Remodeling gut microbiota by *Streptococcus thermophilus* 19 attenuates inflammation in septic mice

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Abstract

Background: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and is the leading cause of death in burn patients. S. *thermophilus* 19 is highly effective probiotic strains with well-studied health benefits, but its role in protecting viscera against injury caused by sepsis and the underlying mechanism is poorly understood.

Methodology/Principal Findings: We tested the utility of S. *thermophilus* 19 in attenuating inflammation in *vitro* and *vivo* of a Lps-induced sepsis mouse model. We also evaluated the influence of sepsis and S. *thermophilus* on microbial community.In *vitro*, S. *thermophilus* 19 can decrease the expression of inflammatory factors. Additionally, in a lipopolysaccharide (LPS)-induced septic mouse model, mice administered the probiotics 19 was highly resistant to LPS and exhibited decreased expression of inflammatory factors compared to LPS-treated control mice. A MiSeq-based sequence analysis revealed that gut microbiota alterations in mice intraperitoneally injected with 1 mg/ml LPS were mitigated by the administration of oral probiotics 19. Furthermore, changes in the levels of inflammatory factors in different organs related to different gut microbiota alterations were observed.

Conclusions/Significance: Together these findings indicate that S. *thermophilus* 19 may be a new avenue for interventions against inflammation caused by sepsis and other systemic inflammatory diseases.

Author summary

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and is the leading cause of death in burn patients, responsible for up to 50 to 60% of burn injury deaths. Although our understanding of sepsis has increased substantially in recent years, it is still reported to be the leading cause of death in seriously ill patients, and the incidence of sepsis has increased annually. Therefore, new insights into the causes of sepsis are urgently needed.Here, we use Miseq method to determine the composition of gut microbiota.We also use a probiotic (*Streptococcus thermophilus* 19) to investigate the therapeutic effect on sepsis. We found that the

exceptional efficacy of probiotic *Streptococcus thermophilus* 19 in attenuating the inflammation in septic mice by modifying the structure and function of gut microbiota. Our study proposed oraling *Streptococcus thermophilus* 19 might be a new avenue for interventions against inflammation caused by sepsis and other systemic inflammatory diseases.

64 **Introduction**

65 Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection
66 and is the leading cause of death in burn patients, responsible for up to 50 to 60% of burn injury
67 deaths[1, 2]. Although our understanding of sepsis has increased substantially in recent years, it
68 is still reported to be the leading cause of death in seriously ill patients, and the incidence of
69 sepsis has increased annually. Therefore, new insights into the causes of sepsis are urgently
70 needed.

71 The gut microbiota is a complex ecosystem consisting of trillions of bacteria that live in
72 the digestive tracts of humans and other animals[3]. Growing evidence supports the key role of a
73 healthy gut microbiota in promoting and maintaining a balanced immune response and in the
74 establishment of the gut barrier immediately after birth[4, 5]. Moreover, a dysbiotic state of the
75 gut microbiota can lead to dysregulation of various processes, which can in turn contribute to the
76 development of autoimmune conditions[6]. For instance, the presence or overabundance of
77 specific types of bacteria may contribute to inflammatory disorders such as IBD[6]. Additionally,
78 metabolites from certain members of the gut flora may influence host signaling pathways,
79 contributing to disorders such as colon cancer and obesity. Sepsis is an extreme response to
80 inflammation that has profound effects on all parts of the body. For decades, the gut has been
81 regarded as the motor of sepsis[7], and it has recently been shown that a healthy gut microbiota
82 has a protective role during systemic inflammation. Thus, we hypothesized that intestinal
83 bacteria play an important role in sepsis since the gut microbiota is associated with many
84 diseases.

85 Probiotics are live microbes that have beneficial effects on human and animal health
86 when ingested in sufficient amounts[8]. Probiotics play an important role in maintaining the
87 normal microbiota composition and have been used to treat or prevent a number of gut health
88 disorders, such as irritable bowel syndrome, hypercholesterolemia, gastritis, gut infection,
89 parasitic infestation, hypersensitivity (including food allergies), and even certain types of cancers
90 (e.g., colorectal cancer)[9, 10]. The use of microbes as probiotics also hold potential for oral
91 health in preventing and treating oral infections, dental plaque-related diseases, periodontal
92 diseases and halitosis. Furthermore, probiotics can alleviate inflammation associated with some
93 human diseases by promoting changes in the gut microbiota composition[11, 12]. *Streptococcus*
94 *thermophilus* is a highly effective probiotic that has well studied health benefits, including the
95 production of antibiotics that prevent infections from pneumonia-causing microbes and C.
96 *difficile* and can help to prevent ulcers[13-15].
97 In this study, we used a coculture system (probiotics and RAW264.7 cells) to assess the
98 ability of probiotics to decrease the expression of inflammatory factors. We showed that
99 *Streptococcus thermophilus* 19 can decrease the inflammation induced by LPS in RAW264.7
100 cells. Furthermore, we investigated the ability of *Streptococcus thermophilus* 19 to protect mice
101 against LPS-induced inflammation and gut microbiota alterations when administered as a
102 probiotic. We observed that the administration of *S. thermophilus* 19 as probiotics could alter the
103 gut microbiota composition of untreated mice or mice with LPS-induced sepsis, with the
104 symptoms of sepsis mitigated in the latter group. Moreover, the levels of several inflammatory
105 factors in various organs were correlated to a diverse gut microbiota composition. We

106 hypothesize that the supplementation of diets with probiotics protects visceral organs by
107 reducing inflammation through alterations in the gut microbiota after sepsis.

108 **Results**

109 ***Probiotics decrease the expression of inflammatory factors in vitro***

110 To assess the influence of the assayed probiotics on the expression of inflammatory factors, we
111 developed a coculture system (probiotics and RAW264.7 cells). After incubating for 6 hours,
112 total RNA was extracted and the expression of inflammatory factors was assessed via
113 quantitative RT-PCR. The LPS treatment increased the expression of inflammatory factors
114 compared to the untreated group. After coculturing with probiotics, we observed a reduction in
115 inflammatory factor expression, particularly when cells were incubated S. *thermophilus* 19
116 (Figure. 1). Moreover, we also observed a reduction in CD86 expression when cells treated with
117 S. *thermophilus* 19 (Figure. S1).Therefore, S. *thermophilus* 19 was chosen for further study.

