

## Supplemental Information Inventory:

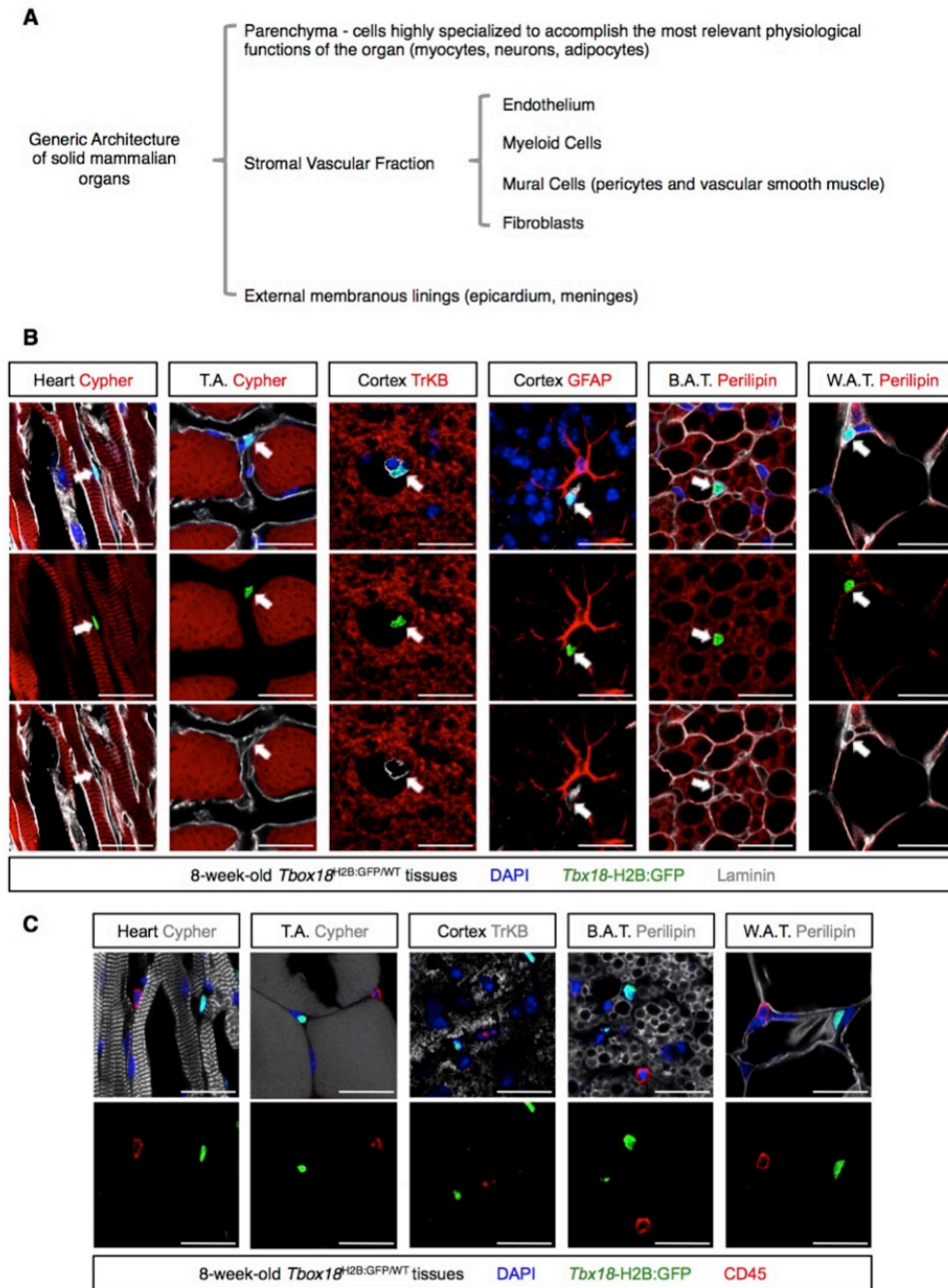
### Supplemental Figures:

- **Figure S1, Related to Figure 1** - Interstitial *Tbx18*-GFP<sup>+</sup> cells did not belong to parenchymal or myeloid lineages.
- **Figure S2, Related to Figure 2** - Interstitial *Tbx18*-GFP<sup>+</sup> cells were mural cells (CD146<sup>+</sup>, NG2<sup>+</sup>) and did not express markers of fibroblasts
- **Figure S3, Related to Figure 2** – Extended cell surface antigen profile of *Tbx18*-GFP<sup>+</sup> mural cells.
- **Figure S4, Related to Figure 3** – in vitro, *Tbx18*-GFP<sup>+</sup> cells behaved as Mesenchymal Stem Cells
- **Figure S5, Related to Figure 4** – Highly efficient labeling of mural cells by *Tbx18*-CreERT2
- **Figure S6, Related to Figure 6** – Mural cells were not progenitors of cardiomyocytes
- **Figure S7, Related to Figure 4, 5 and 6** – Conditional ablation of *Tbx18* does not increase plasticity of mural cells.

