Supporting Information

Bacteroides uniformis degrades β-glucan to promote *Lactobacillus johnsonii* improving indole-3-lactic acid levels in alleviating colitis

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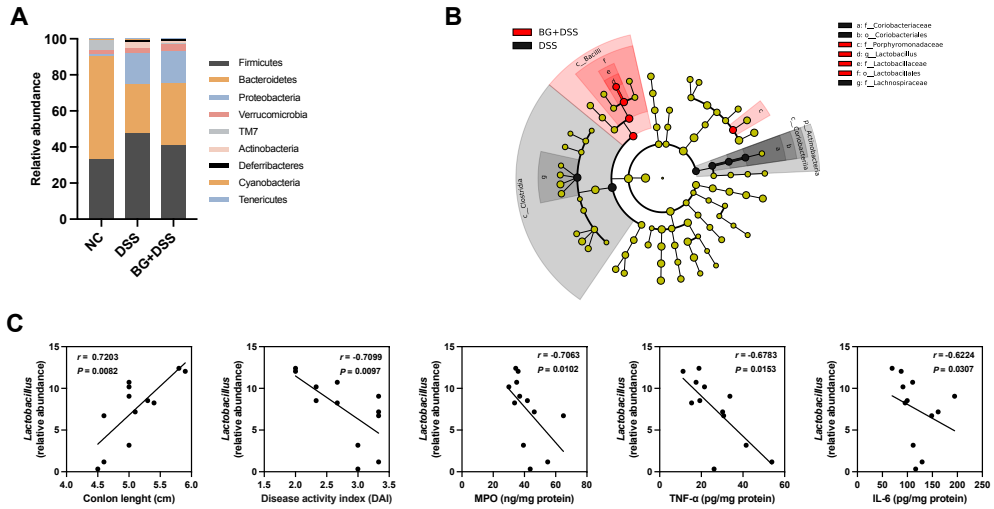

Figure S1. The effect of BG on gut microbiota

(A) Relative abundance at phylum level.

(B) Cladograms generated from LEfSe analysis.

(C) Spearman correlation between the abundance of *Lactobacillus* and DAI, colon length, colonic TNF-α, MPO, and IL-6.

BG, β-glucan; LEfSe, linear discriminant analysis effect size; MPO, myeloperoxidase.

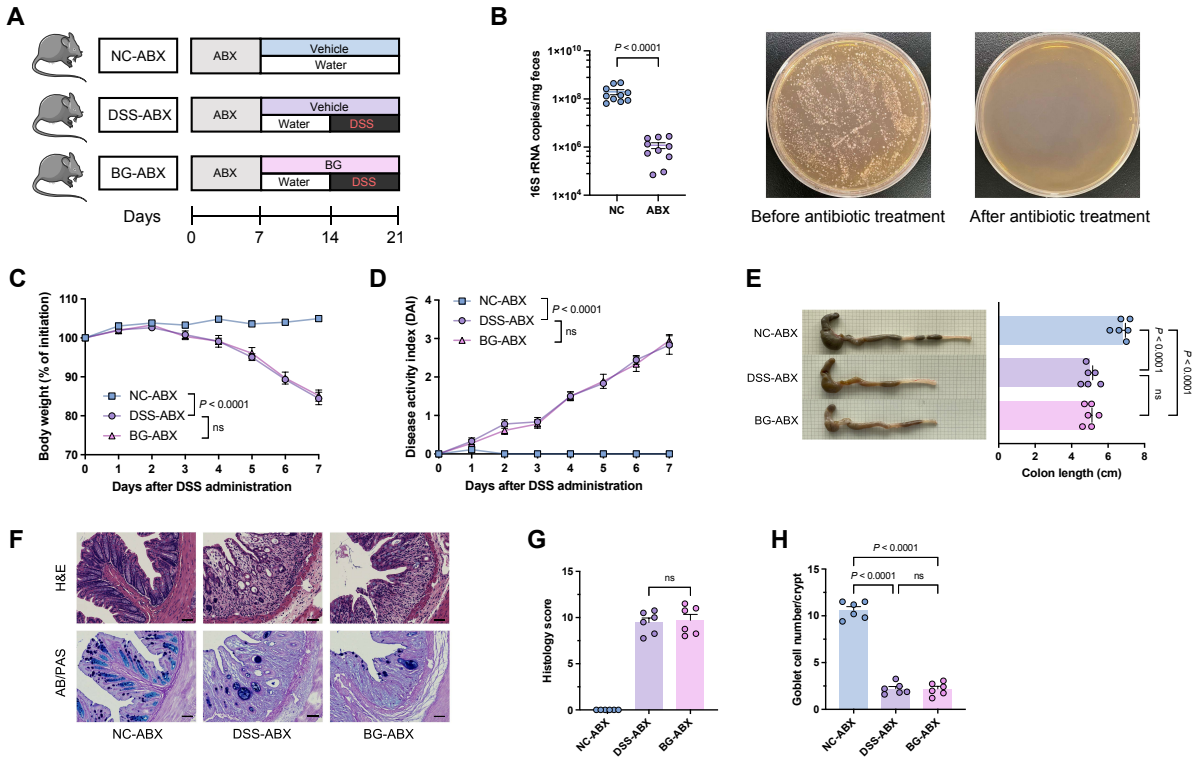

Figure S2. Microbiota depletion with antibiotics eliminated the alleviating effect of BG on colitis

(A) Experimental schema for (B–H). Mice were treated with antibiotic cocktails for 7 days to deplete gut microbiota, and then given with BG from day 7 to day 21 and 3% (w/v) DSS from day 14 to day 21.

