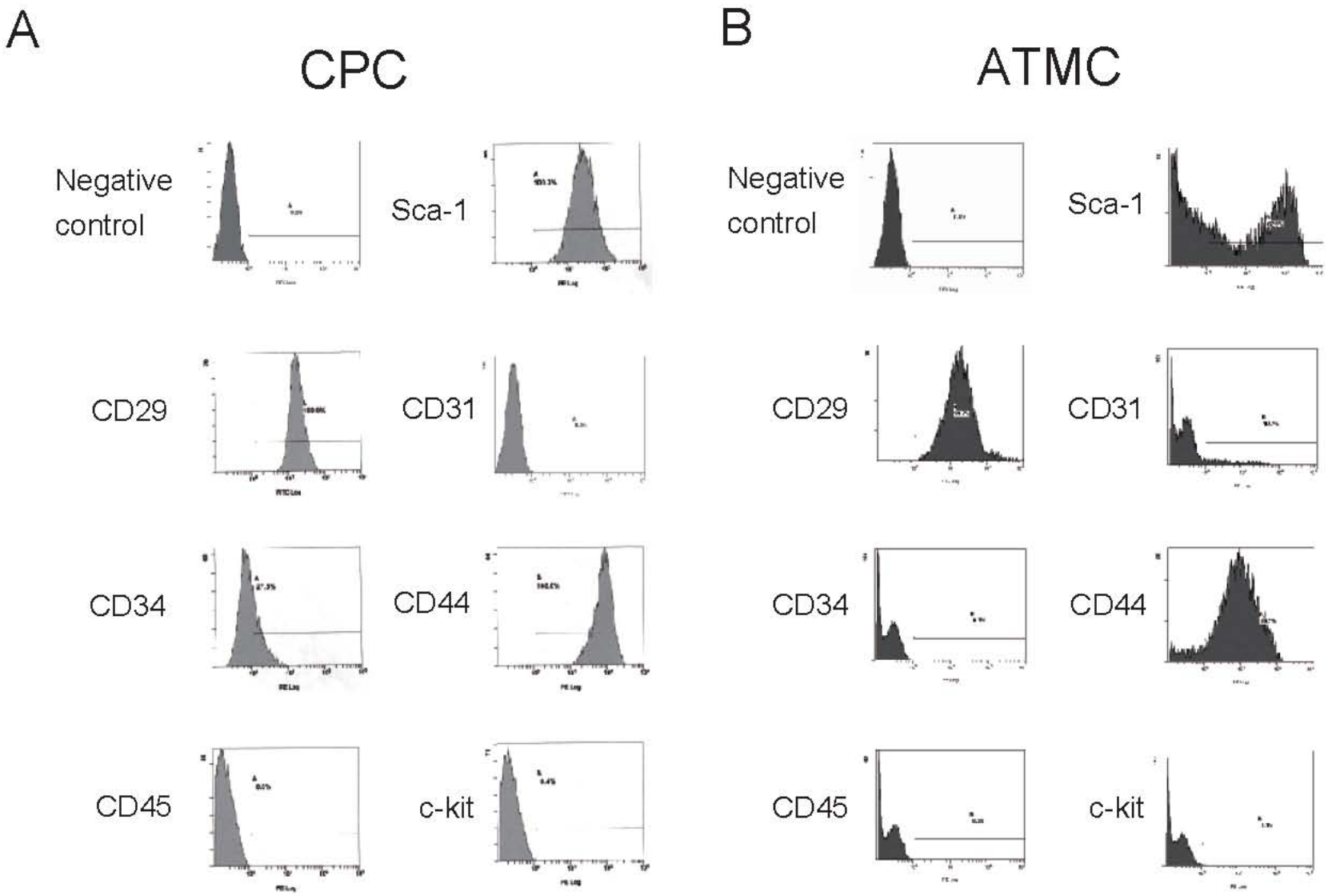
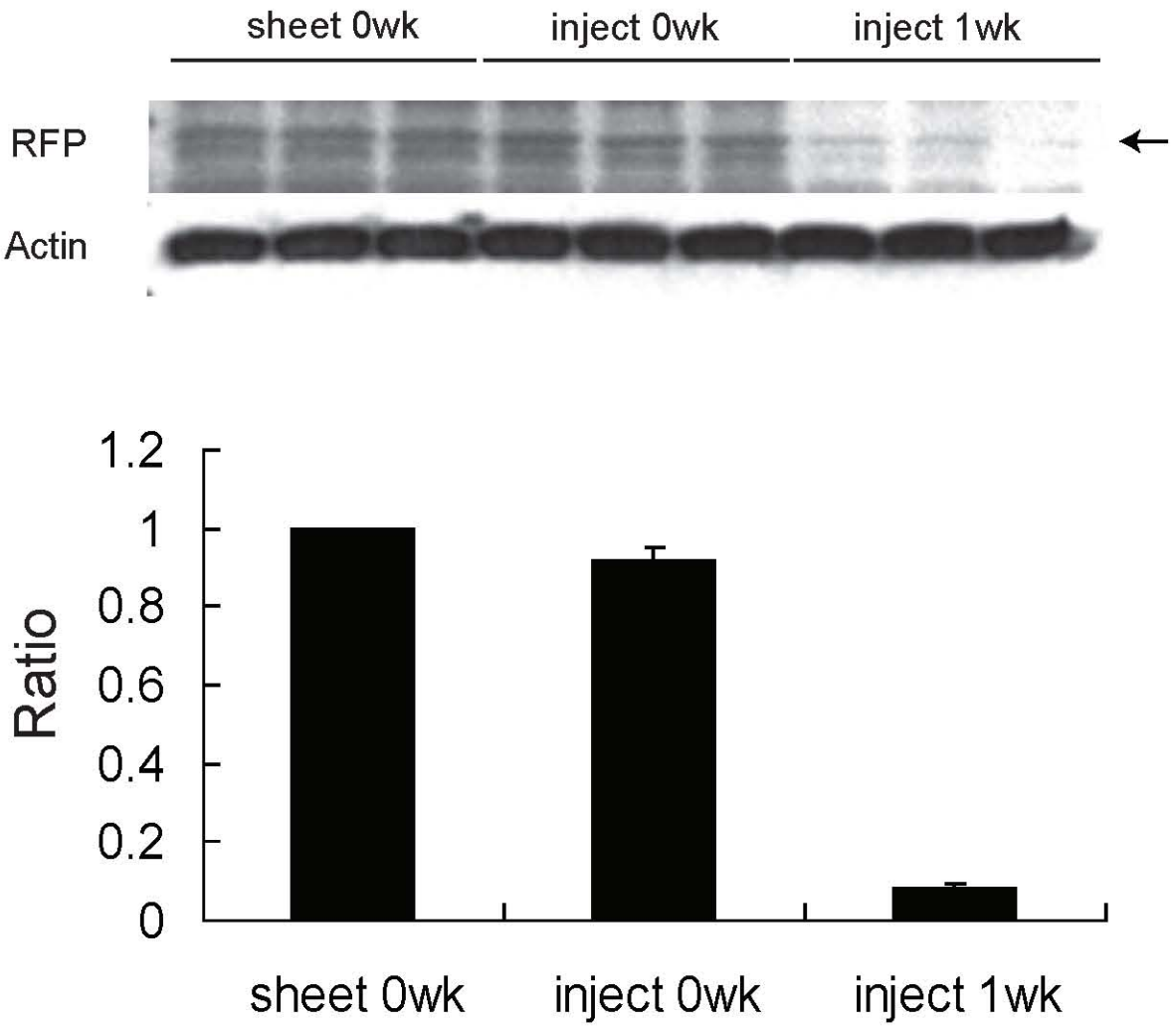
Supplemental Figure 1



