# Annotation cells

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## Contents

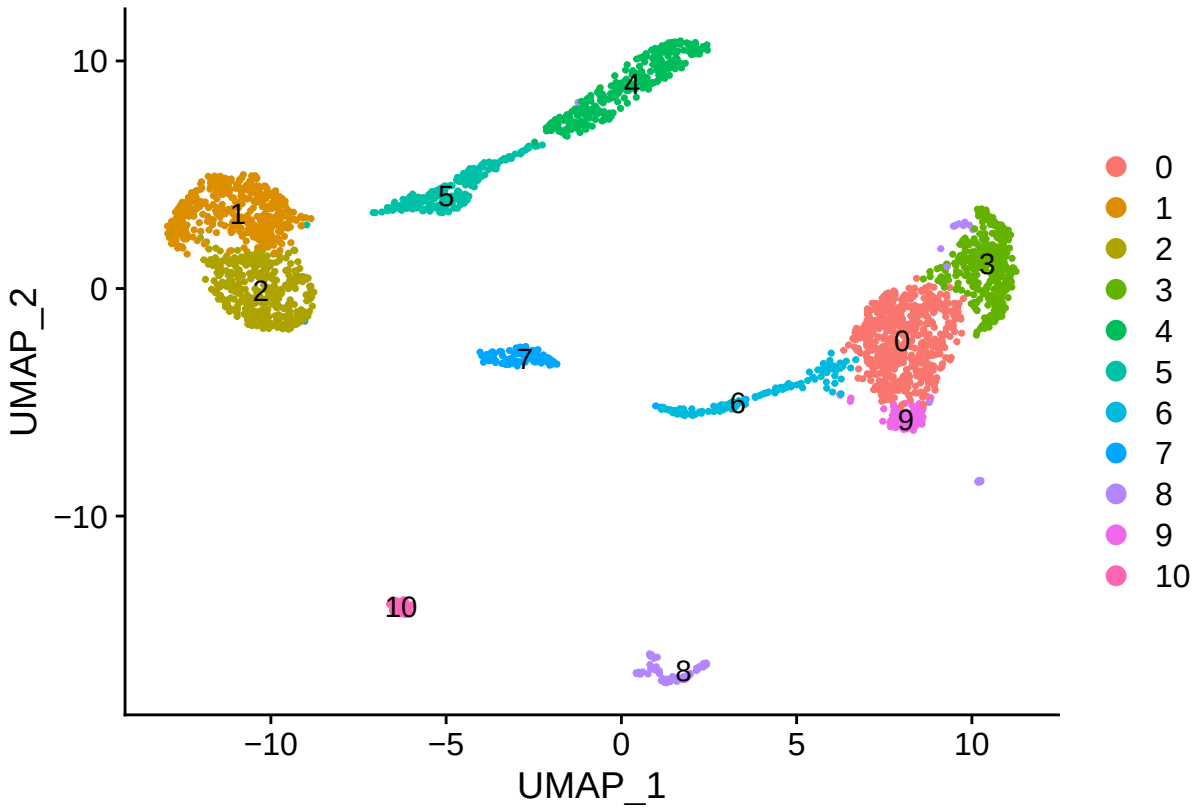


## Load Packages

```
suppressMessages(library(dplyr))
suppressMessages(library(Seurat))
suppressMessages(library(patchwork))
suppressMessages(library(ggplot2))
suppressMessages(library(stringr))
suppressMessages(library(dittoSeq))
suppressMessages(library(ComplexHeatmap))
```

