

Metabolic network analysis

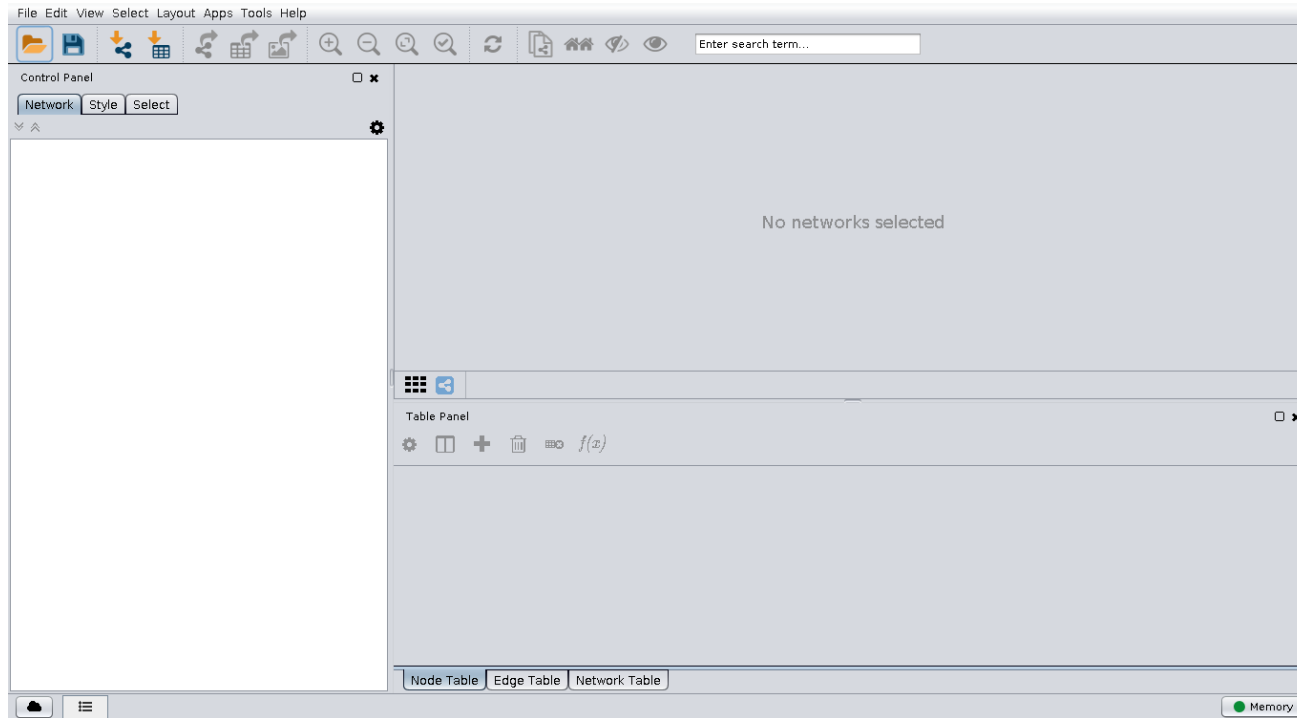
Alexey Sergushichev

Sep 23, Nice

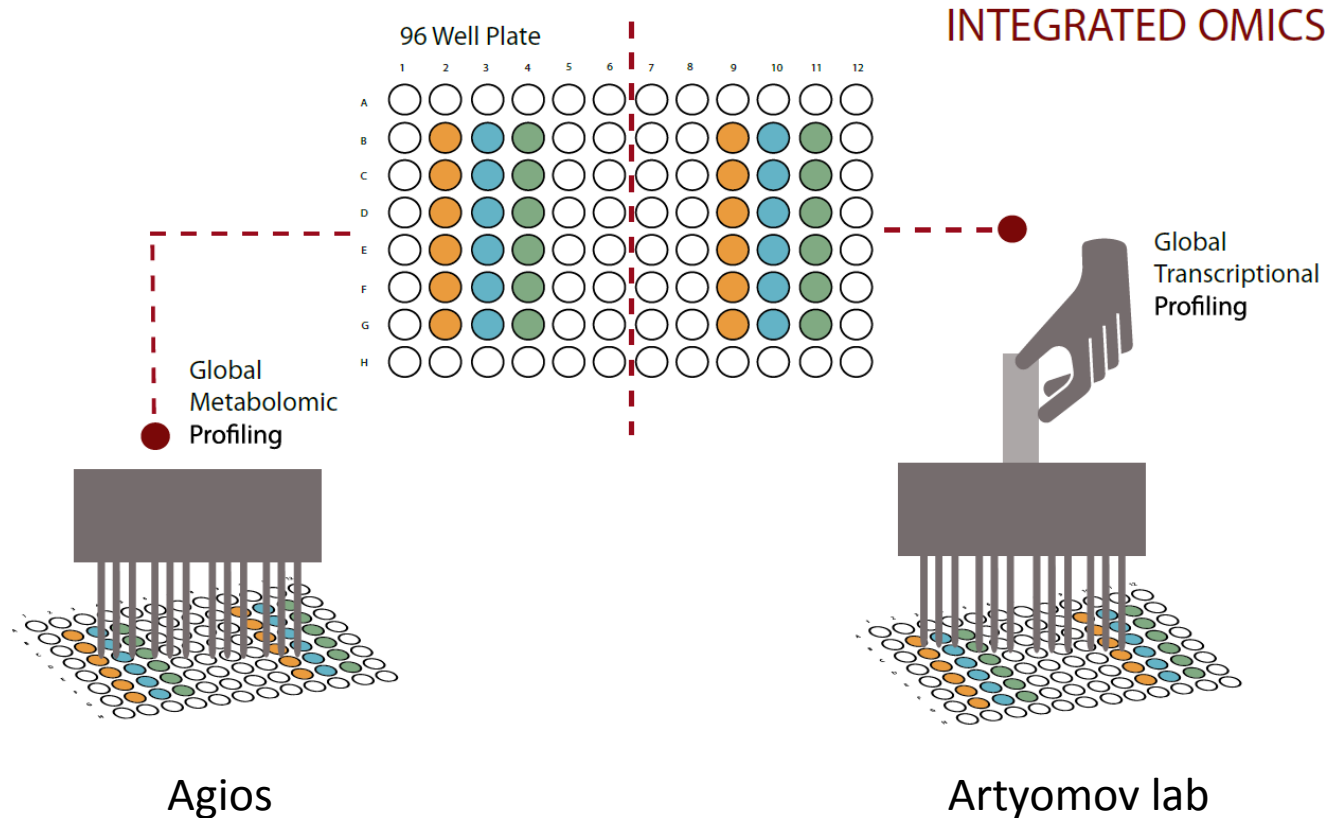
Outline

- ✓ Exploring gene expression datasets
- ✓ Simple analysis methods
- ✓ **Working with public datasets**

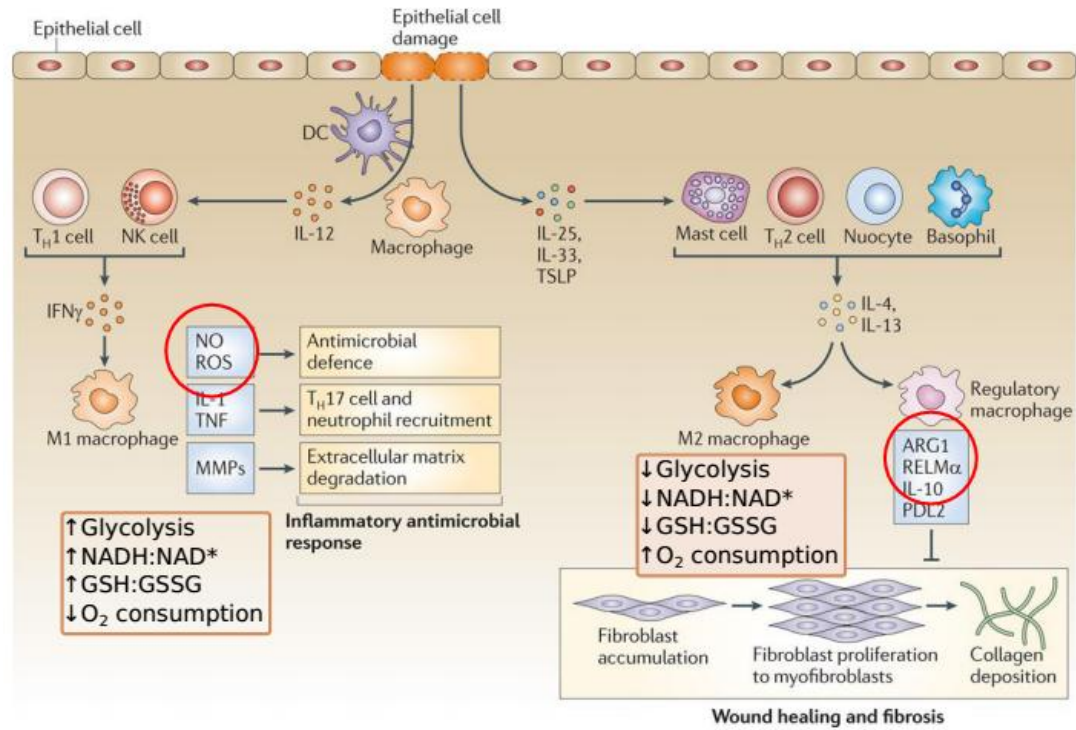
Let's open Cytoscape



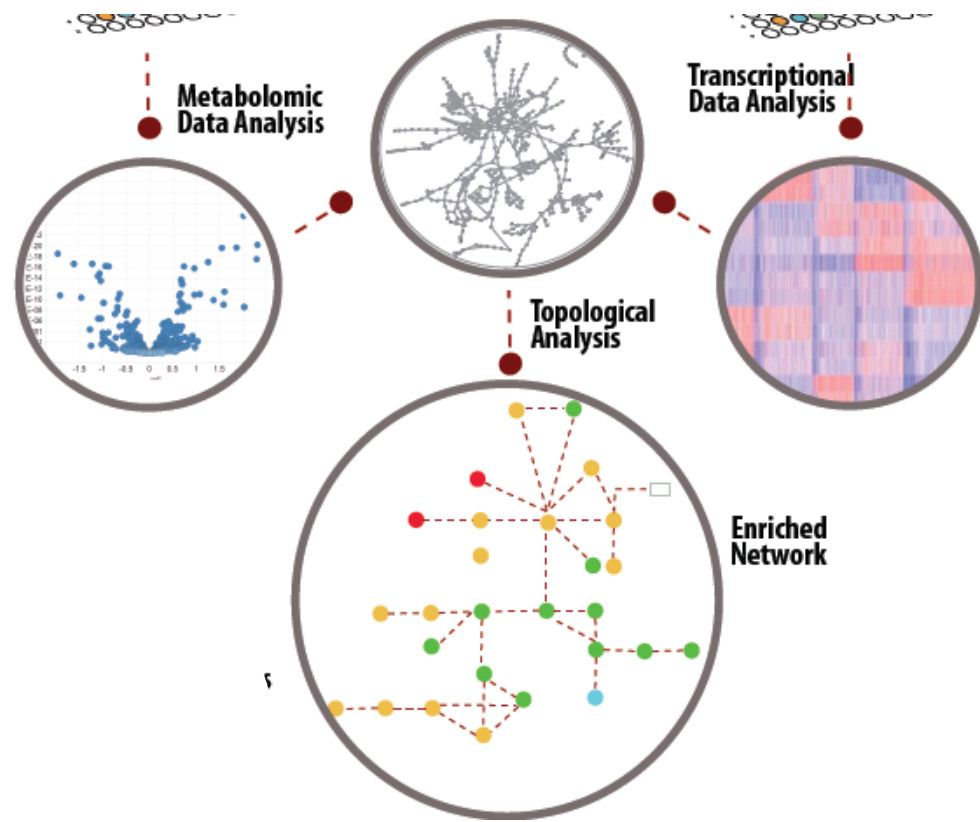
Metabolic&transcriptional profiling pipeline



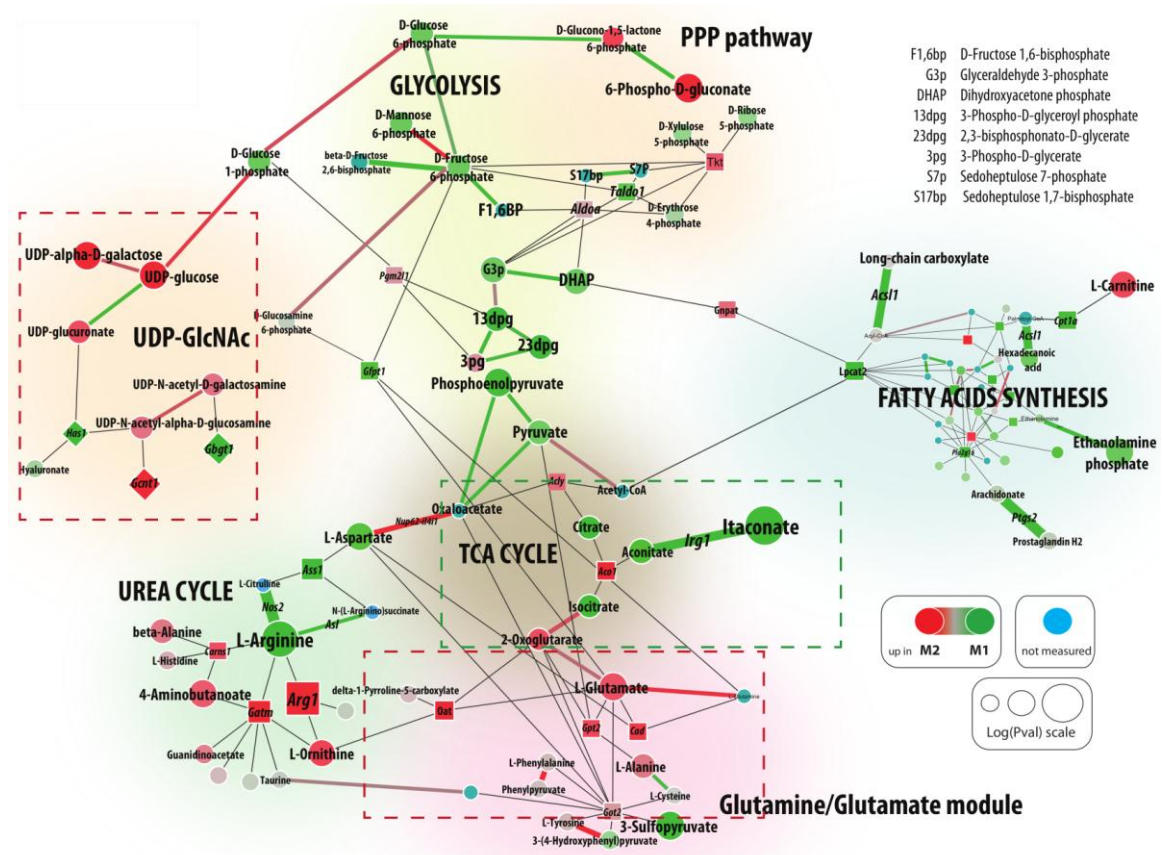
Macrophages polarization goes with high metabolic regulation



