Supplementary materials

Supplementary Figures 1**:** Additional microbial diversity analyses

Supplementary Figure 2: Complete gel images and original blots

Supplementary Table 1: Demographics and clinical characterization of healthy population cohort.

Supplementary Table 2: Animal weights and experimental details

Supplementary Methods: Extended experimental protocols

Additional file 1: Additional microbial diversity analyses

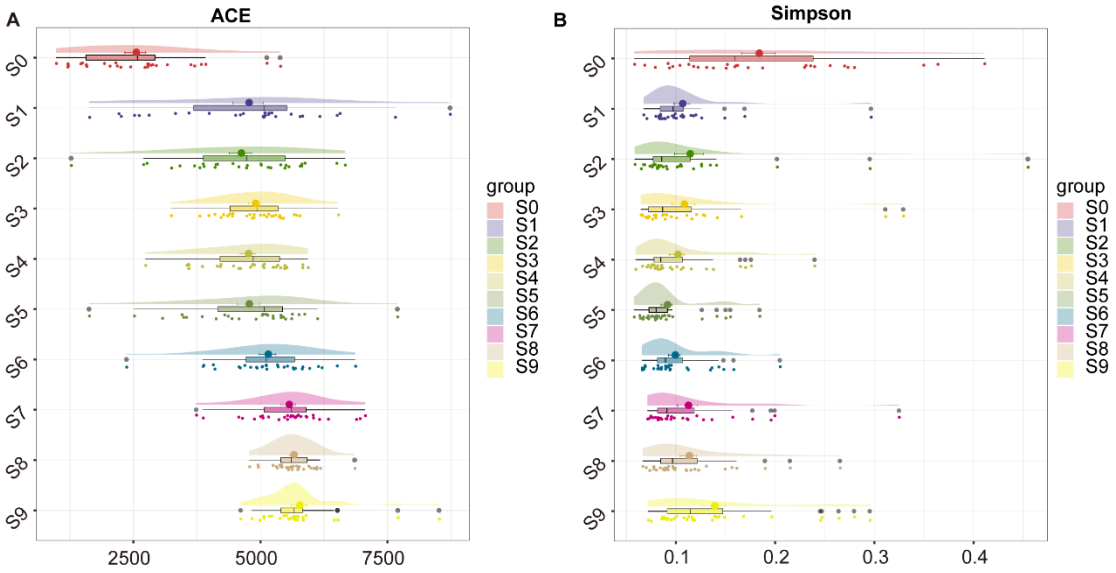


Figure S1. The core gut microbiome altered significantly during the ageing process of the healthy ageing Chinese populations. (A). The ACE index; (B). The Simpson index.

