

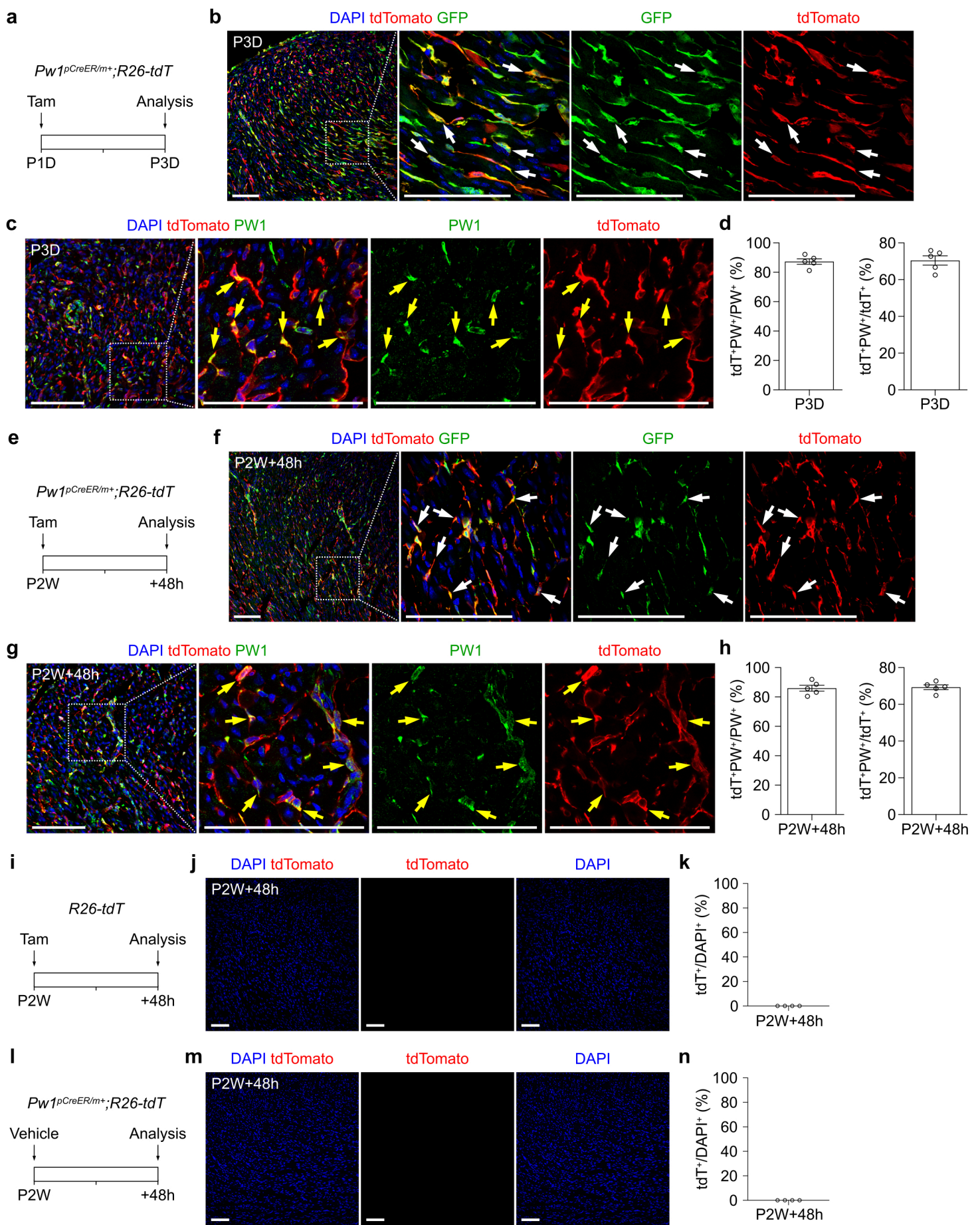


Fig. S1 Genetic targeting of cardiac PW1⁺ cells in *Pw1^{pCreER/m+}* mice. **a, e** Schematics of the experimental strategy for labeling PW1⁺ cells in hearts of *Pw1^{pCreER/m+};R26-tdT* pups. **b, f** Immunostaining for tdTomato and GFP in heart sections from *Pw1^{pCreER/m+};R26-tdT* mice at the indicated time points. White arrows indicate tdTomato⁺GFP⁺ cells. Manders' correlation coefficients: **b** (M1: 0.757; M2: 0.768), **f** (M1: 0.548; M2: 0.918). Scale bars, 100 μ m. **c, g** Immunostaining for tdTomato and PW1 in heart sections from *Pw1^{pCreER/m+};R26-tdT* mice at the indicated stages. Yellow arrows indicate tdTomato⁺PW1⁺ cells. Manders' correlation coefficients: **c** (M1: 0.350; M2: 0.502), **g** (M1: 0.500; M2: 0.743). Scale bars, 100 μ m. **d, h** Quantification of tdTomato⁺PW1⁺ cells. *n* = 5 biological samples per group; both sexes included. **i, l** Schematic of the experimental strategy for assessing reporter leakage in *R26-tdT* (**i**) and *Pw1^{pCreER/m+};R26-tdT* (**l**) mice. **j, m** Immunostaining for tdTomato in heart sections from *R26-tdT* (**j**) and *Pw1^{pCreER/m+};R26-tdT* (**m**) mice. Scale bars, 100 μ m. **k, n** Quantification of tdTomato⁺ cells in hearts of *R26-tdT* (**k**) and *Pw1^{pCreER/m+};R26-tdT* (**n**) mice. *n* = 4 biological replicates per group; both sexes included.

