Supplementary Figure 1

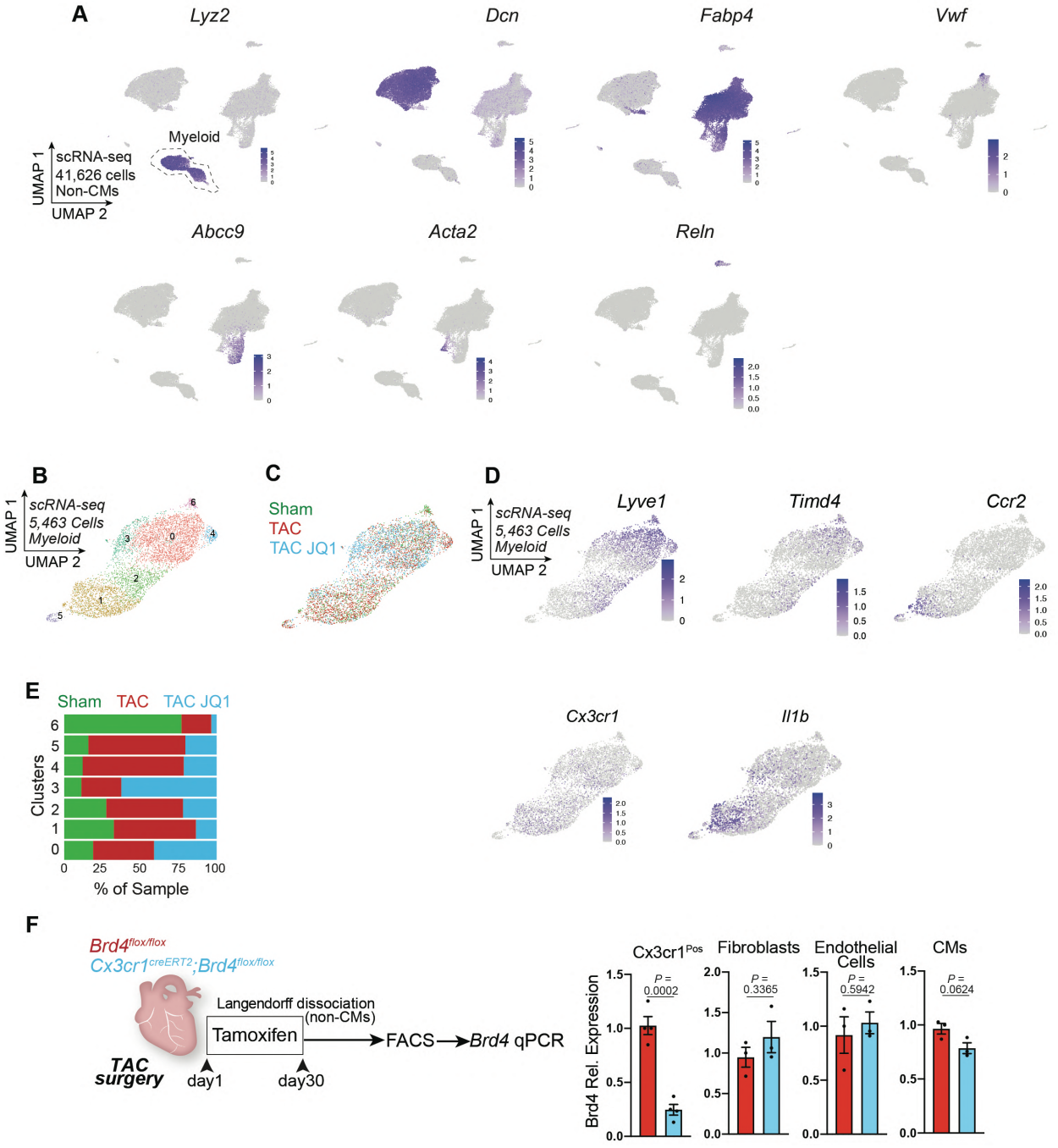

Figure S1: Single-cell RNA-seq in heart failure with BET inhibition identifies a highly dynamic Cx3cr1-expressing monocyte/macrophage subpopulation

A. *Expression Feature Plots (scRNA-seq) of cell population markers in non-CM cells.* ***B,C****. UMAP plot (scRNA-seq) of myeloid cells colored by cluster (B) and sample identity (C).* ***D.*** *Expression Feature Plots (scRNA-seq) of Lyve1, Timd4, Ccr2, Cx3cr1 and Il-1b in myeloid cells.* ***E****. Sample distribution within clusters in myeloid cells.* ***F****. Schematic of experimental settings for conditional Brd4 deletion in Cx3cr1^{Pos} cells. Brd4 expression was measured by qPCR in sorted CX3CR1^{Pos}, fibroblasts (mEF-SK4^{Pos}, CD45^{Neg}, CD31^{Neg}), endothelial cells (mEF-SK4^{Neg}, CD45^{Neg}, CD31^{Pos}) and in unsorted CMs.* ***F****, Data are mean ± s.e.m. Unpaired, two-tailed Student's t-test.*

