Title: Liver sinusoidal endothelial transcription factors in metabolic homeostasis and disease

Running title: LSEC TFs in liver homeostasis & disease

Authors: Dorka Nagy[1], Hannah Maude[1], Graeme M. Birdsey[2], Anna M. Randi[2], Inês Cebola[1*]

Affiliations:

1 – Section of Genetics and Genomics, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK.

2 – National Heart and Lung Institute, Imperial College London, London, UK.

Correspondence: Inês Cebola, i.dos-santos-cebola@imperial.ac.uk

Keywords: Liver, Endothelial cell, Transcription factors, NAFLD, Gene transcription.

Word count: 7,110 words.

Abstract

Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells that form the liver microvasculature. LSECs maintain liver homeostasis, scavenging bloodborne molecules, regulating immune response, and actively promoting hepatic stellate cell quiescence. These diverse functions are underpinned by a suite of unique phenotypical attributes distinct from other blood vessels. In recent years, studies have begun to reveal the specific contributions of LSECs to liver metabolic homeostasis and how LSEC dysfunction associates with disease aetiology. This has been particularly evident in the context of non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome, which is associated with loss of key LSEC phenotypical characteristics and molecular identity. Comparative transcriptome studies of LSECs and other endothelial cells, together with rodent knockout models have revealed that loss of LSEC identity through disruption of core transcription factor activity leads to impaired metabolic homeostasis and to hallmarks of liver disease. This review explores the current knowledge of LSEC transcription factors, covering their roles in LSEC development and maintenance of key phenotypic features, which, when disturbed, lead to disruption of liver metabolic homeostasis.

Introduction

Liver sinusoidal endothelial cells (LSECs) represent a unique and highly specialized endothelial cell (EC) population that forms the liver microvasculature, known as the hepatic sinusoids. LSECs are the most abundant non-parenchymal cell type in the liver, representing 15-20% of all liver cells, but only ~3% of its volume (Blouin et al., 1977, Poisson et al., 2017). In the past two decades, LSECs have emerged as central players in the onset and progression of liver disease, having important implications for metabolic and endocrine health. LSEC dedifferentiation is associated with metabolic conditions such as insulin resistance and non-alcoholic fatty liver disease (NAFLD), a progressive disease that affects ~30% of the world's population (Younossi et al., 2023). In the NAFLD spectrum, non-alcoholic steatohepatitis (NASH) is characterized by steatosis, liver fibrosis, hepatocellular damage, and is accompanied by metabolic and endocrine dysfunction (Benedict and Zhang, 2017). NASH is the major cause of liver fibrosis and significantly increases the risk for chronic liver disease complications, namely cirrhosis and hepatocellular carcinoma, and is a leading cause for liver transplantation (Younossi et al., 2023, Bataller and Brenner, 2005).

Even though LSECs were first isolated five decades ago (Wisse, 1970) and have since been morphologically and functionally characterised (Poisson et al., 2017), the profiling of their transcriptomic and epigenomic features has lagged behind due to difficulties in isolating enough cells and their rapid dedifferentiation in culture (Géraud et al., 2010). The predominance of hepatocytes within the liver tissue (~80% of its mass) has made it difficult to assess and analyse LSEC-specific aspects of gene expression, chromatin accessibility, and transcription factor (TF) occupancy using bulk liver tissue. With advances in cell isolation techniques and single-cell sequencing, the quality and quantity of LSEC-specific data have increased in recent years, along with the identification of key developmental drivers and markers of LSEC identity.

Important progress has also been made in identifying the alterations in the LSEC transcriptome that promote LSEC dysfunction and accompany disease progression, as revealed

by animal models and, more recently, by single-cell gene expression profiling of both mouse and human liver tissue. This now substantial body of work pinpoints specific LSEC TFs as conductors of the transcriptional changes associated with the onset and progression of metabolic liver disease. In this review we discuss the contribution of LSEC TF activity to maintain metabolic homeostasis and how specific LSEC TFs have been implicated in liver disease. Given the intricate relationship between liver metabolism and endocrine health, the relevance of LSEC TFs is expected to extend to this field as well.

LSEC phenotypical properties and function in physiological conditions and disease

The phenotypical features of LSECs have been extensively reviewed in the context of liver physiology (Poisson et al., 2017) and are not the primary focus of the present review. Nevertheless, we provide here a brief overview of their major distinctive features to contextualise phenotypes observed upon ablation of LSEC TFs. Reflecting their various specialized functions, LSECs lack basal lamina, and display fenestrae (large diaphragm-free pores organized into sieve plates) to aid macromolecule transport towards the perisinusoidal space and subsequently towards the hepatocytes (**Figure 1**) (Poisson et al., 2017). Unlike other types of vascular endothelial cells, their cell junctions are loosely organized resulting in a discontinuous endothelial monolayer. Another important feature of LSECs is the high concentration of scavenger receptors on the cell surface (e.g. *STAB1*/*2*) , which enable efficient metabolite clearance and tackling of the viral and bacterial influx from the gut (Sørensen et al., 2015). LSECs display dynamic immune functions and present antigens to $CD8^+$ naïve T cells, contributing to tolerogenic response, while they can also promote T cell activation and local inflammatory response when antigen concentration increases (Burgdorf et al., 2007, Limmer et al., 2000). LSECs also exhibit anti-thrombotic and anti-fibrotic phenotypes, and are involved in the regulation of angiogenesis and vascular tone in homeostatic conditions (Lafoz et al., 2020, Poisson et al., 2017).

During NAFLD progression, LSECs undergo morphological changes that indicate loss of their specialized phenotype and dedifferentiation. This phenomenon is called sinusoidal capillarization and involves loss of fenestrae (defenestration) and formation of basal lamina (Hammoutene and Rautou, 2019). The timing of sinusoidal capillarization during NAFLD progression is still subject to debate. Miyao and colleagues reported that capillarization was an early event during disease progression, appearing already with simple steatosis in two different mouse models (the choline-deficient, L-amino acid-defined and high-fat diet models) and preceding the activation of Kupffer cells and hepatic stellate cells (Miyao et al., 2015), both of which are critical events in fibrosis (Hernandez-Gea and Friedman, 2011). In contrast, a more recent study by Kus et al. has challenged this view, reporting that while LSECs showed inflammatory response in early NAFLD pathogenesis, there was no sinusoidal capillarization, but instead increased fenestrae diameter in high fat diet-induced liver steatosis with no immune activation or fibrosis (Kus et al., 2019). These opposing results likely reflect differences in the experimental models employed and encourage further investigation in rodent models, but also in human specimens. Besides defenestration and capillarization, LSECs display regenerative angiocrine signalling after acute injury, undergoing a fibrogenic switch if the injury is sustained over time (Ding et al., 2014). Furthermore, during the early phase of liver regeneration after hepatectomy, the downregulation of Angiopoietin-2 in LSECs leads to hepatocyte proliferation through releasing the angiocrine proliferative brake. In later stages of regeneration, Angiopoietin-2 expression recovers, enabling angiogenesis in the newly formed tissue (Hu et al., 2014).

LSEC marker genes

The specialized LSEC phenotype is the result of a unique transcriptional, and presumably epigenomic signature. It must be noted that LSECs share some important features with other ECs, like the expression of pan-endothelial TFs such as ERG (Dufton et al., 2017). Lymphatic ECs in particular share salient transcriptional features with LSECs, including the expression of

lymphatic marker genes *LYVE1* and *VEGFR3* (Strauss et al., 2017, Inverso et al., 2021, Aizarani et al., 2019), and the expression of *Maf* in both EC types (Gómez-Salinero et al., 2022). These and other common features between LSECs and other liver ECs have made the isolation of pure LSEC populations difficult in the past. Therefore, characterising the healthy LSEC transcriptional profile can help identify marker genes for use in LSEC isolation. The definition of marker genes differs between studies, but broadly refers to genes enriched in the cell type of interest, often including genes involved in specialised cell functions. LSEC marker genes have been defined by comparing LSECs to other EC types, or to other liver cell types, as is the case in single-cell analysis of liver tissue. These comparisons identified different marker genes (**Figure 2**). For instance, *GATA4* was not identified as a marker gene of LSECs in single-cell studies of liver tissue (Guilliams et al., 2022, Aizarani et al., 2019, Ramachandran et al., 2019, Andrews et al., 2022, MacParland et al., 2018), because hepatocytes also express *GATA4* at relatively high levels. However, *GATA4* is typically not expressed in other ECs and has therefore been identified as an LSEC marker gene in several EC-based studies (de Haan et al., 2020, Géraud et al., 2017, Winkler et al., 2021). Measuring the expression of marker genes can also help to characterise the loss of LSEC identity in disease models or patient biopsies and in developing better *in vitro* systems to study disease. Despite the established roles of LSECs in liver physiology and disease, what truly constitutes an LSEC transcriptional profile, and which are the marker genes of this cell population is still the focus of active research and debate. In this section, we provide an overview of the progress made in the identification of LSEC marker genes, with a focus on TFs (**Box 1**).

Box 1: Transcription factors in a nutshell

Transcription factors (TFs) are proteins that bind DNA in a sequence-specific manner and regulate gene transcription. Through binding to specific target regions, which are commonly located in *cis*-regulatory elements such as promoters, enhancers, or silencers, TFs can activate or repress gene expression in response to regulatory cues. Such regulatory cues can be diverse

and include both intrinsic (e.g. developmental processes) and extrinsic signals (e.g. signalling cascades activated by nutrients), making TFs centrepieces in the coordination of complex and dynamic gene regulatory networks. They can either recruit other TFs and/or transcriptional complexes or disable those functions by occupying target regions (Lambert et al., 2018).

Different TF families are characterised by a preferred DNA recognition sequence or motif. The current approaches to identify the consensus motif of specific TFs involve either the analysis of high-throughput binding assays or chromatin immunoprecipitation followed by sequencing (ChIP-Seq). The results of these experiments have been compiled in several databases that host vast collections of known TF motifs for vertebrates (e.g. JASPAR, Hocomoco, SwissRegulon). However, the presence of a binding motif does not necessarily translate into TF occupancy, which also depends on conditions including (1) the concentration of the TF in the nucleus, (2) the binding of necessary TFs and co-factors to nearby sequences, and (3) the specificity of the TF, in other words its ability to distinguish between a high versus a low affinity binding site (Zabet and Adryan, 2015). Most TFs also require chromatin to be accessible at their binding site, free from nucleosomes and thus exposing the motif for recognition. However, a subset of TFs termed pioneer TFs can induce chromatin remodelling: they modify chromatin architecture by binding condensed, nucleosome-bound chromatin and displacing nucleosomes, enabling other TFs and transcriptional machinery to bind to the DNA (Zaret, 2020). Pioneer TFs are particularly important in development and lineage identity, with the first pioneer TFs (GATA4 and FOXA1) being identified in the context of hepatic specification (Bossard and Zaret, 1998, Gualdi et al., 1996). Regions of accessible chromatin may also have varying levels of activity, reflected by different degrees of deposition of histone marks and DNA methylation. These chromatin features may in turn affect TF binding frequency. In addition to these mechanisms, the activity of TFs is heavily influenced by their post-translational modifications and by protein-protein interactions with binding partners (Jolma et al., 2015). Altogether these factors contribute to the binding of one TF to the

DNA, but it must be noted that TFs usually work as part of an intricate system of coordinated action by multiple TFs.

