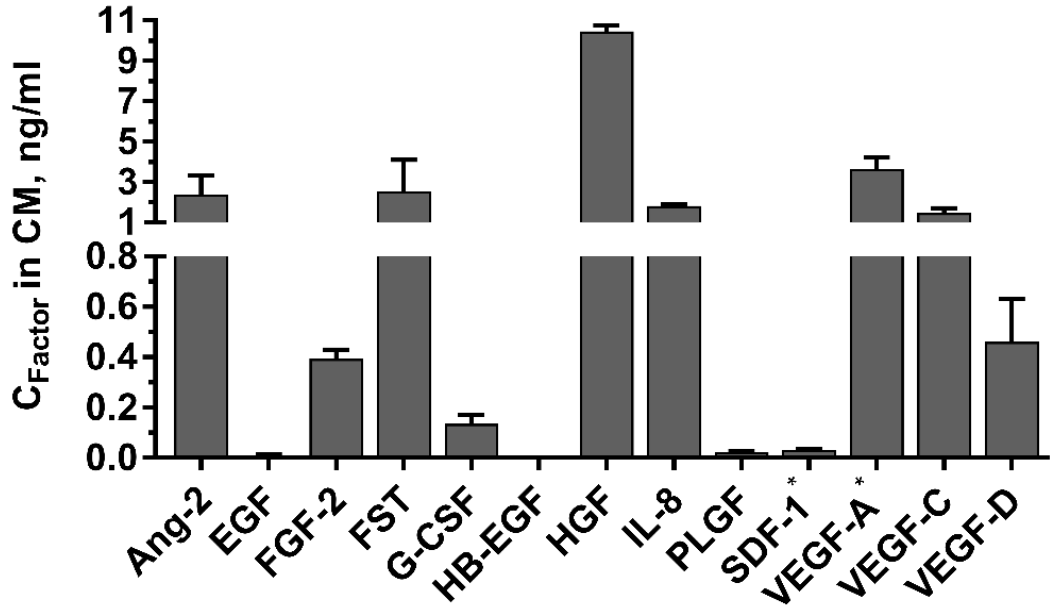
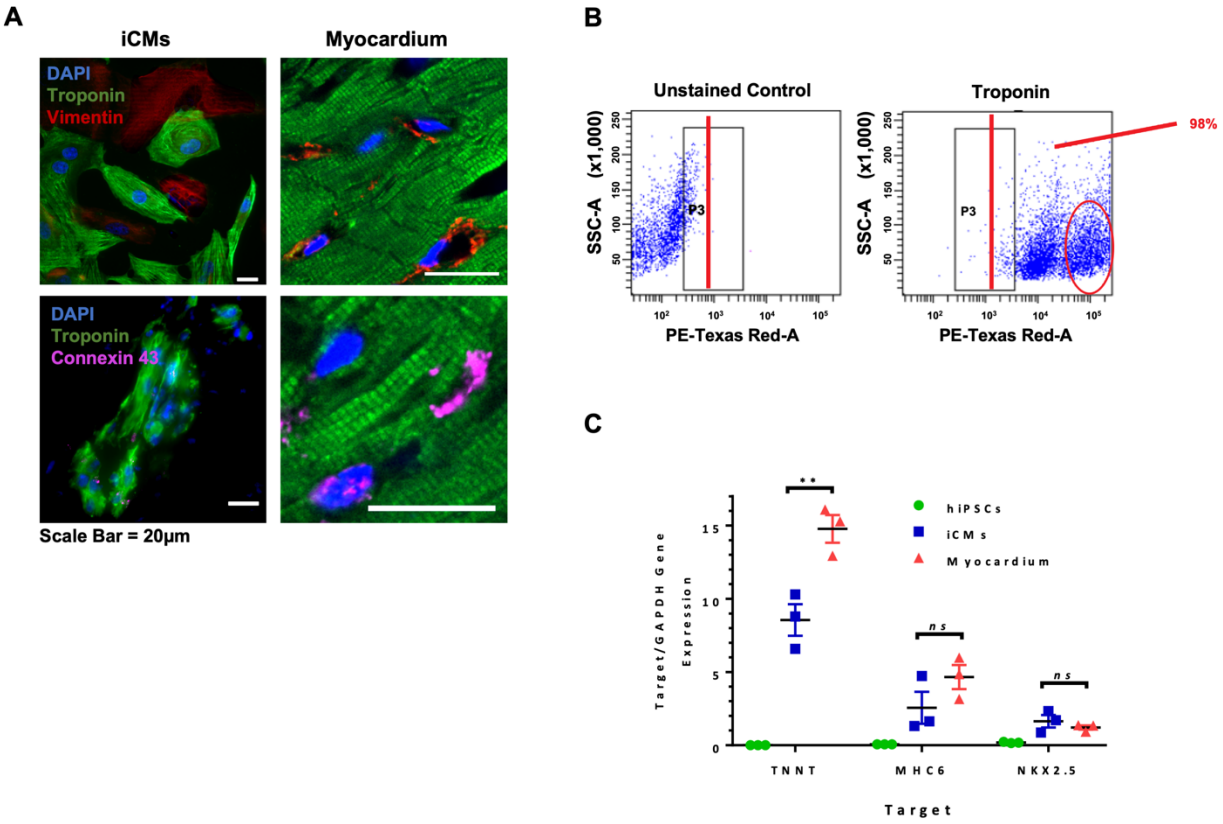
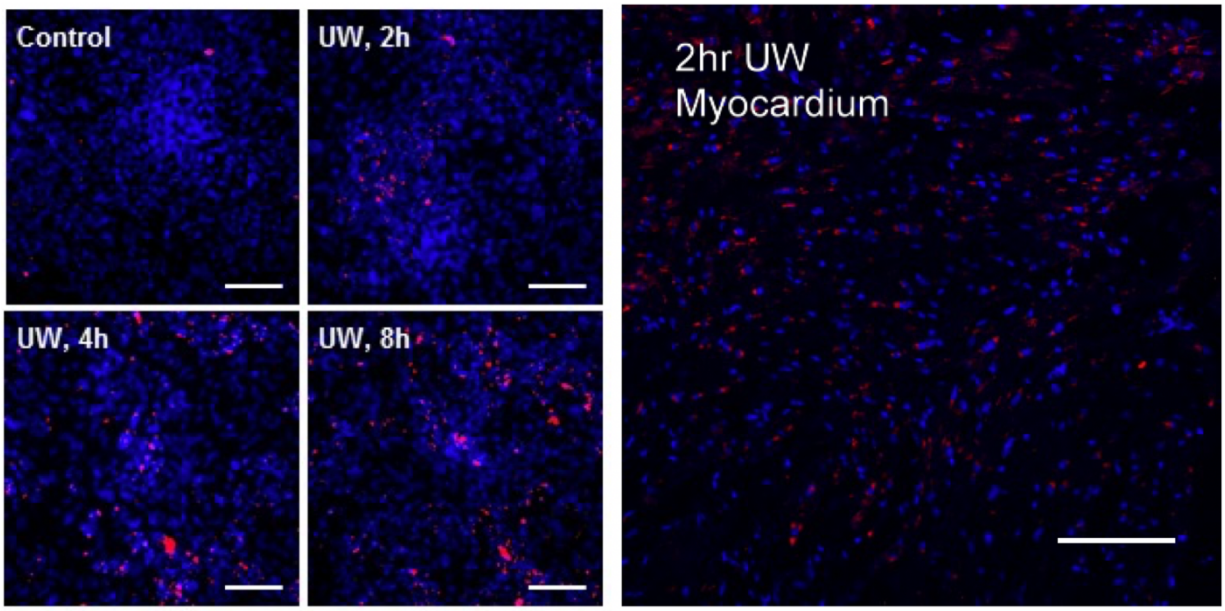
Supplemental Materials:



