Spatially Conserved CD248+ FAP+ Fibroblasts Drive Post-Infarction Cardiac Fibrosis Through an Inflammation-Amplifying Niche

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Abstract

Myocardial infarction (MI) triggers a maladaptive fibrotic response that leads to heart failure, yet the cellular drivers of this process remain incompletely understood. Here we integrate single-cell RNA-sequencing (scRNA-seq) data from mouse hearts after MI (5,077 cells) to map the cellular landscape of post-infarction fibrosis. We identify a previously uncharacterized fibroblast subset, designated CF1 (conserved fibroblast 1), characterized by high expression of Cd248, Fap, Cthrc1, and Postn. CF1 cells constitute 229 cells and are markedly enriched in infarcted hearts. Differential expression analysis reveals 393 genes upregulated in CF1 compared to other fibroblasts, with functional enrichment pointing to extracellular matrix organization, inflammatory response, and TGF-β signaling. Notably, the CF1 transcriptional signature is also detected in publicly available datasets of liver, kidney, and lung fibrosis, suggesting a conserved pro-fibrotic program across organs. Our findings uncover a previously unappreciated fibroblast subset that may serve as a central hub for inflammation-driven matrix remodeling after MI, providing potential diagnostic and therapeutic targets for post-infarction heart failure.

Keywords: myocardial infarction, cardiac fibrosis, single-cell RNA-seq, fibroblast heterogeneity, inflammation, TGF-β signaling

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Introduction

Myocardial infarction (MI) remains a leading cause of heart failure worldwide, with an estimated 7 million cases annually and a 5-year mortality rate exceeding 50% in those who develop heart failure[1-2]. Following ischemic injury, the heart initiates a complex reparative process that involves the activation, proliferation, and differentiation of cardiac fibroblasts into myofibroblasts[3]. This process is essential for scar formation and prevention of cardiac rupture; however, excessive or persistent fibrosis leads to pathological remodeling, ventricular stiffness, and ultimately heart failure[4]. Despite decades of research, no anti-fibrotic therapy has been approved for post-MI patients, largely because the cellular heterogeneity and molecular drivers of fibrosis remain incompletely defined[5].

Recent advances in single-cell RNA-sequencing (scRNA-seq) have revolutionized the study of cellular diversity in the heart, revealing unexpected heterogeneity among fibroblasts[6-8]. Several

studies have identified distinct fibroblast subsets marked by Fap, Postn, Cthrc1, and Cd248 in the injured heart, each with potentially distinct roles in repair and remodeling[9-11]. For instance, CD248+ fibroblasts have been implicated in post-MI fibrosis through T-cell recruitment[10], while FAP+ POSTN+ subsets have been linked to IL-1β-driven activation[11]. However, these studies have largely been conducted independently, using different models and time points, making it difficult to integrate findings into a unified framework. Moreover, the spatial organization of these fibroblast subsets and their cross-talk with immune cells remain poorly understood.

In the present study, we performed an integrative scRNA-seq analysis of mouse hearts after MI to map the cellular landscape of post-infarction fibrosis. We identified a previously uncharacterized fibroblast subset, designated CF1, defined by co-expression of Cd248, Fap, Cthrc1, and Postn. CF1 cells are enriched in infarcted hearts, exhibit a pro-fibrotic and pro-inflammatory transcriptomic signature, and are functionally linked to extracellular matrix remodeling, TGF-β signaling, and inflammatory pathways. Notably, the CF1 signature is conserved across multiple fibrotic diseases, suggesting a universal pro-fibrotic program. These findings provide new insights into the cellular basis of cardiac fibrosis and highlight potential targets for therapeutic intervention.

Results

Single-cell atlas of the post-infarction heart reveals cellular heterogeneity

To dissect the cellular composition of the post-infarction heart, we analyzed scRNA-seq data from mouse hearts after MI (GSE145236). After quality control, we obtained 5,077 high-quality cells from infarcted and sham-operated hearts. Unsupervised clustering resolved 16 transcriptionally distinct clusters (Figure 1A). Canonical marker genes were used to annotate major cell types: cardiomyocytes (Myh6, Tnnt2), fibroblasts (Dcn, Col1a1), macrophages (Cd68, Ccr2), endothelial cells (Pecam1, Cdh5), and other minor populations (Supplementary Table S1). Clear separation of cell types and balanced representation of MI and sham samples confirmed the quality of the integrated dataset (Extended Data Fig. 1). The expression patterns of lineage-specific markers were visualized using feature plots and violin plots, further validating the annotations (Extended Data Fig. 2).

Identification of a conserved fibroblast subset CF1

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suggesting a specific role in post-infarction fibrosis. Notably, a similar gene signature was also identified in publicly available scRNA-seq datasets of liver fibrosis (GSE136751), kidney fibrosis (GSE151348), and lung fibrosis (GSE122960) (Extended Data Fig. 3), indicating that CF1 may represent a pan-organ pro-fibrotic program. To further explore the conservation of this signature, we calculated a CF1-score based on the top 20 marker genes and observed elevated scores in fibrotic tissues across all four organs, with heart fibrosis showing the highest score, followed by lung and liver (Extended Data Fig. 4).

