



# Canadian Bioinformatics Workshops

[www.bioinformatics.ca](http://www.bioinformatics.ca)

[bioinformaticsdotca.github.io](http://bioinformaticsdotca.github.io)

Supported by



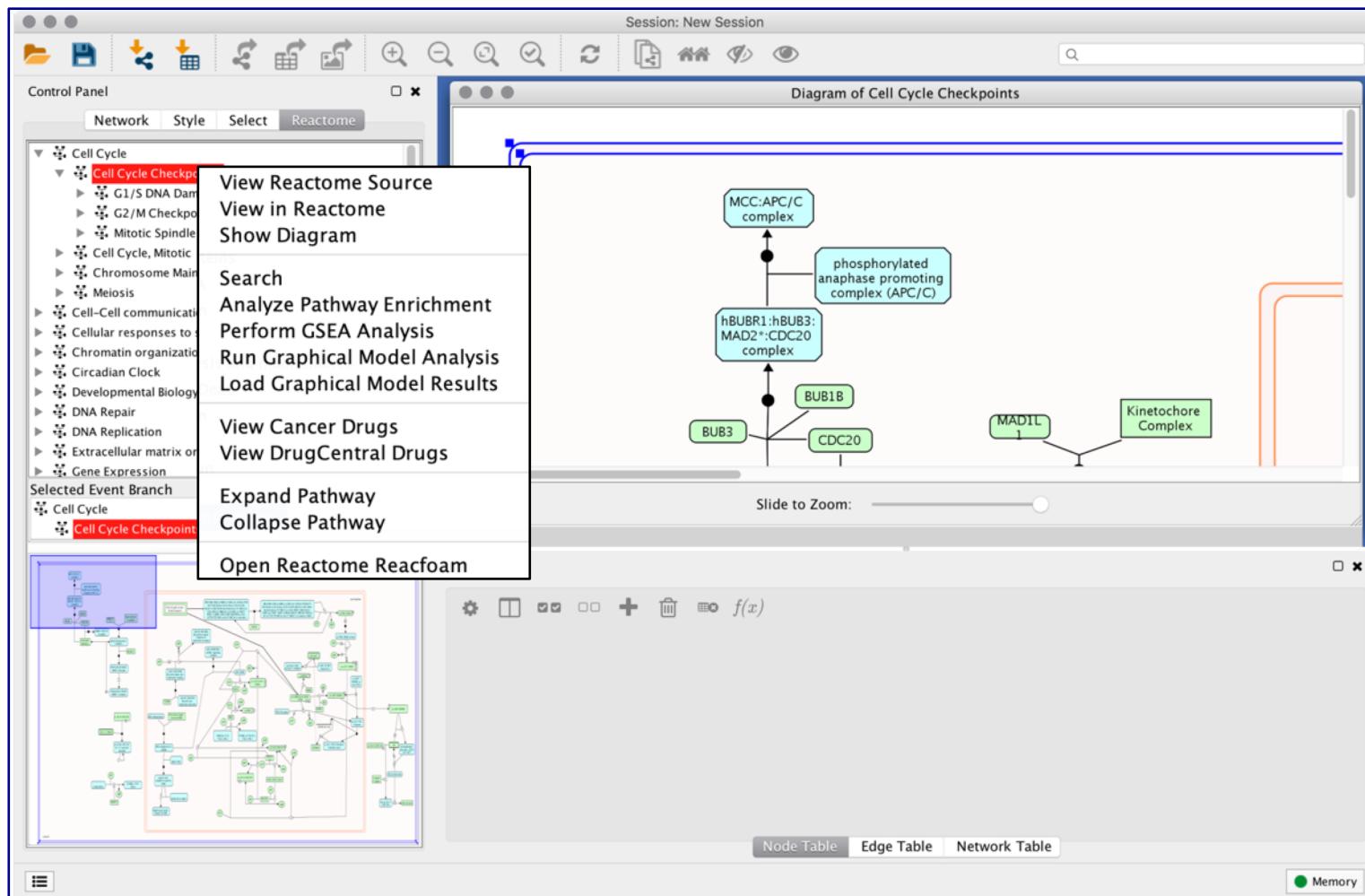
# Learning Objectives of Module

- Be able to perform pathway and network-based data analysis using the ReactomeFIViz app.

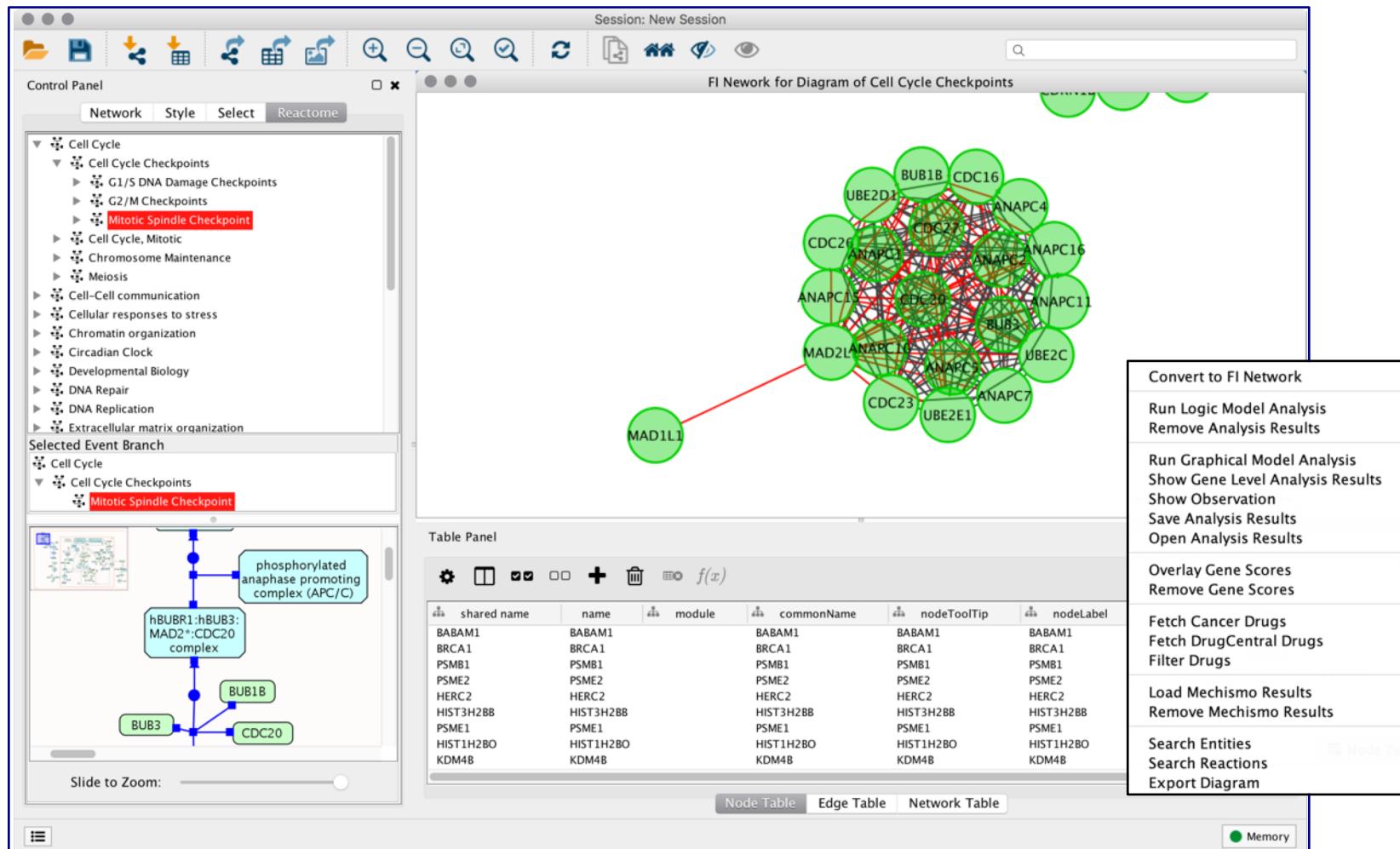
# Major Features in ReactomeFIViz

- Pathway and Network Analysis Features:
  - Support pathway enrichment analysis: Binomial test and GSEA.
  - Integration of Reactome Voronoi map for pathway holistic view.
  - Perform Boolean network and PGM-based pathway modeling
  - Construct FI subnetwork.
  - Support for HotNet analysis.
  - Perform network clustering.
  - Overlay cancer gene index, gene and cancer drug annotations.
  - Support to mouse pathways and FI network.
  - scRNA-seq data analysis and visualization.
  - Perform survival analysis.

