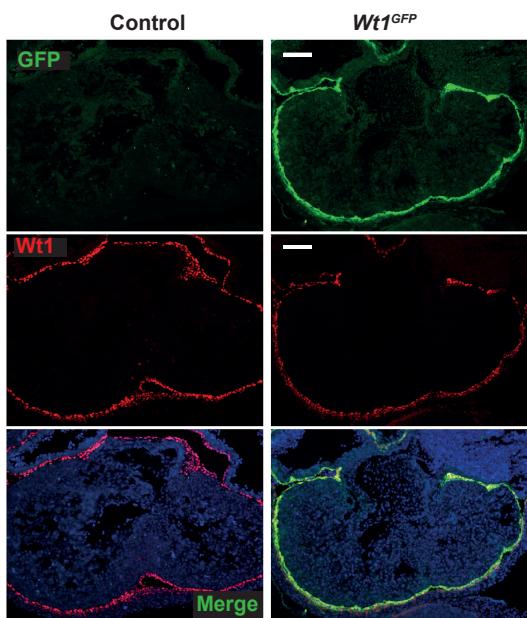


Fig. S1

A



B

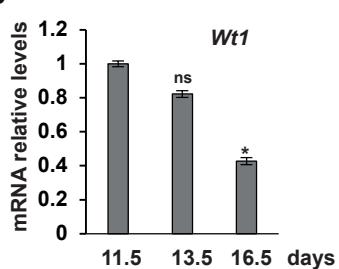
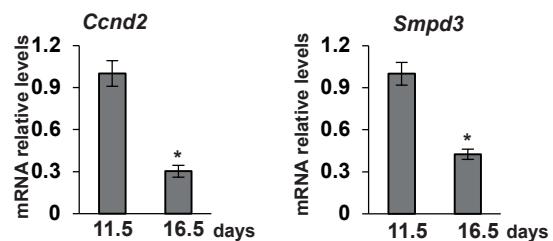


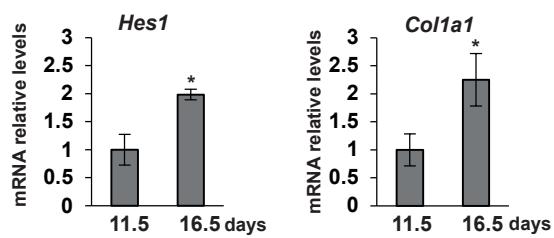
Figure S1. Immunofluorescence analyses of *Wt1* and GFP in heart sections of *Wt1*^{GFP} mice. (A) Immunofluorescent staining for GFP (green) and *Wt1* (red) and nuclear Hoechst stain (blue) using sections from E11.5 wild type littermates and *Wt1*^{GFP} embryonic hearts, where the double staining for *Wt1* and GFP can clearly be seen in the epicardium. (B) qRT-PCR analysis of *Wt1* in *Wt1*^{GFP++} FACS-sorted epicardial enriched cells from *Wt1*^{GFP} mice at different days of development. Error bars represent ± s.e.m. and p-values are based on *P< 0.05, one-way ANOVA followed by Tukey's post hoc test. RNA from FACS-isolated cells from at least 10 embryonic hearts was pooled by embryonic stage. Three replicates per condition were used for the analysis. Scale bars, 100μm.