### Supplemental Tables:

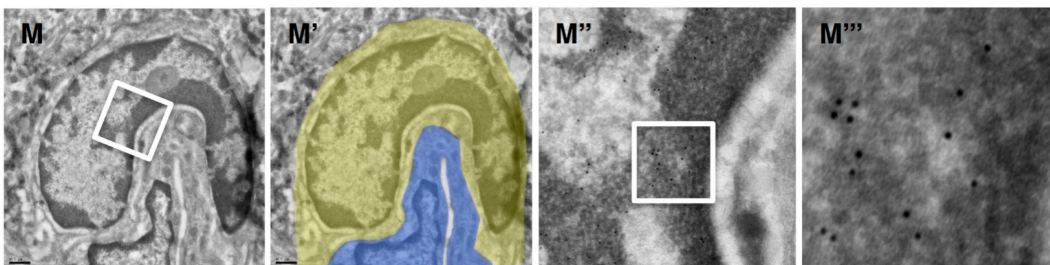
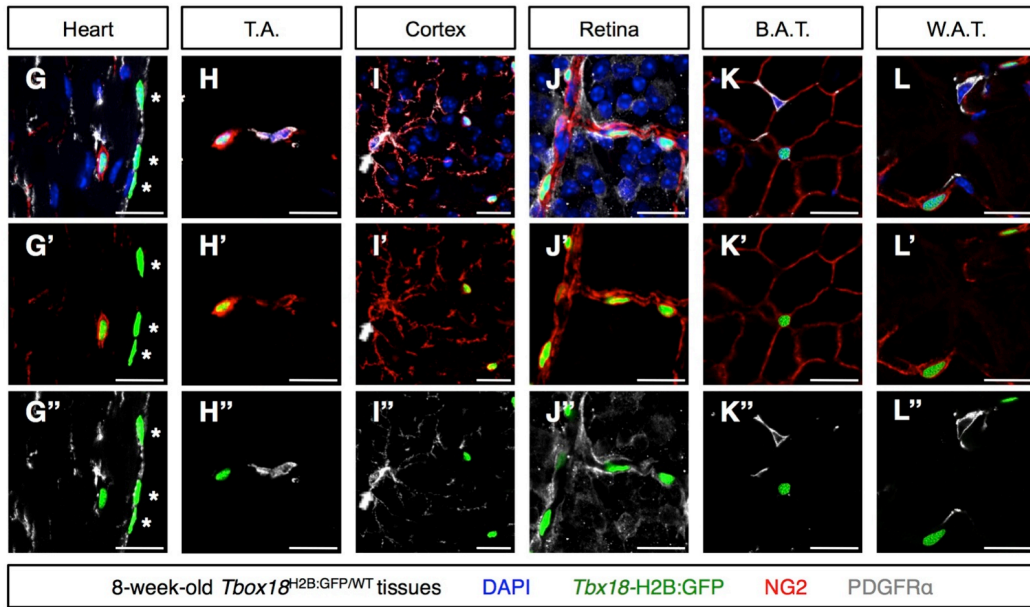
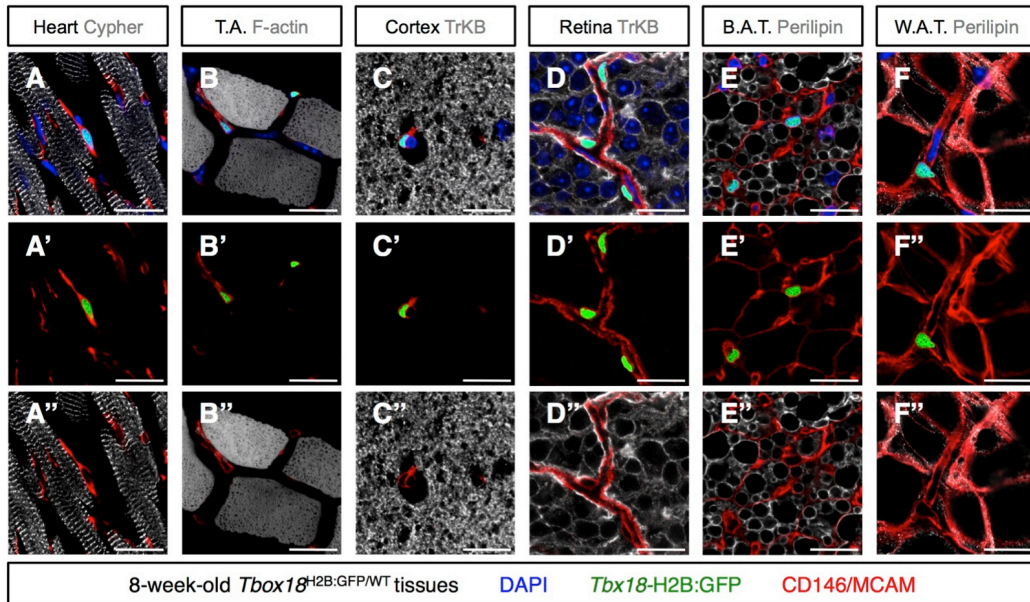
- **Table S1, Related to Figure 2** – Summarized cell surface antigen profile of parenchymal and stromal cell types.
- **Table S2, Related to STAR Methods (Method details)** – list of primers used for genotyping transgenic mice.

