

PBMC transcriptomics

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```
library(Seurat)
#####
# Wrapper function
# Read in each sample, annotate with day
SRRs2Seurat <- function(x) {
  data <- Read10X(data.dir = as.character(x$path))
  obj <- CreateSeuratObject(data,
                            project =
                            "PBMC")

  obj <- AddMetaData(obj, metadata = c(as.character(x$day),
                                       x$lib,
                                       paste(
                                         as.character(x$day),
                                         x$lib, sep = "_")
                                       ),
  col.name = c("day", "lib","day_lib"))

  # % mito reads
  obj[["percent.mt"]] <- PercentageFeatureSet(obj, pattern = "^MT-")

  # simple QC: >200 features/cell, less than 7k features/cell and <12.5% mitochondrial reads.
  obj <- subset(obj, subset = nFeature_RNA > 200 & nFeature_RNA < 7000 & percent.mt < 12.5)
}

#####
# Build a dataframe with sample annotation:

SRRtable <- cbind("path" = c("cellranger/TurnerSRR11233662/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233620/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233619/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233618/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233617/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233661/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233660/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233659/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233658/outs/filtered_feature_bc_matrix/",
                             "cellranger/TurnerSRR11233657/outs/filtered_feature_bc_matrix"),
  "day" = c("d60",
           "d0",
           "d5",
           "d12",
```

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    "d28",
    "d0",
    "d5",
    "d12",
    "d28",
    "d60"),
"lib" = c("PBMC",
        "PBMC",
        "PBMC",
        "PBMC",
        "PBMC",
        "IgD_negative_enriched_PBMC",
        "IgD_negative_enriched_PBMC",
        "IgD_negative_enriched_PBMC",
        "IgD_negative_enriched_PBMC",
        "IgD_negative_enriched_PBMC"))

```

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SRRtable <- data.frame(SRRtable)
SRRtable

SRRtable$day <- factor(SRRtable$day, levels = c("d0", "d5", "d12", "d28", "d60"))

# Make a list
pbmc.list <- list()

## we know from the VDJ_fate_mapping analysis that we need to focus on IgD- enriched B cells (as these are the ones we want to analyze)
## This imports just the IgD- samples:
SRRtable <- SRRtable[SRRtable$lib == "IgD_negative_enriched_PBMC",]

# Loop for each entry in SRR table to do the import to Seurat.
for(i in 1:nrow(SRRtable)){

  pbmc.list[[i]] <- SRRs2Seurat(SRRtable[i,])

}

names(pbmc.list) <- paste(SRRtable$day, SRRtable$lib, sep = "_")

for (i in 1:length(pbmc.list)) {
  pbmc.list[[i]] <- NormalizeData(pbmc.list[[i]], verbose = FALSE)
  pbmc.list[[i]] <- FindVariableFeatures(pbmc.list[[i]], selection.method = "vst",
                                         nfeatures = 2000, verbose = FALSE)
}

