

Supporting Information for

**Protective effects of oligopeptides from two sea cucumber species
against diet-induced hyperuricemic and renal inflammation in mice**

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Supplementary Table S1. Primers used in this study.

Gene	Primer
β -actin	CAGGCATTGCTGACAGGATG / TGCTGATCCACATCTGCTGG
PRPS	CGAGTCTTCTCGGCCAACTC / CGAGTCTTCTCGGCCAACTC
PNP	AGGCCCCAACTTTGAGACTG / ACAGTGCCTTGCGACGATAA
XOD	TATGCATCCAAGGCTGTCGG / GATCCACACAAGCGTTTCGG
OAT4	GCATAATACCGAGGGGCCAT / CCGTCTGCCGAATCATTGTG
GLUT9	GTCGGCATGGGTTTCCAGTA / GGAAGGCAGCTGAGATCTGG
URAT1	ACACAGCCAGTCTCTTGATGGAGTG / CCGTGATGAGCCAGCGTGCC
ABCG2	TTCTGGTGGAGAGAAACGCC / TCTGGGGCCACAACCTGTAGA
MRP4	TCAGCAACTCGTCTTCCCAC / GGCCAGTGCAGATACATGGT
OAT1	GCTGGTACTCCTCCTCTGGA / TCCATGAC CAGCCCGTAGTA
IL-1 β	TGTTGCCCTCAGCAGTAAGG / GCACCAGCACATTGCTTTGA
IL-2	ACCCACTTCAAGCTCCACTT / TCCTGGGGAGTTTCAGGTTC
IL-6	GCCAGAGTCCTTCAGAGAGA / GGAGAGCATTGGAAATTGGGG
TGF- β	AGCCGGCAAAGGAGCAG / CCAAGAAGTCCCCACGTCTC
IL-10	AAGGGTACTTGGGTTGCCA / GAAATCGATGACAGCGCCTC
TLR4	GGACTATGTGATGTGACCATTGAT / TTATAGATACACCTGCCAGAGACA
MyD88	CTACAGAGCAAGGAATGTGACT / ACCTGATGCCATTTGCTGTCC
NF-kB	GTCTCCGTGAGCTGCTG / GGGATGGCCTCAAGAAGGAG

Supplementary Table S2. The alpha-diversity of each group. The data are presented as the mean $\pm$ SD (n=8). Different letters correspond to statistically significant differences ($p<0.05$) between groups.

	Observed_species	Shannon	Simpson	Chao1
Control	992.50 $\pm$ 80.63 ^a	7.27 $\pm$ 0.67 ^a	0.97 $\pm$ 0.02 ^a	1275.72 $\pm$ 50.49 ^a
Model	567.00 $\pm$ 123.47 ^b	4.29 $\pm$ 0.73 ^b	0.81 $\pm$ 0.08 ^b	883.24 $\pm$ 184.36 ^b
EH-JAP	695.75 $\pm$ 46.92 ^c	5.49 $\pm$ 0.35 ^c	0.90 $\pm$ 0.03 ^a	1084.89 $\pm$ 73.06 ^c
EH-LEU	636.00 $\pm$ 140.09 ^c	5.15 $\pm$ 0.74 ^c	0.88 $\pm$ 0.08 ^b	927.35 $\pm$ 156.87 ^b

Supplementary Table S3. Relative abundance of the top four phyla. The data are presented as the mean $\pm$ SD (n=8). Different letters correspond to statistically significant differences ($p<0.05$) between groups.

