Supplementary Figure 1. Ectopic calcification in skeletal muscle and cardiac muscle of WT and dKO mice. Quantification of the area of ectopic calcification in skeletal muscle and cardiac muscle of WT and dKO mice. n=6 for both WT and dKO mice. ** indicates p<0.05.

Supplementary Figure 2. Increased accumulation of RhoA expressing cells in ectopic calcification sites in the hearts of dKO mice. Trichrome staining (A), HE staining (B), and immunostaining of dKO heart section with RhoA antibody (C) showed the increased accumulation of RhoA+ cells at the sites of ectopic calcification. The merged images of B and C is shown in (D).
Supplementary Figure 3. Identity of RhoA-expressing (RhoA+) cells in dKO skeletal muscle. Quantification of the number of RhoA+ cells positive with CD68 (marker for macrophages), PDGFR-α (marker for mesenchymal stromal cells/MSCs), and Pax7 (marker for muscle progenitor cells). n=6 dKO mice (8-week old). **indicates p<0.05.

Supplementary Figure 4. CD68+ cells at ectopic calcification sites are positive of RhoA expression. (A) Immunostaining of CD68 and RhoA in the cardiac muscles of WT and dKO mice, showing whether CD68+ cells are positive with RhoA expression or not. (B) Quantitation of the number of CD68+ cells positive of RhoA in dKO muscle with EC, dKO muscle without EC, and WT muscle. n=6 for both WT and dKO mice. ***indicates p<0.05.
Supplementary Figure 5. Sorting of CD68+ cells from muscle-derived cells of WT and dKO mice. *Fluorescence activated cell sorting* (FACS) of CD68+ cells shows much higher number and ratio of CD68+ cells among all the muscle-derived cells from gastrocnemius muscle of dKO mice, compared to that of WT mice.
Supplementary Figure 6. *In vivo* inhibition of RhoA/ROCK signaling in dKO mice improved dystrophic phenotypes in hearts. Alizarin Red staining showed reduced calcification in heart tissue with Y-27632 treatment. Trichrome staining showed reduced fibrosis formation in heart tissue with Y-27632 treatment. Immunostaining with CD68 and RhoA antibodies showed reduced number of CD68+/RhoA+ cells after Y-27632 treatment. Immunostaining of cardiac muscle with RhoA- and CD163-specific antibodies revealed that the ratio of CD163+ cells (M2 macrophage) to RhoA+ cells (mainly M1 macrophage) increased after Y27632 treatment, suggesting that the M1 to M2 phase transition of macrophages could have occurred in the Y-27632-treated dystrophic muscles.