

Supplementary Data

Table S1. Preparation of Congo red experimental working liquid.

NaOH (mol/L)	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
10 mg/mL GFP (mL)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
300 µmol/L Congo red (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Deionized water (mL)	1.6	1.45	1.3	1.15	1.0	0.85	0.7	0.55	0.4	0.25	0.1
1.0 mol/L NaOH (mL)	0	0.15	0.3	0.45	0.6	0.75	0.9	1.05	1.20	1.35	1.5

Table S2. Preparation of Congo Red blank control working fluid.

NaOH (mol/L)	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
300 µmol/L Congo red (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Deionized water (mL)	2.5	2.35	2.2	2.05	1.9	1.75	1.6	1.45	1.3	1.15	1.0
1.0 mol/L NaOH (mL)	0	0.15	0.3	0.45	0.6	0.75	0.9	1.05	1.20	1.35	1.5

Table S3. PCR primer sequences for DNA detection.

Amplified fragments	Primer sequence
V3–V4	341F (5'-CCTACGGGNGGCWGCAG-3') 805R(5'-GACTACHVGGGTATCTAATCC-3')

Table S4. Base size distribution in sequencing at 0 h and 48 h.

	<200	200-300	300-400	400-500	>=500	Total
0h	5	149	259	1068801	0	1069214
48h	18	543	143	1141057	0	1141761

Table S5. Effective sequencing data after quality control and chimera filtering

Sampl e	Raw_Tag s	Raw_Base s	Valid_Tag s	Valid_Base s	Valid %	Q20 %	Q30 %	GC %
INL6	60982	30.49M	48010	20.15M	78.73	97.67	93.52	55.19
INL5	81162	40.58M	64380	26.93M	79.32	97.77	93.82	54.97
INL4	86662	43.33M	81532	33.58M	94.08	98.54	95.54	57.80
INL3	79443	39.72M	65075	27.41M	81.91	98.06	94.45	55.19
INL2	87996	44.00M	83079	34.19M	94.41	97.89	93.42	57.92
INL1	80664	40.33M	65772	27.69M	81.54	97.91	94.19	55.12
GFP6	75970	37.98M	59759	25.27M	78.66	98.01	94.35	54.45

								54.4
GFP5	74102	37.05M	58422	24.70M	78.84	97.91	94.05	7
								54.3
GFP4	80424	40.21M	63098	26.65M	78.46	97.86	93.99	5
								54.5
GFP3	80803	40.40M	63761	26.96M	78.91	97.95	94.25	3
								54.3
GFP2	78584	39.29M	60085	25.43M	76.46	98.02	94.37	6
								54.4
GFP1	84488	42.24M	67549	28.35M	79.95	97.32	92.80	7
								54.9
BLK6	83119	41.56M	60528	25.83M	72.82	97.29	92.06	1
								54.8
BLK5	82157	41.08M	57978	24.73M	70.57	98.32	95.03	3
								54.8
BLK4	86228	43.11M	62248	26.56M	72.19	98.34	95.11	5
								54.8
BLK3	64510	32.26M	59836	25.52M	92.75	98.34	95.10	4
								54.8
BLK2	83987	41.99M	59196	25.25M	70.48	98.42	95.30	1
								54.8
BLK1	86254	43.13M	61453	26.22M	71.25	97.73	93.14	4

Table S6. Annotation information of key metabolites between the BLK and GFP groups

Name	gamma-Aminobutyric acid	L-4-Hydroxyglutamate semialdehyde	Adenine	Guanine	Indoxyl	L-Tryptophan	5-Hydroxy-L-tryptophan
m/z	102.06	148.06	136.06	152.06	132.04	205.10	221.09
RT	0.82	0.62	1.25	1.25	2.96	2.13	2.01
	89778946	10671354	75724890	29834816	1576723.	29417028	10274065
GFP1	.92	8.53	.22	.33	17	8.85	.63
	86252093	11967904	62663009	25828232	1602287.	29231501	10512759
GFP2	.53	6.31	.64	.73	41	6.56	.80
	87510473	11833893	62726607	28790203	1501204.	29726305	12078685
GFP3	.91	1.73	.98	.83	65	1.33	.06
	87077341	11606377	68845668	27460155	1736533.	26061505	12001727
GFP4	.05	1.96	.94	.22	39	3.64	.70
	80074257	11551212	92097217	33862118	1496655.	25948952	18655811
GFP5	.43	2.08	.79	.15	63	3.09	.14
	90887135	10805786	97871227	34465684	1781078.	31753877	17627793
GFP6	.40	3.44	.09	.56	62	2.94	.23
	33291520	34299917	14141858	1912971.	4968089.	23634933	5708477.
BLK1	.64	.03	1.72	69	67	.15	21
	34160056	29653316	15949228	588075.0	4265803.	47419299	5793590.
BLK2	.77	.45	5.77	1	71	.43	58

