

CITE-seq based plots for Gearty et al. (2021)

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These plots are based on scRNA-seq/CITE-seq data obtained from sorted CD8+ T cells from pancreatic lymph nodes (pLN). The data was obtained from three technical replicates (`Sample`): “pLN_1”, “pLN_3”, “pLN_4” that correspond to three different library prep and sequencing runs. Each of the experiments had CD8 T cells from multiple mice, whose identity was derived via antibody-based sample tagging and can be found in the `SingleCellExperiment`'s `colData` (meta data) via the `Mouse` entry. CD44, CD62L etc. correspond to the normalized CITE-seq values for the respective antibodies

The `SingleCellExperiment` object and all other R objects needed to recreate this document are stored in the Box; the `BiocFileCache` package must be installed to make use of that.

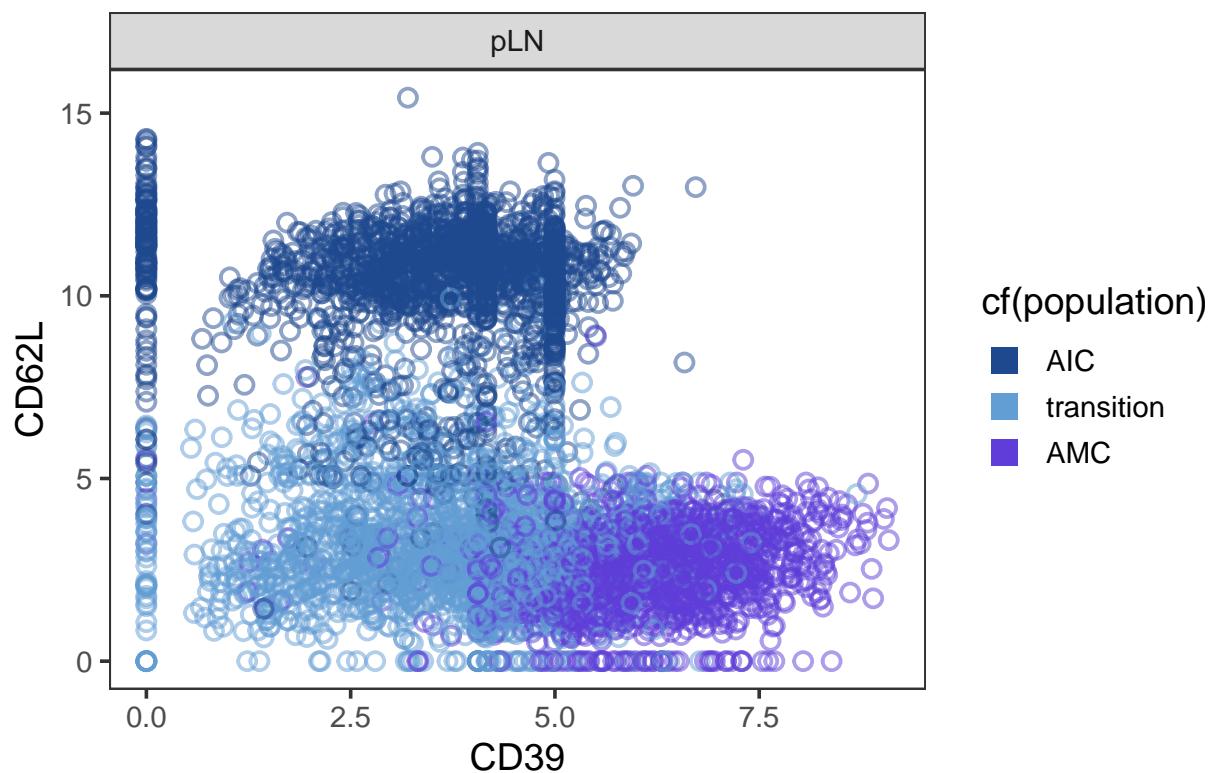
```
f1s <- fread("data/data_links.txt", header=TRUE)
sln <- load_RDSdata_from_Box(shared_link = f1s[object == "SCE"]$link)

## Cached data here: ~/Library/Caches/BiocFileCache/1863d738f6ed0_2wvbdfs3vja2cnlckcqp20eu1693o8o.rds
sln

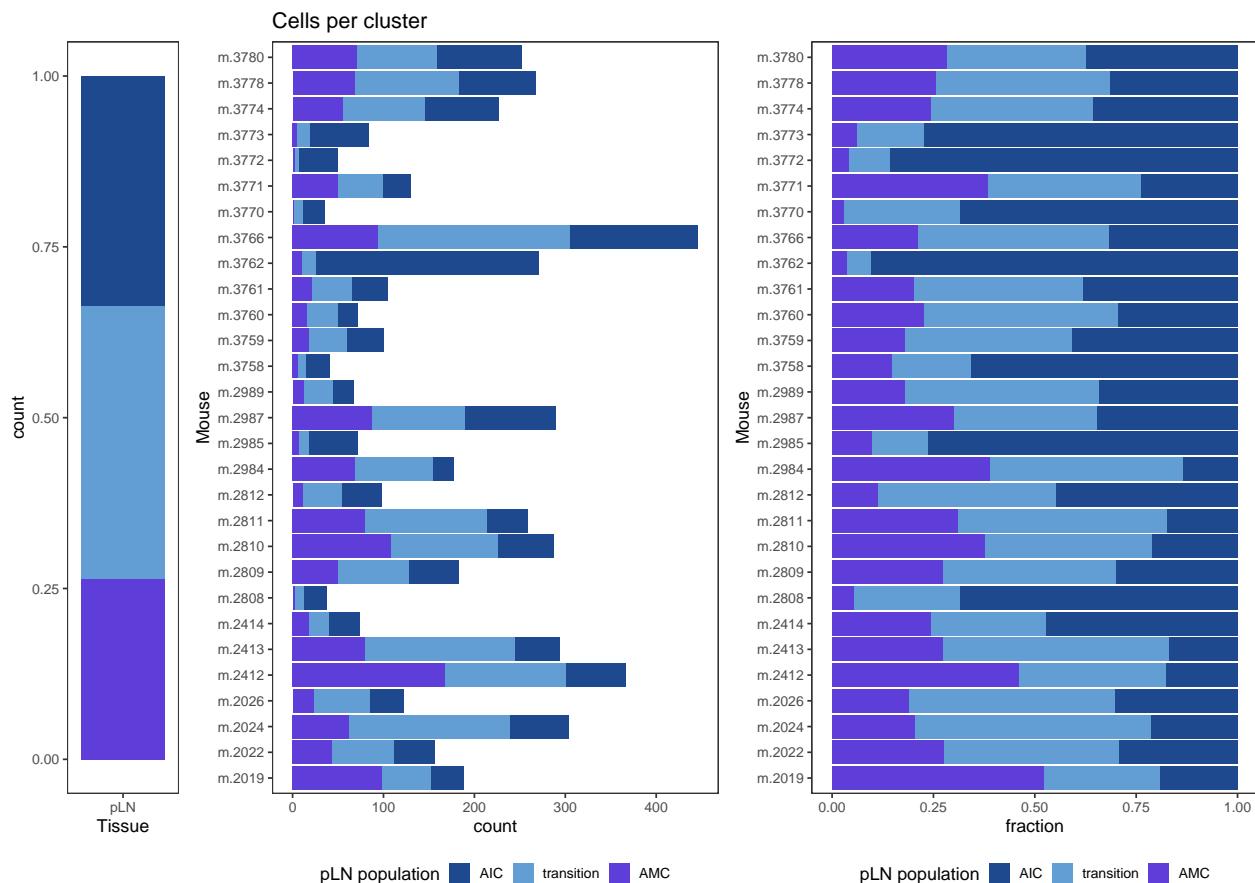
## class: SingleCellExperiment
## dim: 14546 5055
## metadata(0):
## assays(2): counts logcounts
## rownames(14546): Mrpl15 Lypla1 ... CAAA01147332.1 AC149090.1
## rowData names(2): ID Symbol
## colnames(5055): pLN1_2 pLN1_4 ... pLN4_1045 pLN4_1047
## colData names(28): cell Sample ... TSCANAMC population
## reducedDimNames(4): UMAP PCA_corr diffusionMap PCA
## altExpNames(0):
```

