

# PAPER TITLE TO BE DEFINED (in common.yaml)

6-Merge two experiment

2021-11-23 14:13:31 +0100

## Abstract

Lung interstitium macrophages (IMs) are non-alveolar resident tissue macrophages which contribute to the lung homeostasis. These cells were reported to be heterogeneous by our group and other teams, which contains two main distinct subpopulations: CD206+ IMs and CD206- IMs. However, the exact origin of IMs and the transcriptional programs that control IM differentiation remains unclear. In recent report, we analyzed the refilled IMs in the course of time after induced IM depletion with single-cell RNA sequencing (10X Genomics Chromium) and bulk RNA sequencing.

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## 1 Description

## 2 Load data

```
library(Seurat)
library(ggplot2)

imdtr1 <- readRDS("../.../IM_DTR_exp1/analyses/20210114_cell_id/results.
    withSingleR.Rds")
imdtr2 <- readRDS("../.../IM_DTR_exp2/analyses/20210409_immune_cell_id/
    immune.cellType_filtered.withSingleR.seuratObject.Rds")
```

## 3 Merge Exp1 and Exp2

Prepare metadata:

```
imdtr1$orig.ident <- "IM-DTR1"
imdtr2$orig.ident <- "IM-DTR2"

imdtr1$cell.type0 <- "CD45+"

imdtr1$cell.type2 <- imdtr1$RNA_snn_res.0.3

imdtr1$treatment <- ifelse(imdtr1$treatment=="Cre+", "96hDT", no =
    noTreatment)
imdtr2$treatment <- sub(imdtr2$treatment, pattern = "HT[0-9]-",
    replacement = "")
```

Now merge:

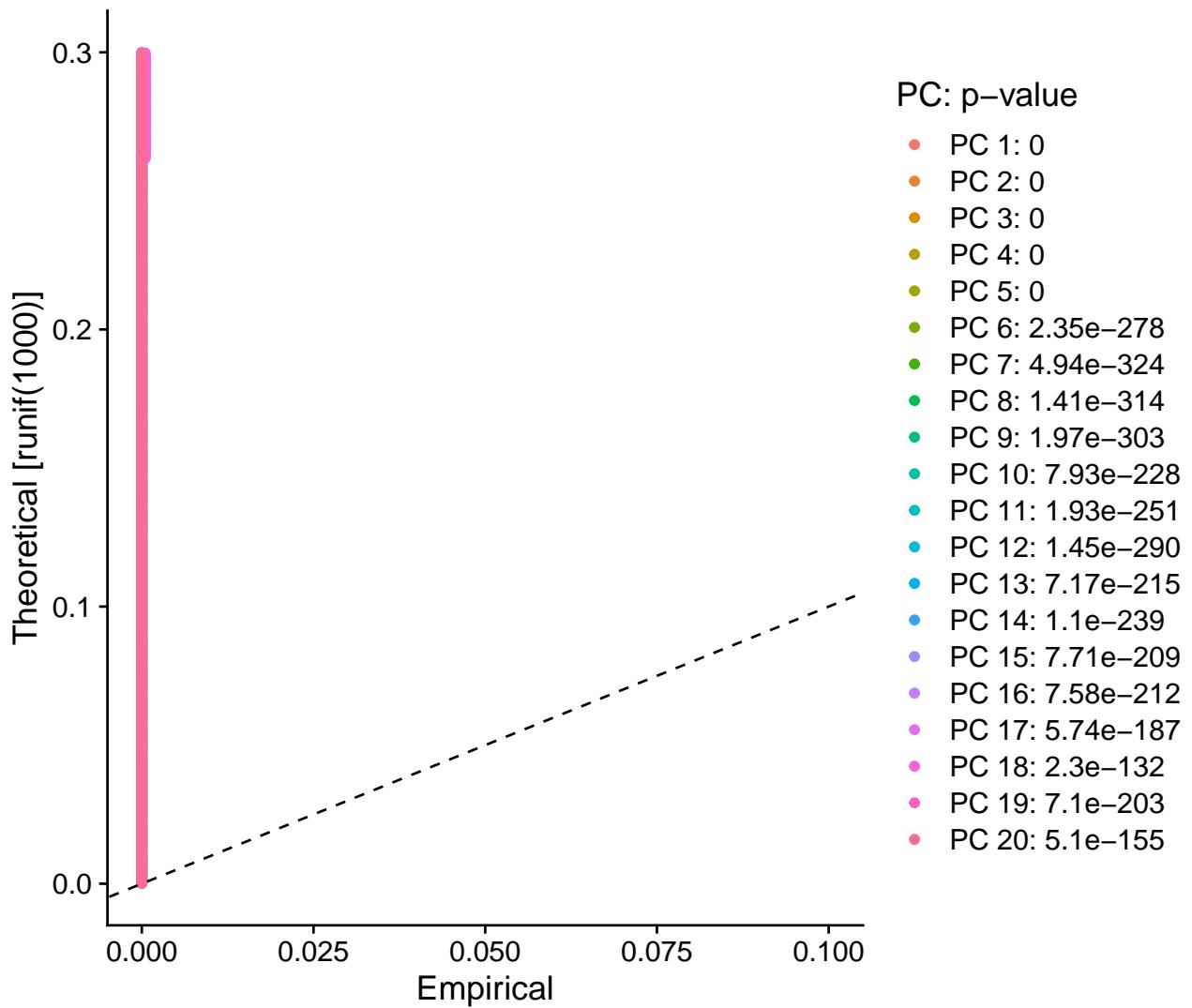
```
imdtr3 <- merge(imdtr1, imdtr2, project = "IM-DTR")
```

Normalize and process:

```
imdtr3 <- NormalizeData(imdtr3)
imdtr3 <- FindVariableFeatures(imdtr3, selection.method = "vst", nfeatures
    = 2000)
imdtr3 <- ScaleData(imdtr3, features = rownames(imdtr3))
```

```
imdtr3 <- RunPCA(imdtr3, features = VariableFeatures(imdtr3))
```

```
imdtr3 <- JackStraw(imdtr3, num.replicate = 100)
imdtr3 <- ScoreJackStraw(imdtr3, dims = 1:20)
JackStrawPlot(imdtr3, dims = 1:20)
```



Samples from Exp1 and Exp2 are so similar.

