Supplementary Information

Fucoidan Alleviates Kidney Fibrosis by Shaping the Gut Microbiota and Modulating Tryptophan Metabolism

Ying Zhang[1,2#], Jinxin Li[2#], Wanting Chen[3], Fengxia Qin[1], Yuan Tian[2], Zunjian Zhang[3], Fengguo Xu[1,2,3*], Pei Zhang[1,2,3*]

[1] Affiliated Jiangning Hospital of Chinese Medicine, China Pharmaceutical University, Nanjing, 211100, China

[2] School of Pharmacy, China Pharmaceutical University, Nanjing, 210009, China

[3] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, 210009, China

[#] These authors contributed equally to this work.

* Correspondence to be addressed to:

Fengguo Xu, Tel.: +86 25-83271021, Email: fengguoxu@cpu.edu.cn

Pei Zhang, Tel.: +86 25-83271021, Email: peizhang@cpu.edu.cn

Extended methods

Sample Preparation

Metabolites in kidney tissue were extracted using a precooled solvent mixture of acetonitrile (ACN, Merck, Darmstadt, Germany), methanol (MeOH, Merck, Darmstadt, Germany), and ultra-pure water (Millipore, Watford, UK) in a 2:2:1 (v/v/v) ratio. A tissue-to-solvent ratio of 1:30 (w/v, mg/μL) was used for extraction. Samples were centrifuged at 14,000 rpm at 4 °C for 10 min. A 100 μL aliquot of the supernatant from each sample was transferred to a new tube and completely dried under a stream of nitrogen at 37 °C using an MD200-1 Sample Concentration system (Allsheng, Hangzhou, China). All dried samples were stored at -80 °C until chemical derivatization and analysis.

Chemical derivatization

Two distinct derivatization procedures were employed to cover a broader range of metabolites.

Amino and phenol submetabolome. For each dried sample, 20 μL of ACN-H2O (1:1, v/v), 100 μL of carbonate buffer (composed of $NaHCO_3$ and Na_2CO_3 (Nanjing Chemical Reagent, Jiangsu, China); pH 10), and 100 μL of 20 mM dansyl chloride (Dns-Cl, J&K Chemical Ltd, China) solution were added. The mixture was incubated at 35 °C for 15 min. To quench the reaction, 40 μL of 100 mM NaOH solution (Nanjing Chemical Reagent, Jiangsu, China) was added and incubated at 35 °C for 10 min. Excess alkaline was then neutralized by adding 40 μL of 425 mM formic acid (Merck, Darmstadt, Germany).

Carboxyl submetabolome. Dried samples were reconstituted with 20 μL of ACN-MeOH-H_2O (2:2:1, v/v/v), 40 μL of MeOH (Merck, Darmstadt, Germany), 20 μL of 6 mM HATU (J&K Chemical Ltd, China) solution, and 20 μL of 12 mM dansyl piperazine (Dns-PP) solution. After thorough mixing, the derivatization reaction was incubated at 55 °C for 60 min.

Instrumental analysis

Metabolite quantification was performed using a Shimadzu Nexera UPLC system interfaced with an 8060 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source. The autosampler was maintained at

4 °C. Chromatographic separation was achieved on an Agilent Zorbax Eclipse XDB-C18 column (2.1 × 100 mm, 1.8 μm, Agilent Technologies, Santa Clara, CA) maintained at 50 °C. The mobile phase consisted of 0.1% formic acid in H_2O (v/v) (Mobile Phase A) and methanol (Mobile Phase B). The flow rate was set at 0.4 mL/min, and the injection volume was 5 μL.

Specific gradient programs were used for the two derivatization methods. Dns-Cl labelled metabolites: The gradient was as follows: 0-2 min, 30% B; 2-5 min, 30-52% B; 5-15 min, 52-65% B; 15-20 min, 65-78% B; 20-23 min, 78% B; 23-27 min, 100% B; and 27-29 min, 30% B. Dns-PP labelled metabolites: The gradient was as follows: 0-2 min, 30% B; 2-5 min, 30-52% B; 5-15 min, 52-65% B; 15-20 min, 65-78% B; 20-22.5 min, 78% B; 22.5-29 min, 90% B; 29-38 min, 100% B; and 38-40 min, 30% B.

The mass spectrometer was operated in scheduled multiple reaction monitoring (MRM) mode with positive ion detection. Optimal parameters were: spray voltage, 4.5 kV; nebulizing gas, 3 L/min; drying gas, 15 L/min; heat block temperature, 400 °C; and desorption line temperature, 250 °C.

Table S1. Primer sequences

Species	Gene name	Sequence
Mus	*GAPDH*	*F:5'-AGGTCGGTGTGAACGGATTTG-3'* *R:5'-TGTAGACCATGTAGTTGAGGTCA-3'*
Mus	*Acta2*	*F:5'-GTCCCAGACATCAGGGAGTAA-3'* *R:5'-TCGGATACTTCAGCGTCAGGA-3'*
Mus	*Col1A1*	*F:5'-GATGACGTGCAATGCAATGAA-3'* *R:5'-CCCTCGACTCCTACATCTTCTGA-3'*
Mus	*Col3A1*	*F:5'-CTGTAACATGGAAACTGGGGAAA-3'* *R:5'-CCATAGCTGAACTGAAAACCACC-3'*
Mus	*Vimentin*	*F:5'-CGGCTGCGAGAGAAATTGC-3'* *R:5'-CCACTTTCCGTTCAAGGTCAAG-3'*
Mus	*Fibronectin*	*F:5'-ATGTGGACCCCTCCTGATAGT-3'* *R:5'-GCCCAGTGATTTCAGCAAAGG-3'*
Mus	*TGF-β1*	*F:5'-ACTGGAGTTGTACGGCAGTG-3'* *R:5'-GGGGCTGATCCCGTTGATTT-3'*
Mus	*IDO1*	*F:5'-TCCGGTCACGAATGTGGAAC-3'* *R:5'-AAGCTGCCCGTTCTCAATCA-3'*
Mus	*IDO2*	*F:5'-CCTTGGATGGAAATTGCCCTCAG-3'* *R:5'-CGCTGCTCACGGTAACTCTTTAG-3'*
Mus	*KMO*	*F:5'-ATACCATGGTCGCCTTCACC-3'* *R:5'-TGCACAAGTAGGTAGGTGCC-3'*
Mus	*KYNU*	*F:5'-CTCATACTGTCAAGCCTGCGTTAG-3'* *R:5'-GAGGGTTTGAAATTCGGAATCCATTG-3'*
Mus	*KYAT1*	*F:5'-CCAGTCTTTAAAGGGCACACAG-3'* *R:5'-CCTGAGTCCCACACACAGTTCT-3'*
Mus	*KYAT3*	*F:5'-CGATGAGGTTTATGAATGGCTTGTC-3'* *R:5'-CCAGCACTTCCTATTGTTATTGTTCTC-3'*
Mus	*TPH1*	*F:5'-GCGAAGGAAGACGTTATGGA-3'* *R:5'-GTCTTTGAAGCCAGGGTGGT-3'*
Homo	*GAPDH*	*F:5'-GGAGCGAGATCCCTCCAAAAT-3'* *R:5'-GGCTGTTGTCATACTTCTCATGG-3'*
Homo	*Col1A1*	*F:5'-GCTCGTGGAAATGATGGTGC-3'* *R:5'-ACCCTGGGGACCTTCAGAG-3'*
Homo	*Fibronectin*	*F:5'-CGGTGGCTGTCAGTCAAAG-3'* *R:5'-AAACCTCGGCTTCCTCCATAA-3'*
Homo	*TGF-β1*	*F:5'-GGCCAGATCCTGTCCAAGC-3'* *R:5'-GTGGGTTTCCACCATTAGCAC-3'*
Homo	*Vimentin*	*F:5'-GAGGAAGCCGAAAACACCCT-3'* *R:5'-ACGCATTGTCAACATCCTGTC-3'*

