Supporting Information for

Gut microbiota mediated the protective effects of tuna oil on collagen-induced arthritis in mice

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Table S1. Compositions of fatty acids in tuna fish oil detected via GC-MS.

Compound	Content (%)
Methyl tetradecanoate	3.60
Pentadecanoic acid	1.18
Hexadecanoic acid	16.94
9-Hexadecenoic acid	5.26
7-methyl-6-hexadecenoic acid	2.60
Heptadecanoic acid	1.31
Methyl 9-heptadecenoate	0.59
Methyl stearate	5.98
Methyl-10-*trans*,12-*cis*-octadecadienoate	1.22
Methyl-9,12-heptadecadienoate	0.67
Methyl-stearidonate	1.18
Methyl-9-eicosenoate	1.29
5,8,11,14-Eicosatetraenoic acid	3.67
EPA	7.43
n-Hexadecanoic acid	1.38
Methyl-4,7,10,13,16-docosapentaenoate	4.05
Methyl-6,9,12,15,18-heneicosapentaenoate	1.37
DHA	40.28

Table S2. Scoring system for arthritis severity

Severity score	Degree of inflammation
0	No evidence of erythema and swelling
1	Erythema and mild swelling confined to the tarsals or ankle joint
2	Erythema and mild swelling extending from the ankle to the tarsals
3	Erythema and moderate swelling extending from the ankle to metatarsal joints
4	Erythema and severe swelling encompass the ankle, foot and digits, or ankylosis of the limb

Table S3. Primers used in this study

Gene	Forward primer	Reverse primer
TNF-α	CCCTCCTAACCCGTTTTGCT	GACGTGGAACTGGCAGAAGA
IL-1β	ATGTATGCCTACTCGTCGGG	GAGGTCGGTCTCACTACCTG
IL-10	TGAGGCGCTGTCATCGATTT	ATGGCCTTGTAGACACCTTGG
IL-17A	TGTGTCAATGCGGAGGGAAA	TGAAAGTGAAGGGGCAGCTC
ALP	AAGACGTACAACACCAACGC	TGAAGCAGGTGAGCCATAAG
RunX2	CCGTTACTTAGAGCAGTCTGTCGTC	AGAAGAGGAAGAGGAAGAGGAAGAGG
BMP2	ATCCAGTCTTGCCGCCTCCA	TCTTCGCCTCCTCCTCCTTCTC
Wnt1	CGAAACCGCCGCTGGAACT	GGAGGTGATTGCGAAGATAAACG
catenin	CAGGAATGAAGGCGTGGCAACA	GGCACCAATGTCCAGTCCAAGAT
Sclerostin	GTGCAAGTGCAAGCGCCTCA	GCTCCGCCTGGTTGGCTTTG
TRAP	CCAGCGACAAGAGGTTCC	AGAGACGTTGCCAAGGTGAT
cath-K	CGAAAAGAGCCTAGCGAACA	TGGGTAGCAGCAGAAACTTG
CTR	TGGTTGAGGTTGTGCCCA	CTCGTGGGTTTGCCTCATC
OPG	GACCCCAGAGCGAAACACG	GGCACAGCAAACCTGAAGAA
RANKL	TGAAACTCACAGCCCTCT	TAAGCATCGGAATACCTC
TRAF6	GATCGGGTTGTGTGTGTCTG	AGACACCCCAGCAGCTAAGA
NF-κb	CCACAAGACAGAAGCTGAAG	AGATACTATCTGTAAGTGAACC
Claudin-1	CCCTTCAGCAGAGCAAGGTT	TAGGGCAACCAAGTGCCTTT
Claudin-2	AACAGCCCAAAGCCAAGAGT	CCCTGATGGGTTGGTCTTCC
Occludin	AGACCTGATGAATTCAAACCCAAT	GTTTAGGCTTCCTCCAAAGAGC
ZO-1	AGCAAGCCTTCTGCACATCT	TCGGGTTTTCCCTTTGAAGAGT
β-actin	TGTGATGGTGGGAATGGG	TGCCAGATCTTCTCCATGTC

Table S4. Bacterial taxonomic profiling at the phylum level. Data are represented as the mean.

