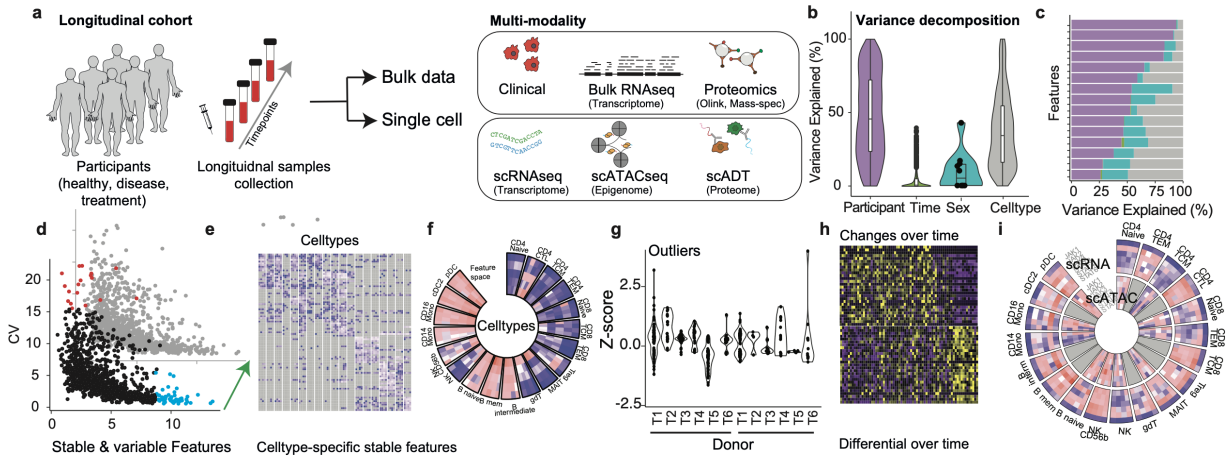
PALM (Platform for Analyzing Longitudinal Multi-omics data) package

12/16/2021

Contents

1 Introduction

PALM (`Platform for Analyzing Longitudinal Multi-omics data`) is a platform for anayzing longitudinal data from bulk as well as single cell. It allows to identify inter-, intra-donor variations in genes over longitudinal time points. The analysis can be done on bulk expression dataset without known celltype information or single cell with celltype/user-defined groups. It allows to infer stable and variable features in given donor and each celltype (or user defined group). The outlier analysis can be performed to identify techinical/biological perturbed samples in donor/participant. Further, differential analysis can be performed to deciher time-wise changes in gene expression in a celtype.



General workflow and analysis schema of **PALM**. It can work with longitudinal data obtained from bulk such as clinical, bulk RNAseq, proteomic or single cell dataset from scRNAseq, and scATACseq.

2 Install package and load library

To install library, simply run

```
install.packages("PALM_0.1.0.tar.gz", repos = NULL, type ="source")
library("PALM")
```

```
library(PALM)
#> Loading required package: grid
#> Loading required package: ggplot2
#> Loading required package: reshape2
#> Loading required package: ComplexHeatmap
#> ========================================
#> ComplexHeatmap version 2.4.3
#> Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
#> Github page: https://github.com/jokergoo/ComplexHeatmap
#> Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
#>
#> If you use it in published research, please cite:
#> Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional
#>   genomic data. Bioinformatics 2016.
#>
#> This message can be suppressed by:
#>   suppressPackageStartupMessages(library(ComplexHeatmap))
#> ========================================
#> Loading required package: circlize
#> ========================================
#> circlize version 0.4.11
#> CRAN page: https://cran.r-project.org/package=circlize
#> Github page: https://github.com/jokergoo/circlize
#> Documentation: https://jokergoo.github.io/circlize_book/book/
#>
#> If you use it in published research, please cite:
#> Gu, Z. circlize implements and enhances circular visualization
```

```
#>   in R. Bioinformatics 2014.
#>
#> This message can be suppressed by:
#>   suppressPackageStartupMessages(library(circlize))
#> ========================================
#> Loading required package: cowplot
#> Loading required package: pheatmap
#> Loading required package: tidyverse
#> -- Attaching packages --------------------------------------- tidyverse 1.3.0 --
#> v tibble  3.1.6     v dplyr   1.0.7
#> v tidyr   1.1.4     v stringr 1.4.0
#> v readr   1.4.0     v forcats 0.5.0
#> v purrr   0.3.4
#> -- Conflicts ------------------------------------------ tidyverse_conflicts() --
#> x dplyr::filter() masks stats::filter()
#> x dplyr::lag()    masks stats::lag()
#> Registered S3 method overwritten by 'spatstat':
#>   method     from
#>   print.boxx cli
```

3 Tutorials

3.1 Tutorial-1: Plasma proteome [Bulk dataset]

This tutorial allows users to explore bulk plasma proteome measured from 6 healthy donors over 10 time-points. Plasma proteomic data available at github. 1. Olink_NPX_log2_Protein.Rda (Normalized protein expression data) 2. data_Annotation.Rda (clinical metadata). Longitudinal dataset includes 6 donors (3 male and 3 females). PBMC samples were collected from 6 donors over 10 weeks. To interrogate longitudinal data, please follow following steps.

3.1.1 Load Library

```
#Load Library and other vizualization packages
library("PALM")
library("Hmisc")
library("ggpubr")
```

3.1.2 Load data and assign paramaters

The annotation table `metadata` must consist of column `Sample` (Participant sample name), `PTID` (Participant), `Time` (longitudinal time points). The datamatrix is an Expression data frame, where rows represents gene/proteins and column represents participant samples (same as annotation table `Sample` column).

```
#Load Plasma proteome data (longitudinal)
load("data/Olink_NPX_log2_Protein.Rda")
#Load metadata
load("data/data_Metadata.Rda")
#assign rownames with sample name
row.names(ann) <- ann$Sample
```

```
#Parameters
metadata=ann
datamatrix=data
featureSet=c("PTID", "Time") #variation attributed to traits
```

3.1.3 Sample overlap

The data matrix were overlapped with metadata for selecting available samples only.

```
overlap <- intersect(metadata$Sample, colnames(datamatrix))
metadata <- metadata[overlap,]
datamatrix <- data.frame(datamatrix, check.names = F, stringsAsFactors = F)
datamatrix <- datamatrix[,overlap]
```

3.1.4 Remove genes with >40%NAs (optional)

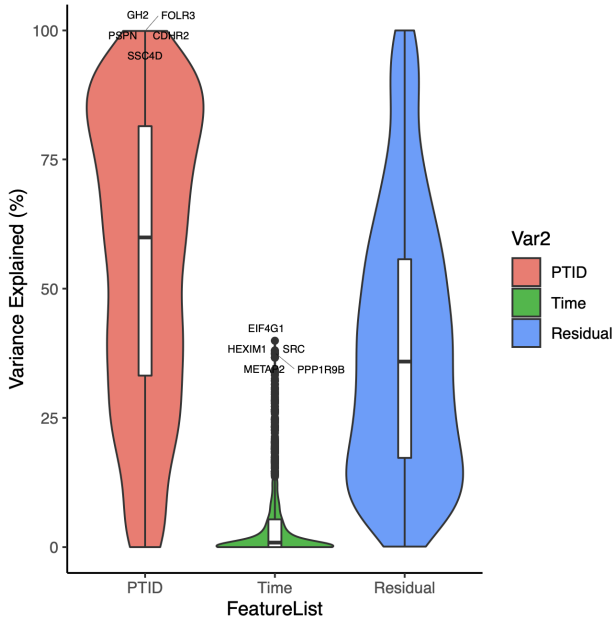
For downstream analysis select genes/proteins with less than 40% of missing values. Users can select cut-off for missing values as necessary.

```
row.na <- apply(datamatrix,1,function(x) { sum(is.na(x)) })
row.non.na <- row.na[row.na < (0.4*ncol(datamatrix))] #select features with NAs <40%
datamatrix <- datamatrix[names(row.non.na),]
rowN <- data.frame(row.names(datamatrix), stringsAsFactors = F)
```

3.1.5 Features contributing towards donor variations (Variance decomposition)

To perform variance decomposition apply `lmeVariance` function with input metadata, and datamatrix. The `featureSet` is a list of variables to which freaction variance explained by each gene is attributed. `meanThreshold` defines the minimum average expression threshold to be used for ongitudinal dataset. Here we used normalized protein expression 1 based on mean expression profile of each gene across longitudinal samples. Residuals suggest the variance can not be explained by available feature set. The variance explained by each gene towards the featureSet of interest given in percentage.

```
lmem_res <- lmeVariance(ann=metadata, mat=datamatrix,
                  featureSet=featureSet, meanThreshold=1)
```

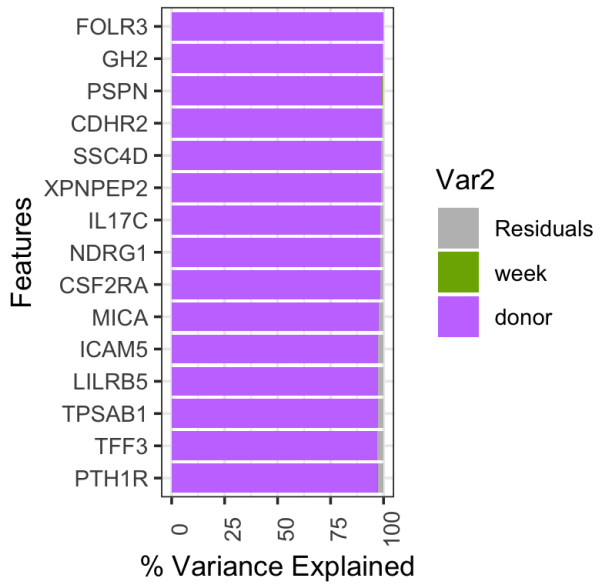



```
res <- lmem_res[,c("PTID","Time","Residual")]
colnames(res) <- c("donor","week","Residuals")
res <- res*100 #in percentage
head(res)

#Features      donor      week  Residuals
#FOLR3   99.90070 0.0000000 0.09930098
#GH2     99.49856 0.0000000 0.50144042
#PSPN    99.26882 0.1021076 0.62906798
#CDHR2   99.07933 0.1157406 0.80493162
#SSC4D   98.82794 0.0000000 1.17206000
#XPNPEP2 98.67628 0.0000000 1.32372323
```

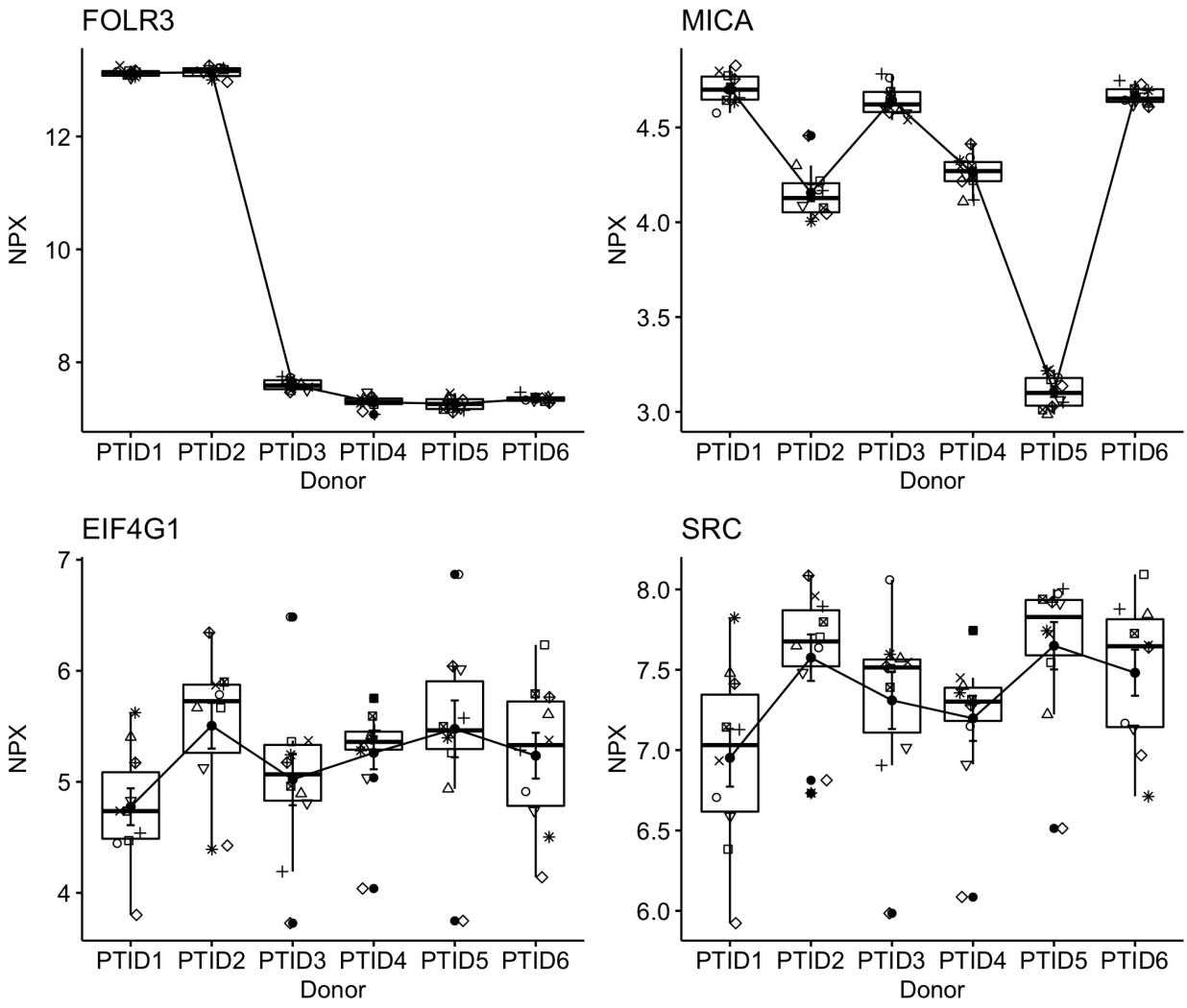
3.1.6 Donor-specific variance contrubuting features

```
df1 <- filter(res, donor>week & Residuals < 50)
df1 <- df1[order(df1$donor, decreasing = T),]
df <- melt(data.matrix(df1[1:15,]))
df$Var2 <- factor(df$Var2, levels = rev(c("donor","week", "Residuals")))
df$Var1 <- factor(df$Var1, levels = rev(unique(df$Var1)))
p1 <- ggplot(df, aes(x=Var1, y=value, fill=Var2)) +
    geom_bar(stat="identity", position="stack") +
    scale_fill_manual(values = c("donor"="#C77CFF", "celltype"="#00BFC4",
    "week"="#7CAE00", "Residuals"="grey")) +
    labs(x="Features", y="% Variance Explained") +
    theme_bw() + theme(axis.text.x = element_text(angle=90, hjust = 0.5,
    vjust = 1),legend.position = "right") +
    coord_flip()
print(p1)
```



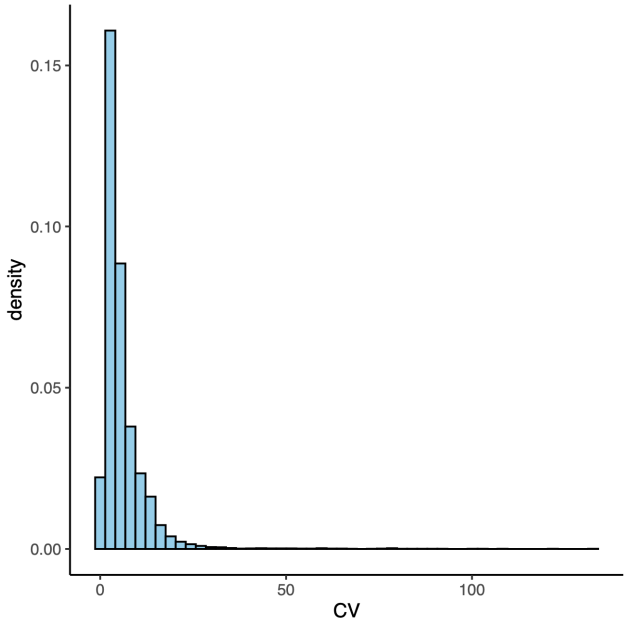
3.1.7 Plot the Top variables

```
genelist <- c("FOLR3", "MICA", "EIF4G1", "SRC")
uniSample <- as.character(unique(metadata$PTID))
splots <- list()
for(i in 1:length(genelist)) {
  geneName <- genelist[i]
  df <- data.frame(exp=as.numeric(datamatrix[geneName,]), metadata, stringsAsFactors = F)
  df$PTID <- factor(df$PTID, levels = uniSample)
  plot1 <- ggpubr::ggline(df, x = "PTID", y = "exp",
            add.params = list(shape="Time"), add = c("mean_se", "jitter", "boxplot"),
            ylab = "NPX", xlab = "Donor", title = geneName, legend = "none",
            outlier.shape = NA) + scale_shape_manual(values = 0:10)
  splots[[i]] <- plot1
}
plot_grid(plotlist=splots, ncol= 2, align="hv")
```

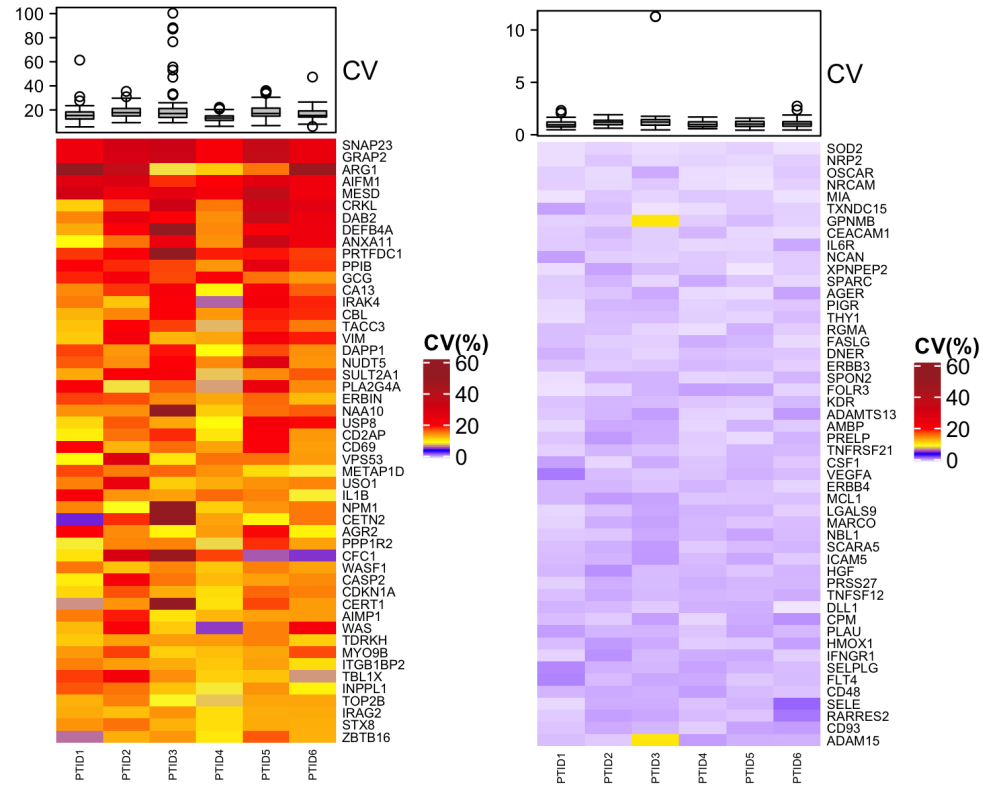


3.1.8 Intra-donor variations over time

```
#CV vs Mean
cv_res <- cvCalcBulk(mat=datamatrix, ann=metadata, meanThreshold=1,
                     cvThreshold=5)
```



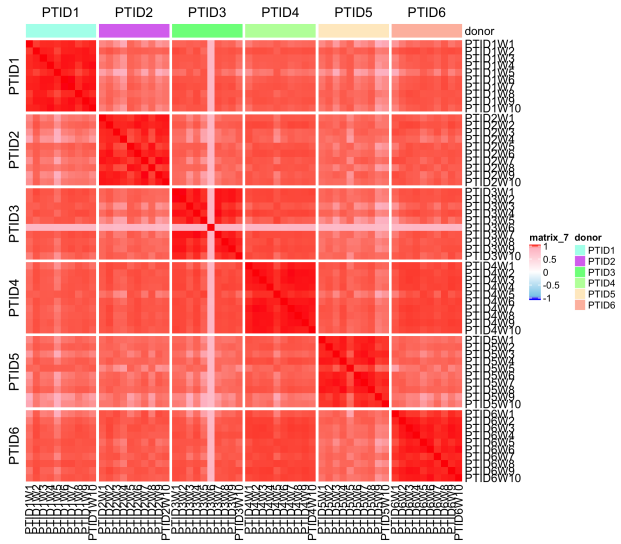

```
CV <- cv_res$CV
variable_genes <- cv_res$variable_genes
stable_genes <- cv_res$stable_genes
```



3.1.9 Outlier analysis

Perform the sample correlation to find out overall correlation between longitudinal samples.

