SUPPLEMENTAL MATERIAL

Supplemental Methods

In vitro wound healing assay.

PDMS micropillars with fabricated photolithography were used to quantify in vitro cellular wounding and closure over time. A negative SU-8 photoresist was spun on a silicon wafer to reach 2000 micrometer thickness that was developed with UV illumination with a shadow mask of 500 micrometer diameter and 3000 micrometer spacing. This silicon micropillar mold was treated with tridecafluoro- 1,1,2,2- tetrahydrooctyl- 1- trichlorosilane for 30 min before casting with PDMS at 65 degrees Celsius overnight at the mixing ratio of 10:1. Resulting PDMS was treated with trichlorosilane and second casted for desired micropillar development. *Mapk14*$^{fl/fl}$ MEFs treated with AdβGal or AdCre were seeded at 10^6 cells/mL and incubated for an hour. Then the PDMS micropilllar device was positioned to create high throughput wounds in each of the monolayers. After removal of PDMS micropillars, an automated microscopy system with x-y-z memorable stage and temperature/CO_2 controlled chamber was utilized to create time-lapse videos of wound closures for tracking single cell migration into the wound for 14-24 hrs.

Proliferation assays.

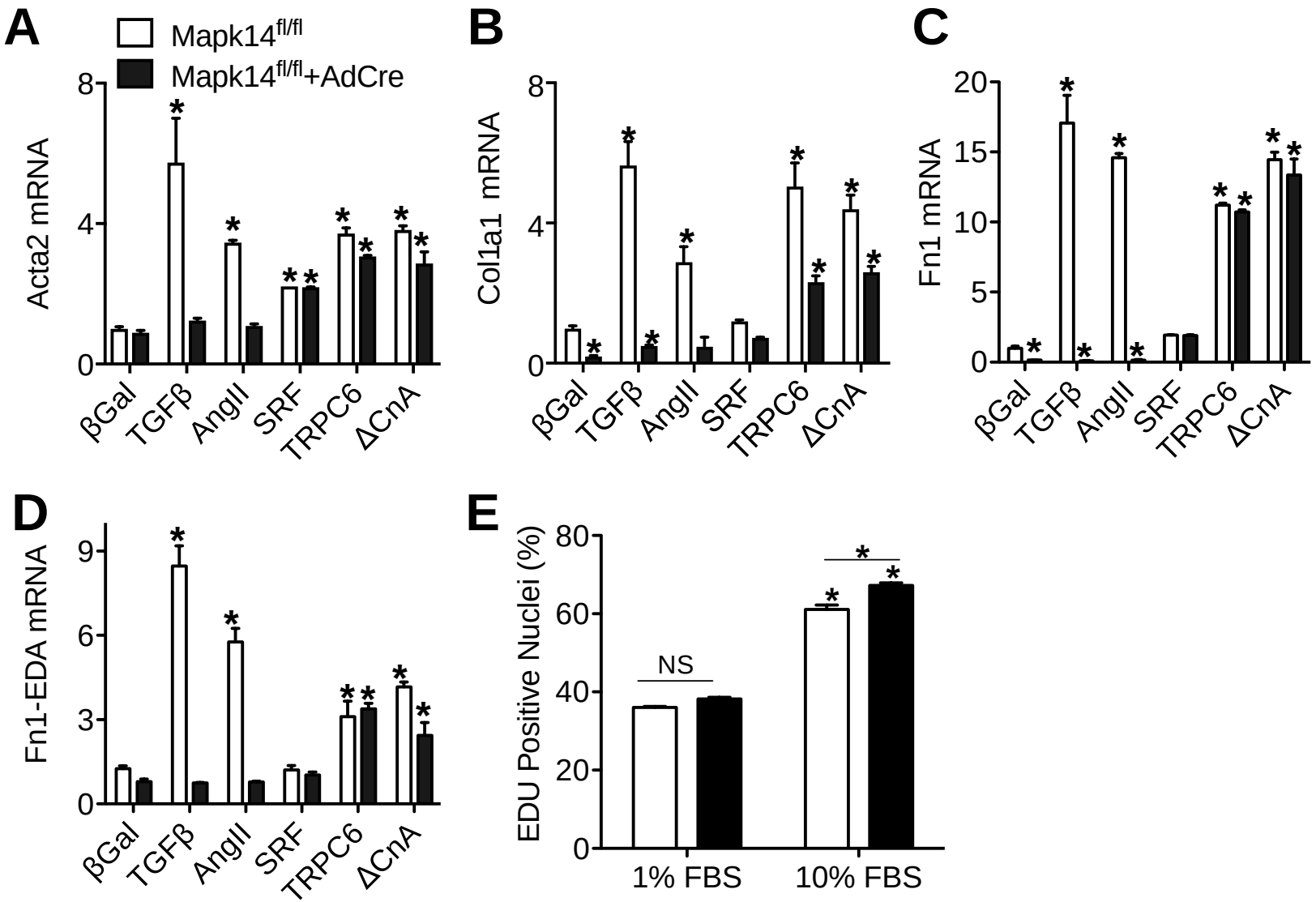
MEFs or flow cytometry sorted cardiac fibroblasts were cultured for 24 hours in 10 μM 5-ethynyl-2'deoxyuridine (EDU) and DMEM supplemented with penicillin-atreptomycin, nonessential amino acids, and either 1% fetal bovine serum (FBS), 1% FBS + TGFβ (10 ng/ml, R&D Systems) , or 10% FBS. To detect EDU incorporation a Click-iT EDU imaging kit (Invitrogen) was used. Per the manufacturer's instructions fibroblasts were fixed with 4% paraformaldehyde and permeabilized with phosphate buffered saline with 0.5% Triton X-100. Click-iT buffers were prepared fresh and the subsequent reaction performed in the dark. Hoechst staining was performed to label all nuclei. The coverslips were imaged and analyze for the percent EDU positive nuclei.