There have been several attempts to describe the transcriptional features of LSECs, particularly to pinpoint the set of LSEC lineage-determining TFs (**Figure 2**). A major challenge in the study of LSECs is their rapid *in vitro* dedifferentiation. This dedifferentiation was demonstrated at the transcriptional level in a comparative analysis of freshly isolated rat LSECs versus LSECs cultured for 42 hours, which revealed 465 genes downregulated by culture, including the TFs *Gata4*, *Tcfec*, and *Maf* (Géraud et al., 2010). In a recent study, de Haan et al. described an LSEC fingerprint comprised of 27 LSEC-enriched genes, defined as genes enriched in LSECs vs heart and brain ECs, plus three previously identified important genes (*LYVE1, STAB1,* and *LSIGN*). This LSEC fingerprint included seven genes encoding TFs: *GATA4, TCFEC, MAF, ZEB2, MEIS2, HOXB5,* and *CUX2* (de Haan et al., 2020).

As mentioned above, it is challenging to isolate pure LSEC populations due to overlapping expression of markers across different types of ECs. de Haan et al. used a pan-endothelial marker, *Tie2*, to isolate mouse liver ECs, reporting a purity of >99% for the microvascular marker CD36 and low expression of lymphatic EC markers (de Haan et al., 2020). However, CD36 is less abundant in central venous LSECs than in periportal LSECs (Su et al., 2021) and therefore may not be an ideal total-LSEC reporter for cell sorting. Similarly, other markers used for cell isolation may have varying expression along the periportal-pericentral axis, a phenomenon known as zonation (Ben-Moshe and Itzkovitz, 2019) (**Box 2**), which raises an important consideration around the methods used to isolate LSECs for molecular characterisation.

Box 2: **Liver zonation**

The liver is structurally organised into lobules: hexagonal units in which oxygen and nutrient-rich blood enters via the portal triad, flows through the sinusoids, and exits via the central vein. As blood passes through the sinusoid, oxygen and nutrients are exported into the LSECs and liver parenchyma, while metabolism products and other cell exports enter the blood (**Figure 1**). This structure creates a gradient of oxygen, nutrients, and signalling molecules including Wnt morphogens, a phenomenon called zonation. Importantly, zonation manifests gradual changes at the gene expression level of LSECs and hepatocytes across the periportal-pericentral axis (Paris and Henderson, 2022). Although the zonation gradient is continuous, the sinusoid is often referred to as distinct periportal, midzonal and pericentral zones. It was recently estimated that 67% of LSEC-expressed genes exhibit significant zonation (Inverso et al., 2021), including genes involved in peptide hormone and xenobiotic metabolism, response to gut-derived toxins, canonical Wnt signalling, as well as the binding and uptake of ligands by scavenger receptors (recently reviewed in (Paris and Henderson, 2022)). Zonation is a mechanism essential for liver metabolic homeostasis and its loss has been linked with pathophysiological settings, including fibrosis and cirrhosis (Su et al., 2021).

Recent years have seen the application of sequencing at single-cell resolution, including single-cell RNA-seq (scRNA-seq), which can be used to profile a complex pool of cells and capture cell-to-cell heterogeneity. scRNA-seq can be applied to liver tissue or to pre-sorted ECs, with similar cells clustered by computational algorithms based on shared transcriptomic features. Thus far, most scRNA-seq studies of LSECs have relied on marker gene expression for EC-enrichment purposes given their small contribution to liver cell mass. These studies have identified sets of genes that distinguish LSECs from other liver cell types (Ben-Moshe and

Itzkovitz, 2019, Saviano et al., 2020) as well as genes with heterogeneous expression across LSEC sub-populations and that are zonated (Su et al., 2021, Inverso et al., 2021).

Several scRNA-seq studies of the liver have highlighted TFs with enriched expression in LSECs. A seminal study of healthy and cirrhotic human liver using scRNA-seq identified a cluster of LSECs with high expression of TFs including *GATA3/4, STAT2/3, MEIS1, TBX2, NR5A2, NR2F1, ELK1, ETS1, HIF1A, SREBF1, RARB, IRF1, ATF3, MLXIP, XBP1, FOXO1, MEF2C, CEBPB, JUND, BACH1*, and *JUN* (Ramachandran et al., 2019). Many of these are recognised core LSEC TFs, yet the lack of standardized scRNA-seq methods and the high transcriptional heterogeneity of LSECs means that it is still difficult to reach a consensus list of marker genes (**Figure 2**).

In addition to markers of mature LSECs, some interesting results have come from the application of single-cell methods to different stages of liver development. For example, the Rafii team observed that LSECs constitute “the most transcriptional diverse” liver endothelial cell type when comparing developmental stages (Gómez-Salinero et al., 2022). In this study, the authors employed scRNA-seq on $CD45^{neg}CD31^{+}$ cells to characterize the mouse liver endothelium throughout embryonic (E12-E18) and postnatal (P2, P8, P15, and P30) development, describing marker genes for a total of 10 LSEC clusters. The genes *Cd34*, *Pgk1,* and *Mif* were reported as markers of undifferentiated LSEC; whilst *Aqp1*, *Mrc1*, *Fcgr2b*, *Clec4g*, and *Kit* were markers of adult differentiated LSEC.

Another important angle to the identification of LSEC markers is the possibility of improving the understanding of liver pathophysiological processes. Studies using the carbon tetrachloride (CCl_4) liver injury model have revealed that the transcriptional profile of LSECs is markedly changed during liver injury with over 7,000 differentially expressed genes (Manicardi et al., 2021). The transcriptional changes included increased expression of genes encoding secretory proteins (e.g. *Cxcl10, Inhbb, Tpbpb*, and *Il4ra*) and decreased expression of membrane and transport-related genes (Manicardi et al., 2021), possibly revealing LSEC de-differentiation

under stress. Single-cell transcriptomics has proved to be a powerful tool to investigate liver disease states as well, revealing for instance that cirrhotic livers show a substantial depletion of LSECs along with the appearance of cells with distinct transcriptional signatures, as revealed by studies in both human (Ramachandran et al., 2019) and mouse liver (Su et al., 2021, Xiong et al., 2019). Su et al. observed that periportal LSECs may be the most vulnerable to injury, implicating in this process the TFs *Klf2* and *Klf4,* and components of the AP-1 complex, with reduced expression in cirrhotic mouse LSECs (Su et al., 2021).

The transcription factors that orchestrate LSEC transcriptional identity

The specialized LSEC phenotype we described above is likely the result of the orchestrated action of multiple TFs (**Box 1**) that are responsible for initiating and maintaining the LSEC-specific gene expression program. The sinusoidal endothelium develops from the mesenchyme of the septum transversum (mesoderm) and differentiates under the transcriptional control of GATA4 and c-MAF (Asahina et al., 2011, Géraud et al., 2017, Gómez-Salinero et al., 2022). Interestingly, these two TFs remain central to the maintenance of LSEC function and identity in adulthood. A less investigated but seemingly important TF is MEIS2, which has been identified by a few studies in recent years as a main regulator of LSEC fate (de Haan et al., 2020, Liang et al., 2022). Overexpression of this TF triad was shown to be partially successful in promoting an LSEC-like gene program in human umbilical vein endothelial cells (HUVECs), inducing the expression of a number of LSEC markers, albeit with incomplete recapitulation of LSEC functionality (de Haan et al., 2020). Whole transcriptome analyses were not carried out to investigate the extent to which the co-expression of GATA4, c-MAF and MEIS2 induced an LSEC transcriptional profile (de Haan et al., 2020). Other TFs are therefore likely required for the complete establishment of the LSEC transcriptional program (Danoy et al., 2020). Plausible candidates are SPI1 and ZEB2, which have been implicated in the regulation of genes related to LSEC immune and angiogenic functions, respectively (De Smedt et al., 2021, de Haan et al., 2021). Besides these LSEC-

enriched TFs, pan-endothelial TFs also play key roles in LSEC identity and function. ERG, as the better-established example of a pan-endothelial TF, is essential for LSEC homeostasis, protecting from liver fibrosis (Dufton et al., 2017). In this section, we describe in more detail the TFs that have been observed to drive the LSEC transcriptional machinery and discuss how they have been implicated in liver metabolic disease.

1. c-MAF

MAF factors, including c-MAF, are subunits of the activator protein 1 (AP-1) transcriptional complex and have a strong preference for nucleosome-depleted regions (Grossman et al., 2018). c-MAF, encoded by the *MAF* gene in humans, has been identified by several studies as a TF of central importance in LSEC development and function (Gómez-Salinero et al., 2022, Géraud et al., 2010, de Haan et al., 2020). Recently, Gómez-Salinero et al. identified c-Maf as the top-enriched TF in liver ECs using publicly available scRNA-seq datasets representing multiple mouse tissues (Gómez-Salinero et al., 2022). *Maf* was shown to increase expression continuously throughout mouse liver embryonic and postnatal development by scRNA-seq analysis (Gómez-Salinero et al., 2022). This contrasted with *Gata4*, which showed uniform expression across liver EC types and developmental stages. Thus, despite the important roles of these two TFs, their specific contributions to the regulation of LSEC homeostasis seem to be distinct. scRNA-seq analysis of adult human liver ECs further revealed that *MAF* expression is higher in the liver sinusoid compared to either portal or central vein ECs (Gómez-Salinero et al., 2022).

c-Maf has a key role in regulating LSEC maturation and identity. Gómez-Salinero et al. also observed increased co-expression of *Maf* with the LSEC markers *Mrc1* and *Fcgr2b*: endothelial-specific ablation of *Maf* at E12-14, postnatally and in adult mice led to a reduction in the expression of *Mrc1* and *Fcgr2b* (Gómez-Salinero et al., 2022). RNA velocity analysis revealed that postnatal c-Maf deletion led to an immature EC phenotype, with decreased expression of LSEC markers and overexpression of arterial genes, such as *Cd34*, *Ly6a*, *Aplnr*, and *Cd9*, which

associated with the retention of liver haematopoiesis. In adult mice, deletion of c-Maf also led to decreased expression of LSEC markers, but this time it associated with increased expression of genes characteristic of portal vein ECs (Gómez-Salinero et al., 2022).

c-Maf also seems to be required for phenotypical specification along the periportal-pericentral axis, as its deletion during embryonic development resulted in an aberrant zonation phenotype and in the appearance of EC clusters detected by scRNA-seq that were not present in wild type mice (Gómez-Salinero et al., 2022). In adult mice, c-Maf removal resulted in a mild liver zonation phenotype with expansion of hepatocytes expressing glutamine synthase, an enzyme known to be exclusively expressed in the pericentral zone (Gebhardt et al., 2007), even though other zonation markers such as E-cadherin or Cyp2E1 were not affected (Gómez-Salinero et al., 2022). Consistent with the role of c-Maf in regulating and maintaining LSEC maturation, its expression in liver ECs was diminished upon induction of fibrosis with CCl_4 for a month (Gómez-Salinero et al., 2022). LSEC-specific c-Maf deletion alone did not induce fibrosis in mice, but its combination with CCl_4 resulted in an exacerbation of the fibrotic phenotype (Gómez-Salinero et al., 2022). These observations in murine models suggest that c-MAF loss may be a contributing factor to human NASH. Furthermore, the implications of c-MAF in liver disease are likely not restricted to the endothelial compartment, as c-MAF is also highly expressed in Kupffer cells and scar-associated macrophages (Ramachandran et al., 2019, Guilliams et al., 2022).