Mimicking FACS plots

ADT: CD62L vs. CD39



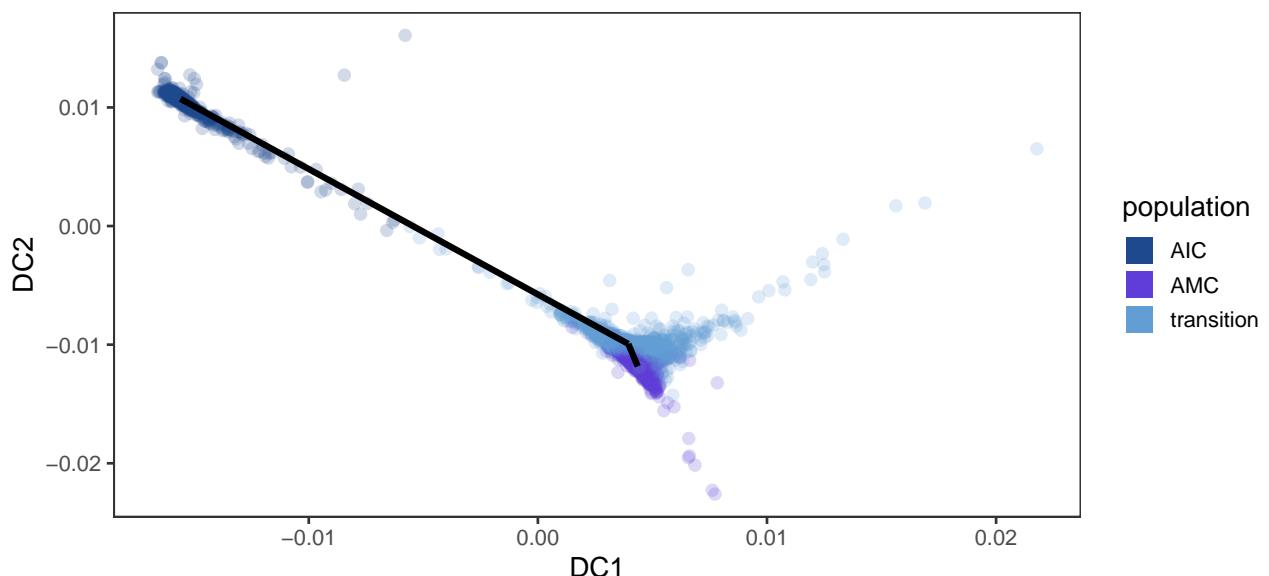
Cells per cluster per mouse



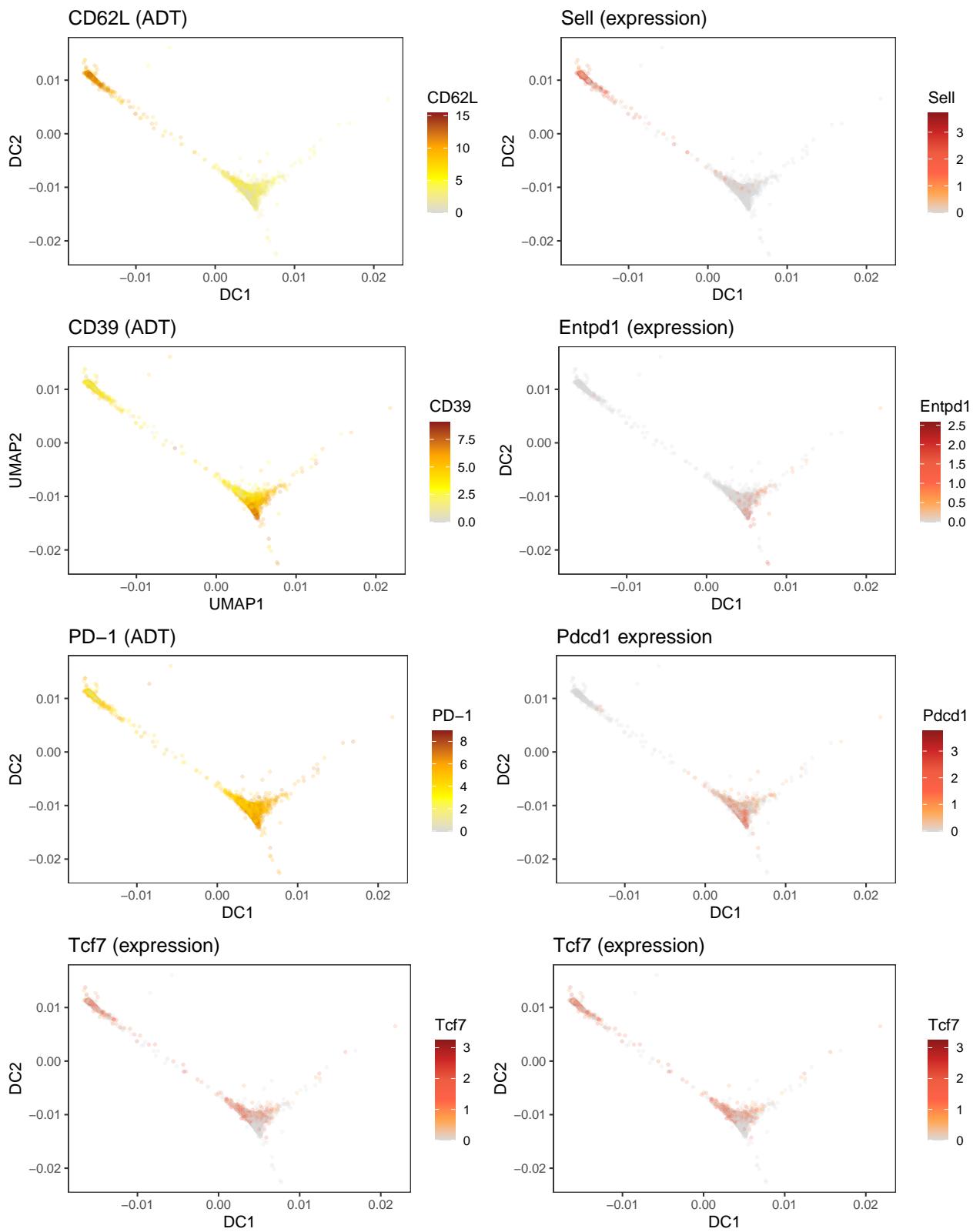
Diffusion map + trajectory

```
## Cached data here: ~/Library/Caches/BiocFileCache/1863d6a6e0b13_rakg00duv7j5v18e9jw2jq93px18713h.rda
```

