

Supplementary information

Integrated Analysis of Serum and Intact Muscle Metabonomics Identify Metabolic Profiles of Cancer Cachexia in a Dynamic Mouse Model

Yang QuanJun^{1#}, Yang GenJin^{2#}, Wan LiLi¹, Huo Yan¹, Han YongLong¹, Lu Jin¹, Chen

PengGuo¹, Li Jie¹, Huang JinLu¹, Guo Cheng^{1*}

1, Department of Pharmacy, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, P. R. China

2, School of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China

[#] Contributed equally for the manuscript

*Corresponding author:

Guo Cheng, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, No. 600, Road Yishan, Shanghai, 200233, China. Phone # +86 21 24058098; E-mail: guopharm@126.com.

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Fig. S1. The 2D score plot of the seven groups analyzed with MetPA. M1-M7 represent the groups from day 9 to day 21 (every other day), corresponding to the groups of G1 to G7. Each group was 6 mice other than 8 mice for the control group. It was noted that the development from procachexia toward severe cachexia showed a dynamic track.

Fig. S2. The VIP and Coefficients plot of dynamic cachexi model mice. M1-M7 represent the groups from day 9 to day 21 (every other day), corresponding to the groups of G1 to G7.

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Table S1. Statistics results of serum metabolites in dynamic cachexia model animal.

Table S2. Statistics results of intact muscle gastrocnemius metabolites in dynamic cachexia model animal

Table S3. Analysis of the altered metabolic pathways of cancer cachexia. The Total is the total number of compounds in the pathway; the Hits is the actually matched number from the user uploaded data; 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.

