Supplementary materials

Untargeted metabolomic analysis using LC–TOF/MS and LC–MS/MS for revealing metabolic alterations linked to alcohol-induced hepatic steatosis in rat serum and plasma

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Supporting information Text S1

Parameter settings for XCMS processing depend on the instrument platform in which the data were acquired. The parameters as follows: centWave for feature detection (Δ*m/z*= 5 ppm, minimum peak width = 10 s, and maximum peak width = 60 s); obiwarp settings for retention-time correction (profStep = 0.5); and parameters for chromatogram alignment, including mzwid = 0.025, minfrac = 0.5, and bw = 5.

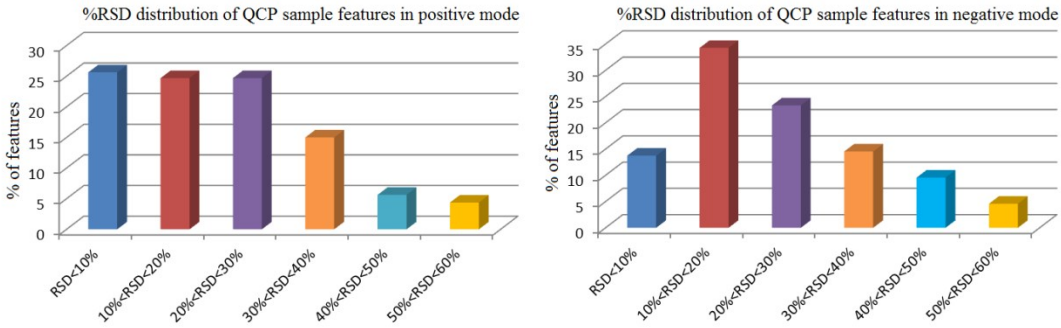

Fig.S1. The %RSD distribution of all features detected from QCP sample in positive mode and negative mode, respectively. The 80%-rule was applied on the data and features having %RSD values higher than 60% were excluded because these are likely to have large RSDs and may not be a true reflection of the variation between subjects.

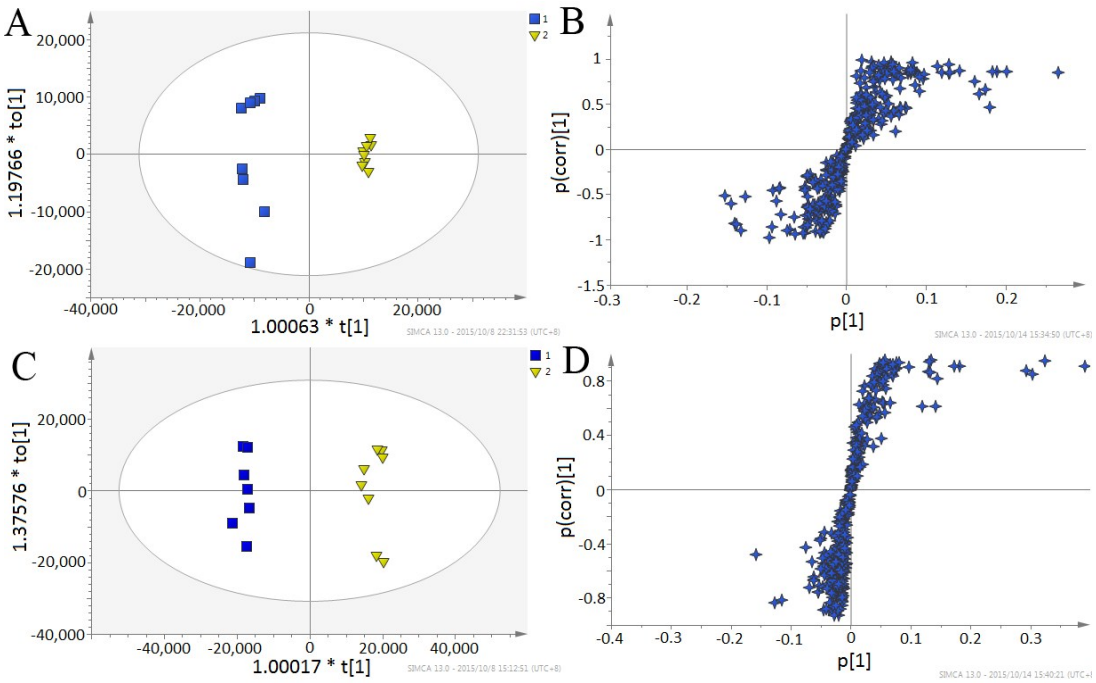

Fig. S2. Score plots of OPLS-DA in positive-ion mode (A) with the statistical parameters (R^2X = 0.597, R^2Y = 0.989, Q^2 = 0.902, p[CV-ANOVA] = 4.3 × 10^{-4}) and in negative-ion mode (C) with the statistical parameters (R^2X = 0.760, R^2Y = 0.984, Q^2 = 0.957, p[CV-ANOVA] = 1.2 × 10^{-5}) between the normal rat serum (blue squares) and AHS rat serum (yellow inverted triangle). The corresponding S-plot from OPLS-DA model of the two groups in positive-ion mode (B) and in negative-ion mode (D).

