

Supplementary information

Phospholipid-rich krill oil promotes intestinal health by strengthening beneficial gut microbial interactions in an infectious colitis model

Fang Liu*, Allen D. Smith, Thomas T. Y. Wang, Quynhchi Pham, Pengfen Hou

Lumei Cheung, Haiyan Yang*, Robert W. Li*

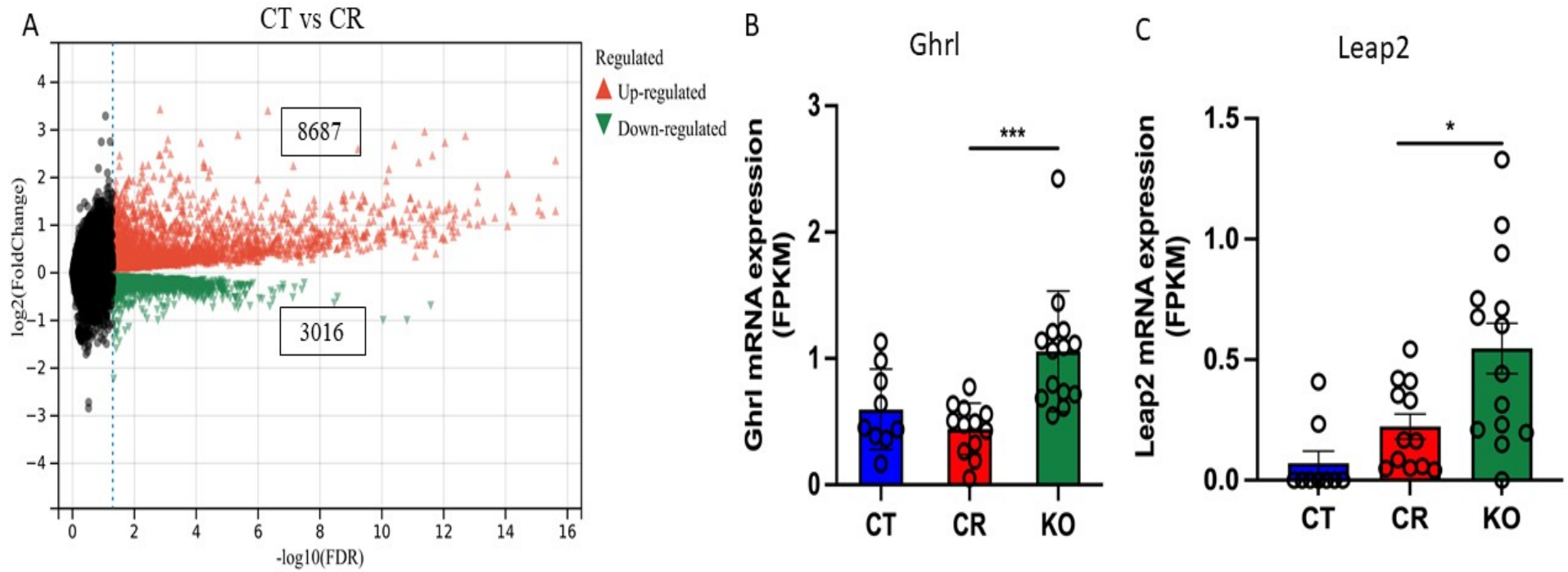
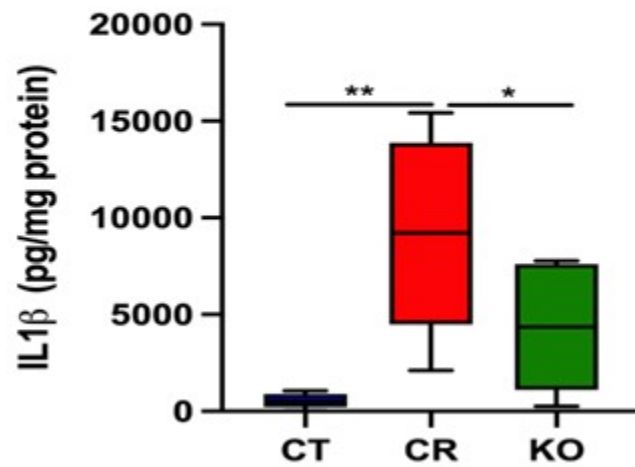


