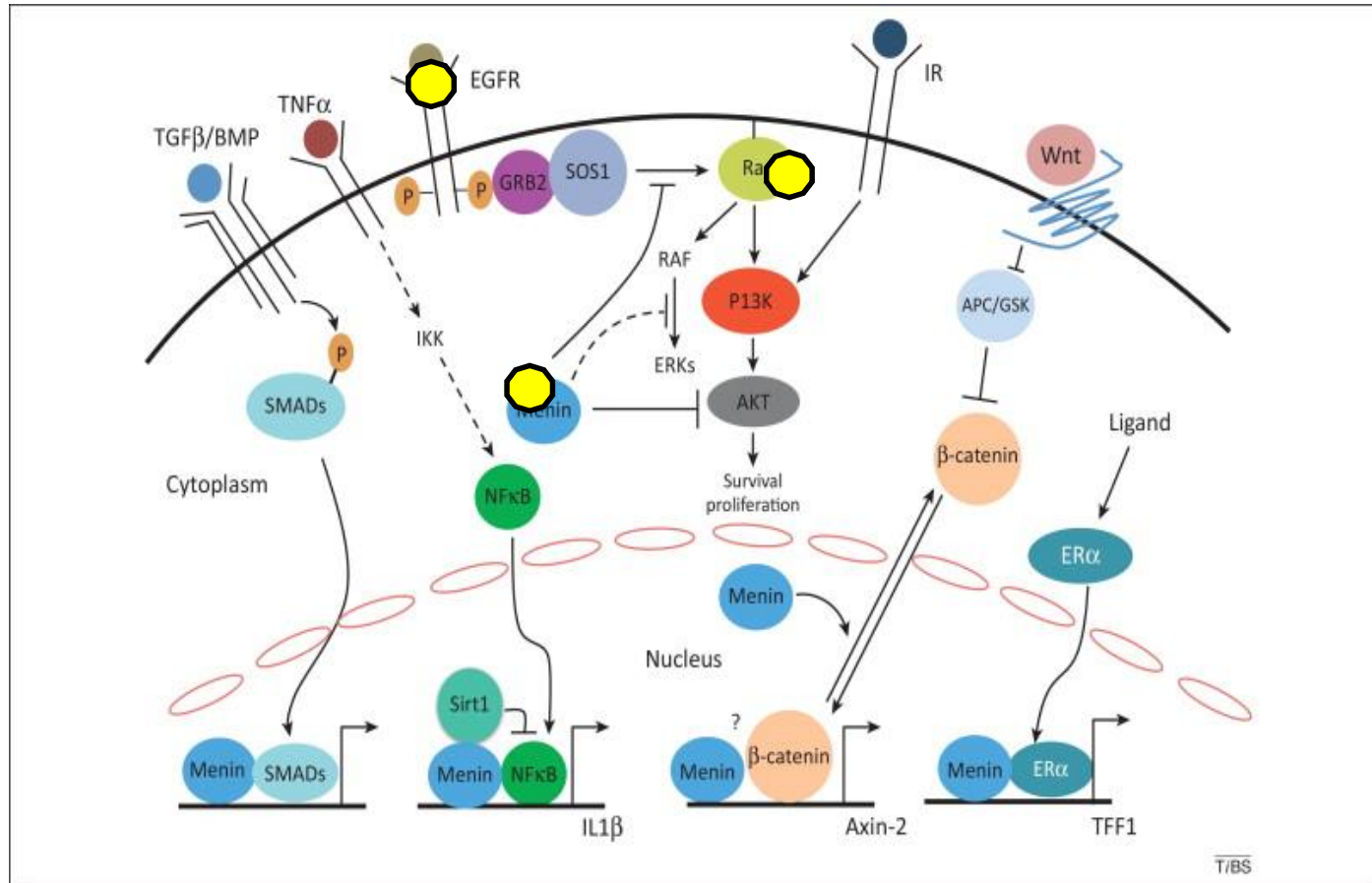

From genes to pathways: pathway quantification with ROMA

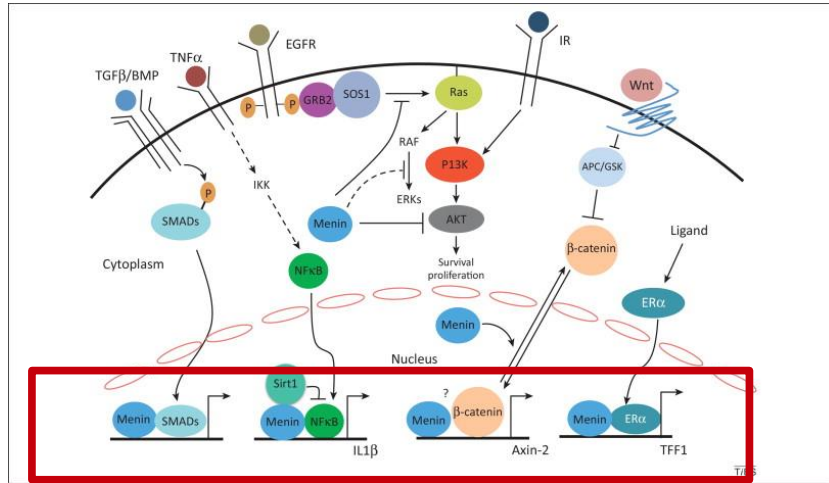
A Montagud, L Albergante, U Czerwinska, A Zinovyev, L Martignetti
U900 Computational Systems Biology of Cancer team
Institut Curie

In cancer the same biological process can be affected by damages in different individual genes

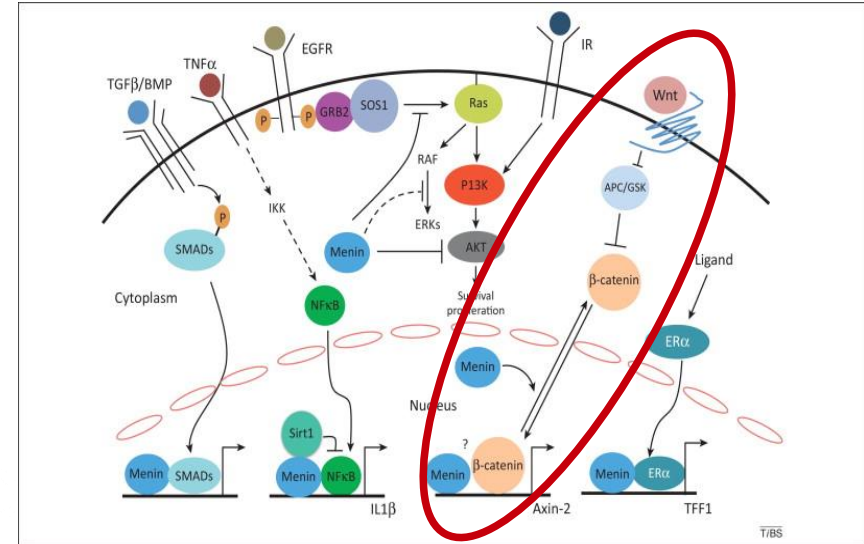


 alterations

Reasoning in terms of active/inactive gene-sets rather than single differentially expressed genes



**Gene set = target genes
co-regulated by the same TFs**



**Gene set = genes involved in a
common signalling pathway**

Pathway-level analysis :

- make use of **existing knowledge** (e.g. public database, literature, etc.)
- try to "**separate scales**", identify and **retain coarse-grained** variables that are essential for the problem

Quantification of gene-set activity

- **Single biomarker** gene expression as a proxy of the whole gene-set
- **Mean/Median expression** of the genes in the set

Some drawbacks :

- Different genes do not contribute in the **same way/strength** to the activity of the gene-set
- Some genes can **correlate negatively** with the activity of the gene-set

Alternative :

Gene set activity as a **linear combination** of individual gene expression

$$A_j = \sum \alpha_i x_{ij}$$

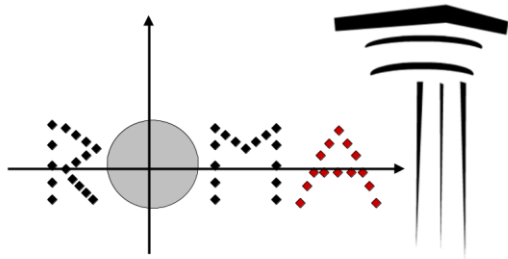
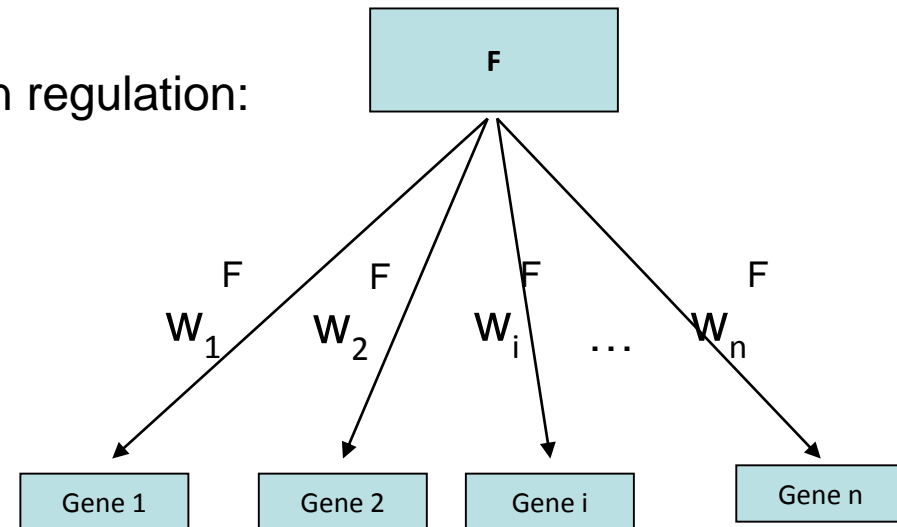
Fan J et al, Nature Methods 2016

Tomfohr et al, BMC Bioinformatics 2005

Quantification of gene-set activity by PCA

The **uni-factor linear model** of gene expression regulation:

$$x(\text{gene}_i, S_j) \sim w_i^{(F)} A_j^{(F)}$$



ROMA: Representation and Quantification of Module Activity from Target Expression Data

