AF0016-50ul	Affinity Biosciences	Jiangsu, China
Phospho-HIF1A (Ser641/Ser643) antibody	AF0062	Affinity Biosciences	Jiangsu, China
HIF1A Antibody	AF1009	Affinity Biosciences	Jiangsu, China
GSK3 beta Antibody	AF5016	Affinity Biosciences	Jiangsu, China
P-GSK3 beta (Ser9) Ab	AF2016	Affinity Biosciences	Jiangsu, China
Multi-rAb HRp-Goat anti-Mouse Recombinant (H + L)	RGAM001	proteintech	Shanghai, China
Multi-rAb HRp-Goat anti-Rabbit Recombinant (H + L)	RGAR001	proteintech	Shanghai, China

Supplementary Legends

Figure S1

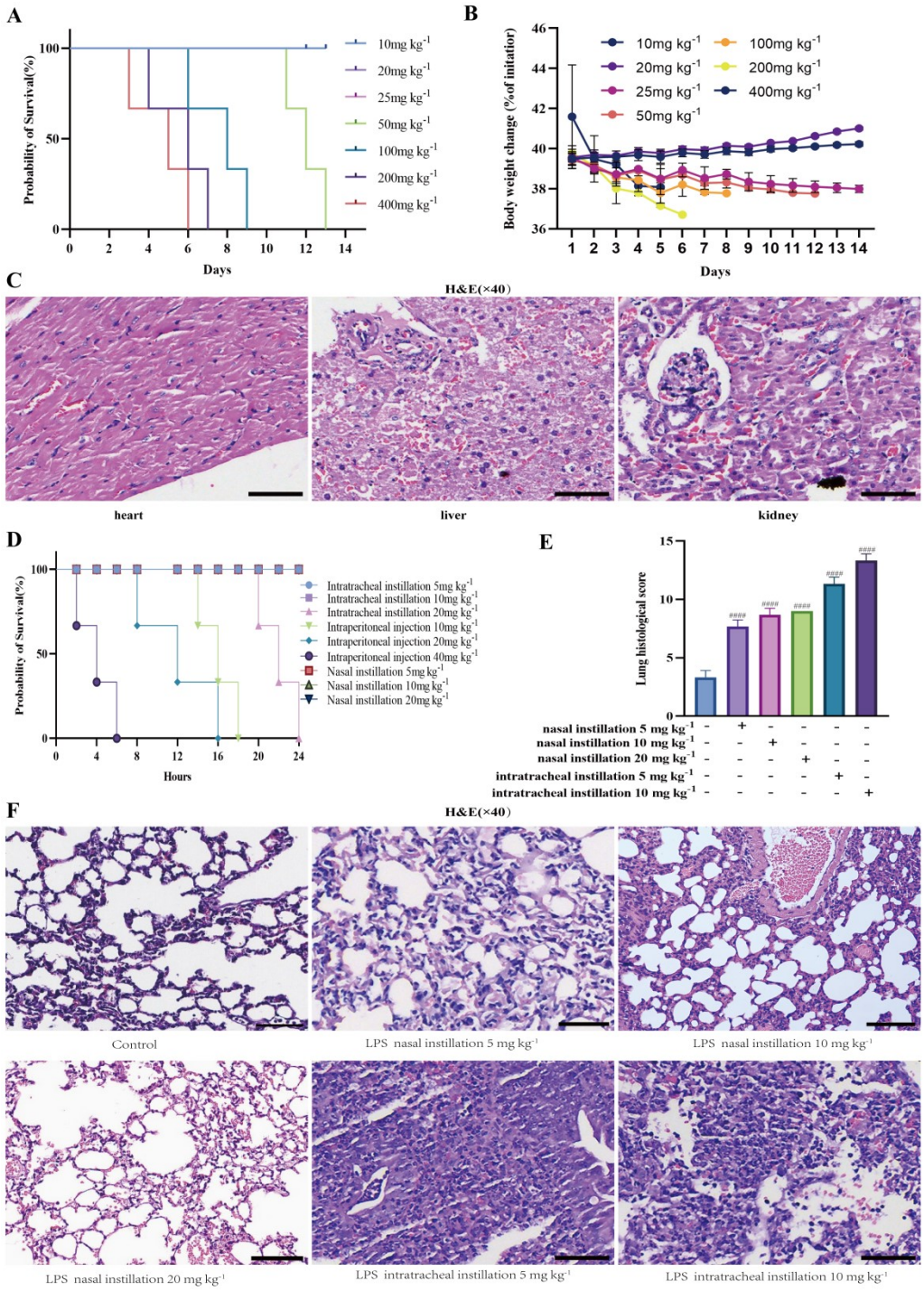

Figure S1. Dose selection of EGCG and LPS.