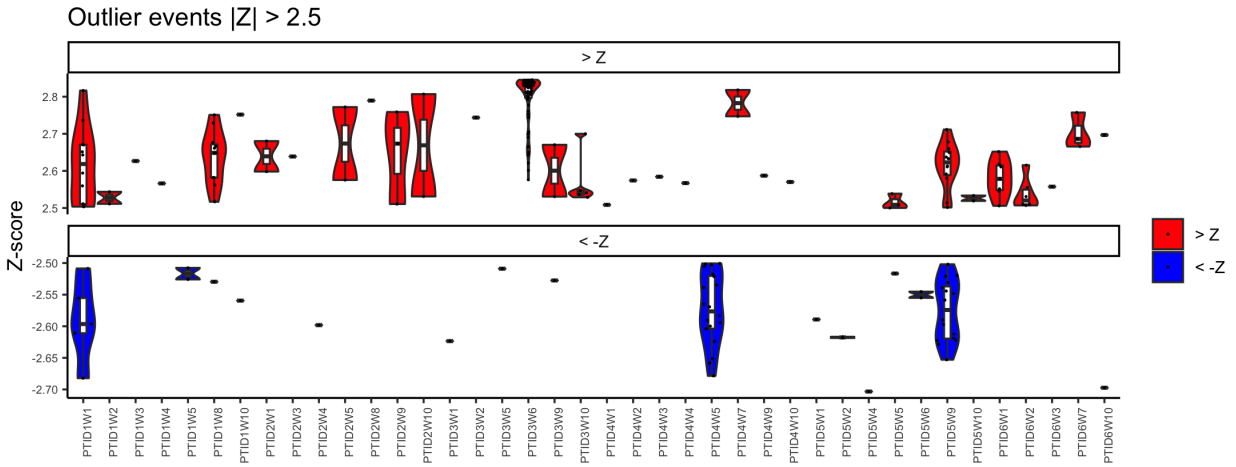
```
#Sample variability (Correlation)
cor_res <- sample_correlation(data=datamatrix, column_sep = "W",
            method="spearman")
```

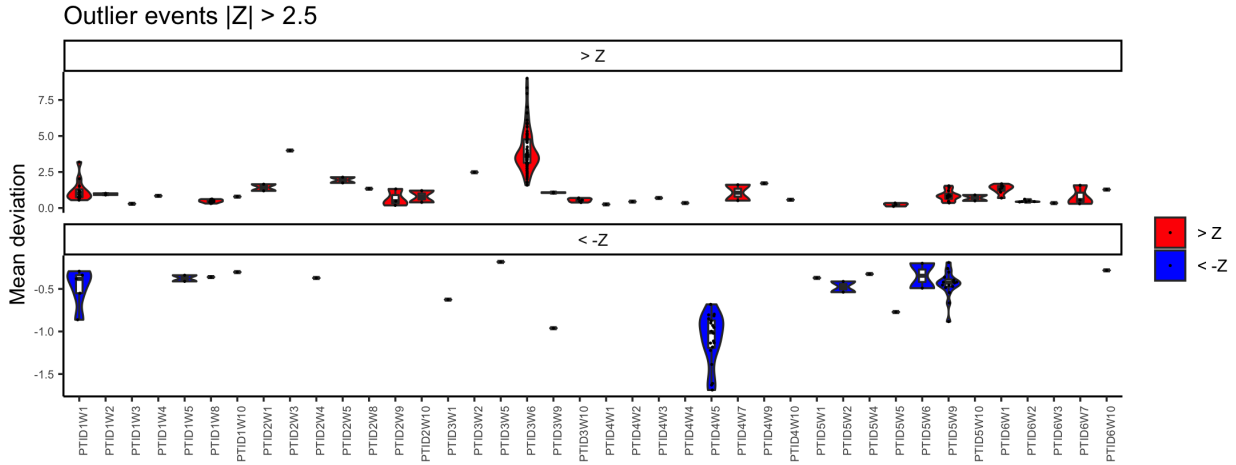


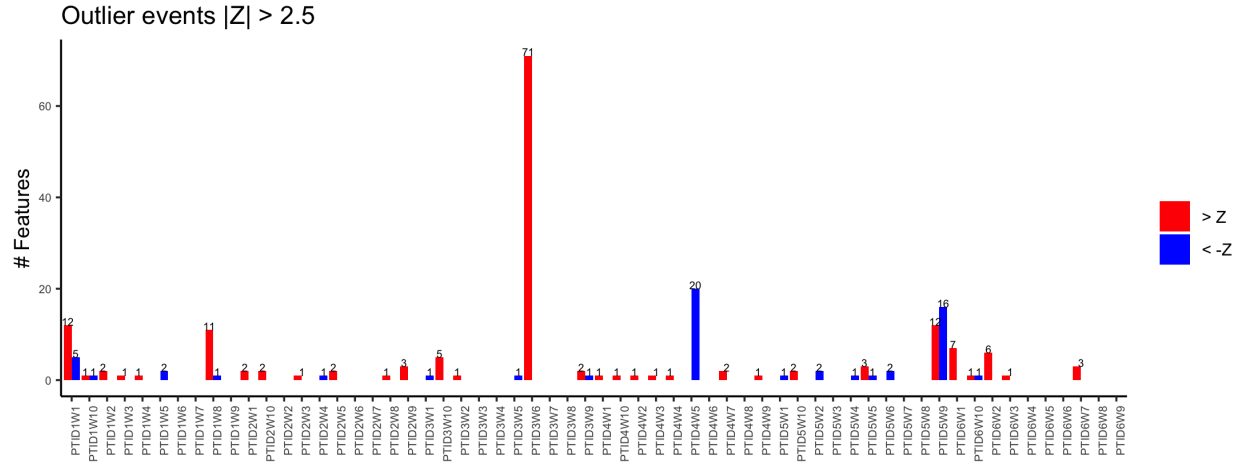

```
#Detect outliers (if any)
outlier_res <- outlierDetect(ann=metadata, mat=datamatrix, z_cutoff=2)

head(outlier_res)
#  exp  Sample  PTID Time    Sex      gene  meanDev        z
#PTID3W643 10.729880 PTID3W6 PTID3   W6 Female     IFI30 8.340474 2.845471
#PTID3W627 10.133645 PTID3W6 PTID3   W6 Female     DPEP2 6.595705 2.844629
#PTID3W631 10.188437 PTID3W6 PTID3   W6 Female      FCAR 7.004890 2.844607
#PTID3W626  7.656418 PTID3W6 PTID3   W6 Female     DPEP1 4.574449 2.844574
#PTID3W685  9.129149 PTID3W6 PTID3   W6 Female TNFRSF13C 6.605767 2.844410 2.844410
#PTID3W652  8.031215 PTID3W6 PTID3   W6 Female   KIR2DL3 5.156982 2.844018

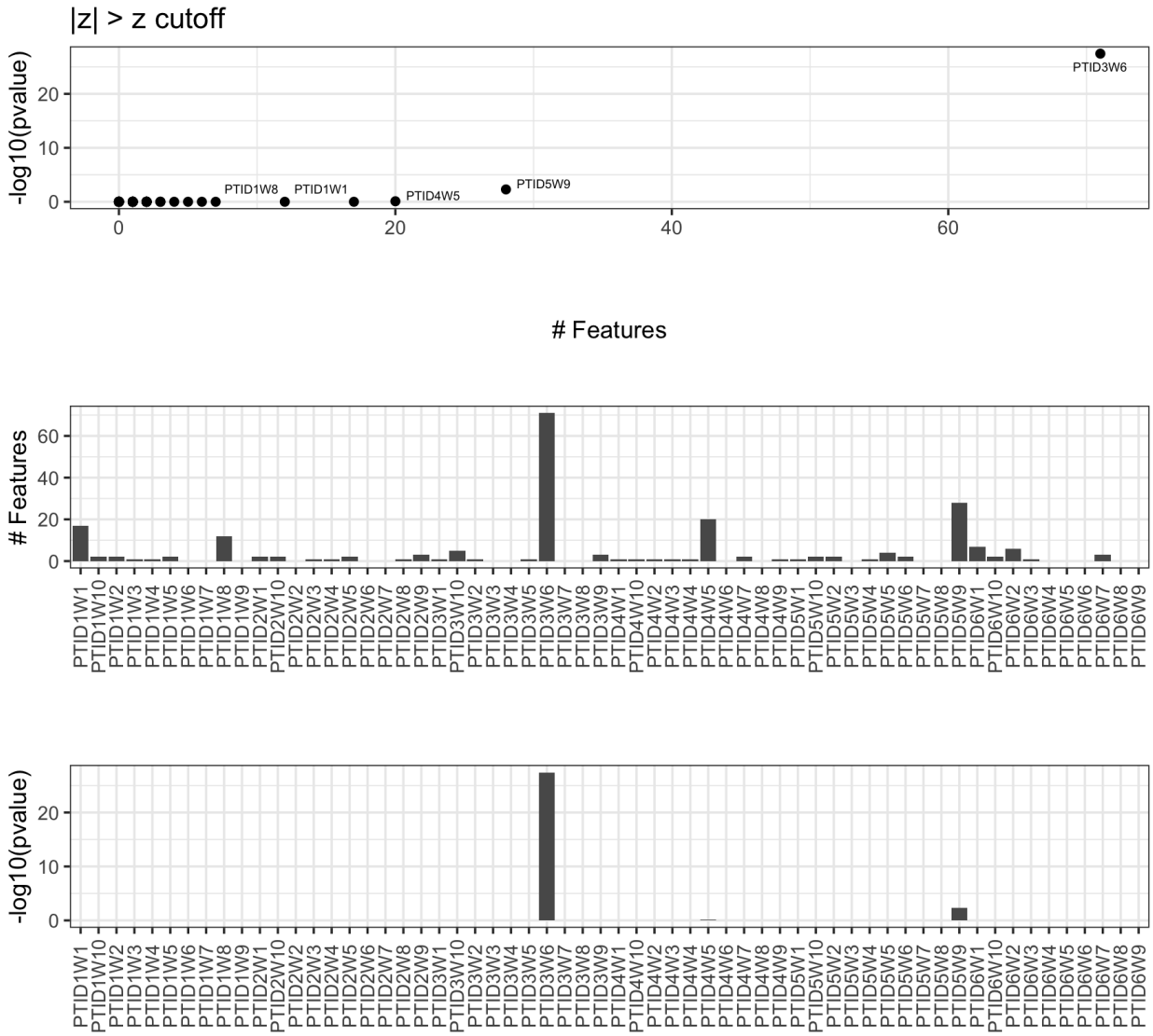
#Stringent SD
outlier_res <- outlierDetect(ann=metadata, mat=datamatrix, z_cutoff= 2.5)
df <- data.frame(table(outlier_res$Sample))
df <- df[order(df$Freq, decreasing = T),]
head(df)
#Var1 Freq
#PTID3W6   71
#PTID5W9   28
#PTID4W5   20
#PTID1W1   17
#PTID1W8   12
#PTID6W1    7
```







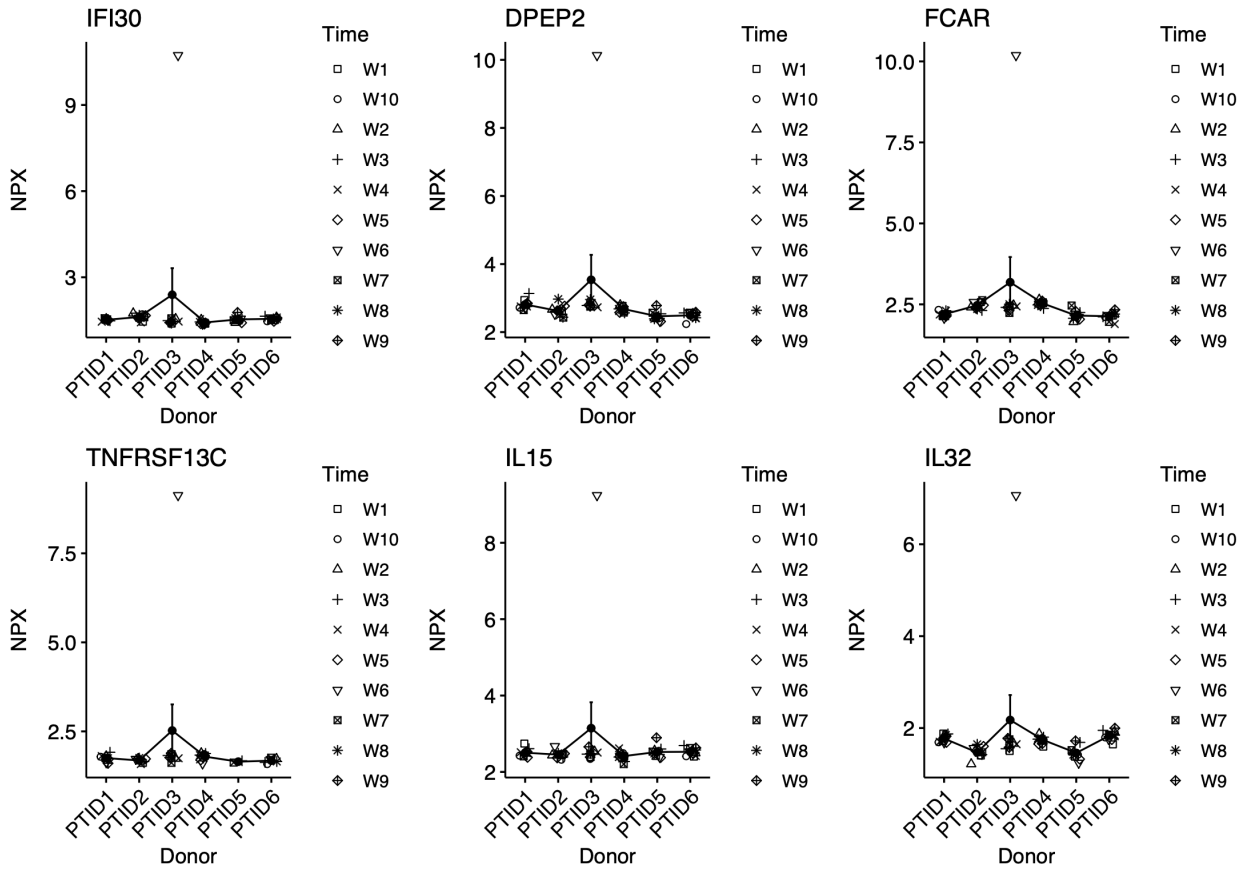

```
#Calculate p value
outlierDetectP(outlier_events=outlier_res, z_cutoff=2.5, nGenes=1042)
```




```
#### Gene plot (probable outliers)
plots <- genePlot(ann=metadata, data=datamatrix, geneName="IFI30")
plots[[5]]

#or
genelist <- c("IFI30", "DPEP2","FCAR", "TNFRSF13C", "IL15", "IL32")
splots <- list()
for(i in 1:length(genelist)) {
   geneName <- genelist[i]
   df <- data.frame(exp=as.numeric(datamatrix[geneName,]), metadata, stringsAsFactors = F)
   df$PTID <- factor(df$PTID, levels = uniSample)
   plot1 <- ggline(df, x = "PTID", y = "exp", add.params = list(shape="Time"),
   add = c("mean_se", "jitter"), ylab = "NPX", xlab = "Donor",
   title = geneName, legend = "right", outlier.shape = NA) +
   scale_shape_manual(values = 0:10) +
   theme(axis.text.x = element_text(angle=45, hjust = 1,
   vjust = 1))
```

```
   splots[[i]] <- plot1
}
plot_grid(plotlist=splots, ncol= 3, align="hv")
```



3.2 Tutorial-2: scRNA longitudinal data (n=4 and 6 weeks follow-up)

This tutorial allows users to explore single cell RNAseq data measured from 4 healthy donors over 6 time points (week 2-7). Single cell data available at GSE190992. (1) pbmc_longitudinal_data (Normalized scRNA seurat object) (2) data_Annotation.Rda (clinical metadata). Longitudinal data set includes 4 donors and 24 samples. To infer iner-donor, intra-donor variations, and stable features, please follow following steps.