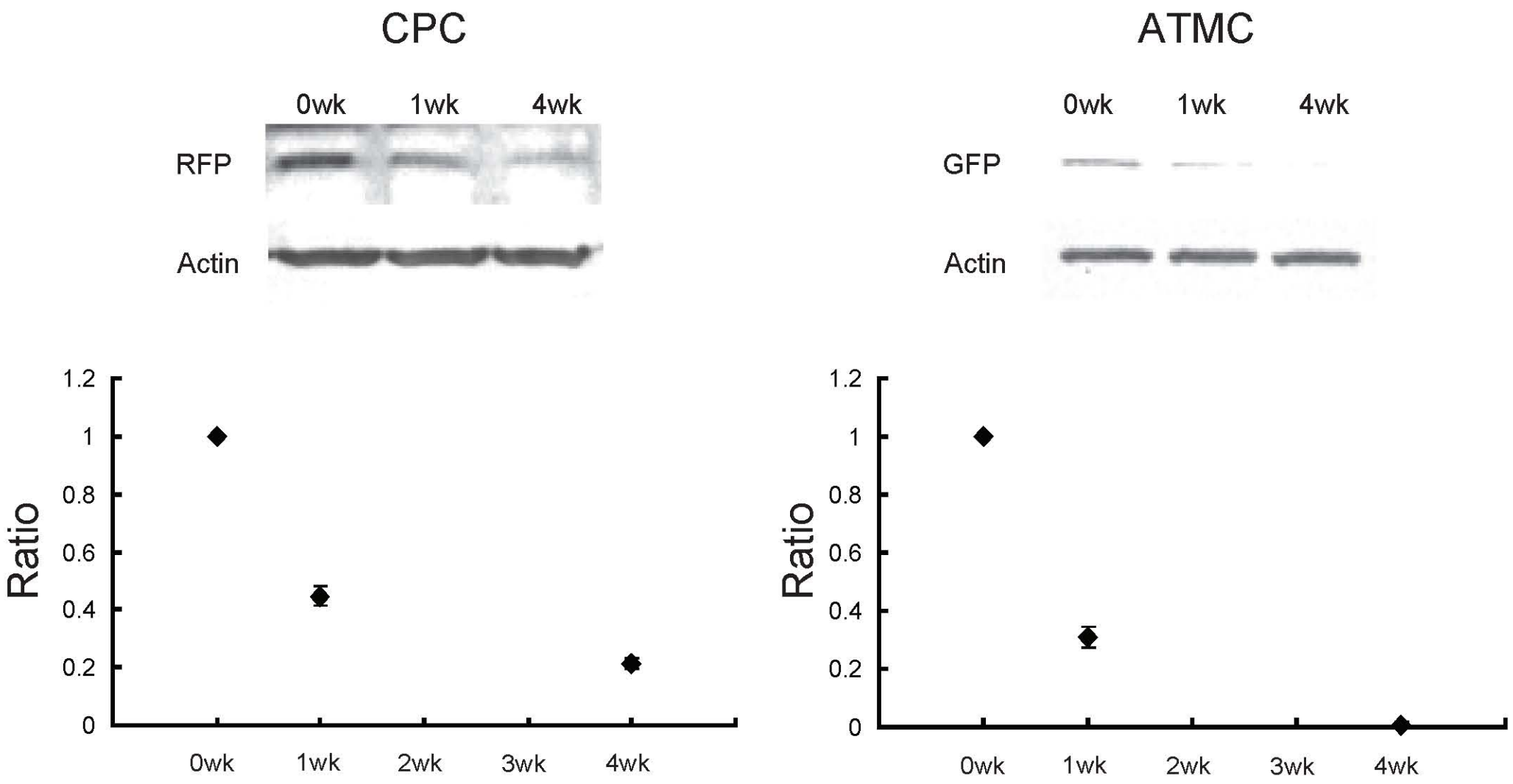
Flow cytometric analysis of CPC and ATMC. Representative images of cell surface antigens of CPC (A) and ATMC (B). All CPC expressed Sca-1, while about 60% of ATMC expressed Sca-1. Other cell surface antigens expression was almost similar between them.

