Supplemental Methods

Microbiome analysis

The genomic DNA of gut microbiota was extracted from murine feces and 454-barcode pyrosequencing of microbial 16S rRNA genes was performed as described previously.[31, 32]

Microbial genomic DNA was extracted using a phenol-chloroform standard protocol with vigorous shaking with 0.1mm zirconia/silica beads.

The V1–V2 region of the 16S rRNA gene was amplified using forward primer (5′-CCATCTCATCCCTGCGTGTCTCCGACTCAGNNNNNNNNNNagrgtttgatymtggctcag-3′) containing the 454 primer A, a unique 10-bp barcode sequence for each sample (indicated in N), and 27Fmod (5′-agrgtttgatymtggctcag) in which the third base A in the original primer 27F was changed to R, and reverse primer (5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGtgctgcctcccgtaggagt-3′) containing the 454 primer B and reverse primer 338R (5′-tgctgcctcccgtaggagt). PCR was performed with Ex Taq polymerase (Takara Bio) on a 9700 PCR system (Life Technologies). PCR products of approximately 370 bp were purified by AMPure XP magnetic purification beads (Beckman Coulter), and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies). Mixed samples were prepared by pooling approximately equal amounts of PCR amplicons from each sample and subjected to 454 GS FLX Titanium or 454 GS JUNIOR (Roche) sequencing according to the manufacturer's instructions.

We developed an analysis pipeline for 454 barcoded pyrosequencing of PCR amplicons of the V1-2 region amplified by 27Fmod-338R primers. First, 16S reads were

assigned to each sample based on the barcode sequence information. Second, 16S reads that did not have PCR primer sequences at both sequence termini and those with an average quality value < 25 were filtered out. Third, 16S reads containing possible chimeric sequences that had BLAST match lengths of < 90% with reference sequences in the database were removed. Finally, filter-passed reads were obtained for further analysis by trimming off both primer sequences.

The filter-passed 16s rRNA reads were analyzed using QIIME and RDP-classifier. We used 16S sequences for operational taxonomic unit, UniFrac distance analysis and Chao1 rarefaction diversity measurement for each sample. The microbiome data have been deposited at the DDBJ database (http://getentry.ddbj.nig.ac.jp/) under accession number DRA002254.

CE-TOFMS measurement

A quantitative analysis of charged metabolites by capillary electrophoresis with electrospray ionization time-of-flight mass spectrometry (CE-TOFMS) was performed as described previously.[33, 34]

Plasma (50 μL) was immediately plunged into methanol (450 μL) containing internal standards (20 μM each of methionine sulfone [Wako] for cations, MES [Dojindo] and CSA [D-Camphol-10-sulfonic acid, Wako]). Then, de-ionized water (200 μL) and chloroform (500 μL) were added. The solution was centrifuged at 4600× g for 5 min at 4 °C, and the upper aqueous layer was centrifugally filtered through a Millipore 5000 Da cutoff filter (Millipore) to remove proteins. The filtrate was lyophilized and dissolved in 25 μL of water containing reference compounds (200 μM each of 3-aminopyrrolidine [Sigma Aldrich] and trimesate [Wako]) prior to CE-TOFMS

