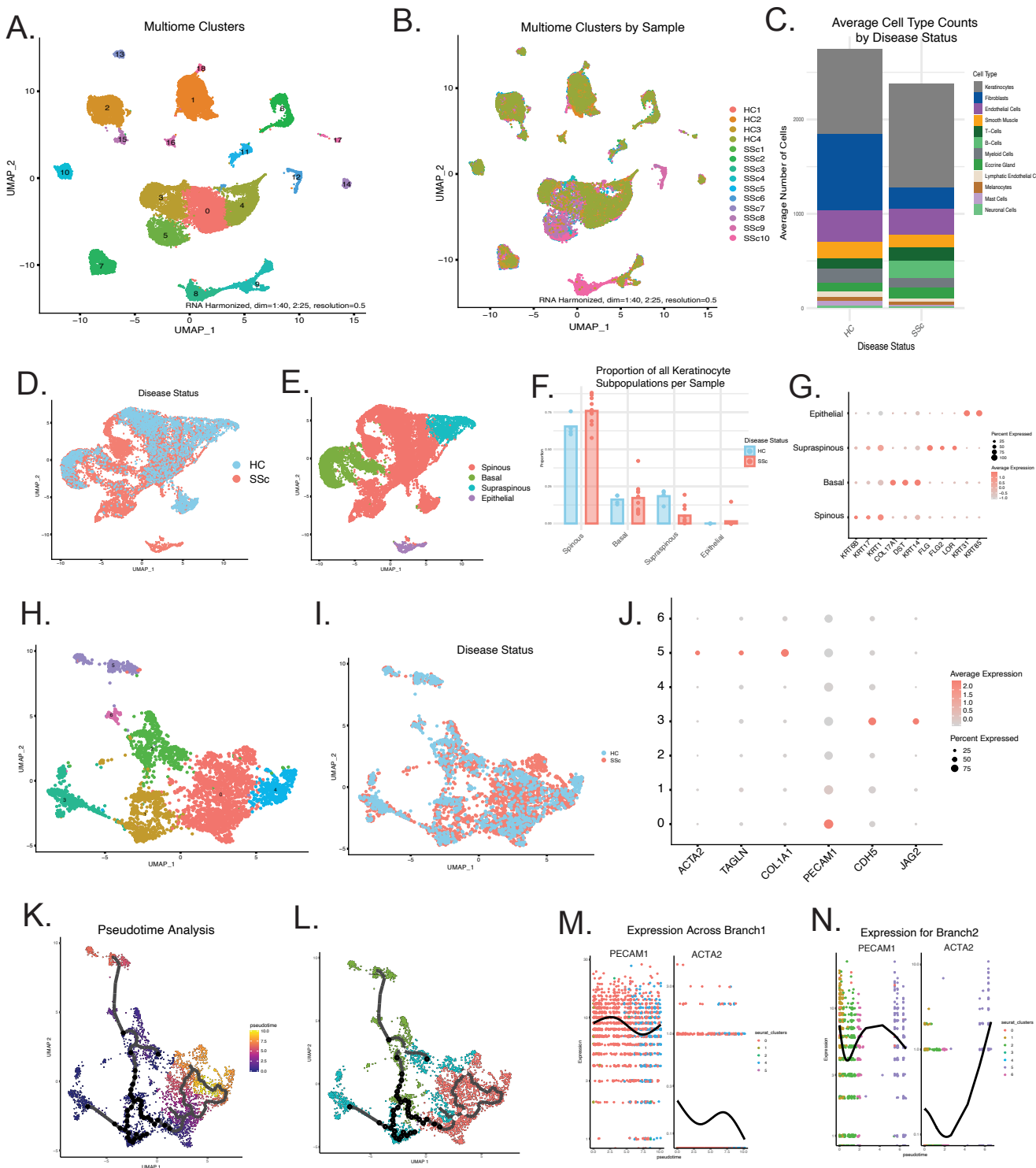
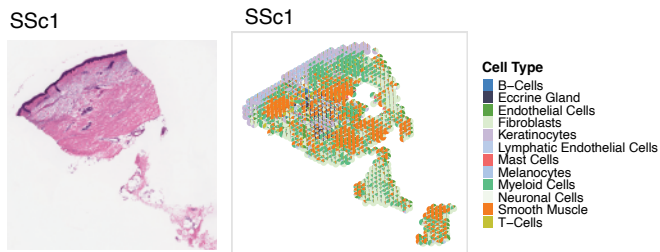


Supplementary Figures



Supplementary Figure 1: Multiome unsupervised clustering. A) Nuclei clustered and colored by unsupervised labels for initial cell type assessment. Data were harmonized for batch correction. Dimensions for RNA and ATAC data. B) Nuclei colored by sample origin name, demonstrating even distribution of cell types by original sample ID. C) Total nuclei counts differences by diseases for all cell types. D) UMAP of keratinocyte subclustering colored by disease status. E) UMAP of keratinocyte subpopulations named through canonical subtype. F) Proportion of each keratinocyte subpopulation to all keratinocytes shows no significant difference in canonical subclusters by disease. G) Differentially expressed canonical markers (top 2) for each keratinocyte subtype. H) UMAP of endothelial subclusters. I) UMAP of endothelial cell subclustering colored by disease status. J) Expression patterns of profibrotic markers (ACTA2, TAGLN, COL1A1) and classical endothelial cell markers (PECAM1, CDH5, JAG2) across all endothelial cell clusters. K) Pseudotime analysis with assigned root node, circled in green, across the endothelial cell subclusters, indicating divergent branches of trajectory. L) Branches identified to separate out potential endothelial cell lineages. M) Expression of PECAM1 and ACTA2 across the pseudotime space of Branch1. N) Expression of PECAM1 and ACTA2 across the pseudotime space of Branch2.