Table S2. Metabolite retention time and MRM parameters

No.	Metabolite	Abbreviation	MW	RT (min)	Parent ion (m/z)	Fragment (m/z)	IS RT (min)	IS Parent ion (m/z)	IS Fragment (m/z)
1	Ethanolamine	EA	61.08	5.75	295.00	156.60	5.40	301.00	160.10
2	Putrescine	Put	88.15	17.40	555.20	170.10	16.75	567.20	176.10
3	L-Histidine	His	155.15	18.45	622.70	169.70	17.91	634.70	176.00
4	L-Arginine	Arg	174.20	3.75	408.10	170.20	3.33	414.10	176.20
5	L-Asparagine	Asn	132.12	3.95	366.10	170.10	3.72	372.10	176.10
6	L-Glutamine	Gln	146.14	4.52	380.20	169.50	4.07	386.20	176.00
7	L-Serine	Ser	105.09	5.09	338.70	169.50	4.66	344.70	176.00
8	L-Glycine	Gly	75.07	5.71	309.00	156.50	5.33	315.00	160.10
9	L-Threonine	Thr	119.12	6.08	353.20	157.20	5.76	359.20	160.10
10	L-Alanine	Ala	89.09	6.53	323.20	156.50	6.22	329.20	160.10
11	L-Proline	Pro	115.13	8.09	349.00	169.50	7.73	355.00	176.25
12	L-Methionine	Met	149.21	8.75	383.00	169.50	8.35	389.00	176.00
13	L-Valine	Val	117.15	9.04	351.10	156.70	8.62	357.10	160.10
14	L-Tryptophan	Trp	204.23	9.61	438.20	170.10	9.09	444.20	176.10
15	L-Isoleucine	Ile	131.17	11.18	365.00	156.50	10.71	371.00	160.10
16	L-Leucine	Leu	131.17	11.42	365.00	156.50	10.97	371.00	160.10
17	L-Phenylalanine	Phe	165.19	11.50	399.10	169.50	10.92	405.10	176.00
18	L-Ornithine	Orn	132.16	14.99	599.20	170.00	14.18	611.20	176.00
19	L-Lysine	Lys	146.19	15.91	613.20	170.10	15.17	625.20	176.10
20	L-Tyrosine	Tyr	181.19	20.11	648.20	170.10	19.79	660.20	176.10
21	L-Aspartic acid	Asp	133.10	3.95	367.37	170.10	3.42	373.37	176.10
22	L-Glutamic acid	Glu	147.13	5.30	381.40	169.50	4.93	387.40	176.00
23	L-Citrulline	Cit	175.19	4.91	409.45	170.10	4.52	415.45	176.10
24	Nε-Methyl-L-lysine	MeLys	160.21	18.41	626.85	170.15	17.93	638.85	176.15
25	Nε-Nε-Nε-Trimethyl-L-lysine	TMeLys	188.27	3.46	422.05	170.15	3.06	428.05	176.15

No.	Metabolite	Abbreviation	MW	RT (min)	Parent ion (m/z)	Fragment (m/z)	IS RT (min)	IS Parent ion (m/z)	IS Fragment (m/z)
26	Nε-Acetyl-L-lysine	AceLys	188.22	6.16	421.75	170.15	5.92	427.75	176.15
27	NG-NG'-Dimethyl-L-arginine	SDMA	202.25	4.49	435.80	170.15	4.19	441.80	176.15
28	NG, NG-Dimethyl-L-arginine	ADMA	202.25	4.33	435.80	170.15	4.01	441.80	176.15
29	NG-Methyl-L-arginine	MeArg	188.23	4.12	422.00	170.15	3.77	428.00	176.15
30	Kynurenine	KYN	208.21	8.89	442.20	170.15	8.48	448.20	176.15
31	Xanthurenic acid	XA	205.17	9.51	439.10	170.10	9.21	445.10	176.10
32	6-Hydroxymelatonin	HMT	248.28	11.74	482.20	170.05	11.41	488.20	176.05
33	5-Hydroxyindoleacetate	HIAA	191.18	11.92	425.10	170.10	11.62	431.10	176.10
34	N-Acetyl-serotonin	NAS	218.25	12.02	452.15	170.05	11.74	458.15	176.05
35	Tryptamine	Trpm	160.22	13.25	394.20	170.05	12.71	400.20	176.05
36	3-Hydroxyanthranilic acid	HAA	153.14	15.54	387.20	170.10	15.23	393.20	176.10
37	Kynurenic acid	KA	189.17	17.14	423.15	170.15	16.89	429.15	176.15
38	3-Hydroxy-DL-kynurenine	HK	224.21	20.62	691.20	170.05	20.34	703.20	176.05
39	Serotonin	5-HT	176.22	20.79	643.20	170.05	20.50	655.20	176.05
40	Indole-3-carboxaldehyde	IAld	145.16	18.68	379.15	169.50	18.46	385.15	176.10
41	5-Hydroxy-L-tryptophan	HTP	220.23	18.48	687.30	170.20	18.01	699.30	176.20
42	Xanthine	Xan	152.11	7.26	386.30	170.00	7.10	392.30	176.00
43	Hypoxanthine	Hyp	136.11	8.45	369.90	169.70	8.25	375.90	176.00
44	Cytosine	Cyt	111.10	6.87	345.00	170.15	6.67	351.00	176.15
45	5-Methylcytosine	MeCyt	125.13	7.73	358.95	170.20	7.50	364.95	176.20
46	2'-Deoxycytidine	dC	227.22	6.46	461.00	112.15	6.32	467.00	112.15
47	5-Methyl-2'-deoxycytidine	MedC	241.24	6.65	475.00	126.20	6.52	481.00	126.20
48	Cytidine	Cyd	243.22	5.75	477.10	112.10	5.59	483.10	112.10
49	5-Methylcytidine	MeCyd	257.24	5.92	491.00	126.20	5.77	497.00	126.20
50	Adenine	Ade	135.13	9.33	369.00	170.20	9.08	375.00	176.20
51	1-Methyladenine	MeAde	149.15	5.91	382.70	170.10	5.84	388.70	176.10
52	Adenosine	Ado	267.24	5.33	500.95	136.20	5.21	506.95	136.20

