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Supplemental Information

Transient secretion of VEGF protein from transplanted hiPSC-CMs enhances engraftment and improves rat heart function post MI

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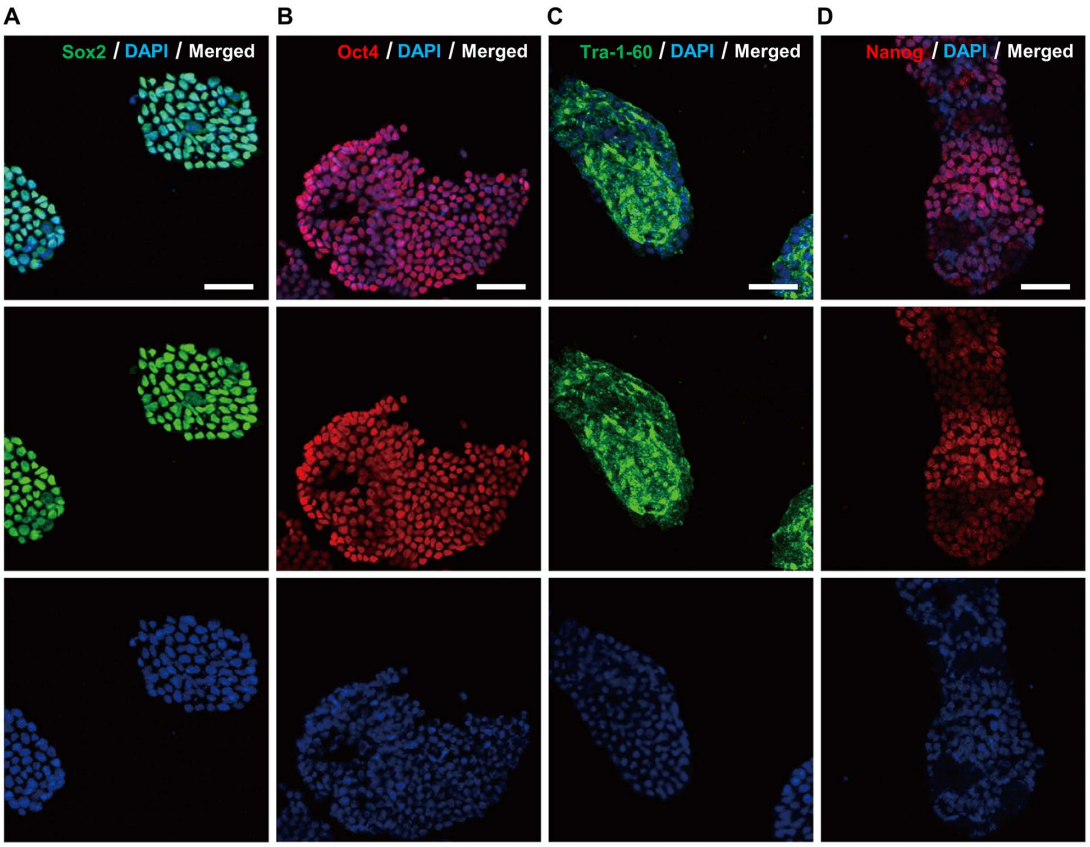

Figure S1. Representative immunohistochemistry staining of iPSCs expressing pluripotency markers.

(A-D) Representative immunostainings of iPSCs expressing Sox2 (A), Oct4 (B), TRA-1-60 (C), and Nanog (D). Scale bar, 25μm.

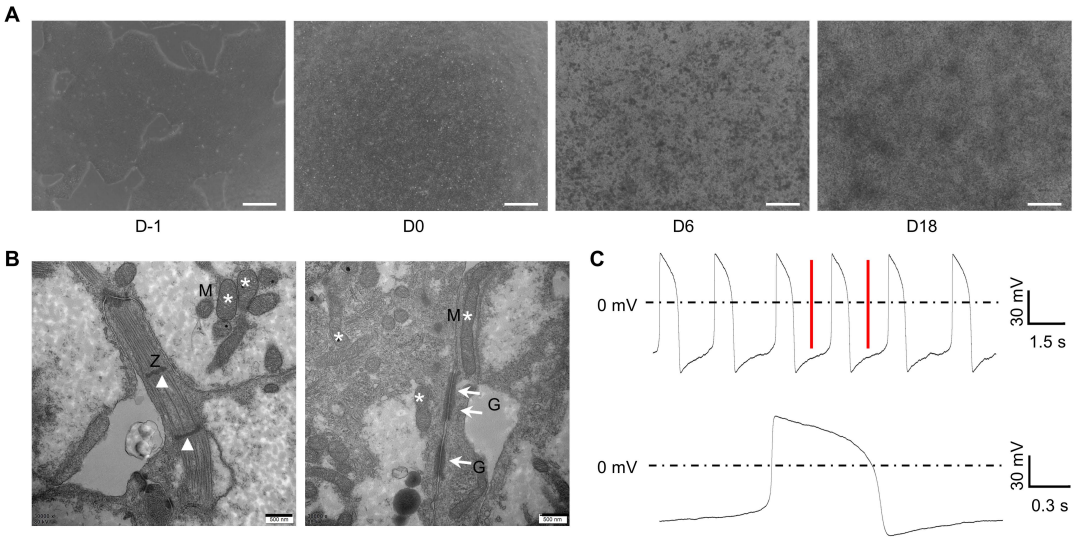

Figure S2. Characteristics of differentiating iPSCs and iPSC-derived cardiomyocytes (iPSC-CMs)

(A) Representative images of iPSCs before the start of differentiation and at several stages after initiation of cardiac differentiation. Scale bar, 500nm. (B) Transmission electron microscope of iPSC-CMs reveal structural details of the sarcomere, Z-line, mitochondria, and gap junctions. Z, white arrowhead indicates Z line; M, white asterisk indicates mitochondria; G, white arrows indicate gap junction. Scale bar, 500nm. (C) Patch clamp recordings showcase action potentials of a single cardiomyocyte. A single action potential magnifies the waveform. The dotted line indicates that the resting potential at 0 mV.

