Mafb-restricted local monocyte proliferation precedes lung interstitial macrophage differentiation

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 rely on either monocyte engraftment and differentiation, or RTM self-renewal. Here, we developed an inducible mouse model of lung interstitial macrophage (IM) niche depletion and repopulation to investigate IM development in vivo. Using time-course single-cell RNA-sequencing analyses, bone marrow chimeras and gene targeting, we found that engrafted Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner before their differentiation into RTM. We further showed that the switch from monocyte proliferation towards IM subset specification was controlled by MafB, while c-Maf specifically regulated the identity of the CD206+ IM subset. Our data shed new light on the transcriptional regulation of IM development and 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.[1]

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")
```

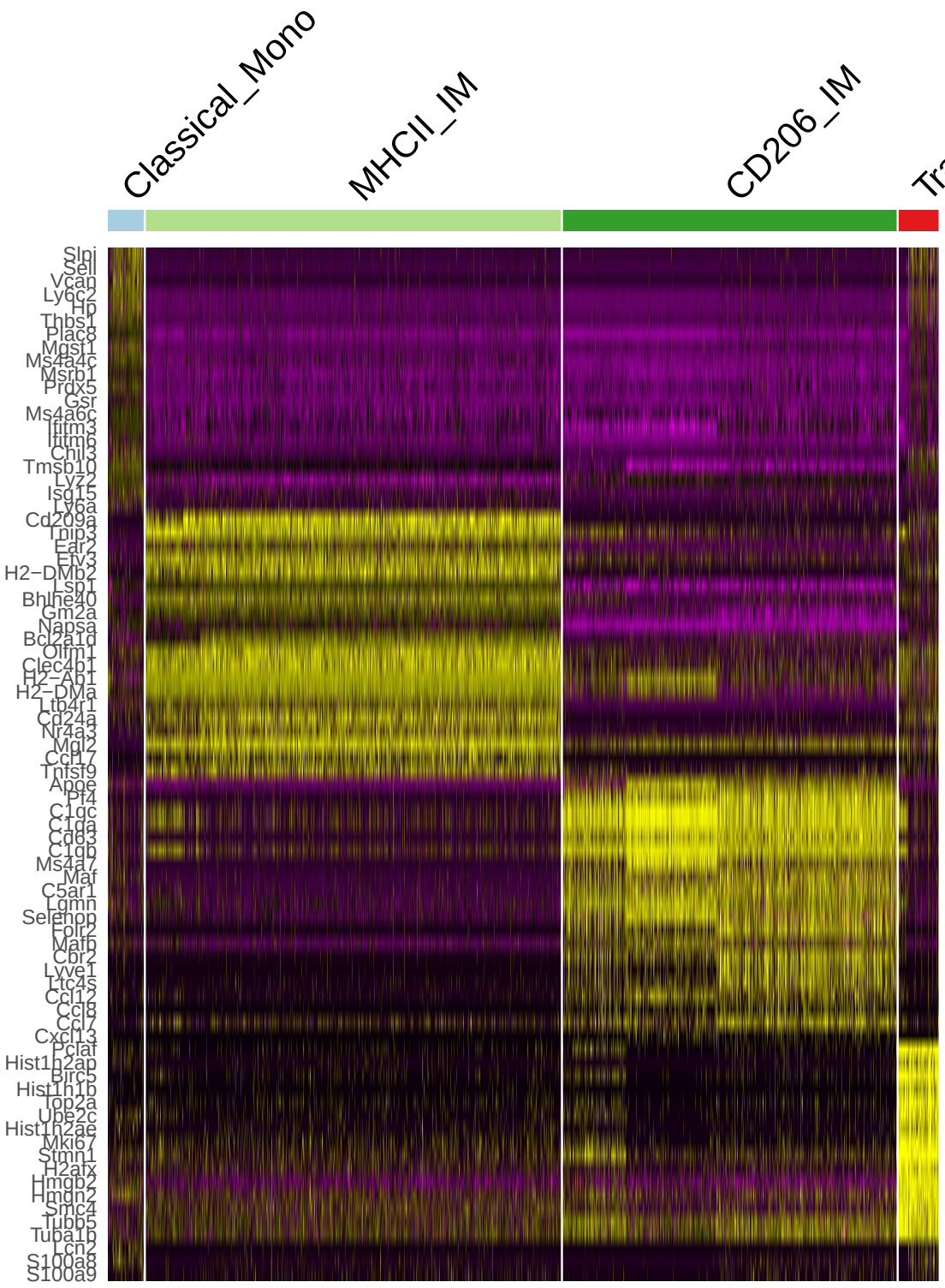
2.1 Subset the cMo population with only the differentiating cells

