Spatial proteogenomics reveals distinct and evolutionarily conserved hepatic macrophage niches

Graphical abstract

Highlights
- Spatial proteogenomic single-cell atlas of healthy and obese murine and human liver
- Validated flow cytometry and microscopy panels for all hepatic cells
- LAMs are differentially located in the lean and obese liver
- Evolutionary conserved BMP9/10-ALK1 axis is essential for KC development

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In brief
By combining single-cell and -nucleus sequencing with spatial mapping of RNA and proteins, this vast spatial proteogenomic atlas of healthy and obese human and mouse livers presents methods to identify and localize all hepatic cells and provides insights into hepatic myeloid cells, including identification of reliable surface markers for isolation and localization of hepatic macrophages, characterization of lipid-associated macrophages in both healthy and steatotic livers, determination of a key regulatory axis of Kupffer cell development, and identification of a conserved core gene expression signature of Kupffer cells across 7 species, including chickens and zebrafish.

Guilliams et al., 2022, Cell 185, 379–396
January 20, 2022 © 2021 The Authors. Published by Elsevier Inc.
https://doi.org/10.1016/j.cell.2021.12.018
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Spatial proteogenomics reveals distinct and evolutionarily conserved hepatic macrophage niches

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.Summary

The liver is the largest solid organ in the body, yet it remains incompletely characterized. Here we present a spatial proteogenomic atlas of the healthy and obese human and murine liver combining single-cell CITE-seq, single-nuclei sequencing, spatial transcriptomics, and spatial proteomics. By integrating these multi-omic datasets, we provide validated strategies to reliably discriminate and localize all hepatic cells, including a population of lipid-associated macrophages (LAMs) at the bile ducts. We then align this atlas across seven species, revealing the conserved program of bona fide Kupffer cells and LAMs. We also uncover the respective spatially resolved cellular niches of these macrophages and the microenvironmental circuits driving their unique transcriptomic identities. We demonstrate that LAMs are induced by local lipid exposure, leading to their induction in steatotic regions of the murine and human liver, while Kupffer cell development crucially depends on their cross-talk with hepatic stellate cells via the evolutionarily conserved ALK1-BMP9/10 axis.

.Introduction

The immense advances in single-cell transcriptomics have enabled a better understanding of the cellular composition of different organs across species. However, we still lack information regarding how these cells are organized in their distinct microenvironmental niches. Moreover, the specific cell-cell interactions determining the identity of individual cells within...
tissues remain to be defined (Guilliams and Scott, 2017; Lindeboom et al., 2021). While the spatial organization of hepatocytes within the liver is understood (Halpern et al., 2017), that of nonparenchymal liver cells remains unclear. This is the case for the mouse liver, but even more so for the human liver, where the identity and the precise localization of most hepatic cells is unknown. Moreover, the link between the transcriptome and the proteome has not been studied, resulting in a lack of reliable surface markers to identify these cells by flow cytometry and confocal microscopy. Here, we used proteogenomic techniques including cellular indexing of transcriptomes and epitomes by sequencing (CITE-seq) and spatial approaches to identify all cells and their specific locations within the healthy and obese livers of mice and humans. By doing so, we have developed strategies for the identification and further study of hepatic cells. Demonstrating the usefulness of this approach, we identify the evolutionary conserved and spatially restricted signals driving the distinct hepatic macrophage phenotypes.

RESULTS

A practical proteogenomic atlas of the murine liver

To generate a proteogenomic atlas of the liver, we first examined the optimal method for retrieving all hepatic cells. Using the murine liver, we compared single-cell RNA sequencing (scRNA-seq) using cells isolated via ex vivo or in vivo enzymatic digestion with single-nuclei RNA sequencing (snRNA-seq) (Figures S1A–S1C). We did not observe any differences in the number of genes/cell between the two digestion methods (Figures S1D and S1E), but snRNA-seq typically yielded a lower number of genes/cell (Figures S1D and S1F). This did not prevent distinct cell types from being identified in the snRNA-seq dataset as both scRNA-seq and snRNA-seq identified highly expressed genes in each population. However, expression was often higher in the scRNA-seq (Figure S1F). Additionally, we observed a signature of digestion-associated genes and snRNA-seq-associated genes across cell types (Figure S1F). While in terms of genes/cell snRNA-seq is inferior to scRNA-seq, this method best recapitulated the cell frequencies observed in vivo (Figures S1D–S1N). As each method has advantages and disadvantages, the optimal method to use depends on the biological question being addressed. Here, as we sought to generate a proteogenomic atlas of all hepatic cells, we thus opted to use a combination of all protocols. To investigate mRNA and protein expression at single-cell resolution, we used CITE-seq (Stoeckius et al., 2017), staining a selection of the scRNA-seq samples with 107–161 oligo-conjugated antibodies (Figure 1A). Data were pooled together for a single analysis where, with TotalVI (Gayoso et al., 2021), both the protein and mRNA profiles were considered for clustering (Figure 1B). Analysis of the differentially expressed genes and proteins (DEGs/DEPs; Figures S2A and S2B; Table S1) identified 17 cell types (Figure 1B), which were differentially represented with each isolation method (Figure S2C). Addition of antibodies in the CITE-seq analysis enabled surface markers for all cells to be identified, including VSIG4 and FOLR2 for Kupffer cells (KCs) (Figure S2B), without affecting the quality of the transcriptomic data (Figure 2D). This analysis identified one subset of KCs. However, 2 subpopulations termed KC1 and KC2 have recently been described (Blieriot et al., 2021; Simone et al., 2021). As our CITE-seq analysis identified that the markers used to identify KC2s, namely CD206 and ESAM, are largely expressed by liver sinusoidal endothelial cells (LSECs) (Figure S2E), we next sought to determine if we had previously removed any potential KC2s in our initial QC steps as LSEC-KC doublets. To examine this, we generated a UMAP of samples containing CD206 and ESAM in the CITE-seq panel and performed a minimal QC filtering on number of genes and percentage of mitochondrial genes. In this UMAP, we found multiple subpopulations including 3 populations of KCs and 2 populations of B cells (Figure S2F). To determine if any of these populations could be KC2s, we harnessed the power of CITE-seq to recreate the gating strategy recently proposed to identify KC2s (Blieriot et al., 2021). Converting the CITE-seq data into an FCS file and analyzing this in FlowJo identified 2 of the KC populations to be KC2s expressing CD206 and ESAM, and these cells were present in a similar ratio among KCs as reported by Blieriot et al. (Figure S2G). However, employing the same gating strategy with the B cells also enabled a B cell2 population to be identified (Figure S2G). The KC2 and B cell2 populations also expressed other protein markers associated with LSECs including CD26, CD31, and CD38, suggesting that these may be doublets (Figure S2H). Consistent with this, we did not uncover any DEGs
specifically expressed in the KC2s or B cell2s, rather than these cells had an intermediate profile between either KC1s or B cell1s and LSECs (Figure S2J), as would be expected for doublets. Finally, we perfused the livers with antigen fix to inflate the LSECs to be able to distinguish more readily between KCs and LSECs and performed confocal microscopy. 3D reconstruction of these images indicates that CD206 expression is observed primarily in the LSECs with which the KCs are intertwined. These microscopy images also show that KCs express some CD206, however, consistent with our CITE-seq analysis, this was observed across the KC population rather than in a KC subset further suggesting the existence of only one KC population (Figure S2J).

With this in mind, we decided to continue to apply our initial strict QC controls eliminating these potential doublet sets from further analyses.

Distinct spatial orientation of hepatic myeloid cell subsets
To locate the cells identified we performed spatial transcriptomics analysis using Visium. For this, we cut the liver in two distinct orientations to profile both the liver tissue and the capsule (Figures 1C and S2K). We ordered each Visium spot along a spatial trajectory, and annotated portal, periportal, mid, and central zones based on known hepatocyte zonation markers (Halpern et al., 2017; Figures 1D, 1E, and S2L) and confirmed this annotation using confocal microscopy (Figure S2M). By using the reference sc/snRNA-seq data, we then deconvolved each spot into its constituent cell types and investigated how cell abundance changed with zonation (Figures 1F, 1G, and S2N). Validating this approach, cholangiocytes mapped specifically to the portal zones (Aizarani et al., 2019), while KCs were preferentially located in periportal and mid zones (Bonnardel et al., 2019; Gola et al., 2021). While KC location is zonated, we did not identify a strong zonation pattern in the gene expression profiles of KCs (data not shown). We further identified B cells, T cells, endothelial cells (ECs), and stromal cells (SCs) across all zones, while conventional dendritic cells (cDCs), were found at the portal vein (PV), with a minor presence at the central vein (CV) (Figures 1F, 1G, and S2N).

To validate these locations at single-cell resolution, we next sought to identify the best cell-specific surface markers that would also work by confocal microscopy. As the fixation step utilized for confocal microscopy often affects the integrity of protein epitopes, it is not possible to predict which antibodies will work spatially on fixed tissue slices. Therefore, to simultaneously screen multiple antibodies to identify those working by microscopy, we performed a second Visium analysis which we complemented with 100 oligo-conjugated antibodies, chosen based on the 1939 2013 2017 2019 2021

Figure 1. A proteogenomic atlas of the healthy murine liver

(A) Hepatic cells were isolated from healthy C57B/6 mice by ex vivo (5 mice, 15 samples) or in vivo (5 mice, 19 samples) enzymatic digestion. Alternatively, nuclei were isolated by tissue homogenization (4 mice, 12 samples). Live cells/intact nuclei were FACs-purified. For cells, total live, live CD45+, live CD45- , live hepatocytes, or myeloid cells (live CD45+, CD3- , CD19- , B220- , NK1.1- ) were sorted. 18 samples (7 ex vivo, 11 in vivo) were also stained with a panel of 107–161 barcode-labeled antibodies for CITE-seq analysis. All datasets were pooled together and after QC 185,894 cells/nuclei were clustered using TotalVI.

(B) UMAP of sc/snRNA-seq data.

(C) Tissue and capsule images from Visium analysis with clusters overlaid.

(D) UMAP of zonation of Visium spots (left) and origin of the cells (right).

(E) Zonation pattern mapped onto tissue slice.

(F) Indicated cell signatures from sc/snRNA-seq mapped onto the Visium zonation data.

(G) mRNA zonation pattern in Visium highly multiplexed protein analysis and VSIG4-ADT expression pattern (left) and zonated expression patterns of indicated antibodies (right).

(H) MICS analysis of indicated proteins.

(I) Molecular Cartography of indicated genes and cell types.

(K) mRNA (Xcr1, Fth1, Mafb, and Clc10a) and protein (MHCIi and F4/80) expression in the same tissue slice. Scale bars, 50 μm. PV, portal vein; CV, central vein. Arrows indicate specific cell types, colors correspond to cell type/markers. Images are representative of 2–4 mice. See also Figure S2 and Tables S1, S2, S3, and S5.
Taken together, by combining multiple spatial transcriptomic and proteomic approaches, we located all the cells within the murine liver and identified additional heterogeneity within the myeloid cells, not revealed when examining the sc/snRNA-seq dataset in isolation. This highlights the power of combining single-cell and spatial proteogenomic techniques to investigate cellular heterogeneity.

**Refined analysis of myeloid cells identifies three subsets of hepatic macrophages**

To better understand the non-KC macs, we zoomed in on myeloid cells (cDCs, KCs, monocytes, and monocye-derived cells) in our sc/snRNA-seq analysis defining 11 populations (Figures 2A–2C; Table S2). This included KCs, 3 populations of non-KC macs and cells that had a profile intermediate between monocytes and patrolling monocytes or macs, termed transitioning monocytes. Closer inspection of the non-KC macs identified cluster7 as peritoneal macs (Figure 2B). The DEGs between the remaining populations suggested that cluster7 likely resembles capsule macs (Sierra et al., 2017), expressing Cd207 and Cx3cr1 while cluster8 resembles Gpnmb+Spp1+ lipid-associated macrophages (LAMs) we recently described in the fatty liver (Remmerie et al., 2020; Figures 2A–2D). Conversion of the CITE-seq data into an FCS file allowed an in silico gating strategy to be defined (Figure S3A). Validating this, we utilized the strategy to FACS-purify the populations and assess gene expression (Figures S3B–S3D). Fitting with a recent report (Jin et al., 2021), washing the liver prior to digestion enriched the peritoneal macs in the wash fraction, demonstrating these were contaminants on the liver surface rather than being present in the liver tissue itself (Figure S3E). While the CITE-seq markers did not discriminate between cluster7 and cluster8, adding CD207 to the panel enabled the non-KCs to be divided into CD207+ and CD207− macs (Figure S3F). Fitting with their designation as capsule macs, the relative abundance of CD207+ macs was increased if we dissected and digested the capsule (Figure S3F). However, although Molecular Cartography confirmed the presence of Cd207+ macs in the capsule, it also revealed Cd207+ macs at the CV, which were rarely found at the PV (Figures 2E–2H and S3G–S3J). Thus, cluster7 consists of both capsule and CV CD207+ macs. This finding further demonstrates the need for spatial approaches to confirm cell identities. Molecular Cartography also identified macs at the PVs and CVs expressing Ccr2 and Chil3 (Figures 2G, 2H, S3H, and S3J), resembling transitioning monocytes (cluster11). Finally, a population of Gpnmb−expressing macs were found to be enriched around the bile ducts (Figures 2G, 2H, S3H, S3K, and S3L). As Gpnmb expression is cluster8 specific (Figure 2B), and these cells resemble LAMs (Remmerie et al., 2020), we termed these cells bile-duct LAMs.

**KC and LAMs are functionally distinct in the homeostatic murine liver**

We next sought to investigate the differences between KCs and LAMs. Analysis of GO terms associated with biological processes for these cells suggested that KCs may play a role in regulating humoral responses, while LAMs were more broadly associated with immune responses (Figures 2I and 2J). Consistent with this, in the 100-plex protein microscopy data we noted that a significant proportion of the B cells present were interacting with KCs, which was not observed with T cells (Figure 2K). This suggests cross-talk between these two populations, potentially linked to the high expression of the B cell chemokine Cxcl13 by murine KCs (Table S2). To assess the inflammatory nature of LAMs compared with KCs, we FACS-purified the cells and performed qPCR analysis to examine expression of various cytokines. To enable LAM purification, we eliminated the capsule prior to digesting the tissue. Fitting with the GO analysis, LAMs expressed more Il1b at steady state compared with KCs (Figure 2L). However, despite this, upon in vivo TLR4 stimulation, they were less responsive than KCs, both in terms of pro- and anti-inflammatory cytokines (Figure 2L), possibly indicative of LPS tolerance. This may result from their location at the PV and hence exposure to blood from the intestine, although this remains to be tested. Taken together this highlights the distinct nature and functions of these cells; however, further research is required to determine the precise roles of these cells.

**Macrophage subsets reside in distinct spatial niches**

As all the mac populations are in close contact with CD45− cells in their local environment (Bonnardel et al., 2019; Figure S3M), we further analyzed the CD45− cells, identifying multiple subsets of ECs and SCs and a gating strategy to distinguish them (Figures 3A–3C and S4A–S4C; Table S3). ECs could be further subdivided into 4 distinct clusters and analysis of their locations allowed them to be identified as CV ECs (cluster10), LSECs...
(cluster9), PV ECs (cluster11), and lymphatic ECs (LECs; cluster12) (Figures 3D, 3E, S4D, and S4E). As Visium found fibroblasts at both the PVs and CVs (Figure 3D), and as a previous report has suggested the presence of distinct subsets within these cells (Dobie et al., 2019), we further zoomed in on the SCs to better assess their heterogeneity (Figures 3F, 3G, S4A–S4C, and S4F; Table S4). This revealed subsets of mesothelial cells and fibroblasts restricted to the capsule (Figures 3H and S4C, and S4F; Table S4). This revealed subsets of mesothelial blasts at both the PVs and CVs (Figure 3D), and as a previous report has suggested the presence of distinct subsets within SCs to better assess their heterogeneity (Figures 3F, 3G, S4A–S4C, and S4F; Table S4). This revealed subsets of mesothelial cells and fibroblasts restricted to the capsule (Figures 3H and S4C, and S4F; Table S4). This revealed subsets of mesothelial blasts at both the PVs and CVs (Figure 3D), and as a previous report has suggested the presence of distinct subsets within SCs to better assess their heterogeneity (Figures 3F, 3G, S4A–S4C, and S4F; Table S4). This revealed subsets of mesothelial cells and fibroblasts restricted to the capsule (Figures 3H and S4C, and S4F; Table S4).

An in silico gating strategy for murine hepatic lymphoid cells

Finally, in addition to providing gating strategies for myeloid and CD45+ cells, we also wanted to investigate if the CITE-seq data would allow us to develop similar strategies for the lymphoid populations. To achieve this, we re-clustered the T cells, NK cells, ILCs, B cells, and pDCs from Figure 1B identifying 12 distinct populations (Figure S5A). Analysis of the DEGs highlighted that while B cells, NK cells, ILC1s, and pDCs were distinct populations, there was considerable overlap between the transcriptomic profiles of the T cells (Figure S5B; Table S5). However, by analyzing the DEPs we were able to define distinct subsets including naive CD4 and CD8 T cells, TRegps, T17s, CTls, and T17s (Figures S5A and S5C; Table S5). Moreover, we were able to design a gating strategy to isolate the distinct populations (Figure S5D).

A practical proteogenomic atlas of the healthy human liver

To determine the degree of conservation between the mac subsets and their different microenvironmental niches between the mouse and the human liver, we next generated a proteogenomic atlas of the human liver using sc/snRNA-seq and CITE-seq on 19 liver biopsies (Figures 4A, 4B, S6A, and S6B; Tables S1 and S5). Of these, most were histologically healthy with only 5 patients showing >10% hepatic steatosis in the absence of any significant fibrosis (Table S6). Cellular proportions varied according to the isolation technique used, and while there was some variability between patients, this was not linked to the surgery (Figures S6C–S6E). Further confirming the lack of fibrosis in the steatotic livers, we did not detect any increase in CTLs (Figures S5E–S5I; Table S5), which have been shown to correlate with non-alcoholic steatohepatitis (NASI) (Haas et al., 2019). A significant increase in CTLs was detected in the setting of murine NASH induced by feeding a western diet (WD) for up to 36 weeks, demonstrating that this is not due a limitation in detecting these differences using CITE-seq (Figures S5J–S5M; Table S7). As Visium reliably located murine hepatic cells, we used this to locate the cells of the human liver in 4 biopsies (Figure 4C). However, the Visium spots from patients with >10% steatosis were found to cluster separately from the healthy samples (<10% steatosis; Figures 4D and 4E). We therefore used the healthy samples to calculate a baseline zonation and then transferred this trajectory onto the steatotic samples (Figures 4F and S6F). This identified the steatosis to be predominantly present in regions expressing peri-central zonation genes like CYP2E1. This zonation pattern was further validated using Molecular Cartography (Figure S6G). This fits with previous clinical studies demonstrating peri-central steatosis to be most common in non-alcoholic fatty liver disease (NAFLD) patients, especially in early disease (Chalasani et al., 2008; Kleiner and Makkoluf, 2016). However, as these peri-central regions are larger than in the healthy controls, it could also imply that the presence of steatosis alters expression of the zonated hepatocyte genes, but this remains to be tested. Notably, the overall cellular distribution was not impacted by the presence of steatosis, although neutrophils and monocytes and monocyte-derived cells were preferentially localized peri-centrally in the steatotic patients correlating with the presence of steatosis (Figure 4G). 

Evolutionarily conserved transcriptomic and proteomic identity of KCs

To date, no validated markers of bona fide human KCs have been described. Explaining the difficulty to accurately define human KCs, we found monocytes and macs formed a single continuum in the human sc/snRNA-seq data, preventing a simple definition of human KCs (Figure 4B). Notably, a similar continuum from monocyte to KCs was also observed in the NASH murine liver (Figures S5J and S6J–S6N, Table S8). Consistent with our previous report (Remmerie et al., 2020), we observed both long-term resident Timd4-expressing KCs and recently recruited Timd4−
monocyte-derived KCs (moKCs; Figures S6J–S6N). As the presence of such a continuum in the human liver suggests that there may also be monocyte contribution to the KC pool in the healthy human liver, we next zoomed in on myeloid cells to examine this, identifying 10 clusters (Figures 4H, S6G, and S6P; Table S2). To define the KCs, we examined expression of the top 25 murine KC genes by these clusters, which identified cluster 10 to be the genuine human KCs (Figure S6Q). Unlike in mice, these were preferentially located in the mid zone (Figure S6R). Cluster 9 also expressed many of these genes but lacked TIMD4 (Figure S6Q), suggesting that these cells may be recently recruited moKCs. The presence of moKCs in the liver is consistent with reports that host-derived macs are identified in transplanted liver donors (Bittmann et al., 2003; Pallett et al., 2020) and suggests that the KC population may be a mix of embryonic and monocyte-derived cells. Although not the case at mRNA level, VSIG4 was found to be the best human KC protein marker in the CITE-seq data, while FOLR2, CD163, and CD169 were also identified as useful markers of these cells for flow cytometry and confocal microscopy on frozen and paraffin sections (Figures 4I and S6S–S6X). Co-staining human livers for VSIG4 protein and KC-specific CD5L mRNA and MICS 100-plex protein analysis also confirmed the mid-zonal localization of KCs (Figures 4J and S6U). To assess if KC identity was further conserved in evolution, we profiled macaque, pig, hamster, chicken, and zebrafish (Figure S6A). KCs were identified using the human-human antibodies or scRNA-seq (hamster, chicken, and zebrafish), or nuclei were isolated for snRNA-seq (macaque). Total live cells (hamster, chicken, and pig), likely due to the conserved expression of core KC transcription factors (Figure S7J), were then examined. As we next zoomed in on myeloid cells to examine this, to identify most of the other hepatic cells across species on the basis of conserved genes (Figures S7N and S7O). cDC2s were the main exception to this, as specific cDC2 marker genes were not conserved across all species (Figure S7O).

