

### 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) AIFI-scATAC-PBMC-FinalData.Rda (2) [AIFI-Metadata.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("PALMO")
library("Hmisc")
library("ggpubr")
library("cowplot")
```

#### 3.3.2 Load data and assign paramaters (Time <30sec)

```
#scATAC object
```

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

```
load("data/AIFI-scATAC-PBMC-FinalData.Rda")
datamatrix <- log2(scatac_gm+1)
```

```
#Load annotation data
load("data/AIFI-Metadata.Rda")
```

#### 3.3.3 Create PALMO object (Time <30sec)

```
#Create PALMO object
palmo_obj <- createPALMOobject(anndata=ann, data=datamatrix)

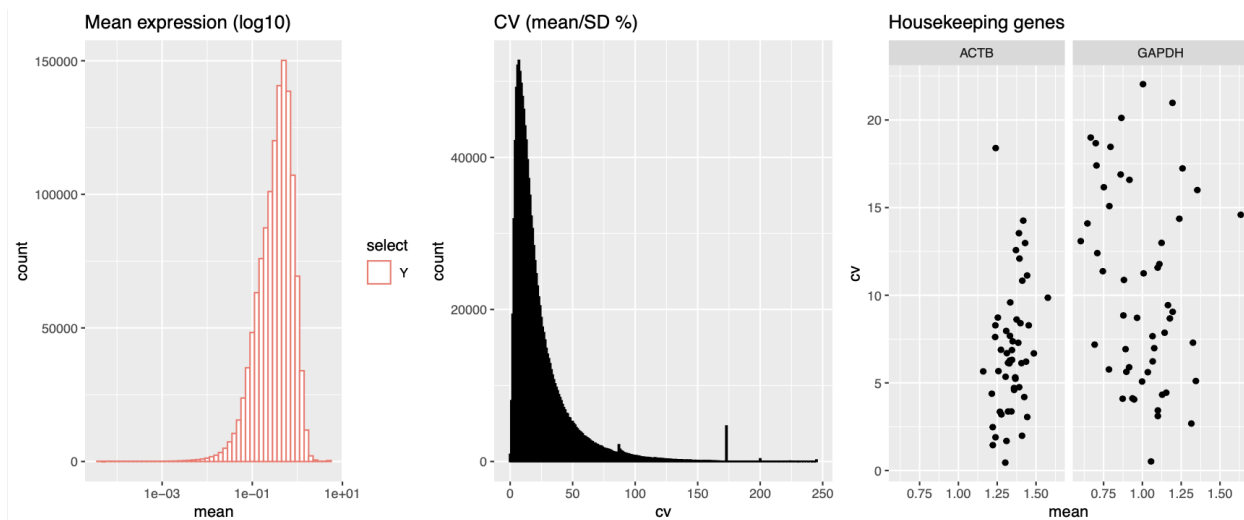
#Assign Sample, PTID and Time parameters
palmo_obj <- annotateMetadata(data_object=palmo_obj,
                             sample_column= "Sample", donor_column= "PTID",
                             time_column= "Time")

#Sample overlap and final matrix
palmo_obj <- mergePALMOdata(data_object=palmo_obj, datatype="singlecell")

