

SUPPLEMENTAL MATERIAL

Cardiac stem cell isolation and characterization. hCPCS are isolated from heart tissue obtained from patients undergoing surgery for Left Ventricular Assist Device (LVAD) implantation as previously described¹. The tissue is minced in small pieces and digested enzymatically in collagenase 150U mg/ml (Worthington Bio Corp, Lakewood, NJ) for 2 hours at 37°C. The cells are then passed through 100µm and 50µm filters (BD biosciences CA). The cells are then centrifuged at 1200rpm for 5 min. The pellet is re-suspended in hCPCs media: Hams F12 (Fisher), 10% FBS, 1% PSG, 5mU/ml human erythropoietin (Sigma), 10ng/ml basic FGF (Peprotech), and incubated overnight at 37°C in CO₂ incubator. The next day the cells are collected and passed through 100µm and 50µm filters and centrifuged at 1200rpm for 5 min. The pellet is incubated with c-kit labeled beads (miltenyi biotec, CA) and sorted according to manufacturer's protocol. The cells are then seeded until few cells adhere to the bottom of the dish and expanded clonally.

Cardiac progenitor cells have been isolated as previously published by our group². Briefly, CPCs were isolated from the hearts of male FVB mice by labeling with CD117 microbeads and cultured in mouse CPC medium according to the protocol used previously³. CPCs were characterized by FACS analysis for c-kit expression. Briefly, CPCs were fixed in PFA, washed and blocked in horse serum followed by incubation with c-kit antibody (ab5506, 1:50) for 30min. Cells were further labeled with secondary antibody (Donkey anti-rabbit Cy5), resuspended in horse serum and analyzed immediately by FACS. Freshly isolated cells from whole mouse hearts expressed c-kit (76.4%) and Sca-1 (92.5%). Early passage cells express c-kit (68.3%) in hCPCe and (69.1%) in hCPCeβct. Additionally, CPCs were negative for hematopoietic markers CD45, CD34, CD31, TER-119, CD3, CD90⁴. Cultured CPCs are positive for Ki67 (a marker of cell cycling), nucleostemin (a marker of pluripotent stem cells) and GATA-4 under normal conditions². Dexamethasone treatment of CPCs can induce expression cardiomyocyte specific

factors such as MEF-2c, troponin T and smooth muscle actin^{2, 5}. Adoptive transfer of c-kit+ CPCs in mouse model of myocardial infarction results in long term improvement cardiac function concurrent with differentiation of adoptively transferred cells into cells of all three cardiac lineages i.e. myocyte, endothelial cells and smooth muscle cells⁶. Medium used to culture cardiac stem cells consisted of DMEM-F12 with 10% FBS, 1% PSG, 0.02ng/ml bFGF (Peprotech #100-18B), 0.4µg/ml EGF (Sigma #E9644), 1000U/ml LIF (Chemicon #ESG1107), and 1X ITS (Lonza #17-838Z).

Protein Isolation and Immunoblot. CPCs are plated in 6-well dishes (50,000 cells/ well), harvested in 50µl sample buffer, boiled and sonicated. Protein lysates are run on 4-12% NuPage Novex Bis Tris gel (Invitrogen) and transferred on polyvinylidene fluoride (PVDF) membrane, followed by blocking in 8% skim milk in Tris-buffered saline Tween-20 (TBST) solution for 1 hr at room temperature and then incubation with primary antibodies overnight at 4°C (Table S2). Secondary antibodies are applied for 1 hr at room temperature after several washes with TBST solution. Fluorescence signal is detected and quantified using Typhoon 9400 fluorescent scanner together with Image Quant 5.0 software (Amersham Biosciences).

