Supporting Information

Lin et al. 10.1073/pnas.0910430107

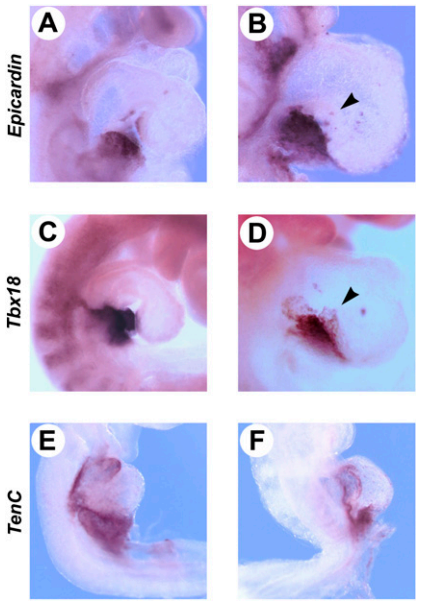

Fig. S1. Proepicardial markers are expressed in *Raldh2*$^{-/-}$ embryos. Whole-mount in situ hybridization was performed with *Epicardin* (*A* and *B*), *Tbx18* (*C* and *D*), and *TenC* (*E* and *F*). All genes are expressed to similar exents in *Raldh2*$^{-/-}$ embryos (*B*, *D*, and *F*) when compared with WT (*A*, *C*, and *E*), despite the heart looping defect. Arrowheads point to proepicardial cells colonizing the heart tube.

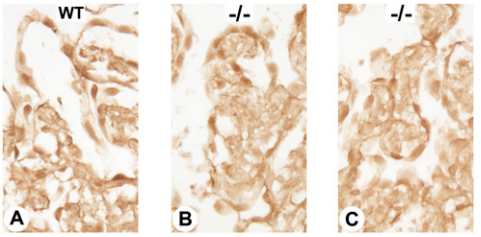

Fig. S2. Immunohistochemistry analysis of β-catenin in E12.5 WT (*A*) and *Raldh2*$^{-/-}$ hearts (*B* and *C*) after short-term RA rescue that show similar patterns of nuclear localization among cells of the trabecular myocardium.

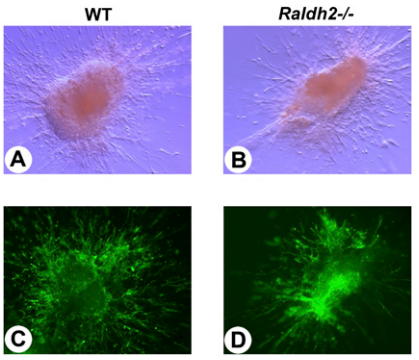

Fig. S3. Epicardial cells from *Raldh2*$^{-/-}$ mutants behave normally in epicardial invasion assays. Epicardial/subepicardial explants from E12.5 WT (*A* and *C*) and short-term rescued *Raldh2*$^{-/-}$ mutants (*B* and *D*) were cultured for 60 h on collagen gels (*Materials and Methods*). Epithelial-mesenchymal transformation was assessed as the invasion of cells into the gel (*A* and *B*) and the formation of vessel-like structures by immunostaining for smooth muscle α-actin (*C* and *D*).

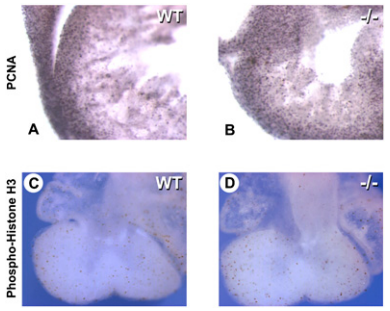

Fig. S4. Proliferating cell nuclear antigen (PCNA) (*A* and *B*) and phospho-histone H3 (*C* and *D*) immunochemistry analysis on E12.5 vibratome sections and E11.5 whole hearts, respectively. *Raldh2*$^{-/-}$ mutants (*B* and *D*) were obtained after short-term rescue (E7.5–8.5).

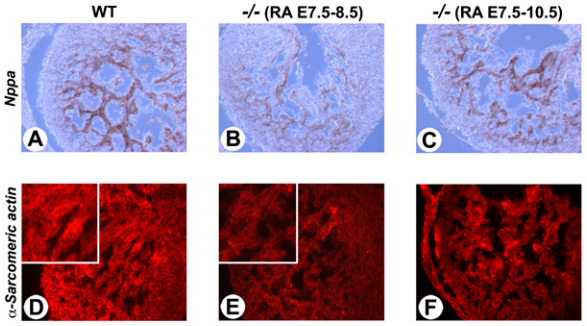

Fig. S5. RA deficiency impairs early differentiation of embryonic cardiomyocytes. (*A*–*C*) In situ hybridization shows decreased *Nppa* expression throughout the myocardium of short-term rescued mutants (*B*), whereas levels are almost normalized after longer-term rescue (E7.5–10.5) of the mutants (*C*). A similar observation was made by immunofluorescence analysis of α-sarcomeric actin (*D*–*F*).

