SUPPLEMENTAL MATERIAL

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1. Supplementary Materials and Methods

Mice

All animal procedures were performed according to protocols approved by the Icahn School of Medicine at Mount Sinai's Institutional Care and Use Committee. We used male and female CFW mice. Before surgery, mice were anesthetized with a cocktail of 100 mg/kg ketamine and 10 mg/kg xylazine. For the protein expression assay, we injected 100 µg of Luc, Sphk1 or S1pr2 modRNA in 60 µl citrate buffer directly into the myocardium during open-chest surgery, as described previously.[27] MI was induced by permanently ligating the LAD. The left thoracic region was shaved and sterilized. After intubation, the heart was exposed by left thoracotomy. The LAD was ligated with a suture. When required, we injected 100 µg modRNA into the infarct border zone immediately after LAD ligation. The thoracotomy and skin were sutured closed in layers.

Excess air was removed from the thoracic cavity, and the mouse was removed from ventilation when normal breathing was established. Hearts from sham-operated mice were collected immediately after open-heart surgery without LAD ligation.

ModRNA Synthesis

Clean PCR products generated with plasmid templates (GeneArt, Thermo Fisher Scientific), were used as the template for mRNA (a complete list of the open reading frames used in this study is provided in **Supplementary Table 1**). ModRNAs were generated by transcription *in vitro* with a customized ribonucleoside blend of anti-reverse cap analog, 30-O-Me-m7G(50) ppp(50)G (6 mM, TriLink Biotechnologies); guanosine triphosphate (1.5 mM, Life Technologies); adenosine triphosphate (7.5 mM, Life Technologies); cytidine triphosphate (7.5 mM, Life Technologies) and N1-methylpseudouridine-5-triphosphate (7.5 mM, TriLink Biotechnologies). The mRNA was purified with the Megaclear kit (Life Technologies), and treated with Antarctic Phosphatase (New England Biolabs) and then repurified with the Megaclear kit. The mRNA was quantified on a Nanodrop spectrometer (Thermo Scientific), precipitated with ethanol and ammonium acetate and resuspended in 10 mM Tris-HCl and 1 mM EDTA. This protocol has been described in detail elsewhere.[26]

***In vitro* transfection with modRNA**

Using a 24-well plate, we complexed 2.5 µg per well of mRNA encoding nGFP, AC, Sphk1, Sphk2 or S1pr2 with RNAiMAX (Life Technologies) and used the resulting complex to transfect neonatal rat or hPSC-derived CMs in accordance with the instructions provided by the RNAiMAX manufacturer. For immunofluorescence staining,

cells were washed once with PBS 18 hours after transfection, then fixed by incubation for 10 minutes in 4% PFA and washed three times with PBS. For western blot analysis, cells were washed once with PBS and lysed with lysis buffer (Sigma). To induce cell death by hypoxia, cells were placed in a hypoxia chamber for 48 hours and then harvested by treating the plates with 0.25% trypsin (Sigma) for FACS analysis.

Neonatal rat CM isolation

Neonatal rat ventricular CMs were isolated from three- to four-day-old Sprague-Dawley rats (Jackson ImmunoResearch Laboratories) via multiple rounds of digestion with 0.1% collagenase II (Invitrogen) in PBS. After each digestion, the supernatant was collected in horse serum (Invitrogen). The total cell suspension was centrifuged at 300 x *g* for 5 minutes. The supernatants were discarded and cells were resuspended in DMEM (Gibco) supplemented with 0.1 mM ascorbic acid (Sigma), 0.5% insulin-transferrin-selenium (100x), penicillin (100 U/mL) and streptomycin (100 mg/mL). Cells were plated in plastic culture dishes for 90 minutes until most of the non-myocytes were attached to the dish whilst the myocytes remained in suspension. Myocytes were then used to seed 24-well plates at a density of 1 x 10^5 cells/well. Neonatal rat CMs were incubated for 48 hours in DMEM supplemented with 5% horse serum and then transfected with modRNAs, as described above.

RNA isolation

Total RNA was isolated with the RNeasy mini kit (QIAGEN) and DNA was degraded by treatment with TURBO DNase (Invitrogen).

Real-time qPCR analyses

Total RNA was reverse-transcribed with Superscript III reverse transcriptase (Invitrogen), according to the manufacturer's instructions. Real-time qPCR analyses were performed on a Mastercycler Realplex 4 Sequence Detector (Eppendorf) with the HotStart-IT SYBR Green qPCR master mix (x2) (Affymetrix). Data were normalized relative to 18srRNA or GAPDH. Fold-changes in gene expression were determined by the ddCT method. The PCR primer sequences are listed in **Supplementary Table 8**.

RNA sequencing

The Epigenomics Core at Cornell Medical College prepared poly(A)-tailed RNA with the mRNA Seq Sample Prep Kit (Illumina) and then created libraries for HiSeq2000 sequencing (Illumina). We used single 50 bp reads for sequencing. We obtained a mean of 30 million reads per sample, with a mean quality score of 35.2. We used Partek flow software for data analysis. RNA-Seq reads were aligned to mm10 with STAR version 2.53a. Read counts were generated by applying the Partek E/M algorithm to UCSC RefSeq 2017-08-02. Counts were normalized with TMM algorithm and the Partek flow GSA algorithm was used for statistical analysis of the pathways altered in the heart post MI. We used the enricher tool for gene ontology (GO) analysis using the Fisher exact test with gene set terms from KEGG pathways database. The RNSseq data used in this study are available using accession # GSE138201.

Western blotting

Heart lysates were thawed and subjected to SDS-PAGE in 12% precast Nupage Bis/Tris gels (Invitrogen) under reducing conditions in MES running buffer (Invitrogen). The resulting bands were transferred onto a nitrocellulose membrane (Bio-Rad) by blotting in a semidry transfer apparatus with Nupage-MOPS transfer buffer (Invitrogen). The membrane was blocked by incubation with TBS/Tween containing 5% dry milk powder and incubated with specific primary antibodies overnight at 40°C. It was then washed in TBS/Tween and incubated with rabbit or goat secondary antibodies conjugated to horseradish peroxidase for 1 hour at room temperature. Antibody binding was detected with an enhanced chemiluminescence (ECL) detection system (Pierce). We used prestained protein standards (Amersham) to determine molecular weight. A complete list of the antibodies used in this study is provided in **Supplementary Table 8**.

***In vivo* inhibitor assay**

0.1ml of ARN14974 in DNSO (10mg/ml) or DMSO was mixed with 15% polyethylene glycol, 15% Tween-80 and 70% saline to get a final concentration of 1mg/ml. 10 ml/kg (10mg/kg) was injected IP at the time of LAD ligation and 7 hours post ligation. 24 hours post LAD ligation, hearts were harvested, fixed and stained with TUNEL to assess cell death levels in the LV.

***In vitro* inhibitor assays**

All inhibitors were dissolved in DMSO, ARN14974 and B13 10mg/ml, SK1-II 5mg/ml. The inhibitors were added to nrCM media to assess their effect on cell death 24 and 48 hours later under either normal or anoxic conditions. The following chemical inhibitors of AC and Sphk were used: B13 (Echelon, (1R,2R)-B13, CAY10466) targeting AC), ARN14974 (Cayman Chemical, (Acid ceramidase inhibitor 17a) targeting AC), SK1-II (Millipore Sigma, (2-(p-Hydroxyanilino)-4-(p-chlorophenyl) thiazole, HCl, SK Inhibitor I) targeting Sphingosine kinase).

Analyzing sphingolipid composition

Mass spectrometry was used to determine the sphingolipid composition of frozen mouse hearts crushed with a pestle; both had been stored at -80°C. The powdered heart was transferred to cold Eppendorf tubes and snap frozen in liquid N_2. Small volumes of Tris (20 mM, pH = 7.8) were added to the tubes, which were then subjected to sonication (10 min, 4°C) for protein and lipid extraction. The tubes were centrifuged to remove the solid material, and the supernatants were transferred to clean tubes. Aliquots containing the equivalent of 1 mg total protein, (BCA protein assay, Pierce™ A53226, 560 nM) were subjected to sphingolipid analysis in conical flasks (15 mL). Extraction solution (2 mL, ethyl acetate:isopropanol:water = 5:2.3:1) and internal standards (50 µL, Stony Brook) were added to the flasks, which were then sonicated (3 seconds), vortexed (1 minute) and centrifuged (5 minutes at 600 x *g*). Extracts were transferred to glass test tubes and a second extraction procedure was performed. The solvent was removed at 37°C under a stream of N_2 gas. Samples were resuspended in 150 µL of mobile phase B (methanol, formic acid (0.2%) and ammonium formate (1 mM)

for analysis, following the previously published method,[62] at the Stony Brook Lipidomics facility.

AC activity assay

AC activity in heart tissue lysate was analyzed by HPLC-MS/MS. Briefly, the lysate was incubated in AC buffer with N-palmitoyl ceramide-D31 (PCD, Avanti Lipids®-868516). This ceramide was selected for these assays in order to monitor palmitic acid-D31 (PAD) production without interference from the palmitic acid (m/z = 255.2) ubiquitously detected in negative mode. The reaction was quenched and the proteins were simultaneously precipitated. The proteins were removed by centrifugation and the supernatant was transferred to a sample vial for analysis, which was performed on an HPLC machine connected to a MS/MS detector-monitoring PAD in negative mode. PAD (Sigma®-68277) was quantified with an 18-point calibration curve (2 to 100 nM) in assay conditions. Samples without proteins or without substrate were analyzed as negative controls. The heart apex was lysed in Cytobuster™ (Millipore®-71009) lysis buffer. The resulting suspension was subjected to sonication and centrifuged (300 x *g*, 10 minutes, 4°C). The supernatants were used as total protein lysates (3.8 µg/µL). Aqueous solutions of sodium citrate (Sigma®-71402, 25 mL, 400 mM); sodium phosphate (Sigma®-S5136, 25 mL, 400 mM); citric acid (Sigma®-C0706, 50 mL, 300 mM) and NaCl (100 mL, 300 mM) were prepared and mixed to generate the AC buffer (50 mM sodium citrate, 50 mM sodium phosphate, 75 mM citric acid, 150 mM NaCl, pH = 4.5). For the AC assay, PCD (8.5 µL, 2.5 mM) was added to a tube and the solvent was evaporated off in a Speedvac™. PCD was reconstituted in Nonidet™ P40 (Sigma®- 98379, 2 µL, 10% in H_2O); FBS (1 µL) and AC-buffer (97 µL) to produce the

PCD stock (213.3 µM). PCD stock (15 µL) and cell lysate (5 µL) were combined to constitute the reaction mixture (PCD 160 µM, 0.2% Nonidet, 0.75% FBS), which was then incubated at 37°C for 24 hours. A solution of acetonitrile:ethanol (1:1, 80 µL) was used to quench the reaction and precipitate proteins. The tube was centrifuged (450 x *g*, 10 minutes), and the supernatant was transferred to a sample vial for injection. Three controls were used: protein controls in Cytobuster™ lysis buffer free of extract with and without PCD stock solution, a substrate control consisting of cell lysate and a control stock solution without PCD. For HPLC-MS/MS conditions, chromatography was performed on an HPLC machine (Shimadzu®) equipped with two pumps (LC20AD), an autosampler (SIL20AC, 25 µL injection volume, 15°C) and a column oven (CTO20AC, 40°C) containing a Luna-C18(2) column (Phenomenex®-00D-4252-B0, 100 Å, 5 µm, 100 x 2 mm) equipped with a guard column (Phenomenex®-AJ0-7606). The solvents used were mixtures of acetonitrile:water:isopropanol (A = 10:88:2, B = 93:5:2) supplemented with ammonium formate (10 mM). We used a normal flow rate (1.0 mL/min) with scheduled changes to the percentage of solvent B (0% isocratic for 0-1 min, 1-100% gradient over 1-4 min, 100% isocratic for 4-7.5 min and 0% isocratic for 7.5-9.5 min for post-equilibration). The detector (Sciex® 5500) was optimized for electrospray ionization of PAD (*t* = 5.1 min, MRM = 286.4-286.4, CUR = 35, CAD = Low, IS = -4500 V, TEM = 200°C, GS1 = 40, GS2 = 60, EP = -10 V, DP = -150 V, CE = -13 V, CXP = -18 V) in negative mode. Analyst® software (1.6.3, build 1569) was used for quantification.

