

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

11-Cell cycling in transit cells

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## 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 Prepare data

```
suppressMessages(  
  {  
    library(Seurat)  
    library(dplyr)  
    library(RColorBrewer)  
    library(clusterProfiler)  
    library(ggplot2)  
    library(monocle3)  
  }  
)  
  
so <- readRDS("../8-SCENIC_analysis/only_IM_differentiation.with_SCENIC.  
  seuratObject.Rds")
```

### 2.1 Subset the cMo population with only the differentiating cells

```
# here's the cells that were used for monocyte-IM differentiation analysis  
:  
cds <- readRDS("../9-Monocle_analysis_and_pseudotime_estimation/Mono_to_IM  
  .cds")  
  
# subset with the cell names  
so <- subset(so, cells = colnames(cds))  
  
# check cell number  
ncol(so) == ncol(cds)  
  
## [1] TRUE
```

## 3 DE genes in transit cells

```
pal <- c("#A6CEE3", "#B2DF8A", "#33A02C", "#E31A1C")  
all_cluster.markers <- FindAllMarkers(so, verbose = FALSE)  
top20 <- all_cluster.markers %>% group_by(cluster) %>% top_n(n = 20, wt =  
  avg_log2FC)  
DoHeatmap(so, features = top20$gene, group.colors = pal) + NoLegend()
```



```
0.01), ]
write.csv(de.transit, file = "./DE-Genes_in_Transit_cells.csv", quote =
FALSE)
```

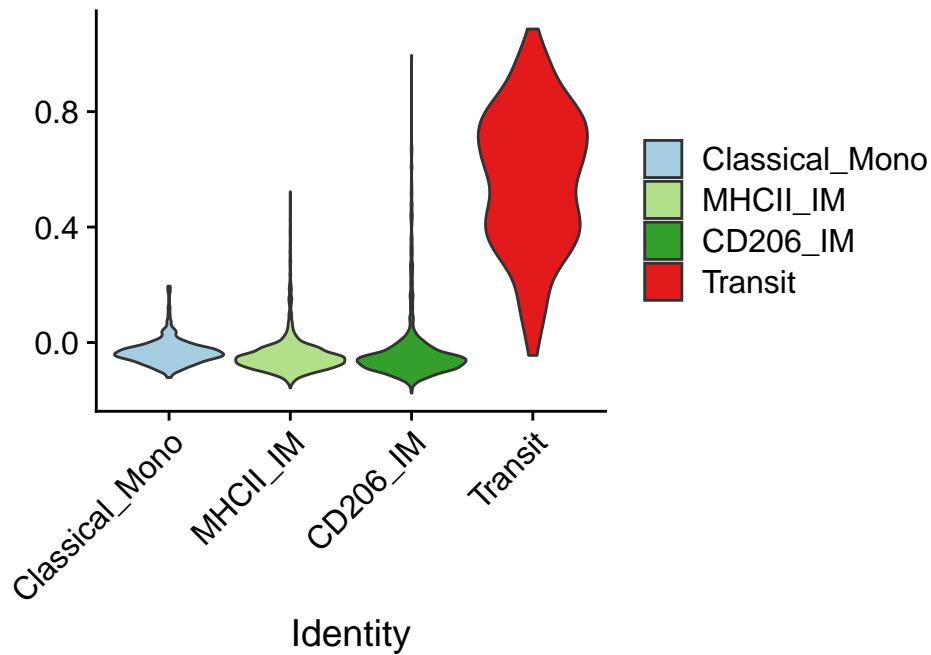
```
suppressMessages({
  de_entrez <- bitr( geneID = de.transit$gene, fromType = "SYMBOL", toType
    = "ENTREZID", OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
})
```

```
suppressMessages({
result.enrichGO <- enrichGO(de_entrez, OrgDb = "org.Mm.eg.db", ont = "BP")
result.enrichGO <- replaceEntrezID(result.enrichGO, organism = "mmu")
})
write.csv(result.enrichGO@result, file = "./enrichGO_DE_genes_transit_
  cells.csv")
result.enrichGO@result
```

```
## # A tibble: 4,744 x 9
##   ID      Description GeneRatio BgRatio   pvalue p.adjust   qvalue
##   <chr>   <chr>         <chr>   <chr>     <dbl>   <dbl>     <dbl> <chr>
##   <int>
## 1 G0:00~ chromosome~ 99/851    324/23~ 1.77e-63 8.41e-60 6.50e-60
  Birc5/~    99
## 2 G0:00~ nuclear di~ 95/851    418/23~ 2.84e-48 6.73e-45 5.20e-45
  Birc5/~    95
## 3 G0:00~ nuclear ch~ 77/851    262/23~ 5.49e-48 8.68e-45 6.71e-45
  Birc5/~    77
## 4 G0:00~ sister chr~ 65/851    181/23~ 7.73e-47 9.17e-44 7.09e-44
  Birc5/~    65
## 5 G0:01~ mitotic nu~ 76/851    268/23~ 4.05e-46 3.84e-43 2.97e-43
  Birc5/~    76
## 6 G0:00~ organelle ~ 97/851    472/23~ 3.60e-45 2.85e-42 2.20e-42
  Birc5/~    97
## 7 G0:00~ mitotic si~ 59/851    151/23~ 4.32e-45 2.93e-42 2.27e-42
  Birc5/~    59
## 8 G0:00~ cell cycle~ 80/851    415/23~ 3.39e-35 2.01e-32 1.55e-32
  Birc5/~    80
## 9 G0:00~ mitotic ce~ 75/851    376/23~ 4.45e-34 2.34e-31 1.81e-31
  Birc5/~    75
## 10 G0:00~ DNA replic~ 61/851    244/23~ 1.03e-33 4.89e-31 3.78e-31
  Pclaf/~    61
## # ... with 4,734 more rows
```