Integrating metabolic and transcriptional data




M1 vs M2 module




Nucleic Acids Research


GAM: a web-service for integrated transcriptional and metabolic network analysis


Alexey A. Sergushichev ; Alexander A. Loboda; Abhishek K. Jha; Emma E. Vincent; Edward M. Driggers; Russell G. Jones; Edward J. Pearce; Maxim N. Artyomov


Nucleic Acids Res (2016) 44 (W1): W194-W200. DOI: <https://doi.org/10.1093/nar/gkw266>

Published: 20 April 2016 **Article history** ▼

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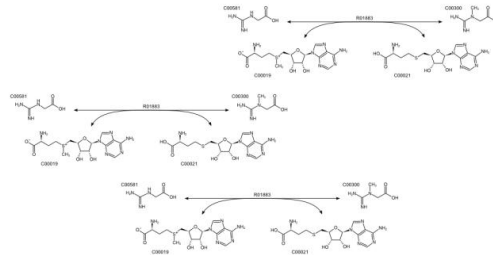
The analysis outline

KEGG database

REACTION: R01786	
Entry	R01786 Reaction
Name	ATP-alpha-D-glucose 6-phosphotransferase
Definition	ATP + alpha-D-Glucose <=> ADP + alpha-D-Glucose 6-phosphate
Reaction	C0002 + C0067 <=> C0008 + C0068
EC	2.7.1.1
Pathway	<ul style="list-style-type: none"> map00001 Glycolysis / Gluconeogenesis map00002 Galactose metabolism map00020 Starch and sucrose metabolism map00026 Amino sugar and nucleotide sugar metabolism map00100 Metabolic pathways map00109 Biosynthesis of secondary metabolites map00120 Microbial metabolism in diverse environments map00122 Carbon metabolism
Orthology	<ul style="list-style-type: none"> K00844 hexokinase [EC:2.7.1.1] K00845 glucokinase [EC:2.7.1.2] K12457 glucokinase [EC:2.7.1.2]

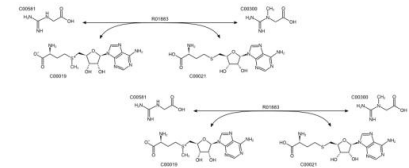
A

Species-specific reactions

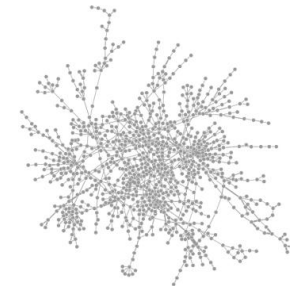


B

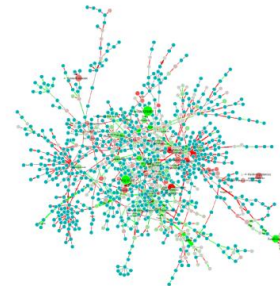
Data-specific reactions



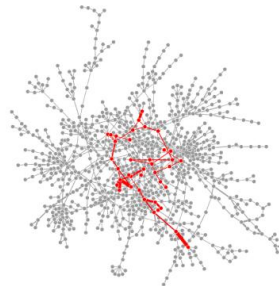
C



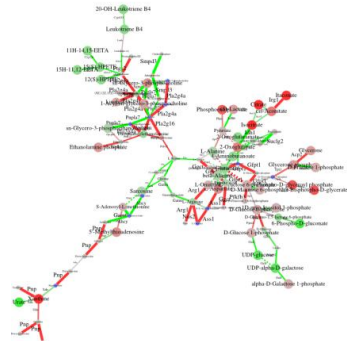
D



E



F



Final module

Most regulated subgraph

Scored reaction graph

Reaction graph

Let's go to Shiny GAM

<https://artyomovlab.wustl.edu/shiny/gam/>

Shiny GAM: integrated analysis of genes and metabolites

Work Help About

Like Shiny GAM? Check out Phantasus where you can do differential expression and submit the results to Shiny GAM

Reset all

Example DE for genes

Example DE for metabolites

Select an organism

Mouse

File with DE for genes

Browse... No file selected

File with DE for metabolites

Browse... No file selected

Interpret reactions as

nodes

Collapse reactions

Run step 1, autogenerate FDRs and run step 2

or

Step 1: Make network

Differential expression for genes

There is no genomic data

Network summary

There is no built network

Differential expression for metabolites

There is no metabolic data

Load the example data

Check both

Shiny GAM: integrated analysis of genes and metabolites

Work
Help
About

Like Shiny GAM? Check out Phantasus where you can do differential expression and submit the results to Shiny GAM

Reset all

Example DE for genes

Example DE for metabolites

Organism: Mouse

Interpret reactions as

edges

Use RPAIRs

Run step 1, autogenerate FDRs and run step 2

or

Step 1: Make network

Differential expression for genes

- name : Ctrl.vs.MandLPSandIFNg.gene.de.tsv
- length : 16829
- ID type : RefSeq

Top DE genes:

ID	pval	log2FC	baseMean
NM_008730	2.89e-42	-12.39	490
NM_172621	3.85e-30	12.64	1388
NM_013653	2.16e-29	8.58	3164
NM_001004174	1.34e-26	8.07	3670
NM_011198	1.80e-26	7.98	1857
NM_021274	2.17e-26	8.02	3065

Not mapped to Entrez: 73
 Top unmapped genes: [show](#)

Differential expression for metabolites

- name : Ctrl.vs.MandLPSandIFNg.met.de.tsv
- length : 2119
- ID type : HMDB

Top DE metabolites:

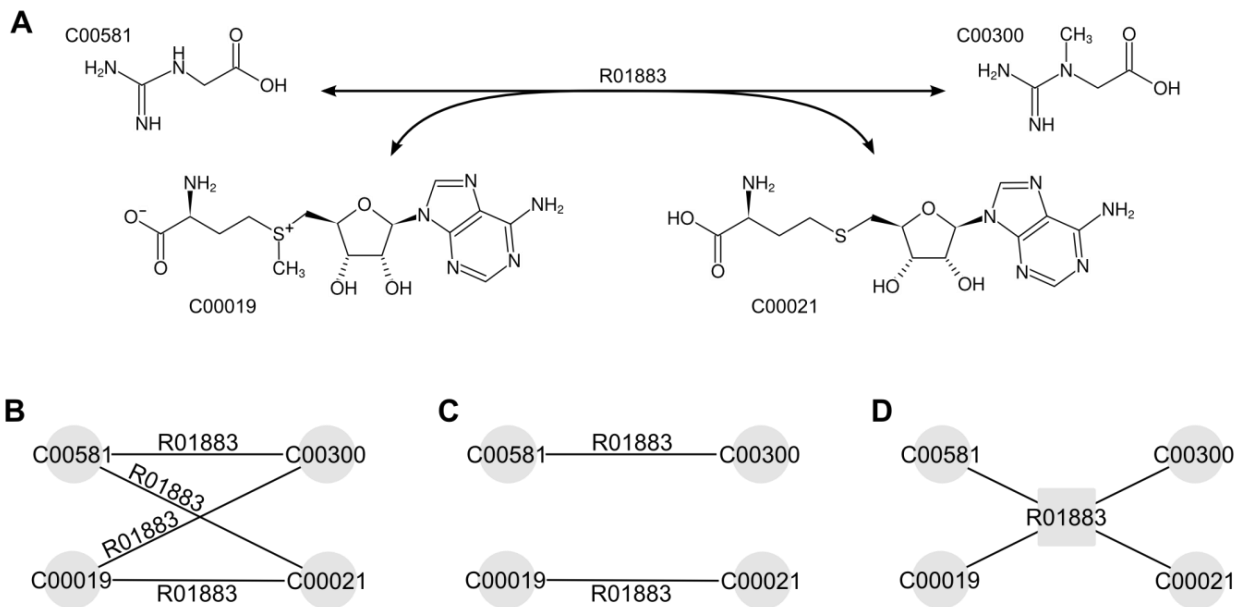
ID	pval	log2FC	baseMean
HMDB00634	8.83e-34	3.12	17.1
HMDB00620	8.83e-34	3.12	17.1
HMDB02092	8.83e-34	3.12	17.1
HMDB00749	8.83e-34	3.12	17.1
HMDB10720	5.93e-31	2.51	16.0
HMDB03407	5.93e-31	2.51	16.0

Not mapped to KEGG: 570
 Top unmapped metabolites: [show](#)

Network summary

There is no built network

There are multiple ways to represent reactions as a graph



Reactions as edges

Reaction as nodes

Create a network

Shiny GAM: integrated analysis of genes and metabolites

Work Help About

Like Shiny GAM? Check out Phantasus where you can do differential expression and submit the results to Shiny GAM

Reset all

Example DE for genes

Example DE for metabolites

Organism: Mouse

Interpret reactions as

edges

Use RPAIRs

Run step 1, autogenerate FDRs and run step 2

or

Step 1: Make network

Differential expression for genes

- name : Ctrl.vs.MandLPSandIFNg.gene.de.tsv
- length : 16829
- ID type : RefSeq

Top DE genes:

ID	pval	log2FC	baseMean
NM_008730	2.89e-42	-12.39	490
NM_172621	3.85e-30	12.64	1388
NM_013653	2.76e-29	8.58	3164
NM_001004174	1.34e-26	8.07	3670
NM_011198	1.80e-26	7.98	1857
NM_021274	2.17e-26	8.02	3065

Not mapped to Entrez: 73

Top unmapped genes: [show](#)

Differential expression for metabolites

- name : Ctrl.vs.MandLPSandIFNg.met.de.tsv
- length : 2119
- ID type : HMDB

Top DE metabolites:

ID	pval	log2FC	baseMean
HMDB00634	8.83e-34	3.12	17.1
HMDB00620	8.83e-34	3.12	17.1
HMDB02092	8.83e-34	3.12	17.1
HMDB00749	8.83e-34	3.12	17.1
HMDB10720	5.93e-31	2.51	16.0
HMDB02407	5.93e-31	2.51	16.0

Not mapped to KEGG: 570

Top unmapped metabolites: [show](#)

Network summary

- number of nodes : 1645
- number of edges : 1649

Download XGMML

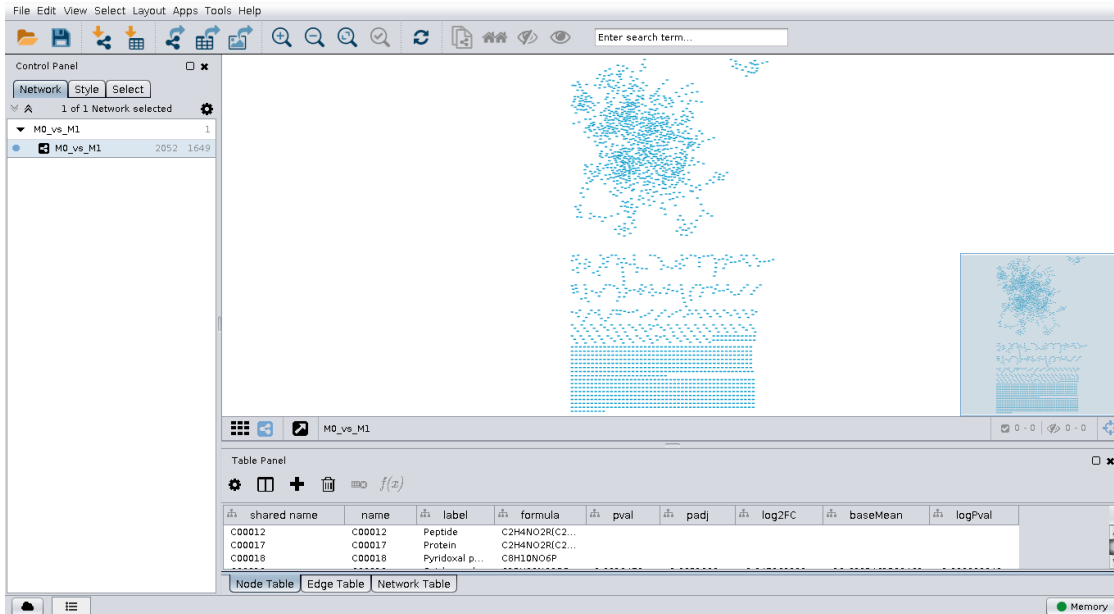
Reactions as edges

Download the network

Click "Make network"

Cytoscape: loading network

- ✓ Start New Session > From Network File > network.mmu.xgmml
 - or File > Import > Network > File
- ✓ Layout > Apply Preferred Layout (F5)



The screenshot displays the Cytoscape application window. The main area shows a network graph with numerous blue nodes and edges. The Control Panel on the left indicates that one network is selected, named 'MO_vs_M1'. Below the graph, the Table Panel is visible, showing a table with columns for node properties.

shared name	name	label	formula	pval	padj	log2FC	baseMean	logPval
C00012	C00012	Peptide	C2H4NO2R[C2...					
C00017	C00017	Protein	C2H4NO2R[C2...					
C00018	C00018	Pyridoxal p...	C8H11NO6P...					