A) Survival rates under treatment with different doses of EGCG (N = 3). B) Daily changes in body weight after EGCG treatment. C) Pathological damage of the heart, liver, and kidneys in mice treated with EGCG at a dose of 25 mg kg^{-1}. D) Survival rates under treatment with different doses of LPS. E) Histological scores of lung tissues. F) Pathological changes in the lungs after treatment with LPS at different doses and *via* different treatment methods. All data were normalized with reference to

the control group. Data are presented as Means ± SEMs (N = 3, $^{\#\#\#\#}p < 0.0001$, compared to the control group).

Figure S2

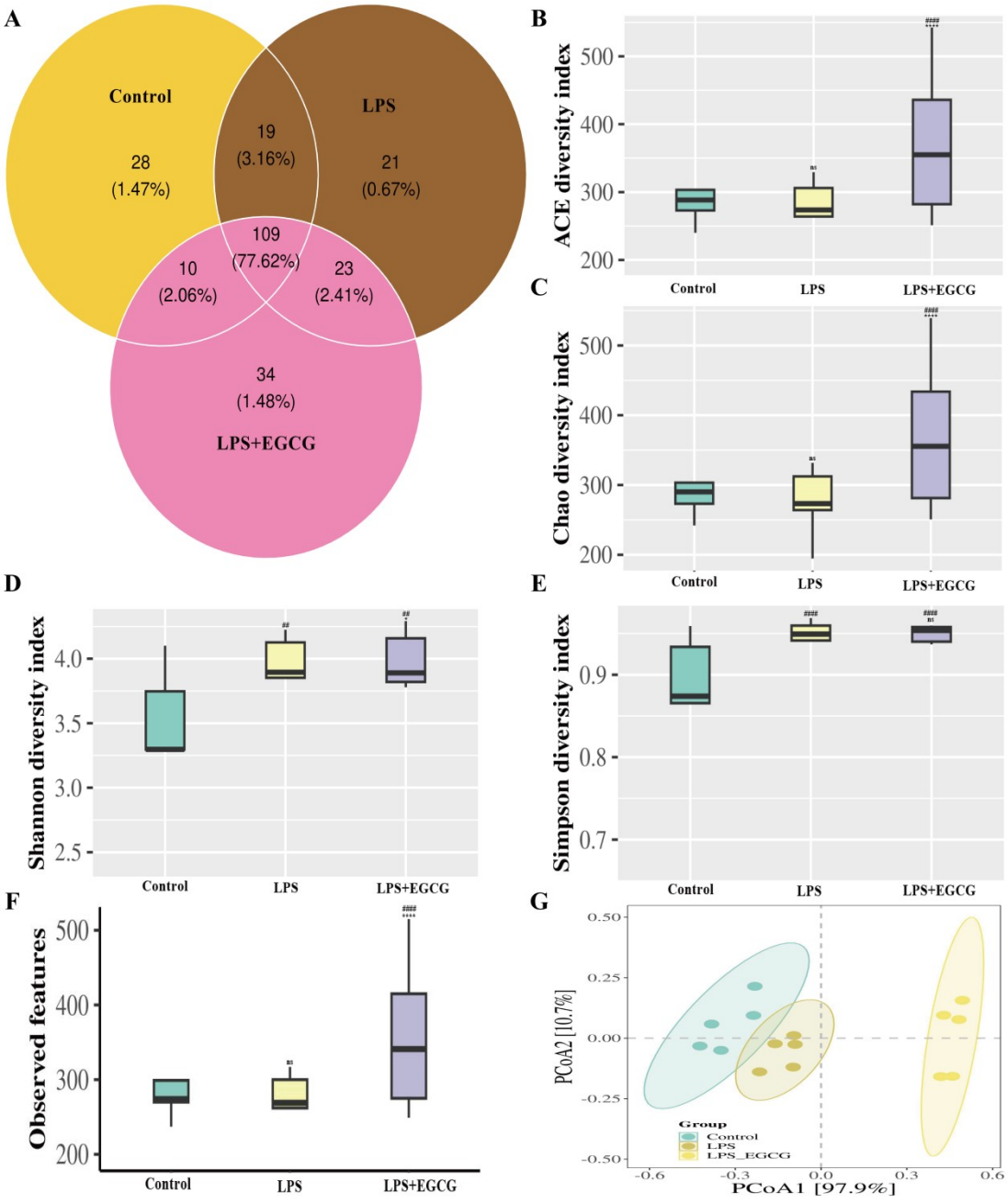

Figure S2. EGCG regulates the diversity and significance of gut microbiota in mice.

