

# Supplementary material

**Partial mural cell ablation disrupts coronary vasculature integrity and induces systolic dysfunction.**

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## Supplemental tables

### A/ List of primary antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
mouse PECAM-1 (CD31)	BMA Biomedicals	T-2001	2 µg/mL
mouse PODXL	R&D systems	AF1556	4 µg/mL
mouse Cy3-anti-smα-actin	Sigma	C6198	5 µg/mL
mouse NG2	Chemicon	AB 5320	
mouse ACTN2	Sigma	A 2172	
mouse VIM	Cell signaling technology	5741	
mouse ICAM-1 (CD54)	BD Pharmingen	550287	2.5 µg/mL
mouse VCAM-1	Invitrogen	14-1061-82	
mouse CDH5	R&D systems	AF1002	
mouse FGB	Abcam	ab227063	
mouse COL1A1	Abcam	ab21285	
mouse CX43	Sigma	C6219	
mouse CD41	Proteintech	18308-1-AP	3.5 µg/mL
mouse Myh7	Atlas antibodies	HPA001239	1 µg/mL
mouse Desmin	DB Biotech	DB148-0.1	0.5 µg/mL
mouse CD45	BD Pharmingen	550539	0.625 µg/mL
mouse CD68	Biolegend	137001	5 µg/mL
mouse CD3	Santa-Cruz Biotechnology	sc-1127	
mouse B220	R&D systems	MAB1217	
mouse COL4A1	Abcam	ab19808	
Mouse HIF1A	R&D systems	AF1935	
GFP	Novus	NB100-1770SS	
GFP	Invitrogen	A6455	
mouse ATP2A2	Badrilla	A010-80	
phospho-PLN (Ser16)	Badrilla	A010-12	
phospho-PLN (Thr17)	Badrilla	A010-13	
total-PLN	Badrilla	A010-14	
phospho-RYR2 (Ser2814)	Badrilla	A010-31	
α-tubulin	Sigma	T5168	
human CDH5	Cell signaling technology	2500	
human ICAM-1	Santa-Cruz Biotechnology	sc-8439	

B/ List of secondary antibodies

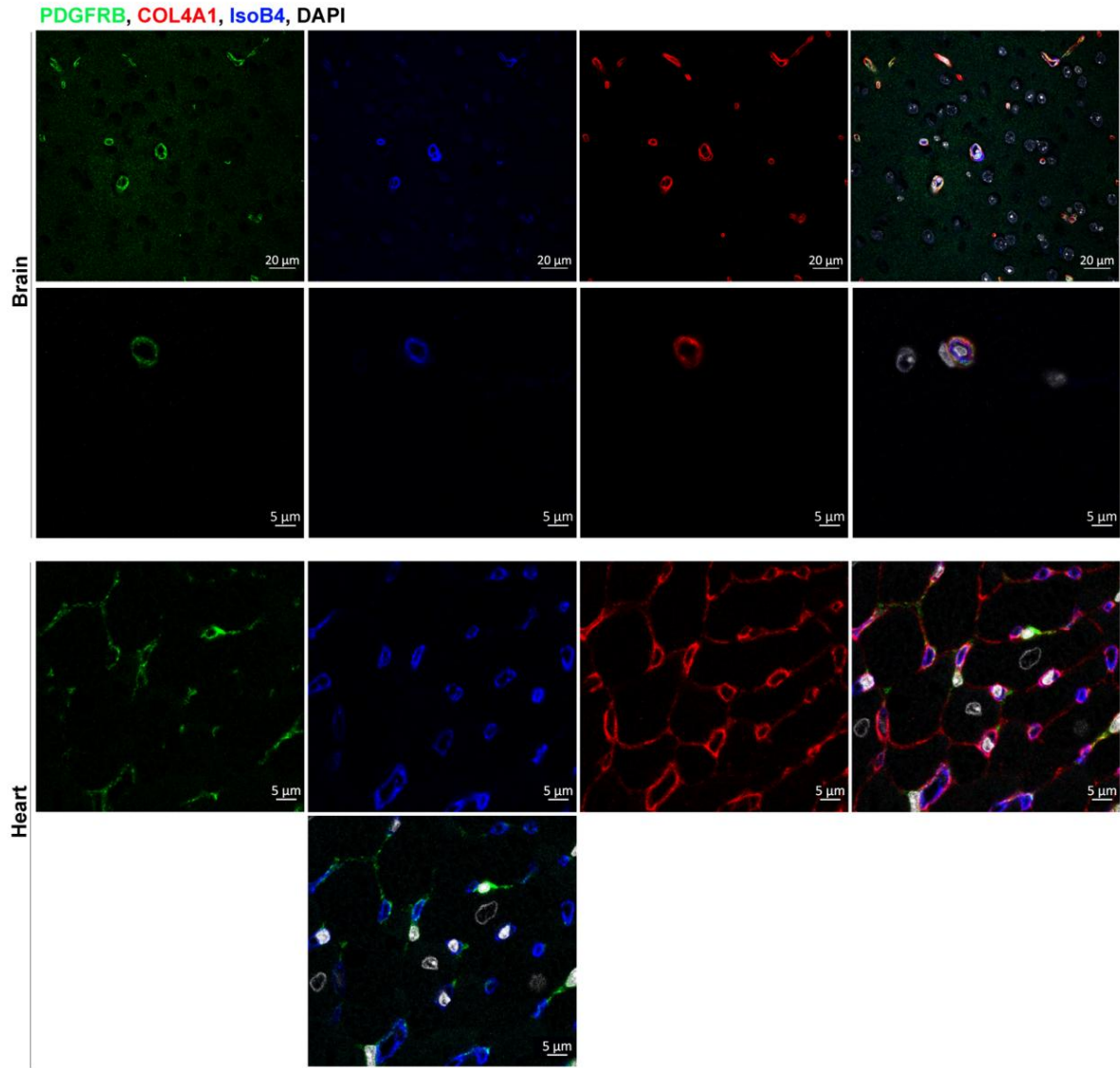
<b>Target antigen</b>	<b>Conjugate</b>	<b>Vendor or Source</b>	<b>Catalog #</b>	<b>Working concentration</b>
Rat IgG	Biotin	Jackson ImmunoResearch	712-065-153	Dilution 1/500
Rabbit	Biotin	Jackson ImmunoResearch	711-065-152	Dilution 1/500
Goat IgG	Alexa Fluor 568	Invitrogen	A-11057	10 µg/mL
Rat IgG	Alexa Fluor 647	Invitrogen	A-48265	10 µg/mL
Hamster IgG	Biotin	Jackson ImmunoResearch	127-065-160	Dilution 1/500
Goat IgG	Alexa Fluor 488	Invitrogen	A-11055	10 µg/mL
Rabbit IgG	Alexa Fluor 488	Invitrogen	A-21206	10 µg/mL
Rabbit IgG	Alexa Fluor 568	Invitrogen	A-10042	10 µg/mL

