Supporting Information

Table S1. The primer sequence of target gene and housekeeping gene.

Gene	Primer Sequence (5'-3')	GenBank NO.
IL-6	F: CCCACCAAGAACGATAGTCA R: CTCCGACTTGTGAAGTGGTA	NM_001314054.1
TNF-α	F: CAGGCGGTGCCTATGTCTC R: CGATCACCCCGAAGTTCAGTAG	NM_001278601.1
IL-10	F: CAAGGAGCATTTGAATTCCC R: GGCCTTGTAGACACCTTGGTC	NM_010548.2
Shank3	F: GATCTGCCATCCCTACAAC R: AGCTAAGGGTGAGCTAGGAT	NM_021423.4
Pten	F: TGGATTCGACTTAGACTTGACCT R: GCGGTGTCATAATGTCTCTCAG	NM_008960.2
Claudin-1	F: CATCAATGCCAGGTATGAATT R: TGTTGGGTAAGAGGTTGTTT	NM_016674.4
GAPDH	F: AGGTCGGTGTGAACGGATTTG R: GGGGTCGTTGATGGCAACA	NM_001289726.2

Figure S1

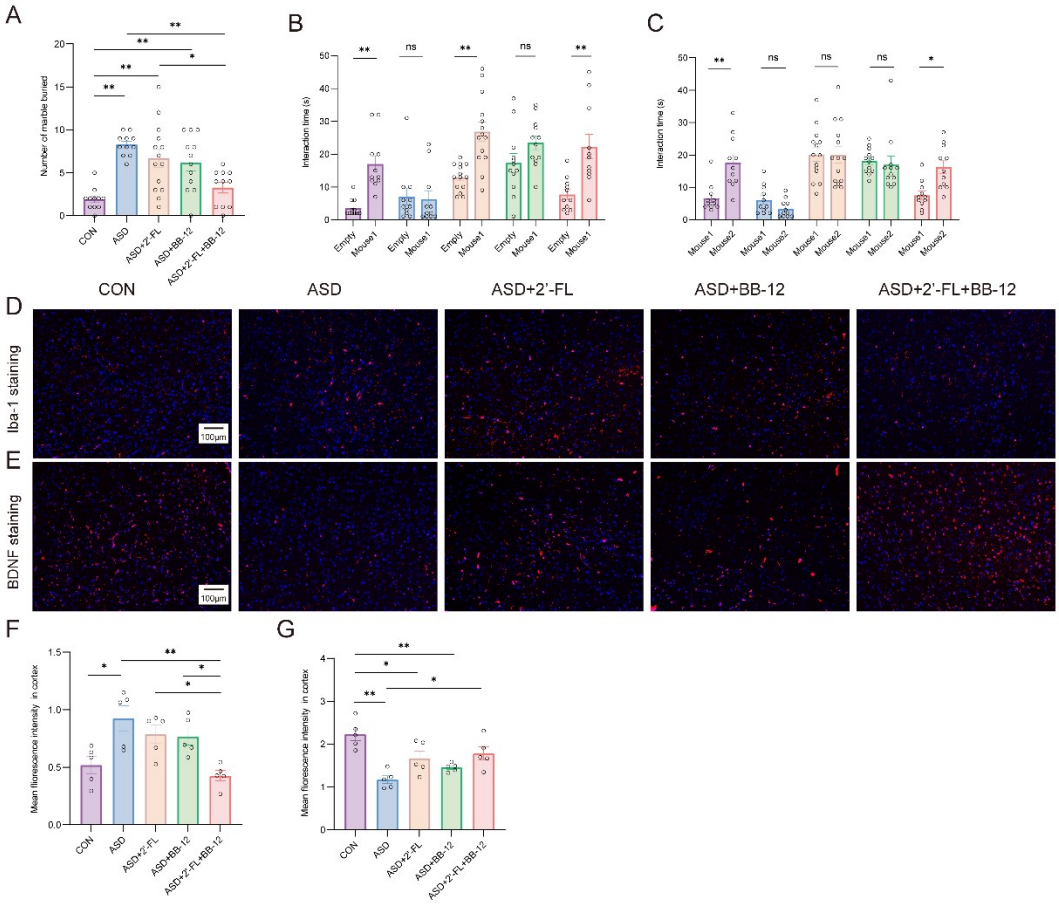

Figure S1 Synbiotic intervention improved behavioral impairment of VPA-induced ASD mice.

