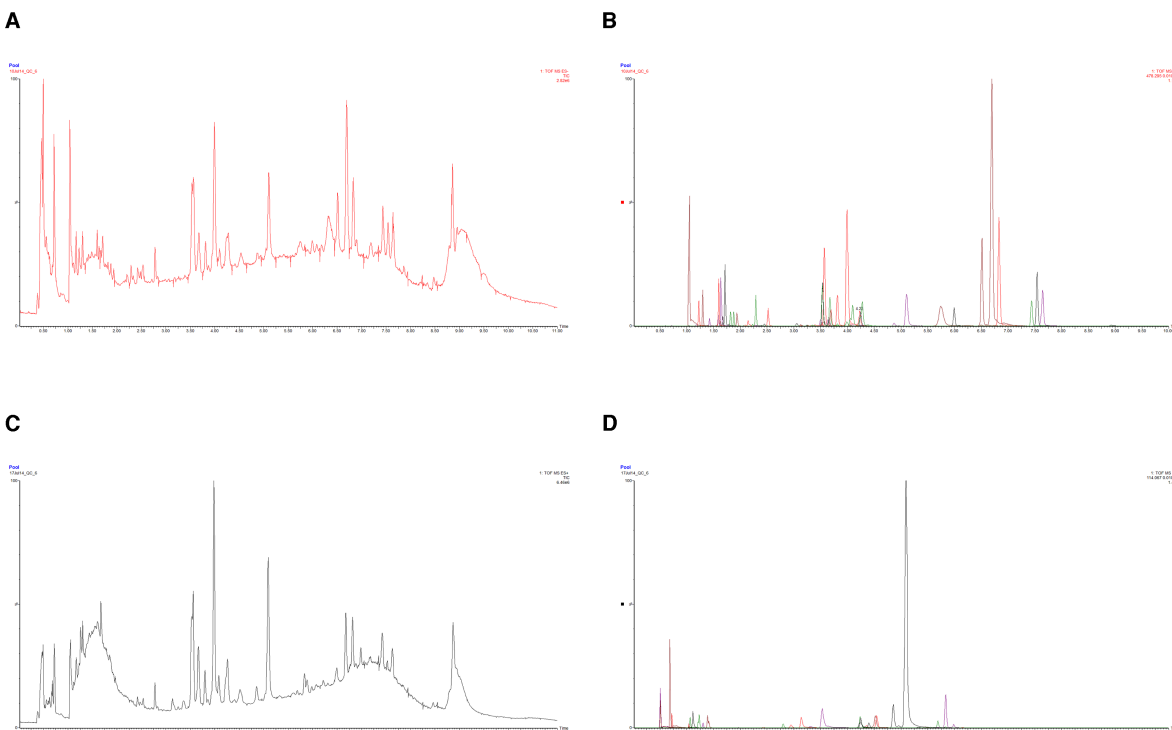


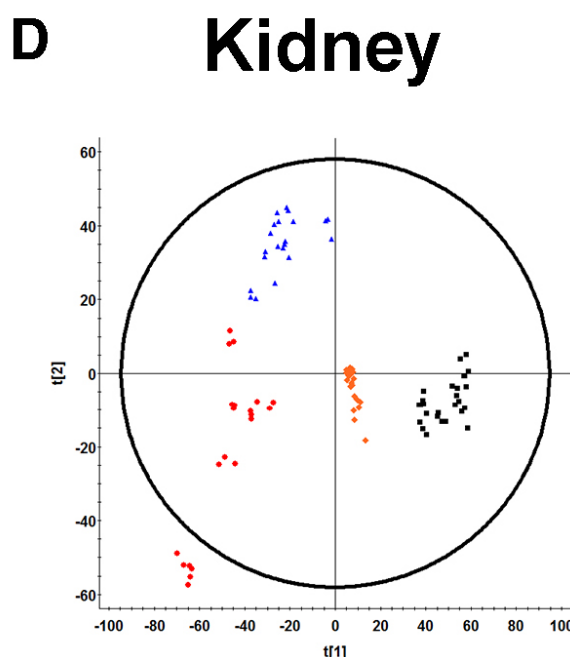
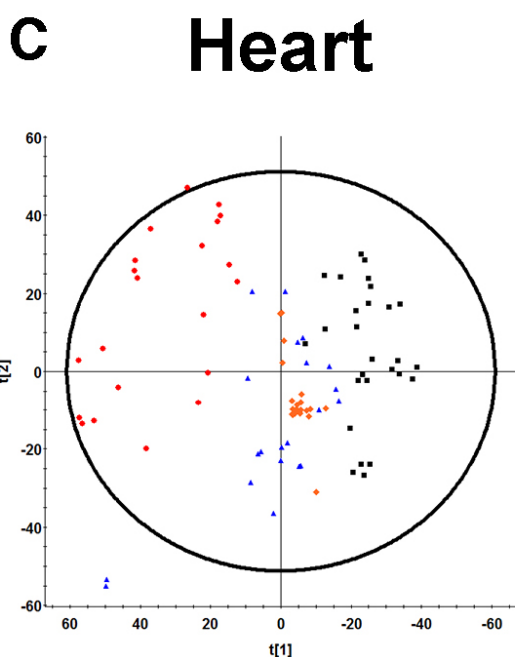
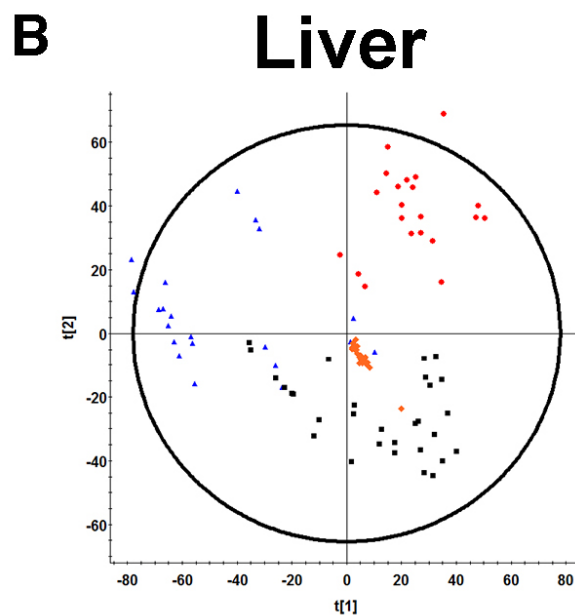
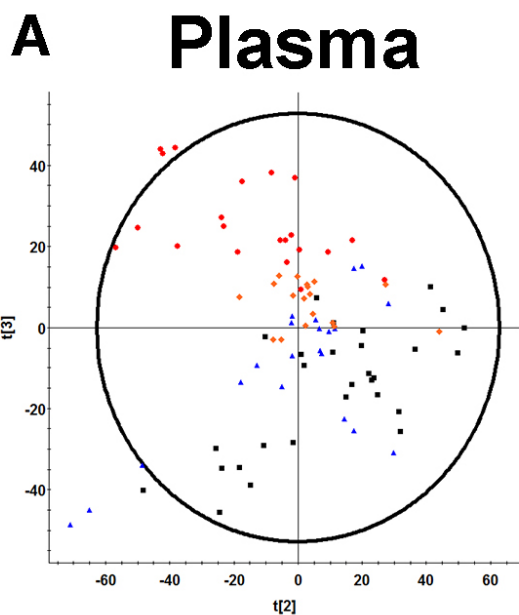
Untargeted plasma and tissue metabolomics in rats with chronic kidney disease given AST-120.

