Supplemental Information

Metabolic characteristics of lethal ventricular tarchyarrhythmia (LVTA) by non-targeted metabolomics----A study from two LVTA rat models

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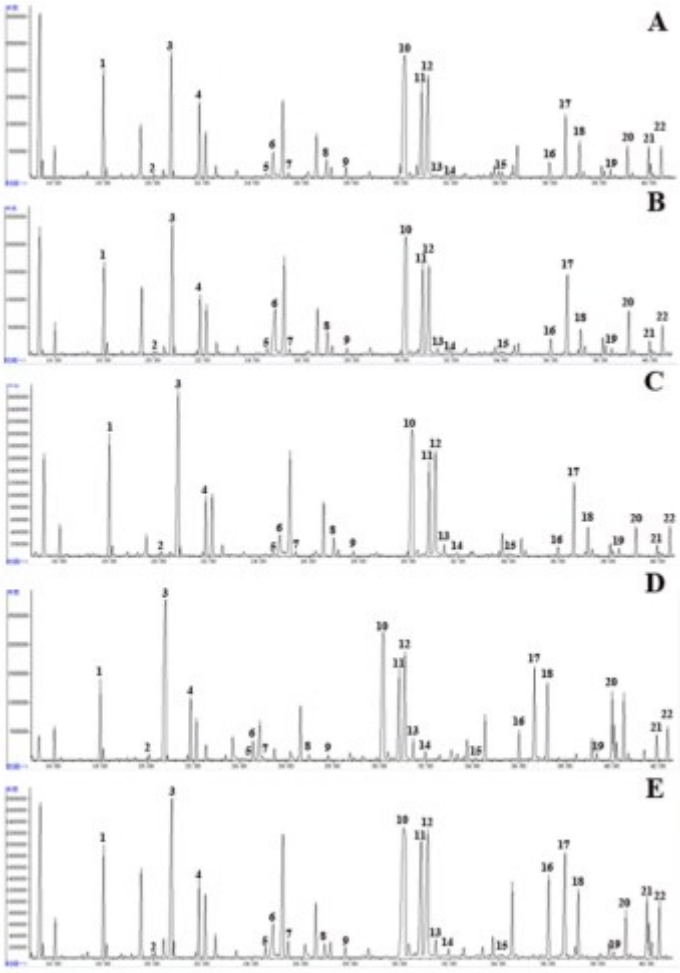

Fig. S1 Protocols for metabolomic analysis of myocardium profiling using GC/MS. A： CAL-N； B：CAL-M；C：CAL-T；D：control group for ACO-LVTA-SCD；E：ACO-LVTA-SCD. Spectrum is different for each group (1: carbonate, 2: ethanolamine, 3: lactic acid, 4: alanine, 5: valine,

6: urea, 7: leucine, 8: succinic acid , 9: fumaric acid, 10: phosphoric acid, 11: proline, 12: creatinine, 13: serine, 14: threonine, 15: aspartic acid, 16: hypoxanthine, 17: glutamic acid, 18: hexadecanoic acid, 19: lysine, 20: glutamine, 21: linoleic acid, 22: stearic acid)

