SUPPLEMENTAL MATERIAL

Supplemental Methods:

Generation of Lentiviral vectors
Bicistronic lentiviral vectors were generated by introducing murine AKT cDNA fused to a 3X nuclear localization sequence as well as a myc tag. The control construct CPCe, expresses eGFP off an internal ribosomal entry site (IRES), while the nuclear targeted AKT construct termed CPCeA, expresses nuclear targeted AKT from a myeloproliferative sarcoma virus LTR-negative control region deleted (MND) promoter as well as eGFP off an IRES.

Cardiac Progenitor Cell Isolation, Cell culture, and Lentiviral Infection
CPCs were isolated from 10-12 week old male FVB mice and cultured in cardiac stem cell (CSC) media: DMEM F-12, 10% FBS, 1% PSG, 1X ITS (Lonza, 17-838Z), .4mg/ml EGF (Sigma, E9644), .02ng/ml bFGF (Peprotech, 100-18B), and 1000U/ml LIF (Chemicon #ESG1107). CPCs were plated in 96-well flat bottom plates and transduced with lentivirus (eGFP or nuclear AKT) at an MOI of 10 overnight. CPCs were washed 18 hours later and fresh CSC media was added. Cells were expanded and analyzed by flow cytometry to determine the percentage of eGFP+ cells. Cells were incubated with Akt inhibitor V (10mmol/L) for 7 days with and without Dexamethazone where indicated. Differentiation media consisted of aMEM, 10% FBS, 1% PSG, and 10^{-8}mol/L Dexamethazone.

Western blot:
CPCe and CPCeA were plated in 6-well dishes (50,000 cells/well), harvested in sample buffer, boiled, and sonicated before running on 4-12% Bis-Tris gels. Primary antibodies were incubated overnight at 4°C in 7% milk. Secondary antibodies were used at 1:4000 dilutions and incubated at room temperature for two hours. Membranes were washed in 1X (Tris Buffered Saline and Tween) TBST and scanned on a Typhoon.

Myocardial Infarction, Injections, Echocardiography, and Hemodynamics:
Ten to twelve week old female FVB mice were anesthetized under isoflurane, intubated, and ventilated. A thoracotomy was performed and the LAD ligated. Vehicle (n=18), CPCe (n=16), or CPCeA (n=18) 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. Sham animals (n=8) were used as controls. 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 CPCeA). Closed chest hemodynamic assessment was performed on anesthetized mice (3% Chloral hydrate, 300mg/kg) 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. Hemodynamic assessment was performed under chloral hydrate sedation and heart rate of all animals was required to be between 400-500 beats

per minute for study inclusion. As a consequence of exclusion criteria, fewer animals were analyzed in hemodynamic study compared to echocardiography which accounts for minor discrepancies between end-point results between hemodynamic and echocardiography assesments.

Histology and Embedding of heart tissue
Briefly, hearts from saline, CPCe and CPCeA injected mice were retroperfused through the abdominal aorta with potassium chloride to arrest in diastole and fixed in 10% formalin for 24-hours, after which hearts were changed to 70% ethanol. Hearts were embedded in paraffin and 5 mm sections were cut.

Deparaffinization
Three changes for 5 minutes in xylene, followed by 3 changes for 3 minutes in 100% ethanol, followed by 2 changes for 3 minutes in 95% ethanol, followed by 1 change in 70% ethanol, followed by 5 minutes in deionized water.

Antigen Retrieval
Sections were placed in plastic coplin jars containing 10mmol/L citrate buffer (pH 6.0) and heated in the microwave on full power for 3 minutes. Power setting on microwave was then changed to 50% power for and additional 12 minutes. Every 3 minutes, microwave was stopped and coplin jars containing slides were refilled with citrate buffer to keep the level above the tissue sections. Extra citrate buffer was heated in the microwave alongside slides. Slides were allowed to cool and washed 3 times in deionized water, followed by equilibration in 1X Tris-NaCl (TN) buffer (150mM NaCl/100mM Tris pH 7.5). Proceed to blocking step (if not quenching) with Tris-NaCl-Blocking buffer (TNB) (.1M Tris, pH 7.5+.15M NaCl + .5% Blocking reagent from Perkin Elmer catalog # FP1020).