analysis. All CE-TOFMS experiments were performed using the Agilent CE capillary electrophoresis system (Agilent Technologies), the Agilent G3250AA LC/MSD TOF system (Agilent Technologies), the Agilent 1100 series binary HPLC pump, the G1603A Agilent CE-MS adapter, and the G1607A Agilent CE-ESI-MS sprayer kit.
Cationic metabolites were separated in a fused silica capillary (50 μm i.d. × 100 cm) filled with 1 M formic acid as the electrolyte. A sample solution was injected at 50 mbar for 3 s (3 nL) and 30 kV of voltage was applied. The capillary temperature and the sample tray were set at 20 °C and below 5°C, respectively. Methanol water (50% v/v) containing 0.1 μM Hexakis (2,2-difluorothoxy) phosphazene was delivered as the sheath liquid at 10 μL/min. ESI-TOFMS was operated in the positive ion mode, and the capillary voltage was set at 4 kV. A flow rate of heated dry nitrogen gas (heater temperature 300°C) was maintained at 10 psig. In TOFMS, the fragmentor, skimmer and Oct RFV voltages were set at 75, 50, and 125 V, respectively. Automatic recalibration of each acquired spectrum was performed using reference masses of reference standards; ([^{13}C isotopic ion of protonated methanol dimer (2MeOH + H)]$^+$, *m*/*z* 66.0632) and ([Hexakis (2,2-difluorothoxy)phosphazene + H]$^+$, *m*/*z* 622.0290). Exact mass data were acquired at a rate of 1.5 spectra/s over a 50–1000 *m*/*z* range.
Anionic metabolites were separated in a COSMO(+), chemically coated with a cationic polymer, capillary (50 μm i.d. × 100 cm) (Nacalai Tesque) filled with 50 mM ammonium acetate solution (pH 8.5) as the electrolyte. A sample solution was injected at 50 mbar for 30 s (30 nL) and −30 kV of voltage was applied. A platinum electrospray ionization spray needle was replaced with the original Agilent stainless steel needle. A 5 mM ammonium acetate in 50% (v/v) methanol-water containing 0.1 μM Hexakis (2,2-difluorothoxy) phosphazene was delivered as the sheath liquid at 10 μL/min.

ESI-TOFMS was operated in the negative ion mode, and the capillary voltage was set at 3.5 kV. In TOFMS, the fragmentor, skimmer and Oct RFV voltages were set at 100, 50, and 200 V, respectively. Automatic recalibration of each acquired spectrum was performed using reference masses of reference standards; ([^{13}C isotopic ion of deprotonated acetic acid dimer ($2CH_3COOH-H)]^-$, *m*/*z* 120.0384) and ([Hexakis(2,2-difluorothoxy)phosphazene + deprotonated acetic acid($CH_3COOH-H)]^-$, *m*/*z* 680.0355). Other conditions were as the same as in cationic metabolite analysis.

Time course analysis of fecal microbiome after the adenine feeding

Seven-weeks male C57BL/6 mice were fed a 0.2% adenine containing diet for 6 weeks. Feces were collected at 1, 2, 4 and 6 weeks. Samples of 0-week were collected from the other 7-weeks male C57BL/6 mice. Microbiome analysis was performed as described above.

Measurement of *p*-cresyl sulfate

Plasma *p*-cresyl sulfate was analyzed based on a previous study.[35] Briefly, liquid chromatographic separation was performed using an Agilent 1290 Infinity LC system (Agilent Technologies) on a Waters Atlantis dC18 (2.1 mm × 150 mm, 3 μm) column that was maintained at 40°C. The mobile phase consisted of 5 mM ammonium acetate as solution A and acetonitrile as solution B. The initial gradient condition was 5% B for 3 min followed by a linear gradient up to 90% B over the next 17 min, and then retained at 90% B for 5 min. The flow rate was 0.2 mL/min. Sample preparation were identical with CE-MS analysis, and 1 μL of sample which diluted ten-times with Milli-Q water were injected into the column.

ESI-MS/MS analysis was carried out using an Agilent 6490 Triple Quadrupole Mass Spectrometor (Agilent Technologies) equipped with Agilent Jet Stream source in the negative ion mode. The source parameters were: gas temperature 300°C, gas flow 12 L/min, nebulizer 30 psi, sheath gas temperature 400°C, sheath gas flow 12 L/min, capillary voltage 3,000 V, nozzle voltage 2,000 V. The multiple reaction monitoring (MRM) mode was used in this study. The MRM parameters, Q1 *m/z*, Q3 *m/z* and collision energy were as follows: 187, 107.1 and 20 for *p*-cresyl sulfate, 231.1, 80 and 36 for camphor 10-sulfonic acid (internal standard). The dwell time for mass transition and cell accelerator voltage were 250 ms and 7 V, respectively.

Measurement of acetate

Acetate analysis using CE-ESI-TOFMS was performed as described previously.[36]

References

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