## Methods

### *Assessment of peripheral blood contamination in FNA passes*

After the FNA procedure, 5 µL of each aspirate was lysed with 45 µL of 1x red blood cell (RBC) lysis buffer (BD Bioscience) and the optical density (OD) was measured at 450 nm (OD450) (OD450) as a proxy for the amount of RBC contamination in the FNA. As a reference blood contamination was also evaluated by the relative frequencies of major T cell populations, determined by flow cytometry. Aspirates with the lowest OD value were selected for downstream analysis.

### *Red blood cell depletion of fine needle aspirates and peripheral blood*

RBCs in the FNA were removed using magnetic bead-based depletion. Briefly, 12.5 µL of EasySep RBC Depletion Reagent (STEMCELL Technologies Inc.) was added to each FNA pass and incubated for 5 min before placing the sample on the magnet for 5 min (EasySep violet magnet, STEMCELL Technologies Inc.). The FNA cell suspension was poured into another 5 mL polypropylene tube and the procedure was repeated. The RBC depleted cell suspension was then manually counted using trypan blue and a haemocytometer (Neubauer Improved, INCYTO).
Paired whole blood was diluted to 1000 µL using MACS buffer (PBS, 1% BSA and 6 mM EDTA), and RBCs were depleted by addition of 25 µL of EasySep RBC Depletion Reagent.) and incubated for 5 min before placing the sample on the magnet for 5 min (EasySep violet magnet, STEMCELL Technologies Inc.). The blood cell suspension was poured into another 5 mL polypropylene tube and the process was repeated. The RBC depleted cell suspension was then manually counted using trypan blue and a haemocytometer (Neubauer Improved, INCYTO). Magnetic bead RBC depletion retained granulocytes that are normally lost via density gradient peripheral blood mononuclear cell (PBMC) preparation.

For PBMCs, approximately 50 mL of blood was collected in 8.5ml acid-citrate-dextrose (ACD) tubes by standard venipuncture at the time of FNA collection. PBMCs were isolated by standard density gradient centrifugation using Ficoll.

***Flow cytometric analysis to quantify peripheral immune cell contamination***

After the OD450 of individual FNA passes were collected and RBCs were depleted, we quantified the frequency of naïve CD8 and CD4 T cells and mucosal-associated invariant T cells in individual FNA passes. Each FNA pass was stained with a viability dye and the following antibodies for 30 min at 4 °C to identify the above-mentioned immune cell populations: CD3, CD4, CD8, Valpha7.2, CD161, CCR7 and CD45RA. The frequency of each population was calculated as a percent of total T cells in the FNA (CD3+) and plotted against OD450 values.

***Cryopreservation of PBMC and FNAs***

Any remaining FNA cells and PBMCs were cryopreserved in KnockOut serum Replacement (KO serum, Gibco) with 10% DMSO. Briefly, freezing media A (KO serum alone) and freezing media B (KO serum with 20% DMSO) were prepared on the day of collection. After centrifuging the cells for 5 min at 300g, supernatant was removed and cells were resuspended in freezing media A. An equal volume of freezing media B was added drop by drop, with gentle mixing, giving a final DMSO concentration of 10%. The samples were aliquoted into 1.5 mL cryovials and cryopreserved by cooling to -80 °C in ''Mr. Frosty'' freezing containers and then moved for long-term storage in -150 °C freezers or liquid nitrogen.

***Seq-Well $S^3$ transcriptomic profiling***

The Seq-Well $S^3$ protocol was performed as detailed previously, with several adjustments to improve clinical utility[1]. After RBC depletion, cells were diluted to a concentration of 75,000 cells/mL when possible. A suspension of 200 µL was then loaded onto Seq-Well $S^3$ arrays pre-loaded with mRNA capture beads, by adding them dropwise in a zig-zag pattern. When the starting cell suspension was already more dilute, cells were not centrifuged to avoid loss or damage. Instead, an appropriate amount of volume was added in the same dropwise fashion to achieve the same total cell number. This resulted in a larger volume of cell suspension on top of the array, and extra care was taken to make sure that the solution remained on top of the array.

Following membrane sealing for 30 min at 37 °C, samples underwent one of two possible paths. Fresh samples were directly processed on site through cell lysis, hybridization, bead

isolation, and reverse transcription as previously described[1]. Frozen samples were placed horizontally in 50 mL conical tubes and immediately stored at -80 °C. Up to two weeks later, the 'frozen' samples were placed into complete lysis buffer, the top slides were removed, and the samples proceeded with hybridization, bead isolation, and reverse transcription as previously described. Reverse transcription was performed the same for all samples: 30 minutes at room temperature followed by overnight (18 hr) at 52 °C, both with end-over-end rotation (Hula Mixer, Thermo fisher). After reverse transcription, samples underwent exonuclease treatment, second strand synthesis, whole transcriptome amplification, and Illumina Nextera XT Library preparation. Two SPRI bead-based PCR clean-up steps were performed following both the WTA and Illumina Nextera XT Library Prep Kits library preparation steps. Each time, first a 0.6x and then a 0.8x volume ratio cDNA:SPRI-bead was performed. Sequencing was performed on either a NextSeq 500/550 instrument with a High Output Flowcell and a 75-cycle kit (PE 20/50) or a NovaSeq 6000 instrument with a S4S4 Flowcell and a 100-cycle kit. Samples were demultiplexed according to the Illumina protocols and indices used. Samples were sequenced to an average depth of 1M reads per Seq-Well array.

