Supplementary Materials

Xiao and Hill et al.

Supplementary Figure 1

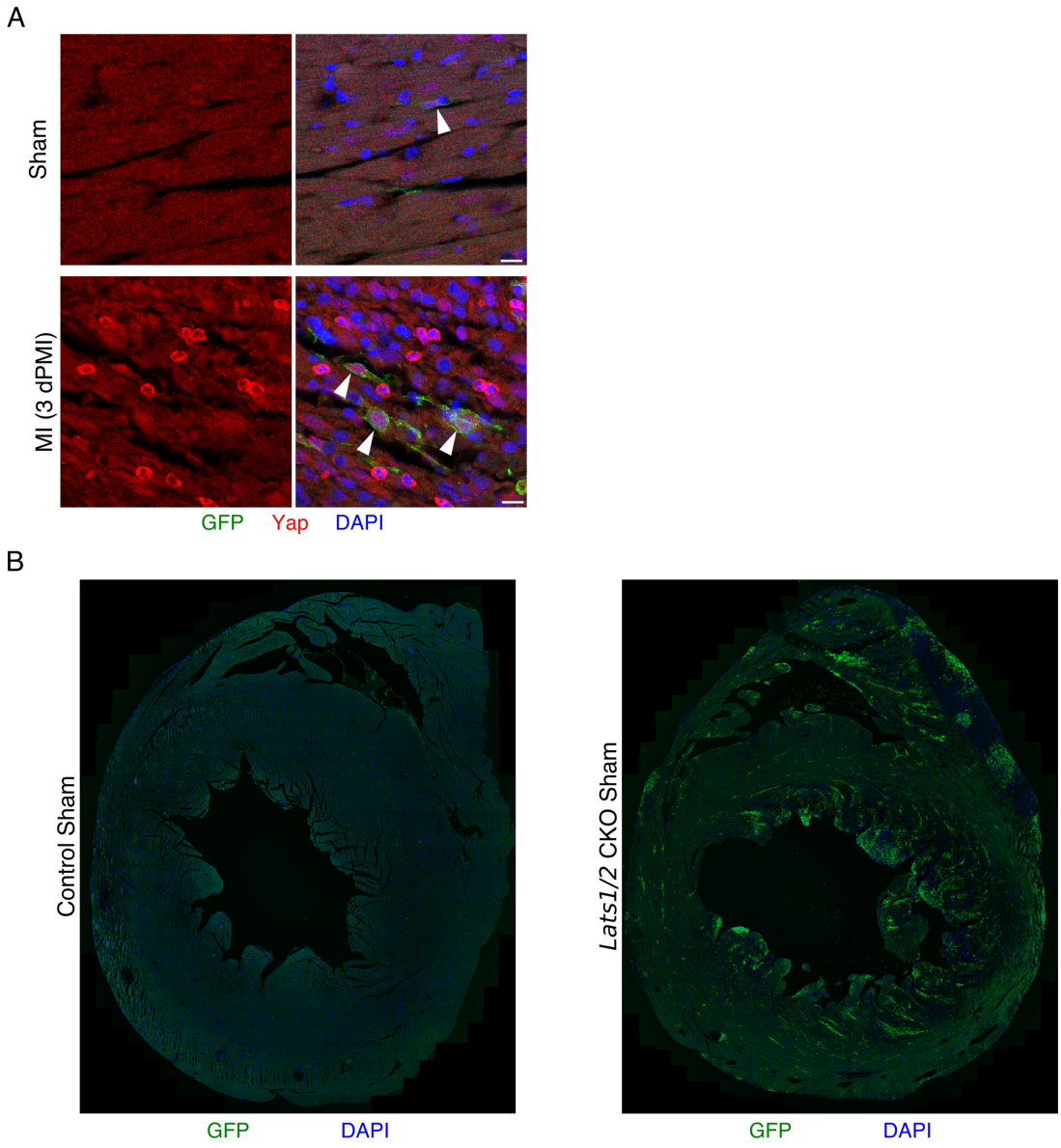


Figure S1. Yap is Activated Following Myocardial Infarction, related to Figure 1.

