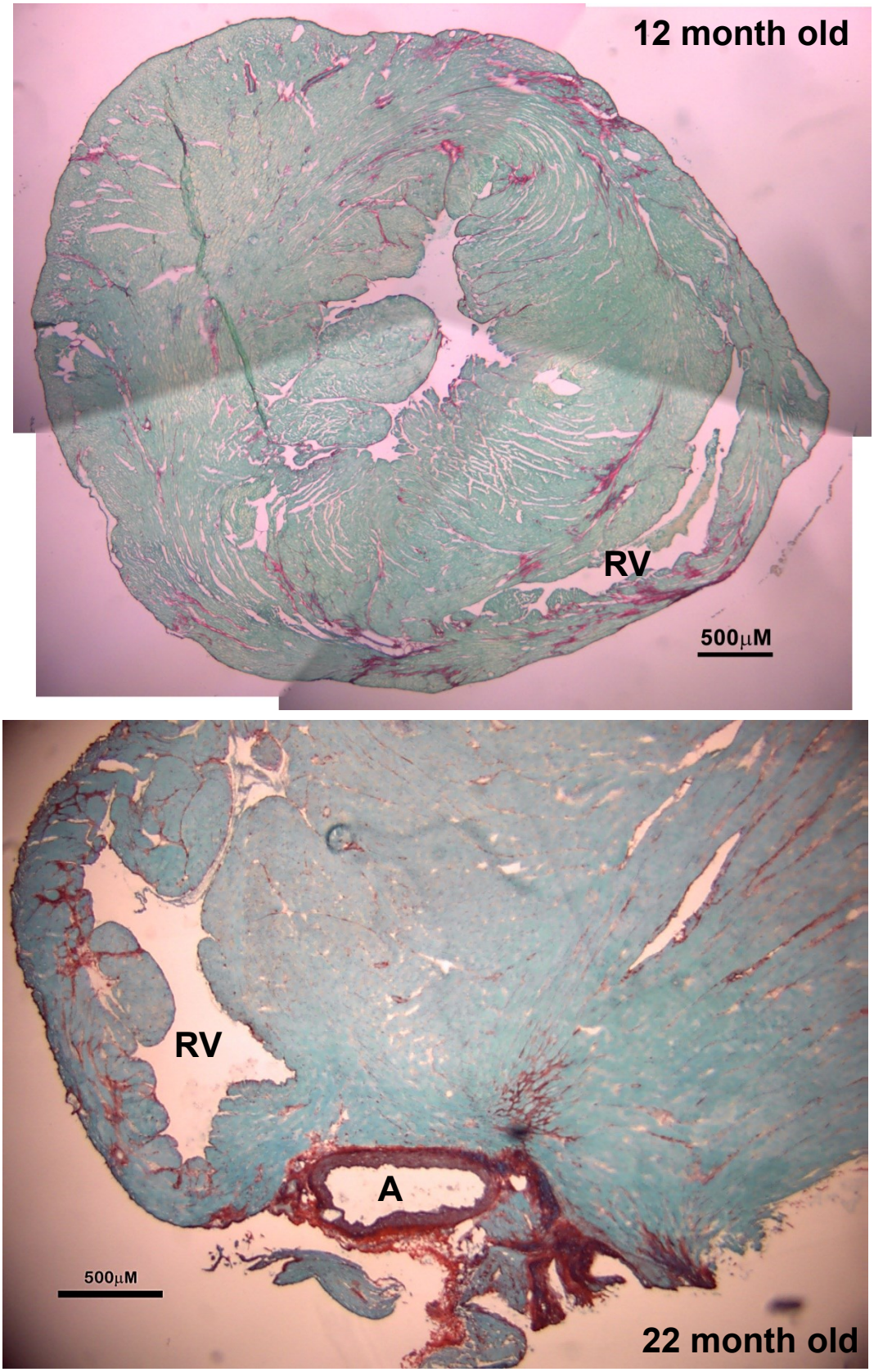
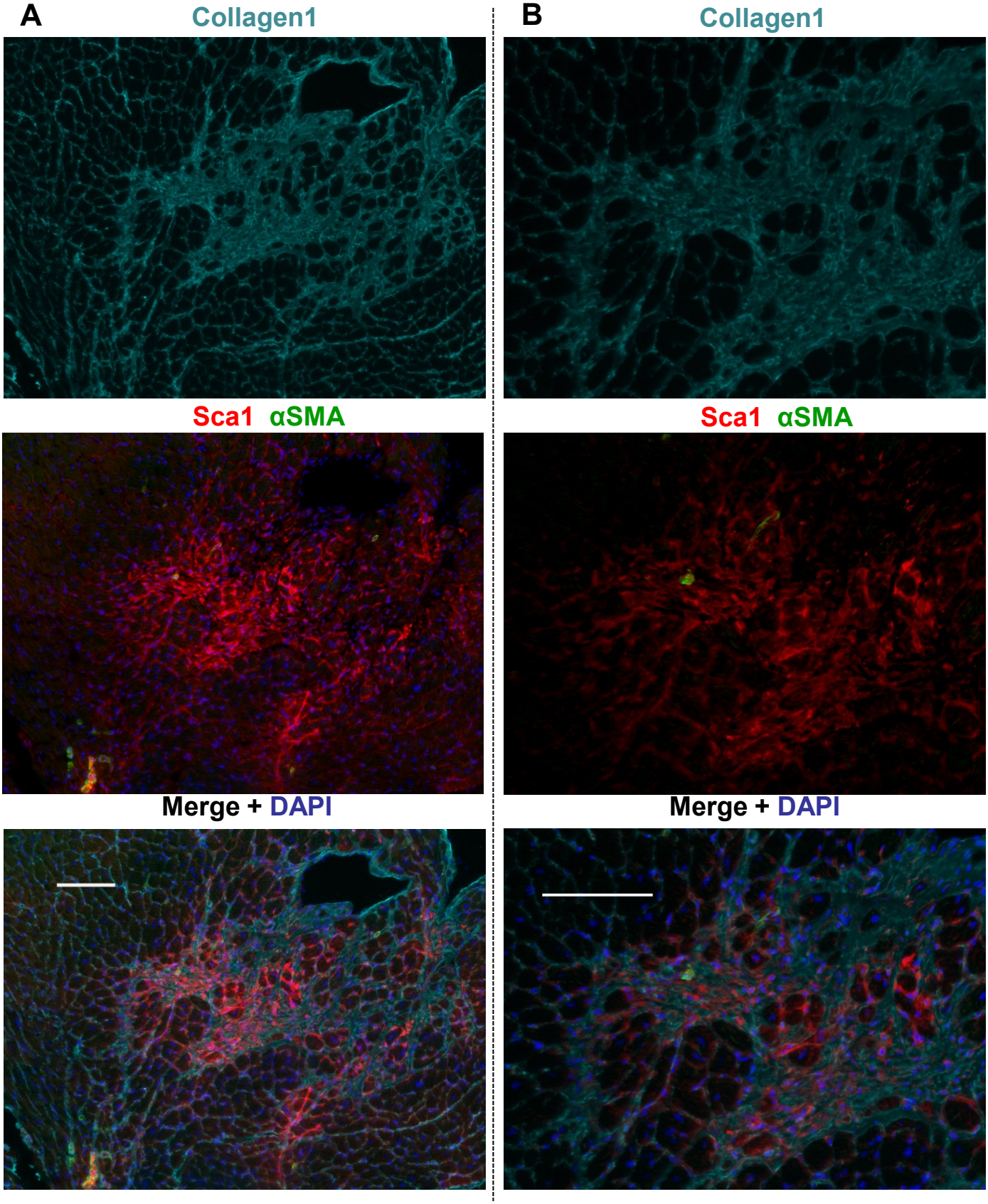
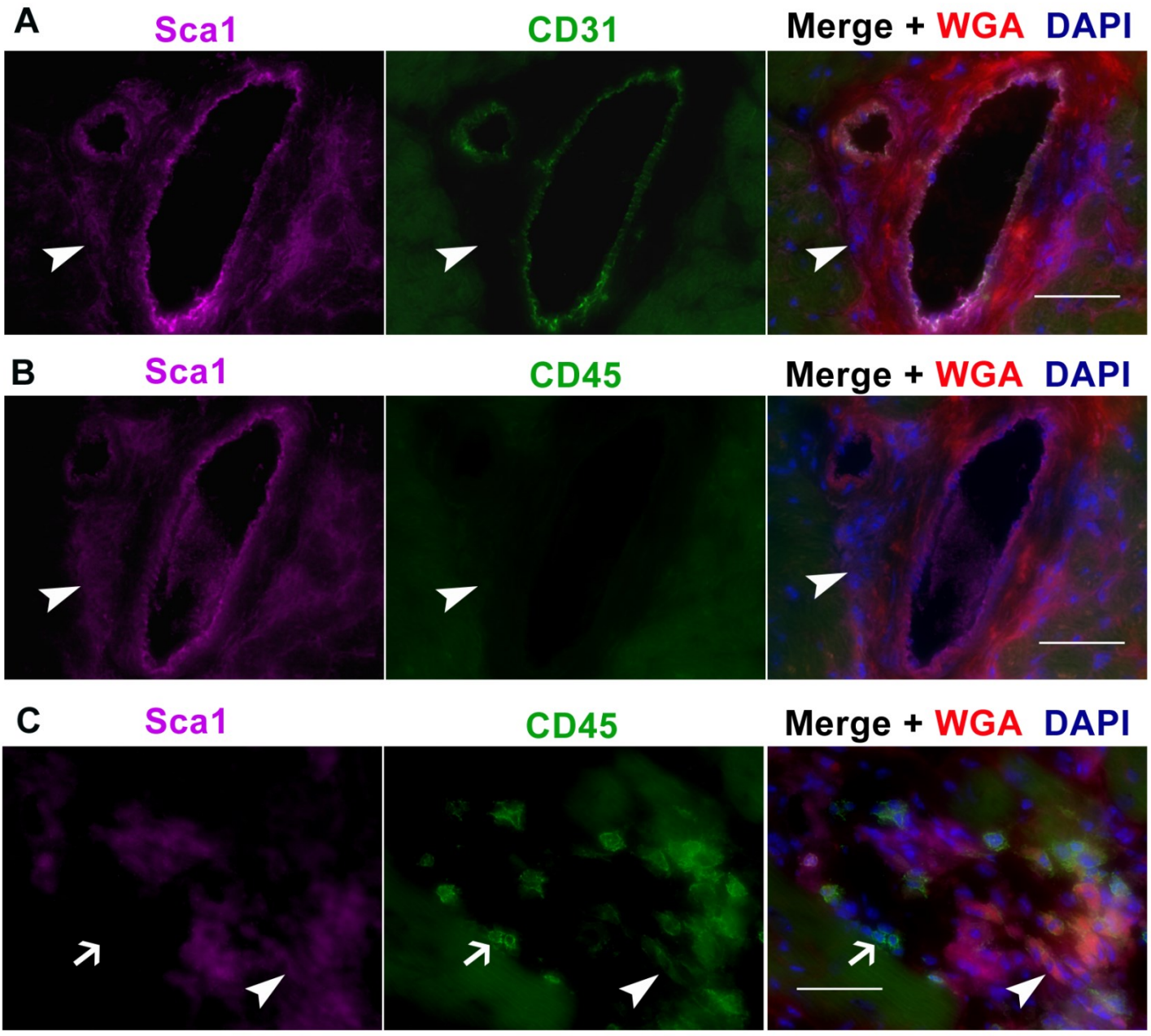


