library(Seurat)

library(dplyr)

library(ggplot2)

library(cowplot)

theme\_set(theme\_cowplot())

WTvsK <- WTvsKO3

Idents(object = WTvsK) <- "orig.ident"

levels(Bcell)

Diff.exp.Bcell <- FindMarkers(Bcell, ident.1 = "Exp1\_WTmice", ident.2 = "Exp1\_KOmice", verbose = FALSE, logfc.threshold = 0.1)

Bcell <- subset(WTvsKO3, idents = c("0", "2"))

write.table(Diff.exp.global4, file = "~/WTvsKO\_Analysis3\_DiffExpBcell.csv", sep = ",")

Neutromarkers <- FindConservedMarkers(WTvsKO, )

table(Neutro@active.ident)

FeaturePlot(SPMLPM, features = "Cd36", cols = c("orange", "black"), reduction = "umap", sort.cell = TRUE,split.by = "orig.ident", pt.size = 1)

VlnPlot(WTvsK, features = "Cd36", split.by = "orig.ident", ncol = 1, pt.size = 0.1)

DotPlot(SPMLPM, features = c("Foxp1", "Myc", "Tcf3", "Foxo1"), split.by = "orig.ident", cols = c("blue", "orange"))

SPM <- subset(WTvsKO3, idents = c("3", "4", "6"))

Idents(object = SPMLPM) <- "orig.ident"

levels(SPM)

Diff.exp.SPM <- FindMarkers(SPM, ident.1 = "Exp1\_WTmice", ident.2 = "Exp1\_KOmice", verbose = FALSE, logfc.threshold = 0.1)

write.table(Diff.exp.SPM, file = "~/WTvsKO\_Analysis3\_DiffExpSPM.csv", sep = ",")

LPM <- subset(WTvsKO3, idents = c("18", "15", "5", "1", "10"))

Idents(object = LPM) <- "orig.ident"

levels(LPM)

Diff.exp.LPM <- FindMarkers(LPM, ident.1 = "Exp1\_WTmice", ident.2 = "Exp1\_KOmice", verbose = FALSE, logfc.threshold = 0.1)

write.table(Diff.exp.LPM, file = "~/WTvsKO\_Analysis3\_DiffExpLPM.csv", sep = ",")

All.genes <- FindAllMarkers(WTvsK)

SPMLPM <- subset(WTvsKO3, idents = c("3", "4", "6", "18", "15", "5", "1", "10"))

WT.data <- Read10X(data.dir = "R:/goldberglab/goldberglabspace/Julie/2.RESULTS/Single cell RNA seq data/Mac36 mice/WT vs KO analysis/WT filtered feature bc matrix/")

KO.data <- Read10X(data.dir = "R:/goldberglab/goldberglabspace/Julie/2.RESULTS/Single cell RNA seq data/Mac36 mice/WT vs KO analysis/KO filtered feature bc matrix/")

WT <- CreateSeuratObject(counts = WT.data, project = "Exp1\_WTmice", min.cells = 5)

WT$KO <- "WTmice"

WT <- subset(WT, subset = nFeature\_RNA > 400)

WT <- NormalizeData(WT, verbose = FALSE)

WT <- FindVariableFeatures(WT, selection.method = "vst", nfeatures = 2500)

KO <- CreateSeuratObject(counts = KO.data, project = "Exp1\_KOmice", min.cells = 5)

KO$KO <- "KOmice"

KO <- subset(KO, subset = nFeature\_RNA > 400)

KO <- NormalizeData(KO, verbose = FALSE)

KO <- FindVariableFeatures(KO, selection.method = "vst", nfeatures = 2500)

immune.anchors <- FindIntegrationAnchors(object.list = list(WT, KO), dims = 1:20)

immune.combined <- IntegrateData(anchorset = immune.anchors, dims = 1:20)

DefaultAssay(immune.combined) <- "integrated"

immune.combined <- ScaleData(immune.combined, verbose = FALSE)

immune.combined <- RunPCA(immune.combined, npcs = 30, verbose = FALSE)

immune.combined <- RunUMAP(immune.combined, reduction = "pca", dims = 1:20)

immune.combined <- RunTSNE(immune.combined, reduction = "pca", dims = 1:20)

immune.combined <- FindNeighbors(immune.combined, reduction = "pca", dims = 1:20)

immune.combined <- FindClusters(immune.combined, resolution = 0.6)