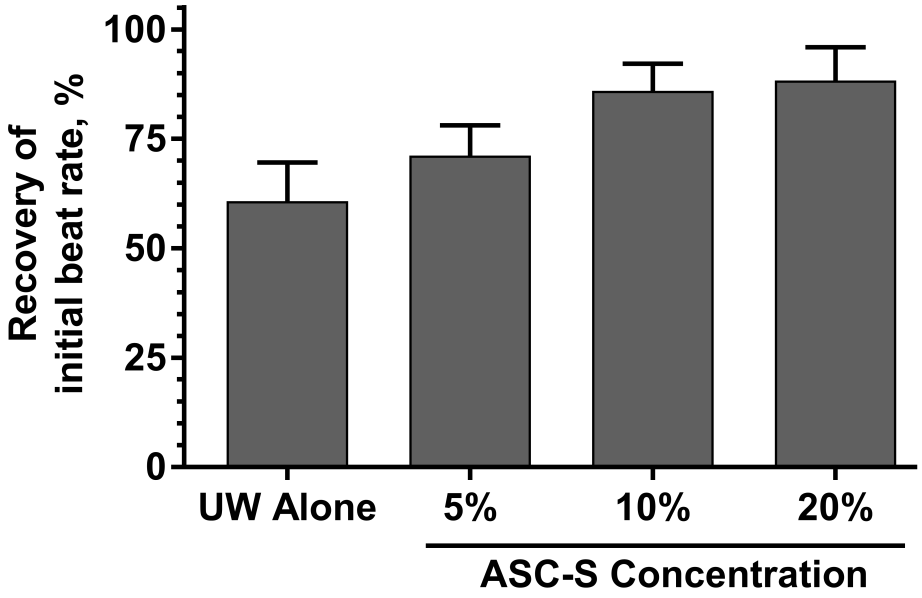
Supplemental Figure 1. Analysis of ASC-S composition. Levels of several growth factors and cytokines in ASC-S were evaluated using the Human Angiogenesis Array & Growth Factor 17-plex Array (Eve Technology; HDAGP17), except for VEGF and SDF-1 levels, which were assessed using standard ELISA kits from RnD Systems.