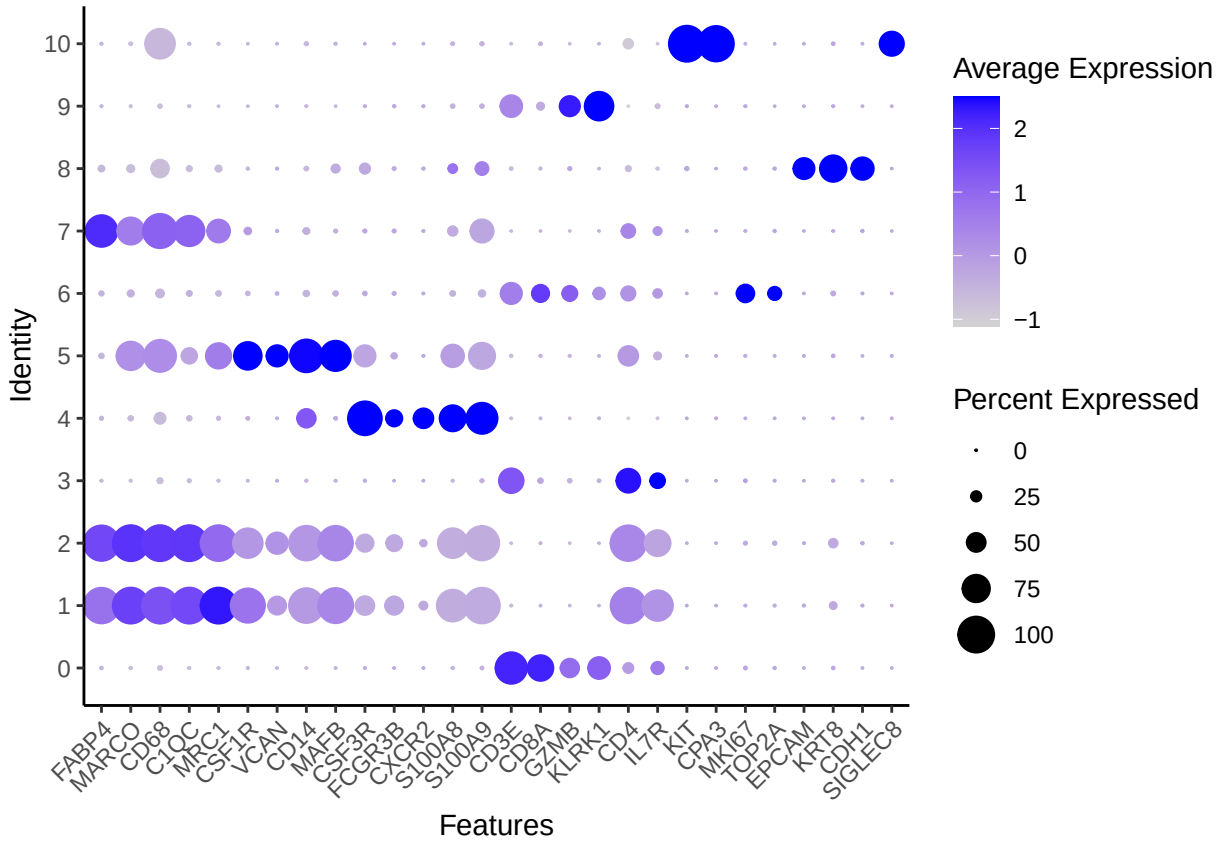
## Load Seurat objects

```
BAL_10x.integrated <- readRDS("../10x-1-Pre_Processing/BAL_10x.integrated_noDB.rds")

DimPlot(BAL_10x.integrated, label = T)
```