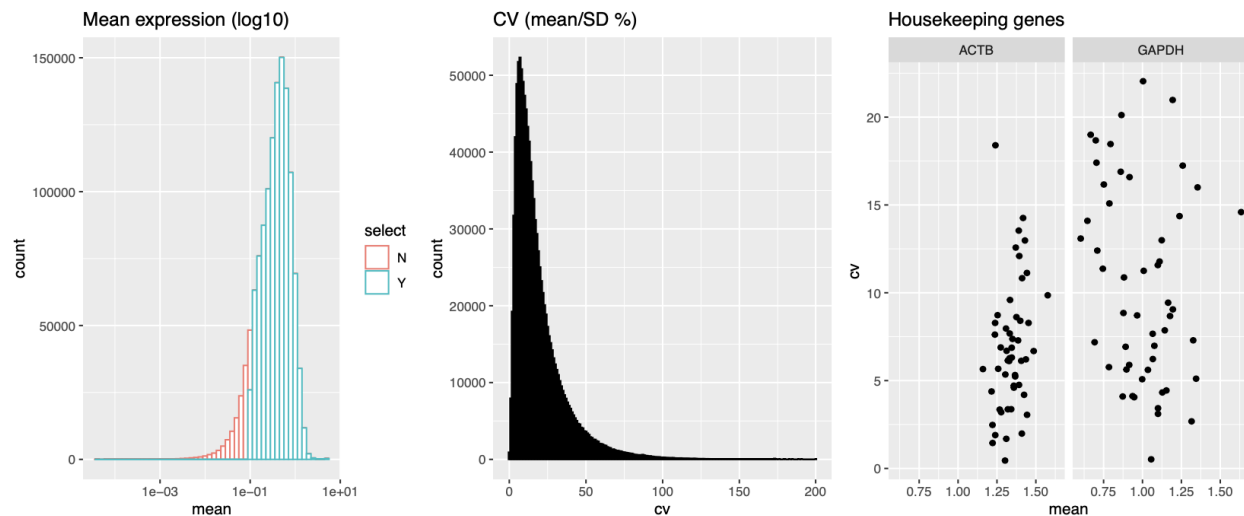
#Check for replicates
palmo_obj <- checkReplicates(data_object=palmo_obj, mergeReplicates = T)
```

#### 3.3.4 CV profile (Time ~2min)

```
palmo_obj <- cvCalcSCProfile(data_object=palmo_obj,
                             housekeeping_genes=c("GAPDH", "ACTB"),
                             fileName="scatac")
```



```
palmo_obj <- cvCalcSCProfile(data_object=palmo_obj,
                             housekeeping_genes=c("GAPDH", "ACTB"),
                             meanThreshold = 0.1,
                             fileName="scatac")
```



```
#Sample Celltype Mean-CV plot
cvSCsampleprofile(data_object=palmo_obj, meanThreshold = 0.1,
                   cvThreshold = 10, fileName="scatac")
#plots saved in output directory
```

### 3.3.5 Features contributing towards donor variations (Time ~ 8min)

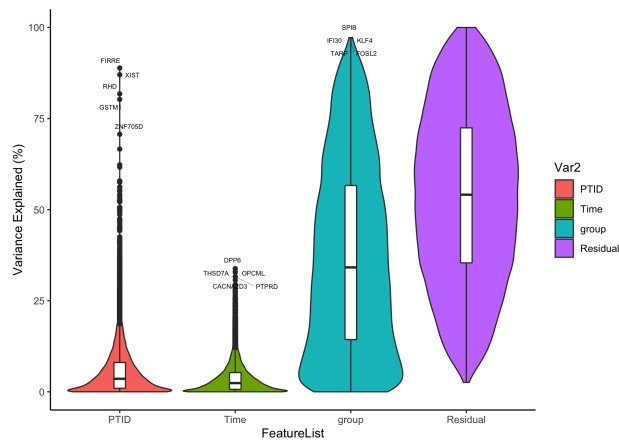
```
#Check the group of interest
head(palmo_obj@curated$anndata)
```

```
#Variance decomposition
featureSet <- c("PTID","Time","group")
palmo_obj <- lmeVariance(data_object=palmo_obj,
```

```

featureSet=featureSet,
meanThreshold=0.1, cl=4,
fileName="scatac")
var_decomp <- palmo_obj@result$variance_decomposition
head(var_decomp[,featureSet])
#           PTID      Time      group
#FIRRE      88.88239 0.2606612 2.8339971
#XIST       87.05064 0.3986387 0.5178851
#RHD        81.77230 0.3641998 1.4574140
#GSTM1      80.29766 0.1786628 6.2277645
#ZNF705D    70.69163 0.4342325 0.4752960
#LOC105376805 66.62977 1.2741294 9.8918653

```

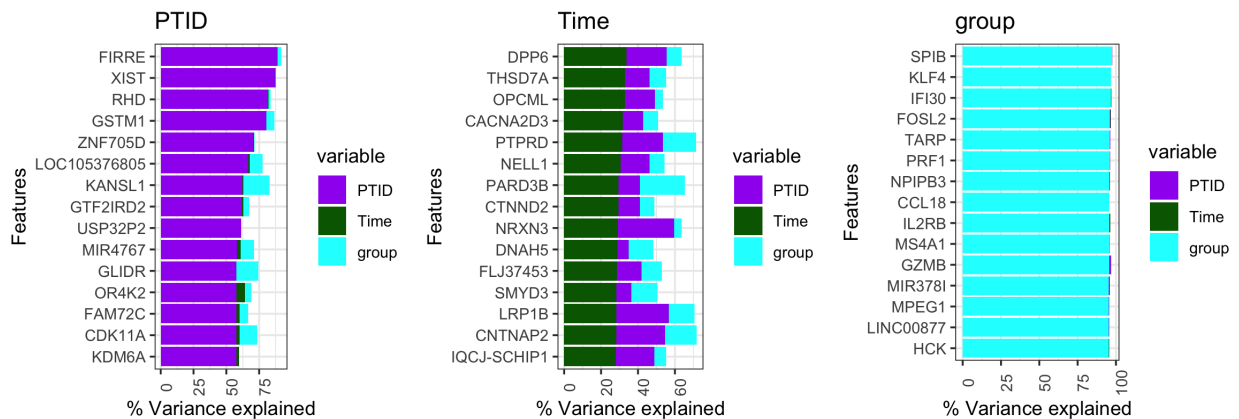


#Variance contributing features