Diffusion map with TSCAN–predicted trajectory



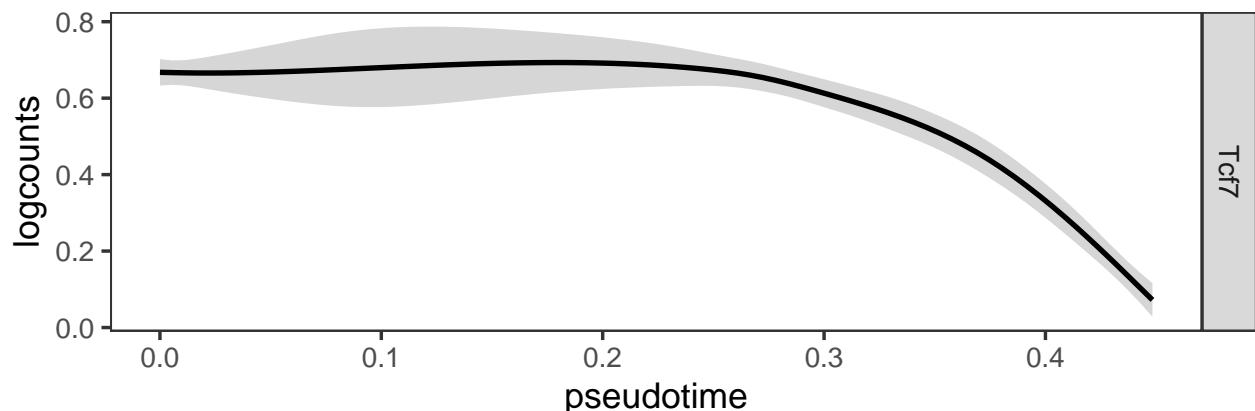
Gene expression in diffusion map



Genes' expression plotted along pseudotime

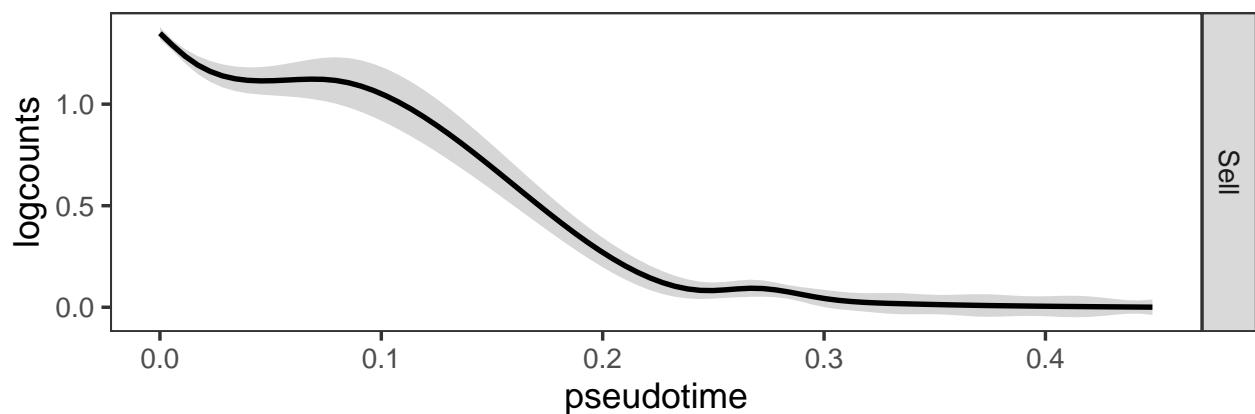
Tcf7

Cells sorted acc. to pseudotime



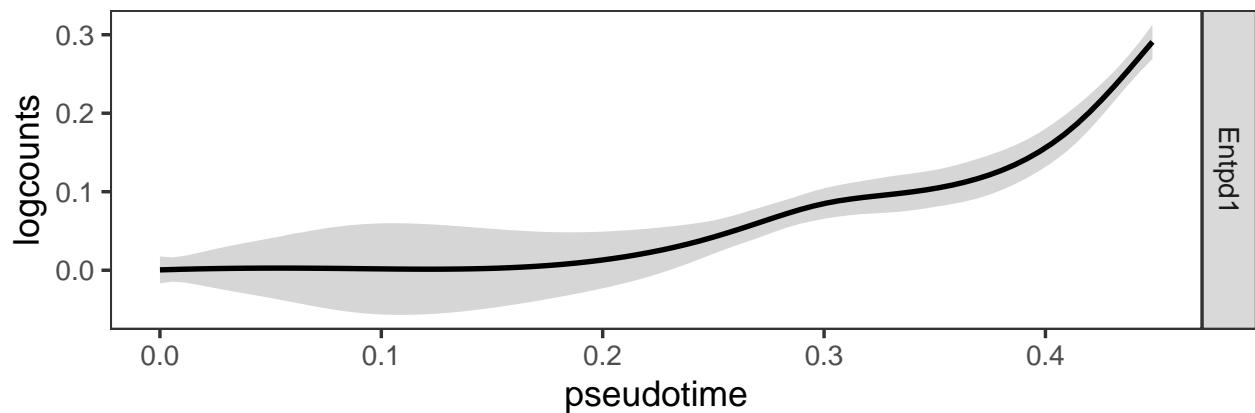
Sell

Cells sorted acc. to pseudotime



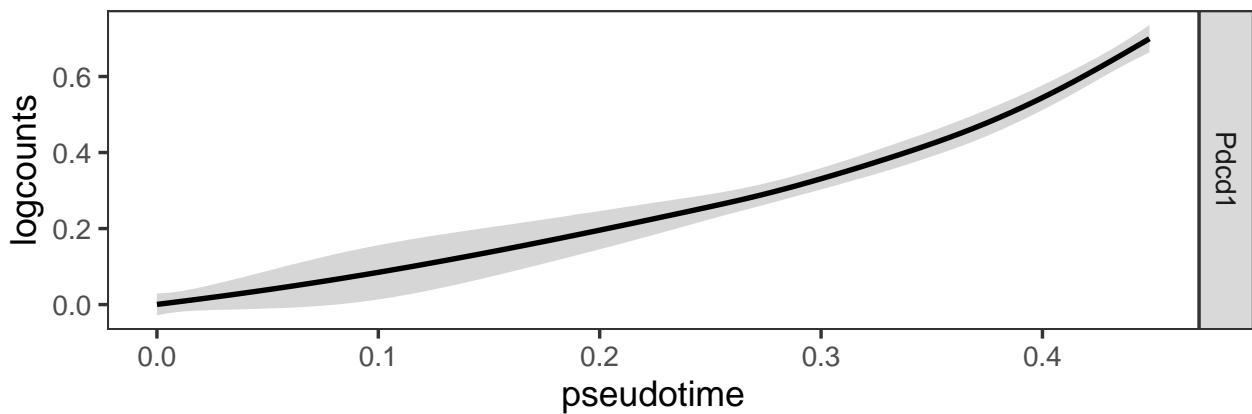
Entpd1

Cells sorted acc. to pseudotime



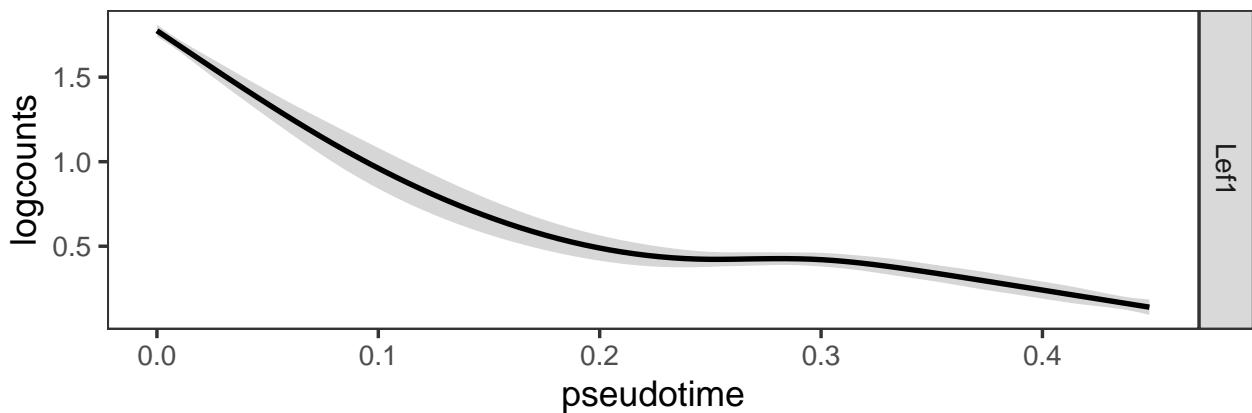
Pdcd1

Cells sorted acc. to pseudotime



