

EMCN_Manuscript_Adult_WT_Choroid

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2024-01-11

First we open the dataset saved in “EMCN_manuscript_data_analysis_2-1-24.Rmd”

```
full.dataset <- readRDS("./WT_8_WT_dataset_for_EMN_MS_2-1-24.rds")
DefaultAssay(full.dataset) <- 'RNA'
```

Now the clusters were identified

```
markers.p56 <- FindAllMarkers(full.dataset, assay = "RNA", test.use = "MAST", logfc.threshold = 1, min
```



```
## Calculating cluster 1
```



```
##
```

```
## Done!
```



```
## Combining coefficients and standard errors
```



```
## Calculating log-fold changes
```



```
## Calculating likelihood ratio tests
```



```
## Refitting on reduced model...
```



```
##
```

```
## Done!
```



```
## Calculating cluster 2
```



```
##
```

```
## Done!
```



```
## Combining coefficients and standard errors
```



```
## Calculating log-fold changes
```



```
## Calculating likelihood ratio tests
```



```
## Refitting on reduced model...
```

```
##  
## Done!  
  
## Calculating cluster 3  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 4  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 5  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...
```

```
##  
## Done!  
  
## Calculating cluster 6  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 7  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 8  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...
```

```
##  
## Done!  
  
## Calculating cluster 9  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 10  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 11  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...
```

```
##  
## Done!  
  
## Calculating cluster 12  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 13  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 14  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...
```

```
##  
## Done!  
  
## Calculating cluster 15  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 16  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 17  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...
```

```
##  
## Done!  
  
## Calculating cluster 18  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 19  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 20  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...
```

```
##  
## Done!  
  
## Calculating cluster 21  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 22  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 23  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...
```

```
##  
## Done!  
  
## Calculating cluster 24  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 25  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...  
  
##  
## Done!  
  
## Calculating cluster 26  
  
##  
## Done!  
  
## Combining coefficients and standard errors  
  
## Calculating log-fold changes  
  
## Calculating likelihood ratio tests  
  
## Refitting on reduced model...
```

```

##  

## Done!

## Calculating cluster 27

##  

## Done!

## Combining coefficients and standard errors

## Calculating log-fold changes

## Calculating likelihood ratio tests

## Refitting on reduced model...

##  

## Done!

## Calculating cluster 28

##  

## Done!

## Combining coefficients and standard errors

## Calculating log-fold changes

## Calculating likelihood ratio tests

## Refitting on reduced model...

##  

## Done!

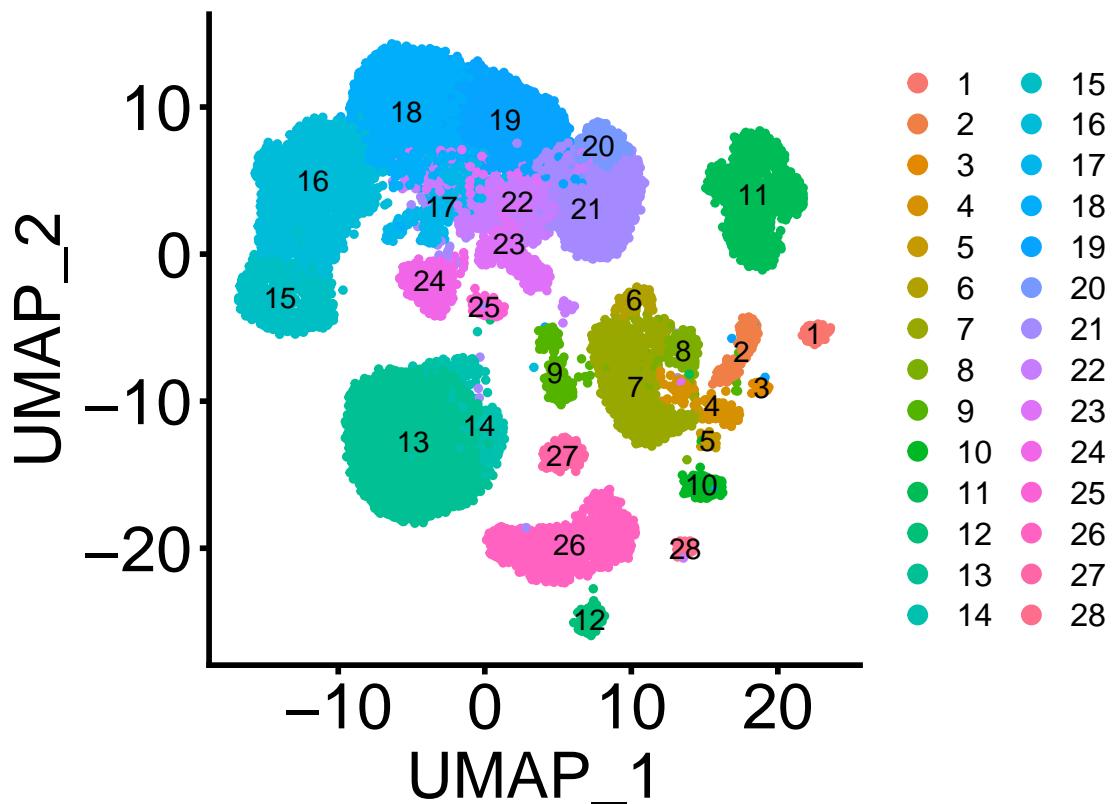
write.csv(markers.p56, file = "./P56_markers_all_2-11-24.csv")

DimPlot(object = full.dataset, reduction = "umap", label = TRUE, pt.size = 1,
        label.size = 4) & coord_fixed() & theme(aspect.ratio=1) &
        theme(plot.title = element_text(size = 26, face = "italic")) &
        theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(
        theme(axis.title = element_text(size = 24), axis.text = element_text(size = 24))

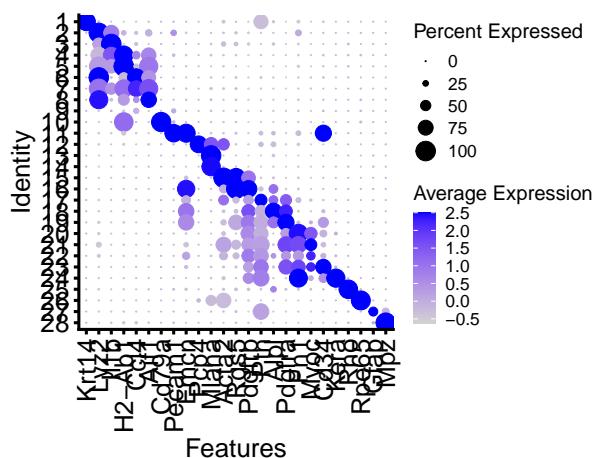
## Warning: The 'size' argument of 'element_line()' is deprecated as of ggplot2 3.4.0.  

## i Please use the 'linewidth' argument instead.