Fig. S2

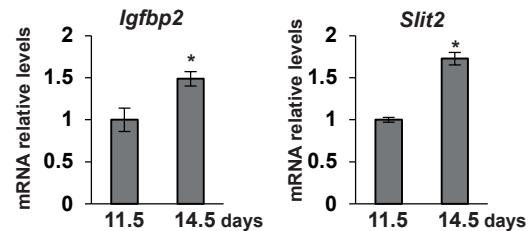
A Cluster 1



Cluster 2



Cluster 3



Cluster 4

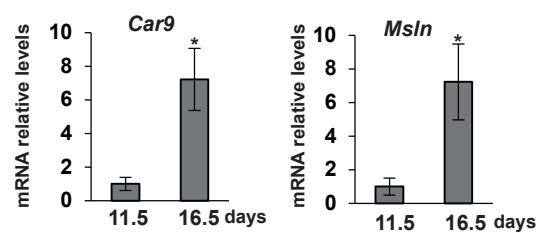


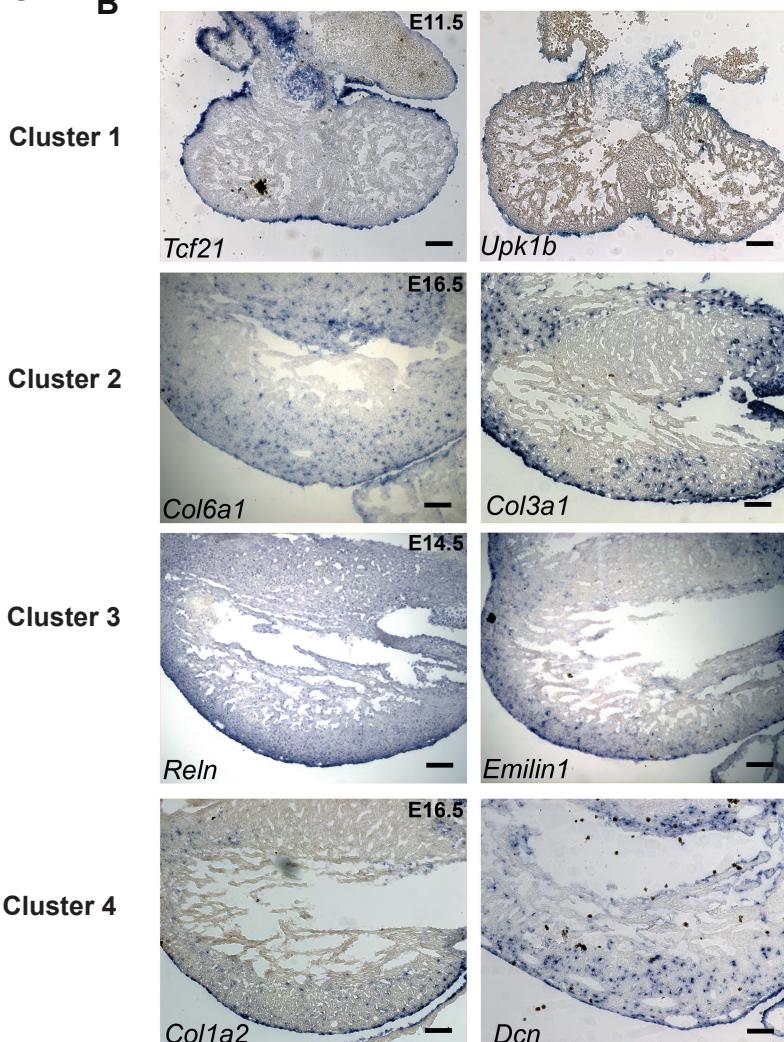
Fig. S2 B

Figure S2. Validation of genes included in different clusters. (A) qRT-PCR analysis of indicated genes in *Wt1GFP⁺⁺* FACS-sorted epicardial enriched cells from *Wt1^{GFP}* mice at different days of development. Two genes per cluster are shown in each panel. Error bars represent \pm s.e.m. and p-values are based on $*P < 0.05$, two-tailed t-tests. RNA from FACS-isolated cells from at least 10 embryonic hearts was pooled by embryonic stage. Three replicates per condition were used for the analysis. (B) *In situ* mRNA hybridisation analyses of indicated genes in embryonic heart sections. Two genes from each cluster were analysed and their expression was found to be highly enriched in the embryonic epicardium. Scale bars, 100 μ m.

