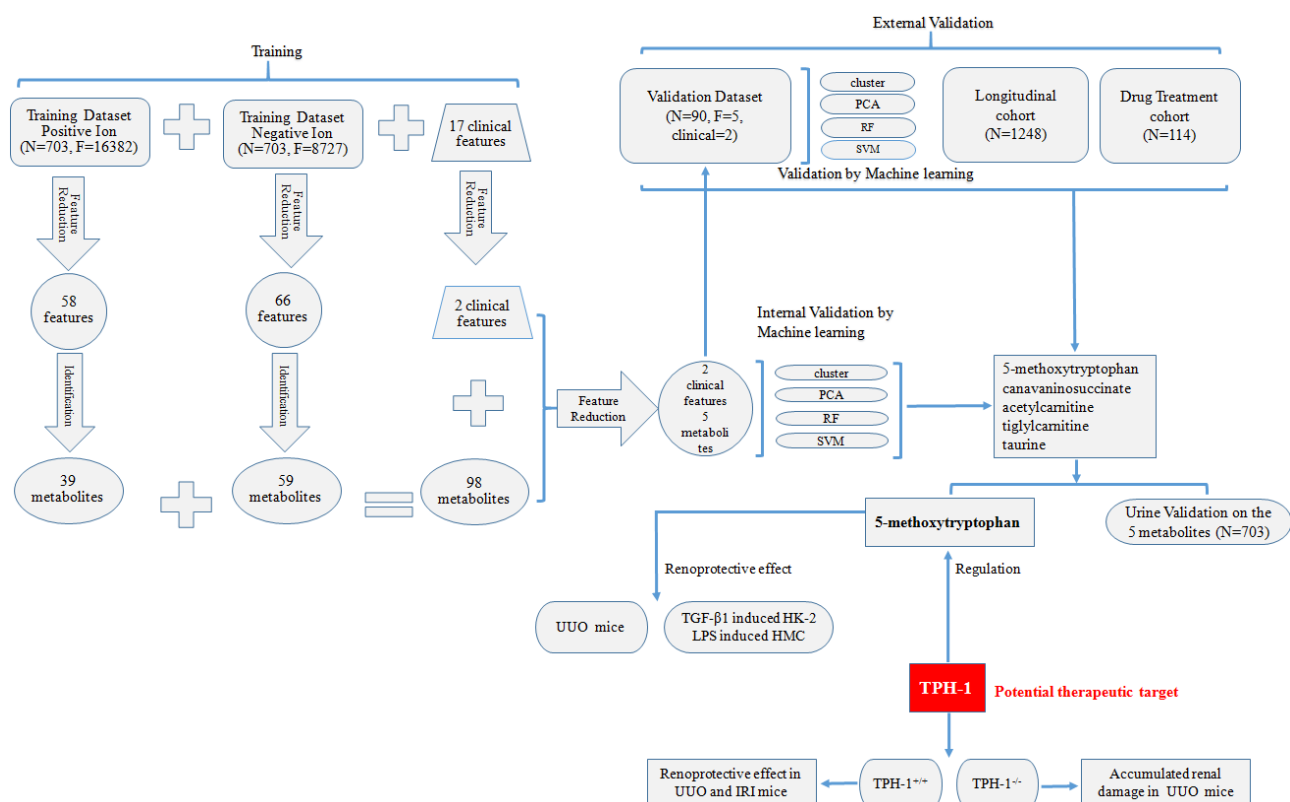
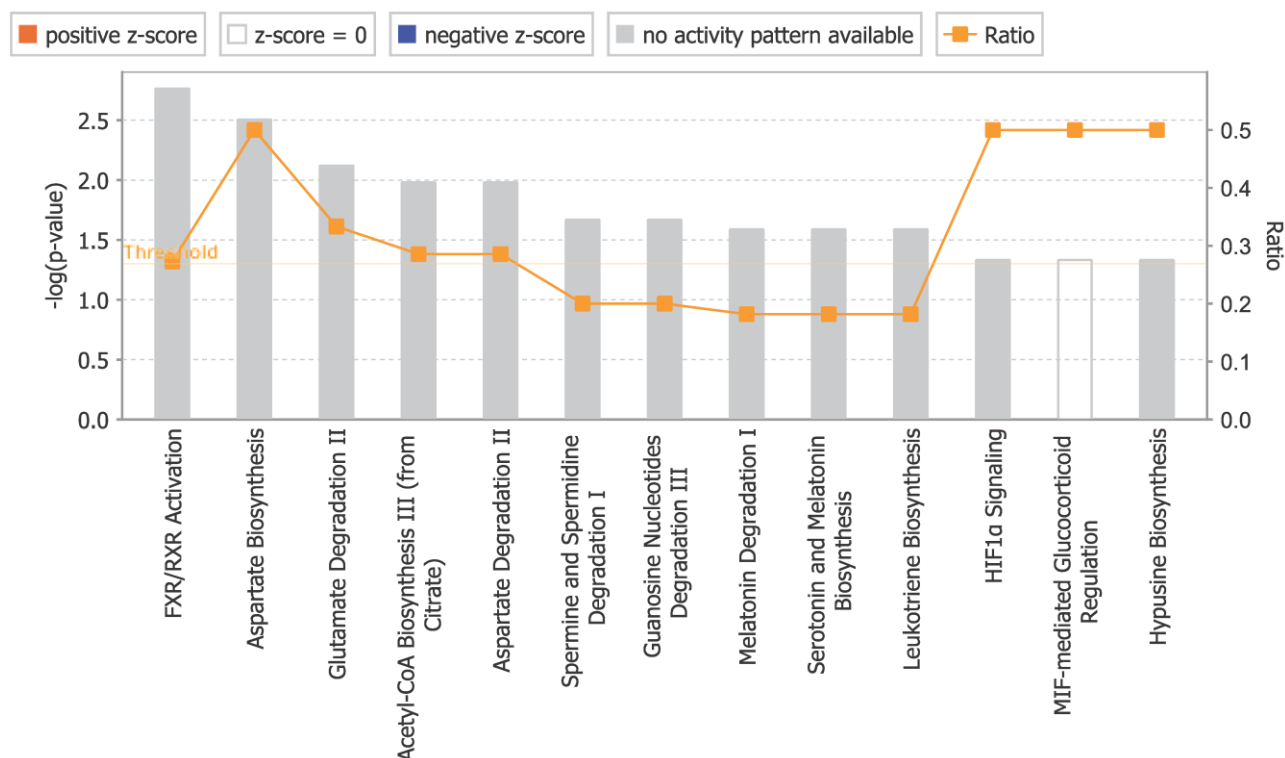


Identification of serum metabolites associating with chronic kidney disease progression and anti-fibrotic effect of 5-methoxytryptophan