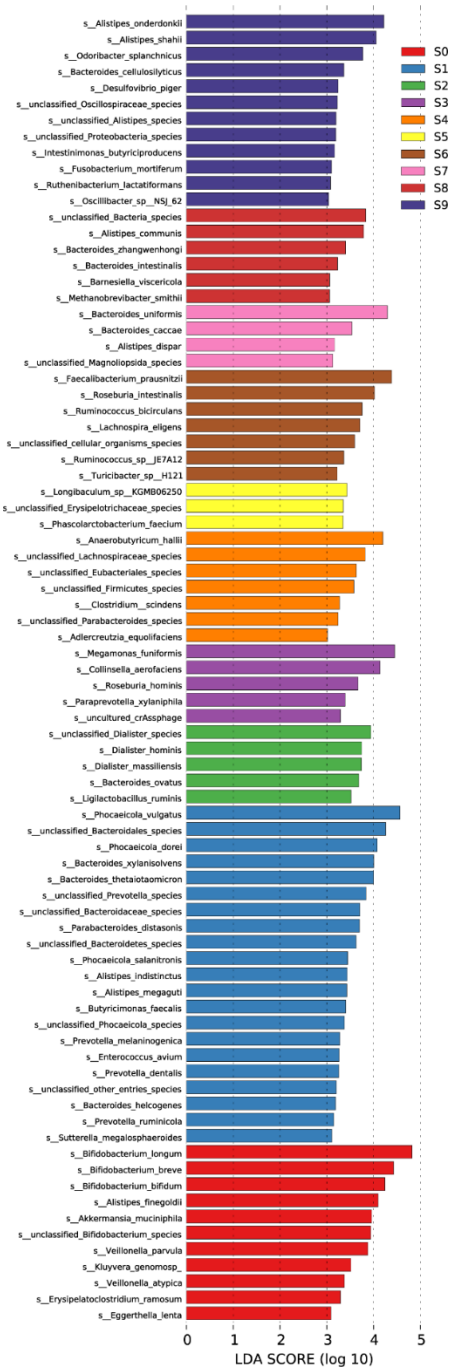


Fig.S2. The LDA results at the species level showed the potential biomarkers for healthy human aging ($p < 0.05$, LDA > 3.0).

