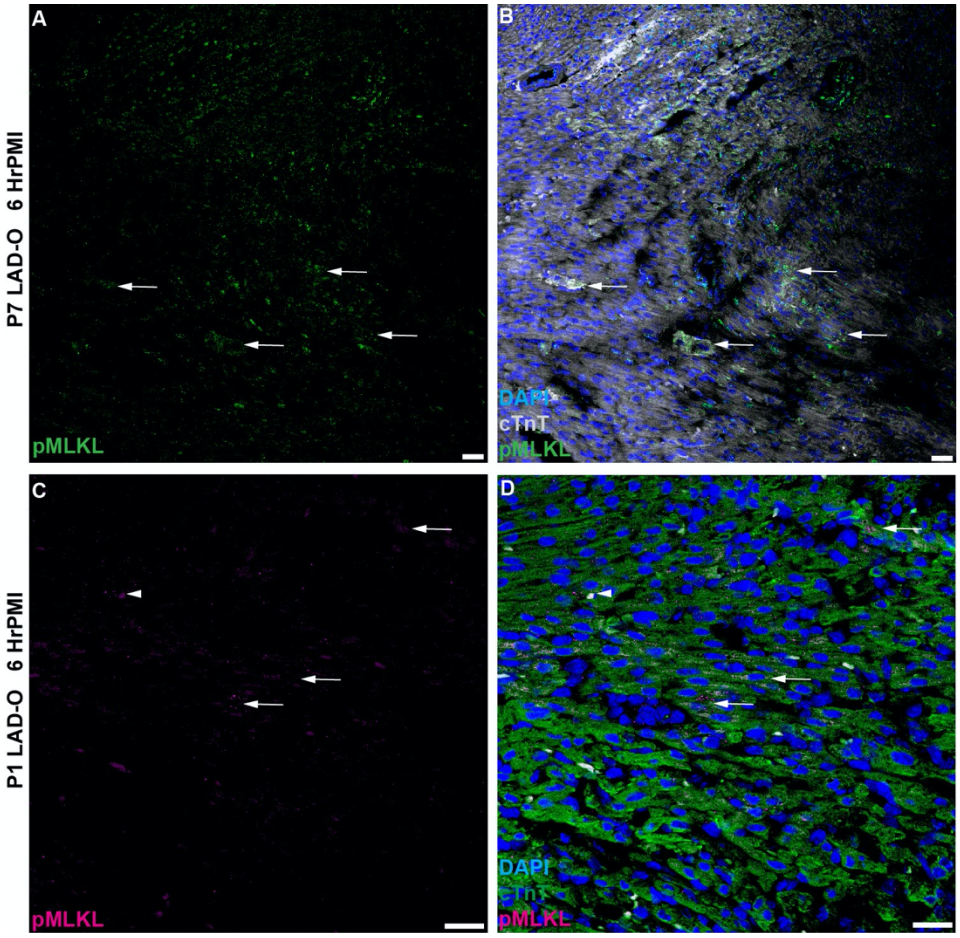

Figure S1. Necroptosis in cardiomyocytes immediately after MI, related to Figure 1. (A, B) Heart tissue section of wild type mouse stained for pMLKL (Green) and cTnT (grey) at 6 hours after P7 LAD-O. (C, D) Heart tissue section of wild type mouse stained for pMLKL (magenta) and cTnT (green) at 6 hours after P1 LAD-O. DAPI in blue. Arrows, cardiomyocytes positive for pMLKL. Scale bar, 25 μm.