3.2.1 Load Library

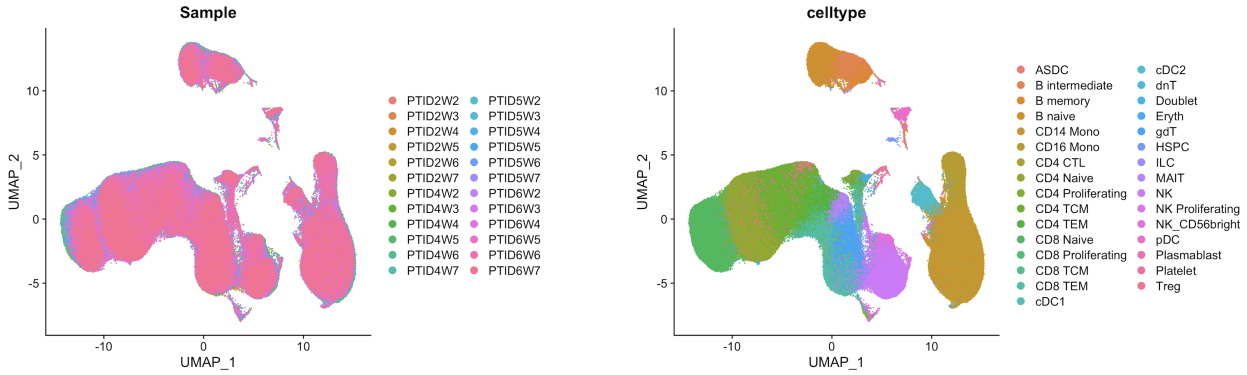
```
#Load Library
library("PALM")
library("Hmisc")
library("ggpubr")
library("Seurat")
```

3.2.2 Load data and assign paramaters

```
#scRNA seurat object
pbmc <- readRDS("data/AIFI-scRNA-PBMC-FinalData.RDS")
```

```
metaData <- pbmc@meta.data
pbmc@meta.data$Sample <- pbmc@meta.data$orig.ident

#UMAP plot
p1 <- DimPlot(object = pbmc, reduction = 'umap', group.by = "Sample", label = F)
p2 <- DimPlot(object = pbmc, reduction = 'umap', group.by = "celltype", label = F)
print(plot_grid(p1, p2, align="hv", ncol=2))
```



3.2.3 Load annotation data

```
#Clinical metadata/annotation
load("data/data_Metadata.Rda")
metadata=ann
row.names(metadata) <- metadata$Sample

#Parameters
dataObj <- pbmc
featureSet <- c("PTID", "Time")
avgGroup <- "celltype"
housekeeping_genes <- c("GAPDH", "ACTB")

#Celltypes observed in dataset
cell_type <- sort(unique(pbmc@meta.data$celltype))
#Celltypes selected for analysis consisting atleast >5% of cells in each celltype.
group_oi <- c("CD4_Naive","CD4_TEM","CD4_TCM","CD4_CTL","CD8_Naive",
              "CD8_TEM","CD8_TCM","Treg","MAIT","gdT",
              "NK", "NK_CD56bright",
              "B_naive", "B_memory", "B_intermediate",
              "CD14_Mono","CD16_Mono",
              "cDC2","pDC")
```

3.2.4 Create output directory

```
outputDirectory <- "output"
filePATH <- paste(getwd(), "/",outputDirectory, sep="")
dir.create(file.path(getwd(), outputDirectory), showWarnings = FALSE)
```

3.2.5 Sample overlap

```
overlap <- intersect(metadata$Sample, dataObj@meta.data$Sample)
```

```
metadata <- metadata[overlap,]
#in-case subset of samples only
dataObj <- subset(x = dataObj, subset = Sample %in% overlap)
```

3.2.6 Aggregate data at celltypes (pseudo-bulk)

3.2.7 For single cell data merge annotation and single cell metadata

```
metaData <- dataObj@meta.data
metadata1 <- metadata[metaData$Sample,]
metaData <- cbind(metaData, metadata1)
dataObj@meta.data <- metaData
```

3.2.8 Define sample group and Calculate average expression

```
dataObj@meta.data$Sample_group <- paste(dataObj@meta.data$Sample,
                            dataObj@meta.data[,avgGroup], sep=":")
dataObj@meta.data$Sample_group <- gsub(" ", "_", dataObj@meta.data$Sample_group)
metaData <- dataObj@meta.data
```

3.2.9 Calculate average expression across group/celltype

```
Idents(dataObj) <- "Sample_group"
table(Idents(dataObj))
scrna_avgmat <- avgExpCalc(dataObj=dataObj, group.by="Sample_group")
```

3.2.10 Keep genes with avgExpression > zero

```
rowDF <- rowSums(scrna_avgmat)
rowDF <- rowDF[rowDF > 0]
mat <- scrna_avgmat[names(rowDF),]
```

3.2.11 Create annotation

```
cn <- data.frame(Sample_group=colnames(mat))
temp <- data.frame(do.call(rbind, strsplit(cn$Sample_group, split = ":")), stringsAsFactors = F)
cn <- data.frame(cn, Sample=temp$X1, group=temp$X2, stringsAsFactors = F)
row.names(cn) <- cn$Sample_group
cn <- merge(cn, metadata, by="Sample", all=TRUE)
cn <- cn[!is.na(cn$Sample_group),]
row.names(cn) <- cn$Sample_group
ann <- cn
ann$Sample_group_i <- paste(ann$group, ann$PTID, sep=":")
rm(cn)
```

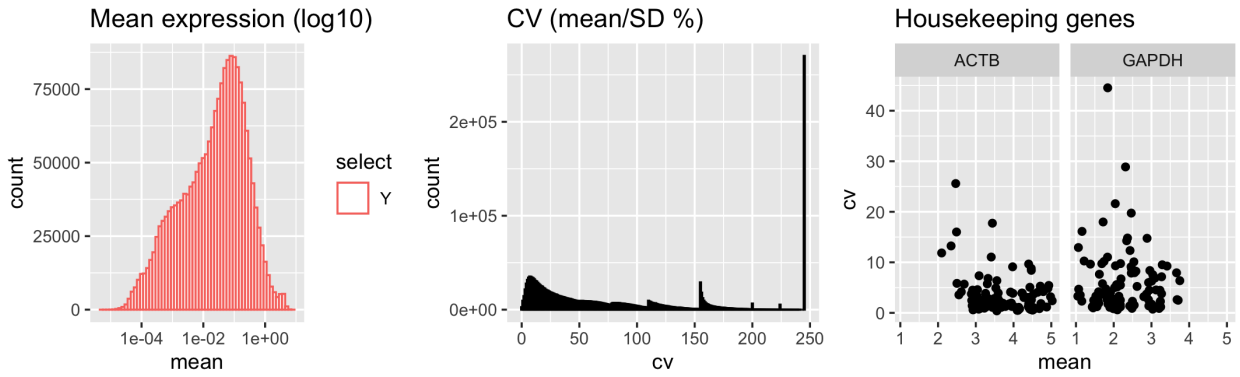
3.2.12 Final matrix

```
Overlap <- intersect(colnames(mat), row.names(ann))
ann <- ann[Overlap,]
mat <- mat[,Overlap]
```

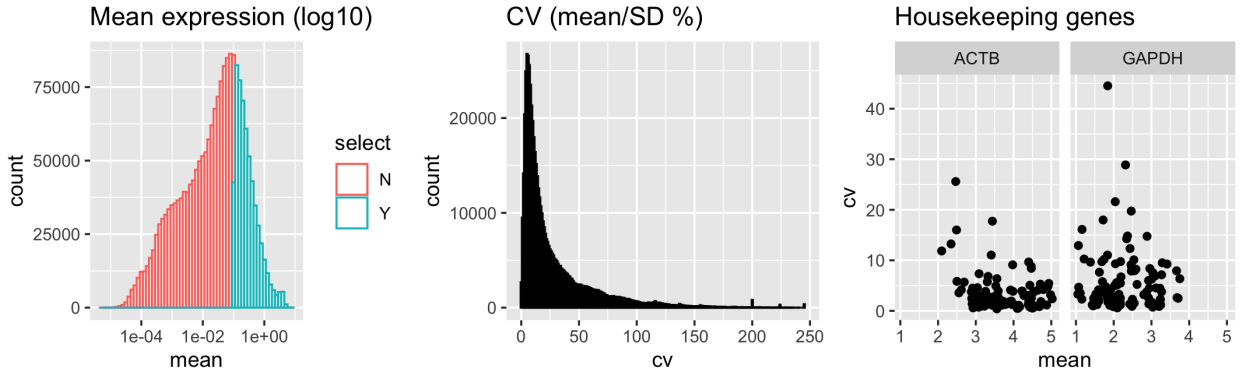
```
print(sort(unique(ann$group)))
#[1] "ASDC"               "B_intermediate"    "B_memory"
#[4] "B_naive"            "CD14_Mono"         "CD16_Mono"
#[7] "CD4_CTL"            "CD4_Naive"         "CD4_Proliferating"
#[10] "CD4_TCM"            "CD4_TEM"           "CD8_Naive"
#[13] "CD8_Proliferating" "CD8_TCM"           "CD8_TEM"
#[16] "cDC1"               "cDC2"              "dnT"
#[19] "Doublet"            "Eryth"             "gdT"
#[22] "HSPC"               "ILC"               "MAIT"
#[25] "NK"                 "NK_CD56bright"     "NK_Proliferating"
#[28] "pDC"                "Plasmablast"       "Platelet"
#[31] "Treg"
```

3.2.13 CV profile

```
#Check the mean expression and CV cross groups (celltypes)
cv_profile <- cvprofile(mat=mat, ann=ann)
```



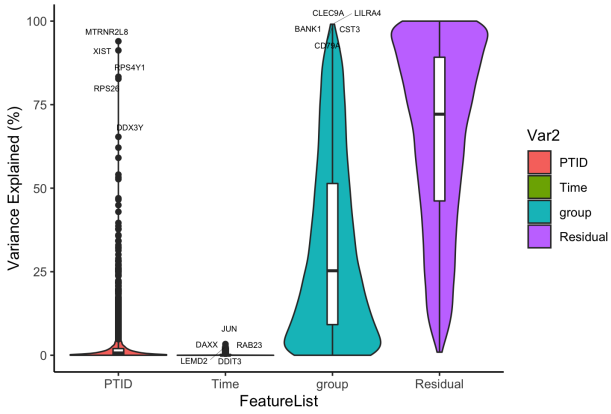

```
#Lowly expressed genes show abnormal CV, which needs to be filtered.
cv_profile <- cvprofile(mat=mat, ann=ann, meanThreshold = 0.1)
```




```
#Sample Celltype Mean-CV plot (check output folder)
cvSampleprofile(mat=mat, ann=ann, meanThreshold = 0.1, cvThreshold = 10)
```

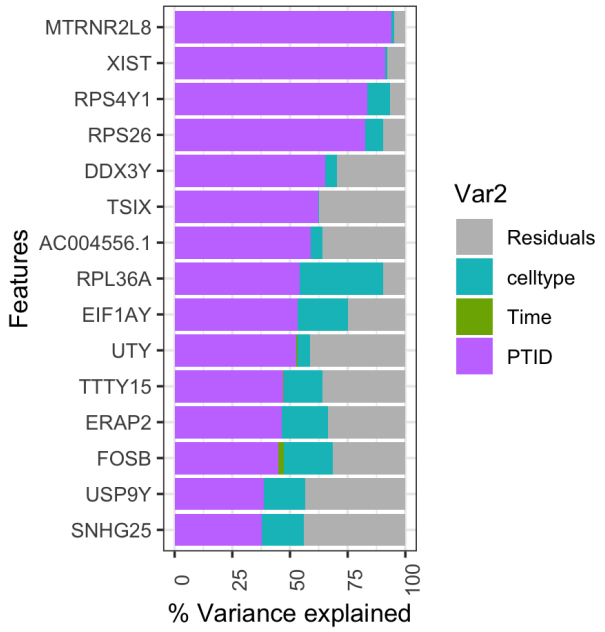
3.2.14 Features contributing towards donor variations

```
#Variance decomposition
lmem_res <- lmeVariance(ann=ann, mat=mat,
            featureSet=c(featureSet,"group"),
            meanThreshold=0.1,
            fileName="scRNA", filePATH=filePATH)
res <- lmem_res[,c("PTID","Time","group","Residual")]
colnames(res) <- c("PTID","Time","celltype","Residuals")
res <- res*100 #in percentage
```

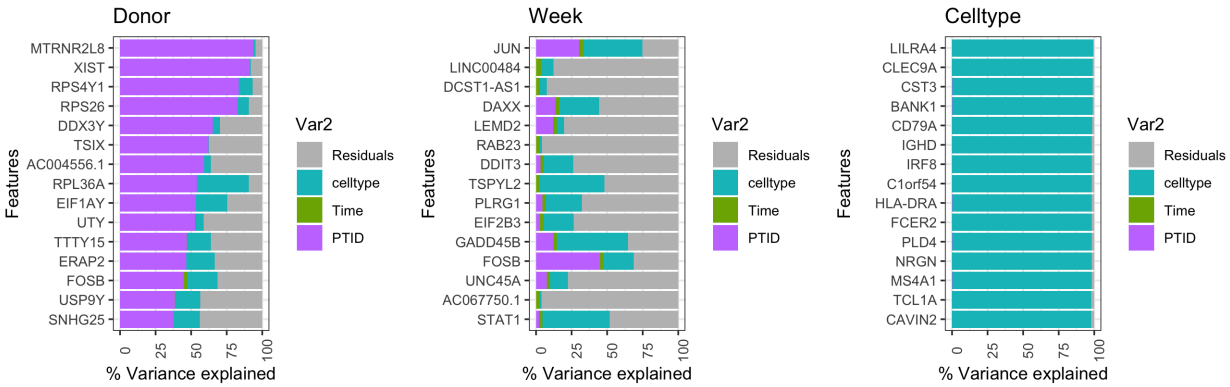


3.2.15 Donor-specific variance

```
#Donor-specific
df1 <- filter(res, PTID>Time & PTID>celltype & Residuals < 50)
df1 <- df1[order(df1$PTID, decreasing = T),]
df <- melt(data.matrix(df1[1:15,]))
df$Var2 <- factor(df$Var2, levels = rev(colnames(res)))
df$Var1 <- factor(df$Var1, levels = rev(unique(df$Var1)))
plot1 <- ggplot(df, aes(x=Var1, y=value, fill=Var2)) +
  geom_bar(stat="identity", position="stack") + labs(x="Features", y="% Variance explained") +
  scale_fill_manual(values = c("PTID"="#C77CFF", "celltype"="#00BFC4", "Time"="#7CAE00", "Residuals"="gr
  theme_bw() + theme(axis.text.x = element_text(angle=90, hjust = 0.5, vjust = 1),legend.position = "rig
  coord_flip()
print(plot1)
```

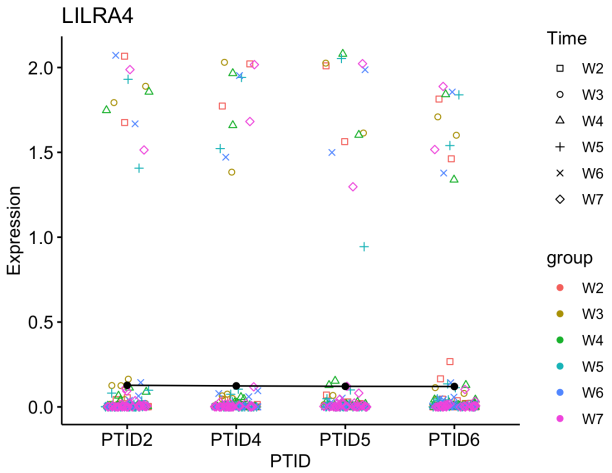



```
#Similar procedure applied to get Time- and celltype-attributed variance features
```

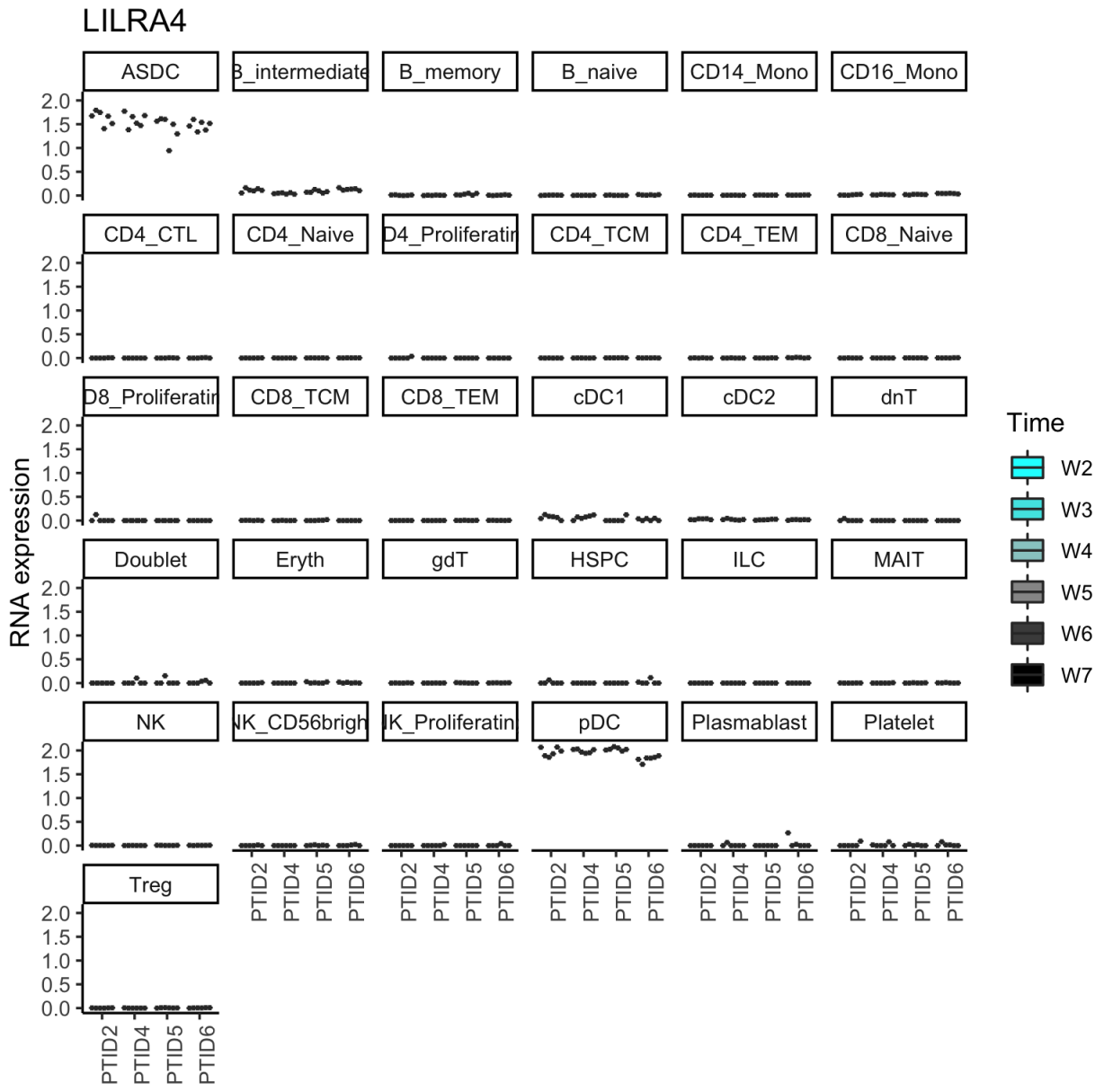


3.2.16 Plot the top features

```
plots <- genePlot(ann=ann, data=mat, geneName="LILRA4", groupName="Time")
print(plots$plot4)
```