Fig. S3

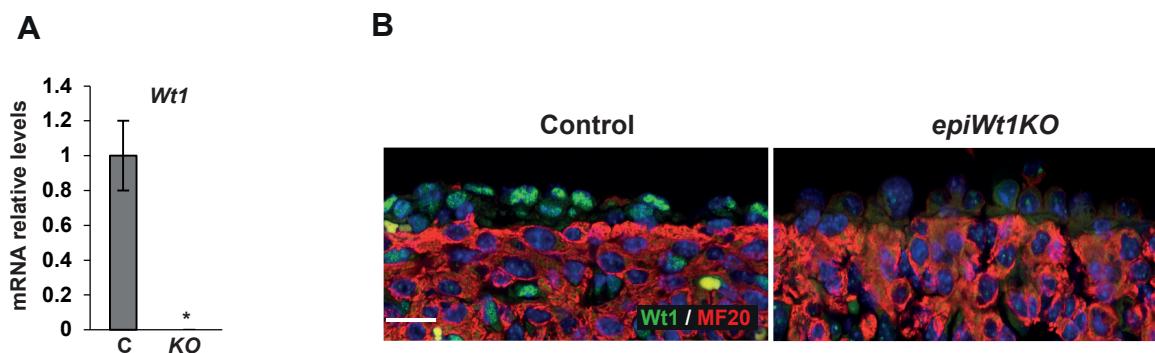


Figure S3. Efficient deletion of Wt1 in the epicardium of epiWt1KO mouse model. (A) qRT-PCR analysis of *Wt1* expression in *Wt1GFP⁺⁺* FACS-sorted epicardial cells from control and *epiWt1KO* ventricles at E13.5. Error bars represent \pm s.e.m. and p-values are based on $*P < 0.05$, two-tailed *t*-tests. RNA from FACS-isolated cells from at least 10 embryonic hearts was pooled to make one replicate. Three replicates per condition were used for the analysis. (B) Immunofluorescent staining for Wt1 (green), the cardiac myosin marker MF20 (red) and nuclear Hoechst stain (blue), in heart sections from a littermate control and *epiWt1KO* hearts at stage E15.5. Scale bar, 20 μ m.

Fig. S4**A**

<i>Gene symbol</i>	KO vs Control
<i>Figf</i>	13.13 ± 3.54*
<i>Car9</i>	9.37 ± 0.85*
<i>Thy1</i>	6.15 ± 2.02*
<i>Cdkn2b</i>	5.43 ± 1.63*
<i>Krt17</i>	3.88 ± 1.59*
<i>Smpd3</i>	3.65 ± 0.43*
<i>Egfr</i>	3.58 ± 1.08*
<i>Slc9a3r1</i>	2.72 ± 0.71*
<i>Krt14</i>	2.41 ± 0.74*
Bmp4	2.39 ± 0.11*
<i>Fah</i>	2.23 ± 0.80
<i>Col1a1</i>	2.21 ± 0.22*
<i>Cldn15</i>	2.21 ± 0.55*
<i>Krt7</i>	2.05 ± 0.29*
<i>Col1a2</i>	1.20 ± 0.30
<i>Col12a1</i>	1.19 ± 0.79
<i>Slit2</i>	0.31 ± 0.08*
<i>Nbl1</i>	0.31 ± 0.28*

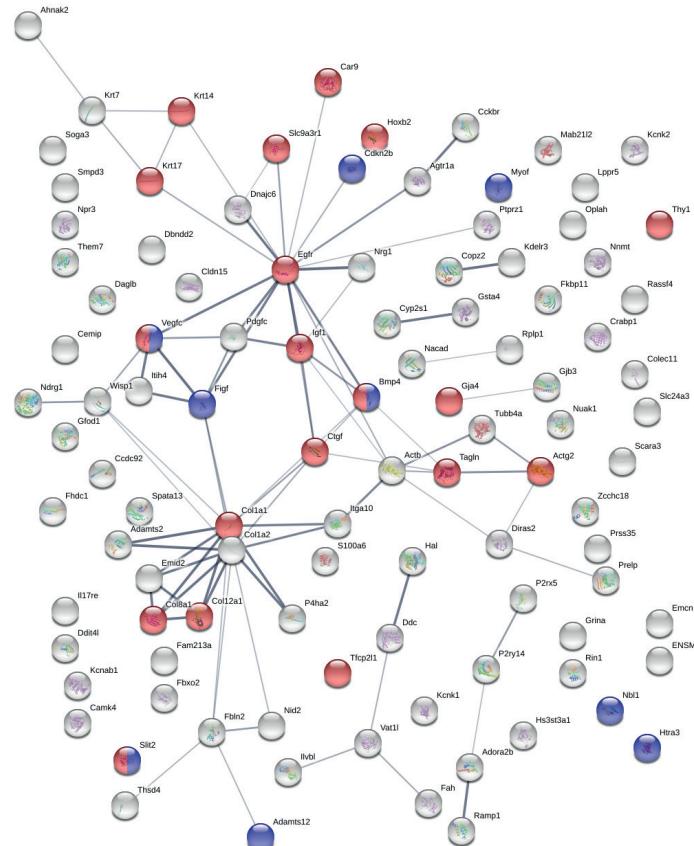
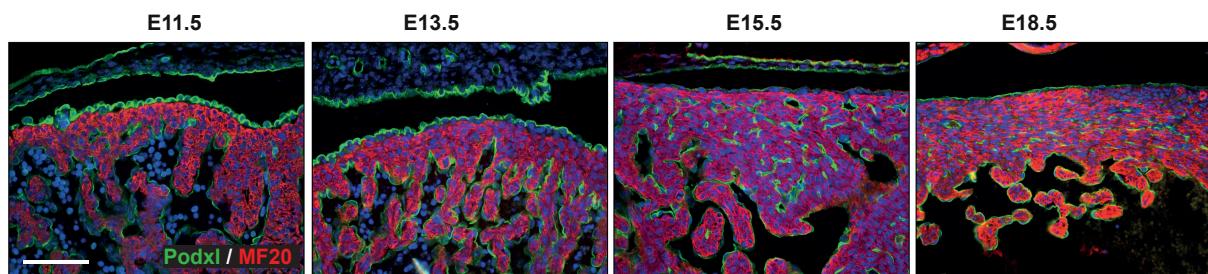
B