A) Venn diagram depicting the intestinal microbiota composition following treatment with EGCG and LPS. B) ACE diversity index among different groups. C) Chao diversity index among different groups. D) Observed features among different groups. E) Shannon diversity index among different groups. F) Simpson diversity index among different groups. G) Analysis of β-diversity of gut

microbiota in different experimental groups of mice based on principal coordinate analysis (PCoA).

(N=8)

Figure S3

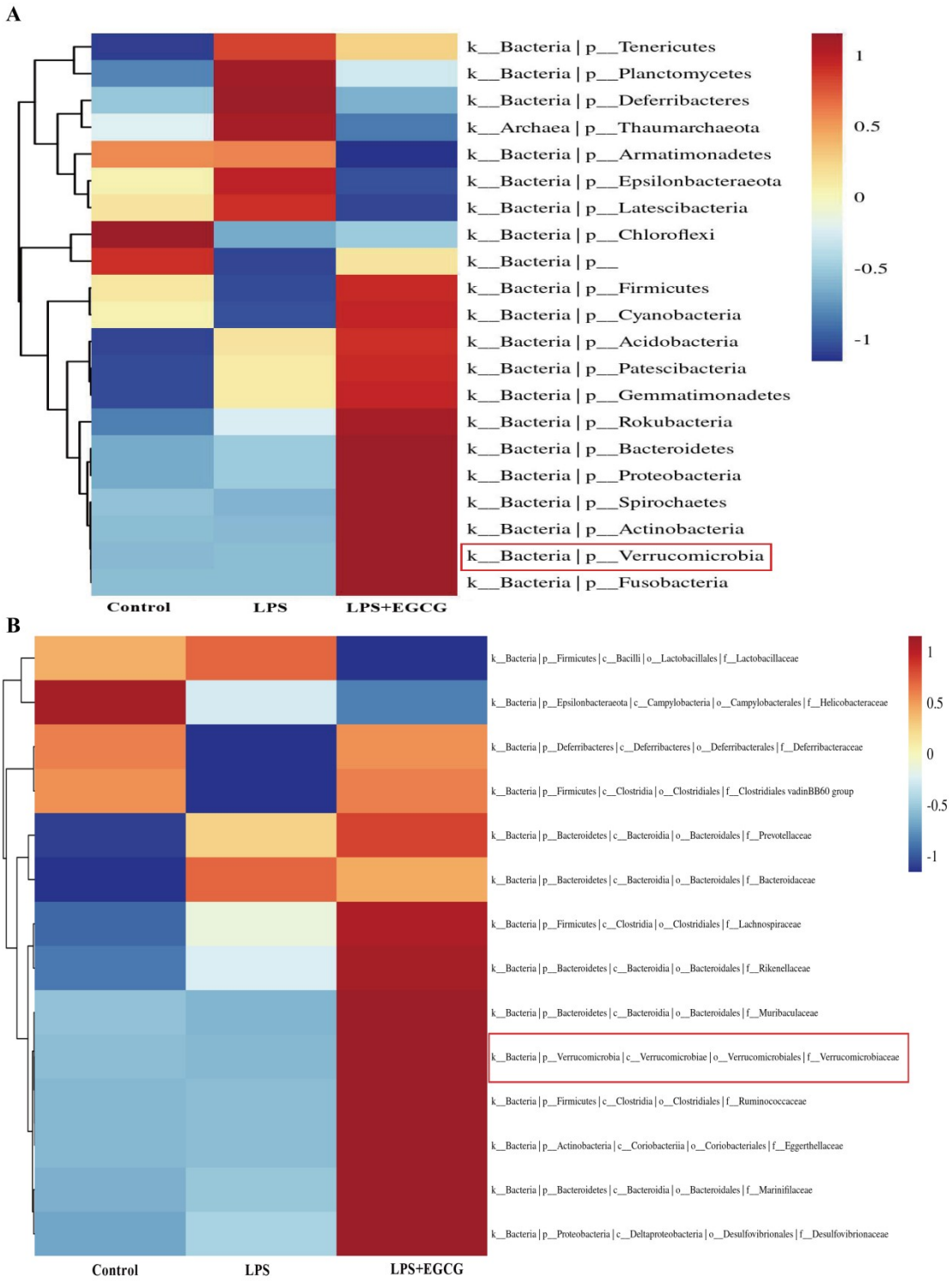

Figure S3. Mice treated with EGCG exhibit a unique gut microbial composition at the phylum and family levels.