```



```
DotPlot(full.dataset, features = c("Krt14", "Lyz2", "Il1b", "H2-Ab1", "Ccl4", "Aif1", "Cd79a", "Pecam1",
                                    "Myoc", "Cd34", "Kera", "Rho", "Rpe65", "Gfap", "Mpz")) + theme(
  theme(aspect.ratio=1) &
    theme(plot.title = element_text(size = 24, face = "italic")) &
    theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(
      color="black", linewidth = 1)) &
    theme(axis.title = element_text(size = 18), axis.text = element_text(size = 18)))
```



First, we merge clusters to generate the final clusters for figure 2.

```

full.dataset <- RenameIds(object = full.dataset,
                           '1' = "Cor. Epi",
                           '2' = "Mono/Mac",
                           '3' = "Mono/Mac",
                           '4' = "Mono/Mac",
                           '5' = "Mono/Mac",
                           '6' = "Mono/Mac",
                           '7' = "Mono/Mac",
                           '8' = "Mono/Mac",
                           '9' = "T/NK",
                           '10' = "B cell",
                           '11' = "Endos",
                           '12' = "Ciliary Epithelium",
                           '13' = "melanocyte",
                           '14' = "melanocyte",
                           '15' = "vSMC",
                           '16' = "Pericyte",
                           '17' = "Stroma",
                           '18' = "Stroma",
                           '19' = "Stroma",
                           '20' = "Stroma",
                           '21' = "Stroma",
                           '22' = "Stroma",
                           '23' = "Sclera",
                           '24' = "Cor. Stroma",
                           '25' = "Rod",
                           '26' = "RPE",
                           '27' = "Astrocyte",
                           '28' = "Schwann"
)
saveRDS(full.dataset, "./full-dataset-annotated-3-21-24.rds")

```

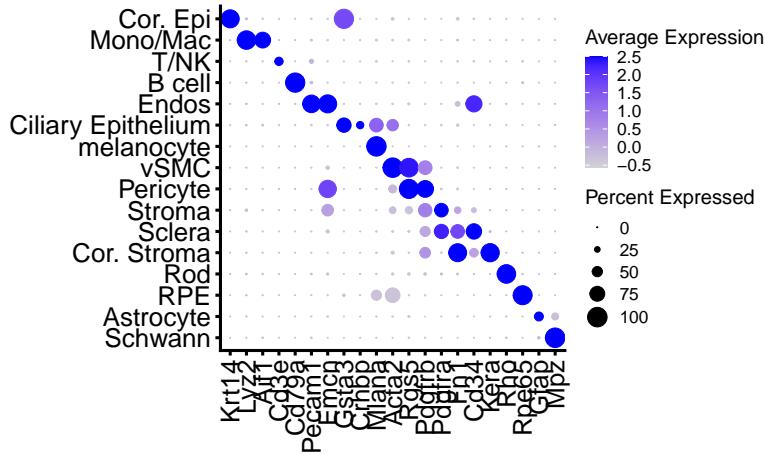
Dot plot of classical markers, streamlined

```

P1 <- DotPlot(full.dataset, features = c("Krt14", "Lyz2", "Aif1", "Cd3e", "Cd79a", "Pecam1", "Emcn",
                                         "Gsta3", "Crhbp", "Mlana", "Acta2", "Rgs5", "Pdgfrb", "Pdgfra",
                                         "Cd34", "Kera", "Rho", "Rpe65", "Gfap", "Mpz")) + theme(axis.text.x =
theme(aspect.ratio=1) &
  theme(plot.title = element_text(size = 24, face = "italic")) &
  theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line
  theme(axis.title = element_blank(), axis.text = element_text(size = 18))

```

P1



```
pdf(file = "./figures/Figure_2_identity-dots.pdf", width = 11, height = 6)
plot(P1)
dev.off()
```

```
## pdf
## 2

P1 <- DimPlot(object = full.dataset, reduction = "umap", label = F, pt.size = 1,
              label.size = 4) & coord_fixed() & theme(aspect.ratio=1) &
              theme(plot.title = element_text(size = 26, face = "italic")) &
              theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(
                color="black", linewidth = 1)) &
              theme(axis.title = element_text(size = 24), axis.text = element_text(size = 24))

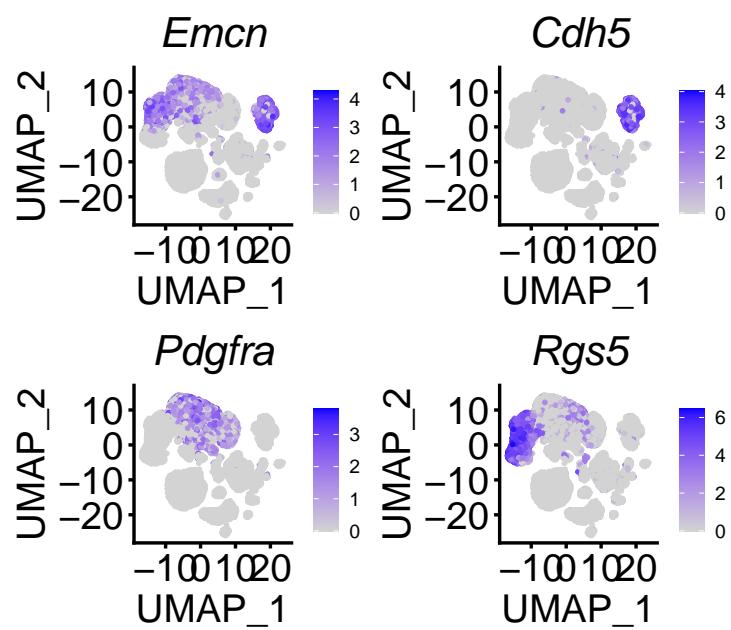
pdf(file = "./figures/Figure_2_UMAP.pdf", width = 6, height = 6)
plot(P1)
dev.off()
```

```
## pdf
## 2
```

Now plot endomucin compared with other markers

```
P1 <- FeaturePlot(full.dataset, features = c("Emcn", "Cdh5", "Pdgfra", "Rgs5"), ncol = 2, pt.size = 1) &
              theme(aspect.ratio=1) &
              theme(plot.title = element_text(size = 26, face = "italic")) &
              theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(
                color="black", linewidth = 1)) &
              theme(axis.title = element_text(size = 24), axis.text = element_text(size = 24))
```

```
P1
```



```

pdf(file = "./figures/Figure_2_b.pdf", width = 10, height = 10)
plot(P1)
dev.off()

