Supplementary Material

Synbiotic formulation of *Lactobacillus reuteri* and inulin alleviates ASD-like behaviors in a mouse model: the mediating role of the gut-brain axis

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Supplementary Table and Figures

Table S1 Formulation of carbon source added to the culture medium

Concentration (g/L)	**5**	**10**	**20**	**30**	**40**
GAM/mL	5	5	5	5	5
Carbon source/g	0.025	0.050	0.100	0.150	0.200

Table S2 Primer sequences used for semi-quantitative RT-PCR analysis

	Forward 5'-3'	**Reverse 5'-3'**
GAPDH	TGGAGAAACCTGCCAAGTATGA	TGGAAGAATGGGAGTTGCTGT
ZO-1	TGGGAACAGCACACAGTGAC	GCTGGCCCTCCTTTTAACAC
Claudin-1	AGCTGCCTGTTCCATGTACT	CTCCCATTTGTCTGCTGCTC
GPR43	GGCTCCCTGCCAACCTGCTG	GTGCACAGGGGCAGGCTGAG
IL-1β	AGCTGGAGAGTGTGGATCCC	CCTGTCTTGGCCGAGGACTA
Tnf-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
IL-6	CCACTTCACAAGTCGGAGGC	GGAGAGCATTGGAAATTGGGGT
IL-10	GCTCCAAGACCAAGGTGTCTACAA	CCGTTAGCTAAGATCCCTGGATCA
ShAnk3	GATCTGCCATCCCTACAAC	AGCTAAGGGTGAGCTAGGAT
SNAP25	CAACTGGAACGCATTGAGGAA	GGCCACTACTCCATCCTGATTAT
PTEN	TGGATTCGACTTAGACTTGACCT	GCGGTGTCATAATGTCTCTCAG

Mecp2	ATGGTAGCTGGGATGTTAGGG	TGAGCTTTCTGATGTTTCTGCTT
BDNF	CTCCGCCATGCAATTTCCACT	GCCTTCATGCAACCGAAGTA
PSD-95	TCTGTGCGAGAGGTAGCAGA	AAGCACTCCGTGAACTCCTG
Mct1	CAGTGCAACGACCAGTGAAG	AGTTGAAAGCAAGCCCAAGA
Gpr109a	GGGTCCTACCTAGCCTGTCC	GGCTAACCCCCTCTTACCAC
Gpr41	GTGACCATGGGGACAAGCTTC	CCCTGGCTGTAGGTTGCATT
Hmgcs2	ATACCACCAACGCCTGTTATGG	CAATGTCACCACAGACCACCAG
Hmgcl	CAGGTGAAGATCGTGGAAGTC	TGGGAGAAACAAAGCTGGTG
Bdh1	GCTTCCTTGTATTTGCTGGC	TTCTCCACCTCTTCACTGTTG
bacteria	ACTCCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGG
L. reuteri	GAAGATCAGTCGCAYTGGCCCAA	TCCATTGTGGCCGATCAG

Table S3 R function envfit was used to coordinates of microbial communities (PCoA)

OUT	plsda1	plsda2	R square	p value	Taxonomy
OTU39	-0.744263178	-0.667886458	0.592218753	0.001	Bacteria;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Desulfovibrio
OTU231	0.165561759	-0.986199424	0.526974891	0.001	Bacteria;Firmicutes;Clostridia;Clostridiales
OTU600	0.889665336	-0.456613172	0.463116376	0.001	Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae
OTU403	0.993594288	-0.113006153	0.429620101	0.001	Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae

OTU116	0.956258367	-0.292523393	0.40501793	0.001	Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae
OTU26	0.983206755	-0.182495143	0.39978134	0.001	Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;Barnesiella;Barnesiella_intestinihominis
OTU31	0.964582904	-0.263779873	0.36640954	0.001	Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella
OTU138	-0.450785064	-0.892632526	0.363562822	0.001	Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae
OTU487	0.993359737	-0.115049695	0.360608945	0.001	Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae
OTU356	0.994762976	0.102208711	0.359997072	0.001	Bacteria;Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae

