Dataset analysis using QIAGEN IPA

GraceChou 周儀柔 資深業務專員

Zoe Huang 黃柔諭　　產品專員

IPA Training Account & Demo Data

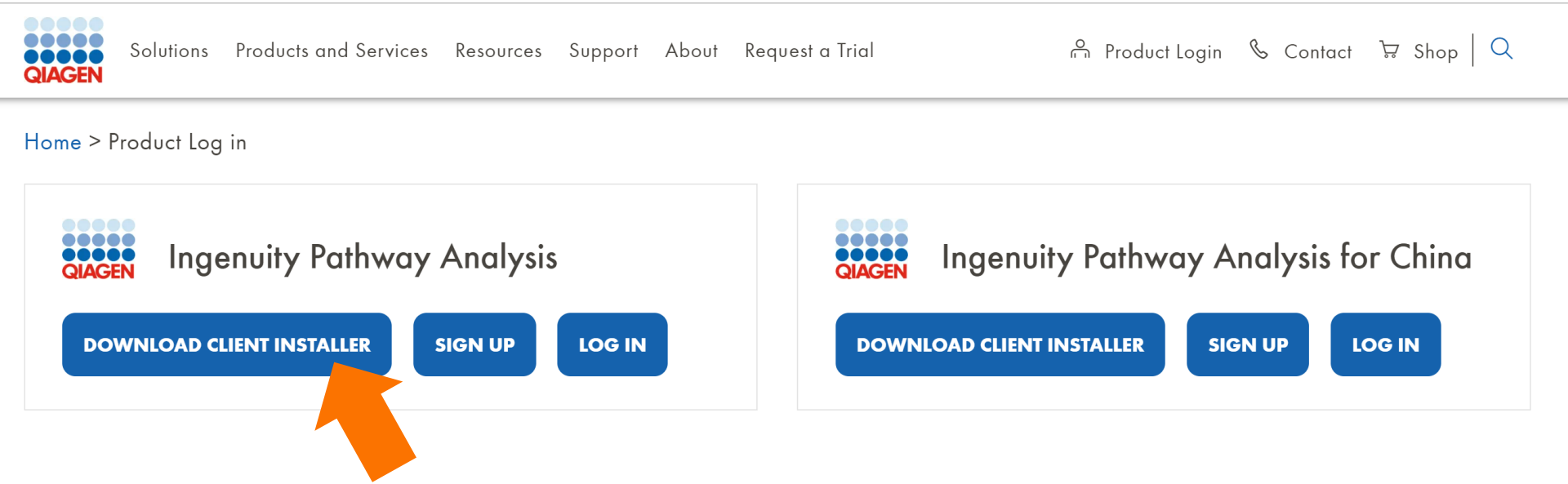
Account: sinicaXX@ingenuity.com

(XX= 1~10)

Password: Qiagen@312

Dataset: https://reurl.cc/9V2Ogj

Client: (IPA Login)



