

1      **Supplementary Information**

3      **Autophagy-driven lipid regulation by an herbal decoction alleviates**  
4      **cardiac lipotoxicity in severe acute pancreatitis**

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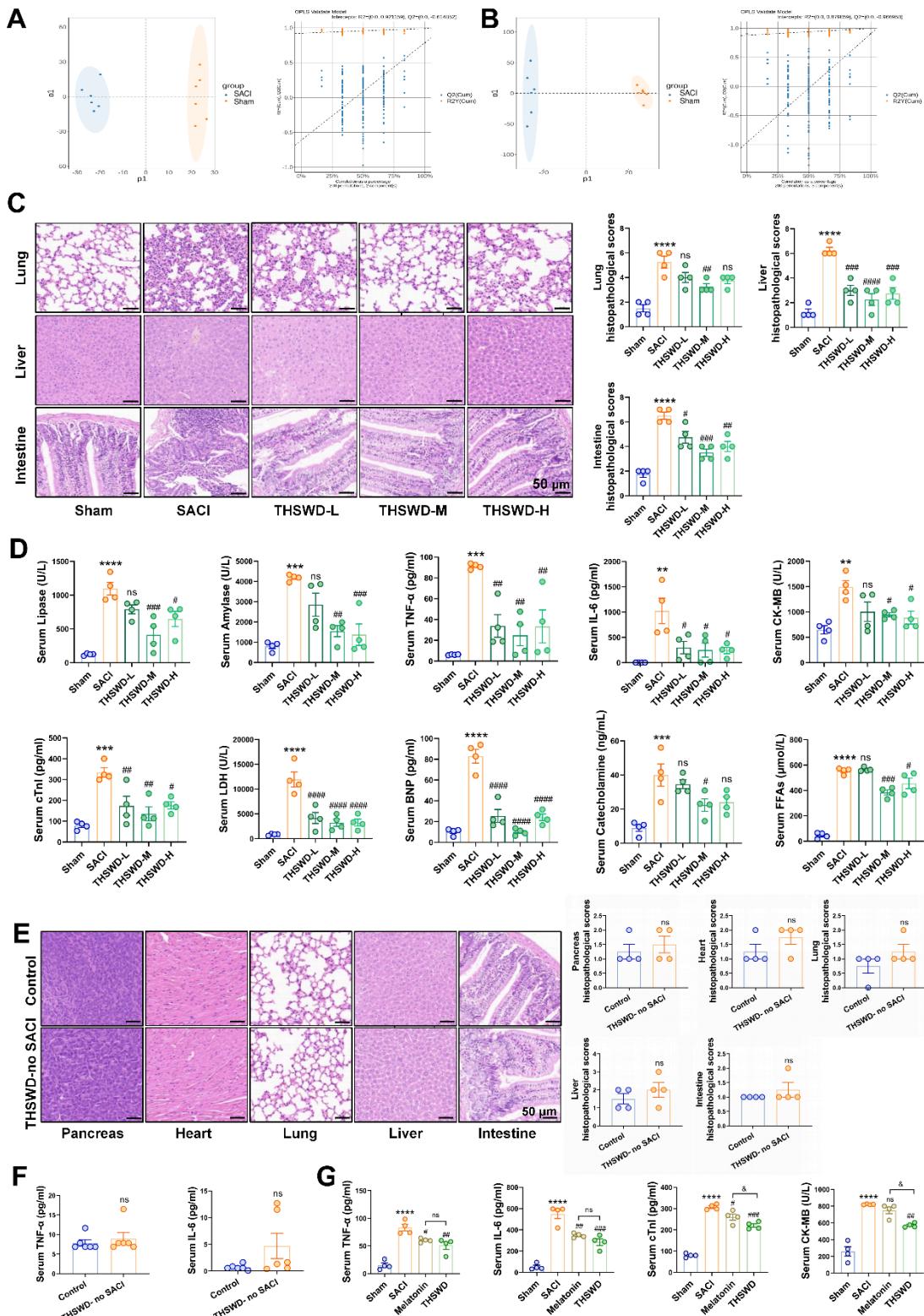
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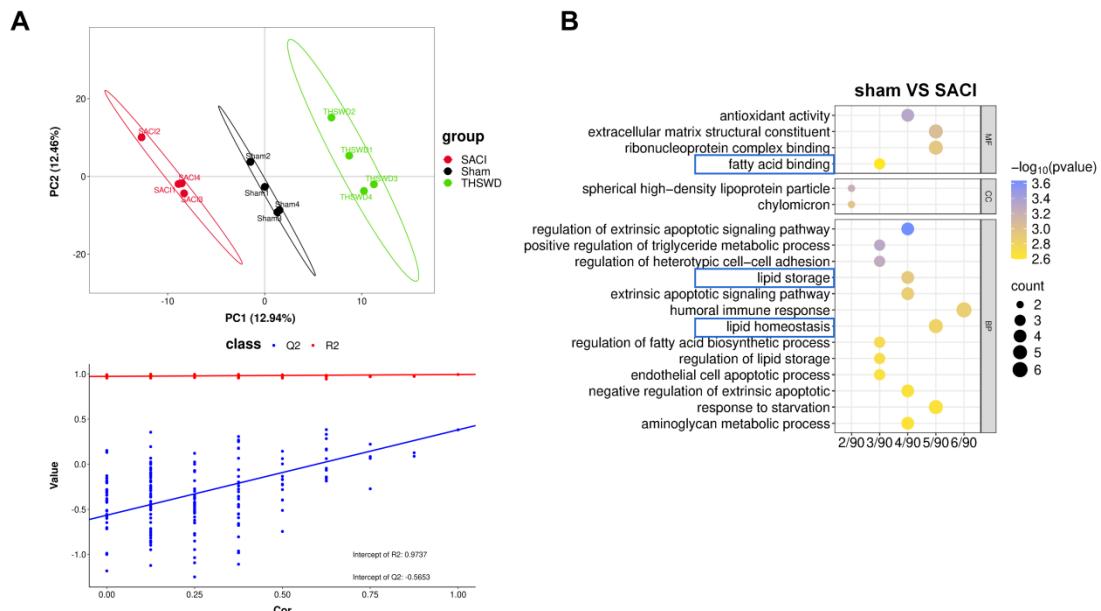
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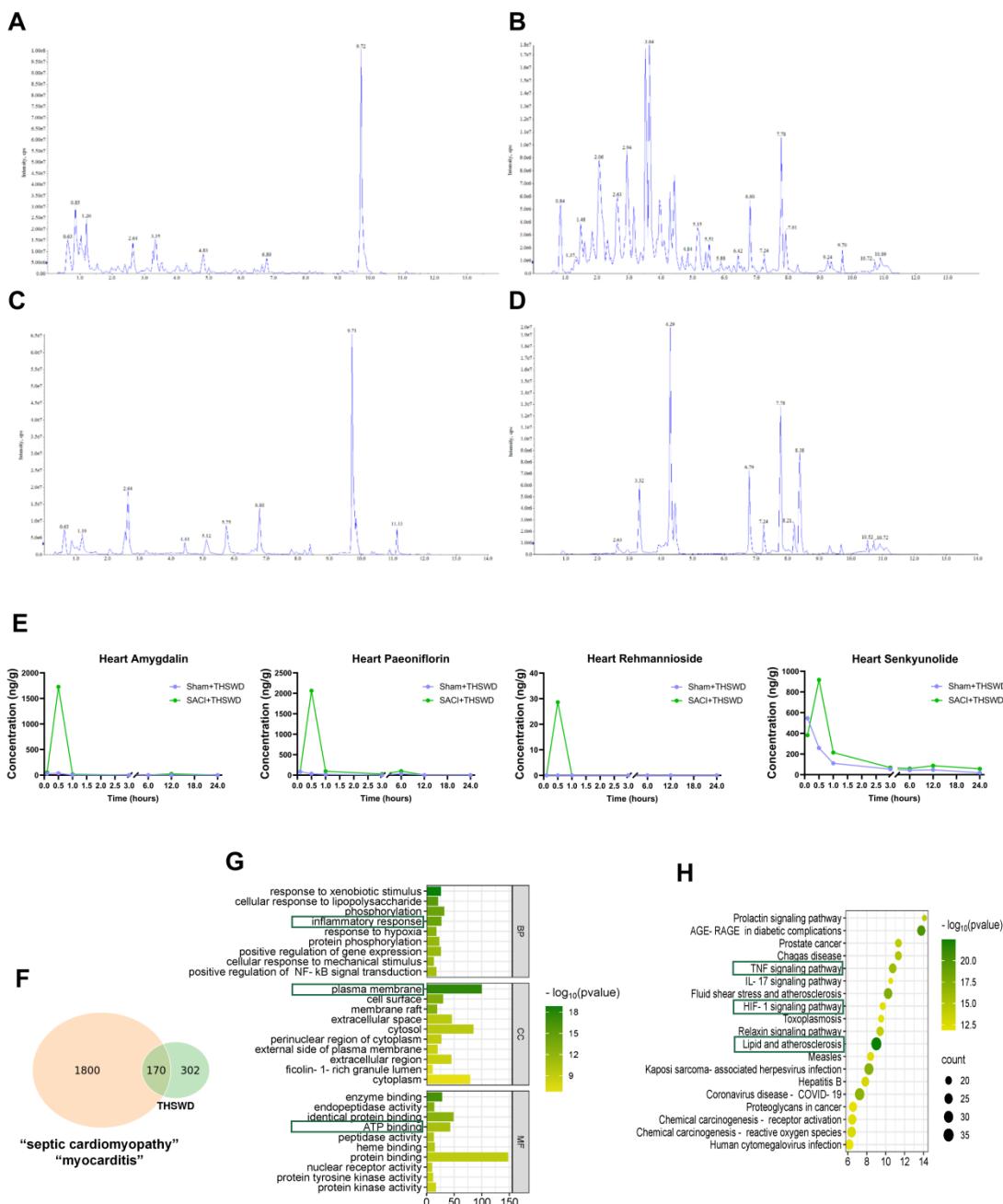
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38 **Figure S1.** THSWD attenuates cardiac tissue changes related to SACI metabolic dysregulation. (A)  
39 Serum and (B) cardiac tissue orthogonal partial least squares discriminant analysis (OPLS-DA)  
40 model results for metabolomics data of the sham, SACI, SACI+THSWD groups (n = 6/group).(C)  
41 Representative images and histopathologic scoring of H&E stained lung, liver and intestine tissue  
42 specimens from uninjured (sham) and SACI mice treated with or without low, medium and high

