SUPPLEMENTAL MATERIAL

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Expanded Methods:

Generation of Lentiviral Vectors:

All constructs are third generation self-inactivating (SIN) lentiviral vectors and incorporate several elements to ensure long-term expression of the transgene. The MND promoter allows for high expression of the transgene, while the LTR allows for long-term expression after repeated passage [1, 2]. The vectors also include (IFN)-β-scaffold attachment region (SAR) element. The SAR element has been shown to be important in keeping the vector transcriptionally active by inhibiting methylation and protecting the transgene from being silenced [3-5]. The human Pim-1 cDNA was amplified out using primers containing EcoR1 restriction sites at both ends in order to facilitate cloning into the lentiviral backbone.

Lentiviral preparation

Lv-egfp and Lv-egfp+Pim1 constructs were co-transfected with packaging plasmids pMDLg/pRRE, pRSV-rev, and VSVG into 293T cells. Media changed 16 hours later and viral supernatant harvested at 24 and 48 hours. High titer virus was achieved using ultracentrifugation and titer calculated using limiting dilutions of viral stock.

Cardiac Stem Cell Medias:

DMEM-F12 with 10% FBS, 1% PSG, .02ng/ml bFGF (Peprotech #100-18B), .4μg/ml EGF (Sigma #E9644), 1000U/ml LIF (Chemicon #ESG1107), and

1X ITS (Lonza #17-838Z). Differentiation media: aMEM, 10% FBS, 1% PSG, .22% sodium bicarbonate, 10nM Dexamethasone (Sigma # D4902), pH 7.2.

Myocardial Infarction, Injections, Echocardiography, and Hemodynamics:

Briefly, 10-12 week old female FVB mice were anesthetized under isoflurane, intubated, and ventilated. A thoracotomy was performed and the LAD ligated. Vehicle, CPCe, or CPCeP were injected by blinded surgeon at five minutes post ligation around border zone in five sites with a total of 100,000 cells per heart. Infarction size was standardized by echocardiography performed on animals imaged along a parasternal short-axis view by M-mode recorded at 3 days post-infarction/injection. Lack of anterior wall motion in conjunction with at least 40% decrease in EF and FS were required for study inclusion. Hemodynamic performance assessed by echocardiography three days post-infarction was not statistically different between infarcted and injected groups (PBS, CPCe, and CPCeP). Closed chest hemodynamic assessment was performed on anesthetized mice prior to insertion of microtip pressure transducer (FT111B, Scisense) into the right carotid artery and advancement into left ventricle. The catheter was connected to an A/D converter (FV892A, Scisense) for data collection. After hemodynamic measurements, hearts were arrested in diastole and perfused with phosphate-buffered formalin.

Antibody Table:

Primary Ab	Species	Dilution	Amplification	Manufacturer
c-kit	Goat	1:40	Yes	R&D Systems
Pim-1	Rabbit	1:500	Yes	CST
Tropomyosin	Mouse	1:75	No	Sigma

GFP	Rabbit	1:500	Yes	Molecular Probes
vWF	Rabbit	1:100	No	Sigma
Mef2C	Rabbit	1:100	No	Invitrogen
Connexin 43	Rabbit	1:100	Yes	Sigma
SMA	Rabbit	1:300	No	Inviva

Expanded Results:

CPCeP differentiate into cardiac, endothelial, and smooth muscle lineages

CPCe and CPCeP co-expressed c-kit and MEF2C, a myocyte-specific transcription factor. Additionally, CPCeP also expressed VWF, an endothelial cell marker (Figure S4A). The endothelial nature of CPCeP was supported by uptake of acetylated-low density lipoprotein (Ac-LDL) showing a small percentage of CPCeP are Ac-LDL+, whereas Ac-LDL was undetectable in CPCe (Figure S4B). The capacity of genetically engineered CPCs to express cardiogenic markers consistent with lineage commitment was examined *in-vitro* by treatment with Dex). Immunostaining for cardiogenic lineages was performed using antibodies to c-kit, MEF2C, VonWillebrands Factor (vWF), and Gata6 to identify progenitor, cardiac, endothelial, and smooth muscle cells, respectively. Markers of all three lineages were detected by immunostaining following Dex treatment of CPCe or CPCeP (Figure S4C).

