



# Dataset analysis using QIAGEN IPA

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1. Data Upload and How to Run a Core Analysis  
Upload experiment data
2. Functional Interpretation in IPA  
Introduction for Analysis Tools
3. Comparison Analyses
4. Q&A

- ID 、 Observation
- Annotated Dataset

- Create Core Analysis
  - Expression type
  - Measurement type
- Create Expression Analysis
  - Criteria
  - Cutoffs

- Summary
- Diseases/Disorders
- Canonical Pathways
- Upstream regulators
- Interaction Network

Dataset Preparation



Analyze Dataset



Expression Analysis

**IDs (required)**

**Ratio, fold change, etc. (recommended)**

**Significance (optional)**

	A	B	C	D	E
	geneid	UCvsNormal.Log2FoldChange	UCvsNormal.pval	52wksVedolizumabvsBaseline.Log2FoldChange	52wksVedolizumabvsBaseline.pval
1	DDX11L1	-0.1067	0.2878	0.1183	0.1624
2	WASH7P	-0.1883	0.0097	0.3063	0.0006
3	FAM138F	-0.0761	0.4699	0.2466	0.0191
4	OR4F5	0.1474	0.5311	0.1713	0.2913
5	LOC729737	0.4789	0.0017	0.029	0.8331
6	LOC100133331	0.4789	0.0017	0.029	0.8331
7	LOC100132062	0.4789	0.0017	0.029	0.8331
8	OR4F29	0.2495	0.2389	0.2181	0.1887
9	JA429831	0.1215	0.3338	0.2556	0.0004

Observation 1      Observation 2

## Common identifier types

- Arrays from Affymetrix, Illumina, etc.
- Gene symbols (Entrez or HUGO)
- Ensembl, RefSeq, UCSC, etc.

## Accepted file formats

- .txt (tab-delimited text files)
- .xls, .xlsx, .csv (Excel tables)
- .diff (Cuffdiff output)

IDs are the only **required** column

**Change measurements** are needed for IPA to make activity predictions

IDs (required)

Ratio, fold change, etc. (recommended)

Significance (optional)

	A	B	C	D
	Proteins	Fold change	P_value	P_value_adjust
1				
2	P00738	0.592740341	0.000671209	0.016736513
3	P01008	0.25826353	0.000155027	0.006454004
4	P01011	0.47378079	0.000628734	0.016577608
5	P04003	0.312321917	2.2507E-05	0.001618456
6	P06681	0.272046102	0.001374078	0.027869114
7	P05155	0.429462469	4.19294E-05	0.002551241
8	P02748	0.580232999	0.002252137	0.038734209
9	P02763	0.555940063	0.00014192	0.006236575
10	Q14520	0.368464274	9.75518E-05	0.004786156
11	Q08380	0.536007179	0.000258392	0.009290371
12	Q9BXR6	0.332814513	0.00075662	0.01813594
13	P03951	0.306633696	0.000594476	0.016236342
14	P08185	0.304349939	1.12204E-05	0.000914984
15	P05090	0.302847519	0.000817844	0.018730825

Observation 1

## Common protein IDs

- Ensembl
- Gene symbols (Entrez or HUGO)
- GenPept and GenBank
- International Protein Index
- UniProt and SwissProt

## UniProt ID conversion tool:

- <https://www.uniprot.org/mapping/>

Phosphorylation changes (ratio, fold change, etc.) and sites are supported, but these columns **must be assigned**



Multiple ID columns

Ratio, fold change, etc. (recommended)

(optional)

Significance (optional)

	A	B	C	D	E	F	G	H
	Pubchem	Kegg	HMDB	CAS	Metabolites	Fold change	P_value	P_value_adjust
1								
2					(2 or 3)-decenoate (10:1n7 or n8)	1.212936133	4.44028E-05	0.000585189
3	6443013	C14762	HMDB0004667	29623-28-7	13-HODE + 9-HODE	0.584109411	0.003698077	0.016919182
4	10111	C02294	HMDB01522	471-29-4	1-methylguanidine	1.219937764	0.015399637	0.049446834
5	5462190	C15606	HMDB0012134	746507-19-7	2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)*	1.566518315	0.002802172	0.013670263
6	80283	C02356	HMDB00452	1492-24-6	2-aminobutyrate	0.633800292	0.011016709	0.038805594
7	10796774		HMDB00317	488-15-3	2-hydroxy-3-methylvalerate	0.997343835	0.006172648	0.024774766
8	11427		HMDB37115	120-91-2	2-hydroxy-4-(methylthio)butanoic acid	1.294720456	0.000305912	0.002622524

Observation 1

## Common metabolite IDs

- CAS registry number
- Human Metabolome Database
- KEGG
- PubChem CID

## Metabolite ID conversion tools:

- <https://biobnet-abcc.ncifcrf.gov/db/db2db.php>
- <https://cts.fiehnlab.ucdavis.edu/batch>
- <http://csbg.cnb.csic.es/mbrole2/conversion.php>

		Observation 1		Observation 2	
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## Analyte identifier REQUIRED to explore enrichment

RNA examples: Gene symbols, array identifiers from Affymetrix, Ensembl, etc.

Protein examples: UniProt, GenPept, Gene symbols, Ensembl. etc.

Metabolite examples: KEGG, CAS registry number, etc. *\*add multiple columns of ids to ensure best mapping*

## Change values needed to calculate activity predictions

Change value examples: fold changes, ratios, etc.

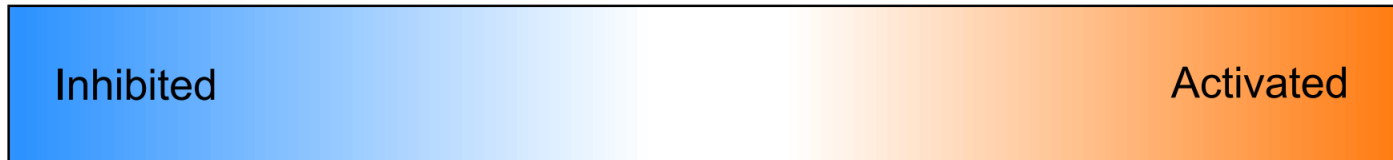
Significance values: P-values *\*optional but recommended to enable filtering for significance*

### Accepted file formats:

- ✓ .txt (tab-delimited text files)
- ✓ .xls, .xlsx, .csv (Excel tables)
- ✓ .diff (Cuffdiff output)

**Multiple comparisons or observations may be uploaded in one file**

## Pathway or gene activity predicted by IPA

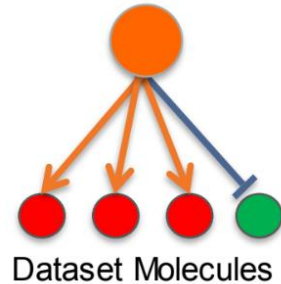


## Actual measurement of gene expression in your dataset

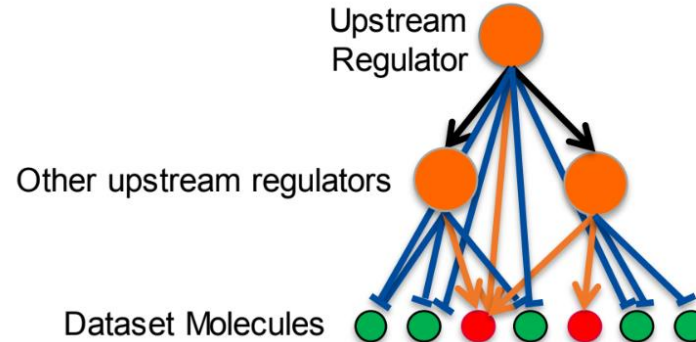




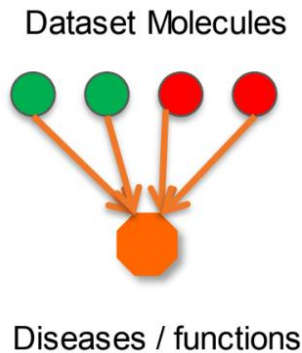
## Upstream Analysis



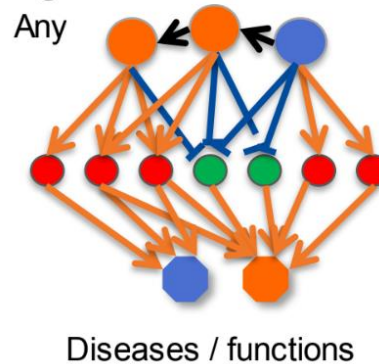
## Mechanistic Network of Upstream Regulators



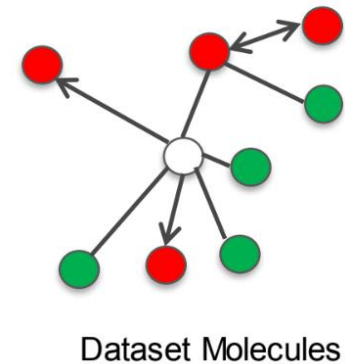
## Function Analysis



## Regulator Effect Network



## Interaction Network

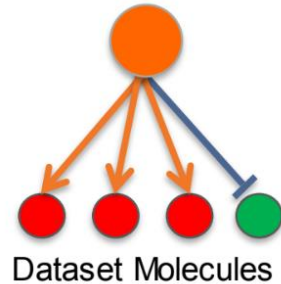


# Live Demo

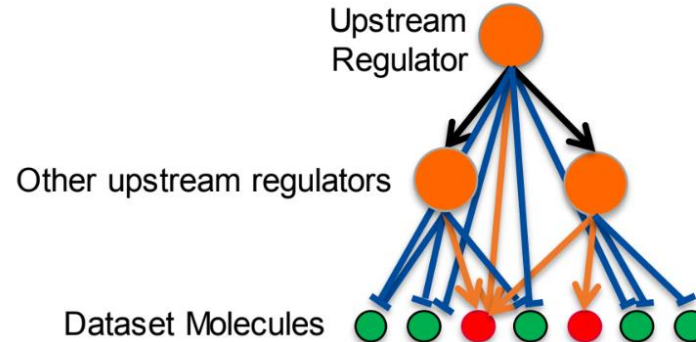
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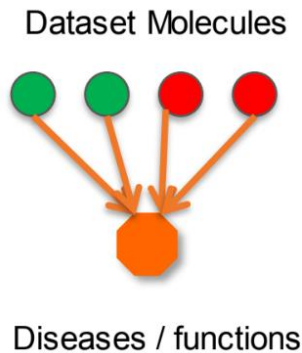
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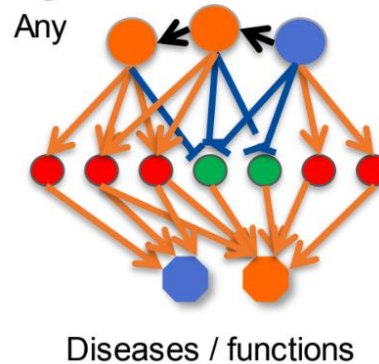
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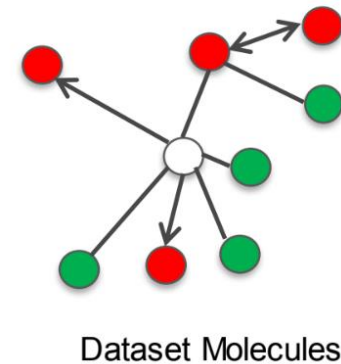
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## Interaction Network



**Graphical Summary:** This feature selects and connects a subset of the most significant entities predicted in the analysis, including the relationship between molecules, functions, diseases and pathways.




**Pathways:** List the Signaling Pathway and Metabolic Pathway affected by the experiment

**Upstream Analysis:** List the upstream molecules related to the changed molecules in the data, and predict whether they are activated or inhibited according to the research literature.

**Diseases & Function:** Present biological functions, diseases and toxicological results affected by molecular changes

**Regulator Effects:** Hypothesize the effects of activation or inhibition of upstream regulators on downstream molecules

**Networks:** Present the network relationship between molecules in the experimental data. And the Build Tool and Overlay Tool can be used to extend and expand knowledge. The above analysis results are important basis for explaining the phenomena observed in the experiment.

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v Top Canonical Pathways				
Name		p-value	Overlap	
EIF2 Signaling	—	4.75E-36	32.1 %	72/224
Regulation of eIF4 and p70S6K Signaling	—	5.64E-16	23.5 %	42/179
ILK Signaling	—	9.78E-16	22.2 %	44/198

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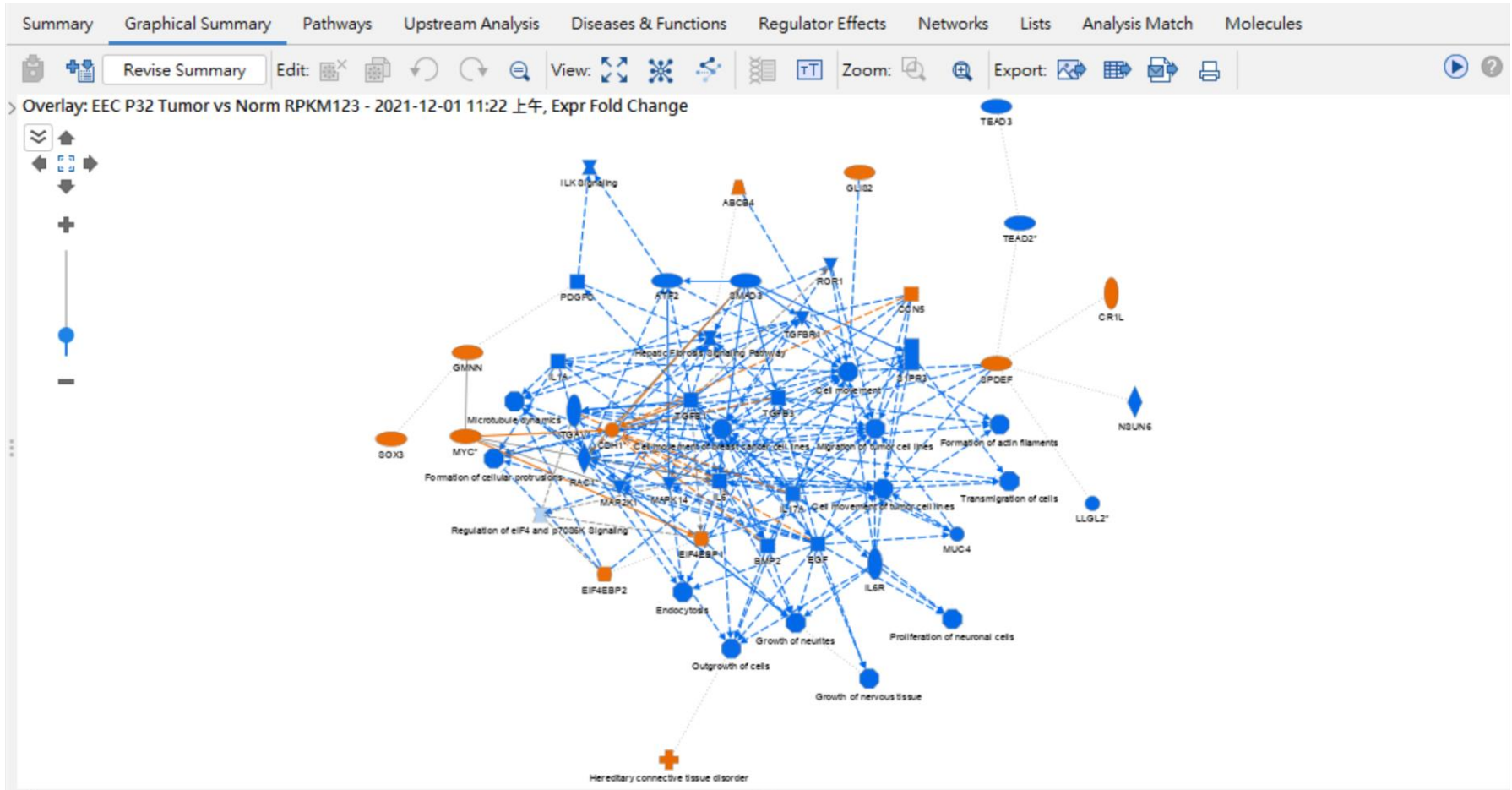
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The Graphical Summary can include entities such as canonical pathways, upstream regulators, diseases, and biological functions. The algorithm that constructs the summary uses machine learning techniques to prioritize and connect entities that are in some cases not yet connected by findings in the QIAGEN Knowledge Graph.



