Supplementary Note SPOTS analysis of murine spleen

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Last Run: March 21, 2022

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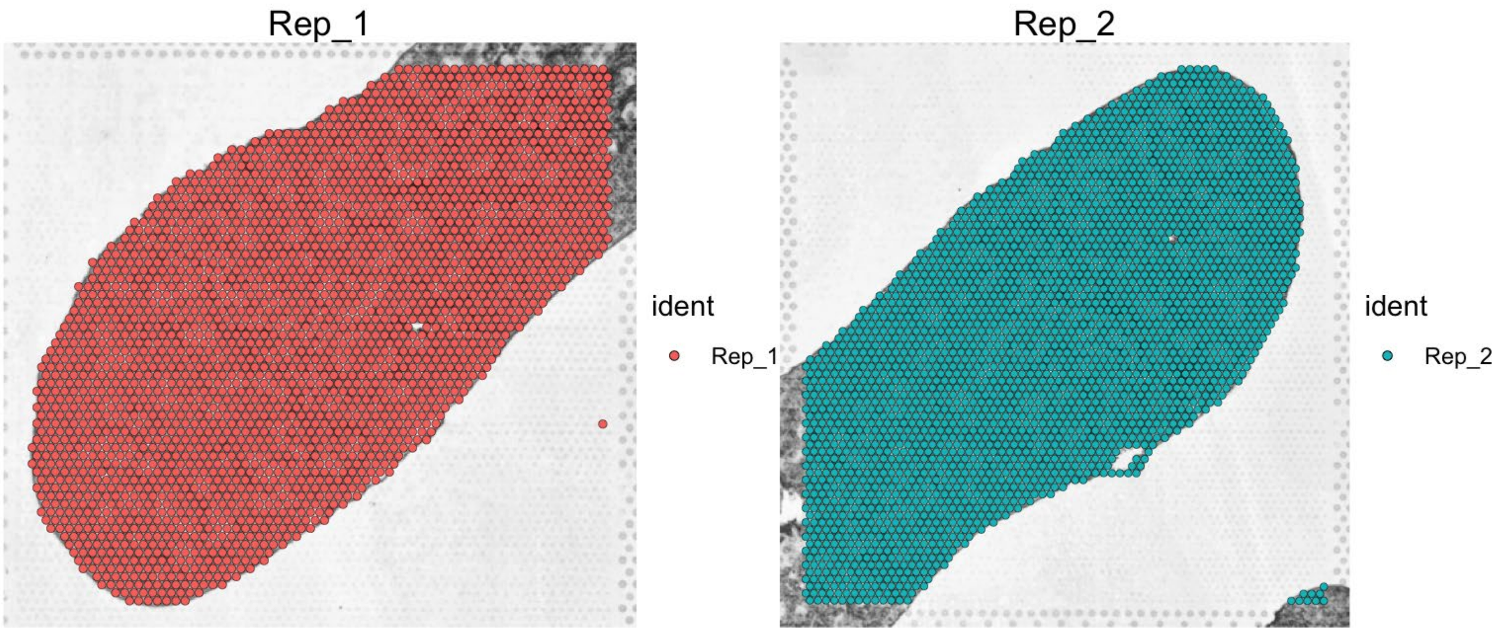
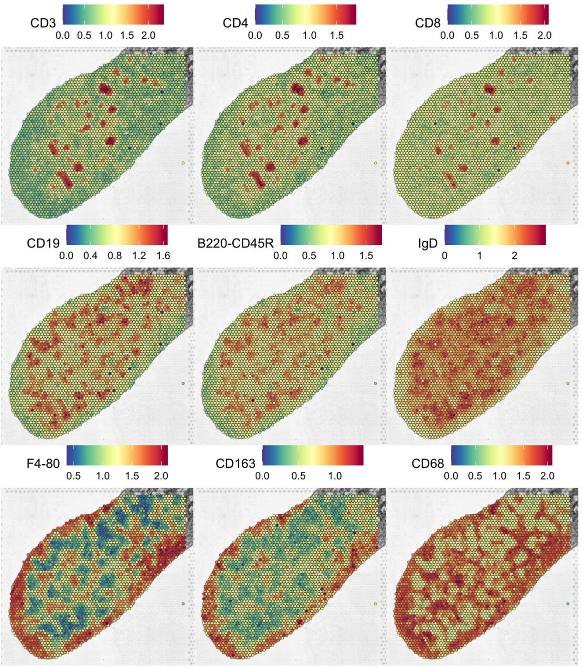
1. Load Data This markdown file contains the scRNA-seq analysis for the paper titled **Integrated protein and transcriptome high-throughput spatial profiling**. The data used for this analysis can be found under the GEO repository GSE198353.

