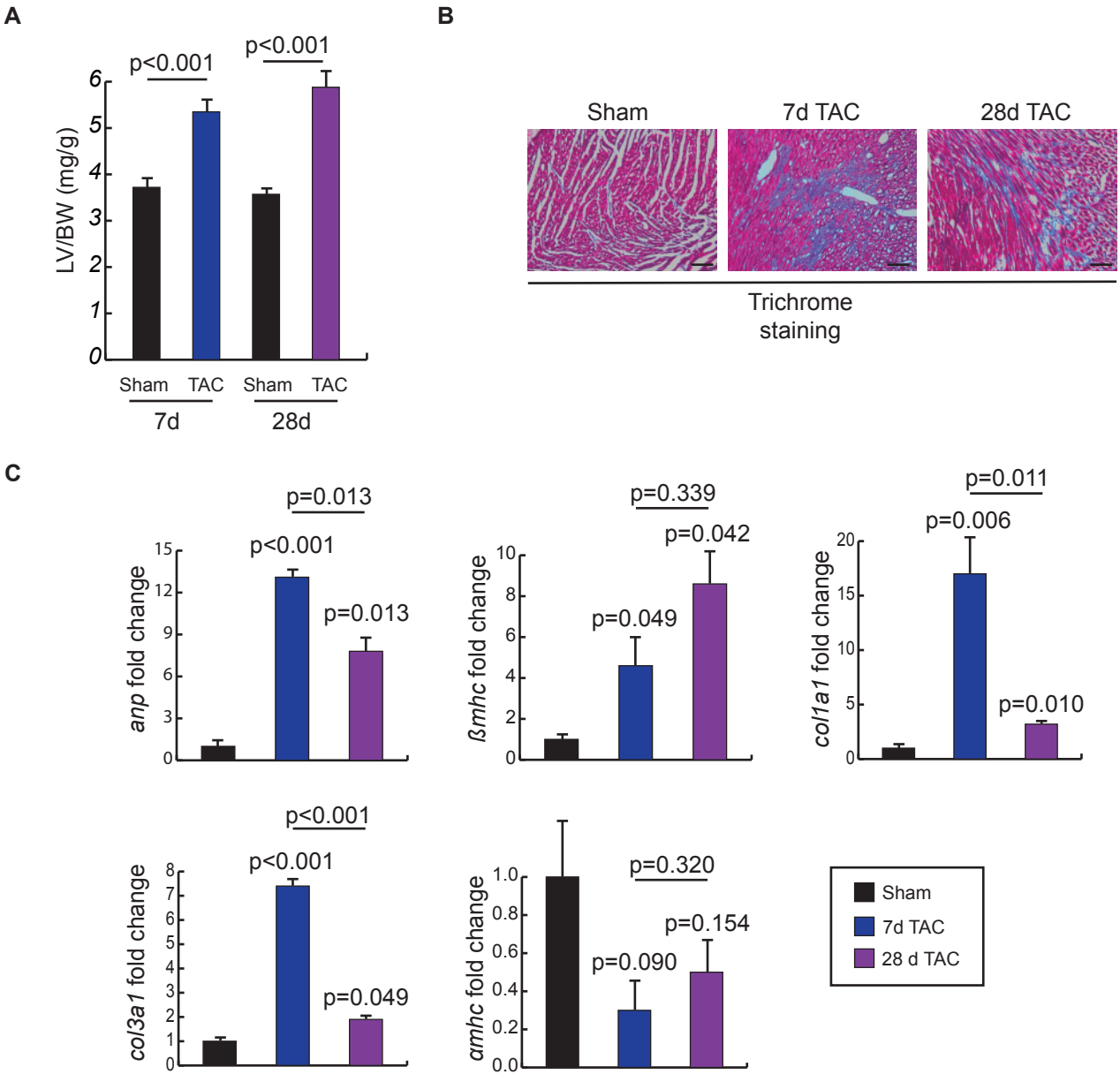
Moore-Morris et al. Supplemental Table 1.

In vivo echocardiographic assessment of cardiac size and function following transaortic constriction (TAC) at 7d and 28d.

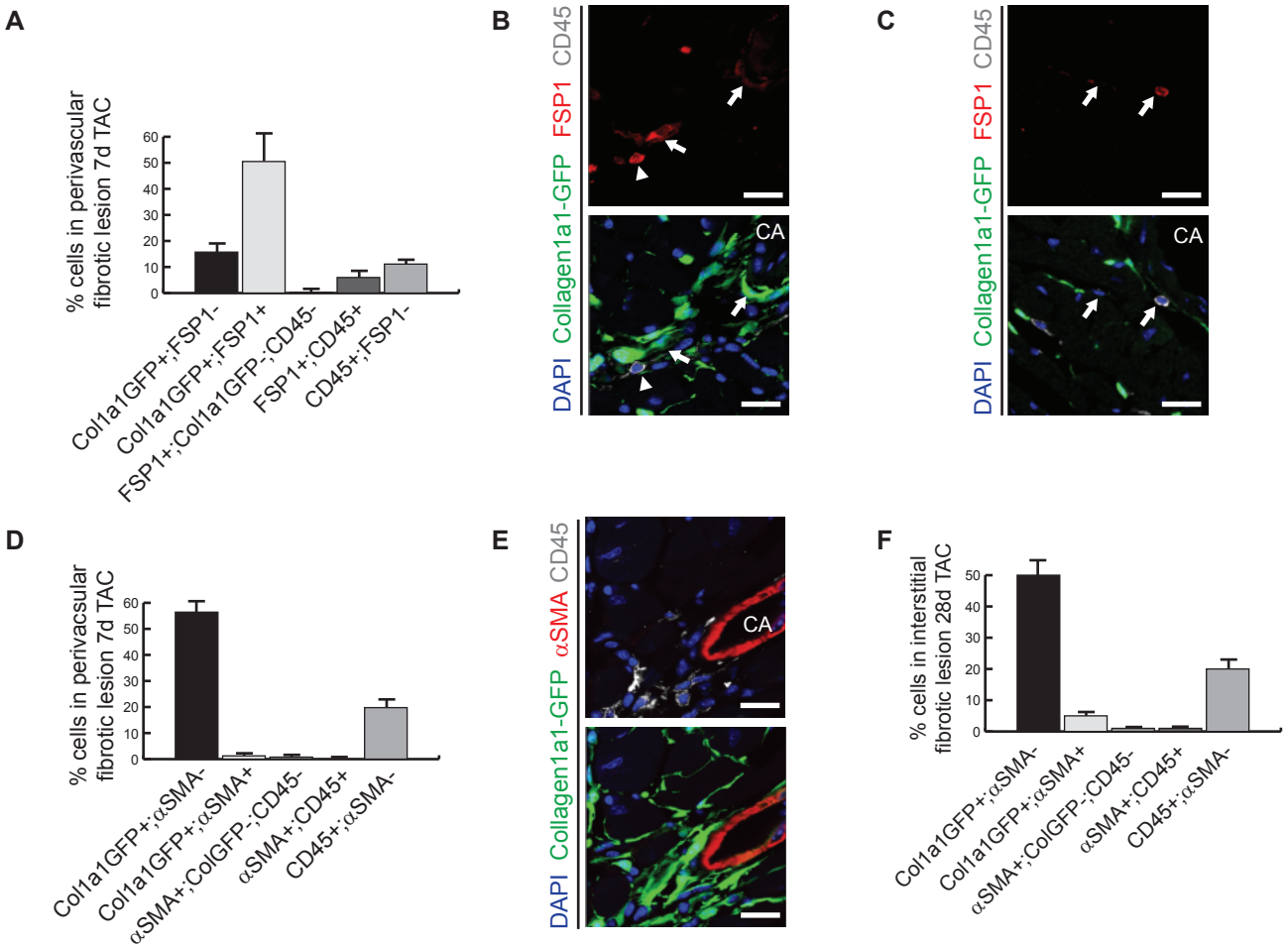
	SHAM 7d N=16	TAC 7d N=15	SHAM 28d N=11	TAC 28d N=16
BW, g	30.00±3.74	28.87±2.80	30.63±2.88	28.5±2.5
HR, bpm	527±72	504±35	535±47	509±50
IVSd, mm	0.70±0.09	1.10±0.17*	0.67±0.05	0.94±0.20*
LVIDd, mm	3.67±0.26	3.59±0.42	3.55±0.35	4.22±0.54*
LVIDs, mm	2.32±0.33	2.29±0.44	2.07±0.07	2.92±0.18*
LVFS	36.9±5.7	36.5±6.78	42.0±5.2	31.6±9.39*
TSPG, mmHg	N/A	81.3±25.5	N/A	86.3±26.5

