# 6-Gene Ontology enrichment

# BAI Qiang\*

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<sup>\*</sup>University Liege, mail qiang.bai@uliege.be

### 1 Description

The DE gene lists used for enrichment analyses were calculated using Seurat function FindMarkers[1] with default arguments except only.pos = TRUE in order to output only positively regulated genes and logfc.threshold of 0.2. KEGG and Gene Ontology enrichment analyses were made using enrichKEGG and enrichGO functions from package clusterProfiler package[2] with default arguments.

## 2 Load data and packages

```
library(Seurat)
library(ggplot2)
library(dplyr)
results <- readRDS(file = "../3-Merge and cell typing/so.merged_clusters.
    seuratObject.Rds")</pre>
```

## 3 DE genes and GO enrichment

#### 3.1 Cluster 1 DE analysis and GO enrichment

The Cluster 1 mentioned in manuscript refers to cluster 0 in the codes following. The same for cluster 2-4 in manuscript, they are cluster 1-3 in the codes.

Calculate the DE genes.

```
de_ident0 <- FindMarkers(results, ident.1 = "0", only.pos = TRUE, logfc.
    threshold = 0.2)
head(de_ident0)</pre>
```

```
# A tibble: 6 x 5
     p_val avg_log2FC pct.1 pct.2 p_val_adj
                                                                                       2
##
     <dbl>
                                                                                       3
                 <dbl> <dbl> <dbl>
                                          <db1>
##
                 0.689 1
                               0.935
                                                                                        4
   1
          0
                                                                                       5
## 2
          0
                 0.682 0.994 0.892
                                              0
## 3
          0
                 0.622 0.998 0.912
                                              0
                                                                                       6
                                              0
## 4
          0
                 0.379 0.993 0.941
                                              0
                                                                                       8
## 5
          0
                 0.733 0.477 0.107
                 0.670 0.994 0.955
## 6
```

Save data

```
write.csv(de_ident0, file = "./Results_lists/DE_cluster1.csv")
```

KEGG/GO enrichment:

```
library(clusterProfiler)

de_entrez.ident0 <- bitr( geneID = rownames(de_ident0), fromType = "SYMBOL",
    toType = "ENTREZID", OrgDb = "org.Hs.eg.db", drop = TRUE ) $ ENTREZID
    result.enrichKEGG.de_ident0 <- enrichKEGG(de_entrez.ident0, organism = "hsa",
    keyType = "ncbi-geneid")</pre>
```

```
result.enrichKEGG.de_ident0@result
```

```
# A tibble: 199 x 9
##
      TD
               Description
                           GeneRatio BgRatio pvalue p.adjust qvalue geneID
   Count
##
      <chr>
               <chr>>
                                       <chr>>
                                                 <dbl>
                                                          <dbl>
                                                                  <dbl> <chr>
                            <chr>>
   <int>
##
   1 hsa05150 Staphylococ~ 12/99
                                      96/8095 1.52e-9 3.02e-7 2.61e-7 712/7~
   2 hsa04940 Type I diab~ 8/99
                                      43/8095 3.83e-8 3.81e-6 3.29e-6 1363/~
##
                                      38/8095 3.06e-7 1.91e-5 1.65e-5 3107/~
##
    3 hsa05330 Allograft r~ 7/99
                                      78/8095 3.85e-7 1.91e-5 1.65e-5 972/3~
    4 hsa04612 Antigen pro~ 9/99
   5 hsa05332 Graft-versu~ 7/99
                                      42/8095 6.28e-7 2.50e-5 2.16e-5 3107/~
##
    6 hsa04610 Complement ~ 9/99
                                      85/8095 8.10e-7 2.69e-5 2.32e-5 712/7~
    7 hsa05320 Autoimmune ~ 7/99
                                      53/8095 3.22e-6 9.14e-5 7.88e-5 3107/~
                                                                                 10
##
##
    8 hsa05416 Viral myoca~ 7/99
                                      60/8095 7.51e-6 1.87e-4 1.61e-4 3107/~
                                                                                 11
    9 hsa04514 Cell adhesi~ 10/99
                                      149/80~ 1.27e-5 2.80e-4 2.41e-4 3107/~
                                      152/80~ 1.51e-5 3.00e-4 2.59e-4 8685/~
## 10 hsa04145 Phagosome
                           10/99
                                                                                 13
## # ... with 189 more rows
                                                                                 14
```

```
## # A tibble: 459 x 9
##
                                              pvalue p.adjust qvalue geneID
      TD
             Description GeneRatio BgRatio
   Count
##
      <chr> <chr>
                          <chr>>
                                    <chr>
                                                <dbl>
                                                         <dbl>
                                                                 <dbl> <chr>
                                                                                3
   <int>
##
   1 GO:00~ peptide bin~ 19/166
                                    308/18~ 4.99e-11
                                                      2.29e-8 1.88e-8 712/86~
    2 GO:00~ amide bindi~ 20/166
                                    381/18~ 2.67e-10
                                                      6.13e-8 5.01e-8 712/86~
##
    3 GO:00~ peptide ant~ 6/166
                                    31/183~ 3.05e- 7 4.67e-5 3.83e-5 3107/3~
##
##
    4 GO:00~ MHC class I~ 4/166
                                    10/183~ 1.30e- 6 1.49e-4 1.22e-4 3122/3~
    5 GO:00~ peptidase r~ 11/166
                                    229/18~ 7.77e- 6 7.13e-4 5.84e-4 26577/~
    6 GO:00~ MHC class I~ 4/166
                                    16/183~ 1.08e- 5 8.26e-4 6.76e-4 972/97~
    7 GO:00~ antioxidant~ 7/166
                                    86/183~ 1.29e- 5 8.44e-4 6.91e-4 4259/8~
                                                                                10
```

```
## 8 GO:00~ enzyme inhi~ 13/166 383/18~ 4.71e- 5 2.70e-3 2.21e-3 10628/~ 11 13  
## 9 GO:00~ amyloid-bet~ 6/166 77/183~ 6.98e- 5 3.23e-3 2.65e-3 712/86~ 12 6  
## 10 GO:00~ MHC protein~ 4/166 25/183~ 7.04e- 5 3.23e-3 2.65e-3 972/97~ 13 4  
## # ... with 449 more rows
```

