SUPPLEMENTAL MATERIAL

Expanded Materials & Methods

Animal Models and Cardiac Physiology

All experiments using animals were approved by the University Committee on Animal Resources at the University of Rochester. Procedures were performed using male and female mice harboring Tcf21^{MerCreMer}, p53^{flox}, and R26R^{mTmG} alleles[26, 62]. The *Tcf21*^{MerCreMer} fibroblast-specific and tamoxifen inducible Cre mouse line[26] was a kind gift from Jeffery Molkentin at the University of Cincinnati Children's Hospital. The p53$^{flox/flox}$ mouse line (Jackson Labs #008462, submitted by Anton Berns[28]) was a kind gift from Archibald Perkins at the University of Rochester School of Medicine and Dentistry. These lines were used to generate p53^{WT}, p53$^{flox/WT}$, and p53$^{flox/flox}$ mice. For lineage tracing experiments, some mice were additionally bred to the *Rosa26*^{mTmG} line, which indelibly labels cells via Cre-mediated recombination. For the Tcf21^{MerCreMer} induction, five once-daily intraperitoneal injections of tamoxifen at 100 mg/kg were performed, and then animals were given a two-week washout period prior to surgery. Some animals were sacrificed after the washout period on the day that surgery would have been performed (called baseline) to evaluate potential effects of p53-CF KO that present on the day of surgery. Transverse aortic constriction (TAC) was performed on isoflurane-anesthetized male and female mice between 12-20 weeks of age via a left thoracotomy by placing a 6-0 silk ligature securely around the trans-aorta and a 27-gauge needle, causing complete aortic occlusion. The needle was then removed, restoring a lumen. A baseline echocardiographic analysis was performed prior to surgery, with subsequent echoes at 7, 14, 21, and 28 days post-TAC. Echocardiographic analysis using M-mode was performed using a Vevo3100 echocardiography machine (VisualSonics, Toronto, Canada) and a linear-array 40MHz

transducer (MS-550D). LV systolic and diastolic measurements were captured in M-mode from the parasternal short axis. No significant difference in cardiac physiology was observed in male and female mice. During the course of TAC, a subset of mice were given an intraperitoneal injection once every other day with B Bromodeoxyuridine (BrdU) at 50 mg/kg in sterile saline to label proliferating cells. For the proliferation time course experiment in Figure 1, these injections were performed either throughout the 28 days, or in weekly spans to label only a discrete week of cell proliferation.

Preparation of single-cell suspensions for sc-RNA sequencing

Two each of $Tcf21^{MerCreMer}$;$p53^{+/+}$;$R26R^{mTmG}$ (p53-CF WT) and $Tcf21^{MerCreMer}$;$p53^{fl/fl}$;$R26R^{mTmG}$ (p53-CF KO) male animals were subjected to the above experimental protocol and sacrificed at 14 days post-TAC. Mice were anesthetized with ketamine/xylazine, then the atria were cut and the heart was perfused with cold PBS via the left ventricular apex. The heart was then removed and atria/vessels were dissected away. Ventricles were minced until smooth then transferred to 3 mL of the cold collagenase/dispase solution with gentle stirring for 60 minutes. Hearts were digested in a solution of collagenase/dispase (3.33 mg/mL final dilution) containing 20 mM butanedione monoxime. Digestion was stopped with 6 mL 0.5 M EDTA and samples were briefly vortexed then placed on ice. Samples were strained through a 75 μM filter in cold HBB buffer (formulation 500 mL HBSS, 10 mL FBS, 1.0 g BSA) and spun at 400g for 5 minutes at 4°C. The supernatant was aspirated and 1 mL ACK lysis buffer was added to eliminate erythrocyte contamination. The sample was vortexed briefly and incubated for 5 minutes at room temperature prior to addition of cold HBSS and a subsequent 400g, 5 minute spin at 4°C. The supernatant was again aspirated and transferred to a tube in 1 mL FACS buffer (formulation

500 mL PBS, 10 mL FBS, 1 mL 0.5 M EDTA) pending flow sorting. 5 minutes prior to flow sorting, DAPI was introduced to label dead cells and provide a negative sorting strategy. Flow sorting was performed on a BD FACSAria II (BD Biosciences) with positive sorting for GFP and negative sorting for tdTomato and DAPI. After sorting, the *Tcf21*$^{+}$ CF were placed on ice and taken directly to the Genomics Research Center at the University of Rochester.

Single cell library preparation and processing

Single cell libraries were generated from Tcf21-lineage cardiac fibroblasts acquired by FACS. Prior to capture using the 10x Genomics Chromium controller (10x Genomics), the number of cells was quantitated (TC20 Automated Cell Counter, Bio-Rad) and cell viability was assessed via the trypan blue exclusion test of cell viability. Only cell populations exhibiting greater than 80% viability were used. All cells were loaded in order to maximize the number of single cells acquired using the Chromium single Cell 3' Reagent Kit. Libraries were prepared according to manufacturer's instructions using the Chromium Single Cell 3' Library and Gel Bead Kit v.2 (10x Genomics). CellRanger v3.1.0 was used to demultiplex each capture, process base-call files to fastq format, and perform 3' gene counting for each individual cell barcode with mouse reference data set (mm10, v 3.1.0).

Cell filtering and cell-type annotation and clustering analysis.

Quality control, identification of variable genes, principal component analysis (PCA) and non-linear reduction using uniform manifold approximation and projection (UMAP) was performed using Seurat (v3.2.3 and R v3.6.1). In order to understand the effect of genotype, the Seurat merge function was used to combined the p53-CF WT and p53-CF KO captures to maintain the variation

introduced by developmental time. Cell cycle scoring was performed and the variation introduced by genes involved in mitochondrial transcription and genes corresponding to cell cycle were regressed out during data scaling. Data was visualized in UMAP space and clusters were defined using default parameters. Seurat was also used to visualize expression of specific genes across all clusters using the VlnPlot, DoHeatMap, and DotPlot functions. clusterProfiler (v3.17.0) was used to visualize gene set enrichment of differentially expressed genes corresponding to various clusters, differential expression across p53-CF KO and p53-CF WT cells within clusters, and differential expression across specific Monocle defined cell states.

