SUPPLEMENTAL MATERIAL

Supplemental Methods

Cell culture

Rat CDCs (rCDCs) were expanded from explanted hearts obtained from 8-week old male WKY rats. Human CDCs (hCDCs) were expanded from human endomyocardial biopsies or myocardial samples, obtained from adult male patients during clinically-indicated procedures after informed consent, under a protocol approved by the Institutional Review Board. The myocardial specimens (both rat hearts and human biopsies) were cut into fragments less than 1 mm^3, washed and partially digested with trypsin (0.05%; GIBCO). These tissue fragments were culture as cardiac explants on fibronectin (20 mg/ml; Sigma) coated dishes in cardiac explant media [CEM; Iscove's Modified Dulbecco's Medium (GIBCO), fetal bovine serum 20% (HyClone, Logan, UT), 100 U/ml penicillin G (GIBCO), 100 U/ml streptomycin (GIBCO), and 0.1 mmol/l 2-mercaptoethanol (GIBCO)]. After a variable period of growth, a layer of stromal-like cells emerged from the cardiac explant over which phase bright cells proliferated. The loosely-adherent cells surrounding the explant (termed cardiac outgrowth) were harvested using mild enzymatic digestion (0.05% trypsin under direct visualization, GIBCO). Cardiac outgrowth could be harvested up to four more times from the same specimen. Harvested cardiac outgrowth was seeded at 50,000 cells/ml on poly-D-lysine coated dishes in CEM. Several days later, cells that remained adherent to the poly-D-lysine coated dishes were discarded, while free-floating cardiospheres were harvested, plated on fibronectin coated flasks and cultured in CEM to generate CDCs.

Flow cytometry

Flow cytometry experiments were performed in order to evaluate surface expression of MHC class I, MHC class II and costimulatory molecules (CD 80, CD86) in human and rat CDCs, both under baseline conditions and after stimulation with 100 ng/ml interferon-γ for 1 day and 7 days. In addition, general phenotypic characterization of CDCs (expression of CD105, c-Kit, CD90, CD31, CD45, CD140b, Discoidin domain-containing receptor 2 [DDR2] and α-smooth muscle actin) was performed. The antibodies are listed in supplemental table 3. Experiments were performed using a benchtop flow cytometer (FACSCalibur; BD Biosciences, San Jose, Ca). Gates were established by 7-amino-actinomycin D fluorescence and forward scatter to exclude dead cells. Fluorescent compensation was performed using single labeled controls. The percentage of positive cells was defined as the percent of the population falling above the 99th percentile of an isotype-matched antibody control cell population. Quantitative analysis was performed using CellQuest software (BD Biosciences).

Mixed-lymphocyte reaction

In order to assess the in vitro immunogenicity of CDCs, one-way mixed lymphocyte reactions were performed. Lymphocytes were isolated from euthanized WKY and BN rat spleens using standard protocols. In brief, spleens were harvested aseptically, mechanically dissociated and filtered through a 100 µm nylon mesh. Erythrocytes were lysed with 0.83% ammonium chloride, cells were washed in RPMI 1640, dead cells were removed by density centrifugation and cell viability was assessed by trypan blue dye exclusion. Stimulating rCDCs and hCDCs were mitotically inactivated with 50 µg/ml mitomycin C (Sigma-Aldrich) in the dark at 37°C for 30 minutes and washed three times with RPMI 1640. 10^4 stimulating CDCs were cocultured with 10^5 responder lymphocytes in 200 µl of culture medium (RPMI 1640 supplemented with 10%

FBS) in 96-well flat-bottom plates for 5 days. The following experimental conditions were tested in quadruplicates: a) rCDCs cocultured with WKY lymphocytes (syngeneic coculture); b) rCDCs cocultured with BN lymphocytes (allogeneic coculture); c) hCDCs cocultured with BN lymphocytes (xenogeneic coculture). All appropriate controls were also tested. BrdU was added to the cocultures for the last 24 hours and responder cell proliferation was assessed by the Cell Proliferation Biotrak ELISA System (GE Healthcare) according to the manufacturer's instructions. Absorbance was measured with a microplate reader (Bio-Rad) at 450 nm. Alloreactive and xenoreactive lymphocyte proliferation is presented as relative proliferative response, normalized to syngeneic coculture proliferation (stimulation index). The cell-free supernatant of the cocultures was collected and the levels of secreted IFN-g, IL-1b, IL-13, IL-4, IL-5, KC/GRO and TNF-a were measured by electrochemiluminescence. The levels of secreted IL-2 were measured using enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's protocols (R&D Systems)

Myocardial infarction and cell injection

Female WKY and BN rats (8-10 week old) underwent left thoracotomy under general anesthesia with 2% isoflurane. MI was produced by permanent ligation of the left anterior descending coronary artery. CDCs (2 million, suspended in120 μl of phosphate-buffered saline [PBS]) or vehicle were intramyocardially injected with a 29-gauge needle at 4 sites along the periphery of the infarct. The following experimental conditions were tested: a) injection of rCDCs into infarcted hearts of WKY rats (syngeneic group); b) injection of rCDCs into infarcted hearts of BN rats (allogeneic group); c) injection of hCDCs into infarcted hearts of BN rats (xenogeneic group); d) a) injection of vehicle into infarcted hearts of WKY rats (control group a); e) injection of vehicle into infarcted hearts of BN rats (control group b). 2 control groups were used in order

to confirm that both strains of rats respond similarly to myocardial infarction. After injections were completed, the chest was closed, anesthesia was discontinued and the animals were allowed to recover. In order to monitor proliferation of both transplanted and endogenous cells, subset of animals was intraperitoneally-injected with BrdU (100mg/kg body weight) daily for either the first week or the second and third week post MI.

Echocardiography

Echocardiography was performed to assess global cardiac function 6 hours (baseline), 3 weeks, 3 months and 6 months after surgery, using the Vevo 770 Imaging System (VISUALSONICS, Toronto, Canada). After the induction of general anesthesia with 2% isoflurane, the hearts were imaged two-dimensionally in the long-axis view (at the level of the greatest LV diameter) and in the short-axis view (at the level of the papillary muscle). Fractional area change (FAC) and left ventricular ejection fraction (LVEF) were measured from the long-axis view while fractional shortening (FS) was measured from the M-mode of the short axis view with Visual Sonics V1.3.8 software.