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Supplemental Figure:

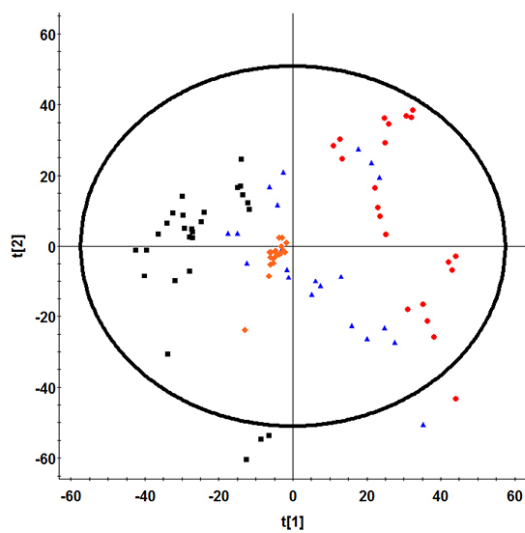


Supplemental Figure S1. Representative chromatograms of total ionic current (TIC) and extracted ions from a pooled rat kidney sample. Negative electrospray ionization mode TIC (A) and overlaid extracted ion chromatograms (B) of metabolites in Table 2 and Supplementary Table S5 from a single injection of pooled rat kidney sample. Positive electrospray ionization mode TIC (C) and overlaid extracted ion chromatograms (D) are also presented for a single injection.

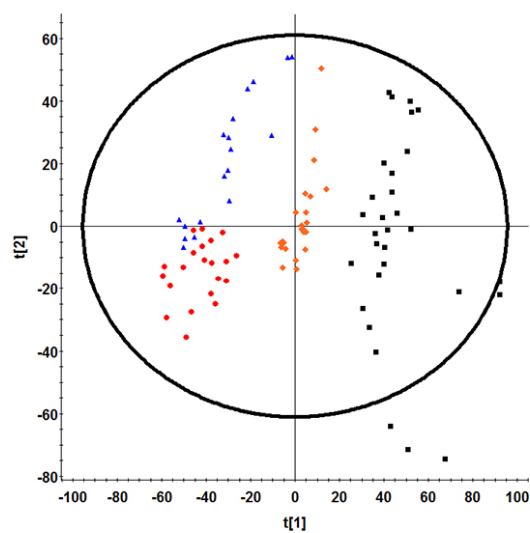


Supplemental Figure S2. Principle component analysis with pooled injections in negative ESI mode. Control (■), CKD (●), CKD+AST-120 (▲) and pooled (◆) negative ESI mode principle component analysis of plasma (A), liver (B), heart (C) and kidney (D) tissue in rats. Triplicate injections are shown.