Loredana Martignetti^{1,2,3,4}, Laurence Calzone^{1,2,3,4}, Eric Bonnet^{1,2,3,4},
Emmanuel Barillot^{1,2,3,4} and Andrei Zinovyev^{1,2,3,4*}

Quantification of gene-set activity by PCA

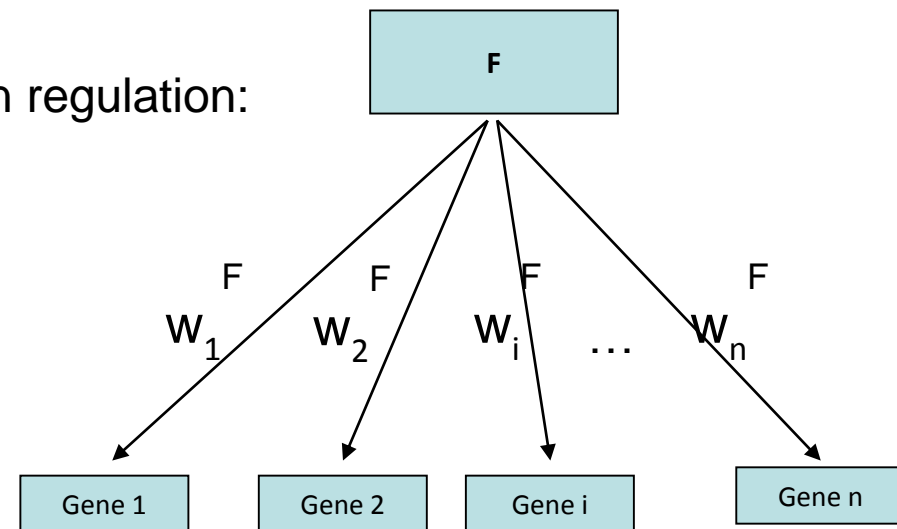
The **uni-factor linear model** of gene expression regulation:

$$x(\text{gene}_i, S_j) \sim w_i^{(F)} A_j^{(F)}$$

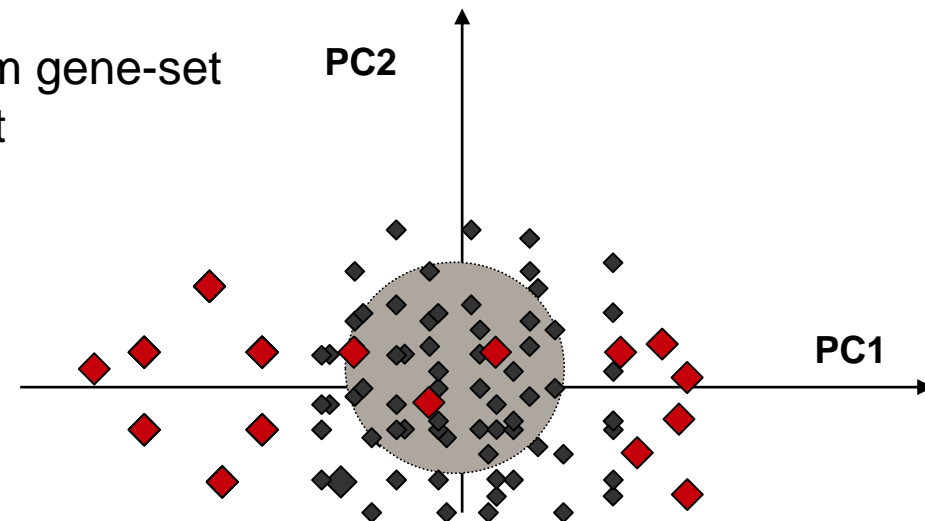
$$X = W D A$$

$$A_j^{(F)} \sim \lambda^{-1} \sum_i w_i^{(F)} x_{ij}$$

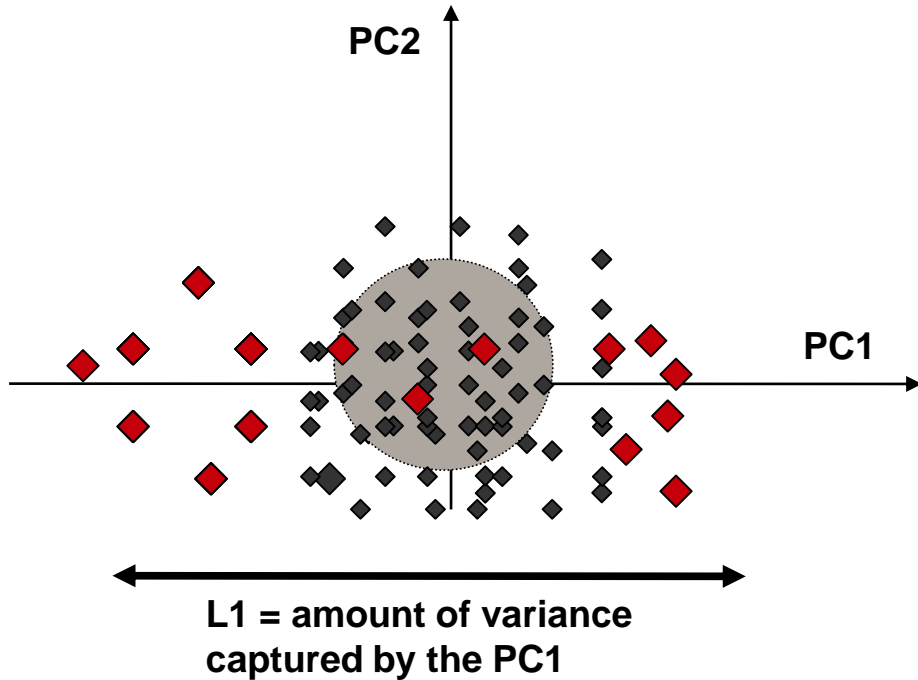
The values $w_i^{(F)}$ and $A_j^{(F)}$ are obtained by the first metagene **PC1 of the gene set** and by the level of this metagene in each sample



◆ Gene from gene-set of interest

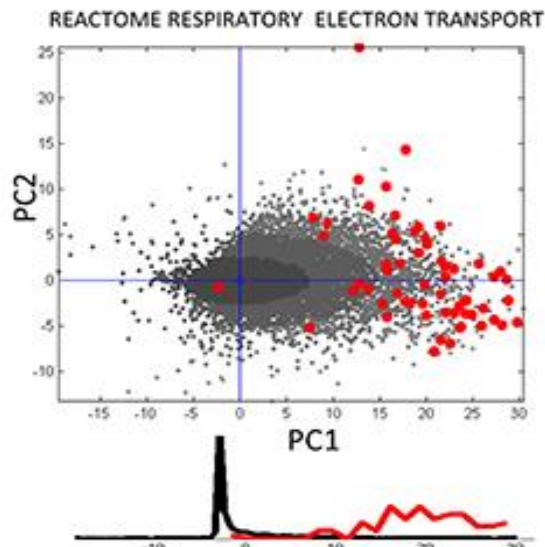
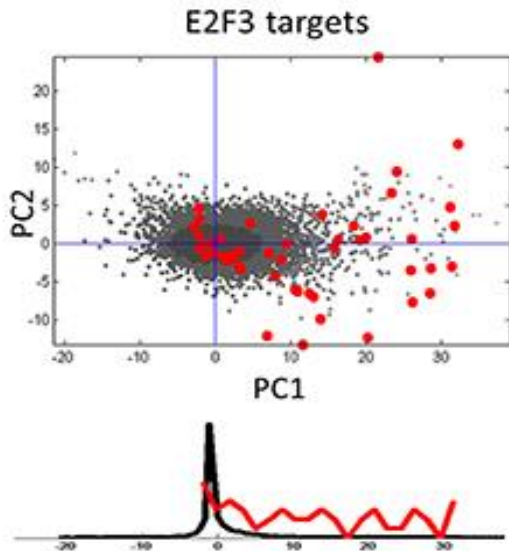


Identification of significantly active/inactive gene-sets by PC1

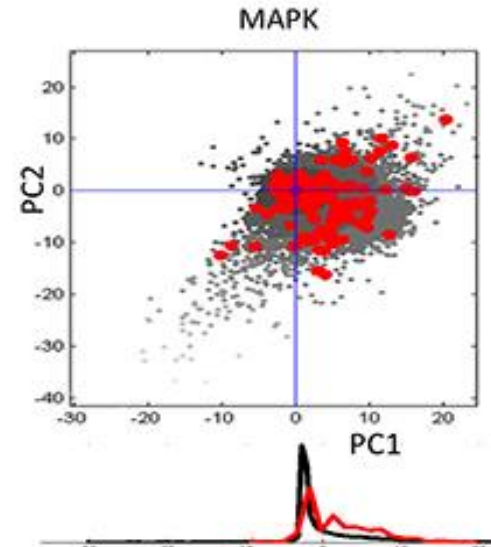


Testing if the PC1 variance L1 of a gene-set **significantly exceeds** the genome-wide **background expectation** = **overdispersion**