Supplemental Figure 2



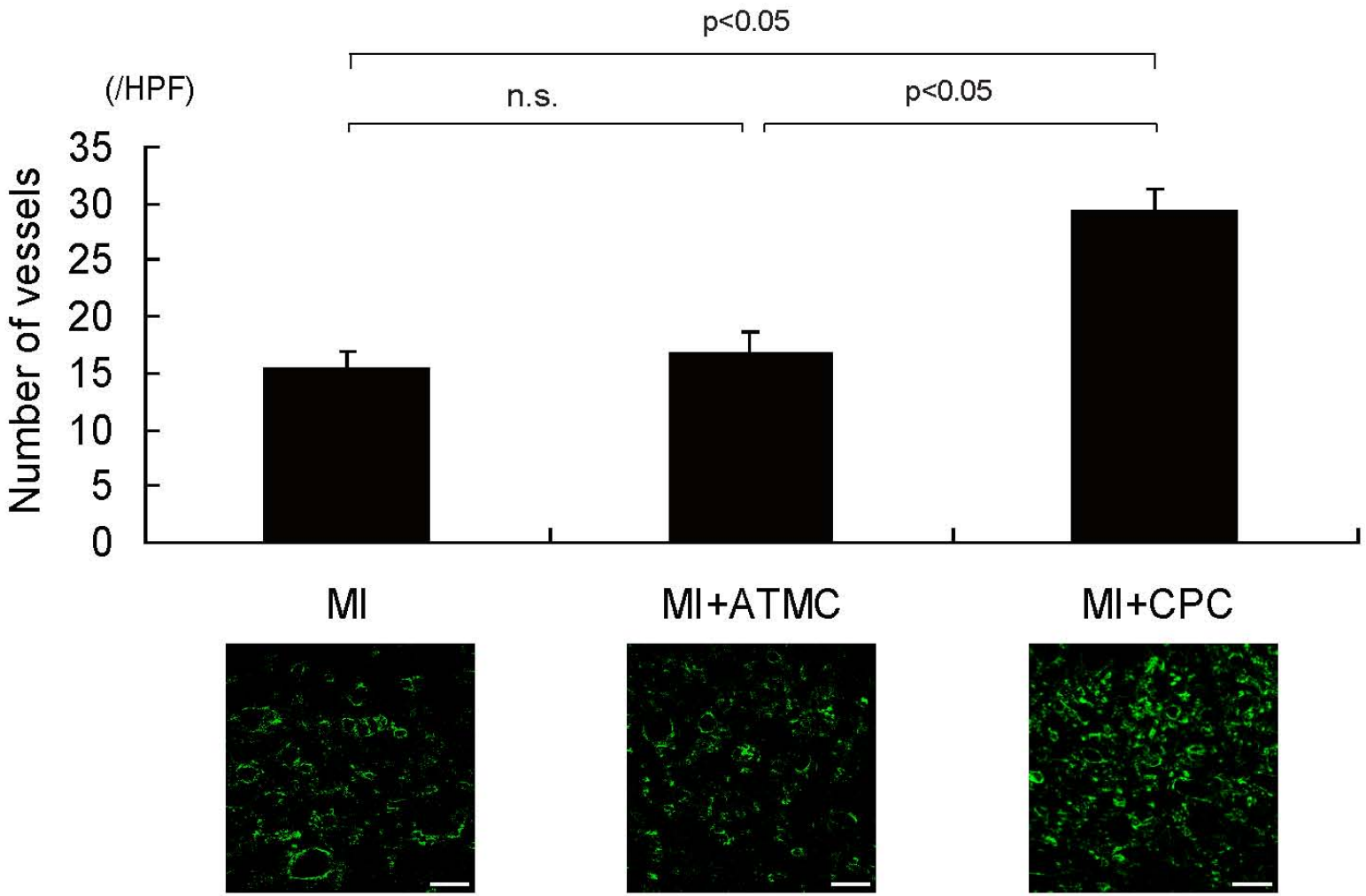
Survival ratio of transplanted cells. Upper panels show representative images of Western blot analysis. The ratio of RFP expression was calculated and is shown in the graph (lower panels, n = 3). The RFP expression in the heart immediate following cell sheet transplantation was used as a control (sheet 0 wk).

Supplemental Figure 3



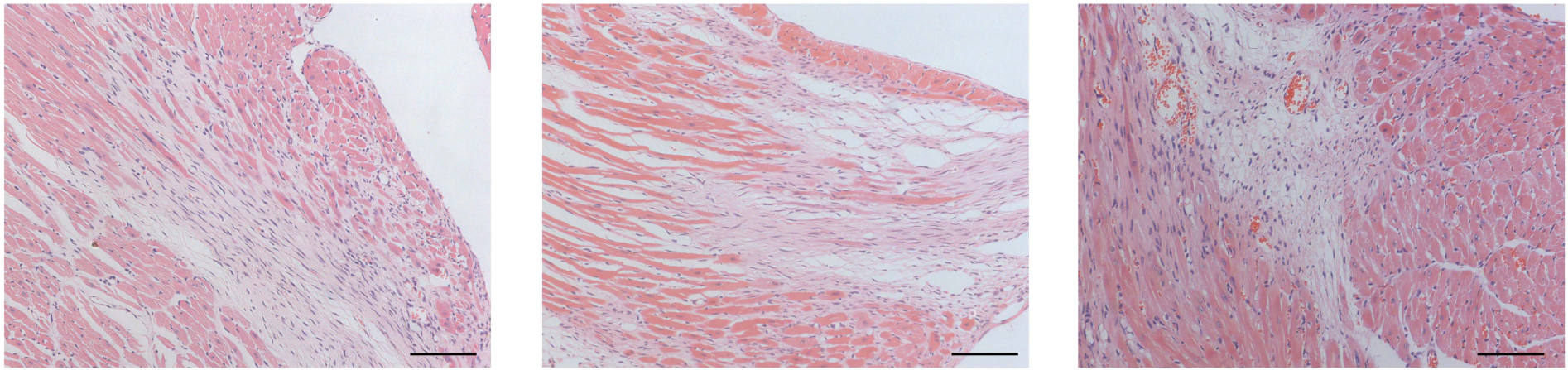
Survival ratio of transplanted cells. Left panels show results of RFP(+) CPC sheet transplantation, and right panels show results of GFP(+) ATMC sheet transplantation. Upper panels show representative images of Western blot analysis. The ratio of RFP or GFP expression in the heart was calculated and is shown in the graph (lower panels, n = 3). Baseline (immediately following transplantation) was shown as 0 wk.

