

SUPPLEMENTAL MATERIAL

The bHLH transcription factor TCF21 inhibits myofibroblast formation and cardiac fibrosis

Contents

Supplemental Methods

Major Resources Table

Figures S1-S7 with legends

Tables S1–S4 are separate EXCEL spreadsheets of large GEO deposited datasets

Supplemental Methods

Animals

All experiments were carried out in accordance with the guidelines and approval by the Institutional Animal Care and Use Committee (IACUC) at Cincinnati Children's Hospital under the protocol IACUC 2021-0047. All mice used were on a *C57/BL6J* background. Both male and female adult mice (8-12-weeks-old at the start of the experiment) were used in all studies, unless otherwise specified. Blinding of experimentation, analysis and randomization of mice to experimental groups was performed when possible. Where possible, littermates were used. Mice were housed in a temperature-controlled vivarium with ad libitum access to food and water under a 12-hour light-dark cycle. All *in vivo* and *in vitro* data points represent individual biological samples. Where indicated, several mice were pooled during sample collection to represent one data point.

To generate cardiac fibroblast gene deleted mice, mice with the MerCreMer allele inserted into the endogenous *Tcf21* locus¹ (*Tcf21*-MerCreMer; *Tcf21*^{MCM} mice), or mice with CreER^{T2} inserted into the start codon of the platelet derived growth factor receptor, alpha polypeptide (*Pdgfra*) gene (*Pdgfra*-CreERT2)² were utilized. For ubiquitous Cre expression, a transgenic CMV-Cre expressing mouse was used from the Jackson laboratory (006054)³. *Tcf21*-loxP targeted mice⁴ were crossed with the indicated Cre expressing line for genetic recombination of the *Tcf21* locus. *Rosa26-loxP-STOP-loxP-enhanced green fluorescent protein (eGFP)* allele knock-in mice were from the Jackson laboratory (012429)⁵.

Generation of inducible TCF21 overexpressing mice

The human *TCF21* cDNA was inserted downstream of the 3'-untranslated region of the mouse *Coll1a1* locus using CRISPR-Cas9 in mouse newly fertilized oocytes. A targeting vector containing 2.5 kb upstream and 3 kb downstream homology arms of the *Coll1a1* locus flanking a CMV early enhancer/chicken β actin (CAG) promoter, lox66, an inverted SV40 poly(A)-addition signal, lox71 and two insulators was generated as previously described⁶. The human *TCF21* cDNA was PCR amplified from plasmid stock MHS6278-202801675 (Horizon Discovery) and inserted in an inverted orientation into the targeting vector using a Mlu I restriction site following the inverted SV40 poly(A) signal. Upon Cre-mediated recombination of the lox66 and lox71 sites, the intervening region is inverted and the *TCF21* cDNA is expressed⁷. To generate *TCF21* targeted mice, fertilized *C57/BL6J* one cell stage zygotes were administered Cas9 protein (1081061, integrated DNA technologies (IDT)), a synthetic sgRNA (5'GGGAGGAAACCTGCCCTTGG) and the targeting vector at a concentration of 40 ng/ μ l, 27 ng/ μ l, and 4 ng/ μ l, respectively, by pronuclear injection. On the same day, approximately 25 injected embryos were transferred to the oviductal ampulla of pseudo-pregnant CD-1

females (strain code 22, Charles River Laboratories). Subsequent pups were genotyped by long-range PCR and Sanger sequencing.

Tamoxifen treatment in mice

To induce genetic recombination of loxP containing alleles in mice in the presence of Cre recombinase, a combination of 400 mg/kg tamoxifen citrate chow (TD.130859, Inotiv; provides ~40 mg/kg body weight per day) was fed for 2 weeks and intraperitoneal (i.p.) injections of tamoxifen (T5648, Sigma-Aldrich) were given as indicated within the figures. In other experiments only i.p. injections of tamoxifen were used. Tamoxifen was prepared fresh every 3 days by pre-dissolving in 100% ethanol at 55 °C (~2 minutes) under agitation and then reconstituting in corn oil (C8267, Sigma-Aldrich) to achieve a 5% w/v ethanol concentration. Tamoxifen was gently shaken at 37 °C for 20 minutes to ensure complete emulsion. Tamoxifen i.p. injections were given for either 3 or 5 consecutive days at a dose of 2 mg/mouse. All mice used in *in vivo* experiments received tamoxifen.

Myocardial infarction

For myocardial infarction (MI) or sham surgeries, adult mice were anesthetized with isoflurane at 3% to obtain a surgical plane of anesthesia. Mice were maintained on a continual flow of 1.5-2.0% isoflurane and monitored for lack of a pain reflex throughout. Mice were intubated and the heart was isolated by an incision in the skin at the third intercostal space and retraction of the pectoral and intercostal muscles. The left coronary artery was isolated and occluded with 8.0 prolene suture (8730H, Ethicon) to induce a MI. For sham surgeries, the procedure was the same except that the artery was not occluded. The muscle and rib cage were closed with 4.0 silk suture (K871H, Ethicon). Analgesia (slow-release buprenorphine, 0.05-0.1 mg/kg) was given immediately after surgery and mice were placed in 30 °C incubators overnight.

Angiotensin II/phenylephrine (Ang II/PE) model of cardiac fibrosis

Ang II/PE was continuously administered to mice via micro-osmotic mini pumps (Alzet, 1002) to induce cardiac injury and fibrosis. Ang II (A9525, Sigma) and PE (P6126, Sigma) were dissolved in 1X Hanks' balanced salt solution (HBSS; SH3058801, Fisher Scientific) at concentrations of 10 mg/mL and 500 mg/mL, respectively. Solutions were combined to achieve a drug infusion rate of 1.5 µg/g/day of Ang II and 50 µg/g/day of PE. Micro-osmotic pumps were filled with the solution using a blunted needle and syringe and placed in HBSS solution overnight at 37 °C. The following day, mice were anesthetized using 3% isoflurane to obtain a surgical plane of anesthesia and maintained by a continual flow of 1.5-2.0% isoflurane with monitoring as above. The pumps were inserted subcutaneously in the back of mice above the thoracic segments of the spine. The 1.5 cm skin insertion site was sutured back together with 4.0 silk suture and mice were administered slow-release buprenorphine (0.05-0.1 mg/kg) and

71 moved to 30 °C incubators overnight. The wound area was treated with antibiotic ointment for
72 2 days following the surgical procedure.

73 *Echocardiography*

74 Pre-shaven mice were anesthetized with 3% isoflurane and maintained at 1.5% isoflurane for
75 echocardiographic measurements. Heart dimensions and function were determined by two-
76 dimensional transthoracic imaging using the VisualSonics 3100 Ultrasound system with a 30
77 MHz transducer. Anesthetized mice were secured to a 37 °C heated platform in the prone
78 position equipped with electrocardiogram sensors for heart rate monitoring (VisualSonics,
79 Fujifilm). Echocardiographic motion (M)-mode measurements were recorded from the
80 parasternal short-axis view at the level of the papillary muscles. Analysis of heart rate, wall
81 thickness, ejection fraction, and end-diastolic/systolic dimensions were analyzed using left
82 ventricular (LV) Trace in the VevoLab VisualSonics analysis package. For MI studies, due to
83 the left ventricular remodeling, the ejection fraction was obtained in brightness (B)-mode using
84 LV trace in the parasternal long axis view. For each animal, a minimum of 3 cardiac cycles
85 were analyzed. For M mode measurements, at least 3 cardiac cycles in 3 different images were
86 analyzed. Echocardiographic measurements and analysis were blinded.

87 *Histological processing of tissue*

88 For general histological analysis of heart tissue, mice were sedated with 3% isoflurane and
89 monitored for the absence of a pain reflex prior to cervical dislocation. The chest was opened
90 to expose the heart and injected with ice cold cardioplegia solution (100 mM KCl, 5% (w/v)
91 dextrose, 30 mM butanedione monoxime in 0.1 M sodium phosphate buffer) and excised.
92 Whole hearts were fixed in 4% v/v paraformaldehyde (PFA)/0.1 M sodium phosphate buffer
93 for 24 hours at 4 °C (for frozen and paraffin embedded tissue) with gentle agitation. For frozen
94 tissue, hearts were rinsed twice in phosphate buffered saline (PBS) for 10 minutes and then
95 immersed in 30% (w/v) sucrose in 0.1 M sodium phosphate buffer overnight prior to embedding
96 in OCT freezing medium (4583, Sakura). Samples were then flash frozen in 2-methylbutane
97 chilled with dry ice. For paraffin embedding, tissue was rinsed twice in PBS for 10 minutes and
98 then immersed in 70% (v/v) ethanol, dehydrated through a series of increasing ethanol
99 concentrations and embedded in paraffin. Tissue blocks were cut into 4 µm (paraffin) and 8 µm
100 (frozen) tissue sections for histological analysis.

101 *Histological staining for fibrosis and tissue morphology*

102 Sirius red staining and hematoxylin and eosin staining of histological sections was performed
103 using the Tissue-Tek Prisma Plus Automated Slide Stainer in the Heart Institute histology core
104 at Cincinnati Children's Hospital Medical Center. For the assessment of fibrosis, heart tissue
105 was cut into 1 mm thick coronal sections using a mouse heart slicer matrix (HSMS001-1,
106 Zivic). Heart slices were frozen in OCT (as above) in molds and cut to reveal multiple regions

within the heart before staining. Slides were imaged using a Leica Aperio slide scanner at x20 in the Cincinnati Children's pathology core and analyzed using a positive pixel count algorithm generated in ImageScope to quantify the total area of fibrotic tissue. For analysis of fibrosis in Ang II/PE treated mice, at least 4 whole heart sections (see **Figure S6**) were analyzed per mouse that were more than 1 mm apart. For analysis of fibrosis after MI, 1 whole heart section in the middle of the heart at the level of the infarction was obtained and analyzed (see **Figure 2J** for a representative image of the tissue analyzed). The best illustrative images were used as representative images.