# Set reference as a day 28 sample (will have some GC-B
# derived, and all other cell types) (the Seurat vignette

```

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# keeps one dataset back to 'project' anchors onto
reference.list <- which(names(pbmc.list) == "d28_IgD_negative_enriched_PBMC")

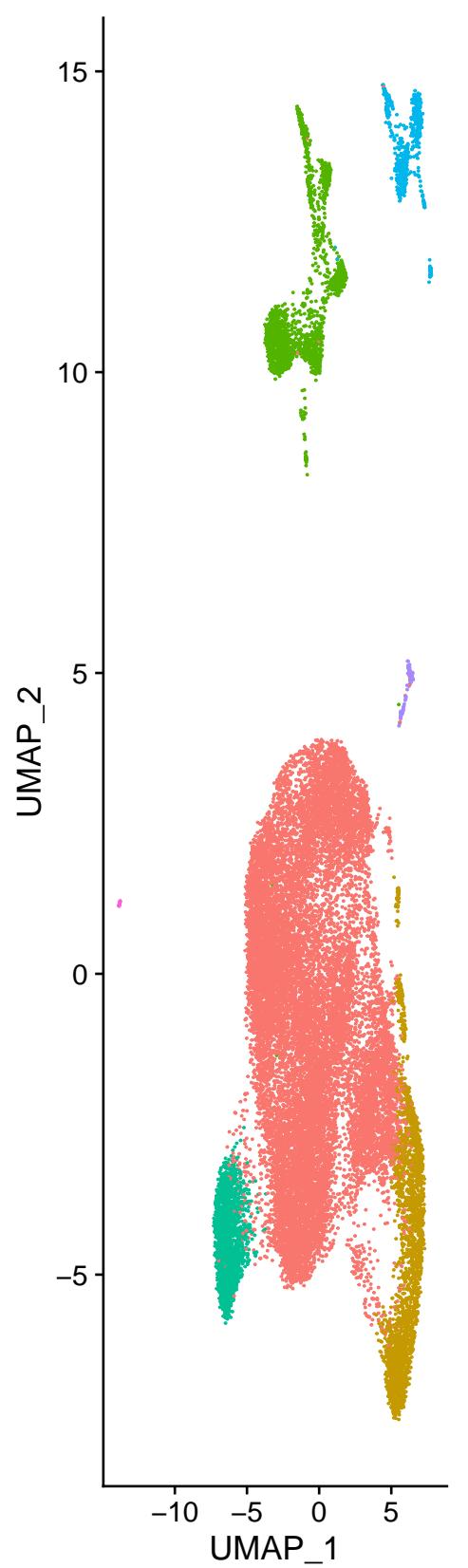
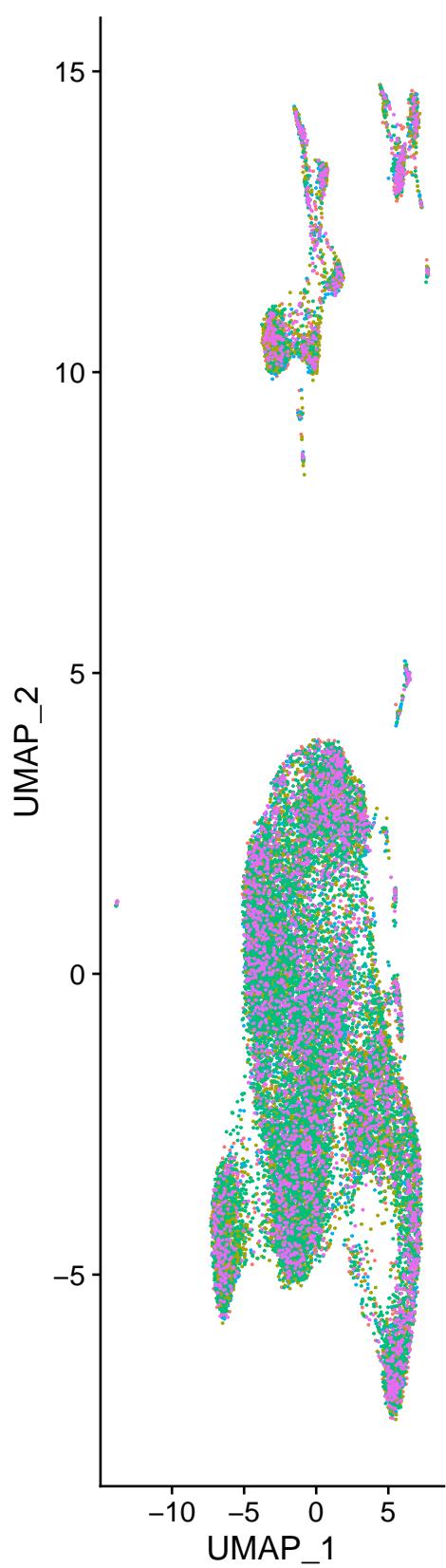
# Find anchors:
pbmc.anchors <- FindIntegrationAnchors(object.list = pbmc.list,
                                         normalization.method = "LogNormalize", reference = reference.list,