Quenching
Quench endogenous tissue peroxidase activity in 3% H2O2/1X TN for 20 minutes.
Wash again in three changes for 3 minutes each of 1X TN. Proceed to blocking.

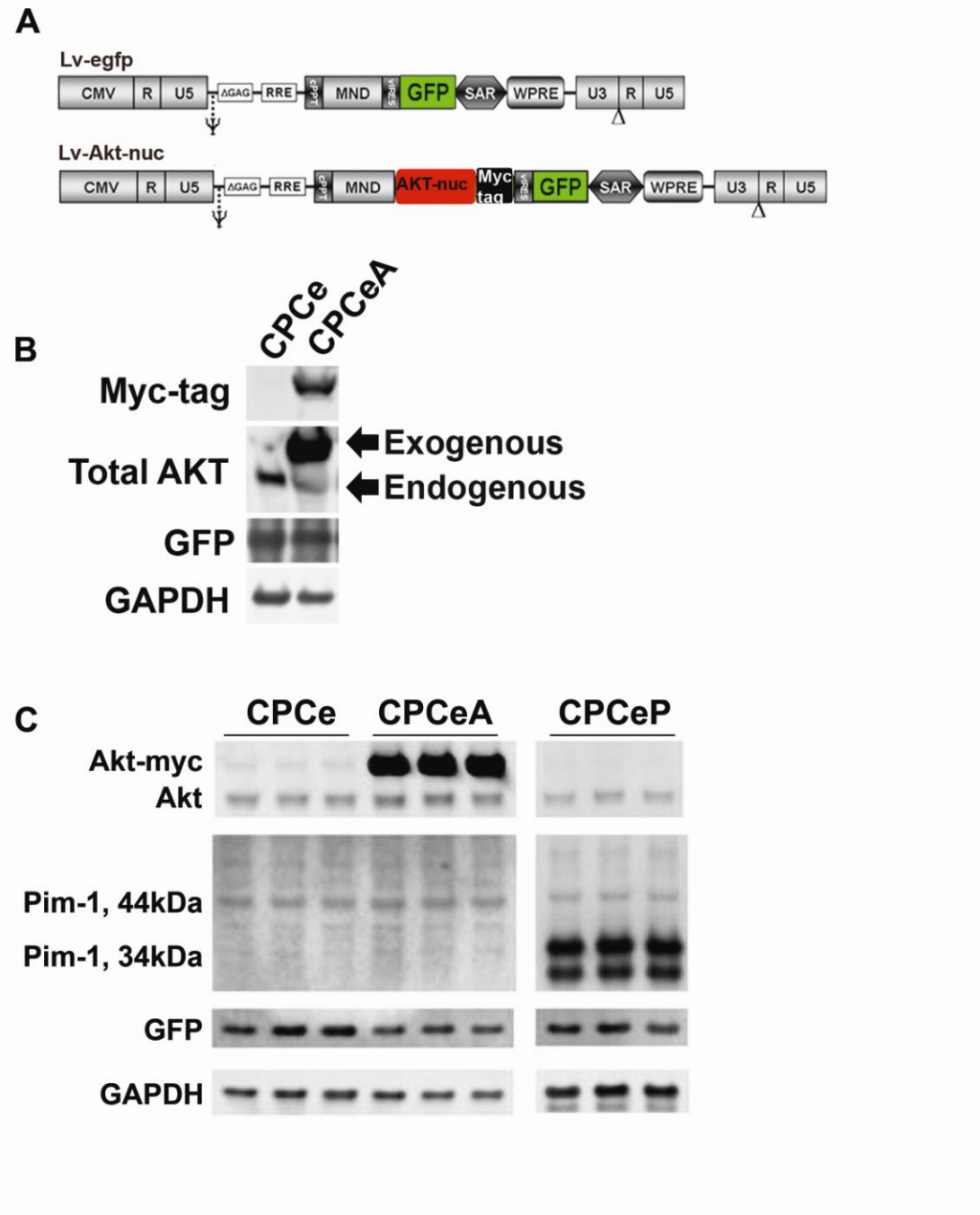
Immunohistochemistry

Application	Antibody	Dilution	Amplify	Company
ICC	c-kit	1:50	no	R&D systems
ICC	GFP	1:500	no	Molecular Probes
IHC	GFP	1:500	yes	Molecular Probes
IHC	c-kit	1:50	yes	R&D systems
IHC	Desmin	1:300	no	Abcam
IHC	Topro-3-iodide	1:300	no	Molecular Probes

