Supporting Information

Ulcerative Colitis Alleviation of Colon-Specific Delivered Rhamnolipid/Fullerene Nanocomposite via Dual Modulation in Oxidative Stress and Intestinal Microbiome

Yuxuan Xia,[a] Liu Hong*[a], Jiayao Zheng,[a] Ziyi Lu,[a] Qiong Zhang,[a] Siyu Chen,[a] Zhi Pang,[b,c] Lei Li,[d] Shumiao Qiao,[e] Qiang Wang,[e] Yonghua Zhou*[e] and Cheng Yang[a]

[a] *Key Laboratory of Synthetic and Biological Colloids, Ministry of Education, School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, China*

[b] *Department of Digestive Disease and Nutrition Research Center, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou 215008, China*

[c] *Department of Gastroenterology, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou 215008, China*

[d] *Wuxi School of Medicine, Jiangnan University, Wuxi 214122, China*

[e] *Key Laboratory of National Health Commission on Parasitic Disease Control and Prevention, Jiangsu Provincial Key Laboratory on Parasite and Vector Control, Jiangsu Institute of Parasitic Diseases and Public Health Research Center of Jiangnan University, Wuxi 214064, China*

Contents

1. Structure determination of RL/C_{60}

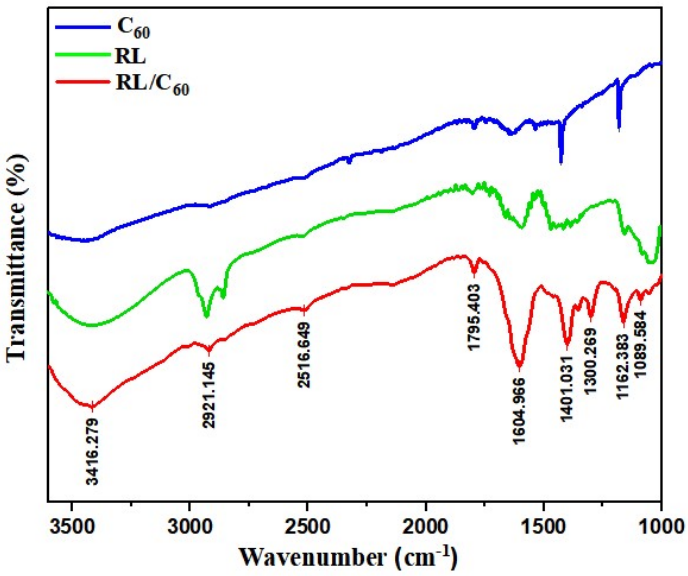

Fig. S1 FT-IR spectrum of C_{60}, RL and RL/C_{60}.

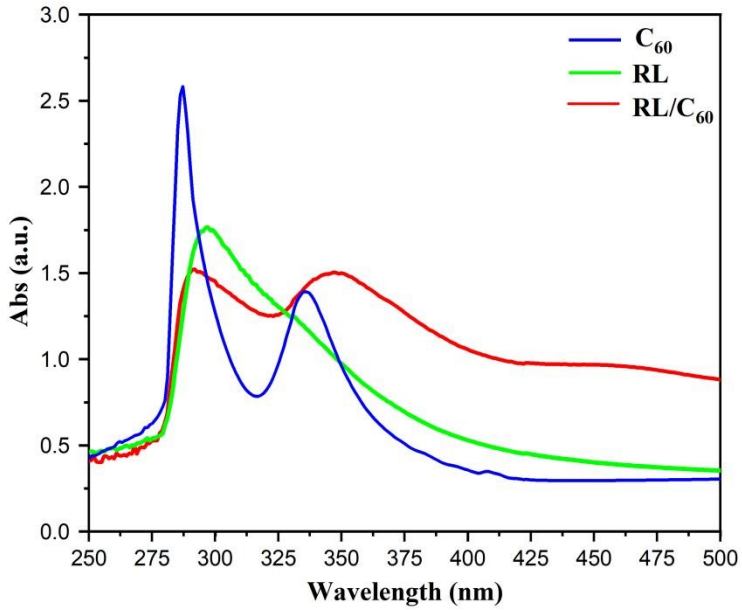

Fig. S2 UV-visible absorption spectra of C_{60}, RL and RL/C_{60}.

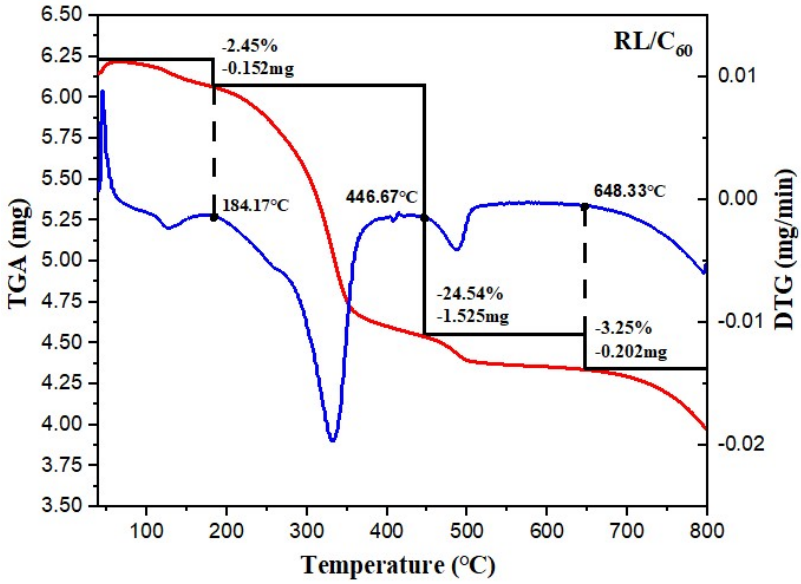

Fig. S3 TGA plots of RL/C_{60} as tested in N_2.

