

Supplementary Information

Sea Cucumber Peptides Alleviate Hyperuricemic Nephropathy via HIF-1 α /NF- κ B/STAT3-SIRT1/p300 Axis-Mediated Metabolic and Epigenetic Modulation

Peiting Lv ^{a, b}, Xinran Liu ^{a, b}, Ximing Yang ^{a, b}, Xue Sang ^c, Lushun Yuan ^d, Shuzhen Cheng ^{a, b*}, Ming

Du ^{a, b, c*}

^a State Key Laboratory of Marine Food Processing & Safety Control, School of Food Science and Technology, Dalian Polytechnic University, Dalian, 116034, China

^b National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China

^c College of Food Science and Engineering, Dalian Ocean University, Dalian 116023, China

^d Department of Vascular Surgery, Intervention Center, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 201620, China

* Corresponding Authors:

Prof. Shuzhen Cheng, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China. Tel: +86-13654980547; E-mail: chengshuzhen0547@yeah.net

Prof. Ming Du, College of Food Science and Engineering, Dalian Ocean University, Dalian 116023, China. Tel: +86-15941154727; E-mail: duming121@163.com

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Supplementary Table S1. Molecular weight distribution of the sea cucumber peptide (SCP) mixture.

Molecular Weight Range (Da)	Percentage of Peak Area (%) (λ 220 nm)	Number-Average Molecular Weight (M_n , Da)	Weight-Average Molecular Weight (M_w , Da)
> 5000	0.91	62536	44650
3000 – 5000	3.42	3712	3788
2000 – 3000	5.86	2373	2404
1000 – 2000	18.33	1333	1383
500 – 1000	29.83	682	707
189 – 500	36.25	314	331
< 189	5.41	94.5 *	94.5 *

Note: Data were obtained by gel permeation chromatography according to the national standard GB/T 22729-2008.

For the fraction with molecular weight < 189 Da, the M_n and M_w were presumed to be 94.5 Da for the calculation of total averages, as per the analytical note in the test report.

Supplementary table 2. The primer sequences

Gene	Forward Sequences (5'-3')	Reverse Sequences (5'-3')
<i>GLUT9</i>	GACCTGGTGGTGACCATCAT	GCAGGTAGAGGCCGAAGAGT
<i>ABCG2</i>	TGGCTGTCATGGCTTCAGTA	GCCACGTGATTCTTCCACAA
<i>URAT1</i>	CTGTCCTGGGCTTCGTCTTC	GGCAGAGGTGACGAAGATGA
<i>LDHA</i>	ATGGCAACTCTAAAGGATCAGC	CCAACCCCAACAACCTGTAATCT
<i>HK2</i>	GAGCCACCACTCACCTACT	CCAGGCATTTCGGCAATGTG
<i>HIF-1α</i>	GAACGTCGAAAAGAAAAGTCTCG	CCTTATCAAGATGCGAACTCACA
<i>PFKFB3</i>	CAGCCACTTCCTCAACACCT	TGCGGTACTCCTCCTTGGTA
<i>p300</i>	GCTTCAGACAAGTCTTGGCAT	ACTACCAGATCGCAGCAATTC
<i>SIRT1</i>	TAGCCTTGTCAGATAAGGAAGGA	ACAGCTTCACAGTCAACTTTGT
<i>STAT3</i>	CAGCAGCTTGACACACGGTA	AAACACCAAAGTGGCATGTGA
<i>NF-κB</i>	AACAGAGAGGATTCGTTTCCG	TTTGACCTGAGGGTAAGACTTCT
<i>β-actin</i>	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

Note : All animal and cell PCR primers in the manuscript are derived from this table.