Cytoscape: editing style

File Edit View Select Layout Apps Tools Help

Control Panel

Network **Style** Select

default

Properties

Def.	Map.	Byp.	
	<input type="checkbox"/>	<input type="checkbox"/>	Border Paint
4.0	<input type="checkbox"/>	<input type="checkbox"/>	Border Width
	<input type="checkbox"/>	<input type="checkbox"/>	Fill Color
30.0	<input type="checkbox"/>	<input type="checkbox"/>	Height
	<input type="checkbox"/>	<input type="checkbox"/>	Label
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Label Color
12	<input type="checkbox"/>	<input type="checkbox"/>	Label Font Size
	<input type="checkbox"/>	<input type="checkbox"/>	Shape
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Size
255	<input type="checkbox"/>	<input type="checkbox"/>	Transparency
70.0	<input type="checkbox"/>	<input type="checkbox"/>	Width
<input type="checkbox"/>	Lock node width and height		

M0_vs_M1

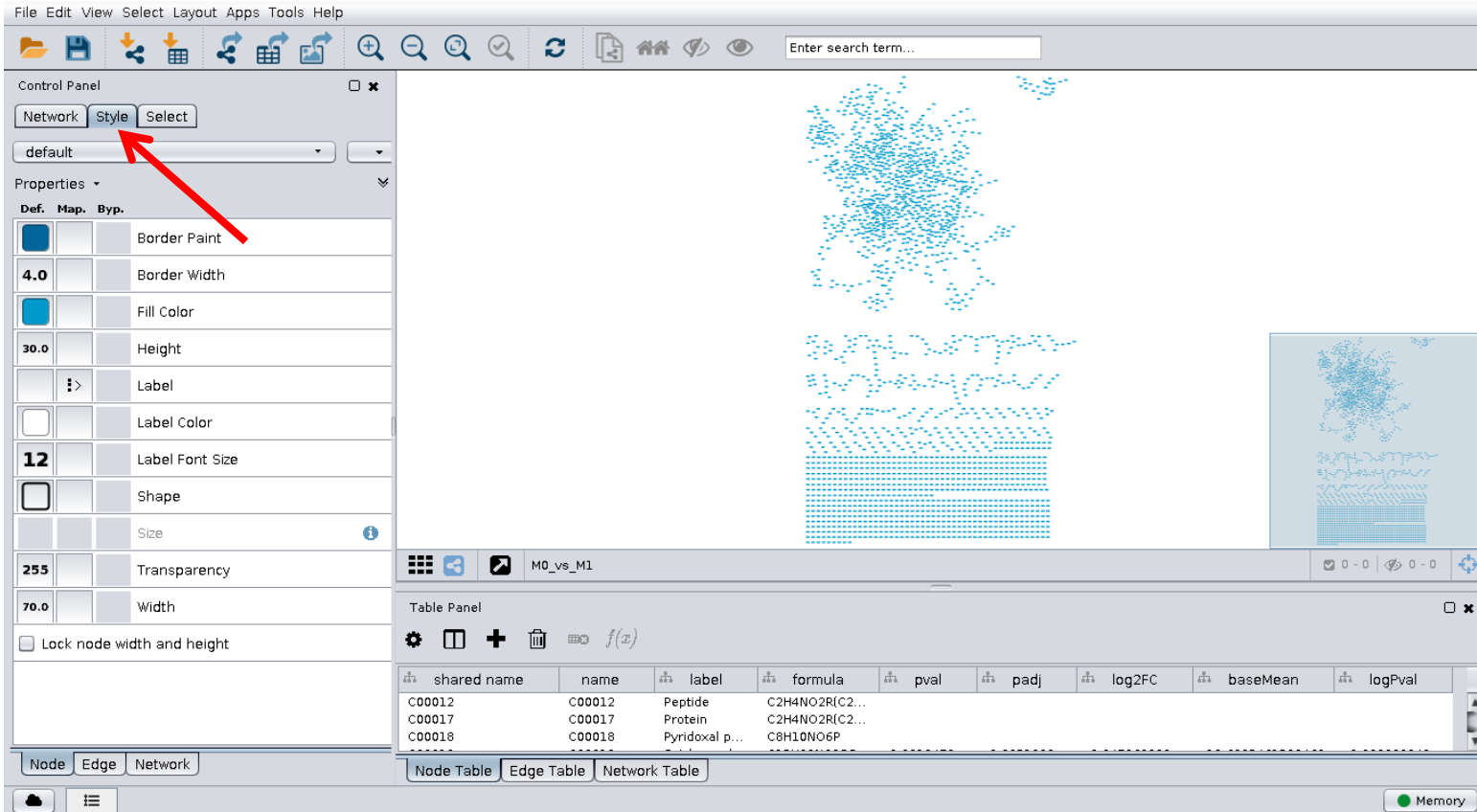
Table Panel

shared name	name	label	formula	pval	padj	log2FC	baseMean	logPval
C00012	C00012	Peptide	C2H4NO2R(C2...					
C00017	C00017	Protein	C2H4NO2R(C2...					
C00018	C00018	Pyridoxal p...	C8H10NO6P					

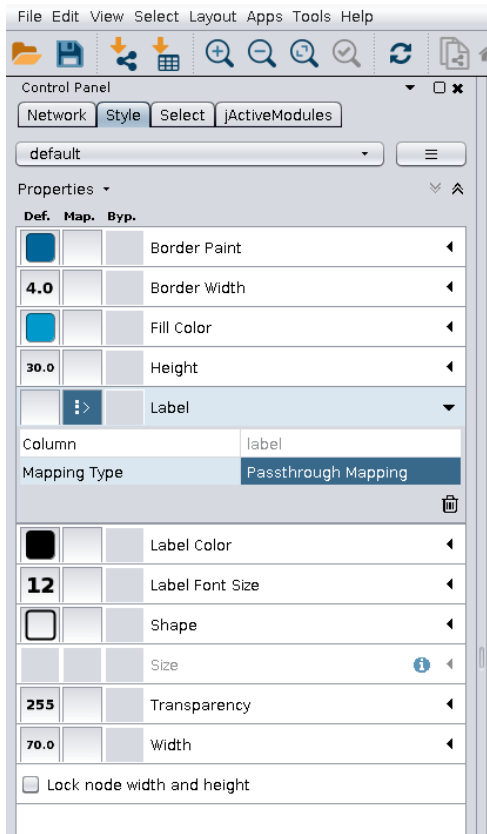
Node Edge Network

Node Table Edge Table Network Table

Memory



Cytoscape: set label style

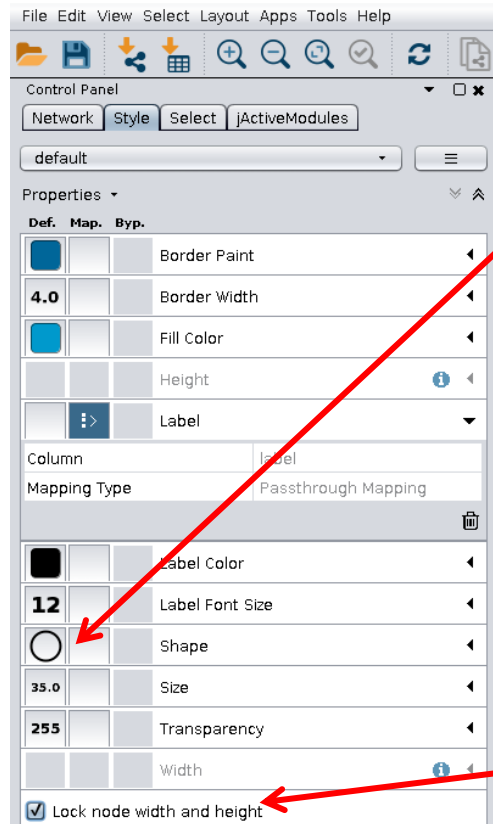


Click to expand option

Set column to "label"

Mapping Type:
"Passthrough Mapping"

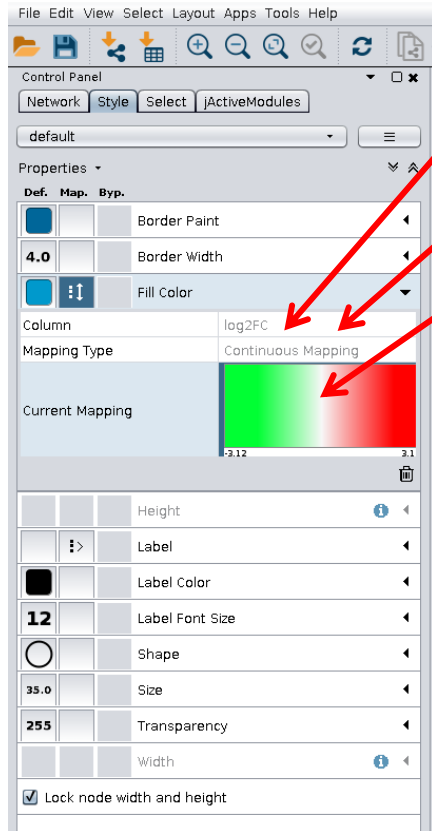
Cytoscape: set shape



Click and set to “Ellipse”

Check “Lock width and height”

Cytoscape: set fill color

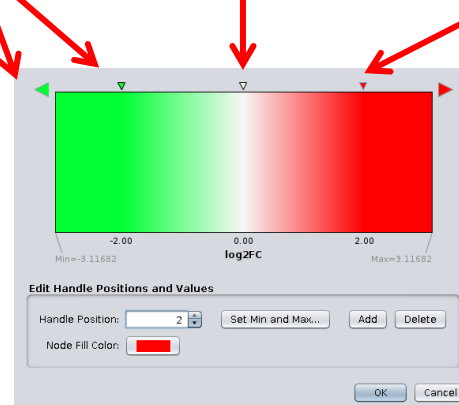


Column: log2FC

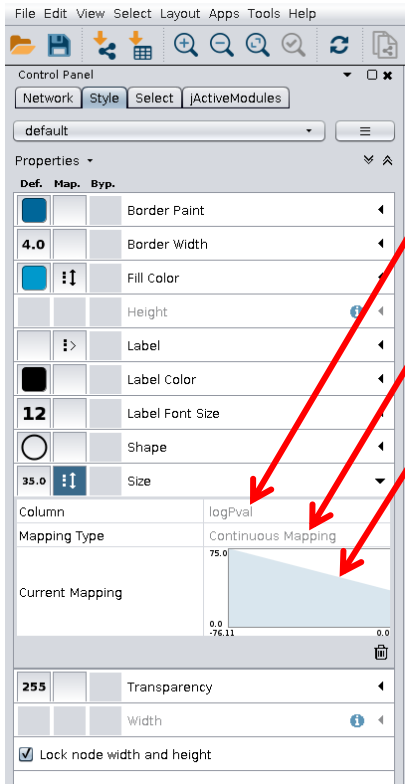
Mapping: continuous

Double click to adjust

Color: Set to zero
green Color: gray Color: red



Cytoscape: set node size

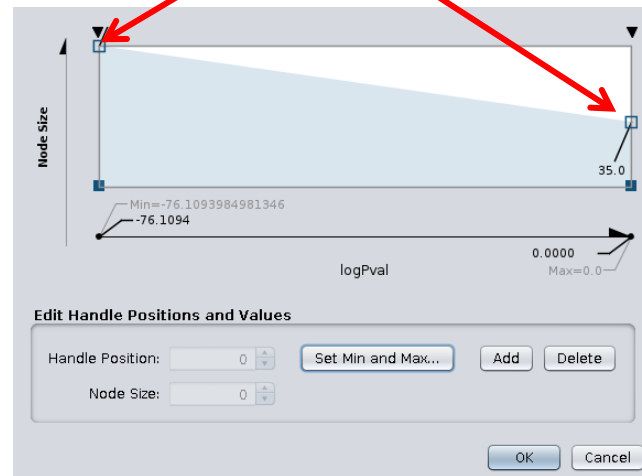


Column: logPval

Mapping: continuous

Double click to adjust

Double click,
set to 75 and 35



Now it's better

File Edit View Select Layout Apps Tools Help

Control Panel

Network Style Select

1 of 1 Network selected

M0_vs_M1 1

M0_vs_M1 2052 1649

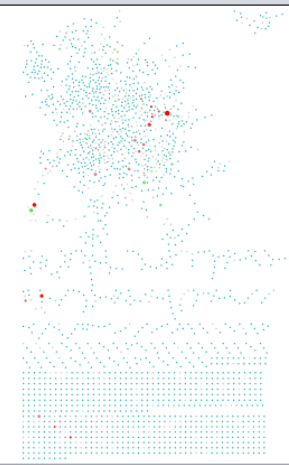


Table Panel

shared name	name	label	formula	pval	padj	log2FC	baseMean	logPval
C00012	C00012	Peptide	C2H4NO2R(C2...					
C00017	C00017	Protein	C2H4NO2R(C2...					
C00018	C00018	Pyridoxal p...	C8H10NO6P					
C00019	C00019	S-Adenosyl...	C15H22N6O5S	0.0010470...	0.0051988...	0.347060000...	13.6905461538462	-9.89938924...
CP0021	C00021	S-Adenosyl...	C14H20N6O5S					

Node Table Edge Table Network Table

Memory

Cytoscape: loading style

- ✓ Download style XML-file from server:
 - http://artyomovlab.wustl.edu/publications/supp_materials/GAM/GAM_VizMap.xml
- ✓ File > Import > Styles > GAM_VizMap.xml
- ✓ Select “GAM” instead of “default”
- ✓ Styles > Options > Make Current Styles Default

Or download from here: 

log₁₀ FDR for genes

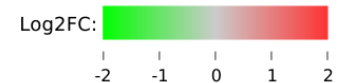
log₁₀ FDR for metabolites


Score for absent metabolites

Autogenerate FDRs

Try to solve to optimality

Step 2: Find module



 Cytoscape VizMap

Cytoscape: loading style (2)

File Edit View Select Layout Apps Tools Help

Control Panel

Network Style Select

GAM_2

Properties

Def. Map. Byp.

Color (Unselected)

Label

Label Color

Label Font Size

Line Type

Source Arrow Shape

Source Arrow Unselected Paint

Stroke Color (Unselected)

Target Arrow Shape

Target Arrow Unselected Paint


Transparency

Width

Edge color to arrows

Node Edge Network

Enter search term...



M0_vs_M1

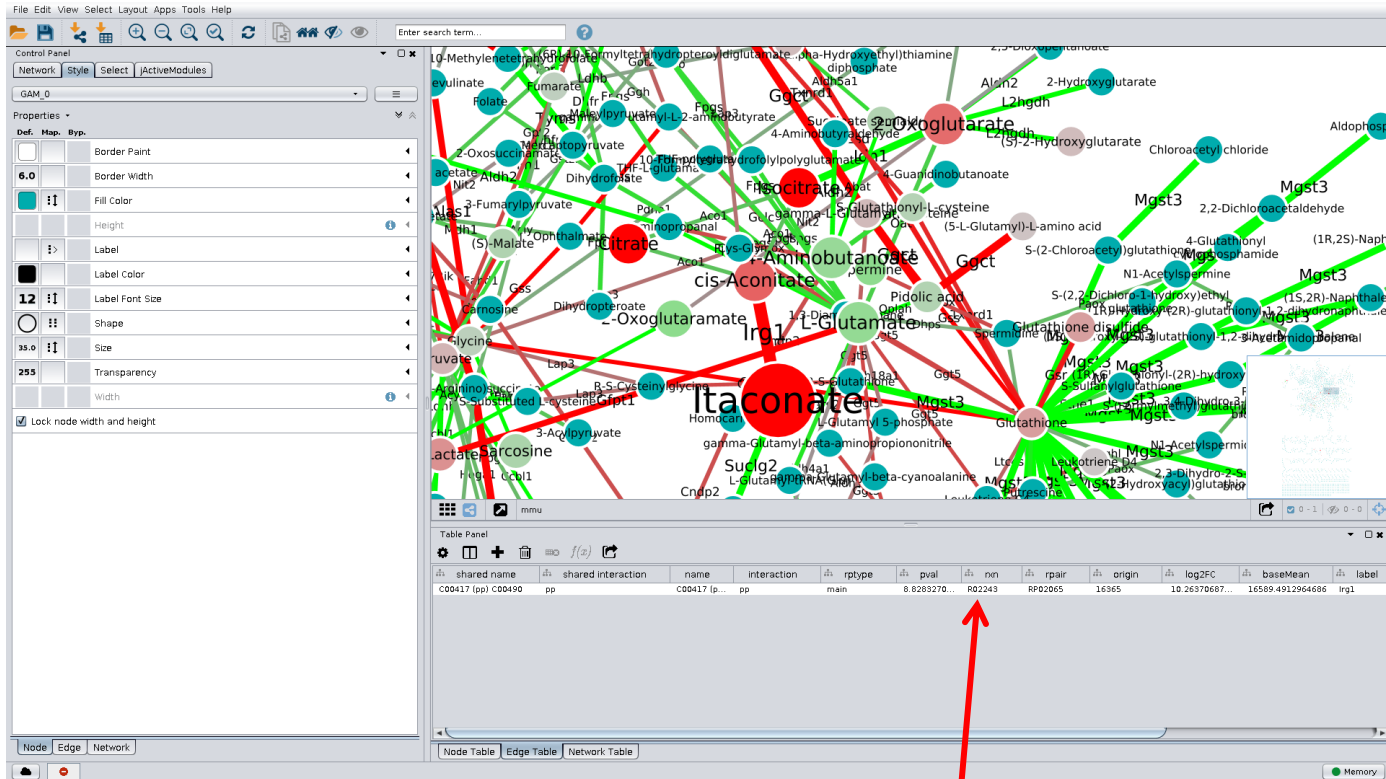
Table Panel

shared name	shared interaction	name	interaction	rxn	gene	symbol	pval		
C00219 (pp)	C00427	pp	C00219 (p...	pp	R01599	19225	Ptgs2	1.7983526...	4.4
C00062 (pp)	C00327	pp	C00062 (p...	pp	R00557	18126	Nos2	3.0458890...	4.7
C00417 (pp)	C00490	pp	C00417 (p...	pp	R02243	16365	Irg1	8.8283270...	2.8
C00154 (pp)	C00249	pp	C00154 (p...	pp	R01280	14081	Acs11	1.3374333...	4.0

