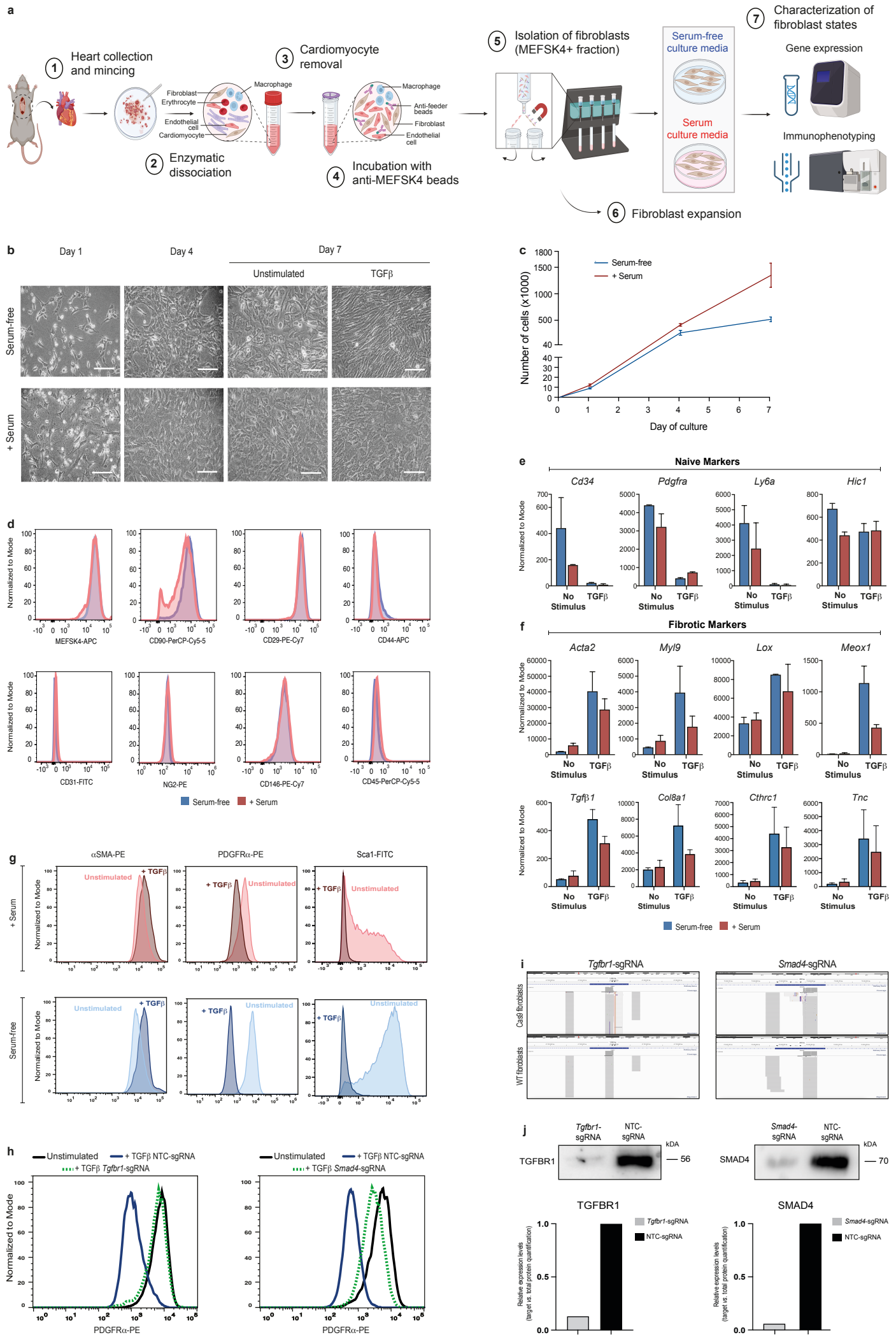
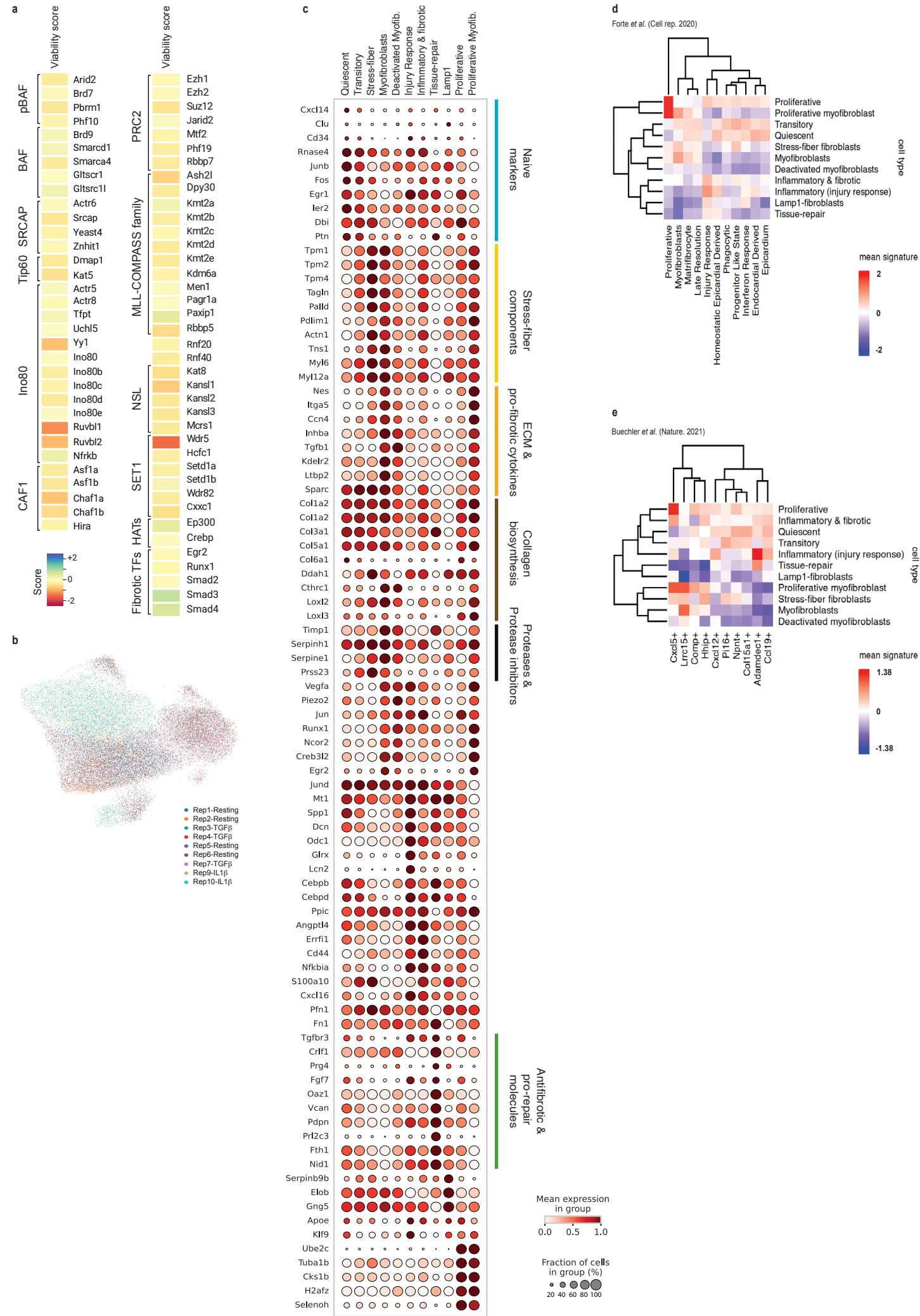


