

Supplementary Materials

Multi-omics and network-based drug repurposing for septic cardiomyopathy

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Method

LC-MS/MS analysis for metabolomics

The sample was injected by a Shimadzu LCMS-8060 system (Japan). Reverse phase chromatographic separation was carried out on an XSelect HSS T3 column (2.1 × 100 mm, 2.5 μm; Waters, USA) at a flow rate of 0.35 mL/min and a temperature of 40°C. The gradient elution employed a mobile phase consisting of (A) 6.5 mM ammonium acetate in water and (B) methanol. The elution gradient was performed as follows: 0-1 min : 2%B ; 1-24 min : 2-95%B ; 24-28 min : 95%B ; 28-29 min : 95-2%B ; 29-34 min : 2%B. MS detection was performed by an electrospray ionization (ESI) source operated with interface voltage of 4.0 kV in positive mode and -3.0 kV in negative mode. The other main parameters were as follows: nebulizing gas, 3 L/min; drying gas, 15 L/min; heat block temperature, 400°C; desorption line (DL) temperature, 250°C; and ion accumulation time 5 ms. The collision energy (CE) and multiple reaction monitoring (MRM) parameters refer to the literature of Xu et al. (*Nat Protoc.* 2020;15(8):2519-2537.) and were provided in **Appendix Data 1**.

RNA sequencing

Heart samples were preserved in RNA stabilization reagents (BioYou, China) and submitted to Shanghai Biotechnology Corporation for RNA-seq. Total RNA was extracted using the MJzol Animal RNA Isolation Kit (Majorivd) and purified using RNAClean XP Kit (Beckman Coulter) and RNase-Free DNase Set (QIAGEN) according to standard operating procedures provided by the manufacturer. The mRNA was enriched using VAHTS® mRNA Capture Beads (Human/Mouse/Rat). The mRNA sequencing libraries were constructed using the VAHTS Universal V6 RNA-seq Library Prep Kit for Illumina® (Vazyme) according to standard operating procedures provided by the manufacturer. The sequencing platform used was Illumina

NovaSeq6000, and the sequencing mode adopted PE150 (Pair-end 150bp). Then apply seqtk to filter poor quality reads and trim poor quality bases from samples. Genome mapping of pre-processed reads was performed applying the spliced mapping algorithm of Hisat2 (version: 2.0.4). The level of gene expression is estimated by the number of reads mapped to the gene region. For RNA sequencing data, edge R (ver. 3.34.1) and limma packages (ver. 3.48.3) in R Studio (ver. 4.1.1) were used for processing. The filterByExpr function in the edgeR package was used to delete low-expression genes, and the data were normalized using the Trimmed Mean of M-values (TMM) method. The preprocessed data were then converted to log 2-count per million (log2CPM). Linear models and empirical Bayesian adjustments were used to evaluate differential expression. The empirical Bayesian parameter method was used to calculate P values and other parameters, and the P values for multiple hypothesis testing were corrected by BH (Benjamini and Hochberg).

HILIC-MS/MS analysis for quantification of amino acids

The sample was injected by a Shimadzu LCMS-8060 system (Japan). Chromatographic separation was carried out using an XBridge BEH Amide column (2.1×100 mm, 2.5 µm, Waters, USA) with a flow rate of 0.3 mL/min at 35 °C. The mobile phase consisted of (A) 5 mM ammonium acetate in water with formic acid adjusting pH to 3.0 and (B) acetonitrile. A 17-min elution gradient was performed as follows: during the first 7 min, the proportion of eluent A was linearly increased from 15% to 18%, then a further increase to 50% A reached in 2 min and kept for 2 min. Finally, the initial conditions were recovered in 1 min and maintained for 5 min. The MS detection was conducted using a Shimadzu 8060 triple quadrupole mass spectrometer (Japan) equipped with an electrospray ionization (ESI) source operated in the positive mode with multiple reaction monitoring (MRM). The MS instrument parameters were as follows: spray voltage 4.0

kV, desorption line temperature 250°C, heat block temperature 40°C, nebulizing gas 3.0 L/min, drying gas 10.0 L/min.