Overdispersed gene sets, $p\text{-value} < 0.01$



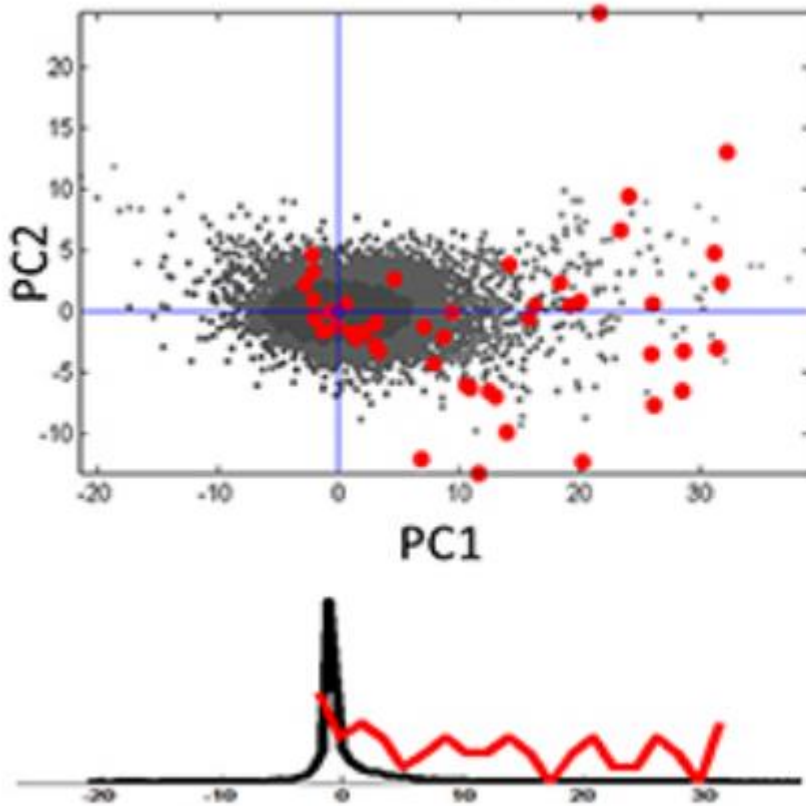
Non-overdispersed



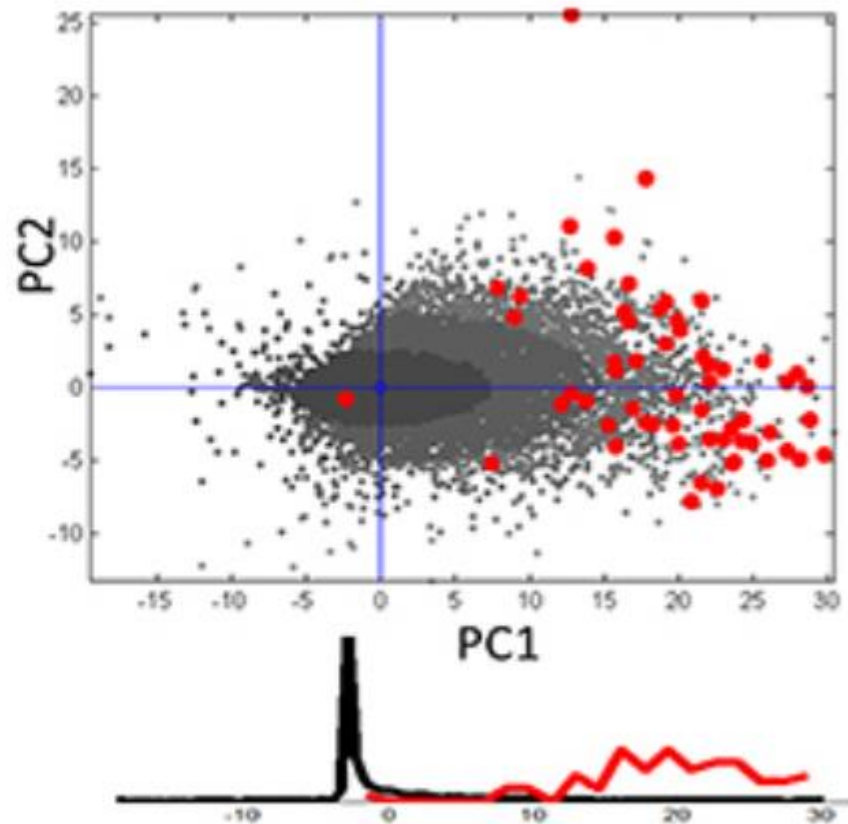
ROMA features: computing PCA with fixed center

Two possible configurations of the **target genes** :

1. **Only some genes** of the modules show overdispersion compared to the background



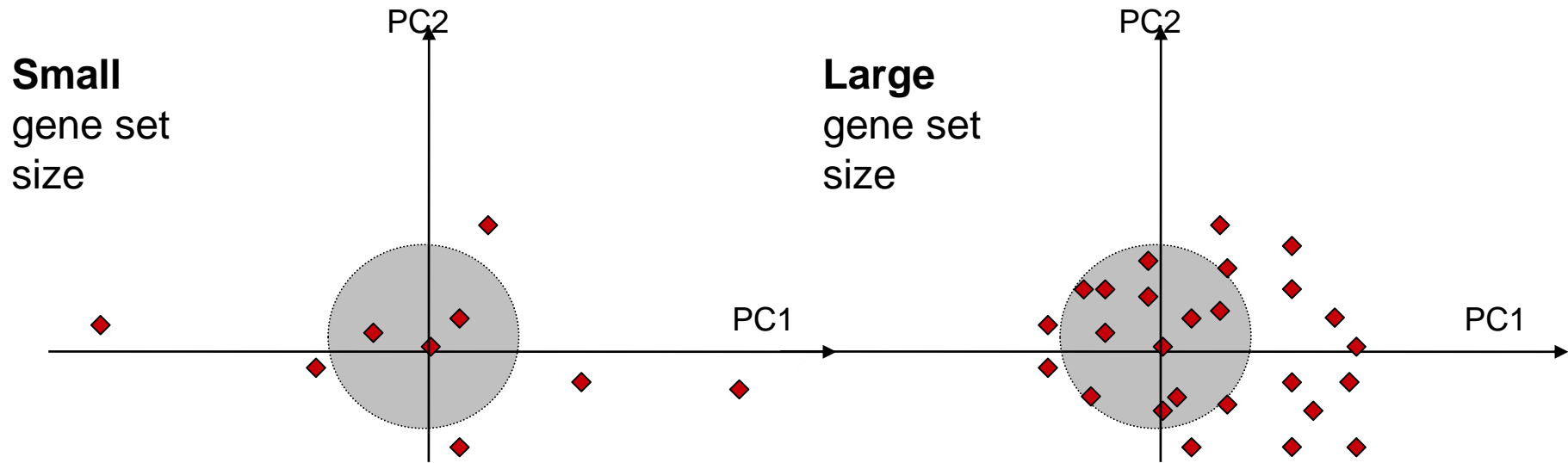
2. **All genes** of the modules are shifted compared to the background genes



The two configurations can be detected in ROMA using **PCA with fixed center**

ROMA features: assessing the statistical significance of gene-set overdispersion

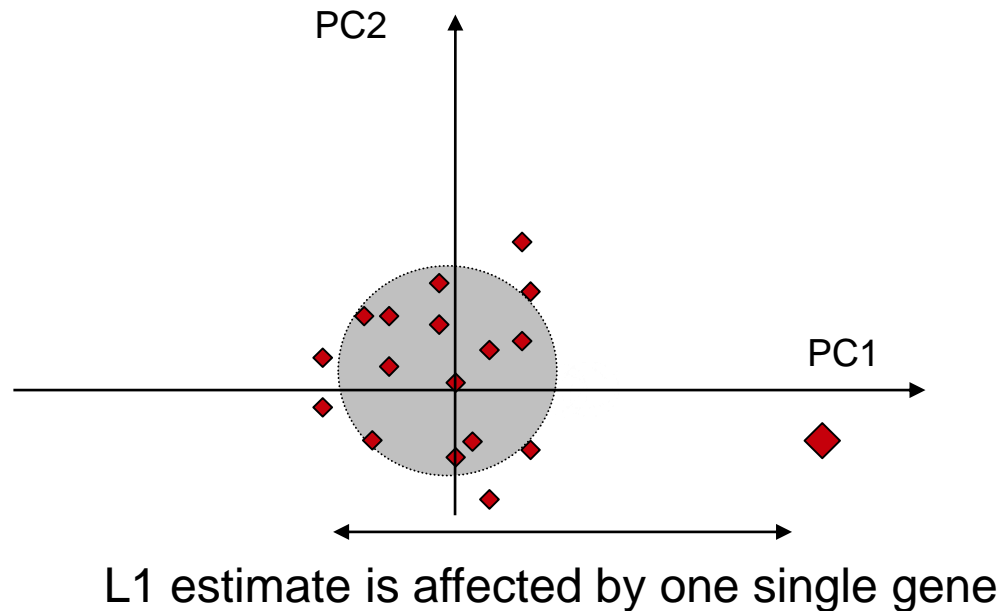
L1 and L1/L2 **strongly depend** on the **size** of the gene set



Statistical significance of L1 and L1/L2 is assessed by **estimating the null distribution** of L1 and L1/L2 from **random set of genes having representative sizes**

