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NMR based metabolomic approach revealed cyclophosphamide-induced systematic alterations in a rat model

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Table S1 ^{1}H-NMR data for endogenous metabolites in rat liver, kidney, and heart.

Keys	Metabolites[a]	δ^{1}H (multiplicity[b])	samples[c]
1	Lipid	0.87(m), 1.27(m)	H, K
2	Valine	0.98(d), 1.04(d)	L, H, K
3	Leucine	0.95(t)	L, H, K
4	Isoleucine	0.93(t), 1.00(d)	L, H, K
5	3-HB	1.20(d)	L, H, K
6	Lactate	1.33(d), 4.12(q)	L, H, K
7	Alanine	1.48(d)	L, H, K
8	Lysine	1.72(m), 1.89(m)	L, H, K
9	Acetate	1.92(s)	L, H, K
10	Arginine	1.70(m), 1.92(m)	L
11	Ornithine	1.75(m), 1.93(m)	L, K
12	Glutamate	2.07(m), 2.35(m)	L, H, K
13	Methionine	2.14(s), 2.63(t)	K
14	Glutamine	2.15(m), 2.44(m)	L, H, K
15	Pyruvate	2.36(s)	K
16	Succinate	2.38(s)	L, H, K
17	GSSG	2.17(m), 2.54(m), 2.95(m), 3.25(m)	L, H
18	Glutathione	2.55(m)	H, K
19	Malate	2.66(d), 2.68(d), 4.31(d)	L, H, K
20	Aspartate	2.67(dd), 2.82(dd)	L, K
21	Dimethylamine	2.72(s)	L, K
22	TMA	2.87(s)	L
23	Trimethylamine	2.89(s)	K
24	Carnitine	3.23(s)	L, K
25	Methanol (residual)	3.35(s)	L, H, K
26	Dimethylglycine	2.91(s), 3.71(s)	H, K
27	Creatine	3.04(s), 3.92(s)	L, H, K
28	Choline	3.20(s), 3.54(m)	L, H, K
29	GPC	3.22(s), 3.68(t)	L, H, K
30	PC	3.21(s)	L, H, K
31	Trimethylamine-N-oxide	3.26(s)	H, K
32	Taurine	3.28(t), 3.42(t)	L, H, K
33	Betaine	3.27(s), 3.91(s)	L, K
34	Glycine	3.56(s)	L, H, K
35	Glycerin	3.56(dd), 3.65(dd)	L, H, K
36	*Myo*-Inositol	3.54(dd), 4.07(t)	K
37	Inosine	6.10(d), 8.22(s), 8.36(s)	L, H, K
38	Threonine	1.33(d), 3.60(d), 4.26(m)	K

39	glucose	5.24(d)	L, H, K
40	Uracil	5.81(d), 7.56(d)	L, H, K
41	Uridine	5.92(d)	L, K
42	Cytidine	6.10(d), 7.85(d)	L, K
43	Fumarate	6.53(s)	L, H, K
44	Tyrosine	6.91(d), 7.19(d)	L, H, K
45	Histidine	7.13(s), 7.90(s)	L, H, K
46	Phenylalanine	7.38(m)	L, H, K
47	Nicotinamide	7.61(dd), 8.72(dd), 8.95(d)	L, H, K
48	Xanthine	7.92(s)	L, H, K
49	Hypoxanthine	8.21(s), 8.22(s)	L, H, K
50	Formate	8.46(s)	L, H, K
51	Inosine monophosphate	6.11(d)	L, H, K

[a]Metabolites: 3-HB: 3-Hydroxybutyrate, GSSG: oxidized glutathione, TMA: trimethylamine, GPC: Glycerophosphocholine, PC: Phosphocholine. [b]s, singlet, d, doublet, t, triplet, q, quartet, m, multiplet, dd, doublet of doublets. [c]L, liver, K, kidney, H, heart.

Table S2 Comparison of normalized integral levels of metabolites in kidney

(As some of the values are too small, all of the data were magnified 100 times).

δ^1H	Metabolites	Control group	CY group	VIP
0.93	Isoleucine	26.34 ± 5.50	19.98 ± 2.83$^{\#}$	1.86
0.96	Leucine	116.83 ±14.79	102.76 ± 6.30$^{\#}$	2.61
1.21	3-HB	6.892 ±1.456	4.71 ± 1.29$^{\#\#}$	1.12
1.33	Lactate	23.57 ± 24.51	81.07 ± 30.40$^{\#\#}$	6.19
1.93	Acetate	103.93 ±15.68	73.85 ± 3.86$^{\#\#}$	4.65
2.14	Methionine	40.34 ± 3.04	36.58 ± 3.36$^{\#}$	1.34
2.15	Glutamine	64.46 ± 5.16	58.56 ± 2.29$^{\#}$	1.79
2.36	Pyruvate	109.13 ± 6.20	99.34 ± 7.60$^{\#}$	2.30
3.04	Creatine	75.00 ±12.12	105.91±16.11$^{\#\#}$	4.58
3.20	Choline	16.84± 2.320	20.87 ± 2.59$^{\#\#}$	1.55
3.21	GPC	288.72 ± 34.83	383.18 ± 46.34$^{\#\#}$	8.10
3.24	Carnitine	96.35 ± 37.90	153.90 ±54.10$^{\#}$	5.34
6.11	Inosine monophosphate	13.97 ± 3.12	8.94 ± 5.00$^{\#}$	1.57
8.23	Hypoxanthine	20.94 ± 2.65	15.35 ± 2.06$^{\#\#}$	1.97