TFs may contribute to gene regulatory programs via different mechanisms and not all TFs can initiate chromatin remodelling as pioneer TFs (**Box1**). Still TFs such as c-MAF can modulate the accessibility of regulatory elements and in this way promote gene programs. Two studies have reported that *in vitro* overexpression of *c-MAF* in HUVECs induced a pro-sinusoidal transcriptional program and the expression of sinusoidal identity genes (de Haan et al., 2020, Gómez-Salinero et al., 2022). Microvascular and LSEC markers *CD36*, *CD26* and *STAB1/2* were induced at the mRNA level, while CD14 and MRC1 induction was also observed at the protein level. RNA-seq analysis of induced LSECs, identified as $CD26^{+}CD36^{+}$, recapitulated broad transcriptional

features of human primary LSECs. Still, the expression of a subset of sinusoidal markers, including *FCGR2B*, was not induced by c-MAF overexpression (Gómez-Salinero et al., 2022).

Until the recent studies investigating the transcriptional properties of LSECs, c-MAF was primarily known as a regulator of lymphocyte differentiation and function (Imbratta et al., 2020). c-MAF has been described to promote chromatin accessibility at its binding sites in subsets of lymphoid cells, which is in line with its prominent developmental role (Parker et al., 2019). While the function of c-MAF in directing LSEC chromatin accessibility has not been investigated, it is plausible to assume it has similar functions in promoting transcriptional programs in the liver endothelium.

The identification of TFs able to induce transcriptional remodelling, such as c-MAF, may be of particular importance to develop *in vitro* models and potential sources of LSECs and other liver cell types for regenerative medicine applications. Encouraging results were already observed in co-cultures of induced LSECs with human primary hepatocyte aggregates, which showed formation of cytoplasmic fenestration gaps in induced LSECs and sustained induction of hepatocyte functionality (CYP1A2 expression and albumin secretion) for at least 28 days (Gómez-Salinero et al., 2022).

2. GATA4

GATA4 is a zinc-finger TF that is crucial in liver development (Watt et al., 2007) and in the maintenance of a differentiated LSEC state. Like other members of the GATA TF family, GATA4 is capable of both activating and repressing gene expression, acting together with co-regulators to assemble a transcriptional complex and recruit chromatin remodelling machinery (GATA transcriptional complexes are described in detail in (Tremblay et al., 2018)). During embryonic development, GATA4 primes liver-specific regulatory elements through its pioneer TF activity (Bossard and Zaret, 1998). In the adult liver, *GATA4* is expressed in LSECs and hepatocytes, albeit its level is lower in hepatocytes.

Comparing freshly isolated rat LSECs to lung microvascular endothelial cells and to LSECs cultured for 42 hours, Géraud et al. identified Gata4 as a TF that is preferentially expressed in LSECs and rapidly lost upon culturing (Géraud et al., 2010). The same group later demonstrated that Gata4 expression in LSEC is essential for foetal development, with all mouse embryos with LSEC-specific *Gata4* deletion showing lethality at E15.5-E17.5 (Géraud et al., 2017). This severe phenotype was associated with very early sinusoidal capillarization, detected at E10.5, and loss of key LSEC identity genes, including *Lyve1* and *Stab2,* as well as upregulation of endothelial genes that are mainly involved in cell junction formation, such as *Cd31* (Géraud et al., 2017). LSEC-specific *Gata4* deletion also led to reduced numbers of stem and progenitor cells in the liver at E11.25, suggesting that Gata4 is necessary for hematopoietic stem cell (HSC) migration into the foetal liver (Géraud et al., 2017). Overexpression of GATA4 in HUVECs suppressed expression of the junctional molecule VE-cadherin, suggesting that GATA4 decreases junctional stability in foetal LSECs and thus the permissiveness of the liver parenchyma for HSCs (Géraud et al., 2017). Whether other angiocrine factors or cytokines also play a role in establishing the liver HSC niche remains unknown.

The studies described above provide evidence that *Gata4* is essential for mouse embryonic development; furthermore, other studies inform about the LSEC *Gata4* role in liver metabolic homeostasis. Deletion of *Gata4* in LSECs at E17.5 using a *Clec4g*-driven Cre recombinase ($Gata4^{LSEC\text{-}KO}$) resulted in impaired hepatocyte function in 3-month-old mice, including elevated aspartate and alanine aminotransferase levels (Winkler et al., 2021), measurements that often correlate with NAFLD in humans (Sookoian et al., 2016). Analysis of livers from $Gata4^{LSEC\text{-}KO}$ mice showed disruption of hepatic zonation and of major metabolic functions, including metabolism of fatty acids, bile acids, and xenobiotics, as well as oxidative phosphorylation. These pathophysiological alterations were accompanied by marked transcriptional remodelling, showing activation of angiogenic and MYC-dependent gene programs (Winkler et al., 2021). Notably, ablation of *Gata4* in LSEC led to downregulation of c-Maf, a result

363 that is consistent with the hypothesis that these two core LSEC TFs are part of the same TF
364 network (Wilkinson et al., 2017).

365 In agreement with the studies of c-Maf deletion models, the early capillarization phenotype
366 observed in *Gata4*$^{LSEC\text{-}KO}$ mice led to the hypothesis that *Gata4* could be important in the
367 prevention of liver fibrosis (Winkler et al., 2021). LSEC-restricted deletion of *Gata4* at E10.5 was
368 associated with increased extracellular matrix (ECM) deposition, as shown by upregulation of
369 ECM-associated genes at the mRNA and protein levels (Géraud et al., 2017); while its deletion
370 at a later developmental stage also led to sinusoidal capillarization and caused perisinusoidal
371 fibrosis in adult mice, with activation and expansion of hepatic stellate cells, and increased
372 presence of infiltrating inflammatory cells (Winkler et al., 2021). Perisinusoidal fibrosis is
373 characteristic of NASH (Takahashi and Fukusato, 2014); and indeed, the LSEC transcriptional
374 profiles of *Gata4*$^{LSEC\text{-}KO}$ mice were similar to those observed in a diet-induced liver fibrosis model
375 (Winkler et al., 2021). Further highlighting the active role of GATA4 in liver metabolic disease,
376 analysis of livers from a diet induced model of NASH showed a strong downregulation of *Gata4*,
377 which was also observed in scRNA-seq from human cirrhotic livers (Winkler et al., 2021).

378 In addition to phenotypical and transcriptomic analyses, the direct interrogation of
379 chromatin activity and TF binding has the potential to provide additional insights into the modes
380 of action of TFs. To unravel the mechanisms of GATA4 activity, Winkler et al. employed ATAC-
381 seq to identify accessible chromatin regions in LSECs from wild type and *Gata4*$^{LSEC\text{-}KO}$ mice
382 (Winkler et al., 2021). This analysis revealed that GATA4 represses a continuous EC gene
383 program via reduction in chromatin accessibility. Integration of these results with whole liver Gata4
384 ChIP-seq implicated chromatin occupancy by Gata4 at sites with reduced accessibility. For
385 instance, *Pdgfb*, which is bound by Gata4 in liver, was upregulated and its promoter became more
386 accessible in LSECs from *Gata4*$^{LSEC\text{-}KO}$ mice (Winkler et al., 2021). This association between
387 Gata4 and *Pdgfb* is quite interesting since platelet derived growth factor (*PDGF*) signalling is

388 associated with stellate cell activation and fibrosis (Bataller and Brenner, 2005). *Pdgfb* is normally
389 expressed in continuous ECs but not in LSECs (Winkler et al., 2021).
390
391 **3. MEIS2**
392 MEIS2 belongs to the TF superclass TALE (three amino acid loop extension), which is a highly
393 conserved family of homeobox proteins (Bürglin, 1997). Several members of the TALE family
394 have been implicated in vertebrate embryogenesis gene programs, driving cell fate specification
395 during segmentation and in later developmental stages (Moens and Selleri, 2006). Mutant mice
396 lacking functional Meis2 displayed embryonic lethality between E13.5-E14.5, showing
397 haemorrhaging and a small liver size compared to healthy controls (Machon et al., 2015).
398 Recent screens for lineage-determining TFs using comparative transcriptomics have
399 suggested MEIS2 to have an important role in the establishment and maintenance of an LSEC-
400 differentiated phenotype, with primary LSECs rapidly losing MEIS2 expression *in vitro* (Liang et
401 al., 2022, de Haan et al., 2020). scRNA-seq analysis indicated that *Meis2* expression and
402 regulatory activity increases along the developmental trajectory, possibly contributing to
403 sinusoidal build-up during embryonic and postnatal development (Danoy et al., 2020).
404 Transcriptomic analysis comparing human pluripotent stem cells (hiPSCs) versus hiPSC-derived
405 LSECs using nanoCAGE revealed that *MEIS2* was upregulated in hiPSC-derived LSECs, along
406 with ERG, c-MAF, SPI1 and other known LSEC TFs (Danoy et al., 2020). In this study, the DNA-
407 binding motif of MEIS2 was identified within the top 10 most important motifs in driving the hiPSC-
408 derived LSEC transcriptional profile. Moreover, a regulatory network analysis implicated MEIS2
409 in the direct regulation of the LSEC marker gene *LYVE1* and of the vascular endothelial growth
410 factor (VEGF) signalling pathway (Danoy et al., 2020). Overexpression studies in HUVECs have
411 also shown that MEIS2 induces the expression of classic LSEC genes, including *F8*, *IL1A*,
412 *CLEC4M*, and *STAB1* (de Haan et al., 2020). Studies in other developmental contexts, namely
413 palatal bone development, suggested that modulation of Meis2 activity associates with changes

