**Supplemental Material**

**Supplemental methods**

**Knockdown of Wnt5a and Wnt11 in differentiated hiPSCs**

Wnt5a and Wnt11 were knocked down in differentiated hiPSCs treated with ISX-9 as described previously [[1](#_ENREF_1)]. Briefly, at day3 of ISX-9 treatment, cells at 80% confluency were transfected with 10nM silencer select siRNA Wnt5a (Sense: UAUCAAUUCCGACAUCGAAtt, Antisense: UUCGAUGUCGGGAAUUGAUAc) and Wnt11 (Sense: ACUUCUGCAUGAAGAAUGATT, Antisense: UCAUUCUUCAUGCAGAAGUCA) using Lipofectamine RNAiMAX (Thermo Fisher Scientific, Cat #13778075). Silencer™ Negative Control No. 1 siRNA was obtained from Thermo Fisher Scientific Silencer siRNA labeling kit (Thermo Fisher Scientific, Cat # AM1632) was used to determine transfection efficiency. After 48h, verification of Wnt5a and Wnt11 knockdown in transfected cells was analyzed by real-time PCR. At day 7, cells were harvested for analysis of cardiac transcription factors expression.

**Immunohistochemistry**

For cell immunocytochemistry, the cells were fixed with 4% paraformaldehyde (PFA) for 10 minutes at room temperature. After washing with PBS, the cells were blocked for 1h minutes at room temperature by a blocking buffer (PBS containing 10% fetal bovine serum and 0.1% triton). Cardiac progenitor cells (CPCs) were immunostained with respective specific primary antibodies Nkx2.5 (abcam, ab91196, 1:300), GATA4 (SC-1237, Santa Cruz, 1:100) and ISL-1 (ab86472, abcam, 1:300). hiPSC-CMs were characterized via the expression of α-sarcomeric actin, cTnT, cTnI, MLC2V and CX43; hiPSC-ECs were characterized via the expression of CD31 and VE-cadherin; hiPSC -SMCs were characterized via the expression of α-smooth muscle actin (SMA) and calponin. Briefly, hiPSC-CMs were immunostained with respective specific primary antibodies against α-sarcomeric actinin (A7811, Sigma, 1:200), cardiac troponin T (13-11, Thermo Fisher Scientific, 1:200), cardiac troponin I (701585, Thermo Fisher Scientific, 1:200), MLC2V (10906-1-AP, Protein Tech Group, 1:200), and CX43 (ab11370, abcam, 1:200). hiPSC-ECs were immunostained with antibodies against EC markers CD31 (ab24590, abcam,1:200) and VE-cadherin (ab33168, abcam, 1:200). hiPSC-SMCs were immunostained with antibodies against SMC markers SMA (ab5694, abcam, 1:500) and calponin (C-2678, sigma, 1:600). The primary antibody-antigen reaction was detected with fluorescently conjugated specific secondary antibodies. Every time after incubation with antibody, the samples were washed three times with PBS. Nucleiwere visualized after staining with 5μg/ml by 4,6'-diamidino-2-phenylindole (DAPI; Life technologies). Fluorescence signals were observed and photographed using fluorescence microscope (Olympus, Tokyo, Japan).

Histological analysis was performed on randomly selected hearts from mice subjected to myocardial infarction (MI) and injected with DPBS, hiPSCs without ISX treatment or CPCs after ISX-9 treatment (n = 6 per group for transplanted cell engraftment analysis; n = 3 per group for CPCs tracking and quantification, TUNEL staining after 3 days of transplantation; n = 3 per group for differentiated CMs tracking and quantification after 3M of transplantation). All hearts were fixed with 4% PFA for 1 hour at room temperature and replaced by 30% sucrose overnight at 4 ℃. These samples were embedded in an optical cutting temperature (OCT) compound (Tissue Tek) and sliced into 5-μm-thick frozen sections. α-sarcomeric actinin (A7811, Sigma, 1:200), human cardiac troponin T not cross reacting with mouse (ab45923, abcam, 1:200) staining, CD31 (MA5-13188, Thermo Fisher Scientific,1:100), α-SMA (ab5694, abcam, 1:300), cardiac troponin I (701585, Thermo Fisher Scientific, 1:200), and human mitochondrial antigen (MAB1273, Millipore sigma,1:200) were carried out. Signals were visualized with DyLight 405 (Thermo Fisher Scientific), Alexa Fluor 647 and Alexa Fluor 488 secondary antibodies (Life technologies). Image acquisition was performed on a confocal microscope (FV1000, Olympus, Japan). Nuclei was counterstained with DAPI or Qnuclear™ Deep Red Stain (Thermo Fisher Scientific). Masson trichrome staining was performed according to the manufacturer’s protocol (HT-15, sigma), n = 5 per group in myocardial fibrosis analysis.