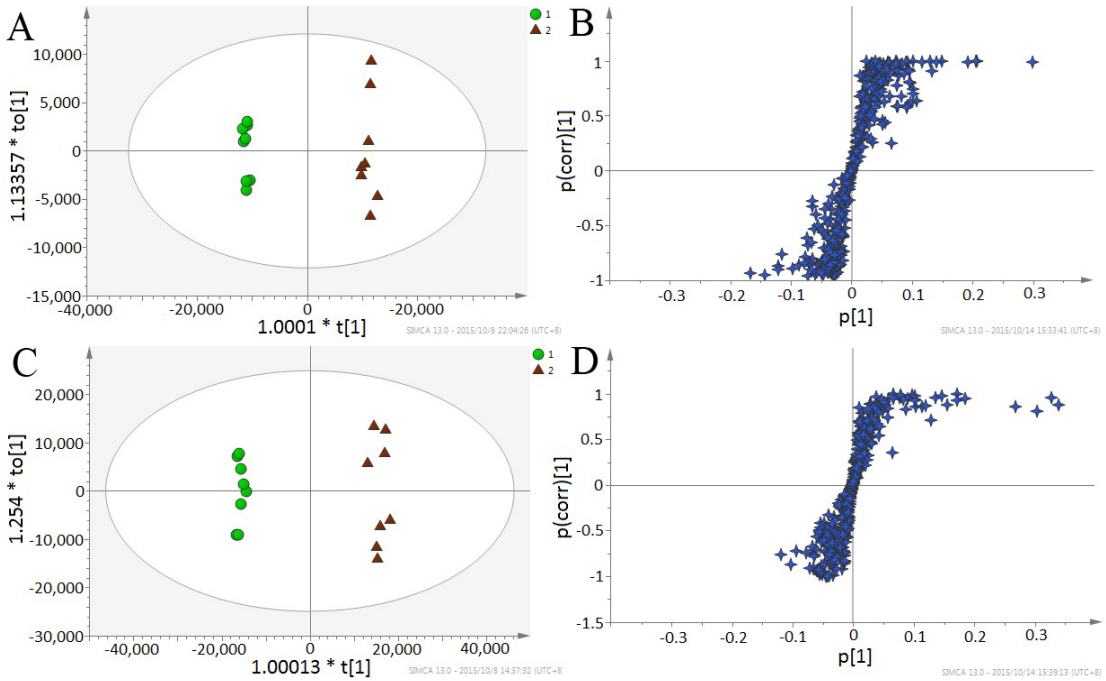

Fig. S3. Score plots of OPLS-DA in positive-ion mode (A) with the statistical parameters (R^2X = 0.539, R^2Y = 0.996, Q^2 = 0.978, p[CV-ANOVA] = 4.6 × 10^{-9}) and in negative-ion mode (B) with the statistical parameters (R^2X = 0.741, R^2Y = 0.994, Q^2 = 0.967, p[CV-ANOVA] = 3.5 × 10^{-6}) between the normal rat plasma (green dot) and AHS rat plasma (brown triangle). The corresponding S-plot from OPLS-DA model of the two groups in positive-ion mode (B) and in negative-ion mode (D).