ROMA features: computing robust PCA

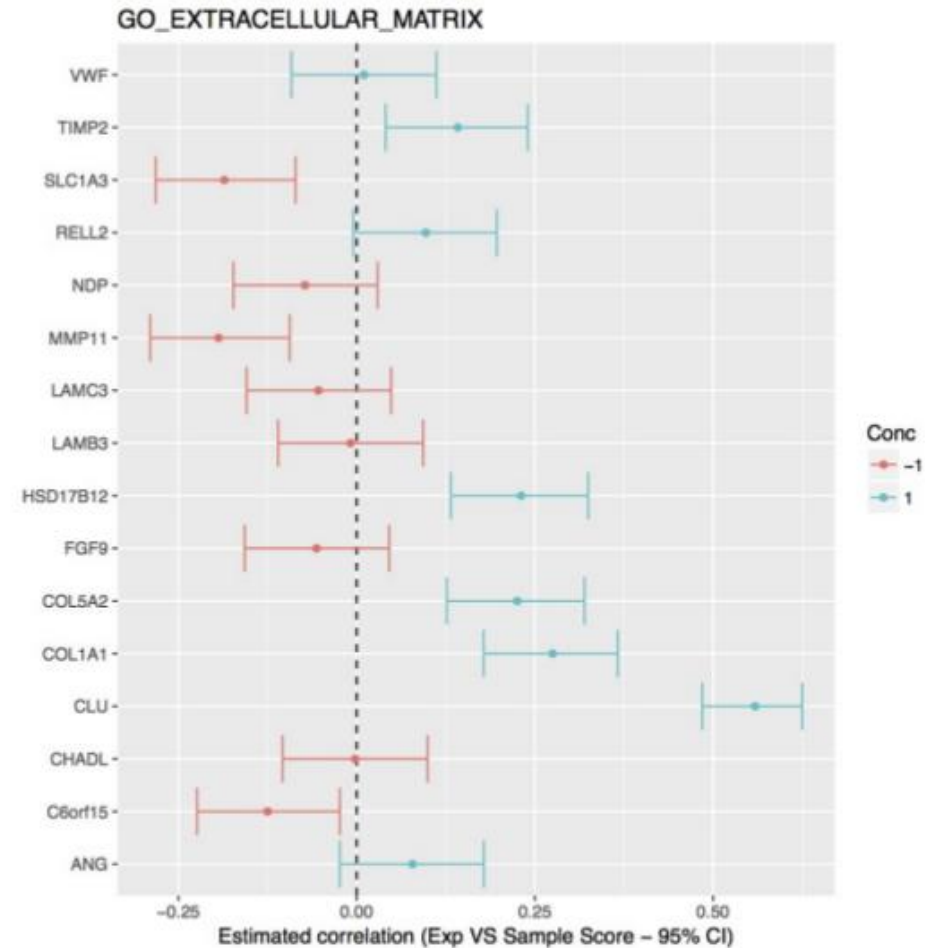
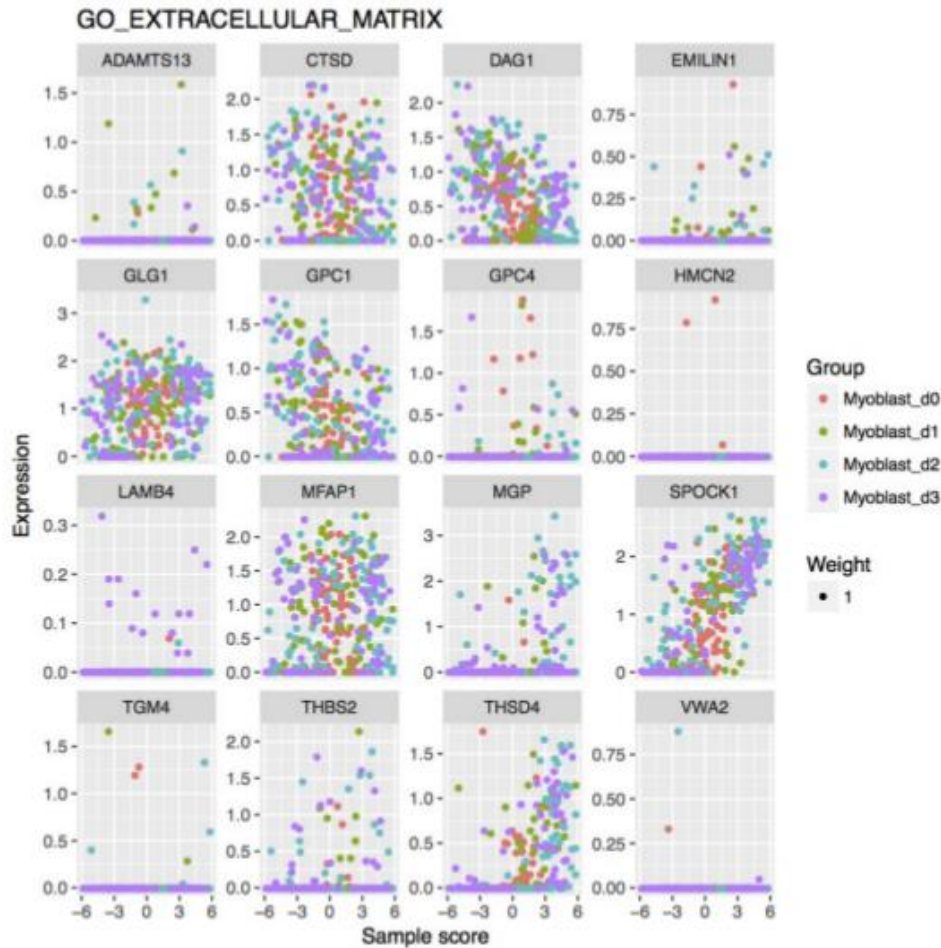
Outlier genes abnormally affecting PC1 are identified by “leave one out” procedure and removed from the gene-set



In ROMA outlier genes are identified by leave-one-out procedure:

- computing L1 n times ($n = \text{gene set size}$) removing at each time one gene in the gene set
- outliers are identified as those genes that dramatically increase L1

ROMA features: orienting PCA



In ROMA PCA is oriented in such a way that **gene projections** are **positively correlated** with **gene expression levels** for most genes

ROMA features:

using weighted gene-sets to include a priori biological knowledge

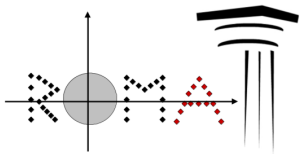
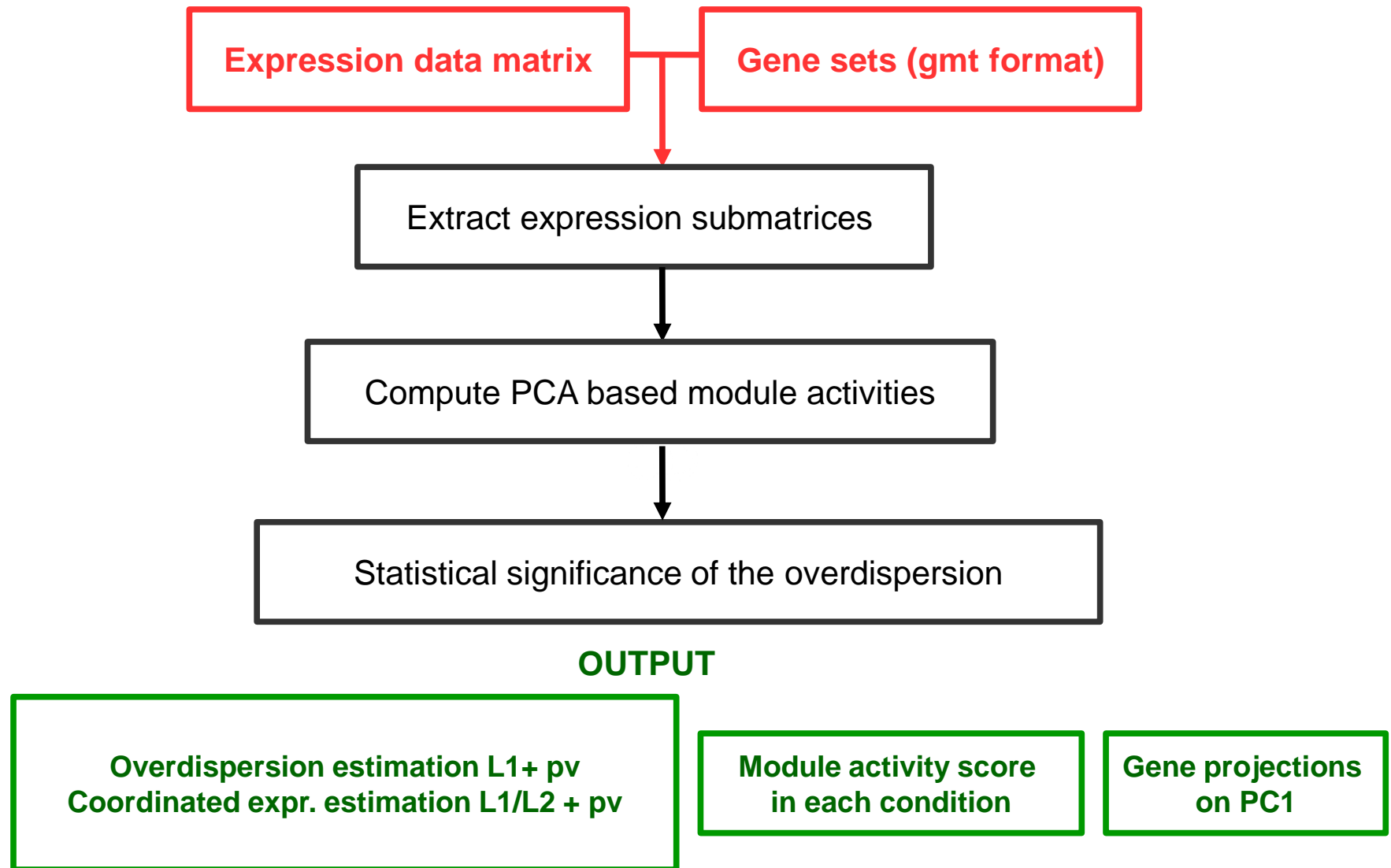
In ROMA, some weights w_g can be assigned by the user
(weighted gmt file)

Example:

positive weights for “positively regulated genes” and negative for
“inhibited genes”

Bigger weights for user-defined « most contributing » genes of the
gene-set

The ROMA algorithm



<https://github.com/sysbio-curie>

LM et al, Front Genet. 2016

**Global gene expression matrix +
M pre-defined gene sets**

	S1	S2	S3
g1	g11	g12	g13
g2	g21	g22	g23
	g31	g32	g33
	...			
gn	gn1	gn2	gn3

**For each
gene set GS**



Global gene expression matrix +
M pre-defined gene sets

	S1	S2	S3
g1	g11	g12	g13
g2	g21	g22	g23
	g31	g32	g33
...
gn	gn1	gn2	gn3

For each
gene set GS



Gene set
expression
submatrix
For GS

	S1	S2	S3
g1	g11	g12	g13
g2	g21	g22	g23
g3	g31	g32	g33
...
gn	gn1	gn2	gn3

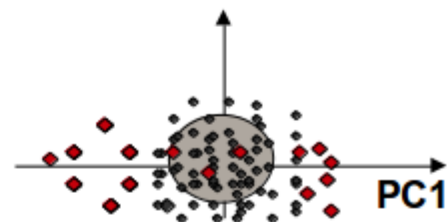


PC1

PC1	S1	S2	S3
w1	a11	a12	a13
w2				
...				
w _m				

Activity levels

Weights



Global gene expression matrix +
M pre-defined gene sets

	S1	S2	S3
g1	g11	g12	g13
g2	g21	g22	g23
	g31	g32	g33
...
gn	gn1	gn2	gn3

Activity
scores

GS1	L1
GS2	L2
	...
GSm	L _m

Gene contributions
to each gene set

	GS1	GS2	...	GSm
g1	w11	w12	...	0
g2	0	0	...	w2m
	0	w32	...	w3m
...
gn	wn1	wn2	...	0