Developmental trajectory and prediction of cell-fate determinants.

The GetAssayData function in Seurat (v3.2.3) was used to extract the raw counts to construct the Monocle object. To construct the trajectory the default functions and parameters as suggested by Monocle (v2.14.0) were used. The resulting Monocle trajectory was colored based on Monocle State, Pseudotime, and Seurat clusters previously identified. A tw2o-proportion test was used to determin significant contributions of either p53-CF KO or p53-CF WT cells to a given state. Monocle states corresponding to cells were exported to Seurat for use in differential expression.

Primary neonatal mouse CF isolations

Primary CF were isolated from neonatal mouse hearts by differential plating as previously described[18]. Briefly, hearts were removed from neonatal mice on the day of birth. Ventricles were isolated and transferred to a solution of 0.8 mg/mL collagenase type II (Worthington Labs) and finely minced. Hearts were then agitated in 10 mL collagenase solution in four cycles of 10 minutes each, collecting cells at each step by centrifugation. Cells were strained through a 70 μm

filter and plated on a 10 cm dish for one hour to enrich adherent cells (non-myocyte fraction). Adherent cells were allowed to expand for 24 hours in low-serum (0.5%), high-glucose DMEM before plating passage one cells for experiments.

Cell culture procedures and assays

Primary neonatal CF isolated as described above were maintained in low-serum (0.5%) high-glucose DMEM to impede spontaneous activation for 24 hours prior to experimentation. For adenoviral deletion experiments, CF from WT or $p53^{flox/flox}$ mice were transduced with adenovirus harboring either β-galactosidase (control) or Cre recombinase (Vector Biolabs). Two distinct regimens were followed: 1) infection with virus for 24 hours, followed by washout and a 24hr (for RNA) or 72 hr (Western blot/immunostaining) period of activation with 10 ng/mL TGF-β1 (R&D Systems) and 1 μM AngII (Sigma-Aldrich); or 2) 72 hours of activation with TGF-β1/AngII, followed by infection with virus for 24 hours prior to RNA or protein analyses. These regimens accomplish p53 deletion in quiescent fibroblasts (Regimen 1) or in activated myofibroblasts (Regimen 2). In a rescue experiment, $p53^{flox/flox}$ CF were transduced +/- Ad/Cre +/- Ad/Cdkn1a. 24 hrs after transduction, CF were treated with 10 ng/mL TGF-β1 (R&D Systems) and 1 μM AngII (Sigma-Aldrich) for an additional 48 hours prior to assay. At all times, CF were maintained at 0.5% serum to reduce spontaneous activation. Hydroxyproline content was measured from CF culture supernatants using the Hydroxyproline Assay Kit (Sigma) according to manufacturer instructions. Cell proliferation was measured using the CyQuant Proliferation Assay (ThermoFisher) according to manufacturer instructions.

Flow cytometry to measure cell cycle

Cells were trypsinized with 2.5% trypsin-EDTA, resuspended in PBS with 0.2% BSA, then fixed with cold 70% ethanol. Cell suspensions were then stained with propidium iodide containing RNase A (BD Pharmingen). Flow cytometry was accomplished using a 3-laser, 12-color BD LSR-II with excitation at 610/20 nm (BD Biosciences). Data were analyzed using FlowJo.

RNA isolation and analyses

RNA isolation and quantitative RT-PCR were performed as previously described[18 4]. For qRT-PCR, RNA was purified using TriZol (Invitrogen) and genomic DNA removed using Turbo DNase (Ambion). 500-1000ng RNA was used to make cDNA using iScript Reverse Transcriptase kit (BioRad). qRT-PCR was performed using a BioRad CFXConnect with SYBR Green (Invitrogen) as the intercalating fluorophore. Threshold cycles were collected and analyzed by the ΔΔCt method. Supplemental Table I contains a list of primer sequences.

Histology and morphometric image analysis

When fully unconscious, the mouse chest cavity was opened and the heart tissue was perfused with calcium-and magnesium-free phosphate-buffered saline (PBS), followed by perfusion with 10% neutral buffered formalin solution and fixation overnight and subsequent histologic processing, paraffin embedding, and sectioning. In order to perform immunofluorescence or PicroSirius Red staining, slides were deparaffinized in a series of xylenes, followed by 3-minute incubations in 100% ethanol (EtOH, 3x), 95% EtOH (1x), and then placed in distilled water.

To evaluate fibrosis, 5 μM sections were generated at ten equally spaced levels from each heart, mounted on slides and stained with PicroSirius Red according to manufacturer instructions

(Abcam, Cambridge, MA). Images were captured at 10x on a BX51 epifluorescence microscope (Olympus, Shinjuku, Japan) using brightfield illumination. To evaluate fibrosis, areas of interest (perivascular and interstitial) were defined in NIH ImageJ (Fiji Version2.0.1). The extent of perivascular fibrosis was determined by calculating the ratio of the area of the positive pixels surrounding the vessel to the total vessel area. The extent of interstitial fibrosis was determined by calculating the number of positive pixels in the non-perivascular area of the section and dividing by the total non-perivascular area.