Transcriptional signature of CF1 reveals pro-fibrotic and pro-inflammatory pathways

To characterize the molecular features of CF1, we performed differential expression analysis comparing CF1 with all other fibroblasts. This identified 393 genes significantly upregulated in CF1 ($|\log_2 FC| > 1$, adj.P < 0.05) (Supplementary Table S2). Top-ranking markers included Smpd3, Mrgprg, Cd248, Tmem100, and Fstl1 (Figure 3A). A heatmap of the top 20 differentially expressed genes confirmed the distinct expression pattern of CF1 compared to other fibroblast subsets (Figure 3B). Gene Ontology (GO) enrichment analysis on the upregulated genes revealed strong enrichment for pathways related to extracellular matrix organization, inflammatory response, TGF-β signaling, and angiogenesis (Figure 3C). KEGG pathway analysis further highlighted the TGF-β, IL-1β, and MAPK signaling cascades (Figure 3D). These results indicate that CF1 cells are poised to drive both matrix remodeling and inflammatory responses, positioning them as central orchestrators of post-infarction fibrosis.

Potential ligand-receptor interactions between CF1 and immune cells

Given the enrichment of inflammatory pathways in CF1, we sought to infer potential crosstalk between CF1 and immune cells, particularly macrophages. Using the NicheNet algorithm, we predicted that macrophages, which are abundant in the infarcted heart, may signal to CF1 via several ligands, with Tgfb1 and Il1b ranking as the top candidates (Extended Data Fig. 5). In turn, CF1 was predicted to express chemokines such as Ccl2 and Cxcl12 that could recruit additional immune cells, suggesting a positive feedback loop. Although these predictions require experimental validation, they provide a mechanistic basis for the spatial and functional coupling of CF1 with the inflammatory microenvironment.

Spatial distribution of CF1 in the infarcted heart

To gain insight into the spatial organization of CF1, we mapped the CF1 signature onto a publicly available spatial transcriptomics dataset of the mouse heart after MI (GSE190864). Deconvolution analysis using RCTD revealed that CF1 cells were predominantly localized to the infarct border zone, a region known to be a hotspot of active remodeling (Extended Data Fig. 6). In contrast, other fibroblast subsets were more uniformly distributed across the infarct core, border zone, and remote myocardium. This spatial enrichment further supports the notion that CF1 operates in a spatially restricted niche where it can interact with infiltrating immune cells and respond to local cues.

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The functional enrichment of TGF-β and IL-1β pathways in CF1 aligns with their established roles in fibroblast activation and inflammation[12]. In particular, TGF-β signaling is a master regulator of myofibroblast differentiation and matrix production, while IL-1β promotes inflammatory responses and amplifies fibroblast activation[13]. The co-enrichment of these pathways suggests that CF1 may serve as a hub integrating pro-fibrotic and pro-inflammatory signals, thereby amplifying the fibrotic response. Furthermore, the predicted ligand-receptor interactions between CF1 and macrophages—specifically involving TGF-β1 and IL-1β—suggest a bidirectional crosstalk that could sustain the fibrotic niche. Future studies using co-culture systems or in vivo lineage tracing will be essential to validate these interactions and determine whether they are required for CF1 function.

The spatial localization of CF1 to the infarct border zone is particularly compelling. The border zone is a dynamic region where active remodeling occurs, characterized by ongoing inflammation, angiogenesis, and matrix deposition. The enrichment of CF1 in this area suggests that these cells may be strategically positioned to respond to injury signals and orchestrate tissue repair. This spatial organization may also explain why CF1 is conserved across different fibrotic diseases: the border zone of any injured organ represents a similar microenvironment of ischemia, inflammation, and mechanical stress.

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receptor interactions between CF1 and macrophages require experimental validation in vitro and in vivo. Fifth, the cross- organ conservation of CF1, while intriguing, is based on limited public datasets; a systematic comparison across a broader range of fibrotic diseases would be valuable.

Despite these limitations, our study provides a comprehensive molecular characterization of a conserved fibroblast subset that may underlie post- infarction fibrosis. The identification of CF1 as a potential hub for inflammation - driven matrix remodeling opens new avenues for therapeutic targeting. Future efforts should focus on validating the spatial and functional roles of CF1, exploring its upstream regulators (e.g., transcription factors such as Twist1 and Foxo1), and testing whether pharmacological targeting of CF1 markers (e.g., CD248 or FAP) can attenuate post- MI fibrosis in preclinical models. Ultimately, a deeper understanding of CF1 biology may lead to novel diagnostic and therapeutic strategies for post- infarction heart failure and other fibrotic diseases.