Phylum	Control (%)	Model (%)	EH-JAP (%)	EH-LEU (%)
Bacteroidetes	63.79 $\pm$ 12.78 ^a	32.50 $\pm$ 9.80 ^b	42.60 $\pm$ 7.69 ^c	44.14 $\pm$ 7.56 ^c
Firmicutes	29.24 $\pm$ 12.85 ^a	61.21 $\pm$ 8.82 ^b	44.91 $\pm$ 6.60 ^c	48.39 $\pm$ 6.90 ^c
Proteobacteria	3.91 $\pm$ 1.81 ^{ab}	2.83 $\pm$ 1.09 ^a	5.52 $\pm$ 2.42 ^b	2.44 $\pm$ 1.07 ^a
Actinobacteria	2.19 $\pm$ 1.15 ^a	2.79 $\pm$ 1.93 ^a	3.51 $\pm$ 1.67 ^a	3.17 $\pm$ 2.48 ^a
others	0.87 $\pm$ 0.69 ^a	1.48 $\pm$ 0.85 ^a	3.47 $\pm$ 1.79 ^b	1.86 $\pm$ 1.74 ^{ab}

Supplementary Table S4. Relative abundance of the top eight genera.

The data are presented as the mean $\pm$ SD (n=8). Different letters correspond to statistically significant differences ($p<0.05$) between groups.

Genus	Control (%)	Model (%)	EH-JAP (%)	EH-LEU (%)
<i>Lactobacillus</i>	52.34 $\pm$ 4.96 ^a	29.49 $\pm$ 7.05 ^b	46.94 $\pm$ 4.68 ^c	48.10 $\pm$ 8.43 ^{ac}
<i>Porphyromonadaceae</i>	9.44 $\pm$ 3.36 ^a	23.10 $\pm$ 4.89 ^b	8.80 $\pm$ 5.81 ^a	7.69 $\pm$ 4.01 ^a
<i>Lachnospiraceae</i>	5.98 $\pm$ 2.20 ^a	4.09 $\pm$ 3.84 ^a	10.13 $\pm$ 2.57 ^b	5.57 $\pm$ 3.76 ^a
<i>Coriobacteriaceae</i>	2.98 $\pm$ 1.63 ^{abc}	5.86 $\pm$ 5.23 ^b	4.66 $\pm$ 3.12 ^c	5.31 $\pm$ 1.54 ^{bc}
<i>Clostridial</i>	7.79 $\pm$ 4.74 ^a	4.39 $\pm$ 2.16 ^a	5.94 $\pm$ 3.18 ^a	5.51 $\pm$ 0.87 ^a
<i>Bacteroides</i>	3.33 $\pm$ 1.91 ^a	11.89 $\pm$ 5.31 ^b	2.87 $\pm$ 1.83 ^a	3.12 $\pm$ 3.55 ^a
<i>Ruminococcaceae</i>	3.32 $\pm$ 1.83 ^a	2.84 $\pm$ 2.22 ^a	2.46 $\pm$ 0.95 ^a	6.05 $\pm$ 1.82 ^b
<i>Streptococcus</i>	5.01 $\pm$ 3.64 ^a	1.07 $\pm$ 1.17 ^{bc}	1.05 $\pm$ 0.86 ^b	2.65 $\pm$ 1.45 ^{ab}
others	9.80 $\pm$ 4.12 ^a	17.26 $\pm$ 4.86 ^b	16.80 $\pm$ 5.36 ^b	16.00 $\pm$ 3.77 ^b

Supplementary Table S5. Key phylotypes of gut microbiota responding to EH-JAP and EH-LEU as determined by redundancy analysis. The data are presented as the mean $\pm$ SD (n=8). Different letters correspond to statistically significant differences ($p<0.05$) between groups.