Remove old calculated neighbor (snn):

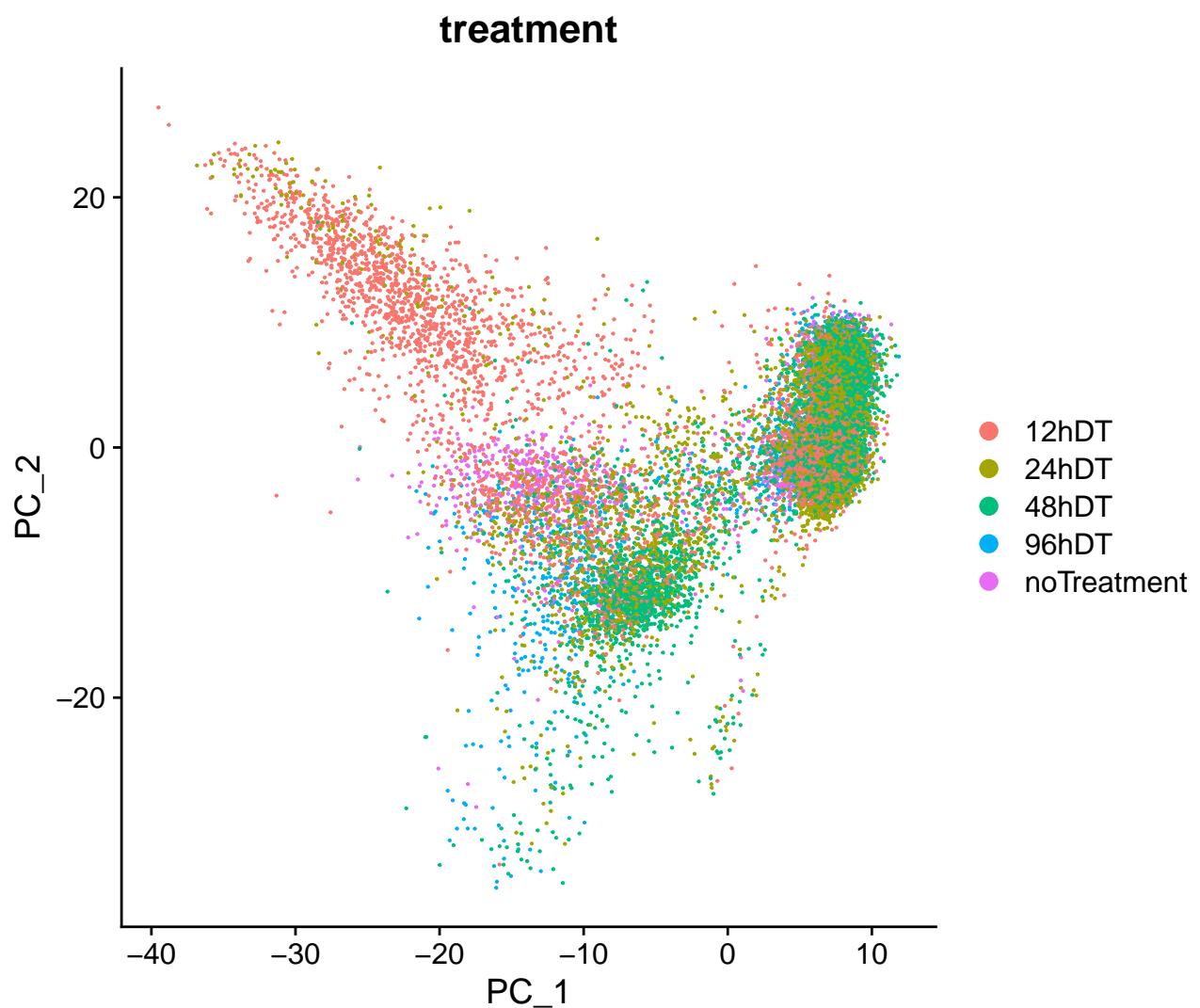
```

meta.names <- names(imdtr3@meta.data)
old.snn <- meta.names[startsWith(meta.names, "RNA_snn") | startsWith(meta.
  names, "res") | startsWith(meta.names, "Clusternames") | startsWith(
  meta.names, "ClusterNames")]
for (i in old.snn) {
  imdtr3[[i]] <- NULL
}

imdtr3 <- RunPCA(imdtr3, features = VariableFeatures(imdtr3))

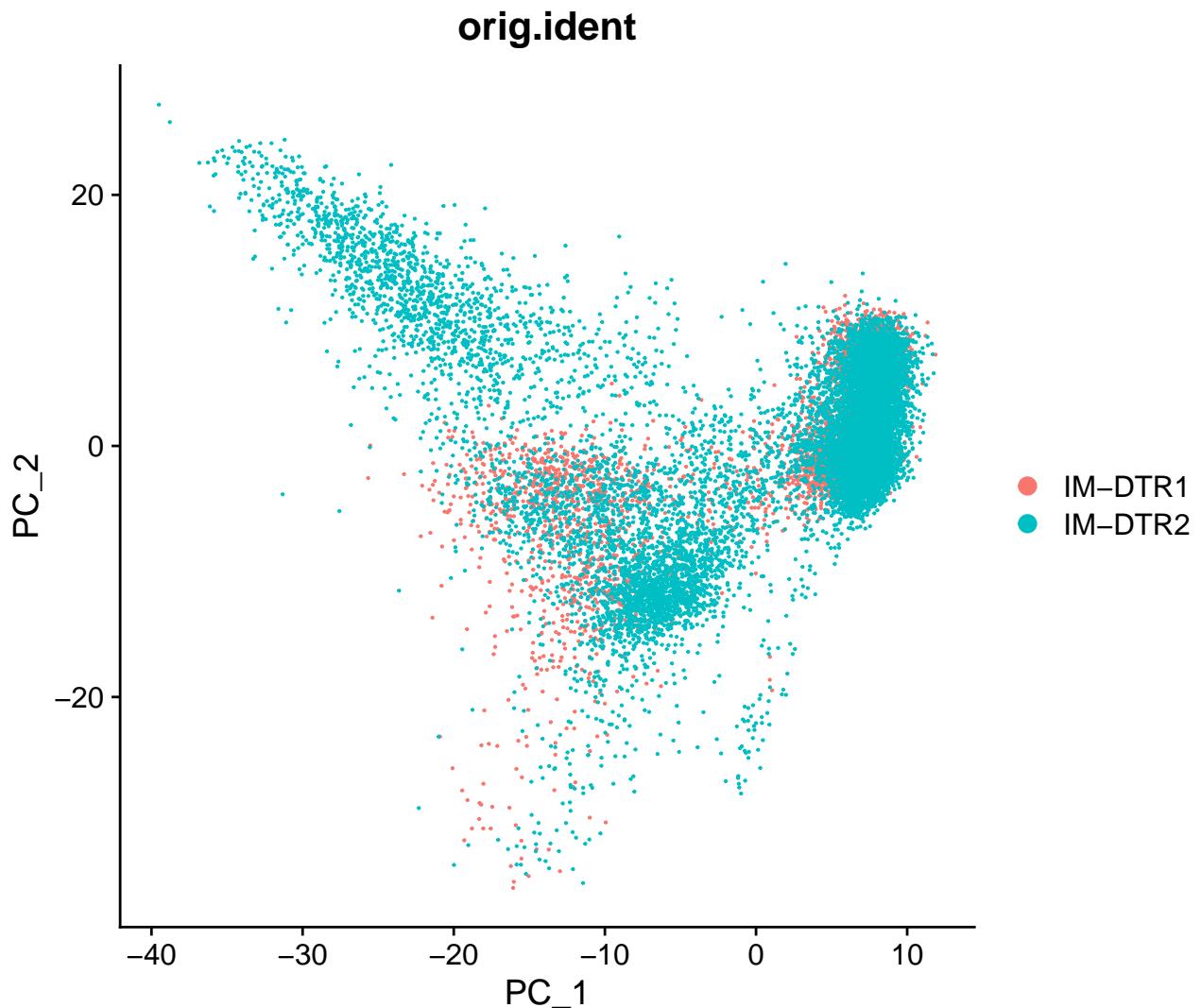
PCAPlot(imdtr3, group.by = "treatment")