**Supplemental Table 1: List of antibodies used for immunostainings**

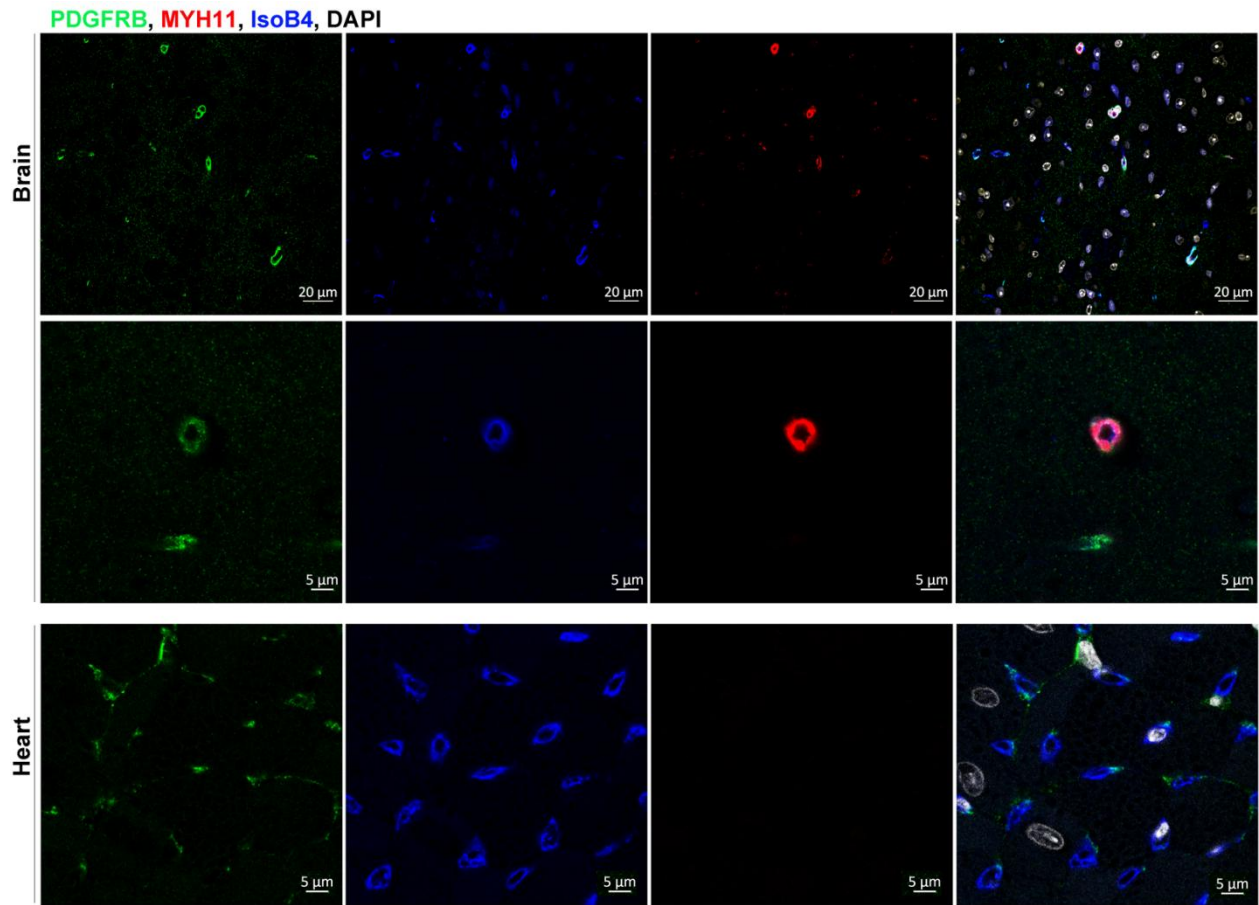
mouse 18S	F	5' -CGCGTTCTATTTTGTGGT-3'
	R	5' -AGTCGGCATCGTTTATGGTC-3'
mouse Actb	F	5' -CCTGAACCCTAAGGCCAACCC-3'
	R	5' -TAGCCCTCGTAGATGGGCAC-3'
mouse Myh6	F	5' -CCCTGGACAAGAAGCAGAGG-3'
	R	5' -TGTTCTCCCGCTTGAAGGTC-3'
mouse Col1a1	F	5' -CAACCTCAAGAAGGCCCTGC-3'
	R	5' -TGTCCAAGGGAGCCACATCG-3'
mouse Col3a1	F	5' -AGCACGAGGTCTTGCTGGAC-3'
	R	5' -ACCAGCTGTACCAGGCTGAC-3'
mouse Tgfb1	F	5' -GCTAATGGTGGACCCGAACAAC-3'
	R	5' -CACTGCTTCCCGAATGTCTGAC-3'
mouse Myh7	F	5' -GGATGACGTCACCTCCAACA-3'
	R	5' -AGATCAGAGCCTCCTTCTCGT-3'
mouse Ctgf	F	5' -GACCCAACCTATGATGCGAGCC-3'
	R	5' -TCCCACAGGTCTTAGAACAGG-3'
mouse Ttn N-2B	F	5' -ACAGTGGGAAAGCAAAGACATC-3'
	R	5' -AGGTGGCCCAGAGCTACTTC-3'
mouse Ttn N2BA	F	5' -GAGACATTGCTCCGCTTTTC-3'
	R	5' -GATCTCCAAAGAGGCTGTC-3'
mouse Atp2a2 (Serca2a)	F	5' -GATCCTCTACGTGGAACCTTTG-3'
	R	5' -GGTAGATGTGTTGCTAACAACG-3'
mouse Cx43 (Gja1)	F	5' -ATCAGGGAGGCAAGCCATGCTCA-3'
	R	5' -ACGTTGGCCCACACCACAAAGA-3'
human ACTB ( $\beta$ -actin)	F	5' -GGAGGAGCTGGAAGCAGCC-3'
	R	5' -GCTGTGCTACGTCGCCCTG-3'
human ICAM-1	F	5' -ACGCCGAGGACAGGGCATT-3'
	R	5' -GGGGCTATGTCTCCCCACCA-3'
human VCAM-1	F	5' -GGCCAGTTGAAGGATGCGGG-3'
	R	5' -AGAGCACGAGAAGCTCAGGAGAA-3'
human CDH2	F	5' -CCGGTTTCATTTGAGGGCAC-3'
	R	5' -CCCATTTGAGGGCATTGGGAT-3'
human IL-6	F	5' -GAAGATTCCAAAGATGTAGCCGC-3'
	R	5' -GGTTGTTTTCTGCCAGTGCCCTC-3'

**Supplemental Table 2: List of primers used for reverse transcription (RT) quantitative polymer chain reaction (qPCR). F: forward; R: reverse**