A. Representative images from cardiac tissue sections after MI or sham operations stained for GFP (green), Yap (red), and DAPI (blue). Cardiac fibroblasts (GFP,

arrowheads) showed increased nuclear Yap activity at 3 dPMI compared with sham cardiac fibroblasts. Scale bar indicates 25 μm.

B. Representative tiled images from uninjured cardiac tissue sections stained for GFP (green), and DAPI (blue).

Supplementary Figure 2

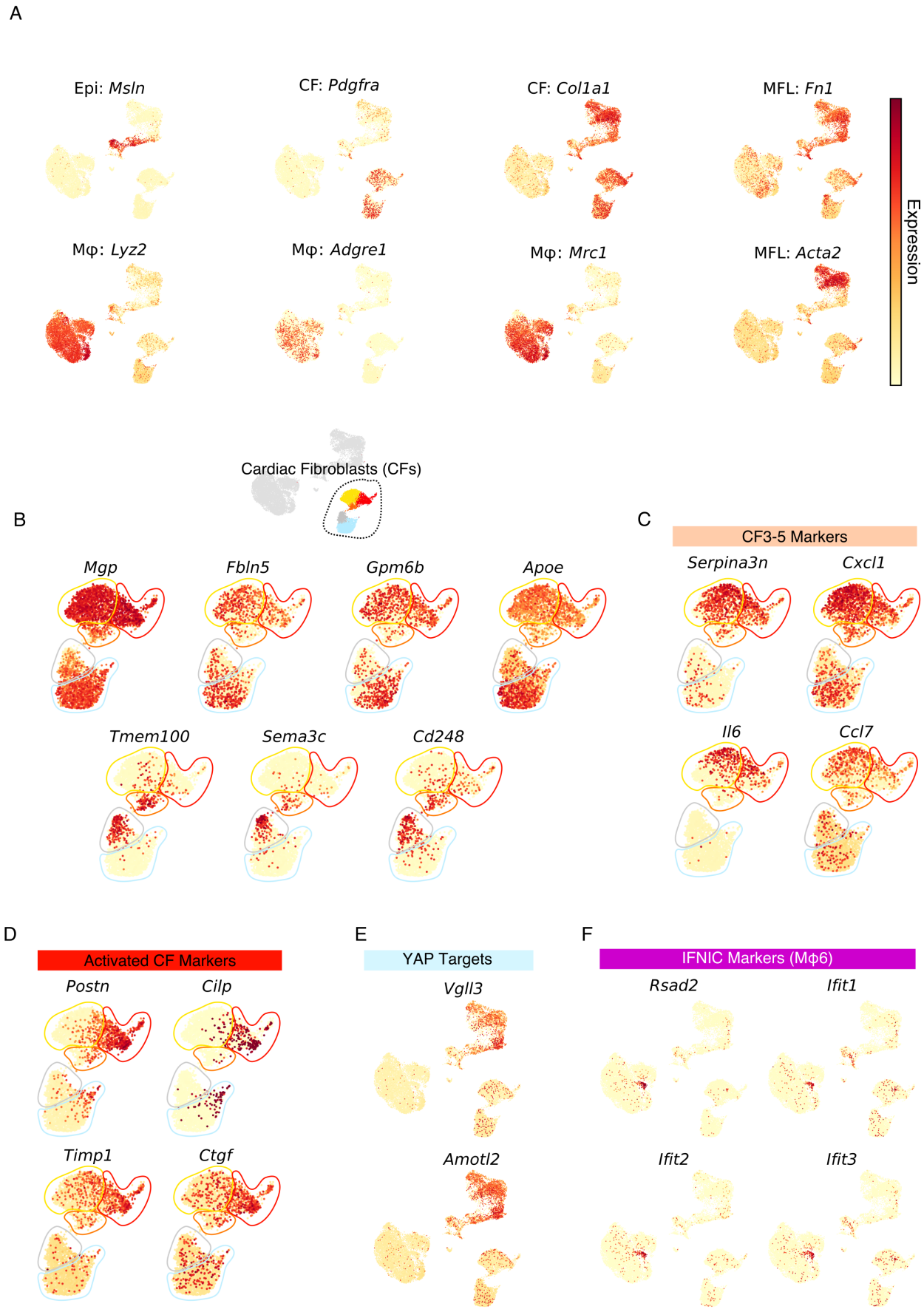


Figure S2. Single-cell RNA-seq Reveals that Hippo Signaling Inhibits the Non-Autonomous Activation of Cardiac Fibroblasts and Myeloid Cells, Related to Figure 2.

A. Feature plot of selected signature gene expression in major cell clusters. High expression shown in red, and cells not expressing gene are tan. UMAP plot originally shown in **Figure 2A**.
B. Feature plot of cardiac fibroblast cluster markers. Fibroblast clusters were extracted from full data set shown in **Figure 2A** for detailed gene profiling. Cell clusters are outlined in same colors shown in **Figure 2A**.
C. Feature plots of cardiac fibroblast markers enriched in non-mutant cardiac fibroblasts derived from *Lats1/2* CKO hearts.
D. Feature plots of cardiac fibroblast markers enriched in activated cardiac fibroblasts (non-mutant) derived from *Lats1/2* CKO hearts.
E. Feature plots of canonical Yap target genes.
F. Feature plots of IFNIC markers.