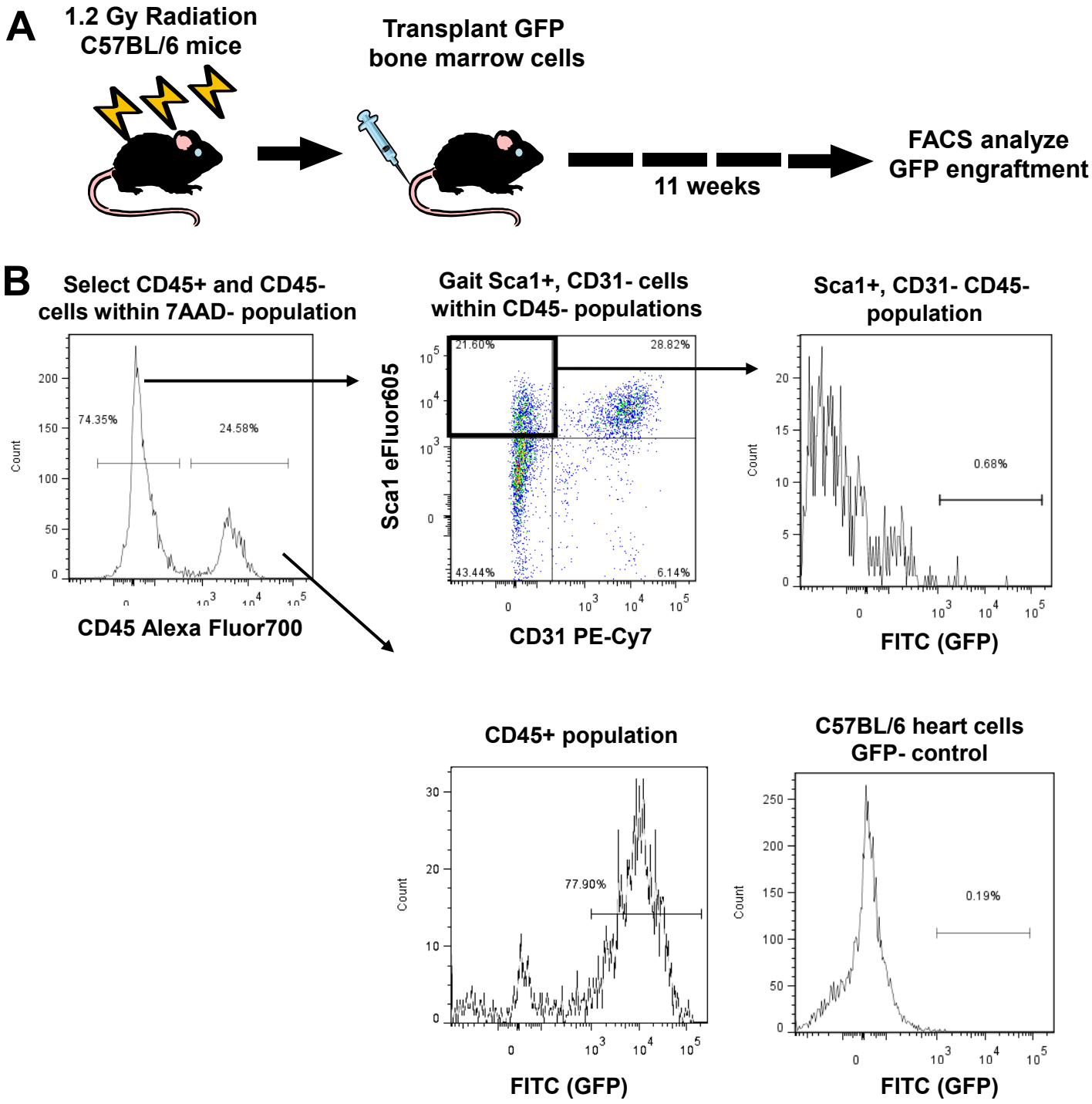
Supplement Fig. 1. Fibrosis in adult and aged *mdx* hearts**.**
Picrosirius red staining of 12 month and 22 month old *mdx* hearts cross section highlights the accumulation of fibrosis (red staining) in the right ventricular (LV) wall and surrounding coronary artery (A). Note for the 12 month old montage 10x photos were merged to represent the entire heart, while the 22 month old is a single 4x photo.



Supplement Fig. 2. Sca1+ cells occupy regions of fibrosis in aged *mdx* hearts
A. Staining for collagen1 in 22 month old *mdx* heart reveals the accumulation of fibrosis and presence of Sca1+ cells in the fibrotic region. The region depicted is in the left ventricle. **B.** Higher magnification highlights the co-localization of Sca1+ cells with collagen 1 staining in the scarred region of heart tissue. Scale bars = 100μm

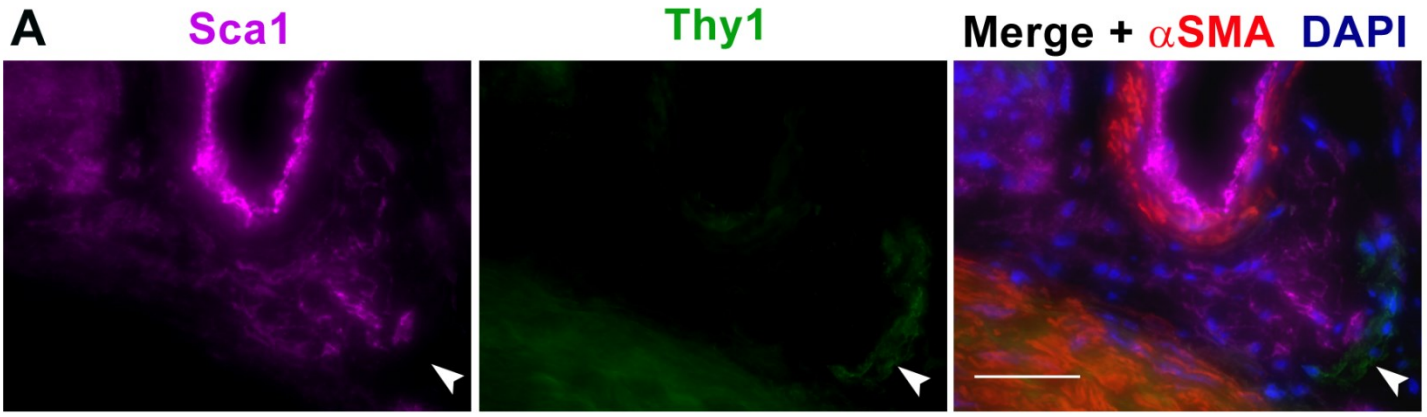


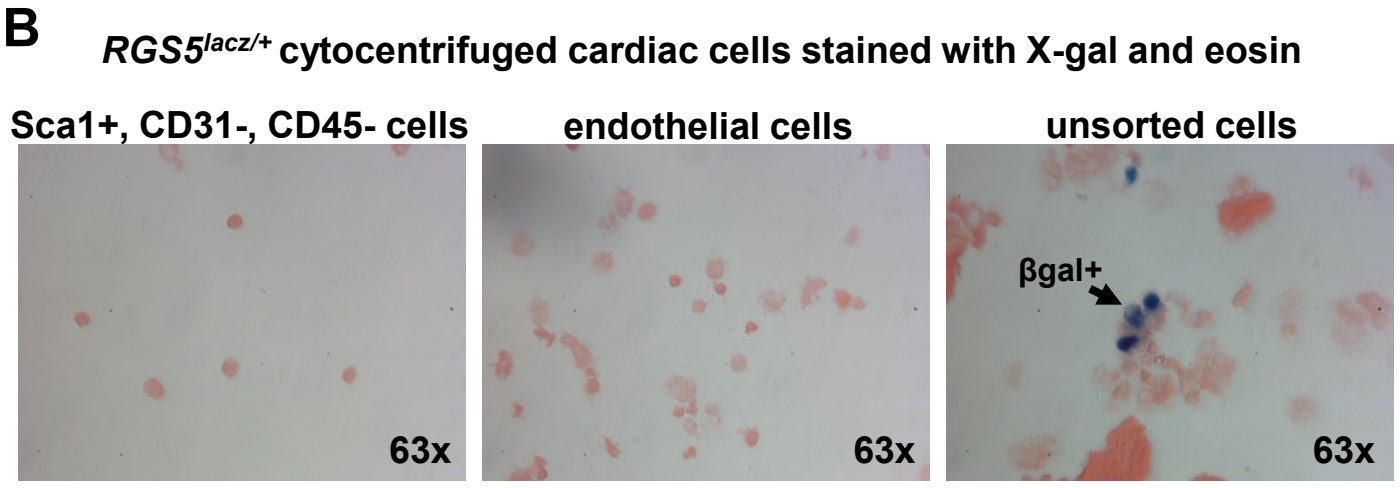
Supplement Fig. 3. Adventitial cells in *mdx* hearts are negative for CD31 and CD45. **A.** Staining for Sca1 and CD31 in 22 month old *mdx* hearts reveals adventitial cells are Sca1+ but CD31- (arrowhead). **B**. An adjacent section of the same vessel shown in A, indicates Sca1+ adventitial cells are also CD45-. **C**. Although adventitial cells were CD45-, we did observe CD45+, Sca1- (arrow) in addition to CD45+, Sca1+ (arrowhead) inflammatory cells in fibrotic areas of the same *mdx* hearts. Sections were also stained with WGA as a vascular / membrane counterstain. Scale bars = 50μm



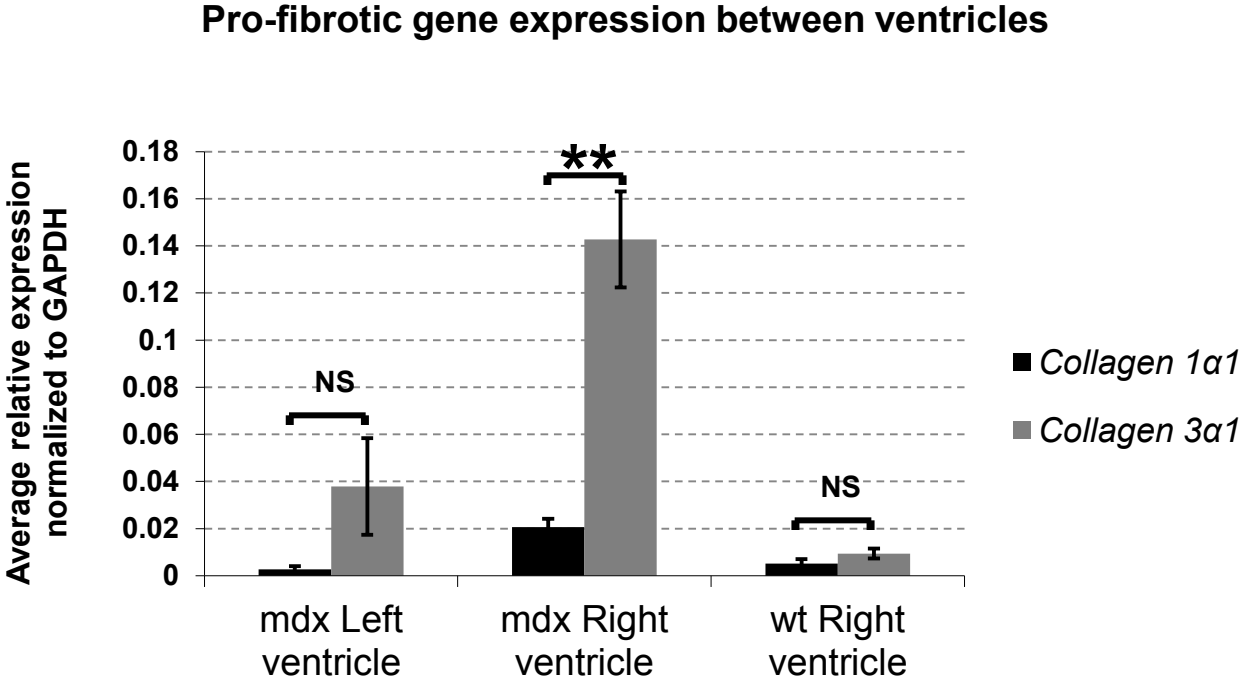
Supplementary Figure 4. Bone marrow derived cells do not contribute to the Sca1+ adventitial cell population in adult hearts.

A. Bone marrow chimeras (n=3) were generated as illustrated using donor bone marrow cells from mice that ubiquitously express EGFP under the chicken actin promoter. Recipients were examined by FACS-analysis 11 weeks post transplant. **B.** Representative FACS plots of cardiac cells analyzed from bone marrow chimers. Cells were first gated as 7AAD- to remove damaged or dead cells that may lose GFP (not shown), then selected as CD45+ or CD45- for GFP analysis. In contrast to CD45+ cells which were mostly GFP+, Sca1+, CD31-, CD45- cells were negative for GFP. Cells isolated from a C57BL/6 heart were used as a negative control for GFP. In all three chimeras, >90% of bone marrow cells were GFP+ by FACS-analysis (not shown).