Figure S4. Validation of genes differentially expressed in *Wt1GFP⁺⁺* epicardial-enriched cells from control and epi*Wt1KO*. (A) qRT-PCR analysis of indicated genes from our microarray analysis of *Wt1GFP⁺⁺* epicardial cells from control and epi*Wt1KO* mice at E13.5. Values are expressed as fold change over control. P-values are based on two-tailed t-tests, *P<0.05. RNA from FACS-isolated cells from at least 10 embryonic hearts was pooled by condition. Three replicates per condition were used for the analysis. (B) STRINGdb interactome of regulated genes, indicating genes involved in the categories of tissue morphogenesis (red) and regulation of cellular response to growth factor stimulus (blue).

Fig. S5

A



B

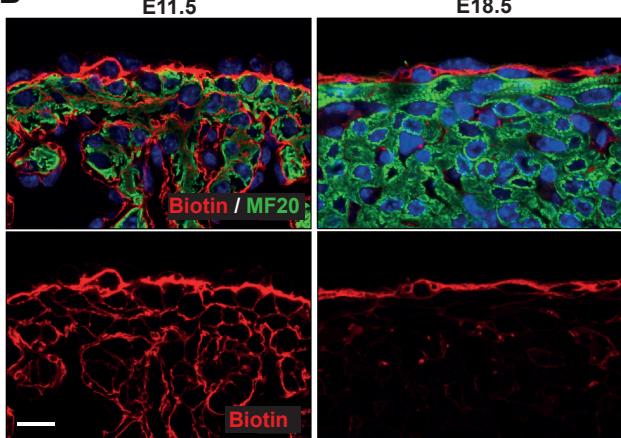


Figure S5. The mature epicardium displays a squamous non-permeable phenotype. (A) Representative low magnification images of the immunofluorescent staining for podocalyxin in green, MF20 (red) and nuclear Hoechst stain (blue), using heart sections at different days of development, from E11.5 to E18.5. Sections of ventricles indicate the epicardial transition from a cuboidal cell shape in the early stages to a flattened shape later on. (B) Biotin permeability assay of the E11.5 and E18.5 epicardium. Biotin was detected with Rhodamine-conjugated streptavidin (red) and shows deep penetrance at E11.5, while biotin staining was confined to the epicardial layer at E18.5. The cardiac myosin marker MF20 was used to stain the myocardium (green) and nuclear Hoechst stain (blue). Scale bars, 100 μ m (A) and 20 μ m (B).