No.	Metabolite	Abbreviation	MW	RT (min)	Parent ion (m/z)	Fragment (m/z)	IS RT (min)	IS Parent ion (m/z)	IS Fragment (m/z)
53	1-Methyladenosine	MeAdo	281.27	3.50	514.80	150.10	3.36	520.80	150.10
54	2′-Deoxyadenosine	dA	251.24	8.89	485.00	119.20	8.65	491.00	119.20
55	Guanine	Gua	151.13	7.81	384.90	170.10	7.59	390.90	176.10
56	Guanosine	Guo	283.24	4.33	517.00	135.10	4.02	523.00	135.10
57	2′-Deoxyguanosine	dG	267.24	8.10	501.00	152.10	7.88	507.00	152.10
58	Uracil	Ura	112.09	8.43	345.95	169.70	8.21	351.95	176.00
59	Uridine	Urd	244.20	6.88	477.95	113.20	6.68	483.95	113.20
60	2′-Deoxyuridine	dU	228.20	8.25	461.95	113.20	8.01	467.95	113.20
61	Thymine	Thy	126.12	10.40	359.90	169.50	10.13	365.90	176.20
62	Thymidine	Thd	242.23	9.20	476.00	127.10	8.94	482.00	127.10
63	4-Aminobutyric acid	GABA	103.12	6.52	337.00	169.50	6.23	343.00	176.00
64	Cholic acid	CA	408.58	23.59	710.00	320.10	23.47	716.00	326.10
65	Chenodeoxycholic acid	CDCA	392.57	24.17	693.80	320.20	24.13	699.80	326.20
66	Deoxycholic acid	DCA	392.57	24.38	694.20	320.20	24.34	700.20	326.20
67	Lithocholic acid	LCA	376.57	25.26	678.05	320.15	25.21	684.05	326.15
68	Glycocholic acid	GCA	465.62	21.97	767.55	320.20	21.83	773.55	326.20
69	Ursodeoxycholic acid	UDCA	392.57	21.89	694.60	320.20	21.77	700.60	326.20
70	Glycodeoxycholic acid	GDCA	449.62	23.89	751.25	320.20	23.86	757.25	326.20
71	Glycochenodeoxycholic acid	GCDCA	449.62	23.72	751.05	320.10	23.68	757.05	326.10
72	Glycoursodeoxycholic acid	GUDCA	449.62	20.48	751.55	320.20	20.36	757.55	326.20
73	L-Carnitine	Car	161.20	4.99	463.10	386.15	4.68	469.10	392.15
74	L-Acetylcarnitine	Car2	203.24	5.43	505.25	386.15	5.27	511.25	392.15
75	Propionylcarnitine	Car3	217.26	6.03	519.30	386.15	5.91	525.30	392.15
76	3-Hydroxybutyryl-carnitine	Car4-OH	247.29	5.72	549.30	386.05	5.58	555.30	392.05
77	Butyrylcarnitine	Car4	231.29	6.60	533.30	386.15	6.59	539.30	392.15
78	Tiglylcarnitine	Car5:1	243.30	6.96	545.10	386.15	6.83	551.10	392.15
79	Isovaleryl-L-carnitine	isoCar5	245.32	7.49	547.35	386.15	7.32	553.35	392.15

No.	Metabolite	Abbreviation	MW	RT (min)	Parent ion (m/z)	Fragment (m/z)	IS RT (min)	IS Parent ion (m/z)	IS Fragment (m/z)
80	Valerylcarnitine	Car5	245.32	7.78	547.15	386.15	7.61	553.15	392.15
81	3-Hydroxyhexanoyl carnitine	Car6-OH	275.34	7.25	577.15	386.15	7.11	583.15	392.15
82	Hexanoylcarnitine	Car6	259.34	9.33	561.15	386.15	9.14	567.15	392.15
83	L-Octanoylcarnitine	Car8	287.40	13.45	588.90	386.15	13.23	594.90	392.15
84	3-Hydroxydodecanoyl-L-carnitine	Car12-OH	359.50	18.27	661.40	386.15	18.16	667.40	392.15
85	Decanoyl-L-carnitine	Car10	315.45	17.51	617.35	386.15	17.36	623.35	392.15
86	trans-2-Dodecenoyl-L-carnitine	Car12:1	341.49	19.31	643.40	386.05	19.21	649.40	392.05
87	Lauroyl-L-carnitine	Car12	343.50	19.84	645.40	386.15	19.75	651.40	392.15
88	cis, cis-5,8-Tetradecandienoyl-L-carnitine	Car14:2	367.52	19.73	669.55	386.15	19.65	675.55	392.15
89	trans-2-Tetradecenoyl-L-carnitine	Car14:1	369.54	21.05	671.55	386.15	20.99	677.55	392.15
90	Myristoyl-L-carnitine	Car14	371.55	21.50	673.45	386.05	21.44	679.45	392.05
91	cis, cis-9,12-Octadecadienoyl-L-carnitine	Car18:2	423.63	23.03	725.55	386.15	22.91	731.55	392.15
92	trans-2-Hexadecenoyl-L-carnitine	Car16:1	397.59	23.09	699.60	386.15	22.97	705.60	392.15
93	3-Hydroxyoctadecanoyl-L-carnitine	Car18-OH	443.66	23.76	745.60	386.15	23.75	751.60	392.15
94	Palmitoyl-L-carnitine	Car16	399.61	23.70	702.25	386.15	23.68	708.25	392.15
95	Oleoyl-L-carnitine	Car18:1	425.64	23.77	727.65	386.15	23.76	733.65	392.15
96	Stearoyl-L-carnitine	Car18	427.66	23.87	729.10	386.05	23.87	735.10	392.05
97	Acetic acid	C2	60.05	7.47	362.20	320.00	7.20	368.20	326.00
98	Propionic acid	C3	74.08	8.91	376.20	320.10	8.58	382.20	326.00
99	Isobutyric acid	C4-2	88.11	10.53	390.30	320.10	10.17	396.30	326.00
100	Butyric acid	C4-1	88.11	10.89	390.30	320.10	10.52	396.30	326.00
101	2-Methylbutyric acid	C5-1	102.13	12.67	403.80	320.10	12.29	409.80	326.10
102	Isovaleric acid	C5-2	102.13	13.03	404.10	320.20	12.65	410.10	326.00
103	Valeric acid	C5-3	102.13	13.54	404.10	320.10	13.17	410.10	326.00