Immunofluorescence

Mouse hearts were harvested and perfused with 4% paraformaldehyde (PFA) in perfusion buffer. Hearts were fixed by overnight incubation in 4% PFA/PBS on a shaker and then washed with PBS for 1 h and incubated in 30% sucrose in PBS at 4°C overnight. Before freezing, hearts were mounted in OCT with polymerization for 30 min. They were then frozen at -80°C. Transverse 10 µm heart sections were cut with a cryostat. These cryosections were washed in PBST and blocked by incubation for 1 h with 5% donkey serum in PBST. Sections were incubated overnight at 4°C with primary antibodies against troponin I, Sphk1 and S1p2. Antibody binding was detected by incubation with fluorescent secondary antibodies (Jackson ImmunoResearch Laboratories). TUNEL staining was performed according to the kit manufacturer's instructions (In-Situ Cell Death Detection Kit, Fluorescein, Cat# 11684795910, Roche). Stained sections were imaged with a Zeiss Slide Scanner on an Axio Scan or Zeiss microscope. TUNEL quantification was then performed on the heart sections with ImageJ software.

For cell immunocytochemistry, isolated CMs were fixed on coverslips by incubation with 4% PFA for 10 minutes at room temperature. Following permeabilization with 0.1% Triton X-100 in PBS for 10 minutes at room temperature, cells were blocked by incubation with 5% donkey serum + 0.1% Triton X-100 in PBS for 30 minutes. Coverslips were incubated with primary antibodies in a damp chamber for 1 hour at room temperature and then with the corresponding secondary antibodies conjugated to Alexa Fluor 488, Alexa Fluor 647 and Alexa Fluor 555, and Hoechst 33342 staining was performed to visualize nuclei (all reagents from Invitrogen). Fluorescence images were

obtained with Zeiss fluorescence microscope at a magnification of 20x. A complete list of the antibodies used in this study is provided in Supplementary Table 2.

Magnetic resonance imaging (MRI)

In a double-blind study (treatment unknown to both the surgeon and the MRI or echography technician), CFW mice (8 weeks old) treated with Luc or AC modRNA were subjected to MRI assessment on day 28 post LAD ligation.[17] We obtained delayed-enhancement CINE images on a 7-T Bruker Pharmascan with cardiac and respiratory gating (SA Instruments). Mice were anesthetized with 1-2% isoflurane in air. ECG, respiratory and temperature probes were placed on the mouse, which was kept warm during scans. Imaging was performed 10 to 20 minutes after IV injection of 0.3 mmol/kg gadolinium-diethylene triamine pentaacetic acid. A stack of eight to ten short-axis heart slices, spanning the apex to the base, were acquired with an ECG-triggered and respiratory-gated FLASH sequence with the following parameters: echo time (TE) 2.7 ms with a resolution of 200 µm x 200 µm; 1 mm slice thickness; 16 frames per R-R interval; 4 excitations with a flip angle of 60°. All DICOM images were loaded into the freely available SEGMENT (http://medviso.com/segment/) cardiac MRI software package. The short-axis and long-axis CINE set series were analyzed for dimensions, volumes, ejection fraction, mass, regional function and chamber indices by an experienced user blind to treatment group. For regional analysis, the heart was divided into four sectors: the anterior, lateral, posterior and septal regions. The MI area was defined as the middle to apical left ventricular anterior wall region, with the anteroseptal region as the adjacent border zone.

Echocardiography (Echo)

In a double-blind study (treatment group unknown to both the surgeon and the MRI or echography technician), CFW mice (8 weeks old) treated with Luc, AC, Sphk1 or AC+Sphk1 modRNAs were treated with Ketamine (50µl per 25g body weight, ketamine concentration 25mg/ml), placed on heating pad and evaluated for heart rate while restrained on a platform with tape. Mice with heart rates between 550-600bpm were subjected to echographic assessments on days 2 and 28 post LAD ligation. Heart function parameters were acquired with a Vivid 7 Dimension (GE Healthcare) machine equipped with an i13L probe set to a frequency of 12 MHz. Fractional shortening was calculated from the dimensions at the end of diastole and systole.

hiPSCs differentiation to hiCMs

The source of the hiPSCs is Adult Human Dermal Fibroblast - P13 (ScienCell Research Laboratories - #2320). The cell line has been reprogrammed by Stemgent mRNA and has a normal female karyotype. hiPSCs were induced into cardiac lineage differentiation as previously described.[63] Briefly, hiPSCs were maintained in E8 media and passaged every 4 to 5 days on Matrigel-coated plates. We generated embryoid bodies (EBs) by treating hiPSCs with 1 mg/ml collagenase B (Roche) for 30 minutes or until the cells dissociated from the plates. Cells were collected by centrifugation at 250 x *g* for 3 minutes and resuspended in small clusters of 50-100 cells by gentle pipetting in differentiation medium containing RPMI (Gibco), 2 mmol/L L-glutamine (Invitrogen), 4 x 10^5 monothioglycerol (MTG, Sigma), 50 mg/mL ascorbic acid (Sigma) and 150 mg/mL transferrin (Roche). The differentiation medium was supplemented with 2 ng/mL BMP4

and 3 mmol thiazovivin (Millipore) (day 0). EBs were maintained in six-well ultra-low attachment plates (Corning) at 37°C under an atmosphere containing 5% CO_2, 5% O_2 and 90% N_2. On day 1, the medium was replaced with a differentiation medium supplemented with 20 ng/mL BMP4 (R&D Systems) and 20 ng/mL activin A (R&D Systems). On day 4, the medium was replaced with differentiation medium supplemented with 5 ng/mL VEGF (R&D Systems) and 5 mmol/L XAV (Stemgent). After day 8, the medium was replaced every 5 days with differentiation medium without supplements.

Flow cytometry

Digestion and staining protocols are as previously published[29] with a few modifications. At 2, 7 and 14 days post MI, hearts of Luc modRNA- or AC modRNA-treated mice were perfused using 10ml of ice-cold PBS. Infarct zones were collected and minced in 1ml of digestion mix containing 3.7 μl DNase (Sigma, D5319); 5 μl Hyaluronidase (12000 U/ml, Sigma, H3506); 10μl Collagenase XI (12500 U/ml, Sigma, C7657); 20 μl HEPES buffer (1M, Corning, 25-060-Cl) and 45 μl of Collagenase I (10000 U/ml, Sigma, C0130) in DPBS and incubated for 1 h at 37°C with agitation (750rpm). Next, cell suspension was triturated and filter through 40μm-cell strainer using FACS buffer (0.5% BSA in PBS) and centrifuged at 340 x g for 7 min at 4°C. Prior to first staining, Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block) was added into cell suspension (1μl per $1*10^6$ cells) and incubated for 5 min at RT following by an antibody mix (B220/CD45R, CD19, CD3, CD90.2, CD49b, CD103, NK-1.1, TER-119 (Biolegend) at 1:300) and incubated on ice for 30min. After washing, second staining was performed for 30 min on ice using

CD11b-APC, CD45-Alexa Fluor 700, F4/80-PE-Cy7, Ly6C-BV510, Ly6G-FITC and MHCII-PE at 1:300 (Biolegend). DAPI, used to mark viable cells, was added to the samples 15 min before acquisition. Data were acquired using AURORA (Cytek) Spectral Flow Cytometer and analyzed using FlowJo software. A detailed list of antibodies is available in Supplementary Table 2, and the gating strategy is shown in Figure 5G.

2. Supplementary Tables

Table 1. Open reading frame sequences used to generate modRNA.

Gene	Open reading frame
ACv1	ATGCCGGGCCGGAGTTGCGTCGCCTTAGTCCTCCTGGCTGCCGCCG TCAGCTGTGCCGTCGCGCAGCACGCGCCGCCGTGGACAGAGGACTG CAGAAAATCAACCTATCCTCCTTCAGGACCAACGTACAGAGGTGCAGT TCCATGGTACACCATAAATCTTGACTTACCACCCTACAAAAGATGGCA TGAATTGATGCTTGACAAGGCACCAGTGCTAAAGGTTATAGTGAATTC TCTGAAGAATATGATAAATACATTCGTGCCAAGTGGAAAAATTATGCA GGTGGTGGATGAAAAATTGCCTGGCCTACTTGGCAACTTTCCTGGCC CTTTTGAAGAGGAAATGAAGGGTATTGCCGCTGTTACTGATATACCTT TAGGAGAGATTATTTCATTCAATATTTTTTATGAATTATTTACCATTTGT ACTTCAATAGTAGCAGAAGACAAAAAAGGTCATCTAATACATGGGAGA AACATGGATTTTGGAGTATTTCTTGGGTGGAACATAAATAATGATACCT GGGTCATAACTGAGCAACTAAAACCTTTAACAGTGAATTTGGATTTCC AAAGAAACAACAAAACTGTCTTCAAGGCTTCAAGCTTTGCTGGCTATG TGGGCATGTTAACAGGATTCAAACCAGGACTGTTCAGTCTTACACTGA ATGAACGTTTCAGTATAAATGGTGGTTATCTGGGTATTCTAGAATGGA TTCTGGGAAAGAAAGATGTCATGTGGATAGGGTTCCTCACTAGAACAG TTCTGGAAAATAGCACAAGTTATGAAGAAGCCAAGAATTTATTGACCA AGACCAAGATATTGGCCCCAGCCTACTTTATCCTGGGAGGCAACCAG TCTGGGGAAGGTTGTGTGATTACACGAGACAGAAAGGAATCATTGGA TGTATATGAACTCGATGCTAAGCAGGGTAGATGGTATGTGGTACAAAC AAATTATGACCGTTGGAAACATCCCTTCTTCCTTGATGATCGCAGAAC GCCTGCAAAGATGTGTCTGAACCGCACCAGCCAAGAGAATATCTCATT