Supplemental Table. RT-PCR primers for mouse p38 isoforms and myofibroblast gene expression.

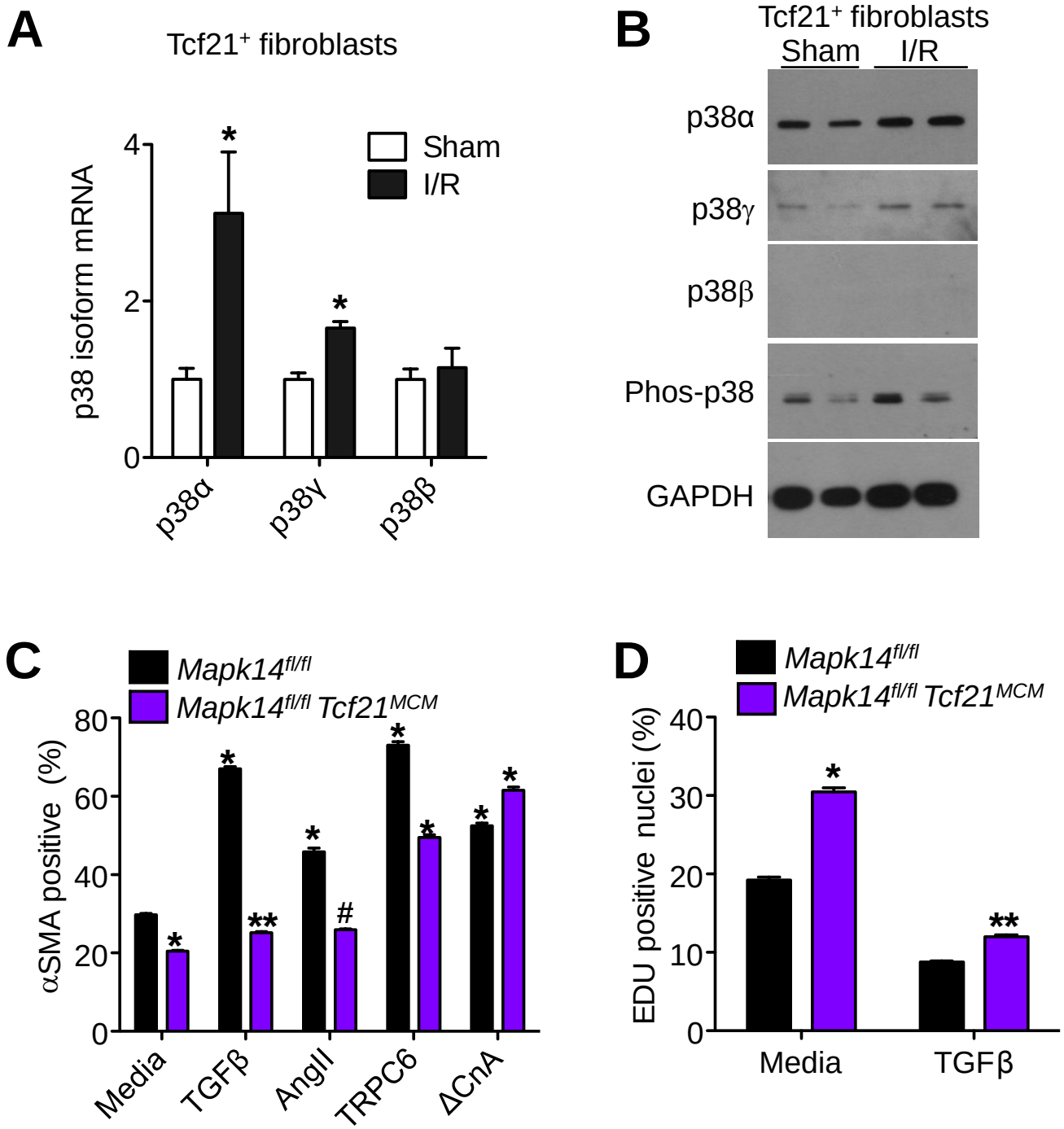
Genes / Isoforms	**Sequence (5'-3')**
Acta2	F*: 5' ACTGGGACGACATGGAAAAG R†: 5' GTTCAGTGGTGCCTCTGTCA
Col1a1	F: 5' GCTCCTCTTAGGGGCCACT R: 5' CCACGTCTCACCATTGGGG
Fn1	F: 5' ATGTGGACCCCTCCTGATAGT R: 5' GCCCAGTGATTTCAGCAAAGG
Fn-EDA Variant	F: 5' CCCACCGTGGAGTATGTGG R: 5' AGCCCTGACACAATCACGGA
P38α	F: 5' CAGGGACCTTCTCATAGAT R: 5' AGGGATAGCCTCAGACC
P38β	F: 5' TGCAAGGAAAGGCCCTC R: 5' AGGCAATGCCTCACTGC
P38γ	F: 5' ATTACTGGGAAGATCCTG R: 5' GTCACAGAGCCGTCTCC
18S RNA	F: 5' GTAACCCGTTGAACCCCATT R: 5' CCATCCAATCGGTAGTAGCG