Figure S1. *Citrobacter rodentium* infection induced a significant alteration in the intestinal transcriptome. A. the volcano plot showing the number of genes significantly regulated by infection. Red: upregulated; Green: downregulated. FDR: False discovery rate. Krill oil increased the expression of both ghrelin receptor (Ghrl, B) and its endogenous antagonist Leap2 (C) by the same extent. CT: Uninfected control. CR: *Citrobacter rodentium* infected mice supplemented with PBS for 26 days. KO: *Citrobacter rodentium* infected mice supplemented with krill oil at 40 mg/Kg BW/day for 26 days. The *P* value was calculated using the Wilcoxon Rank Sum Test (CR vs KO). * $P < 0.05$; *** $P < 0.001$.

A.



B.

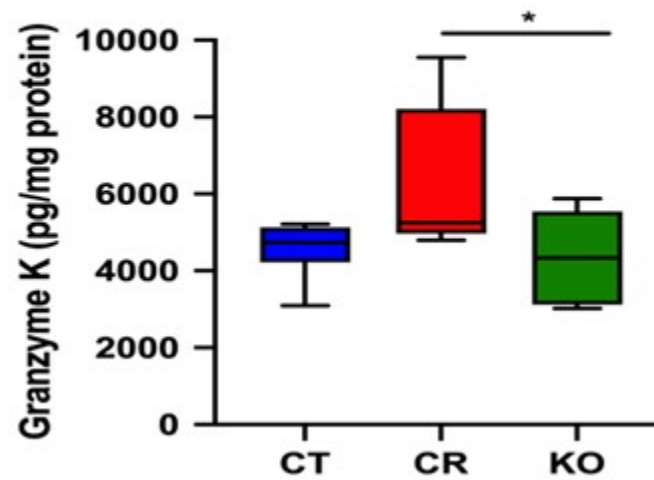


Figure S2. The IL1 β and granzyme K protein levels in the colon homogenate as detected by ELISA assay. A: IL1 β ; B: Granzyme K (GzmK). The protein level, as expressed as pg per mg total protein, was derived from the standard curve in the ELISA kit. CT: Uninfected control. CR: *Citrobacter rodentium* infected mice supplemented with PBS for 26 days. KO: *Citrobacter rodentium* infected mice supplemented with krill oil at 40 mg/Kg BW/day for 26 days. *: $P < 0.05$; **: $P < 0.01$.

