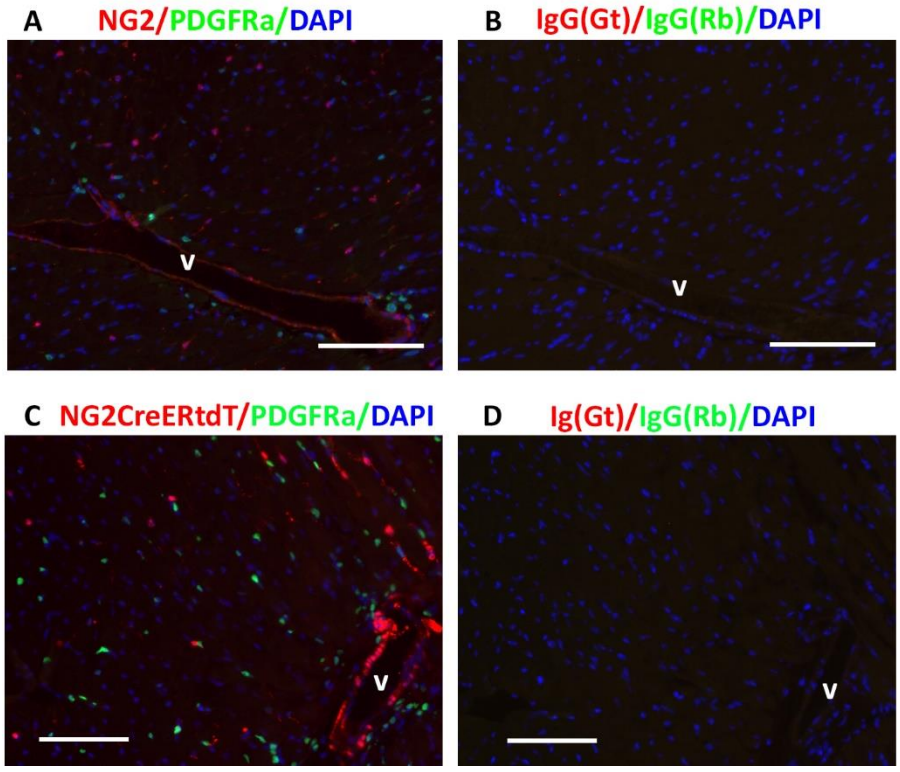
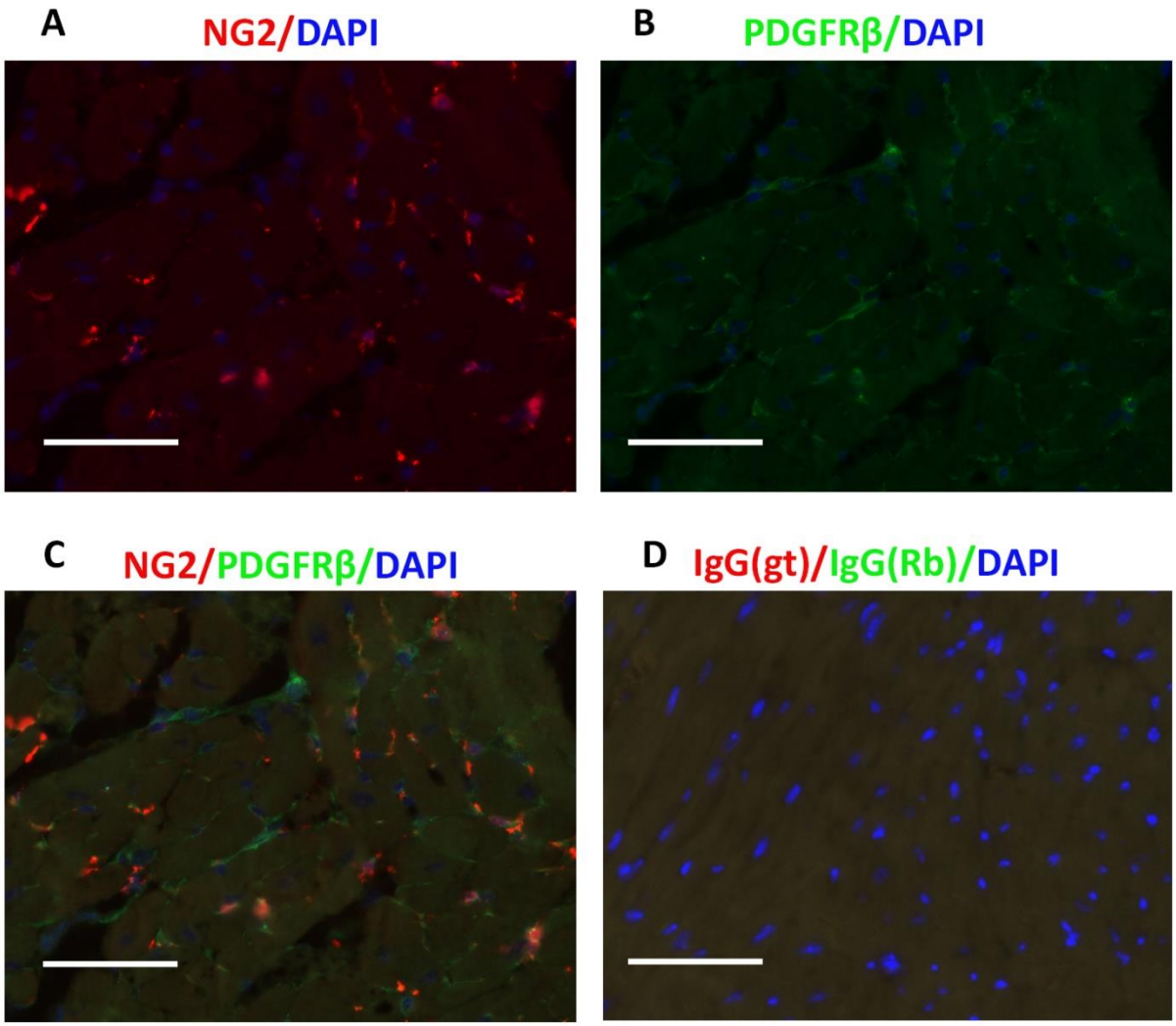
SUPPLEMENTAL MATERIAL