A) Community heatmap analysis at the phylum level. B) Community heatmap analysis at the family level.

Figure S4

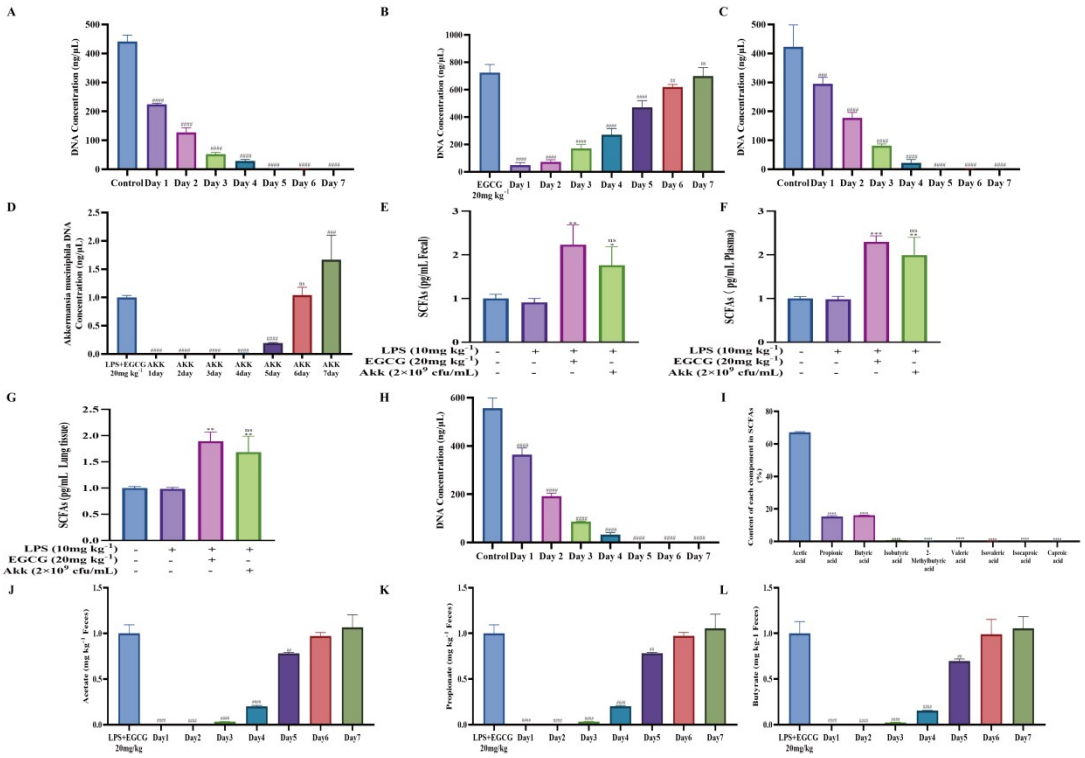

Figure S4. Monitoring the concentrations of total DNA in fecal samples, DNA in AKK bacterial solution, and SCFAs.

A) Detection of fecal DNA in mice of the LPS + FMT group (N=8, $^{\#\#\#\#}p < 0.0001$, compared to the control group). B) Detection of fecal DNA in mice of the LPS + FMT group (N=8, $^{ns}p > 0.0005$, $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$, compared to the EGCG 20 mg kg^{-1} group). C) Detection of fecal DNA in mice of the LPS + AKK group (N=8, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$, compared to the control group). D) RT-PCR was used to detect the DNA concentration of AKK bacterial solution in the feces of mice in the LPS + AKK group (N=8, $^{ns}p > 0.0005$, $^{\#\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$, compared to the LPS + EGCG 20 mg kg^{-1} group). E-G) The levels of SCFAs in feces, serum, and lung tissues were measured using mouse ELISA kits. All data were normalized with reference to the control group. Data are presented as Means ± SEMs (N=8, $^{**}p < 0.01$, $^{***}p < 0.001$, compared to the LPS-induced group; $^{ns}p > 0.05$ compared to the LPS + EGCG 20 mg kg^{-1} group). H) Detection of fecal DNA in mice of the LPS + SCFAs group (N=8, $^{\#\#\#\#}p < 0.0001$, compared to the control group). I) Differences in the relative

abundance of each component of short-chain fatty acids (SCFAs) in the feces of mice in the LPS + AKK treatment group (N=8, $^{****}p < 0.0001$, compared to Acetic). J-L) The concentration of acetic acid, propanoic acid, and butanoic acid in the feces of mice in the LPS + SCFAs group was determined by GC-MS (N=8, $^{ns}p > 0.0005$, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared to the LPS + EGCG 20 mg kg^{-1} group). All data were normalized with reference to the control group or the LPS + EGCG 20 mg kg^{-1} group. Data are presented as Means ± SEMs.