SI Fig. 1



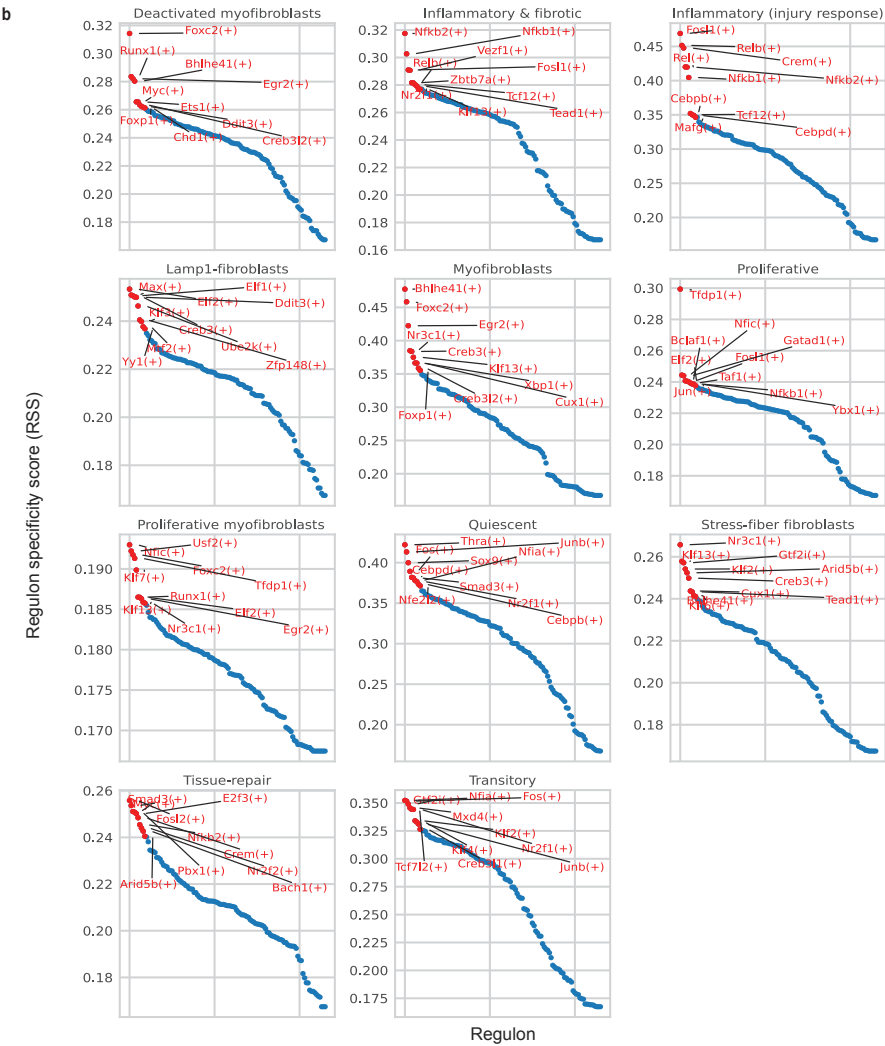
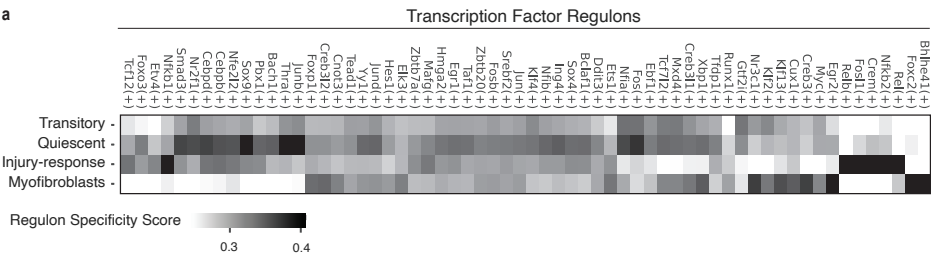
Supplementary Figure 1 | **Characterization of the fibroblast *ex vivo* screen system.** **(a)** Schematic depiction of *ex vivo* murine cardiac fibroblast expansion. Created in BioRender. Gross, T. (2025) <https://BioRender.com/vro6894>. **(b)** Images comparing the morphology of primary murine cardiac fibroblasts in serum-free and serum-containing culture media at days 1, 4 and 7 of culture, and after 24h of TGF- β stimulation at day 7. Scale bars: 30 μ m. **(c)** Cell proliferation curves of cardiac fibroblasts grown in both culture media (n = 3 independent experiments/time-point). **(d)** Representative FACS analysis of fibroblast-stromal (MEFSK4, CD90, CD44, CD29), pericytes (CD146, NG2), endothelial (CD31) and hematopoietic (CD45) cell markers in fibroblasts grown under serum-free (blue) and serum-containing (red) conditions at day 4 of culture (n = 3 independent experiments). **(e-f)** Expression levels of naïve **(e)** and fibrotic **(f)** markers in serum-free (blue) and serum-containing (red) conditions. Values are normalized read counts from two independent experiments. Data are mean \pm SD. **(g)** Representative FACS analysis of intracellular alpha smooth actin (α -SMA), PDGFR α and Sca-1 in unstimulated and TGF- β stimulated fibroblasts grown under serum-free (blue) and serum-containing (red) conditions (n = 3 (for α -SMA and Sca-1) and 4 (for PDGFR α) independent experiments). **(h)** Representative FACS analysis for PDGFR α in Cas9 fibroblasts transduced with NTC sgRNA (blue) and *Smad4* and *Tgfbr1* sgRNAs (green). PDGFR α signal in unstimulated conditions (black) is shown for reference (n = 2 (for *Smad4*) and 3 (for *Tgfbr1*) independent experiments). **(i)** Genome browser snapshots of Indel-seq signal for *Tgfbr1* and *Smad4* loci in Cas9 and wildtype (WT) murine cardiac fibroblasts transduced with *Tgfbr1*- or *Smad4*-sgRNAs. **(j)** Western blot of *Tgfbr1* and *Smad4* in Cas9 fibroblasts transduced with the target-gene or control (NTC) sgRNAs. Relative expression levels were calculated using total protein content loaded stained with Revert700 as a normalization factor. Source data are provided as a Source Data file.

SI Fig 2.



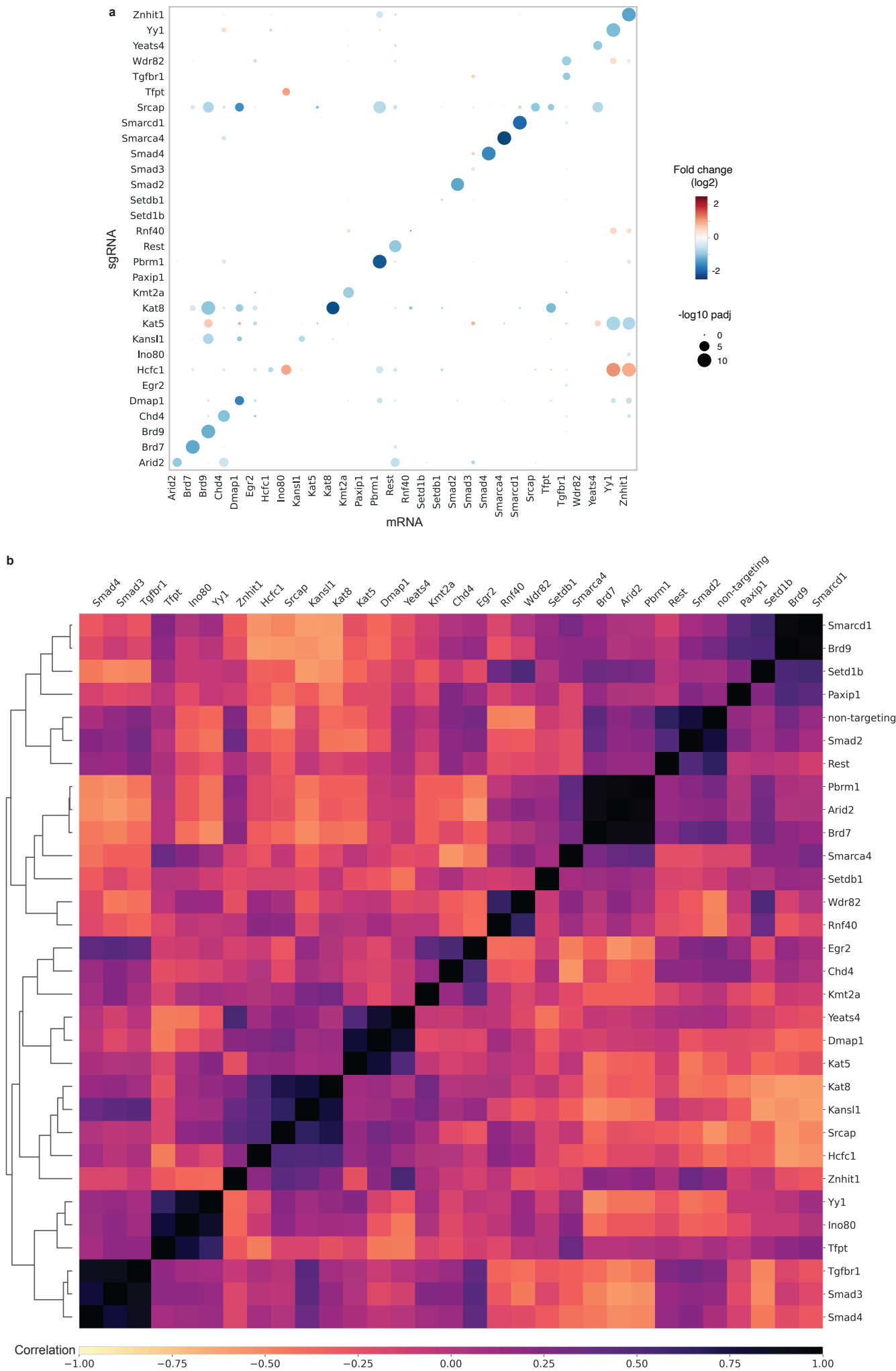
Supplementary Figure 2 | **Single-cell perturbation characterization of *ex vivo* fibroblast cultures.** **(a)** Heatmap showing viability score of Chromatin Factors grouped by Chromatin Complex membership derived from FACS-based CRISPR screen. **(b)** UMAP projection showing the replicates performed for the Perturb-seq screen (n = 4 (for resting), 3 (for TGF- β) and 2 (for IL-1 β) replicates, two independent experiments). **(c)** Plot showing expression of fibroblast markers across fibroblast subpopulations. Markers for naïve fibroblasts, stress-fiber components, ECM and pro-fibrotic cytokines, collagen biosynthesis, proteases and protease inhibitors, antifibrotic and pro-repair molecules and others are included. Dot size represents percentage of cells in the cluster expressing the marker gene. Color represents scaled expression values. **(d-e)** Heatmap showing enrichment of *in vivo* expression signatures shown in Forte *et al.*¹³ **(d)** and Buechler *et al.*¹¹. **(e)** over *ex vivo* fibroblast populations from this study. Source data are provided as a Source Data file.