Figure S1. Isotype-matched controls demonstrate the specificity of fibroblast and pericyte labeling in dual reporter and lineage tracing systems.



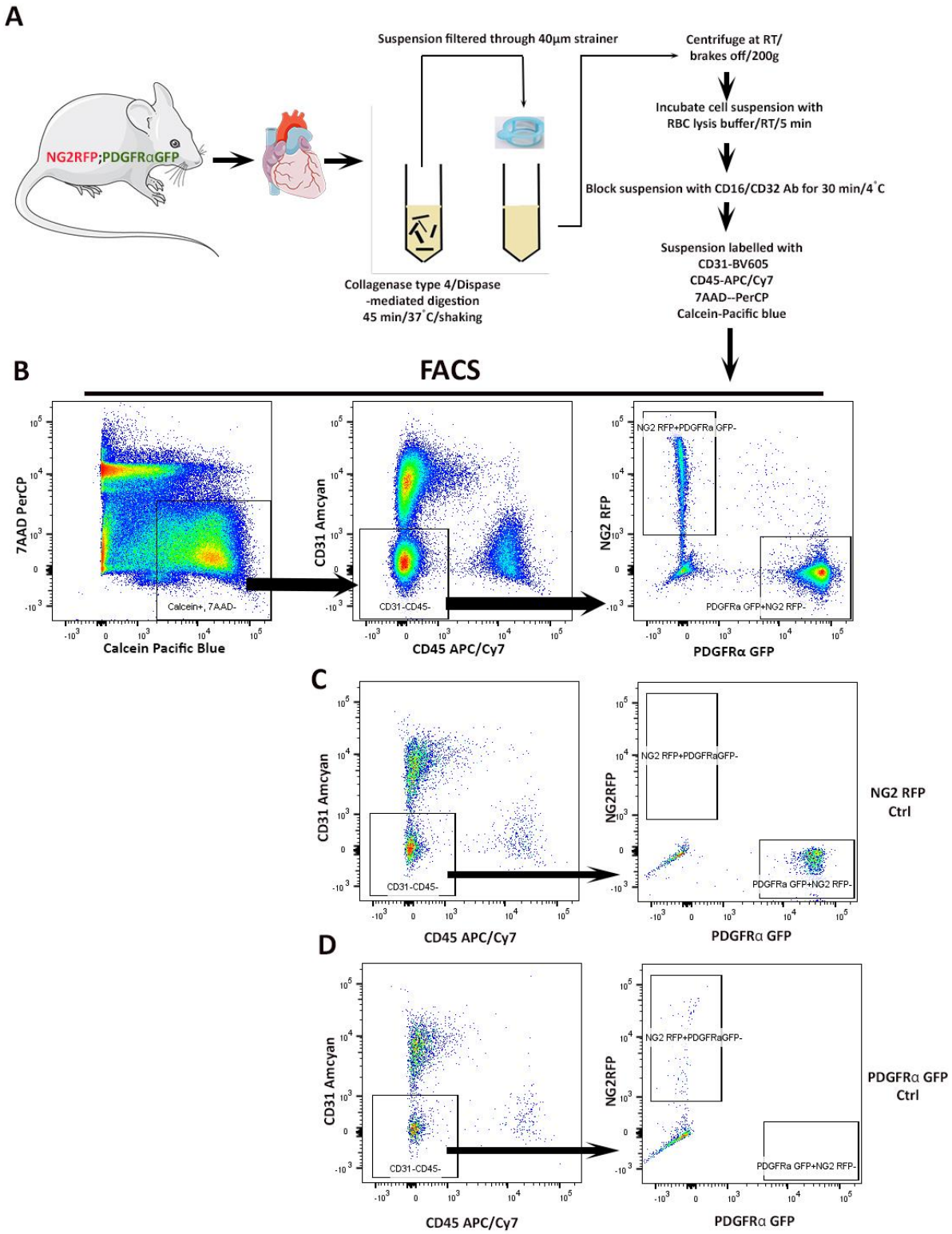
A-B: In myocardial sections from dual reporter NG2^{Dsred};PDGFRα^{EGFP} mice, NG2 labels mural cells (red), whereas PDGFRα stains the nuclei of fibroblasts (green). Isotype-matched controls in a serial section (B) show no staining, demonstrating the specificity of the strategy. Scalebar=80μm. C-D: In a similar manner, isotype-matched controls show the specificity of the lineage tracing approach in PDGRβ$^{iCre/+}$;R26^{tdTomato};PDGFRα^{EGFP} mice. NG2-traced cells appear red, whereas the nuclei of PDGFRα+ fibroblasts are green. An isotype matched control-stained serial section shows no staining. Scalebar=70μm; v, vessel.

