Supplementary Information for

Suppression of inflammation and fibrosis using soluble epoxide hydrolase inhibitors enhances cardiac stem cell-based therapy.

Padmini Sirish, Ph.D.[1,2*], Phung N. Thai, Ph.D.[1*], Jeong Han Lee, Ph.D.[3], Jun Yang, Ph.D.[4], Xiao-Dong Zhang, Ph.D.[1,2], Lu Ren[1], Ning Li, Ph.D.[1], Valeriy Timofeyev, Ph.D.[1], Kin Sing Stephen Lee, Ph.D.[4], Carol E. Nader[1], Douglas J. Rowland Ph.D.[5], Sergey Yechikov Ph.D.[1], Svetlana Ganaga N.P.[6], J. Nilas Young M.D.[6], Deborah K. Lieu Ph.D.[1], Ebenezer N. Yamoah, Ph.D.[3], Bruce D. Hammock, Ph.D.[4], and Nipavan Chiamvimonvat, M.D.[1,2,7]

[1]Division of Cardiovascular Medicine, University of California, Davis, CA
[2]Department of Veterans Affairs, Northern California Health Care System, Mather, CA
[3]Department of Physiology and Cell Biology, University of Nevada, Reno, CA
[4]Department of Entomology and Nematology and Comprehensive Cancer Center University of California, Davis, CA
[5]Center for Molecular and Genomic Imaging, University of California, Davis, CA
[6]Department of Surgery, University of California, Davis, CA
[7]Department of Pharmacology, University of California, Davis, CA

* The authors contributed equally to the work

This PDF file includes:

Supplementary text for SI Materials and Methods
Figs. S1 to S6
References for SI reference citations

Supplementary Information Text

SI Materials and Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the University of California, Davis Institutional Animal Care and Use Committee.

Optical recording of Action Potentials:

HiPSC-CMs were were loaded with 5 μM FluoVolt (Thermo Fisher Scientific) in media with 0.04% Pluronic F-127 and 2 mM probenecid for 15-30 min at 37°C. Action potentials of single cells were recorded in Tyrode's solution with excitation-contraction decoupler blebbistatin (Sigma-Aldrich, St. Louis, MO) at room temperature using a Nipkow disk confocal microscope (Olympus IX71 equipped with Yokogawa CSU10, a 10X microscope objective, and FITC filters) with an EM-CCD camera (Andor iXon 897) at ~100 fps as we have previously described.(1) Images were analyzed using Andor iQ2 software.

Patch-clamp recordings of hiPSC-CMs:

Experiments were performed using hiPSC-CMs treated with ANG II (1 μM for 24 hours, Sigma-Aldrich) and ANG II+TPPU using the conventional whole-cell patch-clamp technique at room temperature.(2) For K^+ current recording, the external solution contained (mM) NMG 130, KCl 5, $CaCl_2$1, $MgCl_2$ 1, Nimodipine 1 μM, glucose 10, HEPES 10, pH 7.4 with HCl. The pipette solution contained (mM) KCl 140, Mg-ATP 4, $MgCl_2$ 1, EGTA 10, HEPES 10, pH 7.4 with KOH. All experiments were performed using 3 M KCl agar bridges. The series resistance was compensated electronically. In all experiments, a series resistance compensation of 90% was obtained. Currents were recorded using Axopatch 200B amplifier (Axon Instruments, Molecular

Devices, San Jose, CA), filtered at 10 kHz using a 4-pole Bessel filter and digitized at sampling frequency of 50 kHz. Data analysis was carried out using custom-written software and commercially available PC-based spreadsheet and graphics software (MicroCal Origin version 6.0, OriginLab Corporation, Northampton, MA).

Histological analyses:

Hearts were excised and perfused in a retrograde fashion with phosphate-buffered solution to wash out blood and fixed in 4% formalin overnight. Hearts were then embedded in paraffin, serial left ventricular cardiac sections of 5 μm in thickness were taken and stained with Mason's trichrome to assess for percent infarct area. Quantification was performed in a blinded fashion.