DimPlot(immune.combined, reduction = "umap", split.by = "KO")

p1 <- DimPlot(immune.combined, reduction = "umap", group.by = "KO")

p2 <- DimPlot(immune.combined, reduction = "umap", label = TRUE)

plot\_grid(p1, p2)

P1 <- DimPlot(immune.combined, reduction = "tsne", group.by = "KO")

P2 <- DimPlot(immune.combined, reduction = "tsne", label = TRUE)

plot\_grid(P1, P2)

Macro.markers <- c("Mafb","Itgam", "Itgax", "Mrc1", "Csf1r", "Cd68","Cd69", "Cd209a", "Ifit1", "Ifitm2", "Ifitm3", "Il6", "Aif1", "Mpeg1", "Arg1", "Retnla", "Adgre1", "Tlr2", "Ly6e")

Lipid.meta.markers <- c("Msr1", "Srebf2", "Abca1", "Scarb1", "Cpt1a")

Other.markers <- c("Selp", "Pf4", "Cd38", "Gzma", "Nkg7", "Epcam", "Cd3d", "Ccr7", "Ccl5", "Sell", "Lyz1", "Cd79a", "Gnly", "Cd74", "Cxcl13", "Adgre1", "Cd63","Stmn1")

plots <- VlnPlot(immune.combined, features = rev(lipid.meta.markers), split.by = "KO", pt.size = 0, combine = FALSE)

CombinePlots(plots, ncol = 2)

DotPlot(immune.combined, features = rev(Macro.markers), cols = c("Blue", "orange"), dot.scale = 5, split.by = "KO") + RotatedAxis()

LPM0.cells <- subset(immune.combined, idents = "LPM main cluster")

LPM0.cells <- subset(immune.combined, idents = "0")

Idents(LPM0.cells) <- "KO"

avg.LPM0.cells <- log1p(AverageExpression(LPM0.cells, verbose = FALSE)$RNA)

avg.LPM0.cells$gene <- rownames(avg.LPM0.cells)

DotPlot(immune.combined, features = c("Cd8b1","Cd4","Ccr7","Csf3r","Ltb","Ms4a1","Cd79a","Cd3g","Cd3d","Cd19","Fcer2a","Cd68", "Cd209a", "Ifit1", "Ifitm2", "Ifitm3", "Il6", "Aif1", "Mpeg1", "Arg1", "Retnla", "Adgre1", "Cd86", "Tlr2", "Ly6e","Selp","mt-Co1","Pf4","Clec9a","Fcer1g","Clec10a","Mrc1","Itgax","Itgam","Tnfrsf11a","Pecam1","Csf1r","Ly6c2","Dusp1","Fos","Cd36","Lyz1","Cd14","Fcgr3", "S100a10", "S100a9", "S100a8", "Csf1r"), cols = c("Blue", "orange"), dot.scale = 5, split.by = "KO") + RotatedAxis()

Macro.markers <- c("Itgam", "Itgax", "Mrc1", "Csf1r", "Cd68", "Cd209a", "Ifit1", "Ifitm2", "Ifitm3", "Il6", "Aif1", "Mpeg1", "Arg1", "Retnla", "Adgre1", "Cd86", "Tlr2", "Ly6e")

p1 <- ggplot(avg.LPM0.cells, aes(WTmice, "KOmice")) + geom\_point() + ggtitle("LPM cluster 0")

p1 <- ggplot(avg.LPM0.cells, aes(WTmice, KOmice)) + geom\_point() + ggtitle("LPM cluster 0")

p1 <- LabelPoints(plot = p1, points = Macro.markers, repel = TRUE)

saveRDS(immune.combined, file = "R:/goldberglab/goldberglabspace/Julie/Single cell RNA seq data/Mac36 mice/WT vs KO analysis/WT vs KO analysis 2/WTvsKOanalysis.rds")

WTvsKOanalysis3 <- readRDS("R:/goldberglab/goldberglabspace/Julie/Single cell RNA seq data/Mac36 mice/WT vs KO analysis/WT vs KO analysis 2/WTvsKOanalysis.rds")

library(Seurat)

DimPlot(object = WTvsKOanalysis3, reduction = "tsne", label = TRUE)

DimPlot(object = WTvsKOanalysis3, reduction = "umap", label = TRUE, group.by [2]="state")

DimPlot(object = WTvsKOanalysis3, reduction = "umap", label = TRUE, group.by [2]"state")

DimPlot(object = WTvsKOanalysis3, reduction = "umap", label = TRUE, group.by [2] = "KO")