	Control (%)	CIA (%)	LD (%)	MD (%)	HD (%)	FMT-control (%)	FMT-CIA (%)	FMT-HD (%)
Firmicutes	50.90	54.76	63.50	48.71	49.39	82.43	63.64	75.78
Bacteroidetes	29.54	38.62	18.99	37.54	35.25	9.43	24.76	14.28
Proteobacteria	4.67	1.31	7.16	1.49	1.11	4.68	6.30	5.01
Actinobacteria	1.40	1.39	2.81	1.73	1.03	1.31	3.33	1.65
Others	13.49	3.91	7.54	10.53	13.23	2.15	1.97	3.27

Table S5. Bacterial taxonomic profiling at the order level in tuna fish oil-treated groups. Data are represented as the mean.

	Control (%)	CIA (%)	LD (%)	MD (%)	HD (%)
Bacteroidales	27.15	42.63	22.43	35.70	45.30
Clostridiales	39.33	14.79	18.19	32.10	14.17
Lactobacillales	8.47	18.87	34.76	10.05	16.08
Saccharibacteria genera incertae sedis	4.38	3.09	5.67	6.50	3.24
Bacteria unclassified	2.19	3.02	1.81	3.60	2.78
Campylobacterales	1.86	0.54	4.50	0.25	0.39
Bacilli unclassified	1.28	2.53	3.07	1.99	10.70
Bacteroidetes unclassified	1.43	1.78	1.04	1.57	1.47
Coriobacteriales	1.51	1.33	2.50	1.64	0.91
Desulfovibrionales	1.89	0.91	0.57	0.99	0.55
Others	10.52	10.50	5.48	5.61	4.42

Table S6. Forty-six key OTUs were identified via RDA in mice that received TO treatments. The red, blue, yellow, and green indicate OTUs that are altered in CIA mice and then restored by three, two, one, and zero groups of TO treatments, respectively. Data are represented as the mean.

	Control (%)	CIA (%)	LD (%)	MD (%)	HD (%)	Genus	Phylum
OTU4598	0.0016	0.0019	0.0093	0.0013	0.0023	*Bacteroidales* unclassified	Bacteroidetes
OTU2906	0.0017	0.0018	0.003	0.0026	0.0032	*Bacteria* unclassified	Bacteria unclassified
OTU3401	0.001	0.0007	0.0015	0.0017	0.0009	*Bacteroidetes* unclassified	Bacteroidetes
OTU813	0.0016	0.0053	0.0032	0.0057	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU899	0.0004	0.0015	0.0008	0.0002	0	*Bacteroidales* unclassified	Bacteroidetes
OTU2393	0.0021	0.0078	0.0028	0.0072	0.0001	*Bacteroidetes* unclassified	Bacteroidetes
OTU1188	0.0027	0.0039	0.0039	0.0026	0.0004	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU2630	0.0005	0.0014	0.0022	0.0017	0.0001	*Bacteria* unclassified	Bacteria unclassified
OTU229	0.0003	0.0052	0.0005	0.0067	0.001	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU400	0.002	0.0014	0.0014	0.0166	0.0003	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU1008	0.0005	0.0011	0.0022	0.0025	0.0001	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU600	0.0001	0.0023	0.0001	0.0041	0	*Barnesiella*	Bacteroidetes
OTU582	0.0009	0.0012	0.0004	0.0041	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU2324	0.0011	0.0007	0.0013	0.0045	0	*Clostridiales* unclassified	Firmicutes
OTU830	0.0007	0.0007	0.0009	0.0003	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU2246	0.0006	0.0055	0.0019	0.0015	0	*Bacteroidales* unclassified	Bacteroidetes
OTU990	0.0003	0.0052	0.001	0.0023	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU567	0.0003	0.0017	0.0009	0.0004	0	*Barnesiella*	Bacteroidetes
OTU352	0.0004	0.0015	0.0005	0.0009	0	*Bacteroides*	Bacteroidetes