	TGAAACCATGTATGATGTCCTGTCAACAAAACCTGTCCTCAACAAGCT GACCGTATACACAACCTTGATAGATGTTACCAAAGGTCAATTCGAAAC TTACCTGCGGGACTGCCCTGACCCTTGTATAGGTTGGTGA
Sphk1	ATCCAGTGGTCGGTTGCGGACGTGGCCTCTTTGGTTTTGTTTTCTCAG CGGGCGGCCCCCGGGGCGTGCTCCCGCGGCCCTGCCGCGTGCTGG TGCTGCTGAACCCGCGCGGCGGCAAGGGCAAGGCCTTGCAGCTCTT CCGGAGTCACGTGCAGCCCCTTTTGGCTGAGGCTGAAATCTCCTTCA CGCTGATGCTCACTGAGCGGCGGAACCACGCGCGGGAGCTGGTGCG GTCGGAGGAGCTGGGCCGCTGGGACGCTCTGGTGGTCATGTCTGGA GACGGGCTGATGCACGAGGTGGTGAACGGGCTCATGGAGCGGCCTG ACTGGGAGACCGCCATCCAGAAGCCCCTGTGTAGCCTCCCAGCAGG CTCTGGCAACGCGCTGGCAGCTTCCTTGAACCATTATGCTGGCTATG AGCAGGTCACCAATGAAGACCTCCTGACCAACTGCACGCTATTGCTG TGCCGCCGGCTGCTGTCACCCATGAACCTGCTGTCTCTGCACACGGC TTCGGGGCTGCGCCTCTTCTCTGTGCTCAGCCTGGCCTGGGGCTTCA TTGCTGATGTGGACCTAGAGAGTGAGAAGTATCGGCGTCTGGGGGAG ATGCGCTTCACTCTGGGCACCTTCCTGCGTCTGGCAGCCCTGCGCAC CTACCGCGGCCGACTGGCCTACCTCCCTGTAGGAAGAGTGGGTTCCA AGACACCTGCCTCCCCCGTTGTGGTCCAGCAGGGCCCGGTAGATGC ACACCTTGTGCCACTGGAGGAGCCAGTGCCCTCTCACTGGACAGTGG TGCCCGACGAGGACTTTGTGCTAGTCCTGGCACTGCTGCACTCGCAC CTGGGCAGTGAGATGTTTGCTGCACCCATGGGCCGCTGTGCAGCTG GCGTCATGCATCTGTTCTACGTGCGGGCGGGAGTGTCTCGTGCCATG CTGCTGCGCCTCTTCCTGGCCATGGAGAAGGGCAGGCATATGGAGTA TGAATGCCCCTACTTGGTATATGTGCCCGTGGTCGCCTTCCGCTTGG

	AGCCCAAGGATGGGAAAGGTGTGTTTGCAGTGGATGGGGAATTGATG GTTAGCGAGGCCGTGCAGGGCCAGGTGCACCCAAACTACTTCTGGAT GGTCAGCGGTTGCGTGGAGCCCCCGCCCAGCTGGAAGCCCCAGCAG ATGCCACCGCCAGAAGAGCCCTTATATG
Sphk2	ATGAATGGACACCTTGAAGCAGAGGAGCAGCAGGACCAGAGGCCAG ACCAGGAGCTGACCGGGAGCTGGGGCCACGGGCCTAGGAGCACCCT GGTCAGGGCTAAGGCCATGGCCCCGCCCCCACCGCCACTGGCTGCC AGCACCCCGCTCCTCCATGGCGAGTTTGGCTCCTACCCAGCCCGAG GCCCACGCTTTGCCCTCACCCTTACATCGCAGGCCCTGCACATACAG CGGCTGCGCCCCAAACCTGAAGCCAGGCCCCGGGGTGGCCTGGTCC CGTTGGCCGAGGTCTCAGGCTGCTGCACCCTGCGAAGCCGCAGCCC CTCAGACTCAGCGGCCTACTTCTGCATCTACACCTACCCTCGGGGCC GGCGCGGGGCCCGGCGCAGAGCCACTCGCACCTTCCGGGCAGATG GGGCCGCCACCTACGAAGAGAACCGTGCCGAGGCCCAGCGCTGGG CCACTGCCCTCACCTGTCTGCTCCGAGGACTGCCACTGCCCGGGGAT GGGGAGATCACCCCTGACCTGCTACCTCGGCCGCCCCGGTTGCTTCT ATTGGTCAATCCCTTTGGGGGTCGGGGCCTGGCCTGGCAGTGGTGTA AGAACCACGTGCTTCCCATGATCTCTGAAGCTGGGCTGTCCTTCAAC CTCATCCAGACAGAACGACAGAACCACGCCCGGGAGCTGGTCCAGG GGCTGAGCCTGAGTGAGTGGGATGGCATCGTCACGGTCTCGGGAGA CGGGCTGCTCCATGAGGTGCTGAACGGGCTCCTAGATCGCCCTGACT GGGAGGAAGCTGTGAAGATGCCTGTGGGCATCCTCCCCTGCGGCTC GGGCAACGCGCTGGCCGGAGCAGTGAACCAGCACGGGGGATTTGAG CCAGCCCTGGGCCTCGACCTGTTGCTCAACTGCTCACTGTTGCTGTG CCGGGGTGGTGGCCACCCACTGGACCTGCTCTCCGTGACGCTGGCC

	TCGGGCTCCCGCTGTTTCTCCTTCCTGTCTGTGGCCTGGGGCTTCGT GTCAGATGTGGATATCCAGAGCGAGCGCTTCAGGGCCTTGGGCAGT GCCCGCTTCACACTGGGCACGGTGCTGGGCCTCGCCACACTGCACA CCTACCGCGGACGCCTCTCCTACCTCCCCGCCACTGTGGAACCTGCC TCGCCCACCCCTGCCCATAGCCTGCCTCGTGCCAAGTCGGAGCTGAC CCTAACCCCAGACCCAGCCCCGCCCATGGCCCACTCACCCCTGCATC GTTCTGTGTCTGACCTGCCTCTTCCCCTGCCCCAGCCTGCCCTGGCC TCTCCTGGCTCGCCAGAACCCCTGCCCATCCTGTCCCTCAACGGTGG GGGCCCAGAGCTGGCTGGGGACTGGGGTGGGGCTGGGGATGCTCC GCTGTCCCCGGACCCACTGCTGTCTTCACCTCCTGGCTCTCCCAAGG CAGCTCTACACTCACCCGTCTCCGAAGGGGCCCCCGTAATTCCCCCA TCCTCTGGGCTCCCACTTCCCACCCCTGATGCCCGGGTAGGGGCCTC CACCTGCGGCCCGCCCGACCACCTGCTGCCTCCGCTGGGCACCCCG CTGCCCCCAGACTGGGTGACGCTGGAGGGGGACTTTGTGCTCATGTT GGCCATCTCGCCCAGCCACCTAGGCGCTGACCTGGTGGCAGCTCCG CATGCGCGCTTCGACGACGGCCTGGTGCACCTGTGCTGGGTGCGTA GCGGCATCTCGCGGGCTGCGCTGCTGCGCCTTTTCTTGGCCATGGA GCGTGGTAGCCACTTCAGCCTGGGCTGTCCGCAGCTGGGCTACGCC GCGGCCCGTGCCTTCCGCCTAGAGCCGCTCACACCACGCGGCGTGC TCACAGTGGACGGGGAGCAGGTGGAGTATGGGCCGCTACAGGCACA GATGCACCCTGGCATCGGTACACTGCTCACTGGGCCTCCTGGCTGCC CGGGGCGGGAGCCCTGA
S1PR2	ATGGGCAGCTTGTACTCGGAGTACCTGAACCCCAACAAGGTCCAGGA ACACTATAATTATACCAAGGAGACGCTGGAAACGCAGGAGACGACCT CCCGCCAGGTGGCCTCGGCCTTCATCGTCATCCTCTGTTGCGCCATT

	GTGGTGGAAAACCTTCTGGTGCTCATTGCGGTGGCCCGAAACAGCAA GTTCCACTCGGCAATGTACCTGTTTCTGGGCAACCTGGCCGCCTCCG ATCTACTGGCAGGCGTGGCCTTCGTAGCCAATACCTTGCTCTCTGGC TCTGTCACGCTGAGGCTGACGCCTGTGCAGTGGTTTGCCCGGGAGG GCTCTGCCTTCATCACGCTCTCGGCCTCTGTCTTCAGCCTCCTGGCC ATCGCCATTGAGCGCCACGTGGCCATTGCCAAGGTCAAGCTGTATGG CAGCGACAAGAGCTGCCGCATGCTTCTGCTCATCGGGGCCTCGTGG CTCATCTCGCTGGTCCTCGGTGGCCTGCCCATCCTTGGCTGGAACTG CCTGGGCCACCTCGAGGCCTGCTCCACTGTCCTGCCTCTCTACGCCA AGCATTATGTGCTGTGCGTGGTGACCATCTTCTCCATCATCCTGTTGG CCATCGTGGCCCTGTACGTGCGCATCTACTGCGTGGTCCGCTCAAGC CACGCTGACATGGCCGCCCCGCAGACGCTAGCCCTGCTCAAGACGG TCACCATCGTGCTAGGCGTCTTTATCGTCTGCTGGCTGCCCGCCTTC AGCATCCTCCTTCTGGACTATGCCTGTCCCGTCCACTCCTGCCCGAT CCTCTACAAAGCCCACTACTTTTTCGCCGTCTCCACCCTGAATTCCCT GCTCAACCCCGTCATCTACACGTGGCGCAGCCGGGACCTGCGGCGG GAGGTGCTTCGGCCGCTGCAGTGCTGGAGGCCGGGGGTGGGGGTG CAAGGACGGAGGCGGGGCGGGACCCCGGGCCACCACCTCCTGCCA CTCCGCAGCTCCAGCTCCCTGGAGAGGGGCATGCACATGCCCACGT CACCCACGTTTCTGGAGGGCAACACGGTGGTCATG
Firefly luciferase	ATGGCCGATGCTAAGAACATTAAGAAGGGCCCTGCTCCCTTCTACCC TCTGGAGGATGGCACCGCTGGCGAGCAGCTGCACAAGGCCATGAAG AGGTATGCCCTGGTGCCTGGCACCATTGCCTTCACCGATGCCCACAT TGAGGTGGACATCACCTATGCCGAGTACTTCGAGATGTCTGTGCGCC TGGCCGAGGCCATGAAGAGGTACGGCCTGAACACCAACCACCGCAT