# A1) Reactome Pathway Enrichment Analysis



# Displaying Reactome Pathways in the FI Network View



# Pathway Enrichment Analysis

Session: New Session

Control Panel

Network Style Select Reactome

FDR: >=0.1 >=0.01 >=0.001 <0.001

Interleukin-7 signaling (FDR: 0.091)

- IL7 binds HGF(495-728)
- IL7R binds JAK1
- IL2RG binds JAK3
- IL7 binds IL7R:JAK1
- IL7:IL7R:JAK1 binds IL7
- IL7R is phosphorylated
- IL7:p-Y449-IL7R:JAK1
- IL7:p-Y449-IL7R:JAK1
- IL7:p-Y449-IL7R:JAK1
- Interleukin-11 receptor alpha
- Interleukin-11 receptor alpha
- IL27 is a dimer of IL27A and IL27B
- IL27 binds IL27RA:gp130

View Reactome Source  
View in Reactome  
Show Diagram  
Search  
**Analyze Pathway Enrichment**  
Perform GSEA Analysis  
Run Graphical Model Analysis  
Load Graphical Model Results  
View Cancer Drugs  
View DrugCentral Drugs  
Expand Pathway  
Collapse Pathway  
Open Reactome Reacfoam

Selected Event Branch

Immune System (FDR: 0.291)

Cytokine Signaling in Immune system (FDR: 0.089)

Signaling by Interleukins (FDR: 0.045)

Interleukin-7 signaling (FDR: 0.091)

Diagram of Interleukin-7 signaling and JAK3 deficiency causes SCID (IL7R)

The diagram illustrates the IL7 signaling pathway. IL7 binds to IL7R, which activates JAK1. This leads to the phosphorylation of IL7R:JAK1. IL7R:JAK1 then activates IL7:IL7R:JAK1. IL7:IL7R:JAK1 activates IL2RG, which binds to JAK3. IL2RG:JAK3 activates STAT3. STAT3 is shown in the cytosol, where it activates various processes like GTP exchange, ATP production, and protein phosphorylation.

Reactome Pathway Enrichment Analysis

Gene Set Loading

Choose a gene set file: hs\_geneNames.txt

Specify file format:  One gene per line  
 Comma delimited (e.g. TP53, EGFR)  
 Tab delimited (e.g. TP53 EGFR)

OK Cancel

Table Panel

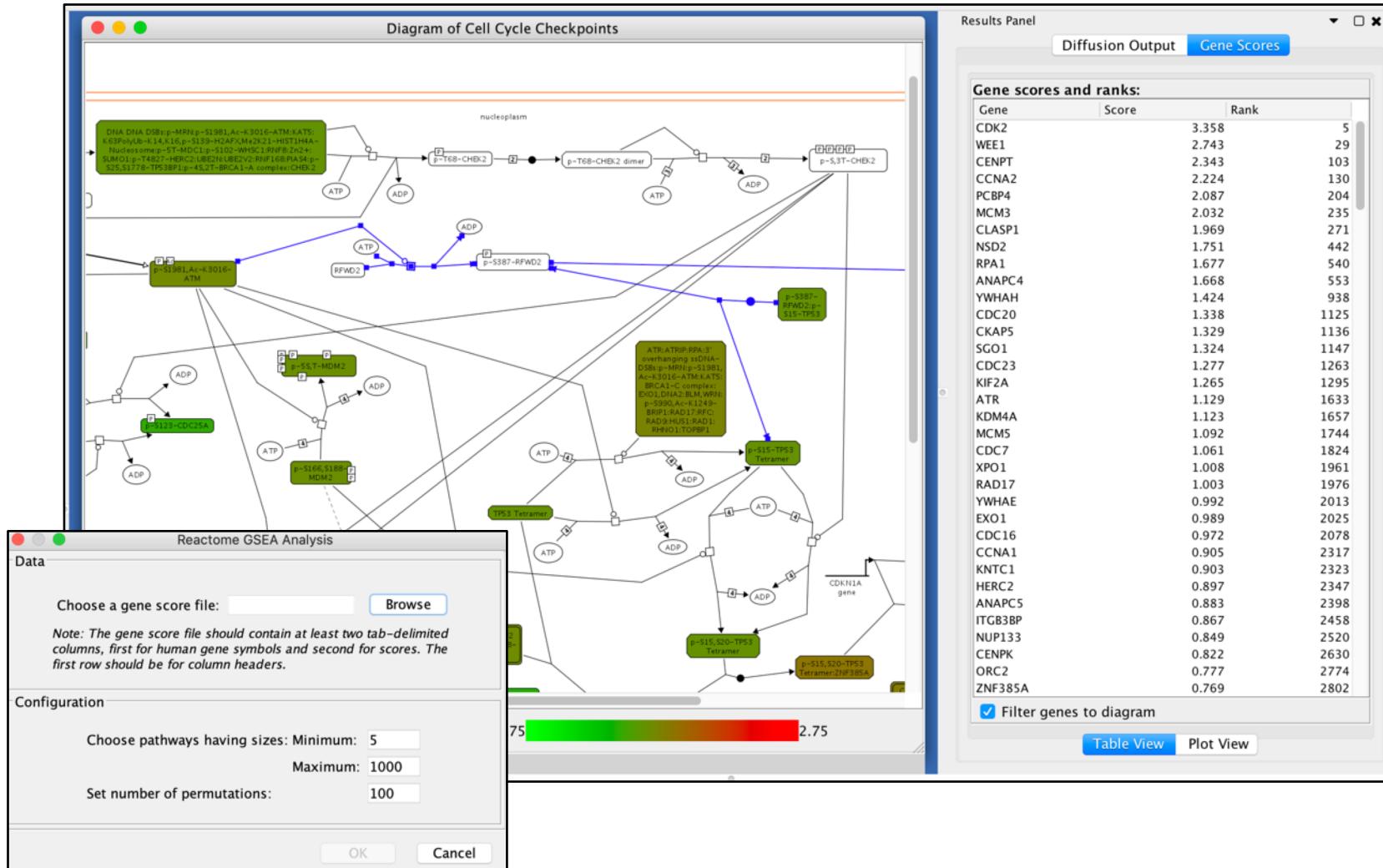
Apply Filters: FDR 1.0

ReactomePathway	RatioOfProteinInPat...	NumberOfProteinIn...	ProteinFromGeneSet	P-value	FDR	HitGenes
Laminin interactions	0.0032	23	5	0.0111	0.0890	ITGA6,LAMC1,LAMB...
Activation of Rac	0.0011	8	3	0.0114	0.0911	ROBO1,SOS1,SLT2
Interleukin-7 signal...	0.0011	8	3	0.0114	0.0911	IL7R,HGF,JAK1
CREB phosphorylati...	0.0021	15	4	0.0115	0.0917	GRIN2B,GRIN2A,AKA...
Mismatch Repair	0.0021	15	4	0.0115	0.0917	RPA1,POLD3,MLH1,...
M Phase	0.0315	225	22	0.0117	0.0937	KNTC1,NDE1,UBC,A...
GABA receptor activ...	0.0074	53	8	0.0124	0.0993	GABBR2,GNAT3,GAB...
Mitotic Prometaphase	0.0139	99	12	0.0131	0.1016	KNTC1,NDE1,PDSSB...
Digestion of dietary ...	0.0004	3	2	0.0132	0.1016	MGAM,LCT
Mitotic Anaphase	0.0227	162	17	0.0136	0.1016	KNTC1,NDE1,UBC,A...
TGF-beta receptor s...	0.0022	16	4	0.0142	0.1016	UBC,PARD3,TGFB2R...

