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## Single cell RNA sequencing of cystic fibrosis liver disease explants reveals endothelial complement activation

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**Keywords:** CFLD; non-cirrhotic portal hypertension; porto-sinusoidal vascular disorders; complement; inflammation.

**List of Abbreviations:** ALD (alcohol-related liver disease), CCA (Canonical correlation analysis), cDC (conventional dendritic cell), CF (cystic fibrosis), CFLD (cystic fibrosis-associated liver disease), CFTR (cystic fibrosis transmembrane conductance regulator), CV (central vein), DAMP (danger-associated molecular pattern), EC (endothelial cell), endMT (endothelial-to-mesenchymal transition), EVs (extracellular vesicles); FEV1 (forced exhaled volume in 1 second), LEC (lymphatic EC), LSEC (liver sinusoidal EC), NAFLD (non-alcoholic fatty liver disease), NCPH (non-cirrhotic portal hypertension), NK (natural killer cells), PAMP (pathogen-associated molecular pattern), PBC (primary biliary cholangitis), PCV (post-capillary vein), pDC (plasmacytoid dendritic cell), PSVD (porto-sinusoidal vascular disorder), scRNA-seq (single cell RNA sequencing).

## Abstract

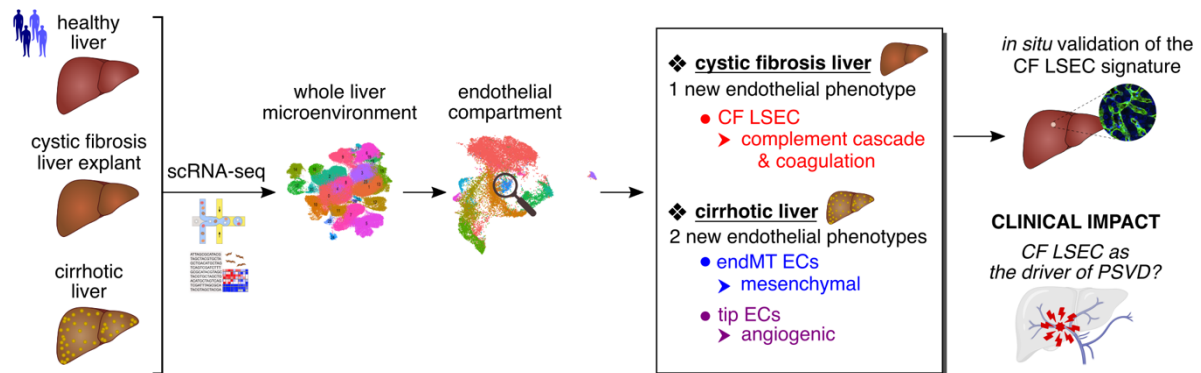
**Background & Aims:** Cystic fibrosis (CF) is considered a multisystemic disorder in which CF-associated liver disease (CFLD) is the third most common cause of mortality. Currently, no effective treatment is available for CFLD because its pathophysiology is still unclear. Interestingly, CFLD exhibits identical vascular characteristics as non-cirrhotic portal hypertension, recently classified as porto-sinusoidal vascular disorders (PSVD).

**Methods:** Since endothelial cells (ECs) are an important component in PSVD, we performed single cell RNA sequencing (scRNA-seq) on 4 explant livers from CFLD patients to identify differential endothelial characteristics which could contribute to the disease. We comprehensively characterized the endothelial compartment and compared it to publicly available scRNA-seq datasets from cirrhotic and healthy livers. Key gene signatures were validated *ex vivo* on patient tissues.

**Results:** We found that ECs from CF liver explants are more closely related to healthy than cirrhotic patients. In CF patients we also discovered a distinct population of liver sinusoidal ECs – coined CF LSECs – upregulating genes involved in the complement cascade and coagulation. Finally, our immunostainings further validated the predominant periportal location of CF LSECs.

**Conclusions:** Our work showed novel aspects of human liver ECs at the single-cell level thereby supporting endothelial involvement in CFLD, and reinforcing the hypothesis that ECs could be a driver of PSVD. Therefore, considering the vascular compartment in CF and CFLD may help developing new therapeutic approaches for these diseases.

## Graphical abstract



## Lay summary

Cystic fibrosis (CF) is a rare genetic disorder that can lead to CF-associated liver disease (CFLD), which is a major cause of mortality in CF patients. In this study we used single cell RNA sequencing to investigate the role of endothelial cells (ECs) in CFLD and found that there is distinct population of ECs upregulating genes involved in inflammation and coagulation. These findings suggest that ECs could be a driver of CFLD and may thus lead to the development of new therapeutic approaches for the disease.

## Introduction

Cystic fibrosis (CF) is a rare, autosomal recessive disorder predominately observed in the Caucasian population with an estimated incidence between 1/3000 - 1/6000 in Europe <sup>1</sup>. CF is caused by bi-allelic pathogenic variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR encodes for a chloride channel with an essential role in the regulation of the secretion and the absorption of anions, and is expressed in a variety of cell types including epithelial and endothelial cells (ECs) <sup>2,3</sup>. CF is considered a multisystemic disorder predominantly inducing lung disease, but also affecting the pancreas, the gastro-intestinal tract and the liver <sup>4-6</sup>.

CF-associated liver disease (CFLD) is the third most common cause of death after respiratory failure and transplant-related complications <sup>7</sup>. Interestingly, with the increase in median survival of the CF population, CFLD is becoming increasingly more frequent <sup>8-10</sup>. While in CF, CFTR modulator therapy shows promising results, not all patients with CFLD are eligible for this therapy and the effect on CFLD itself is still unclear <sup>11</sup>. Although clinically heterogeneous, it is estimated that 20-40% of all CF patients have CFLD, however less than 10% of the CF population suffers from a severe form of the disease with portal hypertension <sup>12-15</sup>. Complicating matters, the pathophysiology of liver disease is still unclear and no effective therapy has been developed to treat CFLD <sup>16,17</sup>.

Currently, two different CFLD pathomechanisms have been reported <sup>4</sup>. The first is based on a biliary hypothesis, focal biliary cirrhosis. Here, CFTR dysfunction in the cholangiocytes results in hyperviscous bile that accumulates and causes bile plugging. The resulting biliary obstructions can induce local and portal inflammation, which may eventually cause focal fibrosis, multilobular fibrosis and cirrhosis <sup>18-20</sup>. Nevertheless, this neonatal cholestasis seems not linked to the development of CFLD later in life <sup>9,21,22</sup>, as CFLD patients usually lack clinical or biochemical features of cholestasis and current treatments to liquify the bile are ineffective in CFLD <sup>16,17</sup>. The second mechanism is based on a vascular hypothesis, first proposed by Witters and colleagues <sup>23,24</sup>. They have shown that CFLD with portal hypertension exhibits identical vascular characteristics as non-cirrhotic portal hypertension (NCPH) <sup>9,23-26</sup>. In addition, the main pathological representation of NCPH, obliterative portal venopathy, has been suggested to be the main cause for CF children and young adults for requiring a liver transplantation <sup>27,28</sup>. Recently, based on anatomic and clinical

observations the term “porto-sinusoidal vascular disorder” (PSVD) was introduced and now includes NCPH <sup>29</sup> (extensively reviewed in <sup>30,31</sup>). Following these recommendations, we will hereafter refer to PSVD when mentioning NCPH.

In all the different etiologies of PSVD a vascular component is present <sup>32</sup>. The exact pathophysiology is still under research but ECs, the cells lining blood vessels, are in the center of the pathogenesis <sup>32,33</sup>. In addition, hepatic vasculature alterations are known mediators of liver disease pathogenesis including inflammation, regeneration, carcinogenesis and portal hypertension <sup>34</sup>. In light of our previous findings where we unravel the role of CFTR in ECs and found endothelial activation and dysfunction <sup>2,35,36</sup>, we performed single cell RNA sequencing (scRNA-seq) to identify vascular defects which could contribute to the PSVD in CFLD.

## Methods

### Patient cohort, sampling and data collection

Four CFLD patients were enrolled in this study from the University Hospitals Leuven. Liver tissue from CF patients was obtained intraoperatively from patients undergoing a combined orthotopic liver transplantation and double lung transplantation at the University Hospitals from Leuven and Ghent. All CFLD patients were diagnosed with portal hypertension based on either splenomegaly with cytopenia, collateral circulation and/or esophageal varices. The demographic and disease characteristics of the CF patients studied by scRNA-seq are listed in Supplemental Table S1.

### Human tissue processing

Following surgical resection, samples from the liver tissue were aliquoted for histological purposes, and immediately processed for scRNA-seq as previously described with minor modifications <sup>37</sup>. After enzymatic tissue digestion, the single-cell suspensions were enriched for CD31<sup>+</sup> ECs using the MACS system (Miltenyi Biotec) according to the manufacturer’s instructions or processed without CD31<sup>+</sup> enrichment. As shown previously, CD31<sup>+</sup> EC enrichment was necessary to capture detailed EC phenotypes after scRNA-seq analysis <sup>38</sup>. The single cell suspensions were converted to barcoded scRNA-seq libraries using the Chromium Single Cell 3’ Library, Gel Bead & Multiplex Kit and Chip Kit (10x Genomics), aiming for 6,000 cells per library. Samples were processed using kits pertaining to V2 barcoding chemistry of 10x Genomics.

Libraries were sequenced on an Illumina HiSeq4000, and mapped to the human genome (build GRCh38) using Cell Ranger software (10x Genomics, version 3.1.1) to generate raw gene expression matrices.

### **Integration of publicly available dataset**

We re-processed the scRNA-seq data on 5 healthy and 5 cirrhotic (2 non-alcoholic fatty liver disease (NAFLD), 2 alcohol-related liver disease (ALD), 1 primary biliary cholangitis (PBC)) livers published by Ramachandran *et al.*<sup>39</sup>. FastQ files were downloaded from the Sequence Read Archive (SRA, NCBI, GSE136103) and subsequently mapped to the human genome (build GRCh38) using Cell Ranger software (10x Genomics, version 3.1.1) to generate raw gene expression matrices.

For further details regarding the materials and methods used, please refer to the Supplementary materials and methods.

## **Results**

### **scRNA-seq and cell typing of liver samples**

We performed scRNA-seq with (n=3) and without (n=2) enrichment for endothelial cells on explant livers from 4 patients with CF undergoing a combined orthotopic liver and double lung transplantation procedure. These samples are referred to as CF. Demographic and clinical data of the patients, including comorbidities and forced exhaled volume in 1 second (FEV1) are summarized in Supplementary information (Supplemental Table S1). After quality filtering, we obtained 34,032 cells with > 200 genes detected.

To compare our CF liver tissue samples to healthy and cirrhotic liver tissue, we re-processed publicly available scRNA-seq data from human livers by Ramachandran *et al.*, consisting of 5 patients with “healthy” liver (n = 43,324 cells) and 5 patients with “cirrhotic” (2x NAFLD, 2x ALD, 1x PBC) liver (n = 46,380 cells)<sup>39</sup>. After quality filtering of the publicly available data, we then merged our CF cells with the healthy and cirrhotic cells yielding a dataset of 123,736 cells in total, of which ~27.5% of cells were from CF. Canonical correlation analysis (CCA)-based integration was performed to remove the patient-specific bias as described previously<sup>40–42</sup> (Fig. 1a, Supplemental Fig. S1a-b), followed by graph based clustering to generate a UMAP per cell type to display the heterogeneity in the liver.