HR, heart rate; LVIDd, LV internal dimension at end-diastole; LVIDs, LV internal dimension at end-systole; TSPG, trans-stenotic systolic pressure gradient as the difference between right carotid and left axillary artery systolic pressures. Results are expressed as mean ± SD. *P < 0.05 by Student's t-test before and after TAC within the same group.

Moore-Morris et al. Supplemental Figure 1

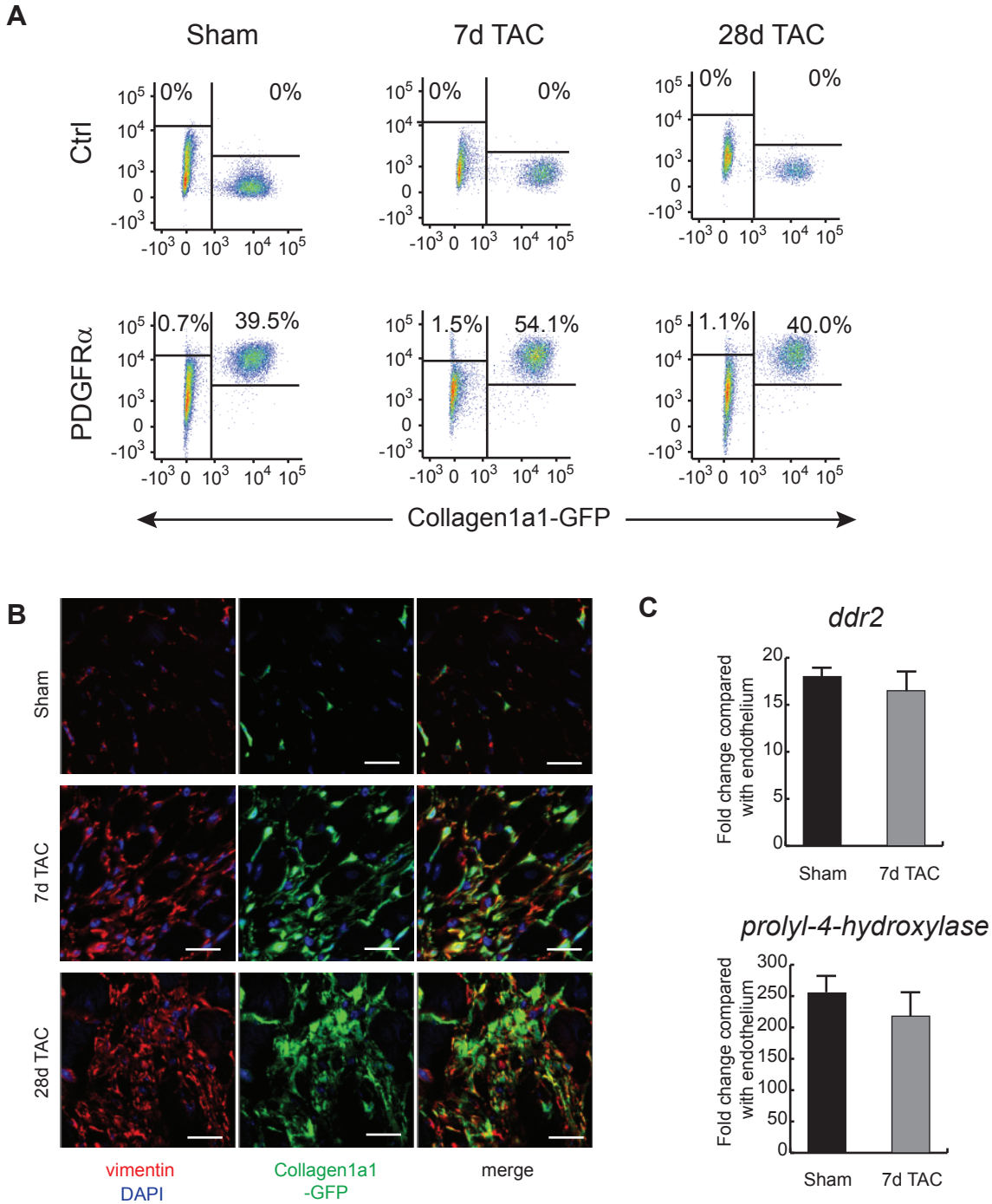


Supplemental Figure 1. Transaortic constriction (TAC) model. (**A**) Left ventricle (LV) weight over body weight (BW). Data was analyzed by Student's t-test (n=6-8 per group). (**B**) Representative trichrome staining of left ventricular wall. (**C**) qRT-PCR analysis of genes associated with hypertrophy/fibrosis. Data was analyzed by unpaired Student's t-test. Scale bars represent 100μm.



