SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Preparation of Minicircles. Minicircles are the product of site-specific intramolecular recombination between the attB and attP sites driven by bacteriophage ΦC31 integrase. The DNA fragment containing enhanced green fluorescent protein (MC-GFP) or HIF1α (MC-HIF1α) were bluntly ligated between the attB and attP sites of the minicircle plasmid. Minicircle DNA plasmids were prepared as described previously[1]. Briefly, *Escherichia coli* Top10 were transformed by parental plasmids. Cells from one transformed colony were inoculated into 5 ml of LB with Kanamycin (50 μg/ml) and incubated at 37°C with shaking at 250 rpm. 8 hours later, the bacteria was amplified by combining 100 μL of culture to every 400 mL LB containing Kanamycin (50 μg/ml) and continued incubation for 16 hours. The next day, a minicircle induction mix comprising of 400 mL fresh LB, 16 ml 1N sodium hydroxide, and 0.4 % L-arabinose were added to the overnight culture and allowed to grow at 32°C for another 8 hours. Minicircles were then isolated using Qiagen's Plasmid Plus Maxi Kit according to manufacturer's protocol.

***In Vitro* Cardiac Differentiation of Sca1$^+$ CPCs.** To induce cardiac differentiation, cultured Sca1$^+$ CPCs were differentiated into cardiomyocytes by culture in Cardiomyocyte Differentiation Medium (Millipore, Temecula, CA) for 12-15 days as previously described[2]. Characterization of the differentiated cardiomyocytes was done by immunostaining of Troponin I (Millipore, MAB1691-50UG) and Actinin (Millipore, MAB1682-25UL) according to manufacturer's instructions.

***In Vitro* and *In Vivo* Optical Bioluminescence Imaging (BLI).** CPCs were plated in increasing numbers in 10 cm dishes and cultured overnight. BLI was then performed the following day using the Xenogen IVIS 200 System (Xenogen, CA) and signals quantification was performed to determine the correlation between signals intensity and cell numbers. For *in vivo* BLI, recipient mice were anesthetized with isoflurane, and were intraperitoneally injected with D-Luciferin (200 mg/kg body weight). Peak signals from a fixed region of interest (ROI) were obtained and signals quantified in photons/s/cm^2/sr as previously described[3].

Echocardiographic Analysis of Left Ventricular Function. Echocardiography was performed before (day -2) and after (days 2, 28 and 42) the LAD ligation using a Vevo 2100 device equipped with 18-38 MHz linear-array transducer with a digital ultrasound system (Visualsonics) (N=10/group). A left ventricular M-mode tracing was obtained using the 2D parasternal short axis imaging as a guide. From these images, fractional shortening (FS) and ejection fraction (EF) were calculated. Left ventricular end-diastolic diameter (EDD) and end-systolic diameter (ESD) were measured and used to calculate fractional shortening (FS) by the following formula: FS (%) = [(EDD - ESD)/EDD] x 100%.

Triphenyltetrazolium Chloride (TTC) Staining for Determination of Infarct Size. Mice were anesthetized and hearts were rapidly removed and perfused with saline to rinse out residual blood (N=6/group). Hearts were then frozen and sectioned into 2 mm transverse sections from apex to occlusion site (4 slices/heart). Heart slices were stained in 1% TTC for 15 min at 37°C and then fixed in 10% formalin overnight. The area of infarction was demarcated as a white area whereas viable myocardium was stained red. Photographs were taken for all sections. Infarct size

was determined by computerized planimetry using the NIH Image J 1.63 software and expressed as a percentage of the left ventricle as previously described[4].

Determination of Capillary Density. The capillary density was determined as described previously[5]. Tissue sections were stained using CD31 antibody (BD Biosciences) (N=6/group). Capillary density in the peri-infarct zone was expressed as capillaries area per field. To obtain the average vessel area per cross-sectional area, a minimum of five individual fields per slide were sampled, and Image J was used to measure the counted field area in each field.

Analysis of Angiogenesis Genes using Laser-Capture Microdissection (LCM). Mice hearts were excised, rinsed, embedded in optimal cutting temperature, and immediately frozen in liquid nitrogen (N=5/group). Ten micron thick tissue sections of left ventricle were prepared on polyethylene napthalate membrane-coated slides (MicroDissect GmbH). For LCM, slides were thawed briefly and air dried 5 minutes before dissection. Green fluorescence observed under laser microscopy was used as a landmark for microdissection. Tissues near to the fluorescence area (engrafted cells) were dissected out by applying Leica LCM Systems (MicroDissect GmbH) into the caps of microcentrifuge tubes as previously described[6].

