Supplementary materials

1. Determination of SAM and SAH levels in the liver using an LC-MS/MS method

1.1 Chromatographic conditions

Column: Shim-pack GIST Amide, 2.1×100 mm, 3.0 μm, SHIMADZU

Mobile phase A (MPA): aqueous solution containing 0.1% formic acid and 10.0 mM ammonium formate

Mobile Phase B (MPB): Acetonitrile

Flow Rate: 0.800 mL/min

Injection Volume: 10.0 μL

Autosampler temperature (Sample Tray Temp): 4 °C

Column Temperature: 35 °C

Elution gradient：

Time (minute)	Module	Function	Value (%)
1.80	Pumps	Pump B Conc.	60.0
2.70	Pumps	Pump B Conc.	60.0
2.80	Pumps	Pump B Conc.	68.0
4.00	System Controller	Stop	

1.2 Mass spectrometry conditions

Ion source: Electrospray Ionization (ESI)

Ionization mode (Ionization mode): Positive

Detection Mode (Mode): Multiple Reaction Monitoring (MRM)

Ion Spray Voltage: 5500.00 V

Turbo Ion Spray Temp: 550.00 °C

Curtain Gas Type: Nitrogen Setting: 40.00 psi

Collision cell gas type (CAD Gas Type): Nitrogen Setting: 9

Nebulizing Gas (Gas1): Nitrogen Setting: 65.00 psi

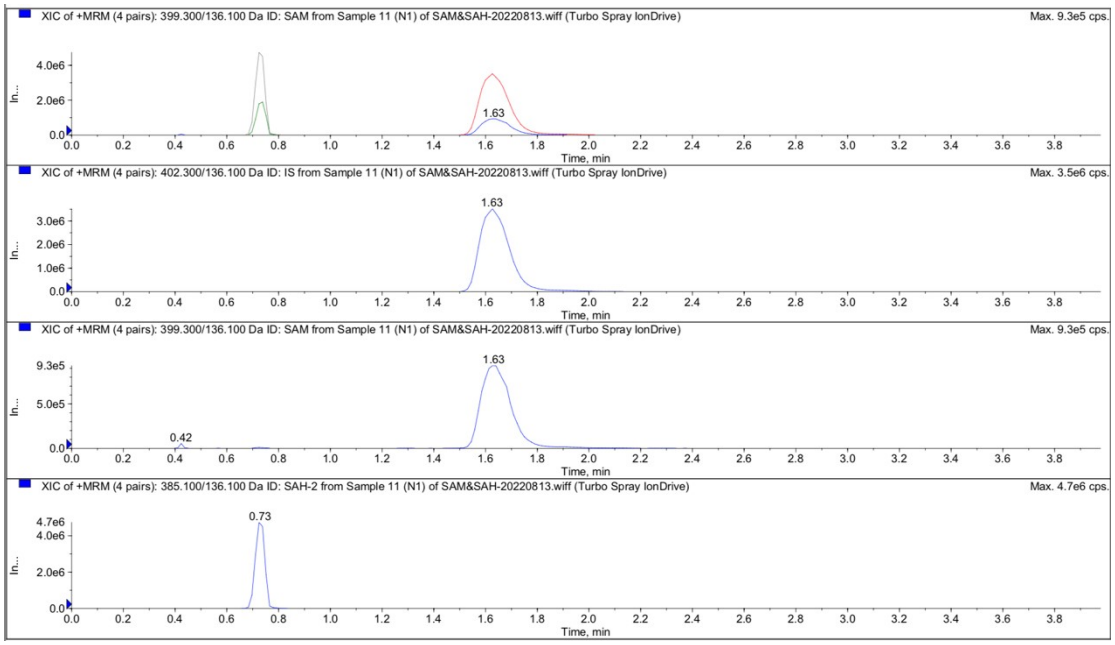
Auxiliary Gas (AuxiliaryGas, Gas 2): Nitrogen Setting: 65.00 psi