Fig. S6

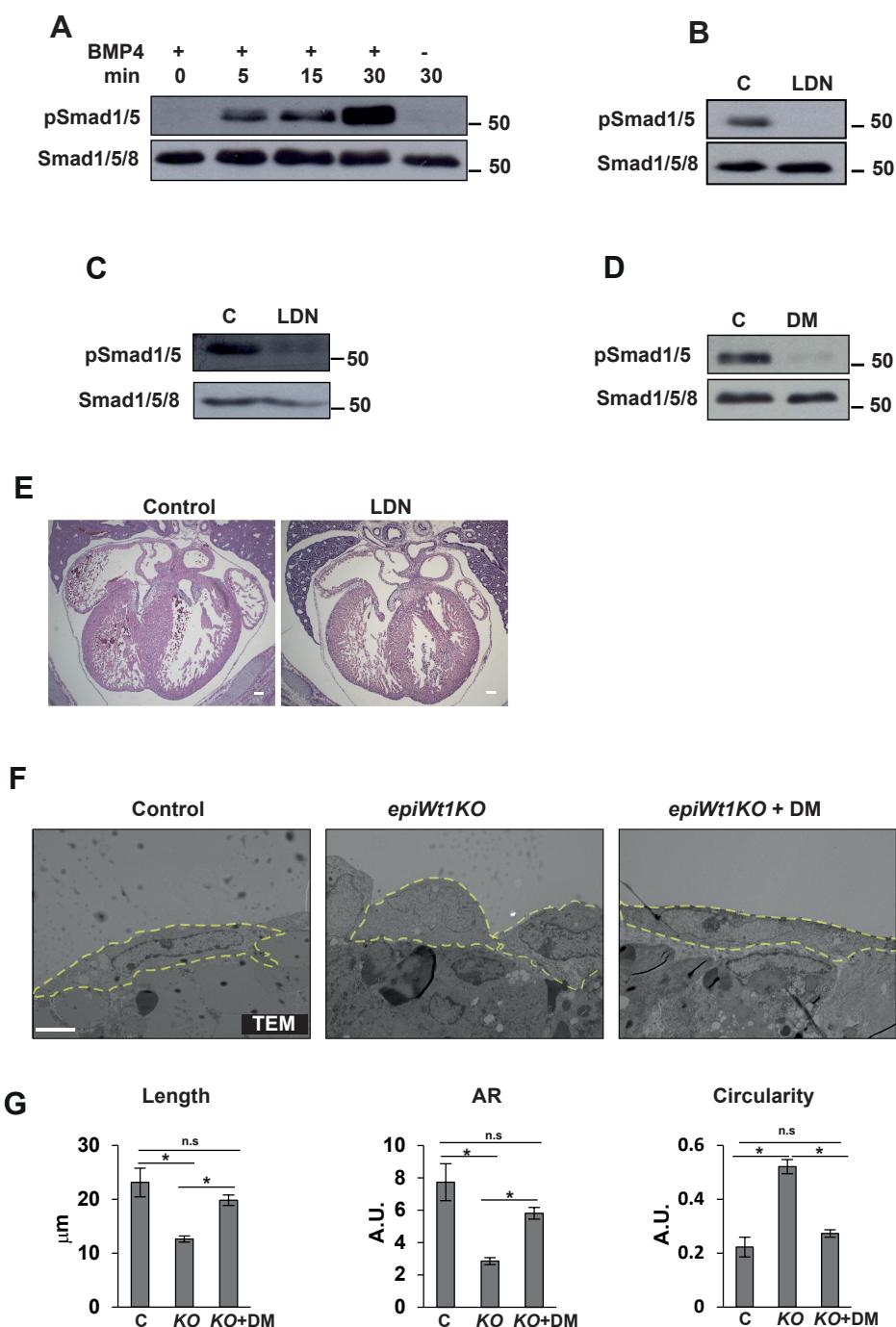


Figure S6. Inhibition of BMP4 signalling rescues the flattening defects in *epiWt1KO* hearts. (A) BMP4 induces the phosphorylation of Smad1 and Smad5 in epicardial cells. Immortalised epicardial cells were pre-treated for 18 hours with dorsomorphin before BMP4 stimulation for indicated time points. Cell lysates were analysed by Western blotting with antibodies against phosphorylated Smad1/5 (P-Smad1/5) and Smad1/5/8 as a loading control. Cell extracts from control (C) and LDN-193189 (LDN)-treated immortalised epicardial cells (B), embryonic heart extracts from control and LDN-193189 (LDN)-treated pregnant mice (C) and cell extracts from DMSO (Control) and dorsomorphin (DM) treated immortalised epicardial cells (D) were analysed by Western blotting with indicated antibodies. (E) H&E staining of E15.5 embryonic heart sections from control and LDN-193189 (LDN)-treated pregnant mice. (F) TEM of control and *epiWt1KO* hearts treated *ex vivo* with DMSO (Control) or dorsomorphin (DM) for 48h. Rescue of the flattening phenotype in epicardial *epiWt1KO* cells in the presence of DM can be observed. (G) Quantification of epicardial cell length, aspect ratio (AR) and circularity are shown as mean \pm s.e.m. Three independent embryos were assessed per condition, and at least 20 epicardial cells were quantitated. Significance was determined by Tukey's multiple-comparison after one-way ANOVA (* $P<0.05$, n.s. = not significant). Scale bars, 100 μ m (E) and 5 μ m (F).

