Supporting Information.

The Development of a Trimodal Contrast Agent for Acoustic and Magnetic Particle Imaging of Stem Cells

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	Total number of nanobubbles	Nanobubbles without iron oxide particles	Free iron oxide particles	% Nanobubbles without iron oxide particles	% Nanobubbles with iron oxide particles
Field of View 1	175	90	0	51%	49%
Field of View 2	60	16	7	27%	73%
Field of View 3	58	9	22	16%	84%
Field of View 4	110	29	13	26%	74%
Field of View 5	29	9	9	31%	69%

Table S1. A comparison of different field of views in TEM imaging. The total number of nanobubbles was counted for five fields of view. The number of nanobubbles without iron oxide particles was counted. The number of free iron oxide particles was also counted. Approximately 70% of the nanobubbles contain iron oxide particles.

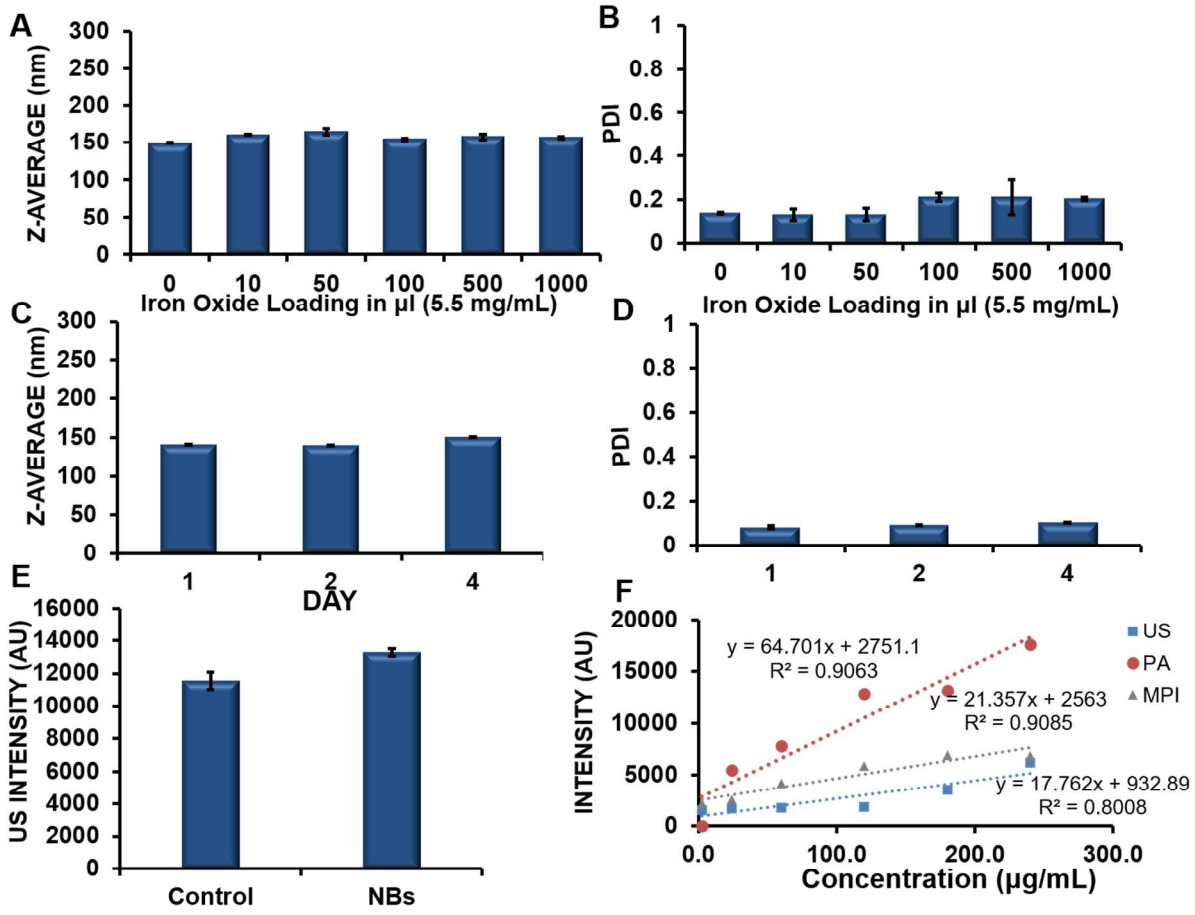

Figure S1. The effect of iron oxide loading on particle size (z-avg) and PDI. **A)** Varying the amount of iron oxide loading (from 0-1000 µl) did not significantly affect the particle size. **B)** Increasing the iron oxide loading (from 