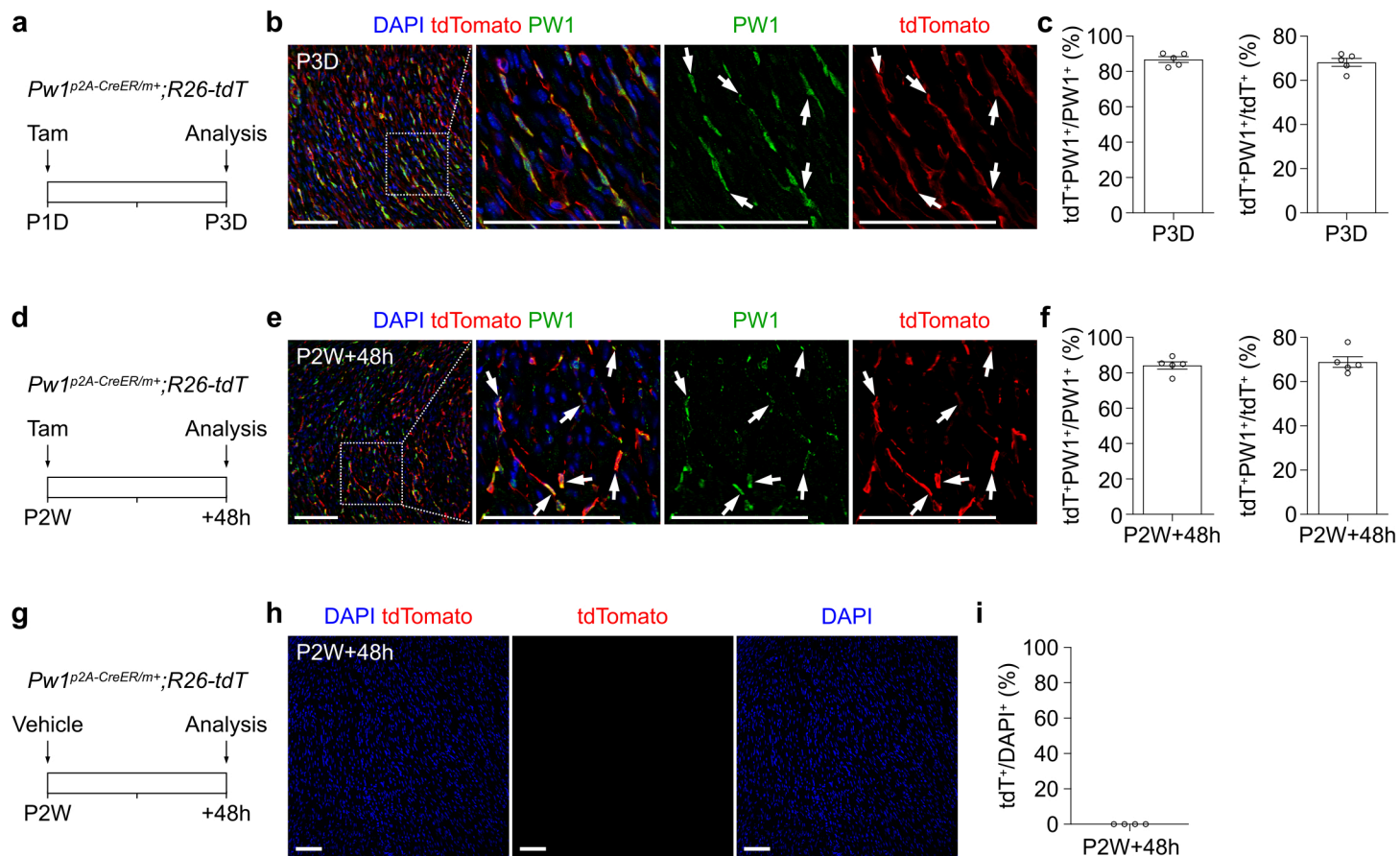


Fig. S2 Genetic targeting of cardiac PW1⁺ cells in *Pw1^{p2A-CreER/m+}* mice. **a, d** Schematics of the experimental strategy for labeling PW1⁺ cells in hearts of *Pw1^{p2A-CreER/m+};R26-tdT* pups. **b, e** Immunostaining for tdTomato and PW1 in heart sections from *Pw1^{p2A-CreER/m+};R26-tdT* mice at the indicated time points. White arrows indicate tdTomato⁺PW1⁺ cells. Manders' correlation coefficients: **b** (M1: 0.635; M2: 0.791), **e** (M1: 0.462; M2: 0.626). Scale bars, 100 μ m. **c, f** Quantification of tdTomato⁺PW1⁺ cells at each developmental stage. $n = 5$ biological samples per group; both sexes included. **g** Schematic of the experimental strategy for assessing reporter leakage in *Pw1^{p2A-CreER/m+};R26-tdT* mice. **h** Immunostaining for tdTomato in heart sections from *Pw1^{p2A-CreER/m+};R26-tdT* mice. Scale bars, 100 μ m. **i** Quantification of tdTomato⁺ cells in hearts of *Pw1^{p2A-CreER/m+};R26-tdT* mice. $n = 4$ biological replicates per group; both sexes included.

