SUPPLEMENTAL FIGURE LEGENDS

Figure S1

A

Injection/Harvest	Survival Rate of *Lats1/2* CKO
E11.5/E14.5	11/11
E11.5/E15.5	7/11
E11.5/E16.5	1/10 * +

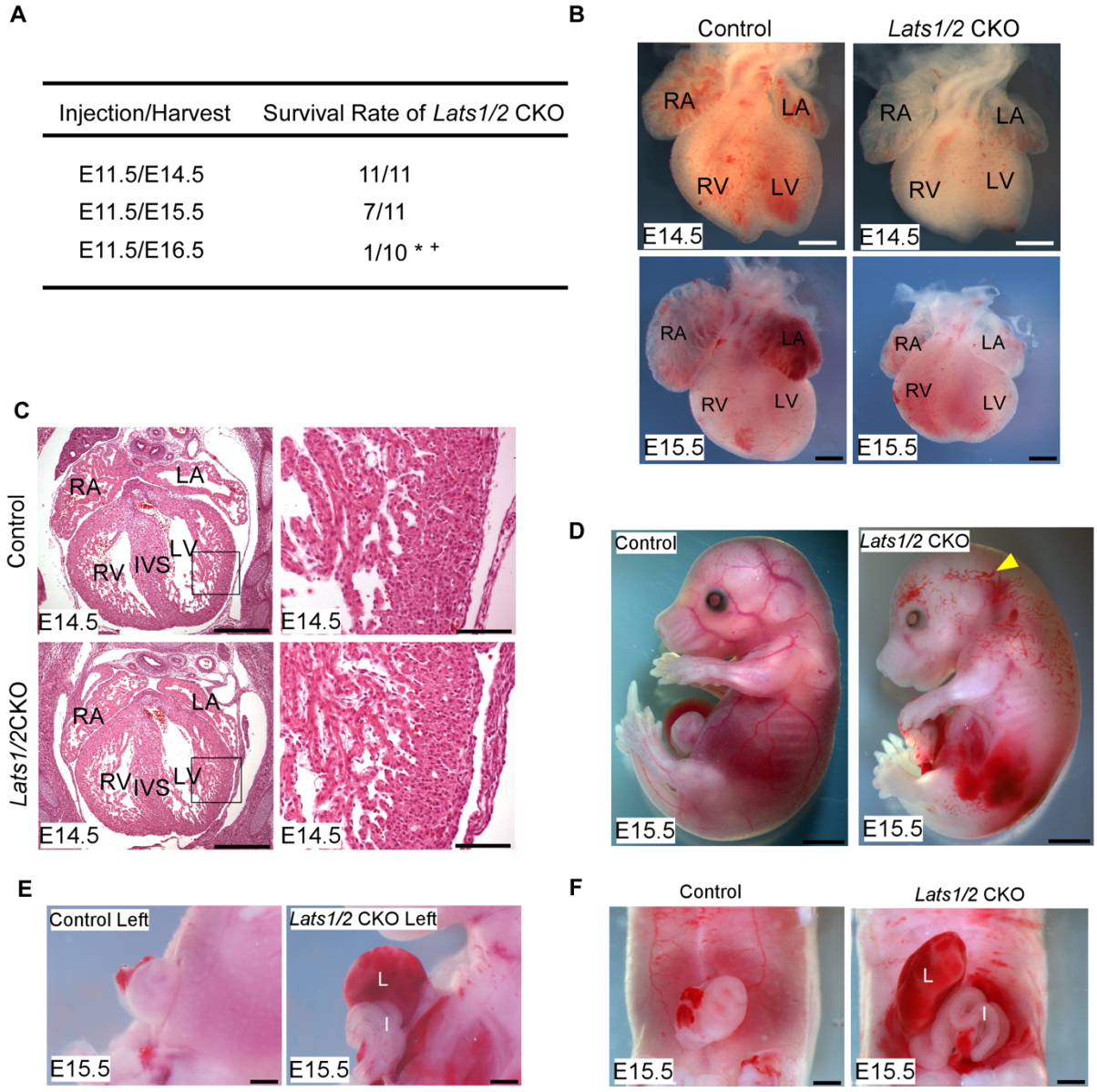
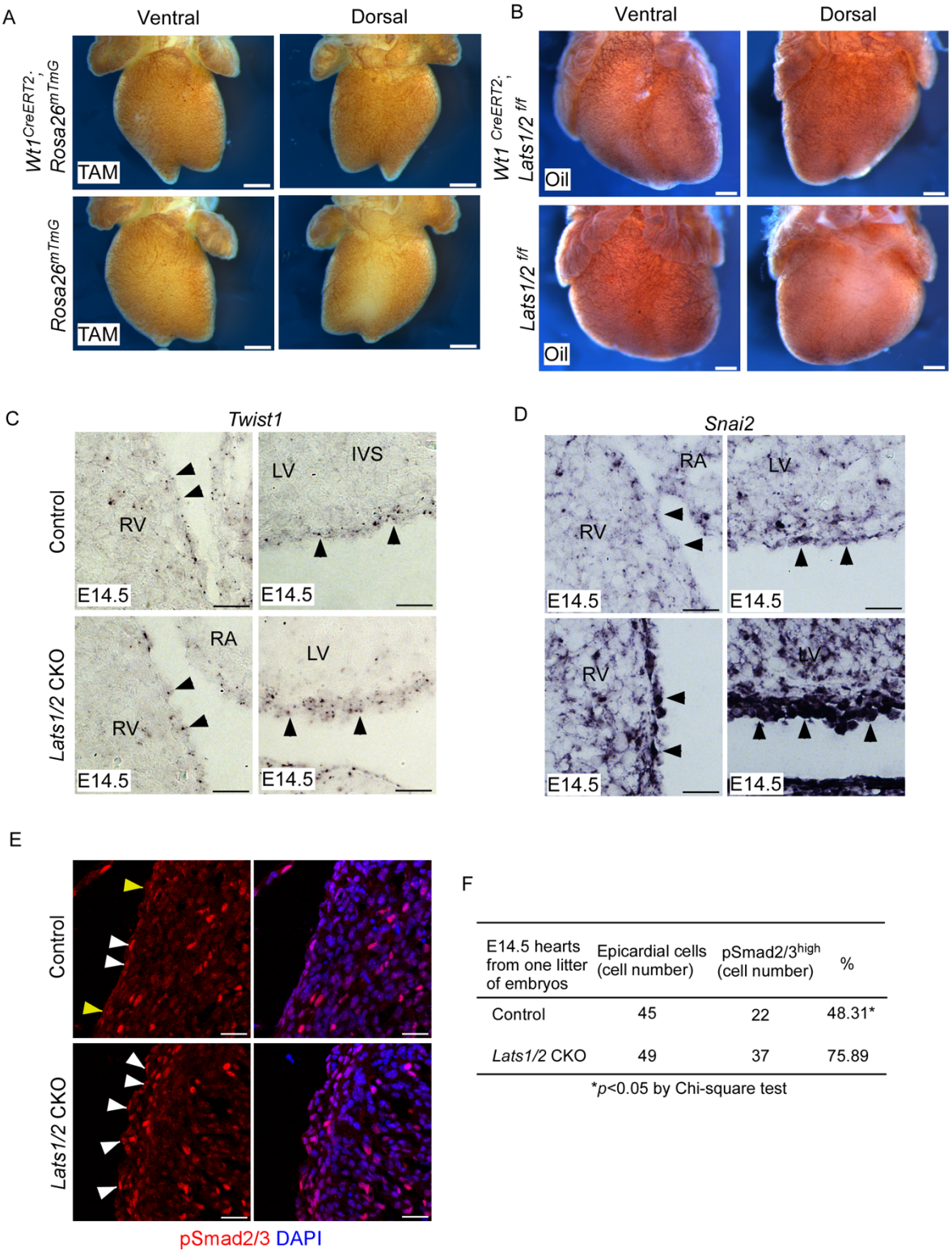