(A) Number of marbles buried in marble burying test. **(B)** In the three-chamber social test, the time spent by the test mice interacting with the empty cup and Mouse 1 was measured. **(C)** In the three-chamber social test, the time spent by the test mice interacting with Mouse 1 and Mouse 2 was measured. **(D)** Representative images of Iba-1 immunofluorescence staining in the brain, the Iba-1 were stained red, and nuclei were counter-stained blue. **(E)** Representative images of BDNF immunofluorescence staining in the cortex, the BDNF were stained red, and nuclei were counter-stained blue. **(F)** The fluorescence intensity of Iba-1 in the cortex. **(G)** The fluorescence intensity of BDNF in the cortex. Data are presented as mean ± SEM and statistical analyses were determined by one-way ANOVA with Tukey's test. *$p < 0.05$, **$p < 0.01$, ns $p > 0.05$.

Figure S2

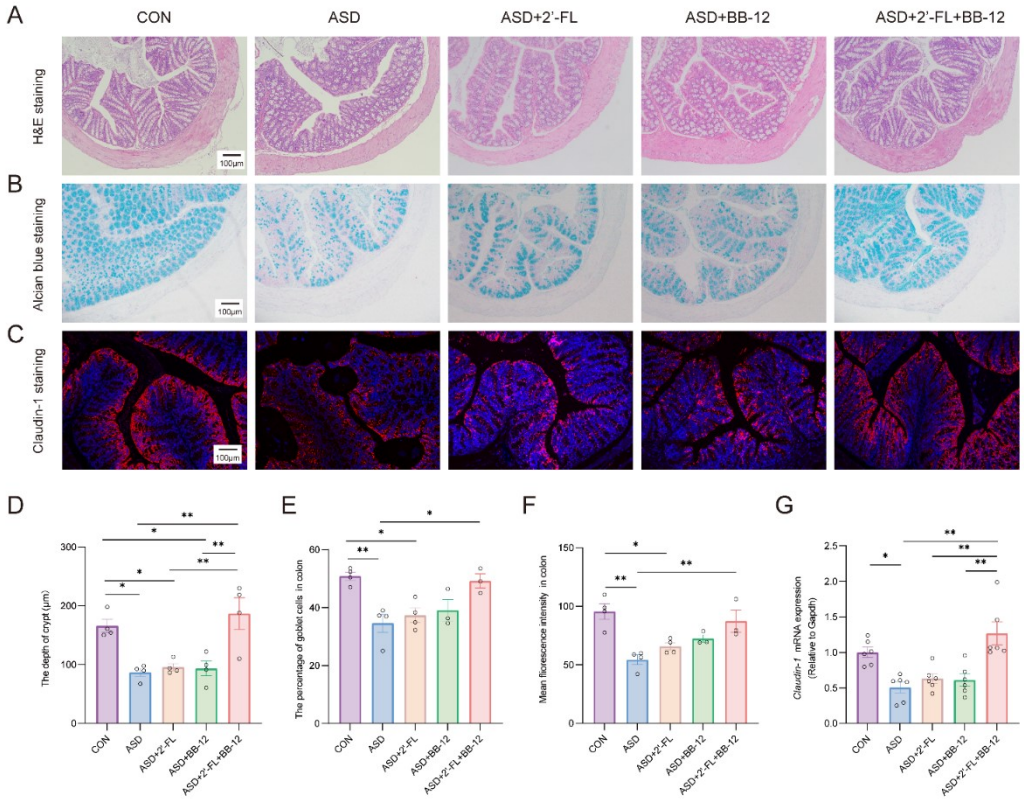

Figure S2 Effects of synbiotic treatment on colon morphology and barrier function in VPA-induced ASD mice.
(A) Representative images of H&E staining of the colon for each group (n=4). **(B)** Representative images of Alcian blue staining of the colon for each group (n=4). **(C)** Representative images of Claudin-1 immunofluorescence staining in the colon, the Claudin-1 were stained red, and nuclei were counter-stained blue (n=3-4). **(D)** Crypt depth based on H&E-stained sections. **(E)** Percentage of Alcian blue-stained area in the colon (%). **(F)** The fluorescence intensity of Claudin-1 in colon. **(G)** The relative mRNA expression levels of *Claudin-1* in the colon of each group. Data are presented as mean ± SEM and statistical analyses were determined by one-way ANOVA with Tukey's test. *$p < 0.05$, **$p < 0.01$.