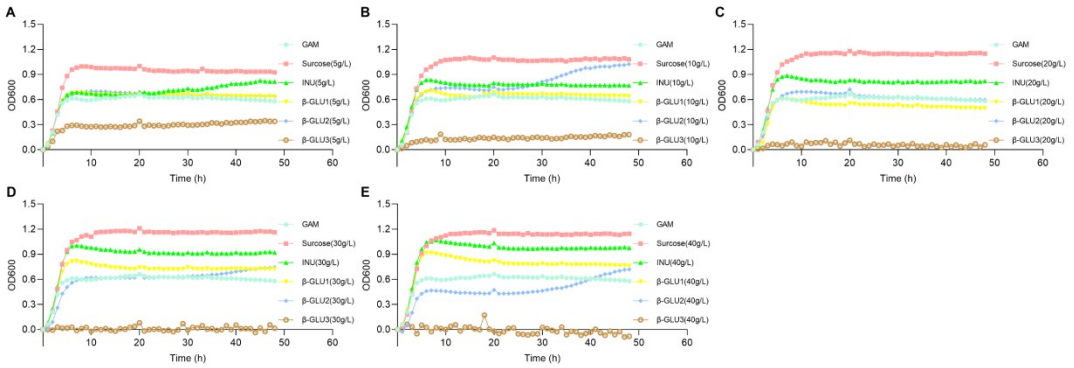

Figure S1 Effect of different carbon sources on the growth curve of *L. reuteri* C501

(A-E) Growth curves of *L. reuteri* C501 containing different concentrations of carbon source, 5 g/L (A), 10 g/L (B), 20 g/L (C), 30 g/L (D), and 40 g/L (E) (n = 3).

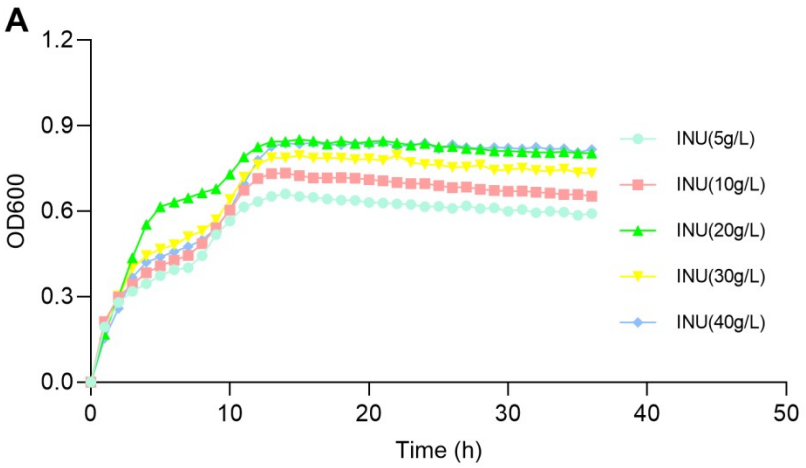

Figure S2 Effect of different doses of inulin on the growth curve of *L. reuteri* C501

(A) Growth curves of *L. reuteri* C501 with different concentrations of inulin (n = 3).

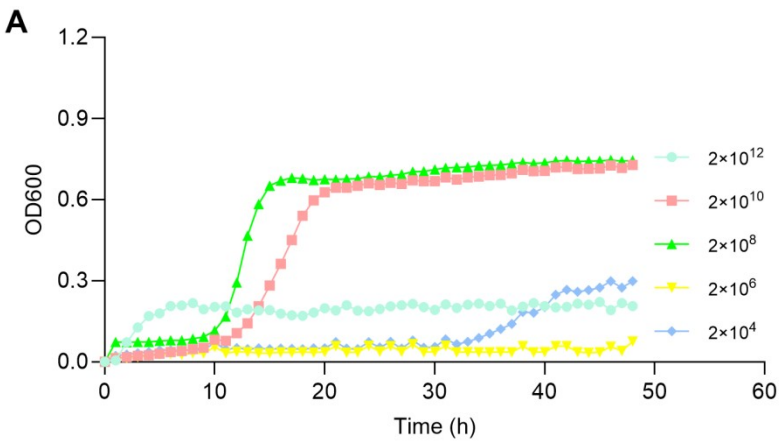

Figure S3 Effect of optimal dose of inulin on the growth curves of *L. reuteri* C501 in different concentrations

(A) Growth curves of *L. reuteri* C501 at 20 g/L inulin on different concentrations (n = 3).

