

5-Scoring Endothelial cells

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Introduction

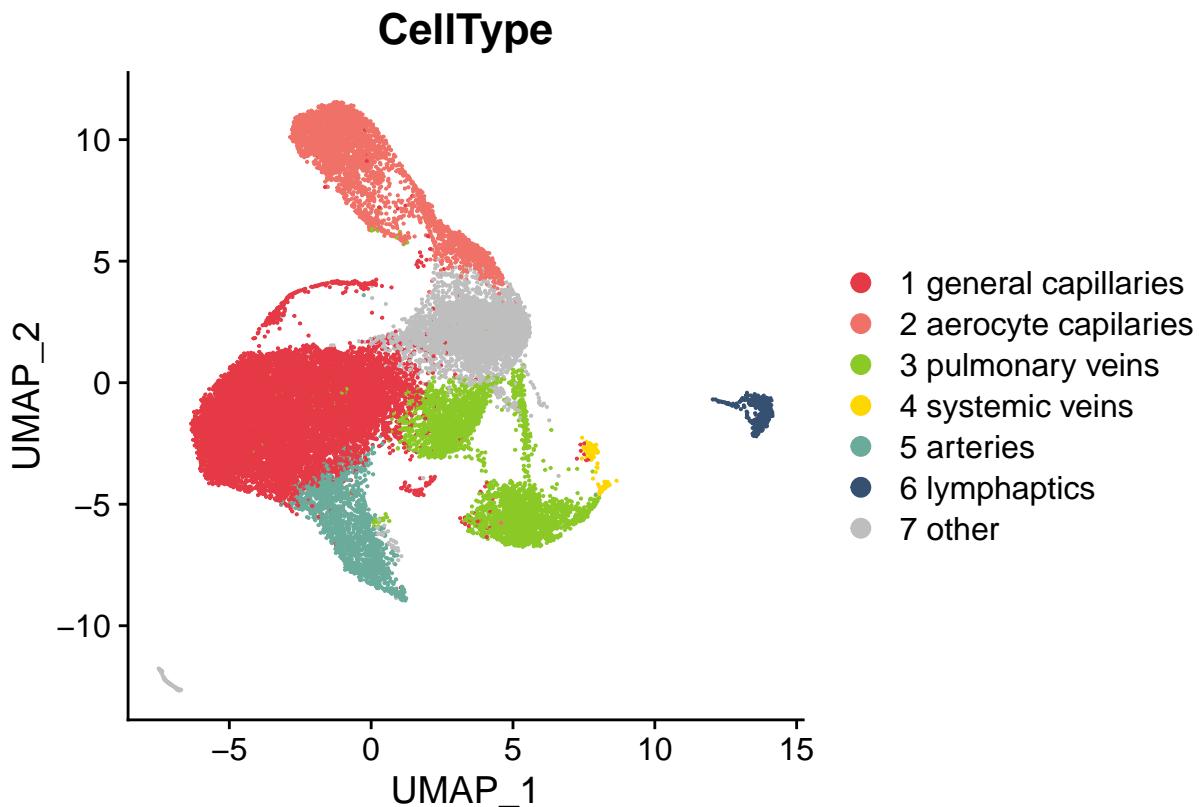
Gene signatures specific of transcriptional change in gCap were obtained by selecting the top 20 genes with the highest absolute log fold-change in each condition (control and MarNeu-targeted). The 2 signatures were then tested on Murine endothelial cells from different tissues. using the AddModuleScore function.

Loading packages

```
suppressMessages({  
  library(dplyr)  
  library(Seurat)  
  library(patchwork)  
  library(ggplot2)  
  library(formatR)  
  library(dittoSeq)  
  library(ggrepel)  
  library(xlsx)  
})
```

Loading Annotated cell

```
endothelial_cells <- readRDS("../02-Clustering_Endo/endothelial_cells_annotated.rds")  
  
color <- c("#E63946", "#F07167", "#8AC926", "#FFD700", "#6AAB9C",  
  "#355070", "gray")  
DimPlot(endothelial_cells, group.by = "CellType", cols = color)
```



Calculate gene signature for the PBS and Treated cells

```
endothelial_cells_subset <- subset(endothelial_cells, CellType %in%  
  "1 general capillaries")  
  
Markers.PBSTreated <- FindMarkers(endothelial_cells_subset, ident.1 = "PBS",  
  ident.2 = c("aCXCR2-D14", "aLy6G-D14"), min.pct = 0.25, group.by = "Condition")  
  
Markers.PBSTreated <- Markers.PBSTreated[order(Markers.PBSTreated$avg_log2FC,  
  decreasing = T), ]  
  
signature_PBS <- rownames(Markers.PBSTreated)[1:20]  
  
signature_treated <- tail(rownames(Markers.PBSTreated), 20)
```