                                         dims = 1:30)

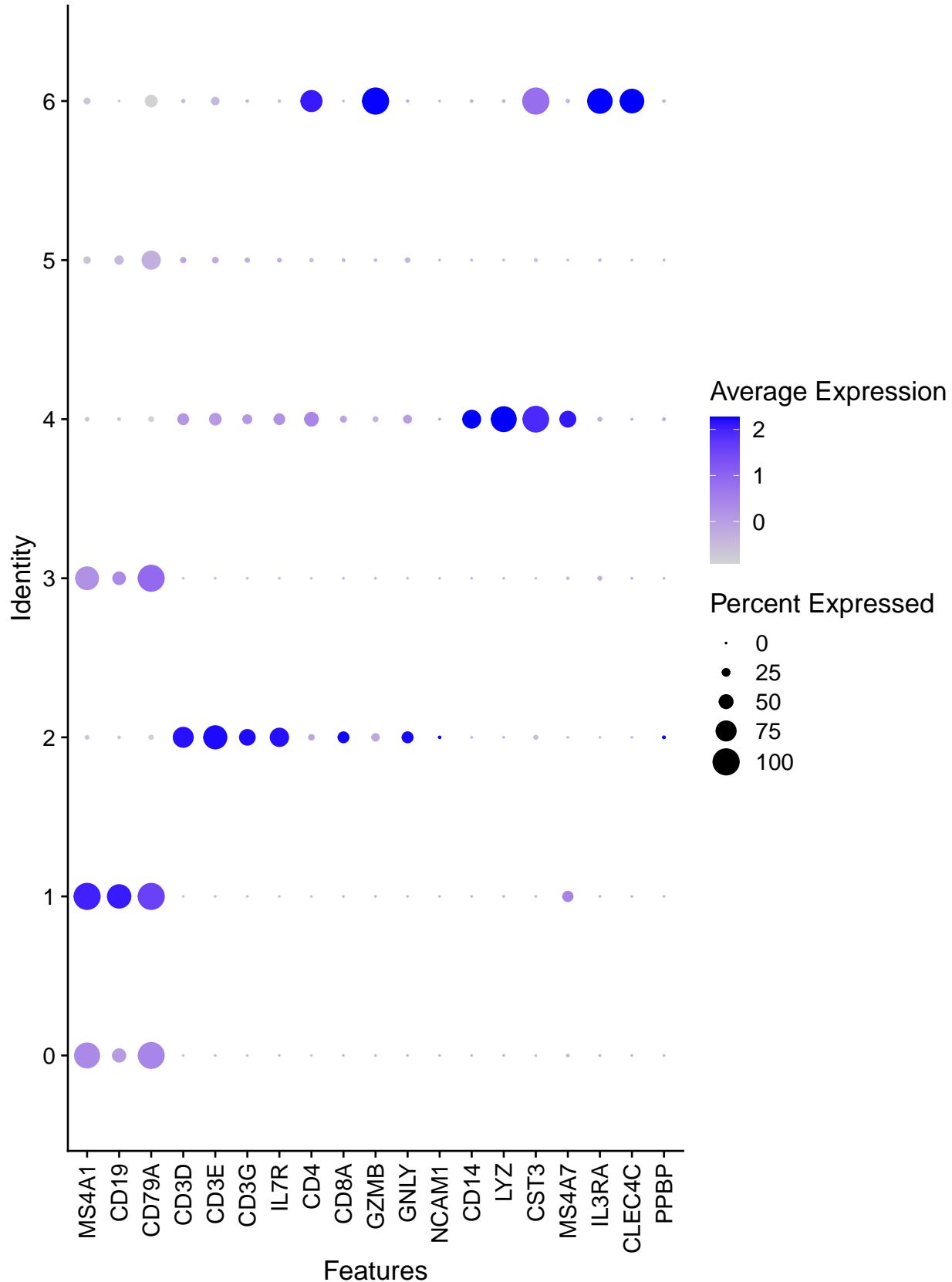
pbmc.integrated <- IntegrateData(anchorset = pbmc.anchors, dims = 1:30)

save(pbmc.integrated, file = "seurat_objects/PBMC_IgD-ve_transcriptomes_AnchorIntegrated.RData")