```



```
PCAPlot(imdtr3, group.by = "orig.ident")
```

1



*Samples from Exp1 and Exp2 are so similar.*

Non-linear reduction:

```
results <- RunTSNE(imdtr3, dims = 1:6)
```

1

Calculate UMAP in 3D:

```
imdtr3.for3d <- RunUMAP(imdtr3, dims = 1:6, n.components = 3L)
```

1

### 3.1 Presentation in 3D

```
library(rgl)
library(RColorBrewer)

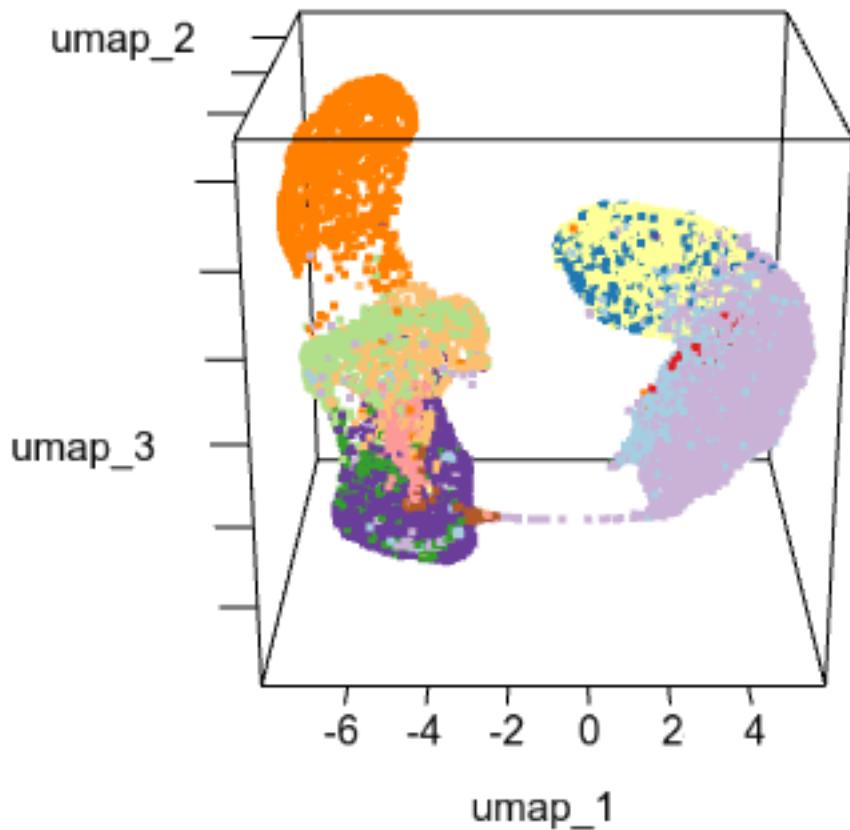
umap_1 <- imdtr3.for3d@reductions$umap@cell.embeddings[,1]
umap_2 <- imdtr3.for3d@reductions$umap@cell.embeddings[,2]
umap_3 <- imdtr3.for3d@reductions$umap@cell.embeddings[,3]

plot3d(x = umap_1, y = umap_2, z = umap_3, col = factor(imdtr3.for3d$cell.type2),
       labels = brewer.pal(length(unique(imdtr3.for3d$cell.type2)),
                           name = "Paired")), size = 3)
```

1  
2  
3  
4  
5  
6  
7  
8

```
rglwidget()
```

9



3d plot shows only one bridge exists.

### 3.2 Cluster cells

```
imdtr3.for3d <- FindNeighbors(imdtr3.for3d, dims = 1:10)
imdtr3.for3d <- FindClusters(imdtr3.for3d, resolution = 0.23)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 15941
## Number of edges: 508837
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.9276
## Number of communities: 7
```

1  
2  
3  
4  
5  
6  
7  
8

```

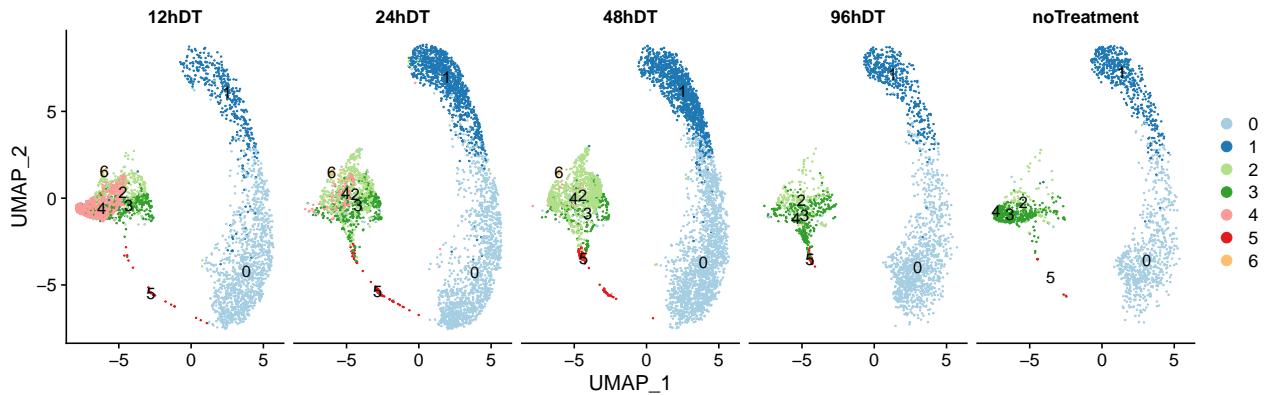
## Elapsed time: 1 seconds

```

```

1 Idents(imdtr3.for3d) <- "RNA_snn_res.0.23"
2 pal <- brewer.pal(length(levels(imdtr3.for3d)), name = "Paired")
3 DimPlot(imdtr3.for3d, label = TRUE, split.by = "treatment", cols = pal,
reduction = "umap")