414 in target gene accessibility at both promoter and distal regulatory regions (Machon et al., 2015).
415 It remains to be investigated if Meis2 regulates gene expression through the same mechanisms
416 during LSEC-specification.
417
418 **4. ERG**
419 The ETS-related gene (ERG) is a lineage-specific master regulator of endothelial gene expression
420 (reviewed in (Shah et al., 2016)). ERG is required during embryonic vascular development,
421 angiogenesis, and for maintenance of vascular homeostasis. ERG expression appears first in the
422 mesoderm during early development (E8.5 in mice) and is maintained throughout adulthood at
423 consistent levels in arterial, venous and microvascular endothelium (Vlaeminck-Guillem et al.,
424 2000). ERG is indispensable for vascular development: constitutive homozygous deletion of
425 endothelial *Erg* causes embryonic lethality in mice (E10.5-12.5) due to disruption of
426 cardiovascular development (Birdsey et al., 2015, Vijayaraj et al., 2012). The crucial role of ERG
427 as a lineage-determining TF is demonstrated by studies where ectopic expression of ERG
428 contributed to cell fate reprogramming. For instance, lentiviral overexpression of *Erg* in embryonic
429 or adult murine somatic fibroblasts along with hematopoietic lineage-determining TFs (GATA2,
430 LMO2, RUNX1c, and SCL) reprogrammed fibroblasts to hematopoietic progenitors (Batta et al.,
431 2014). Expression of ETS factors ERG, FLI1, and ETV2 in combination with TGFβ pathway
432 inhibition was able to reprogram human amniotic cells into vascular endothelium (Ginsberg et al.,
433 2012). Moreover, ectopic expression of the pioneer factor ETV2 (Gong et al., 2022) converted
434 primary human adult skin fibroblasts into functional ECs through activation of *ERG* (Morita et al.,
435 2015). Besides pointing to the importance of ERG as a developmental TF, these studies highlight
436 the potential of combining ERG with other endothelial TFs for the generation and/or improvement
437 of *in vitro* EC models through the induction of endothelial-specific cell fate. This is exemplified by
438 the recent finding that ERG and FLI1 cooperate to activate a vascular gene expression program
439 in adult human mesenchymal stromal cells (Gomez-Salinero et al., 2022).

ChIP-seq analysis of HUVECs revealed ERG binding to multiple sites of accessible open chromatin, including promoters and enhancers (**Box 3**) (Kalna et al., 2019). Globally, ERG binding in HUVECs was greatest at active enhancers; ~67% of ERG bound sites were located at either distal intergenic or intragenic regions, supporting a role for ERG-mediated transactivation of gene expression through EC enhancers (Kalna et al., 2019). In line with its role as a lineage-determining TF, ERG is bound at the vast majority (93%) of HUVEC super-enhancers (Kalna et al., 2019), a class of enhancer clusters that are associated with genes that define cell lineage identity and regulate tissue-specific functions (Hnisz et al., 2013). Notably, siRNA-mediated inhibition of *ERG* in HUVECs led to changes in H3K27ac enrichment at enhancers, and to the redistribution of a subset of core super-enhancers associated with essential endothelial genes (Kalna et al., 2019).

In agreement with many other TFs, ERG can both drive and repress gene expression. Studies have shown that ERG controls transcription through either cooperation or competition with other TFs. For example, ERG cooperates with the TF KLF2 to drive the expression of the anticoagulant cell surface protein Thrombomodulin (TM). Interestingly, this mechanism is organotypic, since it was observed in LSECs, but not aortic EC. Molecular studies showed that ERG is required for KLF2 to access chromatin, by recruiting p300 and mediating H3K27ac in low shear stress conditions, as found in LSECs, but is dispensable in high shear stress conditions, as found in the aorta (Peghaire et al., 2019). An example of competition is the relationship between ERG and SMAD3: here, ERG prevents SMAD3 binding to chromatin at genes driven by the TGFβ-ALK5 pathway, thus promoting homeostasis and preventing endothelial-to-mesenchymal transition (EndoMT) (Dufton et al., 2017). EndoMT is characterised by the loss of endothelial lineage markers, morphology, and function, and is associated with multiple chronic diseases. EndoMT also contributes to LSEC capillarization and ECM production in liver fibrosis (Ruan et al., 2021). In the liver, Dufton et al. showed that EC-specific (constitutive $Erg^{cEC\text{-}Het}$ or inducible *Pdgfb-iCreER-eGFP/*$Erg^{fl/fl}$) deletion of *Erg* in mice led to spontaneous EndoMT and liver fibrogenesis (Dufton et al., 2017). This was associated with disrupted portal tracts and increased

466 periportal collagen deposition – features consistent with a fibrotic phenotype (Dufton et al., 2017).
467 The association between loss of ERG expression and liver fibrogenesis was further supported by
468 the observation that ERG expression is significantly downregulated in human liver fibrosis and
469 cirrhosis (Dufton et al., 2017).
470

Box 3: Enhancers

Enhancers are non-coding *cis*-regulatory elements that activate target gene expression through recruitment of TFs, cofactors (coactivators and corepressors), and basal transcriptional machinery. Enhancers are mostly intergenic or intronic regions of open chromatin that can affect target genes located distally, even a million base pairs away, in an orientation-independent manner (Panigrahi and O'Malley, 2021). Since enhancers are free from nucleosomes, they are accessible to enzymatic action. Frequently used methods to map enhancers are based on profiling chromatin accessibility by ATAC-seq or DNaseI-seq in conjunction with ChIP-seq for active histone marks (e.g. H3K27ac). The most accepted theory of enhancer mechanism is chromatin looping, during which a distal enhancer comes into physical contact with its target gene's promoter region. The activation of gene expression occurs through recruitment and binding of activating transcription factor(s) by the enhancer region, which boosts RNA polymerase II-dependent transcription at the promoter of the target gene. Genetic variants at enhancers can disrupt the binding events of TFs, leading to changed target gene expression. Interestingly, common disease variants have been shown to locate in non-coding regions, with enhancer elements being particularly enriched for them (Maurano et al., 2012). Although enhancers have been investigated for decades, several challenges still hamper the characterization of enhancer role in disease, recently reviewed by Zaugg et al. (Zaugg et al., 2022).

471

5. SPI1

De Smedt et al. designed a computational workflow, CenTFinder, to identify and rank TFs driving lineage specification (De Smedt et al., 2021). This strategy identified SPI1 (also referred to as PU.1), a member of the ETS family, as a regulator of immune response transcriptional programs in LSECs. SPI1 expression is induced during the differentiation of hiPSC-derived LSECs (Danoy et al., 2020); and when overexpressed in stem cells along with ETV2, SPI1 induced an LSEC-like phenotype, including the expression of the LSEC markers *FCGRB2, LYVE1, MRC1, CRHBP, FCN3,* and *OIT3* (De Smedt et al., 2021). Immunostaining and flow cytometry demonstrated upregulation of CD32B and MRC1 in these experiments. However, marker genes that are not related to immune functions, such as *STAB1/2, CLEC4G,* and *CLEC4M*, were not upregulated (De Smedt et al., 2021). The evidence linking SPI1 to liver metabolic disease is still limited, but SPI1 has already been implicated in the regulation of transcriptional changes observed in NASH (Steensels et al., 2020); and the SPI1 binding motif has been reported to be enriched in NAFLD genetic risk variants (Namjou et al., 2019). It remains to be investigated if these associations between SPI1 activity and NAFLD pertain to LSEC and/or other liver cell types. Future studies should also investigate the mechanisms by which SPI1 drives LSEC identity, although it is possible that it acts as a pioneer TF, similar to its function in hematopoietic development, where it opens stem cell heterochromatin (Pham et al., 2013).

6. ZEB2

ZEB2 (also called SIP1: SMAD-interacting protein 1) has only recently been shown to be enriched in liver microvascular ECs (de Haan et al., 2020). However, experimental follow-up for the role of ZEB2 in LSECs showed uniform expression of *Zeb2* in mouse LSECs and non-sinusoidal vessels of the liver (de Haan et al., 2021). Conditional deletion of *Zeb2* in mouse ECs (*Zeb2* EC^{KO}) revealed that loss of *Zeb2* affected *Pdgf*-signalling and angiogenic genes, but *Lyve1* was the only LSEC marker showing decreased expression. Still, the *Zeb2* EC^{KO} mice presented a denser and

irregularly shaped liver microvasculature. Upon CCl_4-induced fibrosis, immunostaining in *Zeb2* EC^{KO} livers showed gain of continuous EC markers (Cd34, Cd31), loss of the LSEC marker Cd32, and formation of basal lamina (de Haan et al., 2021). Considering its important role in other developmental programs (Fardi et al., 2019), these studies uncover of ZEB2 as an interesting candidate for future investigations in LSECs and liver disease.

Future directions

There is an unmet need to identify the drivers of LSEC transcriptional programs and cellular identity to create better *in vitro* model systems, and provide therapeutic targets and biomarkers for liver diseases such as NAFLD. As highlighted by the studies discussed in this review, liver tissue and freshly isolated LSECs are still the gold standards for LSEC expression analysis, with many studies carried out in rodent LSECs. However, this creates limitations in terms of species differences and availability of this difficult-to-obtain cell type, and has important ethical implications. Moreover, the development of reliable LSEC *in vitro* models may improve liver organoid modelling systems and platforms for regenerative therapy. The collected evidence on developmental LSEC TFs and marker genes brings us closer to the possibility of creating a TF overexpression model in more readily available, less differentiated ECs, such as HUVECs. Overexpression of c-MAF, GATA4 and MEIS2 in HUVECs induced partial LSEC phenotype in an additive way, surpassing the level of induction achieved by either of the TFs alone (de Haan et al., 2020). The combination of TF overexpression with the addition of microenvironmental molecules may also be necessary to induce and maintain the LSEC signature. For instance, bone morphogenic protein 9 (BMP9), a circulating endothelial quiescence factor, has been suggested as a necessary maintenance factor for LSEC fenestration (David et al., 2008). *Bmp9* knockout in mice reduced fenestration frequency, opposed to BMP9 treatment in cultured LSEC, which lead to prolonged fenestrated phenotype (Desroches-Castan et al., 2019). Bmp9 treatment also induced c-Maf expression and has been suggested to promote LSEC identity (Desroches-Castan

et al., 2019, Gómez-Salinero et al., 2022). Recently, Gage et al. differentiated LSEC-like cells from hiPSCs by sequentially manipulating signalling molecule levels and pathways, however, some important marker levels (e.g. F8) still remained low in hiPSC-derived LSECs, demonstrating incomplete conversion (Gage et al., 2020). Heterotypic interactions between LSECs and other liver resident cell types are also important for the maintenance of LSEC identity *in vitro*, as it has been exemplified in LSEC-hepatocyte co-culture experiments (Gómez-Salinero et al., 2022) and in more complex systems such as organoids and liver-on-a-chip (Rezvani et al., 2023). Future work should address the completion of the LSEC model based on the overexpression of TFs described in this review and looking in more detail at the cell-to-cell signalling pathways that contribute to specification of the liver endothelium during embryonic and postnatal development.