Node Table Edge Table Network Table

Memory

Zoom in to Itaconate and Irg1



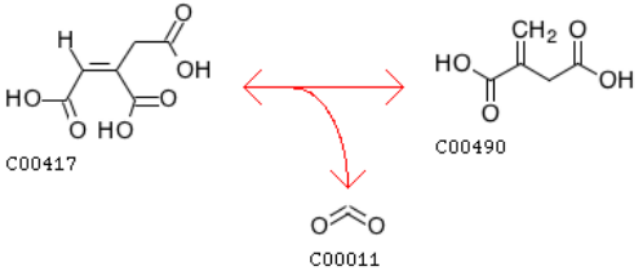
Reaction ID: R02243

Look at Irg1 reaction (R02243) at KEGG



REACTION: R02243

Help

Entry	R02243	Reaction
Name	cis-Aconitate carboxy-lyase	
Definition	cis-Aconitate <=> Itaconate + CO2	
Equation	C00417 <=> C00490 + C00011	
		
Reaction class	RC00667 C00417_C00490	
Enzyme	4.1.1.6	
Pathway	rn00660 C5-Branched dibasic acid metabolism	
Orthology	K17724 aconitate decarboxylase [EC:4.1.1.6]	
Other DBs	RHEA: 15256	

All links

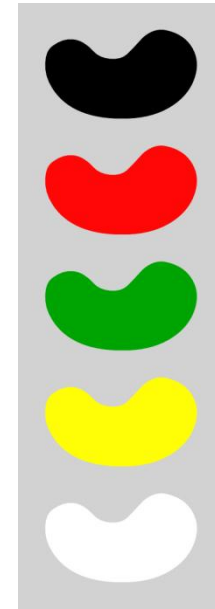
[Ontology \(1\)](#)
[KEGG BRITE \(1\)](#)
[Pathway \(2\)](#)
[KEGG PATHWAY \(2\)](#)
[Chemical substance \(3\)](#)
[KEGG COMPOUND \(3\)](#)
[Chemical reaction \(2\)](#)
[KEGG ENZYME \(1\)](#)
[KEGG RCLASS \(1\)](#)
[Gene \(193\)](#)
[KEGG ORTHOLOGY \(1\)](#)
[KEGG GENES \(192\)](#)
[All databases \(201\)](#)

[Download RDF](#)

DBGET integrated database retrieval system

The network is too big!
Let's try to extract its most
important part.

Jelly beans mining problem



Delicious!

...

OK

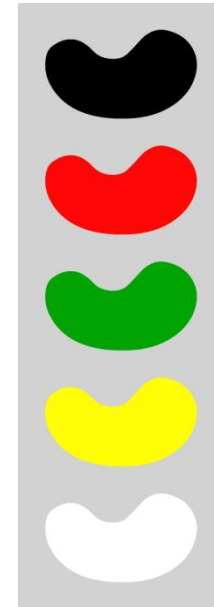
...

Thanks, no

<http://fineartamerica.com/featured/jelly-beans-background-ken-brown.html>

https://sysbio.curie.fr/bionetvisa/BioNetVisA2014_Presentations/BioNetVisA2014_Oral_Presentations/Talk08_Mohammed_ElKarib_BioNetVisa_2014.pdf

Jelly beans mining problem



Delicious!

...

OK

...

Thanks, no

<http://fineartamerica.com/featured/jelly-beans-background-ken-brown.html>

https://sysbio.curie.fr/bionetvisa/BioNetVisA2014_Presentations/BioNetVisA2014_Oral_Presentations/Talk08_Mohammed_ElKarib_BioNetVisa_2014.pdf

Generalized maximum-weight connected subgraph problem (GMWCS)

Definition 2. *Given a connected undirected graph $G = (V, E)$ and a weight function $\omega : (V \cup E) \rightarrow \mathbb{R}$, the Generalized Maximum-Weight Connected Subgraph (GMWCS) problem is the problem of finding a connected subgraph $\tilde{G} = (\tilde{V}, \tilde{E})$ with the maximal total weight*

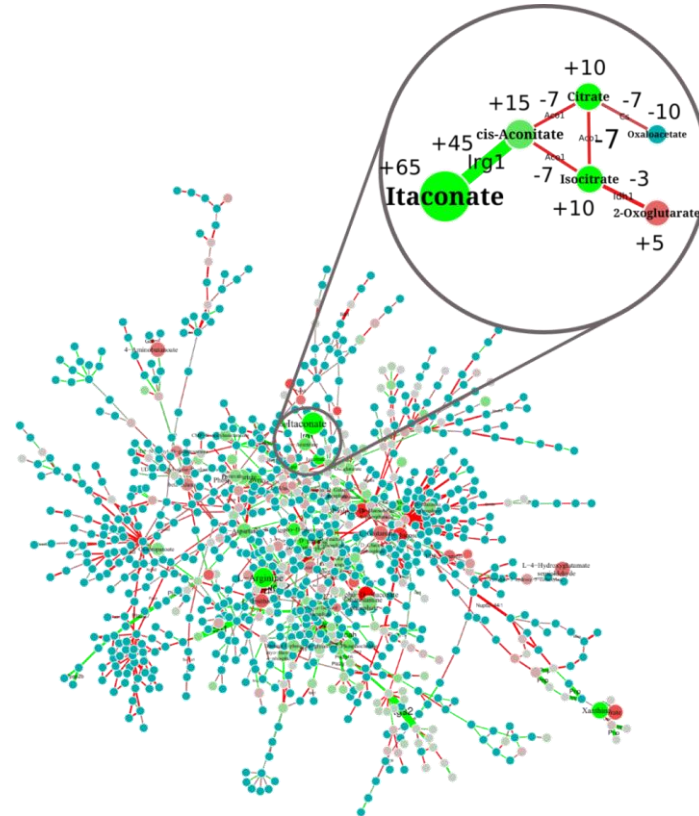
$$\Omega(\tilde{G}) = \sum_{v \in \tilde{V}} \omega(v) + \sum_{e \in \tilde{E}} \omega(e) \rightarrow \max$$



Alexander
Loboda

Network scoring

- ✓ Small p-value -> positive score
- ✓ Close to one p-value -> negative score
- ✓ FDR thresholds control which genes and metabolites have positive score



Assign network scores

Click “Autogenerate FDRs”

The screenshot shows a control panel on the left side of a large, empty network visualization area. The control panel includes the following elements:

- log₁₀ FDR for genes:** A text input field containing the value -2.5.
- log₁₀ FDR for metabolites:** A text input field containing the value -0.9.
- Score for absent metabolites:** A text input field containing the value -11.7.
- Autogenerate FDRs:** A button with a red arrow pointing to it from the text above.
- Try to solve to optimality
- Solver: gmwcs (time limit = 30s)
- Step 2: Find module
- Add trans-edges
- Log2FC:** A horizontal color scale legend ranging from -2 (green) to 2 (red), with 0 being white.
-

The main visualization area is a large, light gray rectangle, currently empty.

Loading module into Cytoscape

- ✓ File > Import > Network > File >
Ctrl.vs.MandLPSandIFNg.gene.de.re.mf=-0.9.rf=-2.5.ams=-11.7.xgmml
- ✓ Layout > Apply preferred layout

M0 vs M1 module

File Edit View Select Layout Apps Tools Help

Enter search term...

Control Panel

Network Style Select ActiveModules

GAM_0

Properties

Def. Map. Byp.

Border Paint

6.0 Border Width

Fill Color

Height

Label

Label Color

12 Label Font Size

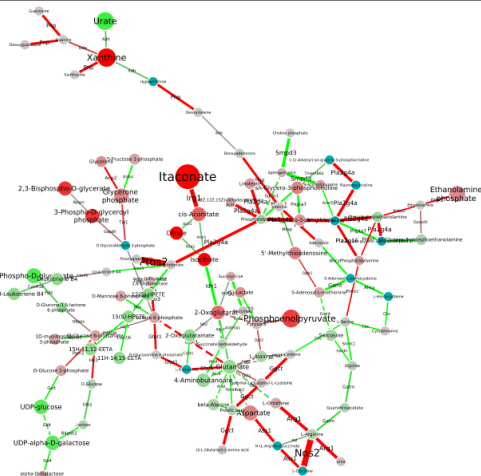
Shape

35.0 Size

255 Transparency

Width

Lock node width and height



Ctrl vs. MandIPSandIFNg. gene. de. re. mf=-0.9. rf=2.5. ams=-11.7

Table Panel

#	shared name	shared interaction	name	interaction	rptype	pval	rxn	rpair	origin	log2FC	baseMean	label
C00019	(pp) C00021	pp	C00019 (p...	pp	main	9.1859397...	R01889	RP00004	14431	-13.39406746...	10.71370663779874	Gamt
C00022	(pp) C00026	pp	C00022 (p...	pp	trans	0.3961281...	R00258	RP05853	108682	1.047952119...	41.878781913922	Gpt2
C00025	(pp) C00026	pp	C00025 (p...	pp	main	0.9529735...	R00667	RP00014	18242	-1.047660662...	352.583713014929	Oat
C00022	(pp) C00041	pp	C00022 (p...	pp	main	0.3961281...	R00258	RP00021	108682	1.047952119...	41.878781913922	Gpt2
C00025	(pp) C00041	pp	C00025 (p...	pp	trans	0.3961281...	R00258	RP00469	108682	1.047952119...	41.878781913922	Gpt2
C00025	(pp) C00049	pp	C00025 (p...	pp	trans	0.4415985...	R00355	RP05883	14719	-0.377343228...	91.3313039144272	Got2
C00029	(pp) C00052	pp	C00029 (p...	pp	trans	0.0869443...	R00955	RP06377	14430	-1.705074309...	32.652023780881	Galt
C00025	(pp) C00064	pp	C00025 (p...	pp	main	5.4249996...	R00768	RP00024	14583	2.465059546...	478.652944814861	Gp11
C00037	(pp) C00065	pp	C00037 (p...	pp	main	0.0786934...	R00909	RP00301	20425	-13.39406746...	2.53965898451015	Shm1
C00022	(pp) C00074	pp	C00022 (p...	pp	main	0.0968186...	R02320	RP00036	18746	0.832820338...	1243.64436660378	Pkm
C00025	(pp) C00077	pp	C00025 (p...	pp	trans	0.0529735...	R00668	RP06031	18242	-1.047660662...	352.583713014923	Oat
C00062	(pp) C00077	pp	C00062 (p...	pp	main	2.8646743...	R00551	RP00066	11846	3.678046434...	125.66010522325	Arg1

Node Edge Network

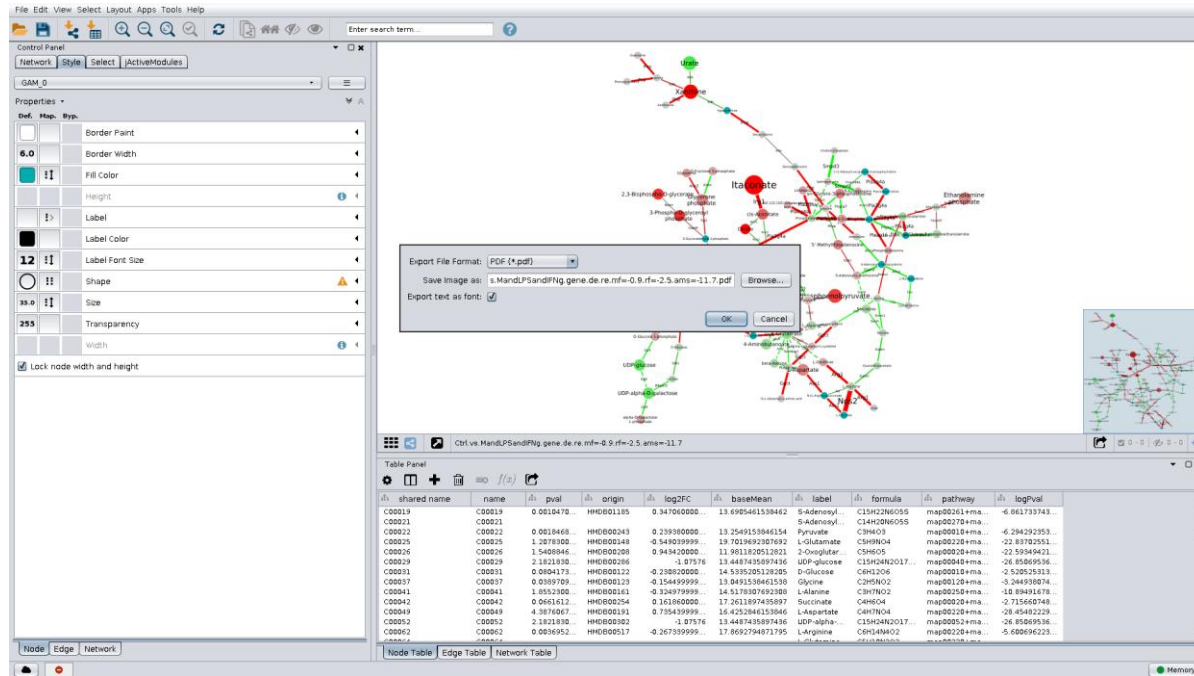
Node Table Edge Table Network Table

Fit Content

Memory

Save the figure

File > Export as image...



The screenshot shows a software interface with a network diagram and a table panel. A dialog box is open for exporting the figure as a PDF.

