Supporting Information 1

Peptides extraction. Eighteen hams were randomly selected as samples. Samples were denuded of extra muscular fat and connective tissues and minced, and then 20 g of M. biceps femoris muscle was homogenized with 80 mL of phosphate buffer (0.2 mmol/L, pH 7.2) in a polytron homogenizer (IKA T25 digital ultra-turrox, IKA, Germany; 4 strokes, 10 s each at 22000 g with cooling in ice). The homogenate was centrifuged at 12000 g for 20 min at 4 °C, and after filtration through tangential flow filtration (TFF) (P/N: S02-E003-05-N, Media/Rating: mPES/3KDa, Surface area: 790 cm^2), the peptides extracted from dry-cured hams were retained by ultrafiltration consisting of fractions of less than 3KDa. The mixture was dried in a rotatory evaporator and stored at −20 °C prior to use.

Peptides separation by size exclusion chromatography. Peptides extracted with a concentration of 0.1 g/mL aliquot were subjected to the SEC using a Sephadex column (10 x 300 mm) the packed with Sephadex™ 10/300 GL (General Electric Company, Marlborough, USA) to fractionate the peptides according to their molecular masses. The separation was performed at a constant flow rate of 0.8 mL/min with 0.01 N HCl at room temperature. The fractions were assayed using an ultraviolet detector (Amersham Biosciences) at 280 nm, and collected in every tube by an automatic fraction collector. Fractions were dried in a vacuum freeze-dryer for further analysis.

Fractionation of peptides by RP-HPLC. The fraction with the highest hepatic protective activity was selected for further fractionation by RP-HPLC. The lyophilized sample was dissolved in 1 mL of distilled water and injected into a HPLC system equipped with a BEH C18 column (1.7 μm, 2.1 × 100 mm, Waters Inc., Milford, MA, USA). The gradient elution

was performed at a flow rate of 0.3 mL/min with eluent A as 0.1% formic acid and eluent B as 100% acetonitrile. The flow gradient was as follows: 0−10 min, 100% A; 10−22 min, 30−80% B; 22−23 min, 100% A. The peptide peaks were monitored at a UV wavelength of 280 nm. The peaks corresponding to peptides were collected as seven fractions and freeze-dried.

Identification of bioactive peptides by LC-MS/MS. The fractionation of the peak with the highest hepatic protective activity from RP-HPLC fractionation was carried out using an Acquity (Waters Inc.) HPLC system equipped with a reversed phase BEH C18 analytical column (1.7 μm, 2.1 × 100 mm, Waters Inc.). The gradient elution was performed at a flow rate of 0.3 mL/min with eluent A as 0.1 % formic acid and eluent B as 100 % ACN. The flow gradient was as follows: 0−10 min, 100 % A; 10−22 min, 30−80 % B; 22−23 min, 100 % A. The column temperature was maintained at 25 °C. The flow entered directly into the MS/MS system for multiple reaction measurement. The recording mass range of precursor ions was m/z 200–4000. Mass Lynx V4.1 was used to operate the instrumental and analyze the mass spectrogram information.

Supporting Information 2

Peptide synthesis. (1) 0.2mmol of resin (2-chlorotrityl chloride resin) was accurately weighed and 3ml of dimethylformamide (DMF) and dichloromethane (DCM) were added for swelling resin for 30 minutes; (2) Place in a 10 mL EP tube according to HCTU: Amino Acid: DIEA: Resin = 4:4:8:1 for later use; (3) Connect the first amino acid (without HCTU), dissolve the amino acid in 3mL DMF, add 132μl DIEA, shake for 3min, pour into the synthesis tube, place the synthesis tube in an air bath shaker at 45 ℃ for 8h; (4) Resin cleaning: first with DMF wash 3 times, then with DCM wash 3

times, and finally with DMF wash 3 times; (5) Sealing with 5% methanol solution: 5ml 1% methanol was placed in the synthesis tube and shaken for 30min; (6) Cleaning resin: repeat the content of Step 4; (7) Remove fmoc: add 20% piperidine reagent and shake for 5 minutes; (8) Connect amino acids: dissolved the weighed amino acids and the condensed mixture in 3mL DMF, then added 132μL DIEA, shaken for 3min, poured into the synthesis tube, shaken for 30min, and repeated steps 6,7,8 to insert the required amino acids in turn to extend the peptide chain; (9) Cleaning resin: repeat the content of Step 4; (10) Drain the resin and cut the peptide with the prepared cutting solution (cutting solution =TFA: Phenof :H_2O:TIPS=88:5:5:2); (11) Press out the solution and concentrate it to 5ml with nitrogen; (12) 35mL ice ether was added to precipitate the peptide, and then centrifuged at 3500g for 15 minutes. The precipitated peptide was the target peptide.