(B) Validation of the depletion effect of antibiotic administration on gut microbiota by determining the bacterial load by RT-qPCR (left) and culturing fecal bacteria in BHI agar plates (right).

(C–D) Body weight (C) and disease activity index (D) were monitored daily after DSS treatment.

(E) Representative photographs (left) and length quantification (right) of colon tissues.

(F) Representative H&E-stained sections (upper) and AB/PAS-stained sections (lower) of the distal colon. Scale bars, 50 μm.

(G) Histology scores were determined from H&E-stained sections.

(H) The number of goblet cells per crypt was determined from AB/PAS-stained sections.

ABX, antibiotic; BHI, brain heart infusion. Data are presented as the mean ± SEM. n = 6 mice per group. Statistical analysis was performed using Mann-Whitney test for (B), two-way ANOVA followed by Tukey' post hoc test for (C)–(D), one-way ANOVA followed by the Tukey' post hoc

test for (E) and (H), *t*-test for (G) which compared only the DSS-ABX group with the BG-ABX group. No significant (ns) for $P > 0.05$.

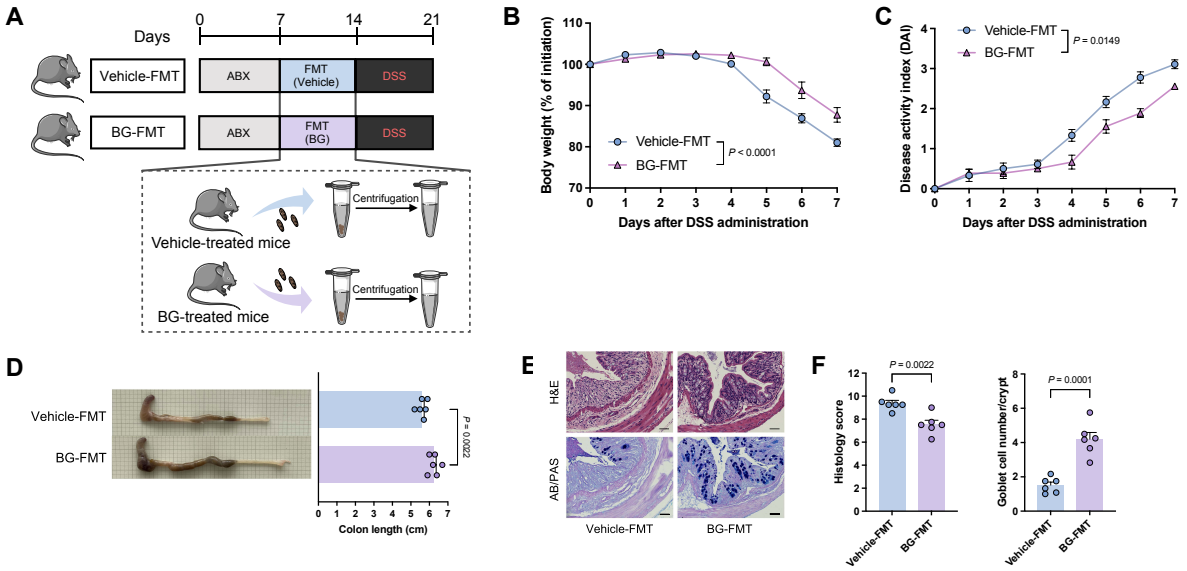

Figure S3. Fecal microbial transplantation of BG-regulated microbiota mitigates DSS-induced colitis

(A) Experimental schema for (B–G). Fecal homogenates from BG-treated or untreated mice were orally transmitted into antibiotic-treated recipient mice. After 7 days of fecal transplantation, the recipient mice were treated with 3% (w/v) DSS for 7 days.

(B–C) Body weight (B) and disease activity index (C) were monitored daily after DSS treatment.

(D) Representative photographs (left) and length quantification (right) of colon tissues.

(E) Representative H&E-stained sections (upper) and AB/PAS-stained sections (lower) of the distal colon. Scale bars, 50 μm.

(F) Histology scores were determined from H&E-stained sections (left) and the number of goblet cells per crypt was determined from AB/PAS-stained sections (right).

Data are presented as the mean ± SEM. n= 6 mice per group. Statistical analysis was performed using two-way ANOVA followed by Bonferroni' post hoc test for (B)–(C), *t*-test for (D)–(F).