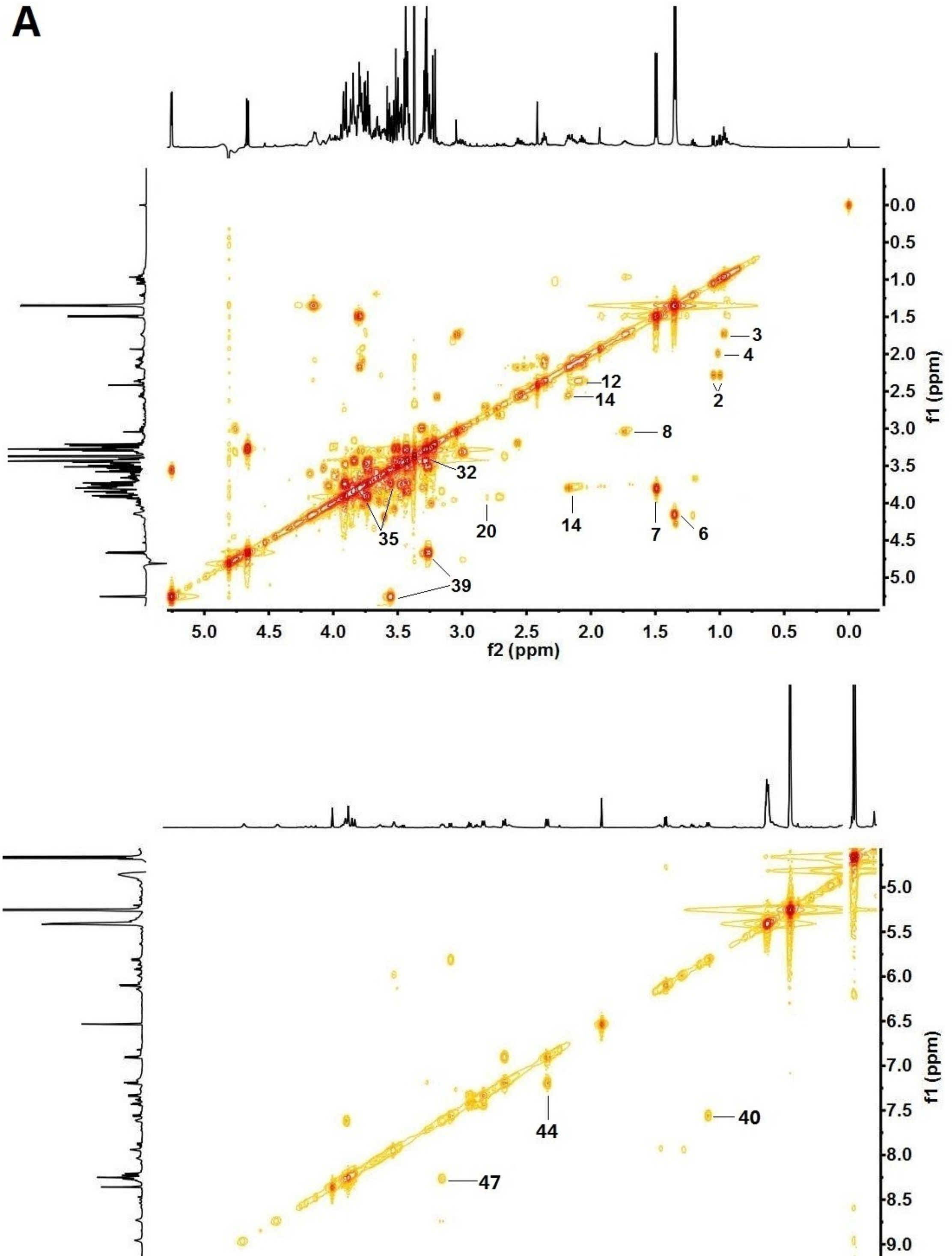
Note: 3-HB: 3-Hydroxybutyrate, GPC: Glycerophosphocholine. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs* control rats.

Table S3 Comparison of normalized integral levels of metabolites in heart

(As some of the values are too small, all of the data were magnified 100 times).

δ^1H	Metabolites	Control group	CY group	VIP
1.49	Alanine	119.58±14.89	97.25 ± 17.71$^{\#}$	3.65
1.93	Acetate	28.29 ± 6.72	35.64± 5.51$^{\#}$	1.99
2.69	Malate	13.99 ± 1.37	9.58 ± 1.46$^{\#\#}$	1.95
3.20	PC	18.72 ± 8.98	33.80 ± 5.34$^{\#\#}$	2.50
3.29	Taurine	280.16 ± 26.29	334.40 ± 41.50$^{\#}$	5.94
7.91	Xanthine	6.78 ± 2.76	12.09 ± 5.11$^{\#}$	1.75

Note: PC: Phosphocholine. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs* control rats.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2.0
1.5
1.0
0.5
0.0
3
4
12
14
2
8
32
35
20
14
7
6
39
f1 (ppm)
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6.0
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7.5
8.0
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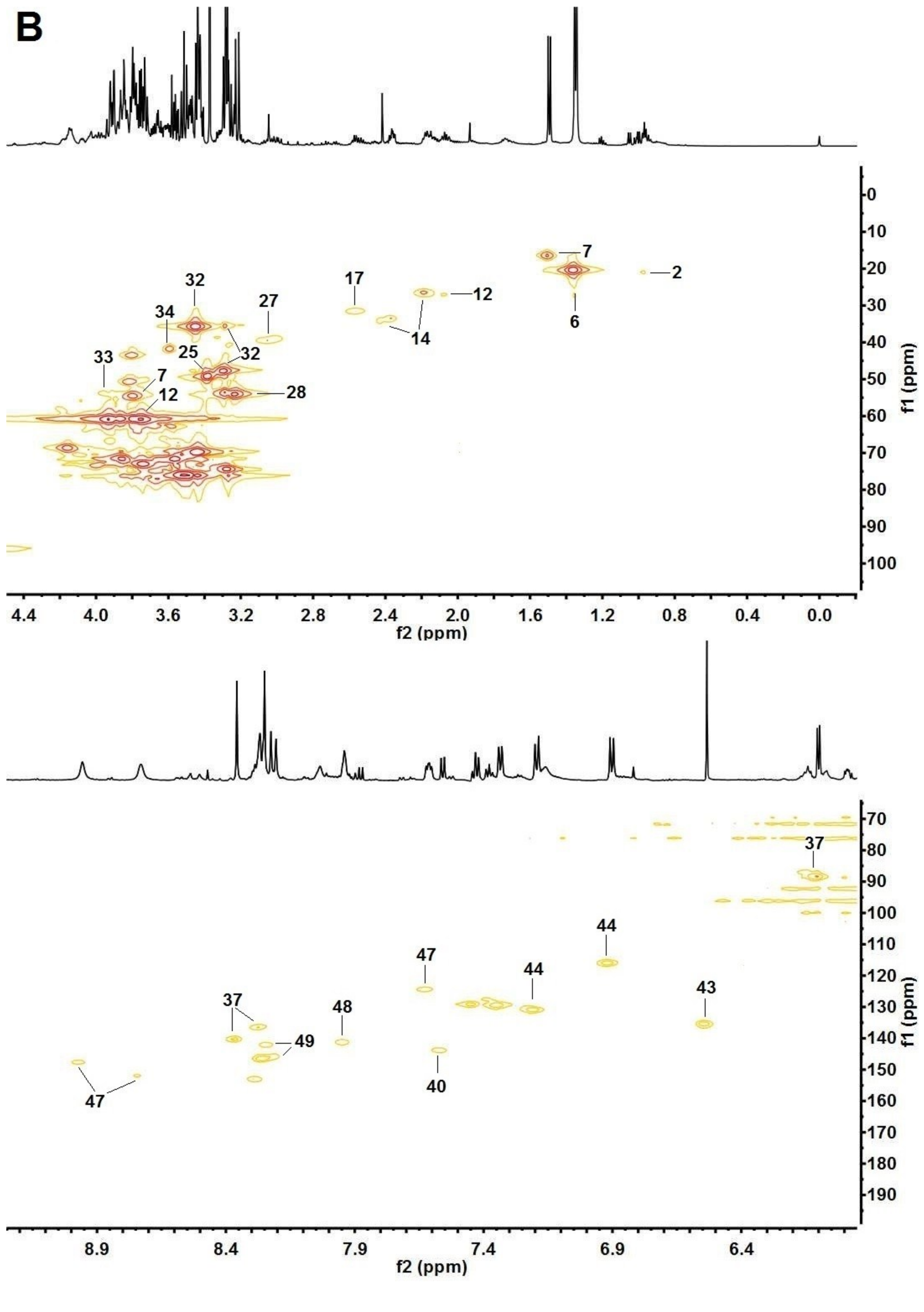
5.2
f2 (ppm)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f2 (ppm)