Figure S1. Related to Figure 1. Knocking out *Lats1/2* leads to embryonic lethality at E15.5.

(A) Cre activity was induced at E11.5 by tamoxifen injection. *Lats1/2* CKO survival rate was at different harvest time series. **P* <0.001 between E16.5 and E14.5, Fisher's exact

test. ^{+}P <0.05 between E16.5 and E15.5, Fisher's exact test. (B-C) Gross heart morphology and H&E stained sections from at E14.5 and E15.5. (D-F) Non-cardiac defects in *Lats1/2* CKO at E15.5, including hemorrhage (yellow arrowheads) and herniated liver (L) and intestine (I). Scale bar: B 400μm; C left panel 500 μm; right panel 100 μm; D 2000μm; E-F 1000μm.

Figure S2



F

E14.5 hearts from one litter of embryos	Epicardial cells (cell number)	pSmad2/3^{high} (cell number)	%
Control	45	22	48.31*
Lats1/2 CKO	49	37	75.89

*p<0.05 by Chi-square test

Figure S2. Related to Figure 1. Control experiments to validate coronary vessel development defects in *Lats1/2* CKO and EMT factors expression in epicardium and EPDC.

(A)*Wt1* $^{CreERT2/+}$; *Rosa26* ^{mTmG} injected with Tamoxifen (TAM) exhibited well-formed coronary vasculature compared with *Rosa26* ^{mTmG} littermate at E14.5. (B) *Wt1* $^{CreERT2/+}$; *Lats1/2* $^{f/f}$ hearts exhibited organized coronary vessel injected with vehicle control peanut oil compared with *Lats1/2* $^{f/f}$ littermate at E15.0. (C-D) EMT factors *Twist1* and *Snai2* detected by *in situ* probe at E14.5. (E) pSmad2/3 activity in epicardium. White arrowheads point to pSmad2/3 high-expressing epicardial cells (pSmad2/3^{high}) and yellow arrowheads point to pSmad2/3 low-expressing epicardial cells. *Lats1/2* CKO hearts exhibited more pSmad2/3 ^{high} epicardial cells. (F) Quantification of pSmad2/3 ^{high} epicardial cells. Scale bar: A-B, 400 μm, C-E, 25 μm.