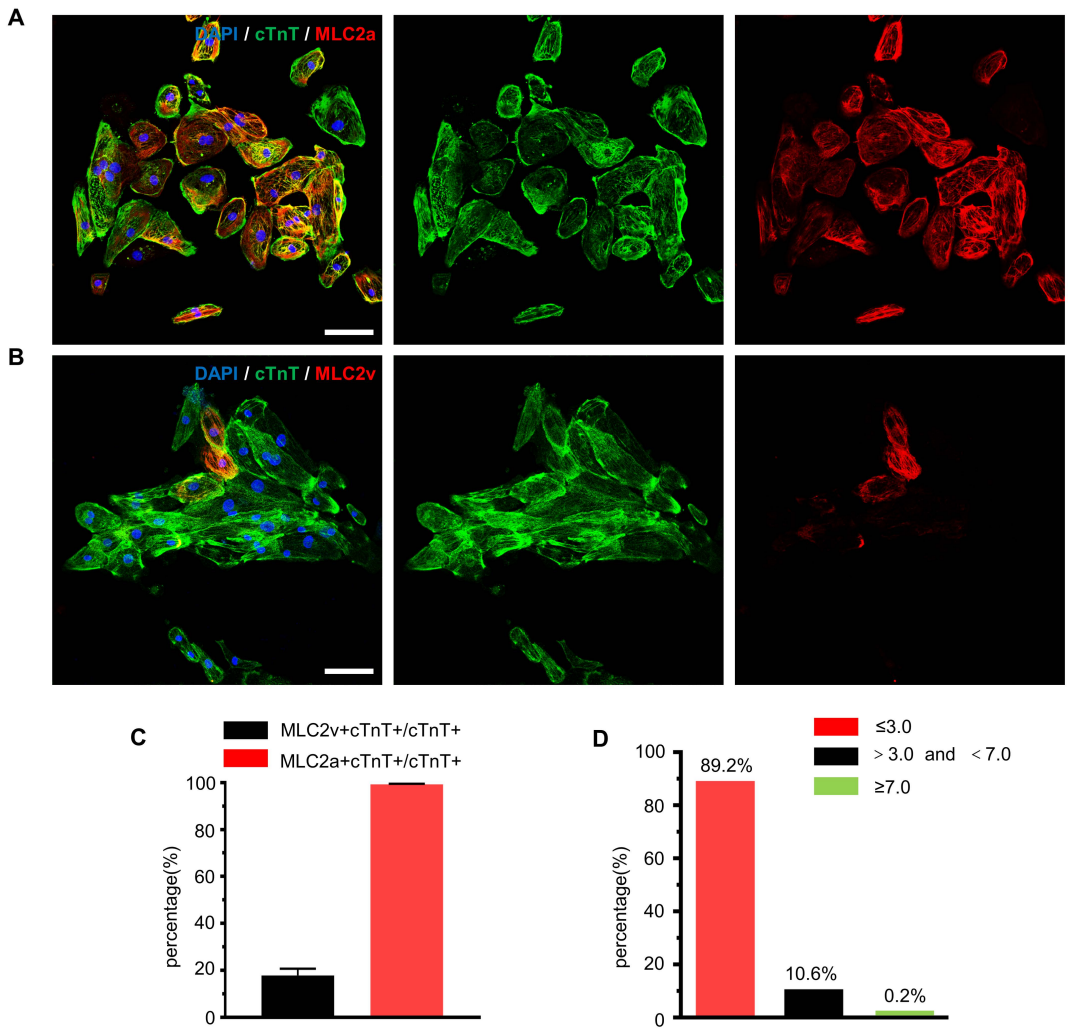

Figure S3. Cardiomyocyte maturation on day 18 of differentiation

(A-B) Representative immunofluorescent images of cardiomyocytes on day 18 of differentiation stained with myocardial structural markers cTnT, MLC2a and MLC2v. A, green, cTnT; red, MLC2a; blue, DAPI; B, green, cTnT; red, MLC2v; blue, DAPI. (Scale bar=75μm). (C) Quantitative analysis of the ratio of MLC2a+cTnT+/cTnT+ and MLC2v+cTnT+/cTnT+ cardiomyocytes. (n=5). (D) Quantitative analysis of the ratio of cardiomyocyte length to width.

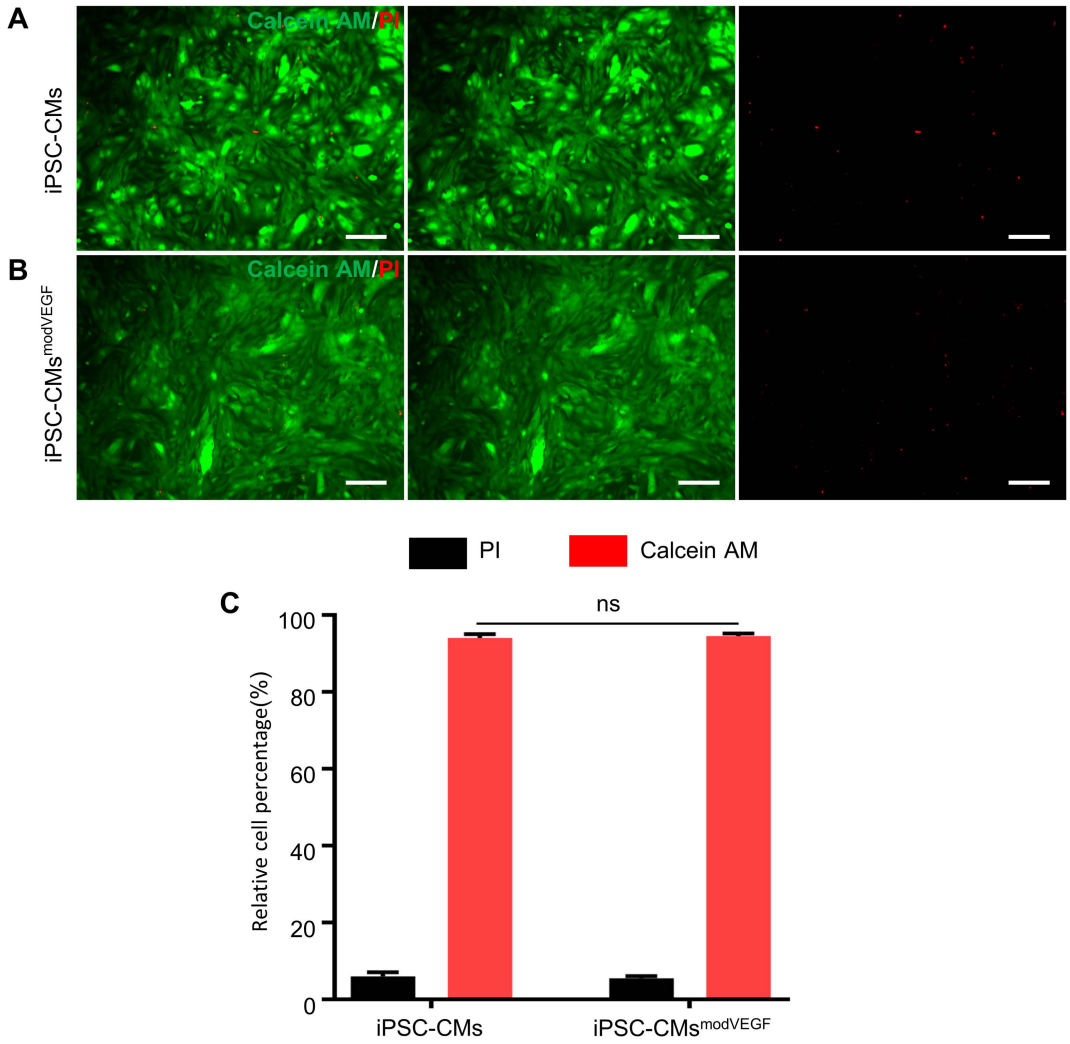

Figure S4. Viability of iPSC-CMs following modRNA transfection

(A-B) Live/dead cell staining 24 hours post-transfection with modRNA: green, Calcein AM, live cells; red, PI, dead cells, Scale bar=150 μm. (C) Quantitative analysis of the ratio of live/dead cells following transfection. (n=5). P values were determined by one-way analyses of variance followed by Bonferroni post-test. (ns indicates $P > 0.05$).