For CHP staining, sections were deparaffinized and rehydrated as described above without antigen retrieval per manufacturer instructions (3Helix). The biotinylated B-CHP peptide was reconstituted as per manufacturer instructions, then heated to 80°C, then quenched in an ice-water bath for 45 seconds prior to pipetting onto the tissue slides. Overnight incubation at 4°C was followed by washing in PBS and secondary staining with AlexaFluor 555-conjugated streptavidin (LifeTech) at 0.005 mg/mL for 1 hr at room temperature per manufacturer recommendations. WGA conjugated to AlexaFluor 647 was also introduced at this step to show tissue architecture.

For p53 detection in sections, we performed immunohistochemistry. Following de-paraffinization, 10 mM citrate buffer (pH 6) was used for antigen retrieval. Peroxidase quenching was performed using 3% hydrogen peroxide. Primary polyclonal rabbit anti-p53 antibodies (Leica NCL-p53-CM5p) were applied to sections in a 1:500 dilution and incubated overnight at 4°C. Secondary biotinylated goat anti-rabbit antibodies (Vector Laboratories BA-1000) were applied in a 1:200 dilution and incubated for 1 hour at room temperature. Binding avidity was increased using a Vectastain ABC Kit (Vector Laboratories PK-4000). Staining was visualized using DAB substrate (Vector Laboratories SK-4100) and counterstained with

Hematoxylin QS (Vector Laboratories H-3404). Stained tissue samples were scanned using an Olympus VS120 automated slide scanner. Image analysis and quantification of p53 IHC staining were performed using Visiopharm (v.2019.07; Hørsholm, Denmark). Blood vessels and adjoining fibrotic tissue were manually selected as ROI. A nuclear quantification APP was run on the ROI to determine the number of DAB-stained (positive) nuclei and hematoxylin-stained (negative) nuclei. Total area of the ROIs was measured and compared, to monitor for selection bias. Statistical analysis was performed using RStudio.

For immunofluorescence, antigen retrieval was performed in pH6 Dako Target Antigen Retrieval buffer (Agilent Technologies) followed by quenching in 3% H_2O_2 in 15mM NaCl/100mM Tris pH 7.5 (TN). Slides were then placed in Blocking Reagent (Perkin Elmer) diluted to 0.5% in TN. Primary antibodies were applied overnight at 4°C at indicated dilutions (Supplementary Table II), then slides were washed in TN buffer followed by secondary antibody incubation for two hours at room temperature. Slides were washed with 1X TN following secondary incubation with the final wash containing 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Thermo Fisher Scientific) for at least 10 minutes to stain for nuclei. Slides were mounted with VECTASHIELD Anti-Face Mounting Media (Vector Labs) before being imaged on an Olympus Confocal Microscope IX81 (Olympus Corporation). Antibodies (primaries and corresponding secondaries) used for immunostaining are listed in Supplemental Table II. Antibody validation was conducted using isotype control immunostaining on an adjacent tissue section on the same slide.

To evaluate the extent of cardiomyocyte hypertrophy, sections were stained with DAPI (to visualize nuclei, 350/50ex-460/50em) and wheat germ agglutinin (to visualize cell borders, 620/60ex-700/75em) scanned at 20x on a BX70 confocal microscope (Olympus, Shinjuku,

Japan). Transverse cardiomyocyte cross-sectional area (CSA) was segmented in Cellpose 2.0 and visualized by overlaid cytoplasmic masks[63]. TissueNet model was trained on ~1000 cells and applied for segmentation. Cellpose masks were processed in Fiji to label regions of interest (ROI) and measured pixels of each ROI. Quantification reflects at least 100-800 cardiomyocytes per mouse, and each datapoint represents the average CSA for an individual mouse.

For staining of cells, cells were cultured as described above, then fixed in 4% paraformaldehyde for 15 minutes at 4°C. Blocking and staining procedures were then followed as described above, and stained cells were left in 1X TN in the dark at 4°C pending imaging. Imaging of cells was performed on an Olympus BX51 epifluorescence microscope (Olympus, Shinjuku, Japan) with a 10x water immersion lens.

Statistical analysis

Replicates in this study are biological (not technical) unless specifically noted otherwise, and data are reported as mean +/- SEM. For imaging analyses, representative images are presented based on the highest quality and most illustrative image that most closely represents the mean value of the data analysis. Statistical analyses are performed using Prism 9.1.1. (GraphPad); R software (version 4.2.3) and the rstatix package (version 0.7.2) was used for nonparametric statistical analyses. Comparison between two groups were performed using unpaired, two-tailed t-tests with Welch's correction for unequal standard deviations. To compare p53 expression we used a 2-sided T-test in R (rstatix::t_test()) assuming equal variance. To compare variance, we ran an F-test (var.test()), and found that p=0.1659, and the F statistic is 4.76, so the sample variance is not statistically different. To compare the differences among three or more groups of data that did not follow a normal distribution, we used the Kruskal-Wallis test,

a nonparametric alternative to the one-way analysis of variance (ANOVA). If the Kruskal-Wallis test showed a significant difference ($p < 0.05$), we performed a post-hoc analysis using Dunn's test, which compares the mean ranks of each pair of groups and adjusts the p-values for multiple comparisons using the false discovery rate (FDR) correction. Geisser-Greenhouse correction was used for repeated measures two-way ANOVA . Post-hoc comparisons were made using the Tukey test for two-way repeated measures ANOVA where three or more groups were compared. $p < 0.05$ is considered significant.