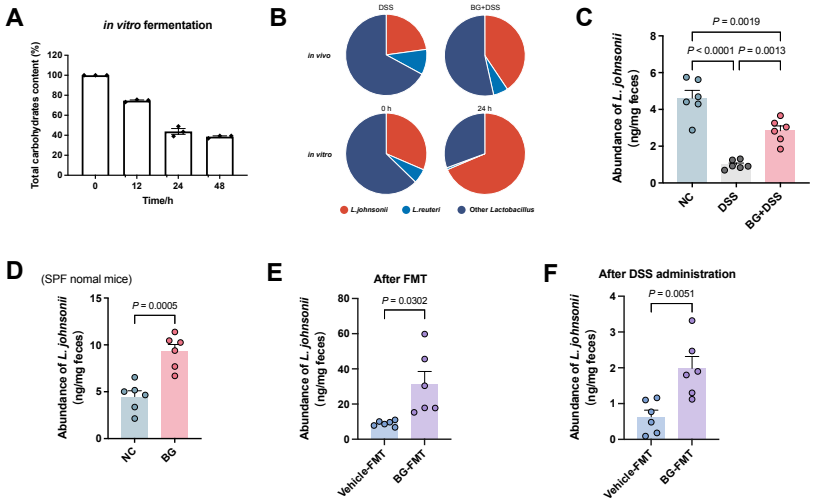

Figure S4. BG has an enrichment effect on *L. johnsonii*

(A) Changes in total carbohydrates during fermentation.

(B) The proportion of *L. johnsonii* and *L. reuteri* to *Lactobacillus* in feces of the DSS and BG+DSS group mice, and the 0 and 24 h fermented samples.

(C) The abundance of *L. johnsonii* in feces of the NC, DSS and BG+DSS group mice.

(D) The abundance of *L. johnsonii* in SPF normal mice feces after 1 week of PBS or BG treatment.

(E) The abundance of *L. johnsonii* after FMT.

(F) The abundance of *L. johnsonii* after FMT following DSS administration.

Data are presented as the mean ± SEM. n= 6 mice per group. Statistical analysis was performed using one-way ANOVA followed by the Tukey' post hoc test for (C), *t*-test for (D) and (F), *t*-test with Welch's correction for (E).