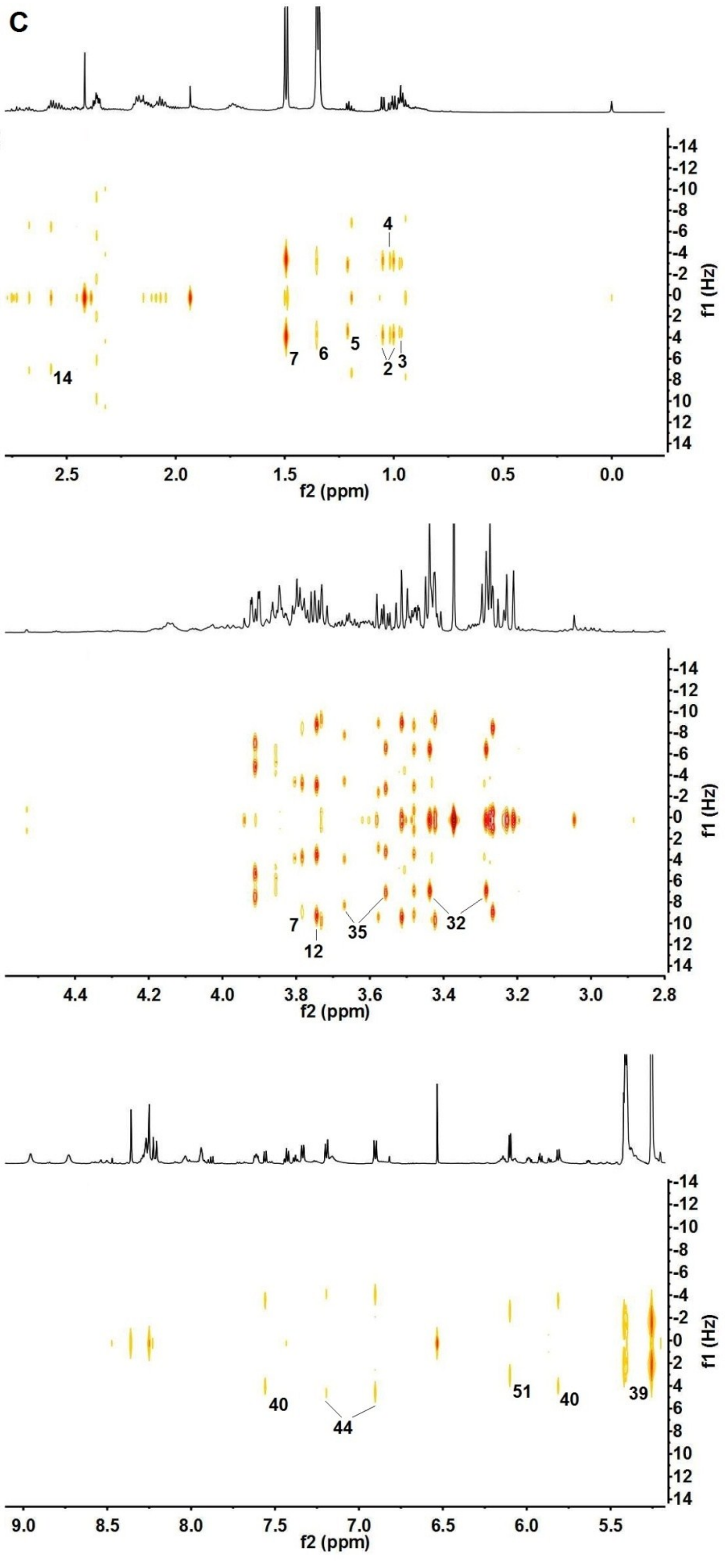
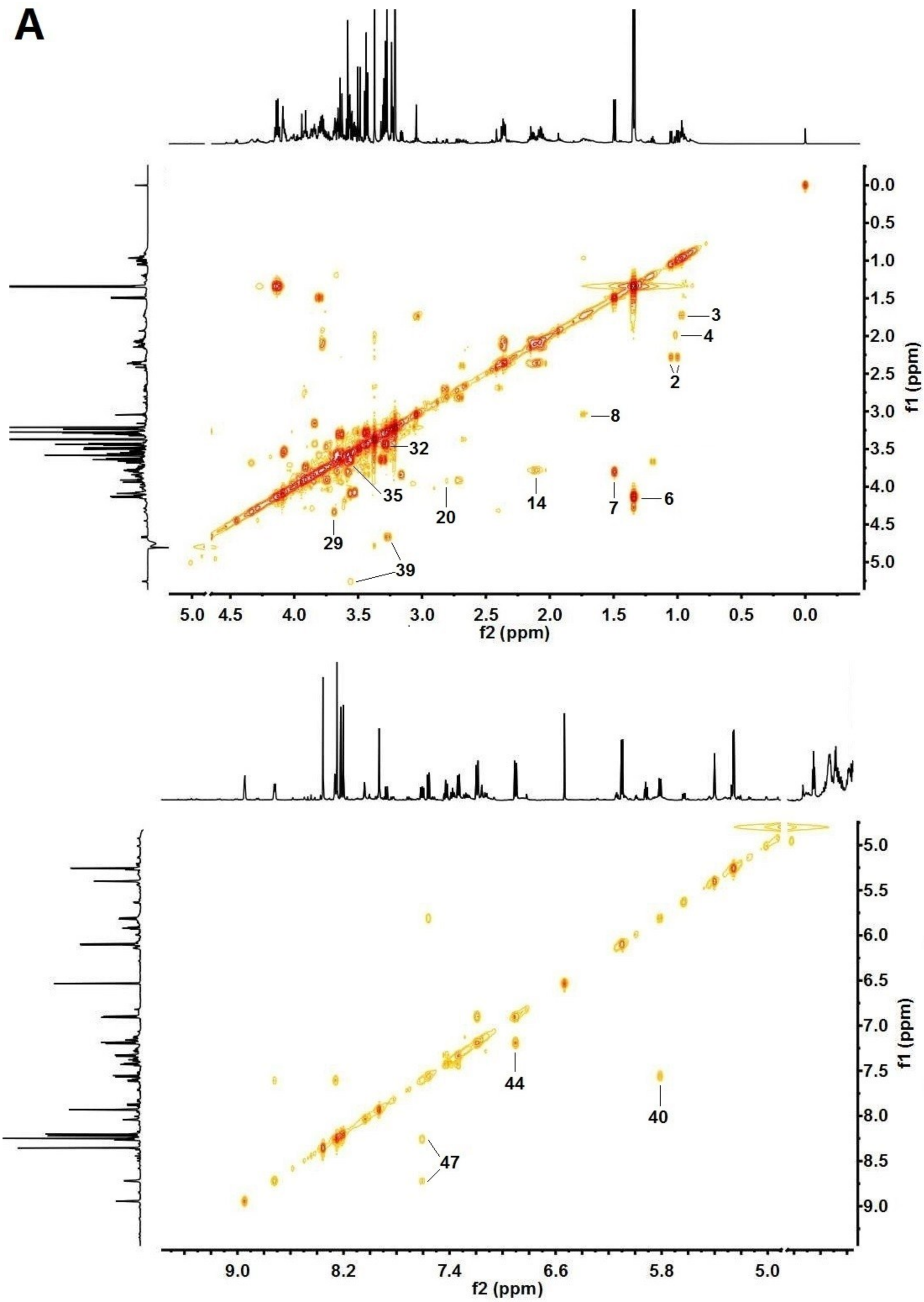