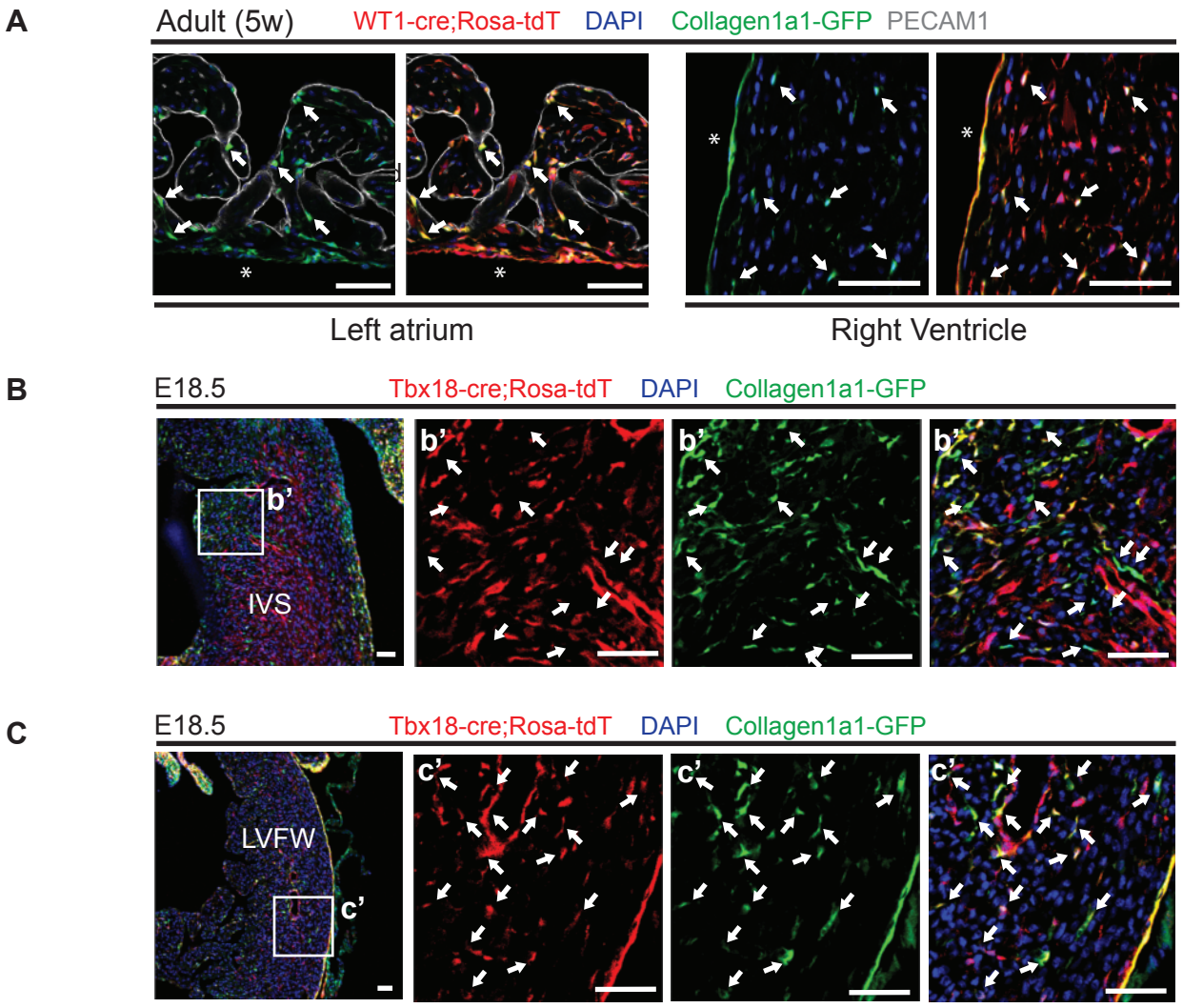
Supplemental Figure 2. Quantification of cell types in fibrotic areas. (**A**) Percentages of cells expressing Collagen1a1-GFP, FSP1 and CD45 in perivascular fibrotic areas. (**B**) Confocal image showing FSP1+ fibroblasts (arrows) and an FSP1+ leukocyte (arrowhead) in a perivascular area. (**C**) Confocal image showing FSP1 labels immune cells (CD45+) and not perivascular fibroblasts in Sham operated mice. (**D**) Percentages of cells expressing Collagen1a1-GFP, αSMA and CD45 in perivascular fibrotic areas. (**E**) Confocal image showing lack of αSMA + fibroblasts in a perivascular area. No Collagen1a1GFP+ cells were CD45+. (**F**) Percentages of cells expressing Collagen1a1-GFP, αSMA and CD45 in interstitial lesions following 28d TAC. Cells were counted in 12 fields each from 2 mice. Histograms represent mean±S.D. Scale bars are 20μm. CA, coronary artery.

Moore-Morris et al. Supplemental Figure 3



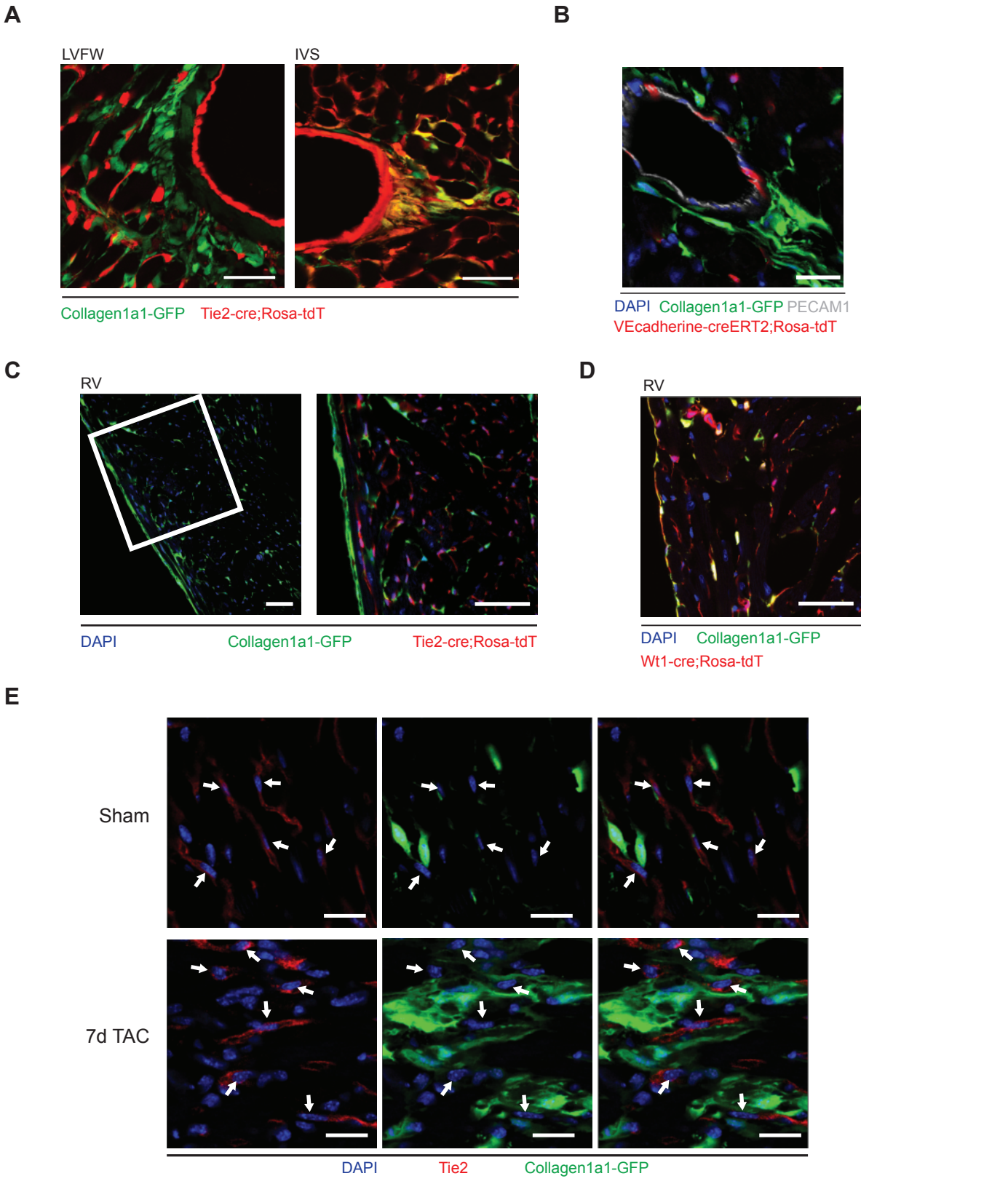
Supplemental Figure 3. Expression of fibroblast markers by Collagen1a1-GFP+ cells (**A**) Representative flow cytometry analysis showing that collagen1a1-GFP+ cells are PDGFRa+ in Sham operated animals and following 7d and 28d TAC. All plots/images are representative of at least 5 animals per group. (**B**) Represesentative images of vimentin staining in Sham operated animals and following 7d and 28d TAC. Scale bars represent 20μm. (**C**) qRT-PCR data showing expression level of DDR2 and Prolyl-4-hydroxylase in Collagen1a1-GFP+ cells versus PECAM1+ endothelial cells sorted from Sham (n=2) and 7d TAC (n=2) hearts.