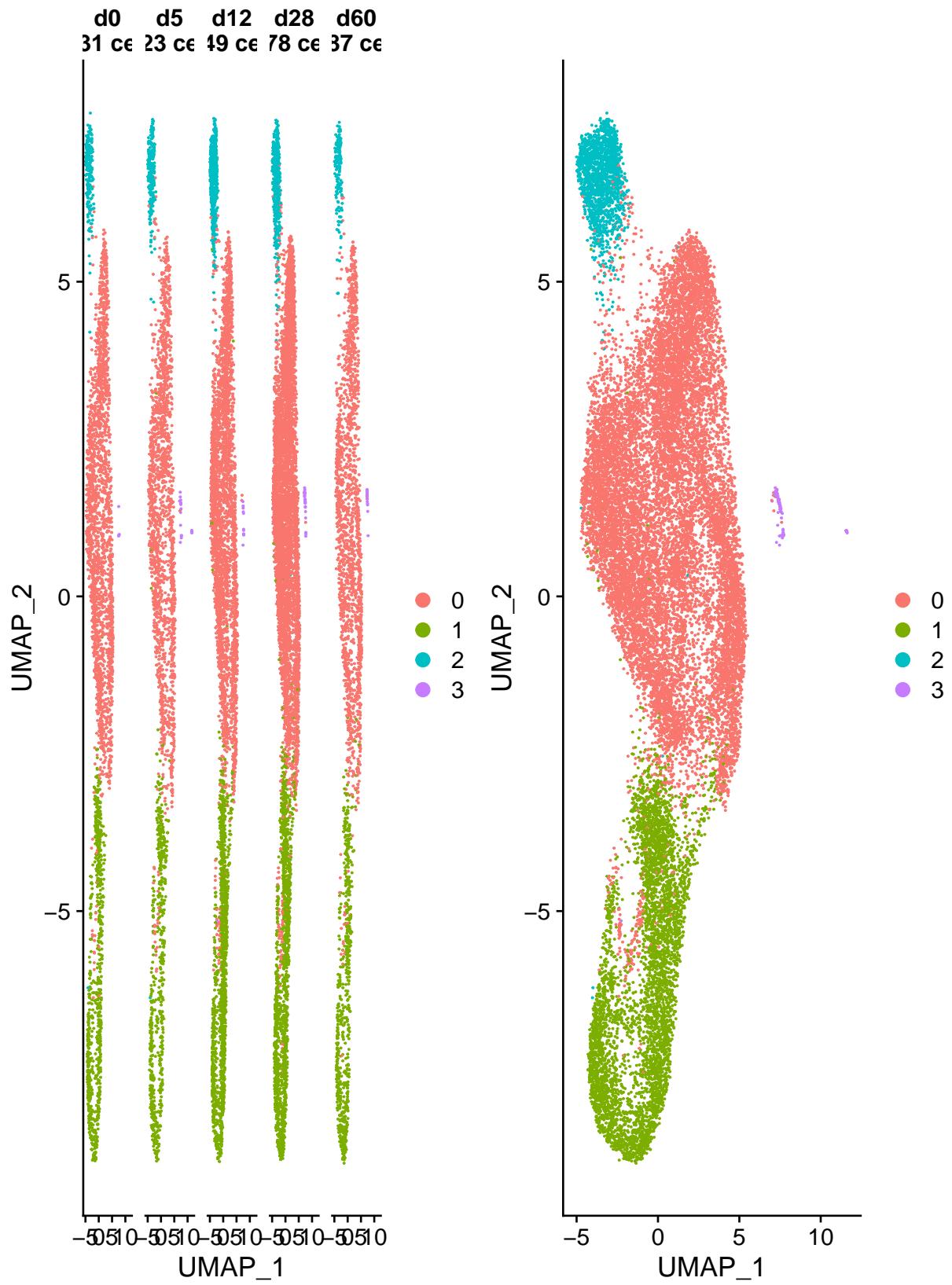
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 24156
## Number of edges: 863299
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.9702
## Number of communities: 7
## Elapsed time: 6 seconds

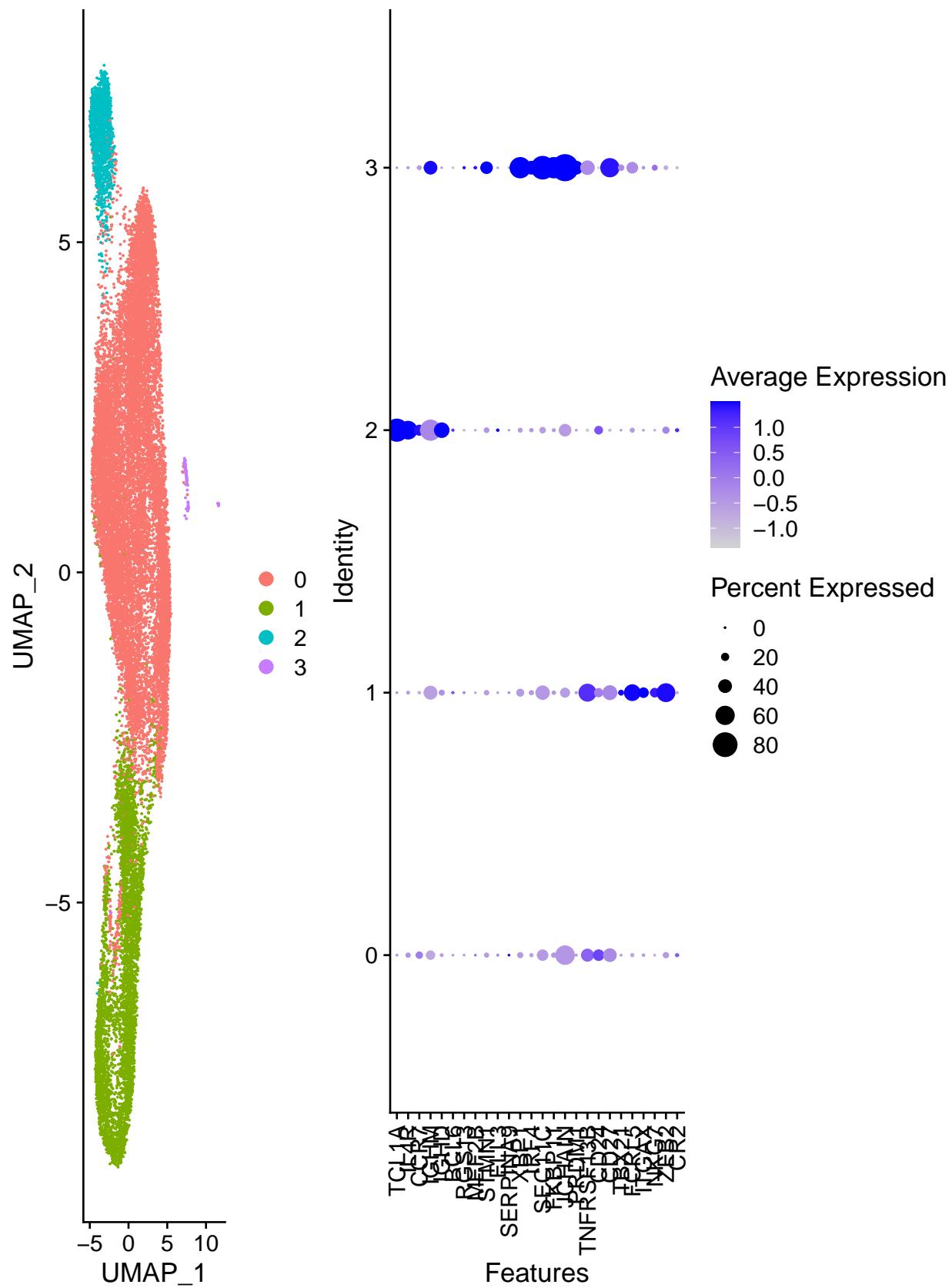
```