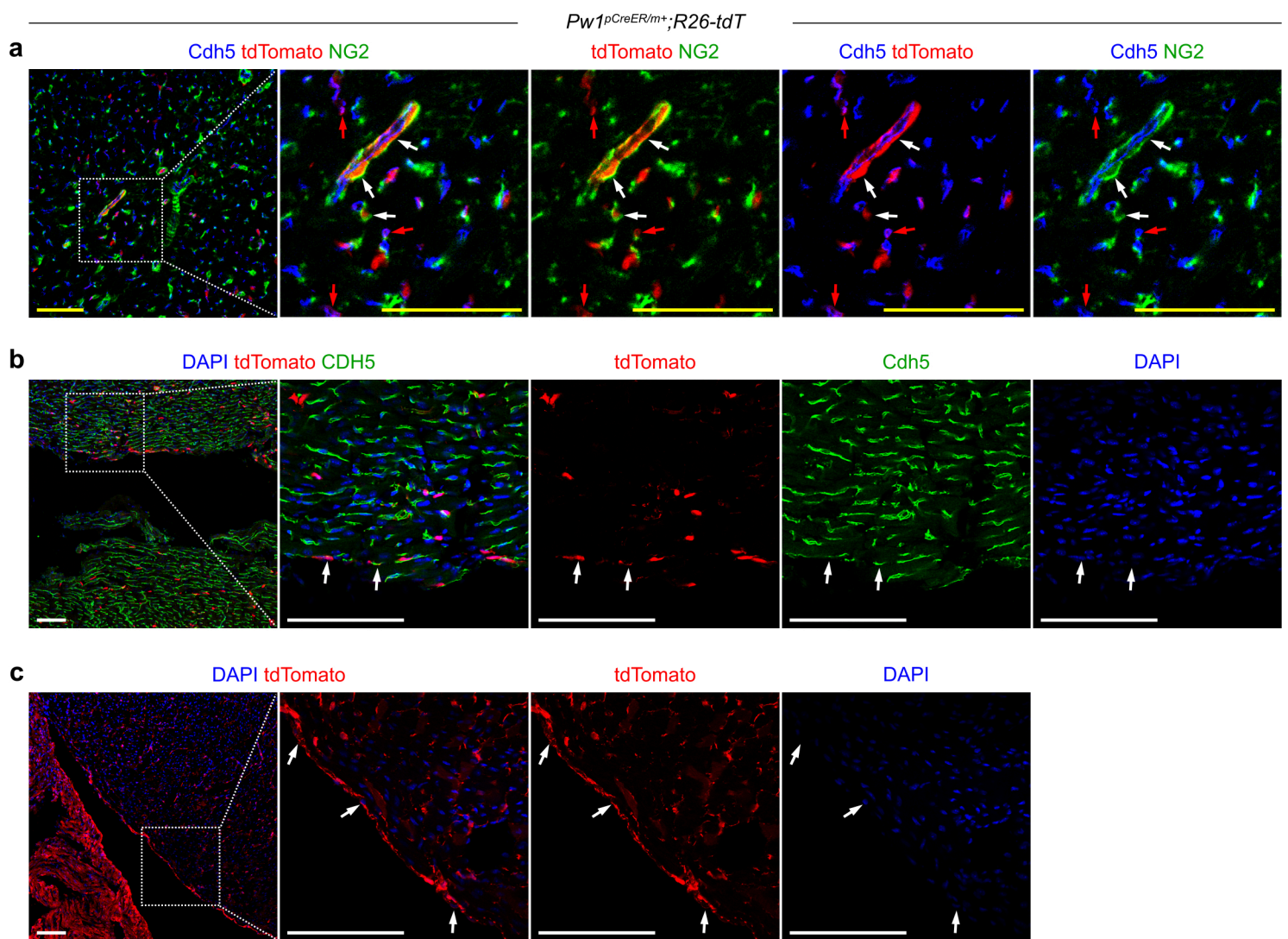


Fig. S3 Additional cell types labeled by tdTomato in *Pw1^{pCreER/m+};R26-tdT* mice at P3W. **a** Immunostaining for Cdh5, NG2 and tdTomato in heart sections from *Pw1^{pCreER/m+};R26-tdT* mice at P3W. White arrows indicate tdTomato⁺ coronary vascular mural cells; red arrows indicate tdTomato⁺ cVECs. **b** Co-staining for Cdh5 and tdTomato. White arrows point to tdTomato⁺ endocardial ECs. **c** tdTomato is detected in epicardial cells. White arrows indicate tdTomato⁺ epicardial cells. Yellow scale bars, 50 μ m; white scale bars, 100 μ m.

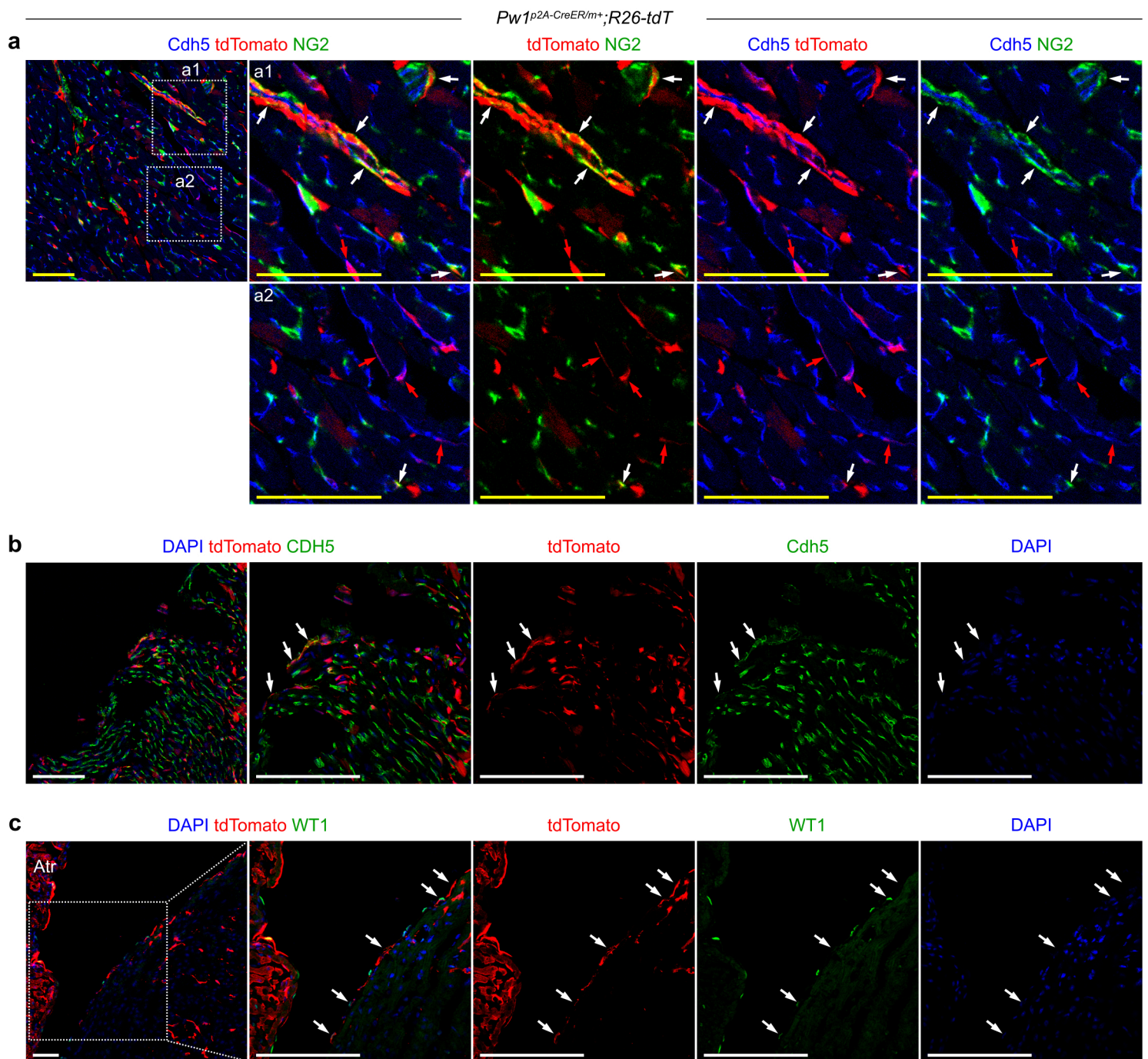


Fig. S4 Additional cell types labeled by tdTomato in *Pw1^{p2A-CreER/m+};R26-tdT* mice at P3W. **a** Immunostaining for Cdh5, NG2, and tdTomato in heart sections from *Pw1^{p2A-CreER/m+};R26-tdT* mice at P3W. White arrows indicate tdTomato⁺ coronary vascular mural cells; red arrows indicate tdTomato⁺ cVECs. **b** Co-staining for Cdh5 and tdTomato. White arrows point to tdTomato⁺ endocardial ECs. **c** Co-staining for WT1 and tdTomato. White arrows indicate epicardial cells. Atr, atrium. Yellow scale bars, 50 μ m; white scale bars, 100 μ m.