Export File Format: PDF (*.pdf)

Save Image as: s.MandPSandPng gene.de.re.rf=0.9.rf=2.5.ams=11.7.pdf

Export text as font:

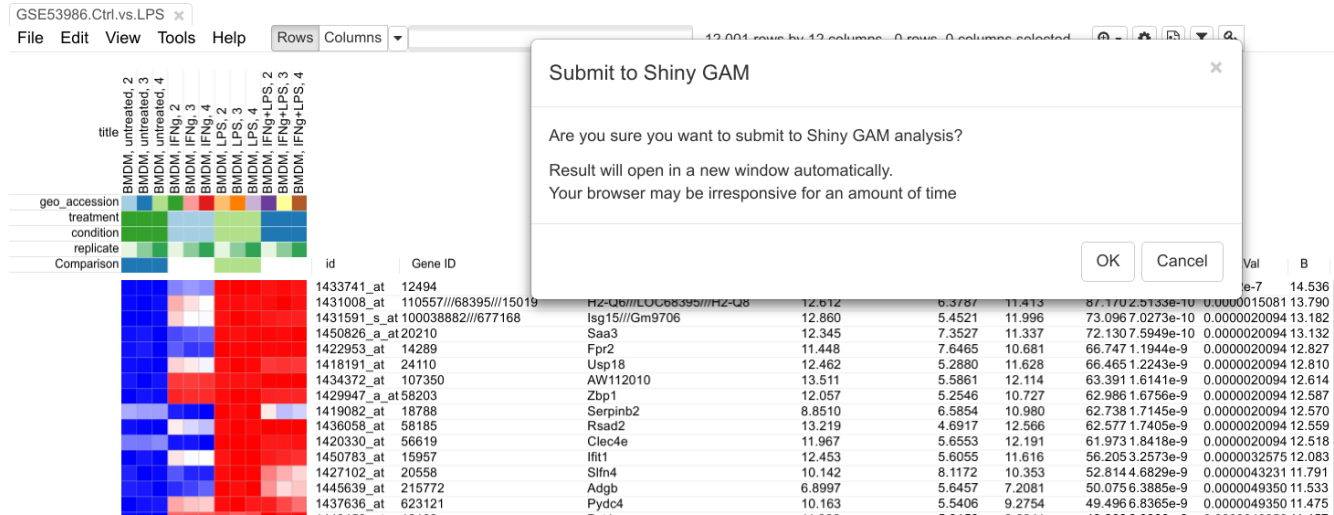
Table Panel:

shared name	name	pval	origin	log2FC	baseMean	label	formula	pathway	logPval
CB0019	CB0019	0.0010470	HM0001185	0.34700000	13.6905461538462	S-adenosyl...	C15H22N6O55	map00261+ma...	-6.861733743
CB0021	CB0021					S-adenosyl...	C14H20N6O55	map00278+ma...	
CB0022	CB0022	0.0018468	HM000243	0.23938000	13.2549153846154	Pyruvate	C3H3O3	map00011+ma...	-6.294292353
CB0025	CB0025	1.2978300	HM000148	-0.549039999	19.7019892307692	L-Glutamate	C5H9NO4	map00220+ma...	-22.83702551
CB0026	CB0026	1.5408046	HM000209	0.94340000	11.901820512031	2-Oxoglutar...	C6H9NO5	map00020+ma...	-22.50969421
CB0029	CB0029	2.1821930	HM000206	-1.075716	13.4447435897436	UDP-glucose	C15H24N2O17	map00040+ma...	-26.85069536
CB0031	CB0031	0.0081753	HM000122	-0.21003000	14.5395205120305	D-Glucose	C6H12O6	map00011+ma...	-2.52053513
CB0037	CB0037	0.0309780	HM000123	0.15649999	13.8461538461538	Glycine	C2H3NO2	map00120+ma...	-3.294938074
CB0041	CB0041	1.8552300	HM000161	-0.324979999	14.5170307692309	L-Alanine	C3H7NO2	map00250+ma...	-10.89431678
CB0042	CB0042	0.0661611	HM000254	0.10300000	17.261897459597	Succinate	C4H5O4	map00020+ma...	-2.71560748
CB0049	CB0049	4.3876067	HM000191	0.735439999	16.4252046153846	L-Aspartate	C4H7NO4	map00022+ma...	-29.45482229
CB0052	CB0052	2.1821890	HM000302	1.075716	13.4447435897436	UDP-alpha...	C15H24N2O17	map00052+ma...	-26.85069536
CB0062	CB0062	0.0036952	HM000517	-0.267939999	17.8652794871795	L-Aspartate	C4H7NO4	map00120+ma...	-6.600694223

PDFs can be opened in Inkscape or Illustrator

Running from Phantasia

- ✔ Open GSE53986.Ctrl.vs.LPS.gct in Phantasia
- ✔ Tools/Submit to Shiny GAM



The screenshot shows the Phantasia interface with a heatmap of gene expression data. The heatmap has columns representing different conditions (e.g., BMDM, IFNg, LPS) and rows representing individual genes. A dialog box titled 'Submit to Shiny GAM' is overlaid on the heatmap, asking for confirmation to submit the data for analysis. The dialog box contains the following text:

Submit to Shiny GAM

Are you sure you want to submit to Shiny GAM analysis?

Result will open in a new window automatically.
Your browser may be irresponsive for an amount of time

OK Cancel

The background heatmap shows a grid of colored cells (blue, red, white) representing gene expression levels across various conditions. The x-axis labels include 'id' and 'Gene ID', and the y-axis labels include 'title', 'geo_accession', 'treatment', 'condition', 'replicate', and 'Comparison'.

Remember that only expressed genes are present

The module

log₁₀ FDR for genes

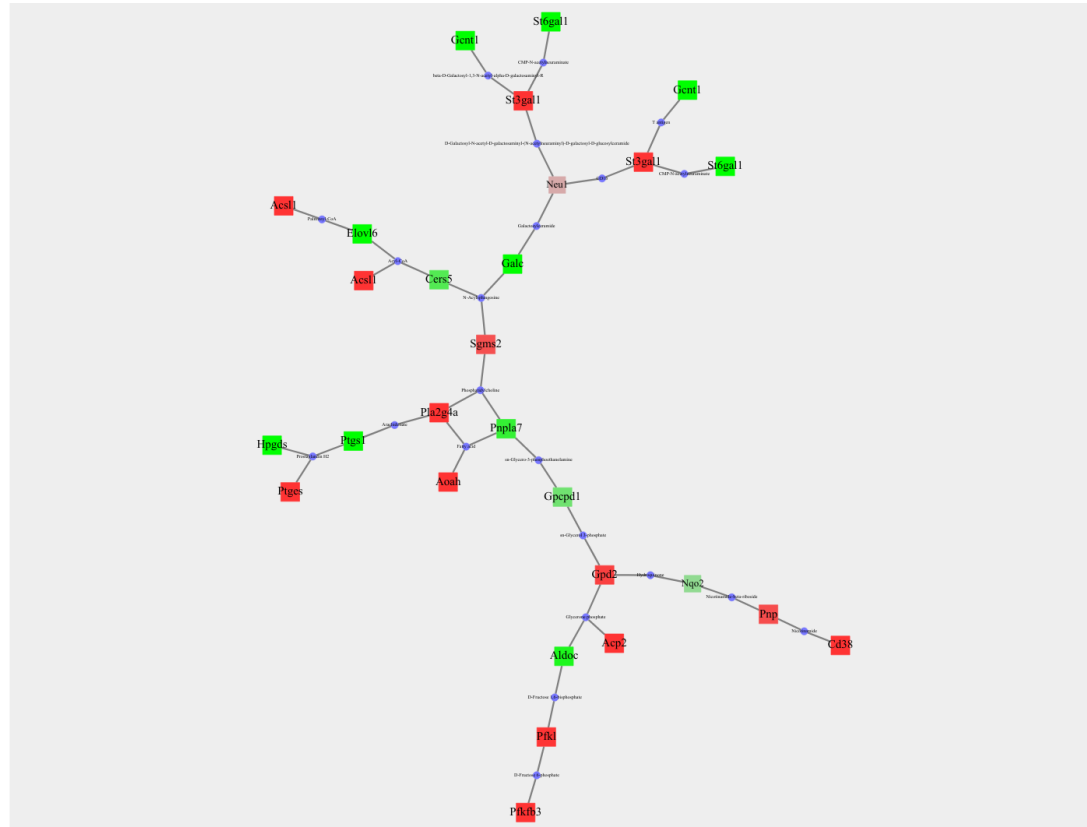
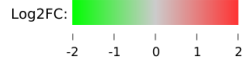
Try to solve to optimality

Solver: Heinz2 (time limit = 30s)

Add common metabolites

Module summary

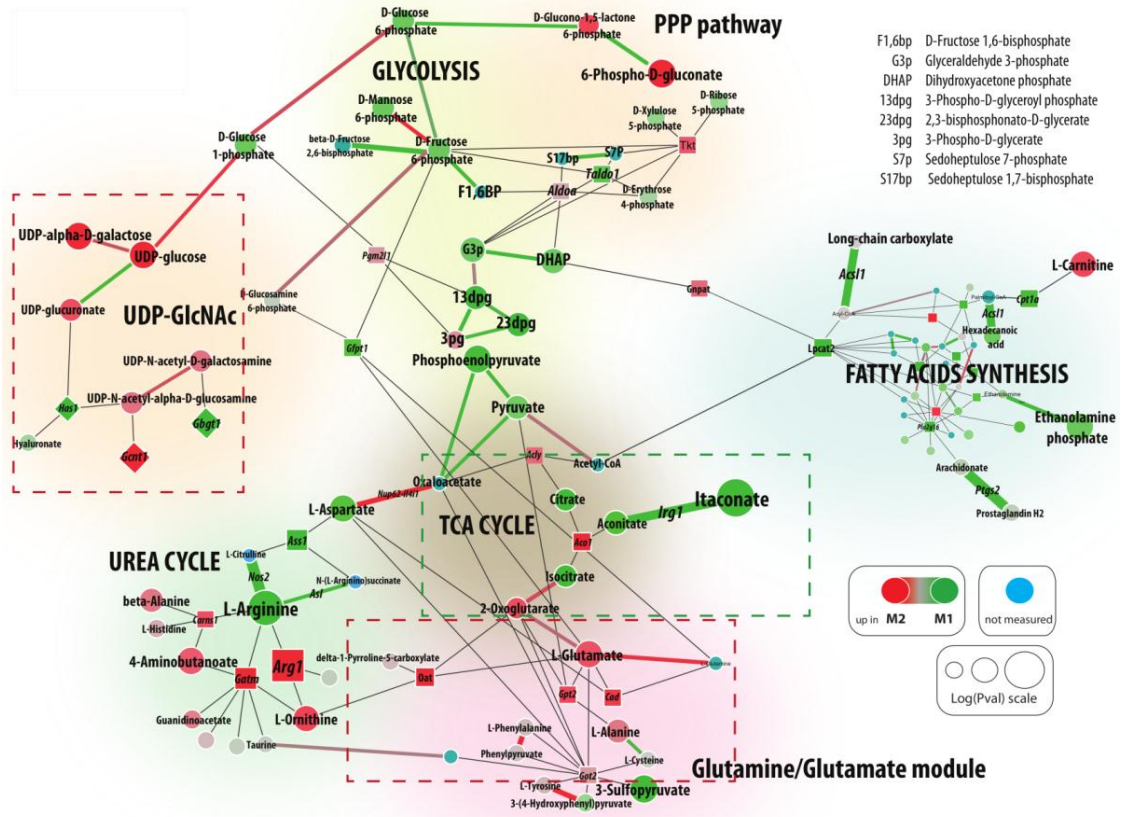
- number of nodes : 50
- number of edges : 50



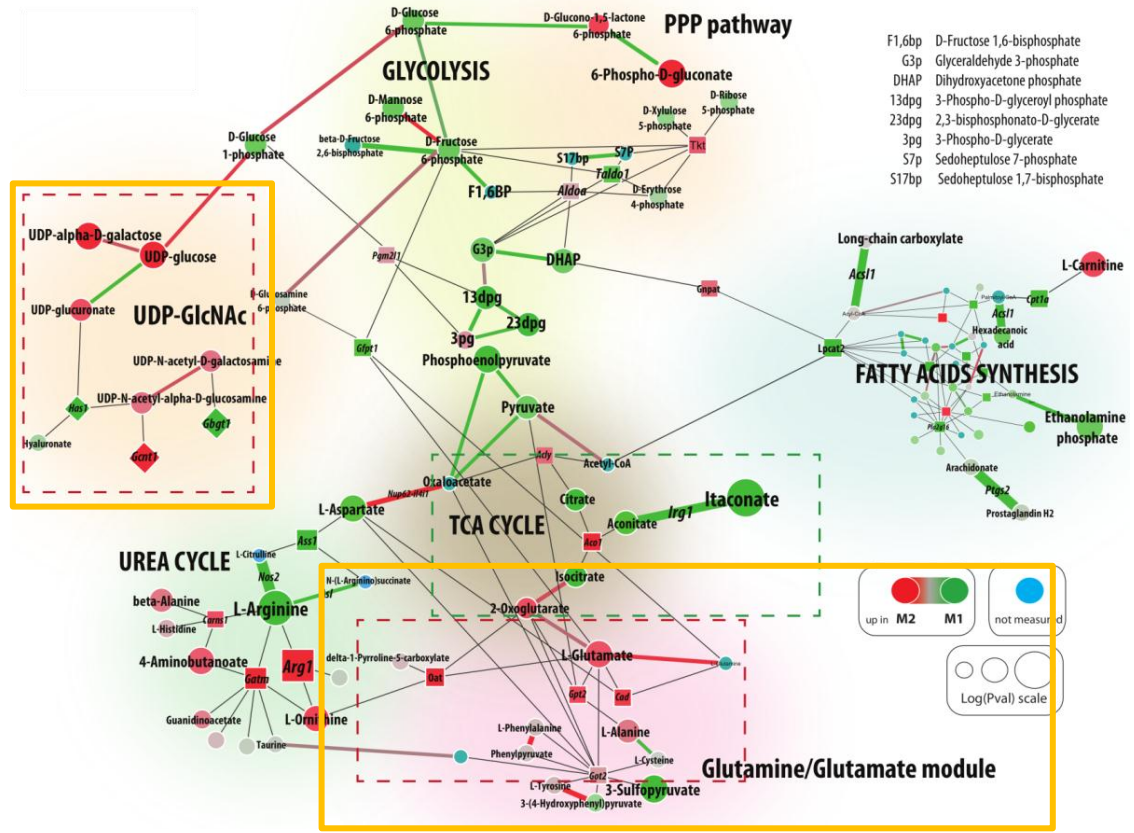
Exercise: upload data manually

- ✓ Open GSE53986.Ctrl.vs.LPS.gct in Excel
- ✓ Save differential expression table as a tab-separated file
- ✓ Upload to GAM

We got the M1 vs M2 module, what's next?