Supplementary Figure 3

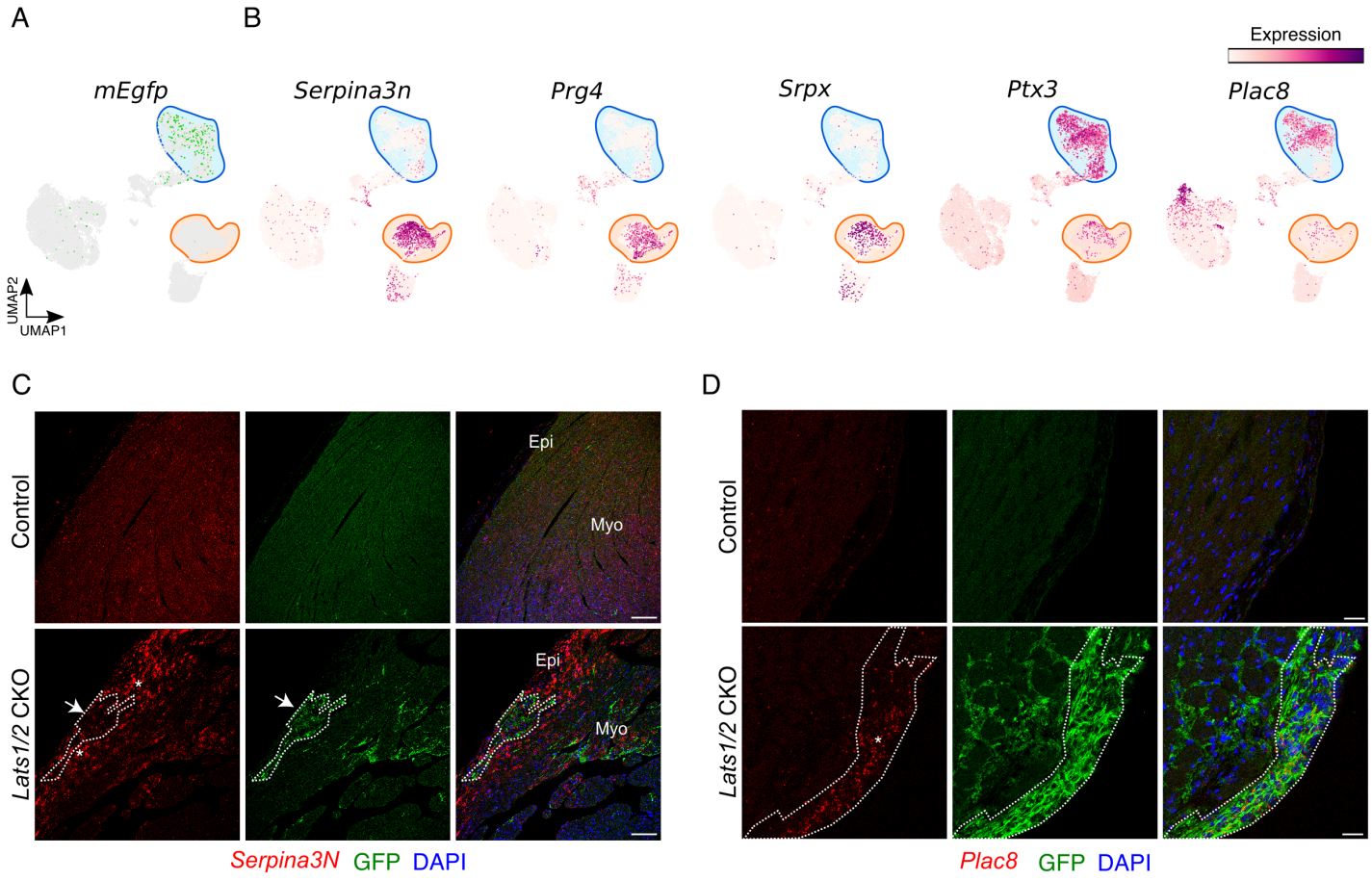


Figure S3. Yap is a Cell-Nonautonomous Regulator of Cardiac Fibroblasts, Related to Figure 3.

A. Feature plots showing expression of *mEgfp*.

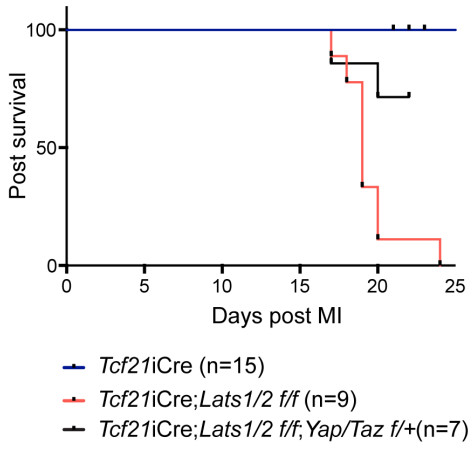
B. Feature plots showing expression of marker genes. MFL clusters are highlighted in blue, and CF3-5 clusters are highlighted in orange.

C. Representative *in situ* hybridization image of *Serpina3N* (red). Cardiac fibroblasts were lineage traced (green). Nuclei were stained with DAPI (blue). Epi, epicardium; Myo, myocardium. Scale bar, 100 um.

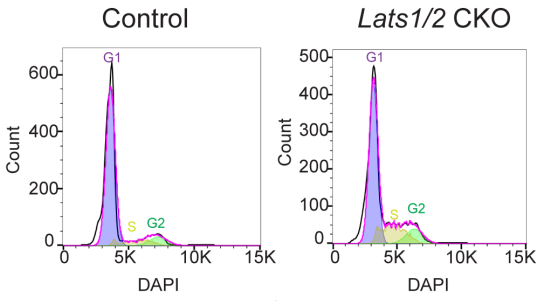
D. Representative *in situ* hybridization image of *Plac8* (red). Cardiac fibroblasts were lineage traced (green). Nuclei were stained with DAPI (blue). Epi, epicardium; Myo, myocardium. Scale bar, 25 um.