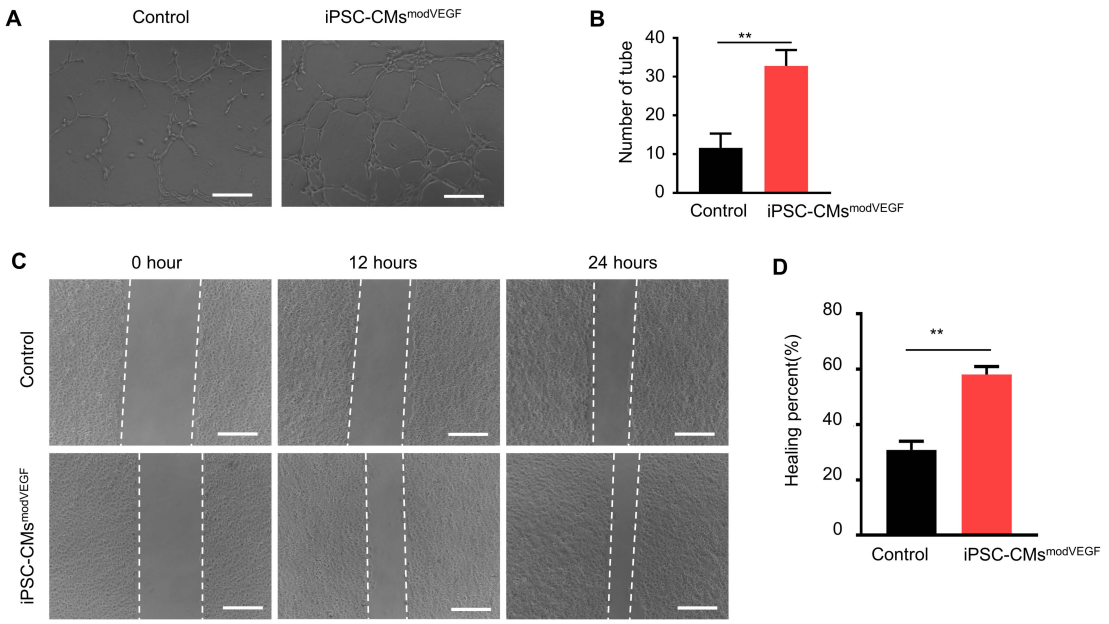

Figure S5. Cell culture supernatant from VEGF-mRNA transfected iPSC-CMs promote functional angiogenic properties

(A) Representative photomicrographs of the tubule formation assay on cultured human umbilical vein endothelial cells (HUVEC), following media changes and (B) quantitative analysis of the number of tubules formed (5 biological repeats for each group). Scale bar=500 μm. (C) Scratch wound healing assay of HUVECs treated with serum lysates from either non-transfected iPSC-CMs (control) or iPSC-CMs transfected with VEGF modRNA and analyzed at 0, 12, 24 hours. Scale bar=500 μm. (D) Quantitative analysis of 24-hour scratch healing rate (5 biological repeats for each group). P values were determined by one-way analyses of variance followed by Bonferroni post-test. (*p <0.05, **p <0.01).