118 ***Food and water intake and animal health conditions***

119 Mice were induced by intravenous injection with different doses of LPS to induce sepsis. All
120 mice died when the concentration of LPS exceeded 2.5 mg/ml, even in mice administered
121 probiotics (data not shown). However, nearly 60% of mice survived when administered
122 probiotics together with 2 mg/ml LPS, whereas only 20% of mice administered the same LPS
123 without probiotics survived. All mice treated with 1 mg/ml of LPS survived (Figure. 2A).

124 We also recorded the total food and water intake and the animal health conditions for all
125 mice. LPS-treated mice with or without probiotics exhibited a reduction in total drinking water
126 and rat chow intake (Figure. 2C). Furthermore, mice treated with the probiotics alone also

127 exhibited decreased water and rat chow intake (Figure. 2C). However, mice treated with LPS lost
128 approximately 10% of their body weight during the 48 hours after injection, while untreated
129 mice did not lose weight. Although all treated groups regained their baseline weight by the third
130 day, mice treated with S. *thermophilus* 19 exhibited high rates of body weight recovery.
131 Meanwhile, mice treated with S. *thermophilus* 19 alone showed no changes in body weight,
132 although they exhibited lower drinking water and food intake (Figure. 2B). The decrease in body
133 weight of the LPS-treated mice could be explained by the LPS-induced inflammation causing a
134 reduction in food and drinking water intake, while the probiotics could alleviate inflammation to
135 promote the recovery in body weight.

136 ***Probiotics alleviate the serum inflammatory factor levels in septic mice***

137 To test whether the assayed probiotics could alleviate inflammation *in vivo*, we induced sepsis in
138 BALB/c (H-2D^{d}) mice with LPS (1 mg/ml) through intraperitoneal injection and treated them
139 with PBS, S. *thermophilus* 19 via oral gavage. We observed a 2-fold increase in TNF-α
140 expression in the sera of mice treated with LPS, while that of IL-1β was less affected (Figure.
141 S2). In mice administered probiotics, the TNF-α level was reduced to that observed in the control
142 group. In contrast, in mice treated with probiotics without the LPS treatment, no significant
143 effect on the serum levels of IL-1β and TNF-α were observed compared to the control group,
144 demonstrating that the probiotics has no influence on the host in the absence of sepsis.

145 ***Probiotics effectively alleviated viscera damage induced by sepsis***

146 Sepsis is an extreme response to inflammation, which has profound effects on all parts of the
147 body. Thus, we investigated the inflammation state of the kidneys, small intestines, liver, heart

148 and lungs of each mouse after the LPS and probiotic treatment. In the small intestines, the LPS
149 treatment dramatically increased the expression of IL-1β, CCL2, TGF-β, IL-6 and TNF-α, while
150 that of IL-10 was decreased compared to the control group. The expression of IL-1β, CCL2,
151 TGF-β, IL-6 and TNF-α were effectively rescued in the mice treated with probiotics compared to
152 the mice treated with LPS alone. Moreover, the expression of TNF-α and IL-1β in the probiotic-
153 and LPS-treated mice and the control mice did not significantly differ, while that of TGF-β and
154 CCL2 exhibited a slight difference. Meanwhile, mice treated only with probiotics exhibited
155 altered TGF-β and CCL2 expression compared to the control group (Figure. 3A, Figure .S3).
156 However, the administration of probiotics in the LPS-treated mice could not mitigate the change
157 in IL-10 expression observed mice treated with LPS alone. In the lungs, the LPS treatment
158 increased IL-1β, TNF-α, CCL2 and IL-6 expression, whereas the expression of IL-1β, TNF-α,
159 CCL2 and IL-6 was decreased in the probiotic- and LPS-treated mice. In addition, the LPS
160 treatment did not affect the expression of IL-10 and TGF-β, while the probiotic treatment
161 decreased the expression of IL-10(Figure. 3A, Figure .S3). In the kidneys, the LPS treatment
162 dramatically increased the expression of IL-1β, CCL2, TGF-β, IL-6 and TNF-α compared to the
163 control group, whereas mice treated with the probiotics exhibited decreased expression of these
164 inflammation factors. However, the expression of IL-10 in mouse kidneys has a crosscurrent
165 compared with that observed in the small intestines (Figure .3A, Figure .S3). Furthermore, the
166 administration of probiotics could decrease the high expression of IL-10 observed in the kidneys
167 due to the LPS treatment. In the livers, the probiotic treatment was also observed to decrease the
168 expression of inflammatory factors (IL-1β, TNF-α, IL-6, and CCL2) induced by LPS (Figure.

169 3A, Figure .S3). The expression of IL-10 in the liver exhibited the same tendency observed in the
170 small intestine. H&E staining (Figure. 3B) revealed that compared with the liver sections in the
171 control group mice, significant congestion of veins and hepatocyte necrosis was observed in the
172 LPS-treated mice, and the loss of intact liver plates and hepatocyte vacuolization was observed.
173 In pulmonary sections, drastic destruction of alveolar structures was detected in the LPS-treated
174 mice, and the effusion in alveoli in these mice was markedly more severe than that observed in
175 the control group mice. Furthermore, tissue infiltration by inflammatory cells was substantially
176 higher in LPS-treated mice than in the control group mice. Cotreatment with probiotics resulted
177 in the restoration of a close-to-normal appearance of liver and lung tissues. Moreover, S.
178 *thermophilus* 19 treatments alone did not affect the liver and lung sections of mice (Figure. 3B).

179 ***LPS altered the gut microbiota structure of mice***

180 In the balance between intestinal microbiota and inflammation, deviations either way may cause
181 corresponding adjustments in the other. To test whether the gut microbiota of mice was altered
182 due to sepsis, we collected the cecal feces of mice and assayed them via MiSeq sequencing to
183 determine the composition of gut microbiota. Mice treated with LPS exhibited decreases
184 intestinal microbiota richness compared to the control group (Chao1 index) while no difference
185 in diversity (Shannon index) between two groups was observed (Figure. 4A -B). Interestingly, 8
186 OTUs were specifically present in the LPS-treatment group compared to the control group, while
187 the control group also contained 8 specific OTUs (Figure. S4). The fecal microbiota of the mice
188 treated with LPS only clustered differently from those of the control group mice, demonstrating
189 the significant effect of LPS on the gut microbiota (Figure. 4C).