Immunofluorescence confocal laser scanning microscopy:

The cardiac sections were deparaffinized with Xylene before rehydrating with serial dilution of ethanol. The sections were blocked with donkey serum and stained with anti-GFP antibody (Thermo Fisher Scientific), and DAPI (1 mg ml^{-1}, Thermo Fisher Scientific). Immunofluorescence-labeled images were obtained using a Zeiss LSM700 confocal laser-scanning microscope (Carl Zeiss, Oberkochen, Germany).

For hiPSC-CMs staining, cultured cells were fixed with 4% paraformaldehyde (PFA), permeabilized with Triton-X100, blocked with goat serum before staining with anti-troponin T antibody (2 mg ml^{-1}, Thermo Fisher Scientific), anti-phalloidin antibody (0.2 mg ml^{-1}, Thermo Fisher Scientific), anti-myosin light chain-2a (MLC2a) and anti- myosin light chain-2v (MLC2v) antibodies. Immunofluorescence-labeled images were obtained using a Zeiss LSM700 confocal laser-scanning microscope.

Flow cytometric analysis of cardiac cells:

Single cell suspensions were obtained from six groups of NSG mice as previously described.(3) The procedure was performed according to the approved UC Davis Animal Care and Use protocol. Briefly, mice were injected with 0.1 ml heparin (1,000 units ml^{-1}) 10 min prior to heart excision, then anesthetized with pentobarbital intraperitoneally (80 mg kg^{-1}). Hearts were removed and placed in Tyrode's solution (mmol l^{-1}: NaCl 140, KCl 5.4, $MgCl_2$ 1.2, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES) 5 and glucose 5, pH 7.4). All chemicals were obtained from Sigma-Aldrich Chemicals unless stated otherwise. The aorta was cannulated under a dissecting microscope and mounted on the Langendorff apparatus. The coronary arteries were perfused in a retrograde fashion with Tyrode's solution gassed with O_2 at 37 °C for 3 min at a flow rate of ~3 ml min^{-1}. The solution was switched to Tyrode's solution containing collagenase type 2 (1 mg ml^{-1}, 330 units mg^{-1}, Worthington Biochemical Corporation, Lakewood NJ). After ~12 min of enzyme perfusion, hearts were removed from the perfusion apparatus and gently teased in high-K^+ solution (mmol l^{-1}: potassium glutamate 120, KCl 20, $MgCl_2$ 1, EGTA 0.3, glucose 10 and HEPES 10, pH 7.4 with KOH). Cells were filtered through 200 µm cell strainer, re-suspended in Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS), fixed with 0.4% PFA, before treating with phytoerythrin-conjugated anti-Thy1.1 (BD Bioscience, San Diego, CA), lineage antibody cocktail (CD3, CD14, CD19, CD20 and CD56, BD Bioscience), anti-CD45 (BD Bioscience, San Diego, CA), anti-troponin T antibody (Thermo Fisher Scientific), anti-myosin heavy chain (Developmental Studies Hybridoma Bank, IA), and proliferation-specific Ki67 antibody (15 µg ml^{-1}, BD Bioscience) in PBS with 5% donkey serum and 20 µg ml^{-1} DNAse-free RNAse (Sigma-Aldrich) overnight at 4°C. Cells were also stained with 40 µg ml^{-1} 7-amino-actinomycin D (7AAD, BD Bioscience, San Jose, CA) to measure the

DNA content. Equal number of myocytes were isolated from sham, sham-TPPU treated, MI, MI+TPPU, MI+Cells, MI+Cells+TPPU-treated hearts and 10,000 to 20,000 cells were analyzed in each run. Data were collected using a standard FACScan cytometer (BD Biosciences) upgraded to a dual laser system with the addition of a blue laser (15 mW at 488 nm) and a red laser (25 mW at 637 nm Cytek Development, Inc, Fremont, CA). Data were acquired using CellQuest software (BD Bioscience) and analyzed using FlowJo software (ver9.4 Treestar Inc., San Carlos, CA). Cells stained with isotype-matched IgG antibodies were used as controls to determine the positive cell population.

To measure oxidative stress, CellRox (Life Technologies, Carlsbad, CA) Deep Red reagent was utilized according to the manufacturers protocol. The CellRox cell-permeable reagents are non-fluorescent while in a reduced state and upon oxidation exhibit strong fluorogenic signal. Apoptosis was measured using Annexin V (Invitrogen, Carlsbad, CA) conjugated to Alexa Fluor 647 according to the manufacturers protocol.