Figure S1

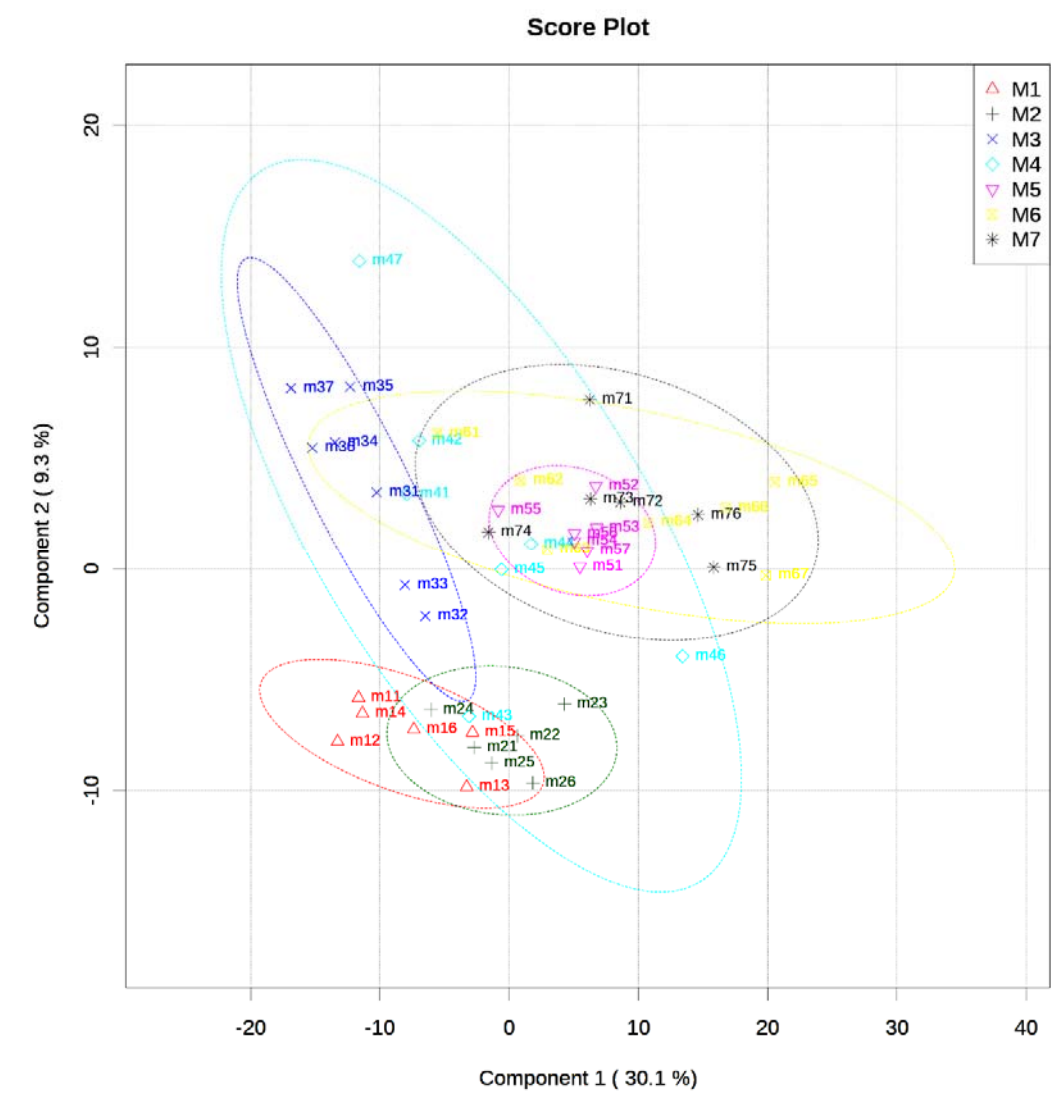


Figure S2

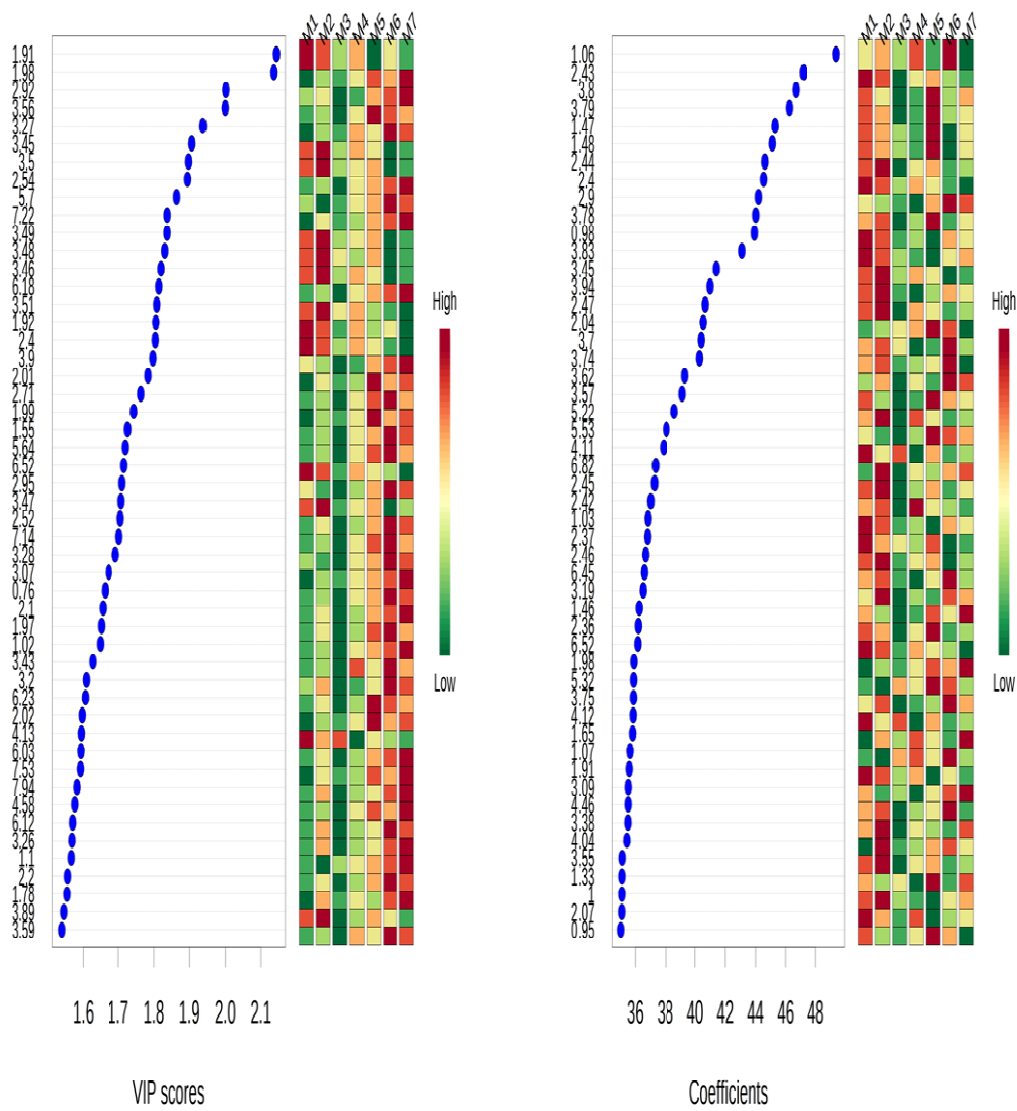


Figure S3

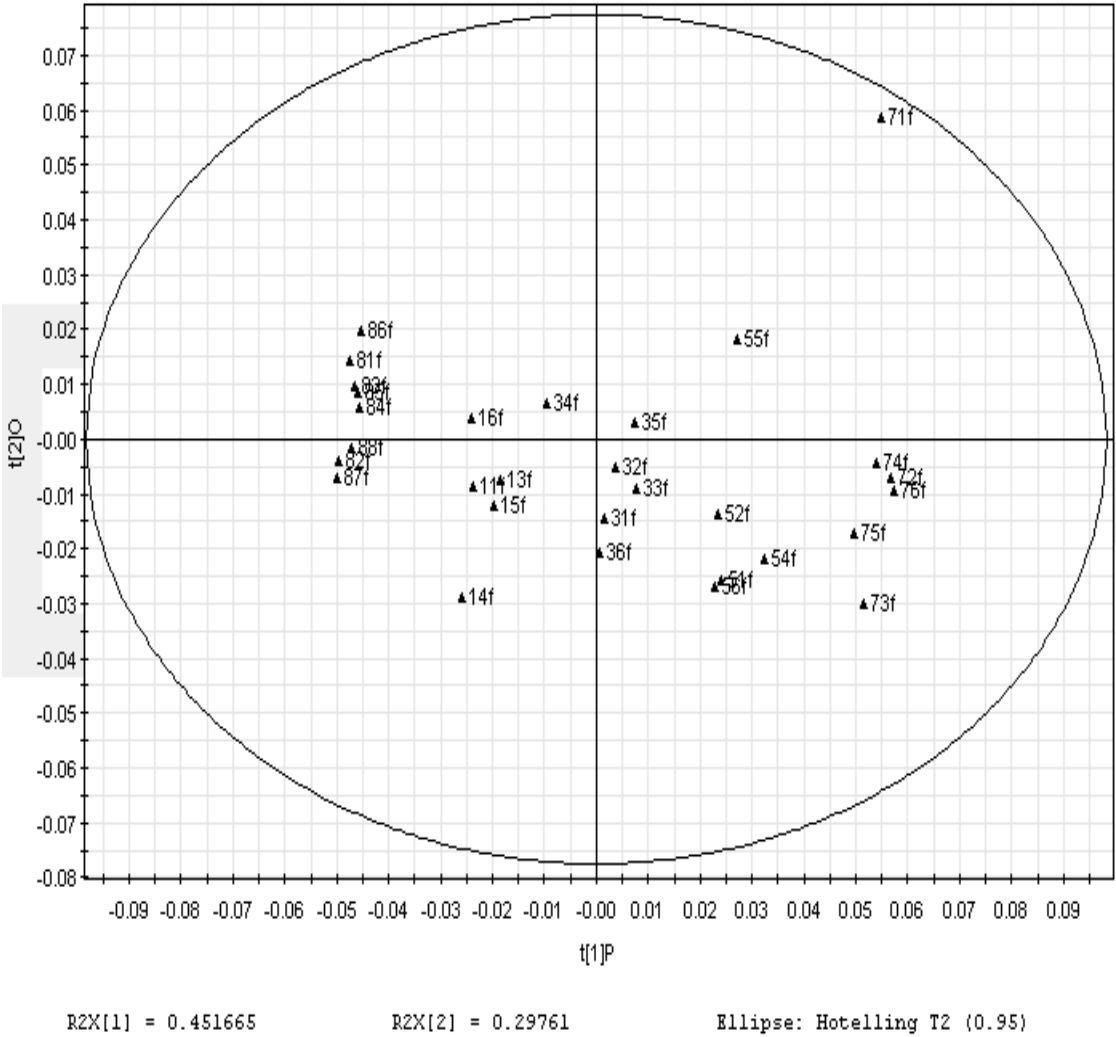


Figure S4