Quantification of engraftment by real time PCR

Quantitative PCR was performed 1 week and 3 weeks post cell injection in order to monitor transplanted cell survival after syngeneic, allogeneic and xenogeneic cell transplantation. We injected cells isolated from male donor WKY rats and male humans in the myocardium of female recipients and quantified absolute cell engraftment by real-time PCR using the (rat and human respectively) SRY gene located on the Y chromosome as target. In brief, the recipient heart was explanted, weighted, homogenized and genomic DNA was isolated using the DNA

Easy minikit (Qiagen), according to the manufacturer's protocol. The TaqMan® assay (Applied Biosystems) was used to quantify the number of transplanted cells with the rat (for syngeneic and allogeneic transplantation) and human (for xenogeneic transplantation) SRY gene as template. A standard curve was constructed with samples derived from multiple log dilutions of genomic DNA, isolated from male rat hearts and samples of male human myocardium, spiked with 50ng of female rat genomic DNA as control. The copy number of the SRY gene at each point of the standard curve was calculated based on the amount of DNA in each sample and the total mass of the rat genome per diploid cell. All samples were tested in triplicates. For each reaction, 50ng of template DNA was used. Real time PCR was performed in an ABI PRISM 7700 instrument. The result from each reaction, copies of the SRY gene in 50ng of genomic DNA, was expressed as the number of engrafted cells/heart by extrapolation to the total DNA content of each heart, taking into account that there is one copy of the SRY gene per transplanted cell.

Histology

Rats were sacrificed 1 week, 3 weeks and 6 months after treatment. Hearts were arrested with KCl solution, explanted, frozen in OCT compound, and sectioned in 5 μm sections on a cryostat. Cryosections were subsequently fixed with 4% paraformaldehyde. Quantitative morphometric analysis with Masson's trichrome staining (6 sections per heart, collected at 400 μm intervals) was performed to examine scar size, infarcted wall thickness and LV remodeling as described previously. In order to evaluate immune rejection, sections (12 sections per heart, collected at 200 μm intervals) were stained with hematoxylin and eosin and evaluated by a blinded cardiac pathologist; in addition immunostaining against immune cell markers (12 sections per heart, collected at 200 μm intervals) was performed. The differentiation of CDCs into myocytes and

endothelial cells was identified by immunostaining (6 sections per heart, collected at 400 µm intervals) with antibodies against GFP, α-sarcomeric actin and von Willebrand factor (vWf). Host cardiomyocyte cell cycle re-entry was evaluated by immunostaining (6 sections per heart, collected at 400 µm intervals) with antibodies against α-sarcomeric actin, Ki67 and BrdU. Recruitment of endogenous progenitors was assessed by immunostaining (6 sections per heart, collected at 400 µm intervals) against c-Kit. Vessel density in the infarct border zone was evaluated by immunostaining (6 sections per heart, collected at 400 µm intervals) with antibodies against vWf. In all sections used for immunohistochemistry, Alexa Fluor conjugated secondary antibodies (Molecular probes) were used and counterstaining with 4,6-diamidino-2-phenylindole (DAPI, Molecular Probes) was performed. Sections were imaged using a confocal laser scan microscope (Leica Microsystems) and images were processed by Leica LAS software suite. All antibodies used for immunohistochemistry are listed in supplemental table 3.

Assessment of systemic immunogenicity and development of memory immune response

In order to assess the systemic immunogenicity of CDC transplantation, levels of circulating inflammatory cytokines were measured in rat sera isolated from recipients of syngeneic, allogeneic, xenogeneic CDCs and controls. The levels of circulating IFN-g, IL-1b, IL-13, IL-4, IL-5, KC/GRO and TNF-α were quantified by electrochemiluminescence.

In order to assess development of humoral memory immune response, recipient rat sera were isolated at 1 week and 3 weeks post transplantation and levels of circulating alloreactive and xenoreactive anti-donor antibodies were quantified by flow cytometry. In brief, rCDCs and hCDCs were incubated with 50 µl of rat serum samples isolated from syngeneic, allogeneic and xenogeneic recipients or with naïve rat serum (isotype control) for 30 min on ice. After washing, cells were incubated with anti-rat IgM and anti-rat IgG antibodies for 30 min on ice. Cells were

analyzed in a benchtop flow cytometer (FACSCalibur; BD Biosciences, San Jose, Ca) and quantitative analysis was performed using CellQuest software (BD Biosciences).

In order to assess development of cellular memory immune response, spleens from allogeneic recipients were harvested at 3 weeks post transplantation. Lymphocytes were isolated and their reactivity against allogeneic donor cells was assessed by one-way mixed lymphocyte cocultures and was compared to that of naïve lymphocytes. The cell-free supernatant of the cocultures was collected and the levels of secreted IFN-g, IL-1b, IL-13, IL-4, IL-5, KC/GRO and TNF-α were measured by electrochemiluminescence. The levels of secreted IL-2 were measured using ELISA kits, according to the manufacturer's protocols (R&D Systems)

Electrochemiluminescence

Electrochemiluminescence was performed to assess levels of inflammatory cytokines in: a) serum samples obtained from rats injected with syngeneic, allogeneic, xenogeneic CDCs or vehicle, at 3 weeks post injection; b) cell culture supernatants obtained from co-cultures of lymphocytes with syngeneic, allogeneic and xenogeneic CDCs. Rat serum samples and cell culture supernatants were assayed for levels of IFN-g, IL-1b, IL-13, IL-4, IL-5, KC/GRO and TNF-α using a commercially available, ruthenium based electrochemoluminescence platform (Meso Scale Discovery, Gaithersburg, MD) following the manufacturer's recommendations. Samples were thawed and centrifuged with 15,000 rcf for 15 min right before use. All samples were run in duplicate using 25 µl of rat serum or supernatant per well. Results were considered valid when recovery (expected concentration divided by calculated concentration multiplied by 100) was 100±20%, percentage of coefficient of variation (CV = average of replicates divided by the standard deviation multiplied by 100) was <20%, intraassay CV was <10% and interassay CV was <20%. A spike-recovery protocol was initiated that allowed control for possible matrix

effects when comparing levels of cytokines between serum and supernatant. Results were considered valid if 85% of the samples of a run met these specifications.