LAM location is altered in the steatotic liver

Alongside KCs, we also identified distinct clusters of macs in the human myeloid cells (Figure 4H). To better understand the nature of these clusters we performed confocal microscopy to examine the specific locations of CD68-VSIG4+ mics in the liver capsule, in close proximity to central and PVs as well as at bile ducts (BDs) (Figures S5A–S5C and S6A). Similar populations were also observed at the PVs and Cvs and at the BDs in the healthy macaque liver (Figure S7M). Examination of the scRNA-seq data and comparison with murine signatures identified immature and mature LAMs, with immature LAMs expressing some monocyte genes (Figures 4H, 5D, S8B, and S8C). Although recently suggested to be specific to fibrotic human livers (Ramachandran et al., 2019), we identified LAMs in all patients profiled with scRNA-seq, but there was a trend toward increased proportions of LAMs in the livers with >10% steatosis (Figure S8D) consistent with the increased population of LAMs in murine NAFLD (Figures S6J–S6N). As in the healthy mouse, Visium identified human LAMs in portal zones of non-steatotic livers. However, in steatotic human livers, LAMs were primarily located peri-centrally, in zones with steatosis (Figure S5E), suggesting that monocytes are recruited to distinct locations in the healthy and obese liver where they then differentiate into LAMs. This altered location of LAMs was further validated by confocal microscopy and Molecular Cartography (Figures 5F–5H). However, this analysis did not identify any capsule macs, suggesting that these cells may be absent from our UMAP, likely as a result of the small amount of capsule tissue on a biopsy. The Mac1 population expressing IGSF21 was present in very low numbers throughout the tissue (Figures 5G and S5H). This coupled with their similar transcriptomic profile to moKCs could suggest that these are moKC precursors as observed in the mouse, but this requires further study. Focusing on the LAMs, the change in their location in the steatotic human liver was also observed in the murine NAFLD model. Here, LAMs were found across portal, periportal, and mid zones (Figures 5I, S8E, and S8F), fitting with the presence of steatosis in these regions and consistent with our previous report (Remmerie et al., 2020). Comparison of DEGs between LAMs in

Figure 4. Identification of bona fide Kupffer cells across species

(A) Cells/nuclei were isolated from liver biopsies (~1–2 mm3; 14 cells, 5 nuclei) from patients undergoing either liver resection, cholecystectomy or gastric bypass. Live cells/intact nuclei were FACS-purified. Either total live, live CD45+, and live CD45−/CD3−/CD19− cells were sorted. 7 cell samples were stained with a panel of 198 barcode-labeled antibodies for CITE-seq analysis. All datasets were pooled together and after QC, 167,598 cells/nuclei were analyzed using TotalVI.

(B) UMAP of sc/snRNA-seq data.

(C) UMAP of Visium data from 4 patient biopsy samples.

(D) Split of Visium spots based on % steatosis.

(E) Healthy and steatotic Visium liver tissue with clusters overlaid and H+E staining to identify steatotic zones.

(F) Zonation of Visium data (top) with zonation pattern mapped onto liver tissue (bottom).

(G) Indicated cell signatures from sc/snRNA-seq mapped onto Visium zonation trajectory, healthy (top), steatotic (bottom).

(H) Myeloid cells (40,821 cells) were isolated from Figure 4B and re-clustered with TotalVI.

(I) Expression of VSIG4 protein (top) and CD5L mRNA (bottom).

(J) Expression of VSIG4, F4/80, FOLRB, and GLUL combined with CD5L/CD5L on murine (left) and human (H25; right) livers. Scale bars, 50 μm. Inset in bottom panels. Scale bars, 20 μm. Images are representative of 2–4 livers.

(K) Livers (2/species) were isolated from healthy macaque, pig, chicken, hamster, and zebrafish. Cells were isolated by ex vivo digestion for CITE-seq (pig: 198 human antibodies) or scRNA-seq (hamster, chicken, and zebrafish), or nuclei were isolated for snRNA-seq (macaque). Total live cells (hamster, chicken, and pig), DsRed “GFP” cells (zebrafish) or nuclei (macaque) were FACS-purified. Following QC, 8,483 nuclei (macaque) or 21,907 (pig), 5,985 (hamster), 7,457 (chicken), and 4,957 (zebrafish) cells were analyzed using TotalVI (pig) or scVI (macaque, hamster, chicken, and zebrafish) (top). KCs were identified using the human-murine KC signature and the signature finder algorithm (Pont et al., 2018) (bottom). See also Figures S6 and S7 and Tables S1, S2, S3, S5, S6, S8, and S9.
standard diet (SD) and WD-fed mice identified that LAMs had a more mature phenotype in WD-fed mice, downregulating their expression of some monocyte genes and increasing their expression of prototypical macrophage markers, consistent with the presence of both immature and mature LAMs in the human liver (Figure 5J). Fitting with a more mature phenotype, WD-derived LAMs also expressed lower levels of Il1b, Tnf, and Iil0 compared with SD LAMs (Figure 5K). While further studies are required to assess the precise functions of these cells in NAFLD, this could further suggest a protective rather than a pathogenic role for LAMs (Daemen et al., 2021).

Potential role for CD45+ hepatic cells in determining macrophage localization

Given the altered localization of KCs in the healthy murine versus human liver and LAMs in the healthy versus steatotic liver of mice and humans, we next sought to investigate how the cells of the mac niches differed in these settings. Analysis of CD45+ cells in the human liver identified similar (sub)populations of ECs, SCs, and hepatocytes as observed in the healthy mouse liver (Figures 6A and 6B; Table S3). However, while the 100-plex MICS analysis detected a second subset of portal fibroblasts expressing DESMIN (Figure S6H), we did not detect these cells in our UMAP, likely due to difficulties detecting DESMIN with snRNA-seq. No significant differences were found in terms of localization of the identified CD45+ cells that could explain the altered location of KCs compared with the mouse (Figures 6C and 6D). With this in mind, we next utilized NicheNet to examine potential ligand-receptor pairs between KCs and CD45+ cells present only in the human that could regulate KC location. This analysis identified CCL23 and CCL14 expression by LSECs and CCL16 by hepatocytes that binds to CCR1 expressed by human KCs but not murine KCs (Figure 6E). Crucially, these ligands were preferentially located peri-centrally (Figure 6F) and thus represent interesting targets for further study regarding the potential regulation of KC location in the human liver.

We next aimed to investigate the role of CD45+ cells in the regulation of LAM location in the healthy versus steatotic liver. Given the low number of steatotic human samples in our sc/snRNA-seq analysis and the variation between patients, we turned to the murine NASH model to investigate this. Zooming in on SCs (Figure 6G; Table S10), we identified that there was an increase in fibroblasts in the WD-fed mice. There was also a considerable overlap between fibroblasts and HSCs in terms of their gene expression profiles suggesting there may be a differentiation trajectory between the HSCs and the fibroblasts, although this remains to be validated in vivo. We also noted a sub-population of fibroblasts expressing Ccl2, a known ligand for CCR2 which is expressed on monocytes recruited to the liver during NAFLD (Remmerie et al., 2020), as well as Cd44 and Vcam1, two genes involved in monocyte recruitment and adhesion (Johnson and Ruffell, 2009; Meerschaert and Furie, 1995). Both fibroblast populations were enriched in the periportal steatotic regions (Figure 6I), in which we also observe enrichment of the LAMs (Figure 5I). We also found high CCL2 and Cd44 expression in human fibroblasts (Table S3), potentially recruiting bileduct LAMs. Together, this suggests that fibroblasts may play an important role in recruiting LAMs.

Differential NicheNet analysis across species reveals a crucial role for the ALK1-BMP9/10 axis in KC development

Having identified a potential role for the mac niche cells in regulating mac subset location, we next sought to determine the involvement of these cells in regulating mac phenotypes. To assess the roles of conserved cell-cell interactions in driving mac heterogeneity across species, we performed a differential NicheNet (Browaeys et al., 2019) analysis between the distinct hepatic macs and the CD45+ cells present in their respective niches focusing on ligands and receptors conserved in both human and mouse. This revealed very few specific ligand-receptor pairs for LAMs (Figure S8G; data not shown), hinting that local factors such as metabolites rather than unique cell-cell interactions may drive the LAM phenotype. Indeed, this would be consistent with the presence of LAM-like cells in multiple tissues including the obese adipose tissue, the brain, the lung, and the heart (Jaitin et al., 2019; Keren-Shaul et al., 2017; Liao et al., 2020; Rizzo et al., 2020). In line with this, BM monocytes cultured with acetylated low-density lipoprotein expressed LAM-associated genes (Figure 7A), demonstrating a dominant role for lipids in inducing the LAM phenotype. Conversely, for KCs, we found multiple ligand-receptor pairs to be conserved between human and mouse (Figures 7B and S8G). One of these, an activin receptor-like kinase (ALK1)-bone morphogenetic protein (BMP)9/10 circuit between KCs (ALK1; encoded by Acrv1) and stellate cells (BMP9/10 encoded by Gdf2/Bmp10 respectively) was found to be conserved.
in all 7 species and was predicted to control the expression of a number of the conserved KC genes (Figures 7B, 7C, and S8H). To validate a role for this axis in KCs, we generated Fcgr1-Cre x Acvrl1fl/fl mice, eliminating ALK1 specifically from CD64-expressing macrophages (Scott et al., 2018). This led to an almost complete loss of VSIG4+ KCs (Figures 8D–8F), demonstrating that evolutionarily conserved ALK1 signaling is crucial for KCs. To determine if ALK1 is required for KC maintenance we generated Clec4f-Dtr x Acvrl1fl/fl mice, eliminating ALK1 only from differentiated KCs (Scott et al., 2018). This revealed a relatively similar phenotype than observed in the Fcgr1-Cre mice, suggesting that ALK1 is also required for KC maintenance (Figure S8I). To directly test the need for ALK1 in KC development, we generated BM chimeras whereby CD45.1 Clec4f-Dtr mice were irradiated with their livers shielded to avoid any radio damage. These mice were then reconstituted with either CD45.2 Fcgr1-Cre x Acvrl1fl/fl or Fcgr1-Cre x Acvrl1fl/fl BM. 4 weeks later, mice were given a single i.p. injection of DT to deplete the KCs and 7 or 13 days thereafter chimerism within the KC population was determined (Figure 7G). We observed that, already by day 7, KO BM-derived cells were almost completely outcompeted by WT BM-derived cells within the KC pool. This was not observed in monocytes but a similar pattern was observed in VSIG4+ macrophages demonstrating that ALK1 is critically required for early KC development (Figure 7H). Finally, while NicheNet predicts that BMP9/10 from stellate cells would signal through ALK1 to induce KC development and maintenance, a prediction in line with our previous NicheNet analysis (Bonnardel et al., 2019), another recent study has suggested that transforming growth factor (TGF)β signaling would be important for KCs (Sakai et al., 2019). This prediction was made on the basis of SMAD4 signaling, which is a common downstream effector of both TGF-β Receptor and ALK1-induced signaling. As TGF-β was also found to participate in an ALK1-TGF-β receptor containing signaling complex (Goumans et al., 2003), we thus examined whether TGF-β signaling is important for KCs. To this end we utilized an ALK1-Fc trap or TGF-β type II receptor (TGFβRII)-Fc trap alongside appropriate isotype controls. ALK1-Fc selectively sequesters BMP9/10 (Desroches-Castan et al., 2021), while TGFβRII-Fc selectively interferes with TGF-β1/TGF-β3 receptor binding (Komesli et al., 2017). Clec4f-Dtr mice were depleted of KCs and simultaneously treated with either receptor-Fc traps or isotype controls and KC development was examined 7 days later (Figure 7I). In line with the chimeric study, treatment with ALK1-Fc significantly abrogated KC development; however, blocking TGFβ signaling had only a minor effect on the proportion of VSIG4+ macrophages (Figure 7J). Altogether this validates the NicheNet prediction and demonstrates that the evolutionarily conserved ALK1-BMP9/10 axis is crucial for the development and maintenance of KCs.

DISCUSSION

To generate a practical cellular atlas of any human tissue and unravel the cell-cell circuits essential for the identities of cells inhabiting that tissue, four key pieces of information are required: (1) an inventory of all cells present, (2) the location of the different cells within the tissue to identify interactions between neighboring cells, (3) an alignment between the human and animal models allowing for any predicted cell-cell interactions to be perturbed, and (4) the identification of reliable antibody-based panels for the efficient screening of different patients and/or transgenic animals. Here, by integrating single-cell and spatial transcriptomic and proteomic data, we provide these 4 pieces of information for the liver and uncover evolutionarily conserved microenvironmental circuits controlling the development of hepatic macrophages.

Unraveling the spatial localization of all hepatic cells, we identify LAMs around the bile ducts in the healthy mouse, human, and macaque liver. However, when steatosis is present, LAMs are preferentially recruited to the steatotic regions of the liver. This spatial information at least partially invalidates the hypothesis that LAM identity is specifically induced by fibrotic SCs (Ramachandran et al., 2019). Rather, our data suggest that LAMs are induced by local lipid exposure. We also provide an alignment of the liver atlas across seven species. This reveals the conserved and unique transcriptomic programs of steady-state KCs and uncovers the spatially restricted and conserved ligand-receptor pairs between KCs and the cells constituting their niche. Underlining the need to first characterize the healthy tissue before attempting to understand how disease perturbs the cells, we identify the DLL-NOTCH interaction to be an evolutionarily conserved cross-talk between homeostatic LSECs and KCs and therefore not unique to hepatocellular carcinoma or fibrosis, as proposed (Ramachandran et al., 2019; Sharma et al., 2020). Similarly, we find that FOLR2 expression is not specific to tumor-associated hepatic macrophages (Sharma et al., 2020) but is expressed by KCs in the healthy mouse and human liver. Finally, we apply a proteogenomic pipeline starting from broad oligo-conjugated antibody panels for both single-cell and spatial profiling. This is crucial as transcriptomic profiling does not always correspond with the ability to detect proteins by flow cytometry or microscopy. By...
screening broadly, we identify the best surface markers for the isolation and localization of hepatic macs and their respective niche cells. This allows both the validation of the spatial location at the single-cell level, and the efficient screening of transgenic mouse models for the loss of KCs. Characterization of Fcgr1-CrexAcvrl1fl/fl mice using our defined panel readily demonstrates the cruciality of the ALK1-BMP9/10 axis in KC development emphasizing that mac-stromal-cell cross-talk goes much further than the exchange of growth factors (Guilliams et al., 2020; Zhou et al., 2018). Moving forward, applying these relatively cheap antibody panels to large patient cohorts or multiple transgenic mouse models should enable any perturbations disturbing liver homeostasis to be efficiently identified.

Limitations of the study
The current study has two main limitations. First, the analysis of the human liver remains restricted by the number of human patients included (19 patients for the sc/snRNA-seq, 4 patients for Visium, and 15 patients by microscopy). While this study provides markers to cheaply and efficiently screen large patient cohorts allowing this analysis to be extended, given the heterogeneity between patients, multiple studies will need to be integrated in a single analysis if we are to be able to interrogate transcriptomic differences in a particular cell subset related to age, sex, ethnicity, or pathological parameters. Second, this study highlights that more research will be needed to fully characterize human SCs. As we could only retrieve these cells through snRNA-seq this means we have not been able to identify good surface markers through CITE-seq. Additionally, for small populations of fibroblasts we did not recover enough nuclei to truly probe their heterogeneity. Better isolation protocols are required to retrieve and enrich these cells from the human liver in order to run a broad panel of CITE-seq markers to identify the different subsets and their corresponding surface markers.

STAR★METHODS
Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY

Figure 7. ALK1-BMP9/10 axis regulates KC development
(A) Mouse BM monocytes were cultured in the presence of CSF1 and indicated concentrations of human ac-LDL, prior to analyzed for expression of indicated genes by qPCR. Data are pooled from 2 experiments. One-way ANOVA with Bonferroni post-test compared with 0 ng/mL.
(B) NicheNet circos plot highlighting conserved ligand-receptor pairs and induced target genes between KCs and indicated niche cells in human and mouse.
(C) Feature plots showing expression of ALK1 (Acvrl1) in human myeloid cells (left) and GDF2/BMP10 in CD45- cells (right).
(D) Livers were harvested from Fcgr1-CrexAcvrl1fl/fl mice or Acvrl1fl/fl or Acvrl1+/+ controls and KCs examined (left) and quantified (right) using VSIG4 expression.
(E) Expression of indicated KC markers by mac populations in Fcgr1-CrexAcvrl1fl/fl or Acvrl1+/+ control mice. Data are pooled from 3 independent experiments with n = 9 per group. Student’s t test.
(F) Expression of indicated markers in livers of Fcgr1-CrexAcvrl1fl/fl or Acvrl1+/+ or Acvrl1+/+ control mice by confocal microscopy. Scale bars, 50 μm. Images are representative of 2 mice per group.
(G) Schematic of chimera experiment setup.
(H) % chimera normalized to levels in blood Ly6Chi monocytes in Clec4f-Dtr mice 7 or 13 days after DT administration following partial irradiation and receiving either Acvrl1fl/fl or Fcgr1-CrexAcvrl1fl/fl BM 4 weeks earlier. Data are pooled from 2 independent experiments with n = 10–12 mice per group.
(I) Schematic of Fc trap experiment setup.
(J) Representative FACS plots showing VSIG4 and CLEC2 expression by total macs. Numbers represent % of total mac population in the indicated gate (left) and % of VSIG4+ and CLEC2+ macs among total CD45+ cells in the different treatment conditions. One-way ANOVA with Bonferroni post-test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. See also Figure S8.
single-cell accelerator program for their help benchmarking technologies; and 10X Genomics for their help setting up the Visium highly multiplexed protein analysis. Finally, we thank the VIB-Ugent animal house staff. BioRender was used to generate some figures.

Funding: Chan Zuckerberg initiative; liver seed atlas grant (M.G. and C.L.S.), FWO SBO; iPSC LiMics (C.L.S., M.G., and Y.S.), ERC consolidator grant; KupferCellNiche, 725924 (M.G.), GOA; BOF18-GOA-024 (M.G. and Y.S.), ERC starting grant; MyFeTvatty, 851908 (C.L.S.), FWO project grant; 3G000519 (C.L.S. and M.G.), FWO PhD fellowship; 1125021N (B.H.), 1181318N (R.B.), 11L2122N (F.F.D.P.), MSCA IF fellowships; MACtivate 101027317 (C.Z.), LiverMacRegenCircuit 844301 (F.F.D.P.).

AUTHOR CONTRIBUTIONS
Conceptualization & data curation: C.L.S. and M.G.; formal analysis: C.L.S., M.G., T.T., L.M., B.H., F.R.S., F.F.D.P., A.R., J.B., W.S., and R.B.; funding acquisition: C.L.S. and M.G.; investigation: C.L.S., M.G., B.H., J.B., V.V.D.B., A.B., F.F.D.P., A.R., C.Z., B.V., T.V., L.M., T.T., R.B., A.H., A.V., F.B., Y.V., E.C., G.F., V.W., A.W., S.K., J.N., K.D., P.G., and S.C.; methodology: C.L.S., M.G., B.H., J.B., L.M., T.T., F.F.D.P., R.B., W.S., A.B., A.G., and S.L.; visualization: C.L.S., M.G., J.B., B.H., L.M., T.T., R.B., and W.S.; resources: C.L.S., M.G., W.S., B.D., and Y.S.; supervision: C.L.S., M.G., H.V.V., L.D., B.D., W.S., and Y.S.; writing: C.L.S. and M.G.

DECLARATION OF INTERESTS
The authors declare no competing interests.

