Supplemental Material

Detailed Methods

Cell isolation and culture

Briefly, small pieces of myocardial tissue (explants) derived from male rats were placed on fibronectin-coated dishes. In the following days, cells exited the explants and formed an adherent monolayer on the dish surface with phase bright cells on top. These cells are harvested using mild enzymatic digestion and transferred to D-poly-lysine coated dishes, where they form three dimensional structures called cardiospheres which are enriched in cardiac progenitors. Cardiospheres are subsequently harvested and grown as monolayers in fibronectin-coated flasks – these cells are called cardiosphere-derived cells (CDCs). CDCs were cultured in IMDM medium (Invitrogen) containing 10% FBS, 10% glutamine and 0.1mM mercaptoethanol, and expanded to 3-5 passages prior to lentiviral transduction.

Lentivirus synthesis

The cDNA encoding the hNIS gene or the cDNA pGL4.10[luc2] encoding firefly luciferase (Promega, Madison, WI, USA) was sub-cloned in place of eGFP into the vector RRLsin18.cPPT.CMV.eGFP.Wpre, resulting in plasmids designated cpPPT.CMV.*h*NIS or pPPT.CMV.*f*luc. Viral vectors were produced by Lipofectamine 2000 (Invitrogen) transfection of 4 lentiviral vector plasmids into HEK293T cells (ATCC, Manassas, VA, USA). Vector-containing supernatant was collected 48 and 72 hours after transfection, filtered, and concentrated (Centricon Plus-70, Millipore, Billerica, MA, USA). Viral titer was assigned on concentrated supernatant by HIV-1 p24 ELISA (Dupont, Wilmington, DE, USA). For genetic labeling, rCDCs were transduced at a multiplicity of infection of 20 yielding transduction efficiencies of >70% for *h*NIS expression and >90% for *f*luc expression. NIS expression was confirmed by immunostaining using a monoclonal mouse anti-*h*NIS antibody (Abcam, Cambridge, MA, USA) and by in-vitro ^{99m}Tc-pertechnetate uptake, while luciferase expression was examined by immunostaining using a polyclonal goat anti-luciferase antibody (Promega) and by an in vitro bioluminescence assay. We have previously demonstrated that transduction of CDCs with firefly luciferase or *h*NIS at an MOI (multiplicity of infection) of 20 does not affect cell proliferation, using in vitro studies.[1, 2] Percentage of transduced cells was calculated by immunostaining prior to cell transplantation.

Flow Cytometry

Annexin V and Propidium iodide were used to identify apoptotic and dead cells, respectively. Annexin V was diluted at a concentration of 1 mg/ml in binding buffer and cells ($1x10^6$ cells) were re-suspended in 1ml of this freshly made solution. Cells were incubated for 10 min in the dark at room temperature then PI solution (0.1ml to give final conc. of 1mg/ml) was added to this solution 5 minutes prior to the analysis with flow cytometry equipment (with 10,000 events collected per sample). Cell debris was excluded by scatter gating and 10,000 gated events were collected per sample using BD Accuri C6 flow cytometer**.** For measurements of cell viability after dissociation, CDCs were trypsinized, counted and used immediately after suspension in media. Trypan blue measurements were compared to measurements by flow cytometry. For measurements of cell viability following suspension for 1hr and 6hrs, CDCs were tyrpsinized, counted and suspended in cell culture medium for 1hrs and 6hrs respectively, in the incubator at 37ºC prior to measurements by flow cytometry.

In vivo SPECT/CT imaging

Male WKY rats underwent left thoracotomy in the 4th or 5th intercostal space under general anesthesia (isoflurane inhalation, 4% for induction and 2% for maintenance). In the myocardial infarction group, the heart was exposed and the left anterior descending coronary artery was

ligated using a 5-0 silk suture. Three million NIS^+ rCDCs suspended in 100µl of IMDM (Invitrogen) were injected directly into the myocardium at three sites in the anterior wall of the left ventricle using a 30G needle. In the non-infarct group, the same procedure was followed as the myocardial infarction group with the exception of the ligation of the left anterior descending coronary artery. Subsequently, the chest was closed with a 3-0 silk suture. $^{99m}TcO_4^-$ (^{99m}Tc labeled technetium-pertechnetate; 555-740 MBq) and $^{201}TlCl$ (^{201}Tl labeled thallous chloride; 37-74 MBq) were injected intravenously via the tail vein immediately after intra-myocardial cell transplantation, to determine stem cell retention and myocardial perfusion. The isoflurane was turned off and the animal was monitored for spontaneous breathing and allowed to fully recover prior to imaging.

