Supplemental Figures and Movies

Supplemental Figure 1. Flow cytometric analysis of GFP^{high} cells in peripheral blood and cardiac graft tissue 2 hours after transplantation of B6 hearts into B6 LysM-GFP recipients. The majority of GFP^{high} cells have high side scatter, express Ly6G and Gr1 and lack expression of CD115. Staining with isotype control antibodies is indicated by crossbars in density plots and by shaded regions in histograms.

Supplemental Figure 2. 2P imaging of explanted heart graft 6 hours after reperfusion. Nontargeted Q-dots (red) were injected intravenously 10 minutes before imaging to label the blood vessels (outlined with white lines). The number of neutrophils (green) increased in heart grafts at 6 hours as compared to 3 hours after reperfusion (Figure 1D). Neutrophils formed dynamic clusters both within vessels (yellow arrowhead) as well as in parenchymal tissue (white arrowheads). Scale bar: 60 μm.

Supplemental Figure 3. Extensive accumulation of neutrophils in the left free ventricular wall of wild-type grafts with formation of dynamic clusters 24 hours after reperfusion. White lines outline coronary veins. Right panels represent three-dimensional rendering of areas depicted in left panels. Scale bar: 60 μm. Relative time is displayed in min:sec.

Supplemental Figure 4. Histological evaluation of B6 heart tissue (A) without removal of pericardium and (B) 2 hours after removal of the pericardium (200x).

Supplemental Movie 1. Time-lapse 2P imaging of explanted heart grafts. Neutrophils (green) migrate and cluster within a heart graft after transplantation-mediated ischemia

reperfusion injury. Blood vessels (red) were labeled by intravenous injection of nontargeted 655-nm Q-dots. Relative time is displayed in hrs:min:sec:msec.

Supplemental Movie 2. Video-rate intravital 2P imaging of a coronary artery. Neutrophil (green) flowing through a coronary artery (red) of a wild-type heart graft after heterotopic transplantation. Vessels were labeled red by intravenous injection of non-targeted 655-nm Q-dots.

Supplemental Movie 3. Time-lapse intravital 2P imaging of intravascular neutrophil behavior. Neutrophils (green) display intraluminal crawling and form cluster in vessels of wild-type heart grafts after transplantation. Bottom left panel shows neutrophils extravasating from discrete locations along the endothelium. Bottom right panel shows neutrophils flowing through capillaries and adhering in larger coronary veins. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm Q-dots. Relative time is displayed in hrs:min:sec:msec.

Supplemental Movie 4. Time-lapse intravital 2P imaging of neutrophil behavior in inflamed ICAM-1-mutant heart grafts. Neutrophils (green) form extensive intravascular clusters within blood vessels (red, 655-nm Q-dots). A few extravascular neutrophils are visible in the lower left region of the movie. Relative time is displayed in hrs:min:sec:msec.

Supplemental Movie 5. Time-lapse intravital 2P imaging of neutrophil behavior in heart graft recipients treated with anti-LFA-1 blocking antibodies. A few neutrophils (green) adhere within blood vessels (red, 655-nm Q-dots). Relative time is displayed in hrs:min:sec:msec.

Supplemental Movie 6. Time-lapse intravital 2P imaging of neutrophil behavior in heart graft recipients treated with anti-Mac-1 blocking antibodies. Neutrophils (green) display intraluminal crawling and clustering within blood vessels (red, 655-nm Q-dots). A few neutrophils are seen extravasating from the vessel near the bottom of the image. Relative time is displayed in hrs:min:sec:msec.