	34892780	30248320	16078056	3490876.	5141903.	25755878	11023864
BLK3	.03	.81	4.48	74	01	.35	.55
	37248383	32573227	14761540	1997076.	5529813.	16403771	5099891.
BLK4	.53	.21	1.35	85	38	.10	48
	32022266	27319232	16139954	2185773.	5501296.	21872101	10025300
BLK5	.70	.44	3.25	23	88	.31	.44
	35956823	30334645	17477501	3181095.	2681805.	11271469	9426386.
BLK6	.46	.34	2.91	03	89	6.17	14
FC	6.71	3.71	0.47	13.50	0.49	2.35	2.42
<i>p</i> value	0.00	0.00	0.00	0.00	0.02	0.02	0.02
VIP	1.58	1.88	1.63	2.66	1.37	2.33	1.05

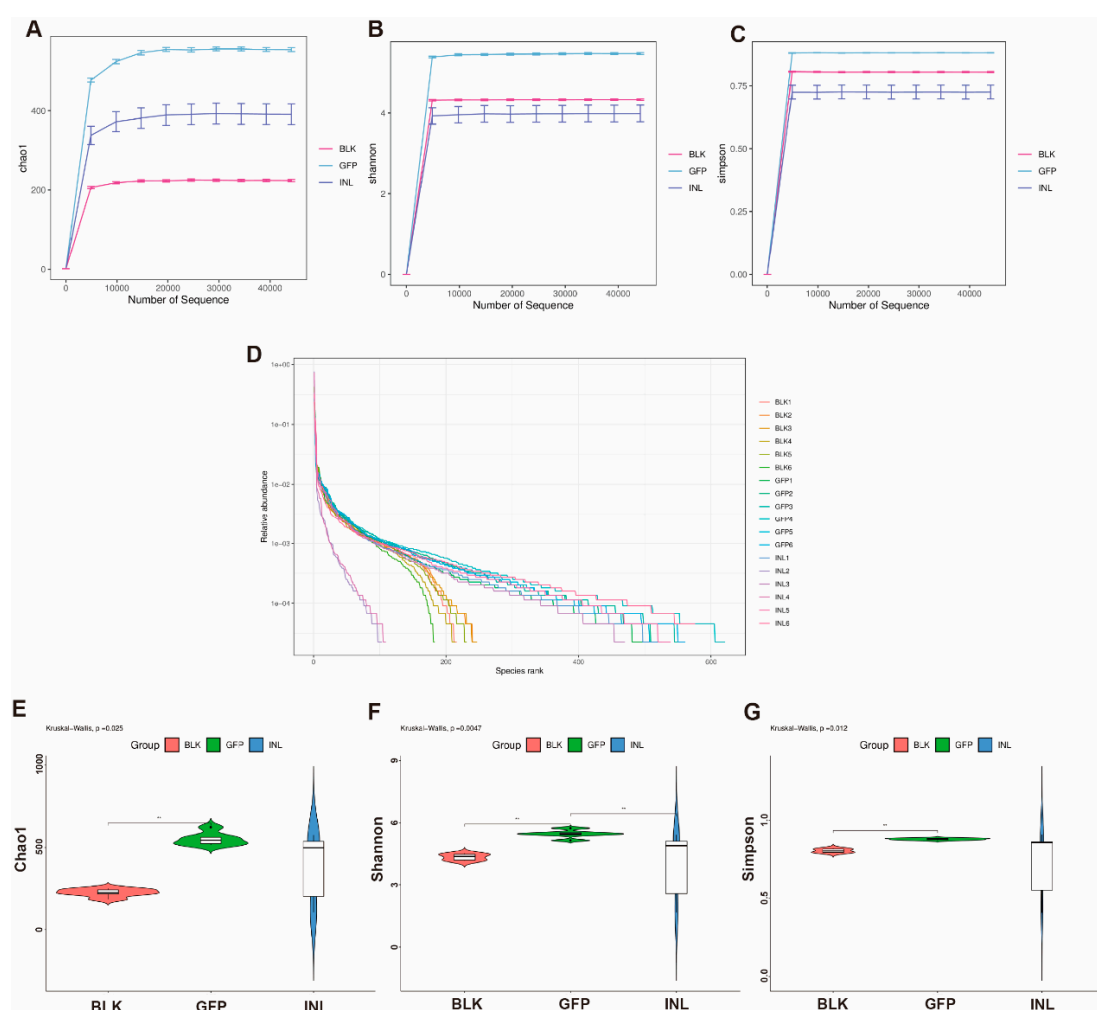


Figure S1. Comparison of α diversity of GFP after 48 h fermentation. (A–C) Rarefaction curves based on Chao1, Shannon, and Simpson indices; (D) Rank–abundance curve; (E–G) Diversity analysis using Chao1, Shannon, and Simpson indices. * $p < 0.05$; ** $p < 0.01$.

