FigureS4_tTF_expression_correlation

tTF expression and correlation rank plots at different axial identities of the nervous system

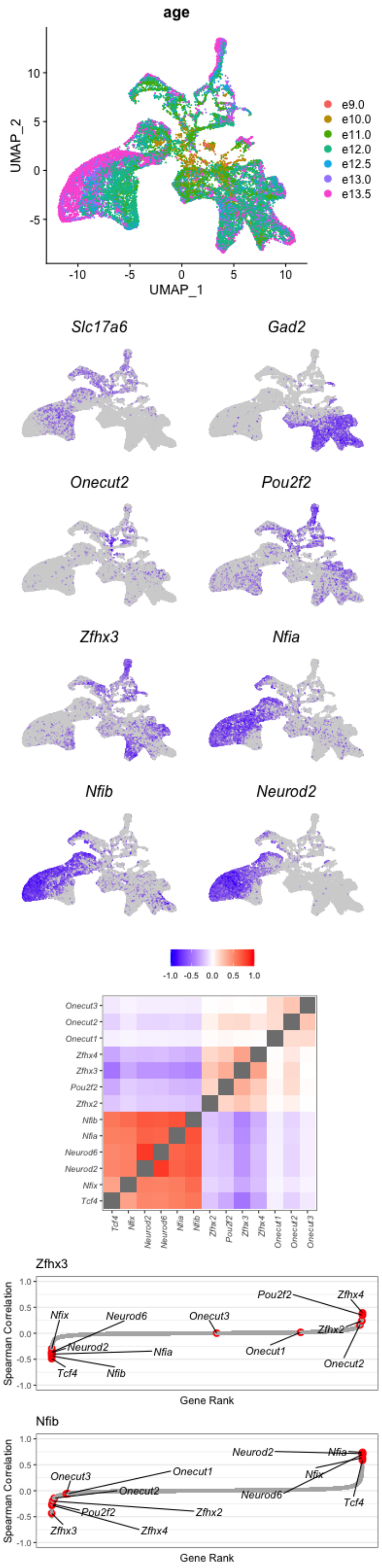
This documents details the steps for the generation of Figure S4 in Sagner et al. 2021.

```
## connect sc.loom file downloaded from mousebrain.org
sc.loom <- connect(filename = paste0(dir, "/input/dev_all.loom"), mode = "r+", skip.validate = TRUE)
```

```
## Warning in initialize(...): Skipping validation step, some fields are
## not populated
```

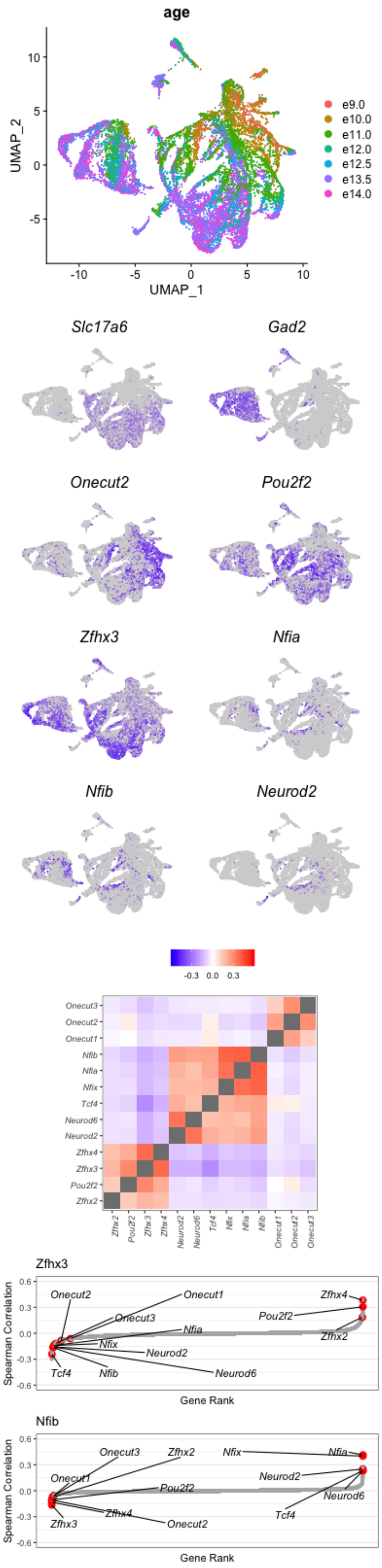
```
## Generate sc.meta file by extracting parameters from connected sc.loom file
sc.meta <- data.frame(
  sc.loom$col.attrs$Age[],
  sc.loom$col.attrs$PseudoAge[],
  sc.loom$col.attrs$Tissue[],
  sc.loom$col.attrs$PseudoTissue[],
  sc.loom$col.attrs$Class[],
  sc.loom$col.attrs$Clusters[],
  10000 / sc.loom$col.attrs$TotalUMI[],
  sc.loom$col.attrs$CellID[]
)

colnames(sc.meta) <- c("age", "pseudoage", "tissue", "pseudotissue", "class", "clusters", "normalizatio
```

```
dir <- "/Users/j76630as/Documents/tTF_paper_2020/scRNAseq/"
setwd(paste0(dir, "/output/"))

corr.mtx.fb <- readRDS("Correlation_matrix_forebrain_neurons.rds")

fb.umaps <- plot.brain.region2(
  tissue = "Forebrain",
  celltype = "Neuron",
  timepoints = c("e9.0", "e10.0", "e11.0", "e12.0", "e12.5", "e13.0", "e13.5", "e14.0"),
  umap.genes = c("Slc17a6", "Gad2", "Onecut2", "Pou2f2", "Zfhx3", "Nfia", "Nfib", "Neurod2"),
  correlation.mtx = corr.mtx.fb,
  correlation.genes = c("Zfhx3", "Nfib"),
  min = -1,
  max = 1,
  labels = c("A", "E", "I", "M")
)

fb.umaps
```


age
UMAP_2
UMAP_1
10
5
0
-5
-10
-5
0
5
10
e9.0
e10.0
e11.0
e12.0
e12.5
e13.0
e13.5
Slc17a6
Gad2
Onecut2
Pou2f2
Zfhx3