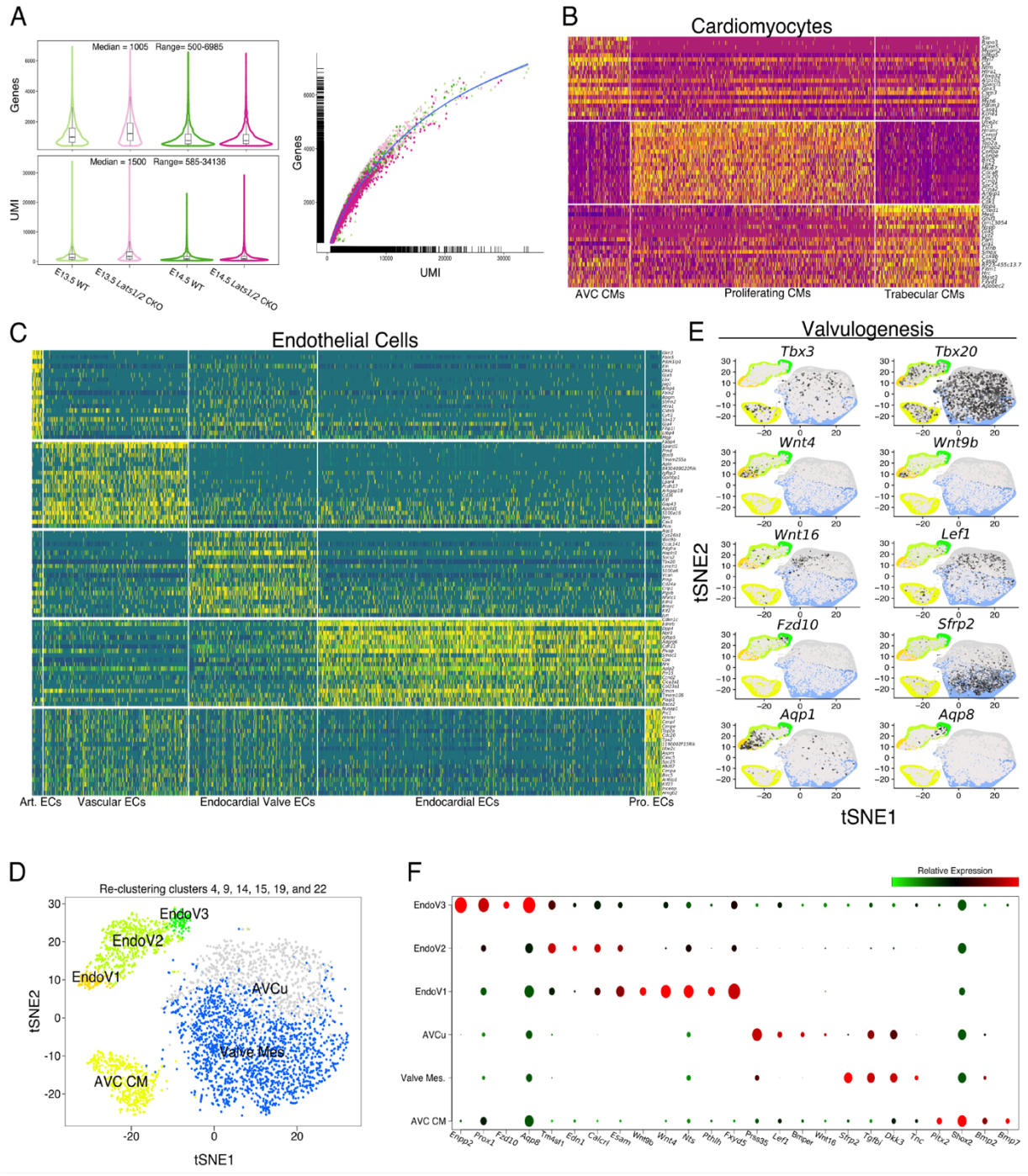
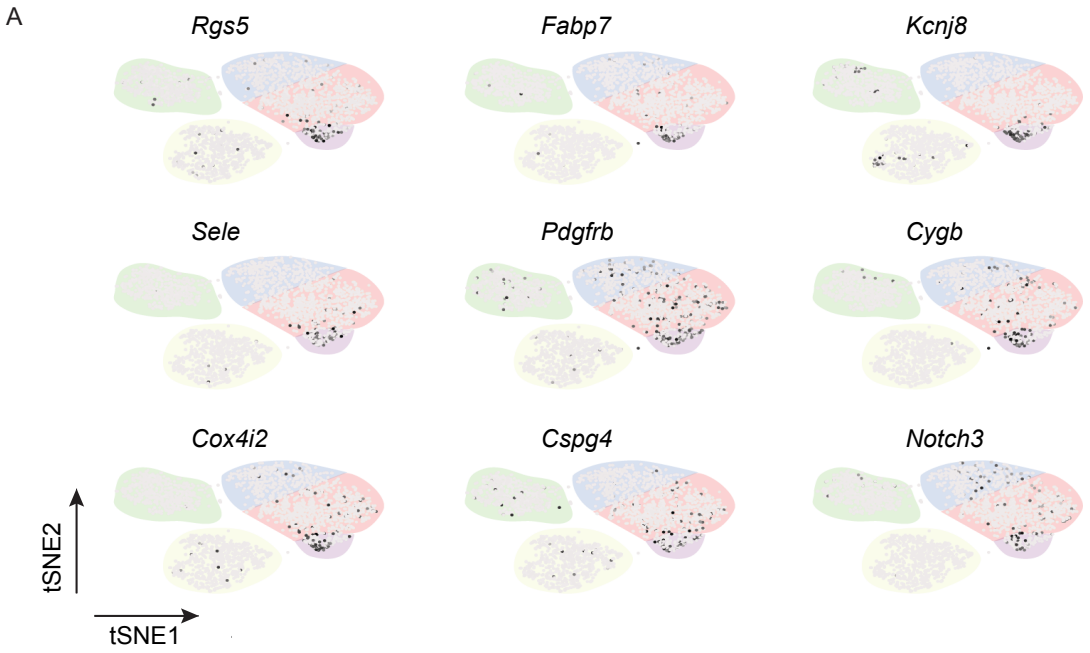