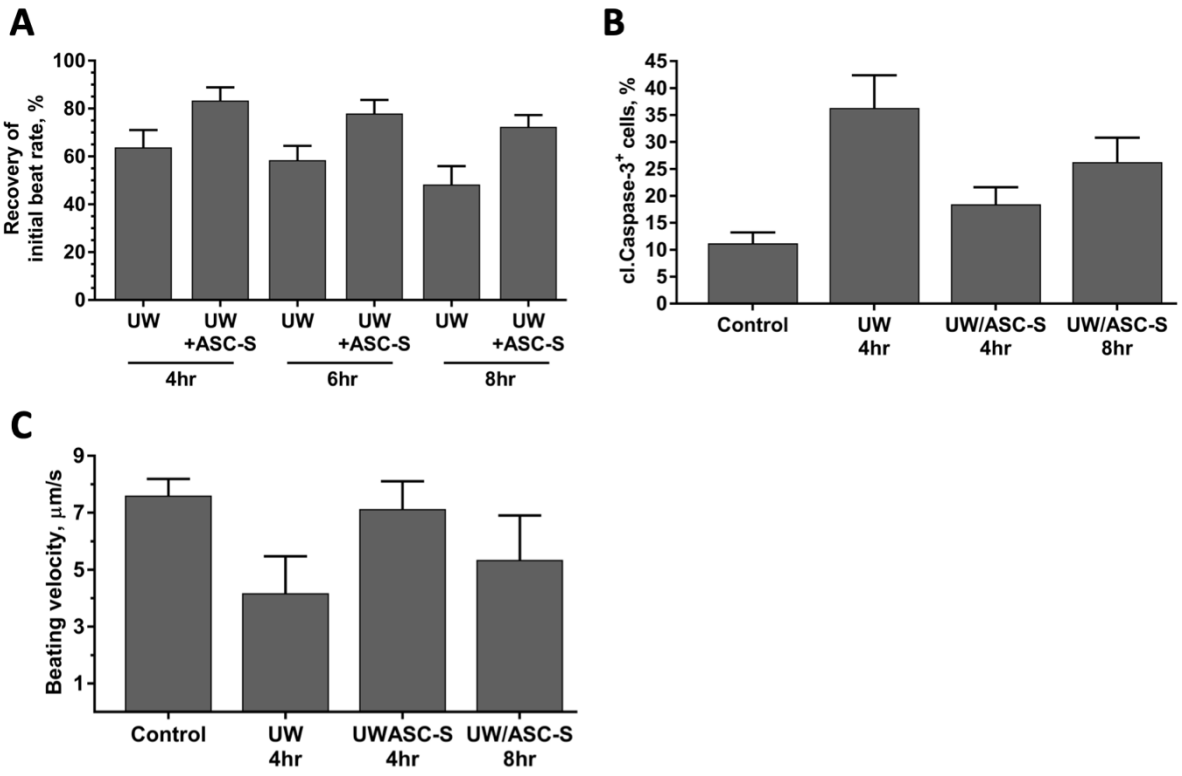
Supplemental Figure 2. iCM phenotype characterization. (A) Representative images of iCM and human myocardium stained for vimentin (red), striated troponin (green), and connexin 43 (magenta). Nuclei were revealed with DAPI (blue). **(B)** Analysis of troponin expression in fully differentiated iCM by flow cytometric analysis. **(C)** mRNA expression of TNNT, MHC6, and NKX2.5 (selected canonical cardiomyocyte markers) in hiPSCs, iCM, and human left ventricle tissue (n=3). One-way ANOVA with Tukey's post hoc test was used for statistical analysis ($^{**}p \leq 0.01$, ns - non significant). Scale Bar = 20μm.



