Supplementary Information

Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes following injury

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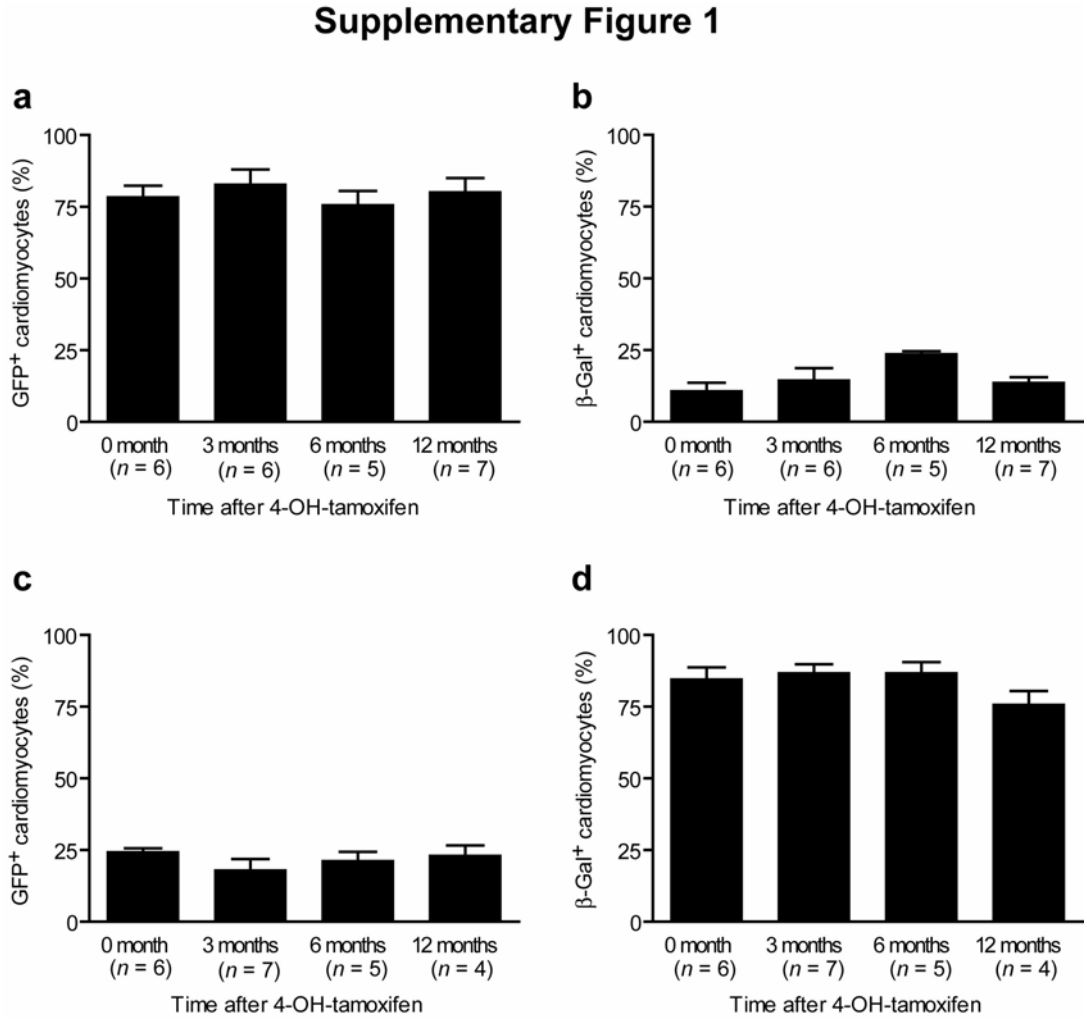
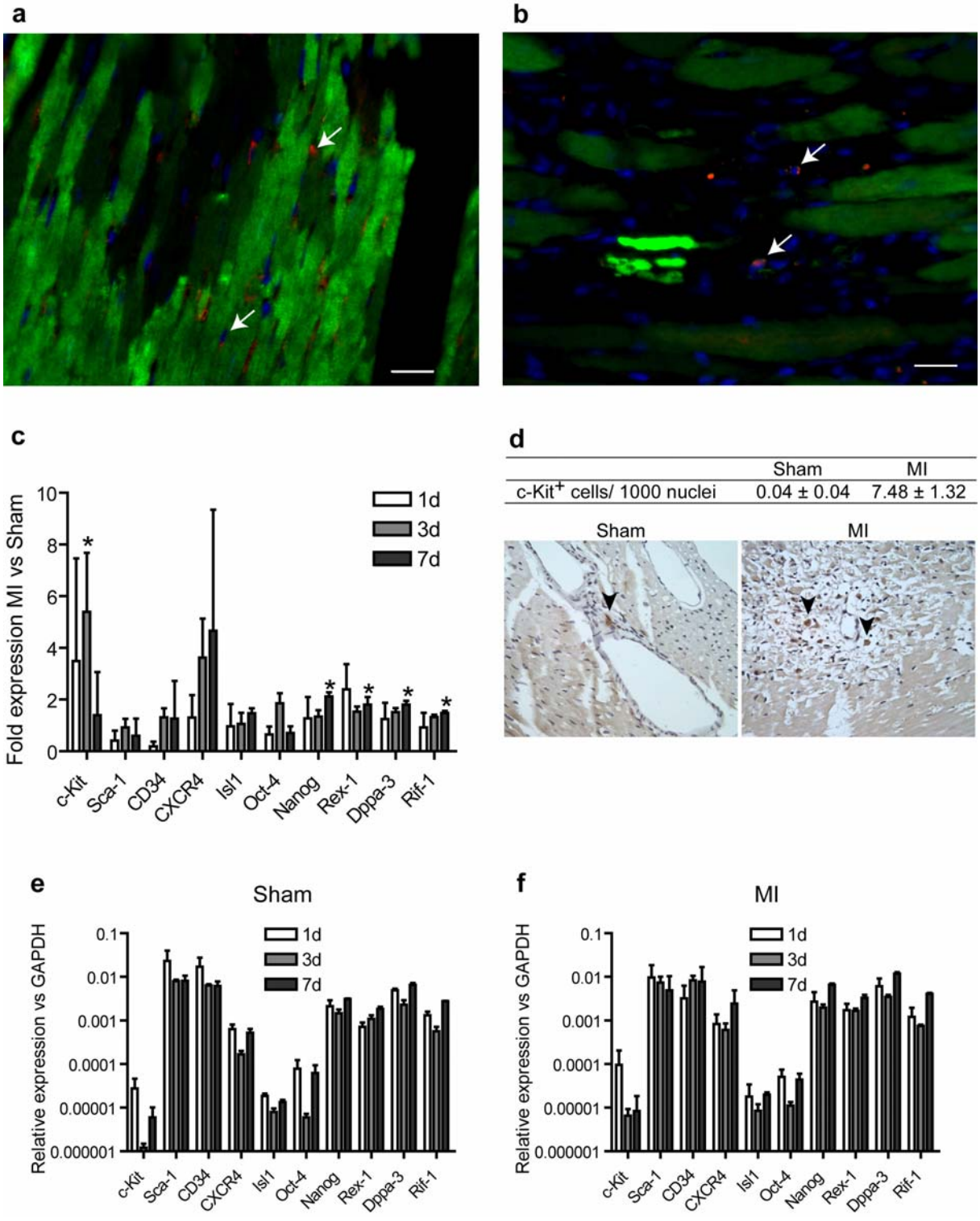