Inlet Voltage (EP): 10.00 V

Collision cell outlet voltage (CXP): 10.00 V

Acquisition Time: 4.00 min

Analytes	MRM	Dwell Time (ms)	（DP） (volts)	CE (volts)	retention time (min)
SAM	399.3→136.1	200	77.0	36.0	1.468
SAH	385.1→136.1	200	74	19	0.732
SAM-d3	402.3→136.1	200	80.0	32.0	1.468



1.3 Solution preparation

(1) Mobile phase A (aqueous solution containing 0.1% formic acid and 10.0 mM ammonium formate) [MPA]: Weigh 630 mg of ammonium formate into a glass bottle filled with 1000 mL of ultrapure water, then add 1.00 mL of formic acid, and mix by shaking. Storage conditions: room temperature. Validity period: 2 weeks.

(2) Mobile phase B (acetonitrile) [MPB]

(3) Acetonitrile: water (30:70, v:v) [R01]: Add 300 mL of acetonitrile and 700 mL of ultrapure water to a glass bottle, shake and mix. Storage conditions: room temperature. Validity period: 2 weeks.

(4) Ammonium formate aqueous solution [R02]: Weigh 11.0 g of ammonium formate into a 50.0 mL polypropylene centrifuge tube filled with 10.0 mL of ultrapure water, and shake. Storage conditions: room temperature. Validity period: 2 weeks.
(5) Methanol:Ethanol (50:50, v:v) [R03]: Add 500 mL methanol and 500 mL ethanol to a glass bottle, shake and mix. Storage conditions: room temperature. Validity period: 30 days.
(6) Acetonitrile:water (70:30, v:v) [R04]: Add 700 mL of acetonitrile and 300 mL of ultrapure water to a glass bottle, shake and mix. Storage conditions: room temperature. Validity: 30 days.

1.4 Sample treatment

This operation is performed under wet ice conditions. The quality control samples and test samples are taken out of the refrigerator and thawed under wet ice conditions. The samples in each vial were vortexed separately before sampling. Using a pipette, add 50.0 μL water to the blank matrix sample, zero concentration sample and blank reagent sample in a 96-well deep-well plate respectively, and calibrate the standard sample. Add the same volume of calibration standard sample, add the same volume of quality control sample to the quality control sample, and add the same volume of test sample to the test sample (where the sample to be diluted is first diluted with an appropriate amount of water). Add 25.0 μL [R01] to the blank matrix sample and blank reagent sample, and add the same volume of internal standard working solution to the other samples. Add 25.0 μL of [R02] to each sample and vortex for 5 min.Add 400 μL of [R03] to each sample and pulse mix at 2000 rpm for 10 min. Centrifuge at 1700 × g for 15 min in a high-speed refrigerated centrifuge at 4 ºC. Transfer 100 μL of the supernatant to another 96-well deep-well plate containing 400 μL R04, and vortex to mix for 5 min. Store the processed samples at the temperature of the autosampler or in a refrigerator at 4 ºC prior to injection for analysis.

Supplementary Table 1. Primer sequences of target genes

Genes	Forward Primer (5’ to 3’)	Reverse Primer (5’ to 3’)
GAPDH	CTTGTGCAGTGCCAGCC	GCCCAATACGGCCAAATCC
CPT1	ACTCCTGGAAGAAGAAGTTCA	AGTATCTTTGACAGCTGGGAC
CPT2	AGTATCTGCAGCACAGCATC	ACTTCTGTCTTCCTGAACTGG
AMPK	TGAAGATCGGCCACTACATC	TTGCCCACCTTCACTTTCC
LKB1	TGGACGTGCTGTACAATGAG	GCACACTGTCCAGCATCTC
SREBP1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
PPARα	AGCTGGTGTAGCAAGTGT	TCTGCTTTCAGTTTTGCTTT
SIRT1	TTGTGAAGCTGTTCGTGGAG	GGCGTGGAGGTTTTTCAGTA
HMGR	TCTTTCCGTGCTGTGTTCTG	TTTTAACCCACGGAGAGGTG
ACC	ACGAGCACACACAGTCCATG	GATGACCTCTGGATGTTCTTG