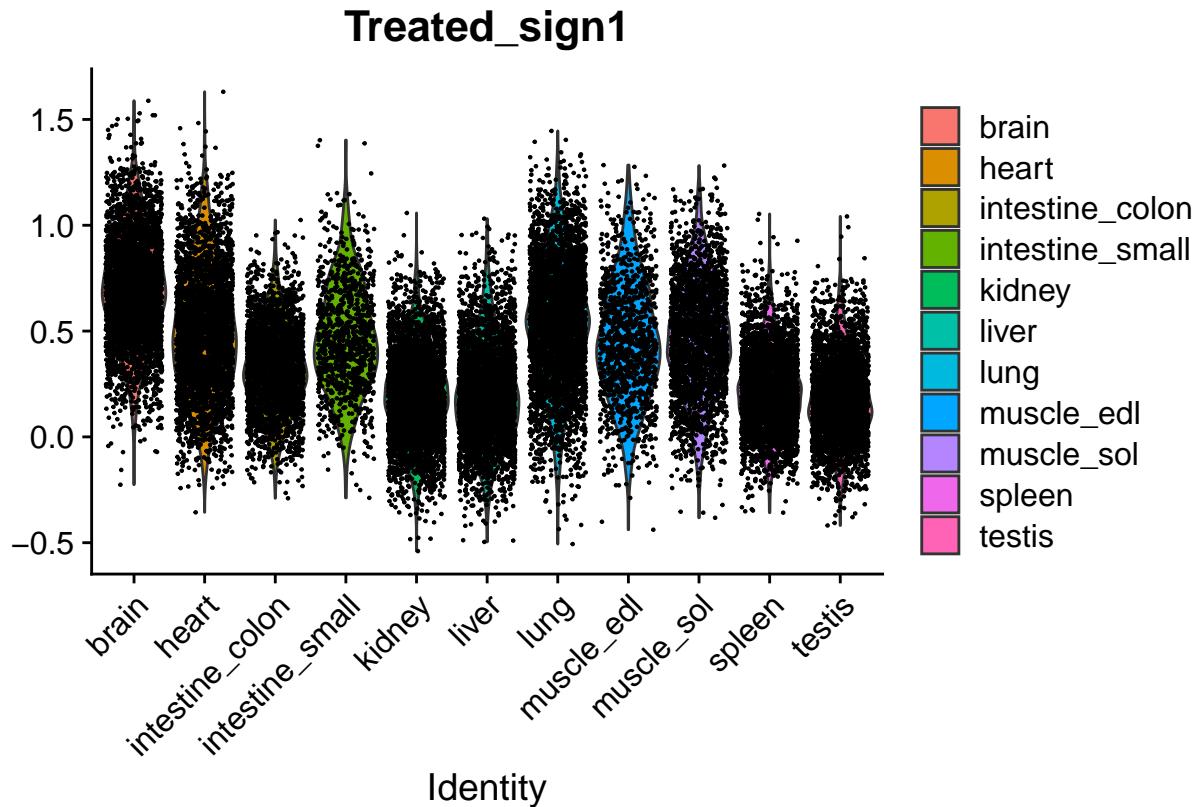
data from reference atlas

The raw counts and the metadata can be downloaded in https://endotheliomics.shinyapps.io/ec_atlas/

```
Count_matrix <- read.csv("Data_endoMuris/Data.csv")  
rownames(Count_matrix) <- Count_matrix[, 1]  
Count_matrix <- Count_matrix[, -1]  
  
metadata <- read.csv("Data_endoMuris/Metadata.csv")  
rownames(metadata) <- metadata$Observation  
  
endo_cells_atlas <- CreateSeuratObject(Count_matrix, project = "Murine Endothelial Cells",  
  meta.data = metadata)  
endo_cells_atlas <- AddMetaData(endo_cells_atlas, metadata)
```

