Supplemental Information

Supplemental methods

Mice. Mice were bred and maintained under pathogen-free conditions at Chonnam National University Medical School animal facilities. Male inbred BALB/C nude mice were purchased from Orient Bio Inc. (Gyenggi-Do, Korea). *Angptl4* knockout mice on a C57BL/6J genetic background were provided by Dr. Min-Seon Kim (University of Ulsan College of Medicine, Korea) (1). All experiments were performed after approval by our local ethical committee at Chonnam National University Medical School (CNU IACUC-H-2016-37).

Cell culture. Human bone marrow-derived mesenchymal stem cells (hMSCs), immortalized by the introduction of telomerase, were kindly provided by Professor Yeon-Soo Kim (Inje University, Seoul, South Korea). hMSCs were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco, 11965-092) with 10% fetal bovine serum. Human adipose tissue-derived mesenchymal stem cells (hATSCs) were obtained in accordance with guidelines approved by Chonnam National University Hospital Institutional Review Board (IRB No. CNUH-2016-266). Freshly excised human fat tissue was obtained from the waste after liposuction. Fat tissues were digested in 0.1% collagenase I (Sigma, C0130) solution for 30 minutes at 37˚C with gentle shaking. After incubation, the digestion mixture was homogenized through a 40-μm nylon mesh and was centrifuged at 1500 rpm for 10 minutes at 4˚C. Harvested cells were cultured in DMEM (Gibco, 11885084) with 10% fetal bovine serum and penicillin/streptomycin. Mouse mesenchymal stem cells (mMSCs) were isolated from bone marrow and cultured (2). Bone marrow cells were collected by flushing the femurs and tibias and were cultured with MesenPRO RS basal with MesenPRO RS growth supplement (Gibco, 12746012), L-glutamine (Gibco, 25030081), 100 mg/mL penicillin and 100 mg/mL streptomycin (Gibco, 15140-122). The culture medium was changed on day 3 to remove non-adherent cells. The medium was subsequently replaced for 4 days, and the mMSCs were used within four passages.

Human macrophages were differentiated from $CD14^+$ monocytes isolated from peripheral blood. Blood sampling was performed under protocols approved by the institutional review board of Chonnam National University Hospital (IRB No. CNUHH-2018-018). Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by density centrifugation by Lymphoprep (stemcell, 07801) according to the manufacturer instructions. $CD14^+$ monocytes were separated from PBMCs by using anti-CD14 mAb-coupled magnetic beads (CD14 MicroBeads; Miltenyi Biotech, 130-050-201) followed by MACS column separation according to the manufacturer's protocol (3). The purity of monocytes was evaluated by fluorescent staining with CD14-APC monoclonal antibody (HCD14, 325608) from Biolegend and FACS analysis (4). Monocytes were seeded into tissue culture plates at a density of 1×10^6 cells per ml and treated for 6 days with 30 ng/mL GM-CSF (Peprotech, 300-03) in RPMI 1640 and differentiated into macrophages for 3 days with LPS (50 ng/mL), IFN-γ (20 ng/mL, ThermoFisher Scientific, PHC4031) and IL-6 (20 ng/mL, Peprotech, 200-06). Human monocytic THP-1 cells, purchased from the Korean Cell Line Bank, were cultured in RPMI 1640 (Gibco, 11875-093) supplemented with 10% fetal bovine serum (Gibco, 16000044), 100 mg/mL penicillin, and 100 mg/mL streptomycin (Gibco, 15140-122). To differentiate THP-1 cells into macrophages, cells were treated with PMA (Sigma-Aldrich, 8139, 200 nmol/L) for 72 hours. For mouse bone marrow-derived macrophages (BMDMs), mononuclear cells were isolated from mouse bone marrow and cultured for 7 days in macrophage differentiation medium (supplemented with 30% L929 cell-conditioned medium, 20% fetal bovine serum, and 50% RPMI-1640). L929 cell-conditioned medium was prepared by growing L929 cells in RPMI-1640 containing 10% fetal bovine serum for 10 days. The medium containing macrophage colony-stimulating factor secreted by the L929 cells was harvested and passed through a 0.22-mm filter. BMDMs were stimulated with LPS 100 ng/mL and IFN-γ 30 ng/mL for 24 hours or 48 hours.

To coculture MSCs and macrophages, MSCs or macrophages were seeded to adhere at 2×10^5 cells/well in 6-well plates. The next day, 0.4-μm-pore size Transwell (Corning Transwell, 3401) inserts were added into the 6-well plates with the hMSCs initially seeded at the bottom well. MSCs or

macrophages were then seeded onto the inserts. After 24 hours or 48 hours, the inserts were discarded, and the cells in the 6-well plate were washed and used for further analysis.

HEK293T cells were cultured in DMEM (Gibco, 11965-092) supplemented with 10% fetal bovine serum and penicillin/streptomycin.

Isolation of neonatal rat cardiomyocytes and cardiac fibroblasts. Primary cardiomyocytes and cardiac fibroblasts were isolated from 2 day-old Sprague-Dawley rats (5). Briefly, neonatal ventricles from neonatal rats euthanized by decapitation were separated and washed in cold-PBS, chopped using a scalpel, and digested with 0.1% collagenase type 2 (210 U/mL, Worthington Laboratories, LS004176) and pancreatin (0.6 mg/mL, Sigma-Aldrich, P3292) for 30 minutes with mild stirring. The supernatant was collected for centrifugation a Percoll gradient (GE-Healthcare, 17-0891-02) at 1000 rpm for 5 minutes. The cardiac cell layer was collected and cultured in a flask with DMEM supplemented with 10% heat-inactivated fetal bovine serum. After 1 hour, the non-adherent cardiomyocyte population was removed from the adherent cardiac fibroblasts. The proliferation culture for cardiac fibroblasts was supplemented with DMEM with 10% fetal bovine serum and 1% PenStrep (50 units/mL penicillin and 50 μg/mL streptomycin). The fibroblasts were used for experiments at passage 2 or passage 3.

Reagents and plasmids. The following reagents were used in this study: LPS (Sigma-Aldrich, L4391), PMA (Sigma-Aldrich, 8139), recombinant human interferon-γ (IFN-γ; ThermoFisher Scientific, PHC4031), recombinant human ANGPTL4 (R&D Systems, 4487-AN), recombinant mouse ANGPTL4 (R&D Systems, 4880-AN), granulocyte macrophage colony-stimulating factor (GM-CSF, Peprotech, 300-03), recombinant human interleukin-6 (IL-6, Peprotech, 200-06), GW4869 (Sigma-Aldrich, 6823-69-4), cytochalasin D (Sigma-Aldrich, 22144-77-0), SR1078 (Tocris Bioscience, 4874), SR1001 (Tocris Bioscience, 4868), SR3335 (Cayman, 293753-05-6), recombinant human TNF-α (R&D Systems, 210-TA), recombinant human interleukin-1β/IL-1F2 (R&D Systems, 201-LB), and angiotensin II (Sigma-Aldrich, A9525).