Statistical Analysis:

Data are presented as mean ± S.E.M. Statistical comparisons were analyzed by one-way ANOVA followed by Bonferroni tests and Tukey's-Kramer honest significant difference analyses for post hoc comparison. Statistical significance was considered to be achieved when $p<0.05$.

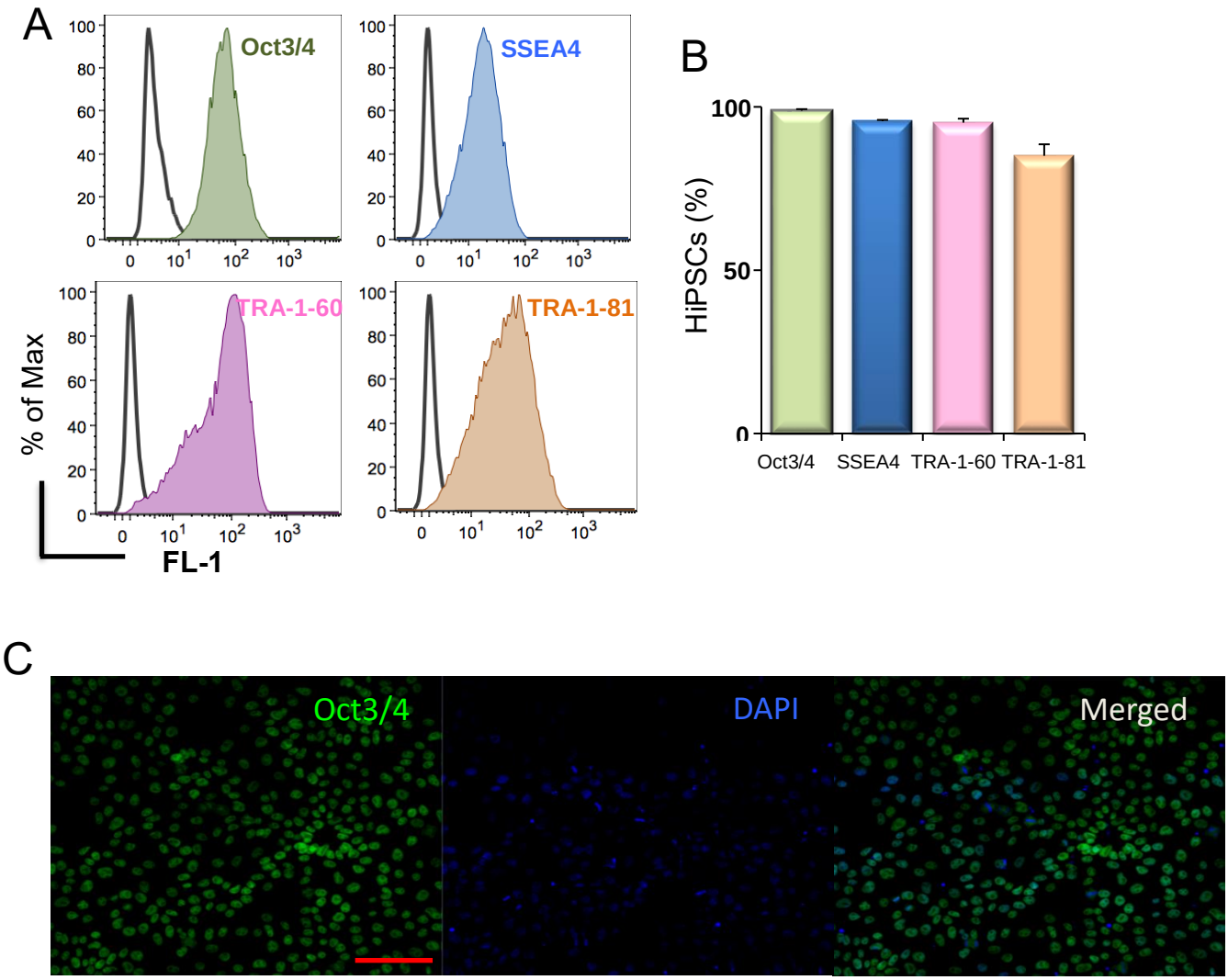

Fig. S1. Expression of pluripotent markers in hiPSCs. (**A, B**) Transduced hiPSCs revealed high protein expression levels of pluripotency markers such as Oct3/4, SSEA4, TRA-1-60 and TRA-1-81 as assessed by flow cytometry. (**C**) Immunofluorescence confocal laser scanning microscopic images of hiPSCs stained positive for pluripotency marker Oct3/4. DAPI (4', 6-diamidino-2-phenylindole) was used to stain the nuclei. Scale bar = 100 μM.