Score Treated

```
endo_cells_atlas <- AddModuleScore(endo_cells_atlas, features = list(signature_treated),  
  name = "Treated_sign")  
  
VlnPlot(endo_cells_atlas, features = "Treated_sign1", group.by = "Tissue")
```



```
# ggsave('endo_atlas_treated.png', width = 12, height = 8)

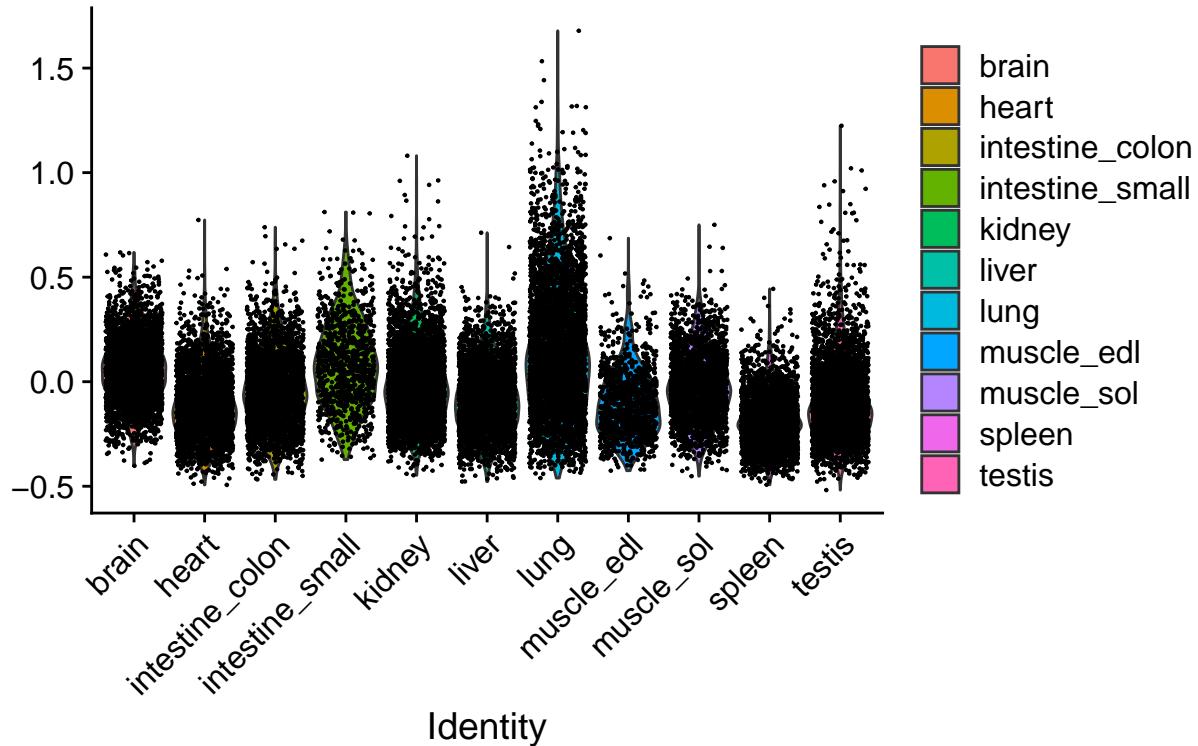
Treated_Score <- FetchData(endo_cells_atlas, vars = c("Treated_sign1",
  "Tissue"))
# write.xlsx(Treated_Score, 'Scoring_endo_treated.xlsx',
# rowNames = T)
```

Score PBS

```
endo_cells_atlas <- AddModuleScore(endo_cells_atlas, features = list(signature_PBS),
  name = "PBS_sign")

## Warning: The following features are not present in the object: Scgb1a1, not
## searching for symbol synonyms
VlnPlot(endo_cells_atlas, features = "PBS_sign1", group.by = "Tissue")
```

PBS_sign1



```
# ggsave('endo_atlas_PBS.png', width = 12, height = 8)

PBS_Score <- FetchData(endo_cells_atlas, vars = c("PBS_sign1", "Tissue"))
# write.xlsx(PBS_Score, 'Scoring_endo_PBS.xlsx', rowNames = T)
```

Endothelial cell scores were subsequently normalized and expressed relative to brain endothelial cells. The results were plotted into violin plot with prism.

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 24.04.2 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/libopenblas-p0.3.26.so;  LAPACK version 3.12.0
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8          LC_NUMERIC=C
## [3] LC_TIME=fr_BE.UTF-8          LC_COLLATE=en_US.UTF-8
## [5] LC_MONETARY=fr_BE.UTF-8       LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=fr_BE.UTF-8          LC_NAME=fr_BE.UTF-8
## [9] LC_ADDRESS=fr_BE.UTF-8         LC_TELEPHONE=fr_BE.UTF-8
## [11] LC_MEASUREMENT=fr_BE.UTF-8    LC_IDENTIFICATION=fr_BE.UTF-8
##
## time zone: Europe/Brussels
## tzcode source: system (glibc)
##
```