```

plots <- variancefeaturePlot(vardata=var_decomp, featureSet=featureSet,
                             cols=c("purple", "darkgreen", "cyan"))
plot_grid(plotlist = plots, ncol=3)

```



#Top genes

```

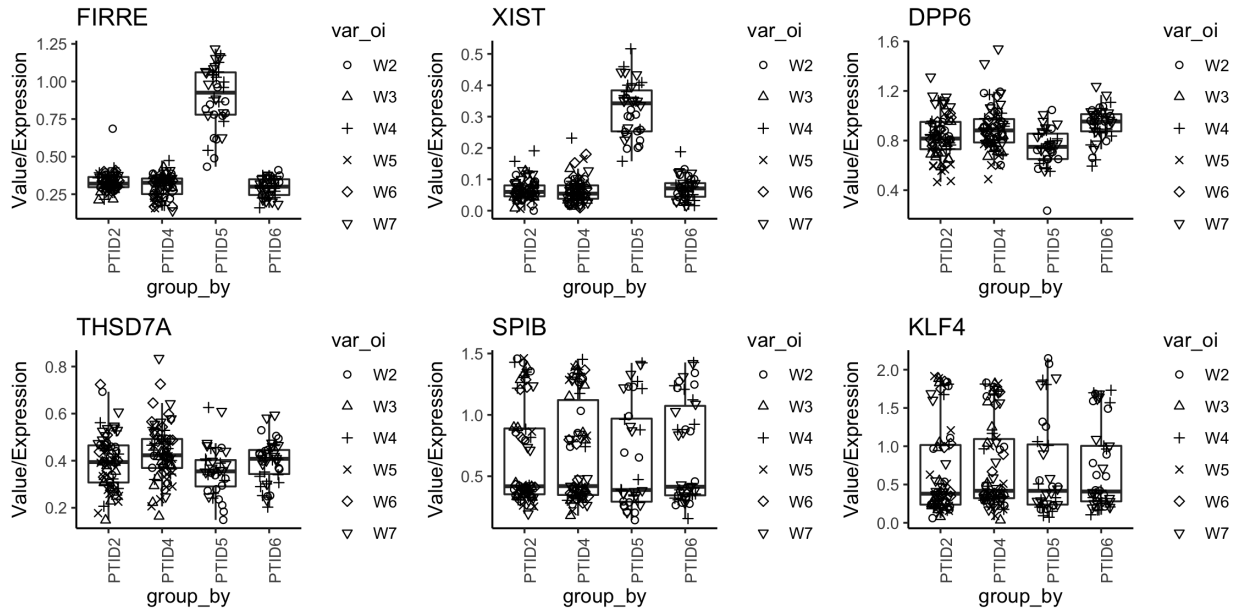
plots <- gene_featureplot(data_object=palmo_obj,
                          featureList=c("FIRRE", "XIST",
                                         "DPP6", "THSD7A",
                                         "SPIB", "KLF4"),
                          x_group_by="PTID", var_oi="Time",

```

```

x_text_angle=90)
plot_grid(plotlist=plots, ncol= 3, align="hv")