Supplementary table 3. The information of antibodies

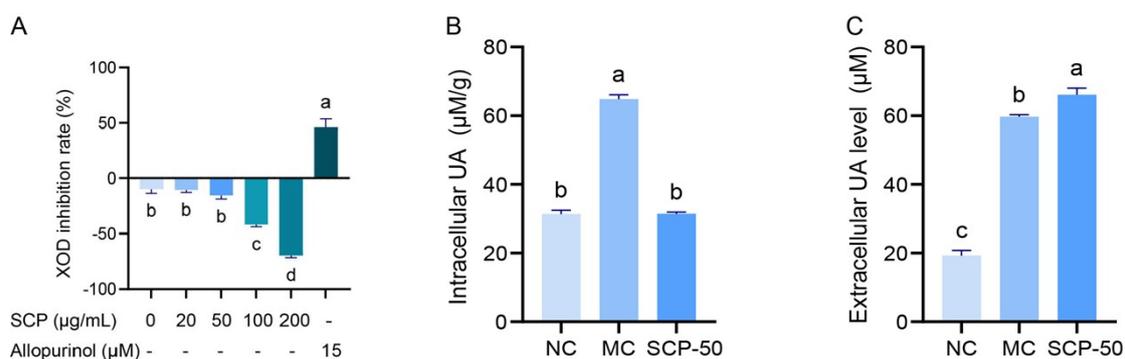
Antibody	Mw (kDa)	Source	Catalog	Manufacturer
Primary Antibody				
β -actin	42	Rabbit IgG	AC026	ABclonal
URAT1	65-79	Rabbit IgG	14937-1-AP	Proteintech
GLUT9	60	Rabbit IgG	26486-1-AP	Proteintech
ABCG2	72	Rabbit IgG	AWA10989	Abiowell
LDHA	35-37	Rabbit IgG	84198-2-RR	Proteintech
PFKFB3	58	Rabbit IgG	13763-1-AP	Proteintech
HK2	102-120	Rabbit IgG	85875-1-RR	Proteintech
P300	264	Rabbit IgG	20695-1-AP	Proteintech
SIRT1	82	Rabbit IgG	RMX00009	Proteintech
Secondary Antibody				
Goat Anti-Rabbit		Goat	SA00001-2	Proteintech

Note: all antibodies used in immunofluorescence staining and immunohistochemical staining of animal

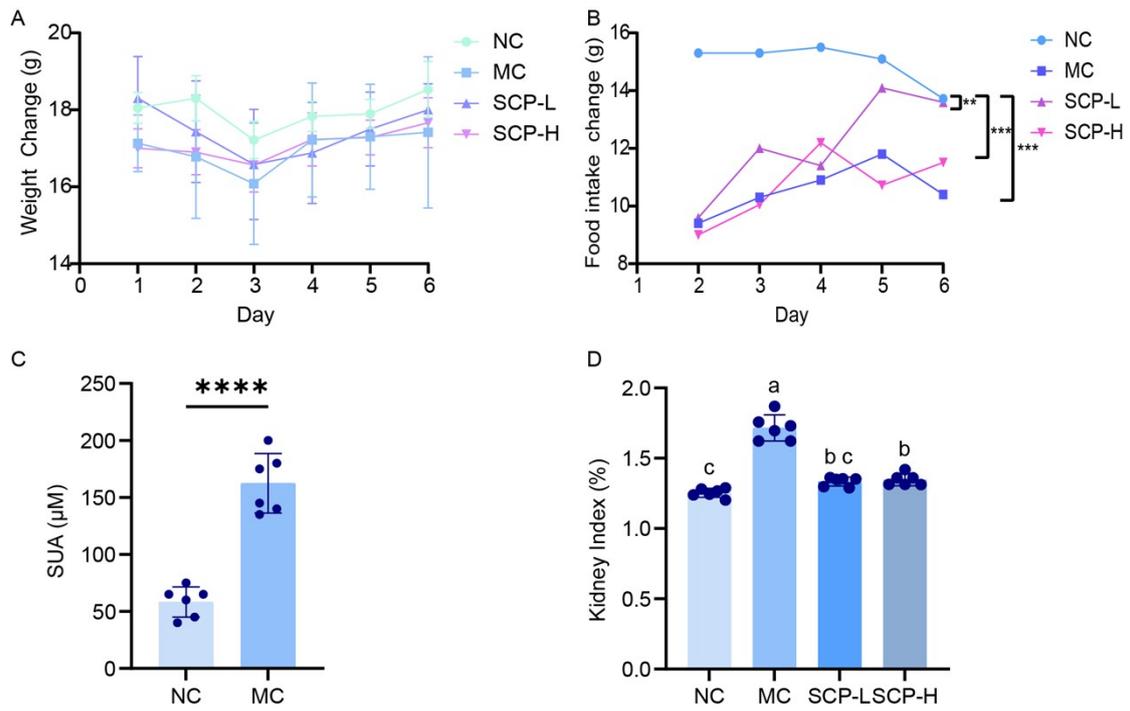
tissues and Western blot experiments in cells in the manuscript are listed in this table.