The synthesised peptides were purified by RP-HPLC equipped with a BEH C18 column (1.7 μm, 2.1 × 100 mm, Waters Inc., Milford, MA, USA). The mobile phases were composed of 0.1% formic acid (solvent A) and 100% acetonitrile (solvent B). The mobile phases were as follows: 0−10 min, 100% solvent A; 10−22 min, 30−80% solvent B; 22−23 min, 100% solvent A, at a flow rate of 0.3ml/min. The separation was monitored at a UV wavelength of 280 nm. The synthesised peptides were identified by liquid chromatography coupled to mass.

Supporting Information 3

Measurement of Antioxidant Activity.

Measurement of Hydroxyl Radical Scavenging Activity. Sample group was a mixture of 0.6 mL of 1,10-phenanthroline monohydrate (5 mmol/L) and 0.4 mL of

phosphate buffer (0.2 M, pH 7.4), and then 0.6 mL of JHP, 0.6 mL of EDTA (15 mmol/L), and 0.6 mL of $FeSO_4$ (5 mmol/L) were mixed thoroughly. After mixing, 0.8 mL of H_2O_2 (0.1 %) was added, and the mixture was incubated at 37 °C for 60 min. Afterward, the mixture absorbance was measured at 536 nm with the multifunctional microplate reader. The control group contained the same solutions as the sample group except deionized water was used instead of sample. The blank group contained the same solution as the control group except deionized water was used instead of H_2O_2. Results were determined according to the equation.

$$hydroxyl\ radical\ scavenging\ activity(\%) = (As - Ac) \div (Ab - Ac) \times 100\%$$

where As, Ac, and Ab represent the absorbance of the sample and the control and blank groups, respectively. BHT was used as a positive control.

Measurement of DPPH Radical Scavenging Activity. One milliliter of the KRQKYD (dissolved in deionized water) was mixed with 1 mL of 0.2 mmol/L DPPH (in 95% ethanol) as sample group; one milliliter of deionized water was mixed with 1 mL of ethanol (95%) as blank group; one milliliter of 0.2 mmol/L DPPH was mixed with 1 mL of ethanol (95%) as control group. The mixture was agitated using vortex and incubated for 30 min at room temperature protected from light and then the absorbance at 517 nm was measured. The scavenging activity of KRQKYD and BHT was calculated using the following equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = 1 - [\ (As - Ac)\ \div (Ab - Ac)\ ]\ \times 100\ \%$$

where As, Ac, and Ab represent the absorbance of the sample, the control, and the blank groups, respectively. BHT was used as a positive control.

Measurement of Fe^{2+} Chelating Ability. Briefly, 1 mL of KRQKYD was mixed with 0.05 mL of $FeCl_2$ (2 mmol/L) and 0.2 mL of ferrozine (5 mmol/L). The mixture was vortexed and kept at room temperature for 10 min prior to measurement of the absorbance at 562 nm (As). Control (Ac) contained everything except using deionized water instead of KRQKYD. The chelating ability was calculated according to the equation

$$Fe^{2+}\ chelating\ ability\ (\%) = (Ac - As) \div Ac \times 100\ \%$$

where As and Ac represent the absorbance of the sample and the control groups. BHT was used as a positive control.

Supporting Information 4

Ethanol liquid diet (directions for preparing 1000 mL)

Ethanol % in the diet (vol/vol)	Dry mix (g)	Maltose dextrin (g)	Water （mL）	95% ethanol (mL)	Calories from maltose dextrin (%)	Calories from ethanol (%)
0	133	91.2	900	0	35.5	0
1	133	77.1	900	10.5	30.0	5.5
3	133	48.7	910	31.6	18.9	16.6
5	133	20.3	910	52.6	7.9	27.6

Ethanol density = 0.789 mL^{-1}; 1g ethanol = 7 kcal; 1 g maltose dextrin (Bio-serv) =3.89 kcal. These water levels are an approximation. The final volume after addition of ethanol should be 1000 mL. Note: Liquid diet is the only source of food and water.

