SUPPLEMENTAL DATA

Knockout of SRC-1 and SRC-3 in Mice Decreases Cardiomyocyte Proliferation and Causes a Noncompaction Cardiomyopathy Phenotype

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Supplemental Video Files (Uploaded):

The real-time heart-beating videos were recorded by the Doppler echocardiography (B-mode) from 5–7-month-old S3F, S1KO;S3F, S3MKO and S1KO;S3MKO mice. Note the typical NCC phenotype of the S1KO;S3MKO heart.

Video File 1. Control heart.avi. This video was recorded from a S3F control mouse.

Video File 2. S1KO S3F heart.avi. This video was recorded from a S1KO;S3F mouse.

Video File 3. S3MKO heart.avi. This video was recorded from a S3MKO mouse.

Video File 4. S1KO S3MKO heart.avi. This video was recorded from a S1KO;S3MKO mouse.

Supplemental Figures:

Suppl. Fig. S1 (Related to Figure 3). Knockout of *SRC-1* and/or *SRC-3* in the myocardial cells

Suppl. Fig. S2 (Related to Figure 3). NCC in S1KO;S3MKO hearts during development

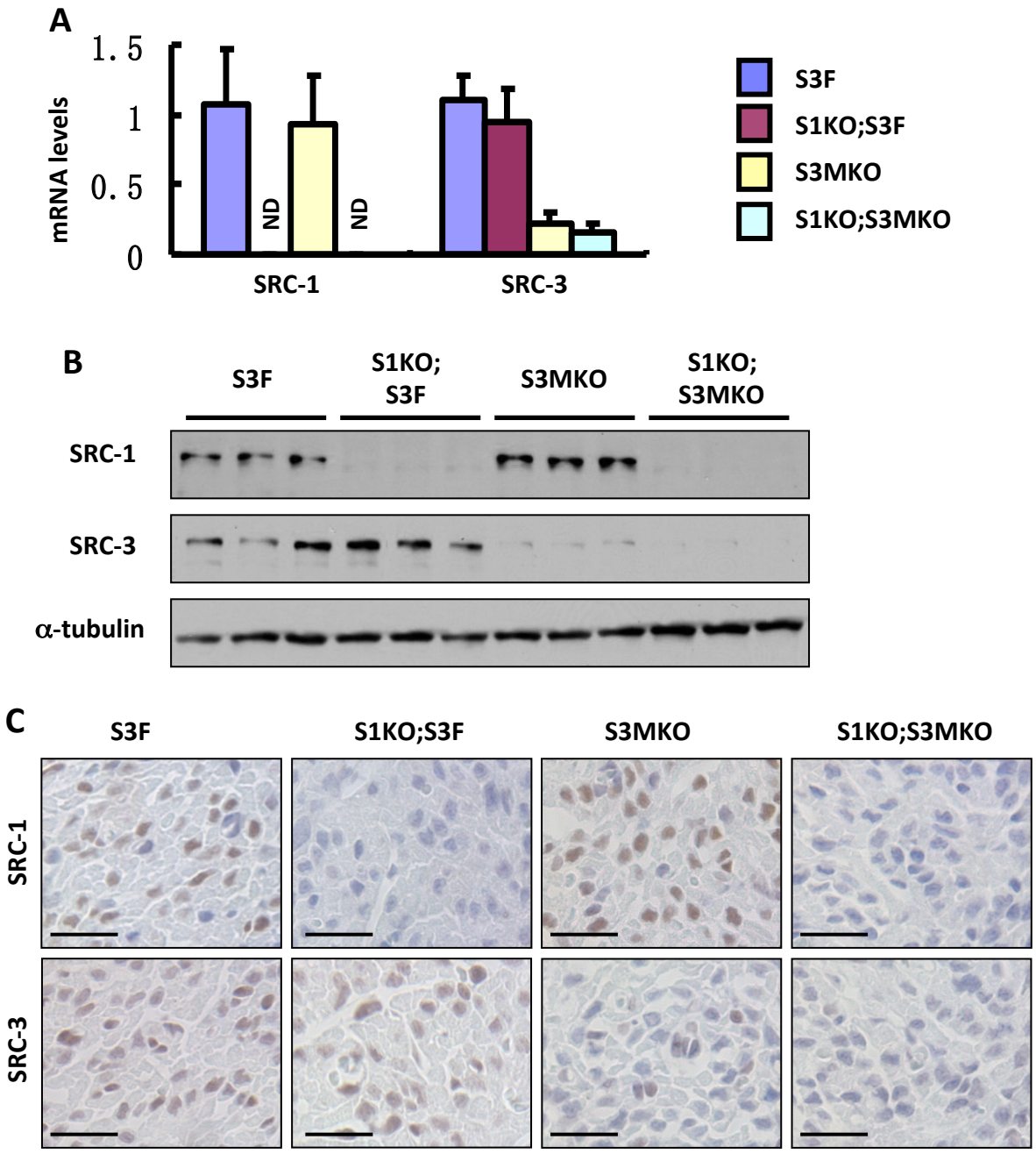
Suppl. Fig. S3 (Related to Figure 5). Analysis of myocardial cell proliferation during heart development

