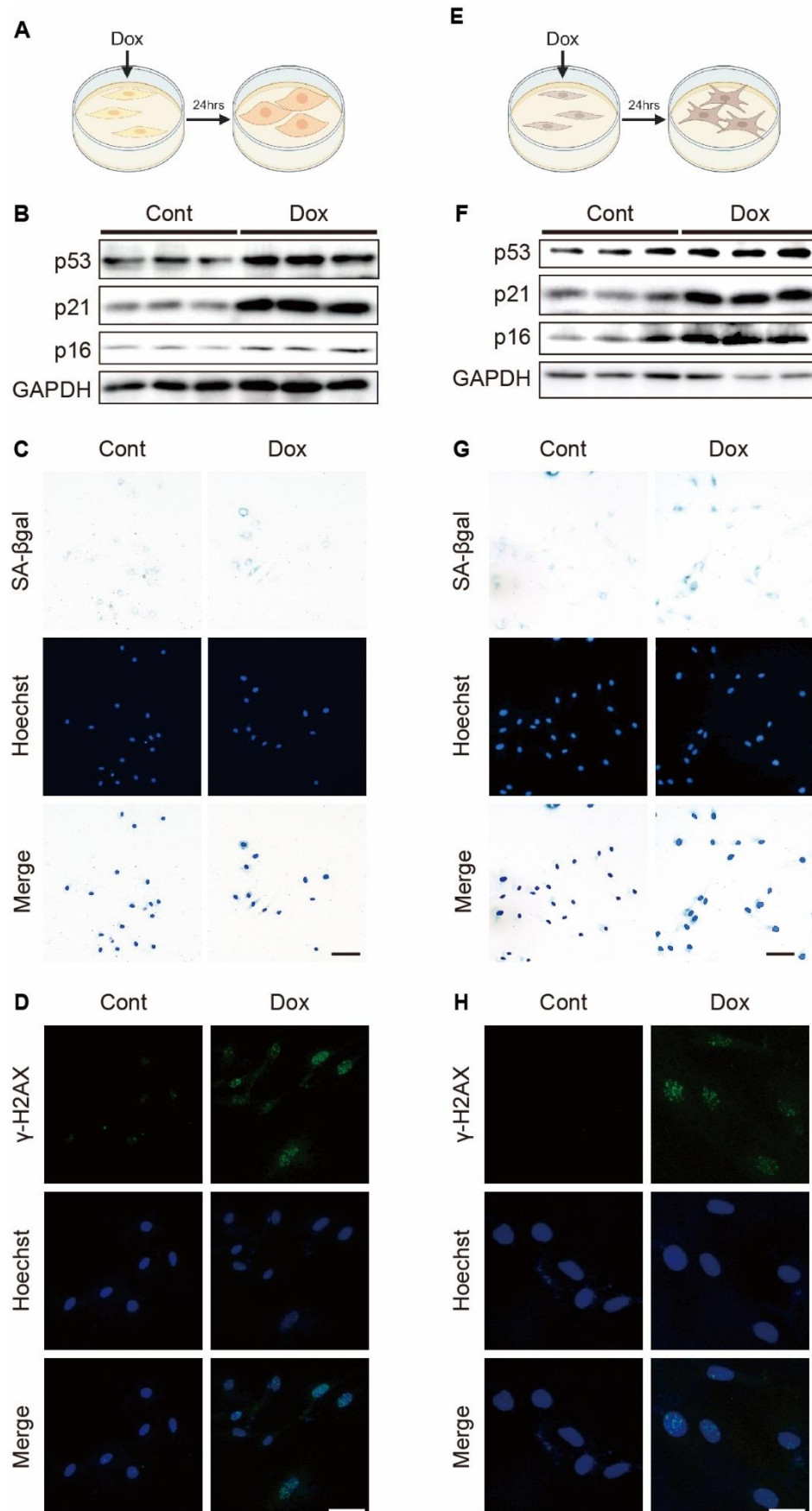


Supplementary Data

The Matricellular Protein CCN5 (WISP2) inhibits Cellular Senescence in Cardiac Myoblasts and Fibroblasts

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and Woo Jin Park

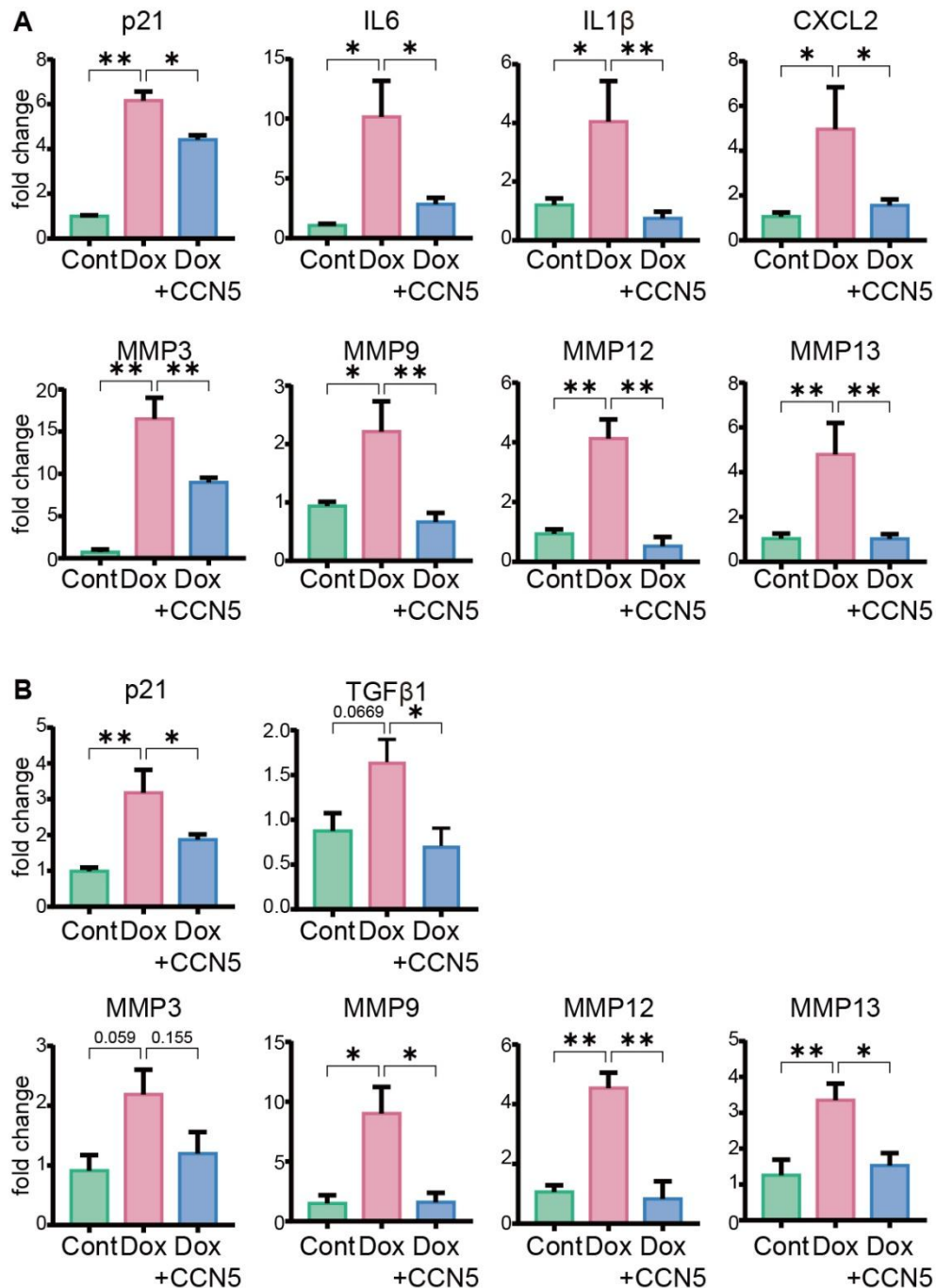
Supplementary Figure 1.