Immunofluorescence

Frozen tissue sections were used for all immunofluorescence analysis. Cut frozen heart tissues were air dried for 10 minutes at room temperature prior to immersion in PBS to remove the OCT. For TCF21 detection, samples were permeabilized with 1% Triton in PBS for 10 minutes. Subsequently, non-specific binding was blocked with 2% BSA (w/v), 0.2% triton (v/v) in 0.1 M sodium phosphate buffer for 1 hour at room temperature in a humidified chamber. Sections were incubated overnight at 4 °C with antibodies against green fluorescent protein (GFP, ab13970, 1:1000, Abcam), smooth muscle alpha-actin (A2547, 1:400, Sigma), periostin (NBP1-30042, 1:300, Novus Biologicals), cartilage oligomeric matrix protein (ab231977, 1:100, Abcam), SM22 (10493-1-AP, 1:200, Proteintech) or TCF21 (HPA013189, 1:200, Sigma). Sections were washed 3 times in PBS for 10 minutes to remove excess antibody and then incubated with the suitable secondary antibody at 1:400 (Invitrogen, Alexa-Fluor® dyes) for 1 hour at room temperature. Nuclei were stained using DAPI 1:5000 (D3571, Invitrogen) for 20 minutes. Sections were rinsed 3 times in PBS prior to mounting with Prolong Diamond Antifade (P36961, Thermo Fisher Scientific). To ensure the validity of the immunofluorescence staining, secondary only controls were initially evaluated on the tissue specimen. Positive and negative control tissue sections were routinely utilized. Cardiac tissue sections from Ang II/PE treated control hearts were used as positive controls for the extracellular expression of COMP and POSTN and fibroblast α SMA expression. Healthy heart tissue was used as a negative control for these proteins (α SMA is also present in smooth muscle cells in these samples). For TCF21 expression, mice with enforced TCF21 expression were utilized as positive controls. Confocal fluorescent images were obtained with a Nikon A1 confocal microscope and images were processed in FIJI/ImageJ. The best illustrative images were used as representative images.

Cardiac fibroblast isolation

Hearts were excised from 8–12-week-old male and female mice, and the ventricles and septum were isolated, rinsed in ice-cold PBS and minced into small 2 mm x 2 mm pieces using sterile micro-scissors. Tissue fragments were digested in HBSS with 2% (v/v) bovine growth serum (BGS) containing type IV collagenase (390 units/mL; LS004188, Worthington) and dispase II

(1.2 units/mL, D4693, Sigma-Aldrich) for 20 minutes at 37 °C under gentle agitation (on a nutator). The digested tissue was triturated repeatedly using a 10 mL pipette (SP210, Alkali Scientific) to promote tissue dissociation and incubated for 2 minutes to collect the sediment. The supernatant containing the liberated cells was passed through a pre-wet 40 µm strainer (76327-098, VWR) over a falcon tube to remove additional debris, diluted with 10% (v/v) BGS in HBSS and kept on ice. This process was repeated 3 times, until all the tissue was adequately digested. At each step, a smaller serological pipette (5 mL then 1 mL) was used to gradually reduce the bore size for dis-aggregation of the tissue. Once the tissue was fully digested, the cell suspension was spun at 290 g for 8 minutes in a centrifuge at 4 °C. The supernatant was removed and the cell pellet was resuspended in 1 mL of pre-warmed red blood cell lysis buffer (R7757, Sigma-Aldrich) and incubated for 1 minute at room temperature. Ten milliliters of 2% (v/v) BGS solution in HBSS was added and the sample was spun at 290 g for 8 minutes in a centrifuge at 4 °C. The final cell pellet containing the cardiac fibroblasts was resuspended in culture medium (DMEM (SH30022FS, Fisher-Scientific) with 10% (v/v) BGS and 1% (v/v) penicillin–streptomycin) and plated on precoated 0.1% gelatin 10 cm plates for 45 minutes to allow fibroblast adherence before removal of unadhered cells during replenishment of the cell culture medium. The medium was replaced the following day and cells were cultured for 2 additional days prior to infection with adenovirus encoding either Cre recombinase or β-galactosidase in 2% BGS containing media overnight. The following day, the cells were washed and maintained in culture medium.

Flow cytometry for cell quantification

Cardiac cell suspensions were prepared as described above. After red blood cell lysis, samples were resuspended in HBSS containing 2% (v/v) BGS and 2 mM ethylenediaminetetraacetic acid (EDTA) (fluorescence activated cell sorting (FACS buffer)) containing fluorophore-conjugated antibodies against cell-surface markers for 30 minutes at 4 °C under gentle rotation. See **Major Resource Table** for a list of antibodies and the concentrations used. Samples were washed twice and resuspended in 500 µl of FACS buffer. All flow cytometry experiments included unstained and single-stained samples to allow for channel compensation. Data were acquired on a BD LSR Fortessa 4-laser cytometer using BD FACSDiva software (BD Biosciences) and data were further compensated and analyzed using FlowJo 10.7. *Tcf21*-lineage traced cardiac fibroblasts were gated as CD45⁻CD31⁻mEF-SK4⁺GFP⁺, endothelial cells were gated as CD45⁻CD31⁺, neutrophils were gated as CD45⁺CD11b⁺Ly6G⁺ and monocytes were gated as CD45⁺CD11b⁺Ly6G⁻Ly6C⁺.

176 *Cell sorting for RNA-sequencing and quantitative real time polymerase chain reaction (qRT-*
177 *PCR)*

178 Cell suspensions were obtained as outlined above in *Cardiac fibroblast isolation*. Whole hearts
179 were used from 8-12-week-old mice. To enrich for fibroblasts, cells were incubated with an
180 antibody against mEF-SK4/Feeder cells conjugated with biotin (1:50, 130-101-875, Miltenyi
181 Biotec) for 20 minutes on a rotating platform at 4 °C in FACS buffer. Five hundred µl of FACS
182 buffer was added and the cells were pelleted at 300 g for 7 minutes at 4 °C. The cell pellet was
183 resuspended in FACS buffer containing anti-biotin microbeads (1:20, 130-101-875, Miltenyi
184 Biotec) and samples were incubated on a rotating platform at 4 °C for 20 minutes. The sample
185 was diluted in 500 µl of FACS buffer, spun down at 300 g for 7 minutes at 4 °C and washed
186 twice. LS columns (130-042-401, Miltenyi Biotec) were mounted on a QuadroMACs magnetic
187 separator (Miltenyi Biotec) and the purified cell population was obtained as per manufacturers'
188 instructions. The eluted cells were spun down at 300 g for 7 minutes at 4 °C and then
189 resuspended in 500 µl of HBSS containing 10% (v/v) BGS and 2 mM EDTA. The cell
190 suspension was filtered through a cell strainer for flow cytometry (08-771-23, Fisher
191 Scientific). DRAQ5 was added to achieve a final concentration of 2 µM (0.2 µL; 65-0880-92,
192 Thermo Fisher Scientific) and the sample was vortexed. After 5 minutes at room temperature,
193 propidium iodide (PI) was added at a final concentration of 3 µM (1 µL; P4864, Sigma-Aldrich)
194 and vortexed. All targeted live nucleated fibroblasts (GFP+, PI-, DRAQ5+ cells) were sorted
195 using a FACSAria™ II flow cytometry system (BD Bioscience). The cells were pelleted and
196 resuspended in 700 µL of QIAzol lysis buffer and passed through a QiaShredder column
197 (79656, Qiagen) for homogenization. The homogenized lysate was frozen at -80 °C for less
198 than 1 week prior to RNA isolation. RNA was isolated using a MiRNeasy micro kit (217084,
199 Qiagen) according to manufacturers' instructions, with the optional DNase step and a minor
200 modification. Specifically, 45 mL of isopropanol was added to buffer RWT instead of the 30
201 mL of ethanol specified in the instructions. To exclude an influence of sex on the transcriptional
202 profile, one sex (males) was selected for RNA-sequencing. For qRT-PCR analysis, a
203 combination of male and female mice was utilized.

204 *Bulk RNA library preparation, sequencing and differential expression analysis*

205 Eluted RNA was shipped to Novogene (California) for quality control analysis prior to library
206 preparation and sequencing. A SMART-seq V4 Ultra Low input RNA kit (Takara Bio) was
207 used to generate non-directional libraries from total RNA. Paired-end 150 base-pair libraries
208 were generated for all samples using a NovaSeq 6000 system. Samples were sequenced at a
209 depth of 40 million reads per sample. Reads were pseudo-aligned and quantified using an index
210 transcriptome version of the GRCm39 mouse genome (NCBI RefSeq GCF_000001635.27)
211 with *Kallisto* using standard settings⁸. Transcript-level abundance estimates were imported and
212 summarized into a counts matrix using the *tximport* package⁹, which were then normalized and

analyzed with DESeq2¹⁰ for differential gene expression. Differentially expressed genes were selected using a Benjamini and Hochberg adjusted p-value below a false discovery rate of <0.1. Heatmaps were generated using the *pheatmap* R package and the volcano plot was generated using the *EnhancedVolcano* R package (<https://github.com/kevinblighe/EnhancedVolcano>).

Gene ontology analysis

For downstream gene ontology analysis, significantly down-regulated and up-regulated genes (cut-offs outlined within the manuscript) were analyzed and annotated using DAVID^{11,12}.

qRT-PCR

For gene expression analysis, an equal amount of RNA was reverse transcribed to complementary DNA (cDNA) using the Verso cDNA synthesis kit (AB1453B, Thermo Fisher Scientific) according to manufacturers' instructions. For amplification, an equal mixture of oligo (dt) and random hexamers was used. qRT-PCR was performed using SsoAdvanced Universal SYBR® Green Supermix (1725274, Bio-Rad) according to manufacturers' instructions in a CFX96 PCR system (1845096, Bio-Rad). An annealing temperature of 60-63°C was used for all primers and with melting curve analysis on all samples. All values were normalized to *Gapdh*. See the **Major Resource Table** for primers used.