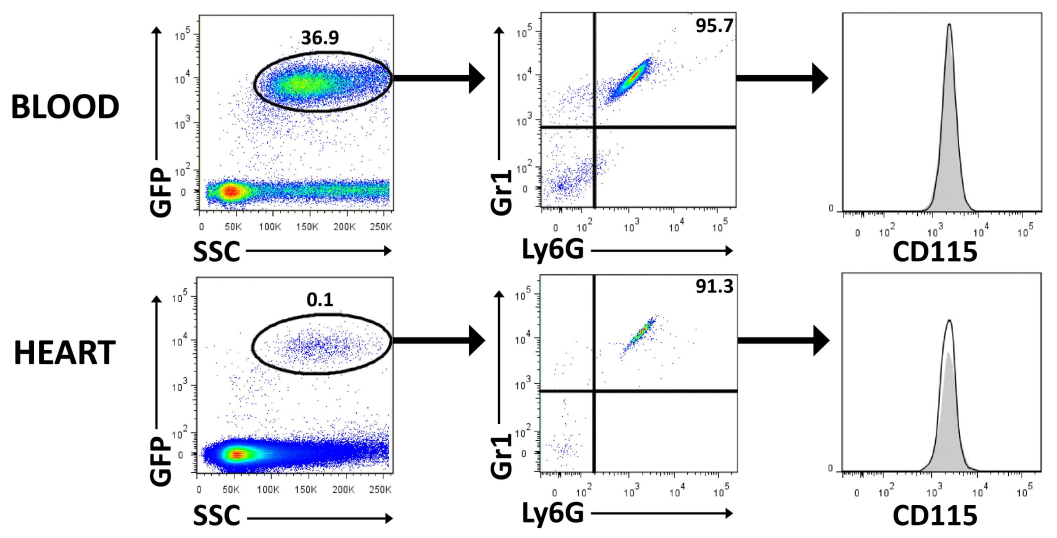
Supplemental Movie 7. Time-lapse intravital 2P imaging of tissue-infiltrating neutrophils forming large dynamic clusters in heart grafts 24 hours after reperfusion. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm Q-dots. Relative time is displayed in hrs:min:sec:msec.

Supplemental Movie 8. Time-lapse intravital 2P imaging of $CX_3CR1\text{-}GFP^{high}$ cells in heterotopic heart transplants. $CX_3CR1\text{-}GFP^{high}$ cells (green) display intraluminal crawling behavior (crawling cells are tracked with a white tail). Yellow tails identify extravasating $CX_3CR1\text{-}GFP^{high}$ cells. Blue tail identifies a $CX_3CR1\text{-}GFP^{high}$ cell that is seen crawling and then extravasating. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm Q-dots. Relative time is displayed in hrs:min:sec:msec.

Supplemental Movie 9. Video-rate intravital 2P imaging of a coronary artery in a beating, intrathoracic native heart. Neutrophils (green) are occasionally captured flowing in the vessel (red, 655-nm Q-dots).

Supplemental Movie 10. Time-lapse intravital 2P imaging of neutrophil behavior in a native beating heart after ischemia reperfusion injury. The left main coronary artery (red, 655-nm Q-dots) was transiently ligated to induce ischemia. The heart was imaged

immediately after the release of the ligature. Neutrophils (green) were seen crawling, extravasating and forming clusters in the myocardial tissue. Relative time is displayed in hrs:min:sec:msec.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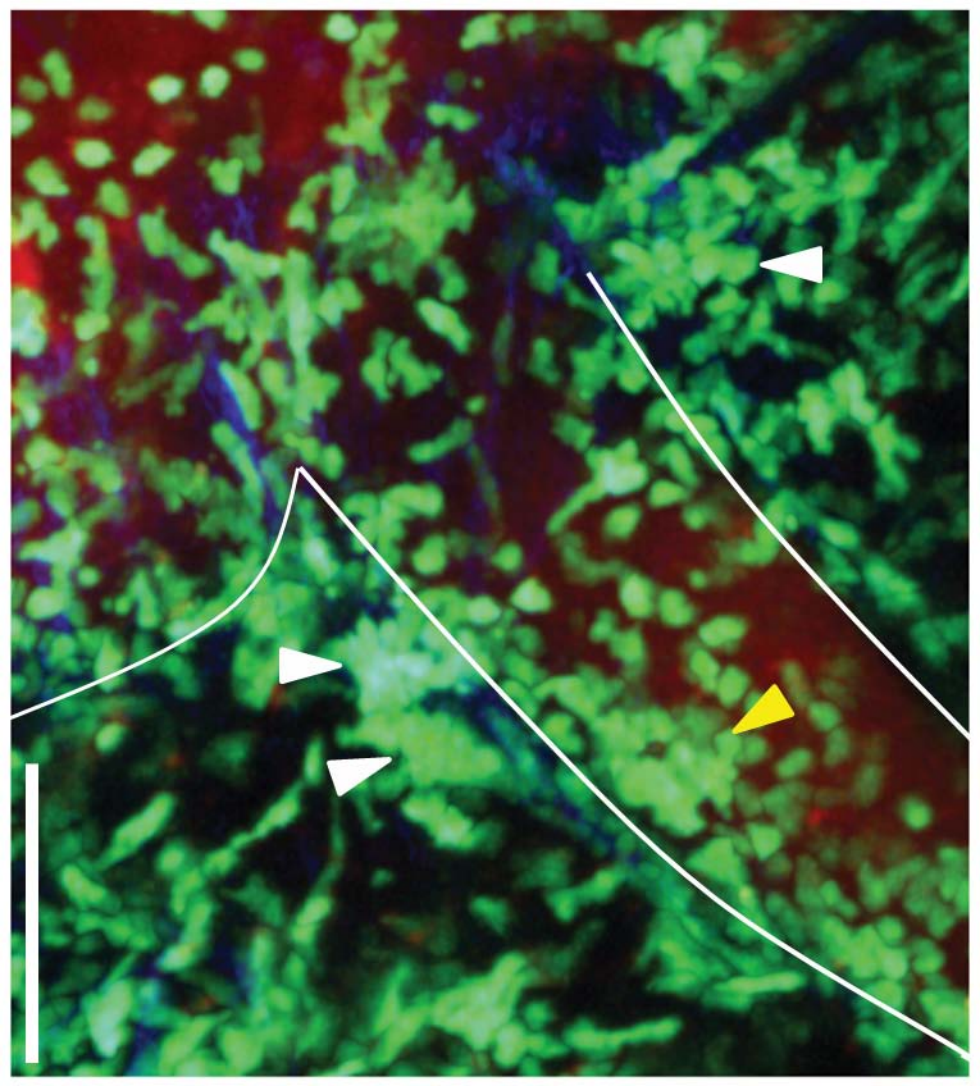