Western Blotting

Western blot analysis was performed to compare the myocardial levels of VEGF, IGF-1 and HGF at various time points post MI among rats treated with syngeneic CDCs, allogeneic CDCs, xenogeneic CDCs and controls. Myocardial samples from the peri-infarct area were collected at 5 minutes, 1 day, 4 days, 7 days and 21 days post MI. Tissue samples were lysed in lysis buffer supplemented with proteinase inhibitors cocktail (Roche) and homogenized with a rotor-stator homogenizer. Homogenates were centrifuged at 12,000 rcf for 15 minutes at 4°C, supernatants were collected and protein content was quantified by Lowry assay (BioRad). The equivalent of 15 μg of total protein per lane was loaded onto 12% Precise Protein gels, and then transferred to PVDF membranes. Membranes were blocked with 5% non-fat milk and incubated overnight with primary antibodies against VEGF, IGF, HGF and GAPDH. Subsequently, the appropriate horseradish peroxidase-conjugated secondary antibodies were used, and then the blots were visualized by using SuperSignal West Femto maximum sensitivity substrate (Thermo Scientific) and exposed to Gel Doc XR System (Bio-Rad Lab. Inc.).Quantitative analysis was performed by ImageJ software, and expressions were normalized to GAPDH.

Supplemental tables

Experiment	**n**
Flow cytometric analysis of rCDCs (Fig 1)	9
Flow cytometric analysis of hCDCs (Fig 1)	9
MLR: responder cell proliferation syngeneic grp (Fig 2b)	6
MLR: responder cell proliferation allogeneic grp (Fig 2b)	6
MLR: responder cell proliferation xenogeneic grp (Fig 2b)	8
MLR: responder cell proliferation sensitized grp (Supp Fig3b)	8
MLR: cytokine levels in supernatant syngeneic grp (ECL+ELISA) (Fig 2c)	25
MLR: cytokine levels in supernatant allogeneic grp (ECL+ELISA) (Fig 2c)	26
MLR: cytokine levels in supernatant xenogeneic grp (ECL+ELISA) (Fig 2c)	21
MLR: cytokine levels in supernatant sensitized grp (ECL+ELISA) (Supp Fig 3c)	28
PCR syngeneic grp (Fig 3C, 3D)	12
PCR allogeneic grp (Fig 3C, 3D)	12
PCR xenogeneic grp (Fig 3C, 3D)	10
Masson’s Trichrome syngeneic grp (Fig 4A-C)	7
Masson’s Trichrome allogeneic grp (Fig 4A-C)	8

Masson’s Trichrome xenogeneic grp (Fig 4A-C)	5
Masson’s Trichrome control grp (Fig 4A-C)	5
Echocardiography syngeneic grp day 0(Fig 4D-F)	23
Echocardiography syngeneic grp 3 weeks(Fig 4D-F)	23
Echocardiography syngeneic grp 3 months(Fig 4D-F)	7
Echocardiography syngeneic grp 6 months(Fig 4D-F)	7
Echocardiography syngeneic grp FAC treatment effect (Fig 4F)	23
Echocardiography allogeneic grp day 0(Fig 4D-F)	21
Echocardiography allogeneic grp 3 weeks(Fig 4D-F)	21
Echocardiography allogeneic grp 3 months(Fig 4D-F)	7
Echocardiography allogeneic grp 6 months(Fig 4D-F)	7
Echocardiography allogeneic grp FAC treatment effect (Fig 4F)	21
Echocardiography control WKY grp day 0(Fig 4D-F)	11
Echocardiography control WKY 3 weeks(Fig 4D-F)	11
Echocardiography control WKY 3 months(Fig 4D-F)	6
Echocardiography control WKY 6 months(Fig 4D-F)	6
Echocardiography control WKY FAC treatment effect (Fig 4F)	11
Echocardiography control BN grp day 0 (Fig 4D-F)	11

Echocardiography control BN 3 weeks(Fig 4D-F)	11
Echocardiography control BN 3 months(Fig 4D-F)	6
Echocardiography control BN 6 months(Fig 4D-F)	6
Echocardiography control BN grp FAC treatment effect (Fig 4F)	11
Echocardiography xenogeneic grp day 0 (Fig 4D-G)	10
Echocardiography xenogeneic grp 3 weeks (Fig 4D-F)	10
Echocardiography xenogeneic grp FAC treatment effect (Fig 4F)	10
H&E syngeneic grp (Fig 5, Supp Fig 5)	13
H&E allogeneic grp (Fig 5, Supp Fig 5)	13
H&E control grp (Fig 5, Supp Fig 5)	13
H&E xenogeneic grp (Fig 5)	9
IHC syngeneic grp (Fig 6, Supp Fig 5)	11
IHC allogeneic grp (Fig 6, Supp Fig 5)	11
IHC control grp (Fig 6, Supp Fig 5)	11
IHC xenogeneic grp (Fig 6)	8
IHC syngeneic grp (Fig 7)	21
IHC allogeneic grp (Fig 7)	21
IHC control grp (Fig 7)	21

Western blot syngeneic grp (Fig 8)	20
Western blot allogeneic grp (Fig 8)	20
Western blot WKY control grp (Fig 8, Sup Fig 6)	15
Western blot BN control grp (Fig 8, Sup Fig 6)	15
Western blot WKY xenogeneic grp (Sup Fig 6)	20
Flow cytometry anti-donor antibodies syngeneic grp (Supp Fig 2)	12
Flow cytometry anti-donor antibodies allogeneic grp (Supp Fig 2)	12
Flow cytometry anti-donor antibodies xenogeneic grp (Supp Fig 2)	14
ECL serum cytokines syngeneic grp (Supp Fig 1)	21
ECL serum cytokines allogeneic grp (Supp Fig 1)	18
ECL serum cytokines xenogeneic grp (Supp Fig1)	20
ECL serum cytokines control grp (Supp Fig 1)	5

Supplemental Table 1. Sample sizes

Name	#1	#2	#3	#4	#5	#6	#7
Age	56	54	52	45	69	62	49
Male/female	M	M	M	M	M	M	M
Weight (kg)	85.7	63.2	71.4	81.2	87.7	68	107
Body mass index	29.6	22.5	26.2	26.4	32.2	25	34.9
Smoker	N	N	Y	N	N	N	Y
Diabetes	N	N	N	N	N	N	Y
Hyperlipidemia	Y	Y	Y	Y	Y	N	Y
Renal dysfunction	N	N	N	N	N	Y	Y
Hypertension	Y	Y	N	Y	Y	Y	Y
Chronic lung disease	N	N	N	N	N	N	N
Peripheral vascular disease	N	N	N	N	Y	N	Y
Cerebrovascular disease	N	N	N	N	N	N	N
Myocardial infarction	Y	Y	Y	Y	Y	N	Y
Coronary artery disease	Y	Y	Y	Y	Y	N	Y
Angina	N	N	N	N	N	N	Y
Arrhythmia	N	N	N	N	N	N	Y
Congestive heart failure	N	N	N	N	N	Y	Y
Classification NYHA (I/II/III/IV)	N	N	N	N	N	IV	IV
Cardiac surgical procedure (CABG/valve/DOR)	N	N	N	N	N	N	N

