

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	No software was used for data collection
Data analysis	FlowJo Software (v10.4) GraphPad Software (v9.1) ImageJ software (v1.53) R (v4.3.2) Cutadapt (v2.10) (v3.4) FastQC software (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) STAR (v2.7.3a) Gencode v45 edgeR (v3.32.1) DESeq2 package (v1.30.0) limma (v3.58.1) bcl2fastq (v2.20) Bowtie2 (v2.3.4.2) (v2.4.2) fgsea (v1.28.0) Trim Galore (v0.6.6) Picard (v2.25.4) ENCODE blacklist (v2.0)

alignmentSieve (v3.5.1)

SAMtools (v1.3.1) (v1.10)

bamCoverage (v3.5.1)

BEDTools (v2.27.1)

MACS (v2.2.7.1)

TOBIAS (v0.13.2)

Cutadapt (v3.4)

featureCounts (v2.0.1)

HOMER (v4.10)

Python (v.3.10.12)

scanpy package (v1.10.3)

ggplot2 (v3.4.4)

ComplexHeatmap (v2.18.0)

pyscenic (v0.12.1)

Code for performing the analyses and generating all figures is available from GitHub (<https://github.com/csb/fibroblast-perturb-seq-analysis>) and has been archived to Zenodo (<https://doi.org/10.5281/zenodo.14717236>).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Bulk expression patterns of murine cardiac fibroblasts: Ruiz-Villalba, A. et al. (GEO accession: GSE132146)

Single-cell expression patterns of in vivo fibroblasts: Forte et al. (ArrayExpress accession: E-MTAB-7895), Koenig et al. (GEO accession: GSE183852), Buechler et al. (<https://fibroXplorer.com>), Amrute et al. (GEO accession: GSE226314), Chaffin et al. (dbGaP accession: phs001539), Fu et al. (Genome Sequence Archive accession: HRA005871), Kuppe et al. (<https://data.humancellatlas.org/explore/projects/e9f36305-d857-44a3-93f0-df4e6007dc97>).

Perturb-seq dataset: GEO accession (GSE261783)

Chromatin accessibility dataset: GEO accession (GSE261740)

ChIP-seq dataset: GEO accession (GSE261741)

Human RNA-seq dataset: GEO accession (GSE280438) and EGA accession (EGAS50000000835)

GSE261740, GSE261741 and GSE261783 subseries belong to the SuperSeries accession id GSE261742 which is a private (access restricted) data for now. If you'd like to access it from GEO please use the private token: gvlscisfvstfe.

GSE280438 is a private (access restricted) data for now. If you'd like to access it from GEO please use the private token: kjqhiyuaupwtjth.

All data is available from <https://doi.org/10.5281/zenodo.14794723>.

Source data are provided with the paper.

Databases used in this study:

GRCh38/mm10 reference genome assembly (GENCODE vM23/Ensembl 98)

GRCh38 reference genome assembly (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.15_GRCh38/seqs_for_alignment_pipelines.ucsc_ids/GCA_000001405.15_GRCh38_no_alt_analysis_set.fna.gz)

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Primary human cardiac fibroblasts from discarded surgical tissue from patients undergoing surgery were obtained from the University of Navarra Clinic (Spain). Samples were anonymized and the researcher had no access to any personal data.

Reporting on race, ethnicity, or other socially relevant groupings

Primary human cardiac fibroblasts from discarded surgical tissue from patients undergoing surgery were obtained from the University of Navarra Clinic (Spain). Samples were anonymized and the researcher had no access to any personal data.

Population characteristics

Primary human cardiac fibroblasts from discarded surgical tissue from patients undergoing surgery were obtained from the University of Navarra Clinic (Spain). Samples were anonymized and the researcher had no access to any personal data.

Recruitment

Primary human cardiac fibroblasts from discarded surgical tissue from patients undergoing surgery were obtained from the University of Navarra Clinic (Spain). Samples were anonymized and the researcher had no access to any personal data.