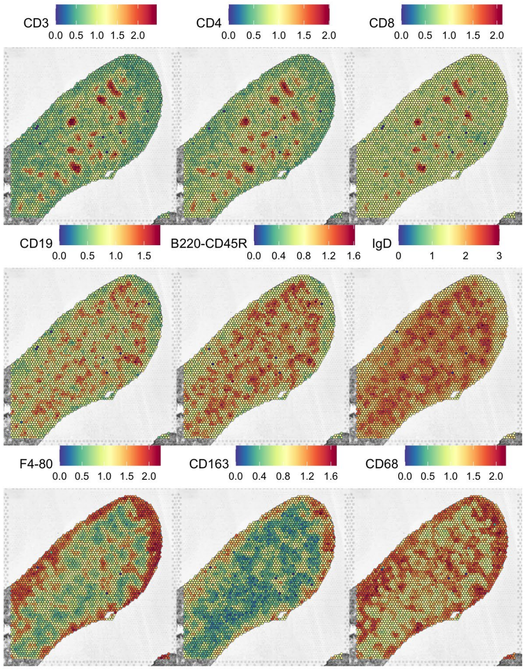
```
# install Seurat v3.0.0
if (!requireNamespace("Seurat", quietly = TRUE) | utils::packageVersion("Seurat") < "4.0.0")
  remotes::install_version("Seurat", version = "4.0.0")

# load data
library(Seurat)
# replicate 1
rep_1_data <- Read10X_h5('spleen_rep_1_filtered_feature_bc_matrix.h5')
rep_1_image <- Read10X_Image('GSE198353_spleen_replicate_1_spatial')
rep_1 <- CreateSeuratObject(rep_1_data$`Gene Expression`, assay = "RNA", project = "Rep_1")
rep_1_CITE <- CreateSeuratObject(rep_1_data$`Antibody Capture`, assay = "CITE", project = "Rep
_1")
rep_1@assays$CITE <- rep_1_CITE@assays$CITE
rep_1$nCount_CITE <- rep_1_CITE$nCount_CITE
rep_1$nFeature_CITE <- rep_1_CITE$nFeature_CITE
rep_1_image@assay <- c("RNA", "CITE")
rep_1_image@key <- "Rep_1"
rep_1@images <- list(Rep_1 = rep_1_image)
# replicate 2
rep_2_data <- Read10X_h5('spleen_rep_2_filtered_feature_bc_matrix.h5')
rep_2_image <- Read10X_Image('GSE198353_spleen_replicate_2_spatial')
rep_2 <- CreateSeuratObject(rep_2_data$`Gene Expression`, assay = "RNA", project = "Rep_2")
rep_2_CITE <- CreateSeuratObject(rep_2_data$`Antibody Capture`, assay = "CITE", project = "Rep
_2")
rep_2@assays$CITE <- rep_2_CITE@assays$CITE
rep_2$nCount_CITE <- rep_2_CITE$nCount_CITE
rep_2$nFeature_CITE <- rep_2_CITE$nFeature_CITE
rep_2_image@assay <- c("RNA", "CITE")
rep_2_image@key <- "Rep_2"
rep_2@images <- list(Rep_2 = rep_2_image)
# merge
spleen <- merge(rep_1, rep_2, add.cell.ids = c("Rep_1", "Rep_2"), project = "Spleen")
SpatialDimPlot(spleen, images = c("Rep_1", "Rep_2"))
```