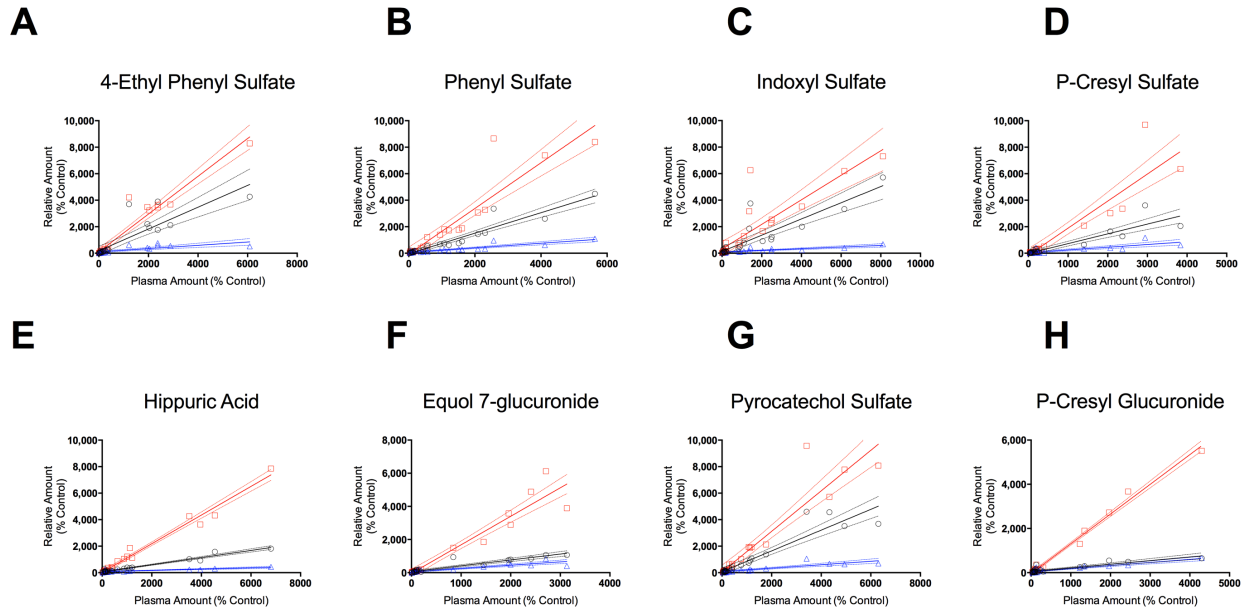
A Heart



B Kidney



Supplemental Figure S3. Principle component analysis with pooled injections in positive ESI mode. Control (■), CKD (●), CKD+AST-120 (▲) and pooled (◆) negative ESI mode principle component analysis of heart (A) and kidney (B) tissue in rats. Triplicate injections are shown.



Supplemental Figure S4. Correlations between plasma and tissues for 8 gut-derived uremic toxins. Plasma levels of 4-ethylphenyl sulfate (A), phenyl sulfate (B), indoxyl sulfate (C), p-cresyl sulfate (D), hippuric acid (E), equol 7-glucuronide (F), pyrocatechol sulfate (G) and p-cresyl glucuronide (H) were correlated to liver (○), heart (◻) and kidney (△) levels for control, CKD and CKD+AST-120 groups. Linear regressions (solid lines) are presented with 95% confidence intervals (dashed lines).

Supplemental Table S5: Summary of metabolites altered in plasma, liver, heart and kidney tissue in rats with CKD compared to control and CKD+AST-120.