Supplemental Figure 4



Lectin perfusiuons assay. At 4 weeks after MI, with or without transplantation, FITC-conjugated lectin was intravenously infused. The number of vessels in the border area was quantified and is shown in the graph (n = 5). HPF, high-power field. Scale bars, 20 μm.

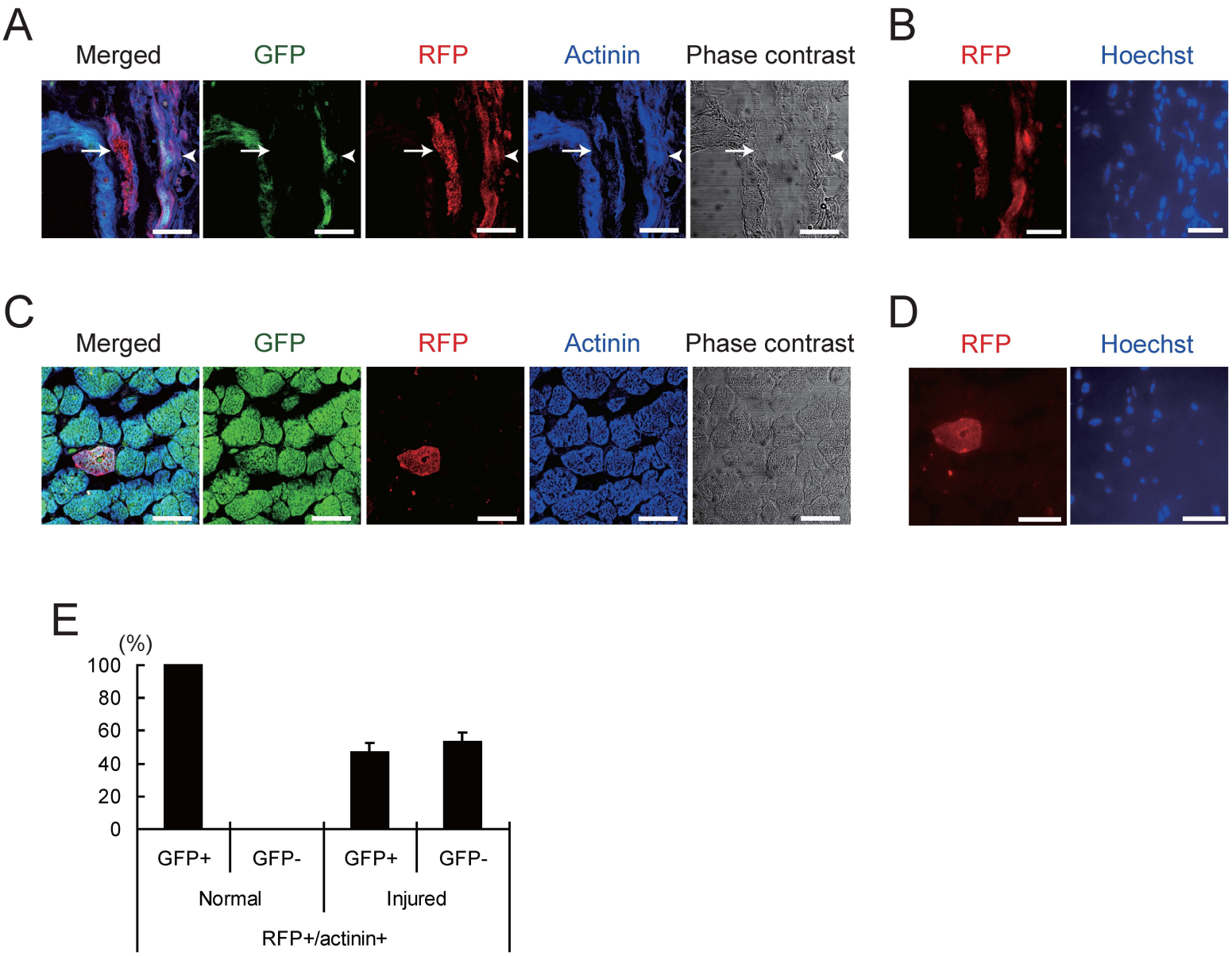
Supplemental Figure 5



MI MI+ATMC MI+CPC

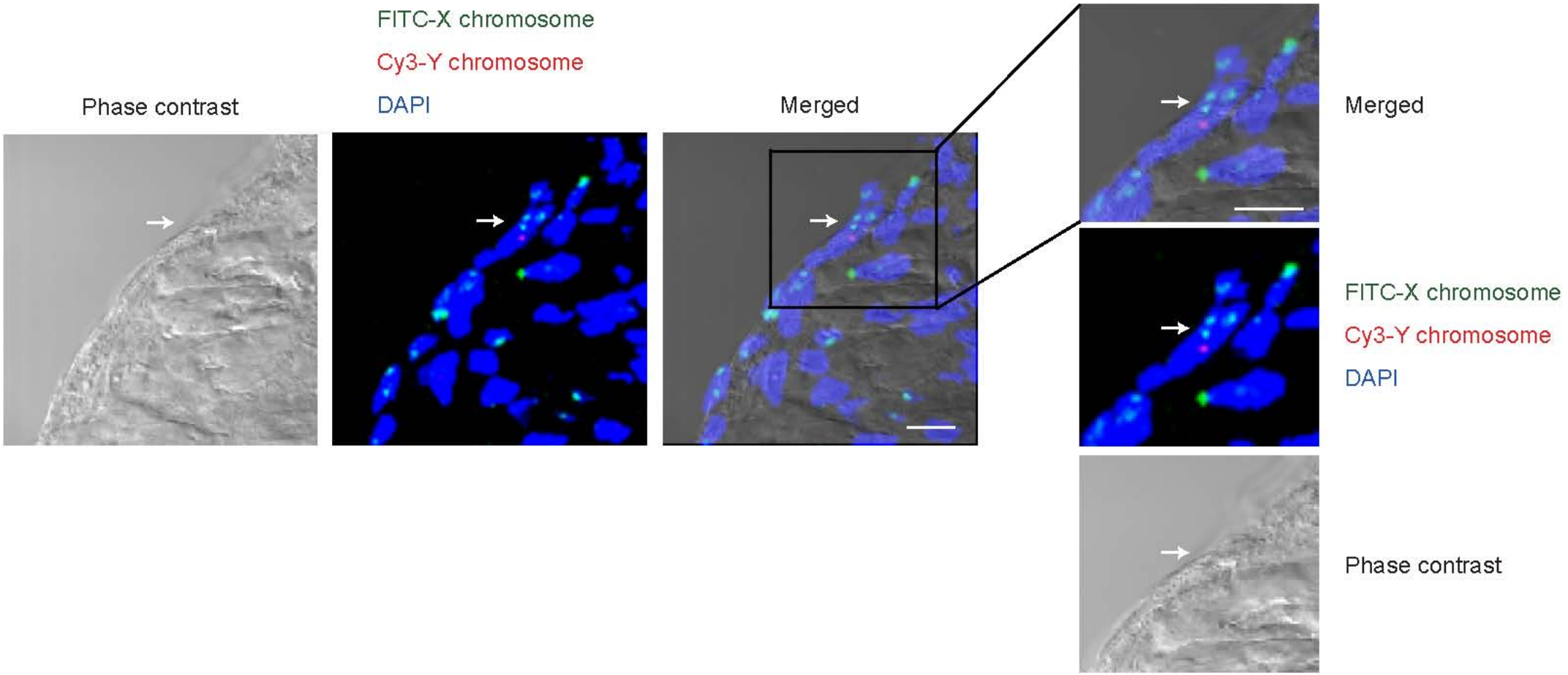
HE staining. Representative images of the border area of H&E staining 4 weeks after transplantation. Scale bars, 100 μm.

Supplemental Figure 6



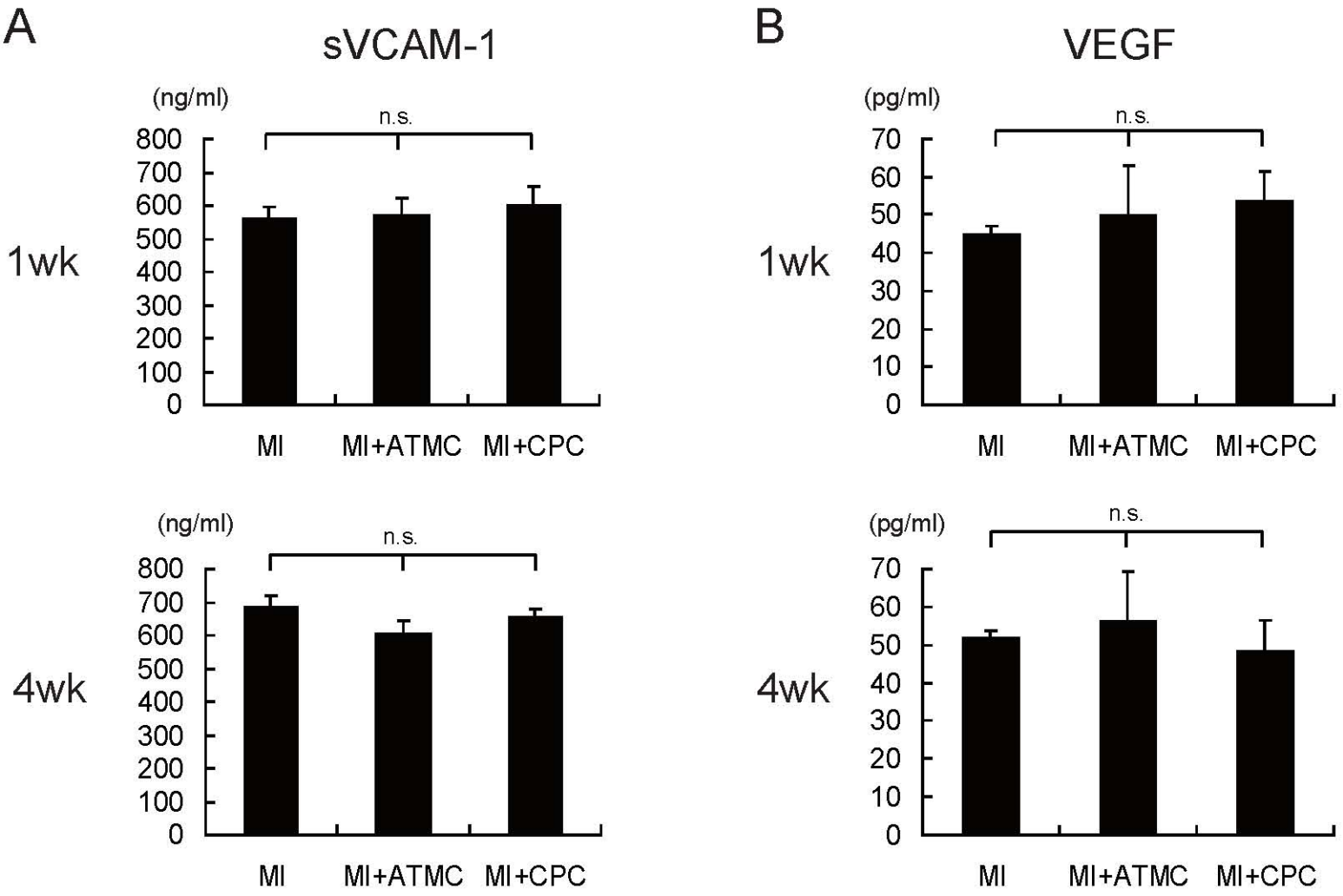
Cell fusion analysis of transplanted CPC. (A, B) Immunofluorescent images of injured area. Some RFP (+) cells (red, arrow) expressed sarcomeric α-actinin (blue, arrow) in a fine striated pattern, but not GFP (green, arrow). Other RFP(+) cells (red, arrowhead) expressed sarcomeric α-actinin (blue, arrowhead) and GFP (green, arrowhead). (A) Confocal laser microscopic images. Images of the same view as (A) were taken by fluorescent microscopy (B). Nuclei were stained with Hoechst 33258. Scale bars, 5 μm. (C, D) Immunofluorescent images of normal area. Some RFP(+) cells (red) expressed sarcomeric α-actinin (blue) and GFP (green). (C) Confocal laser microscopic images. Images of the same view as (C) were taken by fluorescent microscopy (D). Nuclei were stained with Hoechst 33258. Scale bars, 5 μm. (E) The percentage of GFP(+) cells in α-actinin expressing RFP(+) cells were calculated and shown in the graph (n = 2).