In vivo dual isotope SPECT imaging was performed 1hr after injection of ^{99m}Tc-pertechnetate and $^{201}TlCl$. CT imaging was performed prior to SPECT imaging. Both scans were performed on a small animal SPECT/CT system (X-SPECT-CT from Gamma Medica Inc., Northridge, CA) using inhalational isoflurane as the anesthetic agent, administered via a nose cone. Animals were allowed to recover in their cages after completion of imaging on day 0. After 24hrs, the same rats were re-injected with ^{99m}Tc-pertechnetate (555-740 MBq) and $^{201}TlCl$ (37-74 MBq) via the tail vein and *in vivo* dual isotope SPECT-CT imaging was performed. The rats were euthanized after completing the 24hr imaging protocol.

In a second study, rats were serially imaged at 1d, 3-4d and 7d following intra-myocardial transplantation of NIS^+ or $fluc^+$ rCDCs.

SPECT/CT image acquisition and processing: The SPECT module X-SPECT-CT system is composed of two gamma camera heads each consisting of pixelated NaI(Tl) with a total area of 125 mm × 125 mm, divided into 80 × 80 number of pixels with 1.56 mm pitch. Low-energy knife-edge pinhole collimators were used with a pinhole aperture of 1 mm diameter and a focal length of 9cm; a radius-of-rotation of 5.42 cm was used. Each camera head acquired 128 projections over a 180-degree range, with an acquisition time of 30s for each projection for all scans except for the ex vivo heart scans which was changed to 40s per projection.

In the dual isotope SPECT imaging, data were acquired in listmode and were subsequently re-binned into two energy windows ("75 keV +10%/−10%" and "140 keV +10%/−10%") to obtain separate sets of ^{201}Tl and ^{99m}Tc projections. The ^{99m}Tc and ^{201}Tl projection datasets were reconstructed using a 3D pinhole ordered-subset expectation-maximization (OS-EM) imaging reconstruction algorithm with 8 and 4 updates, respectively with an isotropic reconstructed image voxel size of 0.7 mm.

X-ray computed tomography (CT) was performed on the microCT module with an X-ray tube voltage of 75 kVp. A total of 512 projections were acquired over a 360-degree range. The projections with 1,184 × 1,120 isotropic pixels (100 µm) were reconstructed into a CT volume of 512^3 isotropic voxels with 170µm pixel size. The SPECT and CT were then registered using rigid body transform, with pre-set parameters specific to the system.

SPECT image quantification: For absolute quantification, a calibration factor (CF) was calculated from an experimental study by inserting a small hollow sphere filled with a known amount of radioactivity of ^{99m}Tc or ^{201}Tl in water in an average rat-size water-filled cylindrical phantom to simulate a rat scan. Dose-response plot for ^{99m}Tc-pertechnetate was obtained by dual isotope SPECT imaging of varying doses of ^{99m}Tc-pertechnetate and ^{201}Tl and then used to calculate the calibration factor. SPECT data for the phantom were acquired using exactly the same acquisition settings as those used in the animal experiments. In this case, CF (MBq/i.i.) was defined as the quotient of the known activity concentration (MBq/ml) within the radioactive sphere in the phantom divided by the measured mean image intensity (i.i/cm^3) within a ROI drawn over the small sphere in the SPECT image of the phantom.

To quantify the tracer uptake *in vivo*, regions-of-interest (ROI) were manually defined on a region of increased focal tracer uptake and on a contra-lateral normal region of a mid-myocardial section. In the case of no observable increased focal myocardial tracer accumulation, an ROI was placed on the distal anterior wall. The total radioactivity at the region of interest was calculated by the image intensity within the ROI multiplied by the CF. The radioactivity concentration (MBq/mL) within the ROI was calculated by the total activity divided by the volume of the ROI. The background activity was calculated by placing an ROI on the baso-lateral wall of the heart.
Myocardial perfusion polar maps were generated by combining the short-axis image slices through the left ventricular myocardium of the ^{201}Tl SPECT dataset. A perfusion defect was extracted from a threshold of 60% of the maximum intensity of the myocardium.

***Ex-Vivo* SPECT imaging**
The same procedure was adopted as for the in vivo imaging group, except that only ^{99m}Tc-pertechnetate was injected. For the 1hr ex-vivo group, the rats were injected with ^{99m}Tc-pertechnetate (555-740 MBq) immediately after injection of the NIS^{+} rCDCs and imaged 1hr later. Immediately after the SPECT scan, the rat was sacrificed and the heart was rinsed with PBS and washed thoroughly to remove any remaining blood before ex vivo scanning. Imaging parameters were identical to the ones used in the *in vivo* acquisitions, with the exception of the time per projection which was decreased to 40s per projection. For the second group of rats, the same procedure was performed as the 1hr *ex vivo* rats with the exception that the rats were injected with ^{99m}Tc-pertechnetate (555-740 MBq), imaged and sacrificed 24hrs following cell transplantation.