Telomeric length preservation in myocytes derived from CPCeP

Telomere length was measured in myocytes of mice receiving CPCeP in the border zone at 12-weeks after delivery. Presumptive de-novo myocytes derived from CPCeP as evidenced by coincidence of eGFP and tropomyosin labeling showed relatively greater telomeric length compared to resident eGFP- myocytes (Figure S7A). Additionally, cross sectional area of eGFP+ myocytes with long telomeres was significantly smaller compared to those cells with shorter telomeres (Figure S7A). Together, small eGFP+ myocytes with long telomeres

suggests these cells are newly formed young cells presumably originating from the donated cell population.

CPCeP origin for these small eGFP+ myocytes was supported by both immunohistochemical and PCR analyses. Small eGFP+ myocytes show coincident labeling for increased Pim-1 expression. The level of myocyte-specific Pim-1 immunoreactivity in sections from CPCeP injected hearts was clearly greater than that observed for sections from receiving CPCe (Figure S7B). In addition, presence of genomic DNA of male origin was detected in cells (Figure S7C), as well as within the infarct region of female hearts (Figure S7D) by PCR for SRY, a gene located on the Y chromosome. Demonstration of DNA for eGFP encoded by insertion of the Lv-egfp or Lv-egfp+Pim1 sequences into the cellular genome corroborates the presence of cells derived from donor origin in the infarcted region (Figure S7D).

CPCeP have increased proliferation *in-vivo*

At 5-weeks post infarction CPCeP injected animals maintain cardiac function while CPCe injected controls begin to display signs of cardiac failure determined by echocardiography. CPCe or CPCeP injected animals were given BrdU at 5-weeks post-infarction to assess whether sustained cardiac performance in CPCeP animals may be attributed to increased numbers of proliferating cells,. Quantitation of the BrdU+ eGFP+ population demonstrates CPCeP injected animals have a significant ($p<.001$) 3-fold increase in the number of proliferating cells at 5-weeks (Figure S8A). At the 12 week time point

co-expression of PCNA and eGFP were quantitated. Results were similar to that obtained at 5-weeks, whereby CPCeP injected animals had a significant 1.9-fold increase in the number of PCNA+ eGFP+ cells (Figure S8B).

CPCeP and CPCe injected mice were stained with TUNEL and eGFP to address CPCeP resistance to cell death sections from. At 5-weeks no TUNEL+ eGFP+ cells were found in CPCeP or CPCe injected mice. Interestingly CPCeP hearts had a significant 4-fold decrease in TUNEL+ eGFP- cells (Figure S8C). These data suggest injection of CPCeP has a protective effect on surrounding myocardium; however it is not yet known if CPCeP are refractory to apoptosis at time points before 5-weeks.

Supplemental Table:

Fractional Shortening	3 days	7 days	2 weeks	4 weeks	6 week	8 week	10 week	12 week	15 week	18 week	21 week	22 week	23 week	24 week	26 week	28 week	32 week
Sham vs Vehicle	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Sham vs CPCe	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Sham vs CPCeP	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.01	P < 0.001
Vehicle vs CPCe	P > 0.05	P < 0.001	P < 0.001	P < 0.05	P < 0.01	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Vehicle vs CPCeP	P > 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.01	P < 0.001	P < 0.001	P < 0.01
CPCe vs CPCeP	P > 0.05	P > 0.05	P < 0.01	P < 0.001	P < 0.01	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.05	P < 0.01	P < 0.001	P < 0.001	P < 0.001
Ejection Fraction																	
Sham vs Vehicle	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Sham vs CPCe	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Sham vs CPCeP	P < 0.001	P < 0.01	P < 0.001	P < 0.001	P < 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.05	P < 0.01	P < 0.01	P < 0.05	P < 0.05	P < 0.01	P < 0.05	P > 0.05	P < 0.001
Vehicle vs CPCe	P > 0.05	P < 0.001	P < 0.001	P < 0.01	P < 0.001	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Vehicle vs CPCeP	P > 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.01	P < 0.001	P < 0.001	P < 0.001
CPCe vs CPCeP	P > 0.05	P > 0.05	P < 0.01	P < 0.001	P < 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.05	P < 0.01	P < 0.001	P < 0.001	P < 0.001
Anterior Wall Dimension																	
Sham vs Vehicle	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Sham vs CPCe	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Sham vs CPCeP	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Vehicle vs CPCe	P > 0.05	P < 0.001	P > 0.05	P > 0.05	P > 0.05	P < 0.001	P > 0.05	P > 0.05	P < 0.05	P < 0.01	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Vehicle vs CPCeP	P > 0.05	P < 0.001	P < 0.001	P < 0.01	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.05	P < 0.05	P < 0.01	P < 0.001	P < 0.001
CPCe vs CPCeP	P > 0.05	P > 0.05	P < 0.05	P > 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.001	P < 0.01	P > 0.05	P < 0.05	P > 0.05	P < 0.01	P < 0.001

Supplemental Figures:

Figure S1.

