

Spatiotemporal analysis reveals significant overlap of key proepicardial markers in the developing murine heart

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Supplementary Information

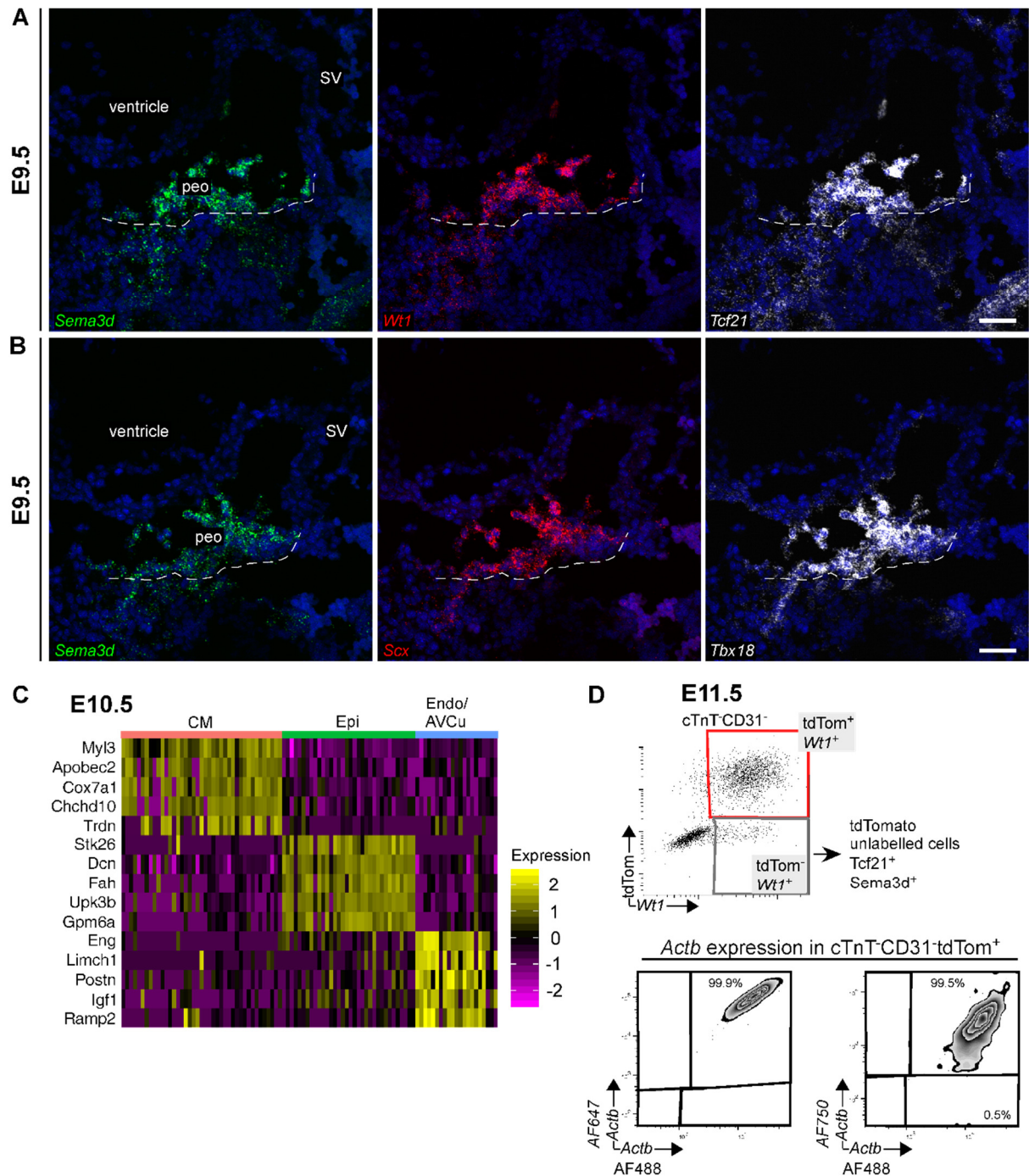


Fig. S1. Gene expression of epicardial markers in the septum transversum region and confirmation of E10.5 scRNA-seq cluster identity.