Subsequent analysis involving dimensionality reduction and clustering identified several clusters (Fig. 1c), which through marker genes (Fig. 1c, Supplemental Fig. S1d-e), and in unbiased way by showing the top 5 upregulated genes per cluster (Fig. 1d), could be assigned to lymphoid cells (T-cells, natural killer cells (NK), B-cells and cytotoxic T/NK-cells), myeloid cells (monocytes/macrophages, neutrophils, mast cells, plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs)), epithelial cells, mesenchymal cells (including hepatic stellate cells and vascular smooth muscle cells), endothelial cells (ECs; blood vascular endothelial cells and lymphatic endothelial cells (LEC)) and cycling cells. We describe each cell type in more detail, highlighting the number of cells and top 50 upregulated genes in Supplemental Table S2.

After integration, as suspected the most abundant cell types were ECs, amounting up to 34.5% (n = 42,732) of all cells. When evaluating the relative enrichment or depletion of all cell types, we found that ECs were more frequent in CF vs. non-CF samples, suggesting our endothelial enrichment protocol was effective (Fig. 1e). On the other hand, T-cells were less abundant in CF versus non-CF, a discrepancy possibly explained by different sample preparation methods as no flow cytometry sorting was performed on CF samples. Notably, B-cells were enriched in “cirrhotic” versus “healthy”, while cDCs were increased in “healthy” versus “cirrhotic” (Fig. 1e). No significant abundance differences were found for the other identified immune and non-immune cell types.

Below, we describe the endothelial heterogeneity in more detail. As before, we performed CCA-based integration but now only on the vascular and LEC clusters to remove any patient-specific bias (Fig 2a, Supplemental Fig. S2a-b)<sup>40–42</sup>.

### **Endothelial heterogeneity in human liver**

After removal of doublets and low quality cells (Supplementary methods), we retrieved 19,286 high quality vascular and lymphatic ECs expressing canonical marker genes *ENG*, *CDH5*, *CLDN5*, *PECAM1* (Supplemental Fig. S2c-d) of which 10,144 were from CF ECs (52.6 %), 5,153 from “cirrhotic” ECs (26.7 %) and 3,989 from “healthy” ECs (20.7%). All ECs were further subclustered into 11 phenotypes: 1 small LEC cluster (*CCL21*, *PROX1*, *PDPN<sup>high</sup>*) and 10 vascular endothelial clusters (Fig. 2b). A list of marker genes used to identify each cellular phenotype is highlighted in Supplementary

information Table S3 (Fig. 2c,d; Supplemental Fig. S2e-f). The annotation was based on the relative abundance of top-ranking marker genes previously described in histological and scRNA-seq studies (Fig. 2c-d, Supplemental Fig. S2d-f)<sup>39,43–51</sup>.

The arterial cluster (artery, *SOX17<sup>high</sup>*) expressed canonical marker genes (*SOX17*, *CXCL12*, *SERPINE2*, *GJA4*) but also revealed high *TMEM100*, *FBLN2* and *SSUH2* expression, which are genes involved in arterial formation, vessel integrity and circumferential stress response respectively (Fig. 2c-d, Supplemental Fig. S2g, Supplemental Table S3)<sup>52–54</sup>.

We identified 1 artery cluster (*SOX17<sup>high</sup>*) and 3 venous EC clusters (*NR2F2<sup>high</sup>*) (Supplemental Fig S2e) having distinct gene signatures: vein, central vein (CV) and post-capillary vein (PCV). Annotation of the CV ECs was based on the previously reported *RSPO3<sup>high</sup>* and *WNT2<sup>high</sup>* expression<sup>39,46</sup>. Additionally, we found *PTGDS*, *LYPD2* and *TAGLN* as most upregulated genes in the CV cluster (Fig. 2c-d, Supplemental Fig. S2g, Supplemental Table S3). We identified a portal vein-like signature expression in two clusters (vein, PCV) based on known venous and portal vein gene expression (*CD320*, *CPE*, *CLU*)<sup>39,46</sup> (Fig. 2c-d, Supplemental Fig. S2f, Fig. S3, Supplemental Table S3).

We could distinguish 2 clusters of liver sinusoidal ECs (LSECs) which are fenestrated and can be distinguished using validated marker genes (*CLEC4G*, *CLEC4M*, *STAB2*, *CD14*) (Fig. 2c-d, Supplemental Fig. S2f, Supplemental Table S3)<sup>34,39,44,45</sup>. As was previously demonstrated, we confirmed that LSECs were enriched for genes with endocytosis and immunoregulatory functions (Supplemental Fig S2h)<sup>34,39,44,45</sup>. Among the two LSEC clusters, one was specifically enriched in CF patients, hence dubbed CF LSEC and extensively characterized in a specific section below.

We also found three clusters with a capillary-like signature (capillary, tip-like and endothelial-to-mesenchymal transition (endMT)) that express well-known capillary markers (*RGCC*, *PLVAP*, *CA4*) (Fig. 2c-d, Supplemental Fig. S2f, Supplemental Table S3)<sup>48–50,55</sup>. The tip-like and endMT clusters are extensively described in the next section.

Finally, proliferating cells (coined cycling cluster) express well known marker genes involved in cell cycle progression and cell division (*MKI67*, *UBE2C*, *TOP2A*, *TYMS*) (Fig. 2c-d, Supplemental Fig. S2f, Supplemental Table S3).

### **Cirrhotic liver diseases induce specific endothelial subpopulations**

Liver diseases can induce hepatic vascular changes including LSEC capillarization. In this process LSECs lose the fenestrae on the sieving plates and develop an organized basement membrane<sup>34</sup>. LSEC capillarization is thought to play a crucial role in fibrogenesis, in part by facilitating hepatic stellate cell activation<sup>34</sup> and undergoing endMT<sup>56</sup>. Upon reanalysis of the data from Ramachandran *et al.*<sup>39</sup>, we found a capillary-like signature expression in three clusters (capillary, tip-like and endMT, see below) in all of which ECs coming from “cirrhotic” livers were overrepresented (Fig. 2e, Supplemental Table S4). Typically, these capillary clusters had increased *CD34* expression, an established marker for chronic liver disease<sup>57–59</sup>, as well as a transdifferentiation signature with the loss of typical LSEC markers *CLEC4M*, *STAB1*, *STAB2* (Fig. 3a)<sup>39,46</sup>. Notably, the capillary signature was characterized by the increased expression of well-known capillary markers (*RGCC*, *PLVAP*, *CA4*) conserved in different organs and species (Fig. 3b)<sup>48–50,55</sup>. These findings are compatible with Ramachandran *et al.*<sup>39</sup>, there denoted as scar-associated endothelial subpopulations, demonstrating the reliability of our analysis. Among the three capillary-like clusters, two have not been described before i.e. an endMT and tip-like cluster.

EndMT is known to play a role in fibrosis in liver disease<sup>60</sup>. One of the capillary clusters had an endMT signature characterized by matrix remodeling associated genes (*FN1*, *COL15A1*, *COL4A1*, *COL4A2*, *SNAI1*)<sup>61</sup> and genes known to promote tissue fibrosis<sup>62,63</sup> and the process of endMT<sup>64</sup> (*IGFBP2*, *IGFBP5*) (Fig. 3c-e, Supplemental Table S3). In addition, and consistent with the role of these microvessels, lipid uptake, transport and metabolism were upregulated in this cluster (*FABP4*, *FABP5*, *LPL*, *CETP*, *PPARG*, *CD300LG*, *MGLL*)<sup>65</sup> (Fig. 3c-e, Supplemental Table S3). Simultaneously, this cluster overexpresses previously described genes which belong to immature ECs found in lung cancer (*RBP7*, *VWA1*, *FLT1*, *ENG*, *PLVAP*, *GSN*) (Fig. 3e, Supplemental Table S3)<sup>49</sup>. Altogether, this endMT cluster, overrepresented in cirrhotic samples, seems to contribute to liver fibrosis and inflammation further aggravating the liver disease.

In the other capillary cluster we found an angiogenic/tip-like signature as we previously described in human lung ECs (*PGF*, *APLN*, *APLNR*, *ANGPT2*, *LXN*, *LOX*, *CXCR4*, *RBP7*, *VWA1*, *LGALS1*) (Fig. 3e)<sup>49</sup>. Gene set enrichment analysis confirmed upregulation in blood vessel formation and extracellular matrix remodeling (Fig. 3f-g).

The tip-like cluster had considerable overlap with the capillary and endMT cluster including fatty acid processing (*FABP4*, *FABP5*, *GPIHBP1*, *MGLL*, *APOD*)<sup>65</sup> and extracellular matrix remodeling (*LAMB1*, *LAMC1*, *LAMA4*, *MMP2*) (Fig. 3e, Supplemental Table S3)<sup>49</sup>. Also a specific pro-inflammatory phenotype was observed with genes specific for T-cell recruitment and signaling (*IL32*, *TNFRSF4*, *CXCR4*, *VSIR*, *THY1*, *LY6H*).

We found an increased fraction of “cirrhotic” ECs in the LEC cluster (Fig. 2e, Supplemental Table S4) thereby supporting earlier findings that chronic liver disease patients have a significantly higher lymphatic vessel density<sup>66</sup>. Given the low relative amount of LECs it was not feasible to reliably identify transcriptomic differences across conditions. Looking at the portal vein-like signature clusters, we identified a PCV-like cluster<sup>49</sup> which was overrepresented by ECs coming from “cirrhotic” ECs (Fig. 2e). The PCV-cluster was characterized by a pro-inflammatory phenotype including high *ACKR1* expression as was previously demonstrated (Fig. 2c-d, Supplemental Fig. S3)<sup>39</sup>. Compared to the vein cluster, this PCV cluster overexpresses the leukocyte adhesion and extravasation genes *SELE* and *SELP* encoding for E-selectin and P-selectin respectively (Supplemental Fig. S3). Other highly expressed genes in this cluster include *POSTN* and *IL6*, genes contributing to a pro-inflammatory phenotype and previously ascribed to post-capillary vein in lung and breast ECs<sup>49,67,68</sup>. Interestingly, the angiogenic inducer *CYR61*, which plays an essential role in physiologic and pathologic neovascularization was most upregulated in the PCV cluster<sup>69,70</sup>.

### **Vascular changes in CFLD**

As PSVD is regarded as a vascular disease and CFLD patients, usually with a well preserved liver function, exhibit obliterative portal venopathy ensuing a liver transplantation<sup>9,23–26,28</sup>, we would expect to observe changes in the endothelial subpopulations. Despite that samples were taken from explanted livers, representing advanced CFLD, it seemed that “healthy” and CF ECs are relatively similar, in contrast to the “cirrhotic” ECs (Fig. 2a, Supplemental Fig. S2a-b). This is in line with the most recent pathophysiological hypothesis<sup>9,16,17,21,22</sup> that cirrhosis is different from CFLD with portal hypertension.