The optimized MRM parameters for fatty acids

Analyte	MW	Precursor ion (m/z)	Product ion (m/z)	CE (eV)
Alanine	89.1	90.1	44.1	-13
Arginine	174.2	175.1	70.1	-22
Aspartic acid	133.1	134.6	134.6	-5
Citrulline	175.2	176.1	70.1	-25
Cysteine	121.2	122.4	75.9	-15
Glutamine	146.1	147.1	84.1	-17
Glutamic acid	147.1	148.1	84.1	-16
Glycine	75.1	76.1	30	-12
Histidine	155.2	156.1	110.2	-14
Isoleucine	131.2	132.2	86.2	-11
Lysine	146.2	147.1	84.2	-17
Methionine	149.2	150.1	61.1	-22
Proline	115.1	116.1	70.1	-16
Serine	105.1	106.1	60.1	-12
Threonine	119	120.1	74.2	-11
Tyrosine	181.2	182.1	91.1	-28
Valine	117.1	118.1	72	-11
γ-Aminobutyric acid	103.1	104.1	87.1	-14
L-Leucine-d3	134.1	135.1	89.2	-11
L-Alanine-d4	93.1	94.1	48.2	-13

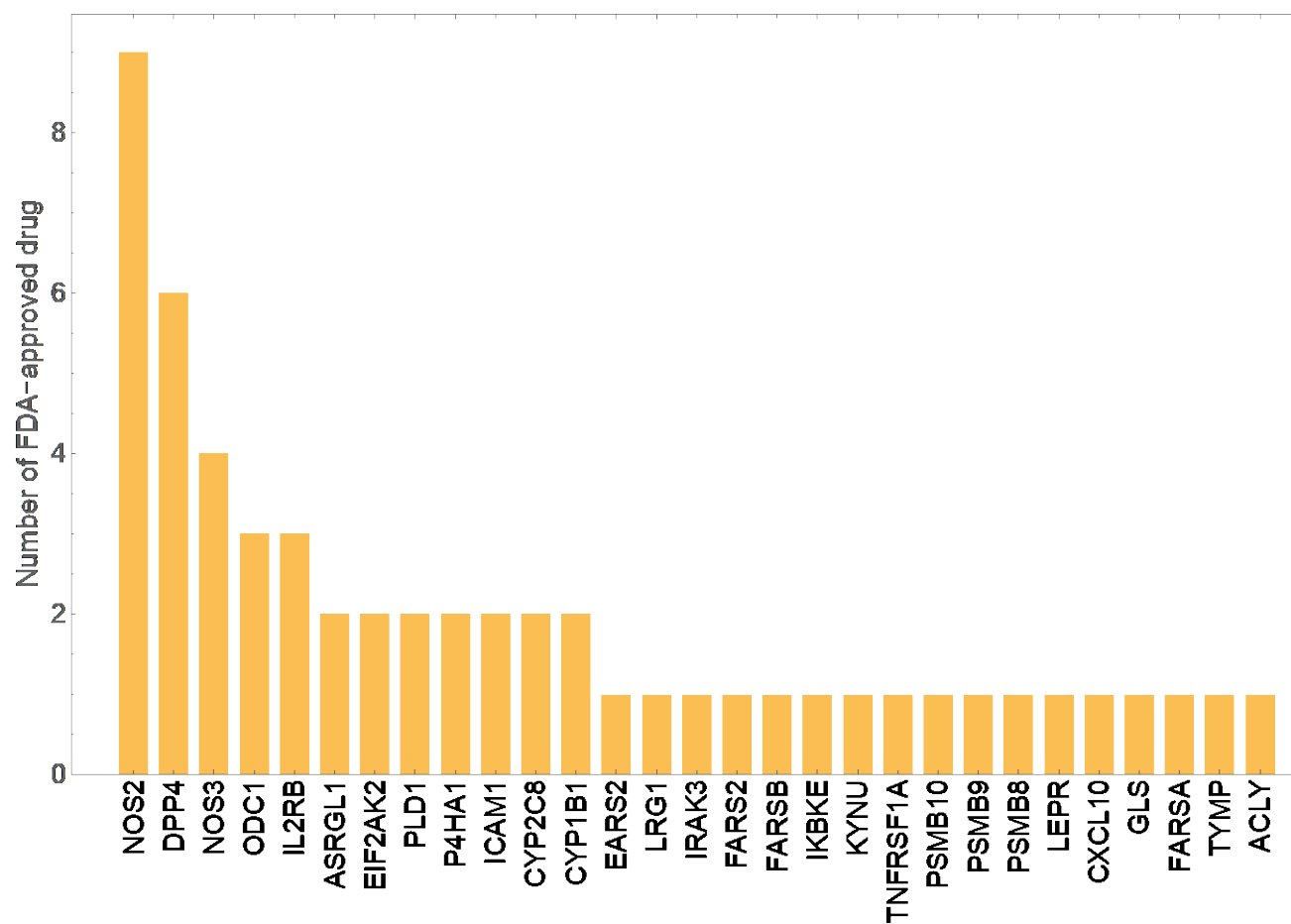


Figure S1. Targetable proteins in SCM-associated module.