Supplemental Tables

Table S1: qRT-PCR Primers

Gene	Fwd Primer Sequence (5'-3')	Rev Primer Sequence (5'-3')
Mouse genes		
Acta2	GTTCAGTGGTGCCTCTGTCA	ACTGGGACGACATGGAAAAG
Ccna2	ACTGCTCGAGGTACATGTGTCTA	AGTCACTAGTCTTACAAGCTGAAC
Ccne1	TGTTTTTGCAAGACCCAGATGA	GGCTGACTGCTATCCTCGCT
Cdkn1a	GTACTTCCTCTGCCCTGCTG	CACAGAGTGAGGGCTAAGGC
Cdkn2a	CATGTTGTTGAGGCTAGAGAGG	CACCGTAGTTGAGCAGAAGAG
Col1a1	TAGGCCATTGTGTATGCAGC	ACATGTTCAGCTTTGTGGACC
Col3a1	TAGGACTGACCAAGGTGGCT	GGAACCTGGTTTCTTCTCACC
Fn1	AGACCTGGGAAAAGCCCTACCAA	ACTGAAGCAGGTTTCCTCGGTTGT
Postn	TGGAAACCATCGGAGGCAAA	TCAAATCTGCAGCTTCAAGG
Trp53	GTCTACGTCCCGCCATAAAA	AGGCAGTGAAGGGACTAGCA

Table S2: Antibody and staining information

Antibody	Manufacturer	Catalog No.	Application	Dilution
Acta2	Sigma-Aldrich	A5228	ICC – Fluor	1:250
BrdU	Novus Biological	NB500-169	IF-Paraffin	1:200
CHP (biotin)	3Helix		IF-Paraffin	1:100
ERG	Abcam	ab92513	IF-Paraffin	1:100
GFP	Torrey Pines Biolabs	TP401	IF-Paraffin	1:100
IsolectinB4-FITC	Sigma-Aldrich	L2895	IF-Paraffin	1:50
Ki67	Novus Biological	NB110-89717	ICC – Fluor	1:100
$p16^{Ink4a}$	Abcam	ab189034	IF-Paraffin	1:100
P53	Leica	NCL-L-p53-CM5p	IHC-Paraffin	1:500
PDGFRα	R&D Systems	AF1062	IF-Paraffin	1:100
SA-555	LifeTech	S21381	IF-Paraffin	1:250
WGA-A647	Thermo-Fisher	W32466	IF-Paraffin	1:100

Supplemental Figures and Figure Legends

Figure S1

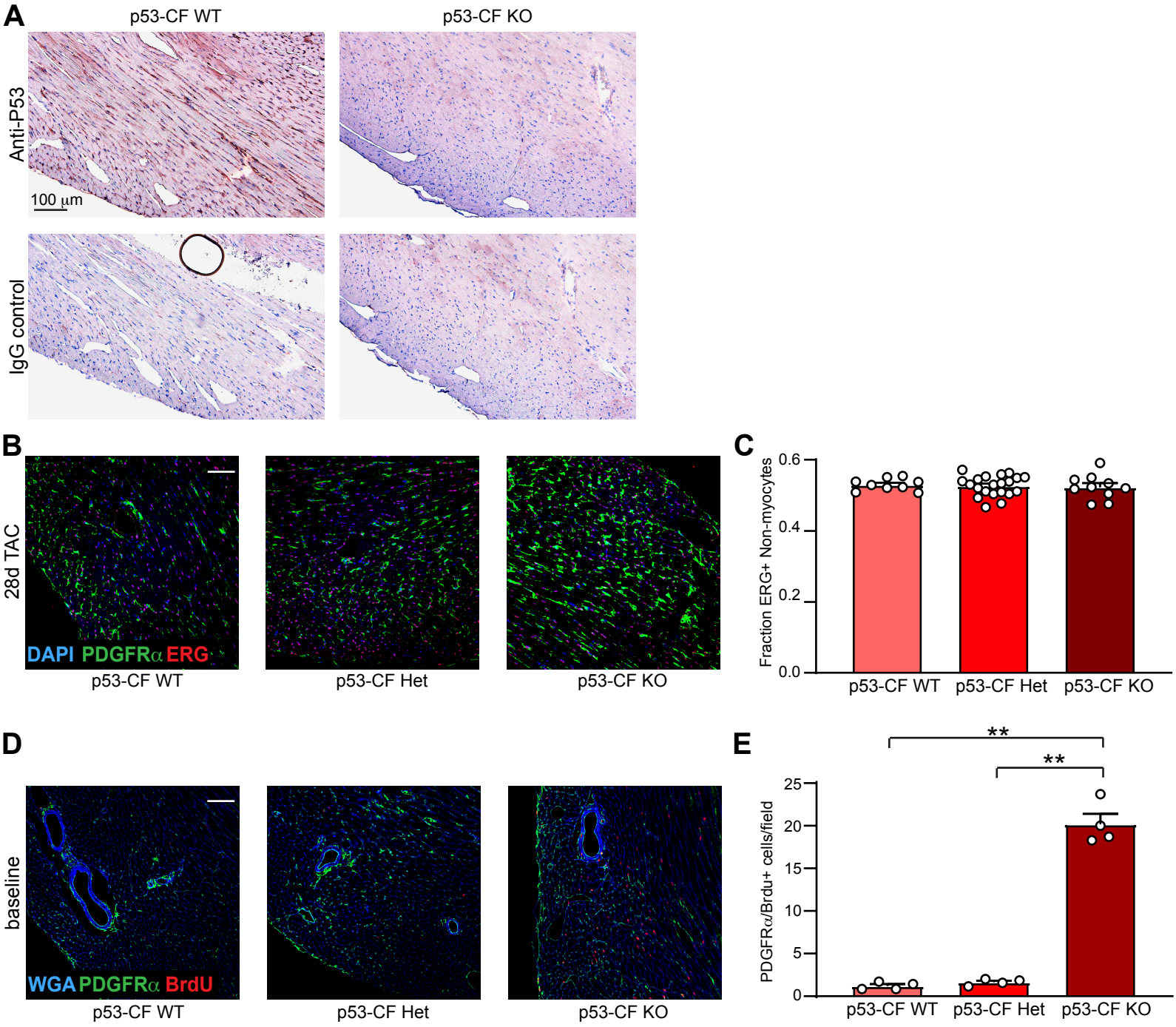

A
p53-CF WT
p53-CF KO
Anti-P53
IgG control
100 μm
B
28d TAC
DAPI PDGFRα ERG
p53-CF WT
p53-CF Het
p53-CF KO
C
Fraction ERG+ Non-myocytes
0.0
0.2
0.4
0.6
p53-CF WT
p53-CF Het
p53-CF KO
D
baseline
WGA PDGFRα BrdU
p53-CF WT
p53-CF Het
p53-CF KO
E
PDGFRα/Brdu+ cells/field
0
5
10
15
20
25
**
**
p53-CF WT
p53-CF Het
p53-CF KO

