

## Appendix E1

### Supplemental Methods

#### Cell Culture and Labeling

Immortalized mouse skeletal myoblasts (C2C12) were grown at 37°C and 5% CO<sub>2</sub> in Dulbecco's Modified Eagle's Medium (Life Technologies, Grand Island, NY) with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, Ga) and 1% penicillin-streptomycin (10 000 U/mL; Life Technologies). C2C12 cells were labeled with either Eu-HP-DO3A (provided by Dr Silvio Aime, University of Turin, Turin, Italy) or saline by using a hypotonic swelling technique (23). Cells were washed twice with sterilized (1×) phosphate-buffered saline, harvested by using 1.5 mL of trypsin–edetic acid solution (Life Technologies), and isolated after centrifugation (1000 revolutions per minute for 5 minutes). Afterward, cells were counted, divided into  $3 \times 10^6$  cells per 1-mL tube, and again centrifuged with subsequent removal of supernatant. Afterward, 500 μL (160 milliosmoles) of a hypotonic solution (100 μL of Eu-HP-DO3A stock [500 mmol/L], 107 μL of phosphate-buffered saline 1×, and 293 μL of distilled H<sub>2</sub>O) was added to each tube. Cells were incubated at 37°C for 30 minutes with agitation every 10 minutes, after which 500 μL of hypertonic and/or restoring solution (400 milliosmoles per liter of phosphate-buffered saline 1×) was added and incubated at 37°C for 30 minutes, with agitation every 10 minutes. Unincorporated agent was removed by washing the cells three times in phosphate-buffered saline 1×, after which cells were resuspended in 50 μL of phosphate-buffered saline 1×.

#### Immunohistochemical Analysis

Immunohistochemical analysis of macrophage cell fraction was performed by using the Metamorph analysis program (Molecular Devices, Sunnyvale, Calif). Boxes measuring 0.15 mm<sup>2</sup> were superimposed at three locations within the graft area in both groups ( $n = 2$  mice per group). The relative fraction of macrophages was calculated by counting the number of nuclei and positively stained cells. 4',6-diamidino-2-phenylindole·2HCl, or DAPI, cell numbers were estimated by using the integrated morphometric analysis plug-in. Images were background corrected to confirm positive F4/80 immunoreactivity. Fluorescent images were obtained by using a C2+ laser scanning confocal microscope (Nikon Instruments, Melville, NY).

#### Supplemental Discussion

In our prior study, we used inductively coupled plasma mass spectrometry to determine that  $2.1 \times 10^{-3}$  ng per cell of europium was present after hypotonic swelling, corresponding to a total delivery of  $5.7665 \times 10^{-7}$  g of Eu-HP-DO3A in  $10^6$  cells. Assuming that we label  $10^6$  cells to the same level and that all  $10^6$  cells rupture and release all of the Eu-HP-DO3A into the bloodstream simultaneously, this would equate to between  $1.9222 \times 10^{-4}$  mg/mL to  $1.15 \times 10^{-4}$  mg/mL. The commercially available preparation of gadolinium (Gd)-HP-DO3A contains 279.3 mg/mL (the molecular weight of gadolinium is about 4% heavier than europium). To perform late gadolinium enhancement imaging in a mouse model, 50 μL of stock solution is injected intravenously,

corresponding to 13.965 mg of Gd-HP-DO3A. When redistributed in the bloodstream, this equates to 4.655 mg/mL to 2.793 mg/mL, both of which are several orders of magnitude higher than the Eu-HP-DO3A scenario described previously.

To image the same section location serially over time, we first measured the distance of the imaging section from the base of the left ventricle and then repeated the same longitudinal offset over time. This enabled us to reliably image the same position within the heart at each time point. Second, we used the geometry of the left ventricular anatomy and the unique substructures, such as the papillary muscles and right ventricular insertion points, as fiducial markers to guide our section placement over time. Figure E6 is the same map as shown in the article, with each of these features highlighted.