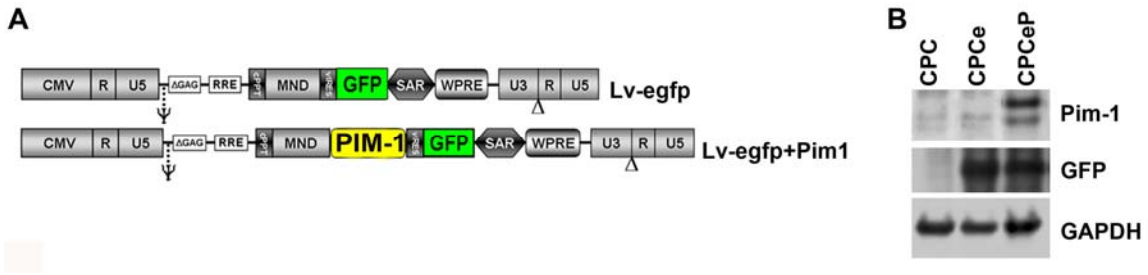


Figure S2.

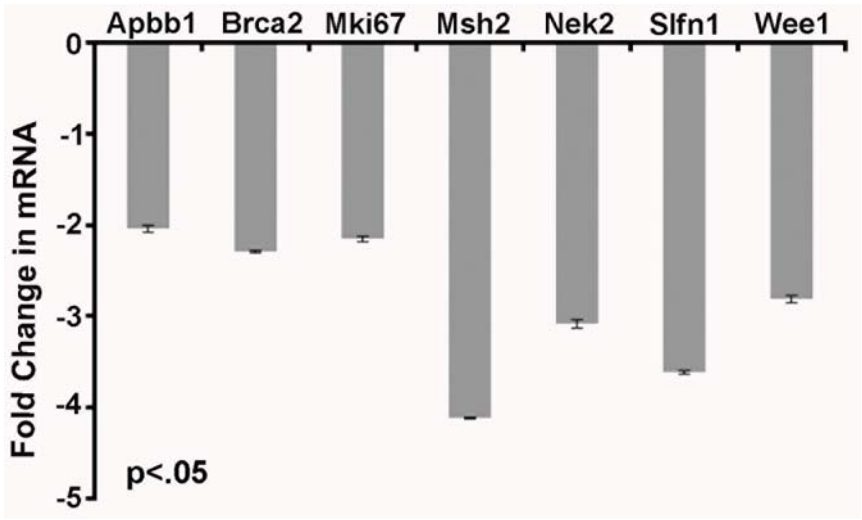
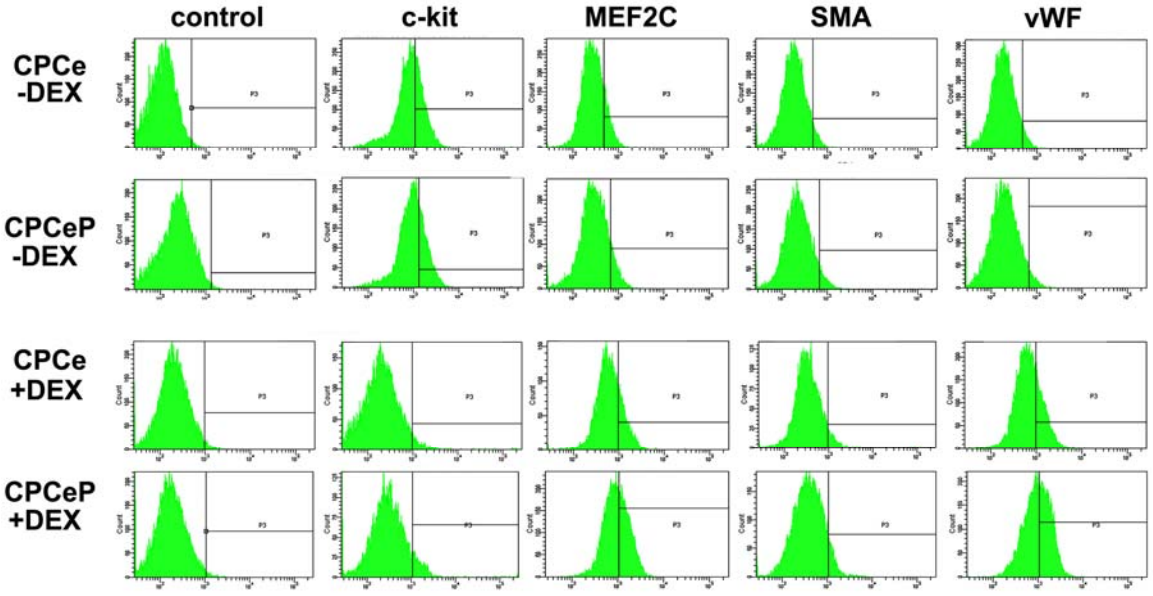