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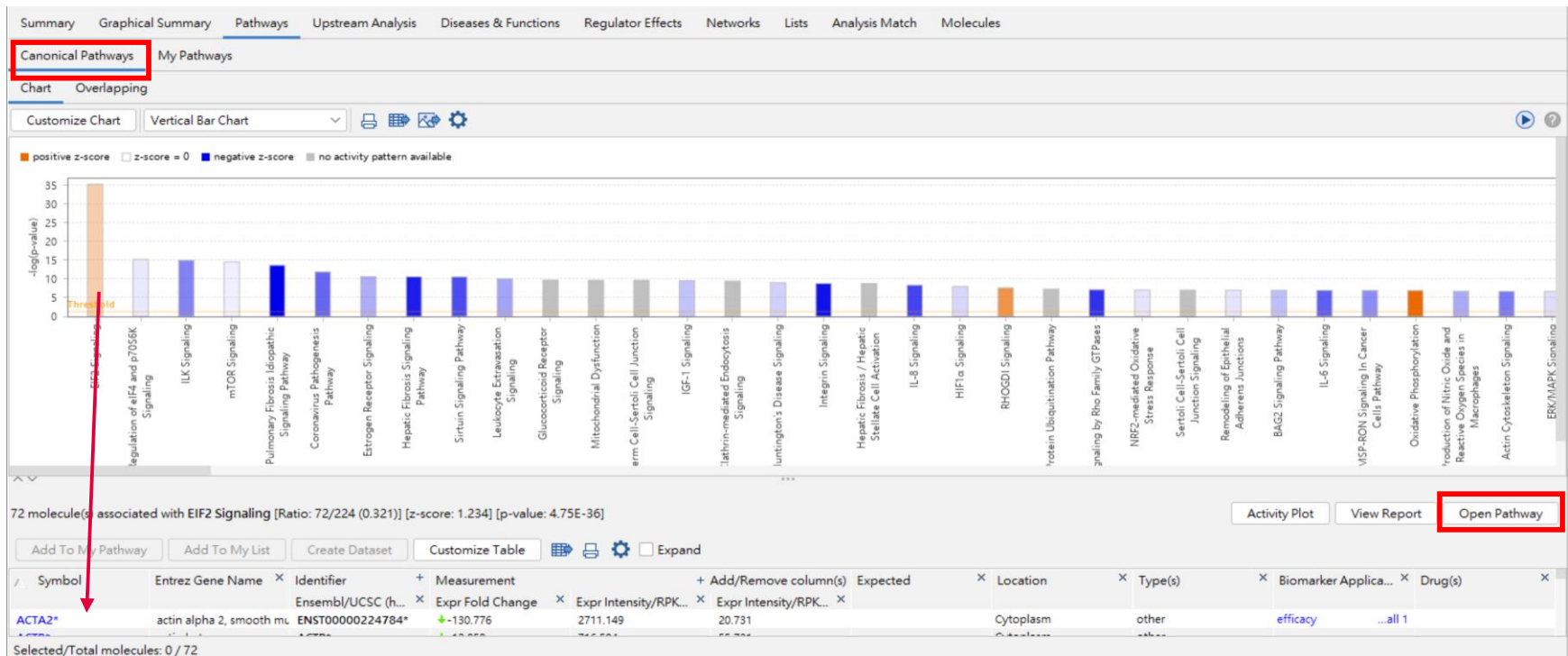
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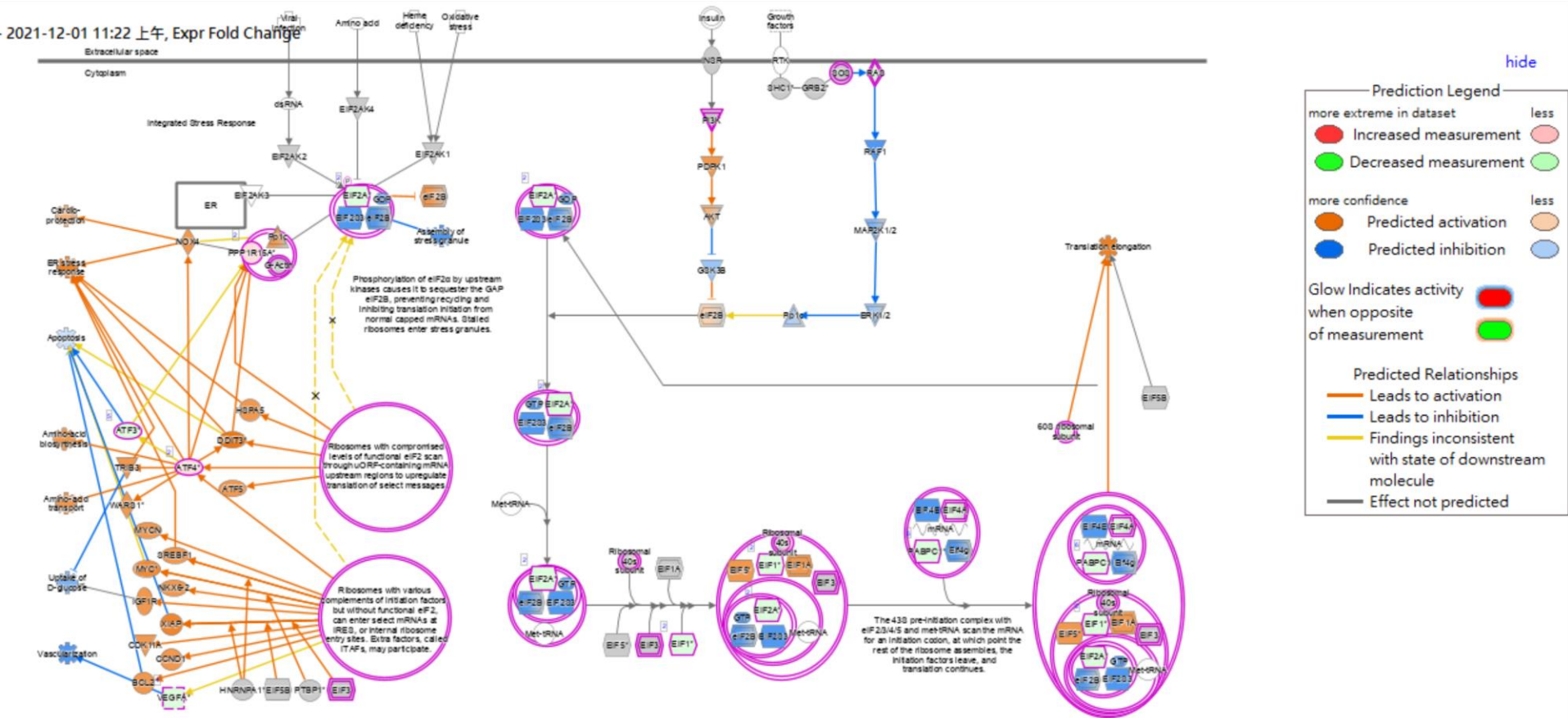
- The affected Signaling Pathway and Metabolic Pathway are arranged in a bar chart according to their significance
- Click the Bar above the name of a specific Canonical Pathway, and the lower window will display the molecular IDs that participate in the composition of the pathway in the dataset
- Click "Open Pathway" to expand the Canonical Pathway, and the molecules in the experimental data will be indicated by colors.








- Click "Open Pathway" to expand the Canonical Pathway, and the molecules in the experimental data will be indicated by colors.



Click on the **View Report** button to display the Canonical Pathway report.



## Canonical Pathway

**Report Date:** 2022-10-18  
**Report Version:**  
**Content Version:** 81348237 (Release Date: 2022-09-15)

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**Canonical Pathway:** **Tumor Microenvironment Pathway**

**Description:** The tumor microenvironment (TME) comprises cancer cells, the cytokine environment, extracellular matrix, immune cell subsets and other components. In this complex network, the pro-tumorigenic immune response, mediated by diverse immunosuppressive cell signaling molecules, plays a pivotal role in driving immune evasion. The tumor not only manages to escape from the host immune system, but it effectively benefits from infiltrating cells by modifying their functions to create the microenvironment favorable for tumor progression.[31500650](#)

The majority of stromal cells within the TME are specific fibroblasts with a myofibroblastic phenotype and are distinguished as cancer-associated fibroblasts (CAFs). These CAFs have a significant impact on cancer progression through remodeling the ECM, inducing angiogenesis, recruiting inflammatory cells, and directly stimulating cancer cell proliferation via the secretion of growth factors, intermediate metabolites and immune suppressive cytokines.[28382138](#)

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells, which are expanded in pathological conditions and up-regulate expression of immune suppressive factors, such as arginase and inducible nitric oxide synthase (NOS2), thus reducing lymphocyte functions. MDSCs also show high expression of indoleamine 2,3-dioxygenase (IDO), an enzyme responsible for the catabolism of tryptophan, which leads to inhibition of T cell proliferation and induces T cell apoptosis. Local hypoxia has been identified as another key regulator that can promote MDSC accumulation.[31430935](#)

The cancer cell-derived cytokines, such as IL-4, IL-13, CSF1, CCL2, induce tumor-associated macrophage (TAM) differentiation. These secrete multiple key proinflammatory cytokines (e.g. IL-1β, IL-6, and TNF-α) which stimulate tumor progression and in parallel inhibit lymphocyte functions through the secretion of IL-10, and also contribute to the expansion of Th17 cells, which induce local inflammation. TAMs also produce vascular endothelial growth factor (VEGF), which stimulate tumor angiogenesis, promoting its invasiveness and metastatic potential.[31500650](#)

Tumor-associated neutrophils (TANs) are associated with aggressive cancer phenotypes, facilitate angiogenesis, promote mutagenesis and suppress the immune system. The migration of TANs from the blood circulation into the TME is stimulated by tumor-derived CXCL8. TANs mainly suppress anti-tumor immunity via interacting with CD8+ T cells, inducing their apoptosis through nitric oxide production.[31430935](#)

**Signaling Pathway Categories:** Cancer

**Top Functions & Diseases:** Cell-To-Cell Signaling and Interaction; Cellular Growth and Proliferation; Lymphoid Tissue Structure and Development

**Molecules:** [show all](#) Accumulation of ATP, Accumulation of myeloid-derived suppressor cells, Adaptive immune response of CD8+ T lymphocyte, Akt, Angiogenesis, Ap1, Apoptosis of CD8+ T lymphocyte, Apoptosis of cytotoxic T cells, Apoptosis of Th1 cells, Apoptosis of tumor cells, ARG1, BAD, BCL2, CCL2, G2ND1, CD274, CD44, Cell viability of tumor cells, GFLAR, collagen type I (family), CSF1, CSF2, CSF3, CSPG4, CTLA4, CXCL12, CXCL8, CXCR4, D-glucose, Development of regulatory T lymphocytes, Differentiation of M2 macrophages, Differentiation of myeloid-derived suppressor cells, Differentiation of Th17 cells, EGF, Epithelial-mesenchymal transition, ERK1/2, FAS, FASLG, fatty acid, Fgf, FN1, Foxo, glutamine, glutathione, HGF, HIF1A, Hypoxia of tumor, ICAM1, IDO, Igf, IL10

[Back to top >>](#)

**Drug Summary** - Overview of drugs targeting molecules in Canonical Pathway

Showing 3 of 1028 row(s) of Drug data. [\(Show All\)](#)

Drug Name	Targets	Actions	Brand Names	Indications/Status
(-)-gossypol	BCL2	inhibitor		adrenal cortex carcinoma/Phase 2 adult Burkitt lymphoma/Phase 1 adult diffuse large-cell lymphoma/Phase 1
1-(3-(1,4-dihydroimidazo[4,5-c]pyrazol-5-yl)-4-methylphenyl)-3-(3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl)urea	RAF1	inhibitor		
1311-chlorotoxin	MMP2	binder		astrocytoma/Phase 1/Phase 2 brain tumor/Phase 1/Phase 2 glioblastoma/Phase 1/Phase 2

[Back to top >>](#)

**Target Information** - Overview of known drug targets in Canonical Pathway

Showing 3 of 112 row(s) of Target data. [\(Show All\)](#)

Target (Gene Symbol)	Entrez Gene Name	Location	Type	Drug(s)	Species
Akt		Cytoplasm	group	afuresertib, Akt inhibitor XI, AT13148, HTBPI, ipatasertib, MSC2363318A, ONC-201, SR-13668, TAS-117, TAS0612	Human, Mouse, Rat
AKT1	AKT serine/threonine kinase 1	Cytoplasm	kinase	A-443654, AKT inhibitor XIII, archexin, BAY1125976, capivasertib, CCT129524, enzastaurin, GSK690693, ipatasertib, LY2780301, miransertib, MK2206, MPT0E028, perifosine, tricinibine, tricinibine phosphate, uposertib, vevorisertib	Human, Mouse, Rat
AKT2	AKT serine/threonine	Cytoplasm	kinase	AKT inhibitor XIII, BAY1125976, CCT128930, CCT129524, enzastaurin, GSK690693, tricinibine, tricinibine phosphate	Human, Mouse, Rat



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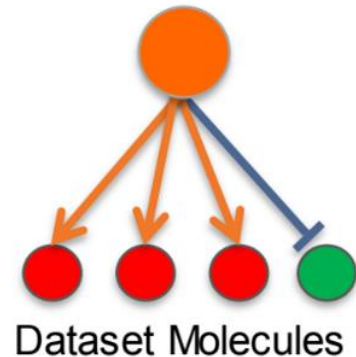
Use experimentally observed relationships (vs. Predicted event) between Upstream Regulators and genes to predict potential regulator and activation.