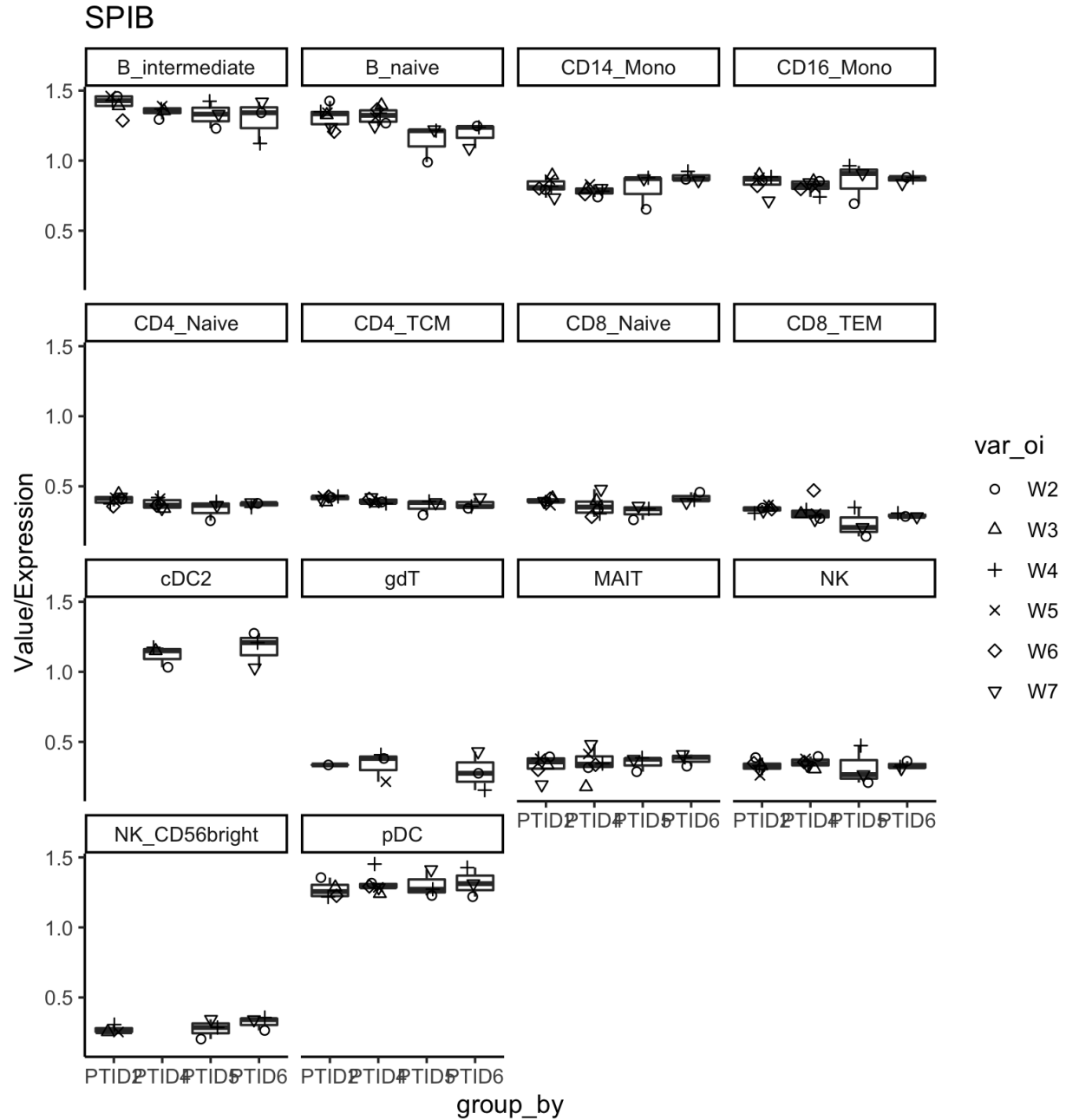
```



```

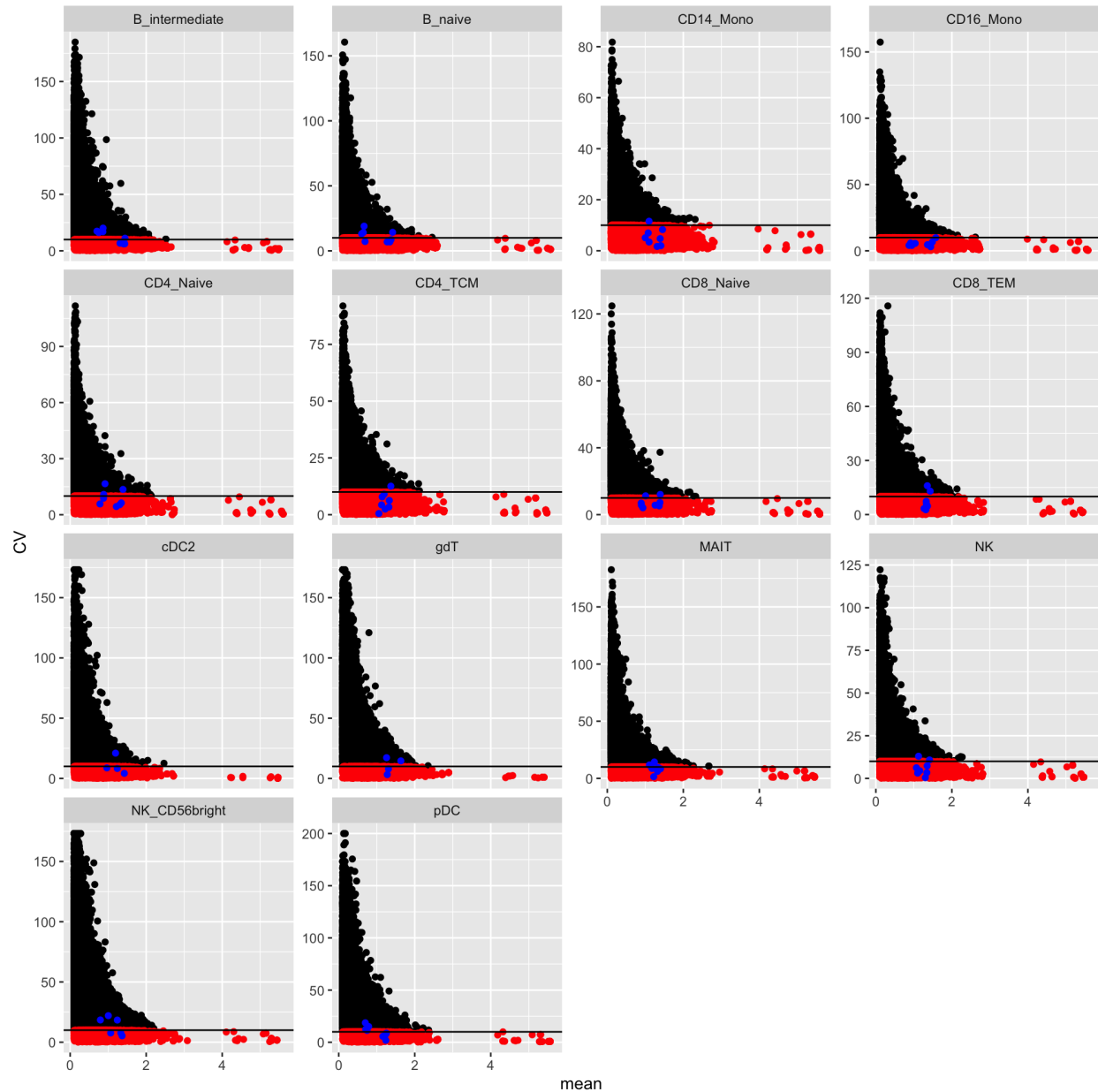
gene_featureplot(data_object=palmo_obj, featureList="SPIB", facet_by="group")

```



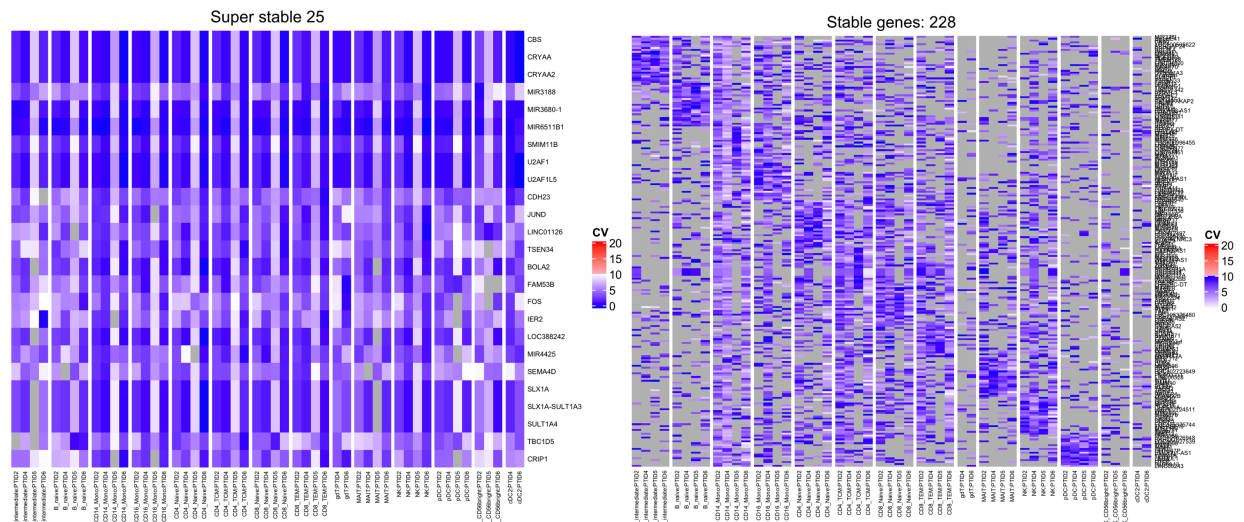
### 3.3.6 Intra-donor variations over time (Time ~ 5min)