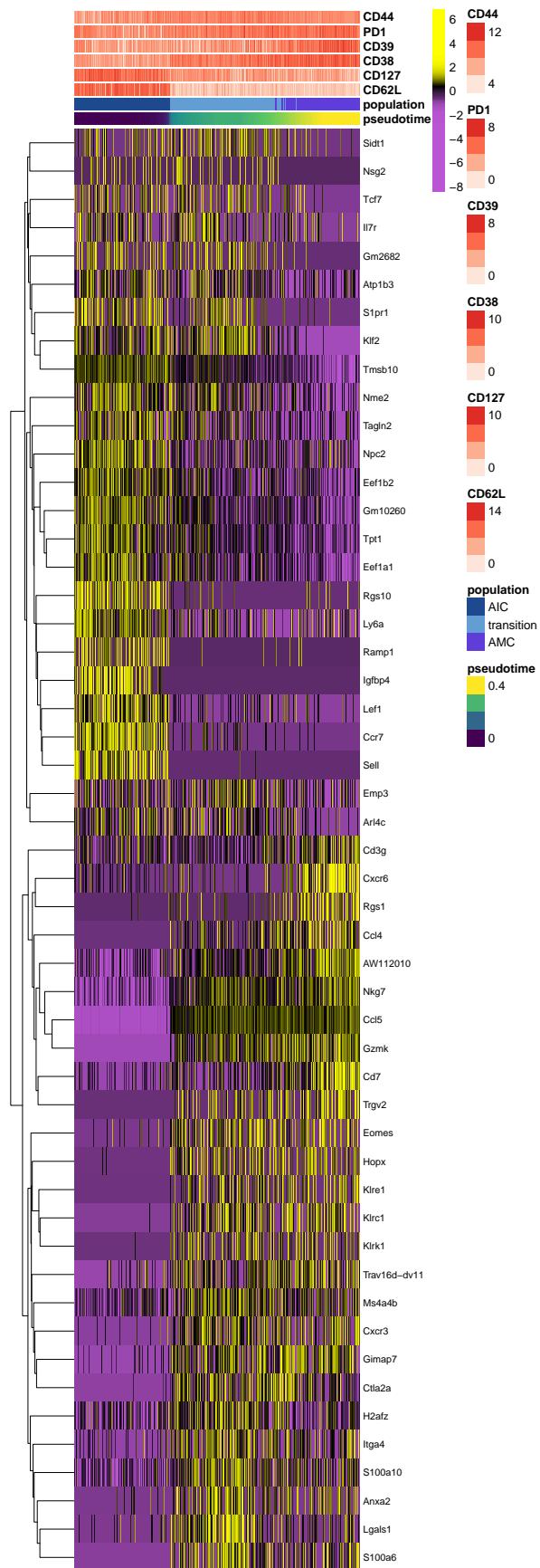
Lef1

Cells sorted acc. to pseudotime



Marker genes between clusters

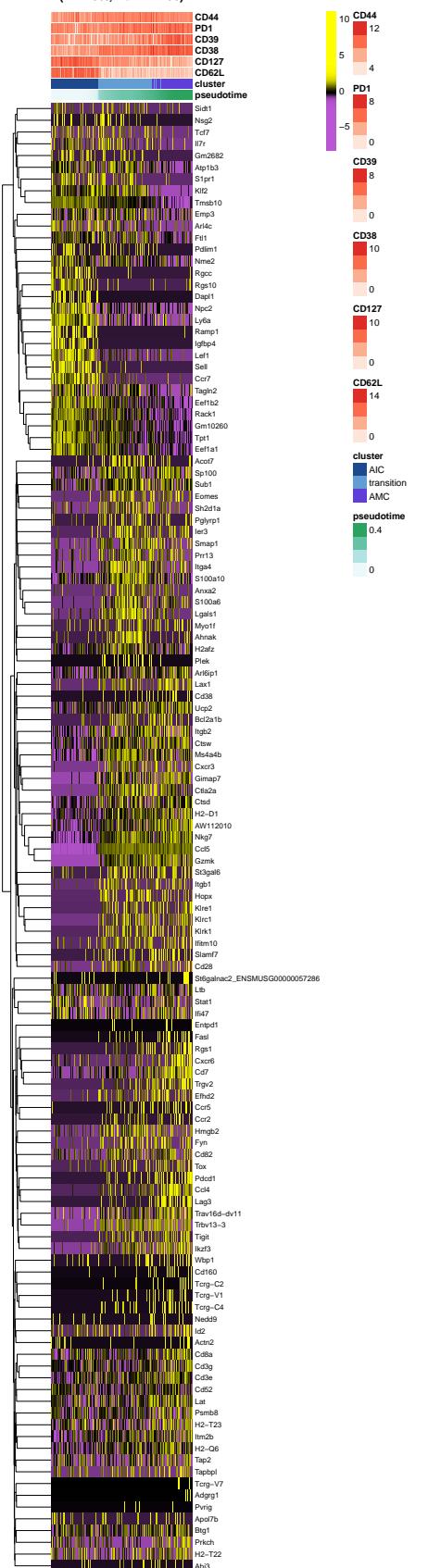
Markers for both directions



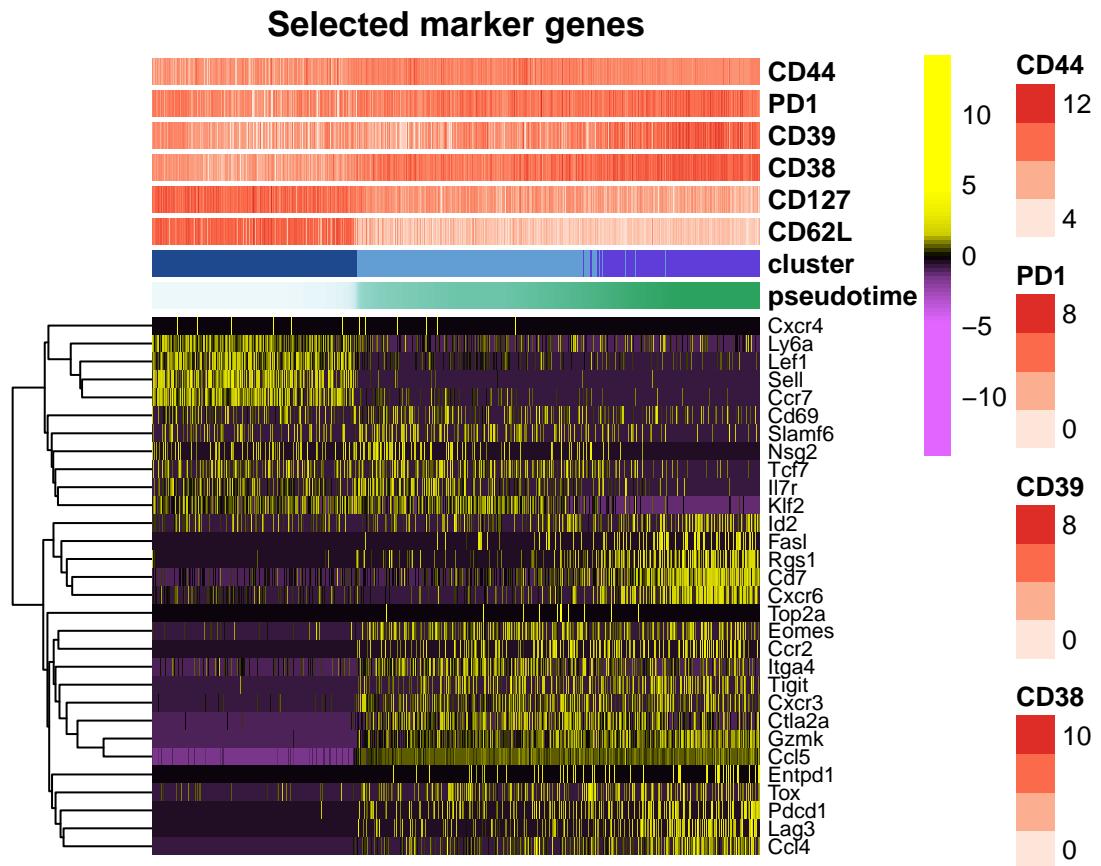
Markers that go up in the respective cluster

We can focus the marker detection to those that go up in an individual cluster.

**Marker genes of the three populations
(FDR 5%, Rank <= 50)**



Sofia's selected genes of interest



GO term enrichments

Ignoring ribosomal genes.