Figure S2. Isotype matched controls show the specificity of NG2(Dsred)/PDGFRβ dual immunofluorescence.



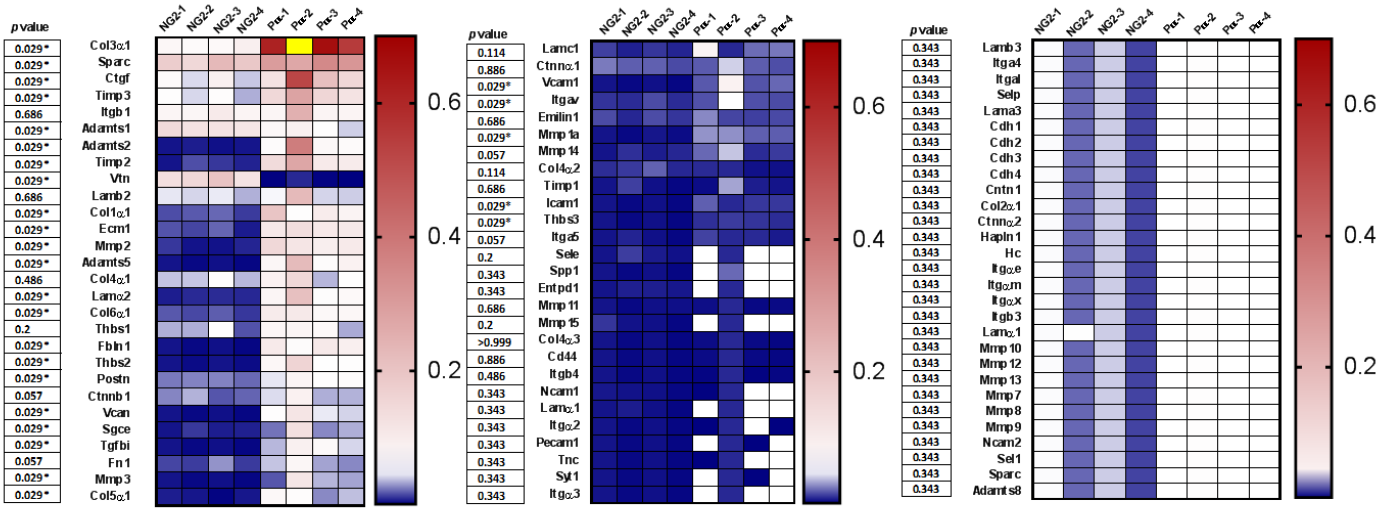
NG2+ positive profiles (red, A) and PDGFRβ-expressing cells (green, B) are identified in the myocardium of $NG2^{Dsred}$ reporter animals. Panel C shows the merged image. D. A section staining with isotype-matched control IgG shows no staining. Scalebar=50μm

Figure S3. Outline of the experimental procedure used to obtain a single cell suspension from dual reporter NG2^{DsRed};PDGFRα^{EGFP} adult mouse heart.