*F, forward primer; †R, reverse primer

Supplemental Figure S1



Supplemental Figure S2



Figures legends for Supplemental Figures 1 and 2

Figure S1**.** p38 regulates myofibroblast-associated matrix gene expression and proliferation in MEFs. RT-PCR for **A**, Acta2, **B**, Col1a1, **C**, Fn1, and **D**, Fn-EDA splice variant mRNA from *Mapk14*$^{fl/fl}$ MEFs 4 days after AdCre infection. MEFs were also given the additional profibrotic agonist TGFβ or adenoviruses shown along the x-axis to alter signaling in these cells. Data are the mean value of biologic triplicates and expressed as ΔΔct values normalized to 18S RNA and expressed relative to Adβgal infected *Mapk14*$^{fl/fl}$ control MEFs. Error bars are SEM, N=3, *P<0.05 vs Adβgal-*Mapk14*$^{fl/fl}$ (Control). **E**, *Mapk14*$^{fl/fl}$ MEFs proliferation with and without AdCre measured by Click-it EDU (5-ethynyl-2'-deoxyuridine) assay 24 hours after treatment with medium containing 1% or 10% fetal bovine serum (FBS). Error bars are SEM, N≥800 nuclei over 3 independent experiments, *P<0.05 vs *Mapk14*$^{fl/fl}$.

Figure S2**.** p38 regulates cardiac fibroblast to myofibroblast differentiation and proliferation capacity. **A**, RT-PCR and **B**, Western blot detecting p38 isoform expression in *Tcf21*$^{+}$ cardiac fibroblasts isolated from sham and I/R hearts 4 days after injury. The p38δ isoform was not detected by RT-PCR or Western. Data are expressed as ΔΔct values normalized to 18S RNA and expressed relative to the sham group. Error bars are SEM, N=3 each group, *P<0.05 vs Sham. **C**, Immunofluorescent-based quantification of the percent cardiac fibroblasts with αSMA^{+} stress fibers isolated and flow sorted from *Mapk14*$^{fl/fl}$ and *Mapk14*$^{fl/f}$ *Tcf21*^{MCM} mice that were tamoxifen-treated prior to isolation to delete p38α protein expression. Cardiac fibroblasts were cultured and infected with the additional adenoviruses or TGFβ shown along the x-axis to alter signaling in these cells. Error bars are SEM, N≥800, *P<0.05 vs Adβgal (Control), **P<0.05 vs *Mapk14*$^{fl/fl}$+TGFβ, #P<0.05 vs *Mapk14*$^{fl/fl}$+AngII. **D**, Cardiac fibroblast proliferation was also measured in these groups by Click-it EDU assay 24 hours after treatment with TGFβ in serum-free medium. Error bars are SEM, N≥800 nuclei over 3 independent experiments, *P<0.05 vs *Mapk14*$^{fl/fl}$ and **P<0.05 vs *Mapk14*$^{fl/fl}$ *Tcf21*^{MCM} media.