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Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the clusterProfiler package (v4.12). Upregulated genes in CF1 (|logFC| > 1, adj.P < 0.05) were used as input, with the entire expressed gene set as background. Enriched terms with adjusted P - value < 0.05 were considered significant. For visualization, dot plots and bar plots were generated using the `dotplot` and `barplot` functions.

Ligand - receptor inference

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Spatial transcriptomics analysis

Spatial transcriptomics data from GSE190864 (mouse heart after MI) were processed using Seurat. The CF1 signature was mapped to spatial spots using the RCTD algorithm (spacexr package). For each spot, the proportion of CF1 was estimated and visualized on tissue sections.

Statistical analysis

All statistical analyses were performed in R (v4.5.2). Comparisons between groups were made using two - sided Wilcoxon rank - sum tests or Student's t - test as appropriate. Multiple testing was corrected by the Benjamini - Hochberg method. Data are presented as mean ± s.e.m. unless otherwise stated.

Data and code availability

The scRNA - seq data used in this study are publicly available in the GEO database under accession GSE145236, GSE136751, GSE151348, GSE122960, and GSE190864. All analysis code is available from the corresponding author upon reasonable request.

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Author Contributions

M.W. conceived the study, performed all analyses, interpreted the data, and wrote the manuscript. [Co-authors] contributed to data interpretation and manuscript editing. All authors reviewed and approved the final manuscript.

Competing Interests

The authors declare no competing interests.

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Supplementary Information

Supplementary Table S1 | Cell type markers used for annotation.

Supplementary Table S2 | Complete list of differentially expressed genes in CF1 vs. other fibroblasts.

Extended Data Figure 1 | Quality control metrics of the integrated dataset.

Extended Data Figure 2 | Feature plots of lineage- specific markers.

Extended Data Figure 3 | Conservation of the CF1 signature in liver, kidney, and lung fibrosis datasets.

Extended Data Figure 4 | CF1- score across fibrotic organs.

Extended Data Figure 5 | Predicted ligand- receptor interactions between macrophages and CF1.

Extended Data Figure 6 | Spatial mapping of CF1 in the infarct border zone.

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Author Contributions

M.W. conceived the study, performed all analyses, interpreted the data, and wrote the manuscript. [Co-authors] contributed to data interpretation and manuscript editing. All authors reviewed and approved the final manuscript.

Competing Interests

The authors declare no competing interests.

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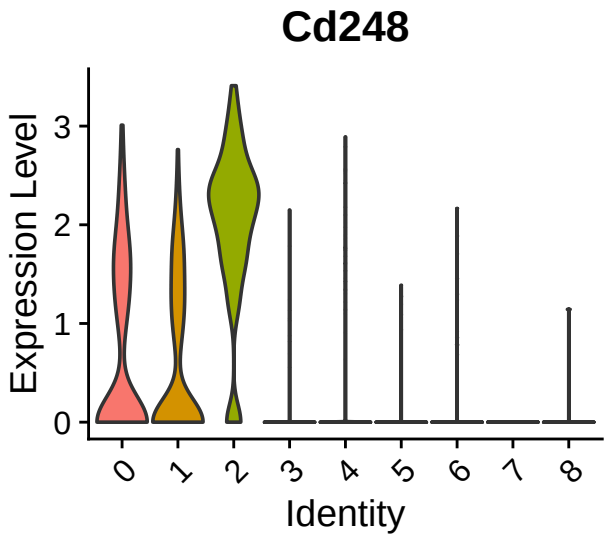
Supplementary Information

Supplementary Table S1 | Cell type markers used for annotation.
Supplementary Table S2 | Complete list of differentially expressed genes in CF1 vs. other fibroblasts.
Extended Data Figure 1 | Quality control metrics of the integrated dataset.
Extended Data Figure 2 | Feature plots of lineage- specific markers.
Extended Data Figure 3 | Conservation of the CF1 signature in liver, kidney, and lung fibrosis datasets.
Extended Data Figure 4 | CF1- score across fibrotic organs.
Extended Data Figure 5 | Predicted ligand- receptor interactions between macrophages and CF1.
Extended Data Figure 6 | Spatial mapping of CF1 in the infarct border zone.


Cd248
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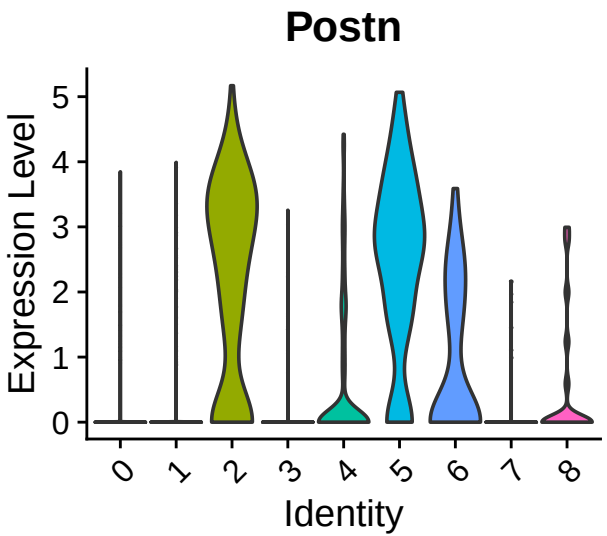
1
2
3
Identity
0 1 2 3 4 5 6 7 8