DimPlot(object = WTvsKOanalysis3, reduction = "umap", label = TRUE, group.by = "KO")

mat <- WTvsKOanalysis3@assays$RNA@counts

write.table(mat, file = "R:/goldberglab/goldberglabspace/Julie/Single cell RNA seq data/Mac36 mice/WT vs KO analysis 2/countmatrix.csv", sep = ",")

write.table(mat, file = "R:/goldberglab/goldberglabspace/Julie/Single cell RNA seq data/Mac36 mice/WT vs KO analysis/KOvsWT\_countmatrix.csv", sep = ",")

DefaultAssay(immune.combined) <- "RNA"

c0.markers <- FindConservedMarkers(immune.combined,ident.1 = 0, grouping.var = "KO", verbose = FALSE)

c1.markers <- FindConservedMarkers(immune.combined,ident.1 = 1, grouping.var = "KO", verbose = FALSE)

c2.markers <- FindConservedMarkers(immune.combined,ident.1 = 2, grouping.var = "KO", verbose = FALSE)

c3.markers <- FindConservedMarkers(immune.combined,ident.1 = 3, grouping.var = "KO", verbose = FALSE)

c4.markers <- FindConservedMarkers(immune.combined,ident.1 = 4, grouping.var = "KO", verbose = FALSE)

c5.markers <- FindConservedMarkers(immune.combined,ident.1 = 5, grouping.var = "KO", verbose = FALSE)

c6.markers <- FindConservedMarkers(immune.combined,ident.1 = 6, grouping.var = "KO", verbose = FALSE)

c7.markers <- FindConservedMarkers(immune.combined,ident.1 = 7, grouping.var = "KO", verbose = FALSE)

c8.markers <- FindConservedMarkers(immune.combined,ident.1 = 8, grouping.var = "KO", verbose = FALSE)

c9.markers <- FindConservedMarkers(immune.combined,ident.1 = 9, grouping.var = "KO", verbose = FALSE)

c10.markers <- FindConservedMarkers(immune.combined,ident.1 = 10, grouping.var = "KO", verbose = FALSE)

c11.markers <- FindConservedMarkers(immune.combined,ident.1 = 11, grouping.var = "KO", verbose = FALSE)

c12.markers <- FindConservedMarkers(immune.combined,ident.1 = 12, grouping.var = "KO", verbose = FALSE)

c13.markers <- FindConservedMarkers(immune.combined,ident.1 = 13, grouping.var = "KO", verbose = FALSE)

c14.markers <- FindConservedMarkers(immune.combined,ident.1 = 14, grouping.var = "KO", verbose = FALSE)

c15.markers <- FindConservedMarkers(immune.combined,ident.1 = 15, grouping.var = "KO", verbose = FALSE)

c16.markers <- FindConservedMarkers(immune.combined,ident.1 = 16, grouping.var = "KO", verbose = FALSE)

c17.markers <- FindConservedMarkers(immune.combined,ident.1 = 17, grouping.var = "KO", verbose = FALSE)

c18.markers <- FindConservedMarkers(immune.combined,ident.1 = 18, grouping.var = "KO", verbose = FALSE)

c19.markers <- FindConservedMarkers(immune.combined,ident.1 = 19, grouping.var = "KO", verbose = FALSE)

c20.markers <- FindConservedMarkers(immune.combined,ident.1 = 15, grouping.var = "KO", verbose = FALSE)

table(immune.combined@active.ident, [immune.combined@meta.data$orig.ident](mailto:immune.combined@meta.data$orig.ident))

immune.combined$celltype.KO <- paste(Idents(immune.combined), immune.combined$KO, sep = "\_")

immune.combined$celltype <- Idents(immune.combined)

Idents(immune.combined) <- "celltype.KO"

Bcell2.response <- FindMarkers(immune.combined, ident.1 = "2\_KOmice", ident.2 = "2\_WTmice", verbose = FALSE)

head(Bcell2.response, n = 15)

immune.combined$celltype.KO <- paste(Idents(immune.combined), immune.combined$KO, sep = "\_")

immune.combined$celltype <- Idents(immune.combined)