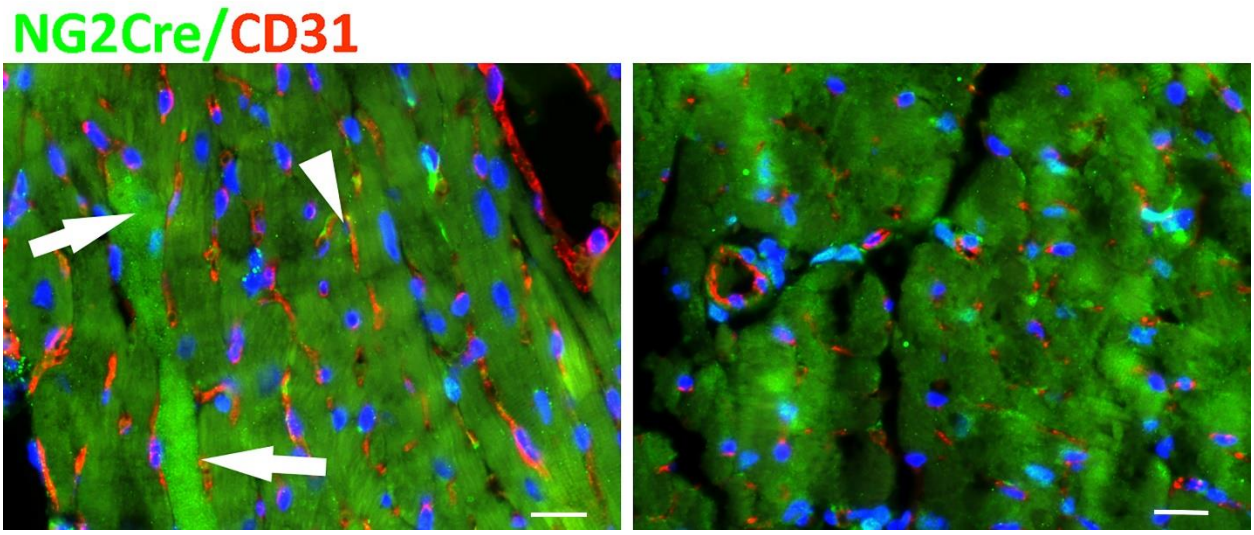
Viable (7AAD-) and metabolically active (calcein+) cells were gated and based on the expression of CD31 and CD45, the non-endothelial and non-hematopoietic cell population was identified. NG2+ and PDGFRα+ populations, subgated from the CD31-CD45- fraction, were FACS-sorted into cell lysis buffer to isolate RNA. C & D. $NG2^{DsRed}$ and $PDGFR\alpha^{EGFP}$ are genetic tags and are identified without the use of antibodies. C represents absence of $NG2^{DsRed}$ population when prepared from $PDGFR\alpha^{EGFP}$ single reporter mouse and D represents the absence of $PDGFR\alpha^{EGFP}$ population when prepared from $NG2^{DsRed}$ mouse model.

Figure S4. NG2+ pericytes and PDGFRα+ fibroblasts exhibit distinct transcriptomic profiles.



