**Supplementary file 4: enrichment analysis of differentially expressed genes in vaccinated subjects**

This analysis is performed using the tmod package

library(tmod)

The csv file generated in the differential expression analysis is imported, genes in this table are ordered by false discovery rate.

#Day 1  
DEA\_Day1<- read.csv2("DEA\_Day1.csv")

The enrichment of the “Li” transcription modules is evaluated applying the CERNO test (an implementation of Fisher’s exact test) to the “genes” column.

D1vsD0\_LI<- tmodCERNOtest(DEA\_Day1$genes, mset="LI")

For each module, the number of significantly up- or down- regulated genes is calculated using a threshold on FDR of 0.05 and with no threshold for log fold change. The list is then converted in dataframe to correct the columns name.

pie.1<- tmodDecideTests(DEA\_Day1$genes, lfc = DEA\_Day1$logFC, pval = DEA\_Day1$FDR, mset = "LI", pval.thr = 0.05, lfc.thr = 0)  
pie.1<- as.data.frame(pie.1)  
colnames(pie.1)<- gsub("X.\*\\.", "", colnames(pie.1))

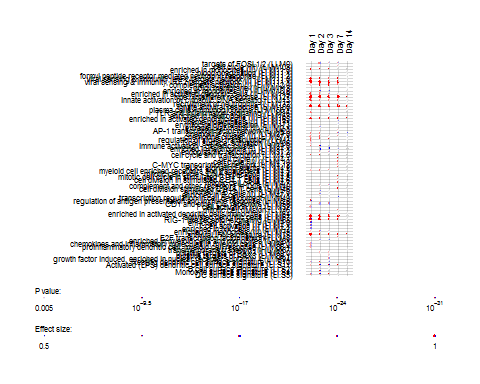
The same analysis was performed for each day until day 14

#Day 2  
DEA\_Day2<- read.csv2("DEA\_Day2.csv")  
D2vsD0\_LI<- tmodCERNOtest(DEA\_Day2$genes, mset="LI")  
pie.2<- tmodDecideTests(DEA\_Day2$genes, lfc = DEA\_Day2$logFC, pval = DEA\_Day2$FDR, mset = "LI", pval.thr = 0.05, lfc.thr = 0)  
pie.2<- as.data.frame(pie.2)  
colnames(pie.2)<- gsub("X.\*\\.", "", colnames(pie.2))  
#Day 3  
DEA\_Day3<- read.csv2("DEA\_Day3.csv")  
D3vsD0\_LI<- tmodCERNOtest(DEA\_Day3$genes, mset="LI")  
pie.3<- tmodDecideTests(DEA\_Day3$genes, lfc = DEA\_Day3$logFC, pval = DEA\_Day3$FDR, mset = "LI", pval.thr = 0.05, lfc.thr = 0)  
pie.3<- as.data.frame(pie.3)  
colnames(pie.3)<- gsub("X.\*\\.", "", colnames(pie.3))  
#Day 7  
DEA\_Day7<- read.csv2("DEA\_Day7.csv")  
D7vsD0\_LI<- tmodCERNOtest(DEA\_Day7$genes, mset="LI")  
pie.7<- tmodDecideTests(DEA\_Day7$genes, lfc = DEA\_Day7$logFC, pval = DEA\_Day7$FDR, mset = "LI", pval.thr = 0.05, lfc.thr = 0)  
pie.7<- as.data.frame(pie.7)  
colnames(pie.7)<- gsub("X.\*\\.", "", colnames(pie.7))  
#Day 14  
DEA\_Day14<- read.csv2("DEA\_Day14.csv")  
D14vsD0\_LI<- tmodCERNOtest(DEA\_Day14$genes, mset="LI")  
pie.14<- tmodDecideTests(DEA\_Day14$genes, lfc = DEA\_Day14$logFC, pval = DEA\_Day14$FDR, mset = "LI", pval.thr = 0.05, lfc.thr = 0)  
pie.14<- as.data.frame(pie.14)  
colnames(pie.14)<- gsub("X.\*\\.", "", colnames(pie.14))

The results were then visualized using the pie plot function, which shows the summary of tmod analysis. The results of CERNO and decide tests are stored in two different lists, which are used for the plot construction.

Panel\_all<- list("Day 1"= D1vsD0\_LI, "Day 2"= D2vsD0\_LI, "Day 3"= D3vsD0\_LI, "Day 7"= D7vsD0\_LI, "Day 14"= D14vsD0\_LI)  
  
Pie\_all<- list("Day 1"= pie.1, "Day 2"= pie.2, "Day 3"= pie.3, "Day 7"= pie.7, "Day 14"= pie.14)  
  
tmodPanelPlot(Panel\_all, pval.thr = 5\*10^-3, pval.cutoff= 10^-31, filter.unknown = T, text.cex = 0.5, clust = NULL, pie = Pie\_all, pie.style = "pie", legend.style = "tall")

## Warning in pvalEffectPlot(me, 10^-mq, row.labels = row.labels, col.labels = col.labels, : Figure too short, the labels will overlap.  
## Consider using smaller text.cex



This analysis was conducted on:

sessionInfo()

## R version 3.5.1 (2018-07-02)  
## Platform: x86\_64-w64-mingw32/x64 (64-bit)  
## Running under: Windows 7 x64 (build 7601) Service Pack 1  
##   
## Matrix products: default  
##   
## locale:  
## [1] LC\_COLLATE=Italian\_Italy.1252 LC\_CTYPE=Italian\_Italy.1252   
## [3] LC\_MONETARY=Italian\_Italy.1252 LC\_NUMERIC=C   
## [5] LC\_TIME=Italian\_Italy.1252   
##   
## attached base packages:  
## [1] stats graphics grDevices utils datasets methods base   
##   
## other attached packages:  
## [1] tmod\_0.40  
##   
## loaded via a namespace (and not attached):  
## [1] Rcpp\_1.0.0 XML\_3.98-1.16 digest\_0.6.18   
## [4] magrittr\_1.5 evaluate\_0.12 stringi\_1.2.4   
## [7] plotwidgets\_0.4 rmarkdown\_1.11 RColorBrewer\_1.1-2  
## [10] tools\_3.5.1 stringr\_1.3.1 tagcloud\_0.6   
## [13] beeswarm\_0.2.3 xfun\_0.4 yaml\_2.2.0   
## [16] compiler\_3.5.1 htmltools\_0.3.6 knitr\_1.21