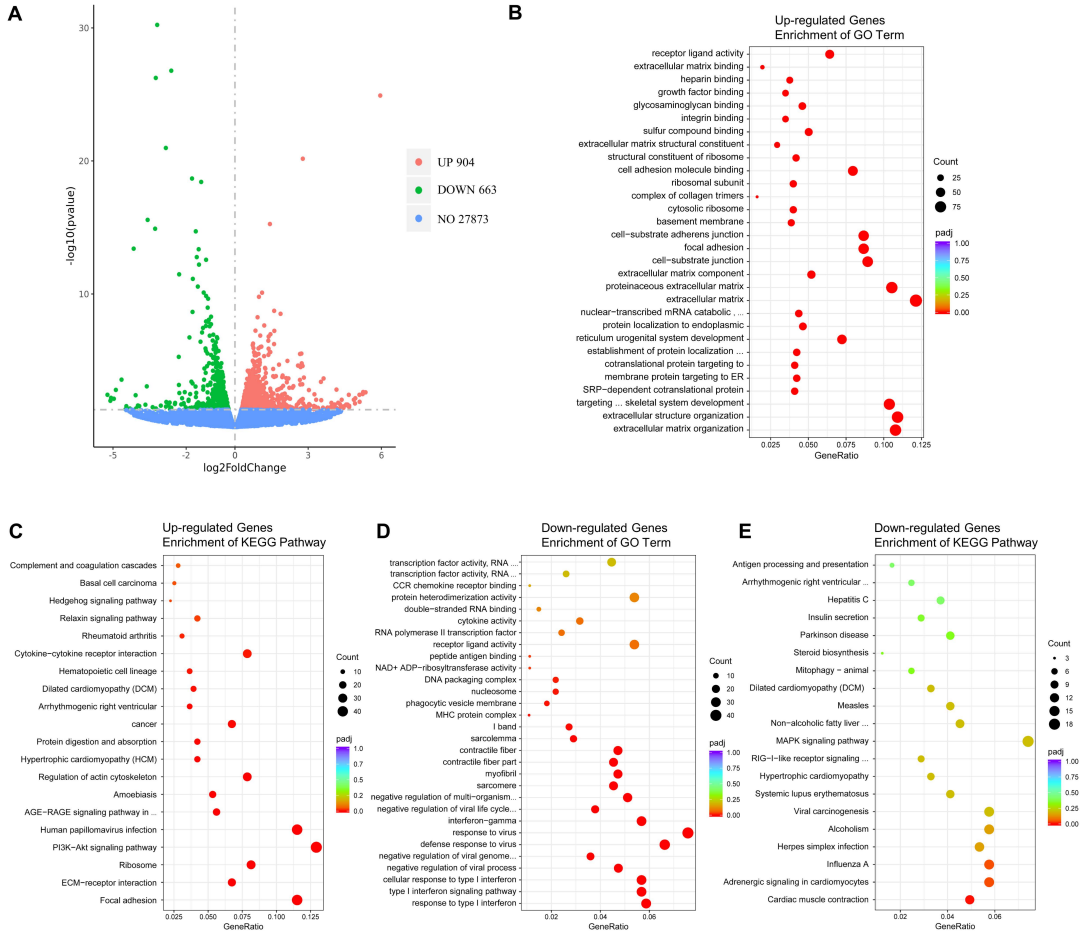

Figure S6. RNA-sequencing data obtained from the modVEGF transfected iPSC-CMs

(A) Volcano plot of all differentially expressed genes on day three post-transfection. UP means up-regulated gene expression, DOWN means down-regulated gene expression, and NO means no differentially expressed gene. (B) GO enrichment analysis of all up-regulated DEGs, and the most significant 30 GO terms were displayed in the bubble chart. The size of the bubble indicates the number of clustered genes, and the color of the bubble indicates the adjusted p-value (padj). (C) KEGG pathway analysis of all up-regulated DEGs, and the bubble chart shows the most significant 20 KEGG pathways. The size of the bubble indicates the number of clustered genes, and the color of the bubble indicates padj. (D) GO enrichment analysis of all down-regulated DEGs, and the bubble chart shows the most significant 30 GO terms. The size of the bubble indicates the number of clustered genes, and the color of the bubble indicates padj. (E)

KEGG pathway analysis of all down-regulated DEGs, and the bubble chart shows the most significant 20 KEGG pathways. The size of the bubble indicates the number of clustered genes, and the color of the bubble indicates padj.

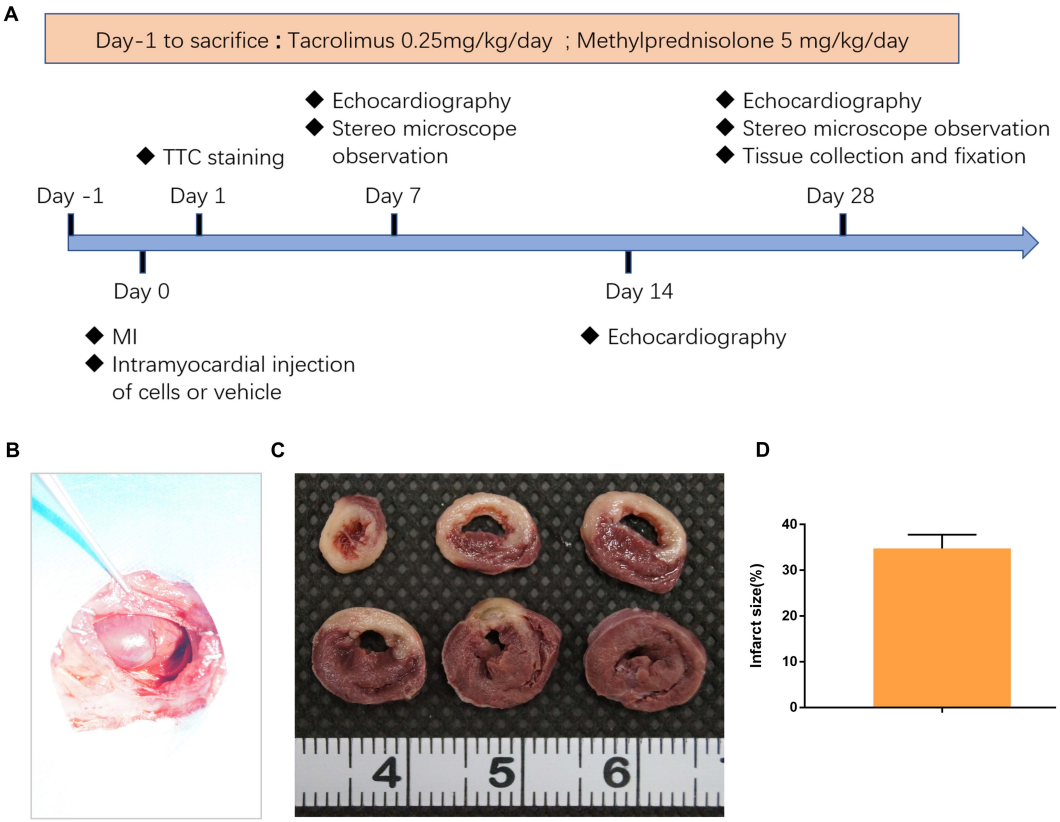

Figure S7. Schematic illustration and overview of the myocardial infarction injury model

(A) Schematic diagram of surgical protocol including in vivo research design and experimental data collection time. (B) Representative image of rat heart after myocardial infarction surgery. (C) Representative TTC staining revealed white ischemic areas where blanching occurred. (D) Comparison of infarcted areas in transversely sectioned hearts at 28 days after the onset of myocardial injury (34.8% ± 2.7%, n = 5; data are means ± SD).