No.	Metabolite	Abbreviation	MW	RT (min)	Parent ion (m/z)	Fragment (m/z)	IS RT (min)	IS Parent ion (m/z)	IS Fragment (m/z)
104	3-Methylvaleric acid	C6-2	116.16	15.66	418.10	320.10	15.29	424.10	326.10
105	Isocaproic acid	C6-3	116.16	16.08	418.10	320.20	15.72	424.10	326.20
106	Hexanoic acid	C6-1	116.16	16.45	418.10	320.00	16.10	424.10	326.00
107	2-Methylhexanoic acid	C7-2	130.18	17.93	432.10	320.20	17.68	438.10	326.20
108	Heptanoic acid	C7-1	130.18	18.78	432.10	320.20	18.56	438.10	326.20
109	2-Ethylhexanoic acid	C8-3	144.21	19.29	446.10	320.20	19.09	452.10	326.20
110	2-Methylheptanoic acid	C8-2	144.21	19.79	446.10	320.10	19.61	452.10	326.10
111	Octanoic acid	C8-1	144.21	20.52	446.10	320.20	20.35	452.10	326.20
112	4-Methyloctanoic acid	C9-2	158.24	21.60	460.10	320.10	21.45	466.10	326.10
113	Nonanoic acid	C9-1	158.24	22.03	460.10	170.00	21.87	466.10	326.20
114	Decanoic acid	C10	172.26	23.70	473.60	170.00	23.63	479.60	176.00
115	Dodecanoic acid	C12	200.32	24.79	501.70	320.30	24.74	507.70	176.00
116	Tridecanoic acid	C13	214.34	25.43	515.90	320.10	25.21	521.90	326.00
117	Myristic acid	C14	228.37	26.21	530.40	320.00	26.13	536.40	326.00
118	cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5	302.45	26.34	604.20	320.00	26.27	610.20	326.00
119	γ-Linolenic acid	C18:3	278.43	26.41	580.00	320.10	26.34	586.00	326.00
120	Palmitoleic acid	C16:1	254.41	26.71	556.50	320.10	26.62	562.00	326.00
121	Pentadecanoic acid	C15	242.40	27.17	544.20	320.00	27.08	550.20	326.00
122	cis-4,7,10,13,16,19-Docosahexaenoic acid	C22:6	328.49	27.19	630.30	157.00	27.09	636.00	326.00
123	Arachidonic acid	C20:4	304.47	27.39	606.20	157.00	27.29	612.20	326.10
124	Linoleic acid	C18:2	280.45	27.45	582.30	263.45	27.36	588.00	326.10
125	Palmitic acid	C16	256.42	28.40	558.20	320.00	28.28	564.00	326.10
126	Oleic acid	C18:1	282.46	29.13	584.30	319.50	28.99	590.30	326.20
127	Stearic acid	C18	284.48	30.65	586.30	320.10	30.59	592.00	326.20
128	cis-11-Eicosenoic acid	C20:1	310.51	30.84	612.30	320.00	30.79	618.30	326.00

No.	Metabolite	Abbreviation	MW	RT (min)	Parent ion (m/z)	Fragment (m/z)	IS RT (min)	IS Parent ion (m/z)	IS Fragment (m/z)
129	Nonadecanoic acid	C19	298.50	31.12	600.50	320.10	31.08	606.50	326.00
130	Erucic acid	C22:1	338.57	31.74	640.50	320.10	31.69	646.50	326.20
131	Heneicosanoic acid	C21	326.56	32.07	628.40	320.20	32.03	634.40	326.20
132	Behenic acid	C22	340.58	32.54	642.00	320.20	32.50	648.00	326.20
133	Nervonic acid	C24:1	366.62	32.67	668.30	320.20	32.61	674.30	326.20
134	Elaidic acid	isoC18:1	282.46	29.48	584.88	320.10	29.34	590.50	326.20
135	2-Oxoadipic Acid	OAA	160.12	19.62	763.45	170.10	19.31	775.45	176.10
136	Indolelactic acid	ILA	205.21	11.19	507.15	170.10	10.86	513.15	176.00
137	5-Methoxyindoleacetic acid	MIAA	205.22	11.20	507.00	320.10	10.87	513.00	326.10
138	Indole-3-Acetic acid	IAA	175.18	12.43	477.20	320.20	12.09	483.20	326.20
139	3-Indolepropionic acid	IPA	189.21	13.60	491.65	320.10	13.26	497.65	326.10
140	2-Picolinic acid	PA	123.11	8.95	425.05	170.10	8.64	431.05	176.10
141	α-Ketoglutaric acid	KGA	146.10	19.69	748.95	320.20	19.38	760.95	326.20
142	Succinic acid	SAD	118.09	18.52	721.45	170.20	18.11	733.45	176.00
143	Fumaric acid	FA	116.07	18.87	719.10	170.00	18.49	731.10	176.00
144	Malic acid	MalA	134.09	17.87	737.40	170.20	17.43	749.40	176.00
145	Pyruvic acid	PyrA	88.06	8.71	390.00	170.20	8.39	396.00	176.00
146	Citric acid	CitrA	192.12	23.88	1096.90	320.20	23.80	1114.90	326.00
147	Ketoleucine	KICA	130.14	16.09	432.25	170.20	15.72	438.25	176.20
148	3-Methyl-2-oxovaleric acid	KMVA	130.14	15.65	432.25	170.00	15.28	438.25	176.00
149	alpha-Ketoisovaleric acid	KIVA	116.12	12.73	418.25	170.20	12.35	424.25	176.20
150	3-Hydroxybutyric acid	BHB	104.10	7.68	406.00	320.20	7.40	412.00	176.00
151	Isocitric acid	isoCitrA	192.12	23.68	1096.80	170.10	23.49	1114.80	176.00

Abbreviations: MW: molecular weight; RT: Retention time; IS: Internal standard.