Figure S5

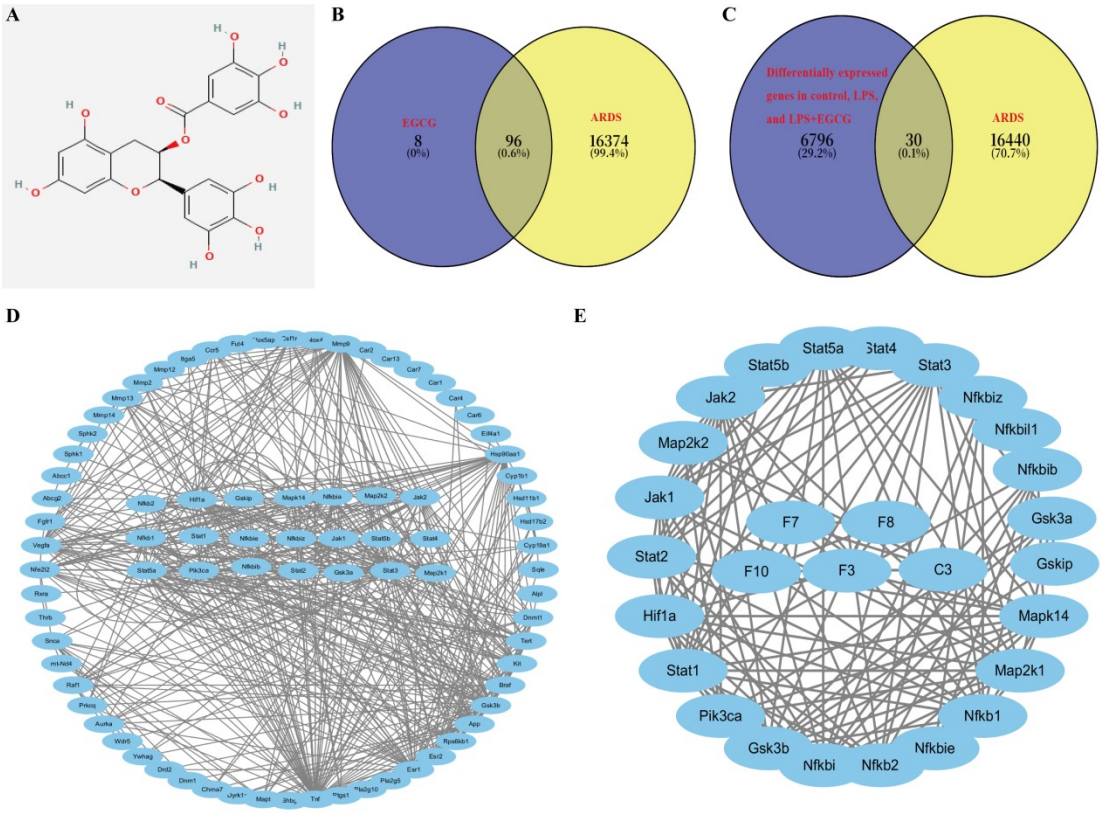

Figure S5. The PPI network and cross-genes at the interface of network pharmacology and transcriptomics.

A) The chemical structure of EGCG was retrieved in the SMILES format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). B) Venn diagram of targets related to EGCG and ARDS in network pharmacology analysis. C) Venn diagram of the differential genes in the control group, LPS group, and LPS + EGCG group and the targets related to ARDS in transcriptomic analysis. D) PPI network map constructed using the intersecting genes obtained by network pharmacology. E) PPI network map constructed using the intersecting genes obtained by transcriptomics.