Activity matrix

	S1	S2	S3
GS1	a11	a12	a13
GS2	a21	a22	a23
	a31	a32	a33
GSm	am1	am2	am3

For each
gene set GS

Global
Results

Gene set
expression
submatrix
For GS

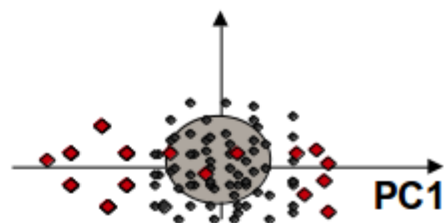
	S1	S2	S3
	g11	g12	g13
	g21	g22	g23
	g31	g32	g33

	gn1	gn2	gn3

	PC1	S1	S2	S3
L1	w1	a11	a12	a13
	w2				
	...				
	w _m				

Activity levels

Weights



How to use ROMA in practice



Java version @ <https://github.com/sysbio-curie/Roma>

Command line usage:

```
java -jar roma_v1.0.jar [required options] [other options]
```



R version @ <https://github.com/sysbio-curie/rRoma>

R version using shiny dashboard @
<https://github.com/sysbio-curie/rRomaDash>

Execute rRoma with command line

Load data

Expression matrix file

Sample annotation file

Testing different signatures for a given pathway (ex: wnt)

```
wntGMT <- ReadGMTFile("Unsigned_wnt_path.gmt")
```

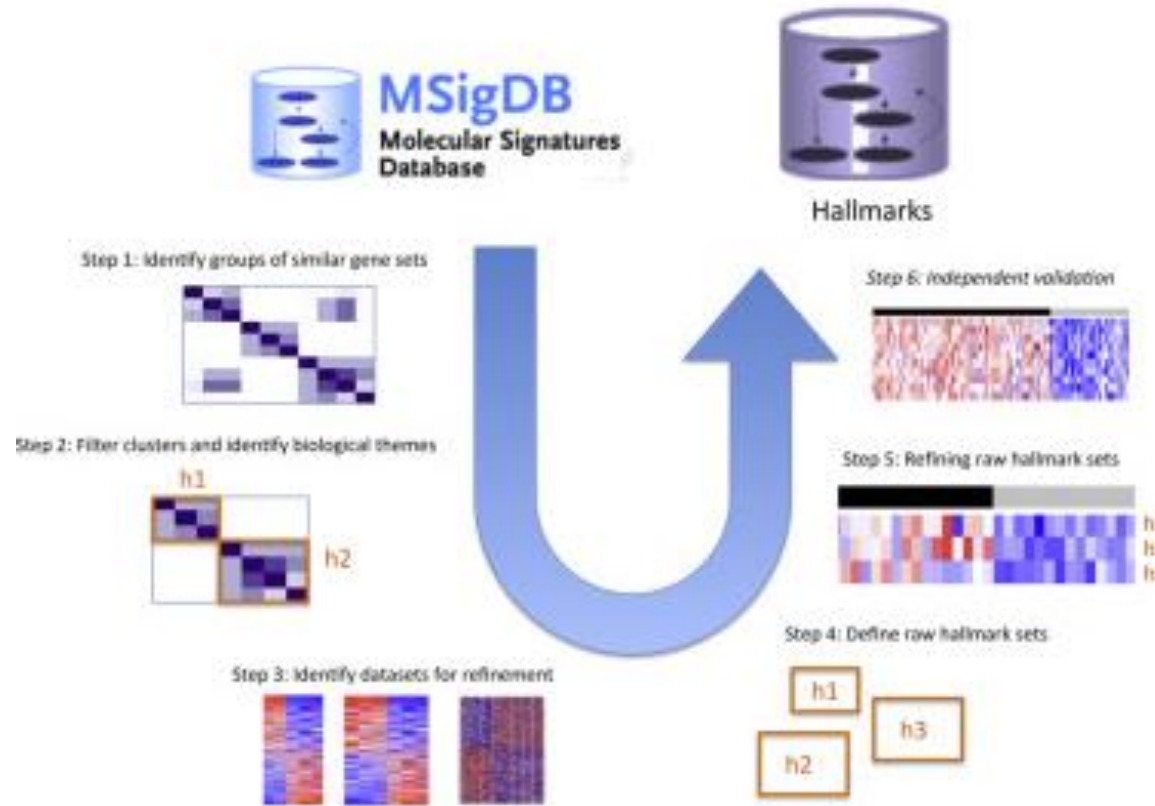
```
Data.wnt <- rRoma.R(ExpressionMatrix = expr,  
                     ModuleList = wntGMT,  
                     FixedCenter = TRUE,  
                     MaxGenes = 1000,  
                     PCSignMode="CorrelateAllWeightsByGene",  
                     PCAType = "DimensionsAreSamples")
```

Results of different signatures of WNT pathway

	L1	ppv L1	L1/L2	ppv L1/L2
WNT_CANONICAL	0.2160966	0.45	1.571934	0.61
Wnt_CELL_MAP	0.3168016	0.18	2.465684	0.10
WNT_NON_CANONICAL	0.2237947	0.34	1.684310	0.48
WNT_pthw_Metastasis	0.3099135	0.04	2.173662	0.14
wnt_IPA	0.3020718	0.00	2.734551	0.01

Two WNT signatures (WNT_pthw_Metastasis,wnt_IPA) perform better than the others

The Molecular Signatures Database (MSigDB) hallmark gene set collection



Hallmark gene sets represent specific well-defined biological states or processes that display coherent expression

<https://software.broadinstitute.org/gsea/msigdb/>

Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. **The Molecular Signatures Database (MSigDB) hallmark gene set collection.** Cell Syst. 2015 Dec 23;1(6):417-425.

Testing a database of signatures (ex: MsigDB Hallmarks)

```
AllHall <- SelectFromMSIGdb("HALLMARK")
```

```
Data.hall <- rRoma.R(ExpressionMatrix = expr,  
                      ModuleList = AllHall,  
                      FixedCenter = TRUE,  
                      MaxGenes = 1000,  
                      PCSignMode="CorrelateAllWeightsByGene",  
                      PCAType = "DimensionsAreSamples")
```

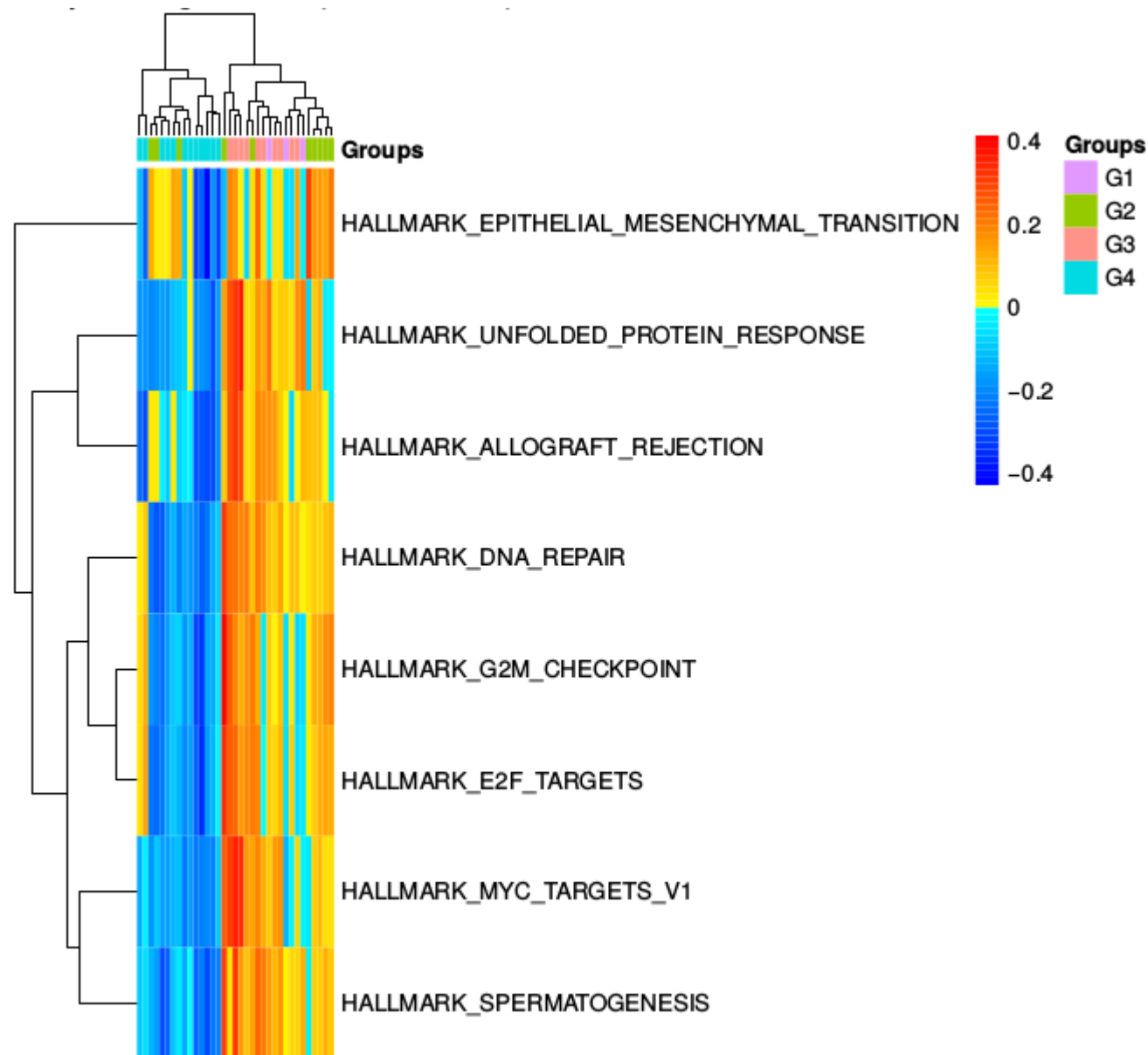
Selecting significantly active/inactive modules

```
AggData.FC <- Plot.Genesets (RomaData = Data.hall,  
  Selected = SelectGeneSets (RomaData = Data.hall,  
    VarThr = 1e-05,  
    VarMode = "Wil",  
    VarType = "Over"),  
  GenesetMargin = 20,  
  SampleMargin = 14,  
  cluster_cols = TRUE,  
  GroupInfo = Group,  
  AggByGroupsFL = c("mean", "sd"),  
  HMTite = "Overdispersed genesets (Fixed center)")
```