```



The cluster 6 could be DCs

```

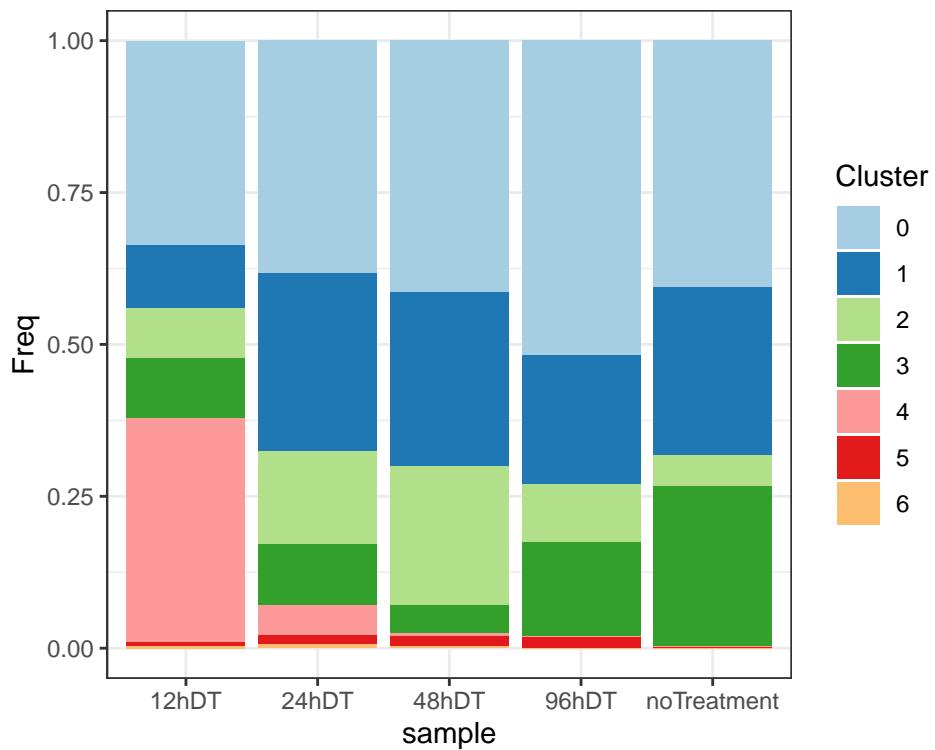
source("../R/SeuratFreqTable.R")
freq.celltype.list <- list(
  `12hDT` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset = treatment
  == "12hDT"), slotName = "RNA_snn_res.0.23"),
  `24hDT` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset = treatment
  == "24hDT"), slotName = "RNA_snn_res.0.23"),
  `48hDT` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset = treatment
  == "48hDT"), slotName = "RNA_snn_res.0.23"),
  `96hDT` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset = treatment
  == "96hDT"), slotName = "RNA_snn_res.0.23"),
  `noTreatment` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset =
  treatment == "noTreatment"), slotName = "RNA_snn_res.0.23")
)

```

```

source("../R/barChart.R")
barChart(freq.celltype.list) + labs(fill = "Cluster") + scale_fill_manual(
  values = pal)

```

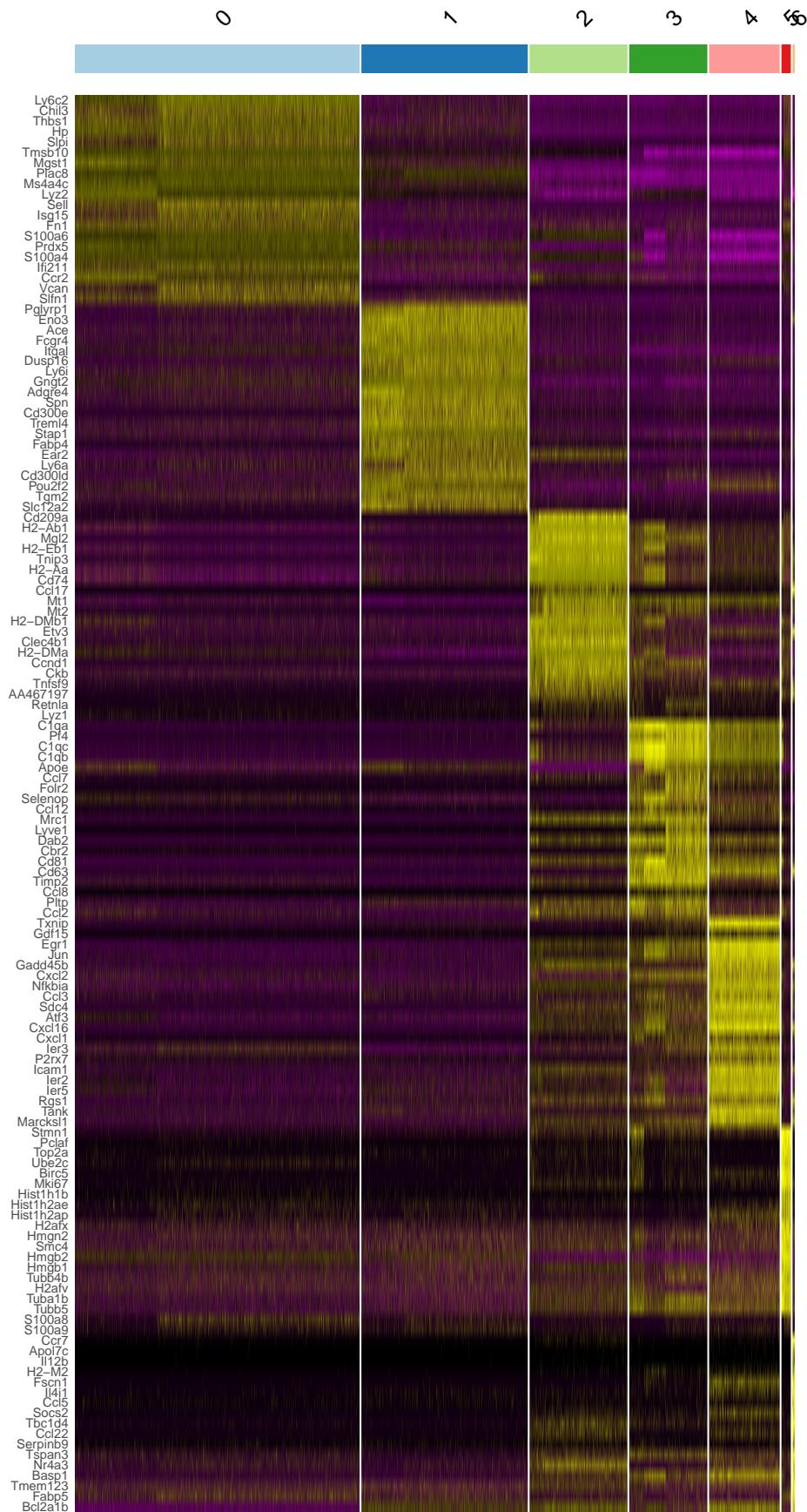


## 4 Population characterization

```

library(dplyr)
all_cluster.markers <- FindAllMarkers(imdtr3.for3d, verbose = FALSE)
top20 <- all_cluster.markers %>% group_by(cluster) %>% top_n(n = 20, wt =
  avg_log2FC)
DoHeatmap(imdtr3.for3d, features = top20$gene, group.colors = pal) +
  NoLegend()

```



Cluster 0: ClaMo Cluster 1:

PatMo Cluster 2: MHCII\_IM Cluster 3: CD206\_IM Cluster 4: Chemoattractant Cluster 5: Transit Cluster  
6: ?