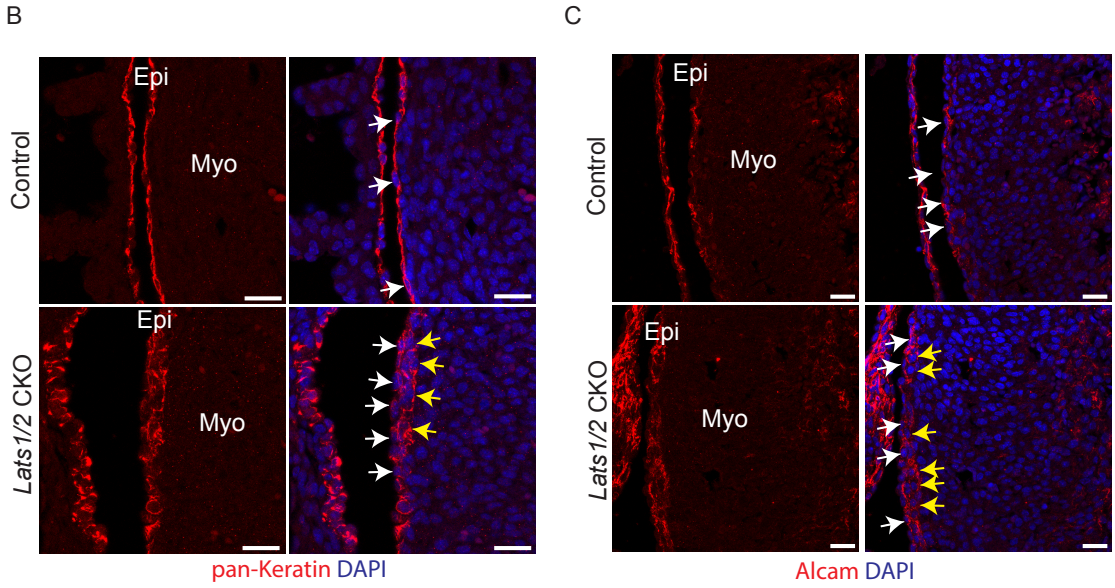

