## **Supporting Information**

# Lactobacillus plantarum 69-2 combined with a-lactalbumin hydrolysate alleviates DSS-induced ulcerative colitis by TLR4/NF-kB inflammatory pathway and gut microbiota in mice

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	Temperature (°C)	pН	Enzymatic hydrolysis time (h)	Enzyme substrate ratio
Alkaline protease	50	10	5	1:50
Neutral protease	50	7	5	1:50
Papain	50	7.5	5	1: 50

Table S1.  $\alpha$ -LA enzymatic hydrolysis conditions

#### Table S2. DAI scoring standard

DAI score	Weight loss rate (%)	Fecal state	Hematochezia status
0	0	Normal	No color in 2 min
1	1-5	Loose	Light green turns to green after 10 sec
2	6-10	Semi-formed with slime	Light green gradually turns blue brown
3	11-15	Loose stools	Gradually turns bluish-brown
4	>15	Watery stool with blood	Immediately turns bluish-brown

Note: Dead mice were scored 4 points for each item.

Extent of disease	Epithelial injury degree	Inflammatory infiltration	Score
no lesion	No obvious damage	Non-infiltration	0
1-25%	Goblet cells were absent	Infiltration around the base of crypt	1
26%-50%	1/3 of the basal epithelium	In filtuate into museularia museace	2
	crypts were destroyed	minutate mo muscularis mucosae	
51-75%	2/3 of the basal enithelium	Infiltration into muscularis	
	crypts were destroyed	mucosae accompanied by mucosal	3
		thickening and marked edema	
76%-100%	All basal epithelial crypts were	Infiltrating submucosa	4
	destroyed	minutung subindeosa	•

#### Table S3. Histopathological scoring criteria of the colon

### Table S4. Primer sequence

Gene	Upstream Primers (5'-3')	Downstream Primers (5'-3')
Claudin-1	AACCCGAAACTGATGCTGTGGATAG	CGCCCTTGGAATGTATGTGGAGAG
ZO-1	AACCCGAAACTGATGCTGTGGATAG	CGCCCTTGGAATGTATGTGGAGAG
MyD88	AGCAGAACCAGGAGTCCGAGAAG	GGGCAGTAGCAGATAAAGGCATCG
TLR4	GAGCCGGAAGGTTATTGTGGTAGTG	AGGACAATGAAGATGATGCCAGAGC
Occludin	CAACGGCAAAGTGAATGGCAAGAG	TCATCCACGGACAAGGTCAGAGG
p-p65	GCGTCTTCCTCCACAGCCATTC	TGTCTCTGTTGGATTGTGCCGAAC
β-actin	CTGAGAGGGAAATCGTGCGTGAC	AGGAAGAGGATGCGGCAGTGG

Sample	Clean_paired_read	GC(%)	Q30(%)
N1	13388	53	94.46%
N2	13209	53	94.51%
N3	14359	53	94.60%
N4	13826	53	95.06%
N5	13246	54	94.59%
D1	13965	52	94.81%
D2	14348	52	94.48%
D3	13738	52	94.92%
D4	13371	52	94.30%
D5	13985	52	95.14%
P1	13983	52	94.34%
P2	14156	52	94.66%
P3	13027	52	95.87%
P4	14008	52	94.67%
P5	13433	52	94.76%
L1	13829	53	95.09%
L2	13280	52	95.10%
L3	13301	53	94.95%
L4	14371	53	94.79%
L5	13253	52	95.29%
PL1	14399	52	94.80%
PL2	13223	53	94.33%
PL3	13205	52	95.05%
PL4	14305	53	93.98%
PL5	13265	52	94.58%
<b>S</b> 1	14023	52	95.43%
S2	13208	52	94.60%
S3	14424	52	94.57%
S4	14102	51	94.50%
S5	13759	52	95.05%

Table S5. Statistical table of different samples

	0	<u>•</u>
SampleID	OTUs	Unique Tags
N1	453	85538
N2	501	86472
N3	509	80096
N4	549	77770
N5	529	79431
D1	387	76677
D2	384	66045
D3	385	75856
D4	382	69363
D5	377	63454
S1	412	81684
S2	406	66954
S3	439	84477
S4	427	76037
S5	427	76009
L1	405	85372
L2	368	70476
L3	375	77261
L4	445	80696
L5	421	74210
P1	378	73547
P2	409	74273
Р3	387	79073
P4	373	73623
Р5	380	74329
PL1	434	75324
PL2	498	78428
PL3	440	74568
PL4	476	81726
PL5	452	82010

Table S6. OTUs and Tags statistics for different samples



Figure S1. Effect of simulated gastrointestinal digestion on antioxidant capacity of α-LA hydrolysate in vitro



Figure S2. PCA analysis of different treatment groups



Figure S3. PCoA analysis of different treatment groups



sample

Figure S4. UPGMA clustering tree



Figure S5. Raw images of the Western blot in Figure 6.