Ion	t_R (min)	Mass (m/z)	Empirical Formula	Mass Error (ppm)	Identity	Tissue	Change compared to Control				Change compared to CKD+AST-120			Metabolite ID Level
							Direction	S-plot VIP	Fold	P-value	Direction	Fold	P-value	
9	1.30	203.0818	C11H11N2O2[H-]	1.5	L-Tryptophan	Plasma	↓	2.86	0.34	2.08E-10	↓	0.68	5.65E-03	1
						Liver	↓	4.62	0.82	7.46E-06	↓	0.91	1.13E-01	
						Heart	↓	3.72	0.72	4.89E-10	↓	0.81	2.83E-08	
						Kidney	↓	6.49	0.41	4.89E-10	↓	0.70	5.80E-04	
10	2.15	514.2833	C26H44NO7S[H-]	1.6	Taurocholic acid	Plasma	↑	1.57	3.88	2.14E-05	↑	1.02	1.00E+00	2
						Liver	↑	10.38	1.68	2.79E-02	↑	1.40	2.52E-01	
						Heart	↑	0.80	1.42	8.94E-02	↓	0.81	3.23E-01	
						Kidney	↓	1.00	0.34	3.45E-03	↓	0.68	8.64E-01	
11	2.21	321.0443	C15H13O6S[H-]	-3.1	Equol 4-Sulfate	Plasma	NA				NA			2
						Liver	↑	10.38	10.38	4.89E-10	↑	160.97	4.89E-10	
						Heart	↑	0.04	1.15	8.36E-01	↑	28.71	4.90E-07	
						Kidney	↓	1.99	0.30	4.61E-09	↑	35.87	4.73E-02	
12	1.42	217.0176	C8H9O5S[H-]	2.3	Tyrosol 4-Sulfate	Plasma	NA				NA			2
						Liver	↑	6.39	129.42	4.99E-10	↑	67.42	7.62E-10	
						Heart	↑	4.85	291.44	4.98E-10	↑	55.28	7.88E-10	
						Kidney	↑	5.40	28.42	4.89E-10	↑	38.61	4.90E-10	
13	2.42	498.2896	C26H44NO6S[H-]	-1.4	Taurodeoxy(cheno)cholic acid	Plasma	NA				NA			3
						Liver	↓	14.06	0.60	6.77E-03	↓	0.59	1.17E-02	
						Heart	↓	0.88	0.63	2.99E-01	↓	0.27	9.09E-10	
						Kidney	↓	1.18	0.28	6.68E-03	↓	0.44	4.40E-01	
14	6.67	303.2335	C20H31O2[H-]	-3.6	Arachidonic Acid	Plasma	NA				NA			2
						Liver	↓	11.59	0.89	7.64E-01	↑	1.10	9.20E-01	
						Heart	↑	7.10	1.05	4.28E-01	↑	1.05	5.32E-01	
						Kidney	↓	1.18	0.85	1.00E-04	↓	0.87	4.81E-03	
15	6.80	279.2332	C18H31O2[H-]	-2.9	Linoleic Acid	Plasma	NA				NA			2
						Liver	↓	9.01	0.95	6.72E-01	↓	0.87	2.81E-02	
						Heart	↑	7.66	1.00	1.00E+00	↑	1.04	8.62E-01	
						Kidney	↓	10.07	0.66	7.63E-08	↓	0.78	1.57E-02	
16	7.40	255.2334	C16H31O2[H-]	0	Palmitic Acid	Plasma	↓	0.90	0.97	9.98E-01	↑	1.00	1.00E+00	1
						Liver	↓	11.99	0.78	8.98E-02	↓	0.55	1.37E-08	
						Heart	↑	0.53	1.16	6.33E-03	↑	1.02	9.79E-01	
						Kidney	↓	4.74	0.66	2.66E-04	↓	0.79	2.07E-01	
17	7.61	281.2491	C18H33O2[H-]	-3.5	Oleic Acid	Plasma	NA				NA			1
						Liver	↓	10.68	0.85	4.12E-01	↓	0.64	1.33E-04	
						Heart	↓	0.36	0.83	8.06E-01	↓	0.89	9.59E-01	
						Kidney	↓	3.63	0.81	1.56E-01	↓	0.73	1.36E-02	
18	5.97	301.2178	C20H29O2[H-]	-3.3	Eicosapentaenoic Acid	Plasma	NA				NA			2
						Liver	↓	9.53	0.84	2.29E-01	↓	0.67	1.05E-04	
						Heart	↑	5.59	2.00	7.96E-08	↑	1.89	2.70E-06	
						Kidney	↓	5.05	0.51	5.69E-10	↓	0.71	1.90E-02	
19	6.51	327.2335	C22H31O2[H-]	3.4	Docosahexaenoic Acid	Plasma	NA				NA			2
						Liver	↓	7.29	0.98	9.91E-01	↓	0.78	1.02E-02	
						Heart	↑	2.67	1.16	4.03E-02	↑	1.09	4.72E-01	
						Kidney	↓	14.17	0.38	4.89E-10	↓	0.75	2.83E-02	