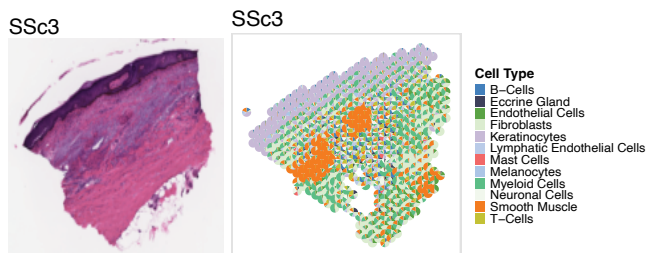
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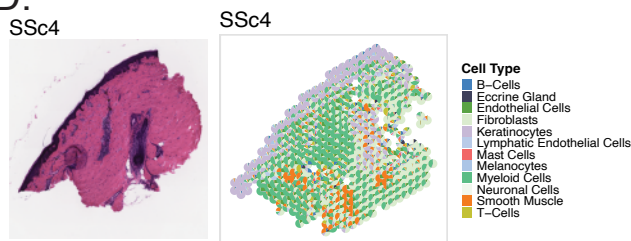
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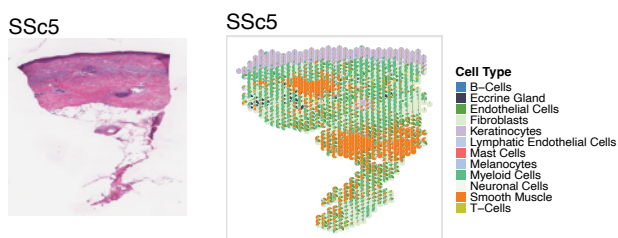
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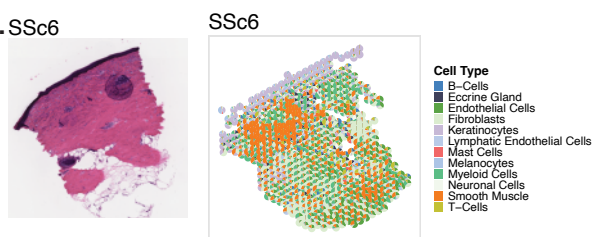
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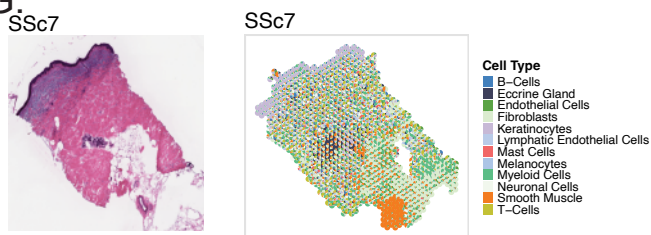
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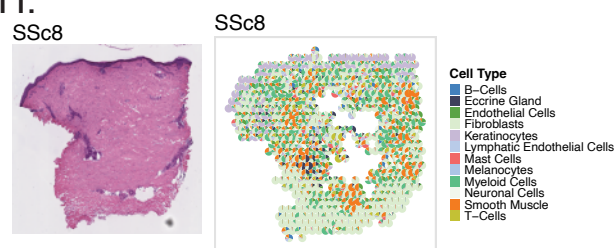
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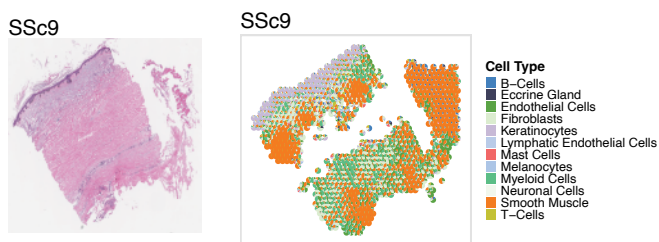
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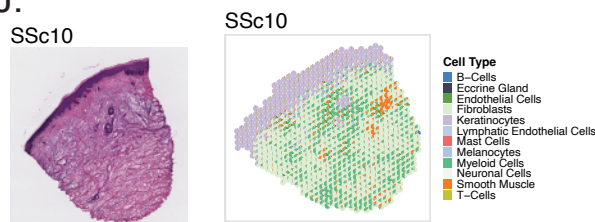
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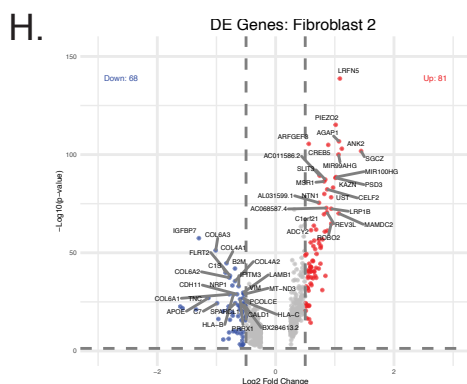
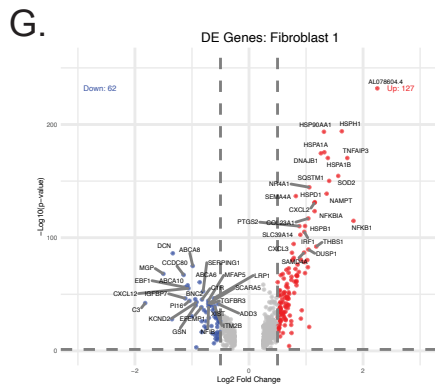
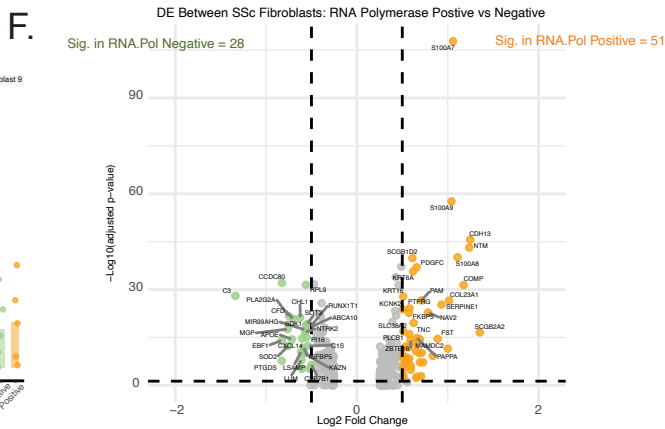
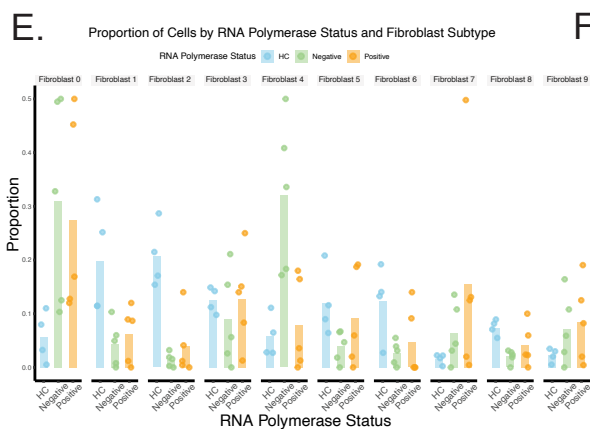
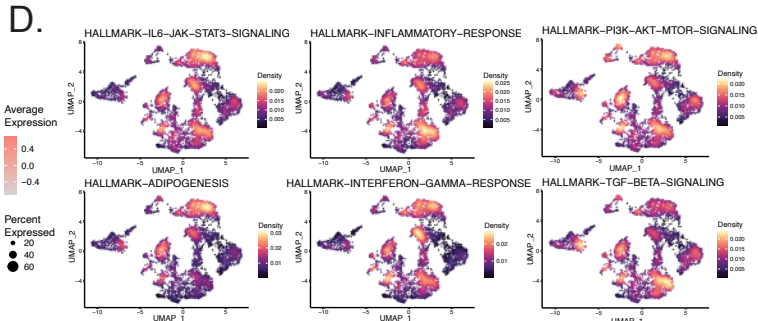
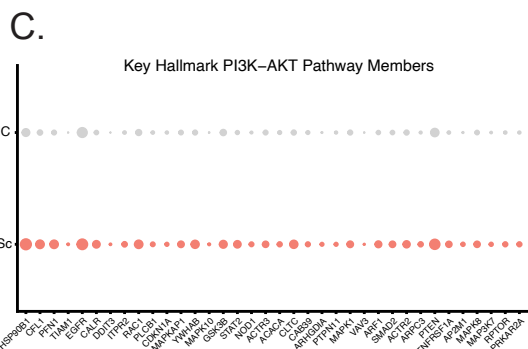
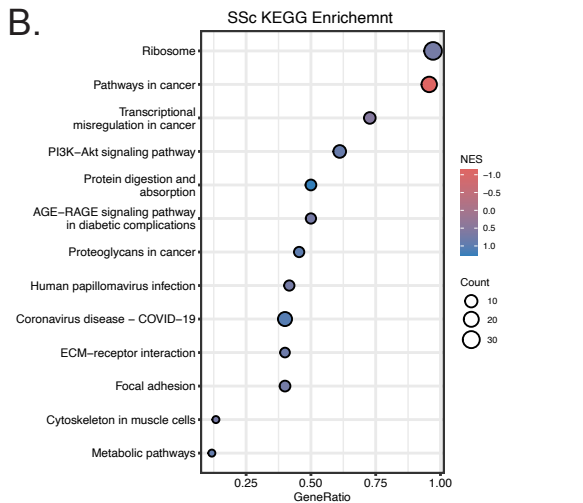
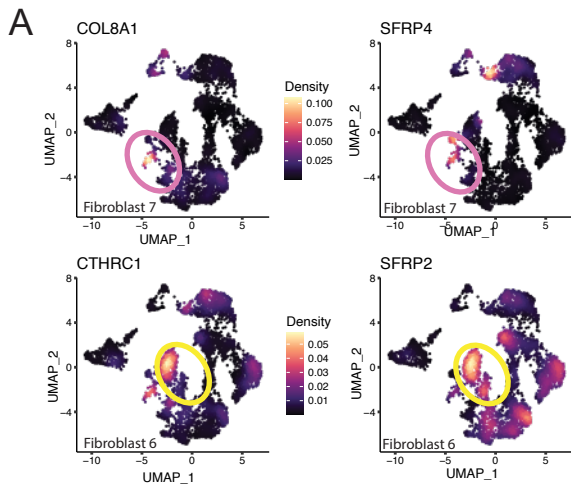
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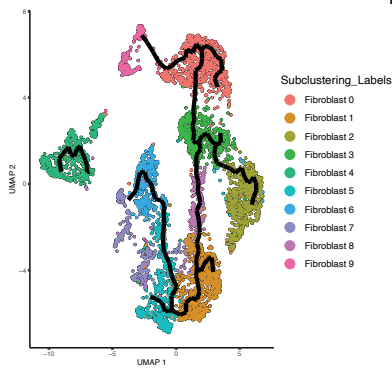
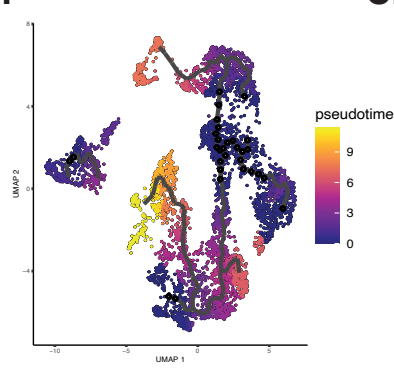
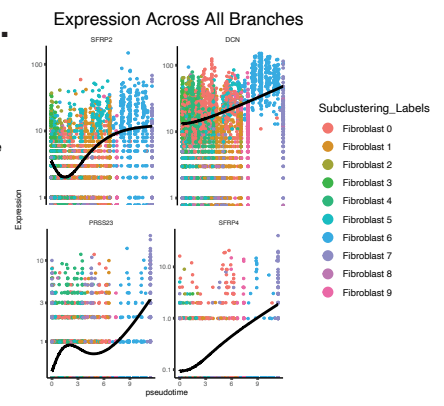
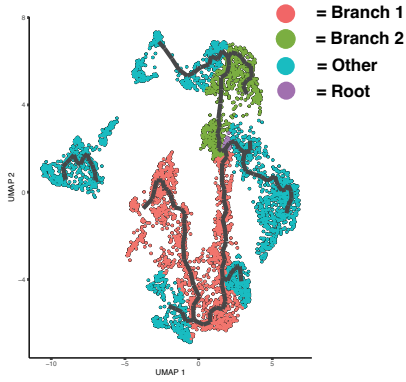
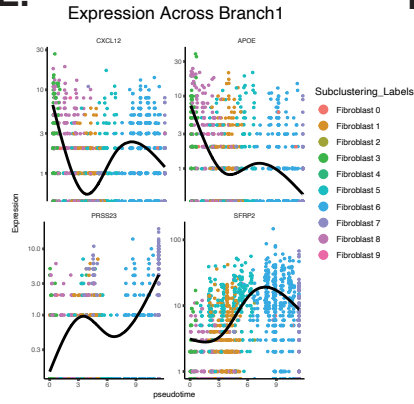
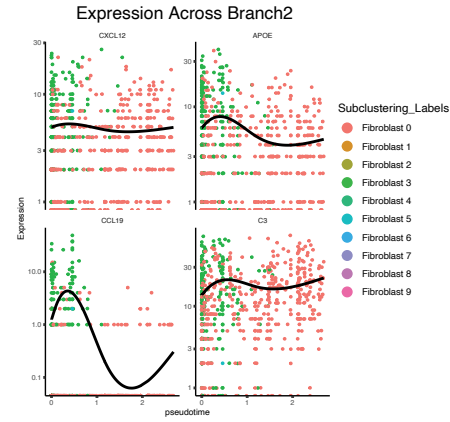
J.