**Figure S1**



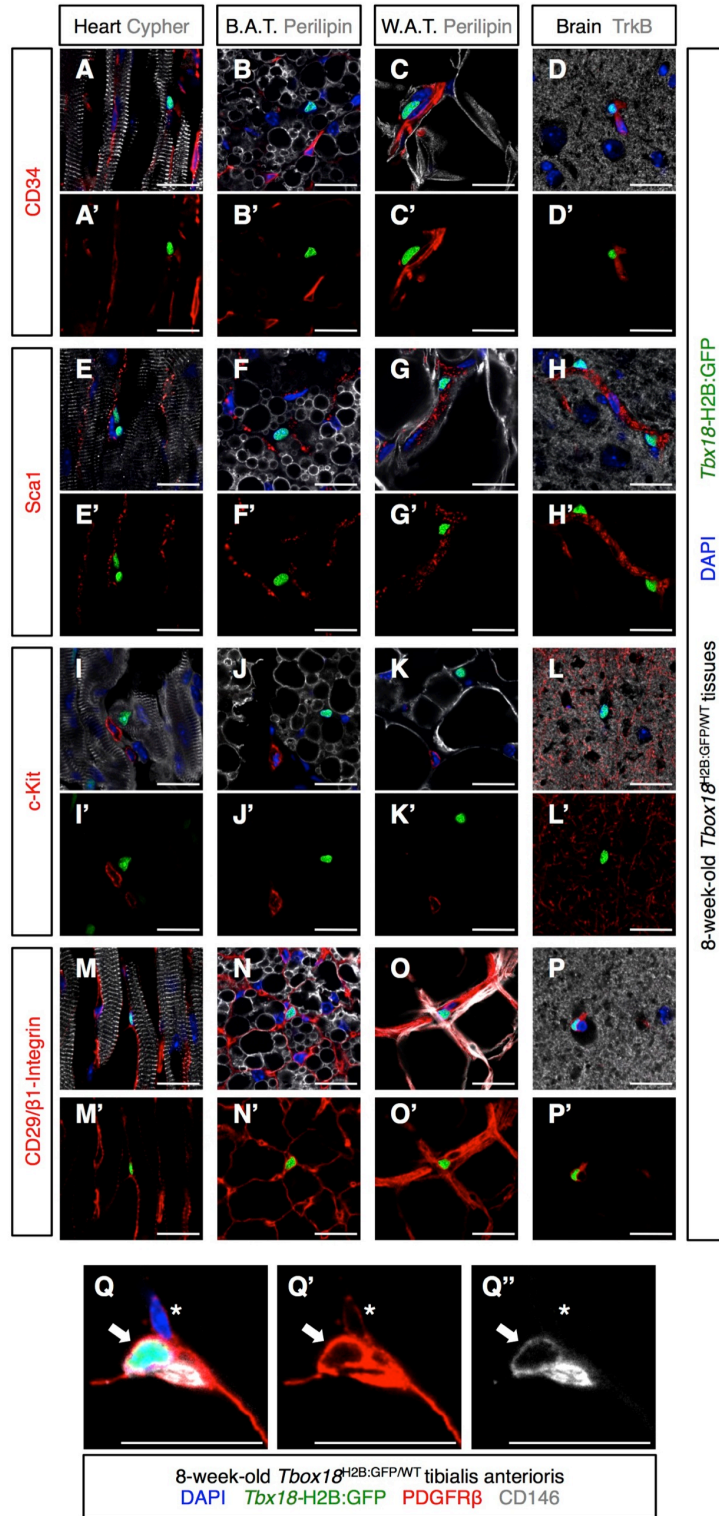
**Figure S1, Related to Figure 1 – Interstitial *Tbx18*-GFP+ cells did not belong to parenchymal or myeloid lineages.** (A) Diagram representing the general architecture of mammalian solid organs. (B) In all tissues analyzed, interstitial *Tbx18*-GFP+ cells did not correspond to parenchymal cells, as denoted by the absence of expression of tissue-specific parenchymal markers (Cypher in cardiac and skeletal muscle, TrkB and GFAP in the central nervous system and Perilipin in adipose depots) and were clearly separated from parenchymal cells by a well-defined basal lamina marked by a pan-Laminin antibody. (C) *Tbx18*-GFP+ cells did not correspond to tissue-resident immune cells, as denoted by absence of expression of the myeloid marker CD45. Bars = 20  $\mu$ m.