Figure S3.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SMA
vWF
CPCe -DEX
CPCeP -DEX
CPCe +DEX
CPCeP +DEX

Figure S4.

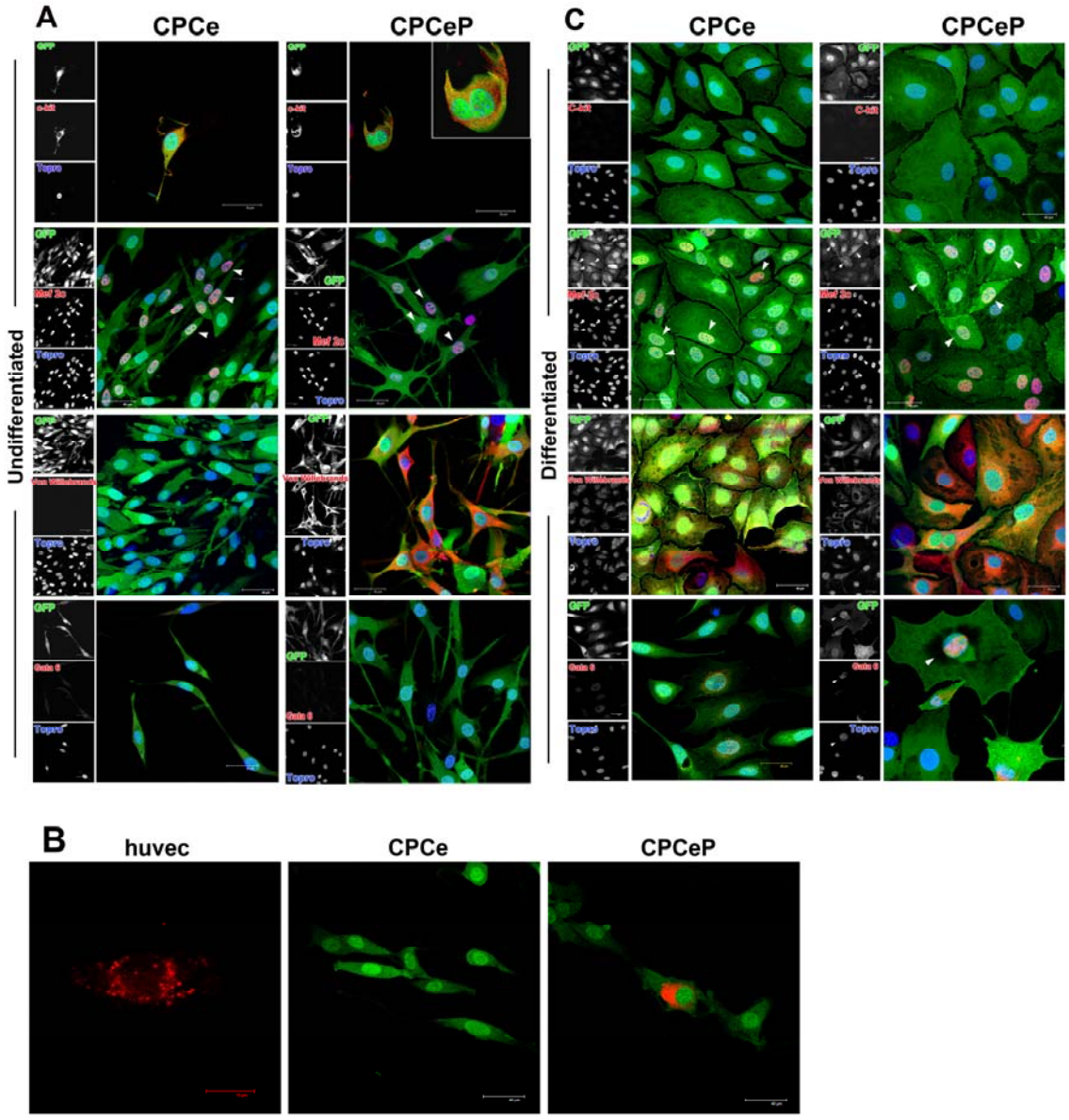

A
CPCe
CPCeP
C
CPCe
CPCeP
Undifferentiated
Differentiated
B
huvec
CPCe
CPCeP

Figure S5.