Supplementary Figure 2: Spot Deconvolution for each sample and histology section. A-J) Pie chart plots for each spot with estimated cell-type proportions computed with CARD using the Multiome data as a reference. Underlying H&E stains for each tissue section sequenced.

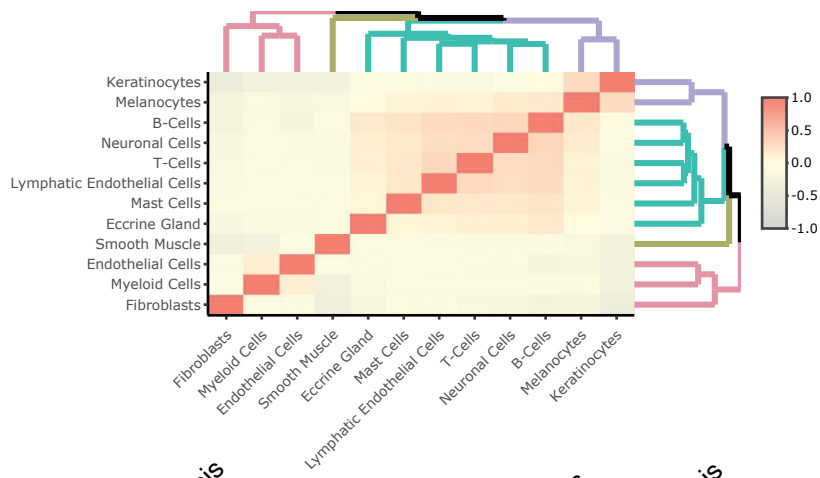


Supplementary Figure 3: Fibroblast Subcluster and pathway interrogation. A) Density plots of common canonical markers of fibroblast subclusters. COL8A1, SFRP4, CTHRC1, and SFRP4 are enriched in Fibroblast 7 and Fibroblast 6. B) GO term analysis, using clusterProfiler, demonstrates enrichment of key KEGG pathways in SSc fibroblasts compared to HC. C) Hallmark geneset PI3K-AKT-MTOR pathway genes differentially expressed between SSc and HC fibroblasts. D) VAM enrichment of key Hallmark genesets across the fibroblast subclusters. E) Proportional comparison of fibroblast subclusters by RNA polymerase autoantibody status. F) Volcano plot of differentially expressed genes of all fibroblasts from SSc samples compared by RNA polymerase status. G) Volcano plot of differential expression results of Fibroblast 1 cluster compared to all other Fibroblast clusters. H) Volcano plot of differential expression results of Fibroblast 2 cluster compared to all other Fibroblast clusters.