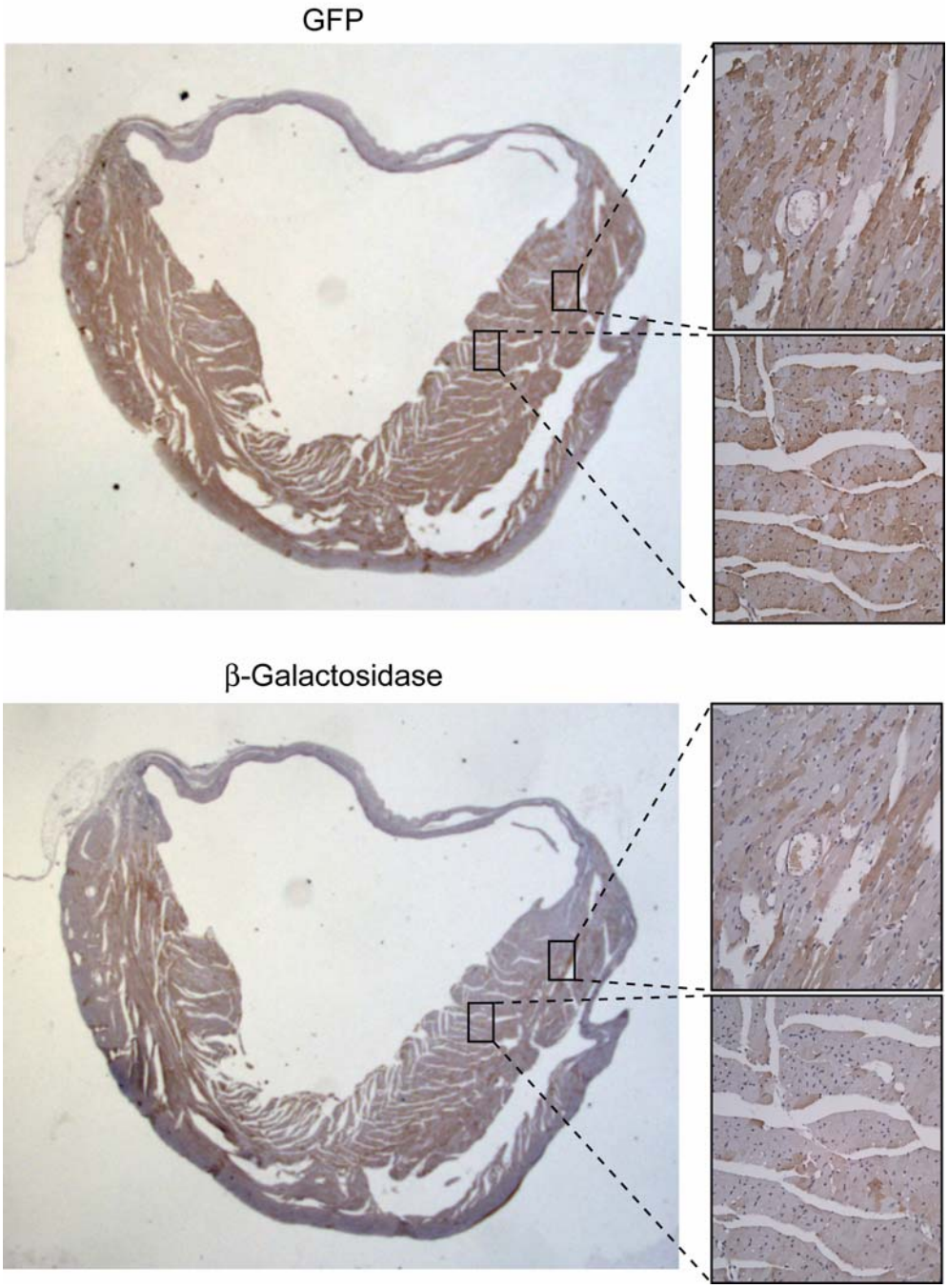
Supplementary Figure 1. Adult mammalian cardiomyocytes are not replaced by stem or progenitor cells during normal aging in right ventricle and atrium. (**a**) Percentage of GFP^+ cardiomyocytes during aging in right ventricle. At 0, 3, 6, and 12 months after the 4-OH-tamoxifen pulse, immunohistochemistry was used to detect GFP. Percentages of GFP^+ cardiomyocytes were 78.5±3.9% at 0 month (n=6), 83.0±5.1% at 3 months (n=6), 75.8±4.8% at 6 months (n=5), and 80.3±4.8% at 12 months (n=7) after the 4-OH-tamoxifen pulse. (**b**) Percentage of β-galactosidase$^+$ cardiomyocytes during aging in right ventricle were 10.9±2.8% at 0 month (n=6), 14.6±4.1% at 3 months (n=6), 23.8±0.8% at 6 months (n=5), and 13.8±1.7% at 12 months (n=7) after the 4-OH-tamoxifen pulse. (**c**) Percentage of GFP^+ cardiomyocytes during aging in atrium were 24.5±1.2% at 0 month (n=6), 18.1±3.7% at 3 months (n=7), 21.4±3.0% at 6 months (n=5), and 23.2±3.4% at 12 months (n=4) after the 4-OH-tamoxifen pulse. (**d**) Percentage of β-galactosidase$^+$ cardiomyocytes during aging in atrium were 84.8±4.0% at 0 month (n=6), 86.9±2.9% at 3 months (n=7), 86.8±3.7% at 6 months (n=5), and 75.9±4.6% at 12 months (n=4) after the 4-OH-Tamoxifen pulse. Mean±SEM.