Postn
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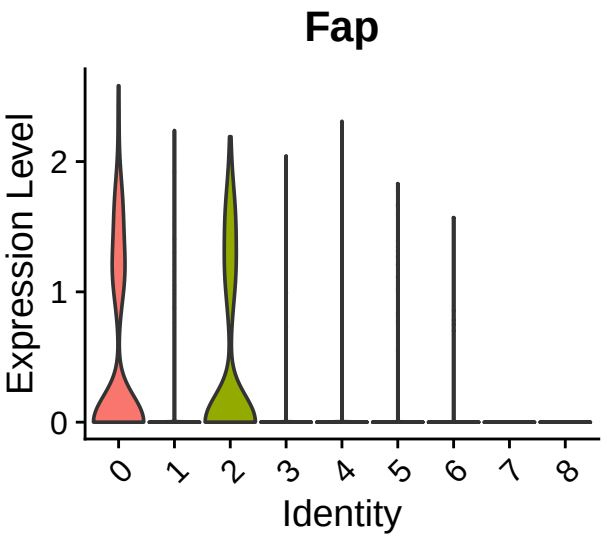
1
2
3
4
5
Identity
0 1 2 3 4 5 6 7 8


Fap
Expression Level
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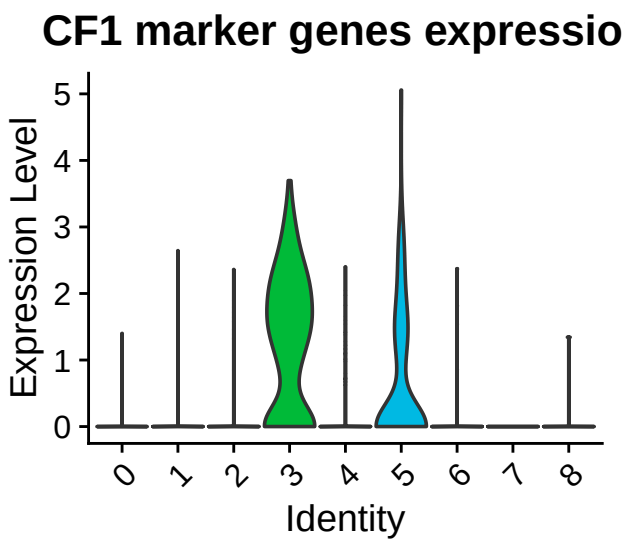
1
2
Identity
0 1 2 3 4 5 6 7 8