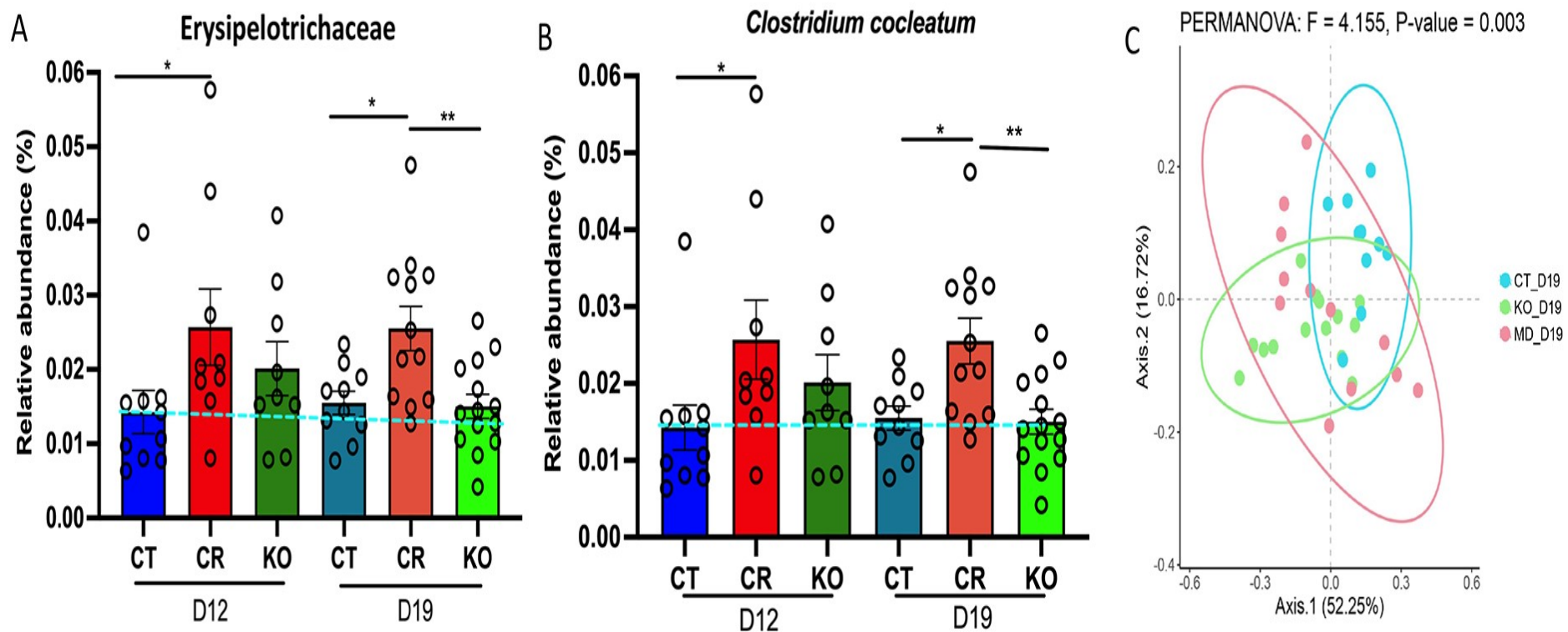


Figure S3. *Citrobacter rodentium* infection induced a marked change in the gut microbial community. CT: Uninfected control. CR: *Citrobacter rodentium* infected mice supplemented with PBS for 26 days. KO: *Citrobacter rodentium* infected mice supplemented with krill oil at 40 mg/Kg BW/day for 26 days. The *P* value was calculated using the Wilcoxon Rank Sum Test (CR vs KO). A: The relative abundance of an unassigned genus in the family *Erysipelotrichaceae*. B: *Clostridium cocleatum*. C: Beta-diversity analyzed using PERMANOVA. D12: 12 days post-infection. D19: 19 days post infection. *: $P < 0.05$; **: $P < 0.01$.