Supplementary Figure 2

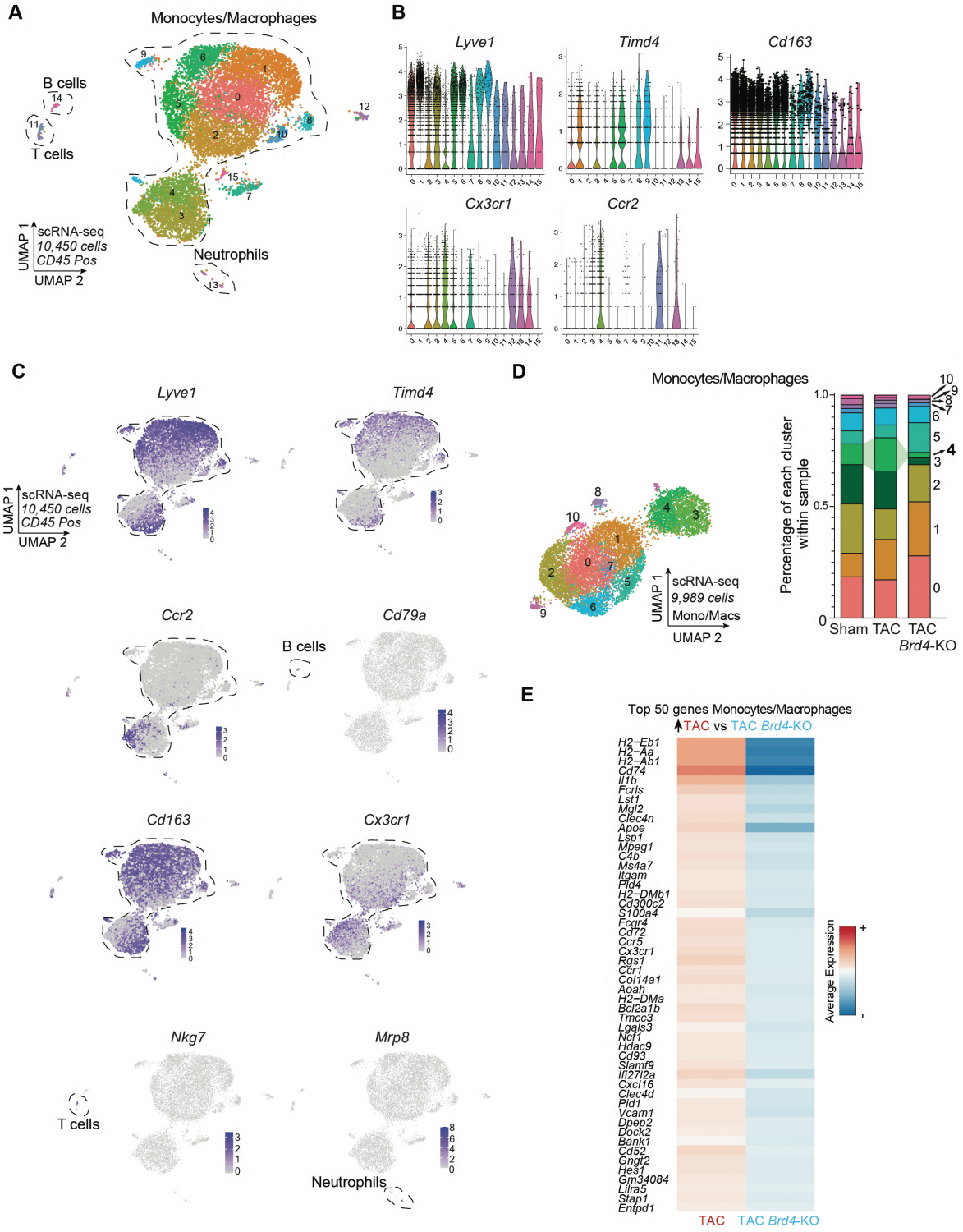

Figure S2: Identification of monocyte/macrophage population with single-cell RNA-seq

A. *UMAP plot (scRNA-seq) of CD45^{Pos} cells colored by cluster identity.* ***B****. Expression Violin Plots of Lyve1, Timd4, Cd163, Cx3cr1 and Ccr2 in CD45^{Pos} cells.* ***C.*** *Expression Feature Plots (scRNA-seq) of Lyve1, Timd4, Ccr2, Cd79a, Cd163, Cx3cr1, Nkg7 and Mrp8 in CD45^{Pos} cells.* ***D.*** *UMAP plot (scRNA-seq) of monocytes/macrophages colored by cluster (left) and cluster composition within samples (right). Changes in cluster 4 are highlighted.* ***E.*** *Heatmap of expression (scRNA-seq) depicting the top 50 genes upregulated in TAC versus TAC Brd4-KO in the monocyte/macrophage population.*