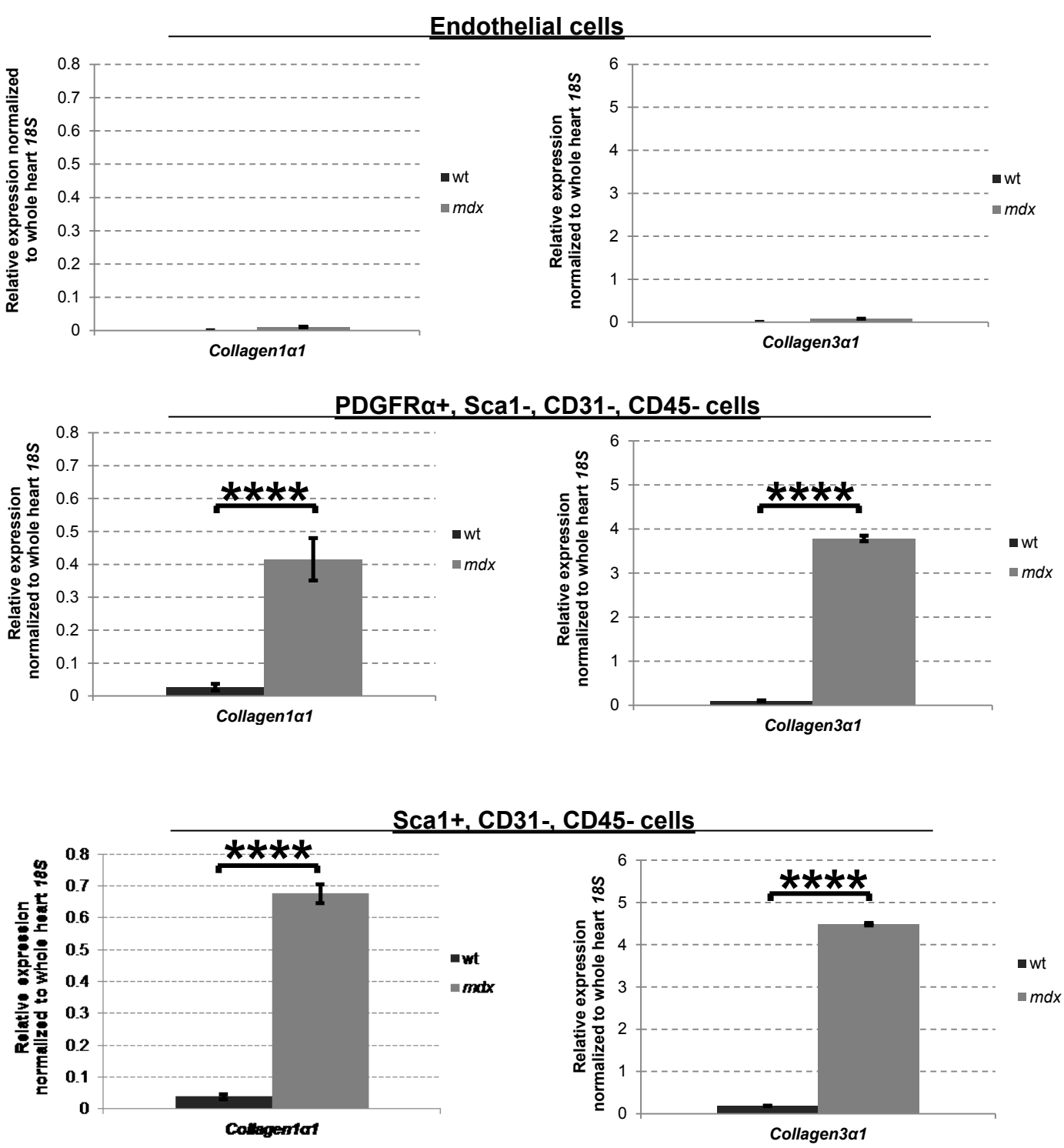




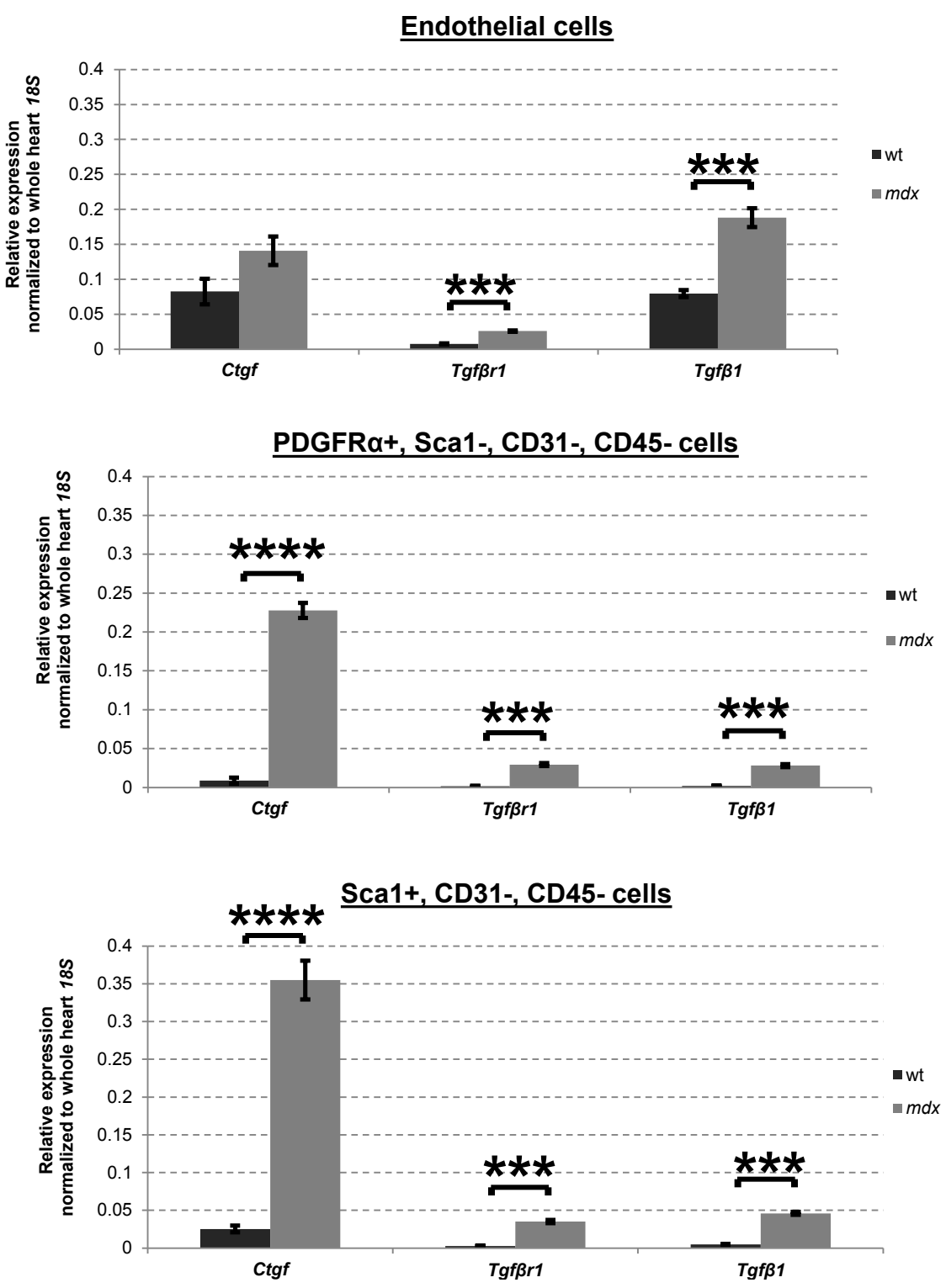
Supplement Fig. 5. Sca1+ adventitial cells are negative for the fibroblast associated marker Thy1 and mural cell reporter *Rgs5*.
A. Staining in *mdx* hearts (22 month old) reveals that Sca1+ adventitial cells are negative for Thy1. In contrast, we did detect Thy1+, Sca1- cells (arrowhead) adjacent to Sca1+. Scale bars = 50μm. **B**. X-gal staining of sorted and unsorted cytocentrifuged cells isolated from hearts of *Rgs5*$^{lacZ+/-}$ mice (n=3, 5.5 month old males). In comparison to unsorted cells used as a positive control, adventitial cells FACS-sorted as Sca1+, CD31-, CD45- were completely negative for X-gal staining. Endothelial cells (Sca1+, CD31+, CD45-) were sorted as a negative control also did not show any X-gal staining. Both populations were cytocentrifuged and stained directly following sorting in parallel with leftover unsorted cells. Following X-gal staining, cells were counterstained with eosin.



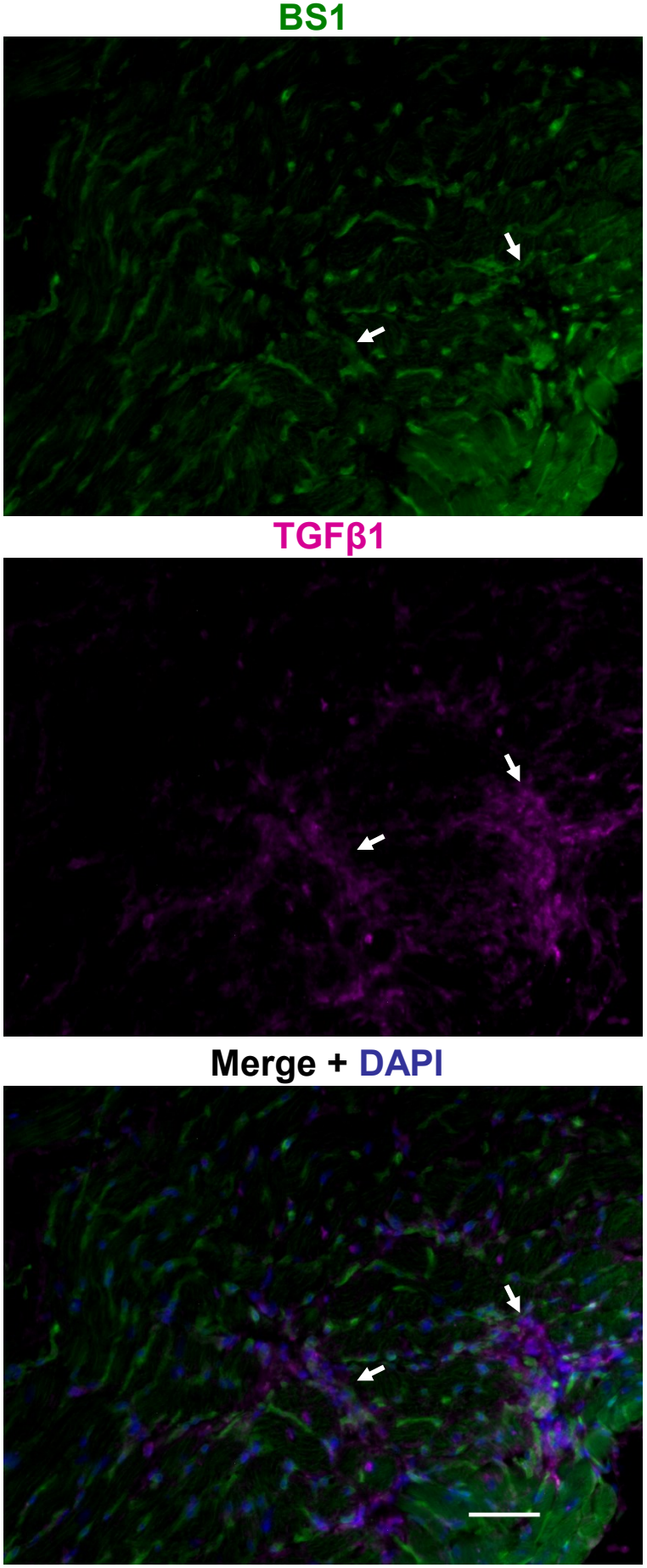
Supplement Fig. 6. *Collagen 3* expression is predominantly elevated in right ventricles of *mdx* hearts.
qRT-PCR analysis of fibrotic genes in *mdx* and wt (n=3 each, all 12 month old males) heart tissue separated into right and left ventricles. In *mdx, Collagen1α1* and *Collagen3α1* expression was elevated right vs. left ventricles. In turn, the expression of *Collagen3α1* significantly greater than *Collagen1α1* in the right ventricles of *mdx* mice but not the left ventricles. The expression of both pro-collagens was also significantly higher ($P<0.05$) in *mdx* vs. wt right ventricles.** $P<0.005$. NS denotes Not Statistically significant.