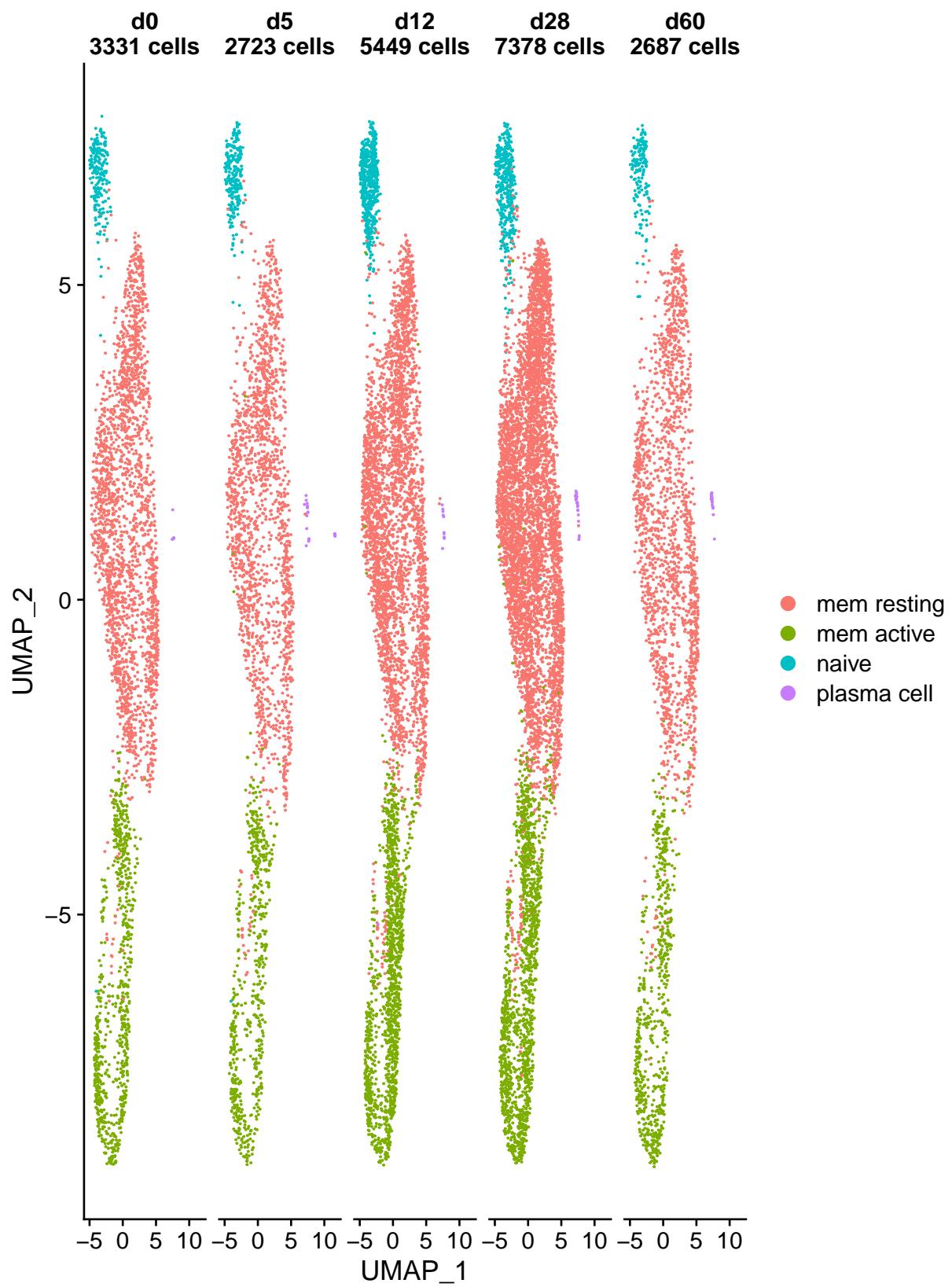




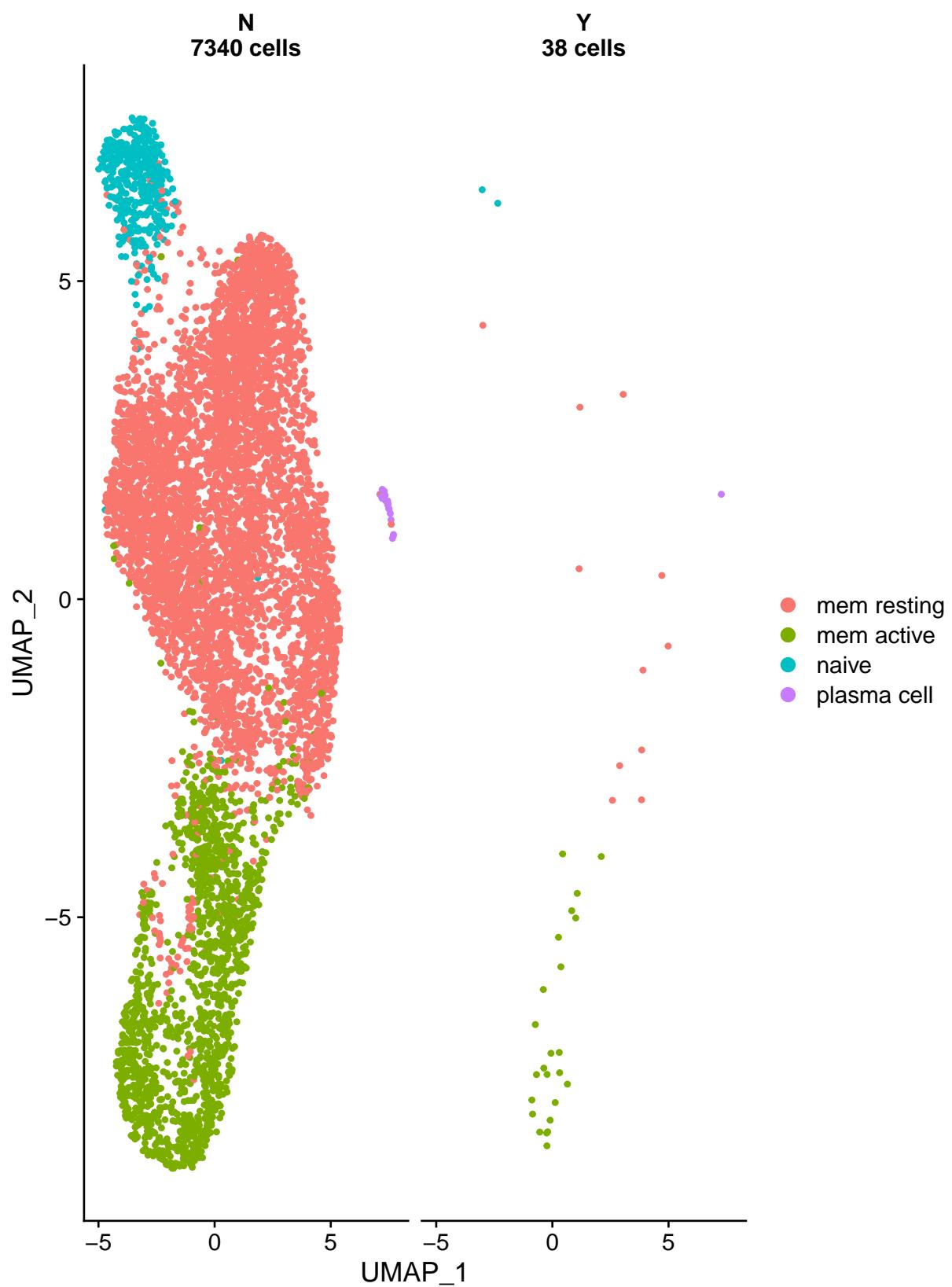
```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 21568
## Number of edges: 704646
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.9613
## Number of communities: 4
## Elapsed time: 6 seconds
```