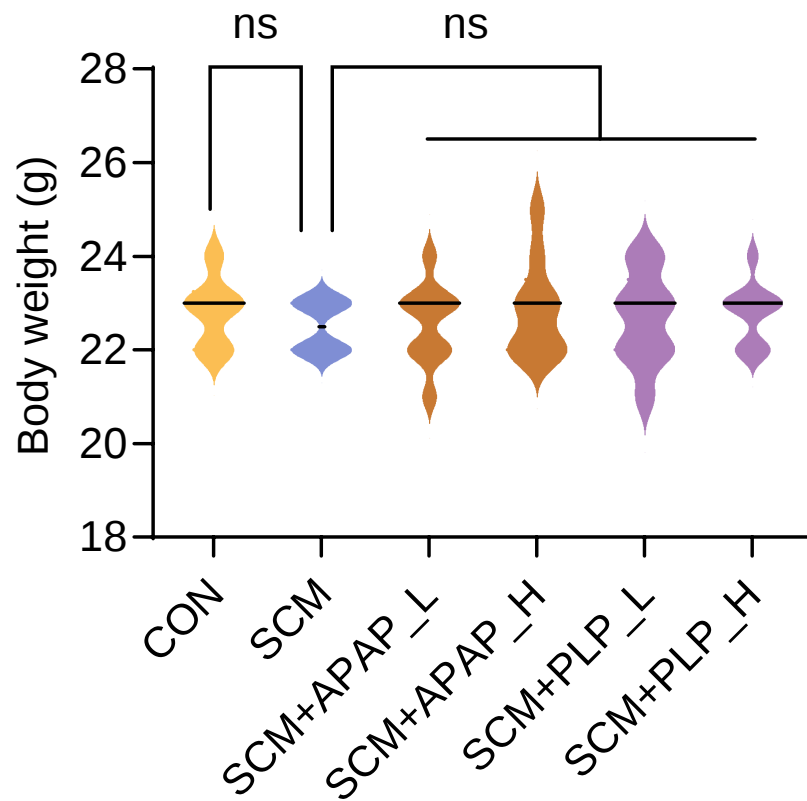


Figure S2. Body weight in each group (CON, n = 6; SCM, n = 6; SCM+APAP_L, n = 9; SCM+APAP_H, n = 9; SCM+PLP_L, n = 9; SCM+PLP_H, n = 9). One-way ANOVA, ns: no significant difference.