***10x genomics transcriptomic profiling***

Samples were prepared as outlined by 10x Genomics Single Cell 3' Reagent Kits v2 user guide. Briefly, samples were washed two times in PBS (Life Technologies) + 0.04% BSA (Miltenyi) and re-suspended in PBS + 0.04% BSA before sample viability was assessed using a haemocytometer (Thermo Fisher Scientific). Following counting, the appropriate volume for each sample was calculated for a target capture of 2000 or 3000 cells. Samples that were too low in cell concentration as defined by the user guide were washed, re-suspended in a reduced volume, and counted again using a haemocytometer prior to loading onto the 10x single cell A chip. After droplet generation, samples were transferred onto a pre-chilled 96-well plate (Eppendorf), heat sealed and incubated overnight in a Veriti 96-well thermocycler (Thermo Fisher). The next day, sample cDNA was recovered using Recovery Agent provided by 10x Genomics and subsequently cleaned up using a Silane DynaBead (Thermo Fisher Scientific) mix as outlined by the user guide. Purified cDNA was amplified for 12 cycles before being cleaned up using SPRIselect beads

(Beckman). Samples were diluted 4:1 (elution buffer (Qiagen) : cDNA) and run on a Bioanalyzer (Agilent Technologies) to determine the cDNA concentration. cDNA libraries were prepared as outlined by the Single Cell 3' Reagent Kits v2 user guide with modifications to the PCR cycles based on the calculated cDNA concentration. The molarity of each library was calculated based on library size as measured with the Bioanalyzer and qPCR amplification data (Sigma). Samples were pooled and normalized to 10 nM, then diluted to 2 nM using elution buffer (Qiagen) with 0.1% Tween20 (Sigma). Each 2 nM pool was denatured using 0.1N NaOH at equal volumes for 5 minutes at room temperature. Library pools were further diluted to 20 pM using HT-1 (Illumina) before being diluted to a final loading concentration of 14 pM. 150 µl from the 14 pM pool was loaded into each well of an 8-well strip tube and loaded onto a cBot (Illumina) for cluster generation. Samples were sequenced on a HiSeq 2500 with the following run parameters: Read 1 – 26 cycles, read 2 – 98 cycles, index 1 – 8 cycles.

***ScRNAseq data preprocessing***

Seq-Well $S^3$ libraries were pre-processed using the DropSeq pipeline with the tools v2.3.0 as previously described[1]. Briefly, pooled libraries were demultiplexed using bcl2fastq v2.20.0.422 with the following settings: *mask_short_adapter_reads=15* and *minimum_trimmed_read_length=35*. Read alignment was done using STAR 2.5 and the human genome assembly reference GRCh38 (hg38). Aligned cell by gene matrices for each sample were merged across all conditions tested and participants. Preprocessing, alignment, and data filtering was applied equivalently to all samples. Cells with less than 500 UMIs or less than 300 genes were removed from downstream analysis.

Data was log-normalized with a scaling factor of 10,000. The top 2,000 most variable genes as determined by the 'vst' method implemented as the FindVariableFeatures function were selected and scaled using a linear model implemented as the ScaleData function, both in the Seurat (v3.1.5) package with version 4.0.2 of R programming language. After, principal component analysis (PCA) was run, the number of significant principal components (PCs) to be used for downstream cell clustering was determined using Jackstraw with a p-value cut-off of 0.05. The best resolution for clustering was determined using an average silhouette scoring

across all clusters, testing 40 resolutions between 0.1 and 2 as previously implemented in Ziegler et. al[2]. Marker genes for each cluster were calculated using the FindAllMarkers function (method = 'wilcox') implemented in Seurat and each cluster was iteratively subclustered further using the same approach. Statistical significance was determined using the Wilcoxon Rank sum test with Bonferroni correction (adjusted p-value < 0.05). Subclustering was stopped when the resulting clusters were not meaningfully different. Clusters were annotated as cell type populations based on the expression of genes that are known markers of specific cells. Final marker genes for each intermediary and refined cell type were determined using FindAllMarkers method = "Wilcox" and can be found in Supplementary Table 1.

***Differential frequency analysis between blood and FNA***

For each annotated cell population, cell type frequencies per participant were calculated and compared between FNA and blood (whole blood or PBMC, except where noted). Participants with '0' cells within a cell population at the main clustering level were excluded from the analysis. Cell type frequencies were calculated per participant as the fraction of cells within a cell population – e.g., the number of *TCL1A*+ naive B cells within the whole B cell population. The Wilcoxon signed-rank test was used to compare the frequency of each cell population across FNA and blood samples and *p*-values were Bonferroni corrected for multiple hypothesis testing.

***Differential expression analysis between blood and FNA***

Differential expression analysis was performed to compare the transcriptomic profiles between blood and liver FNA samples within each cell population using the same subset of cells as described for the differential frequency analysis. For each group, a minimum of 5 cells was required to ensure that the samples size was sufficient for the analysis. Read counts were normalized with $\log_2$(count+1), and normalized values smaller than '1' were set to '0'. The R-package *MAST* was used to obtain hurdle *P*-values which were Bonferroni corrected for multiple hypothesis testing. To remove participant-specific bias in the analysis, participant IDs were treated as covariates. Cohen's D effect sizes were calculated with R-package *effsize*. Significant

genes were determined by corrected *P*-values < 0.05 and Cohen's D cutoff for each cell type were calculated as mean + 2 x standard deviations of Cohen's D for all genes.