Figure S6

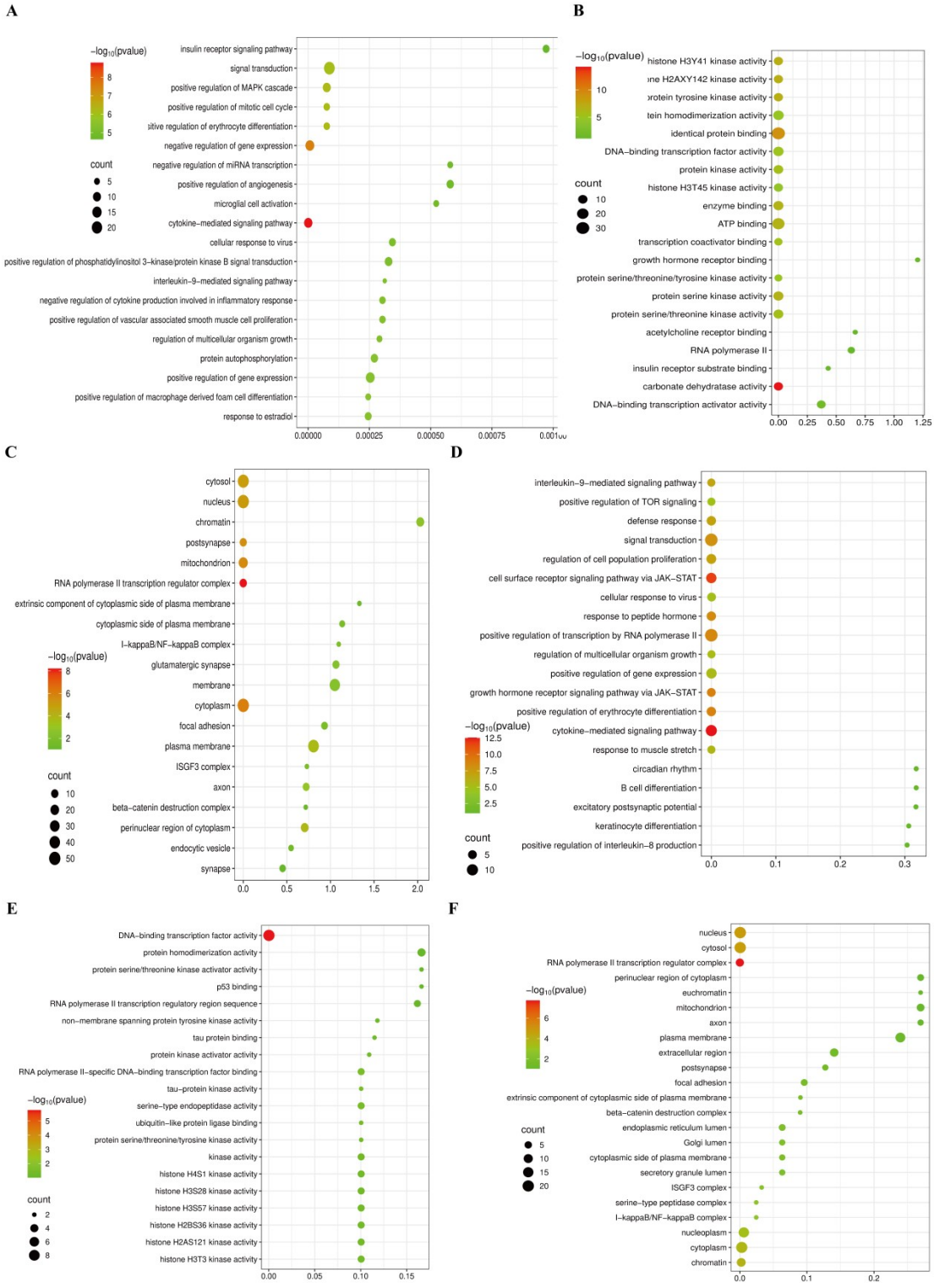

Figure S6. MetaScape analysis of network pharmacology and transcriptomics.

A-C) MetaScape analysis of network pharmacology: BP (A), MF (B), CC (C). D-F) MetaScape enrichment analysis of transcriptomics: BP (D), MF (E), CC (F).