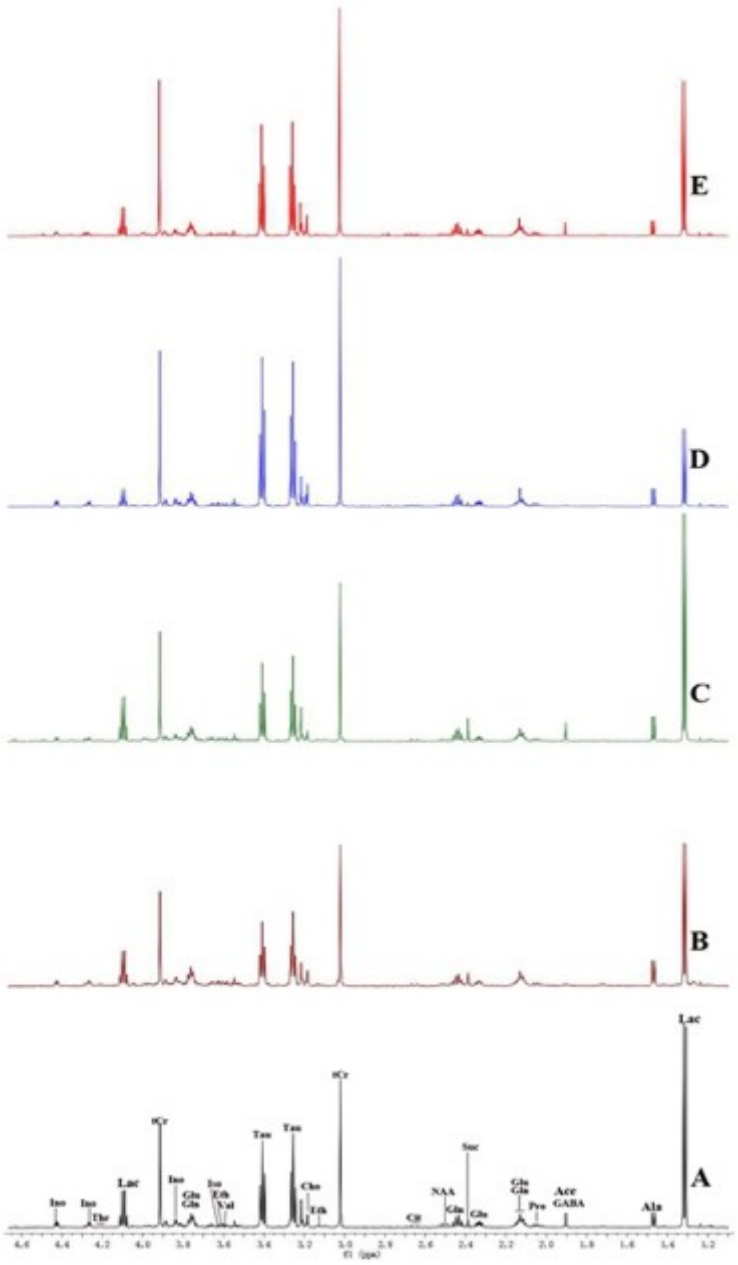

Fig. S2 Protocols for metabolomic analysis of myocardium profiling using 1H-NMR (Bruker BioSpin, 600MHz, noesygppr1d sequence). A： CAL-N；B：CAL-M；C：CAL-T；D：control group for ACO-LVTA-SCD；E：ACO-LVTA-SCD. Each group shows characteristic differences.

