SUPPLEMENTAL MATERIAL

Materials & Methods

Experimental Mice

All animal procedures were approved by the UK Home Office (PPL70/8944). C57BL/6J mice were purchased from Harlan, Blackthorn, Bicester, UK. RFP mice (Stock No: 005884) and leptin receptor deficient mice (Stock No: 00697) were purchased from the Jackson Laboratory, USA. Male wild-type ($Lepr^{+/+}$) and db/db ($Lepr^{-/-}$) mice aged 10-12 weeks were selected for surgery. Heterozygous ($Lepr^{+/-}$) mice were not used. All mice were kept on a chow diet in a 12-hour light and 12-hour dark environment at 25°C in the biological service unit (BSU) at the James Black Centre of King's College London.

Mouse Adventitial Progenitor Cell Culture

The procedure used for adventitial progenitor cell culture was similar to that described previously.[1] In brief, the aortic arch and root, as well as part of the heart from $Lepr^{+/+}$ or $Lepr^{-/-}$ mice, were harvested under sterile conditions. Adventitial tissues were carefully collected under a dissection microscope by removing the aortic media, intima and heart tissue. The adventitial tissues were then cut into pieces and seeded onto a gelatin-coated T25 flask. The flask was incubated at 37℃, 5% CO2 upside down for 2 to 3 hours. After the attachment of adventitial tissues onto the flask, complete stem cell medium (Dulbecco's Modified Eagle's Medium (DMEM ATCC, 30-2002)) with leukemia inhibitory factor (10 ng/mL, Merck Millipore, LIF1050), 2-mercaptoethanol (0.1 mM, GIBCO), 100U/mL penicillin (GIBCO), 100U/mL streptomycin (GIBCO) and 2 mM L-glutamine (GIBCO) was added for 5 days. When the cells reached 90% confluency, they were washed by PBS and trypsinized. The cells were passaged at a ratio of 1:4 every two or three days. Medium was changed every 2 days.

Mouse Vascular Smooth Muscle Cell Culture

The procedure used for vascular smooth muscle cells' culture was similar to that described previously.[1] In brief, the aortic arch and root, as well as part of the heart from $Lepr^{+/+}$ or $Lepr^{-/-}$ mice, were harvested under sterile conditions. Medial tissues were carefully collected under a dissection microscope by removing the aortic adventitia, intima and heart tissue. The media was then cut into pieces and seeded onto a 0.04% gelatin-coated T25 flask. The flask was incubated at 37℃, 5% CO2 upside down for 2 to 3 hours. After the attachment of medial tissues onto the flask, complete medium (Invitrogen 11960085, Dulbecco's Modified Eagle's Medium, high glucose) with 10% fetal bovine serum (GIBICO, 10270), 100U/mL penicillin (GIBCO, 15140122), 100U/mL streptomycin (GIBCO, 15140122) and 2 mM L-glutamine (GIBCO, 25030081) was added for 5 days. When the cells reached 90% confluency, they were washed by PBS and trypsinized. The cells were passaged at a ratio of 1:3 every two or three days. Medium was changed every 2 days.

Sca-1^+ Progenitor Cell Isolation and Culture

When reached 90% confluency, the primary Sca-1^+ adventitial progenitor cells were isolated using the Sca-1^+ microbeads kit (Miltenyi Biotec, Bergisch Gladbach, 130-092-529) according

to the manufacturer's instruction. Sca-1$^+$ progenitor cells were selected using a magnetic cell separator. The Sca-1$^+$ progenitor cells were cultured on 2% gelatin-coated flasks in complete stem cell medium (Dulbecco's Modified Eagle's Medium (DMEM ATCC, 30-2002)) with 10% Embryomax, leukemia inhibitory factor (10 ng/mL) and 2-mercaptoethanol (0.1 mM), penicillin (100 U/mL) and streptomycin (100 mg/mL) for both *in vitro* and *in vivo* studies.

Phenotyping of Cultured Sca-1$^+$ Progenitor Cells And Smooth Muscle Cells

Cells were isolated from the adventitial of Lepr$^{+/+}$ and Lepr$^{-/-}$ (db/db) mice, and kept growing for few passages. The heterogeneous Sca-1$^+$ adventitial cells were purified by applying anti-Sca-1 microbeads. Flow cytometry was performed within 3 passages after sorting. Cultured lepr$^{+/+}$ and Lepr$^{-/-}$ Sca-1$^+$ progenitors were harvested by using scraptase (GenDEPOT, CA110-010). Cells were then centrifuged and resuspended in cold PBS, followed by a 30-minute staining on ice with following antibodies: Anti-Sca-1-FITC (clone D7, abcam, ab25031), Anti-CD45-APC-Cy7 (clone 30-F11, biolegend, 103115), Anti-CD29-PE (clone HMβ1-1, BD Pharmingen, 562801), Anti-CD11b-BV785 (clone M1/70, biolegend, 101243), Anti-CD31-BV510 (clone MEC13.3, BD Horizon, 563089), Anti-CD140a-APC (clone APA5, eBioscience, 17-1401-81), CD105-PE (clone MJ7/18, BioLegend, 120407), CD117(ckit)-PE (clone 2B8, eBioscience, 12-1171-82), CD146-FITC (clone P1H12, eBioscience, 11-1469-41), Flk1-PE (clone AVAS 12α1, BD Pharmingen, 555308), anti-NG2 antibody (rabbit polyclonal, Abcam, ab129051), anti-Lepr (rabbit polyclonal, Sigma, HPA030899). Cells stained with secondary control only were used as negative control. Cells were analyzed with BD accuri C6 or BD LSR fortessa II (Becton Dickinson) flow cytometers.

Transwell Assay

Cell migration assessments were performed using transwell inserts with 8.0 μm micron pore membrane filters in a 24-well plate (#3422, Corning Life Science, USA) as established previously.[2] Sca-1$^+$ progenitor cells were trypsinized and transferred into the upper chamber at 10^5 cells/mL in serum-free media with or without inhibitors (hoelzel-biotech, CYT354 / Santa Cruz, WP1066 / Merck Millipore, PD98059). Meanwhile, the lower chamber was loaded with 800 mL of serum-free media with treatment of leptin (Peprotech, 450-31). After 16-hour incubation, the upper side of the filters was carefully washed using a cotton bud to remove any non-migratory cells. Migratory cells on the underside of the filters were fixed with 4% PFA for 15 minutes, followed by a 15-minutes staining with 1% crystal violet at room temperature. Data is representative of mean cell numbers of migratory progenitor cells in 5 random fields at 20x magnification. For the experiments involving inhibitors or antagonists, Sca-1$^+$ progenitor cells were cultured with the respective chemicals in the upper chamber only.

Scratch-wound Assay

1x10^4 cells per well of Sca-1$^+$ progenitor cells were seeded in a 12-well plate in complete culture media. Once the cells reached 90% confluency, a scratch wound was made from top to bottom using a 1 mL pipette tip. The pipette was kept at a consistent angle and pressure during the scratch to ensure uniformity of width of the scratch. The wells were carefully washed twice using PBS to remove any cell debris caused by the "scratch" procedure.

Treatment and/or inhibitors were added into wells with serum-free media. After 16-hour incubation, cells were fixed with 4% PFA and stained with 1% crystal violet for 15 minutes at room temperature. The migration of Sca-1^+ progenitor cells into the “wound” area was evaluated using a phase contrast microscope. Data indicated are the mean area occupied by migratory progenitor cells in 5 random fields of view at 10x magnification.

RNA Extraction

Cells after overnight starvation in serum-free medium were treated with 100 ng/mL leptin for 5 min, 15 min, 30 min, 1 h and 4 h. Total RNA extraction from Sca-1^+ progenitor cells was performed by applying RNeasy Mini kit (QIAGEN Inc., 74106) According to manufacturer’s instruction, cells were washed twice by PBS, disrupted by proportional amount of RLT lysis buffer and then scraped off from the 6-well plate. The lysate was transferred into a mini QIAshredder spin column and centrifuged at full speed for 2 minutes. High-molecular weight DNA and other substances in lysates were hence removed. Same volume of 70% ethanol was added to the lysate and the mixture was transferred to RNeasy mini column for a 30-second centrifuge at full speed. The flow was discarded and 700μL of RW1 was added to RNeasy mini column. After 30-second centrifuge, the flow was discarded and 500μL of RPE was added to the column twice for washing away the ethanol. The flow was the discarded and the column was centrifuged within a new collection tube for 2 minutes at full speed to ensure no solution outside the column. At last, the RNeasy mini column was transferred into a new 1.5 mL RNA-free tube and 40μL DEPC water (Invitrogen) was added to the membrane of the column, followed by 1-minute centrifuge at full speed.. The RNA concentration was measured by a nanodrop spectrophotometer ND-1000 (Thermo Scientific, UK) at the absorbance at 280nm.

Reverse Transcription (RT)

Reverse transcription was achieved by QuantiTect Reverse Transcription Kit (Qiagen, 205311), according to the manufacturer’s instructions. Briefly, 1 μg of RNA template, 2 μL of gDNA wipeout buffer and enough volume of RNase-free water were mixed in the tube with a total volume 14 μL. The tube was placed in a RT-PCR machine (TECHNE TC-412, Bibby Scientific, UK) at 42℃ for 2 minutes, after which 1 μL of RT enzyme, 1 μL of primer mix and 4 μL of RT buffer, making up to 6μL of reaction volume, was added to the tube mentioned above. Then the mixture was incubated at 42℃ for 15 minutes and subsequently 95℃ for 3 minutes. The cDNA obtained was diluted into 100 μL by using DEPC-treated water, acquiring a final concentration of 10 ng/μL.

Quantitative Real-Time Polymerase Chain Reaction (qPCR)

Quantitative real-time PCR was performed by applying SYBR green system (Qiagen, 204057). The target gene was amplified in a duplex in 20 μL PCR mixtures (10 μL Sybr Green, 2 μL cDNA template, 1.6 μL optimized primers and 6.4 μL DEPC water) which was loaded into a 96-well plate (Eppendorf White, Eppendorf, UK). The plate was centrifuged at 1000 rpm for 5 minutes before running the program in qPCR machine. Ct values were established using EPPENDORF Mastercycler ep realplex. GAPDH served as an endogenous control. Sequences of primer sets used in this experiment are listed in Table S1.

Primers were designed by using DNA Integrated Technologies (IDT). (http://eu.idtdna.com/scitools/Applications/RealTimePCR/)

Conventional Polymerase Chain Reaction (PCR)

Conventional polymerase chain reaction was performed in this project for the identification of $Lepr^{-/-}$ mouse. 50 ng cDNA was amplified to the corresponding DNA by applying Taq DNA polumerase (Invitrogen, 1034253). The products were transferred into 2% agarose gel and observed by the Biospectrum AC Imaging system and Vision worksLS software. All primers for the identification of mice were provided by Jackson laboratory, USA. The sequences of the primers were listed in Table S2.