Other surgical procedures	Y (Colon Resection)	N	N	N	Y (S/P Gastric Resection)	N	N
Other comorbidities	Diverticulitis	N	N	N	Peptic Ulcer Disease	Amyoloidosis, Peptic Ulcer Disease, Cholelithiasis, Nephrolithiasis	Gout
Medications							
Aspirin	Y	Y	Y	Y	Y	N	Y
Clopidogrel	Y	Y	Y	Y	Y	N	N
Statins	Y	Y	Y	Y	Y	N	Y
Amiodarone	N	N	N	N	N	N	N
Angiotensin-converting enzyme inhibitors/ angiotensin receptor blockers	Y	Y	Y	Y	N	N	N
Beta Blocker	Y	Y	Y	Y	Y	Y	Y
Ca++ Blocker	N	N	N	N	N	N	N
Nitrate	N	N	N	N	N	N	N

Supplemental Table 2. Human (xenogeneic) cell donor characteristics

Antigen	Antibody	Procedure
HLA-A,B,C	PE Mouse Anti-Human HLA-ABC (BD Pharmingen)	FC
HLA-A,B,C	APC Mouse Anti-Human HLA-ABC (BD Pharmingen)	FC
HLA-DR,DP,DQ	FITC Mouse Anti-Human HLA-DR,DP,DQ (BD Pharmingen)	FC
CD86	APC Mouse Anti-Human CD86 (BD Pharmingen)	FC
CD80	FITC Mouse Anti-Human CD80 (BD Pharmingen)	FC
RT1A	PE Mouse Anti-Rat RT1A (BD Pharmingen)	FC
RT1D	FITC Mouse Anti-Rat RT1D (BD Pharmingen)	FC
CD80	PE Mouse Anti-Rat CD80 (BD Pharmingen)	FC
CD80	FITC Mouse Anti-Rat CD80 (AbD Serotec)	FC
CD86	FITC Mouse Anti-Rat CD86 (BD Pharmingen)	FC
CD90	PE Mouse Anti-Human CD90 (BD Pharmingen)	FC
CD31	Alexa Fluor 647 Mouse Anti-Human CD31 (BD Pharmingen)	FC
CD45	APC Mouse anti-Human CD45 (BD Pharmingen)	FC
CD105	PE Mouse anti-Human CD105 (R&D systems)	FC
DDR2	Mouse anti-Human CD105 (R&D systems)	FC
αSMA	PE Mouse anti-Human α-SMA (R&D systems)	FC

CD140b	Mouse anti-Human CD140b (BD Pharmingen)	FC
CD90	FITC Mouse anti-Rat CD90 (BD Pharmingen)	FC
CD45	FITC Mouse anti-rat CD45 (BD Pharmingen)	FC
CD31	PE Mouse anti-Rat CD31 (BD Pharmingen)	FC
CD105	Mouse anti-Rat CD105 (Novus Biologicals/Abcam)	FC
DDR2	Rabbit anti-Rat DDR2 (Santa Cruz/Abcam)	FC/ICC
αSMA	Rabbit anti-Rat αSMA	FC
CD140b	Rabbit anti-Rat CD140b	FC
c-Kit	rabbit anti-human/rat/ (Santa Cruz)	FC, IHC
CD3	Rabbit anti-rat CD3 (Abcam)	IHC
CD45RA	Mouse anti-rat CD45RA (Abcam)	IHC
CD45RA	Mouse anti-rat CD45RA (AbD Serotec)	IHC
CD8	Rabbit anti-rat CD8 (Abcam)	IHC
CD4	Mouse anti-rat CD4 (Abcam)	IHC
CD11c	Mouse anti-rat CD11c (Abcam)	IHC
CD68	Rabbit Anti-rat CD68 (Abbiotec)	IHC
CD68	Mouse anti-rat CD68 (AbD Serotec)	IHC
CD8	Mouse anti-rat CD8 (AbD Serotec)	IHC
CD4	Mouse anti-rat CD4 (BD Pharmingen)	IHC

CD11c	Mouse anti-rat CD11c (BD Pharmingen)	IHC
GFP	Goat anti-GFP (Abcam)	IHC
α-smooth muscle actin	Rabbit anti-rat α-smooth muscle actin (Abcam)	IHC
α-sarcomeric actinin	Rabbit anti-rat α-sarcomeric actinin (Abcam)	IHC
Von Willebrand Factor	Rabbit anti-rat vWF (Abcam)	IHC
α-sarcomeric actinin	Mouse anti-rat α-sarcomeric actinin (Sigma)	IHC
α-smooth-muscle actin	Mouse anti-rat α-smooth-muscle actin (Abcam)	IHC
Ki67	Rabbit anti-rat Ki67 (Abcam)	IHC
Ki67	Rabbit anti-rat Ki67 (Thermo)	IHC
BrdU	Mouse anti-BrdU (Roche)	IHC
Goat IgG	Alexa Fluor 488 donkey anti-goat IgG (Molecular Probes)	IHC
Mouse IgG	Alexa Fluor 546 donkey anti-mouse IgG (Molecular Probes)	IHC
Rabbit IgG	Alexa Fluor 647 donkey anti-rabbit IgG (Molecular Probes)	IHC
VEGF	Mouse anti-rat VEGF (Abcam)	WB
IGF1	Mouse monoclonal to IGF1 (Abcam)	WB

HGF	Rabbit anti-rat HGF (Abcam)	WB
VEGF	Mouse anti-rat/human VEGF (Abcam)	WB
IGF1	Goat anti-rat/human IGF1 (Abcam)	WB
HGF	Rabbit anti-rat/human HGF	WB
Mouse IgG	Goat anti-mouse IgG, HRP-conjugated (Cell Signaling)	WB
Rabbit IgG	Goat anti-rabbit IgG, HRP-conjugated (Cell Signaling)	WB
Goat IgG	Donkey anti-goat IgG, HRP-conjugated (Abcam)	WB
Rat IgM	FITC Mouse Anti-Rat IgM (BD Pharmingen)	FC
Rat IgG1/2a	FITC Mouse Anti-Rat IgG1/2a (BD Pharmingen)	FC