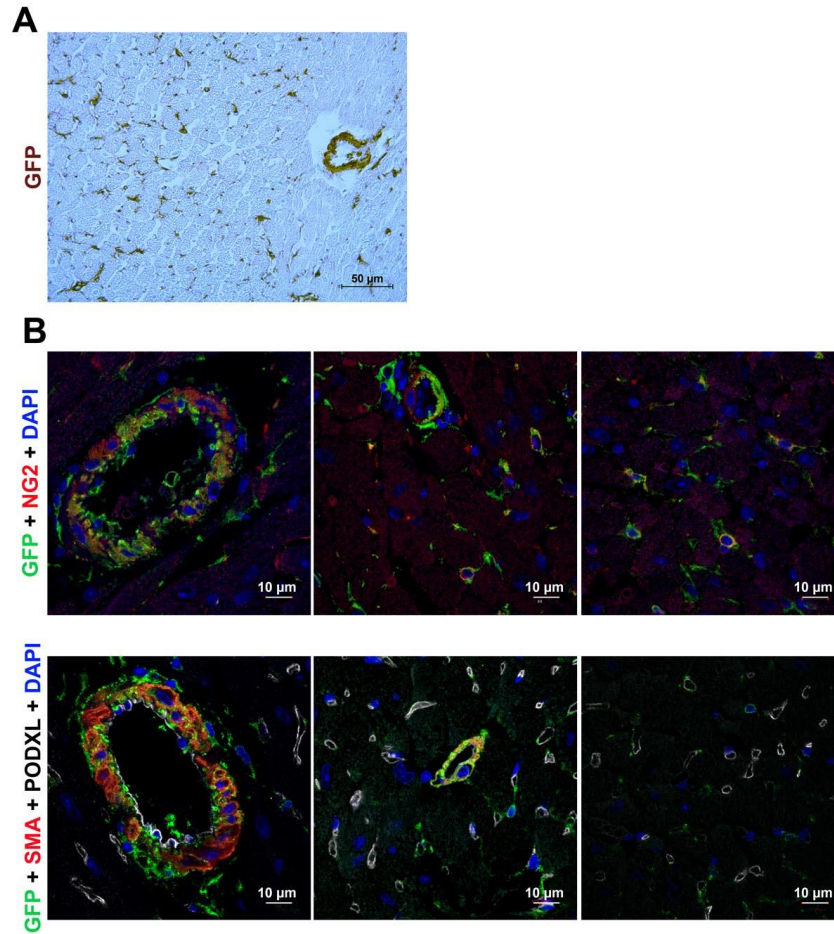
Supplemental figures and figure legends



**Figure S1: Cardiac pericytes are thin-stranded pericytes.** Mouse brain and heart cross-sections were immunostained with anti-PDGFRB antibodies to identify pericytes (in green) and anti-COL4A1 antibodies to identify endothelial basement membrane (in red). ECs were identified using Isolectin B4 (in blue). Nuclei were stained with DAPI (in white).

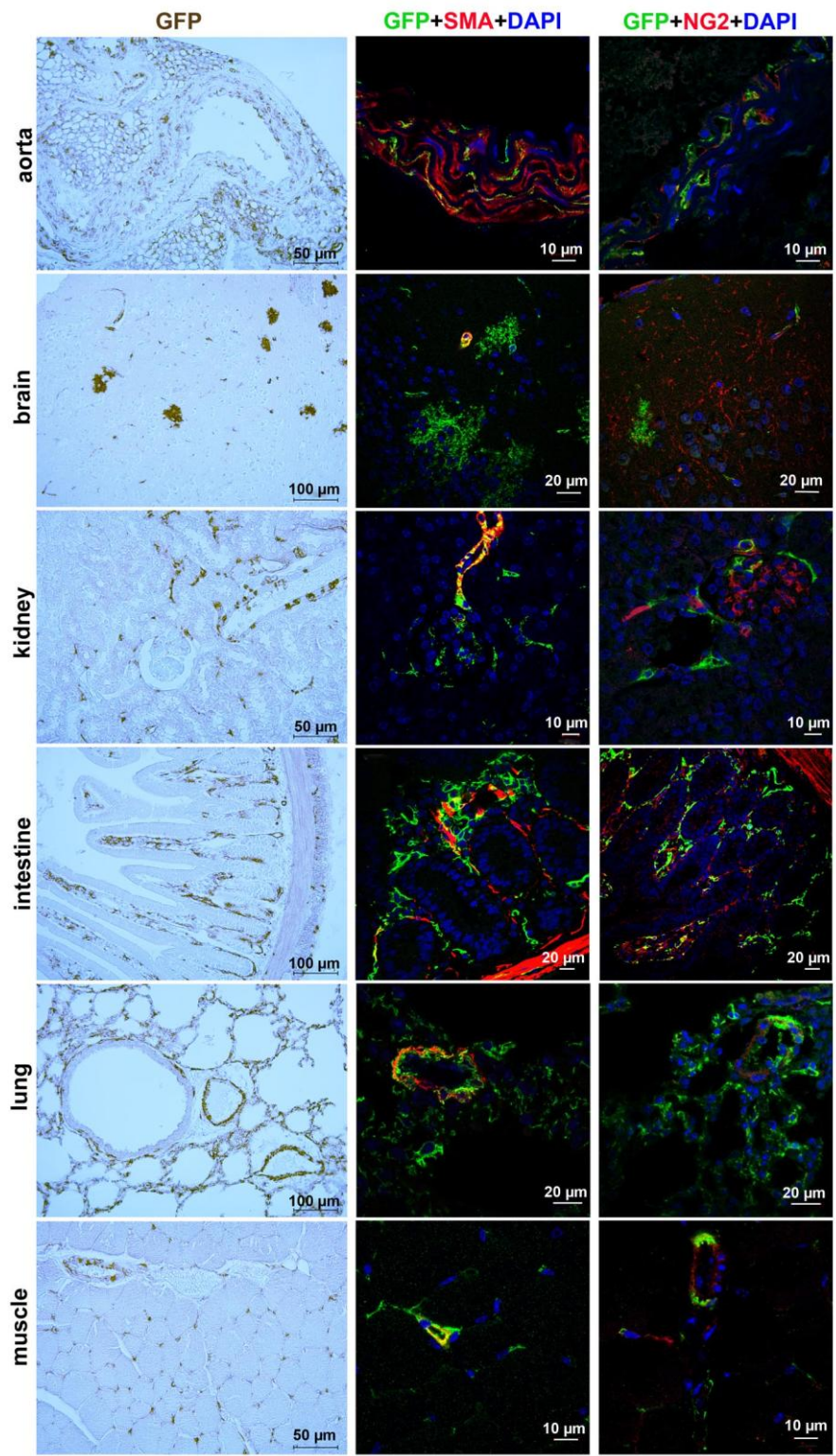


**Figure S2: Cardiac pericytes do not express smMHC:** Mouse brain and heart cross-sections were immunostained with anti-PDGFRB antibodies to identify pericytes (in green) and anti-smMHC antibodies. ECs were identified using Isolectin B4 (in blue). Nuclei were stained with DAPI (in white).