Agenda

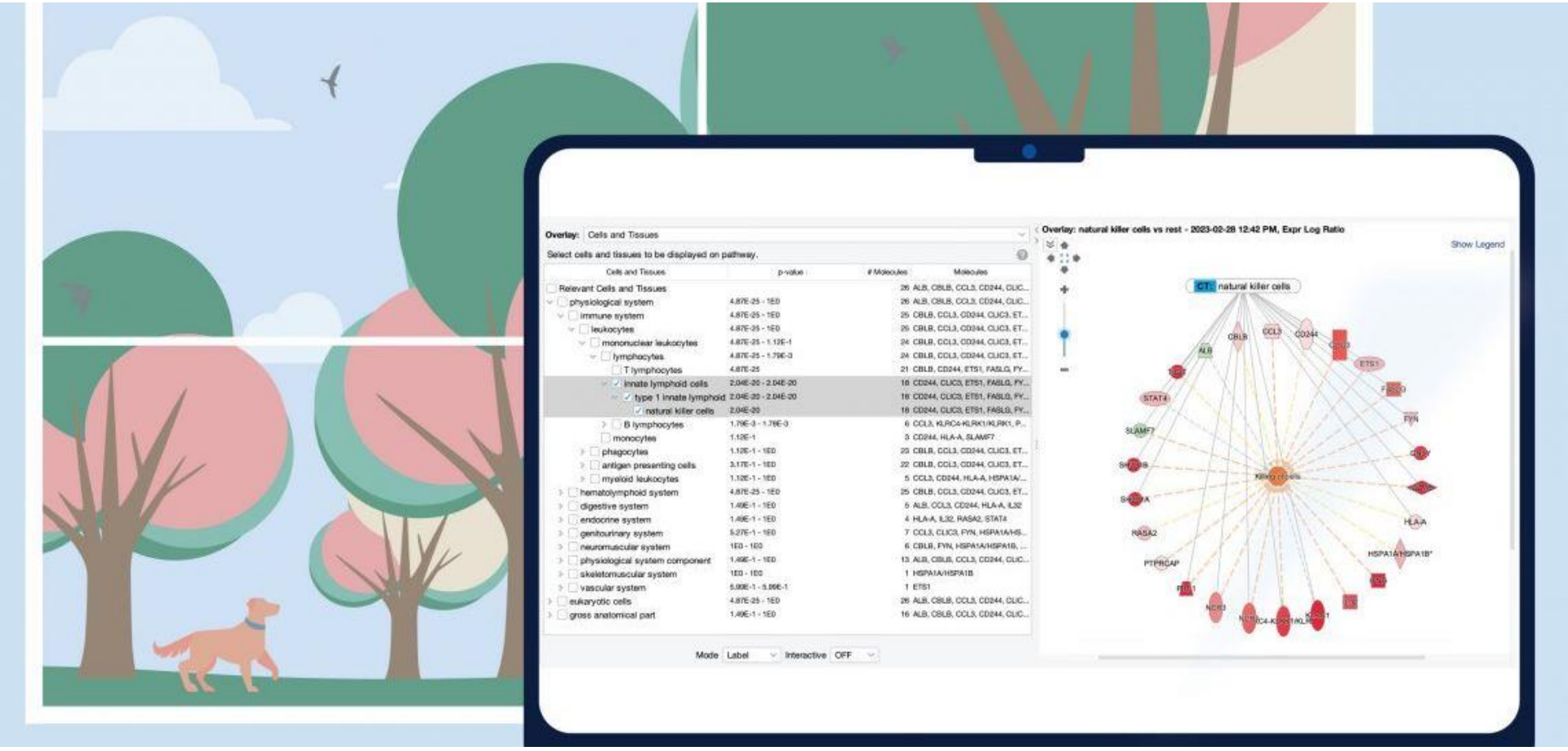
1. What's New in the IPA

2. Data Upload and How to Run a Core Analysis
   Upload experiment data

3. Functional Interpretation in IPA
   Introduction for Analysis Tools

4. Comparison Analyses

5. Q&A



QIAGEN IPA 近期更新內容介紹