2. Data normalization

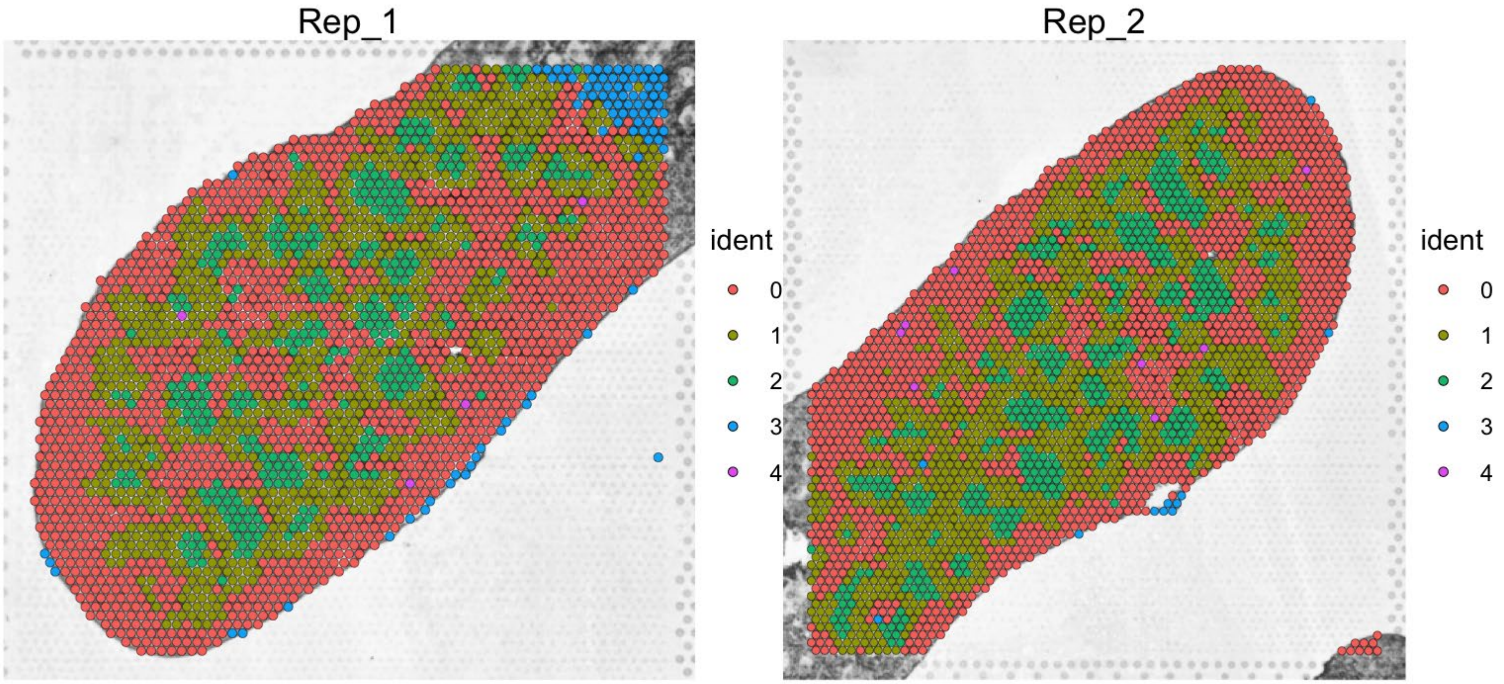
```
DefaultAssay(spleen) = "CITE"
spleen = NormalizeData(spleen, assay = "RNA", verbose = FALSE)
spleen = NormalizeData(spleen, normalization.method = "CLR", assay = "CITE", margin = 2, verbo
se = FALSE)
spleen <- ScaleData(spleen, verbose = FALSE)
p1 <- SpatialFeaturePlot(spleen,features = c("CD3","CD4","CD8",
                                             "CD19","B220-CD45R","IgD",
                                             "F4-80", "CD163", "CD68"), ncol = 3, images = c("
Rep_1"))
p2 <- SpatialFeaturePlot(spleen,features = c("CD3","CD4","CD8",
                                             "CD19","B220-CD45R","IgD",
                                             "F4-80", "CD163", "CD68"), ncol = 3, images = c("
Rep_2"))
p1 - p2
```