Figure S2



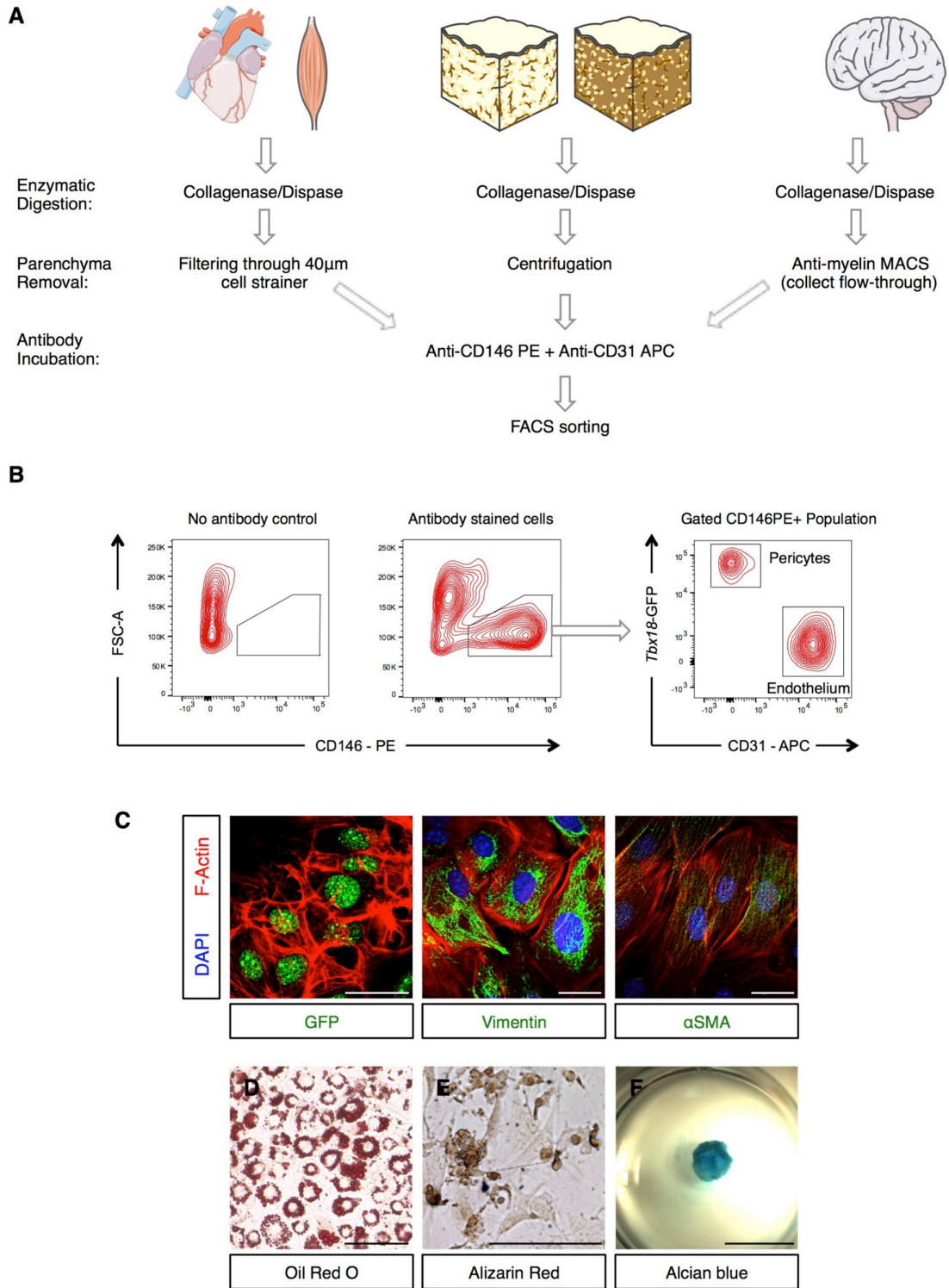
**Figure S2, related to Figure 2 – Interstitial *Tbx18*-GFP+ cells were mural cells (CD146+, NG2+) and did not express markers of fibroblasts.** (A-F) – Confirming their identity as mural cells, interstitial *Tbx18*-GFP+ cells expressed CD146/MCAM (Melanoma Cell Adhesion Molecule), a marker of endothelial cells and mural cells. Importantly, in adipose depots, this molecule was also expressed by brown and white adipocytes (E and F). (G-L) Interstitial *Tbx18*-GFP+ cells also expressed the pericyte marker NG2 and did not express the fibroblast marker PDGFR $\alpha$ . (G) In the heart, NG2 was expressed by interstitial *Tbx18*-GFP+ cells, but not by epicardial cells (asterisks). PDGFR $\alpha$  showed an opposite expression pattern – absent in the majority of mural cells and robustly expressed by epicardium. In histological applications, distinction of *Tbx18*-GFP+ epicardial cells and *Tbx18*-GFP+ mural cells can easily be achieved due to their distinct anatomical locations. In situations where tissue architecture cannot be considered, distinction between these two *Tbx18*-GFP+ populations can be achieved taking advantage of the specific patterns of NG2 and PDGFR $\alpha$  expression reported here. (I) In brain, subsets of interstitial *Tbx18*-GFP+ cells expressed NG2, but it was also possible to identify abundant populations of interstitial *Tbx18*-GFP+ cells negative for this marker. The fact that not all brain pericytes expressed NG2, in conjunction with expression of NG2 by NG2+,PDGFR $\alpha$ + oligodendrocyte progenitors (arrow) rendered NG2 a poor marker for identification of mural cells in brain. (K) In brown adipose tissue, NG2 was also a poor marker for identification of pericytes, as this antigen was also expressed by brown adipocytes. (M-M’’’). Anti-GFP immuno-gold electron microscopy of an ultra-thin section of a *Tbx18*<sup>GFP/WT</sup> brain showing a *Tbx18*-GFP+ pericyte immediately enveloping an endothelial cell. In M’, the endothelial cell has been pseudo-colored blue and the *Tbx18*-GFP+ pericyte yellow. M’’ shows a higher magnification of the region boxed in M. M’’’ shows a higher magnification of the region boxed in M’’, to allow for visualization of the nuclear dots produced by the anti-GFP antibody, indicating active expression of *Tbx18*. Bars = 0.5  $\mu$ m in M and 20  $\mu$ m in all other panels.