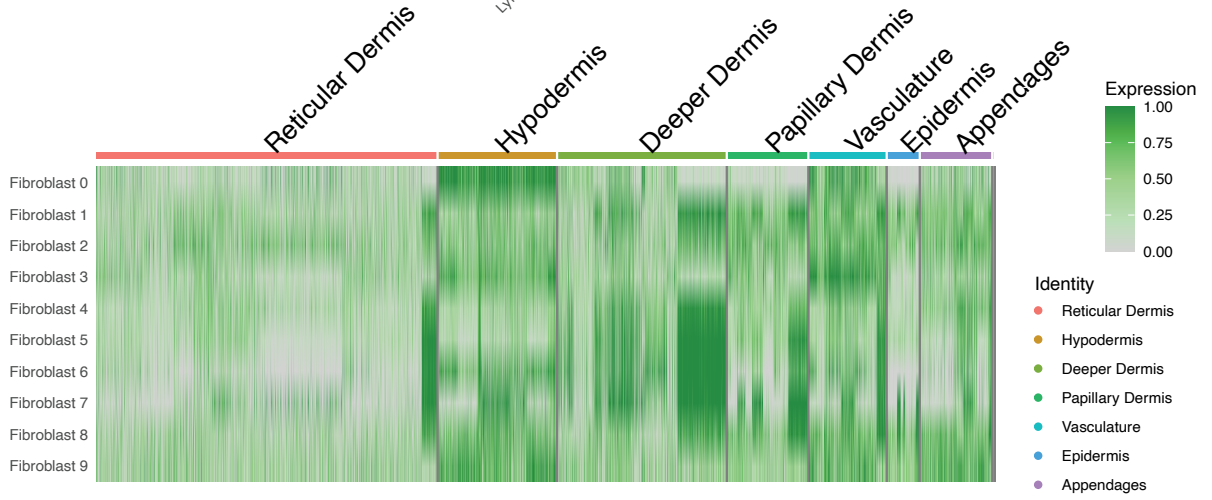
A.**B.****C.****D.****E.****F.**

Supplemental Figure 4: Fibroblast pseudotime analysis shows divergent trajectories. A) Pseudotime path with overlaid with fibroblast subclusters. Fibroblast 3 set as root node given central and basal expression patterns. B) Pseudotime path, with cells colored by resultant pseudotime. Fibroblast 7 and 9 are at the end of the total trajectory path when Fibroblast 3 is set as the root node. C) Gene expression plotted across all pseudotime branches shows an increase in fibrotic markers at the tail end of the pseudotime graph. D) UMAP with pseudotime path and cells marked by key pseudotime branches. E) Key genes expressed across Branch 1. F) Key genes expressed across Branch 2.

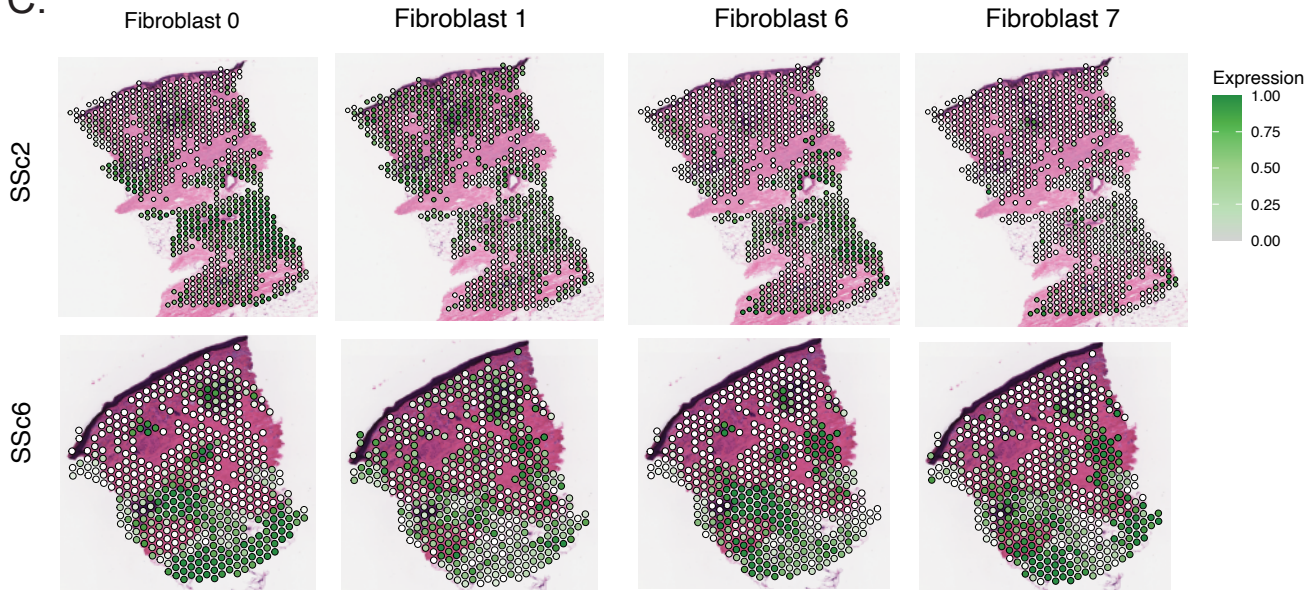
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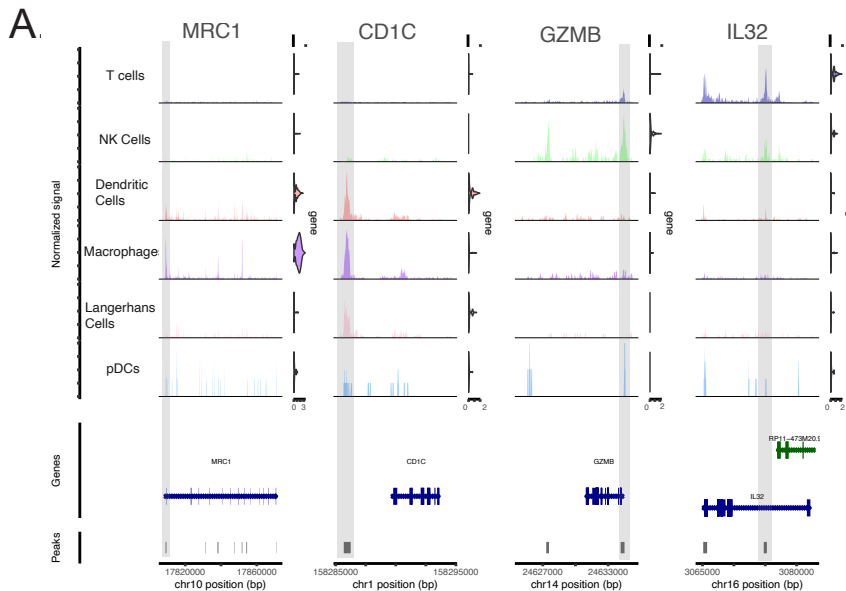
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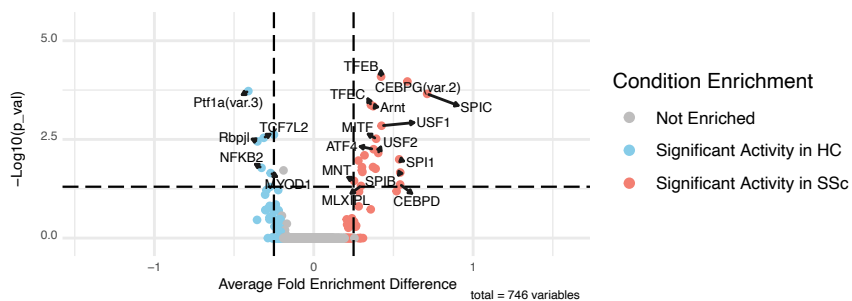
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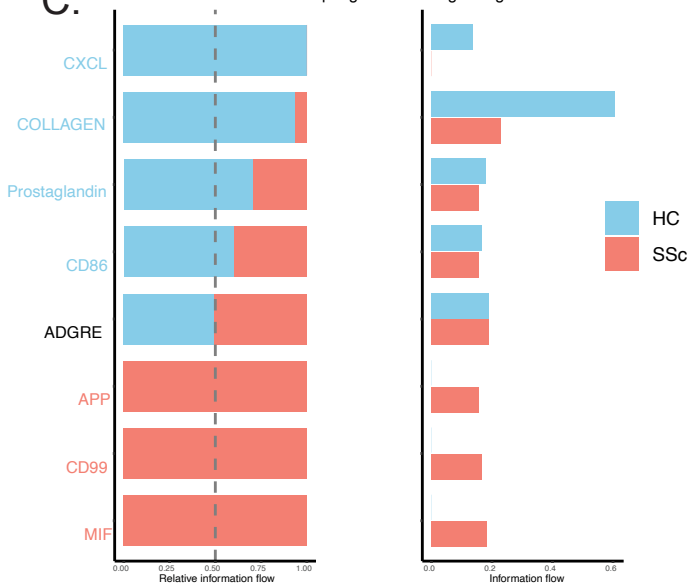
Supplementary Figure 5: Spatial distribution of fibroblast subclusters. A) Correlation of deconvoluted cell types found within the same spots across the all the tissues sections. The most likely cell types to compose spots with fibroblasts, other than other fibroblasts, are endothelial cells or myeloid cells. B) VAM enrichment scores of fibroblast subcluster (in fibroblast containing spots only) genesets, based on differential expression per cluster, across the spatial landscape of two representative sections. C) Heatmap showing VAM enrichment scores for fibroblast subcluster genesets within each region.