43 THSWD doses (THSWD-L, THSWD-M, or THSWD-H) (scale bar = 50  $\mu$ m; n = 4/group). (D)  
 44 Serum lipase, amylase, TNF- $\alpha$ , IL-6, CK-MB, cTnI, LDH, BNP, catecholamine and FFA levels  
 45 measured in these groups (n = 4/group). (E) Representative images and histopathologic scoring of  
 46 H&E stained pancreas, heart, lung, liver and intestine tissue specimens in normal mice treated with  
 47 (THSWD-no SACI) and without THSWD serum (control) (scale bar = 50  $\mu$ m; n = 4/group). (F)  
 48 Serum TNF- $\alpha$  and IL-6 levels detected in these groups (n = 6/group). (G) Serum TNF- $\alpha$ , IL-6, cTnI,  
 49 and CK-MB levels measured in the sham, SACI, SACI+ melatonin, SACI+THSWD groups (n =  
 50 4/group). Results are presented as mean  $\pm$  SEM values. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.005, \*\*\*\*p  
 51 < 0.0001 vs. sham. #p < 0.01, ##p < 0.005, ###p < 0.0001 vs. SACI. ns: nonsignificant vs. SACI.  
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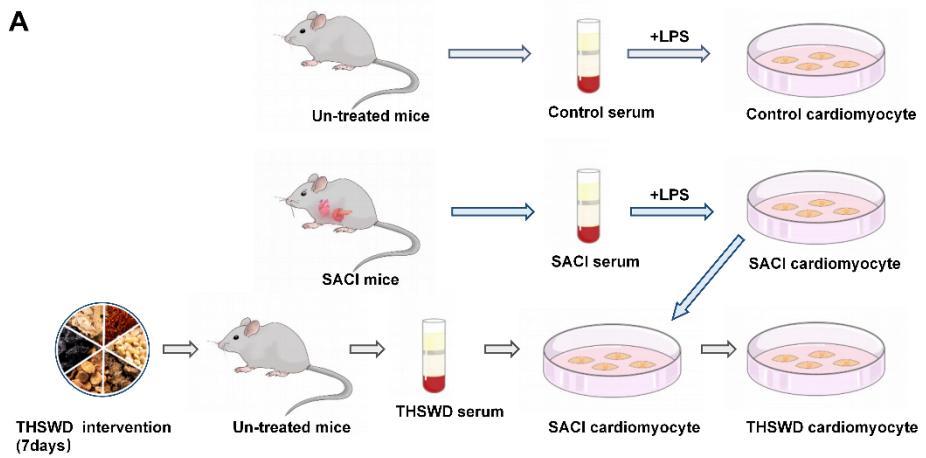


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 54 **Figure S2.** Bioinformatics analysis of Sham, SACI and SACI+THSWD mouse cardiac tissue  
 55 proteomics data. (A) OPLS-DA model of cardiac tissue DEPs detected among the sham, SACI and  
 56 SACI+ THSWD mouse groups (n = 4/group). (B) GO term analysis of DEPs in the cardiac tissues of  
 57 sham vs. SACI mice (n = 4/group).

