

Liver cell-type specific molecular signatures marking transition from advanced fibrosis to cirrhosis in human non-alcoholic steatohepatitis

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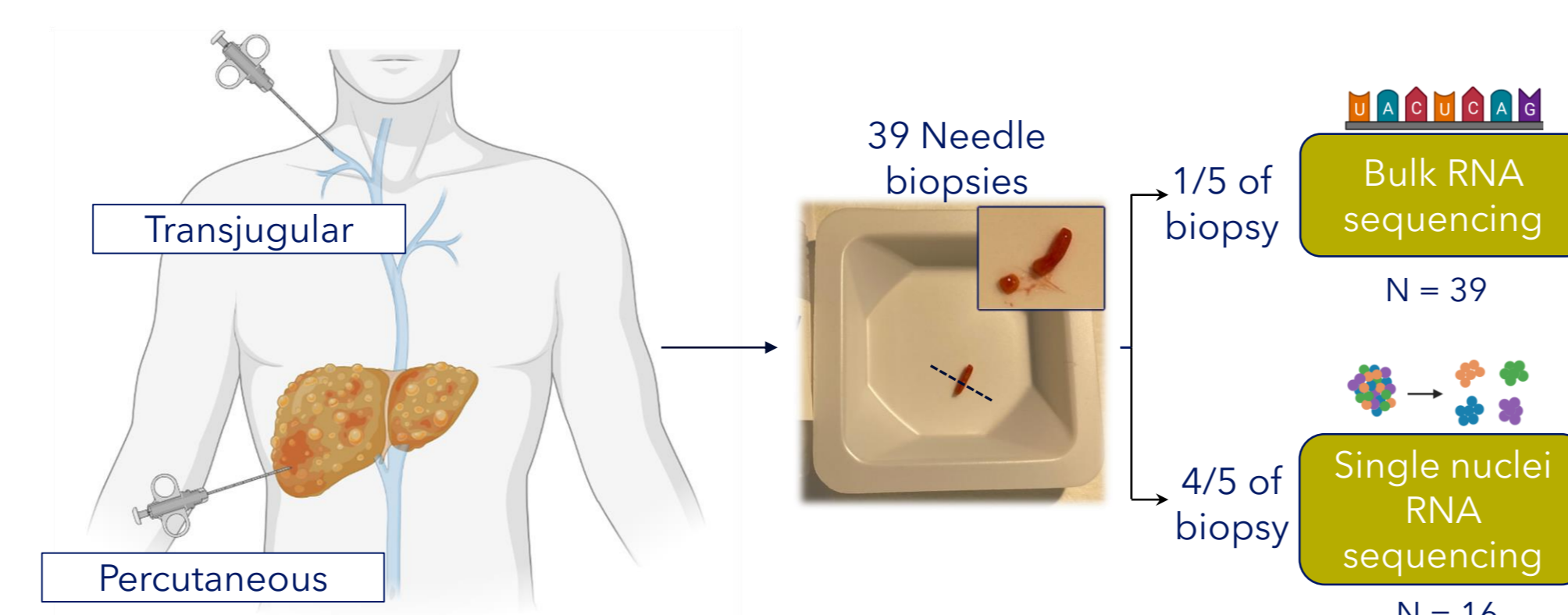
Background & Aim

Hepatic fibrosis is the strongest predictor of morbidity and mortality in non-alcoholic steatohepatitis (NASH), establishing fibrosis as a critical therapeutic target in NASH. To improve treatment outcomes in NASH, it is pertinent to define hepatic cell phenotype-specific molecular signalling mechanisms involved in the progression of fibrosis towards cirrhosis. Using paired bulk and single-nucleus RNA sequencing, we mapped hepatic transcriptome signatures of parenchymal and non-parenchymal cell types across all stages of fibrosis in human NASH.