Sample preparation for single-cell RNA sequencing (scRNA-seq)

For scRNA-seq, the LV was isolated and the infarct area and border zone (or the corresponding tissue area in non-infarcted hearts) was dissected out and cut into 2 mm pieces in HBSS containing 2% (v/v) BGS. To exclude an influence of sex on the transcriptional profile, one sex (males) was selected for analysis. A total of 4 mice were pooled in each group for analysis. To establish a single cell suspension of cardiac interstitial cells for scRNA-seq, a protocol from Forte et al was adopted¹³. Briefly, tissue digestion was performed as described above in the *Cardiac fibroblast isolation* section. After red blood cell lysis, samples were resuspended in 200 µl of dead cell removal microbeads (130-090-10, Miltenyi Biotec) and incubated for 10 minutes at room temperature in the dark. LS columns (130-042-401, Miltenyi Biotec) were placed onto a QuadroMACs magnetic separator and the live cells were eluted through the column according to the manufacturers' instructions. The collected cells were spun down for 8 minutes at 400 g in a 4 °C centrifuge. The resulting cell pellet was resuspended in 500 µl of FACS buffer to prevent cell clumping. DRAQ5 and PI was added as described above. 100,000 live (PI-) nucleated (DRAQ5+) cells were sorted using a FACS Aria™ II flow cytometry system (BD Bioscience).

245 *Single-cell RNA sequencing*

246 Single-cell droplet libraries were generated according to manufacturers' instructions
247 (Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual index), PN-1000268, 10X
248 Genomics; user guide CG000315). All samples had a greater than 70% viability.
249 Approximately 16,000 cells were loaded to recover 10,000 cells. Briefly, gel emulsion beads
250 (GEM) were generated by combining gel beads, master mix containing the cells and
251 partitioning oil and loaded onto a Chromium Next GEM Chip G at a low dilution. Poly-
252 adenylated mRNA from the cell lysate contained in every GEM was reverse transcribed to
253 cDNA adding an Illumina R1 primer sequence, unique molecular identifier (UMI) and the 10X
254 barcode. The barcoded first-strand cDNA was then purified with MyoOne Silane DynaBeads
255 (20000048, 10X Genomics) and amplified using 14 PCR cycles. The full-length barcoded
256 cDNA was then enzymatically fragmented and size selected using SPRIselect (B23317,
257 Beckman Coulter) and adaptor ligated. The cDNA was amplified for library construction
258 adding an Illumina R2 primer sequence, paired-end constructs with the P5 and P7 sequences
259 used for Illumina amplification and sample indexes. Samples were pooled and run on the
260 NovaSeq 6000 sequencer with S4 flow cells using the following sequencing parameters: R1:
261 28 cycles, i7: 10 cycles, i5: 10 cycles, R2: 90 cycles. Raw sequencing reads were processed on
262 Cell ranger 5.0.1 from 10X Genomics. Reads were demultiplexed and aligned to the mm10
263 mouse genome.

264 *Single-cell RNA sequencing analysis*

265 Quality control and clustering of cells was performed with the Seurat V4 R package¹⁴ in
266 RStudio. To exclude low quality cells, cells were filtered based on the number of features
267 (genes; a minimum of 1000 genes and a maximum of 4000), counts (molecules; greater than
268 2000 counts and less than 100,000) and mitochondrial reads (less than 10%) detected per cell.
269 After quality control a total of 25,918 cells were included in the downstream analysis following
270 the Seurat pipeline¹⁴.

271 *Immunoblotting*

272 Immunoblotting was performed on cultured fibroblasts and fibroblasts isolated by magnetic
273 bead sorting with mEF-SK4 as described above in *Cell sorting for RNA-sequencing and*
274 *quantitative real time polymerase chain reaction (qRT-PCR)*. 10X RIPA buffer (20-188, EMD
275 Millipore) was diluted to 1X with deionized water and Halt™ protease and phosphatase
276 inhibitors were added according to manufacturer instructions (78442, Thermo Fisher
277 Scientific). Cultured cells were placed on ice and rinsed twice with ice-cold PBS. RIPA buffer
278 was added to the plates and cells were scraped and collected. For mEF-SK4-sorted fibroblasts,
279 RIPA was added directly to the cell pellet and resuspended. Samples were left on ice for 20
280 minutes, sonicated for 5 minutes and spun down at 14,000 g for 15 minutes at 4 °C to remove

cell debris. The supernatant was transferred to a new tube and assayed for protein concentration using a Direct Detect® spectrophotometer (EMD Millipore). Laemmli loading buffer (0.25% bromophenol blue, 0.5M dithiothreitol (DTT), 50% glycerol, 10% SDS) was added to samples and boiled at 100 °C for 6 minutes. Samples were loaded and run on 12% SDS-PAGE gels and transferred onto Immobilon-FL PVDF membranes (IPFL00010, EMD Millipore). Membranes were incubated in blocking buffer (5% (w/v) bovine serum albumin (BSA) in tris-buffered saline (20 mM Tris, 150 mM NaCl) with 0.1% (v/v) tween (TBST)) for 1 hour prior to antibody incubation overnight in blocking buffer (see **Major Resource Table** for antibodies and the concentrations used). Excess unbound antibody was washed off the membrane by 3 washes in TBST. The membranes were incubated with LI-COR secondary antibodies in blocking buffer for 1 hour, washed 3 times in TBST and imaged on a LI-COR Odyssey CLx.

Chromatin immunoprecipitation (ChIP) sequencing and ChiPmentation

ChIP-Seq was performed as described previously¹⁵. Cardiac fibroblasts were isolated from male and female *Colla1-TCF21* hearts as described above. Each sample prepared consisted of fibroblasts from 3 independent mice. To induce the expression of *TCF21*, cells were infected with an adenovirus encoding Cre recombinase in 2% BGS containing media overnight. Cells were then expanded for 5 additional days to generate 4 confluent 10 cm dishes per sample. Transforming growth factor β 1 (10 ng/mL, 101-B1-010, R&D Systems) was added for the last 24 hours in 2% BGS to further induce fibroblast activation. Cardiac fibroblasts were cross-linked for 10 minutes on ice in fixing solution (0.88% formaldehyde, 0.1 M sodium chloride (NaCl), 1 mM EDTA, 0.5 mM EGTA and 50 mM Hepes-KOH (pH 8.0)). The cross-linking reaction was quenched for 5 minutes at room temperature by adding glycine directly to the cell culture plates to achieve a final concentration of 0.125 M. The cells were washed twice with PBS, scraped and pelleted at 2000 g in a centrifuge at 4 °C. The supernatant was removed and cell pellets were frozen at -80 °C until use. All buffers used in the following procedures apart from the sonication buffer were supplemented with protease inhibitors (PIA32955; Thermo Fisher Scientific) and 0.1 mM sodium butyrate before use. To obtain nuclei, frozen cell pellets were incubated in cell lysis buffer 1 (50 mM Hepes, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40 and 0.25% Triton) for 10 minutes on ice. The lysed cells were Dounce homogenized using a pestle A and then centrifuged at 2000 g for 5 minutes at 4 °C. After centrifugation, the pellet was incubated in cell lysis buffer 2 (200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA and 10 mM Tris pH7.2) at room temperature for 10 minutes to separate the cytoplasm from the nuclei. The samples were Dounce homogenized again using pestle A and then centrifuged at 2000 g for 5 minutes at 4°C to pellet the cell nuclei. The supernatant was removed, and the nuclei were washed and resuspended with cold sonication buffer (TE with 0.1% sodium dodecyl sulfate (SDS)). The samples were sonicated on a Covaris S2 Sonicator (Covaris Inc.) for 5 minutes with SDS (peak power: 105, 10% duty factor and 200 cycles/burst)

until DNA fragments were in the 400-800 base-pair range. Triton, glycerol, NaCl and sodium deoxycholate were added to the chromatin samples to achieve final concentrations of 1%, 5%, 150 mM and 0.1%, respectively. Samples were centrifuged at 14,000 g for 10 minutes at 4 °C and the supernatant containing the chromatin was collected and transferred to a new tube. The chromatin was pre-cleared with 15 µl Protein-A Dynabeads (10001D, Thermo Fisher Scientific) for 45 minutes under rotation at 4 °C, after which the beads were removed. ChIP for TCF21 was performed using an IP-Star Compact automation system (Diagenode) with chromatin solution containing 3 µg of DNA (as measured with Qubit), 3 µg of antibody (HPA013189; Sigma-Aldrich) and 12 µl of Protein-A Dynabeads. The Dynabeads were sequentially washed with buffer 1 (RIPA 150 mM NaCl: 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 0.1% sodium deoxycholate and 1% Triton X-100), buffer 2 (RIPA 250 mM NaCl), buffer 3 (50 mM Tris-HCl, pH 8.0, 2 mM EDTA and 0.2% N-lauroylsarcosine sodium salt) and buffer 4 (Tris-EDTA (TE) and 0.2% Triton X-100) for 15 minutes each. The immunoprecipitated chromatin was then subjected to ChIPmentation on the beads as previously described¹⁶. Tagmented DNA was incubated with proteinase K in elution buffer (TE with 250 mM NaCl and 0.3% SDS) to disrupt the crosslinks, for 4 hours at 65 °C. The DNA was purified using the Qiagen MinElute DNA kit. Library preparation and PCR amplification of the eluted DNA was prepared as previously described¹⁷. Paired-end sequencing was obtained for pooled libraries using a NovaSeq 2000 instrument (Illumina, USA).

ChIP-seq analysis

ChIP-seq data were analyzed with the Trim Galore Chip-seq paired-end reads pipeline (<https://github.com/dairium/workflows/blob/master/workflows/trim-chipseq-pe.cwl>) in the Scientific Data Analysis Platform, SciDAP (<https://scidap.com/>) (Dairium). Adapters were trimmed using Trim Galore (<https://github.com/FelixKrueger/TrimGalore>) and the reads were aligned to the mouse genome assembly mm10/GRCm38 using Bowtie¹⁸. Peak-calling was performed with MACS2¹⁹. The UCSC Genome Browser's bedGraphToBigWig tool was used to produce a bigWig file. Genome browser tracks were created with the genome coverage in BEDTools²⁰ and normalized to total mapped read number and displayed as coverage on IGV browser²¹.

