Targeting Immune-Fibroblast Crosstalk in Myocardial Infarction and Cardiac Fibrosis

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Targeting Immune-Fibroblast Crosstalk in Myocardial Infarction and Cardiac Fibrosis

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Abstract

Inflammation and tissue fibrosis co-exist and are causally linked to organ dysfunction. However, the molecular mechanisms driving immune-fibroblast crosstalk in human cardiac disease remains unexplored and there are currently no therapeutics to target fibrosis. Here, we performed multi-omic single-cell gene expression, epitope mapping, and chromatin accessibility profiling in 38 donors, acutely infarcted, and chronically failing human hearts. We identified a disease-associated fibroblast trajectory marked by cell surface expression of fibroblast activator protein (FAP), which diverged into distinct myofibroblasts and pro-fibrotic fibroblast populations, the latter resembling matrifibrocytes. Pro-fibrotic fibroblasts were transcriptionally similar to cancer associated fibroblasts and expressed high levels of collagens and periostin (*POSTN*), thymocyte differentiation antigen 1 (THY-1), and endothelin receptor A (*EDNRA*) predicted to be driven by a *RUNX1* gene regulatory network. We assessed the applicability of experimental systems to model tissue fibrosis and demonstrated that 3 different *in vivo* mouse models of cardiac injury were superior compared to cultured human heart and dermal fibroblasts in recapitulating the human disease phenotype. Ligand-receptor analysis and spatial transcriptomics predicted that interactions between C-C chemokine receptor type 2 (CCR2) macrophages and fibroblasts mediated by interleukin 1 beta (IL-1β) signaling drove the emergence of pro-fibrotic fibroblasts within spatially defined niches. This concept was validated through *in silico* transcription factor perturbation and *in vivo* inhibition of IL-1β signaling in fibroblasts where we observed reduced pro-fibrotic fibroblasts, preferential differentiation of fibroblasts towards myofibroblasts, and reduced cardiac fibrosis. Herein, we show a subset of macrophages signal to fibroblasts via IL-1β and rewire their gene regulatory network and differentiation trajectory towards a pro-fibrotic fibroblast phenotype. These findings highlight the broader therapeutic potential of targeting inflammation to treat tissue fibrosis and restore organ function.

Keywords: heart failure, fibroblast activator protein, macrophages, C-C chemokine receptor 2, interleukin 1 beta, fibrosis.

Introduction

Tissue fibrosis is an important determinant of organ dysfunction with tremendous clinical impact. Today, there are no therapeutics to target fibrosis. Cardiac fibrosis contributes to life-threatening arrhythmias, heart failure progression, and mortality. While present in nearly all forms of heart disease, cardiac fibrosis is prominent in patients who suffer a myocardial infarction (MI) and those with both ischemic and non-ischemic cardiomyopathies, where fibrosis is associated with loss of viable myocardium[1,2]. Despite advancements in clinical management, MI and chronic cardiomyopathies are among the leading causes of heart failure, associated morbidity, and death worldwide[3,4] and continue to impose staggering financial burden on healthcare systems[3,5–8].

Strategies to therapeutically target cardiac fibrosis have remained elusive. Recent advances in CAR T-cell technology have provided a much needed breakthrough and indicated that selective removal of cardiac fibroblasts that express fibroblast activator protein (FAP) is sufficient to ameliorate fibrosis without impacting the structural integrity of the heart in pre-clinical mouse models[9,10]. While these studies suggested that targeted fibroblast subset depletion may be therapeutically achievable and challenged the dogma that cardiac fibrosis is irreversible, many questions remain to be answered. Numerous studies have elegantly characterized fibroblast subtypes in mouse models of cardiac injury[11–13]. However, the precise fibroblast populations that are responsible for fibrosis in the human heart and mechanisms that orchestrate their emergence and maturation are unknown. Furthermore, it is not clear which of the many available pre-clinical animal and *in vitro* experimental models [14–19] best recapitulate human cardiac fibrosis.

The advent of high-throughput sequencing technologies has enabled high resolution profiling of healthy and diseased tissues to discover novel cell types[20,21]. Numerous single-nucleus (snRNA-seq) studies have studied the healthy and failing human heart[22–26]. However, these studies are performed on frozen tissue and only include transcriptomic information which restrains high-resolution cell phenotyping. Furthermore, the reliance on only nuclear RNA precludes cellular transcriptomic, epigenetic, and proteomic profiling of diseased states. These studies lack the granularity to detect the precise immune and fibroblast populations which emerge after a myocardial infarction and drive cardiac fibrosis. Given the challenges associated with collecting fresh cardiac left ventricle tissue, there is no large-scale study which performs single cell or epitope-based sequencing in human hearts.

126 We performed Cellular Indexing of Transcriptomes and Epitomes by sequencing (CITE-seq)[27] on

127 freshly explanted human hearts and Multiomic (paired single nucleus RNA + assay for

128 transposable accessible chromatin, ATAC) sequencing from non-diseased donors, acutely

129 infarcted, chronic ischemic, and non-ischemic cardiomyopathy patients. We utilized a panel of

130 279 antibodies to phenotype cell states that emerge in disease to comprehensively define human

131 cardiac immune-fibroblast diversity and to uncover cell surface protein targets for diagnostic and

132 therapeutic applications. These studies included fresh cells from acutely infarcted hearts, which

133 represents an important milestone towards characterizing the cellular diversity at early stages of

134 heart failure pathogenesis. Moreover, we evaluated mouse cardiac injury models and human

135 fibroblast cell culture systems to assess the suitability of experimental models to recapitulate

136 human cardiac fibroblast phenotypes. Using cell interaction analysis and spatial transcriptomics

137 in human MI tissue, we identified immune-fibroblast neighborhoods within areas of fibrosis that

138 were predicted to be driven by IL-1β signaling from macrophages to activated fibroblasts via a

139 *RUNX1* gene regulatory network. Finally, we demonstrated that IL-1 signaling to fibroblasts is

140 essential for cardiac fibrosis in mice and elucidated that IL-1 signaling coordinates the emergence

141 and maturation of a pro-fibrotic fibroblast trajectory marked by FAP and THY1 expression that

142 resemble matrifibrocytes. These findings establish a causal role for inflammation in fibroblast

143 lineage specification and tissue fibrosis.

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Results

Multi-omics defines the cellular landscape of human myocardial infarction and heart failure.

To characterize the transcriptional and surface proteomic landscape of human MI and heart failure, we performed CITE-seq in human left ventricle (LV) specimens obtained from 6 healthy donors, 4 acute MI patients (<3 months post-MI), 6 ischemic cardiomyopathy (ICM, >3 months post-MI) patients, and 6 non-ischemic cardiomyopathy (NICM, idiopathic dilated cardiomyopathy) patients (**Fig. 1A**). Donor hearts were obtained from brain dead individuals with no known cardiac disease and normal LV function. Acute MI, ICM, and NICM specimens were obtained at the time of left ventricular assist device (LVAD) implantation or heart transplantation. We included samples from males and females (**Table 1**). Hematoxylin and eosin (H&E) staining showed marked inflammation in acute MI specimens and robust fibrosis in ICM and NICM specimens (**Fig. 1B**).

CITE-seq using 279 surface antibodies was performed on transmural myocardial specimens obtained from the LV anterior wall (**Fig. 1C, Supplementary Fig. 1A**). After pre-processing and application of quality control filters, we obtained high-quality transcriptomic and proteomic reads in 143,804 cells (**Supplementary Fig. 1B-C**) and performed data integration and weighted nearest neighbor (WNN) clustering using RNA and protein expression. Differential gene expression (DGE) was subsequently used to identify 11 distinct cell types (**Fig. 1D, Supplementary Fig. 1D-F**) with canonical gene (**Fig. 1E, Supplementary Fig. 2A**) and protein (**Fig. 1F, Supplementary Fig. 2B**) signatures.

We aobserved marked differences in cell type composition across conditions (**Fig. 1G, Supplementary Fig. 1F**). Increased proportions of myeloid and T-cells and reduced endothelial cell abundance was observed following acute MI with the greatest expansion in myeloid cells occurring within the first week. Accumulation of fibroblasts was seen across pathological conditions beginning 1 week after acute MI. To assess cell specific transcriptional changes in heart failure (HF), we performed pseudobulk DGE analysis at the patient level and observed strong differences between donor and HF (acute MI, ICM, NICM) conditions within fibroblasts, endothelial cells, smooth muscle cells (SMCs) and pericytes, and myeloid cells (**Fig. 1H**).

To dissect the epigenetic landscape of MI and HF, we performed Multiome (paired single nucleus RNA and ATAC) sequencing. After pre-processing and quality control, we used canonical RNA

194   markers to annotate nuclei (**Fig. 1I**). Using RNA derived annotations, we called peaks and found

195   feature marker peaks for the major cell types (**Fig. 1J).** Correspondence between RNA and

196   ATAC-seq modalities was visulaized by constructing a heatmap of the peak-to-gene links split by

197   the two modalities (**Fig. 1K**).

198

199   ***Expansion of pathologic fibroblast subsets in acute MI and chronic heart failure***

200   Unbiased clustering, harmony integration to correct for batch effects, and subsequent DGE

201   identified 13 distinct fibroblast cell states (**Fig. 2A**) marked by classical fibroblast markers (F1,

202   ground state), *ACTA2/TAGLN* (F2, myofibroblasts), *CCL2/THBS1* (F3), *APOE/AGT* (F4),

203   *DLK1/GPX3* (F5), *PTGDS/GPC3* (F6), *APOD/IGFBP5* (F7), *GDF15/ATF5* (F8), *FAP/POSTN*

204   (F9), *ISG15/MX1* (F10), *PLA2G2A* (F11), *PCOLCE2/MFAP5* (F12), and *PRG4/CXCL14* (F13)

205   (**Fig. 2B**). Construction of gene set and surface protein signatures for each fibroblast cell state

206   confirmed clear delineation between fibroblast states (**Supplementary Fig. 3A-D**). Gene

207   Ontology (GO) analysis based on differentially expressed marker genes identified unique pathway

208   enrichment across states (**Supplementary Fig. 4A**). Spatial transcriptomics data from human

209   heart indicated that fibroblast populations were located within defined perivascular and interstitial

210   spatial niches (**Supplementary Fig. 4B – D**)[28].

211

212   Fibroblast cell states differed across donor, acute MI, and chronic heart failure conditions. Donor

213   hearts contained the greatest number of F4 (*APOE/AGT*) and F5 (*DLK1/GPX3*) fibroblasts. We

214   used an existing Visium spatial transcriptomics dataset (Methods) from healthy LV tissue and

215   found strongest enrichment of F4, F5, and F6 fibroblast gene signatures – F9 and F13 showed

216   almost no signal while F2 was enriched in the perivascular space (**Supplementary Fig. 4B, C**).

217   Acute MI and chronic heart failure patients contained the greatest proportion of F9 (*FAP/POSTN*)

218   and F2 (myofibroblasts) (**Fig. 2C**). F9 fibroblasts were strongly enriched for *FAP* at the RNA and

219   cell surface protein levels co-localizing with *POSTN* (**Fig. 2D**). Differential expression analysis

220   from the cell surface protein data identified additional markers for the F2 (F11R) and F9 (THY1,

221   CD276, LAMP1, CD63) states (**Fig. 2D, Supplementary Fig. 3C-D**). In addition to differing

222   transcriptional signatures, pathway enrichment, and protein markers, F2 and F9 fibroblasts

223   resided within distinct regions of the myocardium (**Supplementary Fig. 4D**). F2 fibroblasts were

224   localized in perivascular regions, while F9 fibroblasts were associated with fibrosis and collagen

225   expression. Collectively, these data highlight that myofibroblasts and FAP/POSTN fibroblasts

226   represent distinct disease associated fibroblast populations. Given the fibrosis associated

227 transcriptional signature of F9 fibroblasts and their localization within the infarct and fibrotic
228 regions of the myocardium, we chose to focus our analysis on this population.

229

230 Quantification of the proportion of F9 fibroblasts in individual patients demonstrated consistent
231 increases in acute MI, ICM, and NICM compared to donors (**Supplementary Fig. 4E**).
232 Temporally, the *FAP/POSTN* transcriptional signature peaked early after MI, declined over a 3-
233 month period, and remained chronically elevated thereafter (**Fig. 2E**). To validate our findings, we
234 utilized spatial transcriptomics data from human MI at days 2, 11, 62, and 153 post-MI. Within
235 this dataset, *FAP* expression was most prominent acutely after MI and diminished at later time
236 points **(Fig. 2E**). Immunostaining for FAP in LV sections showed no expression in donors, robust
237 expression following acute MI, and moderate expression in ICM and NICM (**Fig. 2F**). We next
238 performed pseudobulk DGE analysis between donor and diseased fibroblasts and created gene
239 set signatures for donor and diseased fibroblasts using genes with p-adjusted <0.05,
240 baseMeanExpression >500, and log2FC >0.58. The diseased signature localized specifically
241 within F9 fibroblasts, while the donor signature localized within F5, F7, and F11 fibroblasts
242 (**Supplementary Fig. 4F**). KEGG pathway analysis using F9 marker genes (p-adjusted <0.05
243 and log2FC >0.58) revealed enrichment for ECM remodeling, cell-cell adhesion, protein digestion,
244 PI3K-Akt signaling, and AGE-RAGE pathways (**Fig. 2G**).

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246 To assess how cardiac fibroblasts that emerge in acute MI and chronic heart failure compare to
247 fibroblasts from other disease contexts, we utilized a published single-cell dataset that examined
248 diseased fibroblasts in 3 human tissues: pancreas (cancer), intestine (ulcerative colitis), and lung
249 (COVID-19, non-small cell lung cancer, idiopathic pulmonary fibrosis)[29]. We re-normalized the
250 published data with SCTransform, integrated it with our human cardiac fibroblast dataset, and
251 performed clustering using RNA information (**Supplementary Fig. 5A-C**). To assess similarity
252 among integrated fibroblast clusters, we calculated Pearson correlation coefficients between
253 pairwise cell states and found that most cardiac fibroblasts differed from fibroblasts found within
254 other tissues and diseases. F9 fibroblasts were most similar to cancer fibroblasts and F4
255 fibroblasts were similar to IPF fibroblasts (**Fig. 2H**).

256

257 To map fibroblast trajectories in acute MI and heart failure, we undertook two approaches. First,
258 we used scVelo with dynamical modeling to infer directionality in fibroblast cell state
259 differentiation. Projection of the velocity vector field in a force directed layout suggested
260 convergence toward the F2, F4, and F9 fibroblast states (**Fig. 2I**). Next, we used Palantir to predict

261 fibroblast differentiation endpoints, which also identified F2, F4, and F9 as possible terminal states

262 with low entropy values across a range of simulation parameters (**Fig. 2J**). To glean how

263 fibroblasts might evolve over the time course of disease, we plotted donor and diseased fibroblast

264 signatures (**Supplementary Fig. 4F**) versus pseudotime along each predicted lineage (F2, F4,

265 F9). The donor fibroblast signature was most enriched early in pseudotime, while the diseased

266 signature increased over pseudotime in each lineage. *FAP* expression transiently increases along

267 the F2 and F4 lineages and progressively increases along the F9 lineage. Notably, the markers

268 of each population increase over pseudotime in a lineage specific manner (**Fig. 2K**). To dissect

269 putative regulators of the different trajectories, we identified differentially expressed genes and

270 used the resultant gene list to perform a TF enrichment analysis. This approach identified several

271 transcription factors such as (*FBN1, PPRX,* and *FOSX1*) and the previously characterized

272 *MEOX1*[30] as the top TF driving the F9 lineage (**Fig. 2L**). Gene expression density plots showed

273 that *MEOX1* was co-expressed with *RUNX1*, *EDNRA, POSTN* along the F9 lineage (**Fig. 2M**).

274 Furthermore, *RUNX1* and *MEOX1* increased along the F9 lineage but not the other lineages (**Fig.**

275 **2L**). Using PathwayNet we found that *POSTN*, *RUNX1*, and *EDNRA* are predicted to be

276 connected via a transcriptional regulation network (**Supplementary Figure. 4G**). Consistent with

277 this finding, multiome fibroblast ATAC pseudobulk tracts revealed increased chromatin

278 accessibility at the *EDNRA* locus in HF relative to donor fibroblasts (**Fig. 2N**). To explore genetic

279 associations between F9 fibroblasts and HF, we utilized Mendelian Randomization and found an

280 association between genetically predicted *EDNRA* gene expression levels in fibroblasts and HF

281 (beta = 0.052, se = 0.022, and p-value = 0.017) (**Fig. 2O**).

282

283 ***In vivo mouse cardiac injury systems recapitulate human cardiac fibroblast diversity.***

284 To address whether available experimental systems are suitable to study cardiac fibroblasts

285 including the F9 fibroblast, we examined several mouse cardiac injury models and human

286 fibroblast culture systems. First, we sought to address how well a traditional mouse left anterior

287 descending artery (LAD) ligation model mimics human disease using previously published

288 scRNA-seq data obtained from mouse hearts at day 0, 1, 3, 5, 7, 14, and 28 following MI[14]. Raw

289 data was reprocessed using the same computational pipeline employed in our CITE-seq human

290 data to ensure consistency. We first mapped the mouse MI dataset onto the global human CITE-

291 seq reference and detected strong mapping across all populations (**Supplementary Fig. 6A-B**).

292 Consistent with our human data, we observed increase myeloid infiltration on days 1-7 post-MI

293 (**Supplementary Fig. 6C**) and phenotypic shifts within fibroblast, myeloid, and endothelial cell

294 populations (**Supplementary Fig. 6D-E**). We then mapped mouse fibroblasts onto our human

CITE-seq fibroblasts using label-transfer to impute cell state annotation. Mapping scores were strong across all most cell states except for F5, F6, and F7 fibroblasts, which were poorly represented in the mouse data (**Fig. 3A**, **Supplementary Fig. 7A**). When split by time point, there was marked expansion of F9 fibroblasts that peaked on day 7 post-MI and remained elevated compared to sham on d28 post-MI (**Fig. 3B, Supplementary Fig. 7B**).

Next, we assessed other mouse cardiac fibrosis models. We infused angiotensin II and phenylephrine (AngII/PE) via implanted osmotic minipump and performed scRNA-seq at day 28 (**Supplementary Fig. 8A**). After applying QC filters (**Supplementary Fig. 8B**), data normalization, clustering, and cell type annotation (**Supplementary Fig. 8C-D**), we selected the fibroblasts and performed label transfer onto our human cardiac fibroblast CITE-seq dataset (**Fig. 3C, Supplementary Fig. 8E**). Consistent with mouse MI, we recovered most fibroblast cell states except for F5 and F6. We observed expansion of F9 fibroblasts, which were absent in the sham control cohort (**Fig. 3C-E**). We then examined another published scRNA-seq dataset from hearts that underwent transverse aortic constriction (TAC)[30]. We reprocessed the data and performed label transfer to map mouse TAC fibroblasts onto fibroblasts from our human CITE-seq dataset (**Supplementary Fig. 9A-B**). Consistent with mouse MI and Ang II/PE infusion models, we found strong mapping scores for most fibroblast populations except for F5 and F6. Robust expansion of F9 fibroblasts relative to sham was also seen (**Fig. 3C-E, Supplementary Fig. 9A**). Intriguingly, we observed that JQ1 treatment, which resulted in improved LV systolic function and abrogation of fibrosis, was associated with the loss of F9 fibroblasts. Following JQ1 withdrawal, F9 fibroblasts re-emerge suggesting that this is a reversible effect (**Supplementary Fig. 9B**).

To examine whether commonly used human cultured fibroblast systems model human cardiac fibroblasts, we tested three different fibroblast cell preparations: primary human cardiac fibroblasts (PHCF), primary human dermal fibroblasts (PHDF), and immortalized human cardiac fibroblasts (iHCF). Each fibroblast cell line was treated with vehicle, TGF-β, or IL-1β for 72 hours and scRNA-seq performed. Multiple biological and technical replicates were included, individually hash tagged, and pooled to minimize any potential batch effects (**Fig. 3F**). After applying QC filters and data normalization, the data was integrated with harmony (**Supplementary Fig. 10, 11A-B**). To compare human fibroblast preparations to human cardiac fibroblasts, we performed label transfer to map the normalized data onto our human CITE-seq fibroblast dataset and imputed cell annotations. In general, PHCF and PHDF recovered a broader diversity of fibroblast cell states compared to iHCF. However, all cultured cell lines displayed a limited ability to

329   recapitulate fibroblast populations that reside within the human heart. TGF-β treatment led to a
330   modest expansion in F9 fibroblasts in the PHCF and PHDF models, however these cells displayed
331   suboptimal mapping to the human CITE-seq dataset (**Fig. 3G**). To directly compare the ability of
332   mouse cardiac fibroblasts and human cell culture preparations to recapitulate the diversity of
333   human cardiac fibroblasts, we constructed density histograms of the human CITE-seq mapping
334   scores. Fibroblasts recovered from the mouse cardiac injury models showed superior mapping
335   scores relative to the human cell culture preparations (**Fig. 3H, Supplementary Fig. 11C-D**).
336   These data indicate that mouse cardiac injury models are a reasonable experimental approach
337   to explore mechanisms involved in cardiac fibrosis including pro-fibrotic F9 fibroblasts.
338
339   ***Inflammatory signaling within spatially defined cell neighborhoods drives cardiac fibrosis.***
340   To identify cell-cell signaling events that might participate in cardiac fibrosis, we used NicheNet
341   to probe ligand-receptor signaling events enriched in diseased conditions relative to donors. We
342   focused on signals received by fibroblasts from any cell type and identified TGF-β and IL-1β as
343   the strongest predicted signals (**Fig. 4A**). We chose to focus on IL-1β signaling given the
344   availability of FDA approved therapeutics and potential clinical impact. *IL-1β* was specifically
345   expressed in myeloid cells (**Fig. 4B**). Within the myeloid cell compartment, *IL-1β* expression was
346   observed in *CCR2*$^{+}$ monocytes, macrophages, and classic dendritic cells (DC2s) (**Fig. 4C,**
347   **Supplementary Fig. 12A-F**). As an orthogonal approach, we plotted *IL-1β* expression in spatial
348   transcriptomics data obtained from a patient following acute MI (day 2) and observed strong
349   expression in the ischemic zone overlapping with the *CCR2*$^{+}$ monocytes, macrophages, and
350   cDC2s (**Fig. 4C, Supplementary Fig. 12G, H**). IL-1β expression was conserved in mouse CCR2^{+}
351   monocytes and macrophages in the AngII/PE infusion model (**Fig. 4D**). We then leveraged the
352   multiome ATAC-seq information and found that ETS1 and ERG motifs were enriched in HF
353   relative to donor fibroblasts, consistent with the possibility that macrophage-derived IL-1β may
354   signal to fibroblasts in HF (**Fig. 4E**). To further infer macrophage-fibroblast crosstalk at the patient
355   level we regressed macrophage *IL-1β* expression with fibroblast *RUNX1* and *POSTN* expression
356   and observed a strong correlation (**Fig. 4F**).
357
358   To determine whether myeloid cells are spatially localized within regions that contain F9
359   fibroblasts in the diseased human heart, we carried out the following analysis. We performed
360   snRNA-seq in 7 acute MI and ICM LV specimens and integrated the data with our previously
361   published heart failure atlas (consisting of donor and NICM hearts) to generate a comprehensive

single nuclei map of the healthy, injured, and failing human heart than can be used to annotate the spatial transcriptomic data (**Supplementary Fig. 13A**). We identified 13 distinct major cell types as using DGE testing. We then selected representative donor, acute MI, and ICM LV spatial transcriptomic datasets for further analysis. Consistent with our prior analyses, *FAP* expression was increased early after MI within the infarct area and remained elevated in ICM (**Supplementary Fig. 13B**). To localize cell types within space, we first plotted gene signatures for major cell populations and found consistent overlap with anatomic structures (cardiomyocytes, blood vessels, infarct) identified from the H&E images (**Supplementary Fig. 13C**). To capture cell types which co-localize with fibroblasts in acute MI, we used SPOTlight to deconvolute each spot into its relative cell composition using our reference. After deconvolution, we calculated cell-cell correlations and found fibroblasts most strongly co-localize with myeloid cells and T-cells forming an immune-fibroblast niche. SMCs/pericytes and endothelial cells co-localize in distinct perivascular regions (**Fig. 4G**). We applied this approach to multiple donors, acute MI, and ICM patient samples and identified similar niches (**Supplementary Fig. 14-15**). We then plotted gene signatures for fibroblast states that expanded in disease (F2, F9) and found that F9 fibroblasts co-localized with immune-fibroblast niche (*CD68/FAP/CD4*), while F2 fibroblasts were found within the perivascular niche (**Fig. 4H**). Given the strong correlation between macrophage IL-1β and fibroblast *POSTN/RUNX1* expression at the patient level, we created a co-localization plot of *POSTN/RUNX1* in AMI and found enriched NF-kB pathway signaling in corresponding regions (**Fig. 4I**).