Ethics oversight

The use of these samples was approved by the Ethics Committee of Universidad de Navarra.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was used. 2 biologically independent experimental replicates were performed for RNA-seq, FACS-based CRISPR screens, ChIP-seq and ATAC-seq profiling, this is a common standard in the field. For the validation of CRISPR screen hits in cardiac fibroblasts, a minimum of 2 biologically independent replicates were used. In the Perturb-seq screens, we designed our experiments to assay on average 100 cells/ CF-KO, which is an established standard in the field.
Data exclusions	No data were excluded from the analyses except one of the TGF- β replicates of the Perturb-seq screen was removed from downstream analysis due to high mitochondrial content.
Replication	RNA-seq: All analyzed samples were obtained from 2 independent biological replicates or 2 different patients CRISPR screens and validation of candidates: - Screens were performed in 2 independent biological replicates at a 500X CRISPR library coverage - FACS validation of the effects of hits derived from the screens was performed in replicates of at least 2 independent experiments - CRISPR screens and validation experiments were reproducible Perturb-seq - Two experiments (one pilot) were performed in different batches with both showing similar values for transduction efficiency - We analysed the unperturbed patterns (cells with NTC sgRNAs) across batches - For the 15 top CF regulators we performed a pilot experiment, which recapitulated the Perturb-seq experiment presented in the main figures - Overall, we performed 4 replicates for resting conditions, 4 replicates for TGF- β and 2 replicates for IL-1 β . One of the TGF- β replicates was removed from downstream analysis due to high mitochondrial content ATAC-seq of CF-KOs: All experiments were conducted in 2 biological replicates. All attempts of replication were successful ChIP-seq: All experiments were conducted in 2 biological replicates. All attempts of replication were successful Functional in vitro fibrotic assays were performed in cells from 2 independent experiments for aSMA IF (sgRNAs) and apoptosis; and in cells from 3 or more independent experiments for aSMA and H2AZac IF (iKat5), contraction, ELISA and proliferation. All attempts of replication were reproducible and successful
Randomization	Allocation of animals for bulk and Perturb-seq screens was randomized (using always even numbers of males and females in each experimental condition) We have used bulk and single-cell (perturb-seq) to study the roles of CFs in fibrotic transformation of cardiac fibroblasts. Our perturbations were performed in the same cell population (cardiac fibroblasts) producing a pool of different CF mutant cells. The generation of such pool of CF-KOs is already a random process, thus we don't need further randomization.
Blinding	CF-KOs and NTC controls were analyzed side by side and the experimental groups (specific CF-KOs) were experimentally determined based on the expression of sgRNAs (and not assigned a priori). Thus, as groups are defined by the data, investigators could not be blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

Anti-CD31 FITC BioLegend Clone 390
 Anti-CD45 PerCP/Cy5.5 BD Pharmingen Clone 30-F11
 Anti-feeder cells APC Miltenyi Clone MEFSK4
 Anti-CD146 PerCP/Cy5.5 BioLegend Clone ME-9F1
 Anti-CD44 APC Invitrogen Clone KM201
 Anti-CD90.2 PerCP/Cy5.5 BioLegend Clone 30-H12
 Anti-PDGFRa PE BioLegend Clone APA5
 Anti-aSMA PE Sigma Clone 1A4
 Anti-CD29 PE/Cy7 BioLegend Clone HMb1-1
 Anti-SCA1 FITC BD Bioscience Clone E13-161.7
 Anti-NG2 PE Miltenyi Clone 1E6.4
 Anti-H2Az-Ac Diagenode Lot A1738P Ref C15410202
 Anti-mouse CD34 Alexa Fluor 488 BD Biosciences Clone RAM34
 Anti-mouse CD73 PE Biolegend Clone Ty/11.8
 Anti-SMAD4 Cell Signaling Clone D3R4N
 Anti-TGFBR1 Invitrogen Ref PA5-32631
 Anti-KAT5 Abcam Ref ab23886
 Rabbit IgG HRP Ref GENA934

Validation

Anti-CD31 FITC BioLegend Clone 390
 - Validated by the supplier in C57BL/6 mouse splenocytes. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤ 1.0 μg per million cells in 100 μl volume.
 - Used in several publications (see <https://www.biolegend.com/de-de/products/fits-anti-mouse-cd31-antibody-120?GroupID=BLG1566>), among them: Li CM, et al. 2020. Cell Reports. 33(13):108566.

