SUPPLEMENTAL METHODS

Isolation and culture of cardiac progenitor cells from human hearts. Human fetal tissue and atrial biopsies were obtained with approval by the Stanford IRB committee. The human cardiac progenitor cells (hCPCs) were isolated based on a previously described protocol using magnetic separation in conjunction with a Sca-1 antibody [1-3]. Human fetal hearts were subjected to Langendorff perfusion with Tyrode's solution containing collagenase and protease. Atrial biopsies were minced into small pieced followed by collagenase treatment. After cardiomyocyte depletion of the cell suspension, hCPCs were isolated by magnetic cell sorting (MACS, MiltenylBiotec, Sunnyvale, CA) using Sca-1-coupled magnetic beads, according to the manufacturer's protocol. Sca-1^+ cells were eluted from the column by washing with PBS supplemented with 2% FBS and cultured on 0.1% gelatin-coated dishes in M199 (Gibco)/EGM-2 (3:1) supplemented with 10% FCS (Gibco), 10 ng/ml basic fibroblast growth factor (bFGF), 5 ng/ml epithelial growth factor (EGF), 5 ng/ml insulin-like growth factor (IGF-1), and 5 ng/ml hepatocyte growth factor (HGF).

Flow cytometry analysis. Cultured hCPCs were trypsinized and 300,000 cells were used for fluorescence activated cell sorting (FACS) analysis. The cells were washed twice in wash buffer (1% FBS/PBS/0.05M azide) and resuspended in 100 µl wash buffer containing antibody. The cells were incubated on ice in the dark for 30 minutes, washed four times with cold wash buffer, resuspended in 250 µl wash buffer, and analyzed using a Beckman Coulter Cytomics FC500 FACS. Antibodies used were fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated against CD14, CD34, CD45, CD133, CD105 (endoglin), Sca-1, and isotype control IgGs, all from Pharmingen BD.

Lentiviral vector construction. Schematic of lentiviral constructs used in this study are presented in Figure 1A. Lentiviral vectors were cloned used the Enzyme Free Lentivector Kit (System Biosciences, Menlo Park, CA). Four primers were designed for each TK reporter gene to be cloned. Primer 1 (forward) and 2 (reverse) were for amplification. Primers 3 and 4 contained the adaptor sequences GAGGCAGCAGAGACCG and CGAACAGAGAGAGACCG plus the sequence of primers 1 and 2, respectively. The wt-tk and sr39-tk cDNA sequences were kindly provided by Dr. Sanjiv Gambhir (Stanford University School of Medicine) [4]. ΔhTK2 and A168H cDNA sequences were kindly provided by Dr. Juri Gelovani (University of Texas, MD Anderson Cancer Center) [5, 6]. The lentivirus was produced by transfecting 293T packaging cells with Clone-it™ Enzyme free Lentivectors using Lipofectamine 2000 (Invitrogen) in 100-mm dishes. Two 3-dimensional supernatants were collected. The collected supernatant was filtered using Amicon Ultra-15 centrifugal filter devices (Millipore, County Cork, Ireland), and stored at -80ºC.

Quantitative polymerase chain reaction (q-PCR) assay. The transduced hCPCs were analyzed by Quantitative PCR using an ABI StepOnePlus® instrument (Applied Biosystems). The primer pairs which were used to detect puromycin and endogenous standard glyceraldehydes-3-phosphate dehydrogenase (GAPDH) are as followed: 5'-ACCGAGTACAAGCCCACG-3' (PURO-F), 5'-TTGCCGATGTCGAGCC-3' (PURO-R), 5'CCATGGAGAAGGCTGGGG-3'(GAPDH-F), and 5'CAAAGTTGTCATGGATGACC-3' (GAPDH-R). Expression of puromycin was normalized to GAPDH, and results were expressed as change in C_t (ΔC_t) values. PCR reactions were performed on the ABI 7900HT system.

Effect of reporter gene expression on hCPC viability, proliferation, and differentiation potential. The effect of introducing the various TK reporter genes on hCPC viability and proliferation was tested using the Trypan blue exclusion assay and the DHL cell proliferation assay kit (Anaspec), respectively. To test differentiation potential, hCPCs were cultured in the presence of cell culture media supplemented with specific differentiation factors. To induce differentiation into cardiomyocytes, cells were treated with 5 μM 5-azacytidine (Sigma) for 72 hours in differentiation medium (Iscove's Modified Dulbecco's Medium/Ham's F12 (1:1) (Gibco)) supplemented with L-glutamine (Gibco), 2% horse serum, nonessential amino acids, insulin-transferrin-selenium supplement, and 10-4 M ascorbic acid (Sigma). After induction, the medium was changed every three days. For smooth muscle differentiation, CPCs were cultured in poly-D-lysine coated plates in differentiation medium containing 35% Iscove's modified Dulbecco's medium with 10% FBS/65% Dulbecco's modified Eagle medium-Ham F-12 mix containing 2% B27, 0.1 mmol/l 2-mercaptoethanol, 10 ng/ml epidermal growth factor (R&D Systems, Minneapolis, MN), 20 ng/ml basic fibroblast growth factor, 40 nmol/l cardiotrophin-1, 40 nmol/l thrombin (Sigma-Aldrich, St. Louis, MO), 100 U/ml penicillin G, 100 g/ml streptomycin, and 2 mmol/l glutamine. For endothelial differentiation, hCPCs were cultured on fibronectin-coated plates with EGM-2 medium (Lonza, Switzerland) with an extra 20 ng/ml of VEGF (R&D Systems). Then the cells were fixed with 4% paraformaldehyde and processed for immunofluorescence as previously described [7].

***In vitro* [^{18}F]-FHBG accumulation assay.** TK-expressing hCPCs were seeded in 150-mm tissue culture dishes (Falcon) at a concentration of 1×10^6 cells per dish in triplicate and grown until 50%-60% confluent. Then the medium was replaced with 14 mL of medium containing F-18-9-

(4-fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]-FHBG) radiotracer (92.5 kBq/mL at time 0). The cells were subsequently incubated for 15, 30, 60, and 120 min before the monolayers were scraped, transferred into 15-mL tubes, and centrifuged at 1,000 × g for 2 min. A 100 µl aliquot of supernatant was transferred to a pre-weighed scintillation tube, and the rest was removed by aspiration before the cell pellet was snap-frozen on dry ice. The frozen pellets were transferred to pre-weighed scintillation vials, weighed, and thoroughly resuspended in 0.5 mL of Soluene-350 (Perkin Elmer). Radioactive γ-emissions of the medium and the cell pellets were measured on a γ-counter (Cobra Quantum; Packard) to quantify [^{18}F]-FHBG uptake. Activity ratios of the cell pellet to medium ([dpm/g cells]/[dpm/g medium]) were calculated and plotted against time.

Microarray hybridization and data analysis. Total RNA samples were hybridized to Affymetrix GeneChip Human Gene 1.0 ST Arrays, and then normalized and annotated by the Affymetrix® Expression Console™ software. The Pearson Correlation Coefficient was calculated for each pair of samples using the expression level of transcripts which showed a standard deviation of greater than 0.2 among all samples. For hierarchical clustering, we used Pearson correlation for average linkage clustering.

***In vivo* optical bioluminescence imaging (BLI)**. Bioluminescence was measured at day 1, day 7, day 14, day 21 and day 28 using an In Vivo Imaging System 50 (IVISTM; Caliper, Hopkinton, MA) at 37°C. Mice were injected (150 mg/kg body weight, i.p.) with D-luciferin (Caliper) immediately prior to anesthesia. Mice were anesthetized with 2% isoflurane and imaged at 5-min exposure times. Bioluminescent signals were analyzed using Living Image® 2.50.2 software (Caliper) to identify regions of interest, quantitate light emission, and subtract

background luminescence.