Received: July 2, 2021
Revised: November 12, 2021
Accepted: December 13, 2021
Published: January 11, 2022

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# STAR Methods

## Key Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies: Flow cytometry/confocal microscopy** |        |            |
| Rat Monoclonal CD102-FITC (3C4) | Biolegend | 105606; RRID: AB_313199 |
| Rat Monoclonal CD103-BV750 (M290) | BD Biosciences | 747478; RRID: AB_2872154 |
| Rat Monoclonal CD11b-BUV395 (M1/70) | BD Biosciences | 565976; RRID: AB_2721166 |
| Rat Monoclonal CD11b-BV605 (M1/70) | BD Biosciences | 563015; RRID: AB_2737951 |
| Rat Monoclonal MHCI-BUV805 (M5/114,15,2) | BD Biosciences | 748844; RRID: AB_2873247 |
| Rat Monoclonal MHCI-FITC (M5/114,15,2) | Thermo Fisher Scientific | 11-5321-85; RRID: AB_465233 |
| Rat Monoclonal CD14-PE Dazzle 594 (Sa14-2) | Biolegend | 123325; RRID: AB_2721697 |
| Rat Monoclonal CD43-BUV737 (S7) | BD Biosciences | 612840; RRID: AB_2870162 |
| Rat Monoclonal Ly6C-eFluor450 (HK1.4) | eBioscience | 48-5932-82; RRID: AB_10805519 |
| Armenian Hamster Monoclonal CD11c-PE-Cy7 (N418) | eBioscience | 25-0114-82; RRID: AB_49590 |
| Armenian Hamster Monoclonal CD11c-Unconjugated (N418) | Biolegend | 117302; RRID: AB_313771 |
| Rat Monoclonal CD86-BV605 (GL-1) | Biolegend | 105037; RRID: AB_11204429 |
| Rat Monoclonal CD54-PE (YN1/1,7,4) | Biolegend | 116107; RRID: AB_313698 |
| Rat Monoclonal CD54-AF488 (YN1/1,7,4) | Biolegend | 116111; RRID: AB_493494 |
| Rat Monoclonal CD172a-BB630P (P84) | BD Customs | 624294 |
| Mouse Monoclonal XCR1 - BV650 (ZET) | BioLegend | 148220; RRID: AB_2566410 |
| Rat Monoclonal Tim4-PerCP-Cy5.5 (RMT4-54) | eBioscience | 46-5866-82; RRID: AB_2573781 |
| Rat Monoclonal VSG4-PECy7 (NL1A4) | eBioscience | 25-5752-82; RRID: AB_2637431 |
| Rat Monoclonal CD19-PECy5 (1D3) | eBioscience | 15-0193-82; RRID: AB_657672 |
| Rat Monoclonal CD19-Unconjugated (1D3) | BD Biosciences | 553783; RRID: AB_395047 |
| Rat Monoclonal CD26-FITC (H194-112) | BD Biosciences | 559652; RRID: AB_398295 |
| Rat Monoclonal CD38-AF700 (90) | eBioscience | 56-0381-82; RRID: AB_657740 |
| Armenian Hamster Monoclonal CD3e-PECy5 (145-2C11) | TONBO Biosciences | 55-0031; RRID: AB_2621815 |
| Rat Monoclonal CD3-Unconjugated (17A2) | Bioceros | N/A |
| Rat Monoclonal CD45-BV510 (30-F11) | BioLegend | 103138; RRID: AB_2563061 |
| Mouse Monoclonal CD45.1-PE (A20) | BD Biosciences | 553776; RRID: AB_395044 |
| Mouse Monoclonal CD45.1-Unconjugated (A20) | BioLegend | 110702; RRID: AB_313491 |
| Mouse Monoclonal CD45.2-AF700 (104) | eBioscience | 56-0454-82; RRID: AB_657752 |
| Rat Monoclonal CD45R-PE-Cy5 (RA3-6B2) | BD Biosciences | 553091; RRID: AB_394621 |
| Rat Monoclonal Ly6G-BUV563 (1A8) | BD Biosciences | 612921; RRID: AB_2870206 |
| Mouse Monoclonal CD64-BV711 (X54-5/7,1) | BioLegend | 139311; RRID: AB_2563846 |
| Mouse Monoclonal NK1.1-PECy5 (PK136) | BioLegend | 108716; RRID: AB_493390 |
| Rat Monoclonal Ter119-PECy5 (TER-119) | eBioscience | 15-5921-82; RRID: AB_488810 |
| Rat Monoclonal F4/80-BV785 (BM8) | BioLegend | 123141; RRID: AB_2563667 |
| Rat Monoclonal F4/80- AF594 (BM8) | BioLegend | 123140; RRID: AB_2563241 |
| Rat Monoclonal CLEC2-PE (17D9) | BioLegend | 146104; RRID: AB_2562382 |
| Rat Monoclonal FOLRB-PE (10/FR2) | BioLegend | 153304; RRID: AB_2721344 |
| Rat Monoclonal FOLRB-Unconjugated (10/FR2) | BioLegend | 153302; RRID: AB_2687271 |
| Rat Monoclonal SiglecF-BUV395 (E50-2440) | BD Biosciences | 740280; RRID: AB_2740019 |
| Rat Monoclonal CD207-AF647 | Imgenex | DDX0362A647; RRID: AB_1148741 |
| Rat Monoclonal CD206-AF647 | BioLegend | 141712; RRID: AB_10900420 |
| Rat Monoclonal CD326 (EPCAM)-APC (G8.8) | eBioscience | 17-5791-82; RRID: AB_2716944 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Goat Polyclonal Clec4F-Unconjugated | R & D Systems | AF2784; RRID: AB_2081339 |
| Rat Monoclonal Clec4F-Unconjugated (370901) | R & D Systems | MAB2784; RRID: AB_2081338 |
| Goat Polyclonal VSIG4-Unconjugated | R & D Systems | AF4646; RRID: AB_2257239 |
| Goat Polyclonal Lyve1-Unconjugated | R&D Systems | AF2125; RRID: AB_2297188 |
| Donkey Anti-Goat IgG-AF488 | Thermo Fisher Scientific | A-11055; RRID: AB_2534102 |
| Donkey Anti-Goat IgG-AF633 | Thermo Fisher Scientific | A-21082; RRID: AB_2535739 |
| Donkey Anti-Goat IgG-AF647 | Thermo Fisher Scientific | A-21447; RRID: AB_2535864 |
| Donkey Anti-Rat IgG-Cy3 | Jackson ImmunoResearch | 712-166-153; RRID: AB_2340669 |
| Donkey Anti-Mouse IgG–AF555 | Thermo Fisher Scientific | A-31570; RRID: AB_2536180 |
| Donkey Anti-Rabbit IgG-AF647 | Thermo Fisher Scientific | A-31573; RRID: AB_2536183 |
| Donkey Anti-Rabbit IgG-AF680 | Thermo Fisher Scientific | A-32802; RRID: AB_2762836 |
| Goat Anti-Rabbit IgG–AF514 | Thermo Fisher Scientific | A-31558; RRID: AB_2536173 |
| Goat Anti-Hamster IgG-Cy3 | Jackson ImmunoResearch | 127-585-160; RRID: AB_2338999 |
| Goat Anti-Rat IgG-AF568 | Thermo Fisher Scientific | A-11077; RRID: AB_2534121 |
| Goat Anti-Chicken IgY-AF680 | Abcam | Ab175779; |
| Rat Monoclonal CD31-Unconjugated (MEC13.3) | BD Biosciences | 550274; RRID: AB_393571 |
| Rabbit Polyclonal Desmin-Unconjugated | Abcam | ab15200; RRID: AB_301744 |
| Rabbit Polyclonal Glutamine Synthetase - Unconjugated | Abcam | ab73939; RRID: AB_2247389 |
| Goat Anti-Rabbit IgG-AF514 | Thermo Fisher Scientific | A-31558; RRID: AB_2536173 |
| Rabbit Monoclonal GPNMB- unconjugated (EPR18226-147) | Abcam | ab188222; |
| Polyclonal Chicken anti-GFP | Aves Labs | GFP-1010; RRID: AB_2307313 |
| Mouse Monoclonal FOLR2-PE (94b/FOLR2) | Biolegend | 391704; RRID: AB_2721336 |
| Mouse Monoclonal CD45-APCCy7 (2D1) | Biolegend | 368516; RRID: AB_2566376 |
| Mouse Monoclonal CD14-PETexasRed (TuK4) | Thermo Fisher Scientific | MHCD1417; RRID: AB_10373552 |
| Rabbit Polyclonal Glutamine Synthetase - Unconjugated | Abcam | ab73939; RRID: AB_2247389 |
| Goat Anti-Rabbit IgG-AF514 | Thermo Fisher Scientific | A-31558; RRID: AB_2536173 |
| Rabbit Monoclonal CD34-PE (EP373Y) | Abcam | Ab223930; |
| Anti- Mouse CD38 (REA616) FITC | Miltenyi Biotec | 130-122-955; RRID: AB_2811415 |
| Anti-Mouse CD26 (REA1196) PE | Miltenyi Biotec | 130-122-775; RRID: AB_2801934 |
| Anti-Mouse F4/80 (REA126) FITC | Miltenyi Biotec | 130-117-509; RRID: AB_2727970 |
| Anti-Mouse CD5 (53-7.3) FITC | Miltenyi Biotec | 130-102-574; RRID: AB_2658608 |
| Anti-Mouse CD79B (REA1117) PE | Miltenyi Biotec | 130-119-425; RRID: AB_2751702 |
| Anti-Mouse CD45R/B220 (RA3-6B2) FITC | Miltenyi Biotec | 130-118-323; RRID: AB_2751482 |
| Anti- Mouse CD43 (L11) PE | Miltenyi Biotec | 130-102-594; RRID: AB_2661309 |
| Anti-Mouse CCR2 (REA538) PE | Miltenyi Biotec | 130-117-548; RRID: AB_2727981 |
| Anti-Mouse MHCII (M5/114.15.2) FITC | Miltenyi Biotec | 130-123-666; RRID: AB_2802055 |
| Anti-Mouse CD73 (REA778) PE | Miltenyi Biotec | 130-111-331; RRID: AB_2659153 |
| Anti-Mouse CD146 (REA1064) FITC | Miltenyi Biotec | 130-118-252; RRID: AB_2751472 |
| Anti-Mouse CD90.2 (30-H12) PE | Miltenyi Biotec | 130-120-091; RRID: AB_2751997 |
| Anti-Mouse CD138 ( REA104) PE | Miltenyi Biotec | 130-120-810; RRID: AB_2752204 |
| Anti-Mouse CD29 (REA1074) PE | Miltenyi Biotec | 130-119-165; RRID: AB_2751649 |
| Anti-Mouse CD105 (REA1058) FITC | Miltenyi Biotec | 130-118-173; RRID: AB_2733613 |
| Anti-Human CD36 (REA760) PE | Miltenyi Biotec | 130-110-877; RRID: AB_2657728 |
| Anti-Human CD206 (REAL518) APC | Miltenyi Biotec | 130-122-168; RRID: AB_2857557 |
| Anti-Human Collagen IV (REAL567) PE | Miltenyi Biotec | 130-122-866; RRID: AB_2857566 |

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**REAGENT or RESOURCE** | **SOURCE** | **IDENTIFIER**
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Anti-Human CD105 (REA794) PE | Miltenyi Biotec | 130-112-163; RRID: AB_2654424
Anti-Human CD146 (REA773) APC | Miltenyi Biotec | 130-111-323; RRID: AB_2655179
Anti-Human CD90 (REA897) PE | Miltenyi Biotec | 130-114-860; RRID: AB_2726811
Anti-Human DESMIN (REA1134) PE | Miltenyi Biotec | 130-119-490; RRID: AB_2857461
Anti-Human CD74 (REA1103) PE | Miltenyi Biotec | 130-119-203; RRID: AB_2733836
Anti-Human EPCAM (REA764) FITC | Miltenyi Biotec | 130-110-998; RRID: AB_2657493
Anti-Human CD68 (REAL566) PE | Miltenyi Biotec | 130-120-368; RRID: AB_2857592
Anti-Human CD1c (REA694) APC | Miltenyi Biotec | 130-110-537; RRID: AB_2656040
Anti-Human CD3 (REA1151) APC | Miltenyi Biotec | 130-120-269; RRID: AB_2876933
Anti-Human CD5 (REA782) PE | Miltenyi Biotec | 130-110-990; RRID: AB_2658593
Anti-Human CD7 (REA1244) PE | Miltenyi Biotec | 130-124-939; RRID: AB_2819716
Anti-Human CD19 (REAL106) PE | Miltenyi Biotec | 130-122-649; RRID: AB_2784034
Anti-Human CD22 (REA340) APC | Miltenyi Biotec | 130-120-762; RRID: AB_2752186
Anti-Human CD177 (REA258) PE | Miltenyi Biotec | 130-115-253; RRID: AB_2726963
Anti-Human CD63 (REA406) PE | Miltenyi Biotec | 130-121-316; RRID: AB_2857545

**Antibodies: CITE-seq**

TotalSeq-A0001 anti-mouse CD4 (RM4-5) | BioLegend | 100569; RRID: AB_2749956
TotalSeq-A0002 anti-mouse CD8a (53-6.7) | BioLegend | 100773; RRID: AB_2734151
TotalSeq-A0003 anti-mouse CD366 (Tim-3) (RMT3-23) | BioLegend | 119729; RRID: AB_2734178
TotalSeq-A0004 anti-mouse CD279 (PD-1) (RMP1-30) | BioLegend | 109123; RRID: AB_2734169
TotalSeq-A0005 anti-human CD80 (2D10) | BioLegend | 305239; RRID: AB_2749958
TotalSeq-A0006 anti-human CD86 (IT2.2) | BioLegend | 305443; RRID: AB_2734273
TotalSeq-A0007 anti-human CD274 (29E.2A3) | BioLegend | 329743; RRID: AB_2749959
TotalSeq-A0008 anti-human CD273 (24F.10C112) | BioLegend | 329619; RRID: AB_2734321
TotalSeq-A0009 anti-human CD275 (2D3) | BioLegend | 309413; RRID: AB_2734278
TotalSeq-A0010 anti-human CD276 (DCN.70) | BioLegend | 331607; RRID: AB_2734327
TotalSeq-A0012 anti-mouse CD117 (c-kit) (2B8) | BioLegend | 105843; RRID: AB_2749960
TotalSeq-A0013 anti-mouse Ly-6C (HK1.4) | BioLegend | 128047; RRID: AB_2749961
TotalSeq-A0014 anti-mouse/human CD11b (M1/70) | BioLegend | 101265; RRID: AB_2734152
TotalSeq-A0015 anti-mouse Ly-6G (1A8) | BioLegend | 127655; RRID: AB_2749962
TotalSeq-A0016 anti-human Galectin9 (9M1-3) | BioLegend | Barcode: ACTCAGGAGCTCT
TotalSeq-A0020 anti-human CD270 (122) | BioLegend | 318813; RRID: AB_2734293
TotalSeq-A0021 anti-human CD252 (11C3.1) | BioLegend | Barcode: TTATGTCTCGGACT
TotalSeq-A0022 anti-human CD137L (5F4) | BioLegend | 311509; RRID: AB_2734284
TotalSeq-A0023 anti-human CD155 (SKII.4) | BioLegend | 337623; RRID: AB_2749963
TotalSeq-A0024 anti-human CD112 (TX31) | BioLegend | 337417; RRID: AB_2749964
TotalSeq-A0026 anti-human CD47 (CC2C6) | BioLegend | 323129; RRID: AB_2734305
TotalSeq-A0027 anti-human CD70 (113-16) | BioLegend | 355117; RRID: AB_2749965
TotalSeq-A0028 anti-human CD30 (BY88) | BioLegend | 339313; RRID: AB_2749966
TotalSeq-A0029 anti-human CD48 (BJ40) | BioLegend | 336709; RRID: AB_2734342
TotalSeq-A0031 anti-human CD40 (5C3) | BioLegend | 334346; RRID: AB_2749968
TotalSeq-A0032 anti-human CD154 (24-31) | BioLegend | 310843; RRID: AB_2734283
TotalSeq-A0033 anti-human CD52 (H1186) | BioLegend | 316017; RRID: AB_2734292
TotalSeq-A0034 anti-human CD3 (UCHT1) | BioLegend | 300475; RRID: AB_2734246
TotalSeq-A0047 anti-human CD56 (5.1H11) | BioLegend | 362557; RRID: AB_2749970
TotalSeq-A0050 anti-human CD19 (HIB19) | BioLegend | 302259; RRID: AB_2734256