Table S1. Differentially expressed genes in the *Wt1GFP⁺⁺* epicardial-enriched cell population compared to the *Wt1GFP⁺* population of cells.

[Click here to Download Table S1](#)

Table S2. Cluster analyses of modulated genes in the *Wt1GFP⁺⁺* epicardial-enriched cells.

[Click here to Download Table S2](#)

Table S3. Links to *in situ* hybridisations from the Eurexpress database

(www.eurexpress.org) showing the expression of indicated genes in the epicardium.

Gene	Cluster	Link
Upk3b	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_016996&image=01
Bmp4	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_017884&image=01
Cdkn1c	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_018567&image=01
Alcam	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_003463&image=01
Fbln2	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_011955&image=01
Capn6	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_006493&image=01
Gucy1a3	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_012078&image=01
Pmp22	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_017431&image=01
Fn1	1	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_001464&image=01
Adamts2	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_003619&image=01
Col1a1	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_001885&image=01
Col3a1	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_004670&image=01

Col14a1	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_001452&image=01
Hba-a1	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_002328&image=01
S100a1	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_007368&image=01
Dhrs7	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_000191&image=01
Eln	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_004329&image=01
Prnp	2	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_007857&image=01
Slit2	3	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_017890&image=01
Mfap4	3	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_014283&image=01
Reln	3	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_017891&image=01
Col1a2	4	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_004456&image=01
Igfbp5	4	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_001623&image=01
S100a6	4	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_017625&image=01
S100a10	4	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_018301&image=01
Msln	4	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_001942&image=01
Serpinc1	4	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_000667&image=01

Nbl1	4	http://www.eurexpress.org/ee/databases/assay.jsp?assayID=euxassay_000822&image=01
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Table S4. Differentially expressed genes in *Wt1GFP⁺⁺* epicardial-enriched cells in *epiWT1KO* vs control mice.

[Click here to Download Table S4](#)

Table S5. Differentially expressed genes in *Wt1GFP⁺⁺* epicardial-enriched cells in *epiWT1KO* vs control mice that are modulated during development.

[Click here to Download Table S5](#)

Table S6. List of primers used for genotyping.

Name	Sequence	Annealing
<i>Wt1</i> flox F	TGGGTTCCAACCGTACCAAAGA	58
<i>Wt1</i> flox R	GGGCTTATCTCCTCCCATGT	
Cre F	GCATTACCGGTCGATGCAACGAGTGATGAG	58
Cre R	GAGTGAACGAACCTGGTCGAAATCACTGCG	
Cre internal	control	
Fabpi200 F	TGGACAGGACTGGACCTCTGCTTCCTAGA	
Cre internal	control	
Fabpi200 R	TAGAGCTTGCCACATCACAGGTCAATTAG	
<i>Wt1</i> GFP F	GCCTGAAGAACGAGATCAGC	58
<i>Wt1</i> GFP R	GGCAGCTTGAATTCCCTCTCA	
<i>Wt1</i> GFP F2	AGCCTGAAGCTGCTCACATCC	

Table S7. List of antibodies used in this study.

Name	Company	Reference	Dilution	Technique
Sarcomere myosin (MHC)	DSHB	MF20-s	1:20	IF
WT1	Abcam	ab89901	1:1000	IF
PODXL	R&D	AF1556	1:200	IF
PODOPLANIN	Novus bio	NB600-1015	1:100	IF
Donkey anti-rabbit AF488	Invitrogen	A21206	1:800	IF
Donkey anti-goat AF488	Invitrogen	A11055	1:800	IF
Donkey anti-mouse AF594	Invitrogen	A21203	1:800	IF
Goat anti-hamster AF488	Invitrogen	A21110	1:800	IF
Beta-catenin	BD Transduction	610154	1:250	IF
goat anti-rabbit AF488	Life Technologies	A21200	1:400	IF
goat anti-mouse AF546	Life Technologies	A11003	1:400	IF
p-Smad 1/5	Cell Signaling	41D10	1:1000	WB
Smad1/5/8	Santa Cruz	sc-6031-R	1:1000	WB