***Gene set enrichment analysis***

Genes that were differentially expressed between blood and liver FNA, cohen's D > 0.5 were assessed for the overrepresentation of gene sets related to biological states or processes. For this enrichment test, 50 hallmark gene sets with gene symbols were downloaded from the Molecular Signatures Database (MSigDB; http://www.gsea-msigdb.org/gsea/msigdb/genesets.jsp). *P*-values were calculated based on permutations using R-package *fgsea*. The enrichment score (ES) statistic is calculated based on a vector of gene-level signed statistics, such as t-statistic from a differential expression test. FDR was adjusted based on Benjamini Hochberg (BH) correction.

***Statistical Analysis***

GraphPad Prism 9.3.0 (GraphPad Software, San Diego, CA, USA) and R version 4.0.2 was used to evaluate the data. The statistical analysis performed for each experiment and the number of replicates is included in the respective method section and figure legends. P <0.05 was defined as statistically significant. Statistical significance for genes on dot plots and violin plots were done using the Wilcoxon Rank sum test with Bonferroni correction (adjusted p-value < 0.05). Statistical significance for comparison between fresh and frozen samples were done using paired t test. Differences in cell frequency between compartments were determined using the Wilcoxon Signed-Rank test with Bonferroni correction. Statistical significance for genes on volcano plots were done using Cohen's D values which were extracted from hurdle models and p values were adjusted for multiple testing with Bonferroni correction. For GSEA, we used the fold change and adjusted p values obtained from differential expression analysis done on Volcano plots with cohen's D>0.5. FDR was adjusted with Benjamini Hochberg (BH) correction. Each test is indicated in the specific sections and in figure legends for clarity.

**Supplemental Figures**

Supplemental Figure 1. Flow cytometry gating strategy to identify naïve CD4 T cells, naïve CD8 T cells and MAIT cells in liver FNA samples.

Supplemental Figure 2. Seq-Well $S^3$ vs. 10x Genomics quality control metrics. **(a)** Number of transcripts **(b)** number of genes and **(c)** cell captured from liver FNAs by technology. Number of transcripts from each cell showed a significant difference, paired Student's T Test for difference of the mean of medians p=0.044. Number of genes from each cell in liver FNAs showed a significant difference, paired Student's T Test for difference of the mean of medians p=0.013. Number of cells from liver FNA sample showed a significant difference, paired Student's T Test for difference of the mean p=0.039. **(d)** Number of transcripts **(e)** number of genes and **(f)** cell captured from PBMC by technology. Only the number of genes show significant differences in the blood; paired Student's T Test for difference of the mean of medians p=0.034. Seq-Well colors orange, 10x colored in blue.

Supplemental Figure 3. Intrahepatic cell capture using different isolation methods. **(a)** Clustering and annotation of intrahepatic cells isolated by different methods (CD45 sorting, FNAs and tissue digestion) using UMAP dimensionality reduction. (**b)** UMAP dimensionality reduction of intrahepatic cells by different isolation methods**. (c)** Comparison of cell frequencies between different isolation methods. **(d)** Dot plot showing the top 2 cluster defining genes. FNA, fine needle aspirates; pDCs, plasmacytoid dendritic cells.

Supplementary Figure 4. CD4 T cell composition in liver vs blood. **(a)** scRNAseq UMAP for CD4 T cells colored by cluster IDs. **(b)** scRNAseq UMAP colored by cluster and split based on tissue of origin, i.e. liver and blood. **(c)** Comparison of cell frequencies between blood and liver within sample (connected through grey lines) for each CD4 cluster. ITGB1+ and CCR7+ CD4 are significantly more in FNA and blood, respectively. Significance was determined using the Wilcoxon Signed-Rank test with Bonferroni correction (adjusted p-value < 0.05). **d)** Dot plot

showing top 10 marker genes for each cluster ID. **(e)** Violin plot showing the top 5 marker genes for CD4 T cell clusters. Significance was determined using the Wilcoxon Rank sum test with Bonferroni correction (adjusted p-value < 0.05). **(f)** Volcano plots depicting differences in gene expression CD4 T cells between compartments. The R-package *MAST* was used to obtain hurdle *P*-values which were Bonferroni corrected for multiple hypothesis testing. Positive Cohen's D value suggests higher expression in liver. Cohen's D cutoff calculated as mean + 2 x standard deviation of Cohen's D values of all genes. **(h)** Hallmark genesets enriched by CD4 T cell clusters. Normalized enrichment score was calculated based on a vector of gene-level signed statistic and false discovery rate was adjusted based on Benjamini Hochberg (BH) Correction. X-axis represents signed $\log_{10}$ of adjusted P-value for the genesets, and positive value suggests enrichment in liver.