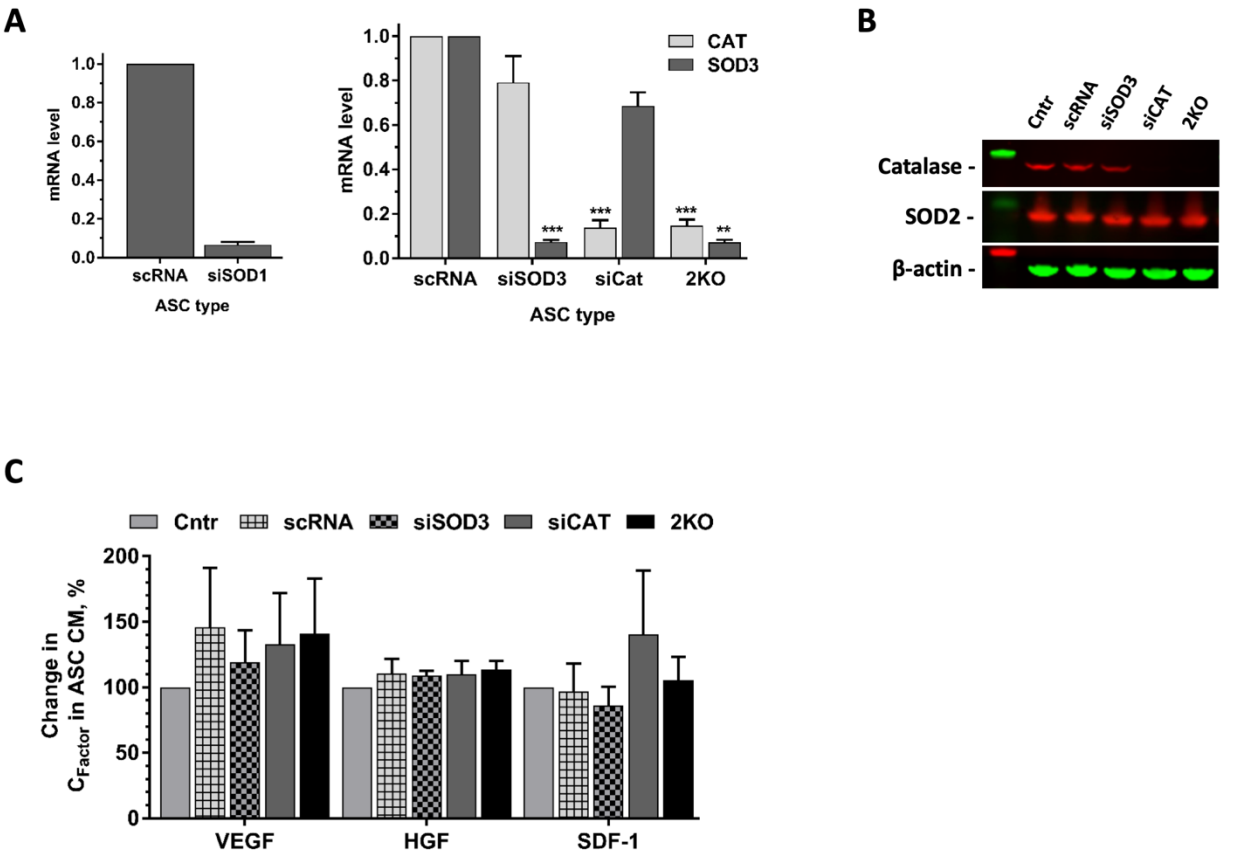
Supplemental Figure 3. Cleaved caspase-3 expression in iCM and human myocardium. Representative images of cleaved caspase-3 (red) expression in iCM after cell exposure to UW for 2-8 hours, followed by 24-hour recovery in FM under standard culture conditions (left four images) and human myocardium after exposure to UW for 2 hours (right). Nuclei were revealed with DAPI (blue). Scale Bar = 100μm.