Table S3. Differential metabolites between UUO and Sham group

No	Metabolite	Abb.	pFDR	FC (UUO/Control)
1	5-Methylcytidine	MeCyd	1.12E-03	27.11
2	1-Methyladenosine	MeAdo	2.66E-04	19.70
3	5-Methoxyindoleacetic acid	MIAA	9.45E-04	16.54
4	2-Oxoadipic acid	OAA	1.38E-03	15.26
5	Indolelactic acid	ILA	8.05E-04	9.50
6	3-Hydroxydodecanoyl-L- carnitine	Car12-OH	3.51E-04	7.41
7	2-Picolinic acid	PA	2.72E-05	7.30
8	5-Hydroxyindoleacetate	HIAA	5.48E-04	6.24
9	3-Hydroxyoctadecanoyl-L-carnitine	Car18-OH	2.14E-04	5.81
10	Palmitoyl-L-carnitine	Car16	2.51E-04	4.99
11	Stearoyl-L-carnitine	Car18	1.80E-04	4.67
12	Cytidine	Cyd	9.50E-04	4.64
13	Kynurenine	KYN	7.53E-05	3.75
14	Nε-Acetyl-L-lysine	AceLys	2.18E-04	3.70
15	3-Indolepropionic acid	IPA	7.97E-03	3.58
16	trans-2-Hexadecenoyl-L-carnitine	Car16:1	2.19E-04	3.26
17	Kynurenic acid	KA	3.25E-04	3.07
18	Butyrylcarnitine	Car4	1.29E-05	3.02
19	Myristoyl-L-carnitine	Car14	5.80E-05	3.00
20	3-Hydroxyhexanoyl carnitine	Car6-OH	9.59E-04	3.00
21	Glycoursodeoxycholic acid	GUDCA	1.88E-03	2.87
22	5-Hydroxy-L-tryptophan	HTP	7.12E-04	2.86
23	Nervonic acid	C24:1	4.81E-04	2.67
24	L-Acetylcarnitine	Car2	7.93E-05	2.49
25	Oleoyl-L-carnitine	Car18:1	4.50E-04	2.39
26	Malic acid	MalA	3.63E-04	2.33
27	Cytosine	Cyt	1.11E-02	2.31
28	NG, NG-Dimethy-L-arginine	ADMA	6.38E-08	2.28
29	Valerylcarnitine	Car5	8.12E-05	2.21
30	Isovaleryl-L-carnitine	isoCar5	1.36E-04	2.19
31	alpha-Ketoisovaleric acid	KIVA	5.84E-03	2.17
32	2′-Deoxyuridine	dU	2.95E-04	2.13
33	3-Methyl-2-oxovaleric acid	KMVA	3.72E-02	2.13
34	L-Citrulline	Cit	1.82E-05	2.11
35	Pentadecanoic acid	C15	3.99E-02	1.97
36	trans-2-Tetradecenoyl-L-carnitine	Car14:1	9.18E-03	1.91
37	L-Ornithine	Orn	1.14E-02	1.90
38	3-Hydroxybutyryl-carnitine	Car4-OH	1.93E-03	1.75
39	6-Hydroxymelatonin	HMT	3.68E-02	1.72
40	4-Aminobutyric acid	GABA	7.52E-04	1.68

No	Metabolite	Abb.	pFDR	FC (UUO/Control)
41	cis, cis-9,12-Octadecadienoyl-L-carnitine	Car18:2	2.30E-03	1.64
42	Oleic acid	C18:1	2.41E-03	0.63
43	Uridine	Urd	1.94E-03	0.53
44	Tiglylcarnitine	Car5:1	6.13E-03	0.52
45	L-Octanoylcarnitine	Car8	4.68E-03	0.48
46	Ethanolamine	EA	1.41E-05	0.47
47	Linoleic acid	C18:2	3.07E-04	0.41
48	Pyruvic acid	PyrA	4.69E-05	0.33
49	Succinic acid	SAD	1.13E-03	0.21
50	Tryptamine	Trpm	4.75E-05	0.16

Abb.: abbreviation; FDR: false discovery rate; FC: fold change.

Table S4. LEfSe analysis at the genus level between Sham and UUO

Group	Biomarker ID	log10 (abundance)	LDA
UUO	*g_Anaerotruncus*	2.71	2.45
UUO	*g_Helicobacter*	3.97	3.56
UUO	*g_Bacteroides*	3.87	3.33
UUO	*g_Parasutterella*	3.74	3.43
UUO	*g_Parabacteroides*	2.72	2.35
Sham	*g_Adlercreutzia*	2.14	2.75
Sham	*g_Allobaculum*	2.44	2.77
Sham	*g_Holdemania*	1.90	2.95
Sham	*g_Dubosiella*	4.98	4.51
Sham	*g_Bacillus*	1.20	3.48

Table S5. LEfSe analysis at the genus level between UUO and FPSH

Group	Biomarker ID	log10 (abundance)	LDA
UUO	*g_Faecalibaculum*	4.43	4.13
UUO	*g_Romboutsia*	3.40	3.03
UUO	*g_Turicibacter*	4.07	3.84
UUO	*g_Enterorhabdus*	3.40	3.04
UUO	*g_Dubosiella*	4.43	4.08
UUO	*g_Parasutterella*	3.74	3.34
UUO	*g_Olsenella*	1.72	3.32
UUO	*g_Bifidobacterium*	4.09	3.82
FPSH	*g_Prevotella*	4.61	4.08
FPSH	*g_Candidatus_Soleaferrea*	1.03	3.56
FPSH	*g_Desulfovibrio*	3.23	2.93
FPSH	*g_Erysipelatoclostridium*	3.52	3.25
FPSH	*g_Akkermansia*	5.04	4.56
FPSH	*g_UCG*	1.77	3.12
FPSH	*g_Butyricicoccus*	2.29	2.45

Supplementary figures

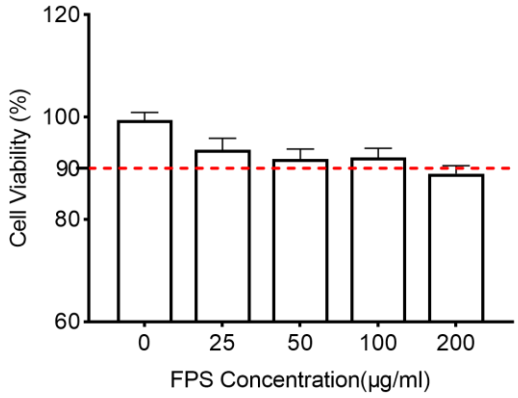


Fig. S1 HK-2 cell viability with FPS treatment.