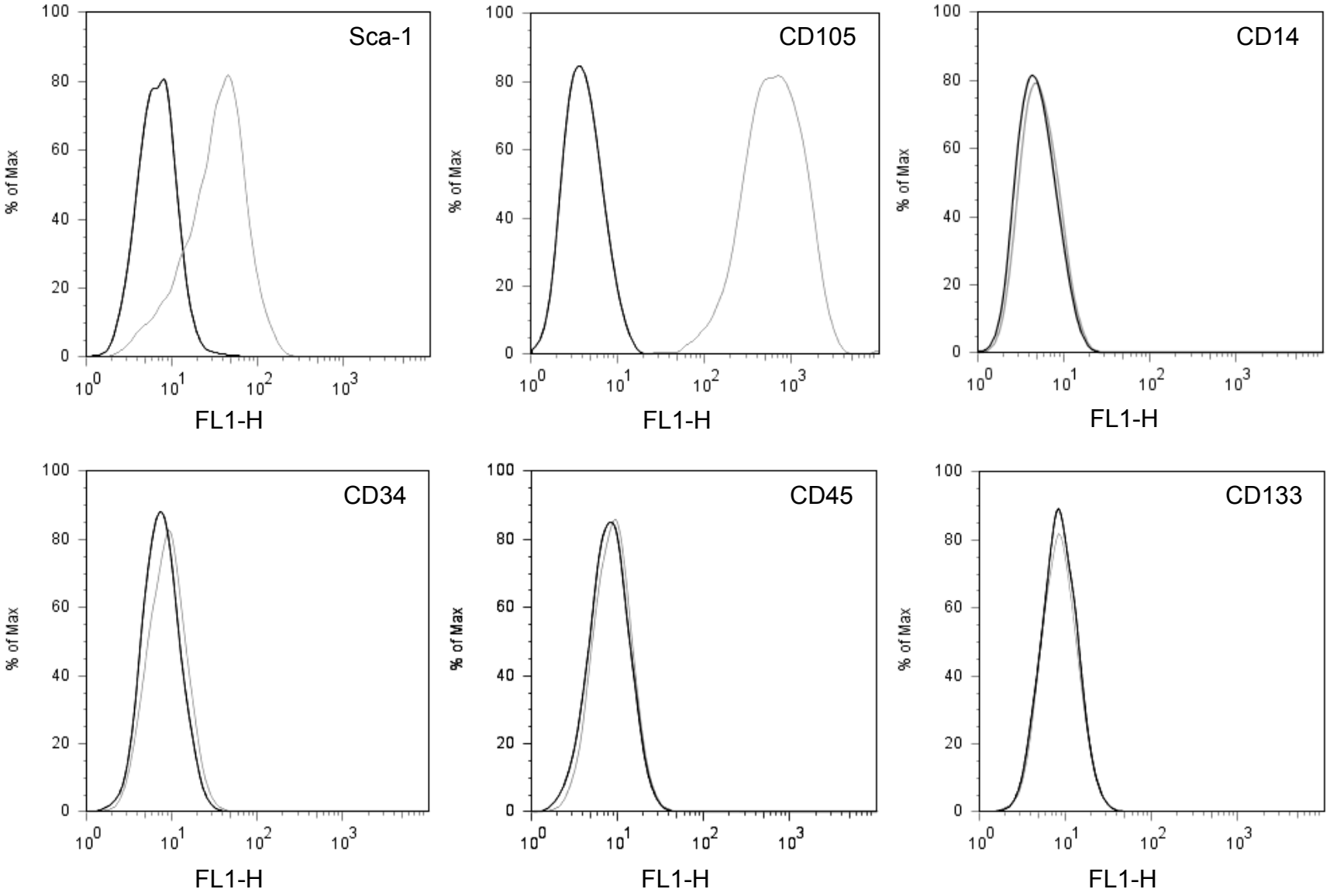
Analysis of left ventricular function with echocardiogram and magnetic resonance imaging (MRI). Echocardiography was performed before (day -7) and after (day 2, week 1, week 2, week 3 and week 4) the LAD ligation. The Siemens-Acuson Sequoia C512 system equipped with a multi-frequency (8-14 MHZ) 15L8 transducer was used by an experienced operator (D.N.) blinded to the group designation. Analysis of M-mode images was performed using Siemens built-in software. Left ventricular contractility was assessed by measuring left ventricular end-diastolic diameter (EDD), end-systolic diameter (ESD), and volume blood at end of diastole (BED), and calculating left ventricular fractional shortening by the formula: LVFS = [EDD-ESD]/BED. The MRI imaging was performed on a Sigma 3.0T Excite HD scanner (GE Healthcare Systems, Milwaukee, Wisconsin) with a Mayo Clinic T/R MRI coil (Mayo Clinic Medical Devices, Rochester, Minnesota) in a subset of animals (n=5/group). Mice were anesthetized with 2% isoflurane with oxygen (1 L/min) and placed in the prone position for imaging. A small animal electrocardiogram and respiratory gating system (Small Animal Instruments, Stony Brook, New York) was used to acquire images as previously described [8].

Analysis of left ventricular function with pressure-volume (PV) loops. At the end of the study (week 8), invasive hemodynamic measurements were performed in a subset of the animals (n = 10/group). Briefly, after midline neck incision, a 1.4 conductance catheter (Millar Instruments, Houston, Texas) was introduced into the left ventricle through the right carotid artery. After stabilization, the signals were continuously recorded at a sampling rate of 1,000/s using PV conductance system coupled to a PowerLab/4SP analog to digital converter (AD

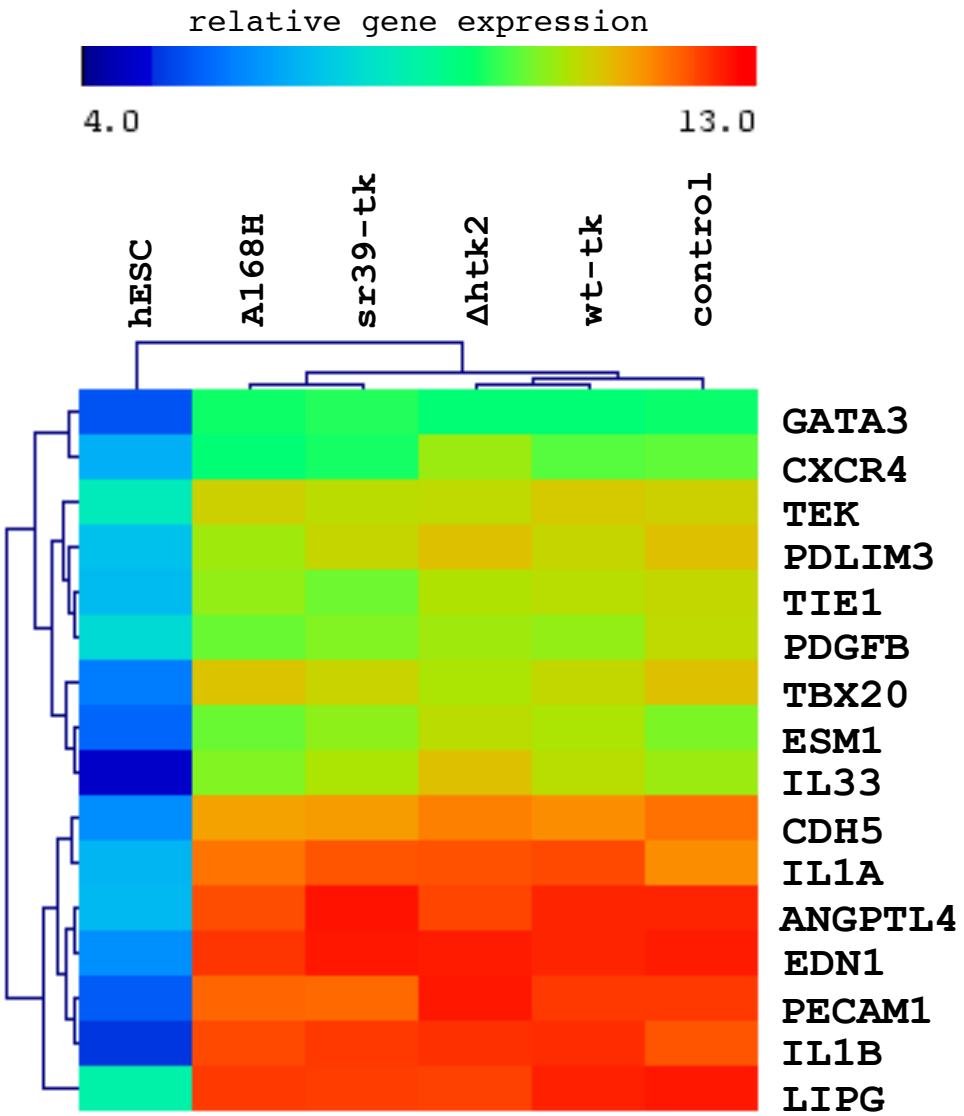
Instruments, Colorado Springs, Colorado). Data were analyzed by using a cardiac PV analysis program (PVAN 3.4, Millar Instruments) and Chart/Scope Software (AD Instruments).