Antibodies used in this study are listed in Supplementary Table 2. RORα construct was purchased from OriGene (RC 202926). ANGPTL4 promoter was purchased from SwitchGear Genomics (S714374). Plasmids used for functional analysis of the ANGPTL4 promoter activity were generated by using pGL3-Basic vector (Promega, E1751) containing a luciferase reporter gene. Serially deleted mutants of the human ANGPTL4 promoter were PCR amplified with the use of restriction enzyme recognition sites (*Nhe 1/Hind III*) from the ANGPTL4 promoter sequence and inserted into the *Nhe 1/Hind III* sites of the pGL3-Basic vector.

Microarray analysis. We analyzed the gene expression of hMSCs by microarray analysis. The quality and quantity of extracted RNA was assessed by using an Agilent 2100 Bioanalyzer, and RNA was amplified by use of Agilent's Low RNA Input Linear Amplification kit PLUS and hybridized to Agilent Rat expression 4X44K (v3).

Quantitative RT-PCR. In all cases, total RNA was extracted with Trizol Reagent (Life Technologies, 15596018) and converted to cDNA by using an Applied Biosystems High-Capacity cDNA Reverse transcription kit (Life Technologies, 4368814), according to the manufacturer's instructions. Real-time PCR was performed using a QuantiTect SYBR Green PCR kit (Qiagen, 204143) and Corbett Research Rotor-Gene RG-3000 Real Time PCR System. Pre-designed primers for human ANGPTL4, CXCL9, CXCL10, CXCL11, CCR7, CD80, TNF, Granulocyte macrophage colony-stimulating factor (CSF2), IL-1β, IL6, IL8, MIP-1α (chemokine(C-C motif) ligand 3; CCL3), MIP-1β (chemokine(C-C motif) ligand 4; CCL4), and RORα were purchased from Bioneer. Mouse primers are listed in Supplemental Table 2.

Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) was performed using the ChIP Assay Kit (Abcam, ab500) according to the manufacturer's instructions. Briefly, 1×10^7 MSCs were fixed with 10% formaldehyde to cross-link histones to DNA. Cells were lysed with lysis buffer (0.3% NP40, 0.1 mmol/L EDTA, 10 mmol/L HEPES pH 7.9, 10 mmol/L KCl) to enrich nuclei.

Chromatin with chromatin shearing buffer was sheared by sonication to an optimal DNA fragment size of 200-1000 bp. The soluble chromatin supernatant was immunoprecipitated with anti-RORα (Abcam, ab60134), negative control (IgG), and positive control (Histone H3). Immunoprecipitated DNA and input DNA were analyzed by real-time PCR, and the results were presented as percentage of input.

Luciferase reporter assay. For luciferase assay, transfections were performed with Lipofectamine 2000 transfection reagent (Invitrogen, 11668-019) in HEK293T cells. The *Renilla* luciferase vector was co-transfected with ANGPTL4 deletion constructs and RORα for control of transfection efficiency. Promoter activities were measured by using the Dual Glo Luciferase Assay kit (Promega, E1960) and a Sirius Luminometer (Berthold Technologies) according to the manufacturer's instructions.

Transfection of plasmid DNA and siRNA. Human RORα plasmid DNA (OriGene, RC202926) was transfected to MSCs by using X-tremeGENE HP DNA Transfection Reagent (Roche, 6366236001). RNA interference was performed according to the manufacturer's protocols. Briefly, siRNA-control (siCon), ANGPTL4 siRNA (siANGPTL4), and RORα siRNA (siRORα) were purchased from Bioneer. Cells were transfected with siRNA using Lipofectamine RNAiMAX transfection reagent (Invitrogen, 13778150) according to the manufacturer's protocols.

Immunoblot analysis. Cells were washed with ice-cold phosphate-buffered saline and lysed in lysis buffer (20 mmol/L Tris-HCl pH 7.4, 0.1 mmol/L EDTA, 150 mmol/L NaCl, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mg/mL leupeptin) on a rotation wheel for 1 hour at 4˚C. After centrifugation at 10,000 ×*g* for 10 minutes, the supernatant was prepared as a protein extract. Equal concentrations of proteins were fractionated by electrophoresis on 8% or 10% acrylamide gels and were transferred onto a polyvinylidene fluoride membrane (Millipore, IPVH00010) membrane, followed by blotting with primary antibody and conjugated antibody with horseradish peroxidase-

conjugated IgG. Protein expression was detected by using Image Reader (LAS-3000 Imaging System, Fuji Photo Film).

Immunocytochemistry. The cells were fixed in 4% paraformaldehyde for 15 minutes. Then the cell membrane was penetrated by 0.25% Triton X-100 for 10 minutes and blocked by normal goat serum for 1 hour, followed by primary antibody staining at 4°C overnight. Subsequently the cells were incubated with secondary antibodies conjugated with Alexa-488 (Molecular Probes, A11034) or Alexa-594 (Molecular Probes, A11037) for 1 hour, followed by mounting with 4,6-diamidino-2-phenylindole (Molecular Probes, D1306).

Enzyme-linked immunosorbent assay. The protein levels of IP-10 (CXCL10, eBioscience, BMS284INST), IL-6 (Invitrogen, BMS603-2), IL-1β (Invitrogen, BMS6002), and ANGPTL4 (RayBiotech, ELH-ANGPTL4 for human ANGPTL4, and Elabscience, E-EL-M0093 for mouse ANGPTL4) were evaluated by using enzyme-linked immunosorbent assay (ELISA) kits. Inflammatory mediators in mouse plasma were evaluated by Multi-Analyte ELISArray kit (Qiagen, 336161).

Cytokine antibody array. Cytokine expression profiles in the supernatants and cell lysates of MSCs were detected by using the human cytokine array panel A (proteome profiler, R&D Systems, ARY005B) according to the manufacturer's instructions. Briefly, cytokine array membranes were blocked in 2 mL of blocking buffer for 1 hour and then incubated with 2 mL of the samples and antibody mixtures overnight at 4˚C. The sample mixtures were then decanted from each container, and the membranes were washed 3 times with 2 mL of wash buffer at room temperature with shaking. The membranes were then incubated in 1:2000-diluted streptavidin-horseradish peroxidase at room temperature for 30 minutes and the membranes were washed thoroughly and exposed to a peroxidase substrate before imaging.

Mouse peritonitis model. This study was reviewed and approved by the Chonnam National University Institutional Animal Care and Use Committee (CNU IACUC-H-2016-37), and all experiments were performed after approval by a local ethical committee at Chonnam National University Medical School. Peritonitis was induced in mice (7-8 weeks of age) with a peritoneal injection of 4% thioglycollate (BD, 211716). Three days later, 1.5 mL of vehicle (growth medium), coculture medium (CCM) or ANGPTL4 protein was injected into the peritoneum, and 15 hours later, mice were injected intraperitoneally with LPS (100 μg) for 4 hours. The peritoneal macrophages were collected from the peritoneal cavity by washing with PBS and adherent macrophages were assessed by further analyses.

Mouse myocardial infarction model. Male inbred Balb/C nude mice (7-8 weeks of age) were purchased from ORIENT BIO Inc. This study was reviewed and approved by the Chonnam National University Institutional Animal Care and Use Committee (CNU IACUC-H-2016-37), and all experiments were performed after approval by a local ethical committee at Chonnam National University Medical School. Mice were anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg), intubated, and mechanically ventilated. The proximal left anterior descending coronary artery was ligated and the mice were randomly divided into three groups. Upon ligation, 1.5 mL of vehicle (growth medium), CCM, or ANGPTL4 protein was injected into the peritoneum at day 0 and 1 after myocardial infarction (MI). Mouse blood samples were collected and transferred into EDTA tubes, and plasma was obtained by centrifugation at 1,500 × *g* at 4°C for 15 minutes.