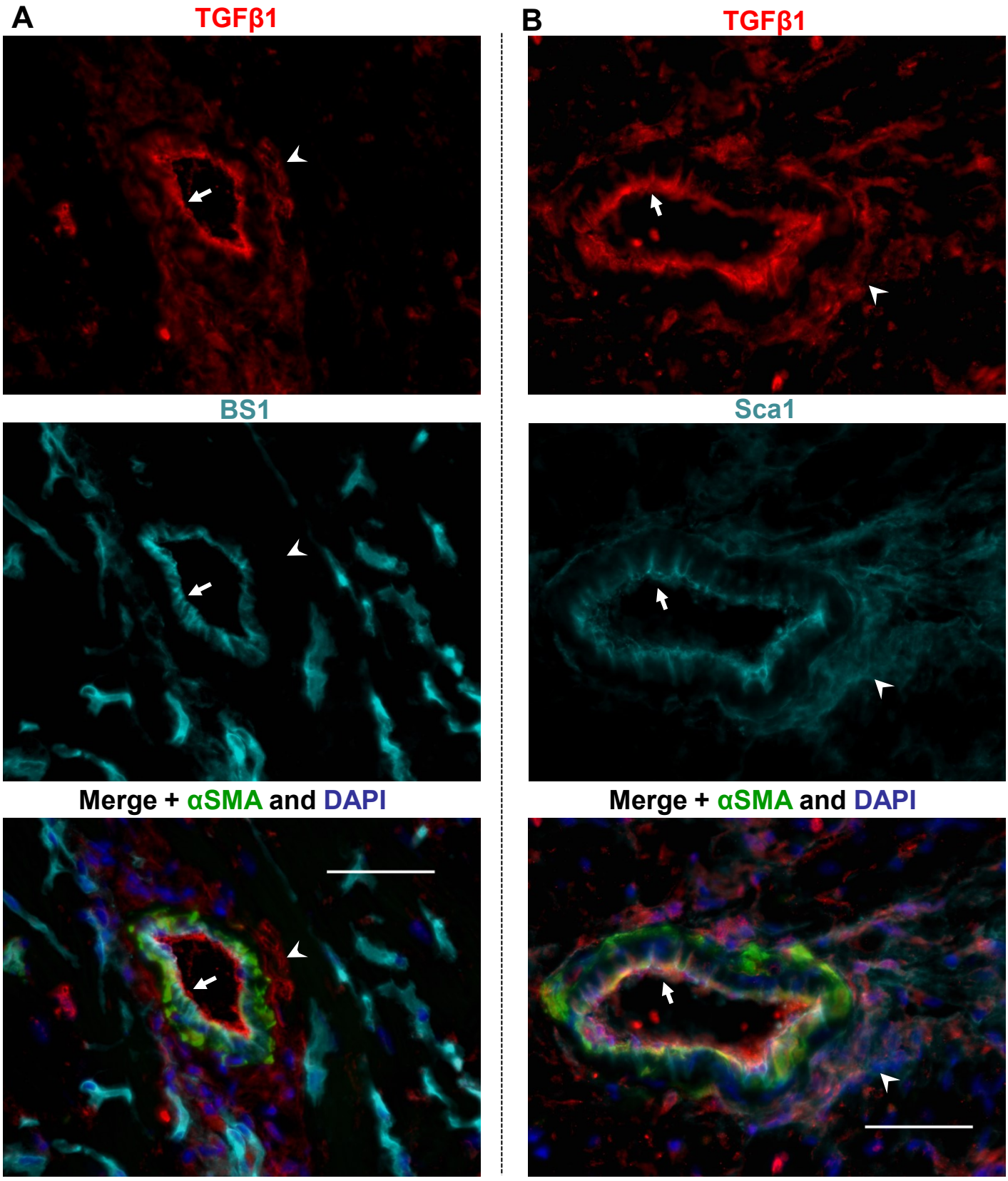
Supplement Fig. 7. *mdx* FACS-sorted cells express greater levels of pro-fibrotic genes. qRT-PCR analysis of the same sorted cell populations depicted in Fig. 3, but normalized *18S* of age matched wt and *mdx* whole hearts, respectively. Data indicates that in comparison to wt, interstitial PDGFRα+, Sca1- and adventitial Sca1+ cells sorted from *mdx* hearts express significantly greater levels *Collagen1α1* and *Collagen3α1* mRNA. This correlates with the development of fibrosis observed in *mdx* but not wt hearts at 12 months of age.**** P<0.005.



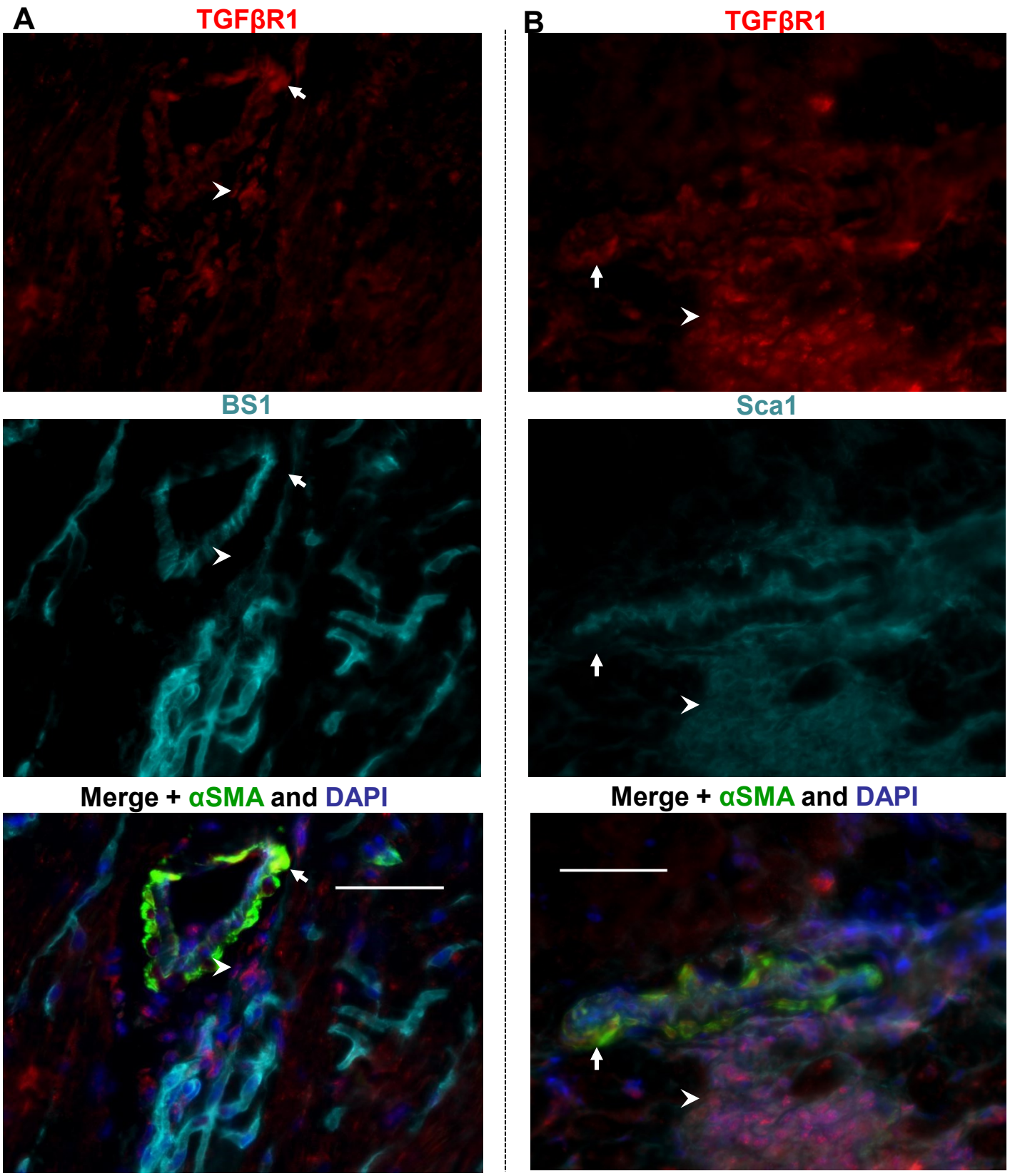
Supplement Fig. 8. *mdx* FACS-sorted cells express greater levels of pro-fibrotic factors. Additional qRT-PCR analysis of the same sorted cell populations depicted in Fig. 3, once more normalized to *18S* of age matched wt and *mdx* whole hearts, respectively. As shown, expression of the majority of these pro-fibrotic genes is elevated in *mdx* cells. Importantly, as indicated in Fig. 3, endothelial cells from *mdx* mice express significantly greater levels of *Tgfβ1* ligand. .*** P<0.005. .**** P<0.005



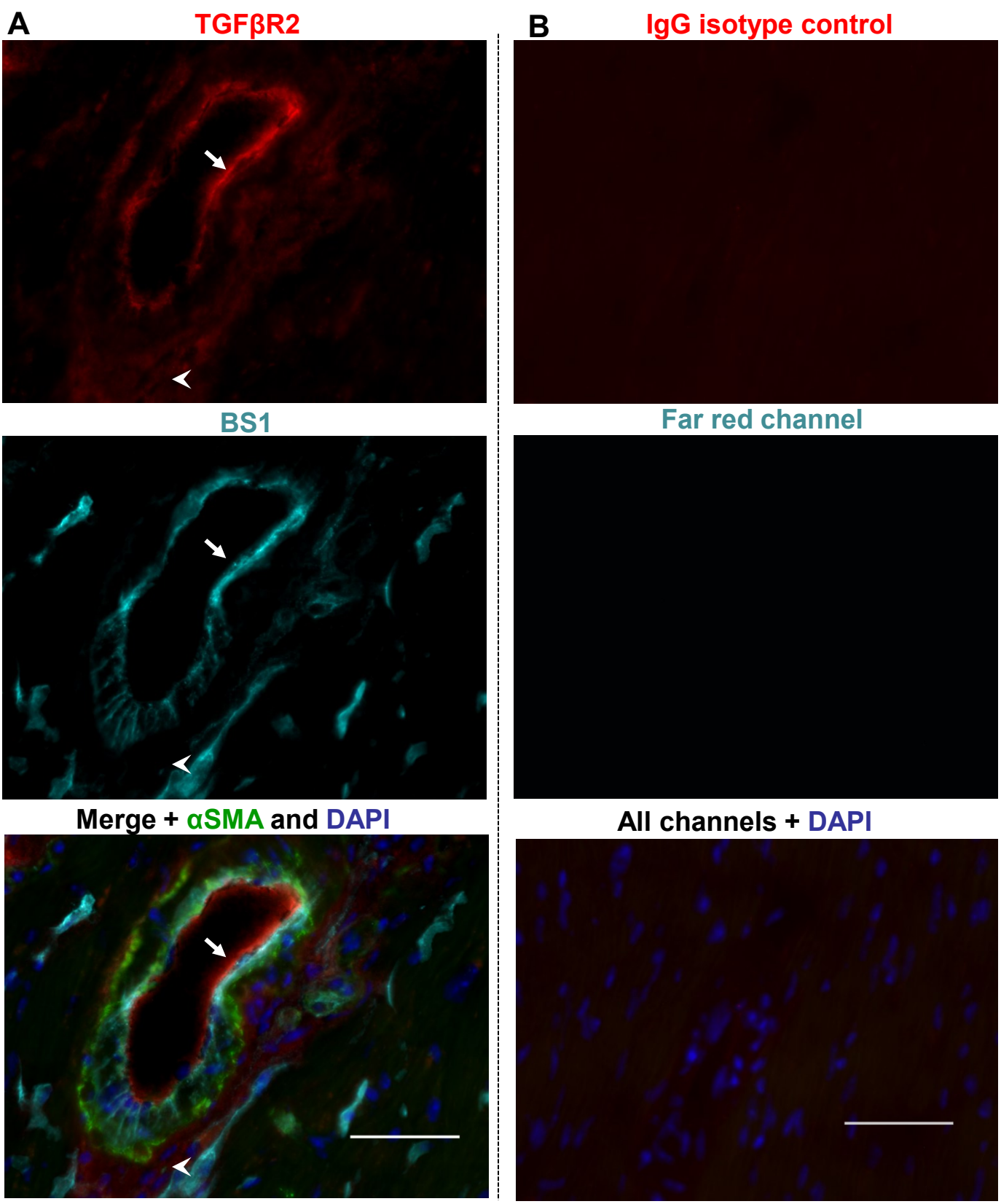
Supplement Fig. 9. TGFβ1 co-localizes with capillaries in *mdx* hearts. Staining for BS1 and TGFβ1 ligand in 12 month old *mdx* hearts confirms co-localization suggesting that microvascular endothelial cells produce and secrete in response to the chronic disease. Control slides stained with an IgG isotype confirmed the absence of non-specific staining. Scale bars = 50μm