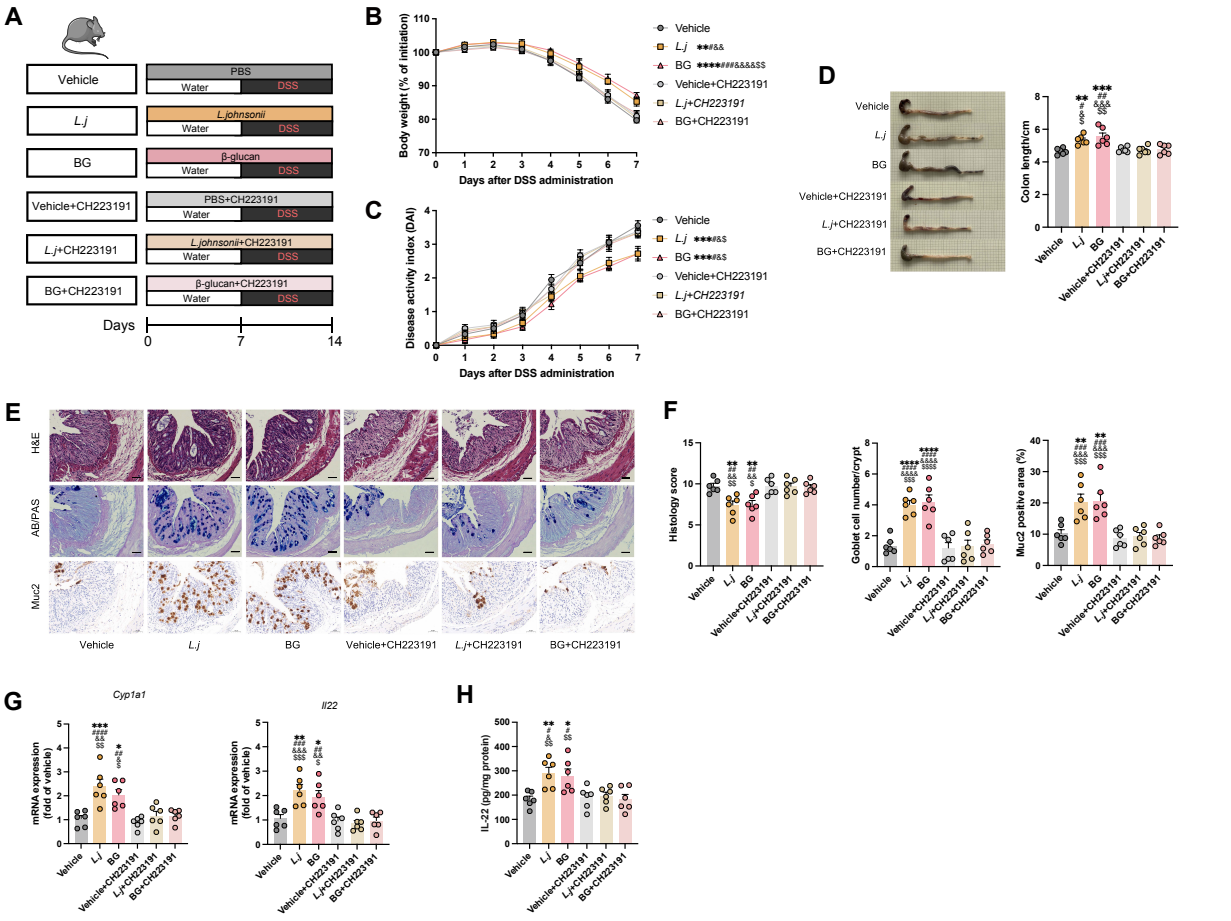

Figure S5. Inhibition of the AhR signaling pathway abolishes the effect of *L. johnsonii* and BG on colitis

(A) Experimental schema for (B–H). Mice were treated with PBS, 1×10^8 CFU of *L. johnsonii*, 400 mg/kg BG, PBS+10 mg/kg of AhR antagonist CH223191, *L. johnsonii*+CH223191, or BG+CH223191, respectively, for 14 days, and 3% (w/v) DSS was added to the drinking water from day 7 to day 14.

(A) Experimental schema of DSS-induced colitis mouse model.

(B–C) Body weight (B) and disease activity index (C) were monitored daily after DSS treatment.

(D) Representative photographs (left) and length quantification (right) of colon tissues.

(E) Representative H&E-stained sections (upper), AB/PAS-stained sections (middle), and immunocytochemistry of Muc2-stained sections (lower) of the distal colon tissue. Scale bars, 50 μm.

(F) Histology score (left) was, the number of goblet cells per crypt (centre), and quantified Muc2 positive area (right) of sections.
(G) Relative gene expression of *Cyp1a1*and *Il22* in colon tissue assessed by RT-qPCR.
(H) Colonic levels of IL-22.
Data are presented as the mean ± SEM. n = 6 mice per group. Statistical analysis was performed using two-way ANOVA followed by Bonferroni' post hoc test for (B)–(C), and one-way ANOVA followed by the Tukey' post hoc test for (D)–(H). Statistical comparisons with Vehicle are represented as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. Statistical comparisons with Vehicle+CH223191 are represented as # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, and #### $P < 0.0001$. Statistical comparisons with *L.j*+CH223191 are represented as & $P < 0.05$, && $P < 0.01$, &&& $P < 0.001$, and &&&& $P < 0.0001$. Statistical comparisons with BG+CH223191 are represented as $ $P < 0.05$, $$ $P < 0.01$, $$$ $P < 0.001$, and $$$$ $P < 0.0001$. *L.j*, *L. johnsonii*; HK-*L.j*, heat-killed *L. johnsonii*; *L.j*-ATCC, *L. johnsonii* ATCC 33200.