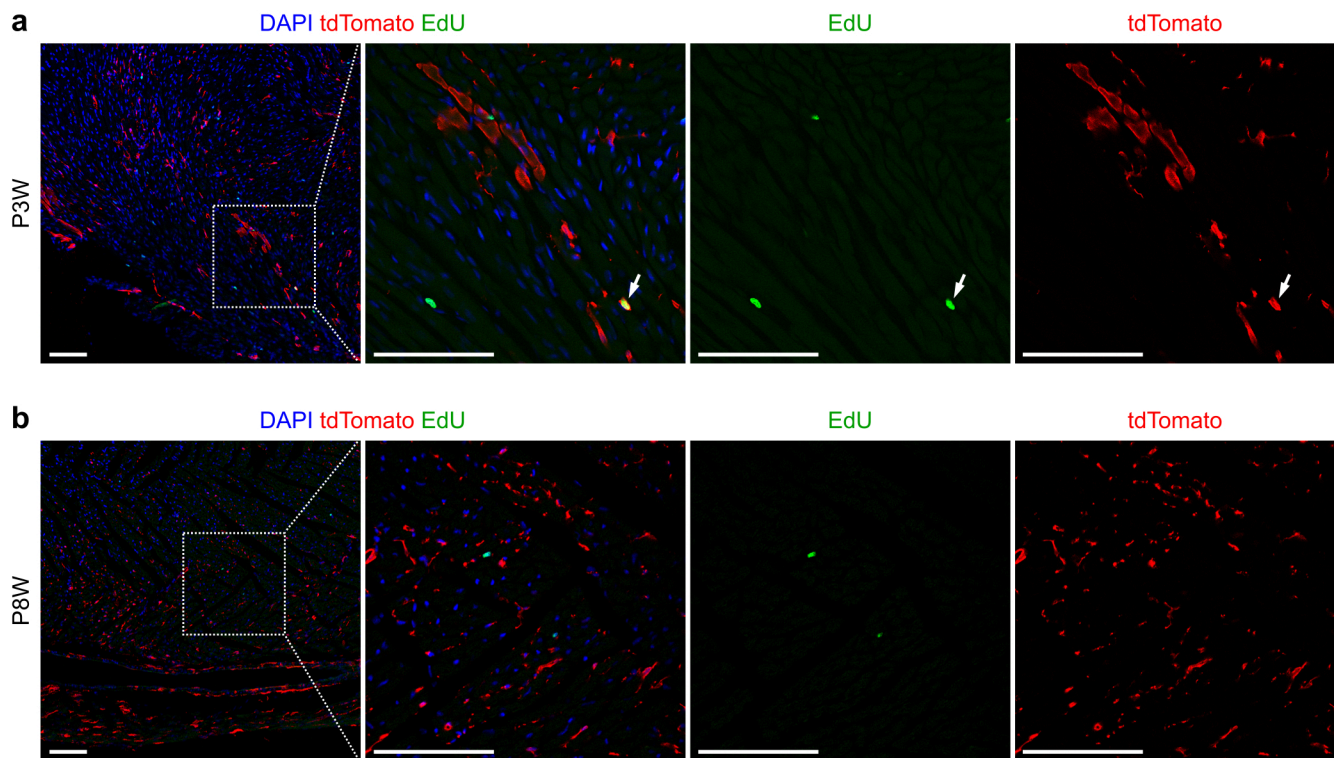


Fig. S5 Proliferation dynamics of PW1-derived cells in postnatal hearts. **a** Co-staining of tdTomato and EdU in heart sections from *Pw1^{pCreER/m+};R26-tdT* mice at P3W. Tamoxifen was administered at P2W. The arrow indicates an EdU⁺tdTomato⁺ cell. **b** Co-staining of tdTomato and EdU in heart sections from *Pw1^{pCreER/m+};R26-tdT* mice at P8W. Tamoxifen was administered at P2W. Mice received an intraperitoneal injection of EdU at (0.02 mg per gram of body weight) 5 hours before sacrifice at each time point. Scale bars, 100 μ m.