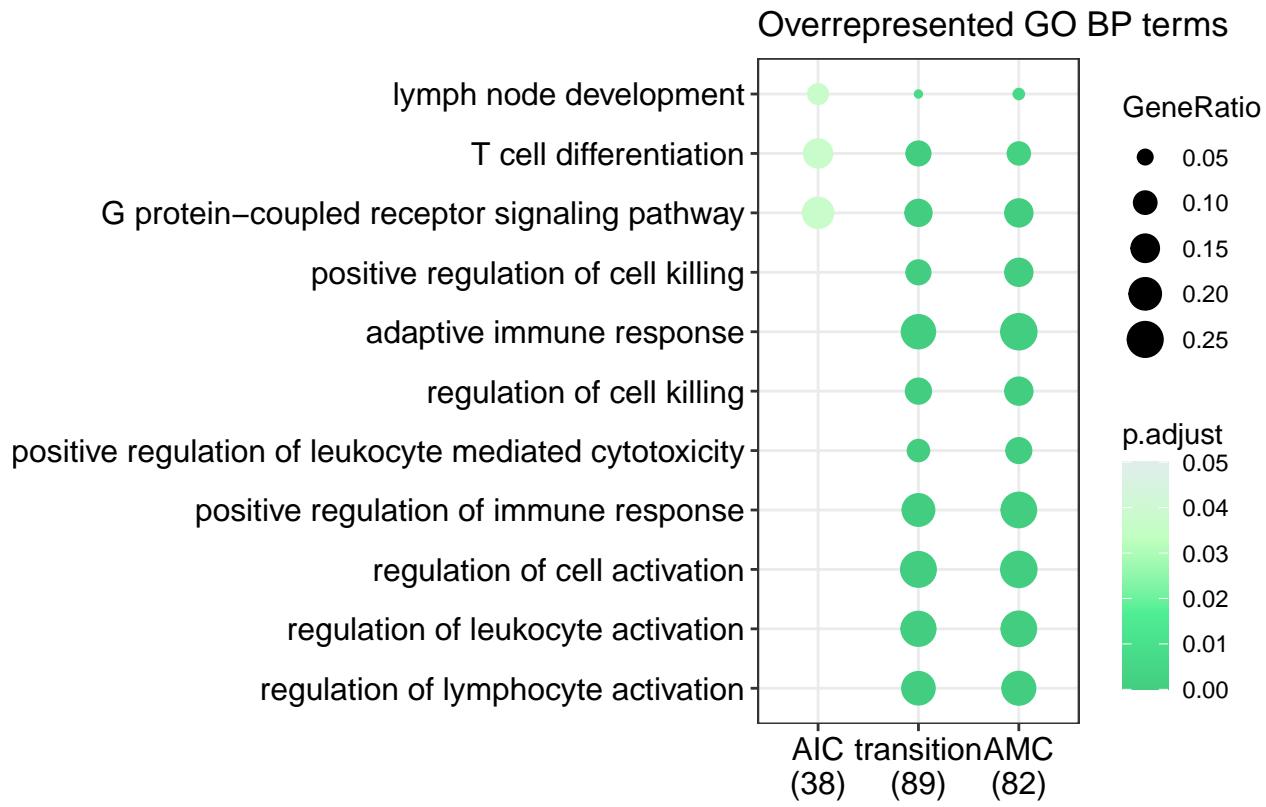
```
## Cached data here: ~/Library/Caches/BiocFileCache/1863d1923013e_8k2cudvwqwx05iywz8d36oof2qavjb8.rda
## Cached data here: ~/Library/Caches/BiocFileCache/7735660e0a40_3zstp6yohqvrc31t26tzs8ox5pr5ukm6.rda
```

GO terms (BP)

Here, we first directly compare the GO term enrichments for the different clusters. Each tab then contains the results of the individual analyses, i.e. one per cell population (AIC, transition, AMC).

If a tab does not contain an extra plot, no enrichments were found for that cell population.

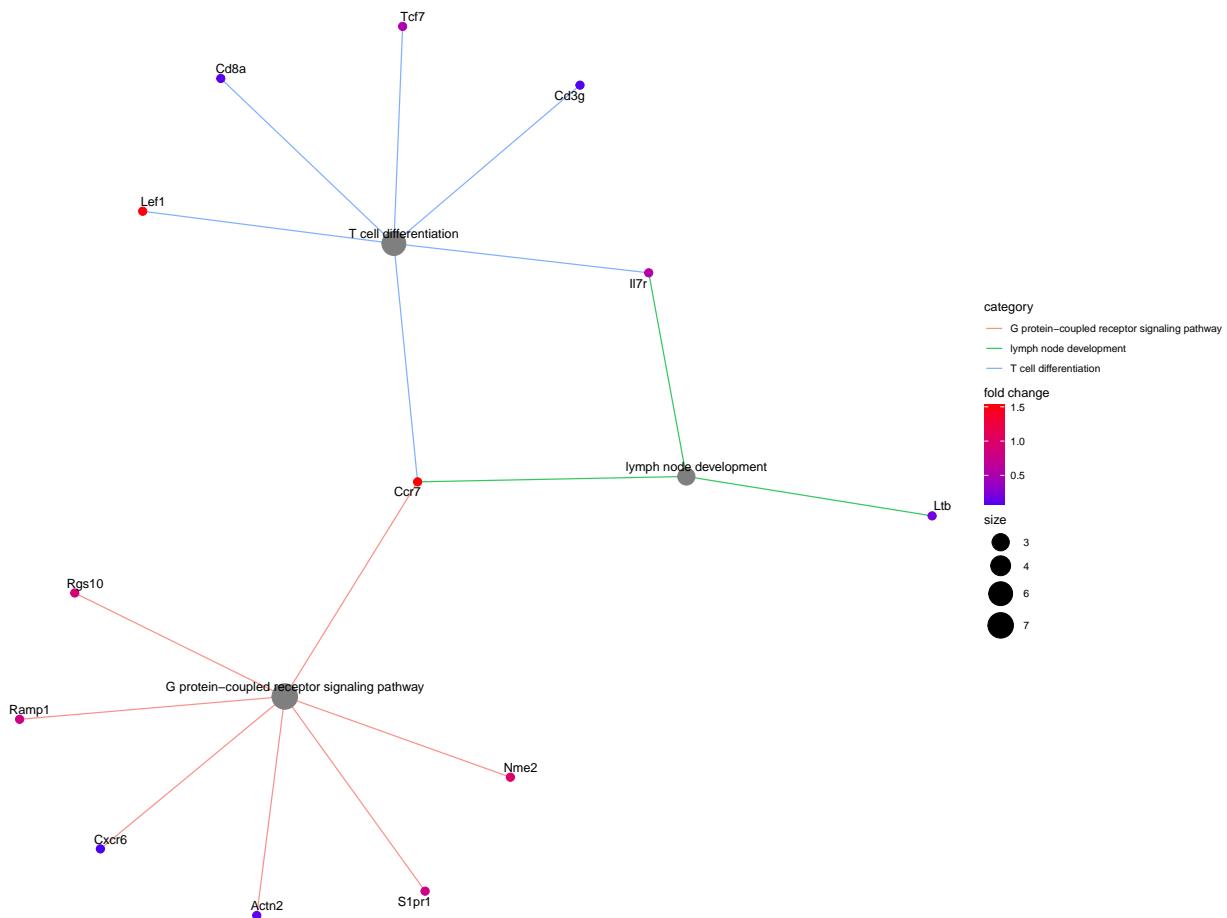
Comparison



- geneRatio should be number of genes that overlap gene set divided by size of gene set

AIC

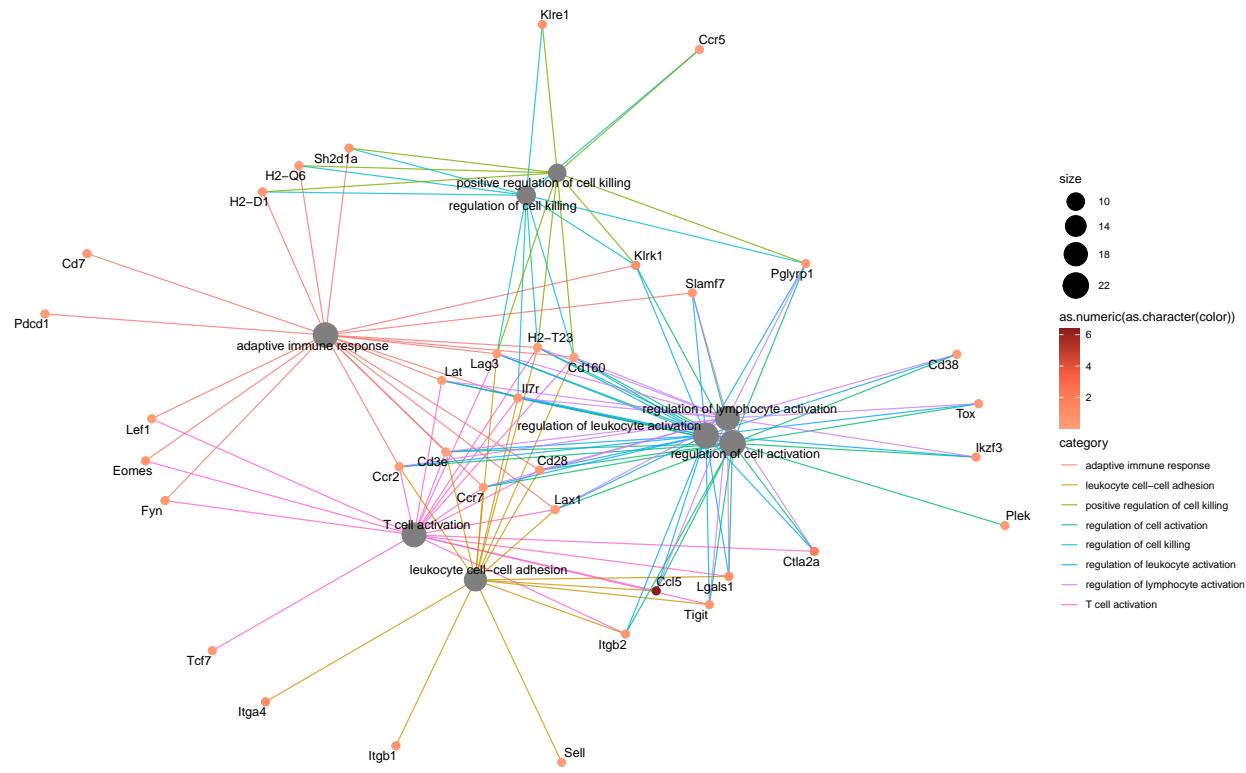
GO BP PWs (AIC)