Supplementary figure 5. NK cell composition in liver and blood. **(a)** scRNAseq UMAP for NK cells colored by clusters. **(b)** Violin plot showing relevant marker genes for NK cell clusters. **(c)** Dot plot showing top 10 marker genes for each cluster ID. Significance was determined using the Wilcoxon Rank sum test with Bonferroni correction (adjusted p-value < 0.05). **(d)** scRNAseq UMAP colored by cluster and split based on tissue of origin, i.e., blood and liver. **(e)** Comparison of cell frequencies between blood and liver within sample (connected through grey lines) for each NK cell cluster. Significance was determined using the Wilcoxon Signed-Rank test with Bonferroni correction (adjusted p-value < 0.05). NCAM1-bright EOMES+ NK cells are significantly more present in liver. **(f)** Volcano plots depicting differences in gene expression in NK cells between compartments. The R-package *MAST* was used to obtain hurdle *P*-values which were Bonferroni corrected for multiple hypothesis testing. Positive Cohen's D value suggests higher expression in blood. Cohen's D cutoff is calculated as mean + 2 x standard deviation of Cohen's D values of all genes. **(g)** Hallmark genesets enriched by NK cell clusters. Normalized enrichment score was calculated based on a vector of gene-level signed statistic and false discovery rate was adjusted based on Benjamini Hochberg (BH) Correction. X-axis represents signed $\log_{10} P_{adj}$ for the genesets and the Positive values indicates enrichment in the liver.

Supplementary figure 6. B cell composition in liver and blood. **(a)** scRNAseq UMAP for B cells colored by clusters. **(b)** Violin plot showing relevant marker genes for B cell clusters. **(c)** Dot plot showing top 10 marker genes for each cluster. Significance was determined using the Wilcoxon Rank sum test with Bonferroni correction (adjusted p-value < 0.05). **(d)** scRNAseq UMAP colored by cluster and split based on tissue of origin. **(e)** Comparison of cell frequencies between blood and liver within sample (connected through grey lines) for each B cell cluster. Significance was determined using the Wilcoxon Signed-Rank test with Bonferroni correction (adjusted p-value < 0.05). All B cell clusters show similar cell frequencies between liver and blood. **(f)** Volcano plots depicting differences in gene expression in B cells between compartments. The R-package *MAST* was used to obtain hurdle *P*-values which were Bonferroni corrected for multiple hypothesis testing. Positive Cohen's D value suggests higher expression in blood. Cohen's D cutoff is calculated as mean + 2 x standard deviation of Cohen's D values of all genes.

Supplementary Figure 7. Monocyte comparison in liver and blood. **(a)** scRNAseq UMAP for monocytes colored by cluster IDs. **(b)** Dot plot showing top 6 cluster defining genes in each cluster. Significance was determined using the Wilcoxon Rank sum test with Bonferroni correction (adjusted p-value < 0.05). **(c)** UMAP dimensionality reduction of monocytes by compartment. **(d)** Frequency of monocytes between blood and liver compartments. Significance was determined using the Wilcoxon Signed-Rank test with Bonferroni correction (adjusted p-value < 0.05). Participants with 0 cells within a cell population were excluded. **(e)** Volcano plots depicting differences in gene expression in monocytes between compartments. The R-package *MAST* was used to obtain hurdle *P*-values which were Bonferroni corrected for multiple hypothesis testing. Positive Cohen's D value suggests higher expression in the liver. Cohen's D cutoff is calculated as mean + 2 x standard deviation of Cohen's D values of all genes. **(f)**. Hallmark genesets enriched in monocyte clusters. Normalized enrichment score was calculated based on a vector of gene-level signed statistic and false discovery rate was adjusted based on Benjamini Hochberg (BH) Correction. X-axis represents signed $\log_{10} P_{adj}$ for the genesets and the Positive values indicates enrichment in the liver. Classical monocytes; Int. Mono, intermediate

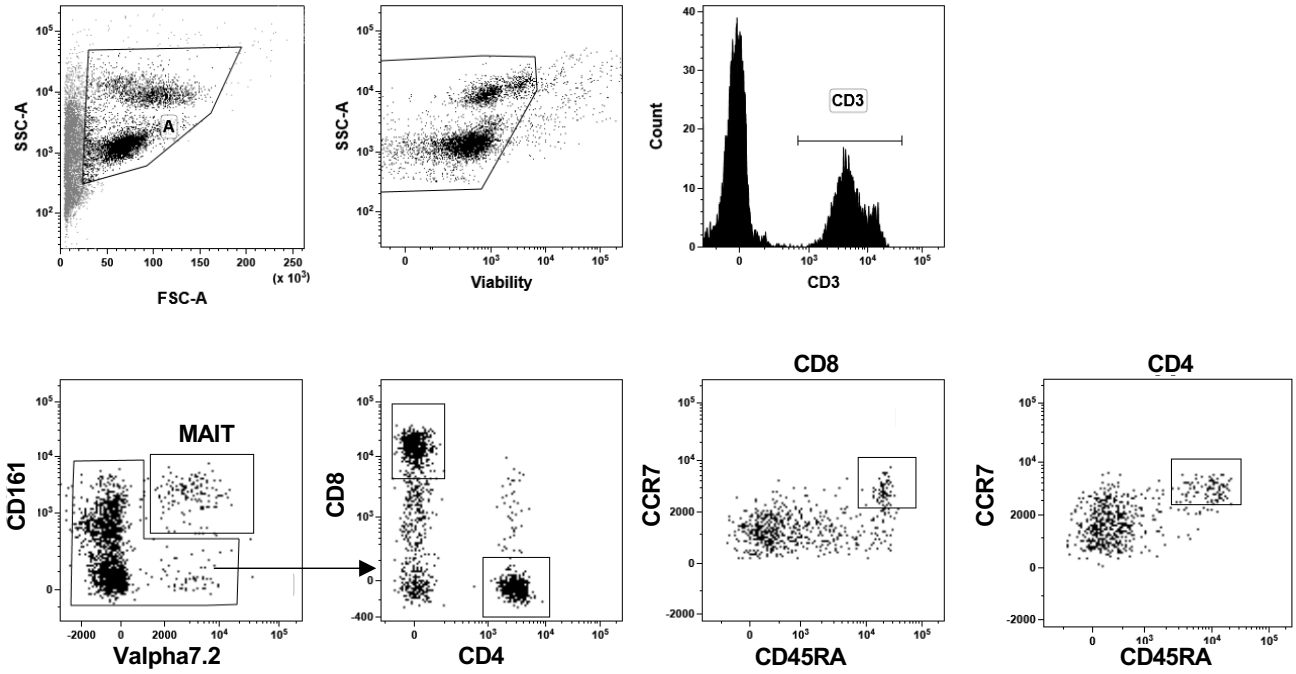
monocytes; ncMono, nonclassical monocytes; IFN-stim. cMono, Interferon stimulated classical monocytes.

