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Irisin promotes cardiac homing of intravenously delivered MSCs and protects against ischemic heart injury

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Experimental methods

Animals

Animal experiments were approved by the Animal Care and Use Committee of the Fourth Military Medical University. All experiments were performed in strict accordance with the National Institutes of Health (NIH) guidelines on the Use of Laboratory Animals (NIH publication no. 85-23, revised 2011). EGFP-expressing transgenic mice on a C57BL/6J background were purchased from The Jackson Laboratory (Stock 003291) [1]. TdTomato-expressing transgenic rats on a Sprague-Dawley background were purchased from Shanghai Model Organisms (NR-KI-190007). Adult (10–12 weeks) C57BL/6J mice, adult (10–12 weeks) Sprague-Dawley rats, and Sprague-Dawley pups (1–2 days) were purchased from the Laboratory Animal Center of the Fourth Military Medical University.

Materials

Recombinant irisin (human, mouse, rat) in monomer was purchased from Aviscera Bioscience (00170-01-10). Recombinant mouse ANGPTL4 was purchased from MedChemExpress (HY-P7508). The lipophilic dye collagenase type I (17018029), collagenase type II (17101015), and CM-DiI (C7001) were purchased from Thermo Fisher Scientific. The cell plasma membrane staining kit with DiO was purchased from Beyotime Biotechnology (C1993S). The red blood cell lysis buffer was purchased from TianGen BioTech (RT122-02).

Animal study protocol

We used a modified surgical model of MI/R for more complete killing of the ischemic zone and greater reproducibility. Mice or rats were anesthetized by inhalation of 1–2% isoflurane, and left anterior descending coronary artery ligation surgery was performed as described previously [2]. After 120 minutes of MI, the slipknot was

released, and the myocardium was reperfused. One day after MI/R, echocardiography was performed. MI/R animals with an LVEF>45% were excluded from the study. Each mouse was injected via the vena angularis with 5×10^5 ADSCs suspended in 100 μL of PBS (containing 0.2 mM EDTA, pH=7.3) at 1 day, 8 days, 15 days, 22 days, and 29 days after the MI/R operation. Each rat was injected via the vena sublingualis with 5×10^6 ADSCs suspended in 300 μL of PBS (containing 0.2 mM EDTA, pH=7.3) at 1 day, 8 days, 15 days, 22 days, and 29 days after the MI/R operation. MI/R control animals received only a saline injection. Sham control animals were subjected to all surgical procedures except for coronary artery ligation.

For the MI/R mouse model, adult male C57BL/6J mice were randomly assigned to the following groups: Sham, MI/R+vehicle, MI/R+ADSC-vehicle (CM-DiI-labeled ADSCs treated with vehicle), and MI/R+ADSC-irisin (CM-DiI-labeled ADSCs treated with 100 ng/mL irisin for 24 hours and then washed with PBS). For CSF2/CSF2RB loss-of-function studies, one group of adult male mice were intramyocardially injected with AAV9-control ($CSF2^{WT}$) or AAV9-CSF2-shRNA ($CSF2^{KD}$) one month before they were divided into the following groups: $CSF2^{WT}$ +sham; $CSF2^{WT}$ +MI/R; $CSF2^{WT}$ +MI/R+ADSC-irisin; $CSF2^{KD}$ +sham; $CSF2^{KD}$ +MI/R; and $CSF2^{KD}$ +MI/R+ADSC-irisin. Another group of mice were randomly subjected to the following five groups: MI/R+ADSC-vehicle (ADSCs were treated with vehicle); MI/R+ADSC-irisin-$CSF2RB^{WT}$ (ADSCs were treated with irisin after they were transfected with scRNA); MI/R+ADSC-irisin-$CSF2RB^{KD}$ (ADSCs were treated with irisin after they were transfected with siRNA against CSF2RB); MI/R+ADSC-irisin+IgG (IgG was intravenously injected with ADSC-irisin); and MI/R+ADSC-irisin+CSF2-BA (the blocking antibody of CSF2 was intravenously injected with ADSC-irisin).

For the MI/R rat model, adult male and female Sprague-Dawley rats were randomly assigned to the following groups: Sham; MI/R+vehicle; MI/R+rADSC-vehicle; and MI/R+rADSC-irisin.

ADSC preparation

ADSCs were isolated from adult male C57BL/6J mice and adult male Sprague-Dawley rats as we previously described [3]. Briefly, inguinal subcutaneous adipose tissue was removed under anesthesia. The adipose tissue was rinsed several times with phosphate buffered saline (PBS). Blood vessels were excised under a dissection microscope. The remaining adipose tissue was cut into fine pieces, digested with 0.1% collagenase type I at 37°C for 60 minutes, and centrifuged at 600 g for 10 minutes. After red blood cell lysis with 1× lysis buffer, the cells were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and F12 medium containing 10% fetal bovine serum (FBS) and penicillin-streptomycin. Six hours after cell plating, the medium was changed to remove nonadherent cells. Adherent cells were cultured in DMEM-F12-10% FBS and split several times for expansion. Cells from passages 2–3 were used in all experiments.

Adenovirus construction and transfection

The recombinant FNDC5 adenovirus was constructed by Likely Biotechnology (Beijing, China). Cells were infected with the FNDC5 adenovirus or adenovirus containing empty plasmids (control) for 24 hours at a multiplicity of infection (MOI) of 20–200. The medium was then replaced with fresh medium, and the cells were cultured for another 24 hours.

Adeno-associated virus serotype-9 construction and intramyocardial injection

Adeno-associated virus serotype-9 carrying CSF2 shRNA (AAV9-CSF2-shRNA) or scramble RNA (AAV9-control) was purchased from GenePharma. One month

before the MI/R operation, AAV9-CSF2-shRNA or AAV9-control (2.6×10^{10} vector genomes per mouse) was intramyocardially injected into the left ventricle free wall at three different sites. The experiments were performed by an investigator who was blinded to the group allocations.

Small interfering RNA (siRNA)-mediated gene knockdown

ANGPTL4 siRNA, CSF2RB siRNA, SOD2 siRNA, and their scramble RNAs were purchased from GenePharma. When ADSCs reached 80% confluence, siRNAs were transfected into the cells with RNAiMAX Transfection Reagent (Thermo Fisher Scientific, 13778075) according to the manufacturer's protocol (final siRNA concentration: 100 nM). After 8 hours of incubation (37°C), the transfection reagent-siRNA mixture was replaced with fresh growth medium. Successful knockdown was confirmed by Western blot analysis of the ANGPTL4, CSF2RB, and SOD2 protein levels.

ADSC labeling

Cultured ADSCs were labeled with the lipophilic dye CM-DiI (5 μM in DMEM-F12-10% FBS) for 20 minutes immediately before intravenous injection. ADSCs were also stained with cell plasma membrane staining kit with DiO, according to the manufacturer's protocol.

Flow cytometric analysis of the cardiac homing of ADSCs

The cardiac homing of ADSCs was evaluated by flow cytometry 1 day after the fifth intravenous injection (30 days after MI/R). Mice were heparinized (10 IU/g body weight), and 5 minutes later, they were anesthetized with an intraperitoneal injection of ketamine/xylazine mixture (100 mg/kg ketamine plus 10 mg/kg xylazine). Single cells were then enzymatically isolated from the hearts in 0.1% collagenase type II (30 minutes at 37°C) as we previously described [4]. A 'myocyte-depleted' cardiac cell

population was prepared by filtering the single cells through a 40-μm mesh and was then stained with a PE/Cy5 anti-mouse/rat CD29 antibody (#102219, Biolegend). A PE/Cy5 Armenian hamster IgG isotype control antibody (#400909, Biolegend) was used as the negative control. The cardiac homing of ADSCs (CM-DiI+/CD29+) was determined with a flow cytometer (Epics MCL, Beckman).

The *in vivo* competitive homing assay

The competitive homing assay [5] was performed after a single intravenous injection (1 day after MI/R), and the distribution of ADSCs in organs was evaluated by flow cytometry. Vehicle-treated ADSCs (ADSC-vehicle) were labeled with DiO (a green dye) and irisin-treated ADSCs (ADSC-irisin) were labeled with CM-DiI (a red dye). Equal number of ADSC-vehicle-DiO and ADSC-irisin-DiI were then mixed before they were intravenously injected into the post-MI/R mice. An aliquot of the cell mixture was kept and analyzed by flow cytometry. The mice were sacrificed 1 day after intravenous injection (2 days after MI/R). The hearts, lungs, and spleens were removed from the mice. The hearts were prepared for the 'myocyte-depleted' cardiac cell population. The lungs were minced and then digested in 0.1% collagenase type II (20 minutes at 37°C). Spleens were homogenized into a single-cell suspension using PBS. After red blood cell lysis with 1× lysis buffer, the lung cells and splenocytes were resuspended in PBS. Flow cytometry assays were performed to detect ADSC-vehicle-DiO and ADSC-irisin-DiI in the 'myocyte-depleted' cardiac cell population and single-cell suspensions of lung and spleen.

Echocardiography

M-mode images of mice and rats subjected to 1–2% isoflurane anesthesia were obtained via a VisualSonics 770 echocardiography machine (Canada) as previously described [6]. Hearts were viewed along the long and short axes between the two

papillary muscles. The LV end-systolic diameter (LVESD) and LV end-diastolic dimension (LVEDD) were measured. The LVEF was automatically calculated by echocardiography software as follows: EF (%)=100×[($LVEDD^3$-$LVESD^3$)/$LVEDD^3$]. Measurements were performed for at least five separate cardiac cycles per mouse. Echocardiography was performed by a single experienced operator in a blinded fashion.

Evaluation of angiogenesis

For the evaluation of angiogenesis, heart sections were deparaffinized and subjected to antigen retrieval in hot citric acid buffer. After cooling, the slides were permeabilized with 0.2% Triton-100 for 15 minutes, blocked with 1% BSA in PBS for 2 hours, and incubated with an anti-CD31 primary antibody at 4°C overnight (#GB13063, Servicebio). A donkey anti-goat antibody conjugated with CY3 (#GB21404, Servicebio) served as the secondary antibody. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, GB1012, Servicebio). Images of immunostained sections were acquired with a Nikon Eclipse C1 microscope and Nikon DS-U3 camera. The myocardial capillary density was quantified by Image-Pro Plus 6.0 software (Media Cybernetics). The representative image for each group was selected based upon the mean value.

Determination of apoptosis

ADSC apoptosis was determined by TUNEL staining with a One Step TUNEL Apoptosis Assay Kit (Beyotime, C1090). Images were acquired with a Nikon Eclipse Ni microscope and Nikon DS-Ri2 camera. Apoptosis of cardiomyocytes in heart tissue was determined with a Roche In Situ Cell Death Detection Kit (Sigma, 11767305001 and 11767291910) according to the manufacturer's protocol. Images were acquired with a Nikon Eclipse C1 microscope and Nikon DS-U3 camera. The index of apoptosis

was determined by the number of TUNEL-positive nuclei/total nuclei. The representative image for each group was selected based on the mean value.

Masson's trichrome staining

Hearts were harvested from anesthetized mice and rats and embedded in paraffin. The heart tissue extending from just distal to the coronary ligation point to the apex was separated into different segments at 200 μm intervals. Serial 5-μm-thick sections were obtained from each segment for Masson's trichrome staining according to the manufacturer's protocol (Sigma, HT15). Microscopic images of mouse heart sections were obtained with a 1.25× object lens (Nikon, Japan). Images of rat heart sections were acquired with a Nikon Eclipse C1 microscope and Nikon DS-U3 camera. For quantification, measurements of 5 transverse heart sections were analyzed. Fibrotic size was determined as the average ratio of the fibrotic area to the LV area (fibrotic size %) with Image-Pro Plus 6.0 software (Media Cybernetics).

CM collection

We employed a modified method to prepare the CM from ADSCs [3]. CM was generated as follows: after growth to 90% confluence in 6-well dishes, ADSCs were pretreated with 100 ng/mL irisin or vehicle for 1 day. The culture medium was washed and replaced with serum-free DMEM/F12 medium. Twenty-four hours later, the serum-free DMEM/F12 medium was collected and centrifuged at 1000 g for 10 minutes to obtain the supernatant (CM).