0-1000 µl) did not significantly affect the PDI. **C**) The particle size (z-avg) did not change as measured by DLS over 4 days in solution. **D**) The PDI of the nanobubbles remained the same after 4 days in solution. **E**) The ultrasound signal of the nanobubbles (NBs) was slightly higher than the ultrasound signal of the control PLGA particle without DiR or iron oxide. **F**) Linear relationship of the 3 modalities showing the dependency concentration of the nanobubble on the imaging intensity. As concentration increases, the imaging intensity increases for each modality.

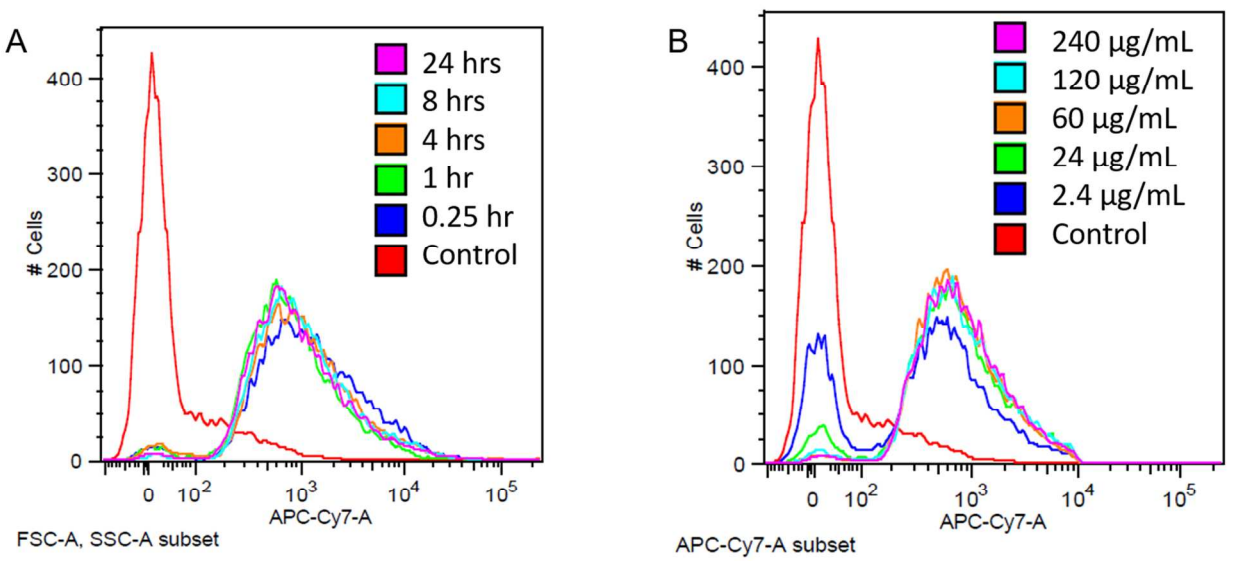

Figure S2. Flow Cytometry data of hMSCs treated with nanobubbles. The control indicates cells not treated with nanobubbles. **A**) Effects of varying incubation time for hMSCs treated with nanobubbles at a concentration of 240 µg/mL. Increasing incubation time from 0.25 – 24 hrs showed higher cell populations labeled. **B**) Effects of varying concentration for hMSCs treated for 8 hours with nanobubbles (µg/mL). Increasing the concentration of nanobubbles showed an increase in the fluorescence of the cells.