Idents(immune.combined) <- "celltype.KO"

c0.response <- FindMarkers(immune.combined, ident.1 = "0\_KOmice", ident.2 = "0\_WTmice", verbose = FALSE)

c01.response <- FindMarkers(immune.combined, ident.1 = "1\_WTmice", ident.2 = "1\_KOmice", verbose = FALSE)

c02.response <- FindMarkers(immune.combined, ident.1 = "2\_WTmice", ident.2 = "2\_KOmice", verbose = FALSE)

c03.response <- FindMarkers(immune.combined, ident.1 = "3\_WTmice", ident.2 = "3\_KOmice", verbose = FALSE)

c04.response <- FindMarkers(immune.combined, ident.1 = "4\_WTmice", ident.2 = "4\_KOmice", verbose = FALSE)

c05.response <- FindMarkers(immune.combined, ident.1 = "5\_WTmice", ident.2 = "5\_KOmice", verbose = FALSE)

c06.response <- FindMarkers(immune.combined, ident.1 = "6\_WTmice", ident.2 = "6\_KOmice", verbose = FALSE)

c07.response <- FindMarkers(immune.combined, ident.1 = "7\_WTmice", ident.2 = "7\_KOmice", verbose = FALSE)

c08.response <- FindMarkers(immune.combined, ident.1 = "8\_WTmice", ident.2 = "8\_KOmice", verbose = FALSE)

c09.response <- FindMarkers(immune.combined, ident.1 = "9\_WTmice", ident.2 = "9\_KOmice", verbose = FALSE)

c10.response <- FindMarkers(immune.combined, ident.1 = "10\_WTmice", ident.2 = "10\_KOmice", verbose = FALSE)

c11.response <- FindMarkers(immune.combined, ident.1 = "11\_WTmice", ident.2 = "11\_KOmice", verbose = FALSE)

c012.response <- FindMarkers(immune.combined, ident.1 = "12\_WTmice", ident.2 = "12\_KOmice", verbose = FALSE)

c013.response <- FindMarkers(immune.combined, ident.1 = "13\_WTmice", ident.2 = "13\_KOmice", verbose = FALSE)

c014.response <- FindMarkers(immune.combined, ident.1 = "14\_WTmice", ident.2 = "14\_KOmice", verbose = FALSE)

c015.response <- FindMarkers(immune.combined, ident.1 = "15\_WTmice", ident.2 = "15\_KOmice", verbose = FALSE)

c016.response <- FindMarkers(immune.combined, ident.1 = "16\_WTmice", ident.2 = "16\_KOmice", verbose = FALSE)

c017.response <- FindMarkers(immune.combined, ident.1 = "17\_WTmice", ident.2 = "17\_KOmice", verbose = FALSE)

c018.response <- FindMarkers(immune.combined, ident.1 = "18\_WTmice", ident.2 = "18\_KOmice", verbose = FALSE)

c019.response <- FindMarkers(immune.combined, ident.1 = "19\_WTmice", ident.2 = "19\_KOmice", verbose = FALSE)

c020.response <- FindMarkers(immune.combined, ident.1 = "20\_WTmice", ident.2 = "20\_KOmice", verbose = FALSE)

**WTvsKO2 <- WTvsKO**

**Idents(object = Neutro) <- "orig.ident"**

**levels(Neutro)**

**Diff.exp.Neutro <- FindMarkers(Neutro, ident.1 = "Exp1\_WTmice", ident.2 = "Exp1\_KOmice", verbose = FALSE)**

**Neutro <- subset(WTvsKO, idents = "18")**

**write.table(Diff.exp.Neutro, file = "~/WTvsKO\_Analysis2\_DiffExpNeutro.csv", sep = ",")**

**Neutromarkers <- FindConservedMarkers(WTvsKO, )**

**table(Neutro@active.ident)**

c0.response <- FindMarkers(immune.combined, ident.1 = "0\_WTmice", ident.2 = "0\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c1.response <- FindMarkers(immune.combined, ident.1 = "1\_WTmice", ident.2 = "1\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c2.response <- FindMarkers(immune.combined, ident.1 = "2\_WTmice", ident.2 = "2\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c3.response <- FindMarkers(immune.combined, ident.1 = "3\_WTmice", ident.2 = "3\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c4.response <- FindMarkers(immune.combined, ident.1 = "4\_WTmice", ident.2 = "4\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c5.response <- FindMarkers(immune.combined, ident.1 = "5\_WTmice", ident.2 = "5\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c6.response <- FindMarkers(immune.combined, ident.1 = "6\_WTmice", ident.2 = "6\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c7.response <- FindMarkers(immune.combined, ident.1 = "7\_WTmice", ident.2 = "7\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c8.response <- FindMarkers(immune.combined, ident.1 = "8\_WTmice", ident.2 = "8\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c9.response <- FindMarkers(immune.combined, ident.1 = "9\_WTmice", ident.2 = "9\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c10.response <- FindMarkers(immune.combined, ident.1 = "10\_WTmice", ident.2 = "10\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c11.response <- FindMarkers(immune.combined, ident.1 = "11\_WTmice", ident.2 = "11\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c12.response <- FindMarkers(immune.combined, ident.1 = "12\_WTmice", ident.2 = "12\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c13.response <- FindMarkers(immune.combined, ident.1 = "13\_WTmice", ident.2 = "13\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c14.response <- FindMarkers(immune.combined, ident.1 = "14\_WTmice", ident.2 = "14\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c15.response <- FindMarkers(immune.combined, ident.1 = "15\_WTmice", ident.2 = "15\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c16.response <- FindMarkers(immune.combined, ident.1 = "16\_WTmice", ident.2 = "16\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