1. Identify potential cell types based on the set of genes on networks and pathways

Overlay :Cell and Tissues

THE HUMAN PROTEIN ATLAS

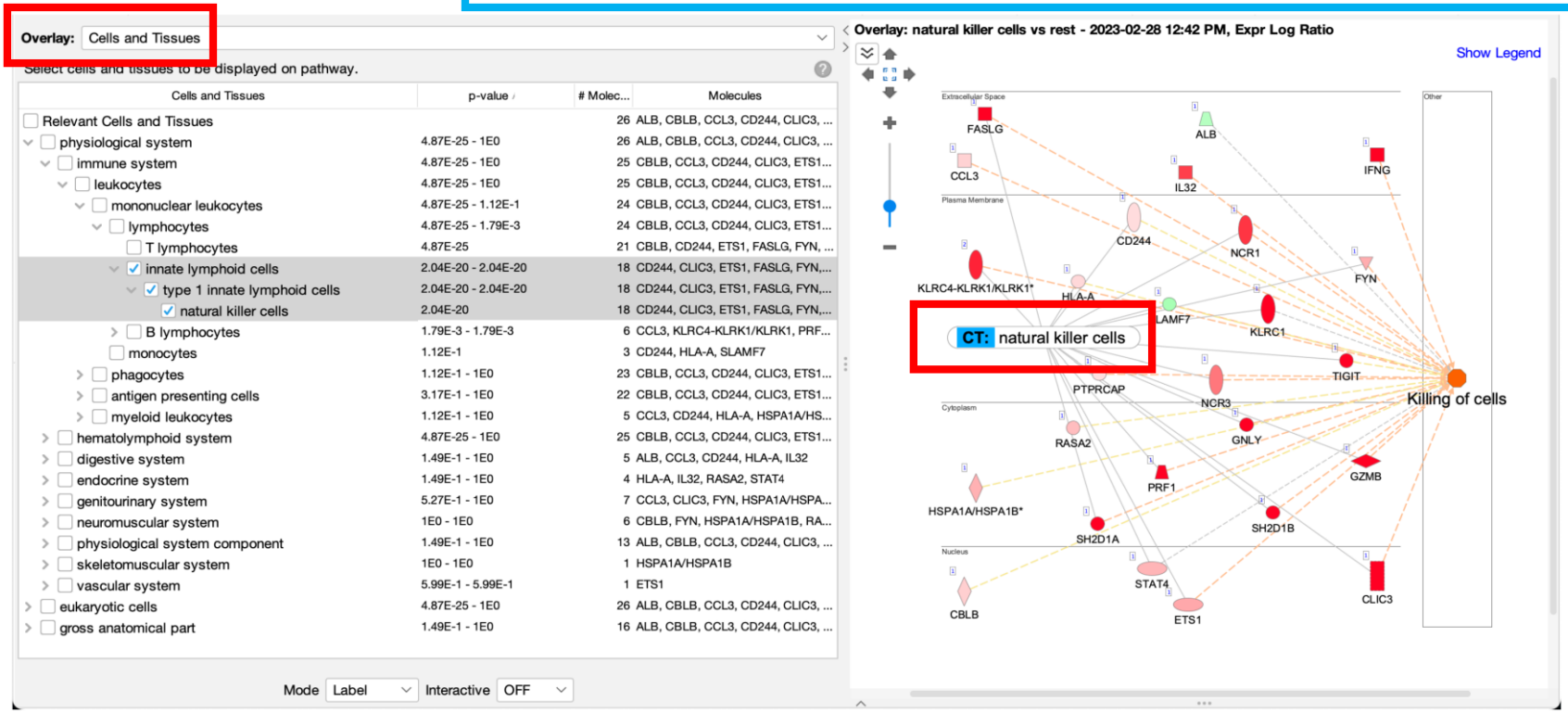
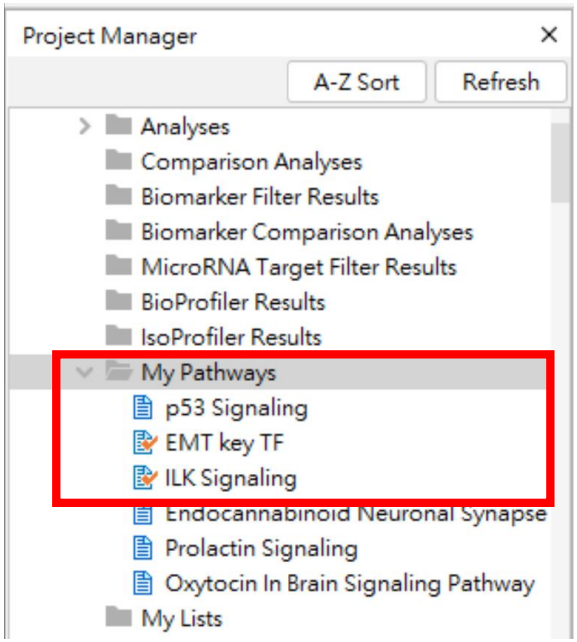


Figure 1. Enrichment of natural killer enriched genes on a network.