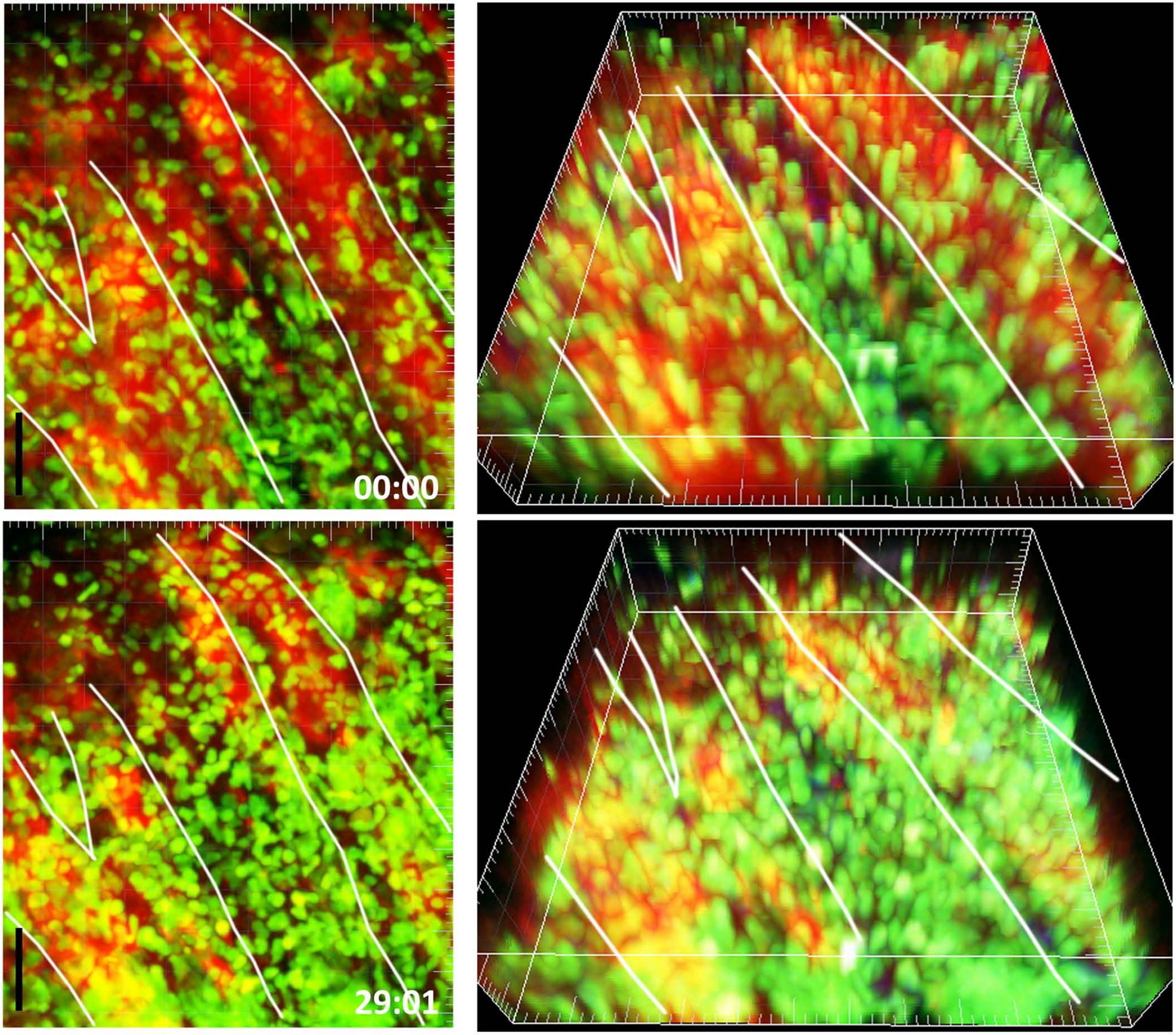
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