Electronic Supplementary Material (ESI) for Food & Function.
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1 Supplementary Methods

1.1 *CUMS procedures and behavioral tests*

CUMS procedure: [1] Forced swimming: a) Fill a glass tank 22 cm deep with water at 23 ± 2 °C; b) Place the animal in the glass tank for 10 min; c) Return the animal to a clean and dry cage with fresh bedding in order to avoid chills and colds. [2] Restraint: a) Place the animal in a 50 mL plastic tube; adjust it with plastic tape on the outside so the animal is unable to move. The tube must have a hole at the far end to allow regular breathing. To place the animal in the plastic tube, it is necessary to place the head of the animal close to the entrance, after which it should enter by itself; b) Wait for 1 h. Although the plastic tube should be sufficient to prevent the animal from coming out is desirable to leave the immobilizer inside the cage; c) Return the animal to its cage. The best way to extract the animal is to make a sudden movement downwards, dropping it into the cage. Try to avoid pulling from the tail. [3] Water deprivation: a) Remove the bottle of water from the cage for 24 h. If the animal house is supervised by staff, indicate that the water/food must not be replaced in that cage; b) Place the bottle of water back after the time point is reached. [4] Isolation: a) Place the animal alone in a new cage; b) Return the animal to the cage with their cage mates. [5] Food deprivation: a) Remove the food from the cage for 24 h; b) Place the food back. [6] Wet bedding: a) Place the animal in a new cage with 200 mL water per 100g bedding for 24 h; b) Return the animal to the cage with their cage mates. [7] No bedding: a) Place the animal in a new cage without bedding for 24 h; b) Return the animal to the cage with their cage mates. [8] Tilt cages: a) Tilt the cages at 45° for 24 h; b) Return the cage to the normal state. [9] Clip tail: a) The plastic clamp is clipped at 2 cm from the end of the tail, sustain 1 min each time, total 3 times a day; b) Return the animal to the cage with their cage mates. [10] Continuous illumination: a) Transfer the cages into another house in a continuous illumination with white light. The illumination intensity is 500 lux; b) After exposed for 24h, return the cage to the normal state. The stressors used in this study have no fatal or disabling risk. More importantly, each stressor is used no more than three times. Although the stressors cause temporary discomfort to animals, they recover quickly when the stress is stopped.

Behavioral tests: For the OFT, mice were assessed for their locomotor activity in a square arena (50 cm×50 cm) enclosed by continuous opaque walls made of plexiglass.

Animals were monitored for 10 minutes, and the time spent in the center zone and active time during the whole period were measured. For the FST, mice were allowed to swim in the water with a temperature of 23-25°C, and the immobility time was recorded during the ten-minute swimming. All mice are trained to swim for 15 minutes the day before the test. Before all the behavioral tests, animals were habituated to the room for one hour. All tests were performed under dim light (60 lux) and monitored by the video tracking system (Ethovision version 13, Noldus, Netherland).

1.2 *Real-time quantitative-PCR*

Each qPCR was performed in a 10 μL reaction volume containing supermix (BIO-RAD, Berkeley, CA, USA), primers (10 pmol), and DNA template (1 μL). The qPCR programme consisted of an initial denaturation step of 3 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 15 s. Melting curve analysis was performed between 65°C and 95°C.

For absolute quantification, a standard curve was prepared using a 10-fold dilution series of DNA isolated from an overnight liquid culture (logarithmic period) of B. breve CCFM1025, for which the number of cells was determined by quantitative culture. The same amount of CCFM1025 bacteria was added to the fecal samples as the amount in the baseline period, and the same DNA extraction steps were performed for the standard samples as those performed for the test samples, to ensure accurate results (Maldonado-Gómez et al. 2016). DNA from the target B. breve strain was used as the positive control, and water, instead of template DNA, was used as the negative control in all PCR runs.

1.3 *LC-MS/MS, GC-MS and HPLC detection methods*

Non-target metabolites: UItiMate-3000 UPLC (1.8 μm, 2.1 × 100 mm T3 column) and Q-Exactive plus mass spectrometer were used to analyze non-target metabolites in feces and serum. The positive ion mobile phase consisted of an aqueous solution containing 0.1% formic acid (phase A) and acetonitrile containing 0.1% formic acid (phase B). Negative ions: 5mM ammonium acetate solution in phase A and acetonitrile in phase B. Gradient elution: 0-1.0 min, 2%B; 1.0-10 min, 98%B; 10-12 min, 98%B; 12 to 15 min, 2%B. The column temperature was 35 °C, the flow rate was 0.3 mL/min, and the injection volume was 1 μL. The temperature of the sample tray was 4 °C. Data

were collected by thermoelectrospray ionization source (ESI) in positive ion and negative ion modes, with the full scan range of 70-1050 m/z. Ion source parameters were set as follows: capillary temperature 250 °C, ESI^- 2500 V, and ESI^+ 3500V.

