

1 [Supplementary information]

2
3 ***Lactobacillus rhamnosus* HDB1258 modulates gut microbiota-mediated immune response**
4 **in mice with or without LPS-induced systemic inflammation**

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Table S1. Primer sequences used in the present study

Gene	Primer	Sequence
TNF- α	Forward	5'-AGCCACGTAGCAAACCACCAA-3'
	Reverse	5'-ACACCCATTCCCTTCACAGAGCAAT-3'
IL-10	Forward	5'-CAGCCGGGAAGACAATAACTG-3'
	Reverse	5'-CCGCAGCTCTAGGAGCATGT-3'
T-bet	Forward	5'-CAACAACCCCTTTGCCAAAG-3'
	Reverse	5'-TCCCCCAAGCAGTTGACAGT-3'
Foxp3	Forward	5'-AGAAGCTGGGAGCTATGCAG-3'
	Reverse	5'-GCTACGATGCAGCAAGCGC-3'

Table S2. Effect of HDB1258 on the gut microbiota composition at the family level in mice

Taxon	Composition (%)			
	NC	SCG	LL	LH
Muribaculaceae	39.62 $\pm$ 21.97 ^a	34.35 $\pm$ 3.45	38.79 $\pm$ 6.03	35.66 $\pm$ 15.38
Lachnospiraceae	22.65 $\pm$ 13.47	14.42 $\pm$ 3.66	12.44 $\pm$ 4.82	25.53 $\pm$ 15.56
Prevotellaceae	11.45 $\pm$ 8.70	21.73 $\pm$ 7.63 [#]	13.06 $\pm$ 5.67	13.98 $\pm$ 8.15
Lactobacillaceae	7.03 $\pm$ 9.75	5.60 $\pm$ 5.53	5.03 $\pm$ 3.03	2.03 $\pm$ 1.71
Ruminococcaceae	5.48 $\pm$ 3.53	8.68 $\pm$ 1.81	6.82 $\pm$ 2.47	5.93 $\pm$ 2.72
Erysipelotrichaceae	3.25 $\pm$ 4.19	3.88 $\pm$ 3.99	6.43 $\pm$ 8.22	0.64 $\pm$ 0.60
Bacteroidaceae	2.69 $\pm$ 1.43	3.32 $\pm$ 1.34	5.49 $\pm$ 2.42 [#]	4.94 $\pm$ 4.53
Helicobacteraceae	2.00 $\pm$ 2.21	0.79 $\pm$ 0.71	0.55 $\pm$ 0.39	1.98 $\pm$ 1.71
Rikenellaceae	1.41 $\pm$ 0.94	1.42 $\pm$ 0.29	1.90 $\pm$ 2.32	1.55 $\pm$ 0.81
Desulfovibrionaceae	1.05 $\pm$ 0.97	0.51 $\pm$ 0.20	0.29 $\pm$ 0.13	1.06 $\pm$ 0.84
FR888536_f	0.58 $\pm$ 0.74	1.22 $\pm$ 1.37	1.32 $\pm$ 1.36	1.51 $\pm$ 1.25
Sutterellaceae	0.55 $\pm$ 0.33	1.10 $\pm$ 0.86	0.51 $\pm$ 0.36	0.86 $\pm$ 0.77
Porphyromonadaceae	0.41 $\pm$ 0.58	0.50 $\pm$ 0.22	0.54 $\pm$ 0.19	1.08 $\pm$ 0.49 [#]
Dehalobacterium_f	0.25 $\pm$ 0.14	0.21 $\pm$ 0.09	0.15 $\pm$ 0.04	0.25 $\pm$ 0.17
Bifidobacteriaceae	0.24 $\pm$ 0.25	0.75 $\pm$ 0.83	0.60 $\pm$ 0.73	0.10 $\pm$ 0.09
AC160630_f	0.23 $\pm$ 0.23	0.15 $\pm$ 0.07	0.86 $\pm$ 0.89	1.25 $\pm$ 1.16 [#]
Deferribacteraceae	0.15 $\pm$ 0.25	0.06 $\pm$ 0.15	0.00 $\pm$ 0.01	0.09 $\pm$ 0.10
Coriobacteriaceae	0.15 $\pm$ 0.13	0.08 $\pm$ 0.05	0.15 $\pm$ 0.10	0.10 $\pm$ 0.05
Akkermansiaceae	0.14 $\pm$ 0.16	0.18 $\pm$ 0.13	4.01 $\pm$ 7.47	0.13 $\pm$ 0.08
Christensenellaceae	0.13 $\pm$ 0.06	0.21 $\pm$ 0.07 [#]	0.22 $\pm$ 0.11	0.36 $\pm$ 0.17 [#]
Clostridiaceae	0.10 $\pm$ 0.07	0.09 $\pm$ 0.07	0.07 $\pm$ 0.06	0.07 $\pm$ 0.06

^amean $\pm$ SD.