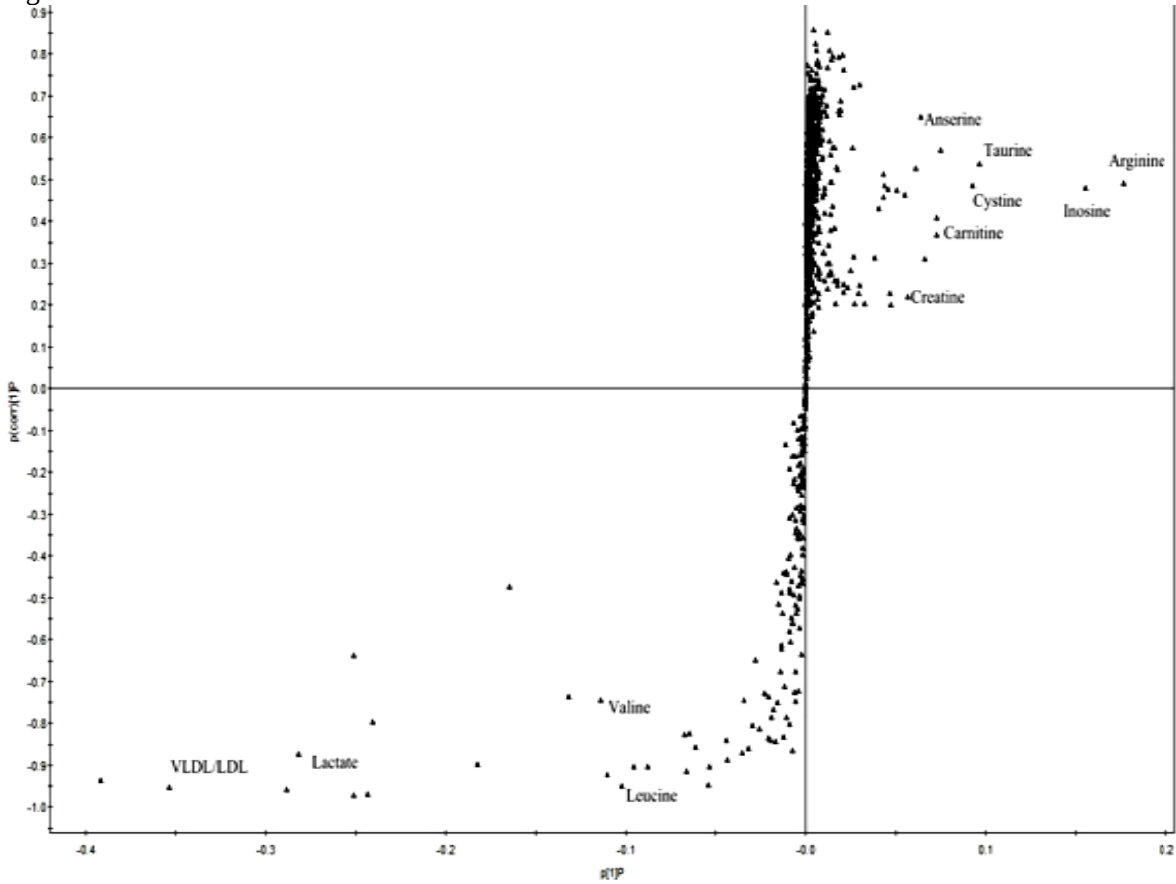


Figure S5

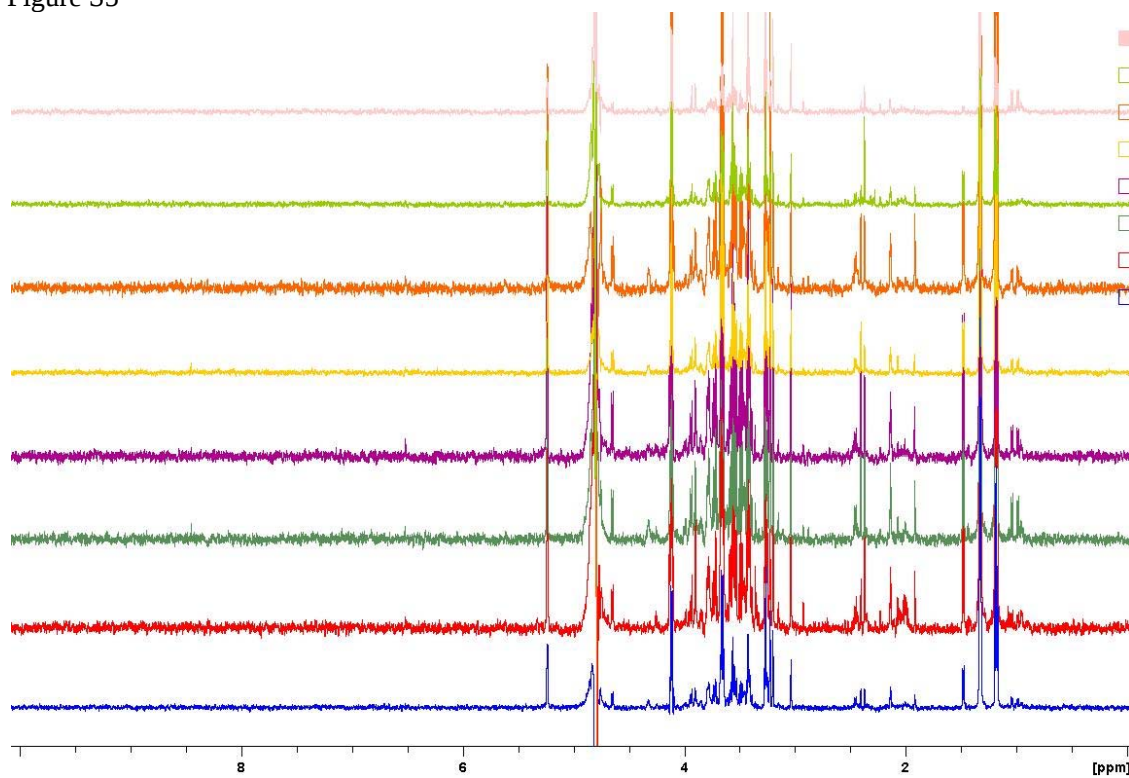


Figure S6

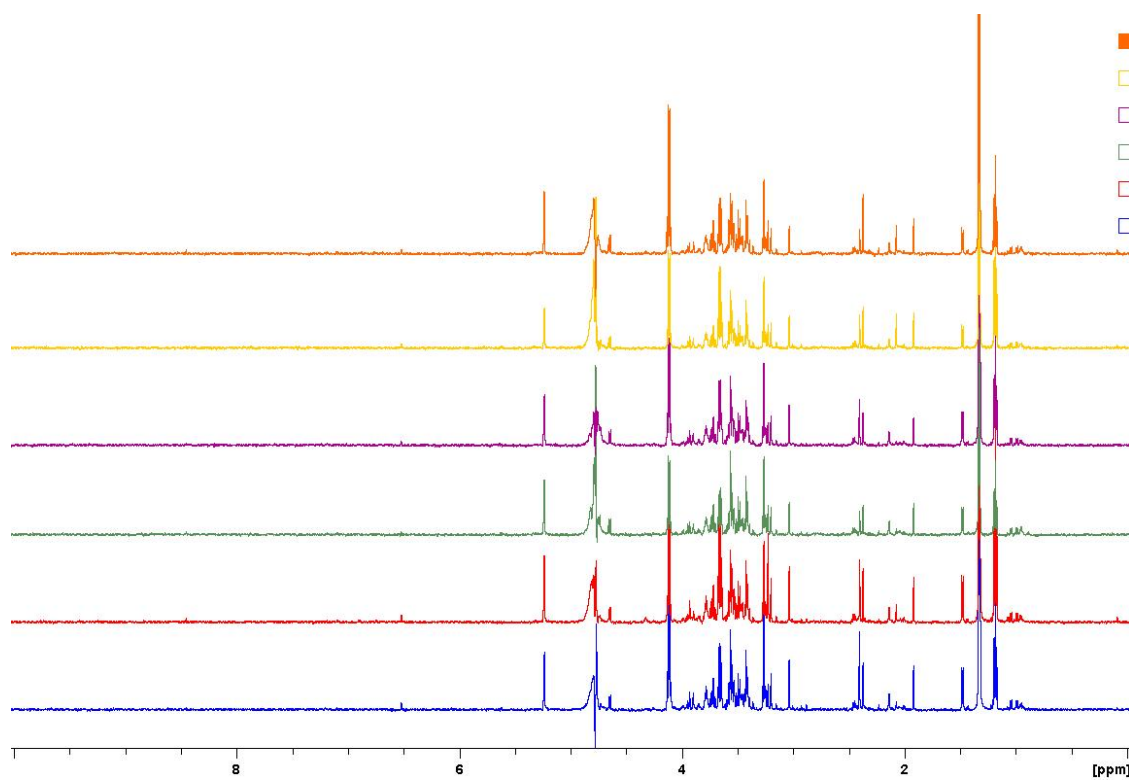


Figure S7

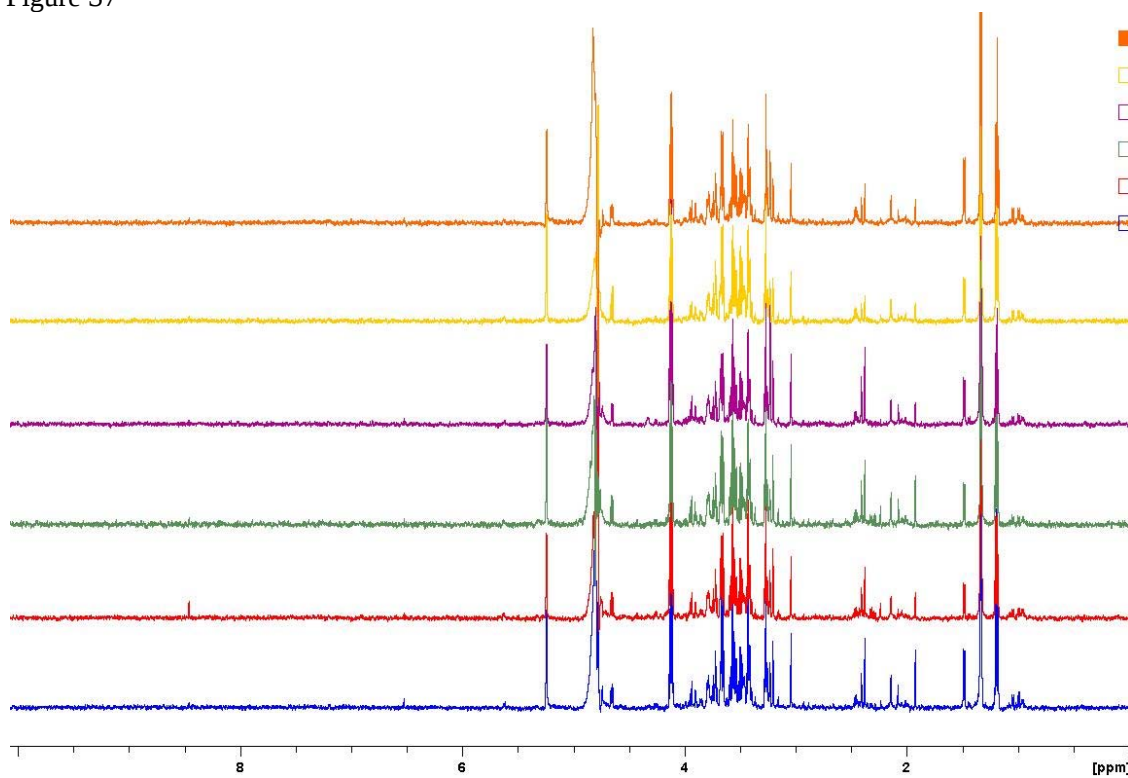