Motif enrichment analysis

Genomic regions of MACS2 called peaks were used for identifying transcription factor binding motifs and de novo motif discovery using HOMER motif analysis²² within SciDAP.

351 *Statistics*

352 Data were plotted using GraphPad Prism version 9 with results expressed as the mean +/-
353 standard error of the mean (SEM). Small sample sizes precluded normality testing, and thus
354 only non-parametric tests were used. For comparisons between 2 independent samples a
355 Kolmogorov-Smirnov (K-S) test was used in GraphPad Prism. For data sets with 2 levels of
356 variation (condition and genotype), the Scheirer–Ray–Hare (SRH) test was used to identify
357 significant effects between conditions, genotype and their interaction on the dependent variable.
358 All SRH tests yielded non-significant p-values for the genotype-by-condition interaction,
359 indicating no significant interaction effects. In cases where the SRH test indicated significant
360 main effects ($P < 0.05$), post-hoc pairwise analysis was performed using a Dunn's test, with the
361 Benjamini-Hochberg method applied for multiple test correction. Statistical analysis was
362 performed in R using the rcompanion and FSA packages. Outliers were not specifically tested
363 and were not removed from any analyses.

Major Resources Table

Genetically Modified Animals

Experimental models	Species	Vendor or Source	Background Strain	Catalog #	Purpose	Persistent ID / URL
B6.129S-Pdgfratm1.1(cre/ERT2)Blh/J	Mouse	The Jackson Laboratory	C57/Bl6J	032770	Fibroblast Cre targeting	RRID:IMSR_JAX:032770
FVB.Cg-Gt(ROSA)26Sortm1(CAG-lacZ,-EGFP)Gih/J	Mouse	The Jackson Laboratory	C57/Bl6J	012429	Lineage tracing	RRID:IMSR_JAX:012429
B6.C-Tg(CMV-cre)1Cgn/J	Mouse	The Jackson Laboratory	C57/Bl6J	006054	Global Cre targeting	RRID:IMSR_JAX:006054
Tcf21-loxP/loxP	Mouse	Susan Quaggin	C57/Bl6J	N/A	Tcf21 deletion	N/A
Tcf21-MerCreMer	Mouse	Michelle Tallquist	C57/Bl6J	N/A	Fibroblast Cre targeting	N/A
Col1a1-TCF21	Mouse	This paper	C57/Bl6J	N/A	Overexpression of TCF21	N/A

Cultured cells

Name	Species	Vendor or Source	Persistent ID / URL
Adult mouse cardiac fibroblasts	Mouse	Mouse primary cells	NA

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Purpose	Persistent ID / URL
Feeder cells-APC (mEF-SK4)	Miltenyi Biotec	130-120-802	1:50	Flow cytometry	RRID:AB_2784336
Feeder cells-biotin (mEF-SK4)	Miltenyi Biotec	130-101-875	1:50	Cell bead sorting	RRID:AB_2660622
Anti-biotin microbeads	Miltenyi Biotec	130-090-485	1:20	Cell bead sorting	RRID:AB_244365
Anti-CD31-PerCP Cy5.5	Biolegend	102420	1:200	Flow cytometry	RRID:AB_10613644
Anti-CD11b-AF700	Biolegend	101222	1:200	Flow cytometry	RRID:AB_493705
Anti-Ly-6C-PE	Biolegend	128007	1:200	Flow cytometry	RRID:AB_1186133
Anti-CD45-BV510	Biolegend	103138	1:200	Flow cytometry	RRID:AB_2563061
Anti-Ly-6G-clone1A8-BV421	BD Bioscience	562737	1:200	Flow cytometry	RRID:AB_2737756
Anti-Tcf21	Sigma-Aldrich	HPA013189	1:1000	Immunoblotting	RRID:AB_10601215
Anti-Tcf21	Sigma-Aldrich	HPA013189	1:200	Immunofluorescence	RRID:AB_10601215
Anti-Tcf21	Sigma-Aldrich	HPA013189	3 µg	Chromatin Immunoprecipitation	RRID:AB_10601215
Anti-GAPDH	Fitzgerald	10R-G109a	1:10,000	Immunoblotting	RRID:AB_1285808
Anti-αSMA	Sigma-Aldrich	A2547	1:1000	Immunoblotting	RRID:AB_476701

Anti-αSMA	Sigma-Aldrich	A2547	1:400	Immunofluorescence	RRID:AB_476701
Anti-SM22	Proteintech	10493-1-AP	1:200	Immunofluorescence	RRID:AB_2199363
Anti-Periostin	Novus Biologicals	NBP1-30042	1:300	Immunoblotting	RRID:AB_1968578
Anti-COMP	Abcam	ab231977	1:100	Immunofluorescence	RRID:AB_3073758
Anti-GFP	Abcam	ab13970	1:1000	Immunofluorescence	RRID:AB_300798
IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody	LI-COR	926-32211	1:10,000	Immunoblotting	RRID AB_621843
IRDye® 680RD Donkey anti-Mouse IgG Secondary Antibody	LI-COR	926-68072	1:10,000	Immunoblotting	RRID AB_10953628
Goat anti-Mouse IgG2a Cross- Adsorbed Secondary Antibody, Alexa Fluor™ 568	Thermo Fisher Scientific	A-21134	1:400	Immunofluorescence	RRID AB_2535773
Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor™ 488	Thermo Fisher Scientific	A-11039	1:400	Immunofluorescence	RRID AB_2534096
Goat anti-Rabbit IgG (H+L) Cross- Adsorbed Secondary Antibody, Alexa Fluor™ 568	Thermo Fisher Scientific	A-11011	1:400	Immunofluorescence	RRID AB_143157

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Catalog number
MGC Human TCF21 Sequence-Verified cDNA		Horizon Discovery	MHS6278-202801675

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
RNA sequencing of cardiac fibroblasts from mice with loss of Tcf21 in the adult heart	GEO database	GSE261166
RNA sequencing of cardiac fibroblasts from mice with loss of Tcf21 in the adult heart in the presence of injury	GEO database	GSE261166

RNA sequencing of cardiac fibroblasts from mice with enforced Tcf21 expression in the adult heart in the presence of injury	GEO database	GSE261166
Single cell RNA sequencing in mice with loss of Tcf21 at baseline and after 3 days of myocardial infarction	GEO database	GSE261150
ChIP-seq of cardiac fibroblasts with enforced Tcf21 expression	GEO database	GSE276785

Chemicals and reagents

Description	Source / Repository	Catalog number	Purpose
Tamoxifen	Sigma-Aldrich	T5648-5G	<i>In vivo</i> recombination
Tamoxifen citrate chow	Inotiv	TD.130859	<i>In vivo</i> recombination
Corn oil	Sigma-Aldrich	C8267	Tamoxifen preparation
Dispase II	Sigma-Aldrich	D4693	Mouse heart digestion
Collagenase Type IV	Worthington	LS004188	Mouse heart digestion
DRAQ5	Thermo Fisher Scientific	65-0880-92	Labeling of isolated nuclei
Propidium iodide	Sigma-Aldrich	P4864	Labeling of live cells
Angiotensin II	Sigma-Aldrich	A9525	Fibrosis induction in vivo
Phenylephrine hydrochloride	Sigma-Aldrich	P6126	Fibrosis induction in vivo
Prolong Diamond Antifade	Thermo Fisher Scientific	P36961	Immunofluorescence
Sucrose	Sigma-Aldrich	S9378	Embedding of tissues
32% paraformaldehyde	Electron Microscopy Sciences	15714	Fixation of tissues
RIPA x10	EMD Millipore	20-188	Preparation of protein lysates
HBSS	Fisher Scientific	SH3058801	Flow cytometry
Cytiva HyClone™ Dulbecco's High Glucose Modified Eagles Medium	Fisher Scientific	SH30022FS	Cell culture
Halt protease and phosphatase inhibitor	Thermo Fisher Scientific	78442	Preparation of protein lysates
Bovine serum albumin, lyophilized powder	Sigma-Aldrich	A6003-25G	Immunohistochemistry blocking buffer
Bovine serum albumin	Fisher Scientific	SH3054103	Tissue culture
Dulbecco's Modified Eagle Medium - high glucose	Sigma-Aldrich	D1145	Tissue culture
Penicillin-streptomycin	Sigma-Aldrich	P0781	Tissue culture
TRIzol	Thermo Fisher Scientific	15596018	RNA isolation
2-propanol	Sigma-Aldrich	34863	RNA isolation
2-methylbutane	Fisher Scientific	M32631-4L	Freezing of OCT medium
Red blood cell lysis buffer	Sigma-Aldrich	R7757	Red blood cell lysis; flow cytometry
Tissue-Tek OCT compound	Sakura	4583	Freezing medium for tissues

Dead cell removal microbeads	Miltenyi Biotec	130-090-10	Single cell RNA sequencing
DAPI	Invitrogen	D3571	Immunofluorescence
Cas9 protein	Integrated DNA technologies	1081061	Generation of Col1a1-TCF21 mice
8.0 prolene suture	Ethicon	8730H	Surgeries
4.0 silk suture	Ethicon	K871H	Surgeries
MyoOne Silane DynaBeads	10x Genomics	20000048	Single cell RNA sequencing
SPRIselect	Beckman Coulter	B23317	Single cell RNA sequencing
Protease inhibitors	Thermo Fisher Scientific	PIA32955	Chromatin immunoprecipitation
Protein-A Dynabeads	Thermo Fisher Scientific	10001D	Chromatin immunoprecipitation
EDTA	Thermo Fisher Scientific	BP24821	Flow cytometry
RNase Free DNase set	Qiagen	79254	RNA isolation
Transforming growth factor β 1	R&D Systems	101-B1-010	Cell culture
Formaldehyde solution	Sigma-Aldrich	252549	Chromatin immunoprecipitation
Protease inhibitor	Sigma-Aldrich	P8340	Chromatin immunoprecipitation
High-fidelity 2x PCR Master Mix	New England Biolabs	M0541S	ChIP-seq
Tn5 Transposase enzyme	Diagenode	c01070012-30	ChIP-seq