Bile acid-targeted metabolites: UltiMate-3000 UPLC (1.8 μm, 2.1 × 100 mm T3 column) and Q-Exactive plus mass spectrometer were used to analyze bile acid-targeted metabolites in feces and serum. The mobile phase consisted of 1 mM ammonium acetate in water (A) and 1 mM ammonium acetate in methanol (B). The gradient elution program was as follows: 0-6min, 20%-60%B; 6-25 min, 60%-100% B; 25-26 min, 100% B; 26-28 min, 100%-50% B; 28-30 min, 50%-20% B; 30 to 32min, 20% B. The column temperature was 35 °C, the flow rate was 0.3 mL/min, and the injection volume was 2 μL. The temperature of the sample tray was 4°C. The thermoelectrospray ionization source (ESI) collected data in negative ion mode and performed full scan at a resolution of 70000 with a scanning range of 50-750 m/z. Ion source parameters were set as follows: ESI^- 2500 V, capillary temperature 325°C. Cholic acid (CA, LOD: 0.0131 ng/mL, RSD: 1.10%), β-muricholic acid (β-MCA, LOD: 0.0700 ng/mL, RSD: 0.74%), chenodeoxycholic acid (CDCA, LOD: 0.0061 ng/mL, RSD: 0.99%), taurocholic acid (TCA, LOD: 0.0034 ng/mL, RSD: 0.89%), Taurine-β-Murine bile acid (T-β-MCA, LOD: 0.0057 ng/mL, RSD: 1.03%), taurochenodeoxycholic acid (TCDCA, LOD: 0.0055 ng/mL, RSD: 1.40%), deoxycholic Acid (DCA, LOD: 0.0063 ng/mL, RSD: 0.98%), ursodeoxycholic acid (UDCA, LOD: 0.0117 ng/mL, RSD: 1.05%), hyodeoxycholic acid (HDCA, LOD: 0.0062 ng/mL, RSD: 1.34%), taurodeoxycholic acid (TDCA, LOD: 0.0034 ng/mL, RSD: 1.19%), tauroursodeoxycholic acid (TUDCA, LOD: 0.0068 ng/mL, RSD: 0.32%).

Trp-Indole targeted metabolites: UltiMate-3000 UPLC (1.7 μm, 2.1 × 100 mm C18 column) and Q-Exactive plus mass spectrometer were used to analyze bile acid-targeted metabolites in feces and serum. The mobile phase consisted of 0.1% (v/v) formic acid aqueous solution (A) and acetonitrile (B). The gradient elution program was as follows: 0-3min, 5%B; 3-9 min, 5%-30% B; 9-15 min, 30%-100% B; 15-16.5 min, 100% B; 16.5-20 min, 100%-5% B. The column temperature was 35 °C, the flow rate was 0.3 mL/min, and the injection volume was 2 μL. The temperature of the sample tray was

4°C. The thermoelectrospray ionization source (ESI) collected data in positive ion mode and performed full scan at a resolution of 70000 with a scanning range of 50-750 m/z. Ion source parameters were set as follows: ESI^+ 3500 V, capillary temperature 320°C.

PUFA-targeted metabolites: QP2010 Ultra GC-MS (0.25 μm, 0.25 mm × 30 m, Rtx-wax column) were used to analyze PUFA-targeted metabolites in serum. The temperature of the gasification chamber was set at 240°C, and the sample was injected with 0.94 mL/min helium (carrier gas) at a split ratio of 10:1. The heating program was as follows: 0-5 min, 40°C; 5-9 min, 40°C-120°C; 9-23 min, 120°C-190°C; 23-28 min, 190°C; 28-34 min, 190°C-220°C, 34-51 min 220°C. The thermoelectrospray ionization source (ESI) collected data in performed full scan with a scanning range of 50-550 m/z.