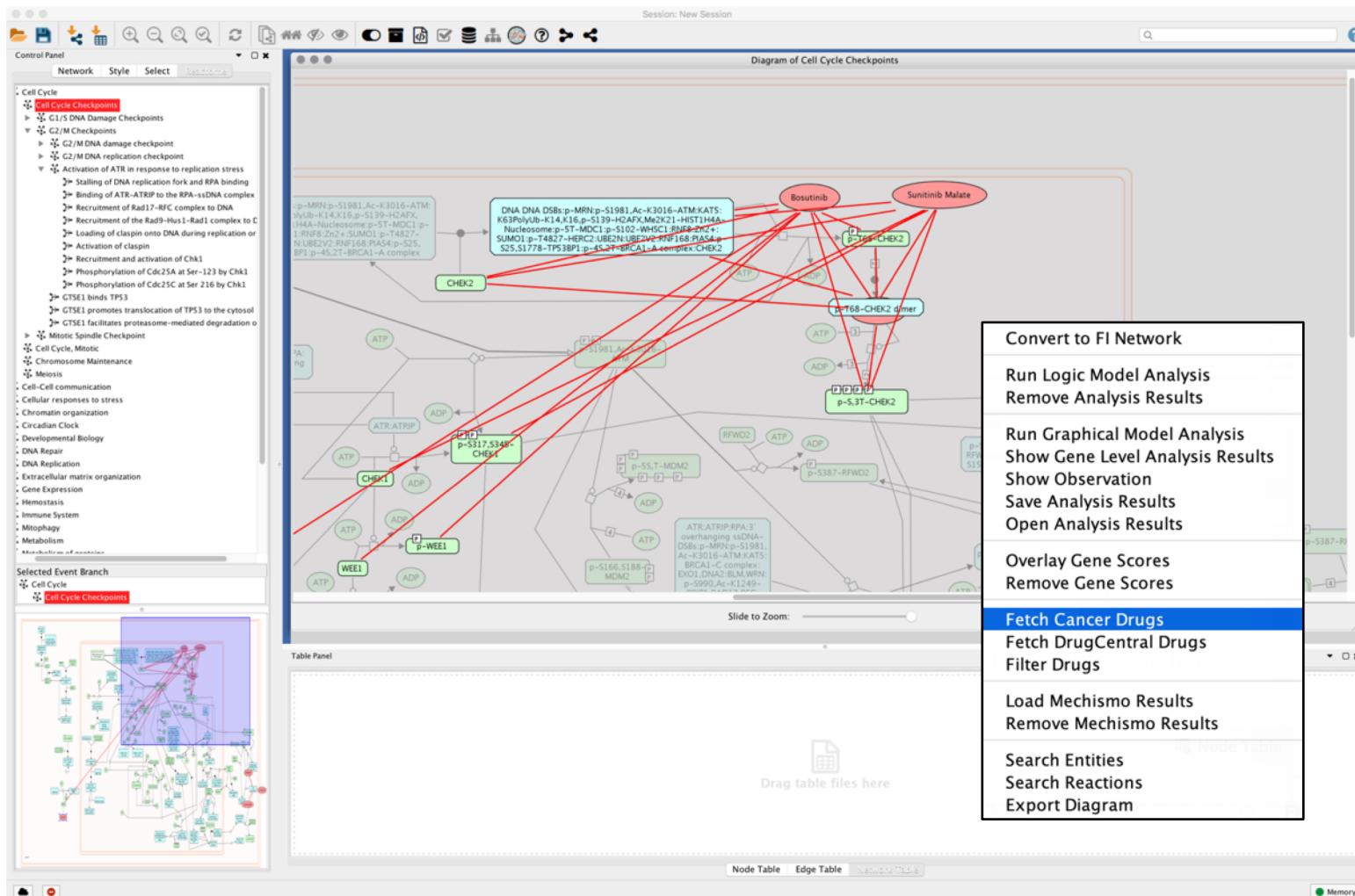
Node Table Edge Table Network Table Reactome Pathway Enrichment

Memory

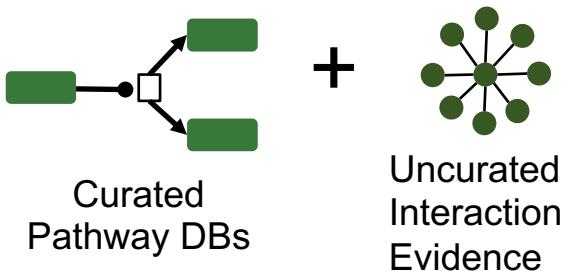
# Gene Set Enrichment Analysis (GSEA)



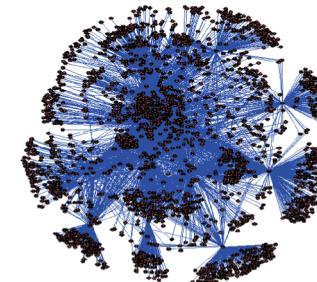
# Visualize Cancer Targetome in Reactome Pathways



# A2) De Novo Subnetwork Construction & Clustering

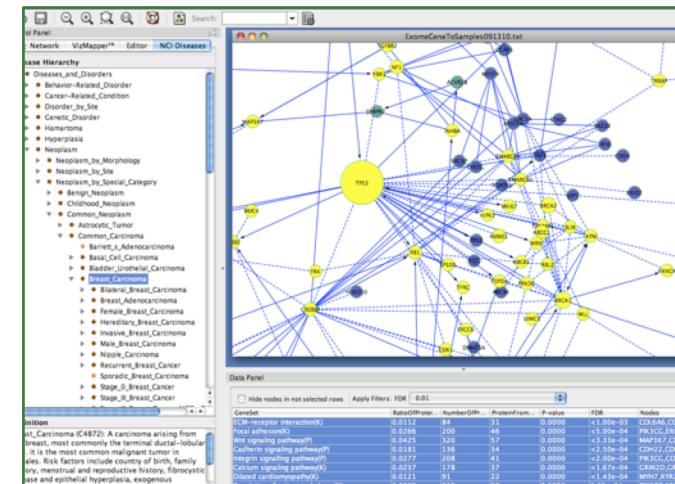
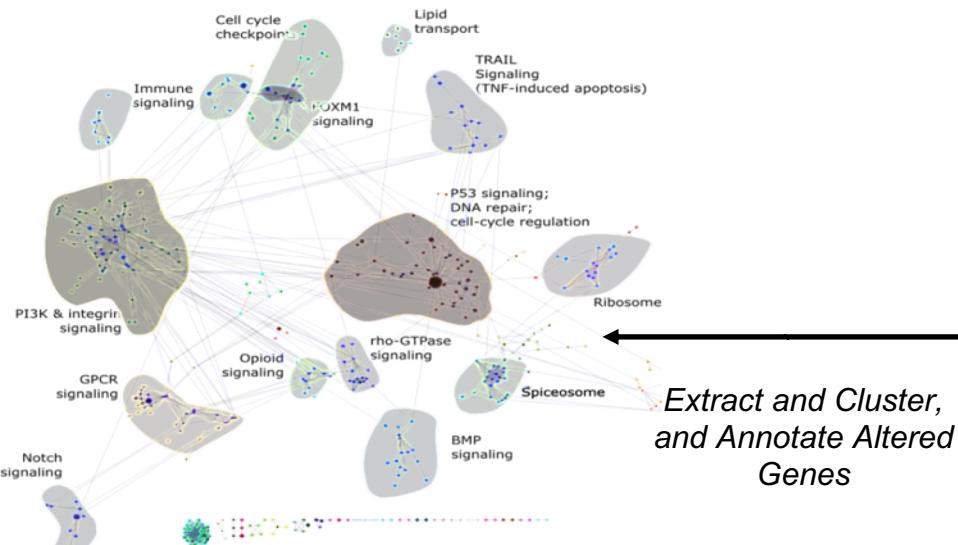


*Machine Learning*



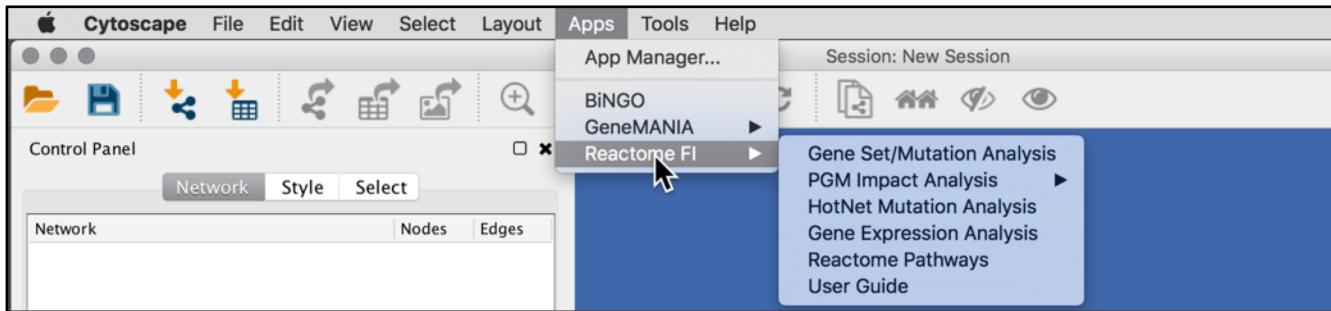
**Reactome Functional Interaction Network**  
(~13K proteins; ~436K interactions)

+



Cytoscape using ReactomeFI Viz app

# Upload your data



- FI plug-in supports four file formats:
  - Simple gene set: one line per gene
  - Gene/sample number pair. Contains two required columns, gene and number of samples having gene mutated, and an optional third column listing sample names (delimited by semi-colon ;)
  - NCI MAF (mutation annotation file)
  - Sample Gene Expression data file

# File Formats

- Choose Plugins, Reactome Fls.