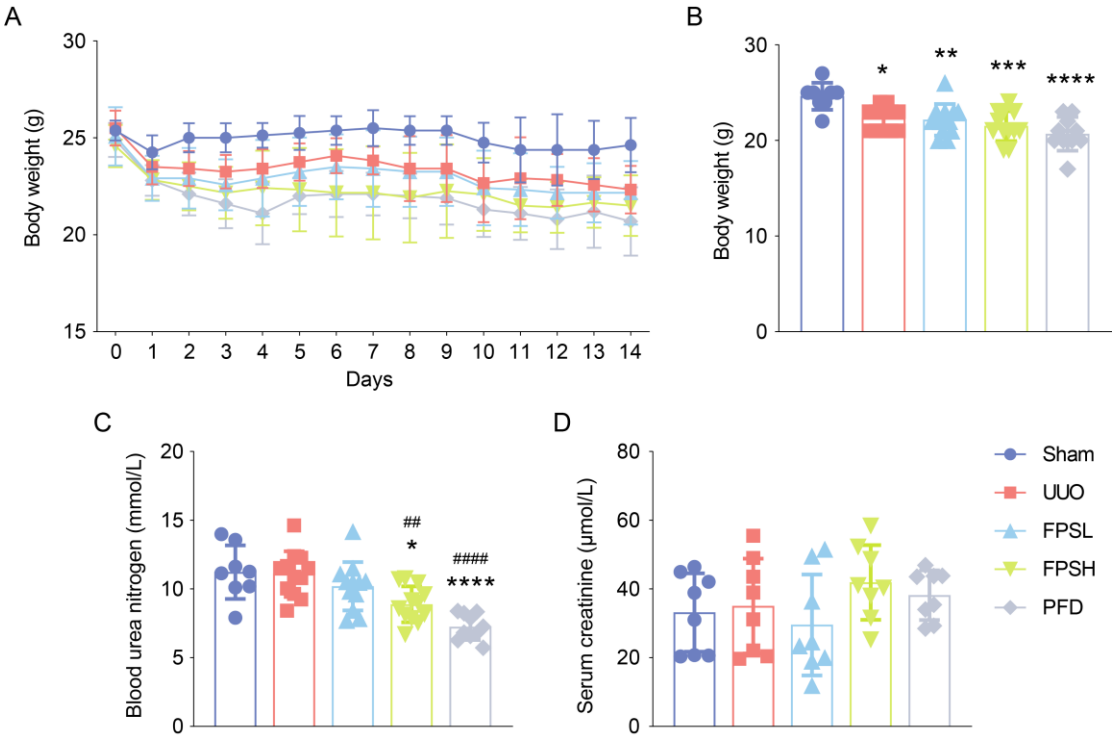


Fig. S2. Body weight and blood biochemical indexes in each group. (A) Daily body weight changes of mice in each group during the experimental period. (B) Comparison of body weight among groups at the end of the experiment. Blood urea nitrogen (C) and serum creatinine (D) levels in each group. Statistical significance is indicated as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ versus the Sham group; $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$ versus the UUO group.

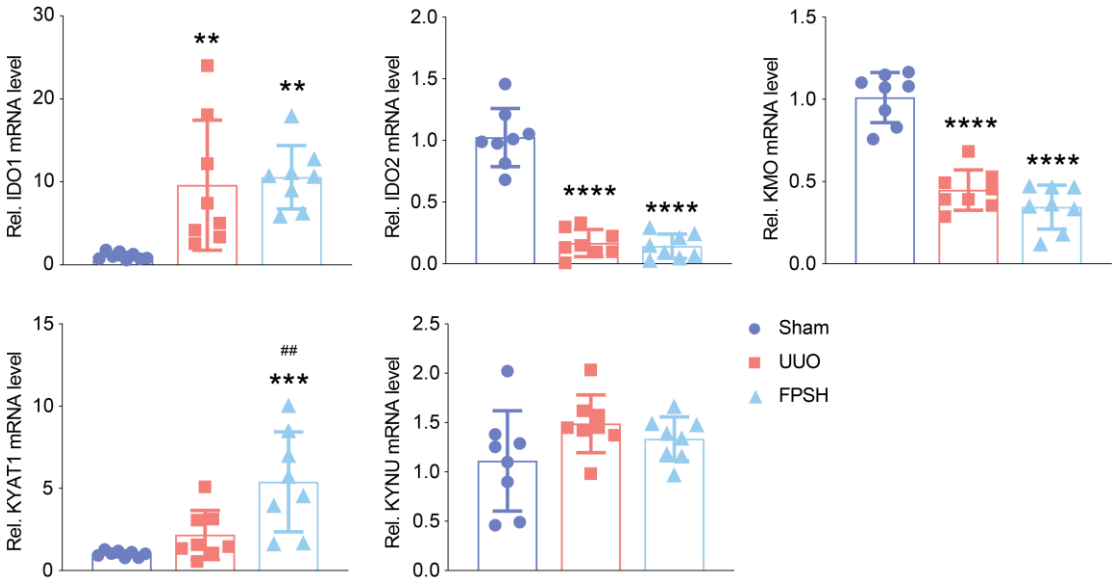
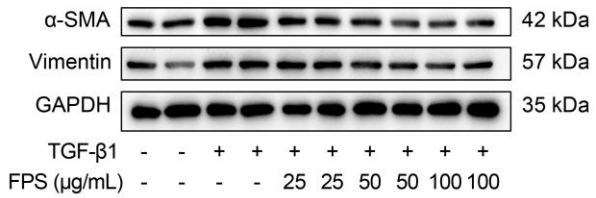
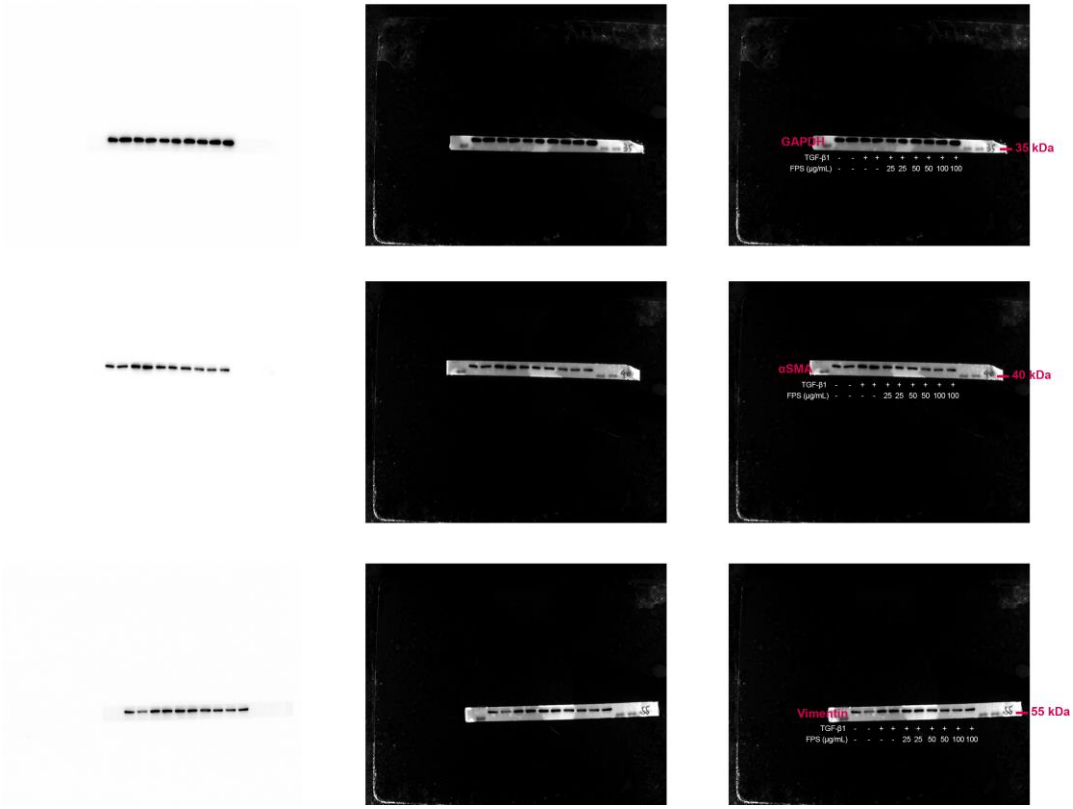