OTUs	Genus	Control (%)	Model (%)	EH-JAP (%)	EH-LEU (%)
OTU05673	<i>Porphyromonadaceae_unclassified</i>	1.67 $\pm$ 0.39 ^a	0.03 $\pm$ 0.05 ^b	0.02 $\pm$ 0.03 ^b	0.15 $\pm$ 0.22 ^b
OTU19609	<i>Porphyromonadaceae_unclassified</i>	1.53 $\pm$ 0.45 ^a	0.05 $\pm$ 0.07 ^b	0.03 $\pm$ 0.04 ^b	0.06 $\pm$ 0.07 ^b
OTU19642	<i>Porphyromonadaceae_unclassified</i>	0.16 $\pm$ 0.16 ^a	0.00 $\pm$ 0.00 ^b	0.00 $\pm$ 0.00 ^b	0.01 $\pm$ 0.02 ^b
OTU33897	<i>Porphyromonadaceae_unclassified</i>	0.38 $\pm$ 0.24 ^{ab}	0.01 $\pm$ 0.02 ^c	0.00 $\pm$ 0.00 ^{ac}	0.02 $\pm$ 0.02 ^b
OTU17976	<i>Porphyromonadaceae_unclassified</i>	0.26 $\pm$ 0.20 ^a	0.04 $\pm$ 0.05 ^b	0.05 $\pm$ 0.03 ^b	0.38 $\pm$ 0.24 ^a
OTU16644	<i>Porphyromonadaceae_unclassified</i>	0.11 $\pm$ 0.15 ^a	0.02 $\pm$ 0.01 ^a	0.02 $\pm$ 0.02 ^a	0.17 $\pm$ 0.12 ^b
OTU00018	<i>Porphyromonadaceae_unclassified</i>	5.11 $\pm$ 2.34 ^a	0.72 $\pm$ 0.28 ^b	1.56 $\pm$ 1.23 ^{bc}	2.37 $\pm$ 1.49 ^c
OTU00024	<i>Porphyromonadaceae_unclassified</i>	0.06 $\pm$ 0.04 ^a	0.00 $\pm$ 0.00 ^b	0.00 $\pm$ 0.00 ^b	0.02 $\pm$ 0.03 ^b
OTU20440	<i>Porphyromonadaceae_unclassified</i>	0.06 $\pm$ 0.03 ^a	0.01 $\pm$ 0.01 ^b	0.00 $\pm$ 0.00 ^b	0.01 $\pm$ 0.01 ^b
OTU25688	<i>Porphyromonadaceae_unclassified</i>	0.17 $\pm$ 0.07 ^a	0.02 $\pm$ 0.02 ^b	0.06 $\pm$ 0.06 ^b	0.26 $\pm$ 0.15 ^a
OTU22251	<i>Barnesiella</i>	0.31 $\pm$ 0.21 ^a	0.02 $\pm$ 0.02 ^b	0.09 $\pm$ 0.10 ^b	0.23 $\pm$ 0.14 ^a
OTU23747	<i>Porphyromonadaceae_unclassified</i>	0.05 $\pm$ 0.02 ^a	0.02 $\pm$ 0.02 ^b	0.02 $\pm$ 0.01 ^b	0.12 $\pm$ 0.05 ^a