***In vitro* cardiomyocyte apoptosis assay**

Both primary NRVCs and iPSC-CMs were used for the *in vitro* cardiomyocyte apoptosis assay. Primary cultures of NRVCs from 1- to 2-day-old Sprague-Dawley pups were prepared as described previously [7]. Human iPSC-CMs were purchased from HELP Therapeutics (#NC20010435). The differentiation and preparation of iPSC-CMs

have been described previously [8]. For the evaluation of cardiomyocyte apoptosis, cultured NRVCs and iPSC-CM were subjected to 6 hours of H_2O_2 (200 μM) before treatment with recombinant irisin (100 ng/mL), ANGPTL4 (2 μg/mL), or CM derived from ADSCs.

Capillary-like tube formation assay

rCAECs were purchased from Procell (CP-R081, Wuhan, China) and cultured in low-glucose DMEM containing endothelial cell growth supplement (#211-GS, Cell Applications), 10% FBS, and penicillin-streptomycin. For the tube formation assay, Matrigel (BD Biosciences) was added to each well of a 48-well plate and allowed to polymerize at 37°C for 30 minutes. rCAECs were treated with CM derived from ADSCs for 24 hours. Images of the formation of capillary-like structures were obtained by computer-assisted microscopy. The total length per field was calculated from five random fields.

Immunohistochemistry

For fixed tissues, wax blocks were cut into 5-μm-thick sections and mounted on glass slides for staining. The slides were deparaffinized and subjected to antigen retrieval in hot citric acid buffer. After cooling, the slides were permeabilized with 0.2% Triton-100 for 15 minutes, blocked with 1% BSA in PBS for 2 hours, and incubated overnight at 4°C with an anti-troponin T mouse monoclonal antibody (Thermo Fisher Scientific, MS-295-P0) (1/1,000). The primary antibody was visualized with a donkey anti-mouse IgG (H+L) secondary antibody conjugated with Alexa Fluor 488 (A77440, Yeasen, China). The frozen sections were fixed in acetone at 4°C for 15 min. The sections were blocked with PBS containing 1% BSA at room temperature. Sections were then incubated overnight at 4°C with an anti-TNNT2 rabbit polyclonal antibody (Affinity Biosciences, DF6261). The primary antibody was visualized with a goat anti-

rabbit IgG (H+L) secondary antibody conjugated with Alexa Fluor 488 (33106ES60, Yeasen, China). Nuclei were stained with DAPI (GB1012, Servicebio). Images were acquired with a Nikon Eclipse C1 microscope and Nikon DS-U3 camera.

RNA sequencing analysis

Differential gene expression analysis was performed using RNAseq at Shanghai Biotree Biological Technology [9]. After treatment with vehicle or irisin for 24 hours, total RNA was extracted from ADSCs via an RNeasy Mini Kit (Qiagen, 74106) according to the manufacturer's protocol. RNA purity was assessed using a NanoPhotometer® spectrophotometer (Implen). RNA integrity was assessed using the RNA Nano 6000 Assay Kit on the Bioanalyzer 2100 system (Agilent Technologies).

A total of 1 μg of RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using the NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) according to the manufacturer's recommendations, and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature conditions in NEBNext First Strand Synthesis Reaction Buffer (5X). First-strand cDNA was synthesized using random hexamer primers and M-MuLV Reverse Transcriptase (RNase H-). Second-strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. The remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of the 3' ends of the DNA fragments, an NEBNext adaptor with hairpin loop structures was ligated to prepare for hybridization. For the preferential selection of cDNA fragments 250~300 bp in length, the library fragments were purified with the AMPure XP system (Beckman Coulter, Beverly, USA). Then, 3 μL of USER Enzyme (NEB, USA) was incubated with size-

selected, adaptor-ligated cDNA for 15 minutes at 37°C and then at 95°C for 5 min prior to performing PCR. Then, PCR was performed with Phusion High-Fidelity DNA polymerase, universal PCR primers and Index (X) Primer. Finally, the PCR products were purified (AMPure XP system), and library quality was assessed on the Agilent Bioanalyzer 2100 system. The library preparations were sequenced on an Illumina NovaSeq platform, and 150 bp paired-end reads were generated.

Differential expression analysis was performed using the DESeq2 R package (1.16.1). DESeq2 provides statistical routines for determining differential expression among digital gene expression data using a model based on the negative binomial distribution. The resulting P-values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value <0.05 found by DESeq2 were assigned as differentially expressed.

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the clusterProfiler R package, during which gene length bias was corrected. GO terms with corrected P values less than 0.05 were considered to be significantly enriched by the differentially expressed genes. The KEGG database is a resource for understanding the high-level functions and utilities of biological systems, such as cells, organisms and ecosystems, based on molecular-level information, especially from large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (http://www.genome.jp/kegg/). We used the clusterProfiler R package to assess the statistical enrichment of differentially expressed genes in KEGG pathways.

Quantitative PCR

Total RNA was extracted from cells via an RNeasy Mini Kit (Qiagen, 74106) according to the manufacturer's protocol. cDNA was prepared from 1 μg of total RNA

using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific, 18080051) according to the manufacturer's protocol. PCR was performed via a 7900HT Fast Real-Time PCR System (Applied Biosystems). Samples were analyzed in triplicate in 10 μL reactions according to the SYBR Green PCR Master Mix protocol (Thermo Fisher Scientific, 4472908). Primers were purchased from TsingKe (listed in Table IV in the Data Supplement). β-actin served as the housekeeping gene. Data were normalized via the standard comparative CT method.

The thermal cycling conditions were as follows: denaturation at 95°C for 5 minutes, followed by 40 cycles of 10 s at 95°C, 20 s at 55°C, and 20 s at 72°C.

Western blot analysis

Total proteins were isolated from cells or heart tissues with 1× lysis buffer (CST #9803) supplemented with a protease inhibitor cocktail (Thermo Fisher Scientific, 78438). A total of 30–70 μg of protein per sample was separated via gel electrophoresis, transferred to a polyvinylidene fluoride membrane, and blocked with 5% milk for 1 hour. The membrane was incubated overnight with primary antibodies at 4°C. After incubation with a secondary HRP-conjugated anti-mouse antibody (Abbkine, A21010, 1/10,000) or anti-rabbit antibody (Abbkine, A21020, 1/10,000) at room temperature for 2 hours, the membranes were exposed to enhanced chemiluminescent (ECL) substrate (Thermo Fisher Scientific, 34096). The Western blot results were quantified by densitometry (Image Lab).

The following primary antibodies were used in this study: anti-FNDC5/irisin antibody (SinoBiogical, 106683-T08); anti-CSF2RB antibody (Santa Cruz, sc-393281); anti-CSF2 antibody (Santa Cruz, sc-32753); anti-cleaved caspase-3 antibody (CST #9661); anti-caspase-3 antibody (CST #9662); anti-phospho-ERK1/2 antibody (CST #9101); anti-ERK1/2 antibody (CST #4695); anti-phospho-Akt^{Ser473} antibody (CST

#4060); anti-AKT antibody (CST #4691); anti-ANGPTL4 antibody (Abcam, ab196746); anti-MCP1 antibody (Affinity Biosciences, DF7577); anti-IL1β antibody (Affinity Biosciences, AF5103); anti-LCN2 antibody (Affinity Biosciences, DF6816); anti-SOD2 antibody (Santa Cruz, sc-133134); anti-TFPI2 antibody (Abcam, ab186747); anti-integrin αV antibody (CST #4711); anti-integrin β1 antibody (CST #34971); anti-integrin β5 antibody (CST #3629); and anti-GAPDH antibody (ProteinTech, 10494-1-AP).

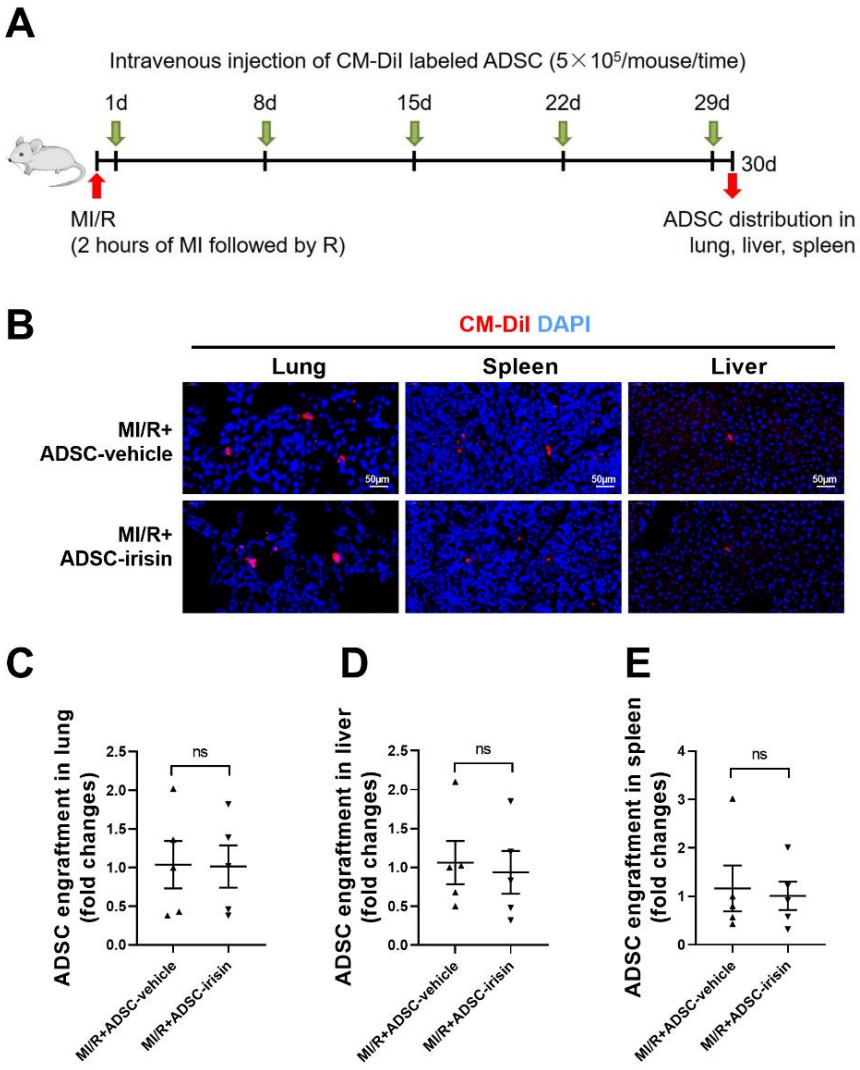

Figure S1. Irisin does not significantly alter the lung, spleen, or liver distribution of intravenously injected ADSCs.

A. Experimental scheme and timeline for *in vivo* mouse studies. At 1, 8, 15, 22, and 29 days after myocardial ischemia/reperfusion (MI/R) operation, 5×10^5 CM-DiI+ ADSCs were infused intravenously via the vena angularis. ADSC distributions in the lung, liver, and spleen were determined 30 days after MI/R.

B. Representative images of CM-DiI-labeled ADSCs in the lung, liver, and spleen 30 days after MI/R. Engrafted ADSCs are CM-DiI positive (red).

C-E. Quantification of CM-DiI-labeled ADSCs in the lung, liver, and spleen (n=5 mice per group).

The data were analyzed by unpaired, 2-tailed Student's t test. ADSC-vehicle, ADSCs treated with vehicle; ADSC-irisin, ADSCs treated with irisin (100ng/mL) for 2 days. ns, not significant.