Moore-Morris et al. Supplemental Figure 4



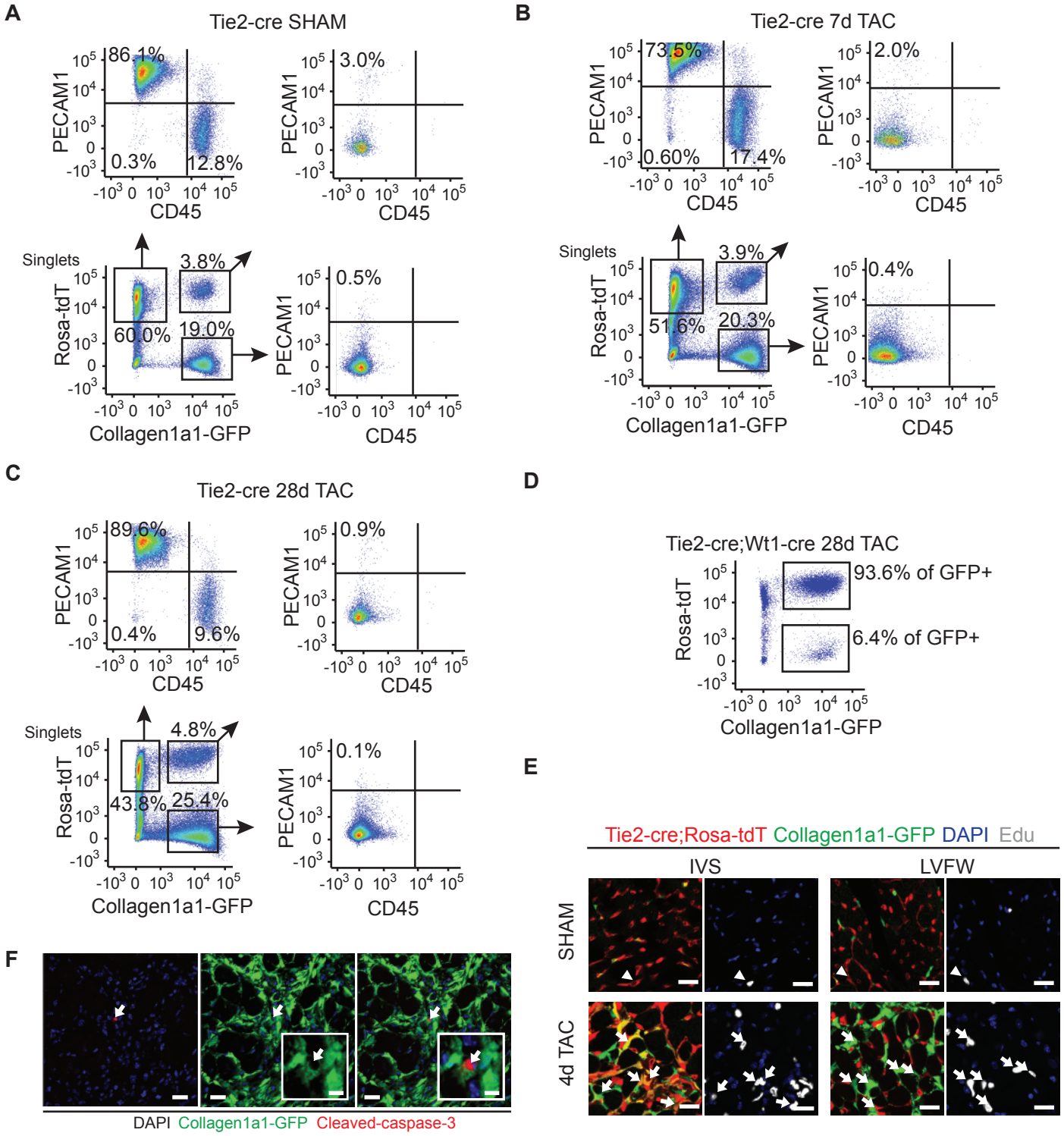
Supplemental Figure 4. Distribution of fibroblasts derived from epicardium. (**A**) Confocal images of adult Wt1-cre lineage traced atria and right ventricle. Fibroblasts were lineage-traced (arrows). (*) Epicardial surface. (**B**) Confocal images of E18.5 Tbx18-cre lineage-traced hearts. As seen with Wt1-cre, many fibrblasts were ot labelled in the interventricular septum (IVS) (arrows). (**C**) A vast majority of fibroblasts were labelled in left ventricular free wall (LVFW) of Tbx18-cre lineage-traced hearts (arrows).Scale bars represent 50μm.