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
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| TotalSeq-A0054 anti-human CD34 (581) | BioLegend | 343547; RRID: AB_2749972 |
| TotalSeq-A0056 anti-human CD269 (19F2) | BioLegend | 357521; RRID: AB_2749974 |
| TotalSeq-A0057 anti-human B2M (2M2) | BioLegend | 316321; RRID: AB_2749975 |
| TotalSeq-A0058 anti-human HLA-ABC (W6/32) | BioLegend | 311445; RRID: AB_2749976 |
| TotalSeq-A0059 anti-human CD90 (5E+10) | BioLegend | 328135; RRID: AB_2832627 |
| TotalSeq-A0060 anti-human CD117 (104D2) | BioLegend | 313241; RRID: AB_2734287 |
| TotalSeq-A0061 anti-human CD10 (HI10a) | BioLegend | 312231; RRID: AB_2832627 |
| TotalSeq-A0063 anti-human CD45RA (HI100) | BioLegend | 304157; RRID: AB_2734267 |
| TotalSeq-A0064 anti-human CD123 (6H6) | BioLegend | 306037; RRID: AB_2749977 |
| TotalSeq-A0065 anti-human CD7 (CD7-6B7) | BioLegend | 343123; RRID: AB_2734345 |
| TotalSeq-A0066 anti-human CD105 (43A3) | BioLegend | 322221; RRID: AB_2703050 |
| TotalSeq-A0067 anti-human CD201 (RCR-401) | BioLegend | 351907; RRID: AB_2749978 |
| TotalSeq-A0070 anti-human/mouse CD49f (GoH3) | BioLegend | 313633; RRID: AB_2734291 |
| TotalSeq-A0071 anti-human/mouse CD44 (IM7) | BioLegend | 103045; RRID: AB_2734154 |
| TotalSeq-A0072 anti-human/mouse CD54 (YN1/1.7.4) | BioLegend | 116127; RRID: AB_2734177 |
| TotalSeq-A0073 anti-mouse/human CD15 (SSEA-1) (MC-480) | BioLegend | 125615; RRID: AB_2800603 |
| TotalSeq-A0074 anti-mouse CD73 (7G3) | BioLegend | 127227; RRID: AB_2749980 |
| TotalSeq-A0075 anti-mouse CD69 (R1-2) | BioLegend | 103623; RRID: AB_2734159 |
| TotalSeq-A0076 anti-mouse CD200 (OX2) (OX-90) | BioLegend | 123811; RRID: AB_2734191 |
| TotalSeq-A0077 anti-mouse CD8a (RPA-T8) | BioLegend | 301067; RRID: AB_2734248 |
| TotalSeq-A0078 anti-mouse CD14 (M5E2) | BioLegend | 301855; RRID: AB_2734254 |
| TotalSeq-A0079 anti-mouse CD16 (3G8) | BioLegend | 302061; RRID: AB_2734255 |
| TotalSeq-A0080 anti-mouse CD123 (6H6) | BioLegend | 302643; RRID: AB_2734258 |
| TotalSeq-A0081 anti-mouse CD45R/B220 | BioLegend | 103263; RRID: AB_2734158 |
| TotalSeq-A0082 anti-mouse CD19 (B4) | BioLegend | 102055; RRID: AB_2749982 |
| TotalSeq-A0083 anti-mouse CD45RO (UCHL1) | BioLegend | 329955; RRID: AB_2734322 |
| TotalSeq-A0084 anti-mouse TIGIT (A15153G) | BioLegend | 372725; RRID: AB_2749976 |
| TotalSeq-A0085 anti-mouse/human CD25 (BC96) | BioLegend | 400199; RRID: AB_2868412 |
| TotalSeq-A0086 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 400285; |
| TotalSeq-A0087 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 123427; RRID: AB_2800875 |
| TotalSeq-A0088 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 135533; RRID: AB_2734198 |
| TotalSeq-A0089 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 117355; RRID: AB_2750352 |
| TotalSeq-A0090 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 123427; RRID: AB_2750540 |
| TotalSeq-A0091 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 101635; RRID: AB_2750358 |
| TotalSeq-A0092 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 101343; RRID: AB_2750532 |
| TotalSeq-A0093 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 143211; RRID: AB_2750541 |
| TotalSeq-A0094 anti-mouse/human/human CD15 (SSEA-1) (MC-480) | BioLegend | 100637; RRID: AB_2749985 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
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| TotalSeq-A0122 anti-human CD326 (9C4) | BioLegend | 324241; RRID: AB_2750362 |
| TotalSeq-A0124 anti-human CD31 (WM59) | BioLegend | 303137; RRID: AB_2750360 |
| TotalSeq-A0132 anti-human EGFR (AY13) | BioLegend | 352923; RRID: AB_2734373 |
| TotalSeq-A0134 anti-human CD146 (P1H12) | BioLegend | 361017; RRID: AB_2750355 |
| TotalSeq-A0136 anti-human IgM (MHM-88) | BioLegend | 314541; RRID: AB_2749992 |
| TotalSeq-A0140 anti-human CD183 (G025H7) | BioLegend | 353745; RRID: AB_2749993 |
| TotalSeq-A0141 anti-human CD195 (J418F1) | BioLegend | 359135; RRID: AB_2749994 |
| TotalSeq-A0142 anti-human CD32 (FUN-2) | BioLegend | 303223; RRID: AB_2749995 |
| TotalSeq-A0143 anti-human CD196 (G034E3) | BioLegend | 353437; RRID: AB_2750534 |
| TotalSeq-A0144 anti-human CD185 (J252D4) | BioLegend | 356937; RRID: AB_2750356 |
| TotalSeq-A0145 anti-human CD103 (Ber-ACT8) | BioLegend | 350321; RRID: AB_2749996 |
| TotalSeq-A0146 anti-human CD69 (FN50) | BioLegend | 310947; RRID: AB_2749997 |
| TotalSeq-A0147 anti-human CD62L (DREG-56) | BioLegend | 304847; RRID: AB_2750365 |
| TotalSeq-A0148 anti-human CD197 (G043H7) | BioLegend | 353247; RRID: AB_2750357 |
| TotalSeq-A0149 anti-human CD161 (HP-3G10) | BioLegend | 339945; RRID: AB_2749998 |
| TotalSeq-A0151 anti-human CD152 (B7) | BioLegend | 369619; RRID: AB_2734423 |
| TotalSeq-A0152 anti-human CD223 (11C3C65) | BioLegend | 369333; RRID: AB_2749999 |
| TotalSeq-A0153 anti-human KLRG1 (SA231A2) | BioLegend | 367271; RRID: AB_2750373 |
| TotalSeq-A0155 anti-human CD107a (HA3) | BioLegend | 328647; RRID: AB_2750351 |
| TotalSeq-A0156 anti-human CD95 (DX2) | BioLegend | 305649; RRID: AB_2750368 |
| TotalSeq-A0158 anti-human CD134 (Ber-ACT35) | BioLegend | 350033; RRID: AB_2783245 |
| TotalSeq-A0159 anti-human HLA-DR (L243) | BioLegend | 307659; RRID: AB_2750001 |
| TotalSeq-A0160 anti-human CD1c (L161) | BioLegend | 331539; RRID: AB_2734326 |
| TotalSeq-A0162 anti-human CD64 (10.1) | BioLegend | 305037; RRID: AB_2750366 |
| TotalSeq-A0163 anti-human CD141 (M80) | BioLegend | 344121; RRID: AB_2783229 |
| TotalSeq-A0164 anti-human CD1d (61.1) | BioLegend | 350317; RRID: AB_2750370 |
| TotalSeq-A0165 anti-human CD314 (ID11) | BioLegend | 320835; RRID: AB_2734298 |
| TotalSeq-A0166 anti-human CD66b (6/40c) | BioLegend | 392905; RRID: AB_2750372 |
| TotalSeq-A0167 anti-human CD35 (E11) | BioLegend | 333407; RRID: AB_2783217 |
| TotalSeq-A0168 anti-human CD57 (QA17A04) | BioLegend | 393319; RRID: AB_2810588 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| TotalSeq-A0169 anti-human CD366 (F38-2E2) | BioLegend | 345047; RRID: AB_2800924 |
| TotalSeq-A0170 anti-human CD272 (M1H26) | BioLegend | 344525; RRID: AB_2750002 |
| TotalSeq-A0171 anti-human/mouse/rat CD278 (ICOS) (C398,4A) | BioLegend | 313555; RRID: AB_2800824 |
| TotalSeq-A0173 anti-mouse CD206 (MMR) (C068C2) | BioLegend | Barcode: TCAACTC GGTTGTC |
| TotalSeq-A0174 anti-human CD58 (TS2/9) | BioLegend | 330919; RRID: AB_2750003 |
| TotalSeq-A0175 anti-human CD96 (NK2.39) | BioLegend | 338419; RRID: AB_2750004 |
| TotalSeq-A0176 anti-human CD39 (A1) | BioLegend | 328233; RRID: AB_2750005 |
| TotalSeq-A0177 anti-human CD178 (NOK-1) | BioLegend | 306413; RRID: AB_2750500 |
| TotalSeq-A0180 anti-human CD24 (ML5) | BioLegend | 311137; RRID: AB_2750374 |
| TotalSeq-A0181 anti-human CD21 (Bu32) | BioLegend | 354915; RRID: AB_2750006 |
| TotalSeq-A0182 anti-mouse CD3 (17A2) | BioLegend | 100251; RRID: AB_2750533 |
| TotalSeq-A0184 anti-mouse CD335 (NKp46) (29A1.4) | BioLegend | 137633; RRID: AB_2734199 |
| TotalSeq-A0185 anti-human CD11a (TS2/4) | BioLegend | 350615; RRID: AB_2734365 |
| TotalSeq-A0186 anti-human IgA (HP6123) | BioLegend | Barcode: AAGATGTCCGAGCAA |
| TotalSeq-A0187 anti-human CD79b (CB3-1) | BioLegend | 341415; RRID: AB_2750347 |
| TotalSeq-A0188 anti-human CD66a_c_e (ASL-32) | BioLegend | 342319; RRID: AB_2783223 |
| TotalSeq-A0189 anti-human CD178 (NOK-1) | BioLegend | 329527; RRID: AB_2750007 |
| TotalSeq-A0190 anti-mouse CD274 (B7-H1, PD-L1) (MIH6) | BioLegend | 153604; RRID: AB_2783125 |
| TotalSeq-A0191 anti-mouse/rat/human CD27 (LG.3A10) | BioLegend | 124235; RRID: AB_2750344 |
| TotalSeq-A0192 anti-mouse CD20 (SA275A11) | BioLegend | 150423; RRID: AB_2734214 |
| TotalSeq-A0193 anti-mouse D357 (GTR) (DTA-1) | BioLegend | 126319; RRID: AB_2734195 |
| TotalSeq-A0194 anti-mouse CD137 (17B5) | BioLegend | 106111; RRID: AB_2783048 |
| TotalSeq-A0195 anti-mouse CD134 (OX-40) (OX-86) | BioLegend | 119426; RRID: AB_2750376 |
| TotalSeq-A0196 anti-human CD235ab (HIR2) | BioLegend | 306623; RRID: AB_2750008 |
| TotalSeq-A0197 anti-mouse CD69 (H1.2F3) | BioLegend | 104546; RRID: AB_2750539 |
| TotalSeq-A0198 anti-mouse CD127 (IL-7Rα) (A7R34) | BioLegend | 135045; RRID: AB_2750009 |
| TotalSeq-A0200 anti-mouse CD86 (GL-1) | BioLegend | 105047; RRID: AB_2750348 |
| TotalSeq-A0201 anti-mouse CD103 (2E7) | BioLegend | 121437; RRID: AB_2750349 |
| TotalSeq-A0202 anti-mouse CD64 (FcγRII) (X54-5/7.1) | BioLegend | 139325; RRID: AB_2750367 |
| TotalSeq-A0203 anti-mouse CD150 (SLAM) (TC15-12F12.2) | BioLegend | 115945; RRID: AB_2783055 |
| TotalSeq-A0204 anti-mouse CD28 (37.51) | BioLegend | Barcode: ATTAAGACGGTGTTG |
| TotalSeq-A0205 anti-human CD206 (15-2) | BioLegend | 321143; RRID: AB_2750510 |
| TotalSeq-A0206 anti-human CD169 (7-239) | BioLegend | 346011; RRID: AB_2750011 |
| TotalSeq-A0207 anti-human CD370 (8F9) | BioLegend | 353807; RRID: AB_2814293 |
| TotalSeq-A0208 anti-human XCR1 (S15046E) | BioLegend | 372613; RRID: AB_2783286 |
| TotalSeq-A0209 anti-mouse TCR (2.11) | BioLegend | 141113; RRID: AB_2800654 |
| TotalSeq-A0210 anti-mouse TCR (P36) | BioLegend | 137507; RRID: AB_2810404 |
| TotalSeq-A0211 anti-mouse TCR (UC3-10A6) | BioLegend | 137709; RRID: AB_2783100 |
| TotalSeq-A0212 anti-mouse CD24 (M1/69) | BioLegend | 101841; RRID: AB_2750380 |
| TotalSeq-A0213 anti-human Notch (MHN1-519) | BioLegend | 352109; RRID: AB_2783247 |
| TotalSeq-A0214 anti-human/mouse integrin β7 (FIB504) | BioLegend | 321227; RRID: AB_2750504 |
| TotalSeq-A0215 anti-human CD268 (11C1) | BioLegend | 316925; RRID: AB_2750502 |
| TotalSeq-A0216 anti-human CD42b (HIP1) | BioLegend | 303937; RRID: AB_2783163 |
| TotalSeq-A0217 anti-human CD54 (HA58) | BioLegend | 353123; RRID: AB_2750384 |
| TotalSeq-A0218 anti-human CD62P (AK4) | BioLegend | 304933; RRID: AB_2750386 |
| TotalSeq-A0219 anti-human CD119 (GIR-208) | BioLegend | 308607; RRID: AB_2750385 |
| TotalSeq-A0221 anti-human IRF5 (11F4A09) | BioLegend | Barcode: GTAGCCCTAGGTTG |
| TotalSeq-A0224 anti-human TCR (IP26) | BioLegend | 306737; RRID: AB_2783167 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
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| TotalSeq-A0225 anti-mouse CD196 (CCR6) (29-2L17) | BioLegend | 129825; RRID: AB_2783083 |
| TotalSeq-A0226 anti-mouse CD106 (429 (MVCAM.A)) | BioLegend | 105725; RRID: AB_2783044 |
| TotalSeq-A0227 anti-mouse CD122 (IL-2Rb) (5H4) | BioLegend | Barcode: GGTATGCCACCTTA |
| TotalSeq-A0228 anti-mouse CD183 (CXR3) (CXR3-173) | BioLegend | Barcode: GTTCAGCCGTAGACT |
| TotalSeq-A0229 anti-mouse CD62P (P-selectin) (RMP-1) | BioLegend | Barcode: TGGTGCCGTAGACT |
| TotalSeq-A0230 anti-mouse CD88 (Ly-3) (YTS156.7.7) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0232 anti-mouse MAdCAM-1 (MECA-367) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0235 anti-mouse TCR (KJ16-133.18) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0236 Rat IgG1, κ Isotype Ctrl (RTK2071) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0237 Rat IgG1, λ Isotype Ctrl (G0114F7) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0238 Rat IgG2a, κ Isotype Ctrl (RTK2758) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0240 Purified Rat IgG2c, κ Isotype Ctrl (RTK4174) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0241 Armenian Hamster IgG Isotype Ctrl (HTK888) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0242 anti-human CD192 (K036C2) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0244 anti-human CD102 (C8R-IC2/2) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0245 anti-human CD106 (STA) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0247 anti-human CD267 (1A1) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0248 anti-human CD62E (HAE-1f) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0249 anti-human CD135 (BV10A4H2) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0250 anti-mouse/human IRF4 (IRF4.3E4) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0251 anti-human CD135 (BV10A4H2) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0252 anti-human FcεR1a (AER-37) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0253 anti-human CD41 (HIP8) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0254 anti-mouse TCR (MR9-4) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0255 anti-human CD137 (4B4-1) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0256 anti-human CD254 (M1H24) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0257 anti-human CD43 (CD43-10G7) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0258 anti-human CD163 (GHI/61) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0259 anti-human CD68 (HB15e) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0260 anti-human CD357 (108-17) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0261 anti-human CD59 (p282 (H19)) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0262 anti-human CD309 (7D4-6) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0263 anti-human CD13 (WM15) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0264 anti-human CD18 (12G5) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0265 anti-human CD2 (TS1/8) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0266 anti-human CD2 (TS1/8) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0267 anti-human CD226 (11A8) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0268 anti-human CD29 (TS2/16) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0269 anti-human CD303 (201A) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0270 anti-human CD49b (P1E6-C5) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0271 anti-human CD81 (5A6) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0272 anti-human CD98 (MEM-10B) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0273 anti-human IgG (M1310G05) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0274 anti-mouse CD195 (CCR5) (HM-CCR5) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0277 anti-mouse CD197 (CCR7) (4B12) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0278 anti-mouse CD223 (LAG-3) (C9B7W) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0279 anti-mouse CD62E (E-selectin) (RME-1/CD62E) | BioLegend | Barcode: GGATTTGTATCTCCC |
| TotalSeq-A0280 anti-mouse Panendothelial Cell Antigen (MECA-32) | BioLegend | Barcode: GGATTTGTATCTCCC |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
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| TotalSeq-A0382 anti-human CD177 (MEM-166) | BioLegend | 315811; RRID: AB_2750554 |
| TotalSeq-A0383 anti-human CD55 (JS11) | BioLegend | 311317; RRID: AB_2750378 |
| TotalSeq-A0384 anti-human IgD (I6E-2) | BioLegend | 348243; RRID: AB_2783238 |
| TotalSeq-A0385 anti-human CD18 (TS1/18) | BioLegend | 302121; RRID: AB_2750382 |
| TotalSeq-A0386 anti-human CD28 (CD28.2) | BioLegend | 302955; RRID: AB_2783159 |
| TotalSeq-A0387 anti-human TSLPR (1D3) | BioLegend | 322907; RRID: AB_2800845 |
| TotalSeq-A0388 anti-mouse CD152 (UC10-4B9) | BioLegend | 106325; RRID: AB_2876417 |
| TotalSeq-A0389 anti-human CD38 (HIT2) | BioLegend | 303541; RRID: AB_2783161 |
| TotalSeq-A0390 anti-human CD127 (A019D5) | BioLegend | 351352; RRID: AB_2734366 |
| TotalSeq-A0392 anti-human CD15 (W6D3) | BioLegend | 323046; RRID: AB_2734304 |
| TotalSeq-A0393 anti-human CD22 (S-HCL-1) | BioLegend | 363514; RRID: AB_2734404 |
| TotalSeq-A0394 anti-human CD71 (CY1G4) | BioLegend | 334123; RRID: AB_2800884 |
| TotalSeq-A0395 anti-human B7H4 (M1H3) | BioLegend | 358114; RRID: AB_2734386 |
| TotalSeq-A0396 anti-human CD26 (BA5b) | BioLegend | 302720; RRID: AB_2734261 |
| TotalSeq-A0397 anti-human CD193 (5e7) | BioLegend | 310729; RRID: AB_2873174 |
| TotalSeq-A0398 anti-human CD115 (4-4D2-1E4) | BioLegend | 347325; RRID: AB_2873237 |
| TotalSeq-A0399 anti-human CD204 (7C9C20) | BioLegend | 371909; RRID: AB_2810584 |
| TotalSeq-A0400 anti-human CD144 (BV9) | BioLegend | 348517; RRID: AB_2783239 |
| TotalSeq-A0401 anti-human CD301 (H037G3) | BioLegend | 354707; RRID: AB_2810566 |
| TotalSeq-A0402 anti-human CD1a (HI149) | BioLegend | 300133; RRID: AB_2783146 |
| TotalSeq-A0404 anti-human CD63 (H5C6) | BioLegend | 353035; RRID: AB_2783249 |
| TotalSeq-A0405 anti-human CD284 (HTA125) | BioLegend | 312817; RRID: AB_2783183 |
| TotalSeq-A0406 anti-human CD304 (12C2) | BioLegend | 354525; RRID: AB_2783261 |
| TotalSeq-A0407 anti-human CD36 (5-271) | BioLegend | 336225; RRID: AB_2800892 |
| TotalSeq-A0408 anti-human CD172a (15-414) | BioLegend | 372109; RRID: AB_2783285 |
| TotalSeq-A0409 anti-human CD85g (17G10.2) | BioLegend | 326411; RRID: AB_2750555 |
| TotalSeq-A0411 Polyclonal Clec4F (Custom) | BioLegend | Barcode: TATGCTGTGGCTATG |
| TotalSeq-A0414 Polyclonal VSIG4 (Custom) | BioLegend | Barcode: ACGTTATACAGGTG |
| TotalSeq-A0415 anti-P2RY12 (S16007D) | BioLegend | 848009; RRID: AB_2783419 |
| TotalSeq-A0416 anti-mouse CD300LG (Nepmucin) (ZAQ5) | BioLegend | 147105; RRID: AB_2783116 |
| TotalSeq-A0417 anti-mouse CD163 (S16007D) | BioLegend | 155303; RRID: AB_2814058 |
| TotalSeq-A0418 anti-human CD243 (4E3.16) | BioLegend | 919407; RRID: AB_2810817 |
| TotalSeq-A0419 anti-human CD72 (3F3) | BioLegend | 316205; RRID: AB_2783189 |
| TotalSeq-A0420 anti-human CD158 (HP-MA4) | BioLegend | 339515; RRID: AB_2800901 |
| TotalSeq-A0421 anti-mouse CD49b (HMa2) | BioLegend | 103523; RRID: AB_2819796 |
| TotalSeq-A0422 anti-mouse CD172a (SIRPα) (P84) | BioLegend | 144033; RRID: AB_2800670 |
| TotalSeq-A0423 anti-human MerTK (S900H11G1E3) | BioLegend | 367617; RRID: AB_2801011 |
| TotalSeq-A0424 anti-mouse CD14 (Sa14-2) | BioLegend | 123333; RRID: AB_2800591 |
| TotalSeq-A0426 anti-mouse CD192 (CCR2) (SA203G11) | BioLegend | 150625; RRID: AB_2783122 |
| TotalSeq-A0427 anti-human FOLR2 (94b/FOLR2) | BioLegend | 391707; RRID: AB_2783289 |
| TotalSeq-A0428 anti-human TIM4 (9F4) | BioLegend | 354009; RRID: AB_2783258 |
| TotalSeq-A0429 anti-mouse CD48 (HM48.1) | BioLegend | 103447; RRID: AB_2800558 |
| TotalSeq-A0430 anti-human CD171 (L1-OV198.5) | BioLegend | 371609; RRID: AB_2801019 |
| TotalSeq-A0432 anti-human CD320 (3F4) | BioLegend | 800319; RRID: AB_2801138 |
| TotalSeq-A0433 anti-human CD325 (8C11) | BioLegend | 350817; RRID: AB_2810555 |
| TotalSeq-A0434 anti-mouse/human ReceptorD4 | BioLegend | Barcode: TCTCTGACCCTGCTT |
| TotalSeq-A0435 anti-mouse/human GABRB3 | BioLegend | Barcode: GTTGTAGCAGCTTT |
| TotalSeq-A0437 anti-mouse/human CD207 (4C7) | BioLegend | Barcode: CGATTGTATCCCT |
| TotalSeq-A0438 anti-mouse/rat KKC2 | BioLegend | Barcode: GAGCTTTGACGGCTT |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| TotalSeq-A0439 anti-mouse CD201 (RCR-16) | BioLegend | 141509; RRID: AB_2800655 |
| TotalSeq-A0440 anti-mouse CD169 (Siglec-1) (N1/12) | BioLegend | 142425; RRID: AB_2783106 |
| TotalSeq-A0441 anti-mouse CD71 (3D6.112) | BioLegend | 113824; RRID: AB_2800574 |
| TotalSeq-A0442 anti-mouse Notch (HMN1-12) | BioLegend | 130617; RRID: AB_2783085 |
| TotalSeq-A0443 anti-mouse CD41 (MWReg30) | BioLegend | 133937; RRID: AB_2800635 |
| TotalSeq-A0444 anti-mouse CXCR4 (L276F12) | BioLegend | 146520; RRID: AB_2800682 |
| TotalSeq-A0445 anti-human TMEM119 (A16075D) | BioLegend | 853303; RRID: AB_2801201 |
| TotalSeq-A0446 anti-human CD93 (VIMD2) | BioLegend | 336121; RRID: AB_2750379 |
| TotalSeq-A0448 anti-mouse CD204 (Msr1) (1F8C33) | BioLegend | 154703; RRID: AB_2783126 |
| TotalSeq-A0449 anti-mouse CD326 (Ep-CAM) (G8.8) | BioLegend | 118237; RRID: AB_2800586 |
| TotalSeq-A0551 anti-mouse CD301a (MGL1) (LOM-8.7) | BioLegend | 145611; RRID: AB_2783114 |
| TotalSeq-A0552 anti-mouse CD304 (Neuropilin-1) (3E12) | BioLegend | 145215; RRID: AB_2750383 |
| TotalSeq-A0554 anti-mouse CD309 (VEGFR2, Flk-1) (89B3A5) | BioLegend | 121921; RRID: AB_2783066 |
| TotalSeq-A0555 anti-mouse CD36 (HM36) | BioLegend | 102621; RRID: AB_2800557 |
| TotalSeq-A0556 anti-mouse CD370 (CLEC9A-DNGR1) (7H11) | BioLegend | Barcode: AACTCAGTGTCGG |
| TotalSeq-A0557 anti-mouse CD38 (90) | BioLegend | 102733; RRID: AB_2750565 |
| TotalSeq-A0558 anti-mouse CD55 (DAF) (Riko-3) | BioLegend | 131809; RRID: AB_2783086 |
| TotalSeq-A0559 anti-mouse CD63 (NVG-2) | BioLegend | 143915; RRID: AB_2783109 |
| TotalSeq-A0560 anti-mouse CD68 (FA-11) | BioLegend | 137031; RRID: AB_2783099 |
| TotalSeq-A0561 anti-mouse CD79b (Igj) (HM79-12) | BioLegend | 132811; RRID: AB_2783087 |
| TotalSeq-A0562 anti-mouse CD83 (Michel-19) | BioLegend | 121519; RRID: AB_2783061 |
| TotalSeq-A0563 anti-mouse CX3CR1 (S011F11) | BioLegend | 149041; RRID: AB_2783121 |
| TotalSeq-A0564 anti-mouse Folate Receptor β (FR-β) (10/FR2) | BioLegend | 153307; RRID: AB_2800690 |
| TotalSeq-A0565 anti-mouse MERTK (Mer) (2B10C42) | BioLegend | Barcode: AGTAGAGCAACTCGT |
| TotalSeq-A0566 anti-mouse CD301b (MGL2) (URA-1) | BioLegend | 146817; RRID: AB_2783115 |
| TotalSeq-A0567 anti-mouse Tim-4 (RMT4-54) | BioLegend | 130011; RRID: AB_2783084 |
| TotalSeq-A0568 anti-mouse/rat XCR1 (ZET) | BioLegend | 148227; RRID: AB_2783120 |
| TotalSeq-A0569 anti-human CD338 (SD3) | BioLegend | 332021; RRID: AB_2783216 |
| TotalSeq-A0570 anti-mouse/rat CD29 (HMj1-1) | BioLegend | 102233; RRID: AB_2783042 |
| TotalSeq-A0571 anti-mouse IgD (11-26,2a) | BioLegend | 405745; RRID: AB_2783321 |
| TotalSeq-A0572 anti-human C5L2 (1D-12) | BioLegend | 342407; RRID: AB_2783226 |
| TotalSeq-A0573 anti-mouse CD140a (AP5) | BioLegend | 135917; RRID: AB_2783094 |
| TotalSeq-A0574 anti-human CD235a (Hil264) | BioLegend | 349117; RRID: AB_2783242 |
| TotalSeq-A0575 anti-human CD49a (T52/7) | BioLegend | 328315; RRID: AB_2783195 |
| TotalSeq-A0576 anti-human CD49d (9F10) | BioLegend | 304337; RRID: AB_2783166 |
| TotalSeq-A0577 anti-human CD73 (AD2) | BioLegend | 344029; RRID: AB_2783228 |
| TotalSeq-A0578 anti-human CD79a | BioLegend | Barcode: CTTATCACCCGCTTT |
| TotalSeq-A0579 anti-human CD9 (H19a) | BioLegend | 312119; RRID: AB_2783182 |
| TotalSeq-A0580 anti-human mast cell tryptase | BioLegend | Barcode: ACTGATAGACCCGCT |
| TotalSeq-A0581 anti-human TCR (3C10) | BioLegend | 351733; RRID: AB_2783246 |
| TotalSeq-A0582 anti-human TCR (B6) | BioLegend | 331433; RRID: AB_2800863 |
| TotalSeq-A0583 anti-human TCR (B3) | BioLegend | 331311; RRID: AB_2783207 |
| TotalSeq-A0584 anti-human TCR (6B11) | BioLegend | 342923; RRID: AB_2783227 |
| TotalSeq-A0586 anti-human CD354 (Trem-26) | BioLegend | 314910; |
| TotalSeq-A0588 anti-human CD202b (HT2) | BioLegend | 334213; RRID: AB_2810511 |
| TotalSeq-A0590 anti-human CD305 (NKTA255) | BioLegend | 342805; RRID: AB_2800911 |
| TotalSeq-A0591 anti-human LOX1 (15C4) | BioLegend | 358611; RRID: AB_2800987 |
| TotalSeq-A0592 anti-human CD158b (DX27) | BioLegend | 312615; RRID: AB_2800818 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| TotalSeq-A0593 anti-human CD203c (NP4D6) | BioLegend | 324627; RRID: AB_2800849 |
| TotalSeq-A0595 anti-mouse CD11a (M17/4) | BioLegend | 101125; RRID: AB_2783036 |
| TotalSeq-A0596 anti-human CD209 (9E9A8) | BioLegend | 330119; RRID: AB_2783206 |
| TotalSeq-A0597 anti-human CD158e1 (DX9) | BioLegend | 312723; RRID: AB_2800819 |
| TotalSeq-A0600 anti-human CD158f (UP-R1) | BioLegend | 341307; RRID: AB_2800904 |
| TotalSeq-A0801 anti-human CD337 (P30-15) | BioLegend | 325221; RRID: AB_2800852 |
| TotalSeq-A0803 anti-mouse CD253 (RIK-2) | BioLegend | 308211; RRID: AB_2800803 |
| TotalSeq-A0804 anti-human CD186 (K041E5) | BioLegend | 356021; RRID: AB_2800961 |
| TotalSeq-A0807 anti-mouse CD200R (OX-110) | BioLegend | 123913; RRID: AB_2800594 |
| TotalSeq-A0808 anti-mouse CD193 (J073E5) | BioLegend | 144523; RRID: AB_2800673 |
| TotalSeq-A0809 anti-mouse CD200R3 (Ba13) | BioLegend | 142209; RRID: AB_2800657 |
| TotalSeq-A0810 anti-mouse CD138 (281-2) | BioLegend | 127027; RRID: AB_2800623 |
| TotalSeq-A0812 anti-mouse CD105 (MJ7/18) | BioLegend | 120421; RRID: AB_2800587 |
| TotalSeq-A0813 anti-mouse CD9 (M23) | BioLegend | 124819; RRID: AB_2800600 |
| TotalSeq-A0814 anti-human CD205 (HD30) | BioLegend | 342211; RRID: AB_2800908 |
| TotalSeq-A0816 anti-human CD271 (ME20.4) | BioLegend | 345123; RRID: AB_2814273 |
| TotalSeq-A0817 anti-human CD109 (W7C5) | BioLegend | 323307; RRID: AB_2800848 |
| TotalSeq-A0819 anti-human CD126 (UV4) | BioLegend | 352813; RRID: AB_2800939 |
| TotalSeq-A0821 anti-human CD164 (67D2) | BioLegend | 324809; RRID: AB_2800850 |
| TotalSeq-A0822 anti-human CD142 (NY2) | BioLegend | 365207; RRID: AB_2801007 |
| TotalSeq-A0824 anti-mouse P2X7R (1F11) | BioLegend | 148711; RRID: AB_2800683 |
| TotalSeq-A0825 anti-mouse CD371 (5D3/Clec12a) | BioLegend | 143407; RRID: AB_2800668 |
| TotalSeq-A0826 anti-human CD307c (HS/FcRL3) | BioLegend | 374411; RRID: AB_2801022 |
| TotalSeq-A0827 anti-mouse CD22 (OX-97) | BioLegend | 126113; RRID: AB_2800614 |
| TotalSeq-A0828 anti-human CD307d (413D12) | BioLegend | 340209; RRID: AB_2800902 |
| TotalSeq-A0829 anti-human CD307e (5096e) | BioLegend | 340307; RRID: AB_2800903 |
| TotalSeq-A0830 anti-human CD319 (162.1) | BioLegend | 331821; RRID: AB_2800872 |
| TotalSeq-A0831 anti-human CD138 (DL-101) | BioLegend | 352325; RRID: AB_2800938 |
| TotalSeq-A0834 anti-mouse CD39 (Duha59) | BioLegend | 143813; RRID: AB_2800669 |
| TotalSeq-A0835 anti-mouse CD314 (NKG2D) (CX5) | BioLegend | 130215; RRID: AB_2814023 |
| TotalSeq-A0836 anti-mouse DR3 (4C12) | BioLegend | 144413; RRID: AB_2814048 |
| TotalSeq-A0837 anti-mouse IL33RA (DIH9) | BioLegend | 145317; RRID: AB_2800680 |
| TotalSeq-A0839 anti-human Ly49H (3D10) | BioLegend | 144715; RRID: AB_2814049 |
| TotalSeq-A0841 anti-mouse Ly49D (4E5) | BioLegend | 138309; RRID: AB_2800647 |
| TotalSeq-A0843 anti-human CD199 (L053E8) | BioLegend | 358919; RRID: AB_2810569 |
| TotalSeq-A0844 anti-human CD45RB (MEM-55) | BioLegend | 310209; RRID: AB_2810471 |
| TotalSeq-A0845 anti-human CD99 (3B2/TA8) | BioLegend | 371317; RRID: AB_2801017 |
| TotalSeq-A0846 anti-mouse CD185 (L138D7) | BioLegend | 145535; RRID: AB_2800681 |
| TotalSeq-A0848 anti-mouse TIGIT (1G9) | BioLegend | 142115; RRID: AB_2800656 |
| TotalSeq-A0849 anti-mouse CD80 (16-10A1) | BioLegend | 104745; RRID: AB_2813935 |
| TotalSeq-A0850 anti-mouse CD49a (Hmz1) | BioLegend | 142613; RRID: AB_2800659 |
| TotalSeq-A0851 anti-mouse CD14 (1B1) | BioLegend | 123529; RRID: AB_2800593 |
| TotalSeq-A0852 anti-mouse CD226 (10E5) | BioLegend | 128823; RRID: AB_2810393 |
| TotalSeq-A0853 anti-human CD371 (50C1) | BioLegend | 353613; RRID: AB_2800948 |
| TotalSeq-A0857 anti-mouse CD34 (HM34) | BioLegend | 128619; RRID: AB_2810392 |
| TotalSeq-A0858 anti-human CD46 (TRA-2-10) | BioLegend | 352415; RRID: AB_2810557 |
| TotalSeq-A0861 anti-human CD151 (50-6) | BioLegend | 350409; RRID: AB_2810554 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
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| TotalSeq-A0862 anti-human CD218a (H44) | BioLegend | 313815; RRID: AB_2810476 |
| TotalSeq-A0863 anti-human CD257 (1D6) | BioLegend | 366509; RRID: AB_2810575 |
| TotalSeq-A0866 anti-human CLEC1B (AYP1) | BioLegend | 372009; RRID: AB_2814333 |
| TotalSeq-A0867 anti-human CD94 (DX22) | BioLegend | 305521; RRID: AB_2814142 |
| TotalSeq-A0868 anti-human IgE (MHE-18) | BioLegend | 325517; RRID: AB_2814186 |
| TotalSeq-A0869 anti-human CD365 (1D12) | BioLegend | 353907; RRID: AB_2814294 |
| TotalSeq-A0870 anti-human CD150 (A12) | BioLegend | 306313; RRID: AB_2814146 |
| TotalSeq-A0871 anti-human CD162 (KPL-1) | BioLegend | 328821; RRID: AB_2814192 |
| TotalSeq-A0875 anti-mouse TLR4 (MTS510) | BioLegend | 117614; RRID: AB_2810352 |
| TotalSeq-A0876 anti-mouse CD300c_d (TX52) | BioLegend | 148005; RRID: AB_2810413 |
| TotalSeq-A0877 anti-mouse JAML (4E+10) | BioLegend | 128507; RRID: AB_2810391 |
| TotalSeq-A0881 anti-mouse CD272 (6A6) | BioLegend | 139113; RRID: AB_2814041 |
| TotalSeq-A0882 anti-mouse PIRA_PIRB (6C1) | BioLegend | 144105; RRID: AB_2810412 |
| TotalSeq-A0883 anti-mouse CD26 (H194-112) | BioLegend | 137811; RRID: AB_2810405 |
| TotalSeq-A0884 anti-mouse DLL1 (HMD1-3) | BioLegend | 128315; RRID: AB_2810390 |
| TotalSeq-A0885 anti-mouse CD270 (HMHC-1B18) | BioLegend | 136307; RRID: AB_2810403 |
| TotalSeq-A0890 anti-mouse 4_1BB (TKS-1) | BioLegend | 107109; RRID: AB_2813955 |
| TotalSeq-A0891 anti-mouse ENPP (YE1/19.1) | BioLegend | 149209; RRID: AB_2814052 |
| TotalSeq-A0892 anti-mouse CD2 (RM2-5) | BioLegend | 100117; RRID: AB_2810312 |
| TotalSeq-A0894 anti-human Ig (MHL-38) | BioLegend | 316531; RRID: AB_2810479 |
| TotalSeq-A0895 anti-mouse/human Mac2 (LM3/38) | BioLegend | 125421; RRID: AB_281384 |
| TotalSeq-A0896 anti-human CD85j (GH1/75) | BioLegend | 333723; RRID: AB_2814225 |
| TotalSeq-A0897 anti-human CD23 (EBVCS-5) | BioLegend | 338523; RRID: AB_2814235 |
| TotalSeq-A0898 anti-human Ig (MHK-49) | BioLegend | 316627; RRID: AB_2814170 |
| TotalSeq-A0899 anti-human HLA-A2 (BB7.2) | BioLegend | 343331; RRID: AB_2810540 |
| TotalSeq-A0900 anti-human CD198 (L263G8) | BioLegend | 360607; RRID: AB_2810572 |
| TotalSeq-A0901 anti-human GARP (7B11) | BioLegend | 352515; RRID: AB_2814283 |
| TotalSeq-A0904 anti-mouse CD31 (990) | BioLegend | 102437; RRID: AB_2810335 |
| TotalSeq-A0905 anti-mouse CD107a (1D4B) | BioLegend | 121635; RRID: AB_2810369 |
| TotalSeq-A0916 anti-mouse CD124 (I015F8) | BioLegend | 144809; RRID: AB_2814050 |
| TotalSeq-A0917 anti-mouse CD95 (SA367H8) | BioLegend | 152614; RRID: AB_2810418 |
| TotalSeq-A0923 anti-human NKp80 (5D12) | BioLegend | 346709; RRID: AB_2814274 |
| TotalSeq-A5106 anti-mouse Ly6D | BioLegend | Barcode: ATGTCTACCTCAA |