Supplementary Table 2. Mobile phase elution gradient

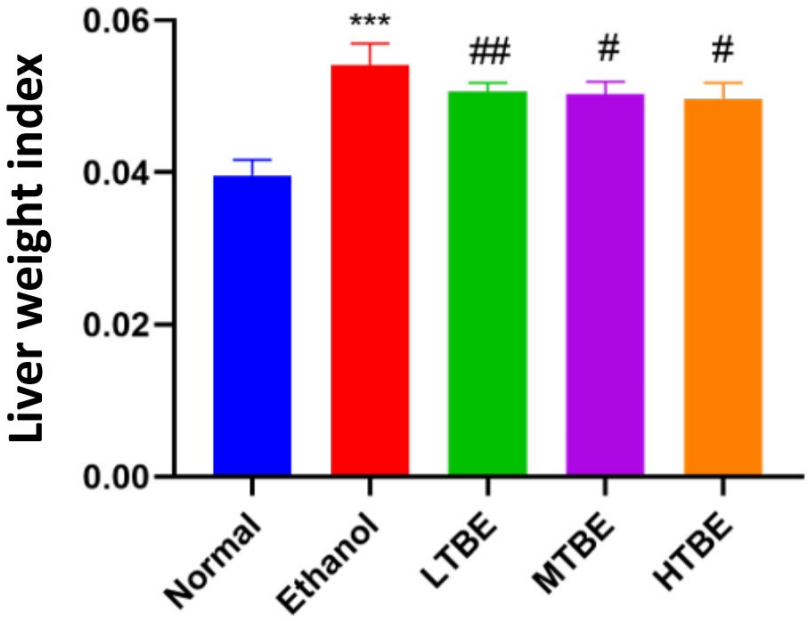
Time (min)	Flow rate (mL/min)	A (%)	B (%)
0	0.4	65	35
4	0.4	40	60
12	0.4	15	85
15	0.4	0	100
17	0.4	0	100
18	0.4	65	35
20	0.4	65	35