Predict activation or inhibition of regulator to explain the changes in gene expression in your dataset.

Calculates two complementary statistical measures:

- Activation z-score
- Overlap p-value

## Upstream Analysis



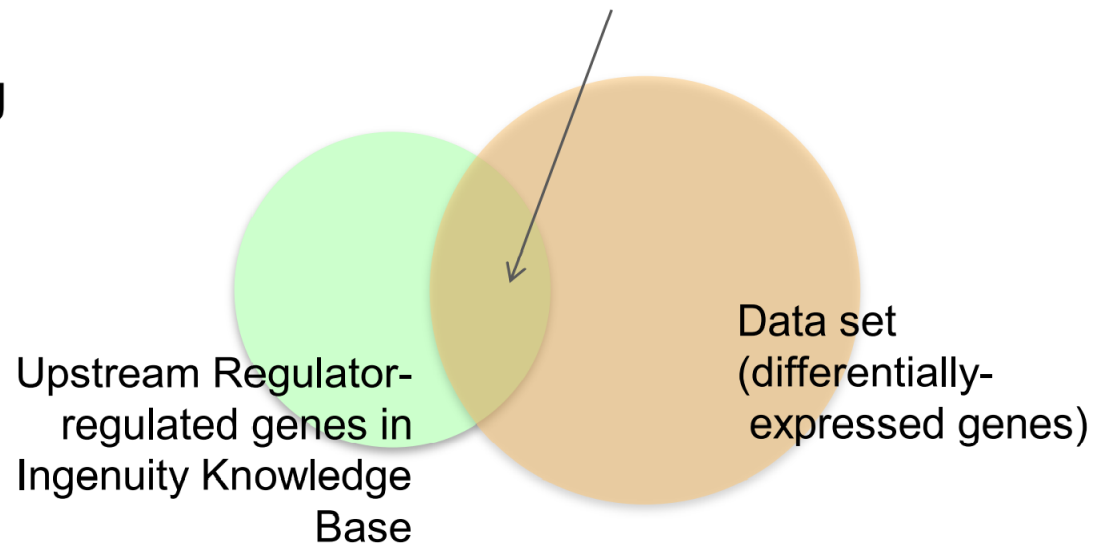
Can we predict the activation state (activated/inhibited) of a potential regulator from expression data?

Approach: Two complementary statistical measures:  
Activation z-score and Overlap p-value

TR → target edge types considered:

- Expression
- Transcription
- Protein-DNA binding

Evaluate the perturbed genes in the dataset that are known targets of a particular regulator



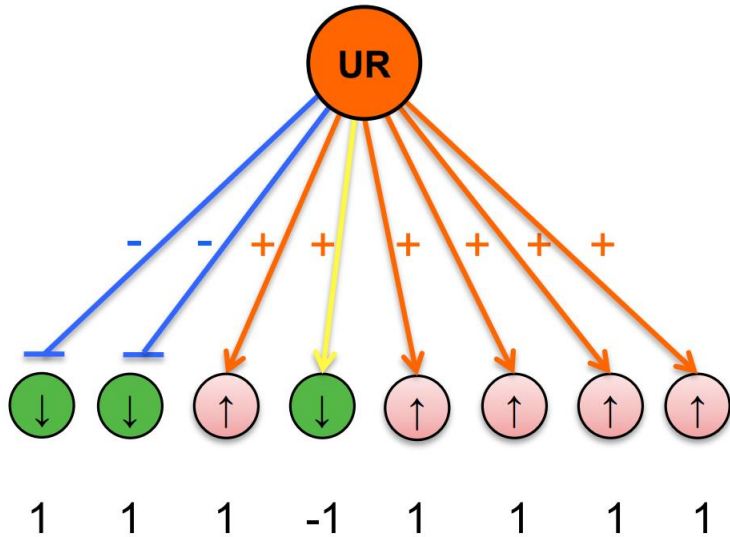
## p-value of overlap

- Null hypothesis: No overlap between molecules from dataset and disease/function/upstream regulator/pathway.
- Calculate using the right-tailed Fisher's Exact Test.
- Significant p-value  $\leq 0.05$

Note: Benjamini-Hochberg correction for multiple testing can be implemented in some cases

## Z-score

- Predicts Activation or Inhibition
- Correlation between what is known (IPA Knowledge Base) and your expression data



← Every possible TF & Upstream Regulator in the Ingenuity Knowledge Base is analyzed

← Literature-based effect TF/UR has on downstream genes

← Differential Gene Expression (Uploaded Data)

← Predicted activation state of TF/UR:  
1 = Consistent with activation of UR  
-1 = Consistent with inhibition of UR

$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}} = (7-1)/\sqrt{8} = 2.12 (= \text{predicted activation})$$

- z-score is a statistical measure of the match between expected relationship direction and observed gene expression
- z-score > 2 or < -2 is considered significant

Note that the actual z-score is weighted by the underlying findings, the relationship bias, and dataset bias

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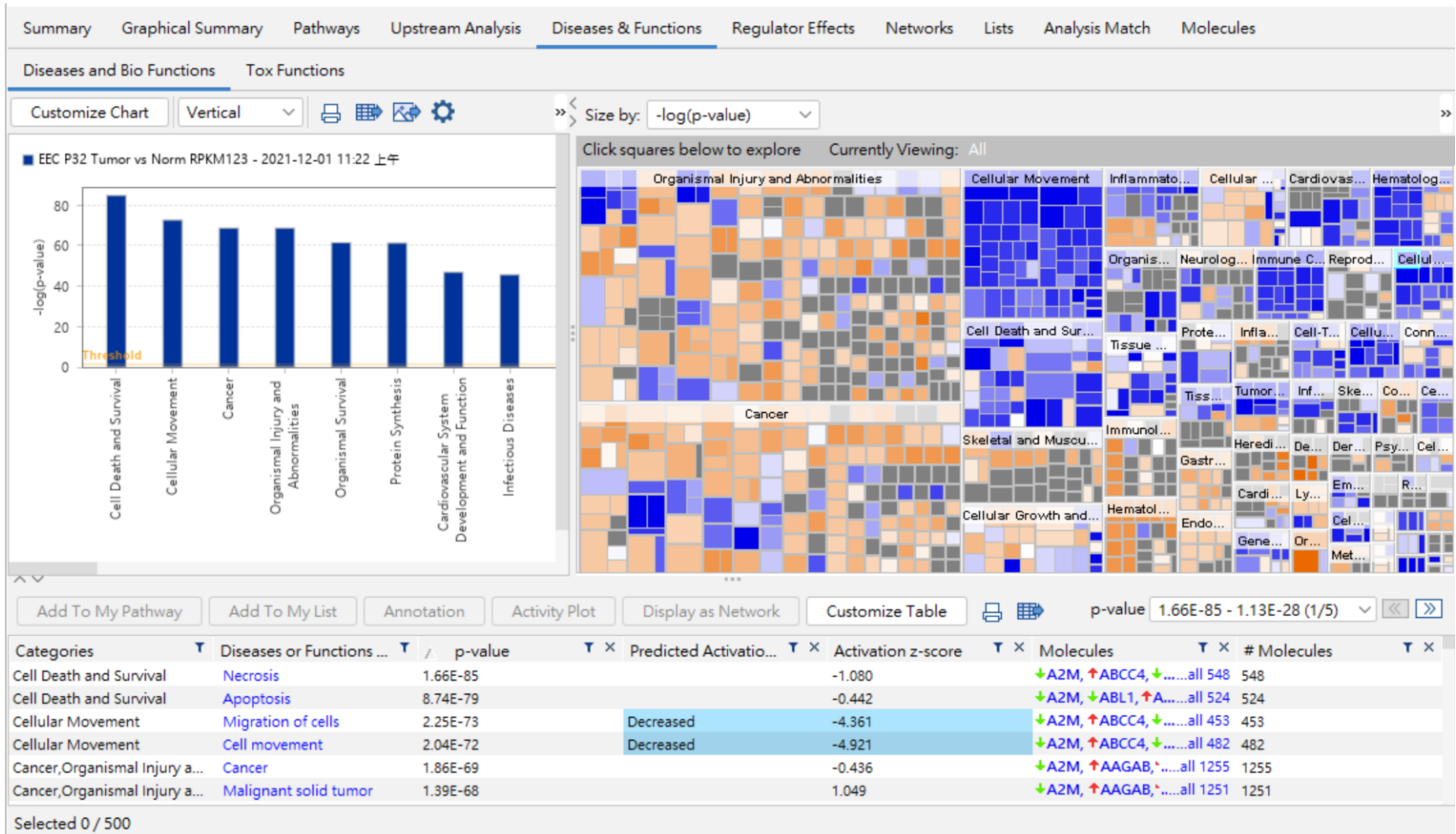
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Identify over-represented biological functions and predict how those functions are increased or decreased in the experiment

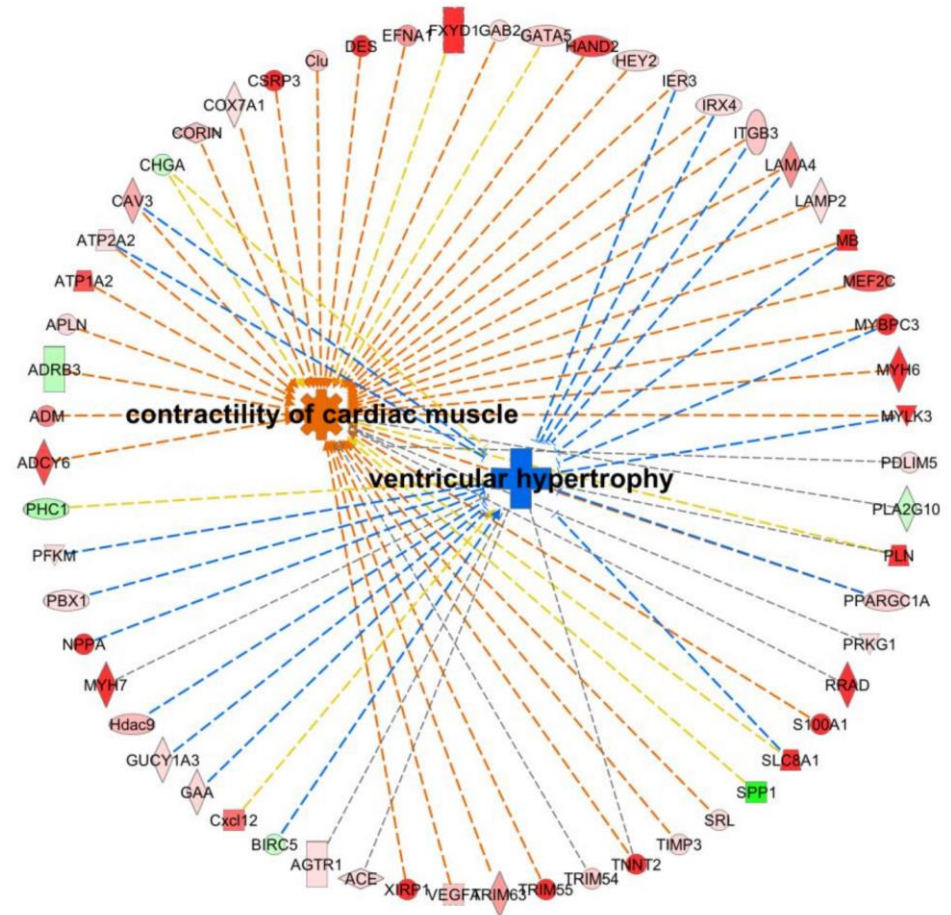
方塊代表受實驗影響的生物功能與疾病，顏色可以用[Color by]指定是z-score,  $-\log(p\text{-value})$ , 或是 # of genes 上色。如果是用z-score上色的話，藍色區塊是預測被減低的功能，橘色則是此功能會增加。是根據實驗資料做出的演算。

個矩形可以經由點擊進入下一層分區：  
Mid-level functional category (level 2)  
與 Specific functions (level 3)

Click to show bar chart



- Powerful functionality enables you to understand causal connections between molecules and diseases.
- Interactive visual exploration of causality between molecules and disease, function, or phenotypes from a network or My Pathway.



provides details associated with the disease or biological function such as molecules associated with that disease or function, known drug targets, drugs known to target those molecules, and more.

## Ingenuity Pathway Analysis



## Disease or Function

[Provide Feedback](#) | [Contact Support](#)

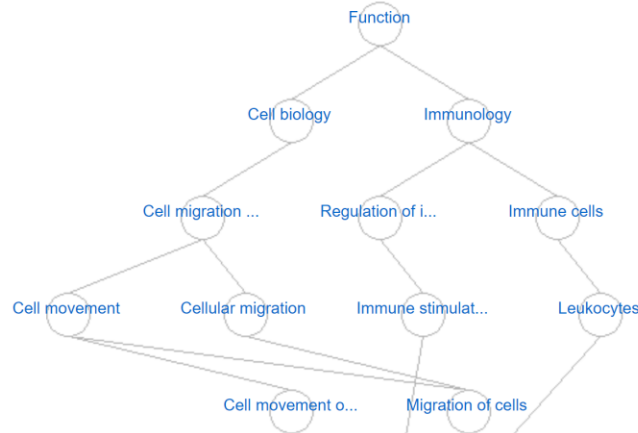
### Disease or Function: Cell movement of leukocytes

**Synonyms:** (Show All) immune cell movement,innate immune cell movement,leukocyte movement,white blood cell movement,cell movement of immune cell,cell movement of immune cells,cell movement of immunological cells,cell movement of innate immune cell,cell movement of innate immune cells,cell movement of leucocytes,cell movement of leukocyte,cell movement of white blood cell,cell movement of white blood cells,movement of leukocytes,movement of immune cell,movement of immune cells,movement of immunological cells,movement of innate immune cell,movement of innate immune cells,movement of leukocytes

**Molecules:** (show all) (+)-butaclamol, (2R\*,4S\*)-N-(1-benzoyl-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)-N-phenylcyclopropanecarboxamide, (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid, methyl ester, (Z,E)-5-(4-ethylbenzylidene)-2-thioxothiazolidin-4-one, (±)-4-DOI, 1,1-diethyl-2-hydroxy-2-nitrosodiazine, 1,10-phenanthroline, 1,2-dimethylhydrazine, 1-(palmitoyl)-2-(5-keto-6-octene-dioyl) phosphatidylcholine, 1-chloro-2,4-dinitrobenzene, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1-naphthylisothiocyanate, 1-o-hexadecyl-2-o-methyl-rac-glycerol, 1-palmitoyl-2-oleoylphosphatidylserine, 10-Cl-BBQ, 11,12-epoxyeicosatrienoic acid, 11-dehydro-thromboxane B2, 12 lipoxigenase, 12(S)-hydroxyeicosatetraenoic acid, 12-hydroxy-17,18-epoxyeicosatetraenoic acid, 12-hydroxyeicosapentaenoic acid, 12S-hydroxy-17R,18S-epoxyeicosatetraenoic acid, 12S-hydroxy-17S,18R-epoxyeicosatetraenoic acid, 13,14-dihydro-15-ketoprostaglandin D2, 15-deoxy-delta-12,14 -PGJ 2, 15-epi-lipoxin A4, 15-hydroxyeicosatetraenoic acid, 15-LOX, 15S-methyl-prostaglandin d2, 16,16-dimethylprostaglandin E2, 17-epi-resolvin D1, 17-octadecynoic acid, 2'-fucosyllactose, 2'3'-cyclic guanosine monophosphate-adenosine monophosphate, 2,4-dinitrofluorobenzene, 2,4-O-di-sulfated iduronic acid, 2-(4-acetoxypheyl)-2-chloro-N-methylethylamine, 2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole, 2-arachidonoylglycerol, 2-mercaptoacetate, 2610528A11Rik, 27-hydroxycholesterol, 3'-O-(4-benzoyl)benzoyladenine 5'-triphosphate, 3,4-dichloroisocoumarin, 3-aminobenzamide, 3-deoxy-2-octulosonic acid(2)-lipid A, 3-hydroxydodecanoic acid, 3M-052, 4-hydroxynonenal, 4-methoxydiphenylmethane, 4-methyl umbelliferone-8-carbaldehyde

[Back to top >>](#)

### Ontology



**Graphical Summary:** This feature selects and connects a subset of the most significant entities predicted in the analysis, including the relationship between molecules, functions, diseases and pathways.