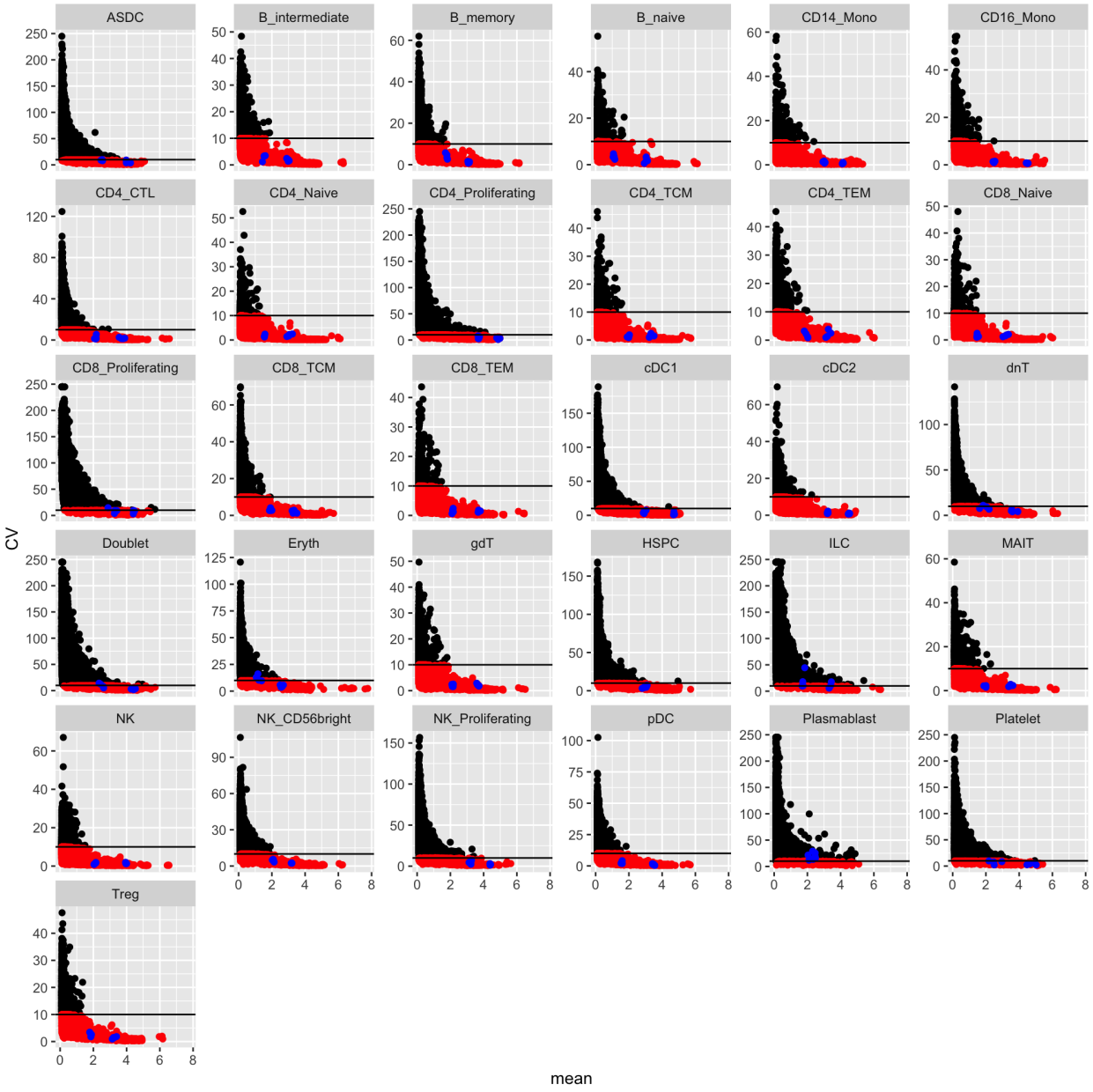

```
plots <- genePlot(ann=ann, data=mat, geneName="LILRA4", groupName="group")
print(plots$plot5)
```



3.2.17 Intra-donor variations over time

```
#Calculate CV
cv_res <- cvCalcSC(mat=mat, ann=ann, meanThreshold=0.1,
          cvThreshold=10, housekeeping_genes=housekeeping_genes,
          filePATH=filePATH, fileName="scrna")

#Plots saved in user-defined output directory
```



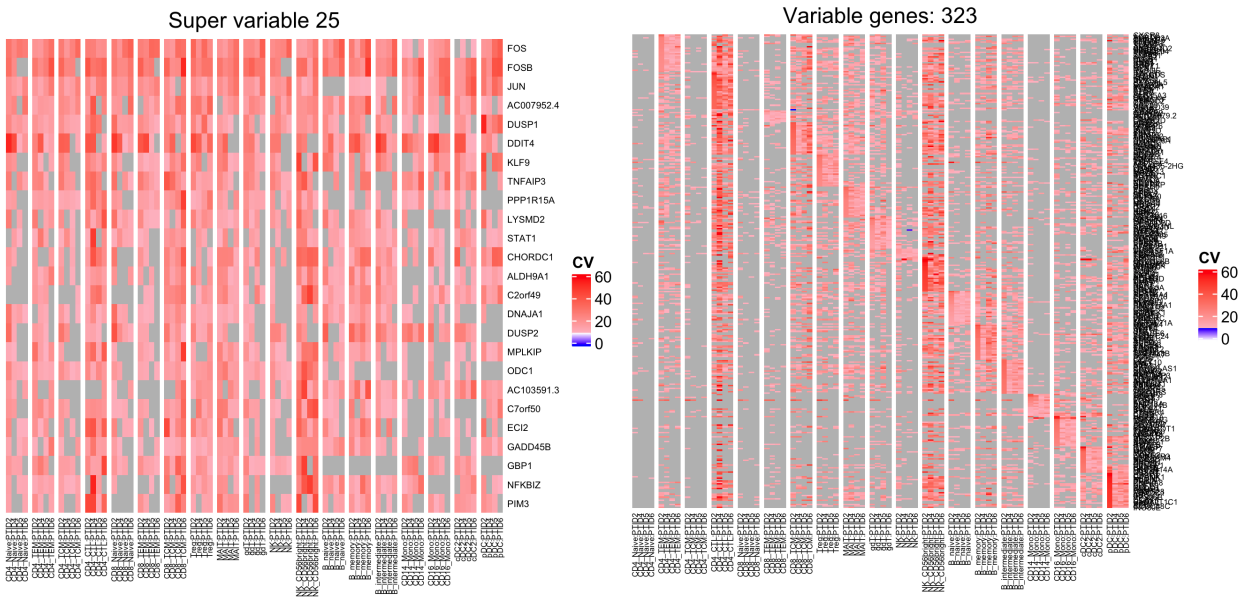
CV profile in celltypes (black) as well as CV for house-keeping genes (blue). Based on CV of house-keeping genes 10% CV cut-off used and genes considered stable elow 10% CV.

3.2.18 Find stable and variable features in longitudinal data

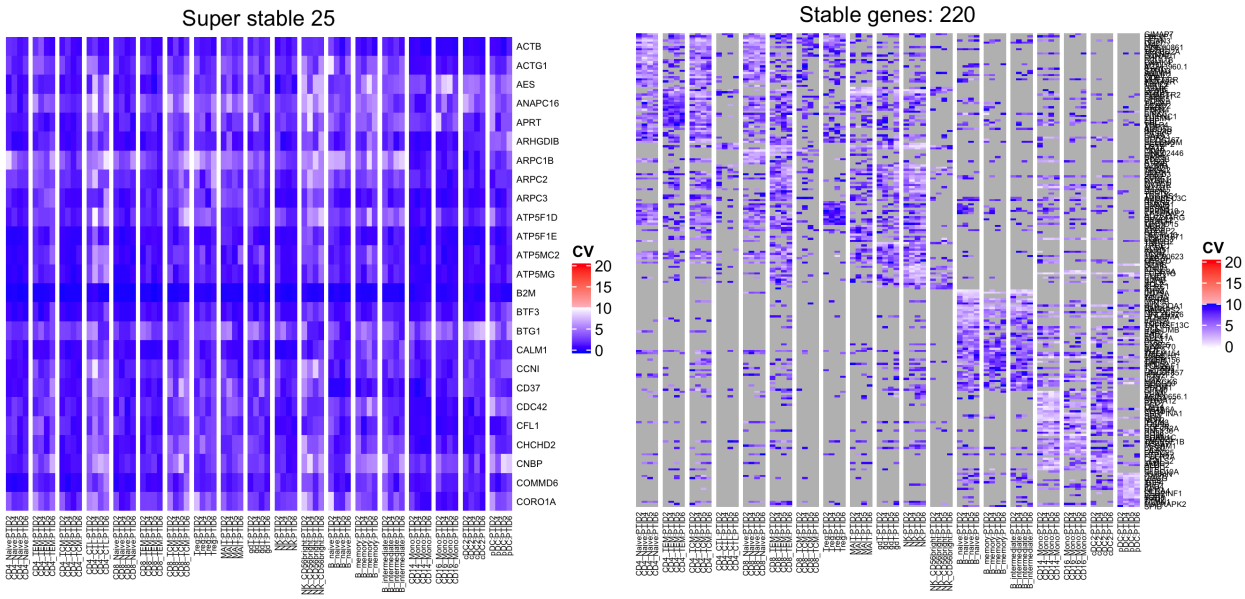
```
donorThreshold <- 4
groupThreshold <- 40 #number of donors * number of celltypes/2 (4x19/2)
topFeatures <- 25
var_gene <- VarFeatures(ann=ann, group_oi=group_oi,
            meanThreshold=0.1, cvThreshold=10,
            donorThreshold=donorThreshold,
            groupThreshold=groupThreshold,
            topFeatures=topFeatures,
            fileName="scrna", filePATH=filePATH)
```

```
stable_gene <- StableFeatures(ann=ann, group_oi=group_oi,
                meanThreshold=0.1,
                cvThreshold=10,
                donorThreshold=donorThreshold,
                groupThreshold=groupThreshold,
                topFeatures=topFeatures,
                fileName="scrna", filePATH=filePATH)
```

Variable genes observed in longitudinal data (CV>10%)

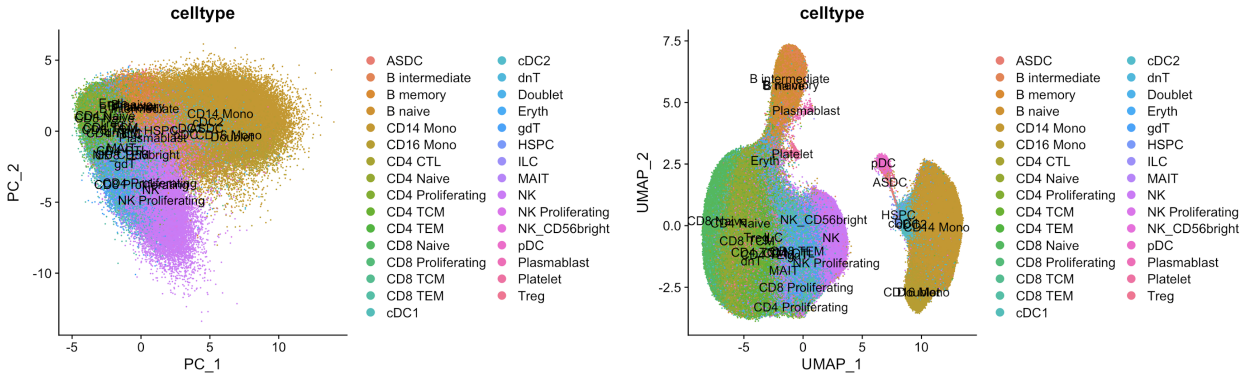


Stable genes observed in longitudinal data (CV<10%)

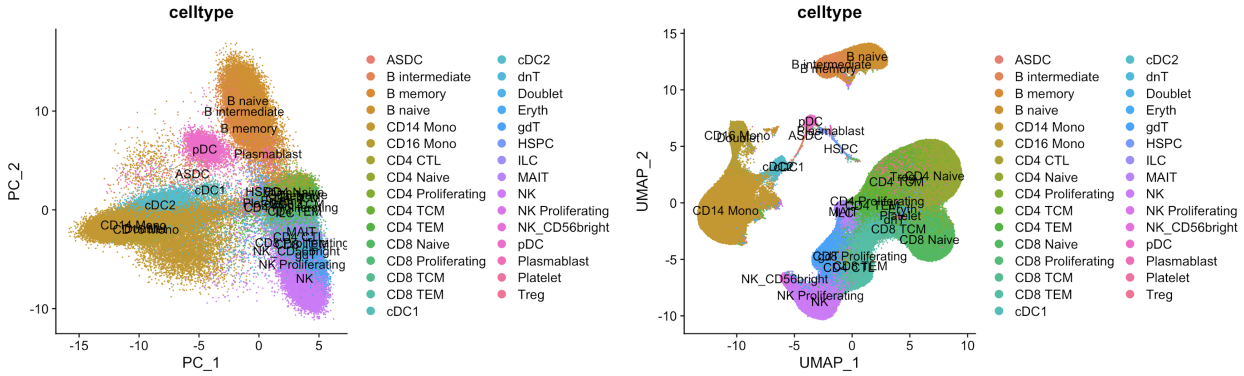


3.2.19 UMAP Plot (Stable/variable)

```
#Top variable and stable features used for UMAP
rnaObj <- dimUMAPPlot(rnaObj=dataObj, nPC=15,
          gene_oi=unique(var_gene$gene), groupName=avgGroup,
          plotname="variable", fileName="scrna",
          filePATH=filePATH)
```

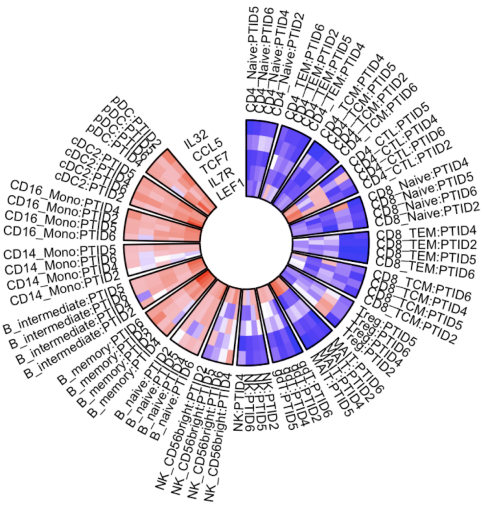



```
rnaObj <- dimUMAPPlot(rnaObj=dataObj, nPC=15,
          gene_oi=unique(stable_gene$gene), groupName=avgGroup,
          plotname="stable", fileName="scrna",
          filePATH=filePATH)
```



3.2.20 Circular gene expression plot

```
load("output/scrna-CV-allgenes-raw.Rda")
geneList <- c("IL32","CCL5","TCF7","IL7R","LEF1") #T-cell
res <- genecircosPlot(data=cv_res, geneList=geneList, groupBy=group_oi)
```



3.3 Tutorial-3: scATAC Longitudinal data (n=4 and 6 weeks follow-up)

This tutorial allows users to explore single cell ATACseq genscore data measured from 4 healthy donors over 6 timepoints (week 2-7). Single cell ATAC data available at GSE190992. (1) pbmc_scatac_archr_genescore_longitudinal_data (2) data_Annotation.Rda (clinical metadata). Longitudinal dataset have 4 donors and 18 samples. To infer the variations at single cell ATAC please follow following steps.