Supplementary Figure 2



Supplementary Figure 2. Evidence for existence of c-Kit+ stem cell pool in adult mouse myocardium. (**a**) Immunofluorescence staining of GFP (green) and Sca-1 (red) in normal myocardium. DAPI = blue. (**b**) Immunofluorescence staining of GFP (green) and c-Kit (red) in normal myocardium. (**c**) Quantitative real-time PCR of myocardium from sham operated animals and infarcted myocardium. mRNA expression of c-Kit increased 5.4 fold (+2.3 (upper SEM), -1.6(lower SEM), p=0.027, n=4) 3 days after myocardial infarction as compared to sham surgery. Other stem cells markers showed a small but significant increase at day 7 after myocardial infarction: Nanog (2.1 +0.2,-0.2 fold induction), Rex-1 (1.8 +0.3,-0.2), Dppa-3 (1.8 +0.2,-0.1), and Rif-1 (1.5 +0.1,-0.1). (**d**) Numbers of c-Kit positive cells per 1000 nuclei in sham and MI operated hearts. Arrows= brown c-Kit positive cells, immunohistochemistry. (**e**) Relative expression of different genes to GAPDH in sham operated mice. (**f**) Relative expression of different genes to GAPDH in the infarcted area. * $P<0.05$. Mean±SEM.

