Supplementary Materials

Therapeutic potential of *Lachnospiraceae* strains in irritable bowel syndrome via differential gut-brain pathways

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The Supplementary materials includes:

Tukey HSD post hoc test, $p < 0.05$).

Supplementary Figure 3. Taxonomic composition of gut microbiota in IBS mice treated with *Lachnospiraceae* strains. (A) Phylum-level bar plot. (B) Genus-level bar plot. (C) Relative abundance of Pseudomonadota and Actinomycetota. (D) Relative abundance of *Clostridia*_UCG-014, *Muribaculaceae, Adlercreutzia,* and *Roseburia*. Note: Data are presented as mean ± SEM. Different letters indicate statistically significant differences among groups (one-way ANOVA followed by Tukey HSD post hoc test, $p < 0.05$).

Supplementary Figure 4. Multi-level correlation analysis of gut-brain axis. (A) Correlation heatmap. (B-E).Correlations between acetic acid and BAs levels (DCA, LCA, UDCA, and TUDCA). (F-K) Correlations of IL-6 with cerebral gene expression (*Tph2*, *Slc6a4*, *Gad1*, *Gabbr1*, GFAP, and *Slc1a2*). $p < 0.05$ was considered statistically significant. Note: (A) Spearman correlation analysis with Benjamini-Hochberg FDR correction; * indicates adjusted $q < 0.05$, $|r| > 0.6$. (B-E) Spearman correlation analysis; *r* and *p* values are indicated in each panel.

Supplementary Table 1. Composition of the synthetic bacterial community (SynCom-23)

Synthetic Bacterial Community (SynCom-23)[a]			
Strain	**concentration**	**Strain**	**concentration**
Bacteroides stercoris MW-001	10^8 CFU/mL	*Bifidobacterium longum* MW-013	10^8 CFU/mL
Bacteroides vulgatus MW-002	10^8 CFU/mL	*Collinsella aerofaciens* MW-014	10^8 CFU/mL
Bacteroides caccae MW-003	10^8 CFU/mL	*Streptococcus luteiensis* MW-015	0.5×10^8 CFU/mL
Bacteroides xylanisolvens MW-004	10^8 CFU/mL	*Streptococcus salivarius* MW-016	0.5×10^8 CFU/mL
Bacteroides uniformis MW-005	10^8 CFU/mL	*Escherichia coli* MW-017	10^8 CFU/mL
Bacteroides	10^8 CFU/mL	*Ruminococcus*	10^8 CFU/mL

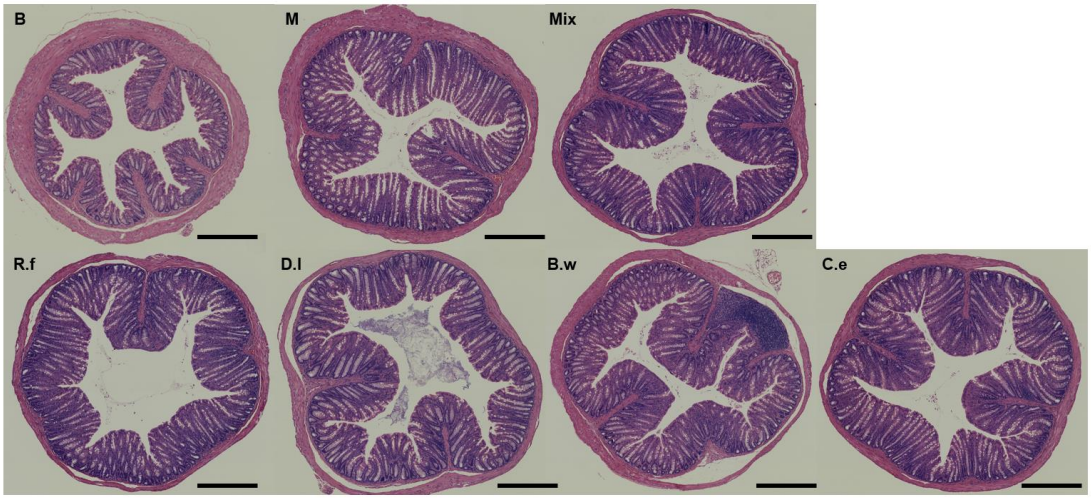
thetaiotaomicron MW-006		*bromii* MW-018	
Bacteroides cellulosilyticus MW-007	10^8 CFU/mL	*Prevotella ihumii* MW-019	0.5×10^8 CFU/mL
Bacteroides dorei MW-008	10^8 CFU/mL	*Prevotella jejuni* MW-020	0.5×10^8 CFU/mL
Bacteroides ovatus MW-009	10^8 CFU/mL	*Phocaeicola dore* MW-021*i*	10^8 CFU/mL
Akkermansia muciniphila MW-010	10^8 CFU/mL	*Eubacterium Siraeum* DSM 15702	10^8 CFU/mL
paraclostridium benzoelyticum MW-011	10^8 CFU/mL	*Clostridium Leptum* DSM 753	10^8 CFU/mL
Agathobacter rectalis MW-012	10^8 CFU/mL		