Commercial assays

Description	Source / Repository	Catalog number	Purpose
Chromium Next GEM single cell 3' kit v3.1	10x Genomics	PN-1000268	Single cell RNA sequencing
Chromium Next GEM chip G single cell 3' kit	10x Genomics	PN-1000120	Single cell RNA sequencing
Dual index kit TT set A	10x Genomics	PN-1000215	Single cell RNA sequencing
SsoAdvanced Universal SYBR Green Supermix	Biorad	1725274	qRT-PCR
Verso cDNA synthesis kit	Thermo Fisher Scientific	AB1453B	qRT-PCR
miRNeasy Micro Kit	Qiagen	217084	RNA isolation
QiaShredder column	Qiagen	79656	RNA isolation
SMART-seq V4 Ultra Low input RNA kit	Takara	634894	RNA library preparation
MinElute DNA kit	Qiagen	28004	Chromatin immunoprecipitation

Oligonucleotides

Description	Source / Repository	Purpose
Tcf21 forward TTCTCCAGGCTCAAGACCAC	DOI: 10.1002/dvg.20750	Tcf21-mcm genotyping
Tcf21 reverse CAAACCCTAGCACAAATCACTCGC	DOI: 10.1002/dvg.20750	Tcf21-mcm genotyping

MerCreMer forward GCTTCCGATATCCAGATCCAGAC	DOI: 10.1002/dvg.20750	Tcf21-mcm genotyping
Tcf21 forward GTGTGCATTTCTGTGGTTGTCTCTG	DOI: 10.1681/ASN.2013121307	Tcf21 floxed allele genotyping
Tcf21 reverse CTGTTGTTTGTGCAGGTGGAGA	DOI: 10.1681/ASN.2013121307	Tcf21 floxed allele genotyping
Pdgfra forward ATCGCATTCCCTTGCAAAAGT	The Jackson Laboratory	Pdgfra-CreERT2 genotyping
Wildtype (pdgfra) forward GCCTTAAGCTGGGACATGCT	The Jackson Laboratory	Pdgfra-CreERT2 genotyping
Pdgfra reverse AGGCCACAGAACATGGAC	The Jackson Laboratory	Pdgfra-CreERT2 genotyping
GFP forward GATCAGCAGCCTCTGTTCCACA	DOI: 10.1002/dvg.20474	R26-GFP genotyping
GFP reverse CGCTGAACTTGTGGCCGTTTAC	DOI: 10.1002/dvg.20474	R26-GFP genotyping
Wildtype for GFP forward AAAGTCGCTCTGAGTTGTTAT	DOI: 10.1002/dvg.20474	R26-GFP genotyping
Wildtype for GFP reverse GGAGCGGGAGAAATGGATATG	DOI: 10.1002/dvg.20474	R26-GFP genotyping
Col1a1-TCF21 wildtype forward GTCCATCCTTCTGAACTCAGCATC	This paper	Col1a1-TCF21 genotyping
Col1a1-TCF21 wildtype reverse TCCCTGGTACCTATGGAGACTGTG	This paper	Col1a1-TCF21 genotyping
Col1a1-TCF21 reverse GGGCTATGAACTAATGACCCCGTA	This paper	Col1a1-TCF21 genotyping
Tcf21 forward CGCTCACTTAAGGCAGATCC	This paper	Tcf21 qRT-PCR
Tcf21 reverse GCTGTAGTTCCACACAAGCG	This paper	Tcf21 qRT-PCR
Tcf21 forward GAGGCAGATCCTGGCTAACG	This paper	Tcf21 human qRT-PCR
Tcf21 reverse AAAGGGCCACGTCAGGTTG	This paper	Tcf21 human qRT-PCR
GAPDH forward TGTCGTGGAGTCTACTGGTG	This paper	GAPDH qRT-PCR
GAPDH reverse ACACCCATCACAACATGG	This paper	GAPDH qRT-PCR
Acta2 forward CTTCGTGACTACTGCCGAGC	This paper	Acta2 qRT-PCR
Acta2 reverse AGGTGGTTTCGTGGATGCC	This paper	Acta2 qRT-PCR
Tagln forward AAACGACCAAGCCTTCTCTG	This paper	Tagln qRT-PCR
COMP fwd AGTTGGCTACATCAGGGTGC	This paper	COMP qRT-PCR
COMP reverse CCGTGTCCAACACCACATTG	This paper	COMP qRT-PCR

Cthrc1 forward CTTGGGAAAATTGCGGAGTGT	This paper	Cthrc1 qRT-PCR
Cthrc1 reverse TCCACAGAGGAAGTACGATGA	This paper	Cthrc1 qRT-PCR
Fmod forward TCCTGTCAACACCAACCTGG	This paper	Fmod qRT-PCR
Fmod reverse AGCTGCTGATGGAGAACTCATTG	This paper	Fmod qRT-PCR
Itga11 forward AGCCTTTGGCCAGGATTCAC	This paper	Itga11 qRT-PCR
Itga11 reverse CCATTGGTTTCCATTGGGGC	This paper	Itga11 qRT-PCR

Recombinant DNA and viruses

Description	Source / Repository	Catalog number	Purpose
Adenovirus beta-galactosidase	Molkentin lab	N/A	Cell transduction
Adenovirus Cre	Molkentin lab	N/A	Cell transduction

Software and algorithms

Description	Source / Repository	Persistent ID / URL	Purpose
Prism 5	GraphPad	https://www.graphpad.com/scientific-software/prism/ ; RRID: SCR_002798	
Fiji	Fiji	RRID:SCR_002285	Image processing
NIS elements	Nikon	RRID:SCR_014329	Image acquisition and processing
Odyssey CLX Imaging system	LI-COR	RRID:SCR_014579	Immunoblot imaging
Vevo 2100	VisualSonics	RRID:SCR_015816	Echo analysis
RStudio	RStudio	RRID:SCR_000432	RNA sequencing analysis
Aperio ImageScope	Leica Biosystems	RRID:SCR_020993	Fibrosis analysis
SciDap	Datirium	https://scidap.com/	ChIP-seq analysis
FlowJo 10.7	FlowJo	RRID:SCR_008520	Flow cytometry analysis

Other

Description	Source / Repository	Catalog/Persistent ID / URL	Purpose
Nikon A1 confocal Laser Microscope	Nikon	RRID:SCR_022628	Image acquisition
Bio-Rad CFX96 Real-Time PCR Detection System	Bio-Rad	RRID:SCR_018064	qRT-PCR
Direct Detect Spectrometer	Millipore Sigma	C134681	Protein quantification
Direct Detect assay-free cards	Millipore Sigma	DDAC00010-8P	Protein quantification
Micro-osmotic pump model 1002	Alzet	0004317	Drug delivery

Mouse heart slicer matrix	Zivic	HSMS001-1	Tissue processing
LS columns	Miltenyi Biotec	130-042-401	Cell sorting
QuadroMACs magnetic separator	Miltenyi Biotec	190-091-051	Cell sorting
Immobilon-FL PVDF membranes	EMD Millipore	IPFL00010	Immunoblotting

ARRIVE GUIDELINES

The ARRIVE guidelines (<https://arriveguidelines.org/>) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

Study Design

Details of the number of animals used, sex, age and groups are included in the manuscript text, figures and supplementary files. In experiments that involved either a myocardial infarction surgery or infusion of angiotensin II and phenylephrine, we observed a maximal loss of 25% of animals from the groups.

Sample Size: The number of mice used in this study reflects the minimum number needed to achieve statistical significance based on previous power analysis and our experience.

Inclusion Criteria The genotype of the mice determined inclusion in the study.

Exclusion Criteria Animals were only excluded from the analysis when there was no evidence of an injury in the model. In some cases, there is an unsuccessful occlusion of the coronary artery during a myocardial infarction surgery, which results in no visible infarct area.

Randomization Randomization of mice was only performed to allocate groups of mice to sham or myocardial infarction surgeries. Other studies compared groups of genetic mice (*Pdgfra-CreERT2* (control) versus *Pdgfra-CreERT2-Tcf21^{fl/fl}* or *Col1a1-TCF21 and Tcf21^{MCM}* (control) versus *Tcf21^{fl/MCM}*). As these studies were based on genotype and not treatment, no randomization was performed.

Blinding All mice were allocated numbers that allowed for blinding of genotypes during the experimental procedures and analysis.

Figure S1

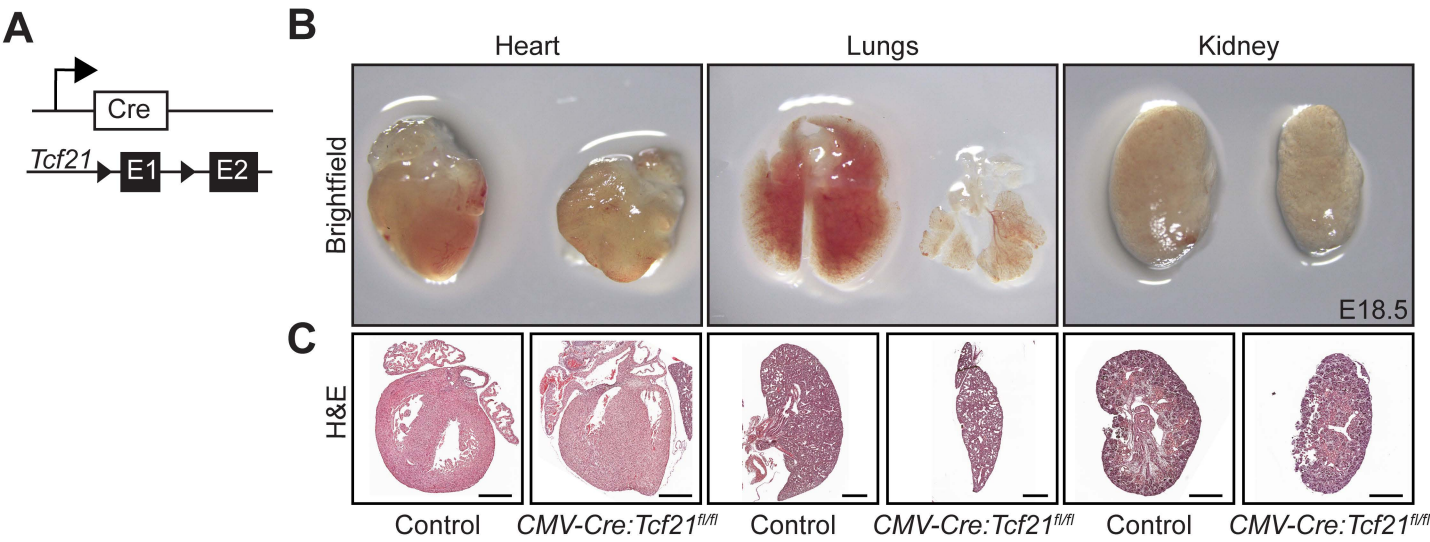


Figure S1. Validation of *Tcf21* loxP targeted allele *in vivo*. (A) A transgenic mouse encoding Cre recombinase under control of a cytomegalovirus promoter was crossed with *Tcf21*-loxP targeted mice to induce ubiquitous early developmental deletion of *Tcf21*. Control mice were *Tcf21*^{fl/fl} only. (B) Stereomicroscope whole mount images and (C) Hematoxylin and eosin-stained (H&E) histological images of the indicated organs at E18.5. The scale bar is 0.5 mm. For "B" the whole mount images of heart, lung and kidney, controls are shown on the left and the *Tcf21* deleted tissues are on the right.