Supplemental Figure 4. ASC-S protects iCM during UW exposure. Recovery of beating rate in iCM incubated in either UW alone or UW supplemented with either 5%, or 10% (standard), or 20% ASC-S at 4^{o}C for 4 hours followed by recovery in standard iCM culture media at 37^{o}C for 24 hours. n=6 for each graph.



Supplemental Figure 5. Long term iCM response to UW incubation and ASC-S intervention. (**A**) Beating rate of iCM incubated in either UW/ASC-BM or UW/ASC-S at 4°C for 4-8 hours followed by recovery in standard iCM culture media (FM) at 37°C for 72 hours. (**B,C**) Prevalence of cleaved caspase+ iCM (**B**) and beating velocity of iCM (**C**) and after cell exposure to UW/ASC-BM for 4 hours or to UW/ASC-S for 4 and 8 hours at 4°C, followed by cell recovery in FM for 72 hours. n=6 for each graph.



Supplemental Figure 6. Assessment of SOD1, SOD3, and Catalase silencing in ASC. (A) Analysis of SOD1, SOD3 and catalase expression in ASC transfected with scrambled RNA or silencing RNA for these factors either individually or in combination performed on day 5 post transfection by RT-qPCR ($^{***}p \leq 0.001$, $^{**}p \leq 0.01$). **(B)** Analysis of catalase and SOD2 protein expression in intact ASC or ASC transfected with either scrambled RNA or silencing RNA for SOD3 and catalase (individually or in combination) performed on day 5 post transfection. Beta-actin was used as loading control. **(C)** Analysis of changes in concentration of vascular endothelial growth factor, hepatocyte growth factor, and stromal derived factor-1 in ASC-S generated by control ASC or ASC transfected with scRNA or siRNA (SOD3 and/or catalase). Expression of various cardio-protective factors was not affected by silencing of SOD3 and/or catalase in ASC.

Supplemental Table 1. qPCR Primer Information

Primer	Target Gene	Target Gene ID	Amplicon Context Sequence
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	ENSG00000111640	GTATGACAACGAATTTGGCTAC AGCAACAGGGTGGTGGACCTC ATGGCCCACAT GGCCTCCAAGGAGTAAGACCC CTGGACCACCAGCCCCAGCAA GAGCACAAGAG GAAGAGAGAGACCCTCACTGC TGGGGAGTCCCTGCCACAC
TNNT2	Troponin T Type 2	ENSG00000118194	GGTCGAACTTCTCTGCCTCCA AGTTATAGATGCTCTGCCACA GCTCCTTGGCCTT CTCCCTCAGCTGATCTTCATTC AGGTGGTCAATGGCCAGCACC TTCCTCCTCTCA GCCAGAATCTTCTTCTTCTTTT CCCGCTCAGTCTGCC
NKX 2-5	NK2 Transcription factor related, locus 5	ENSG00000183072	GGTCGCTGTAGGCACGTGGAT AGAAGGCGGGGGCGGCGGGA AAGGCAGACGC ACACTTGGCCGGTGAAGGCGC GCGGCCCAGCTCTGCGCGCA G
MYH6	Myosin, heavy chain 6, cardiac muscle, alpha	ENSG00000197616	CCTCTCCCTGAGACACGAAGG CGTAGTCGTAGGGATTGTTGG TGACCAGCAGCA TGTCCAGCAACTCCGGCTTCT TGTTGGACAGAATCTGGTAGA AGATGTGGTAGTT TCTCTCAGCTTTCAGC

Supplemental Video 1: Removal of standard media (t = 14 s) from iCMs followed immediately by addition of UW cardioplegic solution (t = 16 s) causing a near immediate cessation of iCM contraction.

Supplemental Video 2: Removal of standard media from iCMs (t = 12 s) followed immediately by addition of UW cardioplegic solution (t = 25 s) causing a near immediate cessation of iCM calcium transient.