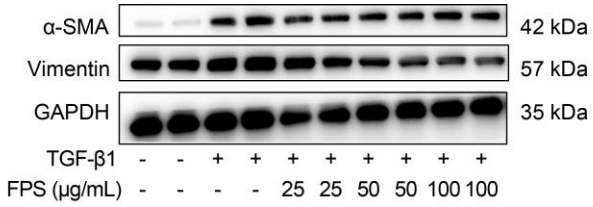
Fig S3. Relative mRNA level of IDO1, IDO2, KMO, KYAT1, and KYNU in kidney. Statistical significance is indicated as follows: $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ versus the Sham group; $^{\#\#}p < 0.01$ versus the UUO group.

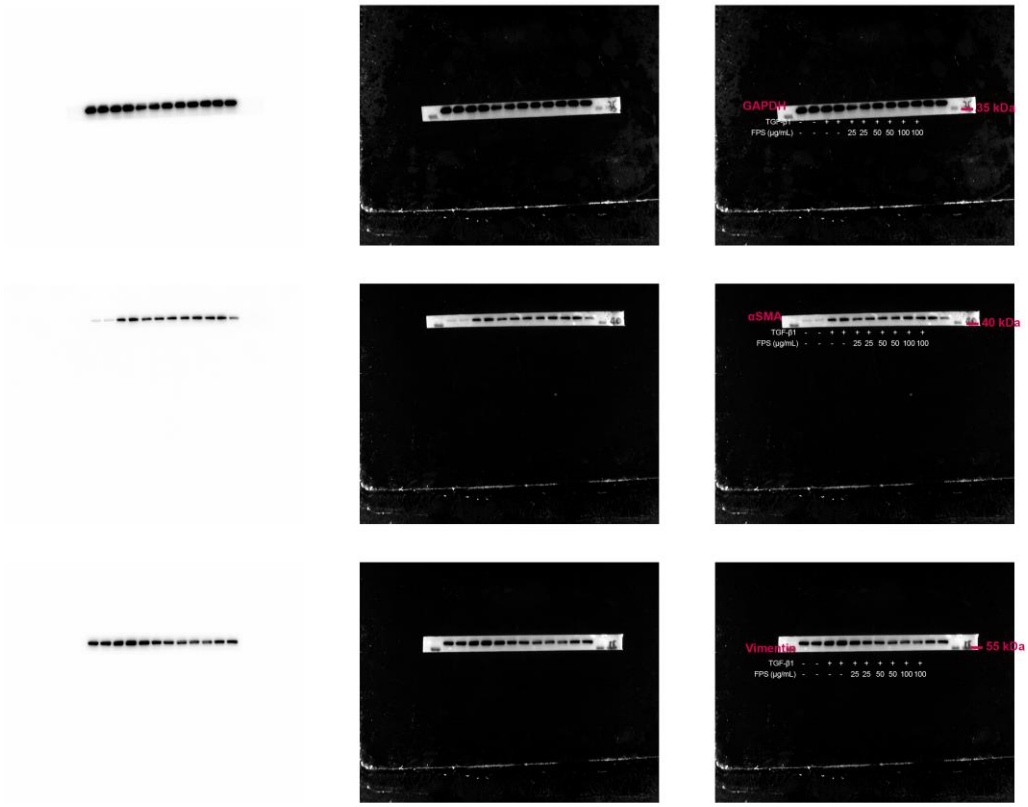
Uncropped and unprocessed western blot images

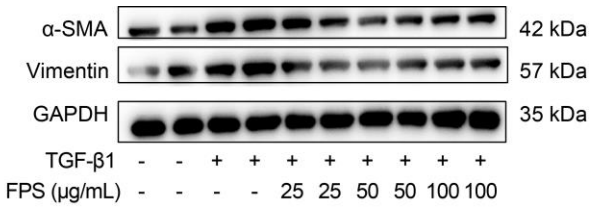
Corresponding to Fig 1. FPS attenuates TGF-β1-induced fibrosis in HK-2 cells. (G) Relative protein expression of α-SMA and Vimentin.