SI Fig 3.



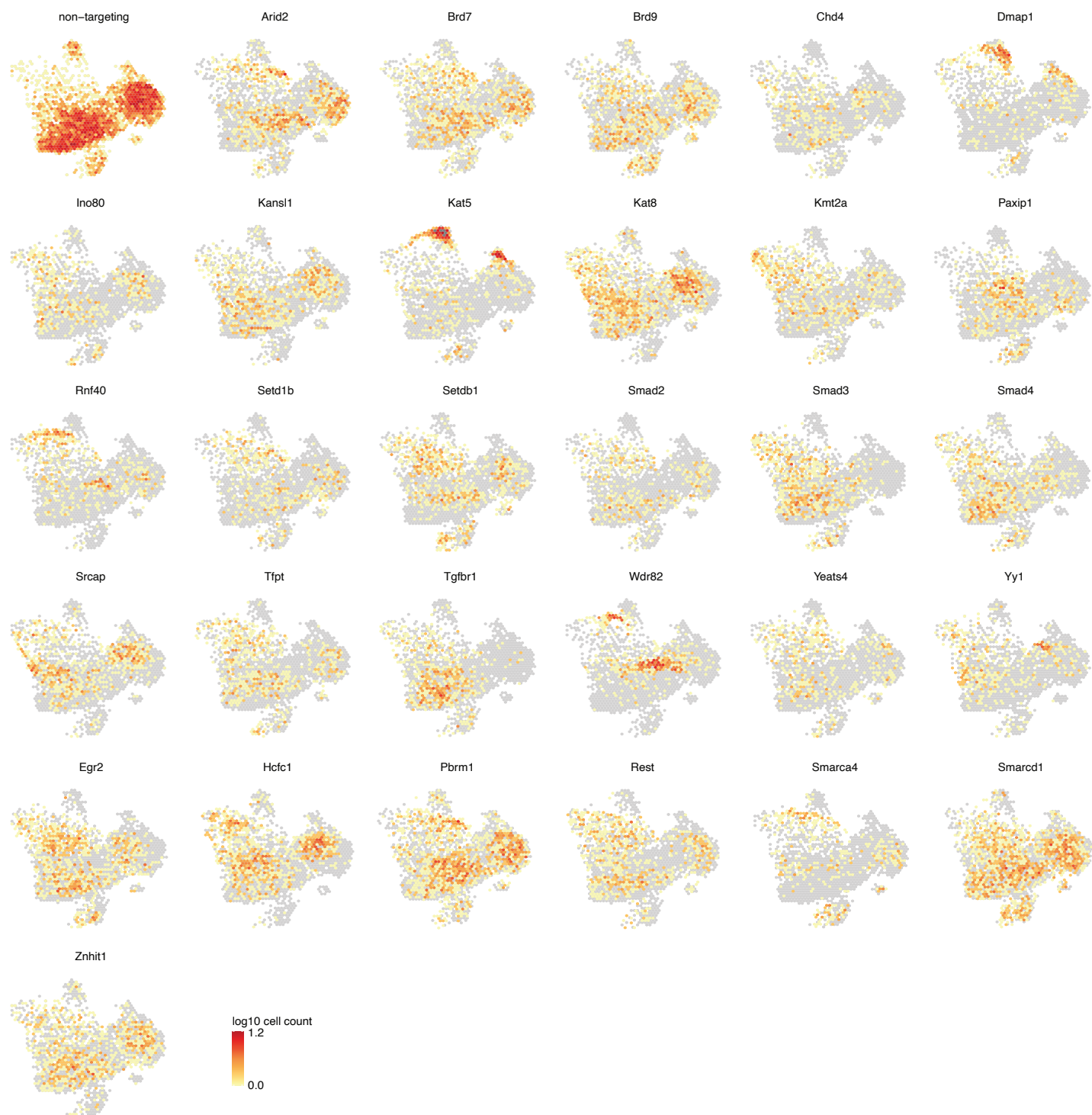
Supplementary Figure 3 | **SCENIC analysis of TF regulons active in the *ex vivo* fibroblast subpopulations characterized in this study.** **(a)** SCENIC analysis of TF regulons active in the *ex vivo* fibroblast subpopulations characterized in this study. **(b)** TF regulons are ordered decreasingly by their regulon specificity scores. The top 10 regulons for each subpopulation are labelled in red.

SI Fig 4.



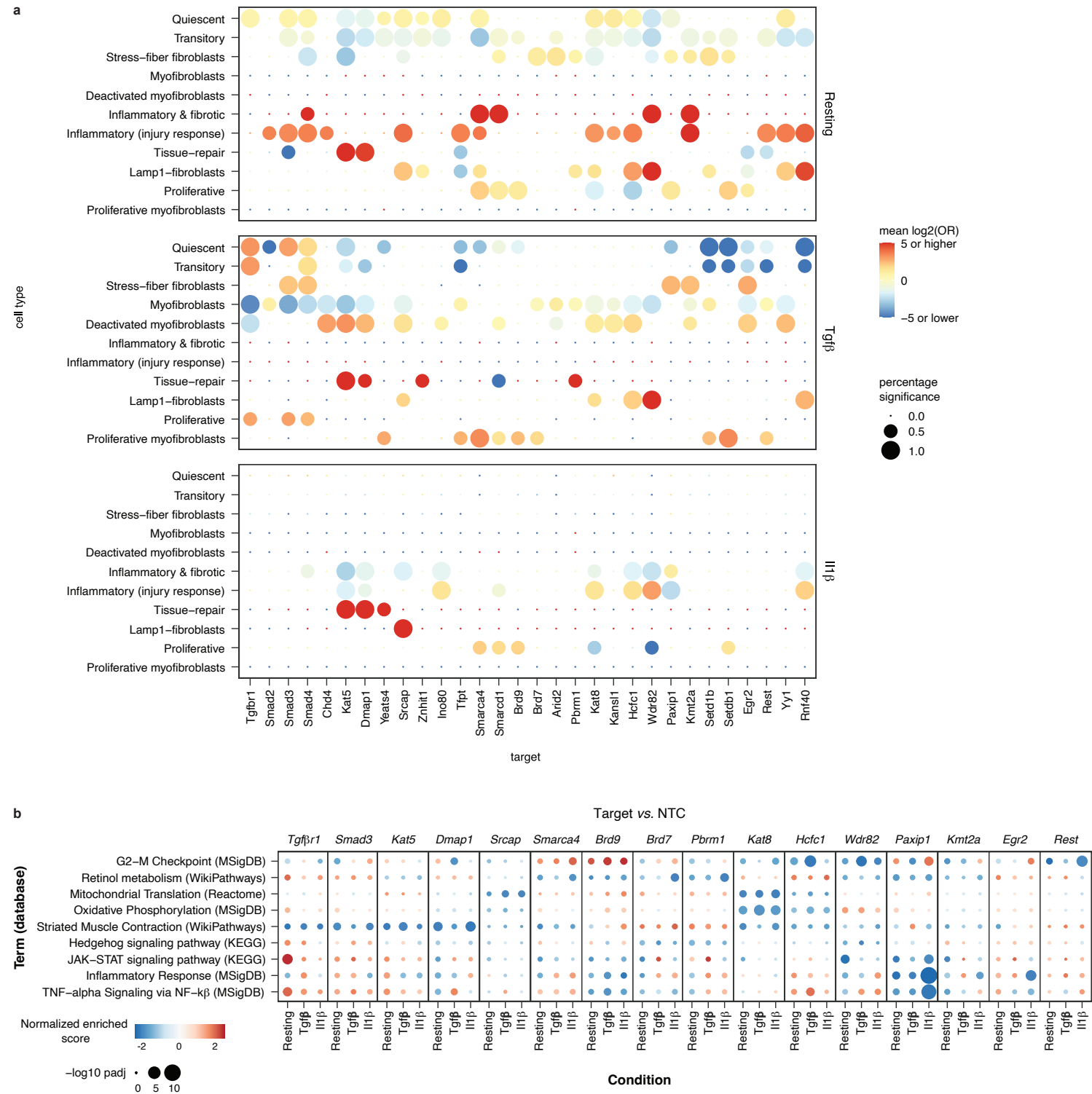
Supplementary Figure 4 | **Single-cell perturbation analysis of epigenetic regulators in primary fibroblasts.** **(a)** Effect of epigenetic perturbations (Y-axis) on the expression of their target genes (X-axis). Differential gene expression in each epigenetic regulator KO was calculated compared to NTC and is represented as \log_2 fold change value. Dark blue to dark red colour gradient denotes lower to higher \log_2 fold changes. **(b)** Correlation plot of gene expression profiles generated through the epigenetic perturbations in primary fibroblasts. Dendrogram showing hierarchical clustering of epigenetic regulator KOs is shown on the left side of the plot. White to black colour gradient denotes lower (-1) to higher (1) coefficients of Pearson. Source data are provided as a Source Data file.

SI Fig 5.



