Fig. S1



Fig. S1 Effects of BCCAO on body weight (**A**), defecation frequency (**B**), length of fecal pellets (**C**), fecal wet weight (**D**), fecal dry weight (**E**), and fecal water content (**F**) in rats (n = 8 Sham, n = 10 BCCAO). The data represent the mean \pm SEM, p < 0.05 was set as the threshold for significance. * p < 0.05 compared to the sham group.

Α



В



Fig. S2 Effects of BCCAO on gut function and microbial ecology. A One of the BCCAO rats was excluded from the intestinal motility test due to intestinal obstruction. **B** The structure of the gut microbiota at the phylum level (n = 15/group).





lassified cultured

1p_g_n

eae 011_group

e_NK4A214_g

Fig. S3 Overall representation of bacterial profiles in the sham and BCCAO rats by linear discriminant effect size (LEfSe) analysis (n = 15/group).



Bacteroidales_S24-7_group Ruminococcaceae Prevotellaceae Erysipelotrichaceae Peptostreptococcaceae Bacteria_unclassified Porphyromonadaceae Mollicutes_RF9_norank Christensenellaceae Coriobacteriaceae Desulfovibrionaceae Acidaminococcaceae Rikenellaceae Enterobacteriaceae Alcaligenaceae Clostridiaceae_1 Family_XIII Gaiellales_uncultured Verrucomicrobiaceae Cyanobacteria_norank Flavobacteriaceae Streptococcaceae Unknown_Family Leuconostocaceae Peptococcaceae Gemmatimonadaceae Spirochaetaceae Enterococcaceae Deferribacteraceae Rhodospirillaceae Bifidobacteriaceae Eubacteriaceae Helicobacteraceae Clostridiales_vadinBB60_group Mitochondria Pasteurellaceae Elusimicrobiaceae Anaeroplasmataceae Vibrionaceae Micrococcaceae Firmicutes_unclassified Veillonellaceae Acidobacteria_norank Thermoanaerobacteraceae Gastranaerophilales_norank

Fig. S4 A heatmap demonstrating the gut microbiota profile at the family level between the sham and BCCAO groups (n = 15/group).



Fig. S6



Fig. S6 Effects of BCCAO on hippocampal neuron, white matter injury and microglial activation. A Bar plots showing the number of NeuN+ cells in different areas of the hippocampus, namaly cornu ammonis (CA) 1, CA2, CA3, and dentate gyrus (DG) (n = 5 Sham, n = 6 BCCAO). **B** Immunofluorescence staining of myelin basic protein (MBP). Scale bar, 1 mm. **C** Representative images of the medial corpus callosum (CC), paramedian CC, internal capsule (IC), and caudoputamen regions and **D** bar plots showing the mean fluorescence intensity (n = 5 Sham, n = 6 BCCAO). Scale bar, 50 μ m. **E** mRNA levels of Iba-1, microglial pro-inflammatory marker (*CD16*), and anti-inflammatory marker (*CD206*) in the hippocampus at day 36 post BCCAO (n = 6/group). The data represent the mean ± SEM, p < 0.05 was set as the threshold for significance. * p < 0.05, ** p < 0.01 compared to the sham group.

Fig. S7



Fig. S7 Fecal microbiota transplantation successfully rebuilds distinct gut microbiota composition. A The structure of gut microbiota at the phylum level successfully reestablished by FMT (n = 10/group). **B** Bar plots showing the relative abundance of f_Ruminococcaceae and f_Prevotellaceae at the family level and g_Bifidobacterium, g Bacteroides, g Clostridium, g Blautia, g Roseburia, g Coprococcus, and g Dorea at the genus level (n = 10/group). S+FMT(B), sham rats received BCCAO-rat-derived fecal microbiota transplantation. S+FMT(S), sham rats received sham-rat-derived fecal microbiota transplantation. B+FMT(B), BCCAO rats received BCCAO-rat-derived fecal microbiota transplantation. B+FMT(S), BCCAO rats received sham-rat-derived fecal microbiota transplantation. The data represent the mean \pm SEM. p < 0.05 was set as the threshold for significance. * p < 0.05, ** p < 0.01, *** p < 0.001 compared to the S+FMT(B) group. & p < 0.05 compared to the S+FMT(S) group. # p < 0.05, # # p < 0.01 compared to the B+FMT(B) group.