NON-STANDARD ABBREVIATIONS AND ACRONYMS

ASC-S: adipose-derived stem/stromal cell secretome

iCM: hiPSC induced cardiomyocyte

UW: University of Wisconsin cardioplegic solution

MSC: mesenchymal stem cell

hiPSC: human induced pluripotent stem cell

K-H: Krebs-Henseleit Buffer

LVDP: left ventricular developed pressure

BDM: basal differentiation media

IHC: immunohistochemistry

SOD1: super oxide dismutase 1

SOD3: super oxide dismutase 3

ROS: reactive oxygen species

TNNT2: cardiac troponin T

DAPI: 4′,6-diamidino-2-phenylindole

RPP: rate pressure product

MHC6: myosin heavy chain 6

FM: full medium

SBR: syncytial beating rate

UW/ASC-BM: University of Wisconsin cardioplegic solution supplemented with adipose stem/stromal cell basal media

UW/ASC-S: University of Wisconsin cardioplegic solution supplemented with adipose stem/stromal cell secretome

Expanded Materials and Methods:

1. Ethics statement

Animal studies were approved by the Institutional Animal Care and Use Committee at Indiana University School of Medicine. The animal procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals. Deidentified human hearts from donors that was deemed unsuitable for transplantation and donated to research were collected through the Indiana Donor Network under a standard protocol for receiving tissue from deceased individuals. All human tissue collection conformed to the Declaration of Helsinki.

2. Ex vivo perfusion of isolated beating mouse hearts (Langendorff)

Hearts were isolated from adult male C57BL/6 mice as previously described.[27] Briefly, mice were heparinized and generally anesthetized with 5% isofluorane via inhalation for 3 minutes until deep anesthesia was reached. Euthanasia was performed by thoracotomy immediately followed by heart excision. Hearts were flushed with Krebs-Henseleit (K-H) buffer to wash out blood and attached to Langendorff apparatus to record pre-cardioplegic baseline cardiac function. Immediately afterwards, 1 ml of cold (4°C) University of Wisconsin (UW) cardioplegic solution (Bridge to Life, USA) alone or with ASC-S was infused into the coronary circulation, after which the arrested hearts were stored in UW solution ± ASC-S at 4°C for 6 hours. After storage, hearts were re-attached to the Langendorff unit, UW solution was flushed out with oxygenated Krebs-Henseleit buffer, and spontaneous contraction was re- established. Heart rate and left ventricular developed pressure (LVDP) were recorded using a PowerLab 8 preamplifier/digitizer (AD Instruments Inc) and the rate-pressure product (heart rate x LVDP) values were used as an index of cardiac function.

3. hiPSC culture

To produce iCM, 1016 SEVA-line hiPSCs, derived from human skin fibroblasts (Harvard Stem Cell Institute, USA), were seeded on Geltrex-coated tissue culture plastic (Thermo Fisher Scientific, USA) and expanded in mTeSR1 media (StemCell

Technologies, CA) supplemented with 100 U/ml penicillin (VWR, USA). At 80% confluence hiPSCs were detached using Accutase (StemCell Technologies), re-seeded into 12-well tissue culture plates at 4 x 10^5 cells/well, and incubated in mTeSR1 media supplemented with protein kinase A inhibitor H89 (StemCell Technologies) for 1 day, followed by daily mTeSR1 media exchanges until cell monolayers reached 95% confluence.