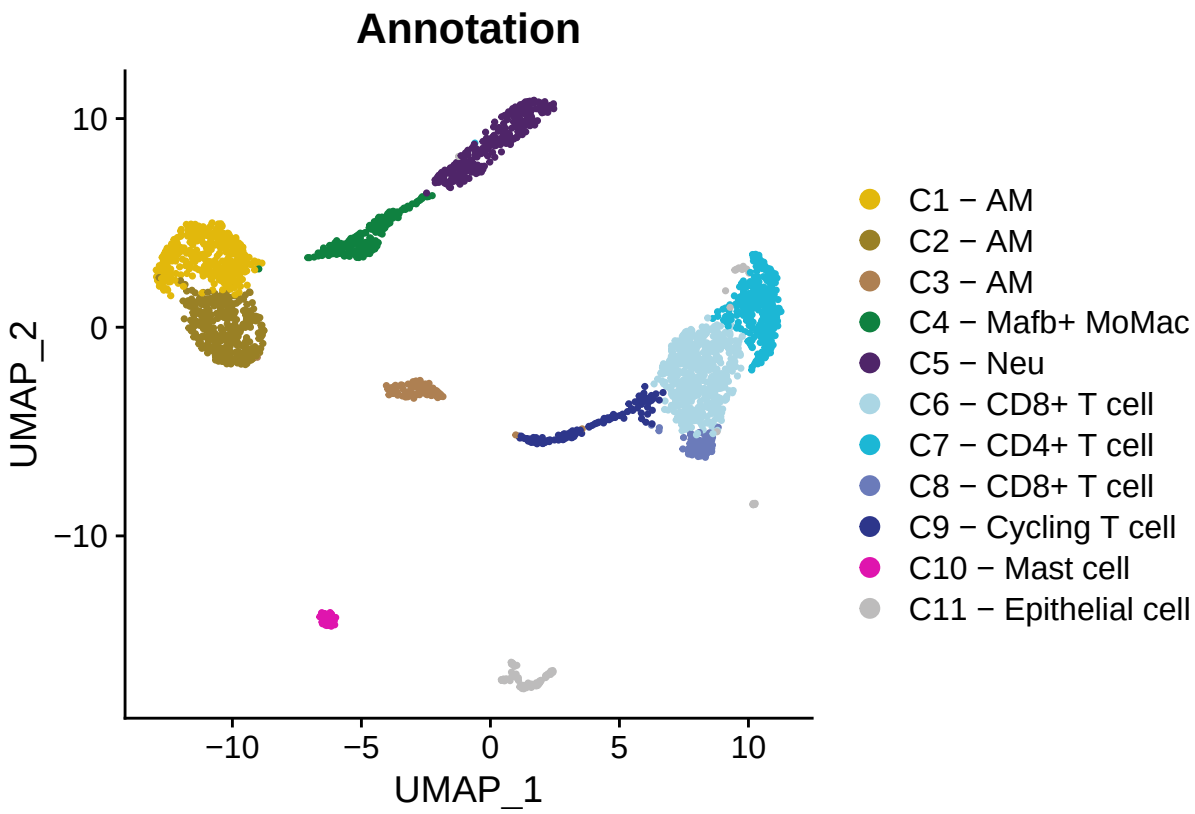

## Annotating clusters

```
markers_neutro <- c("CSF3R", "FCGR3B", "CXCR2", "S100A8",
    "S100A9")
marker_epi <- c("EPCAM", "KRT8", "CDH1", "SIGLEC8")
Marker_Cycling_cell <- c("MKI67", "TOP2A")
markers_AM <- c("FABP4", "MARCO", "CD68", "C1QC", "MRC1")
markers_Mafb_macro <- c("CSF1R", "VCAN", "CD14", "MAFB")
markers_Mast <- c("KIT", "CPA3")
markers_LT <- c("CD3E", "CD8A", "GZMB", "KLGR1", "KLRK1",
    "CD4", "IL7R")

DotPlot(BAL_10x.integrated, features = c(markers_AM,
    markers_Mafb_macro, markers_neutro, markers_LT,
    markers_Mast, Marker_Cycling_cell, marker_epi)) +
    theme_classic() + theme(axis.text.x = element_text(angle = 45,
    hjust = 1))
```