Table S1 Estimation of RL/C_{60} composition based on TGA data

Sample	**Weight loss (%)**			M_{RL}	M_{H2O}
	y_1	y_2	y_3		
	2.45	24.54	73.01	504.66	18
RL/C_{60}	$N_{H2O} = (720*y_1) / (M_{H2O}*y_3)$		$N_{RL} = (720*y_2) / (M_{RL}*y_3)$	N_{C60}	
	1.34		0.48	1	

2. Biocompatibility and antioxidant capacity of RL/C_{60}

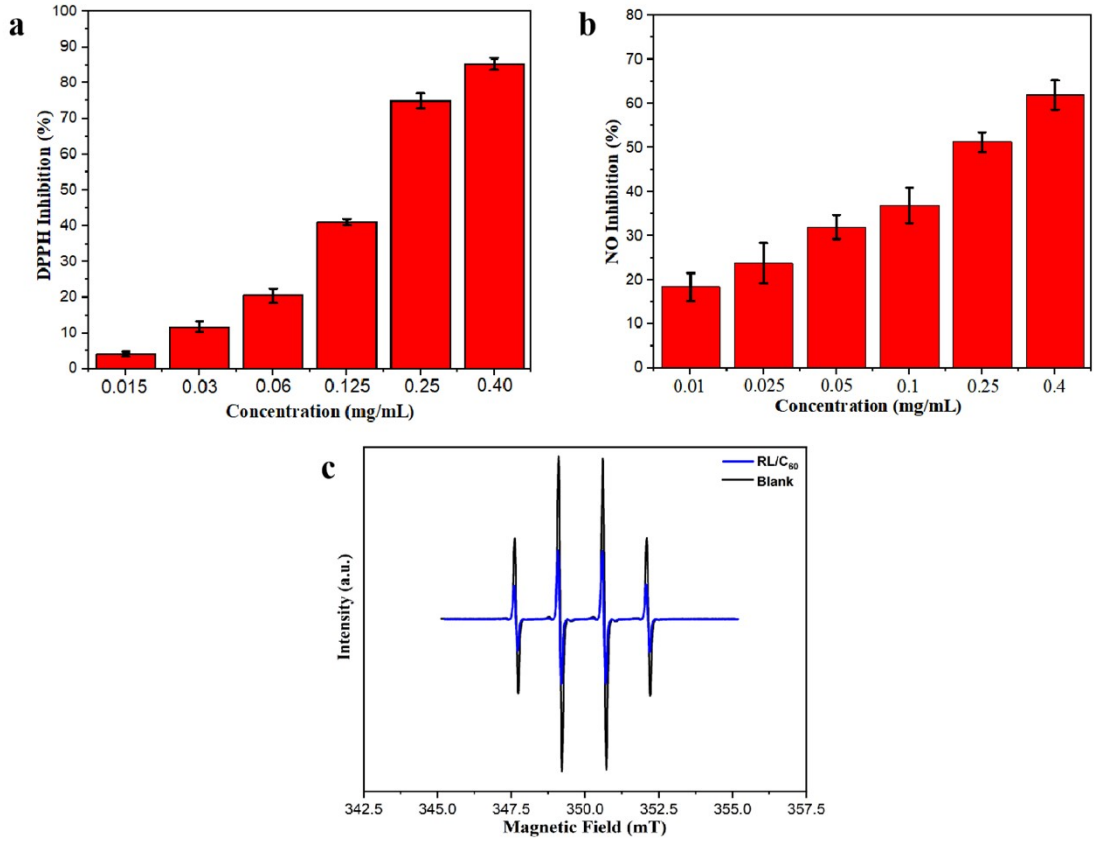

Fig. S4 Radical scavenging ability of RL/C_{60} with different concentrations: (a) DPPH radical (0.015~0.4 mg/mL); (b) ·NO (0.015~0.4 mg/mL); (c) ·OH (0.4 mg/mL).

3. pH responsive behavior of RL/C_{60}

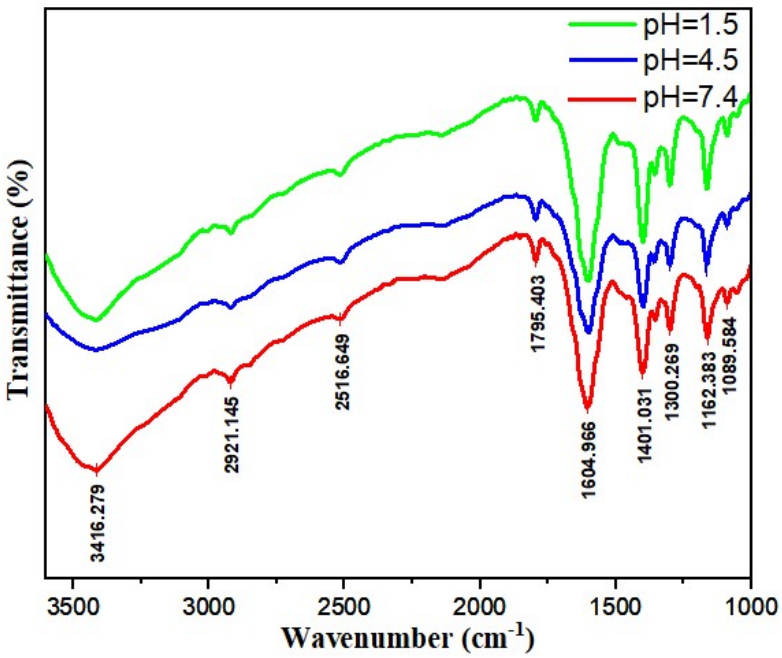

Fig. S5 Stability of RL/C_{60} at different pH conditions as shown by FT-IR spectra.