#### 3.2 Cluster 2 DE analysis and GO enrichment

The Cluster 2 mentioned in manuscript refers to cluster 1 in the codes following.

Calculate the DE genes.

```
de_ident1 <- FindMarkers(results, ident.1 = "1", only.pos = TRUE, logfc.
    threshold = 0.2)
head(de_ident1)</pre>
```

```
## # A tibble: 6 x 5
     p_val avg_log2FC pct.1 pct.2 p_val_adj
                                                                                       2
##
     <dbl>
                                                                                       3
                 <dbl> <dbl> <dbl>
                                          <dbl>
                 0.421 0.848 0.69
## 1
         0
                                                                                       4
## 2
                 0.400 0.776 0.555
                                                                                       5
          0
                                              0
## 3
                 0.387 0.274 0.097
                                              0
                                                                                       6
          0
## 4
          0
                 0.345 0.976 0.977
                                              0
                                                                                       8
## 5
         0
                 0.369 0.272 0.094
                                              0
                 0.556 0.416 0.133
## 6
          0
                                              0
```

Save data

```
write.csv(de_ident1, file = "./Results_lists/DE_cluster2.csv")
```

KEGG/GO enrichment:

```
de_entrez.ident1 <- bitr( geneID = rownames(de_ident1), fromType = "SYMBOL",
    toType = "ENTREZID", OrgDb = "org.Hs.eg.db", drop = TRUE ) $ ENTREZID
result.enrichKEGG.de_ident1 <- enrichKEGG(de_entrez.ident1, organism = "hsa",
    keyType = "ncbi-geneid")</pre>
```

```
result.enrichKEGG.de_ident1@result
```

```
## # A tibble: 200 x 9
##
     ID
               Description
                           GeneRatio BgRatio pvalue p.adjust qvalue geneID
   Count
##
      <chr>
               <chr>>
                             <chr>
                                       <chr>
                                                 <dbl>
                                                           <dbl>
                                                                   <dbl> <chr>
   <int>
    1 hsa04142 Lysosome
                            11/93
                                       128/80~ 2.04e-7 4.08e-5 3.75e-5 58511~
      11
    2 hsa04145 Phagosome
                            9/93
                                       152/80~ 5.74e-5 5.74e-3 5.29e-3 65336~
```

```
##
    3 \text{ hsa} 04966 \text{ Collecting} \sim 4/93
                                        27/8095 2.34e-4 1.19e-2 1.09e-2 24597~
    4 hsa05110 Vibrio chol~ 5/93
                                        50/8095 2.53e-4 1.19e-2 1.09e-2 537/2~
##
    5 hsa05171 Coronavirus~ 10/93
                                        232/80~ 3.09e-4 1.19e-2 1.09e-2 1536/~
    6 hsa00030 Pentose pho~ 4/93
                                        30/8095 3.56e-4 1.19e-2 1.09e-2 5226/~
##
##
    7 hsa03010 Ribosome
                             8/93
                                        158/80~ 4.41e-4 1.26e-2 1.16e-2 6170/~
                                                                                   10
##
    8 hsa05323 Rheumatoid ~ 6/93
                                        93/8095 6.68e-4 1.67e-2 1.54e-2 1435/~
                                                                                   11
                                        70/8095 1.21e-3 2.68e-2 2.47e-2 537/2~
                                                                                   12
##
    9 hsa05120 Epithelial ~ 5/93
## 10 hsa04979 Cholesterol~ 4/93
                                        50/8095 2.51e-3 5.02e-2 4.63e-2 3988/~
                                                                                   13
## # ... with 190 more rows
                                                                                   14
```

```
## # A tibble: 379 x 9
##
      ID
                 Description GeneRatio BgRatio pvalue p.adjust qvalue geneID
   Count
##
      <chr>
                  <chr>>
                              <chr>>
                                         <chr>>
                                                   <dbl>
                                                            <dbl> <dbl> <chr>