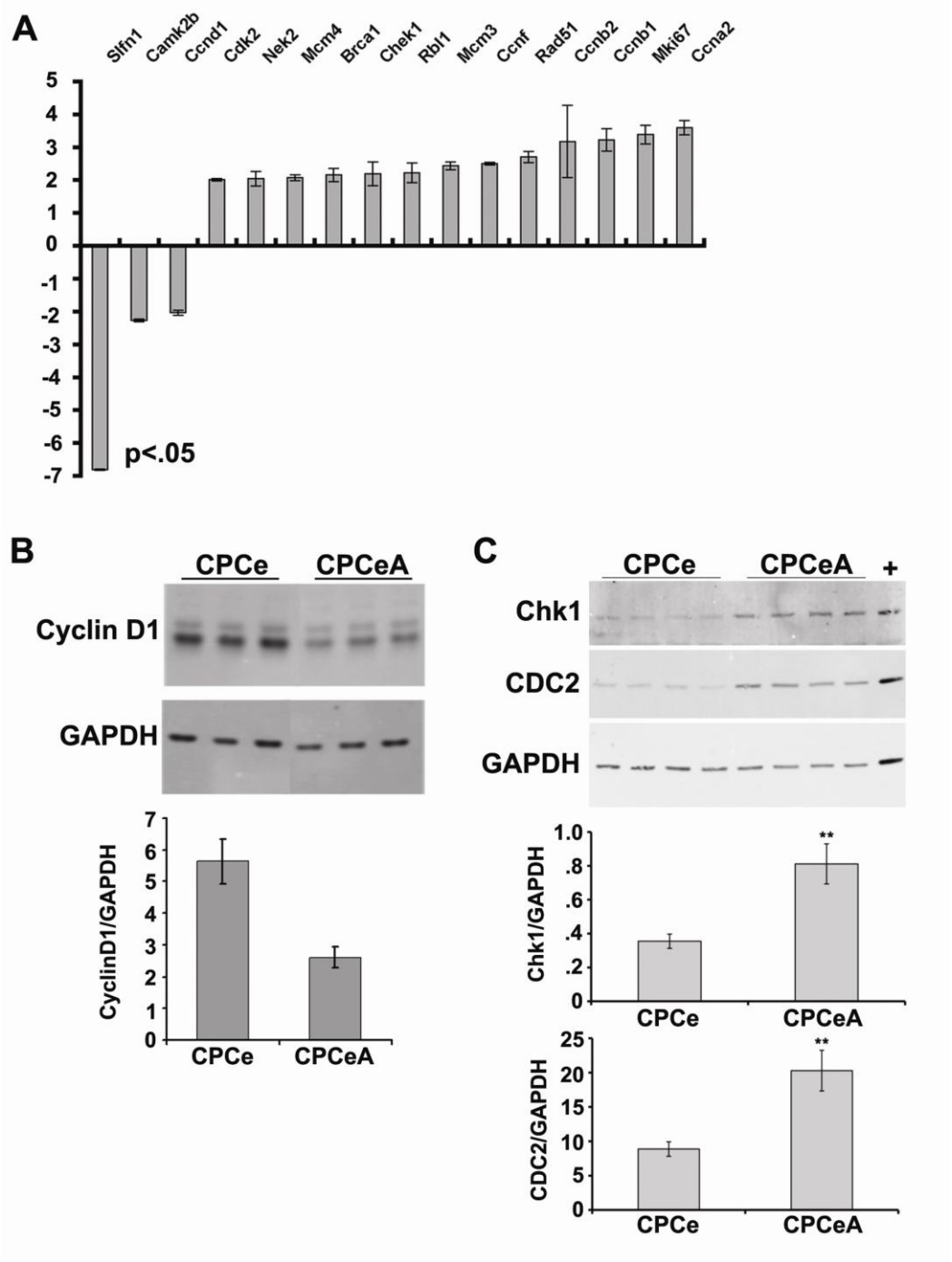
qRT-PCR primer sequences

Primer Name	Forward/ Reverse	Sequence
Troponin T (TnT)	Forward	ACCCTCAGGCTCAGGTTCA
Troponin T (TnT)	Reverse	GTGTGCAGTCCCTGTTCAGA
Fibroblast Growth Factor-2 (FGF-2)	Forward	AAGAGCGACCCACACGTCAAACTA
Fibroblast Growth Factor-2 (FGF-2)	Reverse	TTCATAGCAAGGTACCGGTTGGCA
Follistatin like protein-1 (Fst-1)	Forward	TGTGGTCCTTAGCTGCTCCTGTTT
Follistatin like protein-1 (Fst-1)	Reverse	TCTGGACCAGTTGGCTAAACCACA
Stromal Derived Factor-1 (SDF-1)	Forward	ACCCAAATGCAAAGGCTGAGTGTG
Stromal Derived Factor-1 (SDF-1)	Reverse	AGCTAAGCACTGTTGCAAACCACC
Vascular Endothelial Growth Factor (VEGF)	Forward	TGAGCTTCCTACAGCACAGCAGAT
Vascular Endothelial Growth Factor (VEGF)	Reverse	TTACACGTCTGCGGATCTTGGACA
18S	Forward	CCCCCTCGATGCTCTTAGCT
18S	Reverse	GGGCCTGCTTTGAACACTCTA

Supplemental Figures:

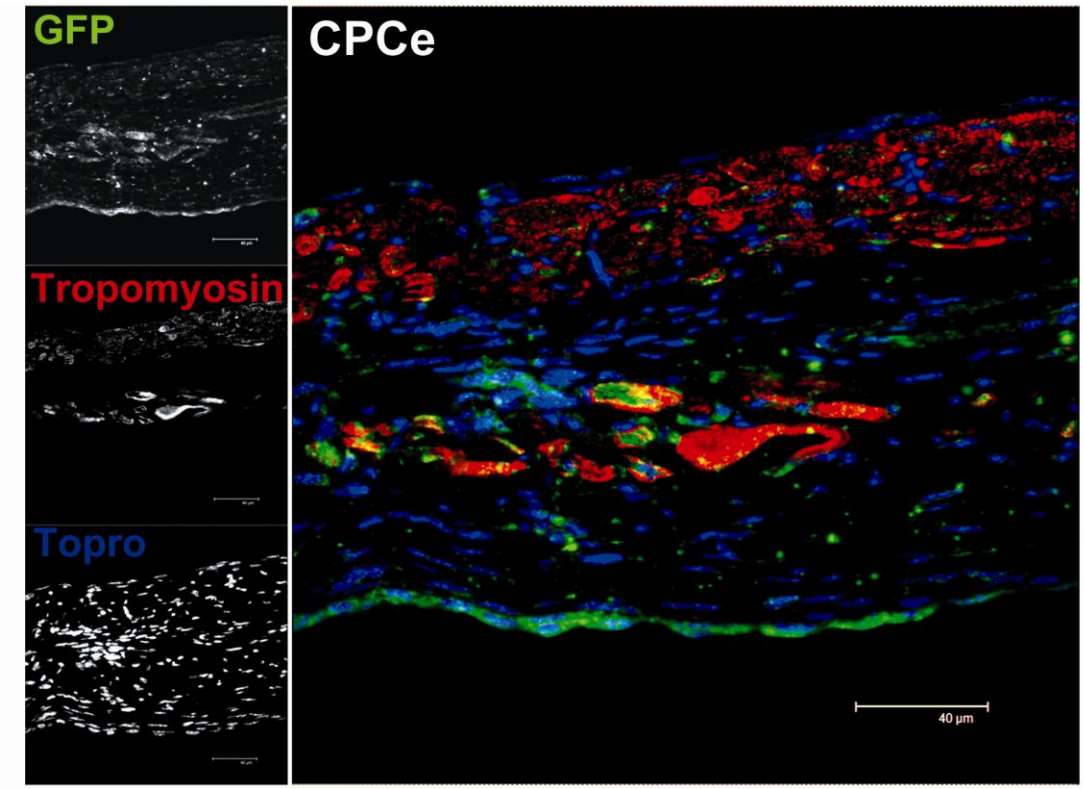


Supplemental Figure I: Overexpression of Nuclear Akt in CPCs
(A) Self-inactivating lentiviral vectors, termed Lv-egfp (GFP control) and Lv-Akt-nuc. (B) Representative immunoblot of CPCe and CPCeA, immunolabeled for myc-tag, Akt1/2, GFP, and GAPDH. (C) Representative immunoblot of CPCe and CPCeA, and CPCeP immunolabeled for Pim-1, Akt1/2, GFP, and GAPDH.

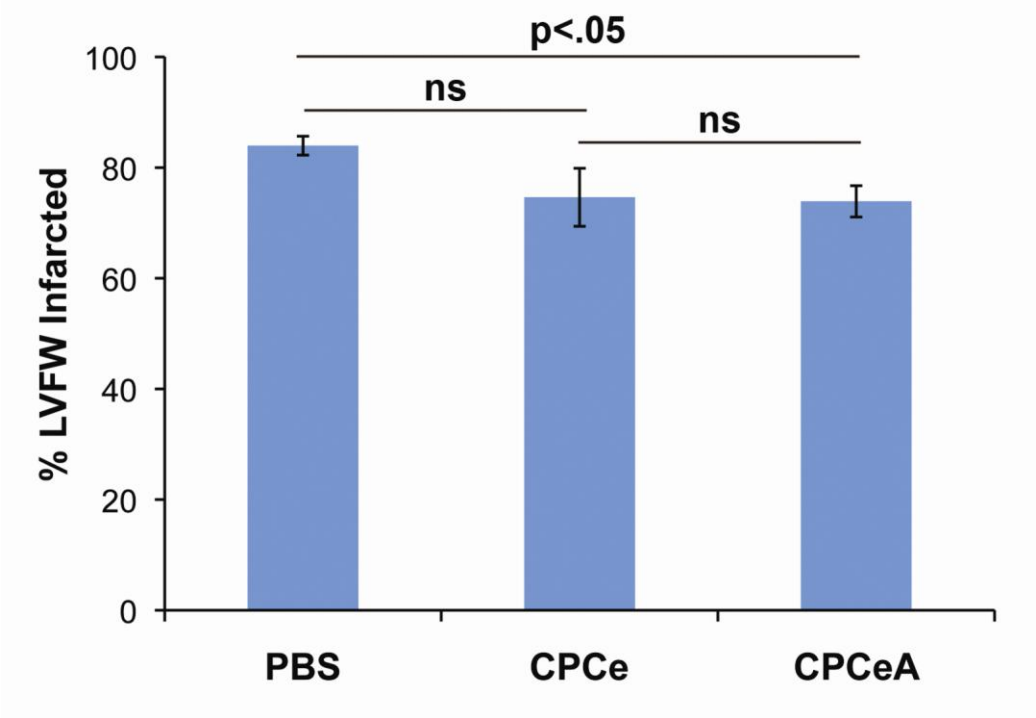


Supplemental Figure II: Nuclear Akt modified CPCs mediate gene expression of cell cycle proteins

(A) Total mRNA was extracted from CPCe (n=3) and CPCeA (n=3) and run on a cell cycle RT2 Profiler Cell Proliferation Array from SuperArray in triplicate. CPCeA and CPCe were normalized to GAPDH. Samples analyzed had ≥2-fold difference from control, with $p<0.05$, (mean ± SEM, n=3). (B-C) Protein expression of Cyclin D1 (B), Chk1 and CDC2 (C) were examined in CPCe and CPCeA (mean ± SEM). *p<.05, **p<.01 compared to CPCe.