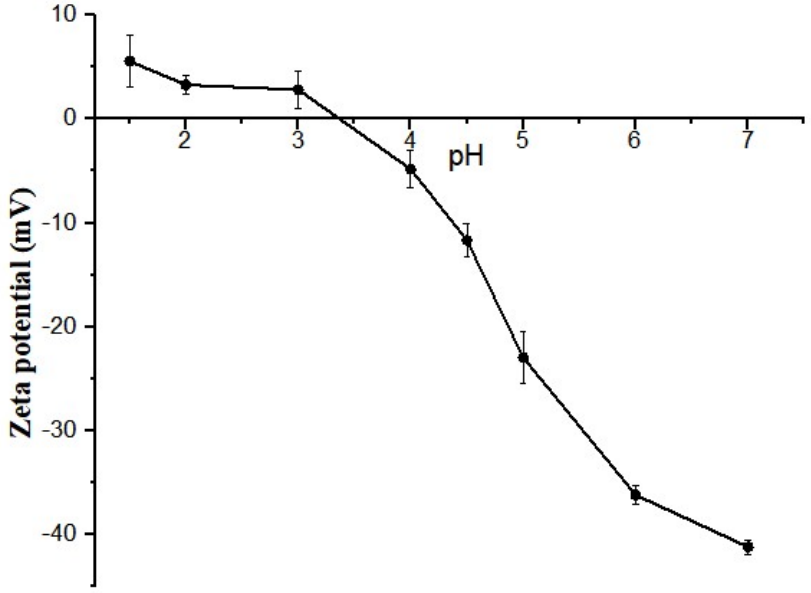

Fig. S6 Zeta potential of RL/C_{60} at different pH conditions

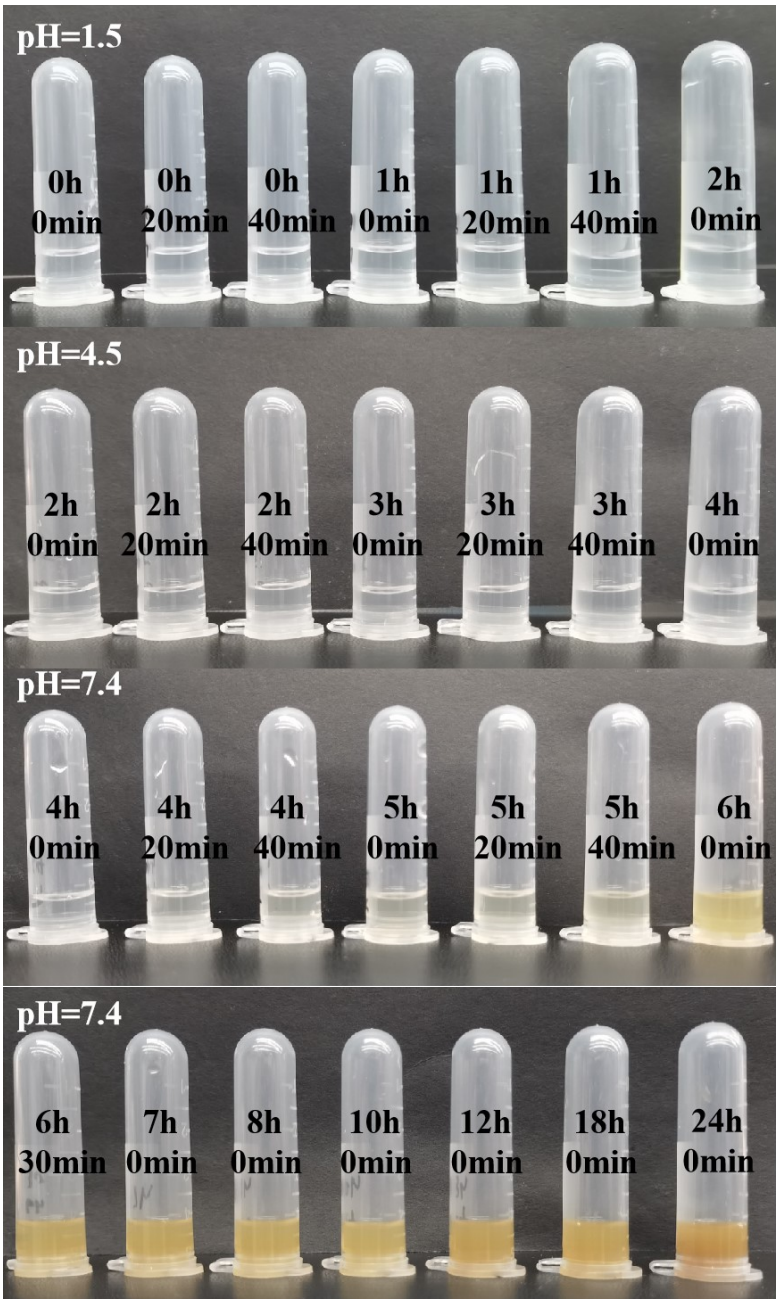

Fig. S7 Visual depiction of RL/C_{60} release behavior at different pH conditions.

4. Evaluation of anti-inflammatory performance of RL/C_{60}

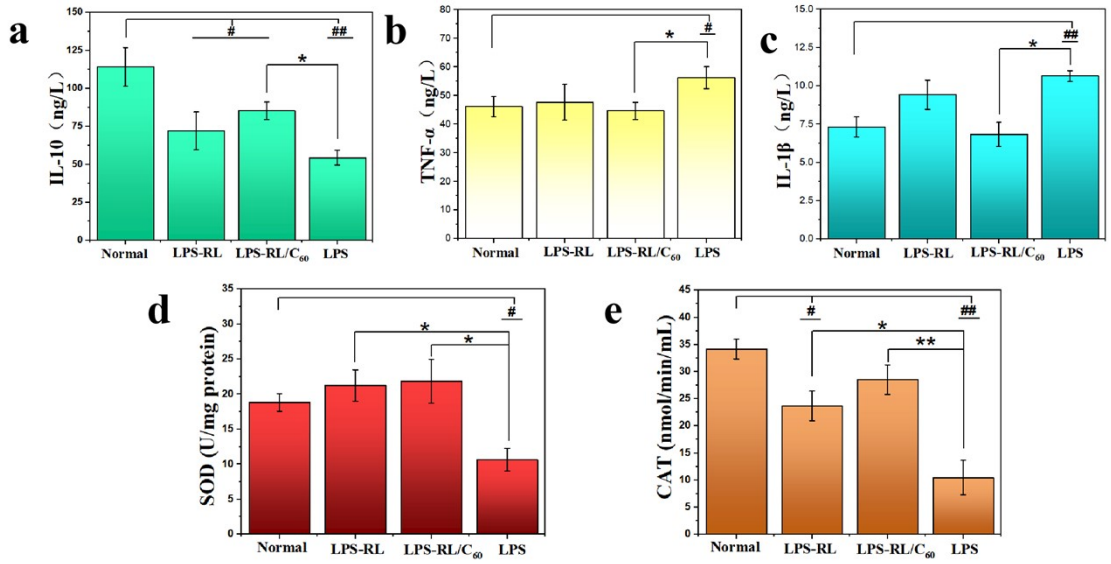

Fig. S8 *In-vitro* antioxidant capacity of RL/C_{60}: (a) IL-10, (b) TNF-α, (c) IL-1β, (d) SOD, (e) CAT. Data are shown as the mean ± S.E.M. (n = 3). Statistical significance was evaluated via one-way ANOVA with Tukey Test. $^{\#}$ P≤0.05, $^{\#\#}$ P≤0.01 vs Normal; * P≤0.05, ** P≤0.01 vs LPS.