20	1.23	218.1037	C9H16NO5[H-]	4.1	D-pantothenic Acid	Plasma	NA				NA			
						Liver	↑	2.92	1.01	9.99E-01	↑	1.09	8.18E-01	2
	1.23	220.1194	C9H18NO5[H+]	4.1		Heart	↓	11.72	0.86	9.58E-01	↓	0.47	1.49E-02	
				Kidney	↓	4.10	0.67	5.19E-10	↑	1.65	3.56E-07			
21	1.88	297.0975	C14H17O7[H-]	-0.3	Phenylethanol Glucuronide	Plasma	NA				NA			
						Liver	↑	4.61	7.91	4.89E-10	↑	27.74	4.89E-10	2
						Heart	↑	6.14	54.66	4.89E-10	↑	46.89	4.89E-10	
Kidney	↑	7.73	6.42	4.89E-10	↑	42.09	4.89E-10							
22	0.70	306.0771	C10H16N3O6S[H-]	3.6	Glutathione	Plasma	NA				NA			
						Liver	↓	5.04	0.84	7.18E-01	↑	1.34	5.63E-01	1
	0.72	308.0923	C10H18N3O6S[H+]	2.3		Heart	↑	7.67	1.70	5.37E-05	↓	0.98	9.94E-01	
				Kidney	↓	0.57	0.07	5.26E-10	↓	0.49	9.37E-01			
23	7.54	331.2647	C22H35O2[H-]	3.0	Adrenic Acid	Plasma	NA				NA			
						Liver	↓	3.43	0.87	7.59E-01	↓	0.56	5.67E-05	2
						Heart	↑	3.96	1.62	8.96E-07	↑	1.30	1.09E-02	
Kidney	↑	10.13	2.57	4.89E-10	↓	0.78	2.79E-06							
24	1.63	245.0124	C9H11O7S (-H2O)[H-]	1.6	3-Methoxy-4-Hydroxyphenylglycol sulfate	Plasma	NA				NA			
						Liver	↑	3.10	18.14	4.89E-10	↑	10.04	4.89E-10	2
						Heart	↑	3.50	106.17	4.89E-10	↑	10.22	4.89E-10	
				Kidney	↑	4.70	7.98	4.89E-10	↑	6.94	4.89E-10			
25	3.98	540.3313	C25H51NO9P[CHOOH-]	2.2	LysoPC(16:0)	Plasma	↑	1.42	2.38	1.30E-04	↑	1.26	4.71E-01	3
						Liver	↑	5.68	1.01	9.92E-01	↑	1.01	9.83E-01	
	3.99	496.3407	C24H51NO7P[H+]	0.8		Heart	↓	10.10	0.91	6.26E-02	↓	0.99	9.98E-01	
				Kidney	↑	1.90	1.70	4.89E-10	↑	1.05	4.07E-01			
26	3.67	564.3316	C26H49NO7P[CHOOH-]	2.7	LysoPC(18:2)	Plasma		NA			NA			
						Liver	↑	2.95	1.02	9.80E-01	↓	0.94	5.02E-01	3
	3.67	520.3412	C26H51NO7P[H+]	1.7		Heart	↓	9.70	0.66	8.72E-10	↓	0.83	4.32E-02	
				Kidney	↓	2.27	0.83	1.69E-02	↑	1.09	6.92E-01			
27	3.63	476.2791	C23H43NO7P[H-]	2.9	LysoPE (18:2)	Plasma	NA				NA			
						Liver	↓	3.22	0.75	1.12E-03	↓	0.63	3.60E-08	3
	3.67	520.3412	C26H51NO7P[H+]	1.7		Heart	↓	6.94	0.60	3.25E-07	↓	0.77	6.21E-02	
				Kidney	↓	3.27	0.38	4.46E-09	↓	0.97	9.99E-01			
28	4.25	436.2837	C21H43NO6P[H-]	2.1	PE(P-16:0/0:0)	Plasma	NA				NA			
						Liver	↓	0.38	0.95	9.93E-01	↓	0.60	1.06E-02	3
						Heart	↓	5.60	0.76	1.59E-05	↓	0.83	2.78E-02	
				Kidney	↑	5.50	2.54	4.89E-10	↑	1.24	5.99E-03			
29	5.66	619.2898	C29H48O12P[H-]	2.4	(PI)20:4)	Plasma	↑		30.87	2.11E-10	↑	3.72	2.93E-07	3
						Liver	↑	1.45	1.25	2.80E-03	↓	0.86	4.56E-02	
						Heart	↓	4.90	0.89	2.22E-01	↓	0.94	8.08E-01	
				Kidney	↓	7.50	0.37	4.89E-10	↓	0.67	8.60E-04			
30	3.57	588.3313	C29H51NO9P[HCOOH-]	2.0	LysoPC(20:4)	Plasma	NA				NA			
						Liver	↑	5.39	1.07	2.52E-04	↑	1.07	1.41E-03	3
	3.57	544.3408	C28H51NO7P[H+]	0.9		Heart	↑	1.40	1.10	6.25E-02	↑	1.21	7.91E-05	
				Kidney	↑	9.70	1.69	4.89E-10	↓	0.99	9.99E-01			
31	4.10	566.3472	C27H53NO9P[HCOOH-]	2.5	LysoPC(18:1)	Plasma	↑		2.71	9.89E-05	↑	1.83	1.74E-02	3
						Liver	↑	3.41	1.14	3.05E-01	↑	1.27	2.54E-02	
	4.08	522.3566	C26H53NO7P[H+]	1.1		Heart	↑	0.58	1.17	6.80E-02	↑	1.20	4.71E-02	
				Kidney	↑	6.66	2.46	4.89E-10	↑	1.04	8.59E-01			
32	5.11	508.3415	C25H51NO7P[H-]	2.4	LysoPC(17:0) or LysoPE(20:0)	Plasma	NA				NA			
						Liver	↑	1.45	1.15	1.47E-02	↑	1.04	8.84E-01	3
						Heart	↓	3.72	0.88	8.49E-01	↓	0.74	2.32E-01	
				Kidney	↑	5.18	1.44	4.89E-10	↑	1.24	1.66E-08			