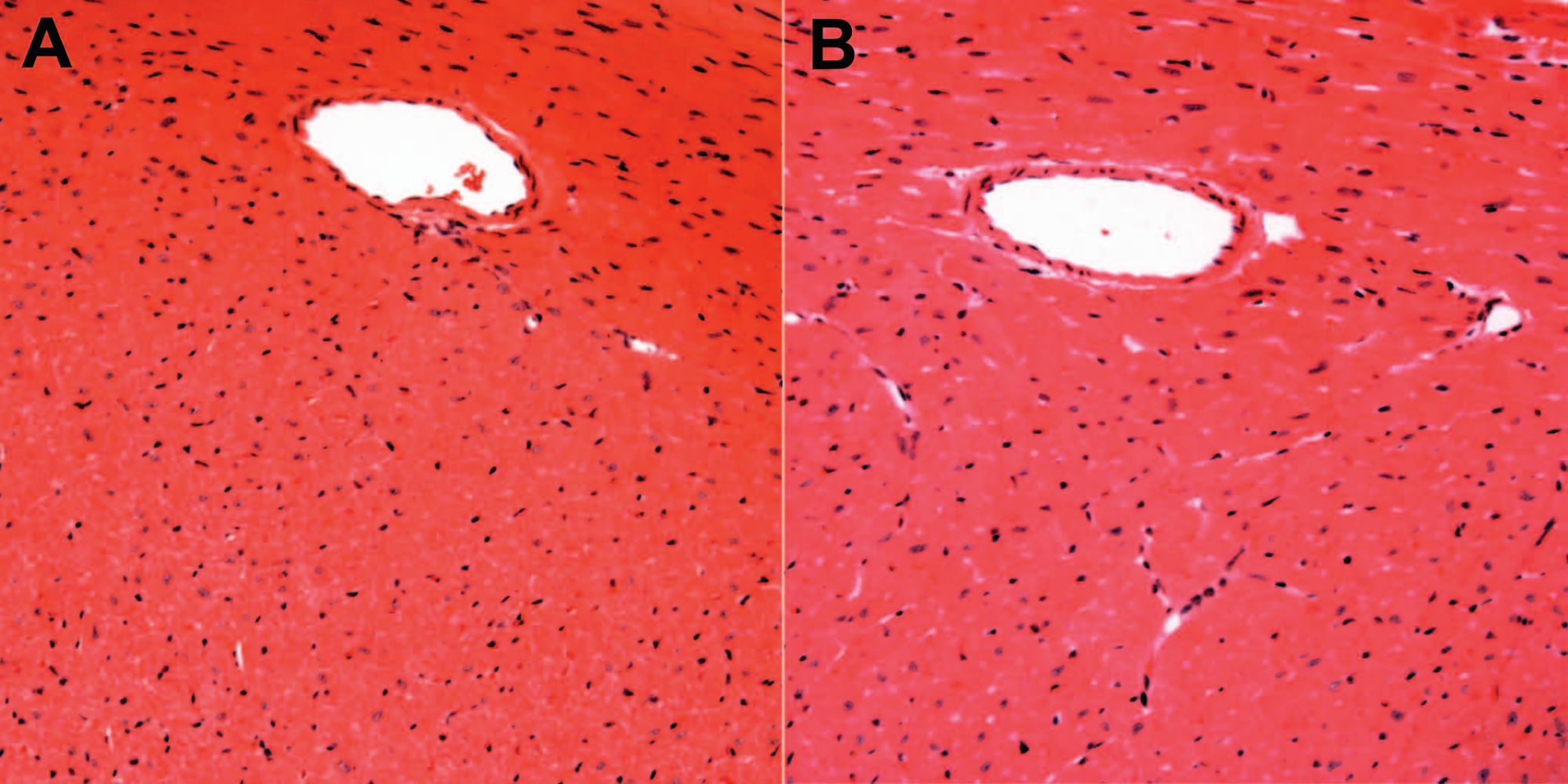
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