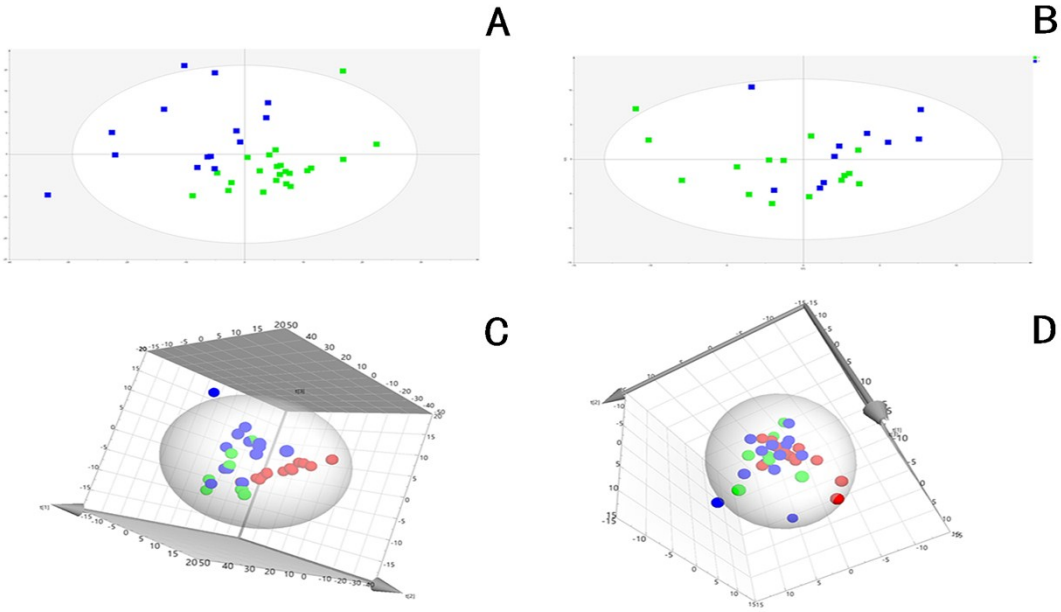

Fig.S3 PCA mode: Two-component plot in ACO-LVTA and its control (green=aconitine, blue=control) from GC-MS (A) and 1H-NMR (B) metabolic profiles; Three-component plot in three CAL-LVTA groups (blue=CAL-T, red=CAL-M, green=CAL-N) from GC-MS (C) and 1H-NMR (D). The cumulative R2Y and Q2 in aconitine-treated model were 0.746 and 0.541, and 0.608 and 0.250 in GC-MS and 1H-NMR modes, respectively. The cumulative R2Y and Q2 in CAL-treated model were 0.899 and 0.746, and 0.641 and 0.10 in GC-MS and 1H-NMR modes, respectively

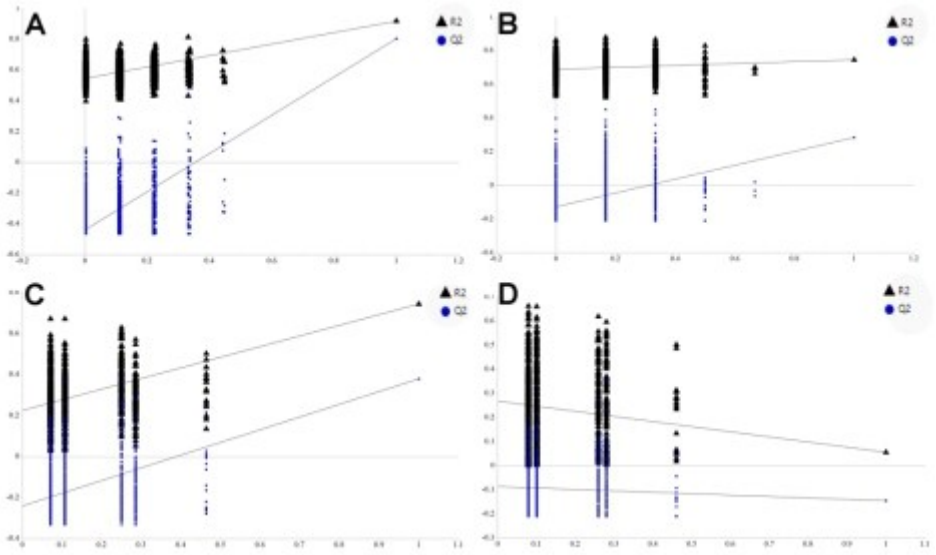

Fig. S4 Validation plots of responses: GC/MS results of (a) ACO-LVTA vs. control, and (b) CAL-N vs. CAL-T; 1H-NMR results of (c) ACO-LVTA vs. its control group and (d) CAL-N vs. CAL-T. The

X axis indicates the correlation coefficient between original and permuted response data. The Y intercepts of the plots for R2Y and Q2Y in each model are represented in number.