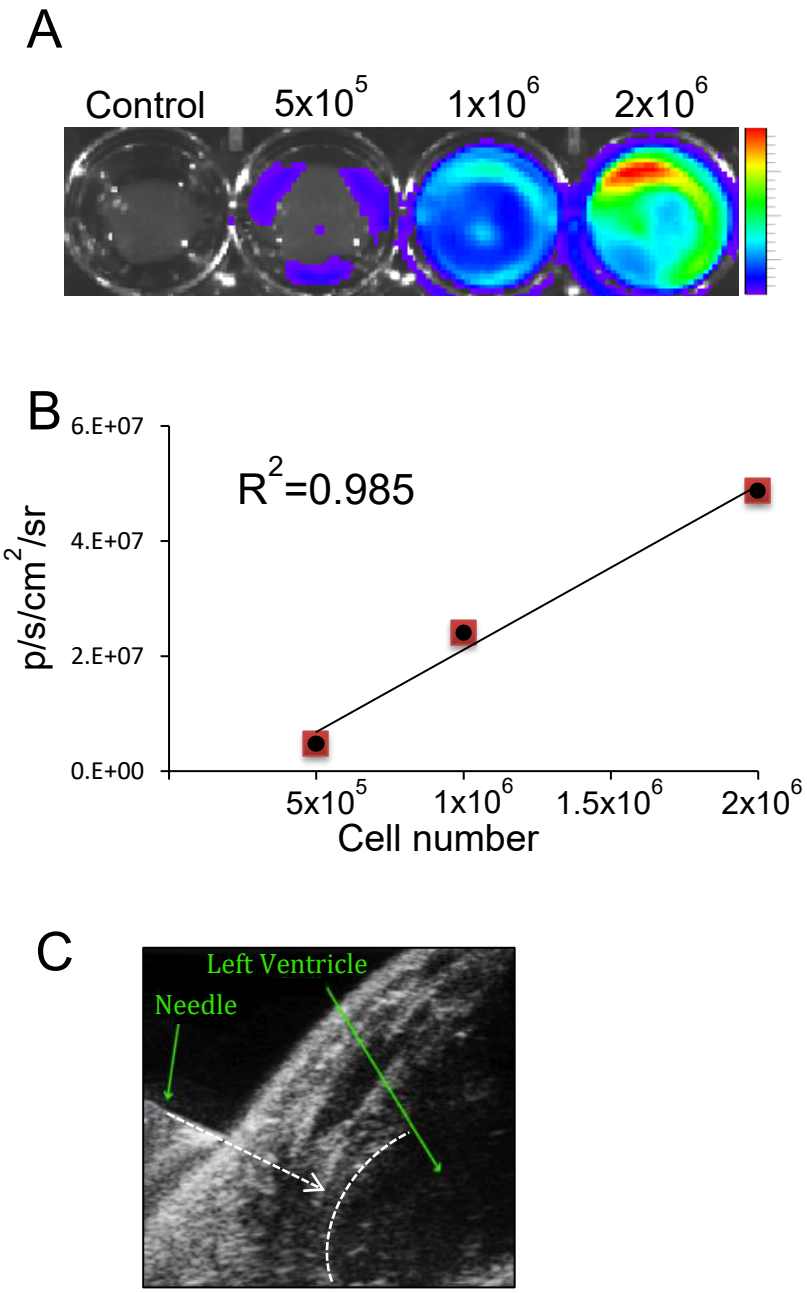

Fig. S2. Expression of firefly luciferace (Fluc) in hiPSC-CMs. (A) Representative examples of *in vitro* bioluminescence imaging of hiPSC-CMs transduced with a double fusion construct of reporter genes; firefly luciferase (Fluc) for bioluminescence imaging and enhanced green fluorescent protein (GFP). **(B)** Quantification of cell numbers based on bioluminescence signals. **(C)** A representative two-dimensional image of ultrasound-guided delivery of hiPSC-CMs into border zone of myocardial infarct. The needle and the left ventricular cavity are labelled in the image.