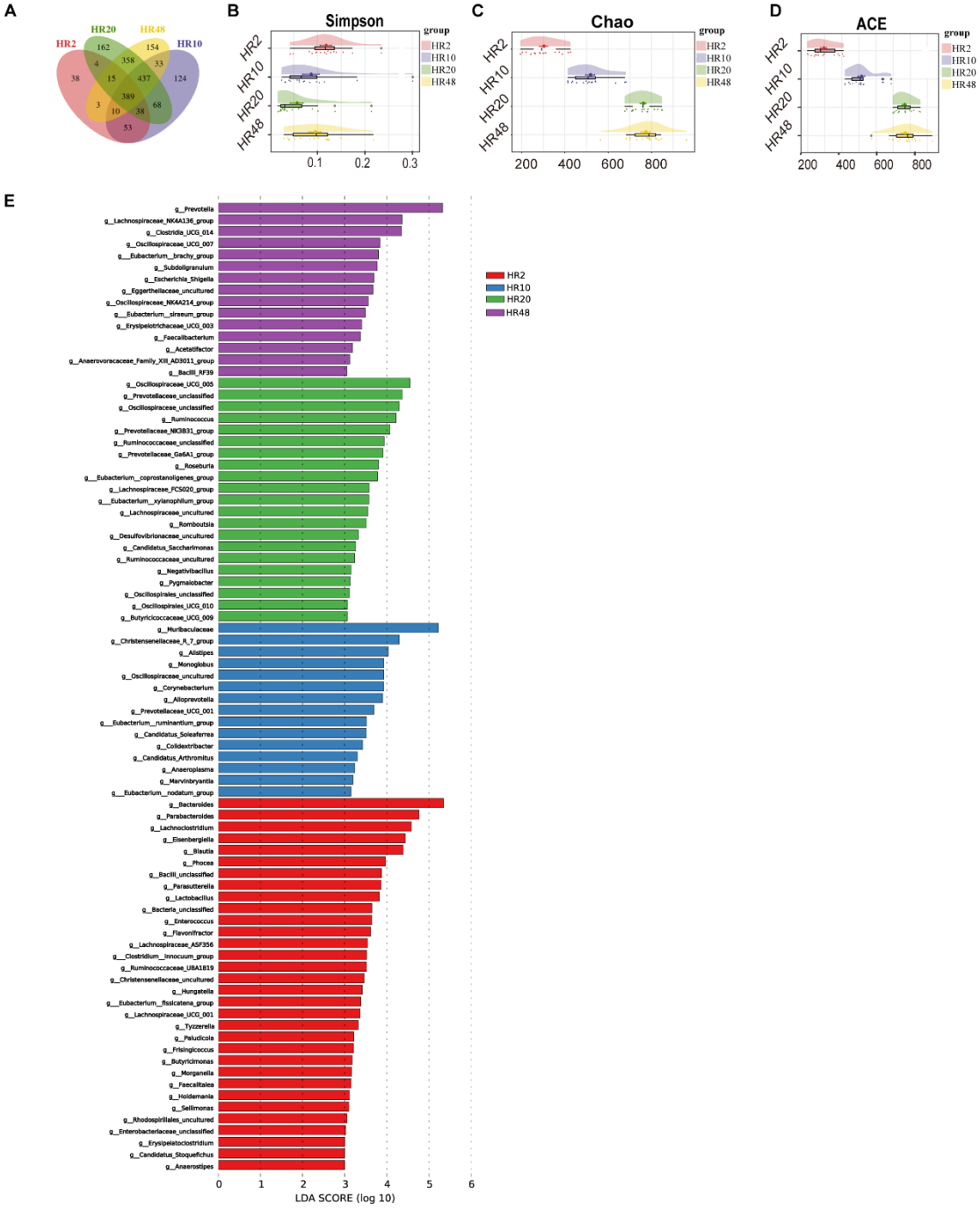


Figure S3. Characteristics of intestinal microflora in healthy rats (HR groups) during the age increasing progression. (A) The Venn diagram indicated that 389 of the 1886 OTUs were shared among the four groups. (B). The Simpson index of the healthy rat cohorts (HR cohorts). (C) Chao index and (D) ACE index. (E). The LDA results at the genus level showed crucial bacteria of gut microbiome related to aging ($p < 0.05$, LDA > 3.0). HR, healthy rat cohorts; LDA, linear discriminant analysis.

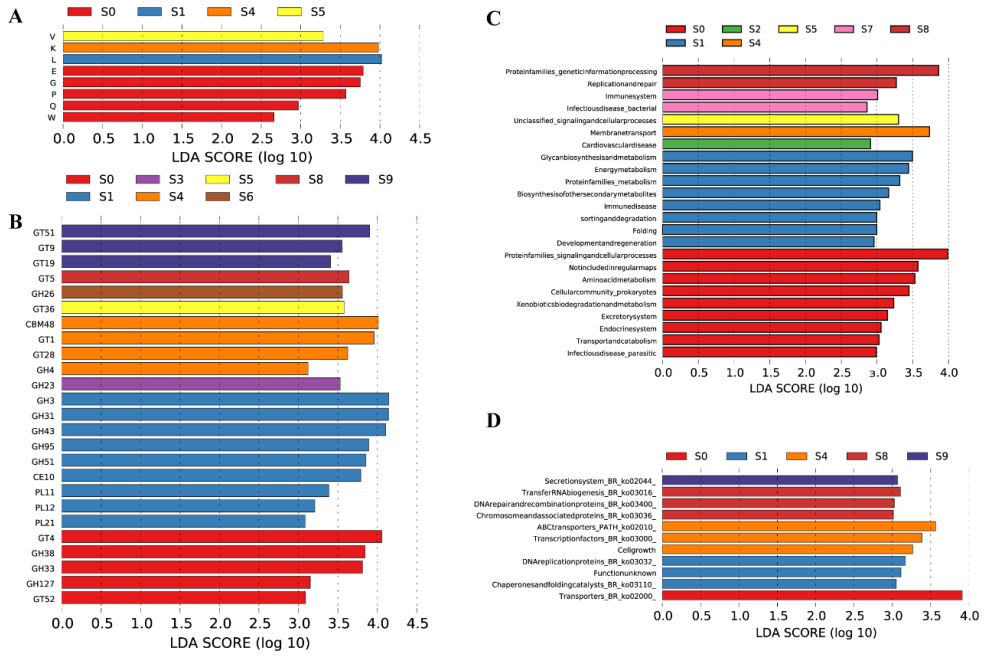


Figure S4. The parallel annotation against KEGG, CAZy, and COGusing PICRUSt2 with default parameters. (A) Functional classification of prokaryotic proteins into COG categories. Bar height represents normalized abundance. (B) Annotation of microbial Carbohydrate-Active Enzymes (CAZy) (GH: glycoside hydrolases; GT: glycosyltransferases). (C) The level2 classification of KEGG pathway analysis showed infectious disease-parasitic, excretory sytem and aminoacid metabolism were obviously enriched in S0 group, While, in S8 group, Einfamilies-genetic information processing and Replication and repair were enriched ($p < 0.05$, LDA > 2.5). (D) The level 3 classification of KEGG pathway analysis revealed the characteristics of metabolic changed during ageing ($p < 0.05$, LDA > 3.0). W: Extracellular structures, Q: Secondary metabolites biosynthesis, transport and catabolism, P: Inorganic ion transport and metabolism, G: Carbohydrate transport and metabolism, and E: Amino acid transport and metabolism; L: Replication, recombination and repair; K: Transcription; V: Defense mechanisms ($p < 0.05$, LDA > 2.5).