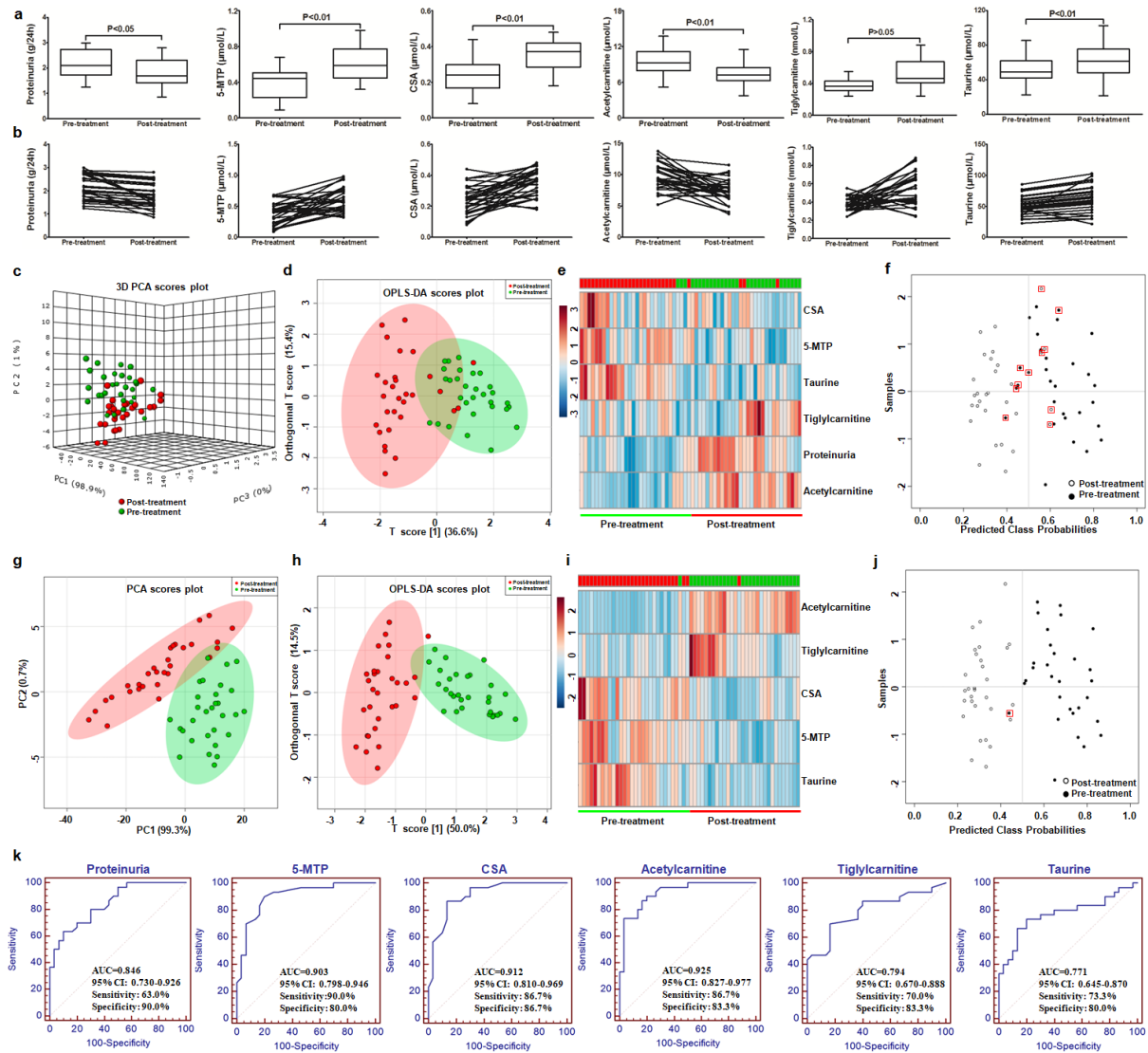
Chen *et al.*

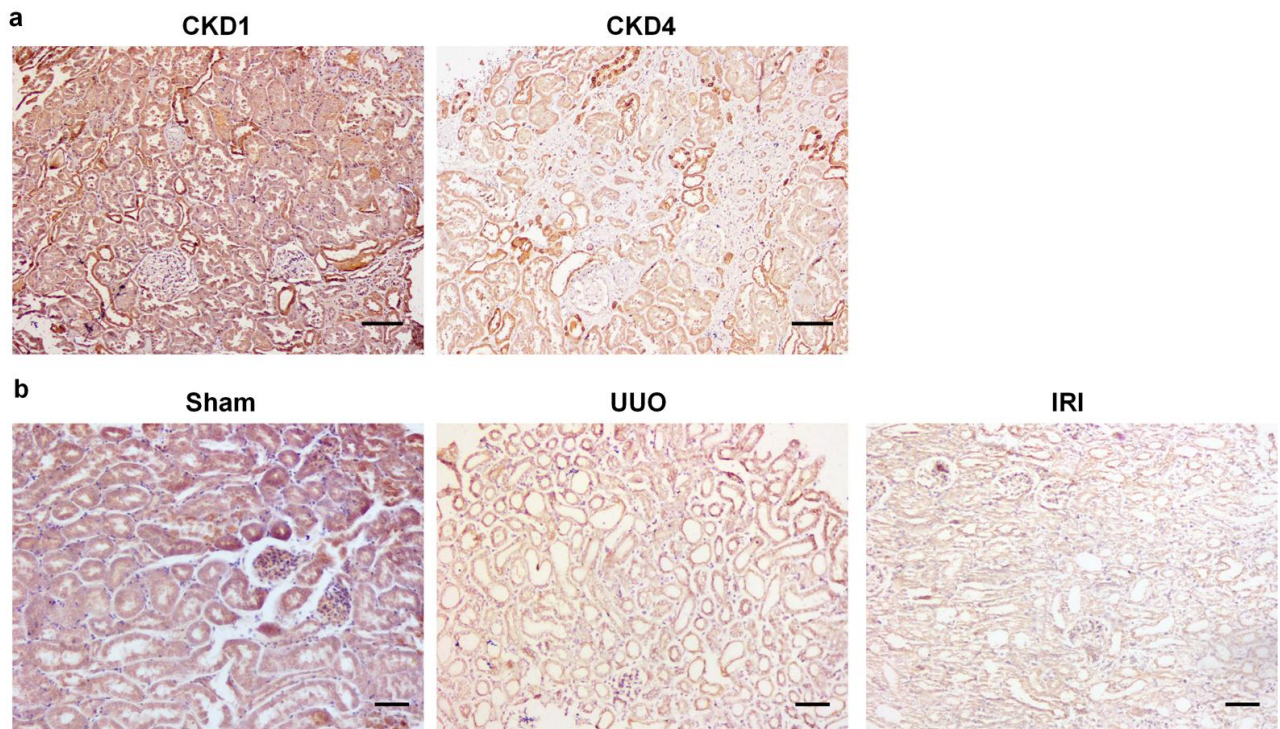


Supplementary Figure 1. The overview of study design. Flow diagram of metabolomic analysis presented the phases of biomarker discover and biomarker validation by a cohort of 90 healthy controls and patients with stages 1-2 CKD; a longitudinal cohort study of 1,248 patients without CKD followed for up to six years; and a pre- and post-drug intervention study of 114 patients with CKD2. Five metabolites, 5-MTP, CSA, acetylcarnitine, tiglylcarnitine and taurine were identified as biomarkers for progressive CKD. 5-MTP was selected for further investigation. 5-MTP and its regulatory TPH-1 enzyme attenuated renal inflammation and fibrosis to reduce renal injury. TPH-1 has the potential to be developed as a new target for new drug discovery and treatment of CKD. RF, Random Forest; SVM, Support vector machine.