Supplemental Figure 7



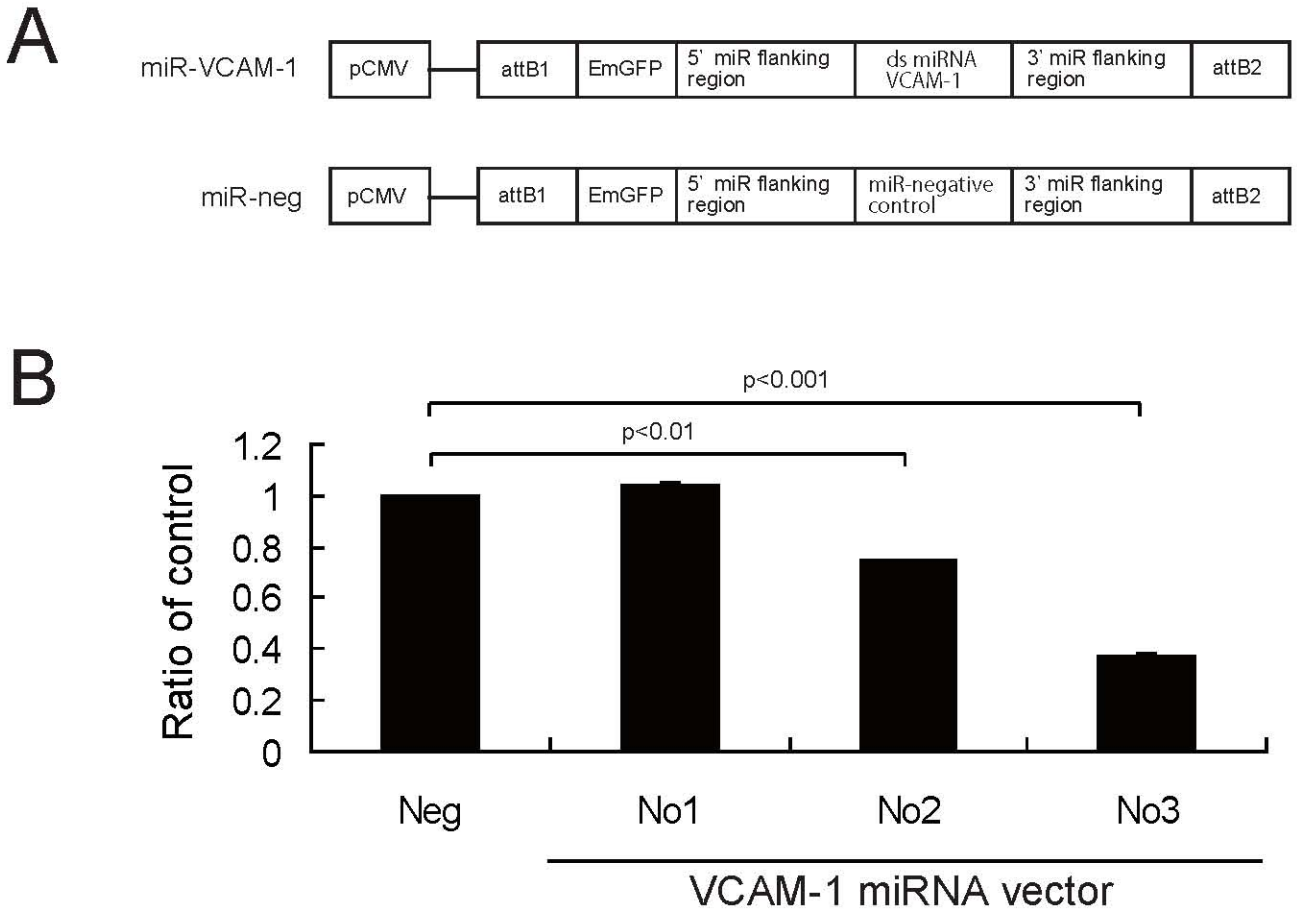
FISH analysis. At 4 weeks after CPC sheet transplantation to the infarcted heart of female mice, cells that contained three X chromosomes (green) and a Y chromosome (red) in the nucleus were detected. The cells also exhibited a fine striated pattern (arrow) in the border area. Scale bars, 20 μm. Nuclei were stained with DAPI (blue).