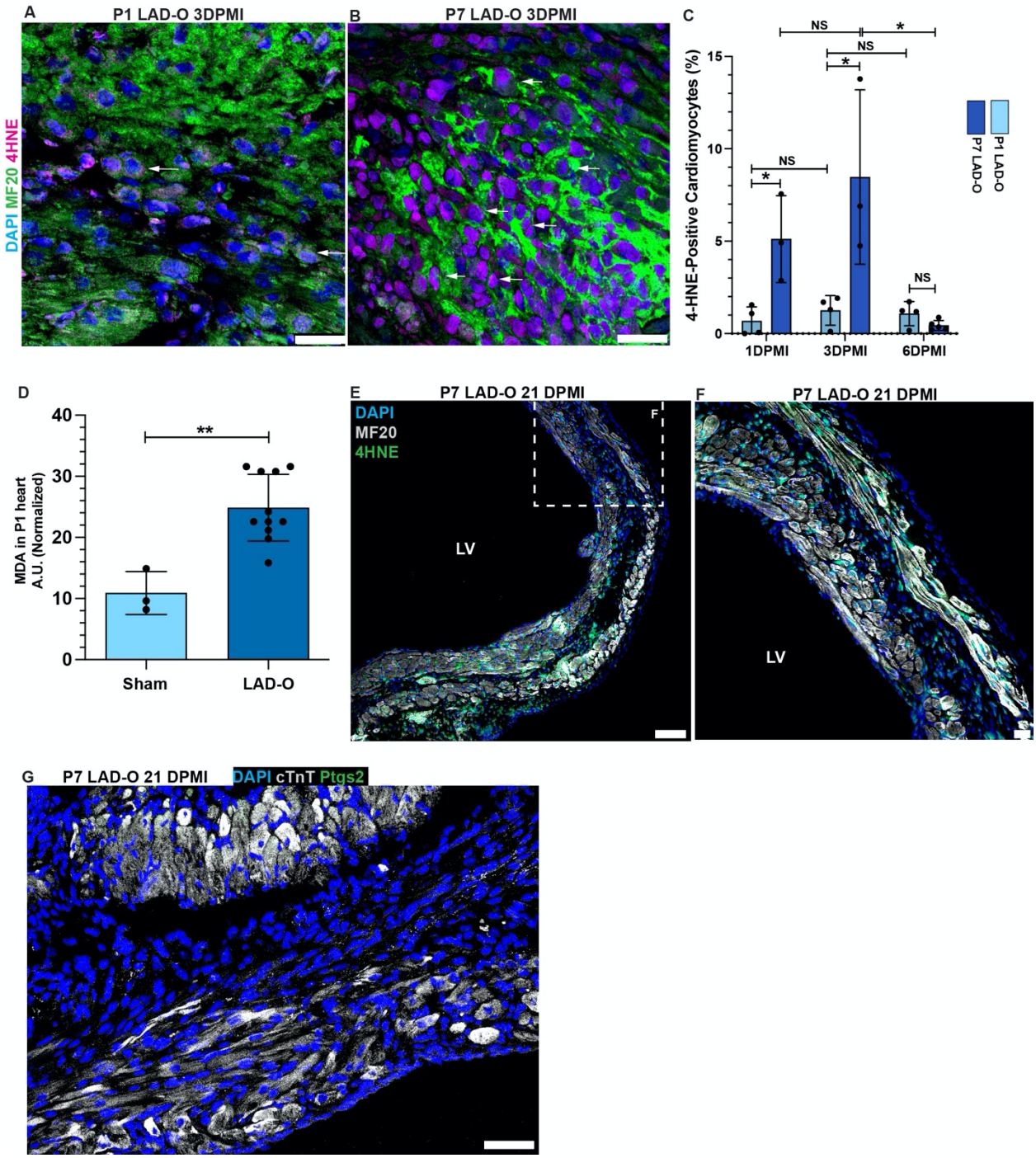

Figure S2. Ferroptosis occurs in cardiomyocytes after MI, related to Figure 1. (A, B) Mouse heart tissue stained for 4-HNE (magenta) and MF20 (green) at 3 days after P1 (A) or P7 (B) LAD-O. Arrows, cardiomyocytes positive for 4-HNE. (C) Ratio of cardiomyocytes positive for 4-HNE at 1, 3 and 6 days after P1 or P7 LAD-O. (D) MDA assay quantified lipid peroxidation level in ventricular myocardium after P1 LAD-O or sham procedure. (E, F) Mouse heart tissue stained for 4-HNE (green) and MF20 (grey) at 21 days after P7 LAD-O. (G) Heart tissue stained for Ptgs2 (green) and MF20 (grey) at 21 days after P7 LAD-O. LV, left ventricle. DAPI in blue. Error bars indicate SD. *, $p<0.05$. **, $p<0.01$. NS, not significant. Scale bar, 25 μm (A, B, F, G), 100 μm (E).