Quantitative Real-Time PCR. RNA was isolated from laser microdissected tissue using miRNeasy Mini Kit (Qiagen), from whole cells using RNeasy Mini Kit (Qiagen) and from exosomes using miRCURY™ RNA Isolation Kit (Exiqon), respectively, according to manufacturer's instructions. Total RNA was reverse transcribed with using the SuperScript first-strand synthesis system for RT-PCR (Applied Biosystems) or using Taqman microRNA reverse

transcription kit (Applied Biosystems). All primers and probes were obtained from Applied Biosystems. Reactions were analyzed using the ABI Prism 7300 sequence detection system and data were normalized to GAPDH or miR-16 levels and quantified using the comparative threshold cycle (Ct) method.

Isolation of Cardiac Endothelial Cells (ECs). Cardiac ECs were isolated from heart explants as described in the main text with slight modifications. Enrichment of ECs was performed using magnetic beads against CD31. Characterization of ECs was performed using an Endothelial Cell Characterization Kit (Millipore) according to manufacturer's instructions. To exclude contamination from serum-derived exosomes, exosome-depleted serum (System Biosciences) was used for propagation of ECs and the collection of conditioned medium (CM) and exosomes. ECs were transfected using Lipofectamine LTX with Plus reagent (Applied Biosystems) with MCs, and using Lipofectamine RNAiMAX reagent with cel-miR-39 (100 nM) and with siRNA targeting nSMase2 (50 nM), miR-126 (50 nM), miR-210 (50 nM), or scrambled (50 nM).

Uptake of Exosomal miRs by CPCs. Following transfection of ECs with cel-miR-39, MC-GFP, MC-HIF or siRNAs, conditioned media or purified exosomes (as indicated in the text) was added to CPCs. Expression of target miRs was then determined by qPCR.

Exosomal Degradation Analysis. Exosomes purified from ECs media were treated with either RNase, Proteinase K or Triton X-100 for 45 minutes at 37ºC. Expression levels of selected miRs were then determined by qPCR.

Electron Microscopy. For transmission electron microscopy, exosomes were fixed in 2% formaldehyde, loaded on 300-mesh formvar/carbon-coated electron microscopy grids (Electron Microscopy Sciences, PA), post-fixed in 1% glutaraldehyde, and then contrasted and embedded as described previously[7]. Transmission electron microscopy images were then obtained using a Jeol 1230 transmission electron microscope operating at 120 kV.

Immunoblotting. A total of 10-50 μg of protein of each sample was loaded onto a 10% Bis-Tris gel (Applied Biosystems). Primary antibodies used are as follows: HIF-1α (Novus Biologicals), CD63 and CD9 (System Biosciences), p-ERK, pan-ERK, p-AKT and pan-AKT (all from Cell Signaling). Equal protein loading was confirmed by α-Tubulin (Cell Signaling).

Dynamic Light Scattering. Dynamic light scattering analysis of exosomes was performed with a Zetasizer Nano ZS (Malvern Instruments, U.K.). Measurements were collected on a continuous basis for 3 minutes in sets of two using two different sets of samples from each exosome preparation.

Exosome Labeling and Uptake Assay. Exosomes were labeled with PKH26 red fluorescent membrane linker-dye (Sigma) according to manufacturer's protocol. Briefly, exosomes were labeled with PKH26 for 5 mins, before stopping the reaction with addition of exosomes-free FBS. Labeled-exosomes were then concentrated through an Ultracel-100K device (Millipore) and added to $Sca1^+$ CPCs in a 24-well plates. Six hours after addition, $Sca1^+$ CPCs were observed by confocal microscopy to visualize for uptake of PKH26-labeled exosomes. Labeling efficiency of

exosomes was also determined by flow cytometry using Exo-FLOW Exosome Purification Kit with slight modifications to manufacturer's instructions (System Biosciences, CA).

Luciferase Reporter Assay. CPCs were transfected with 100 ng of 3'-UTR luciferase reporter vector containing either a miR-126 or miR-210 target seed sequence compared to an empty vector (SwitchGear Genomics). Sixteen hours later, cells were washed and supplemented with conditioned medium as indicated for 24 hours before measuring luciferase activity with LightSwitch Assay Reagent (SwitchGear Genomics).