190 ***Probiotics intervention alters the gut microbiota of mice***

191 To investigate the effect of probiotics on the gut microbiota of mice, we sequenced the gut
192 microbiota of the mice treated with probiotics alone. The diversity of the intestinal microbiota
193 differed for the various probiotics assayed compared to the control group, while no difference in
194 richness was observed among the groups (Figure .5A -B). Nine OTUs were specifically present
195 in the group treated with S. *thermophilus* 19 alone compared to the control group, while the
196 control group also had 5 specific OTUs (Figure.S 4). Moreover, the gut microbiota of mice
197 treated with probiotics alone clustered differently from that of the control group mice (Figure.
198 S5C).

199 ***Oral administration of Probiotics alleviated viscera damage via altering the gut microbiota***

200 We showed that probiotic intervention can attenuate the inflammation in septic mice (Figure. 3
201 and Figure .S3). Furthermore, we previously reported that probiotics can reduce the
202 inflammation induced by Cr(VI) in mice through modifying the gut microbiota. Thus, we
203 hypothesized that the protection of viscera by the probiotic-induced attenuation of inflammation
204 in septic mice is also associated with changes in the intestinal microbiota. To test this hypothesis,
205 we sequenced the 16S rRNA gene variable (V) V3-V4 region of the fecal bacteria samples
206 obtained from S. *thermophilus* 19 treated Lps-treated mice (Lps7) and compared the results to
207 those obtained from the mice treated with LPS alone and the control group. Overall, differences
208 between the S. *thermophilus* 19- and LPS-treated mice were observed (Figure. 5A-B). Mice
209 administered S. *thermophilus* 19 and treated with LPS had 8 specific OTUs compared to mice
210 treated with LPS alone (Figure .S4). Meanwhile, the intestinal microbiota of LPS7 groups

211 clustered differently from mice treated with LPS alone group(Figure.5C), demonstrating the
212 important effect of probiotics. Next, we compared the differences in gut microbiota composition
213 between the probiotic- and LPS-treated mice and the control group mice. Mice treated with LPS
214 and S. *thermophilus* 19 exhibited decreases intestinal microbiota richness compared to the
215 control group (Chao1 index) while no difference in diversity (Shannon index) between two
216 groups was observed (Figure .S6 A - B). The gut microbiota of mice treated with LPS and 19
217 clustered differently from that of the control group mice (Figure.S 6C). Six specific OTUs were
218 identified in the LPS7 group mice and 9 were identified in the control group (Figure .S4). Taken
219 together, these results indicated that all the treatments altered the composition of the gut
220 microbiotas of the assayed mice. Although the composition of the gut bacteria in the mice treated
221 with probiotic and that of the control group differed, the expression of inflammation-associated
222 factors in these mice did not significantly differ. We speculated that the gut microbiota in these
223 exhibited a healthy status, whereas the probiotic and LPS-treated mice had a lower health status.

224 ***Strain-specific effects of probiotics on gut microbiota***

225 Next, sequence data were analyzed at the family and genus levels to investigate the changes in
226 gut microbiota composition. Compared to the control group, LPS-treated mice had lower
227 abundances of bacteria belonging to the phylum *Fusobacteria* and of the genera *Fusobacterium*
228 and *Psychrobacter* (Figure .4D. and Figure. S7). In contrast, higher abundances of bacteria from
229 the genus *Flavonifractor* were observed in the LPS-treated mice. Mice treated with S.
230 *thermophilus* 19 exhibited a decreased abundance of bacteria belonging to the phylum
231 *Bacteroidetes* and an increased abundance of the phylum *Firmicutes* (Figure.S 5D). The details

232 of the changes in microbiota compositions in the 19 treatment mice is shown in Supplementary
233 Figure.7. Compared to the LPS group, LPS-treated mice administered S. *thermophilus* 19
234 exhibited an increased abundance of *Fusobacteria*. In detail, LPS-treated mice administered S.
235 *thermophilus* 19 had lower abundance of *Clostridium*_XIVb and a higher abundance of
236 *Fusobacterium* and *Klebsiella*(Figure.5D and Figure.S7). The changes in the microbiota
237 composition between the LPS7 and control groups is shown in Figure.S 6D and S7.
238 Overall, these data showed that LPS and probiotics significantly impacted the microbiota
239 composition of mice.
240 ***The function of gut microbiota was specifically altered after the administration oral probiotics***
241 Next, we used a Kruskal-Wallis/Wilcoxon rank-sum test to determine how the altered
242 community structure of the gut microbiota affects its function. One KEGG database biochemical
243 pathway was significantly decreased and two were enriched in the LPS-treated mice compared to
244 control group(Figure. 6 A). After oral administration 19, nineteen treated mice were decreased in
245 65 KEGG database biochemical pathways and enhanced for 73 compared to control mice
246 (Figure. 6B). Moreover, 19 LPS-treated mice were enhanced for 2 KEGG database biochemical
247 pathways and were reduced for 3 (Figure.6D). However, the LPS7 mice were enriched in 33
248 pathways and were reduced in 53 compared to the control mice (Figure.6C). Notably, LPS7 mice
249 were decreased in both primary bile acid biosynthesis and secondary bile acid biosynthesis,
250 which have proinflammatory properties compared to the LPS-treated mice. These data suggest a
251 significantly decreased proinflammatory signature, as well as an increased anti-inflammatory
252 capacity of the gut microbiome in probiotic-treated mice. Taken together, the probiotics were

253 observed to reshape the gut microbiota with a distinct composition, network topology and
254 functionality.

255 **Discussion**

256 Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection
257 and often causes multiple organ damage. S. *thermophilus* has been shown to be highly effective
258 probiotic strains with well-studied health benefits. However, the impact of S. *thermophilus* on
259 the gut microbiota composition, and its influence on the inflammation caused by LPS-induced
260 sepsis remains poorly understood. In this study, we utilized a MiSeq sequencing approach to
261 assess how S. *thermophilus* 19 modulate the host fecal microbiota and inflammatory response in
262 an LPS-induced mouse sepsis model. Our results showed that S. *thermophilus* 19 can decrease
263 the expression of inflammatory factors RAW264.7 cells treated with LPS. Moreover, we showed
264 that S. *thermophilus* 19 were able to protect viscera against damage induced by sepsis.
265 Furthermore, S. *thermophilus* 19 could alter the microbiota composition and restore homeostasis
266 of the gut microbiota disrupted by sepsis.