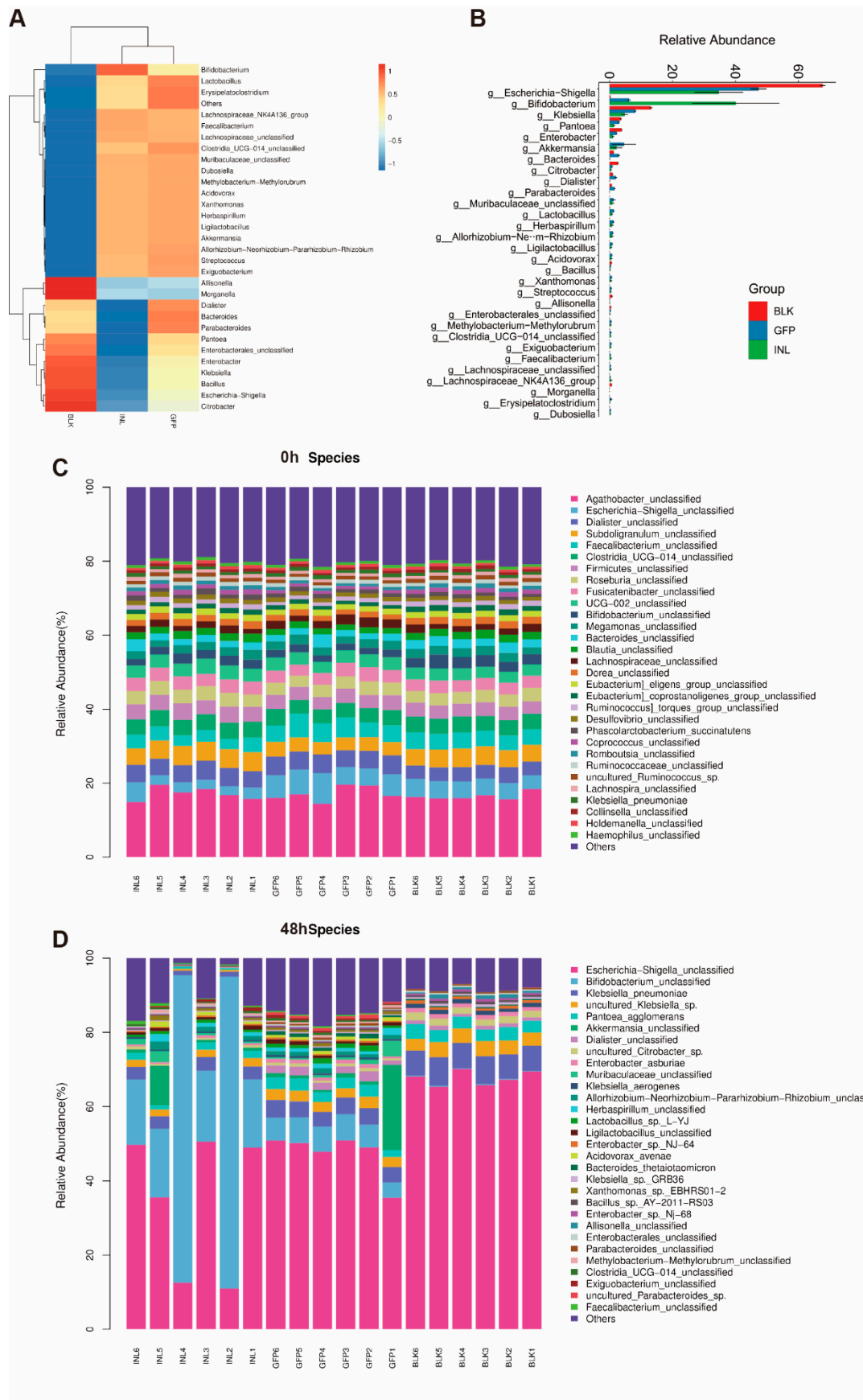


Figure S2. Dominant microbial communities in each group after 48 h of fermentation. (A) Heatmap distribution of microbial abundance in each group; (B) Differences in microbial

communities among the group; (C-D) Relative abundance of gut microbiota at the species level across the BLK, GFPs, and INL groups at 0 h and 48 h.