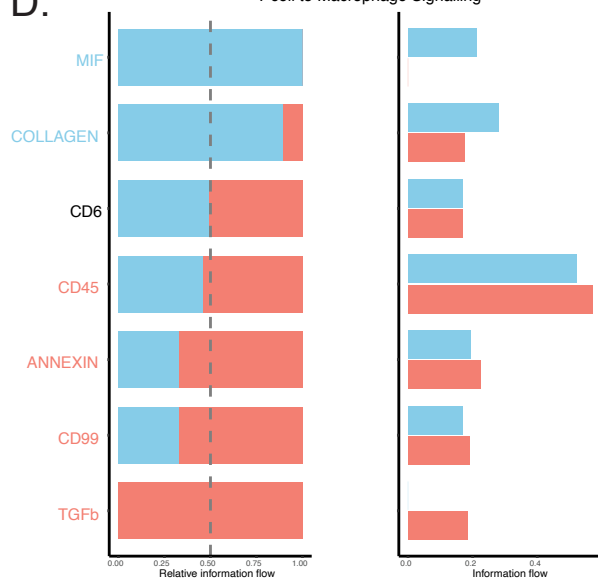
B. Transcription Factor Activity Differential for All Myeloid Cells



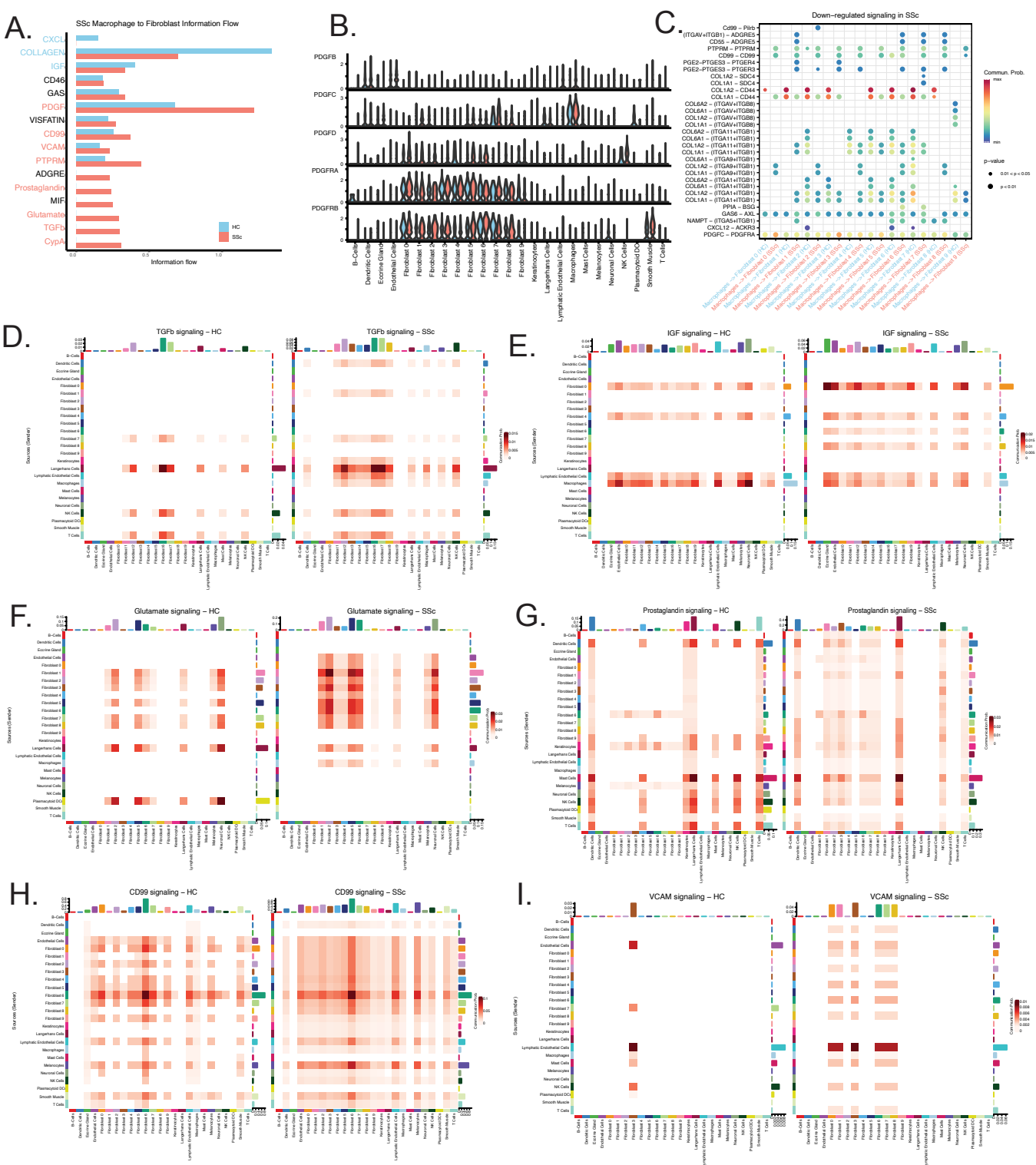
C. Macrophage to T cell Signalling



D. T cell to Macrophage Signalling



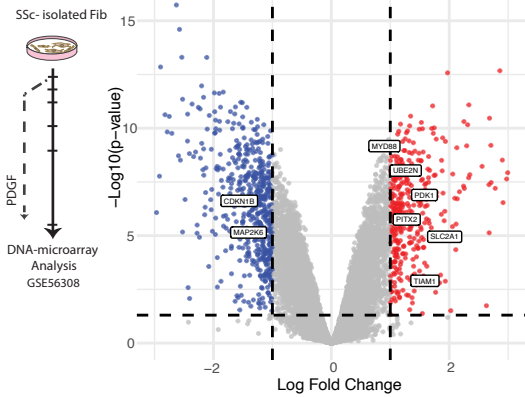
Supplementary Figure 6: Key immune cell subtypes confirmed with ATAC-seq data. A) Immune cell subtypes identified by differentially accessible chromatin regions near canonical genes of interest. Peak before MRC1 is differentially accessible in the macrophages, with matching MRC1 gene expression (right). Peaks near CD1C, GZMB, and IL32 in Dendritic Cells, NK cells, and T-cells. B) Volcano plot of transcription factor enrichment by inferred activity in myeloid cells compared by disease status. C) Ligand-receptor results of macrophage to T cell inferred signaling on a relative and absolute information flow of ligand-receptor pathways. Significant pathways are colored, while pathways not found to be statistically significant compared by disease status. D) Ligand-receptor results of T cell to macrophage inferred signaling on a relative and absolute information flow of ligand-receptor pathways.



