

Supplemental Tables

Table S1 16S rRNA gene sequencing statistics from adult (N), old (O) and L2-treated old (Oa) mice

Sample ID	Reads	OTUs	Chao1	Ace
N1	6785	1687	4277	7309
N2	9879	2145	5039	7777
N4	6574	1858	4659	7829
N5	8494	2218	5351	9217
N6	9019	1757	4344	6630
N7	6443	2051	5099	8532
O1	9814	1730	4779	9100
O2	6297	1446	4470	8120
O3	7773	1429	4072	8137
O4	7520	1700	5066	9765
O5	7186	1971	5831	11323
O7	3472	941	2925	5258
Oa1	6000	1724	5029	10228
Oa2	6565	1396	4012	7037
Oa3	6446	1957	6046	11697
Oa4	5382	1691	4724	8692
Oa5	6765	2050	6612	12813
Oa7	4829	1778	6044	13762

Table S2 The comparison of body weight, spleen index, thymus index, splenic lymphocytes proliferation and natural killer (NK) cell activity among adult (N), old (O) and L2-treated old (Oa) mice

Groups	Weight (g, day 0)	Weight (g, day 28)	Spleen index	Thymus index	Splenic lymphocytes proliferation (OD)	Natural killer (NK) cell activity (%)
N	22.61±1.2 19	24.60±1.3 88	0.0251±0.00 3	0.0153±0.0 05	0.0022±0.001	0.4387±0.109
O	35.59±1.7 53	32.87±1.3 63	0.0189±0.00 3	0.0146±0.0 05	0.1104±0.095 [#]	0.1922±0.082 [#]
Oa	33.43±4.3 25	35.01±4.7 64	0.0238±0.01 0*	0.0142±0.0 03	0.1985±0.091 ^{***}	0.2476±0.092 [#]

*p<0.05, **p<0.01 vs O groups; #p<0.01 vs N groups.

Table S3 Diversity of gut microbiota among adult (N), old (O) and L2-treated old (Oa) mice

groups	OTUs	Chao1	Ace
	mean±SD	mean±SD	mean±SD
N	1652.7±216.7	4795.8±436.2	7882.3±907.1
O	1536.2±354.2	4523.8±983.0	8617.2±2031.4
Oa	1766±228.7	5411.2±982	10704.8±2549.8

Table S4 Family-level comparison of gut microbiota among adult (N), old (O) and L2-treated old (Oa) mice

Bacteria family	O groups (n=6)	Oa groups (n=6)	N groups (n=6)
	mean±SD %	mean±SD %	mean±SD %
Bacteroidaceae	22.13 ± 15.75*	4.97 ± 4.48 ^a	6.70 ± 1.89
Lachnospiraceae	21.16 ± 4.35	27.38 ± 13.38	25.24 ± 4.05
S24-7	18.89 ± 4.20	16.22 ± 5.65	19.48 ± 3.92
Rikenellaceae	11.08 ± 5.21*	13.59 ± 8.10*	20.68 ± 3.12
Ruminococcaceae	4.54 ± 1.62	5.98 ± 3.35	5.59 ± 1.37
Prevotellaceae	11.16 ± 8.35	6.60 ± 7.55	7.37 ± 3.24
Porphyromonadaceae	1.35 ± 0.65*	2.24 ± 0.81 ^{**a}	0.71 ± 0.32
Rhodospirillaceae	0.41 ± 0.22	0.10 ± 0.076 ^{**aa}	0.38 ± 0.19
Erysipelotrichaceae	0.065 ± 0.047	0.15 ± 0.082 ^a	0.13 ± 0.12
Clostridiales_uncultured	0.34 ± 0.23	0.36 ± 0.43	0.22 ± 0.11
Lactobacillaceae	0.11 ± 0.068*	1.53 ± 1.17 ^{**aa}	0.30 ± 0.20
Peptococcaceae	0.033 ± 0.034	0.033 ± 0.031	0.075 ± 0.072
Coriobacteriaceae	0.005 ± 0.008	0.063 ± 0.055 ^a	0.02 ± 0.026
Helicobacteraceae	0.083 ± 0.18	1.86 ± 2.34 ^{*a}	0.018 ± 0.012
Alcaligenaceae	0.017 ± 0.016*	0.055 ± 0.033 ^a	0.047 ± 0.033
Family XIII Incertae Sedis	0.008 ± 0.016	0.007 ± 0.010	0.02 ± 0.027
Enterococcaceae	0.018 ± 0.033	5.45 ± 8.44	0
Streptococcaceae	0.003 ± 0.008*	0.022 ± 0.026	0.0067 ± 0.010

*, p<0.05, **, p<0.01 vs. N groups, respectively. a, p<0.05, aa, p<0.01 vs. O groups, respectively.