```
palmo_obj <- cvCalcSC(data_object=palmo_obj,
  meanThreshold=0.1, cvThreshold=10,
  housekeeping_genes=c("GAPDH", "ACTB"),
  fileName="scatac")
```

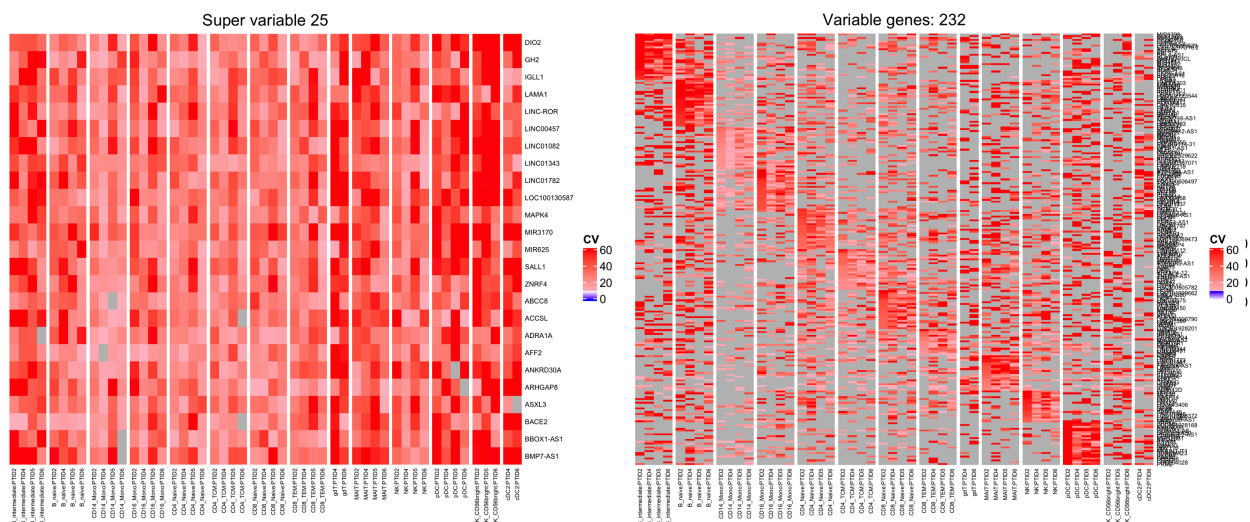


### 3.3.7 Find stable and variable features in longitudinal data (Time 30sec)

```
#Find stable and variable features in longitudinal data
donorThreshold <- 4
groupThreshold <- 28 #number of donors * number of celltypes/2 (4x14/2)
topFeatures <- 25
palmo_obj <- StableFeatures(data_object=palmo_obj,
                             cvThreshold=10,
                             donorThreshold=4, groupThreshold=28,
                             topFeatures=25,
                             fileName="scatac")
stable_gene <- palmo_obj@result$stable_genes
```