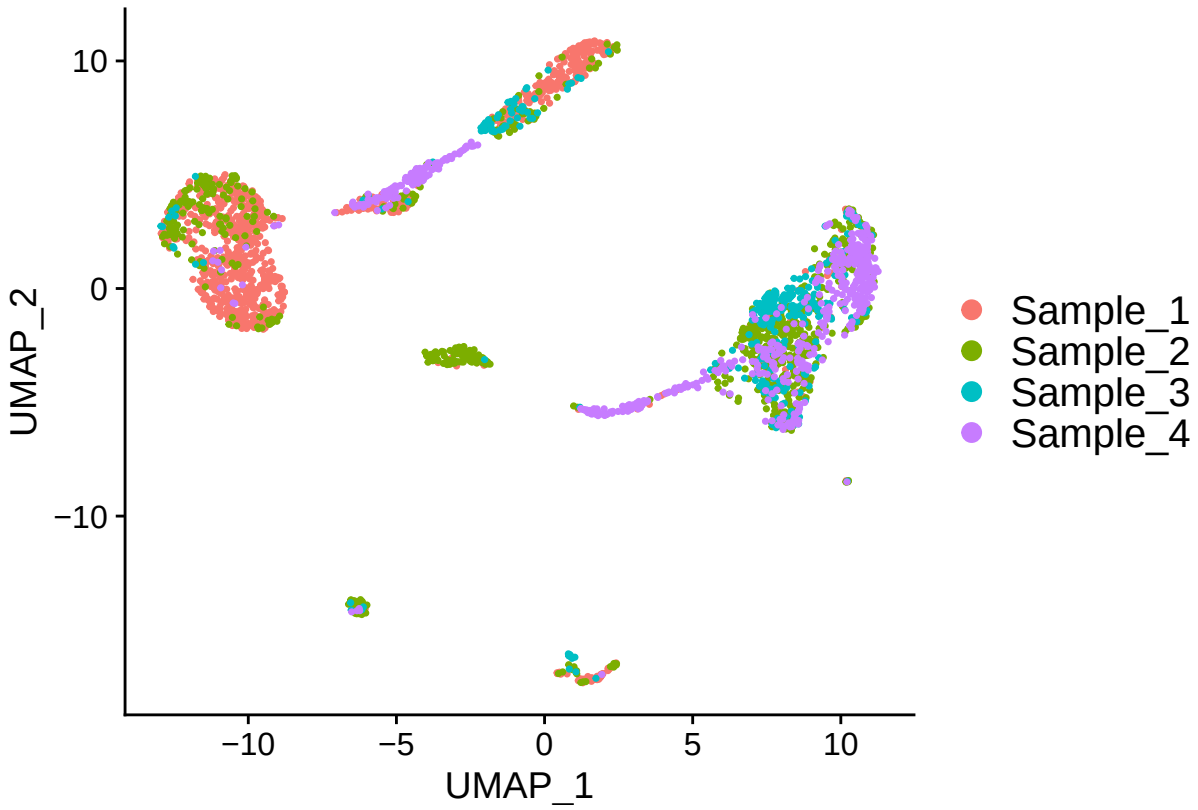



## Annotation

```
BAL_10x.integrated$Annotation <- BAL_10x.integrated$seurat_clusters
BAL_10x.integrated$Annotation <- as.factor(BAL_10x.integrated$Annotation)
levels(BAL_10x.integrated$Annotation) <- c("C6 - CD8+ T cell",
    "C1 - AM", "C2 - AM", "C7 - CD4+ T cell", "C5 - Neu",
    "C4 - Mafb+ MoMac", "C9 - Cycling T cell", "C3 - AM",
    "C11 - Epithelial cell", "C8 - CD8+ T cell", "C10 - Mast cell")

BAL_10x.integrated$Annotation <- factor(BAL_10x.integrated$Annotation,
    levels = c("C1 - AM", "C2 - AM", "C3 - AM", "C4 - Mafb+ MoMac",
        "C5 - Neu", "C6 - CD8+ T cell", "C7 - CD4+ T cell",
        "C8 - CD8+ T cell", "C9 - Cycling T cell",
        "C10 - Mast cell", "C11 - Epithelial cell"))

col <- c("#E2B80C", "#998025", "#AE8052", "#0F8140",
    "#4F2569", "#ABD6E4", "#1CB7D5", "#6B7BBA", "#2D368B",
    "#DF15AE", "#BDBCBC")

DimPlot(BAL_10x.integrated, group.by = "Annotation",
    cols = col)
```



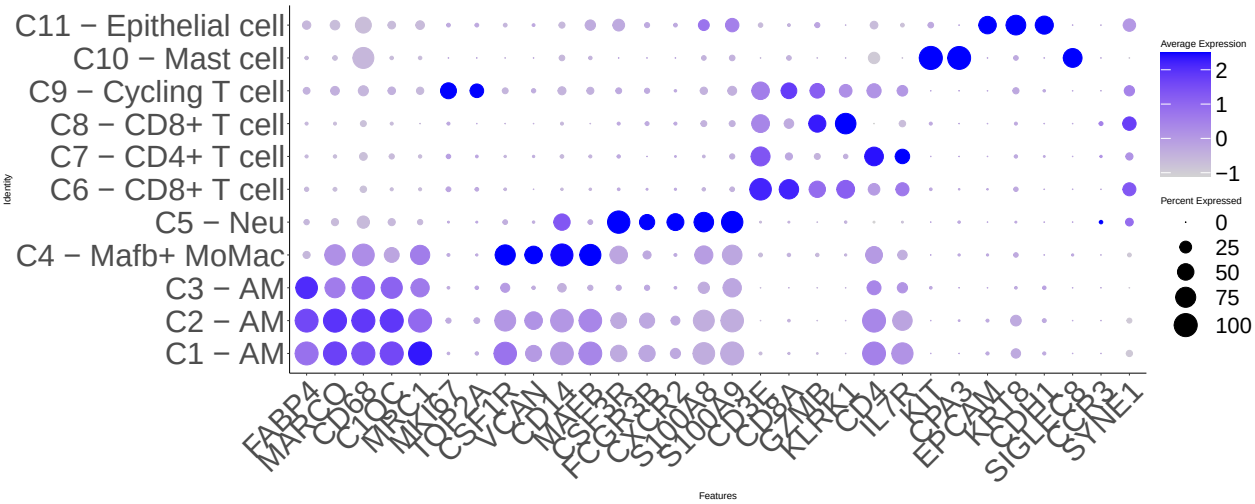

```
# ggsave('Umap_Annotation.pdf', width = 8, height
# = 4)
DimPlot(BAL_10x.integrated, reduction = "umap", group.by = "orig.ident") +
    theme(legend.text = element_text(size = 15)) +
    labs(title = NULL)
```




```
# ggsave('Umap_PerSample.pdf', width = 8, height
# = 4)
```