### Chemicals, peptides, and recombinant proteins

| Chemicals, peptides, and recombinant proteins | Source | Identifier |
|-----------------------------------------------|--------|------------|
| Antigenfix | Diaphth | P0014 |
| β-mercaptoethanol | Sigma-Aldrich | M3148 |
| Calcium chloride dihydrate | Merck | 1023821000 |
| Collagenase A | Sigma-Aldrich | 11088793001 |
| D(-)-Fructose | Merck-Millipore | 1040071000 |
| D(+)-Saccharose | VWR International | PROL27483.294 |
| DAPI | Invitrogen | D1306; RRID: AB_2629482 |
| DMEM | Invitrogen | 41965-039 |
| Dnase I | Sigma-Aldrich | 04 536 282 001 |
| Donkey Serum | Abcam | ab7475 |
| EDTA | Westburg | 51234 |
| EGTA | Sigma-Aldrich | E3889 |
| Eosin | VWR International | MERC 1.15935 |
| FcBlock 2.4G2 | Bioceros | N/A |
| FCS | Bodinco | 5010 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Fixable Viability due Live/Dead - eFluor506 | eBioscience | 65-0866-18 |
| Fixable Viability due Live/Dead - eFluor780 | eBioscience | 65-0865-18 |
| Fixation/Permeabilization Solution Kit | BD Cytofix/Cytoperm | 554714 |
| FoxP3 Transcription factor staining buffer kit | eBioscience | 00-5523-00 |
| Gentamicin | Gibco | 15710-049 |
| GlutaMAX | Thermo Fisher | 35050-038 |
| Gluteraldehyde 25% | Sigma-Aldrich | G5882 |
| Goat Serum | Sigma-Aldrich | G9023 |
| Hematoxylin | VWR International | MERC1.05174 |
| HEPES | Sigma-Aldrich | H3375 |
| Isopropanol | Vel | T0108 |
| Methanol | Merck Millipore | 13680502 |
| Parafomaldehyde 10% | EMS | 15712 |
| Phenol Red | Sigma-Aldrich | P3532 |
| ProLong Diamond | Thermo Fisher | P36970 |
| Rat Serum | Sigma-Aldrich | R9759 |
| Ribonucleoside Vanadyl Complexes | Sigma-Aldrich | R3380-5ML |
| Roche Protector RNAse inhibitor | Sigma-Aldrich | 3335399001 |
| RPMI 1640 | Gibco | 52400-025 |
| Saponin | Sigma-Aldrich | 4521 |
| 10X SDS | Sigma-Aldrich | 71736-100ML |
| Sodium bicarbonate | Sigma-Aldrich | 705219 |
| Sodium chloride | Sigma-Aldrich | 746398 |
| Sodium Dihydrogen Phosphate Monohydrate | Sigma-Aldrich | 1063461000 |
| Sodium phosphate dibasic dihydrate | Sigma-Aldrich | 71643 |
| Tissue-Tek O.C.T | Sakura Finetek | 4583 |
| UltraPure™ Salmon Sperm DNA Solution | Thermo Fisher Scientific | 15632011 |
| Xylene | Prolabo | PROL28973.363 |

Critical commercial assays

| ALinH S Red Taq Mastermix 2x | highQu | HQ.HSM0350 |
| RNEasy Plus Micro Kit | QIAGEN | 74034 |
| SensiFAST cDNA Synthesis Kit | Bioline | BIO-65054 |
| SensiFAST SYBR No-ROX Kit | Bioline | BIO-98020 |
| Visium Spatial Gene Expression Slide and Reagent Kit | 10X Genomics | 1000184 |

Oligonucleotides: qPCR

| Flt3 – qPCR FWD | IDT | GTGACTGCGCCTGGGCTGAACTGAG |
| Flt3 – qPCR REV | IDT | TCAAGGGCGGGTTGTAAGCACTAA |
| Xcr1 - qPCR FWD | IDT | AGAGACACGGAACAGTCAGG |
| Xcr1 - qPCR REV | IDT | TGGCCGTTGCGAGGCCT |
| Cd209a - qPCR FWD | IDT | GCACTCCATCAAGGGTCCT |
| Cd209a - qPCR REV | IDT | CCAACGCTAGGAGCGACCTG |
| Ccr7 - qPCR FWD | IDT | AGAGGCTCAAGACCATGACG |
| Ccr7 - qPCR REV | IDT | TGGAGACCTTGGTGTCGCT |
| Mafb - qPCR FWD | IDT | TGGACTTGTAGCAACCTC |
| Mafb - qPCR REV | IDT | AAGGCATGCAGGACCTGG |
| Cd5i - qPCR FWD | IDT | GAGAGACATGGATGGAAT |
| Cd5i - qPCR REV | IDT | ACCCTTGTTGAGCACCTC |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Cd207 - qPCR FWD    | IDT    | CCGAAGGCCAATTCACAGT |
| Cd207 - qPCR REV    | IDT    | GCGATACGAGAGTTTCTCA |
| Spp1 - qPCR FWD     | IDT    | CCGATGGGATTCCTCATG |
| Spp1 - qPCR REV     | IDT    | GCGAGGAGCCTCTCAG |
| Gata6 - qPCR FWD    | IDT    | CCGAGGAGCCTCTCAG |
| Gata6 - qPCR REV    | IDT    | CCGATGGGATTCCTCATG |
| Gpnmb - qPCR FWD    | IDT    | AGCAACCAATTCAGGG |
| Gpnmb - qPCR REV    | IDT    | CTTCCAGGAAGCTTCAG |
| Pld2q7 - qPCR FWD   | IDT    | CTTCCAGGAAGCTTCAG |
| Pld2q7 - qPCR REV   | IDT    | CCGAGGAGCCTCTCAG |
| Cd9 - qPCR FWD      | IDT    | GCTACTAGCAGCCATG |
| Cd9 - qPCR REV      | IDT    | CACTTGAGTACCAGTTCTC |
| Cd36 - qPCR FWD     | IDT    | GGAGGATACTTCAGGG |
| Cd36 - qPCR REV     | IDT    | GAAACCCACTGAGGAAT |
| Il1b - qPCR FWD     | IDT    | GCAACTTGCTGAACTCA |
| Il1b - qPCR REV     | IDT    | ATCTTTGTTGAGCTCAG |
| Tnf - qPCR FWD      | IDT    | TCTCTTCTTGGGATTCG |
| Tnf - qPCR REV      | IDT    | GGTCTGGGCCATAGAAC |
| Il10 - qPCR FWD     | IDT    | AAGGAGGAGCAGTGAA |
| Il10 - qPCR REV     | IDT    | CAGCCAGACTACAGAC |
| Il18 - qPCR FWD     | IDT    | ACTGTGACAGCGAGTAG |
| Il18 - qPCR REV     | IDT    | AGTGACAGCTTACATAC |
| Actb - qPCR FWD     | IDT    | GCTTTCTAGGGAGACTTCA |
| Actb - qPCR REV     | IDT    | GCCATGGCAATGTTGCTTAT |

Oligonucleotides: Molecular cartography probes

| FLT3 | Resolve Bio. | P0C6W |
| PDGFRB | Resolve Bio. | P0D6V |
| LGR5 | Resolve Bio. | P0E6T |
| OTOA | Resolve Bio. | P0N7M |
| CLEC10A | Resolve Bio. | P1C6X |
| COLEC11 | Resolve Bio. | P1D6W |
| GLS2 | Resolve Bio. | P1E6V |
| KRT19 | Resolve Bio. | P1N6M |
| SDS | Resolve Bio. | P1N7N |
| SIRPA | Resolve Bio. | P2C5Y |
| NGFR | Resolve Bio. | P2D6X |
| HAL | Resolve Bio. | P2E6W |
| GNLY | Resolve Bio. | P2K1J |
| SLC16A9 | Resolve Bio. | P2N7P |
| CD1E | Resolve Bio. | P3C6Z |
| HGF | Resolve Bio. | P3D6Y |
| SLC38A1 | Resolve Bio. | P3N7Q |
| ITGAX | Resolve Bio. | P4422 |
| CD4 | Resolve Bio. | P4A2X |
| ADAMTS12 | Resolve Bio. | P4D6Z |
| SLC40A1 | Resolve Bio. | P4N7R |
| CDKN1C | Resolve Bio. | P5A1X |

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### REAGENT or RESOURCE SOURCE IDENTIFIER