Supplementary Figure 1. Doxorubicin (Dox) induces cellular senescence in H9c2 cardiac myoblasts and fibroblasts.

(A) An experimental scheme is shown. (B) H9c2 cardiac myocytes were treated with Dox. Cell lysates were immunoblotted for senescence marker proteins p53, p21, p16 and GAPDH. Panel B is a composite from separate blots. The full-length blots are presented in Supplementary Fig. 7. (C) H9c2 cells were stained for SA- β -gal activity. (D) H9c2 cells were immunostained with antibodies against γ H2AX (green) and counter-stained with Hoechst (blue) (Original magnification, 200x (20x objective)). (E) An experimental scheme is shown (created using Biorender). (F) Cardiac fibroblasts were treated with Dox. Cell lysates were immunoblotted for senescence marker proteins p53, p21, p16 and GAPDH. Panel F is a composite from separate blots. The full-length blots are presented in Supplementary Fig. 7. (G) Cardiac fibroblasts were stained for SA- β -gal activity. (H) Cardiac fibroblasts were immunostained with antibodies against γ H2AX (green) and counter-stained with Hoechst (blue) (Original magnification, 200x (20x objective)). Scale bar: 50 μ m.

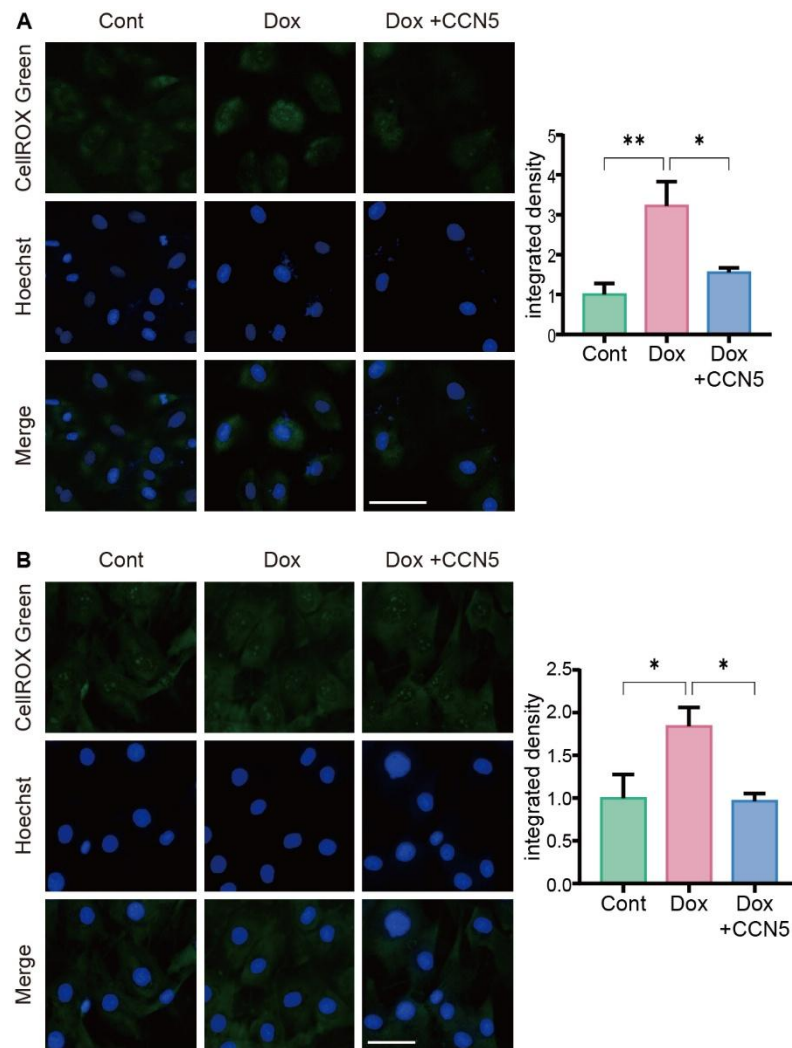
Supplementary Figure 2.



Supplementary Figure 2. CCN5 modulates SASP factors in H9c2 cardiac myoblasts and cardiac fibroblasts.

(A) In H9c2 cells, Dox were treated 24 hours, followed by CCN5 treatment. mRNA expression levels of SASP factors were analyzed by qPCR. (n=4~8) (B) In cardiac fibroblasts, Dox were treated 24 hours, followed by CCN5 treatment. mRNA expression levels of SASP factors were analyzed by qPCR. (n=4~8) Data are shown as the mean $\pm$ SEM. (one-way ANOVA; *p < 0.05, **p < 0.01).