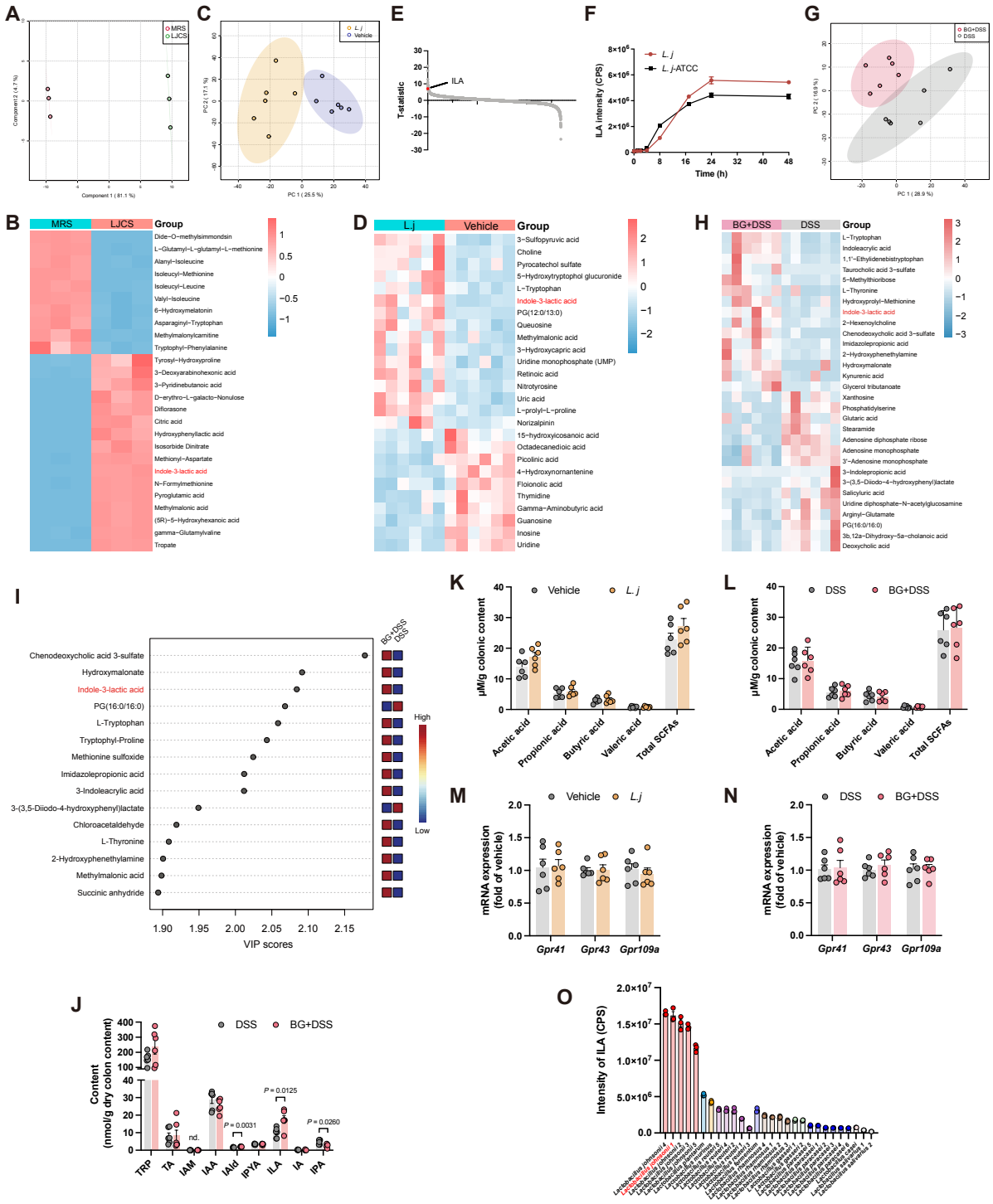
A
MRS
LJCS
Component 1 (81.1 %)
Component 2 (4.7 %)
C
L.j
Vehicle
PC 1 (25.5 %)
PC 2 (17.1 %)
E
ILA
T-statistic
F
L.j
L.j-ATCC
ILA intensity (CPS)
Time (h)
G
BG+DSS
DSS
PC 1 (28.9 %)
PC 2 (16.9 %)
B
MRS
LJCS
Group
Dide−O−methylsimmondsin
L−Glutamyl−L−glutamyl−L−methionine
Alanyl−Isoleucine
Isoleucyl−Methionine
Isoleucyl−Leucine
Valyl−Isoleucine
6−Hydroxymelatonin
Asparaginyl−Tryptophan
Methylmalonylcarnitine
Tryptophyl−Phenylalanine
Tyrosyl−Hydroxyproline
3−Deoxyarabinohexonic acid
3−Pyridinebutanoic acid
D−erythro−L−galacto−Nonulose
Diflorasone
Citric acid
Hydroxyphenyllactic acid
Isosorbide Dinitrate
Methionyl−Aspartate
Indole-3-lactic acid
N−Formylmethionine
Pyroglutamic acid
Methylmalonic acid
(5R)−5−Hydroxyhexanoic acid
gamma−Glutamylvaline
Tropate
D
L.j
Vehicle
Group
3−Sulfopyruvic acid
Choline
Pyrocatechol sulfate
5−Hydroxytryptophol glucuronide
L−Tryptophan
Indole-3-lactic acid
PG(12:0/13:0)
Queuosine
Methylmalonic acid
3−Hydroxycapric acid
Uridine monophosphate (UMP)
Retinoic acid
Nitrotyrosine
Uric acid
L−prolyl−L−proline
Norizalpinin
15−hydroxyicosanoic acid
Octadecanedioic acid
Picolinic acid
4−Hydroxynornantenine
Floionolic acid
Thymidine
Gamma−Aminobutyric acid
Guanosine
Inosine
Uridine
H
BG+DSS
DSS
Group
L−Tryptophan
Indoleacrylic acid
1,1'−Ethylidenebistryptophan
Taurocholic acid 3−sulfate
5−Methylthioribose
L−Thyronine
Hydroxyprolyl−Methionine
Indole-3-lactic acid
2−Hexenoylcholine
Chenodeoxycholic acid 3−sulfate
Imidazolepropionic acid
2−Hydroxyphenethylamine
Hydroxymalonate
Kynurenic acid
Glycerol tributanoate
Xanthosine
Phosphatidylserine
Glutaric acid
Stearamide
Adenosine diphosphate ribose
Adenosine monophosphate
3'−Adenosine monophosphate
3−Indolepropionic acid
3−(3,5−Diiodo−4−hydroxyphenyl)lactate
Salicyluric acid
Uridine diphosphate−N−acetylglucosamine
Arginyl−Glutamate
PG(16:0/16:0)
3b,12a−Dihydroxy−5a−cholanoic acid
Deoxycholic acid
I
BG+DSS
DSS
Chenodeoxycholic acid 3-sulfate
Hydroxymalonate
Indole-3-lactic acid
PG(16:0/16:0)
L-Tryptophan
Tryptophyl-Proline
Methionine sulfoxide
Imidazolepropionic acid
3-Indoleacrylic acid
3-(3,5-Diiodo-4-hydroxyphenyl)lactate
Chloroacetaldehyde
L-Thyronine
2-Hydroxyphenethylamine
Methylmalonic acid
Succinic anhydride
High
Low
VIP scores
K
Vehicle
L.j
μM/g colonic content
Acetic acid
Propionic acid
Butyric acid
Valeric acid
Total SCFAs
L
DSS
BG+DSS
μM/g colonic content
Acetic acid
Propionic acid
Butyric acid
Valeric acid
Total SCFAs
M
Vehicle
L.j
mRNA expression (fold of vehicle)
Gpr41
Gpr43
Gpr109a
N
DSS
BG+DSS
mRNA expression (fold of vehicle)
Gpr41
Gpr43
Gpr109a
J
DSS
BG+DSS
Content (nmol/g dry colon content)
P = 0.0125
P = 0.0031
P = 0.0260
nd.
TRP
TA
IAM
IAA
IAld
IPYA
ILA
IA
IPA
O
Intensity of ILA (CPS)