267 Inflammation and infection are frequently accompanied by an imbalance in the intestinal
268 microflora[16]. A strong inflammatory response may then be mounted against microfloral
269 bacteria, leading to a perpetuation of the inflammation and gut barrier dysfunction[17]. Sepsis is
270 life-threatening organ dysfunction caused by a dysregulated host response to infection, which is
271 often causes a systemic inflammatory response. To assess the relationship between the gut
272 microbiota and sepsis, we induced sepsis in mice through intraperitoneal injection of LPS (2
273 mg/ml) and used a MiSeq sequencing-based approach to evaluate the gut microbiota

274 compositions of the assayed mice. The results showed that the LPS treatment decreased the
275 abundance of *Fusobacteria* and the richness of the intestinal microbiota. Moreover, the
276 abundances of the genera *Fusobacterium*, *Flavonifractor* and *Psychrobacter* were altered in the
277 septic mice. Previous studies showed that shifts in the intestinal *Firmicutes* to *Bacteroidetes*
278 ratio, as well as reduced microbiota diversity[18, 19]. However, these studies had many
279 uncertainties with regard to the variability and temporal nature of sepsis-induced dysbiosis. Thus,
280 we used an LPS-induced sepsis model to investigate the changes in gut microbiota composition
281 to eliminate the influence of other factors. Our results suggest that the genera *Fusobacterium*,
282 *Flavonifractor* and *Psychrobacter* may play important role in the development of sepsis.
283 We observed that LPS significantly upregulates the expression of genes involved in
284 inflammation, especially in the livers, lungs, kidneys and small intestines of mice. Moreover,
285 LPS induced sepsis has been demonstrated to result in the expression of inflammation-related
286 genes in multiple organs[1]. Probiotics are live microbial food supplements or bacterial
287 components that have been shown to have beneficial effects on human health. Additionally,
288 probiotics are often used to treat inflammation-related diseases, such as inflammatory bowel
289 disease, allergic diseases, and acute gastroenteritis. S. *thermophilus* is probiotics that have been
290 used to treat many illnesses. probiotics containing S. *thermophilus* KB19 significantly increased
291 betaine plasma levels in chronic kidney disease[20-23]. Similarly, we observed that S.
292 *thermophilus* decreased the level of inflammatory factors in an LPS-induced sepsis mouse
293 model. In addition, the administration of S. *thermophilus* 19 did not trigger any inflammation or
294 dysbiosis of the gut microbiota, suggesting that they could safely be used to treat sepsis with no

295 obvious harmful side effects. Thus, together with previous results, these results suggest that S.
296 *thermophilus* 19 may be one alternative probiotics for use in sepsis intervention in the future.
297 It has now been recognized that alterations in gut microbiota composition and function
298 appear to be an important mechanism by which probiotics alleviate human disease. Our results
299 showed that the probiotics 19 altered the function of the gut microbiota in mice In particular,
300 mice treated with LPS and probiotics exhibited changes in the function of
301 oxidative-phosphorylation and bile acid biosynthesis, which are important in
302 inflammation-related diseases[24, 25]. Moreover, probiotics also caused other functions of the
303 gut microbiota to change. Meanwhile, mice treated with probiotics alone also exhibited changes
304 in the function of the gut microbiota that may be good for host health by promoting low
305 inflammatory factor expression and a good health state. Taken together, our results indicated that
306 probiotics are good for host health despite the changes they induce in the composition and
307 function of the gut microbiota.
308 In summary, we demonstrated that the probiotics S. *thermophilus* 19 can alleviate
309 inflammation both *in vivo* and *in vitro*. This probiotics reduced the levels of inflammatory factors
310 caused by sepsis, which may occur through multiple targets. For instance, probiotics can resistant
311 some pathogenic bacteria enriched in gut after intraperitoneal injection of LPS, alter the
312 functional potential of intestinal microbes, promote higher intestinal permeability, and alter the
313 composition of the gut microbiota. These results suggest that the probiotics S. *thermophilus* 19
314 may be used to treat to not only sepsis but also other systemic inflammatory diseases
315 (inflammatory bowel disease, systemic inflammatory arthritis, multiple sclerosis and so on).

316 Collectively, the results of our study provide a conceptual framework to further text this
317 hypothesis in humans to treat sepsis and other systemic inflammatory diseases.

318 Method and Materials

319 ***Bacteria and media***

320 L. *plantarum* TW1-1, *Pediococcus acidilactici* XS40, L. *plantarum* DS45, L. *paracasei*
321 LZU-D2, L. *delbruckii*, L. *casei* 18-10, *Streptococcus thermophilus* 19 were provided by Dr.
322 Xusheng Guo (Lanzhou University, Lanzhou, China). The bacterial strains were cultured in De
323 Man, Rogosa, and Sharpe (MRS; Beijing Solarbio Science & Technology, Beijing, China)
324 growth medium with the exception of 19 and XS40, which were cultured in M17 growth medium
325 (MRS; Beijing Solarbio Science & Technology, Beijing, China) supplemented with 1% lactose
326 and MRS medium supplemented with 0.5% glucose, respectively. MRS and M17 agar medium
327 were used to determine the CFU of the assayed probiotic strains.

328 ***In vitro evaluation of inflammatory factors induced by probiotics and flow cytometry***

329 The commercial immortal mouse macrophage cell line RAW264.7 was obtained from the
330 American Type Culture Collection and was grown in Dulbecco's Modified Eagle's Medium
331 (DMEM; Gibco, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum
332 (FBS) under a humidified 10% CO_2 atmosphere at 37°C. The cells were cultured in 12-well
333 culture plates at 1×10^6 cells/well. The bacterial strains were grown in MRS or M17 medium
334 overnight (16 h), after which the cultures were diluted to an optical density (OD) of 0.3, washed
335 with phosphate-buffered saline (PBS; pH 7.4), resuspended in PBS, and were used to infect the
336 RAW264.7 cells at a multiplicity of infection (MOI) of 1:100 (cells/bacteria). The plates were

337 incubated for 6 h at 37°C under a 10% CO_2 atmosphere and samples were collected to assess the
338 levels of inflammatory factors by qRT-PCR and the levels of CD86 and CD206 by flow
339 cytometry. PBS without bacteria was used as negative control.