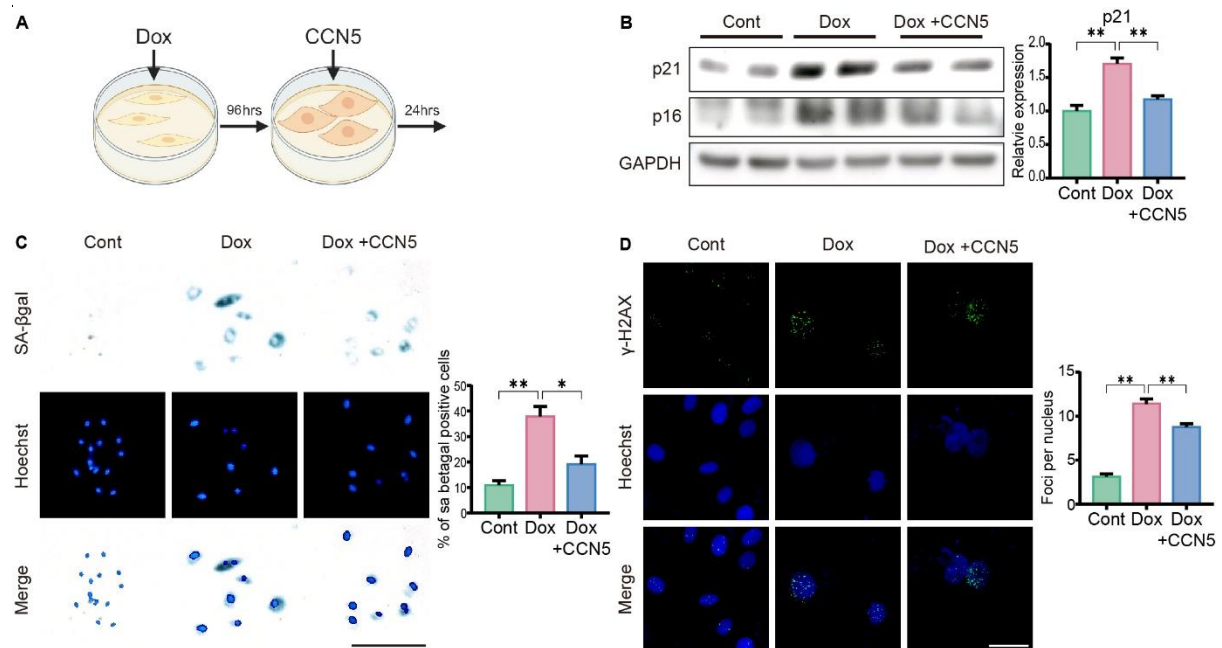
Supplementary Figure 3



Supplementary Figure 3. CCN5 attenuates Dox-induced ROS generation in H9c2 cardiac myoblasts and cardiac fibroblasts.

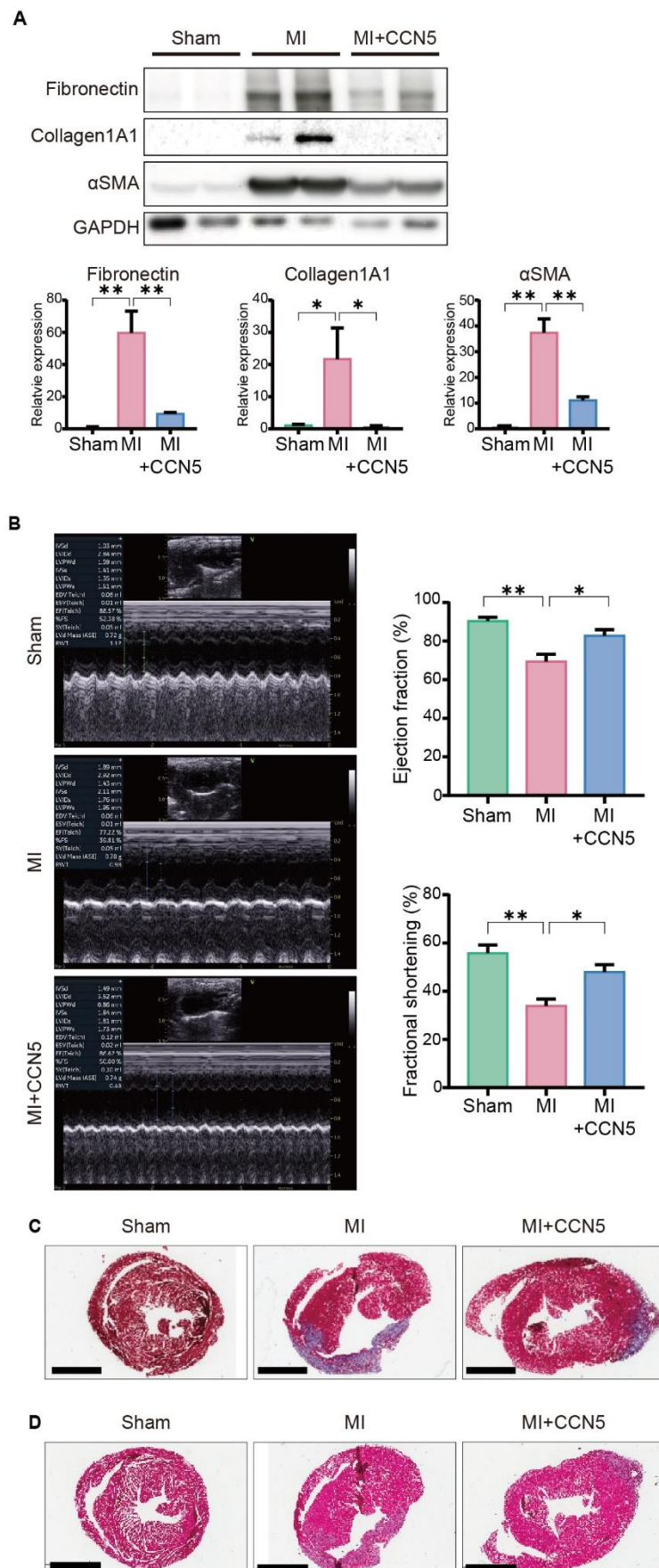
(A) H9c2 cells were treated with Dox (100 nM) for 48 hours, followed by CCN5 treatment. Intracellular ROS levels were measured using CellROX Green reagent. H9c2s were counter-stained with Hoechst (blue) (Original magnification, 200x (20x objective)). (n=4) (B) Cardiac fibroblasts were treated with Dox (100 nM) for 24 hours, followed by CCN5 treatment. Intracellular ROS levels were measured using CellROX Green reagent. Cardiac fibroblasts were counter-stained with Hoechst (blue) (n=4) (Original magnification, 200x (20x objective)). Scale bar: 50 μ m. Data are shown as the mean $\pm$ SEM. (one-way ANOVA; *p < 0.05, **p < 0.01).