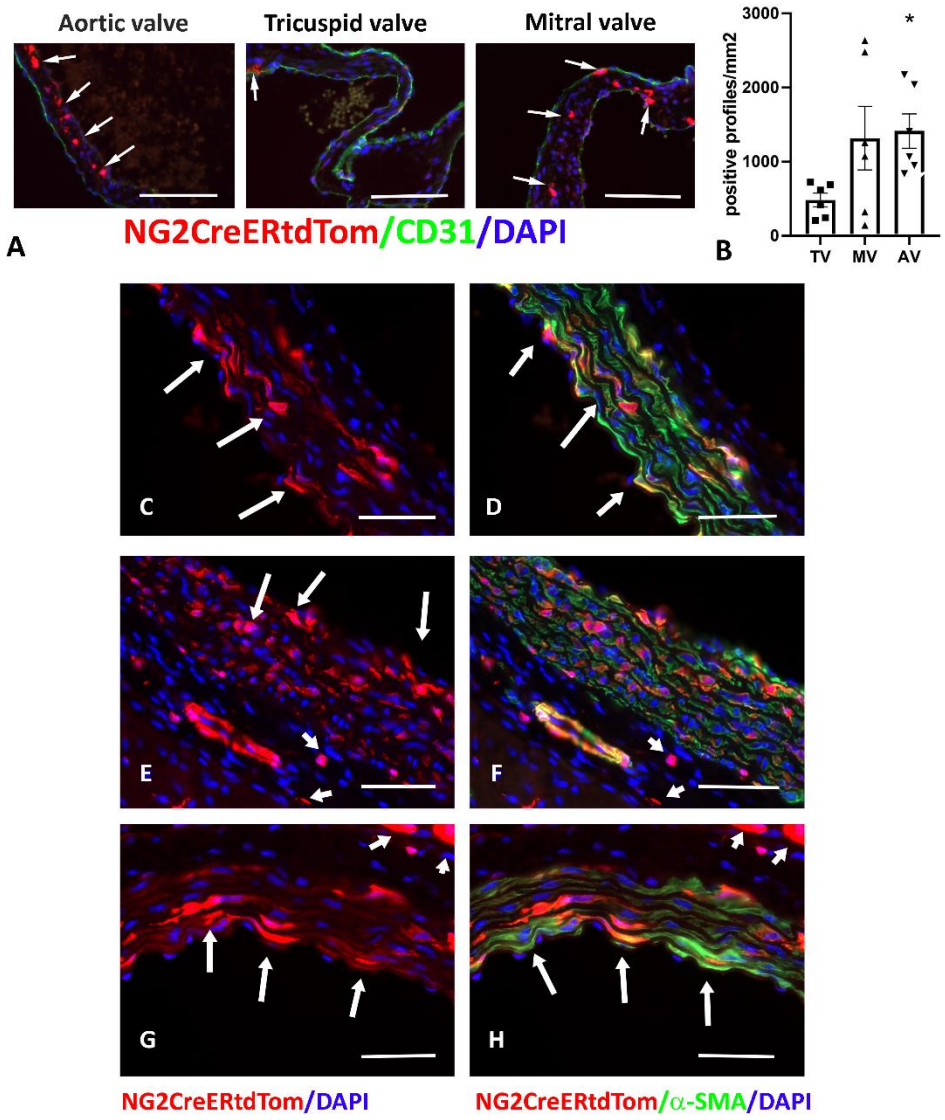
Heat map summarizes the qPCR array data, illustrating differential gene expression of NG2+ pericytes and PDGFRα+ fibroblasts sorted from adult mouse myocardium at baseline and the corresponding p value for each gene (n=4). The sample highlighted in yellow had a very high normalized gene expression value (1.17), that was outside the set range. Statistical comparison was performed using unpaired t-test (for normal distributions), or the Mann-Whitney test for non-Gaussian distributions.

Figure S5. The germline NG2-Cre driver does not specifically label myocardial mural cells.