340 ***Animals and sepsis model***

341 The 7-14-week-old BALB/c (H-2D^d) mice used in this study were originally purchased from the
342 Experimental Animal Center of The Fourth Military Medical University and were bred in our
343 facility under specific-pathogen-free conditions. All animals were maintained under a 12 h
344 light/dark cycle. To induce sepsis, mice were administered different doses of lipopolysaccharide
345 (LPS) by intraperitoneal injection, with a second dose administered 4 days after the first
346 injection. The details of the experimental design are shown in Table 1. The names of the
347 experimental groups were as follows: LPS7 denotes LPS+ S. *thermophilus* 19, 7 denotes S.
348 *thermophilus* 19.

349 ***Ethics Statement***

350 All procedures and protocols used in this study were carried out in compliance with the
351 guidelines of the European Union for handling laboratory animals and were approved by the
352 Ethics Committee of the Fourth Military Medical University.

353 ***Weight, water and food intake measurements and sampling***

354 The body weight, water and food intake, and stool appearance were documented for all groups of
355 mice every other day throughout the experiment. After 1 week, the liver, kidneys, lungs, heart
356 and small intestines were collected from each mouse and were divided into triplicate samples,
357 with one stored in liquid nitrogen, a second stored in RNAiso Plus for RNA extraction, and the

358 third was fixed in 4% (w/v) paraformaldehyde at 4°C for later histological analysis.

359 ***Histology of different tissues***

360 After the animals were sacrificed, different tissue samples were collected. After fixation in 4%
361 paraformaldehyde, the tissue samples were embedded in paraffin and serially cut into 7-mm
362 thick sections. Tissue slides were stained with hematoxylin and eosin (H&E) for histological
363 analysis.

364 ***Microbial DNA extraction and Illumina MiSeq sequencing***

365 Microbial DNA was extracted from the samples using an E.Z.N.A.® Stool DNA Kit (Omega
366 BioTek, Norcross, GA, USA) according to manufacturer's protocols, and the DNA samples were
367 assessed via PCR with the universal 16S rRNA primers 27F/1492R. The DNA concentration and
368 integrity were determined by electrophoresis on 1% agarose gels containing ethidium bromide
369 and spectrophotometrically using an EPOCH instrument (BioTek). After confirmation, the DNA
370 was lyophilized and sent for Illumina MiSeq sequencing and data analysis.

371 The gut microbiota compositions of mice were assessed via Illumina MiSeq sequencing
372 (Genergy Biotech) targeting the V3-V4 region of the bacterial 16S ribosomal RNA gene using
373 the primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R
374 (5'-GACTACHVGGGTATCTAATCC-3'), with an eight-base barcode sequence unique to each
375 sample. The amplicons were extracted from 2% agarose gels and purified using an AxyPrep
376 DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the
377 manufacturer's instructions and were subsequently quantified using a QuantiFluor™-ST
378 instrument (Promega, USA). The purified amplicons were pooled in equimolar ratios and

379 paired-end sequenced (2 × 300) on an Illumina MiSeq platform according to standard protocols.
380 The raw reads were deposited at the NCBI Sequence Read Archive (SRA) database. Operational
381 taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1
382 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME.
383 The taxonomy of each 16S rRNA gene sequence was analyzed using RDP classifier
384 (http://rdp.cme.msu.edu/) against the SILVA (SSU123) 16S rRNA database using a confidence
385 threshold of 70%. The taxonomy of each ITS gene sequence was analyzed using Unite classifier
386 (https://unite.ut.ee/index.php).

387 ***Quantitative RT-PCR for inflammatory factor determination***

388 Total RNA was extracted from different tissues using RNAiso Plus (Takara, Dalian, China) and
389 was subsequently reverse transcribed into cDNA using PrimeScript™ RT.

390 Master Mix using random primers (Takara, Dalian, China) according to the
391 manufacturer's protocol. The expression of inflammatory factor-related genes was analyzed
392 using SYBR® PremixEx Taq™ II and the Bio-Rad CFX system. For real-time PCR, the reaction
393 mixtures contained 1 μL cDNA, 0.4 μL of each primer (10 $mmol^{-1}$), 5 μL of SYBR green PCR
394 Master Mix, and distilled water to a final reaction volume of 10 μL. The Taq DNA polymerase
395 was activated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s, and
396 72°C for 30 s. Quantitative RT-PCR data were normalized to the expression of the housekeeping
397 gene β-actin using the $2^{-\Delta Ct}$ method. The primers used in this study are shown in Table 2.

398 ***Quantification and statistical analysis***

399 Graphpad Prism was used for graphical presentation and statistical analyses. Differences were
400 considered statistically significant at $p<0.05$, and data are presented as the means ± SEM. The
401 number of biological replicates (n) and the number of independent experiments are indicated in
402 the figure legends. The Kruskal-Wallis/Wilcoxon rank-sum test was used to analyze the gut
403 microbiota composition data for all the groups.

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482

483 **Tables**

484 Table1

Group	Treatment Groups(n=8)	Gavaging
1	Control	PBS
2	LPS only	PBS
3	LPS+S. *thermophilus* 19	PBS+S. *thermophilus* 19
4	S. *thermophilus* 19 only	PBS+S. *thermophilus* 19

485

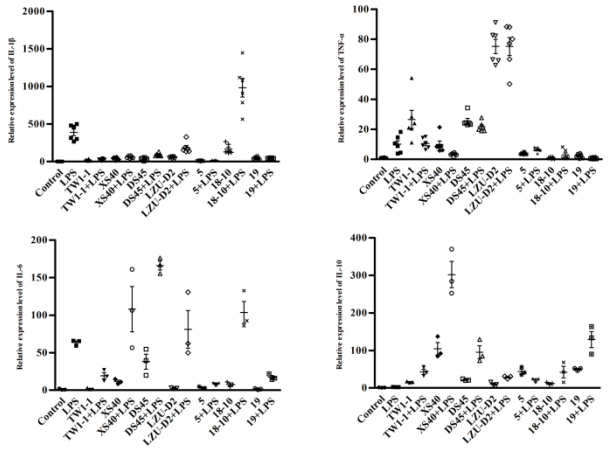
486 LPS: 1mg/ml; S. *thermophilus* 19: $1x10^9$ CFU/ml once every other day in 0.3 ml PBS. Mice

487 received LPS through intraperitoneal injection. Mice received PBS and 19 via gavage.