***In vitro* expression of cytokine proteins by hCPCs after exposure to hypoxia.** hCPCs grown in culture were subjected to a 48 hr hypoxia treatment (5% O_2), after which the supernatant was collected and exposed to the angiogenesis cytokine array (Panomics) according to the manufacturer's instructions. In three separate experiments (each containing duplicate spots), the arrays were hybridized and imaged together. Expression intensities were calculated by adding the total pixel intensity for each spot. Normalization within each array was achieved using positive control spots (8 per array). Protein expression levels were compared between hCPCs grown under normoxic and hypoxic conditions.

Histological examination. Mice were sacrificed and left ventricular (LV) tissue was obtained at 4 weeks after MI. Tissue samples were embedded into OCT compound (Miles Scientific, Elkhart, IN). Frozen sections (5 μm thick) were processed for immunostaining. Anti-cardiac Troponin T antibody (Thermo Scientific) and anti-GFP antibody (Thermo Scientific) were used. To quantify the left ventricle (LV) infarct size, H&E staining was performed in each group (n=5/group). For each heart, eight to ten sections from apex to base (1.2 mm apart) were analyzed. Images were taken for each section to calculate the fibrotic and non-fibrotic areas as well as ventricular and septal wall thickness. The NIH Image J software was used to quantify the infarct zones.



Supplemental Figure 1



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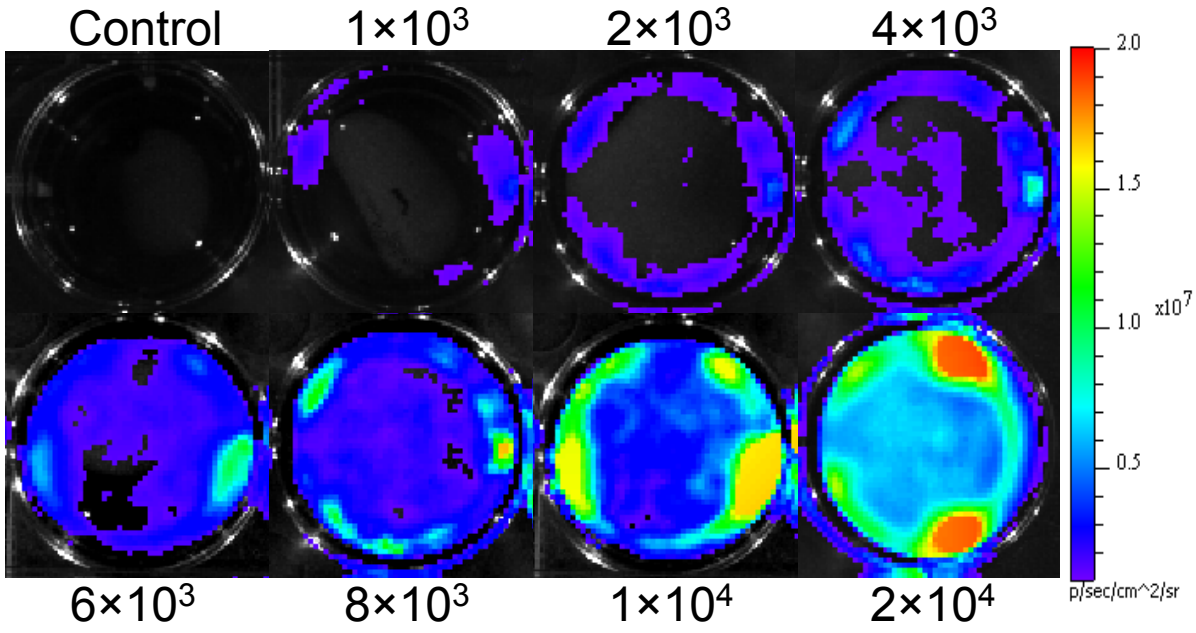

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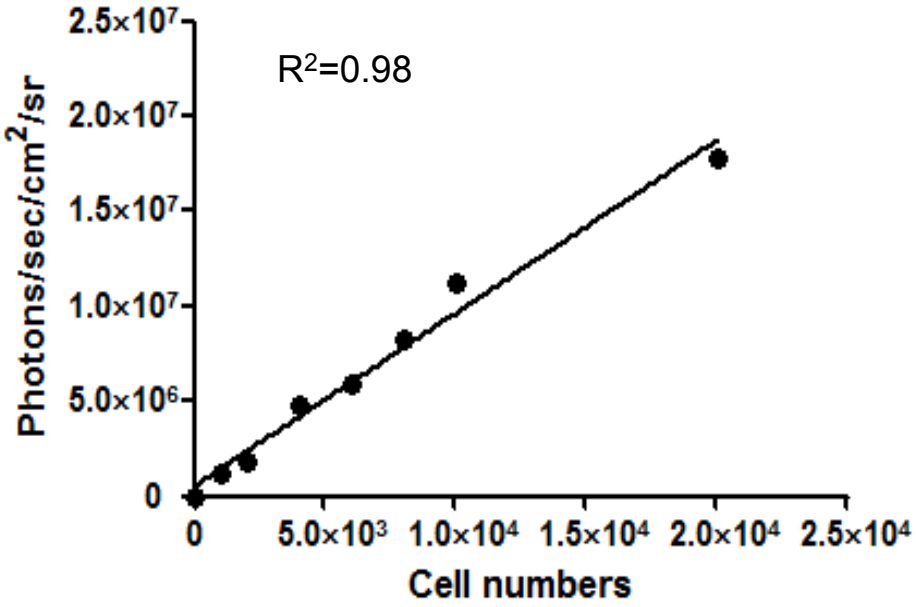

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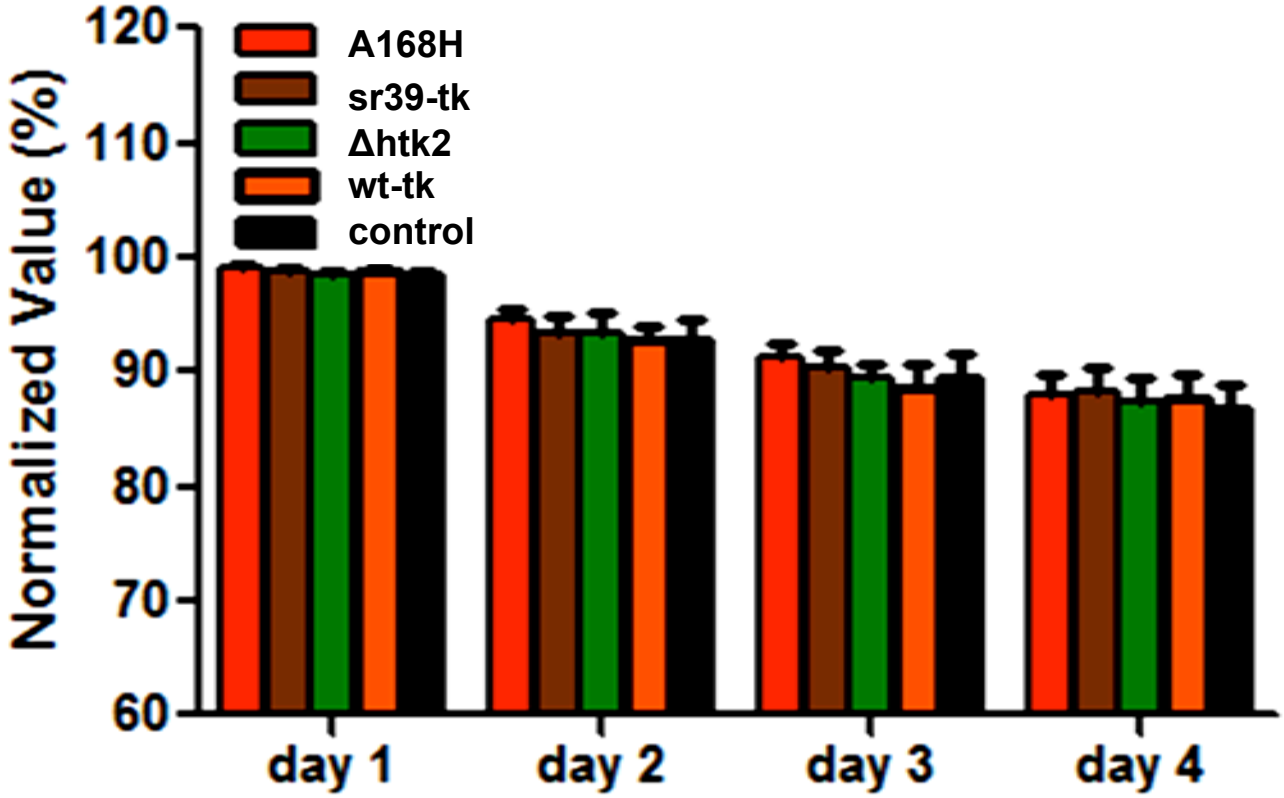