Moore-Morris et al. Supplemental Figure 5



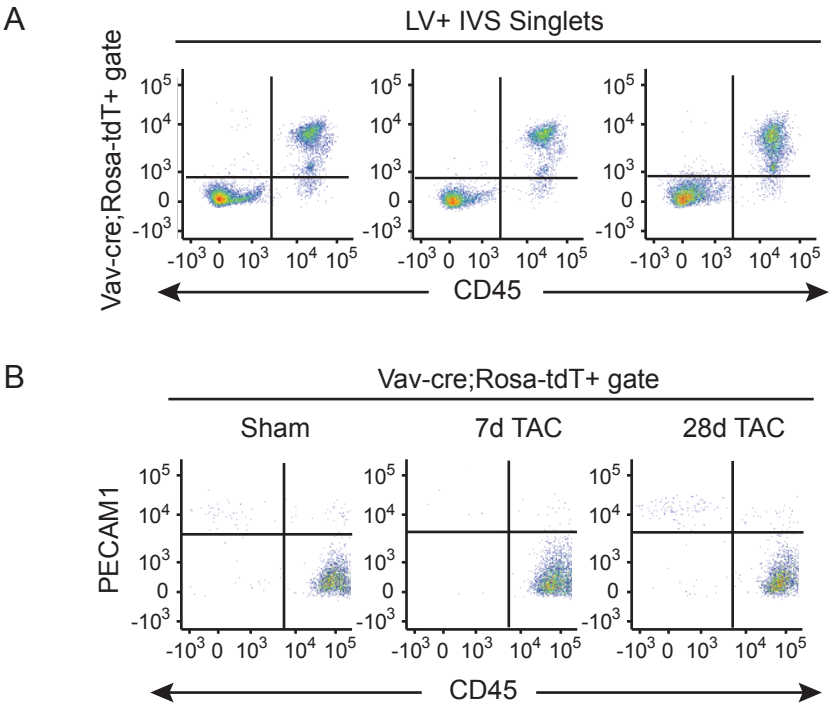
Supplemental Figure 5. Lack of EndoMT following pressure overload. (**A**) Perivascular fibrosis in Tie2cre+/-;Collagen1a1GFP+/-;RosaTdt+/- hearts. Following 7d TAC, as observed for intersititial fibroblasts, perivascular fibroblasts in left ventricular free wall (LVFW) were Tie2cre-RosatdT- whereas perivascular fibroblasts in interventricular septum (IVS) were Tie2cre-RosatdT+. Similar results were observed at 28d TAC. Scale bars represent 50mm. (**B**) Perivascular fibrosis in VE-cadherin lineage traced heart following 7d TAC. PECAM1+ coronary endothelial cells were labelled, but not perivascular fibroblasts. Scale bar 20mm. (**C**) Right ventricle (7d TAC) from Tie2-cre lineage traced heart. (**D**) Equivalent area to inset in (C) in RV of Wt1-creERT2 lineage traced heart. Similar results were observed at 28d TAC. Scale bars represent 50μm. (**E**) Cells expressing Tie2 protein (arrows) were not Collagen1a1-GFP+ fibroblasts in Sham hearts and following 7d TAC. Similar results were observed at 28d TAC. Scale bars represent 20μm.

Moore-Morris et al. Supplemental Figure 6



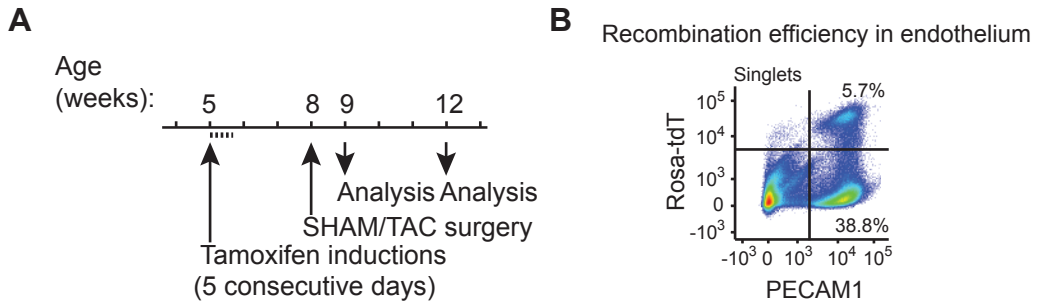
Supplemental Figure 6. Similar responses of Tie2-Cre and non Tie2-Cre derived fibroblasts to pressure overload (**A**) Flow cytometry plot of dissociated left ventricle (LV) + interventricular septum (IVS) from a sham operated Tie2cre+/-;collagen1a1-GFP+/-;Rosa-tdT+/- mouse. Tie2-cre;Rosa-tdT+, Tie2-cre;Rosa-tdT+Collagen1a1-GFP+ and Tie2-cre;Rosa-tdT+Collagen1a1-GFP- populations have been gated and plotted against PECAM1 and CD45 expression. Both Collagen1a1-GFP+ populations are negative for PECAM1 and CD45, except a small subset of collagen1a1-GFP+;PECAM1+ endocardial cells. Tie2-cre;Rosa-tdT+;collagen1a1-GFP- cells consist of PECAM1+ endothelial cells and CD45+ leukocytes. This is also the case following 7d (**B**) and 28d (**C**) TAC. (**D**) Representative flow cytometry plots showing percentages of labelled (Rosa-tdT+) and non-labelled (Rosa-tdT-) CFs in double Tie2-cre;Wt1-cre lineage traced LV + IVS (representative of 3 mice). (**E**) Representative images of IVS and LVFW (left ventricular free wall) of sham operated and TAC operated Tie2-cre+/-;collagen1a1-GFP+/-;Rosa-tdT+/- mice 4d after surgery (PG 76.7±8.2 mmHg, n=3). Hearts were recovered 24h after EdU injection. Rare EdU+ cells (arrowheads) were never fibroblasts in Sham hearts. Equivalent fibrotic areas that contained EdU+ fibroblasts (arrows) were found in IVS and LVFW. Scale bars are 20μm. (**F**) Apoptotic Cleaved-caspase 3+ cells were very rare following TAC and Collagen1a1-GFP- (see inset). The Collagen1a1-GFP reporter is cytoplasmic and nuclear, enabling clear identification of fibroblast nuclei (see nucleus to right of Caspase 3+ cell inset). Scale bars are 20μm except insets (5μm). Image taken from 7d post surgery, similar results were observed following 4d and 28d TAC.

Moore-Morris et al. Supplemental Figure 7.