To examine transcriptional signatures represented in defined niches, we clustered spatial spots within a UMAP embedding and identified 4 spatial clusters (**Fig. 4J**). Cluster 0 was strongly enriched for fibroblast and immune cell markers, clusters 2 and 3 expressed cardiomyocyte markers, and cluster 3 was enriched with SMC and pericyte markers (**Fig. 4K**). Cluster 0 co-localized with regions in space enriched for the F9 gene set score derived from the human CITE-seq dataset (**Fig. 4L**). Co-localization of *CD68/FAP* and *CD68/CD4* was also present in the immune-fibroblast niche. Myofibroblasts (F2) were not present within this niche and instead localized to perivascular regions (**Fig. 4M**). We then used PROGENy to understand what pathways are enriched in the spatial clusters and found enrichment for NFkB signaling (downstream mediator of IL-1β) in the immune-fibroblast niche. TGF-β signaling was most represented in the perivascular niche (**Fig. 4N**). To validate our findings in a broader array of patients, we integrated spatial transcriptomic data across 28 samples in UMAP space from donor, acute MI, and ICM patients. We found high *FAP* and *POSTN* expression in neighborhoods

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396 containing expression of macrophage markers (**Supplementary Fig. 16A-C**). These regions
397 contained the F9 fibroblast signature and were highest in the infarct zone (IZ) followed by the
398 fibrotic zone (FZ) and lowest in donors and the remote zone (RZ) (**Supplementary Fig. 16D**).
399

400 To determine whether IL-1b signaling to fibroblasts is necessary for cardiac fibrosis, we generated
401 mice that lack the IL-1 receptor (IL1R) in fibroblasts (*Il1r$^{flox/flox}$Dermo1-cre* mice[31]). Control and
402 *Il1r$^{flox/flox}$Dermo1-cre* mice either underwent sham surgery or received Ang II/PE eluting osmotic
403 minipumps (**Fig. 4O**). Fibrosis was quantified 4 weeks later by trichrome staining. Ang II/PE
404 infusion resulted in increased interstitial fibrosis in control mice. Consistent with the conclusion
405 that IL-1 signaling to fibroblasts promotes cardiac fibrosis, we observed reduced trichrome
406 staining in AngII/PE treated *Il1r$^{flox/flox}$Dermo1-cre* mice compared to controls (**Fig. 4P-Q**).
407

408 ***T cells expand post-MI and display signatures of activation***
409 Cell composition analysis in the global object showed T cell expansion post-MI and integrated
410 spatial transcriptomic analysis showed co-localization within the immune-fibroblast niche. To
411 further characterize T cell subsets, we leveraged the CITE-seq data and performed WNN
412 clustering (**Supplementary Figure. 17A**). DE analysis showed strong separation cross cell states
413 (**Supplementary Figure. 17B**). All T cells expressed *TCRab* (**Supplementary Figure. 17C**), and
414 we see strong separation of CD4 and CD8 T cells at the protein level (**Supplementary Figure.
415 17C**). Markers of T cell activation such as *CCL3, CCL4, IFNG,* and *TNF* were most enriched in
416 AMI (**Supplementary Figure. 17D**). T cell exhaustion markers such as *CTLA4, PDCD1,
417 HAVCR2*, and *LAG3* were most enriched in the chronic HF patients (**Supplementary Figure.
418 17D**). T cell maturation markers such as *CXCR4* and *CD69* were also enriched in ICM and NICM
419 patients relative to AMI (**Supplementary Figure. 17D**). *TBX21* (necessary for Th1 polarization)
420 was highest in donors while *GATA3* (necessary for Th2 polarization) was highest in AMI and ICM
421 samples (**Supplementary Figure. 17D**). We also plotted the lymphocyte activation signature
422 (*CCL3, CCL4, IFNG,* and *TNF*) in space in an AMI sample and see expression within the areas
423 positive for *CD68* and *FAP/POSTN* (**Supplementary Figure. 17E**).
424

425 ***IL-1β inhibition impedes FAP fibroblast fate specification and maturation***
426 To dissect the therapeutic impact of IL-1β inhibition on fibroblast cell state transitions, we
427 implanted mice with Ang II/PE osmotic minipumps treated animals with an IL-1β neutralizing
428 antibody (anti-IL-1β mAb) or isotype control every 3 days (**Fig. 5A**). Sham mice served as a
429 negative control. To characterize *FAP* fibroblast fate specification we developed a flow cytometry

430   assay to detect FAP cell surface expression on mouse cardiac fibroblasts (**Supplementary**

431   **Figure 18**). Mice were harvested after 7 days on AngII/PE treatment and hearts enzymatically

432   digested for flow cytometry. Quantification of flow cytometry data revealed an increase in the

433   proportion of FAP$^+$ fibroblasts in AngII/PE treated animals that received isotype antibodies

434   compared to corresponding controls. Importantly, we observed a reduction in FAP$^+$ fibroblasts in

435   AngII/PE treated mice that received anti-IL-1β mAb compared to AngII/PE treated mice that

436   received isotype antibody (**Fig. 5B**) demonstrating that endogenous IL-1β signaling regulates

437   FAP$^+$ fibroblast specification.

438

439   To delineate whether IL-1β signaling contributes to FAP$^+$ fibroblast maturation, we sorted FAP$^+$

440   and FAP$^-$ fibroblasts from the hearts of mice that were treated with AngII/PE for 7 days and

441   received either isotype or anti-IL-1β mAb. Sorted fibroblasts were then subjected to scRNA-seq

442   (**Fig. 5A**). Following application of QC filters and data normalization (**Supplementary Fig. 19A-**

443   **B)**, cells were clustered into 10 transcriptionally distinct fibroblast cell states (**Fig. 5C,**

444   **Supplementary Fig. 19B**)*.* Density plot of *Fap/Postn* co-expression showed strong enrichment

445   in clusters F1 and F6 (**Fig. 5D**). GO Biological Pathway analysis identified enrichment for fibrosis

446   associated pathways including ECM organization, integrin signaling, and collagen fibrin

447   organization in mouse FAP$^+$ fibroblast states (**Supplementary Fig. 19C**).

448

449   To unravel phenotypic shifts among fibroblasts following anti-IL-1β mAb treatment, we

450   constructed Gaussian kernel density embedding plots. As anticipated, significantly different cell

451   distributions were found in the FAP$^+$ and FAP$^-$ groups with enrichment of cells in the F1 and F6

452   clusters in the FAP$^+$ fibroblast group. Mice that received anti-IL-1β mAb displayed reductions in

453   FAP$^+$ fibroblasts that localized to cluster F1 compared to corresponding isotype control (**Fig. 5E**).

454   We detected differences in gene expression in both the FAP$^+$ and FAP$^-$ fibroblasts following anti-

455   IL-1β mAb treatment (**Supplementary Fig. 19D**). Within the FAP$^+$ population, isotype treated

456   animals displayed increased expression of *Fap, Thbs4, Isg15, Col1a1, and Cola3.* Conversely,

457   IL-1β mAb treatment increased the expression of myofibroblast markers including (*Tagln*, *Acta2*,

458   and *Svep1*) in FAP$^+$ fibroblasts (**Fig. 5F**). These data suggest that IL-1β mAb treatment may

459   impact the differentiation trajectory of FAP$^+$ fibroblasts. GO biological pathway analysis further

460   suggested that anti-IL-1β mAb treatment impacted genes involved in ECM organization, T cell

461   migration, cytokine signaling, and chemokine production (**Supplementary Fig. 20D**). FAP$^-$

462   fibroblasts showed reduced expression of *ApoE*, *Gdf15*, *Ptgds*, *Ccl2*, and *Prg4* following IL-1β

463   mAb treatment (**Fig. 5F**). To unravel how *Runx1* impacts fibroblast lineage specification, we used

464   CellOracle to construct cell state specific gene regulatory networks and then performed a *in silico*

465   *Runx1* perturbation and the simulated cell identity shifts showed movement away from the F1

466   state (*Postn, Fap*) (**Fig. 5G**).

467

468   To examine the differentiation trajectory of FAP$^+$ fibroblasts, we sub-clustered human FAP$^+$

469   fibroblasts (F9 cell state) from our human CITE-seq dataset at finer resolution. DEG analysis

470   showed distinct cell states within the FAP$^+$ populations (**Fig. 5H-I**). All cells expressing *FAP* and

471   only a subset expressing *POSTN* or *ACTA2* (**Fig. 5J**). To predict differentiated cell states, we

472   used scVelo and observed convergence within the *POSTN* fibroblast (HF2) and *ACTA2*

473   myofibroblast (HF5) clusters (**Fig. 5H**). Next, we mapped the mouse FAP$^+$ fibroblasts onto the

474   human FAP$^+$ fibroblast FDL trajectory and plotted gene signatures enriched in following either

475   isotype or anti-IL-1β mAb treatment. We observed a strong separation triggered by anti-IL-1β mAb

476   treatment (**Fig. 5K**). To interpret the effect of anti-IL-1β mAb treatment within the context of the

477   human FAP$^+$ fibroblast differentiation, we created a heatmap of isotype enriched, anti-IL-1b mAb

478   enriched, and human *POSTN* (HF2) signatures grouped by human FAP$^+$ fibroblast state. We

479   found that the isotype signature corresponds with the human HF2 (*POSTN*) signature, while the

480   anti-IL-1b mAb signature is enriched for alternative human fibroblasts states including

481   myofibroblasts (HF1, HF4, HF5) (**Fig. 5L**). These data further suggest that IL-1b mAb treatment

482   impedes the maturation of FAP$^+$ fibroblasts into *POSTN* fibroblasts.

483

484   To experimentally validate these findings, we performed immunostaining for POSTN in sham

485   hearts and hearts treated with AngII/PE for 7 days. Compared to sham controls, AngII/PE infusion

486   increased the abundance of POSTN$^+$ cells. Consistent with our scRNA-seq data, we observed a

487   reduction in POSTN$^+$ cells in AngII/PE treated hearts from mice that received anti-IL-1β mAb

488   compared to isotype control (**Fig. 5M**). Similarly, quantitative PCR revealed upregulation of *Postn*

489   expression in myocardial tissue obtained from AngII/PE treated mice compared to sham controls.

490   Treatment with anti-IL-1β mAb reduced *Postn* expression induced by AngII/PE infusion within the

491   heart relative to isotype control (**Fig. 5N**).

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Discussion

The advent of single cell multi-omic technologies has afforded the opportunity for the high-resolution detection of novel cell states from healthy and diseased human tissue including the heart[22,23,25,26,28,32]. These human cell atlases have provided new insights into the cellular landscape of HF and identified unexpected cell diversity within the stromal compartment. However, the functional roles of these novel cell states and how they might interact during disease pathogenesis remains to be defined. Among stromal cell types, fibroblasts are of particular interest given their role in tissue fibrosis, arrhythmias, and heart failure[19,33,34]. Little is known regarding the mechanisms and signaling events that orchestrate the specification of pathogenic fibroblasts in the context of human MI and HF. Herein, we utilize CITE-seq and spatial transcriptomics to dissect fibroblast cell states that emerge following acute MI and in chronic HF and identify a fibroblast trajectory marked by *FAP* and *POSTN* expression that are governed by inflammatory cytokines and contribute to cardiac fibrosis.

Unbiased clustering revealed an expansion of fibroblasts expressing *FAP* that peaked early after MI and remained elevated in chronic HF. We used IF and spatial transcriptomics in acute MI hearts[28] to validate expression of FAP at the RNA and protein levels. Recent studies have utilized chimeric antigen receptor (CAR) T cells to target FAP[+] fibroblasts in a mouse model of cardiac injury and shown improved cardiac function and diminished fibrosis[9,10]. This points to a potential pathogenic role of FAP[+] fibroblasts in adverse LV remodeling. Transcriptionally, cardiac FAP[+] fibroblasts resemble cancer associated fibroblasts, which also express *FAP*[3529]. Computational lineage analysis predicted that *FAP* expression marks a fibroblast trajectory that transitions into populations including POSTN[+] matrifibrocytes[11] and myofibroblasts. Unraveling the signals that drive the differentiation and maturation of FAP+ fibroblasts may identify new therapeutic targets to limit fibrosis in the injured and failing heart.

A fundamental question that continues to challenge the field is selection of appropriate model systems to investigate human cardiac fibroblasts. To address this issue, we leveraged publicly available and new scRNA-seq datasets to examine the suitability of mouse cardiac injury models (MI, Ang II/PE infusion, TAC) and human cultured fibroblast platforms. Surprisingly, we found that each of the mouse models contained many of the fibroblast populations found in the human heart and recapitulated expansion of *Fap* and *Postn* expressing fibroblasts following injury. In contrast, primary human cardiac fibroblasts, primary human dermal fibroblasts, and immortalized human cardiac fibroblasts were relatively poor models of human cardiac fibroblast diversity under resting

531 or stimulated (TGF-β[23,36,37], IL-1β[38]) conditions. These findings emphasize the relevance of *in vivo*

532 mouse models in studying cardiac fibrosis and for therapeutic discovery.

533

534 Little is understood regarding the signaling mechanisms that underlie the specification and

535 differentiation of pathological fibroblasts in the heart. Using cell-cell interaction analysis, we

536 identified enriched for IL-1β and TGF-β signaling in fibroblasts in HF relative to donor control

537 conditions. While numerous studies have implicated a role for TGF-β signaling in maladaptive

538 cardiac remodeling and scar formation in HF[23,36], the potential for inflammatory signals to drive

539 fibroblast activation and cardiac fibrosis is less explored. In support of this concept, a large clinical

540 trial targeting the IL-1β pathway with the neutralizing antibody, canakinumab, led to a reduction

541 in ischemic cardiac events[39]. We show that IL-1β is selectively expressed by CCR2^{+} monocytes

542 and macrophages in the human heart consistent with our prior mouse studies[40,41]. Using spot

543 deconvolution and Pearson correlation analysis in spatial transcriptomic data from human hearts,

544 we identified spatial niches of macrophages and T-cells that strongly co-localize with fibroblasts

545 in acute MI. This niche robustly expressed *FAP*, extracellular matrix components associated with

546 fibrosis, and displayed an inflammatory signature. We validated the existence of this immune-

547 fibroblast neighborhood in a larger dataset of 28 patients and observed strong macrophage-

548 fibroblast co-localization in pro-inflammatory niches. Together, these findings suggest that FAP[+]

549 fibroblasts reside within a spatially defined cell neighborhood that contains inflammatory

550 macrophages and that these cell types may communicate via IL-β signaling.

551

552 To verify the importance of IL-1 signaling, we generated mice that lack the IL-1 receptor in

553 fibroblasts and demonstrated a specific requirement for IL-1 signaling to fibroblasts in cardiac

554 fibrosis. Next, we sought to explore the mechanistic implications of inhibiting IL-1β signaling as a

555 therapeutic. We found that anti-IL-1 β mAb treatment abrogated the emergence of FAP[+]

556 fibroblasts. scRNA-seq of cardiac fibroblasts in this model uncovered an unexpected effect on the

557 maturation of the *Fap* fibroblast trajectory. We observed that FAP[+] fibroblasts differentiated into

558 POSTN[+] fibroblasts in isotype control hearts. Strikingly, mice treated with the anti-IL-1 β mAb

559 displayed a differentiation trajectory away from POSTN[+] fibroblasts and towards the myofibroblast

560 lineage. These findings were validated by immunostaining and highlight a central role of IL-1β

561 signaling in pathogenic fibroblast fate specification. They also suggest that IL-1β targeted

562 therapies may not interfere with structural stability of the heart as myofibroblasts are preserved in

563 this context.

564

565 Our study is not without limitations. First, we are not powered to assess the effects of
566 demographics (age, sex, ethnicity) on the cell state diversity post-MI. We included patients of
567 diverse ethnicities, sex, and broad age ranges to remain inclusive in the generation of the human
568 dataset. Second, we have a limited cohort size in the AMI group. Given the challenge acquiring
569 fresh acutely infarcted cardiac tissue, we validated our CITE-seq findings in a broad array of
570 patients through multiple assays such as IF and spatial transcriptomics.

571

572 In conclusion, we utilize multi-omic single cell sequencing to characterize immune-fibroblast cross
573 talk in human myocardial infarction and HF. We identify a macrophage subset in humans which
574 signals to fibroblasts via IL-1 β and modulates fibroblast activation, lineage specification via a
575 *RUNX1* orchestrated gene regulatory network, and cardiac fibrosis. We show *in vivo* IL-1 β
576 signaling in fibroblasts drives activation, specification towards PFs, and is causally linked to
577 fibrosis. Collectively, our findings provide the first single cell characterization of immune fibroblast
578 crosstalk in the human heart, particularly in the early stages of a heart attack and highlight a
579 promising role for immunomodulators in targeting cardiac fibrosis. These findings have broad
580 implications for future therapeutic discovery and the emerging field of cardio-immunology.

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713

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Author Contributions

732

733   JA and isolated cells and made cDNA from human CITE-seq samples. TY made all CITE-seq

734   libraries for sequencing. PM, AB, and AK isolated nuclei for snRNA-seq. JA performed Multiome

735 experiments. JA and XL performed all computational analysis. JA, CK, and SH analyzed spatial
736 transcriptomics data. AF and SS performed human *in vitro* experiments. JA performed all *in vivo*
737 mouse surgeries and downstream experiments. JA, SY, VP, and FK performed
738 immunohistochemistry and analyzed and processed images. VP completed all work associated
739 with the Il1r$^{fl/fl}$ Dermo1cre mice. JA made all figures. JA and KL drafted the manuscript. PL and
740 NS conducted the MR analysis. MF contributed to the experimental design, interpretation, and
741 manuscript production. TY, C-ML, SS and BA contributed to the experimental design, data
742 analysis and interpretation as well as manuscript production. KL is responsible for all aspects of
743 this manuscript including experimental design, data analysis, and manuscript production. All
744 authors approved the final version of the manuscript.
745
746 **Competing Interests**
747
748 XL, TY, SS, AF, MF, C-ML and BA are or were employed by Amgen.
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772 **Materials and Methods**

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774 *Ethical Approval for Human Specimens*

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776 The study is compliant with all relevant ethical regulations and has been approved by the
777 Washington University School of Medicine Institutional Review Board (IRB #201104172).
778 Informed consent was obtained from each patient prior to tissue collection by Washington
779 University School of Medicine and no compensation was provided in exchange for subject
780 participation in the study. All demographic and clinical data has been de-identified and provided
781 in Supplementary Table 1. Patients included in this study span diverse race, age, and sex to
782 provide an inclusive trans-ethic study population.

783

784 *Inclusion Criteria*

785

786 Prior to tissue collection, specific inclusion criteria were employed to ensure well controlled study
787 groups. Any patients with HIV or hepatitis and known genetic cardiomyopathies were excluded
788 from this study. Donor hearts: patients with stable ejection fractions, no know history of cardiac
789 disease and a non-cardiac cause of death/transplant. Acute MI hearts: patients who had a
790 myocardial infarction from coronary artery disease within 3 months from the time of transplant.
791 Ischemic cardiomyopathy: patients who had a myocardial infarction from coronary artery disease
792 greater than 3 months from the time of transplant. Non-ischemic cardiomyopathy: patients with
793 HF independent of ischemic heart disease and no known history of a myocardial infarction.