## Dotplot Annotated

```
DefaultAssay(BAL_10x.integrated) <- "RNA"

Marker_gene <- c("FABP4", "MARCO", "CD68", "C1QC",
    "MRC1", "MKI67", "TOP2A", "CSF1R", "VCAN", "CD14",
    "MAFB", "CSF3R", "FCGR3B", "CXCR2", "S100A8", "S100A9",
    "CD3E", "CD8A", "GZMB", "KLGR1", "KLRK1", "CD4",
    "IL7R", "KIT", "CPA3", "EPCAM", "KRT8", "CDH1",
    "SIGLEC8", "CCR3", "SYNE1")

DotPlot(BAL_10x.integrated, features = Marker_gene,
    group.by = "Annotation") + theme_classic() + scale_size(range = c(0,
    12)) + theme(axis.text.x = element_text(angle = 45,
    hjust = 1), axis.text = element_text(size = 35),
    legend.text = element_text(size = 25), legend.key.height = unit(1,
        "cm"), legend.key.width = unit(2, "cm"))
```

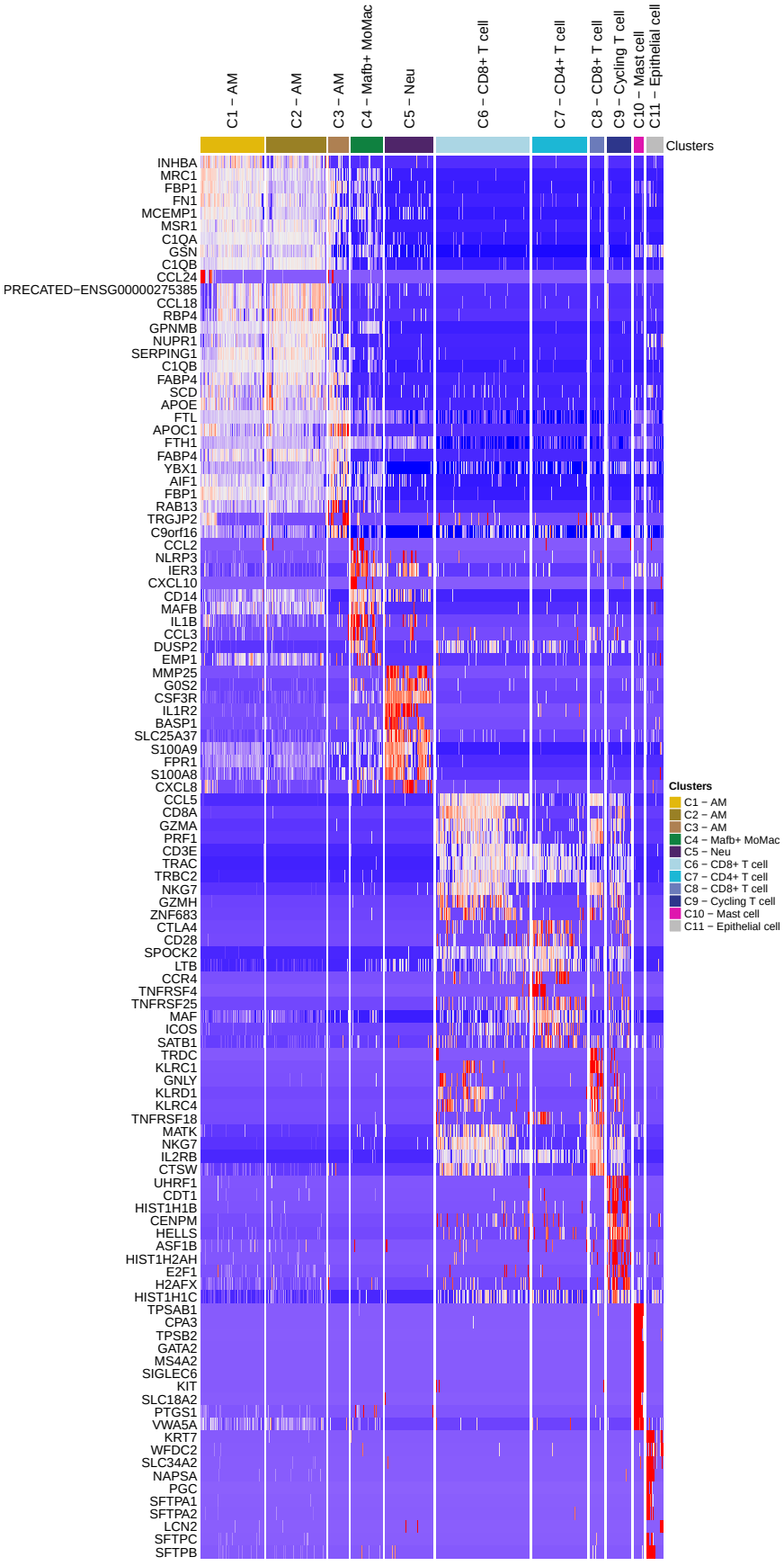



```
# ggsave('Dotplot_Annotated.pdf', width = 35,
# height = 10)
```

```
Idents(BAL_10x.integrated) <- "Annotation"

lavage.markers <- FindAllMarkers(BAL_10x.integrated,
    only.pos = TRUE, min.pct = 0.25)

lavage.markers %>%
    group_by(cluster) %>%
    top_n(n = 10, wt = avg_log2FC) -> top10

mat <- as.matrix(GetAssayData(object = BAL_10x.integrated,
    slot = "data")[as.character(top10$gene), ])

df <- as.data.frame(BAL_10x.integrated$Annotation)
colnames(df) <- "Clusters"
color_df <- list(Clusters = c(`C1 - AM` = "#E2B80C",
    `C2 - AM` = "#998025", `C3 - AM` = "#AE8052", `C4 - Mafb+ MoMac` = "#0F8140",
    `C5 - Neu` = "#4F2569", `C6 - CD8+ T cell` = "#ABD6E4",
    `C7 - CD4+ T cell` = "#1CB7D5", `C8 - CD8+ T cell` = "#6B7BBA",
    `C9 - Cycling T cell` = "#2D368B", `C10 - Mast cell` = "#DF15AE",
    `C11 - Epithelial cell` = "#BDBCBC"))

Heatmap <- Heatmap(t(scale(t(mat))), show_column_names = F,
    column_split = BAL_10x.integrated$Annotation, cluster_column_slices = F,
    cluster_rows = F, top_annotation = HeatmapAnnotation(df = df,
        col = color_df), use_raster = F, show_heatmap_legend = F,
    show_column_dend = F, column_title_rot = 90, row_names_side = "left")

# tidyHeatmap::save_pdf(Heatmap,
# 'Heatmap_10x.pdf', width = 35, height = 50,
# units ='cm')
```