Supplementary Figure 3

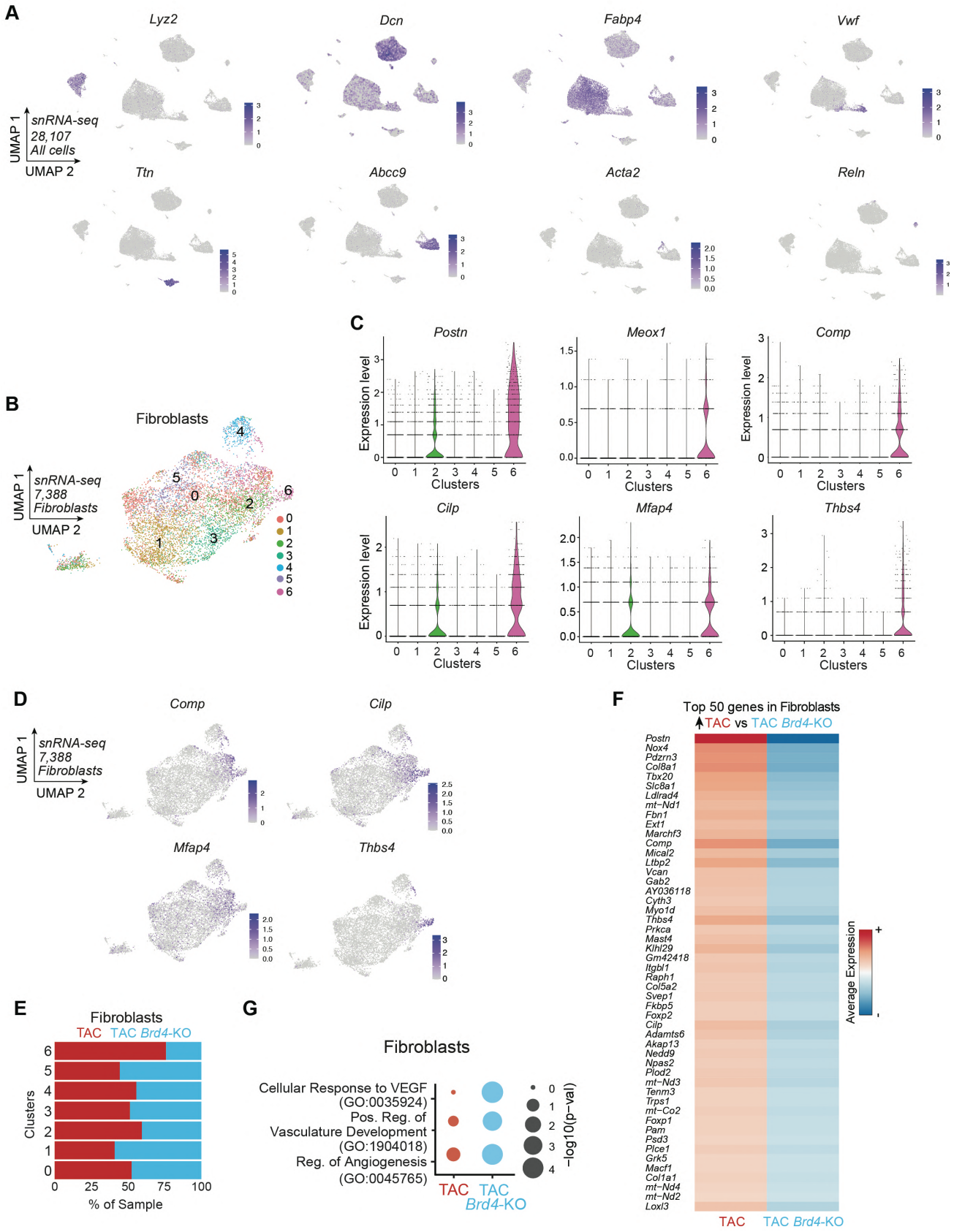

Figure S3: Decreased profibrotic signature in fibroblasts with Brd4 deletion in Cx3cr1-expressing monocytes/macrophages.

A. *Expression Feature Plots (snRNA-seq) of cell population markers in nuclei from cardiac tissue.* ***B****. UMAP plot (snRNA-seq) of fibroblasts colored by cluster identity.* ***C.*** *Expression Violin Plots (snRNA-seq) of Postn, Meox1, Comp, Cilp, Mfap4 and Thbs4 across clusters in fibroblasts.* ***D****. Expression Feature Plots (snRNA-seq) of Comp, Cilp, Mfap4 and Thbs4 in fibroblasts.* ***E****. snRNA-seq sample distribution within fibroblast clusters.* ***F****. Heatmap of expression (snRNA-seq) depicting the top 50 genes upregulated in TAC versus TAC Brd4-KO in fibroblasts.* ***G****. Dot Plot indicating significance (−log10(p−val)) for indicated GO terms in genes upregulated in TAC vs TAC Brd4-KO (red) or upregulated in TAC Brd4-KO vs TAC (blue) in fibroblasts.*