OTU38604	<i>Porphyromonadaceae_unclassified</i>	0.25±0.14 ^a	0.01±0.02 ^b	0.00±0.00 ^b	0.24±0.36 ^{ab}
OTU39302	<i>Porphyromonadaceae_unclassified</i>	0.04±0.03 ^a	0.00±0.00 ^b	0.00±0.00 ^c	0.07±0.04 ^a
OTU31345	<i>Porphyromonadaceae_unclassified</i>	0.15±0.06 ^a	0.00±0.00 ^b	0.00±0.00 ^b	0.07±0.07 ^c
OTU32711	<i>Porphyromonadaceae_unclassified</i>	0.27±0.23 ^a	0.00±0.00 ^b	0.07±0.07 ^c	0.27±0.29 ^c
OTU34632	<i>Barnesiella</i>	0.26±0.22 ^a	0.00±0.00 ^b	0.05±0.05 ^c	0.25±0.30 ^{ac}
OTU20293	<i>Rikenella</i>	0.04±0.05 ^a	0.02±0.02 ^a	0.29±0.21 ^b	2.99±4.91 ^a
OTU22178	<i>Rikenella</i>	0.10±0.06 ^a	0.00±0.00 ^b	0.03±0.02 ^c	0.01±0.01 ^d
OTU32879	<i>Rikenella</i>	0.18±0.09 ^a	0.00±0.00 ^b	0.00±0.01 ^b	0.00±0.00 ^b
OTU20479	<i>Rikenella</i>	0.37±0.33 ^a	0.00±0.00 ^b	0.04±0.05 ^c	0.07±0.10 ^{bc}
OTU22899	<i>Rikenella</i>	0.12±0.04 ^a	0.02±0.03 ^b	0.01±0.02 ^b	0.03±0.03 ^b
OTU25520	<i>Rikenella</i>	0.11±0.06 ^a	0.01±0.02 ^{bc}	0.00±0.00 ^b	0.02±0.02 ^c
OTU26606	<i>Bacteroides</i>	0.00±0.00 ^a	0.09±0.06 ^b	0.02±0.03 ^a	0.00±0.01 ^a
OTU57987	<i>Bacteroides</i>	0.00±0.00 ^a	0.18±0.13 ^b	0.00±0.00 ^a	0.00±0.00 ^a
OTU23864	<i>Bacteroides</i>	0.00±0.00 ^a	0.05±0.03 ^b	0.02±0.01 ^c	0.00±0.00 ^d
OTU31444	<i>Bacteroides</i>	0.07±0.05 ^a	0.08±0.02 ^a	0.14±0.12 ^a	0.01±0.01 ^b
OTU26906	<i>Bacteroides</i>	0.01±0.01 ^a	0.13±0.10 ^b	0.03±0.02 ^c	0.00±0.00 ^d
OTU28645	<i>Bacteroides</i>	0.05±0.03 ^a	0.26±0.19 ^b	0.07±0.06 ^a	0.00±0.01 ^c

OTU28193	<i>Bacteroides</i>	0.01±0.01 ^a	0.17±0.13 ^b	0.04±0.04 ^c	0.00±0.00 ^a
OTU30803	<i>Bacteroides</i>	0.04±0.03 ^a	0.08±0.01 ^b	0.06±0.05 ^a	0.01±0.01 ^c
OTU30477	<i>Bacteroides</i>	0.13±0.08 ^a	0.01±0.01 ^b	0.09±0.10 ^a	0.07±0.07 ^a
OTU16114	<i>Bacteroides</i>	0.03±0.01 ^a	0.00±0.00 ^b	0.05±0.04 ^a	0.06±0.05 ^a
OTU21896	<i>Porphyromonadaceae_unclassified</i>	1.66±0.64 ^a	0.28±0.13 ^b	1.18±0.45 ^c	0.69±0.59 ^{bc}
OTU22302	<i>Bacteroidales_unclassified</i>	0.04±0.01 ^a	0.00±0.00 ^b	0.02±0.02 ^a	0.02±0.02 ^{ab}
OTU11608	<i>Alistipes</i>	0.01±0.02 ^a	0.00±0.00 ^b	0.14±0.09 ^c	0.13±0.09 ^c
OTU19965	<i>Rikenella</i>	0.15±0.12 ^a	0.00±0.00 ^b	0.08±0.06 ^a	0.01±0.01 ^c
OTU20854	<i>Prevotella</i>	0.05±0.08 ^{ab}	0.00±0.01 ^a	0.07±0.06 ^b	0.05±0.04 ^b
OTU15085	<i>Bacteria_unclassified</i>	0.00±0.00 ^a	0.00±0.01 ^a	0.33±0.14 ^b	3.53±4.63 ^{ab}
OTU30208	<i>Bacteria_unclassified</i>	0.37±0.27 ^a	0.13±0.08 ^b	0.61±0.32 ^a	2.87±2.97 ^a
OTU45354	<i>Coriobacteriaceae_unclassified</i>	0.04±0.09 ^{abc}	0.00±0.00 ^a	0.12±0.06 ^b	0.04±0.05 ^c
OTU45737	<i>Coriobacteriaceae_unclassified</i>	0.06±0.05 ^a	0.03±0.01 ^a	0.12±0.07 ^a	0.32±0.25 ^b
OTU00019	<i>Escherichia</i>	0.05±0.05 ^a	0.00±0.00 ^b	0.26±0.14 ^c	0.08±0.06 ^{ad}
OTU00106	<i>Desulfovibrio</i>	0.12±0.13 ^a	0.00±0.00 ^{ab}	0.11±0.12 ^a	0.02±0.01 ^a
OTU46543	<i>Vampirevibrio</i>	0.10±0.07 ^a	0.00±0.00 ^b	0.04±0.02 ^c	0.01±0.01 ^d
OTU45562	<i>Brevundimonas</i>	0.05±0.06 ^a	0.06±0.02 ^a	0.11±0.09 ^a	0.34±0.20 ^b