(A-B) RNA ISH staining of E9.5 embryo cryosections shows overlapping expression of *Sema3d*, (A) *Wt1*, *Tcf21*, (B) *Scx*, and *Tbx18* mRNA only in the proepicardial organ (dashed line) (n=5 embryos). (C) Heatmap of top 5 differentially expressed genes for each cluster in the E10.5 scRNA-Seq. High expression is indicated in yellow. (D) Flow cytometric analysis of *Wt1* and tdTomato at E11.5. Multiplex analysis of *Actb* expression in tdTomato⁺ cells. peo, proepicardial organ; SV, sinus venosus. Scale bars: 50µm in A-B.

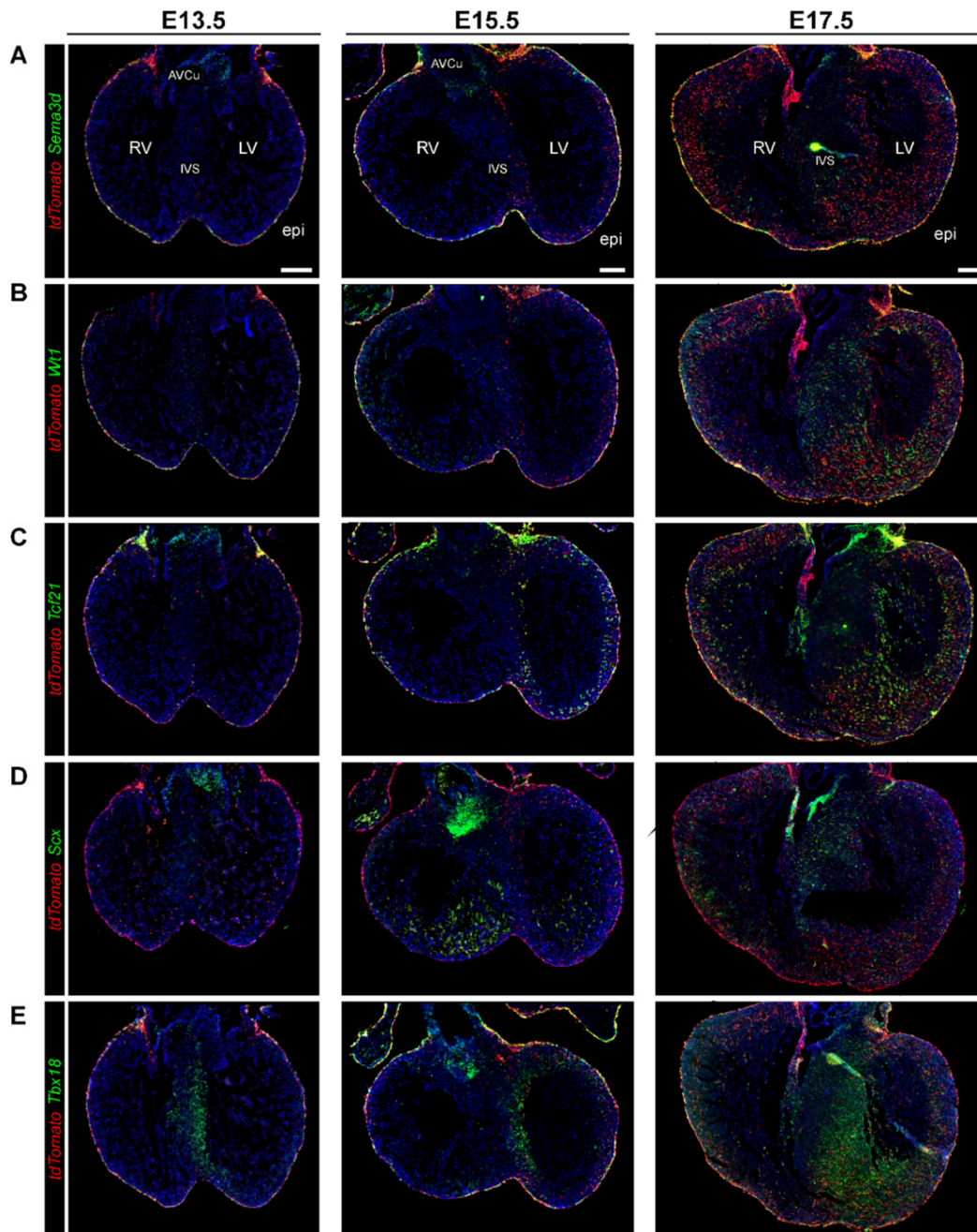


Fig. S2. Non-specificity of the epicardial markers *Sema3d*, *Wt1*, *Tcf21*, *Scx*, and *Tbx18* at later stages of development.