[#]p<0.05 vs. Con.

26 Table S3. Effect of HDB1258 on the gut microbiota composition at the genus level in mice

Taxon Name	Composition (%)			
	NC	SCG	LL	LH
PAC001068_g	14.53 ± 9.62 ^a	15.41 ± 4.18	18.25 ± 4.85	10.62 ± 4.98
Lactobacillus	7.02 ± 9.74	5.58 ± 5.48	5.03 ± 3.03	2.03 ± 1.71
PAC000186_g	6.00 ± 3.88	5.17 ± 2.68	5.15 ± 2.65	4.02 ± 2.75
Prevotellaceae_uc	5.89 ± 5.10	12.63 ± 3.55 [#]	4.90 ± 3.27	1.62 ± 1.35
PAC001485_g	3.65 ± 4.54	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01
Prevotella	3.33 ± 3.69	6.99 ± 3.07	5.61 ± 2.86	3.41 ± 1.66
PAC001112_g	3.24 ± 2.47	1.79 ± 0.28	2.96 ± 1.34	2.60 ± 2.09
PAC000664_g	3.21 ± 2.07	2.54 ± 2.16	2.19 ± 1.28	7.60 ± 7.61
Muribaculum	3.19 ± 3.97	1.81 ± 0.80	1.95 ± 1.91	3.19 ± 1.93
Bacteroides	2.69 ± 1.43	3.32 ± 1.34	5.49 ± 2.42	4.93 ± 4.52
Paraprevotella	2.22 ± 3.13	2.11 ± 1.73	2.34 ± 3.14	8.53 ± 5.95
KE159538_g	2.08 ± 1.974	1.96 ± 1.22	1.98 ± 2.89	3.89 ± 5.80
Helicobacter	2.00 ± 2.21	0.79 ± 0.71	0.55 ± 0.39	1.98 ± 1.71
PAC000198_g	1.94 ± 0.75	2.09 ± 0.66	2.62 ± 1.31	3.51 ± 1.59
PAC002367_g	1.84 ± 3.51	0.03 ± 0.05	0.07 ± 0.06	0.90 ± 1.05
PAC001472_g	1.76 ± 1.15	1.73 ± 0.98	1.34 ± 0.56	0.86 ± 0.71
LLKB_g	1.70 ± 2.12	0.14 ± 0.07	0.38 ± 0.31	0.35 ± 0.40
Pseudoflavonifractor	1.58 ± 1.16	1.48 ± 0.56	0.93 ± 0.47	2.01 ± 0.89
Faecalibaculum	1.45 ± 1.74	3.11 ± 3.52	2.74 ± 3.82	0.13 ± 0.08
Eubacterium_g6	1.30 ± 1.86	0.18 ± 0.11	0.35 ± 0.58	0.27 ± 0.18
Alistipes	1.24 ± 0.90	1.13 ± 0.22	1.58 ± 1.97	1.32 ± 0.77

^amean± SD.

[#]p<0.05 vs. Con.

31 Table S4. Effect of HDB1258 on the gut microbiota composition at the species level in mice

Taxon Name	Composition (%)			
	NC	SCG	LL	LH
PAC001070_s group	6.79 ± 8.19 ^a	3.08 ± 1.57	4.67 ± 4.47	0.58 ± 0.31
Lactobacillus murinus group	5.91 ± 9.02	3.71 ± 4.24	3.73 ± 2.84	1.50 ± 1.60
EU622763_s group	2.69 ± 3.08	6.01 ± 2.85	4.81 ± 2.62	2.59 ± 1.47
EU505186_s	2.66 ± 3.30	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01
PAC001065_s group	2.26 ± 2.43	1.34 ± 0.45	2.22 ± 0.73	1.27 ± 0.82
FJ880724_s	2.00 ± 2.72	1.97 ± 1.59	2.18 ± 2.91	7.73 ± 5.27 [#]
PAC001068_g_uc	1.90 ± 1.56	1.85 ± 0.87	1.95 ± 0.57	1.26 ± 0.85

