

## Supporting Information

### ***Lactobacillus crispatus*-derived Exopolysaccharides with Antibacterial Activity Limit *Salmonella Typhimurium* Invasion by Inhibiting Inflammasome-mediated Pyroptosis**

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**Supplementary Table 1. Primer sequences.**

Primer	Forward sequence	Reverse sequence
<i>16SrRNA</i>	CGATGTCTACTTGGAGGTTGTG	CTCTGGAAAGTTCTGTGGATGTC
<i>sipA</i>	CGCTGTCAGGGGAAATTA	ATTATCGCTTTCTTACCGGC
<i>sipB</i>	GTATGGCAGGCGATGATTGA	ATAAACACTCTTGGCGGTATCC
<i>sipC</i>	GAATAAATCCC GCCGCTTAT	GGTCACTGACTTTACTGCTG
<i>hilA</i>	TATCTCCGGGCAGATGATAC	TCTGAGCAAAAAGATTTCGCAA
<i>invF</i>	ATGAGAATGCTGGGAGAAGA	AATTGGGTGATGTTCTCGTG
<i>Lactobacillus spp.</i>	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG
<i>Bifidobacterium spp.</i>	GCGTGCTTAACACATGCAAGTC	CACCCGTTTCCAGGAGCTATT
<i>Akkermansia</i>	CAGCACGTGAAGGTGGGGAC	CCTTGCGGTTGGCTTCAGAT
<i>Enterobacteriaceae</i>	ATGTTACAACCAAAGCGTACA	TTACCYTGACGCTTAACTGC
<i>Bax</i>	ACCAGCTCTGAACAGATCAT	AGATGGTCACTGTCTGCCAT
<i>Bcl-2</i>	GAGATCGTGATGAAGTACAT	TCAGGTACTCAGTCATCCAC
<i>caspase-3</i>	TGGGACTGATGAGGAGA	ACTGGATGAACCACGAC

**Supplementary Table 2. The Mw, Mn, peak area percentages and PD of EPS 7-4.**

Peak	Mw (Da)	Mn	Peak area (%)	PD index
P1	53,387	35,681	94.39	1.50
P2	4,644	3,687	5.61	1.25

Mw, Molecular weights; Mn, Number-average molecular weight, PD, Polydispersity.

**Supplementary Table 3. Glycosyl composition analysis results of EPS 7-4.**

Name	GalN	Ara	GlcN	Gal	Glc	Man	GlcA
Molar Ratio (%)	4.0	16.0	12.0	7.0	30.8	36.9	7.7
Peak Area	0.666	0.133	1.97	0.522	2.648	1.765	0.662

GalN, Galactosamine hydrochloride; Ara, Arabinose; GlcN, Glucosamine hydrochloride, Gal, Galactose; Glc,