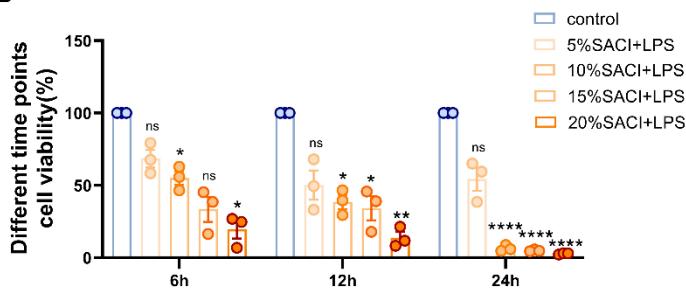


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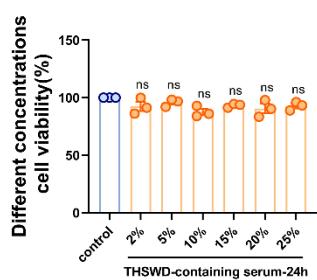
59 **Figure S3.** THSWD regulates cardiac tissue expression of genes associated with lipid storage and  
60 metabolism. (A-B) THSWD extract and (C-D) THSWD serum extract total ion chromatographs  
61 detected in positive (left) and negative (right) detection mode, respectively. (E) Cardiac tissue  
62 concentration-time profiles for six major THSWD components in Sham+THSWD and  
63 SACI+THSWD mice 10 and 30 min, and 1, 3, 6, 12, and 24 h after receiving of a medium THSWD  
64 dose. (F) Venn diagram of the overlap between (left) proteins associated with septic cardiomyopathy  
65 and myocarditis in a GeneCards search using these terms as keywords and (right) potential THSWD  
66 targets identified by screening the 16 THSWD-derived compounds detected in the serum against  
67 the PubChem and Swiss Target Prediction databases. (G) GO term enrichment analysis for these  
68 170 shared protein targets, using the biological process (BP), molecular function (MF) and cellular  
69 component (CC) categories. (H) KEGG analysis of these 170 shared proteins.



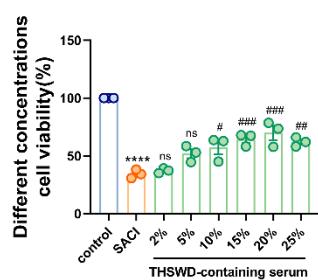
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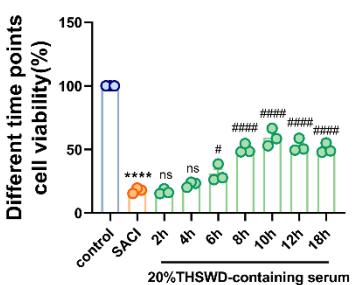
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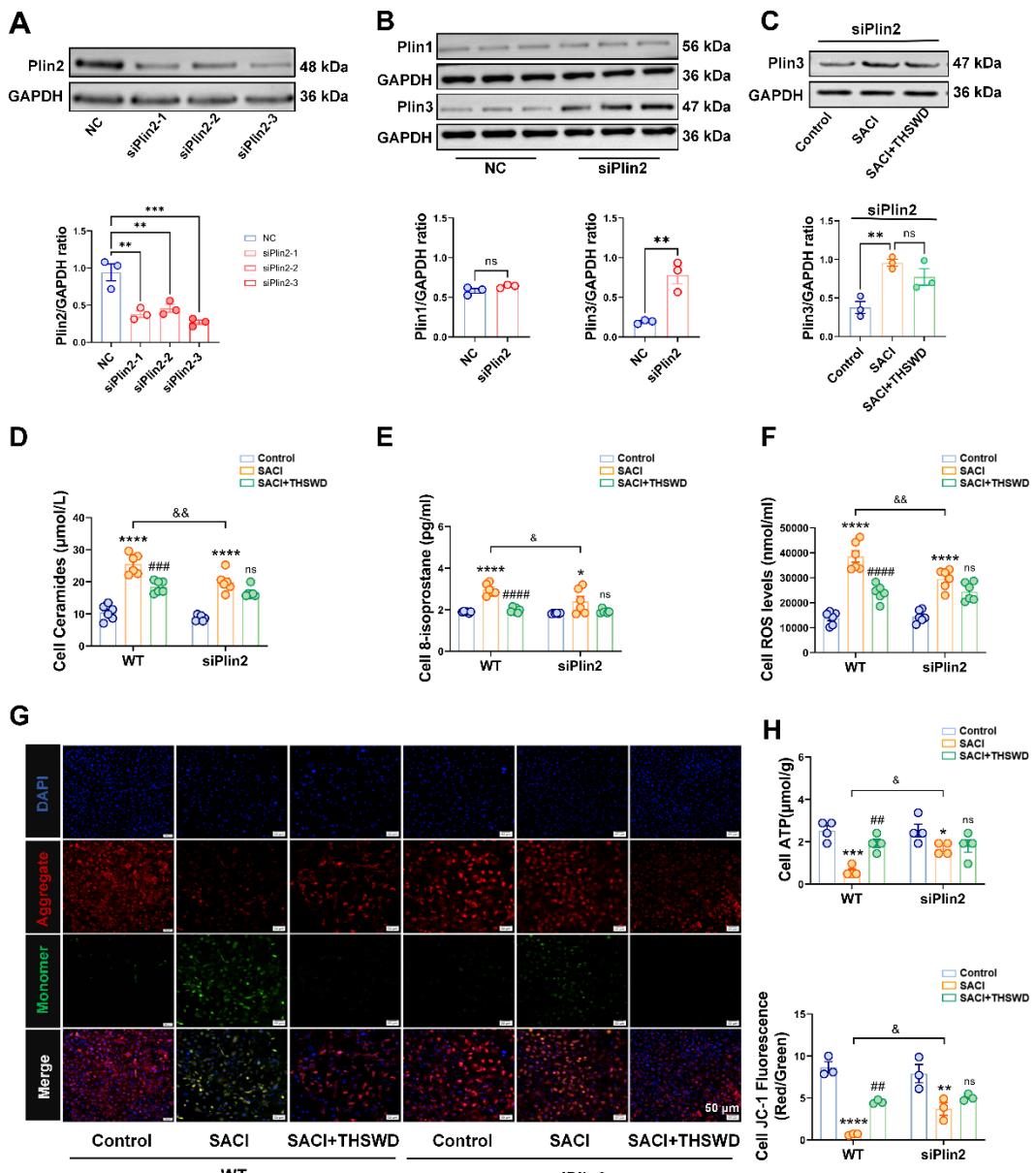