Myocardial Infarction. Surgery is performed on FVB mice including mCPCe=8, mCPCeβct=8. They are anesthetized under isoflourane, intubated and ventilated. Thoracotomy is performed and the heart is exposed between the third and fourth intercostals space. Left Anterior Descending (LAD) artery is ligated permanently with 8/0 suture followed by wound closure. Mice are closely monitored after the surgery and kept on a heating pad throughout the recovery period. Mice are injected at 5 different sites (100,000 cells) at the border zone area after infarction. Quantitation of CPC numbers in Figure 4 represents the number of c-kit+/GFP+ cells

found in the left ventricle at 3 days after transplantation. A rapid loss of transplanted cell occurs as a consequence of physical loss or leakage of cells⁷ reducing the actual number of cells surviving in the myocardium. Furthermore, there is loss of transplanted cells immediately after transplantation in the hostile myocardial environment as shown previously^{8, 9}. Hearts are harvested for histological analysis after 3 days of infarction.

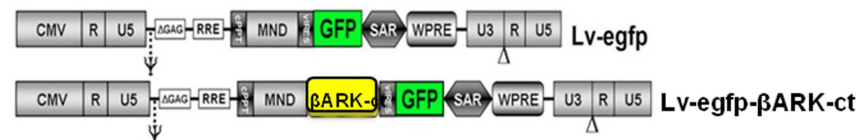
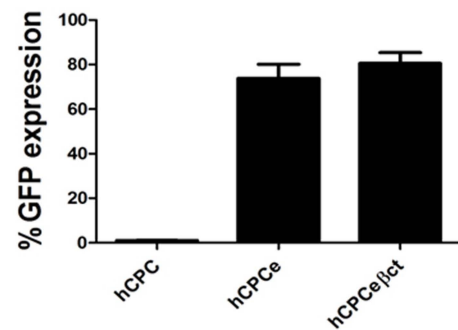
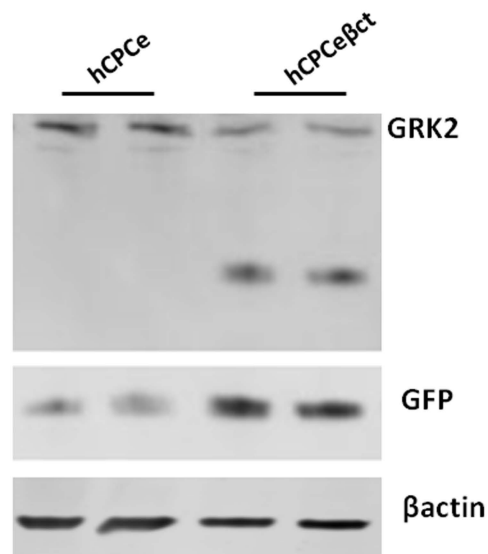
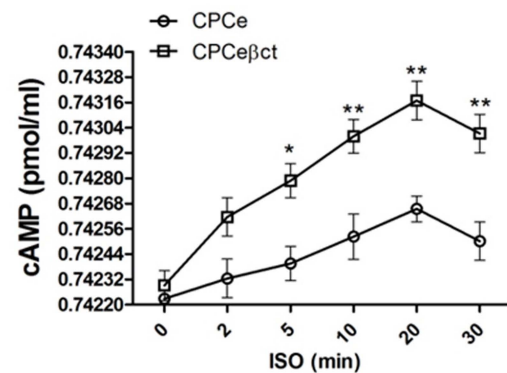
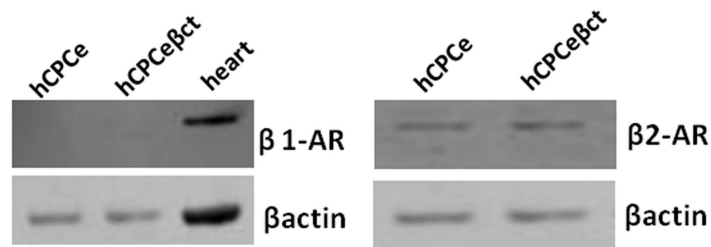
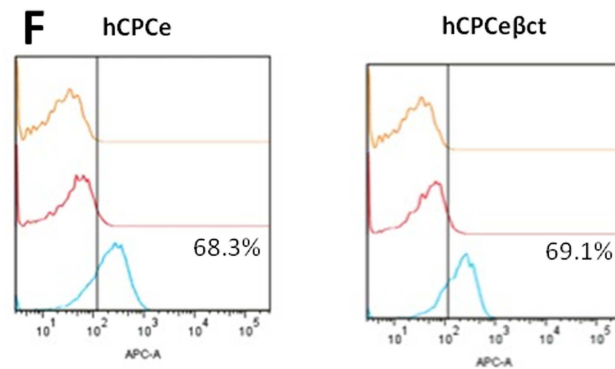
Flow cytometry. Cell death is measured by Annexin V kit (BD Biosciences) according to manufacturer's instructions. Cytometry is performed using a BD FACSAria Flow Cytometer (BD Biosciences).