794

795 *Human single cell isolation for CITE-seq*

796

797 Fresh cardiac left ventricular tissue from ex-planted hearts at the time of transplantation, LVAD
798 cores, or donors declared DCD at Washington University School of Medicine and Mid America
799 Transplant Service were harvested, perfused with cardioplegia, and transported on ice. In donor,
800 ICM, and NICM hearts a section of the LV apex was dissected out; in acute MI hearts a section
801 of the infarct region (identified as a white discoloration) was dissected. Tissues were minced using
802 a razor blade on ice and transferred to a 15 mL conical tube containing 3mL DMEM with 170uL

803   Collagenase IV (250U/mL final concentration), 35uL DNAse1 (60U/mL), and 75uL Hyaluronidase

804   (60U/mL) and incubated at 37 °C for 45 min with agitation. After 45 min, the digestion reaction

805   was quenched with 6 mL of HBB buffer (2% FBS and 0.2% BSA in HBSS), filtered through 40 μm

806   filters into a 50 mL conical tube and transferred back into a 15 mL conical tube to obtain tighter

807   pellets. Samples were then spun down at 4 °C, 1200rpm for 5 min and the supernatant was

808   discarded. Pellet resuspended in 1 mL ACK Lysis buffer (Gibco A10492-01) and incubated at

809   room temperature for 5 min followed by the addition of 9 mL DMEM and centrifugation (4 °C, 5

810   min, 1200 rpm). Supernatant was discarded and the pellet was resuspended in 2 mL FACS buffer

811   (2% FBS and 2mM EDTA in calcium/magnesium free PBS) and centrifugation was repeated in

812   above conditions and supernatant aspirated. The TotalSeq A 277 panel (BioLegend) antibody

813   cocktail was resuspended in 100 uL of FACS buffer and 1 uL each of custom oligo-labeled FAP

814   (Amgen) and LRRC15 (Amgen) were added. The combined 102 uL were used to resuspend the

815   pellet with the addition of 1 uL of DRAQ5 (Thermo Fisher Scientific, 564907) and incubated on

816   ice for 30 min. Solution was washed 3x with FACS buffer following same centrifugation as above

817   and then resuspended in 500 uL of FACS buffer and 1 uL DAPI (BD Biosciences, 564907) and

818   filtered into filter-top FACS tubes. First singlets were gated and subsequent DRAQ5+/DAPI-

819   events were collected in 300 uL cell resuspension buffer (0.04% BSA in PBS) – collected cells

820   were centrifuged as above and resuspended in collection buffer to a target concentration of 1,000

821   cells/uL. Cells were counted on a hemocytometer before proceeding with the 10x protocol.

822

823   *CITE-seq Library Preparation*

824

825   Collected cells were processed using the using the single Cell 3' Kit v 3.1 (10x Genomics PN:

826   1000268). 10,000 cells were loaded onto ChipG (PN:1000121) for GEM generation. Reverse

827   transcription, barcoding, and complementary DNA amplification of the RNA and ADT tags were

828   performed as recommended in the 3' v3.1 chromium protocol. Single-cell libraries were prepared

829   using the single Cell 3' Kit v 3.1 following a modified 3' v3.1 assay protocol (User Guide

830   CG000206) to concurrently prepare gene expression and TotalSeq A antibody derived tag (ADT)

831   libraries as recommended by BioLegend. 1 ul of 0.2uM ADT Additive Primer

832   (CCTTGGCACCCGAGAATT*C*C) and 15 ul of cDNA Primers (PN: 2000089) were used to

833   amplify cDNA. ADT libraries were amplified with a final concentration of 0.25 uM SI

834   Primer (AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGC*T*C)   and

835   0.25 uM TrueSeq Small RNA RPI primer (CAAGCAGAAGACGGCATACGAGAT[6nt

836   index]GTGACTGGAGTTCCTTGGCACCCGAGAATTC*C*A) using 15 cycles. Gene expression

837   libraries were indexed using Single Index Kit T Set A (PN: 2000240). Libraries were sequenced

838   on a NovaSeq 6000 S4 flow cell (Illumina).

839

840   *CITE-seq alignment, quality control, and cell type annotation*

841

842   Raw fastq files were aligned to the human GRCh38 reference genome (v) using CellRanger (10x

843   Genomics, v6.1) with the antibody capture tag for the TotalSeqA 277 + two custom oligo-tagged

844   antibodies. First, protein reads were normalized and de-noised for each sample separately using

845   the dsb package[42] in R v4 with the isotype controls tag to remove noisy cell-to-cell variation.

846   Subsequent quality control, normalization, dimensional reduction, and clustering was performed

847   in Seurat v4.0. Following normalization, quality control was performed and cells passing the

848   following criteria were kept for downstream processing: 500 < nFeature_RNA < 600 and 1,000 <

849   nCount_RNA < 25,000 and percentage mitochondrial reads < 15%. Raw RNA counts were

850   normalized and scaled using SCTransform[43] regressing out percent mitochondrial reads and

851   nCount_RNA. Principal component analysis was performed on normalized RNA counts and to

852   determine the number of PCs to use for further processing the following criteria was applied: PCs

853   exhibit cumulative percent > 90% and the percent variation associated with the PCs as < 5%.

854   Weighted nearest neighbor clustering[44] (WNN) was performed with the significant RNA PCs and

855   dsb normalized proteins (minus isotype controls) directly without PCA as previously outlined with

856   the FindMultiModalNeighbors function in Seurat. Subsequently, a uniform manifold approximation

857   (UMAP) embedding was constructed and FindClusters was used to un-biasedly cluster cells using

858   the SLM modularity optimization algorithm. Clustering was performed for a suite of different

859   resolutions (0.1-0.8 at 0.1 intervals) and differential gene expression using the FindAllMarkers

860   function and a Wilcoxon Rank Sum test with a logFC cut-off of 0.25 and a min.pct cut-off of 0.1.

861   Clusters were annotated using canonical gene and protein markers and subsequent violin plots

862   (RNA) and heatmaps (protein) were created to assess clean separation of clusters into distinct

863   cell types. Previously identified canonical marker genes were also plotted on the UMAP object to

864   further validate cluster annotations. Cell composition in donor, acute MI, ICM, and NICM was

865   calculated as percentages as previously described. Cellular density profiles in the WNN UMAP

866   space were calculated in Scanpy per condition using the tl.embedding_density function. Briefly,

867   a Gaussian density kernel estimation is used to estimate cell density in WNN UMAP space in

868   each condition (donor, acute MI, ICM, and NICM) and density values are scaled between 0-1.

869   Similar approach used for all cell density calculations in manuscript.

870

871 *Cell composition and density shift calculations*

872

873 We used R to compute cell type composition across conditions. To assess shifts in cell density

874 within both the global object and within individual cell types, we converted the .rds object to a

875 .h5ad file format and used scanpy.tl.embedding function which employs a Gaussian kernel

876 density estimation of cell number within the UMAP embedding. Density values are scaled from 0-

877 1 within that category.

878

879 *Pseudobulk Differential Gene Expression*

880

881 We use the DESeq2[45] package to perform pseudobulk DE analysis. After QC, cells were

882 subsetted for each cell population annotated from the global reference, raw counts were

883 aggregated to the patient level, data normalized using a regularized log transform, and differential

884 expression analysis between donor and all HF (acute MI, ICM, NICM) via DESeq2. Genes were

885 deemed statistically significant if adjusted p-value < 0.05 and absolute(log2FC) > 0.58. For

886 constructing pseudobulk donor/HF gene set scores, we only used statistically significant genes

887 with a mean log base expression of > 500.

888

889 *Multiome sample preparation*

890

891 Nuclei were isolated according to 10x Genomics protocol (CG00375; Nuclei Isolation Complex

892 Sample for ATAC GEX Sequencing RevB) and flow cytometry for 7-AAD (Sigma; SML1633-1ML)

893 positive nuclei was used for sorting. Protocol CG000338 from 10x Genomics was used for

894 Chromium Next GEM Single Cell Multiome ATAC + Gene Expression. Briefly, following nuclei

895 isolation, permeabilization was performed, followed by transposition, GEM generation and

896 barcoding using ChipJ (10x Genomics; PN1000234), post-GEM clean up, pre-amplification PCR,

897 cDNA amplification, library construction, and sequencing. Gene expression and ATAC libraires

898 were sequenced to a read depth of 50,000 and 25,000 respectively.

899

900 *Multiome data processing*

901

902 Raw fastq files were aligned to the human GRCh38 reference genome (v) using CellRanger ARC

903 (10x Genomics, v6.1). ArchR (https://www.archrproject.com) was used to process the ATAC

904 fragments and Seurat was used to process RNA. Quality control was performed to keep nuclei

905 with the following: TSS enrichment > 2, nFrags > 1000, 200 < nUMI GEX < 50,000, and percent

906 mito < 5%. Post-QC nuclei were used for doublet removal in ArchR (ATAC information) and then

907 using scrublet (RNA information). Raw RNA counts were normalized and scaled using

908 SCTransform[43] regressing out percent mitochondrial reads and nCount_RNA. Principal

909 component analysis, harmony batch integration (by sample), nearest neighbor clustering, and

910 UMAP embedding construction was then performed in Seurat. Cell types were annotated using

911 different expression and knowledge of canonical gene markers. The RNA annotations and

912 normalized gene expression matrix was added to the ArchR project onto the nuclei with the same

913 barcodes. Using these annotations, pseudobulk replicates were constructed in ArchR followed by

914 peak calling with MACS2. Marker peaks were then defined for the major cell types and a

915 differential accessibility analysis was run between donor and HF samples for motif enrichment.

916 chromVAR was used to perform peak2gene linkage using the ground truth gene expression

917 matrix. Pseudobulk tracts were created in ArchR.

918

919 *Fibroblast, myeloid, and T cell states analysis*

920

921 To cluster cell types into distinct cell states, we subsetted the cell type of interest, re-normalized,

922 computed PCAs, harmony integrated, computed UMAPs, and clustered data at a range of

923 resolutions. DE analysis was then used to identify marker genes for each cell state and a dot-plot

924 or heatmap to assess clustering separation. Using the top marker genes we calculated gene set

925 z-scores and plotted them in UMAP space.

926

927 *Perturbed Disease Associated Fibroblasts*

928

929 Perturbated state human fibroblasts were used from (E-MTAB-10324)[29] processed using the

930 same pipeline as used for human CITE-seq fibroblasts. Seurat was then used to integrate the

931 perturbed state human diseased fibroblasts with human HF CITE-seq fibroblasts. To assess

932 cluster similarity, we used two approaches: (1) built a phylogeny tree using the BuildClusterTree

933 function and (2) computed Pearson correlation coefficients and displayed results as a matrix.

934

935 *Pseudotime Trajectory Analysis*

936

937 Palantir: Palantir was used to perform pseudotime analysis on fibroblasts and macrophage

938 subsets. For the cell type of interest, a normalized count matrix was exported and processed in

939   python via Palantir. A force directed layout was then computed for visualization of trajectories and

940   MAGIC was used to impute data for visualization. For fibroblasts, F1 was used as the starting cell

941   state and for myeloid cells monocytes were used as the starting cells and resident macrophages

942   were excluded from the trajectory analysis. No terminal states were specified. Generalized

943   Additive Models within Palantir was then used to compute gene expression trends along the

944   different fibroblast lineages. Pseudotime, entropy, and FDL embedding values were exported and

945   subsequent plotting for visualization was performed in R/Seurat. scVelo: Using CellRanger

946   aligned BAM files as input, a loom file containing the estimation of spliced and unspliced RNA

947   counts were generated by Velocyto CLI (v0.17.17)[46]. The loom file was further merged with

948   annotated fibroblasts object and pre-processed, RNA velocity was estimated using dynamical or

949   stochastic modeling and the fdl embedding stream graph was generated by scvelo

950   (https://github.com/theislab/scvelo).

951

952   *Pathway Analysis and transcription factor enrichment*

953

954   Statistically significant DE genes were used to perform pathway analysis via EnrichR

955   (https://maayanlab.cloud/Enrichr/). Pathway enrichment values were downloaded as .csv files

956   and plots generated in Prism. GO enrichment analysis to compare cell states was done in R using

957   clusterProlifer[47] compareClusters function using only statistically significant marker genes.

958   PROGENy[48] was used for pathway analysis in the spatial transcriptomics dataset.

959

960   *In Vitro Fibroblast single-cell experiments*

961

962   Human ventricular cardiac fibroblasts (NHCF-V, Lonza, CC-2904), adult human dermal fibroblasts

963   (NHDF-Ad, Lonza, CC-2511), and immortalized human cardiac fibroblasts (iHCF, Applied

964   Biological Materials Inc., T0446) were grown in FibroGRO LS medium (Millipore, SCMF002)

965   supplemented with 2% (NHDF-Ad) or 10% (NHCF-V and iHCF) FBS. Cells were maintained in a

966   humidified atmosphere (95% air, 5% CO_2) at 37°C and passaged every 2-3 days. For cytokine

967   treatment, cells were seeded in 6-well plates and allowed to attach for 24hr. Thereafter, cells were

968   equilibrated for 16 hr in low serum medium (0.2% FBS for NHDF-Ad, and 0.5% for NHCF-V and

969   iHCF), and then treated with 10 ng/mL of recombinant human TGF-b (R&D Systems; 240-B-002)

970   or 10 ng/mL of recombinant human IL-1β (R&D Systems; 201-LB-005) for the indicated times.

971   After trypsinization, cell suspensions were labeled with barcoded lipids from the 3' CellPlex Kit

972   Set A (10x Genomics PN:1000261) following manufacturer's instructions (Protocol

973 CG000391). After labeling, technical replicates were pooled and loaded onto a Chromium
974 Controller (10x Genomics) at a concentration to capture 6-10,000 cells. Single-cell libraries were
975 prepared using the Single Cell 3' Kit v3.1 (10x Genomics PN: 1000268) according to
976 manufacturer's instructions (User Guide CG000388). Gene expression and cellplex libraries
977 were indexed with Dual Index Kit TT Set A and NN Set A indexes (PN: 1000215, 3000482)
978 respectively. Libraries were pooled and sequenced on a NovaSeq 6000 (Illumina).
979

980 *Processing of in vitro single cell data*
981

982 Raw fastq files were aligned to the human GRCh38 reference genome using CellRanger (10x
983 Genomics, v6.1). In vitro single-cell data was processed using the same pipeline as human CITE-
984 seq data for iHCF, NHDF, and NHCF datasets.
985

986 *Reference mapping of in vivo and in vitro data*
987

988 In vitro: The same mapping pipeline was used for iHCF, NHDF, and NHCF datasets. Briefly,
989 SCTransform normalized data was used, the FindTransferAnchors function was used with a spca
990 reference reduction and 50 components. The anchors were then passed into the MapQuery
991 function to impure cell annotations and project the in vitro data into the human CITE-seq fibroblast
992 umap embedding. Mapping scores for each imputed cell state were then plotted in the umap
993 space and a heatmap of average mapping score for each cell state was constructed.
994 In vivo: for the day 28 Ang II/PE, TAC, and MI the mouse genes were first converted into
995 the human analogs using biomaRt, the data was subsequently normalized using SCTransform,
996 and normalized data was used to find anchors and then projected onto the human CITE-seq
997 fibroblast UMAP space as described with the in vitro data. Mapping of in vivo and in vitro used
998 the same pipeline and parameters.
999

1000 *Human single nuclei isolation for snRNA-seq*
1001

1002 Cardiac tissues from LVAD cores at the time of LVAD implant (U/RR-pre) and adjacent to core
1003 samples at the time of explant (U/RR-post) from paired patient were flash frozen using liquid
1004 nitrogen. Identical regions from the apex of LV from explanted donors were used. Single nuclei
1005 suspensions were generated as previously described. In brief, flash frozen sections were minced
1006 with a razor blade, transferred to a Dounce Homogenizer containing 1 mL of lysis buffer (10 mM

1007   Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$ and 0.1% NP-40 in nuclease-free water) on ice.

1008   Samples were homogenized using five strokes, an additional 1 mL of lysis buffer added, and

1009   incubated on ice for 15 min. Samples were then filtered with a 40 um filter, which was rinsed with

1010   1 mL of lysis buffer. The mixture was then centrifuged at 500 *g* for 5 min 4 °C, resuspended in 1

1011   mL nuclei wash buffer (2% BSA and 0.2 U ul^{-1} RNase inhibitor (Thermo Fisher, cat. no. AM2694)

1012   in 1X PBS) and, filtered using a 20 um pluristrainer (Pluriselect, cat. No. SKU43-50020-03).

1013   Filtered solution as centrifuged using the above criteria and resuspended in 300 uL Nuclei Wash

1014   Buffer and transferred into a 5 mL tube for flow cytometry. Subsequently, 1 uL DRAQ5 (5 mM

1015   solution; Thermo Fisher, cat. no. 62251) was added, sample gently vortexed and allowed to

1016   incubate for 5 min prior to sorting. DRAQ5$^+$ nuclei were sorted into 300 uL Nuclei Wash Buffer

1017   using a BD FACS Melody (BD Biosciences) with a 100 uM nozzle. Sorted nuclei were then

1018   centrifuged using the above conditions and resuspended in Nuclei Wash Buffer for a final target

1019   concentration of 1,000 nuclei/uL – nuclei were counted on a hemocytometer. Based on the nuclei

1020   concentration, 10,000 target nuclei were loaded onto a Chip G for GEM generation using the

1021   Chromium Single Cell 5' Reagent v1.1 kit from 10X Genomics. Reverse transcription, barcoding,

1022   complementary DNA amplification and purification for library preparation were performed as per

1023   the Chromium 5' v1.1 protocol at the McDonnel Genome Institute. Sequencing was performed on

1024   a NovaSeq 6000 platform (Illumina) at a target read depth of 100,000 at the McDonnel Genome

1025   Institute.

1026

1027   *snRNA-seq reference map generation*

1028

1029   Nuclei fastq files were aligned to the whole genome pre-mRNA reference generated from the

1030   GRCh38 transcriptome (with the intron flag included) using CellRanger v3 (10X Genomics).

1031   Nuclei were filtered to include those with 1000 < RNA UMI count < 10,000 and mitochondrial

1032   reads < 5%. After initial QC, scrublet was ran on each sample separately in Python with default

1033   parameters to score nuclei and nuclei with a doublet score > 0.2 were excluded from downstream

1034   analysis. We then leveraged supervised doublet removal as previously described on a per cell

1035   type basis before combining all objects. Briefly, clusters were annotated into major cell

1036   populations, each major cell type was subsetted, and re-normalized, PCA, UMAP embedding,

1037   clustering, and DE analysis performed. Sub-clusters which did not express the gene signature of

1038   the cell type or had overlapping genes across different cell types were removed. Post-

1039   contamination, all cell type objects were merged to construct a cleaned object. After QC and

1040   doublet removal downstream analysis was performed in Seurat v4[49]. The cleaned object was

1041 normalized using SCTransform[43] with regressing out mitochondrial percent and RNA UMI counts.

1042 We then computed the principal components and used these to integrate all samples with

1043 harmony[50]. Informed by the ElbowPlot, we used 80 components to construct the UMAP

1044 embedding, find nearest neighbors, and clustered the data at multiple resolutions. We then used

1045 the FindAllMarkers function in Seurat to perform differential expression testing and annotated

1046 clusters into distinct cell types based on canonical gene markers. To define cell states within each

1047 major cell type, we subsetted the major cell populations, re-normalized, re-clustered, re-computed

1048 the UMAP, and annotated cell states. Both the global object and each cell type object was saved

1049 and used for downstream analysis and plotting in Seurat. All differential gene expression used to

1050 identify cell types or cell states was performed using the normalized assay with the FindAllMarkers

1051 function and the Wilcoxon rank sum test with a min.pct = 0.1 and logfc.threshold = 0.25.

1052

1053 *Integration of spatial transcriptomics data*

1054

1055 Visium 10x data from 10x Genomics was used in Supplementary Figure 3

1056 (https://www.10xgenomics.com/resources/datasets/human-heart-1-standard-1-1-0) – processed

1057 as per Seurat v4. Processed .h5ad objects were acquired for all spatial transcriptomic samples

1058 as previously published[51]. Data was re-normalized using SCTransform to keep analysis

1059 consistent with the human data processing. The snRNA-seq reference map was used to impute

1060 voxel annotations from selected spatial transcriptomic samples – mapping scores and gene

1061 signatures were also plotted to validate mapping. SPOTlight[52] was used for spot deconvolution

1062 and Pearson correlation coefficients calculated to identify cells which co-localize in space. To

1063 validate findings from isolated patients in a broader array of patients, the above analysis was

1064 performed on several samples and an integrated UMAP embedding of spatial spots was

1065 constructed for 28 samples using the same analysis pipeline as for snRNA-seq reference map

1066 construction.

1067

1068 *Receptor-ligand interaction analysis*

1069

1070 Using fibroblasts as a receiver, we first used nichenetr (v1.1.0) default pipeline to explore the top

1071 20 ligands from all other non-fibroblast cell types, that are predicted to orchestrate the highest

1072 regulatory potential to regulate downstream target expression in fibroblasts. The regulatory

1073 potential from top 20 ligands on their predicted targets in fibroblasts was represented in a

1074 heatmap. To further capture the disease altered signaling from myeloid cells to fibroblasts, we

1075 extended the analysis to draw information from the differential expression of the ligand-receptor

1076 pairs across HF groups (HF vs healthy) by using the differential NicheNet pipeline. Circos plot

1077 was created to visualize the interaction between ligands from myeloid cells and receptors from

1078 fibroblast cells.

1079

1080 *Mouse strains*

1081

1082 All animal studies were performed in compliance with guidelines set forth by the National Institutes

1083 of Health Office of Laboratory Animal Welfare and approved by the Washington University

1084 institutional animal care and use committee. All mouse strains used in this study (WT, CCR2 GFP)

1085 are on the C57BL/6 background. Il1r$^{fl/fl}$ mice were crossed to the Dermo1 cre to generate

1086 Il1r$^{fl/fl}$Dermo1cre^{+} strain. Il1r$^{fl/fl}$Dermo1cre^{-} mice were used as the control.

1087

1088 *Animal experiments*

1089

1090 8–10-week-old male and female mice were implanted with osmotic minipumps (Alzet model 2001)

1091 and constant infusion of angiotensin II (1.5 ug/g/day) and phenylephrine (50 ug/g/day). Mice were

1092 treated with either an isotype control or an anti-IL-1b neutralizing antibody (Amgen) at 5 mg/kg

1093 via intraperitoneal injection every 3 days starting with a pre-treatment injection one day before

1094 osmotic minipump implantation and then at days 2 and 5. Mice at day 7 as described in the

1095 relevant sections. For the Il1r$^{fl/fl}$Dermo1cre^{+} Ang II/PE experiment 28-day osmotic minipumps

1096 (Alzet model 2004) and mice were sacrificed on day 28 for trichrome staining.