Echocardiography. Cardiac function was assessed by echocardiography. At 2 weeks after injection of MSCs, the mice were anesthetized, intubated, and mechanically ventilated. Their cardiac function, including ejection fraction and fractional shortening, was measured by transthoracic echocardiography (15-MHz linear array transducer system; iE33 system, Philips Medical Systems; Amsterdam, Netherland). Fractional shortening was calculated as 100×(LVDd-LVDs)/LVDd (%).

Histological analyses and immunohistochemistry. For histological studies, the heart tissues were harvested, fixed in formalin, and embedded in paraffin blocks at 14 days after MI and recombinant ANGPTL4 protein injection. Cardiac fibrosis was measured by Masson's trichrome staining. For immunohistochemical analysis, slides were treated with 3% hydrogen peroxide in PBS for 10 minutes at room temperature to block endogenous peroxidase activity. After blocking nonspecific binding with 5% normal goat serum (Sigma-Aldrich, NS02L), the slides were incubated with primary antibody for 18 hours at 4°C. Sections were washed with PBS three times, and then incubated for 1 hour with Alexa-Fluor 488 or 594 secondary antibodies. The images were detected by using a Carl Zeiss confocal microscope, and the images were obtained using Zeiss LSM version 3.2 SP2 software (Carl Zeiss).

Flow cytometric analysis. Single cells were isolated from the heart tissues according to a previously described method with minor modifications (6). Harvested hearts were minced and digested in an enzyme mixture solution containing 675 U/mL collagenase I (Sigma-Aldrich, C0130), 187.5 U/mL collagenase XI (Sigma-Aldrich, C7657), 90U DNase I (Sigma-Aldrich, DN25), and 90 U/mL hyaluronidase (Worthington Laboratories, 2592) in PBS with Ca^{2+}/Mg^{+2} for 60 minutes at 37˚C with gentle shaking. After incubation, the digestion mixture was homogenized through a 70-μm nylon mesh. The digestion mixture was centrifuged at 2000 rpm for 15 minutes at 4˚C and the pellet was suspended in 40% Percoll (GE-Healthcare, 17-0891-02) solution and then carefully flushed with 80% Percoll solution under 40% Percoll solution using a pasture pipette. The tubes were centrifuged at 2200 rpm for 25 minutes at room temperature. After Percoll gradient centrifugation, cardiac leukocytes were collected from the layer between the Percoll solutions. Cells were stained with antibodies at 4°C for 15 minutes simultaneously and were washed, resuspended in staining buffer, and analyzed. The antibodies are listed in Supplemental Table 3. Cell fluorescence was measured with a BD FACSCANTO II (BD Biosciences) and data analysis was performed using FlowJo (Tree Star).

Statistical Analysis. All data are expressed as mean ± SEM from at least three independent experiments. Differences between experimental and control groups were analyzed by Student's *t* test or 1-way

ANOVA with Bonferroni's multiple comparisons test using SPSS (SPSS Inc). A P value less than 0.05 was considered significant.

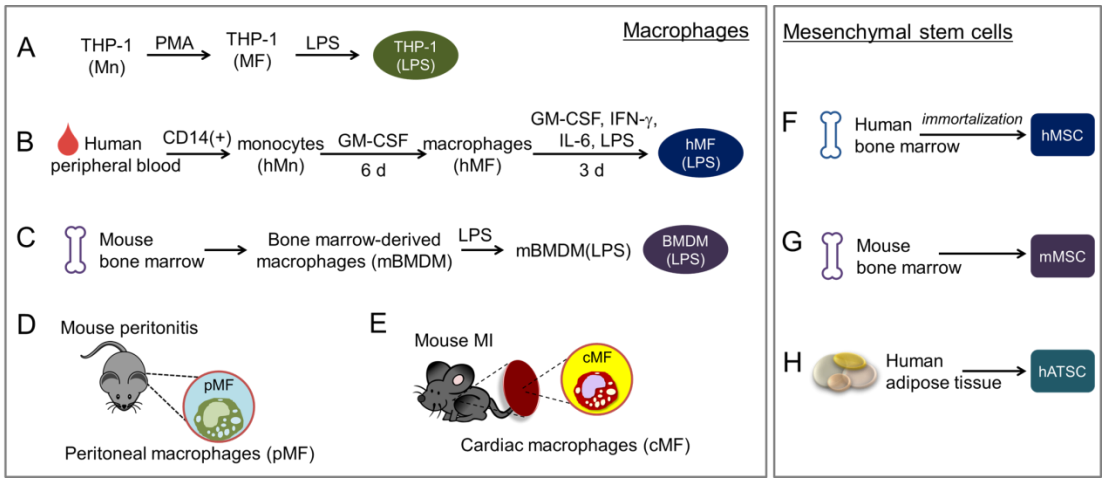

Figure S1. Schematic of macrophages and mesenchymal stem cell types used in this study. (A) Human monocytic THP-1 cells were differentiated to THP-1 macrophages by treatment with PMA for 3 days and then stimulated with LPS. **(B)** Human $CD14^+$ monocytes were isolated from peripheral blood, then differentiated to macrophages by treatment with GM-CSF, and then stimulated with GM-CSF, IFN-γ, IL-6 and LPS. **(C)** Mononuclear cells were collected from mouse bone marrow and differentiated to macrophages (BMDM). **(D)** Peritoneal macrophages (pMF) were isolated from mice with peritonitis. **(E)** Cardiac macrophages (cMF) were isolated from infarcted mouse heart tissue. **(F)** Immortalized human bone marrow-derived mesenchymal stem cells (hMSCs) were provided as described in Materials and Methods. **(G)** Mouse MSCs (mMSCs) were isolated from bone marrow. **(H)** Human adipose tissue-derived MSCs (ATSCs) were isolated from liposuction aspirate fluid.

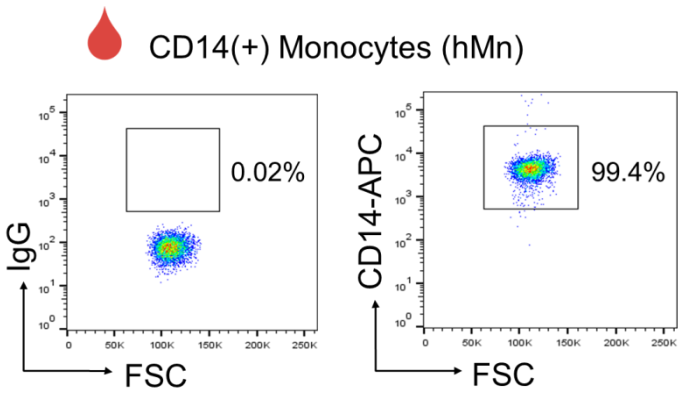

Figure S2. Human macrophages were differentiated from circulating monocytes. Flow cytometry gating strategy for isolating human monocytes. $CD14^{+}$ monocytes were selected for analysis.