Supplementary Figure 4

A



B



C

	Control	*Lats1/2* CKO	P-value
G1	80.73±6.33%	63.9±7.94%	0.018*
S	7.60±1.28%	20.4±2.86%	0.023*
G2/M	8.91±2.11%	12.06±2.55%	0.106
Super G2	1.17±1.02%	2.23±2.37%	0.106

Figure S4. *Lats1/2* Suppress Cardiac Fibroblast Proliferation, Related to Figure 5.

A. Survival curve of control mice (*Tcf21*^{iCre}/+; *Rosa26*^{mTmG}/+) *Lats1/2* CKO (*Tcf21*^{iCre}/+*; Lats1/2*$^{flox/flox}$; *Rosa26*^{mTmG}/+), and *Tcf21*^{iCre}/+*; Lats1/2*$^{flox/flox}$; *Yap/Taz*$^{flox/+}$ mice after myocardial infarction.

B. DNA content and ploidy of cardiac fibroblasts after myocardial infarction**.** Representative histograms from flow cytometry analysis of isolated cardiac nuclei from control and *Lats1/2* CKO hearts, stained with DAPI.

C. Quantification of cardiac fibroblast proliferation after myocardial infarction. Statistical significance was determined by Chi-square test.

Supplementary Figure 5

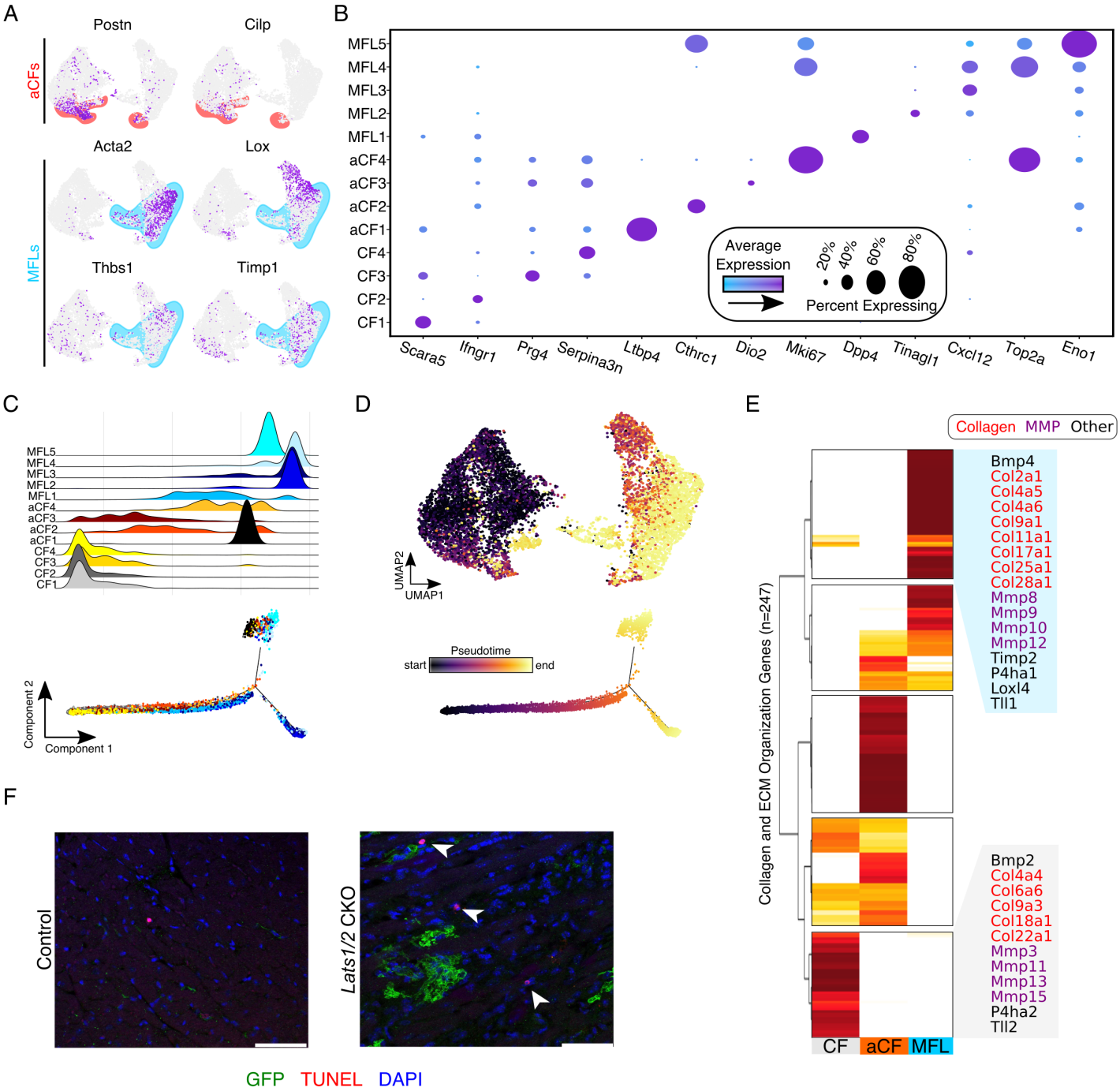


Figure S5. Hippo Signaling Suppresses Myc Expression to Maintain Cardiac Tissue Homeostasis, Related to Figures 6.

A. Feature plots of markers for active fibroblasts (top), and myofibroblasts (bottom). Clusters position in UMAP from **Figure 6A** is shown in colored outlines.

B. Dot plot of marker gene expression for each cardiac fibroblast cluster. Size of dot indicates percentage of cells within a cluster that express a given gene.

C. UMAP plot of cardiac fibroblast single-cell transcriptome experimental identities.

D. Pseudotemporal ordering of cardiac fibroblasts. Top, density plot of cluster compositions across pseudotime. Bottom, ordering of cardiac fibroblasts along a minimum spanning tree (MST). Colored by cell cluster according to **Figure 6A**.

E. Heatmap showing the expression of collagen formation and extracellular matrix organization genes across merged single cell clusters.

F. High magnification image of TUNEL (red) stained cardiac tissue from uninjured control and *Lats1/2* CKO hearts shown in Figure 7L. Lineage traced cardiac fibroblasts are GFP labelled (green). Nuclei stained with DAPI (blue). Scale bar indicates 25 μm.