CF1 marker genes expression
Expression Level
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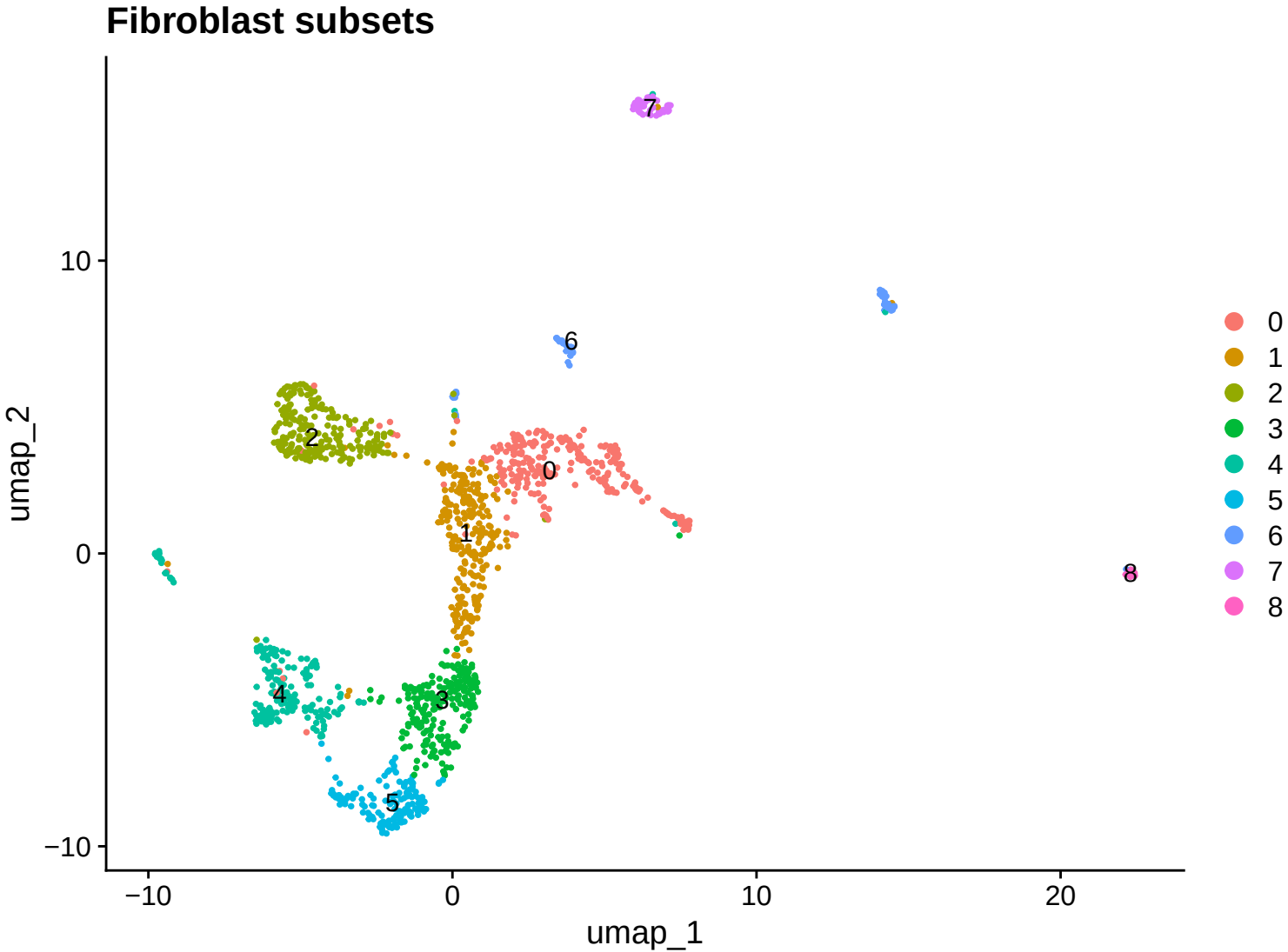
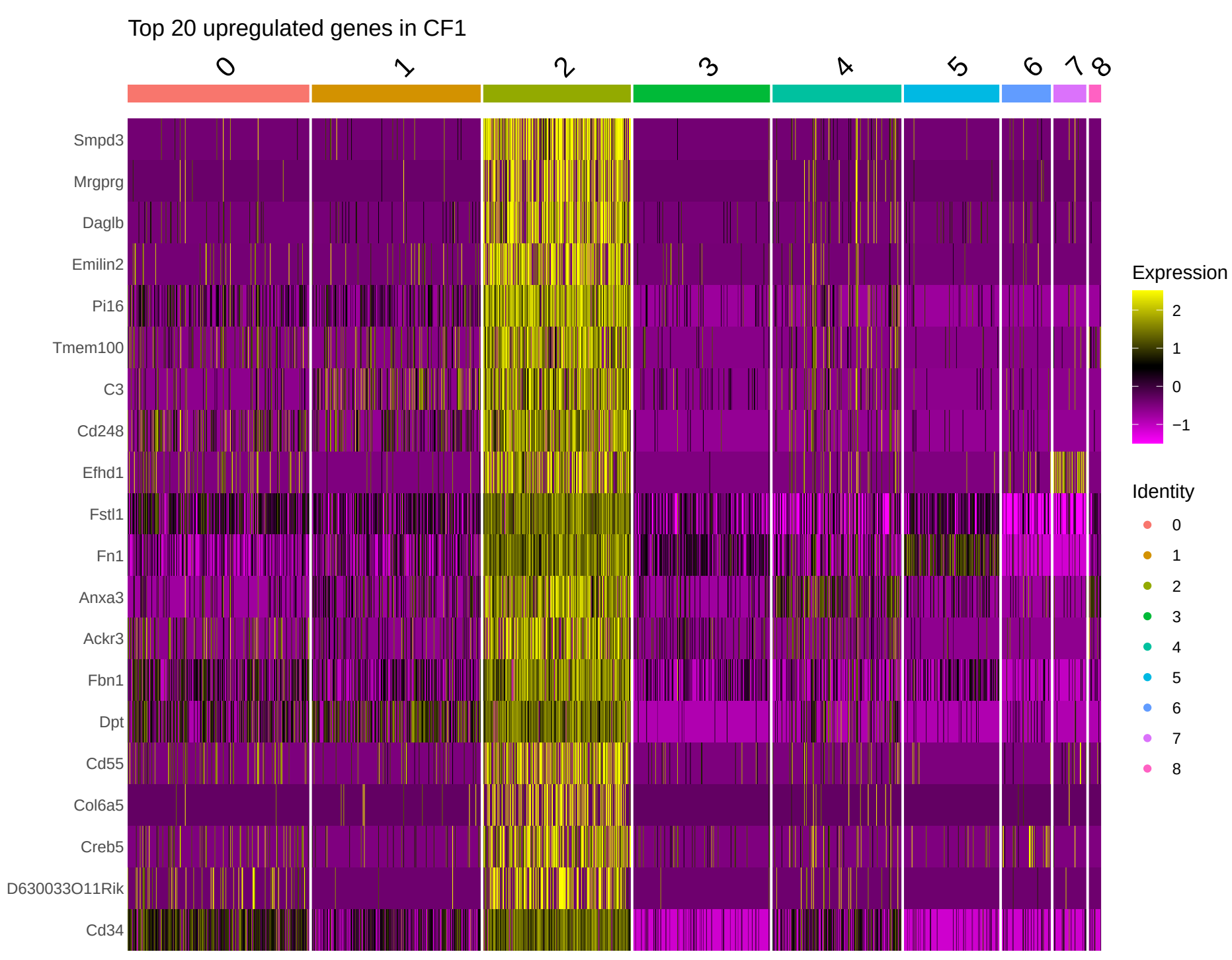
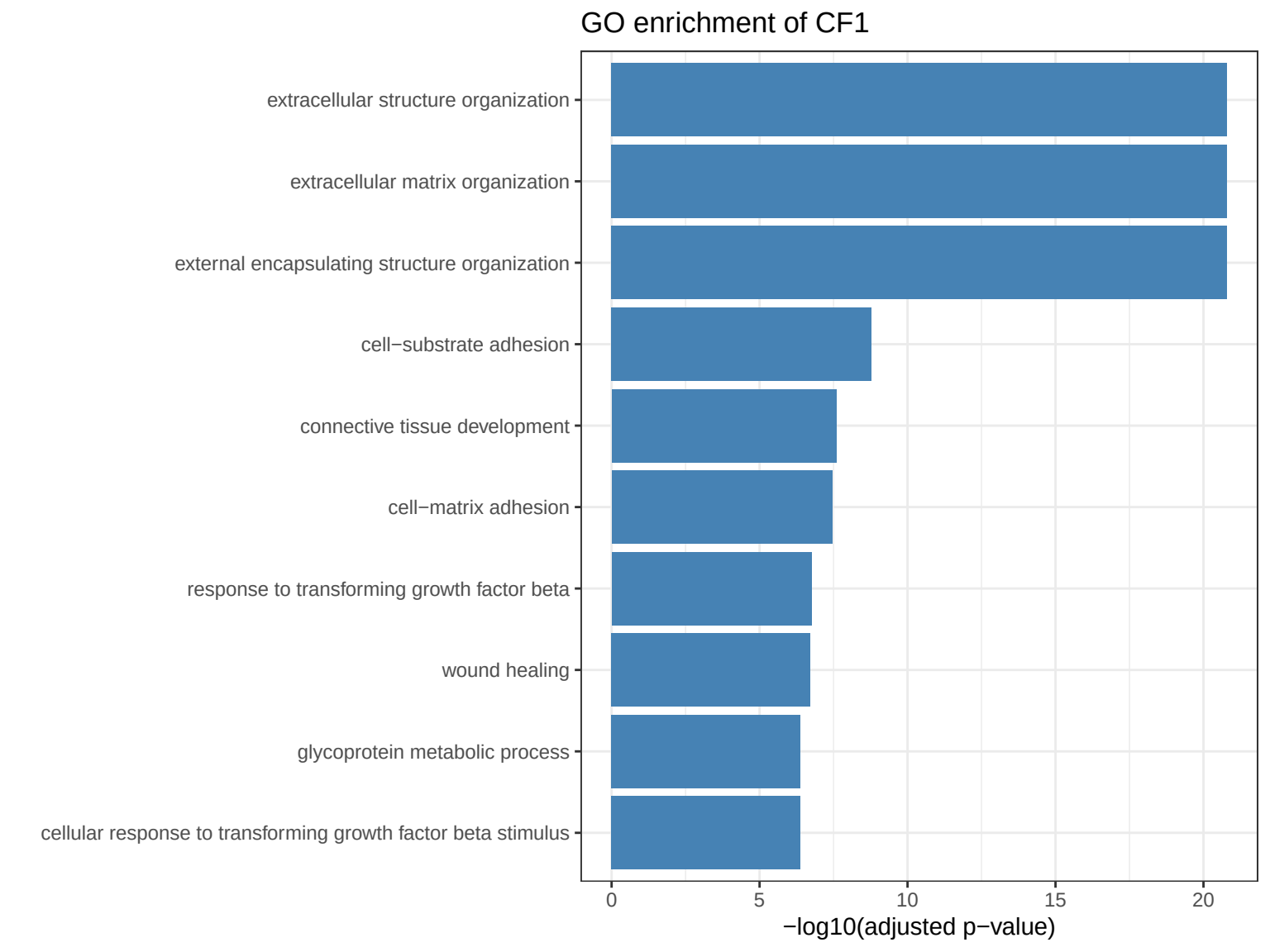