```
palmo_obj <- VarFeatures(data_object=palmo_obj,
  cvThreshold=10,
  donorThreshold=4, groupThreshold=28,
  topFeatures=25,
  fileName="scatic")
var_gene <- palmo_obj@result$var_genes
```

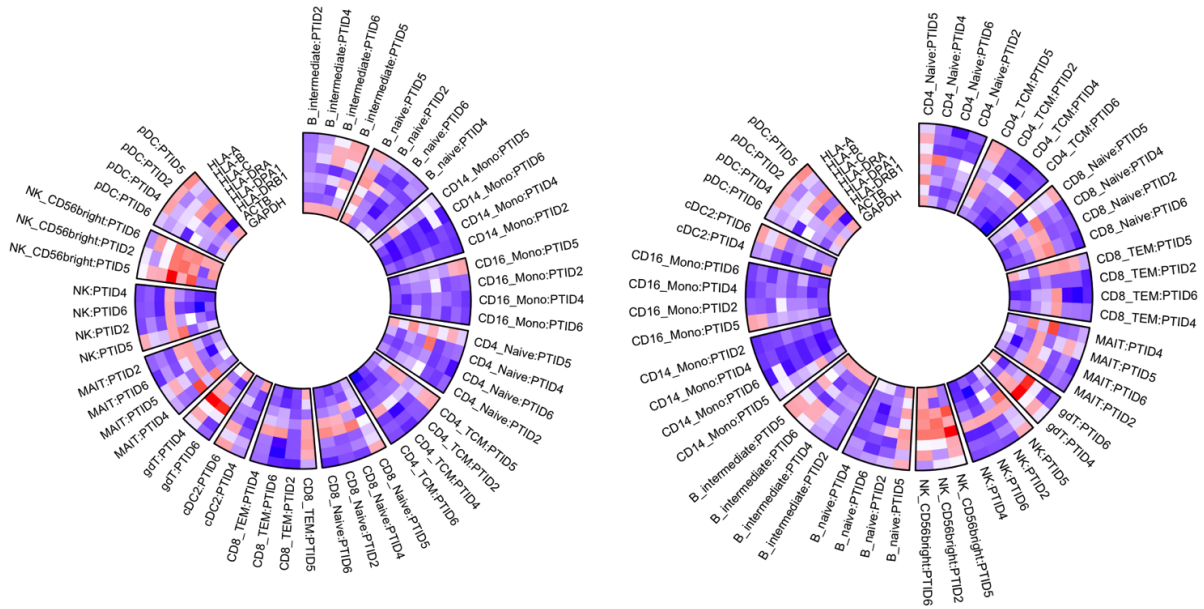


### 3.3.8 Circos CV plot (Time ~ 10sec)

```
geneList <- c("HLA-A","HLA-B","HLA-C","HLA-DRA","HLA-DPA1","HLA-DRB1",
  "ACTB","GAPDH")
plotmatrix <- genecircosPlot(data_object=palmo_obj, geneList=geneList,
  colorThreshold=15)

#order by user-defined group order
celltype_oi <- c("CD4_Naive","CD4_TEM","CD4_TCM","CD4_CTL",
  "CD8_Naive","CD8_TEM","CD8_TCM","Treg","MAIT","gdT",
```