Figure S8

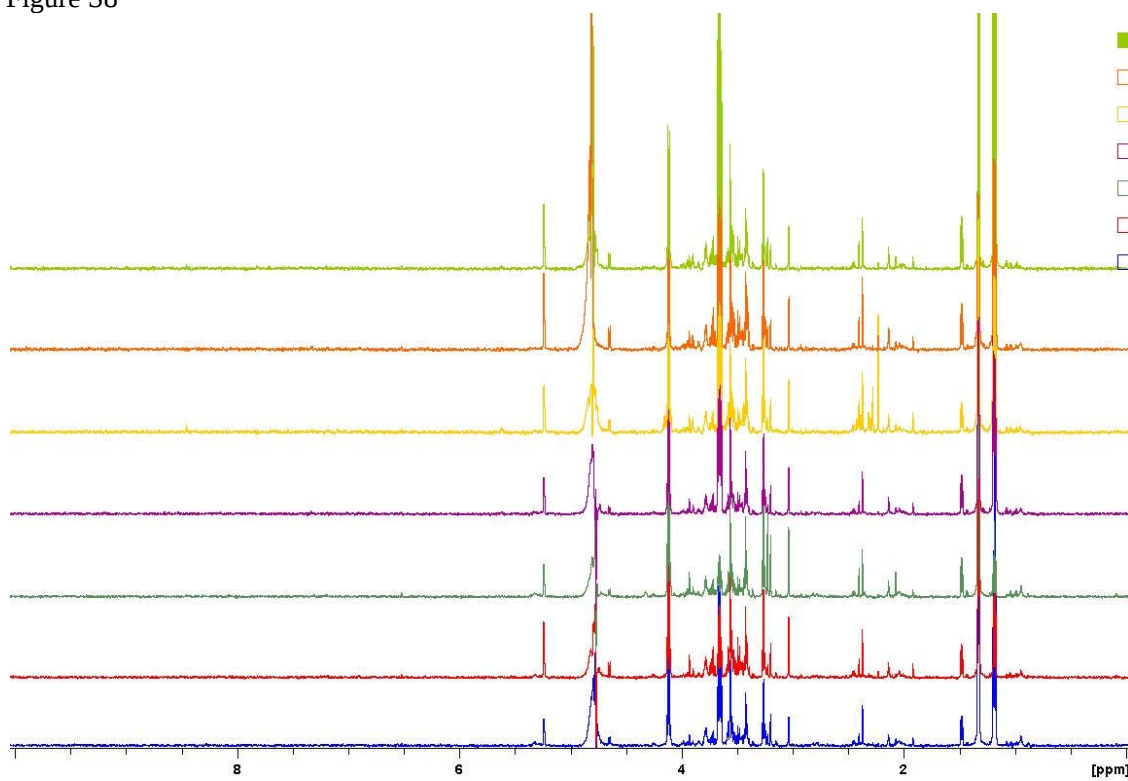


Figure S9

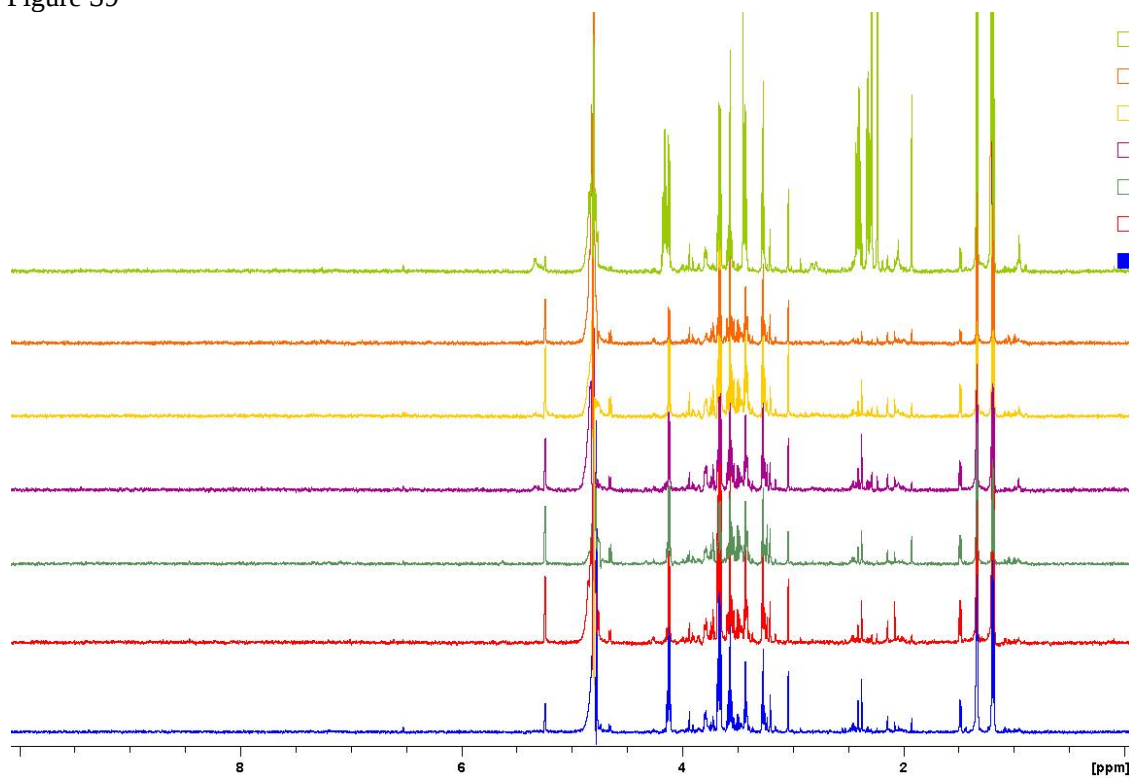


Figure S10

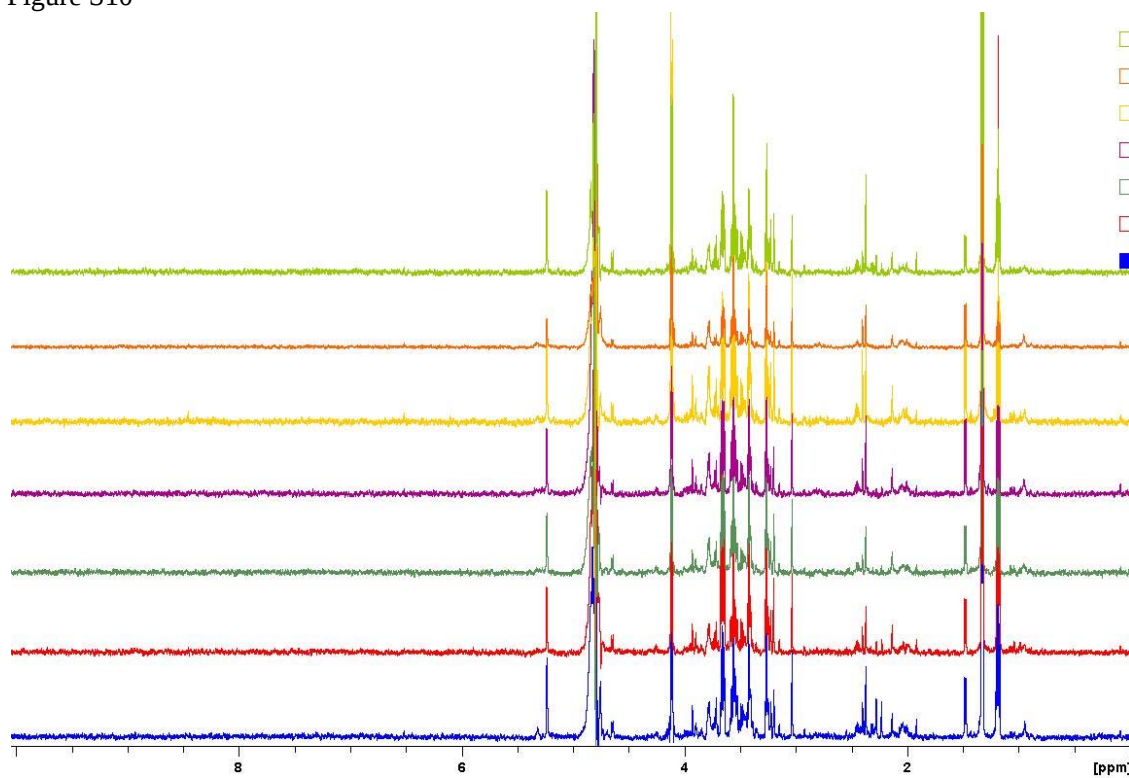


Figure S11