Bile salts: Fortis C18 column (5.8 μm, 4.6 × 250 mm) was used, mobile phase A was acetonitrile-water solution containing 7.5 mM tetrabutylammonium bisulfate (60:40), and mobile phase B was acetonitrile-water solution containing 7.5 mM tetrabutylammonium bisulfate (30:70). The UV detector wavelength was set at 200 nm, the column temperature was 40 °C, the flow rate was 1 mL/min, and the injection volume was 40 μL. The gradient elution program was as follows: 0-30 min, 15%-70%A; 30-31 min, 70%-15%A; 31 to 40 min, 15% A.

2 Supplementary Figures

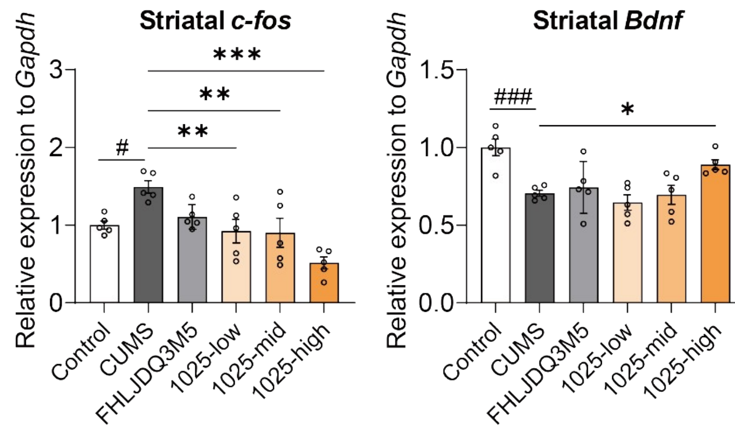

Fig. S1 Gene expression of *c-fos* and *Bdnf* in the striatum.