Oxygen Consumption Measurement. Oxygen consumption was measured using a Seahorse Bioscience XF96 Analyzer. Briefly, cells were seeded 24 hours before the day of experiment with treatments as indicated. Assays were initiated by replacing the growth medium from each well with unbuffered assay medium pre-warmed at 37°C. The cells were incubated at 37°C for 60 min to allow media temperature and pH to reach equilibrium before the first rate measurement. Basal respiration rates is defined as the initial oxygen consumption rate (OCR) taken after equilibration. All data were adjusted for the non-mitochondrial respiration rate (lowest rate after rotenone/antimycin injection). Total cellular protein was measured following each experiment by the Bradford method. All OCRs were normalized to total cellular protein and expressed as fold-change relative to control.

Intracellular Lactate Measurement. CPCs with or without CM treatment were cultured for 16 hours. Upon completion of treatment, cells were lysed, centrifuged, and supernatant was collected for intracellular lactate measurements using Lactate Colorimetric Assay Kit II

(Biovision Inc) according to manufacturer's instructions. Results were normalized against cell number as determined by trypan blue assay using the Countess Automated Cell Counter (Applied Biosystems).

***In Vitro* Hypoxia Assay.** CPCs were exposed to hypoxia (BD GasPak EZ system) for 8 hours in the presence or absence of CM, or the exosome-depleted fraction of EC^{HIF}-CM, or CM derived from ECs transfected with MC-HIF1 and also antagomirs against miR-126 and miR-210. The lactate dehydrogenase (LDH) release, as a marker for cell injury was quantified using CytoTox-ONE Homogeneous Membrane Integrity Assay (Promega) according to manufacturer's protocol.

Uptake of Exosomes by CPCs *In Vivo.* To analyze exosomes uptake by CPCs, cells were injected intramyocardially into NOD/SCID mice following MI (N=3/group). Exosomes pre-labeled with PKH26 were then injected intravenously through the tail vein (10 µg in 50 µl PBS). After 24 hours, hearts were explanted, dissociated into single cells, and analyzed by flow cytometry. Digested cells were first sorted for GFP^+ signals representing transplanted CPCs, followed by $PKH26^+$ signals representing uptake of labeled exosomes *in vivo*. A total of 10, 000 events were recorded in each analysis. Data analysis was performed using FlowJo software (Tree Star, OR).

Direct Effects of Exosomes on CPCs *In Vivo*. To determine whether exosomes can directly confer protection to CPCs *in vivo,* intravenous delivery of saline, EC^{GFP}-derived exosomes (EC^{GFP}-Exo), or EC^{HIF}-derived exosomes (EC^{HIF}-Exo) (20 µg protein in 50 µl PBS) was

delivered through the tail vein concomitantly with transplantation of CPCs following LAD ligation (N=6/group). BLI was then performed to determine cell survival 1 week post-injection.

SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure 1. MC-HIF1 enhances cell survival and promotes cardioprotection. **(A)** Immunostaining of GFP^+ $Sca1^+$ cardiac progenitor cells (CPCs) at 7 days post-injection. **(B)** Representative images of infarct size analysis by tetrazolium chloride staining. **(C)** Representative images of vascularity analysis using an endothelial marker CD31.

Supplemental Figure 2. Uptake of endothelial cell-derived exosomes by CPCs *in vitro*. **(A)** Labeling efficiency of exosomes was determined by flow cytometry. Typical efficiency was around 85%. **(B)** Flow cytometry analysis of exosomal uptake by CPCs. **(C)** Effects of HIF-1 overexpression on the production of exosomes from endothelial cells (ECs). **(D)** Pharmacological activation of HIF-1 increases exosomal miRs expression. $^*P<0.05$ vs. untreated (N=4). **(E)** Expression levels of *Spred1* in CPCs grown in indicated conditions were determined by qPCR. $^*P<0.05$ vs. untreated (N=5); $^\#P<0.05$ vs. EC^{HIF}-Exo (N=5). **(F)** Measurement of intracellular lactate in CPCs grown in conditioned medium from ECs. $^*P<0.05$ vs. EC^{GFP}-Exo (N=4).

Supplemental Figure 3. Schematic diagram describing the modulation of ischemic microenvironment by HIF-1 leads to transfer of exosomal miRs from host endothelial cells to transplanted CPCs and promoting their survival.