```

```

## pdf
## 2

```

To determine if the PGDFrB+ EMCN cells are doublets, we looked for expression of other endothelial markers

```

P1 <- FeaturePlot(full.dataset, features = c("Emcn", "Kdr", "Pecam1", "Tek", "Podxl", "Plvap"), ncol = 2
  theme(aspect.ratio=1) &
  theme(plot.title = element_text(size = 26, face = "italic")) &
  theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(size = 1)) &
  theme(axis.title = element_text(size = 24), axis.text = element_text(size = 24))

```

```

pdf(file = "./figures/Sup figure 1.pdf", width = 12, height = 12)
plot(P1)
dev.off()

```

```

## pdf
## 2

```

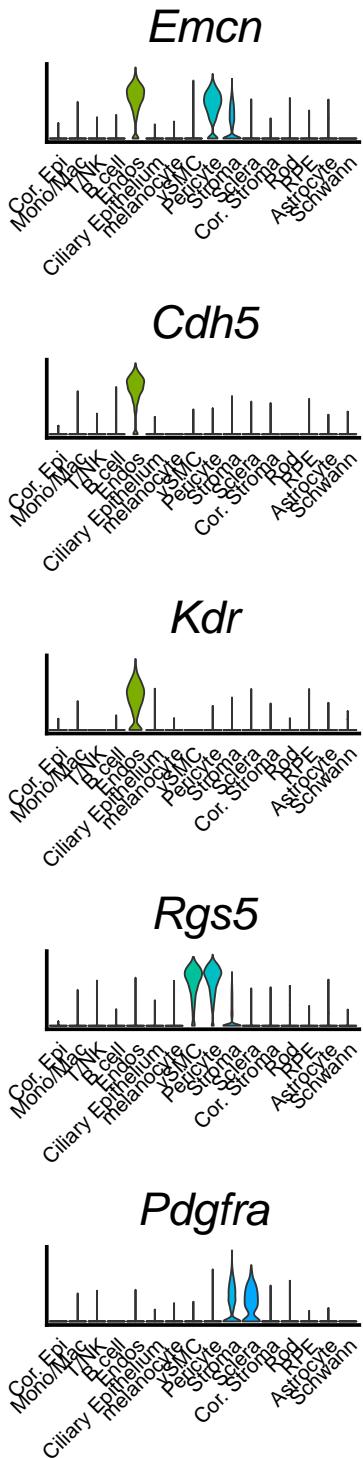
Violin plots

```

P1 <- VlnPlot(full.dataset, features = c("Emcn", "Cdh5", "Kdr", "Rgs5", "Pdgfra"), pt.size = 0, ncol = 2
  NoLegend() &
  theme(aspect.ratio = 0.25) &
  theme(axis.line = element_line(size = 1)) &
  theme(axis.ticks.y = element_blank(), axis.ticks.x = element_blank()) &
  theme(title = element_blank()) &
  theme(axis.title = element_blank(), axis.text = element_text(size = 12),
        axis.title.y = element_blank(), axis.text.y = element_blank()) &
  theme(plot.title = element_text(size = 26, face = "italic")))

```

P1



```

#
# pdf(file = "./figures/Figure_2_vlns.pdf", width = 6, height = 18)
# plot(P1)
# dev.off()

```

Clusters 23 and 24 represent corneal stroma.

15 and 16 vSMCs and pericytes. EMCN seems to be expressed only in pericytes
 Clusters 17-22 are PDGFRA+ stroma cells, clusters 17-19 are EMCN+. How are these cells different?
 First, the stromal clusters were isolated and re-clustered

```

stroma.subset <- subset(full.dataset, idents = c("Stroma"))
DefaultAssay(stroma.subset) <- "integrated"

stroma.subset <- FindVariableFeatures(stroma.subset)

## Warning in FindVariableFeatures.Assay(object = assay.data, selection.method =
## selection.method, : selection.method set to 'vst' but count slot is empty; will
## use data slot instead

## Warning in eval(predvars, data, env): NaNs produced

## Warning in hvf.info$variance.expected[not.const] <- 10^fit$fitted: number of
## items to replace is not a multiple of replacement length

stroma.subset <- RunPCA(stroma.subset, npcs = 50)

## PC_ 1
## Positive: Phlda1, Sparcl1, Adamts1, Ndufs2, Isg15, Junb, Pde4b, Ifit3, Adamts4, Alpl
##     Parp14, Tmem140, Ucp2, Arrdc3, Gbp2, Crem, Cystm1, Iigp1, Gpc3, Nr2f1
##     Phf11d, Ifi47, Rtp4, Ifit1, Cfh, Ifih1, Col23a1, Oasl2, Emcn, Kcnj8
## Negative: Ecrg4, Col12a1, Fmod, Itgb1, Col8a2, Chad, Clec11a, Tnmd, Chil1, Ptgis
##     Penk, Igfbp6, Prelp, Fndc1, Col6a3, Serpinf1, Gas6, Tgfb3, Igfbp2, Omd
##     Comp, Col11a1, Ogn, Clu, Cyp2f2, Tenm3, Sfrp2, Npr3, Htra1, Fbln5
## PC_ 2
## Positive: Ifit1, Gfpt2, Serpine2, Alpl, Ptgs2, Gpc3, Samhd1, Lum, Cyp1b1, Isg15
##     Pdgfra, Igfbp4, Errfi1, Cxcl12, Lsamp, Cygb, Medag, Ugdh, Ifit3, Edn3
##     Rnf213, Cebpd, Oasl2, Zfp3611, Cdhh1, Metrnl, Angpt1, Kitl, Igfbp5, Smoc1
## Negative: Mgll, Vamp8, Cav1, Ptgds, Nceh1, Chchd10, Ninj1, Cox8a, Gpnmb, Utrn
##     Slc24a5, Uqcr11, Efhd2, Scp2, Pmel, Psmd8, Cav2, 5031439G07Rik, Vegfb, Coro1c
##     Msn, Cobll1, Tmem189, Tecpr1, Vamp5, Trpm1, Ostf1, Dhrs3, Ctsd, Nrcam
## PC_ 3
## Positive: Cilp2, Matn4, Angpt17, D030045P18Rik, Mmp3, Cnmd, Sfrp2, Pcolce2, Mmp2, Apod
##     Abi3bp, Sema3a, Ctsk, Cybrd1, Smoc2, Htra1, Oeop, Ptprd, Ccn1, Col1a1
##     Fam180a, Fos, Cpxm2, Cdkn1c, Sparc, Igfbp2, Cyp2f2, Sybu, Cldn10, Egfl6
## Negative: Tnfrsf11b, Hmcn1, Adamts13, Atp8b1, Npr3, Rgs5, Pakap.1, Lmcd1, Gdnf, Stmn2
##     Gdf10, Dpysl3, Tm4sf1, Ank, Cdo1, Phldb2, Ntrk3, Bpgm, Arl6ip5, Anxa3
##     Dclk1, Ctnnal1, Crim1, Slc6a6, Fndc1, Slc12a2, Tagln, Fosl1, Slc39a14, Prss35
## PC_ 4
## Positive: Srxn1, Mthfd2, Eif4ebp1, Mat2a, Fgf2, Fosl1, Pakap.1, Fst, Plaur, Ngf
##     Tnfsf15, Fn1, Psph, Eprs, Igfbp2, Il11, Hspa4, Loxl2, Ell2, Creb3l1
##     Oaf, Phldb1, Lox, Pmepa1, Gdnf, Mir100hg, Ptx3, Gjb3, Flnc, Rnf213
## Negative: Klf2, Fos, Pgf, Hspa1a, Hspa1b, Cygb, Jun, Soc3, Egr1, Ctxn3
##     Pdlim2, Ntn1, Ramp2, Dbp, Ppp1r15a, Kazald1, Angpt1, Fosb, Hs3st3a1, Edn3
##     Zfp3612, Btg2, Atf3, Mfap5, C1qtnf7, Fgfr3, Rhob, Gm973, Tmem119, Ndnf
## PC_ 5
## Positive: Glis1, Dner, Tfap2b, Lrp1b, Ltbp1, Col23a1, Itga8, Rspo2, Rtn1, Xist
##     A2m, Zfp503, Syn2, Sncaip, Col4a5, Apoe, Tagln, Rerg, Chst2, Vegfa