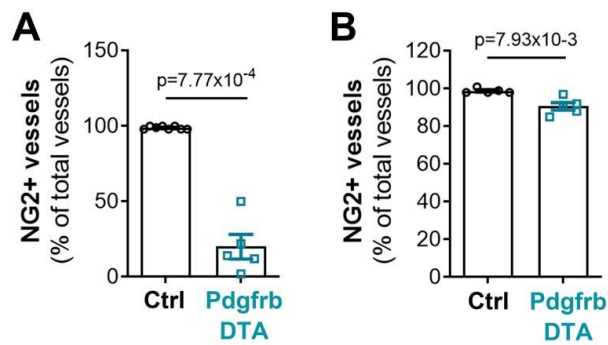
**Figure S3: (A-B): The *Pdgfrb* promoter is active in both cardiac pericytes and SMCs:** *Pdgfrb*-Cre/ERT2; *Rosa*<sup>mTmG</sup> mice were administered with tamoxifen and sacrificed 7 days after the first injection. **(A)** Heart cross-sections were immunostained with anti-GFP antibodies (in brown). **(B)** Heart cross-sections were co-immunostained with anti-GFP antibodies (in green) together with anti-NG2 antibodies (in red) to identify pericytes or anti-SMA (in red) and anti-PODXL antibodies (in white) to identify SMCs and endothelial cells respectively.