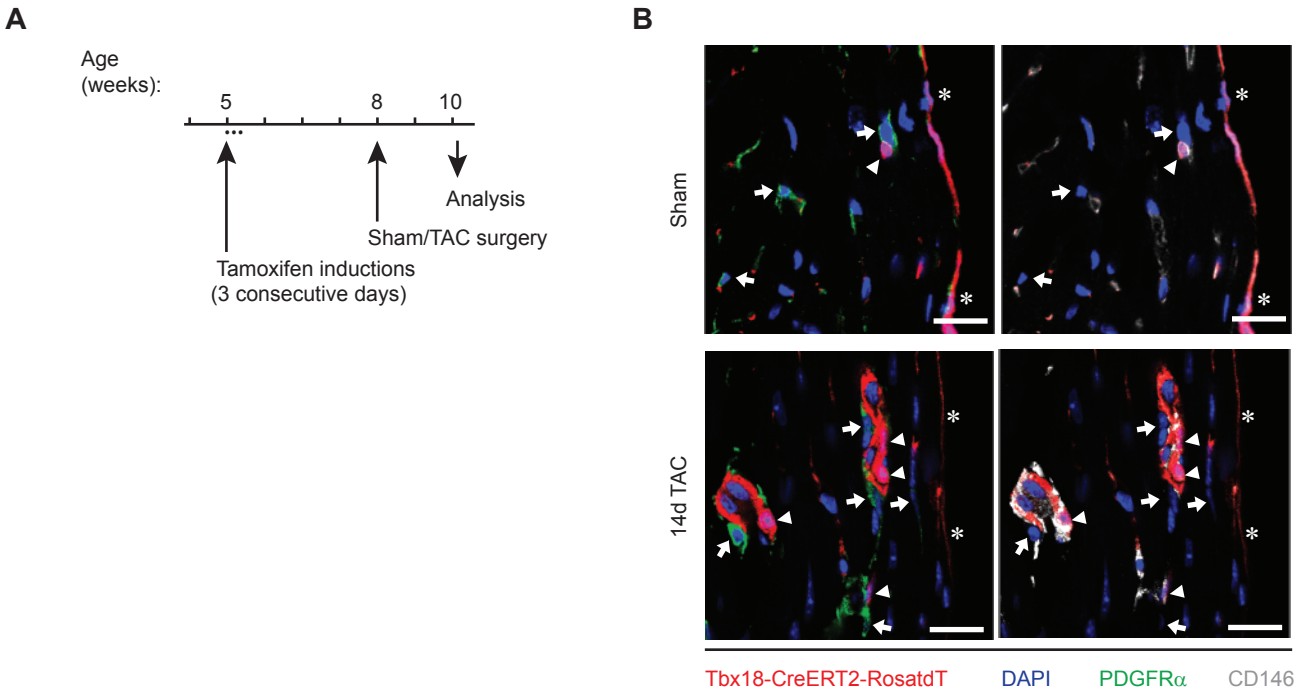
Supplemental Figure 7. Vav-cre labelling following pressure overload. (**A**) Representative flow cytometry plots showing Vav-cre;Rosa-tdT+ and CD45 signals from cell suspensions from LVFW+IVS. >94% of CD45+ leukocytes were labelled in Vav-cre lineage traced mice, with almost no labelling outside of this cellular compartment. (**B**) Vav-cre lineage traced cells consist invariably of CD45+ leukocytes in Sham and following 7d or 28dTAC, with small sub-populations of PECAM1+ endothelial cell. Each plot is representative of at least 3 mice.

Moore-Morris et al. Supplemental Figure 8



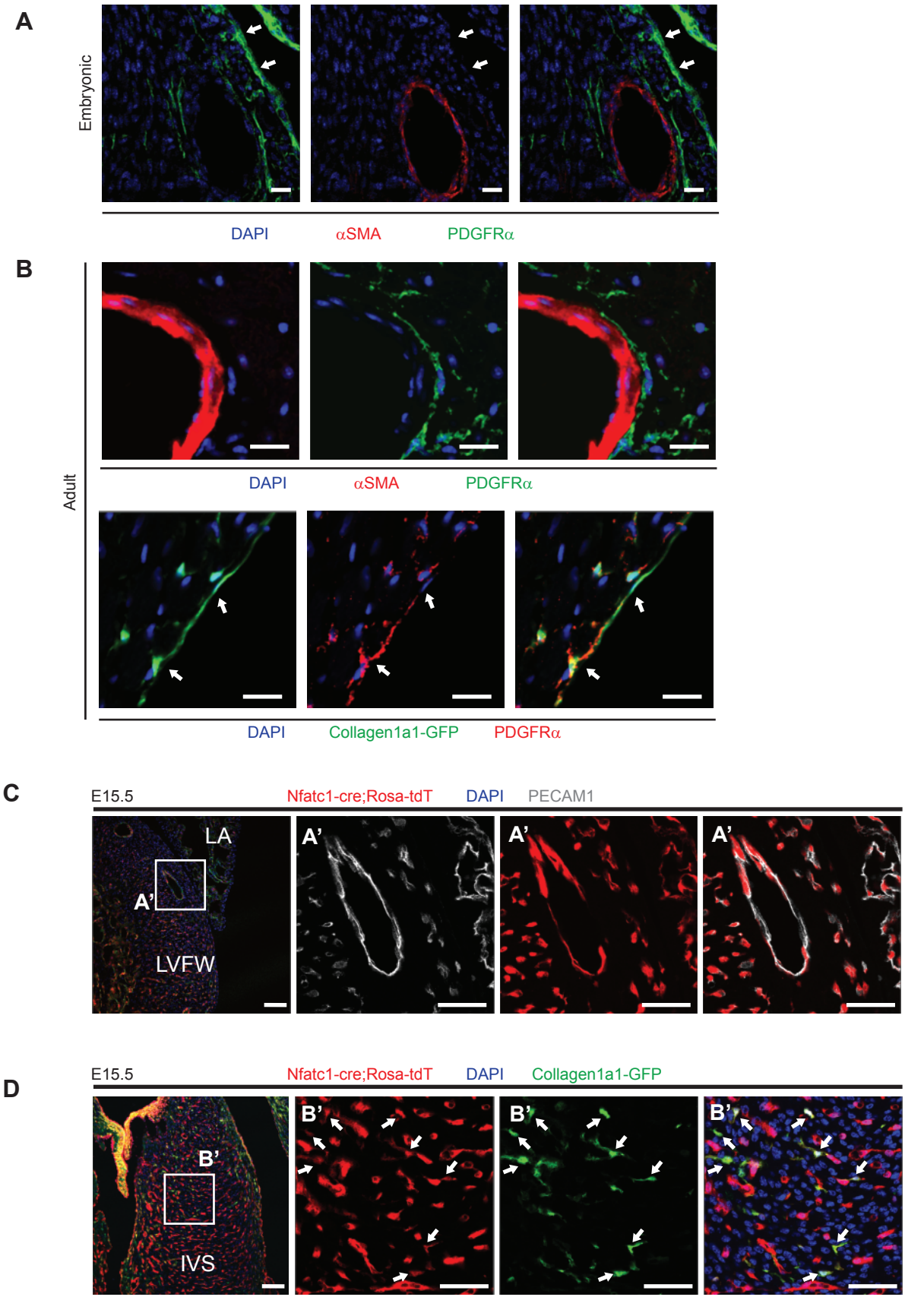
Supplemental Figure 8. Inductions in VE-cadherin-CreERT2 lineage traced mice (**A**) Time line for tamoxifen induction and TAC procedure on VE-cadherin-CreERT2+/-;collagen1a1-GFP+/-;Rosa-Tdt+/- mice. (**B**) Representative flow cytometry plot showing the percentage of PECAM1+ endothelium labeled in left ventricle (LV) + interventicular septum (IVS) by VEcadherin-creERT2 following inductions.