Transition

```
## Scale for 'colour' is already present. Adding another scale for 'colour',
## which will replace the existing scale.
```

GO BP PWs (Transition population)

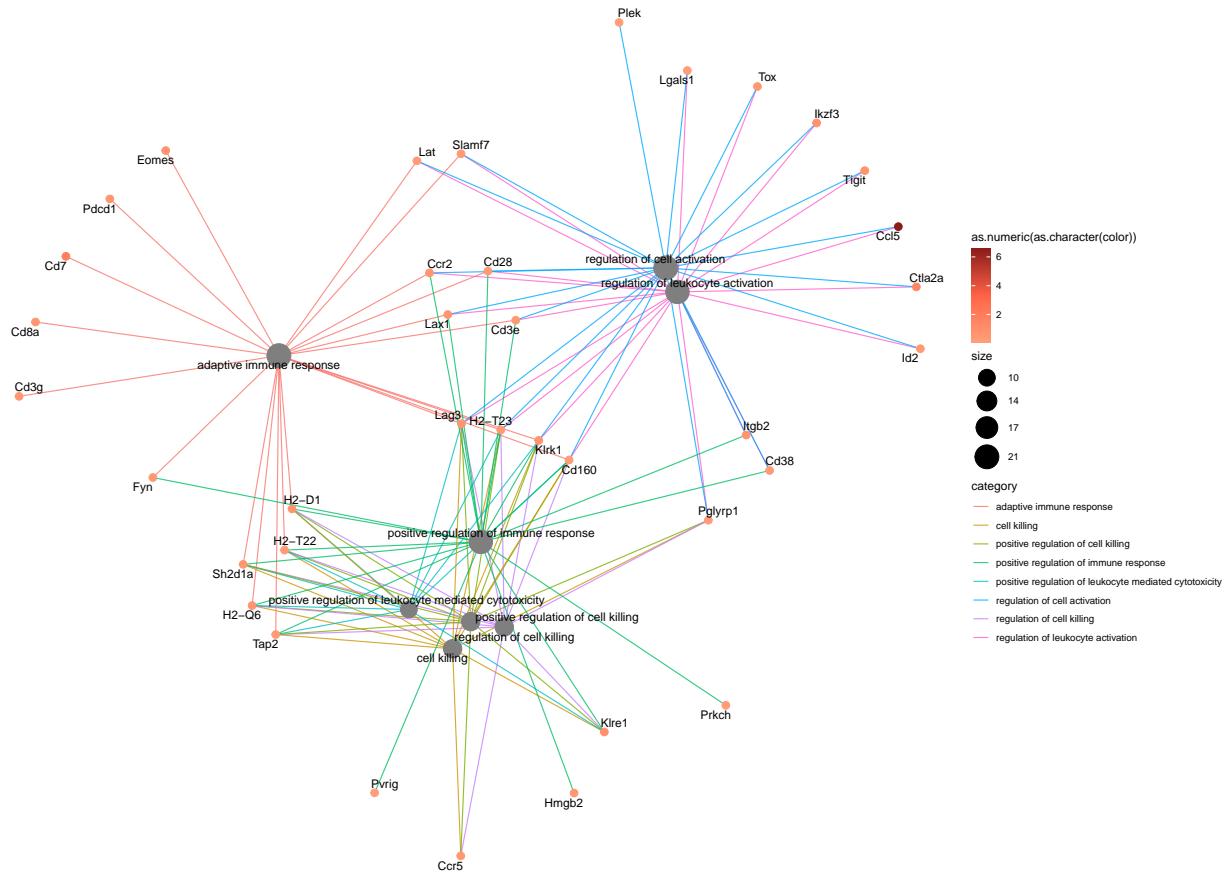


- grey bubbles = name of the gene set at hand (e.g. “translational elongation”)
 - size = number of marker genes that are part of the gene set
- edges = colored by gene set
- nodes belonging to each gene set represent individual genes (e.g. Rack1)
 - their color represents the logFC of that gene in the respective cluster compared to the expression of the gene in all other clusters

AMC

```
## Scale for 'colour' is already present. Adding another scale for 'colour',
## which will replace the existing scale.
```

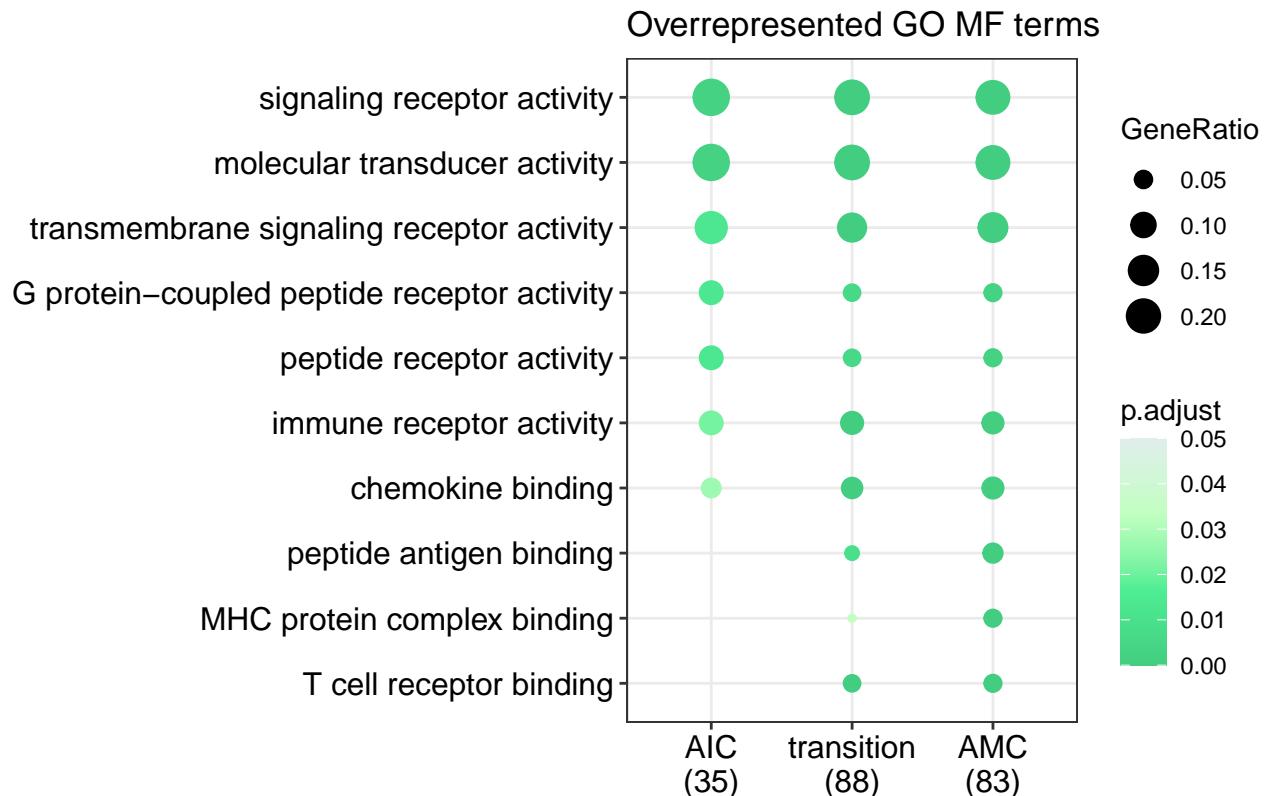
GO BP PWs (AMC)



GO terms (MF)

If a tab does not contain an extra plot, no enrichments were found for that cell population.