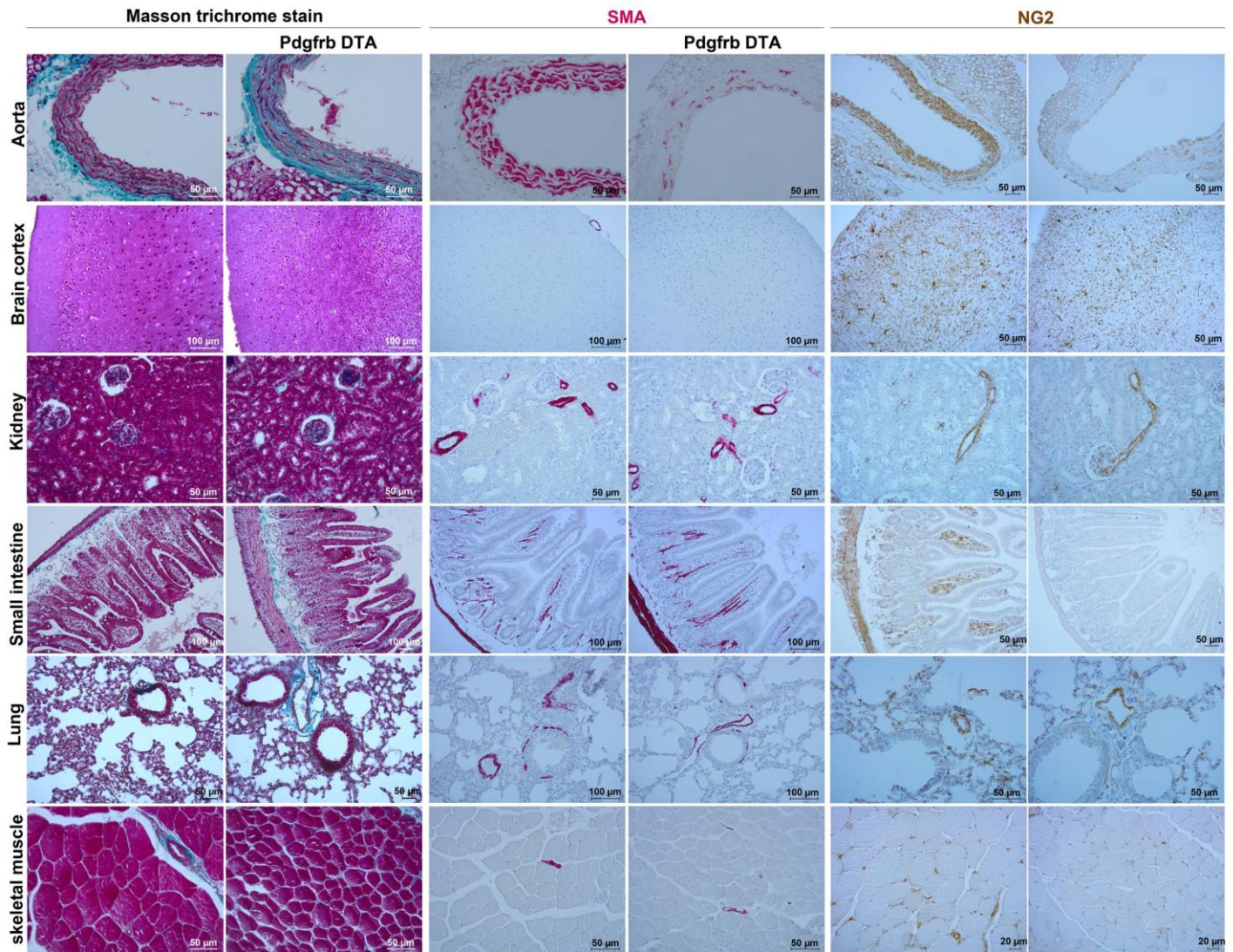




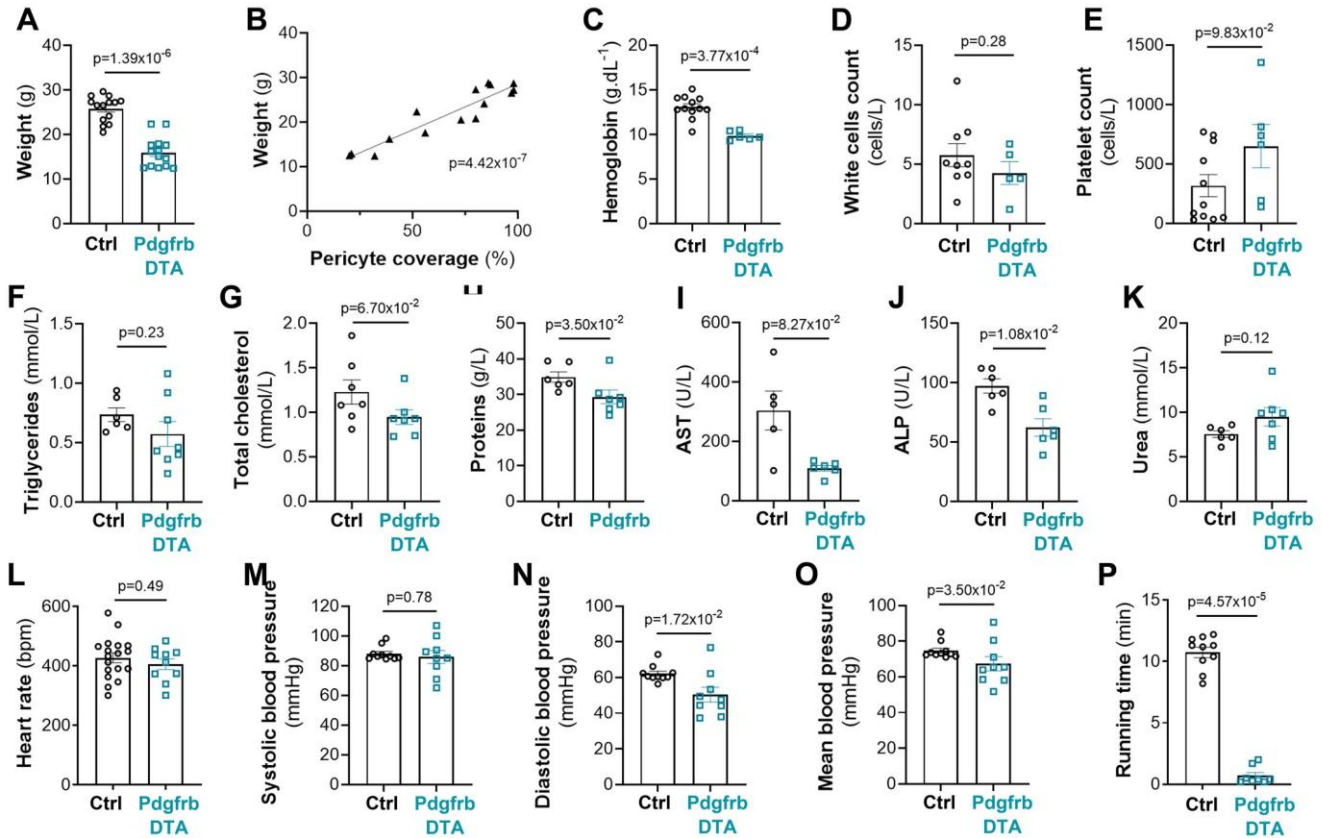
**Figure S4:** *Pdgfrb-Cre/ERT2; Rosa-mTmG* mice were administered with tamoxifen and sacrificed 7 days after the first injection. **(A)** Tissue sections were immunostained with anti-GFP antibodies alone or together with anti-SMA antibodies to identify SMCs or anti-NG2 antibodies to identify pericytes.



**Figure S5: Cardiac pericytes are renewed.** Pdgfrb-Cre/ERT2; Rosa<sup>DTA</sup> (Pdgfrb-DTA) mice were administered with tamoxifen. Mice were sacrificed 7 days (**A**) or 28 days after the first injection (**B**). Heart cross-sections were co-immunostained with anti-NG2 antibodies (in red) together with anti-PODXL antibodies (in green), the percentage of NG2 positive vessel was counted. Mann Whitney tests

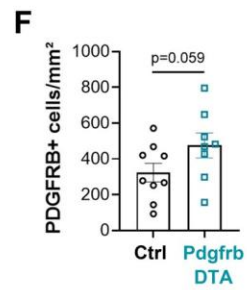
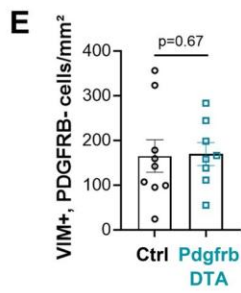
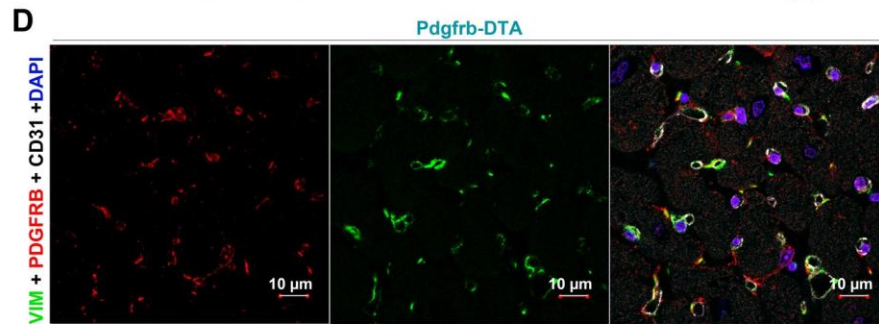
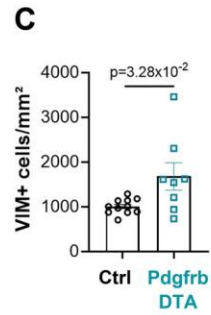
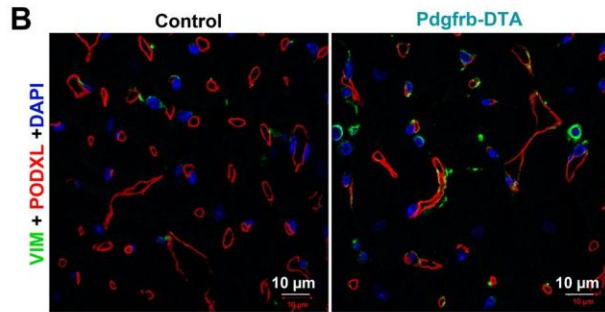
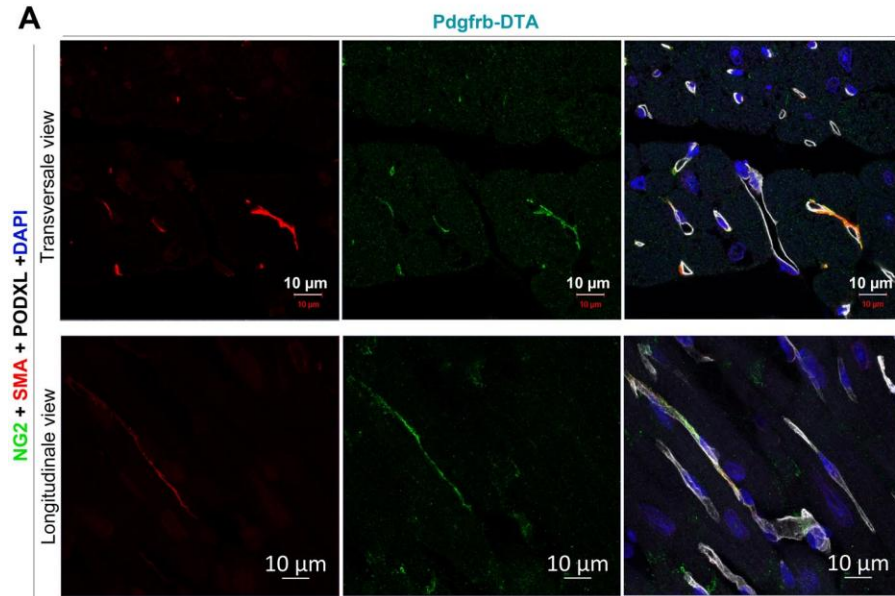


**Figure S6:** Pdgfrb-Cre/ERT2; Rosa<sup>DTA</sup> (Pdgfrb-DTA) mice were administered with tamoxifen and sacrificed 28 days after the first injection. Tissue sections were stained with Masson's trichrome stain, anti-SMA, or anti-NG2 antibodies.