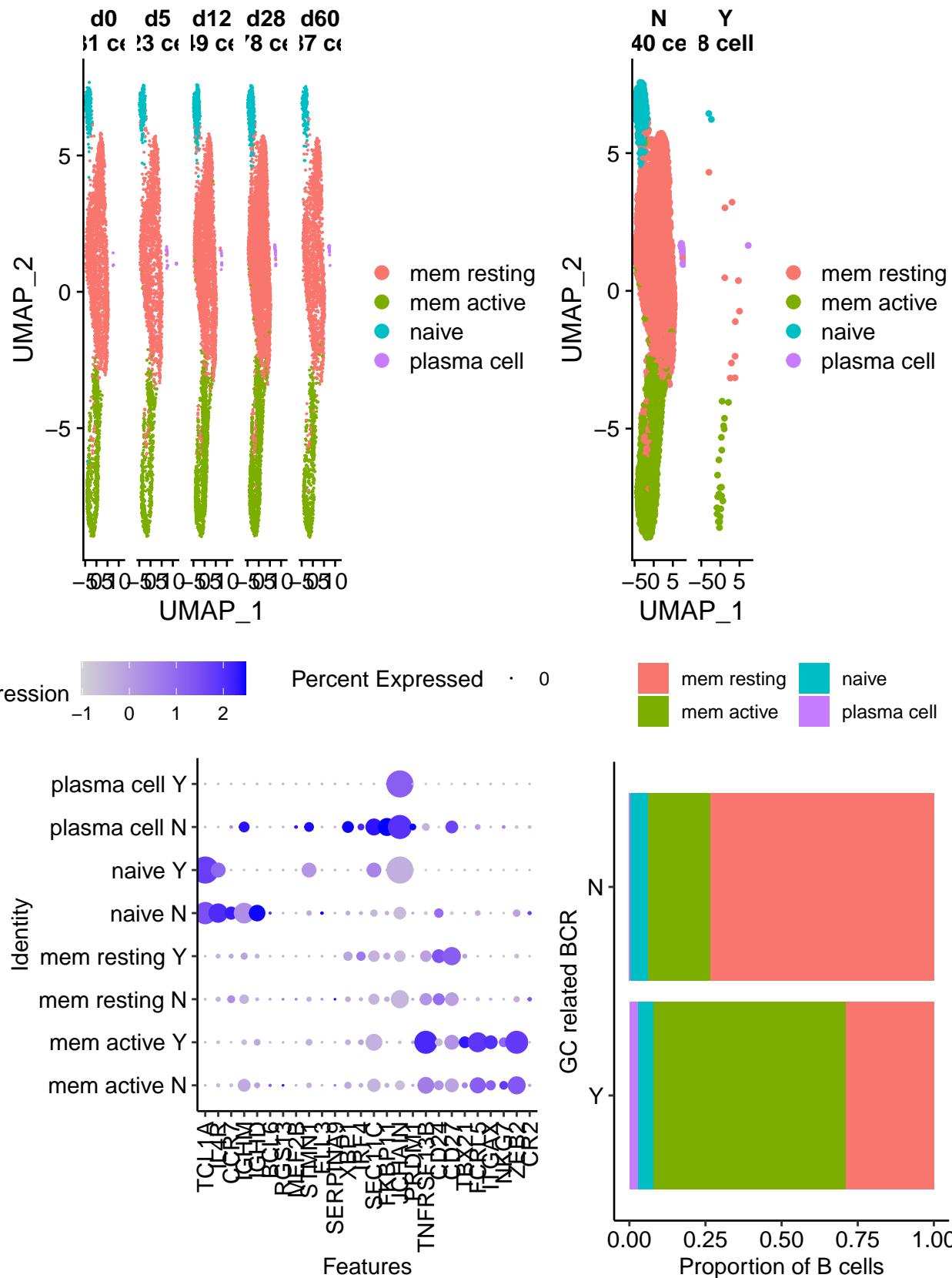






```
##      Mode    FALSE    TRUE
## logical      1     38
##      Mode    FALSE    TRUE
## logical      1     38
##      Mode    TRUE
## logical      8
##      Mode    TRUE
## logical      8
```





