SUPPLEMENTAL MATERIAL

Supplementary Methods

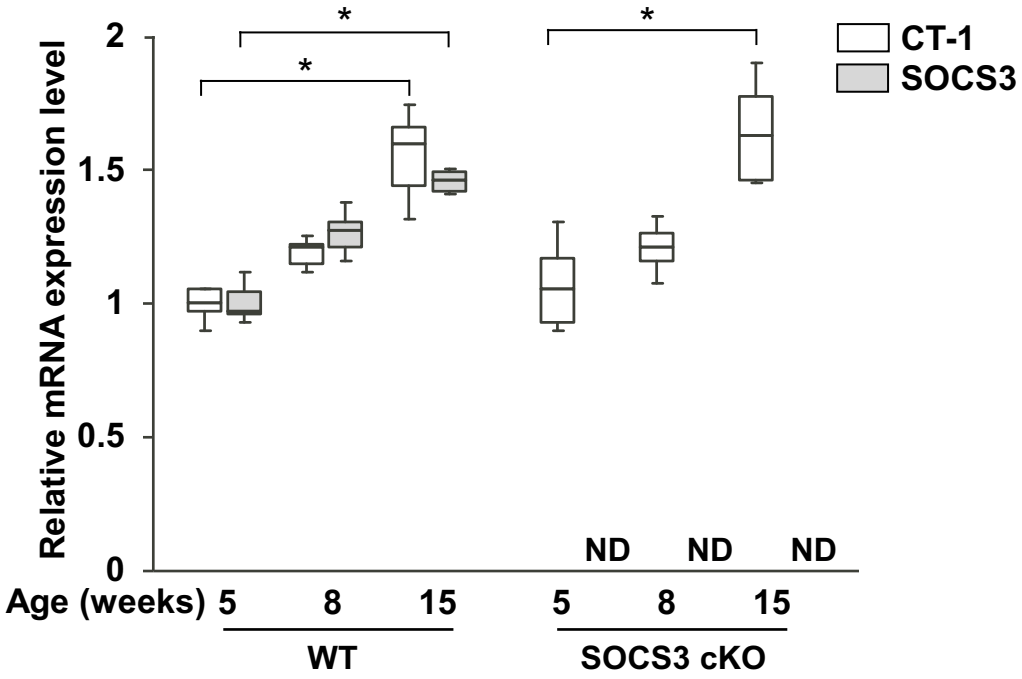
Histology and Transverse electron microscopy (TEM). Hematoxylin and eosin (HE), Masson-trichrome (MT) staining were performed using 10-um paraffin-embedded sections. For TEM, Karnovsky solution, a mixture of 4% glutaraldehyde and 6% formaldehyde was injected into coronary artery by retrograde perfusion to maintain the ultrastructure of the heart.

Quantitative PCR. Total RNA was extracted with Trizol solution (Sigma) followed by reverse transcription with oligo-dT primer using SuperScript III First-Strand (Invitrogen). Quantitative PCR was performed using TaqMan Gene Expression Assay (Applied Biosystems) according to manufacturer's instruction.