Additionally, we found one cluster which was almost unique to CF ECs compared to “healthy” and “cirrhotic” cells (referred to as CF LSEC, Fig. 2e, Supplemental Table S4) and hence can play a role in the pathomechanism. This enriched CF cluster was characterized by typical LSEC marker gene expression (*CLEC4G*, *CLEC4M*, *STAB1*, *STAB2*, *FCN2*) and portal vein markers (*MEG3*, *IL33*, *RFK*, *FLRT2*, *LDB2*) (Fig. 4a, Supplemental S3). In contrast to microvascular cirrhotic ECs, this CF LSEC cluster does not lose key fenestration markers (reminiscent of capillarization), yet it overexpressed genes involved in the coagulation (*EDN1*, *F8*, *ADGRG6*, *TFPI*, *MMRN1*), complement cascade (*FCN2*, *MASP1*, *COLEC11*, *F2R*, *C7*), matrix remodeling and migration (*IGFBP3*, *MATN2*, *BGN*, *MDK*) (Fig. 4a-c). It is well established that the liver complement system is a central player in the innate immune and coagulation system<sup>71,72</sup>. Moreover, many interactions exist between these two cascades which is also reflected in our GSEA analysis (Fig. 4b-c)<sup>71,72</sup>. Interestingly, *CDH11* was highly upregulated in this cluster. This cell-cell adhesion protein is known to contribute to inflammation-driven fibrotic tissue remodeling (Fig. 4a)<sup>73</sup>. Overlapping with the LEC, CF LSEC overexpressed the venous ECs marker *MMRN1*<sup>50,74</sup> known to enhance platelet adhesion and coagulation (Supplemental Table S3)<sup>75</sup>. Among proposed mechanisms for the obliteration of the portal venules are aberrant coagulation processes, vascular remodeling disorders and endothelial injury from immune cells. Interestingly, these mechanisms are upregulated in the CF LSEC cluster, which has an in-between phenotype of LSEC and portal vein marker genes, supporting the pathological evidence of NCPH in CFLD.

In order to validate the biological relevance of our transcriptomic analysis we have verified a selection of the highest upregulated genes of the CF LSEC signature in patient liver tissues (Supplemental Table S1). To confirm endothelial complement activation in CF LSEC, we performed immunostainings and *in-situ* hybridization on CFLD sections for specific CF LSEC markers (*COLEC11*, *MASP1*, *MATN2*, *IGFBP3*). We demonstrate co-localization of CF LSEC markers and *CLEC4G* (an LSEC-specific marker), thereby validating the CF LSEC signature and assuring endothelial involvement (Fig. 4d-e, Supplemental Fig. S4). Furthermore, as compared to “healthy” and “cirrhotic” liver tissues, several CF LSEC markers are more expressed in *CLEC4G*<sup>+</sup> LSECs from the CFLD explants (Fig. 4f, Supplemental Fig. S4), and more specifically in the periportal sinusoids (Supplemental Fig. S4d-f) which corresponds with the observed intermediate transcriptome of CF LSEC. Since excessive

complement activation can cause tissue and organ damage, and may amplify systemic inflammation <sup>71</sup>, our data raises the question whether endothelial complement hyperactivation in CF patients could be the central mediator to induce CFLD.

## Discussion

We performed scRNA-seq on liver explants from human CF patients with liver disease to resolve the vascular niche of CFLD. Thereby we compared the endothelial compartment from CF liver explants with “healthy” and “cirrhotic” livers. First, ECs in CFLD bear more resemblance to “healthy” than “cirrhotic” liver, supporting a different, noncirrhotic pathogenesis. Second, we identified a specific subpopulation of ECs in CFLD patients, which we named CF LSEC, which has an intermediate phenotype of LSEC and portal vein marker genes. The CF LSEC cluster overexpressed genes involved in the complement cascade and coagulation process. Third, immunohistochemistry and *in situ* hybridization on CFLD liver sections confirmed the endothelial specific expression of complement cascade proteins in CF LSEC from CF patients. Moreover, we observed that CF LSEC markers were most expressed on the portal vein and periportal sinusoids which corresponded with the mixed transcriptome of CF LSEC.

It is well-known that excessive complement activation and coagulation can cause liver damage and PSVD, and can act as multiplier of the inflammatory process <sup>71,72</sup>. Additionally, as complement activation can induce coagulation <sup>71,72</sup>, and microangiopathy of the portal vein branches is one of the hallmarks of PSVD <sup>76</sup>, it could be that the CF LSEC population, an intermediate phenotype of LSEC and portal vein – that is unique to CFLD – could represent the driver for PSVD in CFLD. Therapeutic interventions might consist in addressing complement inhibitors or anti-inflammatory molecules to CF LSEC either specifically via nanoparticles or systemically. Moreover, application of our novel CF LSEC cell markers could also potentially assist in histopathology-based CFLD patient identification which could be fundamental for the diagnosis and treatment of CFLD. Circulating biomarkers could be scrutinized by means of extracellular vesicles (EVs), which are nanoscale structures, stable and easily accessible from various biological fluids, and virtually secreted by all cell types <sup>77</sup>. The difficulty of this approach will thus hold in successfully isolate CF LSEC-specific circulating EVs to account for the presence of this novel cell population.

Among PSVD, NCPH is considered a rare disorder representing a heterogeneous group of liver diseases, primarily of vascular origin, leading to portal hypertension<sup>76,78</sup>. It is characterized by intrahepatic portal hypertension in the absence of cirrhosis and the pathogenesis of NCPH is still being investigated. The etiology of NCPH can be divided into five categories: i) chronic infections (endothelial injury from immune cells), ii) exposure to medication/toxins, iii) genetic predisposition, iv) prothrombotic conditions (aberrant coagulation activation or thrombosis), and v) immunologic disorders<sup>33,76,79</sup>. All of these categories can directly influence the endothelium, which is in direct contact with the blood coming from the gut to the liver, and is an important mediator in the aforementioned processes, which are deregulated in CF<sup>2</sup>. Our data further supports the presence of NCPH in CFLD but whether our findings can be expanded to other PSVD disorders remains to be explored.

The gut-liver axis, the anatomical and physiological relationship between the gut and liver, is considered a key factor for the onset and progression of liver diseases<sup>80–82</sup>. More specifically, an increase in intestinal permeability is expected to allow more passage of metabolites, bacteria, pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) from the intestine through the portal vein to the liver. Indeed, microbial dysbiosis together with a more permeable intestinal barrier are known to promote portal hypertension via activation of the complement cascade which consecutively induces proinflammatory and profibrogenic pathways<sup>83</sup>. It has been postulated that the gut-liver axis could be a factor in the pathogenesis of CFLD<sup>84,85</sup>. CF patients with an intestinal obstruction at birth have a higher risk for developing CFLD<sup>9</sup> and CFLD was associated with increased intestinal mucosal lesions, longer small bowel transit time and microbiome alterations regardless of the abnormal intestinal permeability which is present in almost all CF patients<sup>86</sup>. In *in vivo* experiments it was shown that a murine CFTR<sup>KO</sup>-model had increased intestinal barrier permeability and that environmental as well as diet-induced dysbiosis was required to induce liver disease<sup>87–89</sup>. It remains unclear if gut-liver axis could directly induce CFLD, although it has been suggested that the complement cascade could play an essential role in pathogenesis of NCPH<sup>90,91</sup>.

Additionally, upon reanalysis of the data from Ramachandran *et al.*<sup>39</sup>, we found two previously unrecognized capillary populations in cirrhotic liver ECs, namely an angiogenic tip cell and endMT population. The angiogenic population demonstrated an upregulation of genes involved in blood vessel formation and matrix remodeling

required for new blood vessel formation. Previously we also described a similar endothelial population in lung cancer patients <sup>49</sup>. It is evident that angiogenesis is a recurring factor in portal hypertension, liver fibrosis and cirrhosis. Indeed, several factors including hypoxia, elevated oxidative stress, inflammation and shear stress are well-known angiogenic mediators <sup>92</sup>. It is however striking that this population was overrepresented only in cirrhotic ECs and not CF ECs, possibly indicating that CFLD, represented as PSVD, has a different pathological mechanism than cirrhosis.

Liver ECs are able to undergo endMT in response to chronic liver injury <sup>60</sup>. During endMT, ECs lose their key traits and start to express endMT-associated transcription factors and mesenchymal markers. Furthermore, these mesenchymal-like ECs demonstrated overexpression of fatty acid oxidation processing which is a critical regulator of endMT <sup>93</sup>. Our findings confirmed earlier studies that only a small population of ECs in chronic liver disease can undergo endMT <sup>60</sup> as shown in the cirrhotic ECs. Although not statistical, there was a trend toward an increase in the relative fraction of the endMT EC cluster in CF compared to “healthy” liver. As described both in CF epithelium <sup>94</sup> and endothelium <sup>35</sup>, a CFTR impairment results in the induction of a mesenchymal signature. As endMT has been shown to be involved during fibrosis, vascular dysfunction and NCPH, it will be interesting to explore whether it has also a role in the progression of PSVD in CFLD. Indeed, it was hypothesized by Sato and colleagues that endMT could explain the obliterative portal venopathy shown in PSVD <sup>95</sup>. Moreover, we previously showed that *ex vivo* CFTR modulator treatment moderate the pro-inflammatory response and the endMT marker genes induction from CF patient-derived ECs <sup>35,36</sup>. As such, it will be interesting to compare whether patients with CF treated with CFTR modulators present a reduced or different CF LSEC signature.

Despite the clear identification of a characteristic CF LSEC population, absent in “healthy” or “cirrhotic” liver, our study also has limitations. First, our data would benefit from a validation in a larger cohort of patients. Indeed, by scRNA-seq the CF LSEC population could not be identified in 2 patients, albeit validated and present at higher level in immunostaining. This discrepancy could be attributed to the fact that the CF LSEC cluster only represents 2-5% of all ECs, and arise from a relatively small excision piece sampled from the liver explant. Second, although we did not detect sample-specific cell clusters during our analysis, we cannot exclude that any batch effect influencing the general transcriptome is present. The interstudy effect is the

mainstay problem of any scRNA-seq study using publicly available data and is usually introduced by different sample preparation and sequencing methods<sup>96</sup>. Third, on our analysis, we focused on the endothelial population in CF, as they are the presumed driver of NCPH. However, multiple cell types most likely play a role in CFLD as it was demonstrated for liver macrophages in chronic liver diseases<sup>97</sup>. Hence, potential altered interactions between cell types are also worth being investigated to provide a more global view of all cellular changes in CFLD. In addition, it would be interesting to investigate how the CF LSEC signature may evolve in CF patients (before the onset of CFLD, and/or after treatment with CFTR modulators), and whether it is also present - and likely involved - in other PSVDs<sup>31</sup>.

Our work illustrates the power of scRNA-seq to decode the endothelial subpopulations in human “healthy”, “cirrhotic” and CF livers. We characterized previously unrecognized endothelial populations present in “cirrhotic” and CF livers. More specifically, we described two novel capillary populations with an angiogenic and endMT phenotype overrepresented in ECs coming from “cirrhotic” livers. Also, we characterized, *in silico* and *in situ*, a CF LSEC population, which overexpresses genes involved in coagulation and complement cascade. It remains a topic for further study to determine if this population, overabundantly present in CF liver ECs, could have potential clinical implications for CFLD pathogenesis. Nevertheless, it reinforces the hypothesis that CFLD can be present as PSVD of which ECs might represent the drivers of the disease. We previously demonstrated that endothelial CFTR impairment induced EC activation and dysfunction including elevated oxidative stress levels, defective functional autophagy, mitochondrial dysfunction and increased leukocyte adhesion and extravasation<sup>35,36</sup>. Given the role of CFTR in pulmonary arterial hypertension<sup>98–100</sup> and together with our current scRNA-seq findings in CFLD, a similar scRNA-seq study investigating the endothelium in the CF lung seems warranted.