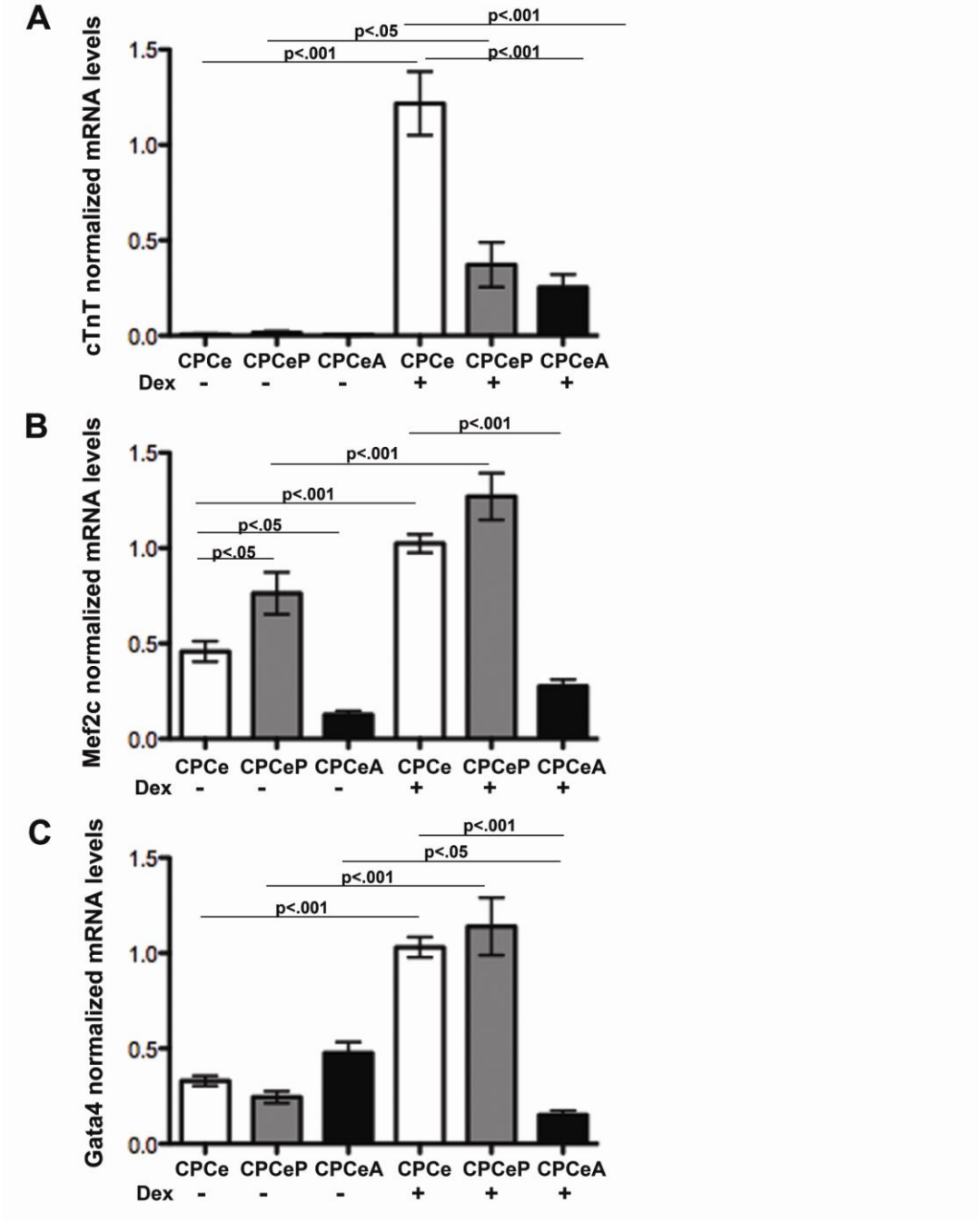


Supplemental Figure III: CPCe acquire markers of cardiac lineage commitment
Hearts from infarcted animals injected with control CPCe were immunostained with GFP (green), Tropomyosin (red) and topro-3-iodide (blue) to mark nuclei.