                                                                                  3
   <int>
    1 GO:0003735 structural~ 8/129
                                                           0.0224 0.0189 6170/~
                                        202/18~ 9.16e-5
##
                                                           0.0224 0.0189 65336~
    2 GO:0009055 electron t~ 6/129
                                        111/18~ 1.33e-4
##
    3 GO:0016209 antioxidan~ 5/129
                                                           0.0224 0.0189 5052/~
##
                                        86/183~ 3.51e-4
    4 GO:0044769 ATPase act~ 3/129
                                                           0.0224 0.0189 24597~
##
                                        20/183~ 3.54e-4
##
    5 GO:0046961 proton-tra~ 3/129
                                        20/183~ 3.54e-4
                                                           0.0224 0.0189 24597~
##
    6 GO:0043021 ribonucleo~ 6/129
                                        133/18~ 3.55e-4
                                                           0.0224 0.0189 4193/~
    7 GO:0015035 protein di~ 3/129
                                        22/183~ 4.74e-4
                                                           0.0236 0.0200 7295/~
                                                                                  10
##
##
    8 GO:0052689 carboxylic~ 6/129
                                        143/18~ 5.22e-4
                                                           0.0236 0.0200 3988/~
                                                                                   11
    9 GO:0019829 ATPase-cou~ 4/129
                                        54/183~ 5.61e-4
                                                           0.0236 0.0200 24597~
                                                                                   12
## 10 GO:0042625 ATPase-cou~ 4/129
                                                           0.0271 0.0229 24597~
                                        58/183~ 7.37e-4
                                                                                   13
## # ... with 369 more rows
                                                                                   14
```

#### 3.3 Cluster 3 DE analysis

The Cluster 3 mentioned in manuscript refers to cluster 2 in the codes following.

Calculate the DE genes.

```
de_ident2 <- FindMarkers(results, ident.1 = "2", only.pos = TRUE, logfc.
    threshold = 0.2)
nrow(de_ident2)</pre>
```

```
## [1] 660
```

Save data

```
write.csv(de_ident2, file = "./Results_lists/DE_cluster3.csv")
```

#### 3.4 Cluster 4 DE analysis

The Cluster 4 mentioned in manuscript refers to cluster 3 in the codes following.

Calculate the DE genes.

```
de_ident3 <- FindMarkers(results, ident.1 = "3", only.pos = TRUE, logfc.
    threshold = 0.2)
head(de_ident3)</pre>
```

```
# A tibble: 6 x 5
##
     p_val avg_log2FC pct.1 pct.2 p_val_adj
                                                                                       2
                                                                                       3
##
     <dbl>
                 <dbl> <dbl> <dbl>
## 1
                 0.244 0.274 0.028
                                                                                       4
         0
                                              0
## 2
         0
                 0.208 0.175 0.002
                                              0
## 3
                 2.34 0.983 0.684
                                              0
                                                                                      6
          0
## 4
          0
                 0.770 0.473 0.014
                                              0
## 5
                 0.319 0.225 0.009
                                              0
                                                                                       8
         0
## 6
                 0.885 0.352 0.003
                                                                                       9
```

Save data

```
write.csv(de_ident3, file = "./Results_lists/DE_cluster4.csv")
```

# 3.5 GO/KEGG enrichment and DE analysis in subpopulations of Cluster 3 after re-clustering

Load Cluster 3 object:

```
results.c2 <- readRDS("../4-Functional characterization of clustered populations/cluster2_clustered.seuratObject.Rds")
```

REMIND: the subpopulations cluster 1-4 (after reclustering the cluster 3) in the manuscript refer to the cluster 0-3 in the following codes.