Figure S1. A) Heart sections were obtained from mice of indicated genotype at 14 days post-TAC (4 weeks post TMX injection). Representative images are shown from sections that were incubated with antibodies directed against p53 (or IgG as control) and counterstained with light eosin. **B)** Representative images of immunofluorescence detection of PDGFRα (green fibroblasts) and ERG (red, endothelial cells) in heart sections of indicated genotype isolated 28 days after TAC. **C)** Quantification of ERG^+ nuclei from images in (B). **D)** Heart sections were obtained from mice of the indicated genotype that were injected with BrdU for the 2 weeks after tamoxifen injection, but without TAC surgery (baseline). Representative images of sections stained with wheat germ agglutinin (WGA, blue), and antibodies directed against PDGFRα (green), and BrdU (red) to measure baseline cardiac fibroblast proliferation. **E)** Quantification of BrdU incorporation into $PDGFR\alpha^+$ cells at baseline from images (D). Scale bar = 100μM (A) or 50μM (B, D). **P<0.01.

Figure S2

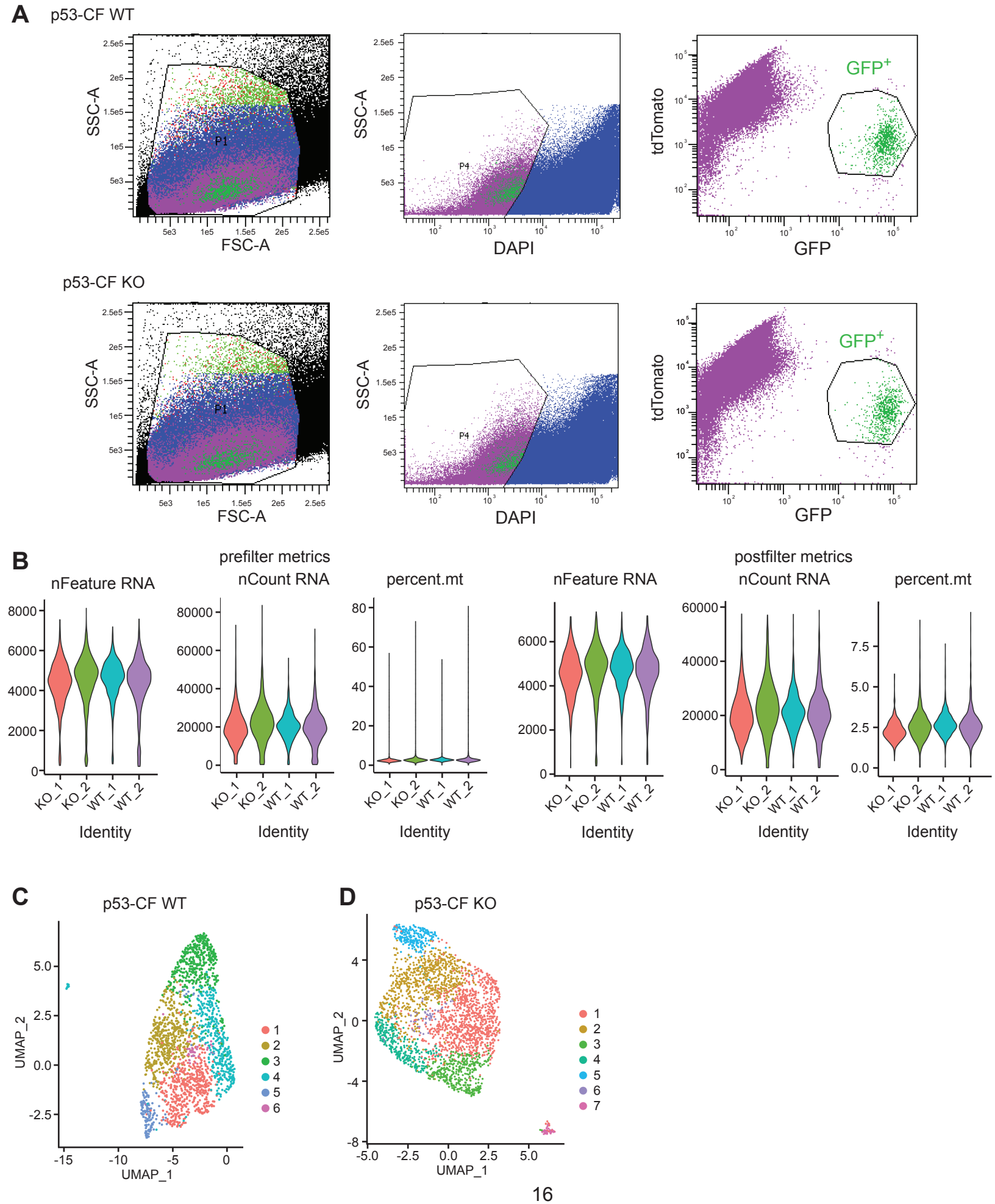
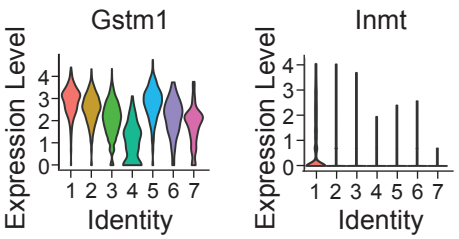