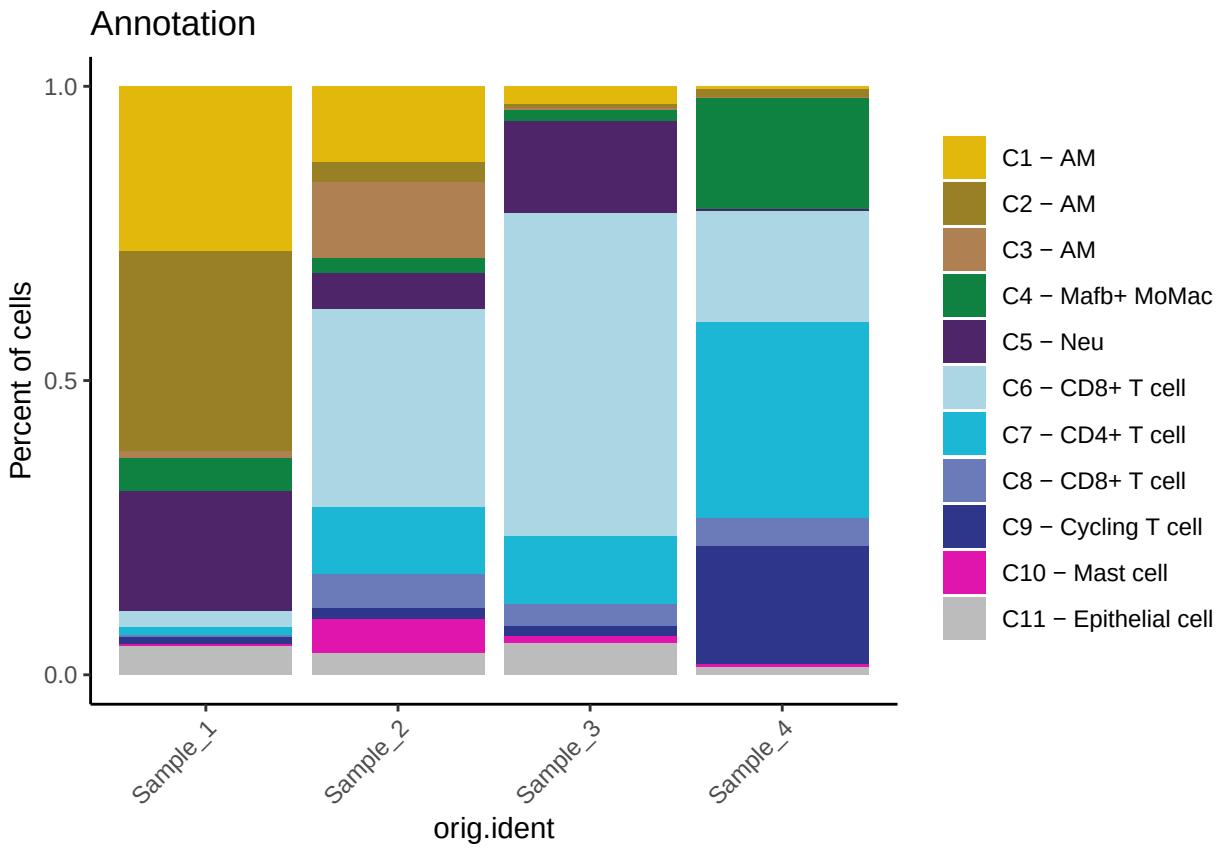
## Heatmap top genes per clusters

```
Heatmap
```