Western Blot Analysis

Femoral artery or Sca-1^{+} progenitor cells with or without treatment were lysed using RIPA buffer (Life Tech, 89901) with phosphatase inhibitor tablets (Roche, 04906845001,) and protease inhibitors (Roche, 11873580001). The lysate was sonicated using a Branson Sonifier 150 at level 1 for 8 seconds twice prior to 45-minutes incubation on ice. The lysate was then centrifuged at 15000 g for 10 minutes at 4 ºC. The supernatant was collected and transferred to a new 1.5 mL tube. The concentration of proteins was measured by performing Biorad Protein Assay (BIO-RAD, 5000006). 20 µg of lysate mixed with SDS loading buffer was loaded into a NuPage 4-12% Bis Tris-gel immersed in NuPage MOPS SDS running buffer, followed by standard Western blot procedures. Primary antibodies against pJAK2 (Cell signaling, 3771S, 1:1000), JAK2 (Cell signaling, 3230, 1:1000), pSTAT3 (Cell Signaling, 9131S, 1:1000), STAT3 (Cell signaling, 4904, 1:1000), pERK 1/2 (Santa Cruz, sc-16982-R, 1:500), ERK 1/2 (Cell Signaling, 4695P, 1:1000), pOBr (Invitrogen, PA5-64638, 1:1000), OBR (Invitrogen, PA1-053,1:1000), pFAK (Abcam, ab39967, 1:600), FAK (Santa Cruz, sc557, 1:600) and GAPDH (Santa Cruz, sc25778, 1:1000) were used to detect the respective proteins. The ECL detection system (Invitrogen, RPN2106) was used to detect the membrane-bound primary antibodies.

MAPK Pathway Phosphorylation Array

The detection of phosphorylated MAPK-related protein was performed by using MAPK pathway phosphorylation array (AAHMAPK1-4, Raybiotech). The proteins of cells with or without treatment were collected by applying lysis buffer provided in the kit. The concentration of cell lysate was measured by using detection buffer provided in the kit. Array membrane was put into a well of the incubation tray and incubated by blocking buffer for 30 minutes at room temperature. Discard the blocking buffer and pipette 1mL of diluted sample into each well and incubate for 2 hours at room temperature. After two times washes, added 1 mL of the well-mixed detection antibody cocktail into each well for 2 hours at room temperature. After two times washes, 2mL of 1x HRP-Anti-Rabbit IgG was added into each well for 2 hours at room temperature. After two times washes, the membrane was well cleaned and incubated with 500 µL of the well-mixed detection buffer for 2 minutes at room temperature. The membrane was analyzed by applying an X-ray film or a chemiluminescence imaging system. The proteins detected in this array were listed below.

Histological Analysis

Femoral artery was fixed with 4% formalin overnight at 4°C prior to a machine-based dehydration. The dehydrated samples were embedded in paraffin and subsequently cut into 5 μm sections. H&E staining was performed using a standard protocol with Hematoxylin and Eosin for morphology analysis.

Cell Proliferation ELISA, BrdU

BrdU assay was performed using cell proliferation ELISA kit (Roche, 11669915001) according to a standard protocol provided by manufactory. Briefly, cells were cultured with leptin for different time points and then labelled with BrdU for 2 hours in a humidified atmosphere at 37°C. Incubation with Fixdenat solution and anti-BrdU POD were subsequently performed. After the three-time washing step, substrate reaction was applied and the absorbance of samples was measured at 450 nm.

Mouse Leptin ELISA

The concentration of serum leptin was measured by applying Mouse/Rat Leptin Quantikine ELISA Kit (R&D systems, # MOB00) according to the standard protocol provided by manufactory. Briefly, blood from wild-type and db/db mice was collected and allowed to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000g. Serum was collected and diluted 20 times for assays. ELISA microplate strips was firstly added 50 μL of Assay Diluent RD1W for each well. 50 μL of standard, control, or samples were then added to each well and incubated for 2 hours at room temperature. After five times wash, 100 μL of Mouse/Rat Leptin Conjugate was added to each well for 2-hour incubation at room temperature. Repeat the washing procedure and add 100 μL of Substrate Solution to each well for 30 minutes at room temperature away from the light. 100 μL of Stop Solution was finally added to each well and the absorbance was determined by using a microplate reader set to 450 nm within 30 minutes. The absorbance at 540 nm or 570 nm was used as wavelength correction.

G-LISA RhoA/Rac1/Cdc42 Activation Assay

The activation of GTPase family was determined by performing G-LISA RhoA/Rac1/Cdc42 Activation Assay (Cytoskeleton, Inc. #BK135) according to the standard protocol provided by manufactory. Briefly, cells were seeded and grown to 70% confluency, followed by the stimulation of leptin from 2 to 30 minutes. Cells were collected and lysed on ice and the concentration of cell lysate was measured by using the detection solution provided in the kit. After adding buffer blank and buffer positive control, 50μl of equalized lysate were added to duplicate wells in an incubation plate. The plate was immediately placed on an orbital shaker at 400rpm for 15 (Rac1 and Cdc42) or 30 (RhoA) minutes in the cold room. After two times wash, 200 μl of room temperature Antigen Presenting Buffer was added to each well at room temperature for 2 minutes. After three times wash, 50 μl of diluted anti-RhoA/Rac1/Cdc42 primary antibody was added to each well on an orbital shaker at 400rpm for 30 (Rac1 and Cdc42) or 45 (RhoA) minutes at room temperature, followed by the incubation of 50 μl of diluted secondary antibody to each well for 30 (Rac1 and Cdc42) or 45 (RhoA) minutes at

room temperature. After three times wash, 50 µl of the mixed HRP detection reagent was added into each well at 37°C for 10-15 min, the reaction of which was stopped by administration of 50 µl of HRP Stop Buffer. The absorbance was measured at 490 nm by using a microplate spectrophotometer. Buffer blank was designated as assay blank.

RFP Labelling Cells

Lentiviral particles were generated by transfecting HEK293T cells with LV H2b_RFP plasmid (Addgene, 26001) and used to label the nucleus of adventitial cells. Sca-1$^+$ progenitor cells were incubated with RFP lentivirus for 10 minutes, and the waste was carefully disposed. Expression of RFP was checked using a fluorescent microscope two days after the treatment with lentivirus.

Mouse Femoral Artery Denudation Injury and Cell Delivery

The procedure used for the mouse model is similar to that established previously.[3] Wild-type or leptin receptor deficient mice (db/db) were anesthetized with ketamine and medetomidine hydrochloride. A groin incision was made under a surgical microscope. An arteriotomy was made in the epigastric branch of femoral arteries. A 0.014' guide wire (Hi-Torque, Cross-it 200XT) was inserted into the femoral artery to the level above the bifurcation of the abdominal aorta. The guide wire was gently pulled back and forth for three times, leading to the endovascular injury of the arteries. After removal of the guide wire, the femoral artery was ligated. Sham-injury arteries without passage of the guide wire were used as a control. After the injury, 1×10^6 (RFP) progenitor cells with or without treatment in 30 µL Matrigel® Basement Membrane Matrix per artery were delivered to the adventitial side of injured artery. The arteries were harvested on days 1, 7, 14 or 28 post-surgery for *En Face*, frozen section or paraffin sections.

Immunofluorescence Staining and *En Face* preparation

Frozen sections were fixed with 4ºC acetone and permeabilized with 0.1% Trixton X-100 in PBS for 30 minutes at room temperature. For *En Face* staining, tissues were fixed with 4% paraformaldehyde for 15 minutes and then permeabilized with 0.5% Trixton X-100 in PBS for 30 minutes. For cell staining in chamber slides (BD Biosciences), the cells were fixed with 4% paraformaldehyde for 15 minutes and then permeabilized with 0.1% Trixton X-100 in PBS for 30 minutes. Tissues or cells were blocked in 5% swine serum for 45 minutes at room temperature prior to their incubation with primary antibodies for either 1 hour at 37 ºC or overnight at 4 ºC. After three washes with PBS, secondary antibodies were administered for 45 minutes at 37 ºC, followed by DAPI (1:5000 in PBS) staining for 5 minutes at room temperature. Frozen section or cells in chamber slides were mounted with fluorescence mounting medium (Dako, S3023). For *En Face* preparation, after the staining with DAPI the vessel was cut open along the long axis with the lumen facing up.[4] The opened vessels were then transferred onto a clean slide and mounted with fluorescence mounting media (Vectashield). A confocal microscope (Leica SP5) and AxioVision Digital Imaging System (Carl Zeiss Ltd) were used for image acquisition. Primary antibodies applied in the present study were Sca-1 (Abcam, ab25031, 1:200), SMA (Sigma, A5228, 1:200), CD31 (Abcam, ab30349, 1:200), leptin (Invitrogen, Pa1-051, 1:200), F4/80 (Abcam, ab6640, 1:200), CD68

(Santa Cruz, sc-9139, 1:200), VE-cadherin (Santa Cruz, sc-6458, 1:200), pFAK (Abcam, ab39967, 1:200), FAK (Santa Cruz, sc557, 1:200), CD45 (Biolegend, 103115, 1:200), OBR (R&D systems, AF497, 1:200), RFP (Abcam, ab62341, 1:200). Slides stained with secondary antibodies only were used as negative control. Secondary antibodies included Thermofisher AlexaFluor Donkey anti-mouse 488 (A-21202) and 594 (A-21203), AlexaFluor Donkey anti-rabbit 488 (A-21206), 594 (A-21206) and 633(A-21070), 647 (A-31573), AlexaFluor Donkey anti-goat 488 (A-11055), 594 (A-11058) and 633 (A-21082), AlexaFluor Donkey anti-rat 488 (A-21208), 594 (A-21209) and 647 (A-21094).

Immunohistochemical Staining

The procedure used in present study is similar to that established previously.[3] Immunohistochemistry was achieved by using vectastain ABC HRP kit (Vector laboratories, PK-6100). Briefly, praffin sections were incubated with SMA (Sigma, A5228, 1:200) primary antibody for 1 hour at room temperature after fixation and blocking. Diluted biotinylated secondary antibody was then applied for 30 minutes at room temperature. After washing, sections were incubated in peroxidase substrate solution until desired stain intensity showed up. The sections were then rinsed, counterstained, cleared and mounted.

Van Gieson Staining

Slides were underwent deparaffin and rehydration, following the immersion in 32 mmol/L of potassium permanganate for 10 minutes. After rinsing in dH_2O, the slides were immersed in 70 mmol/L of oxalicacid dehydrate for 5 minutes, washing in several changes of dH_2O. The slides were again rinsed in 70% ethanol, followed by a 3-hour incubation in Miller's stain. After rinsing in 70% ethanol and dH_2O, the slides were incubated in Van Gieson solution for 5 minutes. After washing in dH_2O, the slides were dried and mounted with mounting medium.

Statistical Analysis

Data represented as the mean and standard error of the mean (S.E.M.) of at least three individual experiments. Data were analyzed using Graphpad Prism 6. For data involved with two groups, unpaired and two-tailed Student's t-test was applied. For data involved with more than two groups, ANOVA test was applied followed by Dunnett's multiple comparison test. The mean of each column with different treatments was compared to the mean of the column named either "Ctr", "Control" or "0". Significance was considered as p-value <0.05.