Table S5 The top 80 OTUs in the fecal microbiota of old (O) and L2-treated old (Oa) mice

OTU name	phylum	class	order	family	genus	species
OTU1	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_acidifaciens
OTU3	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	
OTU5	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_uncultured_Bacteroidaceae_bacterium

OTU6	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU8	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU9	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae		
OTU11	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU12	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_sp_S-18
OTU13	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU14	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus_mouse_gut_metagenome
OTU15	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_acidifaciens
OTU16	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_uncultured_Bacteroidaceae_bacterium
OTU18	Proteobacteria	Epsilonproteobacteria	Campylobacteriales	Helicobacteraceae	Helicobacter	Helicobacter_suncus
OTU19	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_uncultured_Bacteroidaceae_bacterium
OTU20	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU21	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU22	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	
OTU23	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU25	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU26	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Vagococcus	Vagococcus_sp_SSCS12
OTU29	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU32	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU34	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU35	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU36	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	
OTU37	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU38	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU39	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU40	Cyanobacteria					uncultured_rumen_bacterium_4C0d-2
OTU43	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_uncultured_Bacteroidaceae_bacterium
OTU44	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU45	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU49	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU50	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome

OTU51	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_uncultured_Bacteroidaceae_bacterium
OTU52	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU53	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae		
OTU55	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU56	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU58	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae		
OTU60	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU66	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU67	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU68	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU70	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae		
OTU72	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU73	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU74	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		Lachnospiraceae_Clostridium_sp._Clone-27
OTU77	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU78	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU79	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae		
OTU81	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		Lachnospiraceae_Clostridium_sp._Clone-27
OTU83	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU84	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU87	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU88	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	
OTU89	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	
OTU90	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU94	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		Lachnospiraceae_Clostridium_sp._Clone-27
OTU101	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Thalassospira	Thalassospira_uncultured_rumen_bacterium
OTU104	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU113	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU119	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	
OTU145	Bacteroidetes	Bacteroidia	Bacteroidales			

OTU150	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	
OTU153	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_acidifaciens
OTU167	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU176	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU185	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_uncultured_Bacteroidaceae_bacterium
OTU189	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
OTU198	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes_uncultured_Bacteroidaceae_bacterium
OTU200	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus_mouse_gut_metagenome
OTU225	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU227	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU228	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_sp_SLC1-38
OTU230	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU238	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_acidifaciens
OTU248	Bacteroidetes	Bacteroidia	Bacteroidales			S24-7_mouse_gut_metagenome
OTU249	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	Parabacteroides_uncultured_Bacteroidaceae_bacterium

Table S6 Effects of L2 treatment on top 80 OTUs in old mice

OTU name	O1	O2	O3	O4	O5	O7	Oa1	Oa2	Oa3	Oa4	Oa5	Oa7	p value
OTU1	29.8	4.68	15.9	31.1	2.84	9.1	0	0.02	1.49	1.13	3.73	0.12	0.00868
OTU3	1.25	6.21	0.44	1.42	2.21	1.38	1.48	1.63	0.02	0.17	2.1	0.06	0.10427
OTU5	2.47	0.65	0.98	1.72	2.92	2.45	0.05	0.47	0.73	0.15	0.1	0.17	0.00105
OTU6	1.18	14	3.46	0.51	5.54	0.72	1.12	5.67	0.16	1.34	1.01	1.57	0.15345
OTU8	1.31	3.33	5.78	1.06	2.16	2.25	2.12	0.02	0.14	1.11	0.04	1.24	0.01947
OTU9	1.55	2.21	4.54	0.74	0.99	4.69	0.17	0.53	0.06	0.24	0.21	0.97	0.00832
OTU11	0.65	2.49	2.12	0.33	3.19	0.52	1.12	4.22	0.11	0.58	0.4	0.7	0.32806
OTU12	0.82	0.33	0.28	0.23	0.11	0.4	0.05	0.08	0.19	0.07	0.13	0.5	0.07067
OTU13	2.83	3.43	1.17	0	0	1.15	0	0	0.22	0.19	0.87	3.15	0.19376
OTU14	0.06	0.08	0.01	0.01	0.01	0.09	0.23	0.41	0.26	0.19	0.46	0.37	7.2E-05
OTU15	0.3	1.76	1.4	0.35	0.47	0.35	0.02	0.76	5.93	0.17	0.25	0.54	0.30702
OTU16	0.53	0.17	0.08	1.42	0.35	0.66	0.48	0.17	1.92	1.75	0.71	0	0.22579
OTU18	0	0	0	0	0	0	0	0.02	1.37	5.17	0.13	1.33	0.06511
OTU19	0	0	0	0	0.03	0	0.02	0.97	0.74	6.15	1.61	0.12	0.05989
OTU20	0.01	1.78	0.51	3.16	0.13	0	0	0.17	0.79	2.32	0.75	0	0.34589