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## References

1. Scotet, V., L'Hostis, C. & Férec, C. The Changing Epidemiology of Cystic Fibrosis: Incidence, Survival and Impact of the CFTR Gene Discovery. *Genes* **11**, 589 (2020).
2. Declercq, M., Treppe, L., Carmeliet, P. & Witters, P. The role of endothelial cells in cystic fibrosis. *Journal of Cystic Fibrosis* (2019) doi:10.1016/j.jcf.2019.07.005.
3. Cozens, A. L. *et al.* CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *American Journal of Respiratory Cell and Molecular Biology* **10**, 38–47 (1994).
4. Shteinberg, M., Haq, I. J., Polineni, D. & Davies, J. C. Cystic fibrosis. *The Lancet* **397**, 2195–2211 (2021).
5. Bell, S. C. *et al.* The future of cystic fibrosis care: a global perspective. *The Lancet Respiratory Medicine* **8**, 65–124 (2020).
6. Kliegman, R. M. *Nelson Essentials of Pediatrics*. (Elsevier Saunders, St. Louis, 2006).
7. Rowland, M. *et al.* Outcome in patients with cystic fibrosis liver disease. *Journal of Cystic Fibrosis* **14**, 120–126 (2015).
8. Toledano, M. B. *et al.* The emerging burden of liver disease in cystic fibrosis patients: A UK nationwide study. *PloS one* **14**, e0212779–e0212779 (2019).
9. Boëlle, P. *et al.* Cystic Fibrosis Liver Disease: Outcomes and Risk Factors in a Large Cohort of French Patients. *Hepatology* (2018) doi:10.1002/hep.30148.

10. Koh, C. *et al.* Adult-onset cystic fibrosis liver disease: Diagnosis and characterization of an underappreciated entity. *Hepatology* **66**, 591–601 (2017).
11. Levitte, S., Fuchs, Y., Wise, R. & Sellers, Z. M. Effects of CFTR modulators on serum biomarkers of liver fibrosis in children with cystic fibrosis. *Hepatol Commun* **7**, e0010 (2023).
12. Lamireau, T. *et al.* Epidemiology of liver disease in cystic fibrosis: a longitudinal study. *J Hepatol* **41**, 920–925 (2004).
13. Colombo, C. *et al.* Liver disease in cystic fibrosis: A prospective study on incidence, risk factors, and outcome. *Hepatology* **36**, 1374–1382 (2002).
14. Wilschanski, M. & Durie, P. R. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. *Gut* **56**, 1153–1163 (2007).
15. Debray, D., Kelly, D., Houwen, R., Strandvik, B. & Colombo, C. Best practice guidance for the diagnosis and management of cystic fibrosis-associated liver disease. *Journal of Cystic Fibrosis* **10**, S29–S36 (2011).
16. Cheng, K., Ashby, D. & Smyth, R. L. Ursodeoxycholic acid for cystic fibrosis-related liver disease. *Cochrane Database Syst Rev* CD000222 (2014).
17. Colombo, C. *et al.* Ursodeoxycholic acid and liver disease associated with cystic fibrosis: A multicenter cohort study. *Journal of Cystic Fibrosis* (2021)  
doi:<https://doi.org/10.1016/j.jcf.2021.03.014>.
18. Kamal, N., Surana, P. & Koh, C. Liver disease in patients with cystic fibrosis. *Current Opinion in Gastroenterology* **1** (2018)  
doi:[10.1097/MOG.0000000000000432](https://doi.org/10.1097/MOG.0000000000000432).
19. Stauffer, K. Current Treatment Options for Cystic Fibrosis-Related Liver Disease. *International Journal of Molecular Sciences* vol. 21 Preprint at <https://doi.org/10.3390/ijms21228586> (2020).

20. Ooi, C. Y. & Durie, P. R. Cystic fibrosis from the gastroenterologist's perspective. *Nature Reviews Gastroenterology & Hepatology* **13**, 175–185 (2016).
21. Lykavieris, P., Bernard, O. & Hadchouel, M. Neonatal cholestasis as the presenting feature in cystic fibrosis. *Archives of disease in childhood* **75**, 67–70 (1996).
22. Colombo, C. *et al.* Analysis of risk factors for the development of liver disease associated with cystic fibrosis. *The Journal of Pediatrics* **124**, 393–399 (1994).
23. Witters, P. *et al.* Noncirrhotic presinusoidal portal hypertension is common in cystic fibrosis-associated liver disease. *Hepatology* **53**, 1064–1065 (2011).
24. Witters, P. *et al.* Liver disease in cystic fibrosis presents as non-cirrhotic portal hypertension. *Journal of Cystic Fibrosis* **16**, e11–e13 (2017).
25. Hillaire, S. *et al.* Liver transplantation in adult cystic fibrosis: Clinical, imaging, and pathological evidence of obliterative portal venopathy. *Liver Transplantation* **23**, 1342–1347 (2017).
26. Wu, H. *et al.* Obliterative Portal Venopathy Without Cirrhosis Is Prevalent in Pediatric Cystic Fibrosis Liver Disease With Portal Hypertension. *Clinical Gastroenterology and Hepatology* (2018)  
doi:<https://doi.org/10.1016/j.cgh.2018.10.046>.
27. O'Neill, J. P., Clifford, C. S., Nolan, N. P. & McCormick, P. A. Non-cirrhotic portal hypertension (obliterative portal venopathy) is the predominant form of chronic liver disease in cystic fibrosis. *Ir J Med Sci* (2022) doi:10.1007/s11845-022-03153-0.

28. Athwal, V. S., Scott, J. A., Fitzpatrick, E. & Rowland, M. Emerging clinical perspectives in cystic fibrosis liver disease. *Current Opinion in Pulmonary Medicine* (9000).
29. Valainathan, S. R. *et al.* Contrast-enhanced CT and liver surface nodularity for the diagnosis of porto-sinusoidal vascular disorder: A case-control study. *Hepatology* **76**, 418–428 (2022).
30. De Gottardi, A., Sempoux, C. & Berzigotti, A. Porto-sinusoidal vascular disorder. *J Hepatol* **77**, 1124–1135 (2022).
31. De Gottardi, A. *et al.* Porto-sinusoidal vascular disease: proposal and description of a novel entity. *Lancet Gastroenterol Hepatol* **4**, 399–411 (2019).
32. Khanna, R. & Sarin, S. K. Non-cirrhotic portal hypertension - Diagnosis and management. *Journal of Hepatology* **60**, 421–441 (2014).
33. Schouten, J. N., Verheij, J. & Seijo, S. Idiopathic non-cirrhotic portal hypertension: a review. *Orphanet J Rare Dis* **10**, 67 (2015).
34. Gracia-Sancho, J., Caparrós, E., Fernández-Iglesias, A. & Francés, R. Role of liver sinusoidal endothelial cells in liver diseases. *Nat Rev Gastroenterol Hepatol* **18**, 411–431 (2021).
35. Treps, L., Declercq, M., Bousfia, S., Carmeliet, P. & Witters, P. Comparative meta-analysis of cystic fibrosis cell models suggests partial endothelial-to-mesenchymal transition. *Journal of Cystic Fibrosis* (2021)  
doi:<https://doi.org/10.1016/j.jcf.2021.03.019>.
36. Declercq, M. *et al.* Transcriptomic analysis of CFTR-impaired endothelial cells reveals a pro-inflammatory phenotype. *European Respiratory Journal* **57**, 2000261 (2021).

37. Sokol, L. *et al.* Protocols for endothelial cell isolation from mouse tissues: small intestine, colon, heart, and liver. *STAR Protocols* **2**, 100489 (2021).
38. Zeng, Q. *et al.* Understanding tumour endothelial cell heterogeneity and function from single-cell omics. *Nat Rev Cancer* **23**, 544–564 (2023).
39. Ramachandran, P. *et al.* Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* **575**, 512–518 (2019).
40. Bassez, A. *et al.* A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer. *Nature Medicine* **27**, 820–832 (2021).
41. Wauters, E. *et al.* Discriminating mild from critical COVID-19 by innate and adaptive immune single-cell profiling of bronchoalveolar lavages. *Cell Research* **31**, 272–290 (2021).
42. Qian, J. *et al.* A pan-cancer blueprint of the heterogeneous tumor microenvironment revealed by single-cell profiling. *Cell Research* **30**, 745–762 (2020).
43. Strauss, O., Phillips, A., Ruggiero, K., Bartlett, A. & Dunbar, P. R. Immunofluorescence identifies distinct subsets of endothelial cells in the human liver. *Scientific Reports* **7**, 44356 (2017).
44. MacParland, S. A. *et al.* Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. *Nature Communications* **9**, 4383 (2018).
45. Aizarani, N. *et al.* A human liver cell atlas reveals heterogeneity and epithelial progenitors. *Nature* **572**, 199–204 (2019).
46. Ramachandran, P., Matchett, K. P., Dobie, R., Wilson-Kanamori, J. R. & Henderson, N. C. Single-cell technologies in hepatology: new insights into liver

- biology and disease pathogenesis. *Nature Reviews Gastroenterology & Hepatology* **17**, 457–472 (2020).
47. Lalor, P. F., Lai, W. K., Curbishley, S. M., Shetty, S. & Adams, D. H. Human hepatic sinusoidal endothelial cells can be distinguished by expression of phenotypic markers related to their specialised functions in vivo. *World journal of gastroenterology* **12**, 5429–5439 (2006).
48. Dumas, S. J. *et al.* Single-Cell RNA Sequencing Reveals Renal Endothelium Heterogeneity and Metabolic Adaptation to Water Deprivation. *Journal of the American Society of Nephrology* **31**, 118 LP – 138 (2020).
49. Goveia, J. *et al.* An Integrated Gene Expression Landscape Profiling Approach to Identify Lung Tumor Endothelial Cell Heterogeneity and Angiogenic Candidates. *Cancer Cell* **37**, 21-36.e13 (2020).
50. Kalucka, J. *et al.* Single-Cell Transcriptome Atlas of Murine Endothelial Cells. *Cell* **180**, 764-779.e20 (2020).
51. Rohlenova, K. *et al.* Single-Cell RNA Sequencing Maps Endothelial Metabolic Plasticity in Pathological Angiogenesis. *Cell Metabolism* **31**, 862-877.e14 (2020).
52. Somekawa, S. *et al.* Tmem100, an ALK1 receptor signaling-dependent gene essential for arterial endothelium differentiation and vascular morphogenesis. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 12064–12069 (2012).
53. Chapman, S. L. *et al.* Fibulin-2 and fibulin-5 cooperatively function to form the internal elastic lamina and protect from vascular injury. *Arteriosclerosis, thrombosis, and vascular biology* **30**, 68–74 (2010).