Figure S7

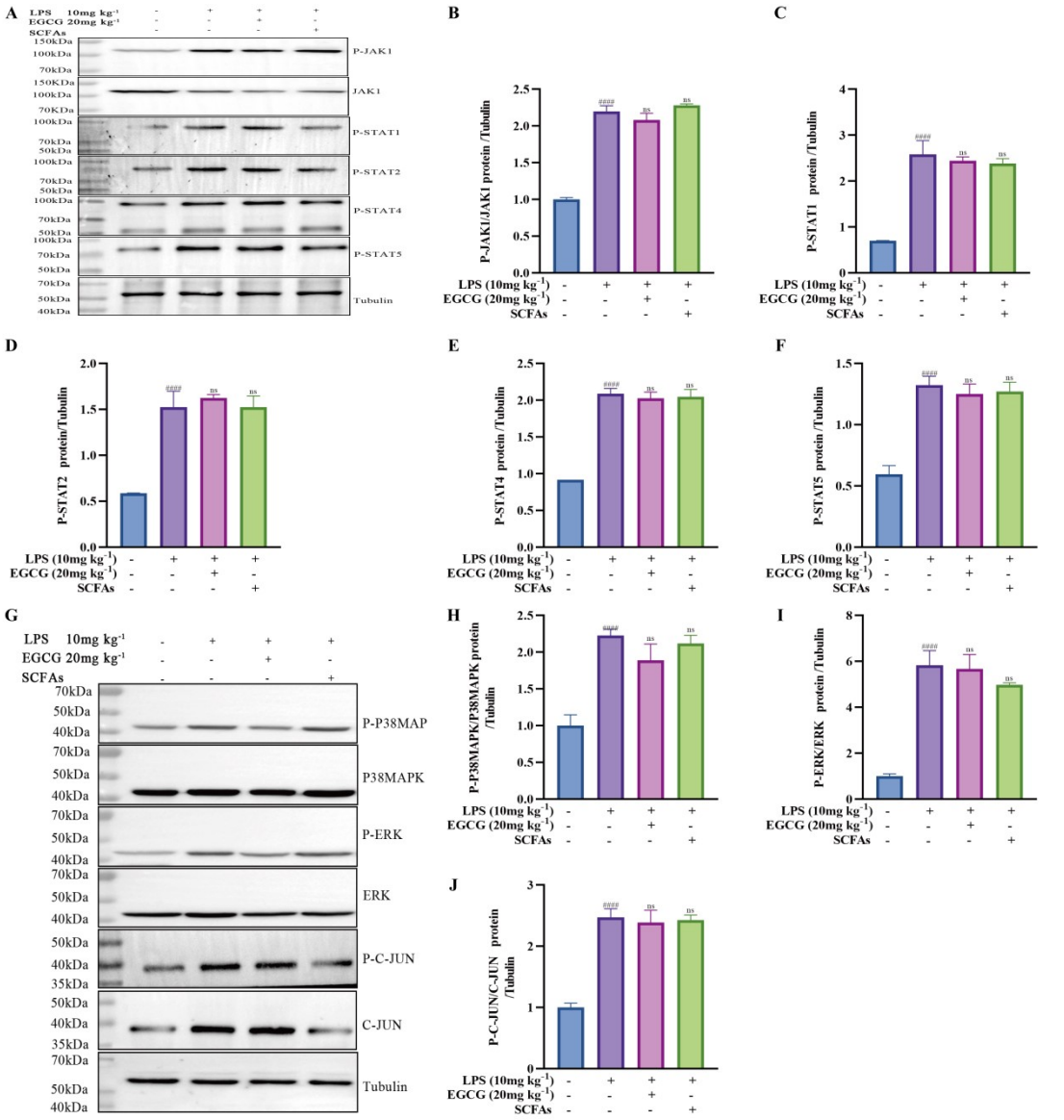

Figure S7. Detection of the activation of inflammatory signaling pathways by SCFAs.

A) The protein expression levels of phosphorylated Janus kinase 1 (P-JAK1), Janus kinase 1 (JAK1), phosphorylated Signal transducer and activator of transcription 1 (P-STAT1), phosphorylated Signal transducer and activator of transcription 2 (P-STAT2), phosphorylated Signal transducer and activator of transcription 4 (P-STAT4), and phosphorylated Signal transducer and activator of transcription 5 (P-STAT5) in the lung tissues of mice in each group were detected by Western blotting. B-F) The relative protein expression levels of P-JAK1/JAK1, P-STAT1, P-STAT2, P-STAT4, and P-STAT5 to Tubulin were measured using ImageJ software. G) The protein expression levels of phosphorylated p38 mitogen-activated protein kinase (P-P38MAPK), p38 mitogen-activated protein kinase (P38MAPK), phosphorylated extracellular signal-regulated kinase (P-ERK), extracellular signal-regulated kinase (ERK), phosphorylated c-Jun (P-C-JUN), and c-Jun (C-JUN) in the lung tissues of mice in each group were detected by Western blotting. H-J) The relative protein expression levels of P-P38MAPK/P38MAPK, P-ERK/ERK, P-C-JUN/C-JUN to Tubulin were measured using ImageJ software. All data are

presented as Means ± SEMs. (N = 8, $^{\#\#\#\#}p < 0.0001$, compared to the control group; $^{ns}p > 0.05$, compared to the LPS-induced group).