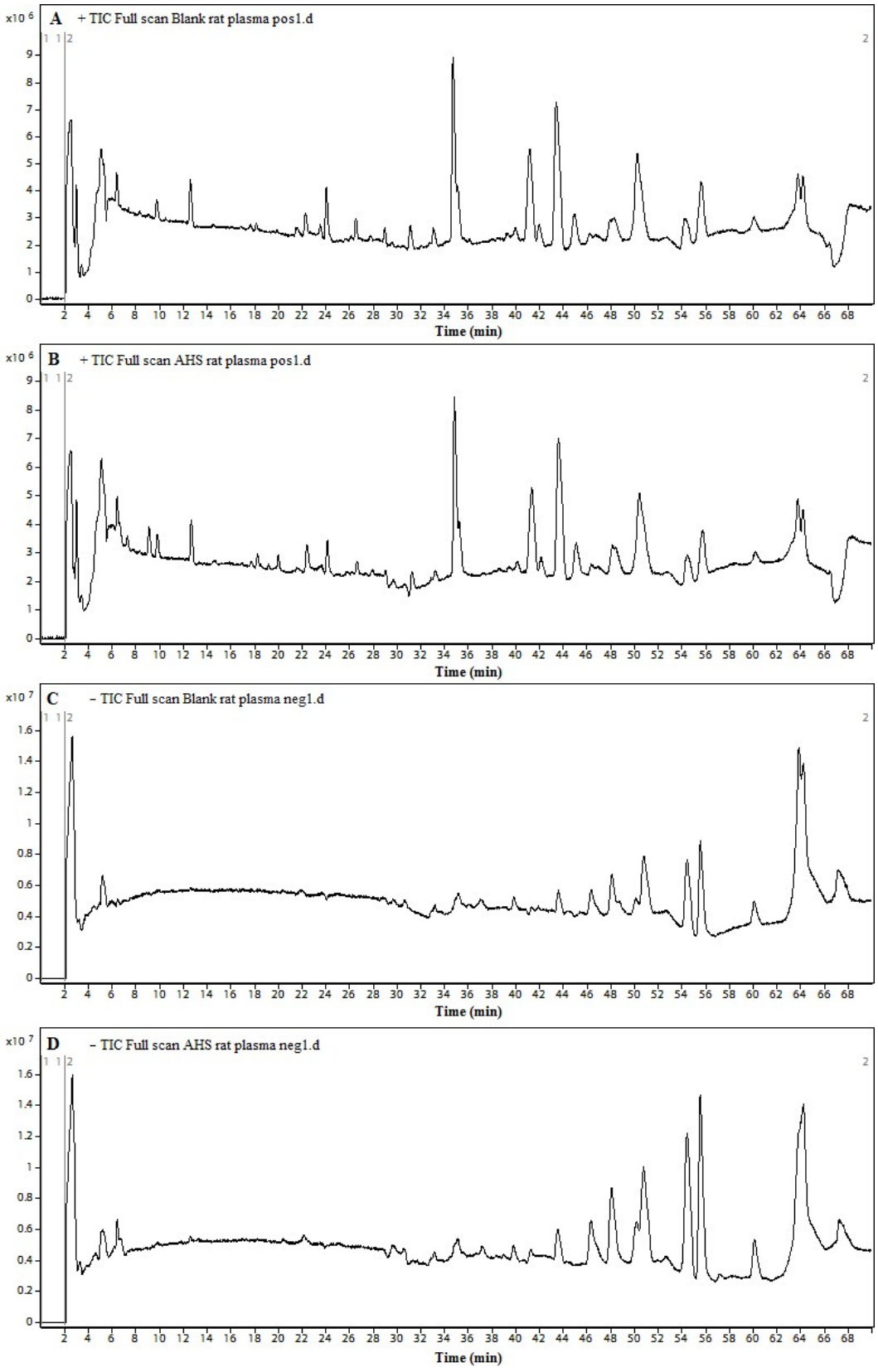

Fig. S4. HPLC-ESI-TOF/MS chromatograms of normal rat plasma and AHS rat plasma in positive-ion mode (A, B) and in negative-ion mode (C, D), respectively.

Table S1: OPLS-DA markers (t_R-*m/z*) of serum and plasma samples in positive-ion mode and negative-ion mode