-> over- or under-underdispersed genesets selected according to VarThr p-value threshold and VarMode = "Wil" or "PPV" for Wilcoxon or permutation test

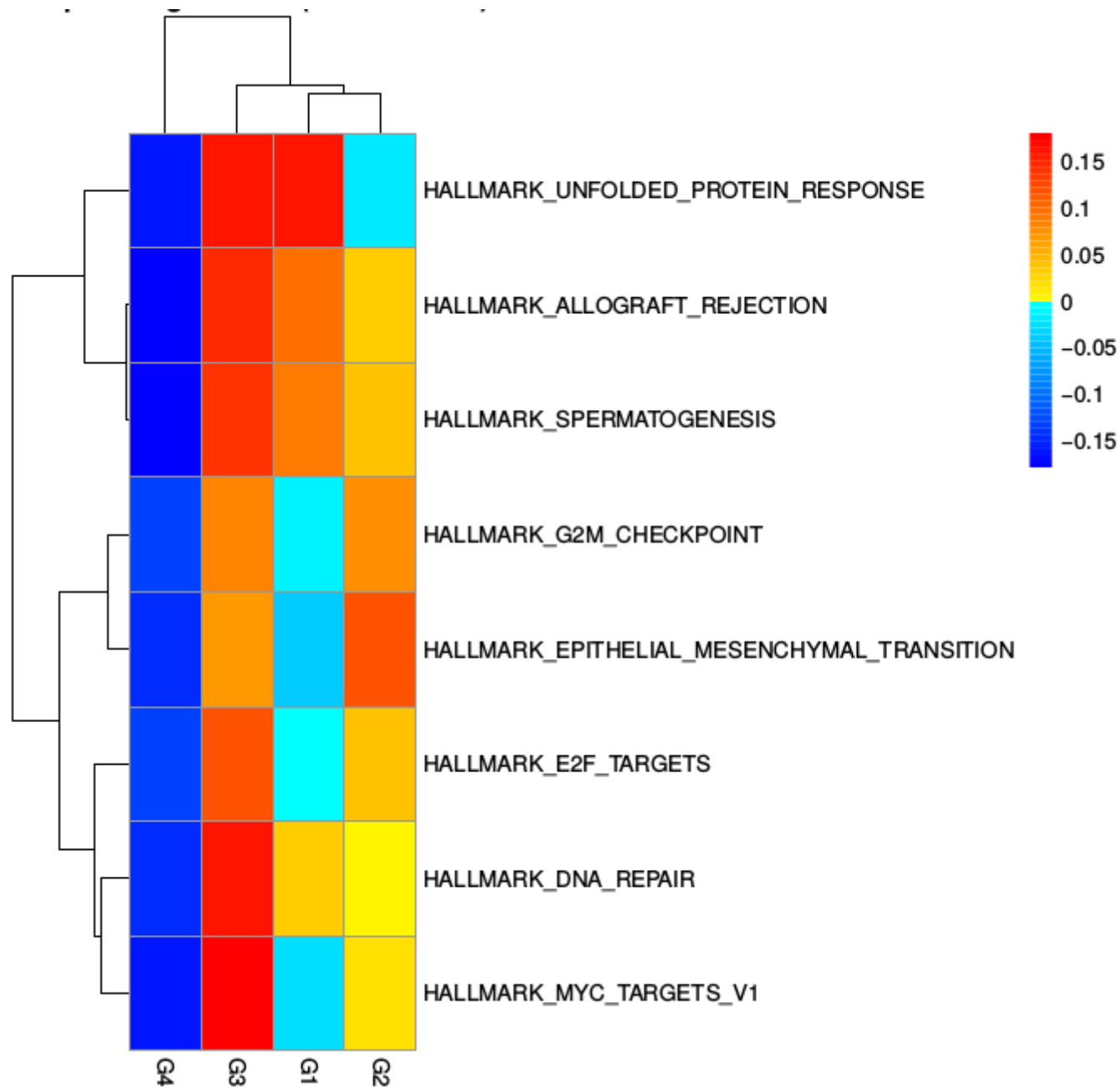
-> Aggregating data by Group

Heatmap of module activity per sample



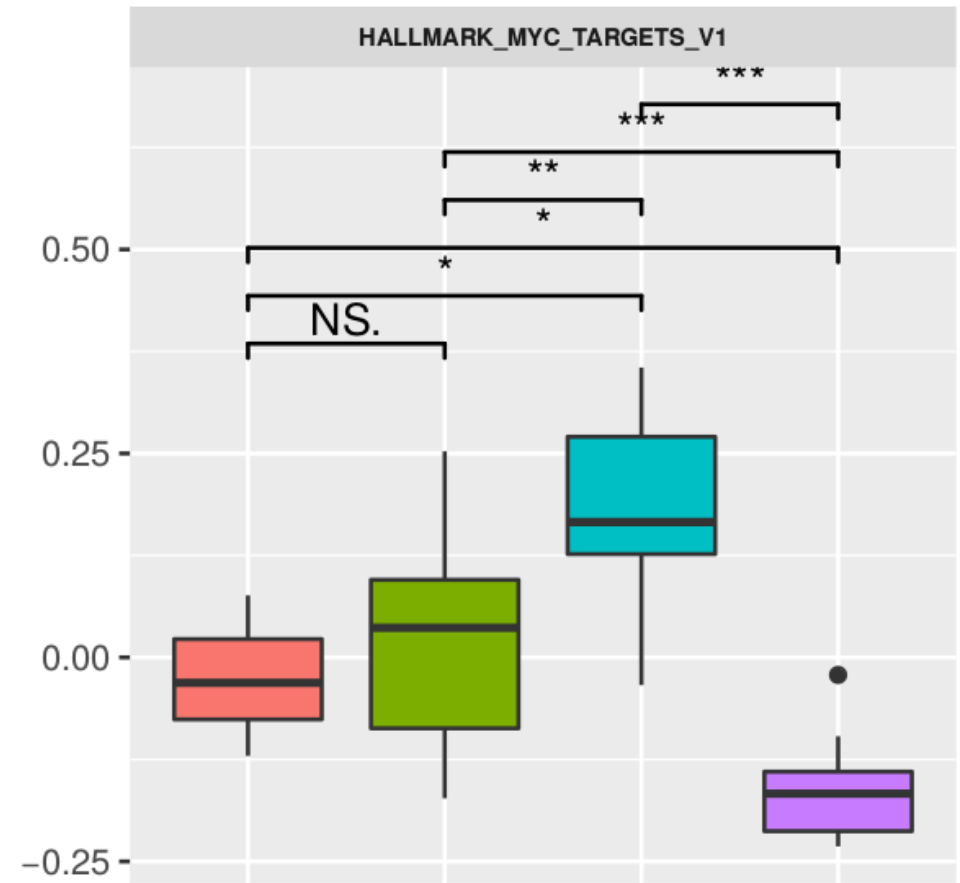
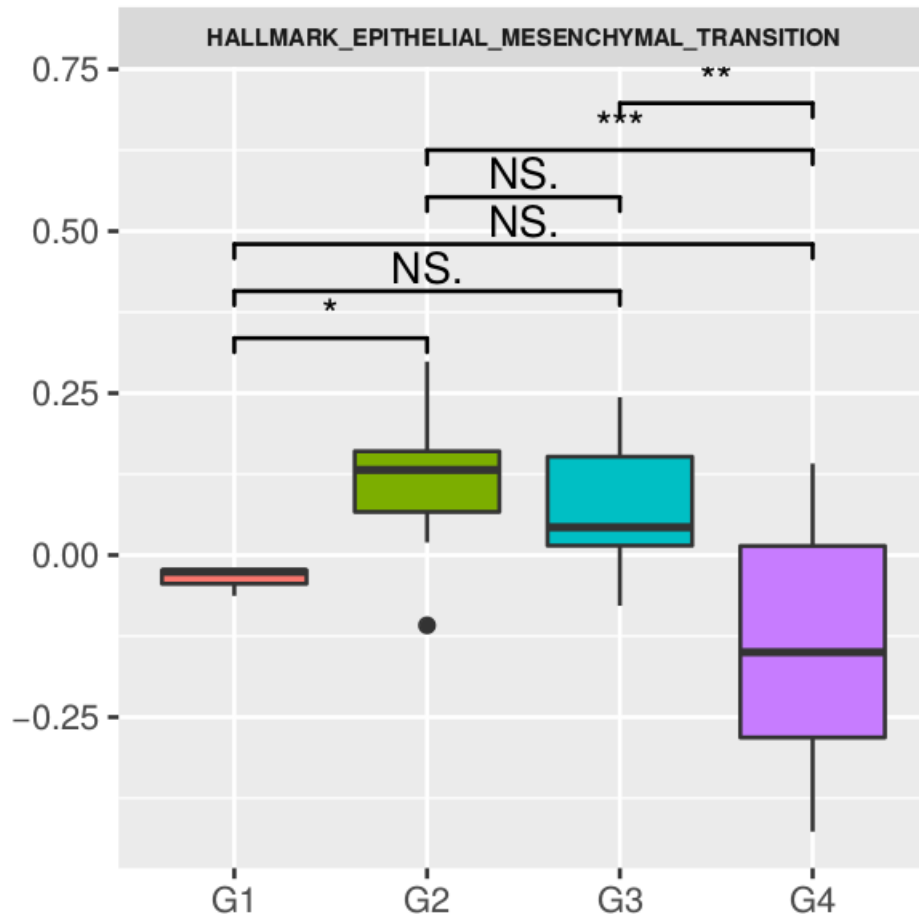
- > EMT active in G2 samples
- > MYC targets activated in G3 samples