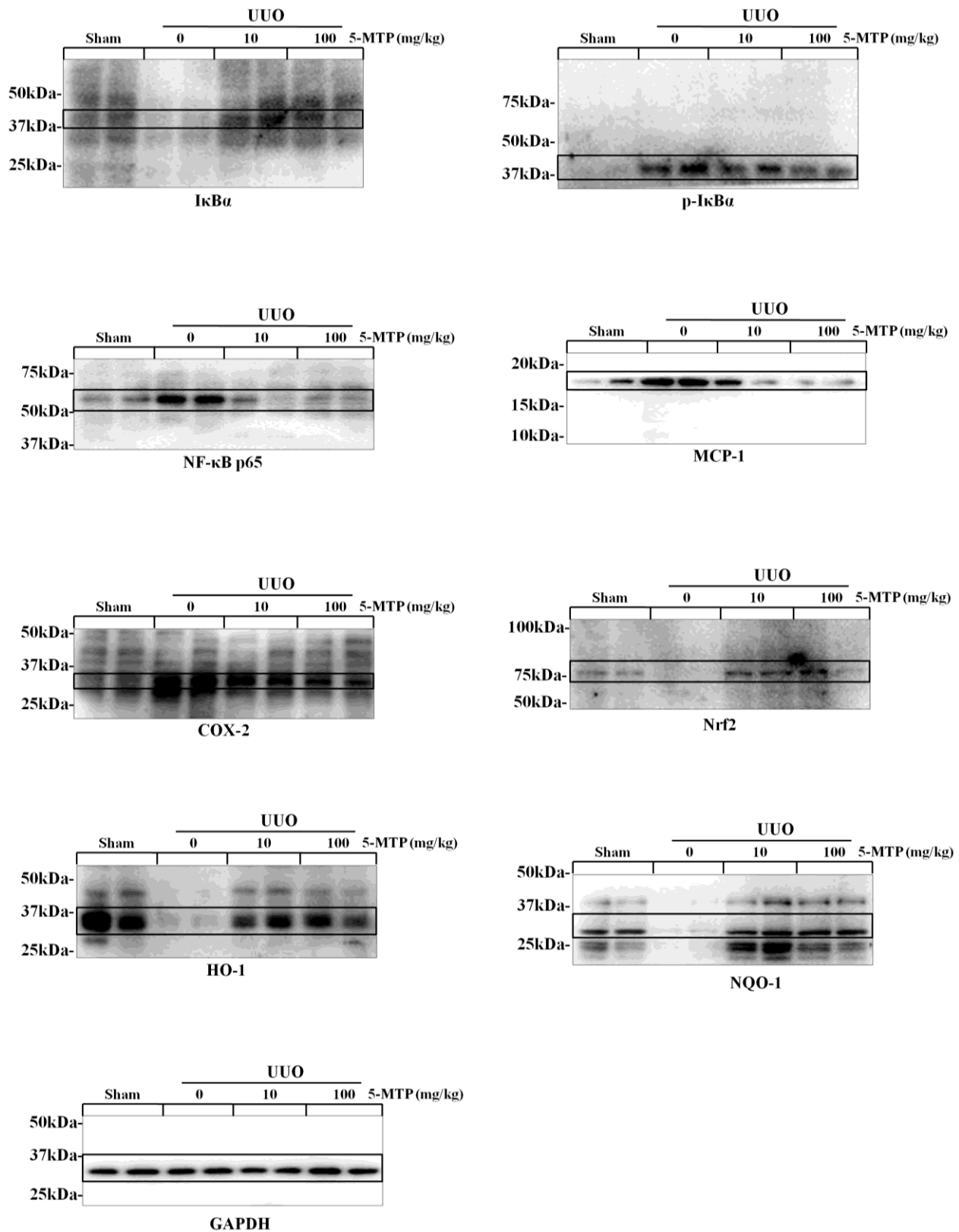


Supplementary Figure 2. The significant Canonical Pathways identified with the 98 metabolites. The pathways are displayed along the x-axis. As the default the y-axis displays the $-\log$ of p-value which is calculated by right tailed Fisher's exact test. Thus taller bars equate to increased significance and the canonical pathways are sorted by significance from left to right. White bars are those with a z-score at or very close to 0. Gray bars indicate pathways where no prediction can currently be made. The orange points connected by a thin line represent the Ratio. The ratio is calculated as follows: # of genes in a given pathway that meet your cutoff criteria, divided by the total # of metabolites that make up that pathway and that are in the reference gene set.

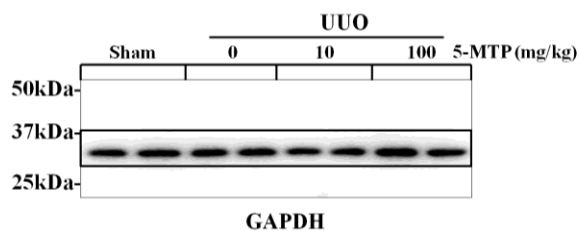
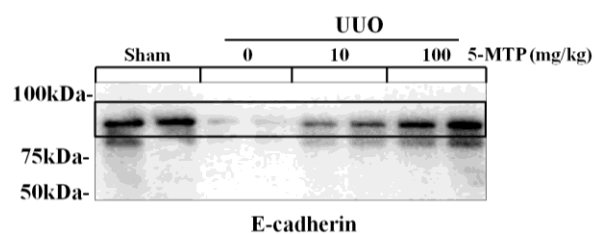
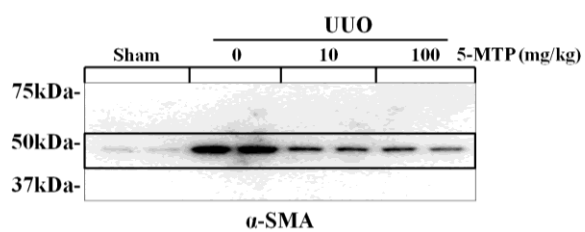
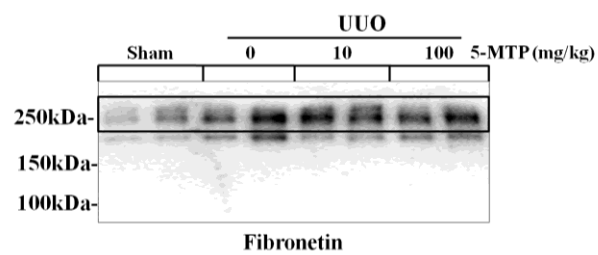
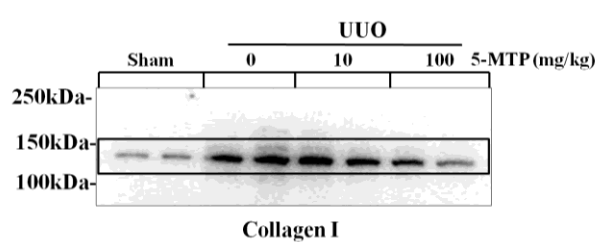