1. Hu Y, Zhang Z, Torsney E, Afzal AR, Davison F, Metzler B, Xu Q. Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in apoe-deficient mice. *The Journal of clinical investigation*. 2004;113:1258-1265
2. Wong MM, Winkler B, Karamariti E, Wang X, Yu B, Simpson R, Chen T, Margariti A, Xu Q. Sirolimus stimulates vascular stem/progenitor cell migration and differentiation into smooth muscle cells via epidermal growth factor receptor/extracellular signal-regulated kinase/beta-catenin signaling pathway. *Arteriosclerosis, thrombosis, and vascular biology*. 2013;33:2397-2406
3. Zeng L, Xiao Q, Margariti A, Zhang Z, Zampetaki A, Patel S, Capogrossi MC, Hu Y, Xu Q. Hdac3 is crucial in shear- and vegf-induced stem cell differentiation toward

endothelial cells. *The Journal of cell biology*. 2006;174:1059-1069

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Supplemental Data Figures

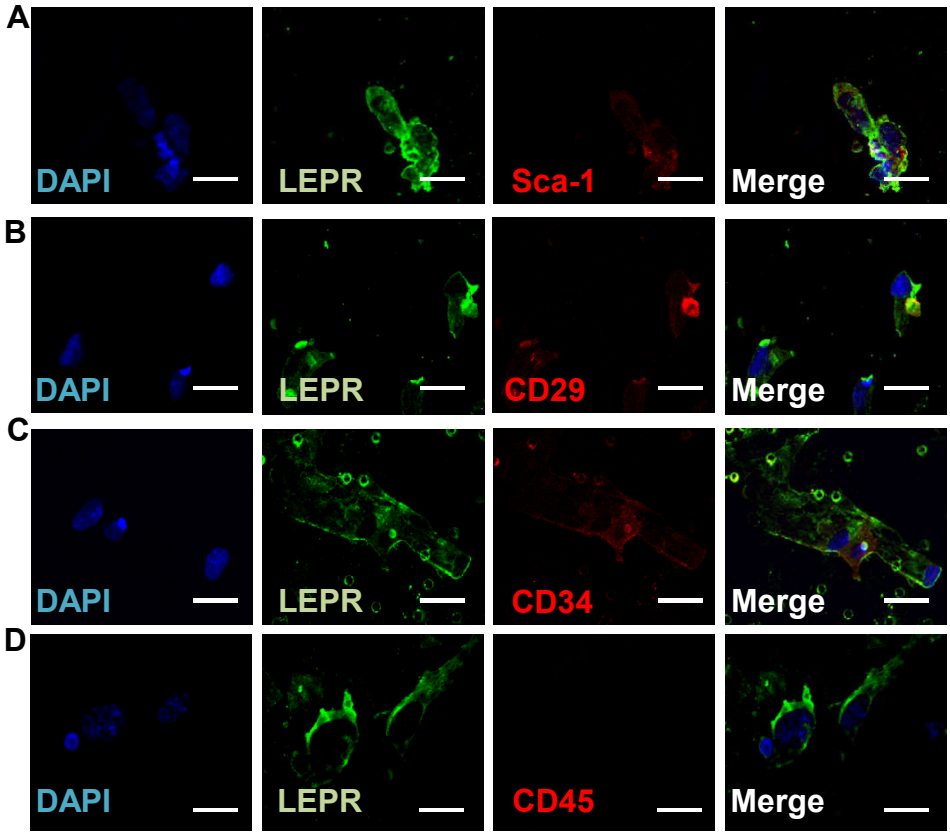


Figure I. The expression of progenitor and hematopoietic markers in leptin-induced migrated cells. Migrated $Lepr^{+/+}$ $Sca\text{-}1^{+}$ progenitor cells after transwell assay were analyzed by immunofluorescence for OBR, Sca-1 (**A**), CD29 (**B**), CD34 (**C**) and CD45 (**D**) (scale bars, 3 μm, n=3).

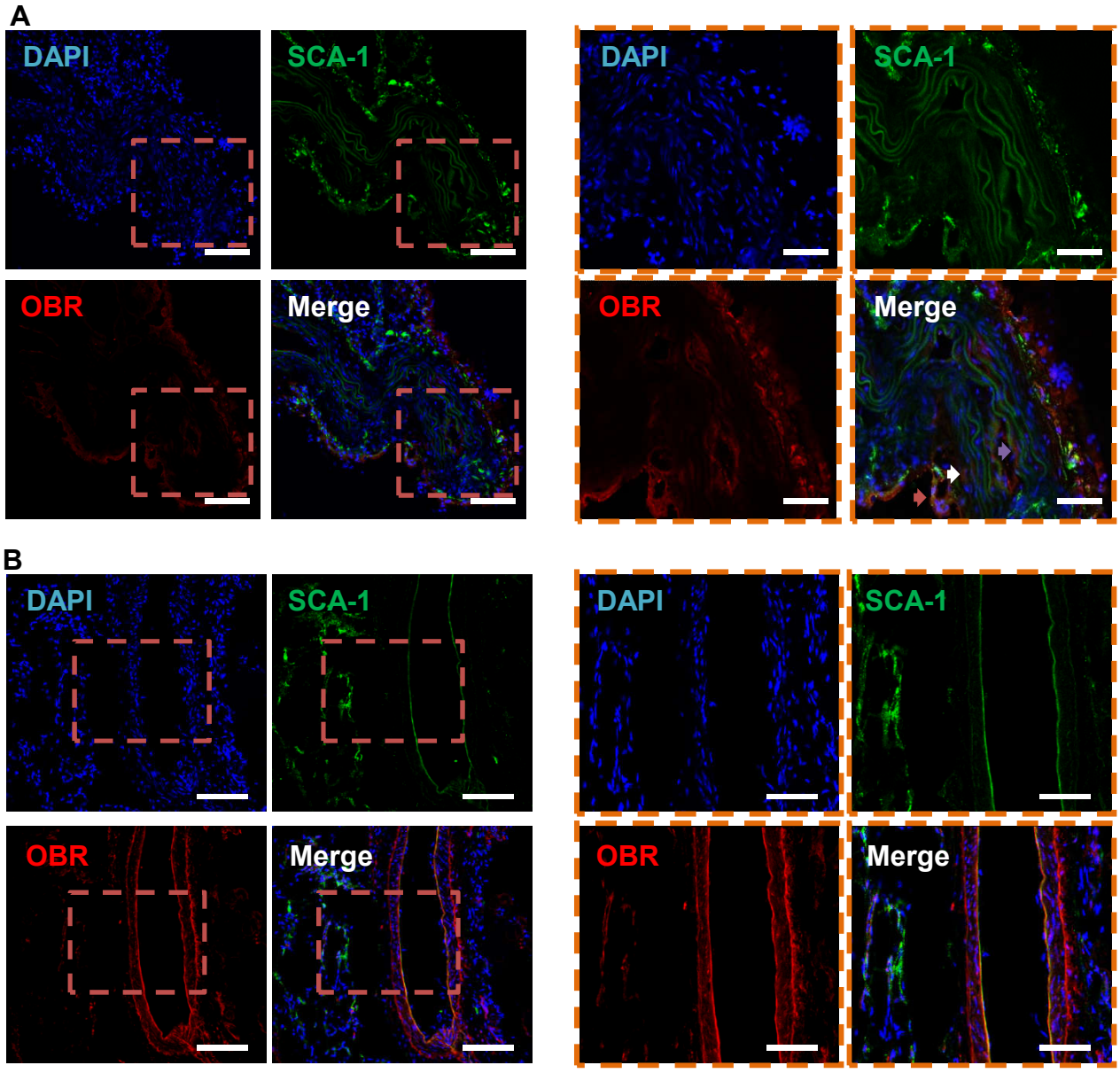


Figure II. Sca-1^{+} OBR^{+} cells reside mainly in the adventitia of aorta and femoral artery. **A**, Cross sections of aorta from wild-type mice were analyzed by immunofluorescence for Sca-1 and OBR (scale bars, 100 and 50 μm, n=4). Red arrow indicated Sca-1^{+} OBR^{+} adventitial cells. White arrow indicated Sca-1^{+} OBR^{+} medial cells. Purple arrow indicated Sca-1^{+} OBR^{+} endothelial cells. **B**, Cross sections of femoral artery from wild-type mice were analyzed by immunofluorescence for Sca-1 and OBR (scale bars, 50 and 25 μm, n=4).

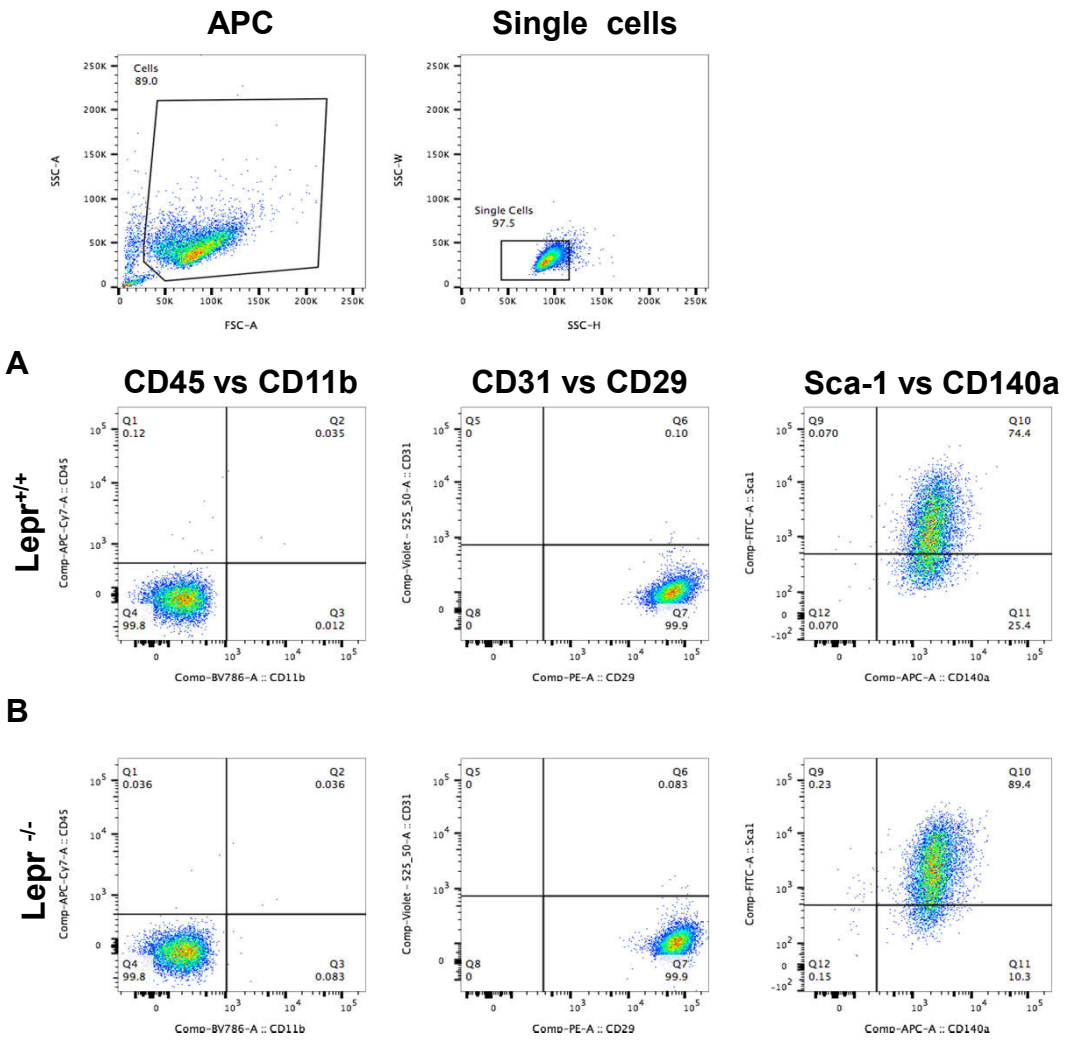


Figure III. Phenotyping of Lepr$^{+/+}$ and Lepr$^{-/-}$ of Sca-1^{+} adventitia progenitor cells. **A**, Phenotyping of Lepr$^{+/+}$ Sca-1^{+} adventitial progenitor cells with primary antibodies of CD45, CD11b, CD31, CD29, CD140a and Sca-1. **B**, Phenotyping of Lepr$^{-/-}$ Sca-1^{+} adventitial progenitor cells with primary antibodies of CD45, CD11b, CD31, CD29, CD140a and Sca-1.