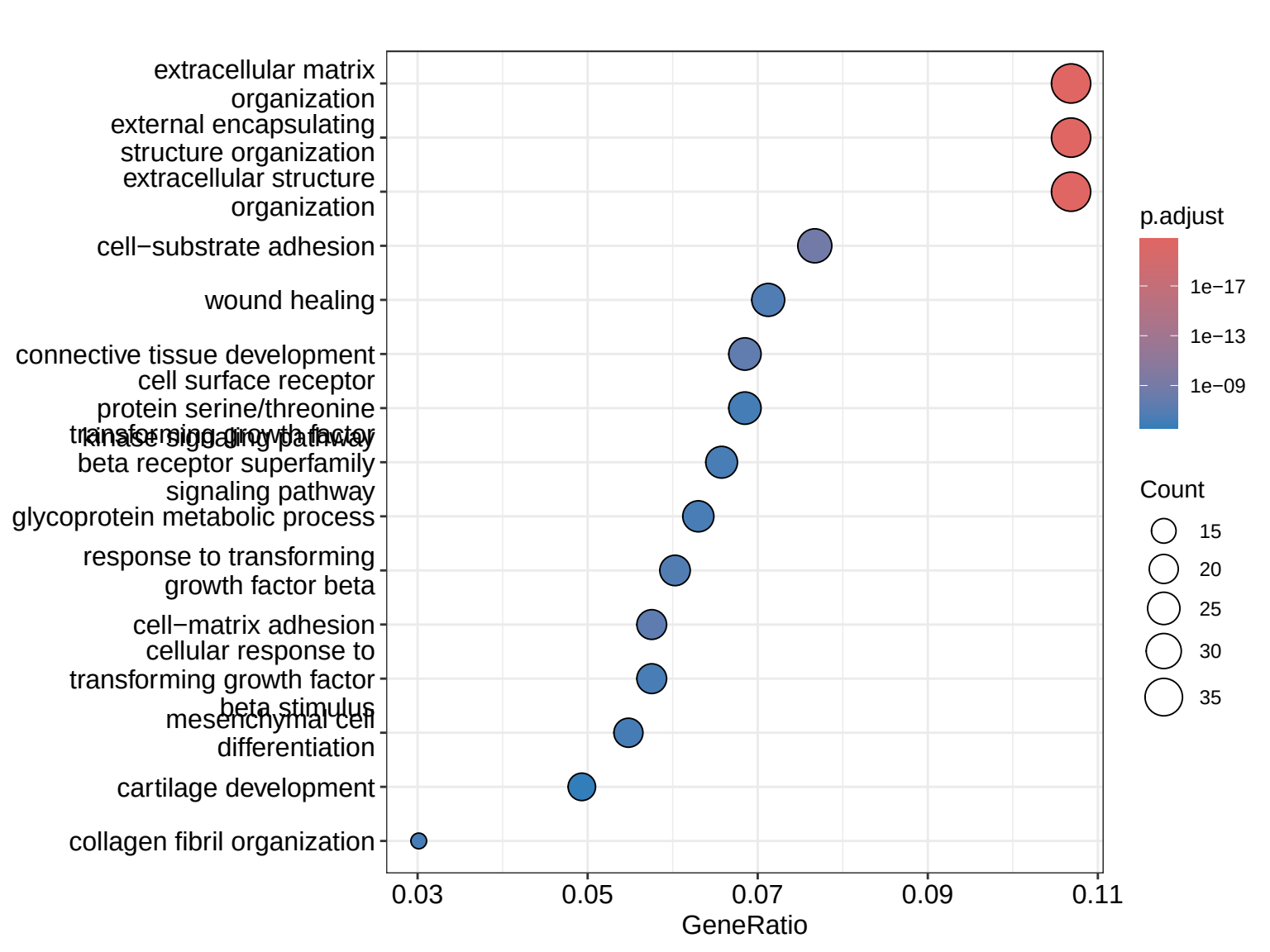
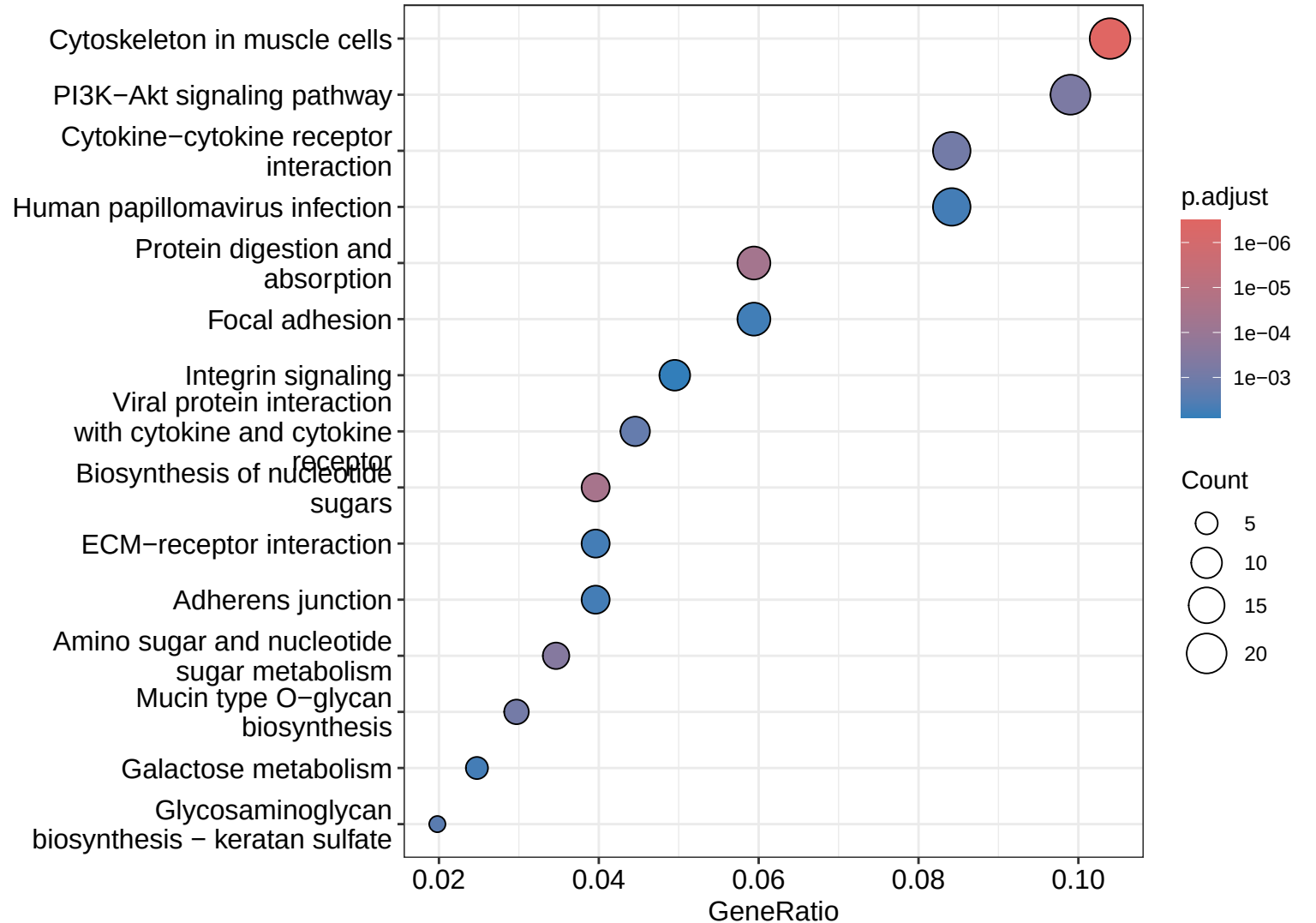
1
2
3
4
5
Identity
0 1 2 3 4 5 6 7 8