54	1.12	257.1149	C11H16N2O5	4.7	1-beta-d-Ribofuranosyl-1,4-dihydronicotinamide	Heart	↓	3.65	0.30	3.01E-08	↓	0.55	3.56E-10	2
						Kidney	↓	10.00	0.07	4.89E-10	↓	1.06	1.00E+00	
55	1.40	246.1712	C12H24NO4[H+]	2.8	2-methylbutyroylcarnitine or pivaloylcarnitine	Heart	↓	9.87	0.26	3.56E-10	↓	0.40	3.56E-10	3
						Kidney	↓	8.48	0.07	4.89E-10	↓	0.64	8.54E-01	
56	3.12	424.3431	C25H46NO4[H+]	0.9	Linoleyl or Linoelaidyl Carnitine	Heart	↓	17.44	0.47	6.26E-10	↓	0.67	1.53E-02	3
						Kidney	↓	7.06	0.33	4.89E-10	↓	0.63	8.63E-04	
57	1.06	262.1661	C12H24O5N1[H+]	2.7	3-Hydroxyisovalerylcarnitine	Heart	↓	6.92	0.42	3.56E-10	↓	0.51	7.99E-08	2
						Kidney	↓	7.61	0.13	4.89E-10	↓	0.70	4.90E-01	

Supplementary Table S6. Metabolite pathway analysis using MetaboAnalyst 3.0

Number	Pathway	Tissue	Total	Hits	Raw p	-LOG(p)	Holm Adjust	FDR	Impact
1	Phenylalanine metabolism	Plasma	45	1	9.27E-10	20.799	6.49E-09	6.49E-09	0.0315
		Liver	45	1	8.50E-11	23.189	1.10E-09	2.89E-10	0.0315
		Heart	45	1	2.52E-08	17.495	3.28E-07	1.26E-07	0.0315
		Kidney	45	1	2.01E-07	15.419	2.41E-06	6.44E-07	0.0315
2	Primary bile acid biosynthesis	Plasma	47	1	2.77E-06	12.795	1.66E-05	6.47E-06	0.00846
		Liver	47	2	0.024685	3.7015	0.14811	0.034971	0.01692
		Heart	47	2	0.017285	4.0579	0.121	0.028809	0.01692
		Kidney	47	2	1.12E-05	11.399	7.84E-05	1.79E-05	0.01692
3	Taurine and hypotaurine metabolism	Plasma	20	1	2.77E-06	12.795	1.66E-05	6.47E-06	0
		Liver	20	1	1.23E-06	13.611	1.47E-05	3.48E-06	0
		Heart	20	1	0.01284	4.3552	0.11015	0.024076	0
		Kidney	20	1	0.010651	4.5421	0.021301	0.011361	0
4	Glycerophospholipid metabolism	Plasma	39	1	0.00018895	8.574	0.00075579	0.00033066	0.00317
		Liver	39	2	0.85934	0.15159	1	0.85934	0.1307
		Heart	39	2	0.012239	4.4031	0.11015	0.024076	0.1307
		Kidney	39	3	2.70E-07	15.124	2.97E-06	7.20E-07	0.14055
5	Fatty acid metabolism	Plasma	50	1	0.25548	1.3646	0.76645	0.25548	0.02959
		Liver	50	1	0.002964	5.8212	0.023712	0.0045808	0.02959
		Heart	50	1	0.0045676	5.3888	0.050243	0.011419	0.02959
		Kidney	50	1	0.0010536	6.8555	0.005268	0.0012041	0.02959
6	Fatty acid biosynthesis	Plasma	49	1	0.25548	1.3646	0.76645	0.25548	0
		Liver	49	2	0.0017773	6.3327	0.015996	0.0033571	0
		Heart	49	2	0.11603	2.1539	0.69617	0.17404	0
		Kidney	49	1	0.0010536	6.8555	0.005268	0.0012041	0
7	Fatty acid elongation in mitochondria	Plasma	27	1	0.25548	1.3646	0.76645	0.25548	0
		Liver	27	1	0.002964	5.8212	0.023712	0.0045808	0