OTU00327	<i>Lactobacillus</i>	0.00±0.00 ^a	0.08±0.05 ^b	0.05±0.05 ^b	0.08±0.11 ^{ab}
OTU00663	<i>Lactobacillus</i>	0.00±0.01 ^a	0.24±0.28 ^b	0.09±0.10 ^b	0.00±0.01 ^{ab}
OTU03910	<i>Lactobacillus</i>	0.00±0.00 ^a	0.56±0.25 ^b	0.17±0.15 ^c	0.61±0.19 ^{ad}
OTU00008	<i>Lactobacillus</i>	0.26±0.13 ^a	35.61±10.13 ^b	17.93±8.77 ^c	32.64±9.79 ^{bd}
OTU03877	<i>Lactobacillus</i>	0.01±0.01 ^a	0.06±0.02 ^b	0.01±0.01 ^a	0.07±0.03 ^b
OTU00004	<i>Lactobacillus</i>	2.03±2.74 ^a	0.64±0.34 ^a	0.96±0.48 ^a	5.19±3.16 ^b
OTU00119	<i>Streptococcus</i>	0.06±0.03 ^{ab}	0.02±0.03 ^c	0.07±0.05 ^{ac}	0.07±0.06 ^b
OTU03231	<i>Erysipelotrichaceae_incertae_sedis</i>	0.02±0.04 ^a	0.01±0.02 ^b	0.28±0.18 ^a	0.15±0.16 ^{ab}
OTU14897	<i>Candidatus_Stoquefichus</i>	0.02±0.02 ^a	0.00±0.00 ^b	0.35±0.24 ^c	0.19±0.31 ^{ac}
OTU45613	<i>Candidatus_Stoquefichus</i>	0.00±0.00 ^a	0.00±0.00 ^b	2.85±3.23 ^c	0.00±0.00 ^{ab}
OTU48104	<i>Clostridia_unclassified</i>	0.10±0.06 ^a	0.00±0.00 ^b	0.62±0.58 ^c	0.01±0.01 ^d
OTU44075	<i>Clostridia_unclassified</i>	0.27±0.18 ^a	0.00±0.01 ^b	0.29±0.16 ^a	0.14±0.19 ^a
OTU43868	<i>Clostridia_unclassified</i>	0.01±0.02 ^a	0.19±0.08 ^b	0.00±0.00 ^c	0.01±0.01 ^a
OTU43493	<i>Clostridia_unclassified</i>	0.05±0.07 ^a	0.01±0.01 ^a	0.19±0.15 ^b	0.42±0.73 ^{ab}
OTU46432	<i>Lachnospiraceae_incertae_sedis</i>	0.01±0.02 ^a	0.00±0.01 ^a	0.09±0.04 ^b	0.01±0.02 ^a
OTU46518	<i>Clostridium XIVa</i>	0.21±0.20 ^a	0.00±0.01 ^b	0.08±0.05 ^a	0.03±0.02 ^c
OTU53513	<i>Lachnospiraceae_unclassified</i>	0.03±0.02 ^a	0.01±0.01 ^b	0.41±0.35 ^c	0.24±0.35 ^{abc}