Anti-CD45 PerCP/Cy5.5 BD Pharmingen Clone 30-F11
 - Validated by the supplier in mouse splenocytes.
 - Used in several publications (see <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/percp-cy-5-5-rat-anti-mouse-cd45.550994>), among them: Ruiz-Villalba, Adrián et al. 2020. Circulation. 14 (19): 1831-1847.

Anti-feeder cells APC Miltenyi Clone MEFSK4
 - Validated by the provider (Miltenyi) in co-cultures of mouse embryonic stem (mES) cells and mouse embryonic fibroblast (mEF) cells.
 - Used in several publications, among them: Ruiz-Villalba, Adrián et al. 2020. Circulation. 14 (19): 1831-1847.

Anti-CD146 PerCP/Cy5.5 BioLegend Clone ME-9F1
 - Validated by the supplier in bEND.3 cells (mouse endothelial cells). Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤ 0.5 μg per million cells in 100 μl volume.
 - Used in several publications (see <https://www.biolegend.com/nl-nl/products/percp-cyanine5-5-anti-mouse-cd146-antibody-7872?Clone=ME-9F1>), among them: Komai Y, et al. 2014. Sci Rep. 4:6175.

Anti-CD44 APC Invitrogen Clone KM201
 - Validated by the supplier (Invitrogen) in C57BL/6 mouse bone marrow cells.

Anti-CD90.2 PerCP/Cy5.5 BioLegend Clone 30-H12
 - Validated by the supplier (BioLegend) in C57BL/6 thymocytes. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤ 0.125 μg per million cells in 100 μl volume.
 - Used in several publications (see <https://www.biolegend.com/nl-be/products/percp-cyanine5-5-anti-mouse-cd90-2-thy1-2-antibody-12938>), among them: Niemann J, et al. 2019. Nat Commun. 10:3236.

Anti-PDGFRa PE BioLegend Clone APA5

- Validated by the supplier (BioLegend) in mouse fibroblast NIH/3T3 cells and used in several publications (see <https://www.biolegend.com/de-de/products/pe-anti-mouse-cd140a-antibody-6253?GroupID=BLG8105>) among them: Buechler MB, et al. 2021. Nature. 593:575.

Anti-aSMA PE Sigma Clone 1A4

- Validated by the supplier (Sigma-Aldrich) and used in 2 publications for flow cytometry (see <https://www.sigmaaldrich.com/ES/es/product/sigma/c6198>)

Anti-CD29 PE/Cy7 BioLegend Clone HMb1-1

- Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis by the provider (BioLegend). For flow cytometric staining, the suggested use of this reagent is = 1.0 µg per 106 cells in 100 µl volume.
- Used in several publications (see <https://www.biolegend.com/fr-ch/products/pe-cyanine7-anti-mouse-rat-cd29-antibody-3505>) among them: Ying Z, et al. 2020. Cell Stem Cell. 26(3):403-419.

Anti-SCA1 FITC BD Bioscience Clone E13-161.7

- Validated by the supplier (BD Bioscience). This antibody is routinely tested by flow cytometric analysis.

Anti-NG2 PE Miltenyi Clone 1E6.4

- Validated by the supplier (Miltenyi Biotec). Mouse brain tissue postnatal day 7 was dissociated using the Neural Tissue Dissociation Kit and the gentleMACS Dissociator. After re-expression of the AN2 antigen, mouse brain cells were stained with Anti-AN2-PE and analyzed by flow cytometry using the MACSQuant Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Anti-H2Az-Ac Diagenode Lot A1738P Ref C15410202

- Validated by the supplier (Diagenode). ChIP was performed on sheared chromatin from 100,000 K562 cells using 1 µg of the antibody using the "iDeal ChIP-seq" kit.
- The optimal antibody amount per IP should be determined by the end-user. They recommend testing 0.5-5 µg per IP.