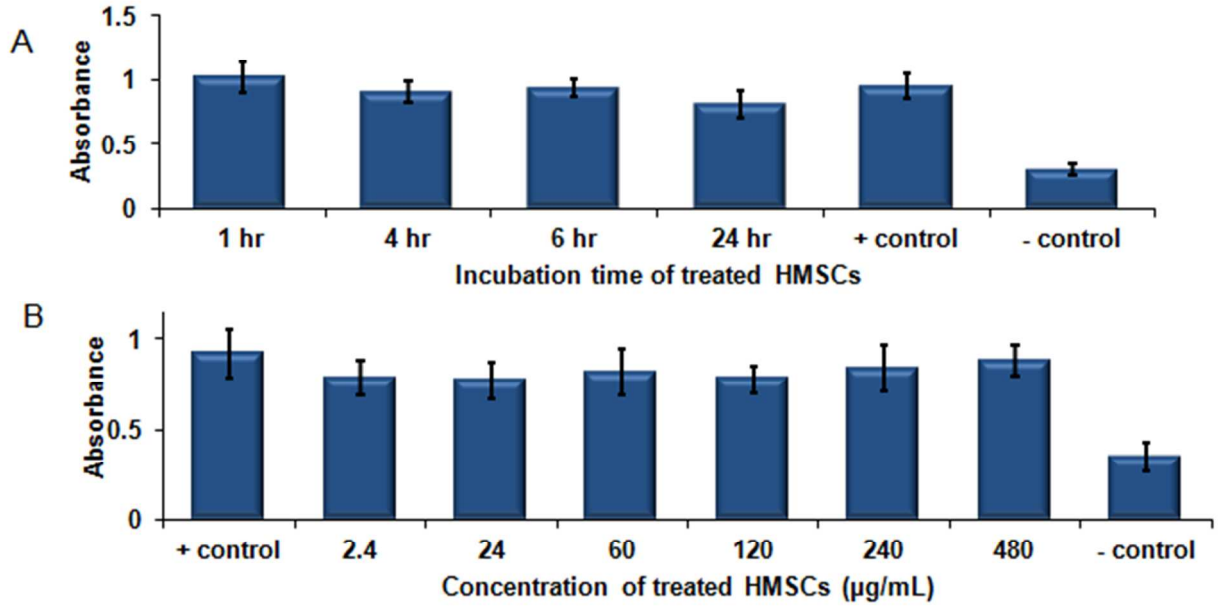

Figure S3. MTT assays of HMSCs treated with nanoparticles. A) Effects of incubation time for HMSCs treated with nanobubbles at a concentration of 240 µg/mL. Cell viability decreased by approximately 21% at 24 h of incubation with the nanobubbles. **B**) Effects of concentration for HMSCs treated for 8 hours at varying concentration with nanoparticles. The concentration up to 480 µg/mL of nanobubbles did not show toxic effects towards cells. The error bars represent the standard error between samples (N=8).