Supplementary Figure 4

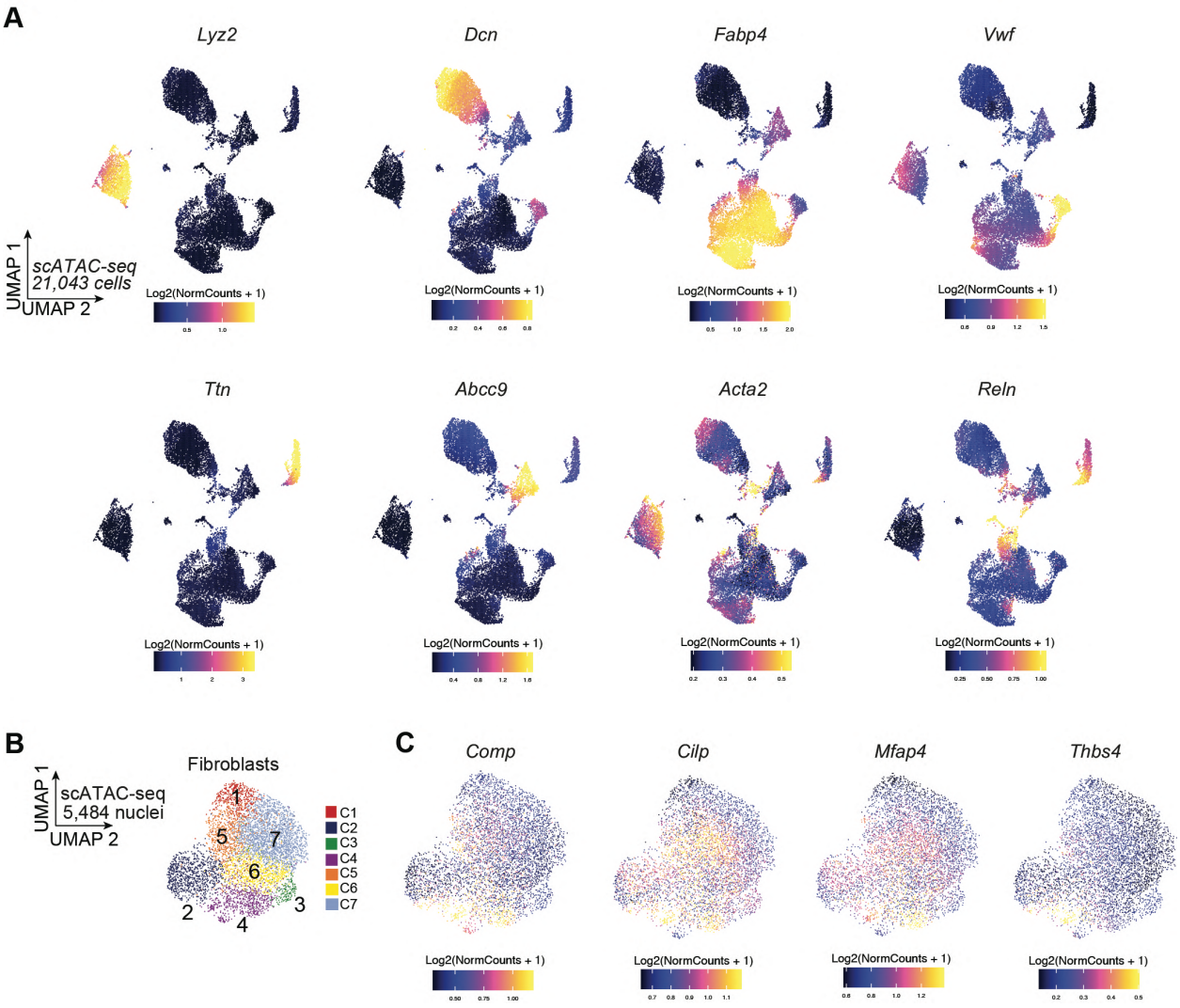

Figure S4: Changes in fibroblast chromatin accessibility with Brd4 deletion in Cx3cr1-expressing monocytes/macrophages

A. *Chromatin accessibility gene score of cell population markers across nuclei from cardiac tissue.* ***B****. UMAP plot (scATAC-seq) of fibroblasts colored by cluster identity.* ***C.*** *Chromatin accessibility gene score of Comp, Cilp, Mfap4 and Thbs4 in fibroblasts.*