Supplementary Figure 6

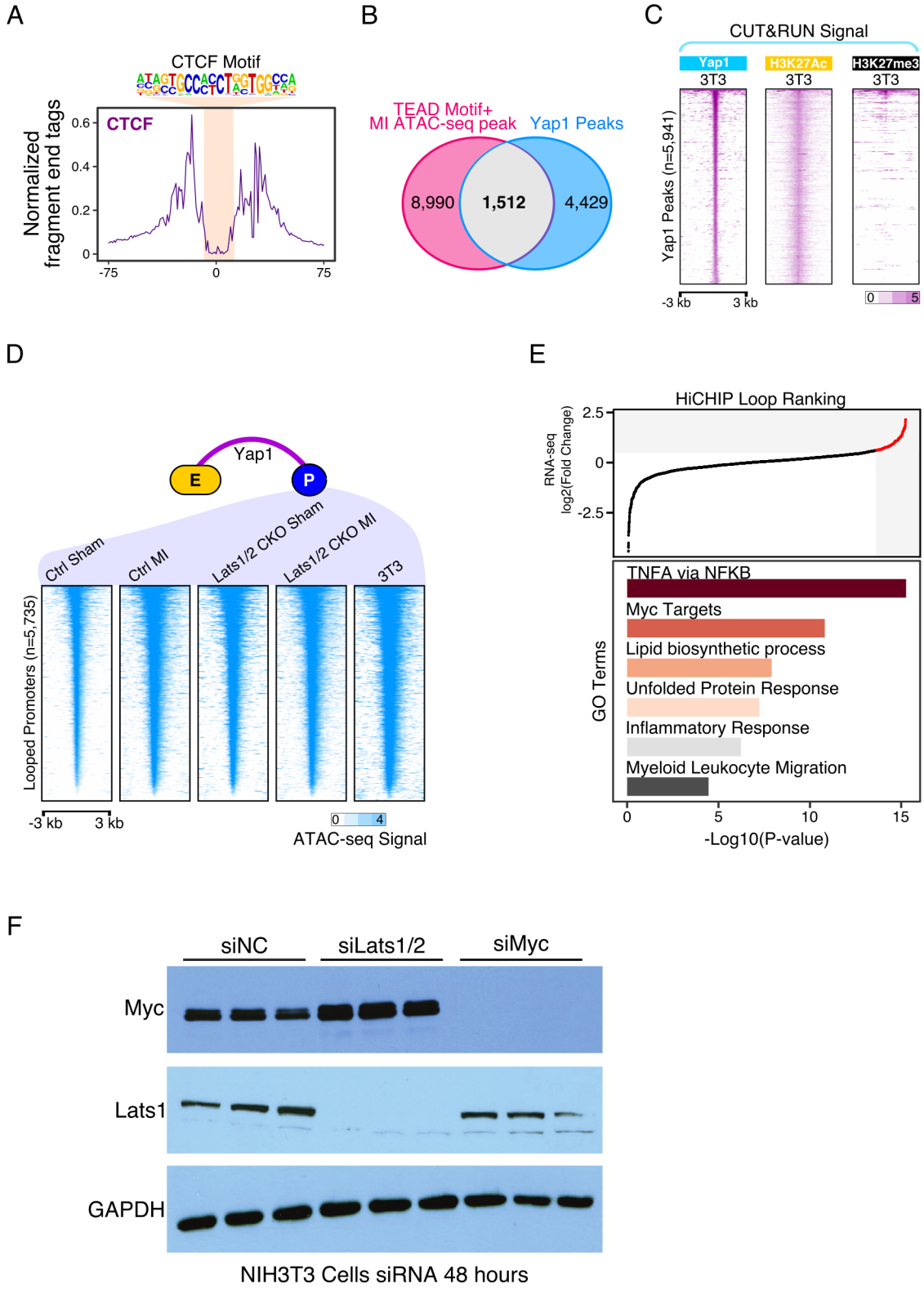


Figure S6. Genome-Wide Myofibroblast Yap Chromatin Occupancy Mapping via CUT&RUN, Related to Figure 7.

A. CUT&RUN footprint analysis for CTCF.

B. Venn diagram showing overlay of TEAD motif containing Fast-ATAC peaks from control cardiac fibroblasts 3 dPMI, and myofibroblast Yap CUT&RUN peaks.

C. Heatmap showing CUT&RUN signal (read depth) across all myofibroblast Yap peaks for indicated histone marks and Yap.

D. Heatmap showing Fast-ATAC signal (read depth) across all Yap-associated and topologically looped promoters. Promoters (n=8,101) were identified in Fig. 2E, as being looped to enhancers. Top, schematic of enhancer-promoter H3K27Ac HiChIP loop interaction for Yap1 occupied anchors.

E. Top, ranking of Yap-mediated H3K27Ac loops by expression in CFs after MI. Red color indicates loops to genes with expression greater than log2 (fold-change) 0.5 compared to sham. Bottom, gene ontology analysis of highlighted looped promoters.

F. Western blot showing MYC, LATS1 , and GAPDH expression in NIH3T3 myofibroblasts 48 hours after siRNA treatment with either control siNC, siLats1/2, or siMyc.