Supporting Information 5

Preparation of Liver and Colon Pathological Sections

Paraffin embedding and section making. Fresh liver and colon tissues were immobilized by soaking in a 4% paraformaldehyde solution for over 12 hours. An anhydrous ethanol solution of 70%, 80%, 90% and 95% vol was prepared using ultraportable water (Mill-Q) for dehydration. The volume ratio of xylene/alcohol solution is 1:1. Take the liver tissue out of the

fixative solution and soak it in 70% ethanol solution overnight. Then, they were soaked in 80%, 90% and 95% ethanol solutions for 1.5 hours, respectively. Finally, use absolute ethanol to soak twice for 30 minutes each to dehydrate the liver tissue. Treat with xylene/alcohol solution for 30 minutes, then use xylene to elute twice, 30-60 minutes each time, until the liver tissue is transparent. In the incubator, embed the transparent tissue, using paraffin I (90min) and paraffin II (120min). Set the paraffin microtome slice thickness to 5μm, and slice the embedded tissue.

Hematoxylin-Eosin Staining. The paraffin-embedded tissue samples are eluted with xylene/alcohol solution for 30 minutes each time. Then, use absolute ethanol to elute 2 times (5 minutes each time), use 95% absolute ethanol solution to elute 2 times (5 minutes each time), the tissue sample is washed, using 80%, 70%, 50% and 30% ethanol solution in sequence (5 minutes each time), and finally washed with tap water for 5 minutes. Place the rehydrated tissue in hematoxylin for staining (5-20min), quickly rinse the section with distilled water (above 30min). After staining with eosin (3-20min), quickly rinse the section with distilled water (above 30 min) and then use 70%, 80%, 90% ethanol solution and absolute ethanol to wash (10s each time), use xylene/alcohol solution to clean for 1 min, and finally use xylene solution to clean 2 times (20 min each time). Place the stained tissue on a glass slide flatly, wipe off the excess xylene around the tissue, cover the cover glass, add an appropriate amount of neutral gum to mount the slide, and place the cover glass obliquely (Avoid bubbles) and let it dry at the end.

Immunofluorescence staining of colon tissue. The frozen tissue sections were rewarmed, and the sections were washed 3 times with PBS solution for 5 minutes each time. Add goat serum (diluted to 10%) so that the serum completely covers the sample, and then block at 37°C for 30

minutes. Add 100 μL of primary antibody diluent (1:100), incubate at 37°C for 1 hour, and place it at 4°C overnight; after rewarming the next day, rinse with 0.01M PBS buffer 3 times, each time 5min; Add 100μL of the secondary antibody diluent (1:200) under dark conditions, incubate at 37°C for 45min, then remove the secondary antibody, add DAPI dye solution (2.5μg/mL), and stain for 20min at room temperature. Rinse 6 times with 0.01M PBS buffer for 5 minutes each time, then mount the slides with anti-fluorescence quencher, and finally place the slices under a fluorescence microscope to observe and collect images. (FITC excitation wavelength 465-495nm, emission wavelength 515-555 nm, green light)

Supporting Information 6

Microbiomic Profiling of Fecal Samples

DNA extraction and PCR amplification. Microbial DNA was extracted from fecal samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30s for annealing at 55 °C, and 45s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase

and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Illumina MiSeq sequencing. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Processing of sequencing data. Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. (ii) Primers were exactly matched allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed. (iii) Sequences whose overlap longer than 10 bp were merged according to their overlap sequence. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%. The results were analysed using the Majorbio Cloud Platform (https://www.majorbio.com).