[a] All strains were isolated in-house from healthy human feces, except for *E. siraeum* DSM 15702 and *C. leptum* DSM 753, which were obtained from DSMZ, Germany.

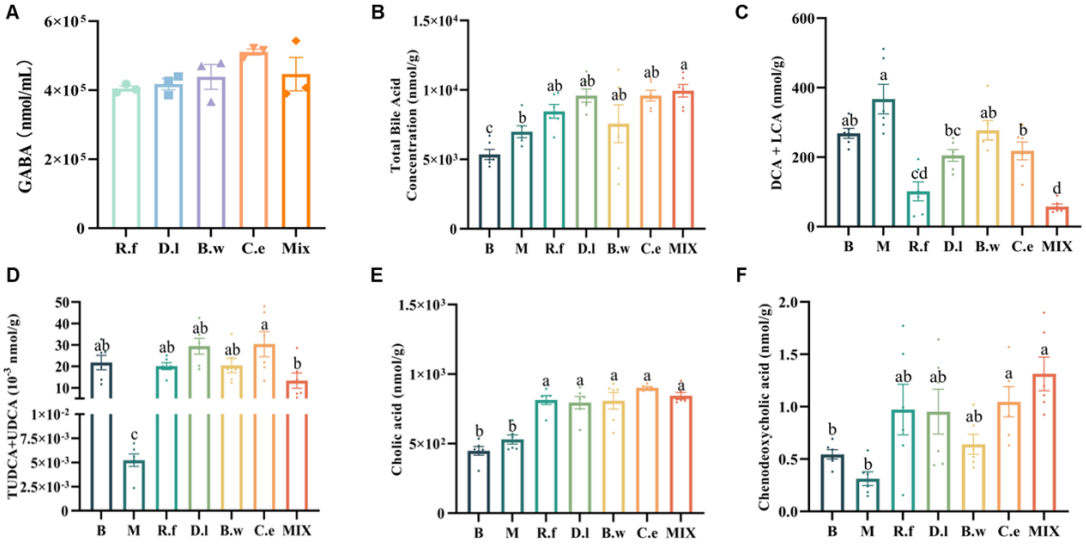
Supplementary Table 2. Primer sequences used for RT-qPCR

Target genes	Primer sequences	References
CGRP	F: GAAGAAGAAGTTCGCCTGCT R: GATTCCCACACCGCTTAGAT	[1]
TRPV1	F: CGAGGATGGGAAGAATAACTCACTG P: GGATGATGAAGACAGCCTTGAAGTC	[2]
ZO-1	F: AGGACACCAAAGCATGTGAG R: GGCATTCCTGCTGGTTACA	[3]
Occludin	F: TTGAAAGTCCACCTCCTTACAGA R: CCGGATAAAAAGAGTACGCTGG	[3]
Claudin1	F: CCTGCCCCAGTGGAAGATTT R: AAACGCAGGACATCCACAGT	[3]
Htr1a	F: ACAGGGCGGTGGGGACTC R: CAAGCAGGCGGGGACATAGG	[4]
Tph2	F: TCCTTTGACCCAAAGACGAC R: TTCAATGCTCTGCGTGTAGG	[5]
Slc6a4	F: GGCTGAGATGAGGAACGAAG R: CTATCCAAACCCAGCGTGAT	[5]