Supplementary Table 3. Mass spectrometry parameters

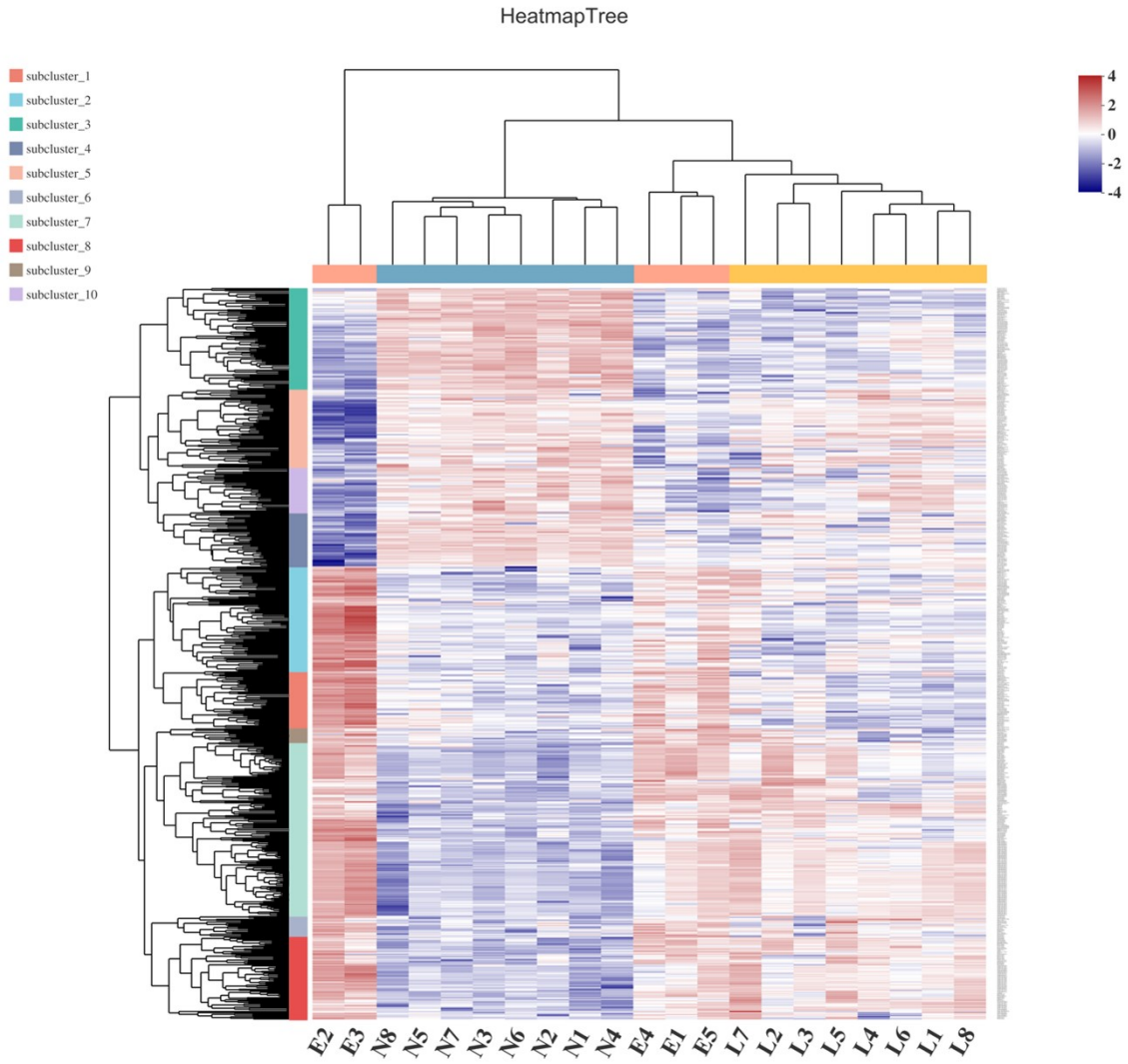
Description	Parameters
Scan type (m/z)	200-2000
Sheath gas flow rate (psi)	60
Aux gas flow rate (psi)	20
Aux gas heater temp (℃)	370
IonSpray Voltage Floating (ESI+) (V)	+3000
IonSpray Voltage Floating (ESI-) (V)	-3000
Normalized collision energy (V)	20-40-60

Supplementary Table 4. Determination of SAM and SAH levels in the liver.