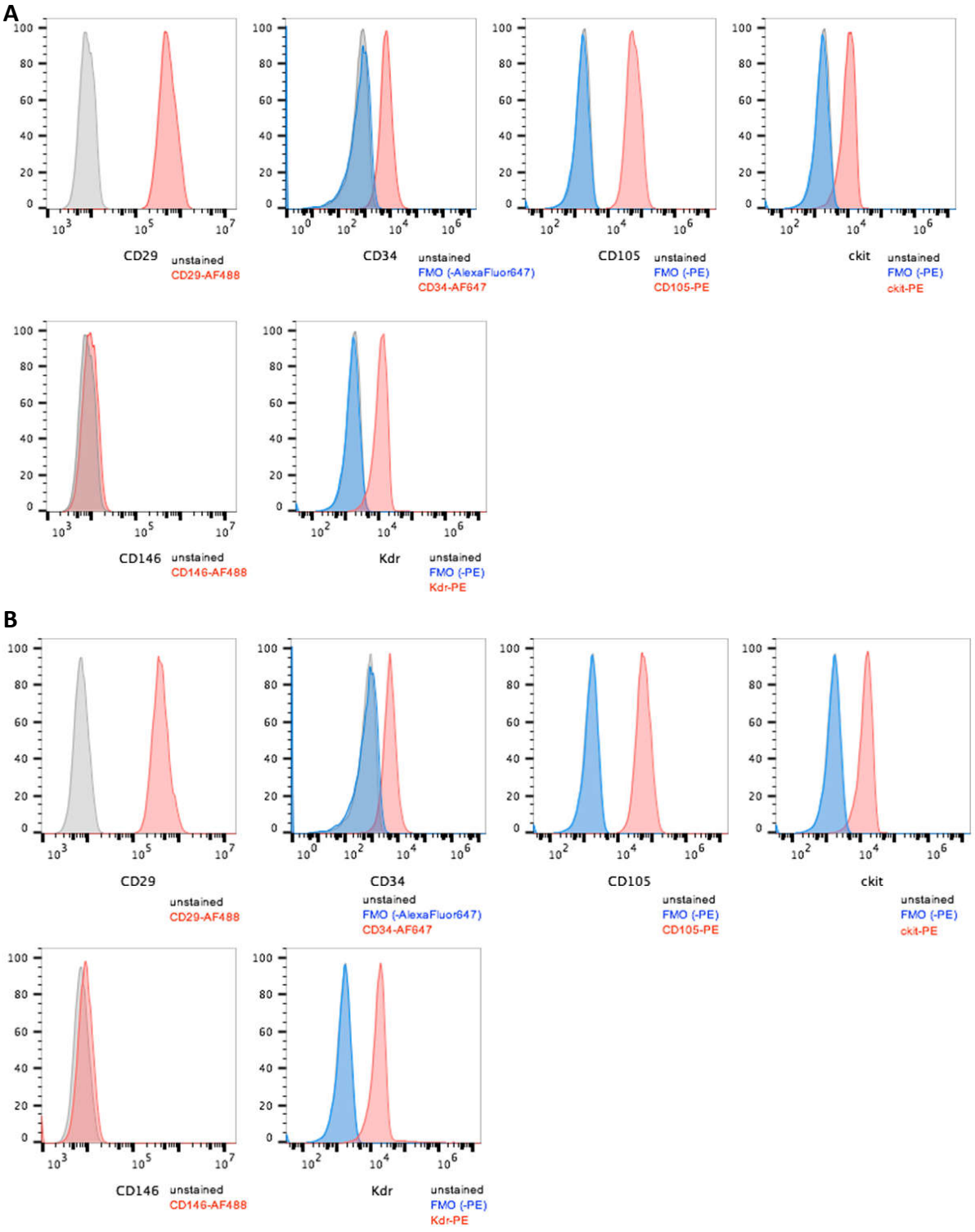


Figure IV. Phenotyping of Lepr$^{+/+}$ and Lepr$^{-/-}$ of Sca-1^{+} adventitial progenitor cells. **A**, Phenotyping of Lepr$^{+/+}$ Sca-1^{+} adventitial progenitor cells with primary antibodies of CD29, CD34, CD105, c-kit, CD146 and Kdr. **B**, Phenotyping of Lepr$^{-/-}$ Sca-1^{+} adventitial progenitor cells with primary antibodies of CD29, CD34, CD105, C-kit, CD146 and KDR.

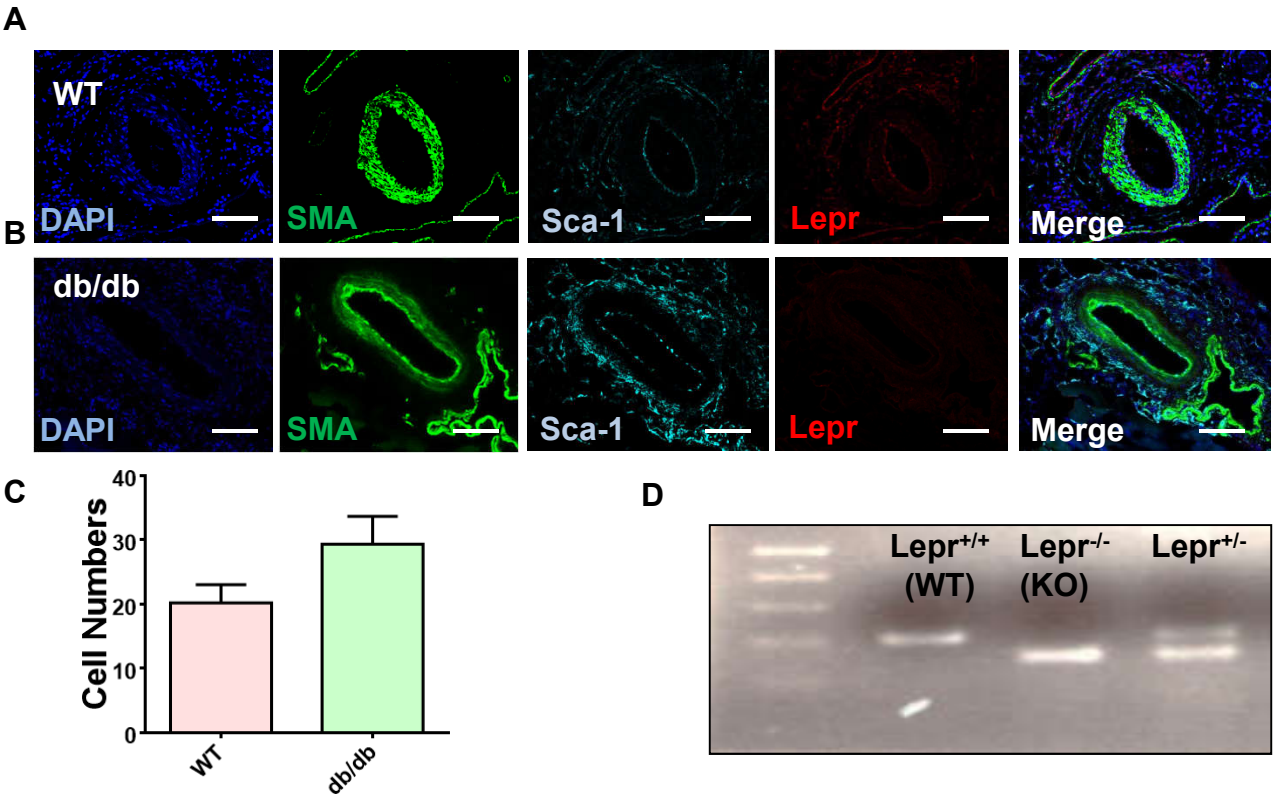


Figure V. The identification of Lepr$^{-/-}$ mice was achieved by applying conventional PCR and immunostaining. **A**, Cross sections of uninjured femoral artery from wild-type were analyzed by immunofluorescence for αSMA, Sca-1 and Lepr (scale bars, 50 μm, n=4). **B**, Cross sections of uninjured femoral artery from db/db were analyzed by immunofluorescence for αSMA, Sca-1 and Lepr (scale bars, 50 μm, n=4). **C**, The semi-quantification of the numbers of Sca-1^{+} adventitial cells was evaluated in the femoral artery of db/db and WT mice (n=5). **D**, Conventional PCR was performed for the identification of every Lepr$^{-/-}$ mice (n=29).

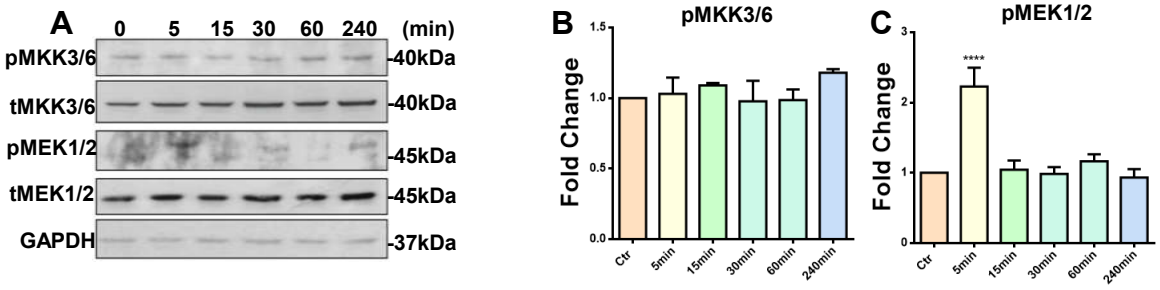
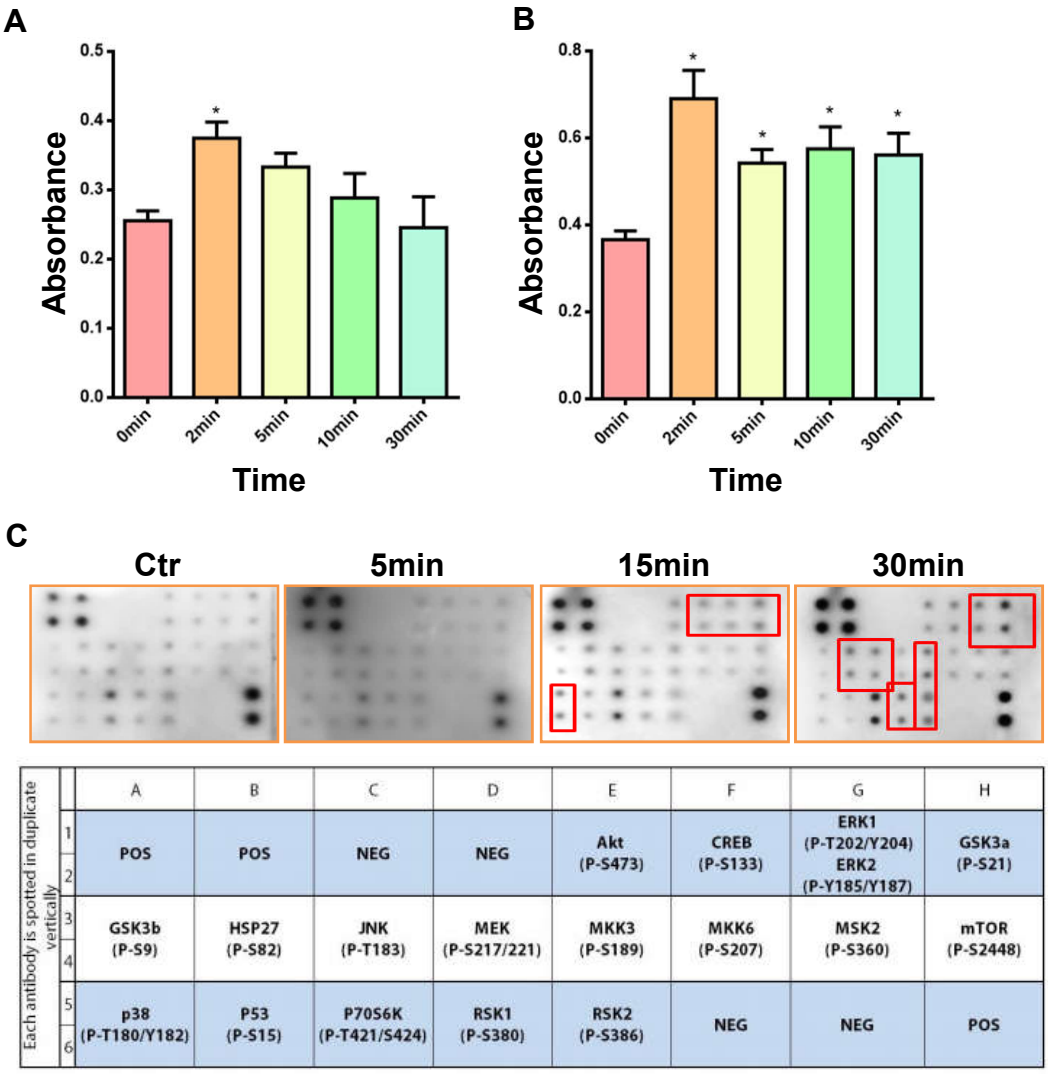


Figure VI. Leptin activated pMEK1/2 but not pMKK3/6 signaling pathways on Sca-1^{+} adventitial progenitor cells. **A,** Western blotting was performed on Sca-1^{+} progenitor cells in response to 100 ng/ml of leptin for the detection of pMKK3/6 (n=3), pMEK1/2 (n=4). **B** and **C**, Quantification of the activation of pMKK3/6 (B) and pMEK1/2 (C).