Features (t_R-*m/z*)	Adduct	Formula	M_W(Da)	Diff. (ppm)	Metabolite	Chemical classe	VIP	Intensity response
Positive-ion mode								
9.77-120.0811	$[M+H]^+$	$C_8H_{10}N$	120.0813	–1.6	Frag of Phe-Phe	Peptide	3.65	Δ
31.21-453.1721	–	–	–	–	DCP	Peptide	3.32	Δ
30.10-437.1983	–	–	–	–	DCP	Peptide	3.31	Δ
9.79-313.1531	$[M+H]^+$	$C_{18}H_{21}N_2O_3$	313.1552	–6.7	Phe-Phe	Peptide	2.72	Δ
41.48-542.3242	$[M+Na]^+$	$C_{26}H_{50}NO_7PNa$	542.3223	3.5	LysoPC (18:2)	Phospholipid	2.68	↑*
45.18-522.3556	$[M+H]^+$	$C_{26}H_{53}NO_7P$	522.3560	–0.8	LysoPC (18:1)	Phospholipid	2.36	↑*
31.76-513.1842	–	–	–	–	DCP	Peptide	2.32	Δ
31.26-504.3089	–	–	–	–	DCP	peptide	2.09	Δ
38.10-539.3192	–	–	–	–	DCP	peptide	1.83	Δ
59.02-782.5683	$[M+H]^+$	$C_{44}H_{81}NO_8P$	782.5700	–2.2	PC (36:4)	Phospholipid	1.55	↑*
43.71-518.3235	$[M+Na]^+$	$C_{24}H_{50}NO_7PNa$	518.3223	2.3	LysoPC (16:0)	Phospholipid	1.51	↑*
50.63-524.3713	$[M+H]^+$	$C_{26}H_{55}NO_7P$	524.3716	–0.6	LysoPC (18:0)	Phospholipid	1.33	↑*
50.81-303.2313	$[M+H]^+$	$C_{20}H_{31}O_2$	303.2324	–3.6	Eicosapentaenoic acid	Fatty acid	1.27	↑*
3.21-175.0244	$[M+Na]^+$	$C_5H_4N_4O_2Na$	175.0232	6.9	Xanthine	Heterocyclic compound	1.17	↑*
5.94-159.0274	$[M+Na]^+$	$C_5H_4N_4ONa$	159.0283	–5.7	Hypoxanthine	Heterocyclic compound	1.14	↑*
59.01-758.5691	$[M+H]^+$	$C_{42}H_{81}NO_8P$	758.5700	–1.2	PC (34:2)	Phospholipid	1.06	↑*
Negative-ion mode								

9.79-311.1402	$[M-H]^-$	$C_{18}H_{19}N_2O_3$	311.1396	1.9	Phe-Phe	Peptide	2.69	△
51.01-279.1978	$[M-H]^-$	$C_{17}H_{27}O_3$	279.1960	6.5	12-HHT	Fatty acid	2.43	↑*
44.95-556.3043	$[M-H]^-$	$C_{28}H_{47}NO_8P$	556.3039	0.7	LysoPC (20:4)	Phospholipid	2.33	↑*
55.71-363.2553	$[M+CH_3COO]^-$	$C_{22}H_{35}O_4$	363.2535	5.0	Arachidonic acid	Fatty acid	2.07	↑*
35.28-317.2127	$[M-H]^-$	$C_{20}H_{29}O_3$	317.2117	3.2	12-HEPE	Fatty acid	2.01	↑*
46.52-301.2173	$[M-H]^-$	$C_{20}H_{29}O_2$	301.2168	1.7	Eicosapentaenoic acid	Fatty acid	1.87	↑*
38.44-319.2293	$[M-H]^-$	$C_{20}H_{31}O_3$	319.2273	6.3	8-HETE	Fatty acid	1.81	↓*
48.31-335.2236	$[M-H]^-$	$C_{20}H_{31}O_4$	335.2222	4.2	5,15-diHETE	Fatty acid	1.72	↓*
43.61-554.3546	$[M+CH_3COO]^-$	$C_{26}H_{53}NO_9P$	554.3458	–2.2	LysoPC (16:0)	Phospholipid	1.62	↑*
41.16-580.3608	$[M+CH_3COO]^-$	$C_{28}H_{55}NO_9P$	580.3614	–1.0	LysoPC (18:1)	Phospholipid	1.57	↑*
54.78-337.2401	$[M-H]^-$	$C_{20}H_{33}O_4$	337.2379	6.5	5,6-diHETrE	Fatty acid	1.55	↑*
39.54-552.3312	$[M+CH_3COO]^-$	$C_{26}H_{51}NO_9P$	552.3301	2.0	LysoPC (16:1)	Phospholipid	1.36	↑*
58.95-834.6011	$[M-H]^-$	$C_{48}H_{85}NO_8P$	834.6013	–0.2	PC (40:5)	Phospholipid	1.29	↑*
43.55-480.3094	$[M-H]^-$	$C_{23}H_{47}NO_7P$	480.3090	0.3	LysoPC (15:0)	Phospholipid	1.11	↑*
50.41-508.3417	$[M-H]^-$	$C_{25}H_{51}NO_7P$	508.3403	2.8	LysoPC (17:0)	Phospholipid	1.02	↑*

Abbreviations: M_W, monoisotopic weight; VIP, variable importance on projection; Phe, phenylalanine; DCP, doubly charged peptide; LysoPC, lysophosphatidylcholine; PC, phosphatidylcholine; HHT, hydroxyheptadecatrienoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; diHETE, dihydroxyeicosatetraenoic acid; diHETrE, dihydroxyeicosatrienoic acid.

*: Response compared to heparinized plasma samples.

△: Detected only in serum.