2. Causally score My Pathways in Core Analysis



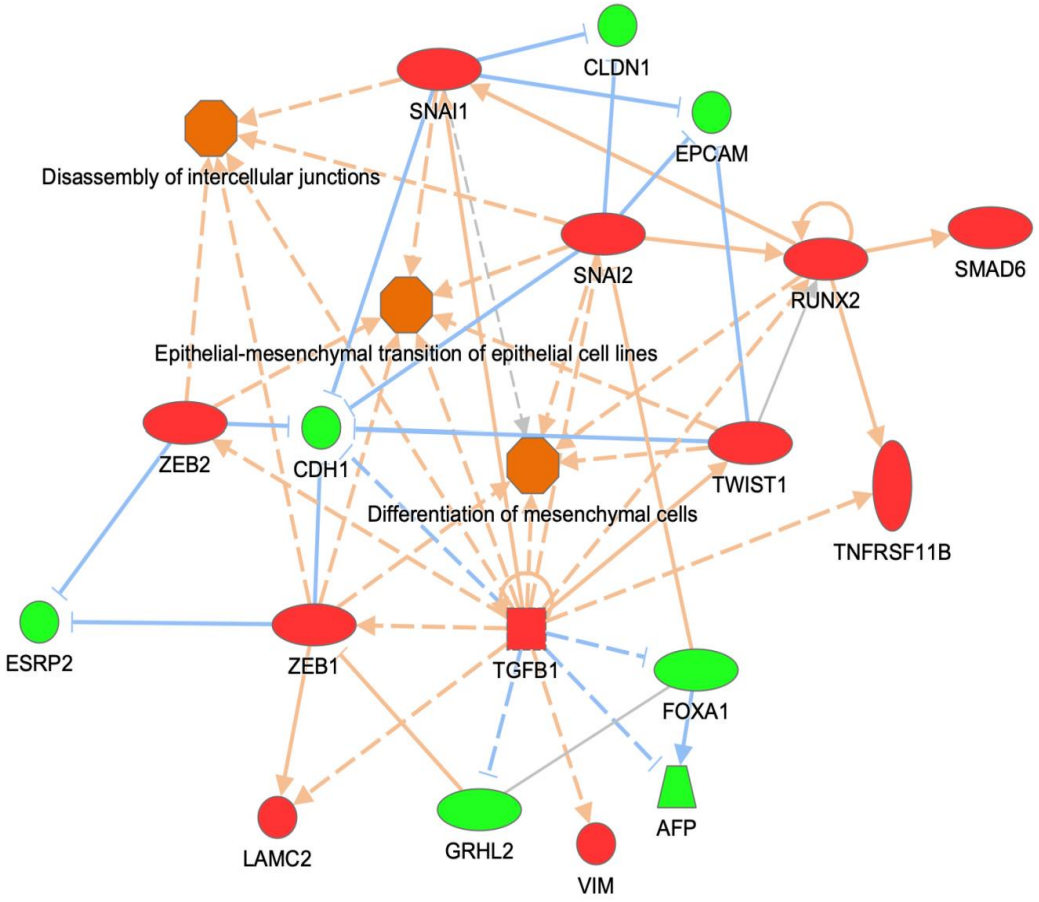
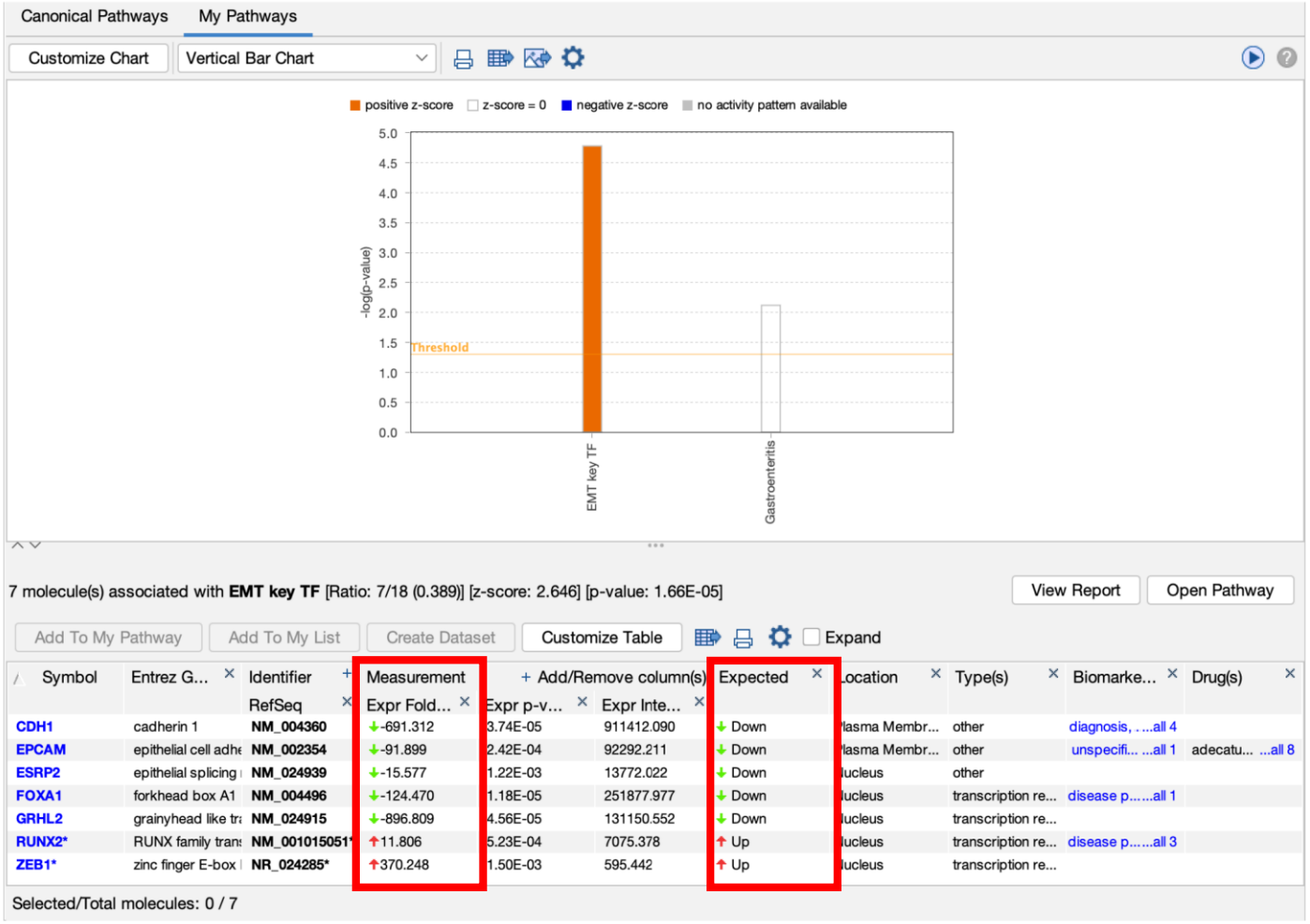