Supplementary Figure 5

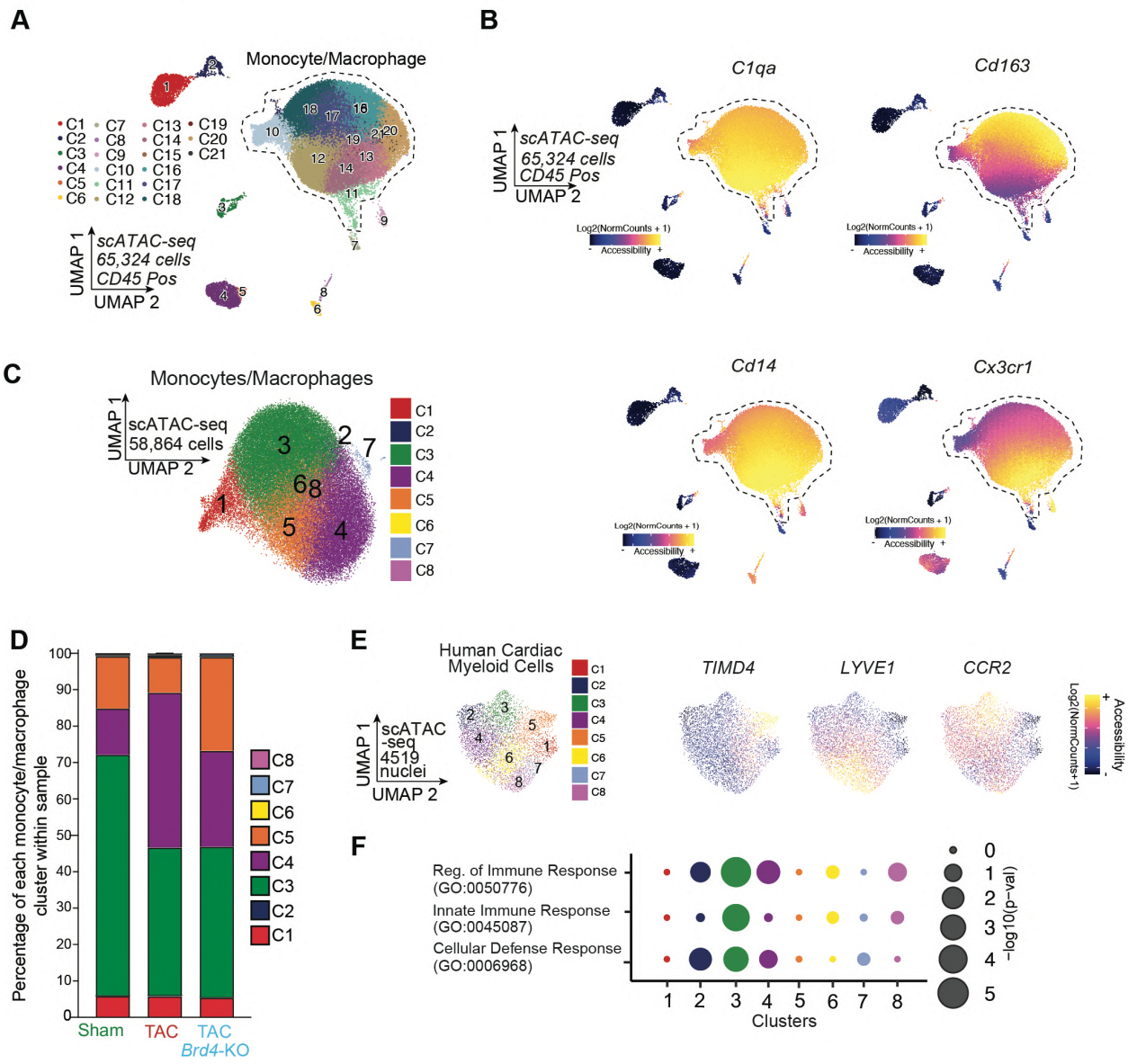

Figure S5: Identification of monocyte/macrophage population with single-cell ATAC-seq

A. *UMAP plot (scATAC-seq) of CD45^{Pos} cells colored by cluster, the clusters encompassing the monocyte/macrophage populations are highlighted.* ***B****. Chromatin accessibility gene score of C1qa, Cd163, Cd14 and Cx3cr1 in CD45^{Pos} cells.* ***C.*** *UMAP plot (scATAC-seq) of monocytes/macrophages colored by cluster identity.* ***D****. Monocyte/macrophage cluster distribution within samples.* ***E****. Chromatin accessibility gene score of TIMD4, LYVE1 and CCR2 in human cardiac myeloid cells.* ***F****. Dot Plot indicating significance (−log10(p−val)) for indicated GO terms across human myeloid cell clusters.*