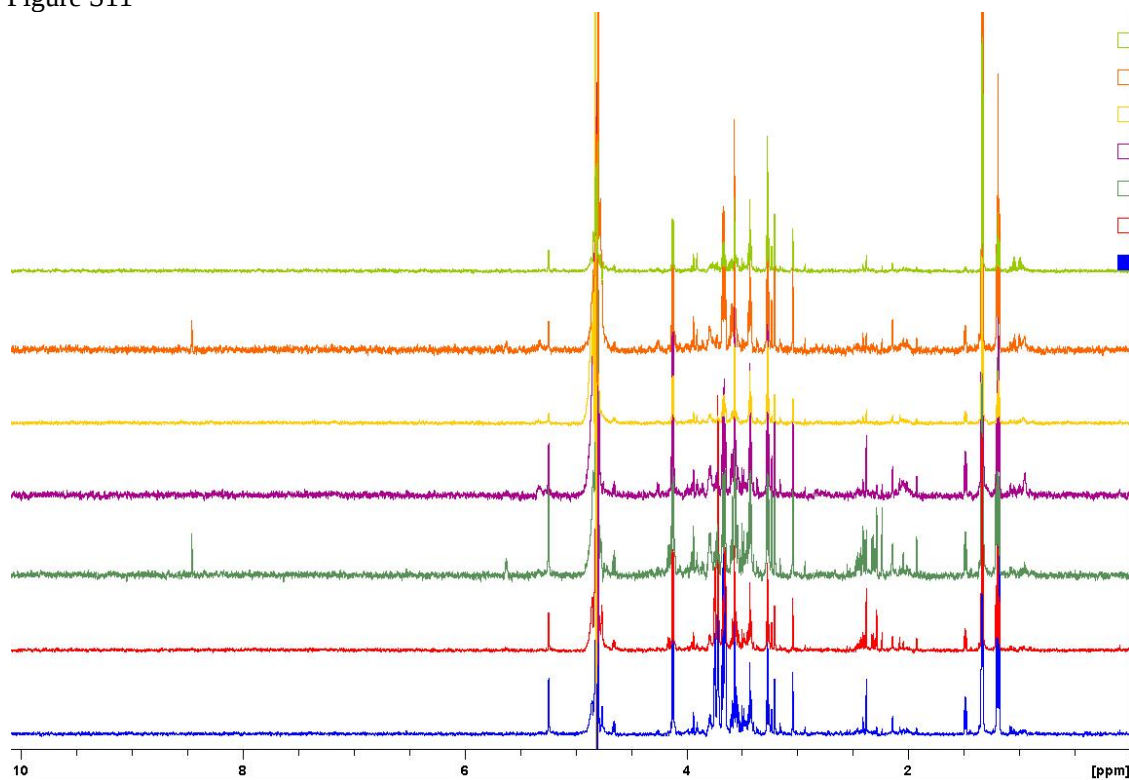


Figure S12

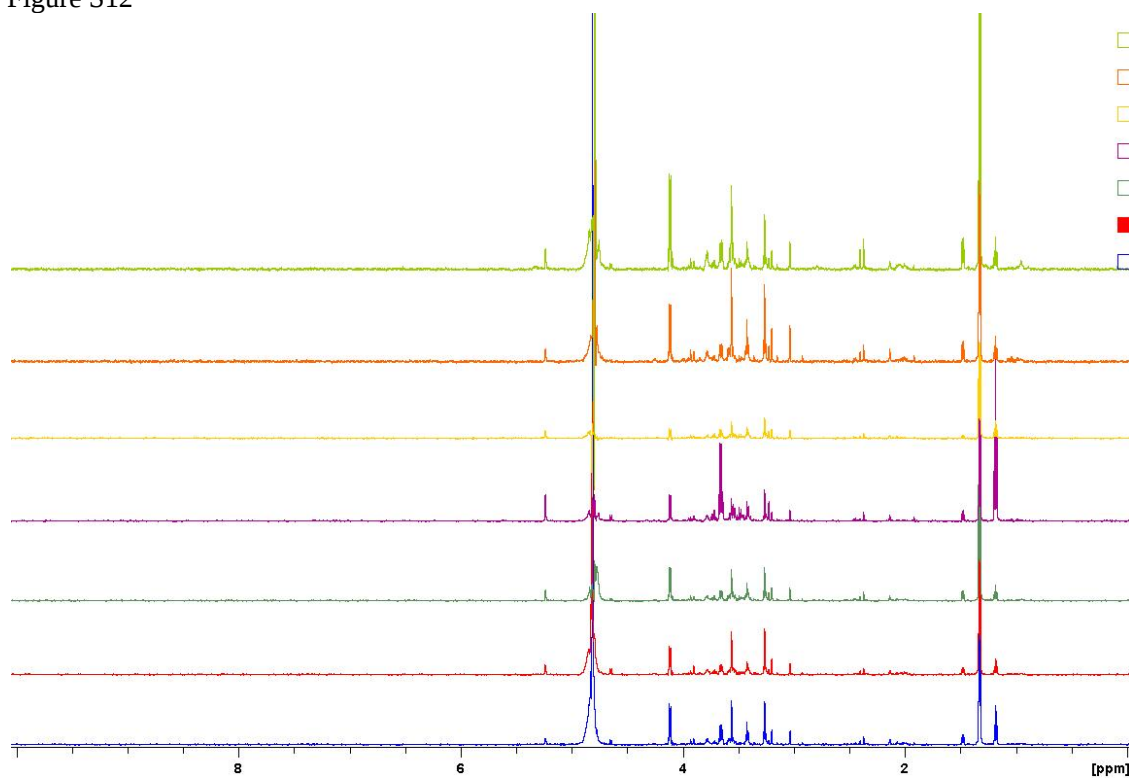


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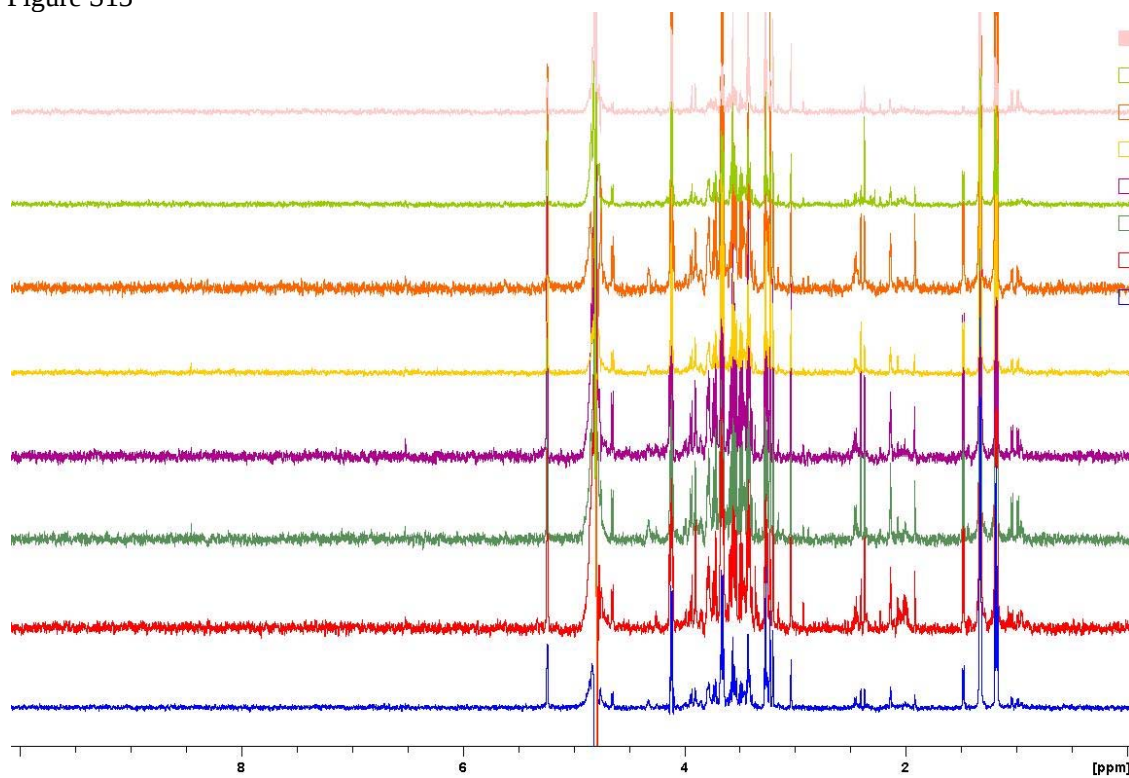