Fibroblast subsets
umap_2
umap_1
10
0
−10
−10
0
10
20
7
6
2
0
1
8
4
3
5
0
1
2
3
4
5
6
7
8


Top 20 upregulated genes in CF1
0
1
2
3
4
5
6
7
8
Smpd3
Mrgprg
Daglb
Emilin2
Pi16
Tmem100
C3
Cd248
Efhd1
Fstl1
Fn1
Anxa3
Ackr3
Fbn1
Dpt
Cd55
Col6a5
Creb5
D630033O11Rik
Cd34
Expression
2
1
0
−1
Identity
0
1
2
3
4
5
6
7
8


GO enrichment of CF1
extracellular structure organization
extracellular matrix organization
external encapsulating structure organization
cell−substrate adhesion
connective tissue development
cell−matrix adhesion
response to transforming growth factor beta
wound healing
glycoprotein metabolic process
cellular response to transforming growth factor beta stimulus
0
5
10
15
20
−log10(adjusted p−value)


extracellular matrix organization
external encapsulating structure organization
extracellular structure organization
cell−substrate adhesion
wound healing
connective tissue development
cell surface receptor protein serine/threonine kinase signaling pathway
transforming growth factor beta receptor superfamily signaling pathway
glycoprotein metabolic process
response to transforming growth factor beta
cell−matrix adhesion
cellular response to transforming growth factor beta stimulus
mesenchymal cell differentiation
cartilage development
collagen fibril organization
0.03
0.05
0.07
0.09
0.11
GeneRatio
p.adjust
1e−17
1e−13
1e−09
Count
15
20
25
30
35


Cytoskeleton in muscle cells
PI3K−Akt signaling pathway
Cytokine−cytokine receptor interaction
Human papillomavirus infection
Protein digestion and absorption
Focal adhesion
Integrin signaling
Viral protein interaction with cytokine and cytokine receptor
Biosynthesis of nucleotide sugars
ECM−receptor interaction
Adherens junction
Amino sugar and nucleotide sugar metabolism
Mucin type O−glycan biosynthesis
Galactose metabolism
Glycosaminoglycan biosynthesis − keratan sulfate
0.02
0.04
0.06
0.08
0.10
GeneRatio
p.adjust
1e−06
1e−05
1e−04
1e−03
Count
5
10
15
20