```
# 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)
```

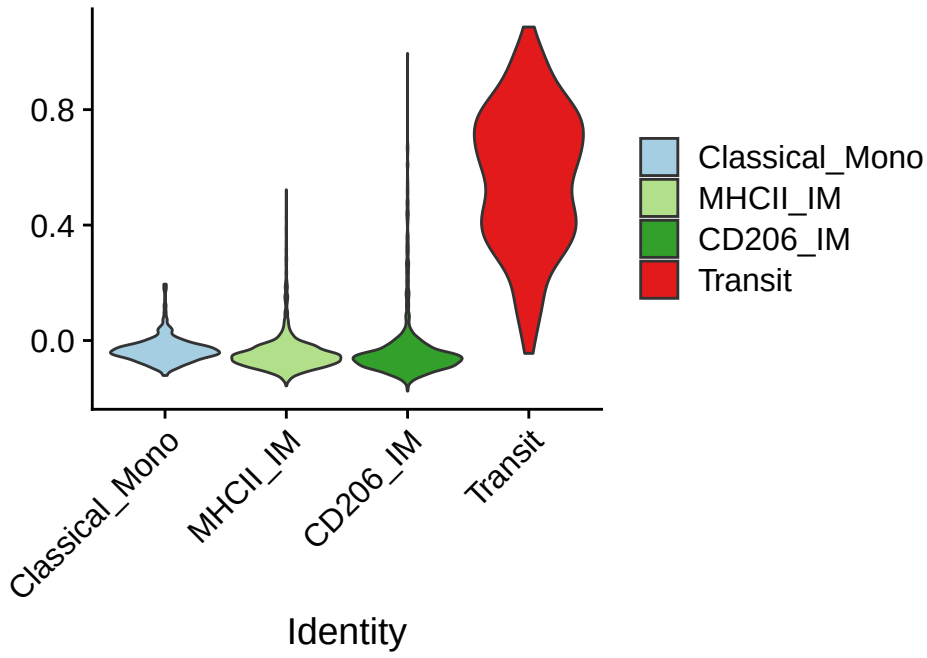
```
suppressMessages({
  de_entrez <- bitr( geneID = de.transit$gene, fromType = "SYMBOL", toType
      = "ENTREZID", OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
})
```

```
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:000~ chromosome~ 99/851    324/23~ 1.77e-63 8.41e-60 6.50e-60
    Birc5~    99
##  2 GO:000~ nuclear di~ 95/851    418/23~ 2.84e-48 6.73e-45 5.20e-45
    Birc5~    95
##  3 GO:009~ nuclear ch~ 77/851    262/23~ 5.49e-48 8.68e-45 6.71e-45
    Birc5~    77
##  4 GO:000~ sister chr~ 65/851    181/23~ 7.73e-47 9.17e-44 7.09e-44
    Birc5~    65
##  5 GO:014~ mitotic nu~ 76/851    268/23~ 4.05e-46 3.84e-43 2.97e-43
    Birc5~    76
##  6 GO:004~ organelle ~ 97/851    472/23~ 3.60e-45 2.85e-42 2.20e-42
    Birc5~    97
##  7 GO:000~ mitotic si~ 59/851    151/23~ 4.32e-45 2.93e-42 2.27e-42
    Birc5~    59
##  8 GO:004~ cell cycle~ 80/851    415/23~ 3.39e-35 2.01e-32 1.55e-32
    Birc5~    80
##  9 GO:004~ mitotic ce~ 75/851    376/23~ 4.45e-34 2.34e-31 1.81e-31
    Birc5~    75
## 10 GO:000~ 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("")
```

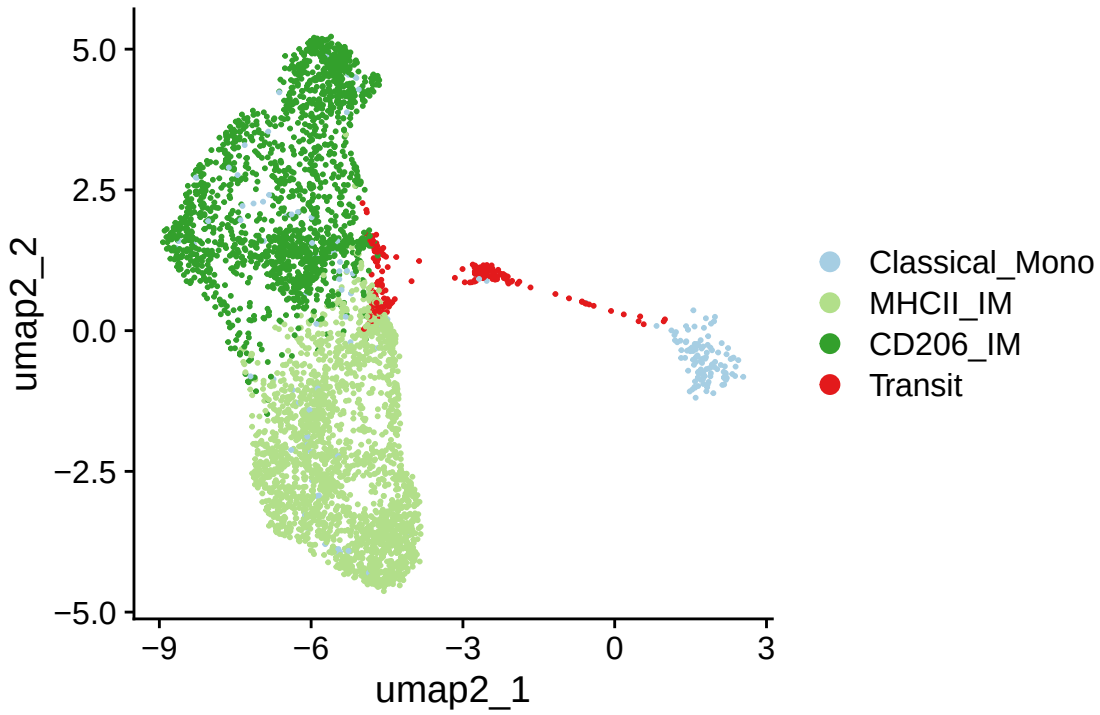