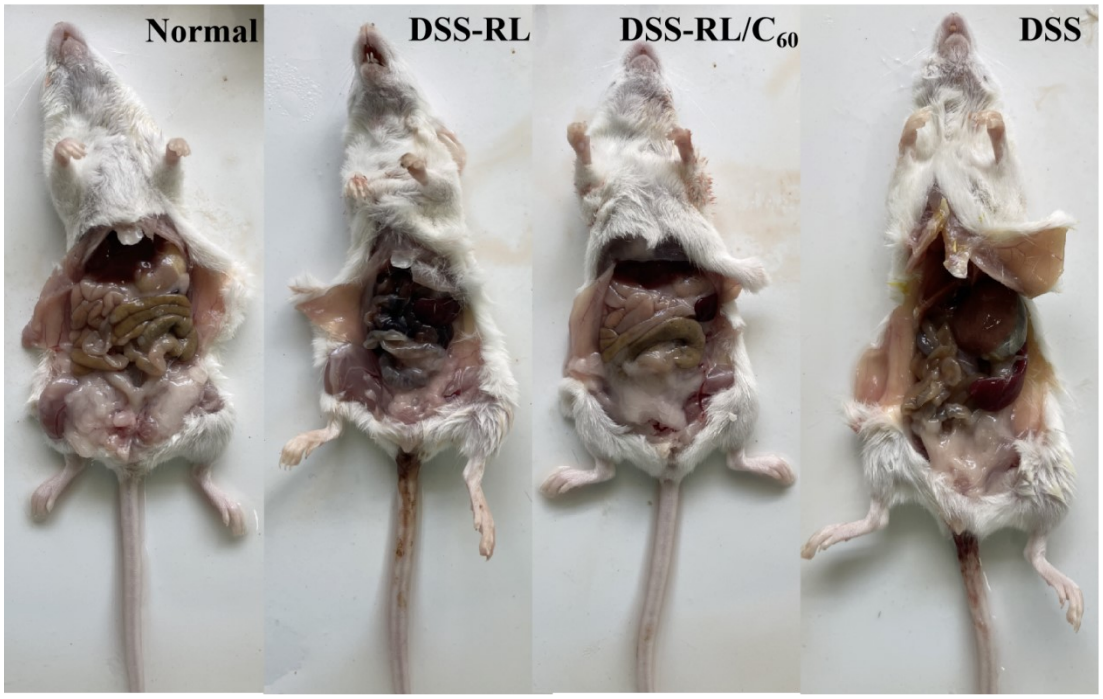

Fig. S9 Anatomical photograph of typical mice in different groups.

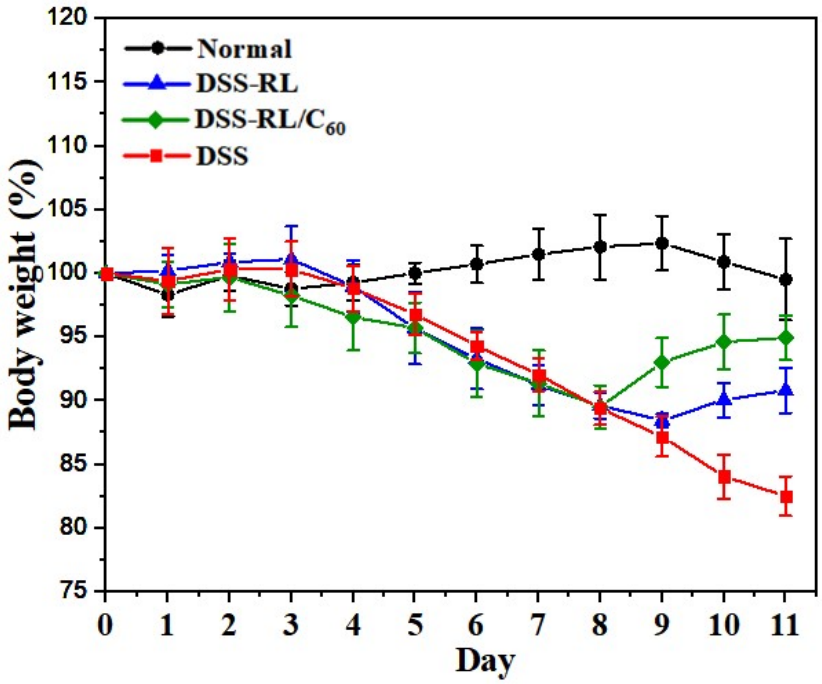

Fig. S10 Daily mice body weight of different groups. Data are shown as the mean ± S.E.M. (n = 4).

Table S2 DAI scoring criteria

Score	Weight Loss (%)	Stool Condition	Hematochezia
0	X<2	normal	no rectal bleeding
1	2≤X<5	softer stool	weak hemoccult
2	5≤X<10	moderate diarrhea	visual blood in stool
3	10≤X<15	diarrhea	fresh rectal bleeding
4	X≥15	/	/

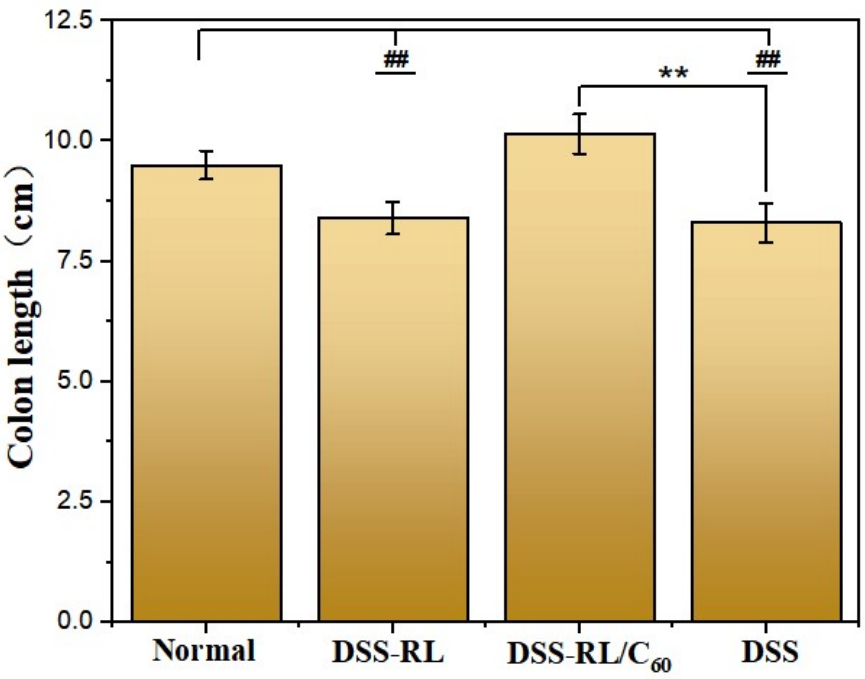

Fig. S11 Comparative diagram of mice colon length in different groups. Data are shown as the mean ± S.E.M. (n = 4). Statistical significance was evaluated via one-way ANOVA with Tukey Test. $^{\#}$ P≤0.05, $^{\#\#}$ P≤0.01 vs Normal; ** P≤0.01 vs DSS.