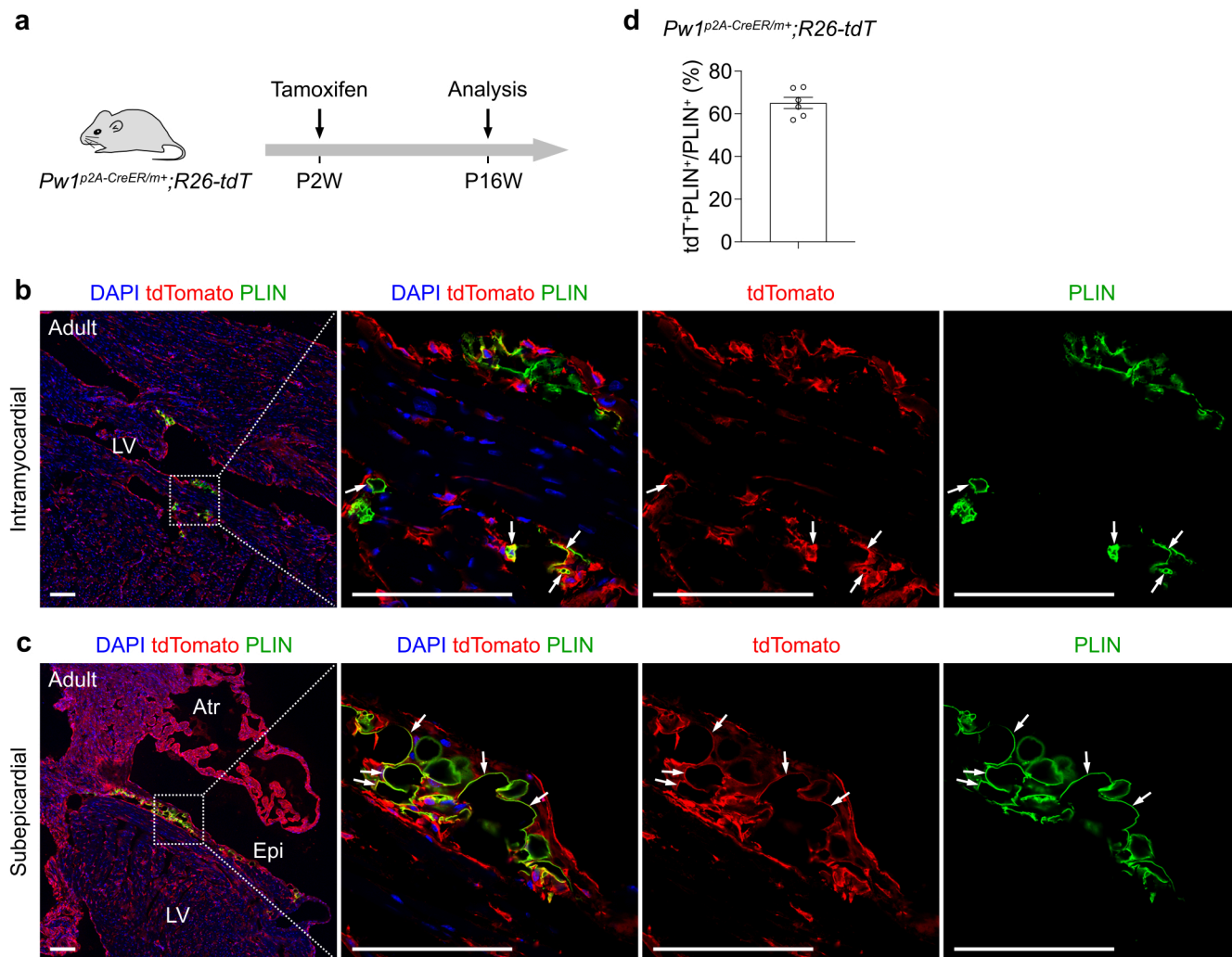


Fig. S6 Contribution of PW1⁺ cells to adipocytes in adult hearts revealed by genetic lineage tracing using *Pw1^{p2A-CreER/m+};R26-tdT* mice. **a** Schematic of the experimental strategy for tracing PW1⁺ cells during postnatal heart development in *Pw1^{p2A-CreER/m+};R26-tdT* mice. **b, c** Immunostaining for tdTomato and PLIN in heart sections from adult *Pw1^{p2A-CreER/m+};R26-tdT* mice. Arrows indicate tdTomato⁺PLIN⁺ adipocytes. LV, left ventricle; Atr, atrium; Epi, epicardium. Scale bars, 100 μ m. **d** Quantification of tdTomato-labeled adipocytes in hearts from *Pw1^{p2A-CreER/m+};R26-tdT* mice. n = 6 mice per group; both sexes included.

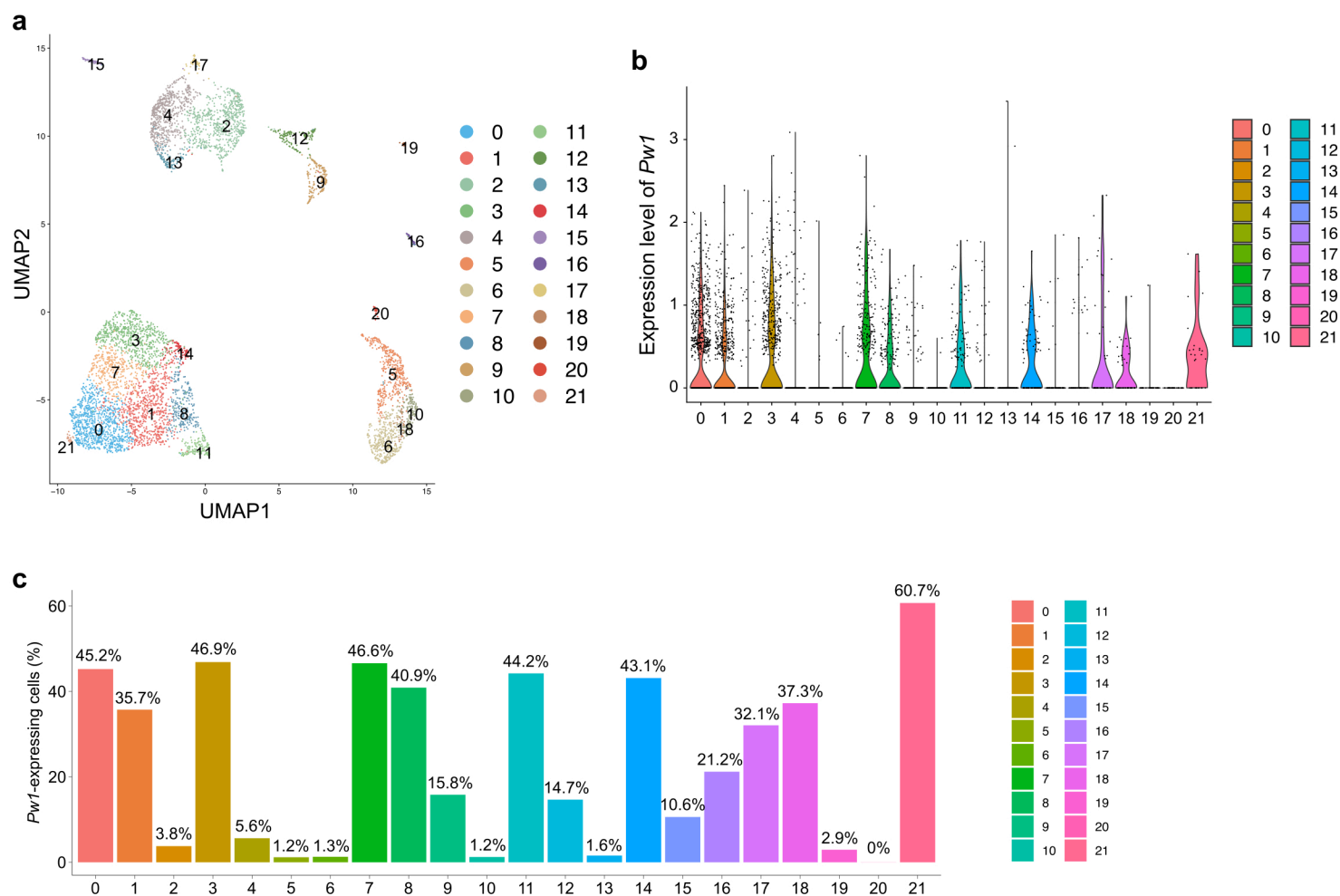


Fig. S7 Expression of *Pw1* across cardiac cell clusters in the adult mouse heart. **a** UMAP visualization of single-cell clusters from 8-week-old mouse hearts. **b** Violin plot showing the expression level of *Pw1* in each cluster. **c** Percentage of cells expressing *Pw1* within each cluster.