Suppl. Fig. S4 (Related to Figure 5). Immunostaining for actinin in primary myocardial cells in culture.

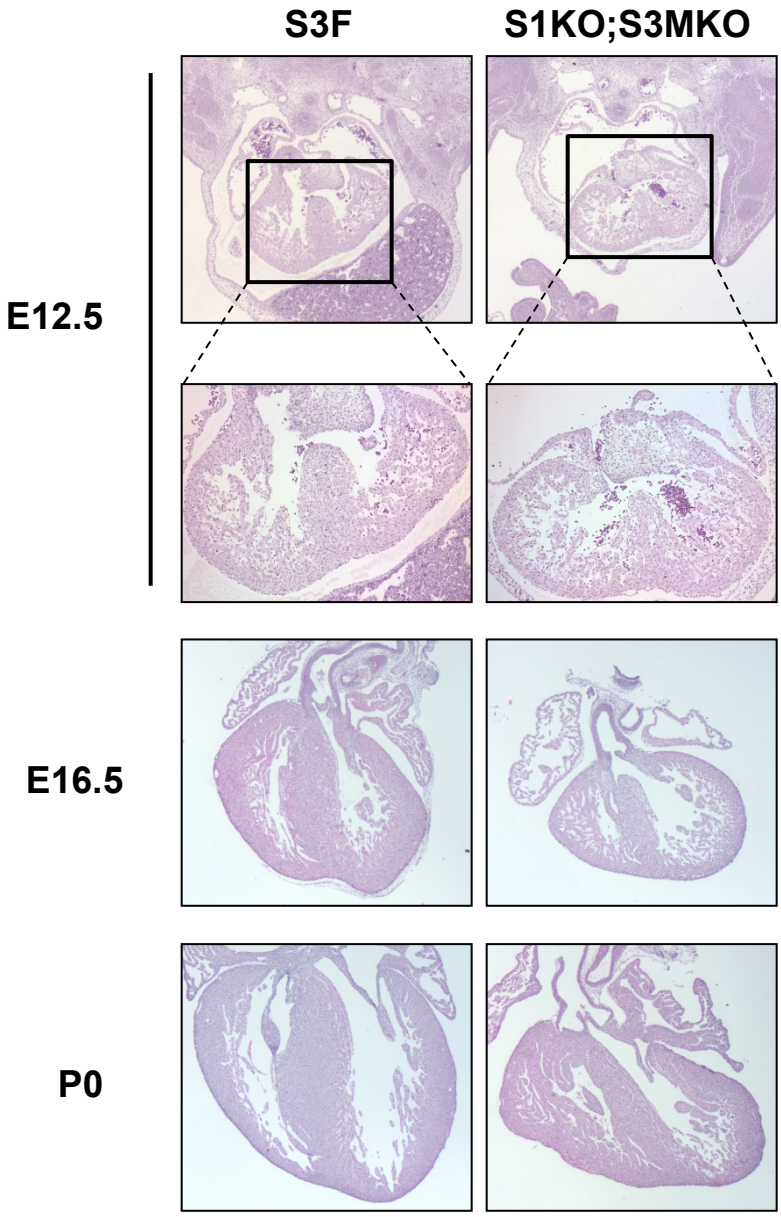
Suppl. Fig. S5 (Related to Figure 5). Adenoviral expression of Cre recombinase to delete the floxed *SRC-3* alleles in cultured primary myocardial cells