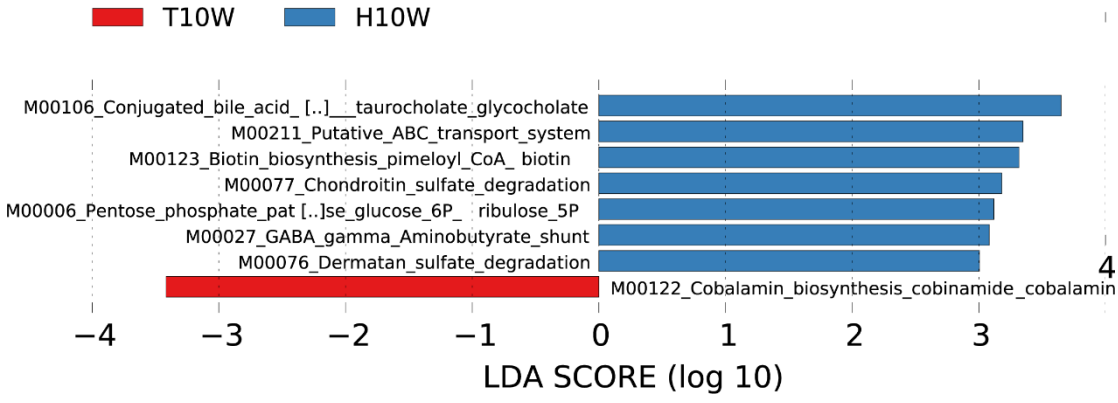


Figure S5. Lefse LDA score of KEGG modules analysis graphically displayed that M00122-Cobalamin-biosynthesis-cobinamide-cabalamin was an important metabolic module in the T10W group, compared with the H10W group ($p < 0.05$, LDA > 3.0).

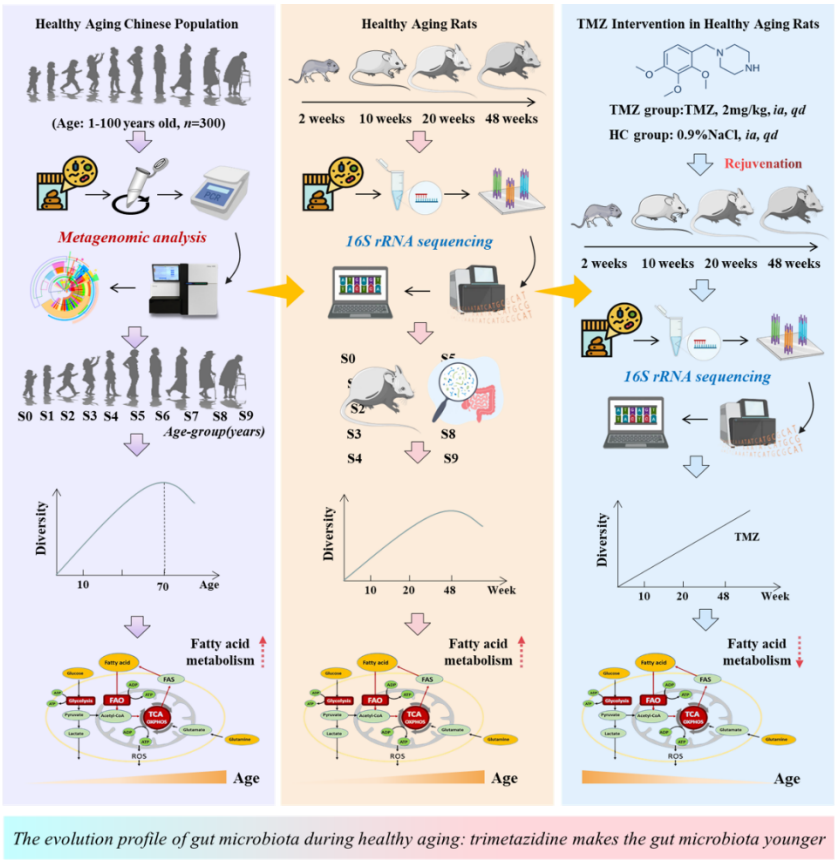
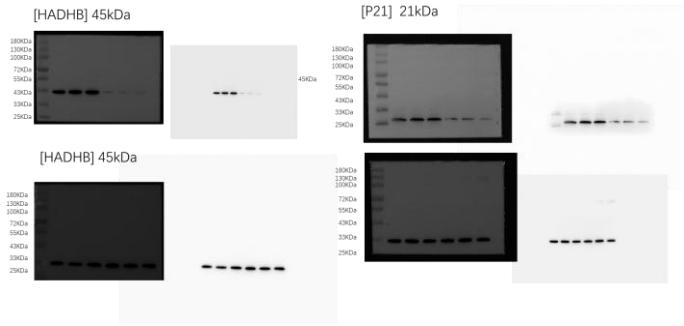