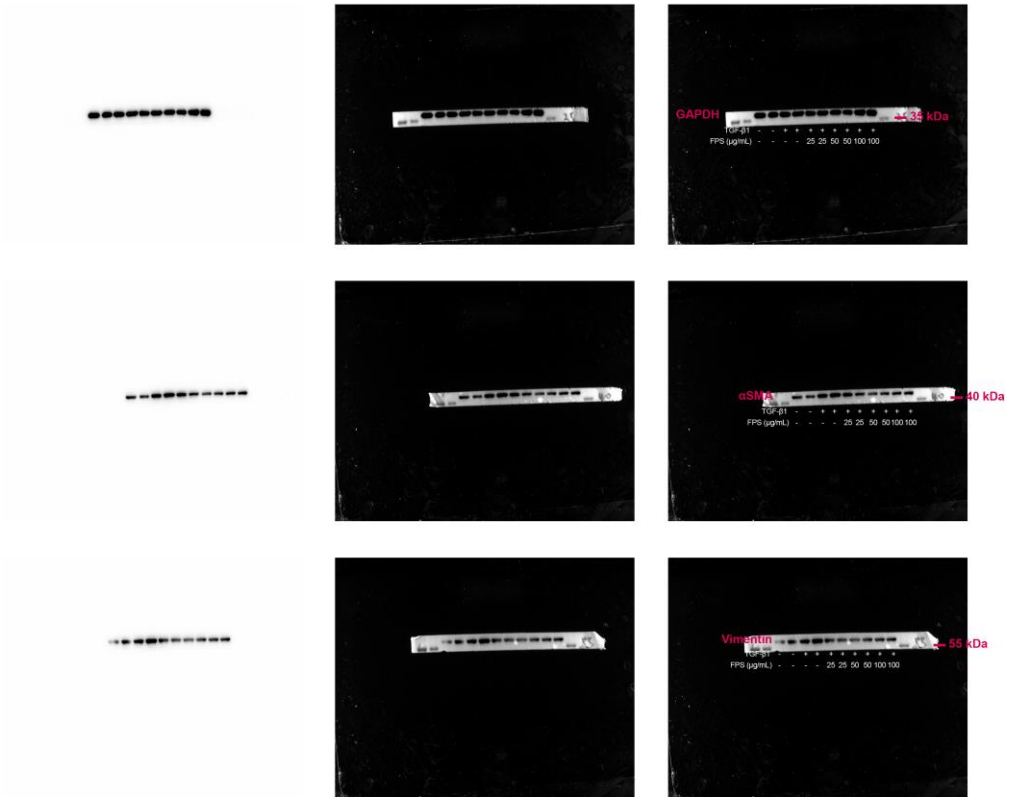




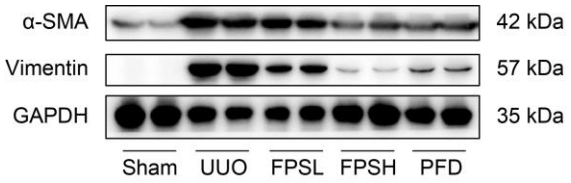

α-SMA
Vimentin
GAPDH
42 kDa
57 kDa
35 kDa
TGF-β1 - - + + + + + + + +
FPS (μg/mL) - - - - 25 25 50 50 100 100

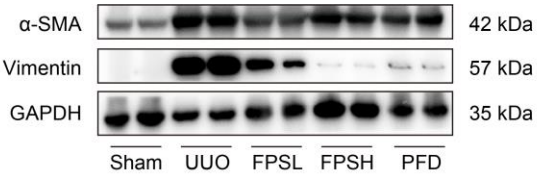

GAPDH
35 kDa
αSMA
40 kDa
Vimentin
55 kDa

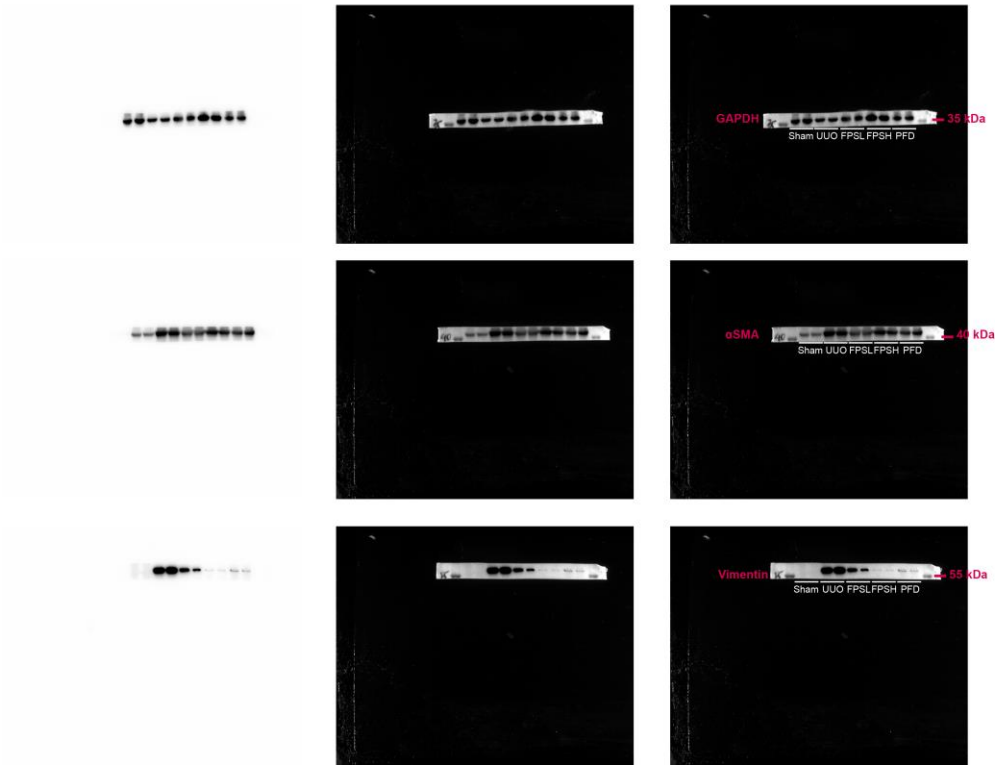

α-SMA
42 kDa
Vimentin
57 kDa
GAPDH
35 kDa
TGF-β1 - - + + + + + + + +
FPS (μg/mL) - - - - 25 25 50 50 100 100

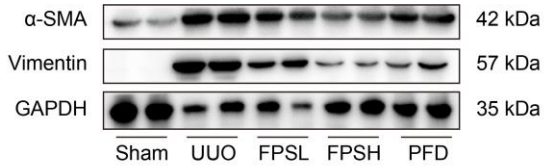

GAPDH
35 kDa
αSMA
40 kDa
Vimentin
55 kDa

Corresponding to Fig. 2. FPS ameliorates UUO-induced renal fibrosis in mice. (H) Relative protein expression of α-SMA and Vimentin.




α-SMA
42 kDa
Vimentin
57 kDa
GAPDH
35 kDa
Sham
UUO
FPSL
FPSH
PFD


GAPDH
35 kDa
Sham UUO FPSL FPSH PFD
αSMA
40 kDa
Sham UUO FPSL FPSH PFD
Vimentin
55 kDa
Sham UUO FPSL FPSH PFD


α-SMA
42 kDa
Vimentin
57 kDa
GAPDH
35 kDa
Sham
UUO
FPSL
FPSH
PFD


GAPDH
Sham UUO FPSLFPSH PFD
αSMA
Sham UUO FPSLFPSH PFD
Vimentin
Sham UUO FPSLFPSH PFD