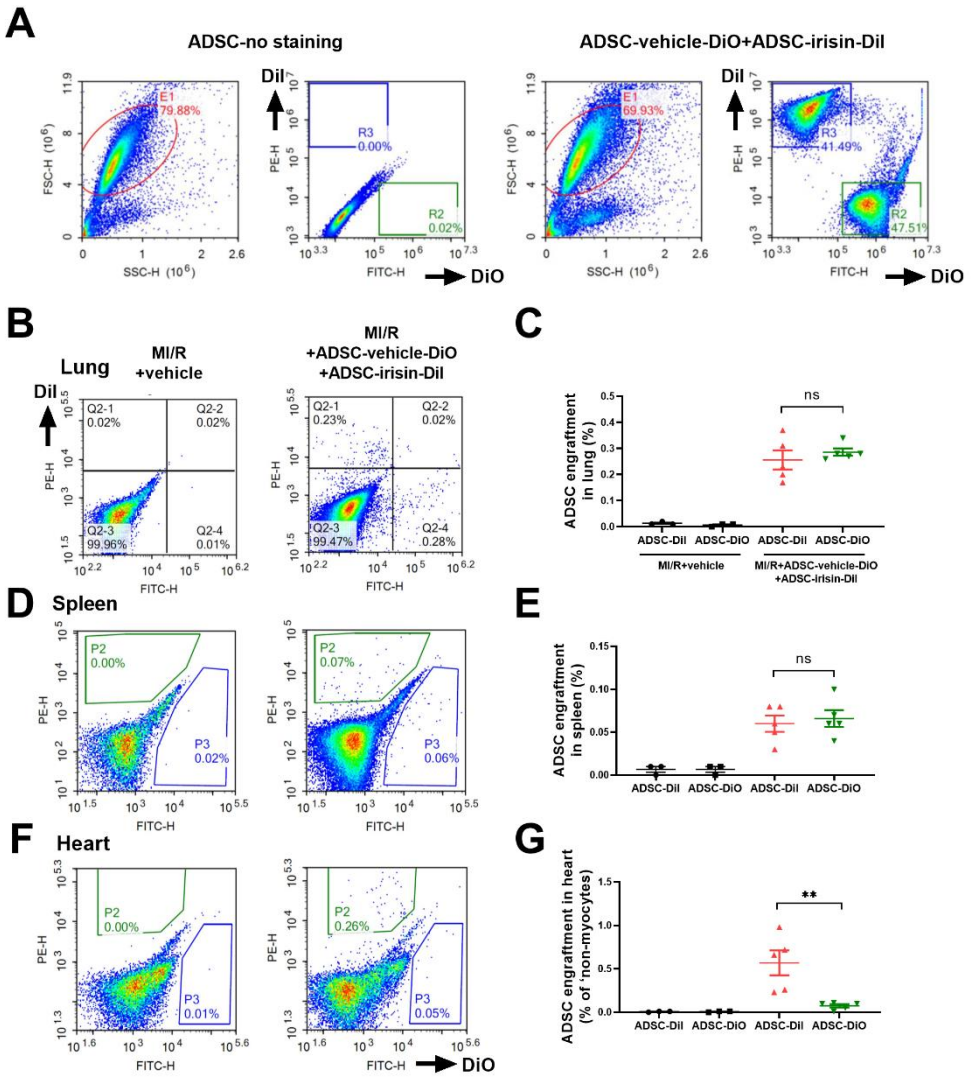

Figure S2. Irisin increases the cardiac engraftment of intravenously delivered ADSCs.

A. Flow cytometric analysis of a cell mixture of ADSC-vehicle with DiO staining (ADSC-vehicle-DiO) and ADSC-irisin with CM-DiI staining (ADSC-irisin-DiI).

B-G. Flow cytometric analysis of ADSC-vehicle-DiO and ADSC-irisin-DiI in lungs (B and C), spleens (D and E), and hearts (F and G) 1 day after intravenous injection (2 days after MI/R). n=3-5 mice per group.

The data were analyzed by one-way ANOVA, followed by a Bonferroni *post hoc* test. **P<0.01; ns, not significant.

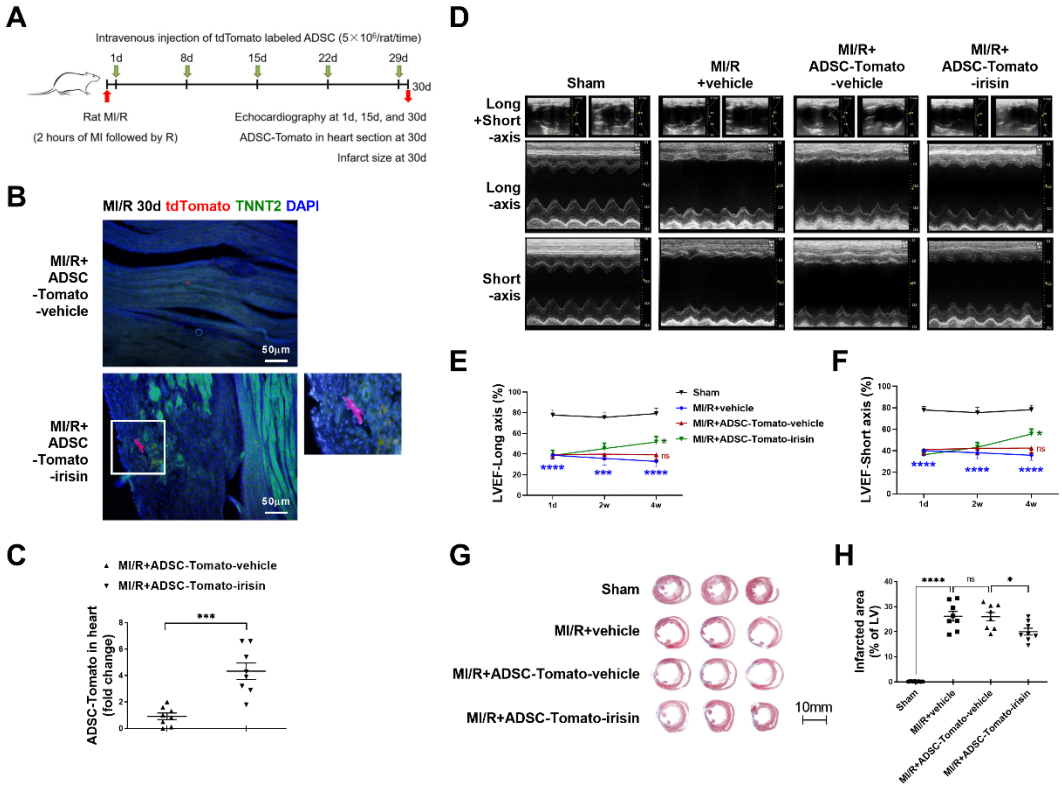

Figure S3. Irisin enhances the post-MI/R cardioprotection of intravenously delivered ADSCs in rats.

A. Experimental scheme and timeline for *in vivo* rat studies. At 1, 8, 15, 22, and 29 days after myocardial ischemia/reperfusion (MI/R) operation, 5×10^6 tdTomato+ ADSCs were infused intravenously (i.v.) injected via the vena sublingualis.

B. Representative images of ADSC-Tomato in the heart 2 days after MI/R. Heart tissue was immunostained for TNNT2 (green) and DAPI (blue). Engrafted ADSCs are tdTomato positive (red). C. Quantification of ADSCs in the peri-infarct area was determined by the number of ADSC-Tomato in a representative section (n=8 mice per group).

D. Representative long-axis and short-axis M-mode echocardiographic images of rats 30 days after MI/R.

E. Left ventricular ejection fraction (LVEF) evaluated by long-axis M-mode echocardiography. F. LVEF evaluated by short-axis M-mode echocardiography. n=6–10. The data were analyzed by 1-way ANOVA, followed by a Bonferroni post hoc test. ****P<0.0001 vs. the Sham group, $^{\#}$P<0.05 vs. the MI/R+ADSC-Tomato-vehicle group.

G. Representative Masson trichrome staining. H. Quantification of the fibrotic area 30 days after MI/R (n=8 rats). The data were analyzed by Kruskal–Wallis test, followed by Dunn *post hoc* tests.

ADSC-Tomato, ADSCs isolated from transgenic rats expressing tdTomato. ADSC-Tomato-vehicle, ADSC-Tomato treated with vehicle; ADSC-Tomato-irisin, ADSC-Tomato treated with irisin (100ng/mL) for 2 days. ns, not significant.

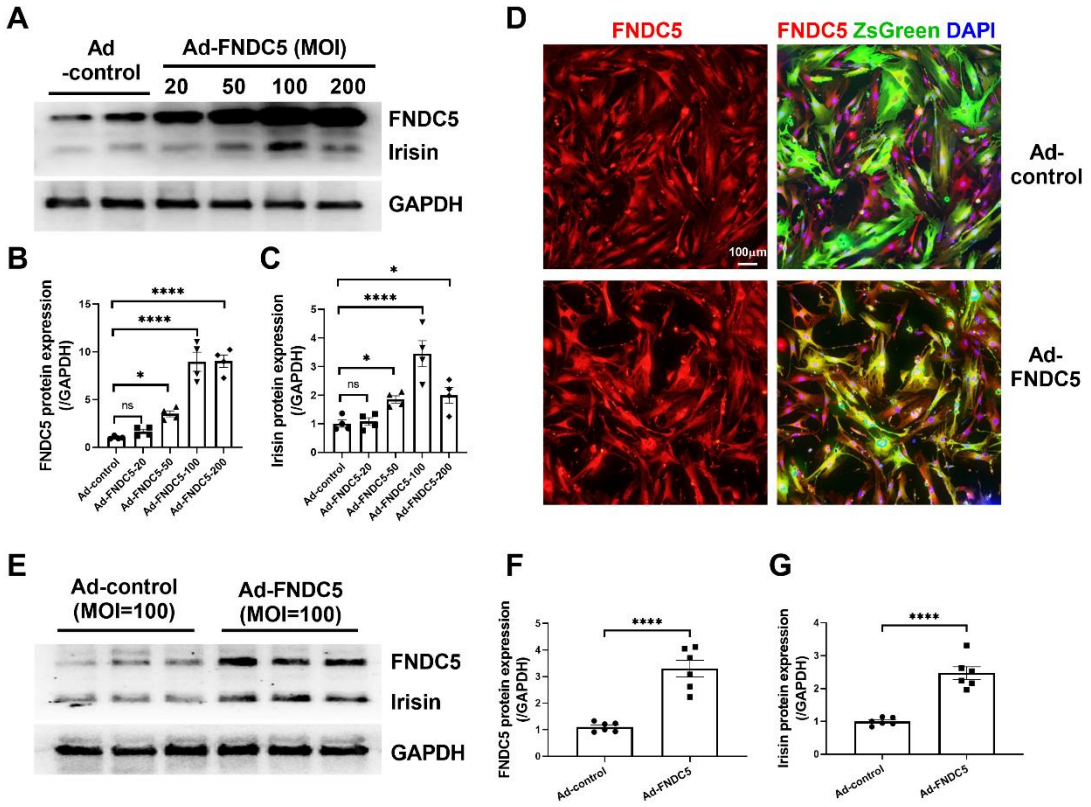

Figure S4. Adenoviruses harboring FNDC5 increase FNDC5 and irisin protein expression in ADSCs.

A-C. Western blot (A) and quantification of FNDC5 (B) and irisin (C) in mouse ADSCs 2 days after transfection with control adenovirus (Ad-control) or adenovirus harboring FNDC5 (Ad-FNDC5). Multiplicity of infection (MOI)=20, 50, 100, 200. n=4.

D. FNDC5 immunostaining in mouse ADSCs 2 days after transfection with Ad-control or Ad-FNDC5.

E-G. Western blots (E) and quantification of FNDC5 (F) and irisin (G) in rat ADSCs 2 days after transfection with Ad-control or Ad-FNDC5. MOI=100. n=6.

The data in F and G were analyzed by unpaired, 2-tailed Student's t test. Other data were analyzed by one-way ANOVA, followed by a Bonferroni *post hoc* test. *P<0.05, ****P<0.0001. ns, not significant.

