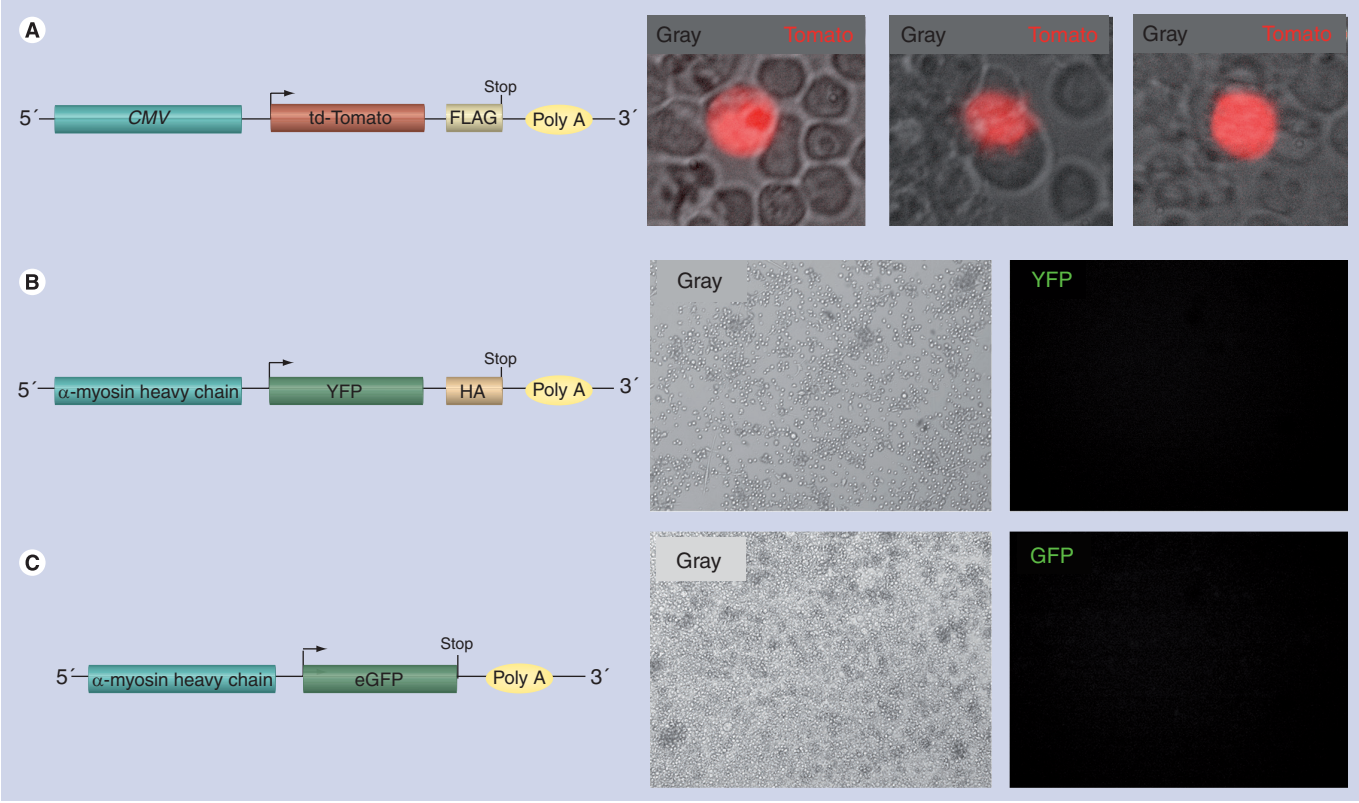
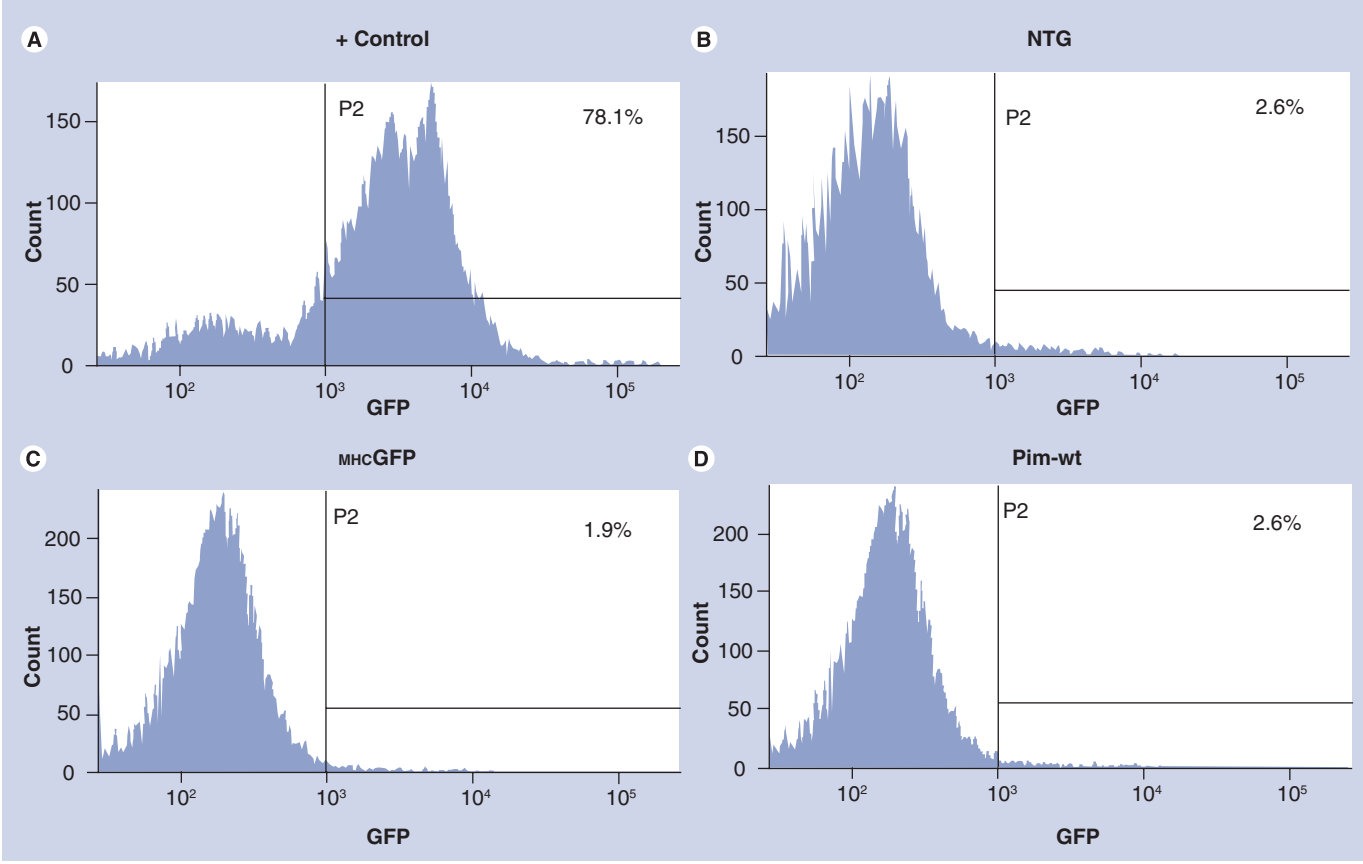