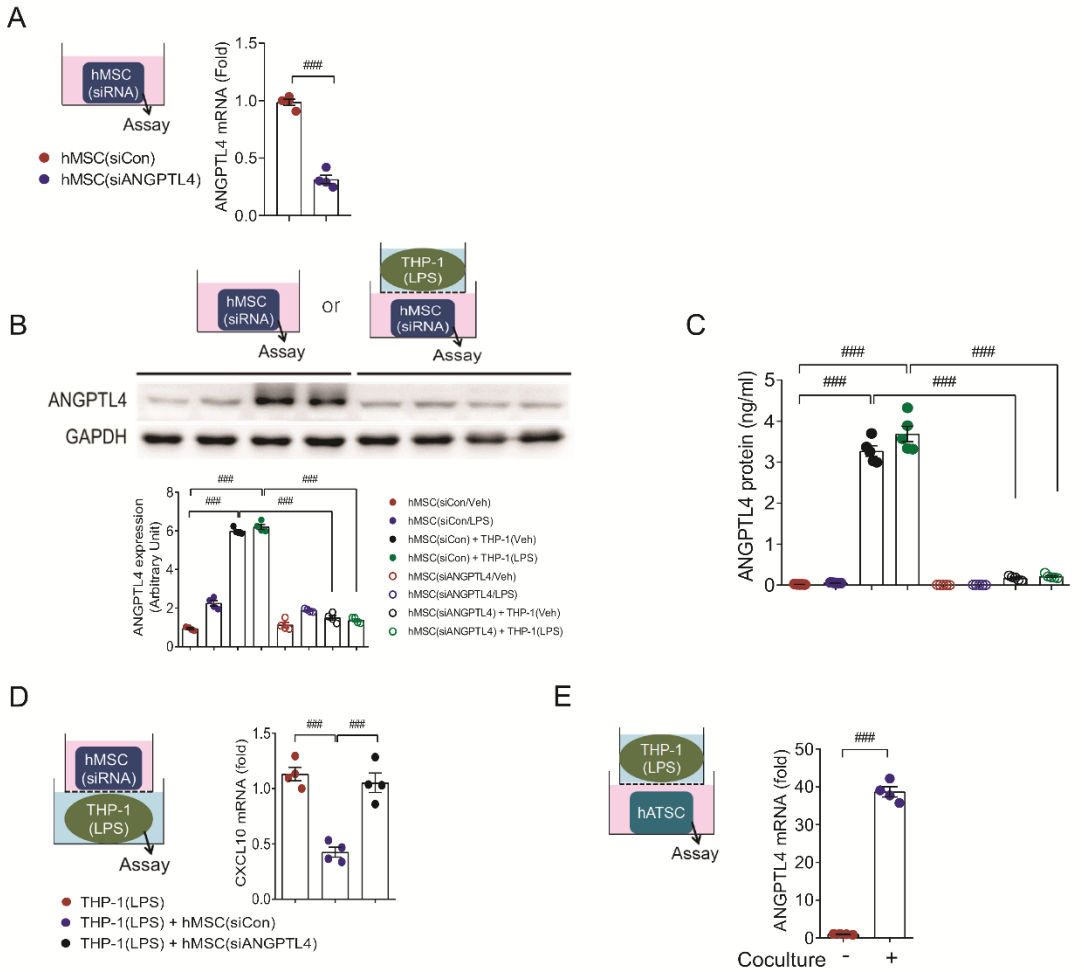

Figure S3. ANGPTL4 knockdown by siRNA in hMSCs did not inhibit CXCL10 induction in macrophages. (E) Human adipose tissue-derived MSCs (ATSCs) showed significant upregulation of ANGPTL4 mRNA by coculture with LPS-stimulated macrophages. ATSCs isolated from human adipose tissue were cocultured with LPS-stimulated THP-1 macrophages for 24 hours, and mRNA of ANGPTL4 was measured by real-time PCR. n = 4. **(A)** ANGPTL4 mRNA was substantially reduced in hMSCs by knockdown with siRNA. n = 4. In hMSCs **(B)** and cultured medium **(C)**, ANGPTL4 protein was upregulated by coculture with either unstimulated THP-1 macrophages or LPS-stimulated THP-1 macrophages. ANGPTL4 knockdown did not result in ANGPTL4 induction even with coculture with THP-1 macrophages. LPS-treated hMSCs did not induce ANGPTL4. n = 5. **(A)** The inhibitory effect of hMSCs on pro-inflammatory CXCL10 in THP-1 macrophages was abolished by coculture with hMSCs with ANGPTL4 knockdown. n = 4. #P < 0.05, ##P < 0.01, ###P < 0.001, ns, not significant (by Student's t test or 1-way ANOVA with Bonferroni's multiple comparisons test).

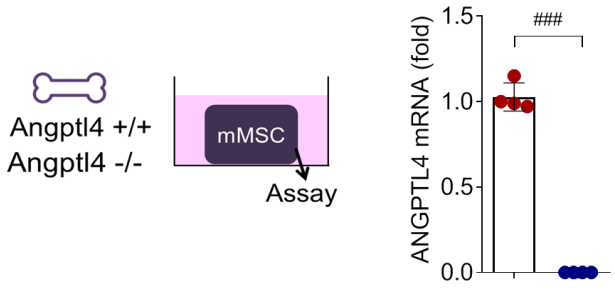

Figure S4. Mesenchymal stem cells were isolated from ANGPTL4 mouse bone marrow. Mouse bone marrow-derived mesenchymal stem cells (mMSCs) were isolated from ANGPTL4 knockout mouse and confirmed by real-time PCR. ###P < 0.001 (by Student's t test).