3.3.1 Load Library

```
#Load Library
library("PALM")
library("Hmisc")
library("ggpubr")
```

3.3.2 Load data and assign paramaters

```
#scATAC object
#Load genescorematrix from archR or relevant tools (Aggregate data at celltypes (pseudo-bulk))
load("data/AIFI-scATAC-PBMC-FinalData.Rda")
boxplot(log2(scatac_gm[,1:50]+1), las=2)

#Load annotation data
load("data/data_Metadata.Rda")
metadata <- ann
row.names(metadata) <- metadata$Sample
```

3.3.3 Parameters

```
atacObj <- log2(scatac_gm+1) #Normalized genescore
featureSet=c("PTID", "Time")
avgGroup="celltype"
housekeeping_genes <- c("GAPDH", "ACTB")
fileName <- "scatac"
#Celltypes to be considered (selected in scRNA)
```

```
group_oi <- c("CD4_Naive","CD4_TEM","CD4_TCM","CD4_CTL","CD8_Naive",
              "CD8_TEM","CD8_TCM","Treg","MAIT","gdT",
              "NK", "NK_CD56bright",
              "B_naive", "B_memory", "B_intermediate",
              "CD14_Mono","CD16_Mono", "cDC2","pDC")
```

3.3.4 Create output directory

```
outputDirectory <- "output"
filePATH <- paste(getwd(), "/",outputDirectory, sep="")
dir.create(file.path(getwd(), outputDirectory), showWarnings = FALSE)
```

3.3.5 Sample overlap

```
#Get the annotation
temp <- data.frame(do.call(rbind, strsplit(colnames(atacObj),
split = ":")), stringsAsFactors = F)
cn <- data.frame(id=colnames(atacObj), Sample=temp$X1, group=temp$X2,
check.names=F, stringsAsFactors = F)
cn <- cn[cn$Sample %in% metadata$Sample,]
overlap <- as.character(unique(cn$Sample))
atac_overlap <- cn$id

#Sample overlap
metadata <- metadata[overlap,]
atacObj <- atacObj[,atac_overlap]
```

3.3.6 Check data

```
#Keep genes with avgExpression > zero
rowDF <- rowSums(atacObj)
rowDF <- rowDF[rowDF > 0]
mat <- atacObj[names(rowDF),]
```

3.3.7 Create annotation

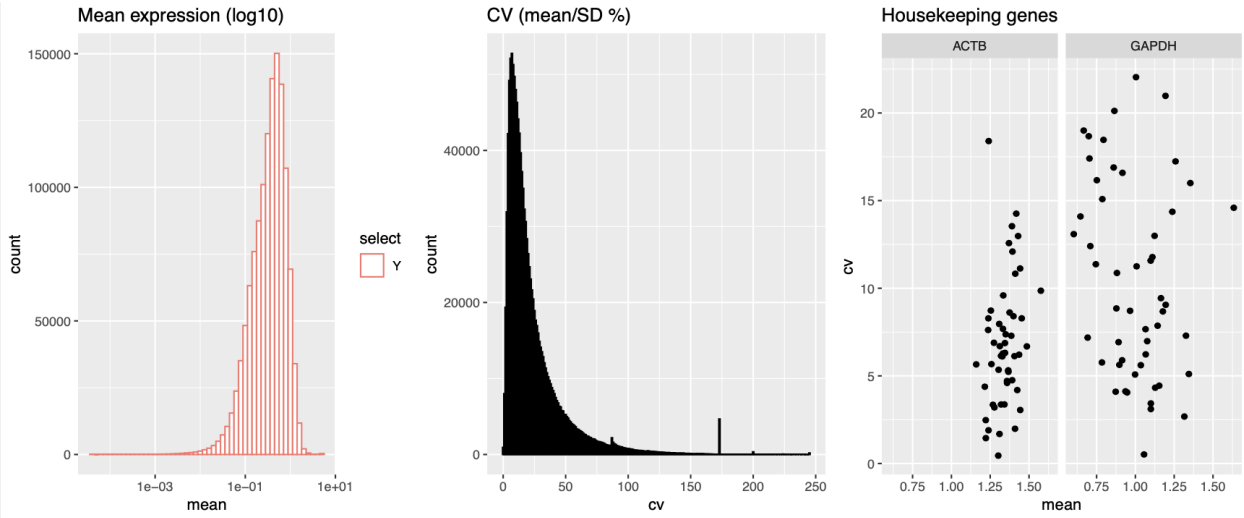
```
cn <- data.frame(Sample_group=colnames(mat))
temp <- data.frame(do.call(rbind, strsplit(cn$Sample_group,
        split = ":")), stringsAsFactors = F)
cn <- data.frame(cn, Sample=temp$X1, group=temp$X2,
        stringsAsFactors = F)
row.names(cn) <- cn$Sample_group
cn <- merge(cn, metadata, by="Sample", all=TRUE)
cn <- cn[!is.na(cn$Sample_group),]
row.names(cn) <- cn$Sample_group
ann <- cn
ann$Sample_group_i <- paste(ann$group, ann$PTID, sep=":")
rm(cn)
```

3.3.8 Final matrix

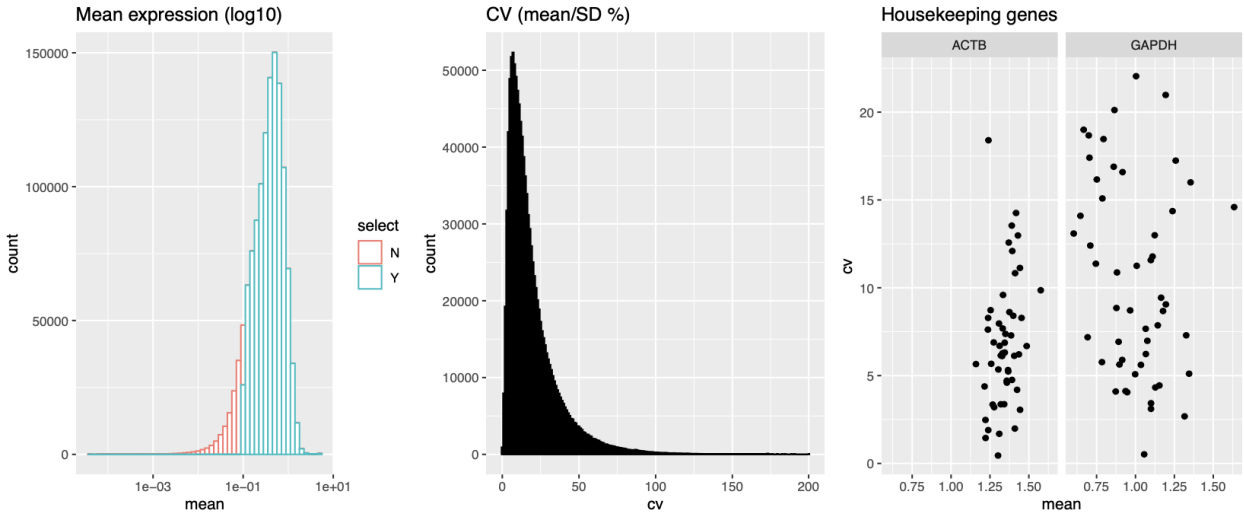
```
Overlap <- intersect(colnames(mat), row.names(ann))
ann <- ann[Overlap,]
mat <- mat[,Overlap]
print(sort(unique(ann$group)))
#[1] "B_intermediate" "B_naive" "CD14_Mono" "CD16_Mono"
#[5] "CD4_Naive" "CD4_TCM" "CD8_Naive" "CD8_TEM"
#[9] "cDC2" "gdT" "MAIT" "NK"
#[13] "NK_CD56bright" "pDC"
```

3.3.9 CV profile

```
cv_profile <- cvprofile(mat=mat, ann=ann, housekeeping_genes=housekeeping_genes)
```



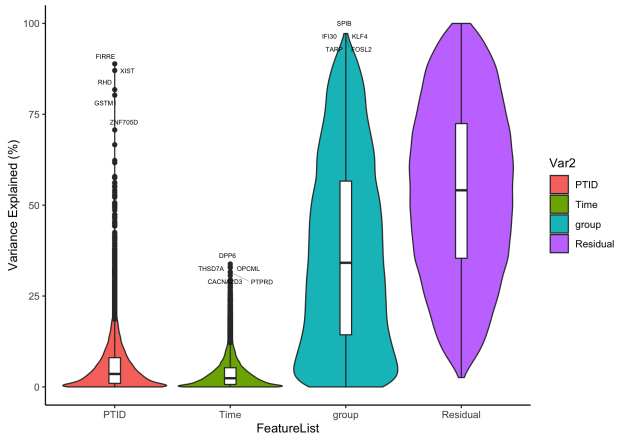

```
cv_profile <- cvprofile(mat=mat, ann=ann, housekeeping_genes=housekeeping_genes, meanThreshold = 0.1)
```



```
#Sample Celltype Mean-CV plot
cv_sample_profile <- cvSampleprofile(mat=mat, ann=ann, meanThreshold = 0.1, cvThreshold = 10)
#plots saved in output directory
```

3.3.10 Features contributing towards donor variations

```
lmem_res <- lmeVariance(ann=ann, mat=mat,
            featureSet=c(featureSet,"group"),
            meanThreshold=0.1,
            fileName=fileName,
            filePATH=filePATH)
res <- lmem_res[,c("PTID","Time","group","Residual")]
colnames(res) <- c("PTID","Time","celltype","Residuals")
res <- res*100 #in percentage
```



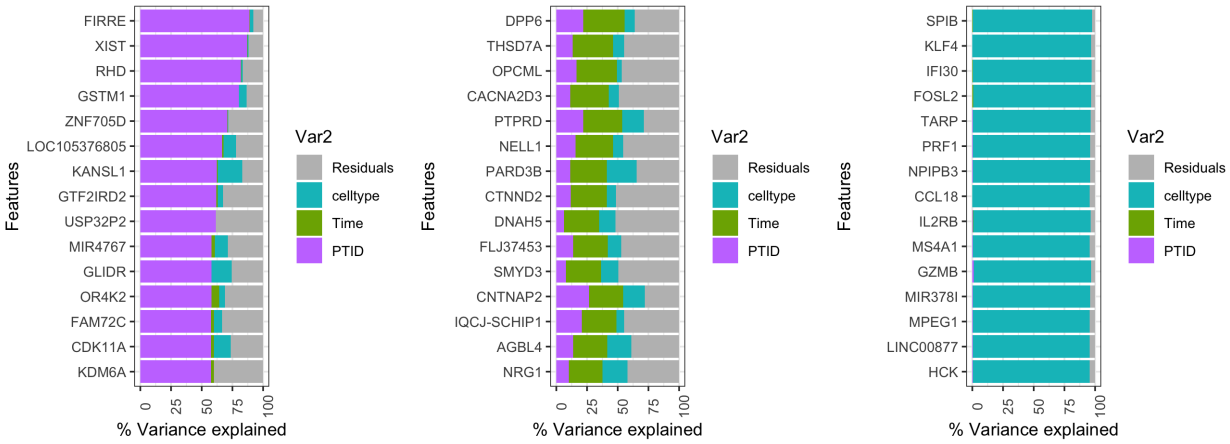

```
#Donor-specific
df1 <- filter(res, PTID>Time & PTID>celltype & Residuals < 50)
df1 <- df1[order(df1$PTID, decreasing = T),]
df <- melt(data.matrix(df1[1:15,])) #select Top15
df$Var2 <- factor(df$Var2, levels = rev(colnames(res)))
df$Var1 <- factor(df$Var1, levels = rev(unique(df$Var1)))
p1 <- ggplot(df, aes(x=Var1, y=value, fill=Var2)) +
  geom_bar(stat="identity", position="stack") +
  labs(x="Features", y="% Variance explained") +
  scale_fill_manual(values = c("PTID"="#C77CFF", "celltype"="#00BFC4",
  "Time"="#7CAE00", "Residuals"="grey")) +
  theme_bw() + theme(axis.text.x = element_text(angle=90, hjust = 0.5,
  vjust = 1),legend.position = "right") +
  coord_flip()

#Time-specific
df2 <- filter(res, PTID<Time & Time>celltype)
df2 <- df2[order(df2$Time, decreasing = T),]
df <- melt(data.matrix(df2[1:15,])) #select Top15
df$Var2 <- factor(df$Var2, levels = rev(colnames(res)))
df$Var1 <- factor(df$Var1, levels = rev(unique(df$Var1)))
p2 <- ggplot(df, aes(x=Var1, y=value, fill=Var2)) +
  geom_bar(stat="identity", position="stack") +
```

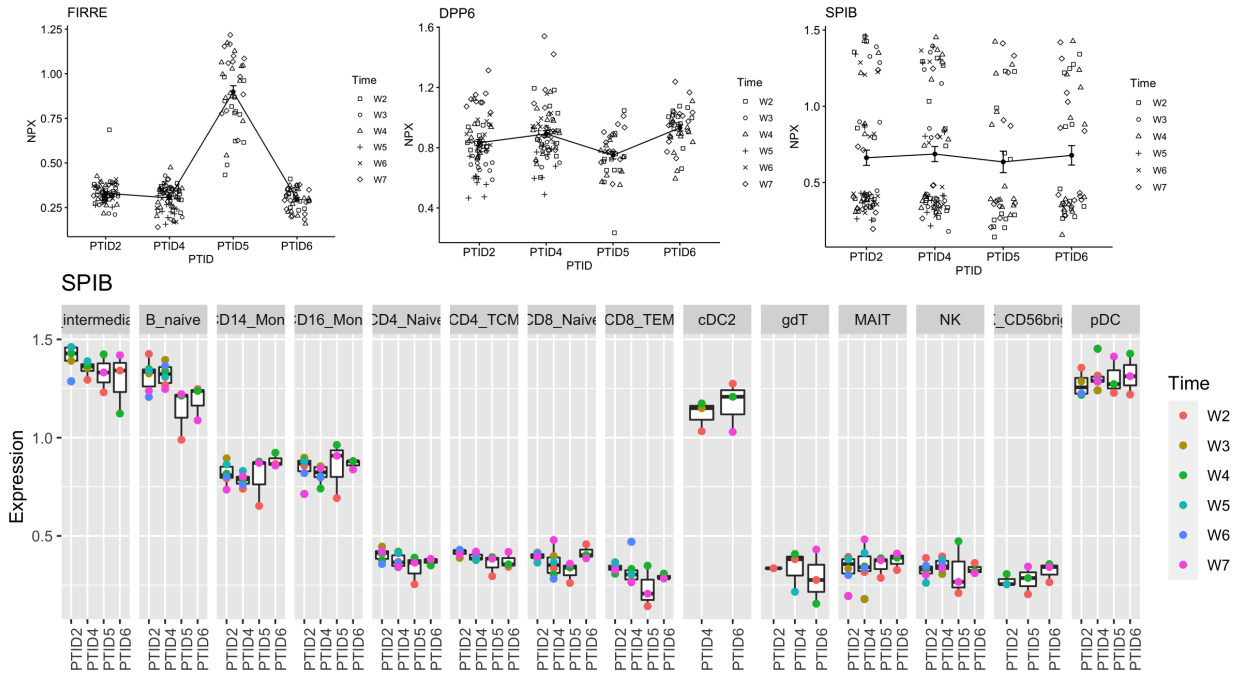
```
  labs(x="Features", y="% Variance explained") +
  scale_fill_manual(values = c("PTID"="#C77CFF", "celltype"="#00BFC4",
  "Time"="#7CAE00", "Residuals"="grey")) +
  theme_bw() + theme(axis.text.x = element_text(angle=90, hjust = 0.5,
  vjust = 1),legend.position = "right") +
  coord_flip()

#Celltype-specific
df3 <- filter(res, celltype>PTID & celltype>Time & Residuals < 50)
df3 <- df3[order(df3$celltype, decreasing = T),]
df <- melt(data.matrix(df3[1:15,])) #select Top15
df$Var2 <- factor(df$Var2, levels = rev(colnames(res)))
df$Var1 <- factor(df$Var1, levels = rev(unique(df$Var1)))
p3 <- ggplot(df, aes(x=Var1, y=value, fill=Var2)) +
  geom_bar(stat="identity", position="stack") +
  labs(x="Features", y="% Variance explained") +
  scale_fill_manual(values = c("PTID"="#C77CFF", "celltype"="#00BFC4",
  "Time"="#7CAE00", "Residuals"="grey")) +
  theme_bw() + theme(axis.text.x = element_text(angle=90, hjust = 0.5,
  vjust = 1),legend.position = "right") +
  coord_flip()

#Plot
plot_grid(p1,p2,p3, align="hv", ncol=3)
```

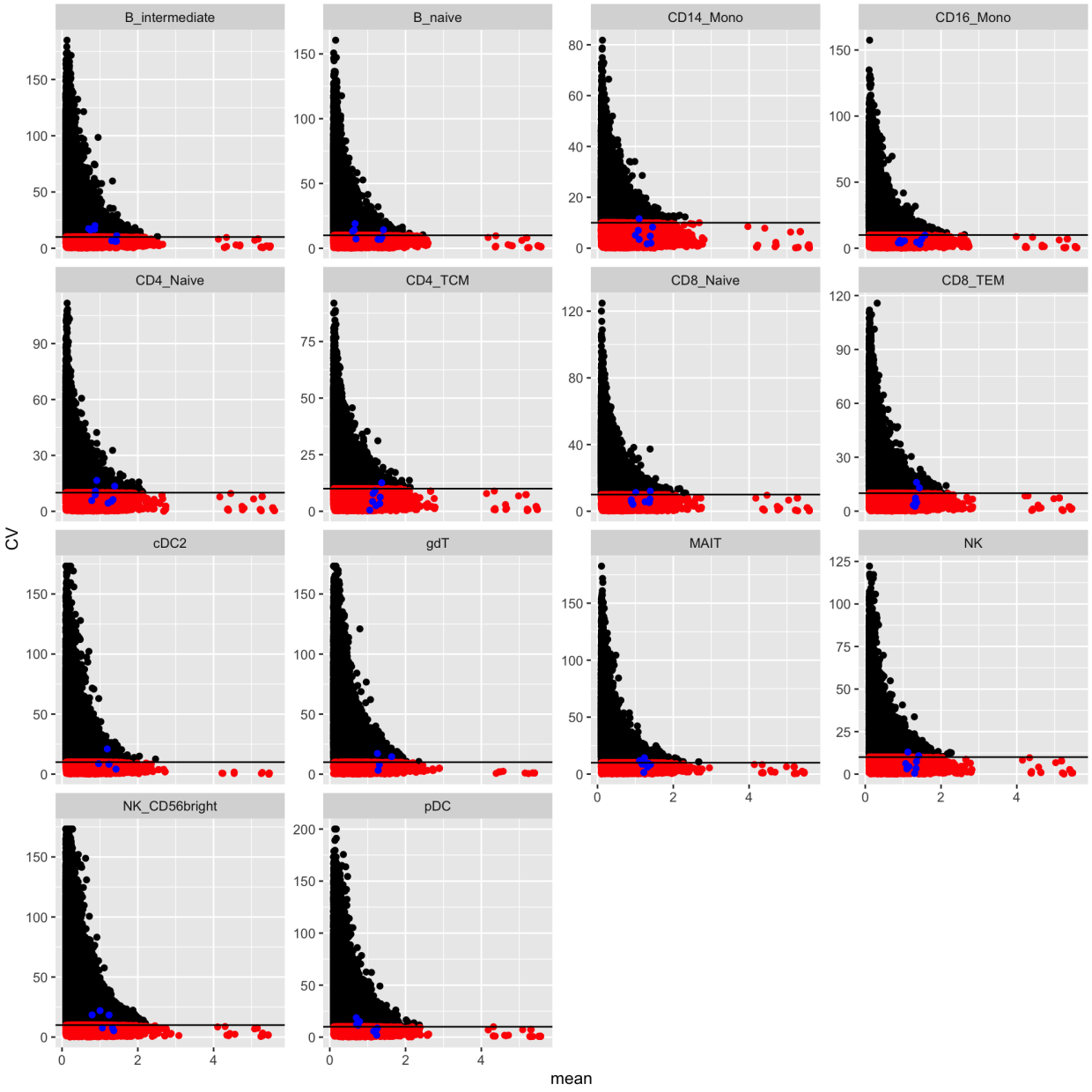



```
#Top genes
plots <- genePlot(ann, mat, geneName="FIRRE", groupName="group")
print(plots$plot1)
print(plots$plot2)
print(plots$plot3)
print(plots$plot4)
print(plots$plot5)
plots <- genePlot(ann, mat, geneName="DPP6", groupName="group")
plots <- genePlot(ann, mat, geneName="SPIB", groupName="group")
```