Calculate the DE genes.

DE genes for each subpopulations:

```
de_subpop0.c2 <- FindMarkers(object = results.c2, ident.1 = "0", only.pos =
    TRUE, logfc.threshold = 0.2, verbose = FALSE)

de_subpop1.c2 <- FindMarkers(object = results.c2, ident.1 = "1", only.pos =
    TRUE, logfc.threshold = 0.2, verbose = FALSE)

de_subpop2.c2 <- FindMarkers(object = results.c2, ident.1 = "2", only.pos =
    TRUE, logfc.threshold = 0.2, verbose = FALSE)

de_subpop3.c2 <- FindMarkers(object = results.c2, ident.1 = "3", only.pos =
    TRUE, logfc.threshold = 0.2, verbose = FALSE)</pre>
```

#### Save data

```
write.csv(de_subpop0.c2, file = "./Results_lists/DE_subpop1_c3.csv")
write.csv(de_subpop1.c2, file = "./Results_lists/DE_subpop2_c3.csv")
write.csv(de_subpop2.c2, file = "./Results_lists/DE_subpop3_c3.csv")
write.csv(de_subpop3.c2, file = "./Results_lists/DE_subpop4_c3.csv")
4
```

Since the subpopulations 2 and 3 are very similar. We would like also to have the DE genes in both subpopulations 2 and 3.

#### **KEGG** enrichment:

```
de_entrez.subpop0.c2 <- bitr( geneID = rownames(de_subpop0.c2), fromType = "</pre>
   SYMBOL", toType = "ENTREZID", OrgDb = "org.Hs.eg.db", drop = TRUE ) $
de_entrez.subpop1.c2 <- bitr( geneID = rownames(de_subpop1.c2), fromType = "</pre>
   SYMBOL", toType = "ENTREZID", OrgDb = "org.Hs.eg.db", drop = TRUE ) $
de_entrez.subpop2.c2 <- bitr( geneID = rownames(de_subpop2.c2), fromType = "</pre>
   SYMBOL", toType = "ENTREZID", OrgDb = "org.Hs.eg.db", drop = TRUE ) $
   ENTREZID
de_entrez.subpop3.c2 <- bitr( geneID = rownames(de_subpop3.c2), fromType = "</pre>
   SYMBOL", toType = "ENTREZID", OrgDb = "org.Hs.eg.db", drop = TRUE ) $
   ENTREZID
result.enrichKEGG.de_entrez.subpop0.c2 <- enrichKEGG(de_entrez.subpop0.c2,
                                                                                  6
   organism = "hsa", keyType = "ncbi-geneid")
result.enrichKEGG.de_entrez.subpop1.c2 <- enrichKEGG(de_entrez.subpop1.c2,</pre>
   organism = "hsa", keyType = "ncbi-geneid")
result.enrichKEGG.de_entrez.subpop2.c2 <- enrichKEGG(de_entrez.subpop2.c2,</pre>
                                                                                  8
   organism = "hsa", keyType = "ncbi-geneid")
result.enrichKEGG.de_entrez.subpop3.c2 <- enrichKEGG(de_entrez.subpop3.c2,
                                                                                  9
   organism = "hsa", keyType = "ncbi-geneid")
                                                                                  10
result.enrichGO.de_entrez.subpopO.c2 <- enrichGO(gene = de_entrez.subpopO.c2,
   OrgDb = "org.Hs.eg.db")
result.enrichGO.de_entrez.subpop1.c2 <- enrichGO(gene = de_entrez.subpop1.c2,
   OrgDb = "org.Hs.eg.db")
```

```
result.enrichGO.de_entrez.subpop2.c2 <- enrichGO(gene = de_entrez.subpop2.c2,
   OrgDb = "org.Hs.eg.db")
result.enrichGO.de_entrez.subpop3.c2 <- enrichGO(gene = de_entrez.subpop3.c2,
                                                                                14
   OrgDb = "org.Hs.eg.db")
write.csv(result.enrichKEGG.de_entrez.subpop0.c2, file = "./Results_lists/
   enrichKEGG_DE_subpop1_c3.csv")
write.csv(result.enrichKEGG.de_entrez.subpop1.c2, file = "./Results_lists/
   enrichKEGG_DE_subpop2_c3.csv")
write.csv(result.enrichKEGG.de_entrez.subpop2.c2, file = "./Results_lists/
   enrichKEGG_DE_subpop3_c3.csv")
write.csv(result.enrichKEGG.de_entrez.subpop3.c2, file = "./Results_lists/
   enrichKEGG_DE_subpop4_c3.csv")
                                                                                6
write.csv(result.enrichGO.de_entrez.subpopO.c2, file = "./Results_lists/
   enrichGO_DE_subpop1_c3.csv")
write.csv(result.enrichGO.de_entrez.subpop1.c2, file = "./Results_lists/
   enrichGO_DE_subpop2_c3.csv")
                                                                                8
write.csv(result.enrichGO.de_entrez.subpop2.c2, file = "./Results_lists/
   enrichGO_DE_subpop3_c3.csv")
                                                                                9
write.csv(result.enrichGO.de_entrez.subpop3.c2, file = "./Results_lists/
   enrichGO_DE_subpop4_c3.csv")
```