Each antibody is spotted in duplicate vertically	A	B	C	D	E	F	G	H
1	POS	POS	NEG	NEG	Akt (P-S473)	CREB (P-S133)	ERK1 (P-T202/Y204) ERK2 (P-Y185/Y187)	GSK3a (P-S21)
2								
3	GSK3b (P-S9)	HSP27 (P-S82)	JNK (P-T183)	MEK (P-S217/221)	MKK3 (P-S189)	MKK6 (P-S207)	MSK2 (P-S360)	mTOR (P-S2448)
4								
5	p38 (P-T180/Y182)	P53 (P-S15)	P70S6K (P-T421/S424)	RSK1 (P-S380)	RSK2 (P-S386)	NEG	NEG	POS
6								

Figure VII. Leptin activated the GTPase Cdc42 and Rac1 on Sca-1^{+} adventitial progenitor cells. **A** and **B**, Quantification of activated GTPase Cdc42 (**A**) and Rac1 (**B**) was evaluated in response to 100 ng/mL of leptin by performing G-LISA G-protein activation assays (n=3). **C**, The expression of MAPK-related protein was detected by applying MAPK protein microarrays over time in response to 100 ng/mL of leptin (n=3). Untreated cells were served as control. All graphs are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

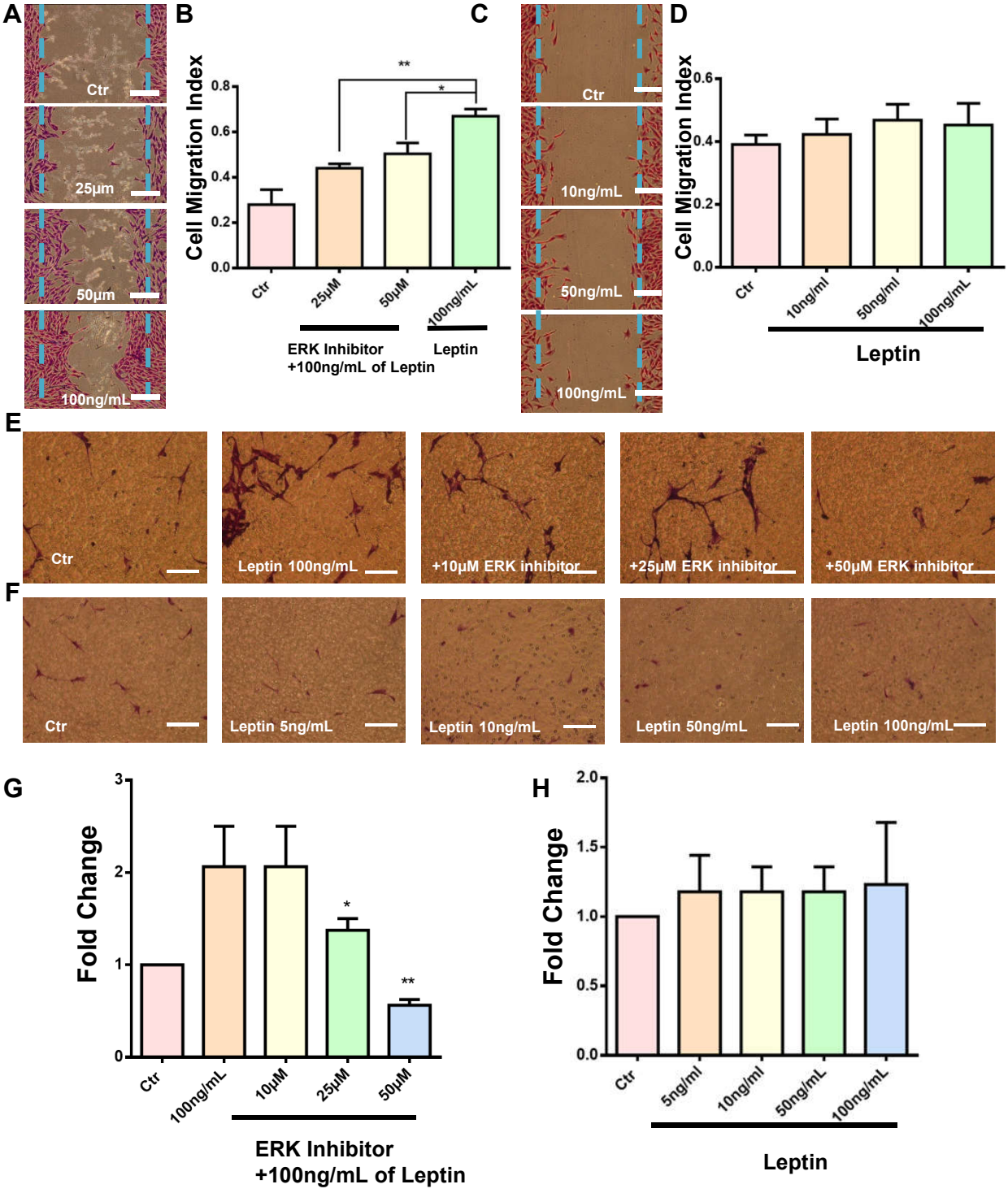

A
Ctr
25μm
50μm
100ng/mL
B
Cell Migration Index
0.0
0.2
0.4
0.6
0.8
**
*
Ctr
25μM
50μM
100ng/mL
ERK Inhibitor
+100ng/mL of Leptin
Leptin
C
Ctr
10ng/mL
50ng/mL
100ng/mL
D
Cell Migration Index
0.0
0.2
0.4
0.6
Ctr
10ng/ml
50ng/ml
100ng/mL
Leptin
E
Ctr
Leptin 100ng/mL
+10μM ERK inhibitor
+25μM ERK inhibitor
+50μM ERK inhibitor
F
Ctr
Leptin 5ng/mL
Leptin 10ng/mL
Leptin 50ng/mL
Leptin 100ng/mL
G
Fold Change
0
1
2
3
*
**
Ctr
100ng/mL
10μM
25μM
50μM
ERK Inhibitor
+100ng/mL of Leptin
H
Fold Change
0.0
0.5
1.0
1.5
2.0
Ctr
5ng/ml
10ng/ml
50ng/mL
100ng/mL
Leptin

Figure VIII. Inhibition of OBR and ERK pathway significantly reduced the migration of Sca-1$^+$ adventitial progenitor cells in response to 100 ng/mL of leptin. **A** and **B**, Migration of Sca-1$^+$ progenitor cells in response to 100 ng/mL of leptin with or without ERK inhibitor was evaluated by applying 1% crystal violet staining after 16-hour incubation (scale bars, 50 μm, n=5). **C** and **D**, Migration of Sca-1$^+$ progenitor cells from db/db mice in response to an increasing gradient concentration of leptin was evaluated by 1% crystal violet staining after 16-hour incubation (scale bars, 50 μm, n=5). **E** and **G**, Chemotaxis of Sca-1$^+$ progenitor cells in response to 100 ng/mL of leptin and ERK inhibitor was documented by using an 8.0 μm transwell system (scale bars, 50 μm, n=5). **F** and **H**, Chemotaxis of Sca-1$^+$ progenitor cells from db/db mice in response to an increasing gradient of leptin in an 8.0 μm transwell system was identified by applying 1% crystal violet staining after 16-hour incubation (scale bars, 50 μm, n=5). Serum-free cultured medium and dimethyl sulfoxide (DMSO) without leptin treatment was used as controls for the migratory assays above. Migration index for transwell and wound healing assays was defined as the mean ratio of treatment to control of cell numbers counted per 5 random fields at 20X magnification. All graphs are shown as mean ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

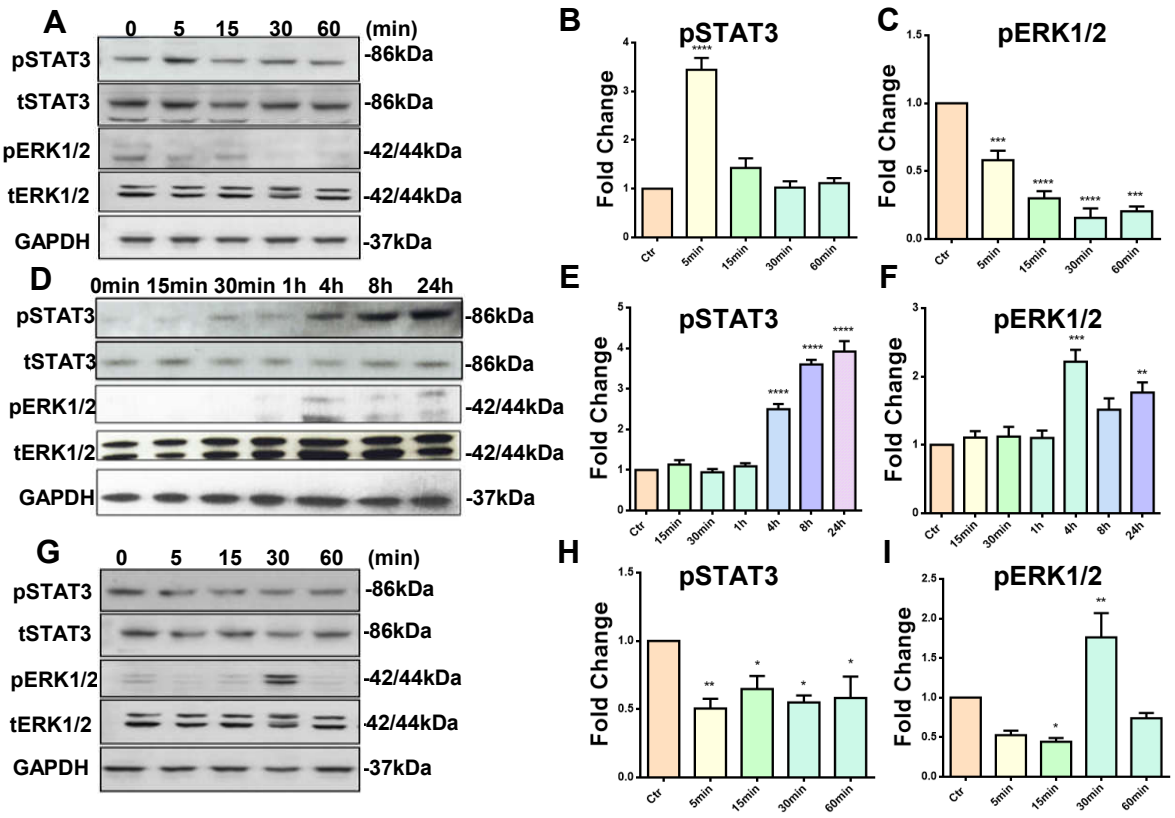


Figure IX. OBR and STAT3 were the upstream of ERK1/2. **A**, Western blotting was performed on Sca-1^{+} progenitor cells in response to 100 ng/ml of leptin with ERK inhibitor for the detection of pSTAT3 (n=8) and pERK1/2 (n=10). **B** and **C**, Quantification of the activation of pSTAT3 and pERK1/2. **D**, Western blotting was performed on Sca-1^{+} progenitor cells in response to 100 ng/ml of leptin with STAT3 inhibitor for the detection of pSTAT3 (n=4) and pERK1/2 (n=4). **E** and **F**, Quantification of the activation of pSTAT3 and pERK1/2. **G**, Western blotting was performed on Lepr$^{-/-}$Sca-1^{+} progenitor cells in response to 100 ng/ml of leptin for the detection of pSTAT3 (n=4) and pERK1/2 (n=4). **H** and **I**, Quantification of the activation of pSTAT3 and pERK1/2.