```
ggsave(filename = "../Figures/VlnPlot_G2MScore_in_IM_differentiation.pdf",
       width = 5, height = 4)
```

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

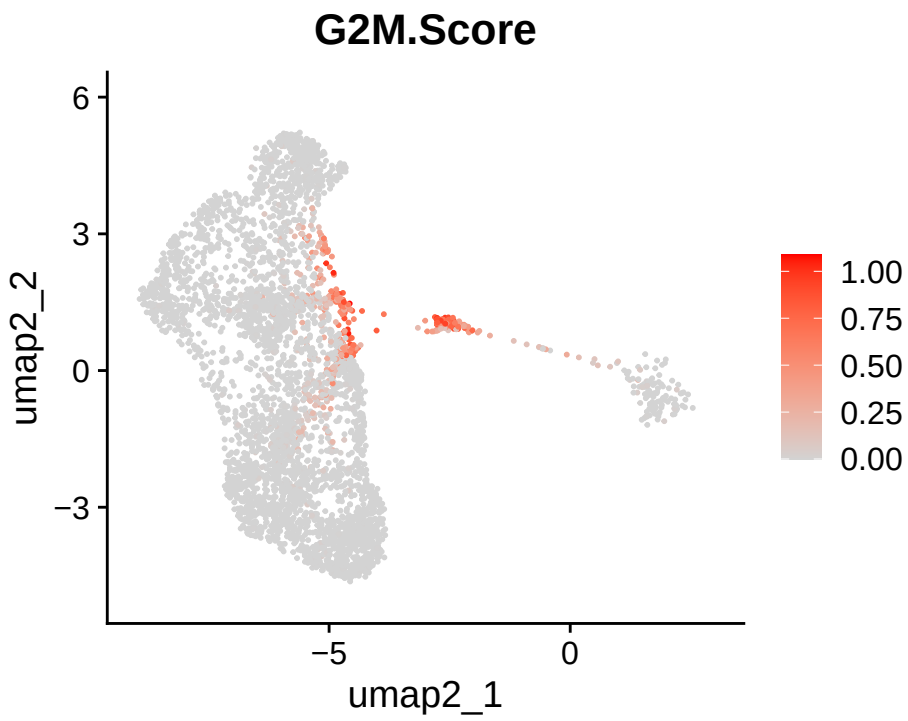
UMAP coordination was calculated and stored in CDS object.