Supplemental Table 3. List of antibodies

Groups	Peri-operative mortality	Longer-term mortality
Syngeneic	4/47 (8.5%)	3/43 (6.9%)
Allogeneic	4/46 (8.7%)	2/42 (4.8%)
Control WKY	2/25 (8.0%)	3/23 (13.1%)
Control BN	3/25 (12.0%)	2/22 (9.1%)
Xenogeneic	4/48 (8.3%)	4/44 (9.1%)

Supplemental Table 4. Peri-operative and longer-term mortality (post-operative period until protocol completion). Ns denote animals that died; (ns) denote mortality rates

	Infarct area			Peri-infarct area			Remote myocardium		
	Patchy infiltration	***Diffuse infiltration***	***Myocyte damage***	***Patchy infiltration***	***Diffuse infiltration***	***Myocyte damage***	***Patchy infiltration***	***Diffuse infiltration***	***Myocyte damage***
Syngeneic (n=4)	**4/48* (2/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**4/48* (2/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**
Allogeneic (n=4)	**4/48* (1/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**4/48* (1/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**
Control (n=4)	**3/48* (1/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**3/48* (1/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**
Xenogeneic (n=4)	**28/48 (4/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**30/48 (4/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**

Supplemental Table 5. Homemade grading system for rejection at 1 week post MI and cell transplantation

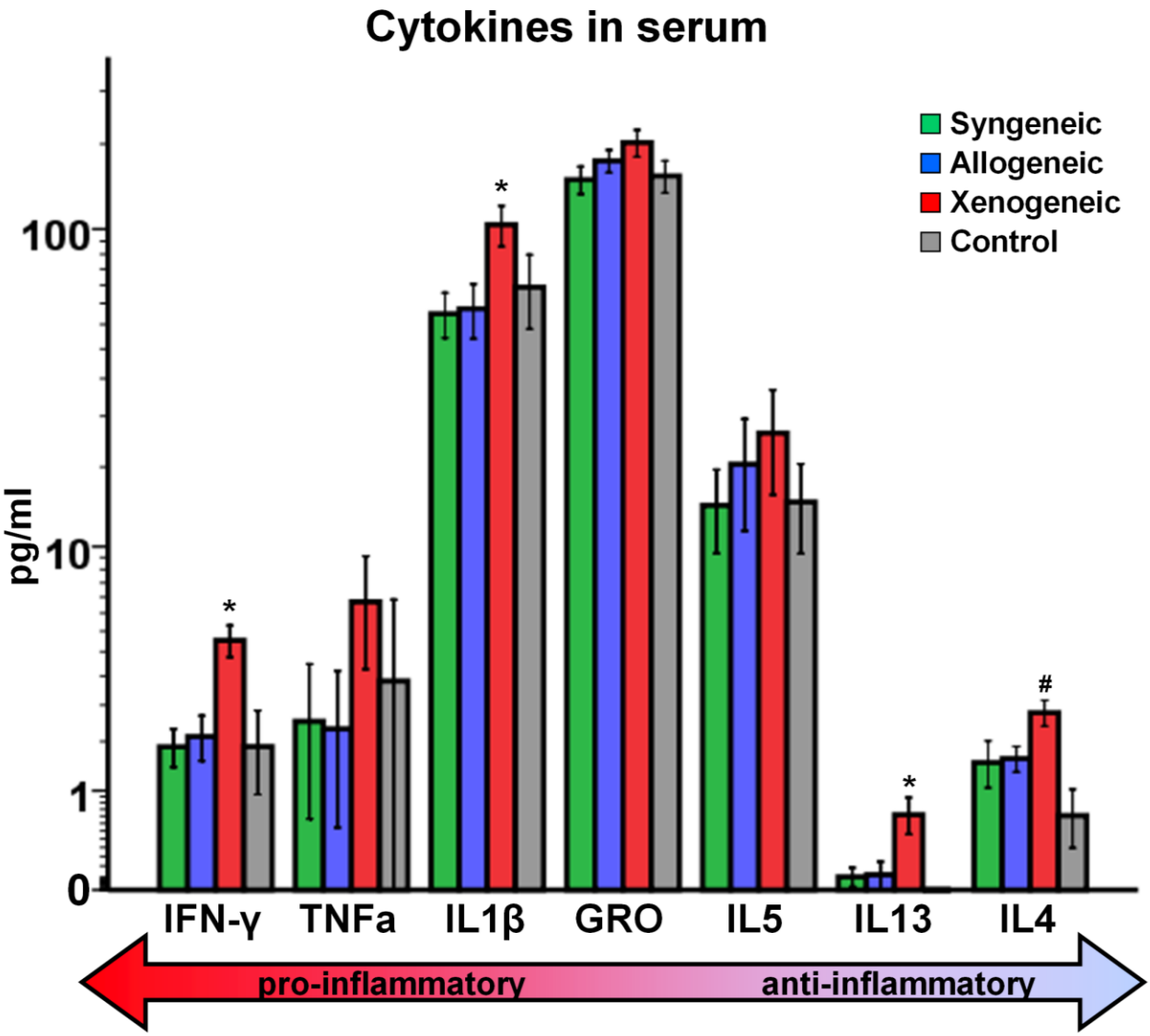
	Infarct area			Peri-infarct area			Remote myocardium		
	Patchy infiltration	***Diffuse infiltration***	***Myocyte damage***	***Patchy infiltration***	***Diffuse infiltration***	***Myocyte damage***	***Patchy infiltration***	***Diffuse infiltration***	***Myocyte damage***
Syngeneic (n=5)	**2/60* (1/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**1/60* (1/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**
Allogeneic (n=5)	**1/60* (1/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60* (1/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**
Control (n=5)	**1/60* (1/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**1/60* (1/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**
Xenogeneic (n=5)	**45/60 (5/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**39/60 (5/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**	**0/60 (0/5)**

Supplemental Table 6. Homemade grading system for rejection at 3 weeks post MI and cell transplantation