Table S8. List of primers used for *in situ* hybridisation probes

Name	Sequence
ISH Tcf21 F	ATGTCCACTGGCTCCCTCAGC
ISH Tcf21 R	CGATGTTAATACGACTCACTATAAGGGAGTCCACACAAGCGGTTGG
ISH Upk1b F	CCTCTTCTGCTTGTCCGTTC
ISH Upk1b R	CGATGTTAATACGACTCACTATAAGGGCAAATCCAACCAGGCAACT
ISH Col6a1 F	GAAATGTGACCCAACCTGGTCAA
ISH Col6a1 R	CGATGTTAATACGACTCACTATAAGGGCAAGCTAACGCCTCTGGGTGTGT
ISH Col3a1 F	TTGGGATGCAGCCACCTTGG
ISH Col3a1 R	CGATGTTAATACGACTCACTATAAGGGTCCTTCATACCAGGGA
ISH Reln F	CACACCGTGGACAAAGCAGTA
ISH Reln R	CGATGTTAATACGACTCACTATAAGGGCAGAGAAACCACACATCATTAGC
ISH Emilin1 F	GCTCCCTGAACCGACTCACT
ISH Emilin1 R	CGATGTTAATACGACTCACTATAAGGAAACTCAGGGCAGCTGAAAAA
ISH Col1a2 F	ATGAATGGGGCAAGACAATC
ISH Col1a2 R	CGATGTTAATACGACTCACTATAAGGATGTCATGGTTCTT
ISH Dcn F	CCTCCTTCTTCCACACCTG
ISH Dcn R	CGATGTTAATACGACTCACTATAAGGCCATAACGGTGATGCTGTTG

Table S9. List of primers used in ChIP.

Name	Sequence	Annealing
Bmp4 prom F	CCCCGCCTCGAAAAGTGG	55
Bmp4 prom R	TGTTCTAACCTCGGAAGCGC	
UTR negative control F	AAGACTGGGGAGGAAGGGAA	55
UTR negative control R	AGGGACGGAGACCAGATACT	

Table S10. List of primers used in quantitative real time PCR

Name	Sequence
Wt1 F	TTCAAGGACTGCGAGAGAAAG
Wt1 R	GGGAAAACCTTCGCTGACAA
Bmp4 F	GAGGAGTTCCATCACGAAGA
Bmp4 R	GCTCTGCCGAGGAGATCA
Nppa F	CACAGATCTGATGGATTCAAGA
Nppa R	CCTCATCTTCTACCGGCATC
Actc1 F	CCGATCGTATGCAAAAGGAA
Actc1 R	CTGGAAGGTGGACAGAGAGG
Actn2 F	CTCGGAGCTCCATCCAGA
Actn2 R	ACTGCTTCAGCTGGTTCATCT
Myh6 F	TGCTCAGAGCTCAAGAAGGAT