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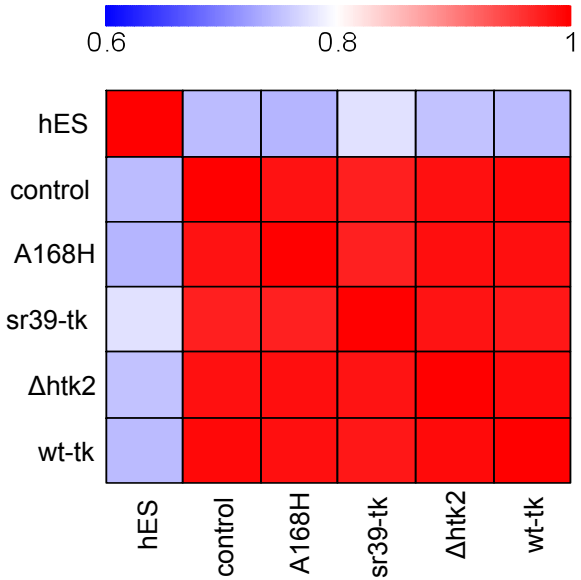

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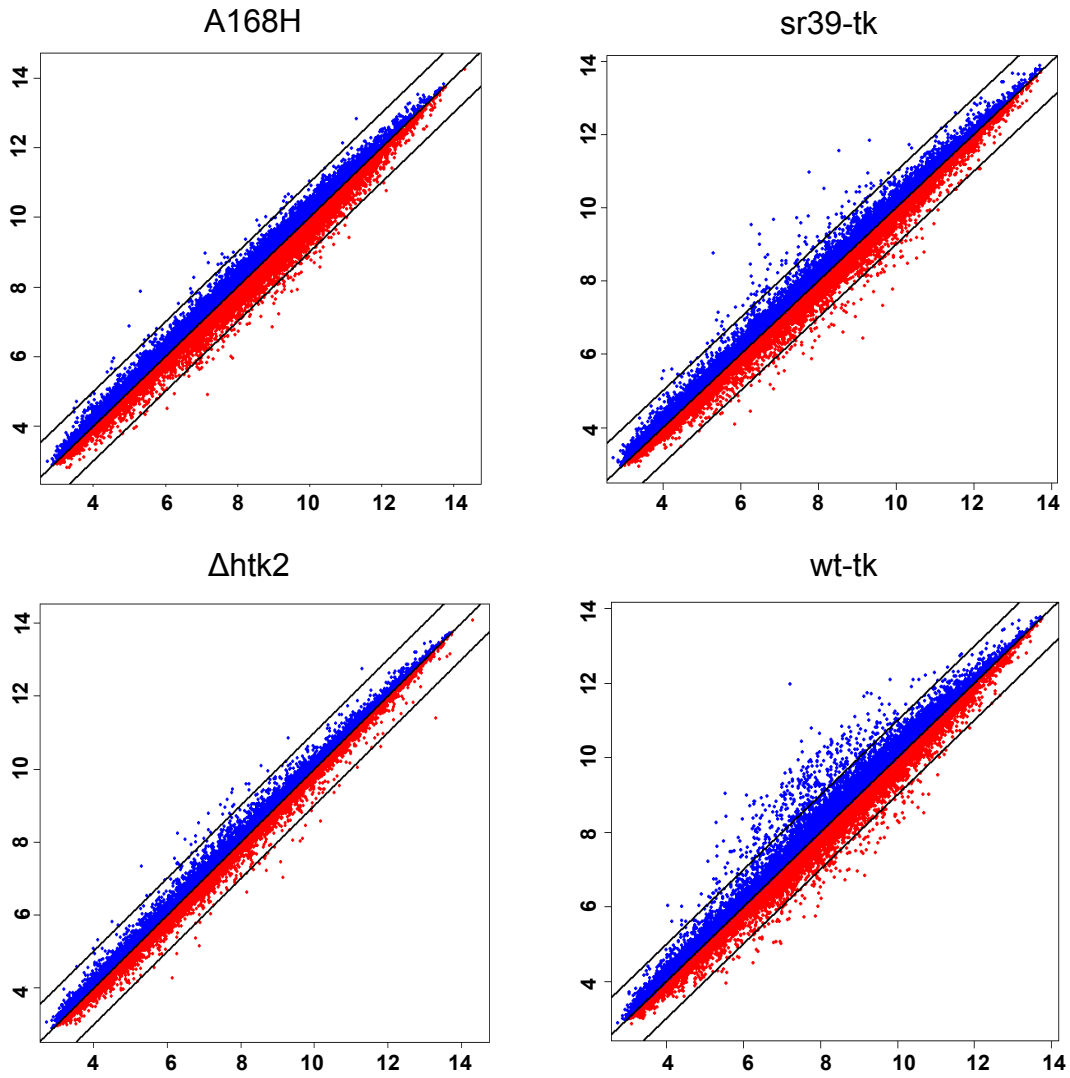

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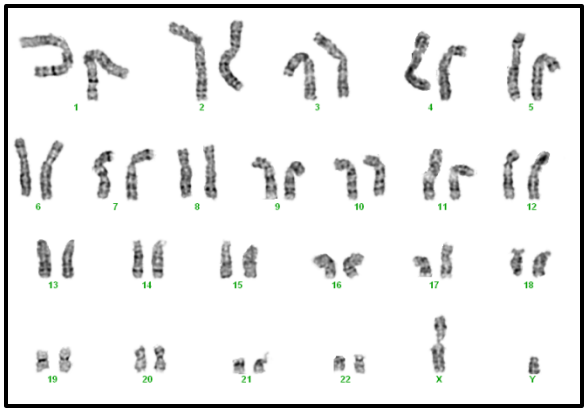

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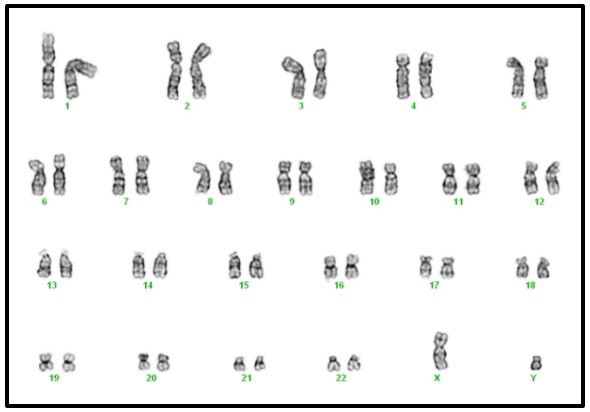

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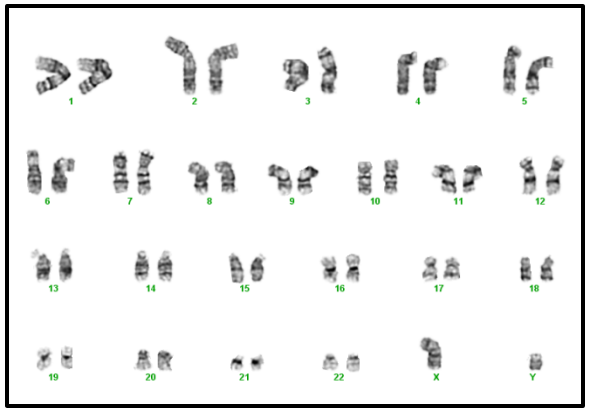