0.0

0.00

0.0



Fig. S8 Effects of FMT on body weight (A), defecation frequency (B), length of fecal pellets (C), fecal wet weight (D), fecal dry weight (E), and fecal water content (F) in recipient rats (n = 10 S+FMT(B) and S+FMT(S), n = 14 B+FMT(B) and B+FMT(S)). The data represent the mean \pm SEM, p < 0.05 was set as the threshold for significance. ** p < 0.01, *** p < 0.001 compared to the S+FMT(B) group. &&& p < 0.001 compared to the S+FMT(B) group.

• S+FMT(B) • S+FMT(S)

• B+FMT(B)

• B+FMT(S)



Fig. S9 Effects of FMT on the number of NeuN+ cells in different areas of the hippocampus, namaly (A) CA 1, (B) CA2, (C) CA3, and (D) DG (n = 6 S+FMT(B) and S+FMT(S), n = 8 B+FMT(B) and B+FMT(S)). The data represent the mean \pm SEM, p < 0.05 was set as the threshold for significance. ** p < 0.01, *** p < 0.001 compared to the S+FMT(B) group. & p < 0.05, &&& p < 0.001 compared to the S+FMT(S) group. # p < 0.05 compared to the B+FMT(B) group.

Fig. S10





Fig. S10 Effects of FMT on white matter injury after BCCAO. A Representative immunofluorescence staining images of MBP in the medial CC, paramedian CC, IC, and caudoputamen regions. Scale bar, 50 μ m. **B** Bar plots showing the mean fluorescent intensity (n = 6/group). The data represent the mean \pm SEM, p < 0.05 was set as the threshold for significance. ** p < 0.01, *** p < 0.001 compared to the S+FMT(B) group. & p < 0.05, && p < 0.01 compared to the S+FMT(S) group.

Fig. S11

• S+FMT(B) • S+FMT(S) • B+FMT(B) • B+FMT(S)



Fig. S11 Effects of FMT on SCFA levels in the hippocampus of recipient rats, including acetic acid (**A**), propionic acid (**B**), isobutyric acid (**C**), butyric acid (**D**), isovaleric acid (**E**), valeric acid (**F**), and hexanoic acid (**G**) (n = 8/group). The data represent the mean \pm SEM, p < 0.05 was set as the threshold for significance. * p < 0.05 compared to the S+FMT(B) group. # p < 0.05 compared to the B+FMT(B) group.

Α



В



Fig. S12 Immunofluorescence staining of the GPR41-a receptor of SCFAs with hippocampal neurons (**A**) and microglia (**B**). Arrows indicate the expression of GPR-41 on microglia. Scale bar, 20 μm.

Fig. S13

0.0



Fig. S13 Effects of long-term SCFA supplementation on body weight (A), defecation frequency (B), length of fecal pellets (C), fecal wet weight (D), fecal dry weight (E), and fecal water content (F) in rats (n = 8 S+NaCl and S+SCFA, n = 12 B+NaCl and B+SCFA). S+NaCl, sham rats treated with sodium chloride (NaCl). S+SCFA, sham rats that received short chain fatty acids (SCFAs) (acetate, propionate and butyrate) supplementation. B+NaCl, BCCAO rats treated with NaCl. B+SCFA, BCCAO rats received SCFAs supplementation. The data represent the mean \pm SEM, p < 0.05 was set as the threshold for significance. ** p < 0.01 compared to S+NaCl group. && p < 0.01 compared to the S+SCFA group.

0.0

0.2

0.0





Fig. S14 Effects of long-term SCFA supplementation on the number of NeuN+ cells in different areas of the hippocampus, namaly (**A**) CA 1, (**B**) CA2, (**C**) CA3, and (**D**) DG (n = 6). The data represent the mean \pm SEM, p < 0.05 was set as the threshold for significance. * p < 0.05, ** p < 0.01, *** p < 0.001 compared to the S+NaCl group. & p < 0.05, && p < 0.01 compared to the S+SCFA group. # p < 0.05 compared to the B+NaCl 37 d group.