Head 10 lines in GO:

For subpop 1:

```
head(result.enrichGO.de_entrez.subpopO.c2@result, 10)
```

```
## # A tibble: 10 x 9
##
      ID
             Description GeneRatio BgRatio
                                               pvalue p.adjust
                                                                  qvalue geneID
   Count
##
      <chr>
             <chr>
                          <chr>
                                     <chr>
                                                <dbl>
                                                          <dbl>
                                                                   <dbl> <chr>
   <int>
##
    1 GO:00~ electron t~ 28/756
                                     111/18~ 5.76e-15 5.00e-12 4.25e-12 1536/8~
##
    2 GO:00~ proton tra~ 25/756
                                    127/18~ 6.00e-11 2.60e- 8 2.21e- 8 6556/9~
    3 GO:00~ proton-tra~ 10/756
                                     19/183~ 8.75e-10 2.53e- 7 2.15e- 7 506/51~
    4 \text{ GO:}00\text{~} cadherin b~ 40/756
                                     332/18~ 1.19e- 9 2.59e- 7 2.20e- 7 6282/7~
    5 GO:00~ actin bind~ 46/756
                                     437/18~ 5.55e- 9 9.63e- 7 8.19e- 7 6275/1~
##
      46
    6 GO:00~ oxidoreduc~ 20/756
                                     109/18~ 1.75e- 8 2.53e- 6 2.15e- 6 1536/4~
                                     26/183~ 3.87e- 8 4.80e- 6 4.08e- 6 506/51~
##
    7 GO:00~ proton cha~ 10/756
                                                                                   10
##
    8 GO:00~ actin fila~ 27/756
                                     206/18~ 1.07e- 7 1.16e- 5 9.88e- 6 10109/~
                                                                                   11
    9 GO:00~ oxidoreduc~ 10/756
                                     29/183~ 1.30e- 7 1.26e- 5 1.07e- 5 5447/1~
                                                                                   12
## 10 GO:00~ cytochrome~ 9/756
                                     28/183~ 1.11e- 6 8.04e- 5 6.83e- 5 1349/1~
                                                                                   13
```

#### For subpop 2:

```
head(result.enrichGO.de_entrez.subpop1.c2@result, 10)
```

```
## # A tibble: 10 x 9
                                                               qvalue geneID
##
             Description GeneRatio BgRatio pvalue p.adjust
   Count
##
      <chr> <chr>
                          <chr>>
                                    <chr>
                                               <dbl>
                                                        <dbl>
                                                                <dbl> <chr>
                                                                                3
   <int>
   1 GO:00~ antioxidant~ 13/305
##
                                    86/183~ 1.68e-9 1.05e-6 8.56e-7 6648/34~
##
    2 GO:00~ chemokine a~ 9/305
                                    49/183~ 9.86e-8 3.08e-5 2.51e-5 6362/63~
                                    70/183~ 2.28e-7 4.74e-5 3.87e-5 6362/63~
    3 G0:00~ chemokine r~ 10/305
##
    4 GO:00~ amide bindi~ 21/305
                                    381/18~ 1.75e-6 2.73e-4 2.23e-4 4311/53~
##
    5 GO:00~ peptide bin~ 18/305
                                    308/18~ 4.34e-6 5.42e-4 4.42e-4 4311/54~
##
                                    229/18~ 7.10e-6 7.39e-4 6.03e-4 710/265~
    6 GO:00~ peptidase r~ 15/305
##
                                    130/18~ 1.12e-5 1.00e-3 8.17e-4 231/864~
                                                                                10
##
   7 GO:00~ oxidoreduct~ 11/305
    8 GO:00~ proteoglyca~ 6/305
                                    36/183~ 2.57e-5 2.00e-3 1.64e-3 1514/34~
                                                                                11
##
                                    212/18~ 5.75e-5 3.99e-3 3.26e-3 2167/34~
    9 GO:00~ carboxylic ~ 13/305
                                                                                12
## 10 GO:00~ endopeptida~ 12/305
                                    192/18~ 9.22e-5 5.71e-3 4.66e-3 710/591~
                                                                                13
      12
```