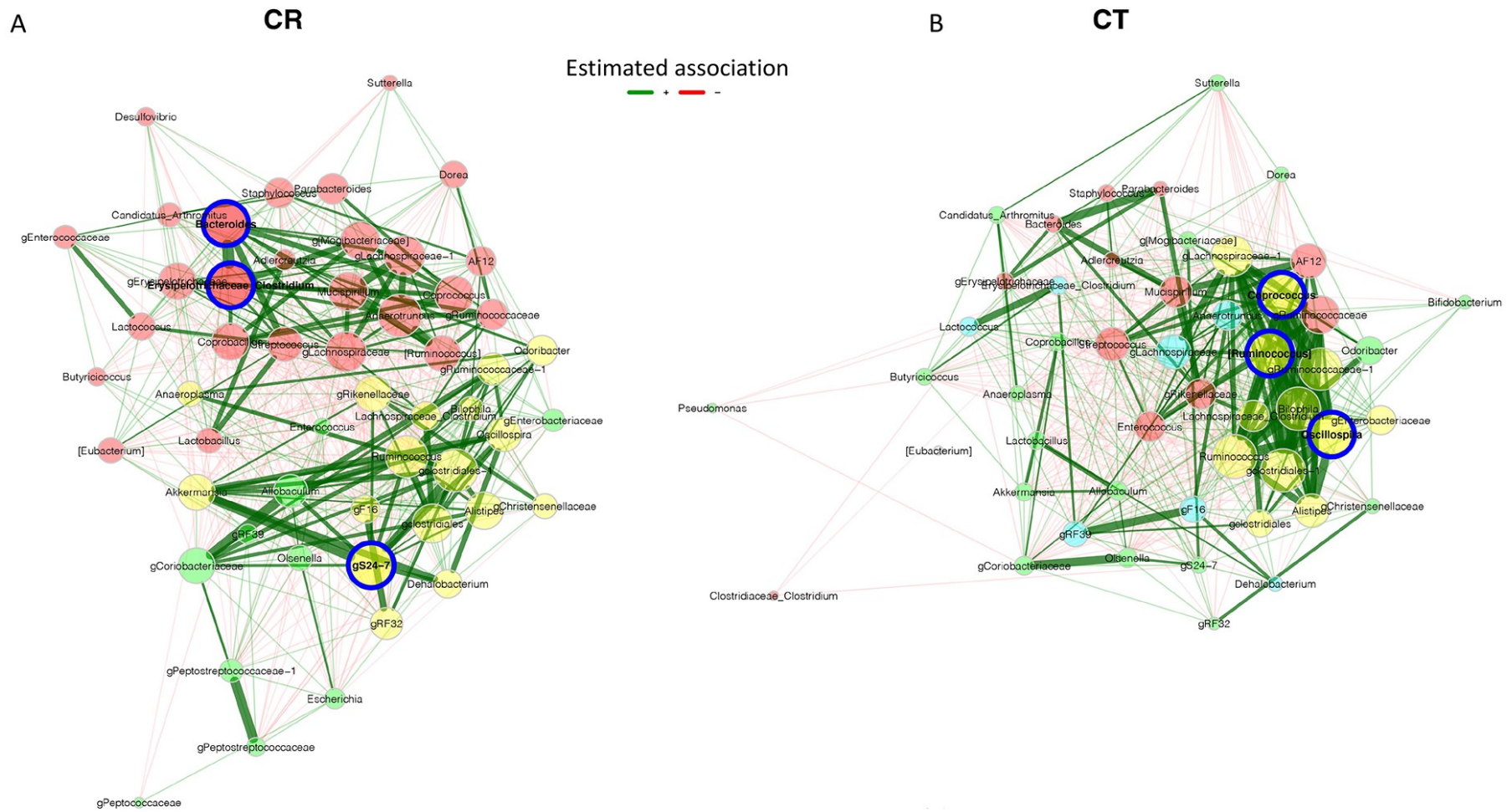


Figure S4. Microbial interactions predicted by the NetCoMi algorithm in gut microbial communities. The node color represents the module. The edge color denotes the direction of the microbial interactions (positive or negative) between nodes in each network. CT: Uninfected mice. CR: *Citrobacter rodentium* infected mice. The bold font with a colored border represents the hub taxa. The width of the edge denotes the strength of each interaction.