Supplementary Figure 6

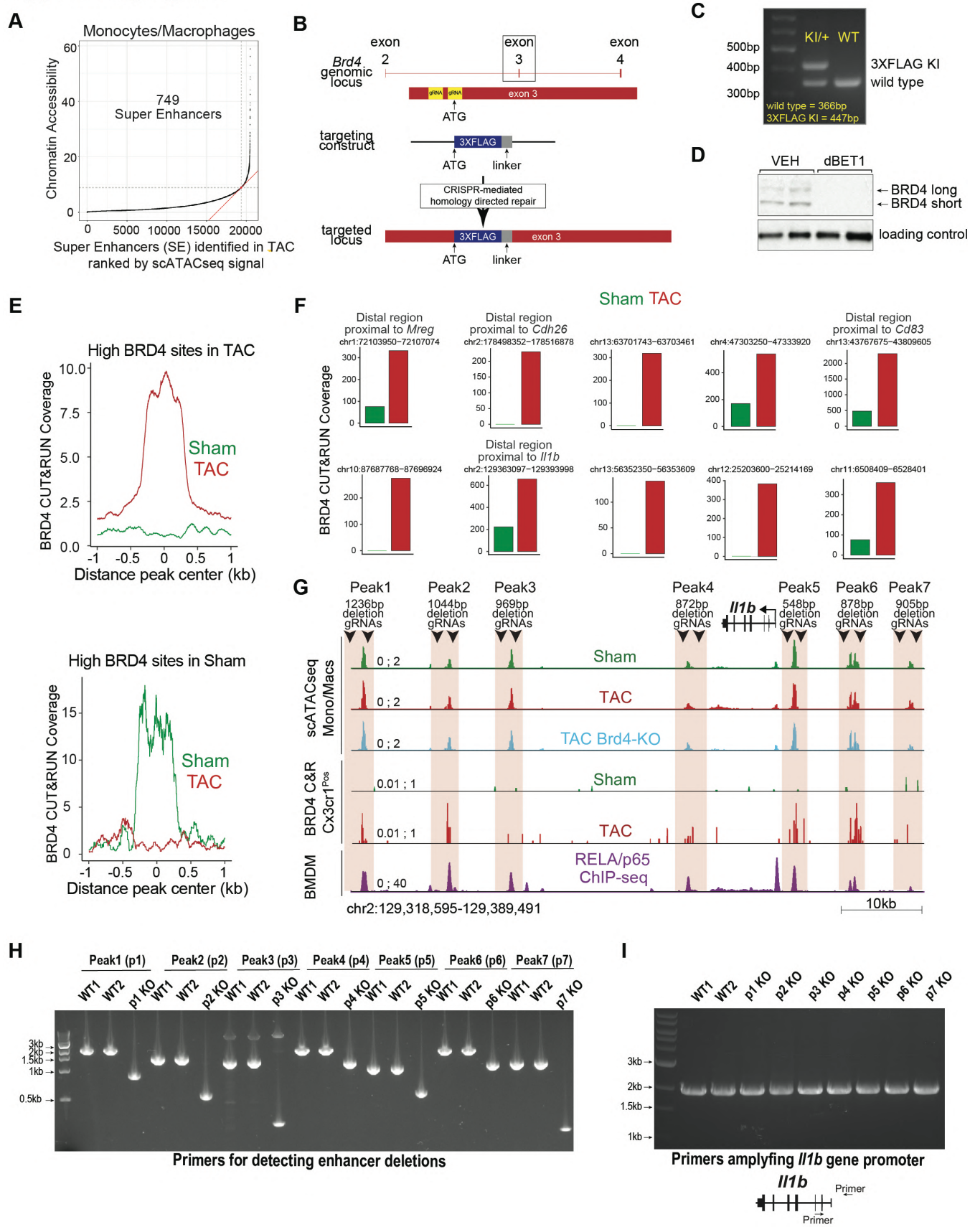

Figure S6: Chromatin accessibility and BRD4 occupancy in Cx3cr1-expressing cells identifies set of highly dynamic monocytes/macrophages distal elements in heart failure

A*. Distribution of accessibility in monocytes/macrophages in the TAC state identifies a class of distal regions (super-enhancers (SE)) for which the accessibility falls over the inflection point of the curve.* ***B****. Schematic of the targeting strategy to generate the Brd4 3xFlag mouse.* ***C.*** *Western blot showing WT and 3xFLAG knock-in bands in WT and* $Brd4^{flag/+}$ *animals.* ***D****. Liver tissue western blot showing expression of endogenous long and short BRD4 isoforms treated with vehicle or with the small-molecule BET protein degrader dBET1*[40]*.* ***E****. Coverage from anti-FLAG Cut&Run in Sham and TAC identifies regions enriched with BRD4 in stress (left) or at baseline (right).* ***F****. Coverage from anti-FLAG CUT&RUN in Sham and TAC in selected ten super enhancer regions.* ***G****. Schematic of the Il1b locus displaying the location of the gRNAs used to delete the 7 distal regions (Peak1 to Peak7).* ***H,I****. Agarose gel electrophoresis to assess distal peak deletions in Il1b locus (H) and unaffected region around Il1b promoter (I) (1813bp) in WT and KO clones.*