#### For subpop 3:

```
head(result.enrichGO.de_entrez.subpop2.c2@result, 10)
```

```
## # A tibble: 10 x 9
                          GeneRatio BgRatio pvalue p.adjust qvalue geneID
##
      TD
             Description
   Count
                                                                                3
##
      <chr> <chr>
                          <chr>>
                                    <chr>>
                                              <dbl>
                                                        <dbl>
                                                                <dbl> <chr>
   <int>
##
   1 GO:00~ virus recep~ 14/531
                                    74/183~ 2.25e-8 8.75e-6 6.91e-6 7852/39~
   2 GO:01~ exogenous p~ 14/531
                                    74/183~ 2.25e-8
                                                     8.75e-6 6.91e-6 7852/39~
   3 GO:00~ cytokine bi~ 18/531
                                    135/18~ 6.99e-8 1.36e-5 1.07e-5 7852/88~
   4 GO:01~ immune rece~ 18/531
                                    135/18~ 6.99e-8
                                                     1.36e-5 1.07e-5 7852/12~
##
   5 GO:00~ proteoglyca~ 9/531
                                    36/183~ 6.23e-7 9.68e-5 7.65e-5 1508/15~
                                    144/18~ 9.40e-7 1.22e-4 9.62e-5 6696/10~
   6 GO:00~ integrin bi~ 17/531
##
   7 GO:00~ phospholipi~ 32/531
                                    454/18~ 3.59e-6 3.99e-4 3.15e-4 19/5360~
                                                                                10
##
```

```
## 8 GO:00~ extracellul~ 10/531 57/183~ 4.81e-6 4.67e-4 3.69e-4 6696/39~ 11 10  
## 9 GO:00~ complement ~ 6/531 21/183~ 2.14e-5 1.84e-3 1.46e-3 966/137~ 12 6  
## 10 GO:00~ phosphatidy~ 21/531 262/18~ 2.68e-5 2.08e-3 1.64e-3 1687/85~ 13
```

For subpop 4:

```
head(result.enrichGO.de_entrez.subpop3.c2@result, 10)
```

```
## # A tibble: 10 x 9
##
      ID
             Description GeneRatio BgRatio
                                              pvalue p.adjust
                                                                 qvalue geneID
   Count
##
      <chr>
             <chr>
                          <chr>
                                    <chr>
                                               <dbl>
                                                         <dbl>
                                                                  <dbl> <chr>
   <int>
##
   1 GO:00~ structural~ 82/934
                                    202/18~ 9.37e-53 7.83e-50 6.95e-50 6141/6~
    2 GO:00~ rRNA bindi~ 25/934
                                    64/183~ 2.04e-16 8.51e-14 7.56e-14 6208/6~
##
      25
    3 GO:00~ MHC class ~ 9/934
                                    10/183~ 2.11e-11 5.87e- 9 5.21e- 9 3112/3~
##
##
    4 GO:01~ immune rec~ 24/934
                                    135/18~ 7.57e- 8 1.58e- 5 1.40e- 5 1235/1~
                                    139/18~ 1.34e- 7 2.24e- 5 1.99e- 5 6208/6~
    5 GO:00~ translatio~ 24/934
##
                                    11/183~ 2.38e- 7 3.32e- 5 2.95e- 5 6125/6~
##
    6 GO:00~ 5S rRNA bi~ 7/934
                                    84/183~ 9.08e- 7 1.08e- 4 9.62e- 5 1915/1~
                                                                                  10
##
    7 GO:00~ translatio~ 17/934
                                    109/18~ 2.30e- 6 2.41e- 4 2.14e- 4 1915/1~
    8 GO:00~ translatio~ 19/934
                                                                                  11
##
    9 GO:00~ kinase reg~ 28/934
                                    216/18~ 5.24e- 6 4.83e- 4 4.29e- 4 5570/5~
                                                                                  12
## 10 GO:00~ enzyme inh~ 41/934
                                    383/18~ 5.82e- 6 4.83e- 4 4.29e- 4 8530/9~
                                                                                  13
      41
```