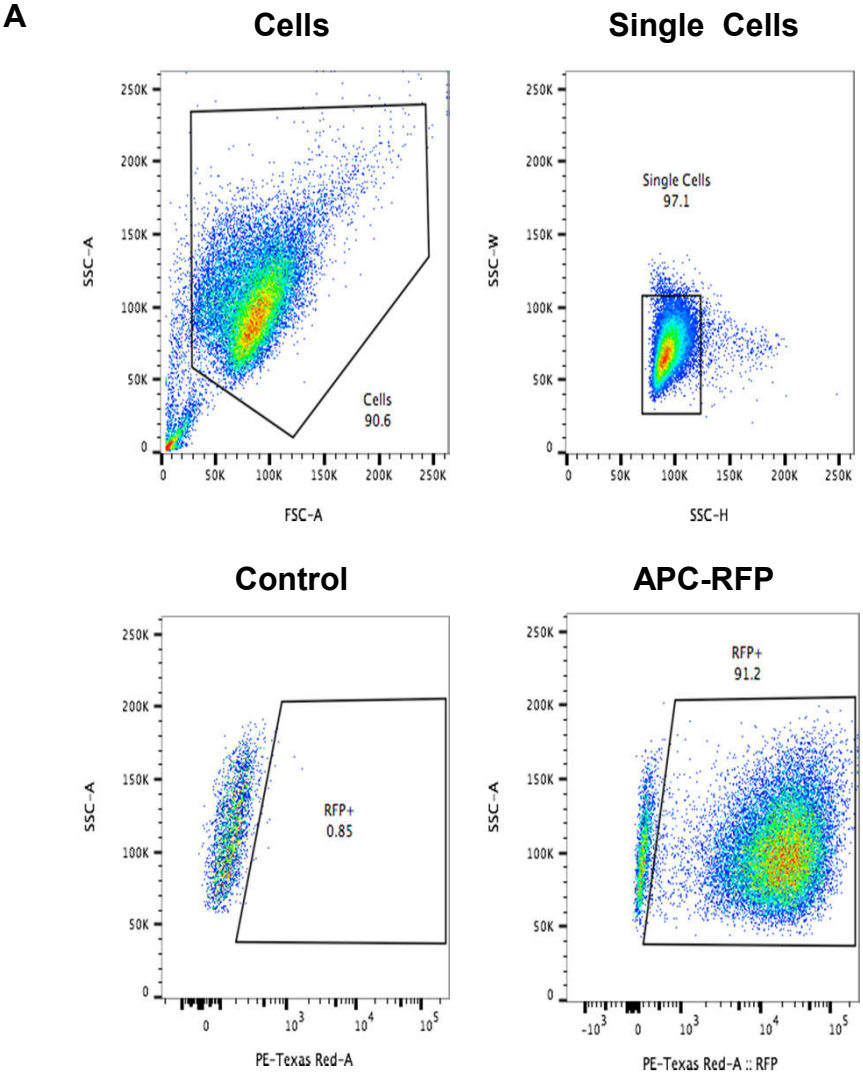


Figure X. Identification of RFP and non-RFP Sca-1$^+$ adventitia progenitor cells after lentivirus transfection. **A**, Confirmation of the existence of RFP in Sca-1$^+$ adventitia progenitor cells with primary antibodies of RFP by applying FACS assay.

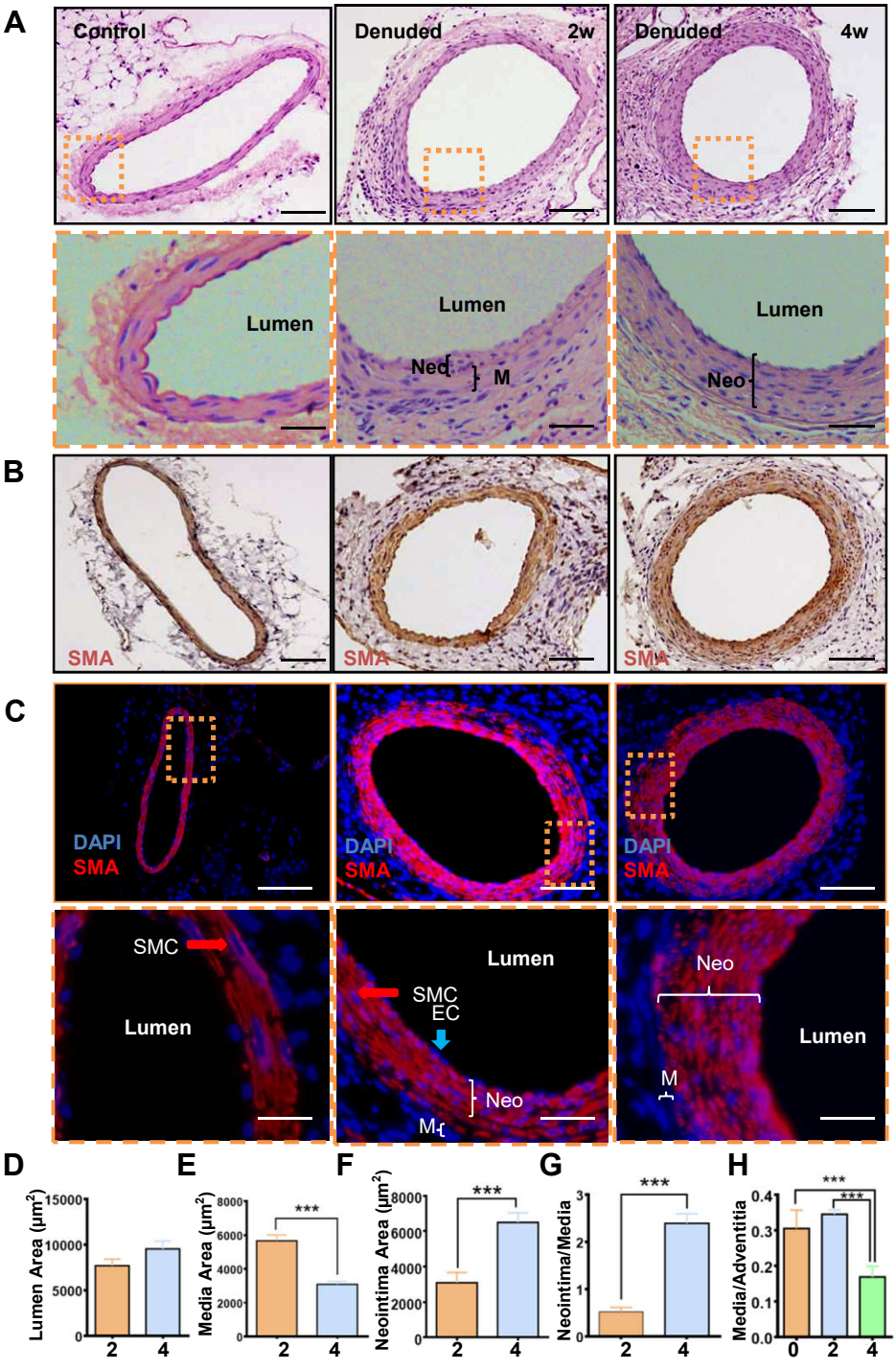

A
Control
Denuded
2w
Denuded
4w
Lumen
Lumen
Ned
M
Lumen
Neo
B
SMA
SMA
SMA
C
DAPI
SMA
DAPI
SMA
DAPI
SMA
SMC
Lumen
Lumen
SMC
EC
Neo
M
Neo
M
Lumen
D
Lumen Area (μm²)
15000
10000
5000
0
2
4
E
Media Area (μm²)
8000
6000
4000
2000
0
***
2
4
F
Neointima Area (μm²)
8000
6000
4000
2000
0
***
2
4
G
Neointima/Media
3
2
1
0
***
2
4
H
Media/Adventitia
0.4
0.3
0.2
0.1
0.0
***
***
0
2
4

Figure XI. Guide-wire injury induced significant neointimal formation 2 weeks and 4 weeks after surgery. **A**, H&E staining of the femoral artery after guide-wire injury was performed (scale bar, 50 and 10 μm, n=10). **B** and **C**, The composition of the neointima was evaluated by immunohistochemistry (B) and immunofluorescence (C) for smooth muscle actin on frozen sections of femoral artery at 2 or 4 weeks after surgery (scale bar, 50 and 10 μm, n=10). **D**, Quantification of lumen area of injured arteries (n=10). **E**, Quantification of media area of injured arteries (n=10). **F**, Quantification of neointimal area of injured arteries (n=10). **G**, Quantification of ratio of neointima to media of injured arteries (n=10). **H**, Quantification of ratio of media to adventitia of controls and injured arteries (n=10). Graphs are shown as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001. Dashed box represented the magnified field. Arrows indicate SMCs or ECs. EC indicates endothelium; SMC, smooth muscle cells; Neo, neointima; M, media; SMA, smooth muscle actin; 2W, 2 weeks; 4W, 4 weeks.