Figure 3. A custom My Pathway with nodes assigned by the user as activated (red) or green (inhibited).

2. Causally score My Pathways in Core Analysis



Symbol	Entrez G...	Identifier	Measurement	Expr Fold...	Expr p-v...	Expr Inte...	Expected	Location	Type(s)	Biomarke...	Drug(s)
		RefSeq									
CDH1	cadherin 1	NM_004360		-691.312	3.74E-05	911412.090	Down	lasma Membr...	other	diagnosis, ...all 4	
EPCAM	epithelial cell adhe	NM_002354		-91.899	2.42E-04	92292.211	Down	lasma Membr...	other	unspecifi... ...all 1	adecatu... ...all 8
ESRP2	epithelial splicing	NM_024939		-15.577	1.22E-03	13772.022	Down	ucleus	other		
FOXA1	forkhead box A1	NM_004496		-124.470	1.18E-05	251877.977	Down	ucleus	transcription re...	disease p.....all 1	
GRHL2	grainyhead like tr	NM_024915		-896.809	4.56E-05	131150.552	Down	ucleus	transcription re...		
RUNX2*	RUNX family trans	NM_001015051		11.806	5.23E-04	7075.378	Up	ucleus	transcription re...	disease p.....all 3	
ZEB1*	zinc finger E-box	NR_024285*		370.248	1.50E-03	595.442	Up	ucleus	transcription re...		