488 Table2

Primer	Sequence(5'-3')
β-actin	GTACGCCAACACAGTGCTG/CGTCATACTCCTGCTTGCTG
IL-1β	GCTTCAGGCAGGCAGTATC/AGGATGGGCTCTTCTTCAAAG
TNF-α	AGAGCTACAAGAGGATCACCAGCAG/TCAGATTTACGGGTCAACTTCACAT
TGF-β	CTTCAATACGTCAGACATTCGGG/GTAACGCCAGGAATTGTTGCTA
IL-6	GAGGATACCACTCCCAACAGACC/ AAGTGCATCATCGTTGTTCATACA
IL-10	GCTCTTACTGACTGGCATGAG/CGCAGCTCTAGGAGCATGTG
CCL2	AGCAGCAGGTGTCCCAAAGA/GTGCTGAAGACCTTAGGGCAGA

489 Primers used in this study.



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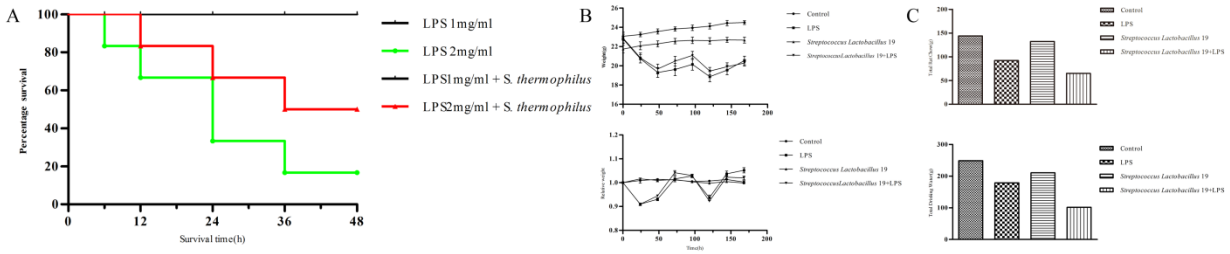

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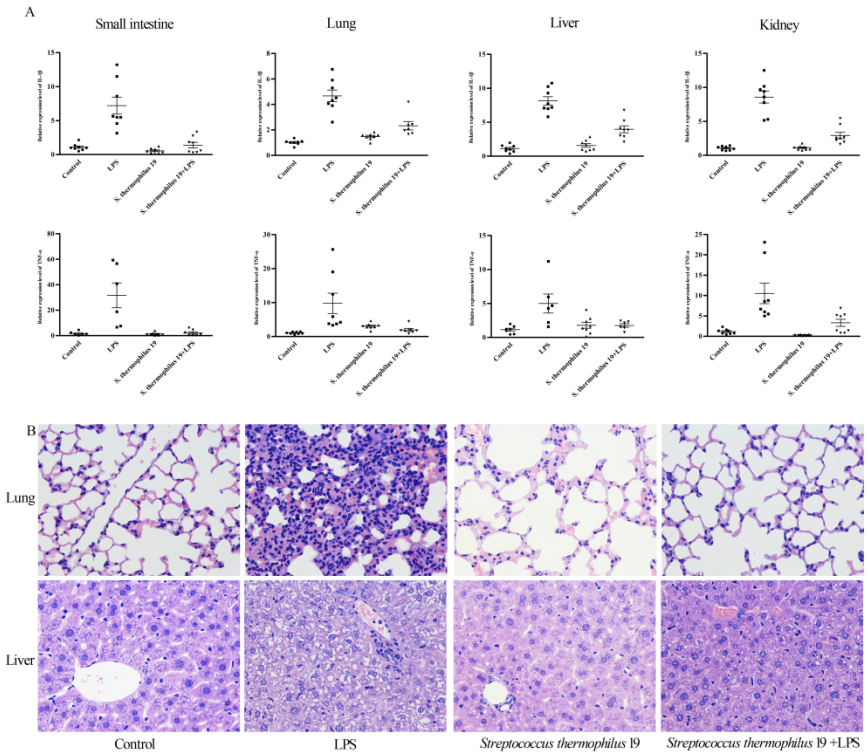

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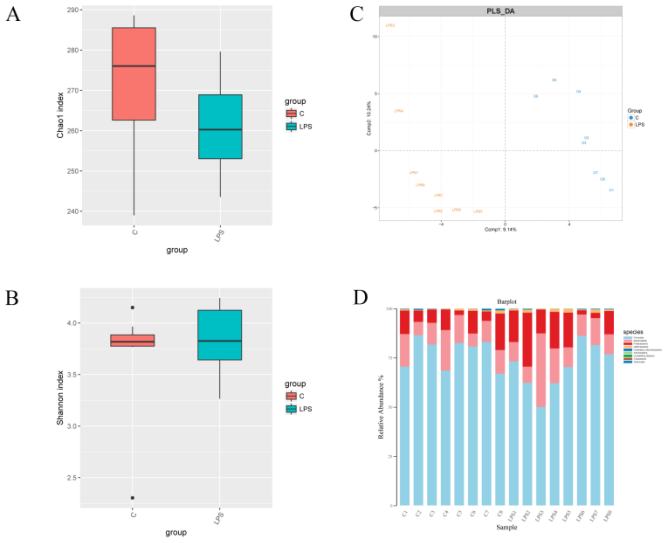