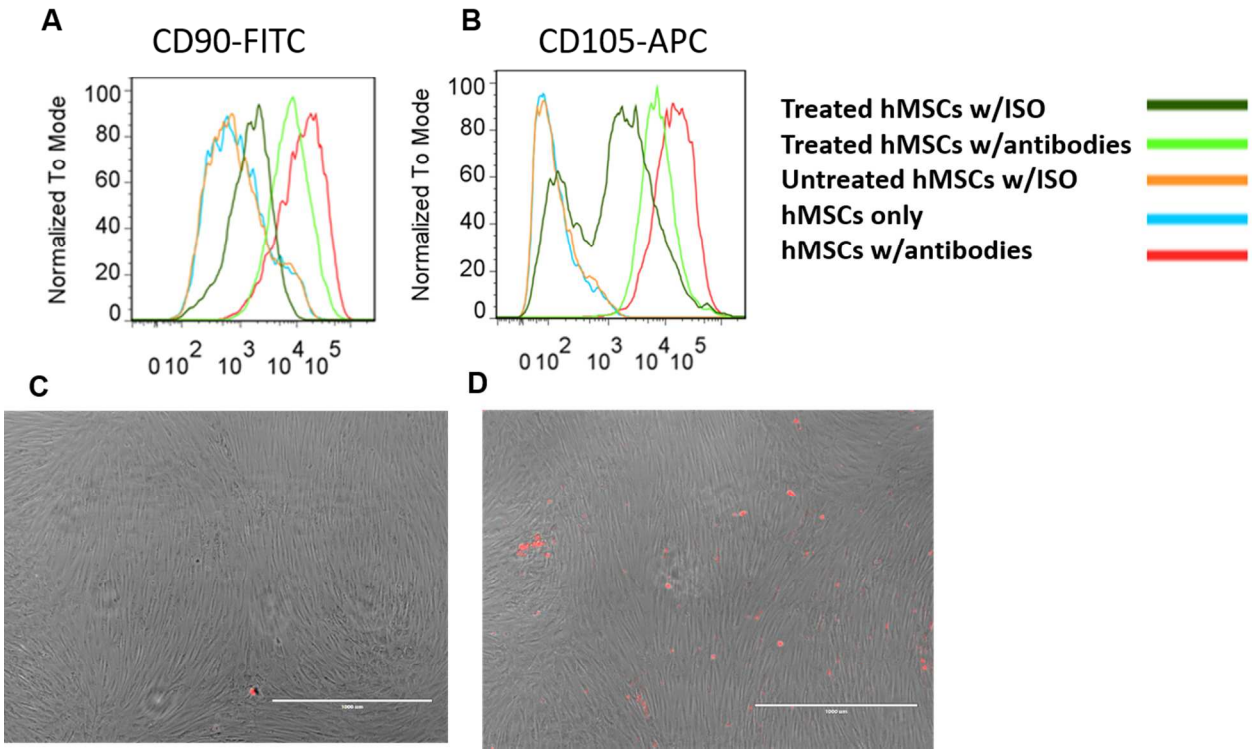

Figure S4. Flow cytometry data and endocytosis mechanism. The nanobubble-treated hMSCs retain characteristic markers as compared to untreated hMSCs. The treated hMSCs showed positive expression for surface markers (**A**) CD90 and (**B**) CD105. Isotype (ISO) controls for unlabeled and labeled hMSCs had low signal. **C**) Cells were treated with Dynasore, an endocytosis inhibitor, labeled with nanobubbles, and washed. Cells treated with this endocytosis inhibitor showed little fluorescence. **D**) Cells were labeled with nanobubbles, washed, and imaged with the Cy 7 filter. The red fluorescence indicates the presence of nanobubbles in the hMSCs. Scale bar = 100 µm.

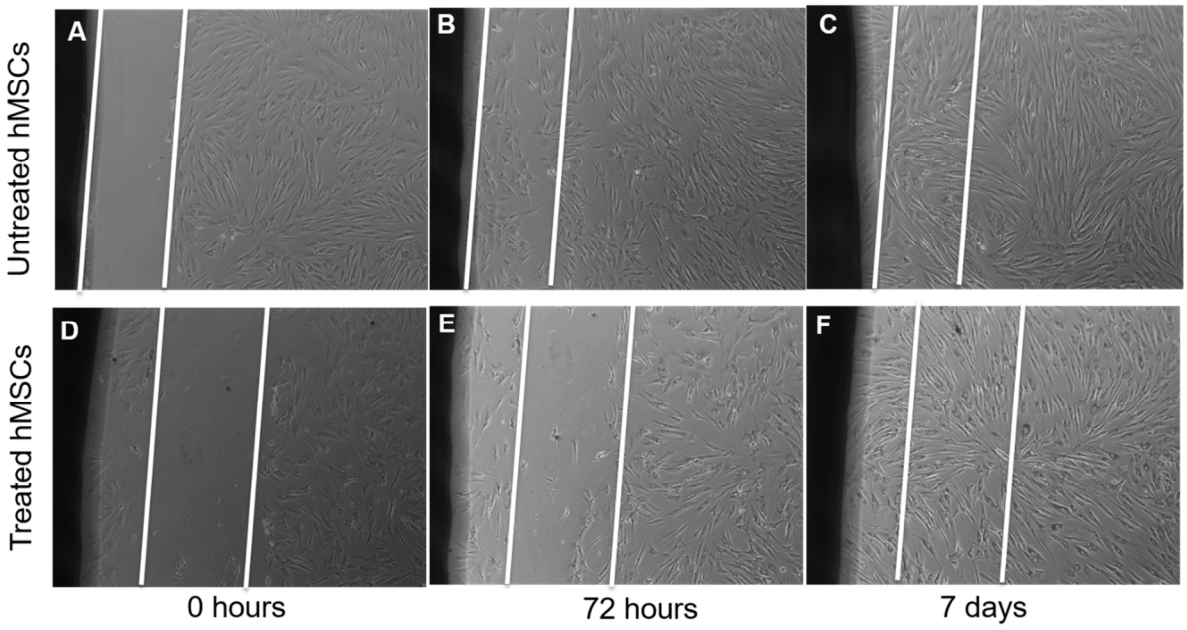

Figure S5. Cell migration assay. The migration ability of hMSCs treated with nanobubbles (240 µg/mL for 6 h) was studied by Leica optical microscopy with a monochrome camera at **(A, C**) 0 hours, (**B, E**) 3 72 hours, and (**C, F**) 7 days. The white line indicates the area where cells were removed. The black area is the fiducial marker used to orient the plate for imaging. The treated hMSCs (**D, E, F**) migrated to an area depleted of hMSCs in 7 days similar to the untreated hMSCs (**A, B, C**).