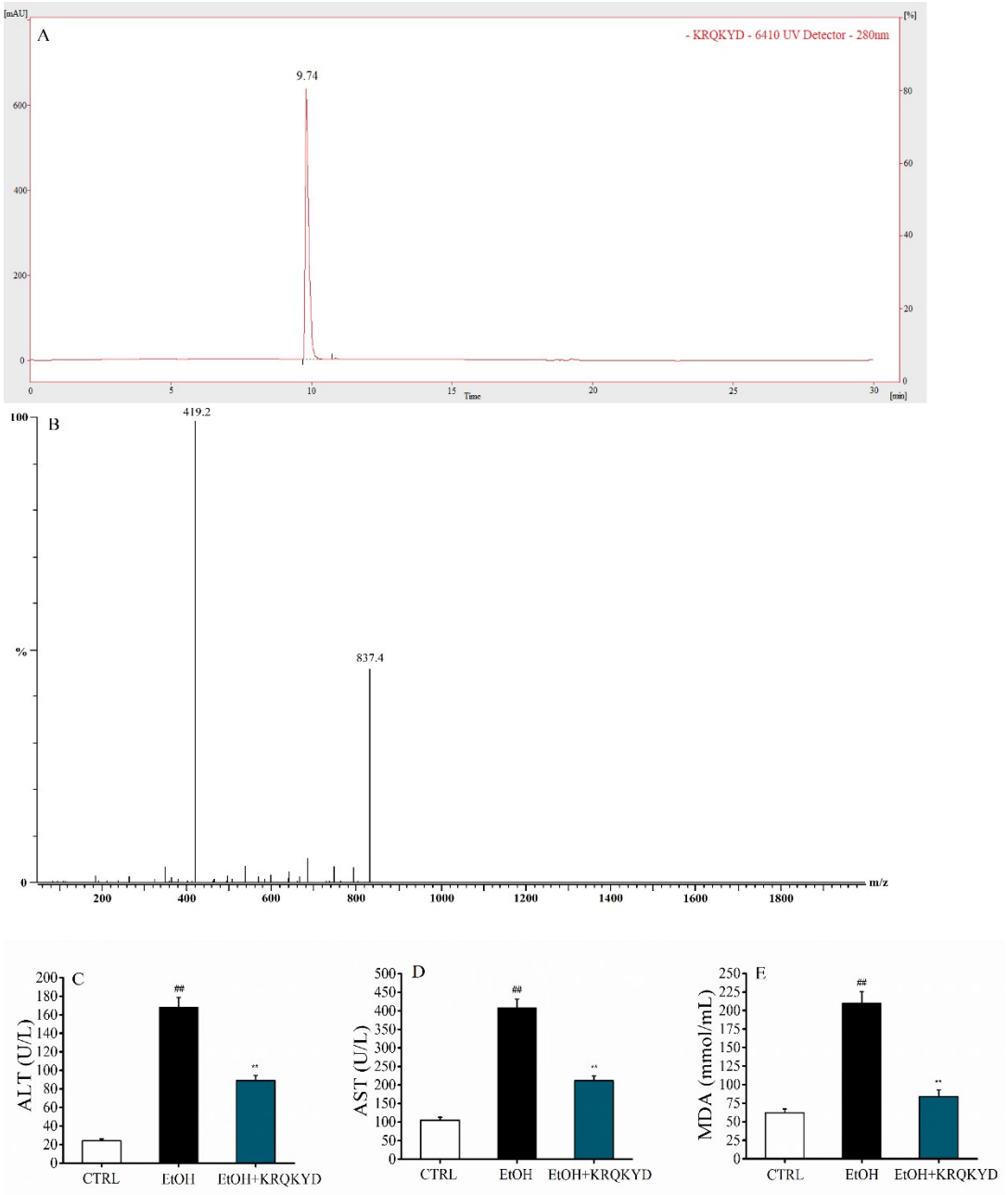
Supporting Information 7

Primers information for PCR

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	ATTCAACGGCACAGTCAAGG	GCAGAAGGGGCGGAGATGA
SCD1	TACACCTGCCTCTTCGGGATT	CACCCCGATAGCAATATCCAGT
FAS	TCAACCATGCCAACCTGAAAAC	AATCACTCCAACGGGCTGAA
ZO-1	ACTCCCACTTCCCCAAAAAC	CCACAGCTGAAGGACTCACA
CYP2E1	GCTGTCAAGGAGGTGCTACT	CCAGTCACGGAGGATACTTAGG
Occludin	CTGTCTATGCTCGTCATCG	CATTCCCGATCTAATGACGC
NRF2	AGATGACCATGAGTCGCTTGC	CCAGCGAGGAGATCGATGAG

Claudin-1	GTTTGCAGAGACCCCATCAC	AGAAGCCAGGATGAAACCCA
SREBP1c	CATAGGGGGCGTCAAACAG	GATGTGCGAACTGGACACAG
HO-1	AAGCCGAGAATGCTGAGTTCA	GCCGTGTAGATATGGTACAAGGA