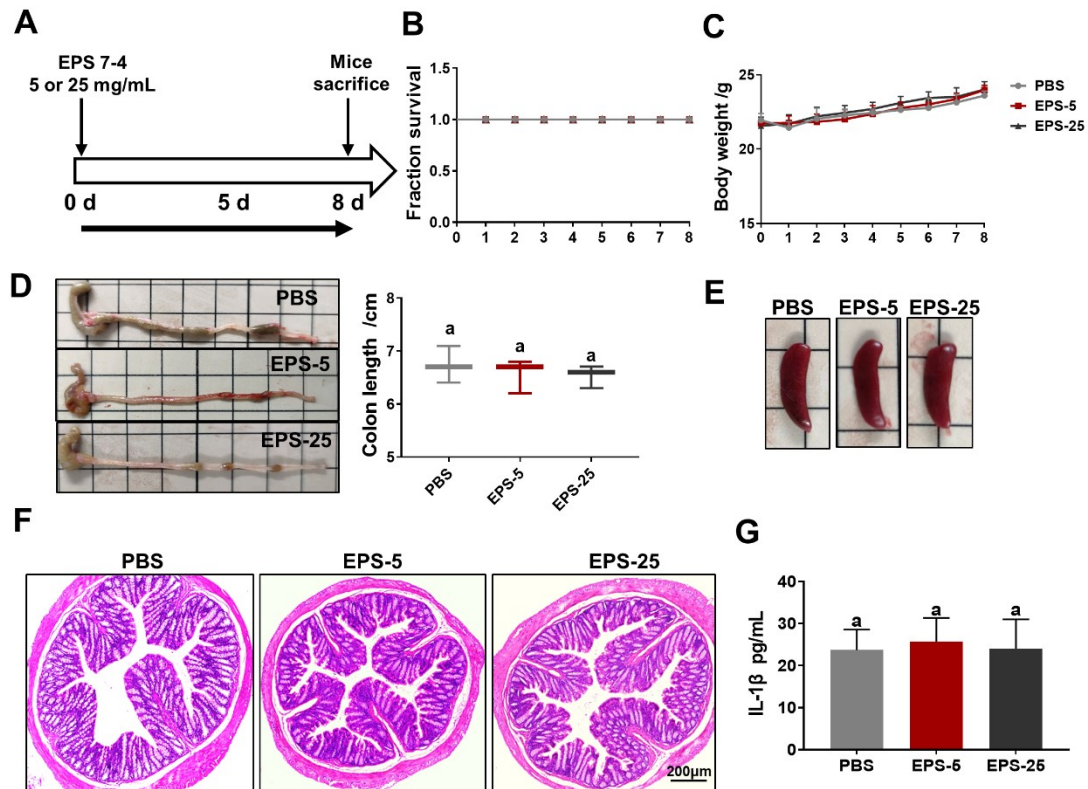
Glucose; Man, Mannose; GlcA, Glucuronic acid.

Supplementary Table 4. The MIC of EPS 7-4 by Broth microanalysis.

Pathogens	Antimicrobial agent						
	EPS 7-4	EPS 7-4	EPS 7-4	EPS 7-4	EPS 7-4	EPS 7-4	EPS 7-4
	(2mg/mL)	(5mg/mL)	(10mg/mL)	(20mg/mL)	(30mg/mL)	(40mg/mL)	(50mg/mL)
<i>E.coli</i> ATCC25922	+	+	+	+	+	+	-
<i>Salmonella typhimurium</i> CVCC542	+	+	-	-	-	-	-
<i>Salmonella enterica</i> ATCC19585	+	+	+	-	-	-	-