Heatmap of module activity per group

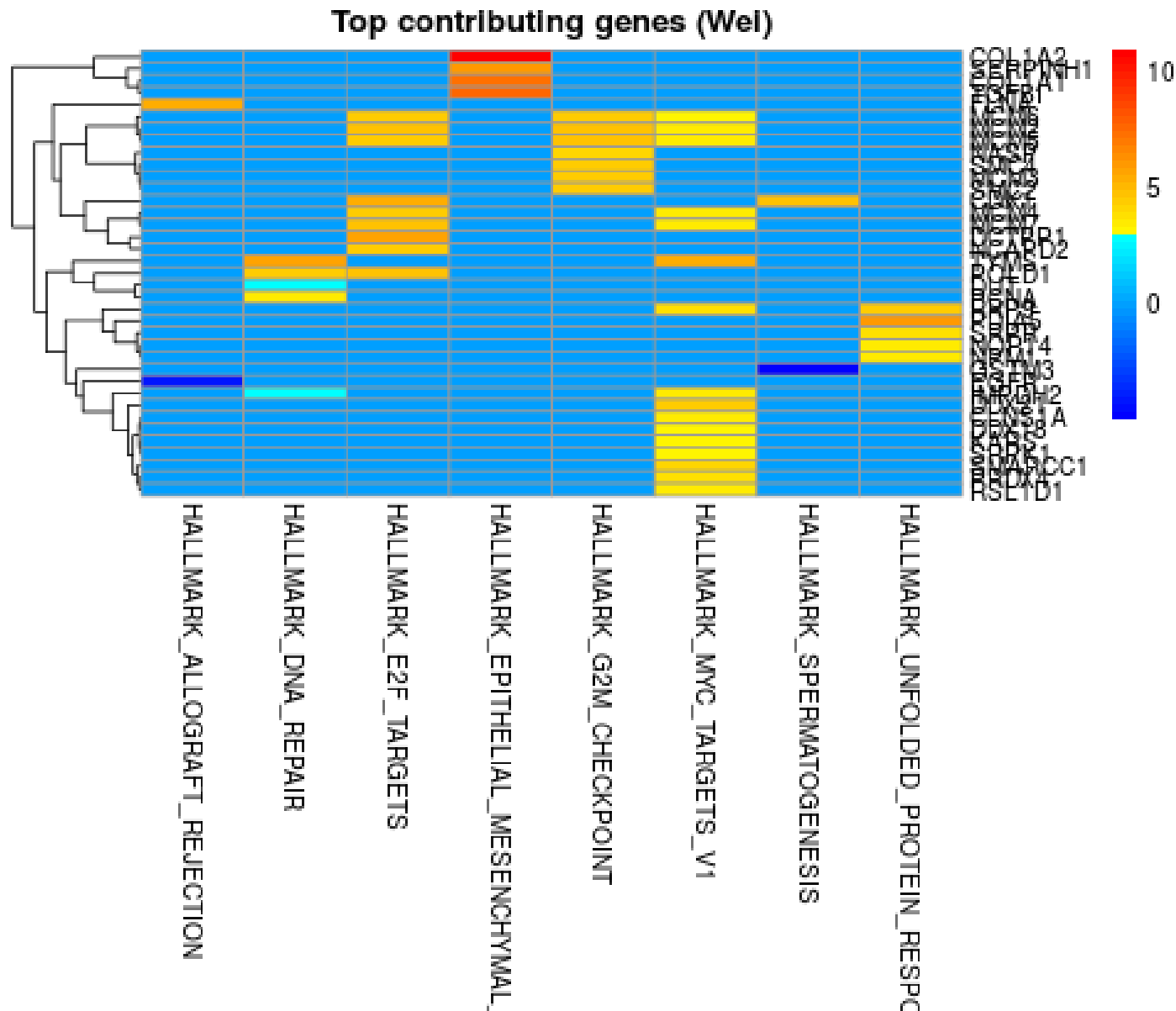


Differential analysis between groups based on module activity

```
CompareAcrossSamples(RomaData = Data.hall,  
  Selected = SelectGeneSets(RomaData = Data.hall,  
    VarThr = 1e-05,  
    VarMode = "Wil",  
    VarType = "Over"),  
  Groups = Group)
```



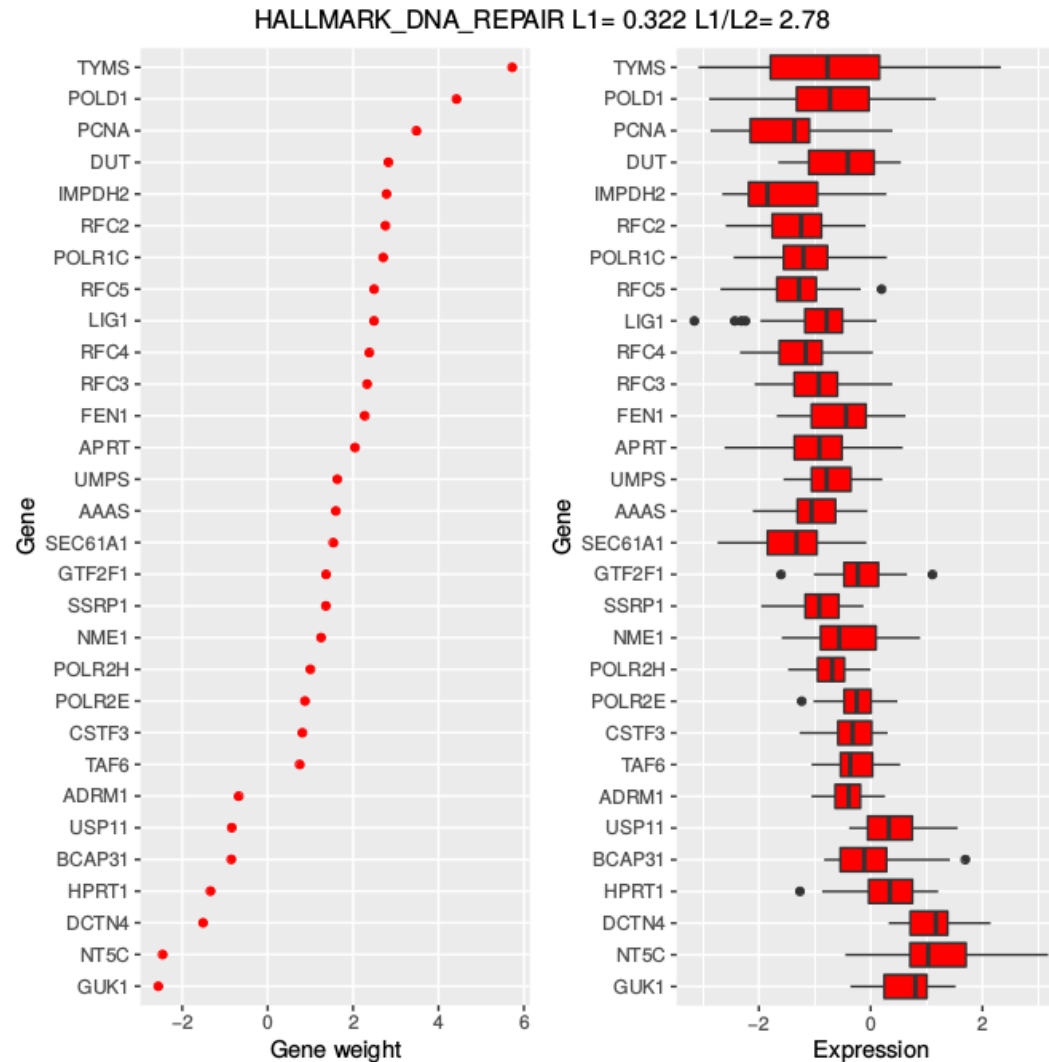
Heatmap of the most contributing genes for significant modules



```
GeneMat <- GetTopContrib(Data.hall,
  Selected = SelectGeneSets(RomaData = Data.hall,
    VarThr = 1e-5, VarMode = "Wil", VarType = "Over"),
  nGenes = .1, OrderType = "Abs", Mode = "Wei", Plot = TRUE)
```