	CGTGGTGTGCTCTGAGAACTCTCTGCAGTTCTTCATGCCAGTGCTGG GCGCCCTGTTCATCGGAGTGGCCGTGGCCCCTGCTAACGACATTTAC AACGAGCGCGAGCTGCTGAACAGCATGGGCATTTCTCAGCCTACCGT GGTGTTCGTGTCTAAGAAGGGCCTGCAGAAGATCCTGAACGTGCAGA AGAAGCTGCCTATCATCCAGAAGATCATCATCATGGACTCTAAGACCG ACTACCAGGGCTTCCAGAGCATGTACACATTCGTGACATCTCATCTGC CTCCTGGCTTCAACGAGTACGACTTCGTGCCAGAGTCTTTCGACAGG GACAAAACCATTGCCCTGATCATGAACAGCTCTGGGTCTACCGGCCT GCCTAAGGGCGTGGCCCTGCCTCATCGCACCGCCTGTGTGCGCTTCT CTCACGCCCGCGACCCTATTTTCGGCAACCAGATCATCCCCGACACC GCTATTCTGAGCGTGGTGCCATTCCACCACGGCTTCGGCATGTTCAC CACCCTGGGCTACCTGATTTGCGGCTTTCGGGTGGTGCTGATGTACC GCTTCGAGGAGGAGCTGTTCCTGCGCAGCCTGCAAGACTACAAAATT CAGTCTGCCCTGCTGGTGCCAACCCTGTTCAGCTTCTTCGCTAAGAG CACCCTGATCGACAAGTACGACCTGTCTAACCTGCACGAGATTGCCT CTGGCGGCGCCCCACTGTCTAAGGAGGTGGGCGAAGCCGTGGCCAA GCGCTTTCATCTGCCAGGCATCCGCCAGGGCTACGGCCTGACCGAG ACAACCAGCGCCATTCTGATTACCCCAGAGGGCGACGACAAGCCTGG CGCCGTGGGCAAGGTGGTGCCATTCTTCGAGGCCAAGGTGGTGGAC CTGGACACCGGCAAGACCCTGGGAGTGAACCAGCGCGGCGAGCTGT GTGTGCGCGGCCCTATGATTATGTCCGGCTACGTGAATAACCCTGAG GCCACAAACGCCCTGATCGACAAGGACGGCTGGCTGCACTCTGGCG ACATTGCCTACTGGGACGAGGACGAGCACTTCTTCATCGTGGACCGC CTGAAGTCTCTGATCAAGTACAAGGGCTACCAGGTGGCCCCAGCCGA GCTGGAGTCTATCCTGCTGCAGCACCCTAACATTTTCGACGCCGGAG

	TGGCCGGCCTGCCCGACGACGATGCCGGCGAGCTGCCTGCCGCCGT CGTCGTGCTGGAACACGGCAAGACCATGACCGAGAAGGAGATCGTG GACTATGTGGCCAGCCAGGTGACAACCGCCAAGAAGCTGCGCGGCG GAGTGGTGTTCGTGGACGAGGTGCCCAAGGGCCTGACCGGCAAGCT GGACGCCCGCAAGATCCGCGAGATCCTGATCAAGGCTAAGAAAGGC GGCAAGATCGCCGTGTAA
nGFP	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCC TGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTC CGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGT GACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGAC CACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTA CGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCAT CGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGG CACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCC GACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAA CATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAAC ACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACC TGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGA TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCG GCATGGACGAGCTGTACAAGGGAGATCCAAAAAAGAAGAGAAAGGTA GGCGATCCAAAAAAGAAGAGAAAGGTAGGTGATCCAAAAAAGAAGAG AAAGGTATAA

Table 2. Antibodies used in this study.

Antigen	Dilution immunostaining (WB) [FACS]	Manufacturer	Catalog #
α-Actinin	1:100	Abcam	ab9465
GFP	1:1000	Abcam	ab13970
Troponin I	1:100	Santa Cruz Biotechnology	SC-15368
CD31	1:100	R&D Biosystems	AF3628
WGA	1:50	Life Technologies	W11261
Caspase 3	(1:50)	Santa Cruz Biotechnology	sc-7148
Caspase 3 p17	(1:50)	Santa Cruz Biotechnology	sc-271028
AC	1:25 (1:100)	Sigma	HPA005468
AC alpha subunit	(1:100)	Santa Cruz Biotechnology	sc-136275
S1pr2	1:50 (1:100)	Novus Biologicals	NBP2-26691SS
Sphk1	1:50 (1:100)	Santa Cruz Biotechnology	sc-48825
Sphk2	1:100 (1:100)	Bioss Antibodies	bs-2653R
B220/CD45R PerCP-Cy5.5	[1:300]	BioLegend	Clone RA3-6B2
CD19 PerCP-Cy5.5	[1:300]	BioLegend	Clone 6D5
CD3 PerCP-Cy5.5	[1:300]	BioLegend	Clone 17A2
CD90.2 (Thy-1.2) PerCP-Cy5.5	[1:300]	BioLegend	Clone 53-2.1
CD49b (pan-NK cells) PerCP-Cy5.5	[1:300]	BioLegend	Clone DX5

CD103 PerCP-Cy5.5	[1:300]	BioLegend	Clone 2E7
NK-1.1 PerCP-Cy5.5	[1:300]	BioLegend	Clone PK136
TER-119/Erythroid Cells PerCP-Cy5.5	[1:300]	BioLegend	Clone TER-119
CD11b APC	[1:300]	BioLegend	Clone M1/70
CD45 Alexa Fluor 700	[1:300]	BioLegend	Clone 30-F11
F4/80 PE-Cy7	[1:300]	BioLegend	Clone BM8
Ly-6C BV510	[1:300]	BioLegend	Clone HK1.4
Ly-6G FITC	[1:300]	BioLegend	Clone 1A8
MHCII PE	[1:300]	BioLegend	Clone M5/114.15.2

Table 3. List of genes involved in sphingolipid metabolism and signaling and their RNA levels and fold-changes in expression 4 and 24 h post MI, relative to the sham-operated control (129 genes added as a separate Excel file).

Table 4. Genes with significantly different expression in hearts treated with AC modRNA vs. Luc control modRNA 4 h post MI (30 genes added as a separate Excel file).

Table 5. Genes with significantly different expression in hearts treated with AC modRNA vs. Luc control modRNA 24 h post MI (299 genes added as a separate Excel file).

Table 6. Genes with significantly different expression levels in hearts treated with AC modRNA vs. Luc control modRNA (1675 genes added as a separate Excel file).

Table 7. GO enrichment analysis 24 hours post MI for hearts treated with AC modRNA.

Gene set	Description	Enrichment score	*P*-value
GO:0048002	Antigen processing and presentation of peptide antigen	21.1029	6.84E-10
GO:0003823	Antigen binding	21.1029	6.84E-10
GO:0005833	Hemoglobin complex	20.8875	8.49E-10
GO:0042611	MHC protein complex	20.8243	9.04E-10
GO:0042605	Peptide antigen binding	19.9564	2.15E-09
GO:0046977	TAP binding	19.208	4.55E-09
GO:0006955	Immune response	19.1991	4.59E-09
GO:0019882	Antigen processing and presentation	19.1353	4.89E-09
GO:0002376	Immune system process	17.936	1.62E-08
GO:0031720	Haptoglobin binding	16.8413	4.85E-08

GO:0051607	Defense response to virus	16.4842	6.93E-08
GO:0002474	Antigen processing and presentation of peptide antigen via MHC class I	15.7855	1.39E-07
GO:0031838	Haptoglobin-hemoglobin complex	15.2437	2.40E-07
GO:0002478	Antigen processing and presentation of exogenous peptide antigen	14.5679	4.71E-07
GO:0009615	Response to virus	13.1906	1.87E-06
GO:0019884	Antigen processing and presentation of exogenous antigen	13.1855	1.88E-06
GO:0042608	T cell receptor binding	12.8124	2.73E-06
GO:0045087	Innate immune response	12.506	3.70E-06
GO:1903901	Negative regulation of viral life cycle	12.129	5.40E-06
GO:0019825	Oxygen binding	11.8701	7.00E-06
GO:0002252	Immune effector process	11.8569	7.09E-06
GO:0002483	Antigen processing and presentation of endogenous peptide antigen	11.5652	9.49E-06
GO:0006952	Defense response	11.3703	1.15E-05
GO:1903900	Regulation of viral life cycle	10.9715	1.72E-05
GO:0042612	MHC class I protein complex	10.7314	2.18E-05

GO:0019883	Antigen processing and presentation of endogenous antigen	10.7314	2.18E-05
GO:0030881	Beta-2-microglobulin binding	10.7314	2.18E-05
GO:0048525	Negative regulation of viral process	10.4834	2.80E-05
GO:0048584	Positive regulation of response to stimulus	10.4587	2.87E-05
GO:0098542	Defense response to other organism	9.99147	4.58E-05
GO:0045088	Regulation of innate immune response	9.74742	5.84E-05
GO:0043900	Regulation of multi-organism process	9.74311	5.87E-05
GO:2000781	Positive regulation of double-strand break repair	9.47654	7.66E-05
GO:0044424	Intracellular part	9.41432	8.15E-05
GO:0044464	Cell part	9.30412	9.10E-05
GO:0005737	Cytoplasm	9.2458	9.65E-05
GO:0045089	Positive regulation of innate immune response	9.12315	0.000109
GO:0019885	Antigen processing and presentation of endogenous peptide antigen via MHC class I	9.11551	0.00011
GO:0009897	External side of plasma membrane	9.09004	0.000113
GO:0034341	Response to interferon-gamma	8.8805	0.000139
GO:0050792	Regulation of viral process	8.75679	0.000157

GO:0050776	Regulation of immune response	8.65487	0.000174
GO:0043901	Negative regulation of multi-organism process	8.60599	0.000183
GO:0070971	Endoplasmic reticulum exit site	8.54454	0.000195
GO:0050778	Positive regulation of immune response	8.42098	0.00022
GO:0031722	Hemoglobin beta binding	8.41449	0.000222
GO:0042613	MHC class II protein complex	8.26225	0.000258
GO:0032182	Ubiquitin-like protein binding	8.2535	0.00026
GO:0051707	Response to other organism	8.24584	0.000262
GO:0045071	Negative regulation of viral genome replication	8.21216	0.000271
GO:0005797	Golgi medial cisterna	8.15608	0.000287
GO:0006950	Response to stress	8.04285	0.000321
GO:1903038	Negative regulation of leukocyte cell-cell adhesion	8.00845	0.000333
GO:0048821	Erythrocyte development	7.97677	0.000343
GO:0007088	Regulation of mitotic nuclear division	7.75156	0.00043
GO:0042287	MHC protein binding	7.48826	0.00056
GO:0043207	Response to external biotic stimulus	7.47071	0.00057
GO:0098797	Plasma membrane protein complex	7.43179	0.000592