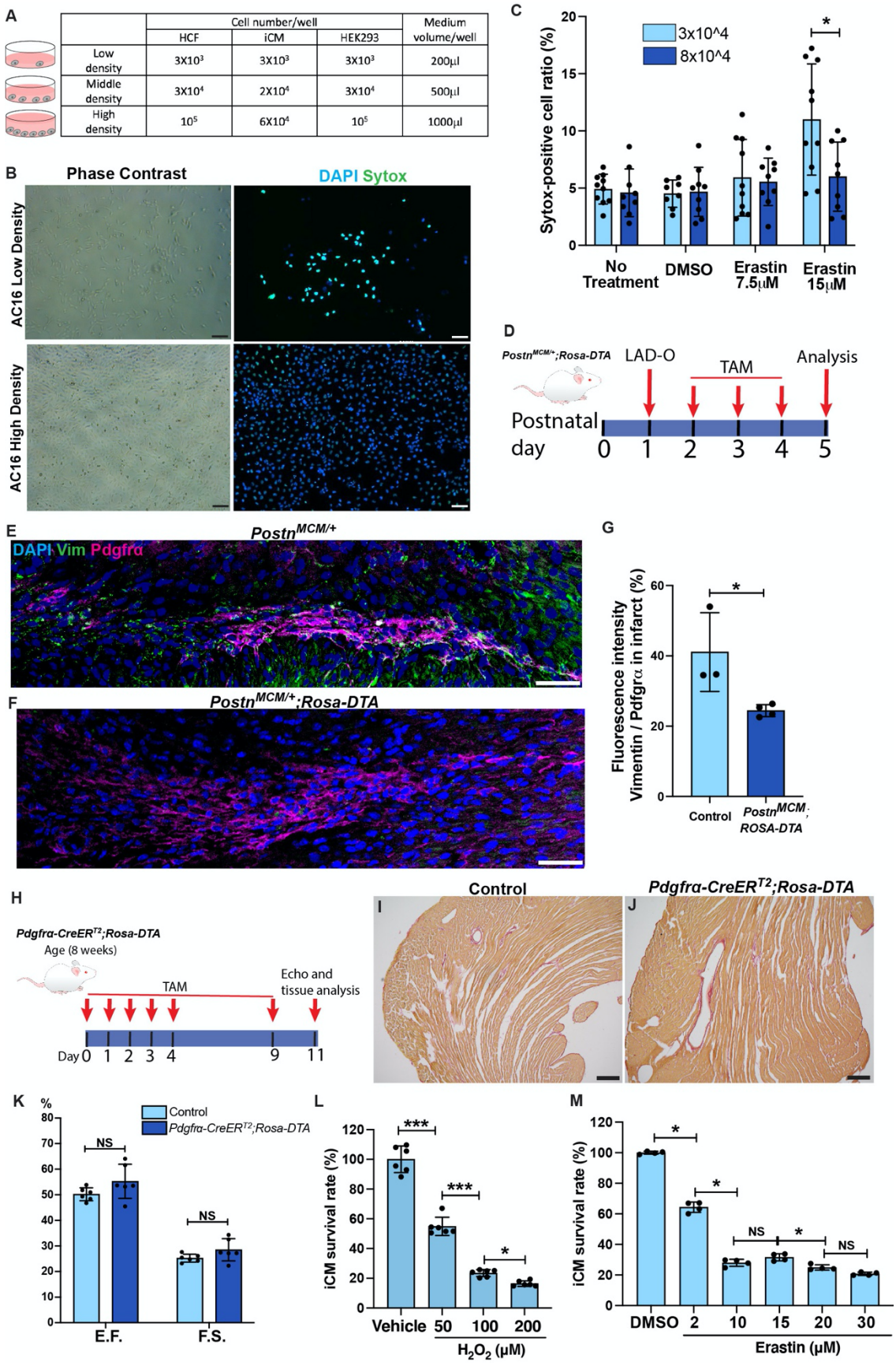
A
Cell number/well
HCF
iCM
HEK293
Medium volume/well
Low density
3X10³
3X10³
3X10³
200µl
Middle density
3X10⁴
2X10⁴
3X10⁴
500µl
High density
10⁵
6X10⁴
10⁵
1000µl
B
Phase Contrast
DAPI Sytox
AC16 Low Density
AC16 High Density
C
Sytox-positive cell ratio (%)
3x10^4
8x10^4
No Treatment
DMSO
Erastin 7.5µM
Erastin 15µM
D
Postn^MCM/+;Rosa-DTA
LAD-O
TAM
Analysis
Postnatal day
0 1 2 3 4 5
E
Postn^MCM/+
DAPI Vim Pdgfrα
F
Postn^MCM/+;Rosa-DTA
G
Fluorescence intensity Vimentin / Pdfgrα in infarct (%)
Control
Postn^MCM; ROSA-DTA
H
Pdgfrα-CreER^T2;Rosa-DTA
Age (8 weeks)
TAM
Echo and tissue analysis
Day 0 1 2 3 4 9 11
Control
Pdgfrα-CreER^T2;Rosa-DTA
I
J
K
%
Control
Pdgfrα-CreER^T2;Rosa-DTA
NS
E.F.
F.S.
L
iCM survival rate (%)
Vehicle
50
100
200
H2O2 (µM)
M
iCM survival rate (%)
DMSO
2
10
15
20
30
Erastin (µM)