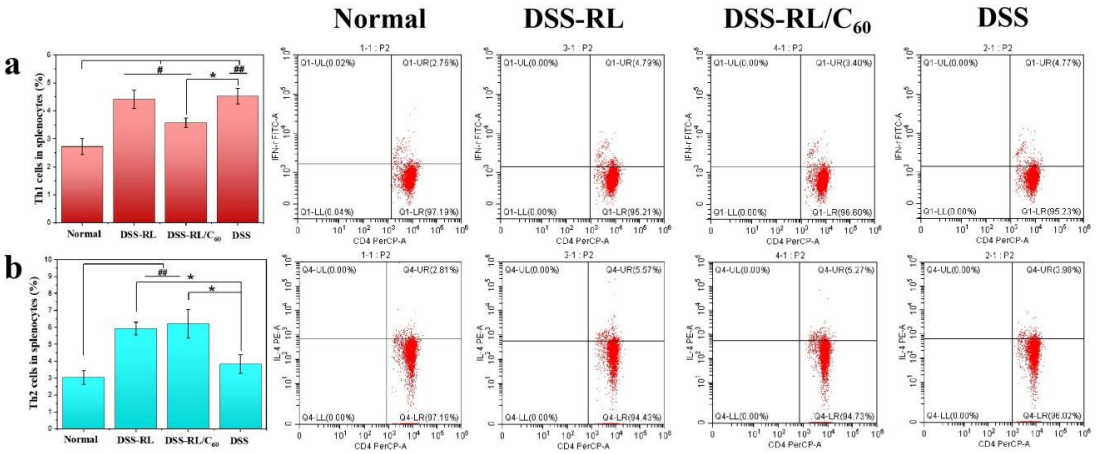

Fig. S12 Th1 and Th2 cells in splenocytes and corresponding flow cytometry data: (a) Th1 cells; (b) Th2 cells. Data are shown as the mean ± S.E.M. (n = 4). Statistical significance was evaluated via one-way ANOVA with Tukey Test. $^{\#}$ P≤0.05, $^{\#\#}$ P≤0.01 vs Normal; * P≤0.05 vs DSS.

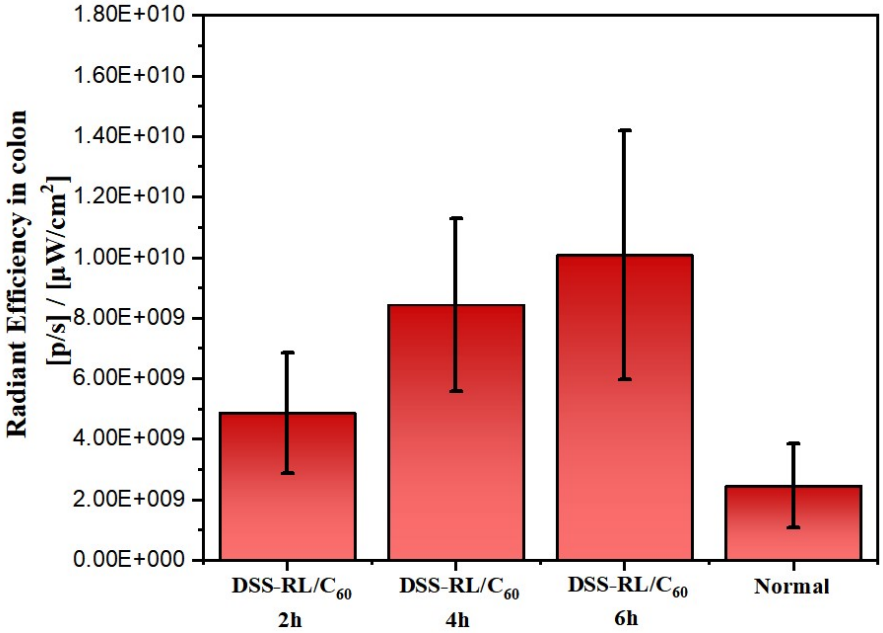

Fig. S13 Colon-targeted delivery of FITC-labeled RL/C_{60}. Data are shown as the mean ± S.E.M. (*n* = 4).

5. Modulation effect of RL/C_{60} on the composition of intestinal microbiota

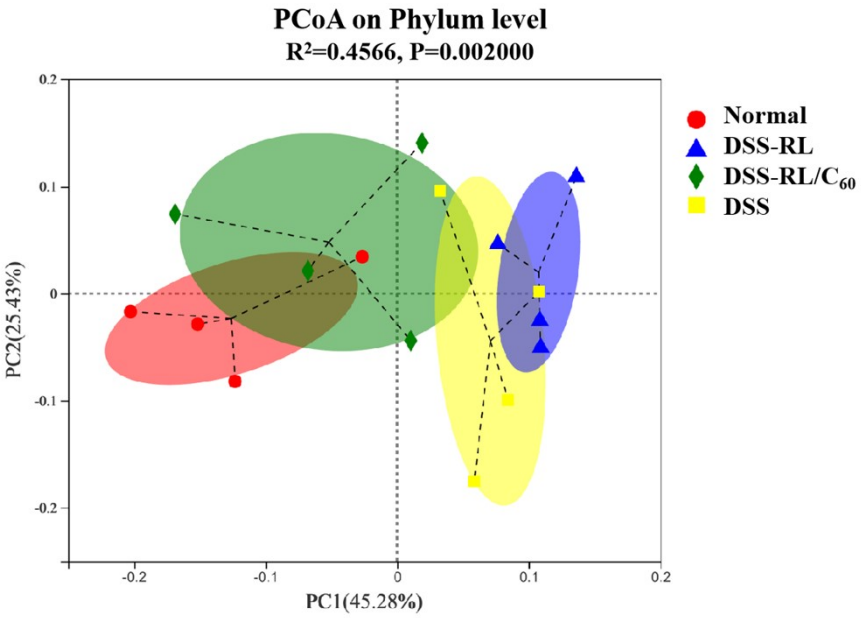

Fig. S14 PoCA diagram of intestinal microflora (phylum level) from different groups.