# Simple Gene List

MSI2  
PTPRT  
PELO  
SLC18A1  
TACC2  
FAM148B  
PRC1  
MSTN  
ATP6V1G3  
APOE  
IMPA2  
AGER  
XPO5  
MEST  
RREB1  
BAT1  
WIP1

## Gene/Sample Number Pairs

Gene	Sample_Number	Samples
IFT88	1	TCGA-10-0937
TACC2	5	TCGA-24-2288;TCGA-09-1662;TCGA-13-0755;TCGA-13-0920;TCGA-24-2035
ADAD2	1	TCGA-24-1419
PODXL2	1	TCGA-24-2290
RTN3	1	TCGA-13-0726
TADA2B	1	TCGA-25-1313
CD274	1	TCGA-25-1627
SULF1	2	TCGA-24-2262;TCGA-09-2056
SYCP2	3	TCGA-24-1563;TCGA-61-2008;TCGA-24-1427
CD200R1L	1	TCGA-13-1488
RCBTB2	1	TCGA-61-2113
TOP2B	1	TCGA-13-0923
C1orf50	1	TCGA-25-2392
ATP9A	5	TCGA-25-1329;TCGA-04-1530;TCGA-24-2262;TCGA-10-0933;TCGA-13-0795
NUDT5	1	TCGA-61-2003
ZNF189	1	TCGA-24-2254
KCNB1	1	TCGA-23-1028
FERMT3	1	TCGA-24-1464
ZCHC2	1	TCGA-61-1998
DDX18	1	TCGA-13-1509
TGM1	2	TCGA-24-0975;TCGA-25-1321
SAMD7	1	TCGA-09-2051
OR2F2	3	TCGA-13-0723;TCGA-24-2267;TCGA-25-2392
ZSWIM3	2	TCGA-24-1466;TCGA-23-1118
EIF2AK2	2	TCGA-59-2355;TCGA-24-1463
KIAA2018	4	TCGA-23-1024;TCGA-04-1367;TCGA-24-2288;TCGA-24-2289
.....		

## NCI MAF (mutation annotation file)

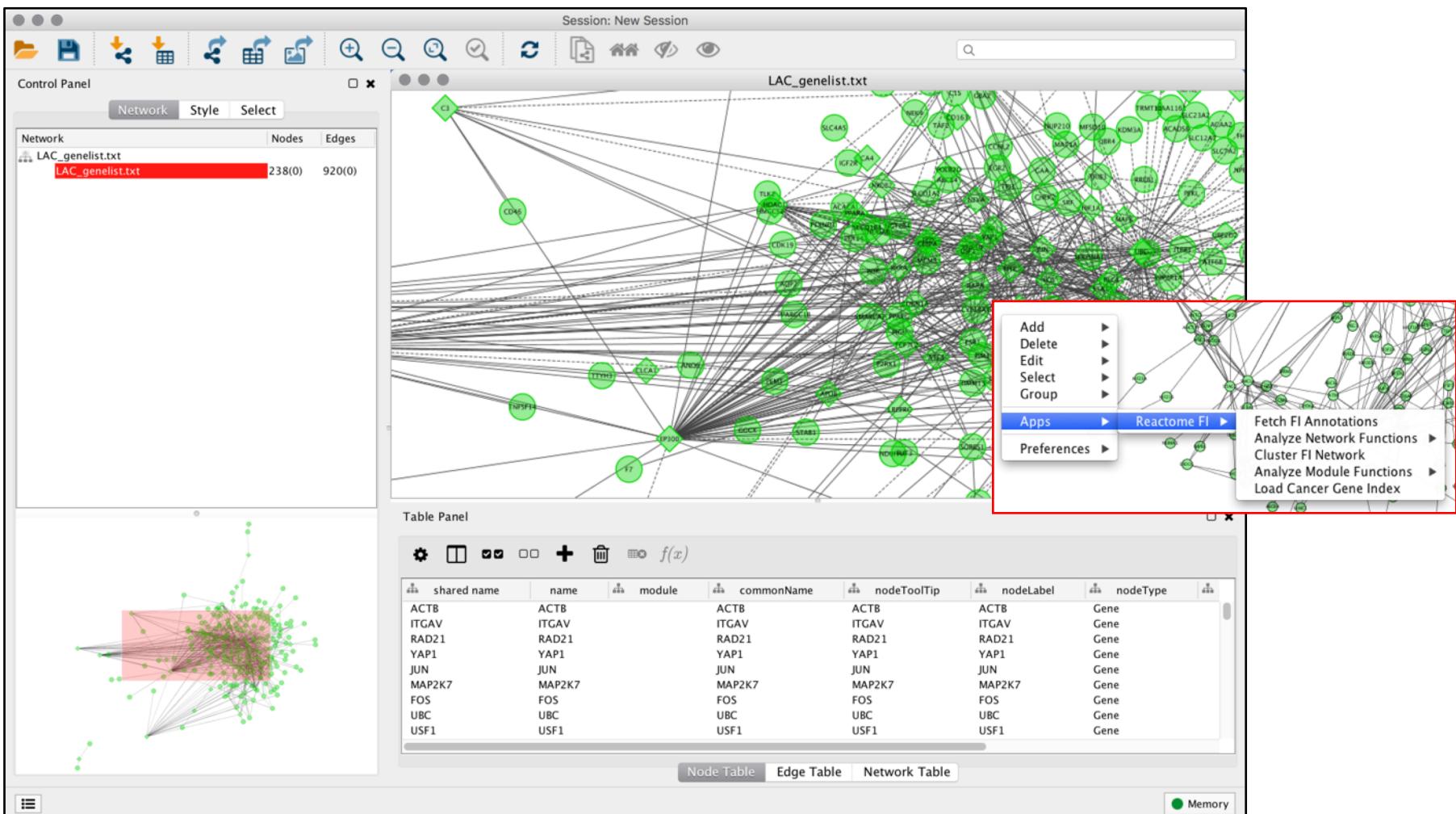
## Microarray (array) data file

gene	Sample 122	Sample 123	Sample 124	Sample 125	Sample 126	Sample 127	Sample 128	Sample 129	Sample 130
IFT8B	1.43351703	0.749571365	0.204170171	1.376375273	0.049501641	0.912377692	0.464660294	0.106486375	0.40767808
KIF9	0.349692236	-0.093947168	-0.568902295	0.177455767	-0.30793794	-0.574121582	-0.772454492	0.850743799	0.19833291
CPSF3	-0.628846878	-0.519774779	0.20101088	-0.388886255	-0.053934584	0.549138111	0.440059517	-0.247094552	0.421708675
TACC2	0.985299196	-0.735255555	0.103645941	-0.057886422	0.730172429	0.554418157	1.280114274	0.466370752	0.95651972
C14orf132	1.249528811	0.108470645	0.524096064	-0.434641864	-1.528261113	-0.467450383	0.715356935	-0.525765954	-0.10693968
RTNE	0.106434333	-0.26693286	-0.329065423	0.545985273	0.105099211	-0.204628824	-0.462552052	1.380114274	-0.58966927
PDXDL2	-0.31340318	-0.39041822	-0.075387324	-0.412394215	0.085789402	-0.073695617	-0.053408711	-0.104692114	0.4380939
CORO1A	-0.24703733	0.664838693	-0.423711396	-0.019075979	0.488162732	-0.435111645	0.596447997	0.463670766	0.05051666
RBM17	-0.360070659	-0.142615078	-0.233914802	0.183969411	-0.130696373	-0.39476802	-0.181893193	-0.62663375	0.99323763
WT1	-0.768491581	-0.694912985	-0.763566041	-0.674252404	-0.168617571	-0.393931693	-0.062419512	-0.358250201	-0.538925163
SULF1	-0.863662291	1.589479478	0.272263522	-0.419487000	0.401966747	0.081472544	-1.178054035	0.327228913	-0.21903657
SYCP2	-0.365295082	0.246319117	-0.107066702	0.891658263	0.914563556	0.091543376	-0.37696798	0.727095265	0.00962058
PHRY	-0.252934465	-0.340129465	0.084219531	0.613340462	-0.8573245	0.479503528	0.229545453	-1.103679457	0.37555123
RCBTB2	-1.216044791	0.113767375	-0.741641062	0.63677998	-0.695953625	-0.46841374	-0.34489208	-0.130543482	-0.572431973
TOPBP2	1.3291376011	1.277684609	0.636481097	0.116284914	0.464606891	0.928925854	-0.479308731	0.39171399	0.050669942
SEC22B	-0.712050905	0.661562362	-0.339431611	0.197245507	-0.277259539	0.413113408	0.588836362	0.6332012077	0.486716031
ATP9A	1.547472859	0.444111338	1.145059578	-0.14324565	0.540051596	0.510070468	-0.233468933	-0.149520936	-0.047580483
TGFBR2	-0.782121280	-0.264862477	-0.132003586	-0.027190120	-0.23982997	0.427719409	-0.37868444	0.110637236	0.170499777
NUDT5	-0.161643202	-0.117594702	0.072831699	-0.025664715	-0.37488737	0.43398528	-0.8989999	0.020300278	0.11232065
VT11B	0.251281715	0.155526231	-0.07352674	0.770902019	-0.368466883	0.168434408	0.191395423	0.967344394	0.2116535981
RP5K6B	-0.387163384	0.639591596	-0.597648114	-0.392291758	0.932165059	0.156370699	0.782463975	0.46043084	-0.674654752
RAGFEP6	0.202545341	0.240920292	0.106670693	0.156485103	0.557135368	-0.0270365	-0.165279317	0.612292366	-0.17859286
PR10596	-0.010404297	0.228155239	0.445509484	0.148635394	0.077918194	-0.376195587	0.138032748	0.03703761	0.03703761
CKN1B	0.093447088	-0.061402128	0.135678483	0.628373548	0.655613085	1.458927537	0.181613556	0.469637643	0.346835207
ALDH9A1	0.702468807	-0.116922973	0.963729452	-0.181038168	0.1083824904	1.698904754	1.844724649	-0.03872325	0.155479589
ZCHC2	0.55864339	0.120509563	0.48570548	-0.479704052	0.47752246	-0.14489382	0.98358135	0.55332533	0.09713821
LCS1272	-0.29141664	-0.264217435	0.134705668	-0.21743876	-0.073822271	-0.699406086	0.071399049	-0.21885858	-0.14635723
DX18	0.349671	0.29483427	-0.00840307	0.105037500	0.140036175	0.07623935	-0.160745532	0.513713364	0.106972508