SessionInfo

```
## R version 3.6.1 (2019-07-05)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: CentOS Linux 7 (Core)
##
## Matrix products: default
## BLAS:    /bi/apps/R/3.6.1/lib64/R/lib/libRblas.so
## LAPACK:  /bi/apps/R/3.6.1/lib64/R/lib/libRlapack.so
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8    LC_NUMERIC=C          LC_TIME=C
## [4] LC_COLLATE=C           LC_MONETARY=C        LC_MESSAGES=C
## [7] LC_PAPER=C              LC_NAME=C            LC_ADDRESS=C
## [10] LC_TELEPHONE=C         LC_MEASUREMENT=C   LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics   grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] forcats_0.4.0  stringr_1.4.0  dplyr_1.0.2   purrr_0.3.3
## [5] readr_1.3.1    tidyverse_1.3.0
## [9] patchwork_1.0.0 cowplot_1.0.0  ggplot2_3.3.2 Seurat_3.2.2
##
## loaded via a namespace (and not attached):
## [1] Rtsne_0.15          colorspace_1.4-1   ggsignif_0.6.0
## [4] deldir_0.1-23       ellipsis_0.3.0    ggridges_0.5.2
## [7] fs_1.3.1            rstudioapi_0.10   spatstat.data_1.4-3
## [10] leiden_0.3.4        ggpubr_0.2.4     listenv_0.8.0
## [13] farver_2.0.1        ggrepel_0.8.1    fansi_0.4.0
## [16] lubridate_1.7.4     RSpectra_0.16-0   xml2_1.2.2
## [19] codetools_0.2-16    splines_3.6.1    knitr_1.26
## [22] polyclip_1.10-0    jsonlite_1.6     broom_0.7.3
## [25] ica_1.0-2          dbplyr_1.4.2    cluster_2.1.0
## [28] png_0.1-7           uwot_0.1.5     shiny_1.4.0
## [31] sctransform_0.3.2.9000 compiler_3.6.1 httr_1.4.1
## [34] backports_1.1.5     assertthat_0.2.1 Matrix_1.2-17
## [37] fastmap_1.0.1      lazyeval_0.2.2   cli_2.0.0
## [40] later_1.0.0         formatR_1.7     htmltools_0.4.0
## [43] tools_3.6.1         rsvd_1.0.2     igraph_1.2.4.2
## [46] gtable_0.3.0        glue_1.4.2     RANN_2.6.1
## [49] reshape2_1.4.3      Rcpp_1.0.3     spatstat_1.64-1
## [52] cellranger_1.1.0    vctrs_0.3.6    gdata_2.18.0
## [55] nlme_3.1-141        lmtest_0.9-37   xfun_0.11
## [58] globals_0.13.1      rvest_0.3.5    mime_0.7
## [61] miniUI_0.1.1.1      lifecycle_0.2.0  irlba_2.3.3
## [64] gtools_3.8.1         goftest_1.2-2   future_1.20.1
## [67] MASS_7.3-51.4       zoo_1.8-8     scales_1.1.0
## [70] hms_0.5.2           promises_1.1.0  spatstat.utils_1.17-0
## [73] parallel_3.6.1      RColorBrewer_1.1-2 yaml_2.2.0
## [76] reticulate_1.14     pbapply_1.4-2   gridExtra_2.3
## [79] rpart_4.1-15         stringi_1.4.3   caTools_1.17.1.3
## [82] rlang_0.4.10         pkgconfig_2.0.3  matrixStats_0.55.0
## [85] bitops_1.0-6         evaluate_0.14   lattice_0.20-38
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## [88] ROCR_1.0-7           tensor_1.5          htmlwidgets_1.5.1
## [91] labeling_0.3          tidyselect_1.1.0    parallelly_1.21.0
## [94] RcppAnnoy_0.0.14      plyr_1.8.5          magrittr_1.5
## [97] R6_2.4.1              gplots_3.0.1.1     generics_0.0.2
## [100] DBI_1.1.0             haven_2.2.0         pillar_1.4.7
## [103] withr_2.1.2           mgcv_1.8-28        fitdistrplus_1.1-1
## [106] survival_2.44-1.1     abind_1.4-5         future.apply_1.6.0
## [109] modelr_0.1.5          crayon_1.3.4       KernSmooth_2.23-15
## [112] plotly_4.9.1           rmarkdown_2.0       readxl_1.3.1
## [115] grid_3.6.1              data.table_1.12.8  reprex_0.3.0
## [118] digest_0.6.23          xtable_1.8-4        httpuv_1.5.2
## [121] RcppParallel_4.4.4     munsell_0.5.0       viridisLite_0.3.0
```