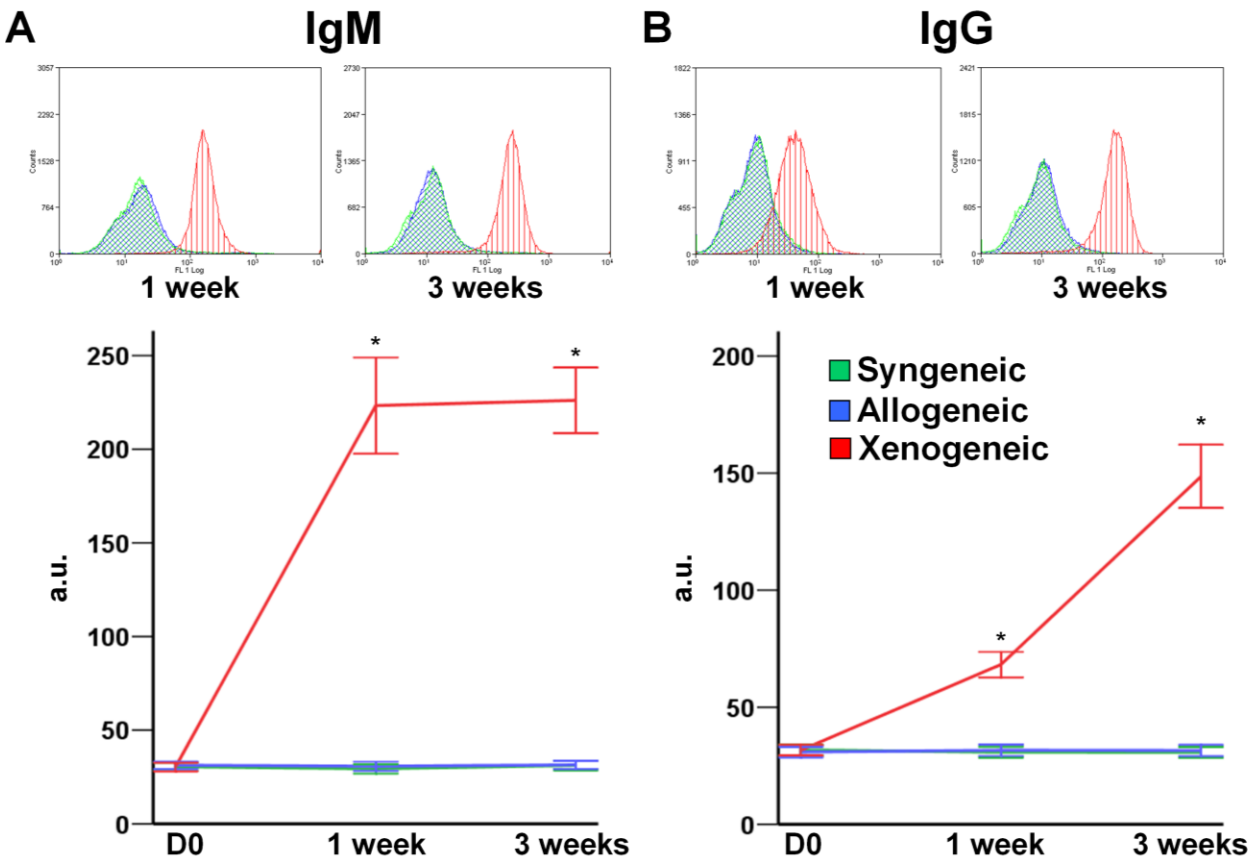
	Infarct area			Peri-infarct area			Remote myocardium		
	Patchy infiltration	***Diffuse infiltration***	***Myocyte damage***	***Patchy infiltration***	***Diffuse infiltration***	***Myocyte damage***	***Patchy infiltration***	***Diffuse infiltration***	***Myocyte damage***
Syngeneic (n=4)	**1/48* (1/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**2/48* (2/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**
Allogeneic (n=4)	**1/48* (1/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**2/48* (1/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**
Control (n=4)	**2/48* (2/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**2/48* (2/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**	**0/48 (0/4)**

Supplemental Table 7. Homemade grading system for rejection at 6 months post MI and cell transplantation