OTU762	0.0009	0.0046	0.0011	0.0006	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU715	0.0006	0.0012	0.0008	0.0002	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU362	0.0002	0.0013	0.0008	0.0004	0.0003	*Bacteroidales* unclassified	Bacteroidetes
OTU922	0.0003	0.0009	0.0009	0.0003	0.0001	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU3489	0.0008	0.0017	0.0003	0.002	0.002	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU3242	0.001	0.0008	0.0006	0.0012	0.0006	*Bacteroidetes* unclassified	Bacteroidetes
OTU3712	0.0007	0.0005	0.001	0.001	0.0009	*Bacteroidales* unclassified	Bacteroidetes
OTU2152	0.0006	0.001	0.0001	0.0002	0.0001	*Lachnospiraceae* unclassified	Firmicutes
OTU644	0.0002	0.0008	0.0002	0.0002	0.0001	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU2578	0.002	0.0007	0.0012	0.0001	0.0001	*Bacteroidales* unclassified	Bacteroidetes
OTU865	0.0018	0.0006	0.0004	0.0005	0.0001	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU2717	0.0007	0.0005	0.0004	0.0001	0.0001	*Ruminococcaceae* unclassified	Firmicutes
OTU1900	0.0041	0.0039	0.0006	0.0033	0.0001	*Lachnospiraceae* unclassified	Firmicutes
OTU720	0.0013	0.0022	0.0007	0.0008	0.0001	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU500	0.0003	0.0013	0.0006	0.0007	0.0001	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU1992	0	0.0008	0.0003	0.0001	0.0003	*Clostridiales* unclassified	Firmicutes
OTU424	0	0.0007	0.0003	0.0012	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU1771	0.0008	0.0002	0.0001	0	0.0001	*Clostridium XlVb*	Firmicutes
OTU1781	0.0005	0.0005	0.0001	0	0	*Clostridium XlVb*	Firmicutes
OTU454	0.0004	0.0007	0.0008	0.0003	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU1161	0.0012	0.0015	0.0005	0.0001	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU845	0.0012	0.0005	0.0007	0.0001	0	*Bacteroidales* unclassified	Bacteroidetes
OTU823	0.0007	0.0006	0.0002	0.0001	0	*Lachnospiraceae* unclassified	Firmicutes
OTU1109	0.0027	0.0001	0.0003	0.0001	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU950	0.0006	0.0004	0.0044	0.0001	0	*Bacteroidales* unclassified	Bacteroidetes

OTU995	0.0003	0.0017	0.0031	0	0	*Porphyromonadaceae* unclassified	Bacteroidetes
OTU920	0.0019	0.003	0.0007	0	0	*Porphyromonadaceae* unclassified	Bacteroidetes

Table S7. Bacterial taxonomic profiling at the order level in FMT treated groups. Data are represented as the mean.

	FMT-control (%)	**FMT-CIA (%)**	**FMT-HD (%)**
Lactobacillales	63.24	38.09	37.32
Clostridiales	18.83	24.89	36.11
Bacteroidales	9.08	24.389	13.81
Campylobacterales	1.89	3.01	1.33
Coriobacteriales	1.25	3.25	1.63
Desulfovibrionales	1.36	1.66	2.62
Saccharibacteria genera incertae sedis	0.72	1.31	1.61
Erysipelotrichales	0.31	0.62	2.30
Deferribacterales	0.90	0.43	0.65
Enterobacteriales	0.75	0.69	0.33
Others	1.67	1.67	2.28

SUPPORTING FIGURE LEGENDS

Figure S1. Schematic representation of the TO treatments (**A**) and its corresponding fecal microbiota transplantation (**B**) in CIA mice.

Figure S2. Effects of different TO treatments and FMT on the spleen index in CIA mice. A. Spleen index in CIA mice received different TO treatments, data are shown as mean ± SD, n=6. **B.** Spleen index in CIA mice received different FMTs, data are shown as mean ± SD, n=5. a-c represent significant differences among groups by one-way ANOVA and Tukey's post-hoc test (p<0.05).