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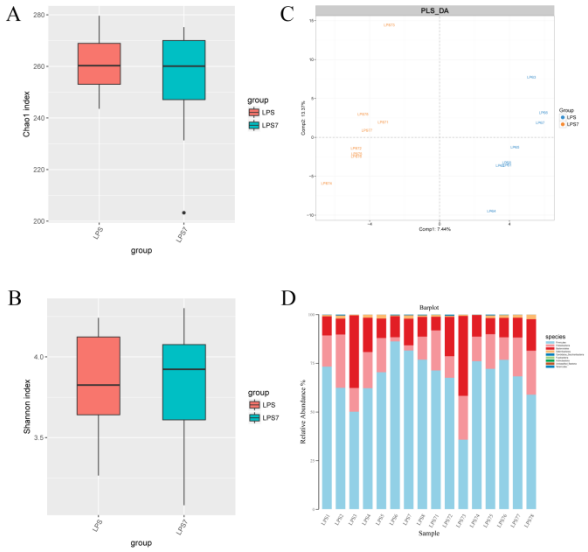

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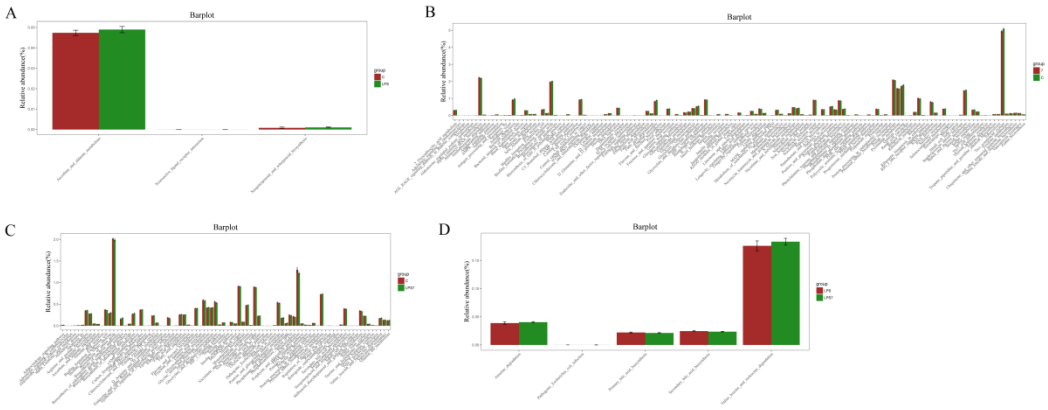

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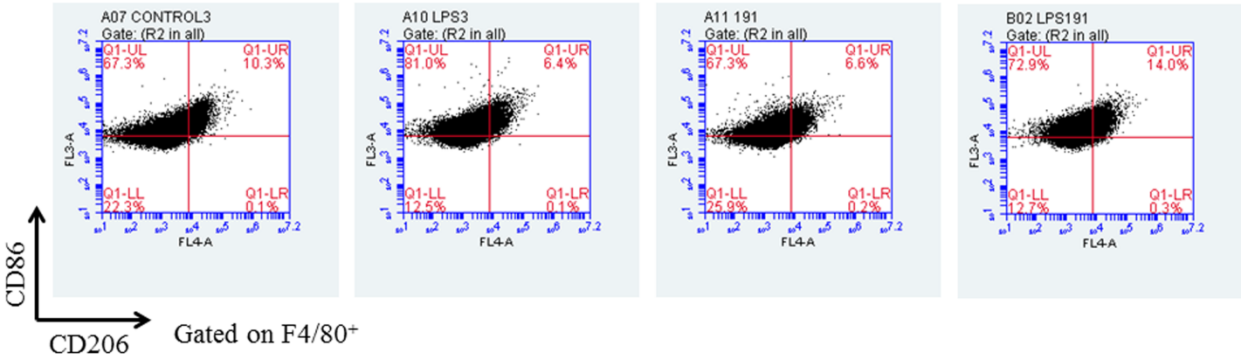

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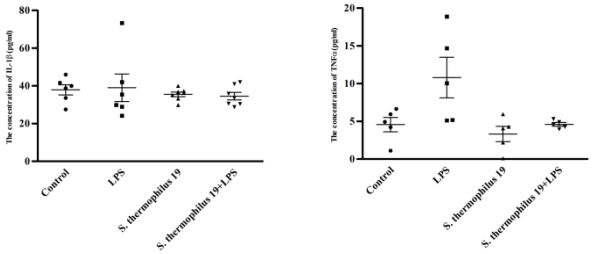

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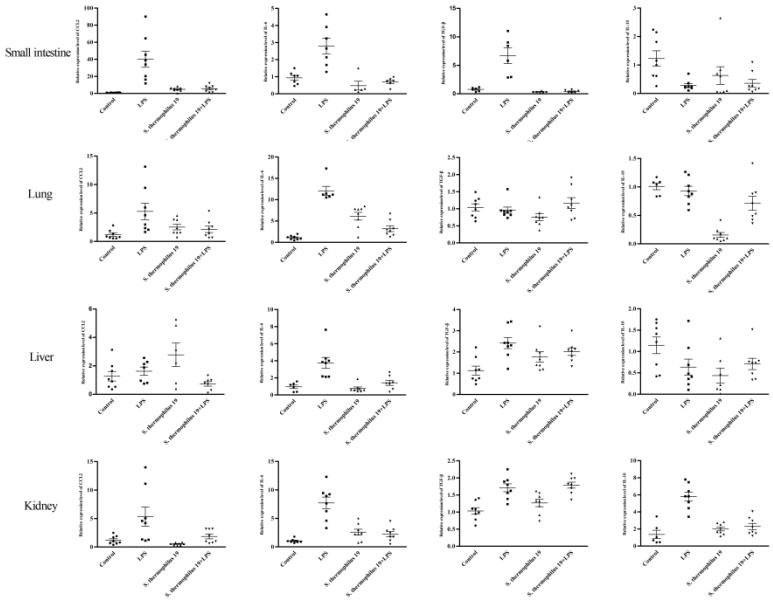

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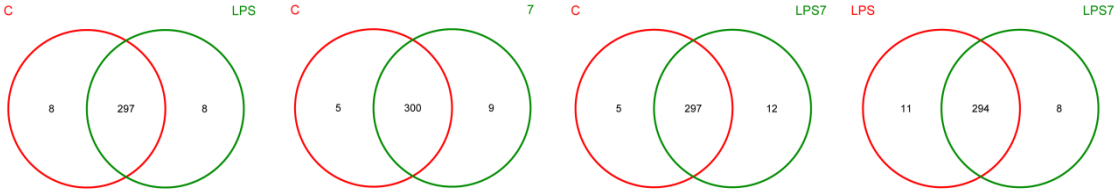