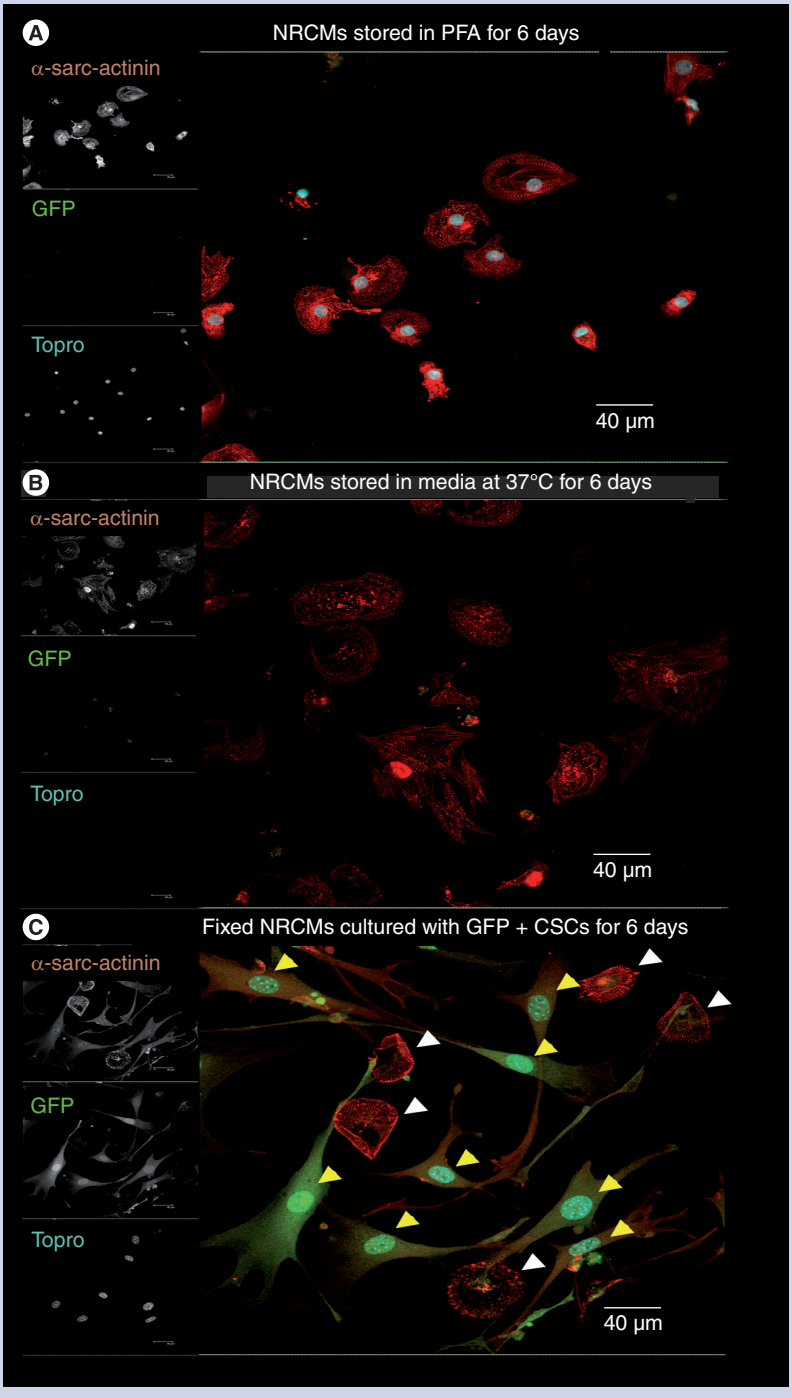