## 4 Cell-cycle score in IM differentiation

```
VlnPlot(so, features = "G2M.Score", cols = pal, pt.size = 0) + ggtitle("")
```

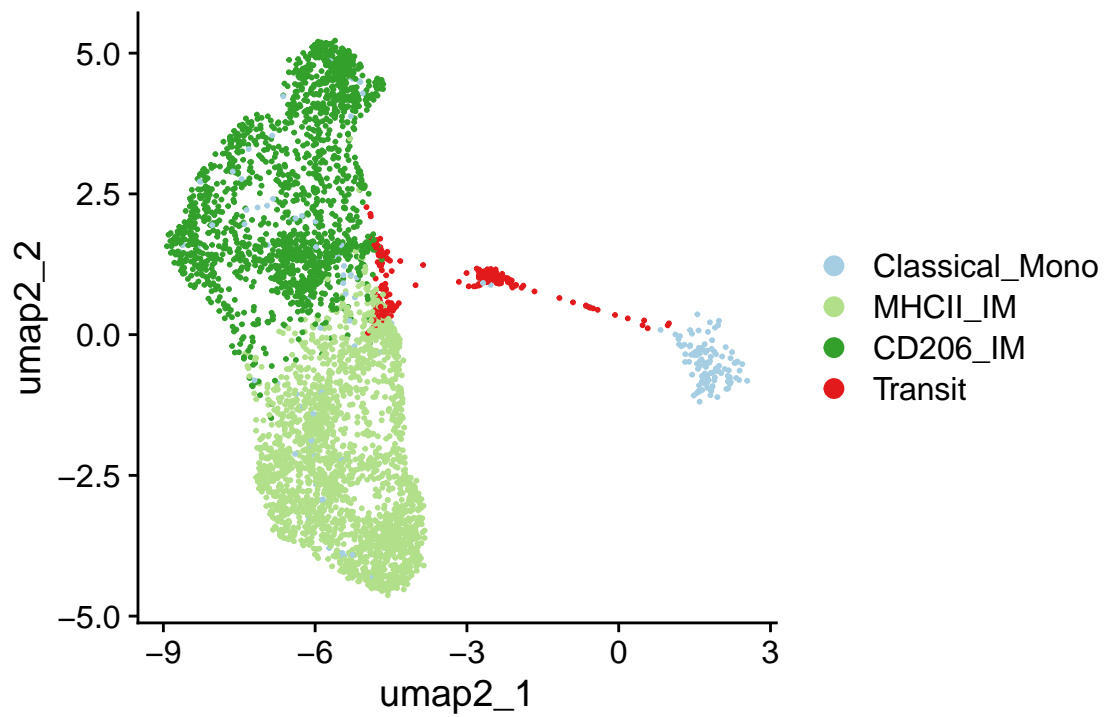


```
ggsave(filename = "../Figures/VlnPlot_G2MScore_in_IM_differentiation.pdf", 1
        width = 5, height = 4) 2
```

## 5 Show cell-cycle score in UMAP plot precalculated in Monocle

UMAP coordination was calculated and stored in CDS object.