Figure S3



**Figure S3, related to Figure 2 – Extended cell surface antigen profile of *Tbx18-GFP+* mural cells.** To be able to compare our results with those of other groups, and to develop a protocol for isolation of mural cells by FACS, we examined whether interstitial *Tbx18-GFP+* cells expressed markers commonly used for isolation of tissue-resident mesenchymal progenitors. In all tissues analyzed, *Tbx18-GFP+* cells never expressed **(A-D)** CD34, or **(E-H)** Sca1, with both markers robustly labeling endothelial cells. **(I-L)** *Tbx18-GFP+* cells also did not express the hematopoietic stem cell marker c-Kit. **(M-P)** *Tbx18-GFP+* cells did express CD29/ $\beta$ 1-Integrin, however, in most tissues this marker proved to be a poor choice for purification of any cellular population as it was broadly expressed by parenchymal cells and cells of the stromal vascular fraction. **(Q)** Example of the immunostaining strategy used for the unequivocal identification of pericytes in tissue sections for quantification purposes. Although at lower levels than mural cells, fibroblasts (asterisk) can also express PDGFR $\beta$  (red). Co-staining with an antibody recognizing CD146 (gray) allowed for discrimination between pericytes (PDGFR $\beta$ , CD146 double positive, arrow) and other stromal cells (PDGFR $\beta^{\text{low}}$ , CD146-). Bars = 20  $\mu$ m.