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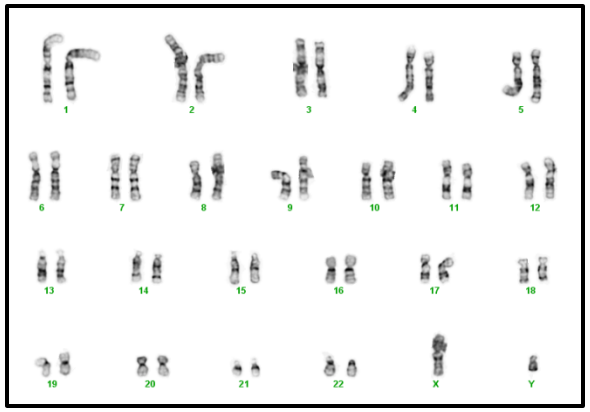

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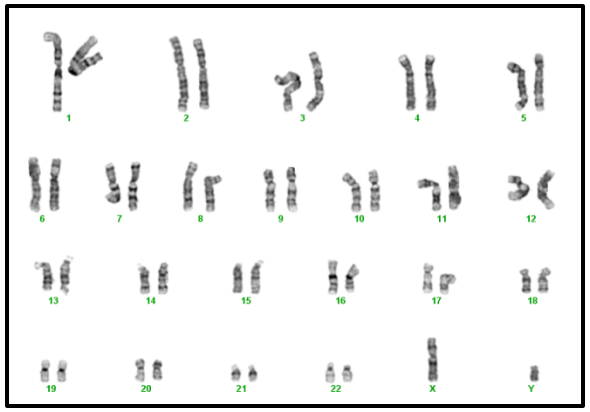
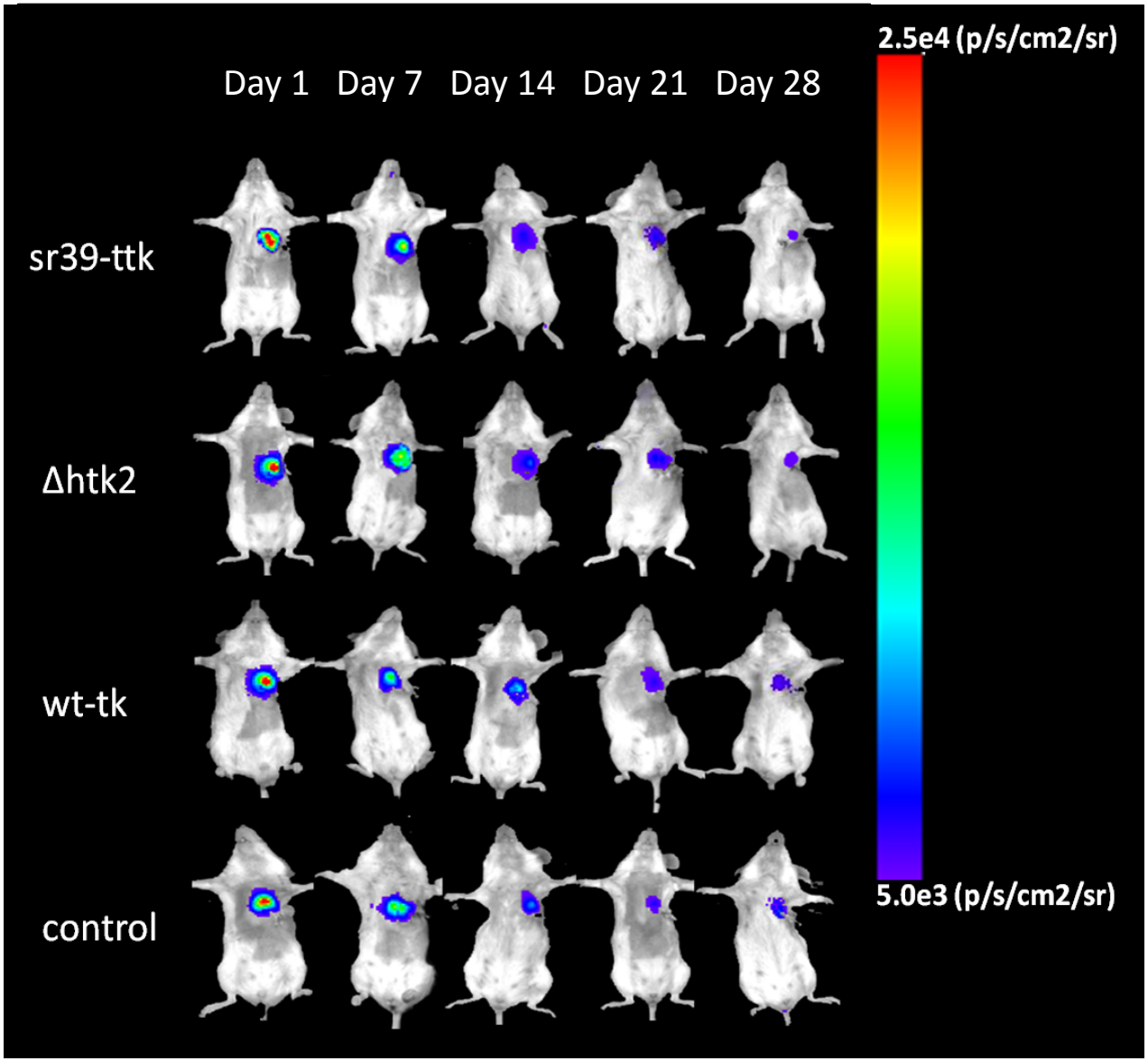


wt-tk

Supplemental Figure 6



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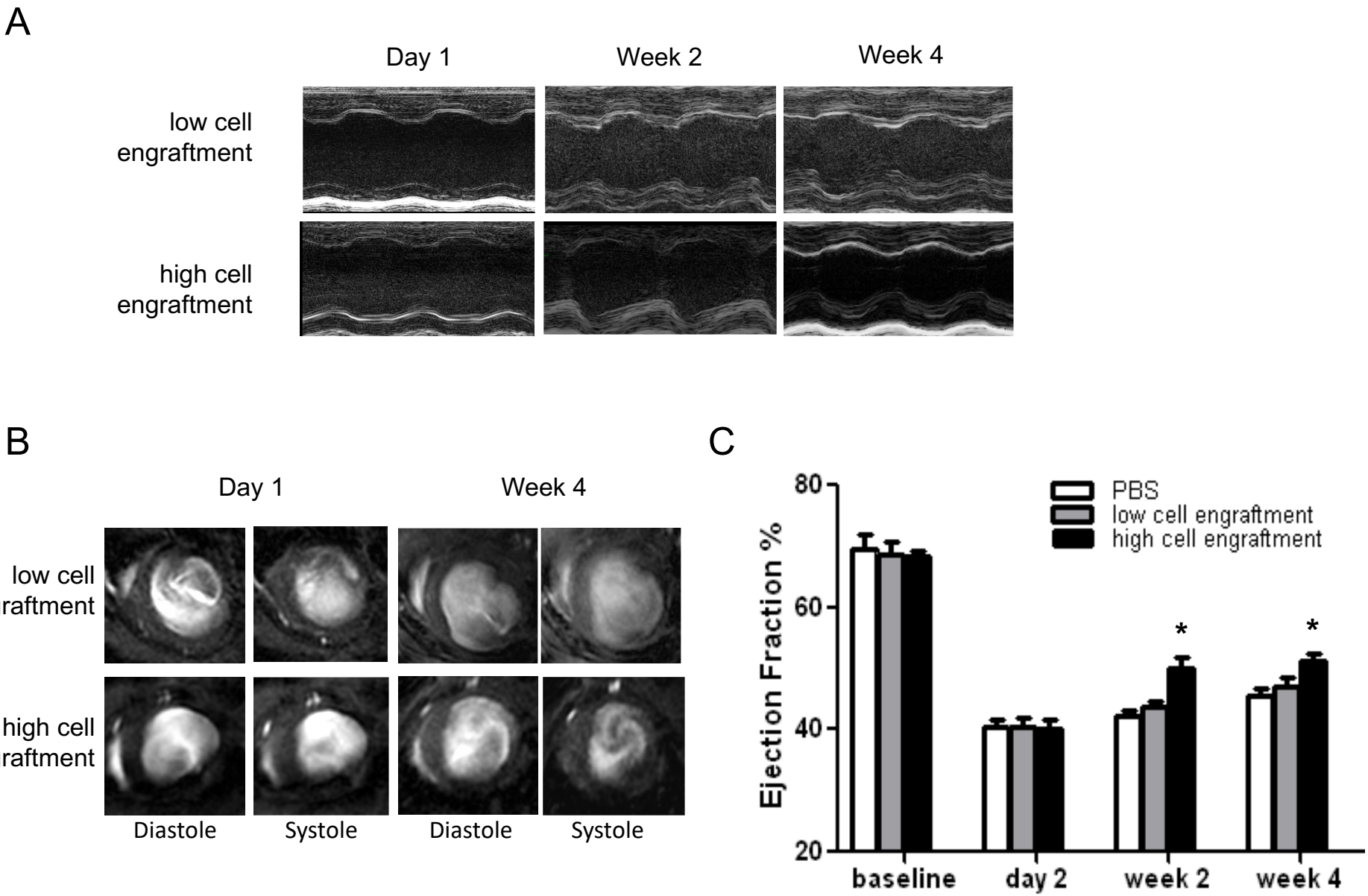