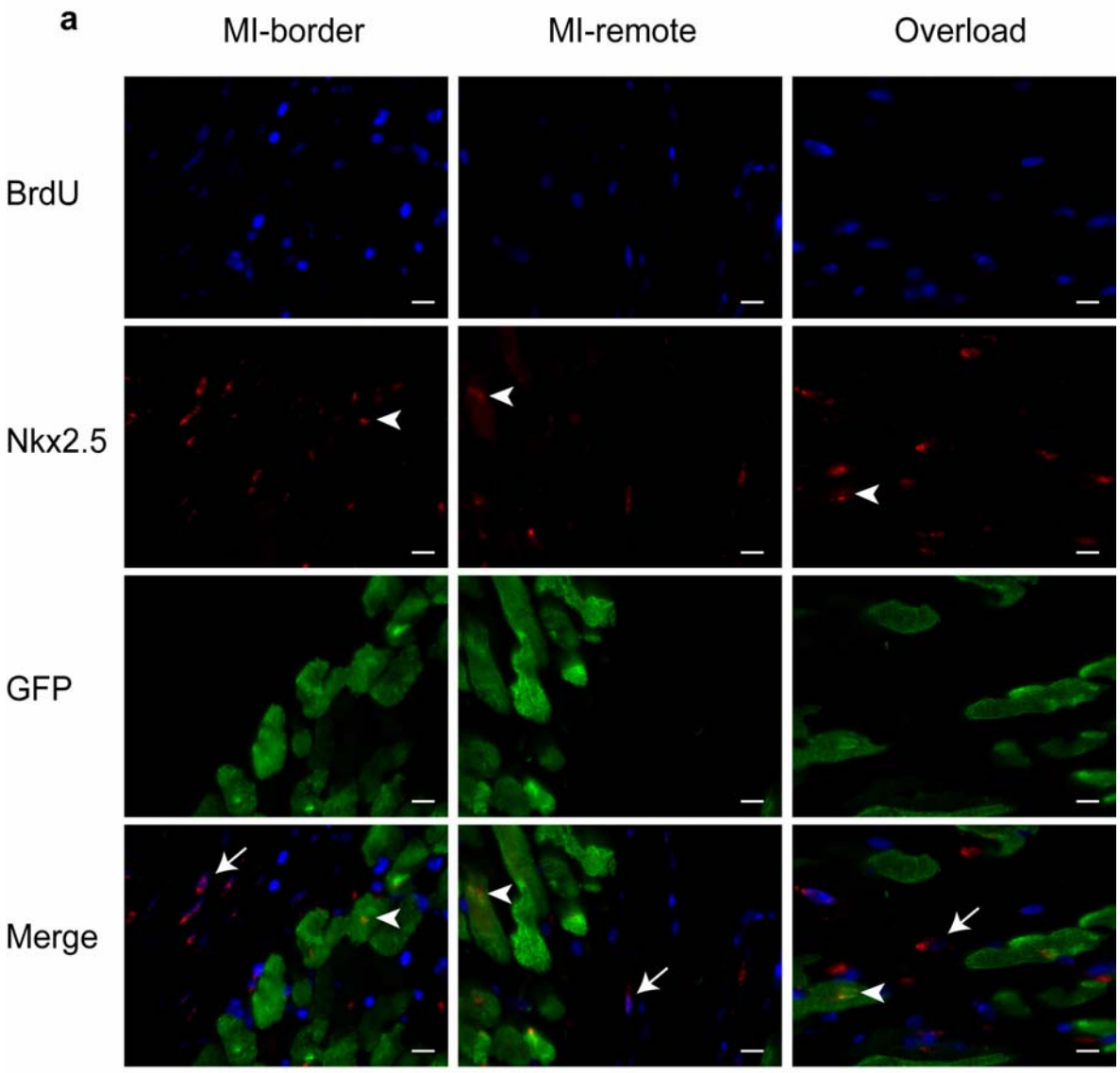
Supplementary Figure 3



Supplementary Figure 3. Images of a representative myocardial infarction. Images at low magnification of GFP or β-galactosidase immunohistochemistry staining (brown) with hematoxylin counterstain. Inserts show border and remote areas; note that these are not adjacent sections.