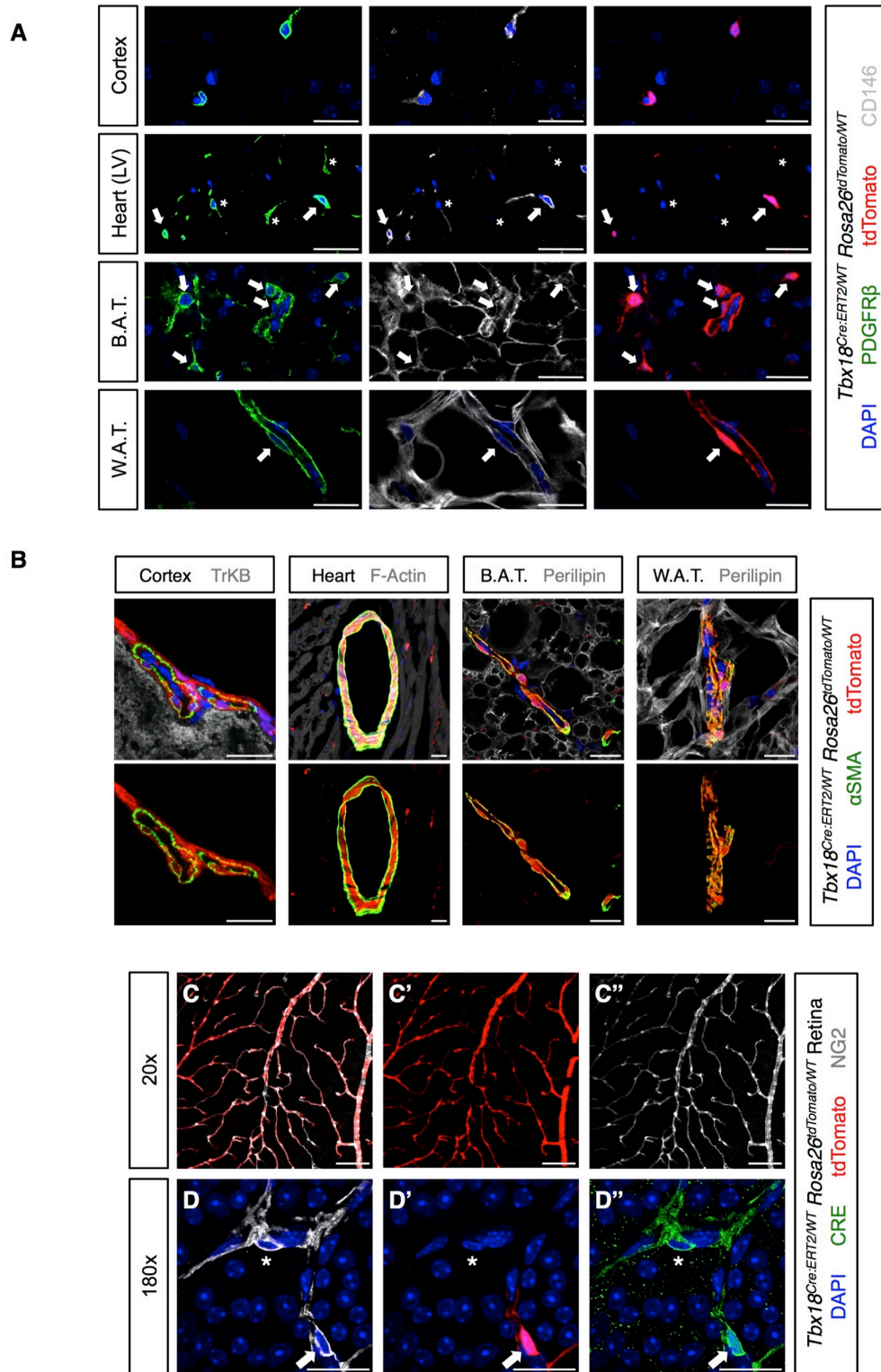
Figure S4



**Figure S4, Related to Figure 3 – In vitro, *Tbx18*-GFP+ cells behaved as Mesenchymal Stem Cells.** (A) A simple protocol for isolation of highly pure populations of mural cells was developed based on labeling of pericytes and vascular smooth muscle by *Tbx18-H2B:GFP* and taking in consideration the detailed characterization of cell surface antigen expression profiles of parenchymal and stromal cells summarized in Table S1. (B) FACS plots demonstrating the two-step strategy used for isolation of highly pure populations of endothelium and mural cells. A well-defined population of CD146+ cells was gated from the stromal vascular fraction and subsequently split into a pure population of GFP+ pericytes and a pure population of CD31+ endothelium. This strategy allowed for exclusion of pericyte-endothelium doublets resulting from incomplete tissue digestion, ensuring sample purity. (C) Cultured mural cells displayed mesenchymal morphology and expression of *Tbx18* (as revealed by GFP immunostaining), Vimentin, and low-levels of Smooth Muscle  $\alpha$ Actinin ( $\alpha$ SMA). (D) Oil red O staining of mural cells cultured in adipogenic medium for 7 days showing efficient differentiation into adipocytes. (E) Alizarin red staining of mural cells cultured in osteogenic medium for 14 days showing deposition of mineralized matrix, indicating differentiation into osteoblastic lineages. (F) Alcian blue staining of a micromass pellet of mural cells cultured in chondrogenic medium for 12 days showing differentiation into cartilage. Bars = 30 $\mu$ m in C; 100 $\mu$ m in D, E; and 500 $\mu$ m in F.