**Murine model of myocardial infarction**

Myocardial Infarction model was induced in 8-9-week-old NOD/SCID mice (The Jackson Laboratory) by left anterior descending artery (LAD) ligation under 2% inhaled isoflurane anesthesia. Briefly, mice anesthetized with isoflurane (5% induction, 1.5-3% maintenance), surgically prepared with analgesics (buprenorphine SR lab, 1.2 mg/kg) before incision. The heart was exposed by left-sided limited thoracotomy and the LAD was ligated with a prolene #8-0 suture. Myocardial ischemia was confirmed by color change of the left ventricular wall.

**Infarct size measurement**

Infarct size was determined as the average of 7 sections sampled at 4-mm intervals from the apex as a ratio of Masson's trichrome-stained area and total left ventricular area. Briefly，the total area of LV and infarct scar were traced by level set and thresholding methods [[2-4](#_ENREF_2)], refined manually in the digital images, and then measured automatically. The level-set method with the ellipse is used in segmentation based on the observation that the epicardium is bounded by a circle-like contour. The level set formulation with shape priors has been demonstrated to be an efficient segmentation method and a number of techniques have been reported [[2](#_ENREF_2)]. In the present study, the general expression for the proposed ellipse refined level set segmentation energy formulation reads as follows,



where Edata represents the chosen data attachment term and Eshape embeds the shape prior. The weight corresponds to a positive hyper-parameter that balances the influence between the two terms. As Wang et al. [[2](#_ENREF_2)] pointed out, Edata can be any of the data attachment terms and in the present study, the narrow band active contour is adopted as the data attachment term. After the segmentation of the epicardium, a color thresholding method was performed to measure the heart tissue and the infarct scar. The infarct size, expressed as a percentage, was calculated by dividing the sum of infarct areas from all sections by the sum of LV areas from all sections (including those without infarct scar) and multiplying by 100.

**Flow Cytometry**

To detect purity of CPCs and hiPSC-CMs, hiPSC-ECs and hiPSC-SMCs, cells were dissociated with Collagen IV (stem cell technologies) for 2h following 10 min accutase digestion. Dissociated cells were fixed and permeabilized with a BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit (BD Biosciences), and then incubated with antibody against Nkx2.5 (abcam, ab91196, 1:200), cTnT (13-11, Thermo Fisher Scientific; 1:200), CD31 and SMA (ab5694, abcam, 1:200) at 4 °C overnight. After three washes, cells were incubated with isotype-matched Alexa Fluorescence-conjugated secondary antibody (Life technologies) for 1hr at room temperature and detected by an LSR II Flow Cytometer (BD Biosciences). Isotype-matched normal IgG was used as negative control.

**Functional Assays of Endothelial cells in vitro**

To analyze tube formation on Matrigel in vitro, hiPSC-ECs were seeded on top of a thin layer of Matrigel at a density of 1.2×105 cells/well of a 24-well plate. After 16h, cells were labeled with calcein AM (Corning) and visualized tube-like structures under the fluorescent microscope. Uptake of acetylated low-density lipoprotein (ac-LDL) was assessed by incubating cells with 5 μg/ml of ac-LDL conjugated with Alexa Fluor-594 (Invitrogen) for 4h. After incubation, cells were counterstained with DAPI to visualize nuclei.

**RNA-sequencing in CPCs generated by ISX-9 treatment**

mRNA-Sequencing transcriptome analysis was performed to reveal differences in gene expression among undifferentiated hiPSCs, DMSO or ISX-9 treated hiPSCs (n = 4). Global miRNA expression profiles in undifferentiated hiPSCs, DMSO or ISX-9 treated hiPSCs were also determined. mRNA-sequencing and miRNA-sequencing were performed by Core Genomics Facility and DNA services facility at University of Illinois at Chicago. Bioinformatic data analysis was conducted by Research Informatics Core at University of Illinois at Chicago. Briefly, total RNA containing miRNA was extracted from cells using Qiagen’s RNasey Plus Mini kit according to manufacturer’s instructions followed by DNAse treatment using RNase-free DNase kit (Qiagen). For mRNA-sequencing, Sequencing libraries were prepared using QuantSeq 3’mRNA-Seq Library Kit (Lexogen Inc, product # 015.96). Input amount for the library preparation was 500 ng of total RNA per sample. Libraries were PCR amplified at 16 cycles and quantified using Qubit dsDNA HS assay. For miRNA-sequencing, Sequencing libraries were prepared using Illumina’s TruSeq Small RNA Core solutions kit + Truseq Small RNA Indices A kit (Illumina product #s respectively: 15016911 and 15016912). Input amount for library preparation was 1 ug of total RNA per sample. Libraries were PCR amplified at 11 cycles and quantified using Qubit dsDNA HS assay. Sequencing libraries were pooled in equimolar concentrations and sequencing was performed on NextSeq 500 (Illumina), single read 75 nt, high output (about 400 million reads per lane).