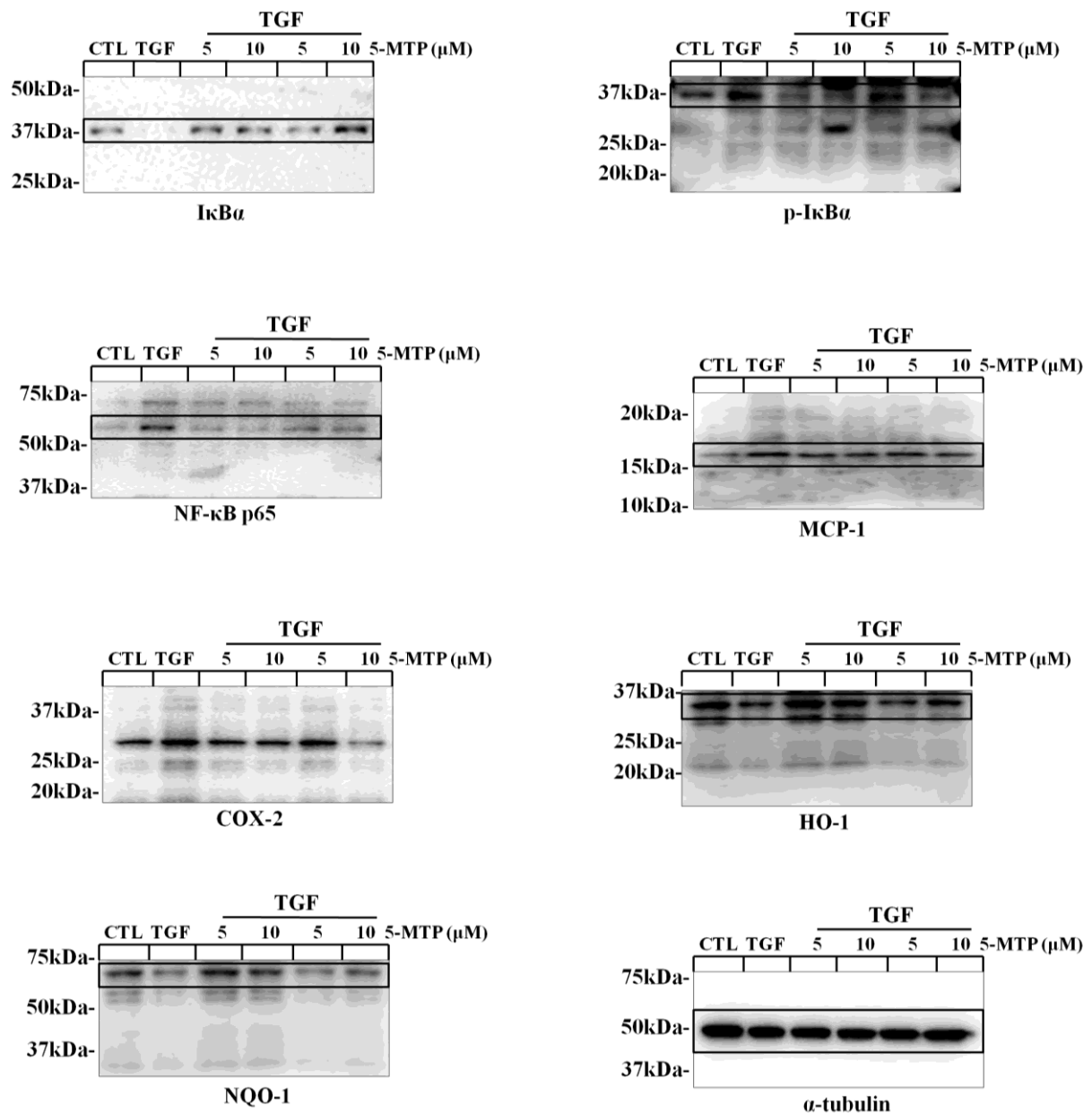
Supplementary Figure 4. TPH-1 staining of kidney tissues. **a** TPH-1 staining of kidney tissues of patients with CKD1 and CKD4, respectively. Scale bar 400 μm . **b** TPH-1 staining of kidney tissues of UUO and IRI mice. In normal station. Scale bar 50 μm .



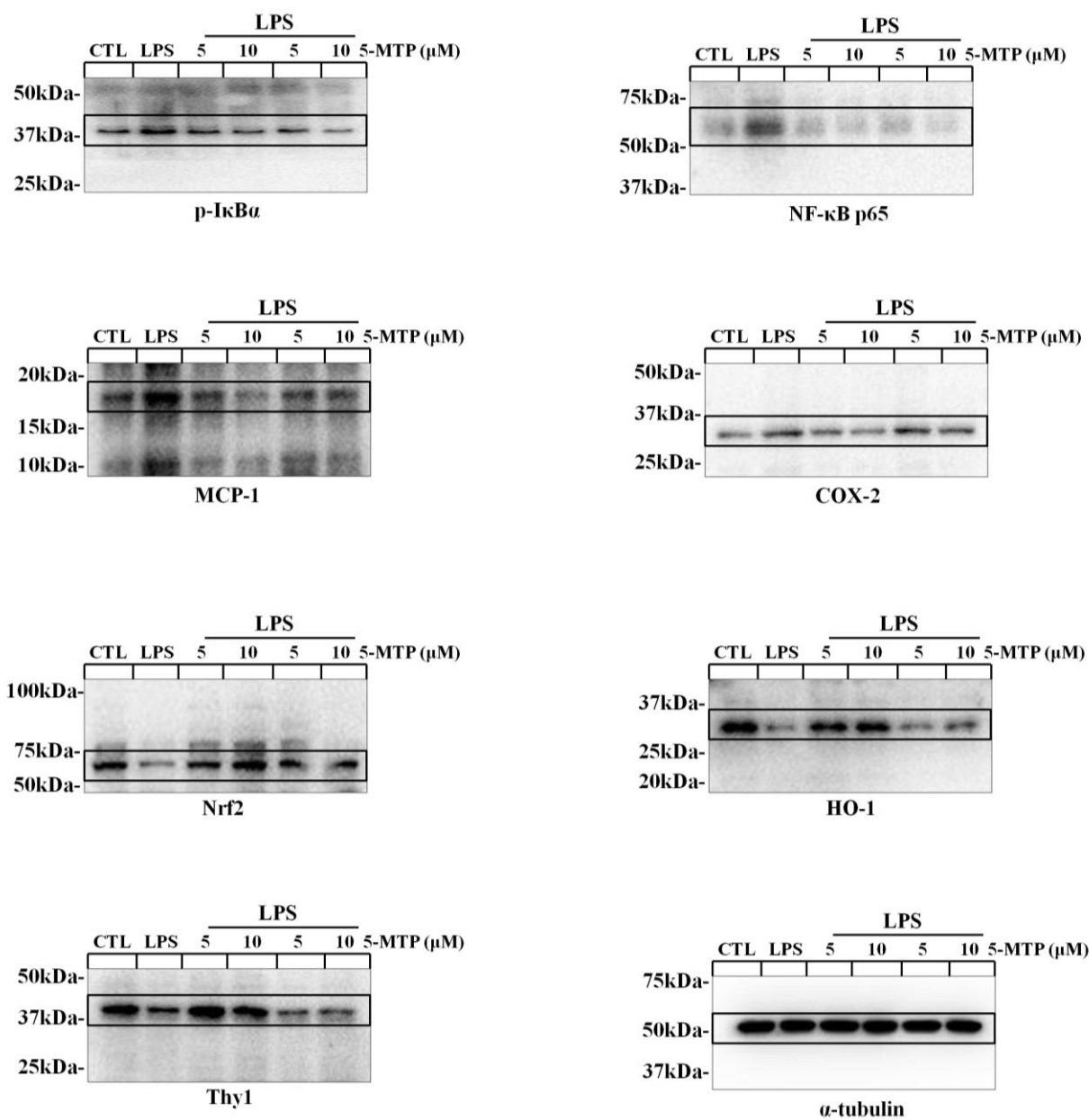
Supplementary Figure 5. Detailed western blots for Figure 6c. The relevant figures are indicated in the blots.