## 4.1 Identify the Cluster 6

GO for cluster 6:

```

library(topGO)
library(org.Mm.eg.db)
allSymbols <- unique(select(org.Mm.eg.db, keys(org.Mm.eg.db), columns = "SYMBOL")$SYMBOL)
extended_signatures_cluster6 <- filter(all_cluster.markers, avg_log2FC > 0.5 & -log10(p_val) > 2 & cluster == "6")
myInterestingGenes <- as.character(extended_signatures_cluster6$gene)

geneList <- factor(as.integer(allSymbols %in% myInterestingGenes))
names(geneList) <- allSymbols

sampleG0data <- new("topGOdata",
                      description = "Simple_session", ontology = "BP",
                      allGenes = geneList,
                      annot = annFUN.org, mapping = "org.Mm.eg.db", ID = "SYMBOL")
resultFisher <- runTest(sampleG0data, algorithm = "classic", statistic = "fisher")
GenTable(sampleG0data, classicFisher = resultFisher, topNodes = 20)

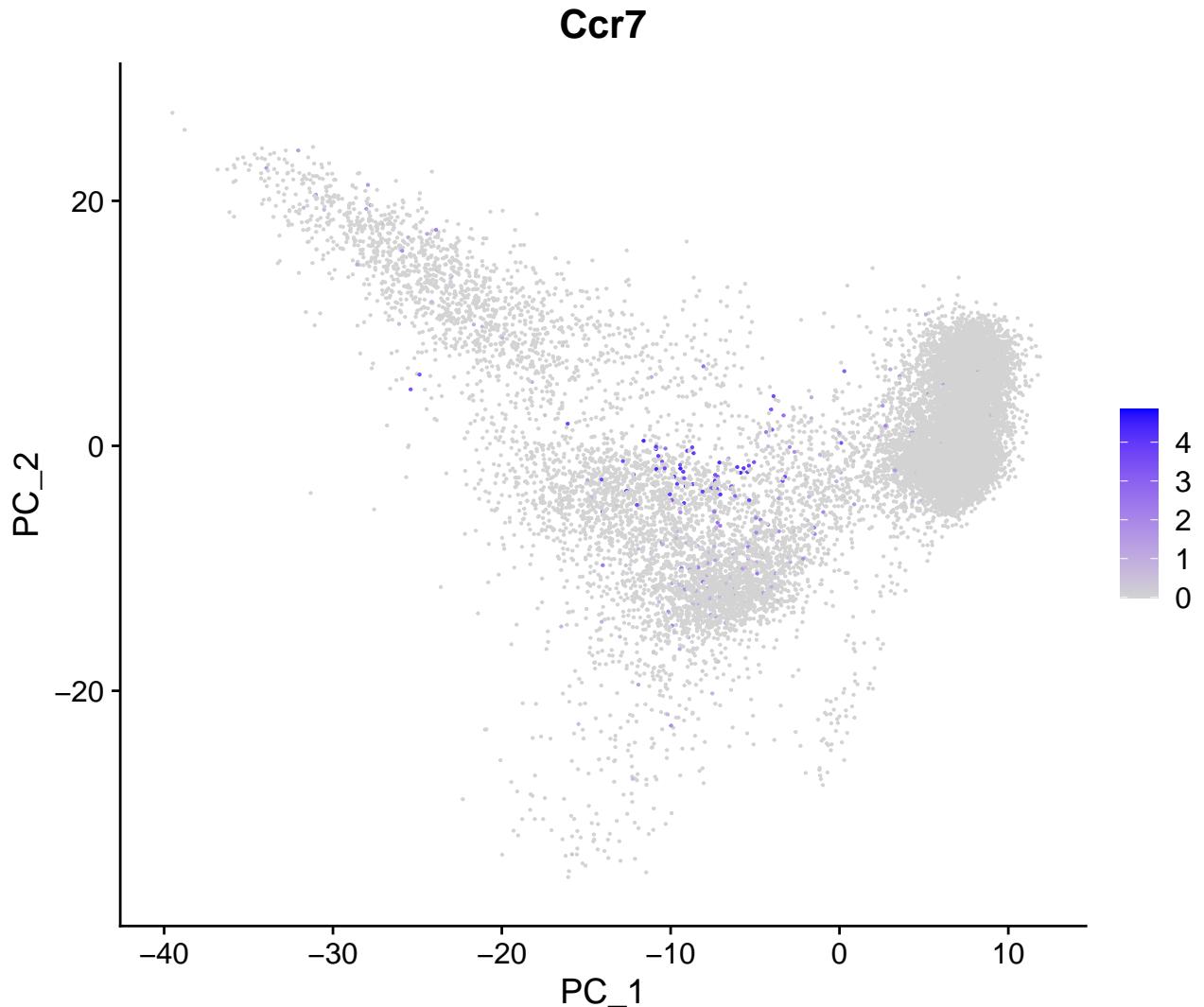
```

	## # A tibble: 20 x 6	## GO.ID	Term	Annotated	Significant	Expected
	## <chr>	## <chr>		<int>	<int>	<dbl> <chr>
1	## <-->	## >				
2	## 1	## GO:0048523	negative regulation ~	4950	173	77.2 2.4e
3	## 2	## GO:0048519	negative regulation ~	5500	181	85.8 8.5e
4	## 3	## GO:0009987	cellular process	17114	343	267. 2.6e
5	## 4	## GO:0048518	positive regulation ~	6358	189	99.2 9.8e
6	## 5	## GO:0065009	regulation of molecu~	2574	109	40.2 3.6e
7	## 6	## GO:0050789	regulation of biolog~	12212	280	191. 1.6e
8	## 7	## GO:0050790	regulation of cataly~	1876	89	29.3 7.2e
9	## 8	## GO:0048522	positive regulation ~	5834	175	91.0 9.1e
10	## 9	## GO:0050794	regulation of cellul~	11652	270	182. 1.6e
11	## 10	## GO:0002376	immune system process	2832	112	44.2 1.8e
12			-21			
13			-21			

## 11 GO:0065007 biological regulation -21	12806	286	200.	1.9e	14
## 12 GO:0040011 locomotion -21	1863	87	29.1	6.6e	15
## 13 GO:0016477 cell migration -21	1501	77	23.4	8.3e	16
## 14 GO:0048870 cell motility -20	1663	81	26.0	1.6e	17
## 15 GO:0051674 localization of cell -20	1663	81	26.0	1.6e	18
## 16 GO:0048856 anatomical structure~ -20	5977	174	93.3	3.9e	19
## 17 GO:0051336 regulation of hydrol~ -20	1002	61	15.6	4.7e	20
## 18 GO:0032502 developmental process -20	6449	182	101.	7.7e	21
## 19 GO:0006928 movement of cell or ~ -19	2101	91	32.8	1.0e	22
## 20 GO:0048583 regulation of respon~ -19	3972	133	62.0	1.9e	23

```
FeaturePlot(imdtr3, features = "Ccr7")
```