For the analysis of 3’mRNA-seq data, the short reads of 12 samples were mapped to the UCSC human hg19 reference genome using BWA mem [[5](#_ENREF_5)].The raw counts of each gene were quantified using featureCounts base on the mapping results, and transcriptome annotation of UCSC hg38. The genes with zero counts across all samples were filtered from further analysis. The raw counts were normalized to count per million (CPM) for each gene within each sample by using R Bioconductor Package [[6](#_ENREF_6)].The Differentially Expressed Genes (DEGs) were identified using edgeR. First, the General Linear Model (GLM) Likelihood Ratio Test (LRT) was performed for identifying DEGs where the mean expression value of a gene in any group is significantly different than in other sample groups. The raw p-values were adjusted by Benjamini–Hochberg correction [[7](#_ENREF_7)].Second, the pairwise comparisons were performed between DMSO vs. hiPSC group, ISX-9 treatment group vs. hiPSC without ISX-9 treatment , and ISX-9 treatment group vs. DMSO. The exact test was performed on each gene between each pair of group samples to identify significant pairwise DEGs [[8](#_ENREF_8)]. The p-values from the exact test were also corrected by Benjamini–Hochberg correction. Likewise, in the analysis of miRNA-seq data, the 3’ short reads of 12 samples were mapped to the NCBI human GRCh38 reference genome using BWA mem [[5](#_ENREF_5)]. The raw counts of transcripts of each miRNA were quantified using featureCounts based on the mapping results, and the miRNA annotation from miRBase [[9](#_ENREF_9)]. The normalization and differential analysis followed the same procedures as in mRNA-seq data analysis. Pathway enrichment analysis was processed using metacore online analysis.

**Transmission Electron microscopy**

The differentiated cardiomyocytes (CMs) were fixed overnight at 4 ℃ in 2.5% (Vol/Vol) glutaraldehyde, 0.1M cacodylate buffer solution and were postfixed with 1% osmium tetroxide in the same buffer, en-block stained with 2% aqueous uranyl acetate, dehydrated in acetone, infiltrated, and embedded in LX-112 resin (Ladd Research Industries, Burlington, VT). Ultrathin 60-nm sections were stained with uranyl acetate and lead citrate. Samples were examined on a JEOL JEM-1220 transmission electron microscope (TEM) (JEOL USA, Inc.)

**Echocardiography**

The mice were anesthetized mildly with inhaled isoflurane (1% isoflurane) and lightly secured in the supine position on a warm pad. After the hair was removed, Acoustic gel was applied and transthoracic echocardiography was performed using [Philips iE33](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&cad=rja&uact=8&ved=0ahUKEwjNssDY1IjPAhUBfCYKHWwtAXoQFggrMAI&url=http%3A%2F%2Fwww.umiultrasound.com%2Fultrasound-machine%2Fphilips%2Fie33&usg=AFQjCNE6_NnFRAWvmRp023YhiHdk8QDY6Q&sig2=riEOHsUaHKIFJW9ZkOo4dA) ultrasound machine with [L15-7io Transducer](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjNssDY1IjPAhUBfCYKHWwtAXoQFggcMAA&url=http%3A%2F%2Fwww.umiultrasound.com%2Fultrasound-transducers%2Fphilips%2Fie33%2Fl15-7io-transducer&usg=AFQjCNG8U1DLyMo-5aIjTPKPaQXiEC3WGQ&sig2=E95vdY4hLBgn_EVAINzvmA). The heart was imaged in the two-dimensional mode in the parasternal long-axis and/or parasternal short-axis views which were subsequently used to position the M-mode cursor perpendicular to the ventricular septum and left ventricle posterior wall, after which M-mode images were obtained. For each animal, measurements were obtained from 4-5 consecutive heart cycles. Measurements of left ventricular end diastolic diameter (LVEDD), and left ventricular end systolic diameter (LVESD) were made from two-dimensionally directed M-mode images of the left ventricle in both systole and diastole [[10](#_ENREF_10)]. The average value from all measurements in each animal were used to determine the indices of left ventricle contractile function, i.e. left ventricle fractional shortening (LVFS) using the following relations LVFS= (LVEDD-LVESD) / LVEDD x 100, ejection fraction (EF) using the following relations EF= [(EDV - ESV) / EDV] x 100. LVFS and EF were expressed as percentages.