Figure S2

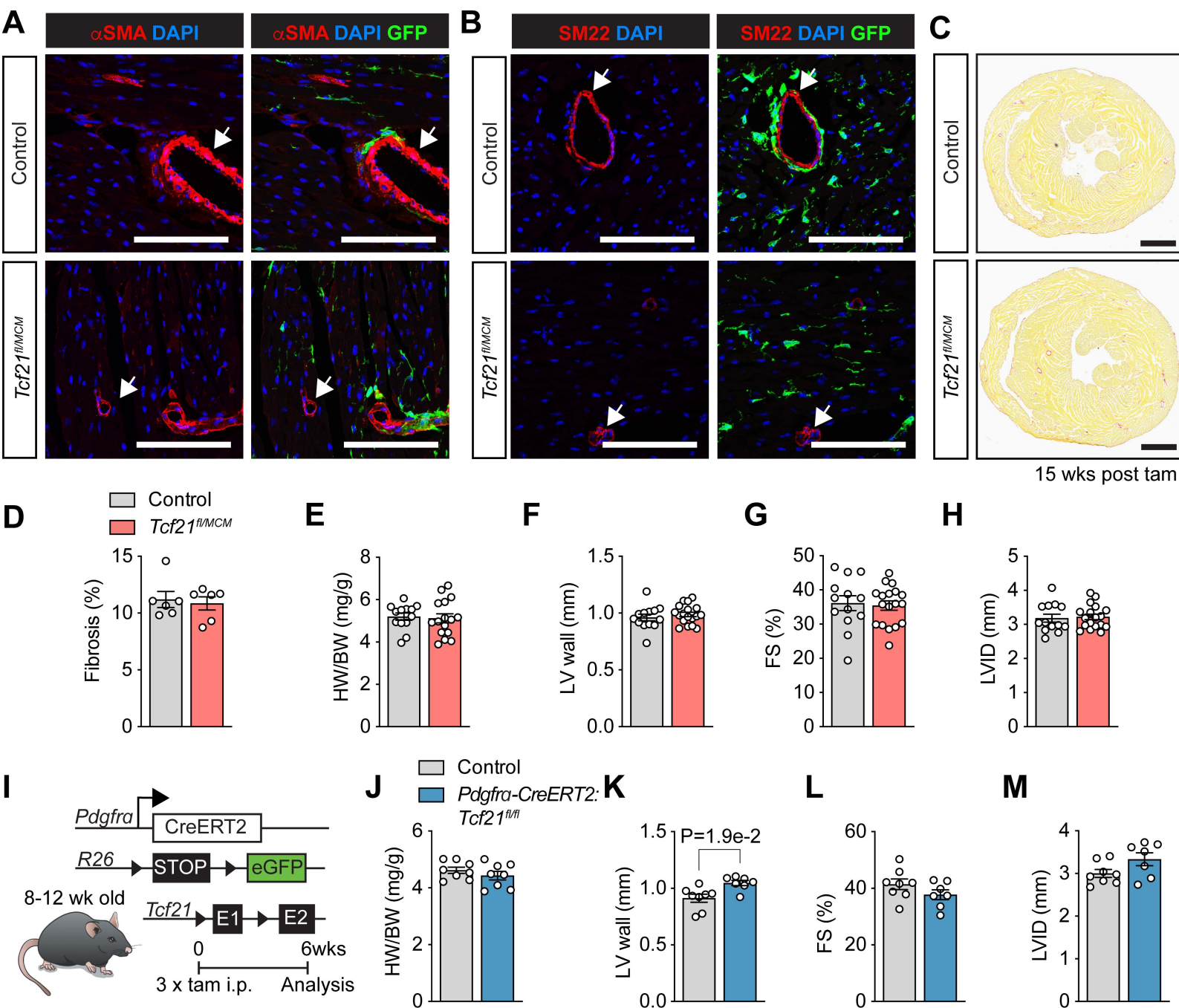


Figure S2. Genetic loss of *Tcf21* does not affect cardiac structure or function. (A) Immunofluorescent stained cardiac histological sections for expression of smooth muscle α actin (α SMA, red) and (B) transgelin (SM22, red) in the indicated groups 15 weeks after tamoxifen (tam) induced genetic recombination. GFP (green) shows the presence of the recombined fibroblasts due to the *Rosa26-eGFP* reporter allele and nuclei are shown in blue with DAPI. *Tcf21^{MCM}* mice were used as controls. The arrowheads show areas of cardiac vessels that are positive for smooth muscle gene expression with α SMA and SM22. Scale bars are 50 μ m. (C) Representative whole heart histological images stained with Sirius red for collagen deposition from the 2 groups of mice shown 15 weeks after tam administration. Scale bars are 1 mm. (D) Quantification of percentage area of tissue fibrosis as shown in "C". (E) Heart weight to body weight ratio (HW/BW) in the same mice described above 15 weeks after genetic recombination with tam in *Tcf21^{fl/MCM}* versus *Tcf21^{MCM}* control mice. (F) Echocardiographic analysis of left ventricular (LV) wall thickness in diastole, (G) fractional shortening (FS) (%) and (H) LV internal diameter in diastole (LVID) in the 2 indicated groups of mice 15 weeks after fibroblast-specific deletion of *Tcf21*. (I) Schematic of gene targeted alleles used to delete *Tcf21* with the *Pdgfra-CreERT2* allele in adult mice. Tam was administered to 8–12-week-old mice for 3 consecutive days by intraperitoneal injection (i.p) and harvested 6 weeks later. (J) HW/BW ratio in *Pdgfra-CreERT2:Tcf21^{fl/fl}* mice versus *Pdgfra-CreERT2* controls. (K) Echocardiographic analysis of LV wall thickness in diastole, (L) fractional shortening (FS) (%) and (M) LV internal diameter in diastole (LVID) in the 2 indicated groups 6 weeks after *Tcf21* gene deletion in fibroblasts. All data shown are mean \pm standard error of the mean. Samples were analyzed by a Kolmogorov-Smirnov test (exact p values are shown; D-H, J-M). All data points in graphs represents biological replicates.

Figure S3

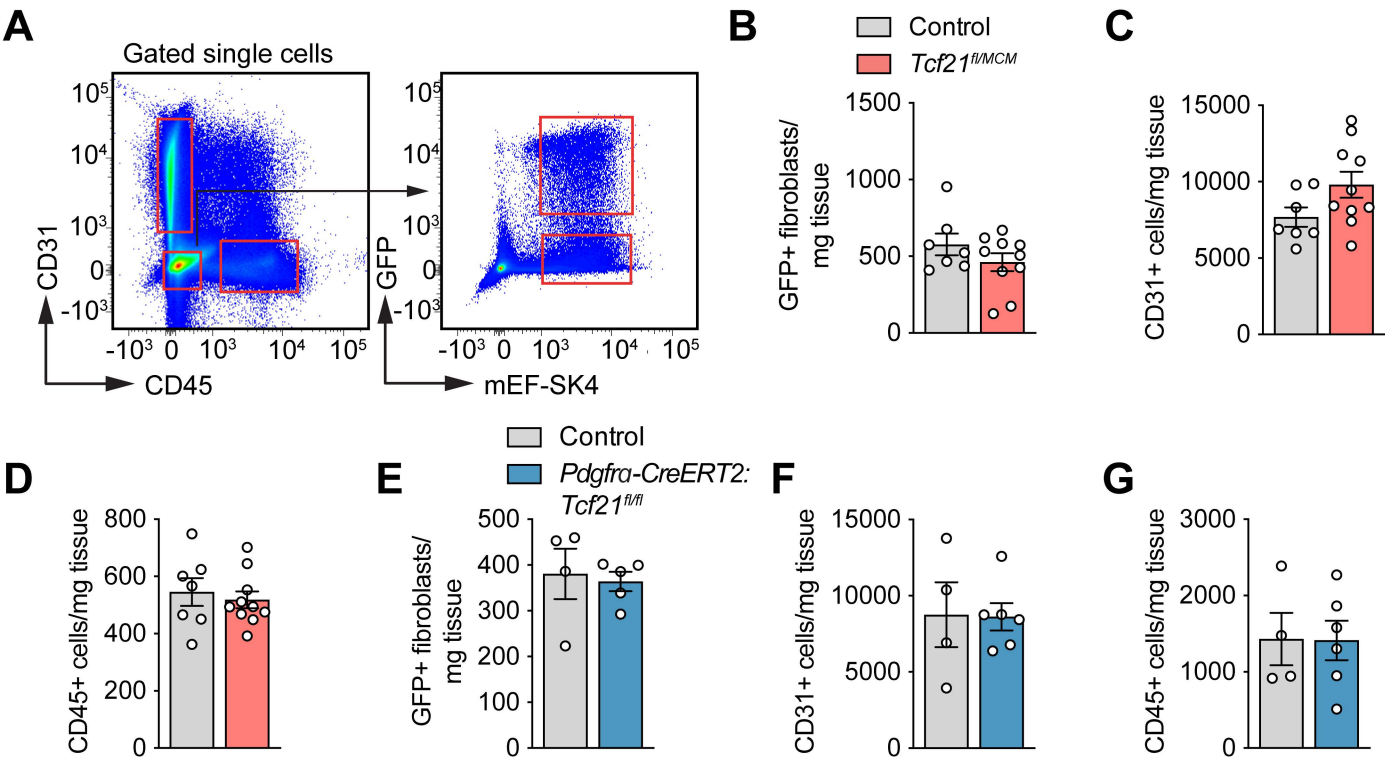
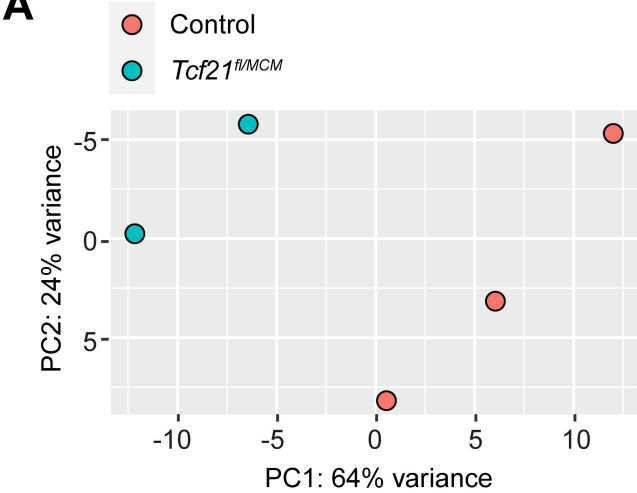