4. Differentiation of hiPSC into induced cardiomyocytes (iCM)

iCM were generated by temporal activation and deactivation of the Wnt signaling pathway following a previously established protocol.[28] Briefly, hiPSCs were incubated in basal differentiation media (BDM(-): RPMI Medium 1640 (Life Technologies, USA), 2% B27 without insulin (Invitrogen, USA), 3.4×10^{-4} % β-mercaptoethanol (Promega, USA), 100 U/ml penicillin, 100 µg/ml streptomycin) supplemented with 12.5 μM Wnt activator CHIR99021 (CHIR, Stemgent, USA) for 24 hours, followed by 2-day incubation in BDM(-) alone. On day 4, cells were exposed to BDM(-) supplemented with 5 μM Wnt inhibitor IWP-4 (Stemgent, USA) with media exchange to BDM(-) on day 6. On day 9, medium was exchanged to BDM(-) with B27 supplement and insulin (BDM(+)), and then exchanged every third day. Spontaneous beating typically emerged between days 12 and 15. Between days 35 and 50, iCM were harvested using 0.25% Trypsin/EDTA (Corning, USA) and reseeded at 10^6 cells/well into fibronectin-coated 24-well tissue culture plates in DMEM supplemented with 10% fetal bovine serum (Hyclone, USA). Twenty-four hours later, media was exchanged to BDM(+) and then changed every third day. To define the proportion of fully differentiated iCM after completion of differentiation protocol, cells were harvested with trypsin, fixed in 4% paraformaldehyde for 15 min, permeabilized with 0.1% Triton X-100 for 10 min, and incubated with TNNT2 antibody (Abcam, USA) for 1 hour, followed by incubation with goat anti-mouse Alexa Fluor 488 IgG (Life Technologies) for 25 min. Cells were analyzed using FACSAria II analyzer (BD Biosciences) equipped with FACSDiva software (BD Biosciences), followed by data analysis using FlowJo 8.7 software (TreeStar).

5. Human heart collection and processing

Hearts deemed unsuitable for transplant were flushed with cardioplegic solution to remove the blood, placed on ice, and brought to the University of Notre Dame. Samples of left ventricle tissue were used for analysis of RNA expression or immunohistochemistry (IHC). Samples used for RNA analysis were initially incubated in RNAlater (Thermo Fisher, USA) at 4°C overnight, and then stored at -80°C. For IHC analysis, tissue samples were fixed in 4% paraformaldehyde at 4°C for 16 hours, washed with PBS, infiltrated with 30% sucrose at 4°C for 16 hours, cryopreserved in OCT medium, and stored at -80°C.

6. ASC conditioned media (ASC-S) generation

Human ASCs were isolated from human subcutaneous adipose tissue, as previously described,[9] and cryo-banked. To generate ASC-S, cells were plated at $5x10^3$ cells/cm^2 and incubated in EGM-2mv (Lonza, Walkersville, MD) for 3 days. After reaching $5.5x10^4$ cells/cm^2 (~90% confluence), the ASC monolayers were incubated under 0.2 ml DMEM/F12 media per cm^2 (CellGro, Manassas, VA) twice for 48 hours. Media from both incubations were combined, concentrated using Amicon Ultra-15 Centrifugal Filter Units with 3kDa NMWL in such a way that 1 ml of concentrated ASC-S was conditioned by $8x10^6$ cells for 48h, and stored at -80°C. In all experiments, ASC-S was diluted tenfold in UW or culture media. ASC-S preparations were characterized based on protein concentration (509 ± 126 µg/ml; Mean±SEM) and expression of selected growth factors and cytokines using Human Angiogenesis Array & Growth Factor 17-plex Array (Eve Technology; HDAGP17) and standard ELISA kits from RnD Systems for VEGF, HGF and SDF-1 (**Supplemental Figure 1**). To test the contribution of selected antioxidant enzymes to ASC effects, cell monolayers were transfected with either superoxide dismutase 1 (SOD1), superoxide dismutase 3 (SOD3), or catalase silencing RNA or with scrambled RNA (all siRNA reagents were from Invitrogen, USA). One day after transfection, the media on the cells was exchanged to DMEM/F12 to produce ASC-S using the same protocol as above.

7. In vitro iCM functional analysis

iCM were passaged after contraction was well-established, to obtain more homogeneous layers. After physiologically relevant beating of reseeded iCM

recommenced (typically between 5-12 days after reseeding), the baseline beat rate was recorded via time-lapse imaging using Axio Observer.Z1 microscope. Subsequently, media on iCM was replaced with UW solution alone or supplemented with various preparations of ASC-S. iCM were incubated in UW ± ASC-S for 2-8 hours either at 4°C or 37°C. After the UW exposure was completed, UW was replaced with either iCM complete culture media or with RPMI alone or supplemented with ASC-S. Time-lapse videos were taken intermittently for 24 hours after UW treatment/cold storage was completed.