Suppl. Fig. S6 (Related to Figure 6). Analysis of cyclin B1, SRC-1, SRC-3 and cyclin E2 expression

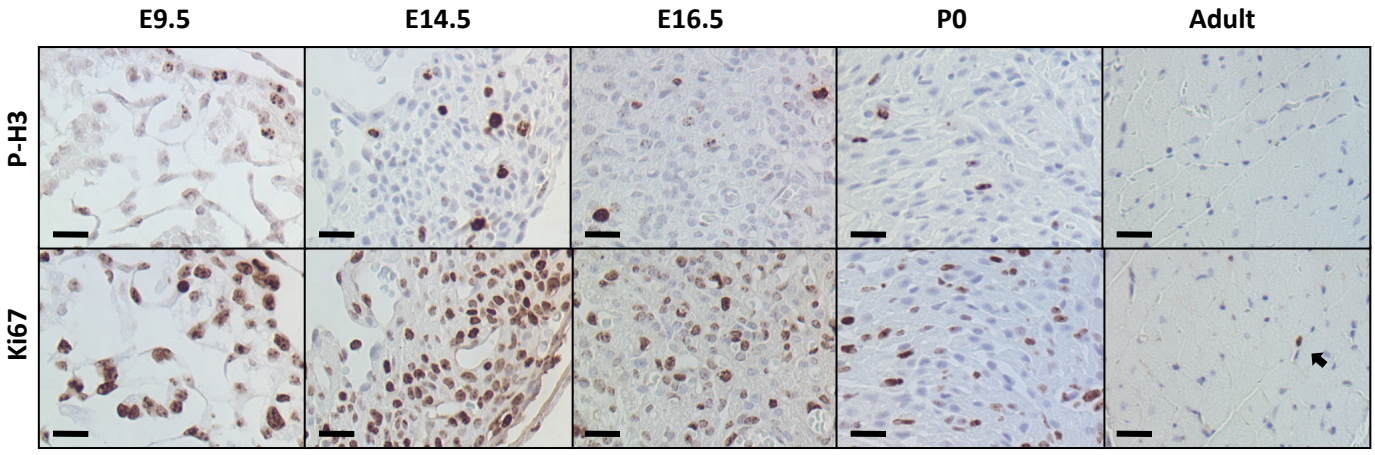
Suppl. Fig. S7 (Related to Figure 6). The relative expression levels of genes relevant to myocardial differentiation



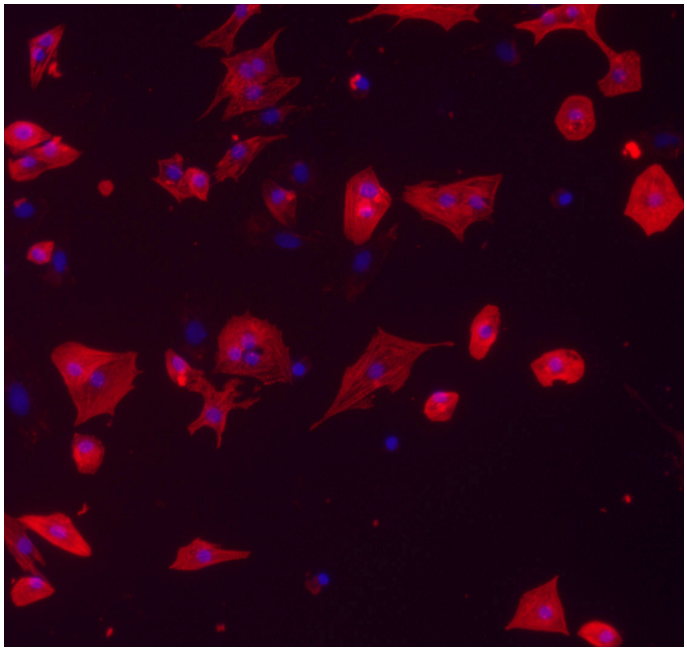
Suppl. Fig. S1. Knockout of *SRC-1* and/or *SRC-3* in the myocardial cells. **A.** Relative mRNA expression levels of *SRC-1* and *SRC-3* in P0 mouse hearts with the indicated genotypes (n = 4–6 for each group). SRC-1 mRNA was not detectable (ND) in S1KO;S3F and S1KO;S3MKO samples. **B.** Western blot analysis of SRC-1 and SRC-3 proteins in the P0 mouse hearts (n = 3) with the indicated genotypes. The α-tubulin served as a loading control. **C.** IHC for SRC-1 and SRC-3 in P0 hearts with the indicated genotypes. The images were taken from the myocardial regions of the ventricular walls. Scale bars, 20 μm.



