SUPPLEMENTARY METHOD

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).

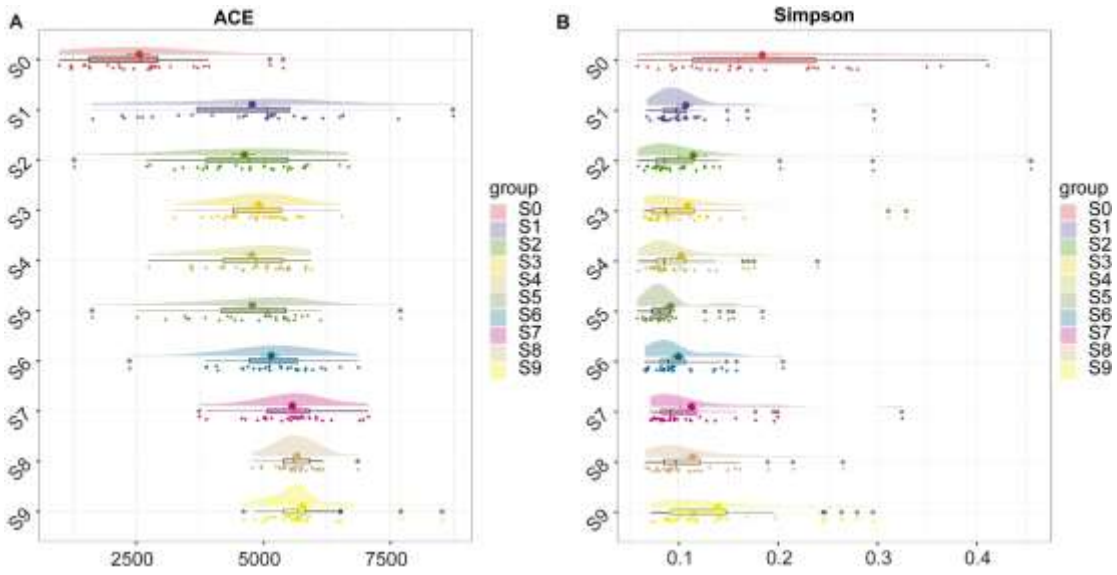
Primer sequences were synthesized by Sangon Biotech Co., Ltd. (Wuhan, China). Sequence (5' to 3') was displayed as follows

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.

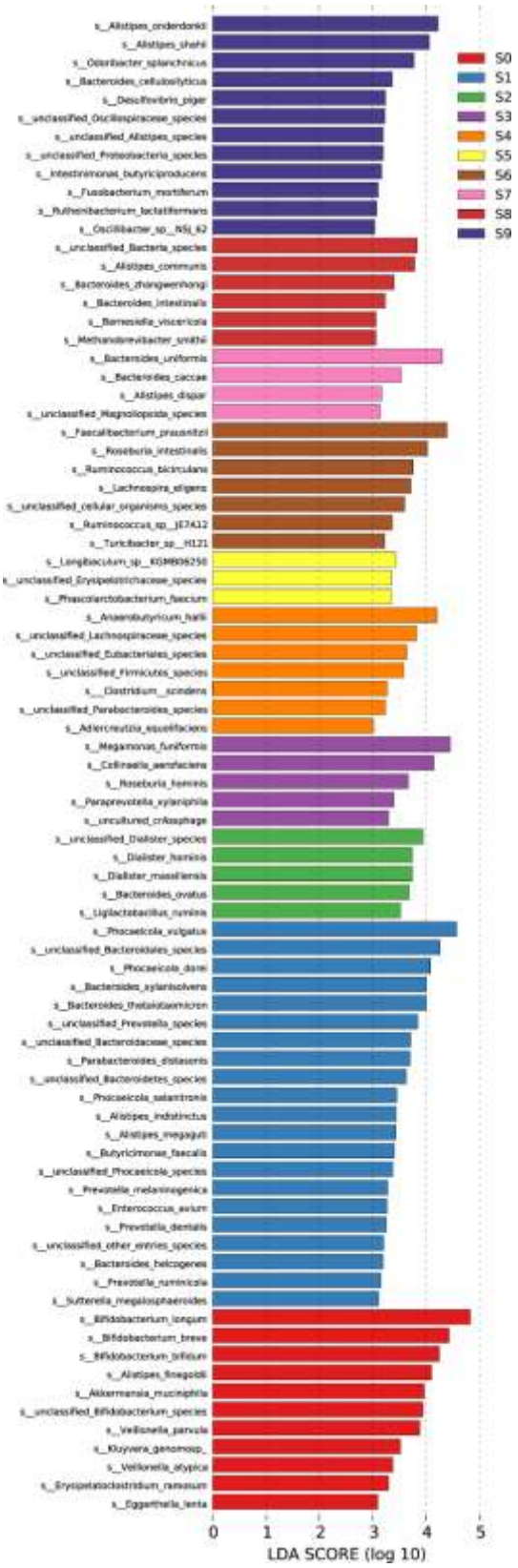
The main exclusion criteria: (1) use of probiotics or prebiotics, fermented dairy products (such as yoghurt), antibiotics (such as penicillin, cephalosporins, tetracycline, etc.) and other medications (steroids, cyclosporine) affecting the flora in the last 3 months; (2) gastrointestinal-related diseases; (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