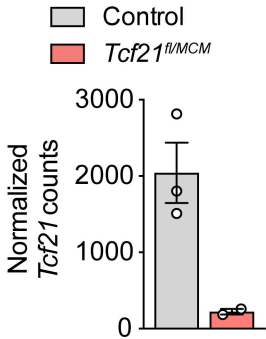
Figure S3. Genetic loss of *Tcf21* does not alter cardiac fibroblast cell content (A) Representative gating strategy and cellular distribution by flow cytometry for total endothelial cells (CD31+), leukocytes (CD45+) and fibroblasts (mEF-SK4+ and GFP+) in left ventricular heart homogenates from *Tcf21^{fl/MCM}* mice versus *Tcf21^{MCM}* mice treated with tamoxifen and harvested 15 weeks later. Both groups of mice had the *Rosa26-eGFP* reporter allele. Quantification of these flow cytometry data for (B) GFP+ fibroblasts, (C) CD31+ cells and (D) CD45+ cells from hearts of the 2 groups of mice. (E-G) Quantification of flow cytometry data from left ventricular homogenates in mice 4 weeks after tamoxifen administration to induce genetic recombination of the *Tcf21* locus using the *Pdgfra-CreERT2* allele. This genetic cross also contained the *Rosa26-eGFP* reporter to permit sorting of recombined fibroblasts. *Pdgfra-CreERT2* mice were used as controls. (E) GFP+ fibroblasts, (F) CD31+ cells and (G) CD45+ cells. All data shown are mean +/- standard error of the mean. Samples were analyzed by a Kolmogorov-Smirnov test (B-G). All data points in graphs represents biological replicates.

Figure S4

A



B



C

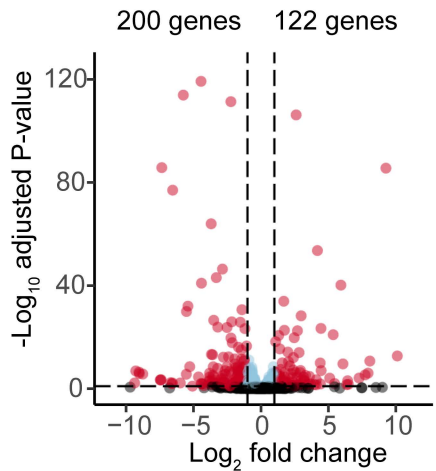


Figure S4. Effect of genetic loss of *Tcf21* on transcriptomic profile in cardiac fibroblasts in response to angiotensin II/phenylephrine (Ang II/PE). (A) Principal component analysis of RNA sequencing data of GFP+ cardiac fibroblasts from hearts of *Tcf21^{fl/MCM}* mice and *Tcf21^{MCM}* controls after 1 week of Ang II/PE stimulation. (B) Normalized *Tcf21* transcript counts. (C) Volcano plot of differentially expressed mRNAs.

Figure S5

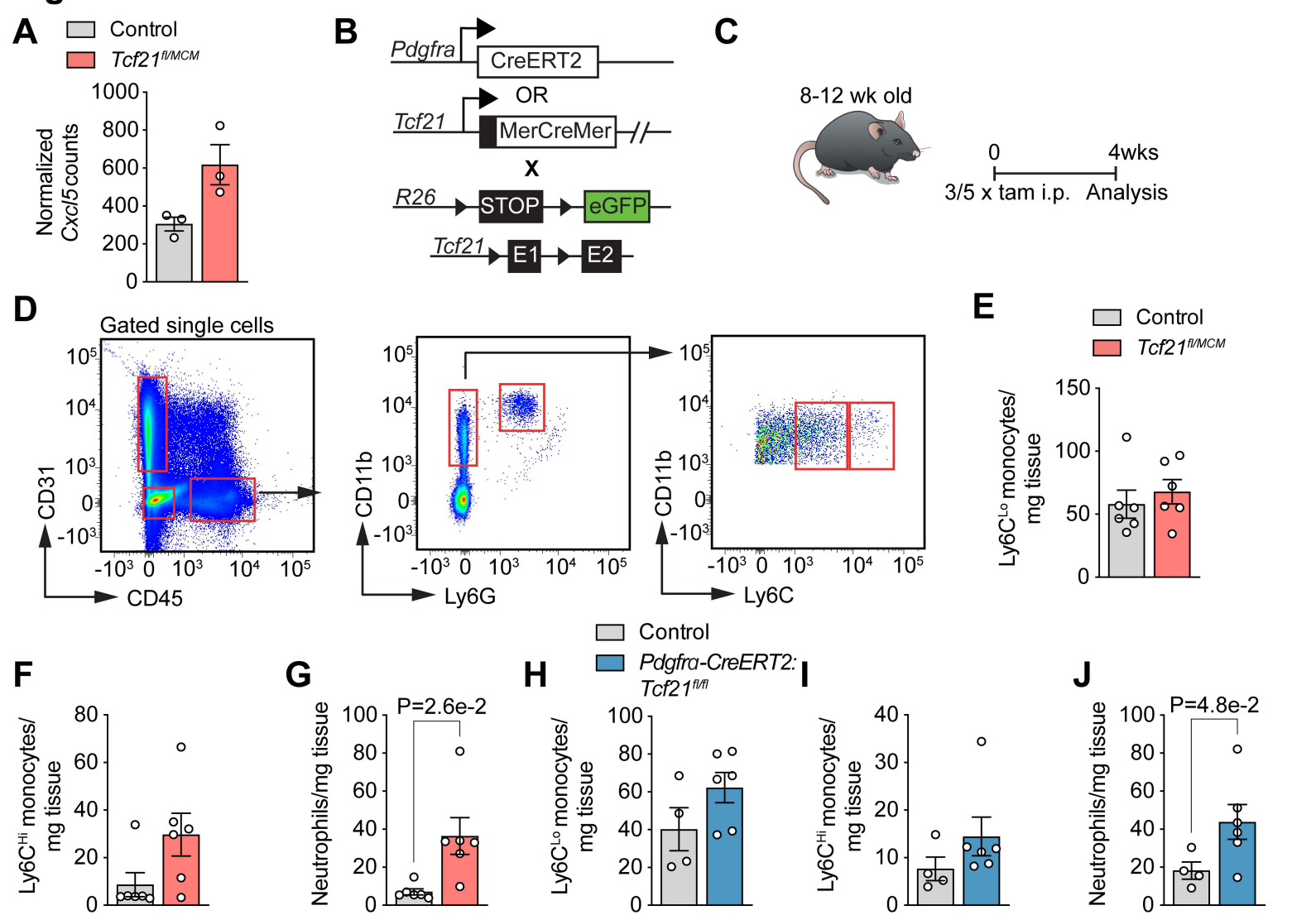


Figure S5. Loss of *Tcf21* alters neutrophil numbers in the uninjured heart. (A) Normalized *Cxcl5* transcript counts as determined by bulk transcriptomic sequencing from hearts of *Tcf21^{fl/MCM}* mice versus *Tcf21^{MCM}* control mice 2 weeks after tamoxifen (tam) treatment. (B) Schematic of mouse genetically modified alleles used and (C) temporal experimental timeline. Tam was administered to 8–12-week-old mice for either 3 or 5 consecutive days by intraperitoneal injection (i.p.) and harvested 4 weeks later. (D) Representative gating strategy and cellular distribution by flow cytometry to quantify monocyte subtypes and neutrophils in left ventricular heart homogenates from *Tcf21* fibroblast-specific deleted mice at baseline. (E–G) Flow cytometry quantification of (E) Ly6C-low (Lo) expressing Cd11b⁺ monocytes, (F) Ly6C-high (Hi) expressing Cd11b⁺ monocytes and (G) neutrophils from hearts of *Tcf21^{fl/MCM}* mice versus *Tcf21^{MCM}* controls. (H–J) Flow cytometry quantification of (H) Ly6C-low (Lo) expressing Cd11b⁺ monocytes, (I) Ly6C-high (Hi) expressing Cd11b⁺ monocytes and (J) neutrophils from hearts of *Pdgfra-CreERT2:Tcf21^{fl/fl}* mice versus *Pdgfra-CreERT2* controls. All data shown are mean \pm standard error of the mean. Samples were analyzed by a Kolmogorov-Smirnov test (exact p values are shown; E–J). All data points in graphs represents biological replicates.