Supplemental Figure 8



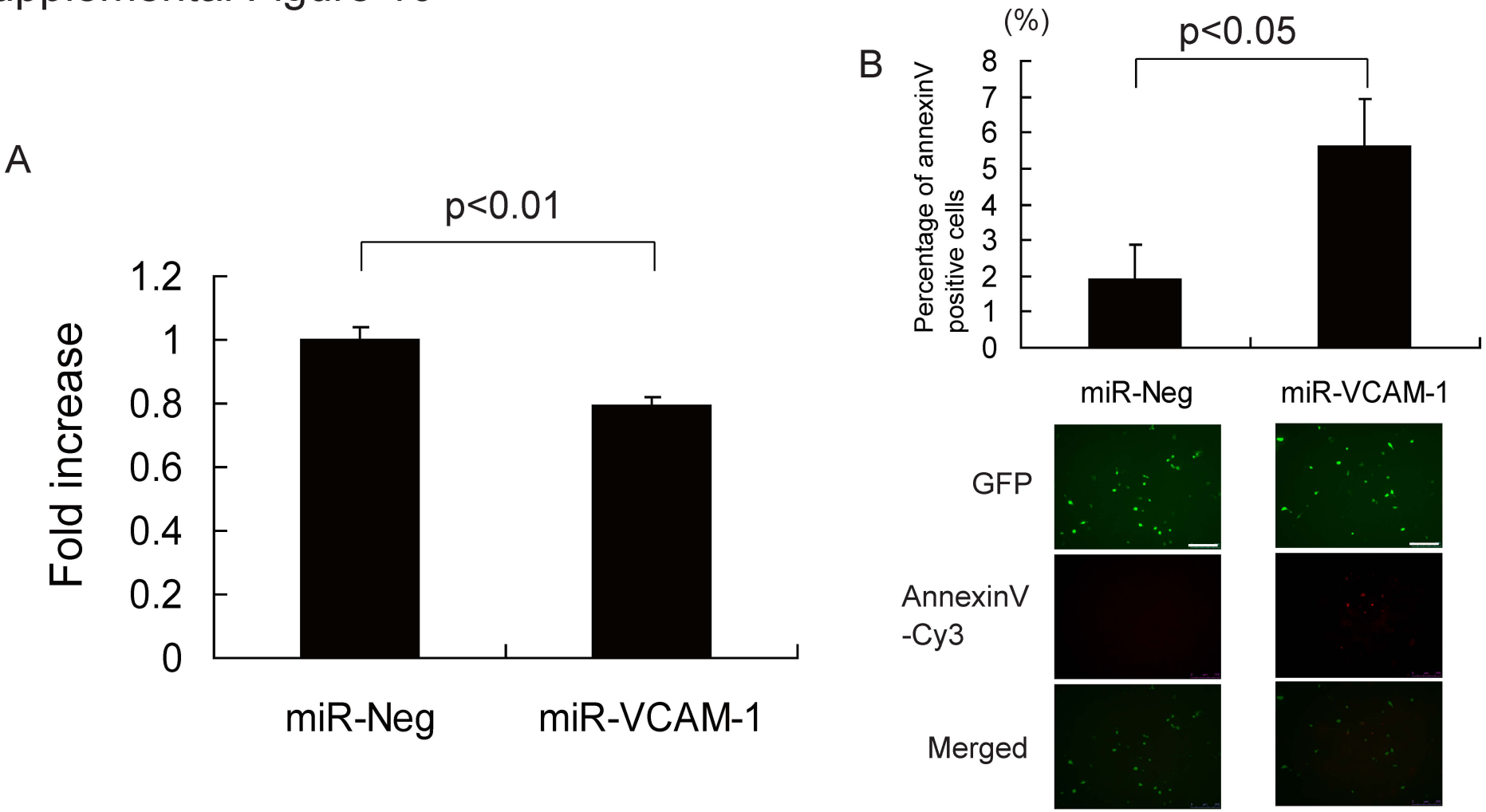
Concentration of sVCAM-1 and VEGF in peripheral blood. (A) Concentration of sVCAM-1 in peripheral blood was measured by ELISA (n = 5). Upper panel, at 1 week. Lower panel, at 4 weeks. (B) Concentration of VEGF in peripheral blood was measured by ELISA (n = 5). Upper panel, at 1 week. Lower panel, at 4 weeks.