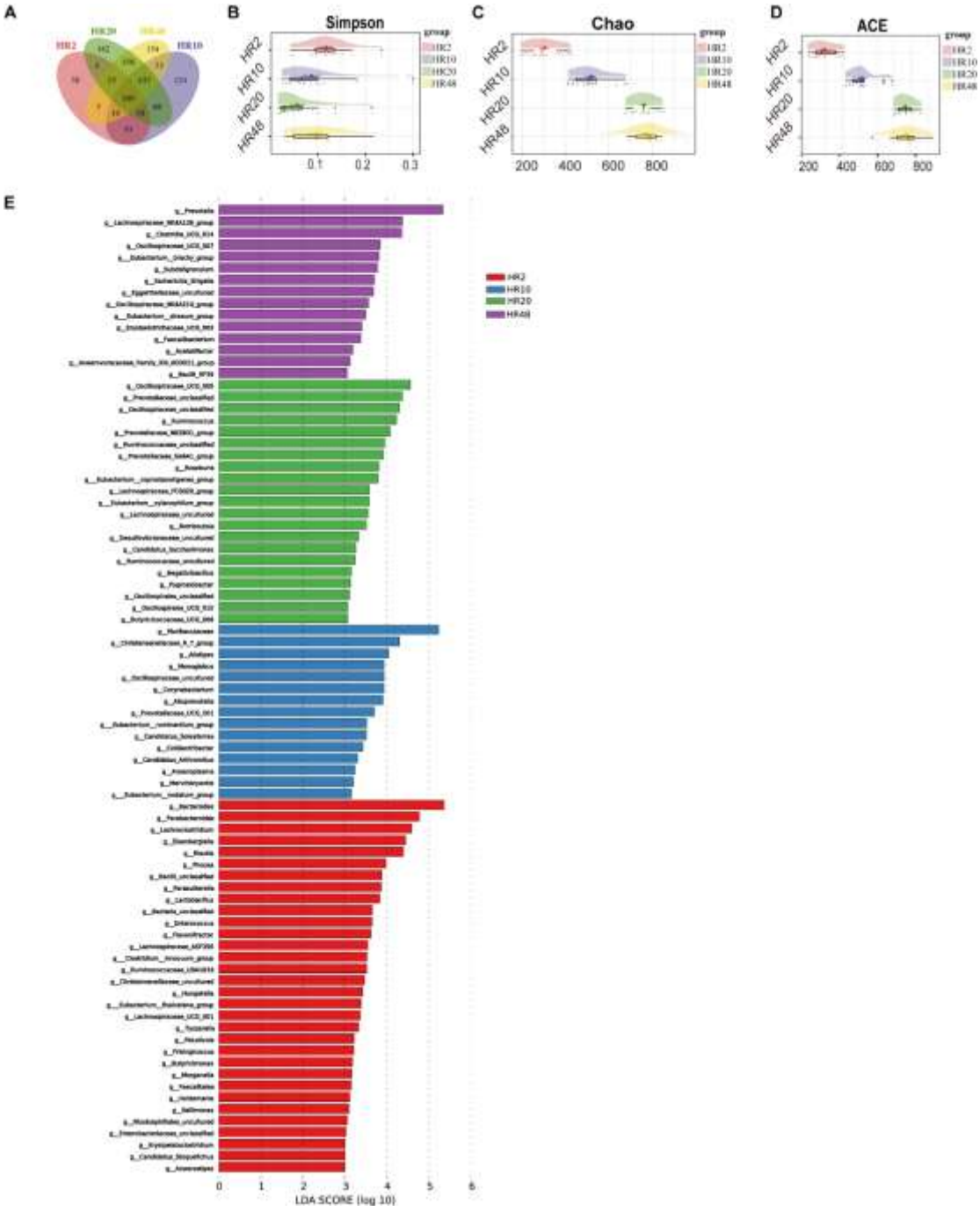
sarcosine-0.1 M phosphate buffer [pH 8.0]) in 2 ml screw-cap tube containing 1g glass beads (0.1mm BioSpec Products, Inc., USA). This mixture was vortexed vigorously then incubate at 70 °C for 1 h. After incubation by bead beating for 10min at maximum speed. DNA was extracted by following the manufacturer's 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.