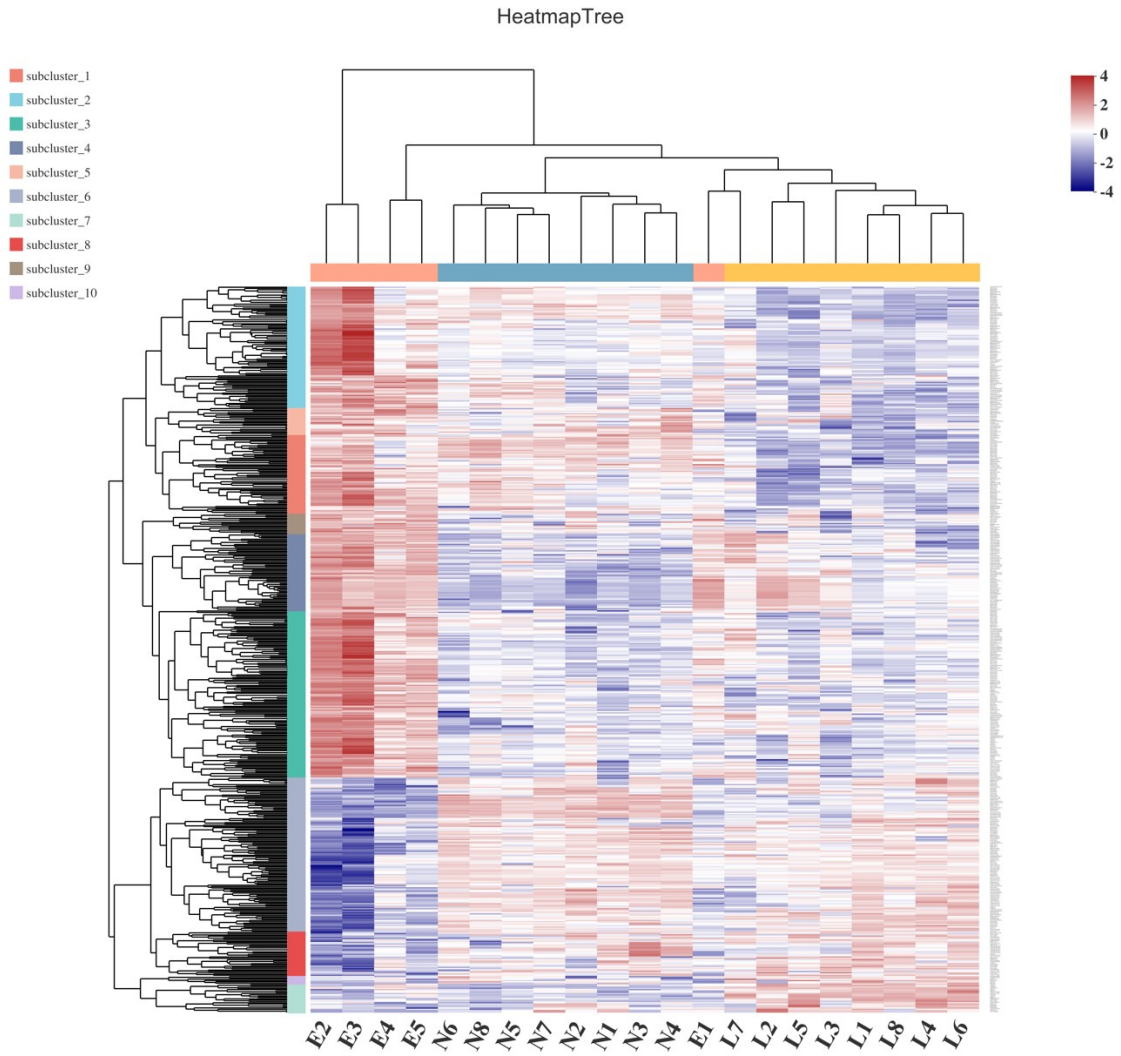
	Normal	Ethanol	LTBE	MTBE	HTBE
SAM (μg/g tissue)	4.27±1.32	2.23±0.66	2.87±1.07	2.40±0.96	2.54±0.72
SAH (μg/g tissue)	12.52±2.87	12.98±3.72	15.96±3.58	12.68±2.85	17.72±3.86



