SUPPLEMENTAL MATERIAL

Endocardial Cushion Morphogenesis and Coronary Vessel Development Require COUP-TFII

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SUPPLEMENTAL METHOD

Luciferase Assay

The pGL3 plasmid containing a 0.9 kb human *Snai1* promoter fragment that included the first conserved Sp1 binding sites was used (*pGL3-hSnai1*) (1) (A gift from Dr. Antonio Garcia de Herreros). HEK 293 cells were transfected with *pGL3-Snai1-Luc* using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Supplemental Table I. The primer sequences for Q RT-PCR

Gene	Primer sequences	Size (bp)	References
hCOUP-TFII	F: GCCATAGTCCTGTTCACCTC R: CTGAGACTTTTCCTGCAAGC	79	(2)
hSnai1	F: GCTGCAGGACTCTAATCCAGAGTT R:GACAGAGTCCCAGATGAGCATTG	130	(3)
h18sRNA	F: TCCGATAACGAACGAGACTC R: CAG GGACTTAATCAACGCAA	81	(4)
ChIP-hSnai1-Sp1-1	F: GGAGACGAGCCTCCGATT R: AGCAGTAGCGCAGAAGAACC	167	
ChIP-hSnai1-Sp1-2	F: GCATGGCTGAGACACAGAAA R: CGATATCCCCGGATTAAAGG	199	
ChIP-hSnai1-Sp1-3	F: AGGAGTGGCCTAACCAGCTT R: CCAAAGCCTCTGATTTCACC	163	
ChIP-Neg	F: AACGGCAGAAGAGAGAACCA R: AAGATGACCCAGGTGAGTGG	105	

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SUPPLEMENTAL FIGURES

Supplemental Figure I. COUP-TFII expression at E10.5.

(A) *Tie2Cre; COUP-TFII* $^{F/+}$ whole mount β-galactosidase stained sagittal sections label endocardial COUP-TFII. COUP-TFII is observed in the endocardium of the atrioventricular cushion toward the atrium. (B) Immunofluorescence for COUP-TFII sagittal sections of E10.5 wild-type embryo. Nuclei were counterstained with DAPI. Scale bar, 100 μm. A, atrium; iAVC, inferior atrioventricular cushion; OFT, outflow tract; sAVC, superior atrioventricular cushion; V, ventricle.

Supplemental Figure II. A decrease in cell proliferation and an increase in apoptosis in Tie2*Cre; COUP-TFII* $^{F/F}$ **mutants.**

(A) Immunohistochemistry of phospho-histone H3 and quantification of phospho-histone H3 positive cells, showing a decrease on cell proliferation in AV cushions of *Tie2Cre; COUP-TFII*$^{F/F}$ embryos at E9.5 compared to the controls. N=3. (B) TUNEL assay and quantification of TUNEL positive cells were performed at E10.5 AV cushions. Control AV cushions are negative for TUNEL assay, while mutant AV cushions are positively stained with TUNEL assay (arrows). N=3. A, atrium and V, ventricle. Error bars indicate standard deviation; **P*<0.05; ****P*<0.001.

Supplemental Figure III. COUP-TFII binds to *Snai1* promoter and activates its transcription.

HEK293 cells were transiently transfected with the *Snail* promoter-luciferase reporter (*pGL3-hSnai1*) with a control vector (Ctrl) or a *COUP-TFII*-expressing vector (COUP-TFII). The results are expressed as relative luciferase activity after correction for protein concentration. COUP-TFII enhances *Sani1* transcriptional activity. Error bars represent standard deviation. ****P*<0.001.

Supplemental Figure IV. COUP-TFII expression is slightly reduced in the epicardium of E11.5 *Gata5Cre; COUP-TFII* $^{F/F}$ **mutants.**

(A) Immunofluorescence for COUP-TFII in transverse sections of E11.5 control and *Gata5Cre; COUP-TFII* $^{F/F}$ embryos. COUP-TFII is expressed in the endocardium and the epicardium (arrows) of controls, whereas COUP-TFII expression is slightly reduced in the epicardium of E11.5 *Gata5Cre; COUP-TFII* $^{F/F}$ embryos, indicating the incomplete deletion of COUP-TFII in the epicardium. (B) Nuclei were counterstained with DAPI. Scale bar, 100 μm.

Supplemental Figure V. Malformation of epicardium in the *COUP-TFII* deficiency hearts.