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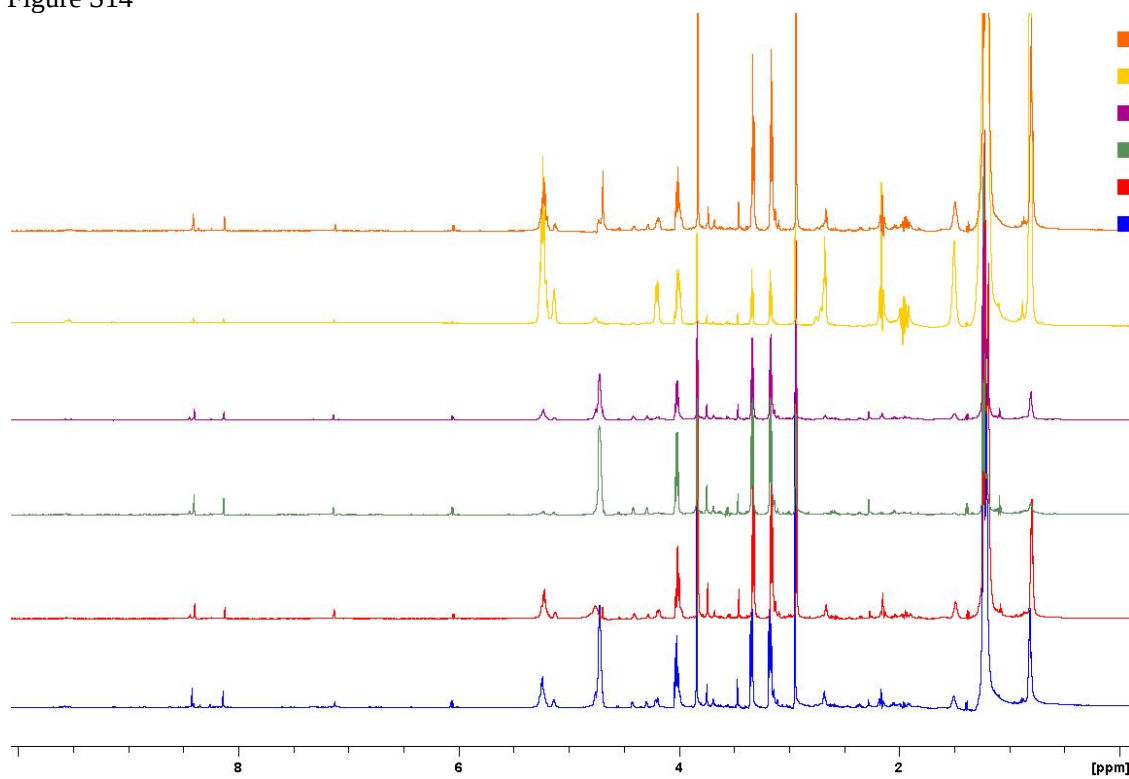