# Gene Set-based Analysis

The screenshot shows the Cytoscape application window. The top menu bar includes Cytoscape, File, Edit, View, Select, Layout, Apps, Tools, and Help. The Apps menu is open, showing the 'Reactome FI' option which is highlighted. A sub-menu for 'Reactome FI' is also open, listing 'Gene Set/Mutation Analysis', 'Reactome Pathways', 'Gene Expression Analysis', 'PGM Impact Analysis', 'Single Cell Analysis', and 'User Guide'. The main workspace displays a network graph with nodes and edges. On the left, there are tabs for 'Network' and 'Style'. A search bar says 'Enter search terms for NDEx...'. Below the search bar is a 'Reactome FI Network Version' dropdown set to 2020. The 'Gene Set Parameters' section includes fields for 'Choose data file' (with a 'Browse' button), 'Or enter gene set:' (with an 'Enter' button), and 'Specify format:' (radio buttons for 'Gene set', 'Gene/sample number pair', and 'NCI MAF (Mutation Annotation File)', with 'Gene set' selected). It also includes a 'Choose sample cutoff:' field set to 2. The 'FI Network Construction Parameters' section contains a checked checkbox for 'Fetch FI annotations' with a note: '\* Annotations may be fetched later.', and two unchecked checkboxes for 'Use linker genes' and 'Show genes not linked to others'. At the bottom are 'OK' and 'Cancel' buttons.

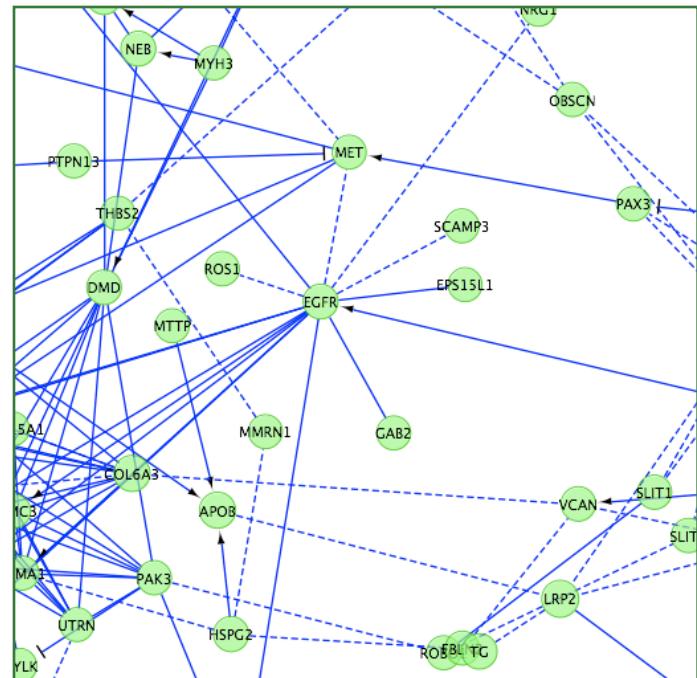
# FI Results Display



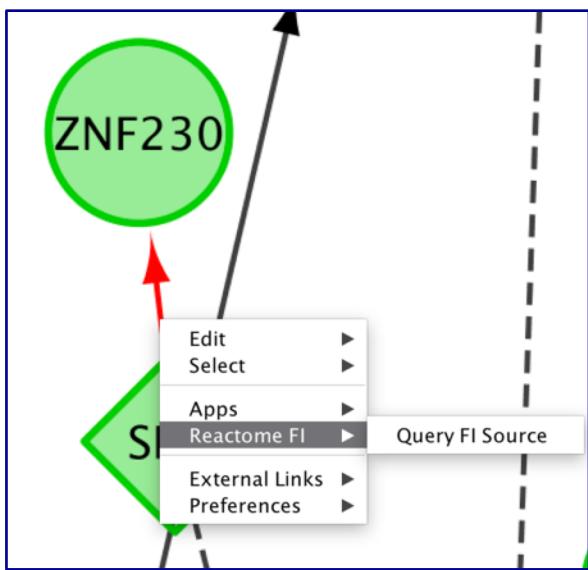
- Constructed network is displayed in the Network View panel using an FI specific visual style

# FI Annotations

- Provides detailed information on selected FIs.
- Three edge attributes are created:
  - FI Annotation.
  - FI Direction.
  - FI Score (for predicted FI).
- Edges display direction attribute values.
  - --> for activating/catalyzing.
  - --| for inhibition.
  - solid line for complexes or inputs.
  - --- for predicted FIs.



# Query FI Source



Annotated FIs

**Interaction Info**  
**Interaction: SIX5 - ZNF230**

**Reactome Sources**

Reactome ID	Type	Data Source
6951674	TARGETED_INTERACTION	ENCODE

**View Reactome Source**

**Reactome Instance View**

classType	TargetedInteraction
dbId	6951674
displayName	SIX5 ZNF230
dataSource	ENCODE
definition	ENCODE proximal_filtered TF/target interaction; supported by GO BP sharing
factor	SIX5
species	Homo sapiens
target	ZNF230