Figure S3. Cell density regulates ferroptosis *in vitro* and *in vivo*, related to Figure 2 and Figure 3.

(A) Schematic of cell density assays, see Figure 2 and 3. (B) AC16 cells cultured at low (3×10^4/well) and high (8×10^4/well) density and treated with erastin. Dying cells were stained with Sytox (green). (C) Ratio of Sytox-positive AC16 cells. (D) Schematic plan for E-G and Figure 2H-P. (E, F) Heart tissue of controls (*Postn*$^{MCM/+}$, E) and *Postn*$^{MCM/+}$*;ROSA-DTA* (F) mice were stained for Vim (green) and Pdgfrα (magenta) at 4 DPMI after P1 LAD-O. (G) Ratio of fluorescence intensity, Vim over Pdgfrα. (H) Schematic plan for I-K and Figure 2R-W. (I, J) Picrosirius red staining of adult control (*Rosa-DTA*, I) and *Pdgfrα-CreER*^{T2}*;ROSA-DTA* (J) mice. (K) Ejection fraction (E.F.) and fractional shorting (F.S.) of control and *Pdgfrα-CreER*^{T2}*;ROSA-DTA* mice. (L) Survival rate of iCM after treatment with 50, 100 or 200 μM of H_2O_2, compared to vehicle (H_2O). (M) Survival rate of iCMs after erastin treatment at 2, 10, 15, 20 or 30 μM, compared to DMSO control. Nuclei stained with DAPI (blue). TAM, tamoxifen. Error bars indicate SD. *, $p<0.05$. ***, $p<0.001$. NS, not significant. Scale bar, 100 μm (B, I, J), 25 μm (E, F).