A
p53-CF WT
SSC-A
FSC-A
P1
DAPI
P4
tdTomato
GFP
GFP+
p53-CF KO
B
prefilter metrics
postfilter metrics
nFeature RNA
nCount RNA
percent.mt
KO_1
KO_2
WT_1
WT_2
Identity
C
p53-CF WT
UMAP_1
UMAP_2
D
p53-CF KO

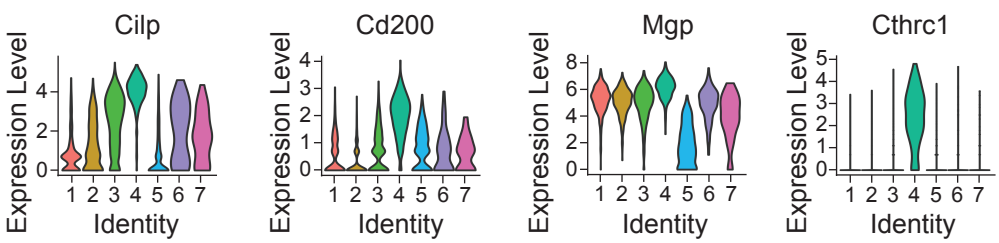
Figure S2. Tcf21^{MerCreMer}; Rosa^{26mTmG}; p53$^{+/+}$ (p53-CF WT) and Tcf21^{MerCreMer}; Rosa26^{mTmG}; p53$^{fl/fl}$ (p53-CF KO) mice were subjected to 5 tamoxifen injections (100 mg/kg) and a 2 week washout prior to TAC surgery. Hearts were isolated at 14 days post-TAC and non-myocytes were isolated for enrichment of Tcf21-lineage cardiac fibroblasts by Fluorescence activated cell sorting (FACS). **A)** Gating plots are shown for one representative p53-CF WT and one representative p53-CF KO mouse. **B)** Pre and post-filtered metrics reveal retained cells after regressing out variability introduced by cell cycle and mitochondrial genes. n= 2 p53-CF WT and n=2 p53-CF KO animals at 14 days post-TAC. **C, D)** Uniform manifold approximation and projection (UMAP) visualization of single cell transcriptomes from p53-CFWT (C) and p53-CF-KO (D) hearts.

Figure S3

A



B



C

C5: vascular	fold enrichment	FDR
positive regulation of MHC class I biosynthetic process	>100	3.74E-02
negative regulation of p38MAPK cascade	>100	4.28E-02
negative regulation of skeletal muscle tissue development	>100	4.33E-02
regulation of MHC class I biosynthetic process	>100	4.38E-02
vasoconstriction	29.45	3.69E-02
cellular response to glucocorticoid stimulus	29.45	3.74E-02
regulation of p38 MAPK cascade	28.73	3.91E-02
negative regulation of DNA biosynthetic process	28.05	4.07E-02
cellular response to corticosteroid stimulus	26.18	4.48E-02

D

C6: immune	fold enrichment	FDR
leading edge cell differentiation	93.17	3.34E-02
cerebral cortex tangential migration using cell-cell interactions	62.11	4.92E-02
substrate-dependent cerebral cortex tangential migration	62.11	4.93E-02
postnatal olfactory bulb interneuron migration	62.11	4.95E-02
osteoclast development	46.58	7.17E-03
negative regulation of monocyte chemotaxis	46.58	7.21E-03
negative regulation of lens fiber cell differentiation	46.58	7.24E-03
bud elongation involved in lung branching	39.93	9.19E-03
regulation of lens fiber cell differentiation	27.95	1.81E-02

Figure S3. Suerat defined Tcf21-lineage cell identities from combined p53-CF WT and p53-CF KO dataset. **A, B)** Violin plot visualization of the relative expression of quiescent fibroblast-enriched (A) and cluster 4 myofibroblast-enriched genes (B). **C, D)** Enriched KEGG pathways representative of Cluster C 5 (C) and C6 (D).

Figure S4

A

GO terms enriched in cell state 1	adjusted p-value
cellular response to cytokine stimulus	2.87E-03
post-translational protein modification	5.36E-03
positive regulation of extrinsic apoptotic signaling pathway via death domain receptors	4.33E-03
negative regulation of dendritic cell apoptotic process	5.67E-03
negative regulation of cell motility	1.05E-02
positive regulation of leukocyte chemotaxis	1.26E-02
regulation of dendritic cell apoptotic process	1.10E-02
peptide metabolic process	9.80E-03
cytokine-mediated signaling pathway	1.13E-02
response to nitric oxide	1.05E-02
embryonic organ morphogenesis	1.08E-02
negative regulation of leukocyte apoptotic process	1.17E-02
negative regulation of cell migration	1.43E-02
cellular protein metabolic process	1.33E-02
platelet degranulation	1.42E-02

B

Transcription factors enriched in cell state 1	adjusted p-value
CEBPD	2.00E-07
OCT4	1.07E-05
NUCKS1	1.00E-05
RELA	7.07E-05
RUNX2	6.43E-05
TCF21	5.36E-05

C

GO terms enriched in cell state 11	adjusted p-value
extracellular matrix organization	0.00E+00
cytokine-mediated signaling pathway	7.00E-07
platelet degranulation	6.00E-05
regulated exocytosis	4.99E-05
cellular response to hypoxia	5.82E-04
regulation of cellular amine metabolic process	6.05E-04
regulation of cellular amino acid metabolic process	5.18E-04
tumor necrosis factor-mediated signaling pathway	1.27E-03
regulation of transcription from RNA polymerase II promoter in response to hypoxia	1.17E-03
regulation of cellular ketone metabolic process	1.48E-03
interleukin-12-mediated signaling pathway	2.05E-03
cellular response to interleukin-12	1.88E-03
post-translational protein modification	2.34E-03
cellular response to tumor necrosis factor	2.21E-03
regulation of transcription from RNA polymerase II promoter in response to stress	3.07E-03