Figure 4. Causally scoring a My Pathway.

3. Set the "User Dataset" as the reference set when uploading a dataset

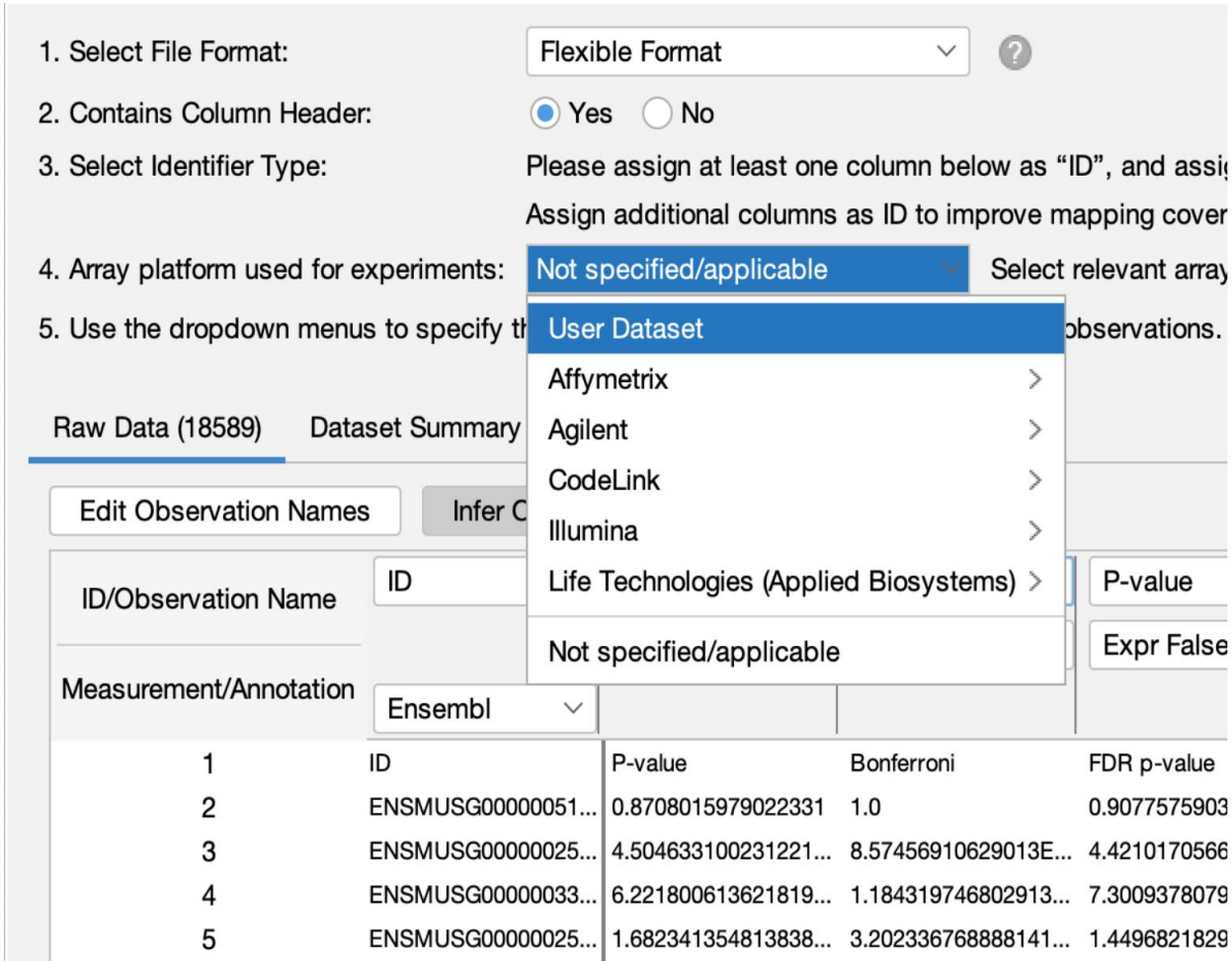


Figure 5. Setting the reference set to User Dataset reference during dataset upload.