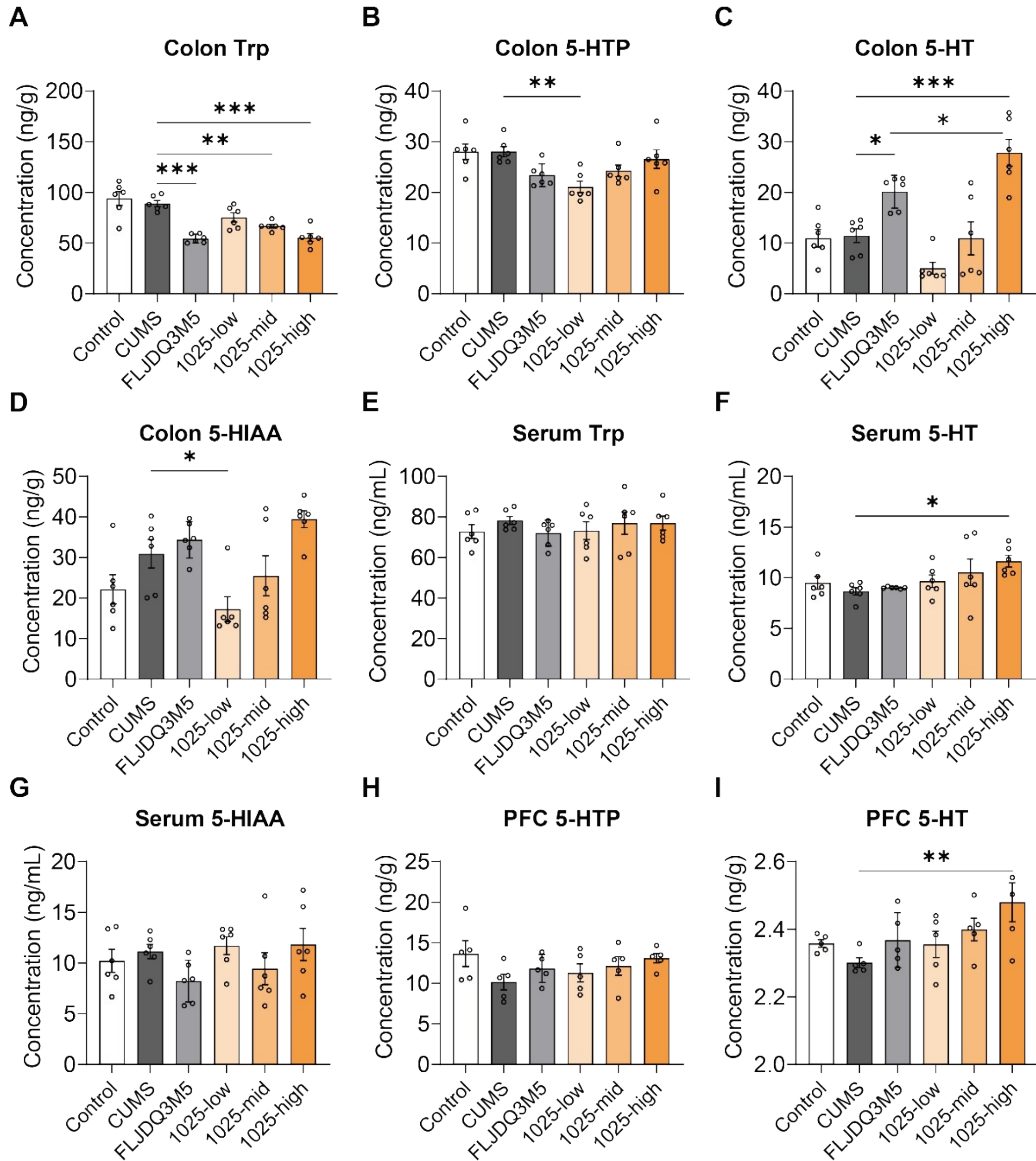

Fig. S2 Regulation of serotonin by *B. breve* CCFM1025 intervention.

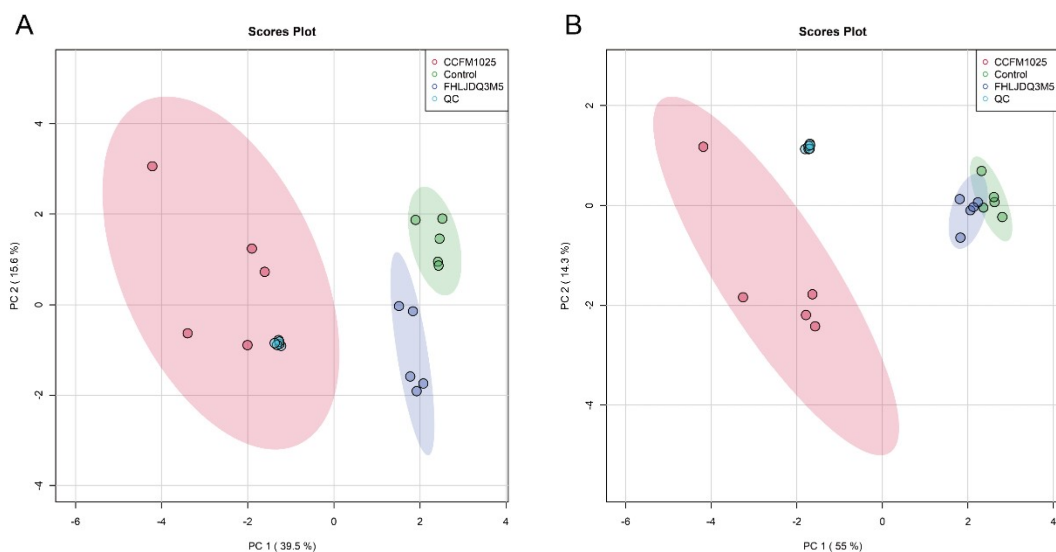

Fig. S3 PCA scatter plots of metabolites. (A) Positive ion scanning modes. (B) Negative ion scanning modes. The PCA analysis was performed based on Euclidean distance.

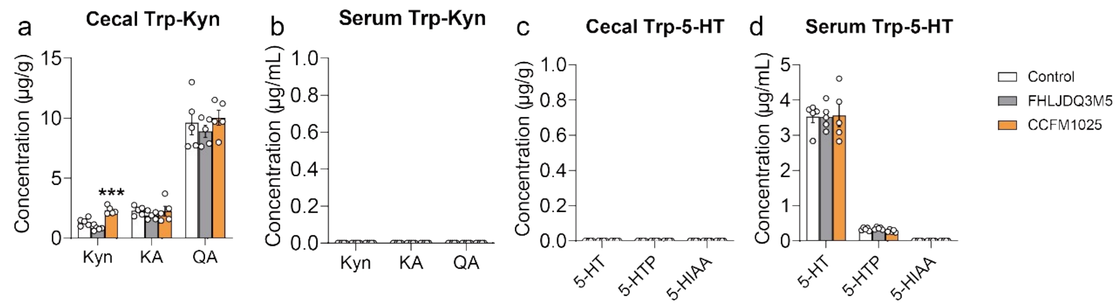

Fig. S4 Targeted detection of tryptophan metabolites. (a-b) Kynurenine metabolites in cecal contents and serum. (c-d) Serotonin metabolites in cecal contents and serum. Sidak-based one-way ANOVA was performed between multiple groups, *** $p < 0.001$ denoting a significant difference between the CCFM1025 group and the FHLJDQ3M5 group.