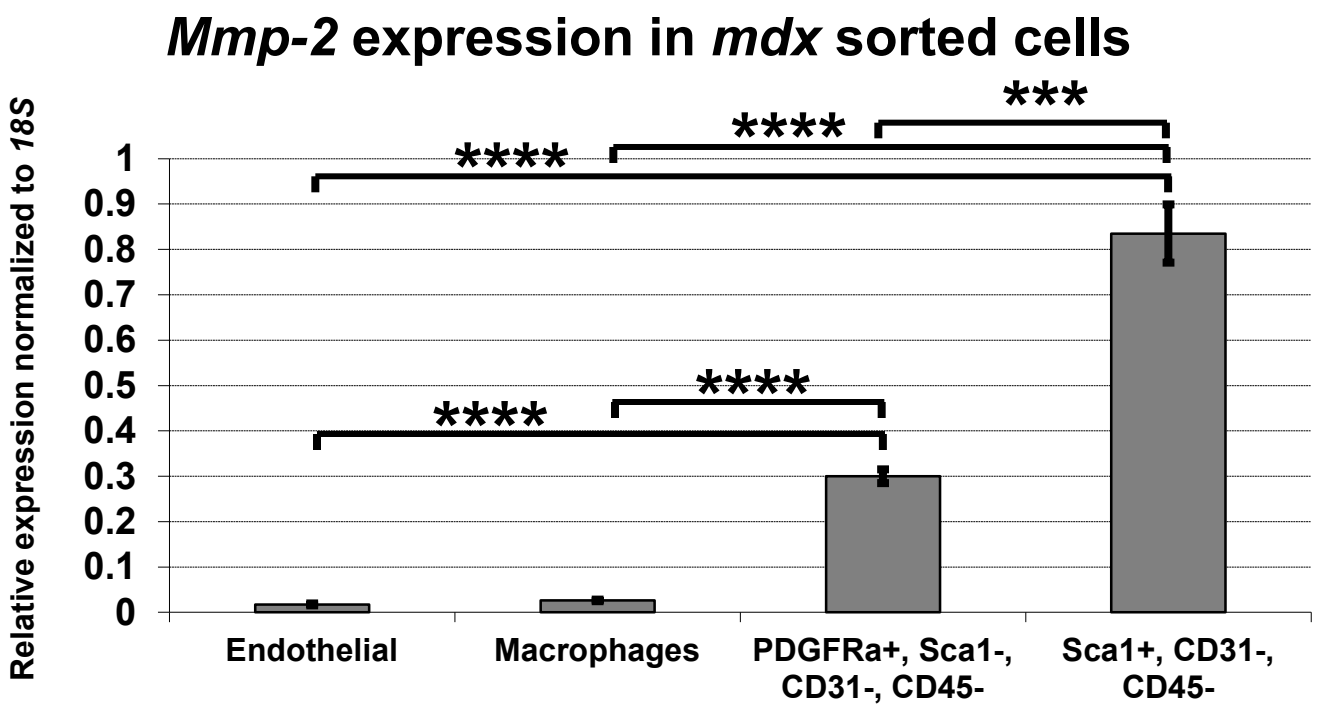
Supplement Fig. 10. Coronary endothelial and adventitial cells stain positive for TGFβ1. **A.** Further histological analysis indicates strong TGFβ1 ligand staining co-localizing with endothelial cells (arrow, BS1+) and adventitial cells (arrow head, BS1-) of *mdx* coronary vessels. **B.** TGFβ1 staining co-localizes with Sca1+ adventitial (arrowhead) and endothelial (arrow) cells. αSMA marks the smooth muscle layer in green. For both staining's sections from a 12 month old male *mdx* heart were used. Scale bars = 50μm

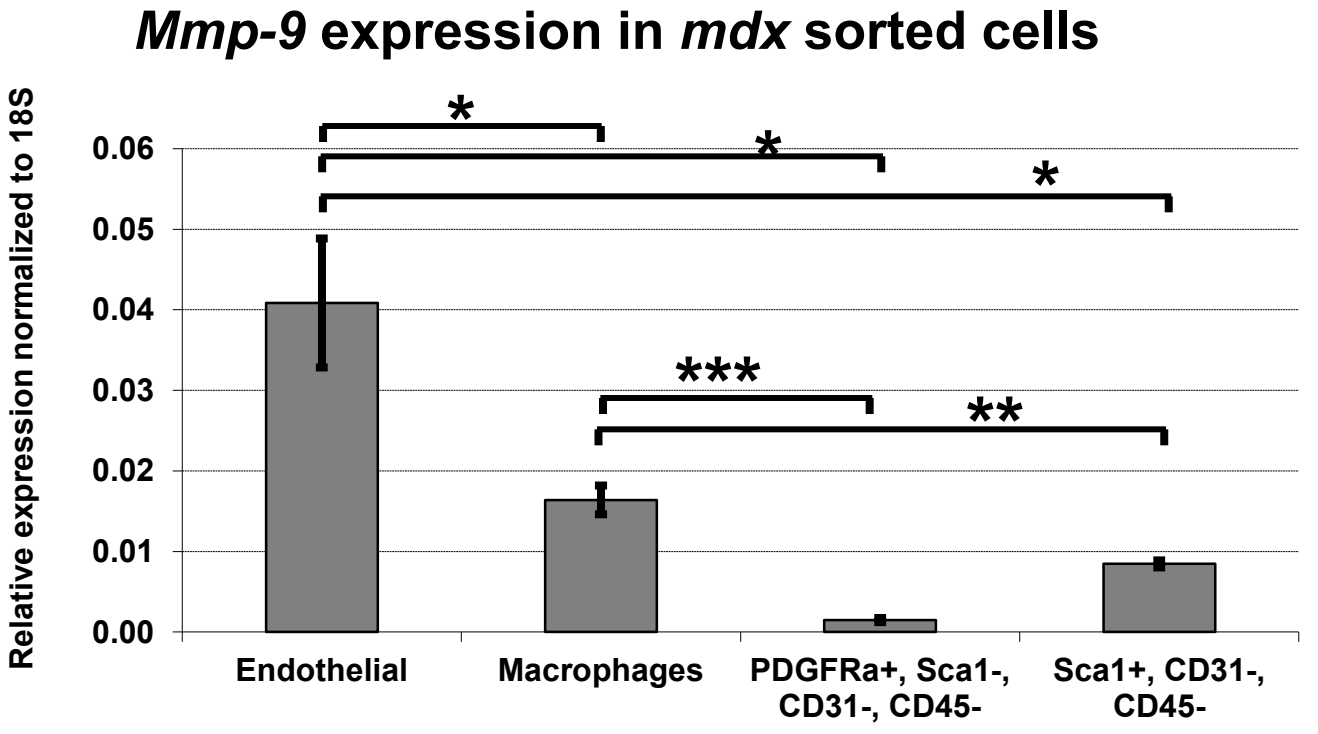


Supplement Fig. 11. TGFβR1 is localized to most coronary vascular cells
A. Further histological analysis in serial sections of the same 12 month old male *mdx* heart depicted in Supplemental Fig. 7, indicates that TGFβR1 is diffuse but more prominent in the vascular smooth muscle (arrow) and adventitial cells (arrowhead) of *mdx* coronary vessels. Once more BS1 labels endothelial cells and αSMA marks the smooth muscle layer in green. **B.** Correspondingly staining for TGFβR1 localizes with Sca1+ adventitial cells (arrowhead) and Sca1- vascular smooth muscle (arrow). Scale bars = 50μm

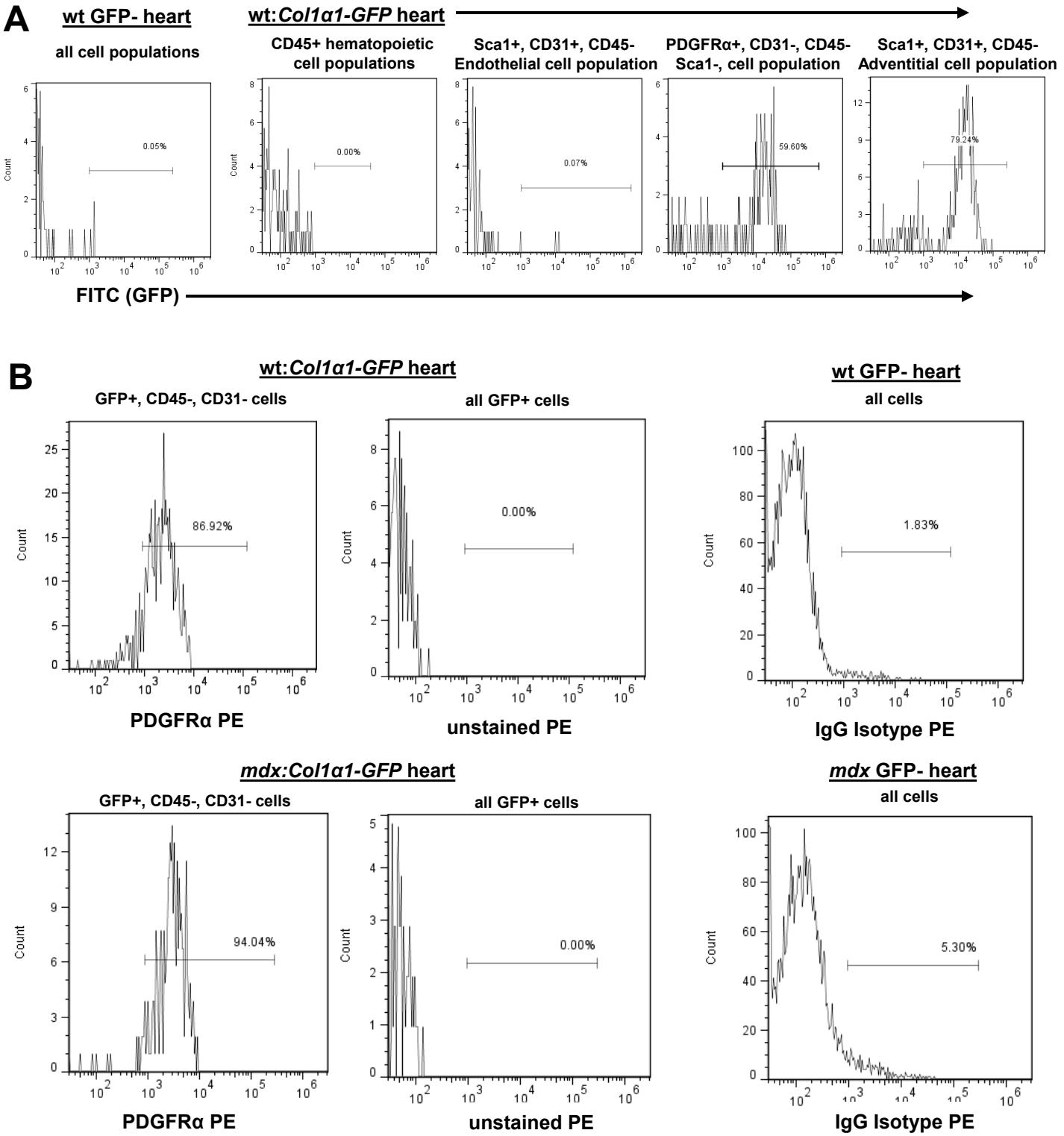


Supplement Fig. 12. Coronary endothelial and adventitial cells stain positive for TGFβR2. **A.** In contrast to TGFβR1 staining (Supplementary Fig. 6B), TGFβR2 was present primarily in the adventitia (arrowhead) and endothelium (arrow) of *mdx* coronary vessels. Once more αSMA marks the smooth muscle layer in green. **B.** Staining for TGFβR2 and those depicted in Supplementary Fig. 9-11, were run in parallel with a rabbit polyclonal IgG isotype control (all the TGFβ antibodies are also rabbit polyclonal) added to serial sections from the same 12 month old male *mdx* heart were used. Scale bars = 50μm

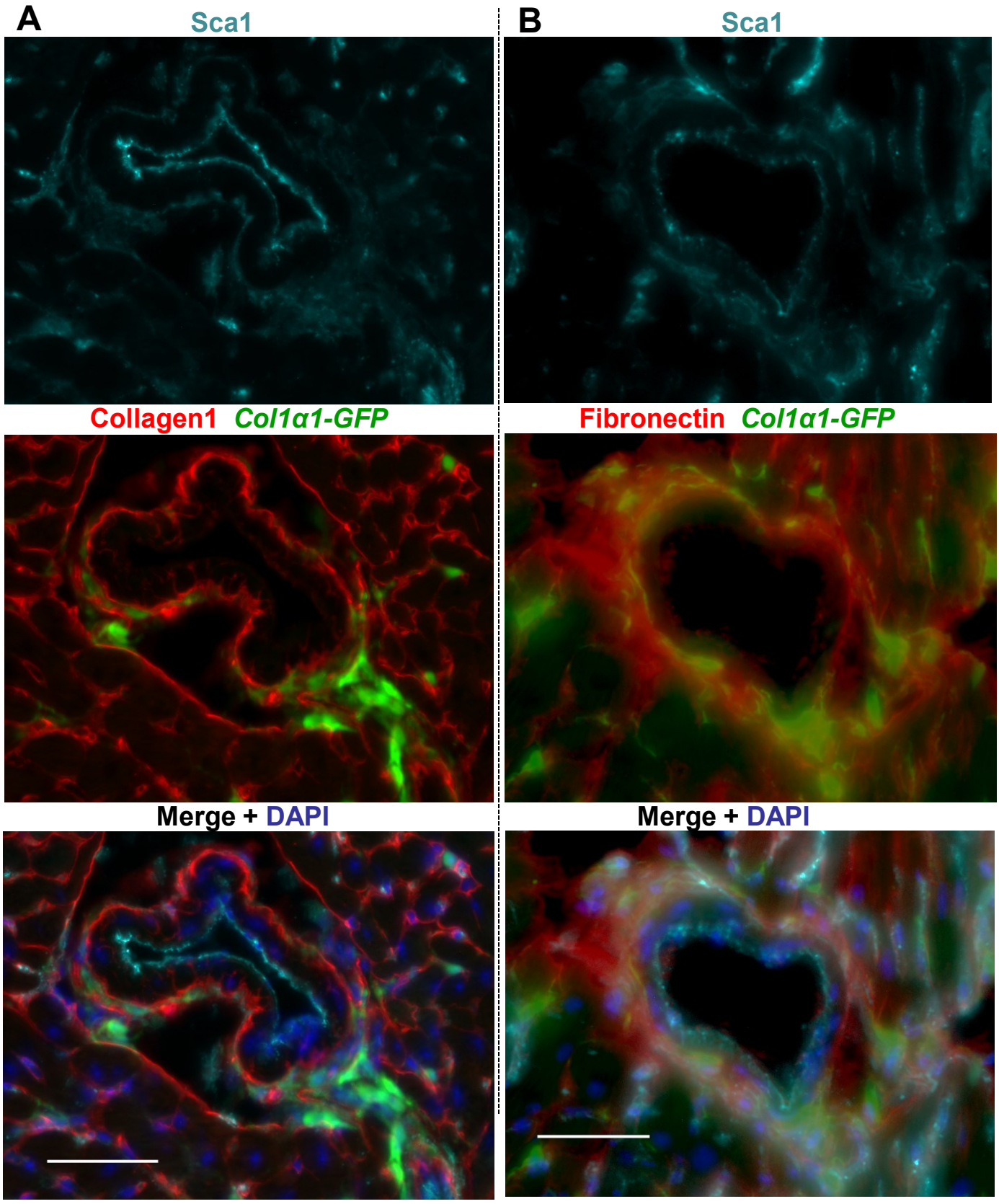




Supplement Fig. 13. FACS-sorted *mdx* adventitial and endothelial cells express significant levels of *metalloproteinase 2* and *9* respectively.
qRT-PCR analysis of cells FACS-sorted from *mdx* hearts (n=4 1 year old males, same samples as Figure 3D/E), reveals Sca1+ adventitial cells express the greatest level of *Mmp*-2 , while endothelial cells express the greatest levels of *Mmp-9*, as compared to the to other populations analyzed. Once more, macrophages were sorted as CD45+, F4/80+, and endothelial cells as Sca1+, CD31+, CD45-. *P<0.05, ** P<0.005, ***P<0.0005, **** P<0.00005

