Supplemental Methods

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Histology and immunofluorescence

For histology and immunofluorescence staining, hearts were fixed in 4% PFA overnight at 4°C and dehydrated in a serial ethanol, xylene and embedded in paraffin. Sections of 7μm thick sections were prepared for staining. Masson's Trichrome staining was performed according to manufacturer's instruction (Sigma, HT15). Antibodies used for immunofluorescence staining were as follows: GFP (1:200, Abcam ab290, ab6673), c-Myc [Y69](Abcam, ab32072), Yap (1:100, Novus, NB 110-58358), pYap(1:100, Cell signaling technologies 4911), α-smooth muscle actin (1:200, Sigma, C6198), Lyz (1:200, Abcam, ab108508). To visualize some antigens, Alexa-647 was employed. When applications required green and red co-staining, sections were pre-treated with 0.3% H_2O_2 in PBS for 20min at room temperature to quench the endogenous GFP and Tomato signals, which come from the *Rosa26*^{mTmG} reporter line. In some cases, Tyramide Signal Amplification Systems (1:100, Perkin Elmer) were used to amplify signal. TUNEL assay was performed according to manufacturer's instruction (Progema, G3250). Immunofluorescence images were captured on a Leica TCS SP5 confocal microscope.

Western Blotting

Mouse fibroblast cells line NIH3T3 was used for western blotting (ATCC®CRL-1658). siRNA used were as follows: Non-targeting siRNA (siNC) (Dharmacon, D-001810-02-05), Mouse Lats1 (siLats1) (Dharmacon,L-063467-00),mouse Lats2 (siLats2)(Dharmacon,L-044602-00) and mouse Myc (Dharmacon, M-040813-02-0005). RNAiMAX (Thermo Fisher Scientific) was used for transfection. Cells were treated with siRNA for 48 hours and harvest for protein detection. Antibody used for Western Blotting were as follows: anti-Myc (Abcam ab32072), anti-Lats1 (Cell Signaling Technology,3477), Gapdh (Abcam ab9485).

FACS analysis for cell cycle scoring

According to DNA content, we assigned cells with 2N and 4N into G1 and G2/M phase respectively, cells with DAPI intensity in between 2N and 4N to S phase, and cell with DNA content more than 4N to super G2 phase. Cardiac fibroblasts were isolated from hearts at 1 week post MI by langendorff perfusion. GFP positive cells were gated for analysis and DAPI were used for analyzing DNA content. FACS were performed on BD Biosciences SORP Aria I and BD Biosciences LSRII and cell cycle modelling were processed with FlowJo software.

RNAscope in situ hybridization

Formaldehyde-fixed paraffin-embedded heart sections were processed for RNA in situ detection using the RNAscope2.5 Assay (Advanced Cell Diagnostics, Inc.) according to the manufacturer's instructions. RNAscope probes used in this study: Serpina3n (430191), and Plac8 (532701).

FAST-ATAC

Briefly, sorted cells were spun down, FACS buffer was removed, the pellet was then re-suspended in a transposase-containing reaction mixture complete with 0.05% digitonin prior to tagmentation at 37^0C with 1000 rpm agitation for 30 minutes. Next, transposed DNA was purified with a Qiagen PCR MinElute kit (Qiagen 28004). Fast-ATAC libraries were purified with a 1.8X SPR purification using AMPure XP beads following PCR amplification.

ATAC-seq Analysis

Reads were mapped to the mouse genome (mm9) using Bowtie2 with default paired-end settings. Next, all non-nuclear and unmapped paired reads were discarded. Duplicated reads were removed with the picard MarkDuplicates function, default settings. Peak calling for differential accessibility analysis was carried out with Macs2 on the merged BAM file, Macs2 callpeak --nomodel –broad. Blacklisted regions, identified by ENCODE, from mm9 were removed from the comprehensive peak file using the bedtools subtract module. Reads were counted for each condition from the comprehensive peak file using the bedtools multicov module. PCA and differential accessibility analysis were performed with the DESeq2 R package using the multicov file as input. Motif enrichment analysis and individual condition peak calling (findPeaks -style factor) was conducted with Homer (findMotifsGenome.pl). Visualization of Fast-ATAC signals was done with Homer, and all reads were normalized by read count, where scores represent read count per bp per 1 x 10^7 reads.