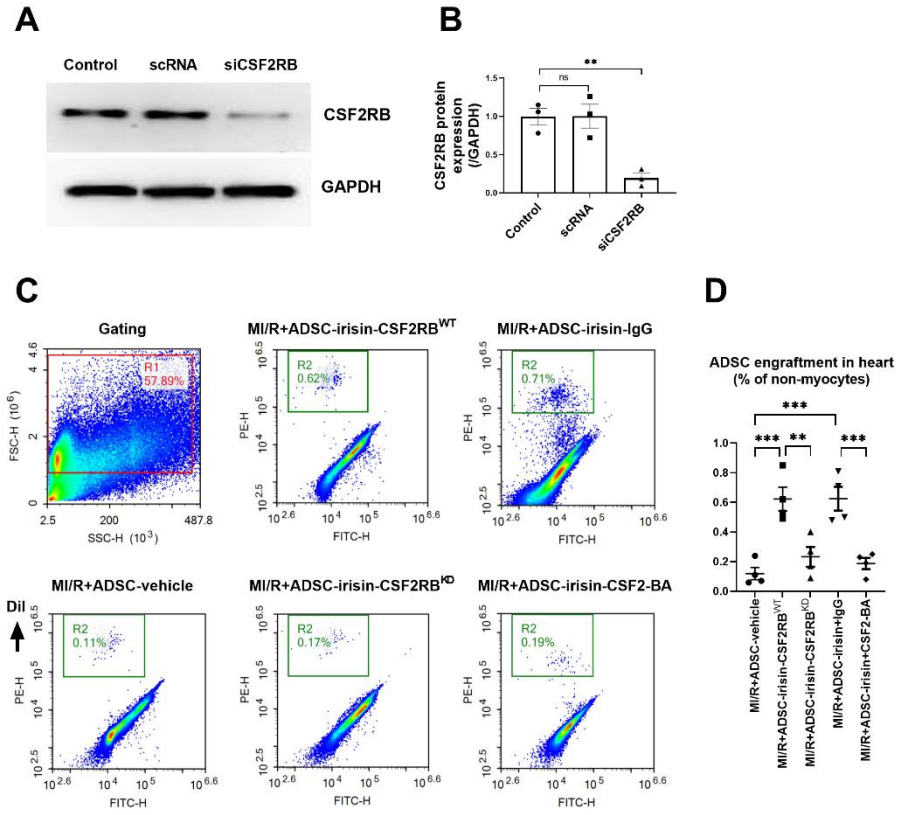

Figure S5. CSF2/CSF2RB is essential for irisin-increased cardiac homing of intravenously delivered ADSCs.

A-B. Western blots and quantification of CSF2RB protein expression in ADSCs. ADSCs were transfected with scRNA or siRNA against CSF2RB (siCSF2RB) for 48 hours. n=3.

C. Flow cytometric analysis of CM-DiI-labeled ADSCs in hearts 1 day after intravenous injection (2 days after MI/R).

D. Myocardial ADSC homing was quantified as the ratio of CM-DiI+ cells to the total number of non-myocytes (n=4 mice).

The data were analyzed by one-way ANOVA, followed by a Bonferroni *post hoc* test. $CSF2RB^{WT}$, CSF2RB scRNA. $CSF2RB^{KD}$, CSF2RB siRNA. CSF2-BA, the blocking antibody of CSF2. **P<0.01, ***P<0.001. ns, not significant.

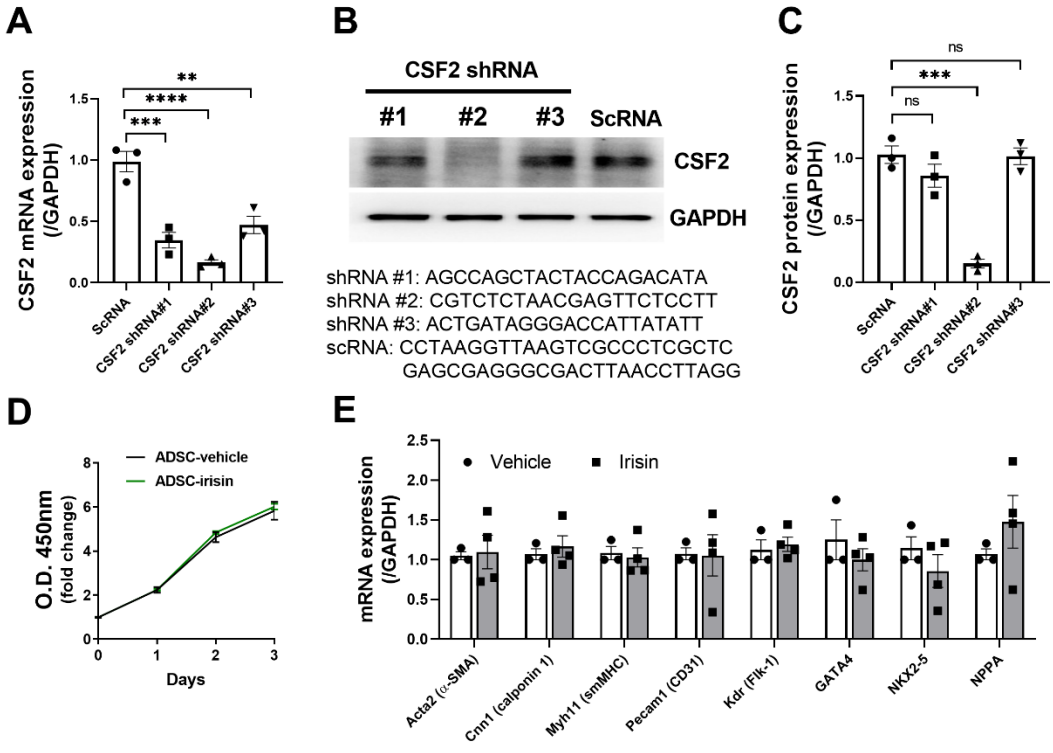

Figure S6.

A. Quantitative PCR analysis of CSF2 mRNA expression in mouse cardiac fibroblasts. Primary cardiac fibroblasts were transfected with 3 CSF2 shRNAs for 36 hours. n=3.

B and C. Western blots and quantification of CSF2 protein expression in mouse cardiac fibroblasts. Primary cardiac fibroblasts were transfected with 3 CSF2 shRNAs for 36 hours. n=3.

shRNA, short hairpin RNA. Data were analyzed by one-way ANOVA, followed by a Bonferroni post hoc test. **P<0.01, ***P<0.001, ****P<0.0001. ns, not significant.

D. ADSC proliferation curves (n=8).

E. Quantitative PCR analysis of fibrogenic (α-SMA, calponin 1, smMHC), vasculogenic (CD31, Flk-1), and cardiogenic (GATA4, NKX2-5, NPPA) gene expression in rat ADSCs treated with vehicle or irisin (100 ng/mL) for 7 days. n=3–4.

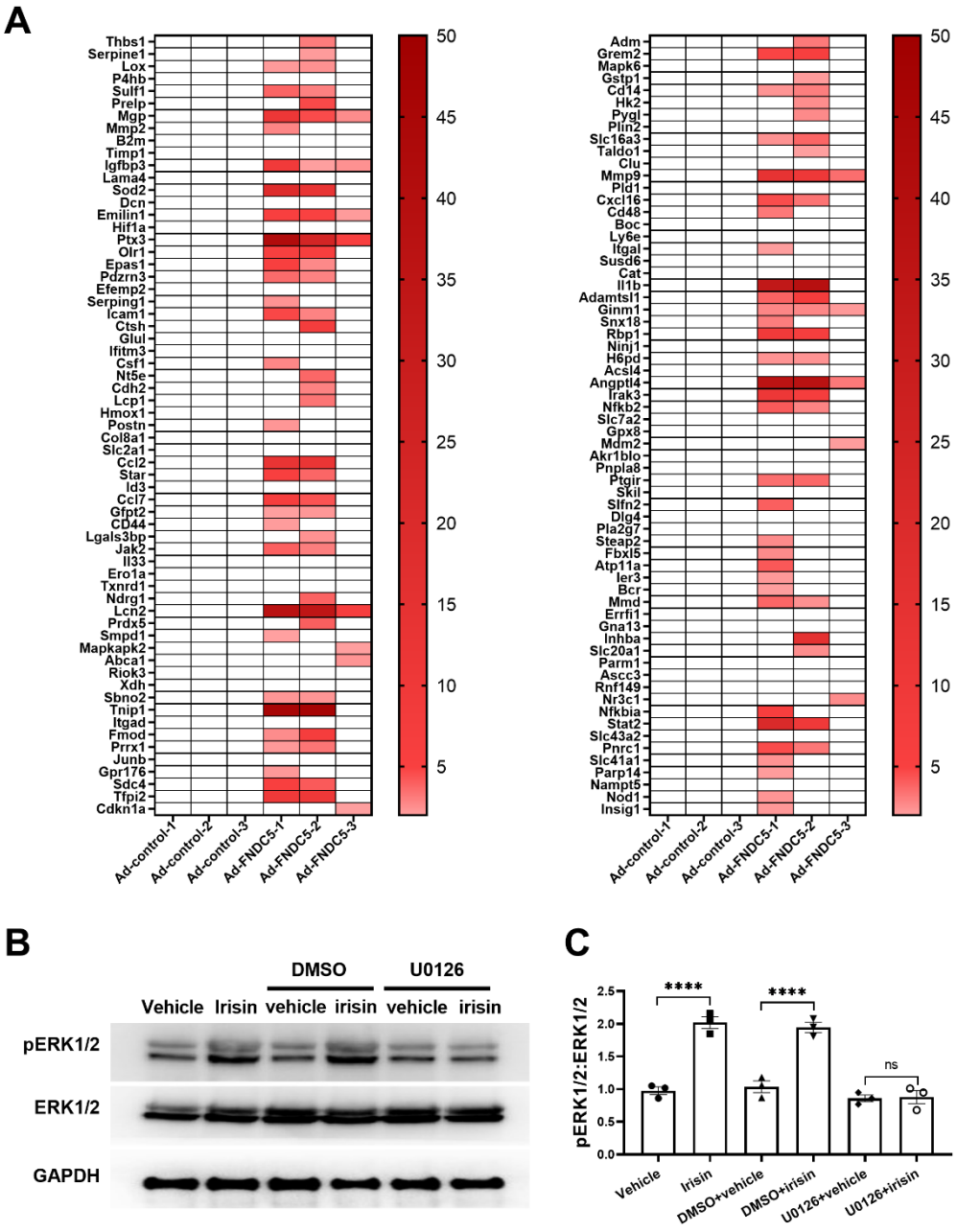

Figure S7.

A. Quantitative PCR analysis of mRNA expression for genes of interest (132 genes in total) in rat ADSCs transfected with adenovirus-control (Ad-control) or adenovirus-FNDC5 (Ad-FNDC5). These 132 genes were increased by irisin, as evidenced by RNA sequencing. n=3.

B and C. Western blots (B) and quantification (C) of pERK1/2:ERK1/2 protein expression in ADSCs. ADSCs were treated with recombinant irisin (100 ng/mL) for 30 minutes. ****P<0.0001; ns, not significant.