Moore-Morris et al. Supplemental Figure 9



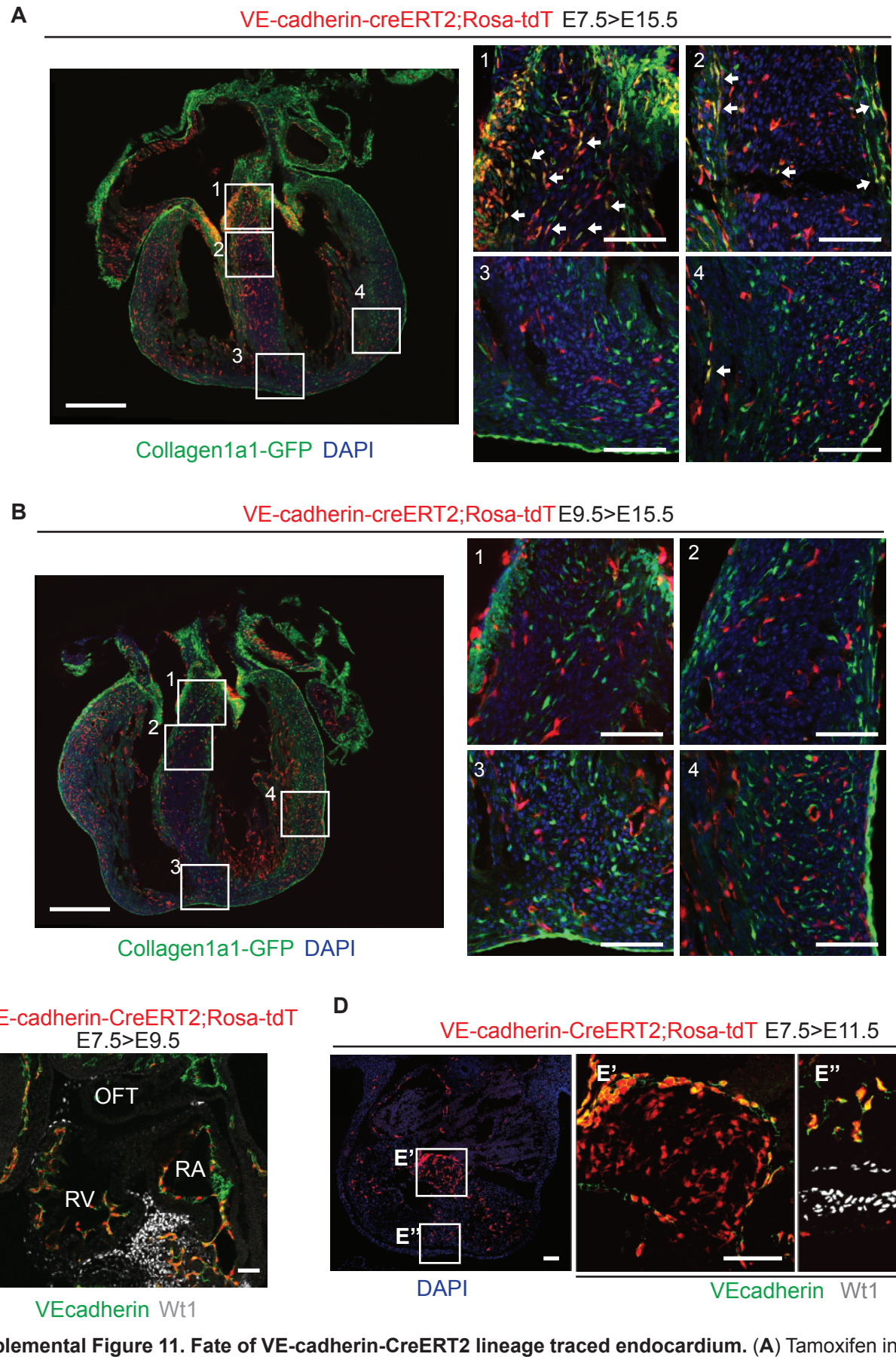
Supplemental Figure 9 Epicardium does not give rise to fibroblasts following pressure overload. (**A**) Time course for tamoxifen inductions and Sham/TAC surgery for Tbx18-CreERT2+/-;Rosa-tdT+/- mice. (**B**) Confocal images showing recombination in epicardium (asterisks) and CD146+ mural cells (arrowheads) but not in PDGFRα+ fibroblasts (arrows) of Sham operated mice and following 14d pressure overload (PG 81±12.5 mmHg, n=3). Scale bars represent 20μm.

Moore-Morris et al. Supplemental Figure 10



Supplemental Figure 10. Lack of PDGFRα staining in vascular smooth muscle/epicardium and confocal images of E15.5 IVS of Nfatc1-cre+/-;Collagen1a1-GFP+/-;Rosa-tdT+/- embyronic heart. **(A)** Embryonic E17.5 left ventricular wall. Epicardium is indicated by arrows. **(B)** Adult smooth muscle (5 weeks old male) is PDGFRα–, wherease epicardial cells (arrows) express PDGFRα. (**C**) LVFW displaying extensive labelling of PECAM1+ vessels (arrows). (**D**) As with Tie2-Cre and VE-cadherin-creERT2, Nfatc1-cre lineage traced fibroblasts were abundant in IVS (arrows). LA, left atrium. Scale bars in A and B represent 20μm, Scale bars in C and D represent 50μm.

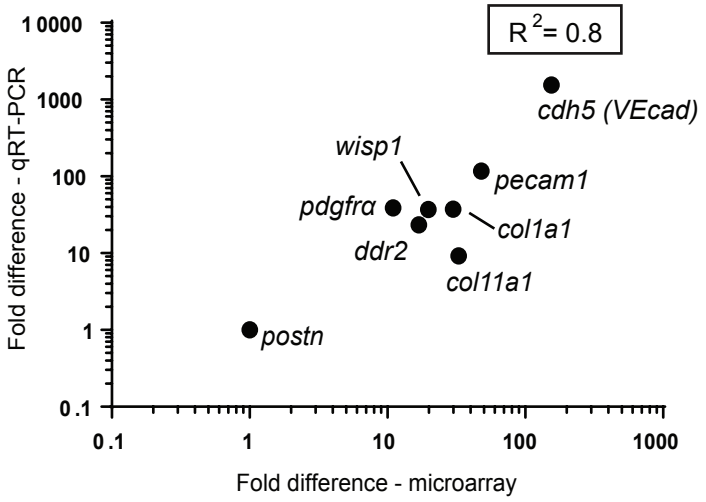
Moore-Morris et al. Supplemental Figure 11



Supplemental Figure 11. Fate of VE-cadherin-CreERT2 lineage traced endocardium. (**A**) Tamoxifen induction at E7.5 in VE-cadherin-creERT2 embryos resulted in labelling of Collagen1a1-GFP+ fibroblasts in the valves and septum (1&2), and labelling of a small number of fibroblasts in the ventricular wall by E15.5 (4, arrows). (**B**) Tamoxifen induction at E9.5 resulted in no labelling of fibroblasts, despite a similar degree of labelling of endothelium. (**C**) Tamoxifen induction in E7.5 embryos resulted in Rosa-tdT+/- lineage labeling of VE-cadherin immunolabeled endothelial/endocardial cells but not Wt1 immunolabeled proepicardial cells at E9.5. (**D**) Inductions at E7.5 and harvesting at E11.5 resulted in labeling of atrioventricular canal cushion (e') and VE-cadherin+ endocardial/endothelial cells, but not Wt1+ epicardial cells (e''). Scale bars are 100μm except 4 chamber views in (A) and (B) that represent 400μm. OFT= Outflow tract, RA= Right atrium.