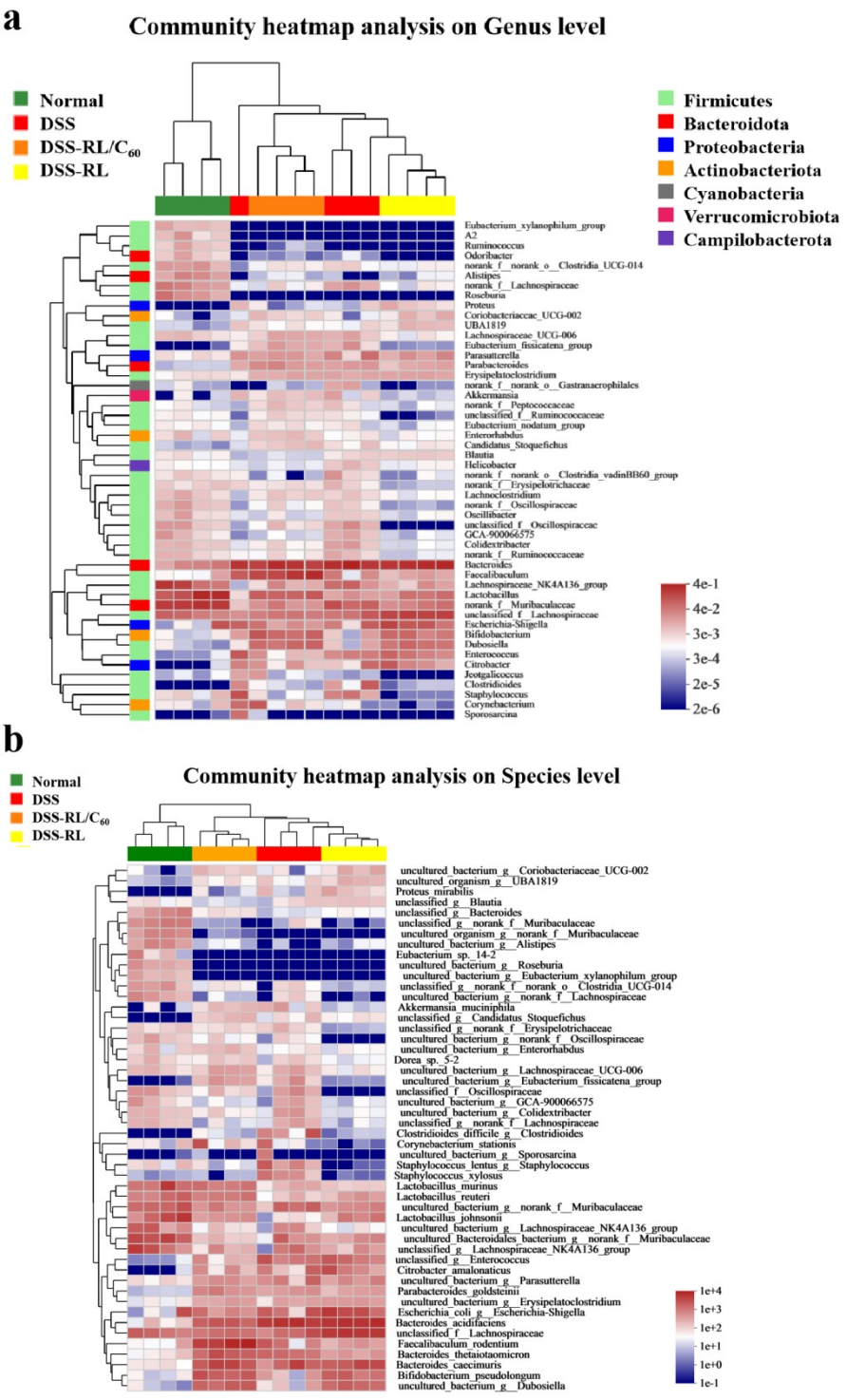

Fig. S15 Intestinal bacteria community heatmap analysis: (a) genus level; (b) species level.