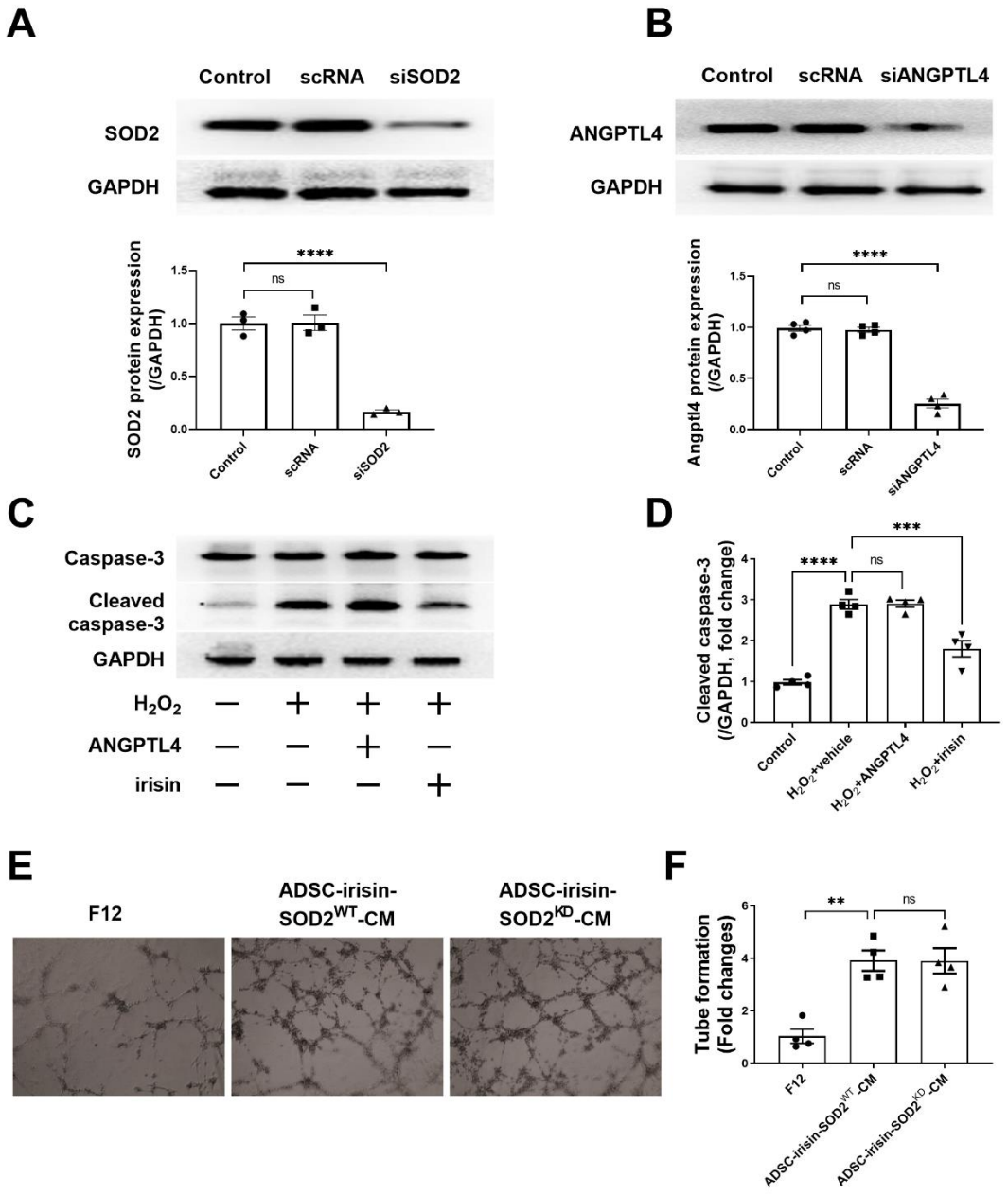

Figure S8.

A. Western blots and quantification of SOD2 protein expression in ADSCs. ADSCs were transfected with scRNA or siRNA against SOD2 (siSOD2) for 48 hours. n=3.

B. Western blots and quantification of ANGPTL4 protein expression in ADSCs. ADSCs were transfected with scRNA or siRNA against ANGPTL4 (siANGPTL4) for 48 hours. n=4.

C and D. Western blots and quantification of cleaved caspase-3 protein expression in NRVCs. NRVCs were treated with recombinant ANGPTL4 (2 μg/mL) or irisin (100 ng/mL) 24 hours before H_2O_2 (200 μM, 6 hours). n=4.

E and F. Tube formation of rCAECs treated with F12, ADSC-irisin-$SOD2^{WT}$-CM, or ADSC-irisin-$SOD2^{KD}$-CM. n=4. $SOD2^{WT}$, SOD2 scRNA; $SOD2^{KD}$, SOD2 siRNA.

**P<0.01, ***P<0.001, ****P<0.0001; ns, not significant. The data were analyzed by one-way ANOVA, followed by Bonferroni *post hoc* test.

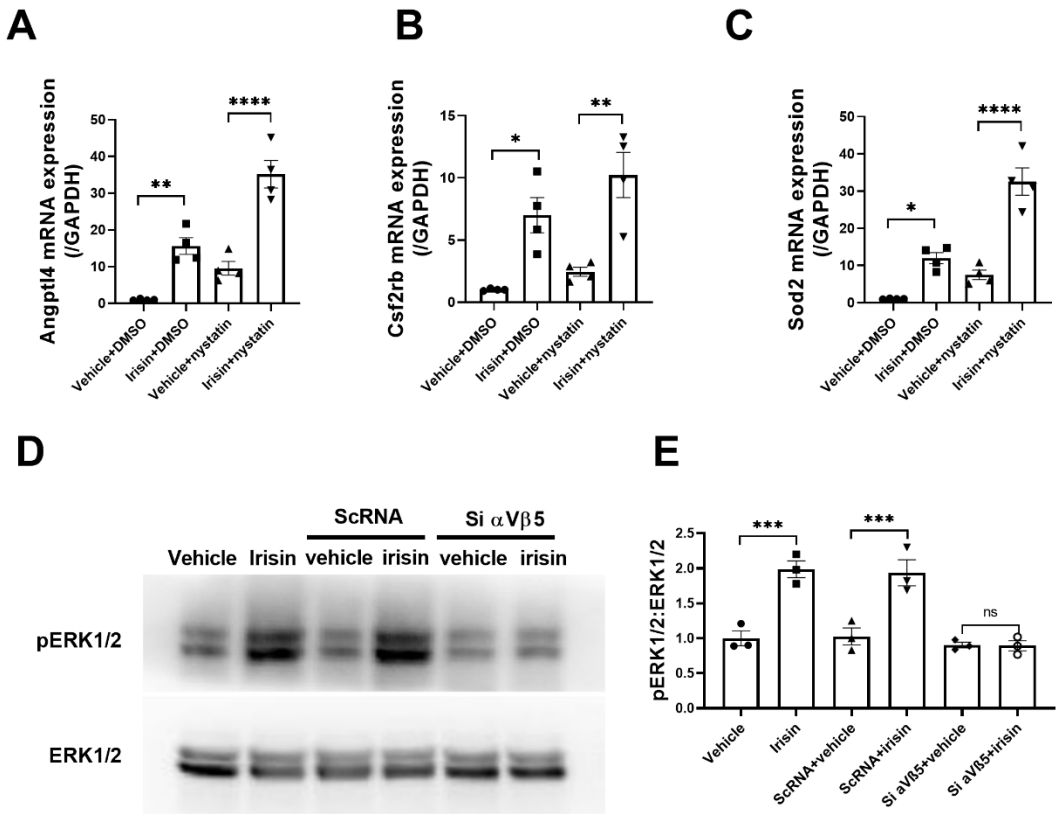

Figure S9.

A-C. Quantitative PCR analysis of Angptl4, Csf2rb, and Sod2 mRNA expression in ADSCs. ADSCs were treated with DMSO or nystatin (5 μM) for 12 hours. n=4.

D and E. Western blots (D) and quantification (E) of pERK1/2:ERK1/2 protein expression in ADSCs. ADSCs were transfected with scRNA or siRNAs against integrin αV and β5 (Si αVβ5) for 48 hours and then treated with recombinant irisin (100 ng/mL) for 30 minutes.

*P<0.05, **P<0.01, ***P<0.0001, ****P<0.0001. The data were analyzed by one-way ANOVA, followed by a Bonferroni *post hoc* test.

Tables

Table I: Genes of the cytokine-cytokine receptor interaction pathway with significantly upregulated expression induced by irisin in rADSCs identified by RNA sequencing (RNAseq) analysis (26 cytokines and 19 cytokine receptors)

Cytokines (Gene ID)	Fold Change (Irisin/Vehicle)	P value	Name
ENSRNOG00000002829	79.14075427	1.15E-06	Ppbp
ENSRNOG00000022242	62.43078777	3.00E-32	Cxcl9
ENSRNOG00000022256	12.27842609	2.57E-32	Cxcl10
ENSRNOG00000022298	119.9730564	6.32E-33	Cxcl11
ENSRNOG00000024899	11.39479722	6.88E-27	Cxcl13
ENSRNOG00000026647	6.610628004	7.37E-67	Cxcl16
ENSRNOG00000028015	4.060679234	1.75E-33	Pf4
ENSRNOG00000011984	3.707080486	4.05E-10	Cxcl14
ENSRNOG00000021851	26.81771201	0.005086	Ccl1
ENSRNOG00000015992	9.680106888	3.32E-07	Ccl20
ENSRNOG00000007159	9.402859941	1.42E-14	Ccl2
ENSRNOG00000011406	6.60562448	2.75E-08	Ccl4
ENSRNOG00000011205	8.384806719	1.43E-10	Ccl3
ENSRNOG00000000239	7.932686519	1.07E-42	Ccl7
ENSRNOG00000010278	51.12247342	7.51E-119	Il6
ENSRNOG00000024390	2.047011185	0.009744	Osm
ENSRNOG00000008525	106.5589005	1.01E-33	Csf3
ENSRNOG00000026805	7.841771392	2.54E-12	Csf2
ENSRNOG00000058856	2.61438999	0.015737	Tslp
ENSRNOG00000007027	8.676243646	4.37E-86	Hgf
ENSRNOG00000004647	2.564628005	1.08E-05	Il10
ENSRNOG00000013269	4.672494347	2.54E-06	Tnfsf10
ENSRNOG00000002968	25.07882016	2.69E-06	Tnfsf4
ENSRNOG00000004575	35.29043015	6.59E-22	Il1a
ENSRNOG00000004649	10.12241892	1.60E-08	Il1b
ENSRNOG00000012509	85.81245466	8.17E-07	Il17f
Cytokine receptors (Gene ID)	**Fold Change (Irisin/Vehicle)**	**P value**	**Name**
ENSRNOG00000010665	10.10604682	2.33E-06	Ccr7
ENSRNOG00000011696	2.012087039	1.04E-05	Lifr
ENSRNOG00000033192	2.070104723	4.31E-11	Osmr
ENSRNOG00000001713	3.074177331	6.03E-06	Il13ra1
ENSRNOG00000007544	47.85899368	0.000197	Il23r
ENSRNOG00000000187	4.002190092	4.50E-22	Csf2rb
ENSRNOG00000047647	168.3991568	3.27E-36	Il2ra
ENSRNOG00000003954	2.191892915	7.49E-17	Il2rg
ENSRNOG00000018706	8.756001075	9.90E-11	Il15ra

ENSRNOG00000057557	18.96913428	0.008953	Prlr
ENSRNOG00000003254	4.280377418	0.006222	Il23a
ENSRNOG00000004332	2.183894984	2.04E-15	Egfr
ENSRNOG00000016308	2.054807912	2.00E-07	Il10ra
ENSRNOG00000028638	1.214787847	0.015096	Il10rb
ENSRNOG00000033984	2.645726785	7.51E-21	Ifnlr1
ENSRNOG00000011517	4.663927188	4.85E-29	Tnfrsf21
ENSRNOG00000016575	6.462754384	1.32E-50	Tnfrsf1b
ENSRNOG00000016782	3.483062639	6.47E-06	Tnfrsf8
ENSRNOG00000016289	3.199449314	4.29E-16	Bmpr1b

Table II: Differentially expressed genes (mean count of >1000) between rADSC-vehicle and rADSC-irisin identified by RNA sequencing (RNAseq) analysis (141 total)