(A) RNA ISH co-staining of *Sema3d* (green) and *tdTomato* (red) at E13.5, E15.5, E17.5 shows overlap in the epicardium. *Sema3d* is also expressed in the atrioventricular cushion (AVCu) and in lymphatics. (B) RNA ISH co-staining of *Wt1* (green) and *tdTomato* (red) at E13.5, E15.5, E17.5 shows overlap in the epicardium. *Wt1* is also expressed in coronary endothelial cells starting E13.5. (C) RNA ISH co-staining of *Tcf21* (green) and *tdTomato* (red) at E13.5, E15.5, E17.5 shows overlap in the epicardium and in EPDCs. *Tcf21* is also expressed in AVCu and in interstitial fibroblasts. (D) RNA ISH co-staining of *Scx* (green) and *tdTomato* (red) at E13.5, E15.5, and E17.5 shows very low expression of *Scx* in the epicardium. *Scx* is highly expressed in the atrioventricular cushion (AVCu). (E) RNA ISH co-staining of *Tbx18* (green) and *tdTomato* (red) at E13.5, E15.5, E17.5 shows overlap in the epicardium. *Tbx18* is also expressed in cardiomyocytes in the septum and left ventricle and in vascular smooth muscle cells in the aorta. AVCu, atrioventricular cushion; RV, right ventricle; IVS, intraventricular septum; LV, left ventricle; epi, epicardium. Scale bars: 200 μ m in A.