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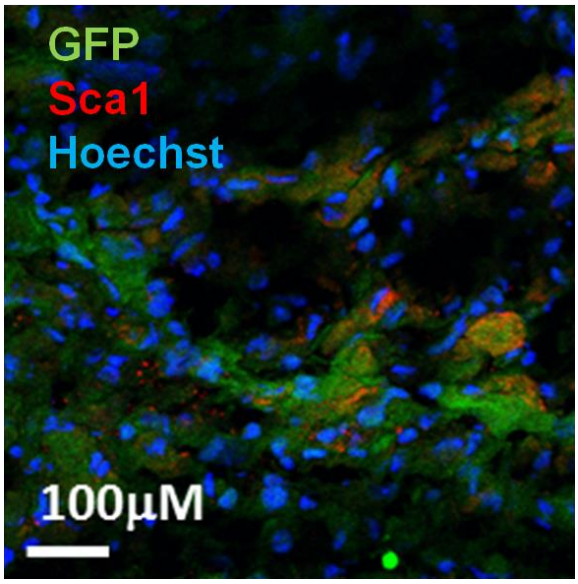
A



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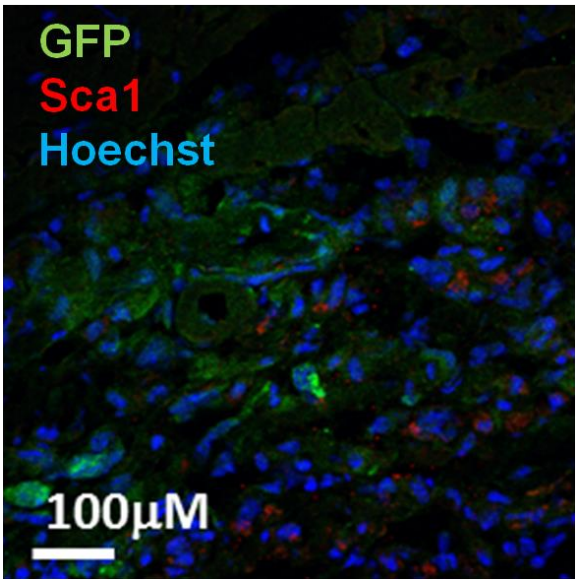

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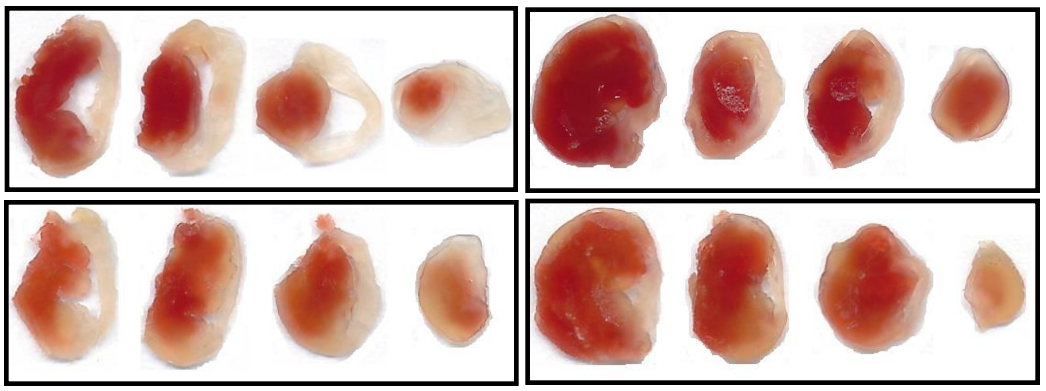