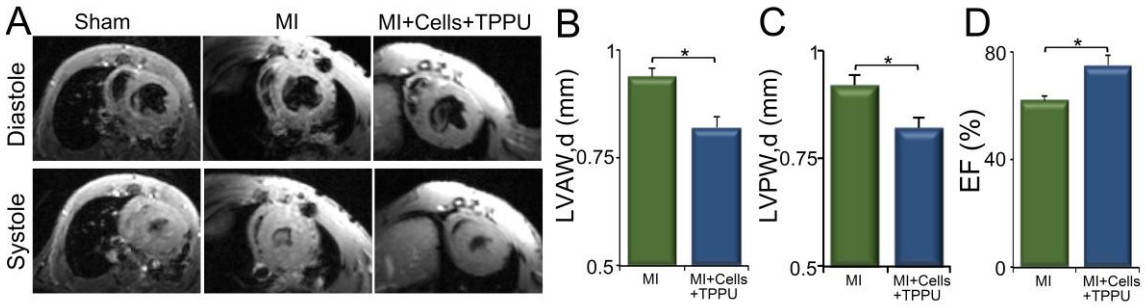

Fig. S3. Cardiac function as assessed by Magnetic resonance imaging (MRI). (A) Representative images from MRI in diastole (top panel) and systole (bottom panel) from Sham-operated, MI and MI+Cells+TPPU animals. Summary data of left ventricular anterior wall (LVAW,d) (B), posterior wall thickness (LVPW,d) during diastole (C), and ejection fraction (D) as assessed by MRI. *$P<0.05$ by ANOVA. Cells = hiPSC-CMs. Data represent Mean±SEM. N = 5 animals per group.