1097

1098 *Fibroblast flow cytometry and sorting for single cell RNA seq analysis*

1099

1100 After ice cold PBS perfusion, heart tissue from mice were minced with a razor blade on ice and

1101 transferred to a 15 mL conical tube containing 3 mL DMEM with 170 uL Collagenase IV (250

1102 U/mL final concentration), 35 uL DNAse1 (60 U/mL), and 75 uL Hyaluronidase (60 U/mL) and

1103 incubated at 37 °C for 45 min with agitation. After 45 min, the digestion reaction was quenched

1104 with 6 mL of HBB buffer (2% FBS and 0.2% BSA in HBSS), filtered through 40 um filters into a

1105 50 mL conical tube and transferred back into a 15 mL conical tube to obtain tighter pellets.

1106 Samples were then spun down at 4 °C, 1200 rpm for 5 min and the supernatant was discarded.

1107 Pellet resuspended in 1 mL ACK Lysis buffer (Gibco A10492-01) and incubated at room

1108 temperature for 5 min followed by the addition of 9 mL DMEM and centrifugation (4 °C, 5 min,

1109   1200 rpm). Supernatant was discarded and the pellet was resuspended in 2 mL FACS buffer (2%

1110   FBS and 2 mM EDTA in calcium/magnesium free PBS) and centrifugation was repeated in above

1111   conditions and supernatant aspirated. Samples were then incubated in Pdpn (BioLegend,

1112   127423, clone 8.1.1), Cd140a (Biolegend, 135911, clone APA5), Cd31 (Biolegend, 102409, clone

1113   390), Fap (Creative Biolabs, HPAB-0171-CN-PE), and Cd45 (Biolegend, 103132, clone 30-F11)

1114   with a 1:200 dilution for 30 min. A no Fap negative control was used which contained all antibodies

1115   except Fap. Solution was washed 3X with FACS buffer following same centrifugation as above

1116   and then resuspended in 500 uL of FACS buffer and 1 uL DAPI (BD Biosciences, 564907) and

1117   filtered into filter-top FACS tubes. Gating was performed as follows: Pdpn+, singlets, Cd45-/Cd31-

1118   , Pdpn+/PDgfra+, and Fap vs Pdgfra gate was used to look at Fap+ fibroblasts. The no Fap

1119   antibody negative control was used to draw the Fap gate.

1120   For the fibroblast scRNA-seq experiment, 4 isotype Ang II/PE and 4 anti-IL-1 mAb + Ang

1121   II/PE mice FAP+ and FAP- fibroblasts were sorted into 300 uL cell resuspension buffer (0.04%

1122   BSA in PBS). Collected cells were centrifuged as above, pooled across mice, and resuspended

1123   in collection buffer to a target concentration of 1,000 cells/uL. Cells were counted on a

1124   hemocytometer before proceeding with the 10x protocol. cDNA construction and library

1125   preparation were performed for 4 libraries (Isotype + Ang II/PE FAP+, Isotype + Ang II/PE FAP-,

1126   anti-IL-1b mAb + Ang II/PE FAP+, and anti-IL-1b mAb + Ang II/PE FAP-. cDNA synthesis, library

1127   construction, and sequencing were performed using the same protocol as the human CITE-seq

1128   data.

1129

1130   *Histology and Immunofluorescence*

1131

1132   Flash frozen LV samples were fixed for 24 hr at 4 °C in 10% neutral buffered formalin, washed in

1133   1X PBS, and embedded in paraffin. Paraffin-embedded sections were cut at an 8 um thickness

1134   using a microtome. Slides were baked at 65 °C for 1 hr, deparaffinized with serial xylene washes,

1135   and rehydrated with ethanol. Then slides underwent methanol treatment (10% MeOH + 3% H_2O_2)

1136   for 20 min at RT followed by 3X TBS-T washes (5 min each). Antigen retrieval was performed

1137   using the AR6 buffer (Ankoya Biosciences, AR600250ML) for 15 min in the microwave and then

1138   cooled to room temperature. Tissue sections were marked with a hydrophobic pen and blocked

1139   in 10% BSA in TBS-T for 30 min at RT. Slides were then stained with the primary antibody diluted

1140   in 10% BSA in TBS-T overnight at 4 °C: FAP (Abcam, ab207178, 1:250) and POSTN (Abcam,

1141   ab215199, 1:250). Next, the primary antibody was detected using Opal Polymer HRP Ms + Rb

1142   (PerkinElmer Opal Multicolor IHC system). The PerkinElmer Opal Multicolor IHC system was

1143 utilized to visualize antibody staining per manufacturer protocol. Slides were imaged using a Zeiss

1144 Axioscan Z1 automated slide scanner. Image processing was performed using Zen Blue and Zen

1145 Black (Zeiss). For POSTN quantification, the whole heart was circled, and an MFI calculated in

1146 sham, isotype + Ang II/PE and anti-IL-1b mAb + Ang II/PE representative sections. For the

1147 Il1r$^{fl/fl}$Dermo1cre$^+$ mice 28-day Ang II/PE infusion, mice were sacrificed on day 28 and FFPE

1148 sections prepared as above. Trichrome staining was performed using Gomori's Trichrome Stain

1149 Kit (ThermoScientific, 87020) and subsequently percent fibrosis was quantified in serial cross-

1150 sections.

1151 For the CCR2 GFP mice, frozen sections were prepared using the following: hearts

1152 perfused with cold PBS, placed in 4% PFA overnight at 4 °C, washed with PBS 3X, infiltrated with

1153 30% sucrose overnight at 4 °C, and embedded in OCT and stored in a -80 °C freezer. Prior to

1154 staining, 10 um sections were cut using a Leica Cryostat, washed with PBS, incubated with 0.25%

1155 Triton-X in PBS at RT for 5 min, blocked in 5% BSA/PBS for 1hr at RT, stained with primary

1156 antibody: anti-GFP (Abcam, ab13970) and IL-1b (R&D systems, AF-401-NA, 1:50) co-stained

1157 with an appropriate secondary antibody, and mounted with DAPI fluoroshield (R&D, F6057).

1158 Slides were then imaged on a Zeiss confocal microscopy and representative images included.

1159

1160 *RT- PCR*

1161

1162 RNA was extracted from flash frozen mouse apex using the Qiagen RNeasy Plus Mini Kit. RNA

1163 concentration was then measured using a nanodrop spectrophotometer and cDNA synthesis was

1164 performed using the HighCapacity RNA to cDNA synthesis kit (Applied Biosystems). Quantitative

1165 real time PCR reactions were prepared with sequence-specific primers with PowerUP™ Syber

1166 Green Master mix (Thermo Fisher Scientific) in a 20 uL volume. Real time PCR was performed

1167 using QuantStudio3 (Thermo Fisher Scientific). mRNA expression was normalized to 36B4. The

1168 Postn primer was purchased from ADT (Fwd: 5'-GGTGCCCTAGAAAGGATCATGG-3'; Rev: 5'-

1169 CAGAGCACTGGAGGGTATTTAG-3').

1170
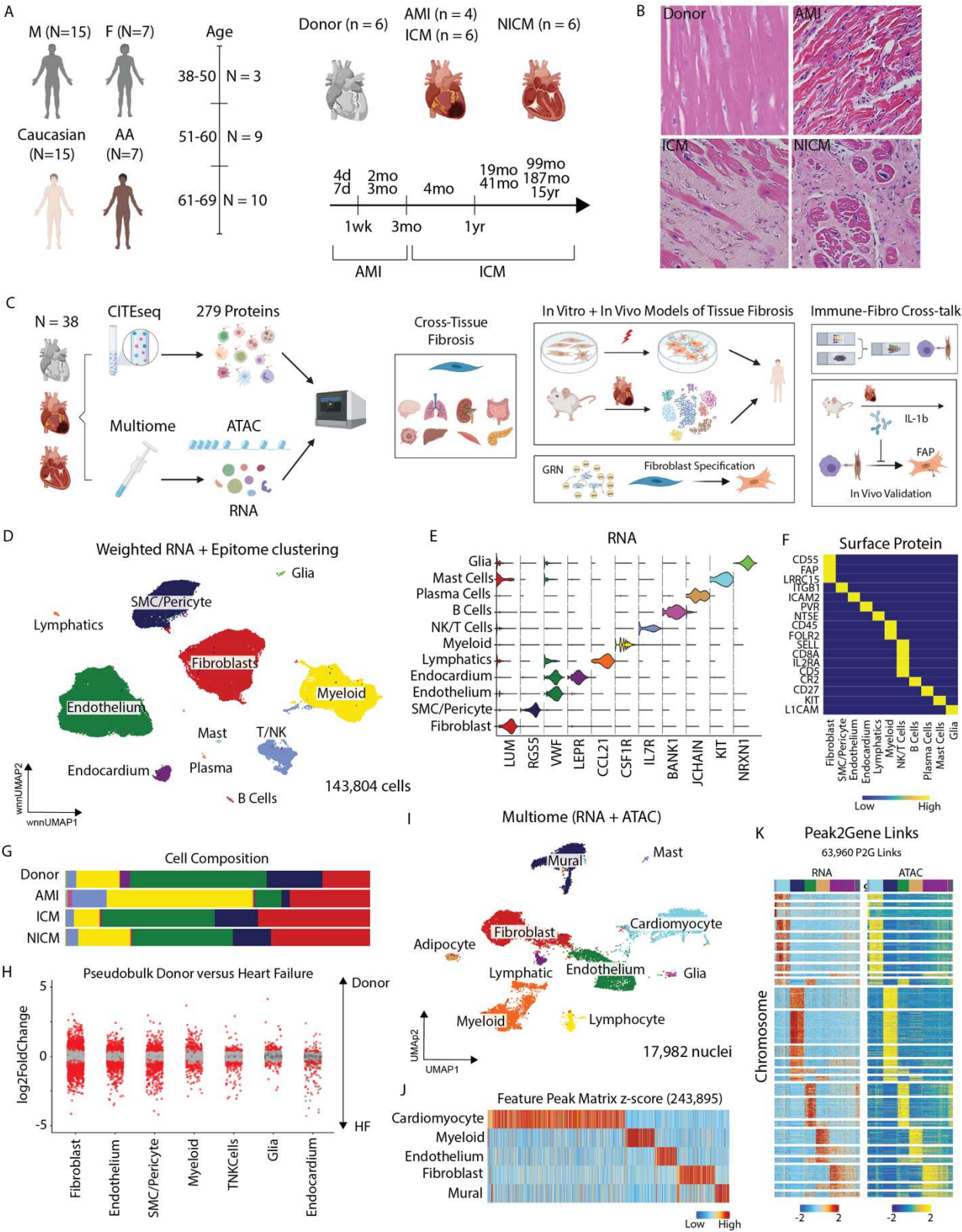
1171 **Data Availability**

1172

1173 Raw sequencing files can be found on the Gene Expression Omnibus super series (GSE218392).

1174 Processed data is available upon request from the authors.

1175

1176 **Code Availability**

1177

1178   Scripts used for analysis in this manuscript can be found at ().

Figure 1

1179
1180

36

1181   **Figure 1.** Study design and integrated multi-omic characterization of human MI and HF. (A)
1182   Multiethnic diverse patient demographics. (B) Representative H&E sections from donor, acute MI,
1183   ischemic cardiomyopathy, and non-ischemic cardiomyopathy. (C) Study design from multi-omic
1184   human sequencing derived hypothesis, cross-tissue fibrosis analysis, *in vitro* and *in vivo*
1185   experimental characterization, immune-fibroblast crosstalk, and *in vivo* validation studies. (D)
1186   UMAP embedding plot with weighted nearest neighbor clustering (RNA and protein) of CITE-seq
1187   data from 22 patients and 143,804 cells. (E) Violin plot for canonical marker genes for cell types.
1188   (F) Heatmap of marker proteins for cell types. (G) Cell cluster composition across conditions. (H)
1189   Pseudobulk differentially expressed genes between donor and HF samples in major cell types
1190   (bottom). Red dots indicate statistically significant genes (log2FC > 0.58 and adjusted p-value <
1191   0.05). (I) UMAP embedding plot of RNA from Multiome (paired RNA and ATAC) specimens from
1192   9 patients and 17,982 nuclei. (J) Marker peaks for the major cell types annotated in (I) with FDR
1193   < 0.1 and log2FC > 0.5. (K) Peak to Gene linkage heatmap using paired RNA and ATAC
1194   information from the same nucleus.
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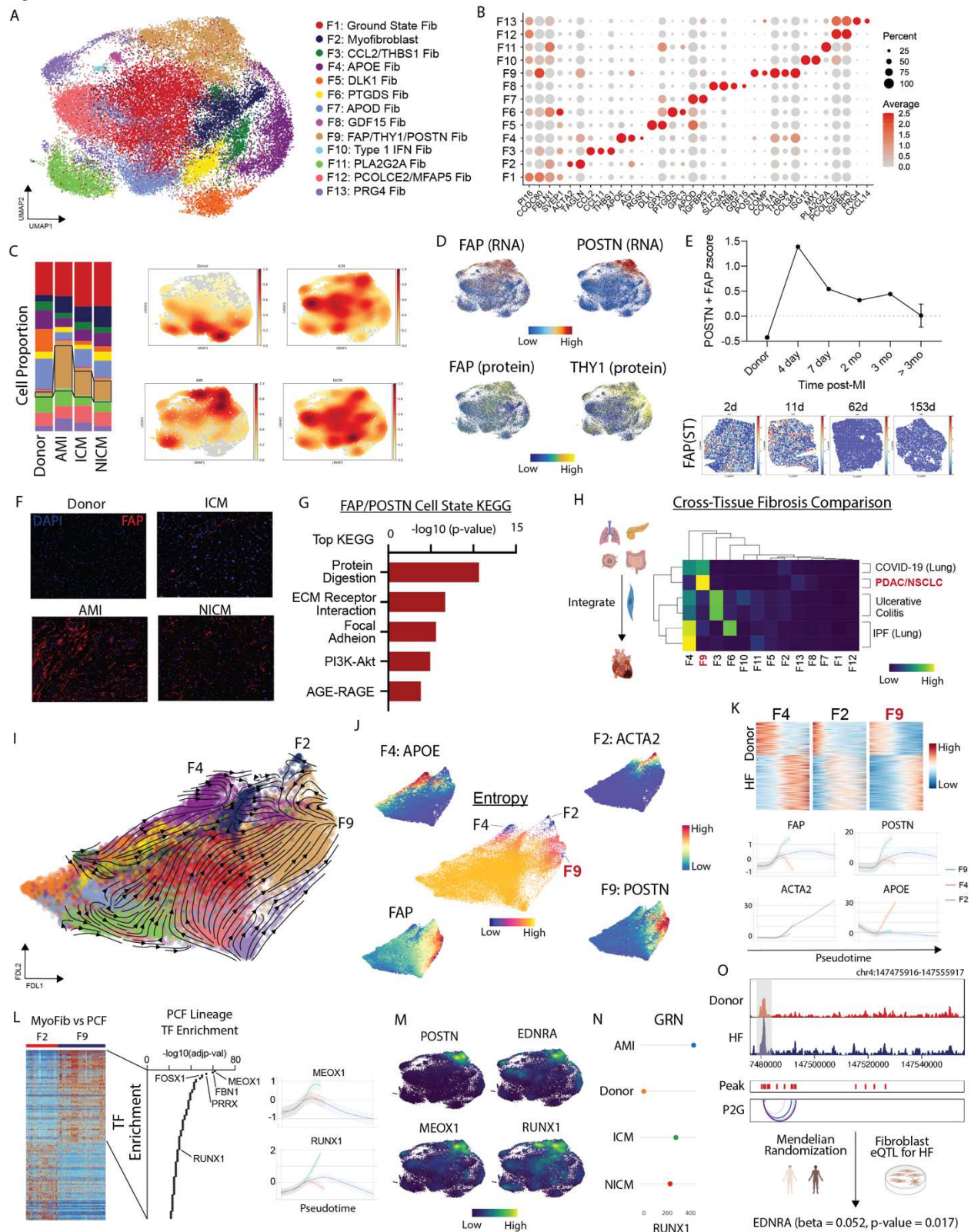1209
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Figure 2

1217   **Figure 2.** FAP fibroblasts expand post-MI into a PF lineage with a *MEOX1/RUNX1* orchestrated

1218   gene regulation network. (A) UMAP embedding of CITE-seq fibroblast cell states. (B) Dot Plot of

1219   marker genes for fibroblast cell states. (C) Fibroblast cell state composition across four groups

1220   (left) and Gaussian kernel density estimation of cells across four groups (right). (D) FAP RNA (top

1221   left), POSTN RNA (top right), FAP protein (bottom left), and THY1 protein (bottom right). (E)

1222   FAP/POSTN z-score in donor, acute MI, and ICM split by time from MI with spatial transcriptomics

1223   samples showing FAP expression at different time points post-MI in human LV specimens. (F)

1224   Immunofluorescence of FAP in donor, acute MI (4 days post-MI), ICM, and NICM left ventricle

1225   myocardium. (G) Top KEGG pathways for FAP/POSTN cell state. (H) Cross-tissue fibrosis

1226   Pearson correlation coefficient between human heart fibroblast cell states and those from other

1227   disease contexts. (I) scvelo analysis in force directed layout embedding and (J) Palantir derived

1228   entropy with terminal cell states noted and marker genes for terminal states in addition to FAP

1229   plotted in FDL embedding highlighting *FAP* derived fibroblasts diverge into myofibroblasts and PFs.

1230   (K) Heatmap of pseudobulk differentially expressed genes between donor and HF and terminal

1231   state marker genes over pseudotime. (L) Heatmap of differentially expressed genes between F2

1232   and F9 fibroblast cell states (left), TF enrichment analysis for F9 lineage using enrichR (TF-Gene

1233   Co-occurrence database) shows key epigenetic regulators of the F9 lineage (middle), and

1234   *RUNX1/MEOX1* gene expression across pseudotime for 3 lineages showing increased

1235   expression along F9 lineage (right). (M) Gene expression density plot in UMAP embedding for

1236   *POSTN*, *RUNX1*, *EDNRA*, and *MEOX1* (markers distinguishing F2 and F9 lineages). (N)

1237   CellOracle gene regulatory network betweenness centrality score for *RUNX1* by HF etiology – a

1238   higher score indicates that the TF has a greater influence on informational flow in the GRN. (O)

1239   ATAC-seq pseudobulk tracts from multiome fibroblasts with peak2gene linkages showing *EDNRA*

1240   locus split by donor and HF; Mendelian Randomization shows that there was a significant positive

1241   association between genetically predicted *EDNRA* gene expression levels and HF in fibroblasts

1242   (beta = 0.052, se = 0.022, and p-value = 0.017).

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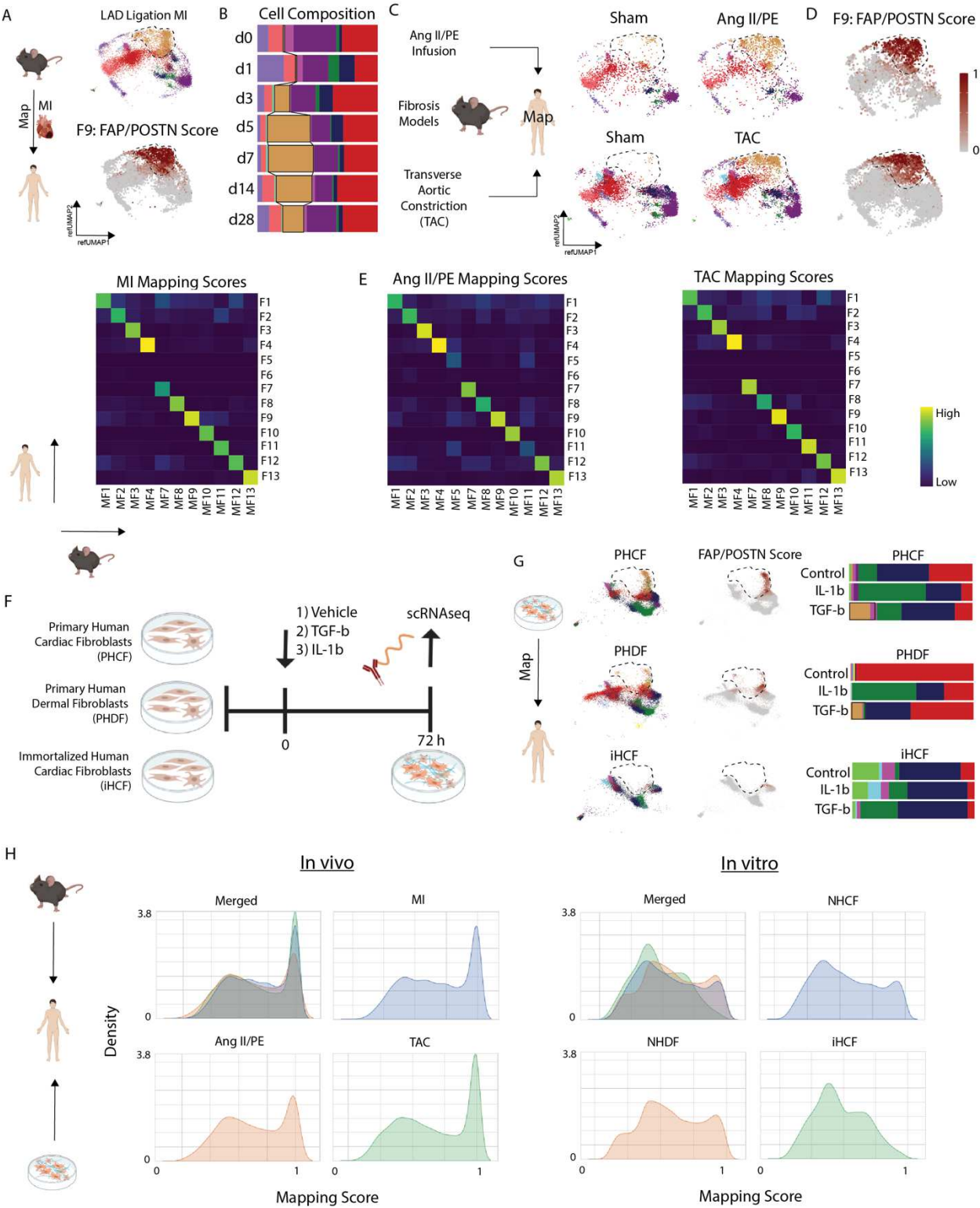
Figure 3

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1254 **Figure 3.** Comparison of *in vivo* and *in vitro* models to study cardiac tissue fibrosis. (A) Reference

1255 mapped mouse MI fibroblasts onto human CITE-seq space with label transfer (top) and prediction

1256 score for cells annotated in POSTN/FAP cluster. (B) Cell composition from label-transferred cell

1257 states post-MI. (C) Reference mapped mouse fibrosis model fibroblasts from Ang II/PE and TAC

1258 split by sham and injury. (D) Prediction score for cells annotated in POSTN/FAP cluster from

1259 fibrosis models. (E) Heatmap of average cell prediction score of human fibroblast cell states (y-

1260 axis) in annotated mouse fibroblasts (x-axis) in MI, Ang II/PE, and TAC. (F) Experimental set-up

1261 of *in vitro* model. (G) Reference mapped *in vitro* cell lines post-stimuli (control, TGF-b, and IL-1b)

1262 onto human cardiac fibroblasts and corresponding cell composition. (H) Density plot of maximum

1263 prediction score for each cell from *in vivo* and *in vitro* systems merged and split by *in vivo* or *in*

1264 *vitro* condition.