Quantification of engraftment by ex vivo luciferase assay: We performed the ex vivo luciferase assay in a separate set of animals, to quantify engraftment of *f*luc^{+} rCDCs in the first 24hrs following transplantation. This assay has high sensitivity and is not affected by conditions such as ischemia/hypoxia, cellular metabolism and diffusion of luciferin to the cell transplantation site, which can all affect the in vivo signal, thus permitting reliable quantification of engraftment. For this purpose, we performed intra-myocardial injection of 1million *f*luc^{+} rCDCs in 12 animals (6 animals without myocardial infarction and 6 animals with infarction). Three animals from each group were sacrificed at 1hr and 24hrs after cell transplantation. A standard curve was constructed to calculate cell number.

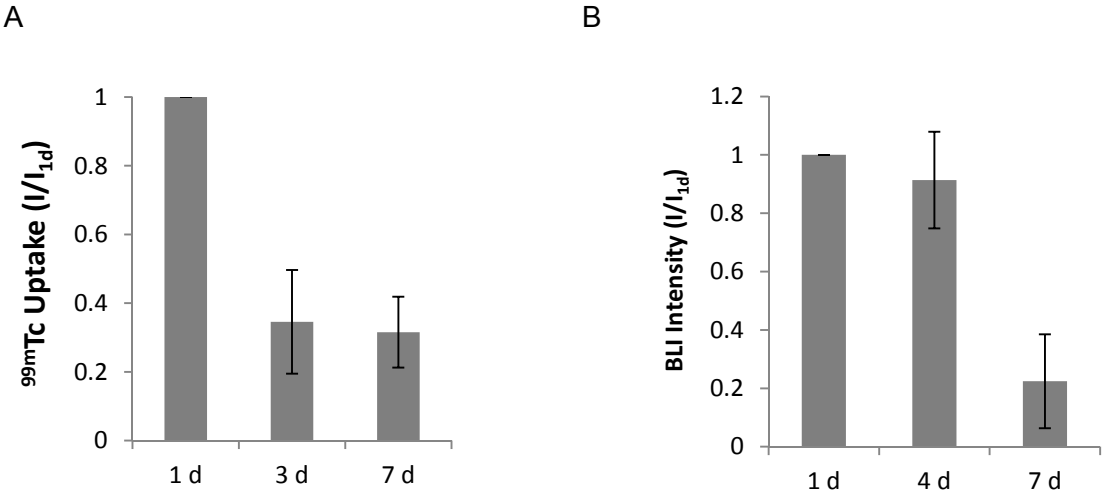
Hearts were harvested and cut into 200mg pieces, from apex to base (4-5 pieces/heart). The tissue samples were mixed with 1ml of lysis buffer (Promega) and 0.5ml of 10% BSA (as a non-specific protease inhibitor). Tissue samples were homogenized by manually grinding (Duall 24 Glass homogenizer, Kontes, Vineland, NJ, USA), the homogenates were collected in 50ml conical tubes (VWR, West Chester, PA, USA) and centrifuged at 25,000 g for 45min, at 4^{o} C. The supernatants were collected and a luciferase assay was performed using a Monolight 2010 luminometer (Analytical Luminescence Laboratories, USA). Twenty microliters of each sample was mixed with 100µl of luciferase assay reagent (Promega) in 75mm glass tubes (VWR) and placed in the instrument (2s measurements). Results were reported as relative light units (RLUs) and converted to cell numbers using the regression equation of the corresponding standard curve.

Standard curve preparation: Luciferase over-expressing rCDCS (from the same isolates that were used for the in vivo experiments) were also used for the standard curve preparation. Different numbers of rCDCs (starting from 500 up to 100,000 in duplicates) were pelleted. Hearts from normal male WKY rats were harvested and cut into small pieces. Cell pellets were lysed (Luciferase lysis buffer, Promega) and 200mg of rat heart tissue was added to the cell lysate. Cell lysates and tissue samples were processed as described above and values