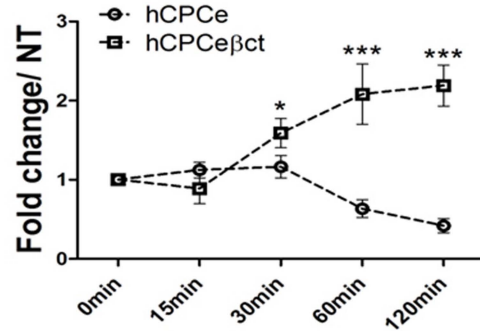
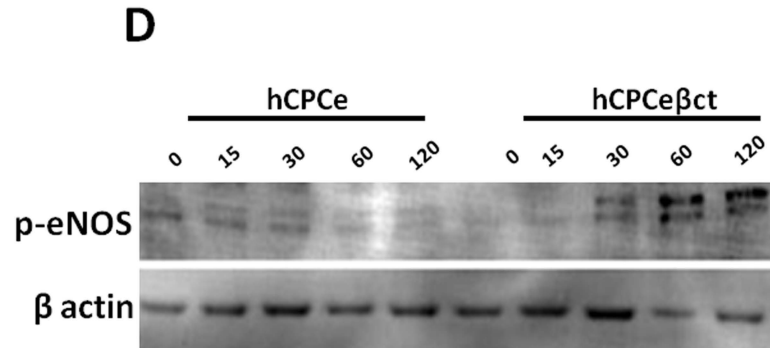
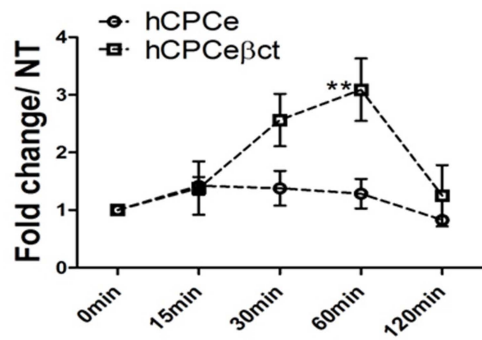
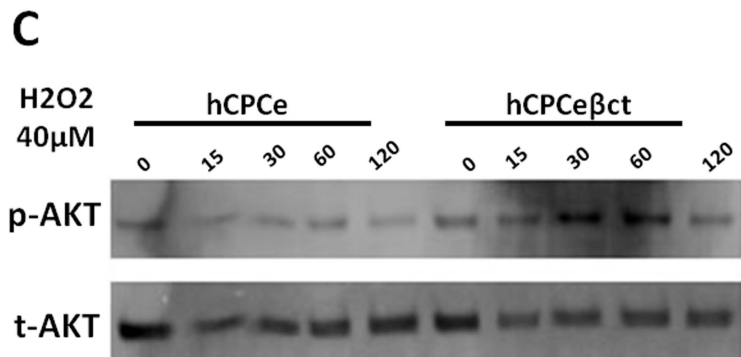
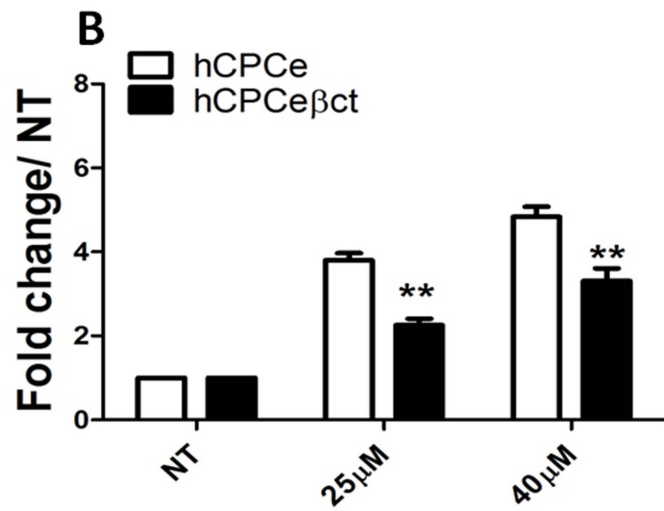
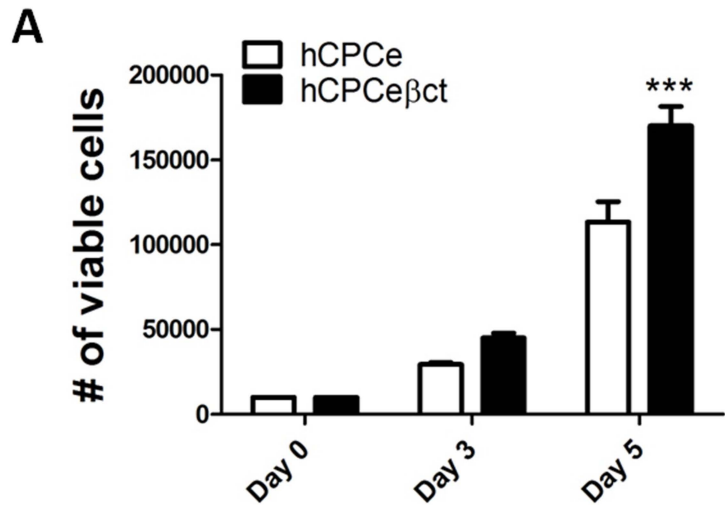
Catecholamine Assay. Plasma epinephrine (Epi) and norepinephrine (NEpi) levels are determined by enzyme-linked immunosorbent assay, performed on mouse plasma samples using the BI-CAT EIA kit from ALPCO Diagnostics (Windham, NH).