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| CPA3                | Resolve Bio. | P5C60     |
| MFAP4               | Resolve Bio. | P5D6L     |
| CXCR6               | Resolve Bio. | P6C61     |
| COL1A1              | Resolve Bio. | P6D60     |
| CD3E                | Resolve Bio. | P6J1Q     |
| TIMD4               | Resolve Bio. | P6N7T     |
| NCR1                | Resolve Bio. | P7A2L     |
| CXCL12              | Resolve Bio. | P7C62     |
| MARCO               | Resolve Bio. | P8C63     |
| MYH11               | Resolve Bio. | P8D62     |
| CD5L                | Resolve Bio. | P9C64     |
| ACTA2               | Resolve Bio. | P9D63     |
| IGFBP3              | Resolve Bio. | PAA11     |
| VSIG4               | Resolve Bio. | PAC65     |
| F8                  | Resolve Bio. | PAD64     |
| NKG7                | Resolve Bio. | PC13E     |
| GPMB                | Resolve Bio. | PCC66     |
| CMTM2               | Resolve Bio. | PDC67     |
| FLT4                | Resolve Bio. | PDD66     |
| CTSG                | Resolve Bio. | PDN7L     |
| CSF1R               | Resolve Bio. | PDR2R     |
| CD79A               | Resolve Bio. | PE13G     |
| EPCAM               | Resolve Bio. | PEW3P     |
| FCGR3B              | Resolve Bio. | PFC69     |
| FCGR3A              | Resolve Bio. | PFT1Q     |
| FOLR2               | Resolve Bio. | PF6E5     |
| LILRA4              | Resolve Bio. | PGC6A     |
| FCGBP               | Resolve Bio. | PGN61     |
| PLA2G7              | Resolve Bio. | PGN72     |
| DPT                 | Resolve Bio. | PGX6T     |
| CLEC4C              | Resolve Bio. | PHC6C     |
| WNT2                | Resolve Bio. | PHD6A     |
| SPN                 | Resolve Bio. | PHM1Z     |
| LHX6                | Resolve Bio. | PJD6C     |
| CD8A                | Resolve Bio. | PJU11     |
| AXL                 | Resolve Bio. | PJR2X     |
| VWF                 | Resolve Bio. | PKD6D     |
| DCN                 | Resolve Bio. | PKY4T     |
| FCN1                | Resolve Bio. | PMC6F     |
| RSPO3               | Resolve Bio. | PDM6E     |
| CCR7                | Resolve Bio. | PMH14     |
| HTRA3               | Resolve Bio. | PMJ68     |
| CCL21               | Resolve Bio. | PMM77     |
| CLEC12A             | Resolve Bio. | PNC6G     |
| GJA5                | Resolve Bio. | PND6F     |
| STAB2               | Resolve Bio. | PNJ69     |
| CD36                | Resolve Bio. | PNM78     |
| CD68                | Resolve Bio. | PPC6H     |
| LYVE1               | Resolve Bio. | PPJ6A     |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| IL7R                | Resolve Bio. | PPK14      |
| CD9                 | Resolve Bio. | PPM79      |
| MSLN                | Resolve Bio. | PQJ6C      |
| ENHO                | Resolve Bio. | PQM7A      |
| TREM2               |Resolve Bio. | PR32Q      |
| SOX9                |Resolve Bio. | PRD6J      |
| RELN                |Resolve Bio. | PRM15      |
| F13A1               |Resolve Bio. | PRM7C      |
| SPP1                |Resolve Bio. | PSD6K      |
| FCER1A              |Resolve Bio. | PSM7D      |
| MAFB                |Resolve Bio. | PTA6P      |
| CD19                |Resolve Bio. | PTH1A      |
| FCGR1A              |Resolve Bio. | PTM7E      |
| C5AR1               |Resolve Bio. | PVA6Q      |
| GDF15               |Resolve Bio. | PVM7F      |
| CD1C                |Resolve Bio. | PWA6R      |
| FCGR2B              |Resolve Bio. | PWG2K      |
| WT1                 |Resolve Bio. | PWD6P      |
| GLUL                |Resolve Bio. | PWG1E      |
| IGSF21              |Resolve Bio. | PWM7G      |
| XCR1                |Resolve Bio. | PXX6S      |
| CYP2E1              |Resolve Bio. | PXD6Q      |
| CD14                |Resolve Bio. | PXS37      |
| CLEC9A              |Resolve Bio. | PYA6T      |
| LILRB5              |Resolve Bio. | PYM7J      |
| THBD                |Resolve Bio. | PZ46V      |
| DES                 |Resolve Bio. | PZC6T      |
| GHR                 |Resolve Bio. | PZD6S      |
| NDST3               |Resolve Bio. | PZM7K      |
| Trem2               |Resolve Bio. | P0E2P      |
| WT1                 |Resolve Bio. | P0E4R      |
| Adgrg6              |Resolve Bio. | P0F4Q      |
| Lira5               |Resolve Bio. | P0M7N      |
| Itgax               |Resolve Bio. | P1C1R      |
| Dcn                 |Resolve Bio. | P1E4S      |
| Mmm1                |Resolve Bio. | P1F4R      |
| Lpl                 |Resolve Bio. | P1M7P      |
| Cd79a               |Resolve Bio. | P2662      |
| Cd36                |Resolve Bio. | P2A5Y      |
| Gpmb                |Resolve Bio. | P2H7T      |
| Marco               |Resolve Bio. | P2M7Q      |
| Cd19                |Resolve Bio. | P3663      |
| Pdgfrb              |Resolve Bio. | P3E4V      |
| Adgra1              |Resolve Bio. | P3F4T      |
| Adamts12            |Resolve Bio. | P3K7S      |
| Mfap4               |Resolve Bio. | P3M7R      |
| Lyve1               |Resolve Bio. | P4961      |
| Axl                 |Resolve Bio. | P4C1V      |
| Olfm13              |Resolve Bio. | P4E2T      |
| Atp6v0d2            |Resolve Bio. | P4K7T      |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Mmp12               | Resolve Bio. | P4M7S      |
| Csar1               | Resolve Bio. | P5K7V      |
| Msln                | Resolve Bio. | P5M7T      |
| Lhx6                | Resolve Bio. | P5T2F      |
| Fn1                 | Resolve Bio. | P6952      |
| Ccr7                | Resolve Bio. | P6K7W      |
| Cd14                | Resolve Bio. | P7K7X      |
| Nrnx1               | Resolve Bio. | P7M7W      |
| Acta2               | Resolve Bio. | P8668      |
| Cd207               | Resolve Bio. | P8K7Y      |
| Cd5f                | Resolve Bio. | P9K7Z      |
| Rapo3               | Resolve Bio. | P9M7Y      |
| Ccr2                | Resolve Bio. | PAF40      |
| Slc40a1             | Resolve Bio. | PAM7Y      |
| Gls2                | Resolve Bio. | PAN7Y      |
| Sds                 | Resolve Bio. | PCG40      |
| Clec10a             | Resolve Bio. | PCK70      |
| Spon2               | Resolve Bio. | PCM7L      |
| Pck1                | Resolve Bio. | PCN7Z      |
| Sirpa               | Resolve Bio. | PDH73      |
| Clec4f              | Resolve Bio. | PDK71      |
| Stab2               | Resolve Bio. | PDM70      |
| Krt19               | Resolve Bio. | PE07N      |
| Siglech             | Resolve Bio. | PEF43      |
| Clic5               | Resolve Bio. | PEK72      |
| Svep1               | Resolve Bio. | PEM71      |
| Cd9                 | Resolve Bio. | PFH75      |
| Col1a1              | Resolve Bio. | PFK73      |
| Timd4               | Resolve Bio. | PFM72      |
| Pecam1              | Resolve Bio. | PFP5Z      |
| Cx3cr1              | Resolve Bio. | PG35J      |
| Ncam1               | Resolve Bio. | PGE46      |
| Colec11             | Resolve Bio. | PGK74      |
| Tmem119             | Resolve Bio. | PGM73      |
| Pdgfra              | Resolve Bio. | PHG45      |
| Cox6a2              | Resolve Bio. | PHK75      |
| Upk3b               | Resolve Bio. | PHM74      |
| Cxcl12              | Resolve Bio. | PJK76      |
| Wnt2                | Resolve Bio. | PJM75      |
| Reln                | Resolve Bio. | PK54J      |
| Fcgr1               | Resolve Bio. | PK86H      |
| Myh11               | Resolve Bio. | PK95F      |
| Des                 | Resolve Bio. | PKK77      |
| Wnt9b               | Resolve Bio. | PKM76      |
| Sox9                | Resolve Bio. | PKT6L      |
| Chi3                | Resolve Bio. | PMH7A      |
| Dpt                 | Resolve Bio. | PMK78      |
| Grip1               | Resolve Bio. | PND2A      |
| Ms4a7               | Resolve Bio. | PNH7C      |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| F13a1               | Resolve Bio. | PNK79      |
| Vsig4               | Resolve Bio. | PPH7D      |
| Flt3                | Resolve Bio. | PPK7A      |
| Spp1                | Resolve Bio. | PQQ4F      |
| Flt4                | Resolve Bio. | PQQ7C      |
| Epcam               | Resolve Bio. | PRD4G      |
| Pipp1               | Resolve Bio. | PRE4F      |
| Fol2                | Resolve Bio. | PRK7D      |
| Cd3e                | Resolve Bio. | PS56T      |
| Clec9a              | Resolve Bio. | PS66S      |
| Ghr                 | Resolve Bio. | PSF4F      |
| Mafb                | Resolve Bio. | PSS66      |
| Gja5                | Resolve Bio. | PTK7F      |
| Cyp2e1              | Resolve Bio. | PVF4H      |
| Sept3               | Resolve Bio. | PW66W      |
| Vwf                 | Resolve Bio. | PWE4K      |
| Lgr5                | Resolve Bio. | PWF4J      |
| Hal                 | Resolve Bio. | PWK7H      |
| Prox1               | Resolve Bio. | PWS25      |
| Xcr1                | Resolve Bio. | PX66V      |
| Hgf                 | Resolve Bio. | PXK7J      |
| Itgae               |Resolve Bio.  | PY86W      |
| Spn                 | Resolve Bio. | PYE7R      |
| Igfbp3              | Resolve Bio. | PYK7K      |
| Cd209a              | Resolve Bio. | PYM6H      |
| Glud                | Resolve Bio. | PZ81R      |
| Itgb7               | Resolve Bio. | PZK7M      |
| Mgl2                | Resolve Bio. | PZM6J      |

**Software and algorithms**

- **Adobe Illustrator**
  - Adobe
  - [www.adobe.com](http://www.adobe.com)

- **Cell Ranger**
  - 10X Genomics
  - [https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger](https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger)

- **FastCAR R Package**
  - N/A
  - [https://github.com/LungCellAtlas/FastCAR](https://github.com/LungCellAtlas/FastCAR)

- **Seurat R Package V3**
  - (Stuart et al., 2019)
  - [https://satijalab.org/seurat/](https://satijalab.org/seurat/)

- **Scater R Package**
  - (McCarthy et al., 2017)
  - [https://bioconductor.org/packages/release/bioc/html/scater.html](https://bioconductor.org/packages/release/bioc/html/scater.html)

- **BioMart**
  - N/A
  - [https://www.ensembl.org/biomart/martview/3e2c65a5e3f783f8c9e5d648e4b64126](https://www.ensembl.org/biomart/martview/3e2c65a5e3f783f8c9e5d648e4b64126)

- **pheatmap R package**
  - N/A
  - [https://rdrr.io/cran/pheatmap/](https://rdrr.io/cran/pheatmap/)

- **ggplot2**
  - Wickham (2016)
  - [https://ggplot2.tidyverse.org](https://ggplot2.tidyverse.org)

- **Scanpy**
  - Wolf et al., 2018
  - [https://scanpy.readthedocs.io/en/stable/](https://scanpy.readthedocs.io/en/stable/)

- **PyTorch**
  - N/A
  - [https://pytorch.org](https://pytorch.org)

- **TotalVI**
  - Gayoso et al., 2021
  - [https://docs.scvi-tools.org/en/stable/user_guide/models/totalvi.html](https://docs.scvi-tools.org/en/stable/user_guide/models/totalvi.html)

- **ScVI**
  - Lopez et al., 2018
  - [https://docs.scvi-tools.org/en/stable/user_guide/models/totalvi.html](https://docs.scvi-tools.org/en/stable/user_guide/models/totalvi.html)

- **NicheNet**
  - Browaeys et al., 2019
  - [https://github.com/saeyslab/nichenet](https://github.com/saeyslab/nichenet)

- **Enrichr**
  - Kuleshov et al., 2016
  - [http://amp.pharm.mssm.edu/Enrichr/](http://amp.pharm.mssm.edu/Enrichr/)

- **FlowJo v10.6.1**
  - FlowJo
  - [https://www.flowjo.com](https://www.flowjo.com)

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RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Martin Guilliams (martin.guilliams@irc.vib-ugent.be).

Materials availability
This study did not generate new unique reagents.

Data and code availability
The datasets generated during this study have been deposited in the Gene Expression Omnibus public database under accession number GSE192742.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

In vivo animal studies
Mice
WT C57Bl/6J mice (Janvier) were used for this study. Male and Female mice were used for all experiments, with the exception of NAFLD experiments were only male mice were used. For NAFLD experiments, mice were put on the SD or WD at 5 weeks of age and sacrificed after either 24 or 36 weeks on the diet as indicated. Fcgr1-Cre mice (Scott et al., 2018) were obtained from Prof. Bernard Malissen, CIML, Marseille and Clec4f-Cre mice (Scott et al., 2016) were crossed with Acvrl1fl/fl mice (Park et al., 2008) obtained from Paul Oh, Barrow Neurological Institute, Florida, USA. Clec4f-Dtr mice (Scott et al., 2016) were crossed to CD45.1 mice (Janvier) for KC depletion and development experiments. All mice were used between 6 and 12 weeks of age unless otherwise stated. All mice maintained at the VIB (Ghent University) under specific pathogen free conditions. All animals were randomly allocated to experimental groups. All experiments were performed in accordance with the ethical committee of the Faculty of Science, UGent and VIB.
Isolation of liver cells
Liver cells were isolated by either ex vivo digestion (all species, except zebrafish) or in vivo liver perfusion (mice only) and digestion as described previously (Bonnardel et al., 2019; Scott et al., 2016). Briefly, for ex vivo digestion, livers were isolated, cut into small pieces and incubated with 1mg/ml Collagenase A and 10U/ml DNAse at 37°C for 20 mins with shaking. For in vivo digestion, after retrograde cannulation, livers were perfused for 1-2mins with an EGTA-containing solution, followed by a 5min (6ml/min) perfusion with 0.2mg/ml collagenase A. Livers were then removed, minced and incubated for 20mins with 0.4mg/ml collagenase A and 10U/ml DNAse at 37°C. All subsequent procedures were performed at 4°C. Samples were filtered over a 100 m mesh filter and red blood cells were lysed. Samples were again filtered over a 40μm mesh filter. At this point in vivo digestion samples were subjected to two centrifugation steps of 1min at 50g to isolate hepatocytes. Remaining liver cells (leukocytes, LSECs and HSCs; in vivo protocol) and total cells from the ex vivo digests were centrifuged at 400g for 5mins before proceeding to antibody staining for flow cytometry. A combination of Collagenase A and DNase were used to digest livers in both protocols to minimize cleavage of surface epitopes.

Dissected livers from 6 months old transgenic zebrafish were triturated and treated with Liberase TM at 33°C for 20 min. Cells were then filtered through 40μm nylon mesh and washed with 2% FBS in PBS by centrifugation. Sytox Red was then added to the samples at a final concentration of 5nM to exclude nonviable cells before proceeding to flow cytometry. DsRed‘GFP’ cells were then FACS-purified.

Isolation of liver nuclei
Nuclei were isolated from snap frozen liver tissue with a sucrose gradient as previously described (Habib et al., 2016). Briefly, frozen liver tissue is homogenized using Kimble Douncer grinder set in 1ml homogenization buffer with RNAse inhibitors. Homogenised

Patient studies
Patient studies were run in collaboration with Ghent University Hospital. Liver biopsies (1–2mm³) were isolated with informed consent from patients undergoing cholecystectomy or gastric bypass. In addition, liver biopsies were isolated from healthy adjacent tissue removed during liver resection due to colorectal cancer metastasis. In most cases, a second biopsy was also taken to evaluate liver histology. A full overview of all patient samples used in this study can be found in Table S6. Paraffin-embedded human liver samples were obtained through collaboration with Dr. Jan Lerut (Université Catholique de Louvain, UCL). All studies were performed in accordance with the ethical committee of the Ghent University Hospital (study numbers: 2015/1334 and 2017/0539).

METHOD DETAILS

Isolation of liver cells
Liver cells were isolated by either ex vivo digestion (all species, except zebrafish) or in vivo liver perfusion (mice only) and digestion as described previously (Bonnardel et al., 2019; Scott et al., 2016). Briefly, for ex vivo digestion, livers were isolated, cut into small pieces and incubated with 1mg/ml Collagenase A and 10U/ml DNAse at 37°C for 20 mins with shaking. For in vivo digestion, after retrograde cannulation, livers were perfused for 1-2mins with an EGTA-containing solution, followed by a 5min (6ml/min) perfusion with 0.2mg/ml collagenase A. Livers were then removed, minced and incubated for 20mins with 0.4mg/ml collagenase A and 10U/ml DNAse at 37°C. All subsequent procedures were performed at 4°C. Samples were filtered over a 100μm mesh filter and red blood cells were lysed. Samples were again filtered over a 40μm mesh filter. At this point in vivo digestion samples were subjected to two centrifugation steps of 1min at 50g to isolate hepatocytes. Remaining liver cells (leukocytes, LSECs and HSCs; in vivo protocol) and total cells from the ex vivo digests were centrifuged at 400g for 5mins before proceeding to antibody staining for flow cytometry. A combination of Collagenase A and DNase were used to digest livers in both protocols to minimize cleavage of surface epitopes.

Dissected livers from 6 months old transgenic zebrafish were triturated and treated with Liberase TM at 33°C for 20 min. Cells were then filtered through 40μm nylon mesh and washed with 2% FBS in PBS by centrifugation. Sytox Red was then added to the samples at a final concentration of 5nM to exclude nonviable cells before proceeding to flow cytometry. DsRed‘GFP’ cells were then FACS-purified.
Mice were euthanized by means of carbon dioxide (CO2) overdose. The liver was excised and consequently trimmed, on ice, to smaller sizes and then stained for protein markers as described above. Probes were hybridized and amplified according to the manufacturer’s instructions. Slides were treated with hydrogen peroxide for 10 min and protease III for 20 min. The recommended Antigen retrieval step was not performed prior to staining.

Visium highly multiplexed protein

Liver slices were prepared as described above for the classical Visium protocol. Slices were dried for 1 min at 37°C and subsequently fixed using 1% paraformaldehyde in PBS. Next, slices were blocked for 30 min (2% BSA, 0.1ug/ul Salmon Sperm, 0.5% Saponin, 10X Visium cDNA libraries were generated according the manufacturer’s instructions. In short: Tissue sections where fixed in 10X Visium capture area. Trimmed tissue pieces were embedded in Tissue-Tek OCT compound (Sakura) and snap frozen in isopentane (Sigma) chilled by liquid nitrogen. Embedded tissue pieces where stored at -80°C until cryosectioning.

Visium highly multiplexed protein

Liver slices were prepared as described above for the classical Visium protocol. Slices were dried for 1 min at 37°C and subsequently fixed using 1% paraformaldehyde in PBS. Next, slices were blocked for 30 min (2% BSA, 0.1ug/ul Salmon Sperm, 0.5% Saponin, 10X Visium capture area. Trimmed tissue pieces were embedded in Tissue-Tek OCT compound (Sakura) and snap frozen in isopentane (Sigma) chilled by liquid nitrogen. Embedded tissue pieces where stored at -80°C until cryosectioning.
immunofluorescence imaging. In brief, staining cycles consisted of the following automated steps: immunofluorescent staining, samples were then processed for a transcriptomic experiment as per manufacturer’s instructions (Visium, 10X Genomics) with modifications to also capture antibody tags. In short, tissue was permeabilized using Tissue Removal Enzyme (Tissue Optimization kit, 10x Genomics) for 9 minutes, as determined by a tissue optimization experiment (10X Genomics, Visium Spatial Tissue Optimization). After reverse transcription, 2 μl of 100 μM FB additive primer (CCTTGACACCAGAATTCC-A) per sample was added to the second strand synthesis mix. During cDNA amplification 1 μl of 0.2 μM FB additive primer (CCTTGACACCAGAATTCC-A) was added. After cDNA amplification, antibody products and mRNA derived cDNA were separated by 0.6X SPRI select. The purified full-length cDNA fraction was quantified by qRT-PCR using KAPA SYBR FAST-qPCR kit on a PCR amplification and detection instrument. After enzymatic fragmentation indexed sequencing libraries were generated via End Repair, A-Tailing, adaptor ligation and sample index PCR. The supernatant containing antibody product was cleaned up by two rounds of 1.9X SPRI select. Next, 45 μl of the purified antibody fraction was amplified with a 96 deep well reaction module: 95°C for 3 min; cycled 8 times: 95°C for 20 s, 60°C for 30 s, and 72°C for 20 s; 72°C for 5 min; end at 4°C. ADT libraries were purified once more with 1.6X SPRI select. Full length cDNA, indexed cDNA libraries and antibody libraries were analyzed using the Qubit fluorometer (Thermo Fisher) and Agilent 2100 Bioanalyzer. The separation of the cDNA and ADT libraries were performed according to the manufacturer’s instructions (10X genomics).

MICS (MACSima™ Imaging Cyclic Staining) technology on the MACSima™ Imaging System by Miltenyi Biotec B.V. & Co. KG

The MACSima™ Imaging System is a fully automated instrument combining liquid handling with widefield microscopy for cyclic immunofluorescence imaging. In brief, staining cycles consisted of the following automated steps: immunofluorescent staining, sampling, washing, multi-field imaging, and signal erasure (photobleaching or REAlease).

Cryosectioned slides on slides were taken out of the -80°C storage and the appropriate MACSWell™ imaging frame was mounted immediately on the slide. An appropriate volume of ice-cold 4% PFA solution was added (according to the MACSWell™ imaging frames datasheet) and incubated for 10 minutes at room temperature. The slide was washed three times with MACSima Running Buffer. After washing the appropriate initial sample volume of MACSima Running Buffer was added (according to the MACSWell™ imaging frames datasheet). Right before the start of the MACSima™ instrument a DAPI pre-staining was performed: the MACSima Running Buffer was removed from the sample to be analysed and stained for 10 min with a 1:10 dilution of a DAPI staining solution (volume depends on working volume for the different MACSWell™ formats, see datasheet). The DAPI staining solution was removed and 3 washing steps were performed (MACSima Running Buffer). Finally, the initial sample volume of MACSima Running Buffer was added. Details of the antibodies used can be found in the key resources table.

Molecular cartography™

Tissue sections

Liver was frozen and sectioned as described above for Visium analysis and liver slices were placed within capture areas on Resolve BioScience slides. Samples were then sent to Resolve BioSciences on dry ice for analysis. Upon arrival, tissue sections were thawed and fixed with 4% v/v Formaldehyde (Sigma-Aldrich F8775) in 1x PBS for 30 min at 4°C. After fixation, sections were washed twice in 1x PBS for two min, followed by one min washes in 50% Ethanol and 70% Ethanol at room temperature. Fixed samples were used for Molecular Cartography™ (100-plex combinatorial single molecule fluorescence in-situ hybridization) according to the manufacturer’s instructions (protocol 3.0; available for download from Resolve’s website to registered users), starting with the aspiration of ethanol and the addition of buffer BST1 (step 6 and 7 of the tissue priming protocol). Briefly, tissues were primed followed by overnight hybridization of all probes specific for the target genes (see below for probe design details and target list). Samples were washed the next day to remove excess probes and fluoroescently tagged in a two-step color development process. Regions of interest were imaged as described below and fluorescent signals removed during decolorization. Color development, imaging and decolorization were repeated for multiple cycles to build a unique combinatorial code for every target gene that was derived from raw images as described below.

Probe Design

The probes for 100 genes were designed using Resolve’s proprietary design algorithm. Briefly, the probe-design was performed at the gene-level. For every targeted gene all full-length protein-coding transcript sequences from the ENSEMBL database were used as design targets if the isoform had the GENCODE annotation tag ‘basic’ (Frankish et al., 2019; Yates et al., 2020). To speed up the process, the calculation of computationally expensive parts, especially the off-target searches, the selection of probe sequences was not performed randomly, but limited to sequences with high success rates. To filter highly repetitive regions, the abundance of k-mers was obtained from the background transcriptome using Jellyfish (Marçais and Kingsford, 2011). Every target sequence was scanned once for all k-mers, and those regions with rare k-mers were preferred as seeds for full probe design. A probe candidate was generated by extending a seed sequence until a certain target stability was reached. A set of simple rules was applied to discard sequences that were found experimentally to cause problems.

After these fast screens, every kept probe candidate was mapped to the background transcriptome using Thermonucleotide-BLAST (Gans and Wolinsky, 2008) and probes with stable off-target hits were discarded. Specific probes were then scored based
on the number of on-target matches (isoforms), which were weighted by their associated APPRIS level (Rodriguez et al., 2018), favoring principal isoforms over others. A bonus was added if the binding-site was inside the protein-coding region. From the pool of accepted probes, the final set was composed by greedily picking the highest scoring probes. Probe details are included in the key resources table.

**Imaging**

Samples were imaged on a Zeiss CellDiscoverer 7, using the 50x Plan Apochromat water immersion objective with an NA of 1.2 and the 0.5x magnification changer, resulting in a 25x final magnification. Standard CD7 LED excitation light source, filters, and dichroic mirrors were used together with customized emission filters optimized for detecting specific signals. Excitation time per image was 1000 ms for each channel (DAPI was 20 ms). A z-stack was taken at each region with a distance per z-slice according to the Nyquist-Shannon sampling theorem. The custom CD7 CMOS camera (Zeiss Axioquant Mono 712, 3.45 μm pixel size) was used.

For each region, a z-stack per fluorescent color (two colors) was imaged per imaging round. A total of 8 imaging rounds were done for each position, resulting in 16 z-stacks per region. The completely automated imaging process per round (including water immersion generation and precise relocation of regions to image in all three dimensions) was realized by a custom python script using the scripting API of the Zeiss ZEN software (Open application development).

**Spot segmentation**

The algorithms for spot segmentation were written in Java and are based on the ImageJ library functionalities. Only the iterative closest point algorithm is written in C++ based on the libpointmatcher library (https://github.com/ethz-asl/libpointmatcher).