Methods

Bulk RNA sequencing (RNAseq) was performed on snap-frozen liver biopsies obtained from a patient cohort of 39 obese individuals with NASH (stages F0-F4) or no/mild NAFLD (macrovesicular steatosis) without fibrosis (control group). Paired single-nucleus (sn) RNAseq analysis was performed on a subset of biopsies (Control, NASH F1, NASH F2-F3, NASH F4; n=4 per group). See study outline in Fig. 1

1 Paired bulk + single nuclei RNA sequencing pipeline



Group	N	M/F	T2DM	HT	DL	Age [yr]	BMI [kg/m ²]	ALT [U/l]	AST [U/l]	Fibrosis score				NAFLD activity score												
										0	1	2	3	4	0	1	2	3	4	5	6	7	8			
Control	12	8/4	5	2	7	55.0±13.0	32.2±8.2	48.0±37.4	30.6±16.2	12	0	0	0	0	4	4	2	2	0	0	0	0	0	0	0	0
F1	10	8/2	6	7	7	50.9±13.5	32.7±4.3	84.3±41.5	53.1±21.4	0	10	0	0	0	0	1	0	2	1	3	2	1	0	0	0	0
F2/F3	11	6/5	8	6	10	53.6±11.8	33.7±5.2	107.0±170.1	70.1±96.3	0	0	7	4	0	1	0	0	1	2	4	1	2	0	0	0	0
F4	6	2/4	4	3	3	65.2±7.5	29.4±4.9	63.3±60.0	73.8±42.9	0	0	0	0	6	1	1	0	2	1	0	1	0	0	0	0	0