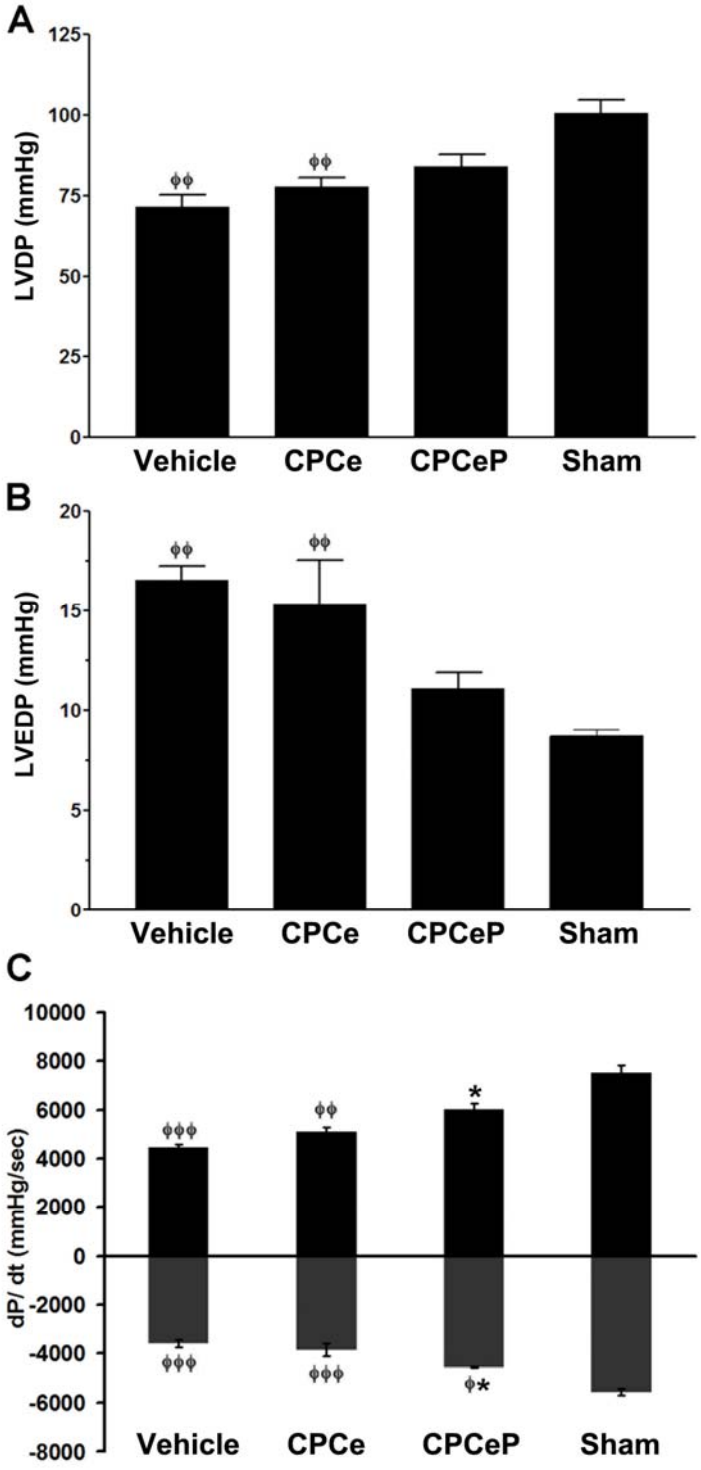

A
LVDP (mmHg)
125
100
75
50
25
0
ϕϕ
ϕϕ
Vehicle
CPCe
CPCeP
Sham
B
LVEDP (mmHg)
20
15
10
5
0
ϕϕ
ϕϕ
Vehicle
CPCe
CPCeP
Sham
C
dP/ dt (mmHg/sec)
10000
8000
6000
4000
2000
0
-2000
-4000
-6000
-8000
ϕϕϕ
ϕϕ
*
ϕϕϕ
ϕϕϕ
ϕ*
Vehicle
CPCe
CPCeP
Sham

Figure S6.