```

```

##      Prrx1, Crlf1, Nr2f1, Myh10, Ctsc, Hs3st3a1, Csf3, Kctd1, Ccdc3, Dnajb4
## Negative: Xdh, Slfn8, Hhip, Ifit1, Igtp, Fmo1, Eif2ak2, Irgm1, Cd34, Samd91
##      Trim25, Mfap5, Timp1, Irgm2, Rnf213, Igfbp6, Map1b, Rsad2, Sema3d, Smoc2
##      Slfn9, Slc39a14, Oasl2, Egfr, Slfn5, Ifih1, Cmpk2, Ifit3, Ddx3y, Tm4sf1

stroma.subset <- RunUMAP(object = stroma.subset, dims = 1:10,
                           min.dist = 0.5, n.neighbors = 800, verbose = FALSE, assay = "integrated")

## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and metric to 'correlation'
## This message will be shown once per session

stroma.subset <- FindNeighbors(object = stroma.subset, dims = 1:10)

## Computing nearest neighbor graph

## Computing SNN

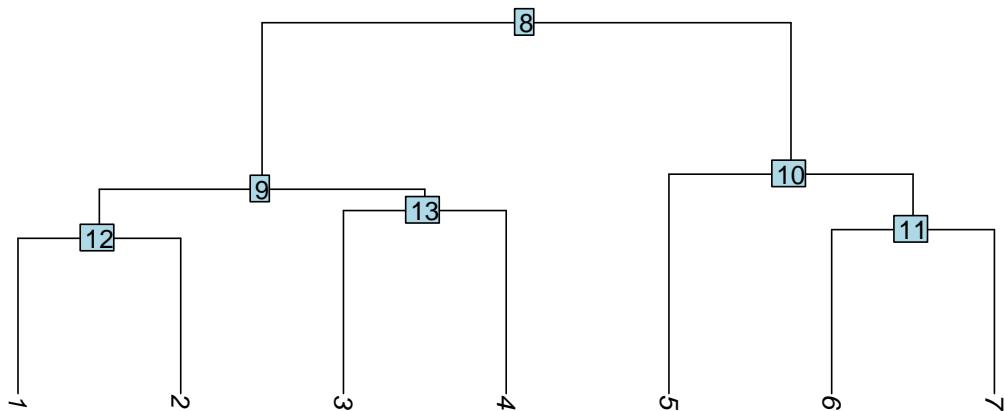
stroma.subset <- FindClusters(object = stroma.subset, resolution = 0.4, verbose = FALSE)

#Reorder cluster identities based on cluster tree
stroma.subset <- BuildClusterTree(stroma.subset, reorder.numeric = TRUE, reorder = TRUE,
                                    verbose = T, assay = "full.dataset", dims = 1:10)

## Reordering identity classes and rebuilding tree

PlotClusterTree(stroma.subset)

```



```

saveRDS(stroma.subset, "./EMCN-stroma-2-21-24.rds")

pdf(file = "./figures/Figure_3_tree.pdf", width = 10, height = 5)
PlotClusterTree(stroma.subset)
dev.off()

## pdf
## 2

DefaultAssay(stroma.subset) <- "RNA"

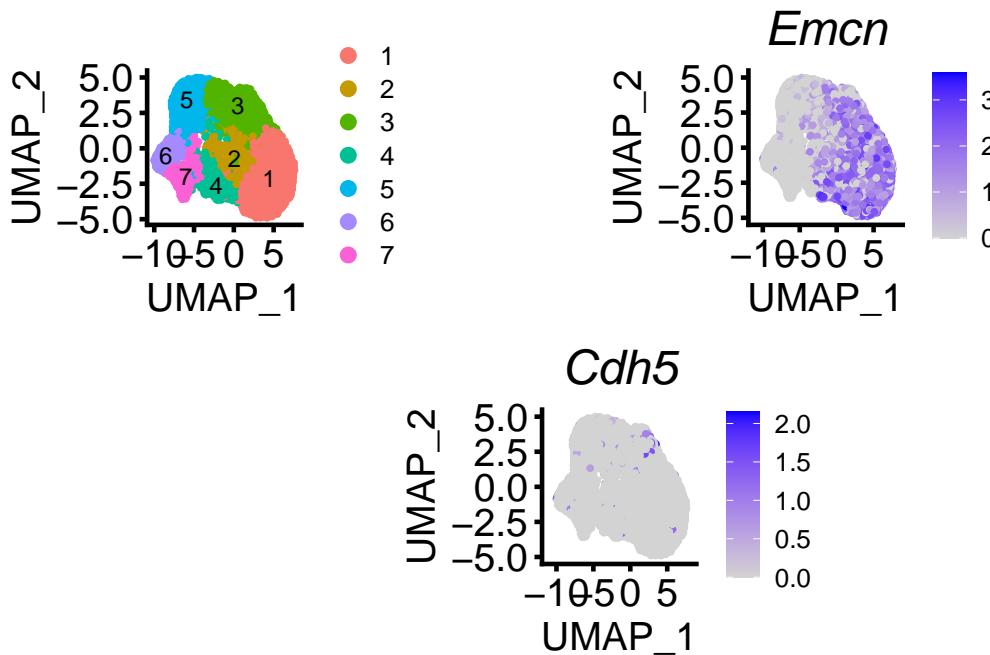
#Plot of cluster identities mapped on full dataset
P1 <- DimPlot(object = stroma.subset, reduction = "umap", label = TRUE, pt.size = 1,
               label.size = 4) & coord_fixed() & theme(aspect.ratio=1) &
               theme(plot.title = element_text(size = 22, face = "italic")) &
               theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(
               theme(axis.title = element_text(size = 18), axis.text = element_text(size = 18))

P2 <- FeaturePlot(stroma.subset, features = "Emcn", pt.size = 1) & coord_fixed() & theme(aspect.rat
               theme(plot.title = element_text(size = 22, face = "italic")) &
               theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(
               theme(axis.title = element_text(size = 18), axis.text = element_text(size = 18))

P3 <- FeaturePlot(stroma.subset, features = "Cdh5", pt.size = 1) & coord_fixed() & theme(aspect.rat
               theme(plot.title = element_text(size = 22, face = "italic")) &
               theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(
               theme(axis.title = element_text(size = 18), axis.text = element_text(size = 18))

(P1 + P2) / P3