A
Median = 1005 Range= 500-6985
Genes
Median = 1500 Range= 585-34136
UMI
E13.5 WT
E13.5 Lats1/2 CKO
E14.5 WT
E14.5 Lats1/2 CKO
Genes
UMI
B
Cardiomyocytes
AVC CMs
Proliferating CMs
Trabecular CMs
C
Endothelial Cells
Art. ECs
Vascular ECs
Endocardial Valve ECs
Endocardial ECs
Pro. ECs
E
Valvulogenesis
Tbx3
Tbx20
Wnt4
Wnt9b
Wnt16
Lef1
Fzd10
Sfrp2
Aqp1
Aqp8
tSNE2
tSNE1
D
Re-clustering clusters 4, 9, 14, 15, 19, and 22
EndoV3
EndoV2
EndoV1
AVCu
Valve Mes.
AVC CM
tSNE2
tSNE1
F
Relative Expression
EndoV3
EndoV2
EndoV1
AVCu
Valve Mes.
AVC CM
Enpp2
Prox1
Fzd10
Aqp8
Tm4sf1
Edn1
Calcrl
Esam
Wnt9b
Wnt4
Nts
Pthlh
Fxyd5
Psat1
Lef1
Bmper
Wnt16
Sfrp2
Tgfbi
Dkk3
Tnc
Plxn2
Shox2
Bmp2
Bmp7

Figure S3. Related to Figure 2 and 3. Drop-seq library quality control, cardiomyocyte sub-population expression, endothelial cell markers, and valvulogenesis signatures. (A) Quality control metrics for individual Drop-seq experiments. (Left) Violin plots displaying the number of genes and Unique Molecular Identifiers (UMI) per individual cell with total statistics listed at top (combined data set). (Right) Number of genes and UMI per cell plotted against each other. Plots and points all colored according to Fig. 2A. (B) Heatmap of top differentially expressed genes among rare CM cell populations. Highly expressed genes are shown as yellow/orange. (C) Heatmap displaying the top differentially expressed genes across all endothelial-like cells. Highly expressed genes are yellow. (D) Clustering and tSNE visualization of 4,183 cells implicated in valvulogenesis. (E) Feature expression plots of the classic, as well as some novel genes expressed during valvulogenesis. Gene expression is indicated by dark black, and the background color indicates the cluster/cellular identity shown in D. (F) Single-cell markers of cardiac valve development. The relative expression levels of each gene (column) are shown as dots for each of the 6 cardiac valve clusters identified in D. The size of the dot indicates the number of cells expressing the gene per cluster, and the color denotes expression levels with bright red representing the highest level of gene expression.


A
Rgs5
Fabp7
Kcnj8
Sele
Pdgfrb
Cygb
Cox4i2
Cspg4
Notch3
tSNE2
tSNE1


B
Control
Lats1/2 CKO
Epi
Myo
pan-Keratin DAPI
C
Control
Lats1/2 CKO
Epi
Myo
Alcam DAPI

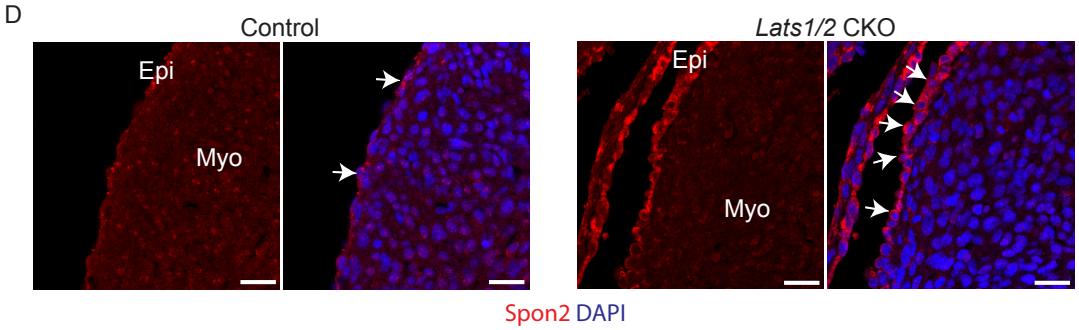

D
Control
Lats1/2 CKO
Epi
Myo
Spon2 DAPI

Figure S4. Related to Figure3 and Figure 4. Gene expression of FB2 and validation of epicardial/subepicardial cell identity. (A) Cluster FB2 feature plot of gene expression projected across the tSNE. Black indicates high gene expression, and cluster identities and boundaries are set in the background and colored according to Figure 3B. (B-C) In addition to epicardium stained with pan-Keratin and Alcam in both control and *Lats1/2* CKO hearts (white arrows), subepicardium of *Lats1/2* CKO hearts exhibited ectopic expression of pan-Keratin and Alcam (yellow arrows). (D) Increased Spon2 expression in *Lats1/2* CKO epicardium compared with wild type.