#### 4 Session information

R session:

```
sessionInfo()
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
                                                                                  2
## Running under: Ubuntu 20.04.3 LTS
                                                                                  3
                                                                                  4
##
## Matrix products: default
           /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
                                                                                  6
## BLAS:
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3
                                                                                  8
##
                                                                                  9
## locale:
                                                                                  10
   [1] LC_CTYPE=en_US.UTF-8
                                    LC NUMERIC=C
```

```
##
    [3] LC TIME=en GB.UTF-8
                                      LC COLLATE=en US.UTF-8
                                                                                      11
##
                                                                                      12
    [5] LC_MONETARY=en_GB.UTF-8
                                      LC_MESSAGES=en_US.UTF-8
                                                                                      13
##
    [7] LC PAPER=en GB.UTF-8
                                      LC NAME=C
                                                                                      14
    [9] LC_ADDRESS=C
                                      LC_TELEPHONE=C
##
##
   [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
                                                                                      15
                                                                                      16
##
                                                                                      17
## attached base packages:
                                                                                      18
   [1] parallel
                  stats4
                             stats
                                        graphics
                                                  grDevices utils
                                                                         datasets
##
   [8] methods
                  base
                                                                                      19
                                                                                      20
##
##
  other attached packages:
                                                                                      21
                                                                                      22
##
    [1] org.Hs.eg.db_3.12.0
                                 AnnotationDbi_1.52.0
                                                           IRanges_2.24.1
                                                                                      23
##
    [4] S4Vectors_0.28.1
                                                          BiocGenerics_0.36.1
                                 Biobase_2.50.0
                                                                                      24
    [7] clusterProfiler_3.18.1 dplyr_1.0.7
##
                                                          ggplot2_3.3.5
  [10] SeuratObject_4.0.2
                                 Seurat_4.0.3
                                                                                      25
##
                                                                                      26
##
                                                                                      27
##
  loaded via a namespace (and not attached):
                                                                                      28
##
     [1] shadowtext 0.0.8
                                 fastmatch 1.1-3
                                                         plyr 1.8.6
##
                                 lazyeval_0.2.2
                                                                                      29
     [4] igraph_1.2.6
                                                         splines_4.0.3
                                                                                      30
##
     [7] BiocParallel 1.24.1
                                 listenv 0.8.0
                                                         scattermore 0.7
##
    [10] digest_0.6.27
                                 htmltools_0.5.1.1
                                                         GOSemSim_2.16.1
                                                                                      31
                                                         fansi 0.5.0
                                                                                      32
##
    [13] viridis 0.6.1
                                 GO.db_3.12.1
                                                                                      33
##
    [16] magrittr_2.0.1
                                                         tensor_1.5
                                 memoise 2.0.0
    [19] cluster 2.1.0
                                                                                      34
##
                                 ROCR_1.0-11
                                                         limma 3.46.0
                                                                                      35
##
    [22] graphlayouts_0.7.1
                                 globals_0.14.0
                                                         matrixStats 0.60.0
##
    [25] spatstat.sparse_2.0-0 enrichplot_1.10.2
                                                         colorspace_2.0-2
                                                                                      36
##
    [28] blob_1.2.2
                                                                                     37
                                 ggrepel_0.9.1
                                                         xfun_0.24
                                                         scatterpie_0.1.6
                                                                                      38
##
    [31] crayon_1.4.1
                                 jsonlite_1.7.2
                                                                                      39
##
    [34] spatstat.data_2.1-0
                                 survival_3.2-7
                                                         zoo_1.8-9
##
    [37] glue_1.4.2
                                 polyclip_1.10-0
                                                         gtable_0.3.0
                                                                                      40
##
    [40] leiden_0.3.9
                                 future.apply_1.7.0
                                                         abind_1.4-5
                                                                                      41
##
    [43] scales_1.1.1
                                 DOSE_3.16.0
                                                         DBI_1.1.1
                                                                                      42