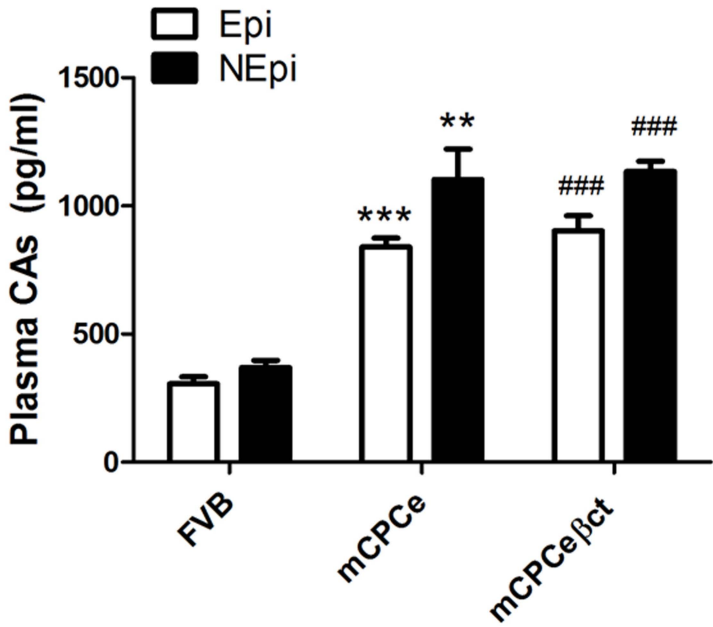
Histology and Staining. Immunostaining of CPCs is performed on cells grown on permanox or glass chamber slides¹⁰. Cells are fixed by 4% paraformaldehyde (PFA), permeabilized in PBS supplemented by 0.2% Triton-X for 10 min, and blocked in PBS supplemented with 10% horse serum for 1 hr. Primary antibodies diluted are applied overnight at 4°C after blocking in PBS with 10% horse serum. The next day, cells are washed with PBS and incubated for 1 h at room temperature with secondary antibodies (Jackson Laboratories) diluted in blocking solution. Sytox Blue or To-Pro (Molecular Probes) is diluted in Vectashield (Vector Labs) mounting media at 1:500 vol/vol and used as nuclear staining.