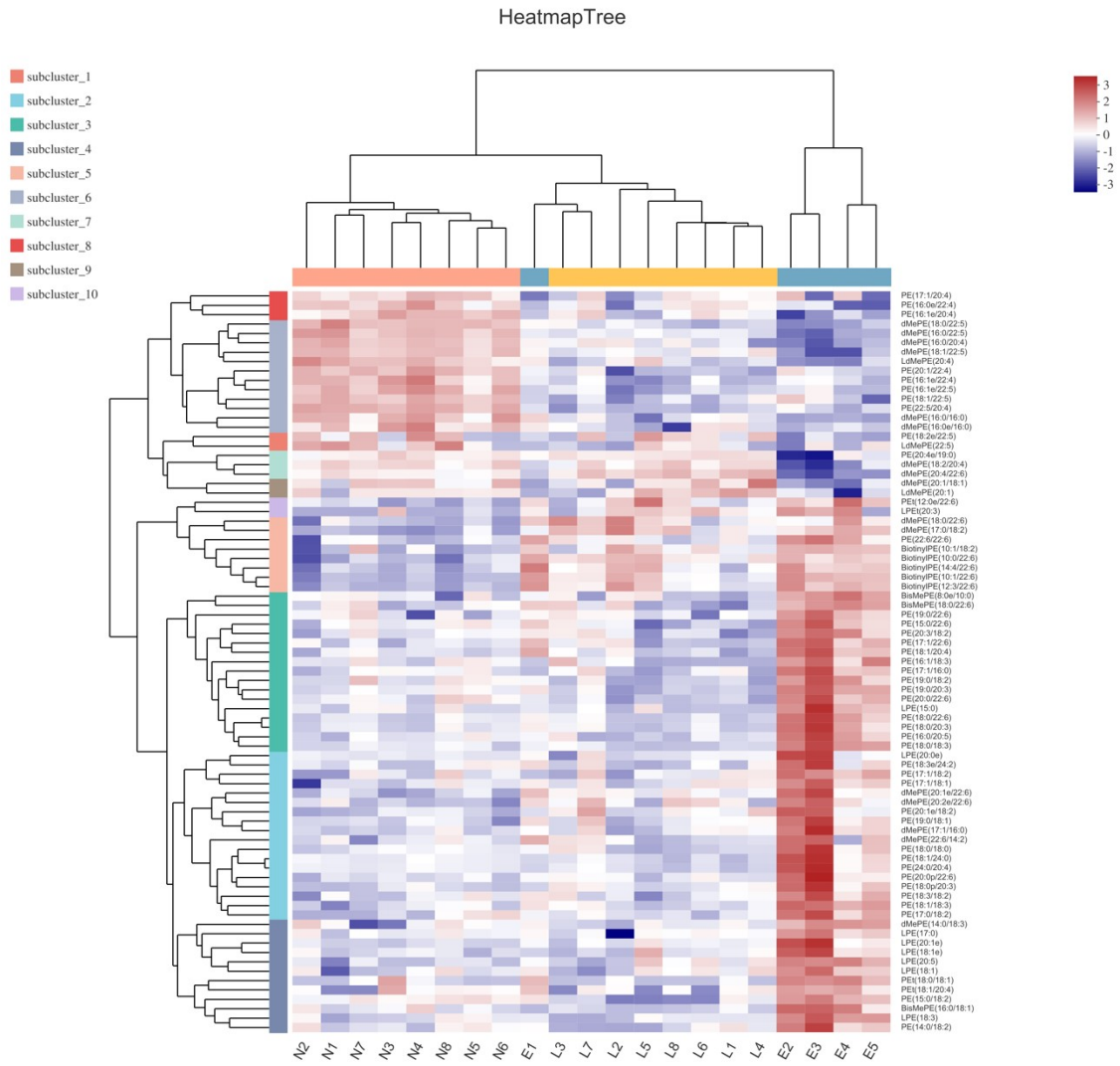
Supplemental Figure 1. Liver weight index. (***p<0.001, compared with Normal group; ## p<0.01, # p<0.05, compared with ALD group)



Supplemental Figure 2. Clustering heat map of differential lipid metabolites (Normal vs ALD, OPLS-DA VIP>1，p<0.05).



Supplemental Figure 3. Clustering heat map of differential lipid metabolites (ALD vs LTBE, OPLS-DA VIP>1，p<0.05).



Supplemental Figure 4. Clustering heat map of differential PC (ALD vs LTBE, OPLS-DA VIP>1，p<0.05).