D

Transcription factors enriched in cell state 11	adjusted p-value
EKLF	0.00E+00
RUNX2	0.00E+00
MYC	0.00E+00
ATF3	2.00E-07
CEBPB	6.90E-06
SMAD2	1.06E-05

Figure S4. Gene ontology enrichment of quiescent and activated cardiac fibroblast states. **A)** Gene ontology (GO) terms enriched in state 1 quiescent cardiac fibroblasts. **B)** Predicted transcription factor upstream regulators that mediate state 1 cell identity. **C)** GO terms enriched in state 11 myofibroblasts. **D)** Predicted transcription factor upstream regulators that mediate state 11 myofibroblast identity.

Figure S5

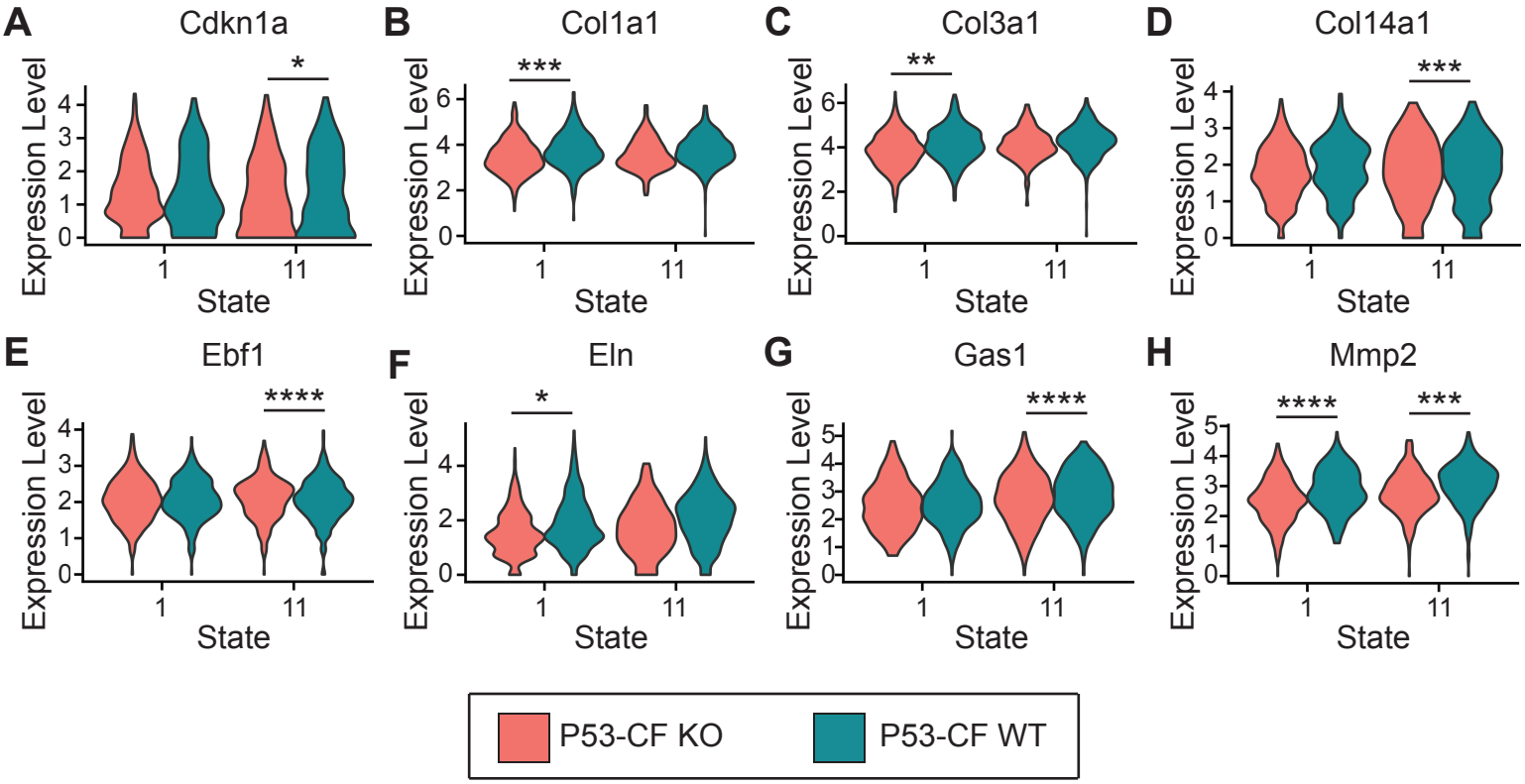

A
Cdkn1a
*
B
Col1a1
***
C
Col3a1
**
D
Col14a1
***
E
Ebf1
****
F
Eln
*
G
Gas1
****
H
Mmp2
****
***
Expression Level
State
1
11
P53-CF KO
P53-CF WT

Figure S5. p53 deletion in cardiac fibroblasts alters expression of genes encoding cell cycle regulators and ECM proteins. **A-H)** Violin plot representation of the expression of select genes in state 1 (quiescent) and 11 (myofibroblast), as a function of genotype. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Figure S6