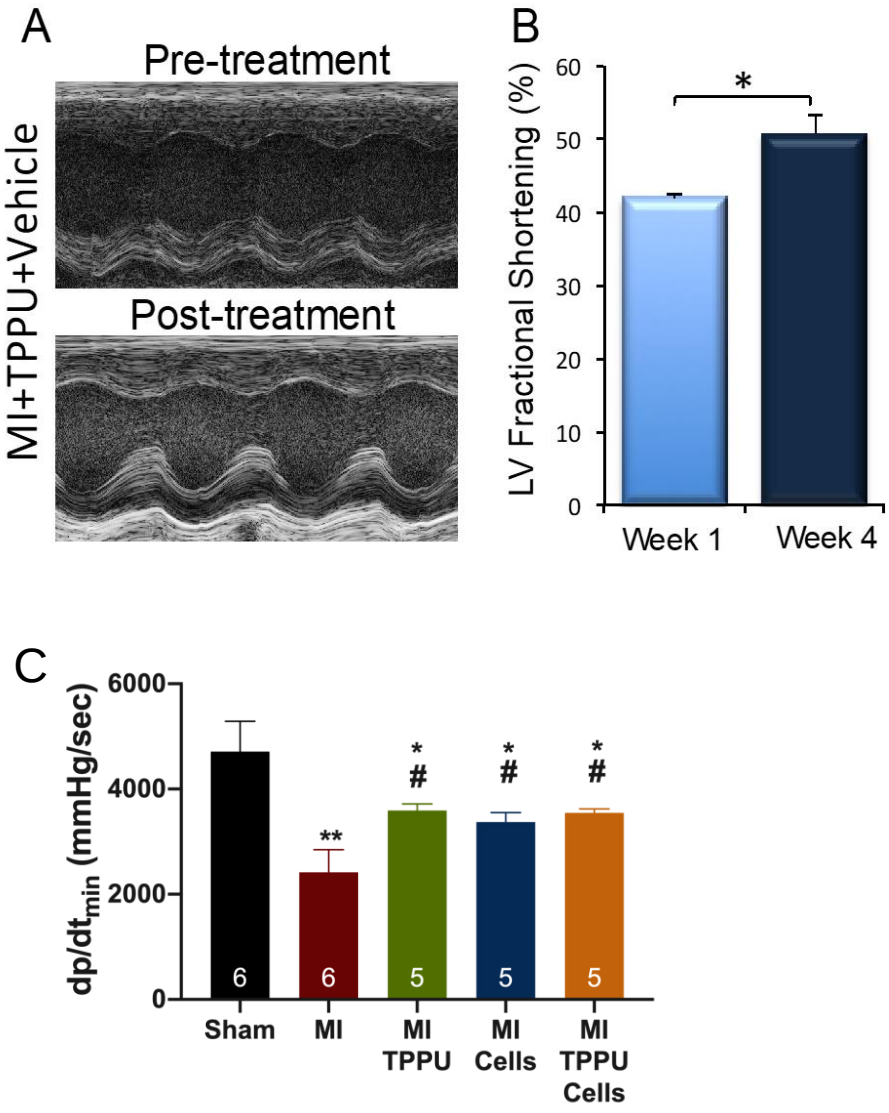

Fig. S4. Cardiac function as assessed by echocardiography. (A) Examples of M-mode echocardiography pre- and post-treatment in the vehicle (phosphate buffered saline, PBS)-treated MI group (MI+TPPU+Vehicle). **(B)** Percentages of fractional shortening (FS) in mice post MI with vehicle injection and TPPU treatment (51±2%) showed a comparable improvement in FS to the MI+TPPU group (55±0.7%) suggesting no adverse effect from the vehicle injection. n = 3 per group. **(C)** Summary data of dP/dt_{min} (Absolute values). These values provide an indication of the heart's diastolic function. * $p<0.05$, ** $p<0.01$ comparing to sham group. # $p<0.05$ comparing to MI group. Mean±SEM.