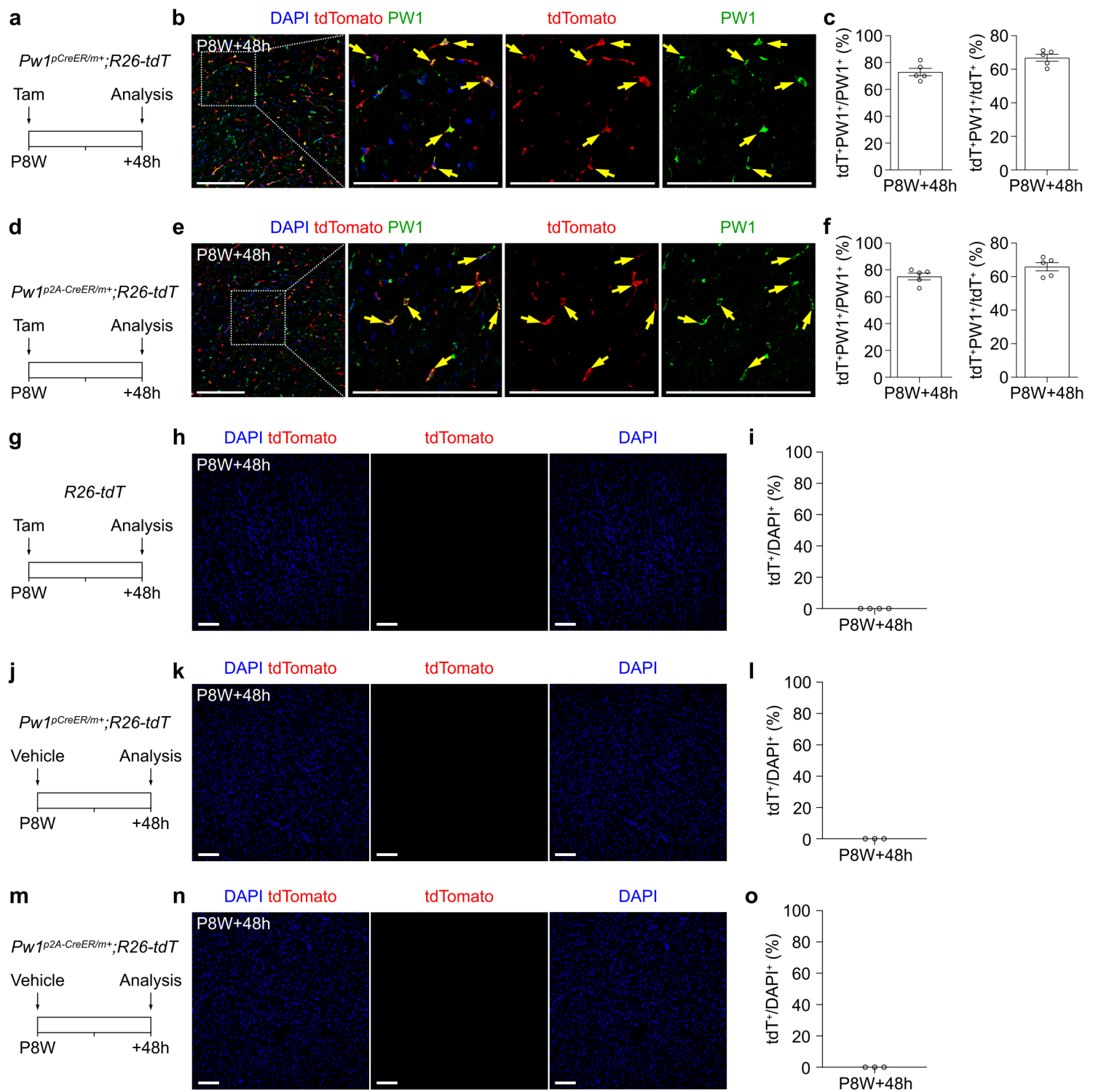


Fig. S8 Labeling of PW1⁺ cells in adult hearts of *Pw1^{pCreER/m+};R26-tdT* and *Pw1^{p2A-CreER/m+};R26-tdT* mice. **a, d** Schematics of the genetic labeling strategy in adult *Pw1^{pCreER/m+};R26-tdT* (**a**) and *Pw1^{p2A-CreER/m+};R26-tdT* (**d**) mice. **b, e** Immunostaining for tdTomato and PW1 in heart sections from adult *Pw1^{pCreER/m+};R26-tdT* (**b**) and *Pw1^{p2A-CreER/m+};R26-tdT* (**e**) mice. Yellow arrows indicate tdTomato⁺PW1⁺ cells. Manders' correlation coefficients: **b** (M1: 0.421; M2: 0.455), **e** (M1: 0.381; M2: 0.532). Scale bars, 100 μ m. **c, f** Quantification of tdTomato⁺PW1⁺ cells in adult *Pw1^{pCreER/m+};R26-tdT* (**c**) and *Pw1^{p2A-CreER/m+};R26-tdT* (**f**) mice. $n = 5$ biological samples per group; both sexes included. **g, j** and **m** Schematic of the experimental design for assessing reporter leakage in adult *R26-tdT* (**g**), *Pw1^{pCreER/m+};R26-tdT* (**j**) and *Pw1^{p2A-CreER/m+};R26-tdT* (**m**) mice. **h, k** and **n** Immunostaining for tdTomato in heart sections from adult *R26-tdT* (**h**), *Pw1^{pCreER/m+};R26-tdT* (**k**) and *Pw1^{p2A-CreER/m+};R26-tdT* (**n**) mice. Scale bars, 100 μ m. **i, l** and **o** Quantification of tdTomato⁺ cells in hearts of adult *R26-tdT* (**i**), *Pw1^{pCreER/m+};R26-tdT* (**l**) and *Pw1^{p2A-CreER/m+};R26-tdT* (**o**) mice. $n = 3$ or 4 biological replicates per group; both sexes included.

