Monocytes can Proliferate in Vacant Tissue Niches prior to Differentiation into Macrophages

11-Cell cycling in transit cells

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Abstract

Resident tissue macrophages (RTM) are differentiated immune cells populating distinct niches and exhibiting important tissue-supportive functions. RTM maintenance is thought to depend either on monocyte engraftment and differentiation, or on the self-renewal of mature RTM. Here, we discovered that monocytes can re-enter cell cycle and proliferate locally before their differentiation into RTM. We developed a mouse model of inducible lung interstitial macrophage (IM) depletion in which the vacant niche is repopulated by BM-derived monocytes giving rise to fully differentiated IM subsets. By performing time-course single-cell RNA-sequencing analyses of myeloid cells during niche refilling, we found that few Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner. We further showed that the transcription factor MafB restricted such proliferation and was essential to mediate RTM specification and identity in our model. Our data provide evidence that, in the mononuclear phagocyte system, self-renewal is not merely restricted to myeloid progenitor cells and mature macrophages, but is also a tightly regulated capability of mature monocytes developing into RTM in vivo.

Contents

1 Description

In the DT treatment time-course analysis, we found transit population had up-DE genes of cell cycle. Here we focus on the cell cycle of this transit populaiton. The cell cycle score

The GO enrichment analysis was made using clusterProfiler package (Yu et al., 2012).

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")
```

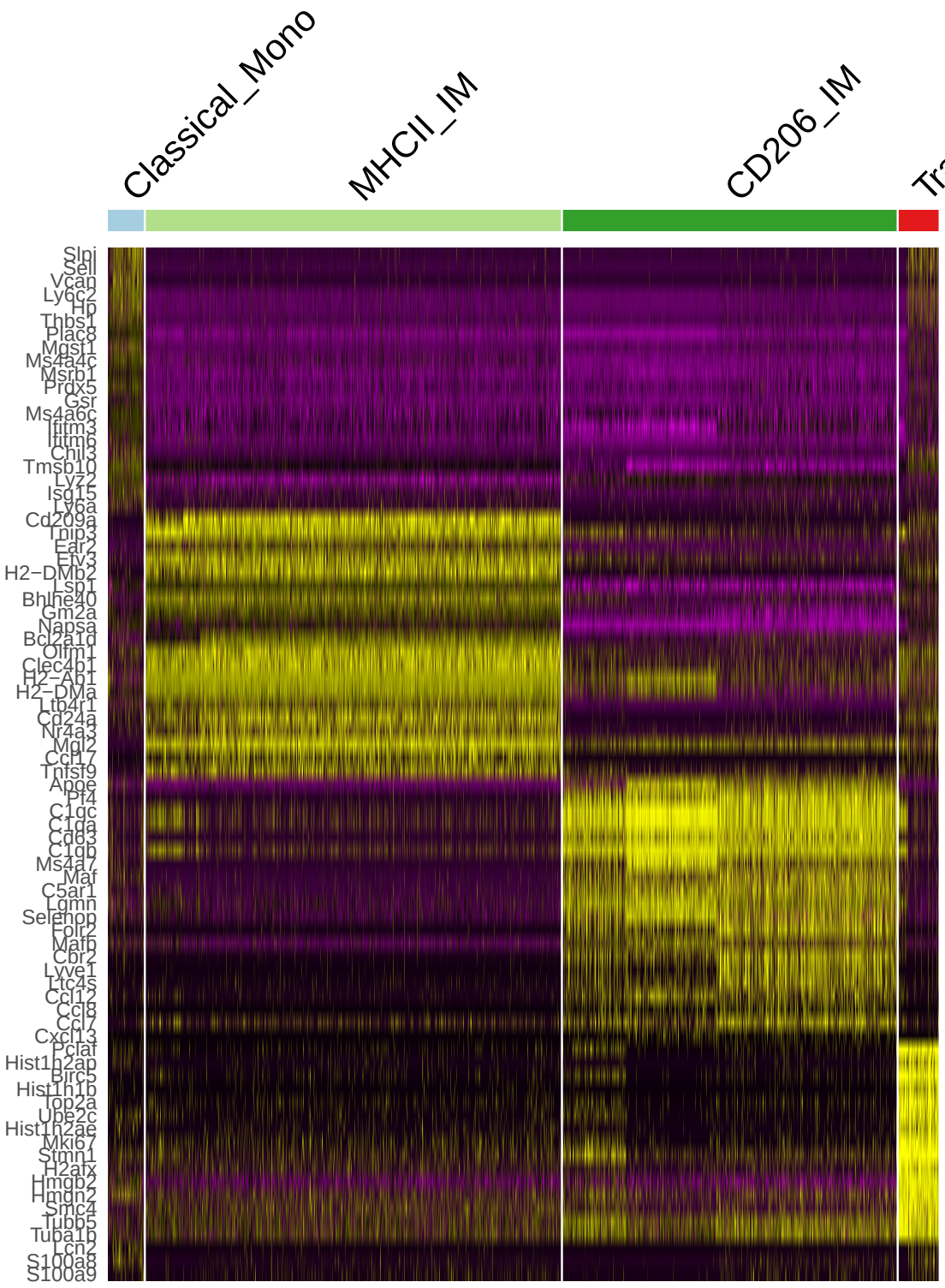
2.1 Subset the cMo population with only the differentiating cells