Differential network

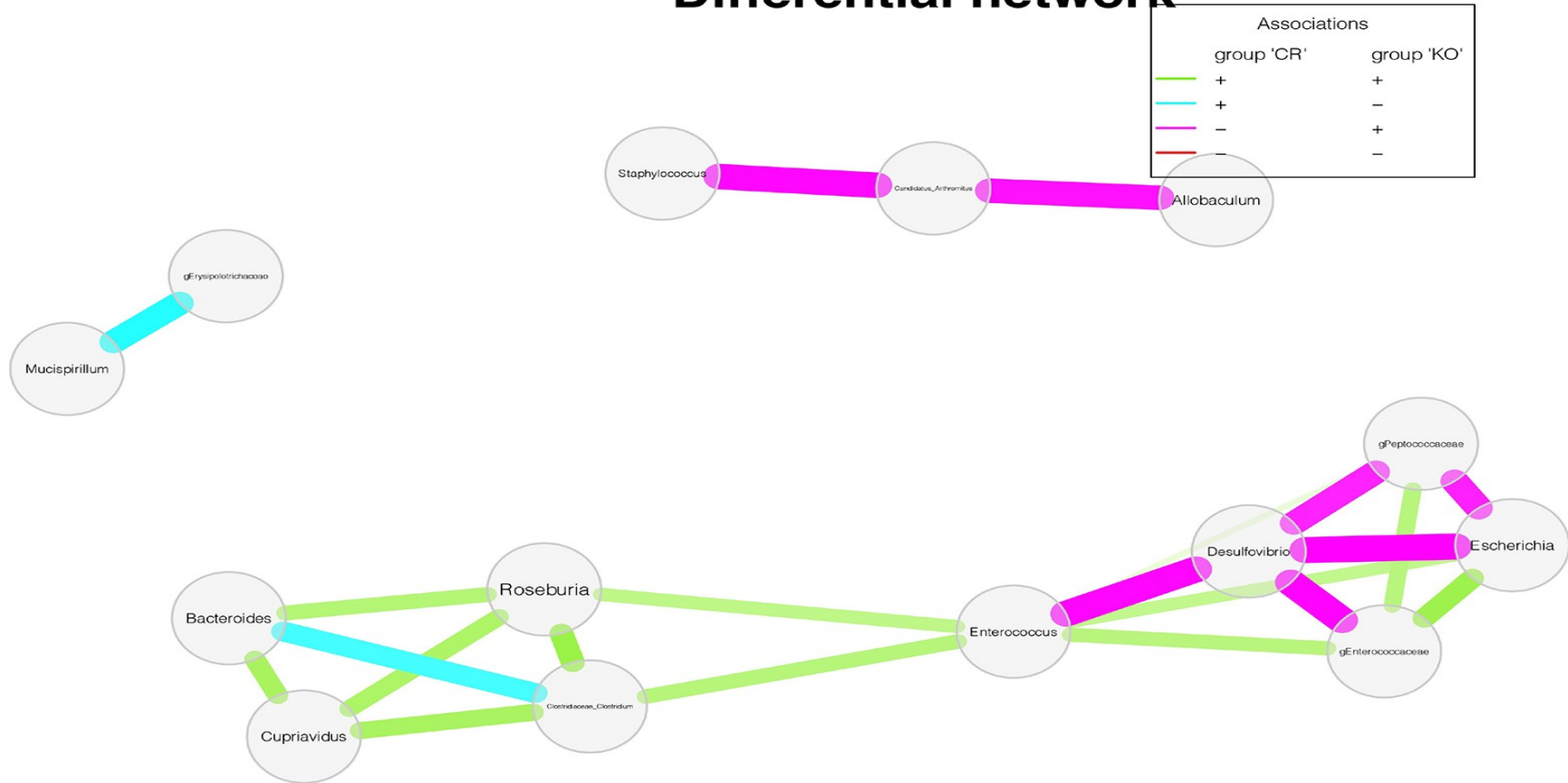


Figure S5. Differential network analysis. The color of the edges represents the differential interactions (associations) between the nodes as specified in the legend.