It is clear from previous studies that the spatiotemporal expression pattern of LSEC TFs is diverse. In terms of cellular specificity, c-MAF, GATA4, MEIS2 and ZEB2 are LSEC-enriched TFs. We refer here to LSEC-enriched TFs in opposition to LSEC-specific because even though the expression of these TFs is higher in LSECs in comparison to other ECs and/or other liver cells, their expression is not exclusively detected in LSECs. As we noted previously, c-MAF is expressed in LSECs, Kupffer cells and macrophages, while GATA4 is an LSEC-hepatocyte shared TF. The temporal expression patterns can also differ between TFs, as illustrated when comparing *Gata4* and *Maf* during embryonic development: Gata4 expression is constant throughout development, whereas Maf expression increases (Gómez-Salinero et al., 2022). These features have implications for the potential roles of these and other TFs in liver pathophysiology and suggest that dysfunction of at least some of these LSEC core TFs may lead to pleotropic effects across multiple cell types.

This review highlights a variety of methods used to study LSECs, particularly at the transcriptional level. The integration of different datasets has been important for addressing the limitations of any one method – this was shown recently in the differences of single-cell technologies (single-nuclei- vs. scRNA-seq) for capturing zonated LSECs (Andrews et al., 2022).

In addition to differential gene expression, other mechanisms may drive zonated transcriptional networks. For instance, differences in translation rates or protein stability may create disconnection between mRNA and protein levels for certain TFs along the periportal-pericentral axis (Inverso et al., 2021). Similarly, differential DNA methylation of TF binding sites may contribute to the zonated activity of LSEC TFs, as has been observed in human hepatocytes (Brosch et al., 2018). Thus, future single-cell multi-omics analyses may reveal additional layers of the LSEC regulome. This and other new advances, including spatial transcriptomics (Hu et al., 2022), and proteomics (Inverso et al., 2021) are sure to add new dimensions to our understanding of LSEC biology.

Cis-regulatory networks involve the coordinated activity of multiple TFs that bind to promoters and enhancers to activate broad gene programs. The liver has been extensively studied in the contexts of health and disease, particularly NAFLD, to show that there is impaired hepatic TF activity in liver metabolic disease (reviewed in (Cebola, 2020)). However, as mentioned above, such studies focused on bulk liver tissue analyses and did not have the granularity needed to capture LSEC-specific TF activity. Thus, future studies employing a combination of better *in vitro* models and single-cell omics are expected to contribute to the better characterisation of the cistromes and transcriptional networks under the control of specific LSEC TFs. It is equally important that we seek to understand how LSEC TFs work in a coordinated fashion to promote the different LSEC phenotypical attributes, especially those implicated in disease. A fundamental and still unanswered question is "How does the pan-endothelial TF ERG act with LSEC-enriched TFs to drive the LSEC transcriptional profile?". Are these TFs part of a single *cis*-regulatory network, whereby for instance ERG controls the expression of *GATA4*? Or are these TFs part of separate transcriptional networks and thus in charge of regulating distinct sets of genes in LSECs? We predict that the application of single-cell ATAC-seq (Cusanovich et al., 2015) and single-cell CUT&Tag (Bartosovic et al., 2021), among other methods, will accelerate such findings and provide answers to some of these questions. Finally, more efficient

and standardized data processing protocols along with advanced data integration methods, including the application of deep learning algorithms (Ma and Xu, 2022), will further enable to new discoveries into the functions of LSEC TFs and their specific contributions to liver homeostasis and disease.

It is well established that genetic variants can contribute to a higher risk of developing NAFLD and other liver metabolic diseases. Some variants may affect TF activity by altering their protein sequence, which may lead for example to changes in DNA-binding affinity. A recent genome-wide association study (GWAS) for NAFLD in a histology-characterised cohort, identified a missense variant in the *PYGO1* gene, which encodes a TF from the canonical Wnt signalling pathway (Anstee et al., 2020). Wnt signalling is essential for appropriate sinusoidal differentiation (Birdsey et al., 2015) and its aberrant activation has been associated with fibrosis (Inverso et al., 2021). It would therefore be interesting to characterise Wnt signalling and TF activity when PYGO1 carries the recently identified NAFLD risk allele.

Most common disease-associated genetic variants however do not affect protein coding sequences, and are instead located within cell type-specific enhancers (Maurano et al., 2012). These common variants may act through disrupting TF binding events, which could lead to changes in how genes are regulated or how they respond to metabolic cues. Given the central role of LSECs in regulating metabolic homeostasis, it is possible that LSECs are also key players in liver disease genetic susceptibility. The assignment of such non-coding variants to their target genes and thus to affected pathways remains challenging, but it is increasingly obvious that it is one of the next steps in identifying disease effector genes for common pathologies. Thus, the combined analysis of LSEC transcriptomic and epigenomics datasets should be considered in the future investigation genetic factors of liver disease.

Conclusions

Healthy, functioning LSECs play a key role in protecting the liver from inflammation and fibrosis, and even contribute to regeneration of the liver in early stages of fibrosis or in hepatectomy. In this review, we collected the available evidence for the roles of six key LSEC TFs, which initiate and drive LSEC development and are important for the maintenance of liver metabolic homeostasis. Given the many challenges of working with primary ECs, we propose that these LSEC TFs should be harnessed to develop better LSEC *in vitro* modelling systems, along with other currently known LSEC marker genes (**Figure 2**) and secreted factors. There are still significant gaps in our understanding of LSEC TF activity, which we envision will be tackled in future studies, deploying state-of-the-art approaches such as the epigenomic profiling of complex tissue samples with single-cell resolution.

Declaration of interest

The authors declare no conflict of interest.

Funding

DN is recipient of a British Heart Foundation 4-year PhD studentship (FS/4yPhD/F/20/34128). IC is recipient of a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (224662/Z/21/Z) and a Research Grant by the Royal Society (RGS\R1\221086). GMB and AMR are supported by the British Heart Foundation (PG/20/16/35047; RG/17/4/32662). The authors are also supported by the National Institute for Health Research (NIHR) Imperial Biomedical Research Centre.

Figure legends

Figure 1. Position, phenotype, and marker fingerprint of LSECs in homeostasis and disease. The liver's structural unit is the liver lobule (top left) where the portal triad and the central vein are connected by sinusoids. Liver sinusoids are the scene for uptake of nutrients derived from the intestines via the portal vein, where there is also an influx of pathogens and signalling molecules from the gut bacteria (top right). Oxygen-enriched blood enters from the portal artery and mixes with the portal vein blood. The mixed blood flows through the sinusoid where it is filtered by the liver sinusoidal endothelial cells (LSECs) and macromolecules and nutrients are transported towards the hepatocytes, while waste products from the liver parenchyma are transported into the sinusoidal blood. This mechanism creates a gradient of oxygen, nutrients, and signalling molecules along the periportal-pericentral axis (**Box 2**). LSECs, through their unique phenotypic and functional properties, play a key role in several aspects of liver homeostasis (see section ***LSEC phenotypical properties and function in physiological conditions and disease***). Damaged LSECs display several changes in their phenotype and function (bottom right) such as loss of fenestrae, formation of basal lamina, decreased endocytic activity, deposition of extracellular matrix, and activation of Kupffer cells. These changes affect macromolecule transport towards the parenchyma, microbe and virus uptake, and can lead to sustained inflammation and fibrosis. The bottom left pictograms highlight some important, known LSEC marker genes and transcription factors, and their loss in liver disease.

Figure 2. Marker genes of LSECs identified across eight independent studies employing different experimental methods and/or analysis. The summary of rodent and human datasets presents genes identified by at least 4 independent studies. The genes are ranked by the number of identifying studies. scRNA-seq studies were considered if they provided marker gene sets for clusters defined as LSECs. Ramachandran et al. provided a pre-filtered LSEC marker list (n=80) (Ramachandran et al., 2019). For the remaining scRNA-seq studies, we obtained the top 100

genes ranked by fold-change of LSECs vs. other liver cell types (Guilliams et al., 2022). In studies that defined two (MacParland et al., 2018) or three (Aizarani et al., 2019) LSEC clusters, the final list combined the top 100 genes from each of those clusters. Haan et al. identified 27 LSEC-enriched genes by comparing LSECs with heart and brain ECs (de Haan et al., 2020). Géraud et al. provided a 46 gene marker list based on gene expression comparison between EC groups (Géraud et al., 2010). De Smedt et al. developed a computational workflow to identify TFs central in differentiation and specification (CenTFinder). The application of CenTFinder to a series of LSEC gene expression datasets resulted in a list of 80 putative marker genes (De Smedt et al., 2021). On the right, we present scaled mean protein expression for LSECs across 4 zones (portal node (PN), periportal (PP), pericentral (PC), central vein (CV)) (n=4 samples per zone) (Inverso et al., 2021). Genes/proteins not reported in the datasets described are shown with a small empty dot. The full list of marker genes is available in **Supplementary Table 1**.

References

AIZARANI, N., SAVIANO, A., SAGAR, MAILLY, L., DURAND, S., HERMAN, J. S., PESSAUX, P., BAUMERT, T. F. & GRÜN, D. 2019. A human liver cell atlas reveals heterogeneity and epithelial progenitors. *Nature,* 572**,** 199-204.

ANDREWS, T. S., ATIF, J., LIU, J. C., PERCIANI, C. T., MA, X. Z., THOENI, C., SLYPER, M., ERASLAN, G., SEGERSTOLPE, A., MANUEL, J., CHUNG, S., WINTER, E., CIRLAN, I., KHUU, N., FISCHER, S., ROZENBLATT-ROSEN, O., REGEV, A., MCGILVRAY, I. D., BADER, G. D. & MACPARLAND, S. A. 2022. Single-Cell, Single-Nucleus, and Spatial RNA Sequencing of the Human Liver Identifies Cholangiocyte and Mesenchymal Heterogeneity. *Hepatol Commun,* 6**,** 821-840.

ANSTEE, Q. M., DARLAY, R., COCKELL, S., MERONI, M., GOVAERE, O., TINIAKOS, D., BURT, A. D., BEDOSSA, P., PALMER, J., LIU, Y.-L., AITHAL, G. P., ALLISON, M., YKI-JÄRVINEN, H., VACCA, M., DUFOUR, J.-F., INVERNIZZI, P., PRATI, D., EKSTEDT, M., KECHAGIAS, S., FRANCQUE, S., PETTA, S., BUGIANESI, E., CLEMENT, K., RATZIU, V., SCHATTENBERG, J. M., VALENTI, L., DAY, C. P., CORDELL, H. J. & DALY, A. K. 2020. Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort[☆]. *Journal of Hepatology,* 73**,** 505-515.