**Supplemental references**

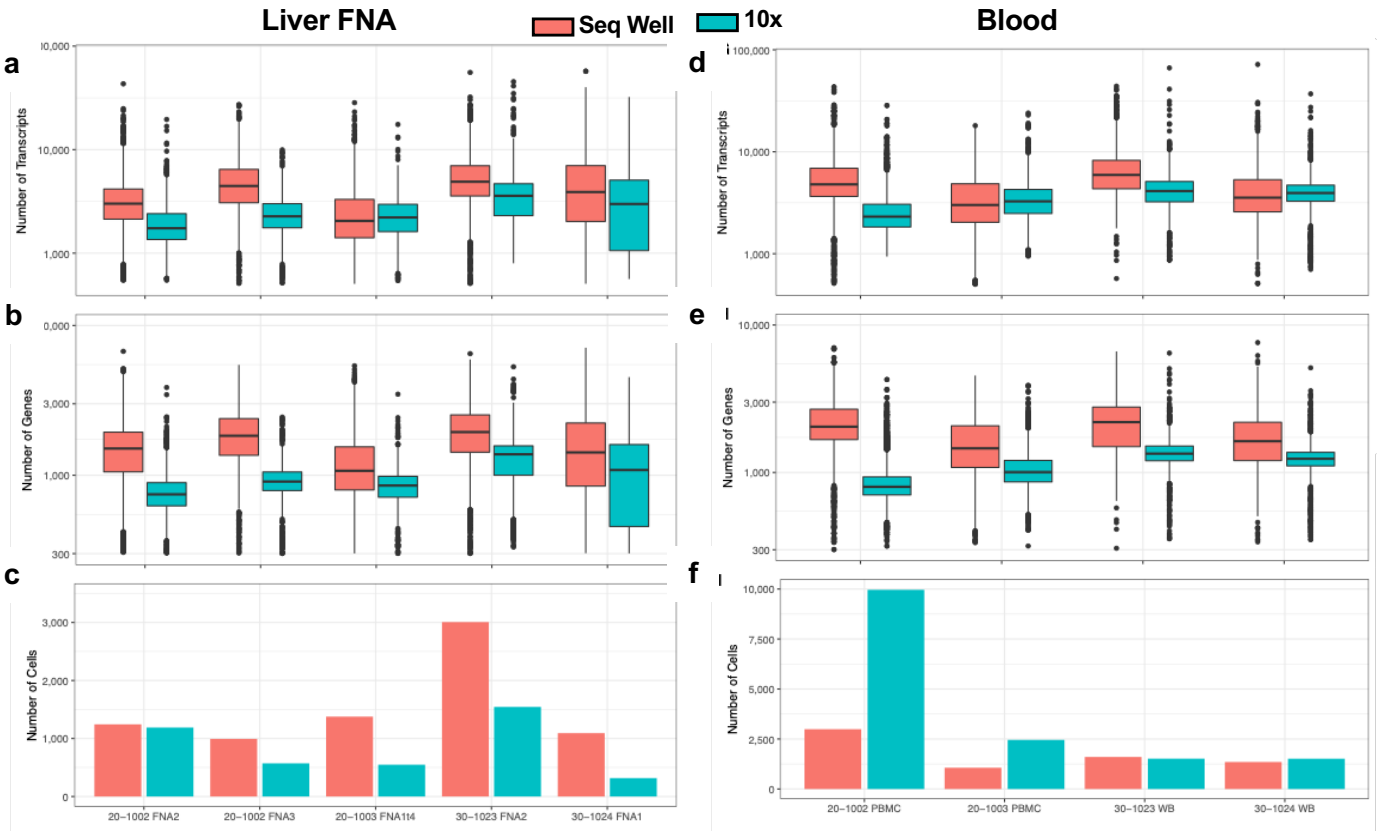
[1] Hughes TK, Wadsworth MH, 2nd, Gierahn TM, Do T, Weiss D, Andrade PR, et al. Second-Strand Synthesis-Based Massively Parallel scRNA-Seq Reveals Cellular States and Molecular Features of Human Inflammatory Skin Pathologies. Immunity 2020;53:878-894 e877.

[2] Ziegler CGK, Miao VN, Owings AH, Navia AW, Tang Y, Bromley JD, et al. Impaired local intrinsic immunity to SARS-CoV-2 infection in severe COVID-19. Cell 2021;184:4713-4733 e4722.