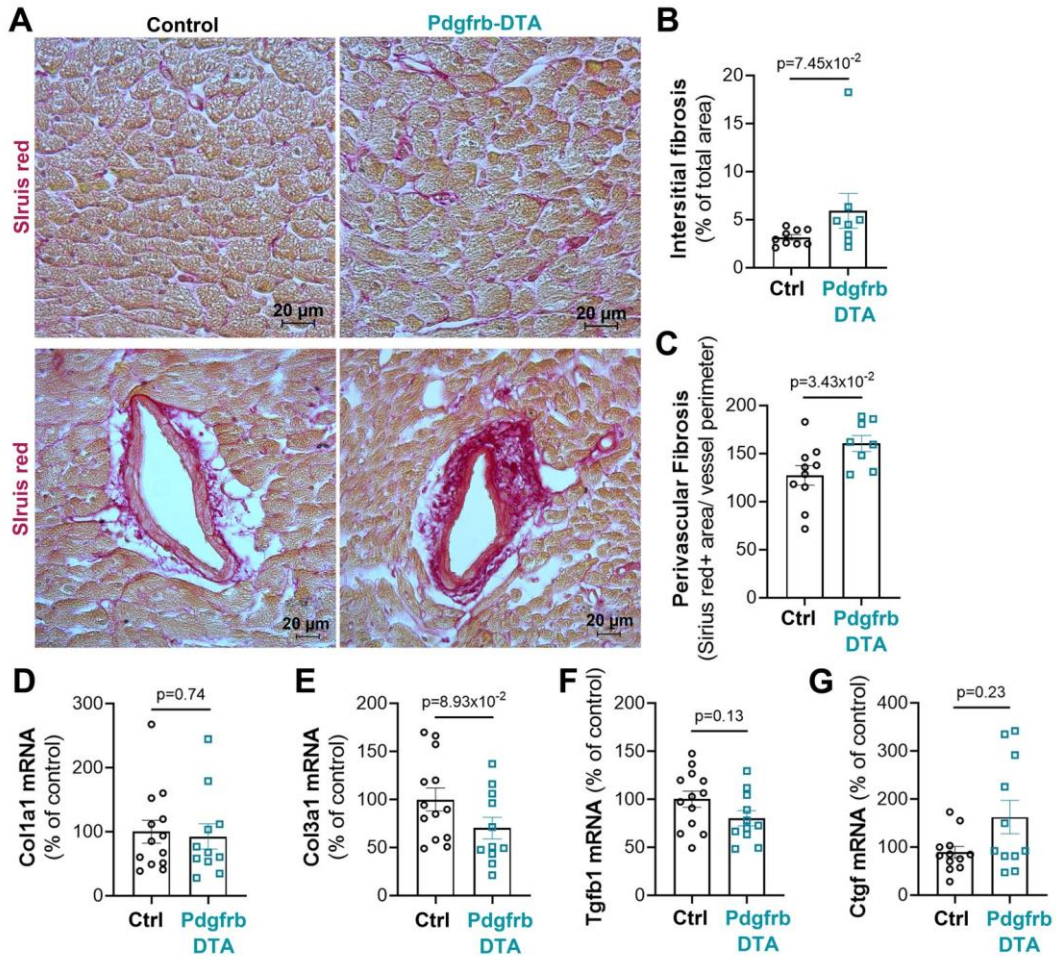
**Figure S7:** Pdgfrb-Cre/ERT2; Rosa<sup>DTA</sup> (Pdgfrb-DTA) and Rosa<sup>DTA</sup> (Control) mice were administered with tamoxifen. Mice were sacrificed 28 days after the first injection. (A) The weight of mice was measured and (B) correlated with pericyte coverage. (C) Hemoglobin content, (D) white blood cells count and platelet count (E) was calculated in total blood samples. (F) Triglycerides, (G) total cholesterol; (H) proteins, (I) Aspartate aminotransferase (AST), (J) Alkaline phosphatase (ALP) and (K) urea levels were measured in plasma samples. (L) Heart rate, (M) systolic, (N) diastolic, and (O) mean blood pressures were measured via left ventricular catheterization. (P) Exercise tolerance on a treadmill was assessed. Mann Whitney tests