1

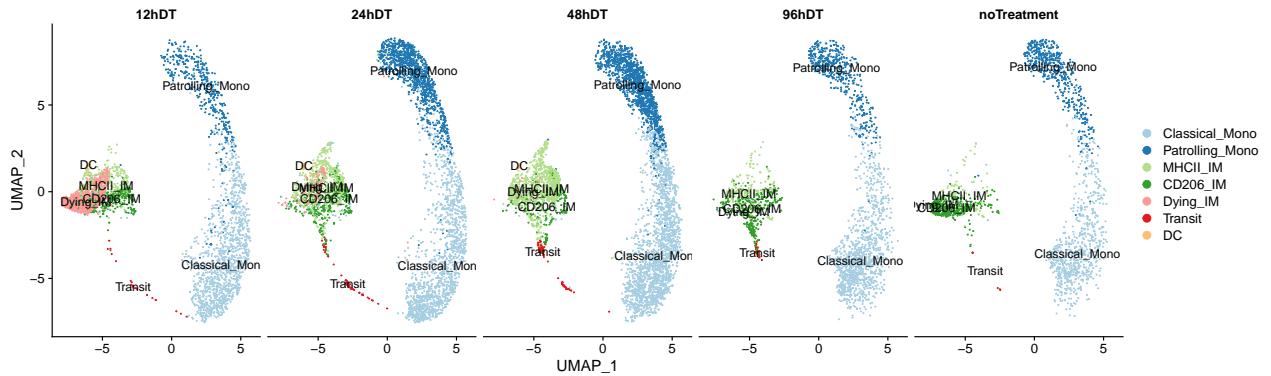


Cluster 0: ClaMo Cluster 1: PatMo Cluster 2: MHCII\_IM Cluster 3: CD206\_IM Cluster 4: Dying\_IM  
Cluster 5: Transit Cluster 6: DC

change the idents

```
levels(imdtr3) 1
## [1] "0"          "1"          "2"          "3"
## [5] "4"          "5"          "CD206_IM"    "
"chemoattractant"
## [9] "Classical_Mono" "MHCII_IM"    "Patrolling_Mono" "Transit" 3

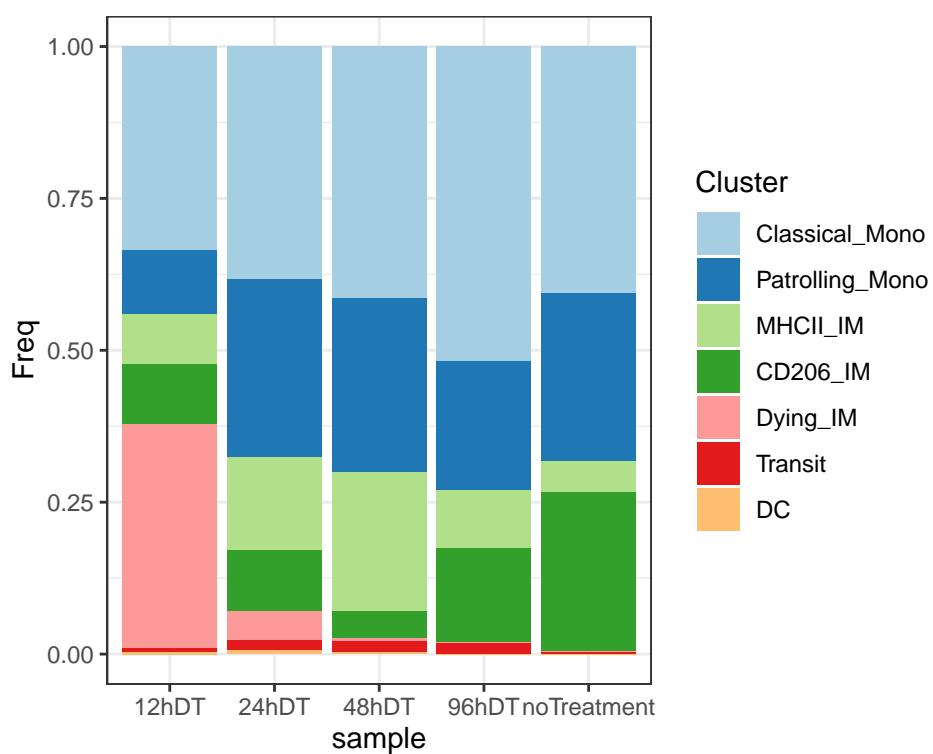
imdtr3.for3d$cell.type2 <- factor(Idents(imdtr3.for3d), labels = c(" 1
"Classical_Mono", "Patrolling_Mono", "MHCII_IM", "CD206_IM", "Dying_IM",
"Transit", "DC"))
Idents(imdtr3.for3d) <- "cell.type2" 2
DimPlot(imdtr3.for3d, label = TRUE, split.by = "treatment", cols = pal) 3
```



```

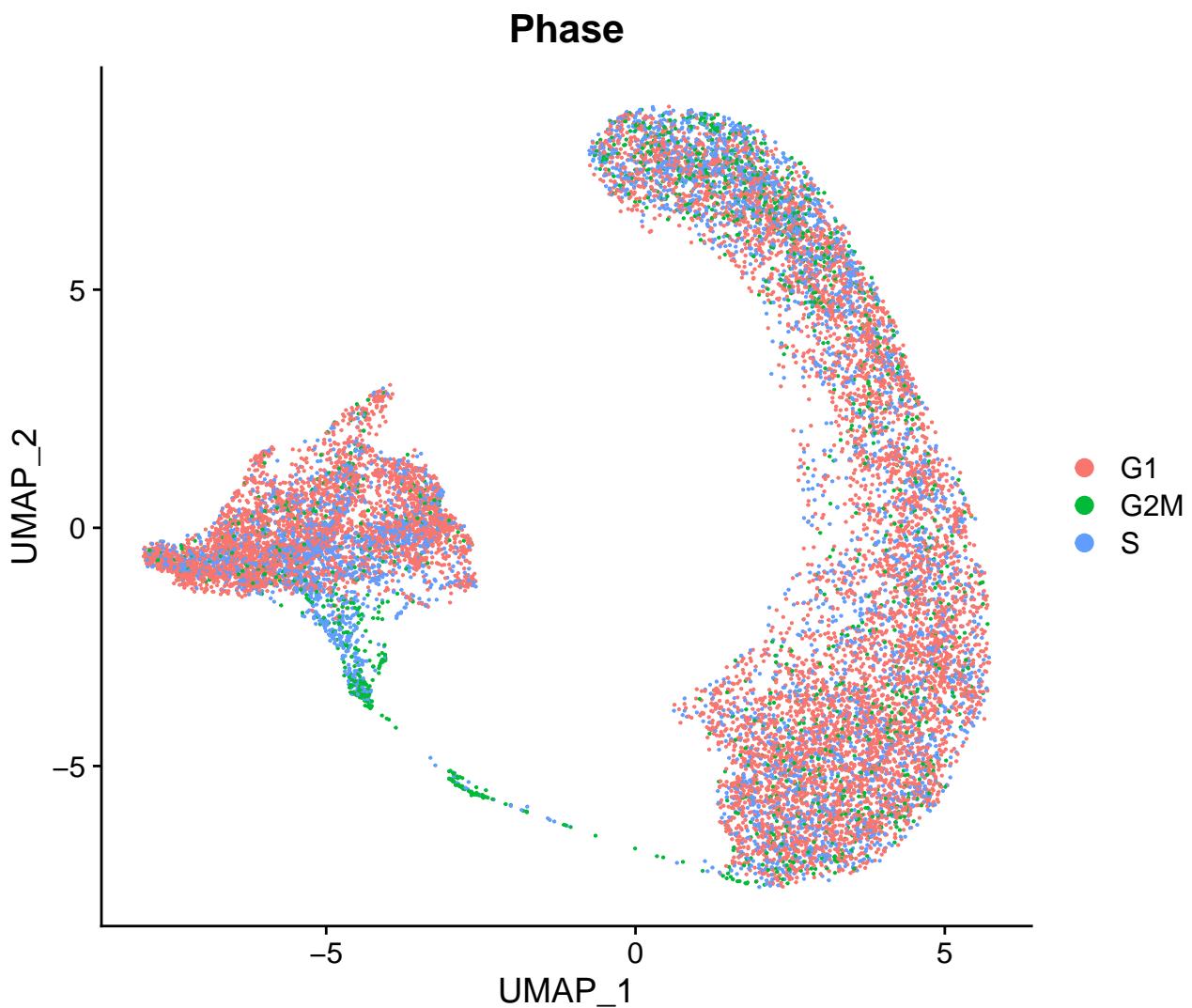
freq.celltype.list <- list(
  `12hDT` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset = treatment
    == "12hDT"), slotName = "cell.type2"),
  `24hDT` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset = treatment
    == "24hDT"), slotName = "cell.type2"),
  `48hDT` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset = treatment
    == "48hDT"), slotName = "cell.type2"),
  `96hDT` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset = treatment
    == "96hDT"), slotName = "cell.type2"),
  `noTreatment` = Seurat2CellFreqTable(subset(imdtr3.for3d, subset =
    treatment == "noTreatment"), slotName = "cell.type2")
)

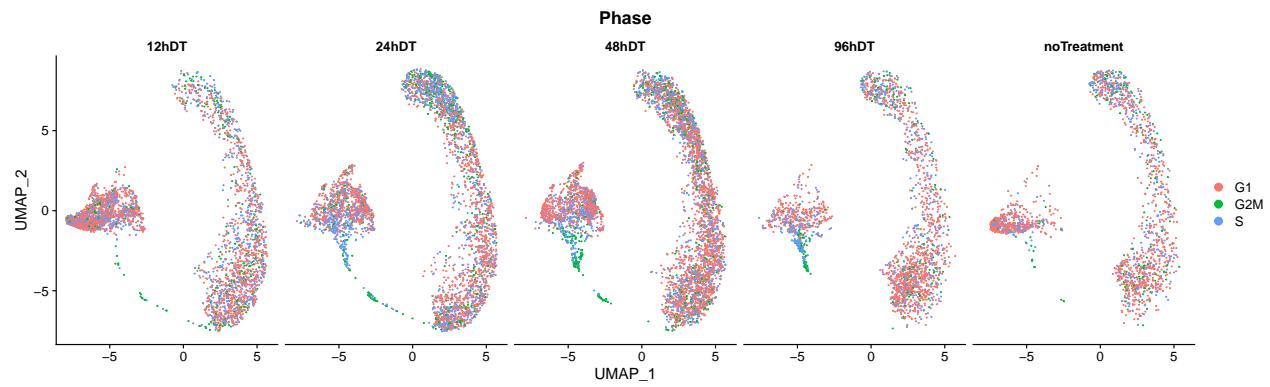
barChart(freq.celltype.list) + labs(fill = "Cluster") + scale_fill_manual(
  values = pal)
  