Figure S3. Effects of TO treatments on cytokine profiles in the spleen of CIA mice. mRNA levels of TNF-α (**A**), IL-1β (**B**), IL-17A (**C**) and IL-10 (**D**). Data are shown as mean ± SD, n=6. a-d represent significant differences among groups by one-way ANOVA and Tukey's post-hoc test (p<0.05).

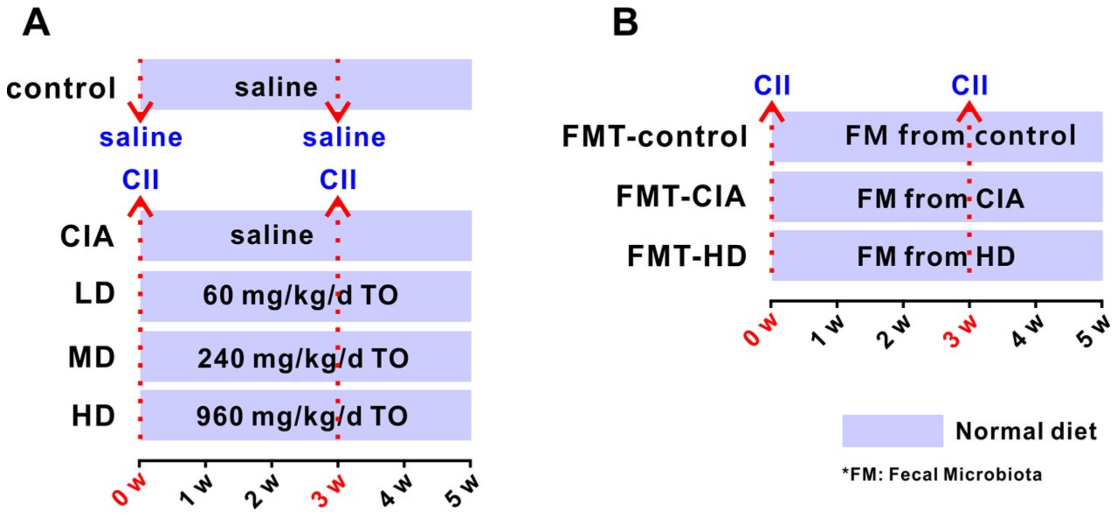
Figure S4. mRNA levels of gene OPG (A) and RANKL (B). Data are shown as mean ± SD, n=6. a-b represent significant differences among groups by one-way ANOVA and Tukey's post-hoc test (p<0.05).

Figure S5. Effects of different TO treatment on mRNA gene levels of pro-inflammatory and anti-inflammatory cytokine in CIA mice. A. mRNA gene levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-17A) and anti-inflammatory cytokine (IL-10) in the colon; **B.** mRNA gene levels of pro-inflammatory and anti-inflammatory cytokine in the cecum. Data are shown as mean ± SD, n=6. a-d represent significant differences among groups by one-way ANOVA and Tukey's post hoc test (p<0.05).

Figure S6. TO affected the mRNA levels of genes claudin-2 and ZO-1 in the colon (A) and cecum (B). Data are shown as mean ± SD, n=6. a-d represent significant differences among groups by one-way ANOVA and Tukey's post hoc test

($p<0.05$).

Figure S7. Alpha diversity assessed by observed species (A) and Simpson (B). Data are shown as mean ± SD, n=6