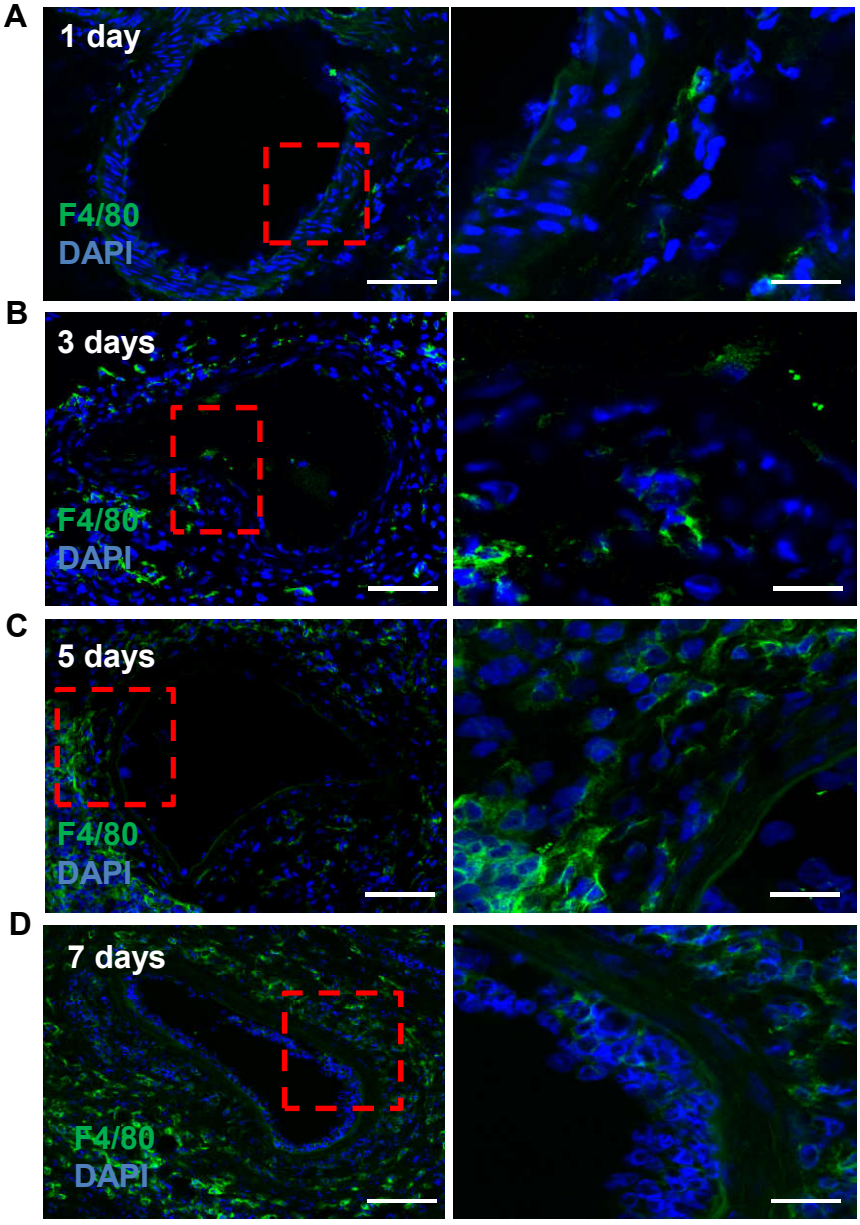


Figure XII. The expression of macrophage marker F4/80 was upregulated after surgery around the injured artery. Expression of macrophage (Alexa 488; green) in injured artery was analyzed on day 1(**A,** n=10), 3(**B,** n=7), 5(**C**, n=5) and 7(**D**, n=10) post-operation by performing immunofluorescence. (scale bars, 50 and 10 μm). Dashed box represented the magnified field.

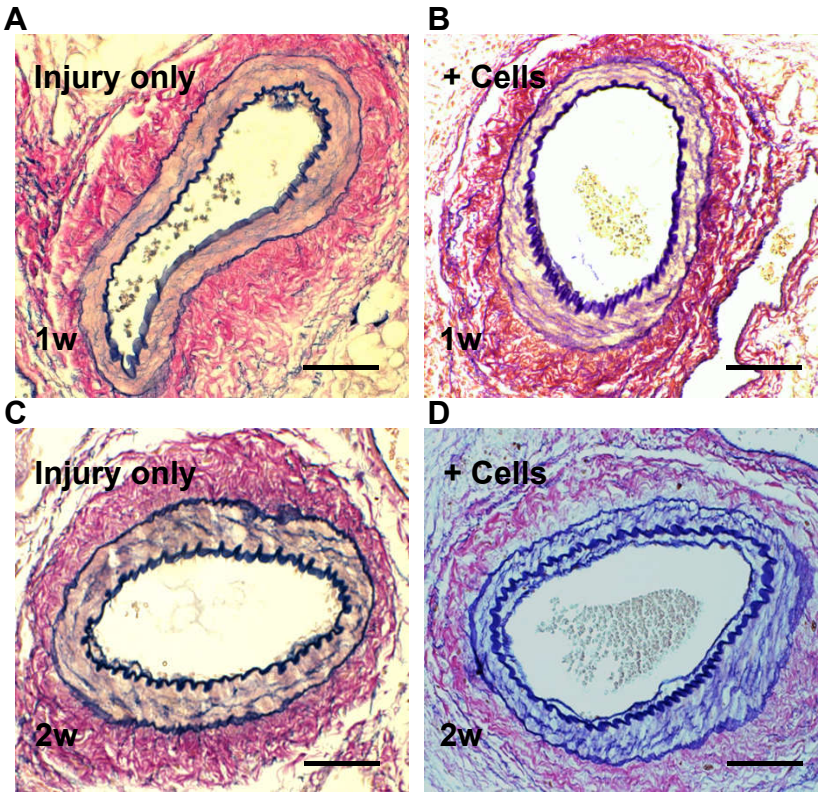


Figure XIII. Transplantation of exogenous Sca-1^{+} progenitor cells did not affect the extent of fibrosis during the neointimal formation. **A**, Cross sections of injured femoral artery at 1 week post-surgery were analyzed by Elastica-van Gieson staining (scale bars, 50 and 10μm, n=3). **B**, Lepr$^{+/+}$ Sca-1^{+} progenitor cells were seeded on the adventitial side of injured artery in wild-type mice. Cross sections of femoral artery at 1 week post-surgery were analyzed by Elastica-van Gieson staining (scale bars, 50 and 10 μm, n=3). **C**, Cross sections of injured femoral artery at 2 week post-surgery were analyzed by Elastica-van Gieson staining (scale bars, 50 and 10μm, n=3). **D**, Lepr$^{+/+}$ Sca-1^{+} progenitor cells were seeded on the adventitial side of injured artery in wild-type mice. Cross sections of femoral artery at 2 week post-surgery were analyzed by Elastica-van Gieson staining (scale bars, 50 and 10μm, n=3). Images shown are representative of at least 3 independent experiments.

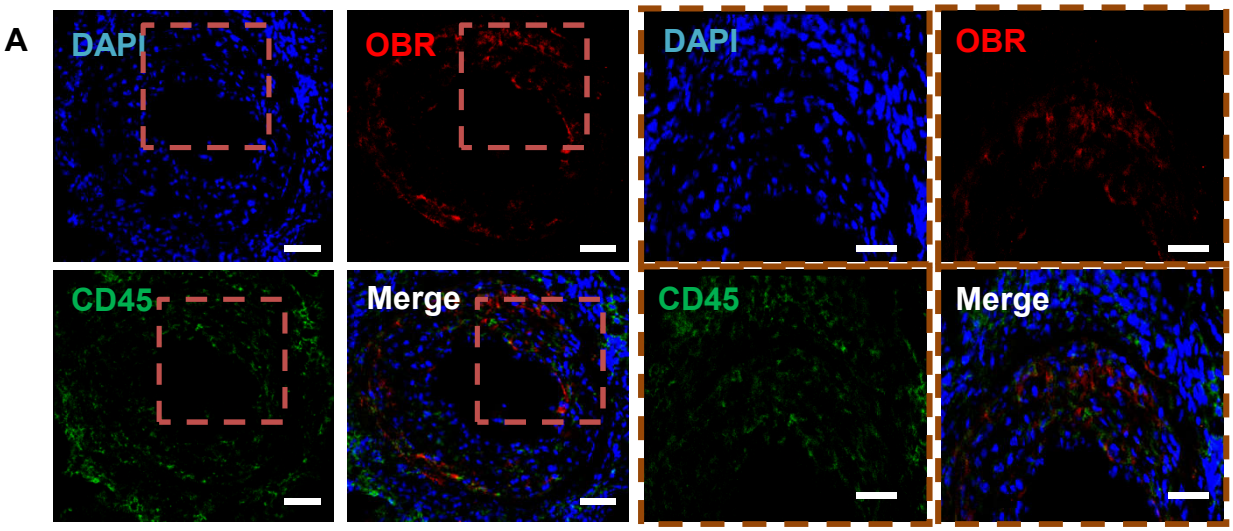


Figure XIV. Both adventitial Sca-1^{+} cells and hematopoietic cells contributed to neointimal formation. **A**, Lepr$^{+/+}$ Sca-1^{+} progenitor cells were seeded on the adventitial side of injured artery in db/db mice. Cross sections of femoral artery from db/db mice at 2 weeks after the surgery were analyzed by immunofluorescence for CD45 and OBR (scale bars, 50 and 10μm, n=4). Dashed box represented the magnified field. Images shown are representative of at least 3 independent experiments.

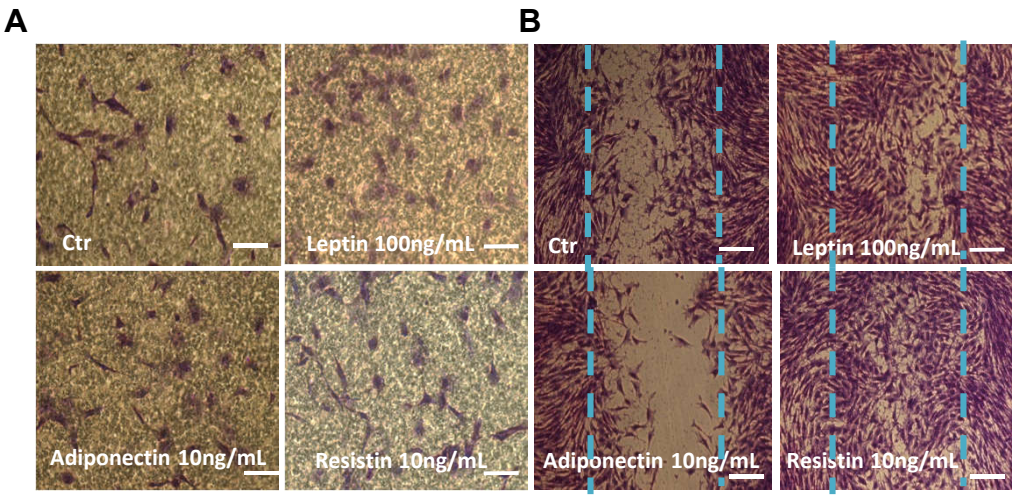


Figure XV. The migration of Sca-1⁺ adventitial progenitor cells could be inhibited or induced by different adipokines. **A**, Chemotaxis of Sca-1⁺ progenitor cells in response to 10 ng/mL of adiponectin (n=3), 10 ng/mL of resistin (n=3) and 100 ng/mL of leptin (n=6) was evaluated by applying 1% crystal violet staining after 16-hour incubation (scale bars, 50μm). **B**, Migration of Sca-1⁺ progenitor cells in response to 10 ng/mL of adiponectin (n=3), 10 ng/mL of resistin (n=3) and 100 ng/mL of leptin (n=6) was documented by using an 8.0 μm transwell system. Serum-free cultured medium and dimethyl sulfoxide (DMSO) without treatment was used as controls for the migration assays above.