GO:0002682	Regulation of immune system process	7.41261	0.000604
GO:0002579	Positive regulation of antigen processing and presentation	7.3327	0.000654
GO:0042824	MHC class I peptide loading complex	7.3258	0.000658
GO:0046978	TAP1 binding	7.3258	0.000658
GO:0046979	TAP2 binding	7.3258	0.000658
GO:0002282	Microglial cell activation involved in immune response	7.3258	0.000658
GO:0002477	Antigen processing and presentation of exogenous peptide antigen via MHC class Ib	7.3258	0.000658
GO:0002481	Antigen processing and presentation of exogenous protein antigen via MHC class Ib, TAP-dependent	7.3258	0.000658
GO:0002484	Antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway	7.3258	0.000658
GO:0002485	Antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TAP-dependent	7.3258	0.000658
GO:0002697	Regulation of immune effector process	7.31249	0.000667

GO:0032481	Positive regulation of type I interferon production	7.23164	0.000723
GO:0002230	Positive regulation of host defense response to virus	7.19722	0.000749
GO:0003725	Double-stranded RNA binding	7.1669	0.000772
GO:0043903	Regulation of symbiosis, encompassing mutualism through parasitism	7.15468	0.000781
GO:0002764	Immune response-regulating signaling pathway	7.15151	0.000784
GO:0051784	Negative regulation of nuclear division	7.13614	0.000796
GO:0061515	Myeloid cell development	7.13614	0.000796
GO:0098552	Side of membrane	7.11603	0.000812
GO:0045069	Regulation of viral genome replication	7.09476	0.000829
GO:2000779	Regulation of double-strand break repair	7.0429	0.000874
GO:0048583	Regulation of response to stimulus	6.98493	0.000926
GO:0009607	Response to biotic stimulus	6.9418	0.000967
GO:0019886	Antigen processing and presentation of exogenous peptide antigen via MHC class II	6.8513	0.001058
GO:0005815	Microtubule organizing center	6.81559	0.001097
GO:0032728	Positive regulation of interferon-beta production	6.80268	0.001111

GO:0051250	Negative regulation of lymphocyte activation	6.8017	0.001112
GO:0050868	Negative regulation of T cell activation	6.79369	0.001121
GO:0042825	TAP complex	6.64257	0.001304
GO:0045356	Positive regulation of interferon-alpha biosynthetic process	6.64257	0.001304
GO:0002476	Antigen processing and presentation of endogenous peptide antigen via MHC class Ib	6.64257	0.001304
GO:0031721	Hemoglobin α binding	6.64257	0.001304
GO:0009407	Toxin catabolic process	6.64257	0.001304
GO:0090487	Secondary metabolite catabolic process	6.64257	0.001304
GO:0140104	Molecular carrier activity	6.63921	0.001308
GO:0032183	SUMO binding	6.63921	0.001308
GO:0071346	Cellular response to interferon-gamma	6.60763	0.00135
GO:0043065	Positive regulation of apoptotic process	6.53846	0.001447
GO:0051783	Regulation of nuclear division	6.53322	0.001454
GO:0032735	Positive regulation of interleukin-12 production	6.44985	0.001581
GO:1902187	Negative regulation of viral release from host cell	6.44262	0.001592

GO:0002504	Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	6.44262	0.001592
GO:0002495	Antigen processing and presentation of peptide antigen via MHC class II	6.44262	0.001592
GO:0009967	Positive regulation of signal transduction	6.43139	0.00161
GO:0043068	Positive regulation of programmed cell death	6.42308	0.001624
GO:0031349	Positive regulation of defense response	6.3557	0.001737
GO:0002683	Negative regulation of immune system process	6.25759	0.001916
GO:0002684	Positive regulation of immune system process	6.25123	0.001928
GO:0045354	Regulation of interferon-alpha biosynthetic process	6.14165	0.002151
GO:2001034	Positive regulation of double-strand break repair via nonhomologous end joining	6.14165	0.002151
GO:0002428	Antigen processing and presentation of peptide antigen via MHC class Ib	6.14165	0.002151
GO:0030578	PML body organization	6.14165	0.002151
GO:0032020	ISG15-protein conjugation	6.14165	0.002151
GO:1990111	Spermatoproteasome complex	6.14165	0.002151
GO:0005488	Binding	6.08803	0.00227

GO:0031347	Regulation of defense response	6.05617	0.002343
GO:0045839	Negative regulation of mitotic nuclear division	6.03176	0.002401
GO:0030554	Adenyl nucleotide binding	6.00878	0.002457
GO:0005524	ATP binding	5.95293	0.002598
GO:0002577	Regulation of antigen processing and presentation	5.92738	0.002665
GO:0003950	NAD+ ADP-ribosyltransferase activity	5.92738	0.002665
GO:0009986	Cell surface	5.85756	0.002858
GO:0002695	Negative regulation of leukocyte activation	5.83136	0.002934
GO:0016740	Transferase activity	5.80548	0.003011
GO:0051704	Multi-organism process	5.77316	0.00311
GO:0050691	Regulation of host defense response to virus	5.75049	0.003181
GO:0004601	Peroxidase activity	5.75049	0.003181
GO:0051534	Negative regulation of NFAT protein import into nucleus	5.74608	0.003195
GO:0002730	Regulation of dendritic cell cytokine production	5.74608	0.003195
GO:0005828	Kinetochore microtubule	5.74608	0.003195
GO:0044389	Ubiquitin-like protein ligase binding	5.69979	0.003347

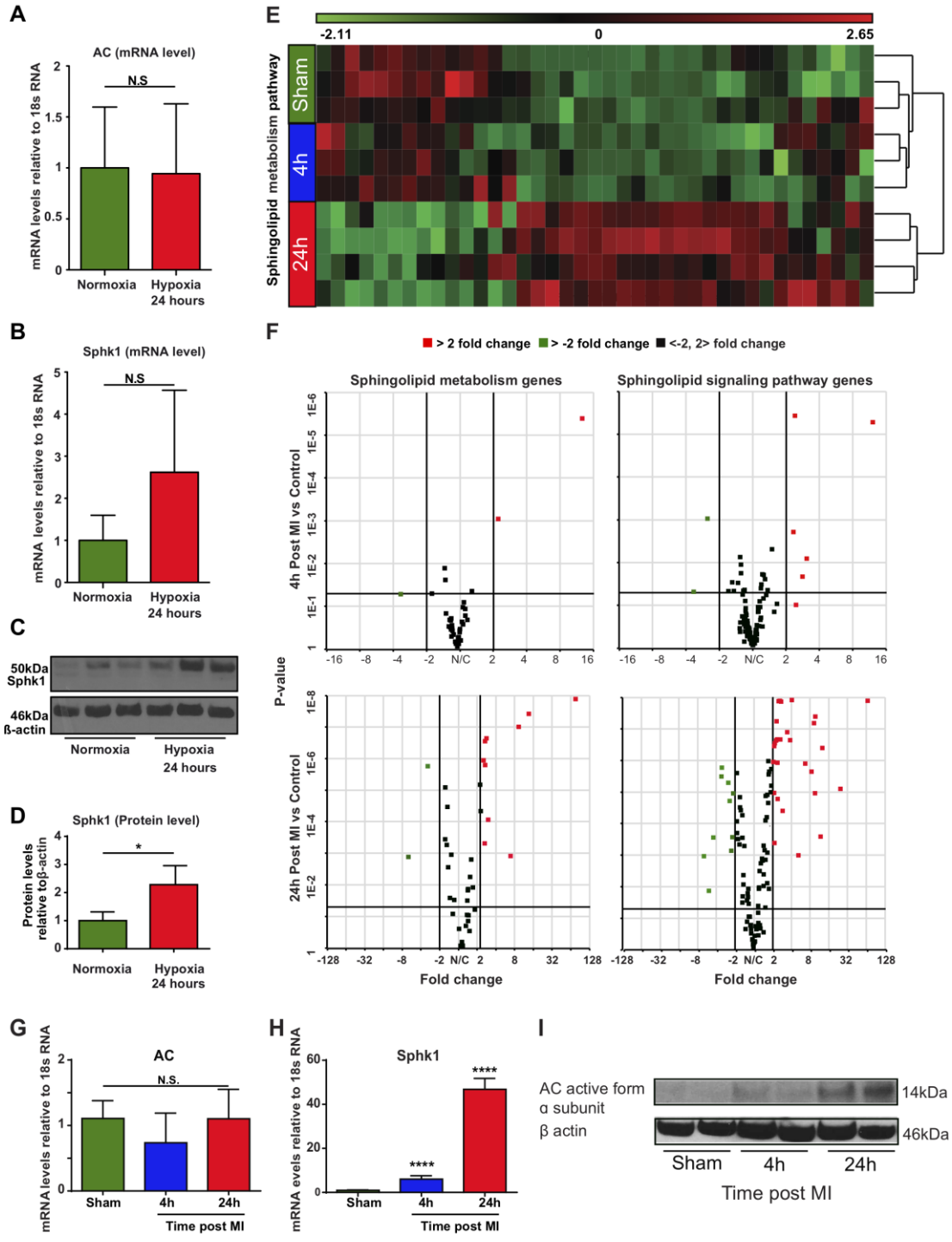
GO:0048522	Positive regulation of cellular process	5.68587	0.003394
GO:0022408	Negative regulation of cell-cell adhesion	5.68149	0.003408
GO:0005886	Plasma membrane	5.66448	0.003467
GO:0033218	Amide binding	5.6501	0.003517
GO:0001774	Microglial cell activation	5.63272	0.003579
GO:0001916	Positive regulation of T cell-mediated cytotoxicity	5.63272	0.003579
GO:0035455	Response to interferon-alpha	5.63272	0.003579
GO:0005813	Centrosome	5.57439	0.003794
GO:0032559	Adenyl ribonucleotide binding	5.51499	0.004026
GO:0048518	Positive regulation of biological process	5.50622	0.004061
GO:0032727	Positive regulation of interferon-alpha production	5.49711	0.004099
GO:0016684	Oxidoreductase activity, acting on peroxide as acceptor	5.49235	0.004118
GO:0051310	Metaphase plate congression	5.49235	0.004118
GO:0048519	Negative regulation of biological process	5.489	0.004132
GO:0042277	Peptide binding	5.48282	0.004158
GO:0010942	Positive regulation of cell death	5.46894	0.004216
GO:0002821	Positive regulation of adaptive immune response	5.45732	0.004265

GO:0002250	Adaptive immune response	5.45532	0.004274
GO:0005515	Protein binding	5.4539	0.00428
GO:0050688	Regulation of defense response to virus	5.42095	0.004423
GO:0042289	MHC class II protein binding	5.41949	0.004429
GO:0042609	CD4 receptor binding	5.41949	0.004429
GO:0045359	Positive regulation of interferon-beta biosynthetic process	5.41949	0.004429
GO:0080009	mRNA methylation	5.41949	0.004429
GO:0030492	Hemoglobin binding	5.41949	0.004429
GO:0070098	Chemokine-mediated signaling pathway	5.41088	0.004468
GO:0031985	Golgi cisterna	5.41088	0.004468
GO:0002831	Regulation of response to biotic stimulus	5.39583	0.004535
GO:2001020	Regulation of response to DNA damage stimulus	5.36171	0.004693

Table 8. Primer sequences for qPCR.