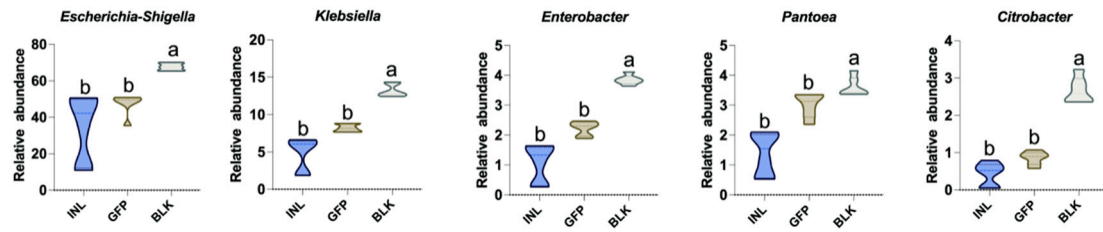


Figure S3. Five key genera in the BLK group.

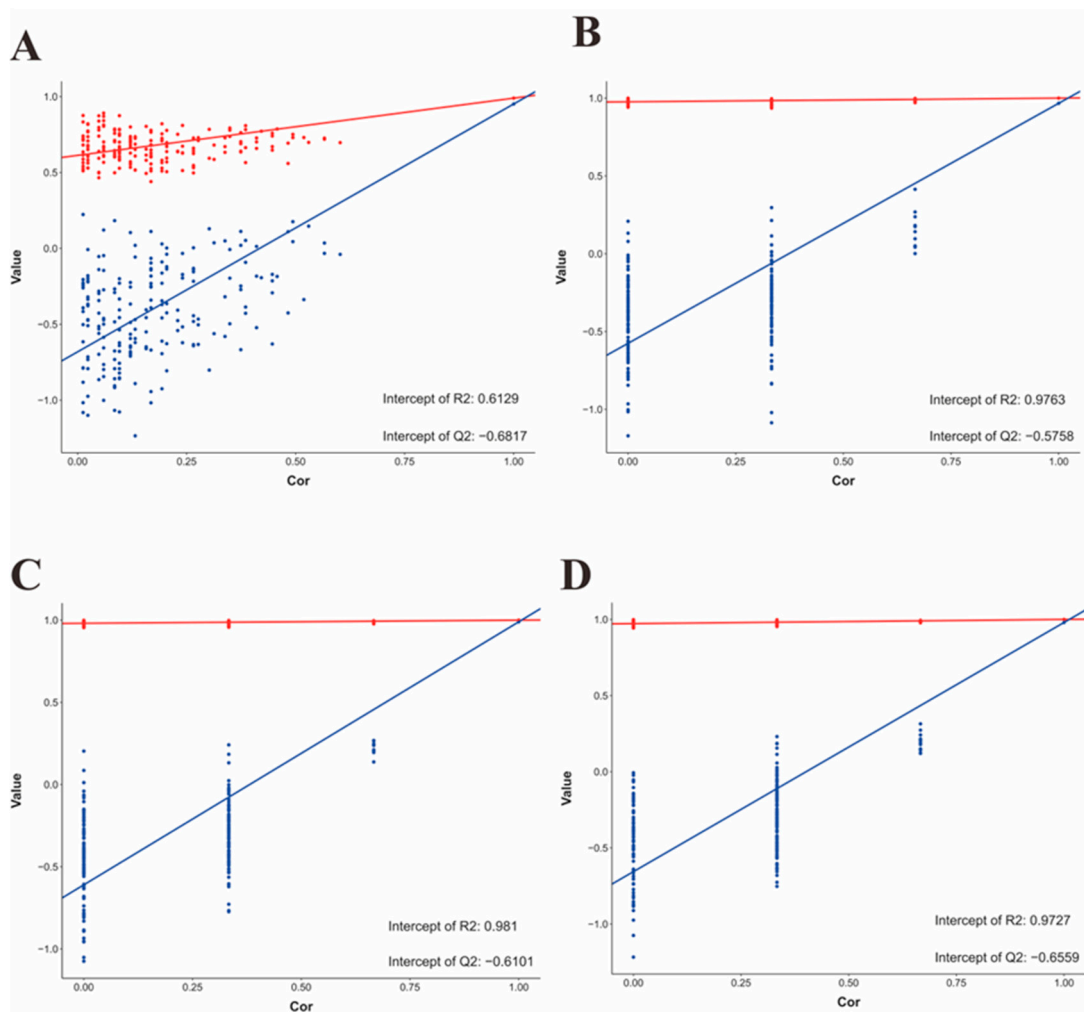


Figure S4. Permutation test of PLS-DA scores among comparison groups. (A) GFP vs. BLK vs. INL; (B) GFP vs. BLK; (C) INL vs. BLK; (D) GFP vs. INL.