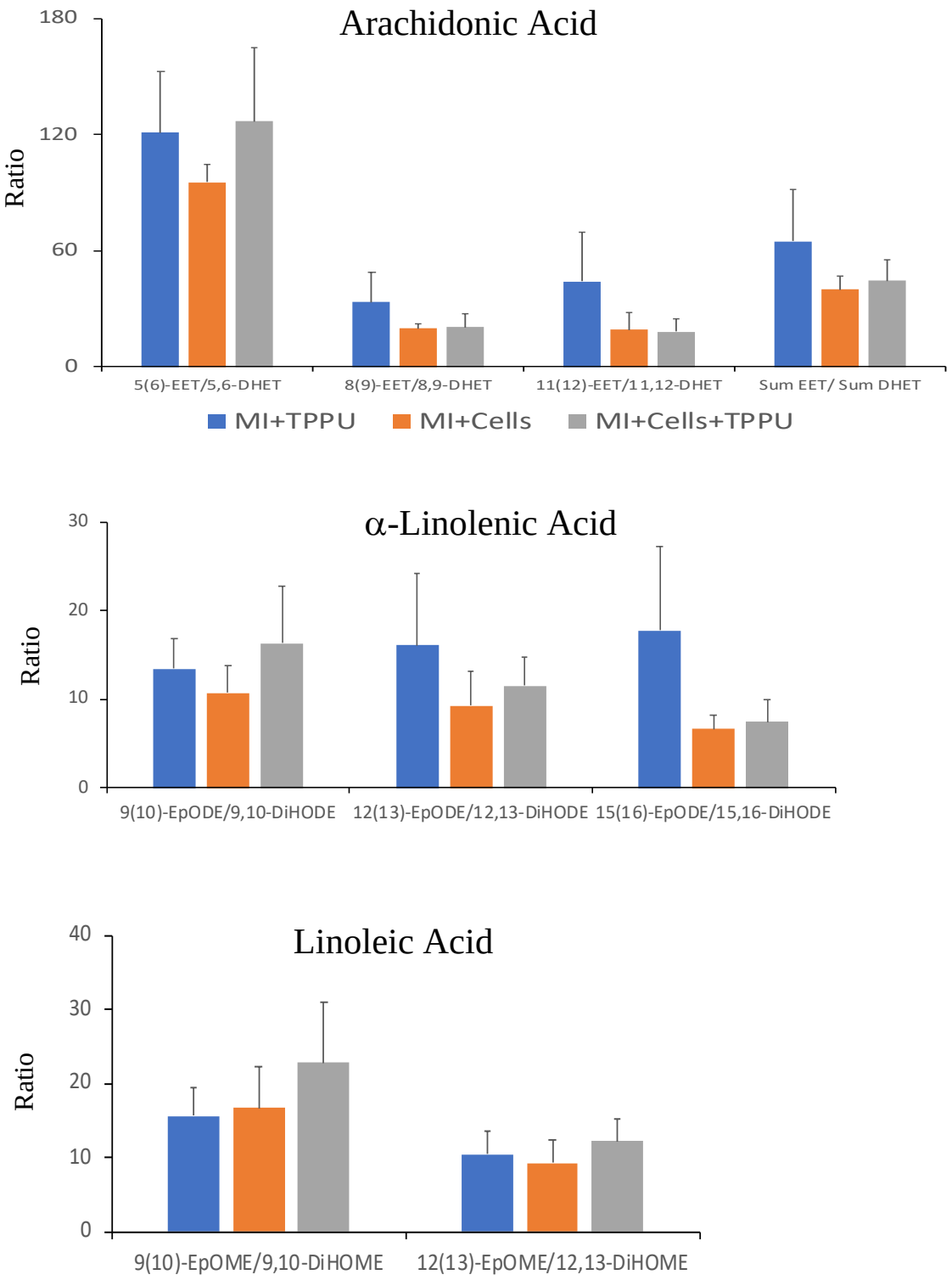

Fig. S5. Oxylipin profiling of Arachidonic acid (Top panel), α-Linolenic acid and Linoleic acid from the three groups of animals including MI+TPPU, MI+Cells and MI+TPPU+ Cells at 3 wk of follow-up. Data shown represent mean ± SE.

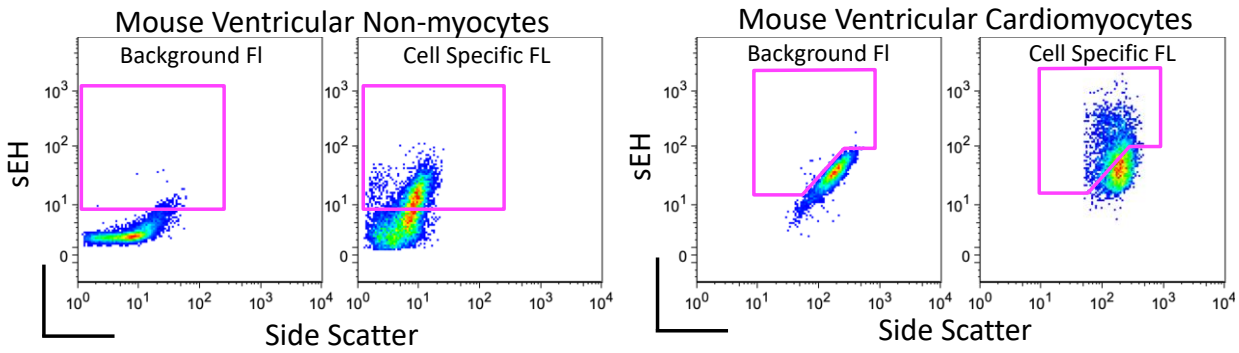

Fig. S6. Presence of sEH enzyme in mouse ventricular cells. Representative flow cytometry images demonstrating the presence of sEH enzyme in mouse ventricular non-myocyte cells (left panels) and cardiomyocytes (right panels)**.**

References

1. Yechikov S, Copaciu R, Gluck JM, Deng W, Chiamvimonvat N, Chan JW, et al. Same-Single-Cell Analysis of Pacemaker-Specific Markers in Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte Subtypes Classified by Electrophysiology. *Stem Cells.* 2016;34(11):2670-80.
2. Li N, Liu JY, Timofeyev V, Qiu H, Hwang SH, Tuteja D, et al. Beneficial effects of soluble epoxide hydrolase inhibitors in myocardial infarction model: Insight gained using metabolomic approaches. *J Mol Cell Cardiol.* 2009;47(6):835-45.
3. Sirish P, Lopez JE, Li N, Wong A, Timofeyev V, Young JN, et al. MicroRNA profiling predicts a variance in the proliferative potential of cardiac progenitor cells derived from neonatal and adult murine hearts. *J Mol Cell Cardiol.* 2012;52(1):264-72.