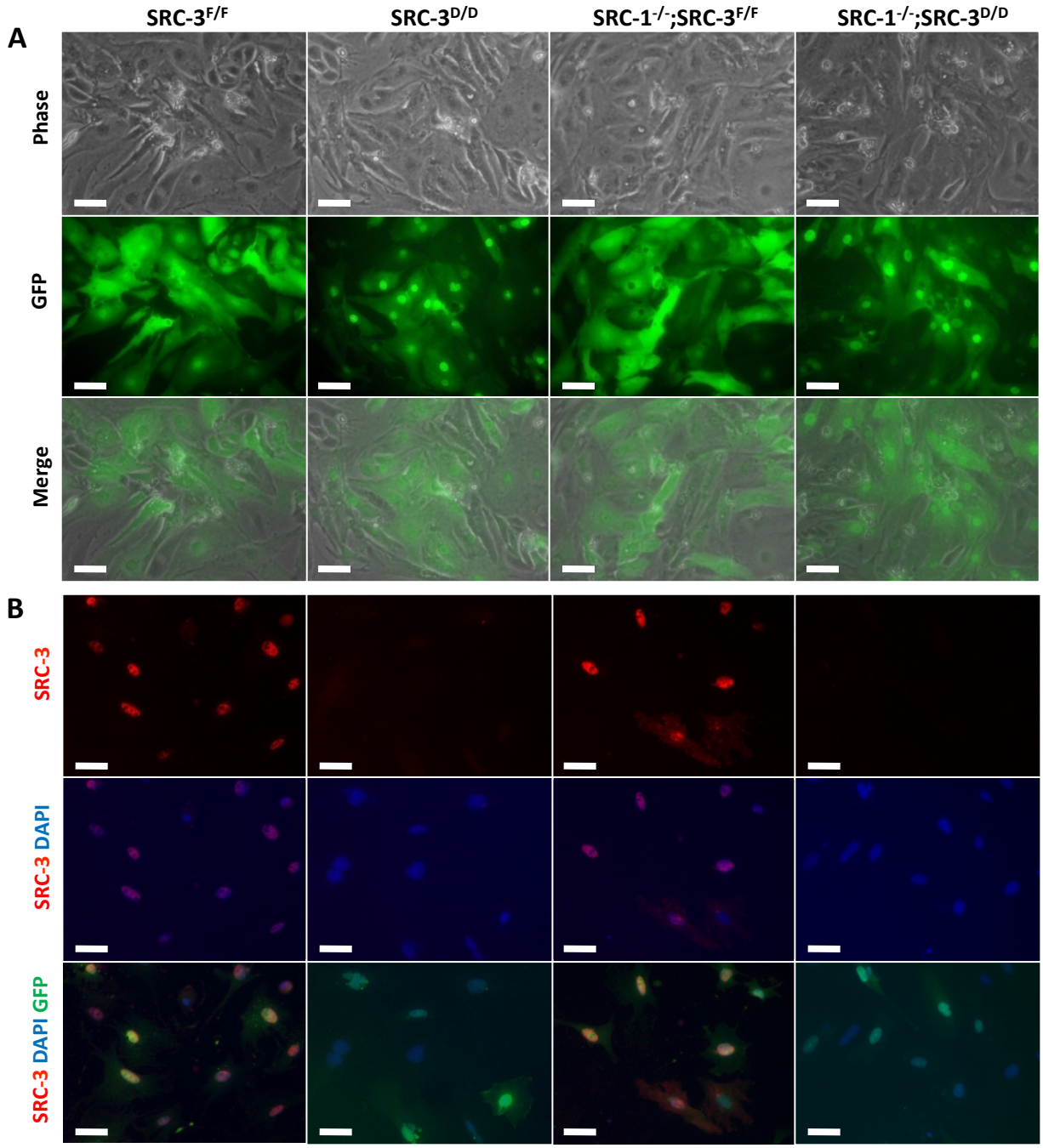
Suppl. Fig. S2. NCC in S1KO;S3MKO hearts during development. H&E stained heart sections prepared from S3F and S1KO;S3MKO mouse hearts during cardiac development at E12.5, E16.5 and P0. At E12.5, S1KO;S3MKO heart showed mild NCC phenotype with a less compacted septums. NCC phenotype was more prominent in the E16.5 and P0 hearts.