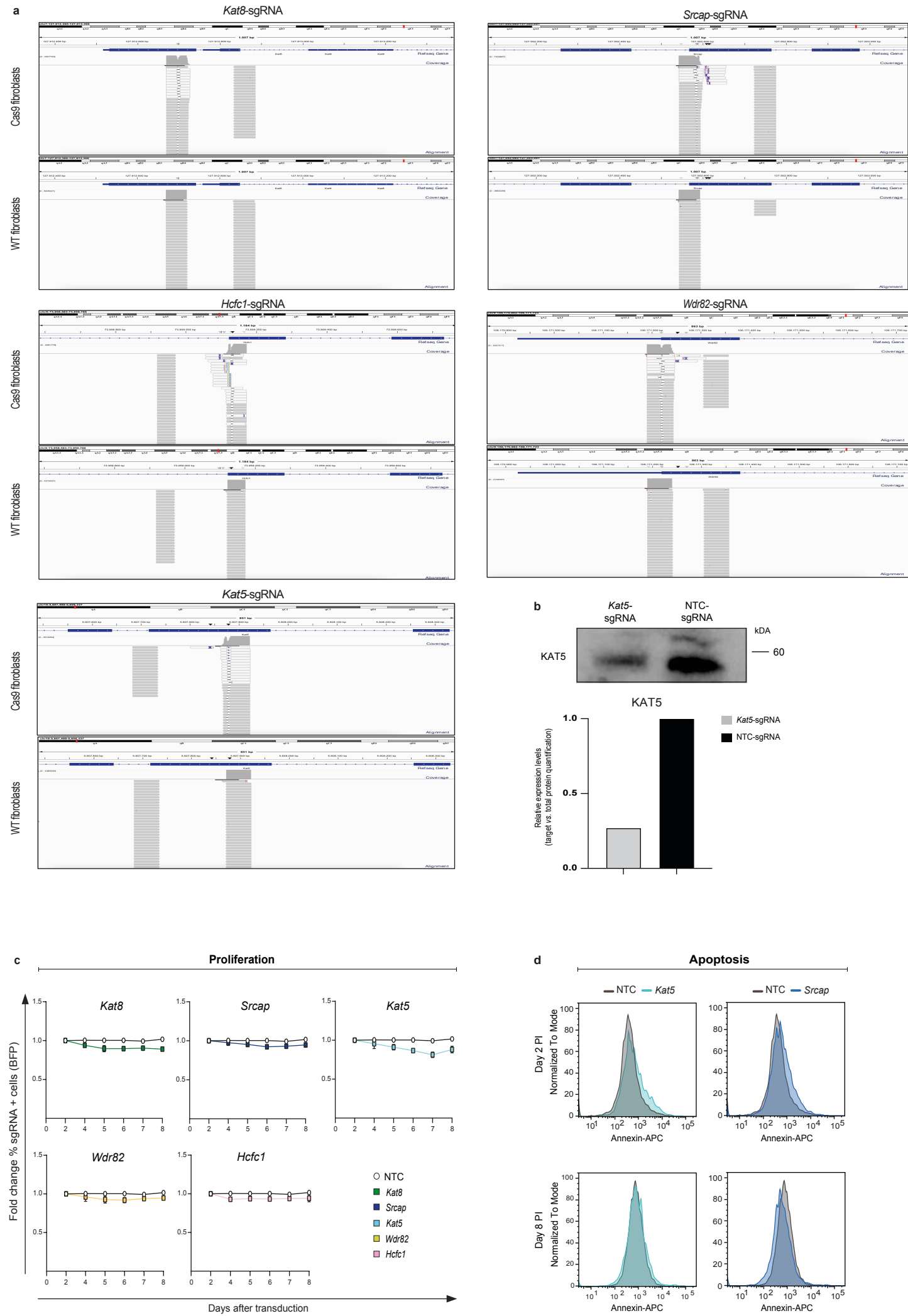
Supplementary Figure 5 | **UMAPs showing the distribution of unperturbed fibroblasts (non-targeting) and *Tgfbr1*, *Smad2*, *Smad3*, *Smad4* and ChrF-KOs in *ex vivo* fibroblasts.** Source data are provided as a Source Data file.

SI Fig 6.



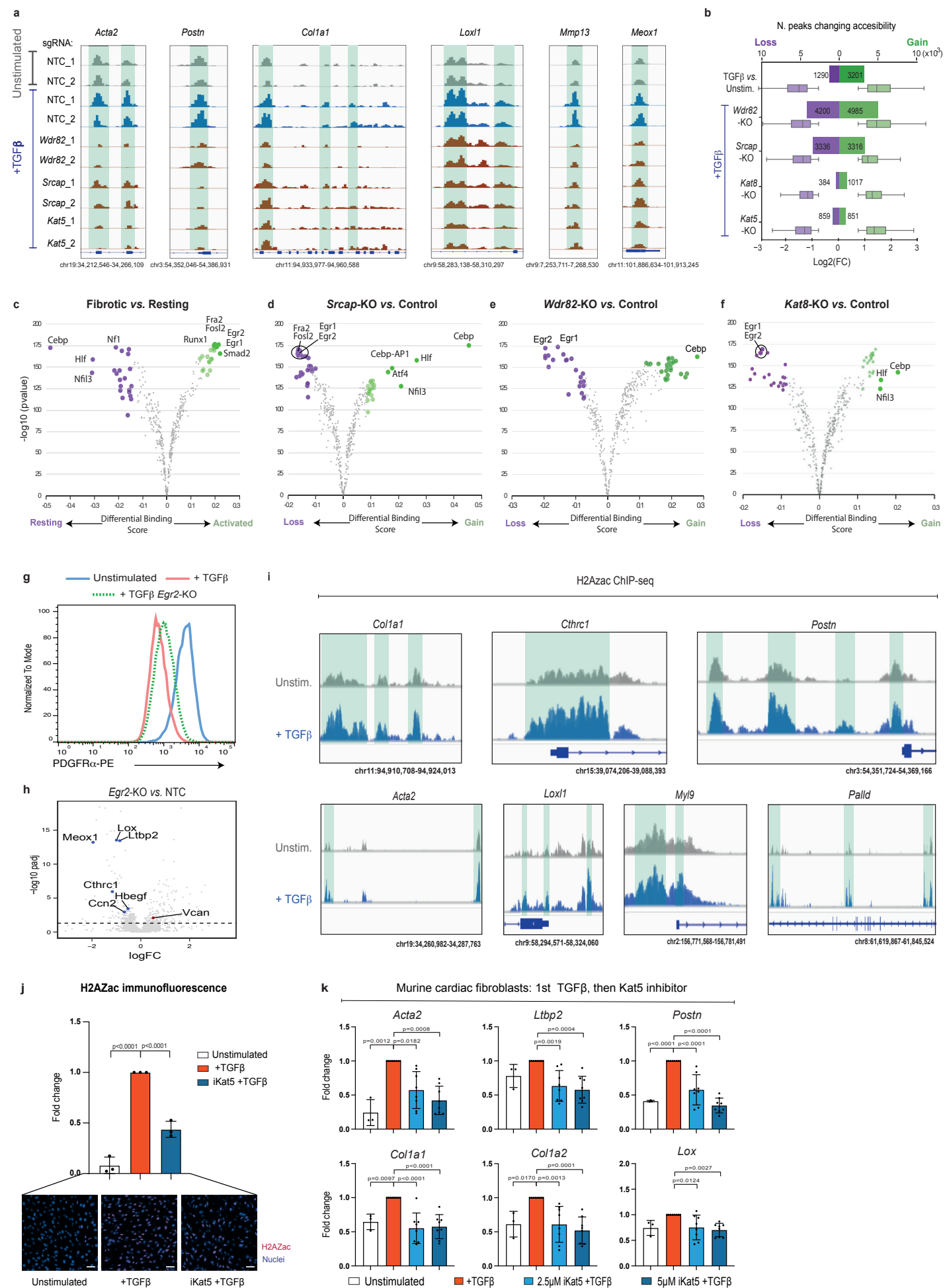
Supplementary Figure 6 | **Single-cell perturbation analysis of epigenetic regulators in primary fibroblasts identifies regulators of specific fibroblast states.** **(a)** Enrichment analysis of cells with specific epigenetic perturbations across the different fibroblast states identified in resting, TGF- β and IL-1 β conditions. Dot color and size relate to the log2 odds ratio (from Fisher's exact test) and the percent of significant enrichments (one test was performed per NTC), respectively. The analysis is based on measurements of two merged sgRNAs per target. p-values were adjusted for multiple comparisons via the Benjamini-Hochberg method. **(b)** Gene set enrichment analysis (GSEA) of differentially expressed genes in across representative knockouts. The color of each dot represents normalized enrichment score, the size represents the $-\log_{10}$ adjusted p-value (adjusted for multiple comparisons via the Benjamini-Hochberg method). Normalized enrichment scores were calculated via an adaptive multilevel splitting Monte Carlo approach as implemented in the R package fgsea. Source data are provided as a Source Data file.

SI Fig 7.