54. Huang, Z. *et al.* Reconstruction of a lncRNA-Associated ceRNA Network in Endothelial Cells under Circumferential Stress. *Cardiology research and practice* **2020**, 1481937 (2020).
55. Adams, T. S. *et al.* Single Cell RNA-seq reveals ectopic and aberrant lung resident cell populations in Idiopathic Pulmonary Fibrosis. *bioRxiv* 759902 (2019) doi:10.1101/759902.
56. Ruan, B. *et al.* Capillarized Liver Sinusoidal Endothelial Cells Undergo Partial Endothelial-Mesenchymal Transition to Actively Deposit Sinusoidal ECM in Liver Fibrosis . *Frontiers in Cell and Developmental Biology* vol. 9 1599 Preprint at (2021).
57. Cui, S. *et al.* Enhanced CD34 expression of sinusoid-like vascular endothelial cells in hepatocellular carcinoma. *Pathology International* **46**, 751–756 (1996).
58. Ohmori, S. *et al.* High expression of CD34-positive sinusoidal endothelial cells is a risk factor for hepatocellular carcinoma in patients with HCV-associated chronic liver diseases. *Human Pathology* **32**, 1363–1370 (2001).
59. Gligorijevic, J., Djordjevic, B., Petrovic, A., Radicevic, A. & Stojanovic, S. Expression of CD34 in cirrhotic liver: Reliance to dedifferentiation. *Vojnosanitetski pregled* **67**, 459–462 (2010).
60. Ribera, J. *et al.* A small population of liver endothelial cells undergoes endothelial-to-mesenchymal transition in response to chronic liver injury. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **313**, G492–G504 (2017).
61. Evrard, S. M. *et al.* Endothelial to mesenchymal transition is common in atherosclerotic lesions and is associated with plaque instability. *Nature Communications* **7**, 11853 (2016).

62. Chin, C. *et al.* Loss of IGFBP2 mediates alveolar type 2 cell senescence and promotes lung fibrosis. *Cell Rep Med* **4**, 100945 (2023).
63. Nguyen, X.-X., Muhammad, L., Nietert, P. J. & Feghali-Bostwick, C. IGFBP-5 Promotes Fibrosis via Increasing Its Own Expression and That of Other Pro-fibrotic Mediators . *Frontiers in Endocrinology* vol. 9 601 Preprint at (2018).
64. Li, T. *et al.* IGFBP2: integrative hub of developmental and oncogenic signaling network. *Oncogene* **39**, 2243–2257 (2020).
65. Chi, J.-T. *et al.* Endothelial cell diversity revealed by global expression profiling. *Proceedings of the National Academy of Sciences* **100**, 10623 LP – 10628 (2003).
66. Tamburini, B. A. J. *et al.* Chronic Liver Disease in Humans Causes Expansion and Differentiation of Liver Lymphatic Endothelial Cells . *Frontiers in Immunology* vol. 10 1036 Preprint at (2019).
67. Geldhof, V. *et al.* Single cell atlas identifies lipid-processing and immunomodulatory endothelial cells in healthy and malignant breast. *Nat Commun* **13**, 5511 (2022).
68. Barnes, T. C., Anderson, M. E. & Moots, R. J. The Many Faces of Interleukin-6: The Role of IL-6 in Inflammation, Vasculopathy, and Fibrosis in Systemic Sclerosis. *International Journal of Rheumatology* **2011**, 721608 (2011).
69. Babic, A. M., Kireeva, M. L., Kolesnikova, T. V & Lau, L. F. CYR61, a product of a growth factor-inducible immediate early gene, promotes angiogenesis and tumor growth. *Proceedings of the National Academy of Sciences* **95**, 6355 LP – 6360 (1998).

70. Hilfiker, A. *et al.* Expression of CYR61, an Angiogenic Immediate Early Gene, in Arteriosclerosis and Its Regulation by Angiotensin II. *Circulation* **106**, 254–260 (2002).
71. Thorgersen, E. B. *et al.* The Role of Complement in Liver Injury, Regeneration, and Transplantation. *Hepatology (Baltimore, Md.)* **70**, 725–736 (2019).
72. Amara, U. *et al.* Interaction between the coagulation and complement system. *Advances in experimental medicine and biology* **632**, 71–79 (2008).
73. Schroer, A. K. *et al.* Cadherin-11 blockade reduces inflammation-driven fibrotic remodeling and improves outcomes after myocardial infarction. *JCI Insight* **4**, (2019).
74. Schupp, J. C. *et al.* Single Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis. *American Journal of Respiratory and Critical Care Medicine* (2020) doi:10.1164/rccm.202004-0991OC.
75. Leatherdale, A. *et al.* Multimerin-1 Binds to Specific Motifs in Vessel Wall Collagens and Contributes to Thrombosis: Novel Insights Regarding the Mechanisms That Support Human and Mouse Platelet Adhesion. *Blood* **130**, 549 (2017).
76. Khanna, R. & Sarin, S. K. Non-cirrhotic portal hypertension - Diagnosis and management. *Journal of Hepatology* **60**, 421–441 (2014).
77. Kara-Terki, L., Treps, L., Blanquart, C. & Fradin, D. Critical Roles of Tumor Extracellular Vesicles in the Microenvironment of Thoracic Cancers. *Int J Mol Sci* **21**, 6024 (2020).
78. Riggio, O. *et al.* Idiopathic noncirrhotic portal hypertension: current perspectives. *Hepat Med* **8**, 81–88 (2016).

79. Kage, M. Pathology of idiopathic non-cirrhotic portal hypertension. *Hepatology International* **11**, 409–411 (2017).
80. Tripathi, A. *et al.* The gut-liver axis and the intersection with the microbiome. *Nature reviews. Gastroenterology & hepatology* **15**, 397–411 (2018).
81. Wiest, R., Albillos, A., Trauner, M., Bajaj, J. S. & Jalan, R. Targeting the gut-liver axis in liver disease. *Journal of Hepatology* **67**, 1084–1103 (2017).
82. Ohtani, N. & Kawada, N. Role of the Gut–Liver Axis in Liver Inflammation, Fibrosis, and Cancer: A Special Focus on the Gut Microbiota Relationship. *Hepatology Communications* **3**, 456–470 (2019).
83. Simbrunner, B., Mandorfer, M., Trauner, M. & Reiberger, T. Gut-liver axis signaling in portal hypertension. *World journal of gastroenterology* **25**, 5897–5917 (2019).
84. Debray, D. *et al.* Cystic fibrosis–related liver disease: research challenges and future perspectives. *Journal of pediatric gastroenterology and nutrition* **65**, 443–448 (2017).
85. Fiorotto, R. & Strazzabosco, M. Pathophysiology of Cystic Fibrosis Liver Disease: A Channelopathy Leading to Alterations in Innate Immunity and in Microbiota. *Cellular and Molecular Gastroenterology and Hepatology* **8**, 197–207 (2019).
86. Flass, T. *et al.* Intestinal Lesions Are Associated with Altered Intestinal Microbiome and Are More Frequent in Children and Young Adults with Cystic Fibrosis and Cirrhosis. *PLOS ONE* **10**, e0116967 (2015).
87. Fiorotto, R. *et al.* Loss of CFTR affects biliary epithelium innate immunity and causes TLR4-NF-κB-mediated inflammatory response in mice. *Gastroenterology* **141**, 1498-1508.e15085 (2011).

88. Fiorotto, R. *et al.* The cystic fibrosis transmembrane conductance regulator controls biliary epithelial inflammation and permeability by regulating Src tyrosine kinase activity. *Hepatology (Baltimore, Md.)* **64**, 2118–2134 (2016).
89. Debray, D. *et al.* Diet-Induced Dysbiosis and Genetic Background Synergize With Cystic Fibrosis Transmembrane Conductance Regulator Deficiency to Promote Cholangiopathy in Mice. *Hepatology Communications* **2**, 1533–1549 (2018).
90. Sarin, S. K. *et al.* Immunological profile of patients with non-cirrhotic portal fibrosis. *Journal of gastroenterology and hepatology* **5**, 425–431 (1990).
91. Goel, A. *et al.* What makes non-cirrhotic portal hypertension a common disease in India? Analysis for environmental factors. *The Indian journal of medical research* **149**, 468–478 (2019).
92. Coulon, S. *et al.* Angiogenesis in chronic liver disease and its complications. *Liver Int* **31**, 146–162 (2011).
93. Xiong, J. *et al.* A Metabolic Basis for Endothelial-to-Mesenchymal Transition. *Molecular Cell* **69**, 689-698.e7 (2018).
94. Quaresma, M. C. *et al.* Mutant CFTR Drives TWIST1 mediated epithelial-mesenchymal transition. *Cell Death Dis* **11**, 920 (2020).
95. Sato, Y. & Nakanuma, Y. Role of endothelial-mesenchymal transition in idiopathic portal hypertension. *Histology and histopathology* **28**, 145–54 (2013).
96. Haghverdi, L., Lun, A. T. L., Morgan, M. D. & Marioni, J. C. Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors. *Nature Biotechnology* **36**, 421–427 (2018).
97. Guilliams, M. & Scott, C. L. Liver macrophages in health and disease. *Immunity* **55**, 1515–1529 (2022).

98. Declercq, M., Treps, L., Bousfia, S., Carmeliet, P. & Witters, P. Endothelial CFTR dysfunction and its involvement in the pathogenesis of pulmonary arterial hypertension. *European Respiratory Journal* **58**, 2101645 (2021).
99. Yang, Q. *et al.* The causality between CFTR and pulmonary hypertension: insights from Mendelian randomization studies. *Hypertension Research* (2021) doi:10.1038/s41440-021-00680-8.
100. Le Ribeuz, H. *et al.* Involvement of CFTR in the pathogenesis of pulmonary arterial hypertension. *European Respiratory Journal* 2000653 (2021) doi:10.1183/13993003.00653-2020.

## Figure legends

### Figure 1: scRNA sequencing of CFLD patients.

a) Schematic overview illustrating the isolation, cell enrichment method and scRNA-seq analysis of the study workflow. b) UMAP representation of the clustering into cell types of 123,736 cells isolated from healthy (n = 5), cirrhotic (n = 5) and CFLD (n = 4) human livers. c) Dot plot of selected marker genes for each cluster. d) Heatmap of the top 5 upregulated genes per cell cluster with exemplar genes labelled (right). e) Relative contribution of each condition to all cell types. P values were assessed by a one-way ANOVA. \*P < 0.05, \*\*P < 0.01, n numbers of scRNA-seq samples for healthy (9), CF (5) and cirrhotic (8).

### Figure 2: Identifying endothelial subpopulations.

a) UMAP representation of the EC clustering per condition; healthy, cirrhotic and CF. b) UMAP representation of the clustering into endothelial subtypes of 19,286 ECs isolated from healthy (n = 5), cirrhotic (n = 5) and CFLD (n = 4) human livers. c) Heatmap of the top 5 upregulated genes per EC subcluster with exemplar genes labelled (right). d) Dot plot of selected marker genes for each cluster. e) Relative contribution of each condition to all cell types. P values were assessed by a one-way ANOVA. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. n for healthy (9), CF (5) and cirrhotic (8).