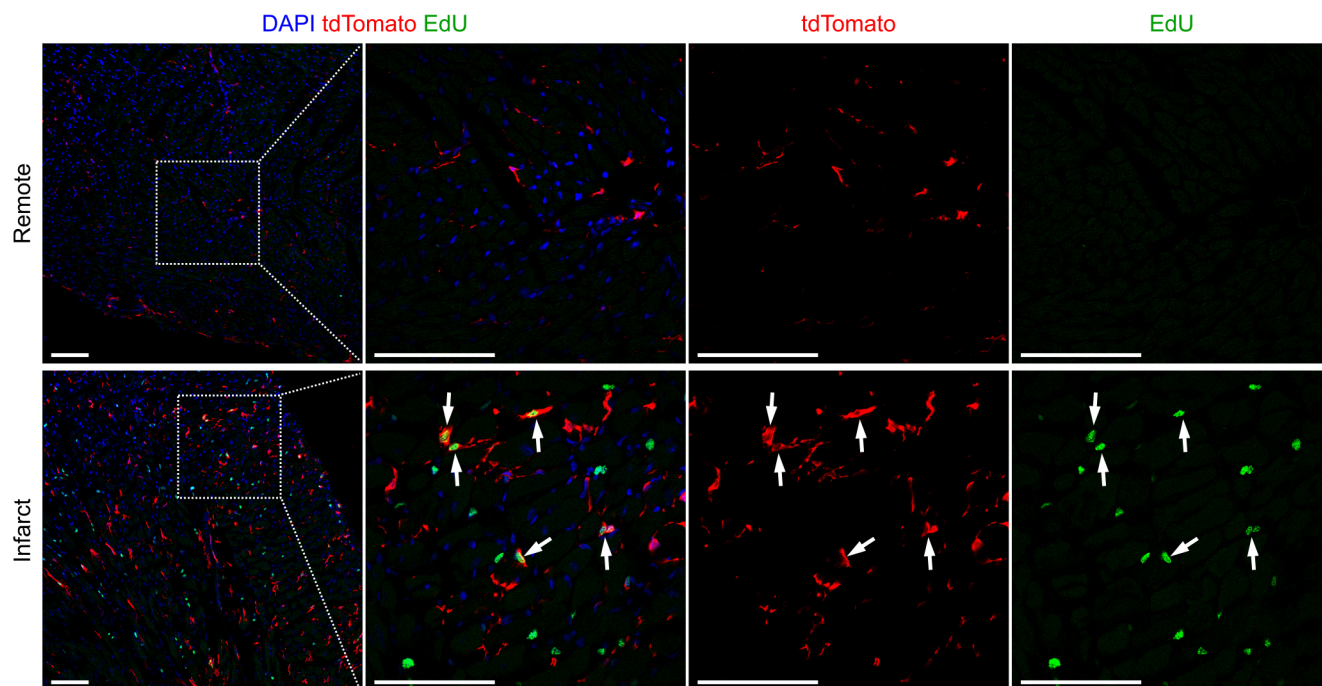


Fig. S9 Proliferation of PW1-derived cells after MI. Immunostaining for tdTomato and EdU in heart sections from *Pw1^{pCreER/m+};R26-tdT* mice at 3 days post-MI. Five hours prior to sacrifice, mice received an intraperitoneal injection of EdU at a dose of 0.02 mg per gram of body weight. Arrows indicate EdU⁺tdTomato⁺ cells within the infarct region. Scale bars, 100 μ m.

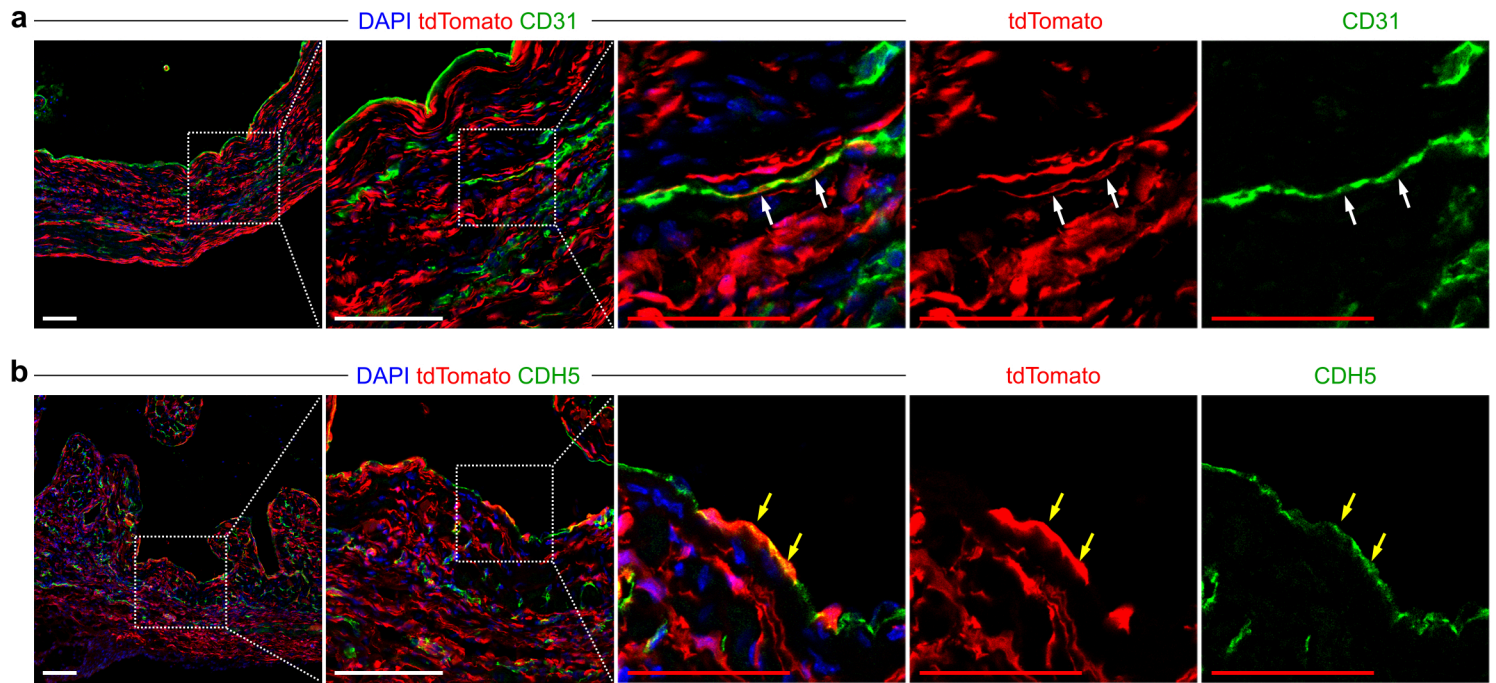


Fig. S10 tdTomato labels a subset of cVECs and endocardial ECs in infarct regions. **a** Immunostaining for tdTomato and CD31 in heart sections from *Pw1^{pCreER/m+};R26-tdT* mice at 14 days post-MI. White arrows indicate tdTomato-labeled cVECs. **b** Immunostaining for tdTomato and CDH5 in heart sections from *Pw1^{pCreER/m+};R26-tdT* mice at 14 days post-MI. Yellow arrows indicate tdTomato-labeled endocardial ECs. White scale bars, 100 μ m; red scale bars, 50 μ m.