UDP-GlcNAc and Glutamine modules



Test glutamine involvement by using media without one



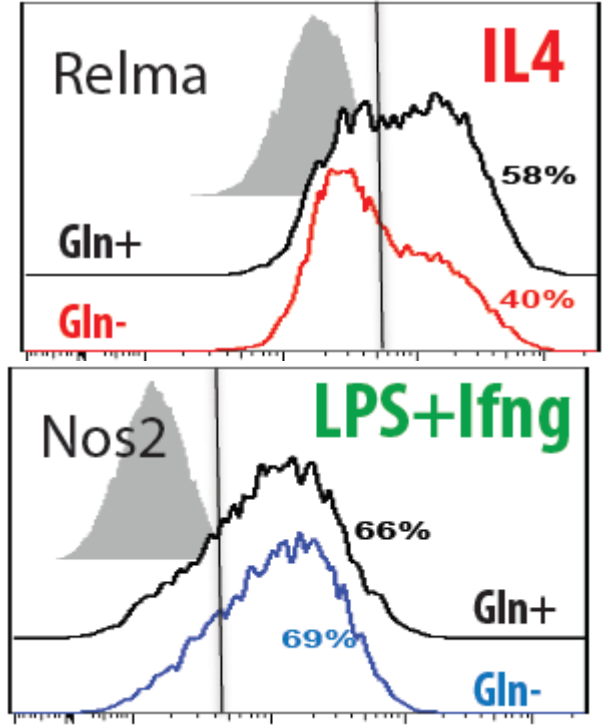
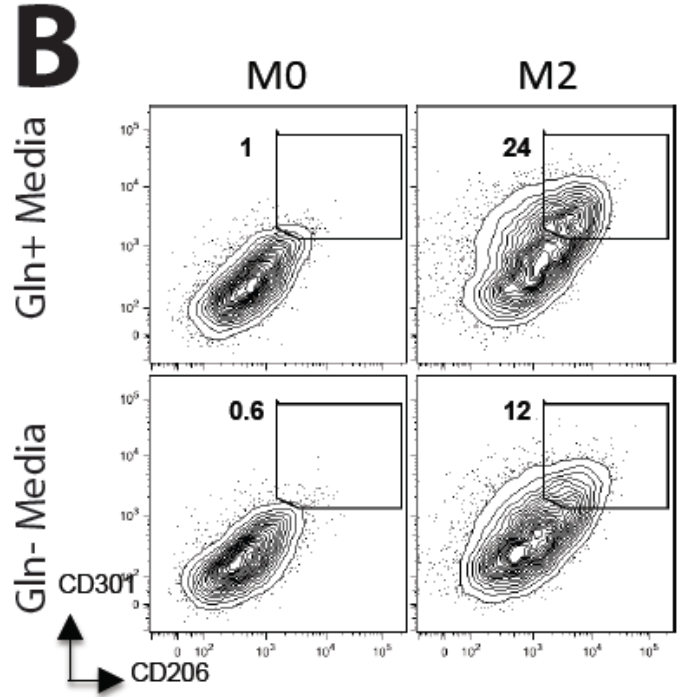
- complete RPMI 1640
- 10 mM glucose
- 2 mM L-glutamine,
- 100 U/mL of penicillin/streptomycin
- 10% FCS

Test glutamine involvement by using media without one

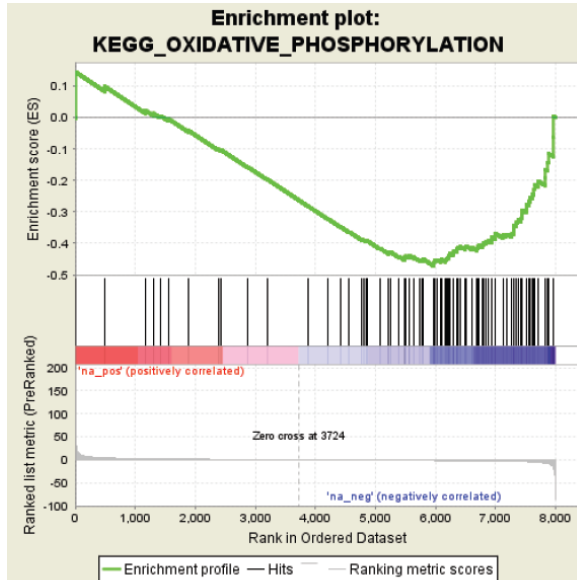


- complete RPMI 1640
- 10 mM glucose
- 2 mM L-glutamine,
- 100 U/mL of penicillin/streptomycin
- 10% FCS

Glutamine withdrawal leads to defect in M2 polarization

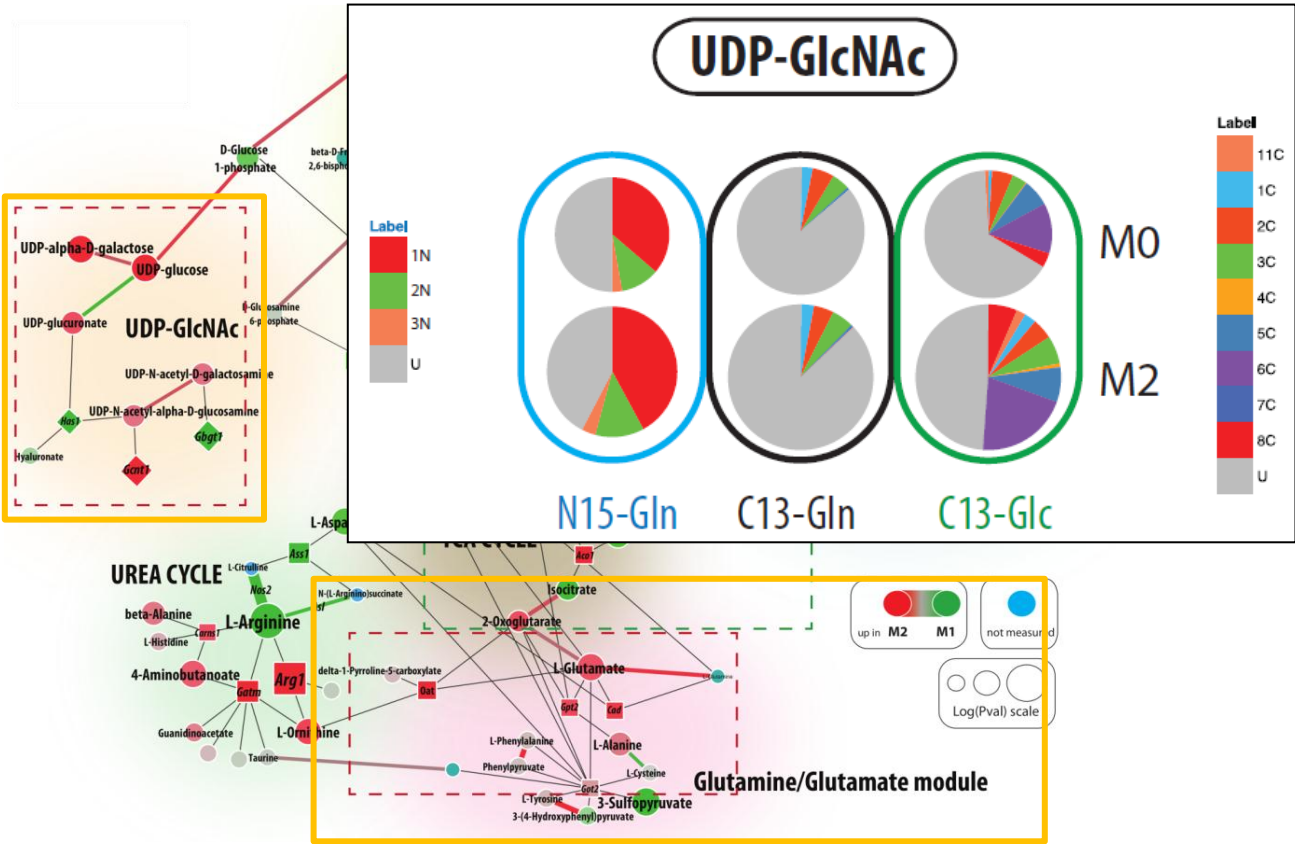


Glutamine withdrawal affect TCA cycle in M2

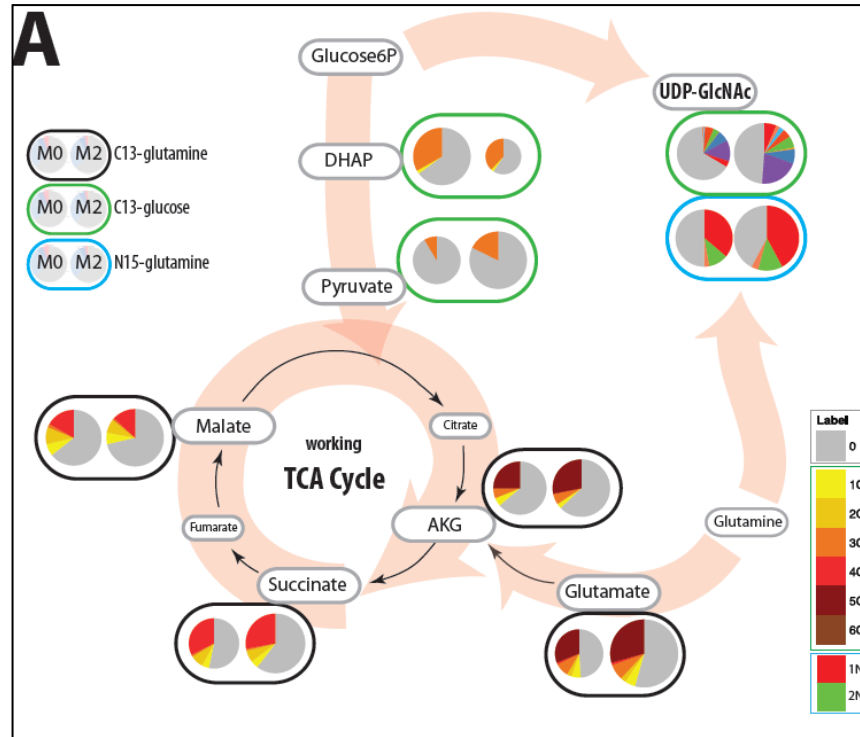


**Oxidative phosphorylation
is impaired upon
glutamine withdrawal in M2 cells**

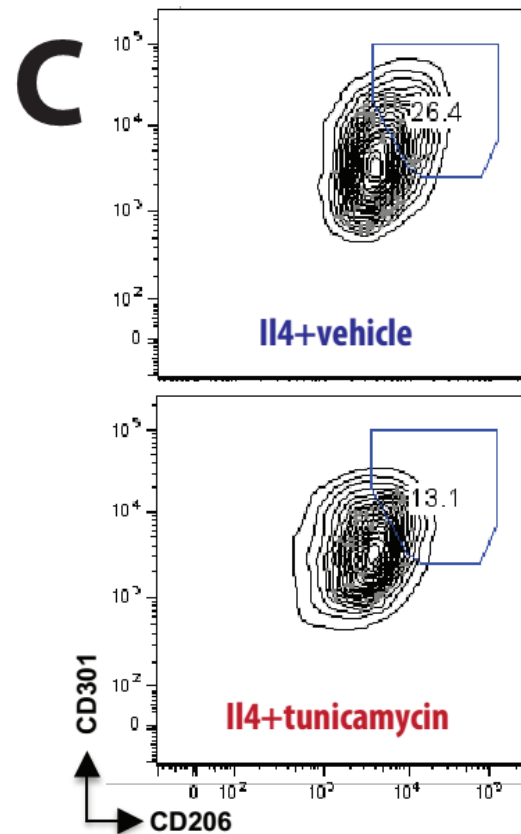
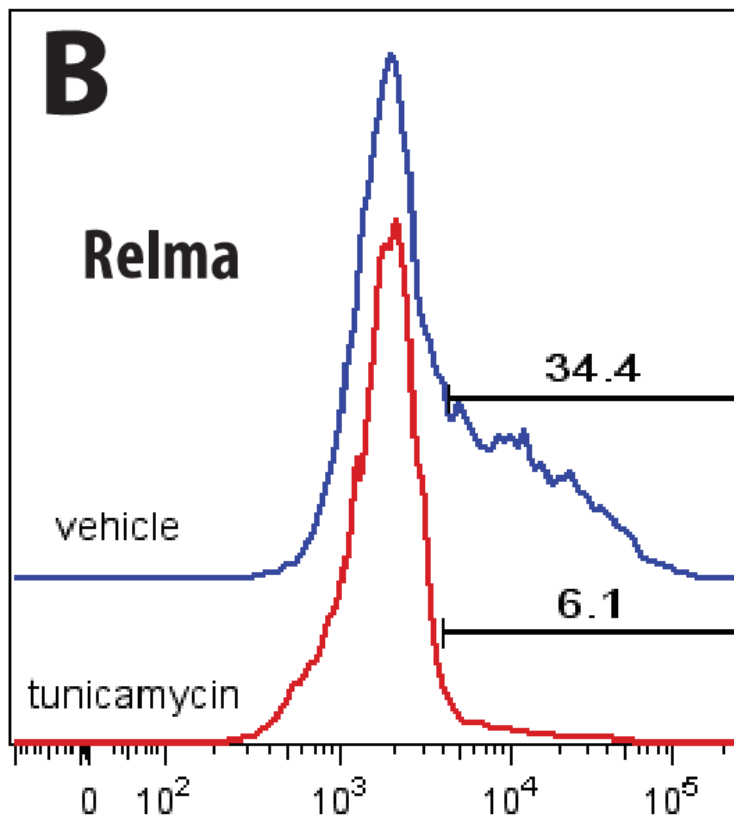
Glutamine feeds into UDP-GlcNAc

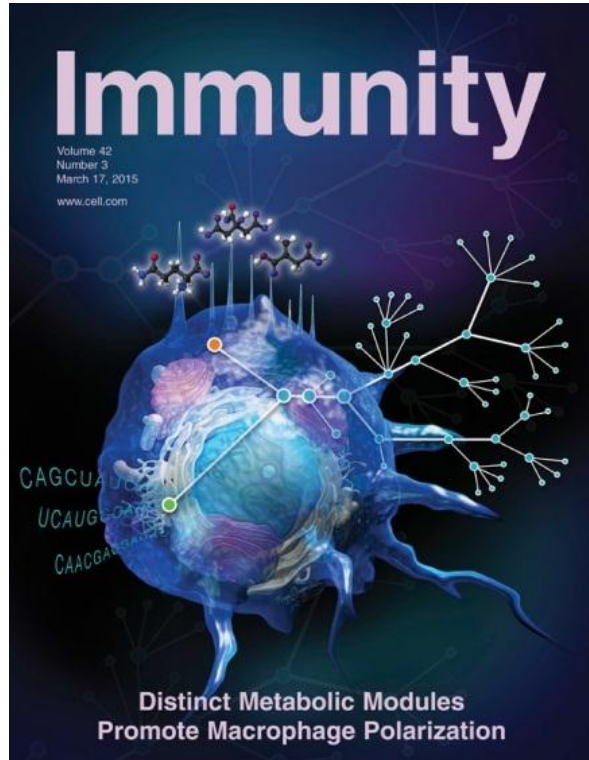


Major metabolic fluxes in M2 macrophages



N-glycosylation inhibition blocks M2 polarization








Immunity


Volume 42, Issue 3, 17 March 2015, Pages 419–430



Article

Network Integration of Parallel Metabolic and Transcriptional Data Reveals Metabolic Modules that Regulate Macrophage Polarization