```
plotmatrix <- genecircosPlot(data_object=palmo_obj, geneList=geneList,  
                             group_oi=celltype_oi, colorThreshold=15)
```





## 6 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] PALMO_0.99.0
#>
#> loaded via a namespace (and not attached):
#> [1] readxl_1.3.1 backports_1.2.0
#> [3] circlize_0.4.11 plyr_1.8.6
#> [5] igraph_1.2.8 lazyeval_0.2.2
#> [7] splines_4.0.3 listenv_0.8.0
#> [9] scattermore_0.7 GenomeInfoDb_1.24.2
#> [11] ggplot2_3.3.5 digest_0.6.28
#> [13] htmltools_0.5.2 fansi_0.5.0
#> [15] magrittr_2.0.1 tensor_1.5
#> [17] cluster_2.1.0 ROCR_1.0-11
#> [19] ComplexHeatmap_2.4.3 globals_0.14.0
#> [21] readr_1.4.0 modelr_0.1.8
#> [23] matrixStats_0.61.0 colorspace_2.0-2
#> [25] rvest_0.3.6 blob_1.2.1
#> [27] ggrepel_0.9.1 haven_2.3.1
#> [29] xfun_0.25 dplyr_1.0.7
#> [31] RCurl_1.98-1.2 crayon_1.4.2
#> [33] jsonlite_1.7.2 lme4_1.1-25
#> [35] spatstat_1.64-1 spatstat.data_2.1-0
#> [37] survival_3.2-7 zoo_1.8-9
#> [39] glue_1.5.0 polyclip_1.10-0
#> [41] gtable_0.3.0 zlibbioc_1.34.0
#> [43] XVector_0.28.0 leiden_0.3.9
#> [45] DelayedArray_0.14.1 GetoptLong_1.0.4
#> [47] SingleCellExperiment_1.10.1 future.apply_1.8.1
#> [49] shape_1.4.5 BiocGenerics_0.34.0
#> [51] abind_1.4-5 scales_1.1.1
#> [53] pheatmap_1.0.12 DBI_1.1.0
#> [55] miniUI_0.1.1.1 Rcpp_1.0.7
#> [57] viridisLite_0.4.0 xtable_1.8-4
#> [59] clue_0.3-57 reticulate_1.22
#> [61] stats4_4.0.3 htmlwidgets_1.5.4
```

```

#> [63] httr_1.4.2
#> [65] ellipsis_0.3.2
#> [67] factoextra_1.0.7.999
#> [69] farver_2.1.0
#> [71] uwot_0.1.10
#> [73] deldir_1.0-6
#> [75] tidyselect_1.1.1
#> [77] reshape2_1.4.4
#> [79] munsell_0.5.0
#> [81] tools_4.0.3
#> [83] broom_0.7.2
#> [85] evaluate_0.14
#> [87] fastmap_1.1.0
#> [89] goftest_1.2-3
#> [91] fs_1.5.0
#> [93] purrr_0.3.4
#> [95] pbapply_1.5-0
#> [97] nlme_3.1-149
#> [99] xml2_1.3.2
#> [101] plotly_4.10.0
#> [103] spatstat.utils_2.2-0
#> [105] tweenr_1.0.1
#> [107] statmod_1.4.35
#> [109] forcats_0.5.0
#> [111] Matrix_1.3-4
#> [113] vctrs_0.3.8
#> [115] lifecycle_1.0.1
#> [117] GlobalOptions_0.1.2
#> [119] bitops_1.0-7
#> [121] cowplot_1.1.1
#> [123] GenomicRanges_1.40.0
#> [125] patchwork_1.1.1
#> [127] promises_1.2.0.1
#> [129] gridExtra_2.3
#> [131] parallelly_1.28.1
#> [133] boot_1.3-25
#> [135] assertthat_0.2.1
#> [137] MAST_1.14.0
#> [139] SeuratObject_4.0.2
#> [141] GenomeInfoDbData_1.2.3
#> [143] mgcv_1.8-33
#> [145] hms_0.5.3
#> [147] tidyverse_1.3.0
#> [149] minqa_1.2.4
#> [151] Rtsne_0.15
#> [153] Biobase_2.48.0
#> [155] lubridate_1.7.9
RColorBrewer_1.1-2
Seurat_4.0.0
ica_1.0-2
pkgconfig_2.0.3
dbplyr_1.4.4
utf8_1.2.2
rlang_0.4.12
later_1.3.0
cellranger_1.1.0
generics_0.1.1
ggridges_0.5.3
stringr_1.4.0
yaml_2.2.1
knitr_1.30
fitdistrplus_1.1-6
RANN_2.6.1
future_1.23.0
mime_0.12
compiler_4.0.3
png_0.1-7
reprex_0.3.0
tibble_3.1.6
stringi_1.7.5
lattice_0.20-41
nloptr_1.2.2.2
pillar_1.6.4
lmtest_0.9-39
RcppAnnoy_0.0.19
data.table_1.14.2
irlba_2.3.3
httpuv_1.6.3
R6_2.5.1
KernSmooth_2.23-17
IRanges_2.22.2
codetools_0.2-16
MASS_7.3-53
SummarizedExperiment_1.18.2
rjson_0.2.20
sctransform_0.3.2
S4Vectors_0.26.1
parallel_4.0.3
rpart_4.1-15
tidyr_1.1.4
rmarkdown_2.5
ggforce_0.3.2
shiny_1.7.1

```