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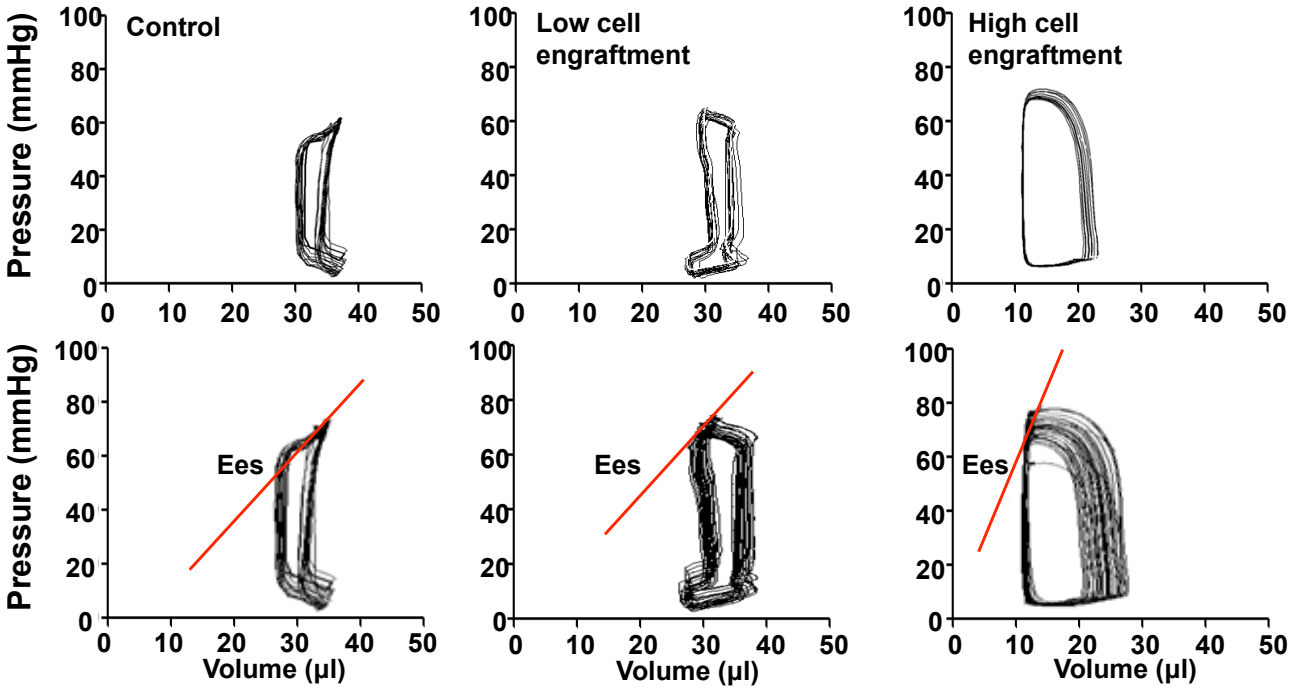

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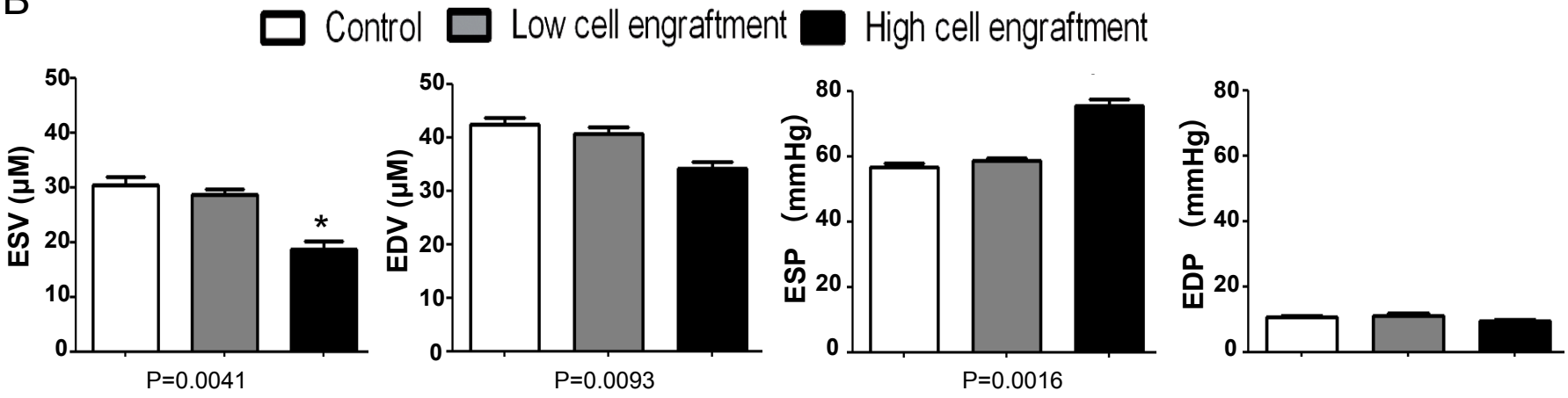

Supplemental Figure 9

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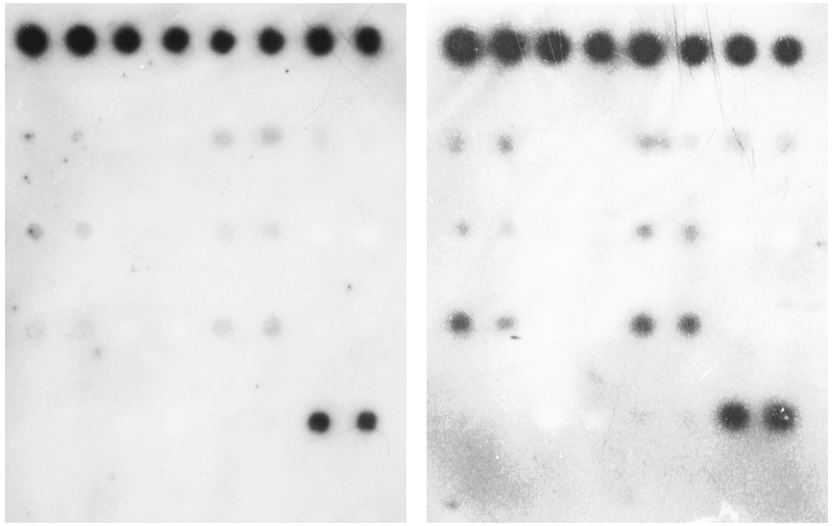
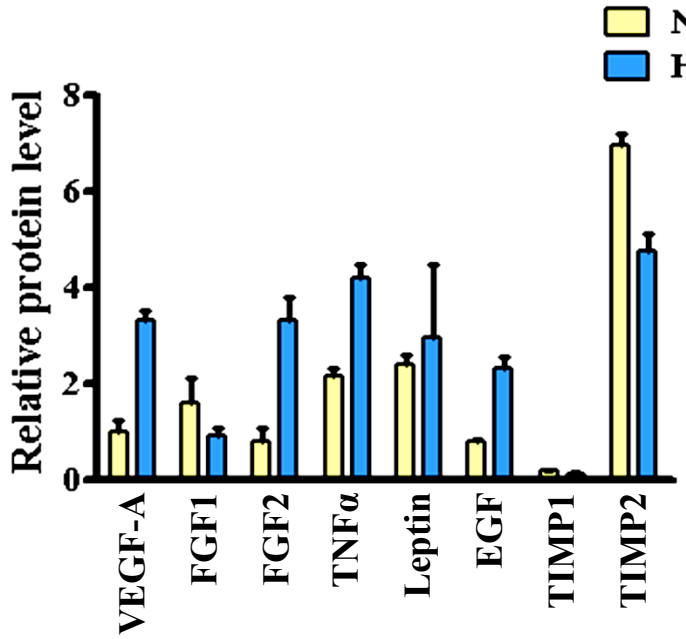