Figure S6. The flowchart of the research design

Supplementary Figure 2: Complete gel images and original blots



Supplementary table 1. Demographics and clinical characterization of healthy population cohort.

Demographics and clinical characterization of study groups										
Value(s) for indicated group										
Variables	S0(n=30)	S1 (n=30)	S2 (n=30)	S3 (n=30)	S4 (n=30)	S5 (n=30)	S6 (n=30)	S7 (n=30)	S8 (n=30)	S9 (n=30)
Sex, Male, no (%)	14(46.7)	20(66.7)	14 (46.7)	17(56.7)	18(60.0)	17(56.7)	15(50.0)	12(40.0)	10（33.3）	13（43.3）
Age-year										
Mean (SD)	NA	12.5(0.31)	21.57(0.28)	32.43(0.26)	44.3(0.48)	54.73(0.32)	64.71(0.54)	73.87(0.38)	82.13(0.34)	93.9(0.43)
Range	1.0-1.0	10.0-19.0	20.0-29.0	30.0-39.0	40.0-49.0	50.0-59.0	60.0-69.0	70.0-79.0	80.0-89.0	90.0-99.0
Body mass index, kg/m2										
Mean (SD)	NA	21.28(0.40)	24.74(0.61)	24.60(0.63)	25.97(0.65)	24.82(0.77)	24.54(0.59)	23.36(0.51)	24.43(0.49)	20.9(0.37)
Laboratory results, Mean (SD)										
White blood cells(10*9/L)	NA	5.49(0.28)	6.42(0.29)	7.24(0.32)	6.03(0.27)	6.1(0.29)	6.11(0.33)	6.04(0.23)	6.53(0.29)	6.66(0.35)
Red blood cells（10*12/L）	NA	4.90(0.09)	4.74(0.07)	4.71(0.09)	4.74(0.10)	4.68(0.06)	5.45(0.25)	4.90(0.10)	4.73(0.07)	4.86(0.11)
Blood hemoglobin（g/L）	NA	151.43(2.72)	142.99(2.91)	144.40(2.87)	144.07(2.94)	144.60(2.28)	146.53(2.67)	144.27(6.02)	147.37(2.18)	150.33(2.96)
Blood Platelet count（10*9/L）	NA	230.40(7.01)	277.3(10.11)	264.30(12.01)	255.27(12.58)	226.10(9.75)	223.33(8.94)	232.87(10.03)	218.3(9.83)	244.6(8.52)
Alanine aminotransferase (IU/L)	NA	23.0(2.21)	16.8(1.99)	32.1(6.66)	19.2(1.70)	22.67(2.85)	20.2(1.7)	26.33(3.07)	23.63(2.22)	23.83(2.50)
Glutamyl transpeptidase (IU/L)	NA	26.30(2.34)	21.97(1.66)	24.23(2.80)	19.97(0.94)	23.63(1.65)	20.0(1.02)	24.07(1.72)	21.93(0.77)	22.10(1.44)
Serum creatinine （mmol/L）	NA	69.63(2.58)	61.68(1.89)	70.07(2.74)	70.9(2.49)	69.87(2.9)	66.87(2.83)	67.37(2.83)	72.17(2.48)	74.33(2.37)
Triglyceride（mmol/L）	NA	1.82(0.22)	0.89(0.07)	1.32(0.18)	2.35(0.5)	1.63(0.23)	2.2(0.5)	1.64(0.15)	1.52(0.16)	1.21(0.13)
Total cholesterol（mmol/L）	NA	4.8(0.19)	4.34(0.13)	4.45(0.13)	4.86(0.19)	4.87(0.14)	4.88(0.17)	4.69(0.17)	4.79(0.18)	4.49(0.1)
Blood glucose（mmol/L）	NA	5.4(0.16)	4.69(0.1)	5.00(0.07)	5.86(0.35)	5.41(0.20)	5.38(0.15)	5.93(0.23)	5.86(0.22)	5.13(0.08)