Nfia
Nfib
Neurod2
-1.0 -0.5 0.0 0.5 1.0
Onecut3
Onecut2
Onecut1
Zfhx4
Zfhx3
Pou2f2
Zfhx2
Nfib
Nfia
Neurod6
Neurod2
Nfix
Tcf4
Tcf4
Nfix
Neurod2
Neurod6
Nfia
Nfib
Zfhx2
Pou2f2
Zfhx3
Zfhx4
Onecut1
Onecut2
Onecut3
Zfhx3
Spearman Correlation
1.0
0.5
0.0
-0.5
-1.0
Nfix
Neurod6
Onecut3
Pou2f2
Zfhx4
Zfhx2
Neurod2
Nfia
Onecut1
Onecut2
Tcf4
Nfib
Gene Rank
Nfib
Spearman Correlation
1.0
0.5
0.0
-0.5
-1.0
Neurod2
Nfia
Onecut3
Onecut1
Nfix
Onecut2
Neurod6
Tcf4
Pou2f2
Zfhx2
Zfhx3
Zfhx4
Gene Rank

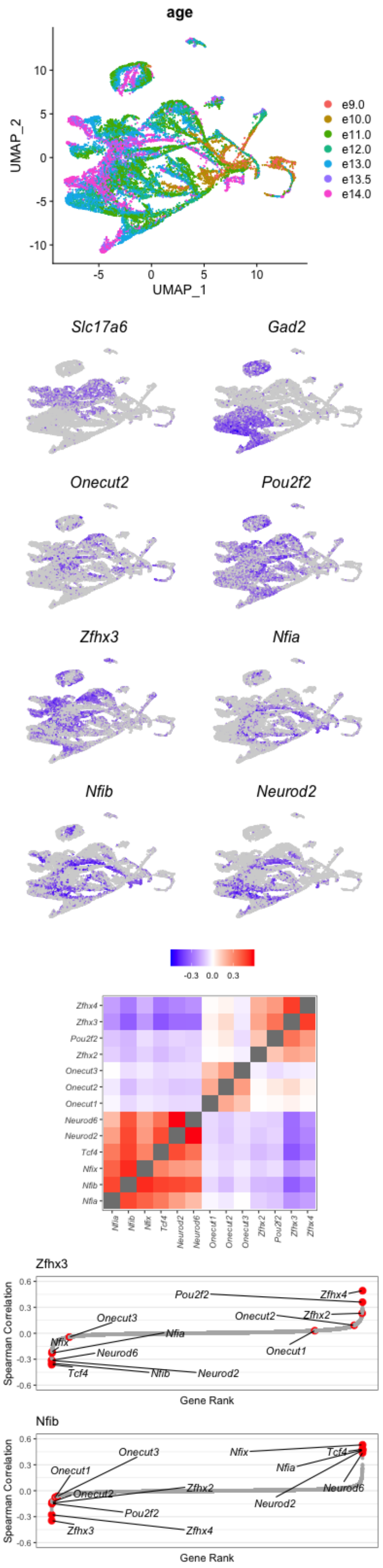
```
remove(corr.mtx.fb)
```

```
dir <- "/Users/j76630as/Documents/tTF_paper_2020/scRNAseq/"
setwd(paste0(dir, "/output/"))

corr.mtx.mb <- readRDS("Correlation_matrix_midbrain_neurons.rds")

mb.umaps <- plot.brain.region2(
  tissue = "Midbrain",
  celltype = "Neuron",
  timepoints = c("e9.0", "e10.0", "e11.0", "e12.0", "e12.5", "e13.0", "e13.5", "e14.0"),
  umap.genes = c("Slc17a6", "Gad2", "Onecut2", "Pou2f2", "Zfhx3", "Nfia", "Nfib", "Neurod2"),
  correlation.mtx = corr.mtx.mb,
  min = -0.6,
  max = 0.6,
  correlation.genes = c("Zfhx3", "Nfib"),
  labels = c("B", "F", "J", "N")
)

mb.umaps
```


age
UMAP_2
UMAP_1
10
5
0
-5
-10
e9.0
e10.0
e11.0
e12.0
e12.5
e13.5
e14.0
Slc17a6
Gad2
Onecut2
Pou2f2
Zfhx3
Nfia
Nfib
Neurod2
-0.3
0.0
0.3
Onecut3
Onecut2
Onecut1
Nfib
Nfia
Nfix
Tcf4
Neurod6
Neurod2
Zfhx4
Zfhx3
Pou2f2
Zfhx2
Zfhx3
Spearman Correlation
Gene Rank
0.6
0.3
0.0
-0.3
-0.6
Onecut2
Onecut1
Onecut3
Nfia
Nfix
Neurod2
Tcf4
Nfib
Neurod6
Zfhx4
Pou2f2
Zfhx2
Nfib
Onecut3
Zfhx2
Nfix
Nfia
Onecut1
Pou2f2
Neurod2
Neurod6
Zfhx4
Zfhx3
Onecut2
Tcf4

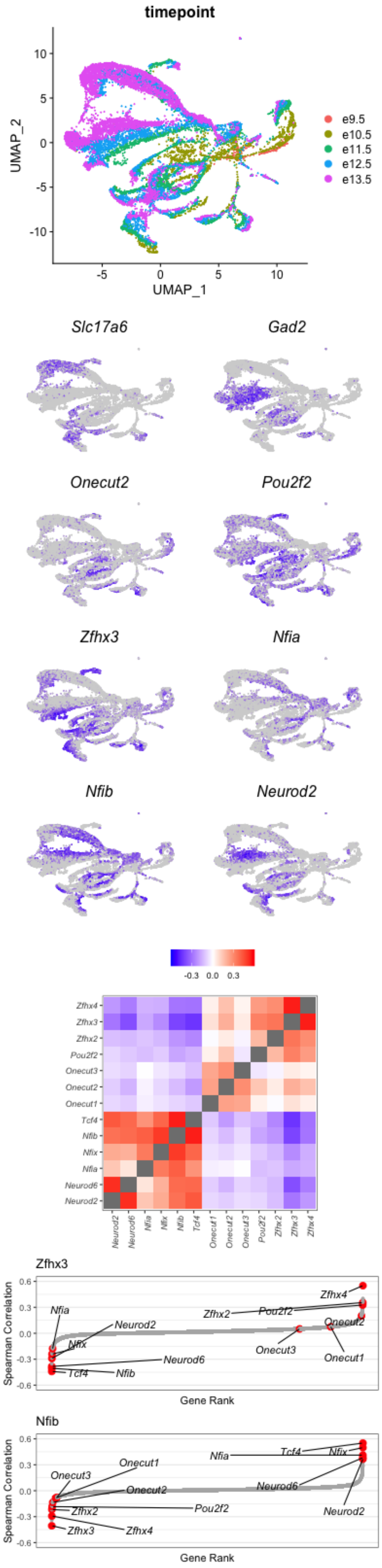
```
remove(corr.mtx.mb)
```

```
dir <- "/Users/j76630as/Documents/tTF_paper_2020/scRNAseq/"