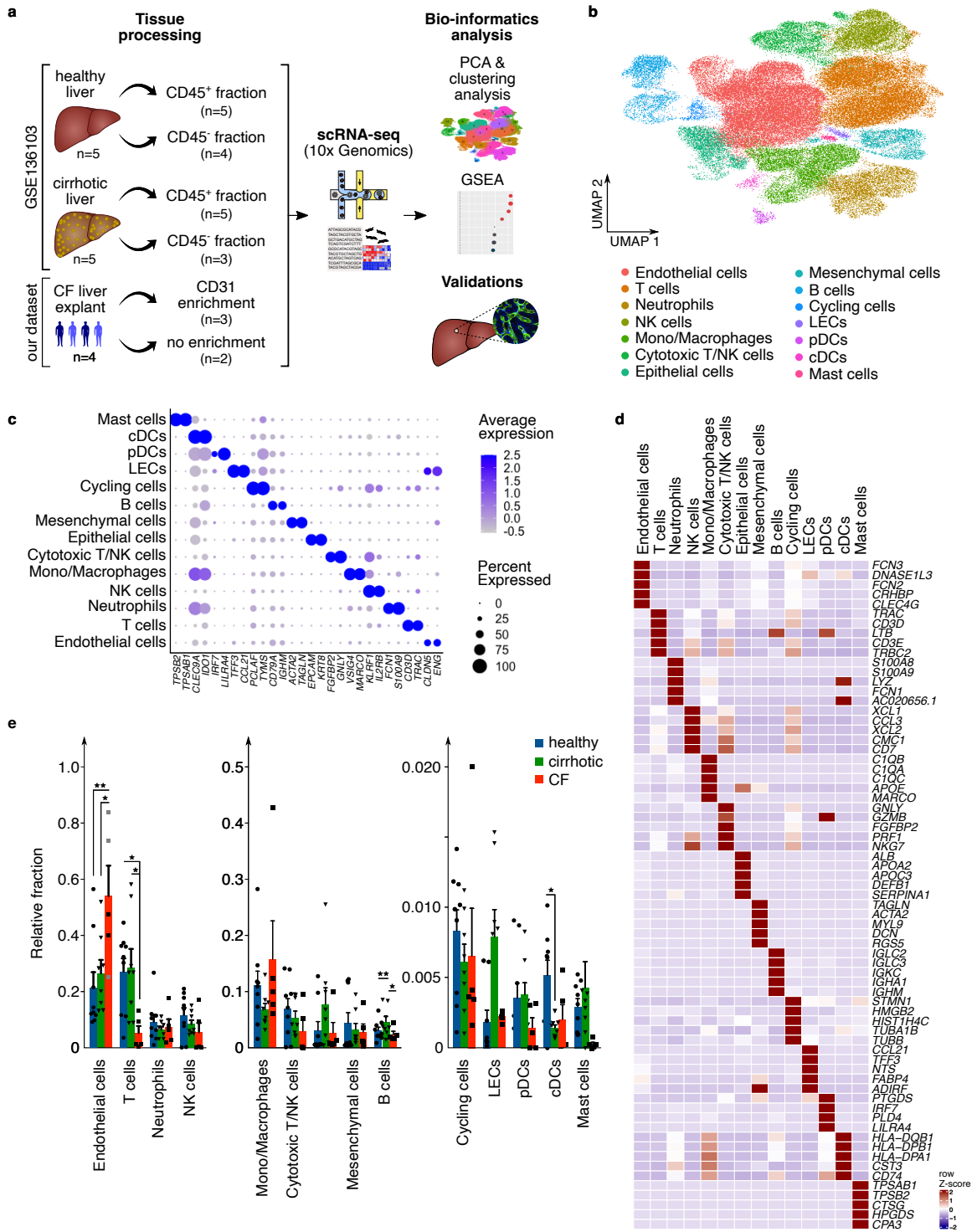
### Figure 3: Characterizing capillary subpopulations.

a) Violin plots of the LSEC transdifferentiation signature (*CD34*, *CLEC4M*, *STAB1*, *STAB2*). b) Heatmap of selected upregulated marker genes for the capillary, endMT and tip cluster with exemplar genes labelled. c,d) GSEA on DEGs in the endMT cluster using hyperR for REACTOME (c) and GO biological processes (d) gene sets (FDR < 0.2). Each dot represents a gene set, color-coded for its enrichment significance and is scaled by its size. e) Heatmap of selected upregulated marker genes for the endMT and tip cluster with exemplar genes labelled. f,g) GSEA on DEGs in the tip cluster using hyperR for GO biological processes (f) and REACTOME (g) gene sets (FDR < 0.2). Each dot represents a gene set, color-coded for its enrichment significance and is scaled by its size.

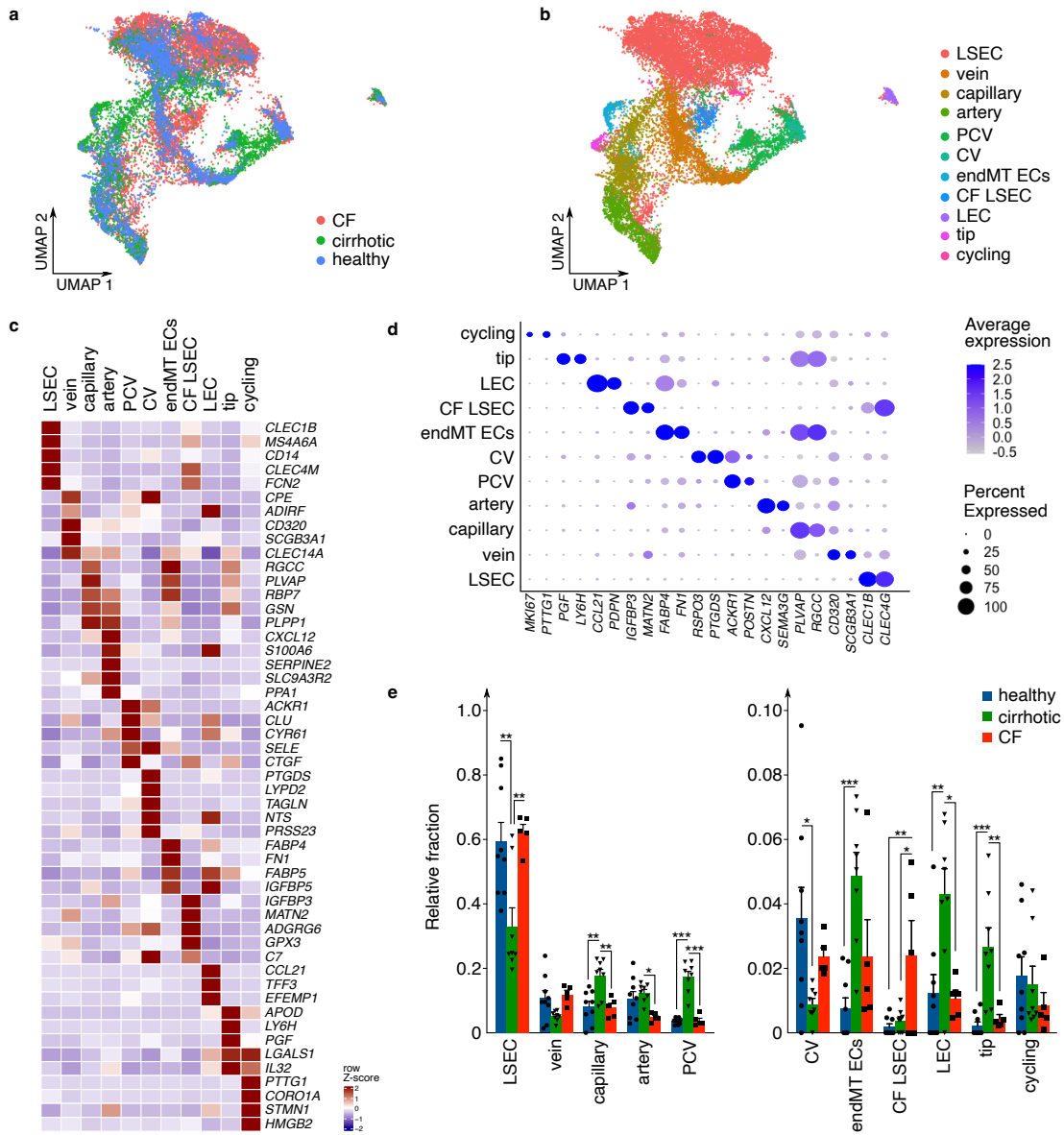
### Figure 4: Characterizing the CF LSEC population.

a) Heatmap of selected upregulated marker genes for the CF LSEC cluster with exemplar genes labelled (right). b,c) GSEA on DEGs in the CF LSEC cluster using hyperR for REACTOME (b) and GO biological processes (c) gene sets (FDR < 0.2). Each dot represents a gene set, color-coded for its enrichment significance and is scaled by its size. d,e) Representative confocal images of CFLD sections from the portal area stained for Hoechst (nuclei; blue), CLEC4G (LSEC vessels; green) and (d) CF LSEC marker gene by *in situ* hybridization (gray), or (e) CF LSEC markers (red). CF: n=5. Scale bars: (d) 25  $\mu$ m and (e) 50  $\mu$ m. f) Staining intensity quantification of CF LSEC markers expressed in the LSECs from the portal area in healthy (n=10), cirrhotic (n=25) and CFLD (n=25) liver acquisitions from healthy (n=2), cirrhotic (n=5) and CFLD (n=5) patients. A.U., arbitrary units. P values were assessed by a one-way ANOVA. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