Supplementary Table 2: Animal weights and experimental details

Supplementary Methods: Extended experimental protocols

Consent Informed

Informed consent form for scientific research

(Translated from Chinese)

Dear participants,

We are from Department of Infectious Diseases, the First Affiliated Hospital of Zhengzhou University. We will free of charge help you monitor your healthy condition and record your clinical information and healthy/disease status or disease progression process. The collected fecal samples from participants in hospital will be used for scientific research. These results and data from the hospital electronic medical records will provide auxiliary data for clinical diagnosis and treatment, and will be used for scientific research. Thank you for your corporation.

Number:	Diagnosis:

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except our research team.

The knowledge that we get from doing this research will be shared with you through community meetings before it is made widely available to the public. Confidential information will not be shared. There will be small meetings in the community and these will be announced. After these meetings, we will publish the results in order that other interested people may learn from our research.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant/_____

Signature of Participant _____

Date _____

Day/month/year

（Participants under the age of 18 are signed by their parents or legal guardians ）

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ AND

Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. We will free of charge help you monitor your healthy condition and record your clinical information and healthy/disease status or disease progression process.
2. These data from hospital electronic medical records will be used for scientific research.
3. The collected fecal samples will be used for scientific research.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Chemicals and reagents/Materials:

Cell lysis buffer for Western and IP (beyotime, P0013); Protease and phosphatase inhibitor cocktail for general use, MS-safe, 50X (beyotime, P1048); BCA Protein Assay Kit (Solarbio, PC0020). Anti-GAPDH antibody (Proteintech, Wuhan, China, 60004-1-Ig). HADHB Polyclonal antibody (Proteintech, Wuhan, China, Cat No. 29091-1-AP). p21 Polyclonal antibody (Proteintech, Wuhan, China, Cat No. 28248-1-AP).

Primer sequences were synthesized by Sangon Biotech Co., Ltd. (Wuhan, China). Sequence (5' to 3') was displayed as follows:

RAT-HADHB-F: act gga gca aat ggc caa ac;

RAT-HADHB-R: acg gcc atc agt cag gaa ag;

RAT P21 F: ggc tca gga gtt agc aag ga;

RAT P21 R: gca tcg tca aca ccc tgt ct;

RAT P16 F: gtc gta ccc cga tac agg tga;

RAT P16 R: gca cca tag gag agc agg ag;

RAT GAPDH F: ctc agt tgc tga gga gtc cc;

RAT GAPDH R: att cga gag aag gga ggg ct;

The main exclusion criteria: (1) use of probiotics or prebiotics, fermented dairy products (such as yoghurt), antibiotics (such as penicillin, cephalosporins, tetracycline, etc.) and other medications (steroids, cyclosporine) affecting the flora in the last 3 months; (2) gastrointestinal-related diseases; (3) other chronic diseases (cardiovascular and cerebral vascular diseases, diabetes mellitus, rheumatoid arthritis, neurodegenerative diseases, and allergic diseases); and (4) subjects during pregnancy

and lactation. Animal experiments were approved by the Animal Care and Use Committee of Zhengzhou University and all experimental procedures involving animals were strictly followed in accordance with the Guide for the Care and Use of Laboratory Animals.