setwd(paste0(dir, "/output/"))

corr.mtx.hb <- readRDS("Correlation_matrix_hindbrain_neurons.rds")

hb.umaps <- plot.brain.region2(
  tissue = "Hindbrain",
  celltype = "Neuron",
  timepoints = c("e9.0", "e10.0", "e11.0", "e12.0", "e12.5", "e13.0", "e13.5", "e14.0"),
  umap.genes = c("Slc17a6", "Gad2", "Onecut2", "Pou2f2", "Zfhx3", "Nfia", "Nfib", "Neurod2"),
  correlation.mtx = corr.mtx.hb,
  min = -0.6,
  max = 0.6,
  correlation.genes = c("Zfhx3", "Nfib"),
  labels = c("C", "G", "K", "O")
)

hb.umaps
```


age
UMAP_2
UMAP_1
10
5
0
-5
-10
-5
0
5
10
e9.0
e10.0
e11.0
e12.0
e13.0
e13.5
e14.0
Slc17a6
Gad2
Onecut2
Pou2f2
Zfhx3
Nfia
Nfib
Neurod2
-0.3
0.0
0.3
Zfhx4
Zfhx3
Pou2f2
Zfhx2
Onecut3
Onecut2
Onecut1
Neurod6
Neurod2
Tcf4
Nfix
Nfib
Nfia
Nfia
Nfib
Nfix
Tcf4
Neurod2
Neurod6
Onecut1
Onecut2
Onecut3
Zfhx2
Pou2f2
Zfhx3
Zfhx4
Zfhx3
Spearman Correlation
0.6
0.3
0.0
-0.3
-0.6
Pou2f2
Zfhx4
Onecut3
Onecut2
Zfhx2
Nfix
Nfia
Onecut1
Neurod6
Tcf4
Nfib
Neurod2
Gene Rank
Nfib
Spearman Correlation
0.6
0.3
0.0
-0.3
-0.6
Onecut3
Nfix
Tcf4
Onecut1
Nfia
Onecut2
Zfhx2
Neurod6
Neurod2
Pou2f2
Zfhx3
Zfhx4
Gene Rank

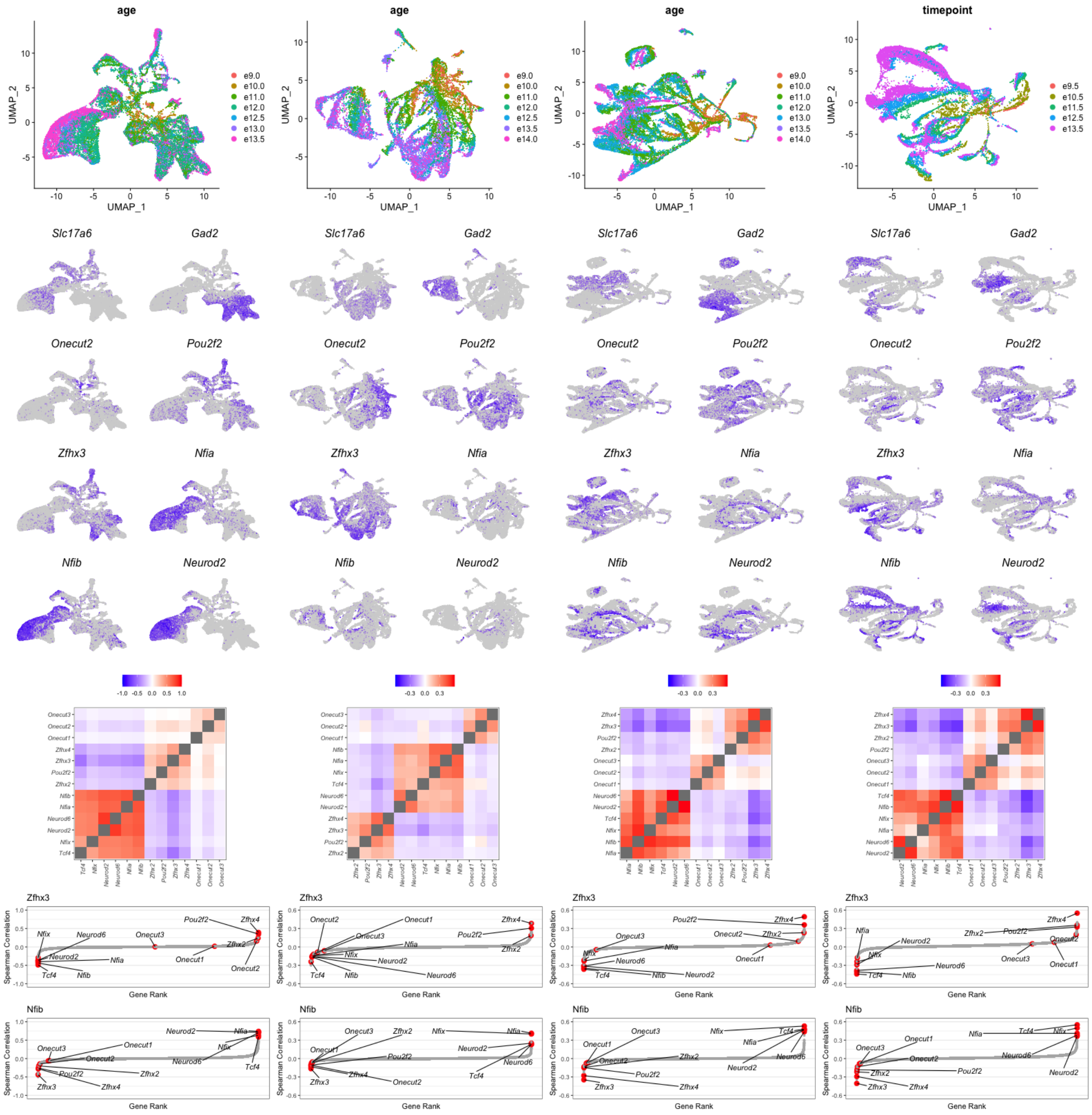
```
remove(corr.mtx.hb)
```

```
dir <- "/Users/j76630as/Documents/tTF_paper_2020/scRNAseq/"
setwd(paste0(dir, "/output/"))

corr.mtx.sc <- readRDS("Correlation_matrix_spinal_cord_neurons.rds")

## Load spinal cord scRNAseq data
eset <- readRDS(paste0(dir, "/input/m_neural.rds"))
eset <- eset$expressionSet

rownames(Biobase::pData(eset)) <- gsub("-", ".", rownames(Biobase::pData(eset)))
colnames(Biobase::exprs(eset)) <- gsub("-", ".", colnames(Biobase::exprs(eset)))

## functions for converting ensemblIDs into real gene names and vice versa

convert.to.ensemblID <- function(genes) {