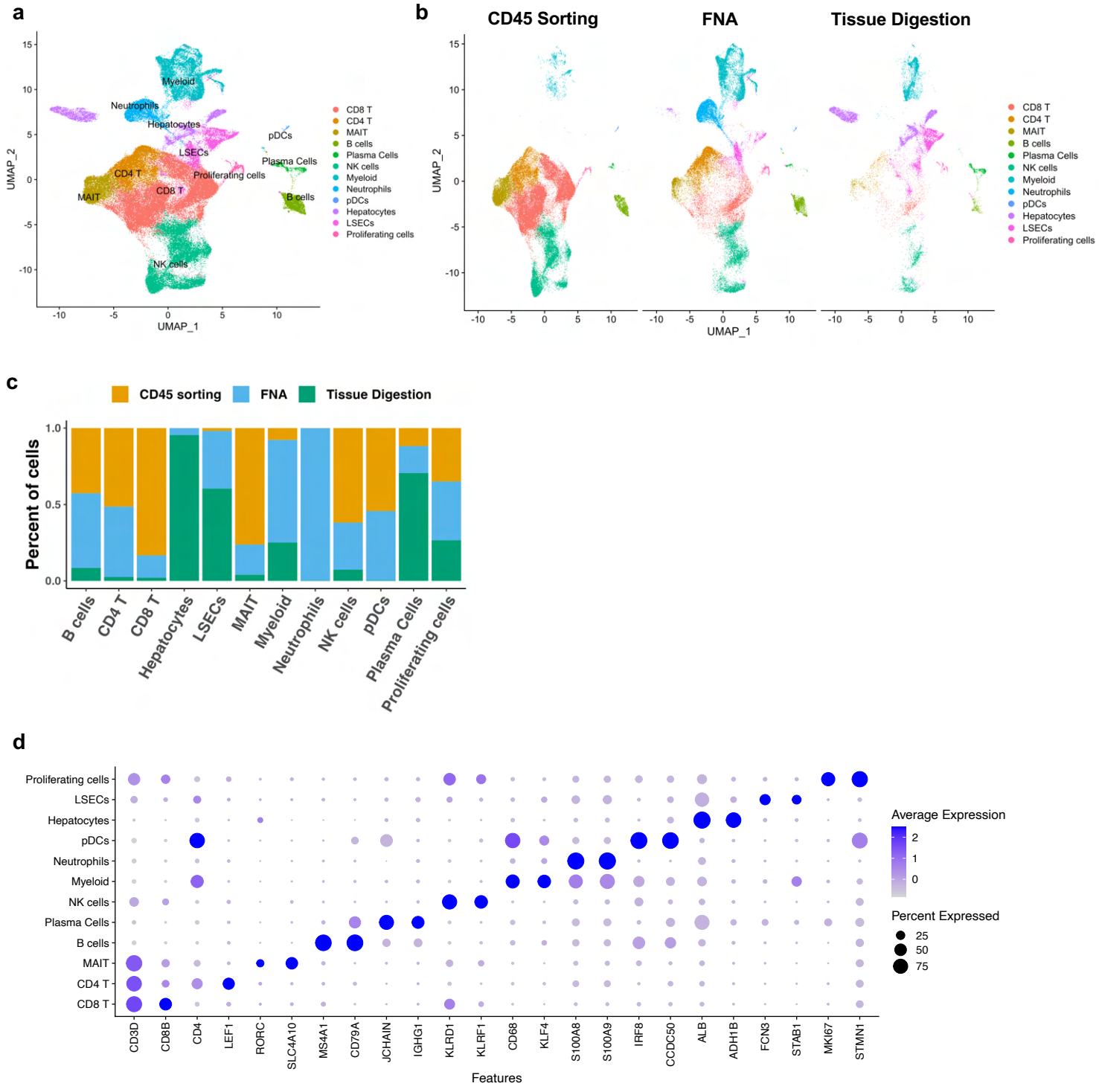
## Suppl Fig 1. FNA Quality Control Gating Strategy