Figure S6. Analysis of the non-target metabolome of culture supernatant and colonic contents of *L. johnsonii*-treated mice

(A–B) Metabolomic profile (A) and heat map (B) of significantly altered metabolites in cultured supernatant of *L. johnsonii* and MRS medium. Metabolites with VIP value >1 and FDR-adjusted $P < 0.05$ were considered to be significantly different. n = 3 per group.

(C–E) Mice were treated with PBS or *L. johnsonii* as in Figure 3A. The colonic contents were collected for untargeted metabolomics profiling.

(C) PCA of metabolomic profiling.

(D) Heat map of significantly altered metabolites of colonic contents from PBS- and *L. johnsonii*-treated mice. Metabolites with VIP value >1 and FDR-adjusted $P < 0.05$ were considered to be significantly different.

(E) T-statistics of all detected metabolites by untargeted metabolomics.

(F) The production of ILA by *L. johnsonii* with incubation time.

(G–I) Mice were treated with PBS or BG as in Figure 1A. The colonic contents were collected for untargeted metabolomics profiling.

(G) PCA of metabolomic profiling.

(H) Heat map of significantly altered metabolites. Metabolites with VIP value >1 and FDR-adjusted $P < 0.05$ were considered to be significantly different.

(I) VIP plot showing the top 15 altered metabolites in descending order of importance.

(J) The levels of tryptophan and related metabolites in colonic content of PBS- and BG-treated mice.

(K) The concentrations of SCFAs in colonic contents of mice gavaged with *L. johnsonii* or PBS.

(L) The concentrations of SCFAs in colonic contents of mice gavaged with BG or PBS.

(M) Relative gene expression of SCFA-related receptor in colon of mice gavaged with *L. johnsonii* or PBS.

(N) Relative gene expression of SCFA-related receptor in colon of mice gavaged with BG or PBS.

(O) The production of ILA by different *Lactobacillus* strains. *Lactobacillus johnsonii* 1 represents *L. johnsonii* used in the mechanistic studies, and *Lactobacillus johnsonii* 5 represents *L. johnsonii* ATCC33200.

L.j, *L. johnsonii*; LJCS, *L. johnsonii* culture supernatant; BG, β-glucan. Data are presented as the mean ± SEM. n = 6 mice per group. Statistical analysis was performed using *t*-test for (J)–(N).