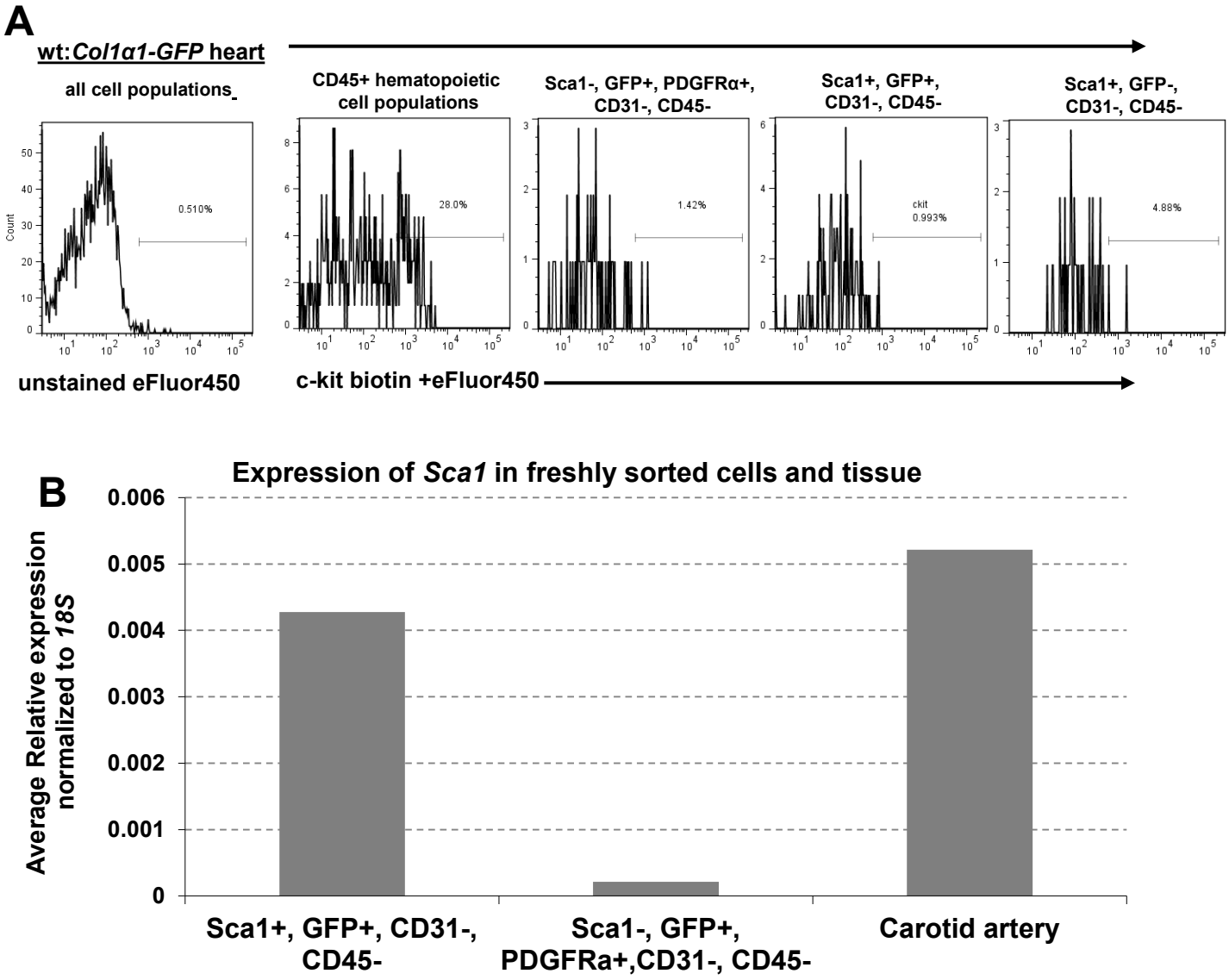


Supplement Fig. 14. The majority of Sca1+ adventitial cells express *Col1α1-GFP* in wt hearts. **A.** Analogous to our analysis of GFP in *mdx* hearts, we analyzed cells from age matched wt:*Col1α1-GFP* hearts (n=3 GFP+ and n=1 GFP- control, 4 month old males) as shown using the same gating strategy represented in Figure 3A. **B.** FACS-analysis of wt and *mdx:Col1α1-GFP* cardiac cells indicated that the majority of GFP+ cells are also PDGFRα+ (left histograms). This is neither due to bleed-through of GFP signal into PE as indicated by the unstained controls (middle histograms, nor due to non-specific binding which was minimal in both wt and *mdx* heart cell suspensions (right histograms).

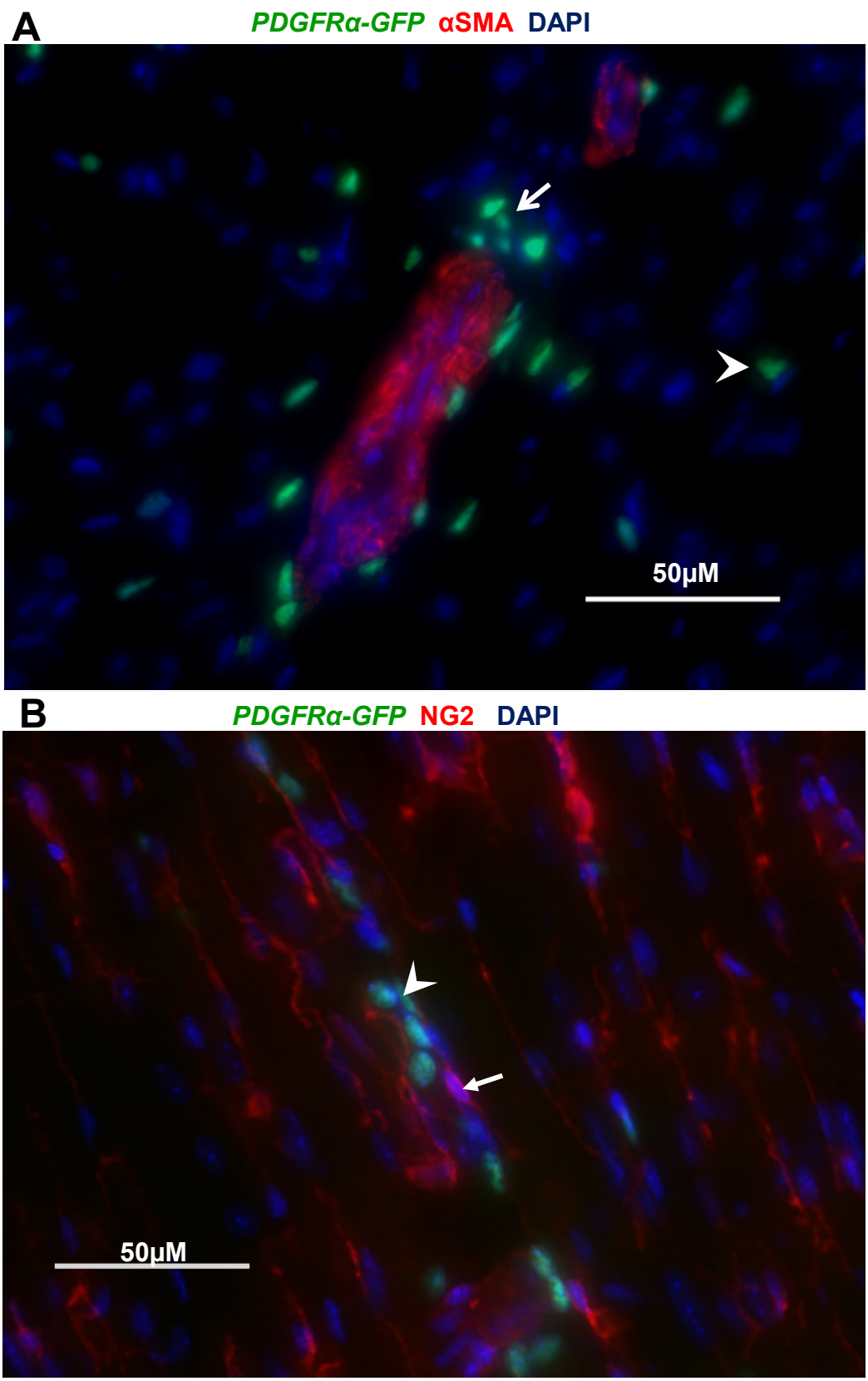


Supplement Fig. 15. Adventitial Sca1+, *Col1α1-GFP*+ cells co-localize with type I collagen and fibronectin in *mdx* hearts.

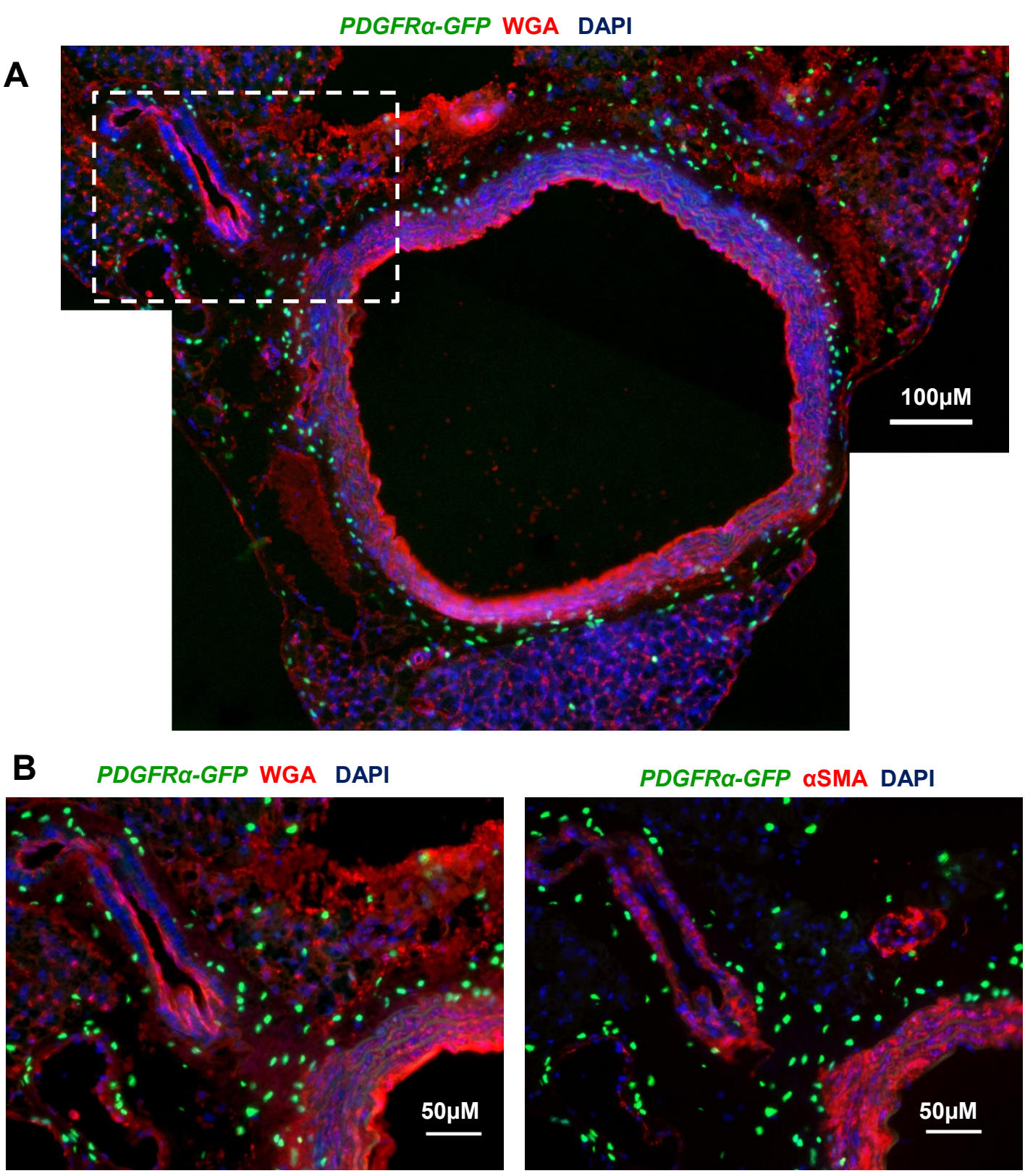
A. Staining for collagen1 in sections from a 4 month old *mdx:Col1α1-GFP* heart, highlights the presence of type I collagen around Sca1+, *Col1α1-GFP*+ adventitial cells. **B.** Fibronectin also stained brightly around Sca1+, *Col1α1-GFP*+ adventitial cells in a serial section of the same *mdx:Col1α1-GFP* heart.