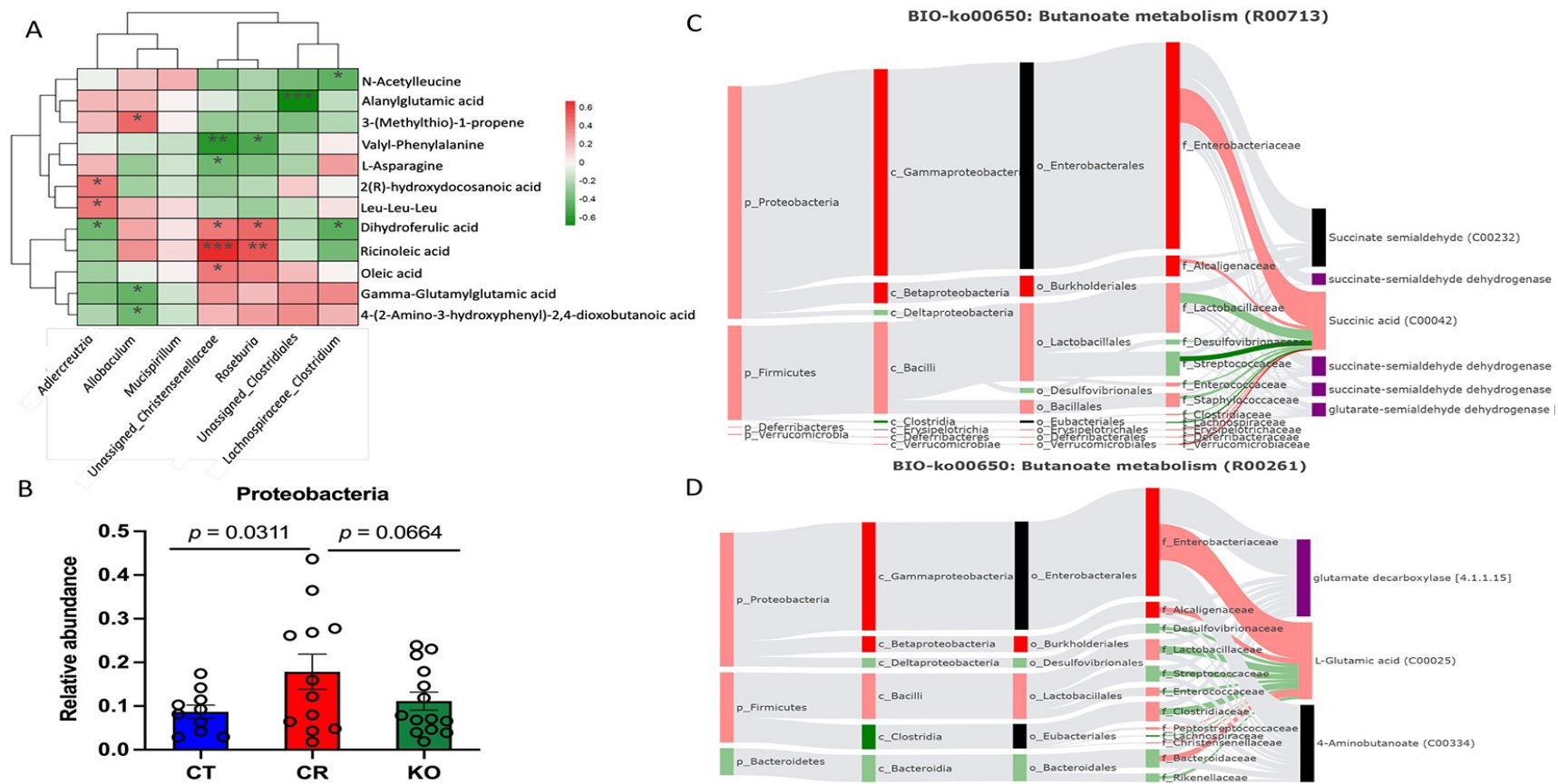


Figure S6. The correlation between select gut luminal metabolites and microbial genera modulated by krill oil in infectious colitis mice. **A:** The correlation between select metabolites and microbial taxa detected using Spearman rank correlation. **B:** Krill oil supplementation reduced the abundance of a pro-inflammatory phylum, Proteobacteria, which was predicted to be involved in multiple metabolic pathways by the MetOrigin algorithm. **C:** Association networks between metabolites and gut microbiota features (taxa) in the butyrate metabolism pathways (KEGG ko00650) based on the origin of metabolites using the Sankey network algorithm. **C:** The metabolic reaction R00713 and **D:** The metabolic reaction R00261. The red/green bands indicate the direction of the statistical correlations (red = positive and green = negative) and the dark color represents the statistically significant correlations ($P < 0.05$).

Table S1. Composition analysis of krill oil used in the study.