8. Beating velocity assay

A previously developed block-matching algorithm[25] was applied to analyze contractility of iCM. Briefly, the time-lapse videos of iCM were recorded at 30 frame per second and then exported as a sequence of single-frame images. The images were then divided into $N \times N$ pixel blocks. The contractility of iCM was detected by tracking the movement of each individual block. This procedure was repeated for each individual block in the image to generate an array of motion vectors demonstrating the beating of iCM for each time frame. This was done for all frames of the time-lapse video to create a time series of iCM beating velocity vectors. The peak velocity for each vector determined over time was then averaged with that of all other vectors within each beating cluster to yield a single value representing each such active syncytium.

9. Reactive oxygen species accumulation assay

Accumulation of intracellular reactive oxygen species (ROS) was assessed in iCM that were exposed to UW solution alone or with ASC-S for 4 hours, and allowed to recover in iCM complete culture media. The level of superoxide or hydroxyl radicals in the cells was determined at specified timepoints with ROS and H_2O_2 Assay kits (Cat#: CA-R933, Cat#: CA-H501, eEnzyme, USA), by following the manufacturer's protocol with minimal modifications. Briefly, 30 minutes prior to each measurement, media on the cells was replaced with the activity assay solution. After 30 minutes incubation at 37°C, three randomly selected fields of view were imaged for each sample using the Zeiss

microscope and the averaged intensity of the fluorescent signal was used to define the representative ROS activity at each timepoint.

10. RNA and Prootein expression analysis

Total RNA was isolated from confluent beating iCM and human left ventricle tissue using RNAeasy Plus Mini Kit (Qiagen, Germany). cDNA was synthesized using the iScript™ Advance cDNA kit for RT-qPCR (Bio-Rad, USA), and expression of selected genes was evaluated using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) and primers provided in Supplementary Table 1. Gene expression was normalized to the level of the housekeeping gene GAPDH, and presented as ΔC_T.

To evaluate the efficiency of silencing of antioxidant enzyme expression, total RNA was isolated from ASC at day 5 post-transfection using NucleoSpin RNA II kit (Clontech, Mountain View, CA) followed by a reverse transcription reaction with iScript RT kit (Bio-Rad, Hercules, CA). qPCR reactions were performed using TaqMan Fast Advanced qPCR mix with target specific probes (Invitrogen) for SOD1, SOD3, catalase and β-actin (housekeeping gene). Efficiency of silencing was also confirmed on protein level. Cell lysates were fractionated on SDS–polyacrylamide and transferred onto nitrocellulose membranes. Membranes were incubated with SOD2, SOD3, catalase and beta-actin IgG (Cell Signaling) followed by incubation with IRDye® 800CW anti-mouse or IRDye® 680RD anti-rabbit IgG, and imaging on Odyssey CLx Imaging system (all from Li-Cor).

11. Immunohistochemistry

iCM were fixed with 4% paraformaldehyde for 20 minutes. iCM and myocardium cryosections were permeabilized with 0.1% Triton X-100 for 30 minutes, incubated with 10% goat serum (Sigma-Aldrich, USA) for 2 hours to block nonspecific binding, followed by incubation with cardiac troponin T (TNNT2), vimentin, connexin-43 (all from Abcam, United Kingdom), or caspase-3 (Cell Signaling Technology, USA) antibody at 4°C overnight, followed by incubation with secondary antibodies tagged with Alexa Fluor 594 or Alexa Fluor 488 (Life-Technologies, USA) at 4°C for 6 hours. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) staining. Stained samples were evaluated using a Nikon Confocal Ni-E Upright Research Microscope or Zeiss Axio Observer.Z1

fluorescence microscope equipped with a Hamamatsu digital camera. Acquired images were processed using Nikon NIS-Elements software (for the confocal microscope) or Zeiss Zen software (for the fluorescent microscope).

iCM, before and after UW exposure, and samples of human left ventricle tissue were stained for cleaved caspase-3 (Cell Signaling Technology) using Alexa Fluor 547-tagged goat anti-rabbit as a secondary antibody. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) staining. To determine the percent of caspase-3 positive cells, three randomly selected fields of view were imaged and the number of caspase-3+ nuclei was determined and normalized to the total number of nuclei in each image.

12. Statistical Analysis

Data are presented as an average ± standard error of the mean. A one-way ANOVA with Tukey's post hoc test was performed for all experiments. All p values are two-sided and statistical significance was defined as $p < 0.05$ and sample size $(n) \geq 3$ for all experiments.

13. Data Availability Statement

The data from this study is available from the corresponding authors upon reasonable request.