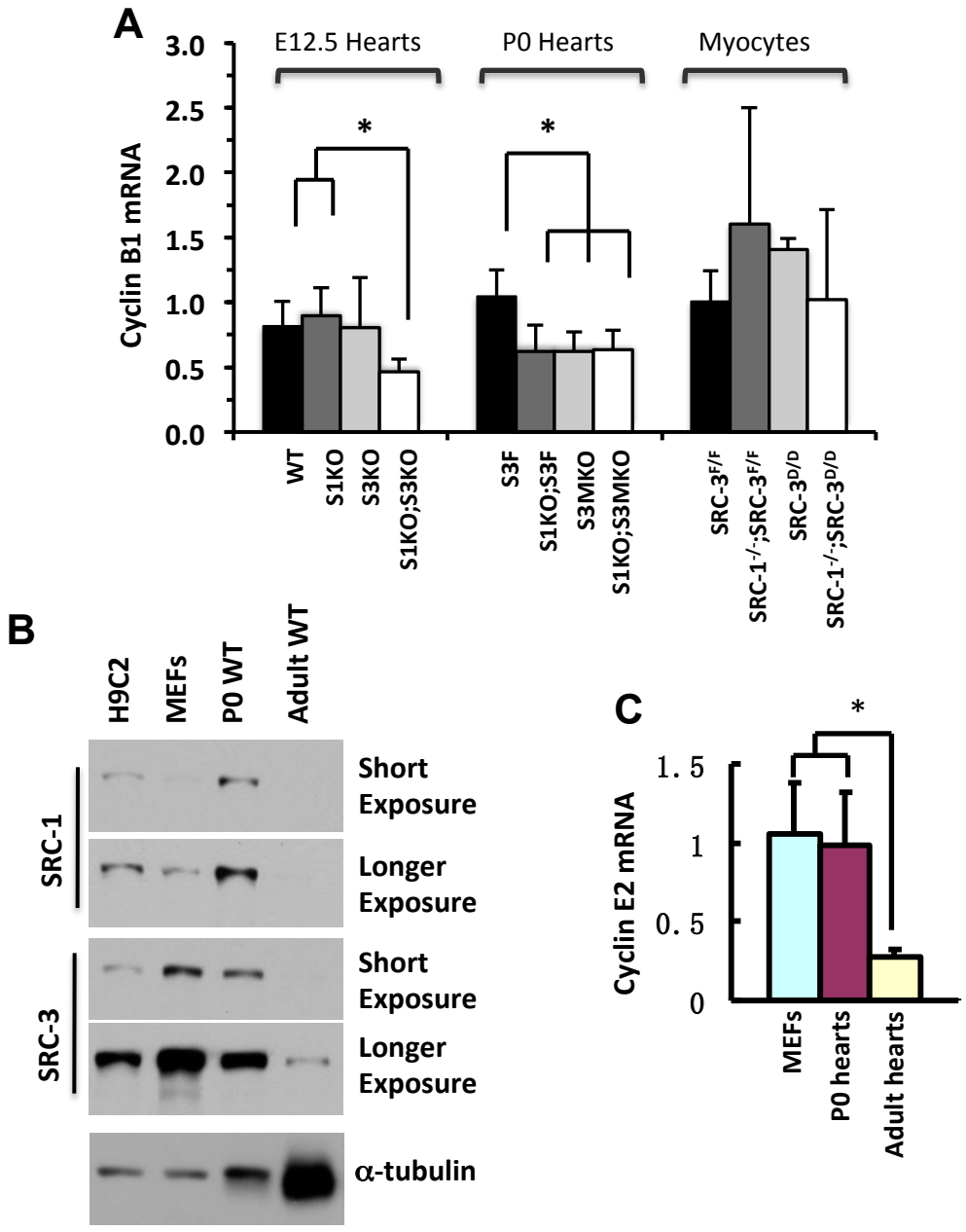
Suppl. Fig. S3. Analysis of myocardial cell proliferation during heart development. The G2/M phase proliferating myocardial cells in the heart tissue sections prepared from normal mice at the indicated developmental stages were detected by IHC for phosphorylated histone H3 (P-H3) (brown color in the upper panels). The proliferating myocardial cells in these sections were also detected by IHC for Ki67 (brown color in the lower panels). The images were taken from the ventricle wall areas. The arrow in the lower right panel indicates a non-myocardial cell positive to Ki67 IHC. Scale bars, 20 μm.