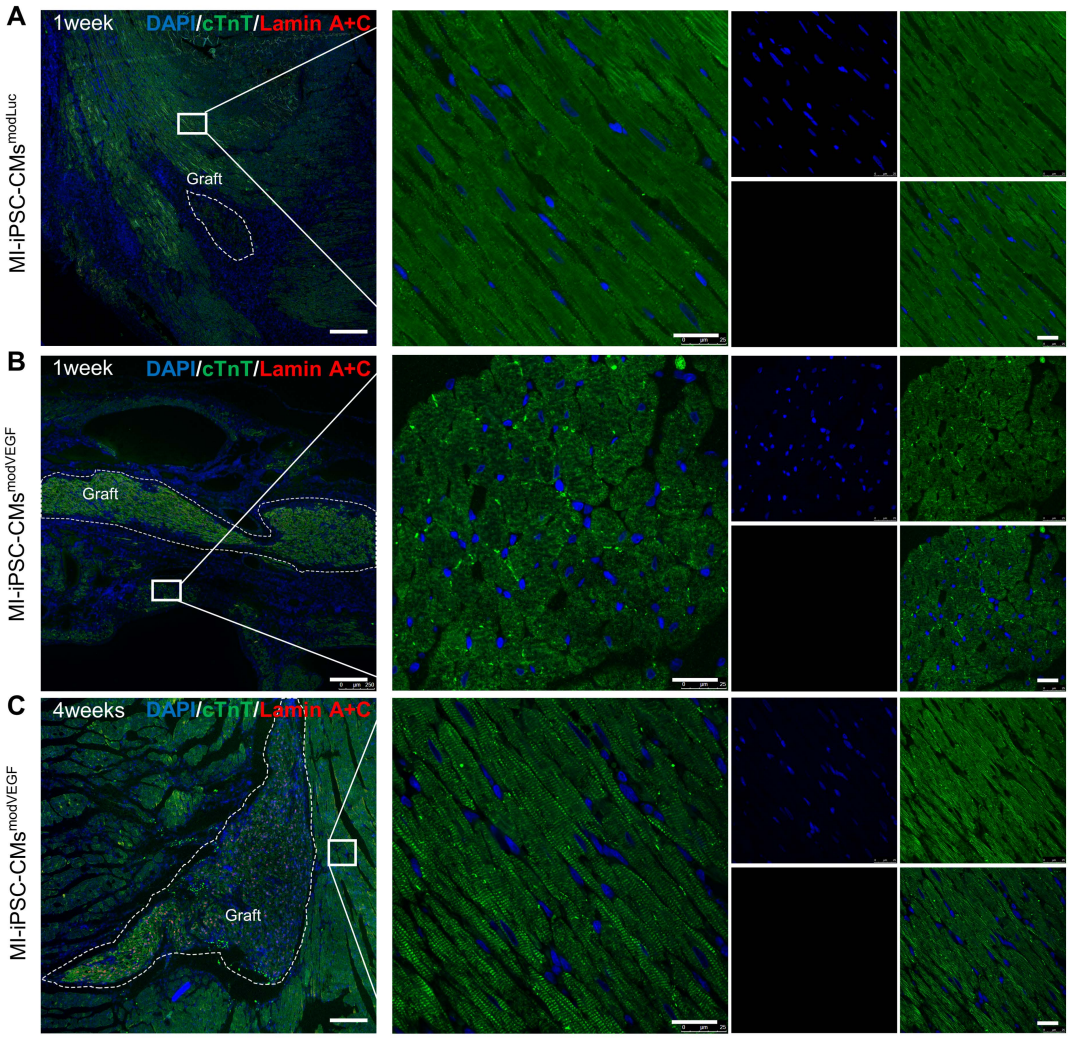

Figure S8. Identification of localized human cardiac muscle islands in the rat myocardium

Representative confocal immunofluorescence images of rat hearts subjected to myocardial infarction and transplantation of iPSC-CMs 1 week (A-B) or 4 weeks (C) after engraftment: green, cTnT; red, Lamin A/C; blue, DAPI. Note: The images in the left panel in Figure S8A-C are reproduced from the left panel images shown in Figure 2B-D to further illustrate the surrounding environments of the engrafted areas. Scale bar on the left: 250 μm. Scale bars (zoomed snapshot): 25 μm.

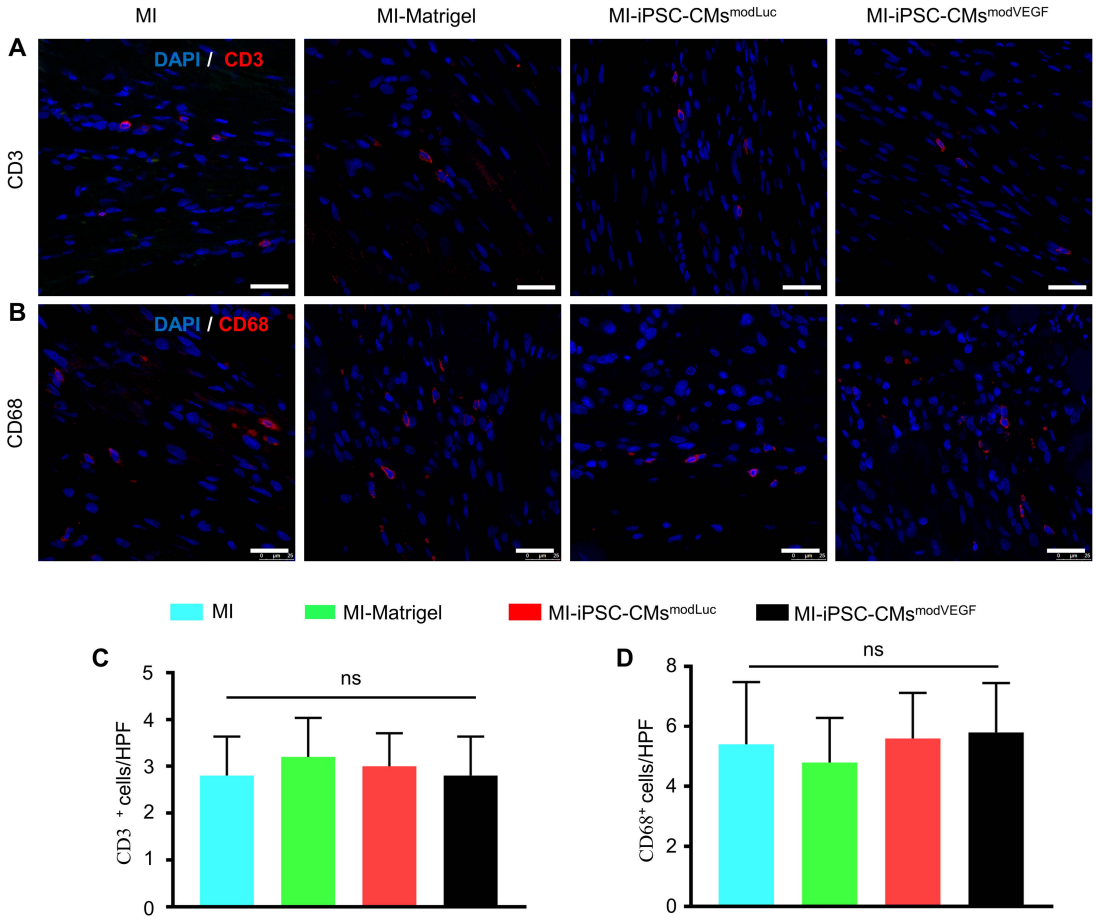

Figure S9. Minimal immune responses after iPSC-CM transplantation

(A-B) Representative confocal images of CD3 and CD68 expression at 4 weeks post-transplantation (scale bar=25 μm). (C) Quantitative analysis of CD3+ immune cell numbers per high powered field (HPF) (n=5). (D) Quantitative analysis of CD68+ immune cell numbers per HPF (n=5). P values were determined by one-way analyses of variance followed by Bonferroni post-test. (ns indicates $P > 0.05$).