Supplementary Figure 4



Supplementary Figure 4. CCN5 inhibits cellular senescence of H9c2 cardiac myoblasts in long-term Dox treatment. (A) An experimental scheme is shown. (B) H9c2 cells were treated with Dox (100 nM) for 4 days. Media containing Dox was refreshed every 48 hours. CCN5 was treated for the 24 hours. Cell lysates were immunoblotted for senescence marker proteins p21, p16 and GAPDH. (n=4) (C) H9c2 cells were stained for SA-β-gal activity. Positive cells were counted in random fields and plotted as a percentages of total cell number (n=3) (D) H9c2 cells were immunostained with antibodies against γH2AX (green) and counter-stained with Hoechst (blue). γH2AX foci were counted in random fields and plotted as a percentage of total nuclei (n=4) (Original magnification, 200x (20x objective)). Scale bar: 50 μm. Data are shown as the mean ± SD. (one-way ANOVA; *p < 0.05, **p < 0.01).

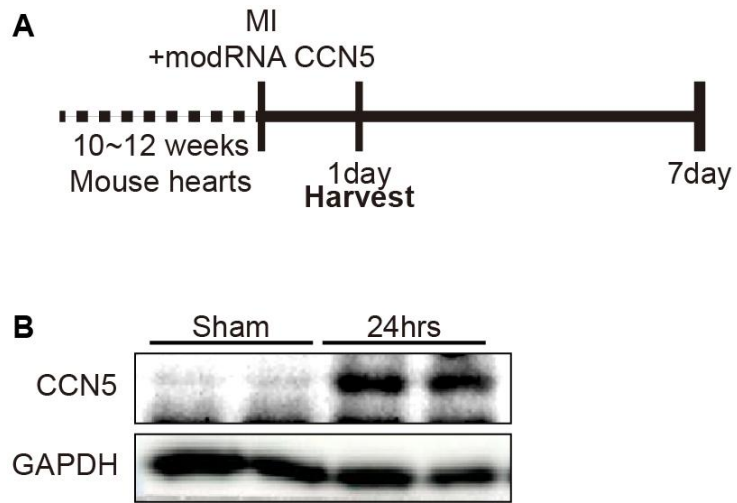
Supplementary Figure 5



Supplementary Figure 5. Validation of myocardial infarction (MI) model.

(A) Heart extract was immunoblotted for senescence marker proteins fibronectin, collagen1A1 and α SMA. Protein levels were quantified and normalized to GAPDH (n=7). Panel A is a composite from separate blots for clarity. The uncropped blots are presented in Supplementary Fig. 7. (B) Echocardiographic analysis showing Fractional Shortening (FS) and Ejection Fraction (EF) in Sham group and MI group. (n=4) (C) Masson's Trichrome and (D) H&E staining confirming fibrotic remodeling across the MI groups. Scale bar: 2mm. Data are shown as the mean $\pm$ SEM. (one-way ANOVA; *p < 0.05, **p < 0.01).

Supplementary Figure 6.



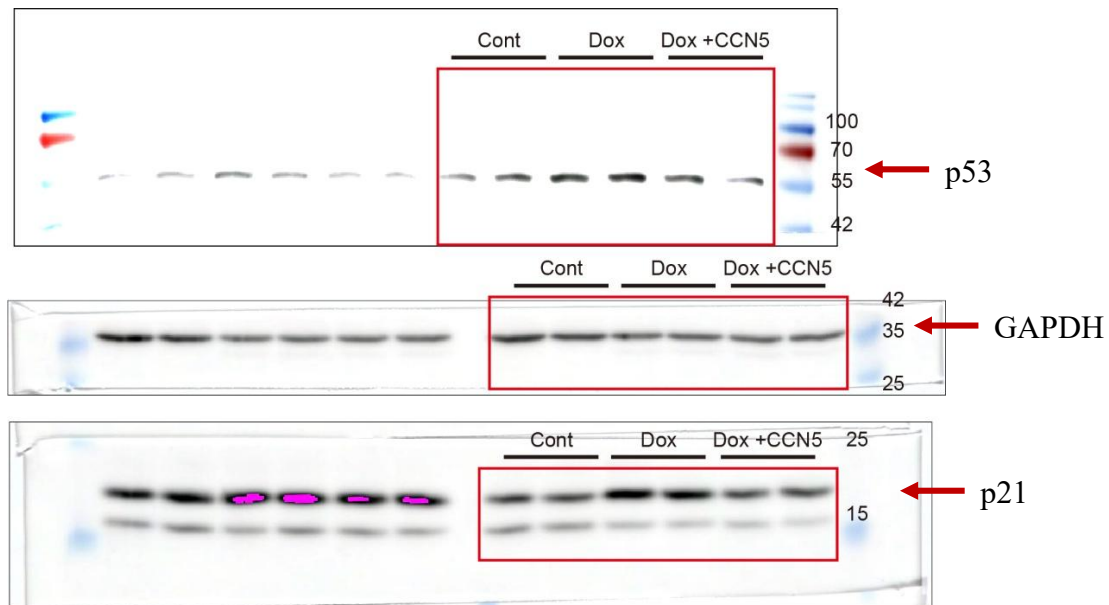
Supplementary Figure 6. ModRNA-CCN5 was expressed via intramyocardial injection

(A) An experimental scheme is shown. (B) Heart extract was immunoblotted for CCN5 and GAPDH. Panel B is a composite from separate blots. The full-length blots are presented in Supplementary Fig. 7.