```



```

pdf(file = "./figures/Figure_3_a.pdf", width = 10, height = 5)
plot(P1 + P2 + P3)
dev.off()

```

```

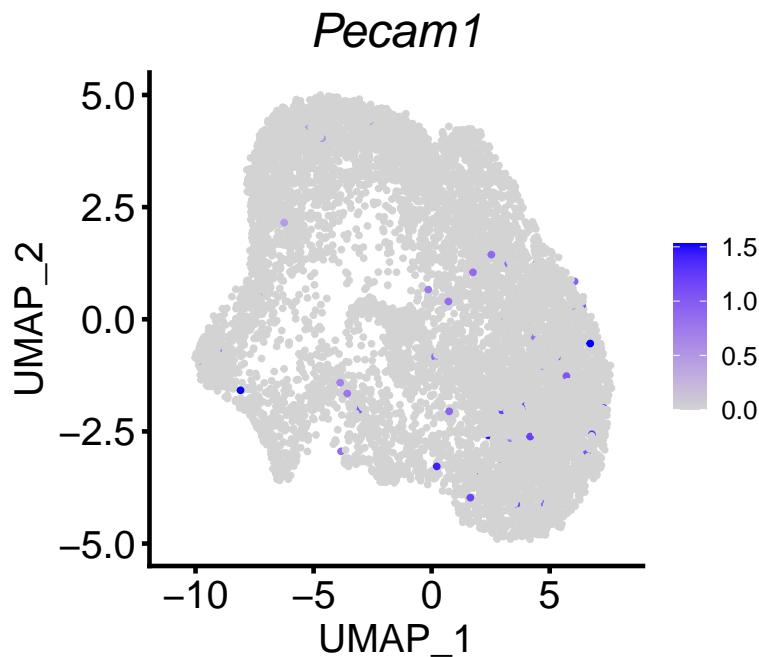
## pdf
## 2

```

```

FeaturePlot(stroma_subset, features = "Pecam1", pt.size = 1) & coord_fixed() & theme(aspect.ratio=1) &
  theme(plot.title = element_text(size = 22, face = "italic")) &
  theme(axis.line = element_line(color="black", linewidth = 1), axis.ticks = element_line(),
  theme(axis.title = element_text(size = 18), axis.text = element_text(size = 18))

```



```

write.csv(FindAllMarkers(stroma_subset, only.pos = T, logfc.threshold = 1), file = "./All_markers_stromal.csv")

```

```

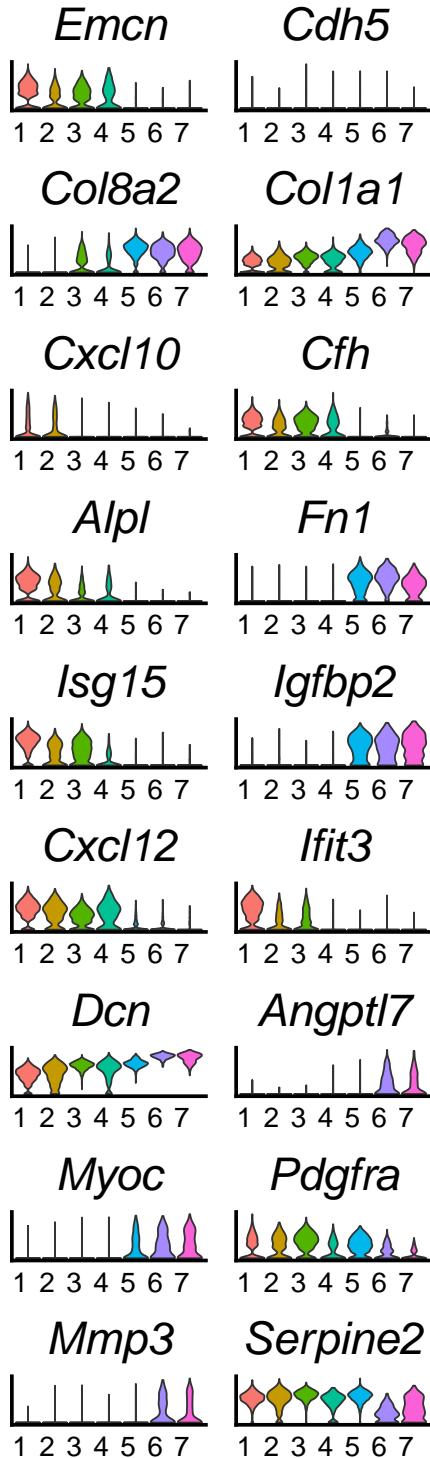
## Calculating cluster 1
## Calculating cluster 2
## Calculating cluster 3
## Calculating cluster 4
## Calculating cluster 5
## Calculating cluster 6
## Calculating cluster 7

```

Violin plots

```
P1 <- VlnPlot(stroma.subset, features = c("Emcn", "Cdh5", "Col8a2", "Col1a1",
                                         "Cxcl10", "Cfh", "Alpl", "Fn1", "Isg15",
                                         "Igfbp2", "Cxcl12", "Ifit3", "Dcn", "Angptl7",
                                         "Myoc", "Pdgfra", "Mmp3", "Serpine2"),
                           pt.size = 0, ncol = 2, assay = "RNA") &
  NoLegend() &
  theme(aspect.ratio = 0.25) &
  theme(axis.line = element_line(size = 1)) &
  theme(axis.ticks.y = element_blank(), axis.ticks.x = element_blank()) &
  theme(title = element_blank()) &
  theme(axis.title = element_blank(), axis.text = element_text(size = 16),
        axis.title.y = element_blank(), axis.text.y = element_blank(),
        axis.text.x = element_text(angle = 0)) &
  theme(plot.title = element_text(size = 26, face = "italic"))
```