Figure S1 2D NMR spectra of liver (A. ^{1}H–^{1}H COSY spectrum; B. HSQC spectrum; C. JRES spectrum)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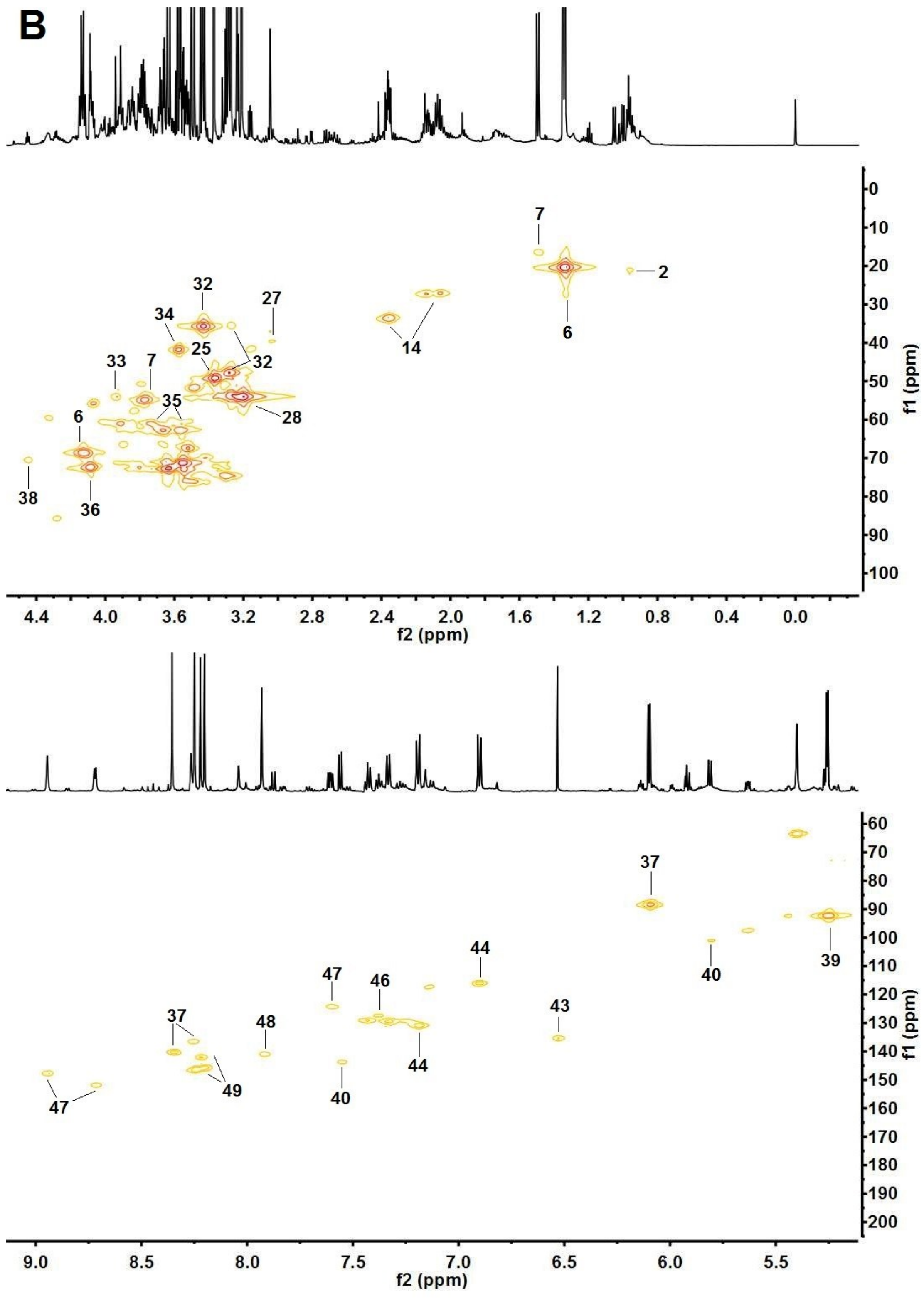
f1 (ppm)