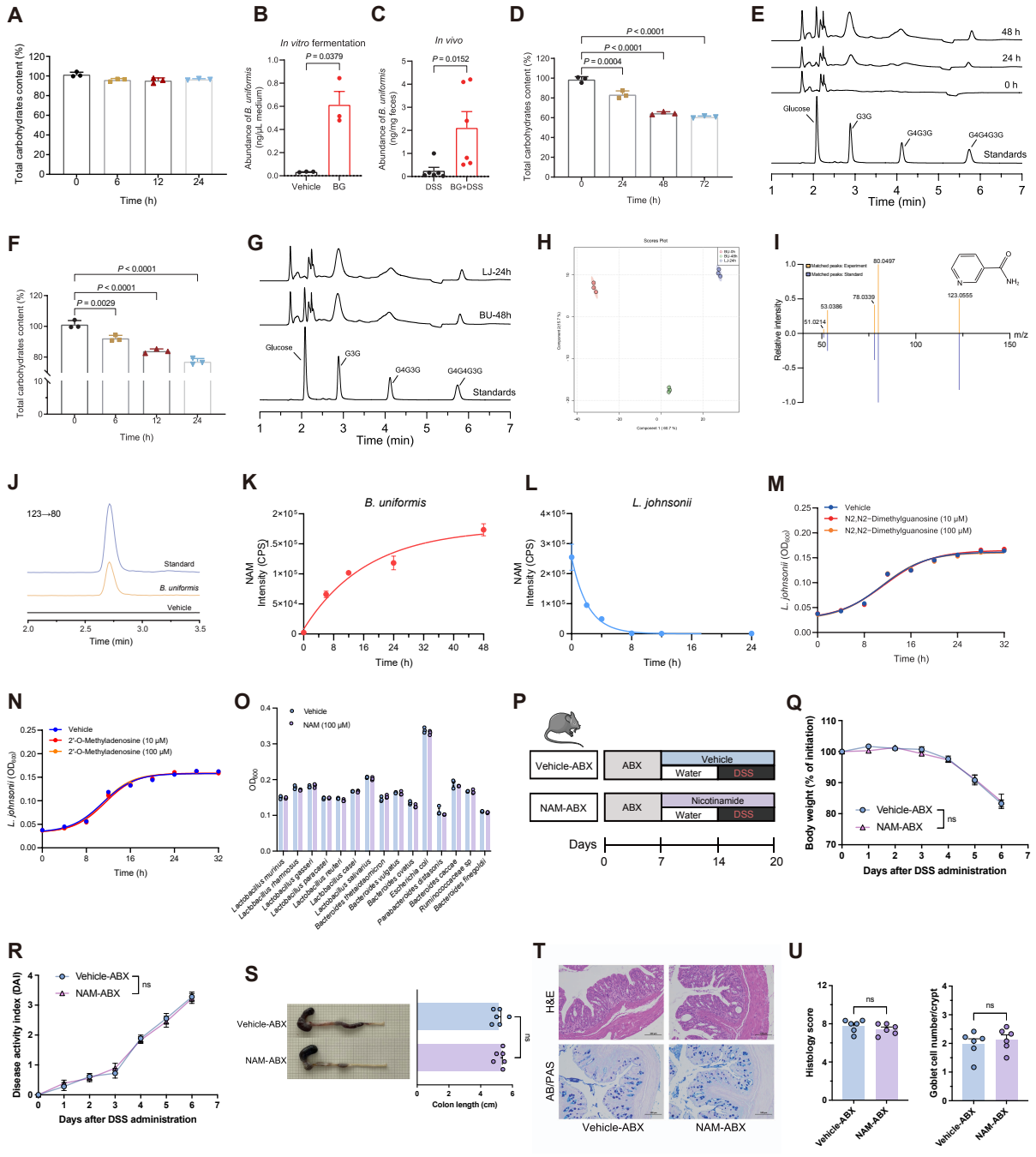

Figure S7. *B. uniformis* promotes the proliferation of *L. johnsonii* (*in vivo* and *in vitro*)

(A–O) *L. johnsonii* can utilize the NAM produced by *B. uniformis in vitro*. For bacteria-related experiments, n = 3 per group; for animal-related experiments, n = 6 per group.

(A) Changes of total carbohydrates of basal medium (BG as the sole carbon source) during the growth of *L. johnsonii*.

(B) The abundance of *B. uniformis* after *in vitro* fermentation with or without BG as the sole carbon source (fecal microbiota was derived from colitic mice).
(C) The abundance of *B. uniformis* in mice feces (BG+DSS and DSS groups).
(D) Changes of total carbohydrates of basal medium (BG as the sole carbon source) during the growth of *B. uniformis*.
(E) The production of carbohydrates with different degrees of polymerization during the growth of *B. uniformis* in basal medium (BG as the sole carbon source).
(F) Changes of total carbohydrates of basal medium (BG as the sole carbon source, with *B. uniformis* pre-culture 48 h) during the growth of *L. johnsonii*.
(G) The production of carbohydrates with different degrees of polymerization during the growth of *L. johnsonii* in basal medium with *B. uniformis* pre-culture 48 h.
(H) PLS-DA analysis of metabolic profile in the supernatant of BU-0h, BU-48h, and LJ-24h.
(I) The MS/MS spectra of NAM standard and NAM detected in samples.
(J) Extracted ion chromatograms of NAM from cultured *B. uniformis* compared to the vehicle.
(K–L) The intensity of NAM in culture supernatant of *B. uniformis* (K) and *L. johnsonii* (L) at the indicated time points.
(M–N) Growth curve of *L. johnsonii* with different concentrations of N2,N2-dimethylguanosine (M) or 2'-O-Methyladenosine (N).
(O) Effects of NAM on the growth of different *Lactobacillus* spp. and core intestinal bacteria.
(P–U) NAM alleviates colitis in a gut microbiota-dependent manner. n = 6 mice per group.
(P) Experimental schema for (Q–U). Mice were treated with antibiotic cocktails for 7 days to deplete gut microbiota, and then given with NAM from day 7 to day 21 and 3% (w/v) DSS from day 14 to day 21.
(Q–R) Body weight (Q) and disease activity index (R) were monitored daily after DSS treatment.
(S) Representative photographs (left) and length quantification (right) of colon tissues.
(T) Representative H&E-stained sections (upper) and AB/PAS-stained sections (lower) of the distal colon. Scale bars, 100 μm.
(U) Histology scores were determined from H&E-stained sections (left) and the number of goblet cells per crypt was determined from AB/PAS-stained sections (right).
Data are presented as the mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by the Tukey' post hoc test for (A), (D), and (F), *t*-test with Welch's correction (B)–(C),