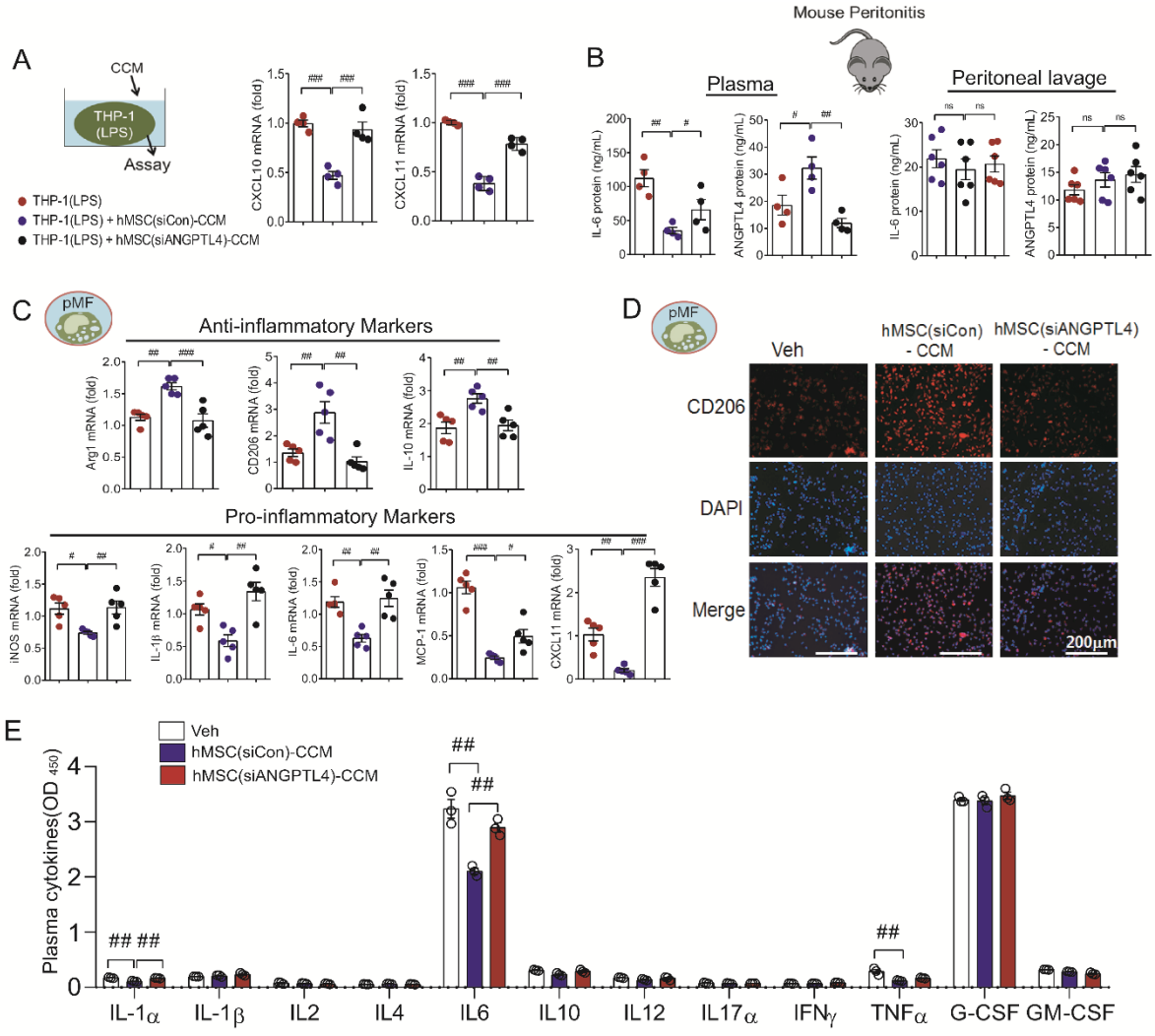

Figure S5. Coculture medium of MSCs and macrophages suppresses peritoneal macrophage activation dependently on ANGPTL4. (A) Coculture medium (CCM) was collected from hMSCs cocultured with THP-1 macrophages. Before coculture, hMSCs were transfected with siRNA to knockdown ANGPTL4. In LPS-stimulated THP-1 macrophages, pro-inflammatory genes CXCL10 and CXCL11 were not reduced by treatment with CCM from hMSCs with ANGPTL4 knockdown. n = 4. **(B)** Peritonitis was induced by thioglycollate injection for 3 days. CCM from MSCs transfected with control siRNA or ANGPTL siRNA was injected into the peritoneum followed by LPS stimulation. ANGPTL4 and IL6 levels of mice with peritonitis were measured by ELISA in blood (n = 4) and peritoneal lavage (n = 6). **(C)** Pro-inflammatory and anti-inflammatory genes of peritoneal macrophages were assessed. n = 5. **(D)** CD206, an anti-inflammatory marker, was visualized by immunofluorescence staining in peritoneal

macrophages. **(E)** Inflammatory mediators in the plasma were analyzed by multiplex ELISA. n = 3. #P < 0.05, ##P < 0.01, ###P < 0.001, ns, not significant (by Student's t test or 1-way ANOVA with Bonferroni's multiple comparisons test).

Abbreviation: Veh, vehicle; Arg1, Arginase 1.

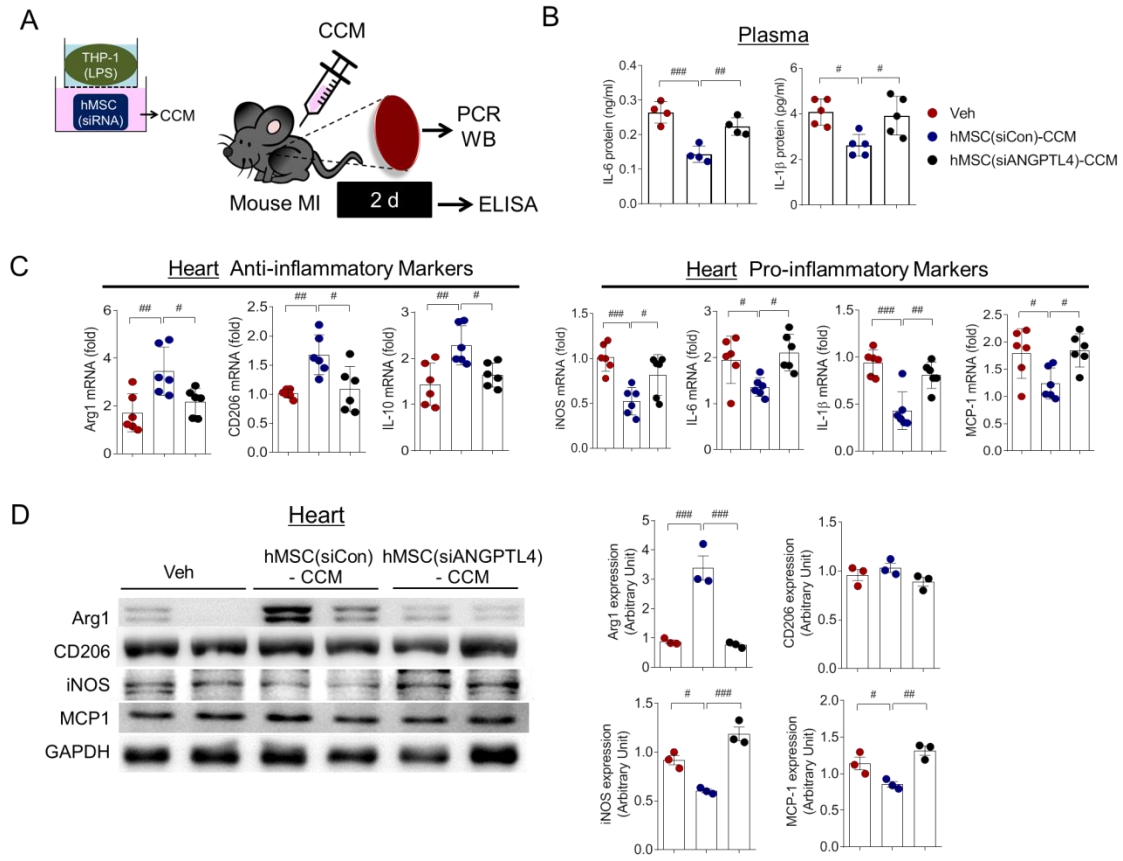

Figure S6. Cardiac inflammation was not attenuated by coculture medium collected from MSCs with ANGPTL4 knockdown. (A) MI was induced by coronary artery ligation, and then coculture medium (CCM) from hMSCs transfected with either siRNA control or siANGPTL4 was injected peritoneally. Heart tissues and blood were collected after 2 days for further studies. **(B)** Circulating IL-6 (n = 4) and IL-1β (n = 5) were not reduced in the ANGPTL4 knockdown hMSC-CCM group. **(C)** The expression levels of inflammatory markers in the heart tissue were assessed by real-time PCR. n = 6. **(D)** Anti-inflammatory proteins Arg1 and CD206, and pro-inflammatory iNOS and MCP1 were assessed by Western blot. n = 3. Intensity quantification is representative of mean ±SEM. Anti-inflammatory activity was not observed in the ANGPTL4 knockdown hMSC-CCM group. #P < 0.05, ##P < 0.01, ###P < 0.001, ns, not significant (by 1-way ANOVA with Bonferroni's multiple comparisons test).