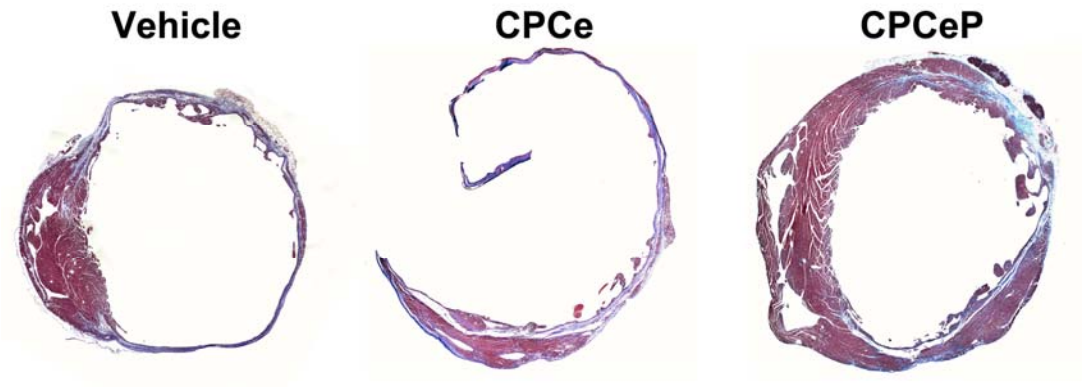


Figure S7.