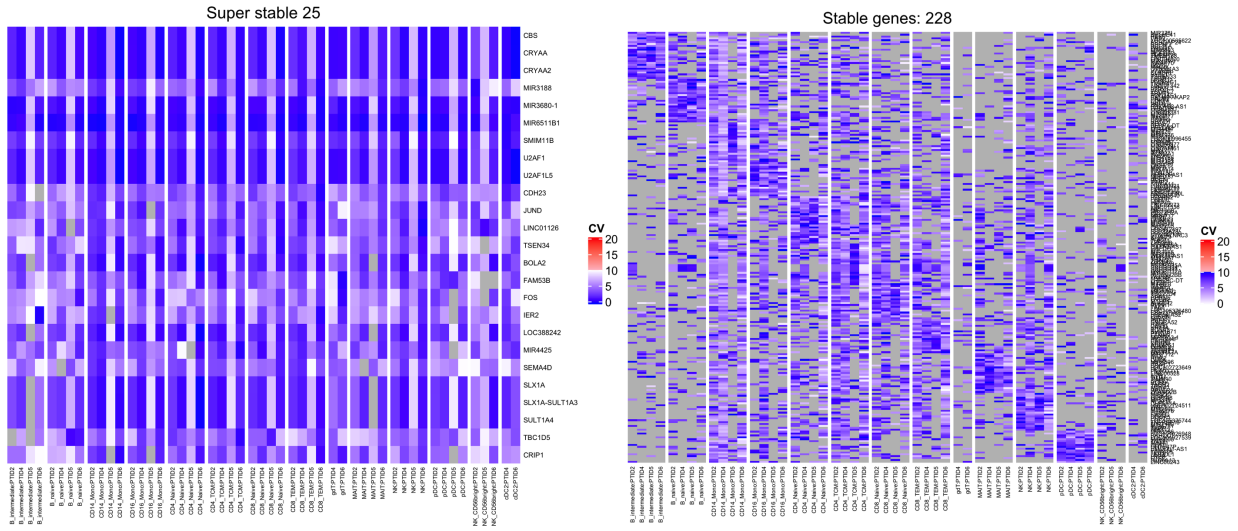
3.3.11 Intra-donor variations over time

```
cv_res <- cvCalcSC(mat=mat, ann=ann,
                   meanThreshold=0.1,
                   cvThreshold=10,
                   housekeeping_genes=housekeeping_genes,
                   filePATH=filePATH, fileName=fileName)
```

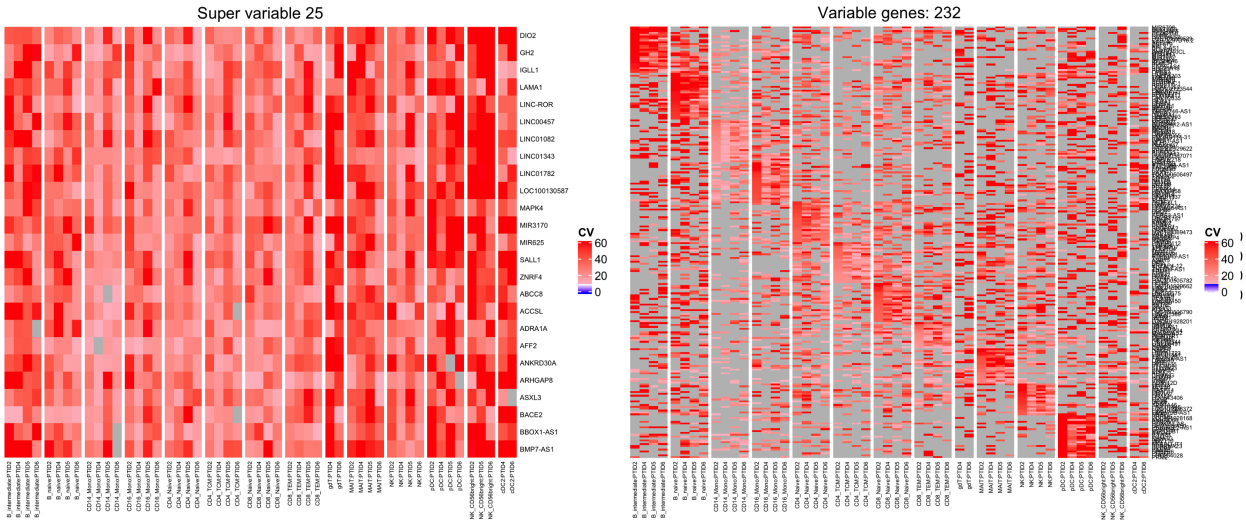


3.3.12 Find stable and variable features in longitudinal data

```
donorThreshold <- 4
groupThreshold <- 28 #number of donors * number of celltypes/2 (4x14/2)
topFeatures <- 25
stable_gene <- StableFeatures(ann=ann,
                meanThreshold=0.1,
                cvThreshold=10,
                donorThreshold=donorThreshold,
                groupThreshold=groupThreshold,
                topFeatures=topFeatures,
                fileName=fileName,
                filePATH=filePATH)
```



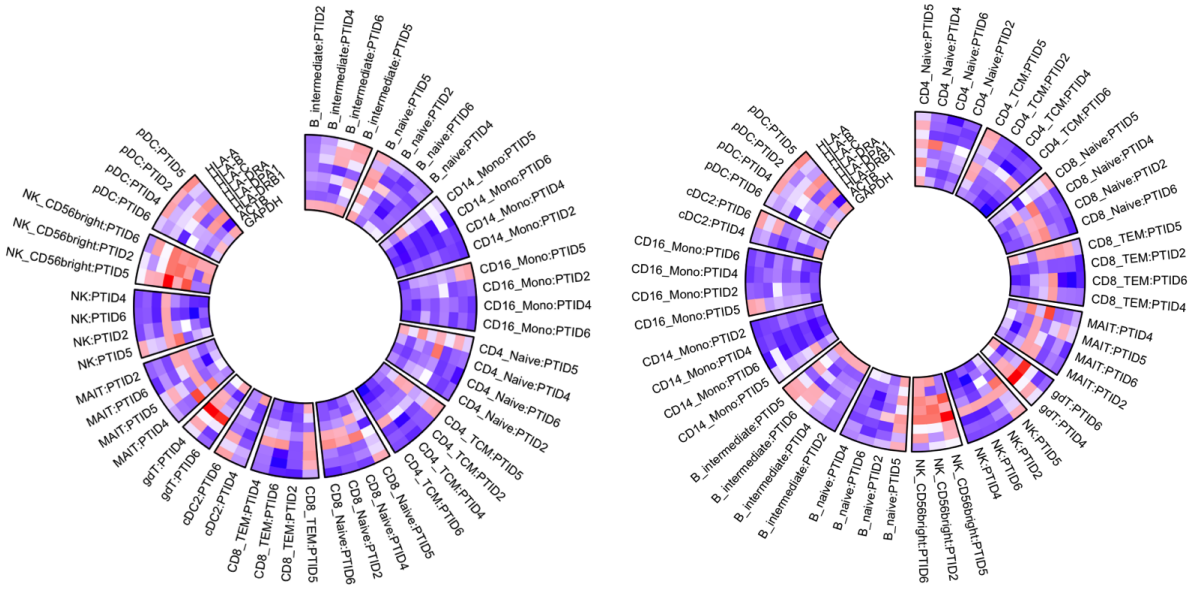

```
var_gene <- VarFeatures(ann=ann,
                meanThreshold=0.1,
                cvThreshold=10,
                donorThreshold=donorThreshold,
                groupThreshold=groupThreshold,
                topFeatures=topFeatures,
                fileName=fileName,
                filePATH=filePATH)
```



3.3.13 Circos CV plot

```
load(paste(filePATH,"/",fileName,"-CV-allgenes-raw.Rda", sep=""))
geneList <- c("HLA-A","HLA-B","HLA-C",
              "HLA-DRA","HLA-DPA1","HLA-DRB1",
              "ACTB","GAPDH")
res <- genecircosPlot(data=cv_res, geneList=geneList, colorThreshold=10)
```

```
#Can use threshold 15 based on housekeeping genes
group_oi <- c("CD4_Naive","CD4_TEM","CD4_TCM","CD4_CTL",
              "CD8_Naive","CD8_TEM","CD8_TCM","Treg","MAIT","gdT",
              "NK", "NK_CD56bright",
              "B_naive","B_memory", "B_intermediate",
              "CD14_Mono","CD16_Mono",
              "cDC2","pDC")
res <- genecircosPlot(data=cv_res, geneList=geneList, groupBy=group_oi, colorThreshold=15)
```



3.4 Tutorial-4: Multi-modal data integration

This tutorial allows users to combine intra-donor variation value between different modalities like scRNA and scATAC data here. Load CV values from scRNA () and scATAC () as described above (check output directory for the files). To integrate variability across modalities, please follow following steps.

3.4.1 Load Library

```
#Load Library
library("PALM")
library("Hmisc")
library("ggpubr")
library("Seurat")
```

3.4.2 Load data

```
#From scRNA analysis obtain the CV data
load("data/result/scrna-CV-allgenes-raw.Rda")
scrna_cv_res <- cv_res

#From scATAC analysis obtain the CV data
load("data/result/scatac-CV-allgenes-raw.Rda")
```

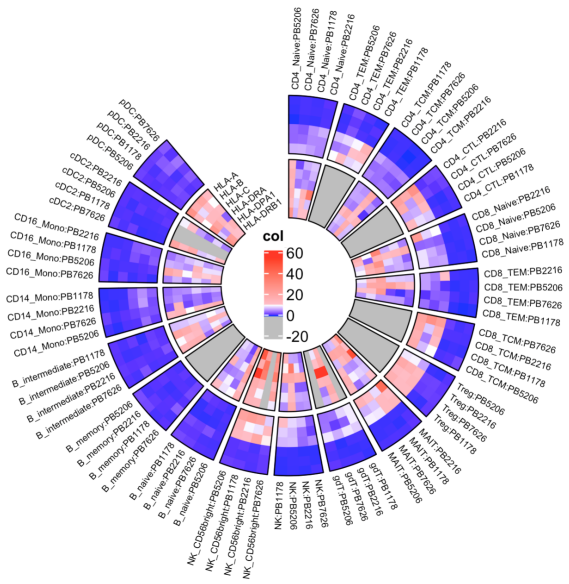
```
scatac_cv_res <- cv_res

#Cell type of interest
cell_type_oi <- c("CD4_Naive","CD4_TEM","CD4_TCM","CD4_CTL",
                  "CD8_Naive","CD8_TEM","CD8_TCM","Treg",
                  "MAIT","gdT", "NK", "NK_CD56bright",
                  "B_naive", "B_memory", "B_intermediate",
                  "CD14_Mono","CD16_Mono",
                  "cDC2","pDC")

geneList <- c("HLA-A","HLA-B","HLA-C","HLA-DRA","HLA-DPA1","HLA-DRB1") #HLAs
```

3.4.3 Run

```
res <- multimodalView(modality1=scrna_cv_res,
               modality2=scatac_cv_res,
               geneList=geneList,
               groupBy=cell_type_oi)
```



3.5 Tutorial-5: CNP0001102 data

This tutorial allows users to explore single cell RNAseq data variability across COVID and FLU donors. PBMC from the patients were collected longitudinally. Single cell data from Zhu et al. 2020 downloaded from here. Metadata is downloaded from table and can be found in the data. To infer variability (inter- and Intra-) and identify stable genes, please follow following steps.

3.5.1 Load Library

```
#Load Library
library("PALM")
library("Hmisc")
```

```
library("ggpubr")
library("Seurat")
```

3.5.2 Load data and assign paramaters

```
#Load scRNA data
pbmc <- readRDS("data/CNP0001102_Final_nCoV_0716_upload.RDS")
pbmc@meta.data$Sample <- pbmc@meta.data$batch
pbmc@meta.data$celltype <- gsub(" ", "_", pbmc@meta.data$cell_type)

#Clinical annotations (Table S1. Clinical data of the enrolled subjects)
metadata <- read.csv("data/CNP0001102-annotation.csv", stringsAsFactors = F)
row.names(metadata) <- metadata$Sample

#Exploring only COVID samples
metadata <- metadata[metadata$PTID %in% c("COV-1", "COV-2", "COV-3", "COV-4", "COV-5"),]
#Exploring only FLU samples
metadata <- metadata[metadata$PTID %in% c("IAV-1","IAV-2"),]

#Parameters
dataObj <- pbmc
featureSet=c("PTID", "Time")
avgGroup="celltype"
housekeeping_genes <- c("GAPDH", "ACTB")
cell_type <- sort(unique(pbmc@meta.data$celltype))
fileName="CNP0001102"
```

3.5.3 Create output directory (optional)

```
outputDirectory <- "output"
filePATH <- paste(getwd(), "/",outputDirectory, sep="")
dir.create(file.path(getwd(), outputDirectory), showWarnings = FALSE)
```

3.5.4 Sample overlap

```
overlap <- intersect(metadata$Sample, dataObj@meta.data$Sample)
metadata <- metadata[overlap,]
#in-case subset of samples only
dataObj <- subset(x = dataObj, subset = Sample %in% overlap)
```

3.5.5 Aggregate data at celltypes (pseudo-bulk)

```
#For single cell data merge annotation and single cell metadata
metaData <- dataObj@meta.data
metadata1 <- metadata[metaData$Sample,]
metaData <- cbind(metaData, metadata1)
dataObj@meta.data <- metaData
```

3.5.6 Define sample group and Calculate average expression

```
dataObj@meta.data$Sample_group <- paste(dataObj@meta.data$Sample,
```

```
dataObj@meta.data[,avgGroup], sep=":")
dataObj@meta.data$Sample_group <- gsub(" ", "_", dataObj@meta.data$Sample_group)
metaData <- dataObj@meta.data
```

3.5.7 Calculate average expression across group/celltype

```
Idents(dataObj) <- "Sample_group"
table(Idents(dataObj))
scrna_avgmat <- avgExpCalc(dataObj=dataObj, group.by="Sample_group")
```

3.5.8 Keep genes with avgExpression > zero

```
rowDF <- rowSums(scrna_avgmat)
rowDF <- rowDF[rowDF > 0]
mat <- scrna_avgmat[names(rowDF),]
```

3.5.9 Create annotation

```
cn <- data.frame(Sample_group=colnames(mat))
temp <- data.frame(do.call(rbind, strsplit(cn$Sample_group, split = ":")),
        stringsAsFactors = F)
cn <- data.frame(cn, Sample=temp$X1, group=temp$X2, stringsAsFactors = F)
row.names(cn) <- cn$Sample_group
cn <- merge(cn, metadata, by="Sample", all=TRUE)
cn <- cn[!is.na(cn$Sample_group),]
row.names(cn) <- cn$Sample_group
ann <- cn
ann$Sample_group_i <- paste(ann$group, ann$PTID, sep=":")
rm(cn)
```

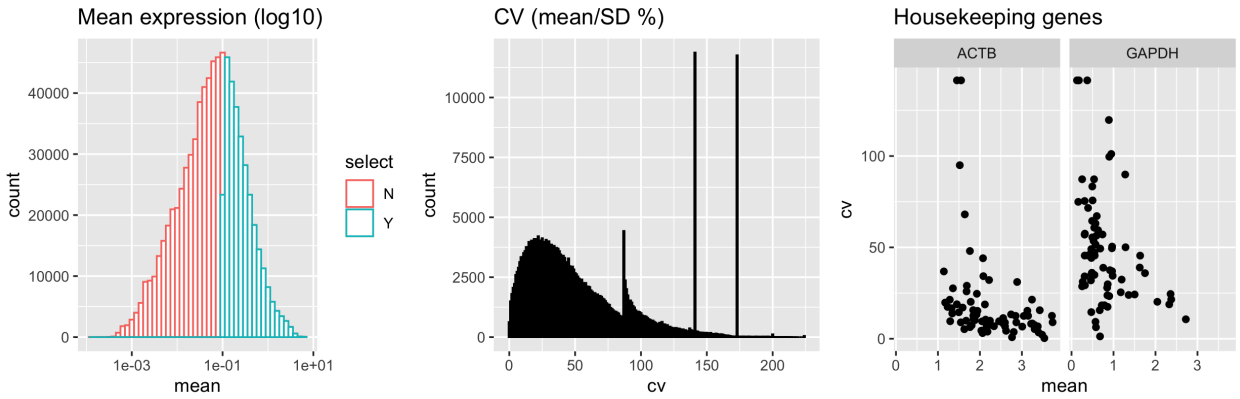
3.5.10 Final matrix

```
Overlap <- intersect(colnames(mat), row.names(ann))
ann <- ann[Overlap,]
mat <- mat[,Overlap]
print(unique(ann$group))
```

```
#[1] "Activated_CD4_T_cells" "Cycling_Plasma"        "Cycling_T_cells"       "Cytotoxic_CD8_T_cells"
#[5] "MAIT"                  "Megakaryocytes"        "Memory_B_cells"        "Naive_B_cells"
#[9] "Naive_T_cells"         "NKs"                   "Plasma"                "Stem_cells"
#[13] "XCL+_NKs"              "DCs"                    "Monocytes"
```

3.5.11 CV profile

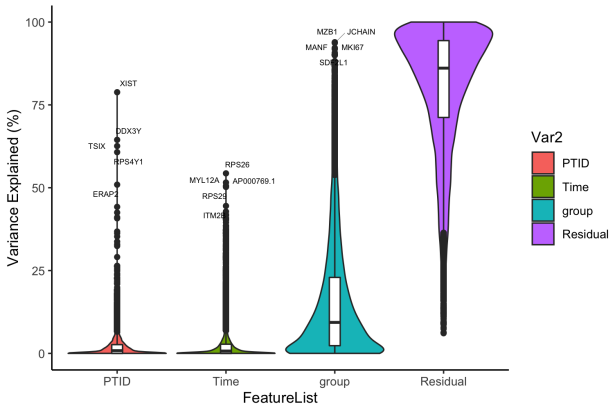
```
cv_profile <- cvprofile(mat=mat, ann=ann, meanThreshold = 0.1)
```




```
#Sample Celltype Mean-CV plot (output directory)
cv_sample_profile <- cvSampleprofile(mat=mat, ann=ann, meanThreshold = 0.1, cvThreshold = 25)
```