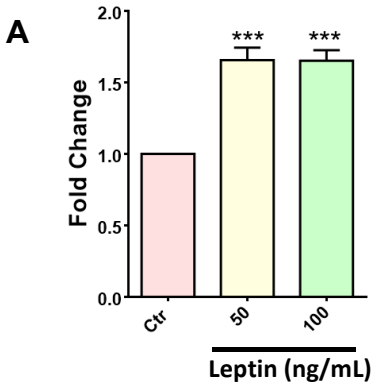


Figure XVI. Leptin could enhance the expression of CXCR5 except for OBR. **A**, Expression of CXCR5 for Sca-1⁺ progenitor cells was evaluated by qPCR 24 hours after the treatment of leptin (n=5). All graphs are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

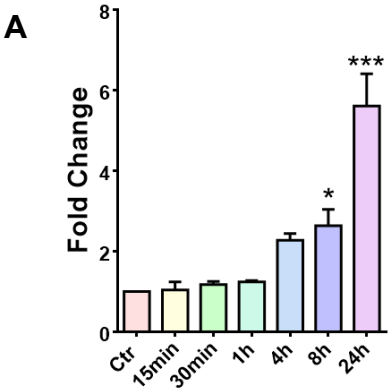


Figure XVII. Leptin might induce the differentiation of Sca-1⁺ progenitor cells towards SMC. **A**, Expression of Calponin (n=5) for Sca-1⁺ progenitor cells was evaluated by qPCR in response to 100 ng/mL of leptin. All graphs are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

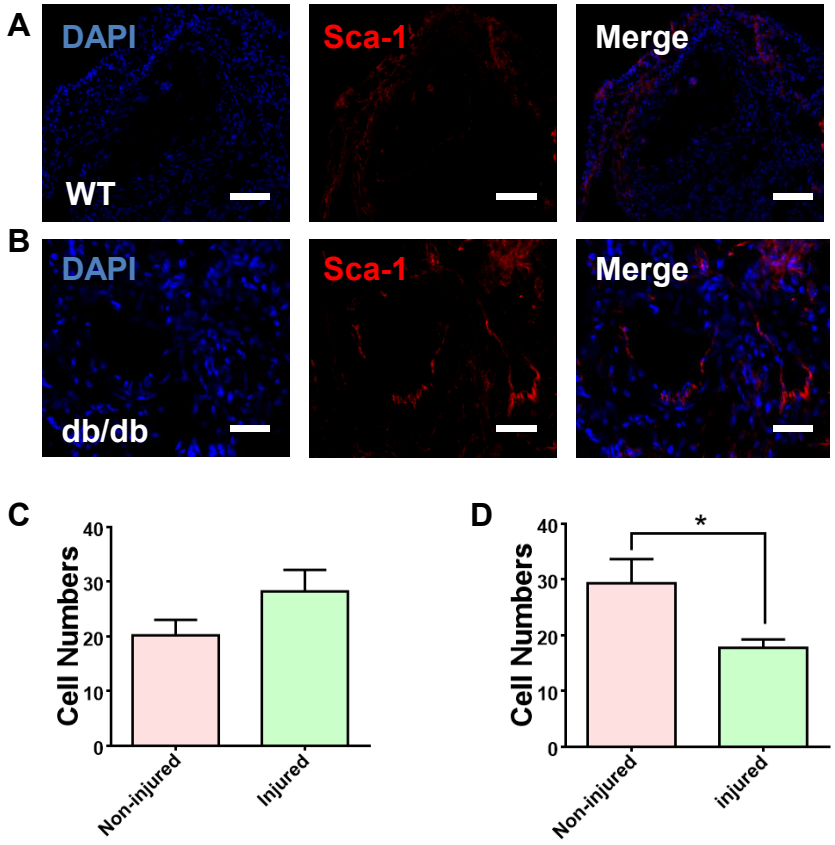


Figure XVIII. Quantification of Sca-1$^+$ cells at two weeks after vessel injury was analyzed in db/db and wild-type. **A**, Cross sections of injured femoral artery from wild-type mice were analyzed by immunofluorescence for Sca-1 (scale bars, 50 μm, n=4). **B**, Cross sections of injured femoral artery from db/db mice were analyzed by immunofluorescence for Sca-1 (scale bars, 50 μm, n=4). **C**, The semi-quantification of number of Sca-1$^+$ adventitial cells was evaluated in wild-type mice before and at two weeks post-surgery (n=4). **D**, The semi-quantification of number of Sca-1$^+$ adventitial cells was evaluated in db/db mice before and at two weeks post-surgery (n=4).

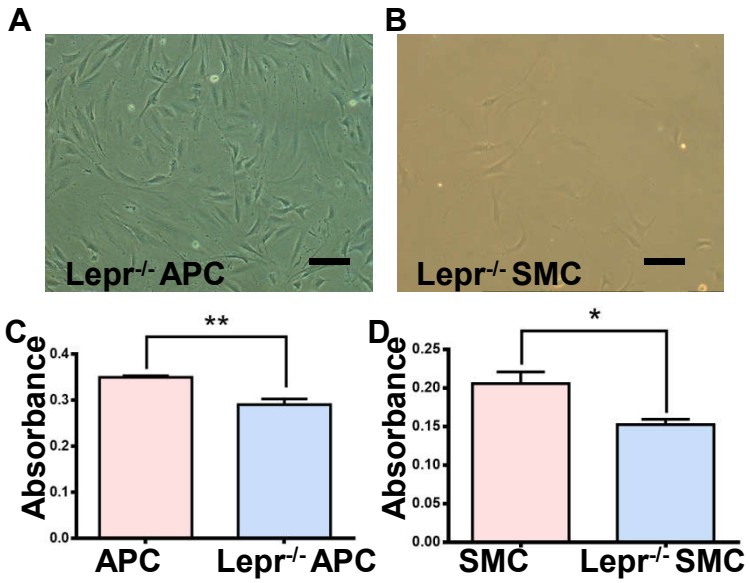


Figure XIX. APCs and SMCs from db/db mice showed a difficulty to expand *in vitro.* **A**, Morphology of Sca-1^{+} progenitor cells from db/db mice. **B**, Morphology of SMCs from db/db mice. **C** and **D**, Proliferation between Lepr$^{-/-}$ or lepr$^{+/+}$ Sca-1^{+} progenitors (**C,** n=4) and SMC (**D,** n=4) was examined by BrdU assay after 16-hour incubation with 100 ng/mL of leptin. Serum-free cultured medium without leptin treatment was used as a control for the migration assays above. Figures shown above are representative of at least 3 separate experiments (scale bars, 50 μm). APC indicates Sca-1^{+} adventitial progenitor cells; SMC indicates smooth muscle cells. All graphs are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

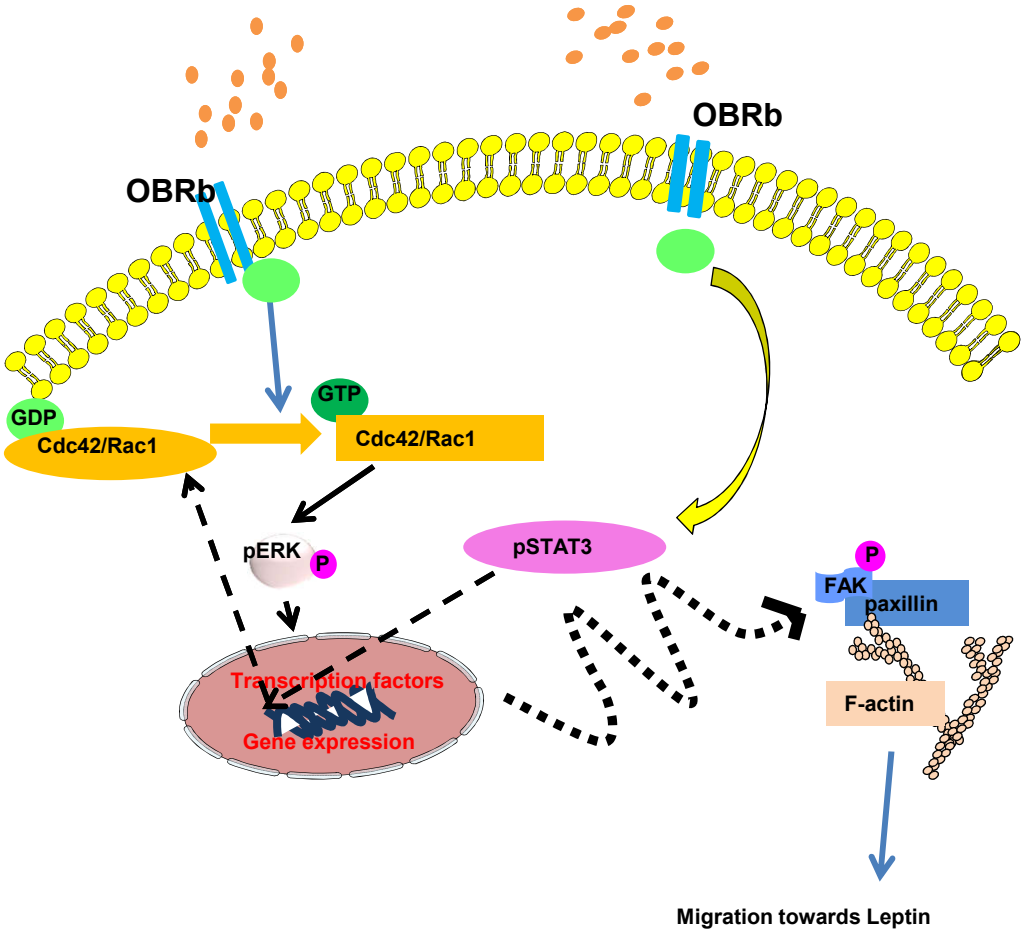


Figure XX. Schematic illustration of the roles of exogenous leptin in enhancing Sca-1^{+} adventitial progenitor cells chemotaxis. Elevated leptin in circulation or femoral artery after guide-wire injury binds to its receptors OBR on the Sca-1^{+} adventitial progenitor cells. The GTPases Rac1 and Cdc42 are activated leading to the phosphorylation of pERK. Leptin also induces the activation of STAT3 pathway and the expression of cytoskeleton related proteins paxillin, vinculin and phosphorylated FAK, which may eventually contribute to cell migration.

Supplemental Data Tables

Table I. Primers used

Name	Sequence	NCBI reference
LEPR	5'>AGCAACTGCATCTCCAGTAATC<3'	NM_146146.2
	5'>GTGGGTTGCTGGTCTGATAAA<3'	
SM22	5'>GATATGGCAGCAGTGCAGAG<3'	NM_011526.5
	5'>AGTTGGCTGTCTGTGAAGTC<3';	
Calponin	5'>GGTCCTGCCTACGGCTTGTC<3'	XM_011242388.2
	5'>TCGCAAAGAATGATCCCGTC<3'	
ADIPOR1	5'>TGTACCCACCATGCACTTTAC<3'	NM_028320.4
	5'>CATCACAGCCATGAGGAAGAA<3'	
ADIPOR2	5'> GTATCCCTGAGCGCTTCTTT <3'	NM_197985.3
	5'> GACTCCGTGGAAGTGAACAA <3'	
CCR1	5'> CATTGTCCATGCTGTGTTTGCCCT <3'	NM_009912.4
	5'>TCTTCCACTGCTTCAGGCTCTTGT <3'	
CCR2	5'> ATCACCATTACACCTGTGGCCCTT <3'	XM_011243064.2
	5'> AGCCCTGTGCCTCTTCTTCTCATT <3'	
CCR7	5'> TCATTGCCGTGGTGGTAGTCTTCA <3'	NM_001301713.1
	5'> ATGTTGAGCTGCTTGCTGGTTTCG <3'	
CCR9	5'> TTGTGTTCATTGTGGGCACCTTGG <3'	NM_001166625.1
	5'> CATCCATTGACCAGCAGCAGCAAT<3'	
CXCR3	5'>TGTAGCCCTCACCTGCATAGTTGT<3'	NM_009910.3
	5'>GTTGTACTGGCAATGGGTGGCATT <3'	
CXCR4	5'>AGCTAAGGAGCATGACGGACAAGT<3'	XM_006529113.3
	5'>AACGCTGCTGTAGAGGTTGACAGT<3'	
CXCR5	5'>AAGCGGAAACTAGAGCCTGGTTCA<3'	NM_007551.2
	5'>ACCATCCCATCACAAGCATCGGTA<3'	
GAPDH	5'>CATGTTCGTCATGGGTGTGAACCA <3'	NM_001289726.1
	5'>ATGGCATGGACTGTGGTCATGAGT<3'	

Table II. Primers used

db/db	5'> AGAACGGACACTCTTTGAAGTCTC<3'	XM_017320002.1
	5'> CATTCAAACCATAGTTTAGGTTTGTGT<3'	