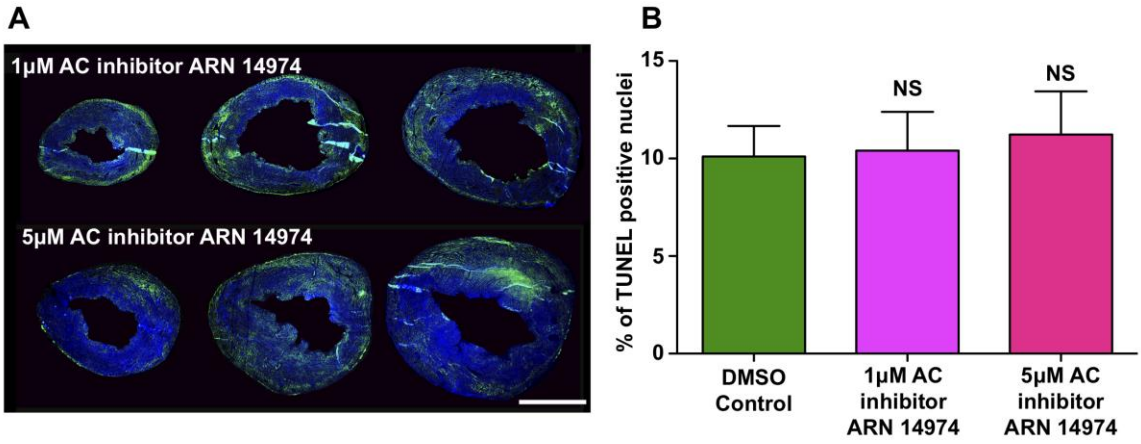
Gene	Forward	Reverse
AC	ACAGGATTCAAACCAGGACTGT	TGGGCATCTTTCCTTCCGAA
Arg1	AAGACAGCAGAGGAGGTGAAGAG	TGGGAGGAGAAGGCGTTTGC
Ngp	GCCTAAAGACTGCGACTTCCT	TGGGTATCCTCTCGACTGCAA
Sphk1	ATACTCACCGAACGGAAGAACC	CCATTAGCCCATTCACCACCTC
Sphk2	CAGAACGACAGAACCATGCC	CCCATTCAGCACCTCGTAAAG
S1PR2	CACAGCCAACAGTCTCCAAA	TCTGAGTATAAGCCGCCCA
18s rRNA	TAACGAACGAGACTCTGGCAT	CGGACATCTAAGGGCATCACAG

3. Supplementary figures



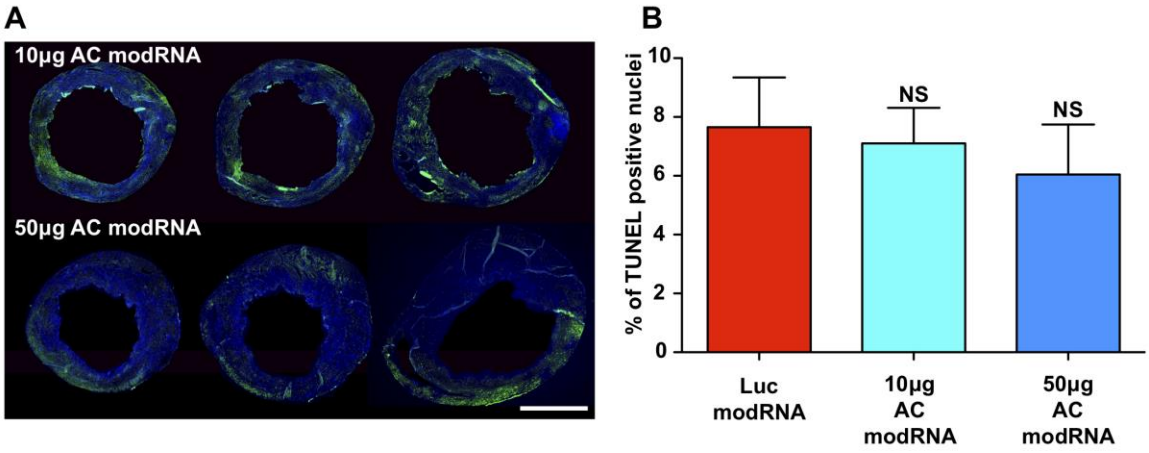
Supplementary Figure S1. Sphingolipid metabolism dynamics in cardiac cells in hypoxic condition *in vitro* and after MI.

Primary cardiomyocytes were isolated from the hearts of two- to three-day-old rats. To evaluate mRNA and protein levels for endogenous AC (mRNA) and Sphk1 (mRNA and protein), we incubated the cells under normoxia (21% oxygen) or hypoxia (<2% oxygen). We isolated mRNA and protein after 24 hours. We determined mRNA levels by RT-qPCR for AC, n=4 (**A**), and Sphk1, n=3 (**B**), and protein levels for Sphk1, n=3 (**C-D**), 24 hours after transfection. **E,** Hierarchical cluster dendrogram for the sphingolipid metabolism transcriptome in sham-operated hearts and in hearts 4 and 24 hours post MI, n=3,3 and 4. **F,** Volcano plot of the sphingolipid metabolism transcriptome and sphingolipid signaling pathway transcriptome at 4 hours and 24 hours post Mi, n=3 and 4. **G-H,** Evaluation of mRNA levels by RT-qPCR for AC, n=4 (**G**), or Sphk1, n=4 (**H**), relative to 18S rRNA in the LV of sham-operated hearts and in hearts 4 and 24 hours post MI. **I,** Western blot of the AC active subunit α in the LV of sham-operated hearts and hearts harvested 4 or 24 hours post MI. ****, $P<0.0001$, *, $P<0.05$, NS, not significant. Two-tailed Student's *t*-tests; (A, B and D), ANOVA test (G-H).



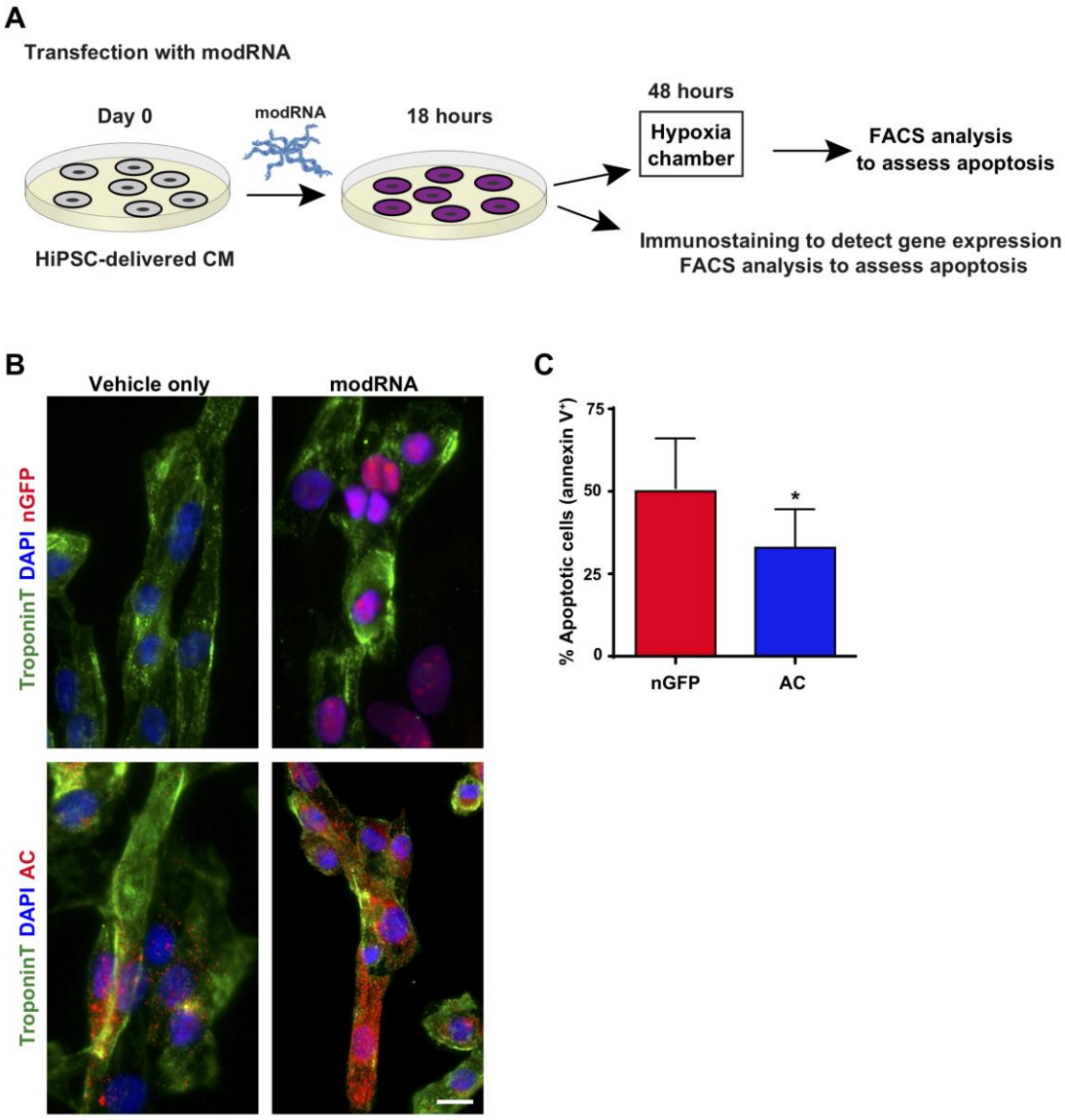
Supplementary Figure S2. The effect of suboptimal treatment with AC inhibitor on cell death after MI.

A, Representative images of heart sections from mice that received suboptimal treatment of AC inhibitor (1 or 5μM), obtained 24 hours post MI. **B,** Quantified cell death levels in the LV after suboptimal treatment with AC inhibitor (1 or 5μM) or DMSO control, 24 hours post MI, n=3. NS, not significant. One-way ANOVA, Tukey's Multiple Comparison Test (B). Scale bar 1mm.