Supplementary Figure 7 | **Validation of the knockout produced with the different guides by CRISPR.** **(a)** Genome browser snapshots of Indel-seq signal for *Kat8*, *Srcap*, *Hcfc1*, *Wdr82* and *Kat5* loci in Cas9 and wildtype (WT) murine cardiac fibroblasts transduced with *Kat8*-, *Srcap*-, *Hcfc1*-, *Wdr82*- or *Kat5*-sgRNAs. **(b)** Western blot of Kat5 in Cas9 fibroblasts transduced with the target-gene or control (NTC) sgRNAs. Relative expression levels were calculated using total protein content loaded stained with Revert700 as a normalization factor. **(c)** Proliferation curves of control fibroblasts and *Kat8*-, *Srcap*, *Kat5*- *Wdr82* and *Hcfc1*-KO fibroblasts. The assay measures the change in the proportion of BFP (sgRNA) expressing cells over time (n = 4 independent experiments). Statistical significance was analyzed by two-way ANOVA followed by Sidak multiple comparisons test. No significant differences were observed at the end-time point. Error bars are SD. **(d)** Representative FACS plots showing apoptosis levels (Annexin V + 7AAD⁺) in unperturbed (NTC) and *Srcap* and *Kat5*-KO *ex vivo* cardiac fibroblasts (n = 2 independent experiments). Source data are provided as a Source Data file.

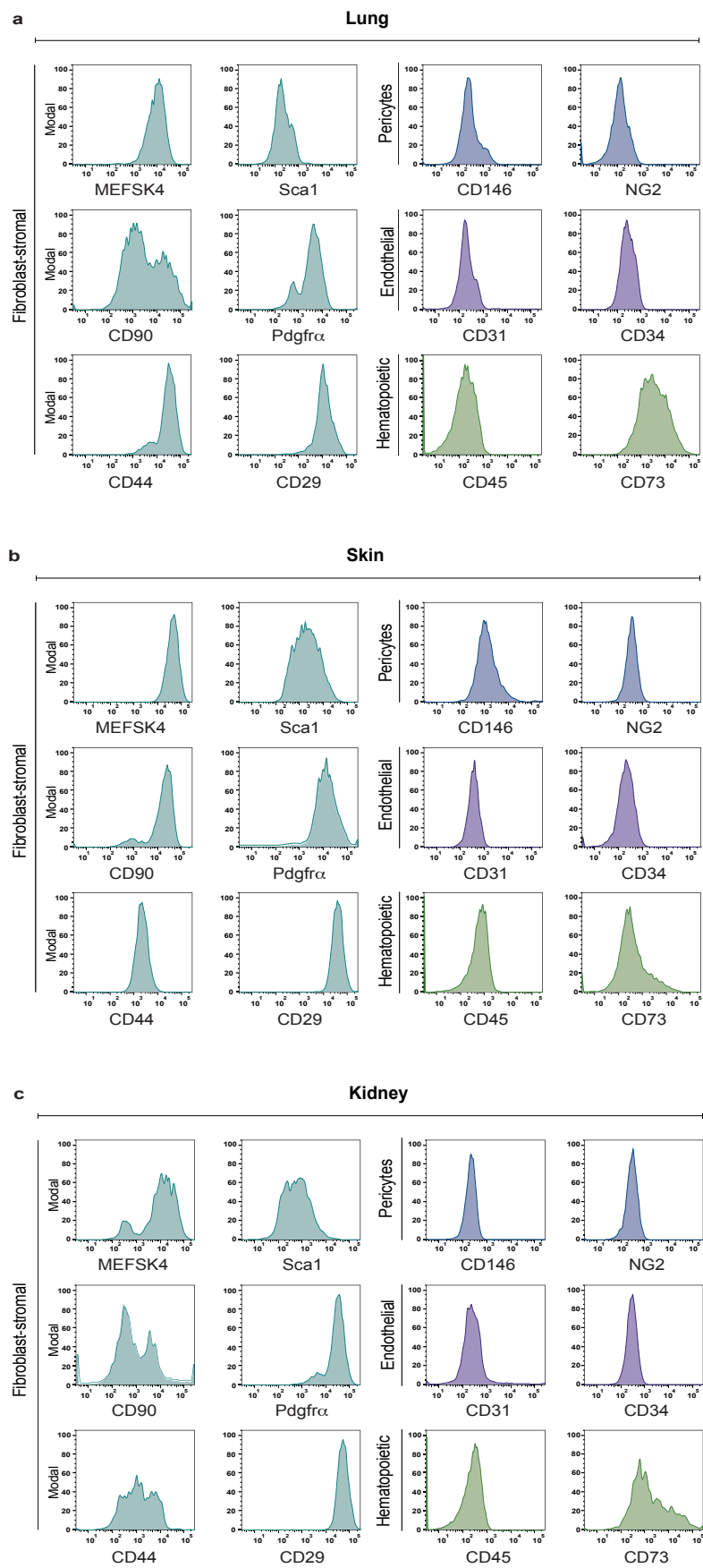
SI Fig 8.



Supplementary Figure 8 | **Epigenetic mechanisms underlying candidates' pro-fibrotic roles.**

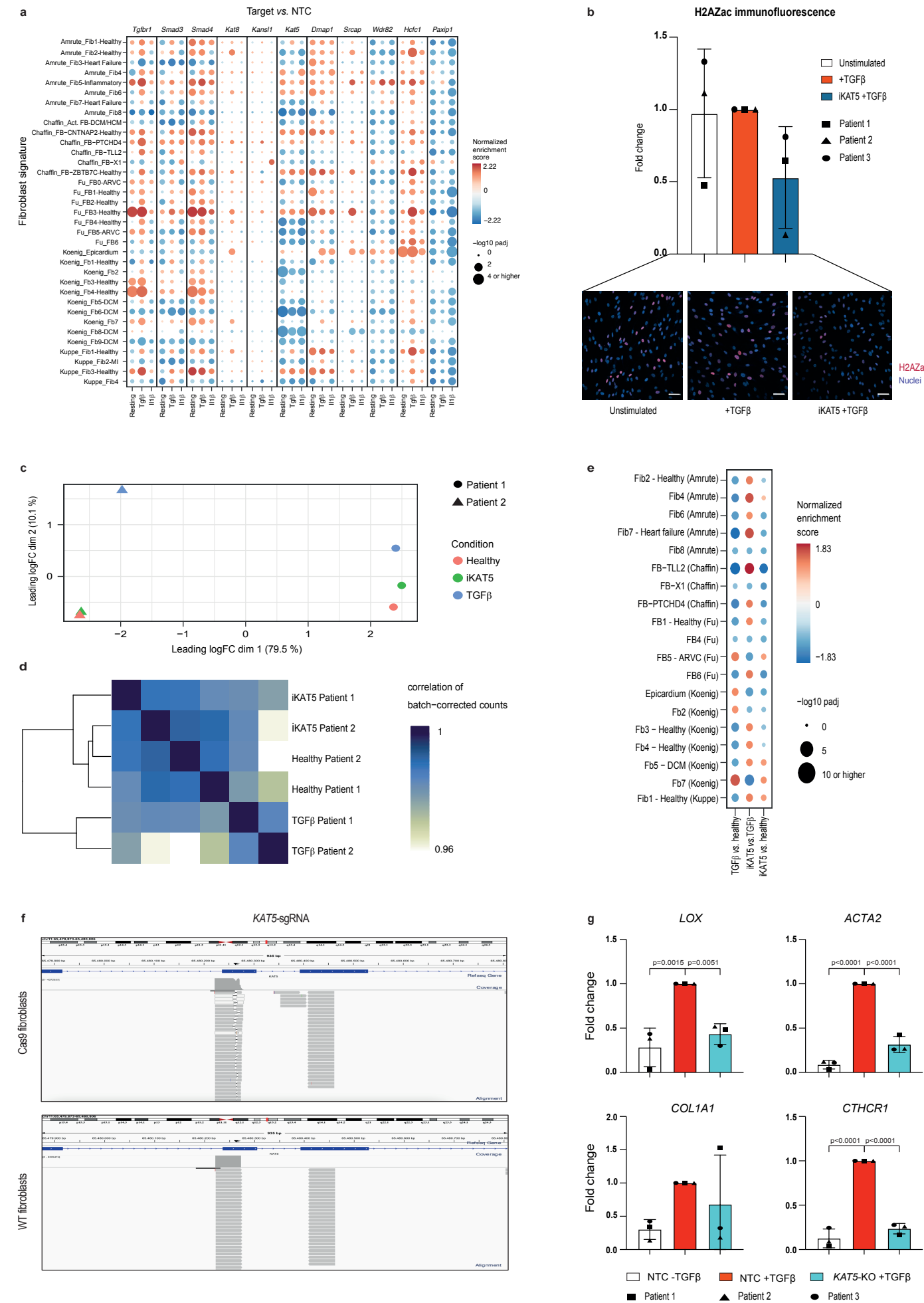
(a) Genome browser snapshots of ATAC-seq signal for fibrotic loci in cardiac fibroblasts: unstimulated (grey), 24h TGF- β -stimulated (blue) and *Wdr82*, *Srcap* and *Kat5* knockouts in TGF- β stimulated conditions (red). Peaks for two replicate samples of each condition are shown. **(b)** Plot showing number of changing peaks ($\log_2\text{FC} > 1$ in samples without replicates, $\log_2\text{FC} > 1$ $\log_2\text{FC} > 0.75$ in samples with replicates)) and fold-change distribution in unstimulated vs. TGF- β -stimulated (24h) unperturbed fibroblasts and in *Wdr82*, *Srcap*, *Kat8* and *Kat5* knockouts. FC values were calculated with DESeq for comparisons with more than one replicate and only peaks with an adjusted P value ≤ 0.01 were considered to measure the number of changing peaks. **(c)** Volcano plot showing differential TF motif footprints between TGF- β -stimulated (24h) and resting conditions. FC and p-values were calculated with TOBIAS. **(d-f)** Volcano plot showing differential TF motif footprints measured with TOBIAS between conditions in TGF- β -stimulated (24h) conditions: **(d)** *Srcap* knockout vs. control fibroblasts (NTC), **(e)** *Wdr82* knockout vs. control fibroblasts (NTC), **(f)** *Kat8* knockout vs. control fibroblasts (NTC). Differential footprinting analysis was performed with TOBIAS **(g)** Representative validation of *Egr2*-KO using PDGFR α FACS readout in Cas9 fibroblasts grown under TGF- β conditions ($n = 2$ independent experiments). **(h)** Volcano plot showing differentially expressed genes derived from Perturb-seq between *Egr2*-KO and control (NTC) fibroblasts in TGF- β -stimulated (24h) conditions. Log-fold changes were obtained via a linear model as implemented in the R package limma. p-values were adjusted for multiple comparisons via the Benjamini-Hochberg method. **(i)** Genome browser snapshots of H2AZac ChIP-seq signal at representative fibrotic loci in unstimulated and TGF- β -stimulated (24h) fibroblasts. **(j)** Quantification of the percentage of H2AZac positive cells, following TGF- β stimulation in control and NU-9056 pre-treated murine cardiac fibroblasts ($n = 3$ independent experiments). Representative images of H2AZac immunostaining assays are shown at the bottom. Scale bars: 50 μm . **(k)** Expression of fibrotic marker gene expression in unstimulated control or following TGF- β stimulation for 24h, followed by treatment with NU-9056 or vehicle for 24h in murine cardiac fibroblasts ($n = 3$ for Mock- and 8 for Mock+, *Kat5* 2.5 μM + TGF β , and *Kat5* 5 μM + TGF β independent experiments). All data are shown as fold change vs. TGF- β values and are mean \pm SD. Statistical significance was assessed using one-way ANOVA (two-sided) followed by Dunnett's multiple comparisons test, or by Kruskal-Wallis test with Dunn's post-hoc test where appropriate. Exact p-values are indicated in the graphs. Source data are provided as a Source Data file.