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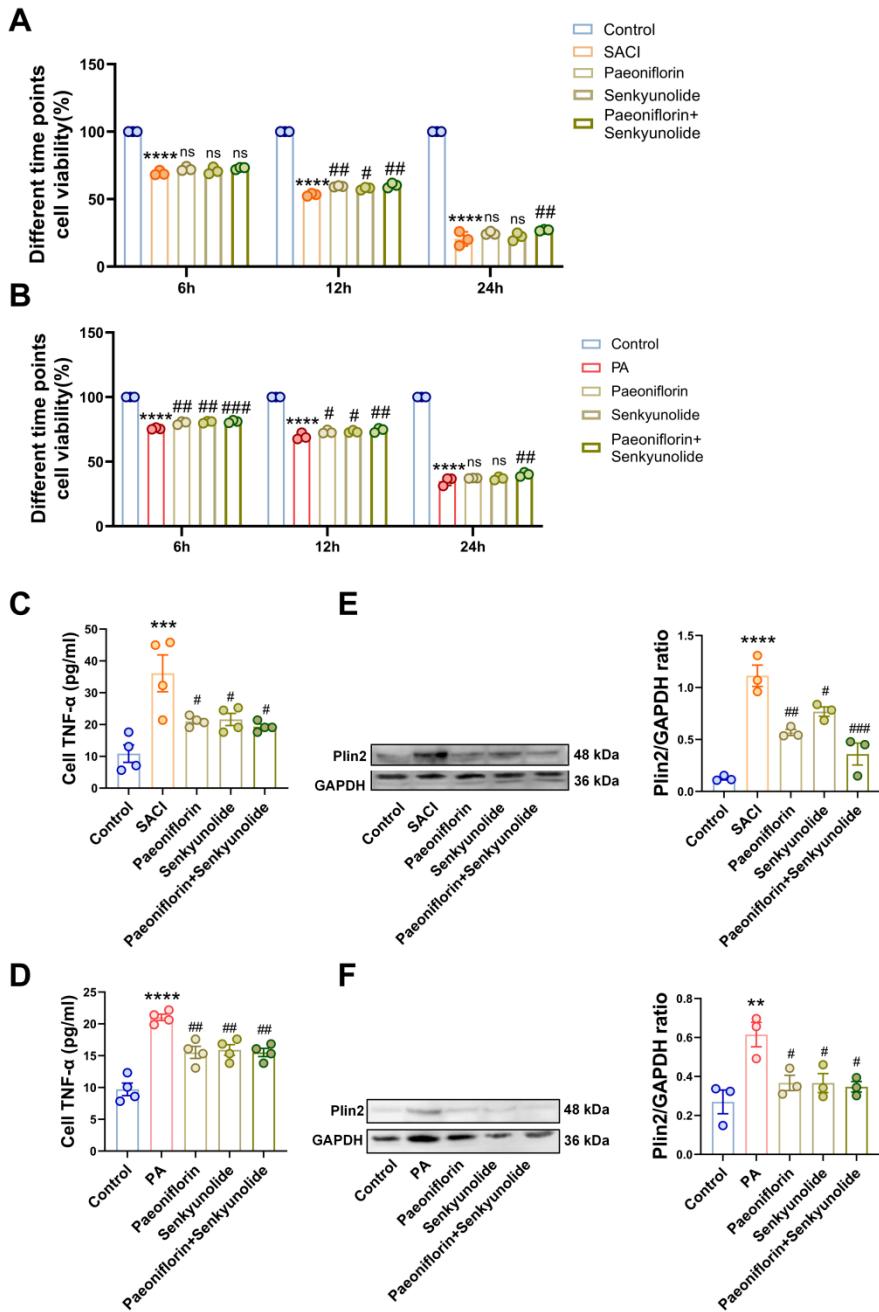
71 **Figure S4.** THSWD serum effects on sham control and SACI cardiomyocyte viability measured by  
72 CCK-8 assay. (A) Schematic of the cell culture protocol. (B) Cardiomyocyte survival after 6, 12,  
73 and 24 h exposure to the indicated concentrations of SACI serum and 1  $\mu$ g/mL LPS (n = 3/group),  
74 where 6 h exposure to 10% SACI serum was selected to produce SACI cardiomyocyte. (C) Sham  
75 control and (D) SACI cardiomyocyte survival after 24 h exposure to the indicated THSWD serum  
76 concentrations (n = 3/group). (E) SACI cardiomyocyte survival after the indicated period of  
77 exposure to 20% THSWD serum (n = 3/group). Results are presented as mean  $\pm$  SEM values. \*p <  
78 0.05, \*\*p < 0.01, \*\*\*\*p < 0.0001 vs. the control; #p < 0.05, ##p < 0.01, ###p < 0.005, #####p < 0.0001  
79 vs. SACI. ns: nonsignificant vs. SACI.