2 Transcriptome signatures of cirrhosis

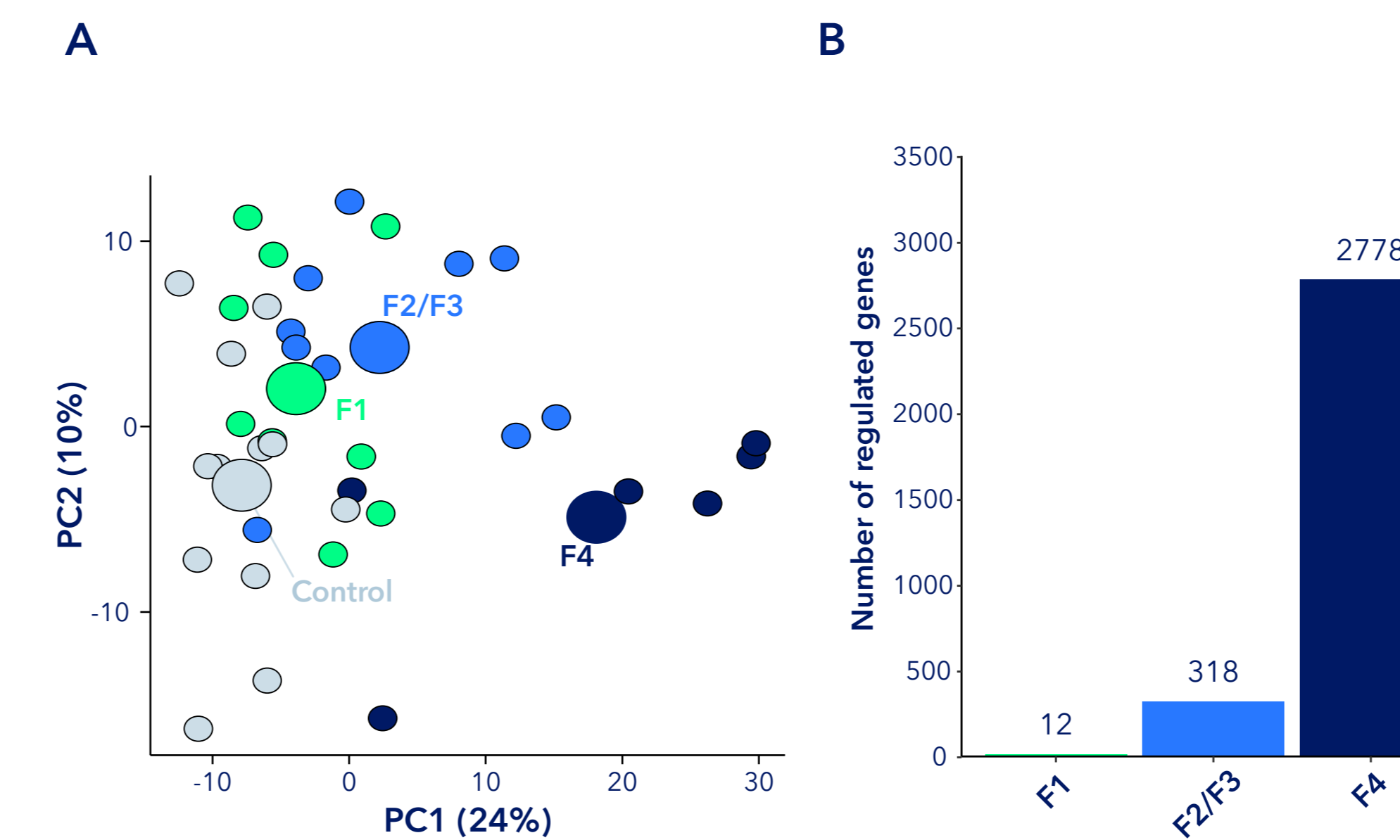


Figure 2. Transcriptome signature of cirrhosis. (A) Principal component analysis (PCA) of top-500 most variable genes. (B) Total number of differentially expressed genes relative to Control group (Padj < 0.05).