```



## 5 Cell-cycle analysis

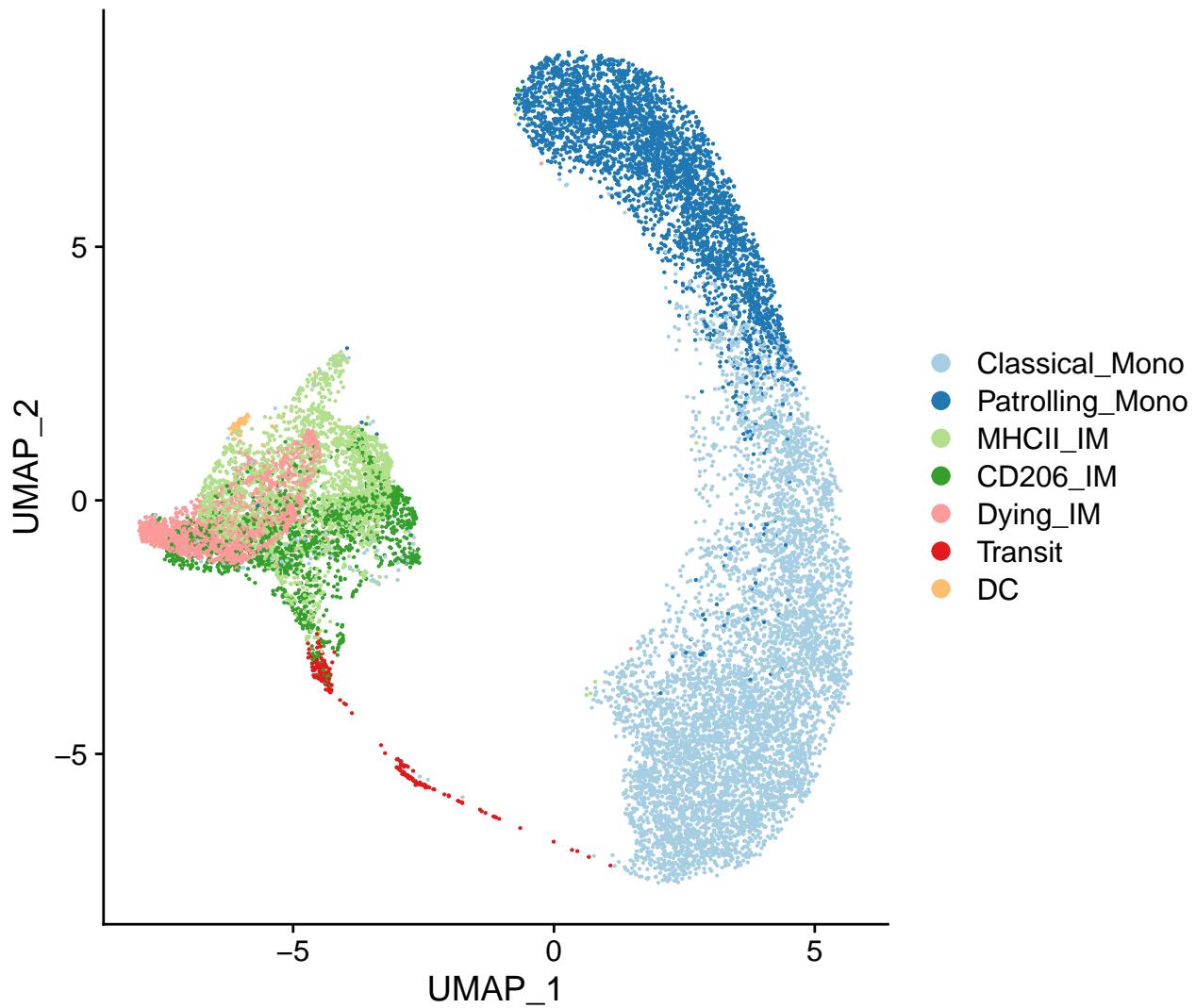
```
library(cowplot)
data("geneinfo_human", package = "nichenetr")
s.genes <- nichenetr::convert_human_to_mouse_symbols(cc.genes.updated.2019
  $s.genes)
g2m.genes <- nichenetr::convert_human_to_mouse_symbols(cc.genes.updated
  .2019$g2m.genes)
imdtr3.for3d <- CellCycleScoring(imdtr3.for3d, s.features = s.genes, g2m.
  features = g2m.genes, set.ident = FALSE)
DimPlot(imdtr3.for3d, group.by = "Phase")
```