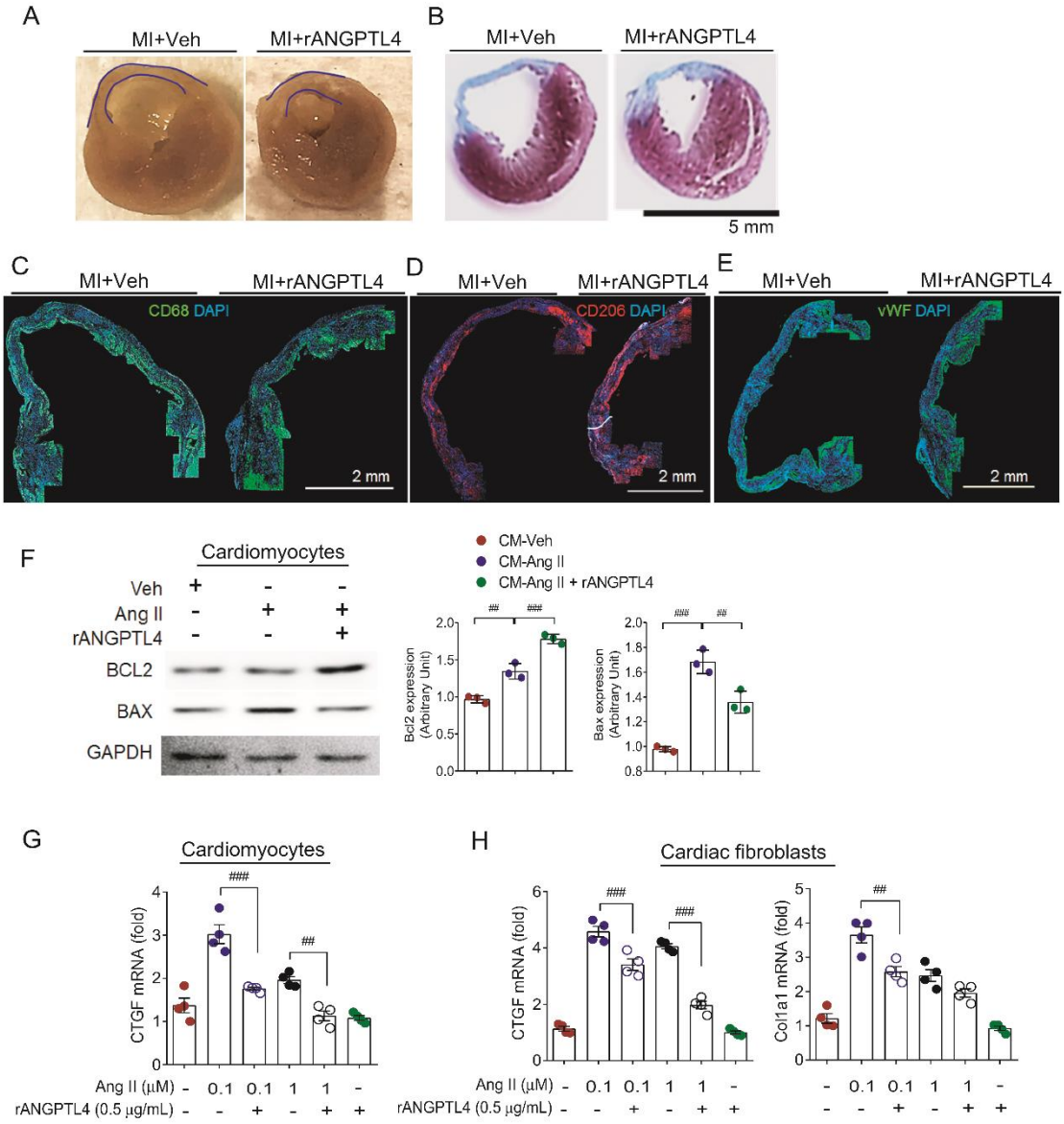

Figure S7. Cardiac inflammation and function were improved by ANGPTL4 treatment in a mouse myocardial infarction model. (A) Representative section images of heart tissue after 2 weeks. The blue line indicates the left ventricular wall thickness. **(B)** Representative images of Masson's trichrome staining-visualized cardiac fibrosis. Scale bar = 5 mm. CD68-positive macrophages **(C)**, anti-inflammatory CD206 **(D)**, and angiogenic vWF **(E)** were stained in the infarcted myocardium from mice treated with vehicle or 200 ng ANGPTL4. Scale bar = 2 mm. **(F)** In angiotensin II (AngII)-stimulated neonatal rat cardiomyocytes, the effect of ANGPTL4 (0.5 μg/mL) on prosurvival BCL2 and apoptotic BAX was examined by Western blot. Intensity quantification is representative of mean ±SEM.

n = 3. **(G)** In AngII-stimulated neonatal rat cardiomyocytes, the effect of ANGPTL4 on profibrotic connective tissue growth factor (CTGF) was assessed by real-time PCR. n = 4. **(H)** In AngII-stimulated cardiac fibroblasts, the effect of ANGPTL4 on collagen type 1 α1 chain (Col1a1) and CTGF was assessed by real-time PCR. n = 4. #P < 0.05, ##P < 0.01, ###P < 0.001, ns, not significant (by 1-way ANOVA with Bonferroni's multiple comparisons test).