		Heart	27	1	0.0045676	5.3888	0.050243	0.011419	0
		Kidney	27	1	0.0010536	6.8555	0.005268	0.0012041	0
8	Pantothenate and CoA biosynthesis	Plasma	NA						
		Liver	27	1	5.37E-11	23.647	8.06E-10	2.28E-10	0.18014
		Heart	27	1	0.50957	0.6742	1	0.58796	0.18014
		Kidney	27	1	8.11E-08	16.328	1.14E-06	3.24E-07	0.18014
9	beta-Alanine metabolism	Plasma	NA						
		Liver	28	1	5.37E-11	23.647	8.06E-10	2.28E-10	0
		Heart	28	1	0.50957	0.6742	1	0.58796	0
		Kidney	28	1	8.11E-08	16.328	1.14E-06	3.24E-07	0
10	Glutathione metabolism	Plasma	NA						
		Liver	38	1	6.60E-06	11.928	7.26E-05	1.40E-05	0.23743
		Heart	38	1	1.30E-08	18.156	1.95E-07	9.77E-08	0.23743
		Kidney	38	1	4.98E-07	14.512	4.98E-06	9.97E-07	0.23743
11	Cysteine and methionine metabolism	Plasma	NA						
		Liver	56	1	6.60E-06	11.928	7.26E-05	1.40E-05	0.00735
		Heart	56	1	1.30E-08	18.156	1.95E-07	9.77E-08	0.00735
		Kidney	56	1	4.98E-07	14.512	4.98E-06	9.97E-07	0.00735
12	Arachidonic acid metabolism	Plasma	NA						
		Liver	62	1	0.027914	3.5786	0.14811	0.036503	0.21669
		Heart	62	1	0.23483	1.4489	1	0.32022	0.21669
		Kidney	62	1	0.00040259	7.8176	0.0024155	0.00058558	0.21669
13	Linoleic acid metabolism	Plasma	NA						
		Liver	15	1	0.035123	3.3489	0.14811	0.042649	0.65625
		Heart	15	1	0.97705	0.023214	1	0.97705	0.65625
		Kidney	15	1	3.04E-06	12.703	2.43E-05	5.41E-06	0.65625
14	Purine metabolism	Plasma	NA						
		Liver	92	1	0.43236	0.8385	1	0.49001	0.00425
		Heart	92	1	0.61199	0.49104	1	0.6557	0.00425
		Kidney	92	1	1.17E-09	20.566	1.76E-08	9.36E-09	0.00425
15	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	Plasma	NA						
		Liver	14	1	0.66349	0.41024	1	0.70496	0.0439
		Heart	14	1	0.0002639	8.2399	0.0031669	0.00098964	0.0439
		Kidney	14	1	2.63E-11	24.361	4.21E-10	4.21E-10	0.0439
16	Ether lipid metabolism	Kidney	23	1	0.37906	0.97007	0.37906	0.37906	0
17	Starch and sucrose metabolism	Liver	50	1	5.46E-18	39.749	9.28E-17	4.64E-17	0.01265
18	Pentose and glucuronate interconversions	Liver	53	1	5.46E-18	39.749	9.28E-17	4.64E-17	0.009

Note: Total is the total number of compounds in the pathway; Hits are the number of compounds matched in that pathway; the Raw p is the original p value calculated from the enrichment analysis; the Holm p is the p value adjusted by Holm-Bonferroni method; the FDR p is the p value adjusted using False Discovery Rate; the Impact is the pathway impact value calculated from pathway topology analysis.