**Close**

Predicted FIs

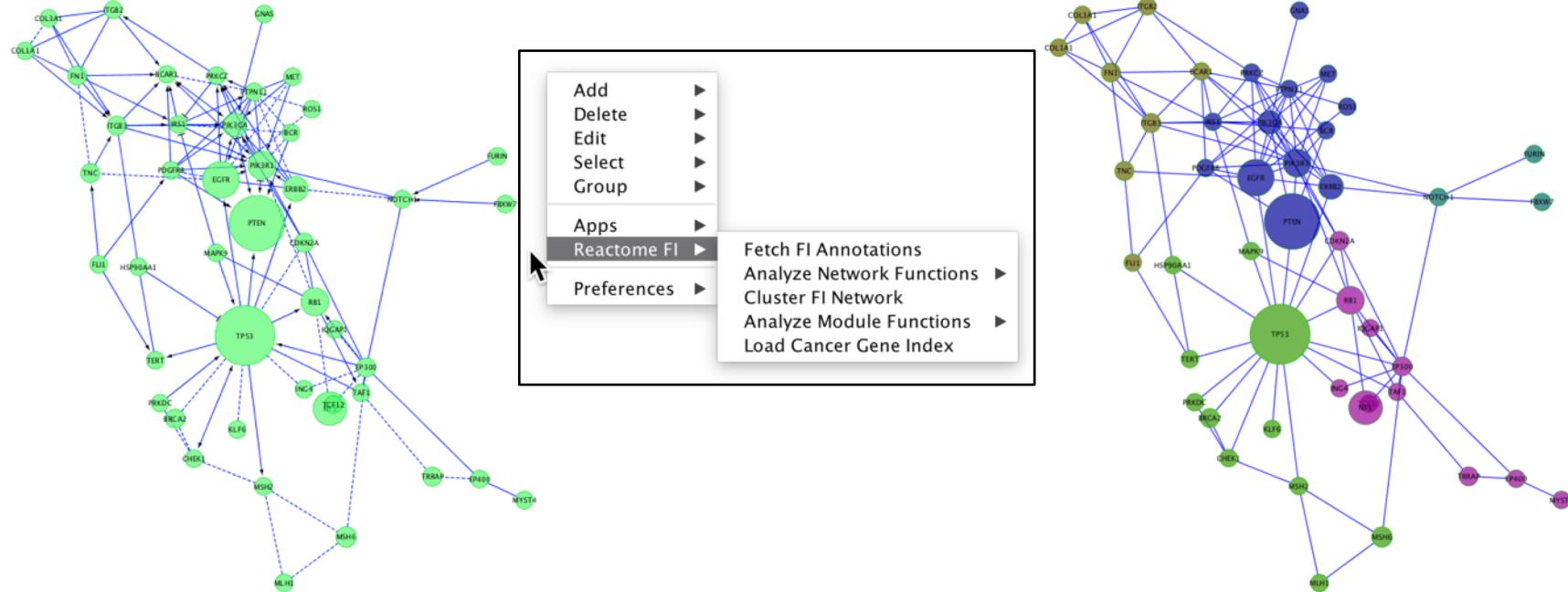
**Interaction Info**  
**Interaction: SNX4 - SNX6**

**Support Evidence**

Predictor	Value
Human Interaction	true
Yeast Interaction	false
Fly Interaction	false
Worm Interaction	false
Mouse Interaction	false
Pavlidis Gene Exp	false
Carlos Gene Exp	false
PfamDomain Interaction	true
GO BP Sharing	true
Score	0.9642255584531738

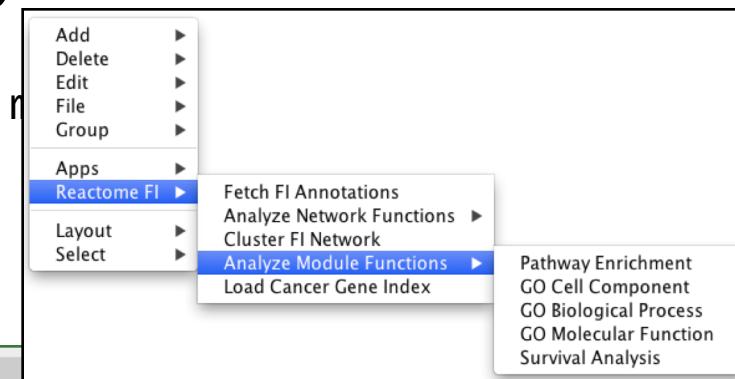
# Cluster FI Network

- Runs spectral partition based network clustering ([Newman, 2006](#)) on the displayed FI network.
- Nodes in different network modules will be shown in different colours (max 15 colours).
- Analyze cancer mutation data with HotNet algorithm ([Vandin, 2012](#))



# Analyze Module Functions

- Pathway or GO term enrichment analysis on individual network modules.
  - Use filter to remove small network modules.
  - Filter by FDR



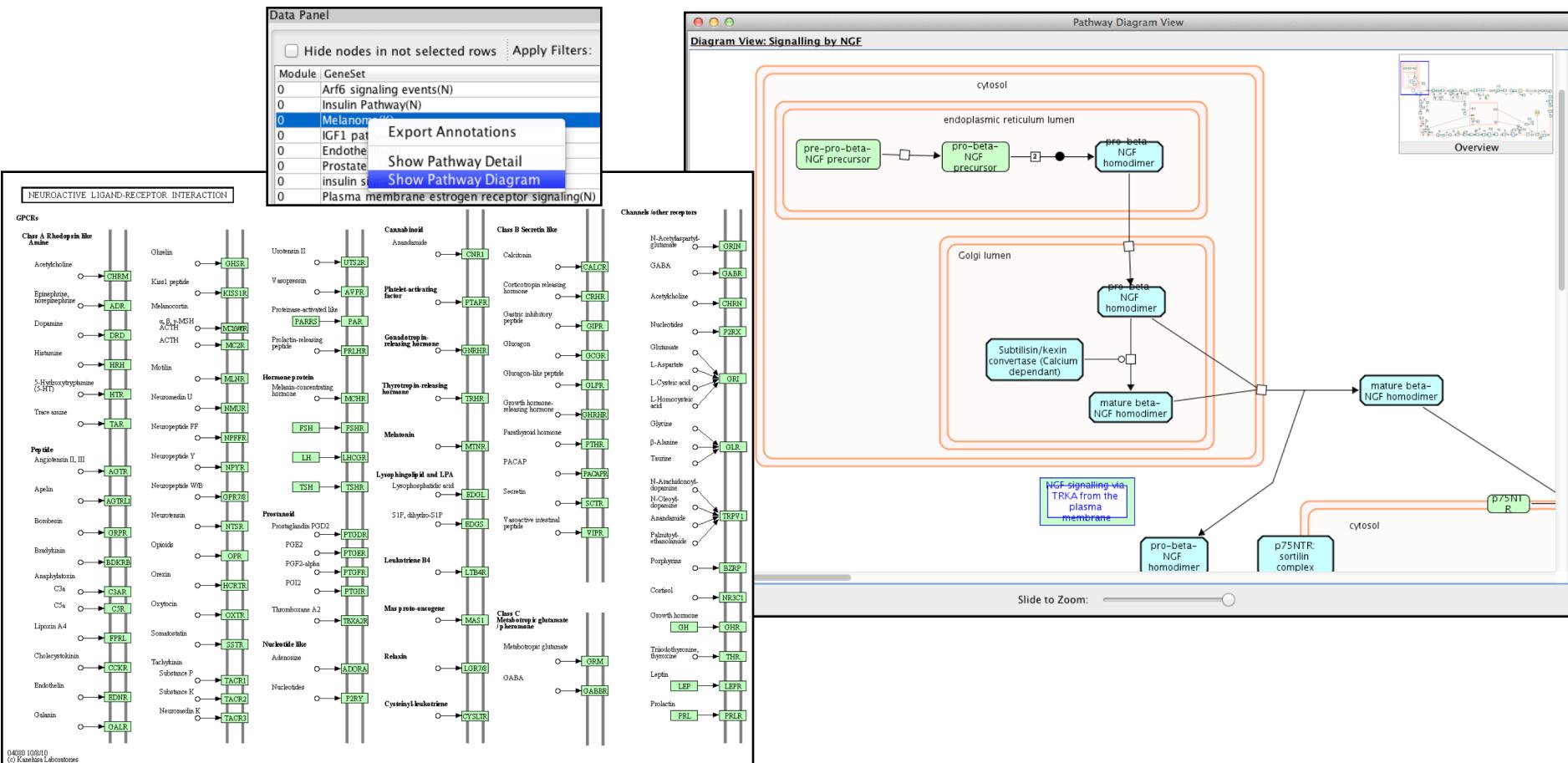
Data Panel

Module	GeneSet	RatioOfProteinInModule	Module Size	Nodes
0	Translation(R)	0.0158	15	RPL18,RPL17,RPL36...
0	Influenza Life Cycle(R)	0.0249	13	RPL18,RPL17,RPL36...
0	Ribosome(K)	0.0117	12	RPL18,RPL17,RPL36...
0	Metabolism of protein...	0.0267	11	RPL18,RPL17,RPL36...
1	M Phase(R)	0.0128	9	ITGB3BP,MAD1L1,CC...
1	Aurora B signaling(N)	0.0052	8	AURKC,AURKB,CDCA...
1	Signaling by Aurora k...	0.0125	7	AURKC,AURKB,CDCA...
2	Glucose Regulation of...	0.0197	148	NDUFB4,NDUFA2,ND...
2	Parkinson's disease(K)	0.0176	132	NDUFB4,NDUFA2,ND...
2	Alzheimer's disease(K)	0.0223	168	NDUFB4,NDUFA2,ND...
2	Huntington's disease(K)	0.0246	185	NDUFB4,NDUFA2,ND...
2	Oxidative phosphoryl...	0.0178	134	NDUFB4,NDUFA2,ND...
2	Electron Transport C...	0.0101	76	NDUFB4,NDUFA2,ND...
2	Metabolic pathways(K)	0.1489	1120	NDUFB4,NDUFA2,ND...
3	Antigen processing a...	0.0104	78	KLRC2,KIR2DS1,HLA...
3	Immunoregulatory int...	0.0153	115	KIR2DS1,HLA-B,HLA...
3	Natural killer cell me...	0.0182	137	KLRC2,KIR2DS1,HLA...