**Pathways:** List the Signaling Pathway and Metabolic Pathway affected by the experiment

**Upstream Analysis:** List the upstream molecules related to the changed molecules in the data, and predict whether they are activated or inhibited according to the research literature.

**Diseases & Function:** Present biological functions, diseases and toxicological results affected by molecular changes

**Regulator Effects:** Hypothesize the effects of activation or inhibition of upstream regulators on downstream molecules

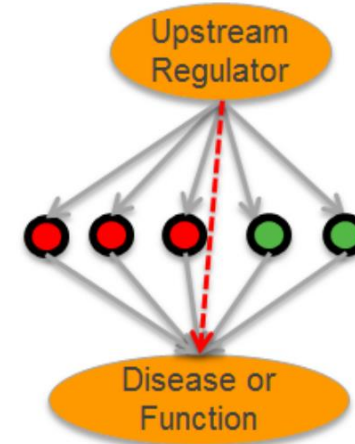
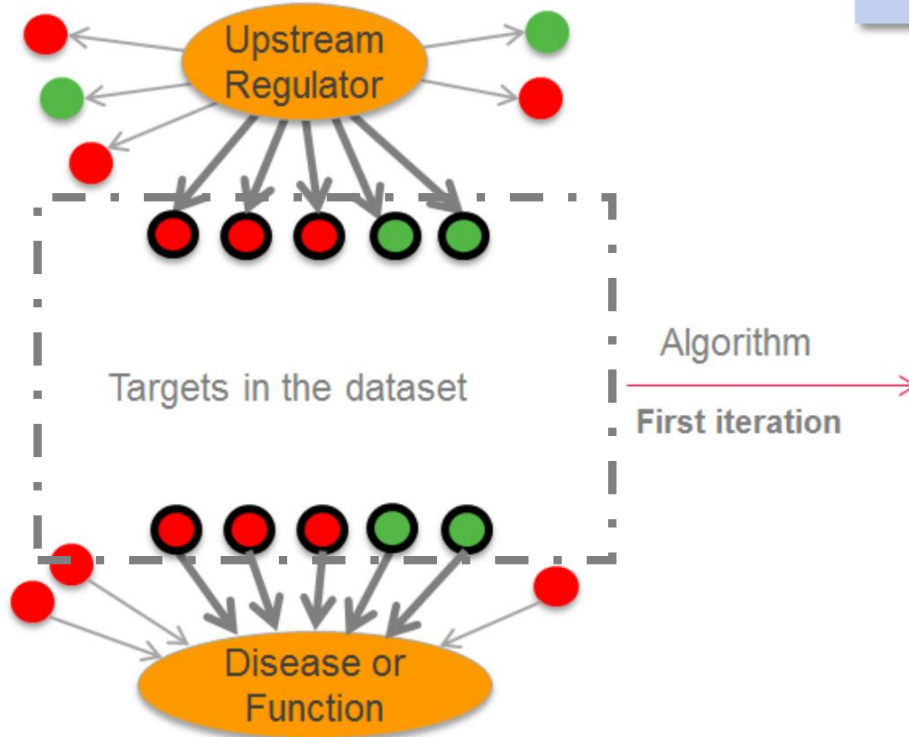
**Networks:** Present the network relationship between molecules in the experimental data. And the Build Tool and Overlay Tool can be used to extend and expand knowledge. The above analysis results are important basis for explaining the phenomena observed in the experiment.

Summary   Graphical Summary   Pathways   Upstream Analysis   Diseases & Functions   Regulator Effects   Networks   Lists   Analysis Match   Molecules			
Export :   			
> Experiment Metadata			
> Analysis Settings			
v Top Canonical Pathways			
Name		p-value	Overlap
EIF2 Signaling	—	4.75E-36	32.1 % 72/224
Regulation of eIF4 and p70S6K Signaling	—	5.64E-16	23.5 % 42/179
ILK Signaling	—	9.78E-16	22.2 % 44/198



## Upstream Regulator Analysis

Hypotheses for how activated or inhibited upstream regulators cause downstream effects on biology

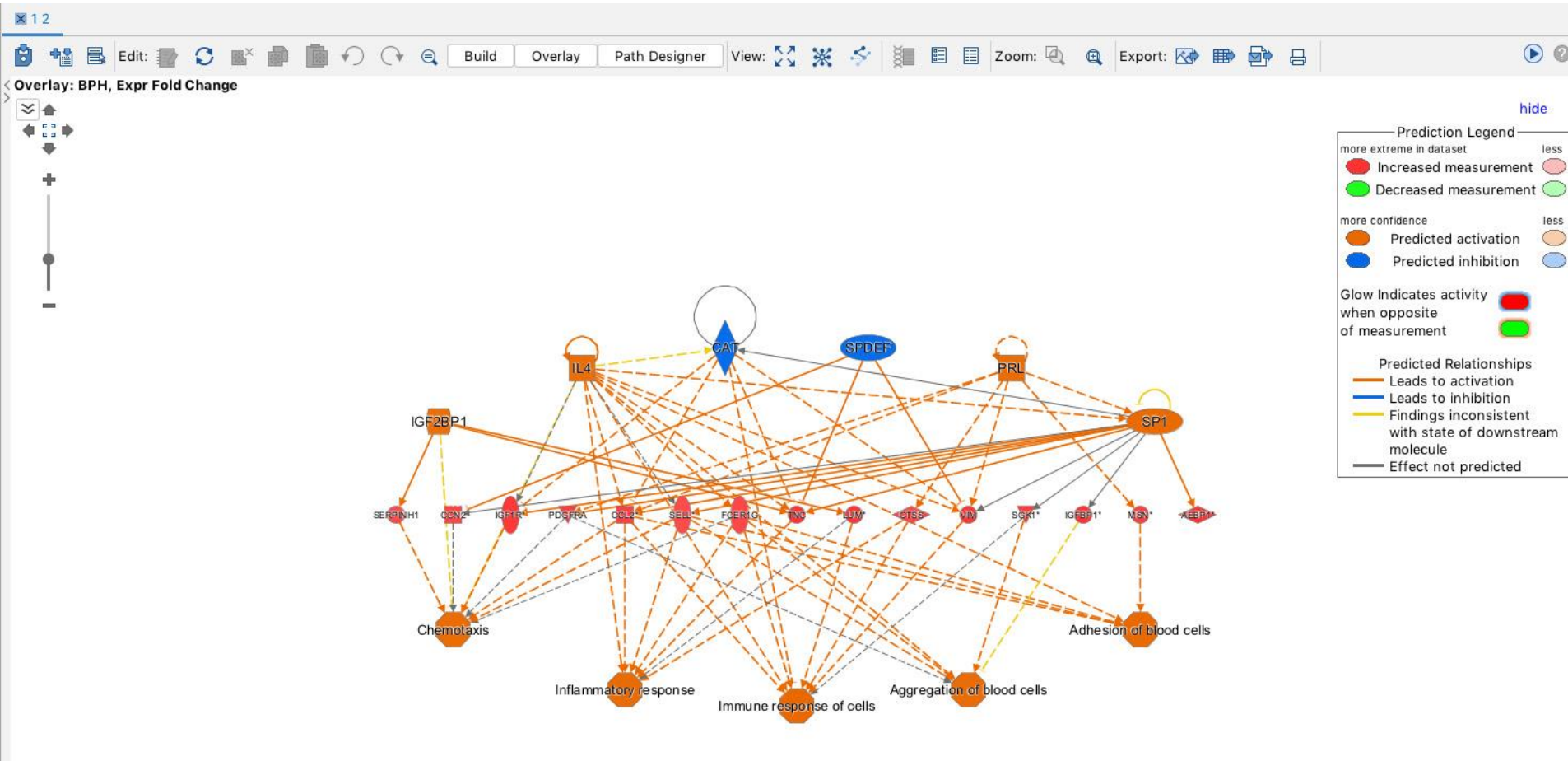


Displays a relationship between the regulator and disease/function if it exists

## Downstream Effects Analysis



Click on the Network ID or Display As Network button to open a Regulator Effects network.



**Graphical Summary:** This feature selects and connects a subset of the most significant entities predicted in the analysis, including the relationship between molecules, functions, diseases and pathways.




**Pathways:** List the Signaling Pathway and Metabolic Pathway affected by the experiment

**Upstream Analysis:** List the upstream molecules related to the changed molecules in the data, and predict whether they are activated or inhibited according to the research literature.

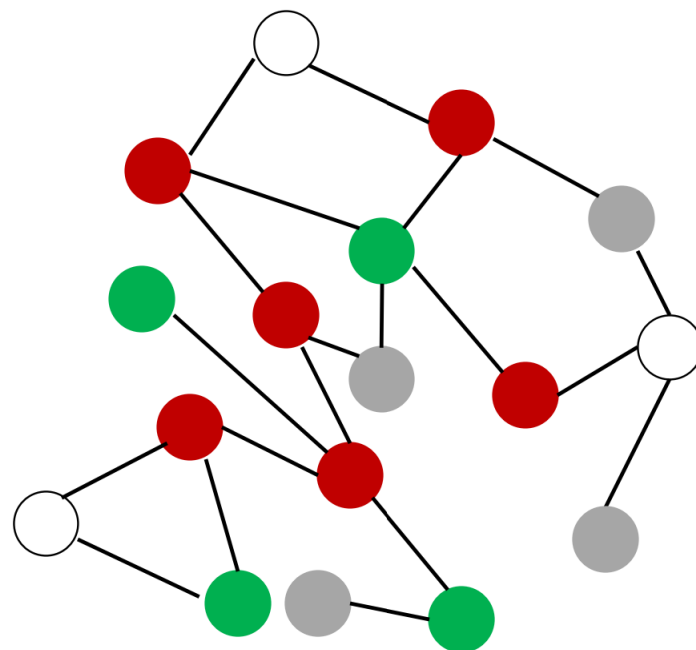
**Diseases & Function:** Present biological functions, diseases and toxicological results affected by molecular changes

**Regulator Effects:** Hypothesize the effects of activation or inhibition of upstream regulators on downstream molecules

**Networks:** Present the network relationship between molecules in the experimental data. And the Build Tool and Overlay Tool can be used to extend and expand knowledge. The above analysis results are important basis for explaining the phenomena observed in the experiment.

Summary   Graphical Summary   Pathways   Upstream Analysis   Diseases & Functions   Regulator Effects   Networks   Lists   Analysis Match   Molecules				
Export :   				
> Experiment Metadata				
> Analysis Settings				
▼ Top Canonical Pathways				
Name		p-value	Overlap	
EIF2 Signaling	—	4.75E-36	32.1 %	72/224
Regulation of eIF4 and p70S6K Signaling	—	5.64E-16	23.5 %	42/179
ILK Signaling	—	9.78E-16	22.2 %	44/198

1. Focus molecules are “seeds”
2. Focus molecules with the most interactions to other focus molecules are then connected together to form a network
3. Non-focus molecules from the dataset are then added
4. Molecules from the Ingenuity’s Knowledge Base are added
5. Resulting Networks are scored and then sorted based on the score



Molecules per Network	Networks per Analysis
35 ▼	25 ▼
35	10
70	25
140	50

## Purpose:

- To show as many interactions between user-specified molecules in a given dataset and how they might work together at the molecular level

## Why are Ingenuity networks biologically interesting?

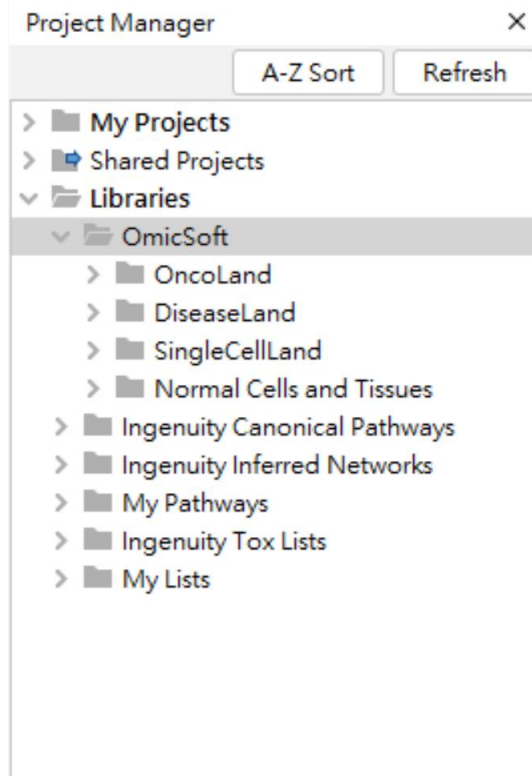
- Highly-interconnected networks are likely to represent significant biological function
- Networks involve molecules you don't see in your data set. This allows genes you have assayed to be linked to metabolites and chemicals that you could not have assayed for, to imply a regulation network that is meaningful.

Automatically discover other IPA Core Analyses with similar (or opposite) biological results as compared to yours, to help confirm your interpretation of the results or to provide unexpected insights into underlying shared biological mechanisms.