  return(unlist(lapply(genes, function(x) {
    return(rownames(Biobase::fData(eset))[which(Biobase::fData(eset)[, "external_gene_name"] == x)])
  })))
}

convert.to.realname <- function(ensemblIDs, eset) {
  return(unlist(lapply(ensemblIDs, function(x) {
    return(Biobase::fData(eset)$external_gene_name[which(rownames(Biobase::fData(eset)) == x)])
  })))
}

## load data into the Seurat package
mat <- Biobase::exprs(eset)
rownames(mat) <- Biobase::fData(eset)[, "external_gene_name"]

seurat <- CreateSeuratObject(counts = mat, meta.data = Biobase::pData(eset), project = "MouseSpinalCord

seurat[["percent.mt"]] <- PercentageFeatureSet(seurat, pattern = "^mt-")

seurat <- seurat %>%
  subset(subset = nFeature_RNA > 600 & nFeature_RNA < 6000 & percent.mt < 6) %>%
  NormalizeData(verbose = FALSE) %>%
  ScaleData(verbose = FALSE) %>%
  FindVariableFeatures(selection.method = "vst", verbose = FALSE) %>%
  RunPCA(npcs = 30, verbose = FALSE) %>%
  RunUMAP(reduction = "pca", dims = 1:30)

sc.umaps <- plot.spinal.cord2(
  seurat.object = seurat,
  celltype = "Neuron",
  timepoints = c("e9.5", "e10.5", "e11.5", "e12.5", "e13.5"),
  umap.genes = c("Slc17a6", "Gad2", "Onecut2", "Pou2f2", "Zfhx3", "Nfia", "Nfib", "Neurod2"),
  correlation.mtx = corr.mtx.sc,
  min = -0.6,
  max = 0.6,
  correlation.genes = c("Zfhx3", "Nfib"),
  labels = c("D", "H", "L", "P")
)

sc.umaps
```


timepoint
UMAP_2
UMAP_1
10
5
0
-5
-10
-5
0
5
10
e9.5
e10.5
e11.5
e12.5
e13.5
Slc17a6
Gad2
Onecut2
Pou2f2
Zfhx3
Nfia
Nfib
Neurod2
-0.3
0.0
0.3
Zfhx4
Zfhx3
Zfhx2
Pou2f2
Onecut3
Onecut2
Onecut1
Tcf4
Nfib
Nfix
Nfia
Neurod6
Neurod2
Neurod2
Neurod6
Nfia
Nfix
Nfib
Tcf4
Onecut1
Onecut2
Onecut3
Pou2f2
Zfhx2
Zfhx3
Zfhx4
Zfhx3
Spearman Correlation
0.6
0.3
0.0