OTU21	0.28	1.16	0	0	1.59	0.49	2.35	0.46	0.05	1.93	1.52	2.09	0.05555
OTU22	2.8	1.41	0.13	0.59	0.26	0.95	0	0.09	0	0	0.19	0.66	0.03233
OTU23	0.63	1.86	0.26	0	0.49	2.74	0	0	0	0	0	0	0.02279
OTU25	0.43	0.1	0.58	0	0.46	4.09	1.32	0.43	0.31	0.61	0.37	1.57	0.40092
OTU26	0	0	0	0	0	0	0.03	13.8	12.5	0	0	0	0.07242
OTU29	0.68	0.78	0.39	1.8	0.01	0.12	0	0	0.43	0.97	0.47	0	0.16441
OTU32	0.47	0.71	1.38	0.24	1.11	1.04	0	0.02	0.42	0.04	0.07	0.02	0.00144
OTU34	0	0	0	0	0	0	0.18	0.32	5.9	0	0.01	0.29	0.13511
OTU35	0.16	0	0	0	0.4	0.09	0.02	0.03	1.49	0.06	0.87	0.6	0.07083
OTU36	0.33	0.33	1.09	0.07	2.32	0.03	0.42	0	0.05	0.04	0	0.14	0.07028
OTU37	0	0	0	0	2.02	0.55	0.02	2.77	0.14	0.11	4.07	0.17	0.17218
OTU38	0	0	0	0	4.16	1.41	0.02	3.78	0.33	0.02	2.19	0.17	0.43659
OTU39	4.34	0	8.66	0	0	0	0.23	0	0	0.02	0.95	0.12	0.10992
OTU40	0.69	0.44	0.64	0.15	0.93	4.61	0.05	0	0.02	0	0	0	0.05014
OTU43	0.96	0.03	0.77	0	3.98	0.98	1.05	0.52	0.51	0.15	1.17	1.24	0.29582
OTU44	0.79	0.02	0	0.08	0	0	1.37	0	0.02	0.06	0	0.62	0.23701
OTU45	0	0	0	0	0	0	1.25	0.69	0.92	0.72	0.24	0	0.00322
OTU49	0	0.03	0	0	0	0	0	0.02	0.26	0	0	3.58	0.15211
OTU50	0.02	0.19	0.26	1.62	0.74	3.74	0.63	0	0	1	0.89	0.25	0.16087
OTU51	0	0	0	0	0	0.03	12.2	0.75	0	0	0.01	0	0.15457
OTU52	2.97	0.52	0.5	1.48	0.08	0.52	3.12	0.02	0.03	1.28	0.03	0.04	0.35434
OTU53	0	0	0	0	0	0	0	5.09	2.25	0	0	0	0.09176
OTU55	0.06	0.81	0.35	1.26	0	0.4	0	0	0.23	0.85	0.37	0	0.17179
OTU56	0.01	0	0	0.7	0.1	1.07	0.02	0.02	0.99	0	0.06	0.02	0.30706
OTU58	0.54	1.27	0.35	0.64	0.35	0.75	0.38	0	0.05	0.11	0.25	0.33	0.00676
OTU60	0.32	0.44	0.76	0.13	0.82	0.35	0.02	0	0.22	0.02	0.04	0.04	0.00237
OTU66	0.23	0.29	0.87	0.11	0.51	0.37	0.02	0	0.26	0	0.04	0.04	0.00846
OTU67	0.17	1.78	0.48	0.05	1.36	0.12	0.17	1.01	0	0.17	0.01	0.33	0.14272
OTU68	0	0	0	0	1.31	0.4	0.07	2.68	0.11	0.06	1.98	0.19	0.15482
OTU70	0	0	0	0	0.47	0.17	0.08	0.06	0.39	0.98	1.67	0.02	0.08065
OTU72	0.15	0.17	0	2.82	0.06	0.46	0.33	0	0.02	0	1.33	0.02	0.26218
OTU73	0	0	0	0	0	0	0.13	0.17	0.16	0.59	0.68	0.06	0.01023
OTU74	0	0.03	0	0	0.22	2.48	0	0	0.42	0	0.9	0.02	0.30226
OTU77	0	0.68	0	1.21	0.18	0.03	0.15	0	0.12	0	0.15	0	0.10059
OTU78	0.31	0.1	0.18	0.29	0.13	0.17	0.05	0.14	0.23	0.07	0.28	0.6	0.35671
OTU79	0.2	0.76	0.46	0.36	0.28	0.23	0.15	0	0.02	0.06	0.06	0.12	0.00257
OTU81	0	0.03	0	0	0	0	0	0	0	3.14	0.99	2.11	0.04257
OTU83	0	0	0	0	0	0	0.1	0.26	0.31	0.69	0.47	0.19	0.00157
OTU84	0	0.79	0	0	0.74	1.21	0	0.02	0.11	0	0.1	0.02	0.04158
OTU87	0.13	0.03	0.08	0.13	0.13	0.09	0.67	0.02	0.06	0.59	0.27	0.17	0.05626
OTU88	0.05	0.22	0.15	0.01	0.18	0.14	0.15	0.41	0.26	0.02	0.18	0.06	0.22288