Figure S8

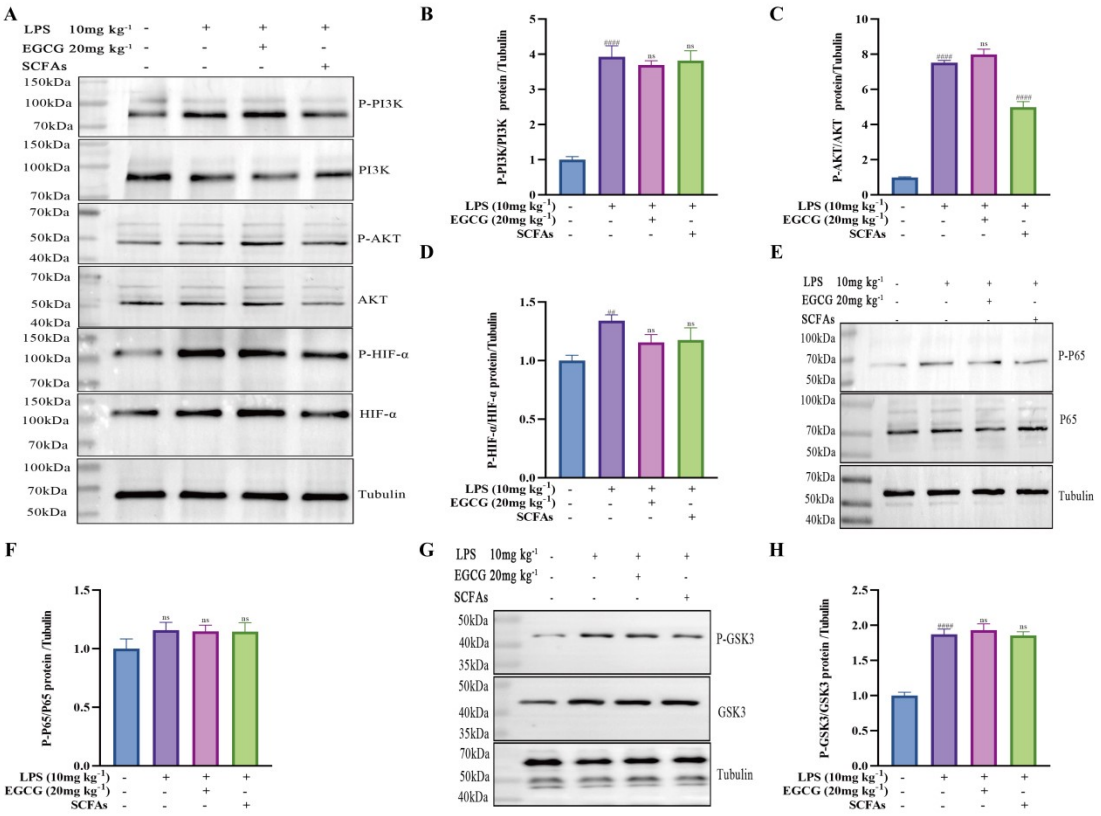

Figure S8. Detection of the activation of inflammatory signaling pathways by SCFAs.

A) The protein expression levels of phosphorylated Phosphoinositide 3-kinase (P-PI3K), Phosphoinositide 3-kinase (PI3K), phosphorylated Protein kinase B (P-AKT), Protein kinase B (AKT), phosphorylated Hypoxia-inducible factor-α (P-HIF-α), and Hypoxia-inducible factor-α (HIF-α) in the lung tissues of mice in each group were detected by Western blotting. B-D) The relative protein expression levels of P-PI3K/PI3K, P-AKT/AKT, P-HIF-α/HIF-α to Tubulin were measured using ImageJ software. E) The protein expression levels of phosphorylated nuclear factor-kappa B p65 subunit (P-P65) and nuclear factor-kappa B p65 subunit (P65) in the lung tissues of mice in each group were detected by Western blotting. F) The relative protein expression levels of P-P65/P65 to Tubulin were measured using ImageJ software. G) The protein expression levels of phosphorylated glycogen synthase kinase 3 (P-GSK3) and glycogen synthase kinase 3 (GSK3) in the lung tissues of mice in each group were detected by Western blotting. H) The relative protein expression levels of P-GSK3/GSK3 to Tubulin were measured using ImageJ software. All data are presented as Means ± SEMs. (N = 8, $^{\#\#\#\#}p < 0.0001$, compared to the control group; $^{ns}p > 0.05$, compared to the LPS-induced group).

Supplementary References

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