OTU43517	<i>Lachnospiraceae_unclassified</i>	0.25±0.36 ^{abc}	0.00±0.00 ^a	0.15±0.14 ^b	0.00±0.00 ^c
OTU44159	<i>Lachnospiraceae_unclassified</i>	0.09±0.13 ^{abc}	0.14±0.07 ^a	0.00±0.00 ^b	0.01±0.01 ^c
OTU43587	<i>Lachnospiraceae_unclassified</i>	0.02±0.04 ^{ab}	0.11±0.07 ^a	0.00±0.00 ^b	0.00±0.00 ^b
OTU52212	<i>Lachnospiraceae_unclassified</i>	0.00±0.00 ^a	0.01±0.02 ^a	0.25±0.28 ^b	0.00±0.00 ^a

Mass spectrometry analysis of a protein complex. The main MS/MS spectrum shows relative intensity (%) vs m/z . Two major peaks are highlighted with red dashed circles and labeled with their m/z values: 553.4009 and 569.3978. To the right, two zoomed-in MS/MS spectra are shown for these peaks.

The top zoomed spectrum is for m/z 569.3978 and shows a peptide sequence G-P-S-G-P-R with b and y fragment ions labeled. The bottom zoomed spectrum is for m/z 553.4009 and shows a peptide sequence G-P-A-G-P-R with b and y fragment ions labeled. Both zoomed spectra show relative intensity (%) vs m/z .

(A) First-order mass spectra of the enzymatically derived oligopeptides. (B) Sequences of GPSGPR, GPAGPR, respectively, as determined by tandem mass analysis.

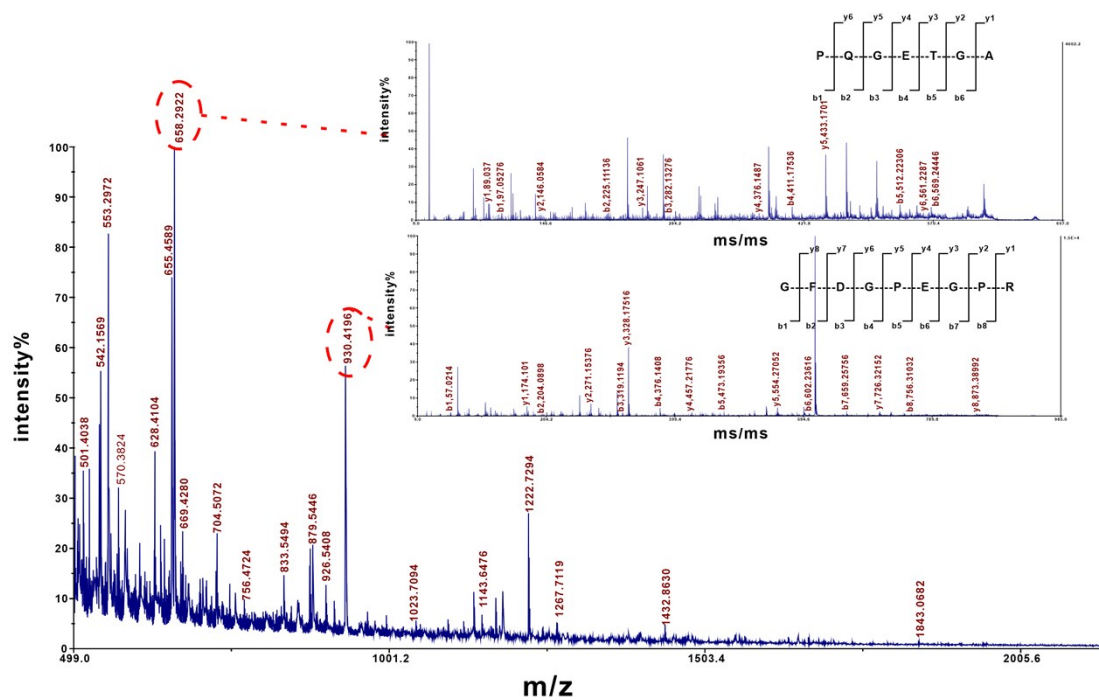


Fig. S2. MALDI-TOF/TOF tandem mass spectra of EH-LEU polypeptides.