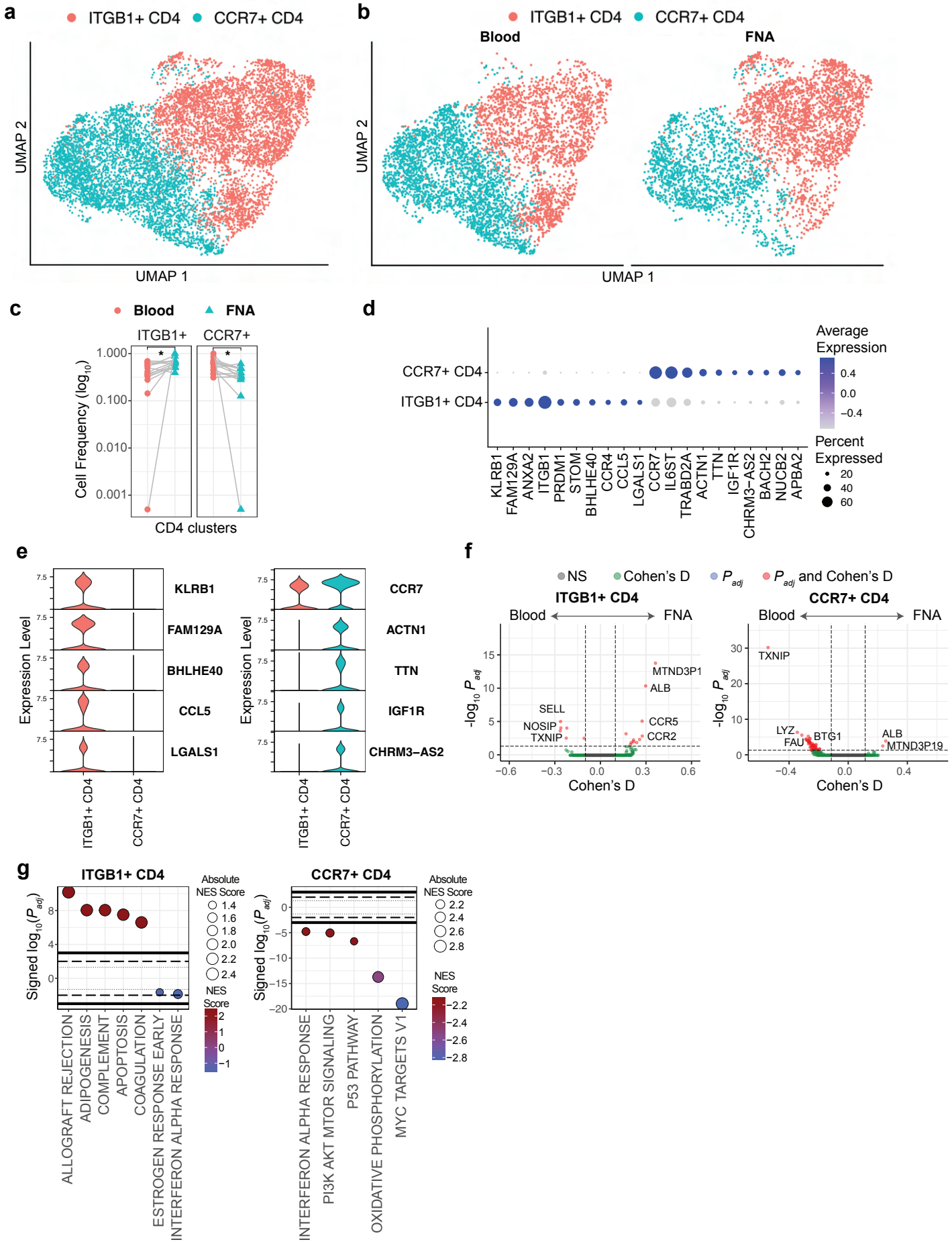
# Suppl Figure 2. scRNAseq QC metrics



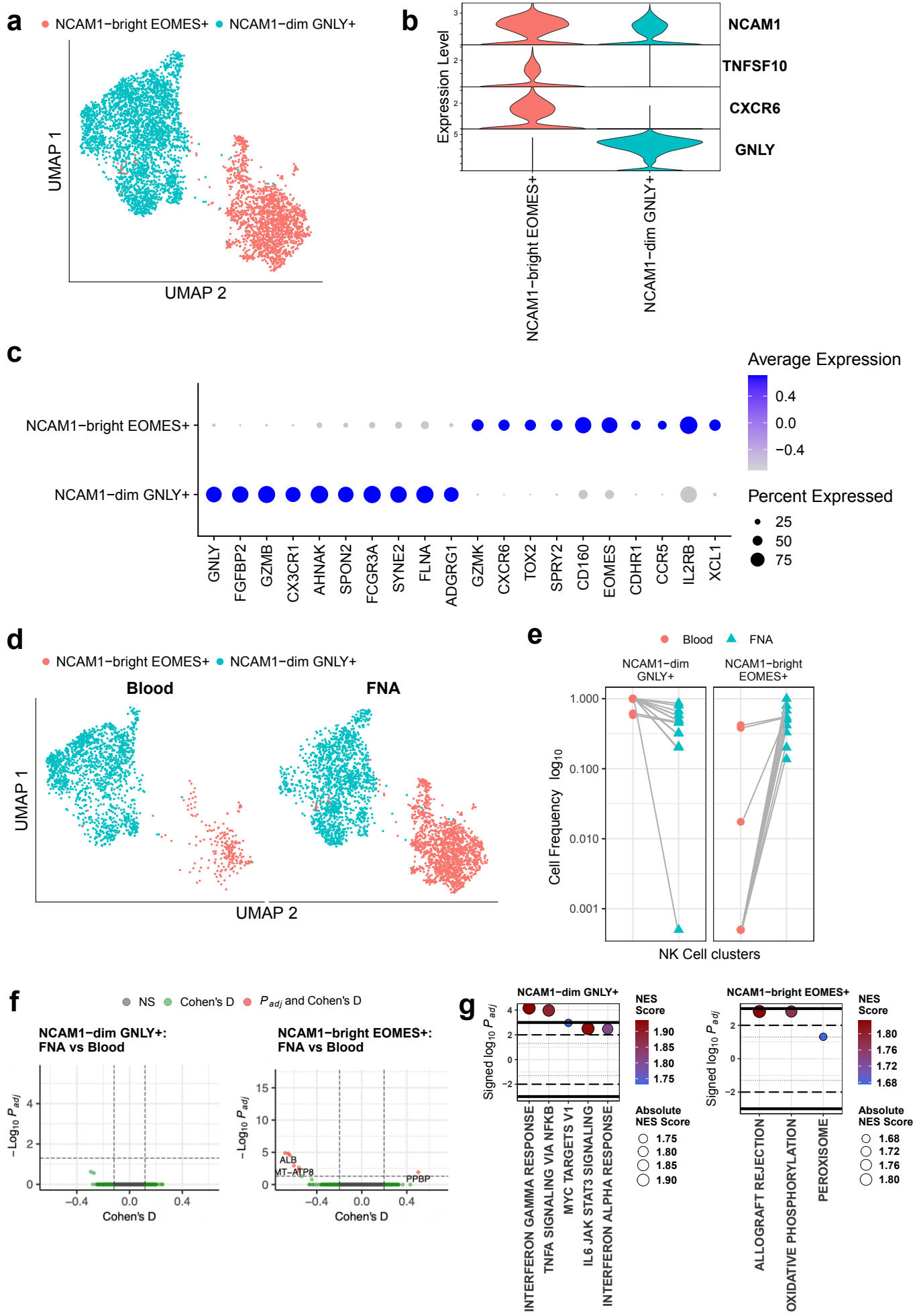
# Suppl Figure 3. Liver cell capture in scRNAseq technologies using different tissue isolation methods