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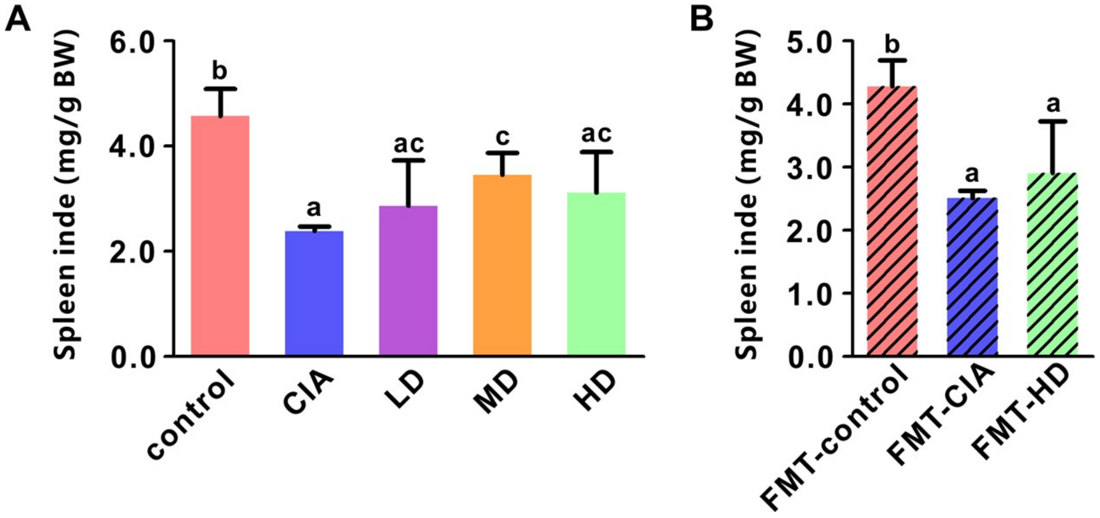

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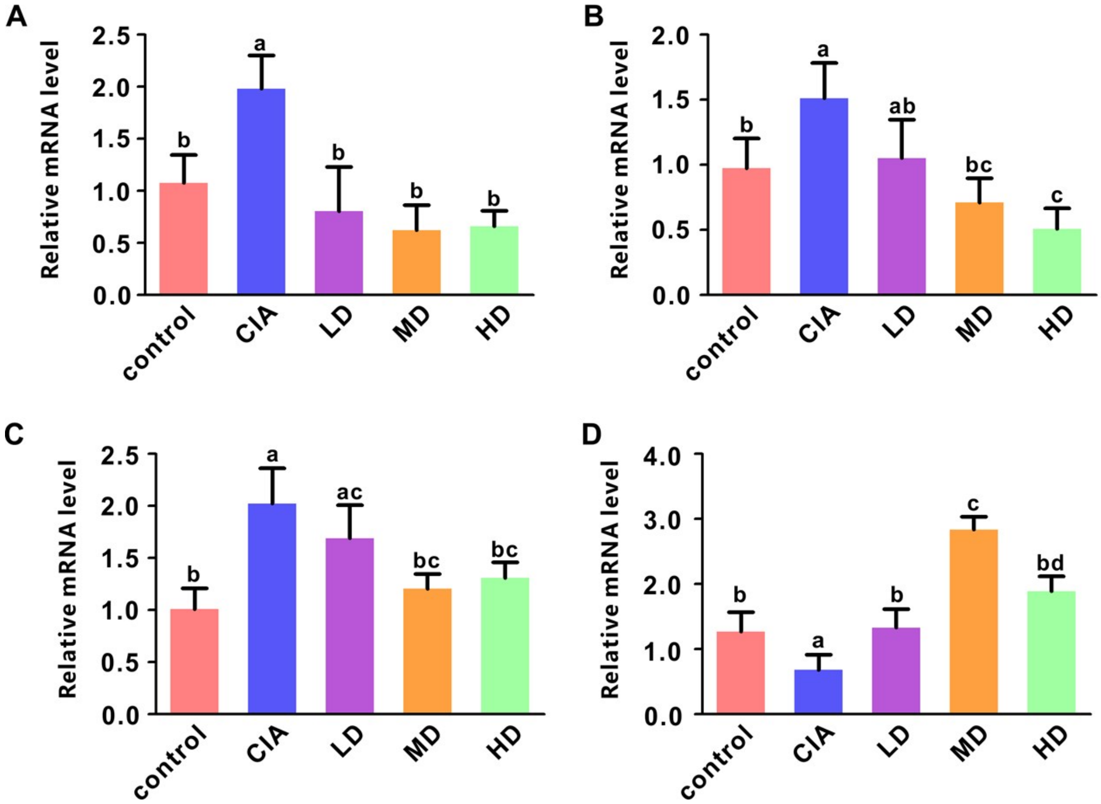