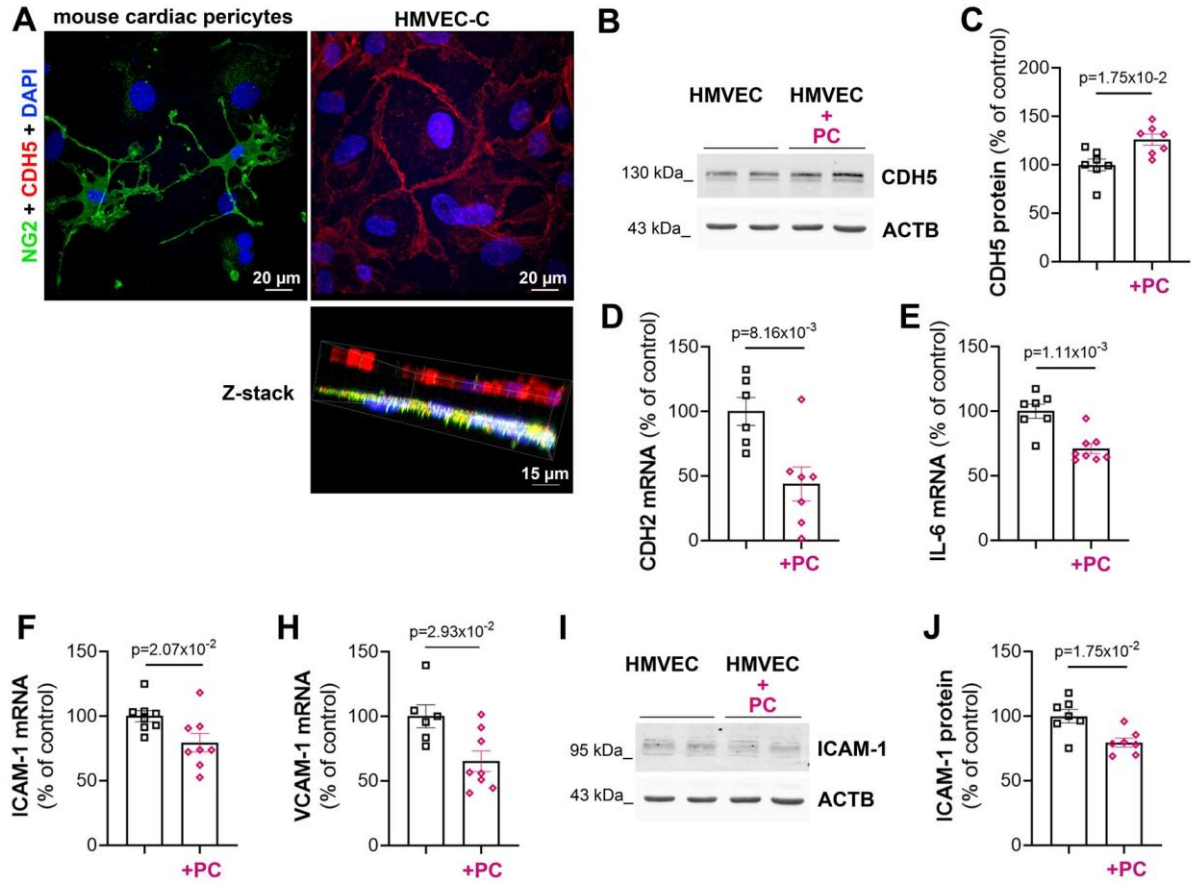


**Figure S8:** *Pdgfrb*-Cre/ERT2; *Rosa*<sup>DTA</sup> (*Pdgfrb*-DTA) and *Rosa*<sup>DTA</sup> (Control) mice were administered with tamoxifen. Mice were sacrificed 28 days after the first injection. **(A)** Heart cross-sections were co-immunostained with anti-NG2 antibodies (in green), anti-SMA (in red), and anti-PODXL (in green) antibodies. **(B)** Heart cross-sections were co-immunostained with anti-VIM (in green), and anti-PODXL (in red) antibodies to identify fibroblasts and ECs respectively. **(C)** The number of VIM+ cells per mm<sup>2</sup> was counted (n=9 and 11). **(D)** Heart cross-sections were co-immunostained with anti-PDGFRB (in red), anti-VIM (in green) and anti-CD31 (in white) antibodies. **(E)** The number of VIM+, PDGFRB- cells per mm<sup>2</sup> was counted (n=9 and 8). **(F)** The number of PDGFRB+ cells per mm<sup>2</sup> was counted (n=9 and 8). Mann Whitney tests