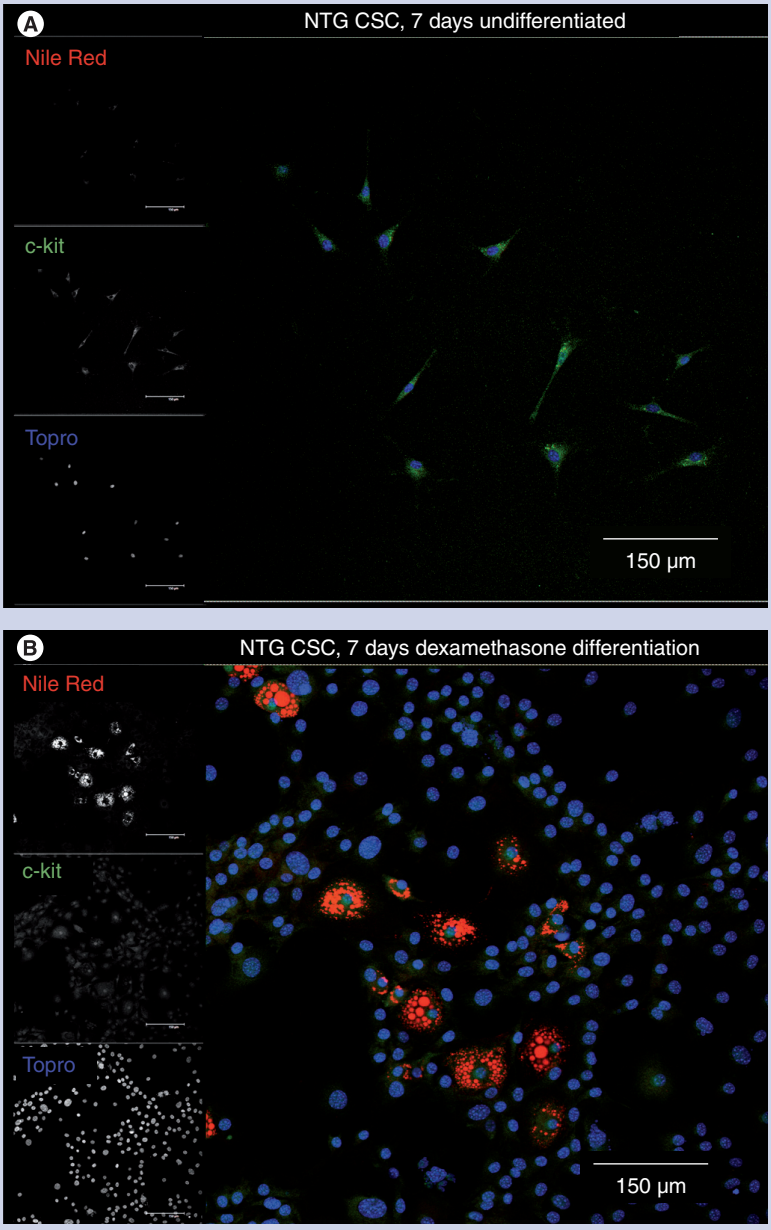
Supplementary Figure 1. The αMHC promoter is not active in primary cultured bone marrow stem cells. Nontransgenic bone marrow stem cells will not express fluorescent markers and tags when transfected with plasmid constructs under the control of the α-myosin heavy chain (αMHC) promoter. Nontransgenic bone marrow stem cells were cultured in growth media, transfected with plasmid constructs and allowed to express for 48 h. **(A)** Cells were transfected with a Tomato-Flag construct under the viral CMV promoter as a control. Td-Tomato protein fluorescence in red and phase-contrast pictures are in gray scale. **(B)** Cells were transfected with a YFP-HA construct under the control of the αMHC promoter. No fluorescence was observed (green) in a wide field of cells (gray scale). **(C)** Cells were transfected with a GFP construct under the control of the αMHC promoter. No fluorescence was observed (green) in a wide field of cells (gray scale).
CMV: Cytomegalovirus; eGFP: Enhanced green fluorescent protein; YFP: Yellow fluorescent protein.