Moore-Morris et al. Supplemental Figure 12

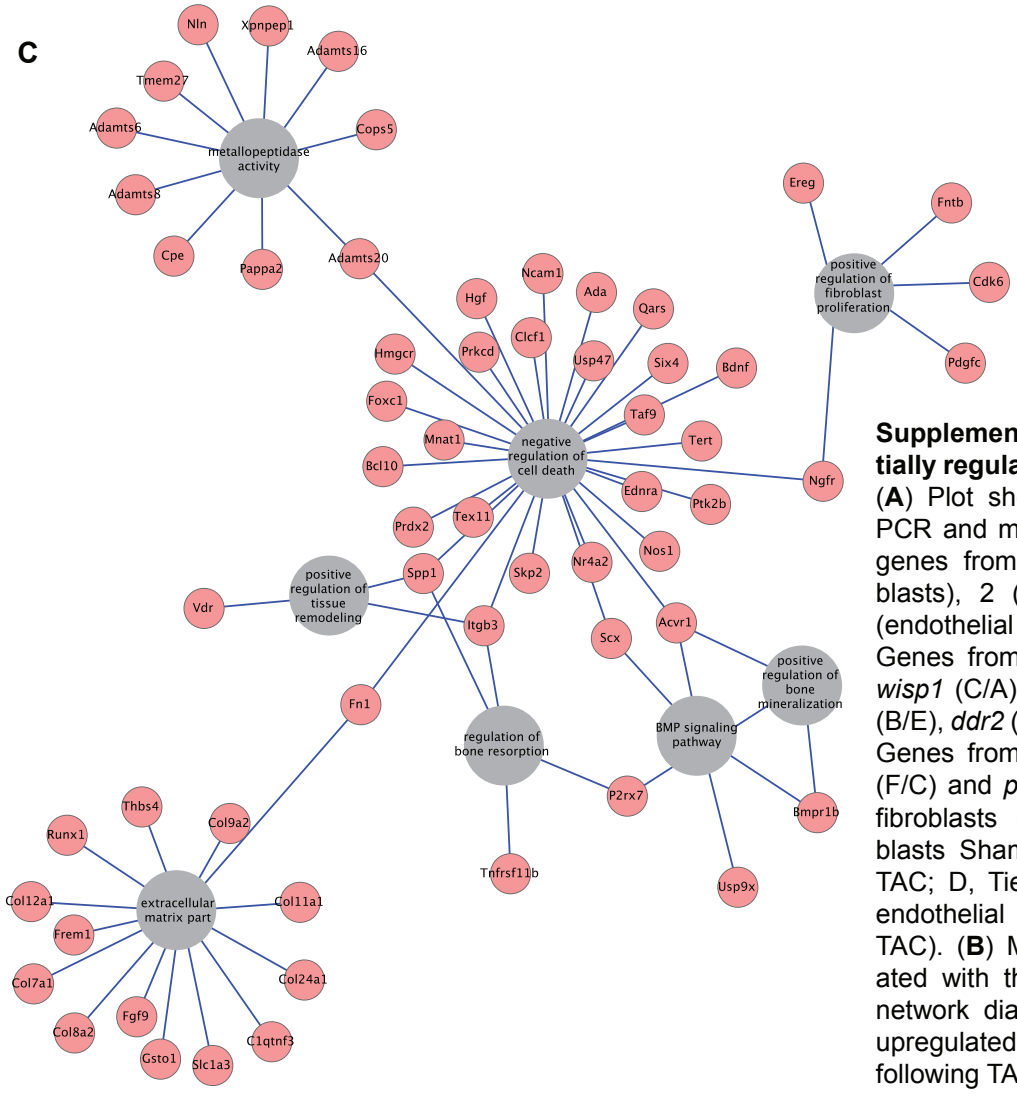
A



B

Cluster	GO Term	Gene number	Permute P value
TAC EFFECT FIBROBLASTS (Cluster 1)	extracellular matrix part(GO:0044420)	14	<0.001
	proteinaceous extracellular matrix(GO:0005578)	17	<0.001
	metallopeptidase activity(GO:0008237)	10	<0.001
	negative regulation of cell death(GO:0060548)	29	<0.01
	positive regulation of fibroblast proliferation (GO:0048146)	4	<0.01
FIBROBLAST SPECIFIC (Cluster 2)	extracellular matrix(GO:0031012)	131	<0.001
	regulation of cartilage development(GO:0061035)	27	<0.001
	pattern binding(GO:0001871)	65	<0.001
	extracellular matrix part(GO:0044420)	65	<0.001
	extracellular matrix organization(GO:0030198)	51	<0.001
ENDOTHELIAL CELL SPECIFIC (Cluster 3)	adherens junction(GO:0005912)	62	0.0325
	regulation of small GTPase mediated signal transduction(GO:0051056)	62	0.0285
	cell-cell adherens junction(GO:0005913)	23	0.004
	cadherin binding(GO:0045296)	13	0.0025
	regulation of angiogenesis(GO:0045765)	40	0.0155
TAC EFFECT FIBROBLASTS AND ENDOTHELIAL CELLS (Cluster 4)	cell cycle(GO:0007049)	127	<0.001
	cell cycle process(GO:0022402)	126	<0.001
	cell division(GO:0051301)	90	<0.001
	chromosome, centromeric region(GO:0000775)	51	<0.001
	chromosome(GO:0005694)	79	<0.001

C



Supplemental Figure 12. Genes differentially regulated following pressure overload. (**A**) Plot showing correlation between q-RT-PCR and microarray fold differences between genes from clusters 1 (TAC effect on fibroblasts), 2 (fibroblast specific genes) and 3 (endothelial specific genes) of Figure 9B. Genes from cluster 1 are *col11a1* (D/B) and *wisp1* (C/A). Genes from cluster 2 are *col1a1* (B/E), *ddr2* (A/E), *pdgfrα* (D/F) and *postn* (D/C). Genes from cluster 3 are *cdh5* (VEcadherin) (F/C) and *pecam1* (E/B) (A, Tie2creRosatdT+ fibroblasts Sham; B, Tie2creRosatdT- fibroblasts Sham; C, Tie2creRosatdT+ fibroblasts TAC; D, Tie2creRosatdT- fibroblasts TAC; E, endothelial cells Sham; F, endothelial cells TAC). (**B**) Most significant GO-Terms associated with the main gene clusters. (**C**) Gene network diagram including genes specifically upregulated in both fibroblast populations following TAC.