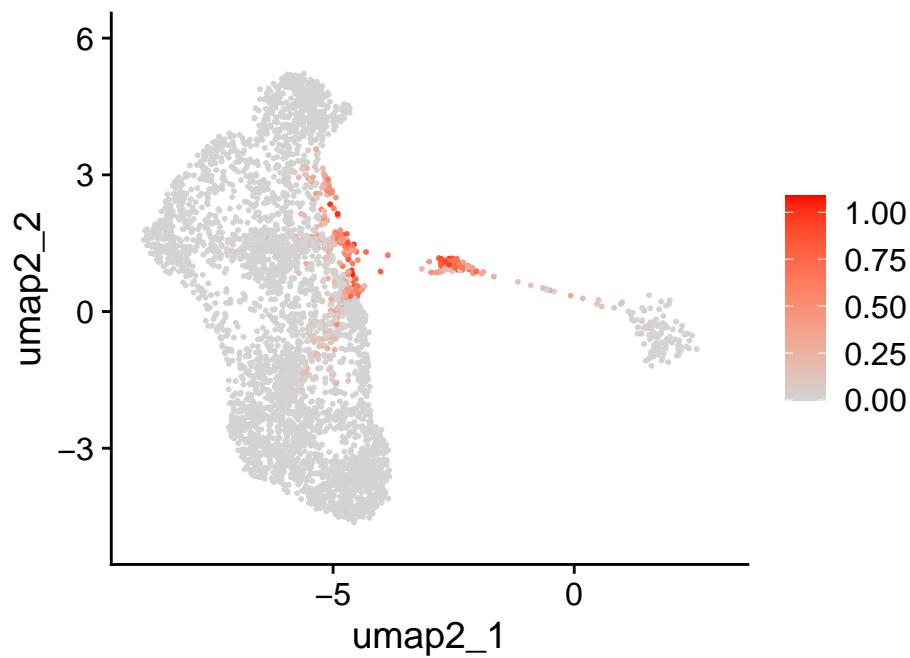
```
so[["UMAP2"]] <- CreateDimReducObject(embeddings = cds@int_ 1
    colData@listData$reducedDims$UMAP,
                                     key = "UMAP_", assay = DefaultAssay( 2
                                     so))
DimPlot(so, cols = pal, reduction = "UMAP2") 3
```



```
ggsave(filename = "../Figures/UMAPplot_IM_diff_PC1_2.pdf",
        width = 6, height = 4)
```

```
FeaturePlot(so, features = "G2M.Score", reduction = "UMAP2",
            cols = c("lightgrey", "red"),
            min.cutoff = 0.)
```

## G2M.Score



```
ggsave(filename = "../Figures/FeaturePlot_G2M_in_IM_diff_PC1_2.pdf",
        width = 5, height = 4)
```

## 6 Session information

R session:

```
sessionInfo()
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.3 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/liblapack.so.3
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_GB.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats4      parallel  stats      graphics  grDevices  utils
##      datasets
## [8] methods    base
##
## other attached packages:
##  [1] org.Mm.eg.db_3.12.0      AnnotationDbi_1.52.0
##  [3] monocle3_1.0.0          SingleCellExperiment_1.12.0
##  [5] SummarizedExperiment_1.20.0 GenomicRanges_1.42.0
##  [7] GenomeInfoDb_1.26.7      IRanges_2.24.1
##  [9] S4Vectors_0.28.1        MatrixGenerics_1.2.1
## [11] matrixStats_0.61.0      Biobase_2.50.0
## [13] BiocGenerics_0.36.1      ggplot2_3.3.5
## [15] clusterProfiler_3.18.1  RColorBrewer_1.1-2
## [17] dplyr_1.0.7             SeuratObject_4.0.4
## [19] Seurat_4.0.5
##
## loaded via a namespace (and not attached):
##  [1] utf8_1.2.2              reticulate_1.22         tidyselect_1.1.1
##  [4] RSQLite_2.2.9           htmlwidgets_1.5.4      grid_4.0.3
##  [7] BiocParallel_1.24.1     Rtsne_0.15             scatterpie_0.1.7
## [10] munsell_0.5.0           codetools_0.2-18       ragg_1.2.1
## [13] ica_1.0-2               future_1.23.0          miniUI_0.1.1.1
## [16] withr_2.4.3            colorspace_2.0-2       GOSemSim_2.16.1
## [19] highr_0.9              knitr_1.36             rstudioapi_0.13
## [22] ROCR_1.0-11            tensor_1.5             DOSE_3.16.0
## [25] listenv_0.8.0          labeling_0.4.2         GenomeInfoDbData_1
##      .2.4
```