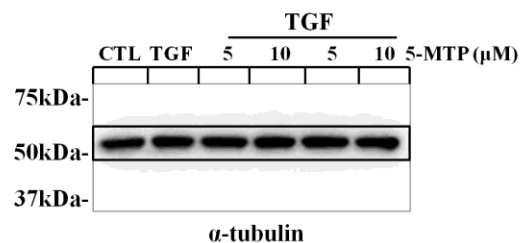
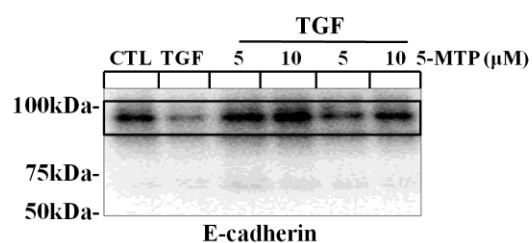
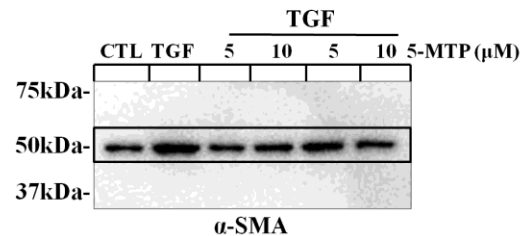
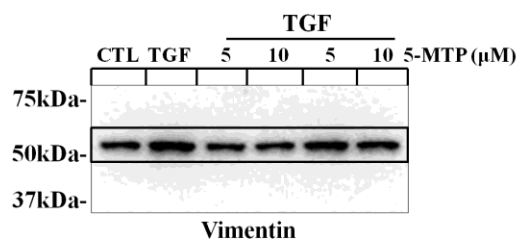
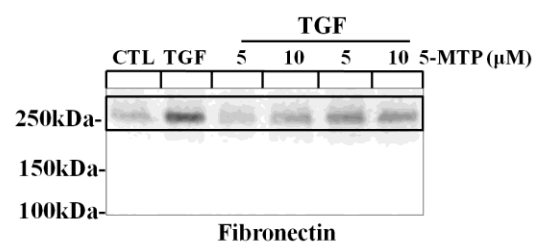
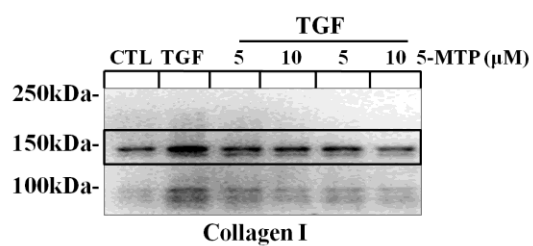
Supplementary Figure 6. Detailed western blots for Figure 6h. The relevant figures are indicated in the blots.



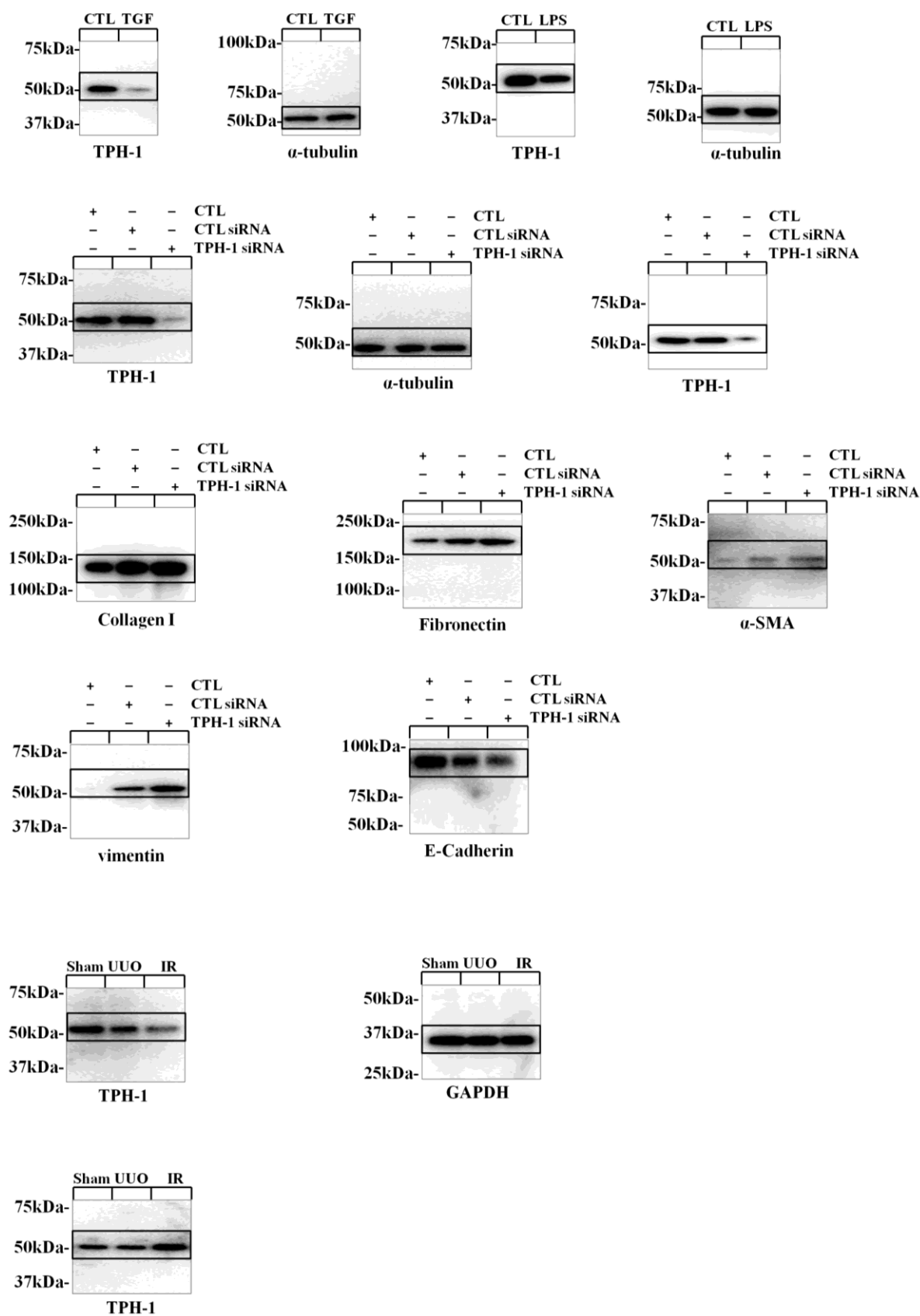
Supplementary Figure 7. Detailed western blots for Figure 7a. The relevant figures are indicated in the blots.