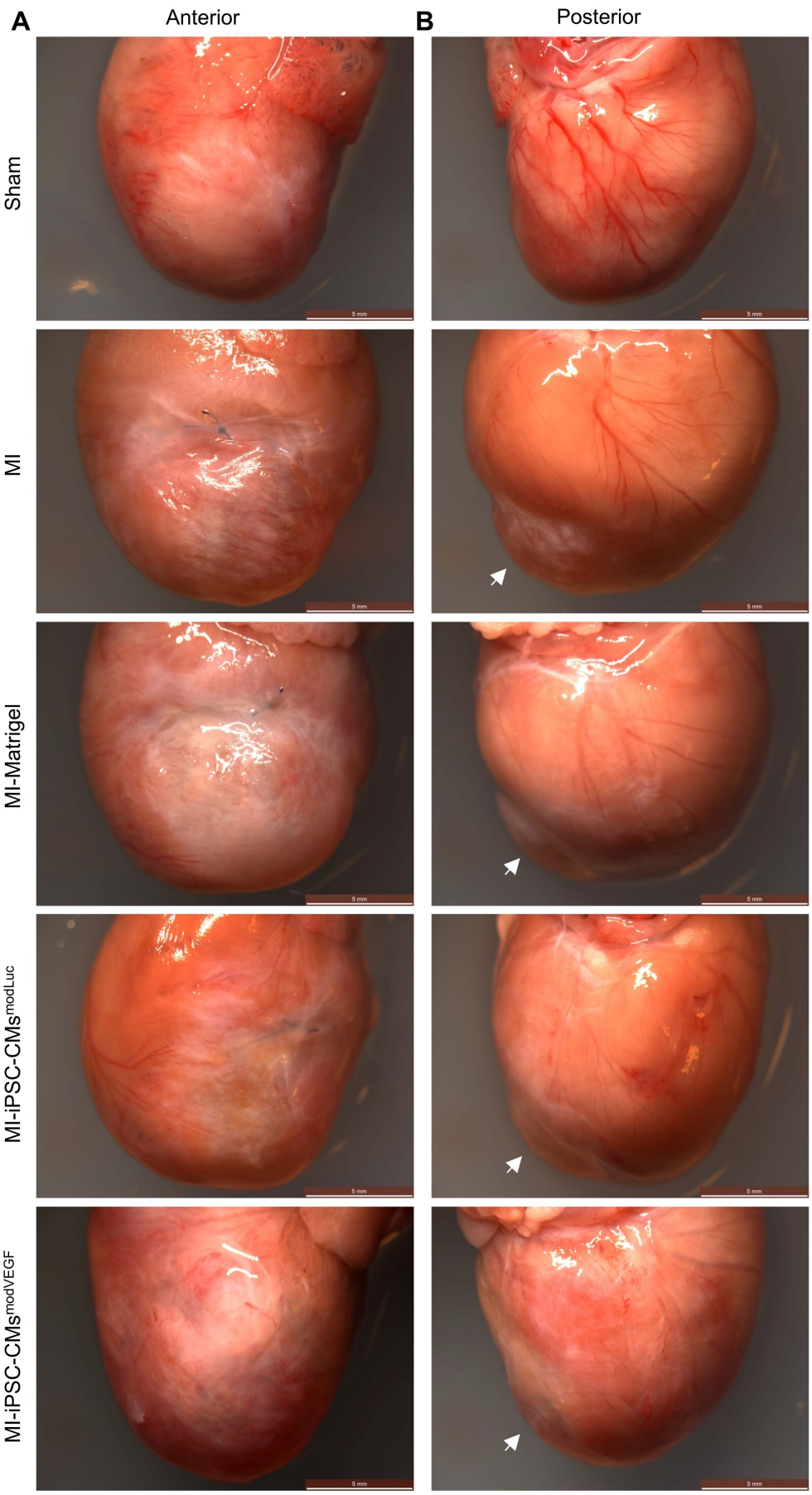
A
Anterior
B
Posterior
Sham
MI
MI-Matrigel
MI-iPSC-CMsmodLuc
MI-iPSC-CMsmodVEGF

Figure S10. Gross morphology of rat heart at 4 weeks after myocardial infarction

Representative digital photographs of whole exposed rat hearts 4 weeks after infarction and iPSC-CMs transplantation, anterior (A) and posterior (B) views. The white arrow indicates the apex from the posterior view.

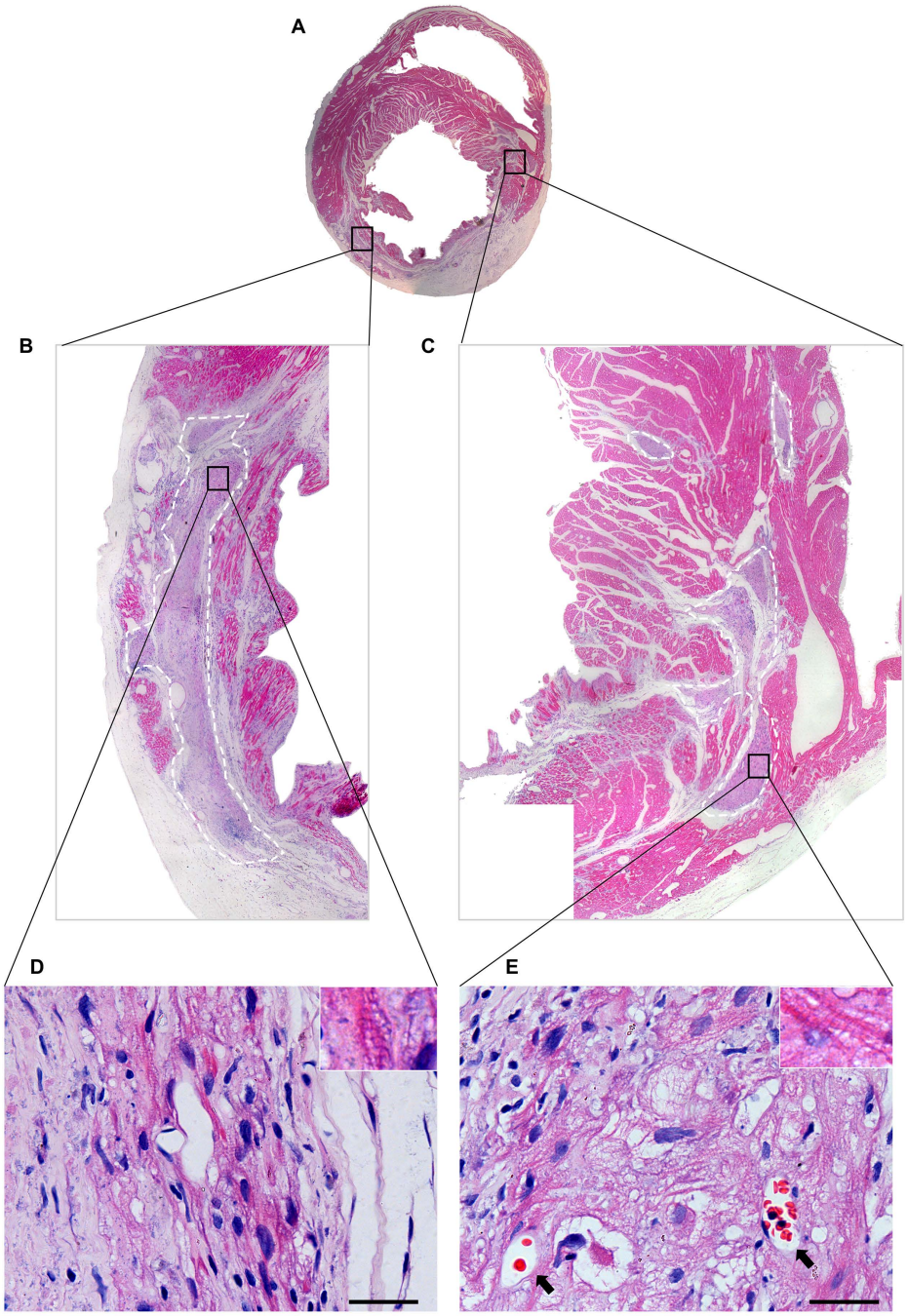

Figure S11. Detection of surviving myocardial grafts in the border zone and infarction zone of the iPSC-CMs^{modVEGF} group at 4 weeks post-transplantation