ASAHINA, K., ZHOU, B., PU, W. T. & TSUKAMOTO, H. 2011. Septum transversum-derived mesothelium gives rise to hepatic stellate cells and perivascular mesenchymal cells in developing mouse liver. *Hepatology,* 53**,** 983-995.

BARTOSOVIC, M., KABBE, M. & CASTELO-BRANCO, G. 2021. Single-cell CUT&Tag profiles histone modifications and transcription factors in complex tissues. *Nature Biotechnology,* 39**,** 825-835.

BATALLER, R. & BRENNER, D. A. 2005. Liver fibrosis. *J Clin Invest,* 115**,** 209-18.

BATTA, K., FLORKOWSKA, M., KOUSKOFF, V. & LACAUD, G. 2014. Direct reprogramming of murine fibroblasts to hematopoietic progenitor cells. *Cell Rep,* 9**,** 1871-1884.

BEN-MOSHE, S. & ITZKOVITZ, S. 2019. Spatial heterogeneity in the mammalian liver. *Nat Rev Gastroenterol Hepatol,* 16**,** 395-410.

BENEDICT, M. & ZHANG, X. 2017. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol,* 9**,** 715-732.

BIRDSEY, G. M., SHAH, A. V., DUFTON, N., REYNOLDS, L. E., OSUNA ALMAGRO, L., YANG, Y., ASPALTER, I. M., KHAN, S. T., MASON, J. C., DEJANA, E., GÖTTGENS, B., HODIVALA-DILKE, K., GERHARDT, H., ADAMS, R. H. & RANDI, A. M. 2015. The endothelial transcription factor ERG promotes vascular stability and growth through Wnt/β-catenin signaling. *Dev Cell,* 32**,** 82-96.

BLOUIN, A., BOLENDER, R. P. & WEIBEL, E. R. 1977. Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. A stereological study. *Journal of Cell Biology,* 72**,** 441-455.

BOSSARD, P. & ZARET, K. S. 1998. GATA transcription factors as potentiators of gut endoderm differentiation. *Development,* 125**,** 4909-17.

BROSCH, M., KATTLER, K., HERRMANN, A., VON SCHÖNFELS, W., NORDSTRÖM, K., SEEHOFER, D., DAMM, G., BECKER, T., ZEISSIG, S., NEHRING, S., REICHEL, F., MOSER, V., THANGAPANDI, R. V., STICKEL, F., BARETTON, G., RÖCKEN, C., MUDERS, M., MATZ-SOJA, M., KRAWCZAK, M., GASPARONI, G., HARTMANN,

H., DAHL, A., SCHAFMAYER, C., WALTER, J. & HAMPE, J. 2018. Epigenomic map of human liver reveals principles of zonated morphogenic and metabolic control. *Nature Communications,* 9**,** 4150.

BURGDORF, S., KAUTZ, A., BÖHNERT, V., KNOLLE, P. A. & KURTS, C. 2007. Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation. *Science,* 316**,** 612-6.

BÜRGLIN, T. R. 1997. Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res,* 25**,** 4173-80.

CEBOLA, I. 2020. Liver gene regulatory networks: Contributing factors to nonalcoholic fatty liver disease. *WIREs Systems Biology and Medicine,* 12**,** e1480.

CUSANOVICH, D. A., DAZA, R., ADEY, A., PLINER, H. A., CHRISTIANSEN, L., GUNDERSON, K. L., STEEMERS, F. J., TRAPNELL, C. & SHENDURE, J. 2015. Multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing. *Science,* 348**,** 910-914.

DANOY, M., POULAIN, S., KOUI, Y., TAURAN, Y., SCHEIDECKER, B., KIDO, T., MIYAJIMA, A., SAKAI, Y., PLESSY, C. & LECLERC, E. 2020. Transcriptome profiling of hiPSC-derived LSECs with nanoCAGE. *Molecular Omics,* 16**,** 138-146.

DAVID, L., MALLET, C., KERAMIDAS, M., LAMANDÉ, N., GASC, J.-M., DUPUIS-GIROD, S., PLAUCHU, H., FEIGE, J.-J. & BAILLY, S. 2008. Bone Morphogenetic Protein-9 Is a Circulating Vascular Quiescence Factor. *Circulation Research,* 102**,** 914-922.

DE HAAN, W., DHEEDENE, W., APELT, K., DÉCOMBAS-DESCHAMPS, S., VINCKIER, S., VERHULST, S., CONIDI, A., DEFFIEUX, T., STARING, M. W., VANDERVOORT, P., CALUWÉ, E., LOX, M., MANNAERTS, I., TAKAGI, T., JAEKERS, J., BERX, G., HAIGH, J., TOPAL, B., ZWIJSEN, A., HIGASHI, Y., VAN GRUNSVEN, L. A., VAN IJCKEN, W. F. J., MULUGETA, E., TANTER, M., LEBRIN, F. P. G., HUYLEBROECK, D. & LUTTUN, A. 2021. Endothelial Zeb2 preserves the hepatic angioarchitecture and protects against liver fibrosis. *Cardiovascular Research,* 118**,** 1262-1275.

DE HAAN, W., ØIE, C., BENKHEIL, M., DHEEDENE, W., VINCKIER, S., COPPIELLO, G., ARANGUREN, X. L., BEERENS, M., JAEKERS, J., TOPAL, B., VERFAILLIE, C., SMEDSRØD, B. & LUTTUN, A. 2020. Unraveling the transcriptional determinants of liver sinusoidal endothelial cell specialization. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* 318**,** G803-G815.

DE SMEDT, J., VAN OS, E. A., TALON, I., GHOSH, S., TOPRAKHISAR, B., FURTADO MADEIRO DA COSTA, R., ZAUNZ, S., VAZQUEZ, M. A., BOON, R., BAATSEN, P., SMOUT, A., VERHULST, S., VAN GRUNSVEN, L. A. & VERFAILLIE, C. M. 2021. PU.1 drives specification of pluripotent stem cell-derived endothelial cells to LSEC-like cells. *Cell Death & Disease,* 12**,** 84.

DESROCHES-CASTAN, A., TILLET, E., RICARD, N., OUARNÉ, M., MALLET, C., BELMUDES, L., COUTÉ, Y., BOILLOT, O., SCOAZEC, J. Y., BAILLY, S. & FEIGE, J. J. 2019. Bone Morphogenetic Protein 9 Is a Paracrine Factor Controlling Liver Sinusoidal Endothelial Cell Fenestration and Protecting Against Hepatic Fibrosis. *Hepatology,* 70**,** 1392-1408.

DING, B. S., CAO, Z., LIS, R., NOLAN, D. J., GUO, P., SIMONS, M., PENFOLD, M. E., SHIDO, K., RABBANY, S. Y. & RAFII, S. 2014. Divergent angiocrine signals from vascular niche balance liver regeneration and fibrosis. *Nature,* 505**,** 97-102.

DUFTON, N. P., PEGHAIRE, C. R., OSUNA-ALMAGRO, L., RAIMONDI, C., KALNA, V., CHAUHAN, A., WEBB, G., YANG, Y., BIRDSEY, G. M., LALOR, P., MASON, J. C., ADAMS, D. H. & RANDI, A. M. 2017. Dynamic regulation of canonical TGFβ signalling by endothelial transcription factor ERG protects from liver fibrogenesis. *Nat Commun,* 8**,** 895.

FARDI, M., ALIVAND, M., BARADARAN, B., FARSHDOUSTI HAGH, M. & SOLALI, S. 2019. The crucial role of ZEB2: From development to epithelial-to-mesenchymal transition and cancer complexity. *Journal of Cellular Physiology,* 234**,** 14783-14799.

GAGE, B. K., LIU, J. C., INNES, B. T., MACPARLAND, S. A., MCGILVRAY, I. D., BADER, G. D. & KELLER, G. M. 2020. Generation of Functional Liver Sinusoidal Endothelial Cells from Human Pluripotent Stem-Cell-Derived Venous Angioblasts. *Cell Stem Cell,* 27**,** 254-269.e9.

GEBHARDT, R., BALDYSIAK-FIGIEL, A., KRÜGEL, V., UEBERHAM, E. & GAUNITZ, F. 2007. Hepatocellular expression of glutamine synthetase: An indicator of morphogen actions as master regulators of zonation in adult liver. *Progress in Histochemistry and Cytochemistry,* 41**,** 201-266.

GÉRAUD, C., KOCH, P.-S., ZIEROW, J., KLAPPROTH, K., BUSCH, K., OLSAVSZKY, V., LEIBING, T., DEMORY, A., ULBRICH, F., DIETT, M., SINGH, S., STICHT, C., BREITKOPF-HEINLEIN, K., RICHTER, K., KARPPINEN, S.-M., PIHLAJANIEMI, T., ARNOLD, B., RODEWALD, H.-R., AUGUSTIN, H. G., SCHLEDZEWSKI, K. & GOERDT, S. 2017. GATA4-dependent organ-specific endothelial differentiation controls liver development and embryonic hematopoiesis. *The Journal of clinical investigation,* 127**,** 1099-1114.

GÉRAUD, C., SCHLEDZEWSKI, K., DEMORY, A., KLEIN, D., KAUS, M., PEYRE, F., STICHT, C., EVDOKIMOV, K., LU, S., SCHMIEDER, A. & GOERDT, S. 2010. Liver sinusoidal endothelium: A microenvironment-dependent differentiation program in rat including the novel junctional protein liver endothelial differentiation-associated protein-1. *Hepatology,* 52**,** 313-326.

GINSBERG, M., JAMES, D., DING, B. S., NOLAN, D., GENG, F., BUTLER, J. M., SCHACHTERLE, W., PULIJAAL, V. R., MATHEW, S., CHASEN, S. T., XIANG, J., ROSENWAKS, Z., SHIDO, K., ELEMENTO, O., RABBANY, S. Y. & RAFII, S. 2012. Efficient direct reprogramming of mature amniotic cells into endothelial cells by ETS factors and TGFβ suppression. *Cell,* 151**,** 559-75.

GOMEZ-SALINERO, J. M., ITKIN, T., HOUGHTON, S., BADWE, C., LIN, Y., KALNA, V., DUFTON, N., PEGHAIRE, C. R., YOKOYAMA, M., WINGO, M., LU, T. M., LI, G., XIANG, J. Z., HSU, Y. S., REDMOND, D., SCHREINER, R., BIRDSEY, G. M., RANDI, A. M. & RAFII, S. 2022. Cooperative ETS Transcription Factors Enforce Adult Endothelial Cell Fate and Cardiovascular Homeostasis. *Nat Cardiovasc Res,* 1**,** 882-899.