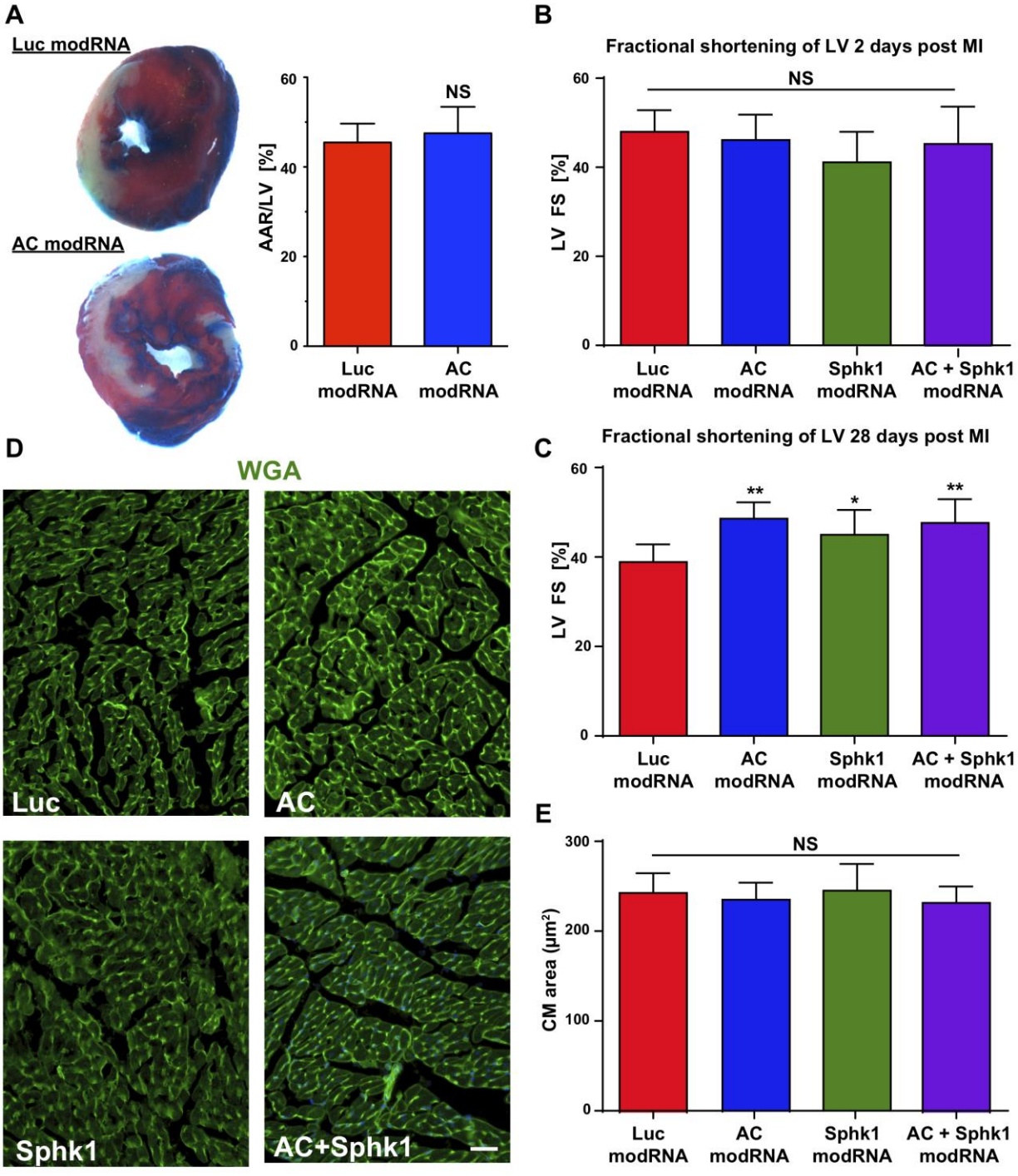
Supplementary Figure S3. The effect of suboptimal treatment with AC modRNA on cell death after MI.

A, Representative images of heart sections from mice that received suboptimal treatment with AC modRNA (10 or 50µg), obtained 48 hours post MI. **B,** Quantified cell death levels in the LV after suboptimal treatment with AC modRNA (10 or 50µ) or 100µ Luc modRNA (control), 48 hours post MI, n=3. NS, not significant. One-way ANOVA, Tukey's Multiple Comparison Test (B). Scale bar 1mm.

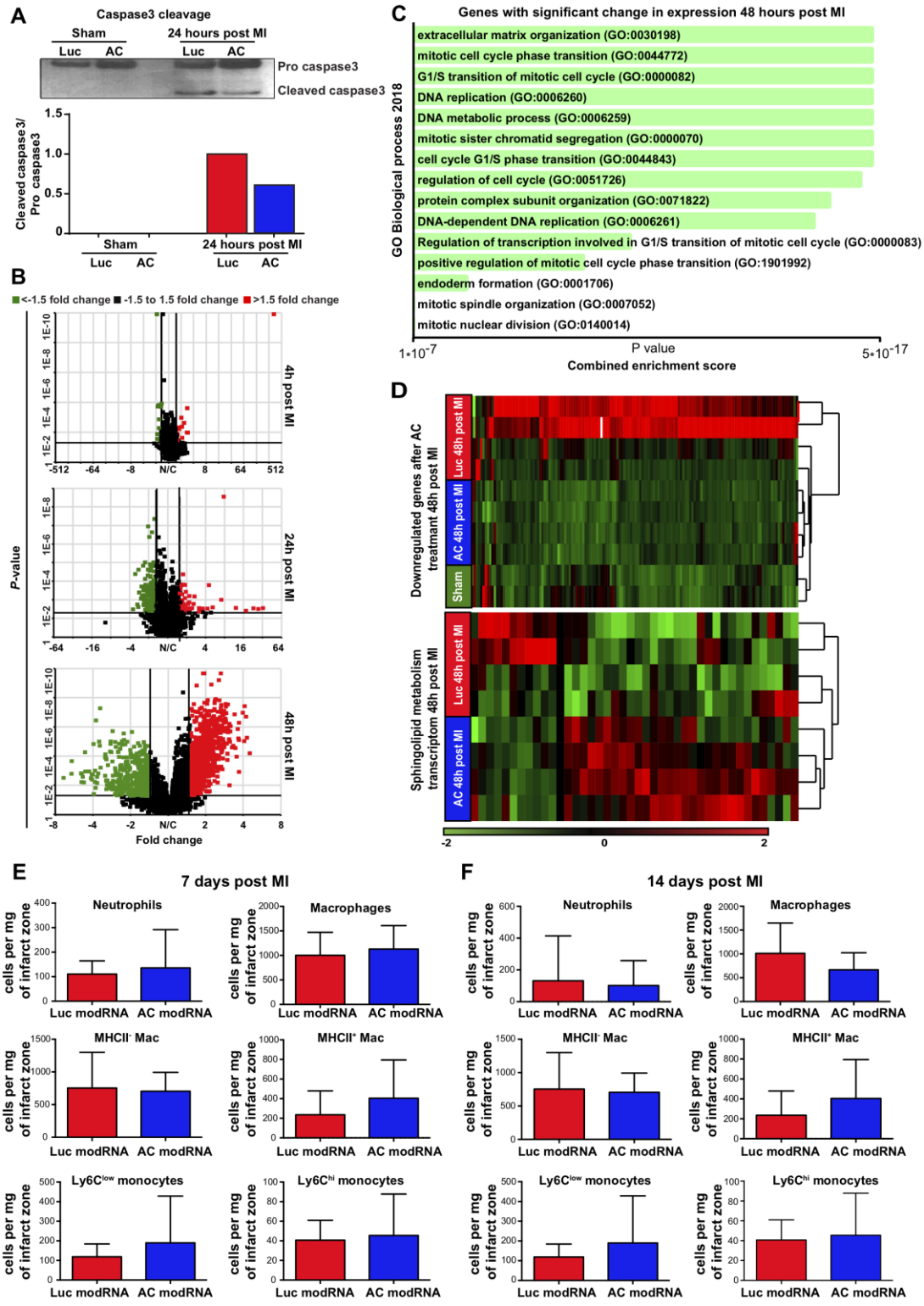


Supplementary Figure S4. Effect of AC modRNA on cell death in human induced pluripotent stem cell-derived CMs (hiCMs) after 48 hours of hypoxia. A, hiCMs were transfected with modRNAs for either nGFP or AC to study the effect of AC overexpression on hiCM cell death. **B,** 18 hours post transfection, the cells were fixed and immunostained to confirm modRNA translation. **C,** Analysis of hiCM cell death after 48 hours culture in hypoxia chamber. nGFP modRNA- or AC modRNA-transfected

hiCMs were stained with Annexin V and analyzed using FACS. *, P<0.05, n=7, two-tailed Student's *t*-tests. Scale bar 10μm.