SI Fig 9.

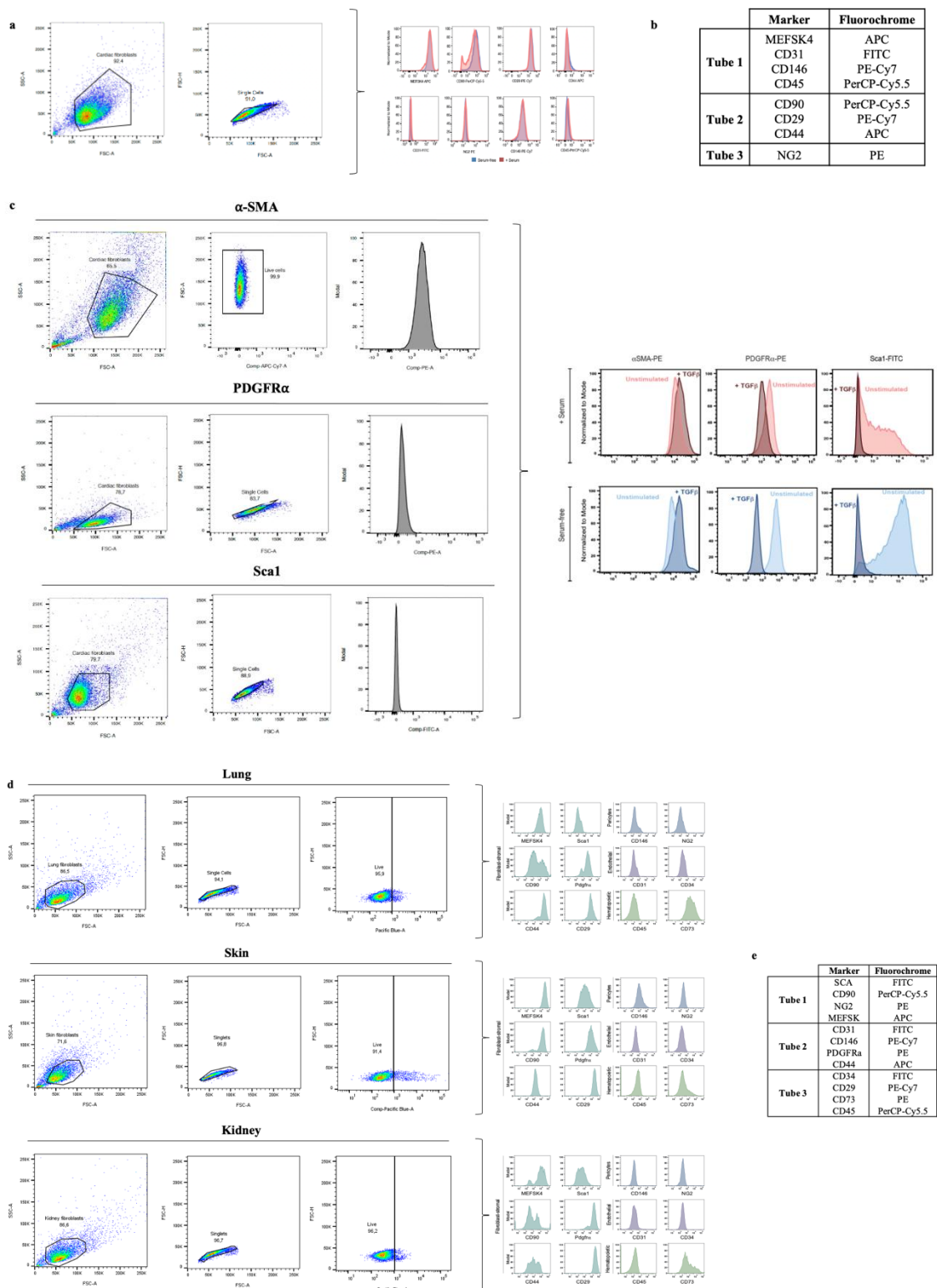


1 Supplementary Figure 9 | **Characterization of lung, skin, and kidney fibroblasts through flow**
2 **cytometry. (a-c)** Representative FACS analysis of fibroblast-stromal (MEFSK4, CD90, CD73,
3 CD44, CD29), pericytes (CD146, NG2), endothelial (CD31, CD34) and hematopoietic (CD45)
4 cell markers in unstimulated lung **(a)**, skin **(b)** and kidney **(c)** fibroblasts at day 7 of culture.
5
6

SI Fig 10.

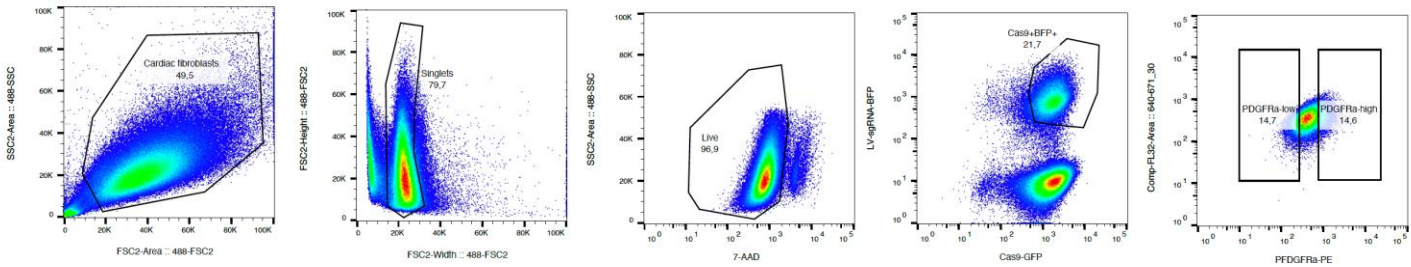


Supplementary Figure 10 | **KAT5 chemical and genetic perturbation attenuates fibrotic responses in human primary cardiac fibroblasts.** (a) Gene set enrichment analysis of differentially expressed genes across representative knockouts. Fibroblast expression signatures are taken from references in Fig. 6a. The color of each dot represents normalized enrichment score, the size represents the $-\log_{10}$ adjusted p-value (adjusted for multiple comparisons via the Benjamini-Hochberg method). Normalized enrichment scores were calculated via an adaptive multilevel splitting Monte Carlo approach as implemented in the R package fgsea. (b) Quantification of the percentage of H2AZac positive cells, following TGF- β stimulation in control and NU-9056 pre-treated human cardiac fibroblasts. The measurements were performed in three patients. Representative images of H2AZac immunostaining assays are shown at the bottom. Scale bars: 50 μm . (c) Principal component analysis of gene expression in patient-derived cardiac fibroblasts. Dimension 1 corresponds to variability of gene expression between patients, while dimension 2 captures effects of TGF- β or KAT5 inhibitor on the transcriptome. (d) Correlation analysis (using Pearson's correlation coefficient) of gene expression demarcates samples treated with TGF- β from unstimulated and KAT5-inhibited samples. (e) Gene set enrichment analysis of differentially expressed genes across treatments. Fibroblast expression signatures are taken from references in Fig. 6a. The color of each dot represents normalized enrichment score, the size represents the $-\log_{10}$ adjusted p-value (adjusted for multiple comparisons via the Benjamini-Hochberg method). Normalized enrichment scores were calculated via an adaptive multilevel splitting Monte Carlo approach as implemented in the R package fgsea. (f) Genome browser snapshots of Indel-seq signal for *KAT5* loci in Cas9 and wildtype (WT) human cardiac fibroblasts transduced with *KAT5*-sgRNA. (g) Gene expression analysis of fibrotic markers in human cardiac fibroblasts depleted for KAT5 using CRISPR/Cas9 and then stimulated with TGF- β . The measurements were performed in cell cultures from three different patients. Each patient is represented by a different symbol. All data are shown as fold change vs. TGF- β values and are mean \pm SD. Statistical significance was assessed using one-way ANOVA (two-sided) followed by Dunnett's multiple comparisons test. Exact p-values are indicated in the graphs. Source data are provided as a Source Data file.