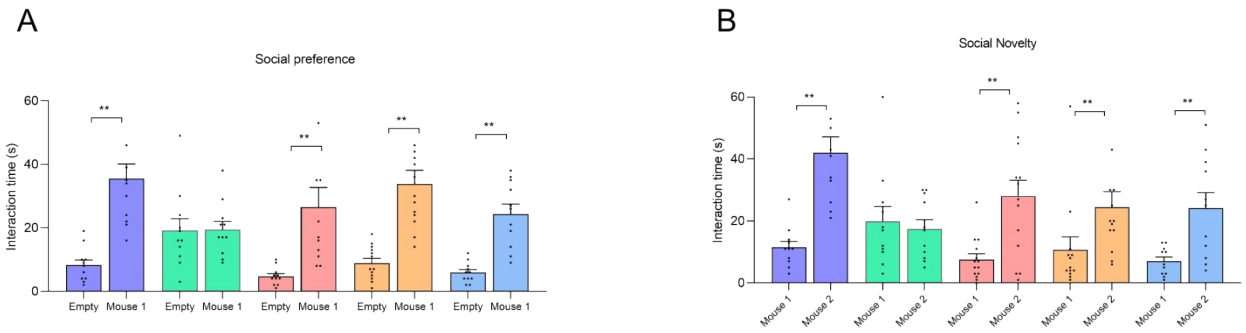

Figure S4 Interaction time in the three-chamber test

（A-B）The original interaction time of each groups for empty wire cup, mouse (mouse) and novel mouse (mouse 2)

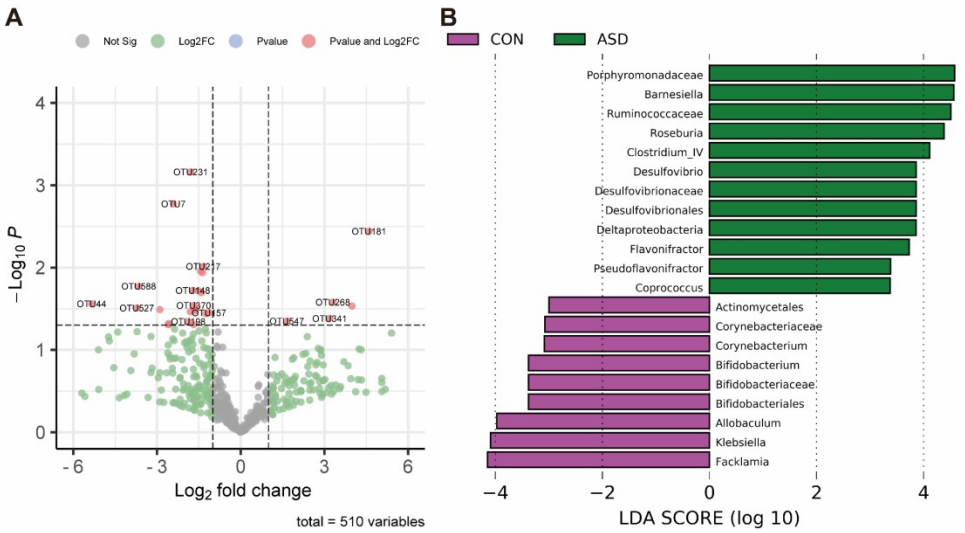

Figure S5 Effects of VPA-induced ASD mice on gut microbiota diversity

(A) Volcano plot of different OTUs between CON and ASD groups (n = 9-10). (B) Linear Discriminant Analysis of CON and ASD groups (n = 9-10).