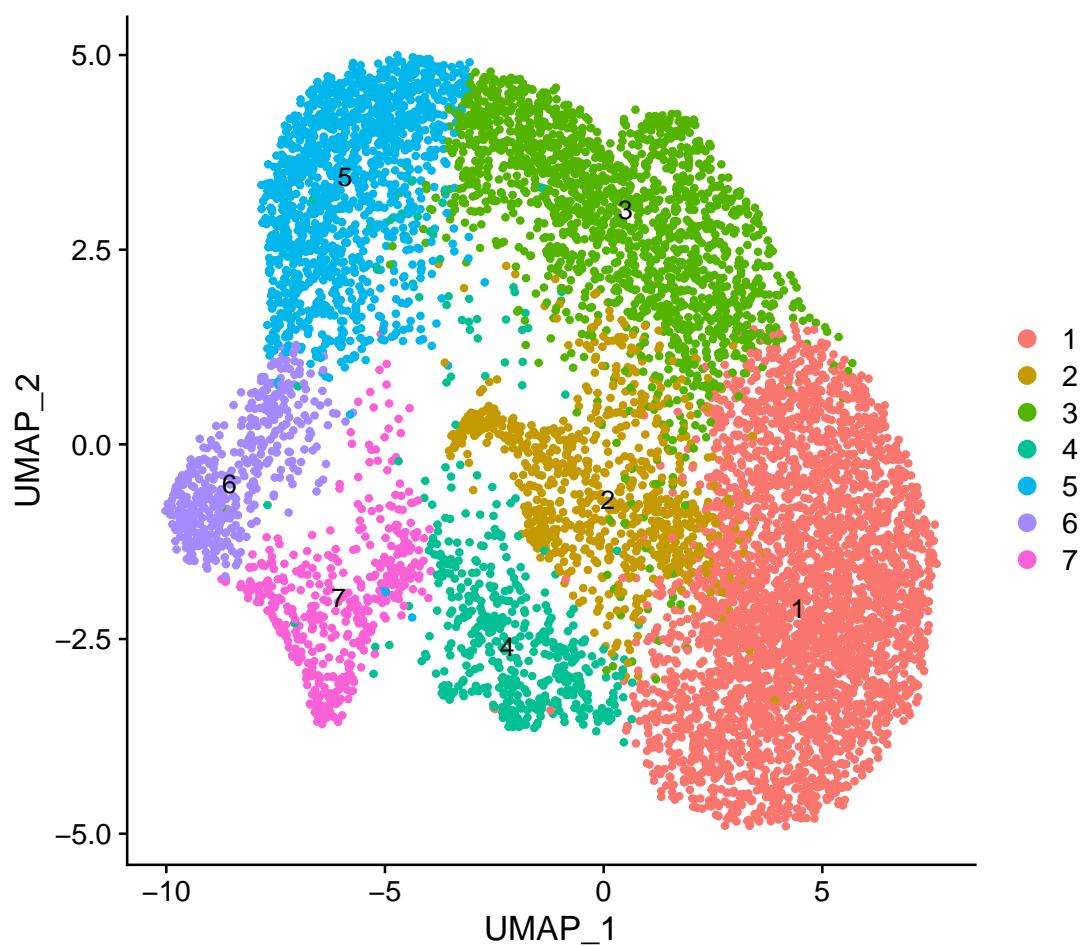
P1



```
pdf(file = "./figures/Figure_3_b.pdf", width = 6, height = 15)
plot(P1)
dev.off()
```

```
## pdf
## 2
```

```
DimPlot(object = stroma.subset, reduction = "umap", label = TRUE, pt.size = 1,  
        label.size = 4) + coord_fixed() + theme(aspect.ratio=1)
```



```

stroma.renamed <- stroma.subset

stroma.renamed$orig.clusters <- Idents(stroma.subset)

Idents(stroma.renamed) <- "Emcn-neg"

stroma.renamed <- SetIdent(stroma.renamed, cells = Cells(subset(stroma.subset, idents = c(1:4))), "Emcn-neg")

stroma.renamed$emcn.status <- Idents(stroma.renamed)

Idents(stroma.renamed) <- stroma.renamed$orig.clusters

Idents(stroma.renamed) <- "emcn.status"

markers <- FindMarkers(stroma.renamed, ident.2 = "Emcn-neg", ident.1 = "Emcn-pos", assay = "RNA", min_pval = 1e-05, n_markers = 1000)

## Done!

## Combining coefficients and standard errors

## Calculating log-fold changes

## Calculating likelihood ratio tests

## Refitting on reduced model...

## Done!

setDT(markers, keep.rownames = "Gene") []

##      Gene      p_val avg_log2FC pct.1 pct.2      p_val_adj
## 1:     Ace 0.000000e+00 -0.5114846 0.018 0.371 0.000000e+00
## 2:    Acta2 0.000000e+00 -1.1778268 0.185 0.648 0.000000e+00
## 3: Adamts1 0.000000e+00  2.7315050 0.817 0.381 0.000000e+00
## 4: Adamts4 0.000000e+00  2.6056075 0.648 0.107 0.000000e+00
## 5:     Adk 0.000000e+00 -0.5637901 0.242 0.718 0.000000e+00
##   ---
## 1868: Hspa1b 3.940822e-22 -0.4033189 0.249 0.360 8.699759e-18
## 1869: Hspa1a 4.619192e-21 -0.6553781 0.214 0.317 1.019733e-16
## 1870: Gfod2 1.425358e-16 -0.2613460 0.049 0.100 3.146621e-12
## 1871: Malat1 1.378321e-13  0.4021334 0.993 0.995 3.042782e-09
## 1872: Klf2 2.703607e-09 -0.2734192 0.404 0.476 5.968482e-05

head(arrange(markers, desc(abs(avg_log2FC))))