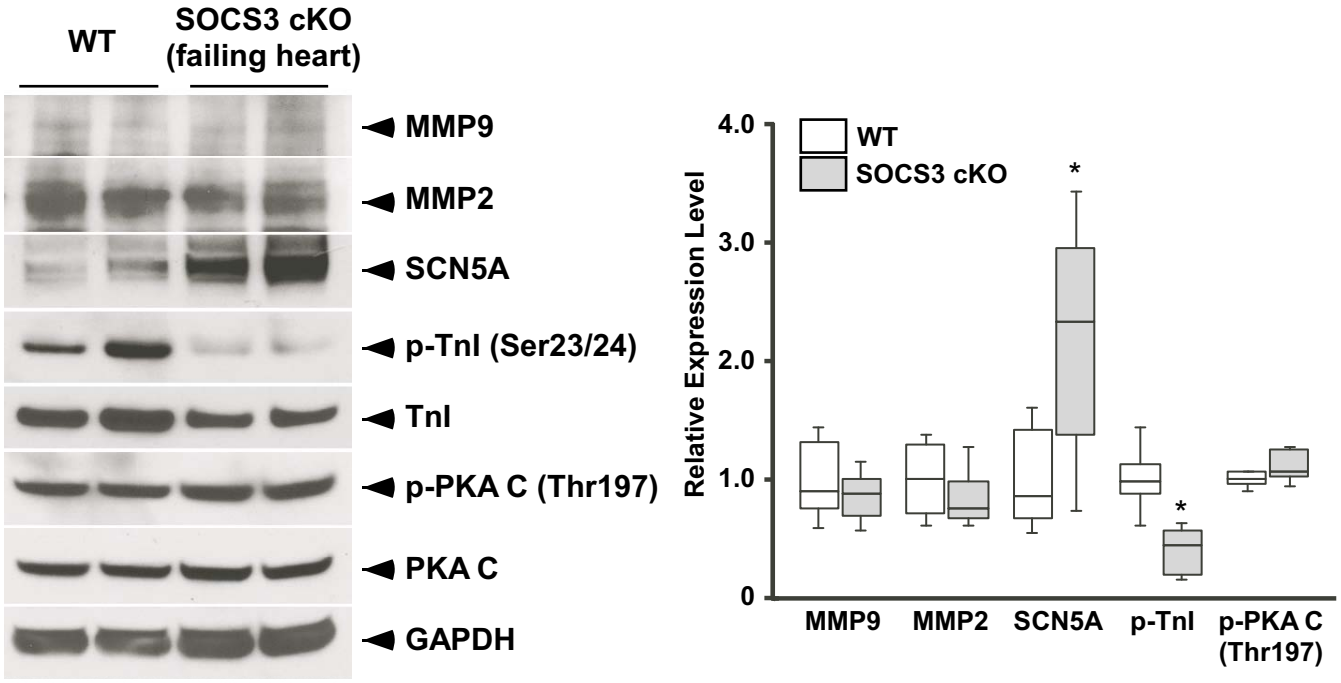
Ca^{2+} transients and sarcomere shortening measurement. After washing with Washing Solution containing 0.5 mM Ca^{2+}, adult ventricular myocytes were incubated in Calcium Transient buffer containing (in mM): 140 NaCl, 5.0 KCl, 1.0 CaCl2,1.2 MgSO4, 10 D-Glucose, 10 HEPES (pH 7.4) for 10 min followed by labeling with 1 μM of Fura-2 AM (Molecular Probe) for 20 min in the buffer. After washing with Calcium transient buffer for 2 times, the cardiomyocytes were field stimulated (square waves, 0.1 mV) at 0.5 Hz until steady state can be achieved. For Ca^{2+} transient measurement, fluorescence was excited at 340/380 nm using a 75W xenon arc lamp on the stage of Nikon Eclipse TE200-U

inverted microscope. The emission wavelength was 535±20 nm. The signal intensity ratio 340/380 nm was calculated as Ca^{2+} concentration (IonWizard, IonOptix). Twitch sarcomere length shortening at 0.5 Hz was detected and measured simultaneously using a video-edge detection system (IonOptix Corp.) The time constant of $[Ca^{2+}]_i$ decline and relaxation were calculated as the time to fall from peak to half its value. For Indo-1 labeling, the cardiomyocytes were incubated with the calcium transient buffer containing 10 μM Indo-1/AM, 1 mg/ml bovine serum albumin, and 0.01% (w/v) Pluronic F-127 for 30 min at room temperature.