(A) Representative photograph of a transverse sectioned rat heart receiving iPSC-CMs^{modVEGF} treatment, stained with hematoxylin and eosin. Note: Figure S11A is reproduced from the image in the bottom row in Figure 4A to illustrate improved muscle wall thickness corresponding to areas of high cell-retention. (B-C) Enlarged view of border zone depicting the surviving grafts. (D-E) The high magnification

microscope images reveal that the grafts have complete cell morphology and sarcomeric structure (inset at upper right), black arrows indicate blood vessel in graft. Scale bar, 20μm.

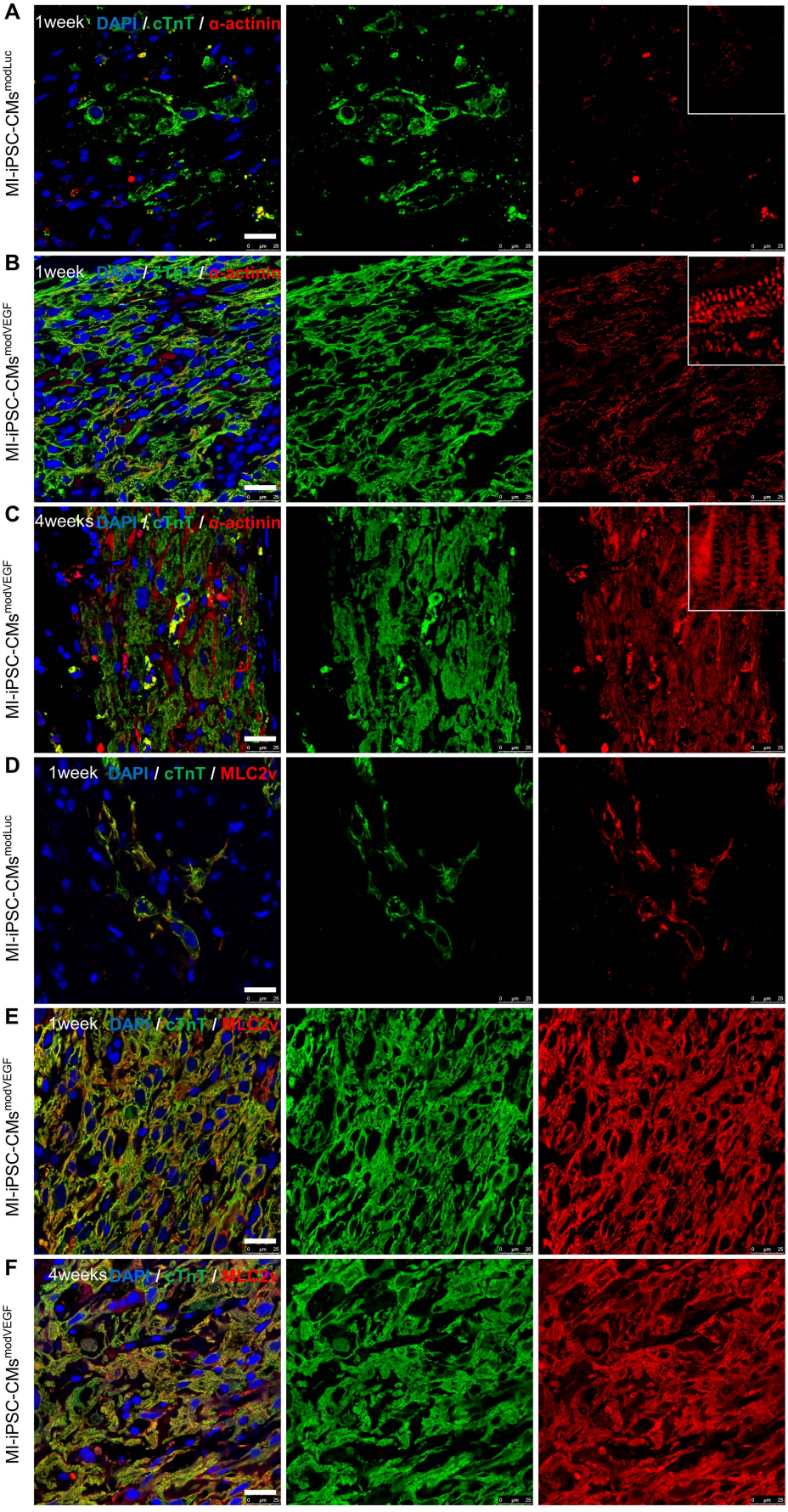
A
1week DAPI / cTnT / α-actinin
MI-iPSC-CMsmodLuc
B
1week DAPI / cTnT / α-actinin
MI-iPSC-CMsmodVEGF
C
4weeks DAPI / cTnT / α-actinin
MI-iPSC-CMsmodVEGF
D
1week DAPI / cTnT / MLC2v
MI-iPSC-CMsmodLuc
E
1week DAPI / cTnT / MLC2v
MI-iPSC-CMsmodVEGF
F
4weeks DAPI / cTnT / MLC2v
MI-iPSC-CMsmodVEGF

Figure S12. Phenotypic profiling of the transplanted and grafted iPSC-CMs

Expression of sarcomeric α-actinin (red) and troponin cTnT (green) in the MI-iPSC-CMs^{modLuc} group (A) and MI-iPSC-CMs^{modVEGF} group (B) 1-week post-transplantation. Scale bar, 25μm. (C) Expression of sarcomeric α-actinin (red) and troponin cTnT (green) in the MI-iPSC-CMs^{modVEGF} group in the grafted region 4 weeks post-transplantation. Scale bar, 25μm. Expression of myosin MLC2v (red) and troponin cTnT (green) in the MI-iPSC-CMs^{modLuc} group (D) and in the MI-iPSC-CMs^{modVEGF} group (E) 1-week post-transplantation. Scale bar, 25μm. (F) Expression of myosin MLC2v (red) and troponin cTnT (green) in the MI-iPSC-CMs^{modVEGF} group in the graft 4 weeks post-transplantation. Scale bar, 25μm.