Figure S15

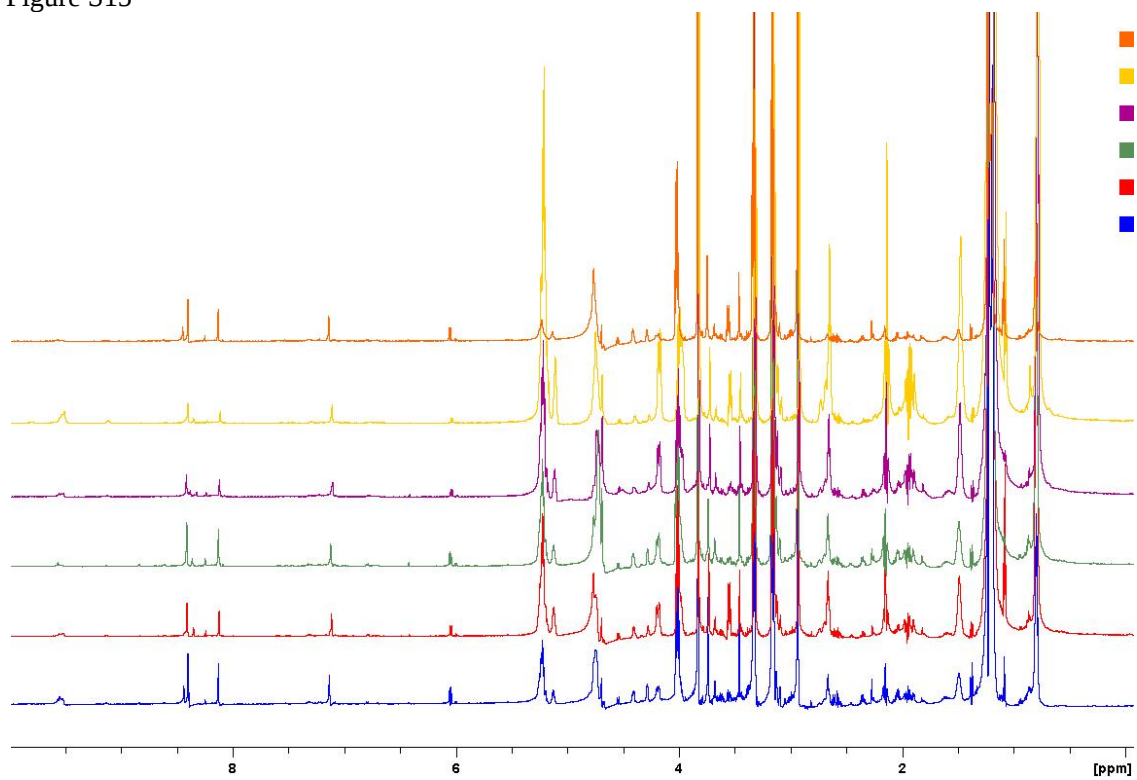


Figure S16

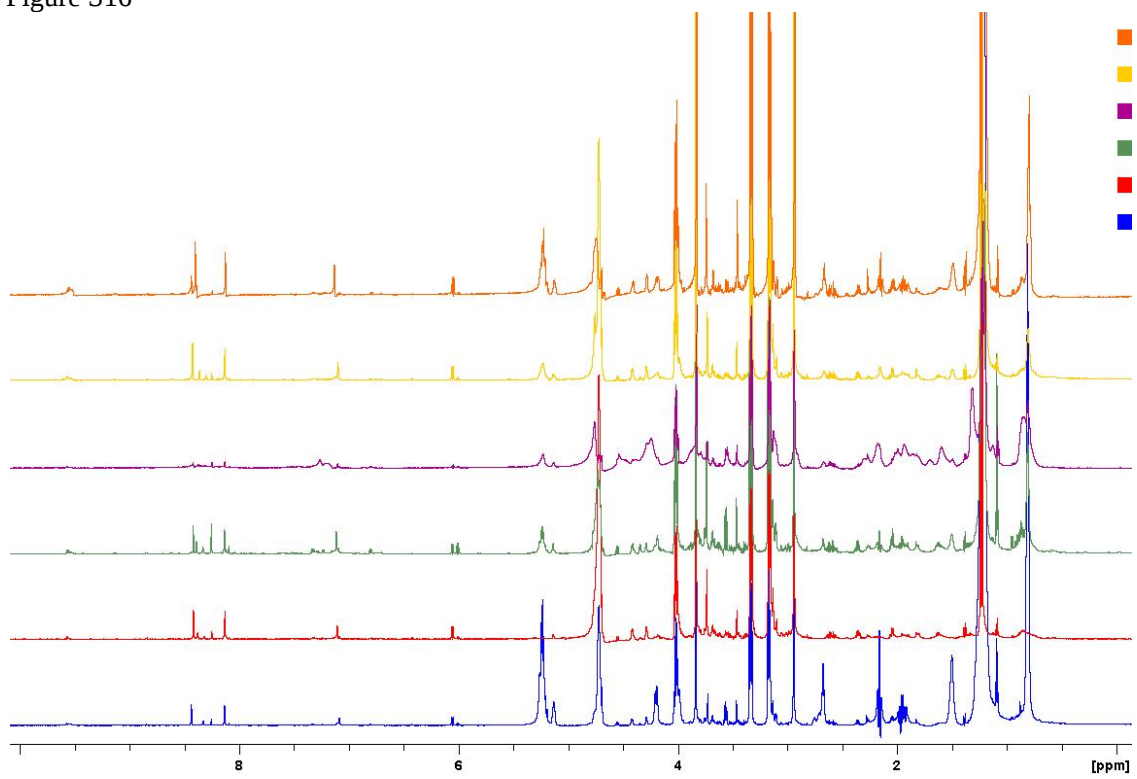


Figure S17

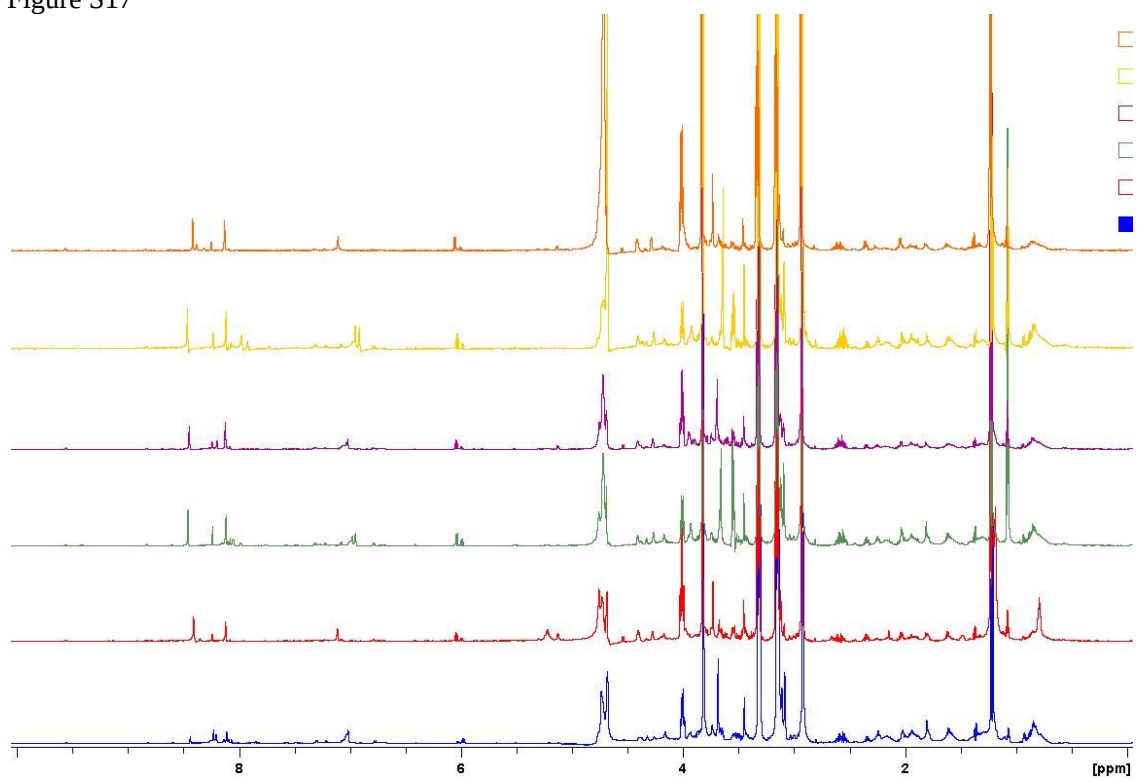


Table S1. Statistics results of serum metabolites in dynamic cachexia model animal

Metabolites	Identification	p.value	-log(p)	FDR	Fisher's LSD
Acetate	1.91(s)	2.71E-08	7.57	1.44E-05	M1 - M3; M1 - M4; M1 - M5; M1 - M6; M1 - M7; M2 - M3; M2 - M5; M2 - M6; M2 - M7; M4 - M3; M4 - M5; M4 - M6; M4 - M7
isocitrate	2.54(m), 4.06(s), 2.97(m), 2.53(m)	5.83E-08	7.23	1.56E-05	M1 - M3; M4 - M1; M5 - M1; M6 - M1; M7 - M1; M2 - M3; M6 - M2; M7 - M2; M4 - M3; M5 - M3; M6 - M3; M7 - M3
Asparagine	2.92(dd), 2.85(dd), 3.99(dd)	9.27E-08	7.03	1.65E-05	M6 - M1; M7 - M1; M2 - M3; M6 - M2; M7 - M2; M5 - M3; M6 - M3; M7 - M3; M6 - M4; M7 - M4; M6 - M5; M7 - M5
2-Hydroxyglutarate	2.00(m), 4.01(dd), 2.22(m)	8.42E-06	5.07	0.0008797	M1 - M3; M5 - M1; M7 - M1; M2 - M3; M5 - M2; M5 - M3; M6 - M3; M7 - M3; M5 - M4; M6 - M4; M7 - M4
Threonate	4.04(m), 3.61(dd), 3.66(dd)	1.02E-05	4.99	0.0008797	M2 - M1; M6 - M1; M2 - M3; M2 - M4; M2 - M5; M2 - M7; M6 - M3; M6 - M4; M6 - M5; M6 - M7
Glycine	3.56(s)	1.16E-05	4.94	0.0008797	M4 - M1; M5 - M1; M6 - M1; M7 - M1; M5 - M2; M6 - M2; M7 - M2; M4 - M3; M5 - M3; M6 - M3; M7 - M3; M5 - M4
Creatine	3.90(s), 3.02(s)	1.92E-05	4.72	0.0012185	M7 - M1; M6 - M2; M7 - M2; M5 - M3; M6 - M3; M7 - M3; M5 - M4; M6 - M4; M7 - M4; M7 - M5; M7 - M6
Glycerol	3.77(m), 3.55(dd), 3.64(dd)	2.15E-05	4.67	0.0012185	M1 - M3; M1 - M4; M2 - M3; M2 - M4; M2 - M6; M4 - M3; M5 - M3; M6 - M3; M7 - M3; M5 - M4; M5 - M6
Taurine	3.45(t), 3.25(t)	2.29E-05	4.64	0.0012185	M1 - M3; M1 - M5; M1 - M6; M1 - M7; M2 - M3; M2 - M4; M2 - M5; M2 - M6; M2 - M7; M4 - M6; M4 - M7
O-Acetylcarnitine	5.65(m), 3.18(s), 2.14(s)	2.90E-05	4.54	0.0013225	M2 - M1; M6 - M1; M2 - M3; M2 - M4; M5 - M3; M6 - M3; M7 - M3; M5 - M4; M6 - M4; M6 - M7
Citrate	2.52(d), 2.69(d)	4.90E-05	4.31	0.0020106	M5 - M1; M6 - M1; M7 - M1; M2 - M3; M6 - M2; M4 - M3; M5 - M3; M6 - M3; M7 - M3; M6 - M4
Methionine	2.13(s), 2.63(t), 3.85(dd)	6.04E-05	4.22	0.0022980	M2 - M1; M7 - M1; M2 - M3; M2 - M4; M5 - M3; M6 - M3; M7 - M3; M7 - M4; M7 - M5; M7 - M6
Glycylproline	1.98(m), 3.94(s), 4.30(dd)	6.64E-05	4.18	0.0023385	M2 - M1; M4 - M1; M5 - M1; M6 - M1; M7 - M1; M5 - M2; M7 - M2; M5 - M3; M6 - M3; M7 - M3
Serine	3.94(dd), 3.84(dd), 3.98(dd)	7.12E-05	4.15	0.0023385	M1 - M3; M1 - M4; M1 - M5; M2 - M3; M2 - M4; M2 - M5; M2 - M6; M2 - M7; M6 - M3; M6 - M4; M7 - M4
N-Carbamoylaspartate	2.47(dd), 2.66(dd)	8.34E-05	4.08	0.0024405	M1 - M3; M1 - M6; M1 - M7; M2 - M3; M2 - M4; M2 - M5; M2 - M6; M2 - M7; M4 - M3; M5 - M3; M4 - M6; M5 - M6
acetoacetate	3.46(s), 2.26(s)	8.97E-05	4.05	0.0024405	M1 - M3; M1 - M4; M1 - M5; M1 - M6; M1 - M7; M2 - M3; M2 - M4; M2 - M5; M2 - M6; M2 - M7; M4 - M6
Trimethylamine N-oxide	3.26(s)	9.65E-05	4.02	0.0024405	M6 - M1; M7 - M1; M2 - M3; M7 - M2; M4 - M3; M5 - M3; M6 - M3; M7 - M3; M6 - M4; M7 - M4; M6 - M5; M7 - M5
Anserine	7.14(w, s), 3.76(s), 8.22(s)	0.000105	3.98	0.0024405	M5 - M1; M6 - M1; M7 - M1; M2 - M3; M4 - M3; M5 - M3; M6 - M3; M7 - M3
cis-Aconitate	5.74(s), 3.07(s)	0.000106	3.97	0.0024405	M1 - M3; M2 - M3; M4 - M3; M5 - M3; M6 - M3; M7 - M3
O-Acetylcarnitine	3.83(dd), 2.13(s), 3.18(s), 3.59(d)	0.000139	3.86	0.0025467	M1 - M3; M1 - M4; M1 - M5; M1 - M6; M2 - M3; M2 - M4; M2 - M5; M2 - M6; M3 - M5; M6 - M5; M7 - M5

(Continued)