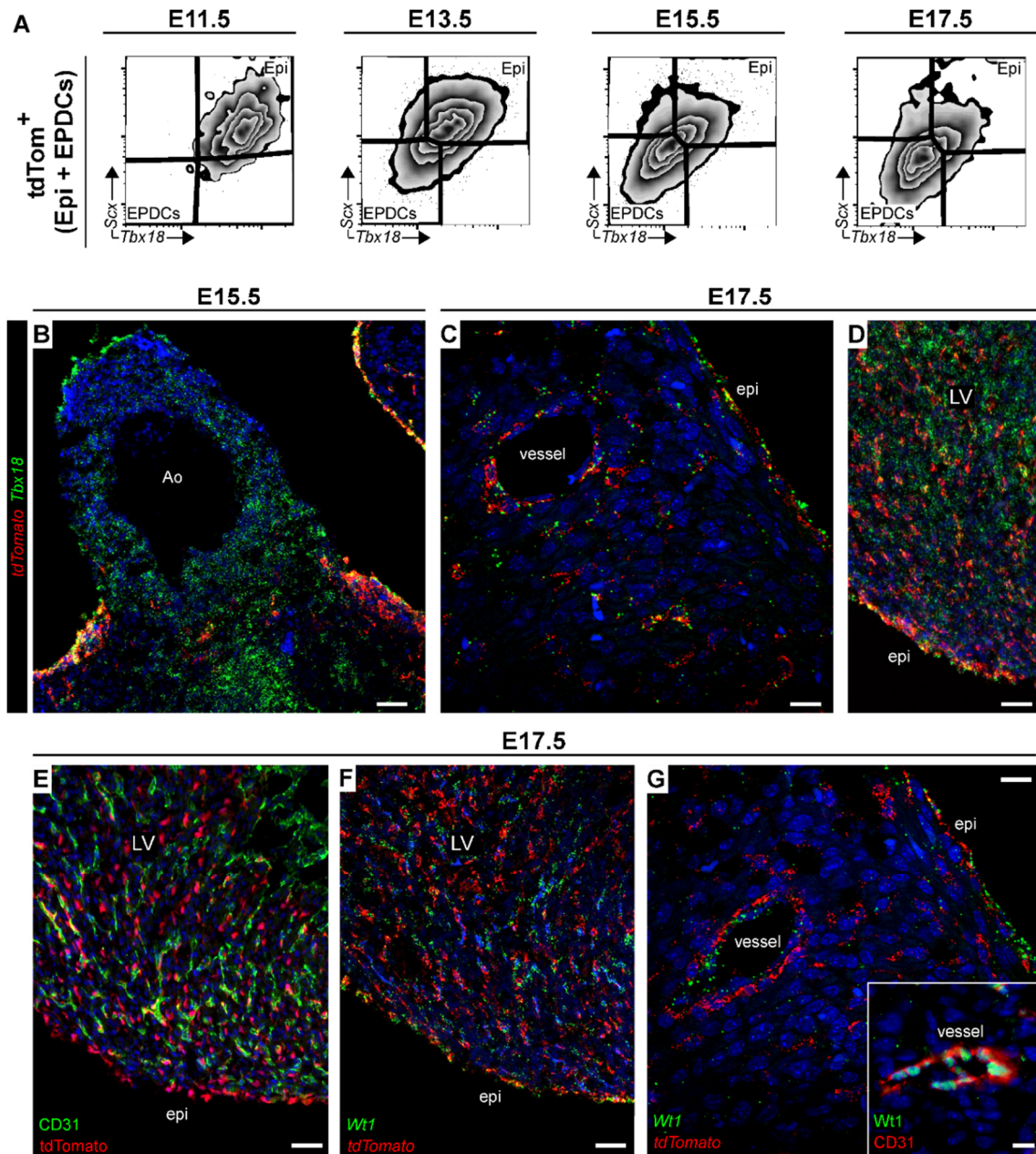


Fig. S3. *Scx* and *Tbx18* expression decreases as epicardial cells transition to EPDCs and *Tbx18* and *Wt1* are expressed *de novo* in the coronary vasculature.

(A) Flow cytometric analysis of *Scx* and *Tbx18* expression in tdTomato⁺ cells, representing the epicardial lineage, shows downregulation of these markers in EPDCs over development. (B-C) RNA ISH co-staining of *Tbx18* (green) and *tdTomato* (red) reveals *Tbx18* expression in vascular smooth muscle cells derived from both non-epicardial and epicardial sources. (D) *Tbx18* is expressed in cardiomyocytes. (E) Co-immunostaining of CD31 and tdTomato shows no contribution of the epicardial lineage to coronary endothelium. (F-G) RNA-ISH co-staining of *Wt1* (green) and *tdTomato* (red) reveals *Wt1* expression in non-epicardial derived coronary endothelium cells. Co-immunostaining of CD31 and WT1 shows overlap in vessels. Epi, epicardium; EPDCs, epicardial-derived cells; Ao, aorta; LV, left ventricle. Scale bars: 50µm in B-G; 10µm in inset G.