f2 (ppm)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f2 (ppm)
f1 (ppm)
f2 (ppm)
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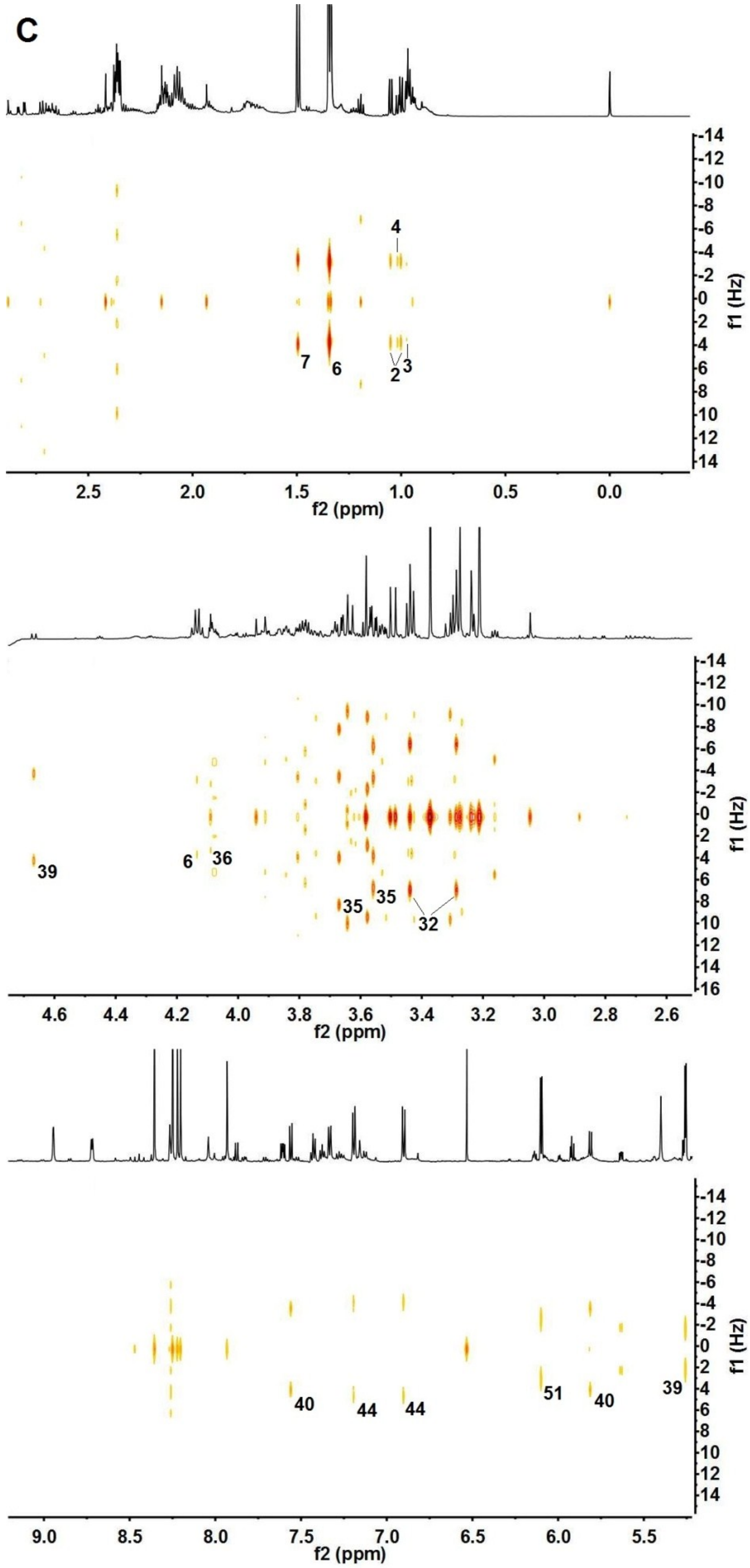
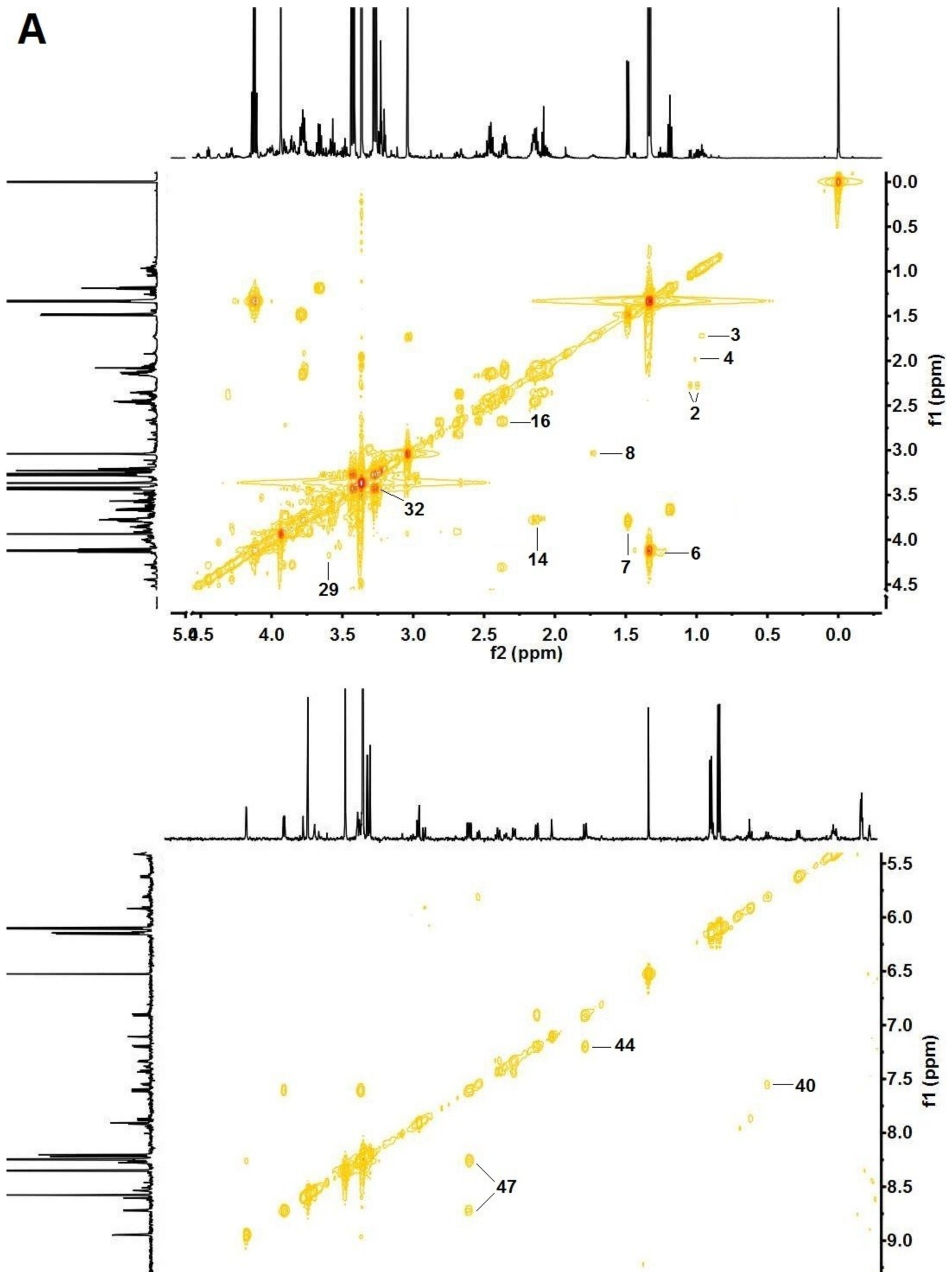