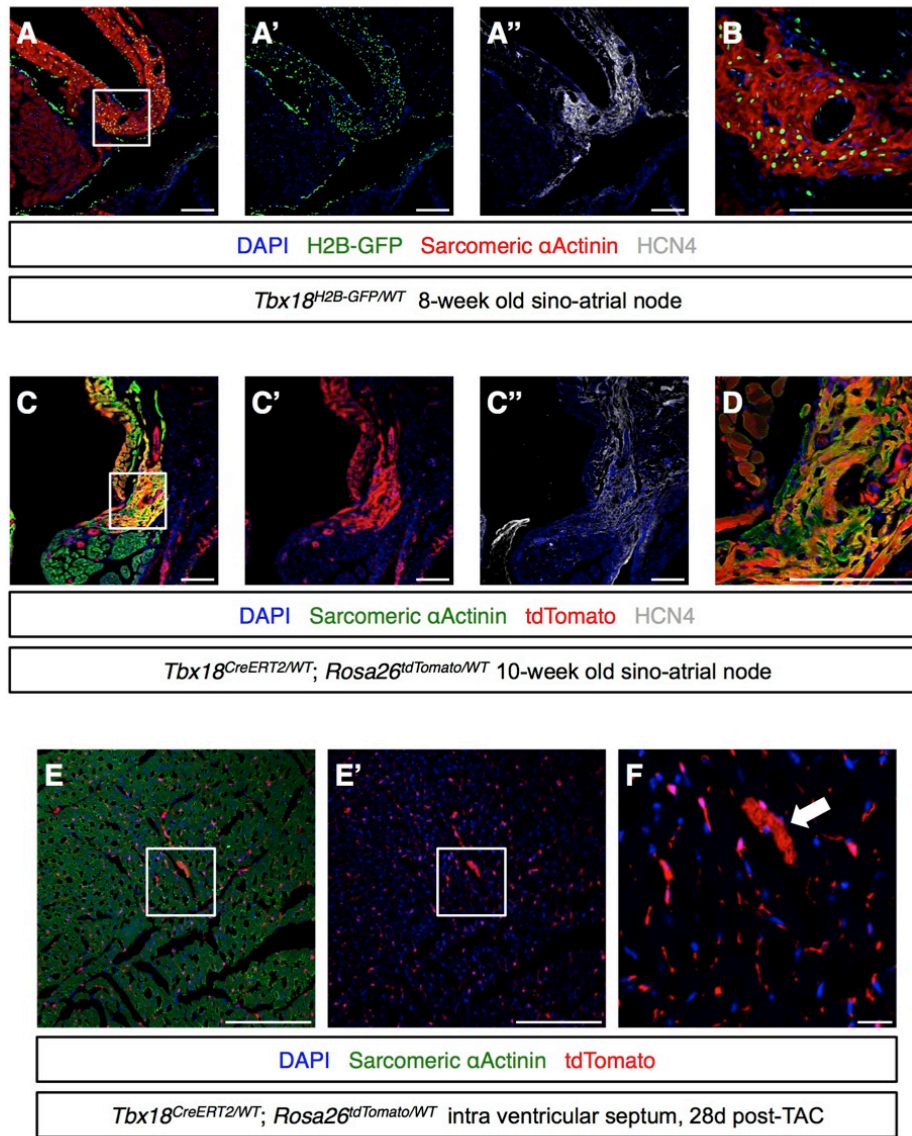


Figure S5



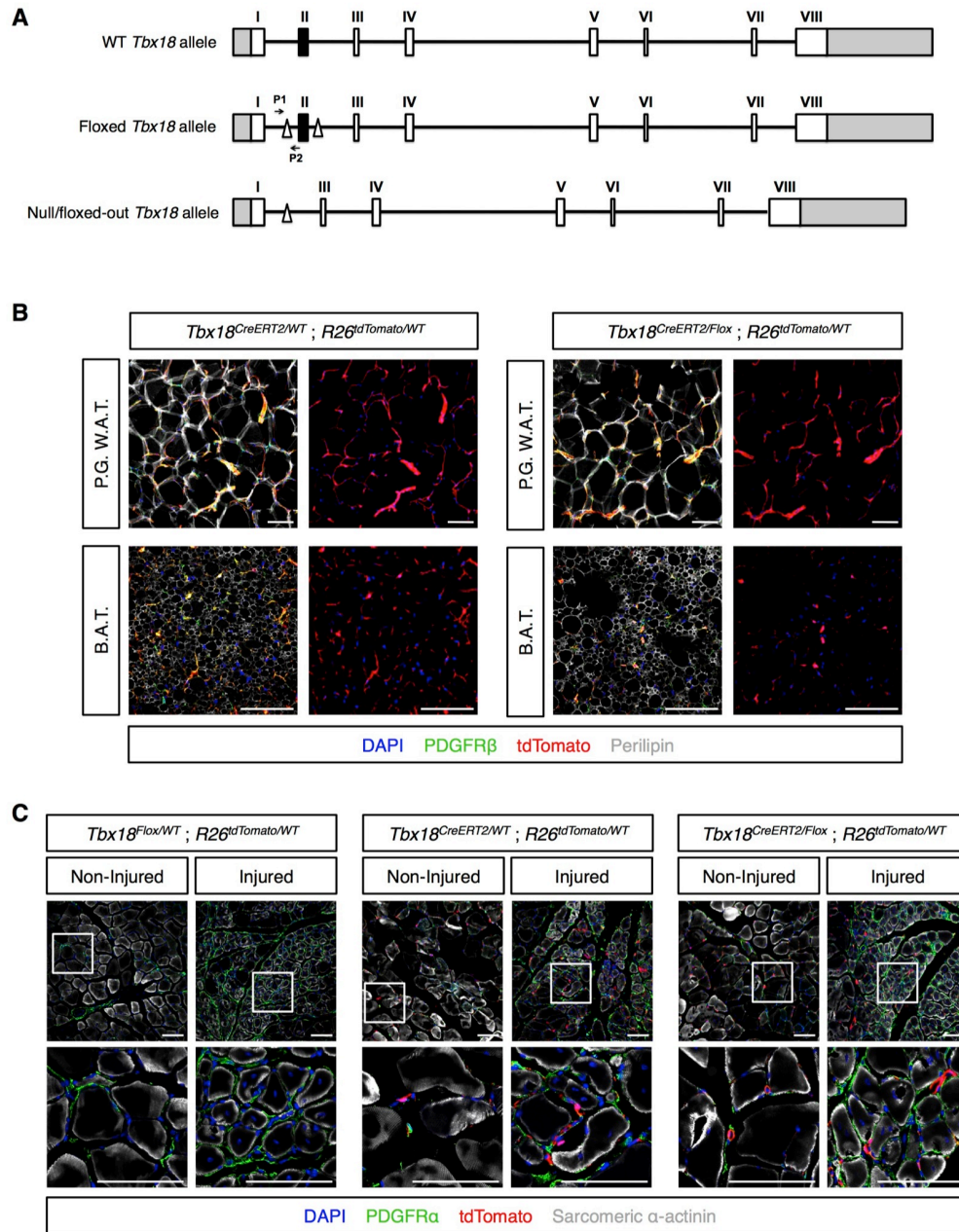
**Figure S5, related to Figure 4 – Highly efficient labeling of mural cells by *Tbx18*-CreERT2.** (A) To quantify the efficiency of pericyte labeling by *Tbx18*-CreERT2, tissues from tamoxifen-induced *Tbx18*<sup>ERT2Cre/Wt</sup>; *Rosa26*<sup>tdTomato/Wt</sup> animals were processed for histological analyses using antibodies recognizing PDGFR $\beta$  (green) and CD146 (far red). Simultaneous use of both pericyte markers was particularly important in heart, where a subset of fibroblasts (asterisks) expressed PDGFR $\beta$  (although at lower levels than pericytes), but not CD146. As such, double positive PDGFR $\beta$ /CD146 pericytes (arrows) could be readily detected. In brain and fat depots, CD146 was dispensable for the identification of pericytes, as these tissues did not have significant numbers of PDGFR $\beta$ <sup>+</sup> fibroblasts. (B) To quantify the efficiency of vascular smooth muscle labeling by *Tbx18*-CreERT2, sections from the same animals were immunostained with an antibody recognizing Smooth Muscle  $\alpha$ -Actinin ( $\alpha$ SMA). Quantification of efficiency of pericyte and vascular smooth muscle labeling can be found in Figure 3 C and D, respectively. (C) Whole mount of *Tbx18*-CreERT2 lineage-traced retina, at time-zero, stained with the mural cell marker NG2. Due to its architecture, the retina allows for ready visualization of the efficiency of recombination produced by the *Tbx18*-CreERT2 line in vessels of distinct caliber. (D) Higher magnification of the same retinal whole mount also displaying immunostaining for Cre recombinase. NG2<sup>+</sup> mural cells (gray) are also positive for CreERT2 recombinase (green). In tdTomato-labeled mural cells, CreERT2 signal is present in both the cytoplasm and nucleus (arrow). However, in non-labeled mural cells (asterisk), CreERT2 recombinase has not translocated into the nucleus, demonstrating lack of CreERT2 activation, rather than lack of expression. This phenomenon was observed in all non-tdTomato lineage traced mural cells throughout the tissue. Bars = 100 $\mu$ m in C and 20 $\mu$ m in all other panels.