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**Figure S5.** Plin2 knockdown mimics THSWD effects to attenuate SACI cardiomyocyte lipotoxicity. (A) Western blot and quantification of Plin2 knockdown in cardiomyocytes treated with the indicated Plin2-specific siRNAs (n = 3/group). (B) Western blot and quantification of Plin1 and Plin3 protein expression in the normal and siPlin2 cardiomyocyte cultures (n = 3/group). (C) Western blot and quantification of Plin3 protein expression in control, SACI, SACI+THSWD cardiomyocyte cultures after knockdown of Plin2 (n = 3/group). (D-E) Ceramides and 8-isoprostanate levels measured in the supernatants of these cultures (n = 6/group). (F) Intracellular ROS concentration in these cultures (n = 6/group). (G) Representative images and quantitation of nuclear (blue) and JC-1 signal that indicates normal (red) and decreased (green) mitochondrial membrane potential ( $\Delta\Psi_m$ ) (scale bar = 50  $\mu$ m; n = 3/group), and (H) cardiomyocyte ATP levels in these cultures (n = 4/group). Results are presented as mean  $\pm$  SEM values. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.005, \*\*\*\*p < 0.0001 vs. Control; #p < 0.01, ##p < 0.005, ###p < 0.0001 vs. SACI; &p < 0.05, &&p < 0.01 vs. WT SACI; ns: nonsignificant vs. siPlin2 SACI.