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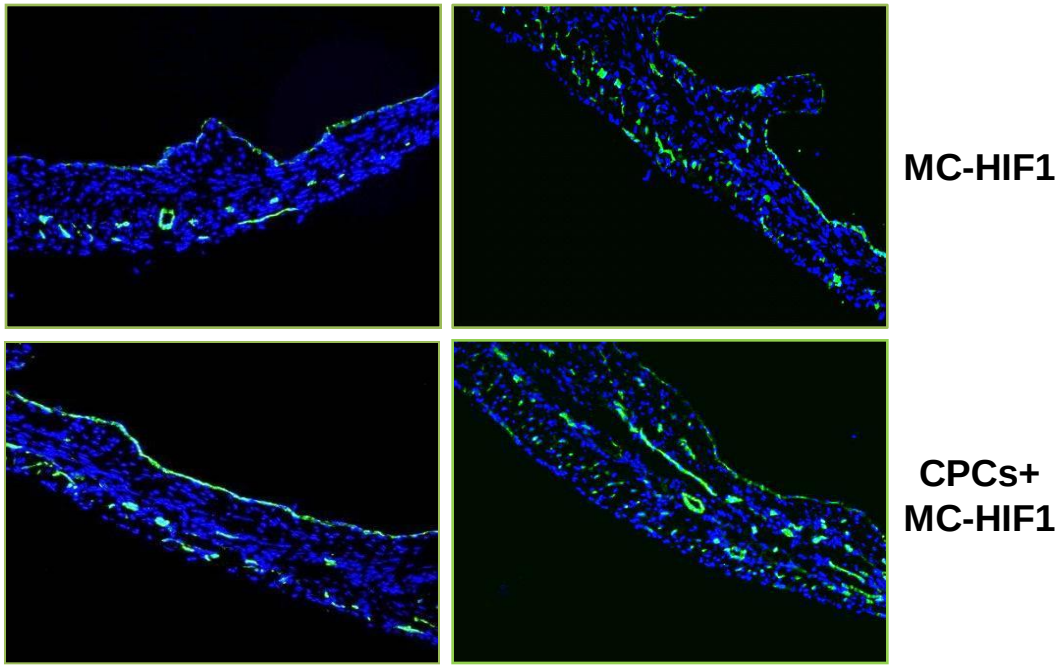
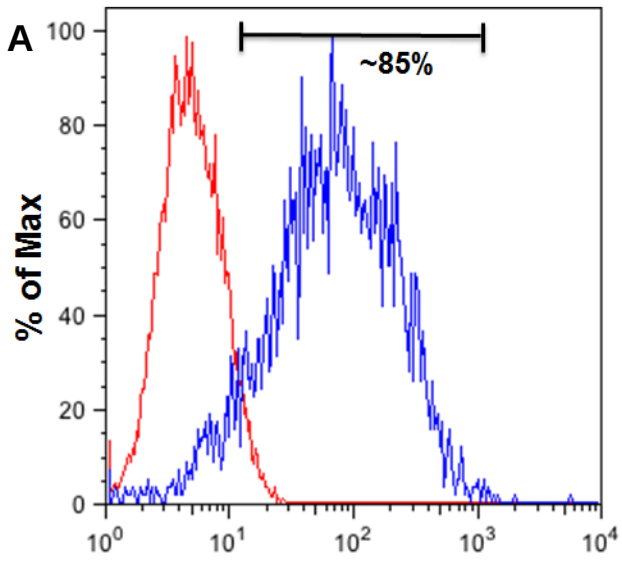