Figure S5

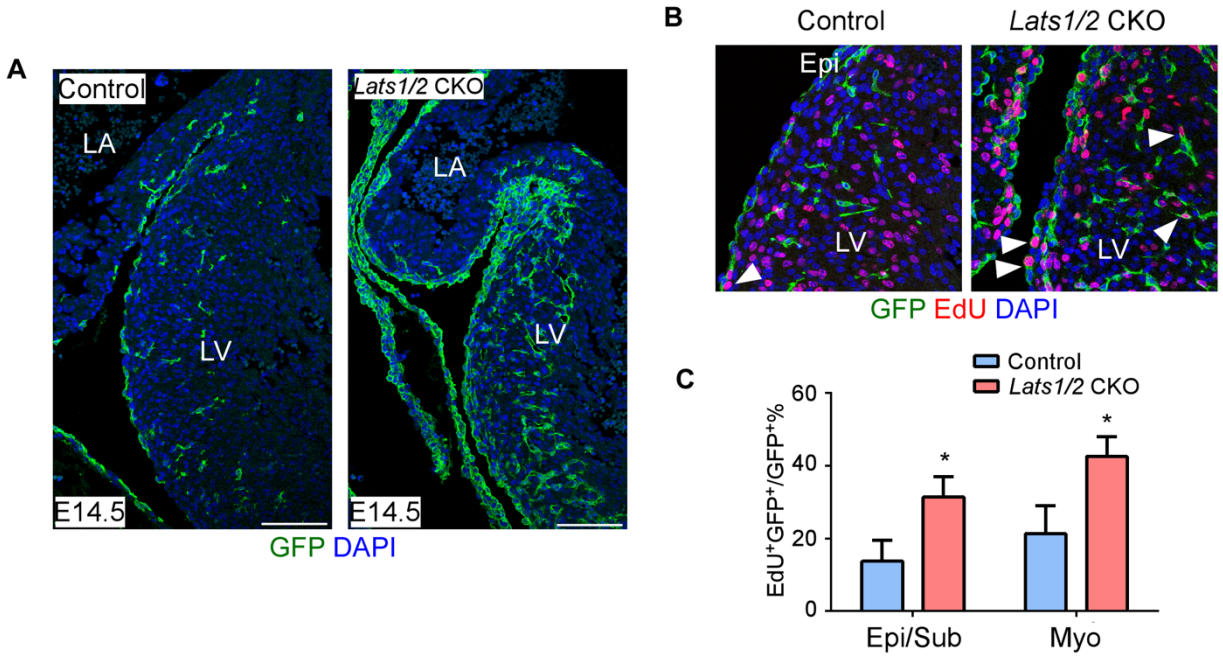


Figure S5. Related to Figure 5. Increased epicardium and EPDC proliferation in *Lats1/2 CKO* hearts

(A) The *Wt1* lineage was traced by GFP expression, which includes epicardium and EPDC. An increased number of epicardium and EPDCs were observed in *Lats1/2* CKO hearts. (B-C) *Lats1/2* CKO exhibited significantly increased EdU labelling in GFP

positive cells. Proliferating epicardial cells and EPDC were indicated as GFP^+ EdU^+ double positive cells(arrowheads). *P<0.05, Mann-Whitney U test. Data shown are means ± SD. Scale bar: 50μm.