Node Attribute Browser Edge Attribute Browser Network Attribute Browser MCL Module Browser Pathways in Modules

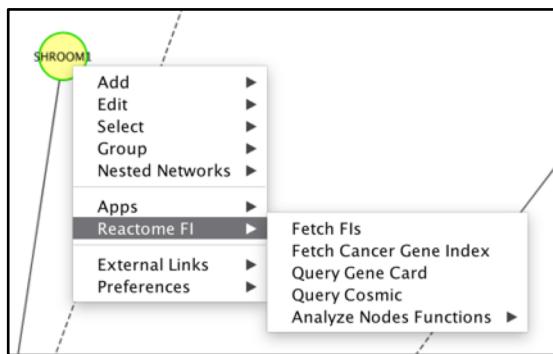
# Show Pathway Diagrams

- Select a pathway in "Pathways in Network/Modules" tabs, right click, select "Show Pathway Diagram"



# NCI Cancer Gene Index

- View detailed annotations for the selected gene or protein.
- Annotations are sortable by PubMed ID, Cancer type, status, and other criteria.



**Cancer Gene Index Annotations for "MAF"**

Order by: PubMedID ▾ Set Filters Current filters: None

Results: 1 to 8 of 8

First Prev Page 1 Next Last

**Cancer type:** tumors  
**Primary NCI role code:** Gene\_Has\_Anomaly  
**Other roles:** not\_assigned  
**Evidence code:** EV-AS-TAS  
**Negation indicator:** no  
**Cellline indicator:** no  
**Status:** finished  
**PubMedID:** [16155016](#)  
**Comment:** Anomalous\_Detail: Chromosomal\_Translocation

Nearly half of tumors are nonhyperdiploid, and mostly have one of five recurrent IgH translocations: 16% 11q13 (CCN D1), 3% 6p21 (CCN D3), 5% 16q23 (MAF), 2% 20q12 (MAFB), and 15% 4p16 (FGFR3 and MMSET).

---

**Cancer type:** tumors  
**Primary NCI role code:** Gene\_is\_Biomarker\_of  
**Other roles:** not\_assigned  
**Evidence code:** EV-EXP-IEP, EV-EXP-IDB  
**Negation indicator:** no  
**Cellline indicator:** no  
**Status:** finished  
**PubMedID:** [15755896](#)

Using gene expression profiling to identify 5 recurrent translocations, specific trisomies, and expression of cyclin D genes. MM tumors can be divided into 8 TC (translocation/cyclin D) groups (11q13, 6p21, 4p16, maf, D1, D1+D2, D2, and none) that appear to be defined by early, and perhaps initiating, oncogenic events.

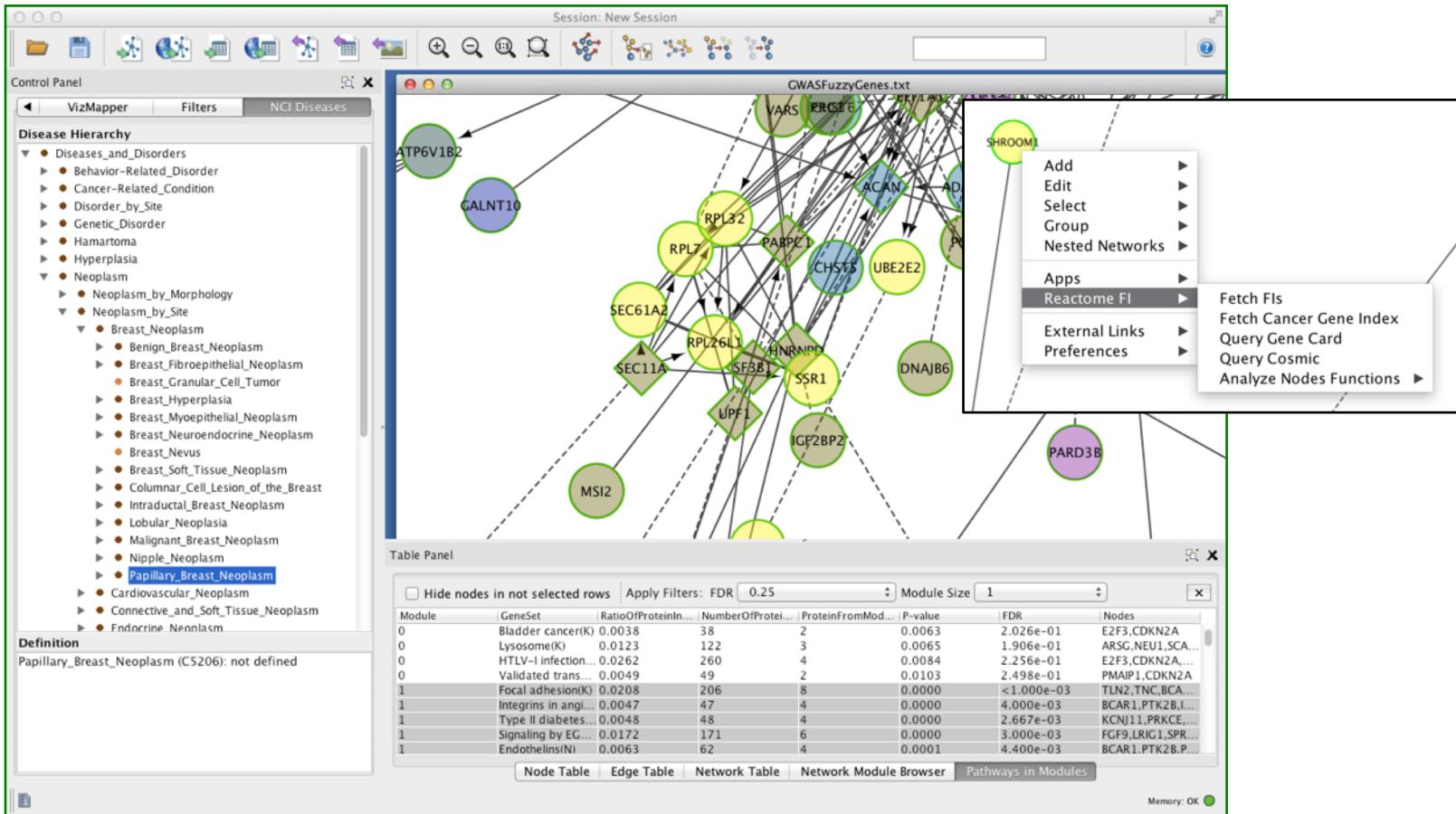
---

**Cancer type:** plasma-cell leukemias (pcl)  
**Negation indicator:** no  
**Cellline indicator:** no  
**Status:** no\_fact  
**PubMedID:** [15543617](#)