2d plot:

```
DimPlot(imdtr3.for3d, cols = pal)
```



```
saveRDS(imdtr3.for3d, file = "./immune_imdtr3.seuratObject.Rds")
```

## 6 Session information

sessionInfo()	1
## R version 4.0.3 (2020-10-10)	1
## Platform: x86_64-pc-linux-gnu (64-bit)	2
## Running under: Ubuntu 20.04.3 LTS	3
##	4
## Matrix products: default	5
## BLAS: /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3	6
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3	7
##	8
## locale:	9
## [1] LC_CTYPE=en_US.UTF-8 LC_NUMERIC=C	10
## [3] LC_TIME=en_GB.UTF-8 LC_COLLATE=en_US.UTF-8	11
## [5] LC_MONETARY=en_GB.UTF-8 LC_MESSAGES=en_US.UTF-8	12
## [7] LC_PAPER=en_GB.UTF-8 LC_NAME=C	13
## [9] LC_ADDRESS=C LC_TELEPHONE=C	14
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C	15
##	16
## attached base packages:	17
## [1] stats4 parallel stats graphics grDevices utils	18
datasets	
## [8] methods base	19
##	20
## other attached packages:	21
## [1] cowplot_1.1.1 org.Mm.eg.db_3.12.0 topGO_2.42.0	22
## [4] SparseM_1.81 G0.db_3.12.1 AnnotationDbi_1.52.0	23
## [7] IRanges_2.24.1 S4Vectors_0.28.1 Biobase_2.50.0	24
## [10] graph_1.68.0 BiocGenerics_0.36.1 dplyr_1.0.7	25
## [13] RColorBrewer_1.1-2 rgl_0.107.10 ggplot2_3.3.5	26
## [16] SeuratObject_4.0.2 Seurat_4.0.3	27
##	28
## loaded via a namespace (and not attached):	29
## [1] utf8_1.2.2 reticulate_1.20 tidyselect_1.1.1	30
## [4] RSQLite_2.2.7 htmlwidgets_1.5.3 grid_4.0.3	31
## [7] Rtsne_0.15 pROC_1.17.0.1 munsell_0.5.0	32
## [10] codetools_0.2-18 ica_1.0-2 future_1.21.0	33
## [13] miniUI_0.1.1.1 withr_2.4.2 colorspace_2.0-2	34
## [16] highr_0.9 knitr_1.33 rstudioapi_0.13	35
## [19] ROCR_1.0-11 tensor_1.5 listenv_0.8.0	36
## [22] labeling_0.4.2 polyclip_1.10-0 bit64_4.0.5	37
## [25] farver_2.1.0 parallelly_1.27.0 vctrs_0.3.8	38
## [28] generics_0.1.0 ipred_0.9-11 xfun_0.24	39
## [31] randomForest_4.6-14 R6_2.5.0 bitops_1.0-7	40
## [34] spatstat.utils_2.2-0 cachem_1.0.5 assertthat_0.2.1	41
## [37] promises_1.2.0.1 scales_1.1.1 nnet_7.3-14	42
## [40] gtable_0.3.0 globals_0.14.0 processx_3.5.2	43
## [43] goftest_1.2-2 timeDate_3043.102 rlang_0.4.11	44
## [46] splines_4.0.3 lazyeval_0.2.2 ModelMetrics_1.2.2.2	45
## [49] checkmate_2.0.0 spatstat.geom_2.2-2 yaml_2.2.1	46
## [52] reshape2_1.4.4 abind_1.4-5 backports_1.2.1	47
## [55] crosstalk_1.1.1 httpuv_1.6.1 Hmisc_4.5-0	48
## [58] caret_6.0-88 DiagrammeR_1.0.6.1 tools_4.0.3	49

##	[61]	lava_1.6.9	ellipsis_0.3.2	spatstat.core_2.3-0	50
##	[64]	proxy_0.4-26	ggridges_0.5.3	Rcpp_1.0.7	51
##	[67]	plyr_1.8.6	base64enc_0.1-3	visNetwork_2.0.9	52
##	[70]	purrr_0.3.4	ps_1.6.0	rpart_4.1-15	53
##	[73]	deldir_0.2-10	pbapply_1.4-3	zoo_1.8-9	54
##	[76]	nichenetr_1.0.0	ggrepel_0.9.1	cluster_2.1.0	55
##	[79]	webshot2_0.0.0.9000	magrittr_2.0.1	data.table_1.14.0	56
##	[82]	RSpectra_0.16-0	scattermore_0.7	lmtest_0.9-38	57
##	[85]	RANN_2.6.1	fitdistrplus_1.1-5	matrixStats_0.60.0	58
##	[88]	hms_1.1.0	patchwork_1.1.1	mime_0.11	59
##	[91]	evaluate_0.14	xtable_1.8-4	jpeg_0.1-9	60
##	[94]	gridExtra_2.3	compiler_4.0.3	tibble_3.1.3	61
##	[97]	KernSmooth_2.23-20	crayon_1.4.1	websocket_1.4.0	62
##	[100]	htmltools_0.5.1.1	mgcv_1.8-33	later_1.2.0	63
##	[103]	tzdb_0.1.2	Formula_1.2-4	tidyR_1.1.3	64
##	[106]	lubridate_1.7.10	DBI_1.1.1	MASS_7.3-53	65
##	[109]	Matrix_1.3-4	readr_2.0.0	cli_3.0.1	66
##	[112]	gower_0.2.2	igraph_1.2.6	pkgconfig_2.0.3	67
##	[115]	foreign_0.8-81	plotly_4.9.4.1	spatstat.sparse_2.0-0	68
##	[118]	recipes_0.1.16	foreach_1.5.1	chromote_0.0.0.9003	69
##	[121]	prodlim_2019.11.13	stringr_1.4.0	digest_0.6.27	70
##	[124]	sctransform_0.3.2	RcppAnnoy_0.0.19	spatstat.data_2.1-0	71
##	[127]	rmarkdown_2.9	leiden_0.3.9	htmlTable_2.2.1	72
##	[130]	uwot_0.1.10.9000	curl_4.3.2	shiny_1.6.0	73
##	[133]	lifecycle_1.0.0	nlme_3.1-152	jsonlite_1.7.2	74
##	[136]	viridisLite_0.4.0	limma_3.46.0	fansi_0.5.0	75
##	[139]	pillar_1.6.2	lattice_0.20-41	fastmap_1.1.0	76
##	[142]	httr_1.4.2	survival_3.2-7	glue_1.4.2	77
##	[145]	fdrtool_1.2.16	png_0.1-7	iterators_1.0.13	78
##	[148]	bit_4.0.4	class_7.3-17	stringi_1.7.3	79
##	[151]	blob_1.2.2	caTools_1.18.2	latticeExtra_0.6-29	80
##	[154]	memoise_2.0.0	irlba_2.3.3	e1071_1.7-8	81
##	[157]	future.apply_1.7.0			82

## 7 References