```

```

##      Gene p_val avg_log2FC pct.1 pct.2 p_val_adj
## 1: Igfbp2    0 -4.247241 0.125 0.854      0
## 2: Ecrg4    0 -4.217623 0.315 0.994      0
## 3: Isg15    0  3.975496 0.813 0.138      0
## 4: Fmod     0 -3.970060 0.179 0.993      0
## 5: Sfrp2    0 -3.761205 0.041 0.874      0
## 6: Phlda1   0  3.617296 0.895 0.279      0

p <- EnhancedVolcano(markers, lab = markers$Gene, x = 'avg_log2FC', y = 'p_val_adj',
                      title = paste("Emcn-neg vs Emcn-pos, stroma"), FCcutoff = 0.2, drawConnectors =
                      pCutoff = 10e-4)

## Warning: One or more p-values is 0. Converting to 10^-1 * current lowest
## non-zero p-value...

ggsave(p, filename=paste("./DE_output/Stroma_reclustered_EMCN-status_volcano_1-12-24.png", sep = "))

## Saving 6.5 x 10 in image

## Warning: ggrepel: 1864 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps

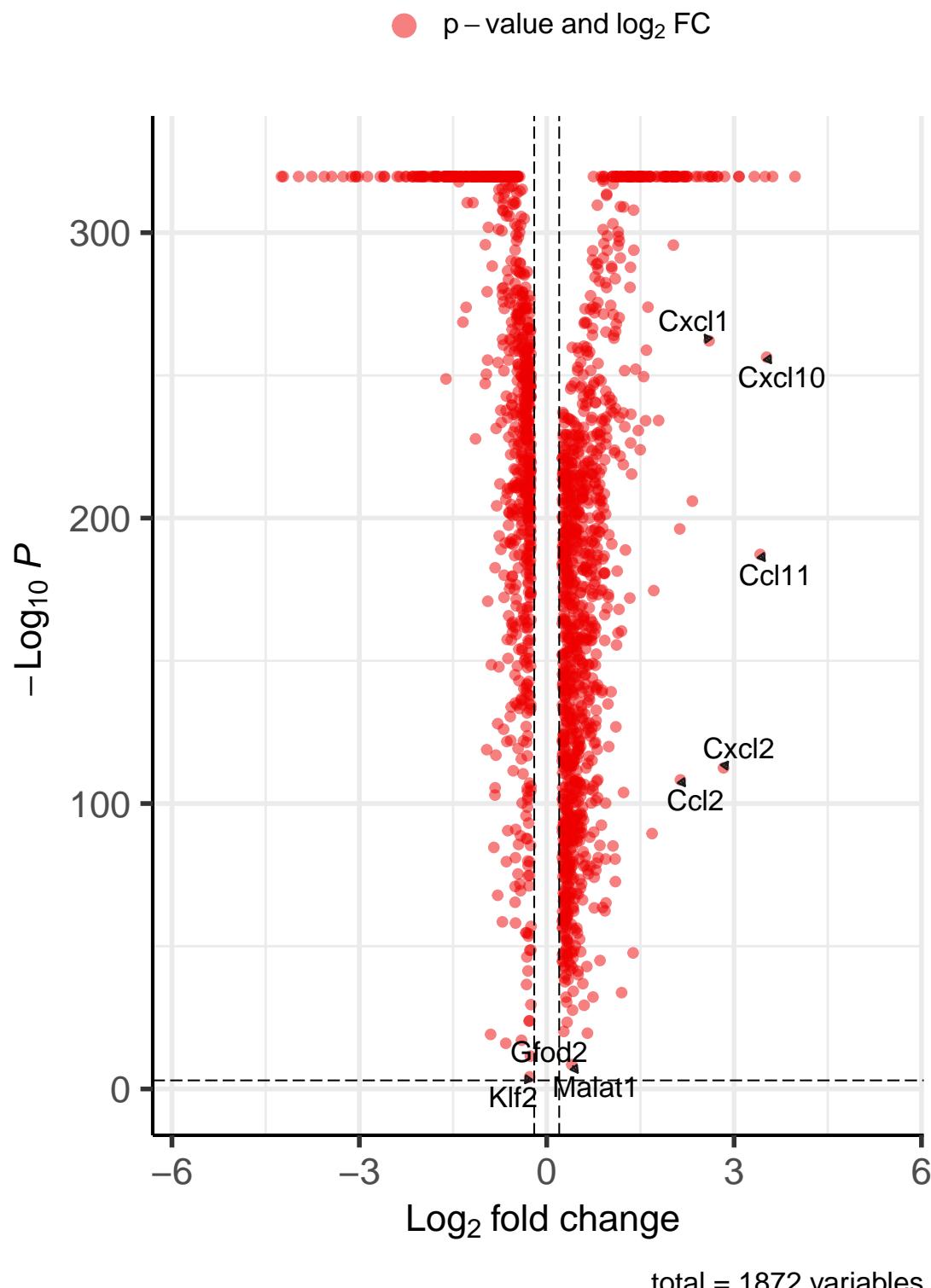
p

```

Warning: ggrepel: 1864 unlabeled data points (too many overlaps). Consider
increasing max.overlaps

Emcn-neg vs Emcn-pos, stroma

EnhancedVolcano



```

write.csv(markers, paste("./DE_output/Stroma_reclustered_EMCN-status_DE_1-2-24.csv", sep = ""))
updated.full <- SetIdent(full.dataset, cells = Cells(stroma.renamed), value = Idents(stroma.renamed))
x <- FindMarkers(updated.full, ident.1 = "Emcn-pos")

```

Find correlated genes

```

# Calculate the correlation matrix between genes

gene_of_interest <- "Emcn"

# Get the expression values of the gene of interest
gene_expression <- stroma.subset@assays$RNA@scale.data[gene_of_interest, ]

# Calculate the correlation between the expression of the gene of interest and all other genes
correlations <- cor(t(stroma.subset@assays$RNA@scale.data), gene_expression)

## Warning in cor(t(stroma.subset@assays$RNA@scale.data), gene_expression): the
## standard deviation is zero

write.csv(correlations, paste("./Stroma_genes_correlated_with_EMCN_DE_1-17-24.csv", sep = ""))
# Calculate the correlation matrix between genes

gene_of_interest <- "Pdgfra"

# Get the expression values of the gene of interest
gene_expression <- stroma.subset@assays$RNA@scale.data[gene_of_interest, ]

# Calculate the correlation between the expression of the gene of interest and all other genes
correlations <- cor(t(stroma.subset@assays$RNA@scale.data), gene_expression)

## Warning in cor(t(stroma.subset@assays$RNA@scale.data), gene_expression): the
## standard deviation is zero

write.csv(correlations, paste("./Stroma_genes_correlated_with_PDGFR_A_DE_1-17-24.csv", sep = ""))
sessionInfo()

## R version 4.2.0 (2022-04-22 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8