Supplemental Figure 2. Cells in the bone marrow do not express α-myosin heavy chain promoter-driven green fluorescent protein. (A) Whole bone marrow from a Sca-1 knockin mouse expressing GFP under control of the endogenous Sca-1 promoter is GFP posititve by flow cytometric analysis. Whole bone marrow from **(B)** NTG, **(C)** $_{MHC}$GFP and **(D)** $_{MHC}$Pim-wt do not express GFP by flow cytometric analysis.
GFP: Green fluorescent protein; MHC: Myosin heavy chain; NTG: Nontransgenic.



Supplementary Figure 3. Postfixation incubation of NRCMs in media causes failure of NRCM nuclei to stain with Topro. (A) NRCM culture fixed and incubated in 4% PFA and stained for α-sarcomeric actinin (red) and Topro (teal). GFP fluorescence was scanned as a control (green). All NRCMs are Topro positive. **(B)** NRCM culture fixed in 4% PFA then incubated in media for 6 days at 37°C and stained for α-sarcomeric actinin (red) and Topro (teal). GFP fluorescence was scanned as a control (green). None of the NRCMs are Topro positive. **(C)** NRCM culture fixed in 4% PFA then incubated in media with GFP plus CSCs for 6 days at 37°C then stained for α-sarcomeric actinin (red) and Topro (teal). GFP fluorescence was scanned (green). None of the GFP-negative NRCMs are Topro positive (white arrows) and all of the GFP positive CSCs are Topro positive (yellow arrows).
CSC: Cardiac stem cell; GFP: Green fluorescent protein; NRCM: Neonatal rat cardiomyocyte; PFA: Paraformaldehye.