Gad1	F: AGGCAGTCCTCCAAGAACCT R: CCGTTCTTAGCTGGAAGCAG	[6]
Gabbr1	F: GA GGACGTGAATAGCCGCAG R: CTGGATCACACTTGCTGTCGT	[7]
Slc1a2	F: GGCAGCTGGGGATGTACA R: ACGCTGGGGAGTTTATTCAAGAAT	[8]
GFAP	F: CCTTCTGACACGGATTTGGT R: TAAGCTAGCCCTGGACATCG	[9]
β-actin	F: GGCTGTATTCCCCTCCATCG R: CCAGTTGGTAACAATGCCATGT	[10]

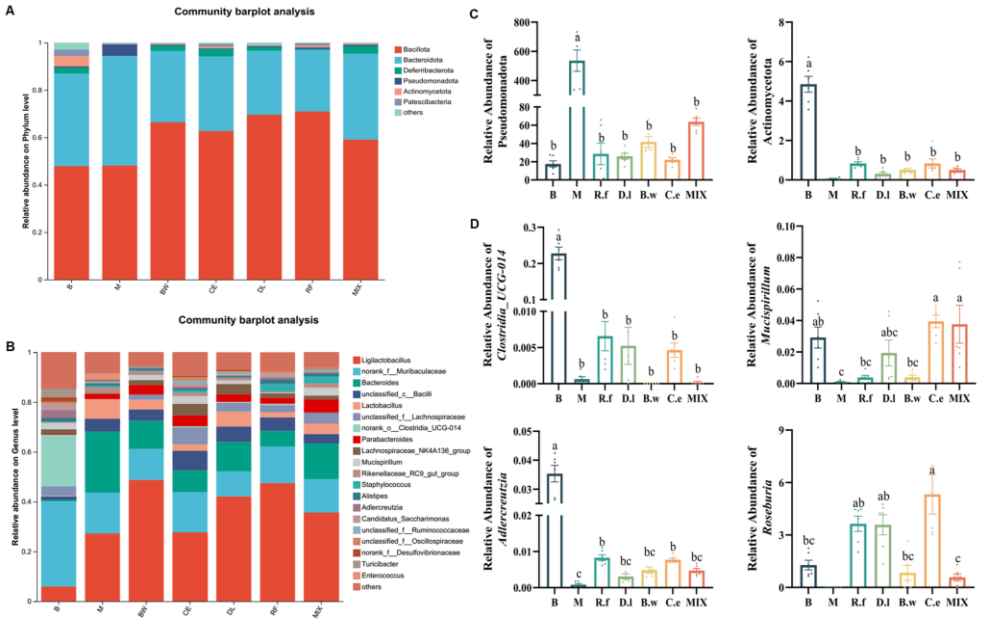


Supplementary Figure 1. H&E staining of colon sections (scale bar = 400 μm).



Supplementary Figure 2. GABA production by *Lachnospiraceae* strains and ileal BAs levels in IBS mice. (A) GABA produced by the four *Lachnospiraceae* strains and

their mixture. (B) Total bile acids (Total BAs). (C) Toxic bile acids: deoxycholic acid (DCA) and lithocholic acid (LCA). (D) Total protective bile acids: ursodeoxycholic acid (UDCA) and tauroursodeoxycholic acid (TUDCA). (E) Cholic acid (CA). (F) Chenodeoxycholic acid (CDCA). Note: Data are presented as mean ± SEM. Different letters indicate statistically significant differences among groups (one-way ANOVA followed by Tukey HSD post hoc test, $p < 0.05$).