Figure S2 2D NMR spectra of kidney (A. ^{1}H–^{1}H COSY spectrum; B. HSQC spectrum; C. JRES spectrum)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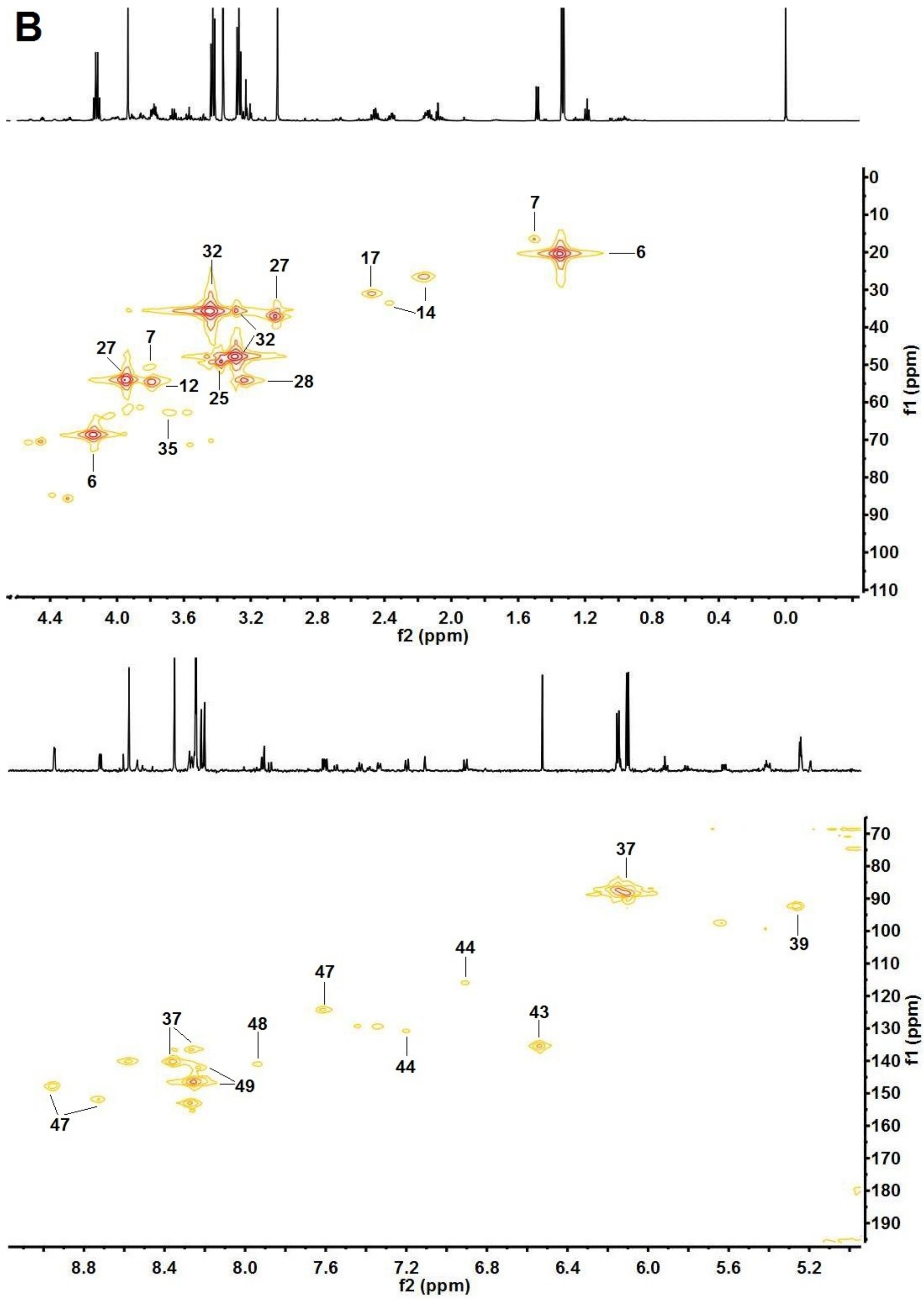
f1 (ppm)
f2 (ppm)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f2 (ppm)