PAC002367_s	1.82 ± 3.47	0.03 ± 0.05	0.07 ± 0.06	0.89 ± 1.05
PAC001267_s	1.82 ± 2.03	0.32 ± 0.17	0.27 ± 0.10	1.02 ± 0.89
PAC001077_s	1.76 ± 2.98	0.17 ± 0.28	1.07 ± 1.33	1.01 ± 0.74
PAC001072_s	1.76 ± 0.88	2.99 ± 0.68#	4.47 ± 1.38#	5.93 ± 2.73#
EF097112_s	1.54 ± 1.46	0.70 ± 0.30	1.46 ± 0.64	0.98 ± 0.67
PAC001064_s	1.44 ± 1.11	0.56 ± 0.20	0.67 ± 0.29	1.35 ± 1.03
Faecalibaculum rodentium	1.43 ± 1.71	3.06 ± 3.45	2.72 ± 3.79	0.13 ± 0.08
Helicobacter rodentium group	1.38 ± 2.11	0.31 ± 0.30	0.33 ± 0.38	1.22 ± 1.04
AB606242_s	1.23 ± 0.11	0.25 ± 0.48	1.13 ± 1.26	4.48 ± 5.25
AB626939_s	1.16 ± 1.93	0.07 ± 0.06	0.27 ± 0.25	0.20 ± 0.37
Bacteroides acidifaciens group	1.13 ± 0.59	1.33 ± 0.63	1.98 ± 1.58	0.84 ± 0.62
PAC001472_g_uc	1.02 ± 1.16	0.49 ± 0.44	0.32 ± 0.17	0.14 ± 0.18
PAC000186_g_uc	0.96 ± 0.68	1.09 ± 0.75	0.85 ± 0.64	0.60 ± 0.44
JQ083736_s	0.96 ± 0.68	1.09 ± 0.75	0.85 ± 0.64	0.60 ± 0.44

^amean± SD.

[#]p<0.05 vs. Con.

Table S5. Effect of HDB1258 on the gut microbiota composition at the family level in mice with LPS-induced systemic inflammation

Taxon Name	Composition (%)			
	NC	LPS	LL	LH
Muribaculaceae	32.26 ± 7.84 ^a	30.50 ± 8.81	33.71 ± 9.51	35.68 ± 8.99
Lachnospiraceae	21.98 ± 7.10	9.54 ± 2.66 [#]	22.25 ± 9.41*	19.35 ± 11.22*
Prevotellaceae	18.13 ± 6.12	21.48 ± 3.32	21.56 ± 8.92	21.97 ± 7.20
Ruminococcaceae	8.83 ± 2.09	11.65 ± 3.22	7.02 ± 2.64*	6.52 ± 2.01*
Lactobacillaceae	5.28 ± 4.67	6.46 ± 3.38	3.20 ± 1.75*	2.99 ± 3.32
Bacteroidaceae	3.73 ± 1.38	4.45 ± 1.54	4.05 ± 1.89	3.99 ± 1.46
Rikenellaceae	2.40 ± 1.27	1.17 ± 0.50 [#]	1.52 ± 0.59	2.21 ± 0.63*
Helicobacteraceae	1.32 ± 0.79	0.21 ± 0.11 [#]	0.70 ± 0.32*	1.65 ± 2.44
Erysipelotrichaceae	1.11 ± 0.93	9.88 ± 4.46 [#]	1.42 ± 2.17*	0.58 ± 0.62*
FR888536_f	0.83 ± 0.98	1.02 ± 0.97	0.40 ± 0.18	1.39 ± 2.33
Porphyromonadaceae	0.69 ± 0.30	0.65 ± 0.24	0.79 ± 0.25	0.64 ± 0.29
Desulfovibrionaceae	0.67 ± 0.32	0.33 ± 0.12 [#]	0.84 ± 0.52*	0.69 ± 0.51*
Akkermansiaceae	0.52 ± 0.88	0.07 ± 0.04	0.09 ± 0.07	0.49 ± 0.41*
Dehalobacterium_f	0.31 ± 0.13	0.14 ± 0.10 [#]	0.31 ± 0.25	0.23 ± 0.16
Sutterellaceae	0.31 ± 0.16	0.95 ± 0.30 [#]	0.37 ± 0.40*	0.41 ± 0.16*
AC160630_f	0.26 ± 0.15	0.29 ± 0.21	0.57 ± 0.32	0.11 ± 0.06