3.5.12 Features contributing towards donor variations

```
#Variance decomposition
lmem_res <- lmeVariance(ann=ann, mat=mat,
                    featureSet=c(featureSet,"group"),
                    meanThreshold=0.1,
                    fileName=fileName,
                    filePATH=filePATH)
res <- lmem_res[,c("PTID","Time","group","Residual")]
colnames(res) <- c("PTID","Time","celltype","Residuals")
res <- res*100 #in percentage
```




```
head(res[order(res$celltype, decreasing = T),])
#         PTID         Time celltype Residuals
#MZB1    3.634604e-08 1.242595e-09 93.91550  6.084498
#JCHAIN  0.000000e+00 0.000000e+00 93.67179  6.328213
#MKI67   3.013979e-01 1.236726e-01 92.19240  7.382530
#MANF    3.197369e-01 0.000000e+00 91.82359  7.856671
#SDF2L1  5.210284e-02 1.019559e-01 90.75454  9.091404
#POU2AF1 2.432974e-01 3.463273e-01 90.24514  9.165233
```

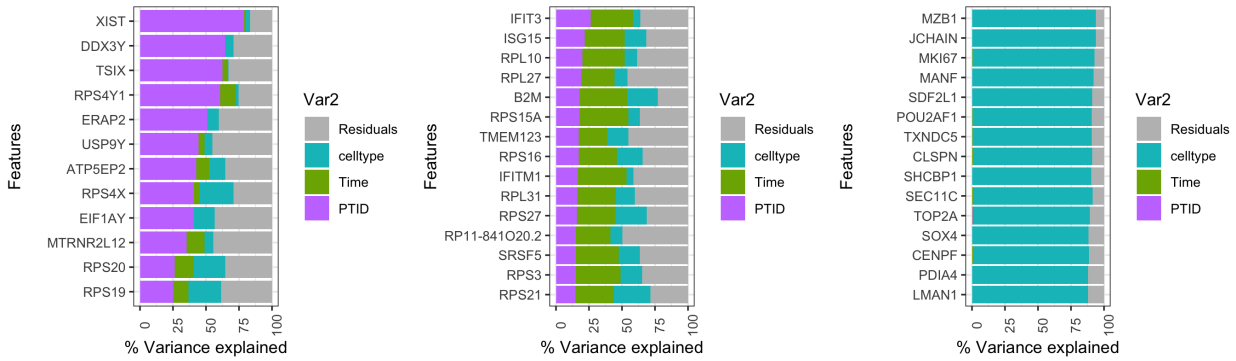
3.5.13 Variance explained

```
#Donor-specific
df1 <- filter(res, PTID>Time & PTID>celltype & Residuals < 50)
df1 <- df1[order(df1$PTID, decreasing = T),]
df <- melt(data.matrix(df1[1:15,]))
df$Var2 <- factor(df$Var2, levels = rev(colnames(res)))
df$Var1 <- factor(df$Var1, levels = rev(unique(df$Var1)))
p1 <- ggplot(df, aes(x=Var1, y=value, fill=Var2)) +
  geom_bar(stat="identity", position="stack") +
  labs(x="Features", y="% Variance explained") +
  scale_fill_manual(values = c("PTID"="#C77CFF", "celltype"="#00BFC4",
  "Time"="#7CAE00", "Residuals"="grey")) +
  theme_bw() + theme(axis.text.x = element_text(angle=90, hjust = 0.5,
  vjust = 1),legend.position = "right") +
  coord_flip()

#Time-specific
df2 <- filter(res, Time>PTID & Time>celltype & Residuals < 50)
#df2 <- res[order(res$Time, decreasing = T),]
#select Top15
df2 <- df2[1:15,]
df <- melt(data.matrix(df2))
df$Var2 <- factor(df$Var2, levels = rev(colnames(res)))
df$Var1 <- factor(df$Var1, levels = rev(unique(df$Var1)))
p2 <- ggplot(df, aes(x=Var1, y=value, fill=Var2)) +
  geom_bar(stat="identity", position="stack") +
  labs(x="Features", y="% Variance explained") +
  scale_fill_manual(values = c("PTID"="#C77CFF", "celltype"="#00BFC4",
  "Time"="#7CAE00", "Residuals"="grey")) +
  theme_bw() + theme(axis.text.x = element_text(angle=90, hjust = 0.5,
  vjust = 1),legend.position = "right") +
  coord_flip()

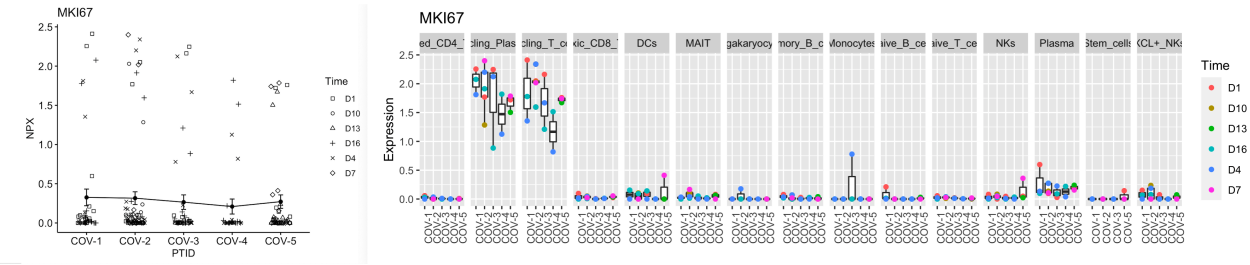
#celltype-specific
df3 <- filter(res, celltype>PTID & celltype>Time & Residuals < 50)
df3 <- df3[order(df3$celltype, decreasing = T),]
#select Top15
df3 <- df3[1:15,]
df <- melt(data.matrix(df3))
df$Var2 <- factor(df$Var2, levels = rev(colnames(res)))
df$Var1 <- factor(df$Var1, levels = rev(unique(df$Var1)))
p3 <- ggplot(df, aes(x=Var1, y=value, fill=Var2)) +
  geom_bar(stat="identity", position="stack") +
  labs(x="Features", y="% Variance explained") +
  scale_fill_manual(values = c("PTID"="#C77CFF", "celltype"="#00BFC4",
  "Time"="#7CAE00", "Residuals"="grey")) +
  theme_bw() + theme(axis.text.x = element_text(angle=90, hjust = 0.5,
  vjust = 1),legend.position = "right") +
  coord_flip()

#Plot
plot_grid(p1,p2,p3, align="hv", ncol=3)
```



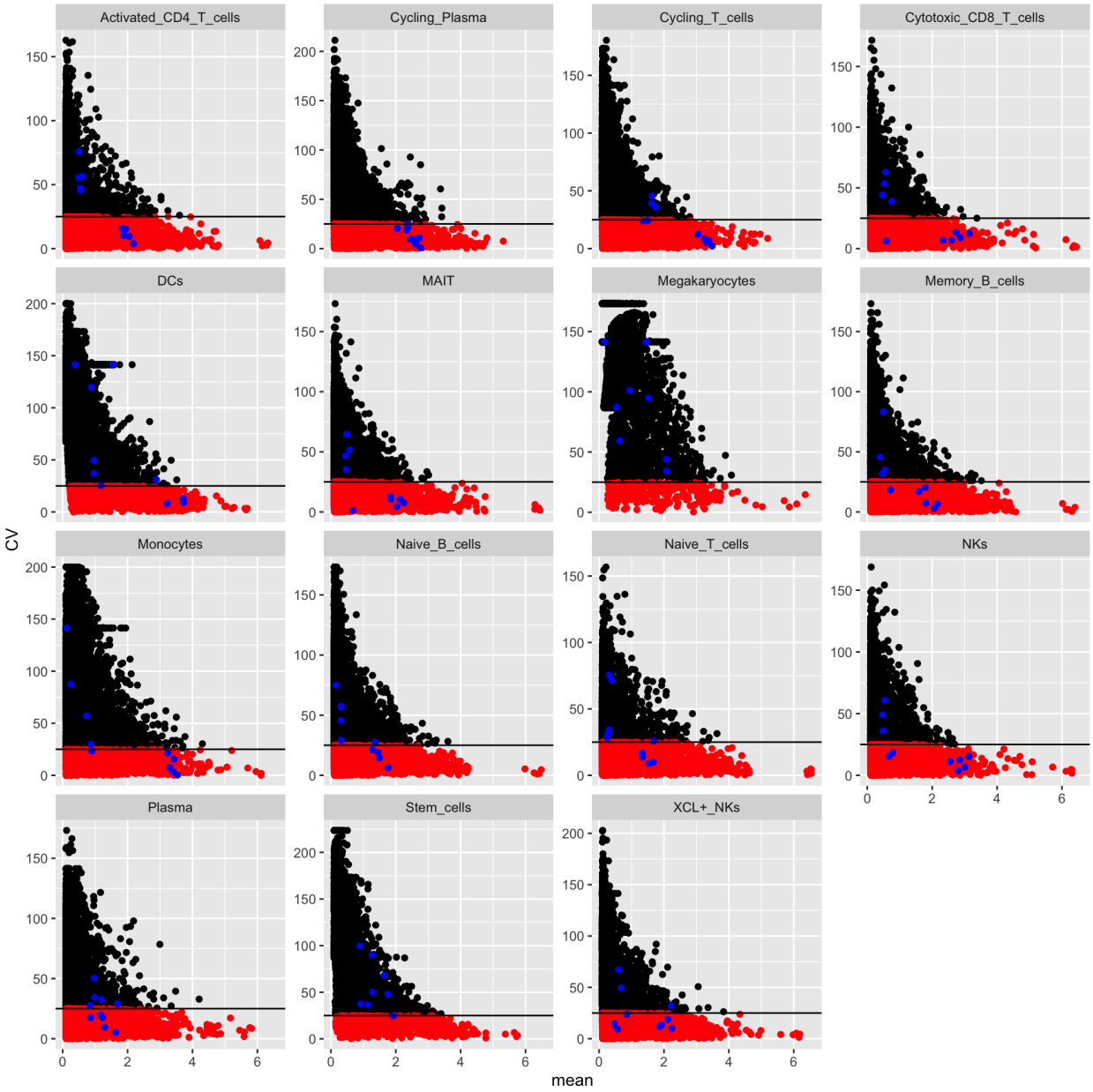
3.5.14 Plot the variables

```
plots <- genePlot(ann, mat, geneName="MKI67", groupName="group")
print(plots$plot1)
print(plots$plot2)
print(plots$plot3)
print(plots$plot4)
print(plots$plot5)
```



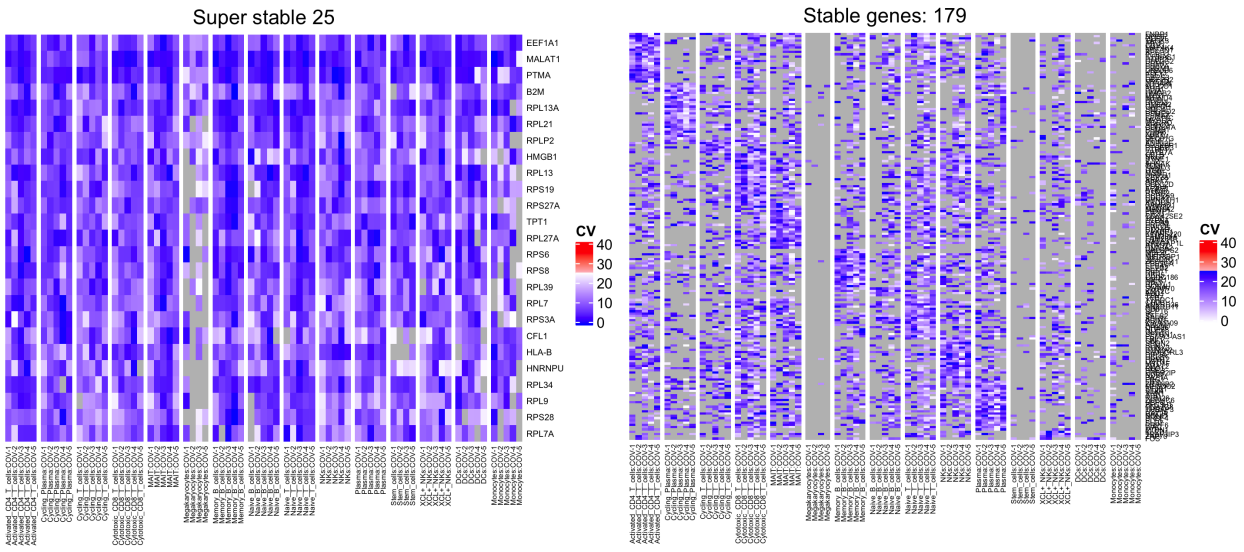
3.5.15 Intra-donor variations over time

```
#Calculate CV
meanThreshold=0.1
cvThreshold=25
cv_res <- cvCalcSC(mat=mat, ann=ann,
                   meanThreshold=meanThreshold,
                   cvThreshold=cvThreshold,
                   housekeeping_genes=housekeeping_genes,
                   fileName=fileName,
                   filePATH=filePATH)
```

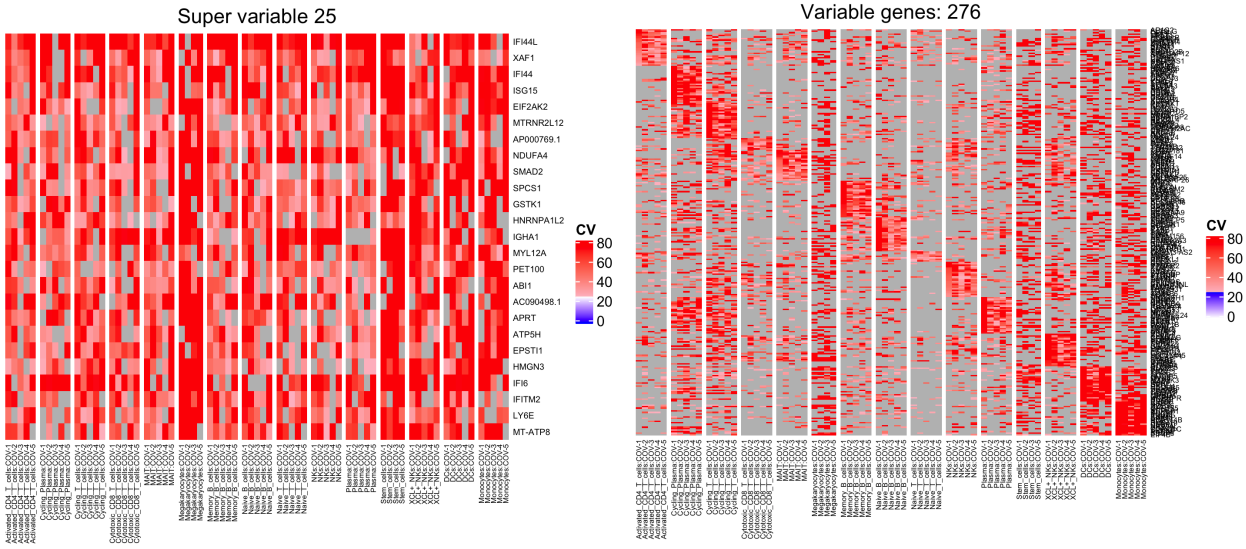


3.5.16 Find stable and variable features in longitudinal data

```
donorThreshold <- 5 #number of donors
groupThreshold <- 38 #number of donors * number of celltypes/2 (5x15/2)
topFeatures <- 25
stable_gene <- StableFeatures(ann=ann,
                              meanThreshold=meanThreshold,
                              cvThreshold=cvThreshold,
                              donorThreshold=donorThreshold,
                              groupThreshold=groupThreshold,
                              topFeatures=topFeatures,
                              fileName=fileName,
                              filePATH=filePATH)
```

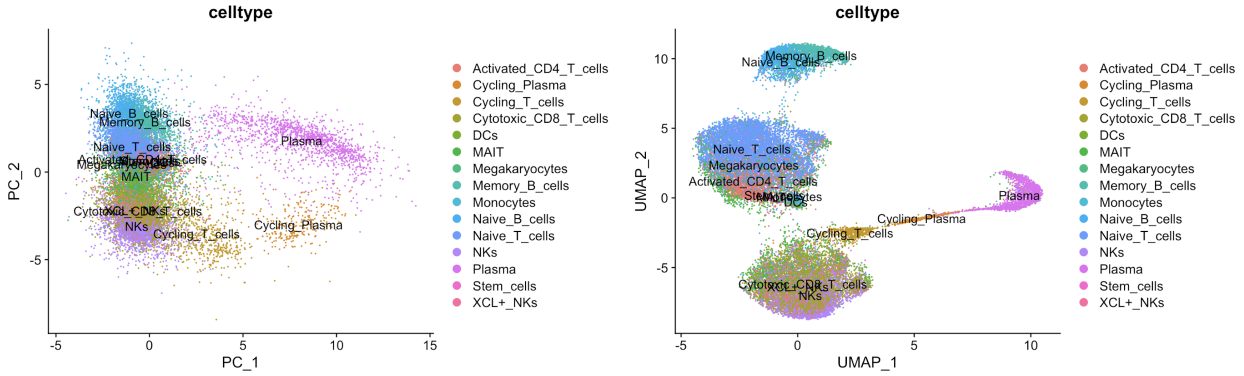



```
var_gene <- VarFeatures(ann=ann,
                        meanThreshold=meanThreshold,
                        cvThreshold=cvThreshold,
                        donorThreshold=donorThreshold,
                        groupThreshold=groupThreshold,
                        topFeatures=topFeatures,
                        fileName=fileName,
                        filePATH=filePATH)
```