Figure S3

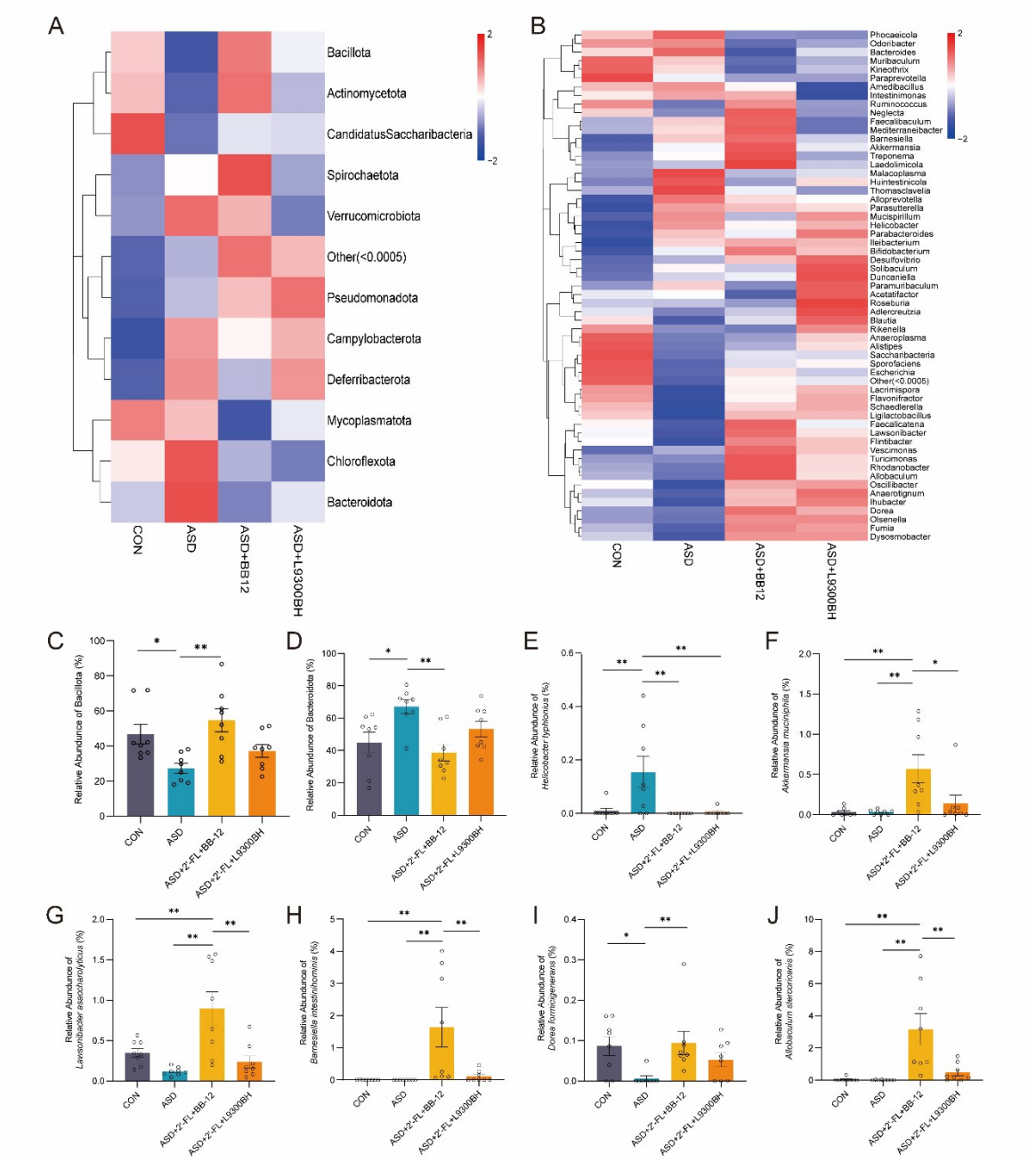

Figure S3 Effects of synbiotic treatment on the colonic microbiota of VPA-induced ASD mice.

(A) A heatmap displaying the relative abundance of gut microbiota at the phylum level across different experimental groups. **(B)** A heatmap displaying the relative abundance of gut microbiota at the genus level across different experimental groups. **(C)** Relative abundance of Bacillota. **(D)** Relative abundance of Bacteroidota. **(E)** Relative abundance of *Helicobacter typhlonius*. **(F)** Relative abundance of *Akkermansia muciniphila*. **(G)** Relative abundance of *Lawsonibacter asaccharolyticus*. **(H)** Relative abundance of *Barnesiella intestinihominis*. **(I)** Relative abundance of *Dorea formicigenerans*. **(J)** Relative abundance of *Allobaculum stercoricanis*. Data are presented as mean ± SEM and statistical analyses were determined by one-way ANOVA with Tukey's test. *$p < 0.05$, **$p < 0.01$.