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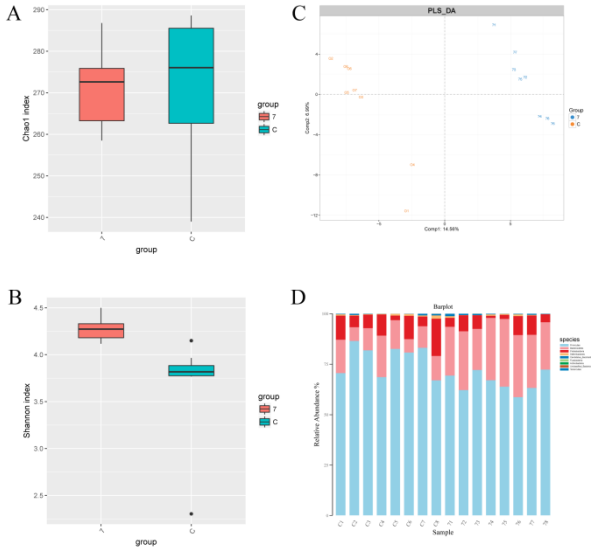

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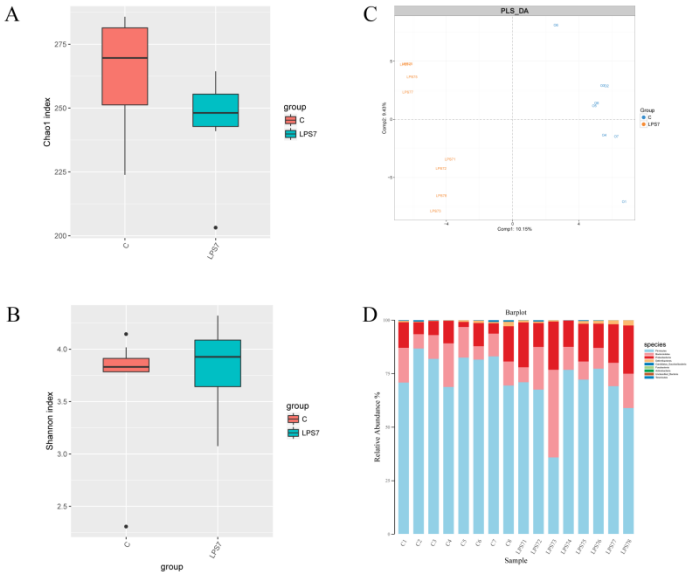

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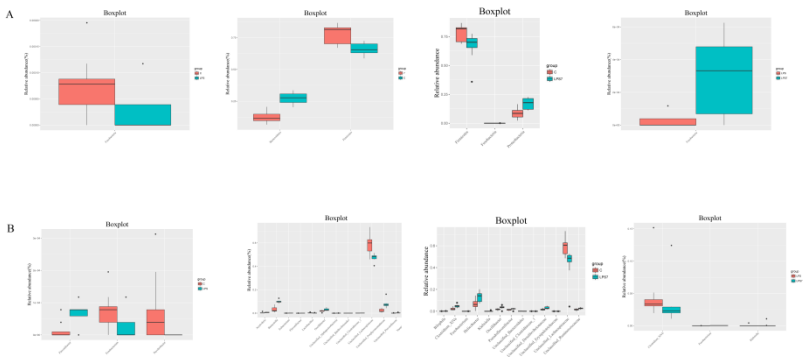

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515 **Figure.S7**

516 **Figure.1**. The expression of inflammatory factors(IL-1β,TNF-α,IL-6 and IL-10) in LPS-treated,

517 probiotics-LPS treated and untreated RAW264.7 cells. Error bars represents SEM.

518 **Figure.2.** Probiotics alleviate the inflammatory caused by LPS-induced sepsis. (A) Survival rates

519 of mice with or without probiotics treatment after 48h stimulation with different dose of LPS. (B)

520 Body weight change and relative weight change (n=8/group). (C) Total rat chow intake and

521 drinking water.

522 **Figure.3.** Probiotics intervention resulted in decreased inflammation in vivo. (A) inflammation

523 factor of Small intestine, lung, liver and kidney. Error bars represents SEM. (B) Hematoxylin

524 and eosin staining of liver, and lung tissues from different groups. Sections were examined and

525 photographed under a microscope.

526 **Figure.4.** LPS induce significant impact on microbiota composition. (A) (B) Fecal microbiota

527 alfa diversity.(C) PLS_DA plot of fecal microbiota of LPS-treated or control mice. (D) The

528 change of gut microbiota at phylum level.

529 **Figure.5.** S. *thermophilus* 19 induce significant impact on microbiota composition compared to

530 LPS-treated mice. (A) (B) Fecal microbiota alfa diversity.(C) PLS_DA plot of fecal microbiota

531 of LPS-treated mice with or without S. *thermophilus* 19 treatment. (D) The change of gut

532 microbiota at phylum level.

533 **Figure.6.** The presence of LPS and S. *thermophilus* 19 induces changes in gut microbiota

534 function. Statistical comparison was performed by first testing normality using

535 Kruskal-Wallis/Wilcoxon rank-sum test. Error bars represents SEM.

536 **Figure.S1.**The expression level of CD86 and CD206 in RAW264.7 cells from different groups

537 gated on F4/80^{+} cells. 19**:** S. *thermophilus* 19, LPS19:LPS+ S. *thermophilus* 19.

538 **Figure.S2.** Levels of IL-1β and TNF-α in blood were determined using commercial ELISA kits.

539 Error bars represents SEM.

540 **Figure.S3.** Probiotics intervention resulted in decreased inflammation in vivo. Inflammation

541 factor(CCL2,IL-6,IL-10 and TGF-β) of small intestine, lung, liver and kidney. Error bars

542 represents SEM.

543 **Figure.S4.** Specifically OTUS existed in different groups.

544 **Figure.S5.** S. *thermophilus* 19 induce significant impact on microbiota composition compared to

545 control mice.

546 **Figure.S6.**The gut microbiota composition between control group and co-treatment(19 and LPS)

547 group.

548 **Figure.S7.** The details of the change of gut microbiota at phylum and genus level in different

549 groups.