+, broth cloudy; -, broth clarify

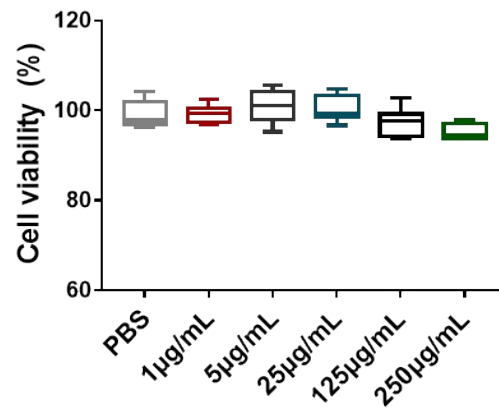
Supplementary Figure 1



**Fig. S1 The effect of EPS 7-4 on mice.**

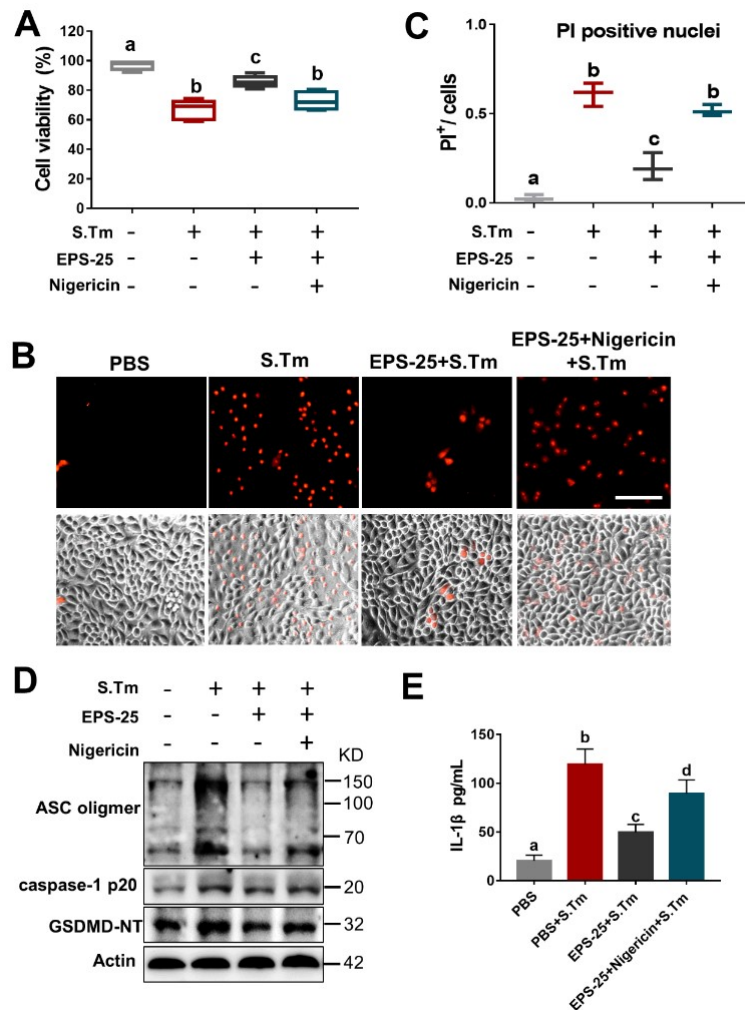
(A) Animal experiment process. (B) Survival rate. (C) Body weight change. (D) Colon image acquisition and length measurement. (E) Spleen image acquisition. (F) Colon histological observation and blindly scored (0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe) for epithelial damage and submucosal edema (Scale bar: 200  $\mu\text{m}$ ). (G) IL-1 $\beta$  level in serum. n = 3.

**Supplementary Figure 2**



**Figure S2. The cytotoxicity of EPS 7-4 on NCM460 cells.**

### Supplementary Figure 3



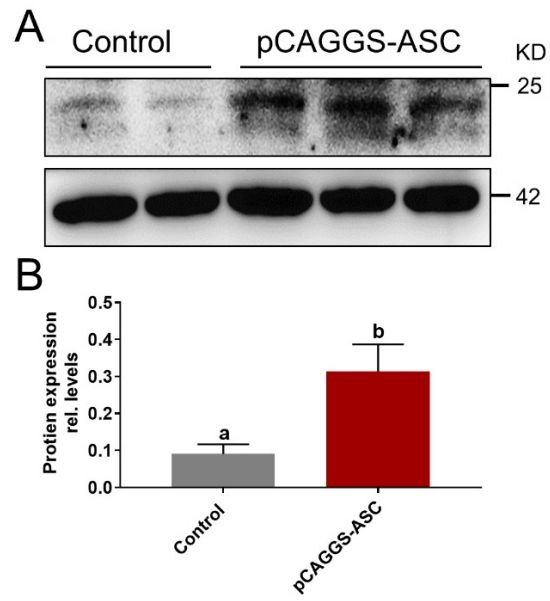
**Figure S3. The inflammasome activation reduces the anti-pyrogenic effect of EPS 7-4.**

(A) Cell viability. (B and C) Fluorescence images of PI (red) staining and PI positive cells rate. Scale bar indicates

500  $\mu$ m. (D) The protein expression levels of ASC oligomerization, caspase-1 p20, GSDMD-NT, and Actin. (E)

IL-1 $\beta$  content on cell supernatants. n = 3.

### Supplementary Figure 4



**Figure S4. The validation of ASC protein over-expression.**

(A and B) Representative protein bands and statistical analysis of ASC. n = 3.