Anti-mouse CD34 Alexa Fluor 488 BD Biosciences Clone RAM34

- Used in several publications (see <https://www.bdbiosciences.com/en-es/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alex-fluor-488-rat-anti-mouse-cd34.567135>), among them: Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature. 2000; 404(6774):193-197.

Anti-mouse CD73 PE Biolegend Clone Ty/11.8

- Validated by the supplier. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
- Used in several publications (see <https://www.biolegend.com/fr-ch/products/pe-anti-mouse-cd73-antibody-4681?GroupID=BLG10609>), among them: Vasanthakumar A, et al. 2020. Nature. 579:581.

Anti-SMAD4 Cell Signaling Clone D3R4N

- Validated by the supplier in extracts from various cell lines. Specificity of the antibody is confirmed by the absence of signal in extracts from HT-29 and COLO 205 cells, both of which contain Smad4 null mutations.
- Used in several publications (see <https://www.cellsignal.com/products/primary-antibodies/smad4-d3r4n-xp-rabbit-mab/46535?index=2&application=all>), among them: Heldin, C.H. et al. (1997) Nature 390, 465-71.

Anti-TGFBR1 Invitrogen Ref PA5-32631

- Validated by the supplier (ThermoFisher Scientific). Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HCT 116 cells were transfected with TGFBR1 siRNA and loss of signal was observed in Western Blot using TGFBR1 Polyclonal Antibody.
- Used in several publications (see <https://www.thermofisher.com/order/genome-database/generatePdf?productName=TGFBR1&assayType=PRANT&detailed=true&productId=PA5-32631>), among them: Lee, TH., Yeh, CF., Lee, YT. et al. Fibroblast-enriched endoplasmic reticulum protein TXNDC5 promotes pulmonary fibrosis by augmenting TGFβ signaling through TGFBR1 stabilization. Nat Commun 11, 4254 (2020).

Anti-KAT5 Abcam Ref ab23886

- The exact immunogen used to generate this antibody is proprietary information.
- Used in several publications (see <https://www.abcam.com/en-us/products/primary-antibodies/kat5-tip60-antibody-ab23886#>), among them: Ortega-Atienza, S., Wong, V. C., DeLoughery, Z., Luczak, M. W., & Zhitkovich, A. (2016). ATM and KAT5 safeguard replicating chromatin against formaldehyde damage. Nucleic acids research, 44(1), 198–209.

Rabbit IgG HRP Ref GENA934

- Validated by the supplier (Cytiva).
- Used in several publications, among them: Emond, R., Griffiths, J. I., Grolmusz, V. K., Nath, A., Chen, J., Medina, E. F., Sousa, R. S., Synold, T., Adler, F. R., & Bild, A. H. (2023). Cell facilitation promotes growth and survival under drug pressure in breast cancer. Nature communications, 14(1), 3851.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK 293T (Sigma, 12022001-DNA-5UG)
Authentication	Purchased from the provider (Sigma) as an authenticated cell line. We did not performed any further authentication and used early passages (p<14) were used for lentivirus production
Mycoplasma contamination	Cell lines were tested negative for Mycoplasma
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells were used

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	B6J.129(Cg)-Gt(ROSA)26Sortm1.1(CAG-cas9*,-EGFP)Fezh/J (stock Jackson #026179) Age: 7-8 weeks Sex: Equal numbers of males and females All animal requisitions, housing, treatments, and procedures were performed according to all state and institutional laws, guidelines and regulations. All studies were fulfilled under the Guidelines of the Care and Use of Laboratory Animals and approved by the Ethics Committee for Animal Research at the University of Navarra and the Government of Navarra
Wild animals	The study does not involve wild animals
Reporting on sex	Equal numbers of males and females were used in the study
Field-collected samples	The study does not involve field-collected samples
Ethics oversight	All studies were fulfilled under the Guidelines of the Care and Use of Laboratory Animals and approved by the Ethics Committee for Animal Research at the University of Navarra and the Government of Navarra