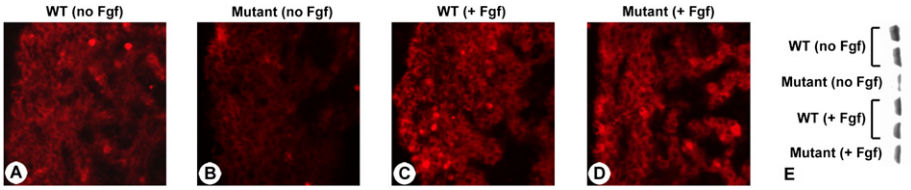

Fig. S6. Exogenous Fgf improves cardiomyocytic differentiation in *Raldh2*$^{-/-}$ heart explants. Hearts were collected from E12.5 WT and *Raldh2*$^{-/-}$ embryos, cultured for 28 h in Transwell inserts in the absence (*A* and *B*) or the presence of exogenous Fgf2 (200 μg/mL), and analyzed by immunohistochemistry (*A*–*D*) or Western blot (*E*) for α-sarcomeric actin. Both assays show increased α-sarcomeric actin levels following Fgf2 administration.

Table S1. Sources and concentrations of antibodies used for immunoreactions

Assay	Antibodies
Western blot	Anti-Fgf2 (1:200) Anti-AKT (1:200) Anti-phosphorylated AKT (1:1,000) Anti ERK1/2 (1:200) Anti-phosphorylated ERK1/2 (1:500) from Cell Signaling Technology Anti-β-catenin monoclonal antibody (1:100) from BIOMOL International, BA1902
Immunohistochemistry	Anti-Fgf2 (1:200 Cell Signaling) Phospho-Akt (1:100 Cell Signaling) β-Catenin (1:200 Cell Signaling) Sarcomeric myosin (MF-20, 1:100 DSHB, University of Iowa) Cardiac troponin-T (CT-3, 1:100 DSHB, University of Iowa) Cardiac α-sarcomeric actin (1:200 Sigma; A2172) Smooth muscle α-actin (1:500 Sigma; A2547 clone 1A4), Goat anti-mouse Ki67 (1:200 BD Sciences #556006) Goat anti-mouse phospho-histone H3 (1:200 Cell Signaling) Rabbit anti-mouse c-Kit (1:400 Santa Cruz Biotechnology SC-5535) Alexa 488-, Alexa 594-, and Cy5-coupled secondary antibodies (Molecular Probes and Jackson ImmunoResearch Laboratories)

Table S2. Primer sequences for real-time quantitative PCR

Gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
Fgf2	GCGACCCACACGTCAAACTA	TCCATCTTCCTTCATAGCAAGGT
Fgf9	ATGGCTCCCTTAGGTGAAGTT	TCATTTAGCAACACCGGACTG
Gli1	TGTGTGAGCAAGAAGGTTGC	GACCATGCACTGTCTTCACG
Gli3	CTTTGCAAGCCAGGAGAAAC	CCCACCCGAGCTATAGTTGT
Shh	AAAGCTGACCCTTTAGCCTA	TTCGGAGTTTCTTGTGATCTTCC
Ptc1	CTCAGGCAATACGAAGCACA	GACAAGGAGCCAGAGTCCAG
Wnt9b	CTGGTGCTCACCTGAAGCAG	CCGTCTCCTTAAAGCCTCTCTG
Gapdh	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
Sca-1	TGGATTCTCAAACAAGGAAAGTAAAGA	ACCCAGGATCTCCATACTTTCAATA
Nkx2.5	GACAAAGCCGAGACGGATGG	CTGTCGCTTGCACTTGTAGC
Gata4	TCTCACTATGGGCACAGCAG	ACAGCACTGGATGGATGGAG
Mef2C	GTCAGTTGGGAGCTTGCACTA	CGGTCTCTAGGAGGAGAAACA
c-Kit	TCATCGAGTGTGATGGGAAA	GGTGACTTGTTTCAGGCAACA
Isl1	CACTATTTGCCACCTAGCCAC	AAATACTGATTACACTCCGCAC
Nppa	ACCCCTCCGATAGATCTGC	TTCGGTACCGGAAGCTGT