3 Perturbation of signaling pathways in cirrhosis

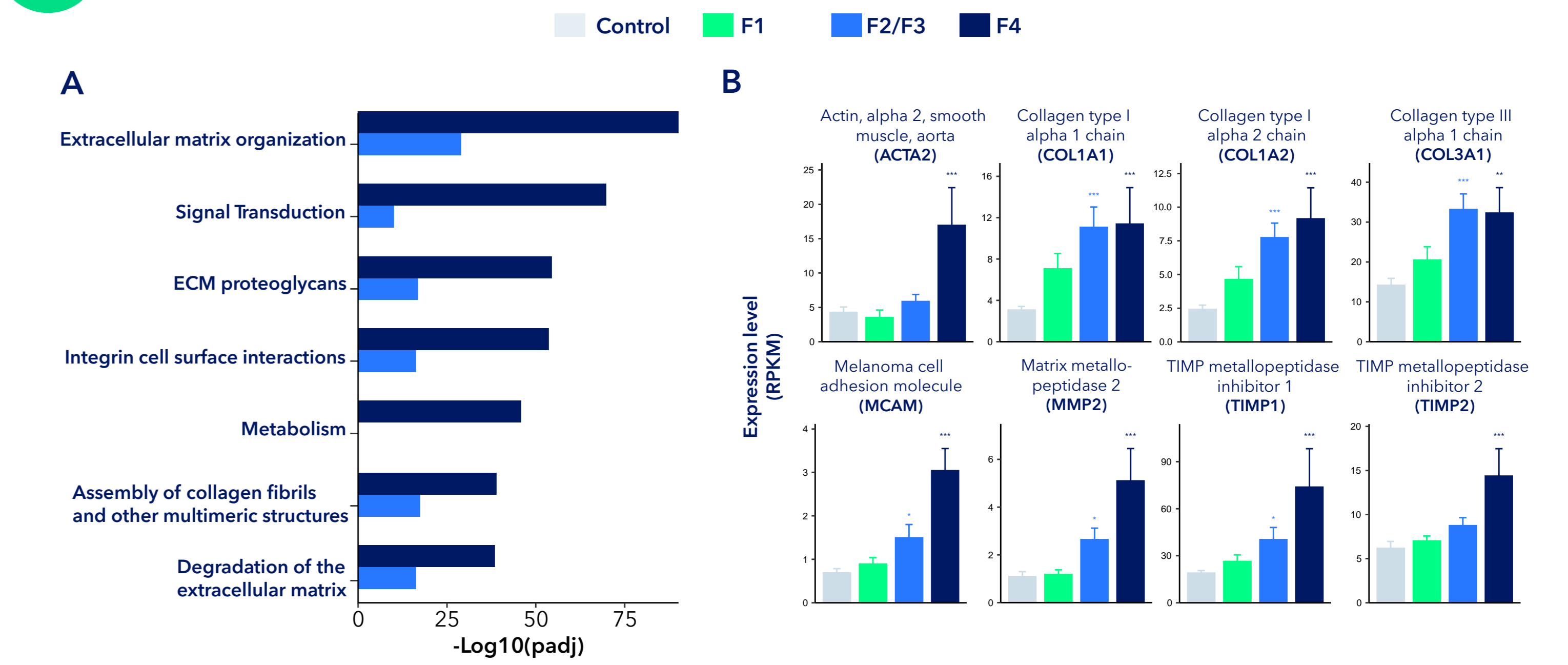


Figure 3. Perturbation of hepatic signaling pathways in NASH-related cirrhosis (A) Unbiased Reactome pathway analysis for comparisons F1, F2/3 and F4 relative to Control. Top-7 most enriched pathways are visualized. (B) Expression levels (reads per kilobase million, RPKM) of selected ECM-associated genes. *: padj<0.05, **:padj<0.01, *** padj < 0.001 relative to Control group. Error bars represent standard error of mean.