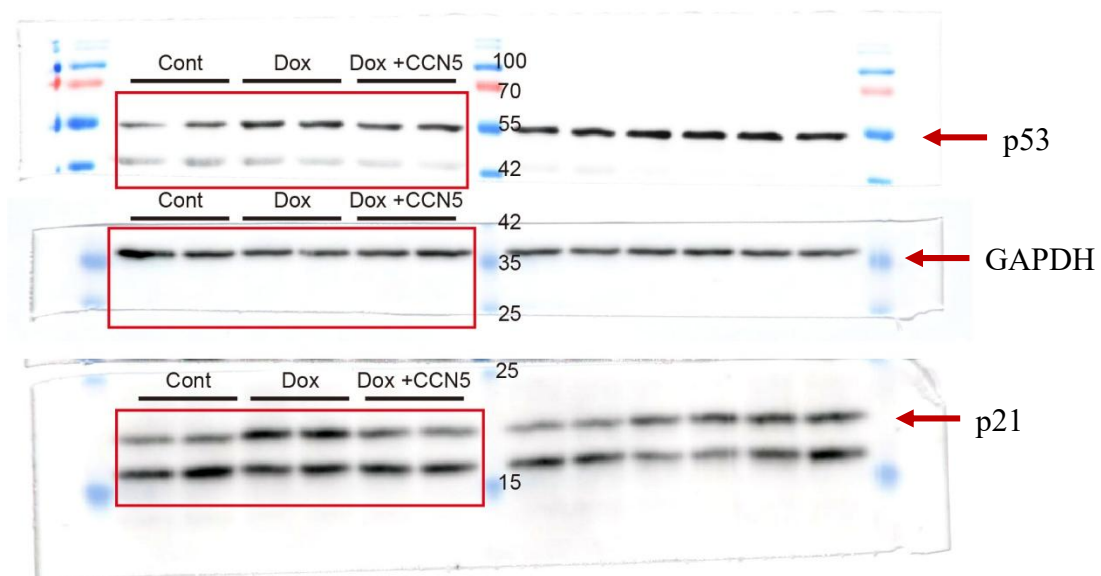
Supplementary Figure 7. Western Blots Uncropped Images

Protein ladder information: PageRuler™ Prestained Protein Ladder, 10 to 180 kDa (CST 26616)

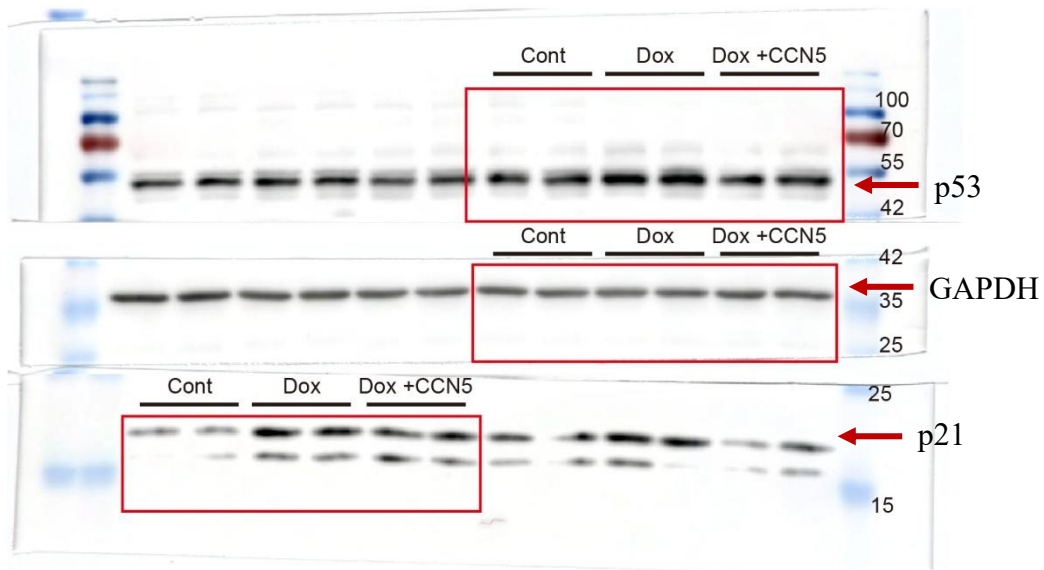
(Figure 1B)



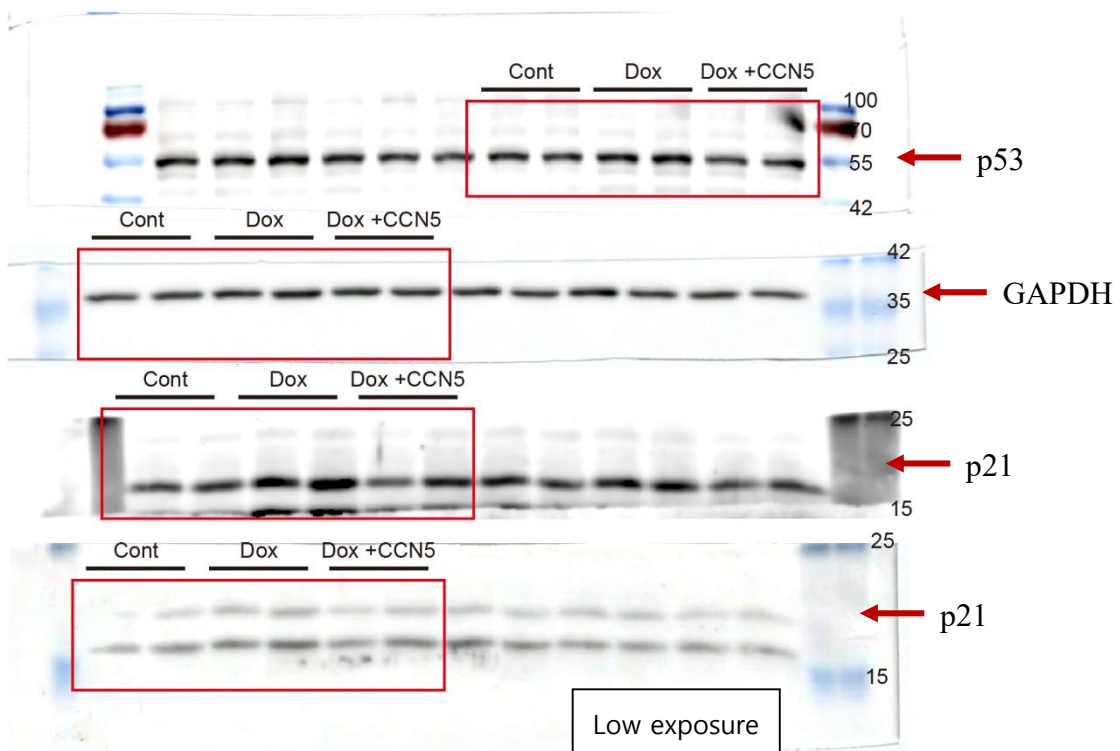
(Figure 2B)