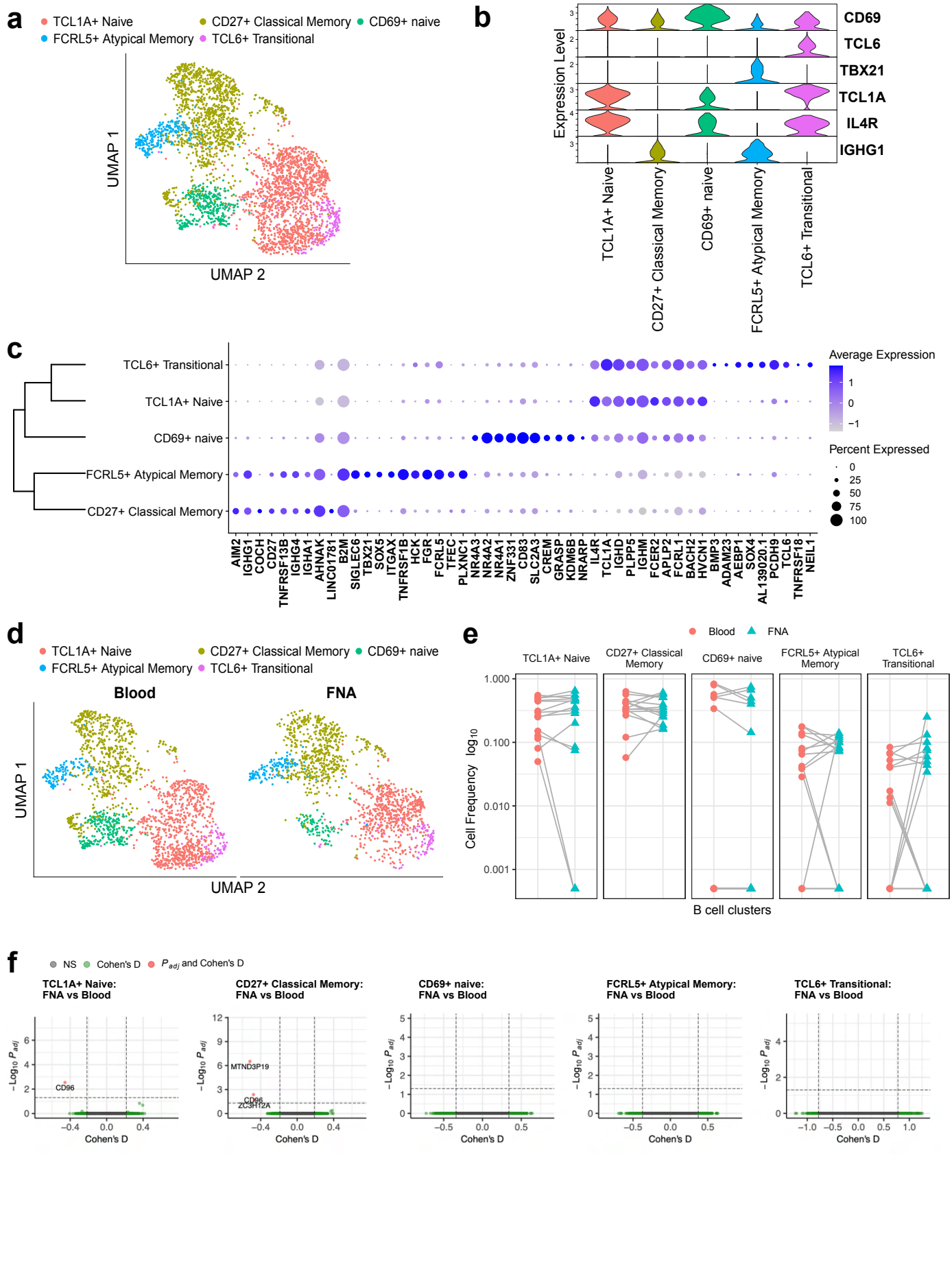
# Suppl Figure 4. CD4+ T cells



## Suppl Figure 5. NK cells



# Suppl Figure 6. B cells



# Suppl Figure 7. Monocytes