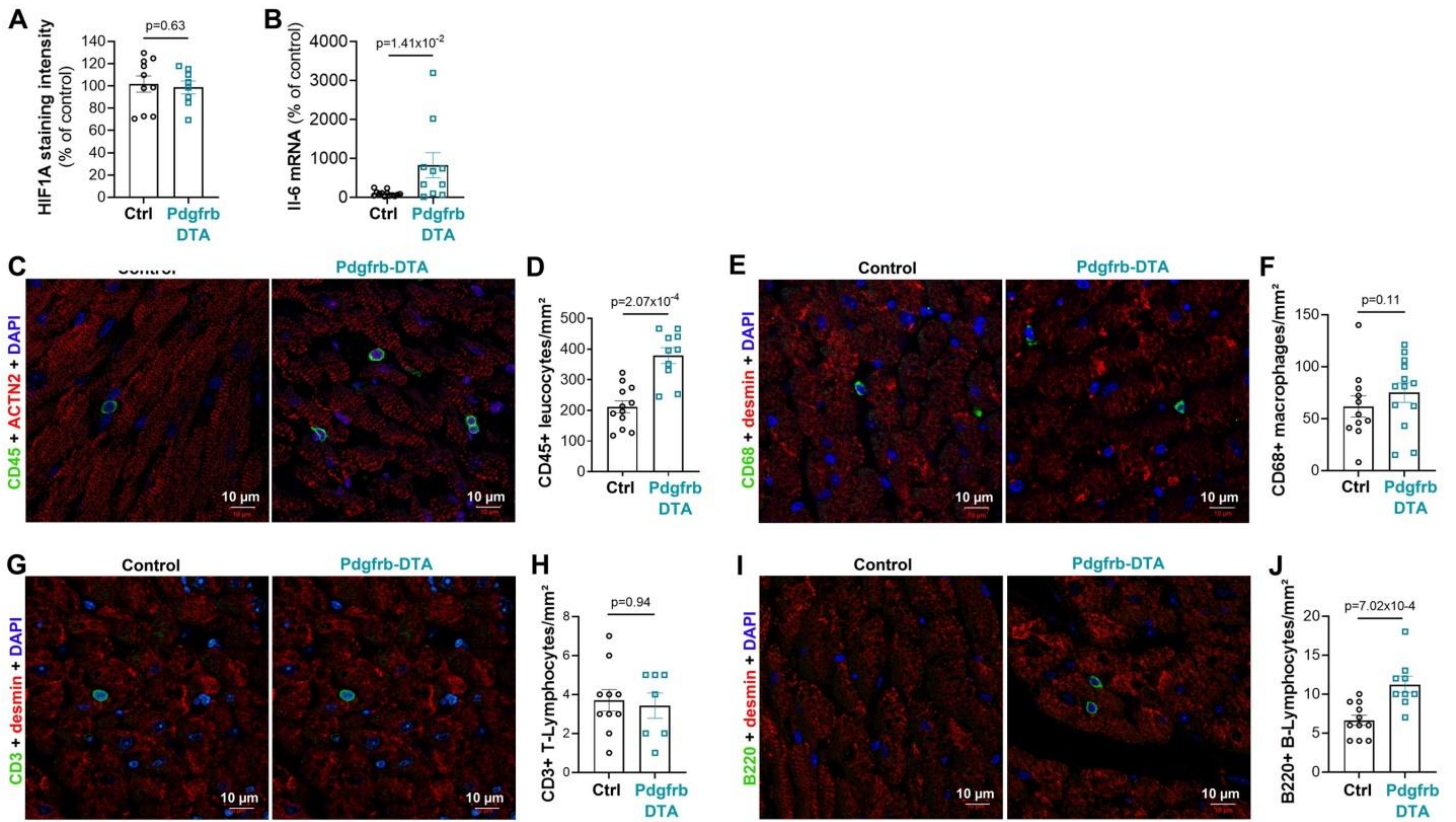




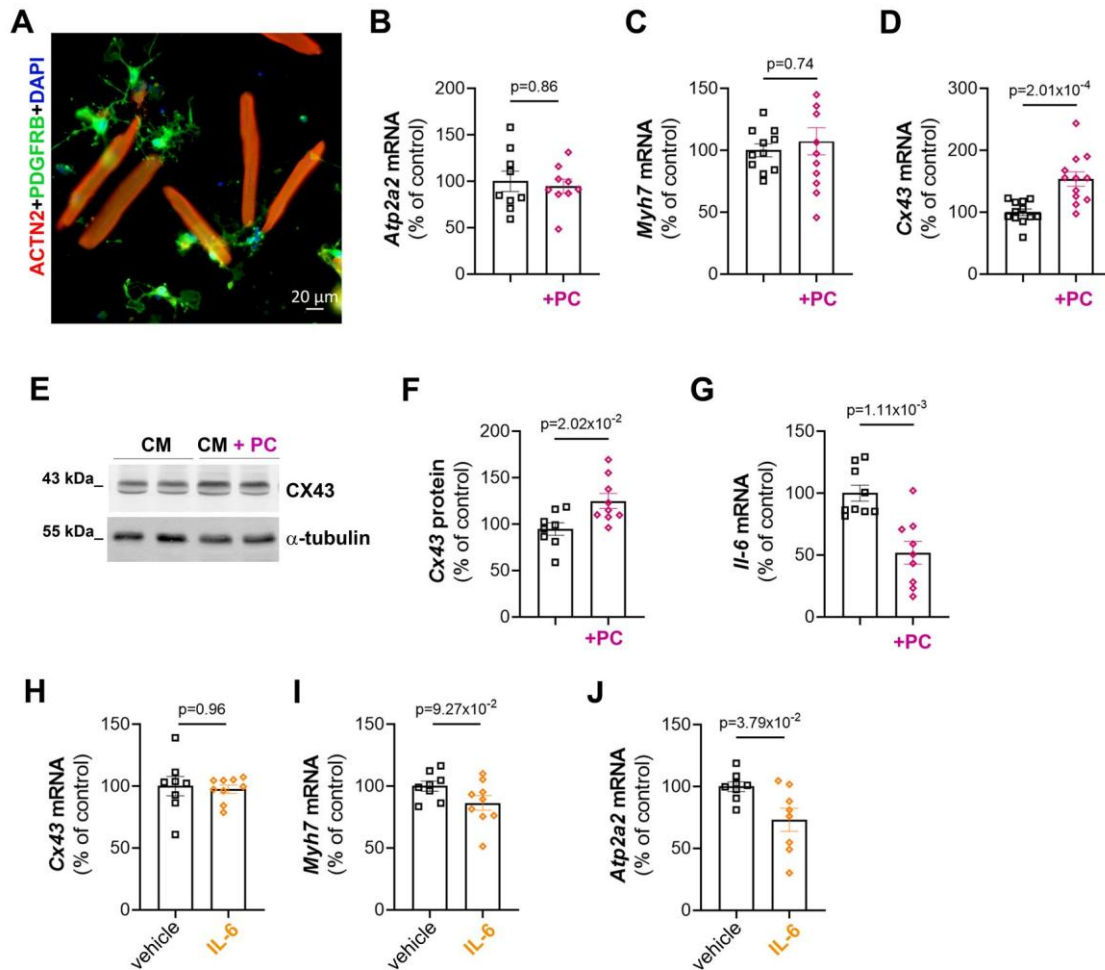
**Figure S9:** Pdgrfb-Cre/ERT2; Rosa<sup>DTA</sup> (Pdgrfb-DTA) and Rosa<sup>DTA</sup> (Control) mice were administered with tamoxifen. Mice were sacrificed 28 days after the first injection. **(A)** Heart cross-sections were stained with Sirius red to identify fibrosis. **(B)** Interstitial fibrosis was quantified using Image J software (n=9 and 11). **(C)** Perivascular fibrosis was quantified using Image J software (n=9 and 11). Col1a1 **(D)**, Col3a1 **(E)**, Tgfb1 **(F)**, and Ctgf **(G)** mRNA were quantified by RT-qPCR in total heart extracts and normalized to 18S rRNA. \*:  $p \leq 0.05$ , ns: not significant (Mann Whitney test).



**Figure S10:** Cardiac HMVECs were co-cultured or not with mouse cardiac pericyte. Each cell type was plated on each side of a 0.4  $\mu$ m pored Transwell<sup>®</sup> membrane for 48 hours. **(A)** Pericytes were immunostained with anti-NG2 antibodies in green and HMVECs were immunostained with anti-CDH5 antibodies in red. **(B)** CDH5 protein level in HMVECs was evaluated via western blot analysis and **(C)** quantified using Image J software. *CDH2* **(D)**, *IL-6* **(E)**, *ICAM-1* **(F)**, and *VCAM-1* **(H)** mRNA levels were quantified via RT-qPCR and normalized to *ACTB* mRNA. **(I)** ICAM-1 protein level in HMVECs was evaluated via western blot analysis and **(J)** quantified using Image J software. Mann Whitney tests



**Figure S11:** Pdgrfb-Cre/ERT2; Rosa<sup>DTA</sup> (Pdgrfb-DTA) and Rosa<sup>DTA</sup> (Control) mice were administered with tamoxifen. Mice were sacrificed 28 days after the first injection. (A) Heart cross-sections were immunostained with anti-HIF1A antibodies. HIF1A staining intensity was quantified using ImageJ software. (B) *Il-6* mRNA level was quantified via RT-qPCR and normalized to 18S rRNA. (C) Heart cross-sections were co-immunostained with anti-CD45 antibodies (in green) and anti-ACTN2 antibodies (in red). (D) The number of CD45 positive leucocytes/mm<sup>2</sup> was quantified using Image J software. (E) Heart cross-sections were co-immunostained with anti-CD68 antibodies (in green) and anti-desmin antibodies (in red). (F) The number of CD68 positive macrophages/mm<sup>2</sup> was quantified using Image J software. (G) Heart cross-sections were co-immunostained with anti-CD3 antibodies (in green) and anti-desmin antibodies (in red). (H) The number of CD3-positive T-lymphocytes/mm<sup>2</sup> was quantified using Image J software. (I) Heart cross-sections were co-immunostained with anti-B220 antibodies (in green) and anti-desmin antibodies (in red). (J) The number of B220 positive B-lymphocytes/mm<sup>2</sup> was quantified using Image J software. Mann Whitney tests



**Figure S12: (A-G)** Mouse adult cardiomyocytes were co-cultured or not with mouse cardiac pericytes for 24 hours. **(A)** Cardiomyocytes were stained anti ACTN2 antibodies (in red) and pericytes were stained with anti-PDGFRB antibodies (in green). **(B)** *Atp2a2*, **(C)** *Myh7*, **(D)** *Cx43* mRNA was quantified via RT-qPCR and normalized to *Myh6* RNA. **(E)** CX43 protein expression was assessed by western blot analysis and **(F)** quantified using Image J software. **(G)** *Il-6* mRNA was quantified via RT-qPCR and normalized to *Myh6* RNA. **(H-J)** Mouse adult cardiomyocytes were treated or not with 10 ng/mL IL-6 recombinant protein for 5 hours. **(H)** *Cx43*, **(I)** *Myh7*, and **(J)** *Atp2a2* mRNA were quantified via RT-qPCR and normalized to *Actb* mRNA. Mann Whitney tests