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>

ChIP-seq

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication.</i>	GSE261741 subserie belongs to the SuperSeries accession id GSE261742 which is a private (access restricted) data for now. If you'd like to access it from GEO please use the private token: gvilsicsfvstfe
Files in database submission	GSM8150687 Unstimulated_H2Az-Ac_Rep2 GSM8150688 TGFb_H2Az-Ac_Rep2 GSM8150689 Unstimulated_H2Az-Ac_Rep1 GSM8150690 TGFb_H2Az-Ac_Rep1
Genome browser session (e.g. UCSC)	<i>Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.</i>

Methodology

Replicates	Every ChIP-seq analysis was performed with two replicate independent ChIP-seq experiments
Sequencing depth	ChIP-seq libraries were sequenced to 50 million reads per sample (paired-end 50 bp) in a NextSeq 2000 system
Antibodies	Anti-H2Az-Ac Diagenode Lot A1738P Ref C15410202
Peak calling parameters	ChIP-seq reads were aligned to the GRCm38/mm10 reference genome assembly using Bowtie2 version 2.4.2, followed by sorting and indexing of alignment outputs via Samtools version 1.10. Peaks were called using MACS version 2.2.7.1 with the parameters --broad flag suitable for histone marks and a broad-cutoff of 0.1.
Data quality	We evaluated the sequence quality of the data with FastQC (v0.11.9). Adapters and bases below a Q20 quality threshold were trimmed using Cutadapt (v3.4), ensuring high-quality read inputs for alignment. Consensus peak regions were derived by merging overlapping peaks across biological replicates with HOMER's mergePeaks function. Peaks at FDR 5% and above 5-fold enrichment: 4660 (pads< 0.05 & lgFC 0.75)
Software	FastQC (v0.11.9) Cutadapt (v3.4) Bowtie2 (v2.4.2) MACS2 (v2.2.7.1) DESeq2 (v1.30.0) HOMER (v4.10) Custom code can be found here: https://github.com/dalameda/Normal_TGfb_H2azAc

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cardiac fibroblasts from ex vivo cultures were harvested by trypsinization, centrifugated at 400 g for 5 min and resuspended into 100 µl of sorting buffer (PBS pH 7.6, 5 mM EDTA, 50 mM Hepes, and 3% FBS) before staining with the respective antibodies against specific surface markers for 30 min at 4°C in the dark, following antibody datasheet and manufacturer instructions. Non-stained cardiac fibroblasts were used as negative control and to set the gating strategy. Immunolabelled cells were washed twice with 2 ml of sorting buffer and resuspended in 300 µl of sorting buffer to be analyzed by flow cytometry. For α-SMA intracellular flow cytometry analyses, Cytotfix/Cytoperm Fixation/Permeabilization kit (BD Biosciences) was used, following manufacturer instructions.
Instrument	Flow cytometry was performed on a FACSCANTO (BD Bioscience) Flow Cytometer, whereas fluorescence-activated cell sorting (FACS) was performed using a FACS Aria Flow Cytometer (BD Biosciences) or a MoFloAstrios Flow Cytometer (Beckman Coulter).
Software	FlowJo (v10.4)
Cell population abundance	The purity of the post sorting fractions was further characterized with single-cell RNA-seq.
Gating strategy	Standard, strict forward scatter width versus area criteria were used to discriminate debris and gate only singleton cells. Viable cells were identified by staining with a viability marker (7-AAD, SYTOX Blue or Zombie NIR). BFP+ cells (containing CRISPR guides) were gated from Cas9 (GFP+) and Non-Cas9 (GFP-) populations. For bulk CRISPR screens, the readout PDGFRa marker was used to discriminate quiescent/resting (15% PDGFRa-high population) from activated/fibrotic (15% PDGFRa-low population) fibroblasts. Non-stained cardiac fibroblasts were used as negative controls and to set the gating strategies.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.