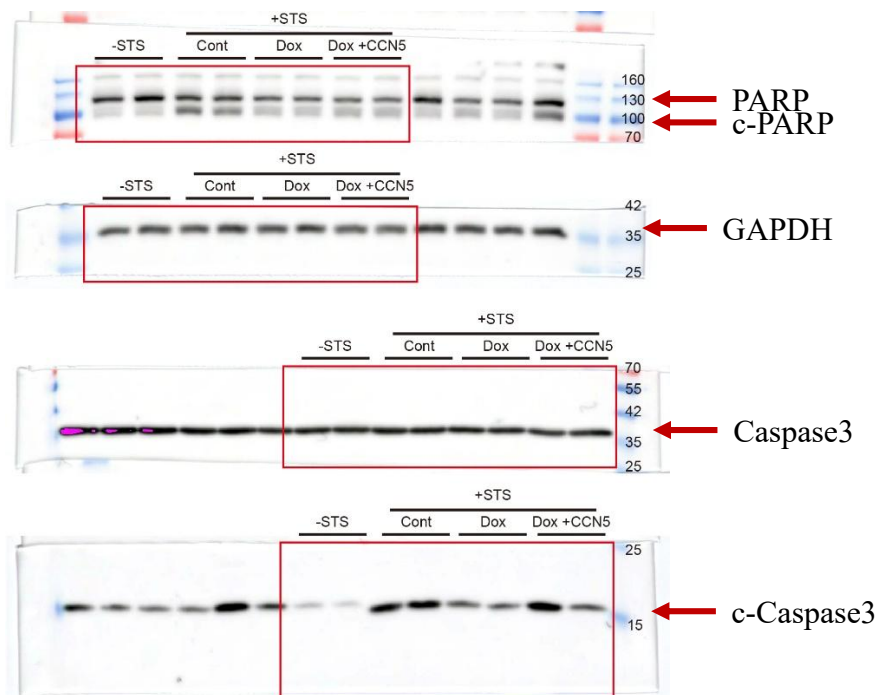
(Figure 3B)



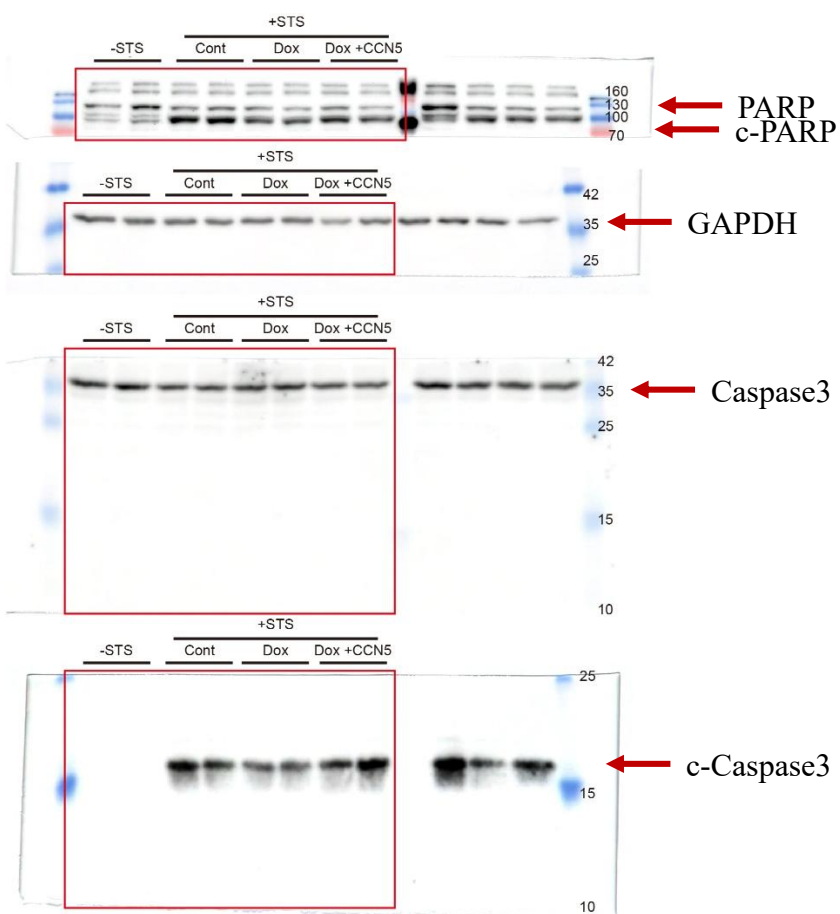
(Figure 4B)