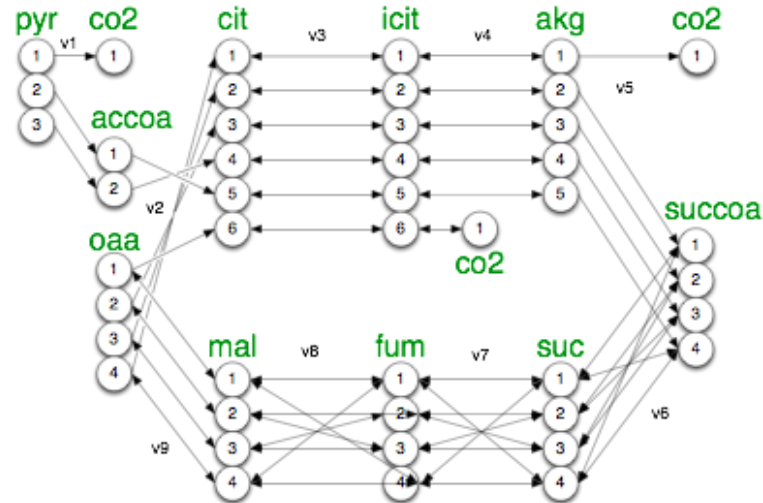
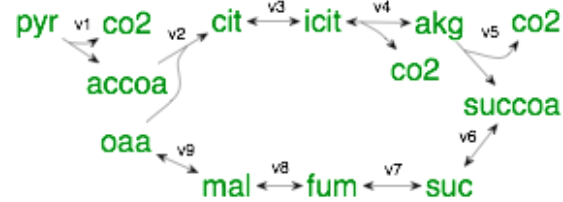
Abhishek K. Jha¹, Stanley Ching-Cheng Huang², Alexey Sergushichev^{2,3}, Vicky Lampropoulou², Yulia Ivanova², Ekaterina Loginicheva², Karina Chmielewski¹, Kelly M. Stewart¹, Juliet Ashall², Bart Everts^{2,5}, Edward J. Pearce^{2,4}, Edward M. Driggers^{1,4,6},  , Maxim N. Artyomov^{2,4},  

 [Show more](#)

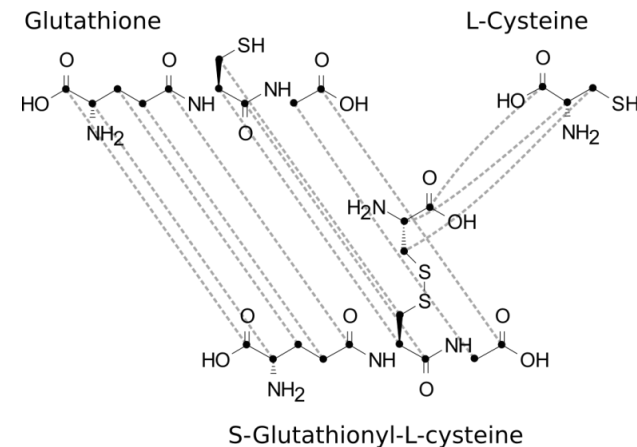
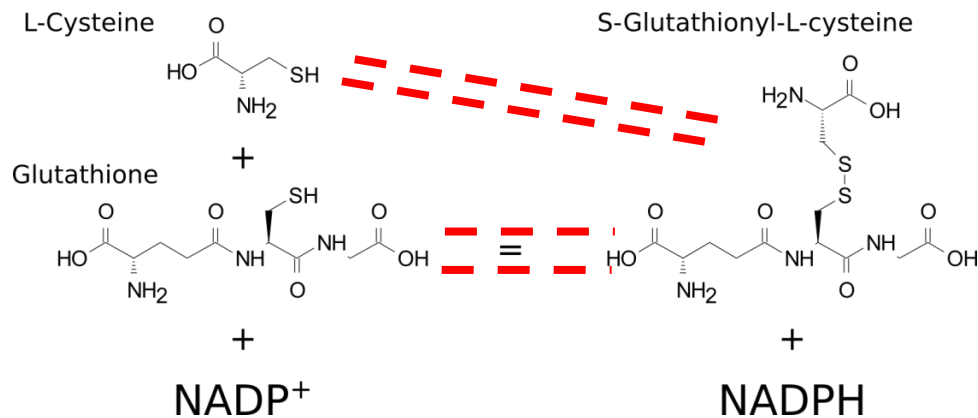
Highlights

- Glutamine deprivation affects M2 polarization but not M1 polarization
- UDP-GlcNAc biosynthesis and N-glycosylation are important for M2 polarization
- There is no reverse or direct flow through Idh or malic enzyme in M1 macrophages
- Aspartate-arginosuccinate shunt connects the NO and TCA cycles in M1 polarization

Atom transition network

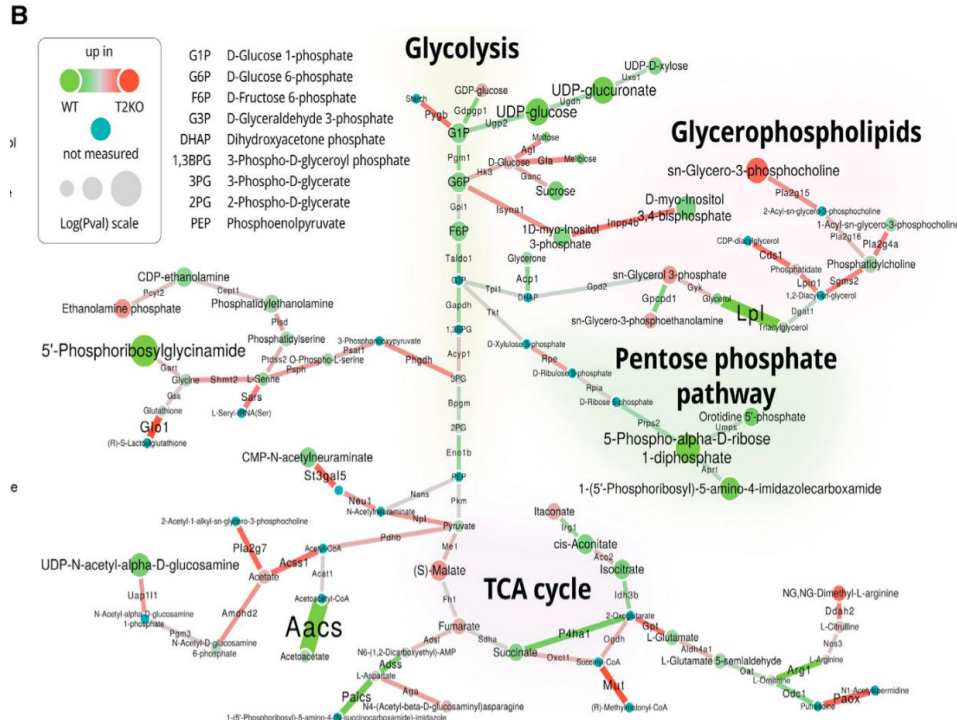


Using atom transitions graph limits “bad” connections



Any path in an atom graph corresponds to a potential reaction flux

Trem2 KO in BMDM leads to decrease in energetic metabolism

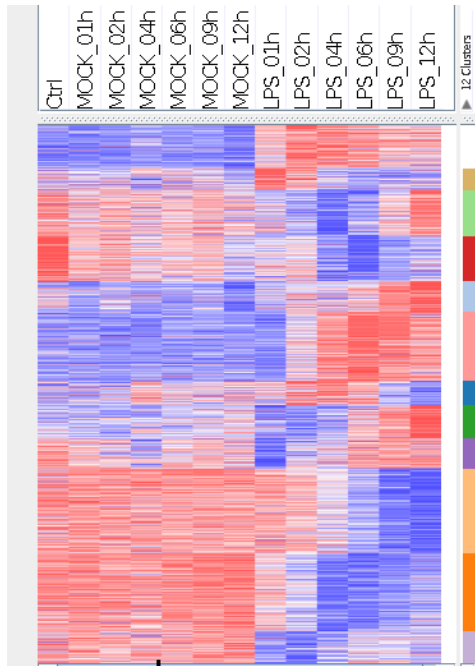


GATOM

- ✓ <https://github.com/ctlab/gatom>
- ✓ Can be installed and ran in R
- ✓ To be available in Shiny GAM

We live in a high-throughput era,
there can be many conditions!

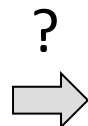
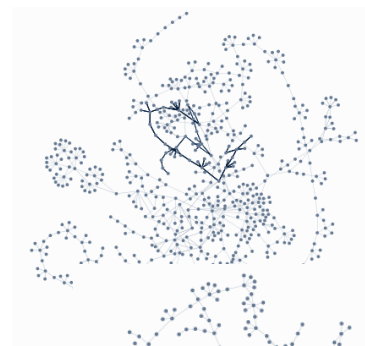
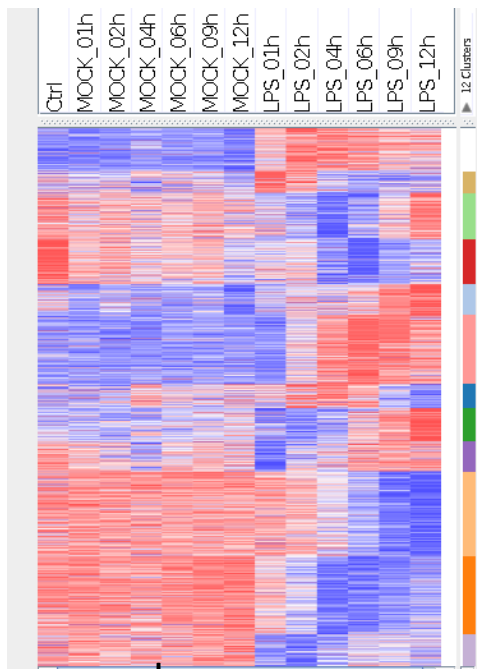
Dendritic cells time-course: many conditions, how to analyze?



*Only enzymes shown

GSE59784, Jovanovic et al, 2015

Can we identify modules for the expression patterns?

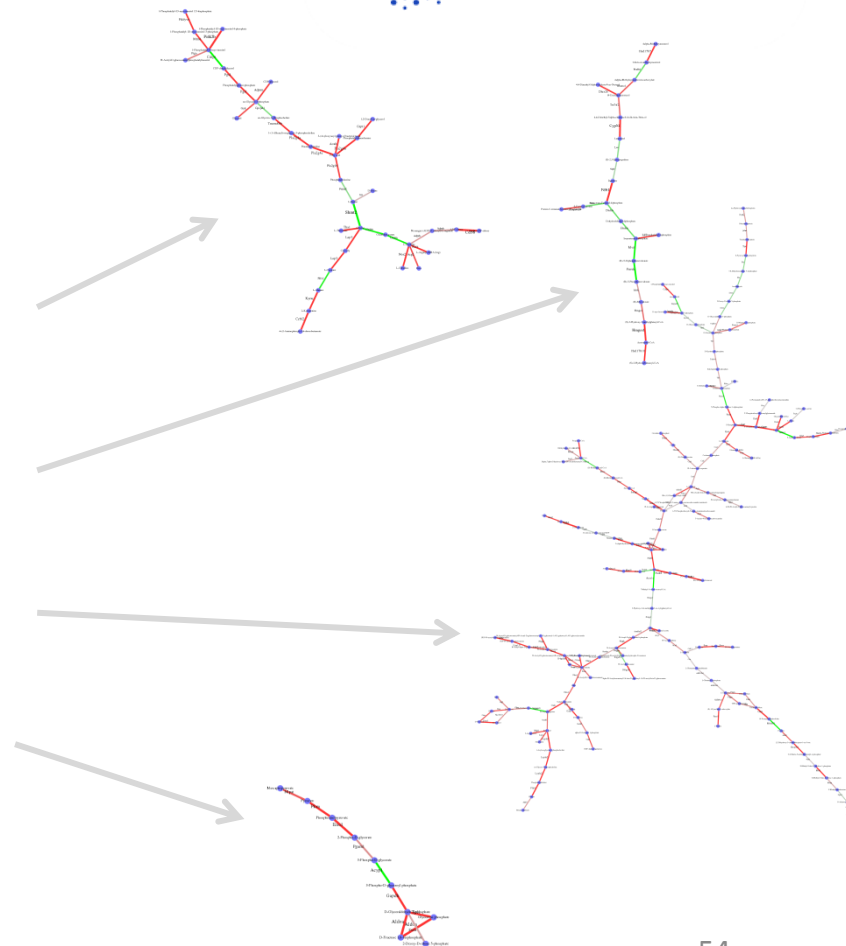
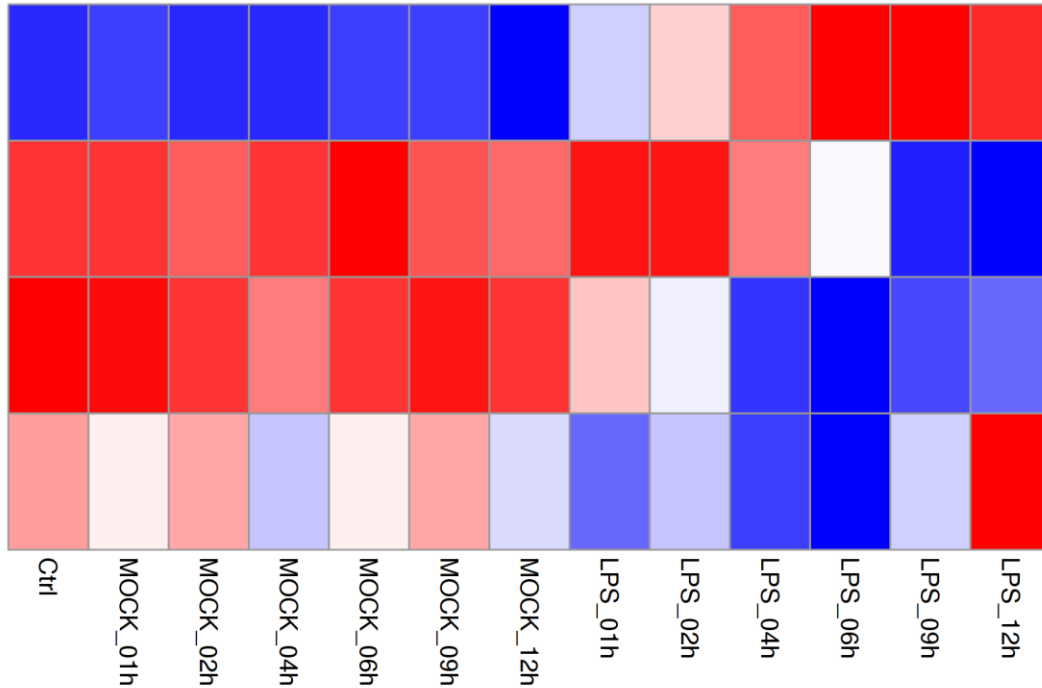