C1 – AM
C2 – AM
C3 – AM
C4 – Mafb+ MoMac
C5 – Neu
C6 – CD8+ T cell
C7 – CD4+ T cell
C8 – CD8+ T cell
C9 – Cycling T cell
C10 – Mast cell
C11 – Epithelial cell
Clusters
INHBA
MRC1
FBP1
FN1
MCEMP1
MSR1
C1QA
GSN
C1QB
CCL24
PRECATED−ENSG00000275385
CCL18
RBP4
GPNMB
NUPR1
SERPING1
C1QB
FABP4
SCD
APOE
FTL
APOC1
FTH1
FABP4
YBX1
AIF1
FBP1
RAB13
TRGJP2
C9orf16
CCL2
NLRP3
IER3
CXCL10
CD14
MAFB
IL1B
CCL3
DUSP2
EMP1
MMP25
G0S2
CSF3R
IL1R2
BASP1
SLC25A37
S100A9
FPR1
S100A8
CXCL8
CCL5
CD8A
GZMA
PRF1
CD3E
TRAC
TRBC2
NKG7
GZMH
ZNF683
CTLA4
CD28
SPOCK2
LTB
CCR4
TNFRSF4
TNFRSF25
MAF
ICOS
SATB1
TRDC
KLRC1
GNLY
KLRD1
KLRC4
TNFRSF18
MATK
NKG7
IL2RB
CTSW
UHRF1
CDT1
HIST1H1B
CENPM
HELLS
ASF1B
HIST1H2AH
E2F1
H2AFX
HIST1H1C
TPSAB1
CPA3
TPSB2
GATA2
MS4A2
SIGLEC6
KIT
SLC18A2
PTGS1
VWA5A
KRT7
WFDC2
SLC34A2
NAPSA
PGC
SFTPA1
SFTPA2
LCN2
SFTPC
SFTPB
Clusters
C1 – AM
C2 – AM
C3 – AM
C4 – Mafb+ MoMac
C5 – Neu
C6 – CD8+ T cell
C7 – CD4+ T cell
C8 – CD8+ T cell
C9 – Cycling T cell
C10 – Mast cell
C11 – Epithelial cell

## Cluster frequency per sample

```
var_order <- c(1, 4, 5, 6, 7, 8, 9, 10, 11, 2, 3)
dittoBarPlot(BAL_10x.integrated, "Annotation", group.by = "orig.ident",
    var.labels.reorder = var_order, color.panel = col)
```




```
# ggsave('bar_freq_10x.pdf')
```

## Saving results for later

```
saveRDS(BAL_10x.integrated, "BAL_10x.annotated_noDB.rds")
```

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 22.04.4 LTS
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblasp-r0.3.20.so;  LAPACK version 3.10.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=fr_BE.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=fr_BE.UTF-8    LC_MESSAGES=en_US.UTF-8
```