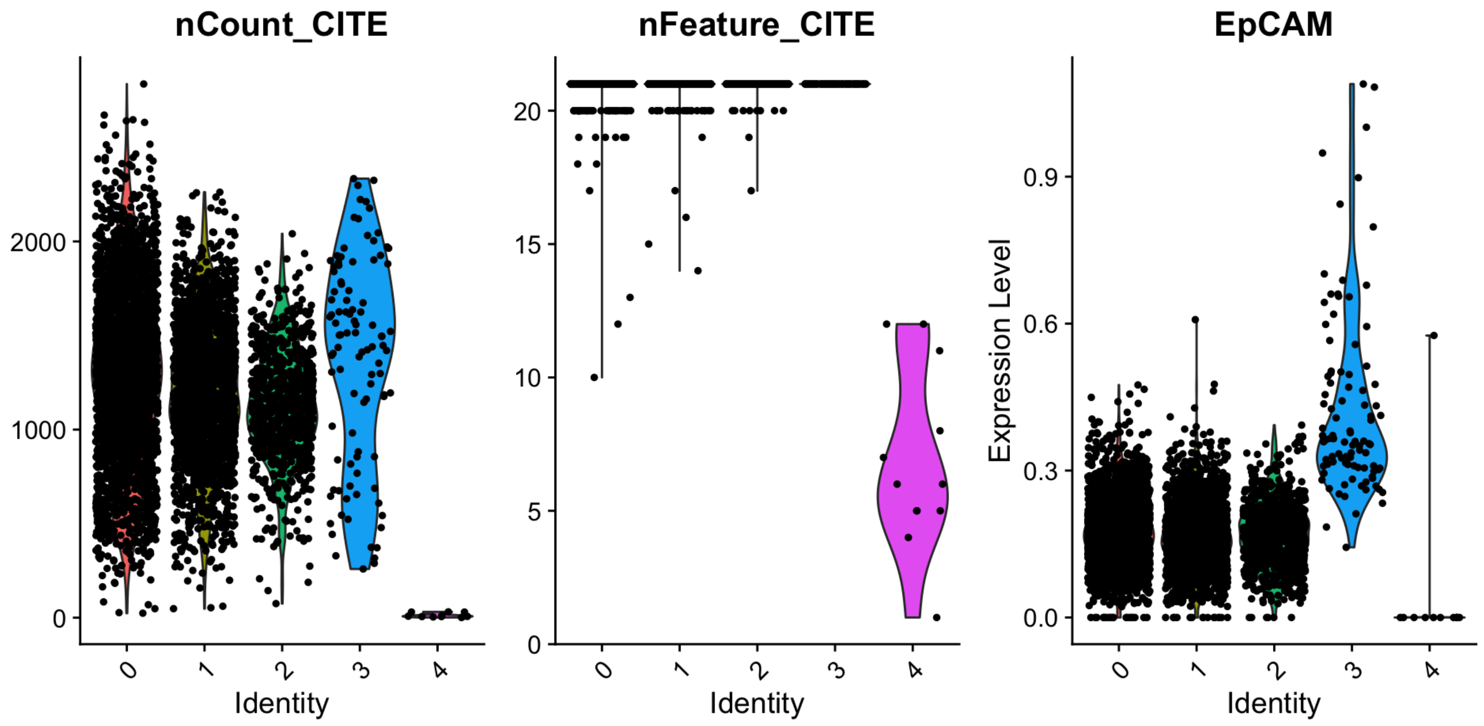


2. Dimensionality Reduction and Clustering

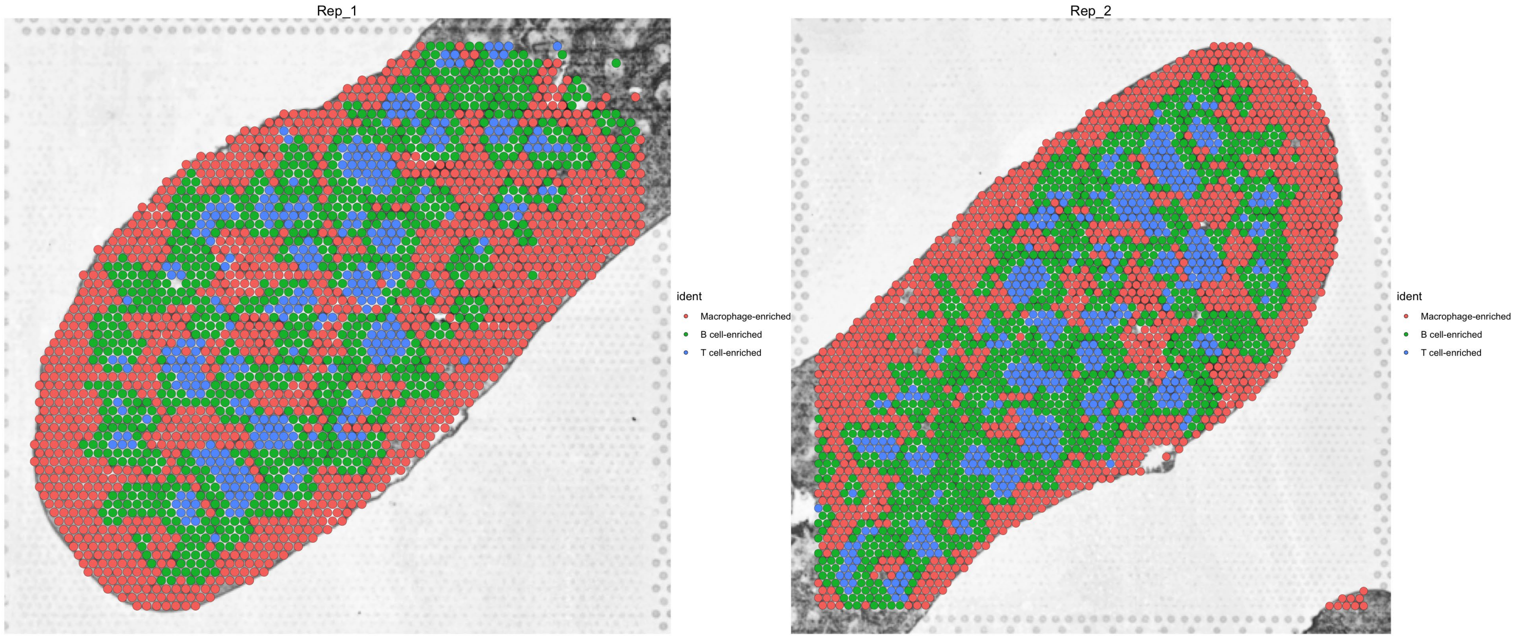
```r
spleen <- RunPCA(spleen, features = rownames(spleen))
spleen <- FindNeighbors(spleen, dims = 1:10)
spleen <- FindClusters(spleen, resolution = 0.2, verbose = FALSE)
SpatialDimPlot(spleen, images = c("Rep_1","Rep_2"))
```



```
# Cluster 4 represents low-quality spatial barcodes
# Cluster 3 represents epithelial enriched tissue borders
VlnPlot(spleen, c("nCount_CITE", "nFeature_CITE", "EpCAM"))
```