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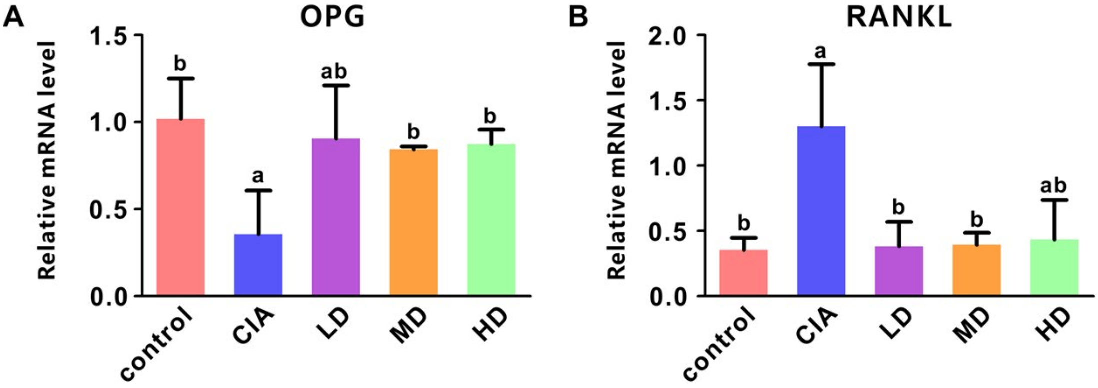

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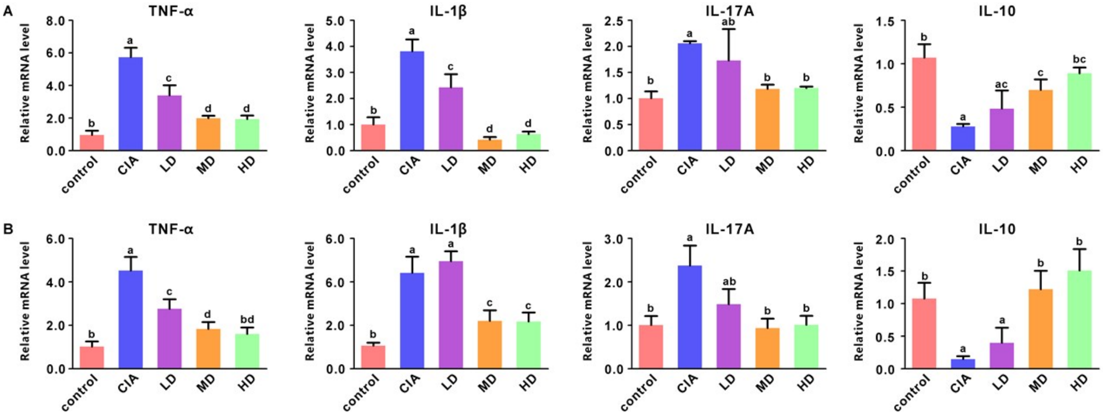