```

## attached base packages:
## [1] stats      graphics   grDevices utils     datasets   methods    base
##
## other attached packages:
## [1] xlsx_0.6.5        ggrepel_0.9.3      dittoSeq_1.12.0    formatR_1.14
## [5] ggplot2_3.4.2     patchwork_1.1.2    SeuratObject_4.1.3 Seurat_4.3.0
## [9] dplyr_1.1.2
##
## loaded via a namespace (and not attached):
##  [1] RColorBrewer_1.1-3      rstudioapi_0.14
##  [3] jsonlite_1.8.7          magrittr_2.0.3
##  [5] ggbeeswarm_0.7.2       spatstat.utils_3.0-3
##  [7] farver_2.1.1           rmarkdown_2.23
##  [9] zlibbioc_1.46.0        vctrs_0.6.3
## [11] ROOCR_1.0-11          spatstat.explore_3.2-1
## [13] RCurl_1.98-1.12        S4Arrays_1.2.1
## [15] htmltools_0.5.5        sctransform_0.3.5
## [17] parallelly_1.36.0      KernSmooth_2.23-22
## [19] htmlwidgets_1.6.2      ica_1.0-3
## [21] plyr_1.8.8             plotly_4.10.2
## [23] zoo_1.8-12            igraph_1.5.0.1
## [25] mime_0.12              lifecycle_1.0.3
## [27] pkgconfig_2.0.3         Matrix_1.6-1
## [29] R6_2.5.1               fastmap_1.1.1
## [31] GenomeInfoDbData_1.2.10 MatrixGenerics_1.12.2
## [33] fitdistrplus_1.1-11    future_1.33.0
## [35] shiny_1.7.4.1          digest_0.6.33
## [37] colorspace_2.1-0       S4Vectors_0.38.1
## [39] tensor_1.5             irlba_2.3.5.1
## [41] GenomicRanges_1.52.0    labeling_0.4.2
## [43] progressr_0.13.0       fansi_1.0.4
## [45] spatstat.sparse_3.0-2  httr_1.4.6
## [47] polyclip_1.10-4       abind_1.4-5
## [49] compiler_4.3.3         withr_2.5.0
## [51] highr_0.10             MASS_7.3-60.0.1
## [53] DelayedArray_0.26.3    tools_4.3.3
## [55] viper_0.4.5            lmtest_0.9-40
## [57] beeswarm_0.4.0          httpuv_1.6.11
## [59] future.apply_1.11.0     goftest_1.2-3
## [61] glue_1.6.2              nlme_3.1-164
## [63] promises_1.2.0.1        grid_4.3.3
## [65] Rtsne_0.16              cluster_2.1.6
## [67] reshape2_1.4.4          generics_0.1.3
## [69] gtable_0.3.3            spatstat.data_3.0-1
## [71] tidyverse_1.3.0          data.table_1.14.8
## [73] XVector_0.40.0          sp_2.2-0
## [75] utf8_1.2.3              BiocGenerics_0.46.0
## [77] spatstat.geom_3.2-4     RcppAnnoy_0.0.21
## [79] RANN_2.6.1              pillar_1.9.0
## [81] stringr_1.5.0            limma_3.56.2
## [83] spam_2.9-1              later_1.3.1
## [85] rJava_1.0-6              splines_4.3.3
## [87] lattice_0.22-5          survival_3.5-8
## [89] deldir_1.0-9            tidyselect_1.2.0

```

```
## [91] SingleCellExperiment_1.22.0 miniUI_0.1.1.1
## [93] pbapply_1.7-2 knitr_1.43
## [95] gridExtra_2.3 IRanges_2.34.0
## [97] SummarizedExperiment_1.30.2 scattermore_1.2
## [99] stats4_4.3.3 xfun_0.39
## [101] Biobase_2.60.0 matrixStats_1.0.0
## [103] pheatmap_1.0.12 stringi_1.8.4
## [105] lazyeval_0.2.2 yaml_2.3.7
## [107] xlsxjars_0.6.1 evaluate_0.21
## [109] codetools_0.2-19 tibble_3.2.1
## [111] cli_3.6.1 uwot_0.1.16
## [113] xtable_1.8-4 reticulate_1.30
## [115] munsell_0.5.0 Rcpp_1.0.11
## [117] GenomeInfoDb_1.36.0 globals_0.16.2
## [119] spatstat.random_3.1-5 png_0.1-8
## [121] ggrastr_1.0.2 parallel_4.3.3
## [123] ellipsis_0.3.2 dotCall64_1.0-2
## [125] bitops_1.0-7 listenv_0.9.0
## [127] viridisLite_0.4.2 scales_1.2.1
## [129] ggridges_0.5.4 crayon_1.5.2
## [131] leiden_0.4.3 purrrr_1.0.1
## [133] rlang_1.1.1 cowplot_1.1.1
```