-0.3
-0.6
Nfia
Neurod2
Nfix
Neurod6
Tcf4
Nfib
Zfhx4
Zfhx2
Pou2f2
Onecut2
Onecut3
Onecut1
Gene Rank
Nfib
Spearman Correlation
0.6
0.3
0.0
-0.3
-0.6
Onecut1
Onecut3
Onecut2
Zfhx2
Pou2f2
Zfhx3
Zfhx4
Nfia
Tcf4
Nfix
Neurod6
Neurod2
Gene Rank

```
remove(corr.mtx.sc)
```

```
cowplot::plot_grid(fb.umaps, mb.umaps, hb.umaps, sc.umaps, ncol = 4)
```



Plot sessionInfo

```
sessionInfo()
```

```
## R version 4.0.4 (2021-02-15)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
## 
## 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_GB.UTF-8/en_GB.UTF-8/en_GB.UTF-8/C/en_GB.UTF-8/en_GB.UTF-8
## 
## attached base packages:
## [1] stats4    parallel  stats     graphics  grDevices utils    
## [7] datasets  methods   base     
## 
## other attached packages:
##  [1] ggrepel_0.9.1               plyr_1.8.6                 
##  [3] pbapply_1.4-3               scater_1.18.6              
##  [5] SingleCellExperiment_1.12.0 SummarizedExperiment_1.20.0
##  [7] GenomicRanges_1.42.0        GenomeInfoDb_1.26.7        
##  [9] IRanges_2.24.1              S4Vectors_0.28.1           
## [11] MatrixGenerics_1.2.1        matrixStats_0.58.0         
## [13] viridis_0.6.0               viridisLite_0.4.0          
## [15] tibble_3.1.1                ggplot2_3.3.3              
## [17] loomR_0.2.1.9000            hdf5r_1.3.2                
## [19] R6_2.5.0                    scales_1.1.1               
## [21] dplyr_1.0.5                 SeuratObject_4.0.0         
## [23] Seurat_4.0.1                slingshot_1.8.0            
## [25] princurve_2.1.6             Biobase_2.50.0             
## [27] BiocGenerics_0.36.1         knitr_1.32                 
## 
## loaded via a namespace (and not attached):
##   [1] backports_1.2.1          igraph_1.2.6            
##   [3] lazyeval_0.2.2           splines_4.0.4           
##   [5] BiocParallel_1.24.1      listenv_0.8.0           
##   [7] scattermore_0.7          digest_0.6.27           
##   [9] htmltools_0.5.1.1        fansi_0.4.2             
##  [11] memoise_2.0.0            magrittr_2.0.1          
##  [13] tensor_1.5               cluster_2.1.2           
##  [15] ROCR_1.0-11              globals_0.14.0          
##  [17] R.utils_2.10.1           spatstat.sparse_2.0-0   
##  [19] colorspace_2.0-0         blob_1.2.1              
##  [21] xfun_0.22                crayon_1.4.1            
##  [23] RCurl_1.98-1.3           jsonlite_1.7.2          
##  [25] spatstat.data_2.1-0      survival_3.2-10         
##  [27] zoo_1.8-9                ape_5.4-1               
##  [29] glue_1.4.2               polyclip_1.10-0         
##  [31] gtable_0.3.0             zlibbioc_1.36.0         
##  [33] XVector_0.30.0           leiden_0.3.7            
##  [35] DelayedArray_0.16.3      BiocSingular_1.6.0      
##  [37] R.cache_0.14.0           future.apply_1.7.0      
##  [39] abind_1.4-5              DBI_1.1.1               
##  [41] miniUI_0.1.1.1           Rcpp_1.0.6              
##  [43] xtable_1.8-4             reticulate_1.18         
##  [45] spatstat.core_2.1-2      rsvd_1.0.5              
##  [47] bit_4.0.4                 htmlwidgets_1.5.3
##  [49] httr_1.4.2                RColorBrewer_1.1-2
##  [51] ellipsis_0.3.1            ica_1.0-2
##  [53] farver_2.1.0              pkgconfig_2.0.3
##  [55] R.methodsS3_1.8.1         scuttle_1.0.4
##  [57] uwot_0.1.10               deldir_0.2-10
##  [59] utf8_1.2.1                AnnotationDbi_1.52.0
##  [61] labeling_0.4.2            