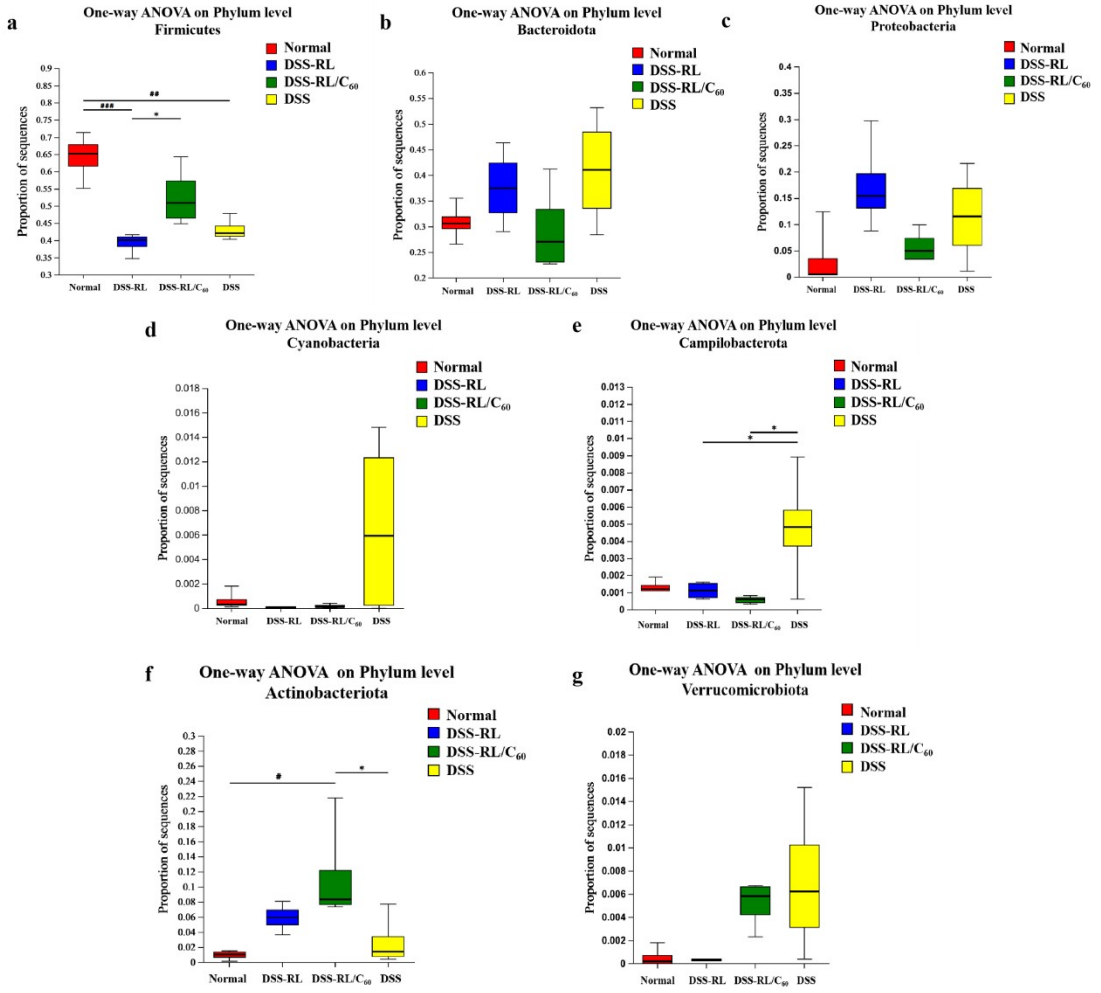

Fig. S16 Comparison of group differences in microflora at phylum level: (a) *Firmicutes*; (b) *Bacteroidota*; (c) *Proteobacteria*; (d) *Cyanobacteria*; (e) *Campilobacterota*; (f) *Actinobacteriota*; (g) *Verrucomicrobiota*. Data are shown as the mean ± S.E.M. (n = 4). Statistical significance was evaluated via one-way ANOVA with Tukey Test. $^{\#}$ P≤0.05, $^{\#\#}$ P≤0.01, $^{\#\#\#}$ P≤0.001 vs Normal; * P≤0.05 vs DSS.

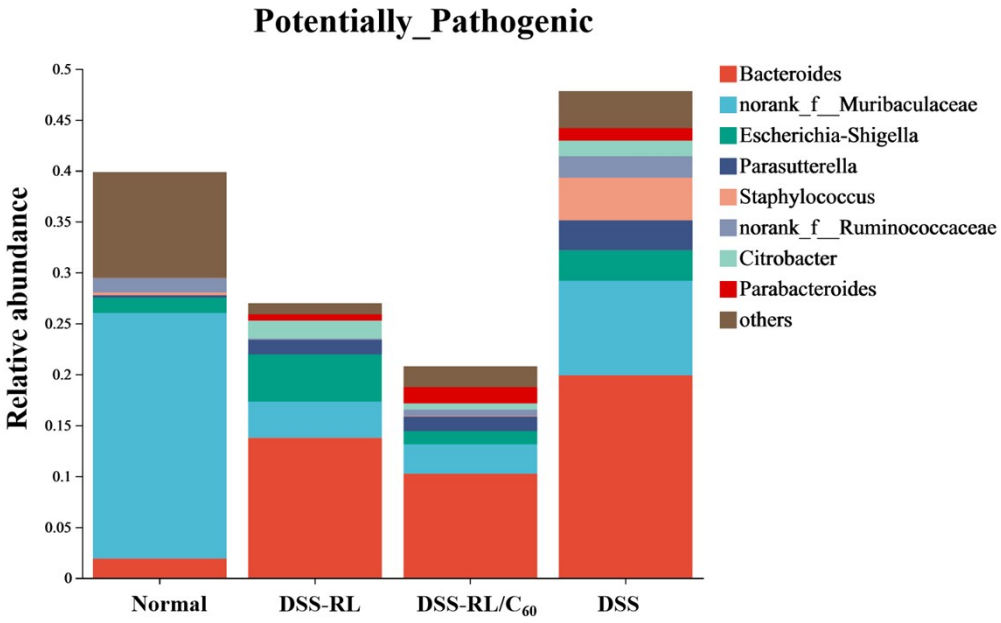

Fig. S17 Potential pathogenic histogram of microbiome in different groups predicted by BugBase.