Expression Analysis - CDDO vs DMSO genes																
Summary Graphical Summary Pathways Upstream Analysis Diseases & Functions Regulator Effects Networks Lists Analysis Match Molecules																
Evaluate Metadata View As Heatmap View Comparison Customize Table																
z-sc... 37.32 - 16.28 (1/448)																
Analysis Name	Project	case...	case...	case...	case...	com...	com...	com...	webl...	CP (...)	UR (...)	CN (...)	DE (z...	z...	z...	z...
32- normal control [lung] NA 12384	MouseDisease	normal control	lung	NA	Treatment vs....	Genotype:Ge...	GSE109776.GP	https://www.n...	52.52	37.59	59.16	37.32				
28- bronchitis;emphysema [lung] NA 12379	MouseDisease	bronchitis;em...	lung	NA	Treatment vs....	Genotype:Ge...	GSE109776.GP	https://www.n...	55.71	45.44	44.72	36.47				
3- chronic obstructive pulmonary disease (COPD) NA 11786	MouseDisease	chronic obstr...	lung	NA	Treatment vs....	SubjectTreat...	GSE87292.GPL	https://www.n...	51.21	46.63	44.72	35.64				
31- normal control [nasal epithelium] NA 11786	MouseDisease	normal control	nasal epithelium	NA	Treatment vs....	SubjectTreat...	E-MTAB-3150.J	http://www.eb...	50.85	40.38	44.72	33.99				
18- normal control [peripheral blood] NA 4945	MouseDisease	normal control	peripheral blo...	NA	Treatment1 v...	SubjectTreat...	GSE131914.GP	https://www.n...	43.55	19.78	59.16	30.62				
1- alcoholic fatty liver [liver] NA 17035	MouseDisease	alcoholic fatty ...	liver	NA	Disease vs. N...	DiseaseState ...	GSE40334.GPL	http://www.nc...	42.11	27.86	44.72	28.67				
947- normal control [stomach] 946	SingleCellMouseUmi	normal control	stomach	CellType1 vs...	epithelial cell v...	GSE108097_UA	https://www.n...	42.11	23.31	44.72	27.54					
1- skin disease [skin] NA 16522	MouseDisease	skin disease	skin	NA	Disease vs. N...	DiseaseState ...	GSE35160.GPL	https://www.n...	60.17	48.90		27.27				
1- normal control [lung] NA 16447	MouseDisease	normal control	lung	NA	Treatment vs....	SubjectTreat...	GSE33512.GPL	https://www.n...	57.24	51.08		27.08				
17- colorectal cancer [colon] NA 409	OncoMouse	colorectal can...	colon	NA	Other Compa...	SubjectTreat...	GSE109520.GP	https://www.n...	37.14		70.71	26.96				
23- normal control [nasal epithelium] NA 11777	MouseDisease	normal control	nasal epithelium	NA	Treatment vs....	SubjectTreat...	E-MTAB-3150.J	http://www.eb...	60.17	44.23		26.10				
10888- pancreas adenocarcinoma (PAAD) [pancreas] LNCSC	pancreas ade... KEAP1	pancreas ade...	pancreas	KEAP1 overe...	Treatment vs...	Treatment =>	GSE70138.GPL	https://www.n...	54.14	48.90		25.76				
25- normal control [nasal epithelium] NA 11779	MouseDisease	normal control	nasal epithelium	NA	Treatment vs....	SubjectTreat...	E-MTAB-3150.J	http://www.eb...	60.17	40.38		25.14				
3- ovarian cancer [ovary] NA 8121	OncoHuman	ovarian cancer	ovary	NA	Disease1 vs...	ExperimentGr...	GSE30274.GPL	https://www.n...	27.85	27.58	44.72	25.04				
7- normal control [peripheral blood] NA 18008	MouseDisease	normal control	peripheral blo...	NA	Treatment1 v...	Dosage:Samp...	GSE52403.GPL	http://www.nc...	37.14	17.38	44.72	24.81				
2- normal control [lung] NA 19305	MouseDisease	normal control	lung	NA	Treatment vs....	Genotype:Su...	GSE65124.GPL	http://www.nc...	61.59	37.59		24.79				
39- normal control [jejunum] NA 16270	MouseDisease	normal control	jejunum	NA	Tissue vs. Tl...	SamplingTime...	GSE32513.GPL	http://www.nc...	32.16	20.85	44.72	24.43				
5- atherosclerosis;hyperlipidemia [liver] NA 19373	MouseDisease	atherosclerosis...	liver	NA	Other Compa...	AnimalStrain[...	GSE66568.GPL	https://www.n...	49.52	46.63		24.04				
27- normal control [nasal epithelium] NA 11781	MouseDisease	normal control	nasal epithelium	NA	Treatment vs....	SubjectTreat...	E-MTAB-3150.J	http://www.eb...	52.52	41.96		23.62				
221- small intestine carcinoid neuroendocrine tumor	OncoHuman	small intestine...	small intestine	luminespb	Treatment vs...	TreatTime:Ex...	GSE96760.GPL	https://www.n...	24.81	23.31	44.72	23.21				
562- normal control [liver] 561	SingleCellMouseUmi	normal control	liver	CellType1 vs...	hepatocyte vs...	GSE108097_UA	https://www.n...	30.64	17.03	44.72	23.10					
3- disease control [liver] NA 952	OncoMouse	disease control	liver	NA	Other Compa...	ExperimentGr...	GSE79084.GPL	https://www.n...	55.71	36.12		22.96				
1- type 1 diabetes mellitus [liver] NA 16835	MouseDisease	type 1 diabet...	liver	NA	Treatment vs....	ExperimentGr...	GSE38138.GPL	https://www.n...	47.34	44.23		22.89				
5- diet induced obesity [hypothalamus] NA 779	MouseDisease	diet induced ...	hypothalamus	NA	Treatment1 v...	SubjectTreat...	GSE102415.GP	https://www.n...	17.62	23.31	50.00	22.73				
2- normal control [large airway epithelium] NA 22527	HumanDisease	normal control	large airway e...	NA	Other Compa...	TissueRegion...	GSE5056.GPL8	http://www.nc...	47.34	42.99		22.58				
4- emphysema [lung] NA 20909	MouseDisease	emphysema	lung	NA	Treatment vs....	TreatTime:Su...	GSE8790.GPL1	http://www.nc...	49.13	40.38		22.38				
6- emphysema [lung] NA 7610	MouseDisease	emphysema	lung	NA	Treatment vs....	SubjectTreat...	GSE76205.GPL	https://www.n...	54.14	34.58		22.18				
3- alcoholic fatty liver [liver] NA 18043	MouseDisease	alcoholic fatty ...	liver	NA	Treatment vs....	Genotype:Su...	GSE52644.GPL	http://www.nc...	47.77	40.38		22.04				
4- normal control [liver] NA 2696	RatDisease	normal control	liver	NA	Treatment vs....	SubjectTreat...	GSE122184.GP	https://www.n...	52.52	34.58		21.78				
1- nonalcoholic steatohepatitis (NASH) [liver] NA 187	MouseDisease	nonalcoholic s...	liver	NA	Treatment vs....	Genotype:Su...	GSE61534.GPL	https://www.n...	52.52	34.58		21.78				
663- normal control [kidney] NA 7126	RatDisease	normal control	kidney	NA	Treatment1 v...	ExperimentGr...	GSE57811.GPL	https://www.n...	21.44	20.85	44.72	21.75				
1186- normal control [liver] NA 4243	RatDisease	normal control	liver	NA	Treatment vs....	ExperimentGr...	GSE57815.GPL	https://www.n...	45.96	40.38		21.58				
16- chronic obstructive pulmonary disease (COPD) NA 20908	HumanDisease	chronic obstr...	small airway e...	NA	Other Compa...	VisitMonth:AQ...	GSE108134.GP	https://www.n...	52.52	32.97		21.37				
3- emphysema [lung] NA 20908	MouseDisease	emphysema	lung	NA	Treatment vs....	TreatTime:Su...	GSE8790.GPL1	http://www.nc...	50.85	34.58		21.36				
1- normal control [large airway epithelium] NA 22526	HumanDisease	normal control	large airway e...	NA	Other Compa...	TissueRegion...	GSE5056.GPL8	http://www.nc...	45.49	39.32		21.20				
65- normal control [liver] NA 6176	MouseDisease	normal control	liver	NA	CellType1 vs...	ExperimentGr...	GSE145820.GP	https://www.n...	34.74		50.00	21.19				
11- normal control [heart left ventricle] NA 16761	MouseDisease	normal control	heart left vent...	NA	Other Compa...	Gender:Genot...	GSE37597.GPL	https://www.n...	45.49	39.01		21.12				
255- Ebola hemorrhagic fever [spleen] NA 4045	MouseDisease	Ebola hemorr...	spleen	NA	Other Compa...	ExperimentGr...	GSE130629.GP	https://www.n...	39.39		44.72	21.03				

Selected 0 / 89515





## DiseaseLandHumanDisease

- MouseDisease
- RatDisease
- LINCS

## OncoLand

- Hematology
- Metastatic Cancer
- OncoHuman (Formerly OncoGEO)
- Pediatrics
- TCGA
- OncoMouse
- ENCODE RNA Binding

## SingleCellLand

- SingleCellHuman
- SingleCellHumanUmi
- SingleCellHumanUmiLite
- SingleCellHumanHCL
- SingleCellMouse
- SingleCellMouseUmi
- SingleCellMouseUmiLite

## Normal Cells and Tissues

- Human Tissues (GTEx)

Total datasets for release: **121,000 +**

## How OmicSoft datasets were analyzed in IPA

### Example of Omicsoft Dataset

Up-regulated



Down-regulated

Filter genes by  $p < 0.01$

(Standard p-value)

If  $< 50$  genes total survive,  
then reject the dataset



If  $> 1000$  genes, then pick  
the top up and down  
genes to get  $\sim 1000$  genes

Maintain balance of up vs  
down

(i.e. 69% up and 31% down)



$\sim 1000$   
Analysis-  
ready genes



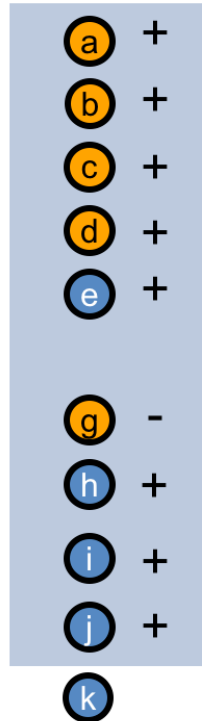
## My Analysis



Match?



## Another analysis



Query  
Upstream Regulator  
signature

Scoring against  
Upstream Regulator  
signature from  
another analysis

$$Z = \frac{\text{matches} - \text{mismatches}}{\text{Square root of all matches}}$$

- z-score is a measure of the match between two patterns
- Assumes the pattern is created from two sets of entities where the sign of the matching entities is random

$$= (8-1)/\sqrt{9} = 2.33 \text{ (raw z-score)}$$

Yes, it matches (because  $z > 2$ )

How signatures are created and compared

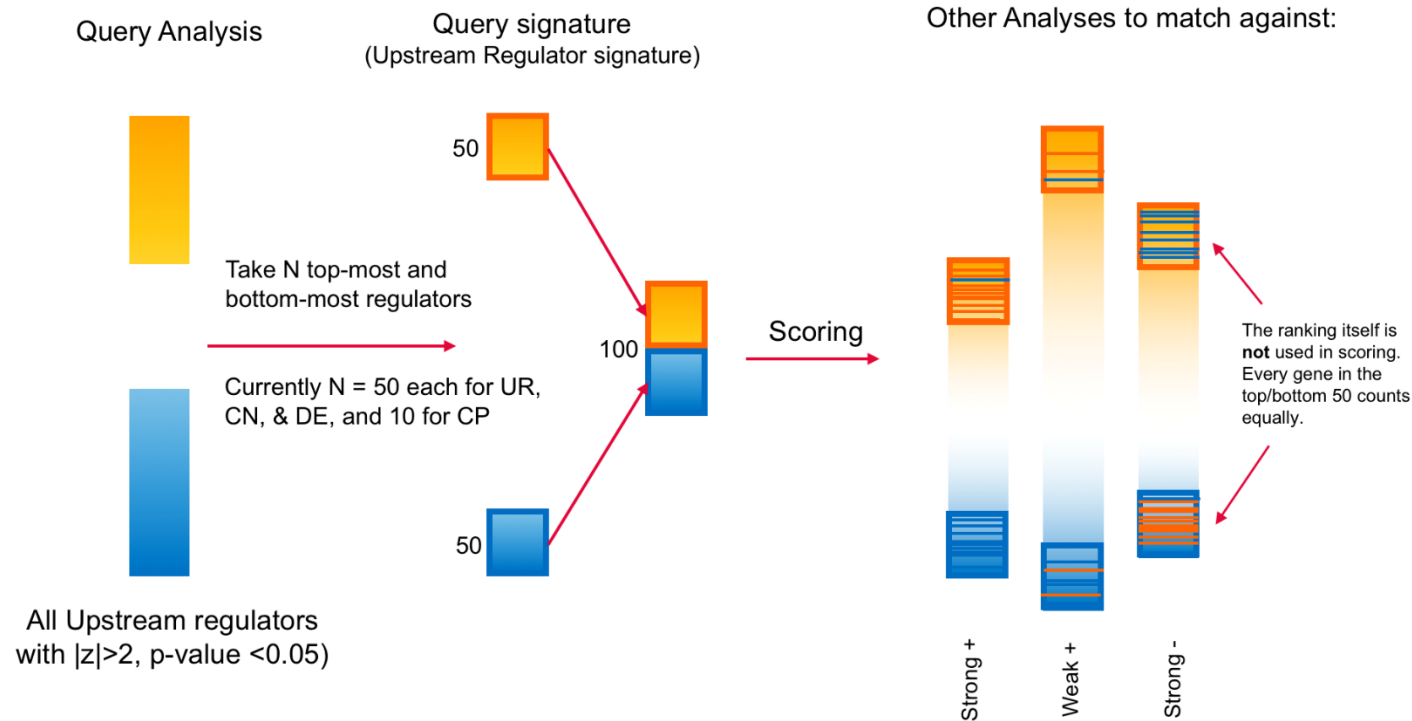
Canonical Pathways (up to 20 pathways)

Upstream Regulators (up to 100 regulators)

Causal Networks (up to 100 master regulators)

Diseases & Functions (up to 100 diseases or functions)

Example



# Live Demo

1. Data Upload and How to Run a Core Analysis  
Upload experiment data
2. Functional Interpretation in IPA  
Introduction for Analysis Tools
3. Comparison Analyses
4. Q&A