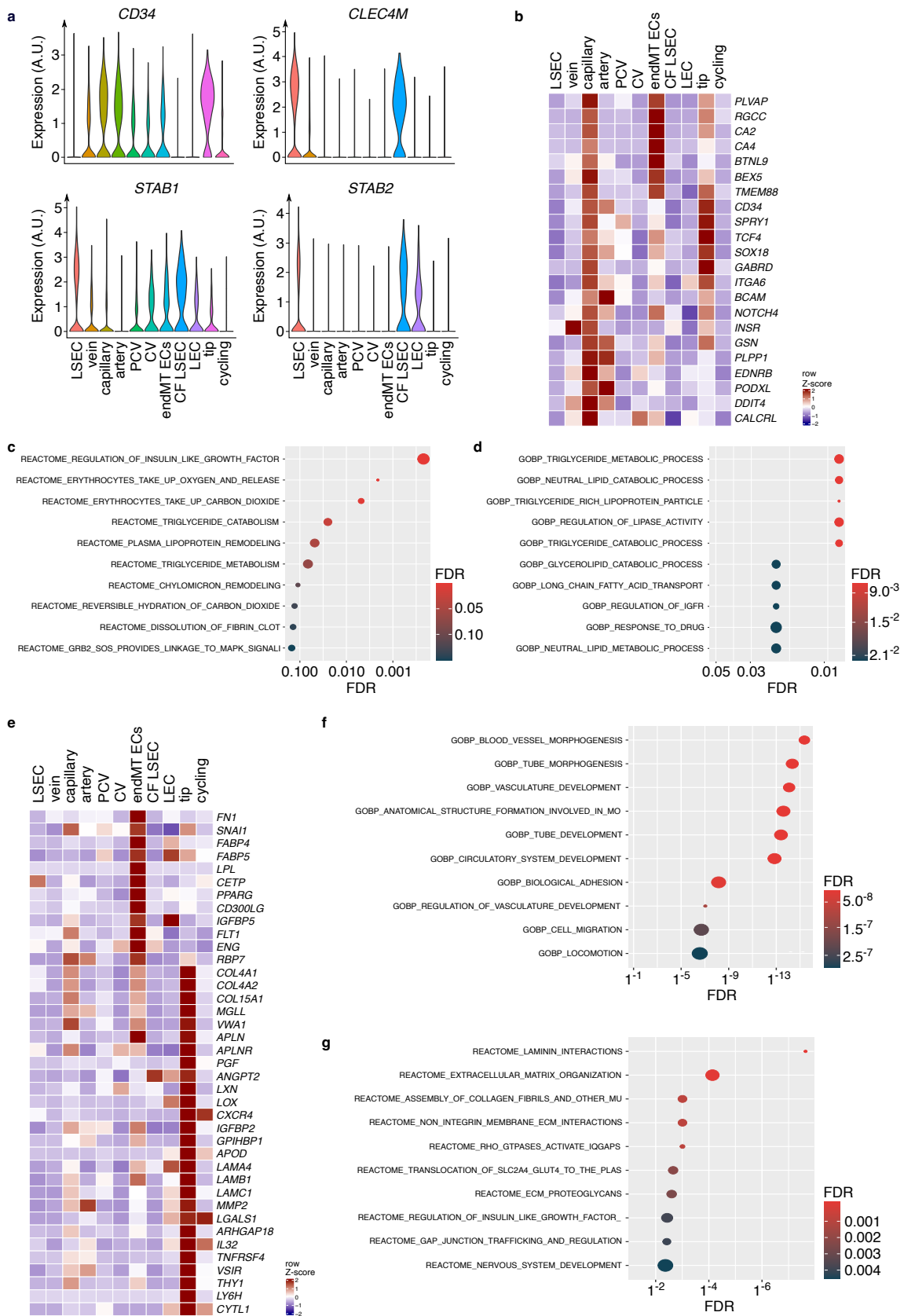
**Figure 1**



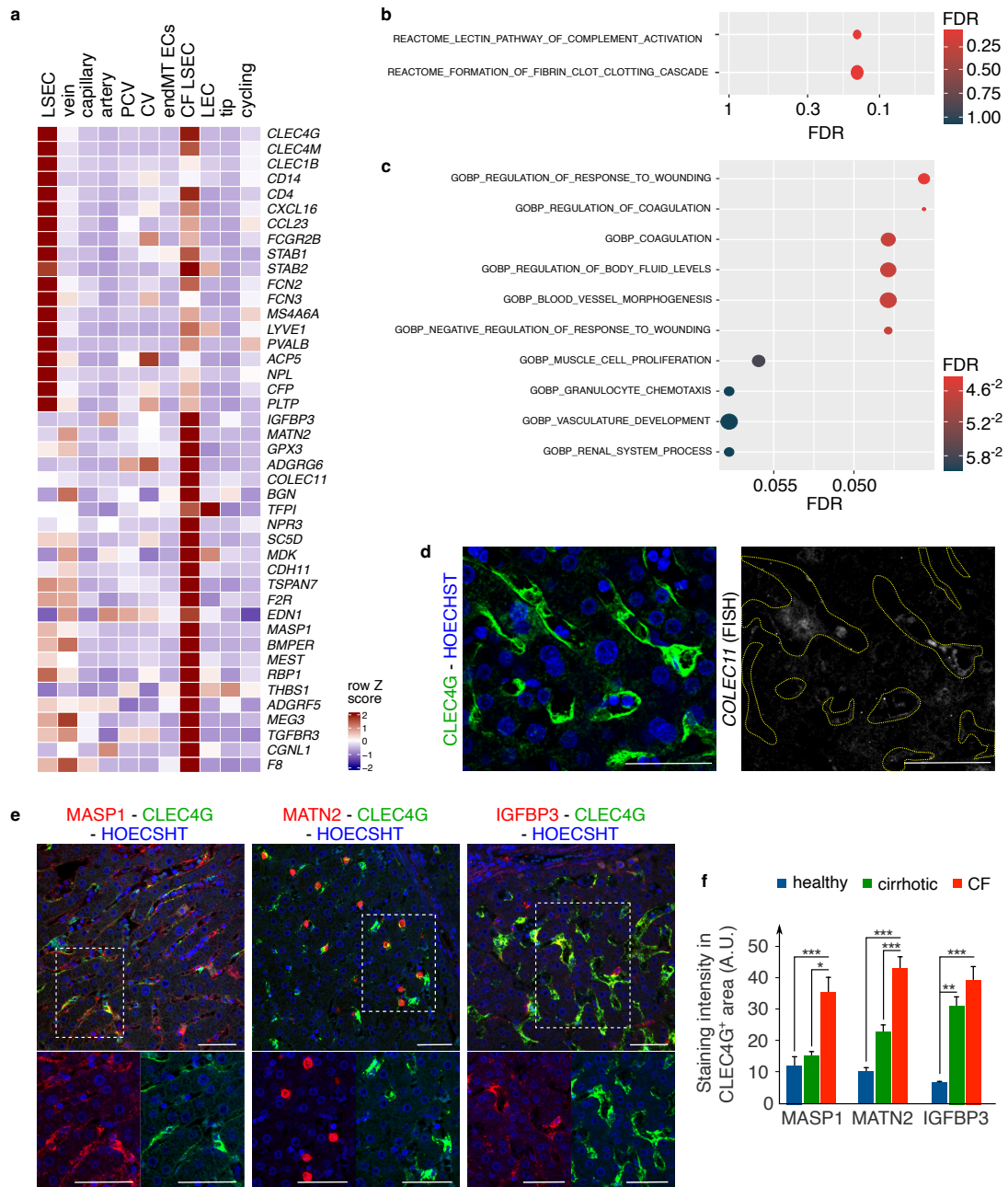
**Figure 2**



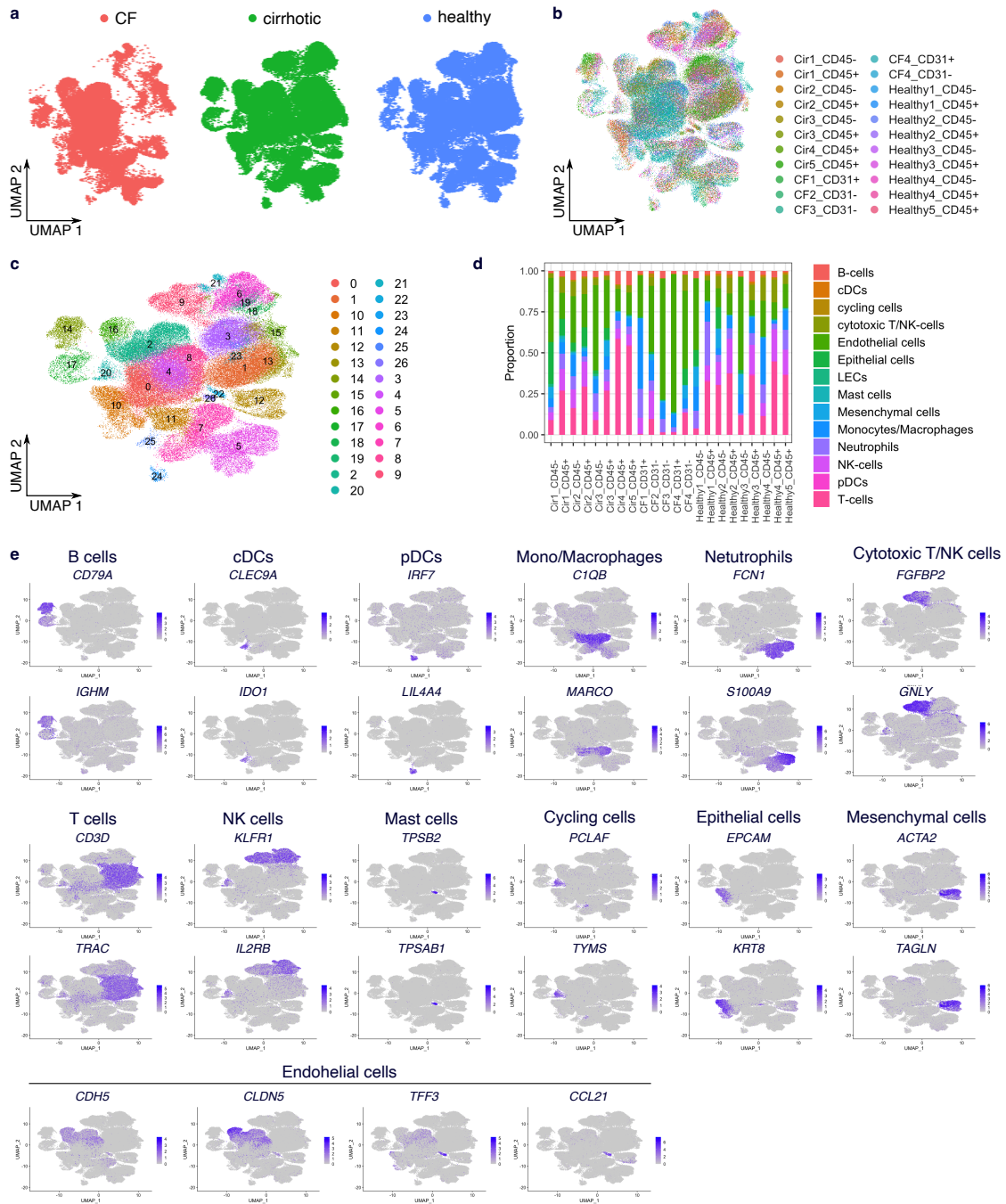
**Figure 3**



**Figure 4**

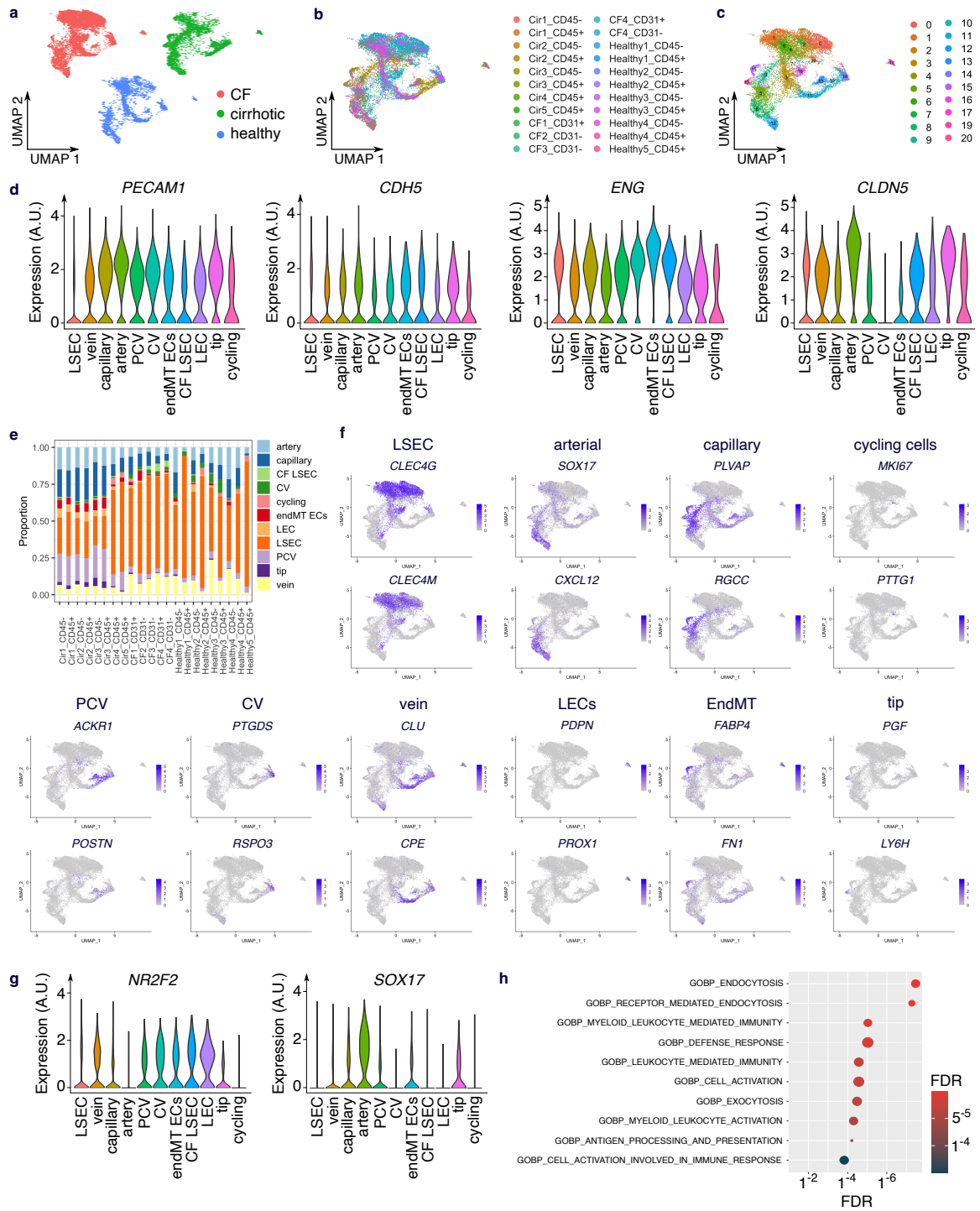


## Supplemental Figure 1



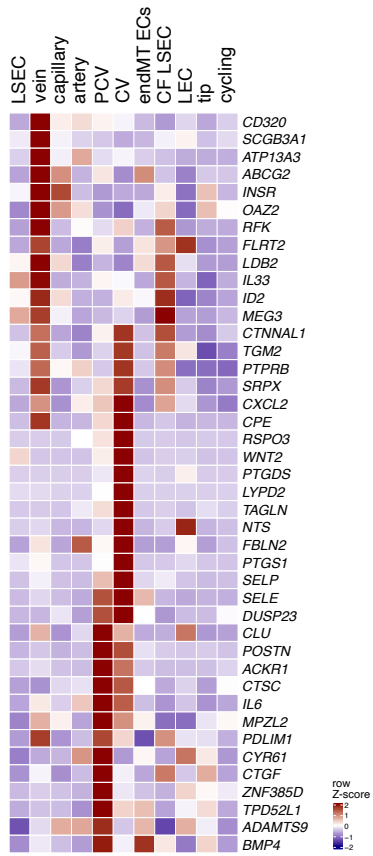
**Figure S1:** scRNA-seq of CFLD patients. a,b) UMAP representation of the clustering of all cells: per condition (healthy, cirrhotic and CF) (a), per sample (b). c) UMAP representation of the clustering prior to the annotation (resolution 0.4). d) Cell type relative distribution across samples. e) UMAP representation showing the expression of 2 marker genes for each cell type.

## Supplemental Figure 2



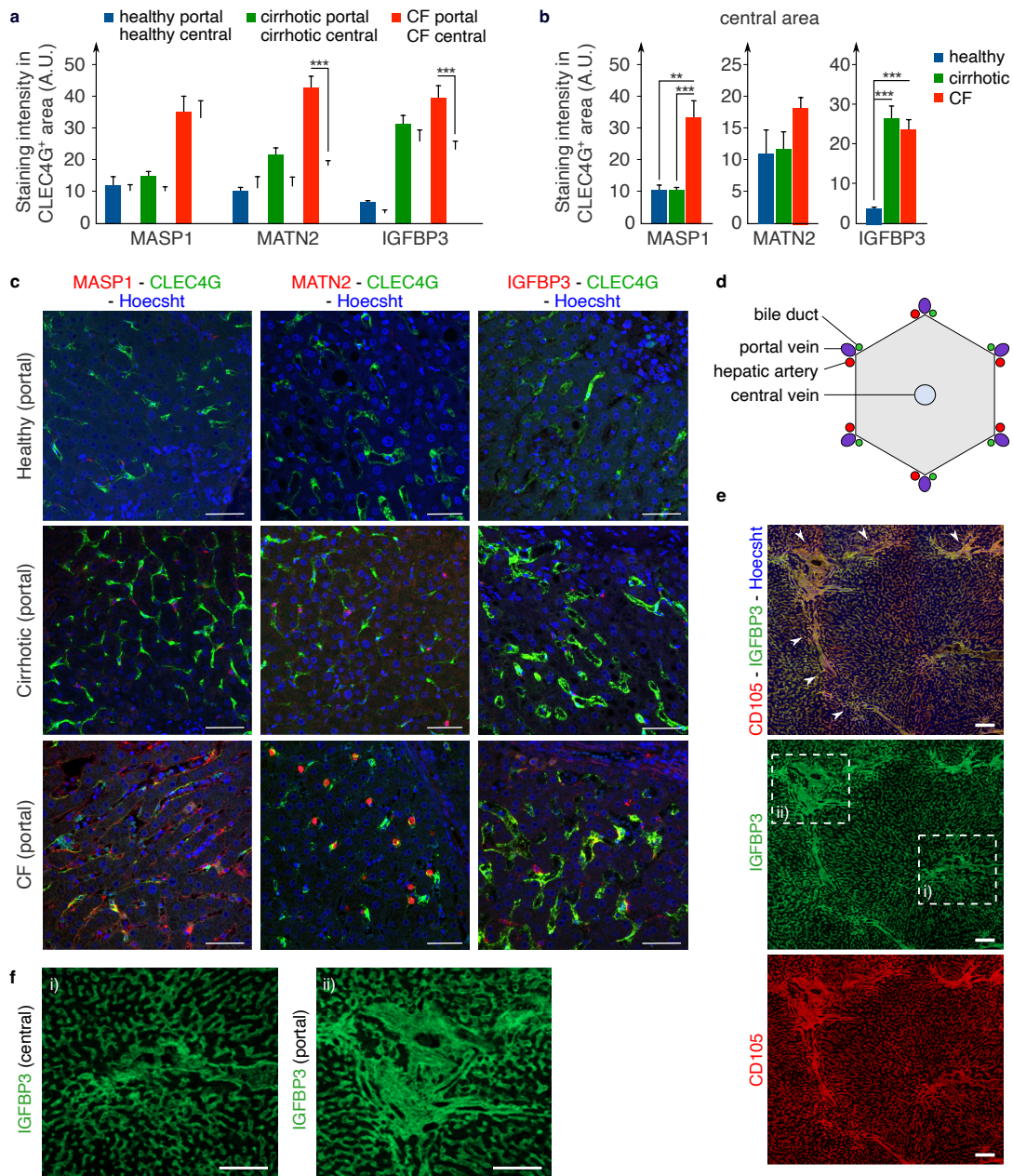
**Figure S2:** Identifying endothelial subpopulations. a,b) UMAP representation of the clustering of the endothelial cells: per condition (healthy, cirrhotic and CF) (a), per sample (b). c) UMAP representation of the clustering prior to the annotation (resolution 1.5). d) Violin plots of the endothelial marker genes (*PECAM1*, *CDH5*, *ENG*, *CLDN5*). e) Endothelial subcluster relative distribution across samples. f) UMAP representation showing the expression of 2 marker genes for each endothelial subtype. g) Violin plots of the venous (left) and arterial (right) marker gene (*NR2F2*, *SOX17*). h) GSEA on DEGs in the LSEC cluster using hyperR for GO biological processes gene sets (FDR < 0.2). Each dot represents a gene set, color-coded for its enrichment significance and is scaled by its size.

### Supplemental Figure 3



**Figure S3:** Identifying venous subpopulations. Heatmap of selected upregulated marker genes for the vein, PCV and CV cluster with exemplar genes labelled.

## Supplemental Figure 4



**Figure S4:** Localization of the CF LSEC populations. a,b) Staining intensity quantification of CF LSEC markers expressed in the LSEC CLEC4G<sup>+</sup> compartment from the (a) portal and (b) central area in healthy, cirrhotic and CFLD liver sections. P values were assessed by a one-way ANOVA. \*\*P<0.01, \*\*\*P<0.001. c) Representative pictures of the portal staining quantification of CF LSEC markers. d) schematic representation of a liver lobule. e) Representative confocal images of CFLD sections stained for Hoechst (nuclei; blue), CD105 (vessels; red) and CF LSEC marker (IGFBP3; green). Arrowheads indicate portal veins. f) Magnification inserts from panel e. Scale bar: 200  $\mu$ m.

Table S1: baseline characteristics of CF patients included in the study					