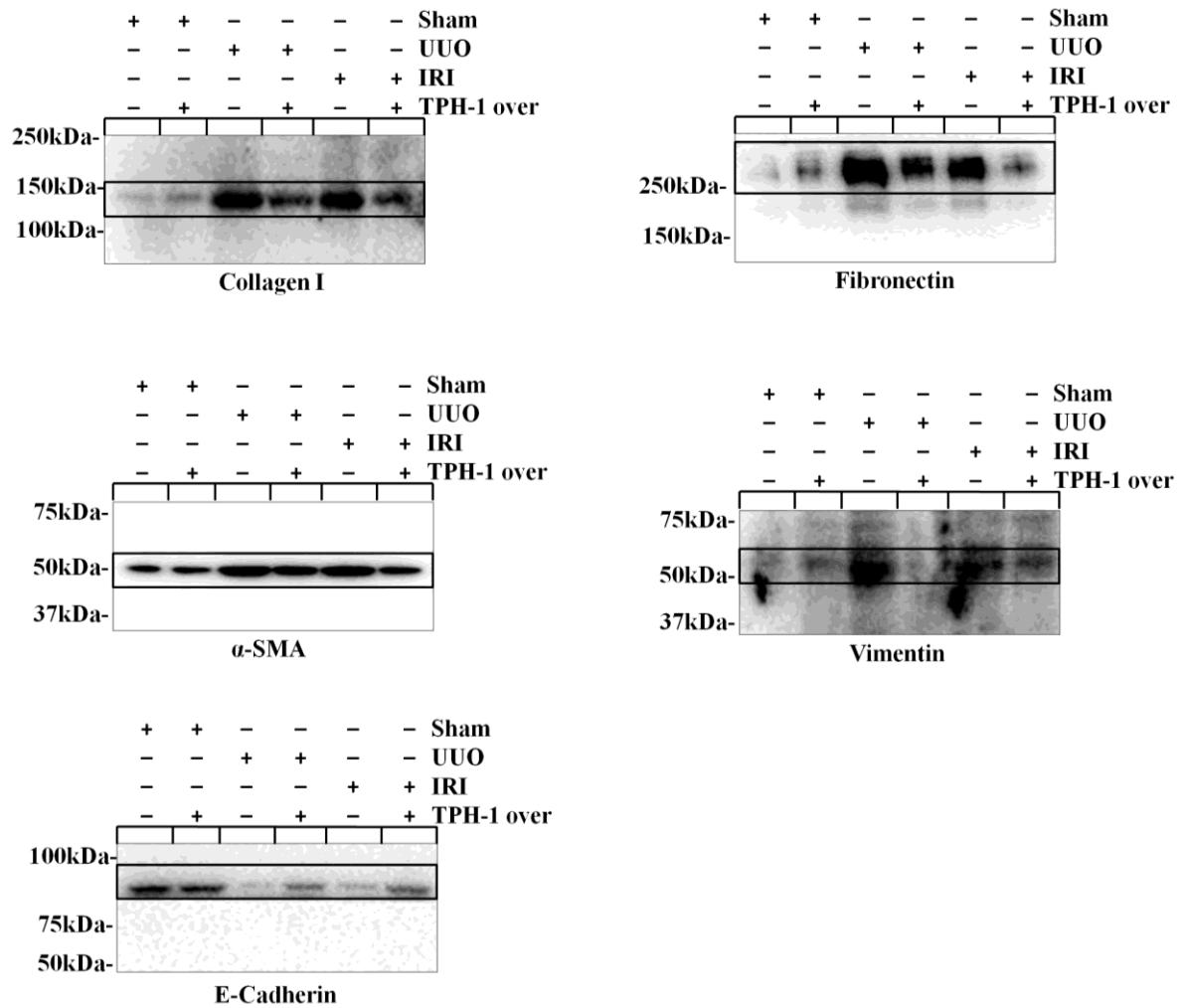
Supplementary Figure 8. Detailed western blots for Figure 7d. The relevant figures are indicated in the blots.



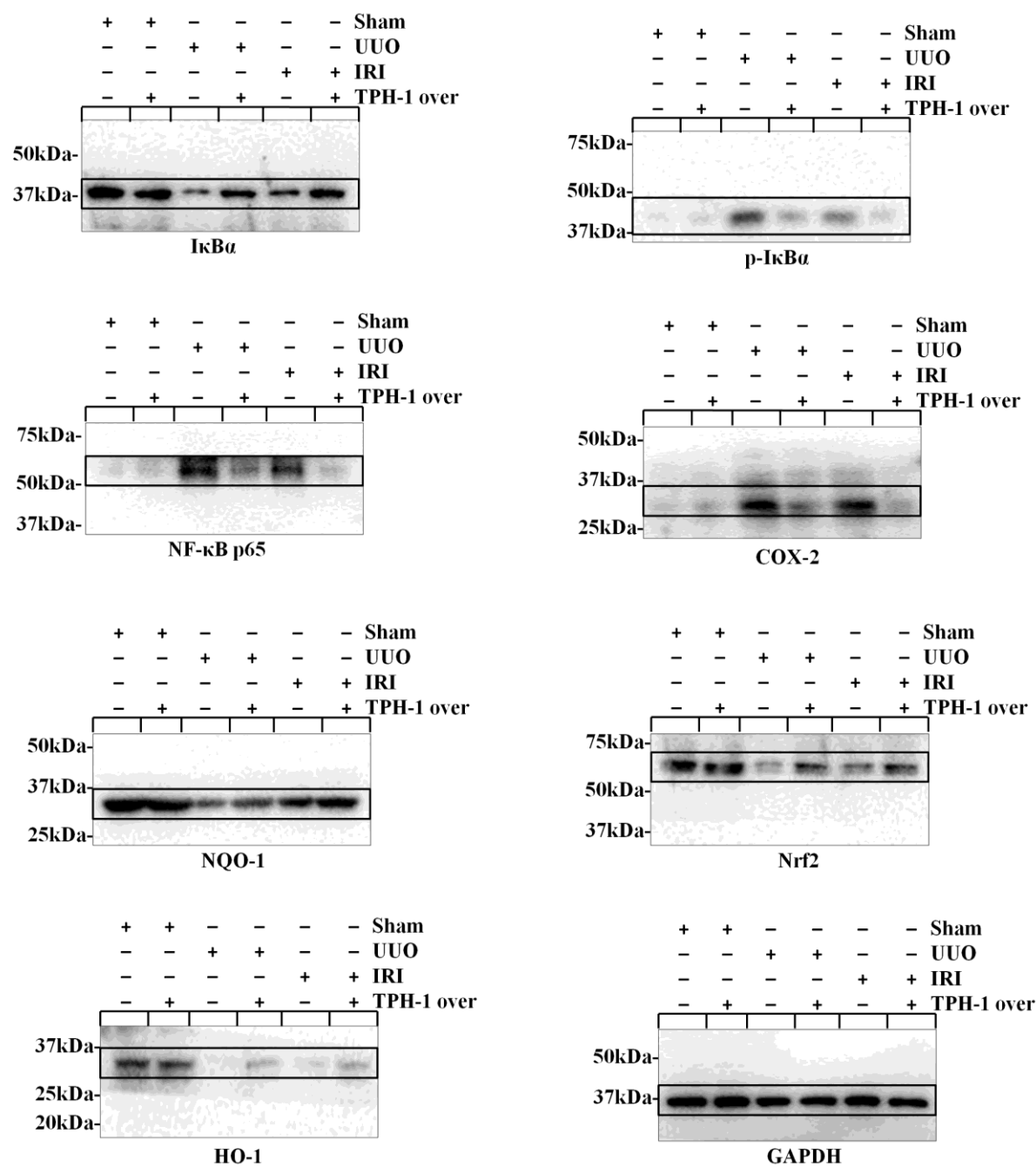
Supplementary Figure 9. Detailed western blots for Figure 7g. The relevant figures are indicated in the blots.