The inhibitory activity of sea cucumber peptide (SCP) at different concentrations was evaluated using an in vitro xanthine oxidase (XO) inhibition assay, with allopurinol serving as the positive control. Experiments were conducted at 37°C. First, 50 µL of the test sample (SCP or allopurinol) was mixed with 50 µL of 0.05 U/mL XO solution and incubated for 10 minutes. Subsequently, 150 µL of 0.5 mM xanthine was added to initiate the enzymatic reaction, which continued for 30 minutes. The reaction was terminated using 80 µL of 1 M HCl. The absorbance of the reaction mixture was measured at 292 nm. Parallel controls containing and lacking enzyme were included in each group to exclude background interference. The xanthine oxidase inhibition rate was calculated using the formula:

$$\text{XOD inhibition rate (\%)} = \frac{A1 - A2}{A3 - A4} \times 100\%$$



Supplementary Figure 1. SCP does not inhibit xanthine oxidase (XOD) activity but reduces intracellular uric acid accumulation in a cellular XOD activity model. (A) XOD Inhibition Rates of Sea Cucumber Peptides at Different Concentrations Compared with Allopurinol. n = 3. Effects of SCP on intracellular (B) and extracellular (C) UA levels in HK-2 cells under adenosine + XOD stimulation. n = 3. Data are presented as mean ± SD. Different lowercase letters above the bars indicate significant differences among groups as determined by one-way ANOVA followed by Tukey's post hoc test (P < 0.05). Values sharing the same letter are not statistically different.



Supplementary Figure 2. Key indicators of mice in each group during HUA induction and SCP