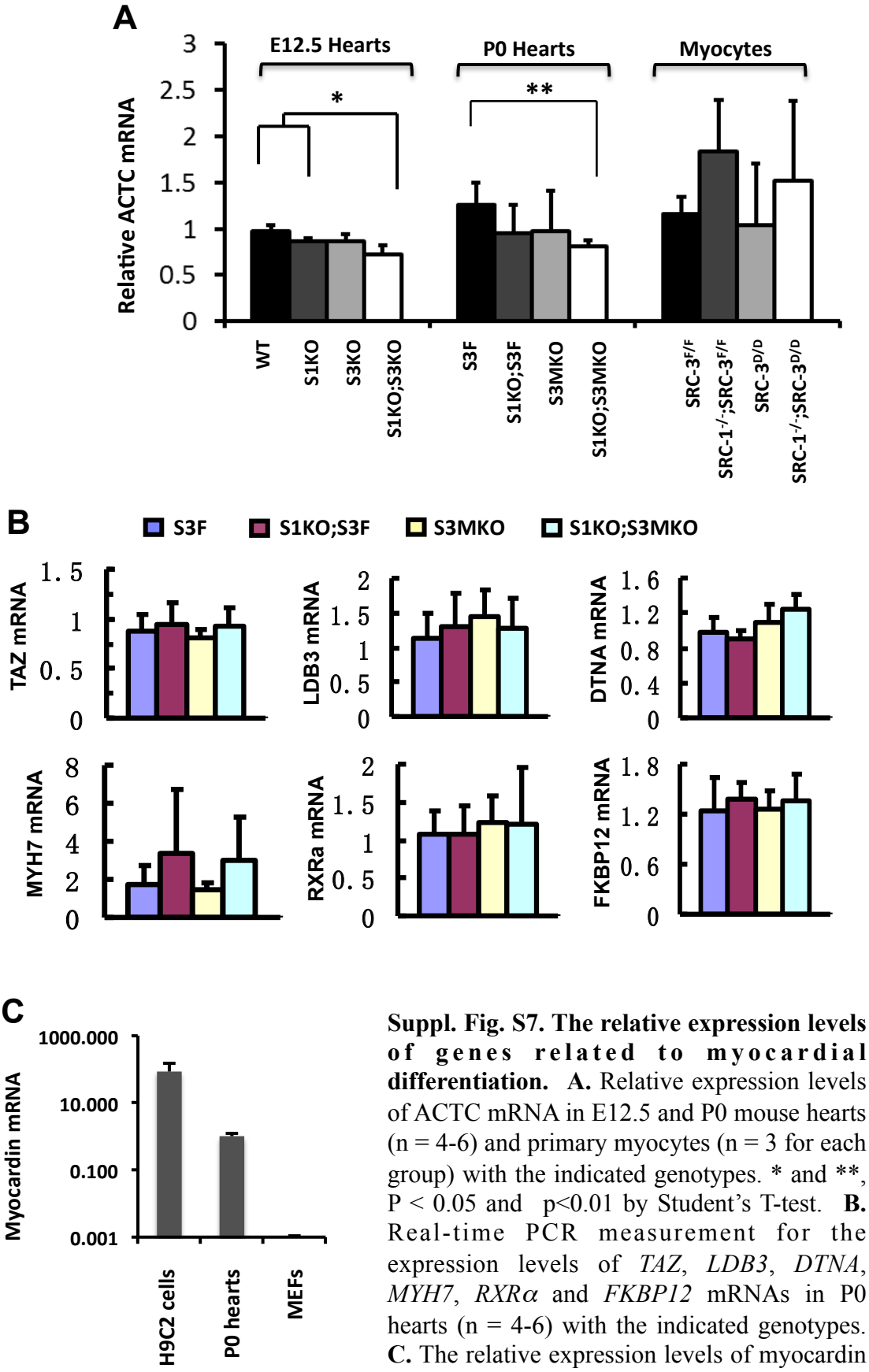
Suppl. Fig. S4. Immunostaining for actinin in primary myocardial cells in culture. The cells were isolated from multiple P0 hearts of newborn *SRC-3$^{f/f}$* mice. Cells were cultured for 7 days before immunofluorescent staining (red) was performed using actinin antibody. Cellular nuclei were stained by DAPI. Ninety-two percent of the total cells in the culture were positive to actinin staining, indicating the majority of the cells that we isolated from these mouse hearts were myocardial cells.