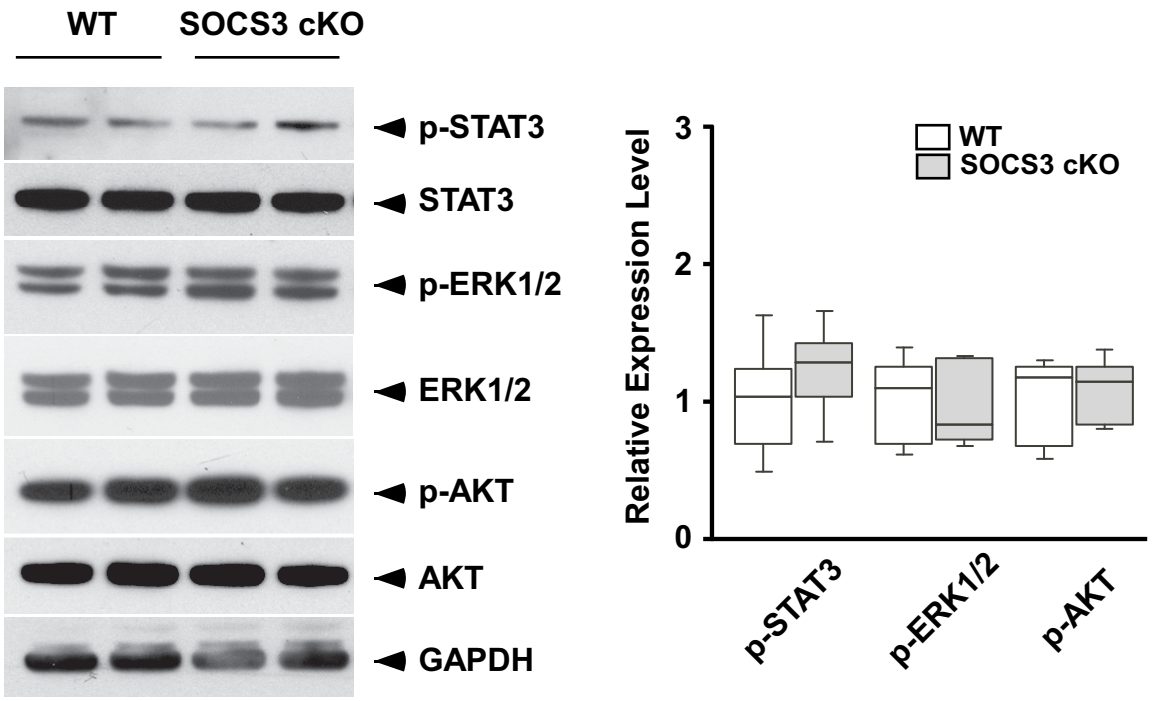
Supplementary Figure 1



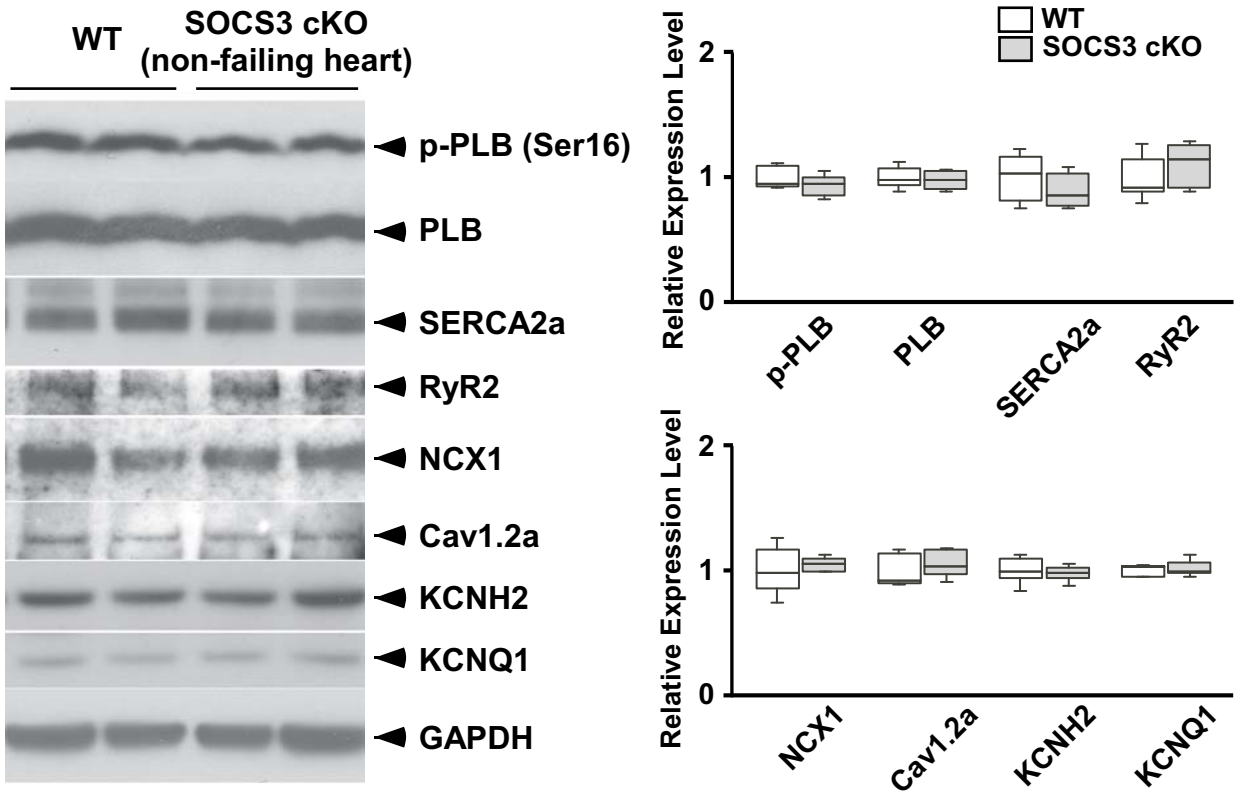
Supplementary Figure 2



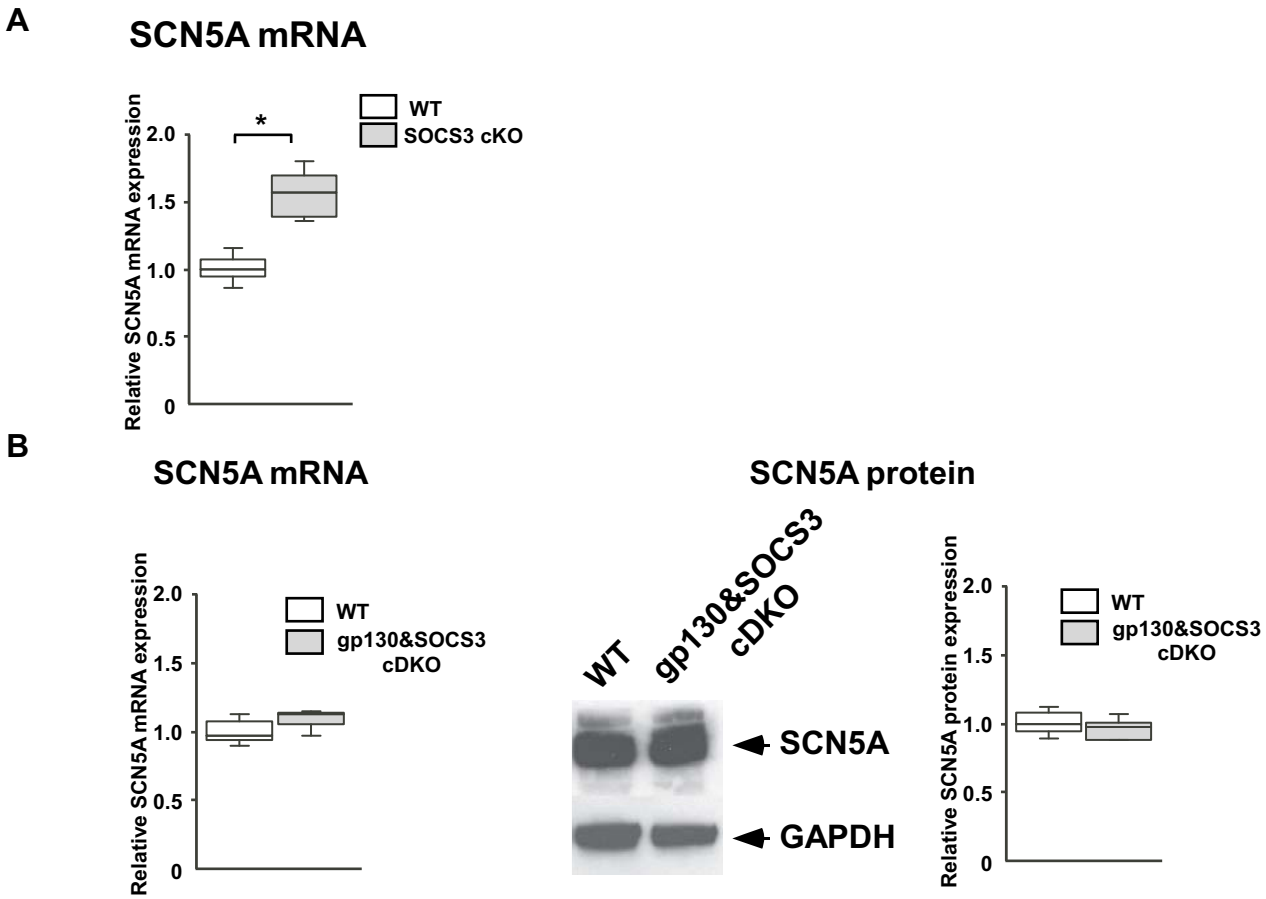
Supplementary Figure 3



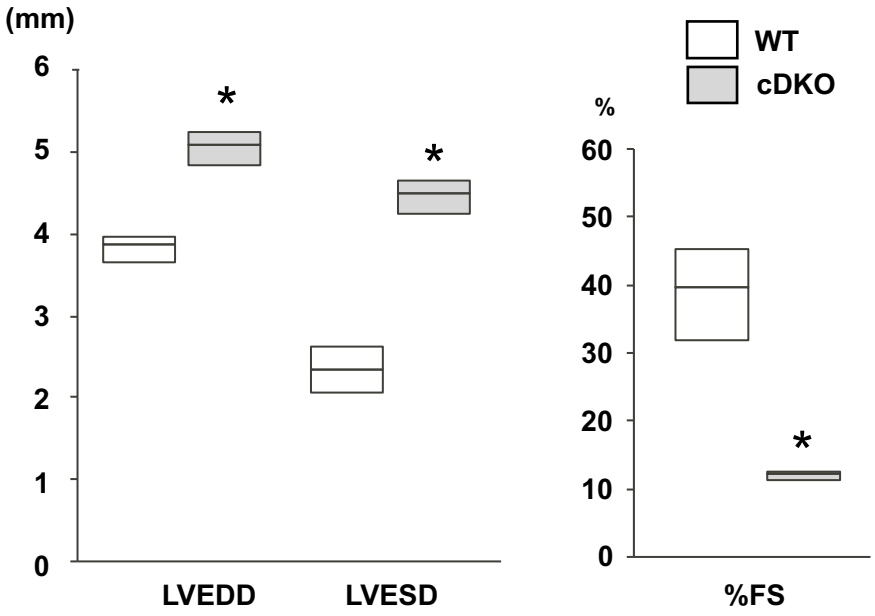
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure legends

Supplementary Figure 1

Postnatal increase in CT-1 and SOCS3 mRNA in the heart. The CT-1 and SOCS3 mRNA expression level in WT and SOCS3 cKO hearts at 5, 8 and 15 weeks of age were examined by quantitative PCR. Results are shown as mean ± SE (n=5 in each group at each time point). * $p<0.05$ comparing 15 weeks of age with 5 weeks of age. ND, not detected.

Supplementary Figure 2