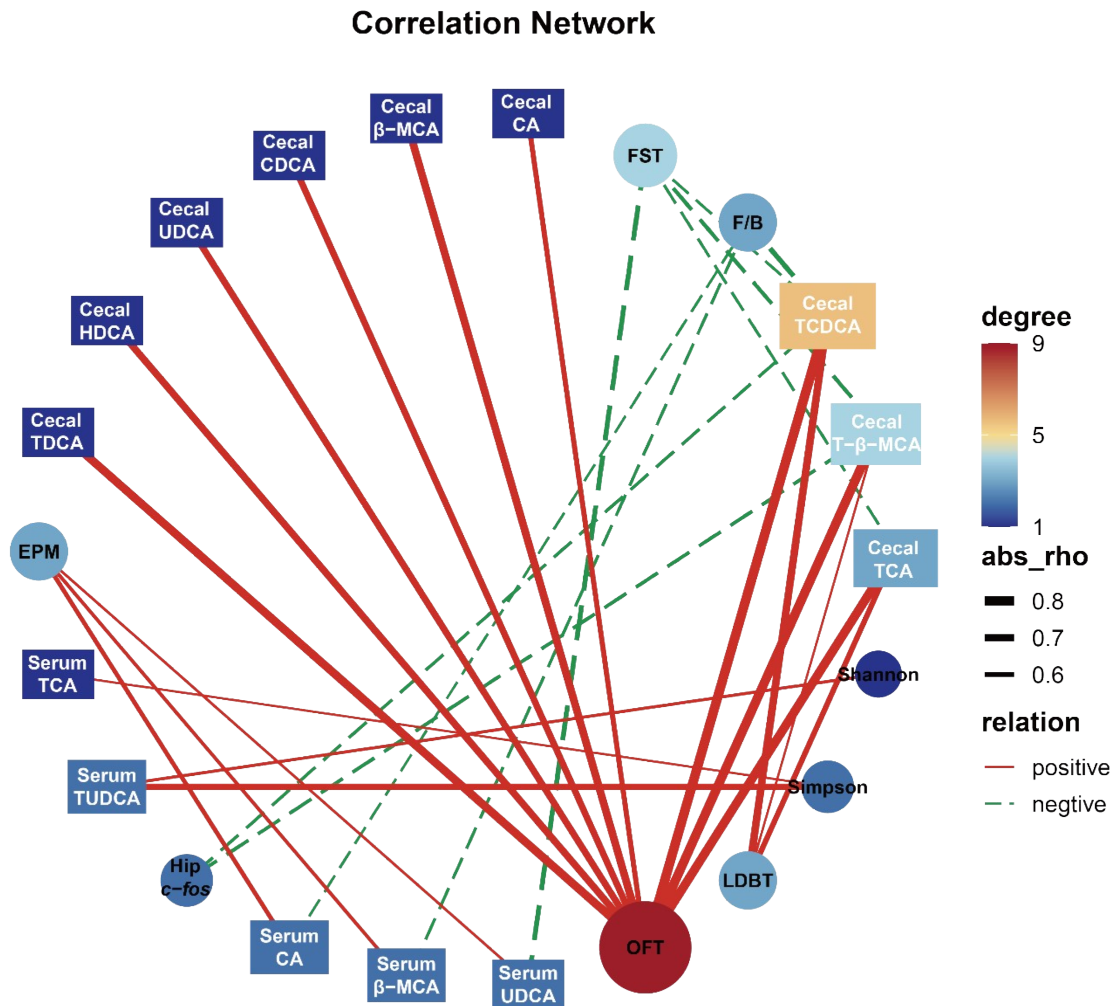

Fig. S5 Correlation Network. The correlation analysis was based on spearman and threshold was set as $P < 0.05$, rho > 0.5 or < -0.5. FST: Forced swim test, F/B: Firmicutes/Bacteroidetes ratio, LDBT: Light dark box test, OFT: Open field test, Hip *c-fos*: Hippocampal *c-fos*, EPM: Elevated plus maze.