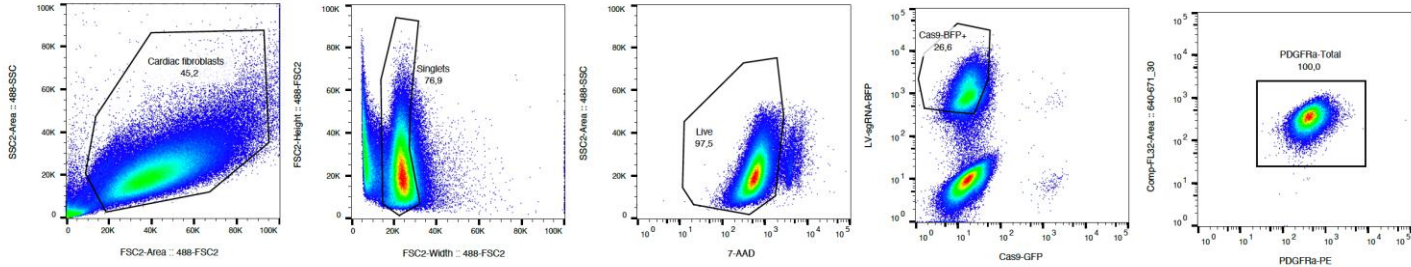


SI Figure 11. Gating strategies corresponding to flow cytometry data presented in SI Fig. 1 d, g and 8. Representative flow cytometry plots showing the sequential gating applied to all samples (a) and antibody/fluorochrome combinations used in each staining tube (b) for the analysis shown in SI Fig. 1d. Representative flow cytometry plots showing the sequential gating applied for the analysis shown in SI Fig. 1g (c) and representative flow cytometry plots showing the sequential gating applied to all samples (d) and antibody/fluorochrome combinations used in each staining tube (e) for the analysis shown in SI Fig. 8.

a

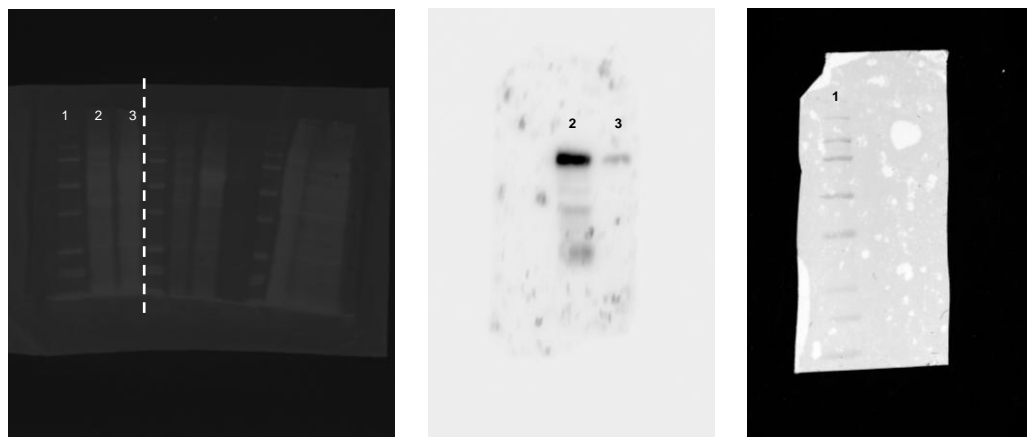


b



SI Figure 12. FACS Readout for Bulk CRISPR screens. a) Gating strategy for separately sorting naïve and fibrotic Cas9 cardiac fibroblasts. **b)** Gating strategy for sorting non-Cas9 (wildtype) cardiac fibroblasts.

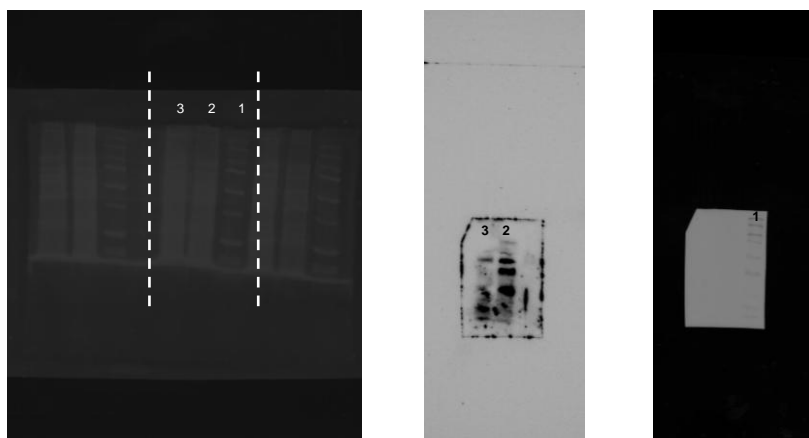
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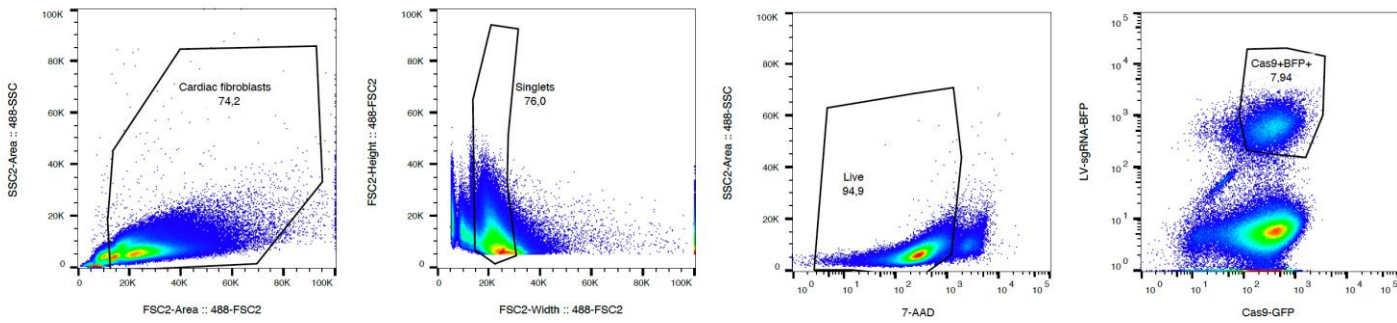
b