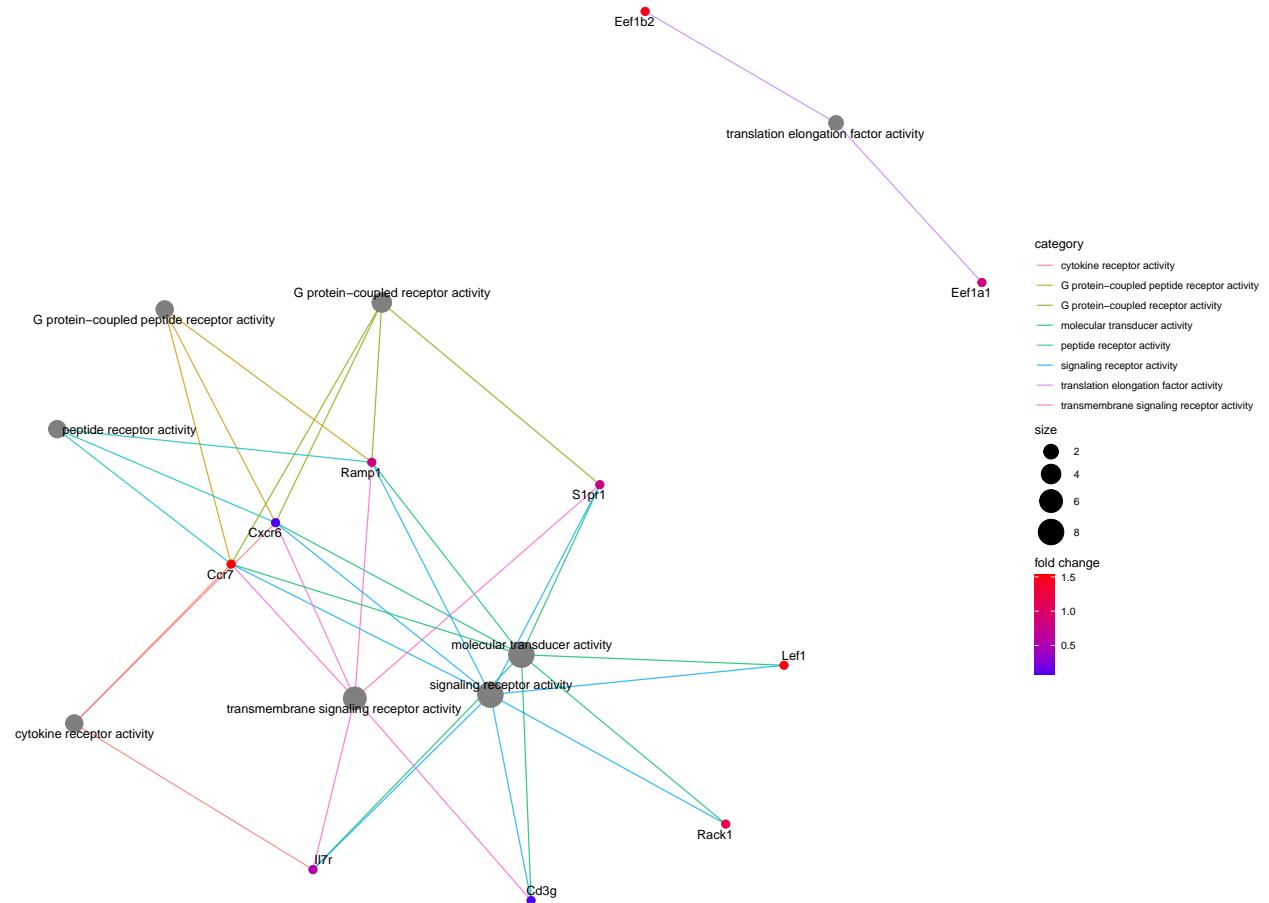
Comparison



- geneRatio should be number of genes that overlap gene set divided by size of gene set

AIC

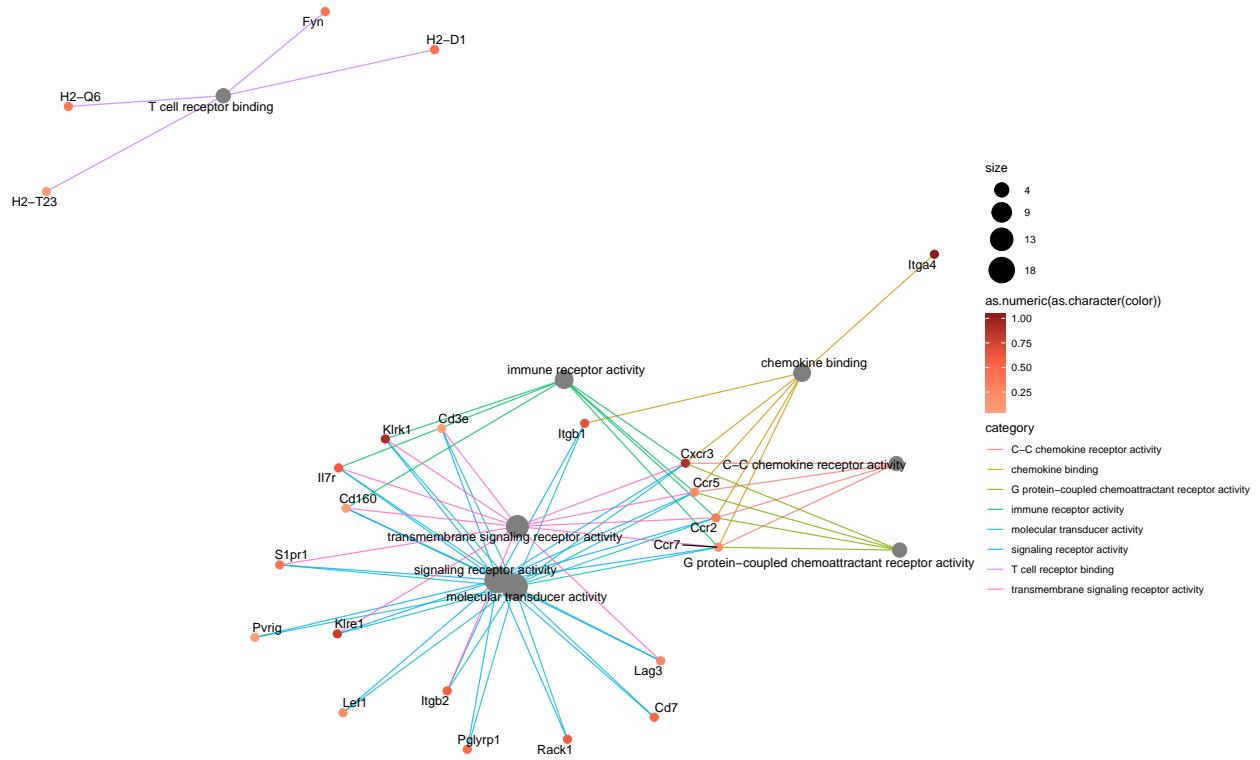
GO MF PWs (AIC)



Transition

```
## Scale for 'colour' is already present. Adding another scale for 'colour',
## which will replace the existing scale.
```

GO MF PWs (Transition population)