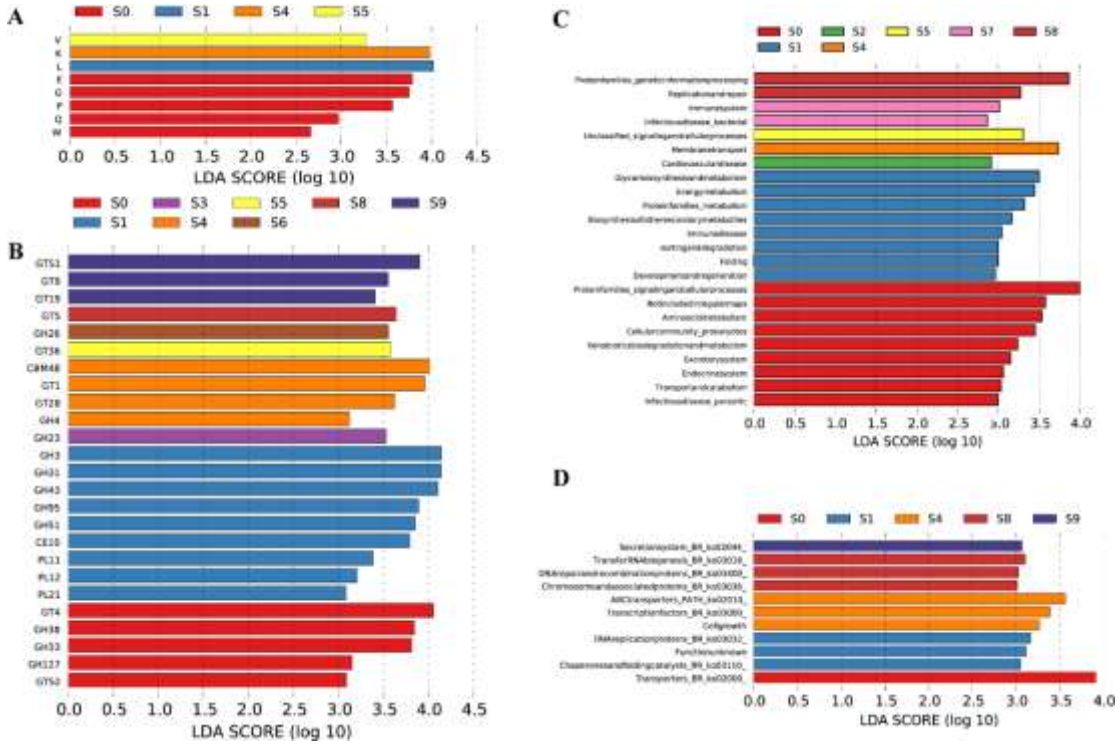
Supplementary Figure 1 The core gut microbiome altered significantly during the ageing process of the healthy ageing Chinese populations. (A). The ACE index; (B). The Simpson index.



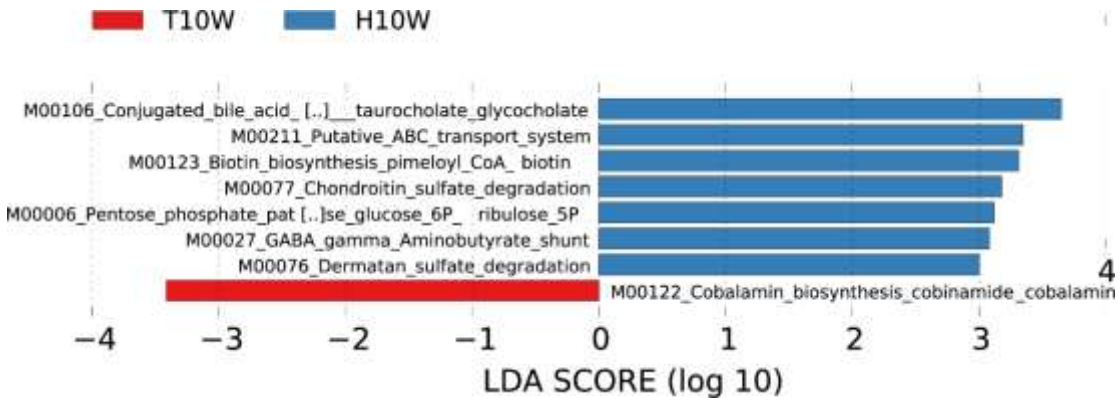
Supplementary Figure 2 The LDA results at the species level showed the potential biomarkers for healthy human aging ($p < 0.05$, LDA > 3.0).



Supplementary Figure 3 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.



Supplementary Figure 4 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). KEGG, Kyoto Encyclopedia of Genes and Genomes); COG, Clusters of Orthologous Groups; CAZy, Carbohydrate-Active Enzymes.



Supplementary Figure 5 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).

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)