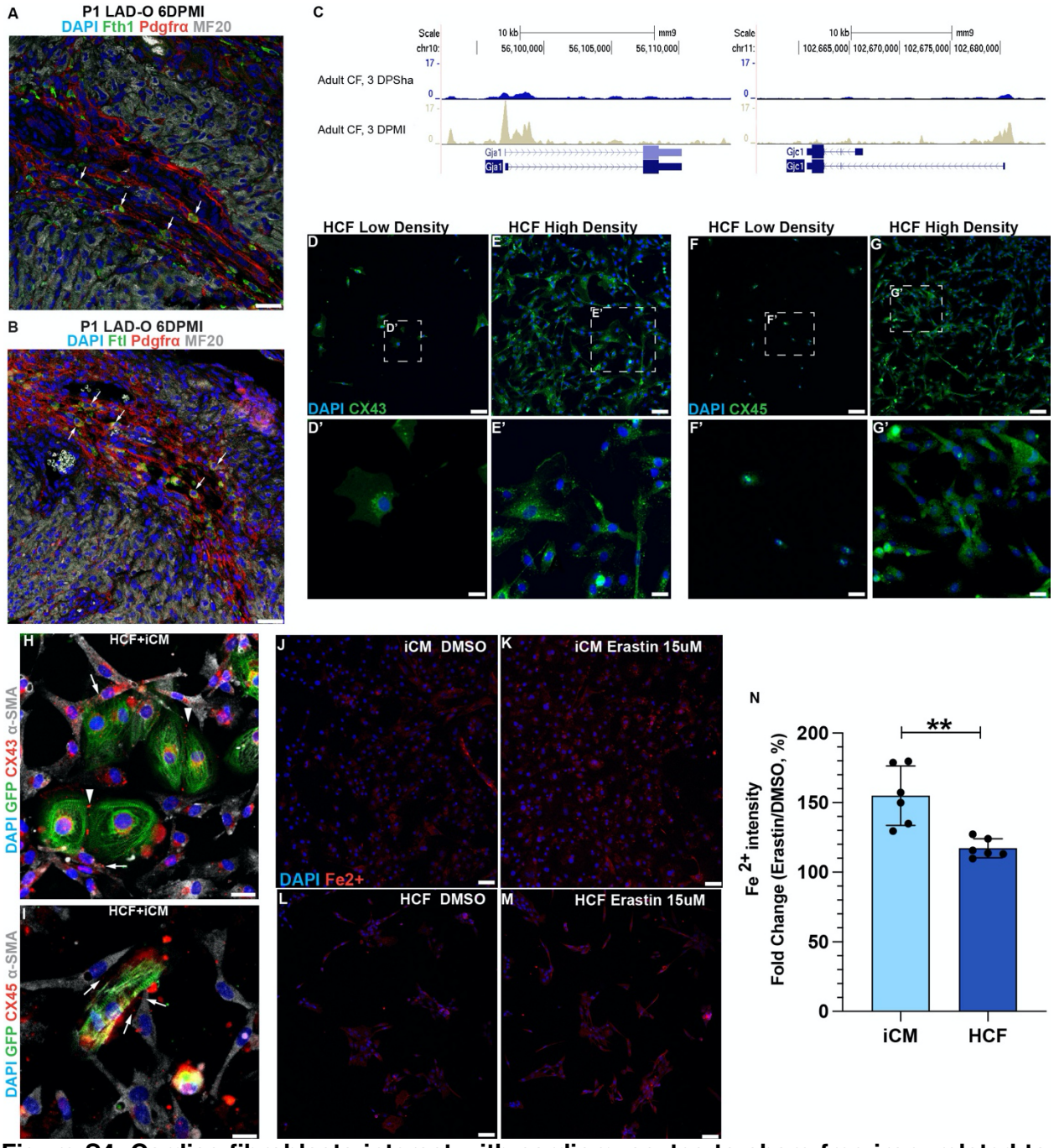

Figure S4. Cardiac fibroblasts interact with cardiomyocytes to share free iron, related to Figure 5. (A, B) Mouse heart sections stained for Fth1 (green, A) or Ftl (green, B), with Pdgfrα (red) and MF20 (grey) at 6 days after P1 LAD-O. Arrows, Pdgfrα-labeled cells positive for Fth1 (A) or Ftl (B). (C) ATAC-Seq shows open chromatin region at loci of *Gja1* (*Cx43*) and *Gjc1* (*Cx45*) in adult cardiac fibroblasts at 3 days after LAD-O or sham procedure. (D-G') HCF cultured in low or high density and stained for CX43 (green, D-E') and CX45 (green, F-G'). (H-I) Co-cultured iCMs (marked by TITIN-GFP, green) and HCFs stained for CX43 (red, H) or CX45 (red, I), with αSMA (grey). Arrowheads in H, gap junctions between iCMs. Arrows in H and I, gap junctions between iCM and HCF. (J-N) iCMs (J, K) and HCF (L, M) stained for Fe^{2+} (red) after DMSO (J, L) or erastin (K, M) treatment. Fold change of Fe^{2+} fluorescent intensity after erastin treatment in

iCM and HCF quantified in N. Nuclei stained with DAPI (blue). Error bars indicate SD. **, p<0.01. Scale bar, 25 μm (A, B, D', E', F', G', H, I), 100 μm (D, E, F, G, J-M).