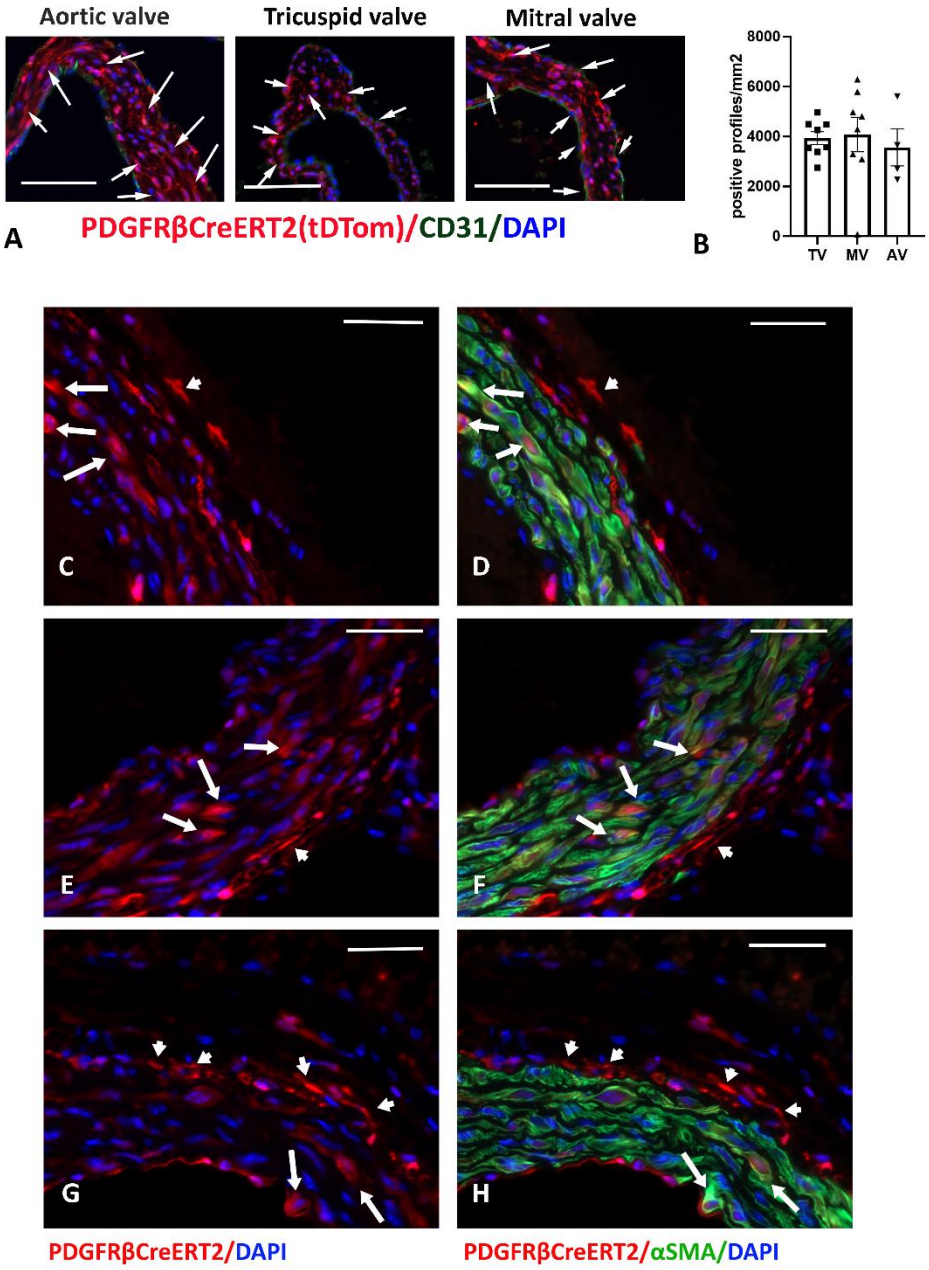
$NG2^{Cre/+};R26^{EYFP}$ mice were generated to trace NG2-derived cells. Dual fluorescence in myocardial sections for EYFP and the endothelial cell marker CD31 (arrrowheads) showed that vascular mural cells could not be specifically identified due to the intense fluorescence in cardiomyocytes (arrows). Images are representative of 6 different experiments. Scalebar=20μm

Figure S6. Labeling of valvular and aortic cells using the inducible NG2CreER^{TM} driver.



A-B, TdTomato staining of myocardial sections in the NG2CreER^{TM} model labeled a significant population of valvular cells, which were more abundant in the aortic valve (AV). **C-H**, Dual fluorescence for a-SMA and tdTomato shows labeling of mural cells in sections from the ascending aorta. ~60% of aortic medial α-SMA+ vascular smooth muscle cells were labeled with the inducible NG2 Cre driver (long arrows). Occasional α-SMA-negative NG2-labeled adventitial cells may represent pericytes of the adventitial vasa vasorum (short arrows). Images are representative of 6 different experiments. Scalebar=100μm. Data are presented as mean $\pm$ SE. Statistical comparison was performed using non-parametric ANOVA (Kruskall-Wallis).

Figure S7. The inducible PDGFRβCreER^{T2} driver labels abundant valvular cells and a large population of aortic adventitial cells.



A-B, tdTomato staining in PDGFRβCre^{ERT2} model labeled a much larger population of valvular interstitial cells when compared to the NG2CreER^{TM} model (shown in Suppl Fig VI). **C-H**, Staining of aortic sections showed that ~70% of aortic αSMA+ vascular smooth muscle cells were labeled using the PDGFRβCre^{ERT2} line (long arrows). Moreover, the inducible PDGFRβ Cre driver also labeled a large population of adventitial aortic cells that were negative for α-SMA (short arrows). These cells had morphological characteristics of fibroblasts. Images are representative of 6 different experiments. Scalebar=100μm. Data are presented as mean ± SE. Statistical comparison was performed using non-parametric ANOVA (Kruskall-Wallis).