Supplementary Figure 7

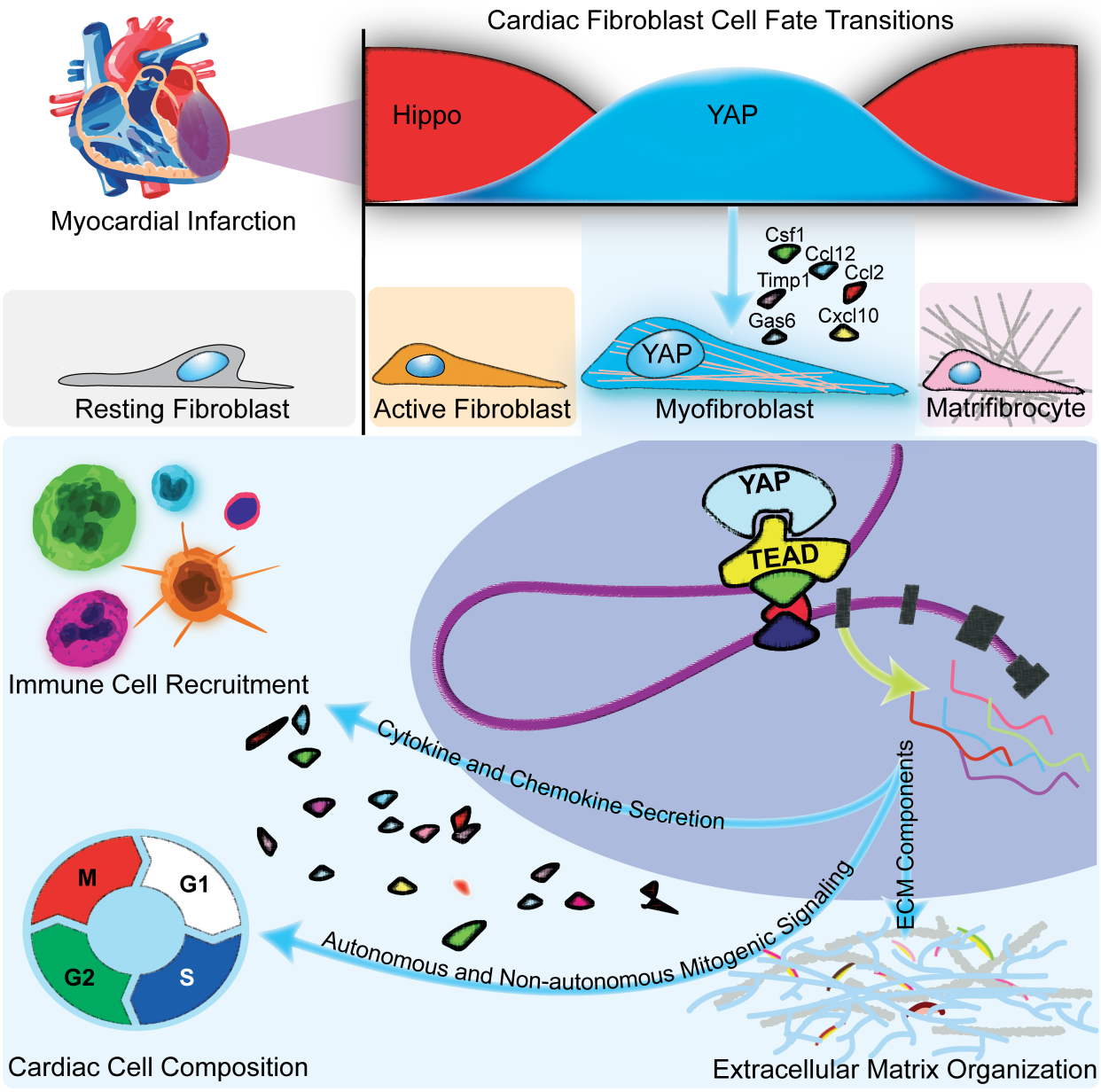


Figure S7. Model of Lats Kinase Regulation of Cardiac Fibroblast Cell Fate Transitions and the Pro-Inflammatory Yap Gene Regulatory Network.