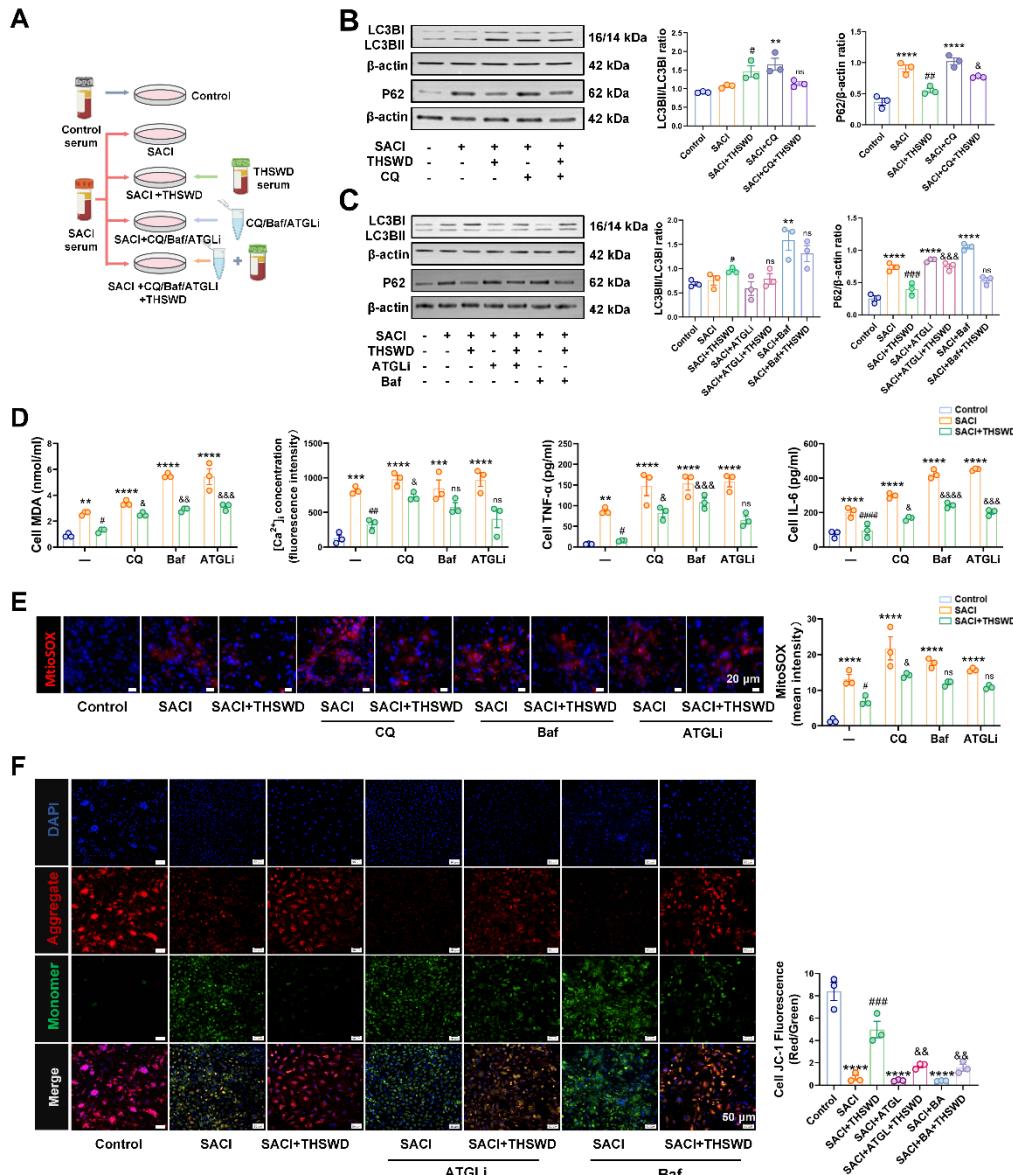


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97 **Figure S6.** The effect of Paeoniflorin and Senkyunolide during continuous SACI serum and  
98 palmitate (PA) exposure. (A) Cardiomyocyte survival after 6, 12, and 24 hours of exposure to  
99 different intervention conditions (n = 3 cells/group), with control group: 10% control group (un-  
100 treated) mouse serum, SACI group: 10% SACI serum + LPS, SACI+ 100  $\mu$ mol/L Paeoniflorin  
101 co-incubated group, SACI+ 1 mmol/L Senkyunolide co-incubated group and SACI+ 100  $\mu$ mol/L  
102 Paeoniflorin+ 1 mmol/L Senkyunolide co-incubated group. (B) Cardiomyocyte survival after 6,  
103 12, and 24 hours of exposure to different intervention conditions (n = 3 cells/group), with control  
104 group: 0.5% BSA group, PA group: 250  $\mu$  M palmitate/BSA solution, PA + 100  $\mu$  mol/L  
105 Paeoniflorin co-incubated group, PA + 1 mmol/L Senkyunolide co-incubated group and PA + 100  
106  $\mu$  mol/L Paeoniflorin+ 1 mmol/L Senkyunolide co-incubated group. (C, D) TNF- $\alpha$  level  
107 measured in the supernatants after 12 h in SACI cardiomyocyte cultures, and after 6 h in PA

108 cardiomyocyte cultures (n = 4/group). (E, F) Western blot and quantification of Plin2 protein  
 109 expression after 12 h in SACI cardiomyocyte cultures, and after 6 h in PA cardiomyocyte cultures  
 110 (n = 3/group). Results are presented as mean  $\pm$  SEM values. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.005,  
 111 \*\*\*\*p < 0.0001 vs. Control; #p < 0.05, ##p < 0.01, ###p < 0.005, #####p < 0.0001 vs. SACI or PA; ns:  
 112 nonsignificant vs. SACI or PA.