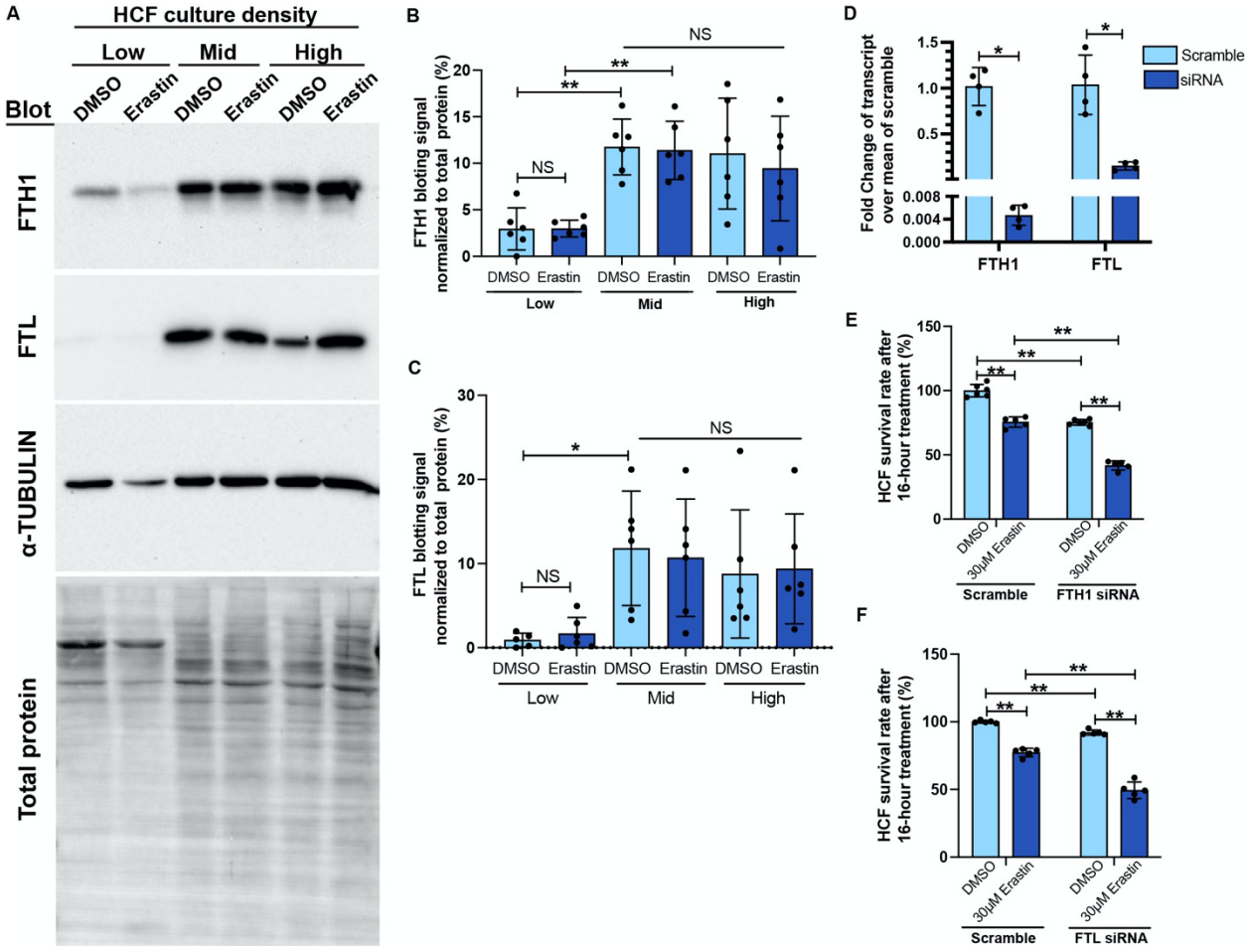

Figure S5. Cardiac fibroblasts increase Ferritin expression to resist ferroptosis, related to Figure 5. (A-C) Western blot of FTH1, FTL and α-TUBULIN in HCFs cultured at low, mid and high density, with DMSO or erastin treatment. Target band signal intensity quantified in B (FTH1) and C (FTL). (D) qPCR shows the knockdown of *FTH1* and *FTL* with siRNA in HCFs. (E, F) Survival rate of HCFs after erastin (30 μM) treatment, with *FTH1* (E) or *FTL* (F) knockdown compared to scramble siRNA. Error bars indicate SD. *, p<0.05. **, p<0.01. NS, not significant.