4 snRNAseq recovers all major liver cell types in NASH patients

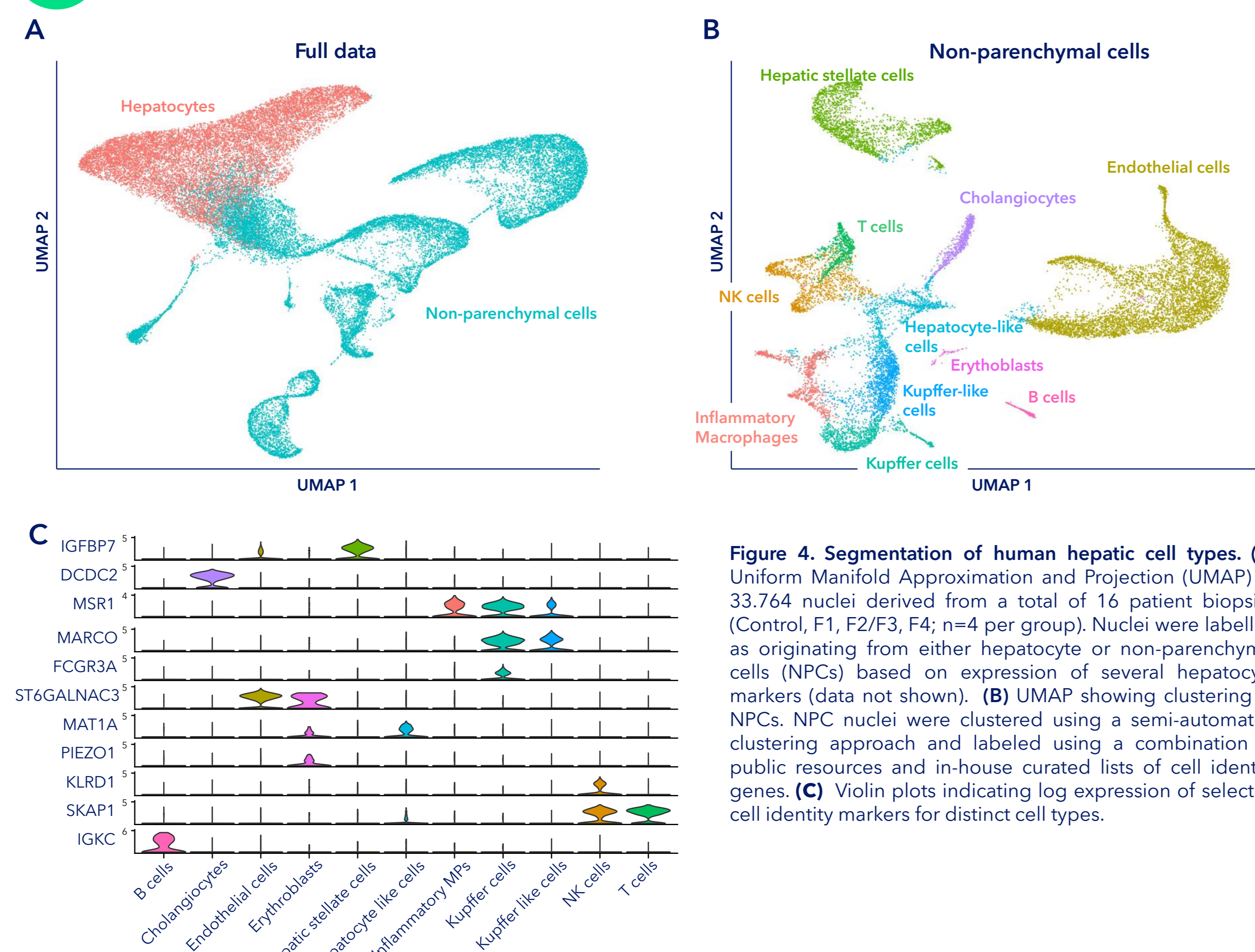


Figure 4. Segmentation of human hepatic cell types. (A) Uniform Manifold Approximation and Projection (UMAP) of 33,764 nuclei derived from a total of 16 patient biopsies (Control, F1, F2/F3, F4; n=4 per group). Nuclei were labelled as originating from either hepatocyte or non-parenchymal cells (NPCs) based on expression of several hepatocyte markers (data not shown). (B) UMAP showing clustering of NPCs. NPC nuclei were clustered using a semi-automated clustering approach and labeled using a combination of public resources and in-house curated lists of cell identity genes. (C) Violin plots indicating log expression of selected cell identity markers for distinct cell types.