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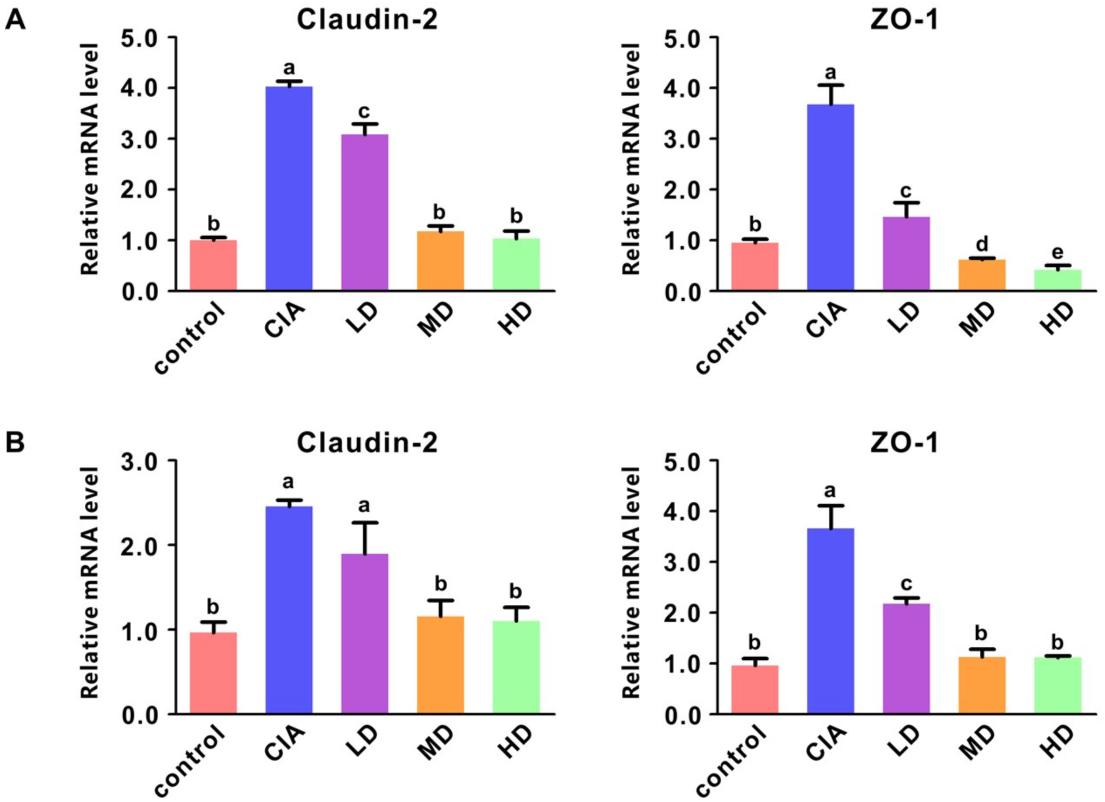

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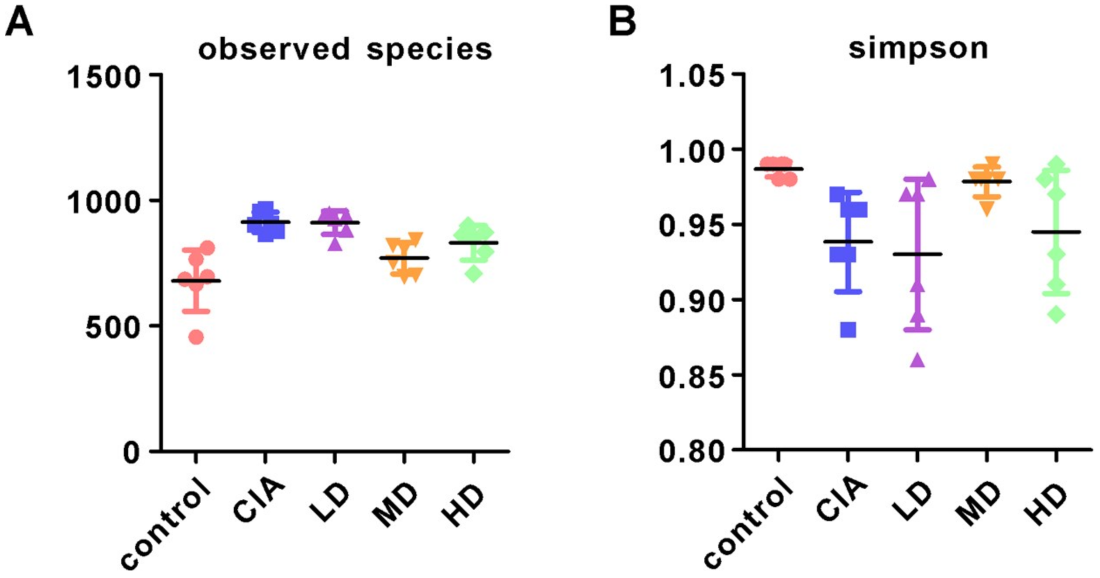

Figure S7.