DROP-seq

Here, cells were co-encapsulated into nano-liter sized droplets containing barcoded microparticles (ChemGenes, catalog number Macosko201110) and lysis buffer using a custom microfluidics device (FlowJEM, Toronto, Canada). After droplet breakage, reverse transcription (Thermo), and exonuclease treatment (NEB) all cDNA was PCR amplified (KAPA), pooled, purified with Ampure XP beads (Beckman Coulter), and ran on a Fragment analyzer (Advanced Analytical Technologies, Inc.) for quality control, quantification, and size determination. Library preparation was performed with the Illumina Nextera XT kit, and libraries were triple purified with Ampure XP beads (Beckman Coulter).

Single Cell RNA-seq Data Analysis

The raw FastQ files were converted to BAMs with Picard tools (MergeSamFiles) and then used is input for STAR alignment, cell barcode correction, and digital gene expression (DGE) matrix generation via the Droplet-based RNA-seq tools software package (available at http://mccarrolllab.com/dropseq/). The minimum gene per cell threshold was set to 500 for inclusion into the final digital expression matrix. Subsequently, DGEs from each experiment were merged and then we imported the comprehensive DGE into Seurat (version 2.3.4) where normalization was performed according to package default settings. Batch effects were corrected for by regressing out the number of molecules per cell, the batch (i.e. orig.ident) and the percentage of mapped mitochondrial reads with the ScaleData function (Seurat package). Next,

principle components analysis (PCA) was performed and significant PCs were used as input for graph-based clustering. Then 2-dimensional visualization of the multi-dimensional data set was done with UMAP. Differential expression of the individual clusters was performed using the likelihood-ratio test for single cell gene expression. To account for over-clustering, clusters that were not transcriptionally distinct were merged. Clusters that represented cell doublets were removed from the final data set. The approximate cell cycle phase of each cell was calculated using Seurat by scoring individual cells on their expression for S-phase, G1, and G2M genes (Kowalczyk et al., 2015). For pseudotemporal analysis, the normalized data from selected clusters were then passed directly into Monocle2 where density peak clustering and downstream analysis was performed. Chi-square statistical analysis between clusters was performed and visualized as described previously (Li et al., 2018). Gene interaction analysis requiring MAGIC analysis was carried out using the Rmagic package (version 1.0.0).

Within the SCENIC computational pipeline, we identified all genes co-expressed with transcription factors using the GRNboost2 fast GRN inferencing algorithm (Friedman, 2002). Next, we performed cis-regulatory motif enrichment analysis on all co-expressed genes. This analysis cataloged putative transcription factor binding sites within the list of co-expressed genes, thereby allowing us to identify potential direct gene targets. Additionally, this enabled us to eliminate false positives and indirect transcriptional targets from the co-expression matrix. All combinations of transcription factors and direct gene targets with significant motif enrichment are referred to as regulons. Finally, the SCENIC AUCell algorithm was applied to calculate the activity of each regulon in every single-cell transcriptome (Aibar et al., 2017). Individual cells

expressing many genes within a given regulon display the highest area under the curve (AUC) score, while those cells expressing few to none receive a low AUC score. The ranked distribution of AUCell scores across all of the cells for a given regulon is used to determine a threshold for active and inactive regulons, thus making the final output binary (active or inactive).

For ligand receptor connectome analysis, we use the scRNA-seq expression matrix to quantify the connections between cells that express ligand genes and cells that express receptor genes by counting the number of these ligand-receptor pairs for each cell to cell permutation. Next, a cell to cell interaction matrix is generated from the sums of these counts. Finally, we created a matrix of ligand-receptor pair connection counts for each permutation of all cell groups and filtered those ligand-receptor pairs that had at least 100 cell-cell connections. We further filtered this ligand receptor connectome to focus on the strongest interactions (greater than log(10)) differentially expressed between indicated cell types.