(A-C) H&E stained images of transverse sections of control *COUP-TFII* $^{F/F}$ and *COUP-TFII* $^{F/-}$ (A), and of control *COUP-TFII* $^{F/F}$ and littermate *CRE-ER*^{T2}*; COUP-TFII* $^{F/F}$ mutant embryos at E11.5 (with Tam administration at E9.5) (B), and of control *COUP-TFII* $^{F/F}$ and *Gata5Cre; COUP-TFII* $^{F/F}$ mutant hearts at E11.5 (C). In control hearts, the epicardium is uniform over the myocardium surface and the subepicaridial spaces become less apparent. The detachments of the epicardium (arrow) from the underlying myocardium are observed in mutant hearts. (D) Immunofluorescence for Wt1 (green) and for pan-cytokeratin (red) in transverse sections of control *COUP-TFII* $^{F/F}$ and littermate *CRE-ER*^{T2}*; COUP-TFII* $^{F/F}$ mutant embryos E11.5 (with Tam administration at E9.5). The number of Wt1 positive epicardial cells is reduced in the mutant hearts as compared to controls. Scale bar, 100 μm; ***P<0.001.

Supplemental Figure VI. *COUP-TFII* deficiency causes abnormal epicardium morphogenesis.

(A, B) Scanning electron microscopy of control and mutant hearts at E12.5. (B) Higher magnification pictures of (A). *COUP-TFII*$^{F/-}$ mutant hearts display abnormal epicardial features at E12.5. (C) The retention of a large sum of cells in sinoatrial region of the heart (arrows) was observed in *COUP-TFII* $^{F/-}$ mutant, but not in the control littermate, suggesting that cell migration in the epicardium is altered in the mutant heart. LV, left ventricle; RV, right ventricle.

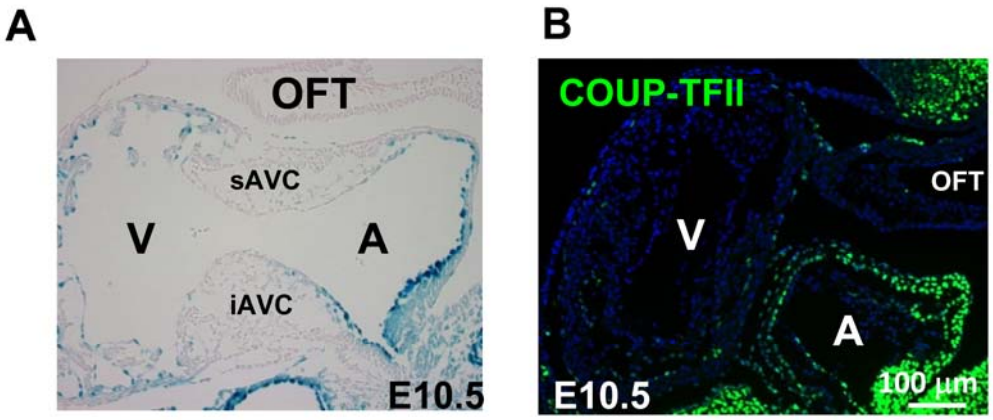
Supplemental Figure VII. Epicardial mesenchymal cells derived from *in vitro* explants are DAPI positive.

(A) E12.5 control epicardial explants were cultured on a collagen gel: a subset of cells delaminated, formed spindle-like shape cells (arrows) and migrated into the collagen gel. (B) DAPI staining revealed epicardial delaminated cells (arrows) invading the collagen gel. Nuclei were counterstained with DAPI.