Christensenellaceae	0.18 ± 0.10	0.22 ± 0.06	0.15 ± 0.06*	0.15 ± 0.08
Bifidobacteriaceae	0.14 ± 0.21	0.35 ± 0.26	0.11 ± 0.14*	0.04 ± 0.08*
Odoribacteraceae	0.14 ± 0.07	0.09 ± 0.10	0.08 ± 0.04	0.31 ± 0.26
Coriobacteriaceae	0.13 ± 0.08	0.08 ± 0.02	0.18 ± 0.07*	0.06 ± 0.04
Clostridiaceae	0.11 ± 0.07	0.15 ± 0.07	0.13 ± 0.07	0.12 ± 0.09

^amean± SD.

[#]p<0.05 vs. Con. *p<0.05 vs.LPS.

Table S6. Effect of HDB1258 on the gut microbiota composition at the genus level in mice with LPS-induced systemic inflammation

Taxon Name	Composition (%)			
	NC	SCG	LL	LH
PAC001068_g	13.47 ± 4.87 ^a	11.04 ± 3.43	13.14 ± 3.86	11.09 ± 3.97
Prevotellaceae_uc	9.13 ± 5.60	9.87 ± 2.71	12.28 ± 6.51	16.62 ± 4.27*
PAC000186_g	6.01 ± 3.94	5.33 ± 1.31	5.12 ± 2.55	5.00 ± 1.54
Prevotella	5.30 ± 2.49	7.97 ± 2.20	6.96 ± 3.42	4.82 ± 3.87
Lactobacillus	5.28 ± 4.66	6.45 ± 3.38	3.20 ± 1.76*	2.98 ± 3.32
KE159538_g	4.55 ± 3.44	0.43 ± 0.51 [#]	4.23 ± 5.43	2.21 ± 1.54*
Bacteroides	3.37 ± 1.37	4.45 ± 1.54	4.05 ± 1.89	3.97 ± 1.45
Paraprevotella	3.33 ± 3.82	3.64 ± 3.64	2.33 ± 3.70	0.53 ± 0.99*
PAC000198_g	2.90 ± 1.49	2.96 ± 1.08	3.02 ± 0.82	3.56 ± 1.25
PAC000664_g	2.78 ± 1.40	1.78 ± 0.99	5.32 ± 5.49	3.28 ± 1.61
PAC002367_g	2.30 ± 2.14	0.82 ± 1.62	1.29 ± 3.20	0.47 ± 0.43
Muribaculum	2.26 ± 1.41	1.86 ± 1.09	2.20 ± 1.73	5.38 ± 1.97*
PAC001112_g	2.17 ± 1.38	2.58 ± 0.74	2.04 ± 0.36	2.85 ± 0.73
Pseudoflavonifractor	2.09 ± 0.69	0.98 ± 0.29 [#]	1.71 ± 0.66*	1.50 ± 0.69
Alistipes	1.96 ± 1.08	1.03 ± 0.52	1.15 ± 0.54	1.90 ± 0.68*
PAC000661_g	1.78 ± 0.88	2.51 ± 1.87	0.79 ± 0.64*	1.36 ± 0.56
Ruminococcus	1.40 ± 1.05	4.90 ± 1.55 [#]	2.13 ± 2.17*	1.44 ± 1.98*
Helicobacter	1.32 ± 0.79	0.21 ± 0.11 [#]	0.70 ± 0.32*	1.65 ± 2.44
Eubacterium_g23	1.17 ± 1.39	1.56 ± 0.92	0.42 ± 0.60*	0.24 ± 0.22*
Oscillibacter	1.07 ± 0.59	0.32 ± 0.13 [#]	0.81 ± 0.46*	1.04 ± 0.58*
LLKB_g	0.89 ± 1.20	0.59 ± 0.35	0.91 ± 1.12	1.04 ± 0.97

^amean± SD.

[#]p<0.05 vs. Con. *p<0.05 vs.LPS.