Metabolites	Identification	p.value	-log(p)	FDR	Fisher's LSD
Glutamate	2.01(m), 3.75(dd), 2.36(m), 2.12(m)	0.000182	3.74	0.002714	M5 - M1; M6 - M1; M7 - M1; M5 - M2; M5 - M3; M6 - M3; M7 - M3; M5 - M4
Choline	3.19(s)	0.000197	3.70	0.002714	M1 - M3; M2 - M3; M2 - M4; M2 - M5; M4 - M3; M6 - M3; M7 - M3; M6 - M5; M7 - M5
Glutamine	2.44(m), 3.77(t), 2.10(m)	0.000207	3.69	0.0027244	M1 - M3; M1 - M6; M2 - M3; M2 - M6; M4 - M3; M5 - M3; M7 - M3; M4 - M6; M5 - M6
2-Oxoglutarate	2.45(t), 2.99(t)	0.000377	3.42	0.0042711	M1 - M3; M1 - M6; M2 - M3; M2 - M4; M2 - M5; M2 - M6; M2 - M7; M4 - M3; M5 - M3; M7 - M3
Pyruvate	2.4(s)	0.000491	3.31	0.004915	M1 - M2; M1 - M3; M1 - M4; M1 - M5; M1 - M6; M1 - M7; M2 - M7; M4 - M7; M5 - M7
Ethanol	3.62(q), 1.17(t)	0.000499	3.30	0.004915	M1 - M3; M6 - M1; M2 - M3; M2 - M5; M4 - M3; M6 - M3; M7 - M3; M4 - M5; M6 - M5; M7 - M5
Arginine	3.25(t), 3.76(t), 1.91(m)	0.00056	3.25	0.0050626	M2 - M1; M6 - M1; M7 - M1; M2 - M3; M4 - M3; M5 - M3; M6 - M3; M7 - M3; M6 - M5
Methionine	2.62(t), 2.11(s), 3.83(dd)	0.000647	3.19	0.0054035	M5 - M1; M6 - M1; M7 - M1; M2 - M3; M4 - M3; M5 - M3; M6 - M3; M7 - M3; M6 - M4
Citrulline	1.55(m), 3.75(t), 3.12(m), 1.84(m)	0.000649	3.19	0.0054035	M2 - M1; M4 - M1; M5 - M1; M6 - M1; M7 - M1; M2 - M3; M4 - M3; M5 - M3; M6 - M3; M7 - M3
Glucose-1-phosphate	5.47	0.000687	3.16	0.005631	M2 - M1; M6 - M1; M2 - M3; M4 - M3; M5 - M3; M6 - M3; M7 - M3; M6 - M4
Carnitine	3.21(s)	0.000781	3.11	0.0061158	M1 - M3; M2 - M3; M2 - M4; M2 - M5; M5 - M3; M6 - M3; M7 - M3; M6 - M4
Succinate	2.43(s)	0.000792	3.10	0.0061158	M1 - M3; M1 - M6; M1 - M7; M2 - M3; M2 - M6; M2 - M7; M4 - M3; M5 - M3; M5 - M7
Glutathione	2.93(m), 2.53(m), 2.14(q), 3.76(d)	0.000814	3.09	0.006201	M2 - M1; M5 - M1; M6 - M1; M7 - M1; M2 - M3; M2 - M4; M5 - M3; M6 - M3; M7 - M3; M5 - M4; M6 - M4; M7 - M4
Creatine	3.01(s), 3.92(s)	0.000986	3.01	0.0069129	M1 - M3; M2 - M3; M2 - M4; M2 - M5; M2 - M6; M2 - M7; M4 - M3; M5 - M3; M6 - M3; M7 - M3
3-Hydroxybutyrate	1.19(s), 4.14(m), 2.29(dd), 2.39(dd)	0.002522	2.60	0.012708	M3 - M1; M4 - M1; M3 - M2; M4 - M2; M3 - M5; M3 - M6; M3 - M7; M4 - M5; M4 - M6; M4 - M7
sn-Glycero-3-phosphocholine	4.35(m), 3.21(s), 3.67(m)	0.002527	2.60	0.012708	M1 - M3; M2 - M3; M4 - M3; M5 - M3; M6 - M3; M7 - M3
Glucose	4.61(d), 5.21(d), 3.39-3.89(m)	0.002657	2.58	0.012783	M1 - M3; M7 - M1; M7 - M2; M5 - M3; M6 - M3; M7 - M3; M7 - M4
trans-Aconitate	6.52(s), 3.37(s)	0.002711	2.57	0.012783	M1 - M3; M1 - M5; M1 - M6; M1 - M7; M2 - M3; M2 - M6; M2 - M7; M4 - M7
Lysine	3.01(t), 3.75(t), 1.72(m)	0.002728	2.56	0.012783	M1 - M3; M1 - M5; M2 - M3; M2 - M5; M4 - M3; M6 - M3; M7 - M3
Ananine	1.47(d), 3.79(dd)	0.002882	2.54	0.012908	M1 - M4; M1 - M6; M2 - M6; M3 - M6; M5 - M4; M5 - M6; M7 - M6
O-Acetylcarnitine	5.61(s), 3.18(s), 2.13(s)	0.006564	2.18	0.019898	M2 - M1; M6 - M1; M2 - M3; M2 - M4; M2 - M5; M2 - M7; M6 - M3; M6 - M4
Leucine	0.98(t), 3.73(dd), 1.7(m)	0.006599	2.18	0.019898	M1 - M3; M1 - M4; M1 - M5; M1 - M7; M2 - M3; M2 - M4; M2 - M5; M2 - M7; M6 - M5
Malate	2.34(dd), 2.66(dd), 4.3(d)	0.006626	2.18	0.019898	M7 - M1; M2 - M3; M4 - M3; M6 - M3; M7 - M3; M7 - M4; M7 - M5

Table S2. Statistics results of intact muscle gastrocnemius metabolites in dynamic cachexia model animal

Metabolites	p.value	-log(p)	FDR	Fisher's LSD
Glycine	1.57E-05	4.8034	0.000189	M3 - M0; M4 - M0; M3 - M1; M4 - M1; M4 - M2; M4 - M3
Creatine	0.000502	3.2995	0.002821	M4 - M0; M4 - M1; M4 - M2; M4 - M3
Lactate	0.000705	3.1517	0.002821	M3 - M0; M4 - M0; M3 - M1; M4 - M1; M3 - M2; M4 - M2
Isoleucine	0.001021	2.9911	0.003062	M4 - M0; M4 - M1; M4 - M2; M4 - M3
3-Methylhistidine	0.001352	2.869	0.003245	M4 - M0; M4 - M1; M4 - M2; M4 - M3
Taurine	0.002754	2.56	0.005509	M4 - M0; M4 - M1; M4 - M2
Valine	0.003991	2.3989	0.006843	M3 - M0; M4 - M0; M4 - M1; M4 - M2
Inosine	0.009456	2.0242	0.012246	M4 - M0; M4 - M2
Glucose	0.012408	1.9063	0.018613	M1 - M4; M2 - M4
3-Hydroxybutyrate	0.016809	1.7745	0.022412	M0 - M3; M0 - M4; M1 - M4; M2 - M4
Leucine	0.02296	1.639	0.027552	M3 - M0; M4 - M0; M4 - M1; M4 - M2
Phenylalanine	0.049316	1.307	0.0538	M1 - M0; M4 - M0

TableS3Analysis of the altered metabolic pathways of cancer cachexia

Pathway Name	Total Cmpd	Hits	Raw p	Holm p	FDR	Impact
Valine, leucine and isoleucine biosynthesis	11	3	0.017441	0.20929	0.025115	0.66666
Synthesis and degradation of ketone bodies	5	2	0.24649	0.69083	0.24649	0.6
Phenylalanine, tyrosine and tryptophan biosynthesis	4	1	0.020409	0.2245	0.027212	0.5
Taurine and hypotaurine metabolism	8	1	0.000202	0.005255	0.000661	0.42857
Phenylalanine metabolism	11	1	0.020409	0.2245	0.027212	0.40741
Glycine, serine and threonine metabolism	31	6	2.1E-05	0.000694	9.94E-05	0.32914
Glycerolipid metabolism	18	1	0.002246	0.04268	0.004493	0.28098
Glyoxylate and dicarboxylate metabolism	18	2	0.19585	0.69083	0.20737	0.25806
Pyruvate metabolism	23	4	1.96E-05	0.000687	9.94E-05	0.235
Citrate cycle (TCA cycle)	20	5	0.00145	0.028991	0.00307	0.22314
Alanine, aspartate and glutamate metabolism	24	5	0.000945	0.02362	0.002788	0.15295
Glycolysis or Gluconeogenesis	26	3	1.98E-05	0.000687	9.94E-05	0.12753
Inositol phosphate metabolism	28	1	0.048726	0.38981	0.058471	0.11163
Cysteine and methionine metabolism	27	2	0.00432	0.073441	0.007497	0.10977
Butanoate metabolism	22	4	0.002628	0.047305	0.00498	0.10145
Arginine and proline metabolism	44	4	0.006133	0.089663	0.009599	0.07598
Primary bile acid biosynthesis	46	2	8.25E-06	0.000297	9.94E-05	0.05952
Starch and sucrose metabolism	19	1	0.001127	0.027054	0.002788	0.03958
Galactose metabolism	26	3	0.001239	0.028199	0.002788	0.03644
Glycerophospholipid metabolism	30	1	0.1044	0.52198	0.11745	0.02315
Glutathione metabolism	26	1	2.76E-05	0.000828	9.94E-05	0.00573

The table below shows the detailed results from the pathway analysis. Since we are testing many pathways at the same time, the statistical p values from enrichment analysis are further adjusted for multiple testings. In particular, the Total is the total number of compounds in the pathway; the Hits is the actually matched number from the user uploaded data; 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.