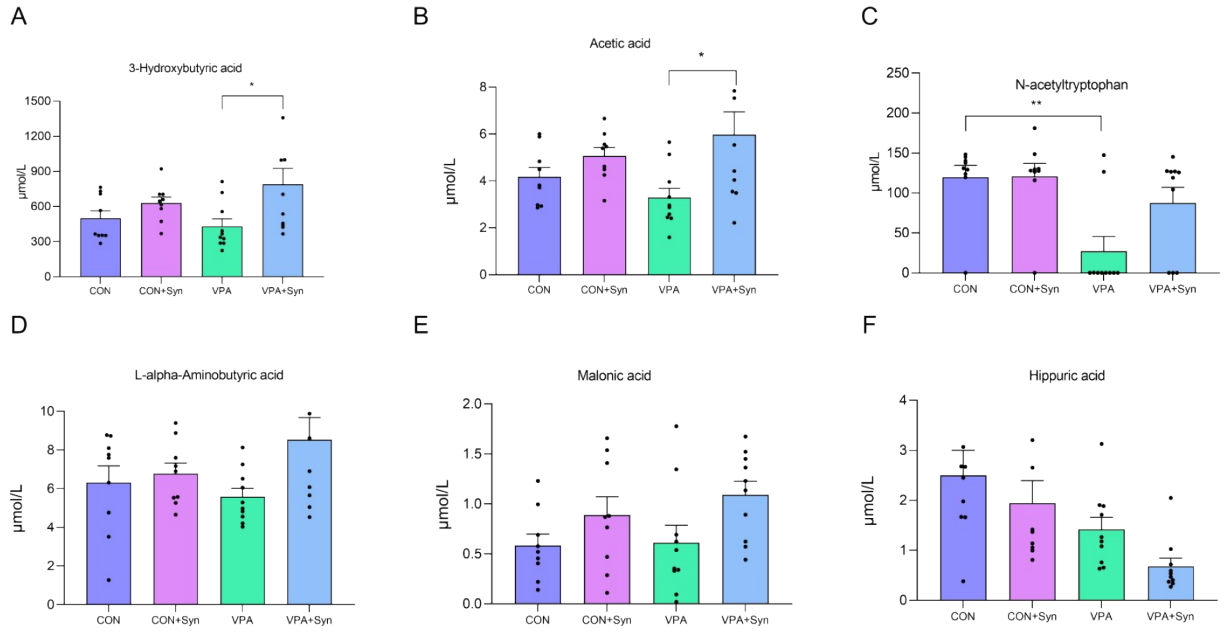

Figure S6 Effects of synbiotic treatment on serum metabolites in VPA-induced ASD mice

(A-I) The concentrations of metabolites Acetic acid, Hippuric acid, L-alpha-Aminobutyric acid, 3-Hydroxybutyric acid, N-acetyltryptophan, and Malonic acid in the serum (n = 9-10).

Data are presented as mean ± SEM and statistical analyses were determined by one-way ANOVA with Tukey's test. *$p < 0.05$, **$p < 0.01$.

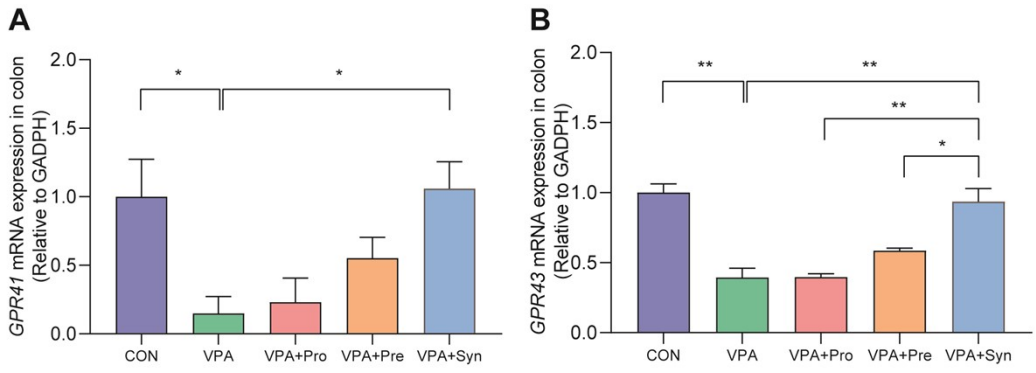

Figure S7 Effects of synbiotic treatment on SCFAs receptors in VPA-induced ASD mice

(A-B) The mRNA expression of *GPR41* and *GPR43* in the colon (n = 3-4).

Data are presented as mean ± SEM and statistical analyses were determined by one-way ANOVA with Tukey's test. *$p < 0.05$, **$p < 0.01$.