Composition	Unit	Content
Total phospholipids	g/Kg KO	500.0
Total Omega-3	g/Kg KO	260.0
EPA	g/Kg KO	160.0
DHA	g/Kg KO	60.0
Choline	g/Kg KO	59.8
Water	g/Kg KO	10.0
Ethanol	g/Kg KO	9.7
Astaxathin (esterified)	mg/Kg KO	529.0
Peroxide	mEq peroxide/Kg	0.2
Viscosity	cP	842.0
Total aerobic count	colony-forming units (cfu)/g KO	10.0
Total coliform	colony-forming units (cfu)/g KO	9.0
E. coli	colony-forming units (cfu)/g KO	0.0
Salmonella	colony-forming units (cfu)/g KO	0.0

Table S2. Microbial taxa with a significantly altered abundance. CT: Uninfected control. CR: *Citrobacter rodentium* infected mice supplemented with PBS for 26 days. KO: *Citrobacter rodentium* infected mice supplemented with krill oil at 40 mg/Kg bodyweight/day for 26 days. The number denotes mean relative abundance \pm SD. LDA: Linear Discriminant Analysis. The *P* value was calculated using the Wilcoxon Rank Sum Test.

Genus	CT	CR	KO	LDA	<i>P</i> value	Significance
<i>Adlercreutzia</i>	0.41 \pm 0.14	0.65 \pm 0.30	0.53 \pm 0.19	3.48	0.0210	CT vs CR
<i>Allobaculum</i>	3.89 \pm 3.89	11.23 \pm 8.31	16.03 \pm 9.22	4.51	0.0479	CT vs CR
<i>Clostridium</i>	0.00 \pm 0.01	0.00 \pm 0.00	0.62 \pm 1.75	3.54	0.0011	CR vs KO
<i>Lachnospiraceae_Clostridium</i>	0.34 \pm 0.15	0.15 \pm 0.09	0.14 \pm 0.14	3.47	0.0024	CT vs CR
<i>Mucispirillum</i>	0.31 \pm 0.44	0.66 \pm 0.68	0.53 \pm 0.33	3.39	0.0347	CT vs CR
<i>Odoribacter</i>	1.35 \pm 0.38	0.73 \pm 0.89	0.69 \pm 0.44	3.52	0.0147	CT vs CR
<i>Roseburia</i>	0.00 \pm 0.00	0.00 \pm 0.00	0.23 \pm 0.30	3.12	0.0025	CR vs KO
<i>Sutterella</i>	0.01 \pm 0.03	0.28 \pm 0.36	0.36 \pm 0.55	3.44	0.0017	CT vs CR
<i>Unassigned Erysipelotrichaceae</i>	1.55 \pm 0.50	2.55 \pm 1.03	1.50 \pm 0.61	3.78	0.0176	CT vs CR & CR vs KO
<i>Unassigned_Christensenellaceae</i>	0.03 \pm 0.02	0.04 \pm 0.04	0.17 \pm 0.09	3.05	0.0001	CR vs KO
<i>Unassigned_Clostridiales</i>	4.53 \pm 3.24	1.80 \pm 1.11	2.42 \pm 1.57	4.10	0.0409	CT vs CR
<i>Unassigned_Clostridiales_1</i>	6.75 \pm 2.73	3.22 \pm 2.04	3.63 \pm 1.46	4.21	0.0056	CT vs CR
<i>Unassigned_Enterobacteriaceae</i>	0.00 \pm 0.01	0.43 \pm 0.61	0.09 \pm 0.15	3.38	0.0049	CT vs CR
<i>Unassigned_Lachnospiraceae</i>	5.02 \pm 1.58	3.57 \pm 1.24	3.47 \pm 1.41	3.84	0.0479	CT vs CR
<i>Unassigned_Mogibacteriaceae</i>	0.13 \pm 0.06	0.34 \pm 0.17	0.21 \pm 0.14	3.50	0.0024	CT vs CR
<i>Unassigned_Peptostreptococcaceae</i>	0.00 \pm 0.00	0.23 \pm 0.43	0.16 \pm 0.27	3.28	0.0053	CT vs CR
<i>Unassigned_Peptostreptococcaceae</i>	0.00 \pm 0.00	0.09 \pm 0.19	0.04 \pm 0.11	3.38	0.0117	CT vs CR
<i>Unassigned_Rikenellaceae</i>	3.87 \pm 1.58	1.77 \pm 1.45	1.54 \pm 0.63	3.96	0.0046	CT vs CR

Table S3. Fecal metabolites significantly affected by krill oil supplementation in infectious colitis mice induced by *Citrobacter rodentium*. The number denotes the normalized ion counts (mean \pm SD). CT: Uninfected control. CR: *Citrobacter rodentium* infected mice supplemented with PBS for 26 days. KO: *Citrobacter rodentium* infected mice supplemented with krill oil at 40 mg/Kg bodyweight/day for 26 days. The *P* value was calculated using the Wilcoxon Rank Sum Test (CR vs KO).