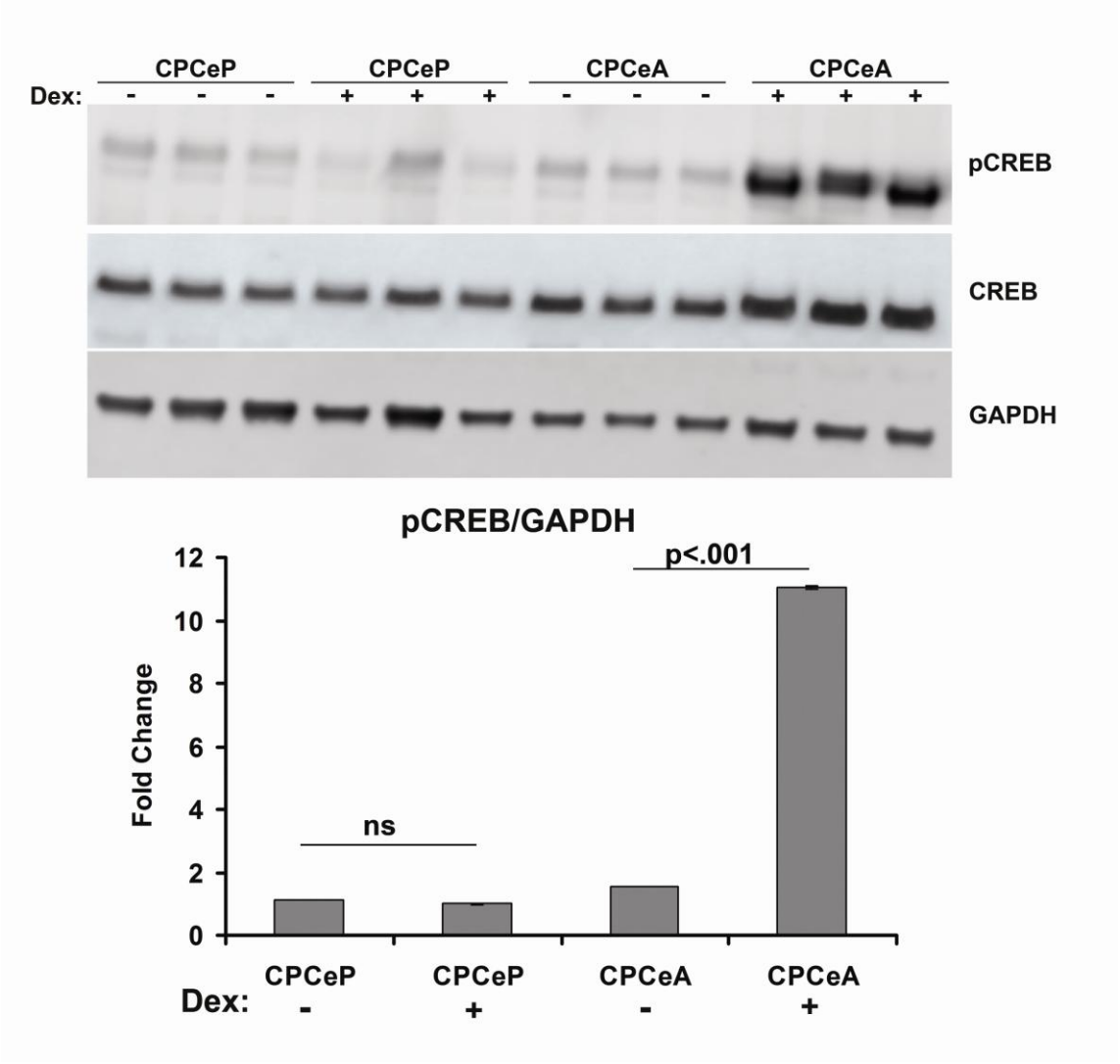


Supplemental Figure IV: CPCeA and CPCe injected hearts have comparable infarct size

Quantitation of infarction area in vehicle (n=3), CPCe (n=3), and CPCeA (n=4) treated hearts 12-weeks post injection (mean ± SEM).



Supplemental Figure V: CPCeA abrogate lineage commitment compared to CPCeP
Transcript levels of cardiac TnT (A), Mef2C (B), and Gata4 (C) were assessed by quantitative real time PCR in CPCe, CPCeP and CPCeA treated with and without Dex.



Supplemental Figure VI: CPCeP do not overexpress phosphorylated CREB after *in vitro* differentiation

Immunoblot and quantitation of phosphorylated CREB in CPCeP and CPCeA treated with and without Dex for seven days.