Supplementary Figure 7: Expanded Ligand-Receptor Analyses. A) Sum of predicted interactions between macrophages and fibroblasts comparing SSc and HC (non-relative). B) Expression of PDGF ligands and receptors included in pathway prediction by all cell types comparing between disease state. C) Most downregulated signaling interactions are by exactly the ligand-receptor pair between macrophages and fibroblasts. Filtered by $p < 0.05$, tested by Wilcoxon rank sum test. D) TGF β signaling interaction probability in all cell types compared by disease state. E) IGF signaling interaction probability in all cell types compared by disease state. F) Glutamate signaling interaction probability in all cell types compared by disease state. G) Prostaglandin signaling interaction probability in all cell types compared by disease state. H) CD99 signaling interaction probability in all cell types compared by disease state. I) VCAM signaling interaction probability in all cell types compared by disease state.

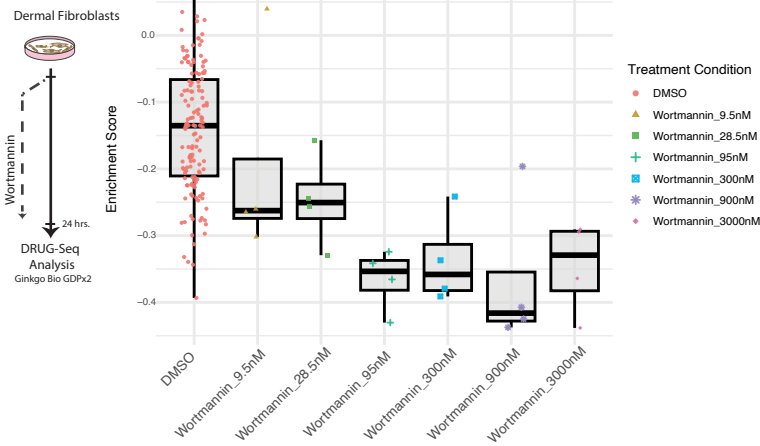
A.

Differentially Expressed Genes After 8 Hours of PDGF



B.

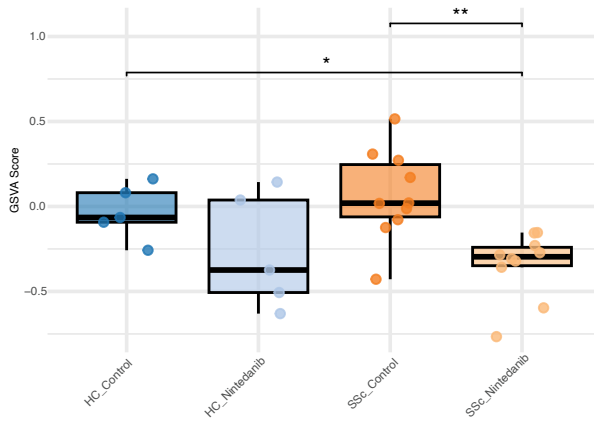
HALLMARK_PI3K_AKT_MTOR_SIGNALING



C.

REACTOME_AKT_PHOSPHORYLATES_TARGETS_IN_THE_NUCLEUS

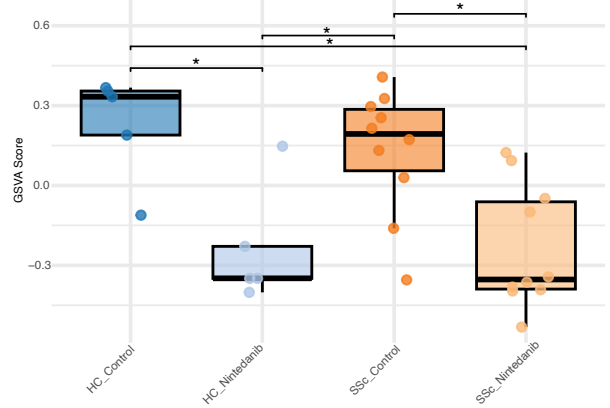
Pairwise comparisons with Wilcoxon test (BH-adjusted p-values)



D.

REACTOME_ECM_PROTEOGLYCANS

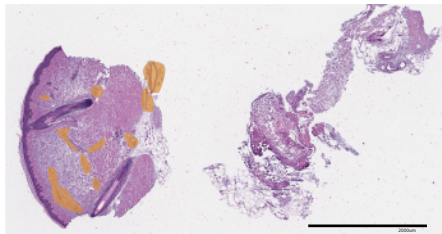
Pairwise comparisons with Wilcoxon test (BH-adjusted p-values)



Supplementary Figure 8: Bulk gene expression analyses. A) Volcano plot with differentially expressed genes comparing eight hours of PDGF exposure to baseline. Differentially expressed PI3K-AKT pathway genes are highlighted. B) Gene set enrichment comparison of DMSO treated fibroblasts or fibroblasts treated with different concentrations of Wortmannin. The Hallmark PI3K-AKT-MTOR pathway is significantly reduced in fibroblasts treated with 28.5 nM and higher concentrations. C) Box plot of GSVA scores for Reactome database's ECM proteoglycans pathway, showing increased expression in untreated 3D tissues with significant reduction upon Nintedanib treatment. D) GSVA scores of Reactome's AKT phosphorylation targets with an SSc-specific response to Nintedanib treatment.

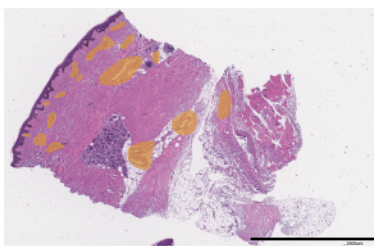
A.

