We measured 11 selected metabolites known to be modulated by the gut microbiome and affecting host behavior, in the collected serum samples via LC-MS (Table S2). Serum-metabolites were extracted by mixing pre-chilled methanol containing isotopic standards (13C11 L-tryptophan, kynurenic acid ring-D5) with serum at the ratio of 2:1 (v:v). The mixture with the final concentration of isotopic standards of 0.5 µg/mL was cooled for 30 min at -20°C before centrifuging at 11,000 g at 4°C for 15 min. The supernatant was filtered through a regenerated carbon filter with a pore size of 0.2 µm. LC-MS analysis of metabolites of interest was performed using a HPLC system (Dionex UltiMate 3000, Thermo Fisher, USA) coupled with a high-resolution mass spectrometer with an electrospray ionization source (Impact II, Bruker, Germany). Reverse phase chromatography was executed using a C-18 column (L × inner diameter 250 cm × 4.6 mm, 5 µm particle size, Agilent P/N 990967-902) held at a constant temperature of 20°C. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). Gradient elution was applied with following conditions: The chromatographic gradient started at 3% B for 3 min, increased linearly to 100% B at 20 min, held at 100% B for 3 min, decreased linearly to 3% B at 27 min, followed by an equilibrating time of 3 min. The flow rate was kept constantly at 0.5 mL/min. The injection volume was 40 µL. Samples were stored in the autosampler at 8°C for a maximum period of 72 h prior to injection. A dilution series of standards with a concentration range from 0.000128 to 10 µg/mL was measured to build the linear regression model. The weighting factor of 1, 1/x, or 1/x2 was selected depending on lowest sum percent relative error (64). The operating parameters of the mass spectrometer were as follows: the spray needle voltage at 3.5 kV, nitrogen was used as nebulizing gas (1.5 bar) and drying gas (5 L/min), and the drying temperature was at 200°C. The data were acquired in Full-MS mode with a scanning range of 50-1,000 m/z and scanning rate of 2 Hz in the positive ion mode. Each measurement included a 30s-segment for automated internal calibration using sodium formate 5 mM. Peak integration of Full-MS data was performed using Skyline (v.21.1). The internal isotopic standards were used for quality control and for normalization purposes.