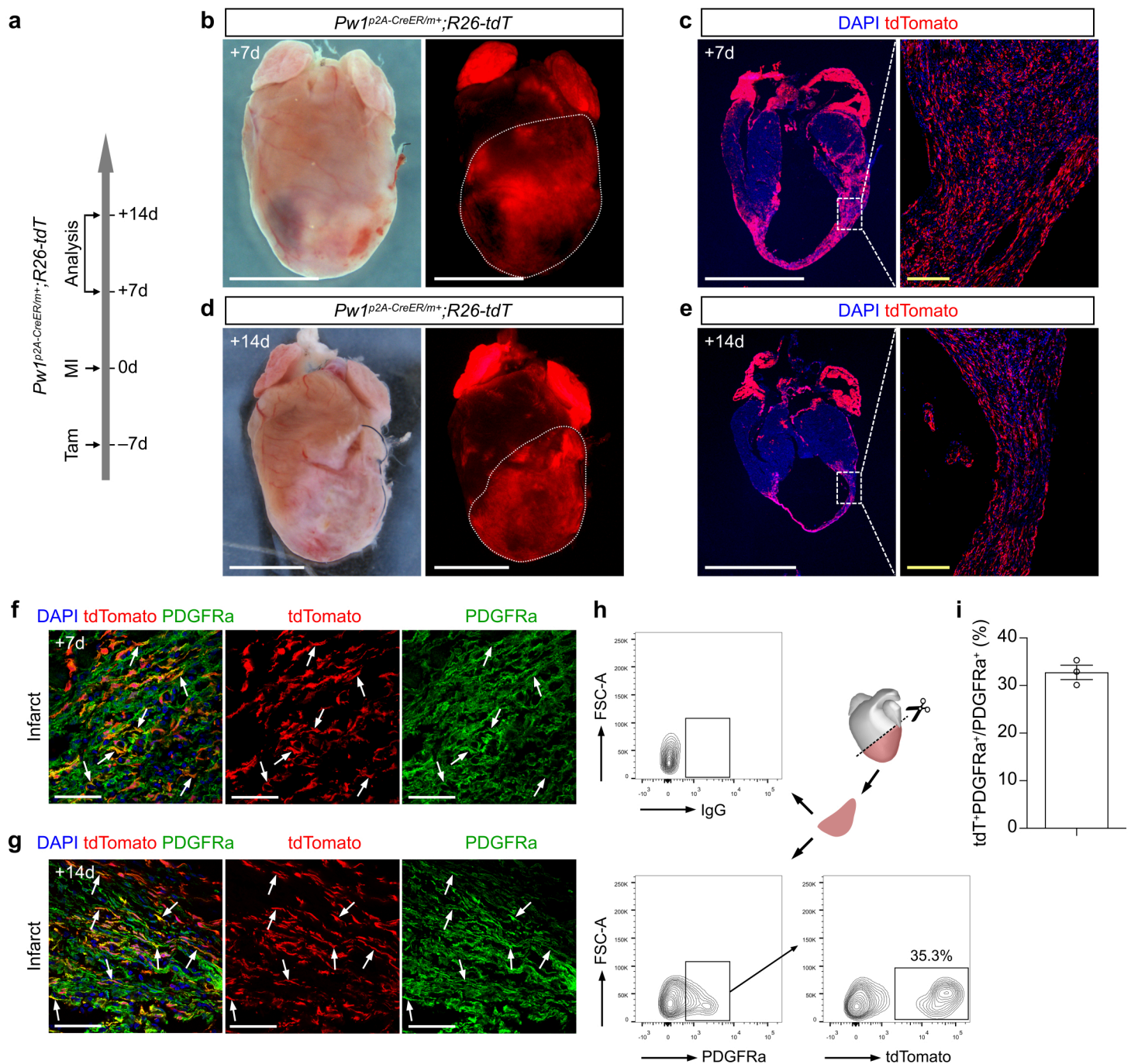


Fig. S11 Lineage tracing of PW1⁺ cells in *Pw1^{p2A-CreER/m+};R26-tdT* mice after MI. **a** Schematic of the experimental strategy. Tamoxifen was administered 7 days before MI; hearts were analysed at 7 and 14 days post-MI. **b, d** Whole-mount images of hearts at 7 and 14 days post-MI. Dashed lines outline infarcted areas. Scale bars, 5 mm. **c, e** Immunostaining for tdTomato in heart sections at 7 and 14 days post-MI. White scale bars, 5 mm; yellow scale bars, 200 μ m. **f, g** Co-staining for tdTomato and PDGFRa in heart sections at 7 and 14 days post-MI. Arrows indicate tdTomato⁺PDGFRa⁺ cells in infarcted regions. Scale bars, 50 μ m. **h** Flow cytometry analysis of the percentage of PDGFRa⁺ myofibroblasts derived from PW1⁺ cells in infarcted regions at 14 days post-MI. **i** Quantification of PW1⁺ cell-derived myofibroblasts in infarcted regions. $n = 3$ biological samples; both sexes included.

Table S1. Cluster-defining markers

Cell populations	Clusters	Cell numbers	Cluster markers					
Fibroblasts			<i>Pdgfra</i>		<i>Col1a1</i>		<i>Col1a2</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	0	904	0.82432389	1.26E-100	0.919692901	7.83E-133	0.986418606	2.90E-143
	1	708	0.78079839	7.27E-83	0.983277827	4.99E-104	1.067419152	1.59E-114
	3	625	1.953409958	8.93E-189	1.235817503	2.92E-130	1.077709103	1.21E-110
	7	369	1.308144117	1.04E-70	0.85490695	2.70E-52	0.835120402	1.62E-48
	8	247	0.291416369	1.01E-11	1.448936635	1.93E-73	1.381915464	3.65E-67
	11	156	/	/	1.53822911	2.42E-37	1.370655223	4.49E-35
	14	102	1.07452285	4.95E-17	0.644129759	1.11E-09	0.534286914	4.24E-06
	21	28	0.564904101	1	0.335270104	1	0.548945667	1
cVECs			<i>Cdh5</i>		<i>Pecam1</i>		<i>Fabp4</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	2	661	2.303100444	0	2.123079977	0	2.727097077	5.75E-300
	4	604	3.144583316	0	2.938567302	0	2.783289746	1.59E-288
	13	127	1.407351673	8.41E-06	1.689185218	0.918098102	1.648702734	2.56E-30
Macrophages			<i>Fcgr1</i>		<i>Cd68</i>		<i>Itgam</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	5	424	3.151187865	6.76E-266	3.087526356	0	2.582025748	6.25E-159
	6	388	3.337107573	0	2.80608728	2.77E-302	3.613108495	0
	10	161	2.659138592	3.20E-147	2.952488074	4.08E-146	2.892961523	8.93E-160
	18	51	1.38427596	1.01E-26	1.483456698	1.27E-28	1.481052291	5.83E-16
	20	34	1.42974033	1.18E-11	2.473644397	7.24E-22	2.336998817	1.57E-18
SMCs			<i>Acta2</i>		<i>Tagln</i>		<i>Myh11</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	9	196	8.17205302	0	7.896058575	3.79E-299	8.848386891	0
Pericytes			<i>Pdgfrb</i>		<i>Cspg4</i>		<i>Notch3</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	12	150	4.153050178	2.19E-112	4.673169781	5.76E-204	4.744119736	1.78E-266
Lymphatic ECs			<i>Prox1</i>		<i>Lyve1</i>		<i>Flt4</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	15	66	6.437139752	1.90E-197	4.072697374	7.39E-65	6.387920993	1.14E-201
Schwan cells			<i>Plp1</i>		<i>Kcna1</i>		<i>Cnp</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	16	66	9.678070709	0	9.905756876	0	5.291690096	2.07E-73
Endocardial ECs			<i>Npr3</i>		<i>Plvap</i>		<i>Cdh11</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	17	53	8.624119587	0	6.450212016	1.40E-80	5.510766051	2.54E-83
B cells			<i>Cd79a</i>		<i>Ms4a1</i>		<i>Cd79b</i>	
			avg_log2FC	p_val_adj	avg_log2FC	p_val_adj	avg_log2FC	p_val_adj
	19	34	10.98806511	0	12.97129962	0	9.639404829	6.55E-255

Note: "/" indicates that the "findallmarker function" did not detect any differential expression of this gene between the current cluster and other clusters.