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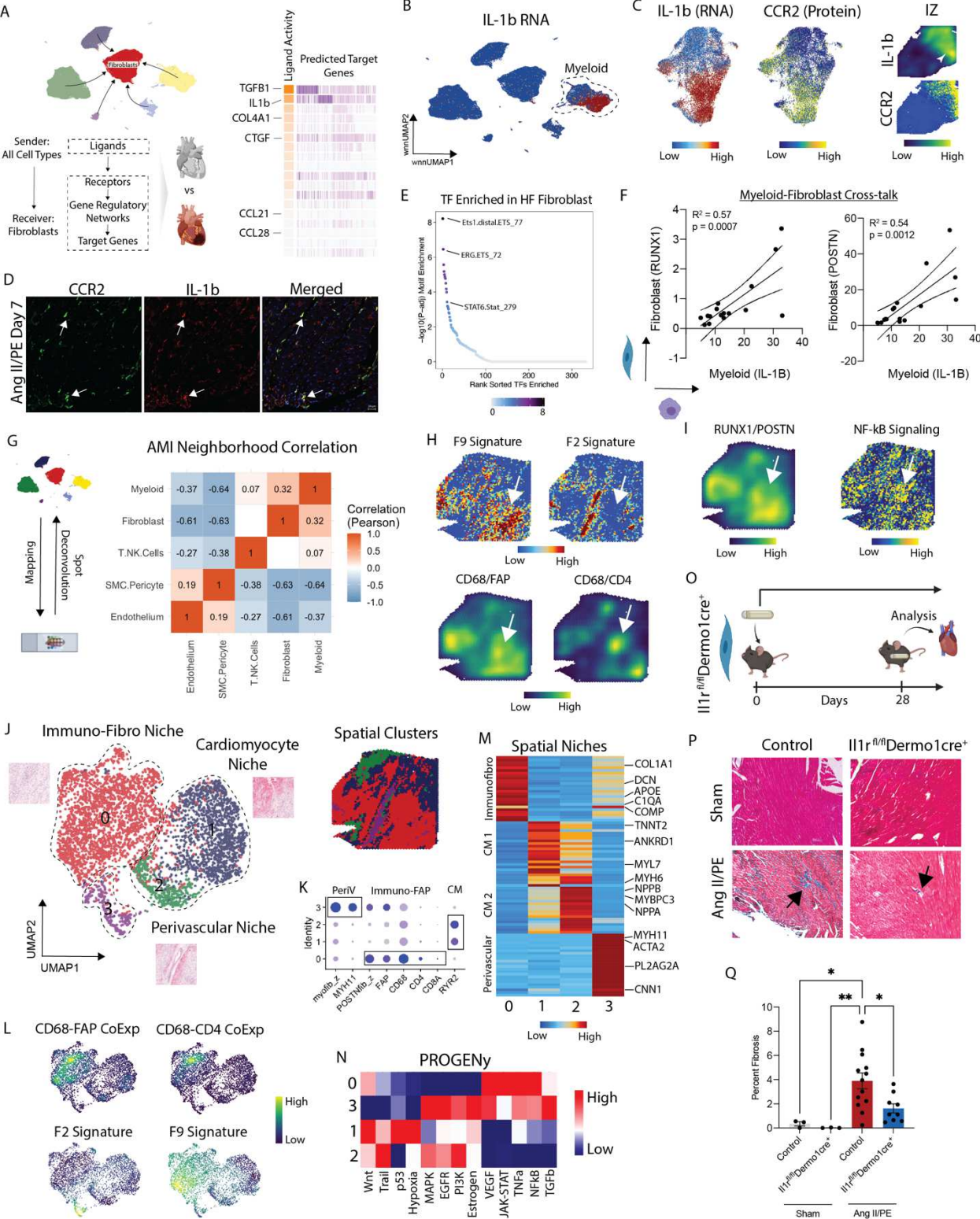
Figure 4

Figure 4. CCR2 macrophages co-localize with and signal to fibroblasts via IL-1b in MI and HF. (A) NicheNet derived predictions of enriched signaling from other cell types to fibroblasts in HF (acute MI, ICM, NICM) relative to donor. (B) IL-1b RNA expression in global CITE-seq UMAP embedding. (C) IL-1b RNA expression in myeloid subsets (left) with corresponding CCR2 protein expression (middle), and IL-1b expression density and imputed CCR2 protein expression in an acute MI IZ spatial transcriptomic sample. (D) Immunofluorescence of IL-1b (red) in CCR2 GFP (green) mice at day 7 post Ang II/PE mini-pump implantation. (E) Rank sorted TF motifs enriched in HF fibroblasts relative to donor fibroblasts from Multiome data show enriched inflammatory signaling. (F) Average expression of IL-1B in macrophages per HF patient versus *RUNX1* (left) and *POSTN* (right) in fibroblasts from the same patient. R^2 indicates the regression coefficient and the p-value tests whether the slope is significantly non-zero. (G) SPOTlight derived cell-cell proportion correlation in acute MI patient. (H) z-score for marker genes in F9 (*POSTN, COMP, FAP, COL1A1, THBS4, COL3A1*) and F2 (*ACTA2, TAGN*) (top) and CD68/CD4 and CD68/FAP joint density embedding plot (bottom). (I) *RUNX1*/*POSTN* co-localization density plot (left) and PROGENy imputed NF-kB pathway score (right). (J) UMAP embedding plot of spatial clusters with three distinct niches and corresponding spatial location of clusters. (K) Dot plot of marker genes and gene set scores for spatial niches. (L) CD68/CD4, CD68/FAP (top) and F2 and F9 fibroblast gene signature (bottom) joint density embedding plot in UMAP embedding. (M) Heatmap of top differentially expressed genes across spatial niches. (N) PROGENy pathway analysis for spatial clusters in (J). (O) Study design for IL-1 receptor deletion in fibroblasts in mice with Ang II/PE minipump implantation, (P) representative images of trichrome staining, and (Q) quantification of fibrosis at day 28.

Figure 5
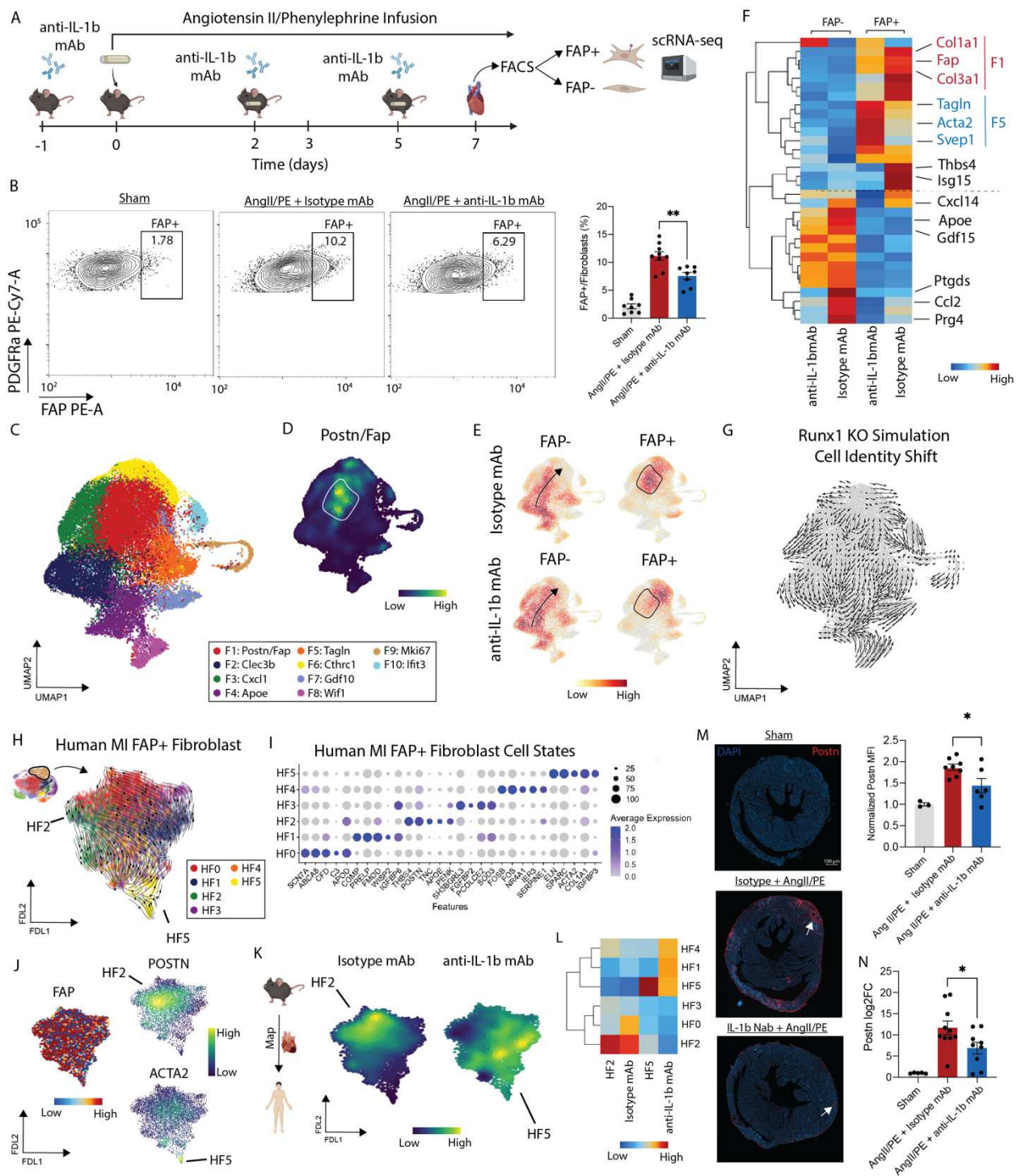
Figure 5. (A) Experimental workflow for cardiac injury experiment with treatment of an IL-1b neutralizing antibody or isotype control. (B) Flow cytometry gating of PDPN vs FAP in a representative sham, Ang II/PE + Isotype, and Ang II/PE + anti-IL-1b mAb mouse heart (left) and FAP+ fibroblasts/total fibroblasts quantified (right). Unpaired t-test and **P = 0.0031 (isotype vs anti-IL-1b mAb). (C) Integrated UMAP for isotype and anti-IL-1b mAb treated mice FAP+/FAP- fibroblasts with annotated clusters. (D) Density plot of Fap/Postn co-localization with area of maximal expression highlighted. (E) Gaussian kernel density plots of 4 conditions in integrated UMAP embedding. (F) Heatmap of key fibroblast cell state genes grouped by four conditions with rows clustered by similarity. (G) CellOracle *Runx1 in silico* knockout simulated cell identify shift shows a shift away from Fap/Postn fibroblasts. (H) scVelo of human FAP+ fibroblasts in a FDL embedding space colored by re-clustered data. (I) Top marker genes for FAP+ human CITE-seq re-clustered data from (F). (J) FAP, POSTN, and ACTA2 expression in human FAP+ fibroblasts in FDL embedding. (K) Mapping mouse differentially expressed signature between FAP+ isotype and anti-IL-1b mAb treated mice. (L) Heatmap of gene set signature for HF2 (*THBS4, POSTN, TNC, APOE,* and *PENK*), isotype treated, HF5 (*ACTA2* and *TAGLN*), and anti-IL-1b mAb treated grouped by FAP+ human fibroblast cell state. (M) IF of *POSTN* in a representative sham, Ang II/PE + Isotype, and Ang II/PE + anti-IL-1b mAb heart (left) with quantification (right). Unpaired t-test and *P = 0.0243. (N) RT-PCR of Postn in mouse hearts split by 3 conditions. Unpaired t-test and *P = 0.0392.

Supplementary Figure 1
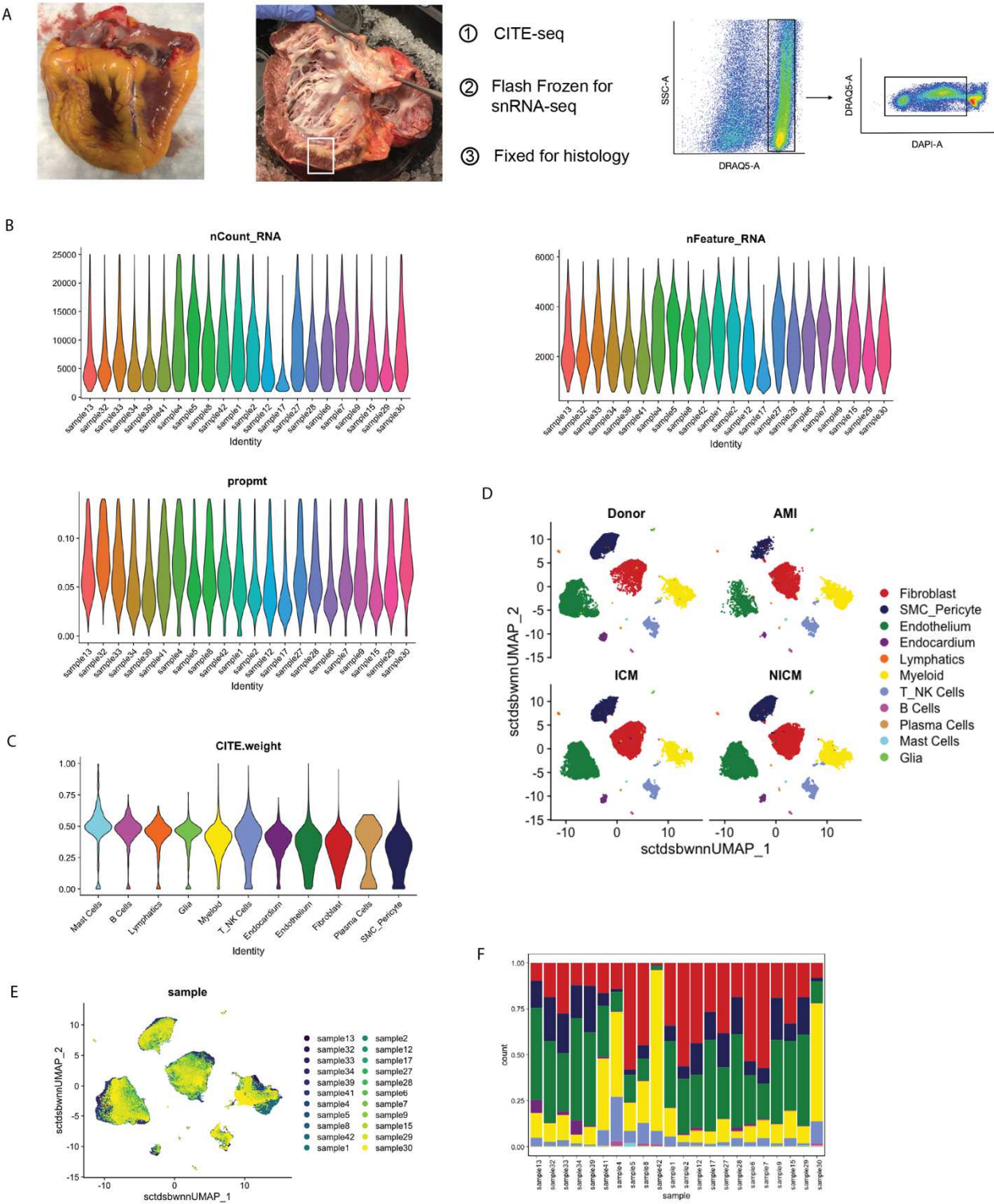
1355 **Supplementary Figure 1**. (A) Explanted heart and dissected region boxed used for CITE-seq,

1356 snRNA-seq, histological analysis with flow cytometry panel to sort live cells. (B) QC metrics post

1357 filtering. (C) CITE-seq protein assay weights used for WNN clustering. (D) Global integrated

1358 UMAP split by 4 conditions. (E) Integrated global UMAP colored by patient sample. (F) Cell type

1359 composition split by each sample.

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Supplementary Figure 2

A

- ● Fibroblast
- ● SMC_Pericyte
- ● Endothelium
- ● Endocardium
- ● Lymphatics
- ● Myeloid
- ● T_NK Cells
- ● B Cells
- ● Plasma Cells
- ● Mast Cells
- ● Glia

Low   High

B

FAP   THY1   ITGB1   CD45

CCR2   ITGAX   CD163   FOLR2

TCRab   CD5   SELL   CD8A

wnnUMAP2
wnnUMAP1

Low   High
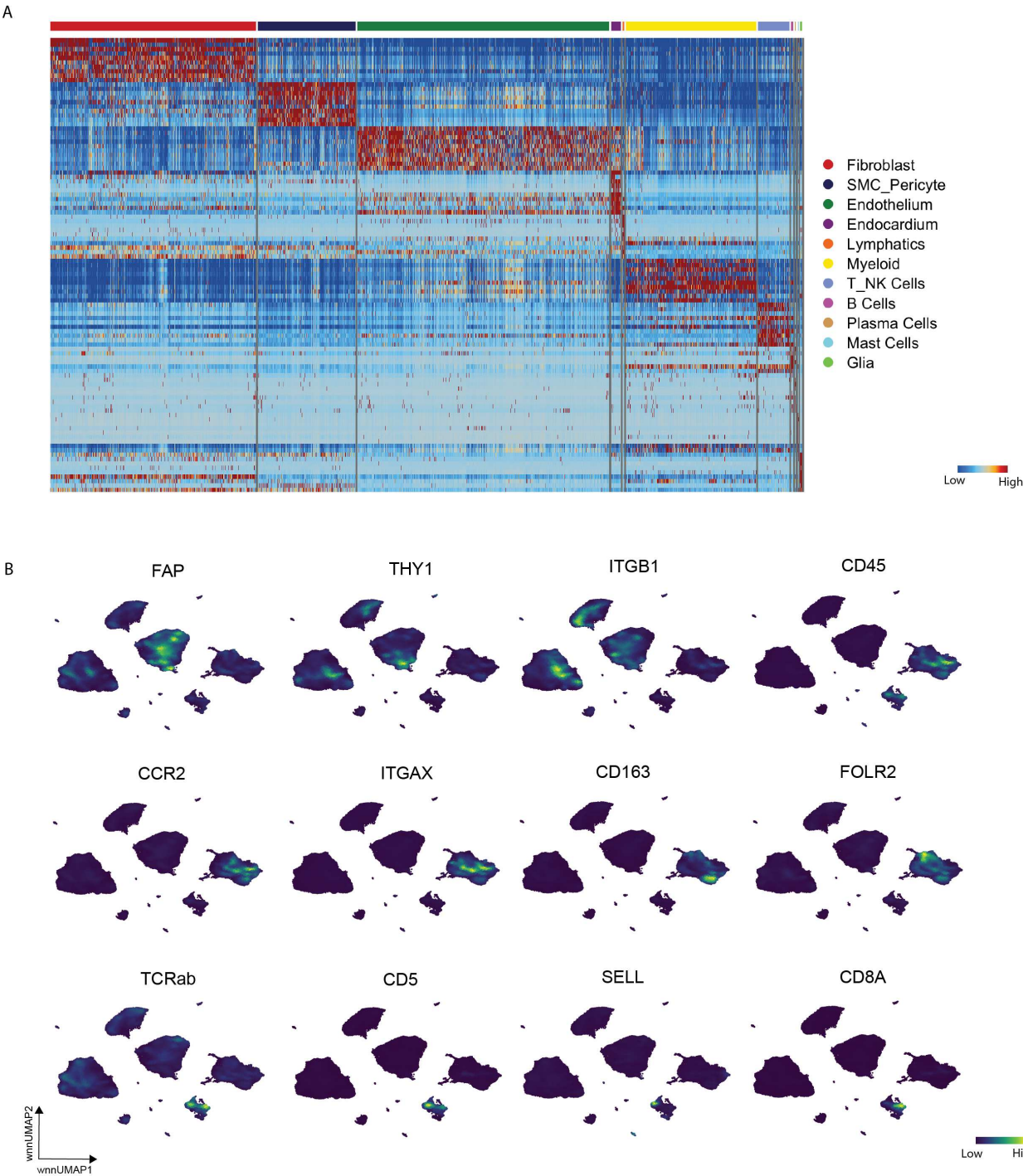
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1395   **Supplementary Figure 2**. (A) Heatmap of top marker genes for each cell type in global UMAP.

1396   (B) Protein expression as a density plot for top CITE-seq protein markers in different cell types.

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Supplementary Figure 3

A

Low High

B

F1: Ground State
PI16
CCDC80
FBLN1
SVEP1

F2: Myofibroblast
ACTA2
TAGLN

F3: CCL2/THBS1
CCL2
CCL11
THBS1

F4: APOE
APOE
AGT
RGS5

F5: DLK1
DLK1
GPX3

F6: PTGDS
PTGDS
GPC3

F7: APOD
APOD
IGFBP5

F8: GDF15
GDF15
ATF5
SLC3A2
TRIB3

F9: POSTN/FAP
POSTN
COMP
FAP
COL1A1
THBS4
COL3A1

F10: IFN
ISG15
MX1

F11: PLA2G2A
PLA2G2A
CFD

F12: PCOLCE2
PCOLCE2
IGFBP6

F13: PRG4
PRG4
CXCL14

Low High

C

THY1 (RNA)   THY1 (protein)   THY1 (protein) density

Low High   Low High   Low High

D

CD276 (protein)   LAMP1 (protein)   CD63 (protein)   F11R (protein)

Low High

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1431   **Supplementary Figure 3**. (A) Heatmap of top marker genes for fibroblast cell states. (B) Gene

1432   set z-scores for fibroblast cell states plotted in UMAP embedding. (C) THY1 RNA, protein, and

1433   protein density plot in fibroblast UMAP space. (D) Density plots for differentially expressed protein

1434   markers in fibroblast UMAP space.