Table S7. Effect of HDB1258 on the gut microbiota composition at the species level in mice

49 with LPS-induced systemic inflammation

Taxon Name	Composition (%)			
	NC	SCG	LL	LH
PAC001070_s group	3.93 ± 1.48 ^a	2.13 ± 2.01	1.83 ± 1.64	1.69 ± 1.53
EU622763_s group	3.73 ± 1.58	6.73 ± 1.74 [#]	5.54 ± 2.95	3.94 ± 3.31
Lactobacillus murinus group	3.52 ± 2.86	5.92 ± 3.37	2.82 ± 1.68*	1.22 ± 0.92*
FJ880724_s	3.03 ± 3.31	3.36 ± 3.34	2.14 ± 3.30	0.51 ± 0.94
PAC001072_s	2.94 ± 1.23	3.01 ± 0.45	4.37 ± 0.55*	3.62 ± 1.27
PAC001696_s	2.54 ± 3.22	0.02 ± 0.03	0.35 ± 0.82	0.27 ± 0.27*
PAC002367_s	2.29 ± 2.14	0.82 ± 1.61	1.29 ± 3.19	0.47 ± 0.43
PAC001065_s group	2.18 ± 0.61	0.86 ± 0.41 [#]	1.84 ± 1.56	1.55 ± 0.76
PAC001068_g_uc	1.62 ± 0.91	0.90 ± 0.40	1.33 ± 0.64	1.14 ± 0.60
PAC000198_s	1.50 ± 1.56	0.39 ± 0.38	0.03 ± 0.05*	0.00 ± 0.00*
PAC002450_s	1.43 ± 1.13	1.35 ± 1.54	1.25 ± 0.77	0.01 ± 0.01*
Bacteroides acidifaciens group	1.34 ± 0.83	1.51 ± 0.20	1.12 ± 0.52	0.54 ± 0.27*
PAC002447_s	1.15 ± 2.93	0.04 ± 0.04	0.00 ± 0.00*	0.00 ± 0.00
PAC001084_s	1.10 ± 0.87	0.54 ± 0.30	1.48 ± 1.06*	1.80 ± 0.75*
EF097112_s	1.05 ± 0.58	1.20 ± 0.76	0.81 ± 0.39	1.01 ± 0.38
PAC001064_s	1.02 ± 0.73	0.92 ± 0.50	1.18 ± 0.36	1.19 ± 0.66
AB599946_s	1.00 ± 0.59	1.09 ± 0.63	0.55 ± 0.38	2.18 ± 0.92*
Lactobacillus reuteri group	0.98 ± 1.11	0.17 ± 0.05	0.23 ± 0.18	0.88 ± 1.30
DQ777952_s	0.94 ± 1.50	0.04 ± 0.04	0.11 ± 0.12	0.03 ± 0.04
PAC001982_s	0.94 ± 1.17	1.49 ± 0.94	0.39 ± 0.60*	0.11 ± 0.12*
PAC000186_g_uc	0.93 ± 0.86	0.99 ± 0.38	0.75 ± 0.46	0.76 ± 0.41

^amean ± SD.

[#]p<0.05 vs. Con. *p<0.05 vs.LPS.