## Single Experiment

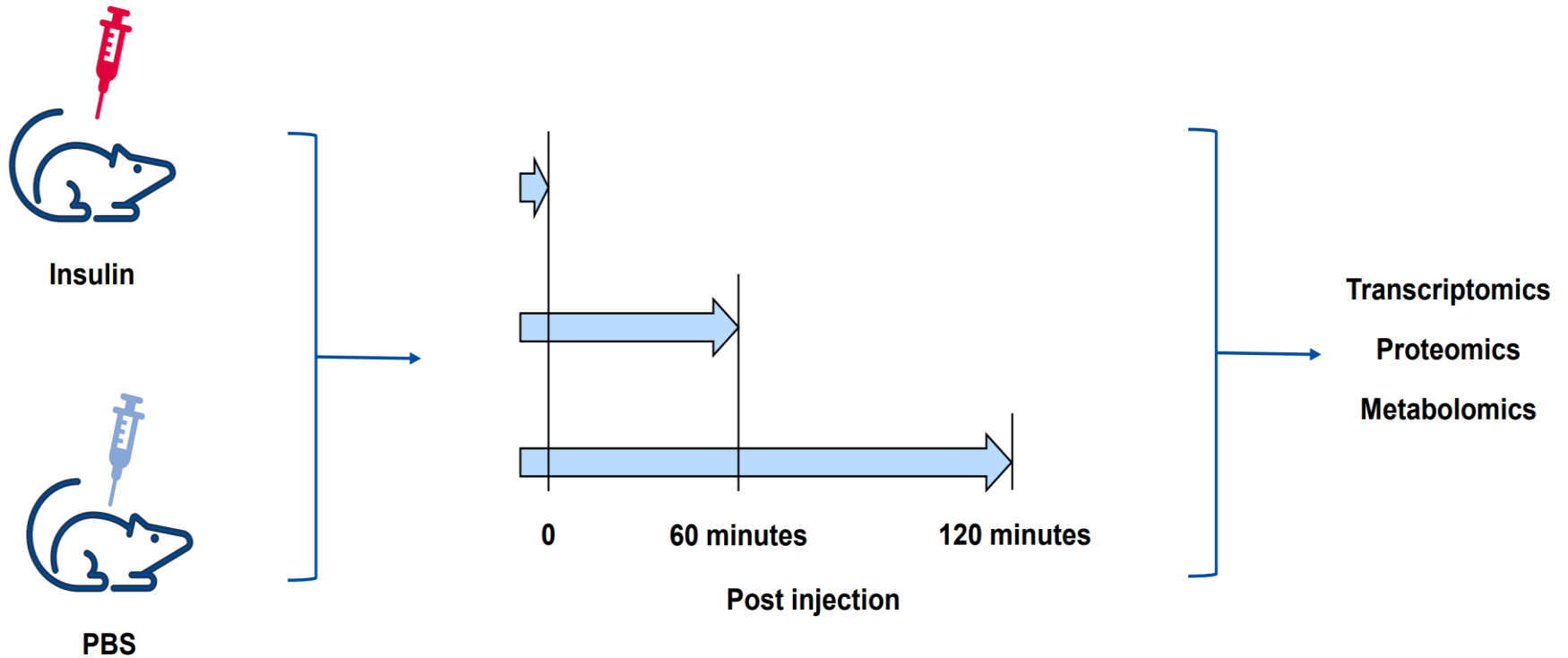
- Time Course
- Dose Response

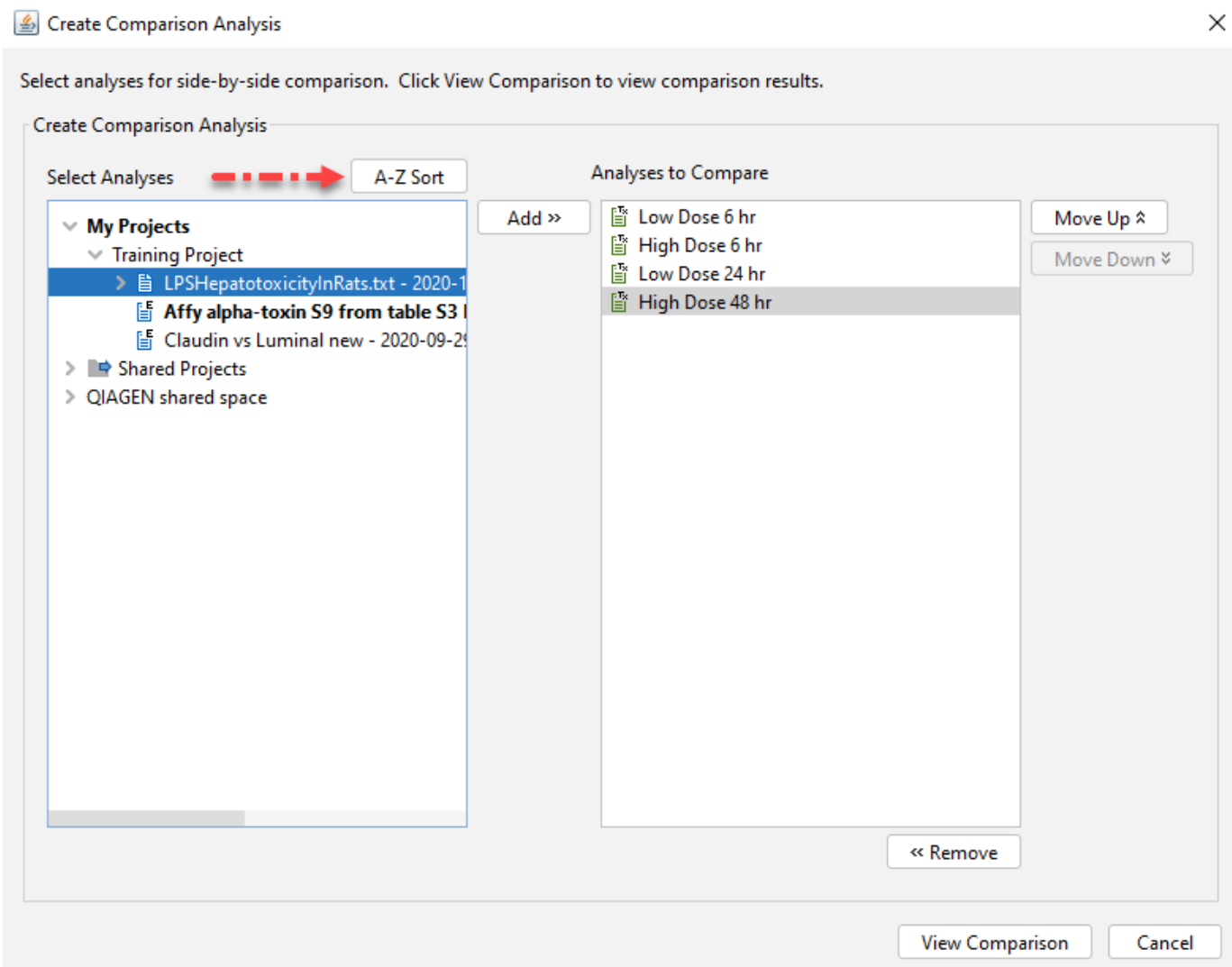
## Multi Experiment

- System biology
- Combining SNP, CNA, mRNA, microRNA, proteomics, etc.

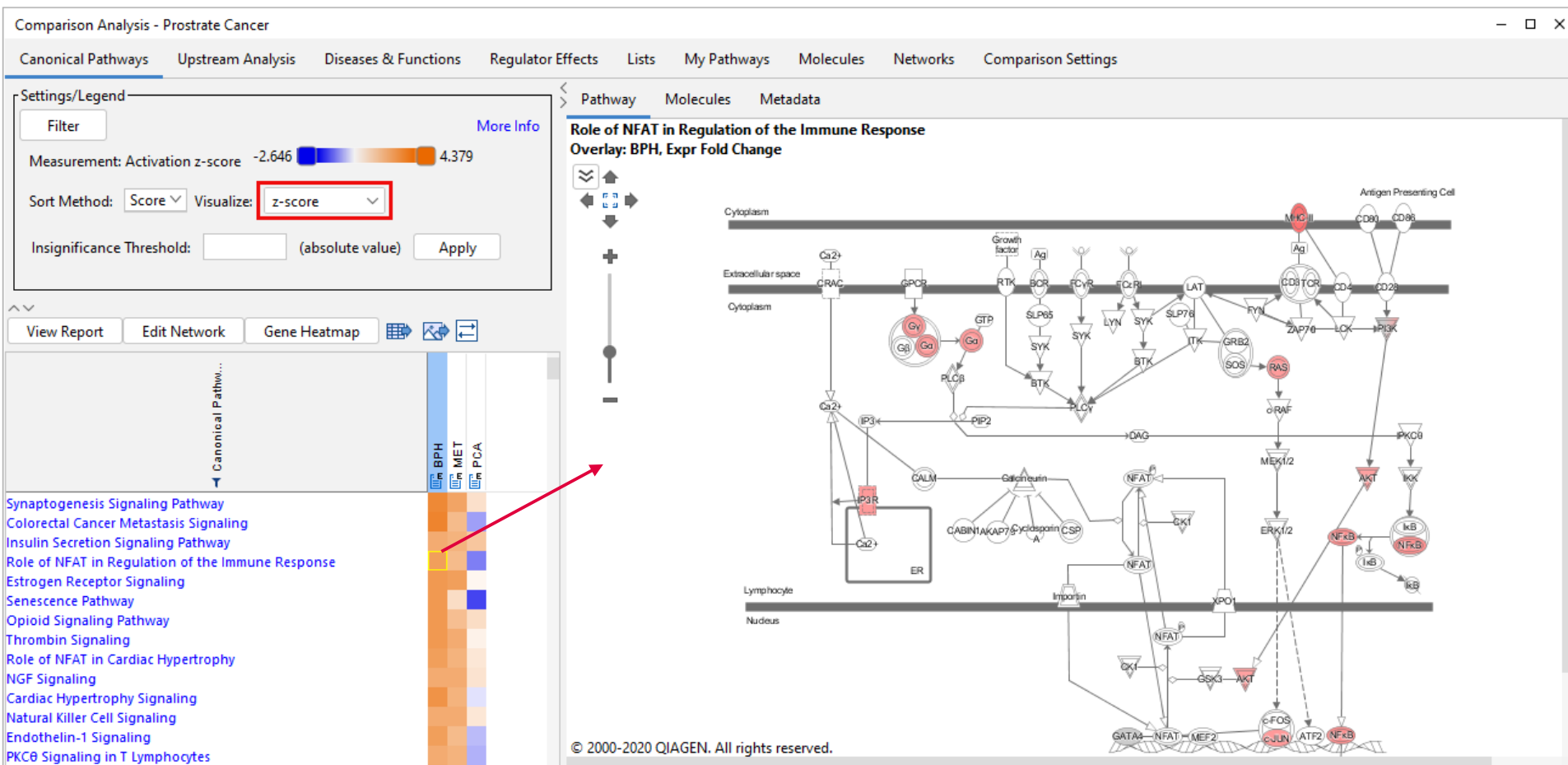
## Set Analysis

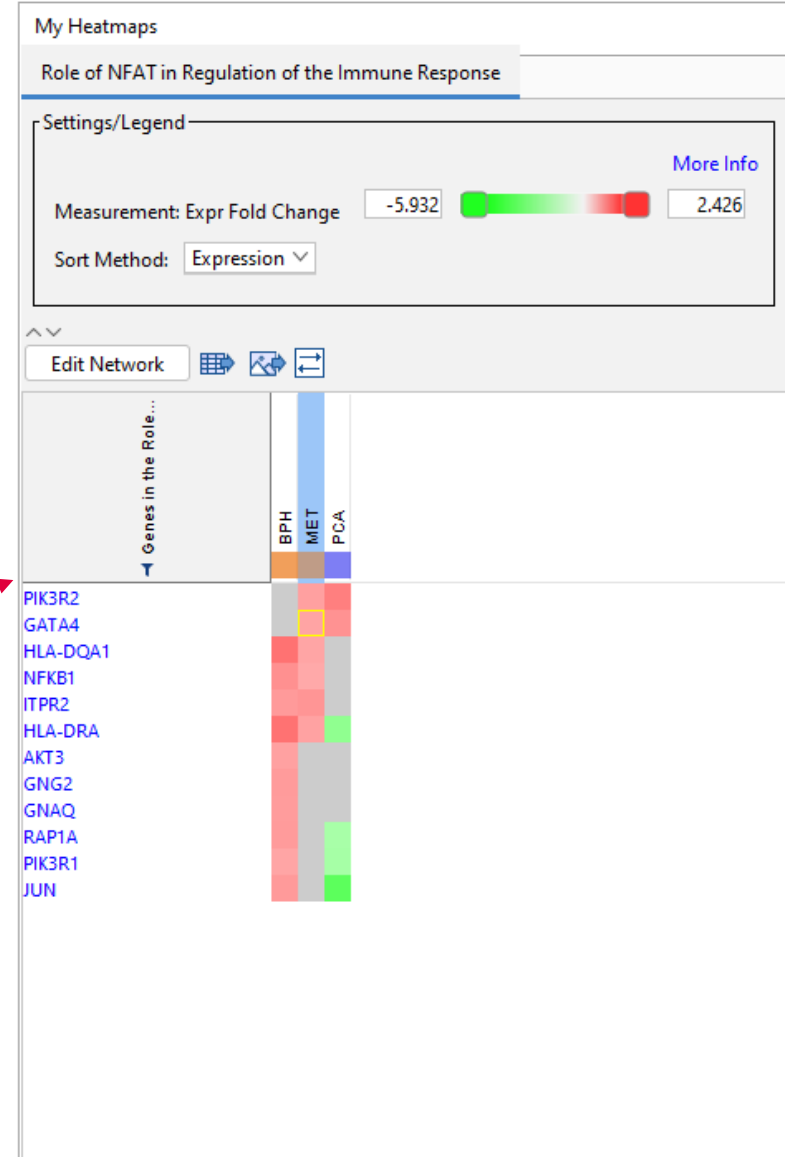
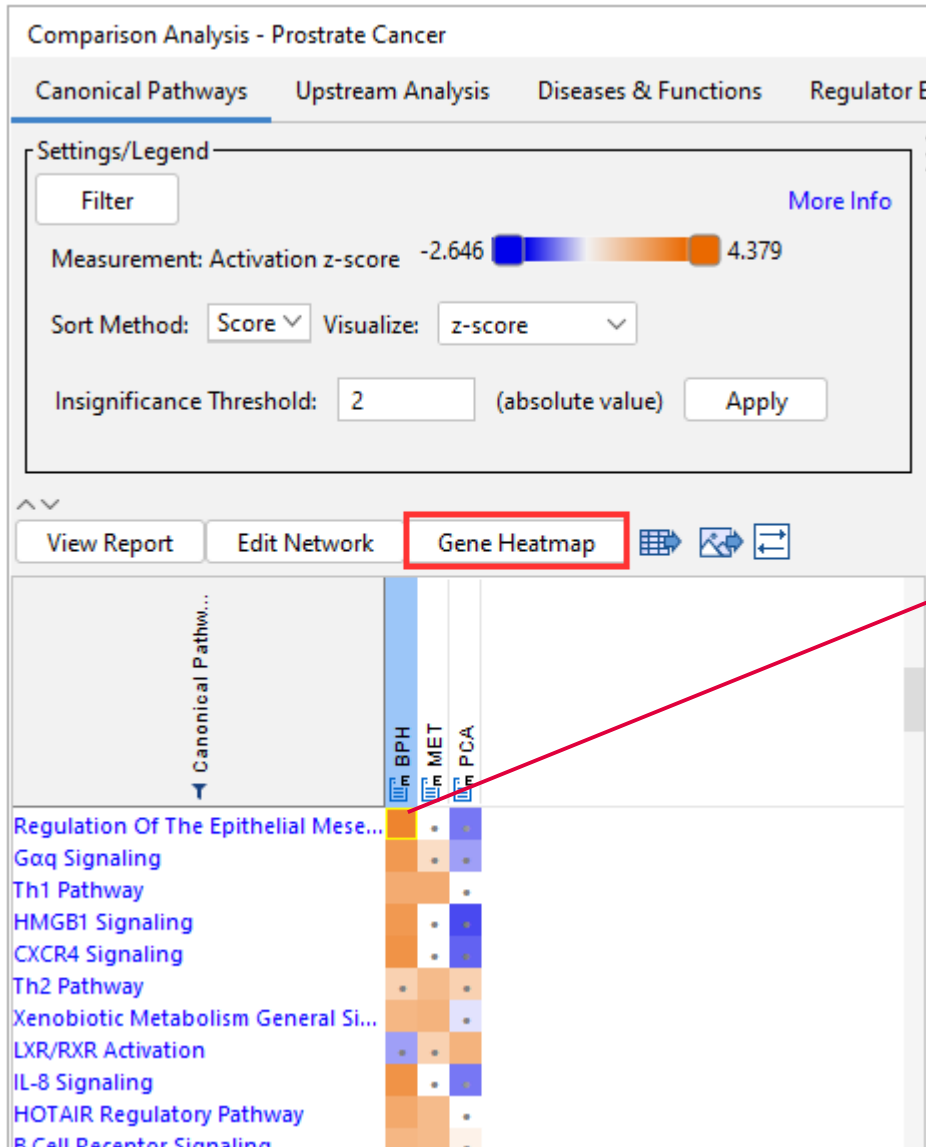
- Exploring Common Molecules across one or more experiment (s)