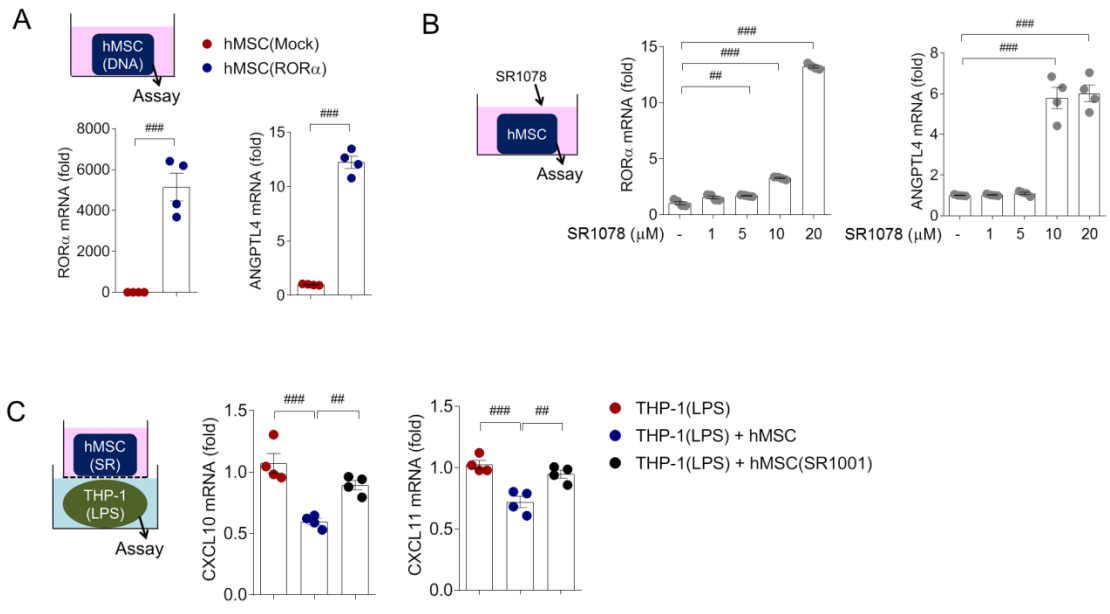

Figure S8. RORα contributed to the effect of ANGPTL4 in MSCs. **(A)** Overexpression of RORα induced ANGPTL4 in MSCs. n = 4. **(B)** RORα and ANGPTL4 were induced by treatment with RORα agonist SR1078 in a dose-dependent manner in MSCs. n = 4. **(C)** MSCs were pretreated with SR1001, an inverse agonist of RORα, before coculture with macrophages. After 24 hours, expression of CXCL10 and CXCL11 mRNA in macrophages was assayed. n = 4. #P < 0.05, ##P < 0.01, ###P < 0.001, ns, not significant (by 1-way ANOVA with Bonferroni's multiple comparisons test).

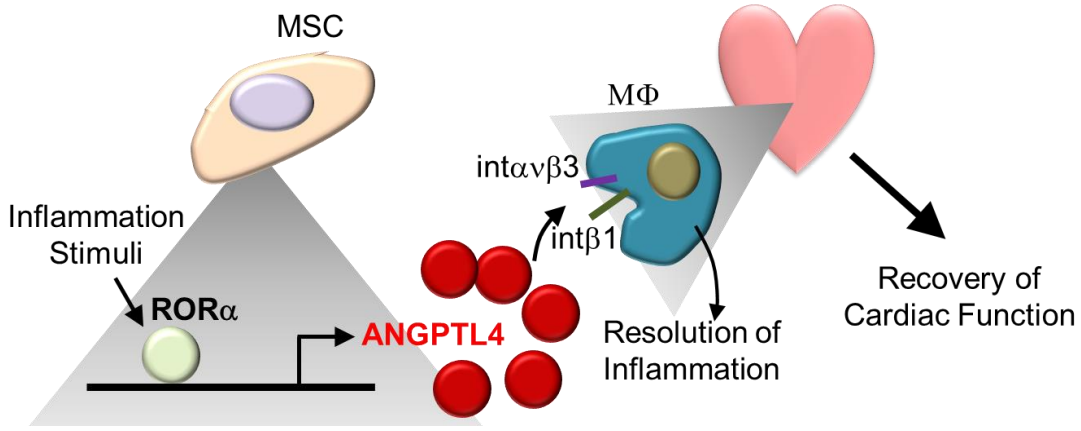

Figure S9. Schematic representation of the relationship between mesenchymal stem cells and inflammatory macrophages during cardiac injury in mice.

Supplemental Table 1. List of highly expressed genes (fold increase vs. mesenchymal stem cell alone) in human mesenchymal stem cells cocultured with either THP-1 macrophages or LPS-stimulated THP-1 macrophages for 24 hours.

Gene symbol	hMSC+THP	hMSC+THP(LPS)	Accession
FRG2B	20.013742	117.79628	NM_001080998
MTSS1	78.99905	115.89478	NM_014751
MLC1	12.910219	110.87978	NM_015166
CSF3	26.063862	103.80238	NM_000759
IL1RN	39.78224	102.29219	NM_173843
IRG1	14.826504	95.768974	XM_001133269
KPRP	8.060947	93.36114	NM_001025231
RSAD2	1.7462701	92.58964	NM_080657
CCL8	1.2673303	92.349686	NM_005623
IL3RA	19.06517	90.95484	NM_002183
CXCL1	61.10998	90.61722	NM_001511
RGS16	52.338238	87.20401	NM_002928
SYT7	56.867867	86.482605	NM_001252065
THBD	12.632683	83.6733	NM_000361
BIRC3	51.4013	79.457306	NM_001165
LCE3D	3.3024528	75.75361	NM_032563
AREG	51.77044	75.7262	NM_001657
STC1	153.46706	75.3867	NM_003155
SSTR2	5.191967	73.438736	NM_001050
PTGS1	48.625225	71.869934	NM_000962
MUC20	2.929484	71.061554	NM_001098516
GBP5	2.5838315	69.04816	NM_052942
ZBP1	0.9983463	68.7907	NM_030776
CCL4	23.309155	65.36921	NM_002984
RRAD	5.3951774	63.416737	NM_004165
AMPD3	45.102108	61.01916	NM_001025390
OR5K2	1.8623062	59.38474	NM_001004737
IL32	13.562407	56.298466	NM_001012633
ISG20	1.3107475	55.649574	NM_002201
MTSS1	55.000267	55.36222	NM_014751
CTSS	11.129068	54.720127	NM_004079
OR10X1	1.2673303	54.15676	NM_001004477
TFPI2	59.33184	48.125156	NM_006528
ANGPTL4	82.74972	45.730583	NM_139314
ESM1	24.153831	43.54558	NM_007036
MYZAP	1.2673303	38.52825	NM_001018100
SSTR2	5.447201	37.957775	NM_001050
MAEL	1.2673303	37.237827	NM_032858
TRIB3	14.507488	37.06422	NM_021158
SLCO4A1	35.19703	27.917551	NM_016354
PRR9	1.2673303	27.13163	NM_001195571
IL18BP	0.7729766	27.042696	NM_173042
TRIM63	20.09952	26.838991	NM_032588
CLCA4	1.2673303	25.301376	NM_012128
FAM65C	56.665775	25.262968	NM_080829
GBP7	1.2673303	25.17499	NM_207398
PTGS1	25.383379	25.114967	NM_000962
C4orf7	2.253574	25.109753	NM_152997
OR8D4	1.2673303	25.089544	NM_001005197
LOC100128977	6.6053166	24.96567	NR_024559
RASGEF1A	3.1363468	24.915886	NM_145313