Metabolite	CT	CR	KO	<i>P</i> value
2(R)-hydroxydocosanoic acid	1654.8 \pm 1675.1	2082.5 \pm 1031.9	667.9 \pm 1095.6	0.0079
3-(Methylthio)-1-propene	1.5 \pm 0.2	2.3 \pm 1.1	1.6 \pm 0.6	0.0197
4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	75.2 \pm 17.8	28.3 \pm 20.4**	55.9 \pm 32.3	0.0357
4-Pyridoxic acid	88.7 \pm 80.5	135.1 \pm 73.7	40.40 \pm 0.4	0.0028
5-Hydroxy-L-tryptophan	27.6 \pm 14.0	23.5 \pm 19.2	42.8 \pm 24.3	0.0159
Acetanilide	414.0 \pm 257.2	150.2 \pm 185.0*	562.7 \pm 372.1	0.0079
Alanylglutamic acid	26.1 \pm 15.2	74.9 \pm 58.9*	40.5 \pm 16.9	0.0357
Alanyltryptophan	3.0 \pm 0.4	2.7 \pm 0.5	3.4 \pm 0.7	0.0159
Dihydroferulic acid	1.7 \pm 0.9	2.3 \pm 1.5	3.4 \pm 0.9	0.0127
Gabapentin	2.1 \pm 0.3	1.9 \pm 0.4	2.5 \pm 0.5	0.0159
Gamma-glutamylglutamic acid	9.2 \pm 6.8	2.1 \pm 1.6*	6.1 \pm 5.3	0.0295
Gamma-glutamylleucine	240.8 \pm 249.0	433.8 \pm 484.8	119.1 \pm 154.2	0.0159
Histamine	12.4 \pm 8.9	43.6 \pm 23.7**	21.2 \pm 18.5	0.0295
L-Asparagine	251.2 \pm 285.8	181.6 \pm 152.6	34.3 \pm 44.8	0.0079
Leu-Leu-Leu	0.4 \pm 0.1	1.1 \pm 1.0**	0.4 \pm 0.1	0.0062
L-iditol	80.1 \pm 49.6	36.7 \pm 43.0	98.7 \pm 65.2	0.0159
N-acetylleucine	122.0 \pm 95.7	336.3 \pm 459.1	95.4 \pm 83.4	0.0242
Oleic acid	17.5 \pm 15.1	12.0 \pm 2.8	14.3 \pm 1.6	0.0430
Phenol	427.5 \pm 257.6	255.1 \pm 204.1	580.0 \pm 369.0	0.0357
Phenylalanylglutamic acid	105.5 \pm 89.1	175.6 \pm 144.1	70.6 \pm 48.3	0.0430
Ricinoleic acid	30.9 \pm 15.8	23.7 \pm 7.3	94.7 \pm 27.1	0.0000
Sulfolithocholic acid	0.5 \pm 0.5	0.4 \pm 0.2	0.3 \pm 0.1	0.0357
Sulfolithocholyglycine	142.5 \pm 266.9	20.5 \pm 11.4	31.0 \pm 11.9	0.0430
Valyl-histidine	4.6 \pm 3.6	5.4 \pm 4.3	2.0 \pm 0.7	0.0159
Valyl-phenylalanine	11.1 \pm 2.4	11.8 \pm 6.8	6.3 \pm 1.9	0.0101

Note: *: *P* < 0.05 (CT vs CR); **: *P* < 0.01 (CT vs CR)