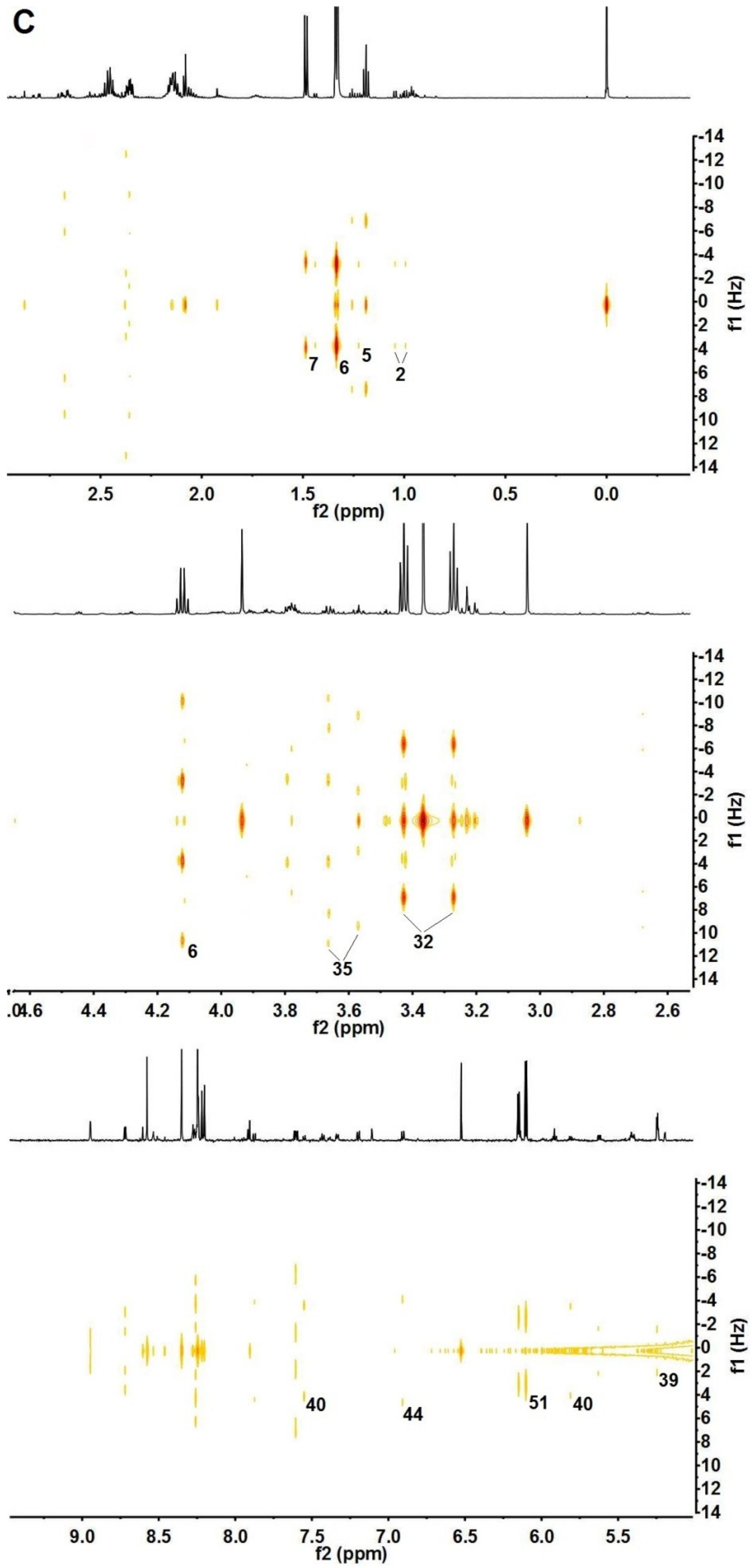

Figure S3 2D NMR spectra of heart (A. ^{1}H–^{1}H COSY spectrum; B. HSQC spectrum; C. JRES spectrum)

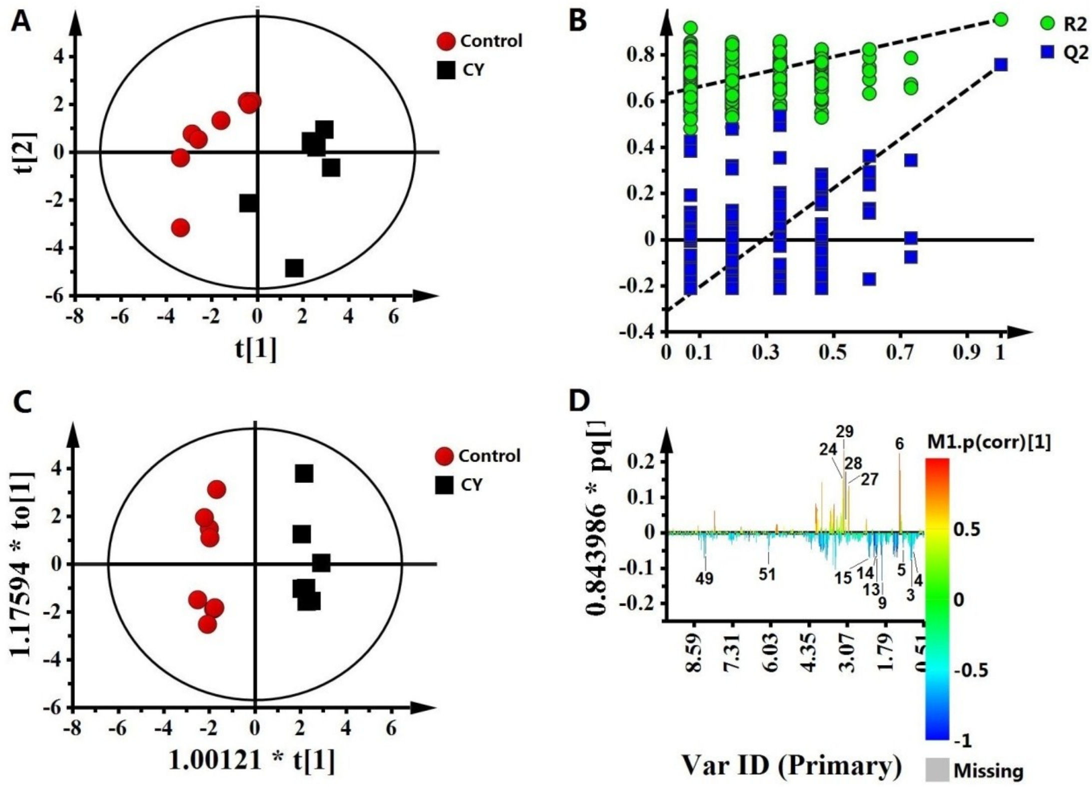

Figure S4 PCA score plot (A), PLS-DA permutation test (B), OPLS-DA score plot (C), and loading plot (D) of rats kidney between Control and CY group.

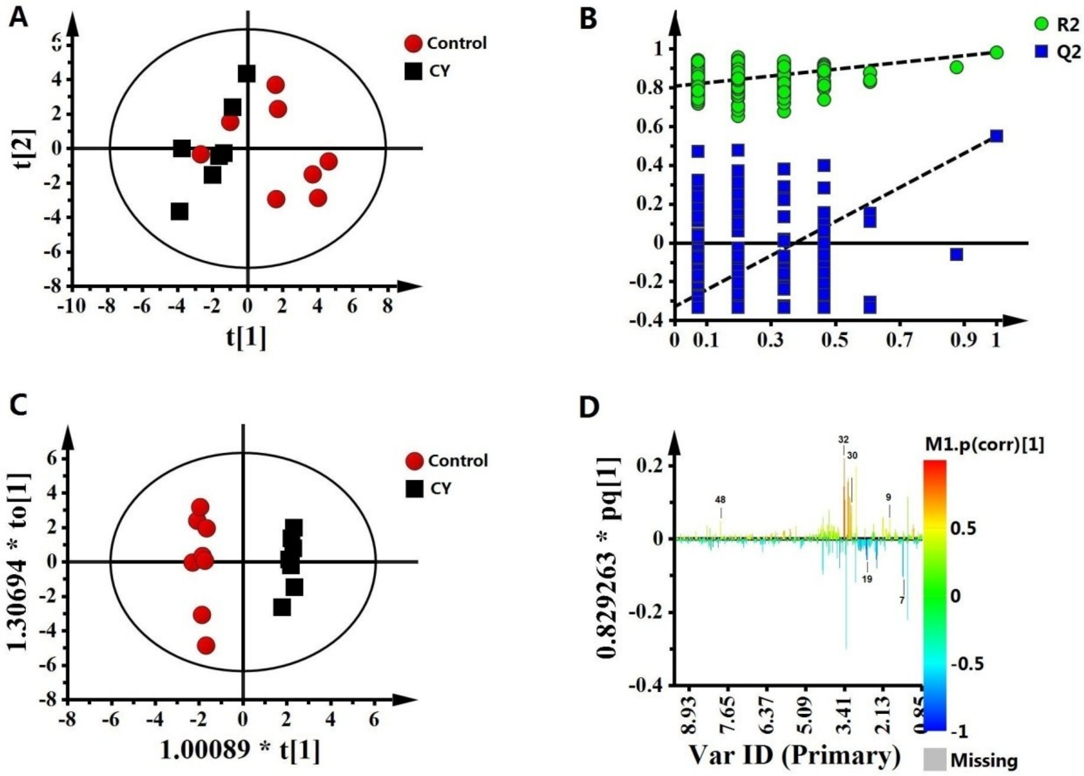

Figure S5 PCA score plot (A), PLS-DA permutation test (B), OPLS-DA score plot (C), and loading plot (D) of rats heart between Control and CY group.