Supplementary Figure 4. Dexamethasone differentiation of cardiac stem cells induce an adipocyte-like phenotype. (A) NTG CSCs stained with Nile Red (red), a red fluorescent lipid dye, c-kit (green) and Topro (blue). NTG CSCs are c-kit positive and show very tiny punctuate staining with Nile Red. **(B)** NTG CSCs that have been differentiated in dexamethosone media for 7 days stained with Nile Red (red), c-kit (green) and Topro (blue). Some differentiated cells have an adiposite-like phenotype with large inclusions of lipid showing Nile Red staining.
CSC: Cardiac stem cell; NTG: Nontransgenic.

Supplementary methods

Bone marrow isolation & culture

Bone marrow cells (BMCs) were flushed from both tibias and femurs of each mouse using phosphate-buffered saline plus 1% fetal bovine serum buffer. BMCs were separated from red blood cells by density centrifugation using Histopaque® reagent according to manufacturer's instructions. The BMCs were either assayed by flow cytometric analysis fresh or cultured in serum-free StemSpan® media supplemented with 1% penicillin/streptomycin/glutamine, 100 ng/ml human stem cell factor, 100 ng/ml Flt-3, 20 ng/ml thrombopoietin, 20 ng/ml IL-3 and 20 ng/ml IL-6.

Supplementary Table 1. Antibodies and dilutions.

Primary antibody	Species	Dilution	Amplification (paraffin only)	Marker	Manufacturer
c-kit	Goat	1:50	Yes	Stem cells	R&D Systems
GFP	Rabbit	1:200	Yes	Tag	Molecular Probes
Pim-1	Rabbit	1:50	Yes		CST
α-sarcomeric-actinin	Mouse	1:100	No	Cardiomyocyte	Sigma
Desmin	Mouse	1:100	No	Cardiomyocyte	Biosource
Myc-tag	Rabbit	1:50	No	Tag	CST
Flag-tag	Mouse	1:100	No	Tag	Stratagene
HA-tag	Mouse	1:100	No	Tag	Santa Cruz

GFP: Green fluorescent protein.

Supplementary Table 2. Primer sequences.

Gene	F/R	5´-Primer sequence-3´	Product length (base pair)	Annealing temperature (°C)
Akt-myc	F	GAAGACATTCTGCGGAACGCCG	634	55
Akt-myc	R	GAGTTTTTGTTCTGCGGCCGTATC	634	55
Pim	F	GCACTCGAGTGCCCATGGAAGT	310	55
Pim	R	TCGATGAGCTTGAGCTCGCC	310	55
GFP	F	ATATCATGGCCGACAAG	190	55
GFP	R	TCTGATCGCGCTTCTCGTT	190	55
GAPDH	F	CAATTCAACGCCACACTCAA	150	55
GAPDH	R	CCTTCTCCATGCTGGTGAA	150	55