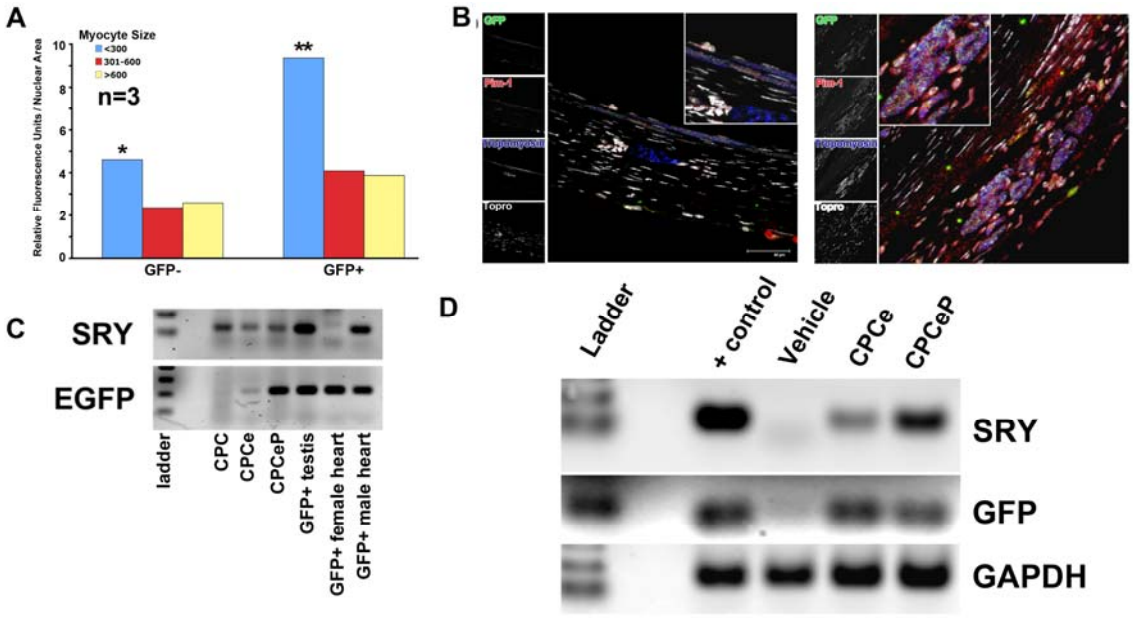
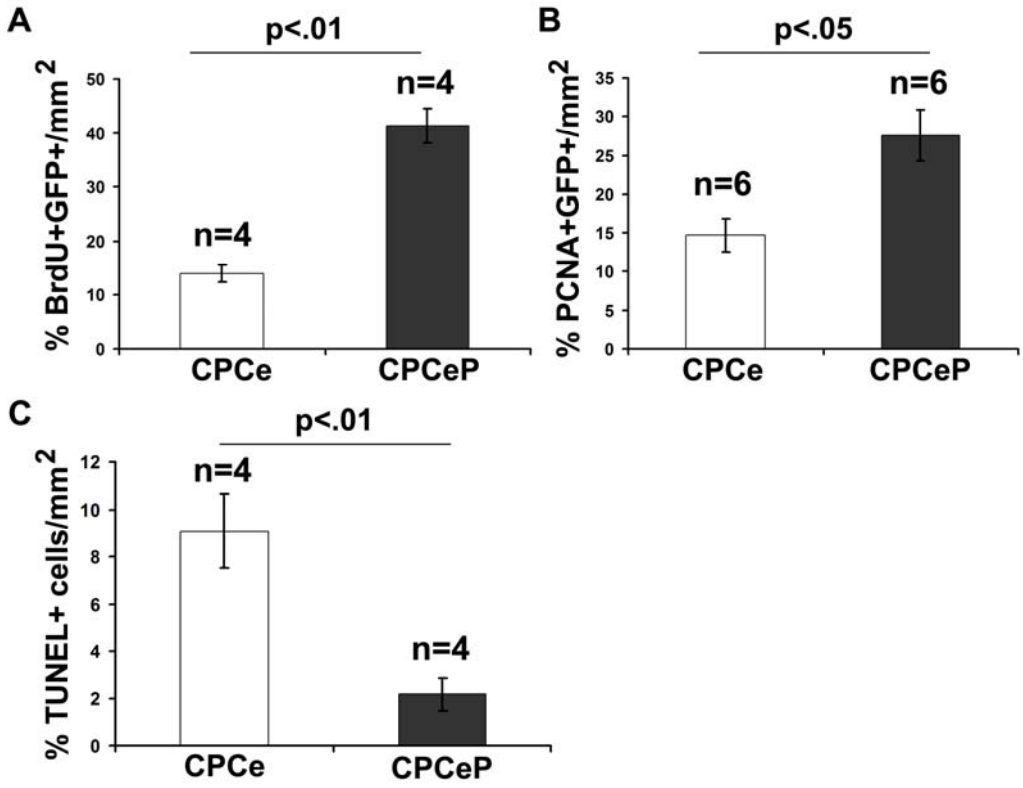


Figure S8.


A
p<.01
% BrdU+GFP+/mm2
n=4
n=4
CPCe
CPCeP
B
p<.05
% PCNA+GFP+/mm2
n=6
n=6
CPCe
CPCeP
C
p<.01
% TUNEL+ cells/mm2
n=4
n=4
CPCe
CPCeP

Supplemental Figure Legends:

Supplementary Table 1. Statistics for 32 week fractional shortening, ejection fraction, and anterior wall dimension in animals that received vehicle, CPCe, or CPCeP.

Figure S1. Lentiviral gene expression in c-kit+ CPCs.

(A) Self-inactivating (SIN) lentiviral vectors, termed Lv-egfp (GFP control) and Lv-egfp+Pim1. **(B)** Representative immunoblot of CPC (n=3), CPCe (n=3), and CPCeP (n=3), immunolabeled for Pim-1, GFP, and GAPDH.

Figure S2. Pim-1 transduced CPCs mediate expression of genes involved in proliferation.

Total mRNA was extracted from CPCe (n=3) and CPCeP (n=3) cells and run on the cell cycle RT^2 Profiler Cell Proliferation Array from SuperArray in triplicate. CPCeP and CPCe were normalized to GAPDH. Samples analyzed had ≥2-fold difference from control, with $p<0.05$, (mean $\pm$ SEM, n=3).

Figure S3. Phenotypic FACS characterization of CPCe and CPCeP *in vitro*.

Flow cytometric analysis of CPCe (n=3) and CPCeP (n=3) treated with and without dexamethasone for seven days.