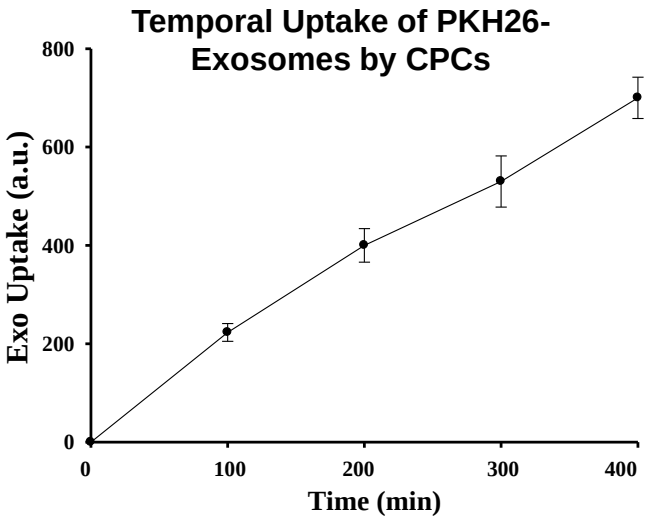
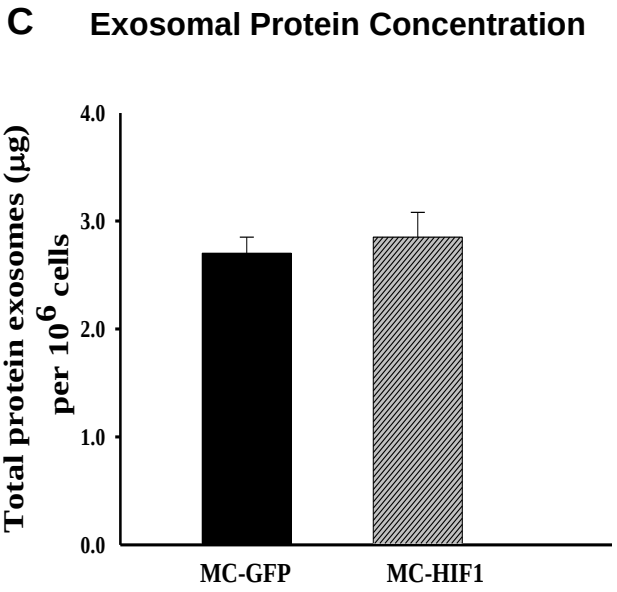
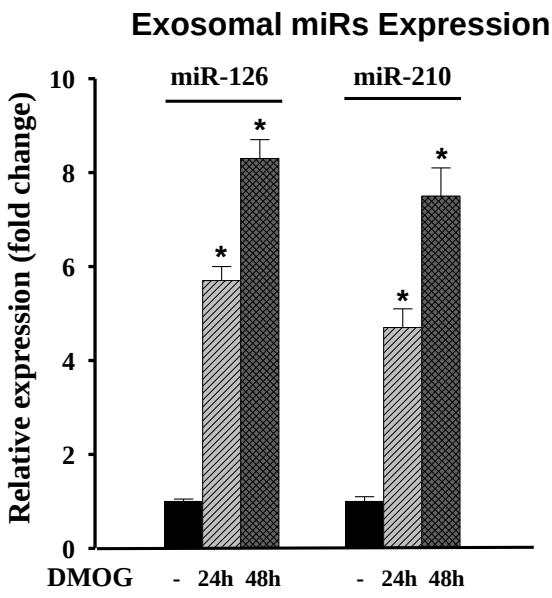