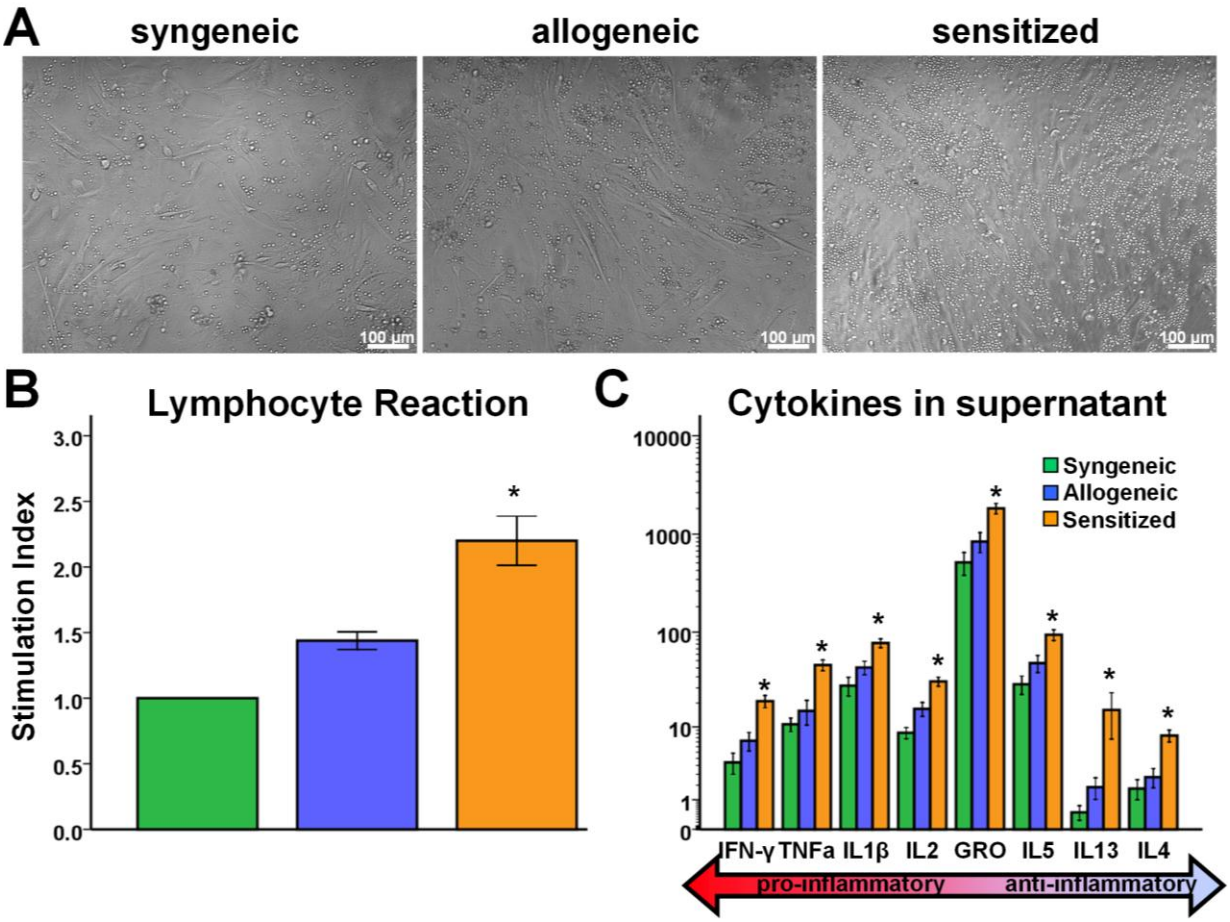
Supplemental Figures



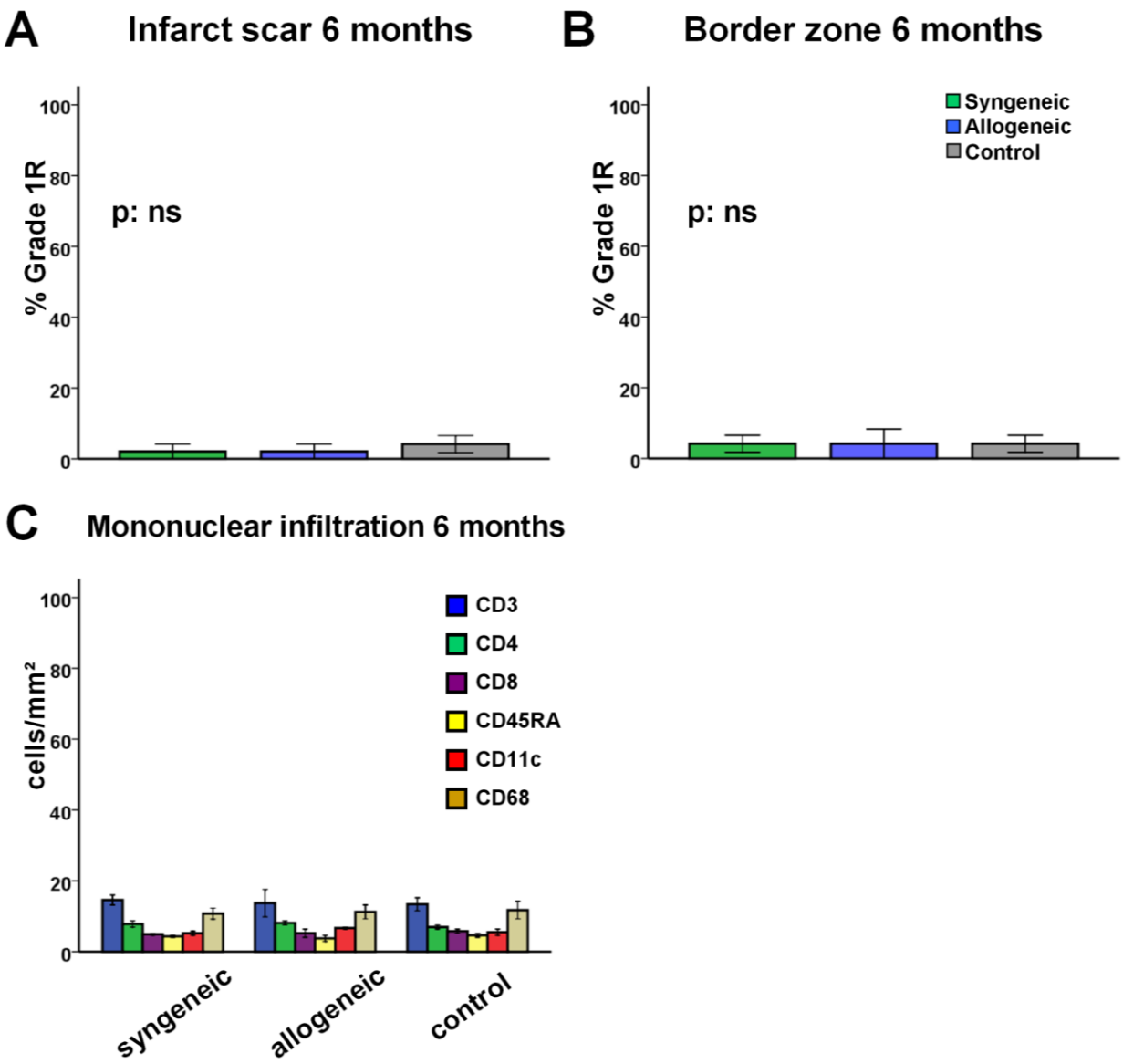
Supplemental Figure 1. Circulating levels of inflammatory cytokines measured with electrochemiluminescence in rat serum samples obtained 3 weeks post-treatment (n=5-21/group). (* $p<0.05$ compared to syngeneic, allogeneic groups; # $p<0.05$ compared to syngeneic, control groups)



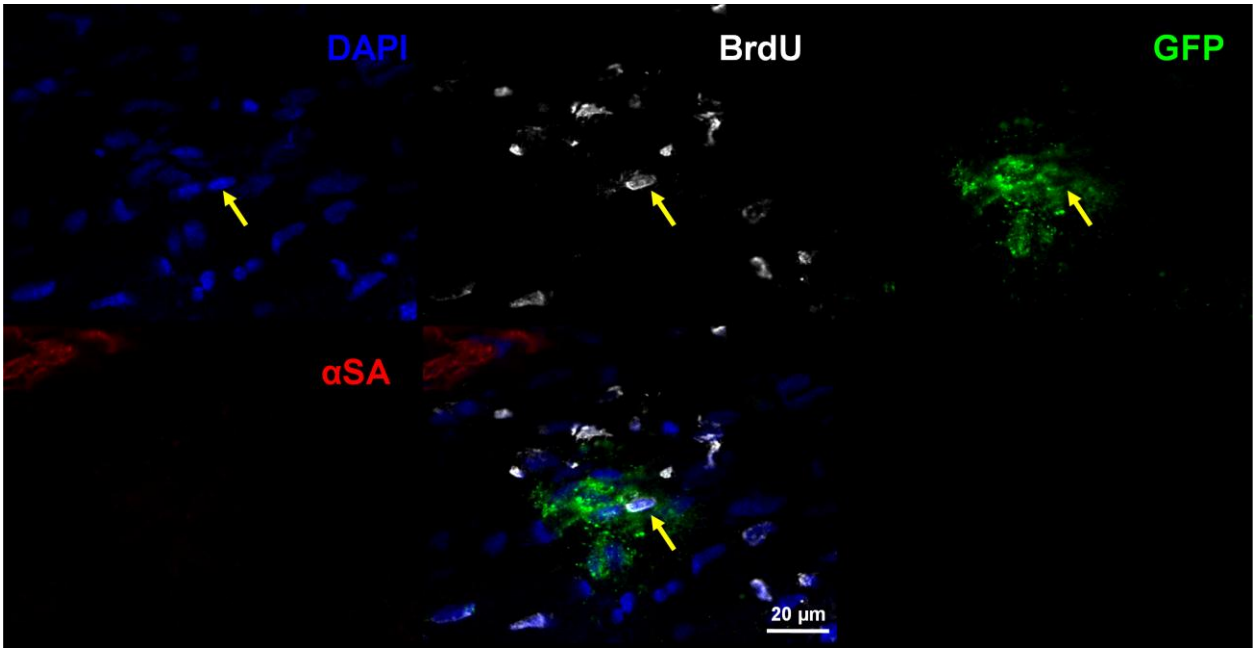
Supplemental Figure 2. Assessment of humoral memory response. Levels of circulating antidonor IgM (A) and IgG (B) antibodies were measured with flow cytometry at 1 week and 3 weeks post treatment (n=4/group at each timepoint). Allogeneic CDC transplantation (contrary to xenogeneic) did not generate detectable levels of circulating anti-donor antibodies. (* $p<0.05$ compared to syngeneic, allogeneic groups)