GÓMEZ-SALINERO, J. M., IZZO, F., LIN, Y., HOUGHTON, S., ITKIN, T., GENG, F., BRAM, Y., ADELSON, R. P., LU, T. M., INGHIRAMI, G., XIANG, J. Z., LIS, R., REDMOND, D., SCHREINER, R., RABBANY, S. Y., LANDAU, D. A., SCHWARTZ, R. E. & RAFII, S. 2022. Specification of fetal liver endothelial progenitors to functional zonated adult sinusoids requires c-Maf induction. *Cell Stem Cell,* 29**,** 593-609.e7.

GONG, W., DAS, S., SIERRA-PAGAN, J. E., SKIE, E., DSOUZA, N., LARSON, T. A., GARRY, M. G., LUZETE-MONTEIRO, E., ZARET, K. S. & GARRY, D. J. 2022. ETV2

functions as a pioneer factor to regulate and reprogram the endothelial lineage. *Nat Cell Biol,* 24**,** 672-684.
GROSSMAN, S. R., ENGREITZ, J., RAY, J. P., NGUYEN, T. H., HACOHEN, N. & LANDER, E. S. 2018. Positional specificity of different transcription factor classes within enhancers. *Proc Natl Acad Sci U S A,* 115**,** E7222-e7230.
GUALDI, R., BOSSARD, P., ZHENG, M., HAMADA, Y., COLEMAN, J. R. & ZARET, K. S. 1996. Hepatic specification of the gut endoderm in vitro: cell signaling and transcriptional control. *Genes Dev,* 10**,** 1670-82.
GUILLIAMS, M., BONNARDEL, J., HAEST, B., VANDERBORGHT, B., WAGNER, C., REMMERIE, A., BUJKO, A., MARTENS, L., THONÉ, T., BROWAEYS, R., DE PONTI, F. F., VANNESTE, B., ZWICKER, C., SVEDBERG, F. R., VANHALEWYN, T., GONÇALVES, A., LIPPENS, S., DEVRIENDT, B., COX, E., FERRERO, G., WITTAMER, V., WILLAERT, A., KAPTEIN, S. J. F., NEYTS, J., DALLMEIER, K., GELDHOF, P., CASAERT, S., DEPLANCKE, B., TEN DIJKE, P., HOORENS, A., VANLANDER, A., BERREVOET, F., VAN NIEUWENHOVE, Y., SAEYS, Y., SAELENS, W., VAN VLIERBERGHE, H., DEVISSCHER, L. & SCOTT, C. L. 2022. Spatial proteogenomics reveals distinct and evolutionarily conserved hepatic macrophage niches. *Cell,* 185**,** 379-396.e38.
HAMMOUTENE, A. & RAUTOU, P.-E. 2019. Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. *Journal of Hepatology,* 70**,** 1278-1291.
HERNANDEZ-GEA, V. & FRIEDMAN, S. L. 2011. Pathogenesis of Liver Fibrosis. *Annual Review of Pathology: Mechanisms of Disease,* 6**,** 425-456.
HNISZ, D., ABRAHAM, B. J., LEE, T. I., LAU, A., SAINT-ANDRÉ, V., SIGOVA, A. A., HOKE, H. A. & YOUNG, R. A. 2013. Super-enhancers in the control of cell identity and disease. *Cell,* 155**,** 934-47.
HU, J., SRIVASTAVA, K., WIELAND, M., RUNGE, A., MOGLER, C., BESEMFELDER, E., TERHARDT, D., VOGEL, M. J., CAO, L., KORN, C., BARTELS, S., THOMAS, M. & AUGUSTIN, H. G. 2014. Endothelial Cell-Derived Angiopoietin-2 Controls Liver Regeneration as a Spatiotemporal Rheostat. *Science,* 343**,** 416-419.
HU, S., LIU, S., BIAN, Y., PODDAR, M., SINGH, S., CAO, C., MCGAUGHEY, J., BELL, A., BLAZER, L. L., ADAMS, J. J., SIDHU, S. S., ANGERS, S. & MONGA, S. P. 2022. Single-cell spatial transcriptomics reveals a dynamic control of metabolic zonation and liver regeneration by endothelial cell Wnt2 and Wnt9b. *Cell Rep Med,* 3**,** 100754.
IMBRATTA, C., HUSSEIN, H., ANDRIS, F. & VERDEIL, G. 2020. c-MAF, a Swiss Army Knife for Tolerance in Lymphocytes. *Frontiers in Immunology,* 11.
INVERSO, D., SHI, J., LEE, K. H., JAKAB, M., BEN-MOSHE, S., KULKARNI, S. R., SCHNEIDER, M., WANG, G., KOMEILI, M., VÉLEZ, P. A., RIEDEL, M., SPEGG, C., RUPPERT, T., SCHAEFFER-REISS, C., HELM, D., SINGH, I., BOUTROS, M., CHINTHARLAPALLI, S., HEIKENWALDER, M., ITZKOVITZ, S. & AUGUSTIN, H. G. 2021. A spatial vascular transcriptomic, proteomic, and phosphoproteomic atlas unveils an angiocrine Tie-Wnt signaling axis in the liver. *Dev Cell,* 56**,** 1677-1693.e10.
JOLMA, A., YIN, Y., NITTA, K. R., DAVE, K., POPOV, A., TAIPALE, M., ENGE, M., KIVIOJA, T., MORGUNOVA, E. & TAIPALE, J. 2015. DNA-dependent formation of transcription factor pairs alters their binding specificity. *Nature,* 527**,** 384-388.
KALNA, V., YANG, Y., PEGHAIRE, C. R., FRUDD, K., HANNAH, R., SHAH, A. V., OSUNA ALMAGRO, L., BOYLE, J. J., GÖTTGENS, B., FERRER, J., RANDI, A. M. &

BIRDSEY, G. M. 2019. The Transcription Factor ERG Regulates Super-Enhancers Associated With an Endothelial-Specific Gene Expression Program. *Circ Res,* 124**,** 1337-1349.

KUS, E., KACZARA, P., CZYZYNSKA-CICHON, I., SZAFRANSKA, K., ZAPOTOCZNY, B., KIJ, A., SOWINSKA, A., KOTLINOWSKI, J., MATEUSZUK, L., CZARNOWSKA, E., SZYMONSKI, M. & CHLOPICKI, S. 2019. LSEC Fenestrae Are Preserved Despite Pro-inflammatory Phenotype of Liver Sinusoidal Endothelial Cells in Mice on High Fat Diet. *Front Physiol,* 10**,** 6.

LAFOZ, E., RUART, M., ANTON, A., ONCINS, A. & HERNÁNDEZ-GEA, V. 2020. The Endothelium as a Driver of Liver Fibrosis and Regeneration. *Cells,* 9**,** 929.

LAMBERT, S. A., JOLMA, A., CAMPITELLI, L. F., DAS, P. K., YIN, Y., ALBU, M., CHEN, X., TAIPALE, J., HUGHES, T. R. & WEIRAUCH, M. T. 2018. The Human Transcription Factors. *Cell,* 172**,** 650-665.

LIANG, Y., KANEKO, K., XIN, B., LEE, J., SUN, X., ZHANG, K. & FENG, G. S. 2022. Temporal analyses of postnatal liver development and maturation by single-cell transcriptomics. *Dev Cell,* 57**,** 398-414.e5.

LIMMER, A., OHL, J., KURTS, C., LJUNGGREN, H. G., REISS, Y., GROETTRUP, M., MOMBURG, F., ARNOLD, B. & KNOLLE, P. A. 2000. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. *Nat Med,* 6**,** 1348-54.

MA, Q. & XU, D. 2022. Deep learning shapes single-cell data analysis. *Nature Reviews Molecular Cell Biology,* 23**,** 303-304.

MACHON, O., MASEK, J., MACHONOVA, O., KRAUSS, S. & KOZMIK, Z. 2015. Meis2 is essential for cranial and cardiac neural crest development. *BMC Developmental Biology,* 15**,** 40.

MACPARLAND, S. A., LIU, J. C., MA, X.-Z., INNES, B. T., BARTCZAK, A. M., GAGE, B. K., MANUEL, J., KHUU, N., ECHEVERRI, J., LINARES, I., GUPTA, R., CHENG, M. L., LIU, L. Y., CAMAT, D., CHUNG, S. W., SELIGA, R. K., SHAO, Z., LEE, E., OGAWA, S., OGAWA, M., WILSON, M. D., FISH, J. E., SELZNER, M., GHANEKAR, A., GRANT, D., GREIG, P., SAPISOCHIN, G., SELZNER, N., WINEGARDEN, N., ADEYI, O., KELLER, G., BADER, G. D. & MCGILVRAY, I. D. 2018. Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. *Nature Communications,* 9**,** 4383.

MANICARDI, N., FERNÁNDEZ-IGLESIAS, A., ABAD-JORDÀ, L., ROYO, F., AZKARGORTA, M., ORTEGA-RIBERA, M., SANFELIU-REDONDO, D., MARTÍNEZ-ALCOCER, A., ELORTZA, F., HESSHEIMER, A. J., FONDEVILA, C., LOZANO, J. J., GARCÍA-PAGÁN, J. C., BOSCH, J., CUBERO, F. J., ALBILLOS, A., VAQUERO, J., FALCÓN-PÉREZ, J. M. & GRACIA-SANCHO, J. 2021. Transcriptomic Profiling of the Liver Sinusoidal Endothelium during Cirrhosis Reveals Stage-Specific Secretory Signature. *Cancers,* 13**,** 2688.

MAURANO, M. T., HUMBERT, R., RYNES, E., THURMAN, R. E., HAUGEN, E., WANG, H., REYNOLDS, A. P., SANDSTROM, R., QU, H., BRODY, J., SHAFER, A., NERI, F., LEE, K., KUTYAVIN, T., STEHLING-SUN, S., JOHNSON, A. K., CANFIELD, T. K., GISTE, E., DIEGEL, M., BATES, D., HANSEN, R. S., NEPH, S., SABO, P. J., HEIMFELD, S., RAUBITSCHEK, A., ZIEGLER, S., COTSAPAS, C., SOTOODEHNIA, N., GLASS, I., SUNYAEV, S. R., KAUL, R. & STAMATOYANNOPOULOS, J. A. 2012.