tidyselect_1.1.1
##  [63] rlang_0.4.10              reshape2_1.4.4
##  [65] later_1.1.0.1             cachem_1.0.4
##  [67] munsell_0.5.0             tools_4.0.4
##  [69] RSQLite_2.2.6             generics_0.1.0
##  [71] ggridges_0.5.3            evaluate_0.14
##  [73] stringr_1.4.0             fastmap_1.1.0
##  [75] yaml_2.2.1                goftest_1.2-2
##  [77] bit64_4.0.5               fitdistrplus_1.1-3
##  [79] purrr_0.3.4               RANN_2.6.1
##  [81] sparseMatrixStats_1.2.1   future_1.21.0
##  [83] nlme_3.1-152              mime_0.10
##  [85] R.oo_1.24.0               compiler_4.0.4
##  [87] beeswarm_0.3.1            plotly_4.9.3
##  [89] png_0.1-7                 spatstat.utils_2.1-0
##  [91] stringi_1.5.3             highr_0.9
##  [93] RSpectra_0.16-0           lattice_0.20-41
##  [95] Matrix_1.3-2              styler_1.4.1
##  [97] vctrs_0.3.7               pillar_1.6.0
##  [99] lifecycle_1.0.0           spatstat.geom_2.1-0
## [101] lmtest_0.9-38             BiocNeighbors_1.8.2
## [103] RcppAnnoy_0.0.18          data.table_1.14.0
## [105] cowplot_1.1.1             bitops_1.0-6
## [107] irlba_2.3.3               httpuv_1.5.5
## [109] patchwork_1.1.1           promises_1.2.0.1
## [111] KernSmooth_2.23-18        gridExtra_2.3
## [113] vipor_0.4.5               parallelly_1.25.0
## [115] codetools_0.2-18          MASS_7.3-53.1
## [117] assertthat_0.2.1          withr_2.4.2
## [119] sctransform_0.3.2         GenomeInfoDbData_1.2.4
## [121] Antler_0.9.0              mgcv_1.8-35
## [123] beachmat_2.6.4            grid_4.0.4
## [125] rpart_4.1-15              tidyr_1.1.3
## [127] DelayedMatrixStats_1.12.3 rmarkdown_2.7
## [129] Rtsne_0.15                shiny_1.6.0
## [131] ggbeeswarm_0.6.0
```