                                                                                      43
##
    [46] miniUI_0.1.1.1
                                 Rcpp_1.0.7
                                                         viridisLite_0.4.0
                                                                                      44
##
    [49] xtable_1.8-4
                                 reticulate_1.20
                                                         spatstat.core_2.3-0
##
    [52] bit 4.0.4
                                                                                      45
                                 htmlwidgets_1.5.3
                                                         httr 1.4.2
##
                                                                                      46
    [55] fgsea_1.16.0
                                 RColorBrewer_1.1-2
                                                         ellipsis_0.3.2
##
    [58] ica 1.0-2
                                 farver 2.1.0
                                                         pkgconfig_2.0.3
                                                                                      47
##
    [61] uwot_0.1.10.9000
                                 deldir_0.2-10
                                                         utf8_1.2.2
                                                                                      48
                                                                                      49
##
    [64] tidyselect_1.1.1
                                 rlang_0.4.11
                                                         reshape2_1.4.4
                                                                                      50
##
    [67] later_1.2.0
                                 munsell_0.5.0
                                                         tools_4.0.3
                                                                                      51
##
    [70] cachem 1.0.5
                                 downloader 0.4
                                                         cli_3.0.1
##
    [73] generics_0.1.0
                                                                                     52
                                 RSQLite_2.2.7
                                                         ggridges_0.5.3
                                                                                      53
##
    [76] evaluate_0.14
                                 stringr_1.4.0
                                                         fastmap_1.1.0
##
                                                                                      54
    [79] yaml_2.2.1
                                 goftest_1.2-2
                                                         knitr_1.33
                                                         tidygraph_1.2.0
                                                                                      55
##
    [82] bit64_4.0.5
                                 fitdistrplus_1.1-5
                                                                                      56
##
    [85] purrr_0.3.4
                                 RANN_2.6.1
                                                         ggraph_2.0.5
                                                                                      57
##
    [88] pbapply_1.4-3
                                 future_1.21.0
                                                         nlme_3.1-152
                                                                                      58
##
    [91] mime_0.11
                                 DO.db_2.9
                                                         compiler_4.0.3
##
    [94] rstudioapi_0.13
                                 plotly_4.9.4.1
                                                         png_0.1-7
                                                                                     59
                                                                                      60
    [97] spatstat.utils_2.2-0
                                 tibble_3.1.3
                                                         tweenr_1.0.2
                                                                                     61
## [100] stringi_1.7.3
                                 lattice_0.20-41
                                                         Matrix_1.3-4
                                                                                     62
## [103] vctrs_0.3.8
                                 pillar 1.6.2
                                                         lifecycle 1.0.0
## [106] BiocManager_1.30.16
                                 spatstat.geom_2.2-2
                                                         lmtest_0.9-38
                                                                                     63
                                                                                     64
   [109] RcppAnnoy_0.0.19
                                 data.table_1.14.0
                                                         cowplot_1.1.1
```

```
## [112] irlba_2.3.3
                                httpuv_1.6.1
                                                        patchwork_1.1.1
                                                                                   65
## [115] qvalue_2.22.0
                                R6_2.5.0
                                                        promises_1.2.0.1
                                                                                   66
                                                                                   67
## [118] KernSmooth 2.23-20
                                                        parallelly_1.27.0
                                gridExtra_2.3
  [121] codetools_0.2-18
                                MASS_7.3-53
                                                        assertthat_0.2.1
                                                                                   68
   [124] withr 2.4.2
                                sctransform_0.3.2
                                                                                   69
                                                        mgcv_1.8-33
                                                                                   70
  [127] grid_4.0.3
                                rpart_4.1-15
                                                        tidyr_1.1.3
                                                                                   71
## [130] rvcheck 0.1.8
                                rmarkdown 2.9
                                                        Rtsne_0.15
## [133] ggforce_0.3.3
                                shiny_1.6.0
                                                                                   72
```

#### References

- 1. Hao Y, Hao S, Andersen-Nissen E, Mauck III WM, Zheng S, Butler A, Lee MJ, Wilk AJ, Darby C, Zagar M, Hoffman P, Stoeckius M, Papalexi E, Mimitou EP, Jain J, Srivastava A, Stuart T, Fleming LB, Yeung B, Rogers AJ, McElrath JM, Blish CA, Gottardo R, Smibert P, Satija R. Integrated analysis of multimodal single-cell data. *Cell* [Internet] 2021; Available from: https://doi.org/10.1016/j.cell.2021.04.048.
- 2. 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; 16: 284–287.