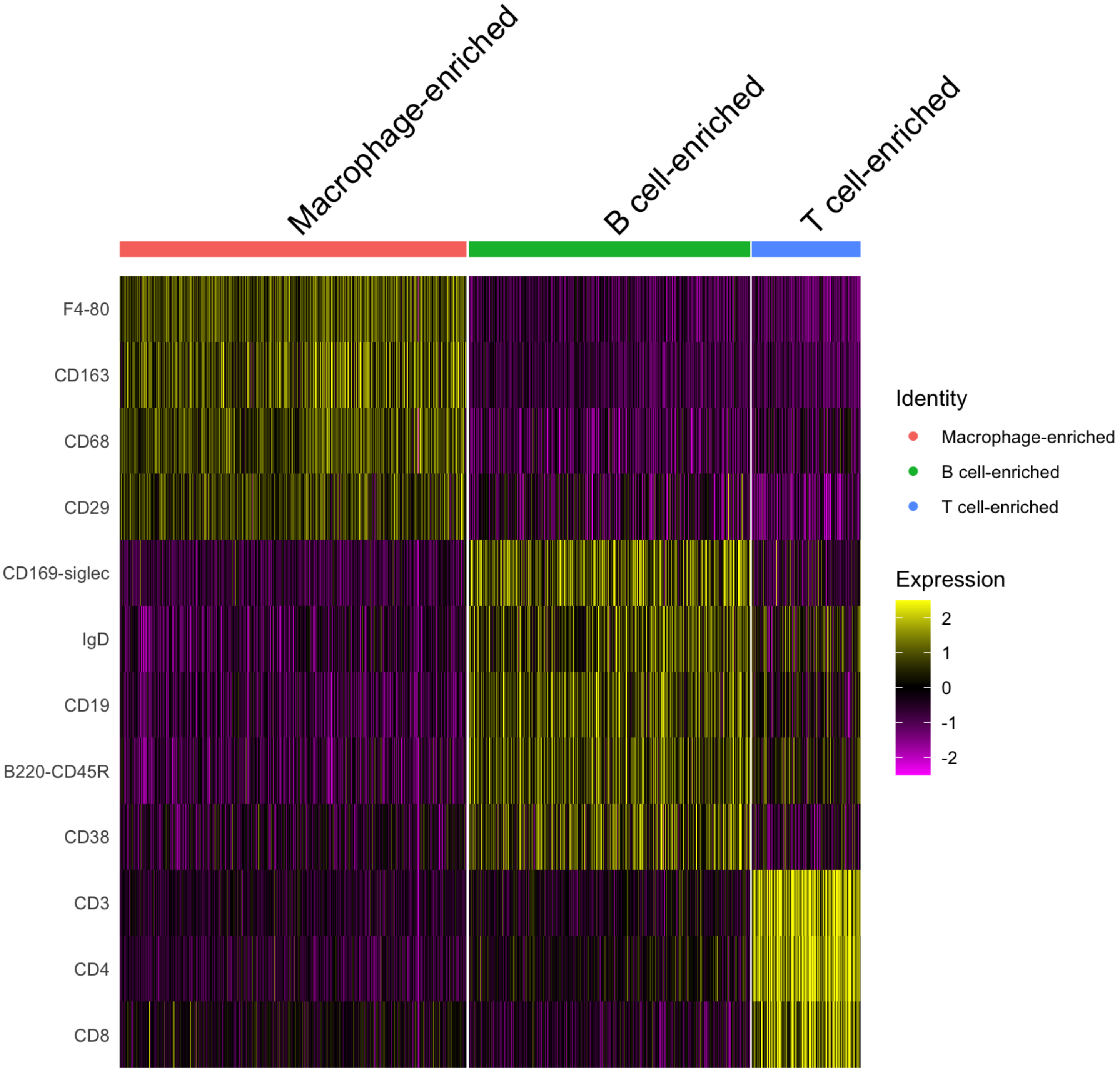


```r
# rename clusters
spleen <- subset(spleen, subset = CITE_snn_res.0.2 %in% c(3,4), invert = TRUE)
spleen <- RenameIdents(spleen, '0' = "Macrophage-enriched", '1' = "B cell-enriched", '2' = "T cell-enriched")
SpatialDimPlot(spleen, images = c("Rep_1","Rep_2"))
```