SYCE3	1.9504256	24.770735	NM_001123225
HLA-DQA1	3.0240445	24.638544	NM_002122
PRAMEF1	1.2673303	24.566725	NM_023013
FAM90A7	1.7931097	24.428045	NM_001136572
TGM2	10.252964	24.40785	NM_004613
GIMAP5	1.9266205	24.201519	NM_018384
DUSP6	20.926975	24.14736	NM_001946
SLITRK4	9.098912	23.873922	NM_173078
IRAK2	14.622249	23.800875	NM_001570
ADAMTS4	22.140358	23.771454	NM_005099
RASGEF1B	1.2673303	23.553087	NM_152545
ZBP1	1.2673303	23.533936	NM_001160419
NOS2	1.2673303	23.419611	NM_000625
LCP2	1.8960947	22.827147	NM_005565
FGF7	16.747692	19.553776	NM_002009
INSL4	1.2673303	19.335701	NM_002195
LOC339685	8.38755	19.215652	NR_038922
DDIT3	6.1530395	19.147018	NM_004083
BST2	0.9471841	18.856667	AK291099
BST2	0.8987768	18.818966	NM_004335
XKRY2	1.2673303	18.78471	NM_001002906
LAMC2	13.116164	18.779324	NM_018891
ATF3	3.8426325	18.688995	NM_001040619
TNFAIP3	10.78105	13.81545	NM_006290
FCRLA	10.494733	13.792599	NM_032738
MREG	10.194867	13.729449	NM_018000
NLRP3	16.090061	13.694563	NM_001079821
LOC284379	2.2892432	12.516126	NR_002938
NFKBIA	7.1327467	6.286956	NM_020529
XIRP2	1.2673303	6.2843423	NM_152381
PLIN2	5.2909994	6.029651	NM_001122
EGR2	2.982881	6.028266	NM_000399
HIVEP2	3.4387348	5.883344	NM_006734
TBRG1	3.253084	5.8817406	NM_032811
SEMA5B	3.2746005	5.8704023	NM_001031702
LIPG	3.4304273	5.478354	NM_006033
LMO4	4.822775	5.477839	NM_006769
NR4A2	4.672957	5.0049267	NM_006186
ZSWIM4	3.914665	4.9976964	NM_023072
LAT2	3.4901059	4.969203	NM_032464
SGTB	2.7406063	4.321316	NM_019072
ABCC9	2.0198138	4.3148656	NM_005691
ZXDA	1.8099318	4.277924	NM_007156
RUNX1	3.19841	4.2706695	NM_001001890
LMLN	1.1112436	4.264184	NM_033029
RORA	5.0155215	3.9810777	NM_134260
ZFHX2	2.9512088	3.9726336	NM_033400
AIFM2	3.1364167	3.9704077	NM_032797
GAB4	1.2673303	3.9665504	NM_001037814

Human bone marrow-derived mesenchymal stem cells (hMSCs) were cocultured with PMA-differentiated THP-1 macrophages. THP-1 macrophages were either unstimulated or LPS-stimulated.

Genes regulated at the mRNA level were examined using an Agilent microarray to compare cDNA expression profiles between hMSCs and macrophage-cocultured hMSCs.

Supplemental Table 2. List of real-time PCR primers for mouse genes

Gene symbol	Sequences
Angptl4	Forward: 5'-GGACCTTAACTGTGCCAAGA-3'
	Reverse: 5'-CGTGGGATAGAGTGGAAGTATTG-3'
Arg 1	Forward: 5'-GATTATCGGAGCGCCTTTCT-3'
	Reverse: 5'-AAGAATGGAAGAGTCAGTGTGG-3'
Ccl2 (MCP-1)	Forward: 5'-CTCACCTGCTGCTACTCATTC-3'
	Reverse: 5'-TTACGGCTCAACTTCACATTCA-3'
CD206 (MRC1)	Forward: 5'-GGCGAGCATCAAGAGTAAAGA-3'
	Reverse: 5'-CATAGGTCAGTCCCAACCAAA-3'
CD163	Forward: 5'-TATGATGGGTGTGAACCACG -3'
	Reverse: 5'-CAGTGAGCT TCCCGTTCACC-3'
Gapdh	Forward: 5'-GGGTGTGAACCACGAGAAATA -3'
	Reverse: 5'-GTCATGAGCCCTTCCACAAT-3'
Il10	Forward: 5'-ACAGCCGGGAAGACAATAAC-3'
	Reverse: 5'-CAGCTGGTCCTTTGTTTGAAAG-3'
Il1β	Forward: 5'-GGTGTGTGACGTTCCCATTA-3'
	Reverse: 5'-TCCTGACCACTGTTGTTTCC-3'
Il6	Forward: 5'-ATCCAGTTGCCTTCTTGGGACTGA-3'
	Reverse: 5'-TTGGATGGTCTTGGTCCTTAGCCA-3'
Nos2	Forward: 5'-TCACCTAGG GCAGCCGA-3'
	Reverse: 5'-TCCGRGGCAAAGCGAGCCAG-3'

Supplemental Table 3. Sources of primary antibodies

Antibody	Supplier	Catalogue #	Application
ANGPTL4	Sigma-Aldrich	SAB1407445	WB 1:1000
ARG 1	Cell Signaling Technology	93668	WB 1:2000
iNOS	Cell Signaling Technology	2977	WB 1:2000
GAPDH	Santa Cruz Biotech	sc-32233	WB 1:2000
IkBα	Cell Signaling Technology	9242	WB 1:2000
MCP 1	Santa Cruz Biotech	sc-52701	IHC 1:200
RORα	Abcam	ab60134	ChIP
	ThermoFisher scientific	PA5-23267	WB 1:1000
Integrin β1	Abcam	ab24693	Neu 20 μg/mL
Integrin αvβ3	Millipore	MAB1976	Neu 20 μg/mL
BAX	Santa Cruz Biotech	sc-526	WB 1:1000
BCL2	Santa Cruz Biotech	sc-492	WB 1:1000
CD68	Biomedicals	T3003	IHC 1:100
	Abcam	ab955	IHC 1:100
von Willebrand factor (vWF)	Sigma-Aldrich	F3520	IHC 1:100
CD206	Abcam	ab64693	IHC 1:100
CD45(30-F11), Pacific Blue	eBioscience	MCD4528	FC
CD11b(M1/70), PE	eBioscience	12-0112	FC
F4/80(BM-8), PerCP-Cyanine5.5	eBioscience	45-4801-80	FC
CD80(B7-1), FITC	eBioscience	11-0801	FC
Ly6C(HK1.4), FITC	BioLegend	128005	FC
CD14(HCD14)-APC	BioLegend	325608	FC
Human IgG Fc(HP6017)	BioLegend	409303	FC

ChIP: Chromatin immunoprecipitation; FC: Flow cytometry; IHC: Immunohistochemistry; Neu: Neutralizing; WB: Western blot

References

1. Kim HK, Youn BS, Shin MS, Namkoong C, Park KH, Baik JH, et al. Hypothalamic Angptl4/Fiaf is a novel regulator of food intake and body weight. *Diabetes.* 2010;59(11):2772-80.
2. Cho DI, Kim MR, Jeong HY, Jeong HC, Jeong MH, Yoon SH, et al. Mesenchymal stem cells reciprocally regulate the M1/M2 balance in mouse bone marrow-derived macrophages. *Experimental & molecular medicine.* 2014;46:e70.
3. Bhattacharjee J, Das B, Mishra A, Sahay P, and Upadhyay P. Monocytes isolated by positive and negative magnetic sorting techniques show different molecular characteristics and immunophenotypic behaviour. *F1000Research.* 2017;6:2045.
4. Zarif JC, Hernandez JR, Verdone JE, Campbell SP, Drake CG, and Pienta KJ. A phased strategy to differentiate human CD14+monocytes into classically and alternatively activated macrophages and dendritic cells. *BioTechniques.* 2016;61(1):33-41.
5. Kim YS, Jeong HY, Kim AR, Kim WH, Cho H, Um J, et al. Natural product derivative BIO promotes recovery after myocardial infarction via unique modulation of the cardiac microenvironment. *Scientific reports.* 2016;6:30726.
6. Sager HB, Hulsmans M, Lavine KJ, Moreira MB, Heidt T, Courties G, et al. Proliferation and Recruitment Contribute to Myocardial Macrophage Expansion in Chronic Heart Failure. *Circulation research.* 2016;119(7):853-64.