Additional file 1 Detailed information for metagenomic shotgun sequencing and GC-TOF-MS analysis.

Metagenomic shotgun sequencing

DNA extraction DNA integrity and quality were verified by electrophoresis on 1.0% agarose gels and Qubit. Next, the genomic DNA was sheared randomly in approximately 500 bp fragments using a Bioruptor Pico (Diagenode, B01060001). And then, the target fragments were subjected to perform DNA end-repair and added a single 'A' nucleotide in the 3' ends of the target DNA fragments.

Raw reads elimination The Illumina raw reads were cleaned by trimming the adapter sequences and low-quality regions (e. g. reads < 50 bp, reads shorter than 60 bp in nucleotide length, and reads mapped to the human genome based on alignment with SOAPaligner).

GC-TOF-MS analysis

Sample processing Faecal sample (50 ± 1 mg) was placed in the 2-mL EP tubes with 0.4 mL extraction liquid (VMethanol: VChloroform = 3:1), then 10 μL of L-2-Chlorophenylalanine (1 mg/mL stock in dH2O) was added as an internal standard followed by vortex-mixing for 30 s. The mixture was homogenised in a ball mill for 4 min at 45 Hz, treated with ultrasound for 5 min (incubated in ice water), and centrifuged at 12,000 rpm at 4℃ for 15 min using a refrigerated centrifuge (Heraeus Fresco17, Thermo Fisher Scientific, MA, US). Then, the sample supernatant (0.35 mL) was transferred into a fresh 2-mL GC/MS glass vial and completely dried in a vacuum concentrator (without heating). The methoxyamination

hydrochloride in pyridine solution (60 μL, 20 mg/mL) was added and incubated for 30 min at 80℃. Next, incubation was performed with 80 μL of the BSTFA (1% TMCS, v/v) at 70℃ for 1.5 h.

The conditions of GC-TOF-MS The column was an Agilent DB-5MS capillary column (30 m length x 0.25 mm i.d. x 0.25 mum film thickness; J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas, with a 3 mL min^{-1} front inlet purge flow and 1 mL min^{-1} gas flow rate through the column. Then, an aliquot (1 μL) of the sample was injected in the splitless mode. The initial temperature was maintained at 80°C for 1 min, then increased to 290°C at a rate of 10°C /min, held at 300°C for 13 min finally. The temperatures of the front injection, transfer line, and ion source were set at 280, 295, and 220°C, respectively. The operation was performed in the electron impact mode (-70 eV). We set the acquisition rate to 10 spectra per second with a mass scan range of 50–600 m/z after a 7.9-min solvent delay.