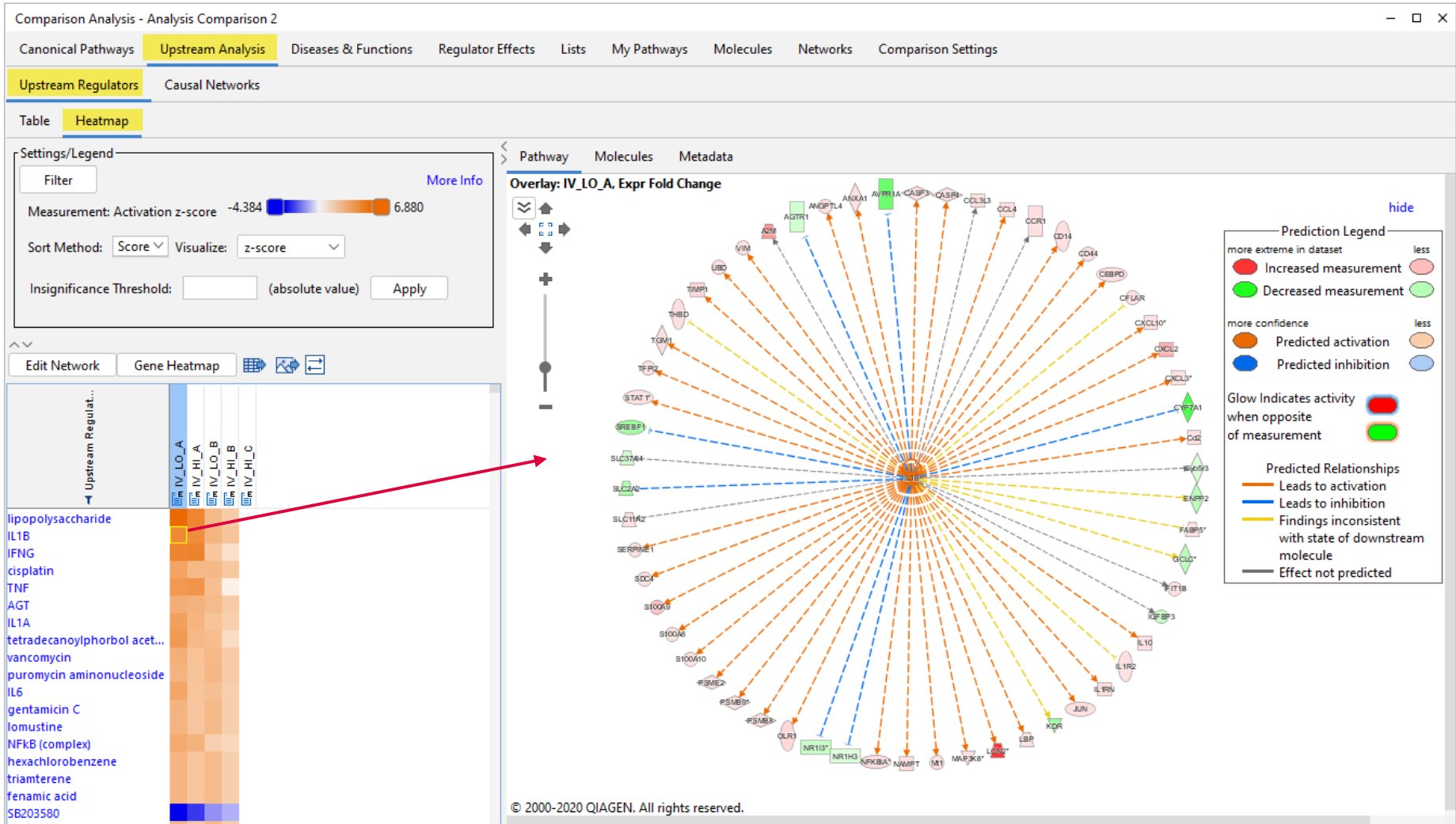


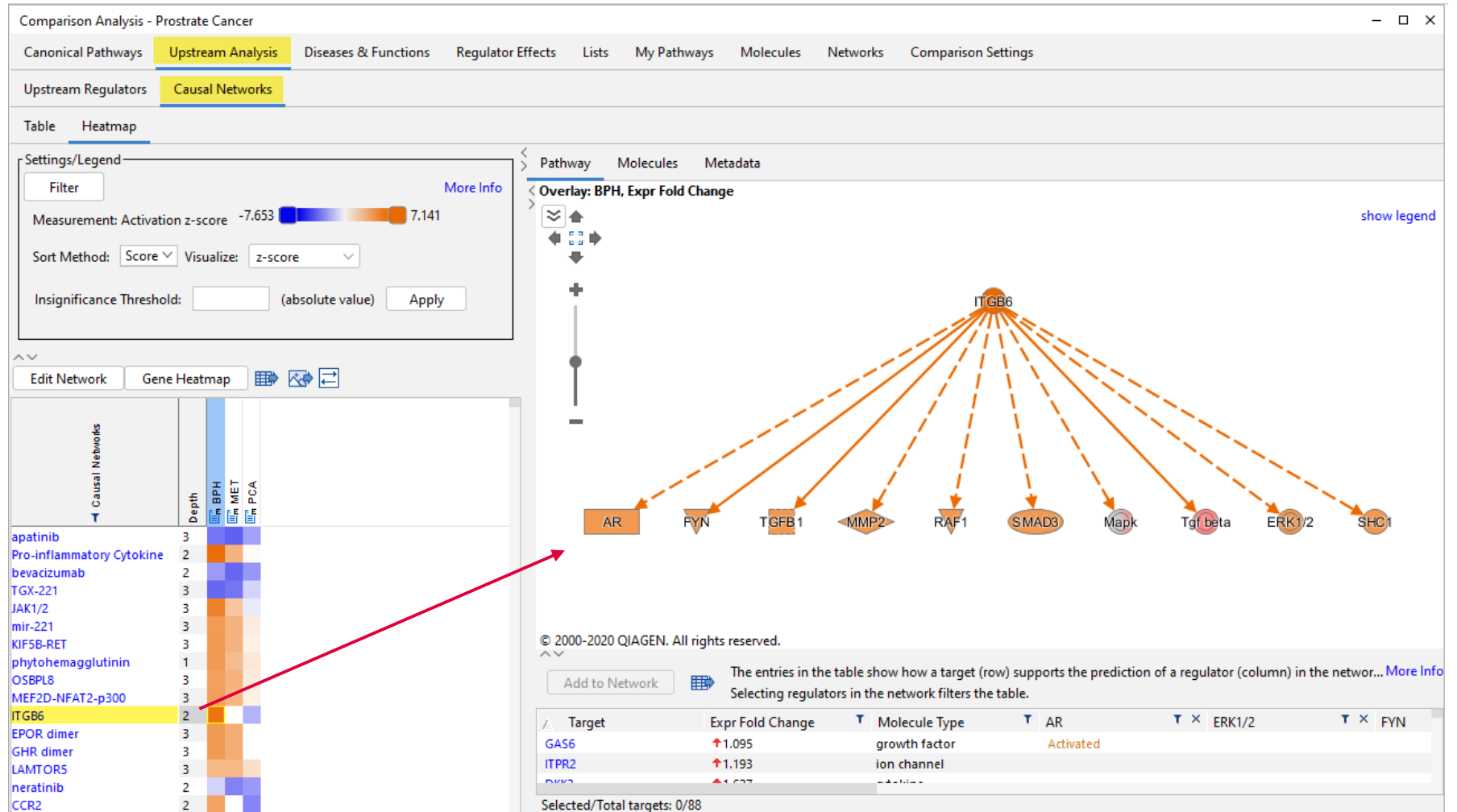


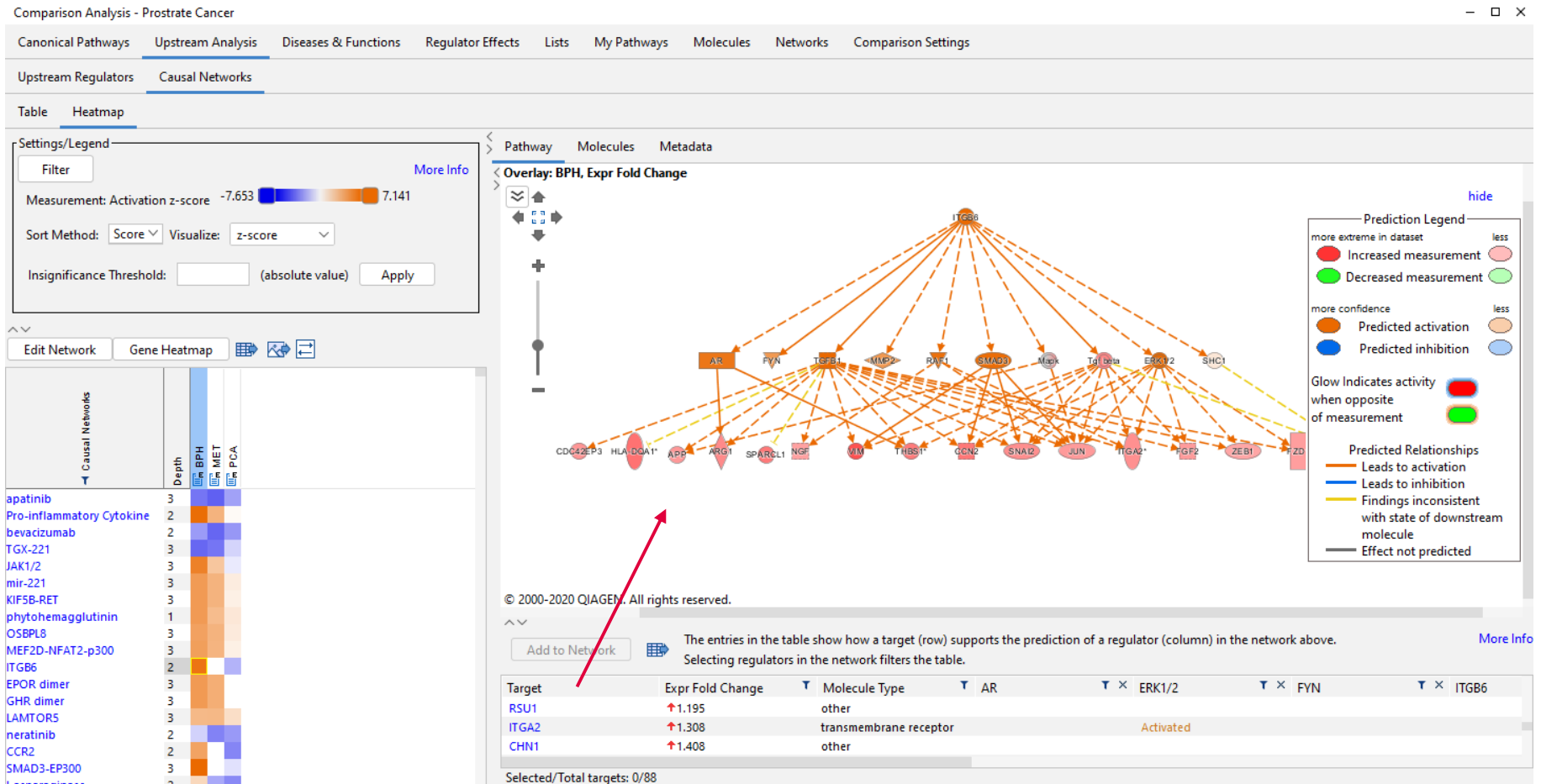


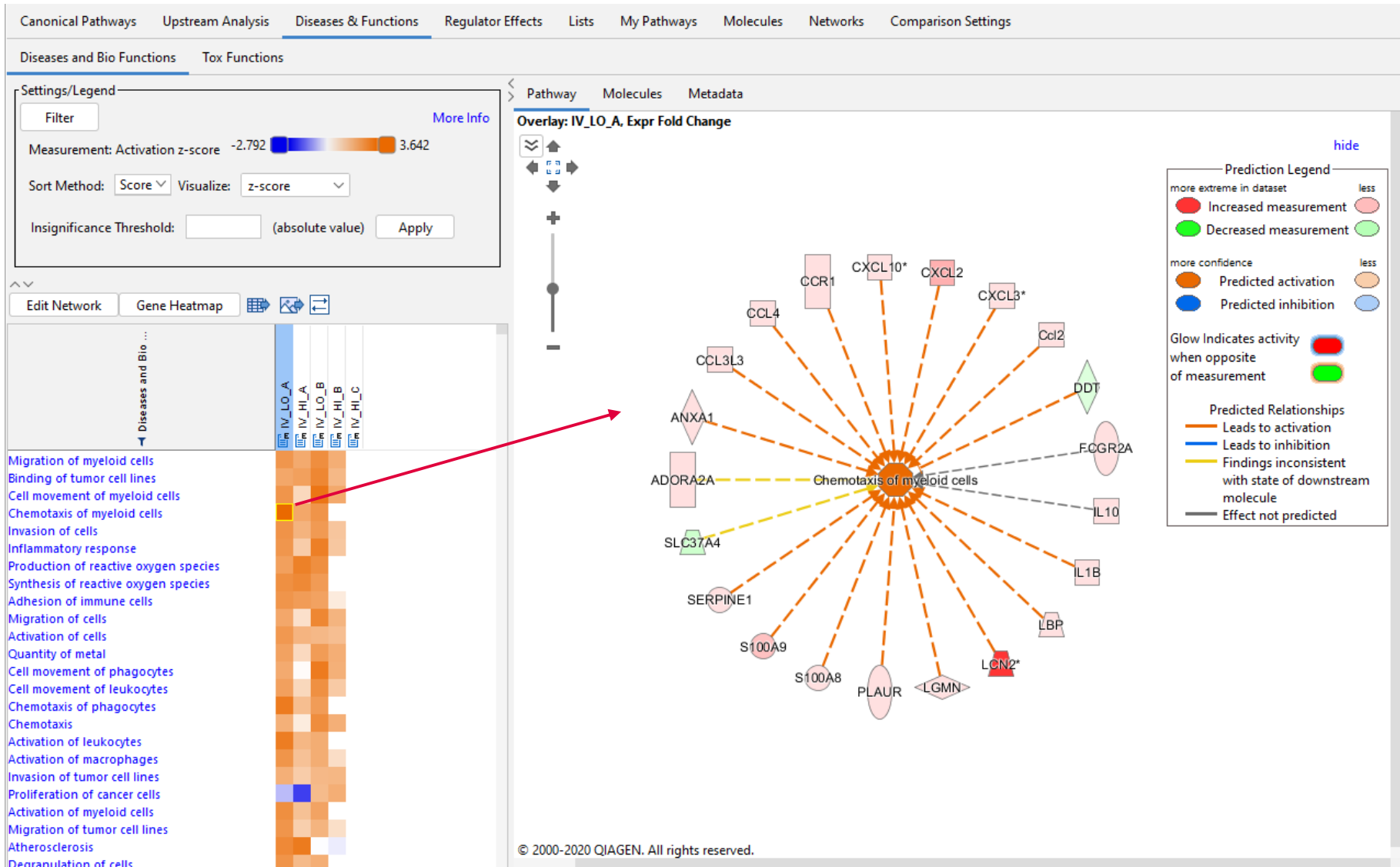














Canonical Pathways   Upstream Analysis   **Diseases & Functions**   Regulator Effects   Lists   My Pathways   Molecules   Netw