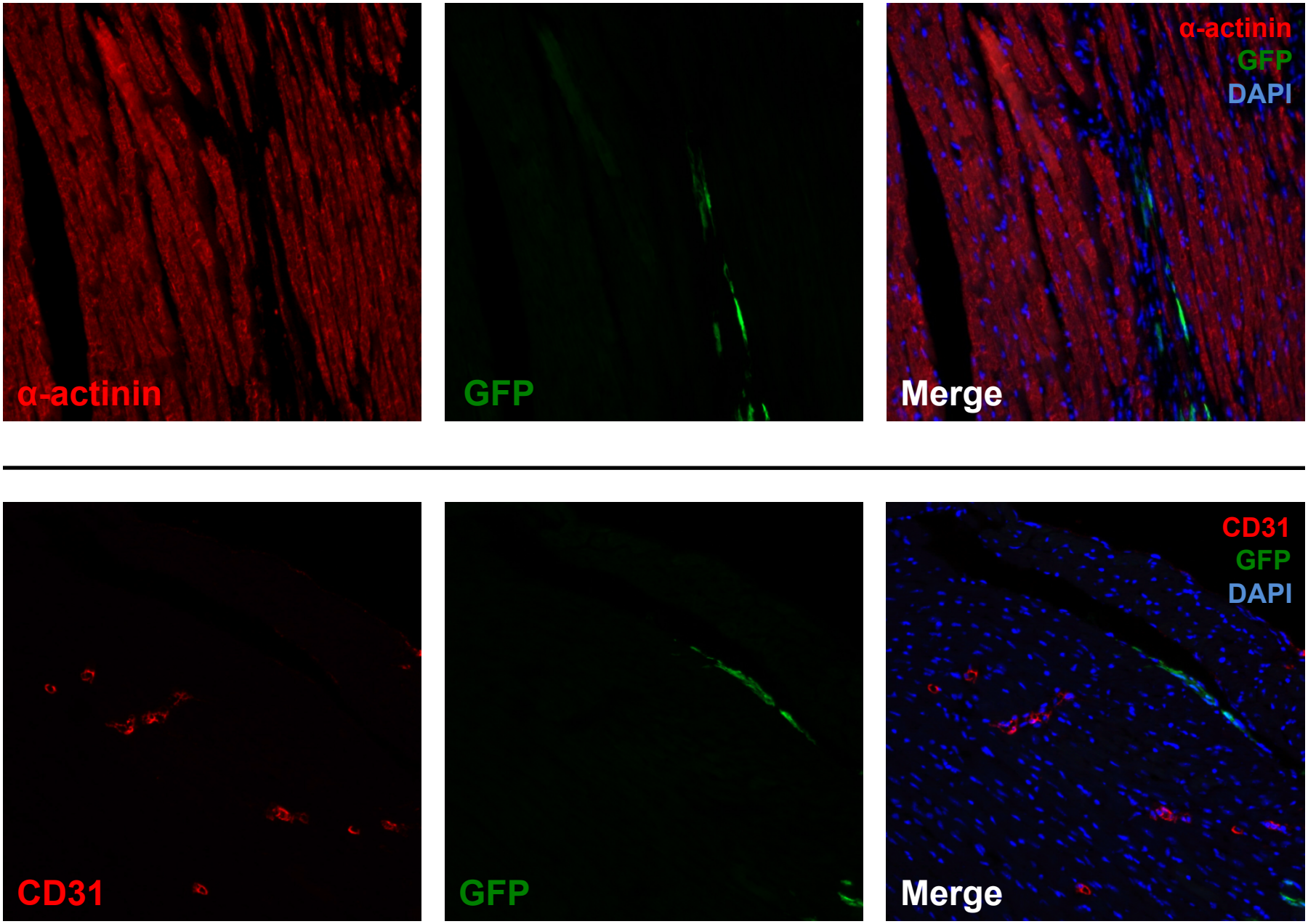
	1 2	3 4	5 6	7 8
A	pos	pos	pos	pos
B	EGF	IL-1-alpha	Leptin	IFN-gamma
C	FGFa	IL-1beta	VEGF	IL-12
D	FGFb	IL-4	TNF-alpha	IP-10
E	G-CSF	IL-6	TGF-alpha	TIMP-1
F	Neg	Neg	TGF-beta	TIMP-2

Activators — Inhibitors

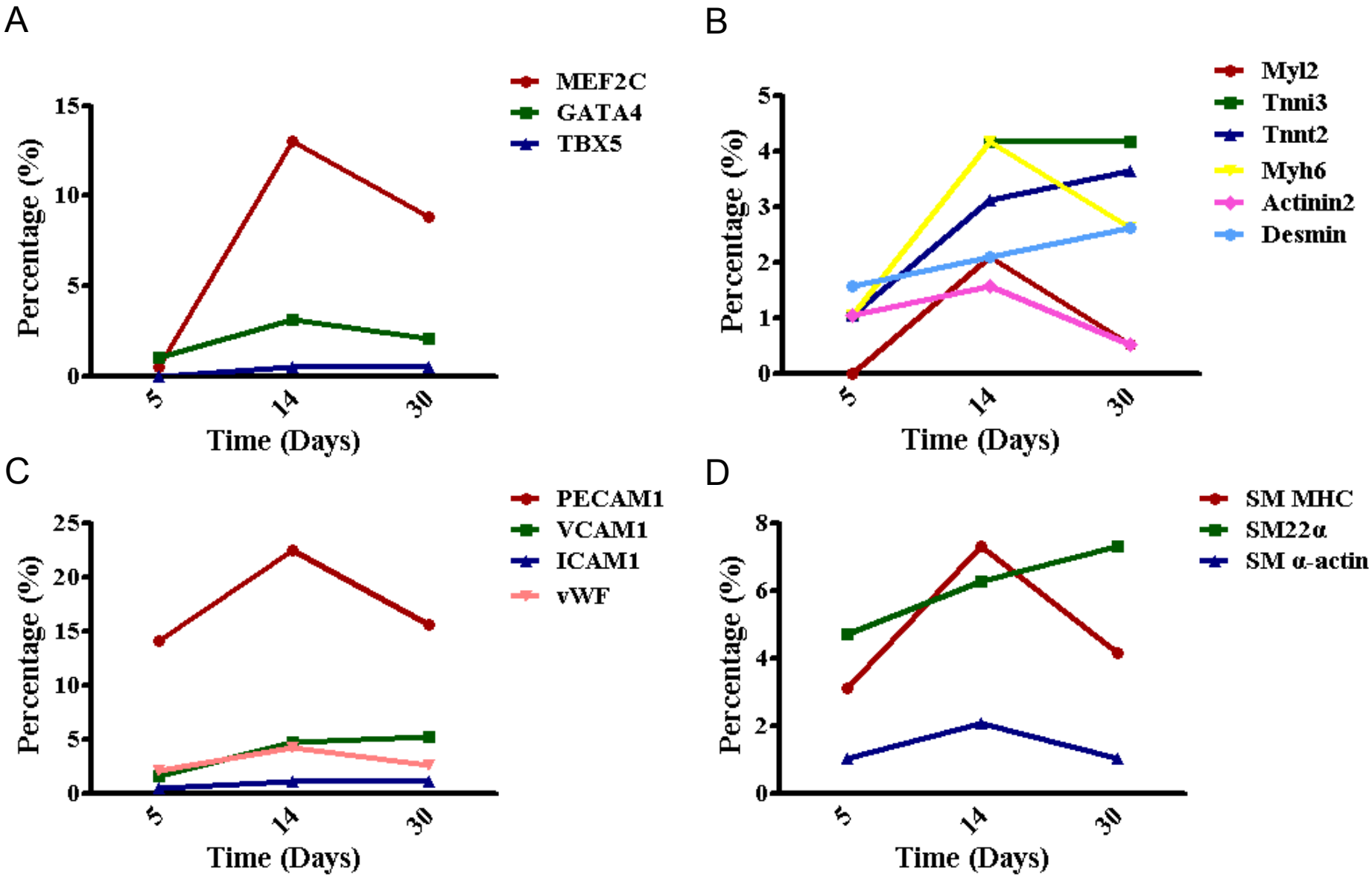
B



Supplemental Figure 10



Supplemental Figure 11



Supplemental Figure 12

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Flow cytometry analysis of cell surface marker expression of cultured hCPCs. Histogram plots are shown with the isotype control in black and the specified marker in gray. hCPCs demonstrate the expected Sca-1^{+}, CD105^{+}, CD45^{-}, CD133^{-}, CD14^{-}, and CD34^{-} profile.