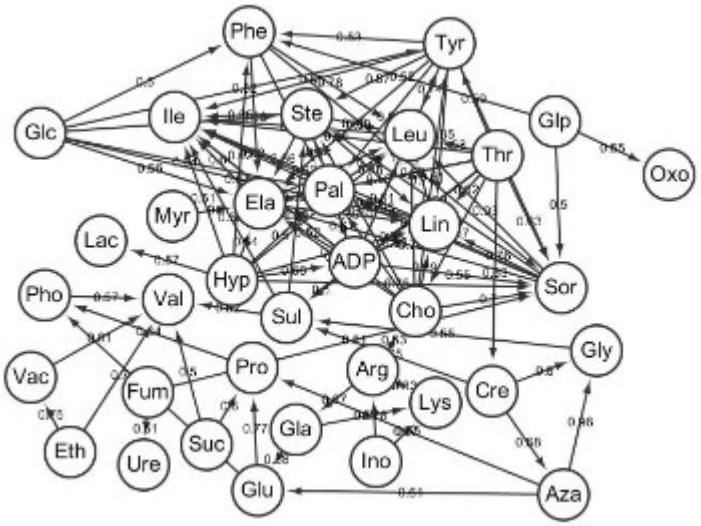

Fig. S5 Metabolic correction network of differential metabolites. Highly correlated metabolites (|correlation coefficient| ≥0.5) are connected with a line showing the correlation coefficient. Lac, lactate; Val, L-valine; Iso, Isoleucine; Leu, L-leucine; Pho, Phosophoric acid; Pro, L-proline; Glu, L-glutamate; Pal, Palmitic acid; Lin, Linoleic acid; Ela, Elaidic acid; Ste, Stearic acid; Suc, Succinate; Sor, Sorbopyranose; Sul, Sulfite; Gly, Glycine; Aza, Azathymine; Phe, L-phenylalanine; Myr, Myristic acid; Cre, Creatinine; Asp, L-aspartate; Ala, L-alanine; Ure, Urea; Ami, 2-amino-4-nitropheno; But, Butanedioic acid; Tyr, Tyrosine; Fum, Fumarate; Cho, Cholesterol; Car, Carbonate; Cho, Choline; Hyp, Hypoxanthine; Gly, Glycerol; Glu, Glucose; Val, Valeridic acid; Lys, Lysine; Glu, Glutamate; Tau, Taurine; Oxo, 2-oxoglutarate; Arg, Arginine; Ino, Inosine; Cre, Creatine; Thr, Threonine; Gly, Glycerophosphocholine; Eth, Ethanol.

Table S1 The Grouping, Feature of ECG and Cause of Death of rats in two LVTA Models

Model I						Model II					
Group	**VT**	**VF**	**VT+VF**	**Score**	**C of D**	**Group**	**VT**	**VF**	**VT+VF**	**Score**	**C of D**
Contr	0	0	0	0	CI	CAL-N	0	0	0	2	H F
Contr	0	0	0	0	CI	CAL-N	0	0	0	3	H F