*Only enzymes shown

GSE59784, Jovanovic et al, 2015

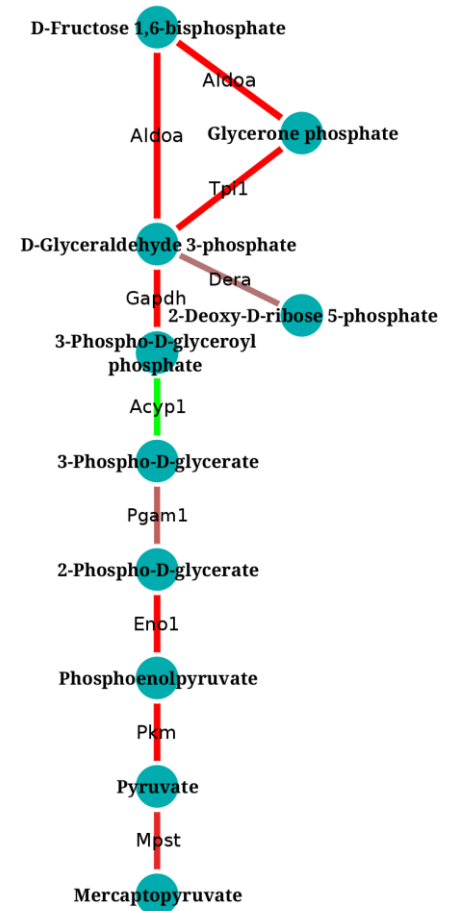
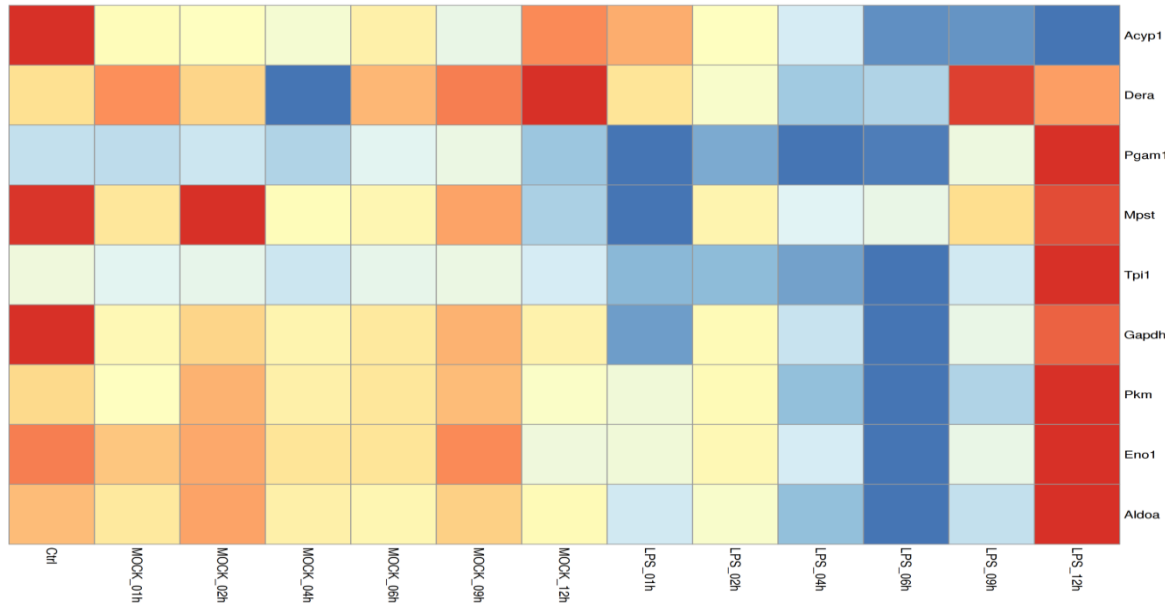
For some of them – yes!

Ctrl 1 2 4 6 9 12



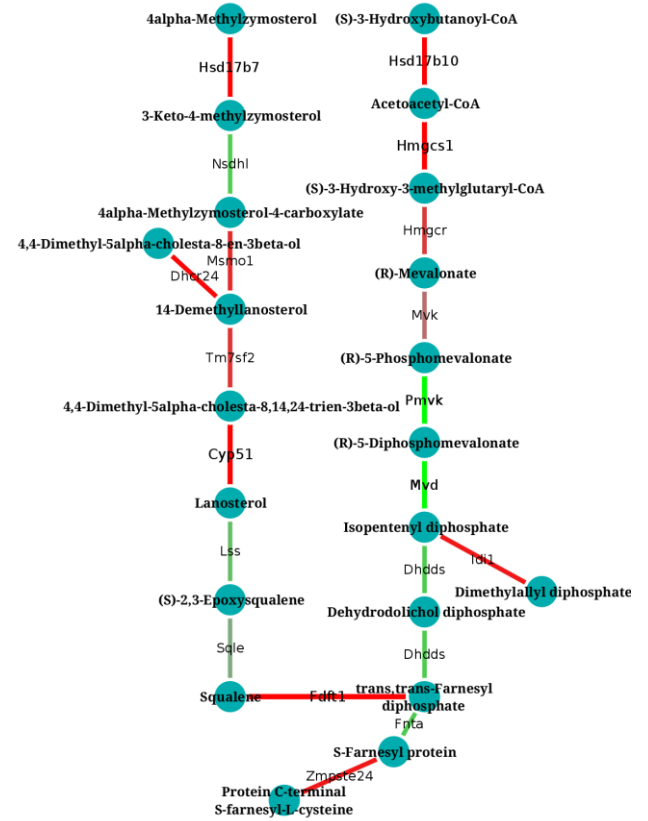
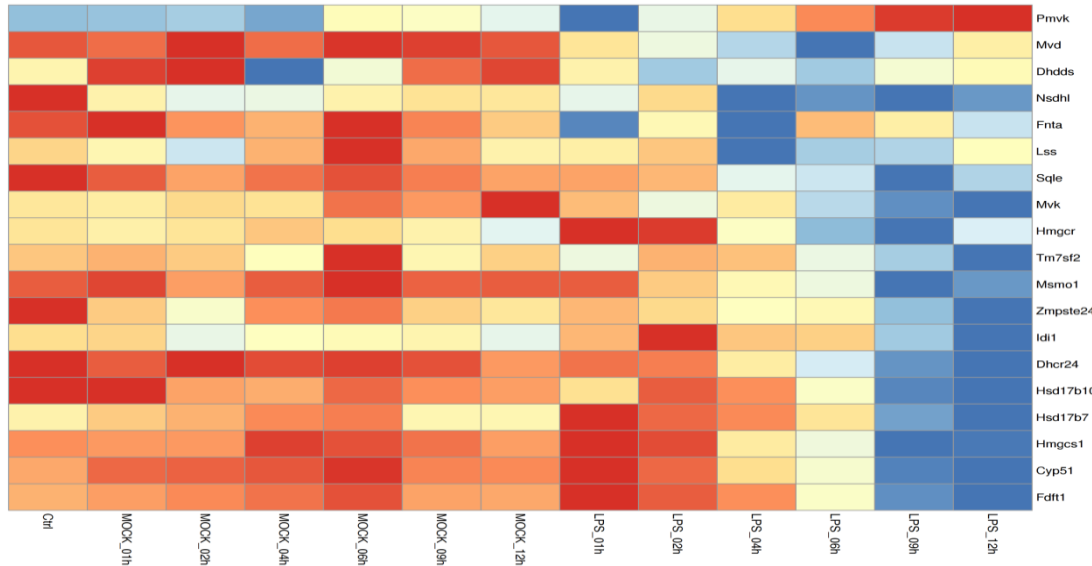
Glycolysis transcriptionally goes up at 12h

Ctrl 1 2 4 6 9 12

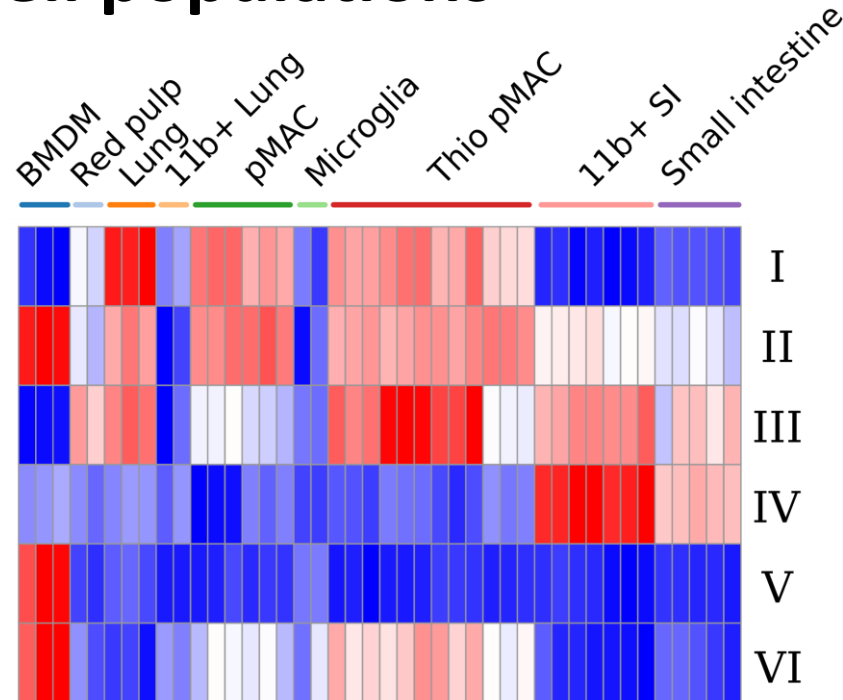


Sterol synthesis/degradation goes down at 6h

Ctrl 1 2 4 6 9 12



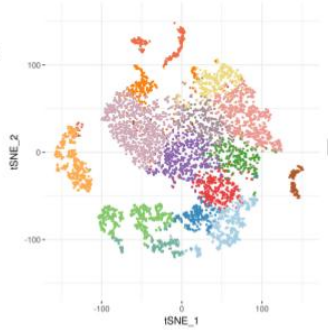
Can find differences in metabolic regulation between cell populations



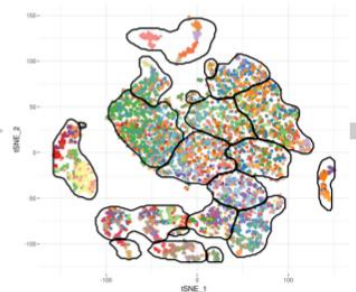
Pipeline for single-cell RNA-seq

Cell type

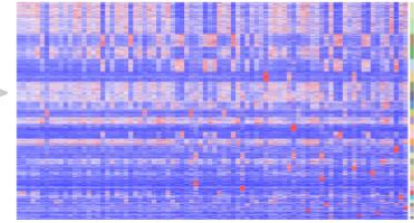
- Enterocyte.Immature.Distal
- Enterocyte.Immature.Proximal
- Enterocyte.Mature.Distal
- Enterocyte.Mature.Proximal
- Enterocyte.Progenitor
- Enterocyte.Progenitor.Early
- Enterocyte.Progenitor.Late
- Enteroendocrine
- Goblet
- Paneth
- Stem
- TA
- TA.G1
- TA.G2
- Tuft



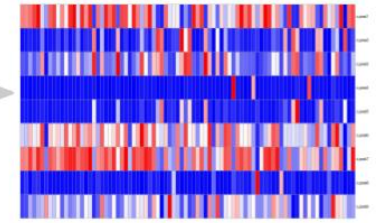
- Dataset for small intestinal epithelium (Haber et al, Nature 2017).
- Total: 7216 cells.



- Cells are fine-clustered into ~100 groups.
- Clustering is independent of cell type annotation.

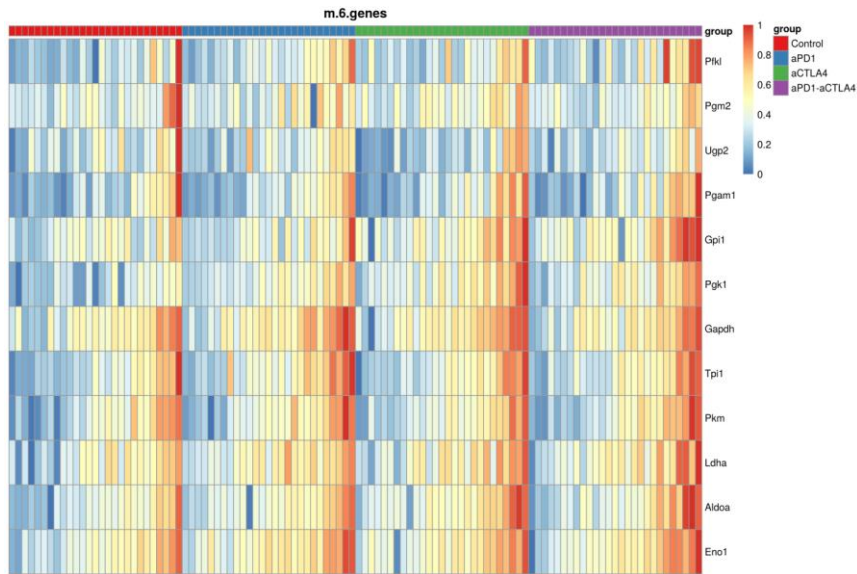


- Gene expression in cell groups are averaged to form a "meta-sample".
- Enzyme are k-means clustered by expression in meta-samples.

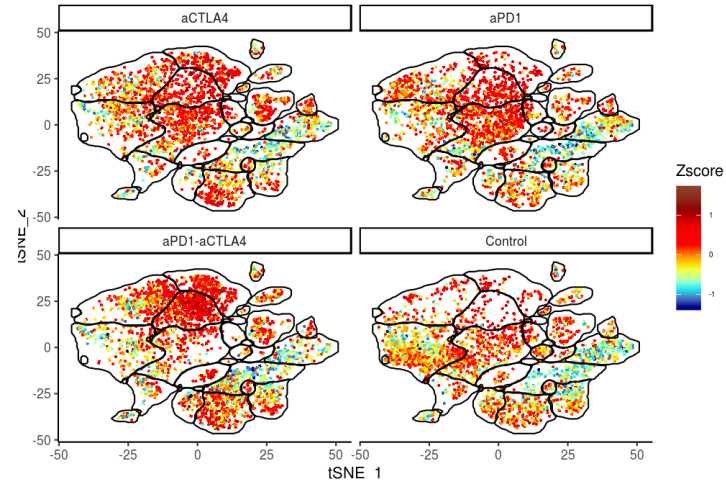


- GAM for multiple conditions is applied to obtain final module patterns.
- In the intestine dataset we ended up with nine modules.

Glycolysis is regulated in the immunotherapy dataset: macrophages getting activated



Average Z score: Pkm, Aldoa, Tpi1, Eno1, Gapdh, Pgam1, Ldha, Pfkfb1, Gpi1, Pfkfb3, Ugp2, Pgm2



Pkm Aldoa Tpi1 Eno1 Gapdh Pgam1
Ldha Pfkfb1 Gpi1 Pfkfb3 Ugp2 Pgm2

Summary

- ✔ Network integration allows to highlight interplay between enzymes and metabolites
- ✔ Network visualization (and some analysis) can be done in Cytoscape
- ✔ Modules need to be interpreted and validated