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Supplementary Figure 4
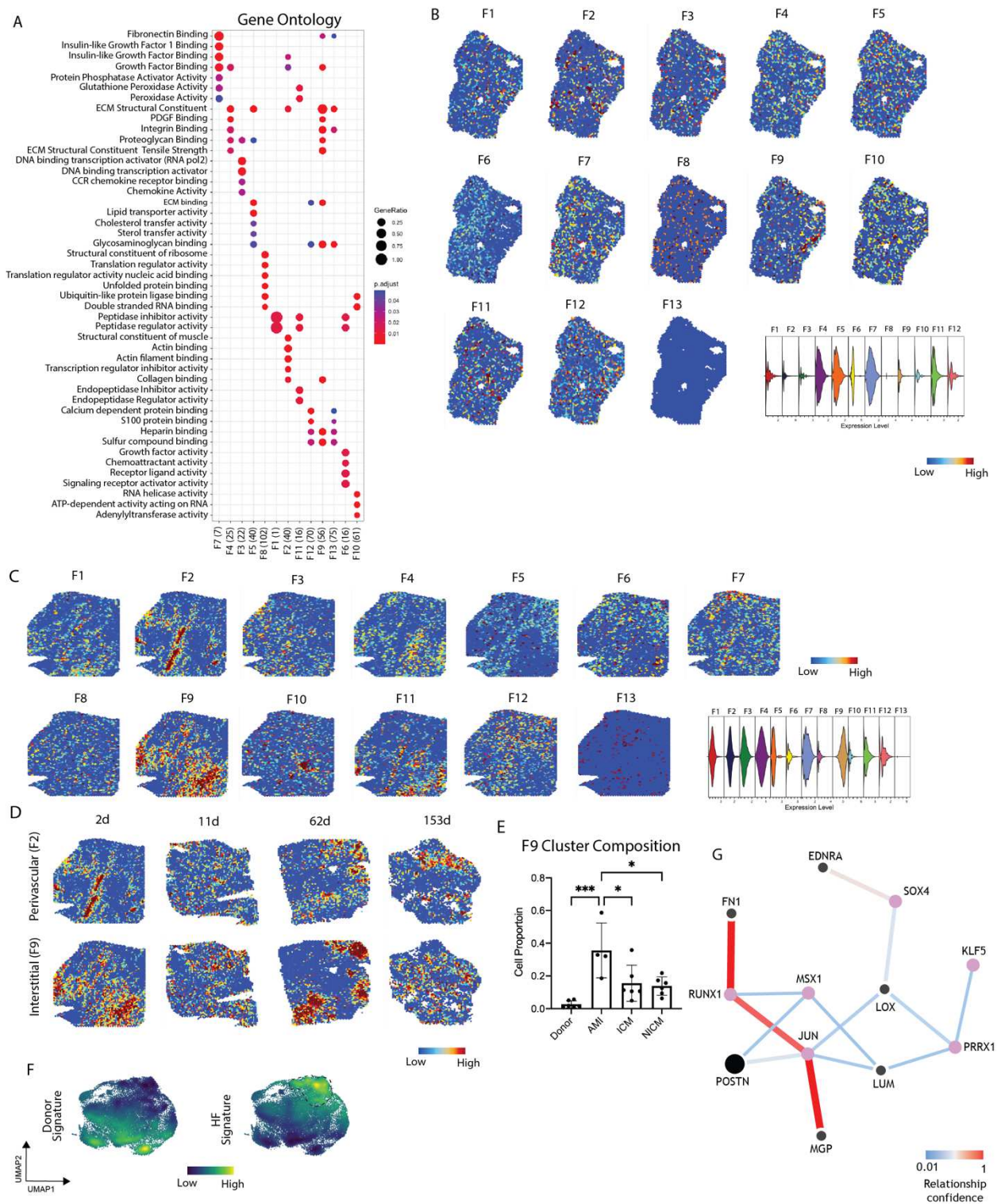
1469   **Supplementary Figure 4**. (A) GO analysis from clusterProfiler for fibroblast cell states. Fibroblast

1470   cell state gene set scores in a spatial transcriptomics (B) donor and (C) AMI sample with

1471   associated violin plot. (D) F2 and F9 gene set score plotted in spatial transcriptomics data across

1472   acute MI and ICM sections. (E) F9: FAP/POSTN cluster composition across four groups. One-

1473   way ANOVA test with multiple comparisons: donor vs acute MI (***$P = 0.0002$), acute MI vs ICM

1474   (*$P = 0.0207$), and acute MI vs NICM (*$P = 0.0115$). (F) Gene set signature kernel density

1475   embedding plot for donor (top) and HF (bottom) – genes are derived from pseudobulk differential

1476   expression analysis between donor and HF. Statistically significant genes (adjusted p-value <

1477   0.05, log2FC > 0.58, and base mean expression > 500). (G) Transcriptional regulation interaction

1478   between *POSTN*, *EDNRA,* and *MEOX1* using PathwayNet (http://pathwaynet.princeton.edu).
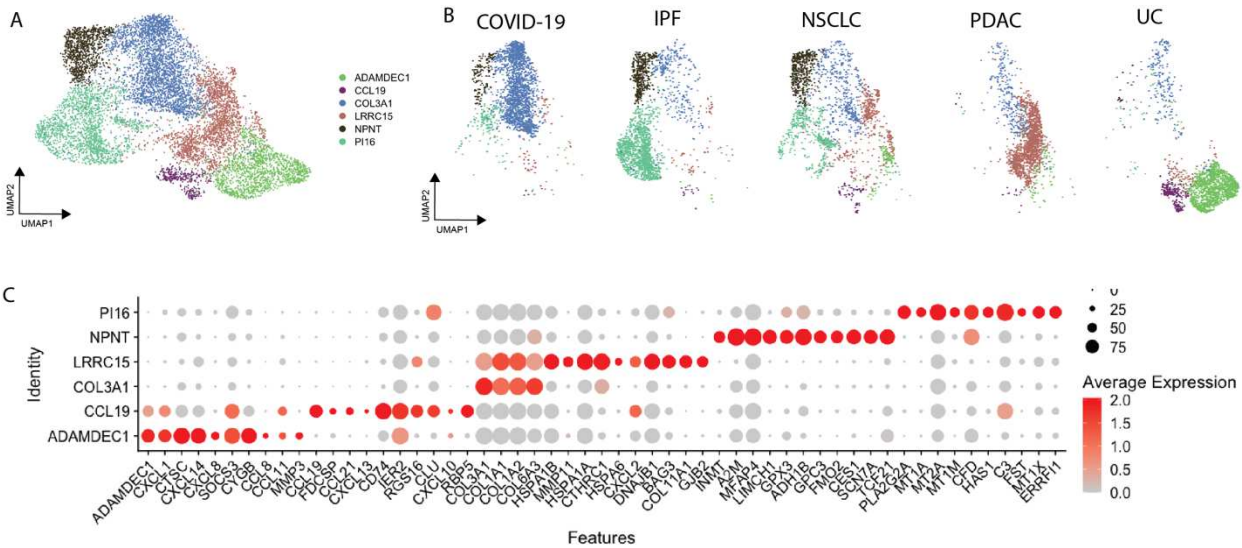
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Supplementary Figure 5

1525   **Supplementary Figure 5**. (A) Integrated UMAP of perturbated pathological fibroblasts and (B)

1526   split by disease category. (C) Dotplot of marker genes for clusters in (A).

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Supplementary Figure 6

A — Mouse MI Mapped to Human CITEseq Ref

B — Mapping Scores

C — Cell Composition

D — Mouse MI Data

E — Human MI Data
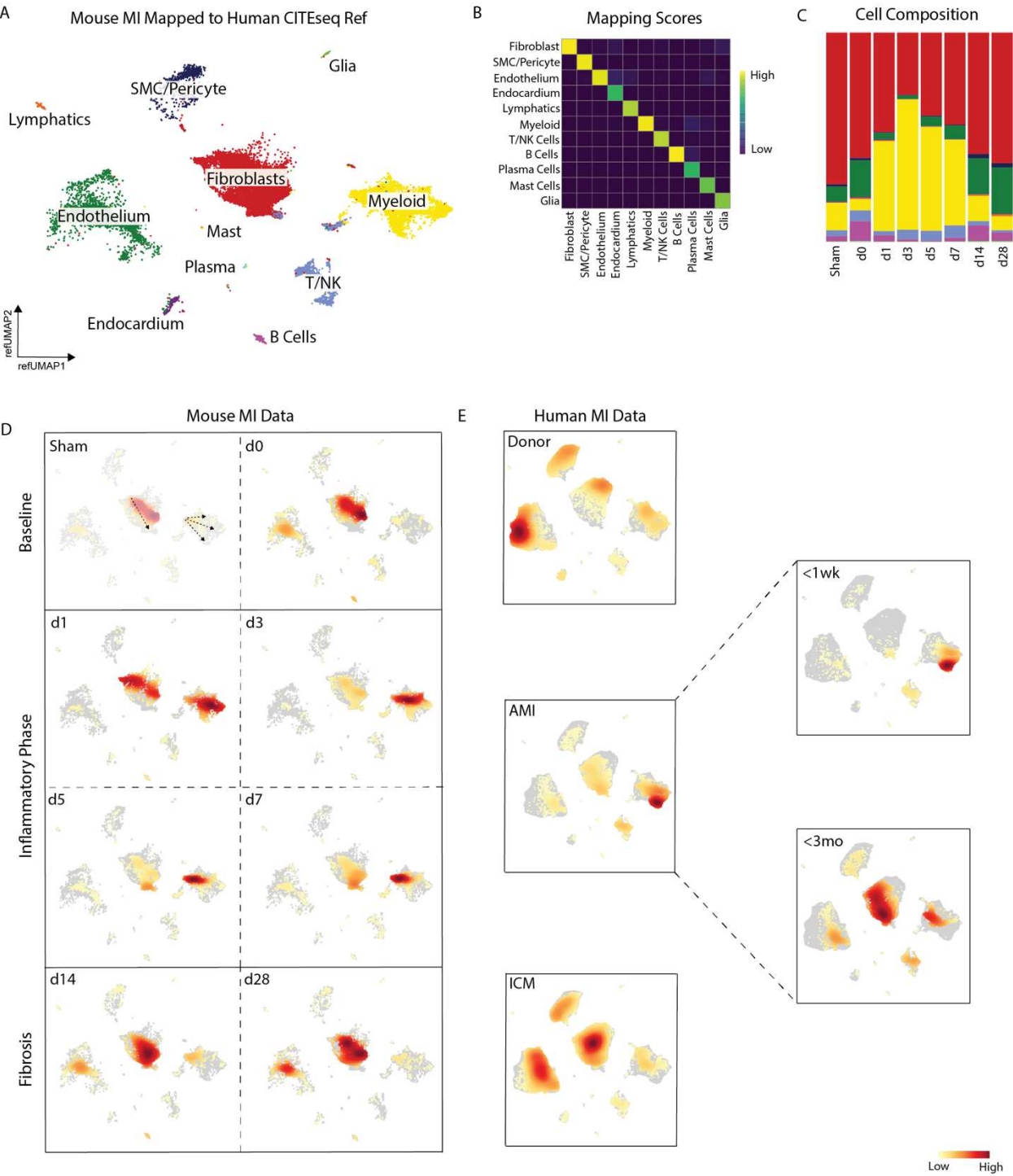
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1558 **Supplementary Figure 6**. (A) Mouse MI data mapped onto human CITE-seq global object. (B)

1559 Seurat reference mapping scores for different cell types. (C) Cell composition from imputed

1560 annotations split by time point. (D) Gaussian kernel density embedding plots in mouse MI data

1561 split by condition and biological phase and (E) human MI data grouped by MI category.

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Supplementary Figure 7

A   Mouse MI Mapping Scores

F5, F6 had no mapping

B   predicted.celltype.l1

- ground state Fib
- Myofibroblast
- CCL2.THBS1 Fib
- APOE Fib
- DLK1 Fib
- PTGDS Fib
- APOD Fib
- GDF15 Fib
- POSTN+ Fib
- Type I IFN Fib
- PLA2G2A Fib
- POLCE2.MFAP5 Fib
- PRG4 Fib
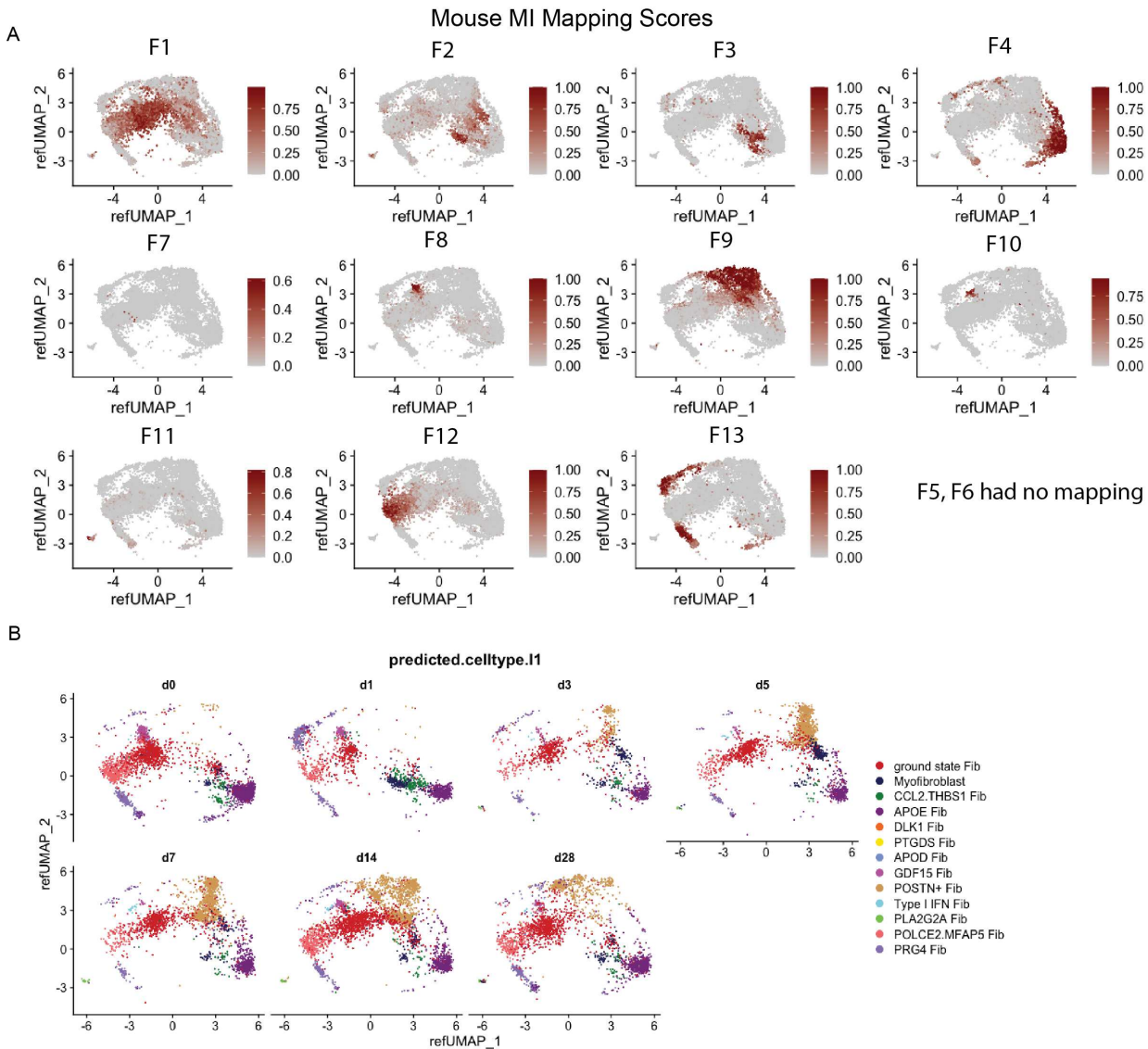
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1602 **Supplementary Figure 7**. (A) Reference mapping scores for mouse MI fibroblasts onto human

1603 heart CITE-seq fibroblast UMAP embedding. (B) Reference mapped data split by MI time point in

1604 human space.

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Ang II/PE Fibroblast Mapping Scores
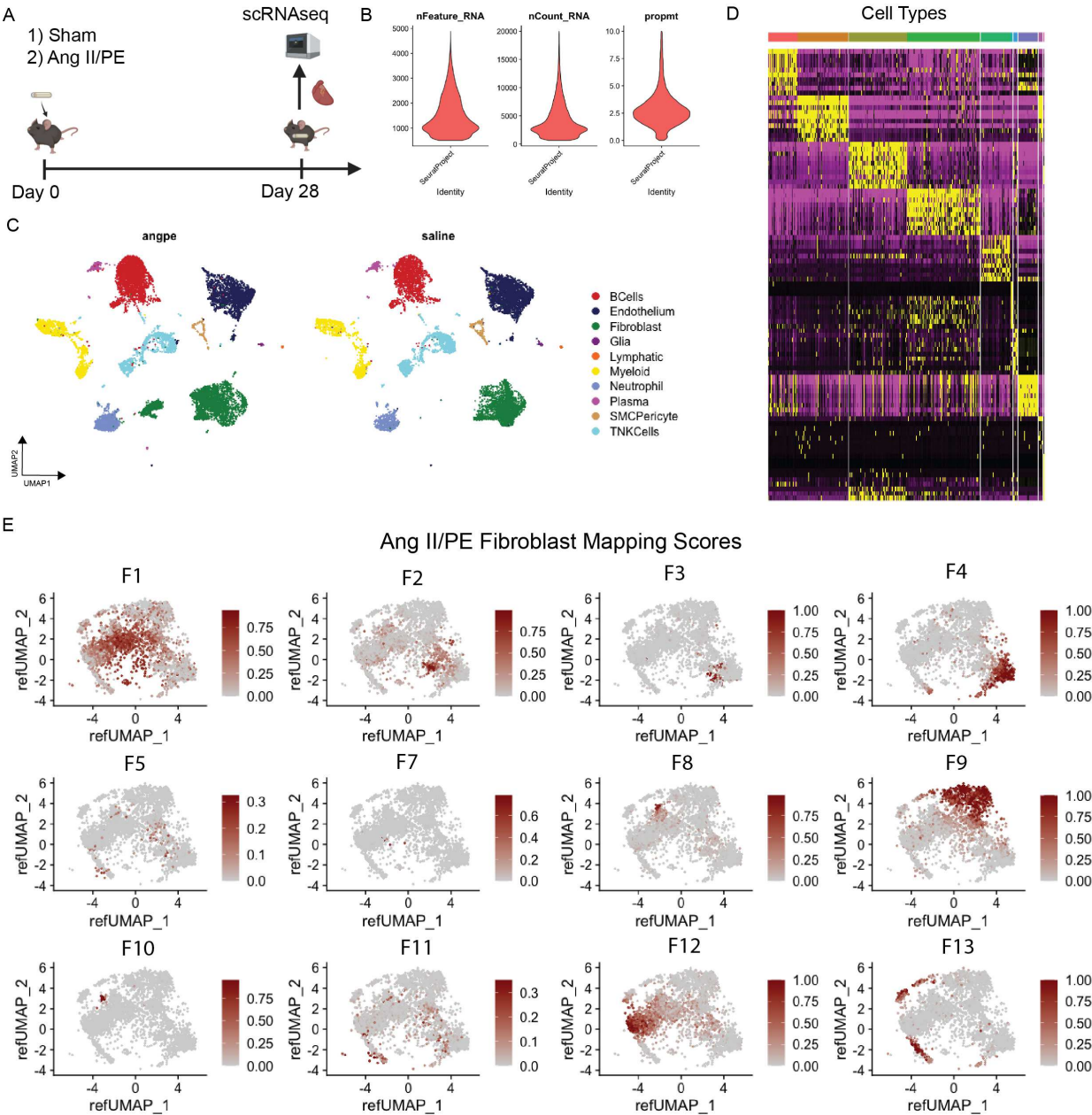
1643   **Supplementary Figure 8**. (A) Experimental design for Ang II/PE 28-day pumps for sequencing.

1644   (B) QC metrics post filtering. (C) Integrated global UMAP split by sham and Ang II/PE at day 28.

1645   (D) Heatmap of top marker genes for clusters from (C). (E) Reference mapping scores for mouse

1646   Ang II/PE and sham d28 fibroblasts onto human heart CITE-seq fibroblast UMAP embedding.

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A

TAC Fibroblast Mapping Scores

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Supplementary Figure 9. (A) Reference mapping scores for mouse TAC fibroblasts onto human

heart CITE-seq fibroblast UMAP embedding. (B) Reference mapped data split by sham, TAC,

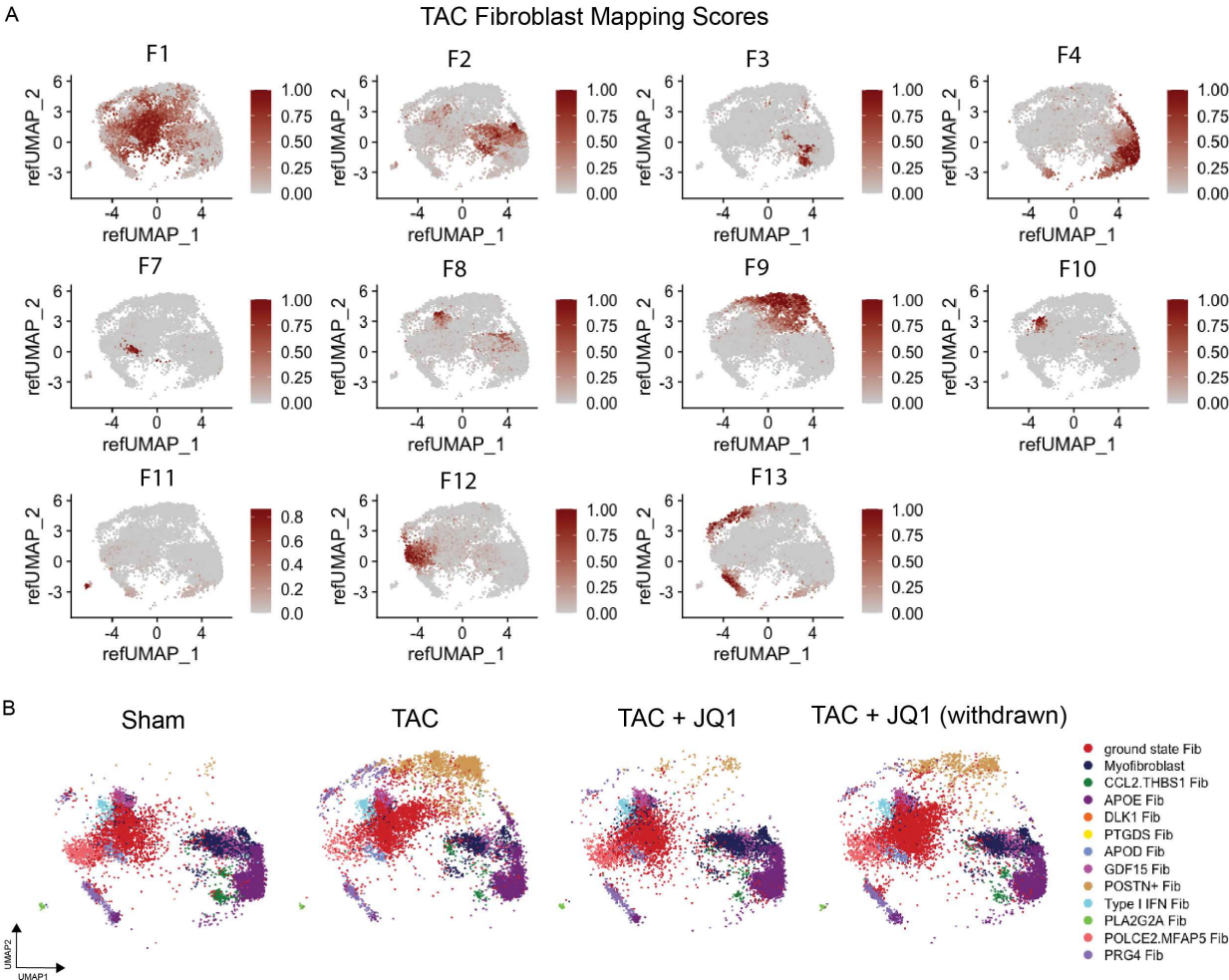TAC + JQ1 treatment, and TAC + JQ1 withdrawn in human space.