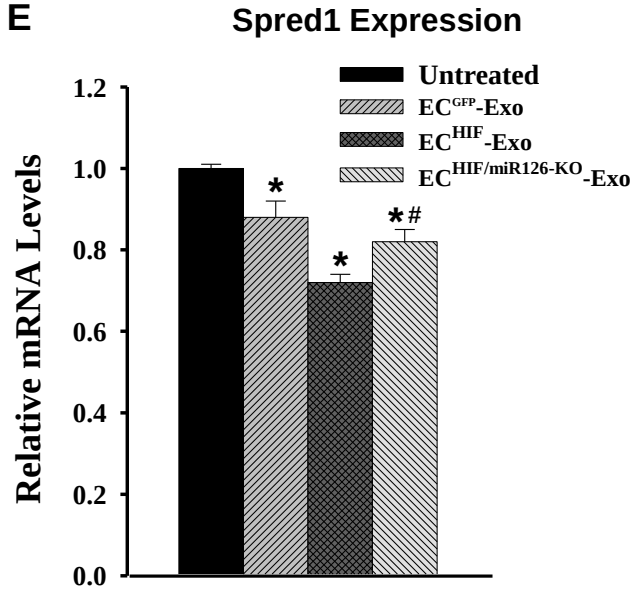
Supplemental Figure 1

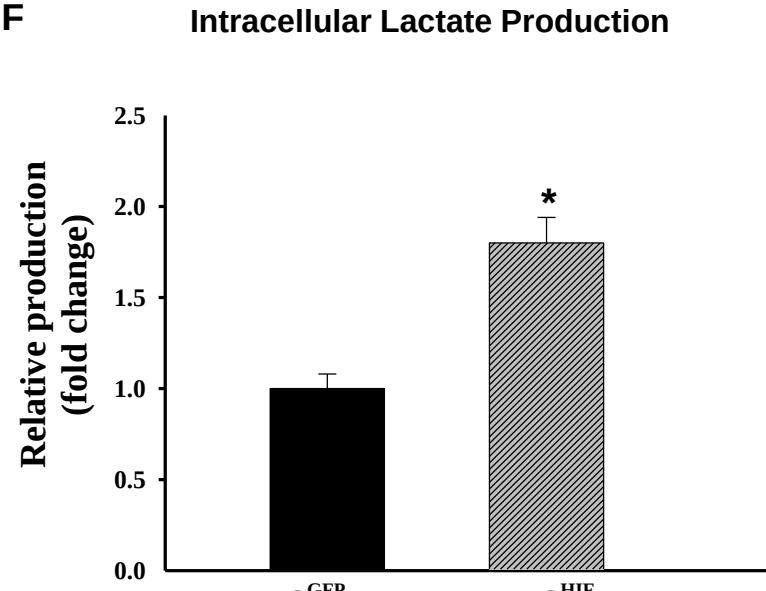












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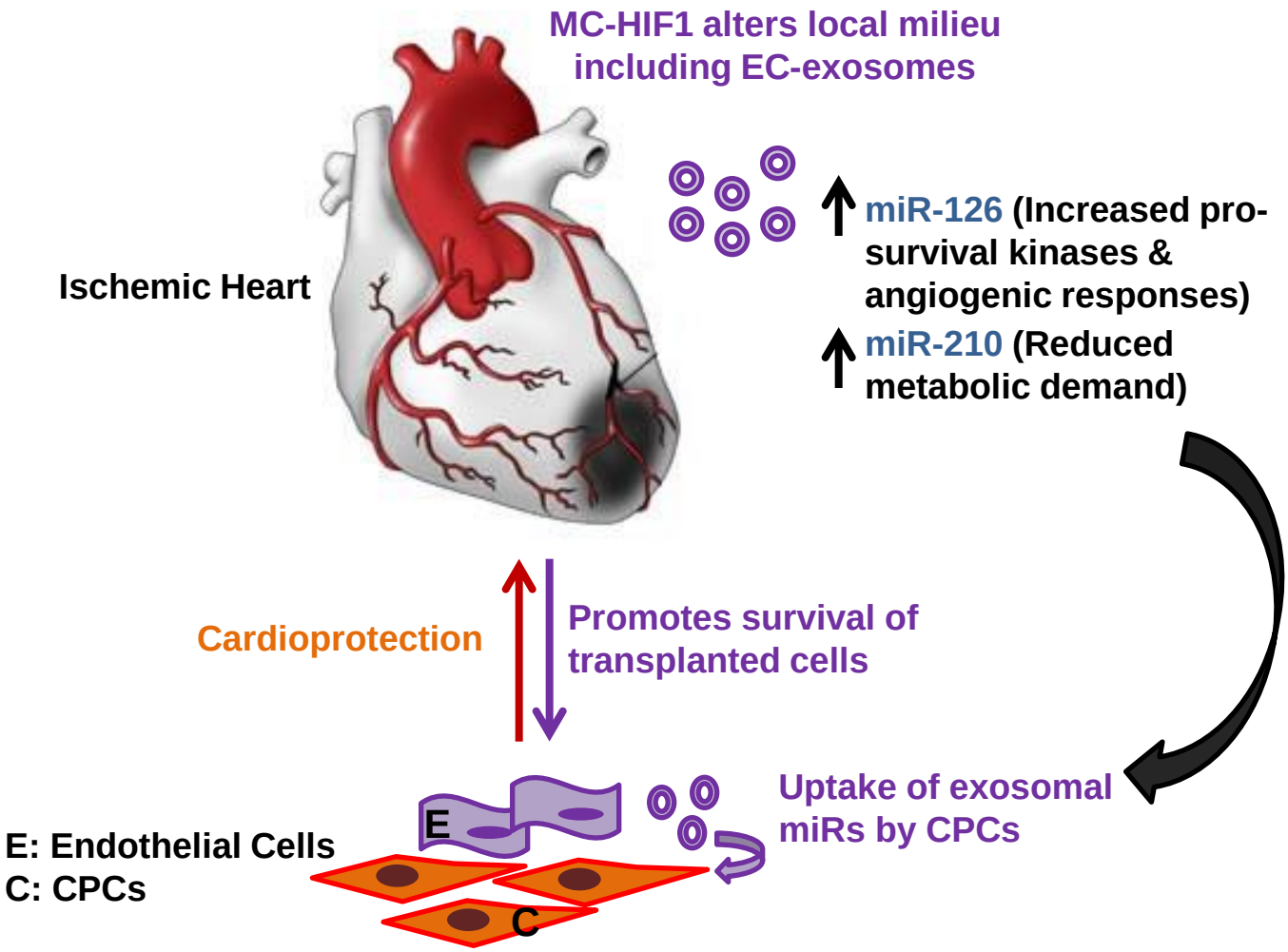

Supplemental Figure 3