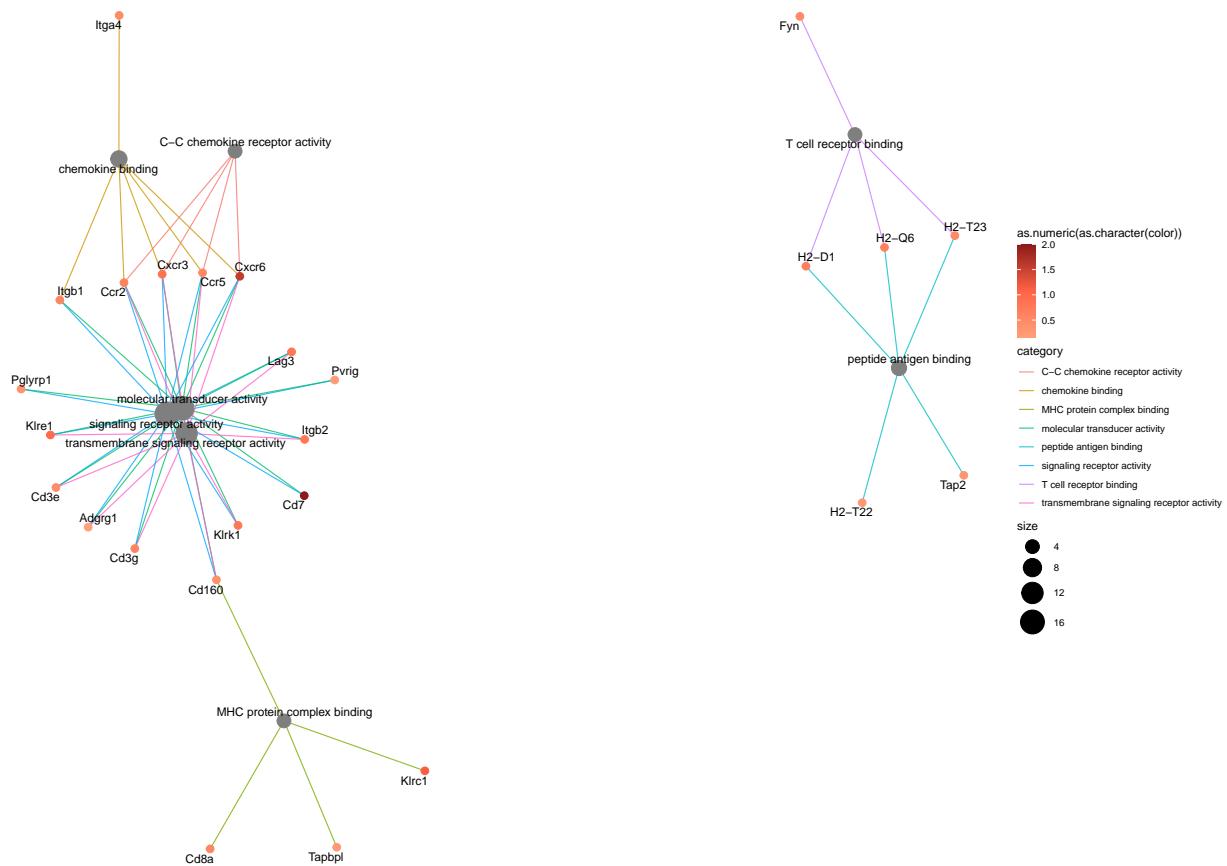


- grey bubbles = name of the gene set at hand (e.g. “translational elongation”)
 - size = number of marker genes that are part of the gene set
- edges = colored by gene set
- nodes belonging to each gene set represent individual genes (e.g. Rack1)
 - their color represents the logFC of that gene in the respective cluster compared to the expression of the gene in all other clusters

AMC

```
## Scale for 'colour' is already present. Adding another scale for 'colour',
## which will replace the existing scale.
```

GO MF PWs (AMC)



SessionInfo

```
## R version 4.0.2 (2020-06-22)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Catalina 10.15.7
##
## Matrix products: default
## BLAS:    /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
## LAPACK:  /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
## [1] org.Mm.eg.db_3.11.4      AnnotationDbi_1.50.3
## [3] BiocFileCache_1.12.1     dbplyr_2.0.0
## [5] clusterProfiler_3.16.1    scABC2_0.3.3
## [7] patchwork_1.1.1          ggplot2_3.3.3
## [9] data.table_1.13.6        magrittr_2.0.1
```

```

## [11] SingleCellExperiment_1.10.1 SummarizedExperiment_1.18.2
## [13] DelayedArray_0.14.1           matrixStats_0.57.0
## [15] Biobase_2.48.0                GenomicRanges_1.40.0
## [17] GenomeInfoDb_1.24.2          IRanges_2.22.2
## [19] S4Vectors_0.26.1             BiocGenerics_0.34.0
##
## loaded via a namespace (and not attached):
##   [1] reticulate_1.18              tidyselect_1.1.1
##   [3] RSQLite_2.2.2                htmlwidgets_1.5.3
##   [5] grid_4.0.2                  BiocParallel_1.22.0
##   [7] Rtsne_0.15                  scatterpie_0.1.5
##   [9] munsell_0.5.0               codetools_0.2-18
##  [11] ica_1.0-2                  statmod_1.4.35
##  [13] scran_1.16.0               future_1.21.0
##  [15] miniUI_0.1.1.1            withr_2.3.0
##  [17] colorspace_2.0-0           GOSemSim_2.14.2
##  [19] knitr_1.30                 rstudioapi_0.13
##  [21] Seurat_3.2.3              ROCR_1.0-11
##  [23] tensor_1.5                 DOSE_3.14.0
##  [25] listenv_0.8.0              labeling_0.4.2
##  [27] urltools_1.7.3            GenomeInfoDbData_1.2.3
##  [29] polyclip_1.10-0           bit64_4.0.5
##  [31] farver_2.0.3              pheatmap_1.0.12
##  [33] downloader_0.4            parallelly_1.23.0
##  [35] vctrs_0.3.8               generics_0.1.0
##  [37] xfun_0.20                 R6_2.5.0
##  [39] ggbeeswarm_0.6.0          graphlayouts_0.7.1
##  [41] rsvd_1.0.3                locfit_1.5-9.4
##  [43] gridGraphics_0.5-1         bitops_1.0-6
##  [45] spatstat.utils_1.20-2      fgsea_1.14.0
##  [47] assertthat_0.2.1          promises_1.1.1
##  [49] scales_1.1.1              ggraph_2.0.4
##  [51] enrichplot_1.8.1          beeswarm_0.2.3
##  [53] gtable_0.3.0              globals_0.14.0
##  [55] goftest_1.2-2             drat_0.1.8
##  [57] tidygraph_1.2.0           rlang_0.4.11
##  [59] splines_4.0.2             lazyeval_0.2.2
##  [61] europepmc_0.4             BiocManager_1.30.10
##  [63] yaml_2.2.1                reshape2_1.4.4
##  [65] abind_1.4-5              httpuv_1.5.4
##  [67] qvalue_2.20.0            tools_4.0.2
##  [69] ggplotify_0.0.5          kableExtra_1.3.1
##  [71] ellipsis_0.3.2           RColorBrewer_1.1-2
##  [73] ggridges_0.5.3            Rcpp_1.0.6
##  [75] plyr_1.8.6                progress_1.2.2
##  [77] zlibbioc_1.34.0           purrr_0.3.4
##  [79] RCurl_1.98-1.2            prettyunits_1.1.1
##  [81] rpart_4.1-15              deldir_0.2-3
##  [83] pbapply_1.4-3             viridis_0.5.1
##  [85] cowplot_1.1.1             zoo_1.8-8
##  [87] ggrepel_0.9.0              cluster_2.1.0
##  [89] scattermore_0.7            DO.db_2.9
##  [91] lmtest_0.9-38             triebeard_0.3.0
##  [93] RANN_2.6.1                ABCutilities_0.3.3

```

```

## [95] fitdistrplus_1.1-3          hms_1.0.0
## [97] mime_0.9                      evaluate_0.14
## [99] xtable_1.8-4                  gridExtra_2.3
## [101] compiler_4.0.2                scater_1.16.2
## [103] tibble_3.0.4                  KernSmooth_2.23-18
## [105] crayon_1.3.4                  htmltools_0.5.1.1
## [107] mgcv_1.8-33                  later_1.1.0.1
## [109] tidyverse_1.1.2               DBI_1.1.0
## [111] tweenr_1.0.1                 rappdirs_0.3.3
## [113] MASS_7.3-53                   Matrix_1.3-2
## [115] igraph_1.2.6                 pkgconfig_2.0.3
## [117] rvcheck_0.1.8                 plotly_4.9.3
## [119] xml2_1.3.2                   viper_0.4.5
## [121] qrng_0.2.1                   webshot_0.5.2
## [123] XVector_0.28.0              rvest_0.3.6
## [125] stringr_1.4.0               digest_0.6.27
## [127] sctransform_0.3.2            RcppAnnoy_0.0.18
## [129] spatstat.data_1.7-0          rmarkdown_2.6
## [131] leiden_0.3.6                 fastmatch_1.1-0
## [133] uwot_0.1.10                  edgeR_3.30.3
## [135] DelayedMatrixStats_1.10.1    curl_4.3
## [137] shiny_1.5.0                  lifecycle_0.2.0
## [139] nlme_3.1-151                 jsonlite_1.7.2
## [141] BiocNeighbors_1.6.0           viridisLite_0.3.0
## [143] limma_3.44.3                pillar_1.4.7
## [145] lattice_0.20-41              fastmap_1.1.0
## [147] httr_1.4.2                   survival_3.2-7
## [149] GO.db_3.11.4                glue_1.4.2
## [151] spatstat_1.64-1              png_0.1-7
## [153] bit_4.0.4                   ggforce_0.3.2
## [155] stringi_1.5.3               blob_1.2.1
## [157] BiocSingular_1.4.0            memoise_1.1.0
## [159] dplyr_1.0.2                  irlba_2.3.3
## [161] future.apply_1.7.0

```