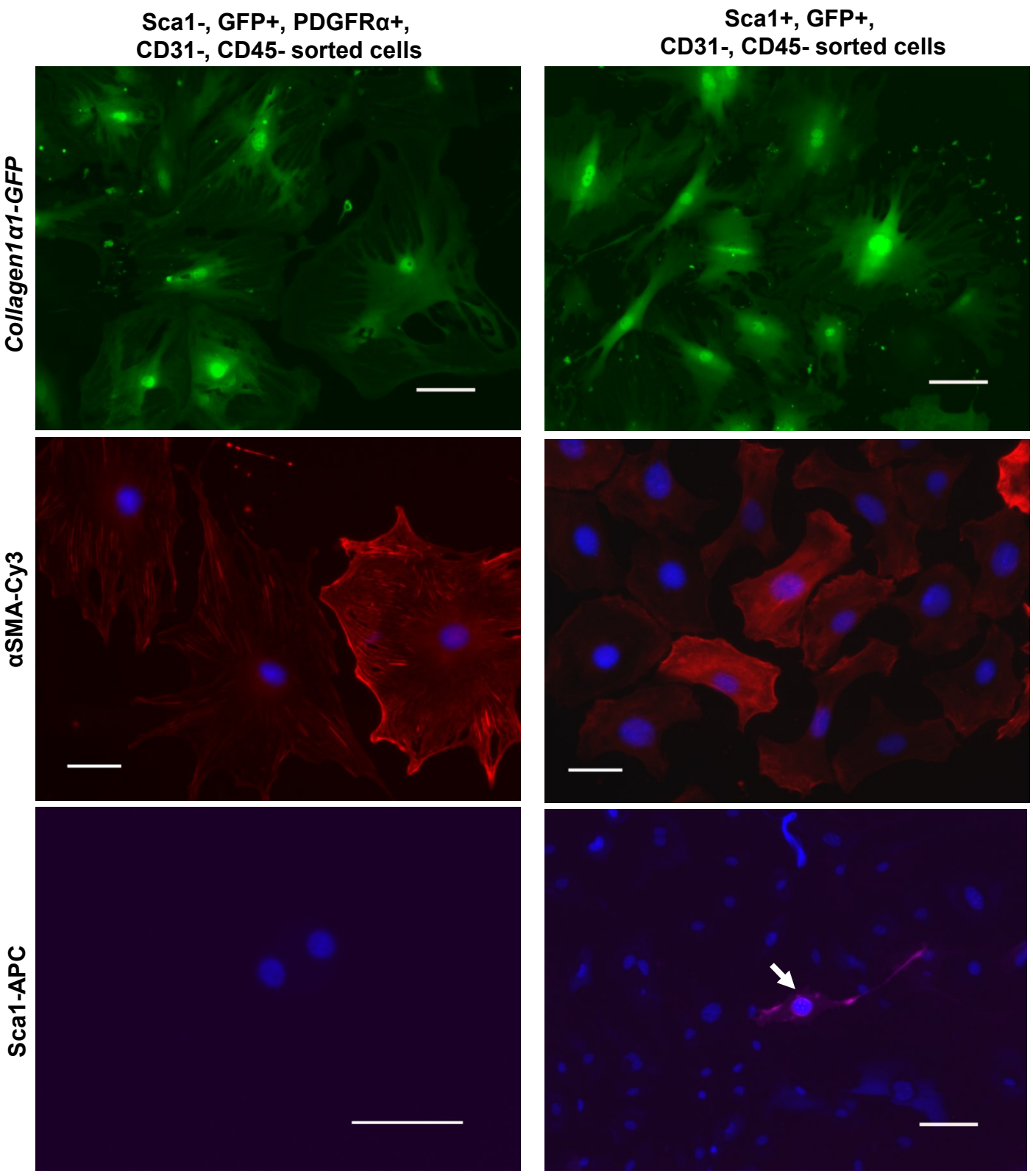
Supplement Fig. 16. *Col1α1-GFP*+ cells are negative for c-Kit but vary in their expression of S*ca1*. **A.** FACS analysis of a wt:*Col1α1-GFP* heart (8 month old male), reveals that only a minority of Sca1+ adventitial cells and Sca1-, PDGFRα+ interstitial fibroblasts are positive for c-Kit. More specifically, GFP+, Sca1+ or Sca1- were largely negative for c-kit, whereas almost 5% of GFP-Sca1+ cells were positive for c-kit. In contrast, almost 30% CD45+ hematopoietic cells were positive for c-Kit. **B.** qRT-PCR analysis for *Sca1* expression in freshly sorted cells isolated from the same wt:*Col1α1-GFP* heart, indicates that GFP+ cells that stain negative for the Sca1 antigen express negligible levels of *Sca1* vs. GFP+ cells that stain positive for the Sca1 antigen. A freshly isolated carotid artery (n=1, wt) which included the adventitia was used as a positive control.



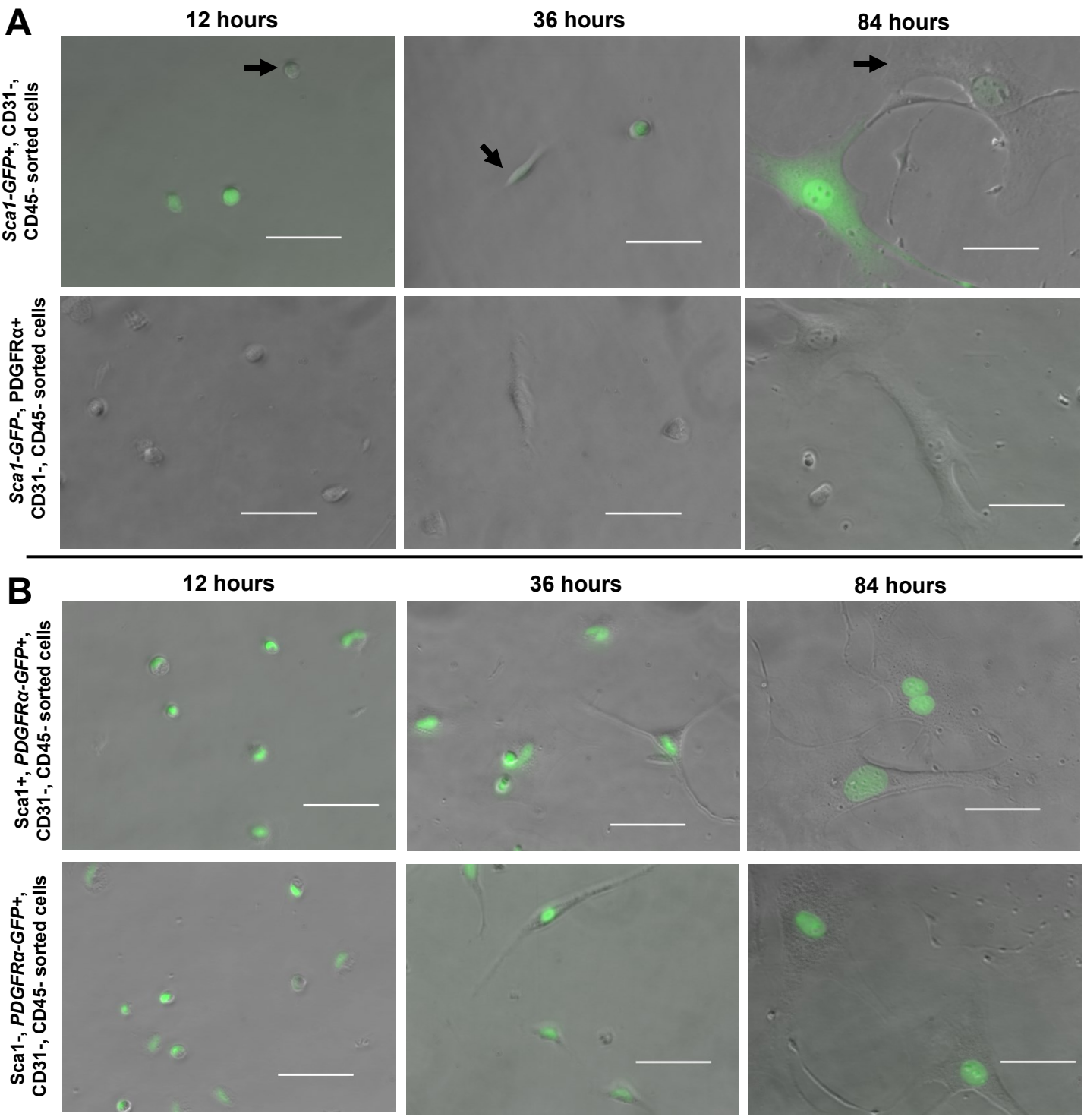
Supplement Fig. 17. Adventitial and interstitial NG2- cells express *PDGFRα-GFP*.
A. Histological analysis of heart sections from a *PDGFRα-GFP* reporter mouse (1.5 month old male), reveals nuclear GFP+ cells are localized to the coronary adventitia, adjacent to αSMA+ smooth muscle cells (arrow), but are also present in the interstitial space (arrowhead). **B.** In turn, cardiac interstitial *PDGFRα-GFP+* cells (arrowhead), stain negative for NG2 but are in close proximity to NG2+ pericytes (arrow).