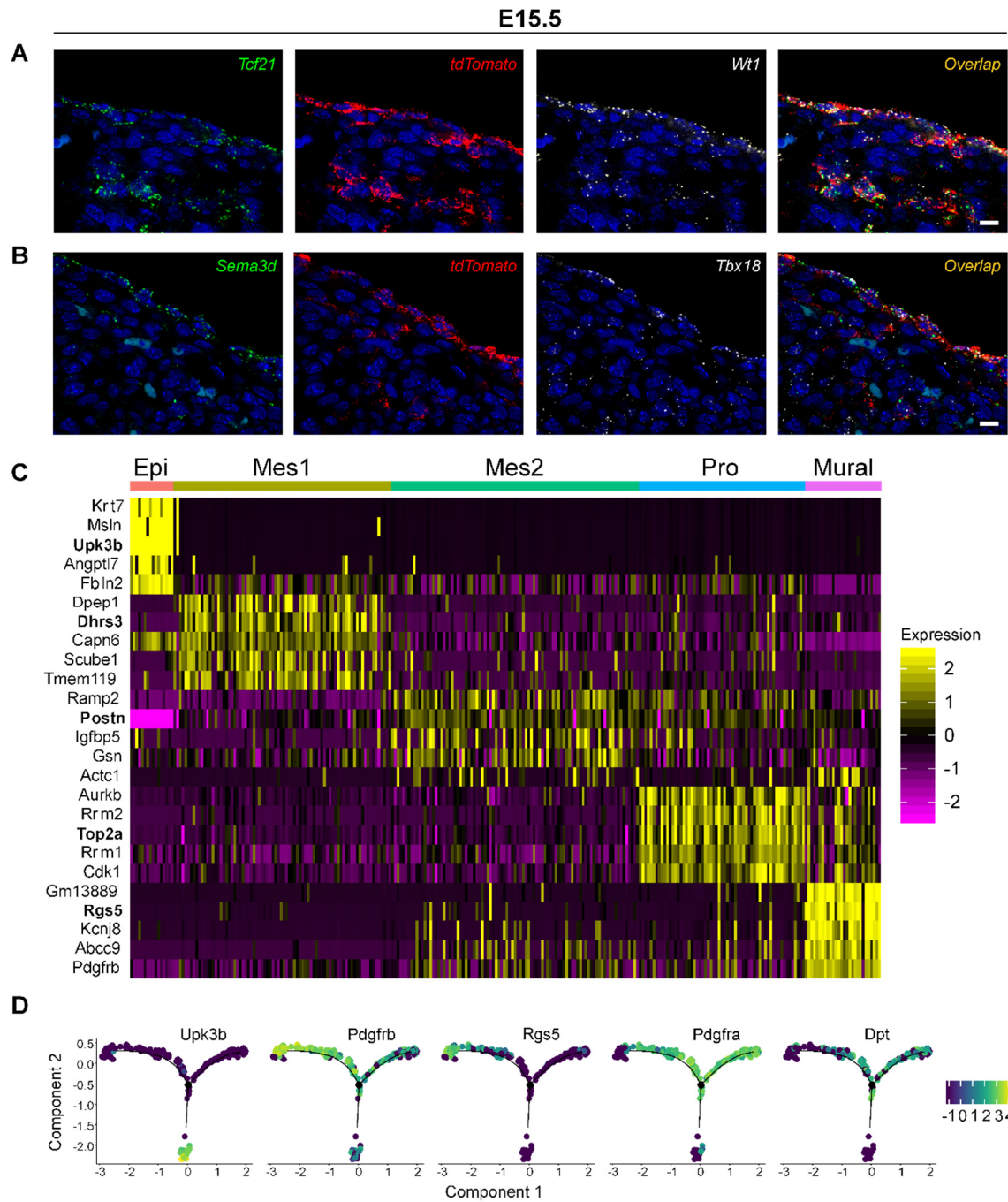


Fig. S4. Expression of the selected epicardial markers at E15.5 and pseudotime representation of cell fate-associated genes.

(A) RNA ISH co-staining of *Tcf21* (green), *tdTomato* (red) and *Wt1* (white) reveals overlap in the epicardial lineage, albeit *Tcf21* expression is low in the epicardium. (B) RNA ISH co-staining of *Sema3d* (green), *tdTomato* (red) and *Tbx18* (white) reveals overlap in the epicardium and reduced expression in EPDCs. (C) Heatmap of top 5 differentially expressed genes per cluster in the E15.5 scRNA-Seq. High expression is indicated in yellow. (D) Visualisation of cluster marker genes in pseudotime reveals one epicardial origin (*Upk3b*⁺) and two potential cell fates, one associated with mural genes (*Pdgfrb* and *Rgs5*) and one associated with mesenchymal/fibroblast fate (*Pdgfra* and *Dpt*).

Table. S1: % Positive expressed (SED) in tdTom+ epicardial population at E11.5

	<i>Sema3d</i>	<i>Wt1</i>	<i>Tcf21</i>	<i>Tbx18</i>	<i>Scx</i>
Number of values	3	3	3	3	3
Minimum	97.30	96.00	94.20	94.90	81.60
25% Percentile	97.30	96.00	94.20	94.90	81.60
Median	97.70	98.20	94.90	98.60	90.10
75% Percentile	98.90	98.20	97.40	99.50	94.40
Maximum	98.90	98.20	97.40	99.50	94.40
Mean	97.97	97.47	95.50	97.67	88.70
Std. Deviation	0.8327	1.270	1.682	2.438	6.514
Std. Error	0.4807	0.7333	0.9713	1.408	3.761

Table. S2: % Positive expressed (SED) *Sema3d* in *Wt1+Tcf21+* epicardial population

	E11.5	E13.5	E15.5	E17.5
Number of values	3	3	2	2
Minimum	98.20	99.50	99.20	96.20
25% Percentile	98.20	99.50	99.20	96.20
Median	98.90	99.70	99.30	96.45
75% Percentile	99.40	99.80	99.40	96.70
Maximum	99.40	99.80	99.40	96.70
Mean	98.83	99.67	99.30	96.45
Std. Deviation	0.6028	0.1528	0.1414	0.3536
Std. Error	0.3480	0.08819	0.1000	0.2500

Table. S3: % Positive expressed (SED) *Scx* in *Tbx18+Sema3d+* epicardial population

	E11.5	E13.5	E15.5	E17.5
Number of values	3	3	2	2
Minimum	82.80	88.70	97.10	93.20
25% Percentile	82.80	88.70	97.10	93.20
Median	93.60	98.80	97.30	94.75
75% Percentile	94.00	99.00	97.50	96.30
Maximum	94.00	99.00	97.50	96.30
Mean	90.13	95.50	97.30	94.75
Std. Deviation	6.354	5.890	0.2828	2.192
Std. Error	3.668	3.400	0.2000	1.550

**SED, Super-Enhanced Dmax Subtraction algorithm calculates percent positives when comparing histograms.