(A) First-order mass spectra of the enzymatically peptides. (B), (C) Sequences of PQGETGA, GFDGPEGPR, respectively, as determined by tender mass analysis.

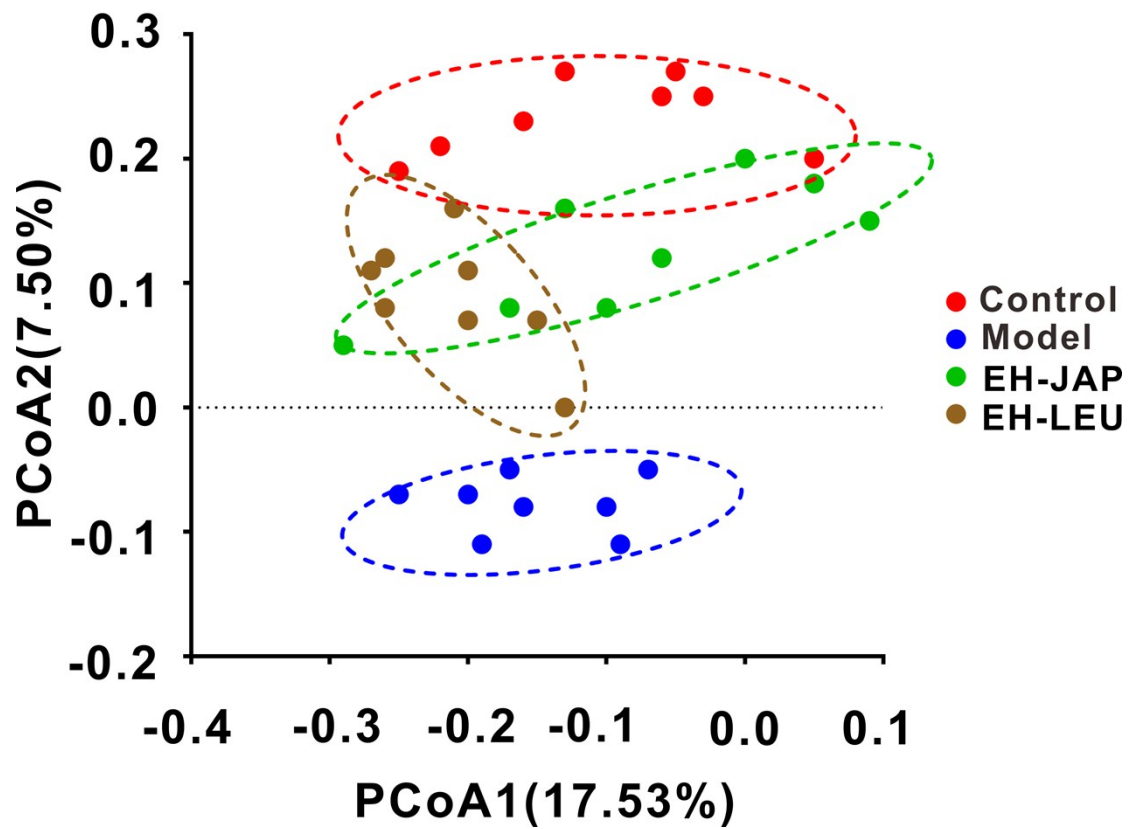


Fig. S3. Unweighted Unifrac PCoA of the gut microbial communities.