```
# 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()
```



GO

analysis of DE genes of transit cells

```
source("../R/entrez2symbol.R")
source("../R/replaceEntrezID.R")

de.transit <- all_cluster.markers[which(all_cluster.markers$cluster=="
    Transit" &
                                        all_cluster.markers$p_val_adj <
```

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

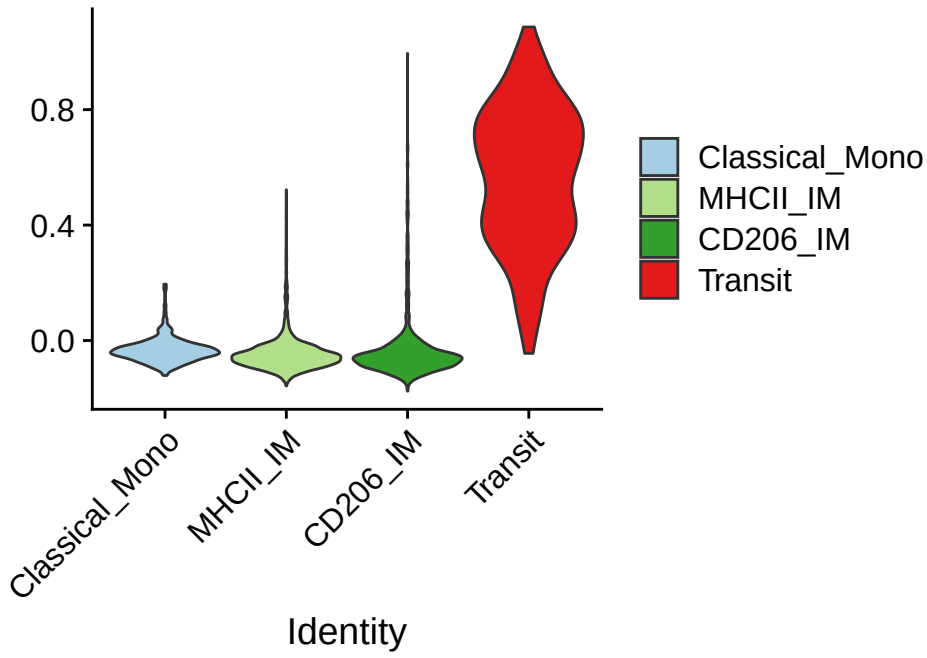
```
suppressMessages({
  de_entrez <- bitr( geneID = de.transit$gene, fromType = "SYMBOL", toType
      = "ENTREZID", OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
})
```