Systematic localization of common disease-associated variation in regulatory DNA. *Science,* 337**,** 1190-5.
MIYAO, M., KOTANI, H., ISHIDA, T., KAWAI, C., MANABE, S., ABIRU, H. & TAMAKI, K. 2015. Pivotal role of liver sinusoidal endothelial cells in NAFLD/NASH progression. *Lab Invest,* 95**,** 1130-44.
MOENS, C. B. & SELLERI, L. 2006. Hox cofactors in vertebrate development. *Developmental Biology,* 291**,** 193-206.
MORITA, R., SUZUKI, M., KASAHARA, H., SHIMIZU, N., SHICHITA, T., SEKIYA, T., KIMURA, A., SASAKI, K., YASUKAWA, H. & YOSHIMURA, A. 2015. ETS transcription factor ETV2 directly converts human fibroblasts into functional endothelial cells. *Proc Natl Acad Sci U S A,* 112**,** 160-5.
NAMJOU, B., LINGREN, T., HUANG, Y., PARAMESWARAN, S., COBB, B. L., STANAWAY, I. B., CONNOLLY, J. J., MENTCH, F. D., BENOIT, B., NIU, X., WEI, W.-Q., CARROLL, R. J., PACHECO, J. A., HARLEY, I. T. W., DIVANOVIC, S., CARRELL, D. S., LARSON, E. B., CAREY, D. J., VERMA, S., RITCHIE, M. D., GHARAVI, A. G., MURPHY, S., WILLIAMS, M. S., CROSSLIN, D. R., JARVIK, G. P., KULLO, I. J., HAKONARSON, H., LI, R., XANTHAKOS, S. A., HARLEY, J. B. & THE E, M. N. 2019. GWAS and enrichment analyses of non-alcoholic fatty liver disease identify new trait-associated genes and pathways across eMERGE Network. *BMC Medicine,* 17**,** 135.
PANIGRAHI, A. & O'MALLEY, B. W. 2021. Mechanisms of enhancer action: the known and the unknown. *Genome Biology,* 22**,** 108.
PARIS, J. & HENDERSON, N. C. 2022. Liver zonation, revisited. *Hepatology,* 76**,** 1219-1230.
PARKER, M. E., BARRERA, A., WHEATON, J. D., ZUBERBUEHLER, M. K., ALLAN, D. S. J., CARLYLE, J. R., REDDY, T. E. & CIOFANI, M. 2019. c-Maf regulates the plasticity of group 3 innate lymphoid cells by restraining the type 1 program. *Journal of Experimental Medicine,* 217.
PEGHAIRE, C., DUFTON, N. P., LANG, M., SALLES-CRAWLEY, I. I., AHNSTRÖM, J., KALNA, V., RAIMONDI, C., PERICLEOUS, C., INUABASI, L., KISELEVA, R., MUZYKANTOV, V. R., MASON, J. C., BIRDSEY, G. M. & RANDI, A. M. 2019. The transcription factor ERG regulates a low shear stress-induced anti-thrombotic pathway in the microvasculature. *Nature Communications,* 10**,** 5014.
PHAM, T. H., MINDERJAHN, J., SCHMIDL, C., HOFFMEISTER, H., SCHMIDHOFER, S., CHEN, W., LÄNGST, G., BENNER, C. & REHLI, M. 2013. Mechanisms of in vivo binding site selection of the hematopoietic master transcription factor PU.1. *Nucleic Acids Res,* 41**,** 6391-402.
POISSON, J., LEMOINNE, S., BOULANGER, C., DURAND, F., MOREAU, R., VALLA, D. & RAUTOU, P.-E. 2017. Liver sinusoidal endothelial cells: Physiology and role in liver diseases. *Journal of Hepatology,* 66**,** 212-227.
RAMACHANDRAN, P., DOBIE, R., WILSON-KANAMORI, J. R., DORA, E. F., HENDERSON, B. E. P., LUU, N. T., PORTMAN, J. R., MATCHETT, K. P., BRICE, M., MARWICK, J. A., TAYLOR, R. S., EFREMOVA, M., VENTO-TORMO, R., CARRAGHER, N. O., KENDALL, T. J., FALLOWFIELD, J. A., HARRISON, E. M., MOLE, D. J., WIGMORE, S. J., NEWSOME, P. N., WESTON, C. J., IREDALE, J. P., TACKE, F., POLLARD, J. W., PONTING, C. P., MARIONI, J. C., TEICHMANN, S. A.

& HENDERSON, N. C. 2019. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature,* 575**,** 512-518.
REZVANI, M., VALLIER, L. & GUILLOT, A. 2023. Modeling non-alcoholic fatty liver disease in the dish using human-specific platforms – strategies and limitations. *Cellular and Molecular Gastroenterology and Hepatology*.
RUAN, B., DUAN, J. L., XU, H., TAO, K. S., HAN, H., DOU, G. R. & WANG, L. 2021. Capillarized Liver Sinusoidal Endothelial Cells Undergo Partial Endothelial-Mesenchymal Transition to Actively Deposit Sinusoidal ECM in Liver Fibrosis. *Front Cell Dev Biol,* 9**,** 671081.
SAVIANO, A., HENDERSON, N. C. & BAUMERT, T. F. 2020. Single-cell genomics and spatial transcriptomics: Discovery of novel cell states and cellular interactions in liver physiology and disease biology. *J Hepatol,* 73**,** 1219-1230.
SHAH, A. V., BIRDSEY, G. M. & RANDI, A. M. 2016. Regulation of endothelial homeostasis, vascular development and angiogenesis by the transcription factor ERG. *Vascul Pharmacol,* 86**,** 3-13.
SOOKOIAN, S., CASTAÑO, G. O., SCIAN, R., FERNÁNDEZ GIANOTTI, T., DOPAZO, H., ROHR, C., GAJ, G., SAN MARTINO, J., SEVIC, I., FLICHMAN, D. & PIROLA, C. J. 2016. Serum aminotransferases in nonalcoholic fatty liver disease are a signature of liver metabolic perturbations at the amino acid and Krebs cycle level1,2. *The American Journal of Clinical Nutrition,* 103**,** 422-434.
SØRENSEN, K. K., SIMON-SANTAMARIA, J., MCCUSKEY, R. S. & SMEDSRØD, B. 2015. Liver Sinusoidal Endothelial Cells. *Compr Physiol,* 5**,** 1751-74.
STEENSELS, S., QIAO, J. & ERSOY, B. A. 2020. Transcriptional Regulation in Non-Alcoholic Fatty Liver Disease. *Metabolites,* 10.
STRAUSS, O., PHILLIPS, A., RUGGIERO, K., BARTLETT, A. & DUNBAR, P. R. 2017. Immunofluorescence identifies distinct subsets of endothelial cells in the human liver. *Scientific Reports,* 7**,** 44356.
SU, T., YANG, Y., LAI, S., JEONG, J., JUNG, Y., MCCONNELL, M., UTSUMI, T. & IWAKIRI, Y. 2021. Single-Cell Transcriptomics Reveals Zone-Specific Alterations of Liver Sinusoidal Endothelial Cells in Cirrhosis. *Cellular and Molecular Gastroenterology and Hepatology,* 11**,** 1139-1161.
TAKAHASHI, Y. & FUKUSATO, T. 2014. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol,* 20**,** 15539-48.
TREMBLAY, M., SANCHEZ-FERRAS, O. & BOUCHARD, M. 2018. GATA transcription factors in development and disease. *Development,* 145.
VIJAYARAJ, P., LE BRAS, A., MITCHELL, N., KONDO, M., JULIAO, S., WASSERMAN, M., BEELER, D., SPOKES, K., AIRD, W. C., BALDWIN, H. S. & OETTGEN, P. 2012. Erg is a crucial regulator of endocardial-mesenchymal transformation during cardiac valve morphogenesis. *Development,* 139**,** 3973-85.
VLAEMINCK-GUILLEM, V., CARRERE, S., DEWITTE, F., STEHELIN, D., DESBIENS, X. & DUTERQUE-COQUILLAUD, M. 2000. The Ets family member Erg gene is expressed in mesodermal tissues and neural crests at fundamental steps during mouse embryogenesis. *Mechanisms of Development,* 91**,** 331-335.
WATT, A. J., ZHAO, R., LI, J. & DUNCAN, S. A. 2007. Development of the mammalian liver and ventral pancreas is dependent on GATA4. *BMC Developmental Biology,* 7**,** 37.

WILKINSON, A. C., NAKAUCHI, H. & GÖTTGENS, B. 2017. Mammalian Transcription Factor Networks: Recent Advances in Interrogating Biological Complexity. *Cell Systems,* 5**,** 319-331.

WINKLER, M., STANICZEK, T., KÜRSCHNER, S. W., SCHMID, C. D., SCHÖNHABER, H., CORDERO, J., KESSLER, L., MATHES, A., STICHT, C., NESSLIN, M., UVAROVSKII, A., ANDERS, S., ZHANG, X.-J., VON FIGURA, G., HARTMANN, D., MOGLER, C., DOBREVA, G., SCHLEDZEWSKI, K., GÉRAUD, C., KOCH, P.-S. & GOERDT, S. 2021. Endothelial GATA4 controls liver fibrosis and regeneration by preventing a pathogenic switch in angiocrine signaling. *Journal of Hepatology,* 74**,** 380-393.

WISSE, E. 1970. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *Journal of Ultrastructure Research,* 31**,** 125-150.

XIONG, X., KUANG, H., ANSARI, S., LIU, T., GONG, J., WANG, S., ZHAO, X.-Y., JI, Y., LI, C., GUO, L., ZHOU, L., CHEN, Z., LEON-MIMILA, P., CHUNG, M. T., KURABAYASHI, K., OPP, J., CAMPOS-PÉREZ, F., VILLAMIL-RAMÍREZ, H., CANIZALES-QUINTEROS, S., LYONS, R., LUMENG, C. N., ZHOU, B., QI, L., HUERTAS-VAZQUEZ, A., LUSIS, A. J., XU, X. Z. S., LI, S., YU, Y., LI, J. Z. & LIN, J. D. 2019. Landscape of Intercellular Crosstalk in Healthy and NASH Liver Revealed by Single-Cell Secretome Gene Analysis. *Molecular Cell,* 75**,** 644-660.e5.

YOUNOSSI, Z. M., GOLABI, P., PAIK, J. M., HENRY, A., VAN DONGEN, C. & HENRY, L. 2023. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology*.

ZABET, N. R. & ADRYAN, B. 2015. Estimating binding properties of transcription factors from genome-wide binding profiles. *Nucleic Acids Res,* 43**,** 84-94.

ZARET, K. S. 2020. Pioneer Transcription Factors Initiating Gene Network Changes. *Annu Rev Genet,* 54**,** 367-385.

ZAUGG, J. B., SAHLÉN, P., ANDERSSON, R., ALBERICH-JORDA, M., DE LAAT, W., DEPLANCKE, B., FERRER, J., MANDRUP, S., NATOLI, G., PLEWCZYNSKI, D., RADA-IGLESIAS, A. & SPICUGLIA, S. 2022. Current challenges in understanding the role of enhancers in disease. *Nature Structural & Molecular Biology,* 29**,** 1148-1158.