Figure S6

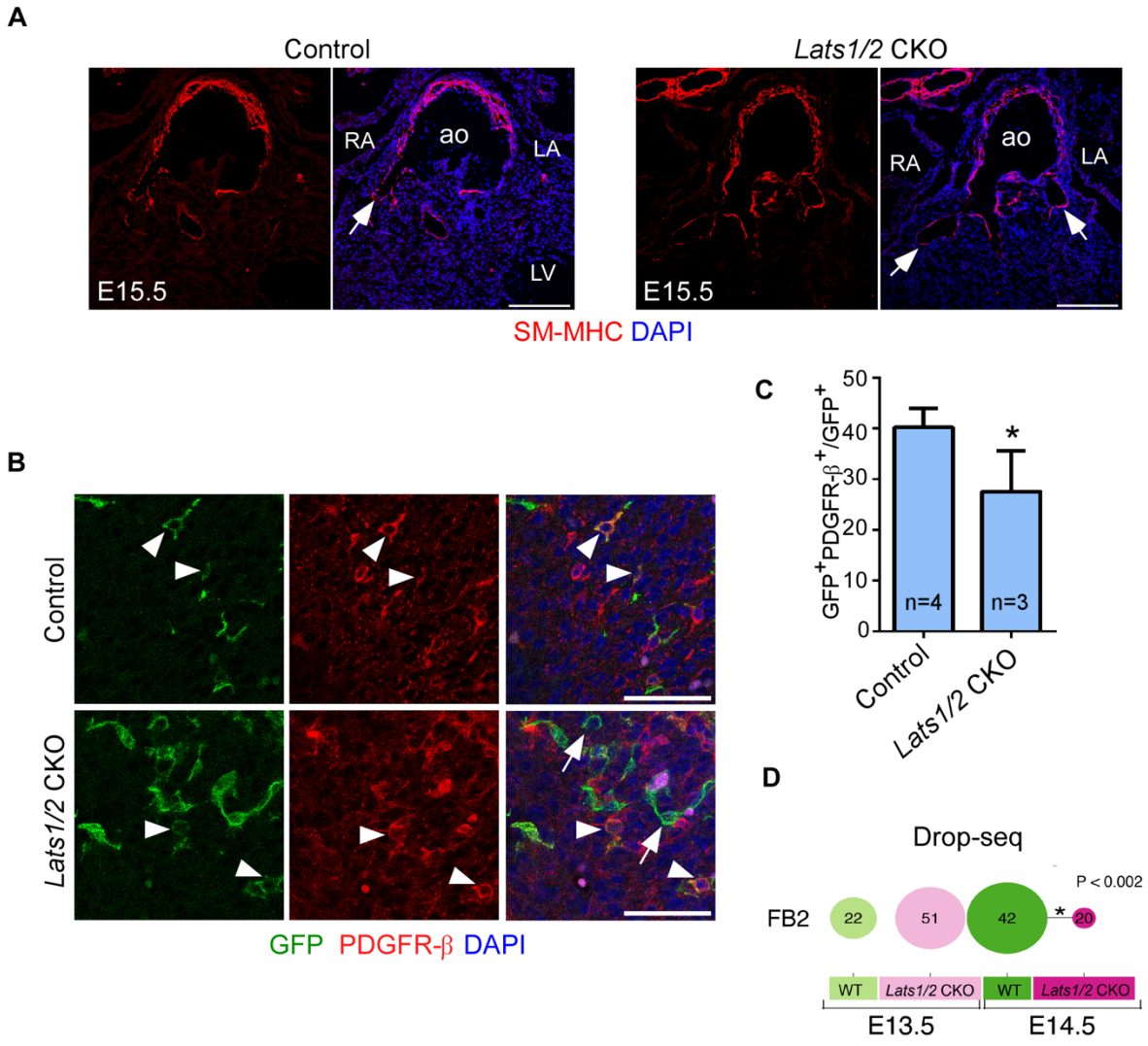


Figure S6. Related to Figure 5. Reduction of epicardial derived coronary artery smooth muscle cells in *Lats1/2* CKO hearts. (A) Mature smooth muscle cells at E15.5 were labelled with SM-MHC. Connection were established between coronary

artery and aorta both in control and *Lats1/2* CKO hearts(arrows). (B) Lineage tracing of epicardial derived coronary artery smooth muscle cell progenitors by co-labelling GFP and PDGFR-β at R14.5.(C) The high magnification views of the yellow boxed area in B. Subepicardium was highlighted with PDGFR-β(yellow arrowheads). *Lats1/2* CKO hearts exhibited accumulation of PDGFR-β^+ cells in subepicardium. (D) The high magnification views of the white boxed area in B. Reduced epicardial derived smooth muscle progenitor cells in *Lats1/2* CKO hearts. (E) Quantification of D. Scale bar, A-D 25μm. Data shown are means ± SD. **P*<0.05, Mann-Whitney U test.