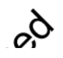
3. Differentially expressed ADTs

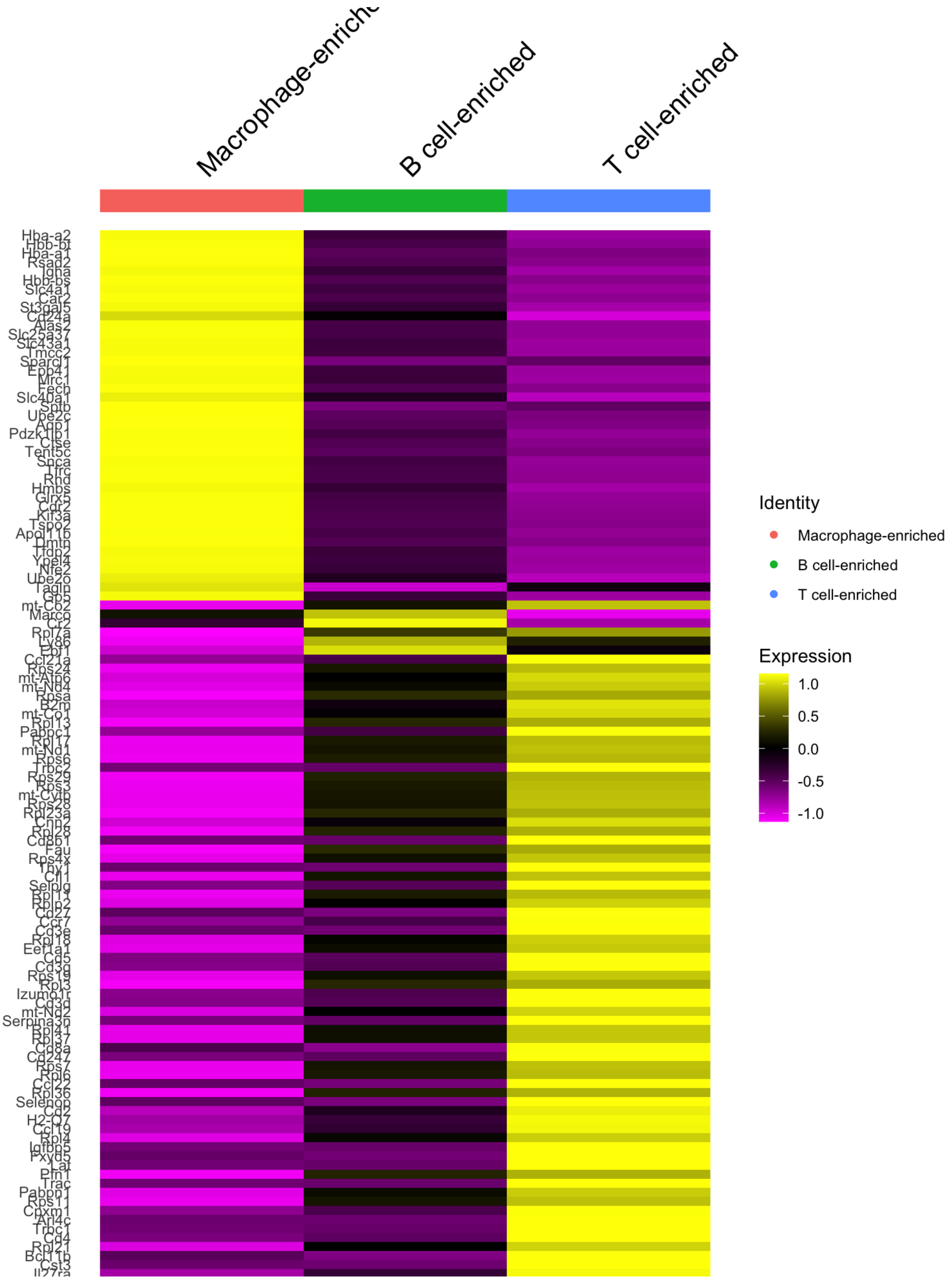
```
adt.markers <- FindAllMarkers(spleen, only.pos = TRUE, logfc.threshold = 0.2, verbose = FALSE)
DoHeatmap(spleen, adt.markers$gene, assay = "CITE", angle = 45)
```



4. Differentially expressed mRNAs

```
DefaultAssay(spleen) = "RNA"
mrna.markers <- FindAllMarkers(spleen, only.pos = TRUE, verbose = FALSE)
spleen.avg <- AverageExpression(spleen, assays = "RNA", return.seurat = TRUE)
DoHeatmap(spleen.avg, mrna.markers$gene, draw.lines = FALSE, assay = "RNA", angle = 45)
```




Macrophage-enrich
B cell-enriched
T cell-enriched
Hba-a2
Hbb-bt
Hba-a1
Rsad2
Igha
Hbb-bs
Slc4a1
Car2
St3gal5
Cd24a
Alas2
Slc25a37
Slc43a1
Tmcc2
Sparcl1
Epb41
Mrc1
Fech
Slc40a1
Spib
Ube2c
Aqp1
Pdzk1ip1
Ctse
Tent5c
Snca
Tfrc
Rhd
Hmbs
Glrx5
Cdr2
Kif3a
Tspo2
Apol11b
Dmtn
Tfdp2
Ypel4
Nfe2
Ube2o
Tagln
Gp5
mt-Co2
Marco
Cr2
Rpl7a
Ly86
Ebf1
Ccl21a
Rps24
mt-Atp6
mt-Nd4
Rpsa
B2m
mt-Co1
Rpl13
Pabpc1
Rpl17
mt-Nd1
Rps6
Trbc2
Rps29
Rps3
mt-Cytb
Rps28
Rpl23a
Cnn2
Rpl28
Cd8b1
Fau
Rps4x
Thy1
Cfl1
Selplg
Rpl11
Rplp2
Cd27
Ccr7
Cd3e
Rpl18
Eef1a1
Cd5
Cd3g
Rps19
Rpl3
Izumo1r
Cd3d
mt-Nd2
Serpina3n
Rpl41
Rpl37
Cd8a
Cd247
Rps7
Rpl6
Ccl22
Rpl36
Selenop
Cd2
H2-Q7
Ccl19
Rpl4
Igfbp5
Fxyd5
Lat
Pfn1
Trac
Pabpn1
Rps11
Cpxm1
Arl4c
Trbc1
Cd4
Rpl21
Bcl11b
Cst3
Il27ra
Identity
● Macrophage-enriched
● B cell-enriched
● T cell-enriched
Expression
1.0
0.5
0.0
-0.5
-1.0