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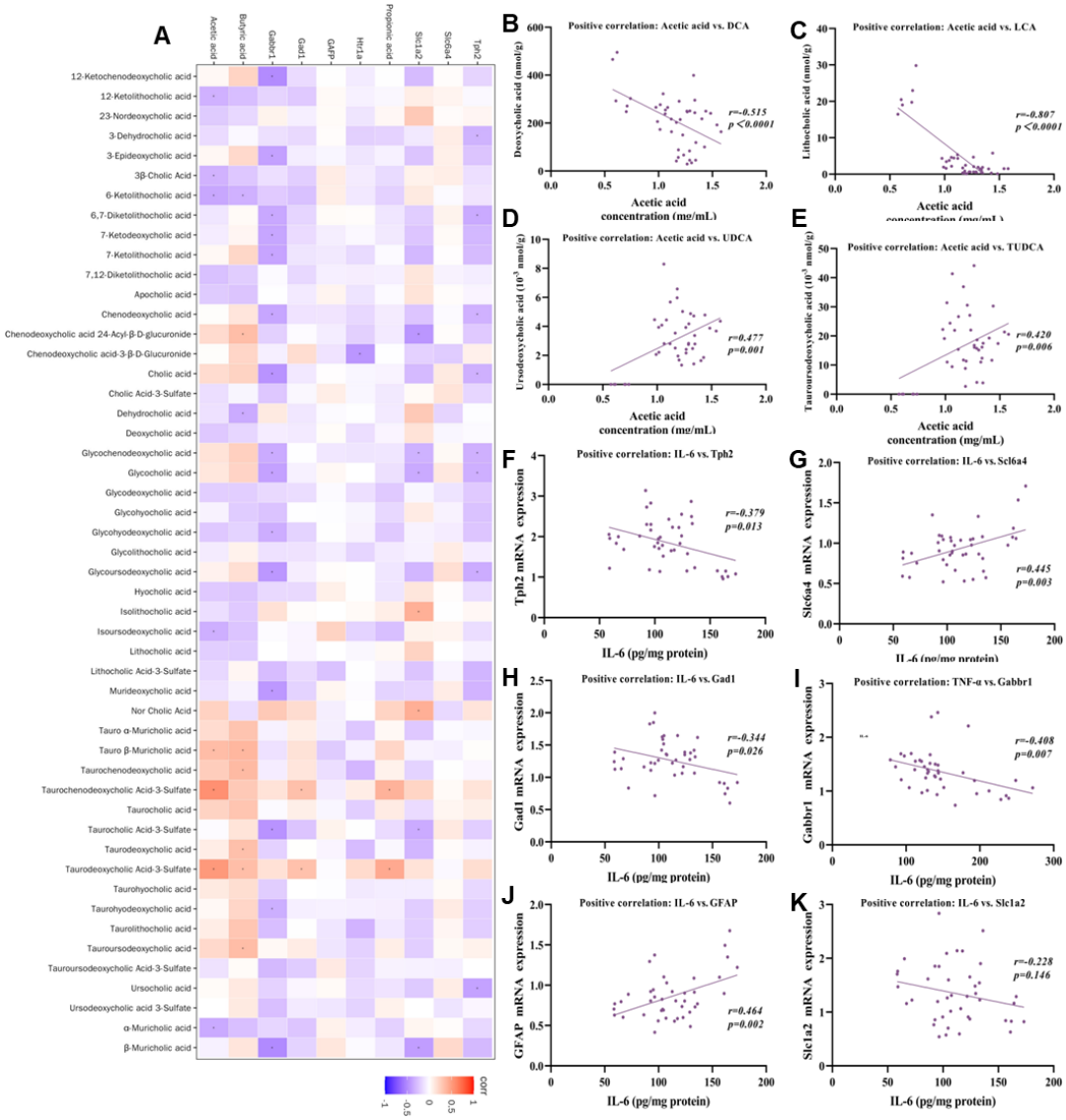

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