##	[28]	polyclip_1.10-0	bit64_4.0.5	farver_2.1.0	43
##	[31]	downloader_0.4	parallelly_1.29.0	vctrs_0.3.8	44
##	[34]	generics_0.1.1	xfun_0.28	R6_2.5.1	45
##	[37]	graphlayouts_0.7.2	bitops_1.0-7	spatstat.utils_2	46
##	[40]	cachem_1.0.6	fgsea_1.16.0	DelayedArray_0.16.3	47
##	[43]	assertthat_0.2.1	promises_1.2.0.1	scales_1.1.1	48
##	[46]	ggraph_2.0.5	enrichplot_1.10.2	gtable_0.3.0	49
##	[49]	globals_0.14.0	goftest_1.2-3	tidygraph_1.2.0	50
##	[52]	rlang_0.4.12	systemfonts_1.0.3	splines_4.0.3	51
##	[55]	lazyeval_0.2.2	spatstat.geom_2.3-0	BiocManager_1.30.16	52
##	[58]	yaml_2.2.1	reshape2_1.4.4	abind_1.4-5	53
##	[61]	httpuv_1.6.3	qvalue_2.22.0	tools_4.0.3	54
##	[64]	ellipsis_0.3.2	spatstat.core_2.3-2	ggribges_0.5.3	55
##	[67]	Rcpp_1.0.7	plyr_1.8.6	zlibbioc_1.36.0	56
##	[70]	purrr_0.3.4	RCurl_1.98-1.5	rpart_4.1-15	57
##	[73]	deldir_1.0-6	pbapply_1.5-0	viridis_0.6.2	58
##	[76]	cowplot_1.1.1	zoo_1.8-9	ggrepel_0.9.1	59
##	[79]	cluster_2.1.0	magrittr_2.0.1	data.table_1.14.2	60
##	[82]	scattermore_0.7	DO.db_2.9	lmtest_0.9-39	61
##	[85]	RANN_2.6.1	fitdistrplus_1.1-6	patchwork_1.1.1	62
##	[88]	mime_0.12	evaluate_0.14	xtable_1.8-4	63
##	[91]	gridExtra_2.3	compiler_4.0.3	tibble_3.1.6	64
##	[94]	KernSmooth_2.23-20	crayon_1.4.2	shadowtext_0.0.9	65
##	[97]	htmltools_0.5.2	ggfun_0.0.4	mgcv_1.8-33	66
##	[100]	later_1.3.0	tidyr_1.1.4	DBI_1.1.1	67
##	[103]	tweenr_1.0.2	MASS_7.3-53	Matrix_1.3-4	68
##	[106]	cli_3.1.0	igraph_1.2.9	pkgconfig_2.0.3	69
##	[109]	rvcheck_0.2.1	plotly_4.10.0	spatstat.sparse_2	70
##	[112]	XVector_0.30.0	yulab.utils_0.0.4	stringr_1.4.0	71
##	[115]	digest_0.6.29	sctransform_0.3.2	RcppAnnoy_0.0.19	72
##	[118]	spatstat.data_2.1-0	rmarkdown_2.11	leiden_0.3.9	73
##	[121]	fastmatch_1.1-3	uwot_0.1.11	shiny_1.7.1	74
##	[124]	lifecycle_1.0.1	nlme_3.1-153	jsonlite_1.7.2	75
##	[127]	viridisLite_0.4.0	limma_3.46.0	fansi_0.5.0	76
##	[130]	pillar_1.6.4	lattice_0.20-41	fastmap_1.1.0	77
##	[133]	httr_1.4.2	survival_3.2-7	G0.db_3.12.1	78
##	[136]	glue_1.5.1	png_0.1-7	bit_4.0.4	79
##	[139]	ggforce_0.3.3	stringi_1.7.6	blob_1.2.2	80
##	[142]	textshaping_0.3.6	memoise_2.0.1	irlba_2.3.5	81
##	[145]	future.apply_1.8.1			82

## 7 References