5 snRNAseq identifies several HSC subpopulations

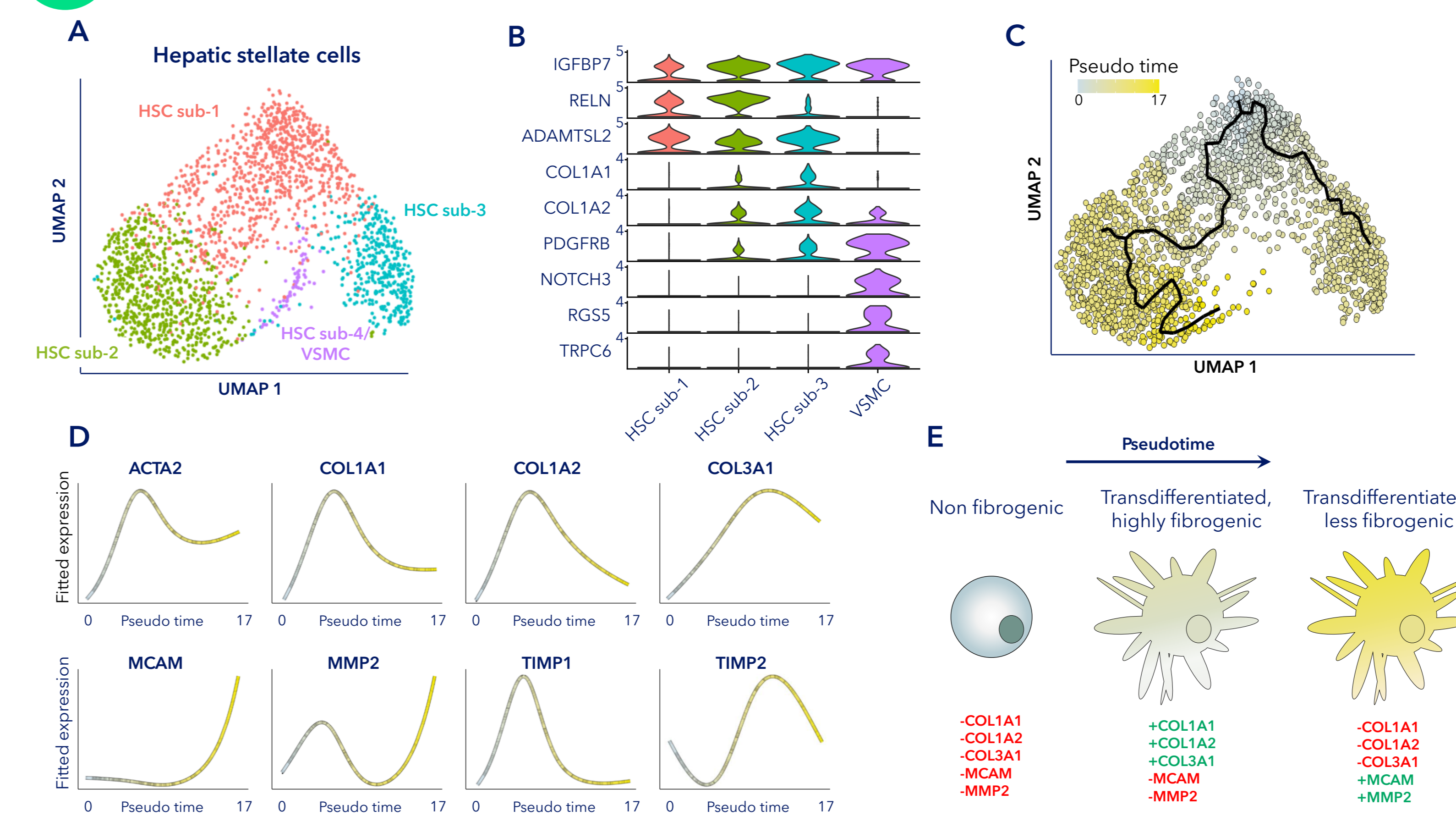


Figure 5. snRNAseq reveals distinct HSC subpopulations in human NASH patients. (A) UMAP showing sub-clustering of the hepatic stellate cell (HSC) cluster shown in Fig. 4B. Here, four distinct HSC subclusters were defined. HSC sub-4 likely represents vascular smooth muscle cells (VSMCs). (B) Violin plot depicting log expression levels of selected HSC genes (IGFBP7, RELN, ADAMTSL2), fibrogenic HSC genes (COL1A1, COL1A2, PDGFRB) and VSMCs (NOTCH3, RGSS, TRPC6). (C) Trajectory analysis on HSC sub-1,2 and 3 using Monocle 3. UMAP with black line illustrating fitted trajectory with HSC sub-1 defining early pseudo-time and nuclei colored by pseudo-time. (D) Modeled expression of selected fibrosis associated genes across HSCs ordered according to pseudo-time. (E) Proposed model for HSC trans-differentiation. Non fibrogenic HSCs undergo trans-differentiation characterized by upregulation of pro-fibrotic collagen genes but have the capacity to develop further into a less fibrogenic state by downregulating collagen mRNA expression while upregulating MCAM and MMP2 genes.

Conclusion

- + We performed paired high-quality bulk and snRNAseq on small liver biopsies from NASH patients
- + NASH patients with cirrhosis demonstrate a unique hepatic transcriptome signature
- + snRNAseq recovers all major human liver cell types
- + Fibrogenic HSCs may potentially differentiate into a less fibrogenic subtype
- + Molecular drivers of HSC trans-differentiation may be targeted to improve outcomes in NASH-related fibrosis

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