Supplementary Figure 4

a

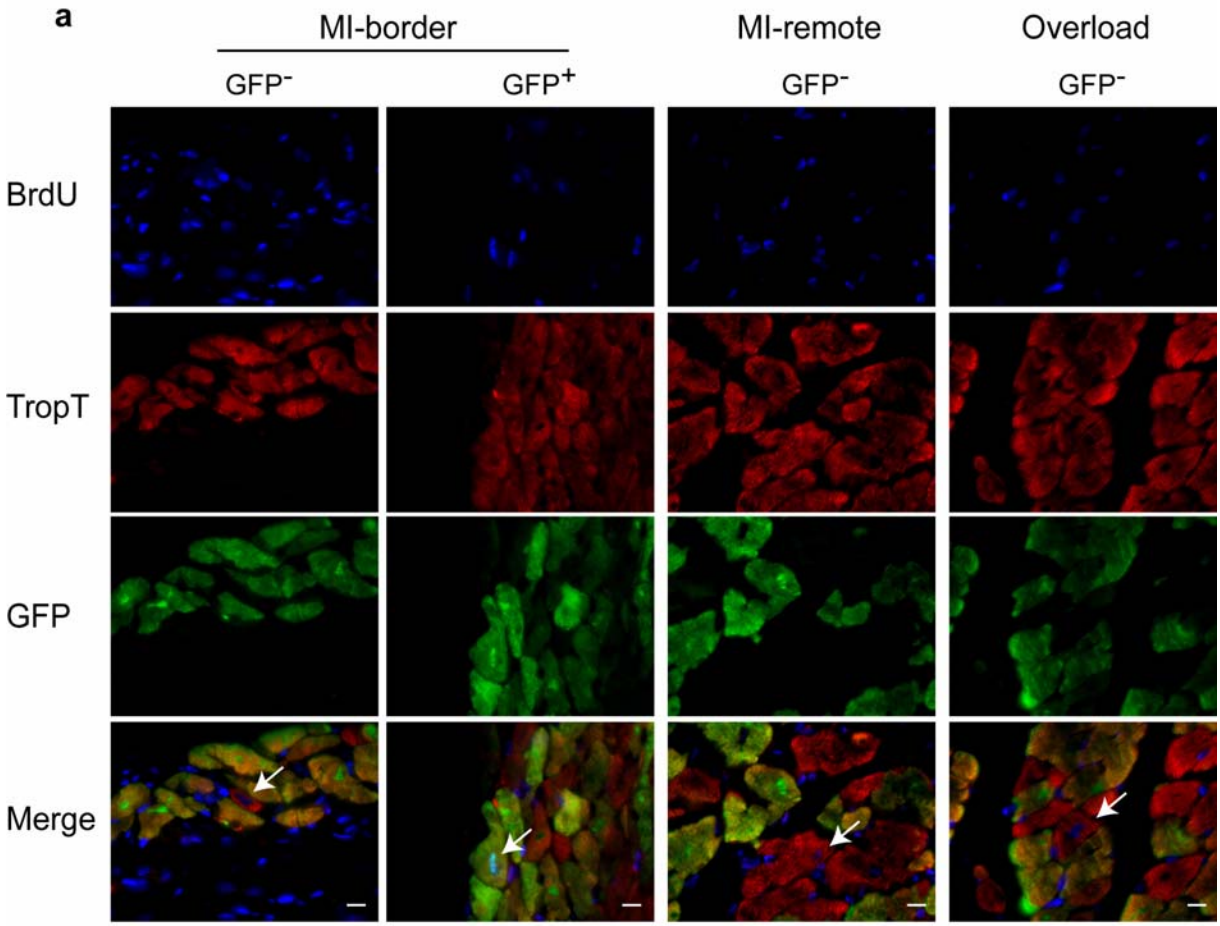


b

(NKX2.5/BrdU^{+} per 1000 Nkx2.5^{+})	10days	30days
Sham	0.0 ± 0.0	0.0 ± 0.0
MI-border	70.6 ±20.0	34.9 ± 7.5
MI-remote	4.2 ± 2.9	2.4 ± 2.4
Overload	2.4 ± 1.5	2.2 ± 0.8

Supplementary Figure 4. Stem or progenitor cells may contribute to the cardiomyocyte pool following myocardial injury. (**a**) BrdU/ Nkx2.5/ GFP staining for cardiomyocytes and/or precursors entering the cell cycle. Shown are representative images of triple staining (Nkx2.5, red; BrdU, blue; GFP, green) in sections from MerCreMer/ZEG mice 10 days after experimental MI or left ventricle overload. All $BrdU^+$ cardiomyocytes were GFP-negative. Arrows indicate $BrdU^+/Nkx2.5^+$ cells. Arrowheads indicate $Nkx2.5^+/GFP^+$ cardiomyocytes. (**b**) Summary of numbers of $BrdU^+$ $Nkx2.5^+$ nuclei per 1000 $Nkx2.5^+$ nuclei of hearts receiving sham operation, MI (both in the border and remote areas), or pressure overload. Scale bars=10 μm.

Supplementary Figure 5



b

(TropT/BrdU$^+$ per 1000 TropT$^+$)	GFP$^-$	GFP$^+$
Sham (n=5)	0.0 ± 0.0	0.0 ± 0.0
MI-border (n=5)	11.5 ± 1.7	0.7 ± 0.3 (P = 0.0002)
MI-remote (n=5)	2.1 ± 0.8	0.0 ± 0.0 (P = 0.047)
Overload (n=5)	1.8 ± 0.8	0.0 ± 0.0 (P = 0.054)

Supplementary Figure 5. Cardiomyocytes positive for BrdU 30 days after surgery. (**a**) BrdU/ Troponin-T/ GFP staining for cardiomyocytes. Shown are representative images of triple staining (Troponin-T, red; BrdU, blue; GFP, green) in sections from MerCreMer/ZEG mice 30 days after experimental MI or left ventricle overload. Most BrdU$^+$ cardiomyocytes were GFP-negative, some BrdU$^+$/Troponin-T$^+$/GFP$^+$ were present in the border zone after myocardial infarction (note GFP expression in the

cardiomyocyte nucleus). Arrows indicate $BrdU^+$/Troponin-T^+ cells. (**b**) Summary of numbers of $BrdU^+$/Troponin-T^+ nuclei per 1000 Troponin-T^+ nuclei of hearts receiving sham operation, MI (both in the border and remote areas), or pressure overload. P values compare $BrdU^+$/Troponin-T^+/GFP^+ to $BrdU^+$/Troponin-T^+/GFP^- cardiomyocytes. Scale bars=10 μm.

Supplementary Table 1: echocardiography data 2 weeks after aortic banding.

	Aortic banding (*n* = 7)	Age-matched controls (*n* = 6)
AWT (mm)	1.62 ± 0.05	0.84 ± 0.04*
PWT (mm)	1.52 ± 0.06	0.83 ± 0.04*
ESD (mm)	1.31 ± 0.26	1.13 ± 0.03
EDD (mm)	2.86 ± 0.19	2.48 ± 0.06

* P<0.0001.