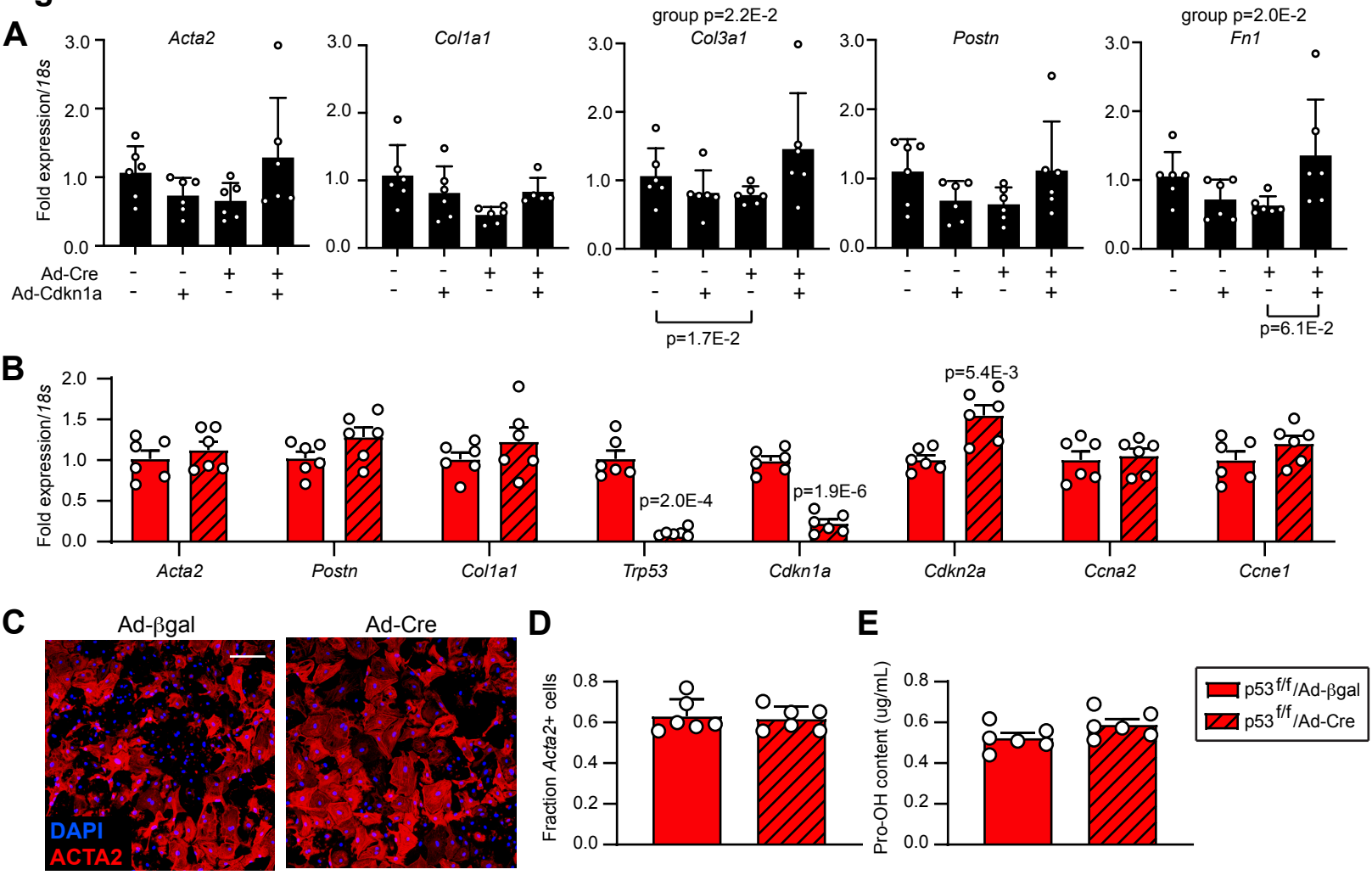

A
Acta2
Col1a1
group p=2.2E-2
Col3a1
Postn
group p=2.0E-2
Fn1
Fold expression/18s
Ad-Cre
Ad-Cdkn1a
p=1.7E-2
p=6.1E-2
B
Fold expression/18s
p=2.0E-4
p=1.9E-6
p=5.4E-3
Acta2
Postn
Col1a1
Trp53
Cdkn1a
Cdkn2a
Ccna2
Ccne1
C
Ad-βgal
Ad-Cre
DAPI
ACTA2
D
Fraction Acta2+ cells
E
Pro-OH content (ug/mL)
p53f/f/Ad-βgal
p53f/f/Ad-Cre

Figure S6. Neonatal cardiac fibroblasts (CF) were isolated from p53-fl/fl mice and treated as follows for indicated experiments. **A)** Cells were treated with adenovirus directing the expression of Cre recombinase and/or Cdkn1a (p21), as indicated for 48 hrs prior to stimulation of the myofibroblast phenotype with TGF-β1 (10ng/mL)/AngII (1μM) treatment for an additional 48 hrs. qRT-PCR is shown for myofibroblast marker genes. Datapoints indicate results of individual biological replicate cell culture well. Data is represented as Mean +/- SEM. Data is analyzed by Kruskal-Wallis test followed by Dunn's test to calculate pairwise comparisons. **B-E)** Cells were treated with adenovirus directing the expression of Cre recombinase as indicated for 72 hrs prior to treatment with TGF-β1 (10ng/mL)/AngII (1μM) treatment for an additional 24 hrs. B) qRT-PCR is shown for candidate proliferation and myofibroblast marker genes. Datapoints indicate results of individual biological replicate cell culture well. Data is represented as Mean +/- SEM. Data is analyzed by unpaired, two-tailed t-test with Welch's correction. C) CF were immunostained with Acta2 to mark myofibroblasts labeled with Dapi to mark nuclei. Scale bar = 50 μm. D) Quantification of fraction $Acta2^{+}$ cells shown in (C). E) Hydroxyproline was detected in conditioned medium of cells of the indicated treatment group.