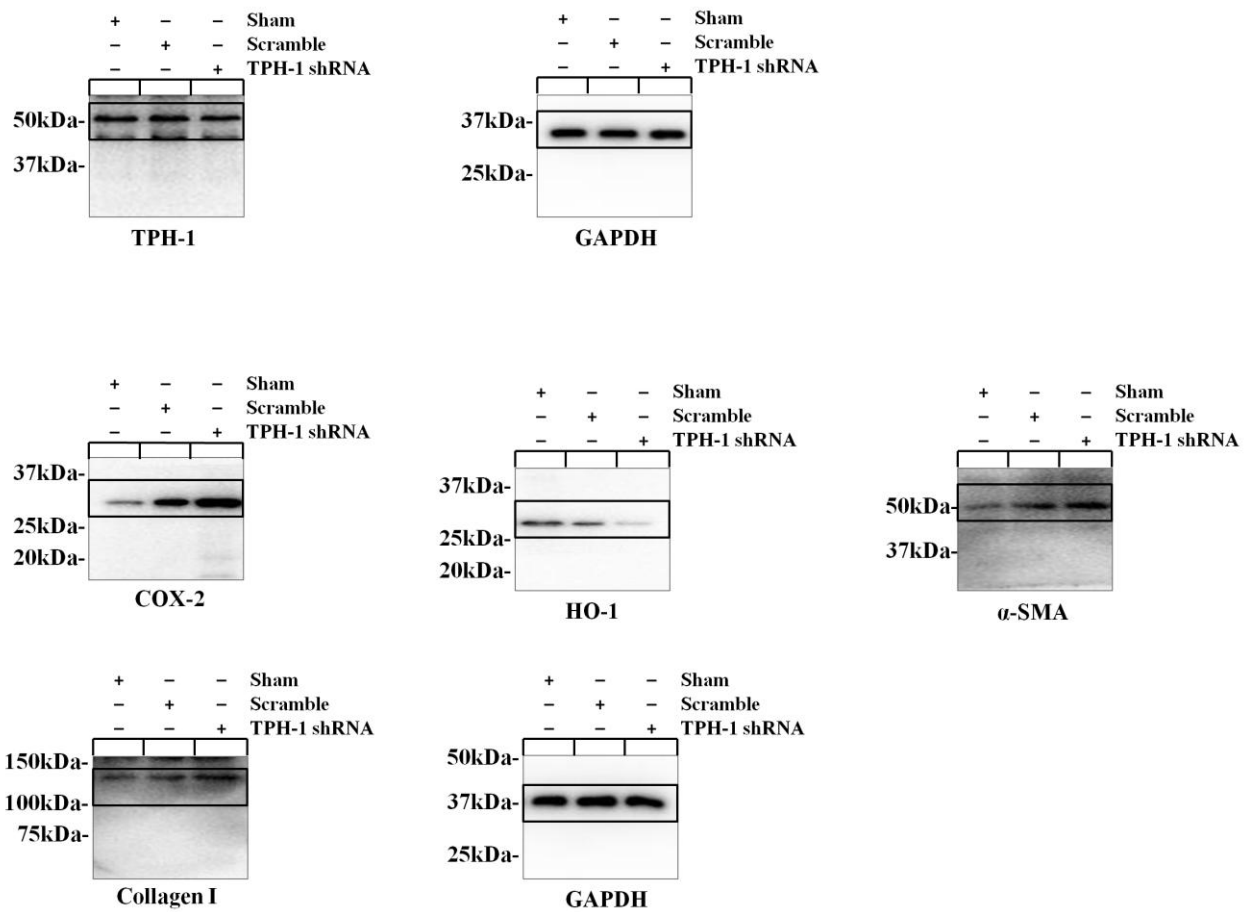
Supplementary Figure 10. Detailed western blots for Figure 8a-c, e and f. The relevant figures are indicated in the blots.



Supplementary Figure 11. Detailed western blots for Figure 8h. The relevant figures are indicated in the blots.



Supplementary Figure 12. Detailed western blots for Figure 8j. The relevant figures are indicated in the blots.



Supplementary Figure 13. Detailed western blots for Figure 8l and n. The relevant figures are indicated in the blots.