OTU89	0	0	0	0	0	0	0.28	0.5	2.19	0.54	1.21	1.1	0.00334
OTU90	0.38	0.03	0.03	0	0.71	0.12	0.02	0	0.25	0	0.1	0.12	0.1591
OTU94	0.01	0	2.73	0.03	0.13	0.2	0.87	0	0	0	0.1	0	0.23271
OTU101	0.26	0.4	0.15	0.32	0.17	0.17	0.15	0	0.02	0.02	0.1	0.06	0.00125
OTU104	0	0.1	0.31	0	0.22	0.09	0	0	0.03	0.02	1.35	0.06	0.298
OTU113	0	0	0.18	0	0	0	0	0	0	0	0.13	1.93	0.17451
OTU119	0	0	0	0	0	0	0.13	4.42	0.05	0	0.01	0.02	0.15732
OTU145	0	0	0	0	0	0	0	2.8	0	0	0.1	0.17	0.14538
OTU150	0.05	0.38	0.13	0	0.25	0.17	0	0.09	0.09	0.02	0.33	0.99	0.29971
OTU153	0.62	0	0.24	0.55	0.03	0.2	0	0	0.06	0.04	0.13	0.02	0.02878
OTU167	0	0	0	0	0	0	3.87	0	0	0	0	0.27	0.1523
OTU176	0.17	0.98	0.08	0	0.03	0.06	0	0	0.05	0.02	0.25	0	0.1601
OTU185	0.33	0	0.19	0.03	0.65	0.14	0	0	0.02	0.13	0.07	0.02	0.04914
OTU189	0.75	0	1.49	0	0	0	0.03	0	0	0.02	0.13	0	0.10473
OTU198	0	0.64	0	0	0.56	0.46	0	0	0	0	0	0.39	0.08423
OTU200	0	0	0	0	0.01	0	0.08	1.61	1.3	0	0	0.14	0.05576
OTU225	0	0	0	0	0.21	0.12	0.03	0	0.11	0.39	0.78	0.02	0.1147
OTU227	0	0	0	0	2.05	0	0	0	0	0	0	1.55	0.42588
OTU228	0	0	0	0	0	0	0	0.15	0	2.21	0	0	0.15248
OTU230	0	0.02	0	0	0.89	0	0	0	0.23	0	0	1.97	0.27844
OTU238	0	0.02	0.03	0	0	0	0.17	0.64	0.7	0	0.06	0.19	0.02148
OTU248	0	0	0	0	0	0	0.42	1.28	0	0	0.13	0	0.08467
OTU249	0	0	0	0	0	0	1.35	0.46	0	0	0	0	0.10298

P<0.05 are highlighted by red.