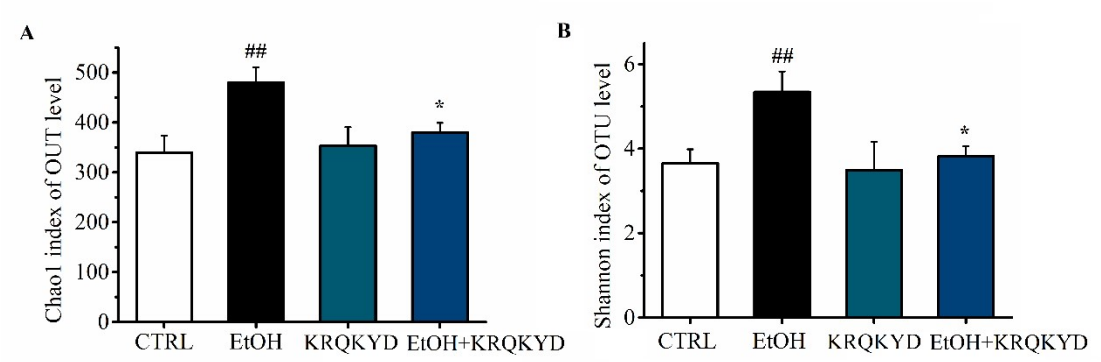
Supporting Information 8



(A) The total particles of KRQKYD in MS/MS spectrum; (B) Mass spectrum of peak at 9.74 min; Effects of KRQKYD on serum concentrations of ALT (C), AST (D) and MDA (E) in male mice fed a control diet or an ethanol-containing diet with or without KRQKYD. Values

are means ± S.E.M; **P < 0.01 vs the EtOH group, ##P < 0.01 vs the control group.

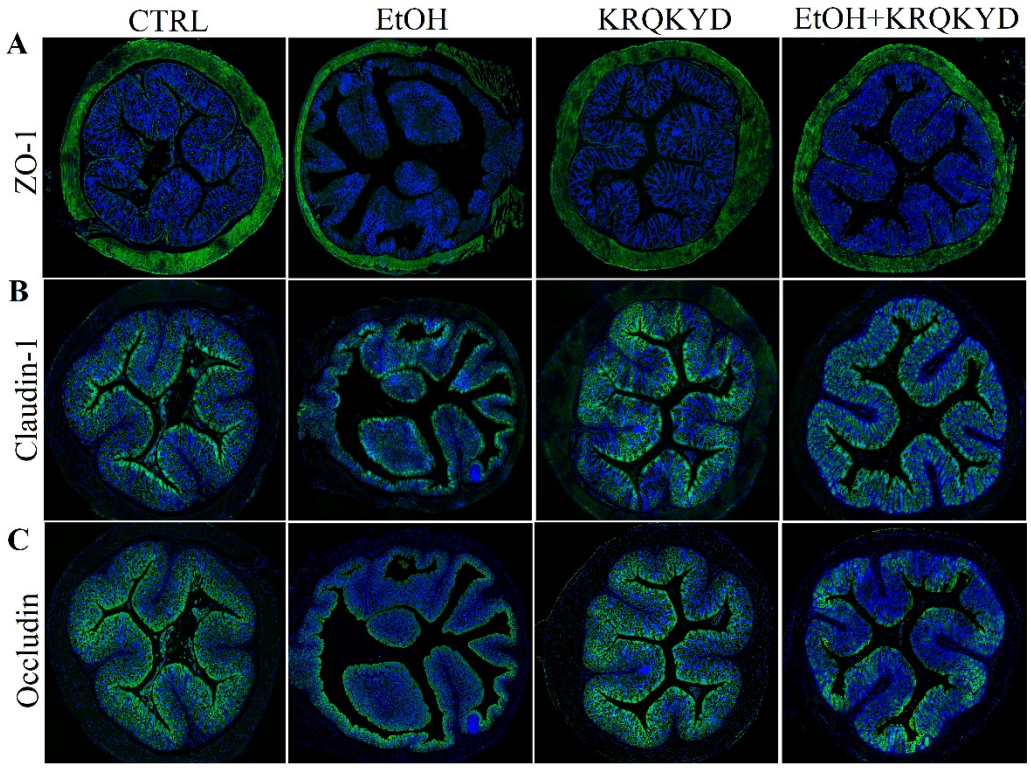
Supporting Information 9



(A) Chao 1 richness index of gut microbiota. (B) Shannon diversity index of gut microbiota.

*P < 0.05 vs the EtOH group, ##P < 0.01 vs the control group.

Supporting Information 10



KRQKYD improved intestinal tight connection in alcohol-treated mice. (A) The tight junction protein expression localization of ZO-1 was detected by immunofluorescence analysis. (B) The tight junction protein expression localization of Claudin-1 was detected by immunofluorescence analysis. (C) The Tight junction protein expression localization of Occludin was detected by immunofluorescence analysis.