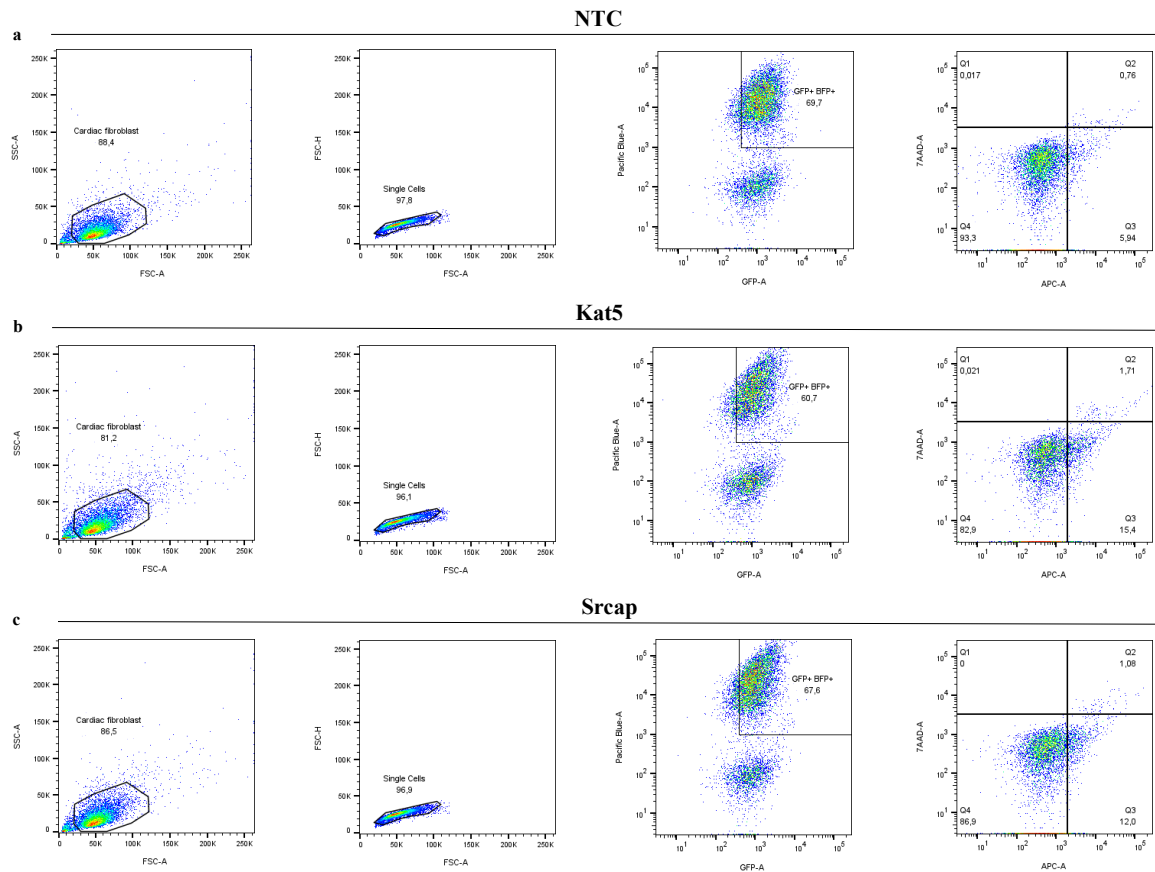
c



SI Figure 13. Uncropped versions of blots for total protein (left), target protein(middle) and Precision Plus Protein Standard (right) of Smad4 (a), Tgfbr1(b) and Kat5 (c)Western Blots. In all the blots shown, lane 1 corresponds to Precision Plus Protein Standard loaded(#161-0376, Bio-Rad), lane 2 corresponds to NTC-sgRNA sample loaded and lane 3 corresponds totarget-sgRNA sample loaded.



SI Figure 14. Perturb-seq gating strategy of Cas9 cardiac fibroblasts.



SI Figure 15. Gating strategy corresponding to the flow cytometry data presented in SI Fig. 7d. Representative flow cytometry plots showing the sequential gating used to analyze Annexin-APC and 7-AAD staining in control and gene-targeted cardiac fibroblasts. **a)** Gating strategy for non-targeting control (NTC) fibroblasts. **b)** Gating strategy for Kat5-targeted fibroblasts. **c)** Gating strategy for Srcap-targeted fibroblasts.

Supplementary Table 1. List of antibodies used for flow cytometry and FACS analyses.

NAME	CLONE	SUPPLIER	DILUTION
CD31-FITC	390	BioLegend	1:200
CD45-PerCP/Cy5.5	30-F11	BD Pharmingen	1:200
Anti-feeder cells-APC	MEFSK4	Miltenyi	1:50
CD146-PE/Cy7	ME-9F1	BioLegend	1:100
CD44-APC	KM201	Invitrogen	1:200
CD90-PerCP/Cy5.5	30-H12	BioLegend	1:200
PDGFRa-PE	APA5	BioLegend	1:200
aSMA-PE	1A4	Sigma	1:100
CD29-PE/Cy7	HMb1-1	BioLegend	1:200
SCA1-FITC	E13-161.7	BD Bioscience	1:300
NG2-PE	1E6.4	Miltenyi	1:50
CD34-FITC	RAM34	BD Bioscience	1:100
CD73-PE	Ty/11.8	BioLegend	1:100
NG2-PE	1E6.4	Miltenyi	1:50

Supplementary Table 2. List of primers used for qPCR analyses in mouse samples.

GENE	SEQUENCE Fw (5' to 3')	SEQUENCE Rv (5'to 3')
<i>Acta2</i>	ATGGAGTCAGCGGGCATC	CGTTCTGGAGGGGCAATGAT
<i>Ltbp2</i>	ACCATCACCACCTCCACTC	CTCGAGTCCTGAAGGCAAAG
<i>Postn</i>	TATGCTCTGCTGCTGCTGTT	CTTTTGGTGCCCAGAATTT
<i>Colla1</i>	GCATCGCCAAGAAGACATCC	CAGATCAAGCATACCTCGGG
<i>Colla2</i>	AGGCAGAGATGGTGTTGATG	AGGGCCAGATGAAACTCCTT
<i>Lox</i>	TGCCAACACACAGAGGAGAG	CTGCCGCATAGGTGTCATAA
<i>Gapdh</i>	ACTTTGTCAAGCTCATTTCC	TGCAGCGAACTTTATTGATG
<i>Rpl4</i>	GCCGCTGGTGGTTGAAGATAA	CGTCGGTTTCTCATTTTGCCC

Supplementary Table 3. List of primers used for qPCR analyses in human samples.

GENE	SEQUENCE Fw (5' to 3')	SEQUENCE Rv (5'to 3')	PROBE (5'to 3')
<i>Col1a1</i>	TTCTGTACGCAGGTGATTG G	GACATGTTTCAGCTTTGTG GAC	/56- FAM/TCGAGGGGCC/ZEN/AAG ACGAAGACATC/3IABkFQ/
<i>Lox</i>	AGTGGCTAAACTCATCCAT ACTG	GCTCAGATTTCCCAAAG AGT	/56- FAM/TGACAACTG/ZEN/TGC CATTCCCAGGA/3IABkFQ/
<i>Acta2</i>	AGAGTTACGAGTTGCCTG ATG	CTGTTGTAGGTGGTTTCA TGGA	/56- FAM/AGACCCTGT/ZEN/TCCA GCCATCCTTC/3IABkFQ/
<i>Cthrc1</i>	CAAGCAGTGTTTCATGGAG TTC	CTTGACAGCATGCATTTTC TGC	/56- FAM/TTGTTTCAGT/ZEN/GGCT CACTTCGGCTAA/3IABkFQ/
<i>Gapdh</i>	ACATCGCTCAGACACCAT G	TGTAGTTGAGGTCAATG AAGGG	/56- FAM/AAGGTCGGA/ZEN/GTC AACGGATTGGTC/3IABkFQ/

Supplementary Table 4. Primer sequences used in this study.

NAME	SEQUENCE (5' to 3')	USE
U6 Forward	TAATTTCTTGGGTAGTTTGCA	Sanger sequencing of sgRNAs
Read1-U6	CTACACGACGCTCTTCCGATCTGTGGAAAGGACGAAACACCG	CRISPR screen
Read2-scaffold	AGACGTGTGCTCTTCCGATCTGCTGTCCCTGTAATAAACCCG	CRISPR screen
P7-index	CAAGCAGAAGACGGCATACGAGAT[i7]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CRISPR screen, INDEL-seq
P5-index	AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT	CRISPR screen, INDEL-seq
P5-i5-Read1	AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTCAGATGTGTAT	ATAC-seq
P7-i7-Read2	CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGGAGATGTG	ATAC-seq
Read1-gDNA_Wdr82	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCCTTCAGCAGCAGCAG	INDEL-seq
Read2-gDNA_Wdr82	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCACGATGGAGTCATCATCG	INDEL-seq
Read1-gDNA_Tgfr1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATGGTCTATATCTGCCATA	INDEL-seq
Read2-gDNA_Tgfr1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCCAACCACTAATTA	INDEL-seq
Read1-gDNA_Smad4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGTAAAGGTGAAGGTGACG	INDEL-seq
Read2-gDNA_Smad4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGCAACAGAGGGTTTAA	INDEL-seq
Read1-gDNA_Srcap	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCATCTTTTTTCCCTTCCT	INDEL-seq
Read2-gDNA_Srcap	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTAGGCACCACCTATAGATG	INDEL-seq
Read1-gDNA_Kat8	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCGCGTGGCGAACCAGAAGT	INDEL-seq
Read2-gDNA_Kat8	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGGGCTTCTCAGCTCCCG	INDEL-seq
Read1-gDNA_Hcfc1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGGGGGTCTGGCCAATGA	INDEL-seq
Read2-gDNA_Hcfc1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACCTACCAGGGGTTTCAG	INDEL-seq
Read1-gDNA_Kat5	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGACTGGCAGTCTGGTGTC	INDEL-seq
Read2-gDNA_Kat5	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGGATAGGCAGCCTCCCA	INDEL-seq
Read1-gDNA_KAT5	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCTATTCTCATAGCCCTGG	INDEL-seq
Read2-gDNA_KAT5	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTGGGGAACCTGGATCTTC	INDEL-seq

Supplementary Table 5. List of primary and secondary antibodies used for Western Blot.

PRIMARY ANTIBODIES			
NAME	CLONE	SUPPLIER	DILUTION
SMAD4	D3R4N	Cell Signaling, 46535	1:1000
TGFBR1	pAb	Invitrogen, PA5-32631	1:500
KAT5	pAb	Abcam, ab23886	1:500
SECONDARY ANTIBODIES			
NAME	CLONE	SUPPLIER	DILUTION
Donkey HRP-conjugated anti-rabbit	pAb	GE Healthcare, NA934	1:5000

Supplementary Table 6. sgRNAs sequences used for human CRISPR/Cas9.

sgRNA sequence	sgRNA ID	Gene ID
CTGAGCGTGAAGGACATCAG	KAT5_H2	<i>KAT5</i>
AGTACCCCTAGGTATGGGGA	NTC_H2	Non-targeting control (NTC)