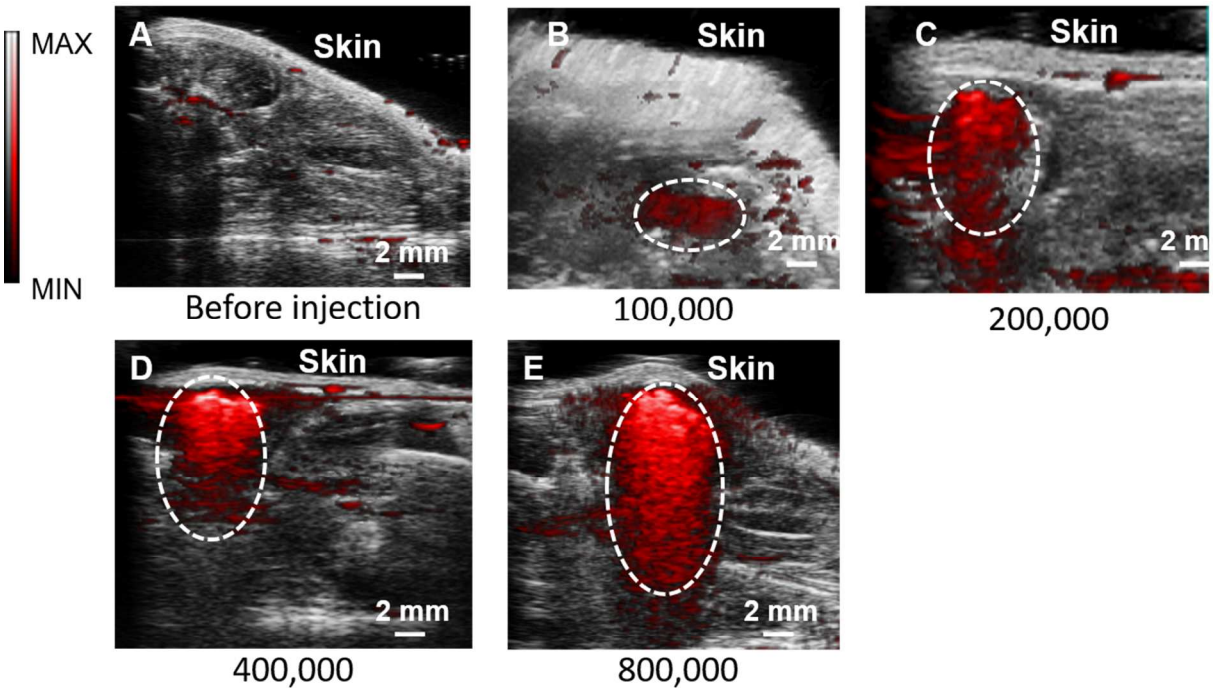

Figure S6. Subcutaneous injections of varying numbers of hMSCs treated with nanobubbles at a concentration of 240 µg/mL for 6 h. **A**) Before injection, **B**) Injection of 100,000 treated hMSCs, **C**) Injection of 200,000 treated hMSCs, **D**) Injection of 400,000 treated hMSCs, **E**) Injection of treated 800,000 hMSCs. The red area within the dotted white circle shows the PA signal from the injected cells.