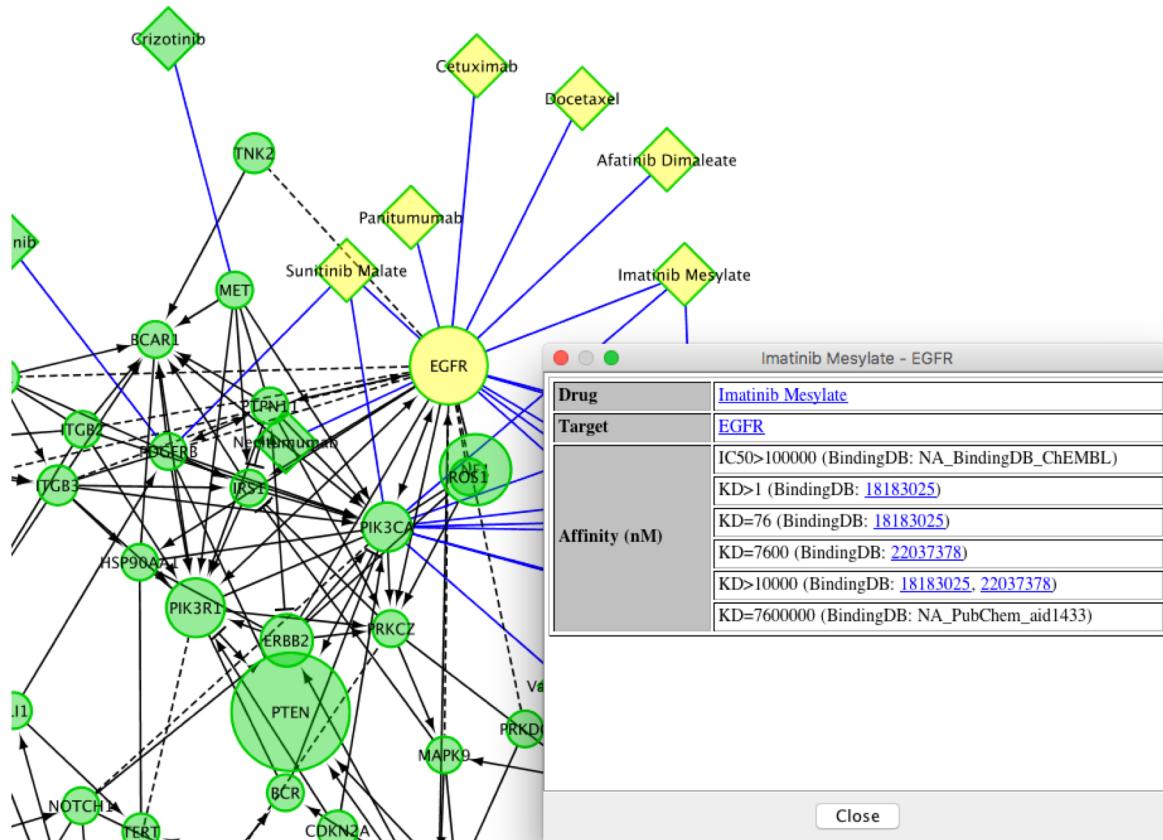
We investigated the expression profiles of the FGFR3/MMSET, CCND1, CCND3, MAF, and MAFB genes, which

# Overlay Cancer Gene Index

- Load the NCI disease terms hierarchy in the left panel.
- Select a disease term in the tree to select all nodes that have this annotation or one of its sub-terms.



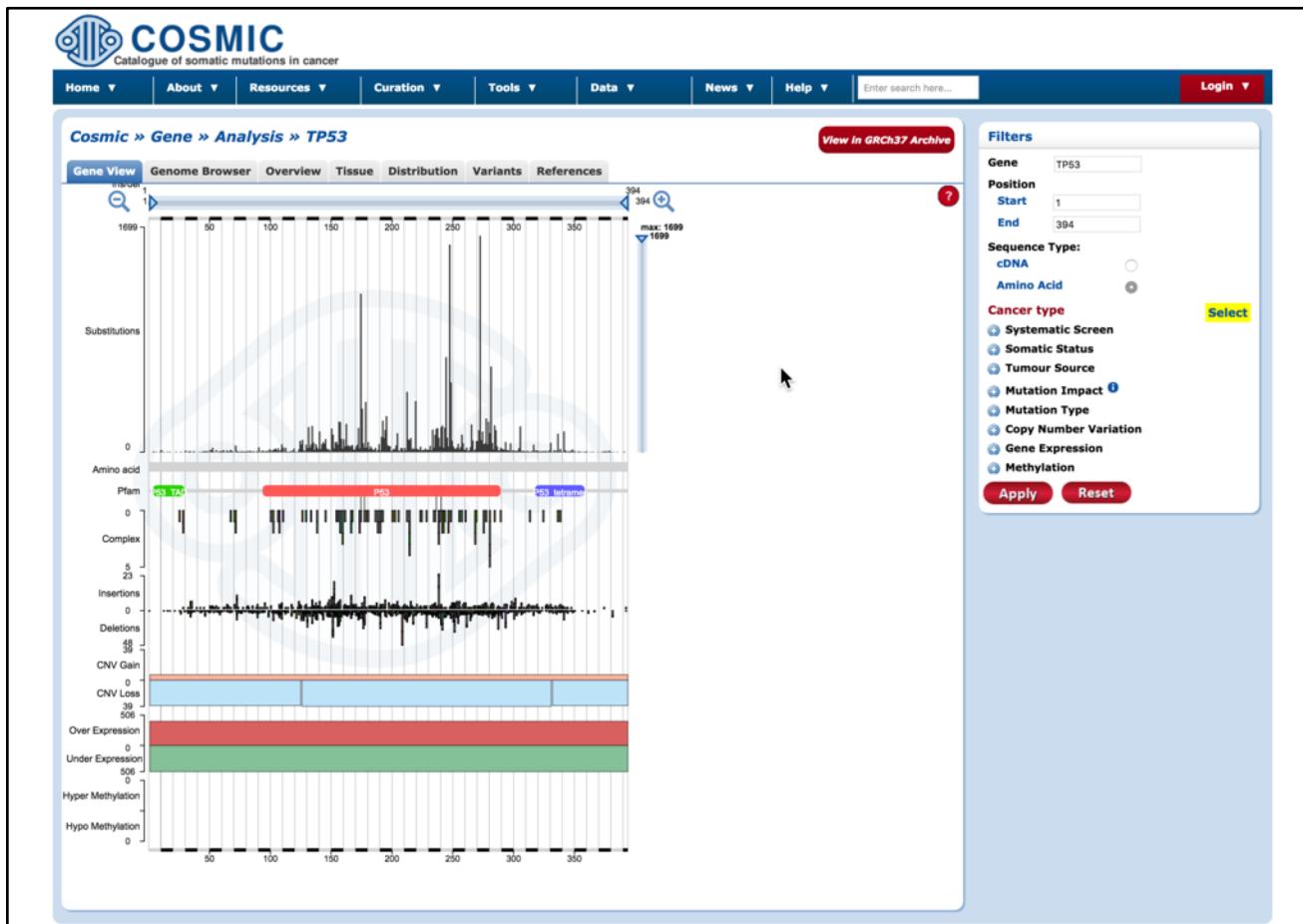
# Visualize Cancer Targetome in the Reactome FI Network



TCGA GBM Mutation Profile

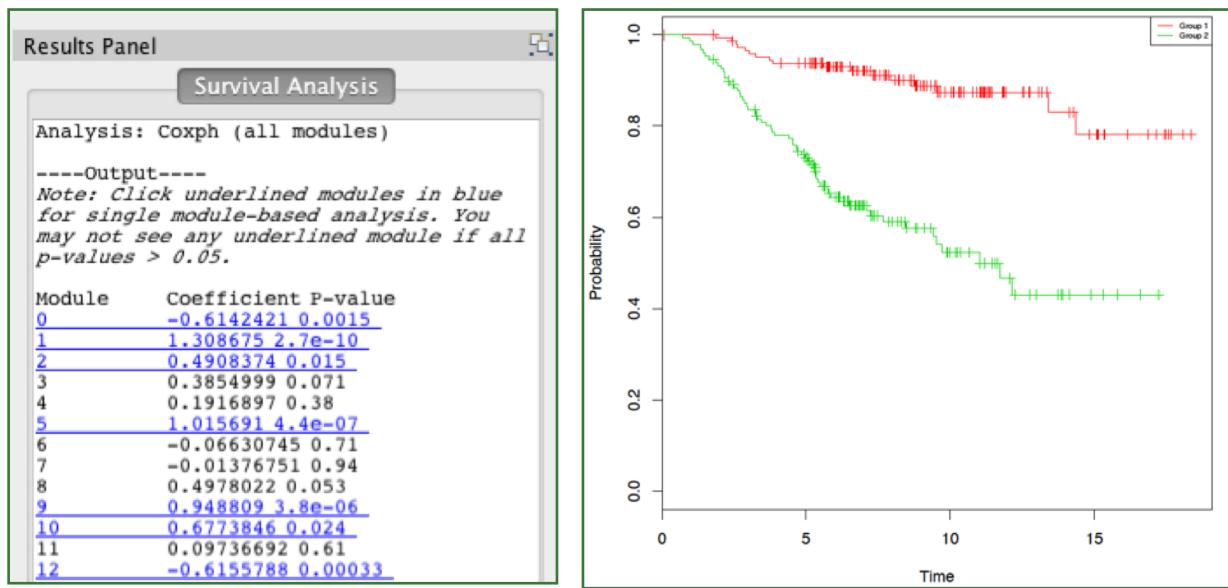
# COSMIC

- View detailed variant annotations for the selected gene or protein from COSMIC database.



# Module Based Survival Analysis

- Discover Prognostic Signatures in Disease Module Datasets.
- Based on a server-side R script that runs either CoxPH or Kaplan-Meyer survival analysis.
- Requires appropriate clinical data file.



# Demos

REACTOMEFIVIZ APP

# We are on a Coffee Break & Networking Session

Workshop Sponsors:

---



Canadian Centre for  
Computational  
Genomics



HPC4Health