```

```

## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
##
## attached base packages:
## [1] stats4      stats       graphics   grDevices  utils      datasets   methods
## [8] base
##
## other attached packages:
## [1] MAST_1.22.0                tibble_3.1.8
## [3] reshape2_1.4.4              purrr_1.0.1
## [5] magrittr_2.0.3              SingleCellExperiment_1.18.1
## [7] SummarizedExperiment_1.26.1 Biobase_2.56.0
## [9] GenomicRanges_1.48.0        GenomeInfoDb_1.32.4
## [11] IRanges_2.30.1             S4Vectors_0.34.0
## [13] BiocGenerics_0.42.0        MatrixGenerics_1.8.1
## [15] matrixStats_0.63.0         edgeR_3.38.4
## [17] limma_3.52.4               Matrix.utils_0.9.8
## [19] Matrix_1.5-3               ashr_2.2-63
## [21] biomaRt_2.52.0             SoupX_1.6.2
## [23] EnhancedVolcano_1.14.0    ggrepel_0.9.3
## [25] intrinsicDimension_1.2.0  yaImpute_1.0-33
## [27] kableExtra_1.3.4           future_1.31.0
## [29] scDblFinder_1.10.0         data.table_1.14.8
## [31] clustree_0.5.0             ggraph_2.1.0
## [33] gridExtra_2.3               ggplot2_3.4.1
## [35] cowplot_1.1.1              sctransform_0.3.5
## [37] gdata_2.18.0.1             dplyr_1.1.0
## [39] SeuratObject_4.1.3          Seurat_4.3.0
##
## loaded via a namespace (and not attached):
## [1] rappdirs_0.3.3              rtracklayer_1.56.1
## [3] scattermore_0.8              ragg_1.2.5
## [5] tidyverse_1.3.0              bit64_4.0.5
## [7] knitr_1.42                  irlba_2.3.5.1
## [9] DelayedArray_0.22.0          KEGGREST_1.36.3
## [11] RCurl_1.98-1.10             generics_0.1.3
## [13] ScaledMatrix_1.4.1          RSQLite_2.3.0
## [15] RANN_2.6.1                  bit_4.0.5
## [17] spatstat.data_3.0-0         webshot_0.5.4
## [19] xml2_1.3.3                 httpuv_1.6.9
## [21] viridis_0.6.2               xfun_0.37
## [23] hms_1.1.2                  evaluate_0.20
## [25] promises_1.2.0.1            fansi_1.0.4
## [27] restfulr_0.0.15             progress_1.2.2
## [29] dbplyr_2.3.1                igraph_1.4.1
## [31] DBI_1.1.3                  htmlwidgets_1.6.1
## [33] spatstat.geom_3.0-6          ellipsis_0.3.2
## [35] deldir_1.0-6                sparseMatrixStats_1.8.0
## [37] vctrs_0.5.2                 ROCR_1.0-11
## [39] abind_1.4-5                cachem_1.0.7
## [41] withr_2.5.0                 grr_0.9.5
## [43] ggforce_0.4.1               progressr_0.13.0
## [45] GenomicAlignments_1.32.1    prettyunits_1.1.1
## [47] scran_1.24.1                goftest_1.2-3

```

```

## [49] svglite_2.1.1           cluster_2.1.4
## [51] ape_5.7                  lazyeval_0.2.2
## [53] crayon_1.5.2            spatstat.explore_3.0-6
## [55] labeling_0.4.2          pkgconfig_2.0.3
## [57] tweenr_2.0.2             nlme_3.1-162
## [59] vipor_0.4.5              rlang_1.0.6
## [61] globals_0.16.2           lifecycle_1.0.3
## [63] miniUI_0.1.1.1          filelock_1.0.2
## [65] BiocFileCache_2.4.0      rservd_1.0.5
## [67] invgamma_1.1             ggrastr_1.0.1
## [69] polyclip_1.10-4          lmtest_0.9-40
## [71] zoo_1.8-11                beeswarm_0.4.0
## [73] ggridges_0.5.4            png_0.1-8
## [75] viridisLite_0.4.1         rjson_0.2.21
## [77] bitops_1.0-7              KernSmooth_2.23-20
## [79] Biostrings_2.64.1          blob_1.2.3
## [81] DelayedMatrixStats_1.18.2 mixsqp_0.3-48
## [83] stringr_1.5.0              SQUAREM_2021.1
## [85] parallelly_1.34.0          spatstat.random_3.1-3
## [87] beachmat_2.12.0            scales_1.2.1
## [89] memoise_2.0.1              plyr_1.8.8
## [91] ica_1.0-3                 zlibbioc_1.42.0
## [93] compiler_4.2.0              dqrng_0.3.0
## [95] BiocIO_1.6.0                RColorBrewer_1.1-3
## [97] fitdistrplus_1.1-8          Rsamtools_2.12.0
## [99] cli_3.6.0                  XVector_0.36.0
## [101] listenv_0.9.0              patchwork_1.1.2
## [103] pbapply_1.7-0              MASS_7.3-58.2
## [105] tidyselect_1.2.0            stringi_1.7.12
## [107] textshaping_0.3.6          highr_0.10
## [109] yaml_2.3.7                 BiocSingular_1.12.0
## [111] locfit_1.5-9.7              grid_4.2.0
## [113] tools_4.2.0                 future.apply_1.10.0
## [115] parallel_4.2.0              rstudioapi_0.14
## [117] bluster_1.6.0              metapod_1.4.0
## [119] farver_2.1.1               Rtsne_0.16
## [121] digest_0.6.31              shiny_1.7.4
## [123] Rcpp_1.0.10                scuttle_1.6.3
## [125] later_1.3.0                RcppAnnoy_0.0.20
## [127] httr_1.4.5                 AnnotationDbi_1.58.0
## [129] colorspace_2.1-0            rvest_1.0.3
## [131] XML_3.99-0.13              tensor_1.5
## [133] reticulate_1.28             truncnorm_1.0-9
## [135] splines_4.2.0              uwot_0.1.14
## [137] statmod_1.5.0              spatstat.utils_3.0-1
## [139] scater_1.24.0              graphlayouts_0.8.4
## [141] sp_1.6-0                   xgboost_1.7.3.1
## [143] plotly_4.10.1              systemfonts_1.0.4
## [145] xtable_1.8-4              jsonlite_1.8.4
## [147] tidygraph_1.2.3             R6_2.5.1
## [149] pillar_1.8.1               htmltools_0.5.4
## [151] mime_0.12                  glue_1.6.2
## [153] fastmap_1.1.1              BiocParallel_1.30.4
## [155] BiocNeighbors_1.14.0         codetools_0.2-18

```

```
## [157] utf8_1.2.3          lattice_0.20-45
## [159] spatstat.sparse_3.0-0  curl_5.0.0
## [161] ggbeeswarm_0.7.1      leiden_0.4.3
## [163] gtools_3.9.4          survival_3.5-3
## [165] rmarkdown_2.20         munsell_0.5.0
## [167] GenomeInfoDbData_1.2.8 gtable_0.3.1
```