SSc1



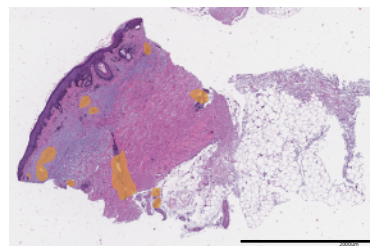
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SSc2



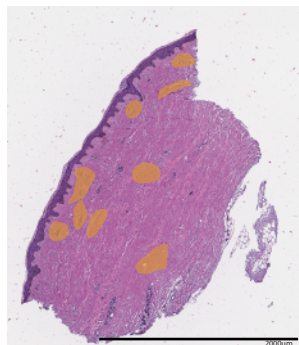
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SSc3



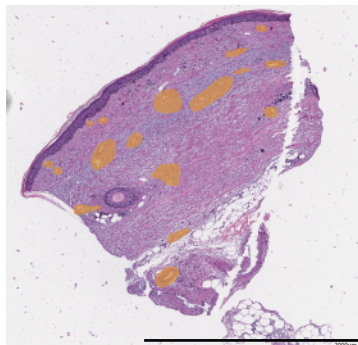
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SSc4



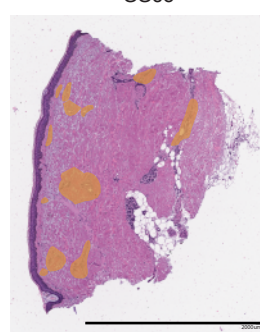
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SSc5



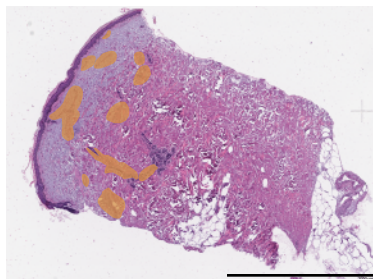
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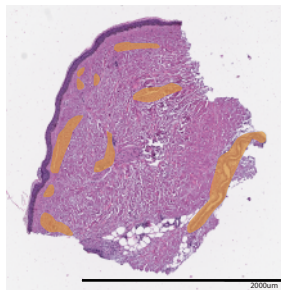
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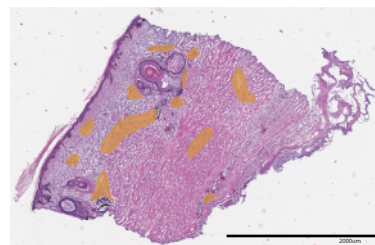
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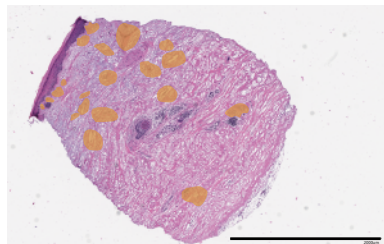
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SSc9

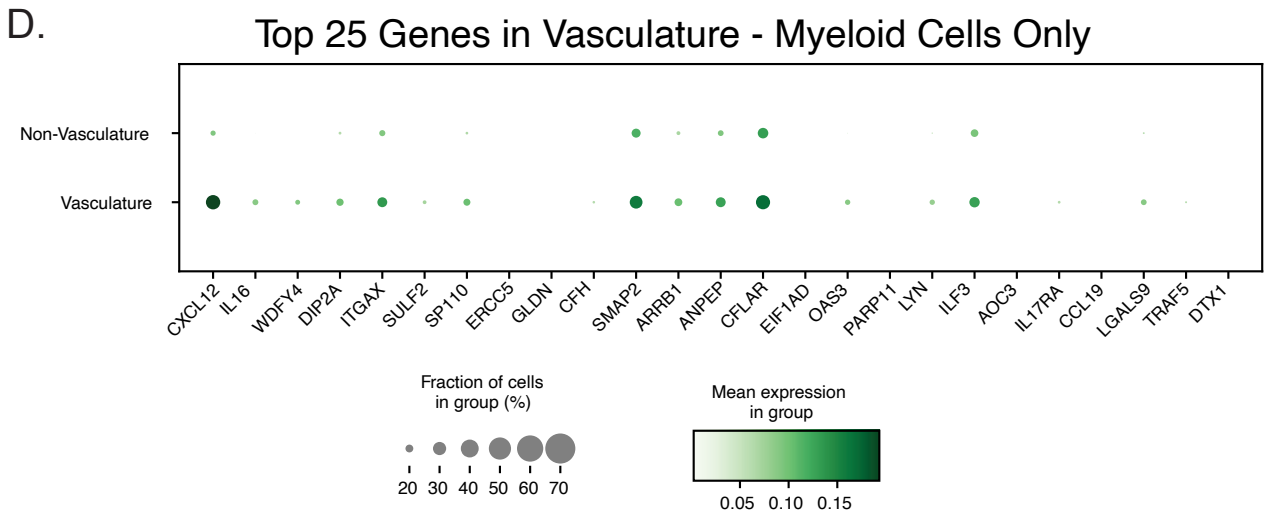
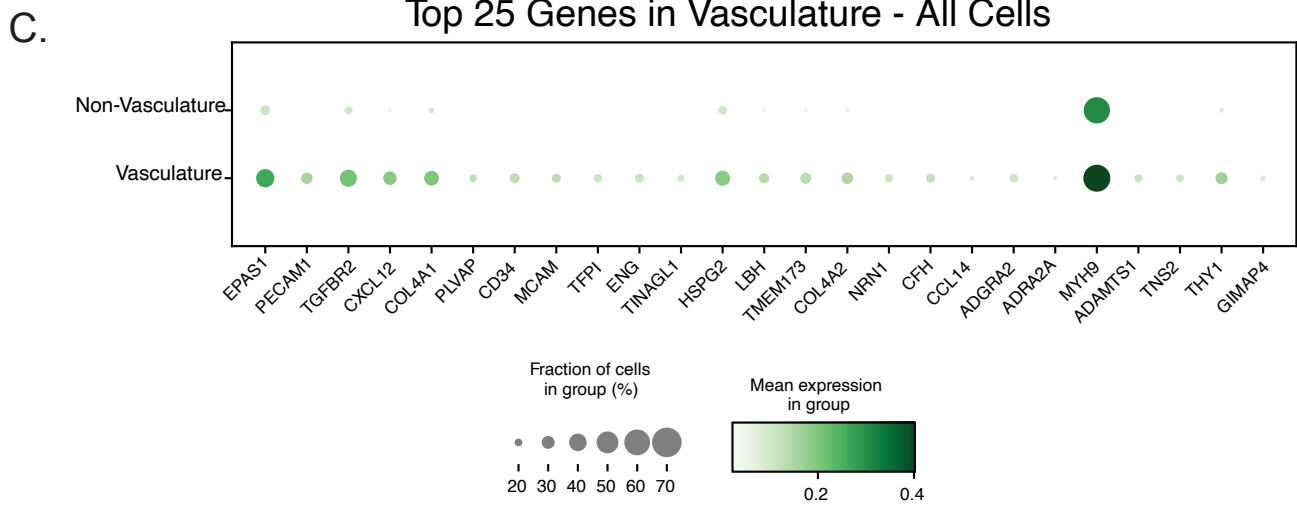
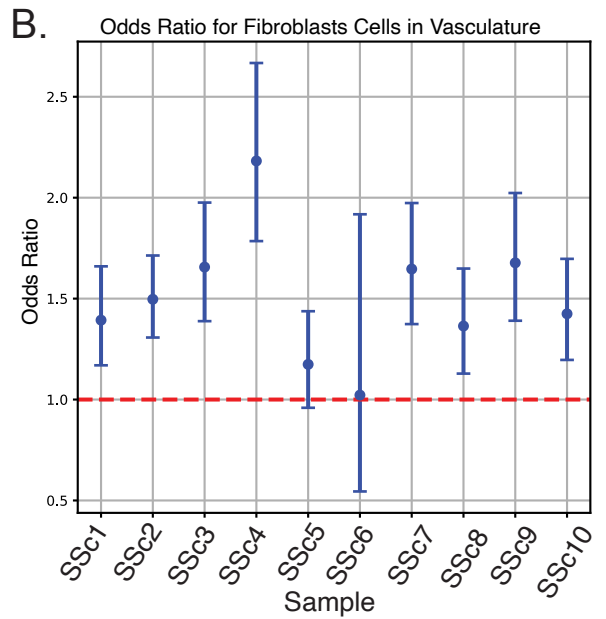
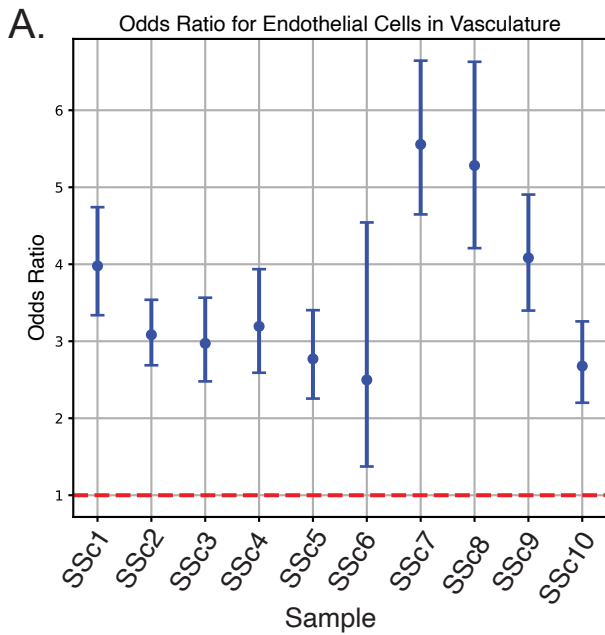