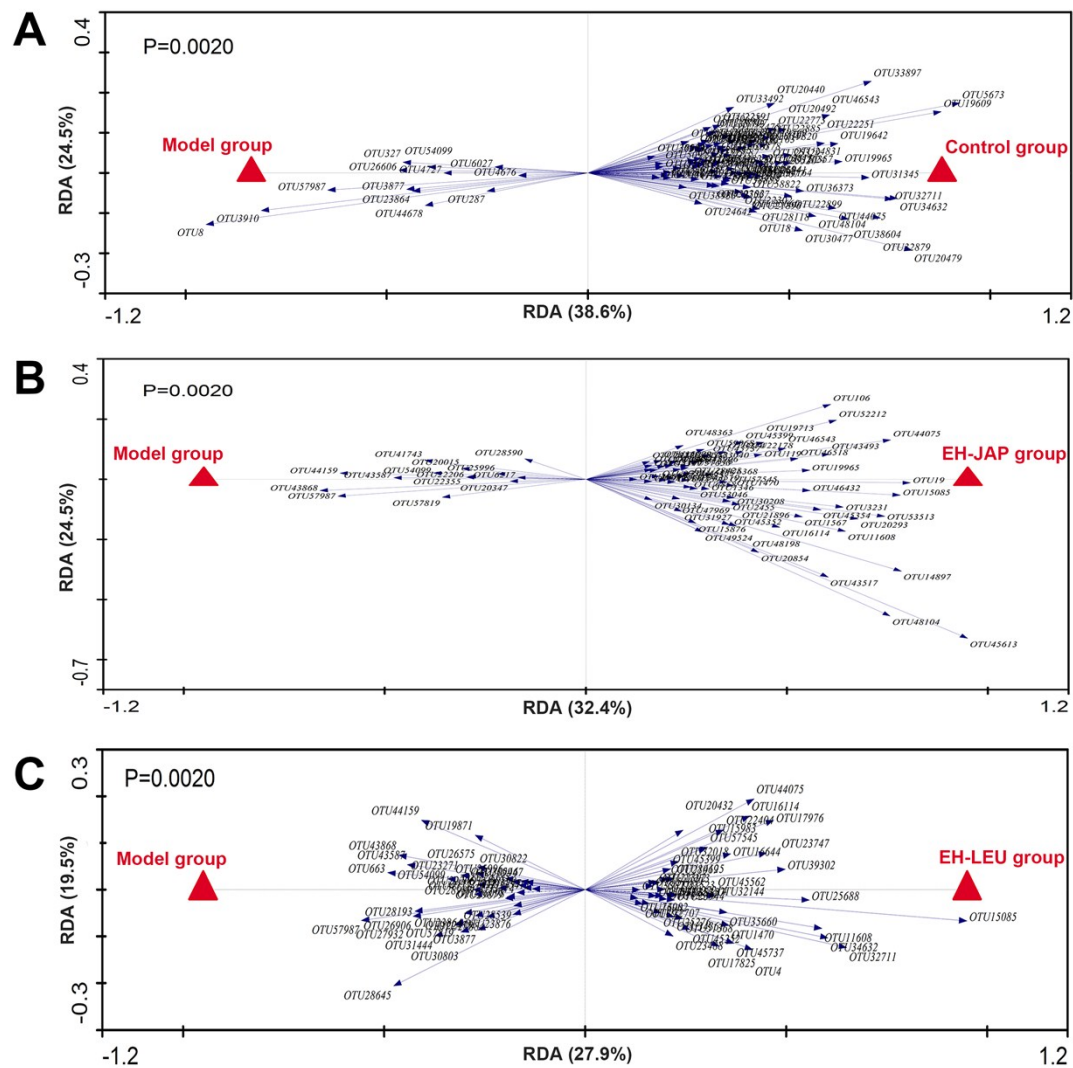


Fig. S4. Original analysis result of redundancy analysis. (A) control vs model group, (B) EH-JAP vs model group, (C) EH-LEU vs model group.