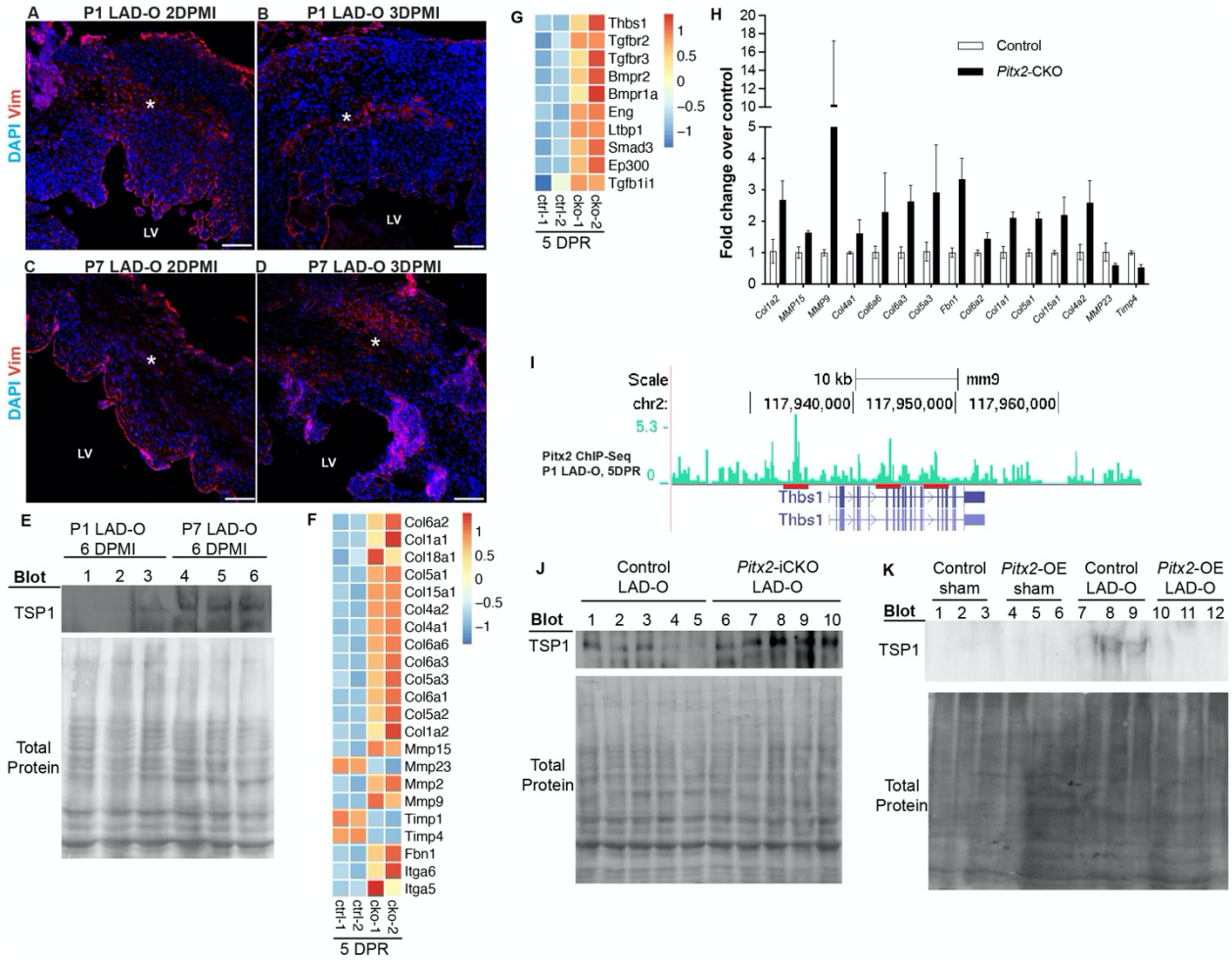

Figure S6. Pitx2 regulates fibrotic gene expression in injured myocardium, related to Figure 6. (A-D) Mouse heart tissue stained for Vim (red) at 2 or 3 days after P1 (A, B) or P7 (C, D) LAD-O. Asterisk, infarct zone. (E) Western blot of Tsp1 in cardiac ventricles at 6 days after P1 or P7 LAD-O. (F, G) Heatmap of fibrosis-relevant genes in control (*Pitx2*$^{f/f}$) and *Pitx2*-CKO (*MCK*^{cre}*;Pitx2*^{ff}) ventricles at 5 days after P1 apex resection. (H) qPCR validation of genes in F and G, $p<0.05$ for all targets. (I) ChIP-Seq shows Pitx2-binding region (red bars) at *Thbs1* locus in regenerative neonatal ventricles. (J) Western blot of Tsp1 in control and *Pitx2*-iCKO ventricles at 3 days after P1 LAD-O. (K) Western blot of Tsp1 in control and *Pitx2*-OE ventricles at 3 days after P7 LAD-O. LV, left ventricle. Nuclei stained with DAPI (blue). Error bars indicate SD. Scale bar, 75 μm (A-D).