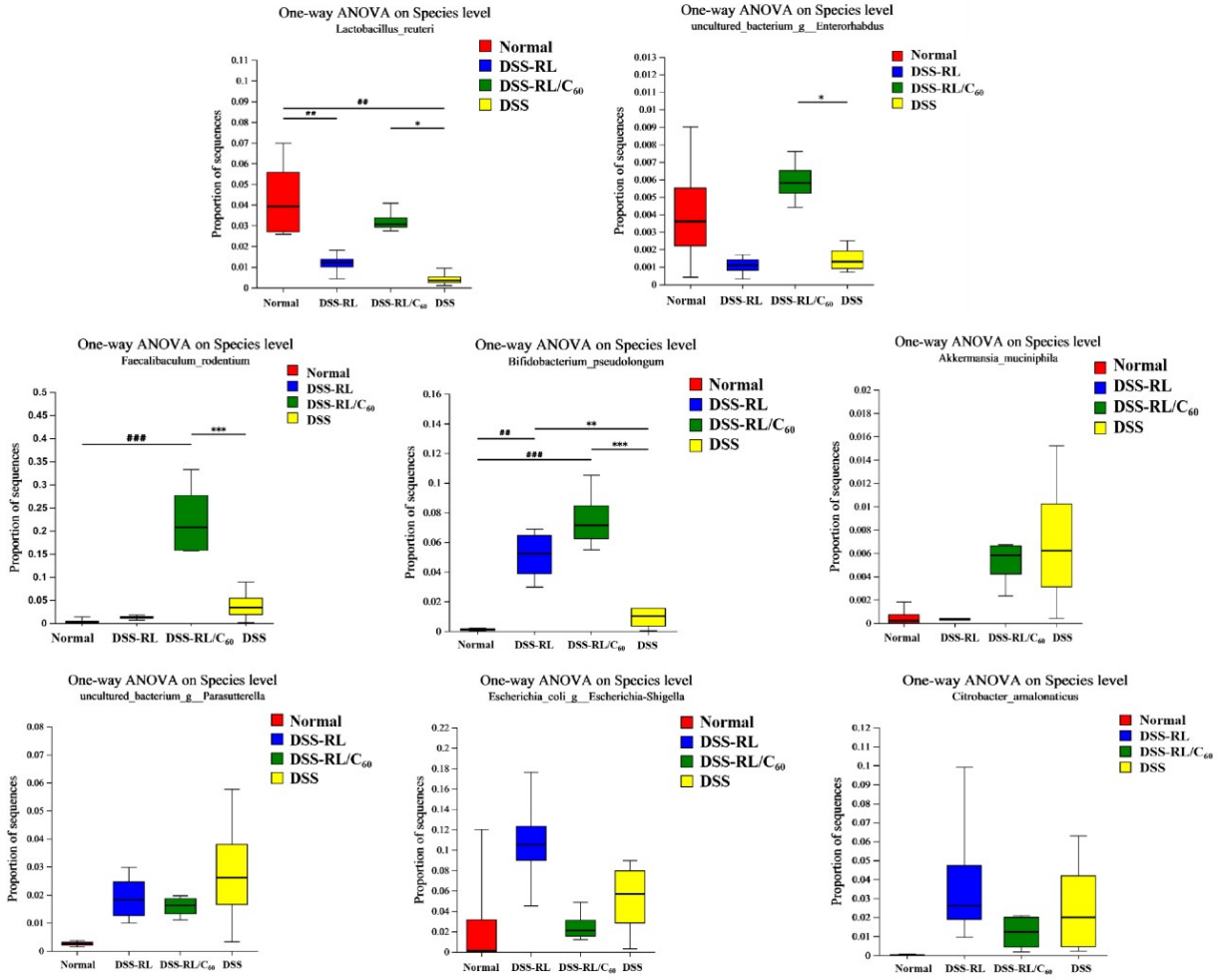

Fig. S18 RL/C_{60} promotes probiotics colonization while suppresses harmful bacteria proliferation. Data are shown as the mean ± S.E.M. (n = 4). Statistical significance was evaluated via one-way ANOVA with Tukey Test. ## P≤0.01, ### P≤0.001 vs Normal; * P≤0.05, ** P≤0.01, *** P≤0.001 vs DSS.

6. Intestinal flora-immunity relationship and its implication on UC treatment

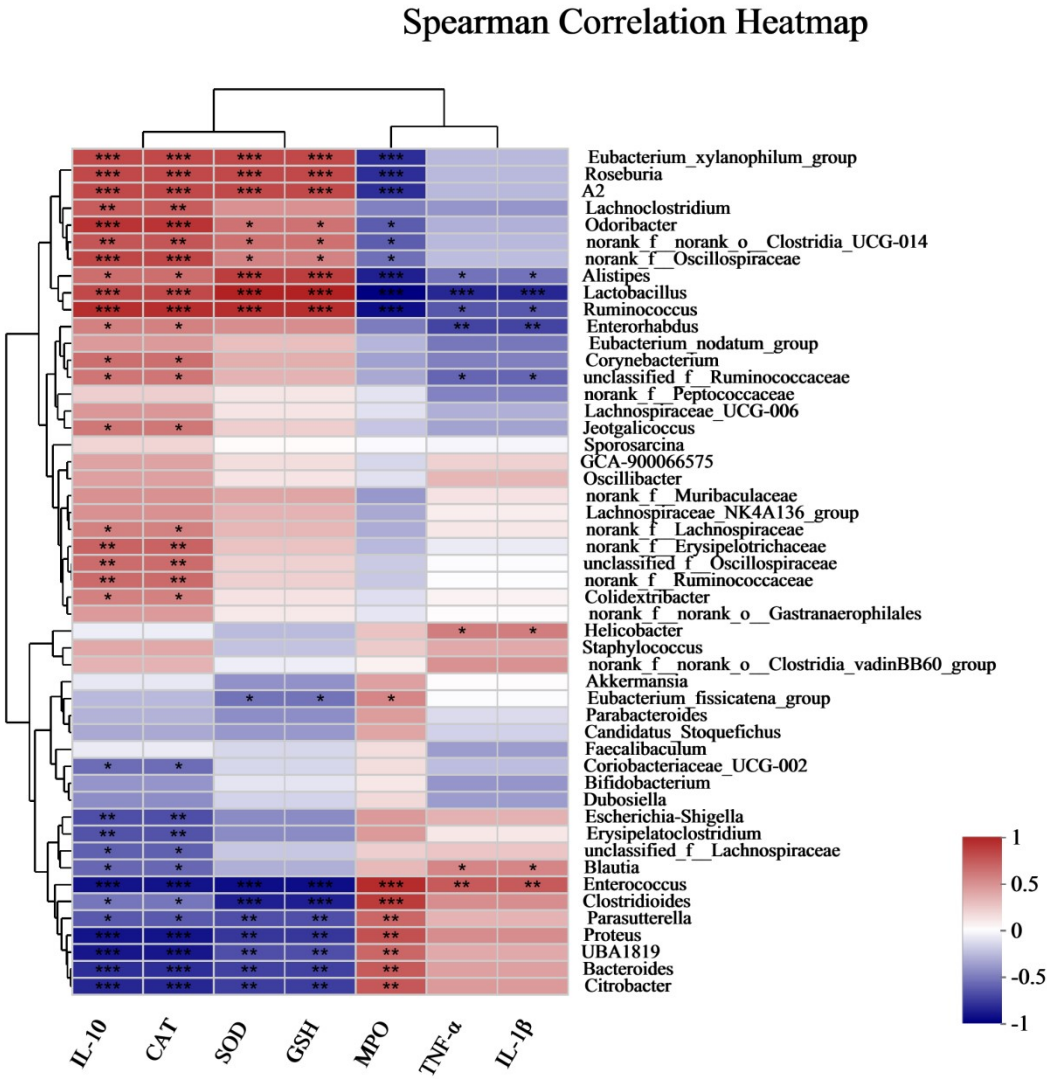

Fig. S19 Correlation heatmap of inflammatory indicators vs. microflora at genus level. Statistical significance was evaluated via one-way ANOVA with Tukey Test. * P≤0.05, ** P≤0.01, *** P≤0.001.

Table S4 SCFAs content in intestinal feces of mice in different group.

Samples	acetic acid	propanoic acid	isobutyric acid	butanoic acid	isovaleric acid	valeric acid	isohexanoic acid	hexanoic acid
Normal-1	4.450	0.730	0.092	0.657	0.074	0.068	0.015	0.019
Normal-2	9.808	0.990	0.089	0.323	0.069	0.039	0.018	0.010
Norma-3	6.420	0.918	0.115	0.990	0.109	0.157	0.022	0.014
DSS-RL-1	1.289	0.473	0.061	0.249	0.079	0.060	0.057	0.009
DSS-RL-2	2.935	1.409	0.204	0.930	0.254	0.197	0.032	0.009
DSS-RL-3	1.677	0.635	0.080	0.328	0.103	0.078	0.067	0.010
DSS-RL/C_{60}-1	8.683	0.757	0.101	0.382	0.118	0.051	0.021	0.018
DSS-RL/C_{60}-2	10.771	0.843	0.113	0.530	0.152	0.043	0.022	0.018
DSS-RL/C_{60}-3	9.589	0.727	0.100	0.475	0.137	0.041	0.016	0.019
DSS-1	1.965	0.929	0.143	0.794	0.139	0.084	0.037	0.009
DSS-2	4.523	1.313	0.162	1.002	0.205	0.158	0.026	0.010
DSS-3	4.893	1.815	0.232	1.169	0.267	0.129	0.044	0.015