**Preprocessing**

As a first step all images were corrected for background fluorescence. A target value for the allowed number of maxima was determined based upon the area of the slice in μm² multiplied by the factor 0.5. This factor was empirically optimized. The brightest maxima per plate were determined, based upon an empirically optimized threshold. The number and location of the respective maxima was stored. This procedure was done for every slice image independently. Maxima that did not have a neighboring maximum in an adjacent slice (called z-group) were excluded. The resulting maxima list was further filtered in an iterative loop by adjusting the allowed thresholds for (Babs-Bback) and (Bperi-Bback) to reach a feature target value (Babs: absolute brightness, Bback: local background, Bperi: background of periphery within 1 pixel). This feature target values were based upon the volume of the 3D-image. Only maxima still in a z-group of at least 2 after filtering were passing the filter step. Each z-group was counted as one hit. The members of the z-groups with the highest absolute brightness were used as features and written to a file. They resemble a 3D-point cloud.

**Final signal segmentation and decoding**

To align the raw data images from different imaging rounds, images had to be corrected. To do so the extracted feature point clouds were used to find the transformation matrices. For this purpose, an iterative closest point cloud algorithm was used to minimize the error between two point-clouds. The point clouds of each round were aligned to the point cloud of round one (reference point cloud). The corresponding point clouds were stored for downstream processes. Based upon the transformation matrices the corresponding images were processed by a rigid transformation using trilinear interpolation.

The aligned images were used to create a profile for each pixel consisting of 16 values (16 images from two color channels in 8 imaging rounds). The pixel profiles were filtered for variance from zero normalized by total brightness of all pixels in the profile. Matched pixel profiles with the highest score were assigned as an ID to the pixel.

Pixels with neighbors having the same ID were grouped. The pixel groups were filtered by group size, number of direct adjacent pixels in group, number of dimensions with size of two pixels. The local 3D-maxima of the groups were determined as potential final transcript locations. Maxima were filtered by number of maxima in the raw data images where a maximum was expected. Remaining maxima were further evaluated by the fit to the corresponding code. The remaining maxima were written to the results file and considered as candidate matches to the corresponding gene. The ratio of signals matching to codes used in the experiment and signals matching to codes not used in the experiment were used as estimation for specificity (false positives).

**Downstream analysis**

Final image analysis was performed in ImageJ using genexyz Polylux tool plugin from Resolve BioSciences to examine specific Molecular Cartography™ signals.

**RNA sequencing, CITE-seq, and qPCR**

**Sorting and RNA Isolation**

40000-160000 cells of interest from livers of the different species were FACs-purified and pelleted by centrifugation at 400g for 5 mins. To ensure sufficient numbers of all cell types were present in our analyses, depending on the sample distinct populations of cells including Live CD45⁺, Live CD45⁻, Hepatocytes, Myeloid cells and Stromal cells were FACs-purified. When CITE-seq was to be performed, cells were then stained with 2.4G2 antibody to block Fc receptors and CITE-seq antibodies for 20mins at 4°C, before being washed in excess PBS with 2% FCS and 2mM EDTA. Antibody details are included in the key resources table. 40000-100000 nuclei were also FACs-purified based on DAPI expression. These were sorted into BSA coated tubes and pelleted by centrifuging for 3 mins at 400g and 5 mins at 600g sequentially. Cells/Nuclei were then resuspended in PBS with 0.04%BSA at ~1000 cells/ml. Cell suspensions (target recovery of 8000-10000 cells) were loaded on a GemCode Single-Cell Instrument (10x Genomics, Pleasanton, CA, USA) to generate single-cell Gel Bead-in-Emulsions (GEMs). Single-cell RNA-Seq libraries were prepared using GemCode Single-Cell 3 Gel Bead and Library Kit (10x Genomics, V2 and V3 technology) according to the manufacturer’s
instructions. Briefly, GEM-RT was performed in a 96-Deep Well Reaction Module: 55°C for 45min, 85°C for 5 min; end at 4°C. After RT, GEMs were broken down and the cDNA was cleaned up with DynaBeads MyOne Silane Beads (Thermo Fisher Scientific, 37002D) and SPRINTselect Reagent Kit (SPRI; Beckman Coulter; B23318). cDNA was amplified with 96-Deep Well Reaction Module: 98°C for 3 min; cycled 12 times: 98°C for 15s, 67°C for 20 s, and 72°C for 1 min; 72°C for 1 min; end at 4°C. Amplified cDNA product was cleaned up with SPRINTselect Reagent Kit prior to enzymatic fragmentation. Indexed sequencing libraries were generated using the reagents in the GemCode Single-Cell 3’ Library Kit with the following intermediates: (1) end repair; (2) A-tailing; (3) adapter ligation; (4) post-ligation SPRINTselect cleanup and (5) sample index PCR. Pre-fragmentation and post-sample index PCR samples were analyzed using the Agilent 2100 Bioanalyzer.

**qPCR**

RNA was extracted from 10000 sorted cells (gated using strategies shown) from livers of C57BL/6 mice using a RNeasy Plus micro kit (QIAGEN). Sensifast cDNA synthesis kit (Bioline) was used to transcribe total RNA to cDNA. Real-time RT-PCR using SensiFast SYBR No-Rox kit (Bioline) was performed to determine gene expression, therefore a PCR amplification and detection instrument LightCycler 480 (Roche) was used. Gene expression was normalized to β-actin gene expression. Primers used in the study can be found in the key resources table.

**RNA sequencing analysis**

sc/snRNA-seq libraries were loaded on an Illumina HiSeq or Illumina NovaSeq 6000 with sequencing settings recommended by 10X Genomics (26/8/0/98 – 2.1pM loading concentration, ADT and cDNA libraries were pooled in a 25:75 ratio). Visium sequencing libraries were loaded on an Illumina NovaSeq 6000 with sequencing settings recommended by 10X Genomics (28/10/10/75 – 2.1pM loading concentration). Sequencing was performed at the VIB Nucleomics Core (VIB, Leuven). The demultiplexing of the raw data was performed using CellRanger software (10x – version 3.1.0; cellranger mkfakaqt which wraps Illumina’s bc2fastq). The reads obtained from the demultiplexing were used as the input for ‘cellranger count’ (CellRanger software), which aligns the reads to the mouse reference genome (mm10) using STAR and collapses to unique molecular identifier (UMI) counts. The result is a large digital expression matrix with cell barcodes as rows and gene identities as columns.

**Preprocessing data**

To remove ambient RNA, the FastCAR R package (v0.1.0) with a contamination chance cutoff of 0.05 was run on the samples separately before merging them. The UMI cut off was determined individually for the different samples, using the CellRanger web_summary output plot (see GitHub). The Scater R package (v1.14.6) was used for the preprocessing of the data. The workflow to identify the outliers, based on 3 metrics (library size, number of expressed genes and mitochondrial proportion) described by the Marioni lab (Lun et al., 2016) was followed. As a first step cells with a value x median absolute deviation (MADs) higher or lower than the median value for each metric were removed. This value was determined individually for the different datasets (see github). Secondly, the runPCA function (default parameters) of the Scater R package was used to generate a principal component analysis (PCA) plot. The outliers in this PCA plot were identified by the R package mvoutlier. By creating the Seurat object, genes that didn’t have an expression in at least 3 cells were removed. To normalize, scale and detecting the highly variable genes, the R package SCTransform (v0.2.1) was used. If batch correction (on sample level) was needed, the NormalizeData (log2 transformation), FindVariableFeatures and ScaleData functions of the Seurat R package (v3.1.2) were used in combination with the Harmony R package (v1.0). The Seurat pipeline was followed to find the clusters and create the UMAP plots. The number of principal components used for the clustering and the resolution were determined individually for the different datasets (see GitHub). On these initial UMAP plots we did multiple rounds of cleaning by removing proliferating and contaminating (e.g. doublets) cells. For non CITE-seq datasets the count data for the clean cells acquired by the previous steps were further processed with the scvi model (scvi Python package v0.6.7) (Lopez et al., 2018). Datasets including Cite-seq samples were further processed with the TotalVI model (Gayoso et al., 2021). The workflows described on scvi-tools.org were followed to generate new UMAPs, DEGs and DEPs. This information was further processed with the pheatmap R package (v1.0.12) to create heatmaps using the normalized values (denoised genes) calculated in the scVI/TotalVI workflow. The plots showing the expression of certain genes or proteins are created with the ggplot2 R package (v3.2.1) with a quantile cut off of 0.01.

For mouse all the ABs from the whitelist (181 ABs) were loaded into TotalVI, while for the other species only the added ABs were loaded into TotalVI. For the ‘human liver-pool of techniques and patients’ we noticed that the batch correction (between samples) faced difficulties for the hepatocytes and stellate cells as the cells all originated from snRNA-Seq samples, while the other cell types originated from both snRNA-seq and scRNA-seq samples. To overcome this issue we randomly allocated 30% of the hepatocytes and 70% of the stellate cells.

Heatmaps were made by scaling the normalized values (denoised values; calculated in the scVI/TotalVI workflow) using the scale_quantile function of the SCORPIUS R package (v1.0.7) and the pheatmap R package (v1.0.12). The plots showing the expression of certain genes or proteins were created based on the normalized values (denoised values) using a quantile cutoff of 0.99 and via either the ggplot2 R package (v3.2.1) or the scanpy.pl.heatmap function of the Scanpy Python package (v1.5.1).

**Conserved human-mouse KC signature**

To find the conserved human and mouse KC markers we started by identifying the human KC markers. We mapped the annotation of the human myeloid UMAP on the human pool of techniques/patients UMAP to identify the real KCs in this last UMAP. The real KCs were identified as the top part of the mac cluster. Using this new annotation we then calculated the DE genes and DE proteins for each cluster. Some genes are listed as marker for multiple clusters, only for the cluster where the gene had the highest score (raw_normalized_mean1/raw_normalized_mean2/`lfc_mean`), the gene was kept as marker. This way we found 110 potential human KC
markers. We then created a heatmap of these 110 genes (using denoised gene values scaled between 0 and 1) and filtered this heatmap by removing the genes where the scaled normalized value was higher than 0.50 in more than 30% of the cells of a certain cell type other than KCs. Except for the macs, we only removed a gene when it had a scaled normalized value higher than 0.50 in more than 70% of the macs. After this filtering we ended up with 36 human KC markers. Next we converted these human gene symbols into MGI IDs via the BioMart tool on the HGNC website (https://biomart.genenames.org/martform/#!/default/HGNC?datasets=hgnc_gene_mart). We found a MGI ID for 30 genes. We then converted these MGI IDs into mouse gene symbols via the MGI webtool (http://www.informatics.jax.org/batch/).

To identify the mouse KC markers we similarly mapped the annotation of the mouse myeloid UMAP on the mouse pool of techniques UMAP to identify the real KCs in this last UMAP. The real KCs matched with the mac cluster. Similarly as in human, the DE genes for each cluster was calculated and genes listed as marker for multiple clusters were dealt with in a similar way. This way we found 264 potential mouse KC markers. We then removed the genes that had a score (raw_normalized_mean1/raw_normalized_mean2*ffc_mean) lower than 10 and ended up with 214 genes. We then created a heatmap of these 214 genes (using denoised gene values scaled between 0 and 1) and filtered this heatmap by removing the genes where the scaled normalized value was higher than 0.50 in more than 30% of the cells of a certain cell type other than KCs. After this filtering we ended up with 68 mouse KC markers. Next we converted these mouse gene symbols into MGI IDs via the MGI webtool (http://www.informatics.jax.org/batch/). We then converted these MGI IDs into human gene symbols via the BioMart tool on the HGNC website (https://biomart.genenames.org/martform/#!/default/HGNC?datasets=hgnc_gene_mart) and ended up with 60 genes.

At this point we found 30 human KC markers and 60 mouse KC markers. In a next step, we only kept the human KC markers that we identified as a Highly Variable Gene (HVG) in the mouse pool of techniques UMAP (29 genes) and the mouse KC markers that were identified as HVGs in the human pool of the techniques UMAP (30 genes). We next put these 20 mouse KC markers in SingleCell-SignatureExplorer (Pont et al., 2019) to see where these genes are enriched in the mouse pool of techniques UMAP. In order to identified as HVGs in the human pool of the techniques UMAP (30 genes). We next put these 20 mouse KC markers in SingleCell-SignatureExplorer (Pont et al., 2019) to see where these genes are enriched in the mouse pool of techniques UMAP. In order to only get an enrichment in the KCs we decided to only use top 10 mouse KC markers (ordered on score), together with Slc40a1 and Hmox1. We then started to add the top human KC markers as long as we keep the enrichment solely in the KCs. This way we ended up with final list of 15 human-mouse conserved KC markers.

We next converted these KC markers into the monkey, pig, chicken or zebrafish orthologs by looking up the human gene symbol on NCBI (https://www.ncbi.nlm.nih.gov/search/) and checking if there is an ortholog of the species of interest listed under the ‘Ortholog’ tab. The found orthologs were then used as input for the SingleCellSignatureExplorer tool.

**Conversion of the CITE-seq data into a flow cytometry file**

The protein normalized values (denoised values; calculated in the TotalVI workflow) were converted into an FCS file using the write.FCS function of the flowCore R package (v1.50.0).

**Preprocessing Visium data**

We first removed per sample all spots that were clear outliers compared to the location of the tissue. Each sample was then normalized individually using the SCTransform function of the Seurat R package (v3.2.3) with default parameters. All samples were then merged with the merge function of the Seurat R package (v3.2.3) with default parameters. Next, we determined the HVGs, created a PCA plot, performed clustering and created an UMAP plot as described in the spatial workflow available on the Seurat website (https://satijalab.org/seurat/articles/spatial_vignette.html). Clusters which showed high mitochondrial gene expression were removed. Spots located at the darker parts of the tissue were also removed as these parts are considered to be dead tissue or of bad quality.

**Modelling of Visium data**

**Probabilistic graphical modeling**

For modelling the cell type composition and zonation, spatial CITE-seq and transcriptomics data were analyzed using probabilistic graphical models, similar to what is used in tools such as cell2location and scVI. In brief, transcriptomics data was modelled as a NegativeBinomial distribution, parameterized with a mean $\mu$ and dispersion $\theta$, the latter optimized as a free parameter for each gene. Visium Highly Multiplexed Protein data was modelled as a mixture of NegativeBinomials, with a shared foreground and a shared dispersion $\theta$. The actual foreground/background signal within a modality was modelled as a latent variable specific for each gene. $\rho$ was modelled as a Normal distribution, transformed into the correct domain using transforms $R \rightarrow R^+: \epsilon, R \rightarrow R^-: \epsilon, R \rightarrow [0,1] : \epsilon$. Free parameters within this model were optimized using gradient descent, with the ELBO as loss function and Adam as optimizer as implemented in Pytorch (Paszke et al., 2019) (pytorch.org). We used a learning rate of 0.01 for variational parameters, and 0.001 for parameters of the amortization functions.
Reference for deconvolution

To calculate the average expression of each gene within a cell type, we used a linear model in which both $\rho$ and $\theta$ were modelled as a latent variable specific for each gene and cell type. The $\rho$ for nuclei were multiplied with a gene-specific correction factor (optimized as a latent variable) that corrected for differences between scRNA-seq and snRNA-seq. Given that spatial transcriptomics data sequences the whole cell, the uncorrected $\rho$ values were used for spatial deconvolution.

Deconvolution

To infer the proportions of each cell type within a spot, we used a model in which the gene expression is modelled as a linear combination of cell type proportions and average expression in each cell type:

$$P_{\text{spot, gene}} = P_{\text{spot, celltype}} \times P_{\text{celltype, gene}}$$

For $P_{\text{celltype, gene}}$ we adapted the values from the reference, but included:

- A capture bias per gene, which corrects for technical and biological differences between spatial and sc/sn-RNA-seq. The capture bias was modelled as a latent variable with prior $\text{Normal}(0, 1)$
- A red blood cell type, which was not included in the reference dataset but nonetheless had a dominant presence in the spatial data. The $\rho$ of this cell type was set to zero for all genes except $\text{Hbb-bt}$, $\text{Hbb-bs}$, $\text{Hba-a1}$, $\text{Hba-a2}$ for mouse and $\text{HBB}$, $\text{HBA1}$, $\text{HBA2}$ for human, which were modelled as free parameters.
- Similarly, the expression of complement factors (C3, C2, C4B/C4b) within hepatocytes was modelled as free parameters.

A background signal shared for all spots was also modelled as follows:

$$P_{\text{spot, gene}} = P_{\text{spot, gene}} \times \text{lib} \times \text{foreground} + P_{\text{background}}$$

With $\text{foreground} \in [0, 1]$ a latent variable specific to each spot and $P_{\text{background}} \in \mathbb{R}$ a latent variable specific to each gene.

A likelihood ratio test was used to assess whether a cell type was significantly present in a spot. Specifically, if $x$ is the gene expression of all genes at a particular spot, we used Monte Carlo samples from the posterior to estimate:

$$\frac{P(x|P_{\text{celltype}})}{P(x|P_{\text{celltype}} = 0)}$$

A cell type was deemed significantly present if the log-likelihood was higher than 10.

Zonation

The zonation of spots was modelled as a univariate latent variable $z \sim \text{Uniform}(0, 1)$ specific to each spot. This latent variable influenced the gene expression $\rho$ using a spline function by using a gaussian basis function ($\sigma = 0.05$) with 10 knots at uniform fixed positions. The coefficients of this spline were modelled as a latent variable specific for each gene, with prior a Gaussian random walk distribution, and the step $\sim \text{Normal}(0, \sigma_{\text{gene}})$. $\sigma_{\text{gene}}$ was determined empirically as 2 times the standard deviation of the log1p transformed expression values in the whole dataset. The variational parameters of the zonation $\mu_z$ and $\sigma_z$ were not optimized directly but were estimated using an amortization function. This amortization function used the count matrix as input, and estimated the variational parameters using the following layers: Linear (with 100 output dimensions), BatchNorm, ReLU, Linear (again with 100 output dimensions), ReLU, and a final Linear layer. This amortization function was used to transfer the zonation onto a different dataset, i.e., 1) to transfer the zonation trained on mouse spatial transcriptomics onto mouse Visium highly multiplexed protein and 2) to transfer the zonation trained on human low steatosis (<10%) onto human high steatosis (>30%).

Differential abundance along zonation

To determine the differential abundance of a cell type across zonation, the significant presence of a cell type within a spot $\in \{0, 1\}$ was modelled using a spline function with the zonation of a cell type as input. The coefficients of this spline function were modelled as a latent variable with the step size $\sim \text{Normal}(0, 1)$. To determine differences in abundance between patients with high and low steatosis, we first modelled the zonation on human data on patients with steatosis < 10%. Potential interaction effects between zonation and steatosis status were then modelled using a spline function with the zonation of a cell type as input. The coefficients of this function were modelled as a latent variable specific for each gene, with prior a Gaussian random walk distribution, and the step $\sim \text{Normal}(0, \sigma_{\text{gene}})$. $\sigma_{\text{gene}}$ was determined empirically as 2 times the standard deviation of the log1p transformed expression values in the whole dataset. The variational parameters of the zonation $\mu_z$ and $\sigma_z$ were not optimized directly but were estimated using an amortization function. This amortization function used the count matrix as input, and estimated the variational parameters using the following layers: Linear (with 100 output dimensions), BatchNorm, ReLU, Linear (again with 100 output dimensions), ReLU, and a final Linear layer. This amortization function was used to transfer the zonation onto a different dataset, i.e., 1) to transfer the zonation trained on mouse spatial transcriptomics onto mouse Visium highly multiplexed protein and 2) to transfer the zonation trained on human low steatosis (<10%) onto human high steatosis (>30%).

Differential NicheNet

To analyze cell-cell communication in the hepatic macro niches, we applied Differential NicheNet, which is an extension of the default NicheNet pipeline to compare cell-cell interactions between different niches and better predict niche-specific ligand-receptor (L-R) pairs. It uses a flexible prioritization scheme that allows ranking L-R pairs according to several properties, such as niche- and region-specific expression of the L-R pair, ligand activity, and level of database curation. This in contrast to the default NicheNet pipeline which prioritizes expressed L-R pairs solely based on ligand activity predictions. All analyses were conducted according to the Differential NicheNet tutorial (https://github.com/saeylslab/nichenet/blob/master/ vignettes/differential_nichenet.md). As input to the Differential NicheNet pipeline, we used the data after normalization via SCTransform and integration of scRNA-seq and snRNA-seq according to the Seurat procedure for integration (Stuart et al., 2019).
For the mouse analyses, Differential NicheNet was first performed for the following 3 niche comparisons: 1) KCs versus central vein macs; 2) KCs versus capsule macs; 3) KCs versus LAMs. Following sender cell types were considered for these niches: KC niche: periportal hepatocytes, periportal LSECs, and periportal stellate cells; Central vein mac niche: central vein ECs and central vein fibroblasts; Capsule mac niche: mesothelial cells and capsule fibroblasts; LAM niche: cholangiocytes and bile duct fibroblasts.

Because of the preferentially periportal localization of KCs in the mouse liver, we also included a ‘region specificity’ factor in the Differential NicheNet prioritization framework. This was done to increase the ranking of ligands that are more strongly expressed in periportal than pericentral niches. Periportal sender cells were determined after subclustering based on the following markers: Hal and Sds for hepatocytes; Mecom, Msrl1, and Efnb2 for LSECs; Ngfr, Igfbp3, and Dach1 for stellate cells.

In the heatmap (Figure S6G), we show the prioritization scores of the top 40 ligands (and their highest scoring receptor) in the KC niche (score averaged over the 3 analyses), and of all the non-KC niche L-R pairs with a prioritization score ≥ the score of the lowest scoring KC L-R pair of this top 40. For each L-R pair/niche combination, we only displayed the score of the sender cell with the highest score (e.g. for the Csf1-Csf1r interaction in the KC niche, the score is shown for the LSEC-KC interaction because that score was higher than for Stellate–KC and Hepatocyte–KC; in the LAM niche, the score of Csf1-Csf1r is shown for the bile duct fibroblast – LAM interaction and not for the cholangiocyte–LAM interaction, etc.).