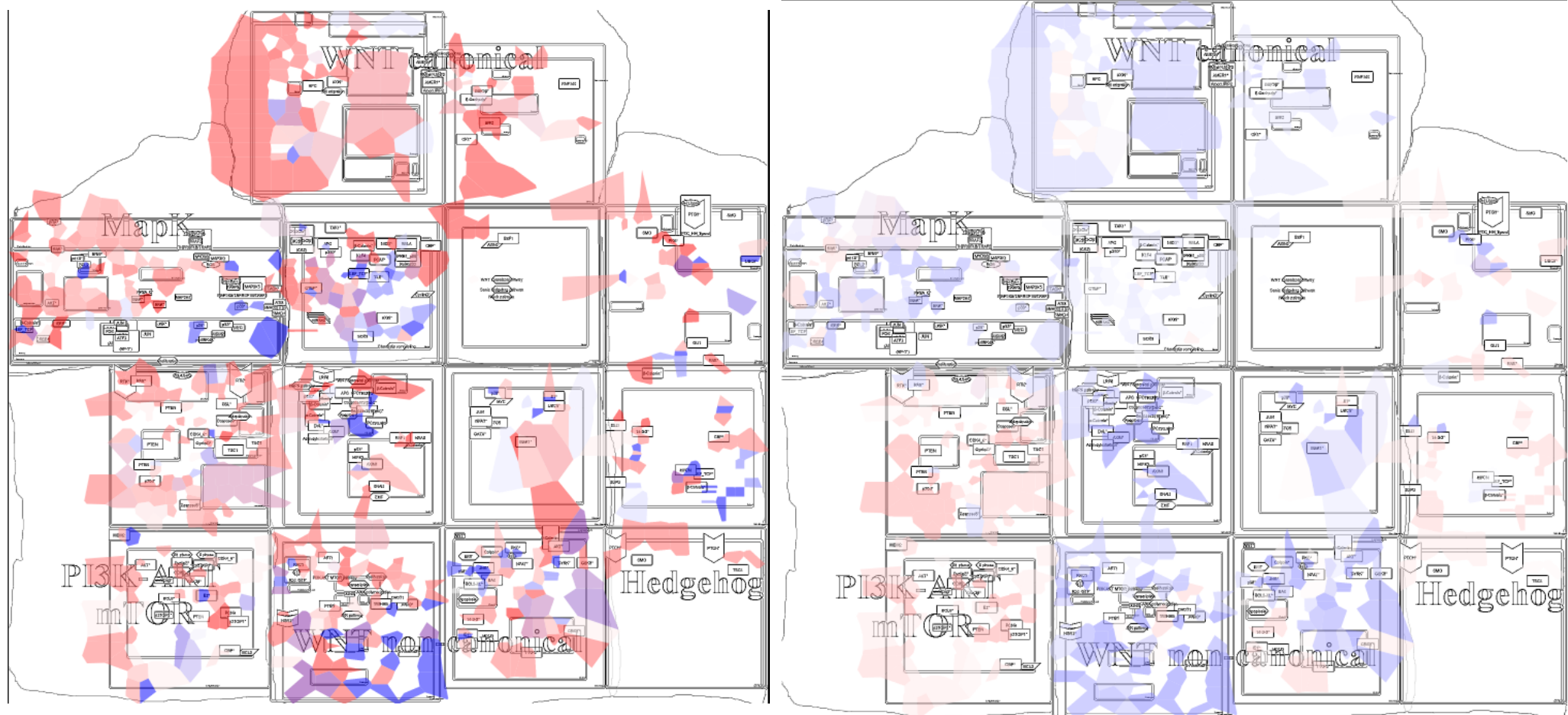
Visualize the most contributing genes for a given module

```
PlotGeneWeight(RomaData = Data.hall, PlotGenes = 30,  
ExpressionMatrix = expr, LogExpression = FALSE,  
Selected = SelectGeneSets(RomaData = Data.hall,  
VarThr = 1e-5, VarMode = "Wil", VarType = "Over"),  
PlotWeighthSign = TRUE)
```

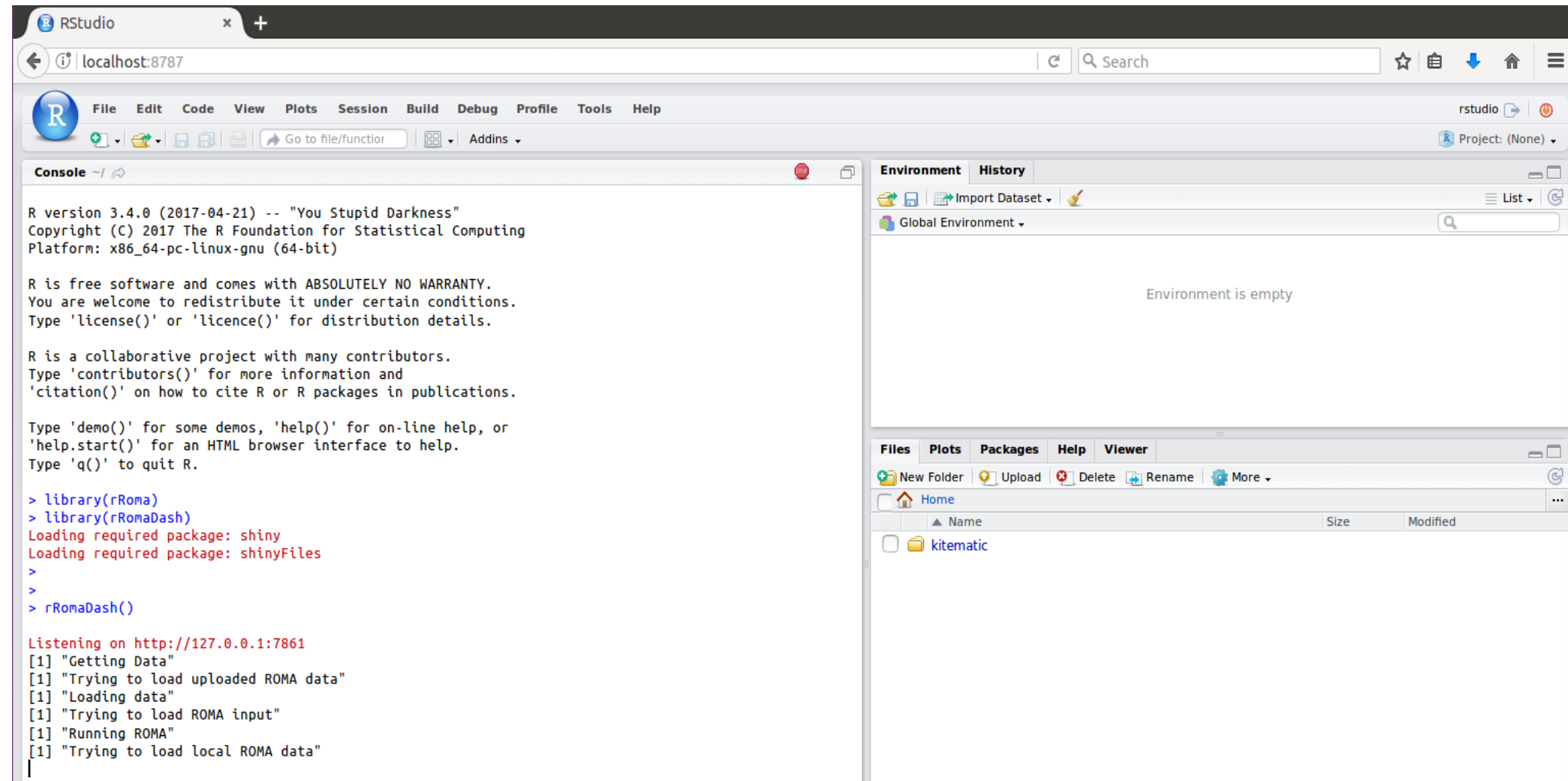


Visualization of ROMA scores on ACSN maps

- > Testing gene sets from ACSN maps
- > Visualizing ROMA scores by Group (creating group-specific maps)



Launch rROMA interface : rROMADash()



The screenshot shows the RStudio environment with the following components:

- Console:** Displays the R version (3.4.0), copyright information, and the execution of the `rRomaDash()` command. The output shows the interface is listening on `http://127.0.0.1:7861` and performing several initialization steps.
- Environment:** Shows the Global Environment, which is currently empty.
- Files:** Shows the file explorer with a folder named `kitematic`.

```
R version 3.4.0 (2017-04-21) -- "You Stupid Darkness"
Copyright (C) 2017 The R Foundation for Statistical Computing
Platform: x86_64-pc-linux-gnu (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

> library(rRoma)
> library(rRomaDash)
Loading required package: shiny
Loading required package: shinyFiles
>
>
> rRomaDash()

Listening on http://127.0.0.1:7861
[1] "Getting Data"
[1] "Trying to load uploaded ROMA data"
[1] "Loading data"
[1] "Trying to load ROMA input"
[1] "Running ROMA"
[1] "Trying to load local ROMA data"
```


Execute rRoma : load data

rRoma dashboard

Analyze Data

Summarize Info

Visualize Results

Save/Load

Execute rROMA

Expression matrix missing
Group information missing
Geneset list loaded

Input

Parameters

Expression matrix

Choose an expression matrix (TSV file)

Browse...

No file selected

Sample groups

Choose a group matrix (TSV file)

Browse...

No file selected

☒ Use groups

Geneset list

Geneset source:

Internal DB

Available geneset list:

Molecular signature DB (v6.0)

Apply

Keywords

hallmark

☐ search all keywords

☐ load weights

Available Genesets:

Show 25 entries

Search:

Names	Genes	Weighted
HALLMARK_TNFA_SIGNALING_VIA_NFKB	200	0
HALLMARK_HYPOXIA	200	0
HALLMARK_CHOLESTEROL_HOMEOSTASIS	74	0
HALLMARK_MITOTIC_SPINDLE	200	0
HALLMARK_WNT_BETA_CATENIN_SIGNALING	42	0
HALLMARK_TGF_BETA_SIGNALING	54	0
HALLMARK_IL6_JAK_STAT3_SIGNALING	87	0
HALLMARK_DNA_REPAIR	150	0
HALLMARK_G2M_CHECKPOINT	200	0
HALLMARK_APOPTOSIS	161	0
HALLMARK_NOTCH_SIGNALING	32	0

Execute rRoma : set parameters

Base parameters

FixedCenter

FALSE

PCSignMode

CorrelateAllWeightsByGene

nSamples

100

GeneOutThr

5

UseParallel

TRUE

nCores

7

ClusType

PSOCK

Advanced parameters

UseWeights

FALSE

SampleFilter

TRUE

MinGenes

10

FullSampleInfo

FALSE

ExpFilter

FALSE

MaxGenes

500

centerData

TRUE

MoreInfo

FALSE

ApproxSamples

5

GeneSelMode

All

GeneOutDetection

L1OutExpOut

PCSignThr

NULL

Ncomp

5

OutGeneNumber

5

CorMethod

pearson

DefaultWeight

1

OutGeneSpace

NULL

Acknowledgments

Computational Systems Biology of Cancer group

Emmanuel Barillot

Luca Albergante

Loredana Martignetti

Urszula Czerwinska

Andrei Zinovyev

Jonas Béal

Inna Kuperstein

Laurence Calzone

Gaelle Letort

Laura Cantini

Christine Lonjou

Mihaly Koltai

Cristóbal Monraz

Maria Kondratova

Resources

<https://github.com/sysbio-curie/rRoma>

<https://github.com/sysbio-curie/rRomaDash>

<https://github.com/sysbio-curie/Roma>

https://github.com/sysbio-curie/Roma_tutorial