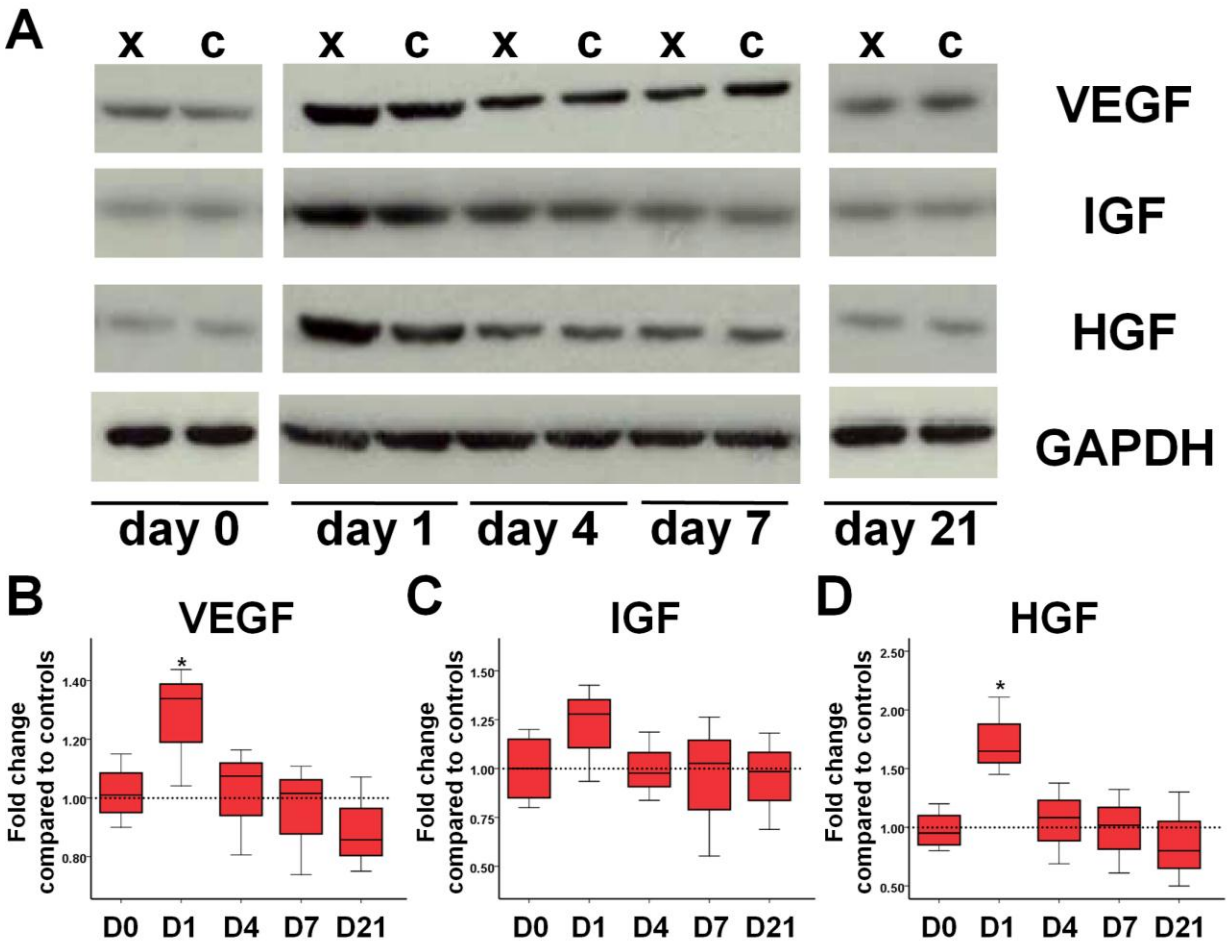
Supplemental Figure 3. Assessment of cellular memory response. (A) Representative images of syngeneic, naïve allogeneic and sensitized allogeneic cocultures. Significant lymphocyte proliferation can be observed in the sensitized allogeneic setting. Quantitative analyses of (B) responder cell proliferation (n= 6-8/group) and (C) inflammatory cytokine secretion (n=21-28/group). (* $p<0.05$ compared to syngeneic, allogeneic groups)



Supplemental Figure 4. Evaluation of immune reaction by H&E staining (A,B) (n=4/group) and immunohistochemistry (C) at 6 months post treatment (n=3/group). The mild immune reaction observed at 3 weeks had completely resolved by 6 months.



Supplemental Figure 5. A cluster of GFP+ CDCs in the peri-infarct area at 3 weeks post treatment. One CDC (arrow) is BrdU+ indicating *in vivo* proliferation.



Supplemental Figure 6. Detection of beneficial paracrine factors by Western blotting in the xenogeneic group. (A) Representative blots demonstrating increased secretion of VEGF, IGF1 and HGF only at day1 post xenogeneic cell transplantation. No difference is observed at later time points. (B-D) Quantitative analysis of myocardial levels of VEGF (B), IGF-1 (C) and HGF (D) post-MI in the xenogeneic group (normalized to the control group; n=3/group at each timepoint). Only the levels at Day 1 are increased, consistent with the rapid clearance of xenogeneic transplanted CDCs (Fig. 3). (* $p<0.05$ compared to control group; x: xenogeneic; c: control)