<i>Myh6</i> R	CCCAGCCATCTCCTCTGTTA
<i>Ttn</i> F	CCGGTGGAAGCTGCTATATT
<i>Ttn</i> R	CCGAGGTTTCACTGCGTA
<i>Upk3b</i> F	CCTGTCCAGATGTGGGATCT
<i>Upk3b</i> R	TCCGAGGATAGTTGAGAGCA
<i>Dcn</i> F	GAGGGAACCTCCACTTGGACA
<i>Dcn</i> R	TTGTTGTGTAAGGTAGACGAC
<i>Tcf21</i> F	TGATTAACCTCTGCCATGAATGA
<i>Tcf21</i> R	AAAAGATACACATTGATAGGCTTTCT
<i>Dlk1</i> F	GATTCTGCGAGGCTGACAAT
<i>Dlk1</i> R	GGTTCCCTGCAGACTCCATT
<i>Upk1b</i> F	CGATTCCACTGTTGTTGC
<i>Upk1b</i> R	GGCGATGCCACACATAC
<i>Nbl1</i> F	CGCACCCCCACTTCTAGG
<i>Nbl1</i> R	CACCAAGGACCCAAAGCAT
<i>Podxl</i> F	TCCTTGTGCTGCCCTCT
<i>Podxl</i> R	CTCTGTGAGCCGTTGCTG
<i>Smpd3</i> F	GATTGACGGCTGTCATTACCT
<i>Smpd3</i> R	ATGTAATGCCCTTGAATGC
<i>Krt8</i> F	AGTCGCCTCCTTCATTGAC
<i>Krt8</i> R	GCTGCAACAGGCTCCACT
<i>Tbx18</i> F	CCGAGACTCTAGGAAC
<i>Tbx18</i> R	TGATGGCCTCGAATGC
<i>Cdkn1c</i> F	TCTCCTTGCTCGTT
<i>Cdkn1c</i> R	GGCATTGTGGGTGTTG
<i>I8s</i> F	CGATTGGATGGTTAGTGAGG
<i>I8s</i> R	AGTCGACCGTCTCAGC
<i>Car9</i> F	GCCCAGAAGAAAACAGTGCT
<i>Car9</i> R	CCAAACCTGGGATCTCAATC
<i>Thy1</i> F	GGTGGCAGAAGAAGACAAGG
<i>Thy1</i> R	CCTTCCTGCACGGACTTAGA
<i>Figf</i> F	GCAACTTCTATGACACTGAAACAC
<i>Figf</i> R	TCTCTCTAGGGCTGCATTGG
<i>Slc9a3r1</i> F	CCCTCAGCAATGGAGAGATAC
<i>Slc9a3r1</i> R	TGGGGCTCTGAAGCTG
<i>Krt7</i> F	GGAGATGGCCAACCACAG
<i>Krt7</i> R	GGCCTGGAGTGTCTCAAACCTT
<i>Krt14</i> F	ATCGAGGACCTGAAGAGCAA
<i>Krt14</i> R	TCGATCTGCAGGAGGACATT
<i>Krt17</i> F	GGAGCTGGCCTACCTGAAG
<i>Krt17</i> R	ACCTGGCCTCTCAGAGCAT
<i>Col12a1</i> F	GACAGATGAGACTACGGACAGTTT
<i>Col12a1</i> R	TGGTCTGTATCTAATCCGATACCTT
<i>Colla2</i> F	CGGAGAAGCTGGATCTGC
<i>Colla2</i> R	CAGGAGGACCCATTACACCA
<i>Fah</i> F	CCTGCAGACTCTTAGACATGGA

<i>Fah</i> R	GATTGGCTCTCCGAATCTGT
<i>Colla1</i> F	CATGTTCAGCTTGTGGACCT
<i>Colla1</i> R	GCAGCTGACTTCAGGGATGT
<i>Cdkn2b</i> F	AATAACTTCCTACGCATTCTGC
<i>Cdkn2b</i> R	CCCTTGGCTTCAAGGTGAG
<i>Cldn15</i> F	GTGGAGACCTTCGGCTTCTT
<i>Cldn15</i> R	AAGGGTCAACCCCAGCAT
<i>Egfr</i> F	GCCACGCCAACTGTACCTAT
<i>Egfr</i> R	GCCACACTTCACATCCTTGA
<i>Slit2</i> F	CAGATCTCTGAACTTGCACCA
<i>Slit2</i> R	TGTGATTTTATTTCCATACAGGACA
<i>Ccnd2</i> F	CACCGACAACTCTGTGAAGC
<i>Ccnd2</i> R	TCCACTTCAGCTTACCCAACA
<i>Cdk4</i> F	AGCGTAAGATCCCCTGCTTC
<i>Cdk4</i> R	ATAGGCACCGACACCAATT
<i>Hes1</i> F	ACACCGGACAAACCAAAGAC
<i>Hes1</i> R	CGCCTTTCTCCATGATAGG
<i>Nfatc1</i> F	CCACGCCCTCTACCAGGTC
<i>Nfatc1</i> R	GGACTTTGGTGTGGACAGG
<i>Colla1</i> F	CATGTTCAGCTTGTGGACCT
<i>Colla1</i> R	GCAGCTGACTTCAGGGATGT
<i>Igfbp2</i> F	GCCCCCTGGAACATCTCTACT
<i>Igfbp2</i> R	TCCGTTCAGAGACATCTTGCA
<i>Ncam1</i> F	AGGGCAAGGCTGCTTCT
<i>Ncam1</i> R	CCCCATCATGGTTGGAGT
<i>Emcn</i> F	GCTCTGGTGGTTGTATCGAA
<i>Emcn</i> R	GCTGATCATTCCGTTTC