```
##  [7] LC_PAPER=fr_BE.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=fr_BE.UTF-8 LC_IDENTIFICATION=C
##
## time zone: Europe/Brussels
## tzcode source: system (glibc)
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
## [1] ComplexHeatmap_2.16.0 dittoSeq_1.12.0       stringr_1.5.0
## [4] ggplot2_3.4.2         patchwork_1.1.2       SeuratObject_4.1.3
## [7] Seurat_4.3.0          dplyr_1.1.2
##
## loaded via a namespace (and not attached):
##   [1] RColorBrewer_1.1-3          shape_1.4.6
##   [3] rstudioapi_0.14             jsonlite_1.8.7
##   [5] magrittr_2.0.3              magick_2.7.5
##   [7] spatstat.utils_3.0-3        farver_2.1.1
##   [9] rmarkdown_2.23              GlobalOptions_0.1.2
##  [11] zlibbioc_1.46.0             vctrs_0.6.3
##  [13] ROCR_1.0-11                 Cairo_1.6-2
##  [15] spatstat.explore_3.2-1      RCurl_1.98-1.12
##  [17] S4Arrays_1.2.1              htmltools_0.5.5
##  [19] sctransform_0.3.5           parallelly_1.36.0
##  [21] KernSmooth_2.23-22          htmlwidgets_1.6.2
##  [23] ica_1.0-3                   plyr_1.8.8
##  [25] plotly_4.10.2               zoo_1.8-12
##  [27] igraph_1.5.0.1              iterators_1.0.14
##  [29] mime_0.12                   lifecycle_1.0.3
##  [31] pkgconfig_2.0.3             Matrix_1.6-1
##  [33] R6_2.5.1                    fastmap_1.1.1
##  [35] clue_0.3-64                 GenomeInfoDbData_1.2.10
##  [37] MatrixGenerics_1.12.2       fitdistrplus_1.1-11
##  [39] future_1.33.0               shiny_1.7.4.1
##  [41] digest_0.6.33               colorspace_2.1-0
##  [43] S4Vectors_0.38.1            tensor_1.5
##  [45] irlba_2.3.5.1               GenomicRanges_1.52.0
##  [47] labeling_0.4.2              progressr_0.13.0
##  [49] fansi_1.0.4                 spatstat.sparse_3.0-2
##  [51] httr_1.4.6                  polyclip_1.10-4
##  [53] abind_1.4-5                 compiler_4.3.3
##  [55] doParallel_1.0.17           withr_2.5.0
##  [57] highr_0.10                  MASS_7.3-60.0.1
##  [59] DelayedArray_0.26.3         rjson_0.2.21
##  [61] tools_4.3.3                 lmtest_0.9-40
##  [63] httpuv_1.6.11               future.apply_1.11.0
##  [65] goftest_1.2-3               glue_1.6.2
##  [67] nlme_3.1-164                promises_1.2.0.1
##  [69] Rtsne_0.16                  cluster_2.1.6
##  [71] reshape2_1.4.4              generics_0.1.3
##  [73] gtable_0.3.3                spatstat.data_3.0-1
```

```
##  [75] tidyr_1.3.0                data.table_1.14.8
##  [77] XVector_0.40.0             sp_2.0-0
##  [79] utf8_1.2.3                 BiocGenerics_0.46.0
##  [81] spatstat.geom_3.2-4        RcppAnnoy_0.0.21
##  [83] foreach_1.5.2              ggrepel_0.9.3
##  [85] RANN_2.6.1                 pillar_1.9.0
##  [87] limma_3.56.2               spam_2.9-1
##  [89] later_1.3.1                circlize_0.4.15
##  [91] splines_4.3.3              lattice_0.22-5
##  [93] survival_3.5-8             deldir_1.0-9
##  [95] tidyselect_1.2.0           SingleCellExperiment_1.22.0
##  [97] miniUI_0.1.1.1             pbapply_1.7-2
##  [99] knitr_1.43                 gridExtra_2.3
## [101] IRanges_2.34.0             SummarizedExperiment_1.30.2
## [103] scattermore_1.2            stats4_4.3.3
## [105] xfun_0.39                  Biobase_2.60.0
## [107] matrixStats_1.0.0          pheatmap_1.0.12
## [109] stringi_1.7.12             lazyeval_0.2.2
## [111] yaml_2.3.7                 evaluate_0.21
## [113] codetools_0.2-19           tibble_3.2.1
## [115] cli_3.6.1                  uwot_0.1.16
## [117] xtable_1.8-4               reticulate_1.30
## [119] munsell_0.5.0              Rcpp_1.0.11
## [121] GenomeInfoDb_1.36.0        globals_0.16.2
## [123] spatstat.random_3.1-5      png_0.1-8
## [125] parallel_4.3.3             ellipsis_0.3.2
## [127] dotCall64_1.0-2            bitops_1.0-7
## [129] listenv_0.9.0              viridisLite_0.4.2
## [131] scales_1.2.1               ggridges_0.5.4
## [133] crayon_1.5.2               leiden_0.4.3
## [135] purrr_1.0.1                GetoptLong_1.0.5
## [137] rlang_1.1.1                cowplot_1.1.1
## [139] formatR_1.14
```