```
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_1.0.8                 SeuratObject_4.0.4
## [19] Seurat_4.1.0
##
## loaded via a namespace (and not attached):
##   [1] utf8_1.2.2             reticulate_1.24        tidyselect_1.1.1
##   [4] RSQLite_2.2.10         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          ragg_1.2.2             codetools_0.2-18
##  [13] ica_1.0-2              future_1.24.0          miniUI_0.1.1.1
##  [16] withr_2.4.3            spatstat.random_2.1-0  colorspace_2.0-3
##  [19] GOSemSim_2.16.1        highr_0.9              knitr_1.37
##  [22] rstudioapi_0.13        ROCR_1.0-11            tensor_1.5
##  [25] DOSE_3.16.0            listenv_0.8.0          labeling_0.4.2
##  [28] GenomeInfoDbData_1.2.4 polyclip_1.10-0        bit64_4.0.5
```

```
##  [31] farver_2.1.0           downloader_0.4         parallelly_1.30.0   44
##  [34] vctrs_0.3.8            generics_0.1.2         xfun_0.29           45
##  [37] R6_2.5.1               ggbeeswarm_0.6.0       graphlayouts_0.8.0  46
##  [40] bitops_1.0-7           spatstat.utils_2.3-0   cachem_1.0.6        47
##  [43] fgsea_1.16.0           DelayedArray_0.16.3    assertthat_0.2.1    48
##  [46] promises_1.2.0.1       scales_1.1.1           ggraph_2.0.5        49
##  [49] enrichplot_1.10.2      beeswarm_0.4.0         gtable_0.3.0        50
##  [52] globals_0.14.0         goftest_1.2-3          tidygraph_1.2.0     51
##  [55] rlang_1.0.1            systemfonts_1.0.4      splines_4.0.3       52
##  [58] lazyeval_0.2.2         spatstat.geom_2.3-2    BiocManager_1.30.16 53
##  [61] yaml_2.3.5             reshape2_1.4.4         abind_1.4-5         54
##  [64] httpuv_1.6.5           qvalue_2.22.0          tools_4.0.3         55
##  [67] ellipsis_0.3.2         spatstat.core_2.4-0    ggridges_0.5.3      56
##  [70] Rcpp_1.0.8             plyr_1.8.6             zlibbioc_1.36.0     57
##  [73] purrr_0.3.4            RCurl_1.98-1.6         rpart_4.1-15        58
##  [76] deldir_1.0-6           pbapply_1.5-0          viridis_0.6.2       59
##  [79] cowplot_1.1.1          zoo_1.8-9              ggrepel_0.9.1       60
##  [82] cluster_2.1.0          magrittr_2.0.2         data.table_1.14.2   61
##  [85] scattermore_0.8        DO.db_2.9              lmtest_0.9-39       62
##  [88] RANN_2.6.1             fitdistrplus_1.1-6     patchwork_1.1.1     63
##  [91] mime_0.12              evaluate_0.15          xtable_1.8-4        64
##  [94] gridExtra_2.3          compiler_4.0.3         tibble_3.1.6        65
##  [97] KernSmooth_2.23-20     crayon_1.5.0           shadowtext_0.1.1    66
## [100] htmltools_0.5.2        ggfun_0.0.5            mgcv_1.8-33         67
## [103] later_1.3.0            tidyr_1.2.0            DBI_1.1.2           68
## [106] tweenr_1.0.2           MASS_7.3-53            Matrix_1.4-0        69
## [109] cli_3.2.0              igraph_1.2.11          pkgconfig_2.0.3     70
## [112] rvcheck_0.2.1          plotly_4.10.0          spatstat.sparse_2   71
    .1-0
## [115] vipor_0.4.5            XVector_0.30.0         yulab.utils_0.0.4   72
## [118] stringr_1.4.0          digest_0.6.29          sctransform_0.3.3   73
## [121] RcppAnnoy_0.0.19       spatstat.data_2.1-2    rmarkdown_2.11      74
## [124] leiden_0.3.9           fastmatch_1.1-3        uwot_0.1.11         75
## [127] shiny_1.7.1            lifecycle_1.0.1        nlme_3.1-155        76
## [130] jsonlite_1.7.3         viridisLite_0.4.0      limma_3.46.0        77
## [133] fansi_1.0.2            pillar_1.7.0           lattice_0.20-41     78
## [136] ggrastr_1.0.1          fastmap_1.1.0          httr_1.4.2          79
## [139] survival_3.2-7         GO.db_3.12.1           glue_1.6.1          80
## [142] png_0.1-7              bit_4.0.4              ggforce_0.3.3       81
## [145] stringi_1.7.6          blob_1.2.2             textshaping_0.3.6   82
## [148] memoise_2.0.1          irlba_2.3.5            future.apply_1.8.1  83
```

References

1. Yu, G., Wang, L. G., Han, Y. & He, Q. Y. ClusterProfiler: An r package for comparing biological themes among gene clusters. *OMICS A Journal of Integrative Biology* (2012) doi:10.1089/omi.2011.0118.