DNA Extraction/Isolation: The sample was suspended in 790 µl of sterile lysis buffer (4M guanidine thiocyanate; 10% N-lauroy l sarcosine; 5% N-lauroy l sarcosine-0.1 M phosphate buffer [pH 8.0]) in 2 ml screw-cap tube containing 1g glass beads (0.1mm BioSpec Products, Inc., USA). This mixture was vortexed vigorously then incubate at 70 °C for 1 h. After incubation by bead beating for 10min at maximum speed. DNA was extracted by following the manufacturer's instructions for bacterial DNA extraction using The E.Z.N.A. ® Stool DNA Kit (Omega Bio-tek, Inc., GA), which excepted lysis steps and stored at -20°C for further analysis. DNA Sample Testing: The detection of DNA samples consists of three main methods; (1) agarose gel electrophoresis (AGE) to analyze the purity and integrity of DNA; (2) Nanodrop to detect the purity of DNA (OD260/280 ratio); and (3) Qubit to accurately quantify the concentration of DNA.

PCR Amplification: The primers F1 and R2 (5'- CCTACGGGNGGCWGCAG -3' and 5'-GACTACHVGGGTATCTAATC- C-3') correspond to positions 341 to 805 in the Escherichia coli 16S rRNA gene were used to amplify the V3~V4 region of each sample by PCR. PCR reactions were run in a T100TM Thermal Cycler PCR system (Bio-Rad Laboratories, Inc., USA) using the following program: 3 min of denaturation at 95 °C followed by 21-cycles of 0.5 min at 94 °C (denaturation), 0.5 min for annealing at 58°C, and 0.5min at 72 °C(elongation), with a final extension at 72 °C for 5min.

Western blot: The hepatic tissues were lysed in RIPA (Merck Millipore, V900854) with phosphatase inhibitors (Roche, 4906845001) and proteinase (Beyotime, ST506) for 30 minutes, and then centrifuged (1,2000r, 10 min, 4 °C). The concentration of total protein was measured by a BCA kit (Beyotime, P0009). Then, 20 µg/10ul of proteins was boiled for 15 min at 100 °C after addition of SDS-PAGE protein loading buffer 5X

(Beyotime, P0015L) and subjected to 10% SDS-PAGE and electrophoretically transferred to PVDF membranes (Bio-Rad, 1620177). After incubation in 5% non-fat milk for 2 h at room temperature, the membranes were incubated overnight with antibodies specific for indicated proteins overnight at 4 °C. Then, the washed membranes were incubated with the peroxidase-conjugated secondary antibody for 1 h at room temperature. The protein bands were detected by an enhanced chemiluminescence reagent (ECL) kit (Thermo Fisher Scientific, 34580) and analyzed by ImageJ 1.52a software. The antibodies used for western blotting are described in Supplementary Table S1.

Quantitative real- time PCR: Total RNA extracted from liver of rats was conducted by a RaPure Total RNA Kit (Yeasen, 10606ES60), and reverse transcription was performed to generated cDNA templated using Hifair II 1st Strand cDNA Synthesis SuperMix (Yeasen, 11123ES60). Quantitative RT-PCR was performed using SYBR Green Master Mix (Yeasen, 11203ES08). Sequence-specific primers were designed using Primer 6 software and synthesized by Shanghai Biotechnology Co., Ltd, and GAPDH was used to normalize the relative level of each transcript. Samples were analyzed in triplicate using a LightCycler 480 System (Roche). Gene expression levels were analyzed using the SYBR qPCR kit on the RT-PCR system. For quantitative analysis, all samples were analyzed using the$2^{-\Delta\Delta Ct}$ based on the Ct values. The specific primer designed for target genes has been given in Table S2.

Immunohistochemical method (IHC): The cells in the logarithmic growth phase were digested for the seed plate, then treated with TGFβ1 (10ng/ml) , NXT629 and αAmyrin (5 μM) for 48 h, and for Cellular fluorescence immunoassay treatment. Covered with the primary antibody (dilution ratio of 1:100), incubate at 4 ◦C overnight, recover the first antibody, and wash with phosphate balanced solution (PBS) three times for 5 min each time. The secondary antibody was diluted with an antibody diluent at a dilution ratio 1:200. Add 250 μl of DAPI staining solution to each hole. The cell slide was removed and placed on another slide. The cell slides were covered with an anti-

fluorescence quenching sealer. The fluorescence was observed using a fully automated intelligent cell imaging system. Fluorescence data were collected using the ImageJ-win 64 software.