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Supplementary Figure 10

1733 **Supplementary Figure 10**. Quality control metrics post filtering grouped by each biological

1734 replicate for each experimental condition (vehicle, TGF-b, and IL-1b) in (A) NHCF, (B) NHDF, and

1735 (C) iHCF. (D) Integrated data from 3 cell lines in a UMAP embedding colored by biological

1736 replicate for each experimental condition.

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Supplementary Figure 11
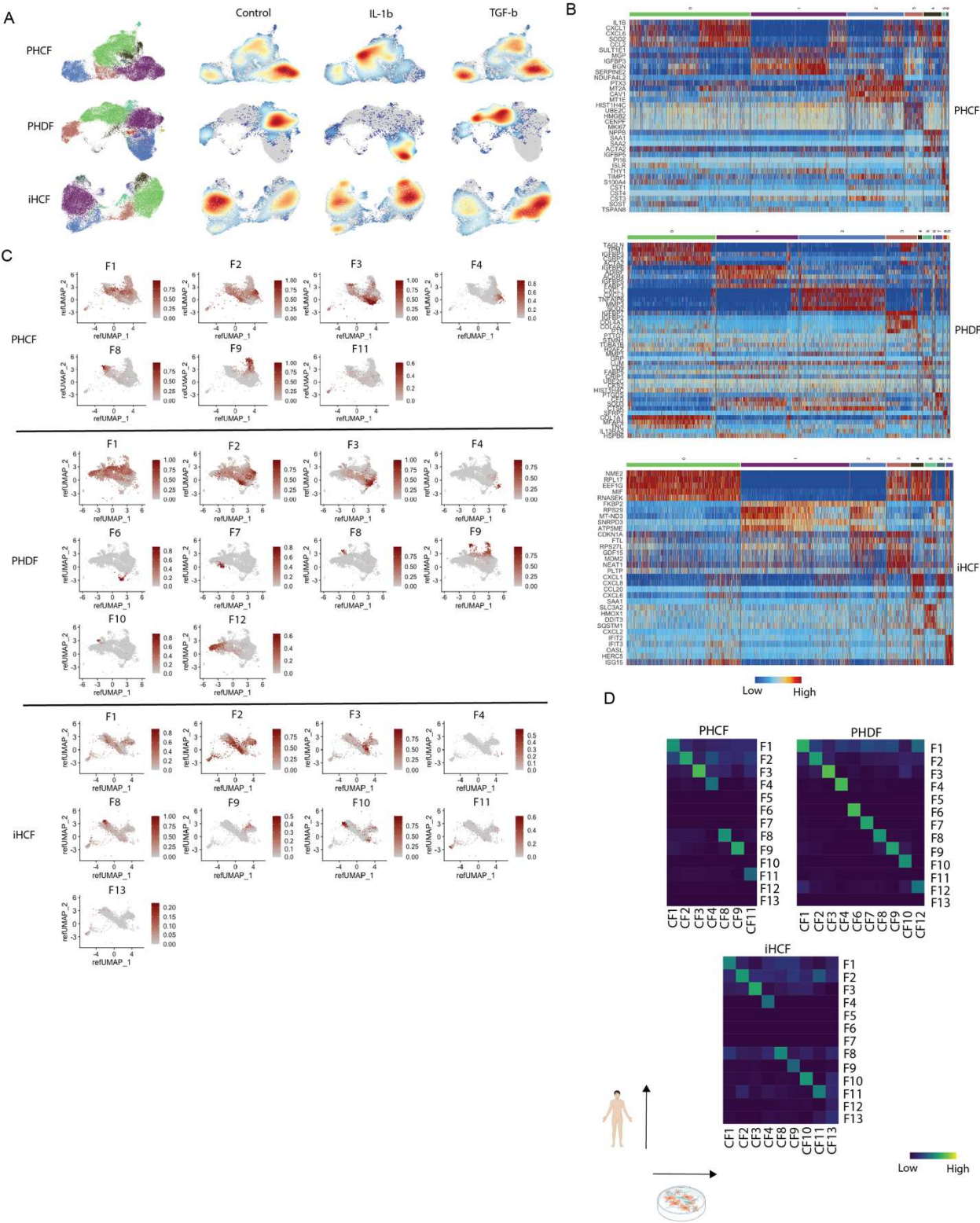
1769 **Supplementary Figure 11**. (A) UMAP embedding with separate clustering in each cell line with
1770 density plots split by experimental condition. (B) Heatmap of marker genes for each cell line for
1771 clusters in (A). (C) Reference mapping scores for *in vitro* fibroblasts onto human heart CITE-seq
1772 fibroblast UMAP embedding. (D) Heatmap of mapping scores with *in vitro* clusters on x-axis and
1773 human heart CITE-seq fibroblasts on y-axis. (E) Human, *in vivo*, and *in vitro* fibroblasts integrated
1774 in a common UMAP space colored by category. (F) Phylogeny tree clustering of categories from
1775 integrated data in (E).

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Supplementary Figure 12

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- Res1
- Res2 (FCGBP)
- Res3 (CCL18)
- Res4 (CCL2)
- IFN I
- IFN II
- TREM2 (SPP1)
- TREM2 (APOE)
- TREM2 (FN1)
- Inflam1 (IL1B)
- Inflam2 (CCL4)
- Early Activation Mac
- Classical Monocyte
- Non-Classical Monocyte
- cDC1
- cDC2 (CCL17)
- cDC2 (MHC Hi)
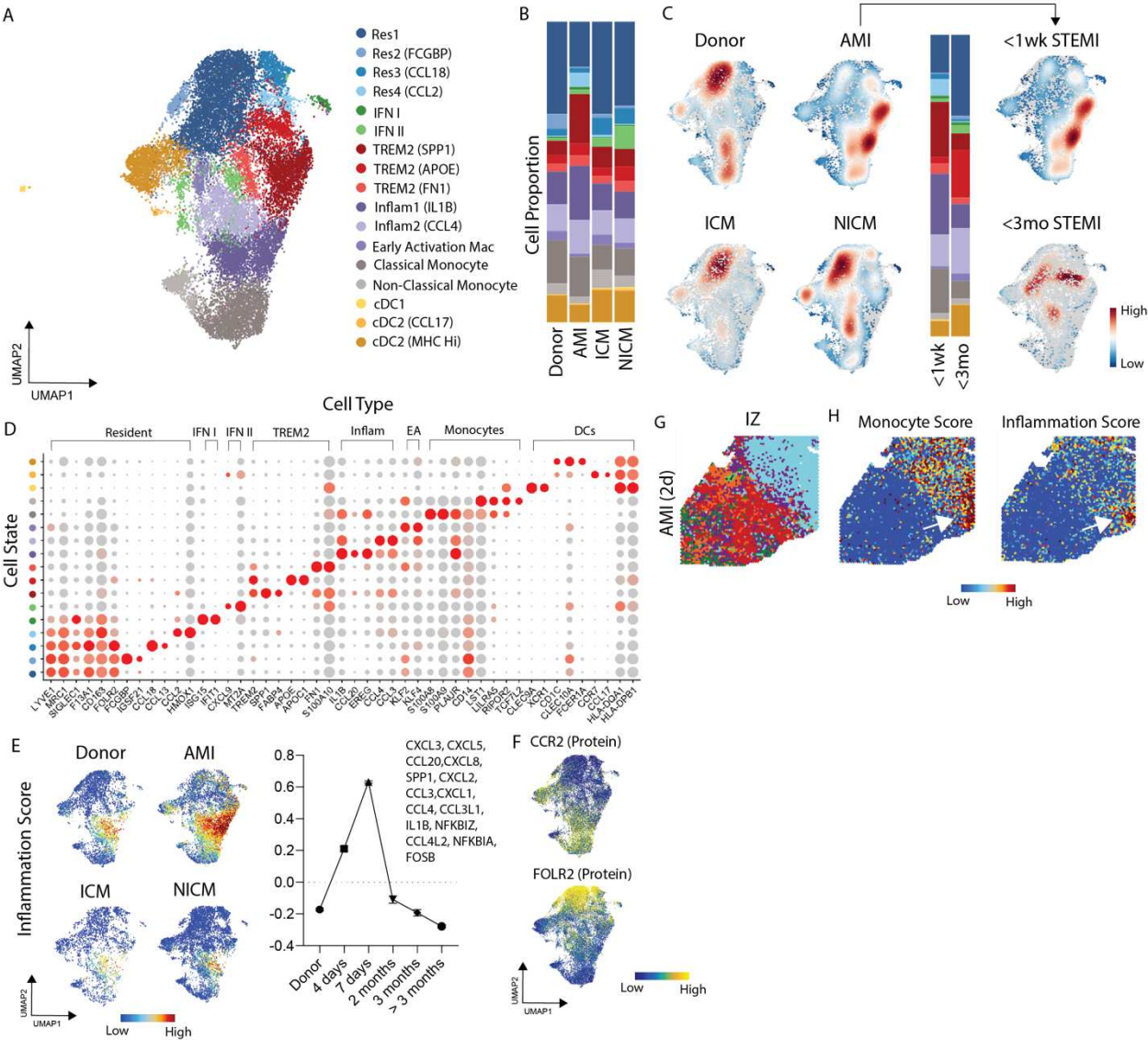
1815   **Supplementary Figure 12**. (A) UMAP embedding plot of myeloid cells with annotated cell states.

1816   (B) Myeloid cell state composition across four groups. (C) Gaussian kernel density estimation of

1817   cells across four groups (left) and split by MI time in acute MI patients with corresponding cell

1818   state composition. (D) Dot Plot of marker genes for macrophages cell states (y-axis) and grouped

1819   by cell type (x-asis). (E) Inflammation gene set score split across 4 groups and (g) grouped from

1820   time post-MI. (F) CCR2 and FOLR2 (protein) expression in UMAP embedding. (G) Spatial

1821   transcriptomic acute MI (2-day post-MI) infarct zone (IZ) sample with label transferred annotations

1822   from snRNA-seq reference map. (H) Monocyte gene set score mapped into space (left) and

1823   inflammation score (right).

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Supplementary Figure 13

A

Integrated Donor, AMI, ICM, NICM snRNAseq Atlas (n = 45)

snRNAseq
AMI/ICM (n = 7)

+

snRNAseq DCM
Atlas (n =38)

- Cardiomyocyte
- Endocardium
- Endothelium
- Epicardium
- Fibroblast
- Glia
- Adipocyte
- Lymphatic
- Mast
- Myeloid
- Pericyte
- SMC
- TNKCells

UMAP2
UMAP1

B

FAP

Donor

AMI

ICM

Low   High

C

Cardiomyocyte

Endo/SMC/Peri

Fibroblast

Myeloid

T-cells

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Low   High
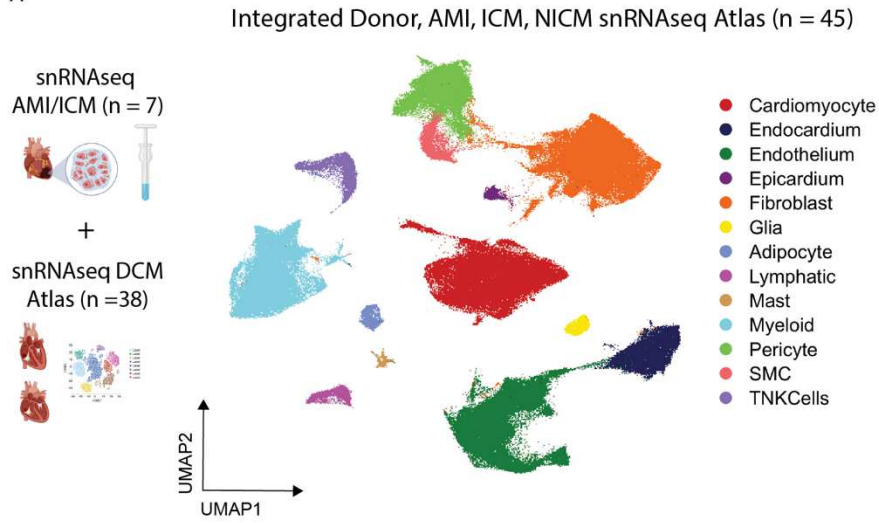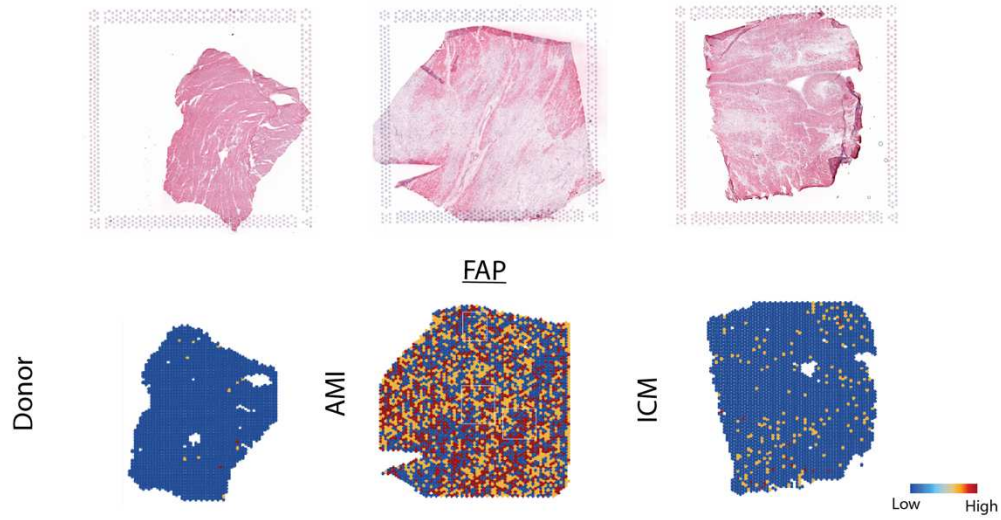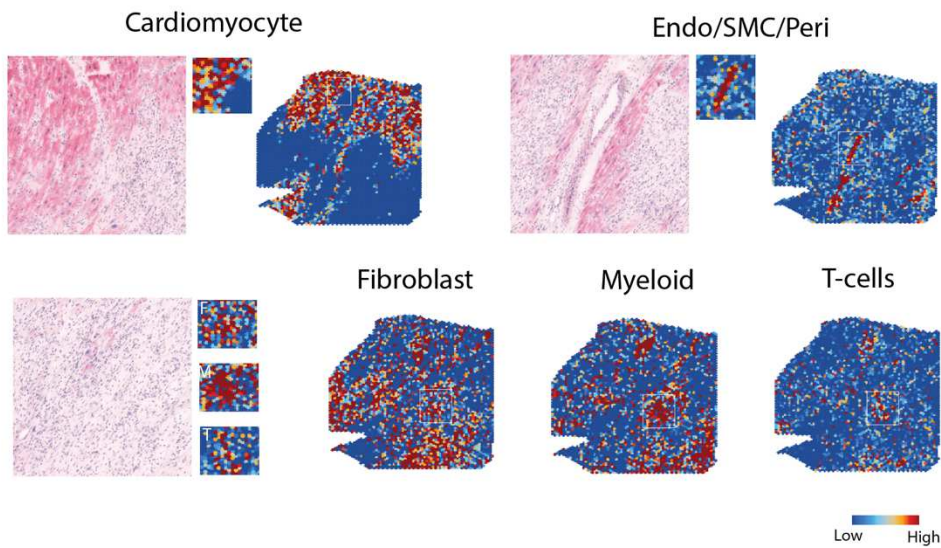
1828 **Supplementary Figure 13**. (A) UMAP embedding of integrated single nuclei RNA-seq data from
1829 donor, acute MI, ICM, and NICM. (B) HE spatial transcriptomics sections and corresponding FAP
1830 RNA expression in space. (C) Gene set signatures for cardiomyocytes,
1831 SMC/endothelium/pericytes, fibroblasts, myeloid cells, and T cells from clusters in (A) plotted in
1832 an acute MI sample.
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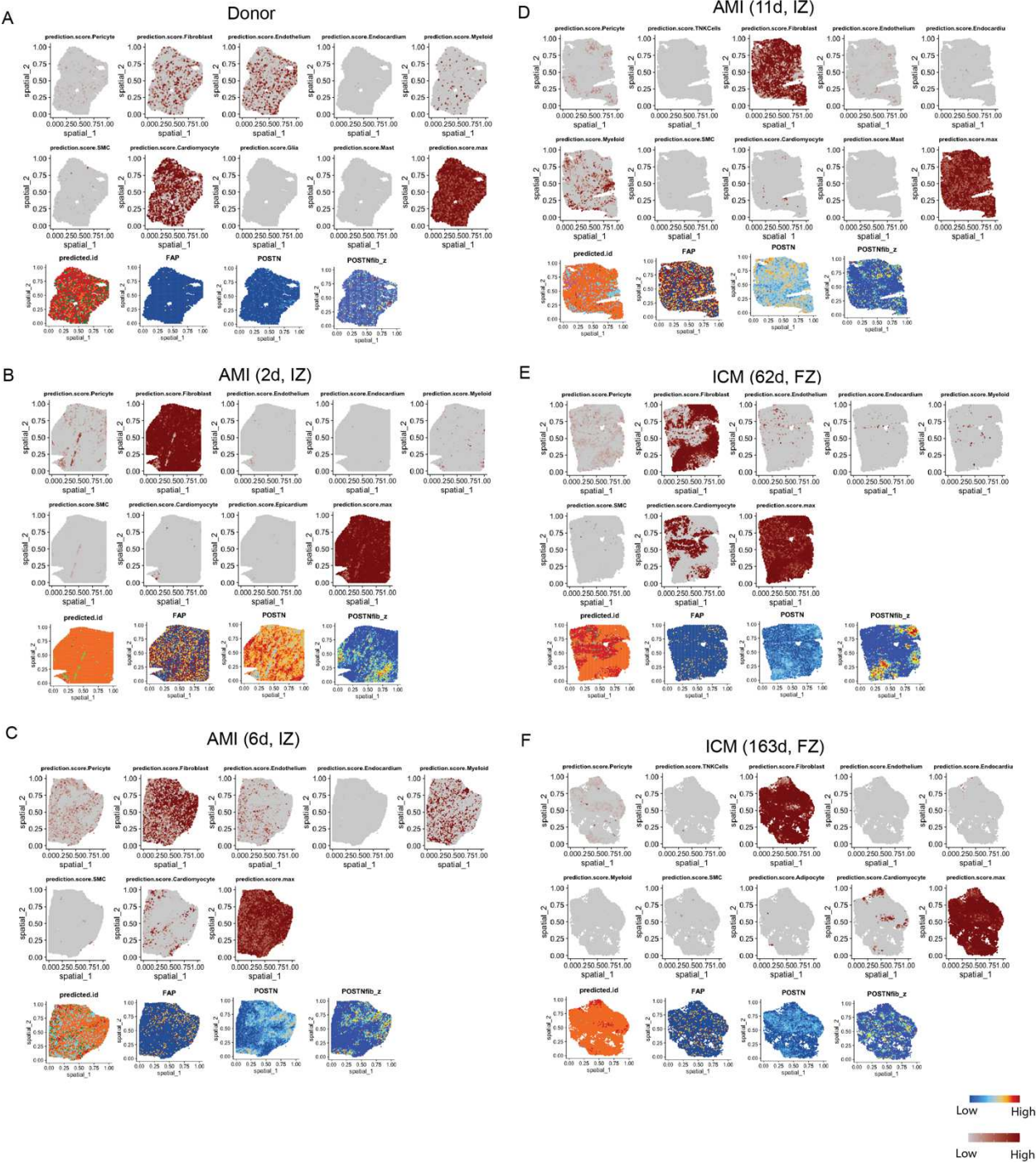
Supplementary Figure 14

1861   **Supplementary Figure 14**. Human snRNA-seq reference map imputed prediction scores,

1862   annotations, FAP expression, POSTN expression, and F9 fibroblast gene set score in (A) donor,

1863   (B) 2d acute MI IZ, (C) 6d acute MI IZ, (D) 11d acute MI IZ, (E) 62d ICM, FZ, and (F) 163d ICM