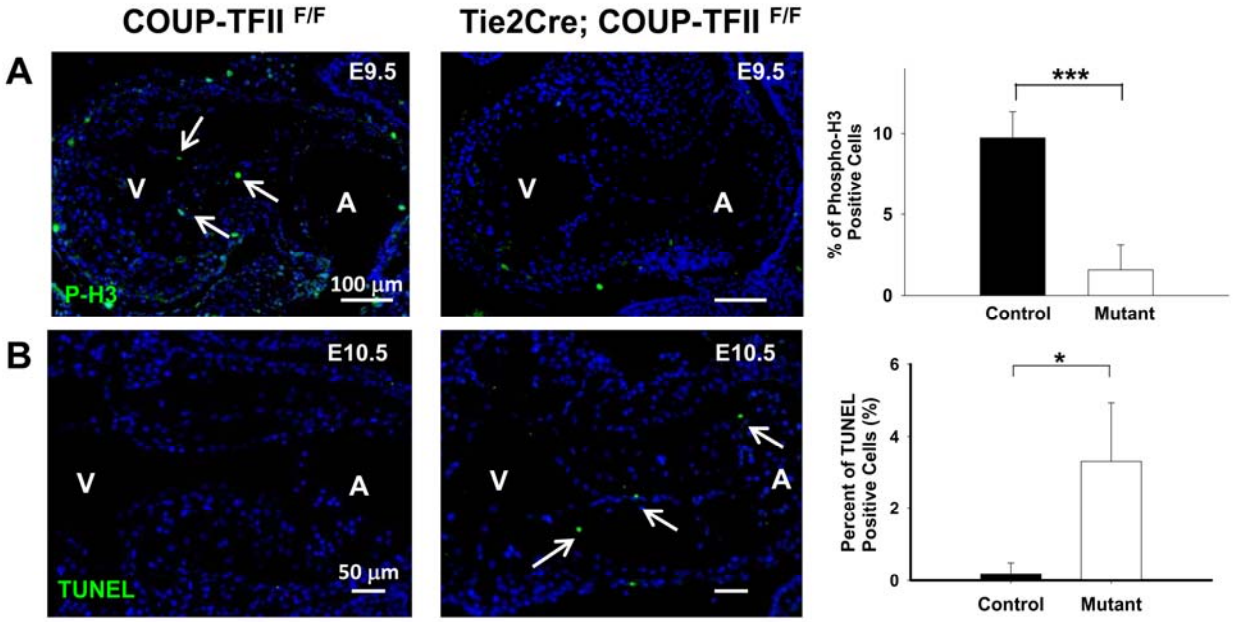
Supplemental Figure VIII. NICD expression is increased in the ventricles of E11.5 *Tie2Cre; COUP-TFII* $^{F/F}$ mutants.

Immunofluorescence for NICD in transverse sections of E11.5 littermate control and *Tie2Cre; COUP-TFII* $^{F/F}$ mutant embryos. Scale bar, 100 μm.