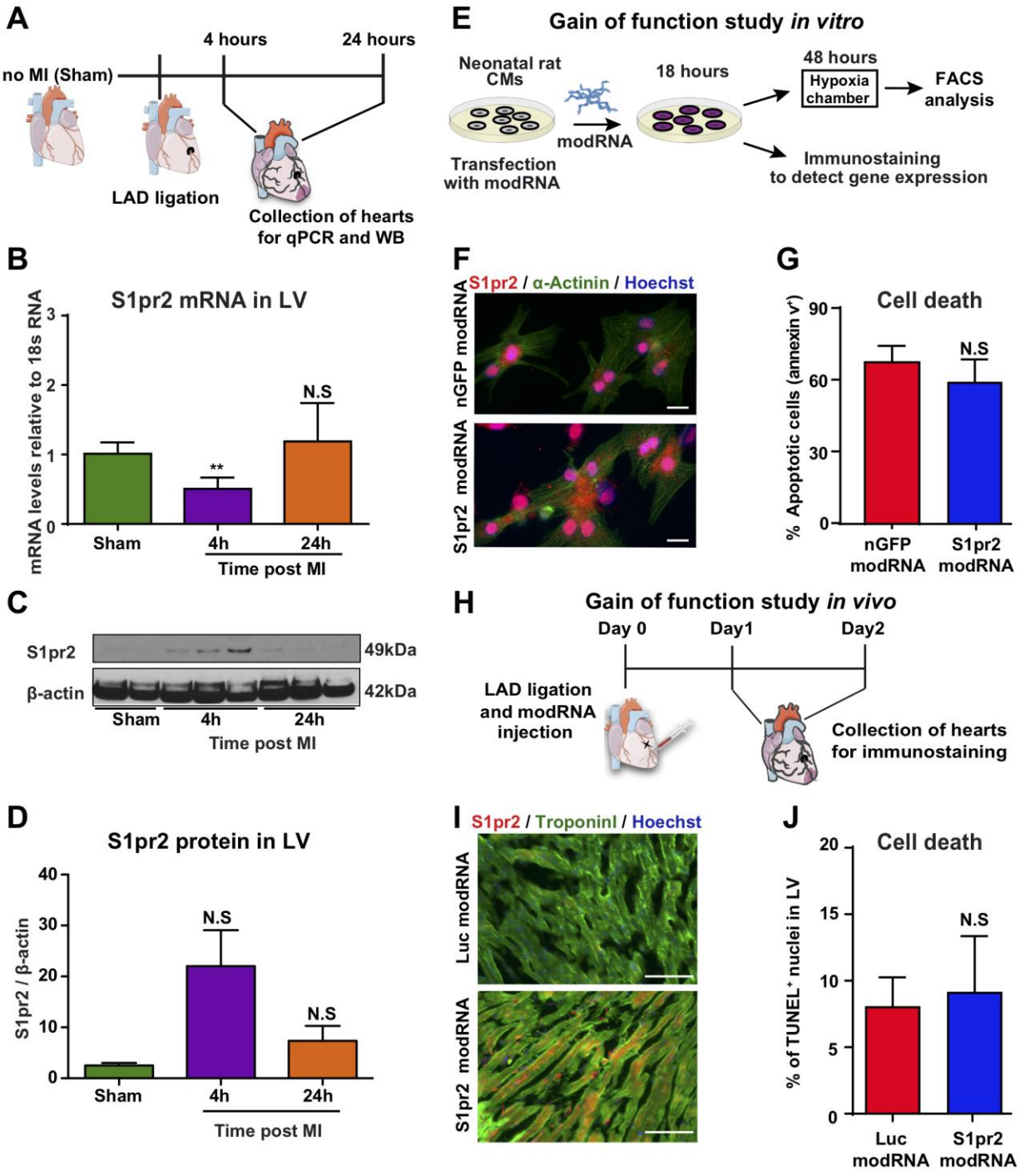


Supplementary Figure S5. Effect of AC modRNA on heart function and CM size post MI. A, Representative images and quantificarion of area at risk from the left ventricle (AAR/LV). The blue-stained areas represent nonischemic tissue, and red stained areas represent the area at risk. Pale areas indicate infarct areas (n=4). **B&C,** Percentage of fractional shortening of LV at 2 and 28 days after MI and modRNA injection. **C-D,** WGA staining was performed 28 days post MI and modRNA injection to assess CM size. **,P<0.01, *, P<0.05, NS, not significant; n=6, one-way ANOVA, Bonferroni post-hoc tests. Scale bar 50μm.



Supplementary Figure S6. Effect of AC overexpression on cell death, global gene expression and composition of immune cell populations in the LV after MI. A,

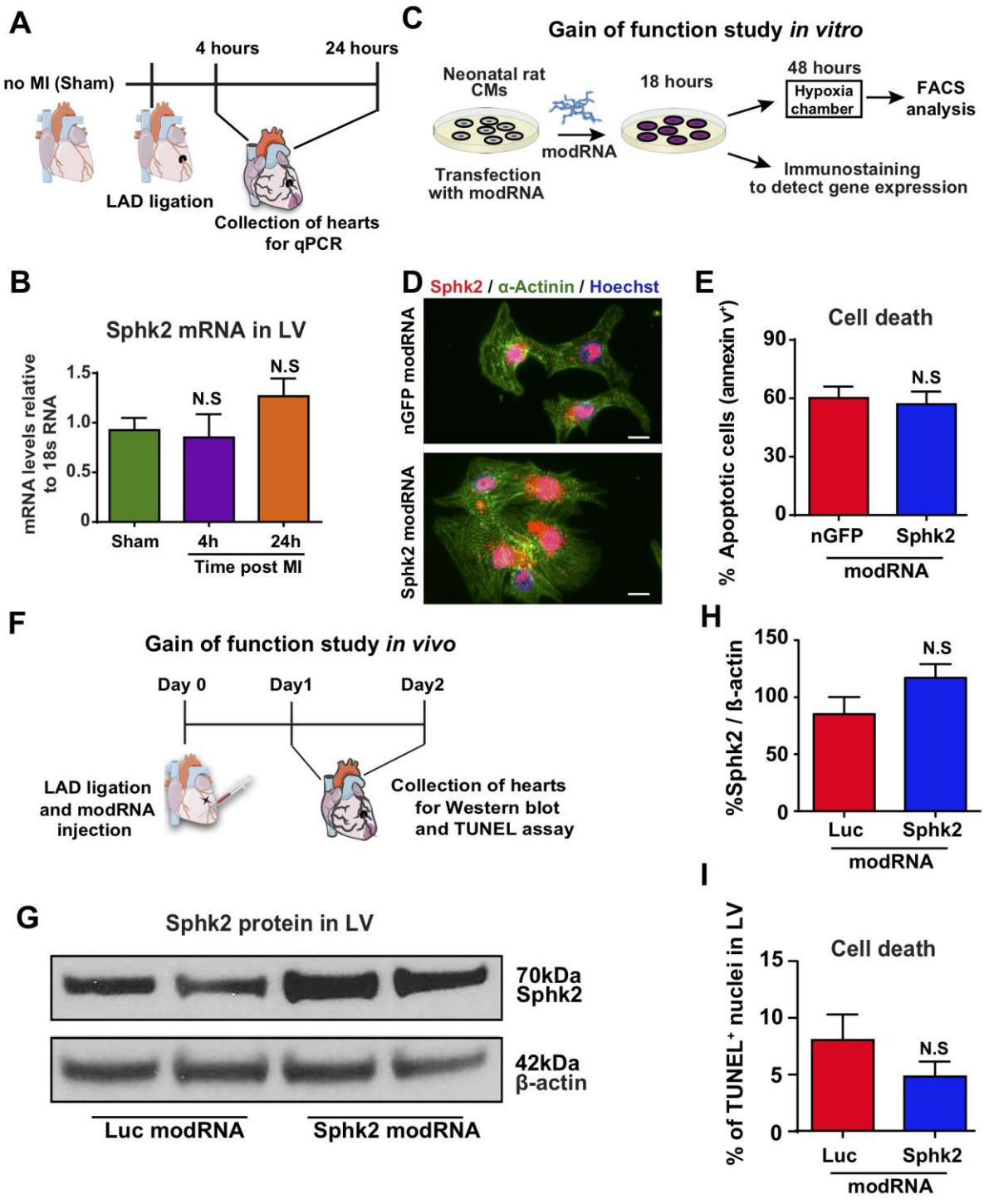
Effects of Luc or AC modRNA-driven overexpression on caspase 3 cleavage in the LV 24 hours post MI. **B,** Volcano plot of the LV transcriptome of mice treated with Luc or AC modRNA; data obtained 4, 24 and 48 hours post MI. **C,** GO enrichment analysis for genes displaying significant (p<0.05) differences in expression between AC modRNA-treated and Luc modRNA-treated hearts 48 hours post MI. **D,** Hierarchical clustering dendrogram of genes upregulated in Luc modRNA-treated mice relative to sham-operated mice 48 hours post MI and genes downregulated in AC modRNA-treated hearts relative to Luc modRNA-treated hearts 48 hours post MI, plus hierarchical clustering dendrogram of the partial transcriptome for sphingolipids 48 hours post MI. **E-F,** Flow cytometric quantification of immune cell populations in the infarct zone of Luc modRNA and AC modRNA-treated hearts at 7 days (**E)** or 14 days (**F**) post MI includes neutrophils, total macrophages, $MHCII^-$ and $MHCII^+$ macrophage subpopulations and $Ly6C^{low}$ and $Ly6C^{hi}$ monocytes. n=2,4,4 (B-D), n=9-12 (E) n=11 (F), Two-tailed Student's *t*-tests (F).



Supplementary Figure S7. Effect of S1pr2 overexpression on cardiac cell death.

A, To analyze changes in S1pr2 receptor expression, hearts were harvested from sham-operated mice or mice 4 hours or 24 hours post MI. Quantification of S1pr2 mRNA (**B**) and protein levels (**C&D**) in the LV of hearts harvested from sham-operated

mice or from mice 4 or 24 hours post MI. **E,** To investigate the effect of S1pr2 overexpression on nrCM cell death, cells were transfected with modRNAs encoding nGFP or S1pr2. **F,** 18 hours post transfection, cells were fixed and immunostained to confirm modRNA translation. **G,** Cell death assessment by FACS analysis of Annexin V^+ cell content within modRNA-transfected cells after 48 hours in a hypoxia chamber. **H,** modRNAs encoding Luc or S1pr2 were injected into mouse hearts at the time of MI. **I,** To confirm S1pr2 overexpression, hearts were collected 24 hours post MI and immunostained. **J,** To evaluate cell death, TUNEL assays were performed on heart tissue collected 48 hours post MI and modRNA injection. **, $P<0.01$, n=4 (B), n=2,3,3 (C&D), n=3 (G&J), n=3 (J). One-way ANOVA, Tukey's Multiple Comparison Test (B&D), Two-tailed Student's t-test (G&J). Scale bar 10μm (F) and 50μm (I).



Supplementary Figure S8. Effect of Sphk2 overexpression on cardiac cell death in ischemia *in vitro* and after MI. A-B, Sphk2 mRNA level was measured using qPCR in hearts harvested from either sham-operated mice or mice 4 and 24 hours post MI. **C,**

nrCMs were transfected with modRNAs for nGFP or Sphk2. **D,** 18 hours post transfection, the cells were fixed and immunostained to confirm modRNA translation. **E,** Cell death assessment of nGFP modRNA- or Sphk2 modRNA-transfected cells by FACS analysis of Annexin V^+ cells after 48 hours in a hypoxia chamber. **F,** modRNAs for Luc or Sphk2 were injected into mouse hearts at the time of MI. **G-H,** 24 hours post MI and modRNA injection, hearts were collected and Sphk2 protein levels were measured using WB. **I,** 48 hours after MI and modRNA injection, hearts were harvested to evaluate cell death using TUNEL assay. NS, not significant, n=4 (B&I), n=3 (E), n=2 (H), n=6 (I). One-way ANOVA, Tukey's Multiple Comparison Test (B), Two-tailed Student’s t-test (E, H&I). Scale bar 5μm.

4. Supplementary Movies

Supplementary Movie 1. Cine MRI of infarcted heart transfected with Luc modRNA one month post MI.

Supplementary Movie 2. Cine MRI of infarcted heart transfected with AC modRNA one month post MI.

5. Author Contributions

Y.H. designed and carried out most of the experiments, analyzed most of the data and wrote the manuscript; A.S.V. developed and performed AC activity assays, prepared samples for sphingolipid analysis and revised the manuscript; E.Y. performed experiments, prepared modRNAs and revised the manuscript; M.M.Z. performed and analyzed experiments regarding immune cell composition in the infarcted heart using FACS, wrote the manuscript and revised the manuscript; E.C. carried out all surgery and echocardiography on mice; N.S. and M.T.K.S prepared modRNAs; R.K. performed qPCR; A.A.K. cut and stained sections; A.M. isolated nrCM, stained slides and revised the manuscript; K.K. and N.G. performed western blots; A.F. analyzed MRI data; M.G.K. revised the manuscript; N.H. cut and stained tissues; E.K. provided advice and analyzed RNAseq data; N.C.G. generated human embryonic stem cell-derived cardiomyocytes; E.S. and R.H. provided advice and revised the manuscript; E.E. designed experiments, analyzed data and wrote the manuscript; L.Z. designed experiments, analyzed data and wrote the manuscript.