Gene ID	Fold Change (Irisin/Vehicle)	P value	Name
ENSRNOG00000045829	2.123140393	2.55E-14	Thbs1
ENSRNOG00000001414	3.794964961	1.18E-30	Serpine1
ENSRNOG00000015036	2.553337851	3.93E-30	Ctgf
ENSRNOG00000014426	2.415426824	1.67E-26	Lox
ENSRNOG00000036689	2.3827209	2.72E-27	P4hb
ENSRNOG00000009037	2.422093523	1.65E-19	Sulf1
ENSRNOG00000003120	3.088727522	4.75E-43	Prelp
ENSRNOG00000005695	8.130157994	2.02E-13	Mgp
ENSRNOG00000014350	2.158863434	1.45E-13	Cyr61
ENSRNOG00000016695	3.690135869	2.71E-58	Mmp2
ENSRNOG00000010947	2.18906125	2.35E-19	Mmp14
ENSRNOG00000017123	4.681066953	1.84E-212	B2m
ENSRNOG00000010208	6.551981115	1.95E-50	Timp1
ENSRNOG00000061910	3.959444431	1.04E-05	Igfbp3
ENSRNOG00000000599	2.000325283	1.98E-26	Lama4
ENSRNOG00000019048	3.083997642	0.000291946	Sod2
ENSRNOG00000004554	9.024900712	5.55E-106	Dcn
ENSRNOG00000008246	3.096440211	1.82E-16	Emilin1
ENSRNOG00000033192	2.070104723	4.31E-11	Osmr
ENSRNOG00000008292	4.500981915	5.22E-63	Hif1a
ENSRNOG00000012280	3.856109334	7.94E-05	Ptx3
ENSRNOG00000056219	2.520122898	3.46E-15	Olr1
ENSRNOG00000021318	2.281308116	7.83E-14	Epas1
ENSRNOG00000057556	2.170829645	1.74E-14	Pdzrn3
ENSRNOG00000020587	2.096719524	3.27E-19	Efemp2
ENSRNOG00000007457	4.272423867	4.01E-49	Serping1
ENSRNOG00000020679	2.731897515	3.04E-31	Icam1
ENSRNOG00000014064	3.069604668	4.61E-47	Ctsh
ENSRNOG00000049560	2.129185669	4.12E-10	Glul
ENSRNOG00000015078	2.51475911	9.04E-42	Ifitm3
ENSRNOG00000018659	2.304451584	3.87E-32	Csf1
ENSRNOG00000011071	3.982595408	6.66E-43	Nt5e
ENSRNOG00000015602	2.447127835	1.57E-23	Cdh2
ENSRNOG00000010319	2.808347245	1.39E-52	Lcp1
ENSRNOG00000014117	5.103462588	1.58E-43	Hmox1
ENSRNOG00000012660	2.555956996	8.69E-39	Postn
ENSRNOG00000039668	2.117682515	9.07E-20	Col8a1
ENSRNOG00000007284	2.87683353	9.20E-24	Slc2a1
ENSRNOG00000007159	9.402859941	1.42E-14	Ccl2
ENSRNOG00000015052	4.078482896	2.08E-27	Star

ENSRNOG00000026124	2.205059155	2.31E-20	Id3
ENSRNOG00000000239	7.932686519	1.07E-42	Ccl7
ENSRNOG00000002810	3.144637479	1.76E-15	Gfpt2
ENSRNOG00000006094	2.664137227	1.45E-40	Cd44
ENSRNOG00000003217	8.316672588	2.07E-258	Lgals3bp
ENSRNOG00000028703	2.656715611	2.76E-41	Slc39a6
ENSRNOG00000054978	2.011544676	1.85E-33	Hist1h1c
ENSRNOG00000059968	3.633766332	1.93E-64	Jak2
ENSRNOG00000016456	9.306631382	2.80E-79	Il33
ENSRNOG00000019587	2.573364088	3.10E-27	Ptprn
ENSRNOG00000006462	2.406939191	3.22E-35	Ero1a
ENSRNOG00000009088	2.243466246	3.00E-33	Txnrd1
ENSRNOG00000007393	2.470140602	8.16E-36	Ndrg1
ENSRNOG00000013973	8.833885756	1.74E-09	Lcn2
ENSRNOG00000021125	3.075576105	5.25E-18	Prdx5
ENSRNOG00000017977	2.314139777	7.96E-16	Smpd1
ENSRNOG00000004726	2.125972599	3.15E-16	Mapkapk2
ENSRNOG00000018126	2.056723332	1.85E-20	Abca1
ENSRNOG00000023376	3.997382435	2.36E-50	Riok3
ENSRNOG00000007081	2.35734155	6.07E-20	Xdh
ENSRNOG00000013987	2.009129906	3.89E-11	Sbno2
ENSRNOG00000010370	3.795503078	1.74E-18	Tnip1
ENSRNOG00000019728	2.398785071	9.24E-17	Itgad
ENSRNOG00000003183	2.813941831	6.68E-18	Fmod
ENSRNOG00000003720	2.508343112	3.24E-29	Prrx1
ENSRNOG00000042838	2.389507841	4.57E-13	Junb
ENSRNOG00000005971	2.499801359	2.50E-23	Gpr176
ENSRNOG00000014297	2.22286935	7.75E-25	Sdc4
ENSRNOG00000010513	6.81433998	2.27E-58	Tfpi2
ENSRNOG00000004332	2.183894984	2.04E-15	Egfr
ENSRNOG00000000521	3.301449646	1.21E-41	Cdkn1a
ENSRNOG00000027030	2.716836302	2.23E-29	Adm
ENSRNOG00000003616	3.879966629	4.31E-43	Grem2
ENSRNOG00000009381	2.589156809	5.64E-58	Mapk6
ENSRNOG00000018237	2.021072988	4.29E-23	Gstp1
ENSRNOG00000017819	2.988973924	1.08E-18	Cd14
ENSRNOG00000006116	2.464474935	1.37E-26	Hk2
ENSRNOG00000006388	2.608609931	2.00E-20	Pygl
ENSRNOG00000007060	2.319236622	1.02E-18	Plin2
ENSRNOG00000036677	2.712847435	3.51E-20	Slc16a3
ENSRNOG00000018367	2.212776124	4.41E-15	Taldo1
ENSRNOG00000016460	4.569960126	1.55E-47	Clu
ENSRNOG00000017539	2.543673393	0.011815667	Mmp9
ENSRNOG00000028156	2.248601129	2.95E-28	Pld1
ENSRNOG00000026647	6.610628004	7.37E-67	Cxcl16
ENSRNOG00000004737	2.078370915	4.06E-25	Cd48

ENSRNOG00000002041	2.994542165	1.08E-14	Boc
ENSRNOG00000007091	7.239120152	1.23E-164	Ly6e
ENSRNOG00000017980	3.965036211	0.00027917	Itgal
ENSRNOG00000045941	2.267418248	4.64E-17	Susd6
ENSRNOG00000008364	2.167035481	5.60E-43	Cat
ENSRNOG00000004649	10.12241892	1.60E-08	Il1b
ENSRNOG00000006956	2.75533061	2.34E-25	Adamtsl1
ENSRNOG00000015239	2.537974234	2.87E-43	Ginm1
ENSRNOG00000010971	3.792222643	1.16E-23	Snx18
ENSRNOG00000013794	3.009467626	8.83E-15	Rbp1
ENSRNOG00000016587	3.212489649	2.58E-31	Ninj1
ENSRNOG00000017523	2.942902216	1.65E-14	H6pd
ENSRNOG00000019180	3.026206776	1.08E-18	Acsl4
ENSRNOG00000007545	10.61149297	8.86E-07	Angptl4
ENSRNOG00000004226	2.450488003	5.57E-07	Irak3
ENSRNOG00000021243	2.503333796	3.17E-15	Siglec1
ENSRNOG00000019311	2.414601263	6.96E-14	Nfkb2
ENSRNOG00000011016	3.843280555	3.21E-48	Slc7a2
ENSRNOG00000010461	2.118692675	6.94E-20	Gpx8
ENSRNOG00000006304	2.505740465	1.75E-43	Mdm2
ENSRNOG00000009734	9.618816039	1.94E-153	Akr1b10
ENSRNOG00000009263	3.259227328	3.45E-61	Ifi27
ENSRNOG00000039091	2.009605377	3.59E-06	Pnpla8
ENSRNOG00000016756	2.141057573	2.85E-31	Ptgir
ENSRNOG00000009899	2.056766989	1.58E-30	Skil
ENSRNOG00000037113	3.203659436	3.91E-37	Slfn2
ENSRNOG00000018526	2.286855202	3.10E-23	Dlg4
ENSRNOG00000025691	2.143018598	1.52E-22	Pla2g7
ENSRNOG00000006076	2.078669801	7.48E-11	Steap2
ENSRNOG00000005261	3.229041205	7.99E-49	Fbxl5
ENSRNOG00000017154	3.123241958	5.28E-57	Atp11a
ENSRNOG00000000827	2.830483379	3.92E-20	Ier3
ENSRNOG00000001304	2.461858462	1.84E-09	Bcr
ENSRNOG00000002436	3.933758869	1.06E-52	Mmd
ENSRNOG00000058186	2.129961253	2.32E-05	Errfi1
ENSRNOG00000036745	2.107358003	1.21E-11	Gna13
ENSRNOG00000018911	4.551977261	4.85E-32	Pfkfb3
ENSRNOG00000014320	7.982588122	1.11E-12	Inhba
ENSRNOG00000018567	4.232721985	7.64E-35	Slc20a1
ENSRNOG00000002579	4.41787323	7.62E-82	Parm1
ENSRNOG00000037604	2.267769699	2.22E-23	Ascc3
ENSRNOG00000003486	2.433506321	2.01E-11	Mnda
ENSRNOG00000013946	2.004282042	5.84E-08	Rnf149
ENSRNOG00000014096	2.175787918	2.27E-14	Nr3c1
ENSRNOG00000007390	5.492658517	2.19E-08	Nfkbia
ENSRNOG00000031081	3.578701679	3.69E-42	Stat2

ENSRNOG00000003835	2.003788172	6.40E-21	Slc43a2
ENSRNOG00000007793	2.36638579	8.08E-14	Pnrc1
ENSRNOG00000004581	2.016797483	2.72E-21	Zdhhc9
ENSRNOG00000042320	2.251495072	5.00E-20	Slc41a1
ENSRNOG00000023334	2.99500111	2.09E-25	Parp14
ENSRNOG00000009754	2.320730631	1.10E-40	Nampt
ENSRNOG00000010629	2.684884951	9.33E-26	Nod1
ENSRNOG00000006646	2.632162641	1.22E-37	Vopp1
ENSRNOG00000006859	2.130044087	6.51E-07	Insig1

Table III: ADSCs expressed 17 α subunits and 8 β subunits of the integrin family

Rat ADSCs			Mouse ADSCs	
Name		**baseMeanA**	**Name**	**baseMeanA**
Integrin α	Itga11	22099.33333	Itga5	12322.87277
	Itga8	8705.333333	Itgav	5350.083136
	Itga5	8095.333333	Itga11	2434.786535
	Itga1	4781.666667	Itga9	1612.118898
	Itgav	4534.333333	Itgam	1197.078009
	Itgad	2485	Itgax	502.7084217
	Itgal	1760.333333	Itga2	387.9117392
	Itgax	1278.666667	Itga1	374.3388785
	Itga3	1113	Itga4	345.0812506
	Itga2	479.6666667	Itgal	308.1341378
	Itga6	458.3333333	Itga3	292.7618817
	Itga9	194.3333333	Itga6	272.4367632
	Itga4	145.3333333	Itga7	219.8717863
	Itga7	49	Itga10	46.71271689
	Itgae	45	Itga2b	44.70433438
	Itga2b	24.66666667	Itga8	13.88560399
	Itga10	1.666666667	Itgae	8.976568867
Integrin β	Itgb1	58930.33333	Itgb1	24950.84227
	Itgb2	5239.666667	Itgb5	6777.456912
	Itgb5	1597.666667	Itgb2	5249.732476
	Itgb3	323	Itgb3	548.8640977
	Itgb8	96	Itgb7	107.5939095
	Itgb4	49	Itgb8	71.40077486
	Itgb6	4.333333333	Itgb4	45.77376534
	Itgb7	1.666666667	Itgb6	0.995259075