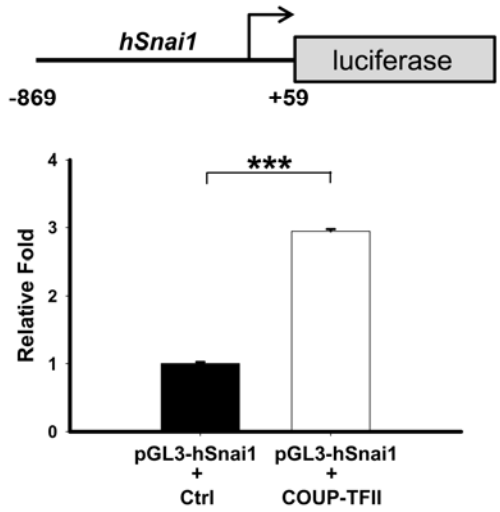
Lin *et al.*, Supplemental Figure I



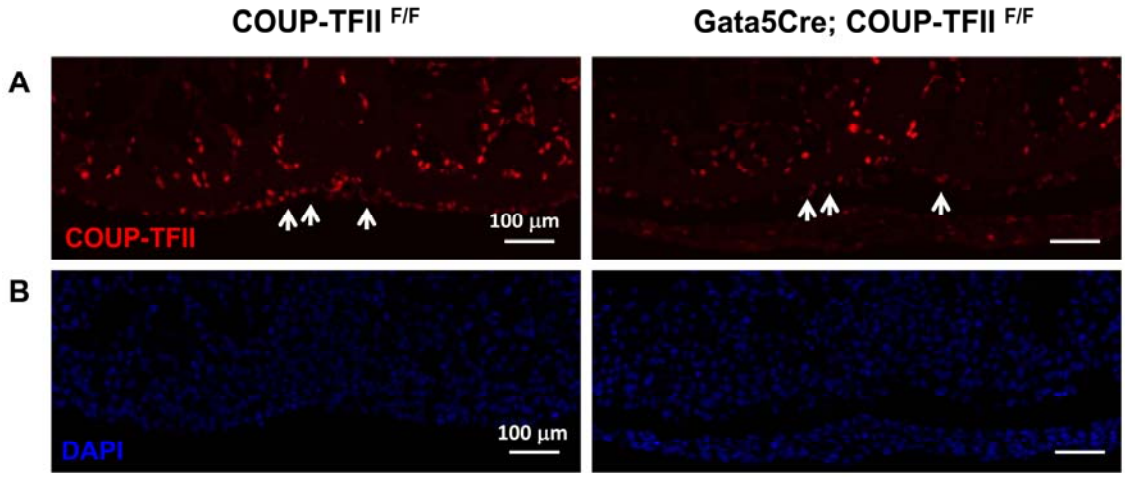
Lin *et al.*, Supplemental Figure II



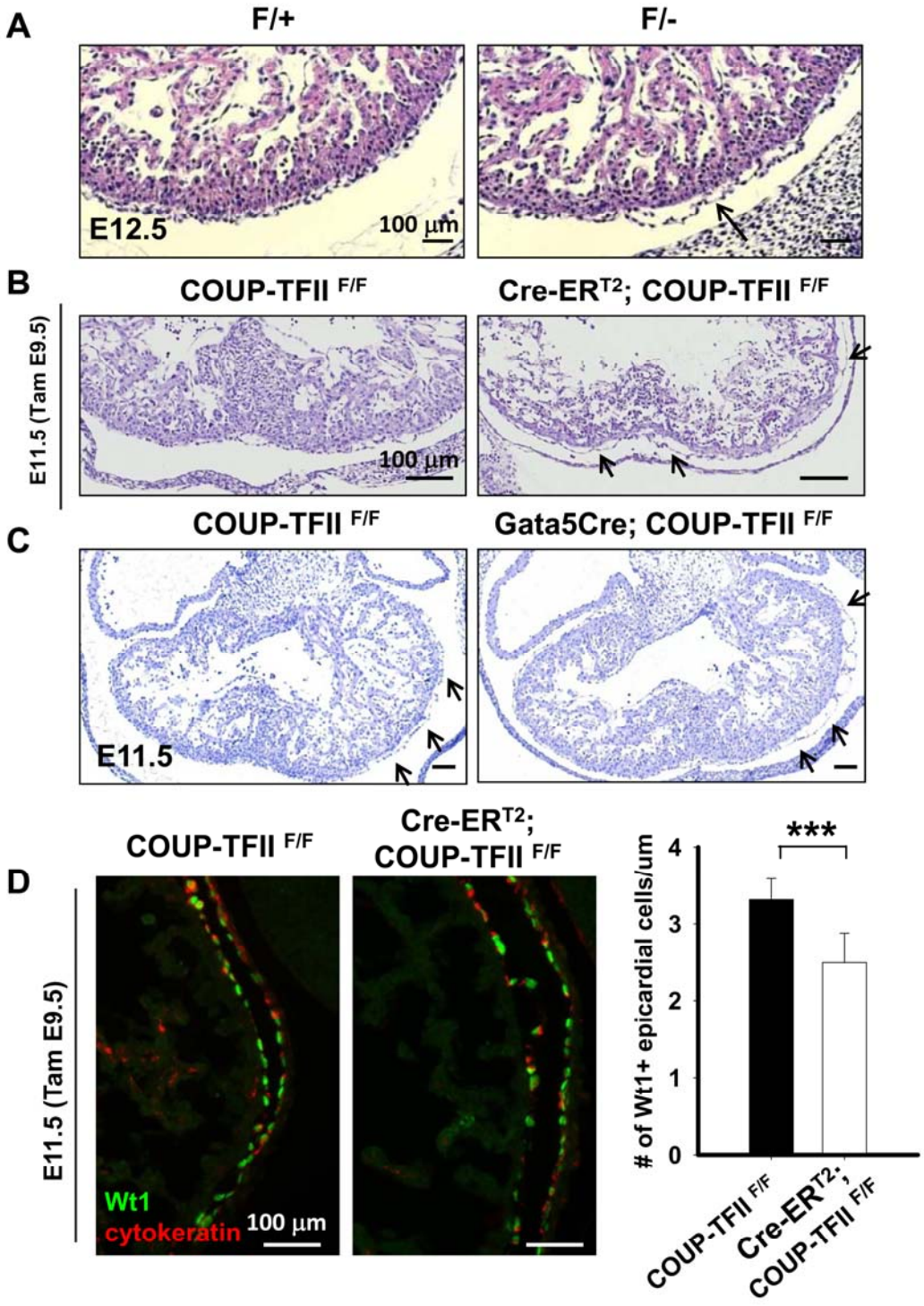
Lin *et al.*, Supplemental Figure III