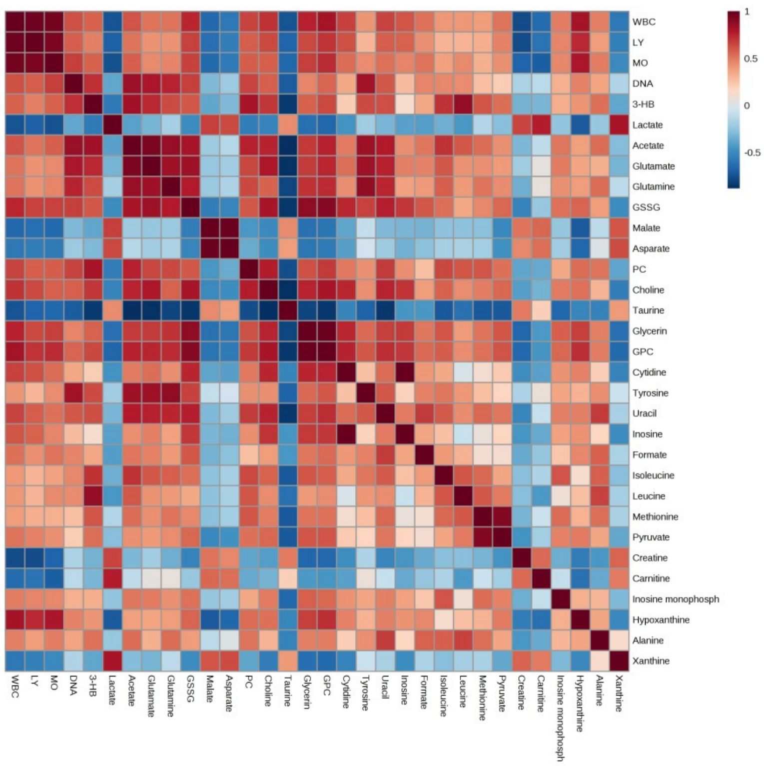

Figure S6 Correlation analysis of WBC, MO, NE, DNA and metabolites. Red and blue represent positive and negative correlations, respectively, the colour scale represents Pearson's correlation coefficients.

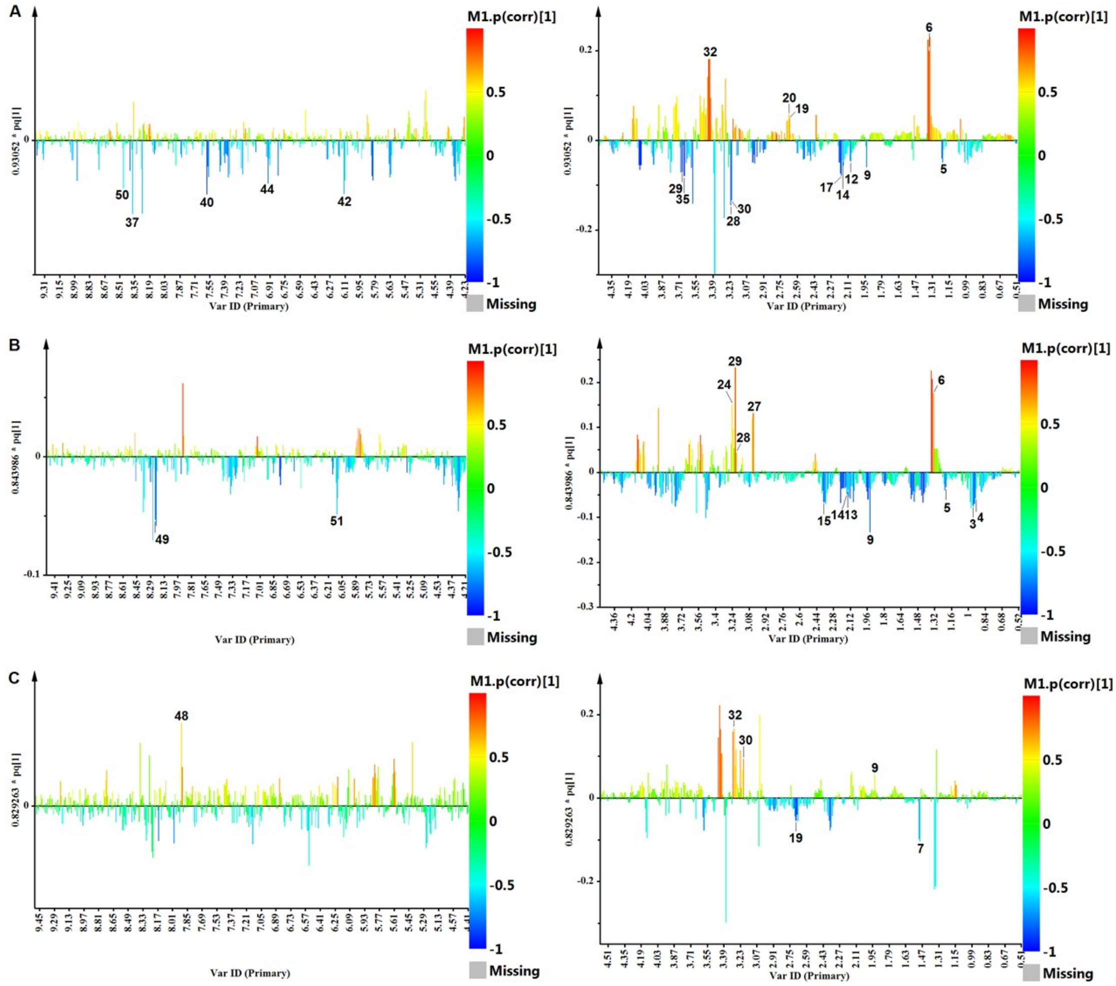

Figure S7 Enlarged loading plots of rats liver(A), kidney(B), and heart(C) between Control and CY group.