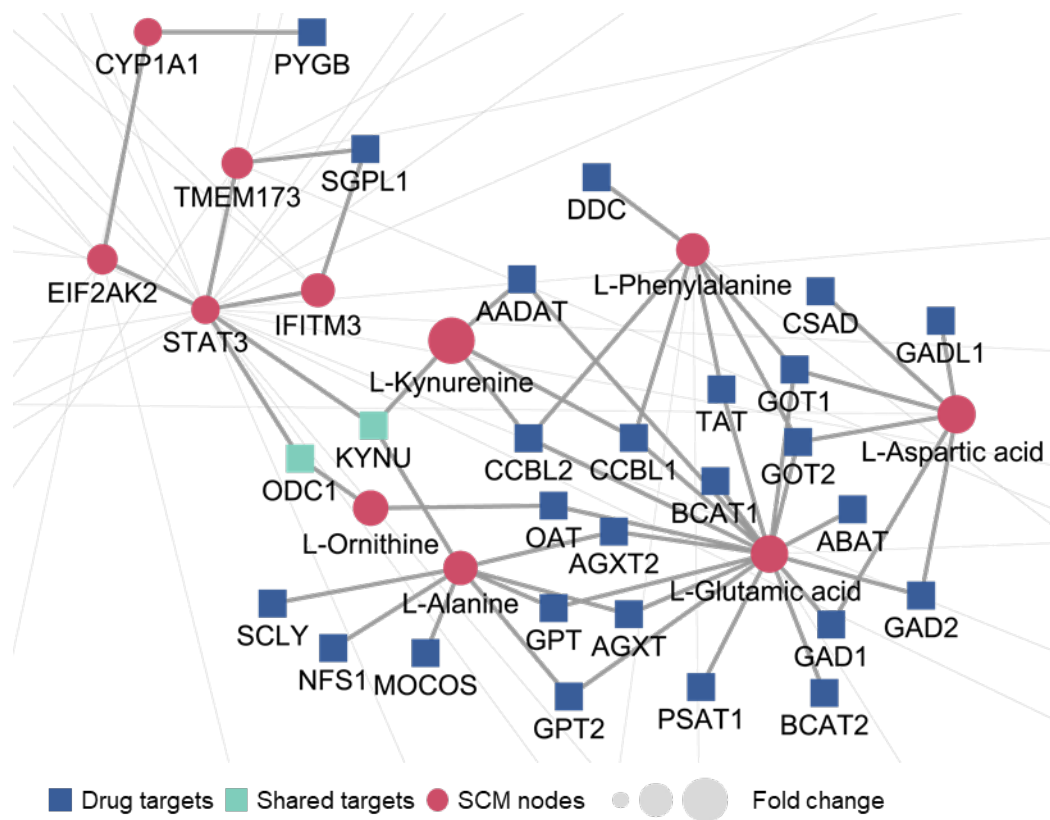


Figure S3. A highlighted subnetwork shows the inferred mechanism-of-action for pyridoxal phosphate's protective effect in septic cardiomyopathy (SCM) network analysis.

Table S1. Information of chemical standards.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
L-Phenylalanine-d5	Macklin	Cat# L874704
Cholic Acid-d4	IsoReag	Cat# IR-14894
L-Methionine-d3	Sigma-Aldrich	Cat# 300616
Fatty acid13:0	Aladdin	Cat# T114032
L-Leucine-d3	Sigma-Aldrich	Cat# 486825
L-Alanine-d4	Cambridge Isotope Laboratories	Cat# DLM-250-1
L-Alanine	Sigma-Aldrich	Cat# A7627
L-Arginine	Aladdin	Cat# A137768
L-Aspartic acid	Aladdin	Cat# A108860
L-Citrulline	Sigma-Aldrich	Cat# 1133842
L-Cysteine	Sigma-Aldrich	Cat# 95437
L-Glutamine	Aladdin	Cat# G105425
L-Glutamic acid	Sigma-Aldrich	Cat# 49449
Glycine	Sigma-Aldrich	Cat# 1295800
L-Histidine	Sigma-Aldrich	Cat# H8000
L-Isoleucine	Sigma-Aldrich	Cat# 58879
L-Lysine	Sigma-Aldrich	Cat# L5501
L-Methionine	Sigma-Aldrich	Cat# M0960000
L-Proline	Aladdin	Cat# P120032
L-Serine	Aladdin	Cat# S137888
L-Threonine	Sigma-Aldrich	Cat# T8625
L-Tyrosine	Sigma-Aldrich	Cat# 93829
L-Valine	Sigma-Aldrich	Cat# 94619
γ -Aminobutyric acid	Aladdin	Cat# A104200

Table S2. Primer sequences used for real-time polymerase chain reaction (RT-PCR).

Gene	Forward Primer	Reverse Primer
<i>Nppb</i>	TATAAAAGGCAGAGGCACCG	AATCATCTGGGACAGCACCT
<i>Bax</i>	CAGTTGAAGTTGCCATCAGC	CAGTTGAAGTTACCATCAGC
<i>Tnfa</i>	AGCATGATCCGAGATGTGGAA	TAGACAGAAGAGCGTGGTGGC
<i>Il-1b</i>	CACCTCTCAAGCAGAGCACAG	GGGTTCCATGGTGAAGTCAAC
<i>Il-6</i>	GTTGCCTTCTTGGGACTGATG	ATACTGGTCTGTTGTGGGTGGT
<i>Nfkb2</i>	GATCTCGACCTCCACCGGAT	TTCCCAGAGTTTCAGACGCC
<i>Ptgs1</i>	GTTTCCCCTGCTGCTGCTC	GGCTGGGGATAAGGTTGGAC
<i>Ptgs2</i>	CTGAAGCCCACCCCAAACAC	TGGGAGTTGGGCAGTCATCA
<i>Ptgs3</i>	GCCCTCTGCCCCGTTAC	GCTTTGCCCTTTCCTTTGTTA
<i>Eif2ak2</i>	CGAGAACAGCACAAACGGTG	GCCTTGTCCTCTTGACTCCG
<i>Stat1</i>	AAGCACCAGAACCGATGGAG	AGCCTCGAGACAGTGCAATC
<i>Stat2</i>	AAGGGGTGGACCTGCAGAAT	TCACCAGCAGTCTTGTTCCG
<i>Stat3</i>	GCTTCTCCTTCTGGGTCTGGC	CCTCCTTCTTTGCTGCTTTCCT
<i>Cxcl10</i>	GGGATCCCTCTCGCAAGAA	CTCAGCGTCTGTTCATGGAAGT
<i>Cxcl11</i>	GGTTCCAGGCTTCGTTATGT	CTTTGTCACAGCCGTTACTC
<i>Gapdh</i>	ATTGTCAGCAATGCATCCTG	ATGGACTGTGGTCATGAGCC