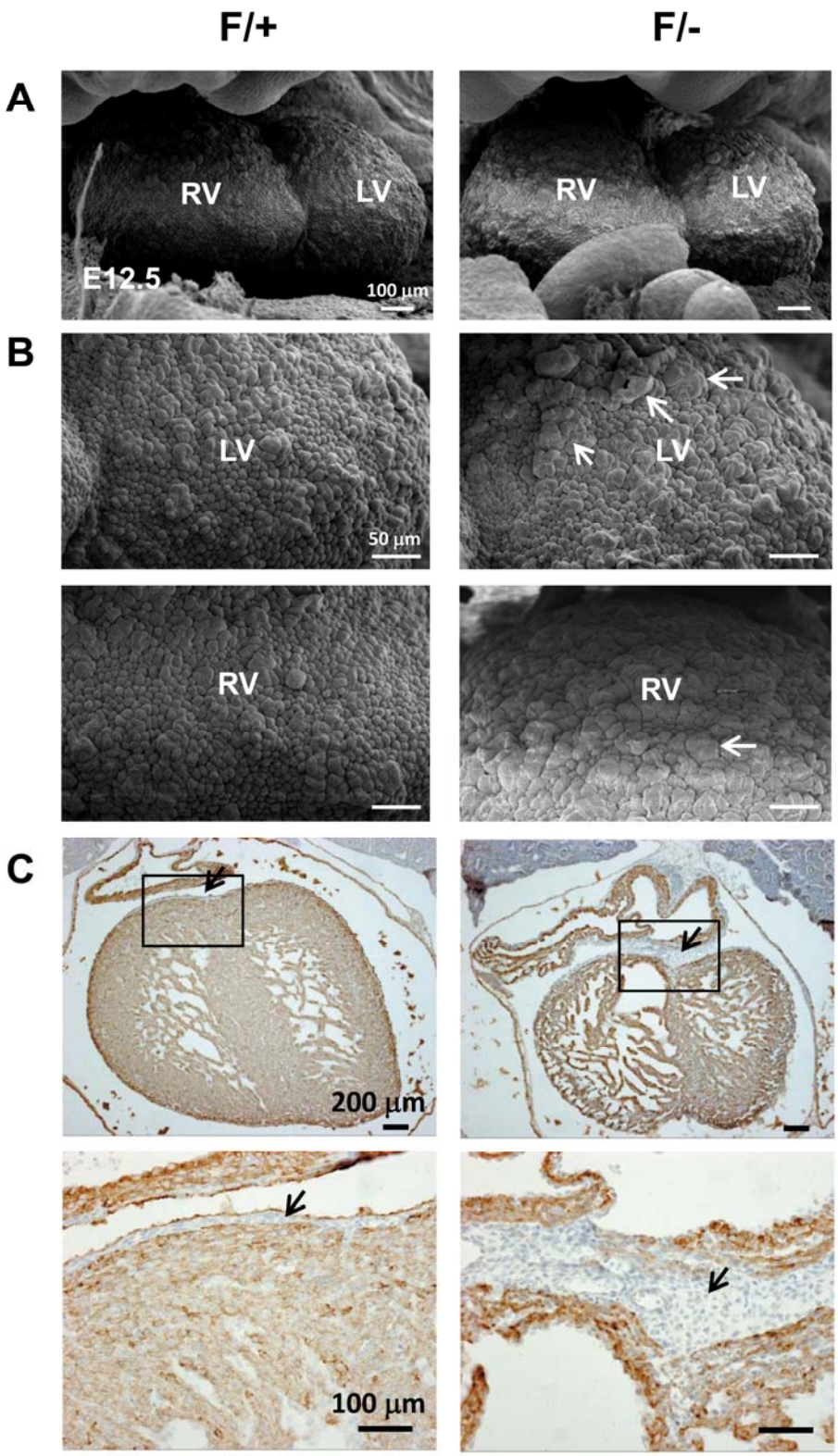
Lin *et al.*, Supplemental Figure IV



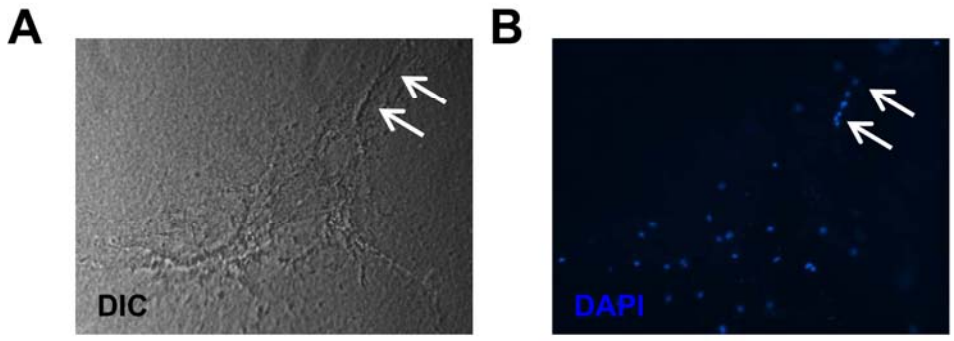
Lin *et al.*, Supplemental Figure V



Lin *et al.*, Supplemental Figure VI



Lin *et al.*, Supplemental Figure VII



Lin *et al.*, Supplemental Figure VIII