Supplemental Figure 9



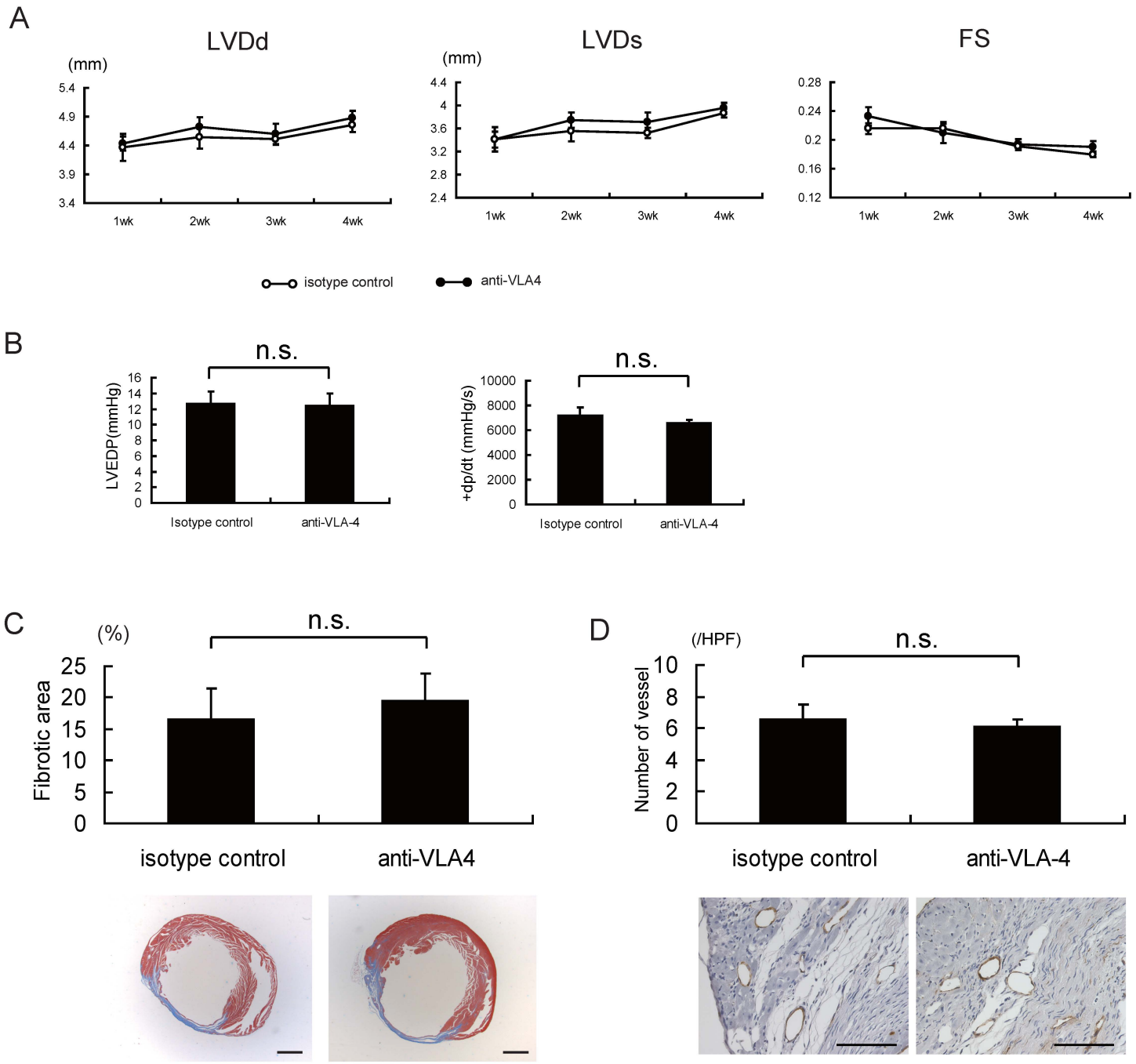
VCAM-1-specific miRNA plasmid vector transfection. (A) Schematic diagrams of miRNA plasmids. (B) mRNA expression of VCAM-1. VCAM-1 mRNA expression in VCAM-1-specific miRNA plasmid vectors transfected CPC were calculated and are shown in the graph (n = 2). Vector No.3 was used for the experiments. (C) CM was collected 3 days after transfection of VCAM-1-specific miRNA plasmid vector (miR-VCAM-1). Reduced sVCAM-1 concentration was observed in CM derived from CPC transfected with VCAM-1-specific miRNA plasmid vector (n = 3). CM derived from CPC transfected with negative control plasmid vector (miR-Neg) was used as a control.

Supplemental Figure 10



VCAM-1 depletion decreases CPC viability. CPC were transfected with miR-negative plasmid vector or miR-VCAM-1 plasmid vector. At 2 days after transfection, CPC viability was measured by MTT assay (A, n = 8) and CPC apoptosis was measured by Annexin V staining (B, n = 8). (A) Viability was significantly decreased in miR-VCAM-1-transfected CPC compared with miR-negative-transfected CPC. (B) Percentage of Annexin V-positive cells in GFP-positive cells were calculated and shown in the graph. Transfected cells expressed GFP, a marker protein of miR-negative vector, or miR-VCAM-1. The percentage of apoptotic cells was significantly increased in miR-VCAM-1-transfected CPC compared with miR-negative-transfected CPC. Lower panels show representative images. Scale bars, 20 μm. Transfection efficiency was similar between each group (miR-Neg, 61.0 ± 2.7 %; miR-VCAM-1, 61.5 ± 2.3 %, n = 8).

Supplemental Figure 11



The roles of VLA-4 signaling on cardiac function in the infarcted heart without cell sheet transplantation. Analysis of cardiac function by echocardiography (A, n = 5) and catheterization (B, n = 5). Anti-VLA-4 Ab (2.5 mg/kg) was intraperitoneally injected daily from 2 to 3 weeks after MI (n = 5). Anti-VLA-4 Ab treatment did not affect cardiac function, such as LVDd, LVDs, FS, LVEDP, and +dp/dt at 3 and 4 weeks after MI. Isotype antibody (2.5 mg/kg) was used as a control (n = 5). (C) Masson trichrome staining. The fibrotic area at 4 weeks after MI was calculated and is shown in the graph (n = 5). Anti-VLA-4 Ab treatment did not affect the fibrotic area of the infarcted heart. Lower panels show representative images. Scale bars, 1 mm. (D) vWF staining. The number of vWF-positive vessels in the border area was quantified and is shown in the graph (n = 5). Anti-VLA-4 Ab treatment did not affect the number of vessels in the border area of the infarcted heart. Lower panels show representative images. Nuclei were stained with hematoxylin. Scale bars, 100 μm.