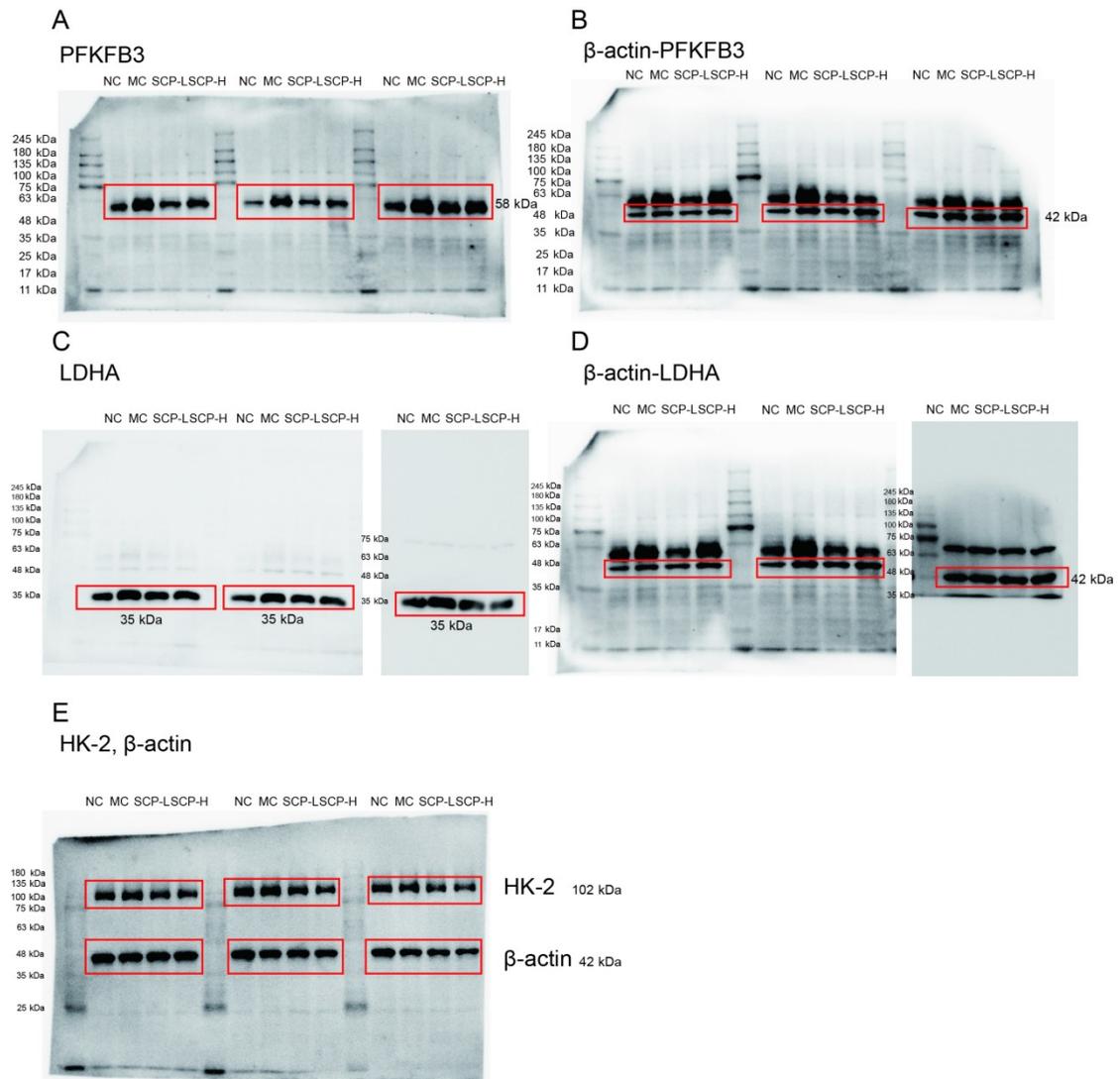
intervention. (A) Body weight changes of mice in each group. n=6. (B) Food intake of mice in each

group. n=1. (C) Food intake of mice in each group. n=6. (D) Kidney index of mice. n=6. Data are

presented as mean \pm SD. Different lowercase letters above the bars indicate significant differences