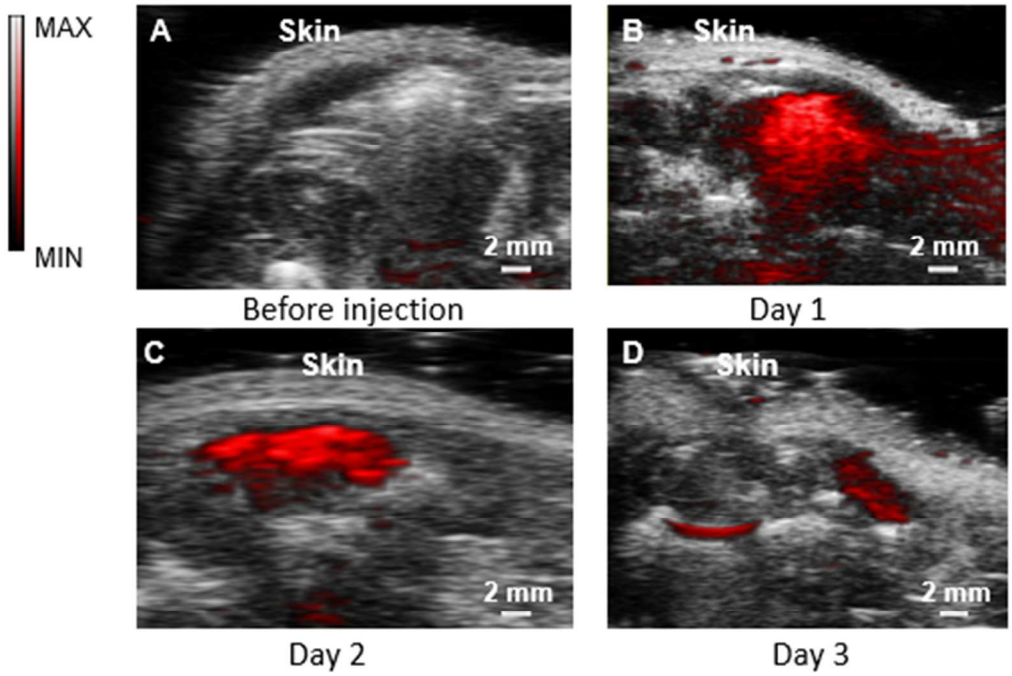

Figure S7. The signal from the nanobubble-treated hMSCs decreased from Day 1 – Day 3 in subcutaneous injections. A) Before injection, **B**) Day 1 of injection, **C**) Day 2 after injection, **D**) Day 3 after injection. hMSCs were treated with 240 μg/mL nanobubbles for 6 h. 400,000 hMSCs were injected.

Video S1. **US guided injection video showing the longitudinal axis view of a live mouse heart.** 800,000 hMSCs treated with 240 μg/mL of nanobubbles for 6 h were injected into the heart muscle. The video shows the increase in ultrasound signal after the injection.

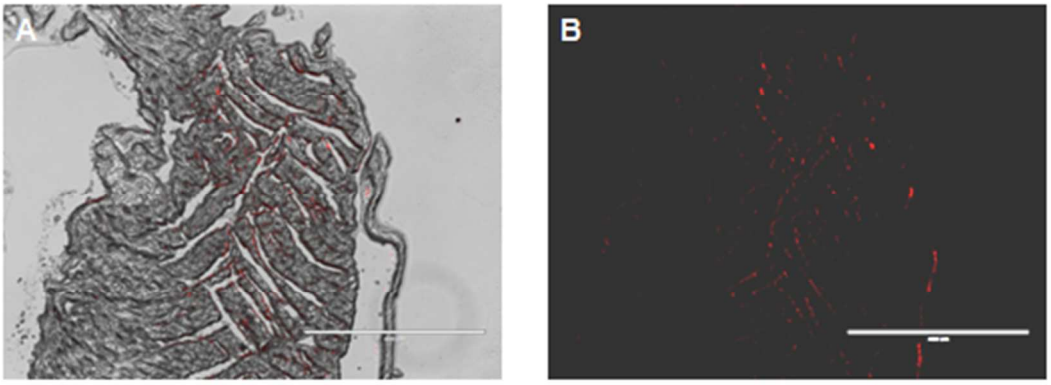

Figure S8. Histology sections show nanobubble-treated hMSCs were injected into mouse cardiac tissue (red dots). A) Overlay microscopy. **B**) Fluorescent microscopy of the nanobubble-injected hMSCs with Cy7 filter. The red fluorescence shows the presence of cells labeled with nanobubbles in the cardiac tissue. 800,000 nanobubble-treated hMSCs (240 μg/mL of nanobubbles for 6 h) were intramyocardially injected into a live mouse. Scale bar = 400 μm.