5. Session info

```
# Session info
print(sessionInfo())
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
## 
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
## 
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
## 
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base     
## 
## other attached packages:
## [1] SeuratObject_4.0.0 Seurat_4.0.1      
## 
## loaded via a namespace (and not attached):
##   [1] nlme_3.1-151          spatstat.sparse_2.0-0 matrixStats_0.57.0    bit64_4.0.5          
   RcppAnnoy_0.0.18      RColorBrewer_1.1-2   
##   [7] httr_1.4.2            sctransform_0.3.2     tools_4.0.3           R6_2.5.0             
   irlba_2.3.3           rpart_4.1-15         
##  [13] KernSmooth_2.23-18    uwot_0.1.10           mgcv_1.8-33           DBI_1.1.0            
   lazyeval_0.2.2        colorspace_2.0-0     
##  [19] withr_2.4.3           tidyselect_1.1.0      gridExtra_2.3         bit_4.0.4            
   compiler_4.0.3        hdf5r_1.3.5          
##  [25] plotly_4.9.2.2        labeling_0.4.2        scales_1.1.1          lmtest_0.9-38        
   spatstat.data_1.7-0   ggridges_0.5.3       
##  [31] pbapply_1.4-3         goftest_1.2-2         stringr_1.4.0         digest_0.6.27        
   spatstat.utils_2.1-0  rmarkdown_2.6        
##  [37] pkgconfig_2.0.3       htmltools_0.5.2       parallelly_1.23.0     limma_3.46.0         
   fastmap_1.1.0         htmlwidgets_1.5.3    
##  [43] rlang_0.4.10          shiny_1.5.0           farver_2.0.3          generics_0.1.0       
   zoo_1.8-8             jsonlite_1.7.2       
##  [49] ica_1.0-2             dplyr_1.0.2           magrittr_2.0.1        patchwork_1.1.1      
   Matrix_1.3-2          Rcpp_1.0.7           
##  [55] munsell_0.5.0         abind_1.4-5           reticulate_1.18       lifecycle_0.2.0      
   stringi_1.5.3         yaml_2.2.1           
##  [61] MASS_7.3-53           Rtsne_0.15            plyr_1.8.6            grid_4.0.3           
   parallel_4.0.3        listenv_0.8.0        
##  [67] promises_1.1.1        ggrepel_0.9.0         crayon_1.3.4          deldir_0.2-3         
   miniUI_0.1.1.1        lattice_0.20-41      
```

```
##  [73] cowplot_1.1.1         splines_4.0.3         tensor_1.5            knitr_1.30           
   pillar_1.4.7          igraph_1.2.6         
##  [79] spatstat.geom_1.65-5  future.apply_1.7.0    reshape2_1.4.4        codetools_0.2-18     
   leiden_0.3.6          glue_1.4.2           
##  [85] evaluate_0.14         data.table_1.13.6     vctrs_0.3.6           png_0.1-7            
   httpuv_1.5.4          polyclip_1.10-0      
##  [91] gtable_0.3.0          RANN_2.6.1            purrr_0.3.4           spatstat.core_1.65-
5  tidyr_1.1.2           scattermore_0.7      
##  [97] future_1.21.0         ggplot2_3.3.3         xfun_0.20             mime_0.9             
   xtable_1.8-4          later_1.1.0.1        
## [103] survival_3.2-7        viridisLite_0.3.0     tibble_3.0.4          cluster_2.1.0        
   globals_0.14.0        fitdistrplus_1.1-3   
## [109] ellipsis_0.3.1        ROCR_1.0-11
```

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