1864   FZ sptial transcriptomic sections.

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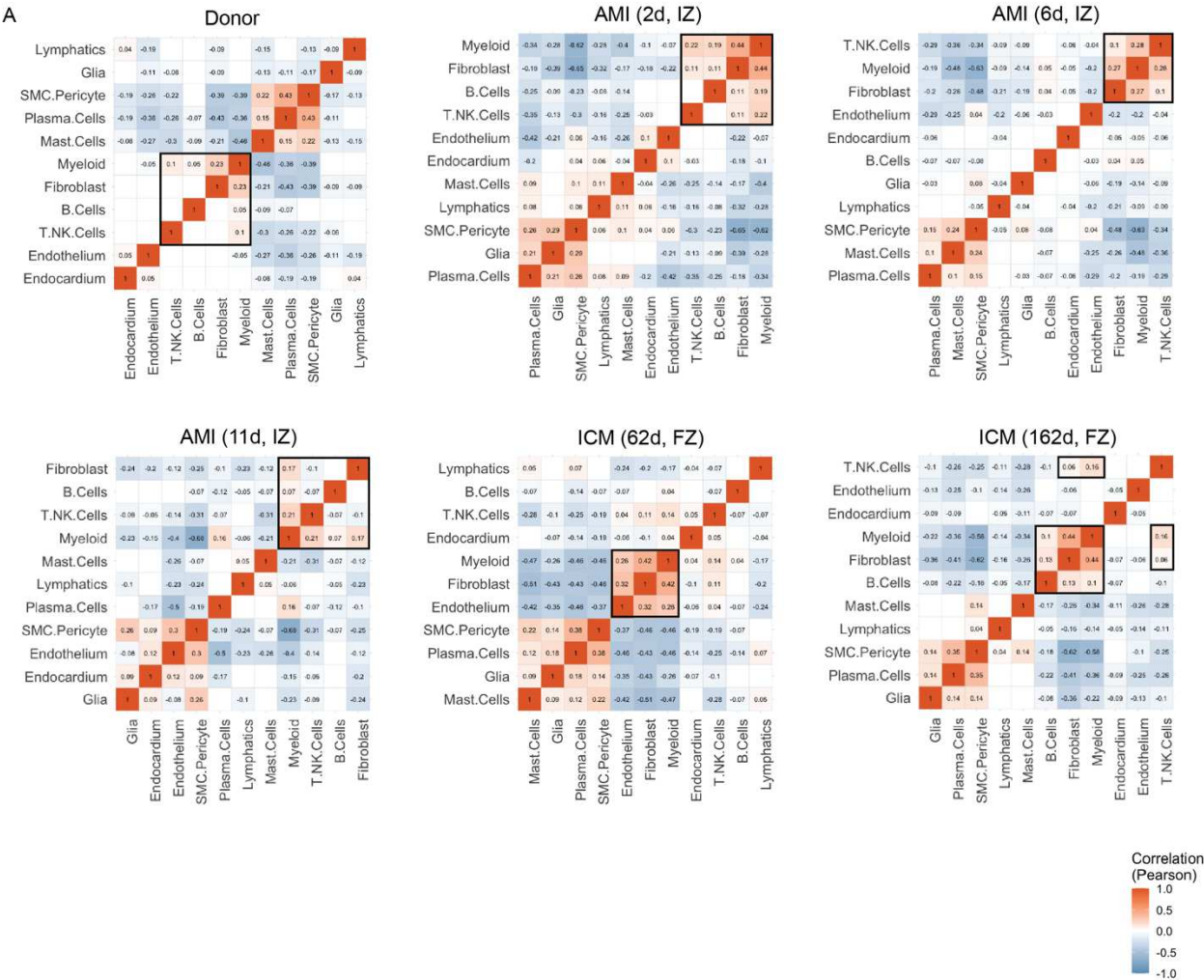
Supplementary Figure 15

1908   **Supplementary Figure 15**. (A) SPOTlight derived cell spot deconvolution Pearson correlation

1909   coefficients for cell neighborhoods in donor, acute MI and ICM spatial transcriptomic sections.

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A

Spatial Neighborhoods
Donor, AMI, ICM

UMAP2

UMAP1

n = 28

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- 12
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- 2
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C

FAP   POSTN   F9

CD68   CD68/FAP

Low   High

B

MYH7
TNNT
COL1A1
SPARC
ACTA1
SERPINE1
MT2A
ANKRD1
FABP4
MX1
ISG15
SPP1
C1QB
HBB
NPPB
TAGLN
ACTA2
MYH11
FN1
S100A9
XIRP2
IGKC

Low   High

D

CD68   F9

Expression Level

Spatial Region

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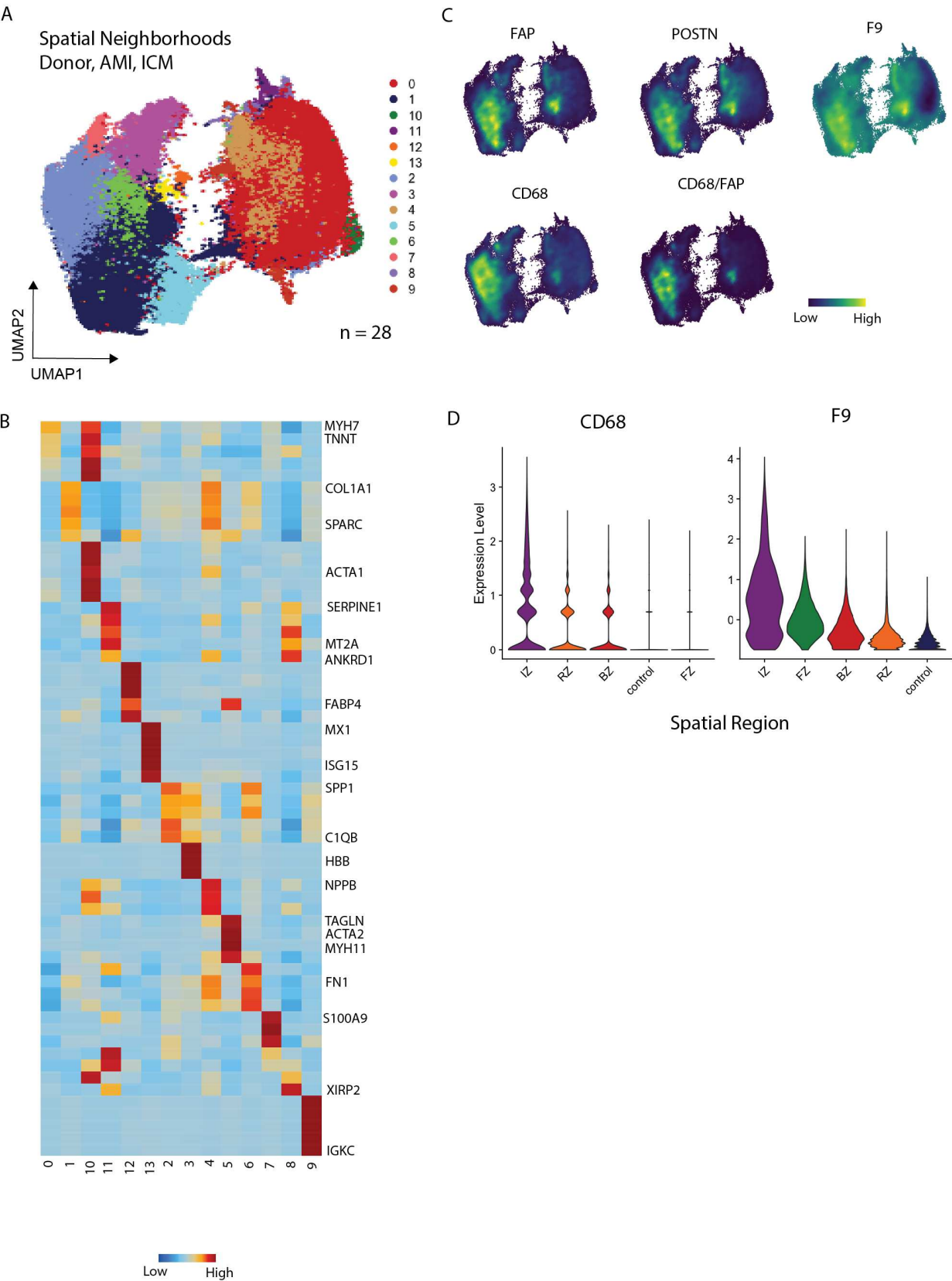
1913 **Supplementary Figure 16**. (A) Integrated UMAP embedding of n=28 spatial transcriptomic
1914 samples spatial spots. (B) Heatmap of average expression for top marker genes for spatial niches
1915 from (A). (C) FAP, POSTN, CD68 expression, CD68/FAP joint, and F9 gene set score density
1916 expression plots in integrated spatial UMAP embedding. (D) CD68 expression and F9 fibroblast
1917 gene set score violin plot split by regions.
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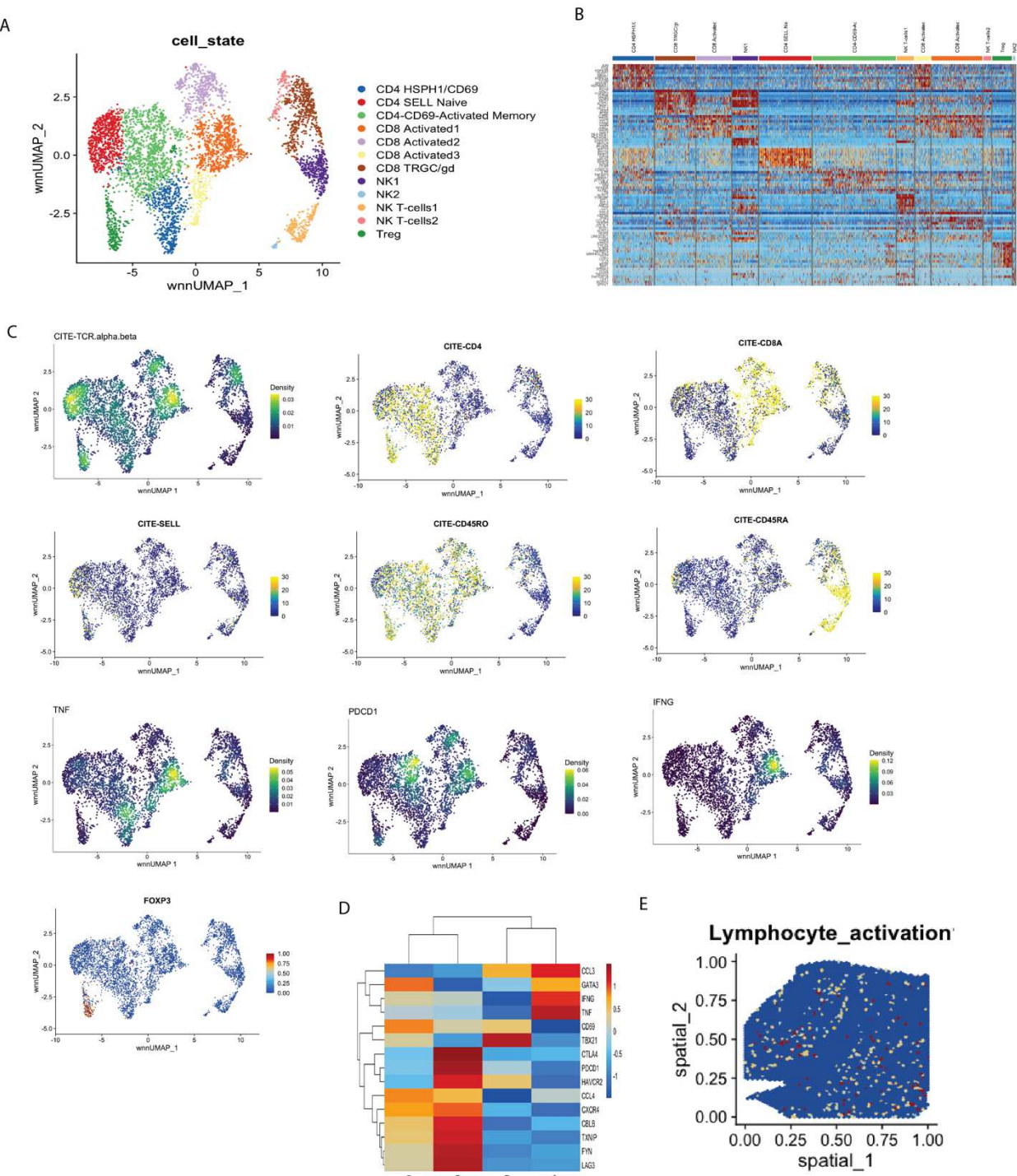
Supplementary Figure 17

1952 **Supplementary Figure 17**. (A) T cell states in WNN embedding space. (B) Top RNA markers for
1953 T cell states from (A). (C) Density, protein, and RNA plots for differentially expressed and key T
1954 cell markers. (D) RNA expression heatmap of key T cell genes (in different phenotypes) grouped
1955 by condition. (E) Lymphocyte activation signature plotted in AMI IZ spatial transcriptomic section.
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Supplementary Figure 18

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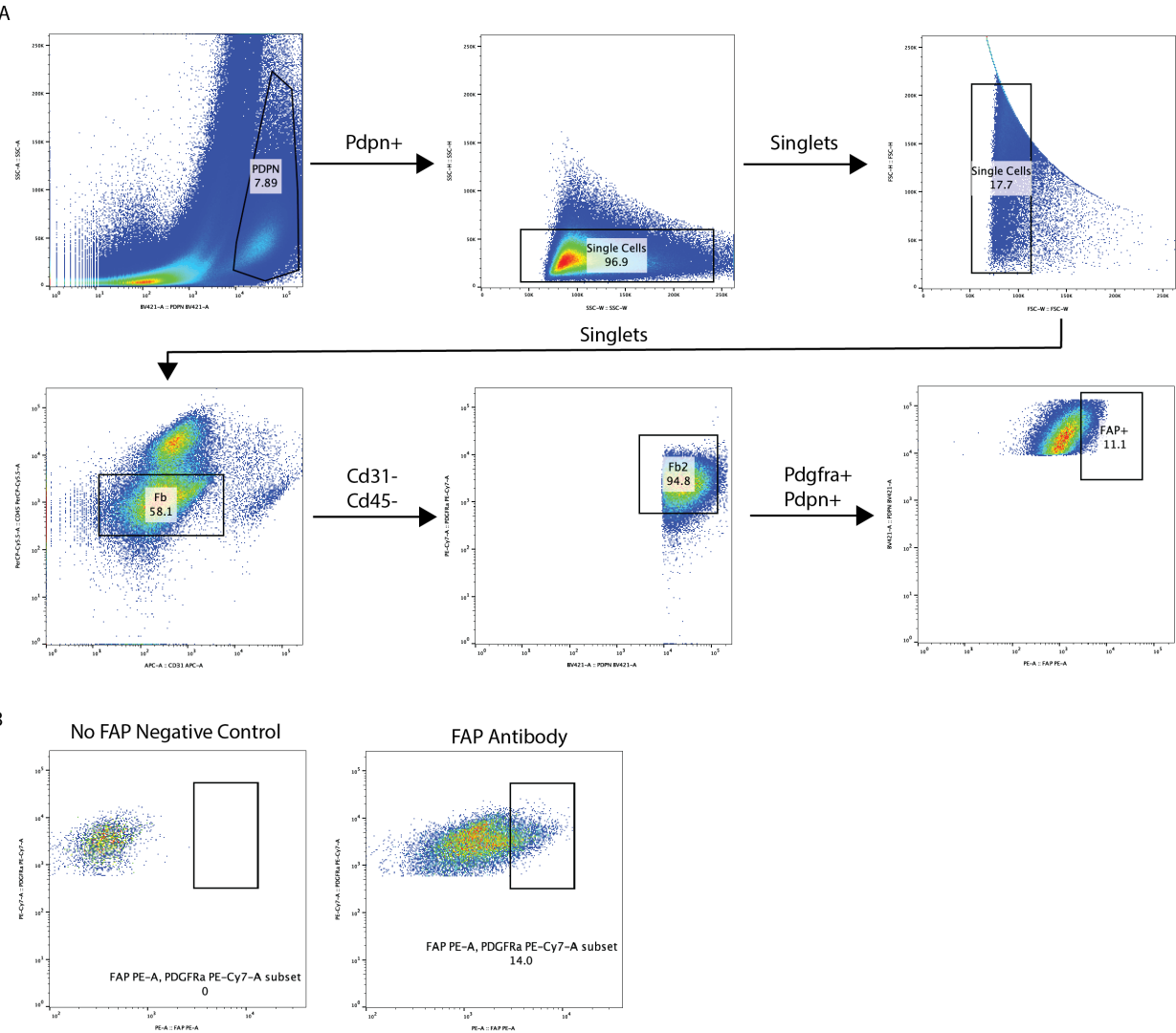
1997   **Supplementary Figure 18**. (A) Flow cytometry gating scheme for fibroblasts. (B) FAP+ gate

1998   construction with a no antibody-stained negative control.

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FAP+ GO BP

extracellular structure organization (GO:0043062)
external encapsulating structure organization (GO:0045229)
extracellular matrix organization (GO:0030198)
positive regulation of hormone secretion (GO:0046887)
endodermal cell differentiation (GO:0035987)
endoderm formation (GO:0001706)
regulation of cell population proliferation (GO:0042127)
integrin-mediated signaling pathway (GO:0007229)
collagen fibril organization (GO:0030199)
cellular protein metabolic process (GO:0044267)

D

Fap+ vs Fap-

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2050   **Supplementary Figure 19**. (A) QC metrics grouped by experimental condition for in vivo sorted

2051   FAP+/- fibroblasts at day 7 in Ang II/PE model with isotype/anti-IL-1b mAb treatment. (B) Heatmap

2052   of top marker genes for clusters in Fig. 7B. (C) Top GO BP pathways enriched in FAP+ fibroblasts

2053   relative to FAP- fibroblasts. (D) DE analysis between FAP+ and FAP- fibroblasts split by isotype

2054   and anti-IL-1b mAb groups dot plot (left) and number of genes up/down quantified (right).

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Supplementary Figure 20

A

Fap+ Ang II/PE day7

B

Fap+ Fibroblast cell state marker genes

C

Fap

Postn

Low   High

D

FAP+ Isotype GO BP

extracellular matrix organization (GO:0030198)
extracellular structure organization (GO:0043062)
external encapsulating structure organization (GO:0045229)
regulation of T cell migration (GO:2000404)
cytokine-mediated signaling pathway (GO:0019221)
positive regulation of bone mineralization (GO:0030501)
positive regulation of ossification (GO:0045778)
positive regulation of biomineral tissue development (GO:0070169)
regulation of chemokine production (GO:0032642)
positive regulation of chemokine production (GO:0032722)

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2104 **Supplementary Figure 20**. (A) Integrated UMAP with cub-clustering of FAP+ fibroblasts at day
2105 7 in Ang II/PE. (B) DotPlot of top marker genes for FAP+ cell states from (A). (C) Fap and Postn
2106 expression in UMAP embedding. (D) GO BP pathways enriched in isotype treated FAP+
2107 fibroblasts relative to FAP+ anti-IL-1b mAb treated mice.
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Supplementary Figure 21

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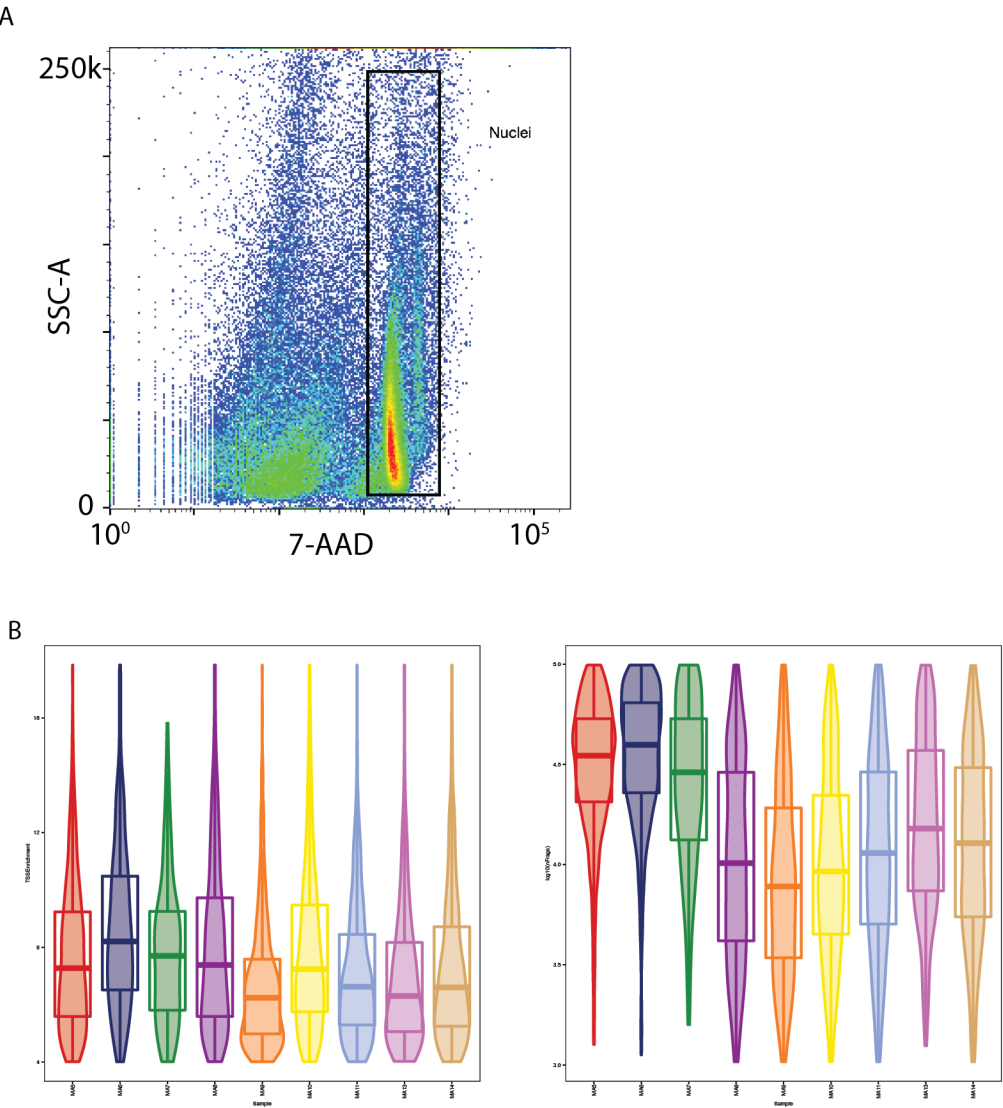
2146   **Supplementary Figure 21**. (A) Flow cytometry gating scheme to isolate nuclei using 7-AAD. (B)

2147   Multiome samples quality control metrics post QC and doublet removal box-violin plot split by

2148   sample; TSS enrichment (left) and log10 (nFrags) calculated in ArchR.

2149

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- table1.csv