Figure S6



**Figure S6, related to Figure 6 – Mural cells were not progenitors of cardiomyocytes.** (A) Sections from a *Tbx18<sup>H2B-GFP/WT</sup>* heart showing robust *Tbx18* expression by cardiomyocytes of the sinoatrial node and venous myocardium. (B) Higher magnification of the area boxed in A. (C) Sections from a *Tbx18<sup>ERT2Cre/WT</sup>; Rosa26<sup>tdTomato/WT</sup>* heart showing robust labeling of sinoatrial node and venous myocardium. Due to this labeling profile, atrial tissues were excluded from our analyses aimed at determining the cellular fate of pericytes post-TAC. (D) Higher magnification of the area boxed in C. (E) Section from a 28-day post-TAC heart displaying one of the very rare events (3 cells out of 3 hearts) where a tdTomato+ cardiomyocyte could be detected. This extremely low rate of cardiomyocyte labeling represented a negligible contribution to the total number of cardiomyocytes in the ventricles and was likely derived from fusion between a cardiomyocyte and an adjacent mural cell. (F) Higher magnification of the area boxed in E. Bars = 20 $\mu$ m in F, and 200 $\mu$ m in all other panels.

**Figure S7**



**Figure S7, Related to Figure 4, 5 and 6 – Conditional ablation of *Tbx18* does not increase plasticity of mural cells.** (A) Diagram representing the newly generated floxed *Tbx18* allele. Sequences of genotyping primers (P1 and P2) can be found in supplemental experimental procedures. Tamoxifen administration to adult *Tbx18<sup>CreERT2/Flox</sup>* animals results in ablation of TBX18 in all *Tbx18*-expressing lineages. (B) – After 6 weeks of high fat diet, *Tbx18* depleted mural cells of distinct fat depots did not display increased adipogenic potential, as revealed by the absence of co-localization between tdTomato labeling and the adipocyte marker Perilipin. (C) – 7 day after skeletal muscle injury induced by BaCl injection, *Tbx18* depleted mural cells of skeletal muscle did not display increased myogenic or fibrotic potential, as revealed by the absence of co-localization between tdTomato labeling and the myocyte marker Sarcomeric Actinin, or the fibroblast marker PDGFR $\alpha$ .

Table S1

Cell type	Antigen												
	Tbx18	CD146 MCAM	CD140a PDGFR $\alpha$	CD140b PDGFR $\beta$	$\alpha$ SMA	NG2	CD29 $\beta$ 1-Integ	CD31 Pecam	CD34	Sca1	CD45	CD117 c-kit	
Cardiomyocytes	-	-	-	-	-	-	++	-	-	-	-	-	
Brown adipocytes	-	++	-	-	-	++	++	-	-	-	-	-	
White adipocytes	-	++	-	-	-	-	++	-	-	-	-	-	
Neurons	-	-	-	-	-	-	-	-	-	-	-	Mosaic	
Endothelium	-	++	-	-	-	-	++	++	++	++	-	N.E.	
Fibroblasts	-	-	++	- or +	-	-	N.E.	-	-	-	-	-	
Leukocytes	-	N.E.	-	-	-	-	N.E.	N.E.	N.E.	N.E.	++	N.E.	
Pericytes	++	++	- or +	++	-	++*	++	-	-	-	-	-	
Vas. Smooth Muscle	++	+	- or +	+	++	+	++	-	-	-	-	-	
Epicardium	++	-	++	-	-	-	++	-	-	-	-	-	
Pia Mater	++	-	++	++	-	-	++	-	-	-	-	-	

**Table S1, Related to Figure 2** – summarized antigen expression profile of parenchymal cells, cells of the stromal vascular fraction and membranous linings (memb.) of organs analyzed (summary of histological data presented in Figures 2, S1, S2, and S3). Note that pericytes and vascular smooth muscle cells can be distinguished from all other cell types based on the simultaneous expression of *Tbx18*-GFP and CD146. This specific expression profile was the basis for the protocol for mural cell purification reported in Figure S4. N.E. = not evaluated; - = not expressed; + = expressed; ++ = strongly expressed. \* = in brain it is possible to find both NG2+ and NG2- pericytes.

Allele(s)	Primers	Amplicon
<i>Tbx18</i> -H2B:GFP	T18_F – GCCAGAGAAAGAGGAAACGGCAAA H2B_R – CTCGAAAATGTCGTTACAAAC	600 bp
<i>Tbx18</i> -CreERT2	T18_F – GCCAGAGAAAGAGGAAACGGCAAA Cre_R – TCCCTGAACATGTCCATCAGGTTC	300 bp
<i>Floxed Tbx18</i>	P1 - AAGTTCTCAGAAAGTGCCTCGCGC P2 - GCCTGGACAGCAGAGGGTAGAGAC	WT=350bp Flox~500bp
<i>Pdgfrb</i> -Cre; <i>Tie2</i> -Cre	Cre – F – AATTTACTGACCGTACACCAAAA Cre – R – CTATTTTCCATGAGTGAACGAAC	449 bp
Rosa26-tdTomato	Tomato_F – CTGTTCTGTACGGCATGG Tomato_R – GGCATTAAGCAGCGTATCC Rosa_WT_F – AAGGGAGCTGCAGTGGAGTA Rosa_WT_R – CCGAAAATCTGTGGGAAGTC	WT = 297bp Tom= 196bp

**Table S2, Related to STAR Methods (Method details)** – list of primers used for genotyping transgenic mice.