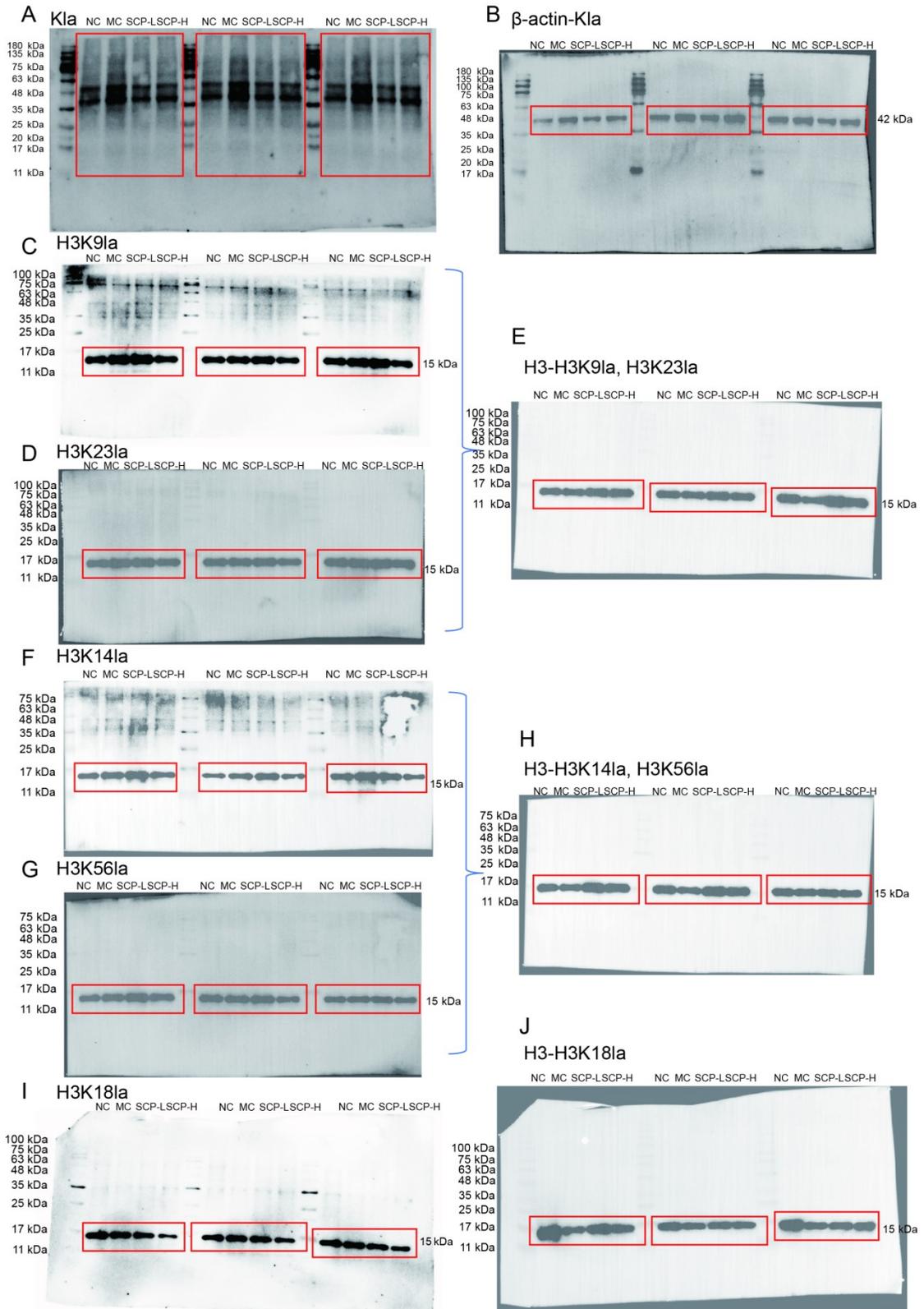
among groups as determined by one-way ANOVA followed by Tukey's post hoc test ($P < 0.05$).

Values sharing the same letter are not statistically different.



Supplementary Figure 3. The full immunoblots of PFKFB3, LDHA and HK2 Western bands.

Western blot analysis of PFKFB3 protein in HK-2 cells stimulated with high uric acid. PFKFB3 protein band (A); corresponding internal control protein band (B). Western blot analysis of LDHA protein in HK-2 cells stimulated with high uric acid. LDHA protein band (C); corresponding internal control protein band (D). Western blot analysis of PFKFB3 protein in HK-2 cells stimulated with high uric acid. HK-2 protein band. HK-2 protein band and corresponding internal control protein band (E).



Supplementary Figure 4. The full immunoblots of K1a, H3K91a, H3K141a, H3K181a, H3K231a and H3K561a Western bands. Western blot analysis of K1a protein in HK-2 cells stimulated with high uric acid. K1a protein band (A); corresponding internal control protein band (B). Western blot

analysis of H3K9la protein in HK-2 cells stimulated with high uric acid. H3K9la protein band (C); Western blot analysis of H3K23la protein in HK-2 cells stimulated with high uric acid. H3K23la protein band (D); corresponding internal control protein band (E). Western blot analysis of H3K14la protein in HK-2 cells stimulated with high uric acid. H3K14la protein band (F); Western blot analysis of H3K56la protein in HK-2 cells stimulated with high uric acid. H3K56la protein band (G); corresponding internal control protein band (H). Western blot analysis of H3K18la protein in HK-2 cells stimulated with high uric acid. H3K18la protein band (G); corresponding internal control protein band (H).