Table IV: Real-time PCR primers

Genes	Species	Forward primer (5'—3')	Reverse primer (5'—3')
Ccr7	Rat	GCTGGTGGTGCTGACATACA	AGCAACATCCCGCTGAAGAA
Lifr	Rat	TTCTGCCGGTTCATCTCCAC	ACCATGACGAGTTGCACCAT
Osmr	Rat	GGCTATGGTAGATGACGCCC	AGTTGCTCTCCACGGATTGG
Il13ra1	Rat	CAAGCCCGGACACCAACTAT	GGAGGACCGGGTTTCACATT
Il23r	Rat	GCTTTTCGGAACCTCATGCC	GGTGCAAGTCATGTTGCCT
Csf2rb	Rat	GGTTGGGGACTACTGCTTCC	CAGGCTGGCTATTGTCCCAA
Il2ra	Rat	ACACAATGGCACATCGGGAA	AAAGTGACCACACCATCGCA
Il2rg	Rat	TACTGGCCTCCCCCATGTTA	GGCATTCTCAAGTAGGGCGT
Il15ra	Rat	GTGACCTGGGTGTAGACTGC	CCTTTGGGTTCACTTTGCCG
Prlr	Rat	ATTATGGTCTGGGCAGTGGC	TTGCGATGGTGGTAGAACCC
Il23a	Rat	GCTTTTCGGAACCTCATGCC	GGTGCAAGTCATGTTGCCT
Egfr	Rat	CTCTGACGGGCTTTGTCACT	CCTCACCATGAGGCAACCTT
Il10ra	Rat	CAGGGCATCCTAAGCACACA	AGGCTTCTGCCAAGGGAATC
Il10rb	Rat	ATGGGCTGCATCTGGTTGTT	TCCTTGCATCTCCACACACG
Ifnlr1	Rat	GTCTAGCCCCACCCAGAAAC	TCAGGCACATCATGGGACAC
Tnfrsf21	Rat	CAGTGAGGCGGGTGTAGTTT	GCTGCCACAAGGAGAGGAAT
Tnfrsf1b	Rat	ACAGAACCGAGTGTGTGCTT	GCCAGGATGCTACAAATGCG
Tnfrsf8	Rat	CTGACCCTGAACCTGTGACC	ACCAGACCCCTGACAGTCTT
Bmpr1b	Rat	CAGAGTGCTGGGCGCATAAT	TGTCTGTCCAGCTTGCTCTC
Thbs1	Rat	AGGCCAAAGACCGGTTTGAT	TGCCAGGCTGGTTATGATCG
Serpine1	Rat	CAACCCACTACGCCTTCACT	TCTGTCTATCTGCTGCCCCT
Ctgf	Rat	ACCCAACTATGATGCGAGCC	GATGCACTTTTTGCCCTTCTTA
Lox	Rat	CAGGCACCGACCTGGATATG	ACTGGCCAGGCAGTTTTCTT
P4hb	Rat	GGTGGACTCAAGCGAAGTGA	AAGAGGACCACCCCATCCTT
Sulf1	Rat	TCCAAGTGAGAGTTGCAGGC	CTGTCCTTTGACGCTTCCCT
Prelp	Rat	CCTGGACAACAACCGCATTC	AGCTTGCTGAATACTCCGGG
Mgp	Rat	CCAGGAAAGAGTCCGGGAAC	TCTTATTTGGCTCCTCGGCG
Cyr61	Rat	GCACCTCGAGAGAAGGACA	CTGGTCAAGTGGAGAAGGGTG
Mmp2	Rat	AGAGGATACCCCAAGCCACT	CAGGAGTCTGCGATGAGCTT
Mmp14	Rat	TCCATAGGTGGGTCCCTTGA	AGCAGATGACCCCATTTGGC
B2m	Rat	GTGTACTCTCGCCATCCACC	GCTCCTTCAGAGTGACGTGT
Timp1	Rat	TGCTCAAAGGATTCGACGCT	AGCAGGGCTCAGATTATGCC
Igfbp3	Rat	GGCCTGACCTACTTGGGAAC	CGCACCGTTATTTGCGACAT
Lama4	Rat	CTGTGTTTTACGTTGGCGGG	CAGCCCTGCTCTGAGTGAAA
Sod2	Rat	CAGACCTGCCTTACGACTATGG	CTCGGTGGCGTTGAGATTGTT
Dcn	Rat	AAACTCCTCAGAGTGCCTGC	CAATACCGGACAGGGTTGCT
Emilin1	Rat	CTCAATCTTACCGCAGCCCA	GCCCCCAAACACACTGAAAC
Hif1a	Rat	TGGTGGCTCAGCAGTCTATTT	GTGCTGTGATCTGGCATTCG
Ptx3	Rat	ACCTCGCTTCTTTCCCTGTG	CATGGTGTGGGGTCCTCG
Olr1	Rat	CATGGGCCCTTTAACTGGGA	GAAACGCCCCTGGTCCTAAA
Epas1	Rat	GGGGTTAAGGAACCCAGGTG	TGTGTTCGCAGGAAGCTGAT
Pdzrn3	Rat	GGCCCTGTGTGGACAATCAA	ATCTTGGTTCGCGGTGTTCT
Efemp2	Rat	TTTCAGATCCGTGCTGGGAA	CCAACATGACCCTTCCCTGAG
Serping1	Rat	CGCCTCTCTGAGCCTGTATG	GGCACTCAAGTAGACGGCAT
Icam1	Rat	CGGTGCTCAGGTATCCATCC	CTGTCTTCCCCAATGTCGCT
Ctsh	Rat	CACCAGTGAAGAACCAGGGG	TCCTTGGCAGCCATGATTGT
Glul	Rat	CCTTTCGGCTGGCCTTCTAA	TCGCAGGGTCCTATCTGGAA
Ifitm3	Rat	CCGTGATCAACATGCCCAGA	GCCTGGGCTCCAGTCATATC
Csf1	Rat	AATCACGAGGACCCACAACC	TCTGTCAGTCTCTGCCTGGA
Nt5e	Rat	TGCATCGATATGGCCAGTCC	TTTGGAATCCATCCCCACCG
Cdh2	Rat	ATAGCCCGGTTTCACCTGAG	CCCAGGCTTTGATCCCTCTG
Lcp1	Rat	TCATCGATGCCATTCAGCCA	GCTTGCTCCTGTGGGACTTA

Hmox1	Rat	ACCAGAGTCCCTCACAGACA	TAAATTCCCACTGCCACGGT
Postn	Rat	GGTGTGAGGGAGACAGCATC	GGTCCGTGAAAGTGGTTTGC
Col8a1	Rat	TAAACCCTGTGAAGAGACGGG	TGGCAGAGGCTTGATTCCAT
Slc2a1	Rat	GGCCTCTACTGCTCAGTGTC	CGGAACAGCTCCAAGATGGT
Ccl2	Rat	TCCTCCACCACTATGCAGGT	TCCAGCCGACTCATTGGGAT
Star	Rat	TGGCTGCCAAAGACCATCAT	GAACTTCCAATGGCGTGCAG
Id3	Rat	ATCTTGCCACAGCCCTCTTC	CTCGACACCCCATTTTCGGA
Ccl7	Rat	TGGACTACGGTCCTAAGGGG	GGAACAAAGTCAATGGGGCG
Gfpt2	Rat	CTGACACCGAGTCCCCTCTA	CTATGAGTAGGGAGCCGGGA
Cd44	Rat	ACAACTTCTGGTCCTGCGAG	TCCCCGTTGAGTTCACTTGG
Lgals3bp	Rat	CTCTGTGCCCACACACTGAT	GGTGACAAAAAGCCGTCCAC
Slc39a6	Rat	GTCAGCTCCGTTGGGTAACA	CCAGCAGGGAACCAATGCTA
Hist1h1c	Rat	GAAGCCAAGCCCAAAGCAAA	CTCTTGGGGGCCTTCTTAGC
Jak2	Rat	GGGTGCCCAGACGAGATTTA	GCCATGTGGCTACAGAGCTT
Il33	Rat	CCCGCCTTGCAAAATCACAA	CAACTGATGCAGCAAACGCT
Ptprn	Rat	CCTGCTGTTGTTGAGCGGC	CCTTGTAAGCGCTGGAGAACT
Ero1a	Rat	CGGAAAGCGGACCCAGTTAT	AACAAGCACATTCCAACCGC
Txnrd1	Rat	TTGGCAAATTTCCGGCAGTG	CGCACTTAGACCAGCAGACA
Ndrg1	Rat	CAACATTTTGCGGTCTGCCA	CCCCATGCCAATGACACTCT
Lcn2	Rat	CCGATGAACTGAAGGAGCGA	CAGACAGGTGGGACCTGAAC
Prdx5	Rat	CTCTTTGGGAATCGTCGGCT	GGCCCTCAGGTAAGGAGGTA
Smpd1	Rat	GCGATCCAGTCCTGTCTACG	GAACCCAGAGAACCGTGGAG
Mapkapk 2	Rat	GGCTTTATTCTGCCCTTGGC	AACAACCTCCACAATGCCCT
Abca1	Rat	CGTTGCTTGACAGCTTAGGG	CACAGACGATTCGGGACACA
Riok3	Rat	TGAAGGAAGTCCACCCATGC	AATTGCACACATCTGGCACG
Xdh	Rat	AGCTCAGCACGGAGATAACG	GGAATCCTGGTGCGGTACAA
Sbno2	Rat	ATCGGTCTGGAGTCTCGGAA	GGCCCTCATTCTCGTCTAGC
Tnip1	Rat	CACACTCACTGGGCACATCT	TTCCATGAGGGTGGCTCAAC
Itgad	Rat	GGTGGGAGGTCTGCTGTTAC	AGGCAGGAGAGTGGATTCCT
Fmod	Rat	CTTGGGATCGGAGATCGGG	GTGGACTTCTGTCACGTGCT
Prrx1	Rat	ACCGAAGCTGGGAGAAAGTT	TGGTCTTCCCTCCAATCCCA
Junb	Rat	AGAGCCTCCCTTGCTCCATA	TGGAAGGGGGCCATGTAAAC
Gpr176	Rat	ATCTCTGACGCCAAGTCTCG	TGACCGTGGTGACGTTGTAG
Sdc4	Rat	CGAGATCGAGCTGGAGTGAC	AATTGTGTGGCCAGTGCAAC
Tfpi2	Rat	TCCACAGTCGGAAACTCTGC	TTCCTCGGGGAACACATTCG
Cdkn1a	Rat	GATCCTGGTGATGTCCGACC	GCGAAGTCAAAGTTCCACCG
Adm	Rat	ACTAGGCAGAACAACTCCAGC	GCTCCGATACCCTGCTGAAA
Grem2	Rat	TGTCCCTGTCAGCAGCAAAT	ATCTTCTGTGTCCGATCCGC
Mapk6	Rat	CAGGAGCTTCTCAGCGTGAT	ATGGGAAAGTGCTTCCTCGG
Gstp1	Rat	GCTATGCCACCGTACACCAT	AAAGCCCTAAAGAGCGACCC
Cd14	Rat	GTTGGGCGAGAAAGGACTGA	GCTCCAGCCCAGTGAAAGAT
Hk2	Rat	GAGAGATCGACATGGGCTCG	TCTCGAAGGAGCCAGTGGTA
Pygl	Rat	GCAGACTACGAAGCCTATGTCA	TTGACCCCATTGCTGGACTC
Plin2	Rat	GGTCAGTGTGTGAGATGGCA	ATCCTTTGCCCCAGTTACGG
Slc16a3	Rat	CTCTTTGTGCCTCCGGTCTT	AGGTAGACAGAGTAGGGCCG
Taldo1	Rat	CCACTGCAACATGACACTGC	CACCCGTGTTACGGAAGGAA
Clu	Rat	AAGCCGCATGGAATGAGACA	AGAGCATCAAGTGCAGGCAT
Mmp9	Rat	TCCAGCATCTGTATGGTCGTG	GCAGTGGGACACATAGTGGG
Pld1	Rat	AGAAAGCTGCCTTGAGGGTC	AGTGCCTAGTGTGGGTAGGT
Cxcl16	Rat	CTTCTGGCACCCAGATACCG	ACTCTGCCCATGACCGATTC
Cd48	Rat	AAGCACCCACTTGCATCGTA	ACTGGTCCTCAGTTTCGTGC
Boc	Rat	CAGCAGACTACACGGAAGCA	AGCAGCTGGTGAGTTGAGTC
Ly6e	Rat	TGTCAACCTTGGCTACACCC	CAAGCTGAGCAGGAGTCCAA
Itgal	Rat	CCCAGGCTGCAGTTATCACA	AACGCATGCCCTAACCTCAA