Suppl. Fig. S5. Adenoviral expression of Cre recombinase to delete the floxed *SRC-3* alleles in cultured primary myocardial cells. **A.** The primary myocardial cells were isolated from the hearts of *SRC-3$^{f/f}$* and *SRC-1$^{-/-}$;SRC-3$^{f/f}$* mice at P0. These cells in culture were infected by either the adenovirus expressing GFP to produce *SRC-3$^{f/f}$* and *SRC-1$^{-/-}$;SRC-3$^{f/f}$* cells or the adenovirus expressing Cre recombinase and GFP to produce *SRC-3$^{d/d}$* and *SRC-1$^{-/-}$;SRC-3$^{d/d}$* cells. The infected cells were imaged under a phase-contrast microscope with bright field for cell morphology (upper panels) or fluorescence field for GFP expression (middle panels). The merged images (lower panels) indicate GFP expression in most cells of all groups. **B.** The cells developed in panel A were analyzed by SRC-3 immunocytofluorescence (red color in all panels), DAPI staining (middle and lower panels) and GFP imaging (lower panels). The merged signals of SRC-3 and DAPI as well as SRC-3, DAPI and GFP indicate efficient knockout of SRC-3 in *SRC-3$^{d/d}$* and *SRC-1$^{-/-}$;SRC-3$^{d/d}$* myocardial cells. Scale bars in panels A and B, 50 μm.



Suppl. Fig. S6. Analysis of cyclin B1, SRC-1, SRC-3 and cyclin E2 expression. **A.** The relative mRNA levels of cyclin B1 in E12.5 and P0 hearts (n = 4-6 for each group) and primary myocytes (n = 3 for each group) with indicated genotypes. **B.** Western blot analysis of SRC-1 and SRC-3 proteins in H9C2 cells, MEFs, and wild type P0 and adult mouse hearts. The α-tubulin served as a loading control. α-tubulin level was higher in the adult heart versus earlier stage hearts when same amount of total protein was loaded. **C.** The relative expression levels of cyclin E2 mRNA in MEFs and P0 and adult mouse hearts. * in panels A and C, $p < 0.05$ by Student's T-test.



Suppl. Fig. S7. The relative expression levels of genes related to myocardial differentiation. **A.** Relative expression levels of ACTC mRNA in E12.5 and P0 mouse hearts (n = 4-6) and primary myocytes (n = 3 for each group) with the indicated genotypes. * and **, $P < 0.05$ and $p<0.01$ by Student's T-test. **B.** Real-time PCR measurement for the expression levels of *TAZ*, *LDB3*, *DTNA*, *MYH7*, *RXRα* and *FKBP12* mRNAs in P0 hearts (n = 4-6) with the indicated genotypes. **C.** The relative expression levels of myocardin mRNA in H9C2 cells, P0 mouse hearts and MEFs.