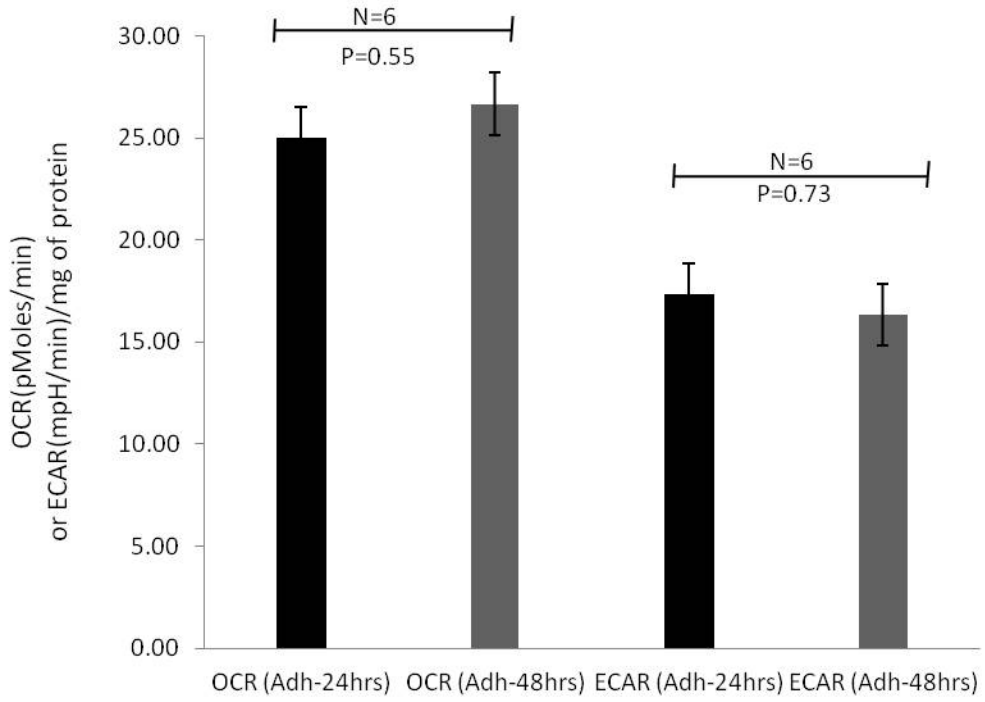
obtained by the luciferase assay were used to construct the standard curve. Luciferase activity demonstrated excellent linear corrrelation to cell numbers.

Quantification of engraftment by quantitative polymerase chain reaction (qPCR): Genomic DNA was isolated from aliquots of the homogenate corresponding to 12.5mg of myocardial tissue, according to the manufacturer's instructions (Qiagen). Real time PCR was performed using the TaqMan® chemistry (Applied Biosystems), with the rat SRY gene as target (forward primer: 5'-GGA GAG AGG CAC AAG TTG GC-3', reverse primer: 5'-TCC CAG CTG CTT GCT GAT C-3', TaqMan probe: 6FAM CAA CAG AAT CCC AGC ATG CAG AAT TCA G TAMRA). For absolute quantification of gene copy number, a standard curve was constructed with samples derived from multiple log dilutions of genomic DNA isolated from male rat CDCs. All samples were spiked with 50ng of female genomic DNA to control for any effects this may have on reaction efficiency in the actual samples. The copy number of the SRY gene at each point of the standard curve is calculated based on the amount of DNA in each sample and total mass of the rat genome per diploid cell. (http:www.cbs.dtu.dk/databases/DOGS/index.html). All samples were tested in triplicate. The qPCR assay was repeated twice with DNA samples isolated on 2 occasions, from each heart. 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, i.e. copies of the SRY gene in 50ng of genomic DNA, was expressed as the number of engrafted cells/heart, by first calculating the copy number of the SRY gene in the total amount of DNA corresponding to 12.5mg of myocardium and then extrapolating to the total weight of each heart.

Supplemental Figures and Figure Legends



Supplemental Figure I: Longitudinal in vivo SPECT/CT imaging of NIS$^+$ rCDCs (A) and BLI of fluc$^+$ rCDCs (B) over 7days revealed progressive cell loss from day1 to 7 post-transplantation (n=3).



Supplemental Figure II: In vitro studies reveal that cellular metabolism is stable after adhesion for 24hrs.

Supplemental References

1. Terrovitis J, Kwok KF, Lautamaki R, Engles JM, Barth AS, Kizana E, Miake J, Leppo MK, Fox J, Seidel J, Pomper M, Wahl RL, Tsui B, Bengel F, Marban E, Abraham MR. Ectopic expression of the sodium-iodide symporter enables imaging of transplanted cardiac stem cells in vivo by single-photon emission computed tomography or positron emission tomography. *J Am Coll Cardiol*. 2008;52:1652-1660
2. Barth AS, Kizana E, Smith RR, Terrovitis J, Dong P, Leppo MK, Zhang Y, Miake J, Olson EN, Schneider JW, Abraham MR, Marban E. Lentiviral vectors bearing the cardiac promoter of the na+-ca2+ exchanger report cardiogenic differentiation in stem cells. *Mol Ther*. 2008;16:957-964