Diseases and Bio Functions   Tox Functions

Settings/Legend

**Filter**

Measurement: Activation z-score -2.792

Sort Method: Score   Visualize: z-score

Insignificance Threshold:   (absolute value)

Edit Network   Gene Heatmap

Diseases and Bio ...

Digestive system cancer  
Digestive organ tumor  
Liver cancer  
Hepatobiliary system cancer  
Gastrointestinal tumor  
Liver tumor  
Gastrointestinal tract cancer

IV\_LO\_A  
IV\_HI\_A  
IV\_LO\_B  
IV\_HI\_B  
IV\_HI\_C

**Filter**

Select Functions and Diseases to Display

- ☐ Bile duct adenocarcinoma
- ☐ Cholangiocarcinoma
- ☐ Hepatitis B surface antigen-negative hepatitis
- ☐ Hepatitis C virus-related hepatocellular carcin
- ☒ Hepatobiliary system cancer
- ☐ Hepatocellular carcinoma
- ☒ Liver cancer
- ☒ Liver tumor
- ☐ Cancer, Gastrointestinal Disease, Inflammatory D
- ☐ Barrett syndrome
- ☐ Cancer, Gastrointestinal Disease, Organismal Inju
- ☐ Colon sporadic adenocarcinoma
- ☒ Digestive organ tumor
- ☒ Digestive system cancer
- ☐ Duodenal neoplasm
- ☒ Gastrointestinal tract cancer
- ☒ Gastrointestinal tumor
- ☐ Metastatic colorectal cancer
- ☐ Pancreatobiliary tumor
- ☐ Cancer, Hematological Disease, Immunological I

Score Filter

p-value Cutoff: 1.3 (log10)

z-score Cutoff:   (absolute value)

B-H p-value Cutoff:   (log10)

OK   Cancel

## Single Experiment

- Time Course
- Dose Response

## Multi Experiment

- System biology
- Combining SNP, CNA, mRNA, microRNA, proteomics, etc

## Set Analysis

- Exploring Common Molecules across one or more experiment (s)

Create New...

- Core Analysis...
- Comparison Analysis...
- Biomarker Filter...
- Biomarker Comparison Analysis...
- MicroRNA Target Filter...
- BioProfiler
- IsoProfiler
- My Pathway
- Path Designer
- Filter Dataset
- Upload Dataset...
- Advanced Search
- Project...
- Compare**
- Import Pathway

## Compare

Select Entities to compare and click Add

▼ **My Projects**

▼ 2020-3

▼ Dataset Files

▼ LPS Hepatotoxicity in Rats.txt

High Dose 24 hr

High Dose 48 hr

High Dose 6 hr

Low Dose 24 hr

Low Dose 48 hr

Low Dose 6 hr

WNV human macrophages TE Ensembl, GSE40718

> LPS Hepatotoxicity in Rats\_Transporter Molecules

Macro\_vs\_Others

Estradiol(E2)treatedMCF7 12hr FC1.5 P<.05 GSE1135

> NRF2-Proteome-modified 2

> ESC vs\_ MES, CP, CM\_ TE mouse cardiomyocytes G

> Welding GSS vs air Illumina MouseRef-8 v2\_0

AB\_T\_vs\_Others

Alpha toxin s9 phosphorylation log ratio PMID 2581

Anti PD-1 Non-Responder vs\_ Responder-TE 2017-0

> APAP Dose + Timecourse

B\_vs\_Others

Claudin vs Luminal RNA-Seq

> Prostrate disease dataset

> Analyses

> BioProfiler Results

> My Pathways

> My Lists

> Training Project

> Shared Projects

Refresh

Add >>

<< Remove

Clear All

Calculate intersections

**Export to image**

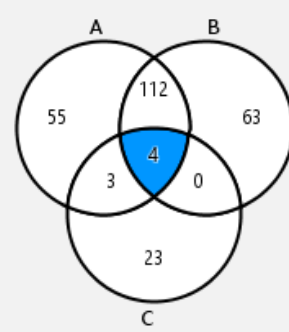
[More Info](#)

Click in the Venn diagram below to compare different sets.  
Keep the Ctrl key down to select multiple areas.

A Low Dose 6 hr (Dataset)

B High Dose 6 hr (Dataset)

C High Dose 24 hr (Dataset)



Entities Comparison Results (4)

A2M
ACLY
CRYL1
S100A9

Add To My Pathway

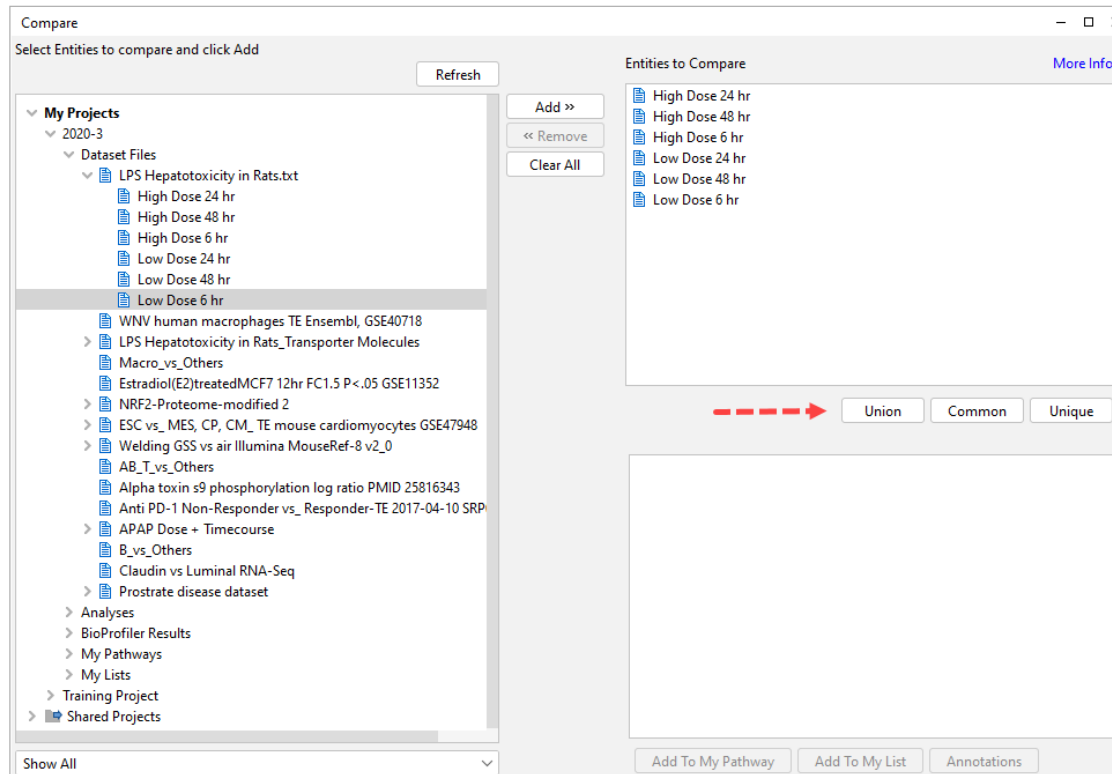
Add To My List

Annotations

Sample to Insight

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- The **Union** operator will display a list of the total population of molecules present in all of the entities (the sum of the molecules).
- The **Common** operator will display the intersection of molecules. In order to appear on this list, a molecule would have to be present on each of the individual entities used in the General Comparison.
- The **Unique** operator will display only the molecules that are present on individual entities. Use the pull-down menu to choose the entity for which you would like to display the results.



To view the molecule details or to create a new dataset from these, click on the **'Annotations'** button.

Compare

Select Entities to compare and click Add

Refresh

My Projects

2020-3

Dataset Files

LPS Hepatotoxicity in Rats.txt

High Dose 24 hr

High Dose 48 hr

High Dose 6 hr

Low Dose 24 hr

Low Dose 48 hr

Low Dose 6 hr

WNV human macrophages TE Ensembl

LPS Hepatotoxicity in Rats\_Transporter M

Macro\_vs\_Others

Estradiol(E2)treatedMCF7 12hr FC1.5 P<.0

NRF2-Proteome-modified 2

ESC vs\_ MES, CP, CM\_ TE mouse cardion

Welding GSS vs air Illumina MouseRef-8

AB\_T\_vs\_Others

Alpha toxin s9 phosphorylation log ratio

Anti PD-1 Non-Responder vs\_ Responde

APAP Dose + Timecourse

B\_vs\_Others

Claudin vs Luminal RNA-Seq

Prostrate disease dataset

Analyses

BioProfiler Results

My Pathways

Show All

Add >>

<< Remove

Clear All

Entities to Compare

More Info

High Dose 24 hr

High Dose 48 hr

High Dose 6 hr

Low Dose 24 hr

Low Dose 48 hr

Low Dose 6 hr

Union

Common

Unique

High Dose 6 hr

Unique to Entity (60)

ACKR3

ACO2

ADH5

AKR1D1

Anp32a

AQP9

ARNTL

ASRGL1

Cald1

CLIP2

CPT2

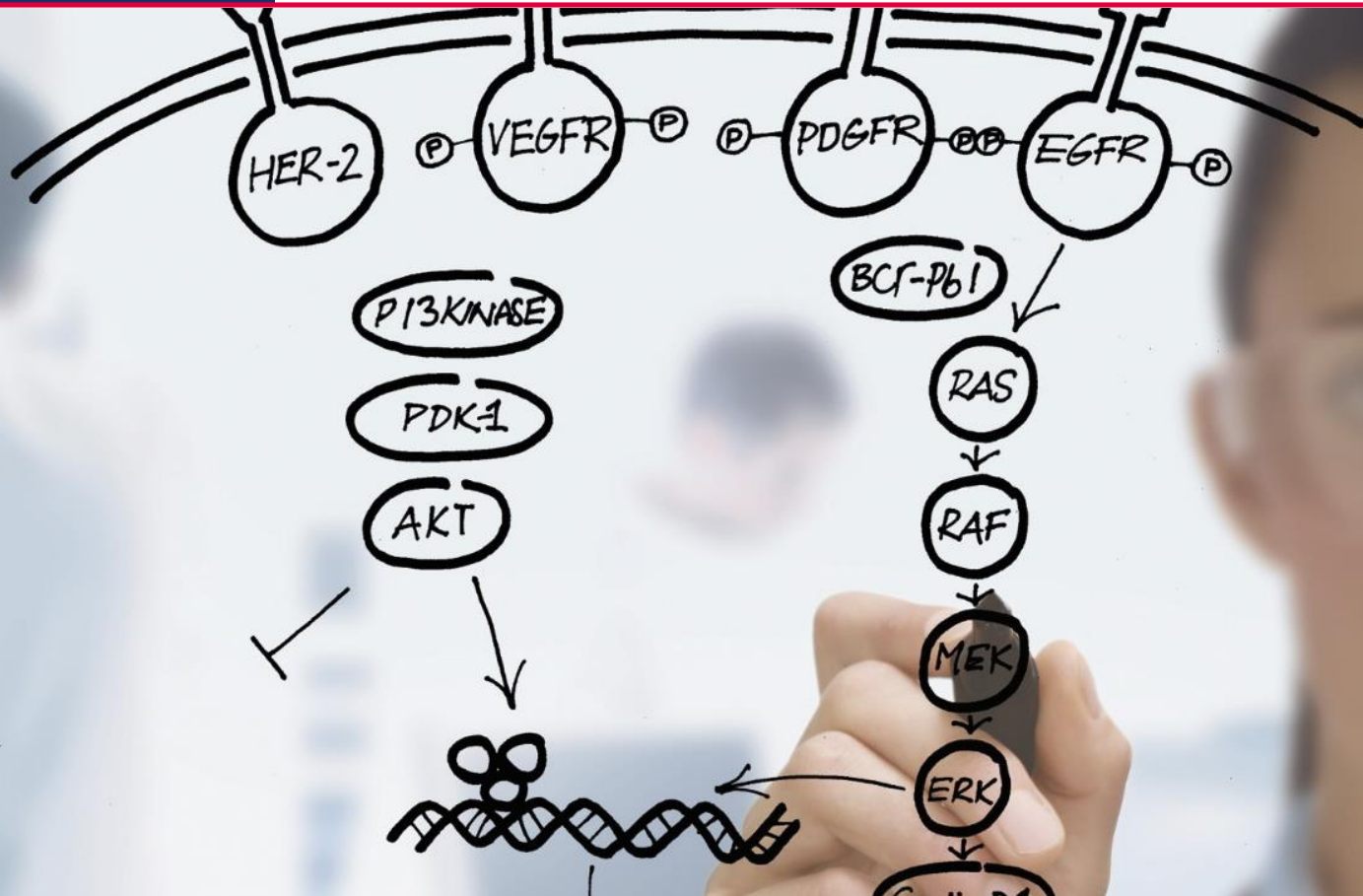
CTH

CYP2F1

Add To My Pathway

Add To My List

Annotations



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