**qRT-PCR**

Total RNA was isolated using RNeasy Mini Kit (Qiagen). Reverse transcription was performed using QuantiTect Reverse Transcription kit (Qiagen) or SuperScript™ IV VILO™ Master Mix (Thermo fisher Scientific Inc). qRT-PCR was performed on real-time system ViiA™ 7 (ABI) or Q3 real-time PCR machine (ABI) using Quantitate SYBR Green real-time PCR method or Taqman probe method as described elsewhere (Primer sequences are shown in Table1). Probes for Wnt3a (Hs00263977\_m1), Wnt5a (Hs00998537\_m1); Wnt11 (Hs01045905\_m1); ISL-1(Hs00158126\_m1); Nkx2.5 (Hs00231763\_m1); Mef2c (Hs00231149\_m1); GATA4 (Hs00171403\_m1); TUBA1A (Hs03045184\_g1) were purchased from Thermo Fisher Scientific GADPH and TUBA1A act as the loading control. The fold change of expression level for each gene was determined by the expression 2-∆∆CT. The final values were averaged and results were represented as fold expression with the standard error of the mean (S.E.M.).

**Supplemental Figures and Figure Legends**

Supplemental Figure 1: hiPSC cell line characterization, pluripotency markers staining and alkaline phosphatase staining.

Supplemental Figure 2: Monolayer hiPSC expressed pluripotency markers.

Supplemental Figure 3: ISX-9 treated cells in mTeSR1 (A) and RPMI/B27; (B) Bright filed, bar=200 µm.

Supplemental Figure 4: NKx2.5, ISL-1, GATA4 staining in mTeSR1, DMSO, ISX-9 (un-differentiation medium).

Supplemental Figure 5: NKx2.5, ISL-1, GATA4 staining in RPMI/B27, DMSO, ISX-9 (differentiation medium).

Supplemental Figure 6: Morphology of differentiating cardiomyocytes (CMs), endothelial cells (ECs) and smooth muscle cells (SMCs), bar = 200 µm.

Supplemental Figure 7: Pathway enrichment analysis of upregulated and downregulated genes in CPCs induced by ISX-9 in comparison with undifferentiated hiPSCs without ISX-9 treatment.

Supplemental Figure 8: (A-B) Silencing efficiency for transient knockdown of Wnt5 and Wnt11 expression using real-time PCR analysis. \* vs. siWnt5 group or siWnt11 group, *P* < 0.05. (C) Representative images of labeling Negative control siRNA (NTsiRNA), siWnt5 and siWnt11 using Cy3 after 48h of transfection in ISX-9 treated hiPSCs, bar = 100 µm.

Supplemental Figure 9: Concentration of cytokines in RPMI/B27, DMSO, ISX-9 treated groups. (\* vs. MOCK group with the same treatment, *P* < 0.05; # vs. DMSO group with the same treatment, *P* < 0.05; & vs. ISX-9 treatment group in normoxic condition; *P* < 0.05).

Supplemental Figure 10: Engrafted CPCs were identified by PKH-26 fluorescence (red fluorescence); muscle fibers were visualized via immunostaining for human specific cTnT or α-sarcomeric actinin (Green fluorescence) at 2M post-MI, bar = 50 µm.

Supplemental Figure 11: Transplantation of hiPSC-CPCs induced vasculogenesis in ischemic heart. Vascular density and arteriole density at 3month post-MI were evaluated in the sections from border zone of infarcted hearts by CD31 (A) and α-SMA (B) staining. CMs were identified by α-sarcomeric actinin staining. Vascular density was determined by counting CD31 positive vascular structures (C) and arteriole density was determined by counting α-SMA positive vascular structures. \* vs. DPBS group, *P* < 0.05; # vs. hiPSC group, *P* < 0.05. Scale bar represents 50 µm.

Supplemental Table

Table 1 Sequence of real-time PCR primers

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Forward 5’-3’** | | **Reverse 5’-3’** | **Tm(℃)** |
| NKX2.5 | CATTTACCCGGGAGCCTACG | GCTTTCCGTCGCCGCCGTGCGCGTG | | 60 |
| Mef2c | AGATACCCACAACACACCACGCGCC | ATCCTTCAGAGAGTCGCATGC | | 60 |
| GATA4 | GGTTCCCAGGCCTCTTGCAATGCGG | AGTGGCATTGCTGGAGTTACCGCTG | | 60 |
| TBX5 | TACCACCACACCCATCAAC | ACACCAAGACAGGGACAGAC | | 60 |
| ISL1 | CACAAGCGTCTCGGGATT | AGTGGCAAGTCTTCCGACA | | 60 |
| GADPH | CATCCATGACAACTTTGGTATC | CACCCTGTTGCTGTAGCCAA | | 60 |

Supplemental video: Beating differentiated cardiomyocytes from CPCs induced by ISX-9 at day 12.

**Supplemental Reference**

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