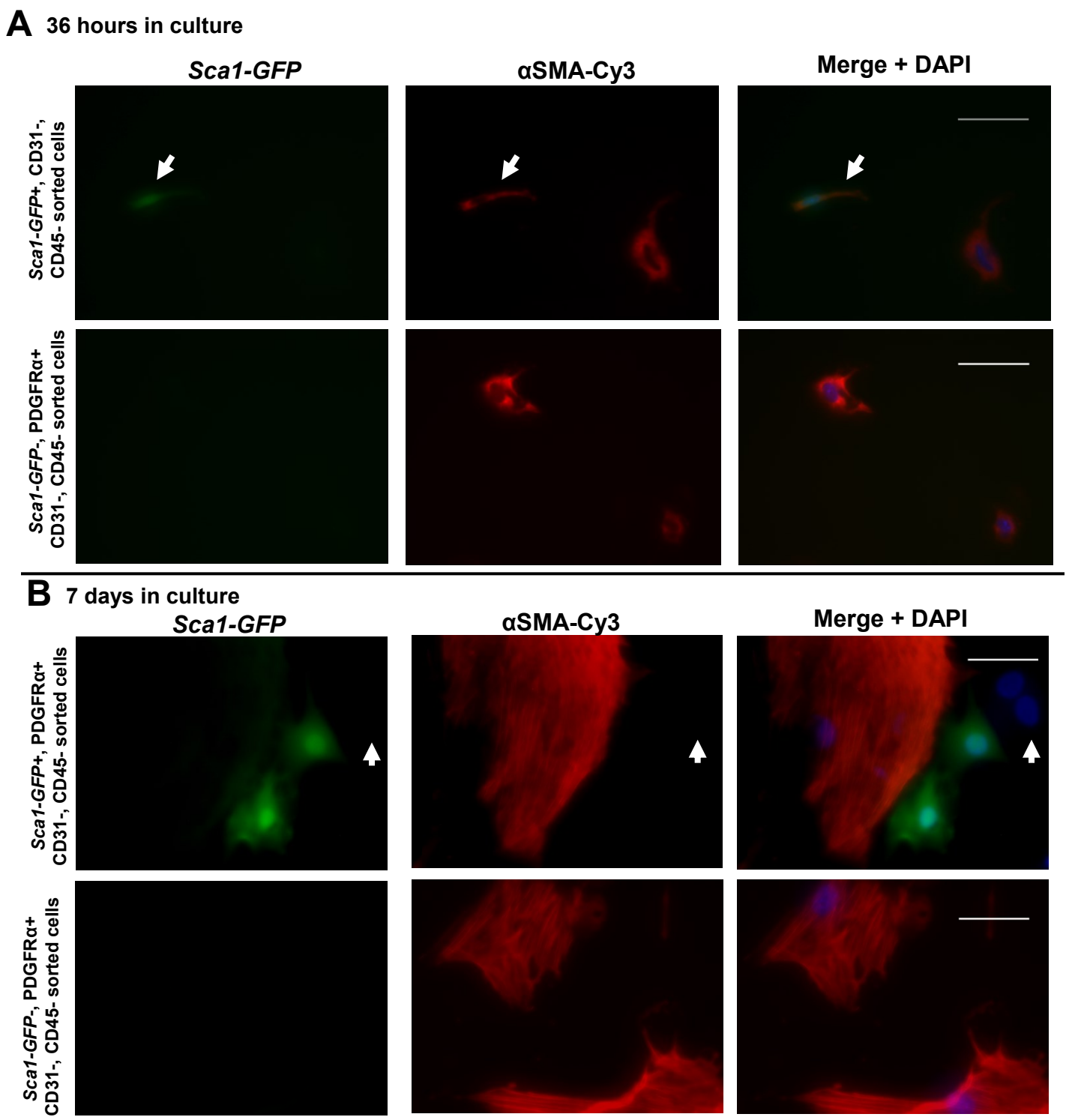
Supplement Fig. 18. *PDGFRα-GFP*+ cells are also present in the macrovascular adventitia. **A.** Cross-sectional montage of the thoracic aorta isolated from the same *PDGFRα-GFP* reporter mouse depicted in Supplementary Fig. 17, reveals PDGFRα+ cells expressing nuclear localized GFP in adventitia of the aorta. Wheat germ agglutinin (WGA) was used as a counterstain to highlight cell membranes and the tissue borders. **B.** Higher magnification photos of the boxed region indicate PDGFRα expressing cells are adjacent to medial smooth muscle cells, which are highlighted by α-smooth muscle actin (αSMAs) staining.



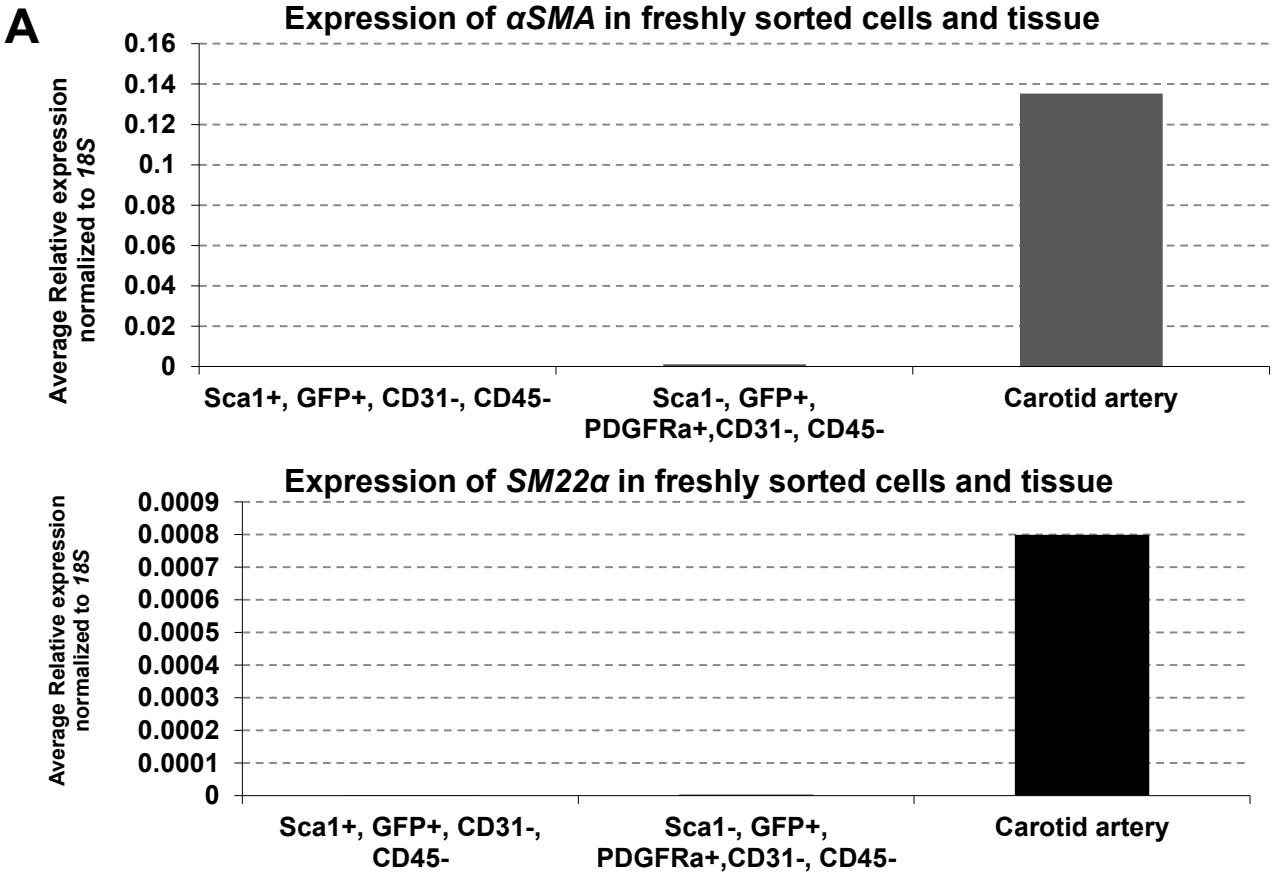
Supplement Fig. 19. Sca1+, *Col1α1-GFP*+ sorted cells express αSMA but lose Sca1 in culture. Cell sorted from 4 month old wt:*Col1α1-GFP* hearts were maintained in culture for 1 month then fixed and analyzed for the presence of GFP, α-smooth muscle actin (αSMA) and Sca1. Both Sca1-, GFP+, PDGFRα+, CD31-, CD45- and Sca1+, GFP+, CD31-, CD45- sorted cells gave rise to αSMA+ cells that remained GFP+ in culture. In contrast, the progeny of cultured Sca1+ sorted cells almost entirely lost the Sca1 antigen; only 1 cell in an entire 12 well plate surveyed was Sca1+ (arrow). In turn, the cultured progeny of Sca1- PDGFRα+ sorted cells remained negative for Sca1. Blue represents DAPI staining merged with αSMA and Sca1 staining's. Scale bars = 100μm.

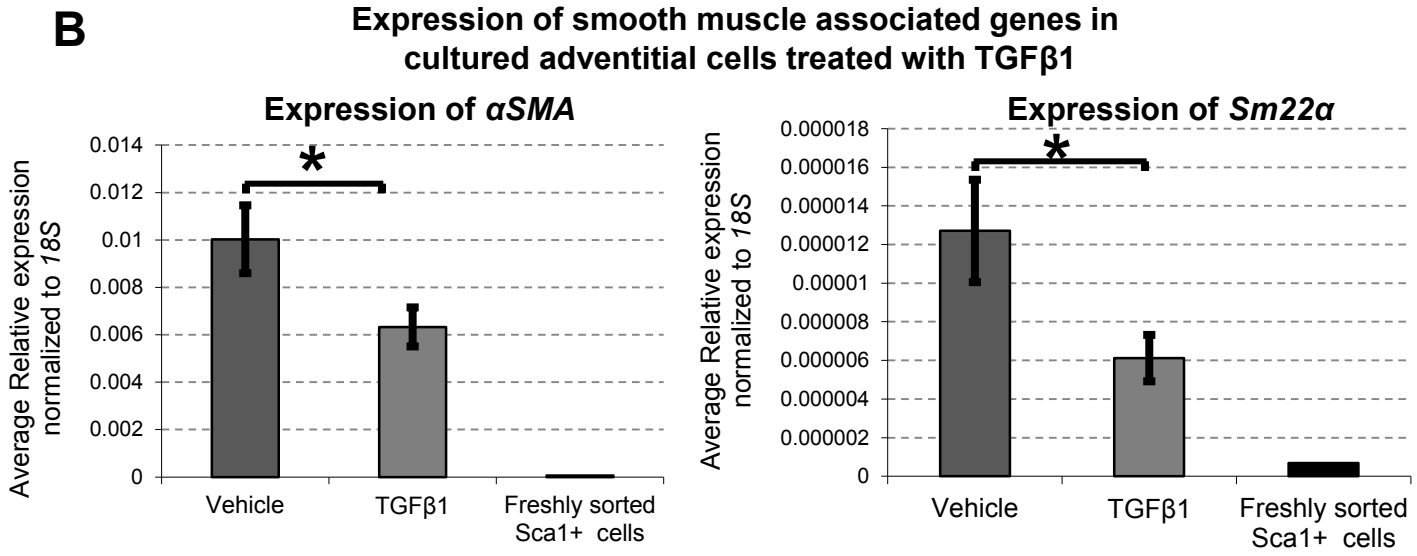


Supplement Fig. 20. *Sca1-GFP* is lost while *PDGFRα-GFP* expression is maintained in culture. **A**. Live imaging of freshly sorted cells captured at 12, 36, and 84 hours following seeding in culture. Sca1+, GFP+, CD31-, CD45- (first row) and Sca1-, GFP-, PDGFRα+, CD31-, CD45- (second row) cells were FACS-sorted from a wt:*Sca1-GFP* heart (8 month old male). Within this timeframe, 2/3rds of *Sca1-GFP*+ sorted cells lost *Sca1* expression by 12 hours of being seeded (third row), as indicated by the absence of cytoplasmic GFP (arrow). By 84 hours in culture 1 in 2 *Sca1-GFP*+ cells lost GFP expression. During this timeframe examined, *Sca1-GFP-* sorted cells remained GFP negative. **B**. In parallel, GFP+, PDGFRα+, CD31-, CD45- (third row) and Sca1-, PDGFRα+, CD31-, CD45- (fourth row) cells were also sorted from a wt:*PDGFRα-GFP* heart (9 month old male). Both Sca1+ and Sca1- sorted cells maintained *PDGFRα* expression in culture, as indicated by the presence of nuclear GFP. Note, each panel is a merge of phase contrast and GFP captured within the same field. Scale bars = 50μm.



Supplement Fig. 21. *Sca1-GFP+* adventitial cells give rise to a heterogeneous population in culture. **A**. The same *Sca1-GFP* FACS-sorted, cultured cells depicted in Supplementary Fig. 13, were fixed and stained for αSMA at 36 hours and 7 days in culture. Cultured cells derived from the sorted Sca1+, GFP+, CD31-, CD45- (first row) and Sca1-, GFP-, PDGFRα+, CD31-, CD45- (second row) populations, both stained positive for αSMA by 36 hours of being cultures. Interestingly, we observed GFP+ (arrow) and negative cells derived from the Sca1+, GFP+, CD31-, CD45- sorted population that appeared to upregulate αSMA . **B**. By day 7 in culture, cells derived from the sorted Sca1+, GFP+, CD31-, CD45- (third row), were heterogeneous as some cells stained brightly positive for αSMA, while some GFP+ and negative (arrow) cells did not show detectable staining. In contrast, cells derived from Sca1-, GFP-, PDGFRα+, CD31-, CD45- sorted cells (fourth row) , were almost unanimously positive for αSMA, but did not appear to expand as the Sca1+ sorted population. Scale bars = 50μm.





Supplement Fig. 22. Adventitial cells upregulate smooth muscle associated genes in culture. **A.** qRT-PCR analysis of freshly sorted cells isolated from a wt:*Col1α1-GFP* heart (n=1, 8 month old male). Both Sca1+ and Sca1-, *Col1α1-GFP*+ cells did not express smooth muscle associated genes smooth muscle *αSM*A and *SM22α*, as compared to a freshly isolated carotid artery (n=1, wt) used as positive control. **B.** qRT-PCR analysis of cultured adventitial cells (grey bars), the same samples represented in Figure 6, indicates that the progeny of Sca1+, *Col1α1-GFP*+ sorted cells express smooth muscle associated genes in culture. This expression is significantly reduced with TGFβ1 treatment. Such data suggests that adventitial cells can adopt a smooth muscle phenotype and revert back to pro-fibrotic state in response to TGFβ1 mediated signaling. Expression values for the freshly sorted Sca1+ parent population shown in A, are depicted for reference purposed as black bars to the right of the values for culture cells. *P<0.05.