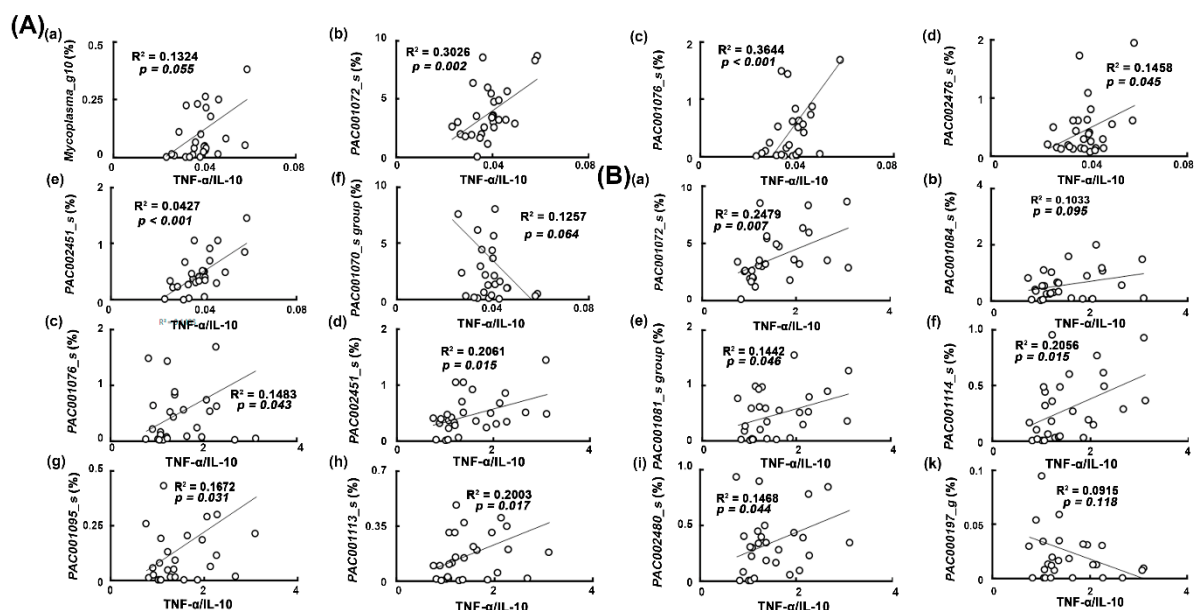


Figure S1. The correlation between gut microbiota (at the species level) and ratio of TNF-α to IL-10 expression in the healthy mice. (A) The correlation between gut microbiota (at the species level) and ratio of TNF-α to IL-10 expression in the colon. (E) The correlation between gut microbiota (at the species level) and TNF-α to IL-10 expression ratio in the spleen.

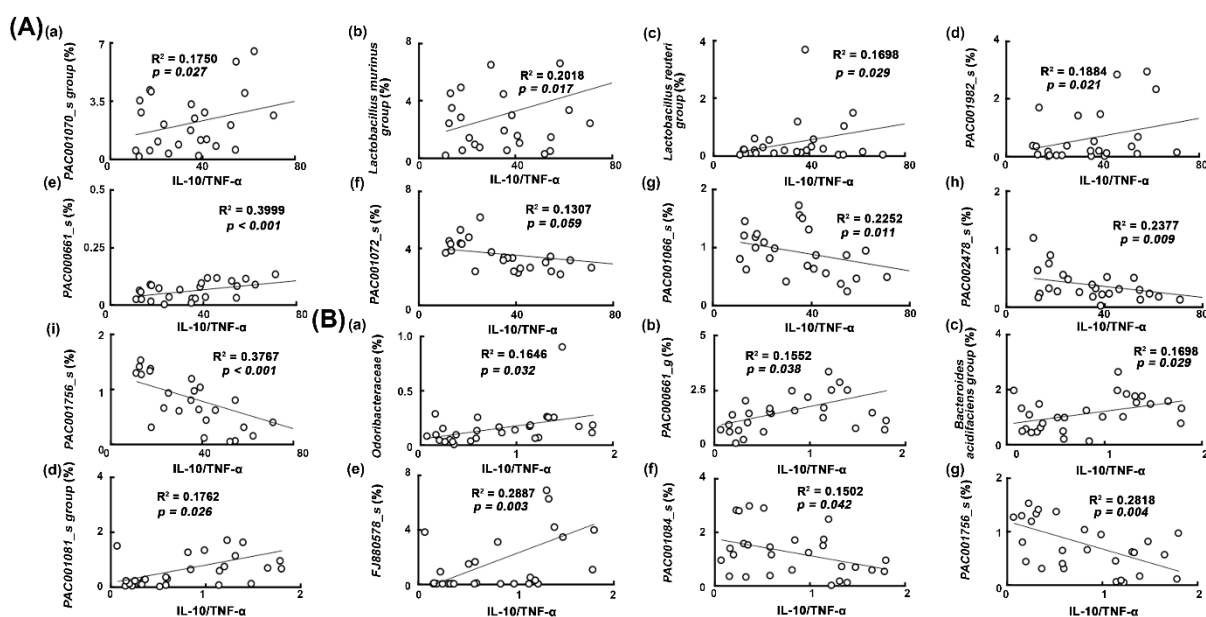


Figure S2. The correlation between gut microbiota (at the species level) and ratio of TNF-α to IL-10 expression in mice with LPS-induced systemic inflammation. (A) The correlation between gut microbiota (at the species level) and ratio of TNF-α to IL-10 expression in the colon. (E) The correlation between gut microbiota (at the species level) and TNF-α to IL-10 expression ratio in the spleen.