Suppl Figure 7

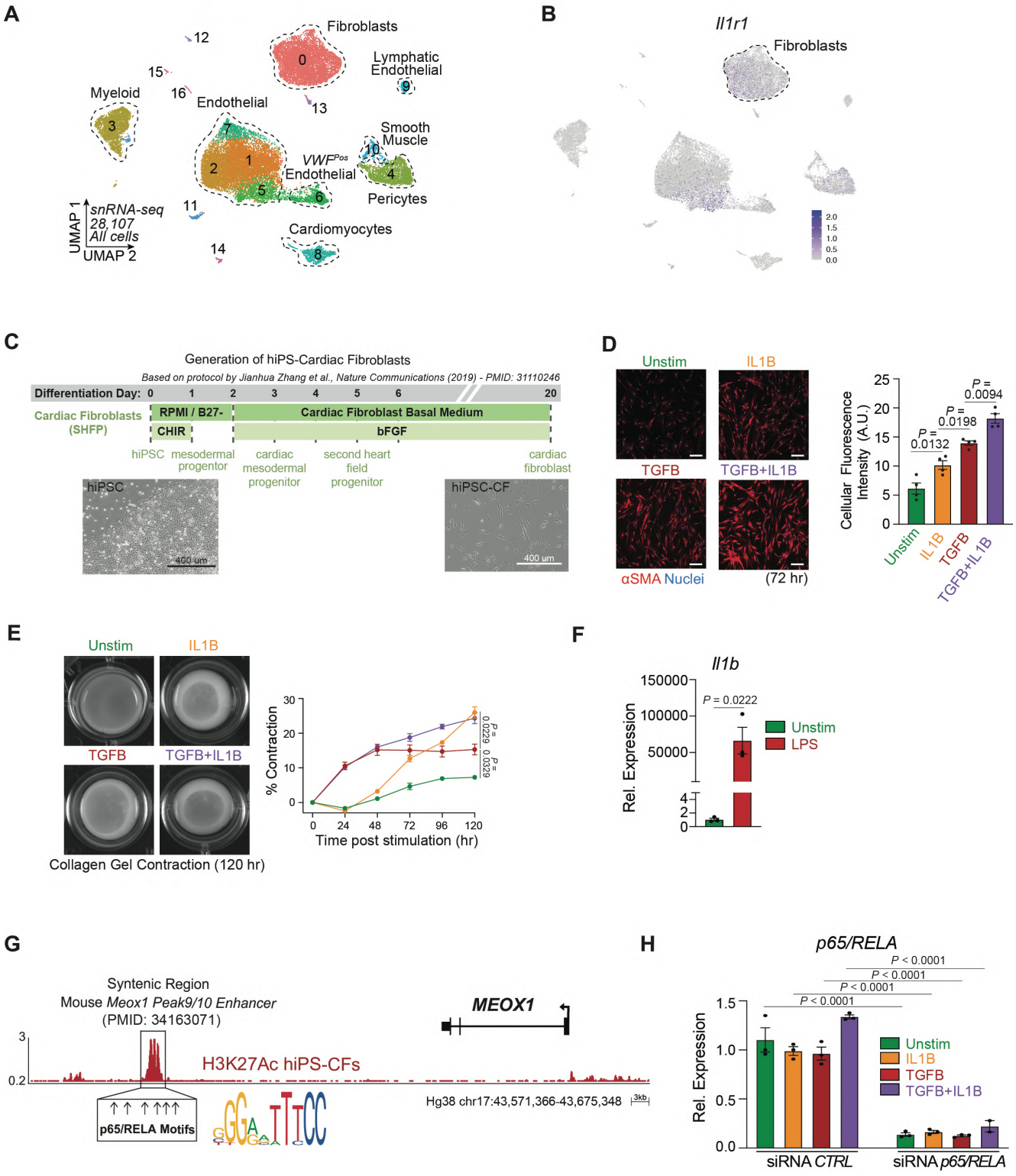

Figure S7: IL1B increases profibrotic response in human induced pluripotent cardiac fibroblasts

A. *UMAP plot (snRNA-seq) of nuclei from cardiac tissue colored by cluster identity.* ***B****. Expression Feature Plot of Il1r1 in nuclei from cardiac tissue. Fibroblasts are highlighted.* ***C.*** *Protocol to generate human induced pluripotent cardiac fibroblasts (iPS-CFs).* ***D****. Immunofluorescence staining of αSMA (left) in Unstimulated iPS-CFs or treated with IL1B, TG-B or TGFB+IL1B. Nuclei are marked by Hoechst. Scale bars, 200 μm. Right, quantification of αSMA staining.* ***E****. Images (left) and quantification (right) of iPS-CFs seeded on compressible collagen gel matrices in unstimulated or with IL1B, TGFB or TGFB+IL1B treatments.* ***F****. Il1b expression by qPCR in Unstimulated and LPS treated Raw264-7 macrophages.* ***G****. Human MEOX1 locus showing H3K27Ac in unstimulated iPS-CFs. The syntenic region of the mouse Meox1 Peak9/10 regulatory element*[3] *is highlighted and the six p65/RELA motifs within the region indicated.* ***H****. p65/RELA expression by qPCR in Unstimulated iPS-CFs or treated with IL1B, TGFB or TGFB+IL1B with control or p65/RELA-targeting siRNAs.* ***D-F,H*** *Data are mean ± s.e.m. One-way (D,H) and Two-way (E) ANOVA followed by Tukey post hoc test. Unpaired, two-tailed Student's t-test (F).*