c17.response <- FindMarkers(immune.combined, ident.1 = "17\_WTmice", ident.2 = "17\_KOmice", verbose = FALSE, test.use = "t", logfc.threshold = 0.15)

head(KO@meta.data)

orig.ident nCount\_RNA nFeature\_RNA KO

AAACCTGAGATCCGAG Exp1\_KOmice 1596 702 KOmice

AAACCTGAGATGTGGC Exp1\_KOmice 11973 2686 KOmice

AAACCTGAGCCAGAAC Exp1\_KOmice 6615 1764 KOmice

AAACCTGAGCTGCGAA Exp1\_KOmice 8350 2321 KOmice

AAACCTGAGGACAGAA Exp1\_KOmice 4040 1536 KOmice

AAACCTGAGGCTCTTA Exp1\_KOmice 9304 2489 KOmice

head(WT@meta.data)

orig.ident nCount\_RNA nFeature\_RNA KO

AAACCTGAGAATTCCC Exp1\_WTmice 4615 1559 WTmice

AAACCTGAGACTGTAA Exp1\_WTmice 11490 2379 WTmice

AAACCTGAGCTAAGAT Exp1\_WTmice 10334 2333 WTmice

AAACCTGAGCTAGTTC Exp1\_WTmice 14218 2879 WTmice

AAACCTGAGCTGCAAG Exp1\_WTmice 21943 3904 WTmice

AAACCTGAGGCACATG Exp1\_WTmice 8450 2023 WTmice

head(immune.combined@meta.data)

orig.ident nCount\_RNA nFeature\_RNA KO integrated\_snn\_res.0.6 seurat\_clusters celltype.KO

AAACCTGAGAATTCCC\_1 Exp1\_WTmice 4615 1559 WTmice 1 1 1\_WTmice

AAACCTGAGACTGTAA\_1 Exp1\_WTmice 11490 2379 WTmice 0 0 0\_WTmice

AAACCTGAGCTAAGAT\_1 Exp1\_WTmice 10334 2333 WTmice 4 4 4\_WTmice

AAACCTGAGCTAGTTC\_1 Exp1\_WTmice 14218 2879 WTmice 13 13 13\_WTmice

AAACCTGAGCTGCAAG\_1 Exp1\_WTmice 21943 3904 WTmice 9 9 9\_WTmice

AAACCTGAGGCACATG\_1 Exp1\_WTmice 8450 2023 WTmice 4 4 4\_WTmice

celltype

AAACCTGAGAATTCCC\_1 1

AAACCTGAGACTGTAA\_1 0

AAACCTGAGCTAAGAT\_1 4

AAACCTGAGCTAGTTC\_1 13

AAACCTGAGCTGCAAG\_1 9

AAACCTGAGGCACATG\_1 4

**A not up-to-date list can be accessed in R through the annotation library**

org.Hs.eg.db (human) and org.Mm.eg.db (mouse). With as.list(org.Hs.egPATH2EG) you will get a list of all pathways (itmes names are KEGG pathway IDs and each item is a vector of NCBI gene IDs that are assigned to the respective pathway). You can save this as a text file in the format you want, e.g. with

library(org.Hs.eg.db)

mapped <- mappedkeys(org.Hs.egPATH2EG)

L <- as.list(org.Hs.egPATH2EG[mapped])

Kegg\_ID <- names(L)

Gene\_IDs <- sapply(L, paste, collapse=",")

write.table(cbind(Kegg\_ID, Gene\_IDs), file="KEGG to Genes.txt", sep="\t", row.names=FALSE, col.names=FALSE)

**Correct export of markers in csv:**

write.table(c1.markers, file = "R:/goldberglab/goldberglabspace/Julie/2.RESULTS/Single cell RNA seq data/Mac36 mice/WT vs KO analysis/WT vs KO anlysis 3/KOvsWT\_c1markers.csv", sep = ",")