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115 **Figure S7.** THSWD attenuates LD-induced cardiomyocyte lipotoxicity by promoting lipophagy. (A)  
 116 Schematic of the cardiomyocyte treatment groups. (B) Western blot and quantification of LC3B and  
 117 P62 expression in sham control, SACI, SACI+THSWD, SACI+CQ (100  $\mu$ M, 1 h before SACI serum),  
 118 and SACI+CQ+THSWD cultures (n = 3/group). (C) Western blot images and quantitation of LC3B  
 119 and P62 expression in control, SACI, SACI+THSWD, SACI+ATGLi (50  $\mu$ M, 6 h with SACI serum),  
 120 SACI+ATGLi+THSWD, SACI+Baf (100 nm, 1 h before SACI serum), SACI+Baf+THSWD  
 121 cultures (n = 3/group). (D) Supernatant MDA levels, intracellular calcium ion ( $[Ca^{2+}]_i$ ) fluorescence  
 122 intensity and supernatant TNF- $\alpha$ , IL-6 levels in these cultures (n = 3/group). (E-F) Representative

123 images and quantitation of (E) mitochondrial superoxide (MitoSOX) signal (red) (scale bar = 20  
124  $\mu\text{m}$ ; n = 3/group) and (F) nuclear (blue) and JC-1 signal for normal (red) and decreased (green)  
125 mitochondrial membrane potential ( $\Delta\Psi\text{m}$ ) in these groups (scale bar = 50  $\mu\text{m}$ ; n = 3/group). Results  
126 are presented as mean  $\pm$  SEM values. \*\*p < 0.01, \*\*\*p < 0.005, \*\*\*\*p < 0.0001 vs. Control; #p < 0.05,  
127 ##p < 0.01, ###p < 0.005, #####p < 0.0001 vs. SACI; &p < 0.05, &&p < 0.01, &&&p < 0.005, &&&&p <  
128 0.0001 vs. SACI+THSWD. ns: nonsignificant vs. SACI.

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**Table S1:** RNAi sequences

si <i>Plin2</i> (ID: 11520)	Sequence
Pair 1	CGGCUACGACGACACCGAATT
	AUCGGUGUCGUCGUAGCCGTT
Pair 2	CCGCUUAUGUCAGUACAAATT
	UUUGUACUGACAUAAAGCGGTT
Pair 3	GGACCAAGUCUGUGGUCAATT
	UUGACCACAGACUUGGUCCCTT

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**Table S2:** Major THSWD components detected by UPLC-MS/MS

	Identification	Molecular weight (Da)	Formula	CAS	THSWD prescription	THSWD-containing serum
1	Rehmannioside D	686.23	C27H42O20	81720-08-3	1305828.45	0
2	Catalpol	362.12	C15H22O10	2415-24-9	15015.67	604.07
3	Angelicin	186.03	C11H6O3	523-50-2	1030663.81	31842.35
4	Ferulic acid	194.06	C10H10O4	537-98-4	2480586.35	4413.51
5	Paeoniflorin	480.16	C23H28O11	23180-57-6	43905372.31	58358.04
6	Paeonilactone B	196.07	C10H12O4	98751-78-1	174809.41	20394.58
7	Paeonilactone C	318.11	C17H18O6	98751-77-0	775683.27	1181.45
8	Albiflorin	480.16	C23H28O11	39011-90-0	47891151.26	85756.58
9	8-Debenzoylpaeoniflorin	376.14	C16H24O10	23532-11-8	100169.47	2502.46
10	Senkyunolide A	192.115	C12H16O2	63038-10-8	435510.96	1026.91
11	Senkyunolide B	204.08	C12H12O3	93236-67-0	916230.74	4238.86
12	Senkyunolide F	206.09	C12H14O3	94530-84-4	693412.62	11855.76
13	Senkyunolide G	208.11	C12H16O3	94530-85-5	1542103.62	3996.31
14	Senkyunolide H	224.10	C12H16O4	94596-27-7	6582007.42	73472.58
15	Senkyunolide I	224.10	C12H16O4	94596-28-8	174224.45	2857.70
16	Z-Ligustilide	190.10	C12H14O2	4431-01-0	249784.81	787.33
17	Amygdalin	457.16	C20H27NO11	29883-15-6	1740063.78	0
18	Picrocrocin	330.17	C16H26O7	138-55-6	108067.40	5153.07

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