Supplementary Figure 8

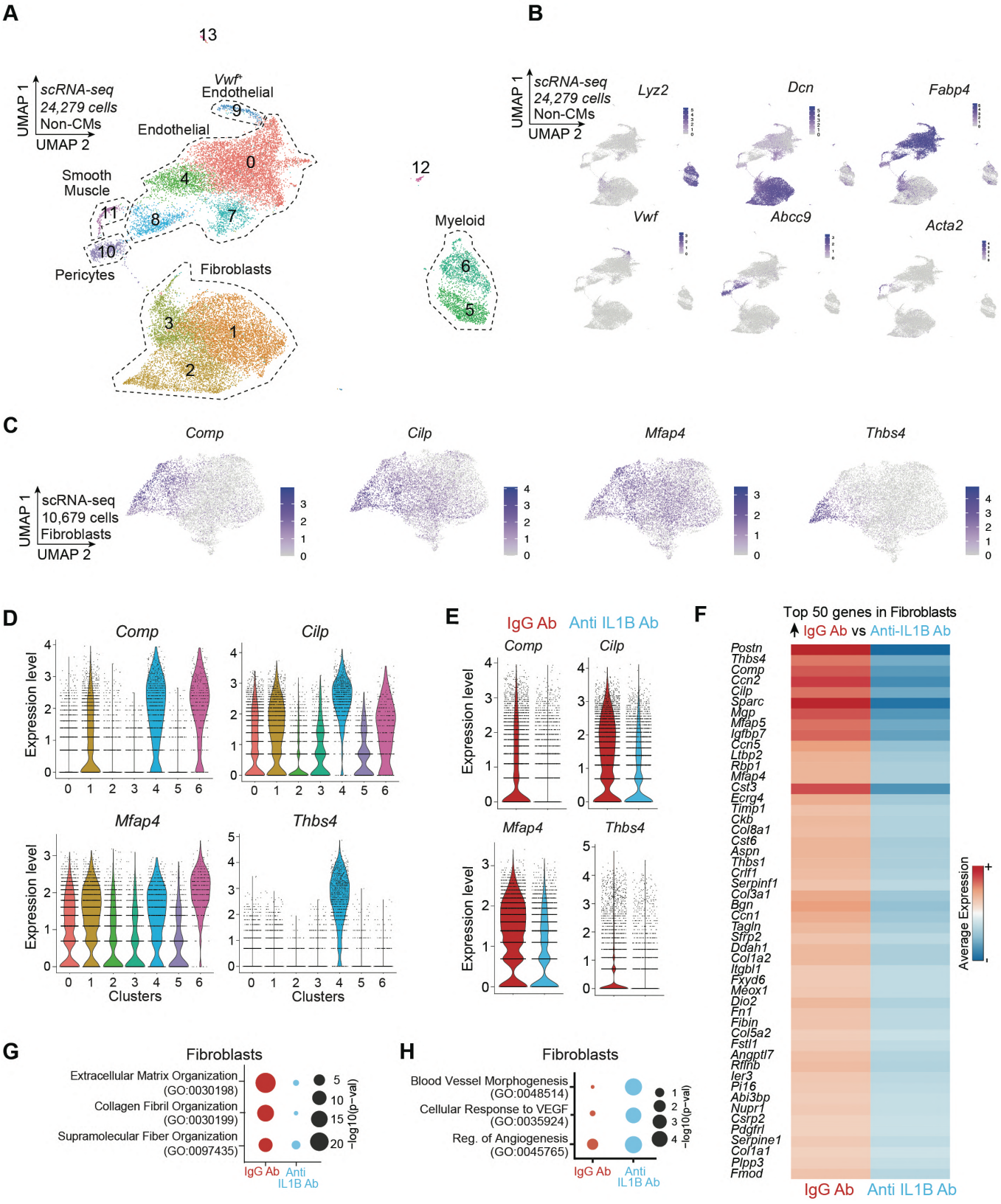

Figure S8: In vivo antibody-mediated IL1B neutralization decreases profibrotic transcriptional signature in fibroblasts

A. *UMAP plot (scRNA-seq) of non-cardiomyocyte cells colored by cluster.* ***B*** *Expression Feature Plots (scRNA-seq) of cell population markers in non-cardiomyocyte cell population.* ***C****. Expression Feature Plots (scRNA-seq) of Comp, Cilp, Mfap4 and Thbs4 in fibroblasts.* ***D****. Expression Violin Plots (scRNA-seq) of Comp, Cilp, Mfap4 and Thbs4 across clusters in fibroblasts.* ***E****. Expression Violin Plots (scRNA-seq) of Comp, Cilp, Mfap4 and Thbs4 across samples in fibroblasts.* ***F****. Heatmap of expression (scRNA-seq) depicting the top 50 genes upregulated in TAC IgG Ab versus TAC Anti-IL1B Ab in fibroblasts.* ***G,H****. Dot Plot indicating significance (−log10(p−val)) for indicated GO terms in genes upregulated in IgG vs Anti-IL1B (red) or upregulated in Anti-IL1B vs IgG (blue) in fibroblasts.*