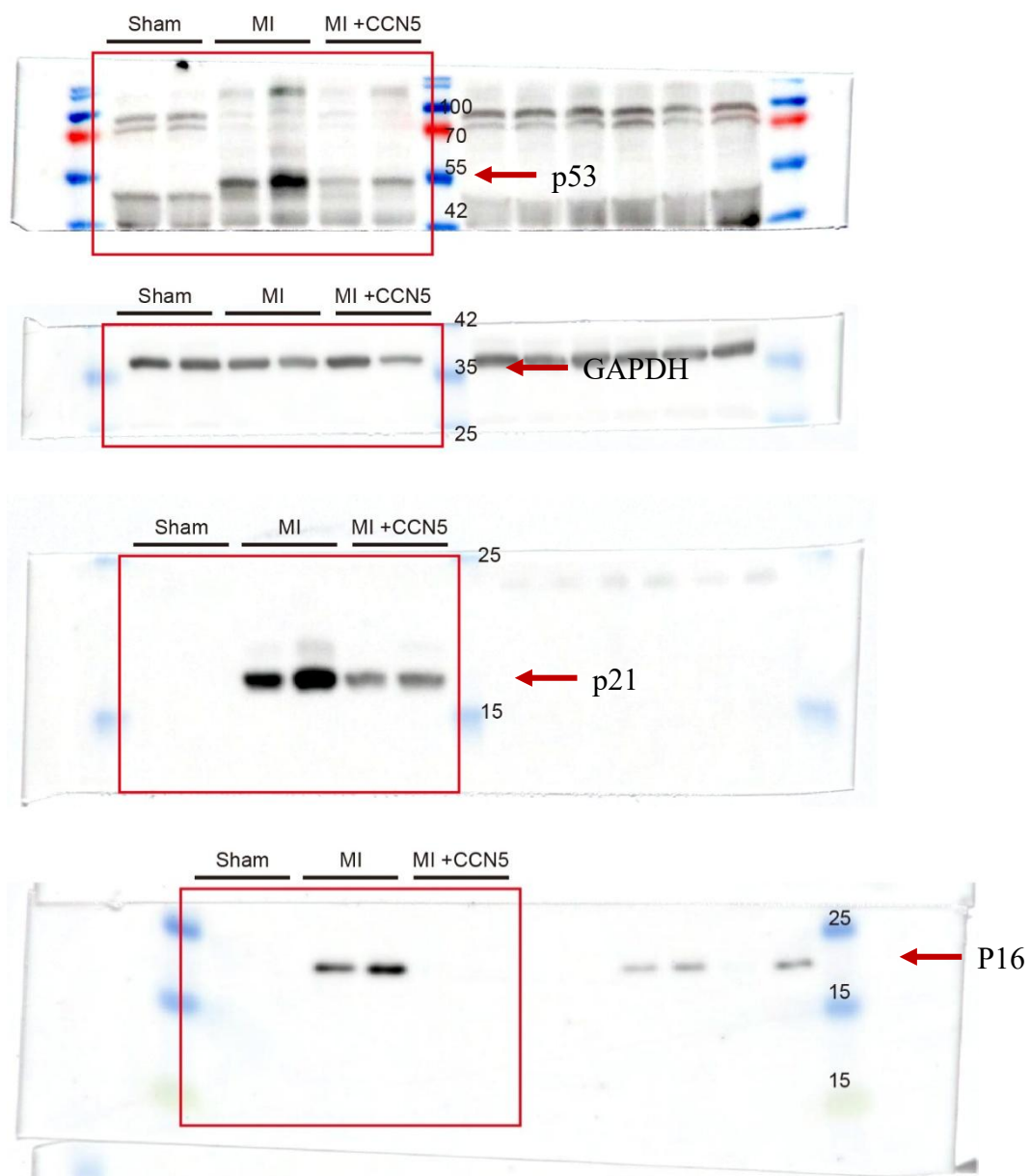
(Figure 5B)



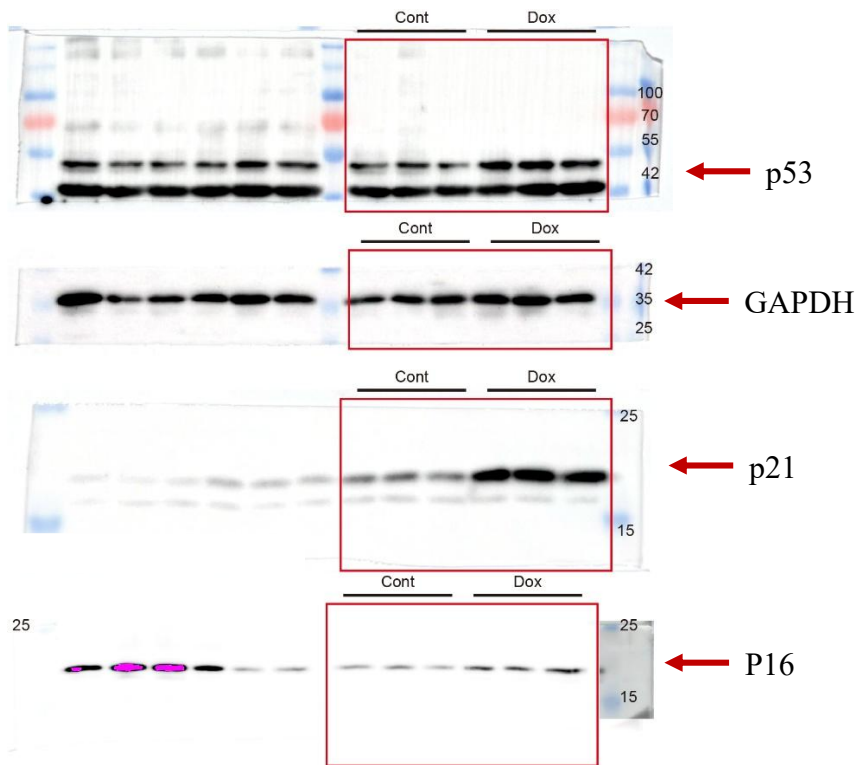
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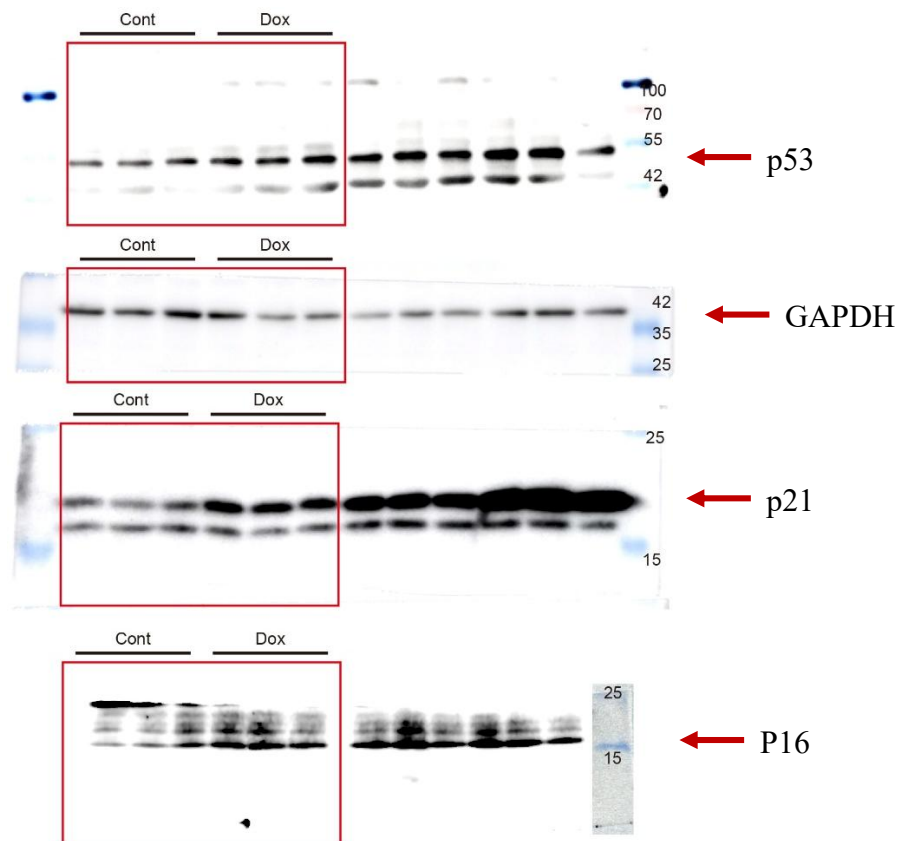
(Figure 6B)