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SSc10

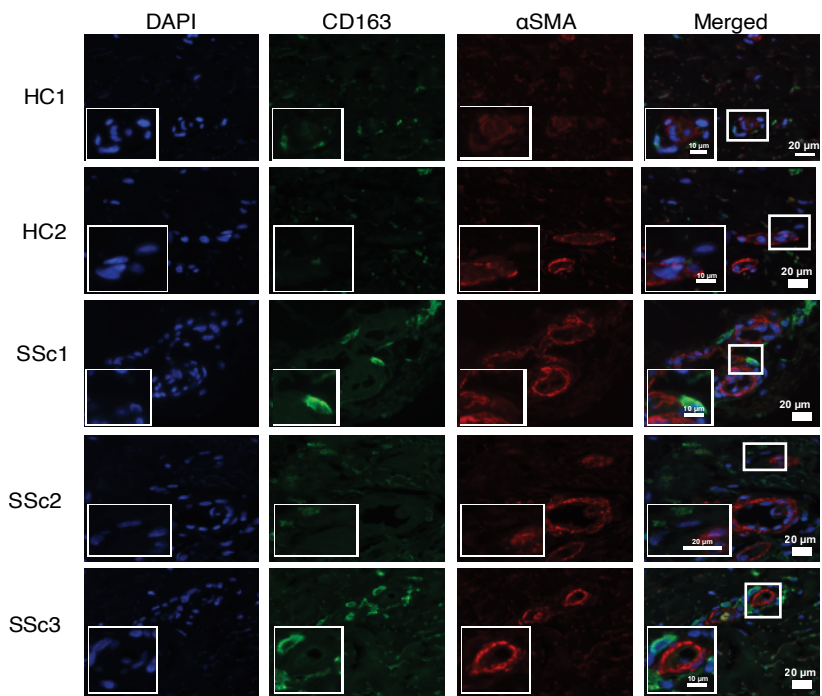


Supplementary Figure 9: Vasculature regions for each tissue section. A-J) Each tissue section used for Xenium assay (stained with H&E) overlayed with manually identified vasculature regions in orange. Bar indicates 2000 μm .

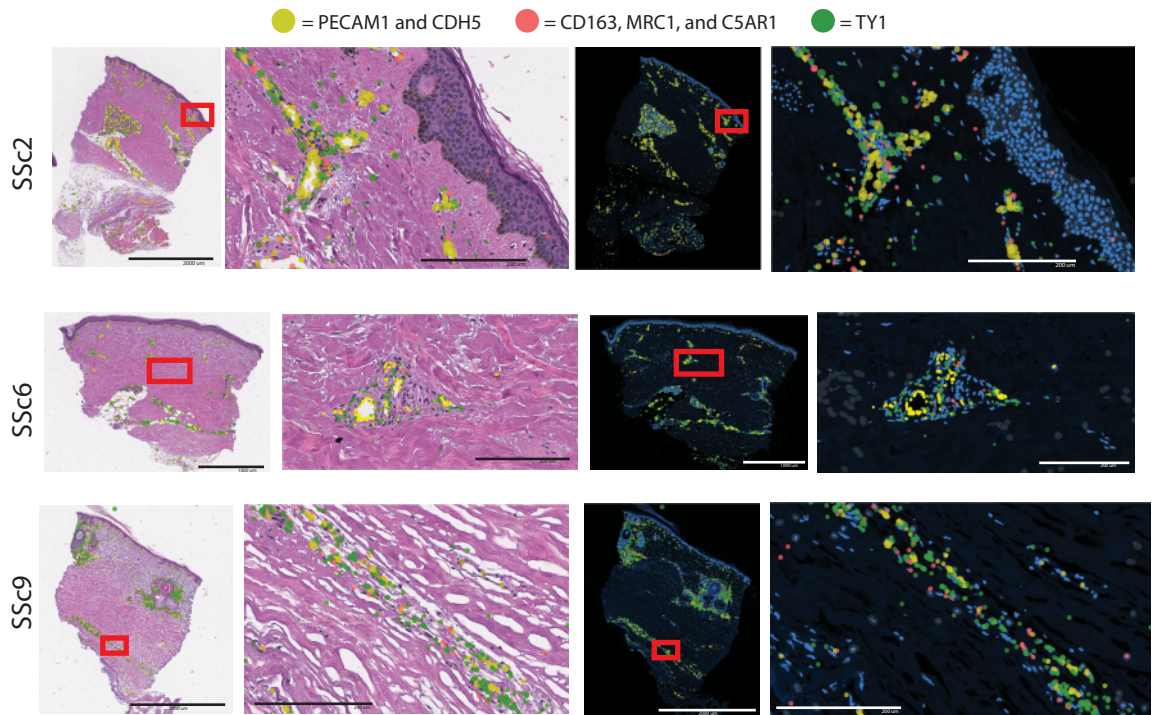


Supplementary Figure 10: Xenium results show the specificity of vasculature regions. A) Odds ratio results show a significant probability of finding endothelial cells in the vasculature regions in all 10 Xenium sections. B) Fibroblasts are often found near vasculature regions as confirmed by odds ratio test, but are often dispersed across the tissue sections. C) 25 differentially expressed genes of fibroblasts in the vasculature region (Wilcoxon Rank Sum Test, sorted by LogFC, all genes $p < 0.05$). D) 25 differentially expressed genes of myeloid cells only in the vasculature region (Wilcoxon Rank Sum Test, sorted by LogFC, all genes $p < 0.05$).

A.



B.



Supplementary Figure 11: Immunofluorescence staining of CD163-positive cells and aSMA-positive cells.

A) All samples included in the dataset include 2 HCs and 3 SSc samples. The size bar represents 20 μm . Inset shows the outlined region, the inset size bar represents 10 μm . B) Xenium overlay results of selected sections. *PECAM* and *CDH5* molecules are pseudocolored yellow to identify endothelial cells, *CD163*, *MRC1*, and *C5AR1* are combined and pseudocolored red to identify macrophages, and *THY1* molecules are pseudocolored green to identify fibroblasts. H&E overview and inlay are displayed alongside DAPI (blue) counterstain overlay and inlay of the same regions. Measurement bars indicated 2000 μm and 200 μm in the overview and inlay, respectively.