Because of the strong concordance between the top-ranked L-R pairs in these 3 non-KC mac niches, it was decided to also conduct a subsequent analysis in which the KC niche is compared against all non-KC hepatic mac niches combined. For this final ‘KC versus all non-KC mac analysis’, KCs were compared to central vein macs, capsule macs, and LAMs together, with the same sender cell types as described here above (but now analyzed together).

For the human analyses, Differential NicheNet was performed to compare the KC niche with the non-KC mac niches (similarly as the final analysis in mouse). For the KC niche, all hepatocytes, LSECs, and stellate cells were selected as sender cells; and KCs as receiver cells. For the non-KC mac niche, cholangiocytes, fibroblasts, and central vein ECs were considered as the sender cells; Mat. LAMs, Imm. LAMs, and Mac1s as the receiver cells (Figure 4H).

To find KC-niche-specific L-R pairs that are conserved across mouse and human, the individual mouse and human prioritization scores were averaged to form a ‘conservation score’. The 40 ligands (and maximally 3 of their highest scoring receptors) with the highest conservation score were selected for further analysis (note: the L-R pair should be expressed by the same sender-receiver pair in both species). In the circos plot (Figure 6C; Gu et al., 2014), only a subset of these top 40 ligands (and maximally 3 of their highest scoring receptors) were shown to keep the figure clearly interpretable. Following ligands were not shown: ITGA9, SEMA6D, JAM3, ITGB1 (stellate cells); ITGA9, F8, CD274, HSP90B1 (LSECs); C5, F9, F2, FGa, TF, TTR, COL18A1, COL5A3, SERPINA1, SERPINC1 (hepatocytes). The depicted target genes are KC-specific in both mouse and human, and a top-predicted target according to the NicheNet ligand-target regulatory potential scores. NR1H3 was manually added as a NOTCH2 target based on recent studies (Bonnardel et al., 2019).

**Staining of human liver paraffin sections**
Resected human liver was fixed in 4% formalin for 24-48h and subsequently embedded in paraffin. Samples were stored for 10-15 years at RT before analysis. Sections of 6 μm thick were cut using a Microm HM360 and mounted on a polarized glass slide. These sections were deparaffinized in xylene and rehydrated in a graded ethanol series. Antigen retrieval was performed by immersing the samples for 5 min in pH 8.3 TRIS-EDTA at 98°C. Slides were then cooled to RT and washed in PBS. Confocal staining was performed as described above.

**Isolation and culture of BM monocytes with acetylated LDL**
BM was isolated from the tibia and femur of mice by centrifugation. Red blood cells were lysed and single cell suspensions were stained with antibodies for flow cytometry. BM monocytes were sorted as live CD45+ CD11b+ Ly6G- Ly6C+ CD115+ cells using a BD FACSARia III. Monocytes were resuspended in DMEM/F12 media supplemented with 10% FCS, 30ng/ml CSF1, 2mM Glutamine and 100U/ml penicillin and streptomycin. 150,000 monocytes were seeded in each well of an adherent 24-well plate pre-coated with bovine collagen type I and cultured overnight (37°C, 5% CO2). The following day 0, 25 or 50ng/ml of ac-LDL was added. Ac-LDL was kindly provided by Sophie Janssens, Ghent, Belgium who received the material from Wilfried Le Goff, Paris, France. 14 hours later by flow cytometry and compared with chimerism levels in blood Ly6C+ monocytes.

**Generation of bone marrow chimeras**
Bone marrow chimeras were generated as described previously (Scott et al., 2016). Briefly, 6-12 week old Clec4f-Dtr mice (CD45.1) were anaesthetized by intraperitoneal administration of Ketamine (150 mg/kg) and Xylazine (10 mg/kg). Mice were lethally irradiated with 8 Gy, with the livers being protected with a 3-cm-thick lead cover. Once recovered from the anesthesia, mice were reconstituted by intravenous administration of 5-10 x 10^6 BM cells from CD45.2 Acvrl1^fl/fl or Fcgr1-CrexAcvrl1^fl/fl mice. 4 weeks after reconstitution mice were administrated a single dose of 500ng DT via intraperitoneal injection to deplete KCs. Chimerism was assessed 7 or 13 days later by flow cytometry and compared with chimerism levels in blood Ly6C+ monocytes.
Administration of Fc traps
Clec4f-Dtr mice were administered 10mg/kg ALK1Fc, TGFβRIIFc or appropriate isotype controls (hlgG1 and mlG2a; Acceleron Pharma) by intraperitoneal injection on days -1, 2, 3 and 5. On Day 0 mice were also administered a single dose of 500ng DT i.p. to deplete KCs. Livers were harvested at day 7 to assess KC development.

QUANTIFICATION AND STATISTICAL ANALYSIS
In all experiments, data are presented as mean ± SEM and/or individual data points are presented unless stated otherwise. Statistical tests were selected based on appropriate assumptions with respect to data distribution and variance characteristics. Details of the precise test used for each analysis can be found in the figure legends. Statistical significance was defined as p<0.05. Sample sizes were chosen according to standard guidelines. Number of animals/patients is indicated as “n”. The investigators were not blinded to the group allocation, unless otherwise stated.

ADDITIONAL RESOURCES
The sc/snRNA-sequencing, CITE-seq FCS files and spatial transcriptomics datasets will be made available for visualization, analysis and download at www.livercellatlas.org.
Cells were isolated from livers of healthy C57BL/6 mice by either ex vivo or in vivo enzymatic digestion. Alternatively, livers were snap frozen and nuclei subsequently isolated following tissue homogenization by a sucrose gradient (3 mice per isolation method). Live cells/intact nuclei were identified and purified using flow cytometry. For the cells, either live CD45+ or live CD45−/CD0 or live hepatocytes were sorted. 1 ex vivo digested sample and 1 in vivo digested sample were also stained with a panel of 107 (ex vivo cells) or 161 (in vivo cells) oligo-conjugated antibodies for CITE-seq analysis. FACS-purified cells/nuclei were loaded onto the 10x chromium platform and scRNA-seq, CITE-seq, or snRNA-seq performed. Following clean up and QC, cells from the same mice were pooled together in the same ratios (CD45+:CD45−/CD0:Heps) as found in the tissue as a whole before sorting, different mice were then pooled together and the data were analyzed using scVI.

Figure S1. Cell types identified in transcriptomic studies depend upon cell/nuclei isolation technique used, related to Figure 1

(A–C) UMAPs showing annotations of cell types and proportions of each cell type as a % of total cells in the UMAP isolated using (A) ex vivo digestion; 13,144 cells, (B) in vivo digestion; 24,014 cells and (C) nuclei; 8,583 nuclei.

(D) Average number of genes/cell in the annotated mac, B cell, hepatocyte, endothelial, and stromal cell populations following each isolation method. *p < 0.05 one-way ANOVA with Bonferroni post-test per cell type.

(E) Correlation plot showing genes captured within the mac population when the liver is digested with the in vitro versus the in vivo digestion protocol.

(F) Correlation plots showing genes captured within the mac, endothelial cell, and hepatocyte populations when cells are isolated using the in vivo digestion protocol or nuclei are isolated.

(G–L) Confocal microscopy images to determine true abundance of (G) stromal cells and cholangiocytes (H) endothelial cells, (I) macs, (J) dendritic cells, (K) B cells, and (L) T cells in vivo. Scale bars, 200 μm.

(M) The percentage of each population was calculated based on the percentage of a given population divided by the total number of nuclei. A threshold was applied to the DAPI channel (picture 1) in ImageJ (picture 2) and nuclei were automatically counted based on the ImageJ “analyze particles” plugin (size). Due to the density of some liver zones, some nuclei were not automatically counted (arrow, picture 3). Those were then manually counted and added to the total number of nuclei. For the populations of interest, cells were counted manually based on specific markers (for example, CD3 for T cells, picture 4). Counting was performed blinded prior to analysis of the sequencing results.

(N) Proportion of indicated cell types as a % of total cells identified in confocal microscopy images. Data are from 3–7 images per cell type taken from 2–4 mice.
Figure S2. Combination of CITE-seq, scRNA-seq, snRNA-seq, and spatial analyses enables identification of all hepatic cell types including bona fide cell doublets, related to Figure 1.

(A and B) Top DEGs (A) and DEPs (B) for cell types from Figure 1B.

(C) Distinct profiles of cells or nuclei within the UMAP depending on isolation protocols; 71,162 cells from ex vivo digestions, 96,066 cells from in vivo digestions, and 18,666 nuclei. Numbers on plots represent numbers of cells/nuclei per population.

(D) Correlation plots showing genes captured within the KC, B cell and neutrophil populations with and without addition of CITE-seq antibodies.

(E) Expression of VSIG4, CD206, and ESAM (protein, top) and Vsig4, Mrc1, and Esam (mRNA, bottom).

(F) UMAP showing clusters of cells when only minimal QC for gene number and % mitochondrial genes is performed; 17,669 cells pooled from 3 samples. Expression of Cd5l, Cd19, and Kdr by the clusters facilitating identification of cell types per annotation.

(G) CITE-seq data from (F) in Flow-Jo showing expression of CD206 and ESAM in total KCs (left) and total B cells (middle). Numbers represent % of entire KC or B cell population. Identified populations were then mapped back onto the original UMAP (right).

(H) Expression of CD31, CD26, and CD38 by indicated populations.

(I) Heatmaps showing expression of top DEGs between KC1s and LSECs (left), KC2s and KC1s + LSECs (middle) and B cell2s and B cell1s + LSECs (right).

(J) 3D reconstruction of murine liver following perfusion with antigen fix to inflate endothelial cells and staining with antibodies against CD31, CD206, and F4/80.

(K) UMAP showing clusters generated from Visium analysis of liver tissue (4 samples) and liver capsule (1 sample).

(L) Top unbiased genes defining zonation trajectory from portal to central vein in Visium.

(M) Expression of Glul and Epcam by confocal microscopy (left), annotation of portal, periportal, mid, and central regions on same tissue section (middle) and overlay of both datasets (right).

(N) Identification of cholangiocyte (left) and cDC (right) signatures on zonated Visium spots.

(P) Molecular Cartography showing expression of indicated zonated hepatocyte mRNAs in liver tissue. Data are representative of 2 mice.

(Q) Expression of Igtae (encoding CD103) in the UMAP of the total liver (left) and flow cytometric analysis of total cDC1s for CD103 and MHCII expression in the healthy murine liver (right).
Figure S3. Validated flow cytometry gating strategy for murine myeloid cells, related to Figure 2

(A) CITE-seq data from the murine myeloid cells in Figure 2A were exported as an FCS file and an in silico gating strategy identified in FlowJo software.
(B) Application of the in silico gating strategy with a 21-color flow cytometry panel. Myeloid cells were pre-gated as live CD45+ lineage cells (Ly6G-CD19−NK1.1−B220−CD3−). Data are representative of 3 experiments with 3–6 mice per experiment.
(C) cDC1s, cDC2s, migratory cDCs (Mig. cDCs), peritoneal macs (Peri. Macs), KCs, and non-KC macs (non-KCs) were FACS-purified using gating strategy in (B), mRNA was isolated and qPCR performed to examine expression of indicated genes defining each population to validate their identity. Data are representative of 2 experiments with n = 3–6.
(D) Putative peritoneal macs were FACS-purified using gating strategy in (B) and expression of Gata6 was examined by qPCR compared with other hepatic myeloid populations. Data are from a single experiment with n = 6.
(E) Peritoneal macs as a % of total macs recovered from the liver using different digestion techniques (in vivo, ex vivo, or capsule) or in supernatants in which livers were washed following removal from the mouse but prior to digestion (wash). Data are from a single experiment with n = 4. *p < 0.05, **p < 0.01 one-way ANOVA with Bonferroni post-test compared with wash data.
(F) Expression of CD14 and CD207 within the non-KC mac population from (B) (left) and % of CD207+ and CD207− populations among total macs in livers digested using the ex vivo or in vivo protocols or in dissected and digested liver capsule (right). Data are representative of two experiments with n = 4–5 mice per experiment. ****p < 0.0001 mixed effects analysis with Tukey's multiple comparison test.
(G) Expression of VSIG4, F4/80, GLUL, and DAPI by confocal microscopy. Insets represent zones featured in Figures 2E, 2G, and S3I.
(H) Molecular Cartography of indicated genes and cell types. Insets represent zones featured in Figures 2F, 2H, and S3J.
(I) Expression of VSIG4, F4/80, GLUL, and DAPI by confocal microscopy at the central vein. Scale bars, 50 μm.
(J) Molecular Cartography of indicated genes and cell types at central vein.
(K) Expression of F4/80, EPCAM, CCR2, GPNMB, and DAPI by confocal microscopy at a portal vein (top) or F4/80 or GPNMB alone (bottom). Scale bars, 25 μm.
(L) Quantification of % of Gpnmb & Trem2 counts over Adgre1 counts in indicated regions of tissue as assessed using Molecular Cartography data. Each dot represents an individual region. *p < 0.05, ****p < 0.0001 one-way ANOVA with Bonferroni post-test.
(M) Expression of DESMIN and F4/80 at the liver capsule and underlying parenchyma (left) or EPCAM, DESMIN and F4/80 at the bile duct by confocal microscopy. PV, portal vein; CV, central vein; HA, hepatic artery; BD, bile duct. Arrows indicate specific cell types, where color corresponds to cell type/markers. All images are representative of 2–6 mice.
Figure S4. Protein markers of murine CD45<sup>+</sup> cell subsets, related to Figure 3
(A) CITE-seq data from the murine CD45<sup>+</sup> cells in Figure 3A were exported as an FCS file and an in silico gating strategy identified in FlowJo.
(B) Gated cell overlay of populations identified using strategy in (A).
(C) Expression of CD90, CD204, CD73, and CD29 markers by indicated cell types.
(D) Expression of indicated protein markers in 60-plex MICS analysis in endothelial cells.
(E) Expression of DESMIN, EPCAM, LYVE1, and CD31 at a portal triad (left) with inset (right).
(F) Expression of indicated protein markers in 60-plex MICS analysis in stromal cells.
(G) Molecular Cartography of indicated genes and cell types at portal vein. PV, portal vein; CV, central vein. Arrows indicate specific cell types, where color corresponds to cell type/markers. All images are representative of 2–6 mice.
**Figure S5. Combination of CITE-seq, scRNA-seq, snRNA-seq, and spatial analyses enables generation of a human liver atlas, related to Figure 4**

(A) Murine lymphoid cells (B cells, T cells, NK cells, ILC1s, pDCs; 27,398 cells) were isolated from Figure 1B and re-clustered with TotalVI.

(B and C) Top DEGs (B) and DEPs (C) for the cell types from Figure S5A.

(D) CITE-seq data from Figure S5A were exported as an FCS file and an in silico gating strategy identified in FlowJo.

(E) Human lymphoid cells (B cells, T cells, NK cells, ILC1s, pDCs; 105,790 cells) were isolated from Figure 4B and re-clustered with TotalVI.

(F and G) Top DEGs (F) and DEPs (G) for the cell types from Figure S5E.

(H) CITE-seq data from Figure S5E were exported as an FCS file and an in silico gating strategy identified in FlowJo.

(I) Proportion of indicated cell types arising from patients with <10% (purple) or >10% steatosis (yellow).

(J) Hepatic cells were isolated from 22 C57BL/6 mice fed either a standard diet (SD) or a western diet (WD) for 24 or 36 weeks to induce NAFLD and NASH by ex vivo (10 samples) or in vivo (12 samples) enzymatic digestion. Alternatively, livers were snap frozen and nuclei isolated by tissue homogenization (14 samples). Live cells/intact nuclei were purified using FACS. For cells, total live, live CD45+ live CD45-, live hepatocytes or myeloid cells (live CD45+, CD3-, CD19-, B220-, NK1.1-) were sorted. 10 samples were also stained with a panel of 107-161 barcode-labeled antibodies for CITE-seq analysis. All datasets were pooled together and after QC 121,980 cells/nuclei were clustered using TotalVI.

(K) Murine lymphoid cells (B cells, T cells, NK cells, ILC1s, pDCs; 21,322 cells) from mice fed the SD or WD for 24 or 36 weeks were isolated from Figure S5J and re-clustered with TotalVI.

(L) Proportion of indicated cell types arising from mice fed the SD (purple) or WD (yellow).

(M) Top DEGs between CTLs isolated from mice fed the SD (purple) or WD (yellow).
Figure S6. Combination of CITE-seq, scRNA-seq, snRNA-seq, and spatial analyses enables generation of a human liver atlas and identification of bona fide human KCs, related to Figure 4.

(A and B) Top DEGs (A) and DEPs (B) for the cell types from Figure 4B.

(C) Distinct profiles of cells or nuclei within the UMAP depending on isolation protocol used; 152,535 cells from *ex vivo* digestions and 15,063 nuclei.

(D) Proportion of each cell type per patient profiled.

(E) Proportion of indicated cell types as a % of total CD45+ cells calculated from *ex vivo* digested samples per surgery type. Ch; cholecystectomy, Re; resection, GB; gastric bypass, *p < 0.05; one-way ANOVA with Bonferroni post-test.

(F) Mapping of Visium UMAP zonation patterns onto tissue sections from patient H35 and H37.

(G) Expression of indicated zonation genes in patients H35–H38 assessed by Molecular Cartography.

(H and I) Expression of indicated proteins by MICS 100-plex protein analysis in the healthy (H) and steatotic (I) human liver.

(J) Murine myeloid cells (cDC1s, cDC2s, Mlg, cDCs, Macs, monocytes, and monocyte-derived cells; 42,922 cells) from mice fed the SD or WD for 24 or 36 weeks were isolated from Figure S5J and re-clustered with TotalVI.

(K) Distribution of cells in UMAP originating from SD- (purple) or WD- (yellow) fed mice.

(L) Proportion of indicated cell types arising from mice fed the SD (purple) or WD (yellow).

(M and N) Flow cytometry analysis of indicated cell populations in SD and WD-fed mice (24 weeks). Representative gating strategies (M) and absolute number of indicated populations (N). *p < 0.05, **p < 0.01 Student’s t test. Data are from 2 independent experiments with n = 5–6 per diet.

(O and P) Top DEGs (O) and DEPs (P) for cell types from Figure 4H.

(Q) Top 25 Murine KC genes as expressed by the human myeloid cell clusters.

(R) Mapping of KC signature onto Visium trajectory for healthy (purple) and steatotic (orange) livers.

(S) Expression of VSIG4 mRNA within human myeloid cells.

(T) Expression of VSIG4 (red) and CD163 (gray, top) or CD169 (gray, bottom) by MICS analysis in healthy human liver.

(U) Representative images showing KC location (red) as assessed by MICS analysis in the healthy (left) and steatotic (right) human liver. PV, portal vein; CV, central vein, dashed line indicates zones of steatosis.

(V) Representative image of CD68 and CD163 staining in 10–15-year-old human liver paraffin sections. Image is representative of 6 different patients.

(W) In silico gating strategy to isolate distinct myeloid cell populations identified from CITE-seq data.

(X) Expression of VSIG4 and FOLR2 by live CD45+ cells also expressing CD14 in indicated human liver biopsies by flow cytometry. Data are representative of 21 biopsy samples analyzed.
Figure S7. Conserved and unique features of KCs across species, related to Figure 4

(A and B) Expression of human-murine KC signature genes across cell types in mouse (A) and human (B).

(C) Unbiased identification of KCs in mouse and human using the human-murine KC signature and the signature finder algorithm (Pont et al., 2019).

(D–H) Annotated UMAPs from indicated species and expression of top KC-specific genes compared with other cells per species.

(I) Expression of previously identified core murine transcription factors (Bonnardel et al., 2019) by KCs across species.

(J) Venn diagram showing convergence and divergence of expression of top 50 KC genes per species across species, see Table S9 for genes lists per species.

(K) Top DEPs (identified with cross reactive human antibodies) in the pig CITE-seq data.

(L) Expression of VSIG4 in the porcine liver by confocal microscopy.

(M) Expression of VSIG4, CD68 (protein), and CD5L (mRNA) in macaque liver. PV, portal vein; HA, hepatic artery; BD, bile duct. All images are representative of 2 livers.

(N and O) Conserved expression of indicated genes across CD45⁺ (N) and CD45⁻ (O) cell types and species.
Figure S8. Evolutionarily conserved signals regulate LAM and KC development, related to Figures 5 and 7

(A) Confocal microscopy of healthy human liver showing expression of indicated markers. Scale bars, 200 μm.

(B and C) Expression of conserved human-murine bile-duct LAM signature in human (B) and mouse (C) hepatic myeloid cells.

(D) Proportion of indicated myeloid cell populations as a % of total myeloid cells in human liver biopsies profiled by scRNA-seq when divided based on presence of steatosis.

(E) Mice were fed a western diet (WD) or standard diet (SD) for 36 weeks to induce NAFLD and Visium analysis was performed. Analysis is pooled from 1 liver slice from the SD condition and 3 liver slices from the WD condition. Shown are cluster and sample annotations.

(F) Zonation of all cell types from Figure S5J in murine NAFLD map (SD&WD).

(G) Differential NicheNet highlighting prioritized conserved (human-mouse) ligand-receptor (LR) pairs between indicated macs and their niche cells. LR pairs are grouped according to the niche cell type with highest ligand expression.

(H) Expression of ALK1 (ACVRL1), BMP9 (GDF2), and BMP10 in human, mouse, and macaque livers where both KCs and stellate cells were profiled.

(I) Livers were harvested from Clec4f-CreAcvrl1fl/fl mice or Acvrl1fl/fl controls and KCs examined (top) and quantified (middle) using VSIG4 expression. Expression of indicated KC markers by mac populations in Clec4f-CreAcvrl1fl/fl or Acvrl1+/+ control mice (bottom). Data are pooled from 2 independent experiments with n = 14 per group. Student’s t test. ****p < 0.0001.

(J) Expression of CD31 (ECs), DESMIN (stromal cells), F4/80 (Macs), and EPCAM (cholangiocytes) by confocal microscopy in Fcgr1-CrexAcvrl1fl/fl mice and Acvrl1+/+ controls. PV, portal vein; CV, central vein. Images are representative of 2 mice per group.