Figure S7

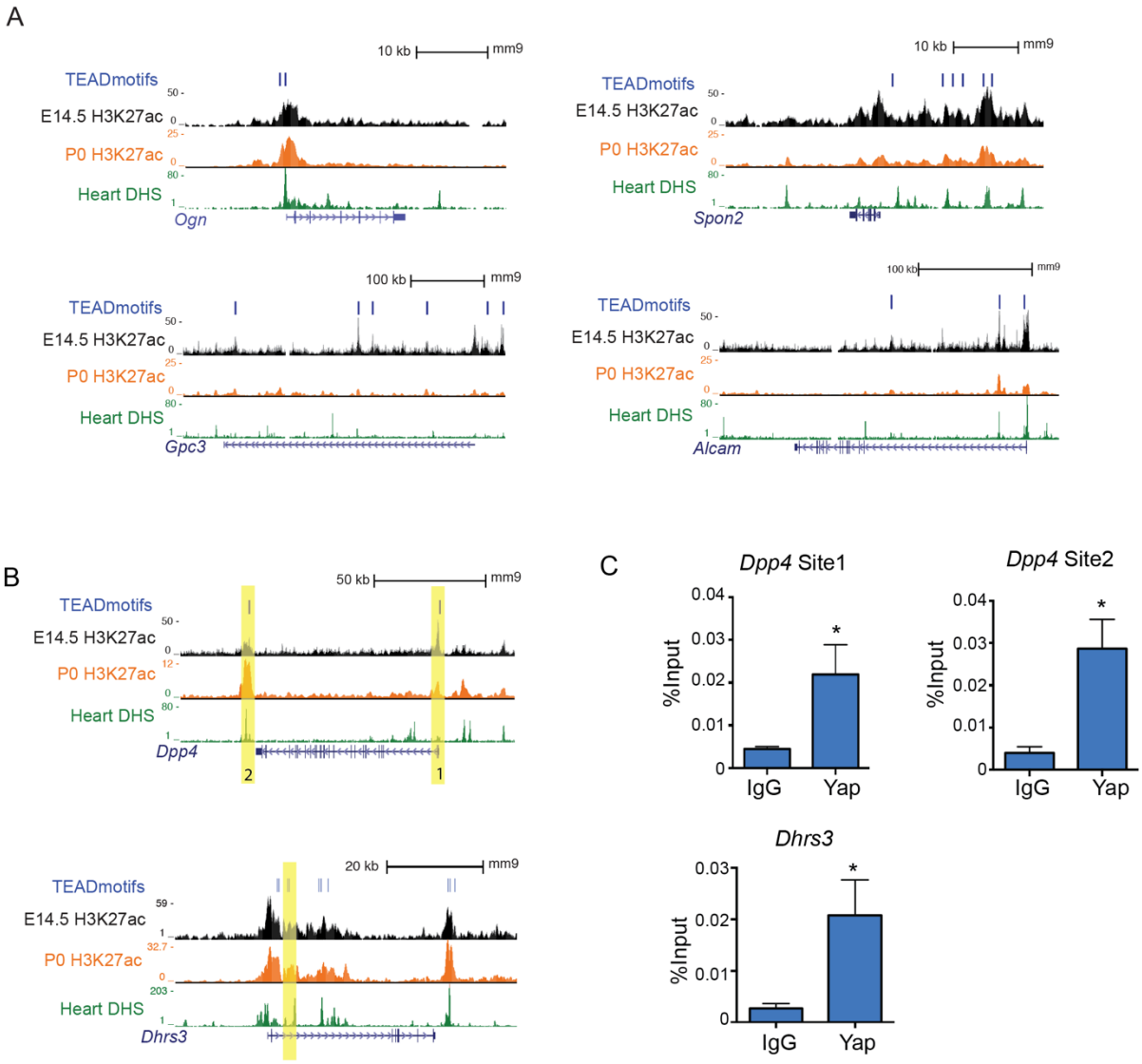

A
10 kb
mm9
TEADmotifs
E14.5 H3K27ac
P0 H3K27ac
Heart DHS
Ogn
Spon2
100 kb
Gpc3
Alcam
B
50 kb
Dpp4
20 kb
Dhrs3
C
Dpp4 Site1
Dpp4 Site2
Dhrs3
%Input
IgG
Yap
D
Wt1 CTnt DAPI

Figure S7. Related to Figure 6. Direct Yap binding to the regulatory regions of the factors regulating extracellular milieu and cell differentiation in *Lats1/2* CKO hearts and cell identity of primary epicardial cell culture.

(A) Numerous TEAD binding motifs were identified at the regulatory regions of genes encoding intercellular factors. (B-C) Yap-TEAD binding sites locates at the regulatory regions of *Dpp4* and *Dhrs3*. Yap ChIP-qPCR, quantified in the bar graphs, demonstrated Yap binding at the yellow highlighted region in the corresponding gene tracks. Data are means ± SD. **P*<0.1 was by Mann-Whitney U test. (D) Cell identity of primary epicardial cell culture at 24-hour after isolation. The culture mainly contains Wt1 positive epicardial cells with minimal $CTnT^{+}$ CMs.