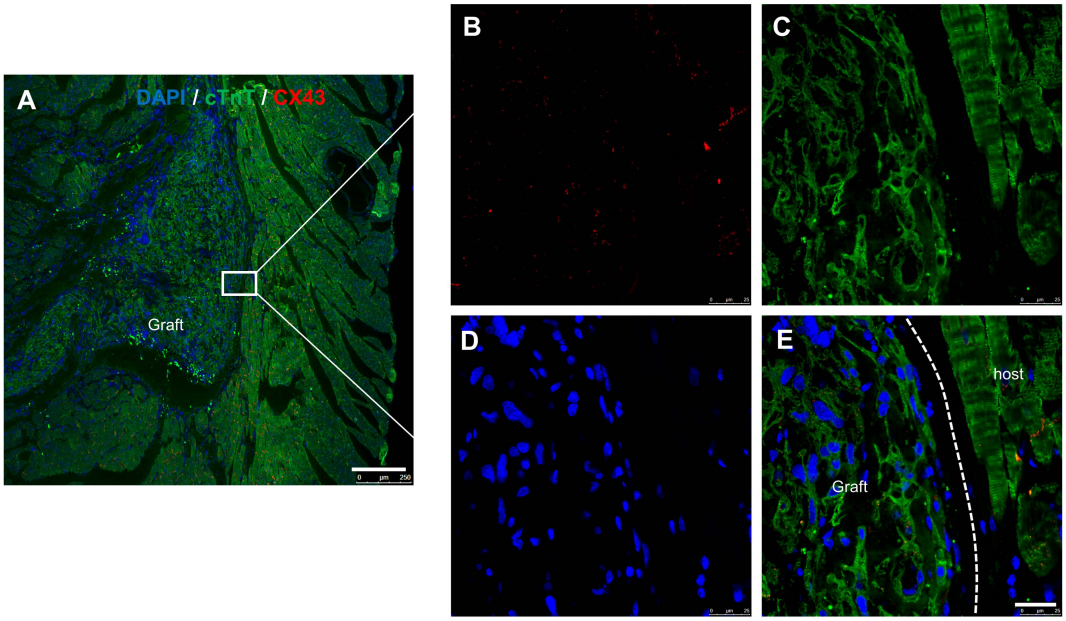

Figure S13. No evidence for gap junction formation between graft and host

(A) Representative confocal pictures of the expression of Cx43 in the myocardial grafts at 4 weeks post-transplantation of iPSC-CMs^{modVEGF}, (scale bar: 250 µm). (B-E) Enlarged view of the border zone between graft and host myocardium, (scale bar: 25 µm), green, cTnT; red, Cx43; blue, DAPI.

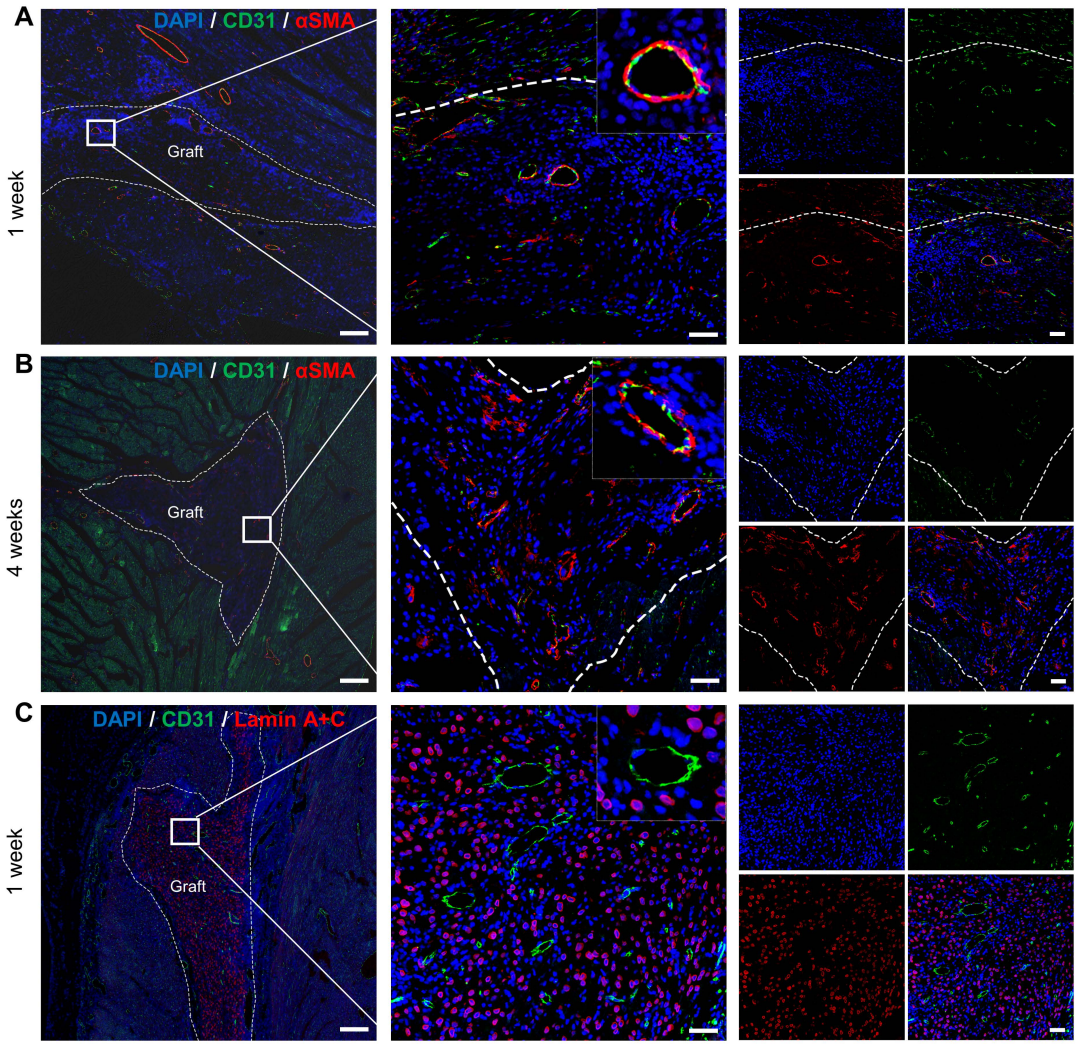

Figure S14. The vascularization of grafts following iPSC-CMs^{modVEGF} treatment

(A) Representative confocal pictures of α-SMA and CD31 expression in the myocardial grafts of iPSC-CMs^{modVEGF} at 1-week post-transplantation (scale bar: 200 μm), zoom in (scale bar: 50 μm). (B) Representative confocal pictures depicting α-SMA and CD31 expression in the engrafted region 4 weeks post-transplantation (scale bar: 200 μm, and enlarged pictures, scale bar: 50 μm). (C) Representative confocal pictures of the expression of Lamin A+C and CD31 in the myocardial grafts at 1-week post-transplantation of iPSC-CMs^{modVEGF} (scale bar: 200 μm, and enlarged pictures, scale bar: 50 μm).