Contr	0	0	0	0	CI	CAL-N	0	0	0	2	H F
Contr	0	0	0	0	CI	CAL-N	0	0	0	2	H F
Contr	0	0	0	0	CI	CAL-N	0	0	0	2	H F
Contr	0	0	0	0	CI	CAL-N	0	0	0	2	H F
Contr	0	0	0	0	CI	CAL-N	0	0	0	2	H F
Contr	0	0	0	0	CI	CAL-T	121	150	271	6	LVTA
Contr	0	0	0	0	CI	CAL-T	135	164	299	6	LVTA
Contr	0	0	0	0	CI	CAL-T	64	23	87	5	LVTA
Contr	0	0	0	0	CI	CAL-T	36	240	276	6	LVTA
Contr	0	0	0	0	CI	CAL-T	210	11	221	6	LVTA
Contr	0	0	0	0	CI	CAL-T	74	254	328	7	LVTA
Contr	0	0	0	0	CI	CAL-T	6	440	446	7	LVTA
ACO	28	107	135	5	LVTA	CAL-T	0	236	236	6	LVTA
ACO	25	97	122	5	LVTA	CAL-T	103	83	186	6	LVTA
ACO	43	96	139	5	LVTA	CAL-T	48	27	75	5	LVTA
ACO	9	126	135	5	LVTA	CAL-T	46	59	105	5	LVTA
ACO	36	112	148	5	LVTA	CAL-T	12	269	281	6	LVTA
ACO	6	101	107	5	LVTA	CAL-T	15	160	175	6	LVTA
ACO	38	50	88	5	LVTA	CAL-T	5	216	221	6	LVTA
ACO	22	112	134	5	LVTA	CAL-N	77	100	177	6	H F
ACO	16	117	133	5	LVTA	CAL-N	2	30	32	4	H F
ACO	42	104	146	5	LVTA	CAL-N	0	10	10	3	H F
ACO	5	110	115	5	LVTA	CAL-N	6	95	101	5	H F
ACO	10	82	92	5	LVTA	CAL-N	67	0	67	4	H F
ACO	29	174	203	6	LVTA	CAL-N	44	27	71	4	H F
ACO	6	150	156	5	LVTA	CAL-N	46	0	46	4	H F
ACO	61	102	163	6	LVTA	CAL-N	58	115	173	6	H F
ACO	32	100	132	5	LVTA	CAL-N	3	0	3	3	H F
ACO	36	87	123	5	LVTA	CAL-N	13	0	13	3	H F
ACO	54	159	213	6	LVTA	CAL-N	107	0	107	5	H F
ACO	9	77	86	5	LVTA	CAL-N	206	72	278	6	H F
ACO	12	109	121	5	LVTA	CAL-N	9	99	108	5	H F
ACO	12	103	115	5	LVTA	CAL-N	8	3	11	3	H F
ACO	14	100	114	5	LVTA	CAL-N	34	39	73	4	H F
ACO	51	92	143	5	LVTA						

Group: ACO, LVTA-SCD induced by aconitine; Contr, Control of LVTA-SCD over-anesthetizing to death. CAL-T, CAL-M, CAL-N are defined the same as the method section. VT, VF,TV+VF stand for the duration (second) of them. Score represents arrhythmia scoring of experimental rats calculated by the Lambeth Conventions. C of D is the abbreviation of "cause of death". CI, central inhibition; LVTA, lethal ventricular tachyarrhythmia; HF, heart failure.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Chemicals

Methanol, acetonitrile, and dichloromethane,pyridine were purchased from Fisher Scientific. Pentobarbital sodium, methoxiamine hydrochloride, D-norleucine (internal standard, IS) and trimethylsilylproprionate (TSP) were purchased from Norell. N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide (MtBSTFA), trimethyl-chlorosilane (TMCS) were purchased from Regis. Deuteroxide was purchased from Sigma-Aldrich. Aconitine was purchased from Nanjing Guangrun Biotech Co. Ltd., China).

Metabolic profiling by GC-MS

Sample preparation: 30 mg of myocardium (the sampling sequence was random, the same for 1H-NMR) was homogenized and extracted with 900 μL of methanol/chloroform (3:1) for seven minutes. After storage at -20℃ for 10 min, the extract was centrifuged at 12 000 g for 10min at 4℃. 200 μL of supernatant was transferred to a GC vial and spiked with an internal standard of D-norleucine (20 μL, 0.5μmol/ml), mixed well and dried under pure nitrogen blowing in a mild flow velocity at 70℃. Then 20μL of methylene chloride was added, and samples were dried again under pure nitrogen to ensure that the samples were completely dry. This dried aliquot was subjected to oximation and derivatization procedures with 30 μL methoxiamine (15mg/ml in pyridine) for 16 h at 20℃, followed by 30μL of MTBSFA (1%TMCS) for 1 h at 20℃. A 1 μL aliquot of the derivatized solution was separated for GC-MS analysis.