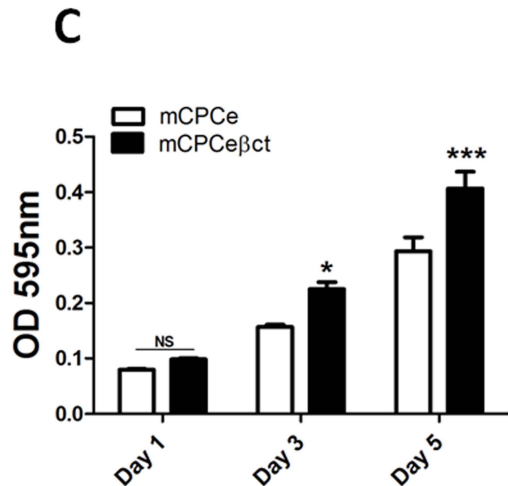
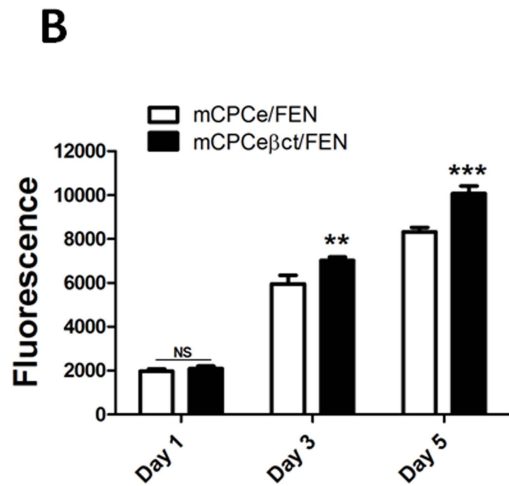
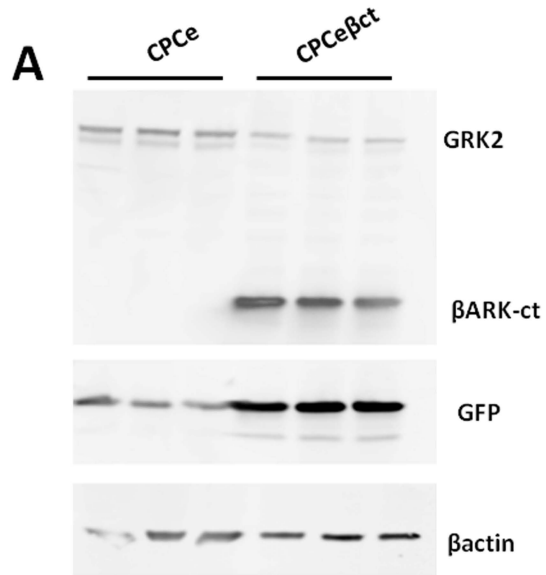
Paraffin heart sections are deparaffinized in xylene and rehydrated through graded alcohols to distilled water. Antigen retrieval is achieved by boiling the slides in 10 mmol/L citrate pH 6.0 for 12–15 min. Slides are washed several times with distilled water and once with TN buffer (100 mmol/L Tris, 150 mmol/L NaCl). Endogenous tissue peroxidase activity is quenched with TN buffer supplemented with 3% H₂O₂ for 20 min whenever necessary. Slides are then washed in TN buffer and blocked in TNB buffer (TSATM kit from Perkin-Elmer) at room temperature for at least 30 min. Primary antibodies are applied overnight at 4°C in TNB buffer. The next day, samples are washed in TN buffer and incubated with secondary antibodies at room temperature in the dark for 1 hr. When amplification of signal is needed, slides are washed in TN buffer and incubated with streptavidin horseradish peroxidase conjugated diluted 1:100 vol/vol in TNB buffer for 30 min at room temperature, and signals are developed using Tyramide substrate diluted 1:50 vol/vol in Amplification Diluent (Perkin-Elmer) for 10 min. Slides are washed in TN buffer and coverslipped using Vectashield in the presence of DNA staining. List of primary and secondary antibodies is reported in Table S1. Quantitation was done by measuring the number of GFP+/B1-AR+ and GFP+/B2-AR+ CPCs/total number of nuclei within a field of view that includes myocytes, endothelial cells as well as CPC nuclei.