	CF patient 1	CF patient 2	CF patient 3	CF patient 4	CF patient 5
<b>Age (yrs)</b>	31	35	44	27	42
<b>Gender (M:F)</b>	M	M	F	F	M
<b>Haemoglobin (g/dl)</b>	12,4	13,5	8,6	11,7	7,9
<b>White Cell Count (x10<sup>9</sup>/l)</b>	9,09	10,01	10,38	7,16	5,39
<b>Prothrombin Time (s)</b>	14,2	12,6	13,3	13,6	13
<b>Creatinine (mg/dl)</b>	0,42	0,66	0,91	0,4	5,01
<b>Na+ (mmol/l)</b>	139	138,1	131	137,2	139,8
<b>Bilirubin (mg/dl)</b>	2,09	0,86	0,85	0,53	0,55
<b>ALT (IU/l)</b>	243	40	6	14	22
<b>ALP (IU/l)</b>	1354	462	283	550	372
<b>INR</b>	1,3	1,1	1,2	1,2	1,1
<b>MELD score</b>	12	7	8	8	21
<b>CFTR genotype</b>	DF508/DF508	394delTT/Y913C	DF508/198+1G>C	DF508/DF508	DF508/DF509
<b>FEV1 (% predicted)</b>	22	42	34	52	35
<b>Portal hypertension</b>	Splenomegaly, colateral circulation, esophageal varices. Hepatic venous pressure gradient 8mmHg.	Post splenectomy, portal hypertension. Hepatic venous pressure gradient 18mmHg.	Splenomegaly, thrombocytopenia, collateral circulation. Hepatic venous pressure gradient 12mmHg.	Splenomegaly, collateral circulation. Hepatic venous pressure gradient 17mmHg.	Splenomegaly, thrombocytopenia, ascites, esophageal varices.
<b>Routine histology</b>	Nodular regenerative hyperplasia, disturbance of microcirculation (absent portal veins, paraportal shunt vessels). Secondary sclerosing cholangitis.	Very variable findings, areas with macronodular architecture, only occasional porto-portal septae. Secondary sclerosing (peri)cholangitis.	Nodular regenerative hyperplasia with vague nodularity with sinusoidal dilatation without fibrosis. Paraportal shunt vessels and sclerosed portal vein branches.	Areas of biliary type fibrosis and areas of nodular regenerative hyperplasia. One area of portal venous thrombosis.	Incomplete septal fibrosis with discrete periportal fibrosis, ductular reaction. Sinusoidal dilation, paraportal shunt vessels, flebosclerosis.
<b>Purpose in the study</b>	scRNA-seq & IHC validation	scRNA-seq & IHC validation	scRNA-seq & IHC validation	scRNA-seq & IHC validation	IHC validation
<b>Enrichment method</b>	CD31 enrichment	no enrichment	no enrichment	CD31 enrichment	n/a
<b>scRNA-seq sample name</b>	CF1_CD31+	CF2_CD31-	CF3_CD31-	CF4_CD31+	n/a