Supplemental Figure 2. Gene expression profiling of hCPC lines transduced with various thymidine kinase reporter genes in comparison to untransduced control hCPCs as well as human embryonic stem cells (hESC). Upregulation of cardiac-specific genes is observed in relation to hESC (H7 line), with no significant differences in gene expression noted between the various hCPC lines transduced with different TK reporter genes.

Supplemental Figure 3. $^{Fluc+/GFP+}$hCPCs demonstrate a linear correlation between bioluminescence imaging (BLI) signal and number of viable cells, allowing for quantitative longitudinal BLI of hCPC transplant in living animals.

Supplemental Figure 4. Viability of hCPCs as assessed by Trypan blue exclusion assay was not affected by stable transduction of thymidine kinase variants. Cell viability values were normalized to those obtained on day 0 of cell culture.

Supplemental Figure 5. Microarray analysis of hCPC transcriptome before and after stable expression of thymidine kinase variants. Stable transduction with thymidine kinase reporter genes does not affect the gene expression profile of hCPCs. **(A)** Cross-correlation coefficients of paired microarray comparisons are >0.97 for all tested hCPC lines. A negative control human

embryonic stem cell line (hES) was included for comparison. **(B)** Scatter plots depicting gene expression fold-changes between paired cell lines. No significant variations in gene expression are noted among the various stably transduced hCPC lines.

Supplemental Figure 6. Normal karyotypes of the hCPC lines used in this study. Normal 46,XY karyotype of parent $^{Fluc+/GFP+}$hCPC line (left). Normal 46,XY karyotypes of hCPC lines stably expressing the A168H, sr39-tk, Δhtk2, and wt-tk variants (right). Stable expression of thymidine kinase reporter gene does not result in any detectable genomic instability.

Supplemental Figure 7. Temporal kinetics of hCPC engraftment monitored using bioluminescence imaging. Representative images of adult SCID beige female mice injected with $1x10^6$ hCPCs stably expressing the sr39-tk, Δhtk2, or wt-tk PET reporter gene variants are shown over a period of four weeks. A substantial decline in BLI signal intensity was observed over the study period, as shown in Figure 4, confirming the results of our PET imaging data acquisition and analysis.

Supplemental Figure 8. Echocardiographic and MRI assessment of cardiac contractility segregated into high vs. low engraftment groups. **(A)** Representative M-mode echocardiogram of hearts in the high vs. low cell engraftment categories. **(B)** Representative cardiac MR images of hearts in the high vs. low engraftment categories. **(C)** Significant improvement in left ventricular ejection fraction (vertical axis) in the high cell engraftment group compared to PBS controls at week 2 (Linear mixed model with high vs low engraftment, time, and group X time interaction as fixed effects, mouse as random effect. $P<0.001$; n=37 total, n=19 in the high cell engraftment

group, n=18 in the low cell engraftment group).

Supplemental Figure 9. Invasive hemodynamic monitoring confirms results of echocardiographic and cardiac MR analyses. **(A)** Pressure-volume (PV) loop recordings of mice in the high and low cell engraftment groups along with PBS controls at week 8 after injection. **(B)** Significant improvement in end-systolic volume (ESV), end-diastolic volume (EDV), and end-systolic pressure (ESP) is seen in the high cell engraftment group, when compared with PBS controls. One-way ANOVA; $P = 0.004$, $P = 0.009$, and $P = 0.002$, respectively. (n=10/group). Dunnett's Multiple Comparison Test was also performed ($P<0.05$ for high cell engraftment vs PBS).

Supplemental Figure 10. Growth factor expression of CPCs increases dramatically in response to hypoxia. **(A)** Raw image of the growth factor antibody array. The media of CPCs grown in normoxic or hypoxic (5% O_2) culture conditions for 48 hours was exposed to the antibody array. A key to the antibody array is displayed to the right. **(B)** Quantification of three antibody array experiments reveals significant increases in the secretion of growth factors such as VEGF-A, FGF2, and EGF in CPCs grown under hypoxic conditions.

Supplemental Figure 11. Immunohistochemical analysis of transplanted GFP^+ hCPCs reveals no evidence of significant differentiation of transplanted cells into cardiomyocyte or endothelial cells lineages. Co-localization is not observed between GFP and α-actinin, a cardiomyocyte marker (top right panel) or between GFP and CD31, and endothelial cell marker (bottom right panel).

Supplemental Figure 12. Cardiac lineage transcript expression over time in transplanted cardiac progenitor cells shows no evidence for significant direct cardiac differentiation. **(A)** Transcriptional markers of early cardiac commitment, such as GATA4 and TBX5, were only marginally positive in isolated GFP^+ CPCs. **(B)** Transcriptional markers of committed cardiomyocytes, such as TNNT2, MYH6, and Actinin2 were positive in <5% of isolated cells. **(C)** Endothelial cell transcripts such as VCAM1, ICAM1, and vWF were also only marginally positive in isolated GFP^+ cells. **(D)** Transplanted cells were also largely negative for smooth muscle markers such as SMA.

REFERENCES

1. Goumans MJ, de Boer TP, Smits AM, van Laake LW, van Vliet P, Metz CH, Korfage TH, Kats KP, Hochstenbach R, Pasterkamp G, Verhaar MC, van der Heyden MA, de Kleijn D, Mummery CL, van Veen TA, Sluijter JP, Doevendans PA. Tgf-β1 induces efficient differentiation of human cardiomyocyte progenitor cells into functional cardiomyocytes in vitro. *Stem Cell Res*. 2007;1:138-149
2. Smits AM, van Vliet P, Metz CH, Korfage T, Sluijter JP, Doevendans PA, Goumans MJ. Human cardiomyocyte progenitor cells differentiate into functional mature cardiomyocytes: An in vitro model for studying human cardiac physiology and pathophysiology. *Nat Protoc*. 2009;4:232-243
3. van Vliet P, Roccio M, Smits AM, van Oorschot AA, Metz CH, van Veen TA, Sluijter JP, Doevendans PA, Goumans MJ. Progenitor cells isolated from the human heart: A potential cell source for regenerative therapy. *Neth Heart J*. 2008;16:163-169
4. Gambhir SS, Bauer E, Black ME, Liang Q, Kokoris MS, Barrio JR, Iyer M, Namavari M, Phelps ME, Herschman HR. A mutant herpes simplex virus type 1 thymidine kinase reporter gene shows improved sensitivity for imaging reporter gene expression with positron emission tomography. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97:2785-2790
5. Ponomarev V, Doubrovin M, Shavrin A, Serganova I, Beresten T, Ageyeva L, Cai C, Balatoni J, Alauddin M, Gelovani J. A human-derived reporter gene for noninvasive imaging in humans: Mitochondrial thymidine kinase type 2. *The Journal of Nuclear Medicine*. 2007;48:819-826

6. Najjar AM, Nishii R, Maxwell DS, Volgin A, Mukhopadhyay U, Bornmann WG, Tong W, Alauddin M, Gelovani JG. Molecular-genetic pet imaging using an HSV1-TK mutant reporter gene with enhanced specificity to acycloguanosine nucleoside analogs. *The Journal of Nuclear Medicine*. 2009;50:409-416

7. Li Z, Lee A, Huang M, Chun H, Chung J, Chu P, Hoyt G, Yang P, Rosenberg J, Robbins RC, Wu JC. Imaging survival and function of transplanted cardiac resident stem cells. *J Am Coll Cardiol*. 2009;53:1229-1240

8. Rosenzweig A. Cardiac cell therapy--mixed results from mixed cells. *N Engl J Med*. 2006;355:1274-1277