TUNEL staining is performed using the In Situ Cell Death Detection Kit, TMR red (Roche Applied Science) according to the manufacturer's directions. Briefly, after permeabilization, samples are incubated with TUNEL reagent for 1 h at 37 °C and mounted in Vectashield for examination by confocal microscopy. Confocal images were acquired using a Leica TCS SP2 confocal laser- scanning microscope (Leica).

A**B****C****D****E****F**







Supplementary Figure Legends

Supplementary Figure 1: Characterization of β ARKct overexpression in human CPCs. A)

Schematic representation of the β ARKct and GFP lentiviral constructs. B) % GFP expression in β ARKct human CPCs compared to GFP engineered cells. C) Human CPCs engineered to express β ARKct (hCPCe β ct). D) cAMP levels in hCPCe β ct compared to hCPCe after acute ISO stimulation. * p <0.05, ** p <0.01, *** p <0.001 significance for hCPCe vs hCPCe β ct. E) Expression of β -adrenergic receptors in CPCs after β ARKct modification. F) c-kit expression in early passage hCPCs by FACS analysis.

Supplementary Figure 2: Enhanced viability and survival in hCPCe β ct in response to oxidative stress. A)

Increased viability in hCPCe β ct compared to hCPCe for 5 days (n=4). B) Cell death assay; hCPCe β ct are resistant to oxidative stress as evidence by decreased Annexin-V+ cells after H₂O₂ (40uM) treatment compared to hCPCe (n=3). C) Increased phosphorylation of AKT and eNOS (D) 30 and 60 min after H₂O₂ treatment in hCPCe β ct compared to hCPCe in the presence of H₂O₂ induced oxidative stress along with blot quantitation (n=3). * p <0.05, ** p <0.01, *** p <0.001 significance for hCPCe vs hCPCe β ct.

Supplementary Figure 3: Serum plasma levels of epinephrine and norepinephrine. Serum

levels of norepinephrine and epinephrine in normal, mCPCe and mCPCe β ct mice. FVB (n=3) mCPCe (n=4), mCPCe β ct (n=4). * p <0.05, ** p <0.01, *** p <0.001 significance for Epi/NEpi levels compared between FVB vs mCPCe, # p <0.05, ## p <0.01, ### p <0.001 Epi/NEpi levels FVB vs mCPCe β ct.

Supplementary Figure 4: β ARKct engineering in mouse CPCs. A) Mouse CPCs

overexpressing β ARKct (CPCe β ct). Increased proliferation (B) and metabolic activity (C) in

CPCe β ct compared to CPCe. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significance for mCPCe/FEN vs mCPCe β ct/FEN.

Table S1**Antibodies**

Application	Antibody	Dilution	Amplify	Company
ICC	GFP	1:200	No	Molecular Probes
ICC	β 1-AR	1:200	No	Abcam
ICC	β 2-AR	1:200	No	Abcam
ICC	c-kit	1:200	No	RND systems
ICC	GRK2	1:100	No	Santa Cruz
IHC	α -sarcomeric actin	1:100	No	Sigma
IHC	PCNA	1:300	Yes	Santa Cruz
IHC	c-kit	1:50	Yes	RND systems
Western blot	eNOS	1:500	No	Cell Signaling
Western blot	phospho-eNOS S1177	1:500	No	Cell Signaling
Western blot	AKt	1:500	No	Santa Cruz
Western blot	Phospho-AKT S473	1:500	No	Cell Signaling
Western Blot	Phospho-GRK2 S670	1:500	No	Millipore
Western Blot	GRK2	1:500	No	Santa Cruz
Western Blot	β actin	1:2000	No	Santa Cruz

References

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