```
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
    geneID  Count
##    <chr>  <chr>       <chr>     <chr>      <dbl>    <dbl>    <dbl> <chr
    >   <int>
##  1 GO:00~ chromosome~ 99/851    324/23~ 1.77e-63 8.41e-60 6.50e-60
    Birc5/~    99
##  2 GO:00~ nuclear di~ 95/851    418/23~ 2.84e-48 6.73e-45 5.20e-45
    Birc5/~    95
##  3 GO:00~ nuclear ch~ 77/851    262/23~ 5.49e-48 8.68e-45 6.71e-45
    Birc5/~    77
##  4 GO:00~ sister chr~ 65/851    181/23~ 7.73e-47 9.17e-44 7.09e-44
    Birc5/~    65
##  5 GO:01~ mitotic nu~ 76/851    268/23~ 4.05e-46 3.84e-43 2.97e-43
    Birc5/~    76
##  6 GO:00~ organelle ~ 97/851    472/23~ 3.60e-45 2.85e-42 2.20e-42
    Birc5/~    97
##  7 GO:00~ mitotic si~ 59/851    151/23~ 4.32e-45 2.93e-42 2.27e-42
    Birc5/~    59
##  8 GO:00~ cell cycle~ 80/851    415/23~ 3.39e-35 2.01e-32 1.55e-32
    Birc5/~    80
##  9 GO:00~ mitotic ce~ 75/851    376/23~ 4.45e-34 2.34e-31 1.81e-31
    Birc5/~    75
## 10 GO: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("")
```

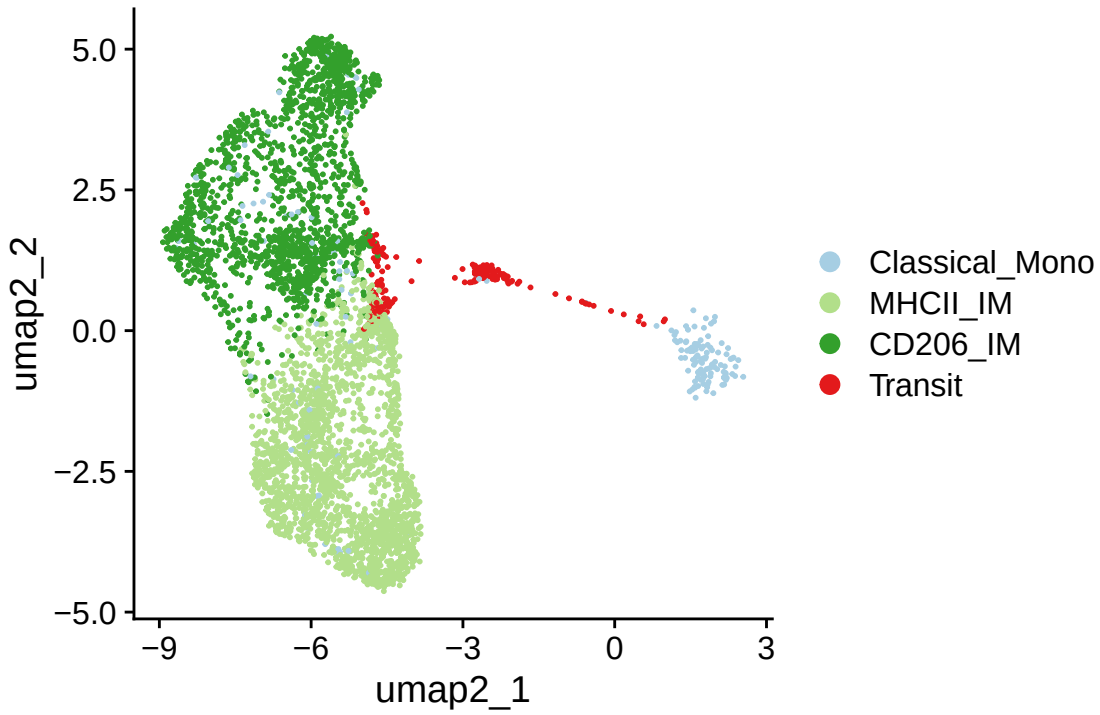



```
ggsave(filename = "../Figures/VlnPlot_G2MScore_in_IM_differentiation.pdf",
       width = 5, height = 4)
```

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

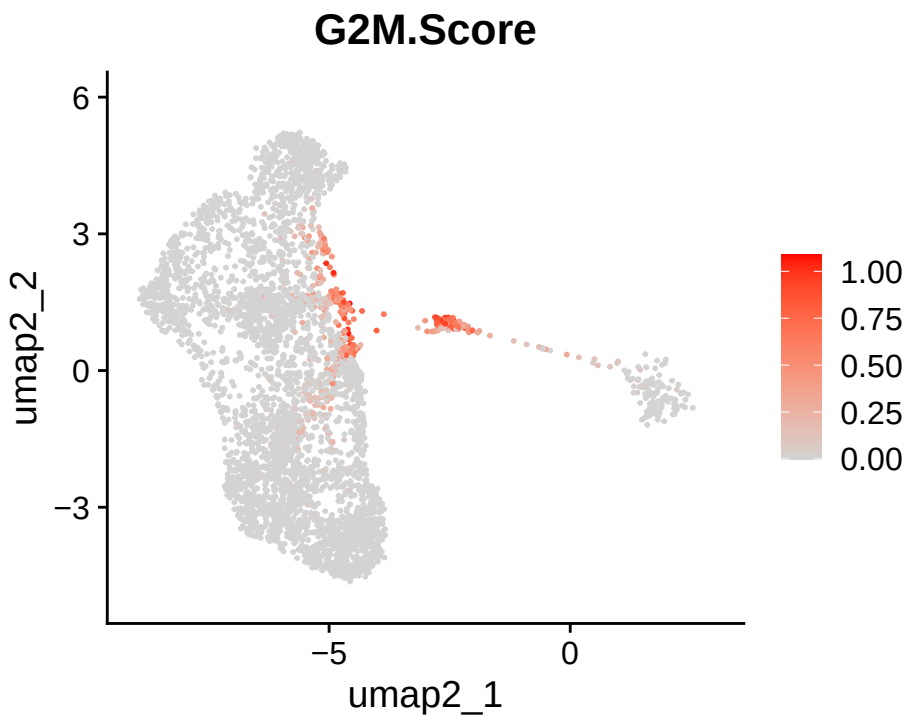
UMAP coordination was calculated and stored in CDS object.

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




```
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.)
```



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

6 Session information

R sesssion:

```
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
    .2-0
##  [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     ggridges_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
    .0-0
## [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          GO.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
```

References

Yu, G., Wang, L.G., Han, Y., and He, Q.Y. (2012). ClusterProfiler: An r package for comparing biological themes among gene clusters. OMICS A Journal of Integrative Biology.