Figure S6

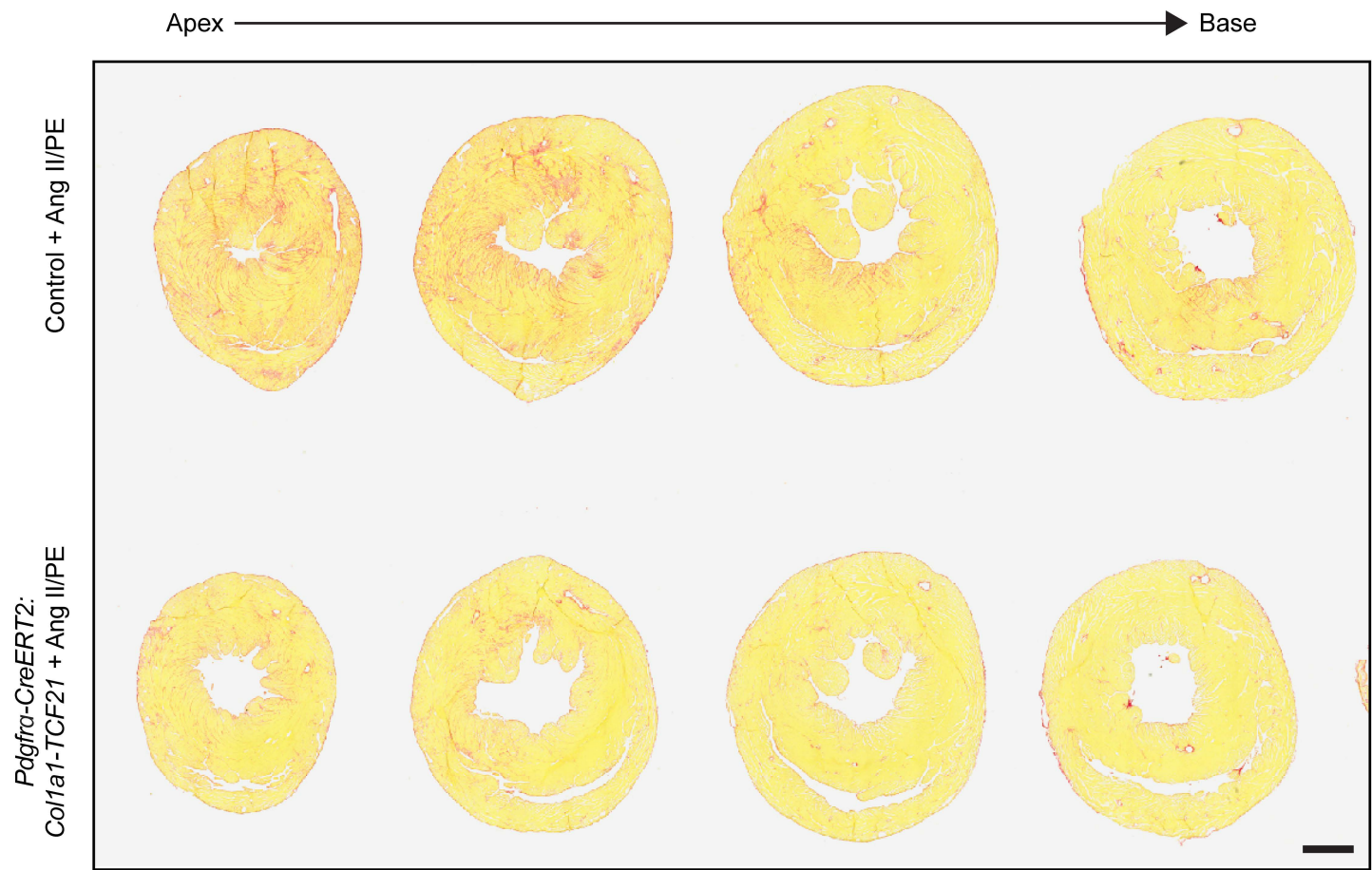


Figure S6. Cardiac fibroblast-specific enforced expression of *TCF21* reduces fibrosis deposition in response to angiotensin II/phenylephrine (Ang II/PE). Histological heart sections stained with Sirius red from hearts of *Pdgfra-CreERT2:Col1a1-TCF21* mice versus *Pdgfra-CreERT2* controls. Images here expand on the analysis shown in Figure 4F - see methods for details in serial sectioning procedure. Scale bar is 1 mm.

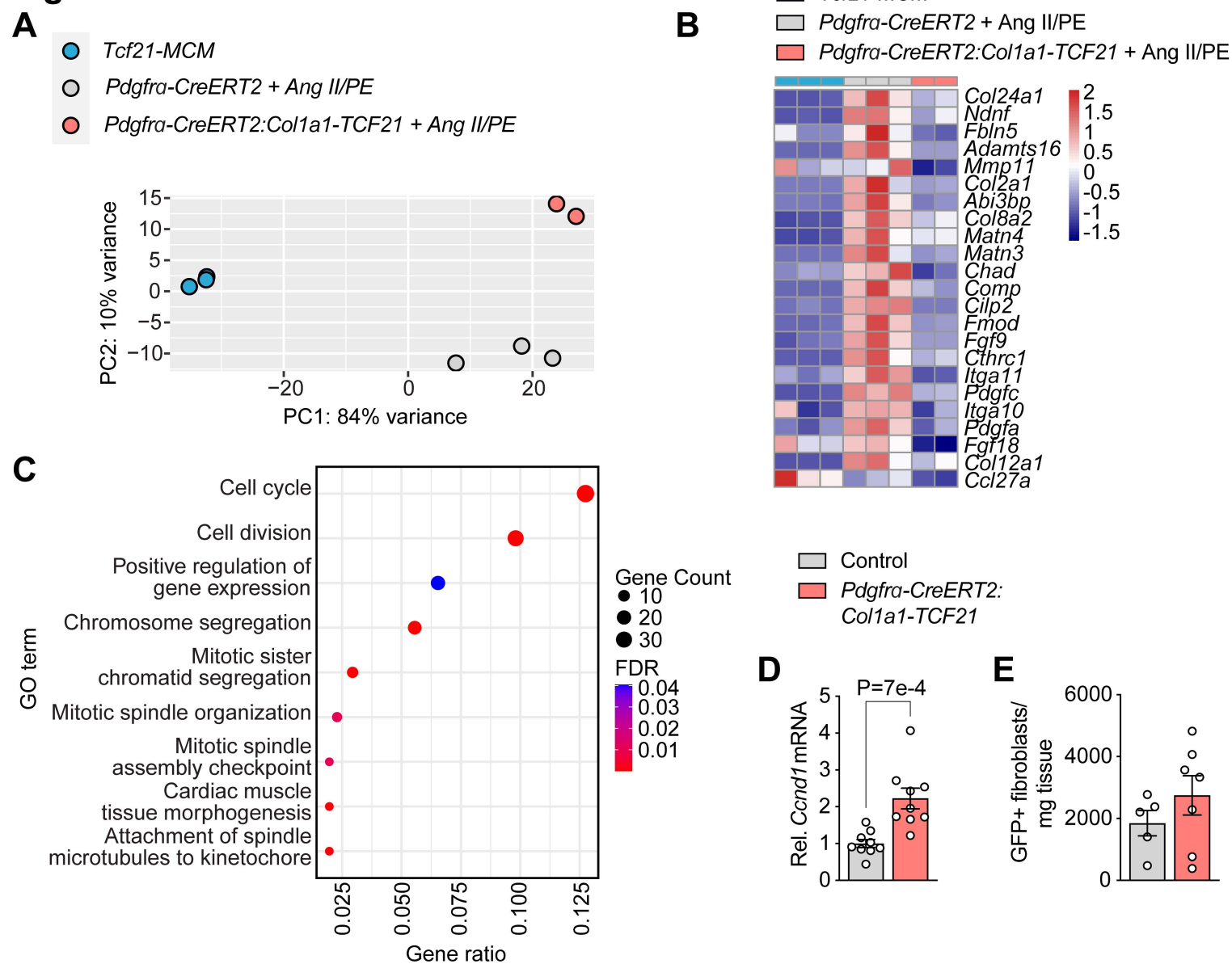
Figure S7

Figure S7. Cardiac fibroblast-specific enforced expression of *TCF21* reduces induction of injury-associated fibrosis gene expression. (A) Principal component analysis of the 3 major groups included in differential gene expression analysis at baseline or with angiotensin II/phenylephrine (Ang II/PE). (B) Heatmap of select z-score normalized genes associated with fibroblast differentiation and fibrosis from the 3 groups of mice shown in the legend with the color scheme above the heatmap for 3, 3, and 2 hearts analyzed. (C) Dot plot of top gene ontology (GO) terms of biological processes with a false discovery rate (FDR) < 0.05 of all upregulated genes with a Log2 fold change (FC) > -1, FDR < 0.1 comparing fibroblasts of *Pdgfra*-CreERT2:Col1a1-TCF21 versus *Pdgfra*-CreERT2 controls following 1 week of Ang II/PE stimulation. (D) Quantitative real time polymerase chain reaction (qRT-PCR) analysis of cyclin D1 expression (*Ccnd1*) in GFP+ cardiac fibroblasts isolated by fluorescence-activated cell sorting of the 2 groups of mice after Ang II/PE stimulation. *Pdgfra*-CreERT2 mice were used as controls. (E) Flow cytometry analysis for fibroblasts (mEF-SK4+ and GFP+) in left ventricular homogenates from *Pdgfra*-CreERT2:Col1a1-TCF21 versus *Pdgfra*-CreERT2 controls after Ang II/PE stimulation. This genetic cross also contained the *Rosa26-eGFP* reporter to permit sorting of recombined fibroblasts. All data shown are mean \pm standard error of the mean. Samples were analyzed by a Kolmogorov-Smirnov test (exact p values are shown; D-E). All data points in graphs represents biological replicates.