Figure S4. Phenotypic characterization of CPCe and CPCeP *in vitro.*

(A) Immunolabeling of CPCe and CPCeP for cardiac (Mef2C), endothelial (VonWillebrands Factor), and smooth muscle (Gata6). White arrowheads indicate cells of interest. In each case eGFP is represented in green, lineage marker in red, and Topro-3-iodide (nuclei) in blue. **(B)** Ac-LDL-DiI uptake assay in CPCe and CPCeP using HUVEC positive control. **(C)** Immunolabeling of CPCe (n=3) and CPCeP (n=3) ran in triplicate after exposure for 7 days to dexamethasone for cardiac (Mef2C), endothelial (VonWillebrands Factor), and smooth muscle (Gata6). White arrowheads indicate cells of interest.

Figure S5. Improved cardiac function in CPCe and CPCeP treated animals.

(A-C) In-vivo hemodynamic measurements of left ventricular developed pressure (DP) **(A)**, left ventricular end diastolic pressure (Ped) **(B)**, and dP/dT maximum and minimum **(C)** assessing cardiac function of sham (n=4), vehicle (n=4), CPCe (n=4), and CPCeP (n=4) injected animals 4 weeks post-intramyocardial injection (mean $\pm$ SEM). $\phi p<.05$, $\phi\phi p<.01$ compared to Sham and $*p<.05$ compared to CPCe.

Figure S6. Decreased fibrosis in heart of CPCeP treated animals.

Representative images of Masson's Trichrome staining in hearts from CPC, CPCe, and CPCeP injected mice.

Figure S7. Preserved telomere length in CPCeP and detection of male SRY gene. (A) Quantitation of telomeric length in small to large border zone myocytes in the hearts of CPCeP injected mice 12-weeks post infarction (mean ± SEM, n=3). **(B)** Myocytes (tropomyosin, blue) immunolabeled for Pim-1 [6], eGFP (green), and nuclei (white) in CPCe and CPCeP treated hearts 12-weeks post infarction. (*p<.05, **p<.01). **(C)** PCR of genomic DNA of lentivirally infected cells, CPCe and CPCeP, to confirm male origin. **(D)** PCR of genomic DNA detecting SRY and eGFP from excised infarct region in CPCe and CPCeP injected hearts 12-weeks post infarction. Scale bars represent 40μm.

Figure S8. Increased proliferation of CPCeP *in-vivo*.

(A) Quantitation of BrdU+ GFP+ colocalization in CPCe (n=4) and CPCeP (n=4) injected animals at 5 weeks post infarction and CPC injection. **(B)** Quantitation of PCNA and GFP colocalization in CPCe (n=6) and CPCeP (n=6) injected animals 12 weeks post infarction and CPC injection. **(C)** Quantitation of TUNEL+ GFP+ cells in CPCe (n=4) and CPCeP (n=4) injected animals at 5 weeks post infarction and CPC injection.

Supplemental References:

1. Miyoshi H, Smith KA, Mosier DE, Verma IM, Torbett BE. Transduction of human CD34+ cells that mediate long-term engraftment of NOD/SCID mice by HIV vectors. *Science.* 1999;283:682-686.

2. Miyoshi H, Blomer U, Takahashi M, Gage FH, Verma IM. Development of a self-inactivating lentivirus vector. *J Virol.* 1998;72:8150-8157.

3. Agarwal M, Austin TW, Morel F, Chen J, Bohnlein E, Plavec I. Scaffold attachment region-mediated enhancement of retroviral vector expression in primary T cells. *J Virol.* 1998;72:3720-3728.

4. Auten J, Agarwal M, Chen J, Sutton R, Plavec I. Effect of scaffold attachment region on transgene expression in retrovirus vector-transduced primary T cells and macrophages. *Hum Gene Ther.* 1999;10:1389-1399.

5. Kurre P, Morris J, Thomasson B, Kohn DB, Kiem HP. Scaffold attachment region-containing retrovirus vectors improve long-term proviral expression after transplantation of GFP-modified CD34+ baboon repopulating cells. *Blood.* 2003;102:3117-3119.

6. Tillmanns J, Rota M, Hosoda T, Misao Y, Esposito G, Gonzalez A, Vitale S, Parolin C, Yasuzawa-Amano S, Muraski J, De Angelis A, Lecapitaine N, Siggins RW, Loredo M, Bearzi C, Bolli R, Urbanek K, Leri A, Kajstura J, Anversa P. Formation of large coronary arteries by cardiac progenitor cells. *Proc Natl Acad Sci U S A.* 2008;105:1668-1673.