Expression levels of indicated proteins were examined in SOCS3 cKO failing hearts (28 weeks old, n=5) by western-blot. Representative western-blot from three independent experiments is shown. *$p<0.05$ comparing SOCS3 cKO with WT.

Supplementary Figure 3

There were no significant differences in STAT3, ERK1/2 and AKT activation between WT and SOCS3 cKO hearts at 10 days post Sham operation (n=5 in each group).

Supplementary Figure 4

The expression level of proteins that can affect Ca^{2+} transients and SR Ca^{2+} content in SOCS3 cKO non-failing hearts. Representative western-blot from three independent experiments is shown (n=5 in each group). RyR; Ryanodine receptor, NCX1; Na^{+}-Ca^{2+} exchanger, Cav1.2a; L-type Ca^{2+} channel, KCNH2; voltage-gated potassium channel, subfamily H, member 2, KCNQ1; voltage-gated potassium channel, KQT-like subfamily, member 1.

Supplementary Figure 5

Gp130 dependent increase in SCN5A mRNA and protein in SOCS3 cKO (20 weeks old). A, The expression level of SCN5A mRNA were evaluated by quantitative PCR in SOCS3 cKO hearts. B, The expression level of SCN5A mRNA and protein were evaluated in gp130 & SOCS3 cDKO hearts by quantitative PCR and western-blot, respectively (n=5 in each group). * $p<0.05$ comparing SOCS3 cKO with WT. Quantitative PCR was performed at least three times with duplicated samples.

Supplementary Figure 6

Cardiac function of cardiac-specific gp130 & SOCS3 KO mice (cDKO) was examined by echocardiography at 33 weeks of age (n=4 in each group). *$p<0.05$ comparing cDKO

mice with WT.