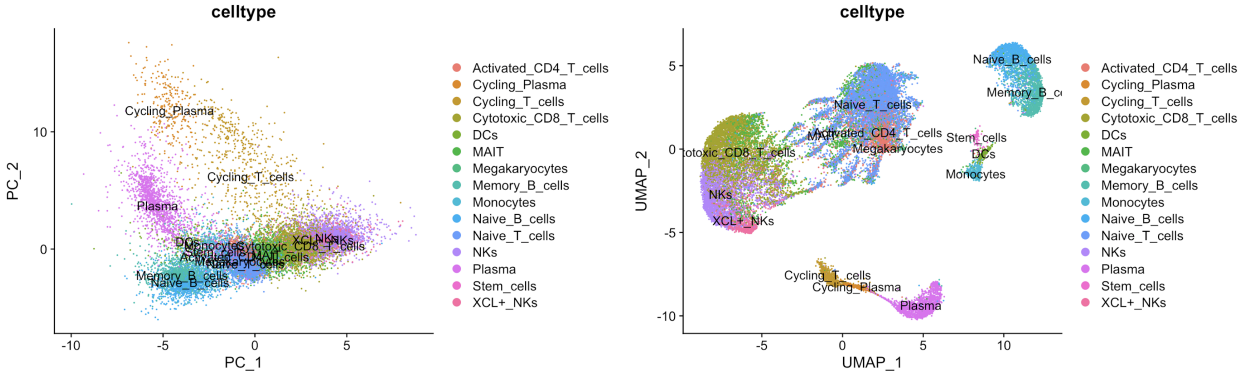


3.5.17 UMAP Plot

```
#Stable genes UMAP
dimUMAPPlot(rnaObj=dataObj, nPC=15,
            gene_oi=unique(stable_gene$gene), groupName=avgGroup,
            plotname="stable", filePATH=filePATH, fileName=fileName)
```

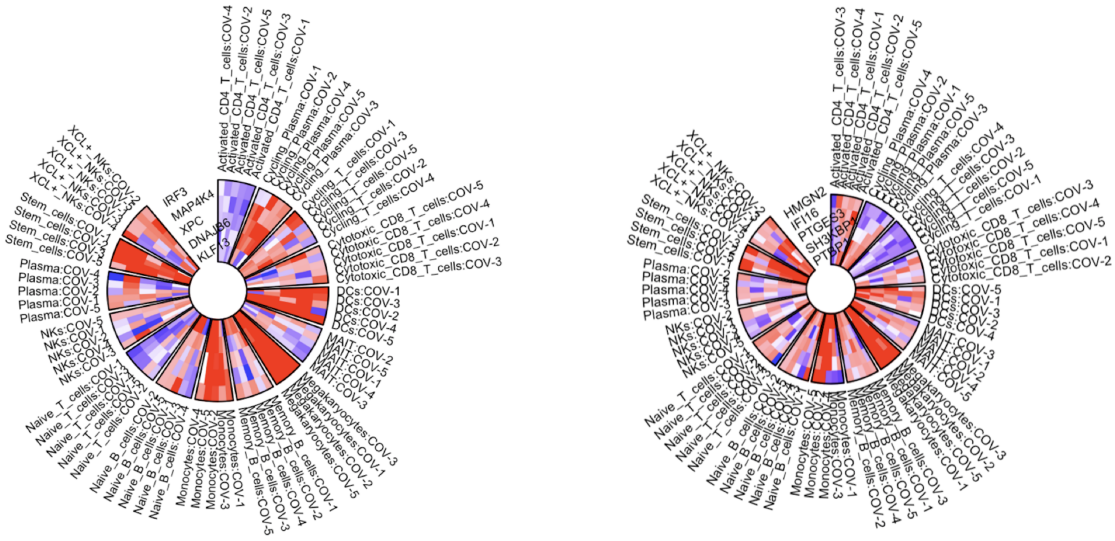



```
#Variable genes UMAP
dimUMAPPlot(rnaObj=dataObj, nPC=15,
            gene_oi=unique(var_gene$gene), groupName=avgGroup,
            plotname="variable", filePATH=filePATH, fileName=fileName)
```



3.5.18 Circos CV Plot

```
load(paste(filePATH,"/",fileName, "-CV-allgenes-raw.Rda", sep=""))
geneList <- c("IRF3","MAP4K4","XPC","DNAJB6", "KLF13") #Activated CD4 T-cells
geneList <- c("HMGN2", "IFI16", "PTGES3", "SH3KBP1", "PTBP1") #Cycling T-cells
res <- genecircosPlot(data=cv_res, geneList=geneList, colorThreshold=cvThreshold)
```



3.6 Tutorial-6: Differential Gene analysis in longitudinal data

This tutorial allows users to identify differential expressed genes in direction of time-points. As an example single cell data from Zhu et al. 2020 downloaded from here. Metadata is downloaded from table and can be found in the data. The dataset consists of 5 Covid-19 donors, 2 Flu donors with longitudinal data and 3 controls. To explore differetial expressed gened in each celltype of each donor we used hurdle model based modeling on input data to retrive the DEGs. To infer DEGs in each celltype towards time progression (timepoints considered as continoues if more than 2), please follow following steps.

3.6.1 load data and clinical metadata

Single cell object CNP0001102

```
pbmc <- readRDS("data/CNP0001102_Final_nCoV_0716_upload.RDS")
#Add column Sample and group as celltype
pbmc@meta.data$Sample <- pbmc@meta.data$batch
pbmc@meta.data$celltype <- gsub(" ", "_", pbmc@meta.data$cell_type)
```

3.6.2 Clinical annotations Table S1. Clinical data of the enrolled subjects

```
metadata <- read.csv("data/CNP0001102-annotation.csv", stringsAsFactors = F)
row.names(metadata) <- metadata$Sample
```

3.6.3 Load library and run

```
library("PALM")
#run
DEGres <- sclongitudinalDEG(ann=metadata, dataObj=pbmc, scassay="RNA", celltypecol="celltype")

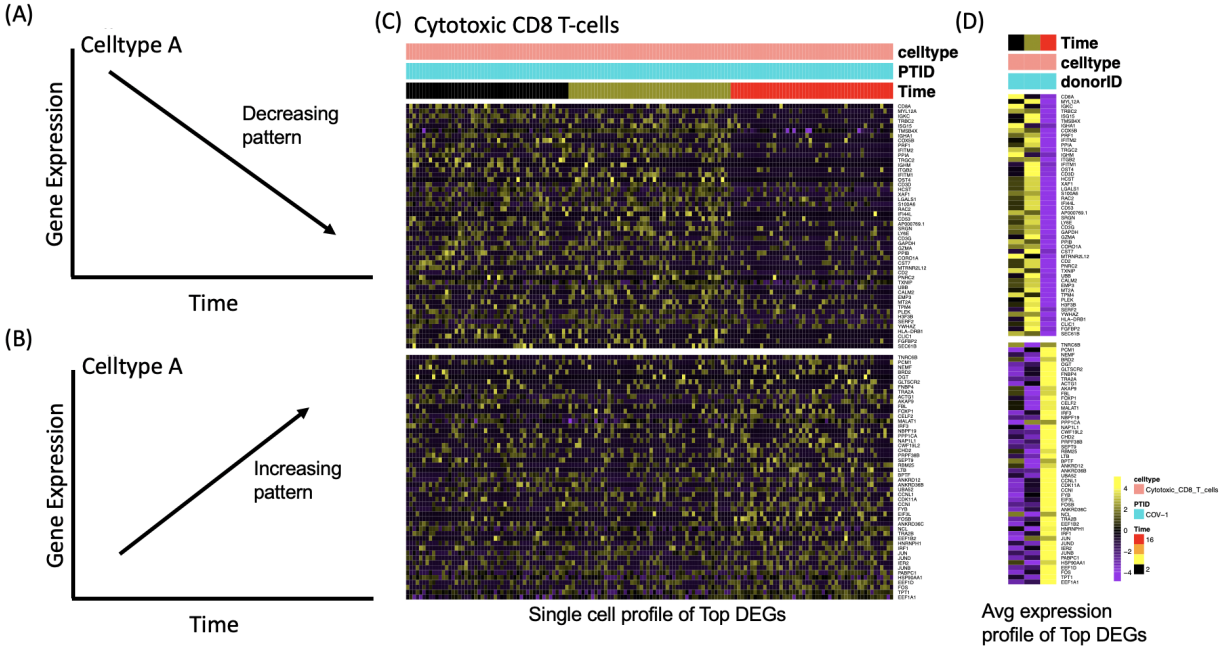
>Fitting a ZLM model for donorID:  COV-1
>Fitting a ZLM model for donorID:  COV-2
>Fitting a ZLM model for donorID:  COV-3
```

```
>Fitting a ZLM model for donorID:  COV-4
>Fitting a ZLM model for donorID:  COV-5
>Fitting a ZLM model for donorID:  IAV-1
>Fitting a ZLM model for donorID:  IAV-2

#Plots can be seen in output directory output
head(DEGres[order(DEGres$coef, decreasing = T),])

#primerid contrast         nomP     coef         adjP donorID celltype dir
#IGHG4   TimeD9 1.701579e-26 3.056092 1.453999e-23   IAV-2   Plasma upregulated at D9
#JCHAIN   TimeD9 8.759407e-32 2.647757 2.245474e-28   IAV-2   Plasma upregulated at D9
#IGHG3   TimeD9 8.470810e-22 2.485250 2.412769e-19   IAV-2   Plasma upregulated at D9
#IGLC2   TimeD9 1.352490e-16 2.289544 8.890022e-15   IAV-2   Plasma upregulated at D9
#IGHG1   TimeD9 1.292885e-16 2.219146 8.608601e-15   IAV-2   Plasma upregulated at D9
#SYNE2   TimeD4 7.249010e-13 2.209541 1.215659e-09   COV-4 XCL+_NKs upregulated at D4
```

General analysis schema and differential results in each donor over timepoints in celltype Cytotoxic CD8 T-cells using `PALM` shown below.



4 Quick Usage

```
#Load libraries
library("PALM")
library("Hmisc")
library("ggpubr")
```

4.1 Plasma proteome

```
#Load Plasma proteomic expression (NPX) data
load("data/Olink_NPX_log2_Protein.Rda")
```

```
#Clinical Metdata/annotation
load("data/data_Metadata.Rda")
#Run longuitudinal analysis
ld_res <- PALM(metadata=ann,
                           data=data,
                           datatype="bulk",
                           omics="plasmaproteome",
                           featureSet=c("PTID", "Time"),
                           meanThreshold=1, cvThreshold=5,
                           NA_threshold=0.4,
                           column_sep="W",
                           method="spearman",
                           clusterBy="donor",
                           z_cutoff=2,
                           doOutlier=TRUE,
                           fileName="proteome",
                           outputDirectory="output")
```

4.2 Single cell RNA data

```
#Seurat object (Check for Sample and column)
pbmc <- readRDS("data/AIFI-scRNA-PBMC-FinalData.RDS")
pbmc@meta.data$Sample <- pbmc@meta.data$orig.ident
#Clinical Metdata/annotation
load("data/data_Metadata.Rda")

#Celltypes observed
cell_type <- c("CD4_Naive","CD4_TEM","CD4_TCM","CD4_CTL","CD8_Naive",
               "CD8_TEM","CD8_TCM","Treg","MAIT","gdT","dnT",
               "CD4_Proliferating", "CD8_Proliferating",
               "NK_Proliferating",
               "NK", "NK_CD56bright",
               "B_naive", "B_memory", "B_intermediate","Plasmablast",
               "CD14_Mono","CD16_Mono",
               "cDC1","cDC2","pDC","ASDC",
               "Platelet","Eryth", "ILC","HSPC","Doublet")
#Selected celltypes (manuscript) based on proportions >5%
group_oi <- c("CD4_Naive","CD4_TEM","CD4_TCM","CD4_CTL","CD8_Naive",
              "CD8_TEM","CD8_TCM","Treg","MAIT","gdT",
              "NK", "NK_CD56bright",
              "B_naive", "B_memory", "B_intermediate",
              "CD14_Mono","CD16_Mono",
              "cDC2","pDC")

#Run longuitudinal analysis
ld_res <- PALM(metadata=ann,
                           data=pbmc,
                           datatype="singlecell", omics="rna",
                           featureSet=c("PTID", "Time"),
                           meanThreshold=0.1, cvThreshold=10,
                           housekeeping_genes=c("GAPDH", "ACTB"),
                           avgGroup="celltype",
                           group_oi=group_oi,
```

```
                            donorThreshold=4, groupThreshold=40,
                            topFeatures=25,
                            method="spearman", column_sep=":",
                            clusterBy="group", z_cutoff=2,
                            fileName="scrna",
                            outputDirectory="output")
```

4.3 Single cell ATAC data

Load genescorematrix from archR or relevant tools (Aggregate data at celltypes (pseudo-bulk))

```
#scATAC object
load("data/AIFI-scATAC-PBMC-FinalData.Rda") #aggregated genescore
data <- log2(scatac_gm+1) #log2
#Load annotation data
load("data/data_Metadata.Rda")

#Run longuitudinal analysis
ld_res <- PALM(metadata=ann,
                            data=data,
                            datatype="singlecell", omics="atac",
                            featureSet=c("PTID", "Time"),
                            meanThreshold=0.1, cvThreshold=10,
                            housekeeping_genes=c("GAPDH", "ACTB"),
                            avgGroup="celltype",
                            group_oi=group_oi,
                            donorThreshold=4, groupThreshold=28,
                            topFeatures=25,
                            column_sep=":", method="spearman",
                            clusterBy="group", z_cutoff=2,
                            fileName="scatac",
                            outputDirectory="output")
```

5 Authors

Suhas Vasaikar, Aarthi talla and Xiaojun Li designed the PALM algorithm. Suhas Vasaikar implemented the PALM package.

6 License

PALM is licensed under the MIT-License.

7 Session info

```
sessionInfo()
#> R version 4.0.3 (2020-10-10)
#> 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] grid      stats     graphics  grDevices utils     datasets  methods
#> [8] base
#>
#> other attached packages:
#>  [1] PALM_0.1.0           forcats_0.5.0        stringr_1.4.0
#>  [4] dplyr_1.0.7          purrr_0.3.4          readr_1.4.0
#>  [7] tidyr_1.1.4          tibble_3.1.6         tidyverse_1.3.0
#> [10] pheatmap_1.0.12      cowplot_1.1.1        circlize_0.4.11
#> [13] ComplexHeatmap_2.4.3 reshape2_1.4.4       ggplot2_3.3.5
#>
#> loaded via a namespace (and not attached):
#>   [1] readxl_1.3.1                backports_1.2.0
#>   [3] plyr_1.8.6                  igraph_1.2.8
#>   [5] lazyeval_0.2.2              splines_4.0.3
#>   [7] listenv_0.8.0               scattermore_0.7
#>   [9] GenomeInfoDb_1.24.2         digest_0.6.28
#>  [11] htmltools_0.5.2             fansi_0.5.0
#>  [13] magrittr_2.0.1              tensor_1.5
#>  [15] cluster_2.1.0               ROCR_1.0-11
#>  [17] globals_0.14.0              modelr_0.1.8
#>  [19] matrixStats_0.61.0          colorspace_2.0-2
#>  [21] blob_1.2.1                  rvest_0.3.6
#>  [23] ggrepel_0.9.1               haven_2.3.1
#>  [25] xfun_0.25                   RCurl_1.98-1.2
#>  [27] crayon_1.4.2                jsonlite_1.7.2
#>  [29] lme4_1.1-25                 spatstat_1.64-1
#>  [31] spatstat.data_2.1-0         survival_3.2-7
#>  [33] zoo_1.8-9                   glue_1.5.0
#>  [35] polyclip_1.10-0             gtable_0.3.0
#>  [37] zlibbioc_1.34.0             XVector_0.28.0
#>  [39] leiden_0.3.9                DelayedArray_0.14.1
#>  [41] GetoptLong_1.0.4            SingleCellExperiment_1.10.1
#>  [43] future.apply_1.8.1          shape_1.4.5
#>  [45] BiocGenerics_0.34.0         abind_1.4-5
#>  [47] scales_1.1.1                DBI_1.1.0
#>  [49] miniUI_0.1.1.1              Rcpp_1.0.7
#>  [51] viridisLite_0.4.0           xtable_1.8-4
#>  [53] clue_0.3-57                 reticulate_1.22
#>  [55] stats4_4.0.3                htmlwidgets_1.5.4
#>  [57] httr_1.4.2                  RColorBrewer_1.1-2
#>  [59] ellipsis_0.3.2              Seurat_4.0.0
#>  [61] factoextra_1.0.7.999        ica_1.0-2
#>  [63] farver_2.1.0                pkgconfig_2.0.3
```

```
#>  [65] uwot_0.1.10                dbplyr_1.4.4
#>  [67] deldir_1.0-6               utf8_1.2.2
#>  [69] tidyselect_1.1.1           rlang_0.4.12
#>  [71] later_1.3.0                munsell_0.5.0
#>  [73] cellranger_1.1.0           tools_4.0.3
#>  [75] cli_3.1.0                  generics_0.1.1
#>  [77] broom_0.7.2                ggridges_0.5.3
#>  [79] evaluate_0.14              fastmap_1.1.0
#>  [81] yaml_2.2.1                 goftest_1.2-3
#>  [83] knitr_1.30                 fs_1.5.0
#>  [85] fitdistrplus_1.1-6         RANN_2.6.1
#>  [87] pbapply_1.5-0              future_1.23.0
#>  [89] nlme_3.1-149               mime_0.12
#>  [91] xml2_1.3.2                 compiler_4.0.3
#>  [93] rstudioapi_0.13            plotly_4.10.0
#>  [95] png_0.1-7                  spatstat.utils_2.2-0
#>  [97] reprex_0.3.0               tweenr_1.0.1
#>  [99] statmod_1.4.35             stringi_1.7.5
#> [101] lattice_0.20-41            Matrix_1.3-4
#> [103] nloptr_1.2.2.2             vctrs_0.3.8
#> [105] pillar_1.6.4               lifecycle_1.0.1
#> [107] lmtest_0.9-39              GlobalOptions_0.1.2
#> [109] RcppAnnoy_0.0.19           bitops_1.0-7
#> [111] data.table_1.14.2          irlba_2.3.3
#> [113] GenomicRanges_1.40.0       httpuv_1.6.3
#> [115] patchwork_1.1.1            R6_2.5.1
#> [117] promises_1.2.0.1           KernSmooth_2.23-17
#> [119] gridExtra_2.3              IRanges_2.22.2
#> [121] parallelly_1.28.1          codetools_0.2-16
#> [123] boot_1.3-25                MASS_7.3-53
#> [125] assertthat_0.2.1           SummarizedExperiment_1.18.2
#> [127] MAST_1.14.0                rjson_0.2.20
#> [129] withr_2.4.2                SeuratObject_4.0.2
#> [131] sctransform_0.3.2          GenomeInfoDbData_1.2.3
#> [133] S4Vectors_0.26.1           mgcv_1.8-33
#> [135] parallel_4.0.3             hms_0.5.3
#> [137] rpart_4.1-15               minqa_1.2.4
#> [139] rmarkdown_2.5              Rtsne_0.15
#> [141] ggforce_0.3.2              Biobase_2.48.0
#> [143] shiny_1.7.1                lubridate_1.7.9
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