two-way ANOVA followed by Bonferroni' post hoc test for (M)–(N), (Q)–(R), and *t*-test for (O), (S)–(U). No significant (ns) for $P > 0.05$. BG, β-glucan; *B. U*, *B. uniformis*; *L. J* +*B. U*, *L. johnsonii* + *B. uniformis*; G3G, laminaribiose; G4G3G, Glcb(1–4) Glcb (1–3) Glc; G4G4G3G, Glcb(1–4) Glcb(1–4) Glcb(1–3) Glc; BU-0h, BU-48h, and LJ-24h are supernatants of the culture medium (marked in Figure 5F); NAM, nicotinamide.

Table S1. Primer sequences for real-time PCR of target genes used in the study

Target genes	Primer sequence
Cyp1a1	forward 5'-GACCCTTACAAGTATTTGGTCGT-3' reverse 5'-GGTATCCAGAGCCAGTAACCT-3'
Il22	forward 5'-ATGAGTTTTTCCCTTATGGGGAC-3' reverse 5'-GCTGGAAGTTGGACACCTCAA-3'
Muc2	forward 5'-AGGGCTCGGAACTCCAGAAA-3' reverse 5'-CCAGGGAATCGGTAGACATCG-3'
B3gnt7	forward 5'-CTTGTGGCTGTCACCGTTTTC-3' reverse 5'-GCTGTTGGGGTTGACCAGAT-3'
Mfsd2a	forward 5'-AGAAGCAGCAACTGTCCATTT-3' reverse 5'-CTCGGCCCACAAAAAGGATAAT-3'
Ccnd1	forward 5'-GCGTACCCTGACACCAATCTC-3' reverse 5'-CTCCTCTTCGCACTTCTGCTC-3'
Oas2	forward 5'-TGCGGAAGTTCCTACTGACC-3' reverse 5'-CCCACCATGTCACTTGTCTTT-3'
Oas1a	forward 5'-GCCTGATCCCAGAATCTATGC-3' reverse 5'-GAGCAACTCTAGGGCGTACTG-3'
Zbp1	forward 5'-AAGAGTCCCCTGCGATTATTTG-3' reverse 5'-TCTGGATGGCGTTTGAATTGG-3'
β-actin	forward 5'-GGCTGTATTCCCCTCCATCG-3' reverse 5'-CCAGTTGGTAACAATGCCATGT-3'

Table S2. Primer sequences for real-time qPCR analysis of target bacteria

Target bacteria	Primer sequence	Ref.
Eubacteria (All bacteria)	forward 5'-ACTCCTACGGGAGGCAGCAG-3' reverse 5'-ATTACCGCGGCTGCTGG-3'	[1]
L. johnsonii	forward 5'-TGGAAACAGATGCTAATACCG-3' reverse 5'-CAGTTACTACCTCTATCTTTCTTCACTAC-3'	[2]
L. reuteri	forward 5'-ACCGAGAACACCGCGTTATTT-3' reverse 5'-CATAACTTAACCTAAACAATCAAAGATTGTCT-3'	[3]
B. uniformis	forward 5'-TCTTCCGCATGGTAGAACTATTA-3' reverse 5'-ACCGTGTCTCAGTTCCAATGTG-3'	[4]

References

1 Haarman, M., Knol, J. Quantitative real-time PCR analysis of fecal Lactobacillus species in infants receiving a prebiotic infant formula. Appl. Environ. Microbiol. 2006; 72(4): 2359-65.

2 Cui, Y., Wei, H., Lu, F., Liu, X., Liu, D., Gu, L., Ouyang, C. Different effects of three selected Lactobacillus strains in dextran sulfate sodium-induced colitis in BALB/c mice. PLoS One. 2016; 11(2): e0148241.

3 Mikelsaar, M., Stsepetova, J., Hütt, P., Kolk, H., Sepp, E., Lõivukene, K., Zilmer, K., Zilmer, M. Intestinal Lactobacillus sp. is associated with some cellular and metabolic characteristics of blood in elderly people. Anaerobe. 2010; 16(3): 240-6.

4 Han, X., Wang, Y., Zhang, P., Zhu, M., Li, L., Mao, X., Sha, X., Li, L. Kazak faecal microbiota transplantation induces short-chain fatty acids that promote glucagon-like peptide-1 secretion by regulating gut microbiota in db/db mice. Pharm. Biol. 2021; 59(1): 1075-85.