Susd6	Rat	CTCTGGAGTCGGTCACAGC	ATGACACCGATCTTCTGGGA
Cat	Rat	CTGACTGACGCGATTGCCTA	GTGGTCAGGACATCGGGTTT
Il1b	Rat	CCTTGTCGAGAATGGGCAGT	TTCTGTCGACAATGCTGCCT
Adamtsl1	Rat	GATACCTTCAGAGCAGCCCTC	CACGTGGATTTCAGCGAAGA
Ginm1	Rat	GGTTGTGGAGCTCGATGGAA	TTGGAAAAATCGGGAGGCGT
Snx18	Rat	TGCCTTCAGACCGGTTGTTT	CCAGCATCTTACCAGGCGAT
Rbp1	Rat	TGACAGGCATAGATGACCGC	TTGGGCCATCCCACTTGTTC
Ninj1	Rat	GACCCCTGCAACTGCTGTAT	TGGGTTTCGGTGGAACTGTC
H6pd	Rat	GCAGACATTCACTGCCCTCT	AGACAGAGCCATCCTGCCTA
Acsl4	Rat	AGTTCGATTGAGCCCAGAGC	AACAGAGAGATCAACAAAGGCT
Angptl4	Rat	ACCCGAAGGATAGAGTCCCC	GCCATCTTGGGAAGCCTCTT
Irak3	Rat	AGAATTGCTGTGGTCCTGGG	GTCCGGGGTCACTTTCTCTG
Siglec1	Rat	TGGAGATCCAGAAGCCGGTA	CCAAGAAAAGTTGGCAGGGC
Nfkb2	Rat	ACGCTCTGCCCCAAATGTTA	CTAGATGCAGTGGGGTTCGG
Slc7a2	Rat	TGGGTTGTCATGGGTTTGCT	TCCCAAGCACACATGTACCC
Gpx8	Rat	ATGGTTCTGTGCACCGTGAT	TCTTGTCTGTGAACCGGCAG
Mdm2	Rat	GCCTGAGTGATGAAGGGCAT	CCAGTTCTCACGAAGGGTCC
Akr1b10	Rat	CATCGTGGTCACAGCCTACA	CTCTTGTATGCGGGAGGGTG
Ifi27	Rat	GCTCCTATCTGGAACGCACA	CAGTGCAGTCTGGGCTAACA
Pnpla8	Rat	CCCCTAGATGAAAGCCGCAA	TGATGGCCTTGAGGAACACA
Ptgir	Rat	GGGAACTGGGAGTTGACTGG	GCAGGTCCTACCGAGTCTTG
Skil	Rat	GTCCGCCAGAACACTGGTTA	ATGGTGGGCAGACCTTTCAG
Slfn2	Rat	TGTCACCAGGAGGATTTGGC	CCGTGTTTGCGAATGCAGAA
Dlg4	Rat	AGGAGTACCCGCTGTAGGG	TCAGGAGAGCCCCCATGC
Pla2g7	Rat	AGCTCGGAAAGAGCAGGTTC	GGATCAAGGGCAATCCCACA
Steap2	Rat	TGTTCTTGCGCTTGTCTTGC	GACCCTCTCAGGGGACAGAT
Fbxl5	Rat	AGCATACAGCTCTGCAGTCTC	CCTGCACTTTTCAGAACGCC
Atp11a	Rat	TCTTCACTGACAAGACCGGC	ACATCGTCCCCACAATGGTC
Ier3	Rat	ATGCAGGGGTGCGAGATATG	GCCACAGACCGTATTCCCAA
Bcr	Rat	ATGACCCGGAAACCTGTGAC	GGAAAGGTACAGTCTGGGGC
Mmd	Rat	CAAGCGCCGTTTCTATCACG	GAATGAGGAACCGTGGTGGT
Errfi1	Rat	CTCCCGTACCCCAAGTCCTA	ATGGCGGTCTCTCAGGTAGT
Gna13	Rat	GGAATGCTTCCGGGGGAAA	ACAGCGAGTCTCCTCATCAC
Pfkfb3	Rat	CGCAATAGTGTCACCCCACT	GGGGGCTCCTCATGTTCTTT
Inhba	Rat	GACCTCATGAGACAAGAGCC	AAGTCGTGTGGTTGCCTTCT
Slc20a1	Rat	TCCTCTGTGGGGTACCATCC	TTCTCAACATCGCCAGGAGC
Parm1	Rat	CACCAGTCCGCCTTTGAAGA	GAGGTAACGGAAGCGGAGAG
Ascc3	Rat	TGGCTGTAAAACGCGTAGGA	TGGCCTGTTTGGGTCACAAT
Mnda	Rat	CCCCGAGTATAAAGGCGGTC	TCACTGGCCAGCAATGACTT
Rnf149	Rat	CACAGGACCTACCCATCCCA	AAGACGGCACTAGCAGATGG
Nr3c1	Rat	TGTGAAAATGGGTCGGTGCT	ACGGTTAATCTGCACAGCCT
Nfkbia	Rat	AGCCCCGAGCATTCTATTGT	CACACTGGGGTCAGCTACAG
Stat2	Rat	CCTTGTGAGCAGGGTTAGCA	CTGCCCTTAAGACCCCTGTG
Slc43a2	Rat	CACCACACTGGAGCCCATAC	ATGCTTGCTGGAGAAGTCCC
Pnrc1	Rat	TATCGCAGCATCCGACACAA	CGTGCCTTAGCACCCACTTA
Zdhhc9	Rat	CATCGTCTATGTGGCCCTCAA	TGTACGGATTCTGCACACGG
Slc41a1	Rat	GGCAGGAGAAAGTCGCTTCA	CACTCGGACCTCTGGAAACC
Parp14	Rat	ATCAGCCACCGCTCAAATCA	TCGGGGAAACACTCAACTCG
Nampt	Rat	TGGGGTGAAGACCTGAGACA	TGGCAGCAACTTGTAGCCTT
Nod1	Rat	TCAGCAATGAAAGGCGGGAT	TCCGAATGTTGGTGACCAGG
Vopp1	Rat	TTCCAGGTCCAGCCCAATTC	CAAAAGGGCCTGTCCATCCT
Insig1	Rat	CACGTCTGGAGCTACCCAAG	CGCCAAATGAGAAGAGCACG
Csf2rb	Mouse	GGTTGGGGACTACTGCTTCC	CAGGCTGGCTATTGTCCCAA
Angptl4	Mouse	CACCCACTTACACAGGCCG	GAAGTCCACAGAGCCGTTCA
Ccl2	Mouse	GGCTCAGCCAGATGCAGTTA	TTCCTTCTTGGGGTCAGCAC
Il1b	Mouse	TGCCACCTTTTGACAGTGATG	TGATGTGCTGCTGCGAGATT

Lcn2	Mouse	CGAGTTACCTCGTCCGAGTG	CAGCCGTCGATACACTGGTC
Mmp9	Mouse	GCAGAGGCATACTTGTACCG	TGATGTTATGATGGTCCCACTTG
Ptx3	Mouse	CAGGAGAGCCGTGACGC	TGTTTCACAACCTGCGGGC
Stat2	Mouse	TCCGCTGTTCGCTATCTTGG	TGCGCCATTTGGACTCTTCT
Tfpi2	Mouse	AGCACTCTCCTCCCTCCAG	AGATCTCTAAATTATTCCCTTGGGC
Tnip1	Mouse	CCTGGGCTGAAGCTAGGC	GTTCAAAAGCTGCGGACACC
Sod2	Mouse	CAGACCTGCCTTACGACTATGG	CTCGGTGGCGTTGAGATTGTT
FNDC5	Mouse	CTGTCTCCAATGTTCCACTTGTCTG	CTTGCCTTTGTTCTTTGAGGCCATC
Actb (β-actin)	Mouse	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Acta2 (a-SMA)	Mouse	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
Cnn1 (Calponin 1)	Mouse	TCTGCACATTTTAACCGAGGTC	GCCAGCTTGTTCTTTACTTCAGC
Myh11 (smMHC)	Mouse	AAGCTGCGGCTAGAGGTCA	CCCTCCCTTTGATGGCTGAG
Pecam1 (CD31)	Mouse	CTGCCAGTCCGAAAATGGAAC	CTTCATCCACCGGGGCTATC
Kdr (Flk-1)	Mouse	TTTGGCAAATACAACCCTTCAGA	GCAGAAGATACTGTCACCACC
Gata4	Mouse	CCCTACCCAGCCTACATGG	ACATATCGAGATTGGGGTGTCT
Nkx2-5	Mouse	TGACCCAGCCAAAGACCCT	CCATCCGTCTCGGCTTTGT
Nppa	Mouse	GCTTCCAGGCCATATTGGAG	GGGGGCATGACCTCATCTT

Supplementary References

[1] W. Yan, Y. Guo, L. Tao, W. B. Lau, L. Gan, Z. Yan, R. Guo, E. Gao, G. W. Wong, W. L. Koch, Y. Wang, X. L. Ma, *Circulation* **2017**, *136* (22), 2162.
[2] R. J. Vagnozzi, M. Maillet, M. A. Sargent, H. Khalil, A. K. Z. Johansen, J. A. Schwanekamp, A. J. York, V. Huang, M. Nahrendorf, S. Sadayappan, J. D. Molkentin, *Nature* **2020**, *577* (7790), 405.
[3] W. Yan, C. Lin, Y. Guo, Y. Chen, Y. Du, W. B. Lau, Y. Xia, F. Zhang, R. Su, E. Gao, Y. Wang, C. Li, R. Liu, X. L. Ma, L. Tao, *Circulation research* **2020**, *126* (7), 857.
[4] W. Yan, F. Zhang, R. Zhang, X. Zhang, Y. Wang, F. Zhou, Y. Xia, P. Liu, C. Gao, H. Wang, L. Zhang, J. Zhou, F. Gao, E. Gao, W. J. Koch, H. Wang, H. Cheng, Y. Qu, L. Tao, *Journal of molecular and cellular cardiology* **2014**, *74*, 183.
[5] E. J. Villablanca, J. R. Mora, *Journal of visualized experiments : JoVE* **2011**, (49).
[6] Y. Xia, F. Zhang, S. Zhao, Y. Li, X. Chen, E. Gao, X. Xu, Z. Xiong, X. Zhang, J. Zhang, H. Zhao, W. Wang, H. Wang, Y. Guo, Y. Liu, C. Li, S. Wang, L. Zhang, W. Yan, L. Tao, *Cardiovasc Res* **2018**, *114* (10), 1335.
[7] W. Yan, H. Zhang, P. Liu, H. Wang, J. Liu, C. Gao, Y. Liu, K. Lian, L. Yang, L. Sun, Y. Guo, L. Zhang, L. Dong, W. B. Lau, E. Gao, F. Gao, L. Xiong, H. Wang, Y. Qu, L. Tao, *Basic research in cardiology* **2013**, *108* (3), 329.
[8] X. Guan, W. Xu, H. Zhang, Q. Wang, J. Yu, R. Zhang, Y. Chen, Y. Xia, J. Wang, D. Wang, *Stem cell research & therapy* **2020**, *11* (1), 73.
[9] J. Pang, X. Qi, Y. Luo, X. Li, T. Shu, B. Li, M. Song, Y. Liu, D. Wei, J. Chen, J. Wang, C. Wang, *Theranostics* **2021**, *11* (5), 2381.