Supplementary Tables

Supplementary Table 1 Clinical and demographic baseline characteristics of healthy controls and CKD patients

Clinical characteristics	Normal	CKD1	CKD2	CKD3	CKD4	CKD5	Effect ^a	P ^b
Sample size	116	120	104	110	119	134	NA	NA
Men (%)	56.8	49.1	48.1	68.1	52.1	47	NA	NA
eGFR (mL/min/1.73 m ²)	103.5±12.9	104.9±13.3	75.4±8.8**	43.9±12.3**	21.0±4.5**	8.9±4.5**	NA	NA
Age (years)	54±14	53±16	52±11	54±15	60±15**	58±15*	0.0104	3.31E-02
BW (kg)	68±11	69±12	72±12**	78±13**	79±11**	80±13**	0.012	1.26E-04
BMI (kg/m ²)	23.7±3.7	24.7±4.1	24.9±5.2	26.4±4.4**	27.3±4.1**	27.9±5.1**	0.0519	1.24E-06
SBP (mm Hg)	121±17	124±18	125±16*	140±27**	138±20**	144±24**	0.0009	1.15E-01
DBP (mm Hg)	76±12	77±12	79±12*	84±17**	75±12	80±16**	-0.0023	3.94E-01
Total protein (g/L)	62.5±3.3	51.0±12.4**	61.9±11.8	66.2±10.1**	61.7±8.9	60.5±9.0	0.0271	1.55E-06
Albumin (g/L)	42.0±3.7	32.1±8.2**	36.2±8.7**	38.0±6.6**	36.1±6.1**	35.4±6.2**	-0.0513	7.62E-11
TC (mmol/L)	4.51±1.30	4.95±2.64**	4.30±1.95	4.61±1.52	4.50±1.49	4.30±1.26	-0.0578	1.50E-02
TG (mmol/L)	1.83±1.45	2.03±1.28	2.11±1.56	1.84±1.30	1.71±1.35	1.43±0.90	-0.0312	2.63E-01
HDL-C (mmol/L)	1.77±0.62	1.72±0.49	1.70±0.49	1.67±0.48	1.64±0.46*	1.59±0.47**	0.0394	5.82E-01
LDL-C (mmol/L)	2.90±1.00	4.48±2.02**	3.22±1.53*	3.65±1.68**	3.72±1.76**	3.72±1.77**	-0.0205	4.70E-01
CREA (μmol/L)	68±13	58±16	92±18**	153±33**	256±60**	598±280**	0.0025	8.71E-24
Urea (mmol/L)	5.5±5.4	4.8±1.4	5.9±2.1	9.2±2.5**	15.9±6.6**	22.3±8.0**	0.0697	2.12E-18
Uric acid (μmol/L)	327±93	323±100	315±76	437±123**	418±111**	423±138**	0.0008	1.35E-02
P/C ratio	0.15±0.04	3.94±3.28**	2.14±1.89**	2.53±2.19**	2.70±2.38**	2.76±2.41**	0.1008	3.06E-05
Proteinuria (g/24h)	0.07±0.02	2.84±2.31**	2.29±2.23**	2.26±2.36**	2.17±2.07**	2.30±2.25**	0.0367	1.90E-01

Associations of clinical variables with CKD stages. Results are expressed as the means ± SD, * $P < 0.05$, ** $P < 0.01$ compared with healthy controls. “NA”, not applicable. P/C, urine protein/urine creatinine; SPB, systolic pressure blood; DPB, diastolic pressure. ^a Positive effect indicates that the clinical variable is positively associated with CKD stage; negative effect indicates that the clinical variable is negatively associated with CKD stage. ^b P value was computed using original regression.

Supplementary Table 2 Final model with 5 metabolites and 2 clinical indexes in patients with CKD five stages

Metabolites	Effect ^a	Std Err ^b	p ^c
5-Methoxytryptophan	-0.0111	0.0015	8.22E-13
Canavaninosuccinate	-0.0069	0.0016	2.99E-05
Acetylcarnitine	0.0072	0.0010	3.19E-13
Tiglylcarnitine	-0.0109	0.0019	7.17E-09
Taurine	-0.0058	0.0011	1.63E-07
CREA	0.0010	0.0001	4.81E-16
Urea	0.0197	0.0036	5.60E-08

Model adjusted R² =94.1%. ^a Positive effect denotes positive association between metabolite/clinical variable and CKD stage; negative effect denotes negative association between metabolite/clinical variable and CKD stage. ^b Std Err, standard error. Std Err was computed from ordinal regression. ^c P values were computed from ordinal regression.

Supplementary Table 3 Clinical and demographic baseline characteristics of healthy controls and CKD patients

Variable	Healthy controls	CKD1	CKD2
Sample size	30	30	30
Men (%)	53.3	56.3	50.0
Age (years)	56±15	58±18	57±16
BMI (kg/m ²)	25.1	25.5	26.4
Hypertension (%)	21	60	63
eGFR (mL/min/1.73 m ²)	98.2±13.1	99.3±14.6	68.7±12.8**
CREA (μmol/L)	67.3±15.2	65.7±13.2	85.4±19.1**
Urea (mmol/L)	5.3±2.1	5.4±1.7	6.2±2.8
Proteinuria (g/24h)	N/A	1.9±1.3**	2.1±1.6**

Results are expressed as the number (%) or means ± SD, * $P<0.05$, ** $P<0.01$ compared with healthy controls. N/A, not applicable. One-way ANOVA followed by Dunnett's *post hoc* test for multiple comparisons was used for three or more groups.

Supplementary Table 4 Association between clinical indexes and eGFR

feature	Effect ^a	Std Err ^b	P ^c
5-Methoxytryptophan	0.0036	0.0006	0.0000
Canavaninosuccinate	0.0004	0.0001	0.0000
Acetylcarnitine	-0.0030	0.0003	0.0000
Tiglylcarnitine	0.0004	0.0001	0.0000
Taurine	0.0004	0.0001	0.0000
CREA	-0.2119	0.0761	0.0066
Urea	-1.1237	0.8026	0.1650
Sex	1.8412	2.9076	0.5282
Age	-0.1648	0.0876	0.0632
BW (kg)	-0.0602	0.1375	0.6625
BMI (kg/m ²)	-2.4467	0.6538	0.0003
SBP (mm Hg)	-0.0675	0.0852	0.4308
DBP (mm Hg)	-0.2064	0.1323	0.1222
Total protein (g/L)	-0.0580	0.1397	0.6791
Albumin (g/L)	0.2700	0.1499	0.0752
TC (mmol/L)	0.6270	0.6248	0.3184
TG (mmol/L)	-1.1422	0.8643	0.1897
HDL-C (mmol/L)	-1.5051	1.1176	0.1815
LDL-C (mmol/L)	0.5331	0.6888	0.4410
Uric acid (μmol/L)	-0.0172	0.0152	0.2633
P/C ratio	-22.0000	6.6573	0.0037
Proteinuria (g/24h)	-1.3152	0.6314	0.0402

^a Positive effect denotes positive association between metabolite/clinical variable and eGFR. Negative effect denotes negative association between metabolite/clinical variable and eGFR. ^b Std Err, standard error. Std Err was computed from ordinal regression. ^c P values were computed from ordinal regression.

Supplementary Table 5 Baseline characteristics of the longitudinal cohort study sample

Clinical Characteristics	Participants who developed CKD	Participants who did not develop CKD
Sample size	31	1217
Age (years)	59.5 ± 7**	55.8 ± 8
Men (%)	54	52
BMI(kg/m ²)	25.4	25.8
Diabetes (%)	12**	4
Hypertension (%)	67**	37
Dipstick proteinuria (%)	42**	29
eGFR (mL/min/1.73 m ²)	87.5 ± 22.7*	96.3 ± 24.4
eGFR at follow-up (mL/min/1.73 m ²)	50.2 ± 9.4**	88.7 ± 20.4

Results are expressed as the number (%) or mean ± SD, * $P < 0.05$, ** $P < 0.01$ compared with participants who did not develop.