Metabolic profiling was analyzed by a GC-TOF MS system consisting of an Agilent 6890N GC system, connected to an Agilent 5975c single quadrupole MSD. A DB-5MS capillary column (30 m×250 μm×0.25 μm film thickness; 5% diphenyl cross-linked 95% dimethylpolysiloxane) (Agilent Technologies, USA) was used for the separation of metabolites. One μL of derivatized sample was injected in splitless mode. High purity helium (99.9996%) was used for the carrier gas at a constant flow rate of 1mL/min. The GC oven temperature was programmed with an initial temperature at 60 °C for 2 min, and then increased lastly to 285 ℃ at 5 ℃/min and maintained for 2 min. The temperature of the injection port, the transfer interface and the EI source were set to 230, 290 and 230 ℃, respectively. The selected mass range was set to 50−600 m/z with electron impact ionization (70 eV), and the

selected scan speed was 0.99 scans per second.

Metabolic profiling by 1H NMR

Sample Preparation: 30 mg of myocardium was disrupted in 0.6 M ice-cold perchloric acid (30 ml/g) by an automated homogenizer for exactly 5 min. After the mixture was vortexed for 30 s and centrifuged for 20 minutes (12,000 rpm) at 4°C in succession, 800 μL of supernatant was collected, and neutralized with ice-cold potassium hydroxide (pH=7), and incubated on ice for 20 min to lower the solubility of chlorate. Then 900 μL of supernatant from each sample was collected, frozen at -80°C, and lyophilized in a Moduloyd freeze dryer (Thermo Electron Company, USA) for 16 h to avoid the appearance of a dominant water signal in the 1H-NMR spectra. The dry powder was dissolved in 550 μL of D_2O with sodium phosphate buffer (pH=7) to minimize variation in sample pH and permit deuterium locking, including the internal standard of deuterated TSP at a known concentration. The resulting sample was vortexed and centrifuged again. Finally, 500 μL aliquots of supernatant were transferred to a 5 mm NMR tube (Wilmad, America) for analysis.

1H-NMR spectra of heart samples were obtained at 600.13 MHz (Bruker Biospin Gmbh, Rheinstetten, Germany, Europe) at 298 K using a NOESYPRID spectrum with water suppression. NOESYPRID spectrum were obtained using the pulse sequence [D1-90°-t-90°-t-90°-ACQ] with a relaxation delay of 4.0 s, a spectral width of 12335.5 Hz, and 32 scans collected into 16 k data points. As with standard 1D spectrum, all data sets were zerofilled to 16 k data points and multiplied by an exponential window function with a line broadening apodization factor of 0.3 Hz prior to Fourier transformation. 1H-NMR spectra were manually phased and baseline corrected and chemical shifts were referenced to TSP (0.0 ppm) using the MestReNova software (version 6.1.0, Mnova). The residual water resonance signal (δ 4.6-5.2) was excluded before analysis. Spectra were “binned” into 0.01 ppm regions over the range of 0.5-8.2 ppm, and then the data matrices covering overall metabolic information were produced in an Excel format, followed by a multivariate analysis.

The normalization of integral values in each spectrum was performed by using a constant sum of all integrals in a spectrum before statistical analyses. Metabolites in the spectra were identified by matching with information in relevant literature and comparison to public databases, such as the

BioMagResBank Database (BMRB, www.bmrb.wisc.edu) and the Human Metabolome Database (HMDB, www.hmdb.ca) .

The relative contents of metabolites were calculated with the following formula according to reference: $Cm=TIm/TItsp\times Np/9\times Ctsp$, where TIm and TItsp stand for the total integrals of metabolite and TSP, respectively. Np and 9 represent for the number of protons of the metabolite and TSP, respectively. Cm and Ctsp stand for the concentrations of metabolites and TSP, respectively.