CUT&RUN

Briefly, 200,000 NIH3T3 cells were washed in wash buffer (20 mM HEPES, pH7.5, 150 mM NaCl, 0.5 mM Spermidine and complete protease inhibitor (EDTA-free, Roche), captured with Concanavalin A beads (Polysciences, Warrington, PA) and incubated with primary antibodies overnight at 4°C. After washing with Dig-wash buffer (20 mM HEPES, pH7.5, 150 mM NaCl, 0.5 mM Spermidine, 0.08% Digitonin and protease inhibitors), cells were resuspended in 50 µL Digwash buffer and 2.5 µL of protein A-MNase (1:10 diluted, batch 6 from Steve Henikoff) and incubated at room temperature

for 10 minutes. Cell pellets were washed again and placed in a 0°C metal block, and 2 mM of CaCl2 was added and incubated for 45 minutes. MNase reaction was terminated by the addition of 2XSTOP buffer and incubated at 37°C 10 minutes. Samples were then digested by proteinase K at 70°C for 10 minutes and DNA was extracted by ethanol precipitation. Library were prepared using KAPA Hyper Prep Kit (KAPA) and custom Y-shaped TruSeq adapters according to the manufacturer's instructions. The antibodies used were anti-Yap (Novus, NB110-58358), anti-H3K27ac (Abcam, ab4729), anti-H3K27me3 (Cell Signaling, 9733S), anti H3K4me3 (Diagenode, C15410003), anti-CTCF (Diagenode, C15410210), anti-GABPA (ProteinTech, 215420-1), Gabpb1 (ProteinTech, 12597-1).

CUT&RUN Data Analysis

Raw paired-end reads were aligned to the mm9 genome according to (Skene et al., 2018). Briefly, fastq files were mapped using Bowtie2 (version v2.2.5) with the following options: --local --very-sensitive-local --no-unal --no-mixed --no-discordant --phred33 -I 10 -X 700. For mapping Yeast spike-in fragments, we used the following options in addition to those stated directly above: --no-overlap --no-dovetail. Peak calling was performed as described in (Liu et al., 2018). In short, peaks were called from aligned BAM files using MACS2 callpeaks with the narrowPeak option and a P-value cutoff of 1e-5. Footprint detection was carried out by aligning all mapped read ends around motif containing peak centers. Peaks were centered around motifs using HOMER. Fragment ends were piled up using scripts previously published and available at: https://github.com/peteskene. For the final Yap CUT&RUN peak calling, mm9 aligned

BAM files derived from both YAP5SA-NIH3T3 cells and control NIH3T3 cells were combined.

H3K27ac HiChIP

H3K27ac HiChIP was performed according to (Mumbach et al., 2017) with only minor modifications. Approximately 15 million NIH3T3 cells were used as input, and MboI digestion was carried out for 2 hours. Cells were sonicated for 10 cycles (30 seconds on, and 30 seconds off) using a Bioruptor Pico instrument (Diagenode). For biotin pull down 150ng of chromatin was used as input, and tagementation was performed using 4 uL of Tn5 transposase. For post-PCR size selection we performed a double-sided size selection with Ampure XP beads (Beckmann-Coulter). All libraries were sequenced on a NextSeq 500 platform.

H3K27Ac HiChIP Analysis

H3K27ac HiChIP paired-end reads were aligned to the mm9 genome using HiC-Pro (Servant et al., 2015). Aligned reads were passed to the hichipper computational analysis pipeline (Lareau and Aryee, 2018), and loop calling was carried out with default parameters. For peak calling, we used “COMBINED, ALL”, and the MboI restriction fragment bed file for the mm9 genome was generated with the HiC-Pro digest_genome.py utility (digest_genome.py -r mboi). DNA loops that passed hichipper quality control were filtered to intrachromosomal loops with a minimum length of 5 Kbp

and a maximum length of 2 Mbp. Tracks containing H3K27ac loop interactions calculated using the hichipper (--make-ucsc flag) were visualized using the WashU Epigenome browser and filtered according to interaction score. Virtual 4C (v4C) profiles were generated by visualizing dumped hic file outputs from Juicer (Durand et al., 2016a, 2016b) with a custom R script according to (Mumbach et al., 2017).

RNA sequencing

For FACS-sorted RNA-seq, GFP positive cells were isolated using Langendorff perfusion of heart 3 days after 6-dose tamoxifen injection. Because of limited GFP cells, sorted cells were directly collected in RLT lysis buffer from RNeasy Plus Micro Kit (Qiagen) and SMART-Seq Ultra Low Input RNA Kit to prepare RNA-seq library (Clonetech Laboratories). RNA-seq was performed on Ion Proton. RNA Seq reads were aligned to mm10 (*Mus musculus* assembly July 2007). Raw read counts were normalized and analyzed for differential gene expression by DESeq2. Metascape (http://metascape.org/) was used for Gene Ontology (GO) analysis to extract the information on gene set and gene network.