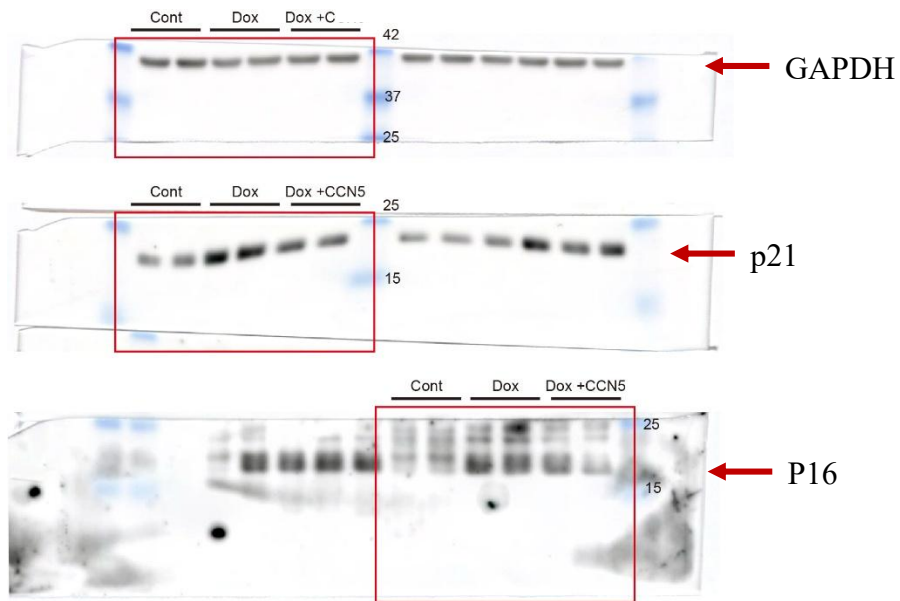
(Supplementary Figure 1B)



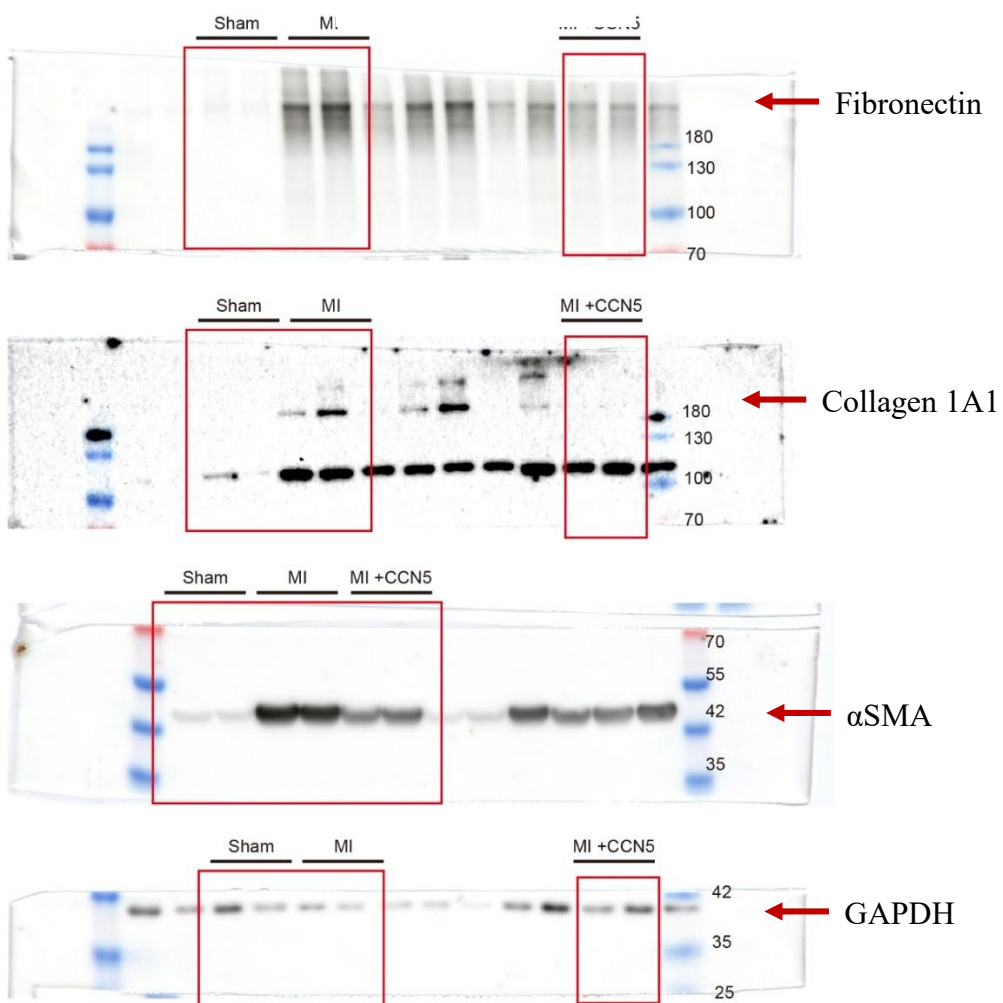
(Supplementary Figure 1F)



(Supplementary Figure 4B)



(Supplementary Figure 5A)



(Supplementary Figure 6B)