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IPA的資料上傳與分析功能介紹

Ingenuity Pathways Analysis的分析的結果回傳

- 與實驗資料相關的生物功能或是疾病分析
- 所影響的Signaling Pathway與Metabolic Pathway以及裡面的組成分子
- 受影響的Transcription regulator的種類以及相關基因與蛋白
- 實驗資料中的分子關係如何形成的網路

分析功能種類:

IPA-Core Analysis: 分析mRNA, miRNA或是protein的實驗資料

IPA-Tox Analysis: 分析後得到毒性學相關結果

IPA-Metabolomics Analysis: 主要用於分析代謝體(Metabolomics)實驗相關資料

Data Upload Workflow

- ID、Observation
- Annotated Dataset

Dataset Preparation

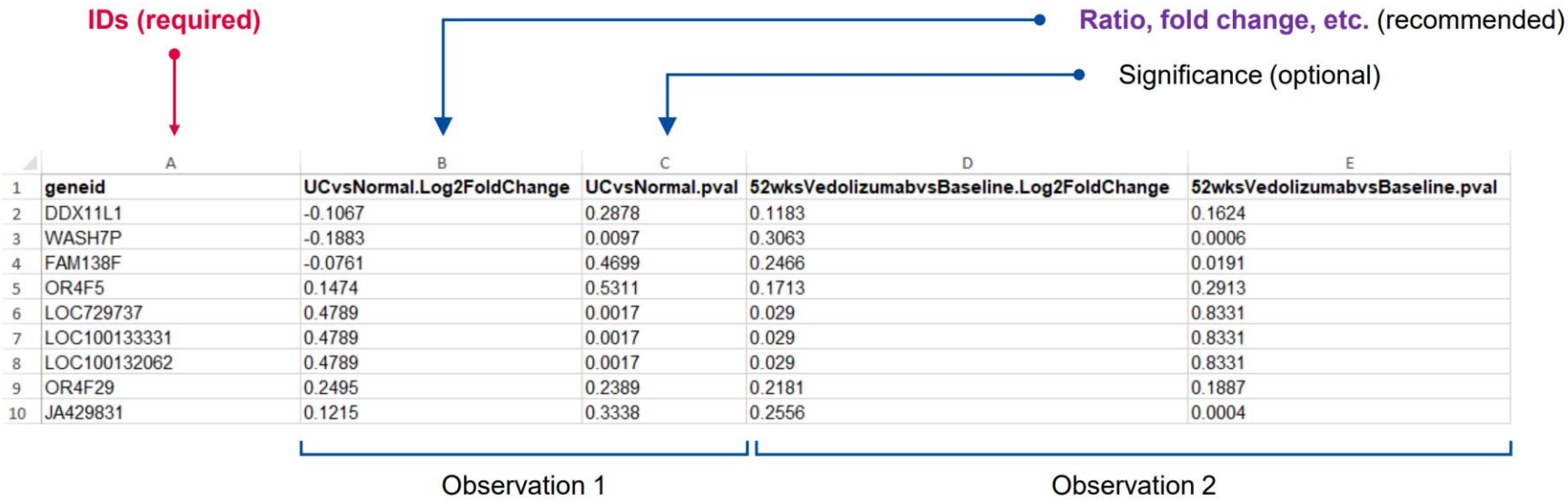
- Create Core Analysis
  - Expression type
  - Measurement type
- Create Expression Analysis
  - Criteria
  - Cutoffs

Analyze Dataset

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

Expression Analysis

Formatting transcriptomics data before uploading to IPA



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

Common identifier types

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

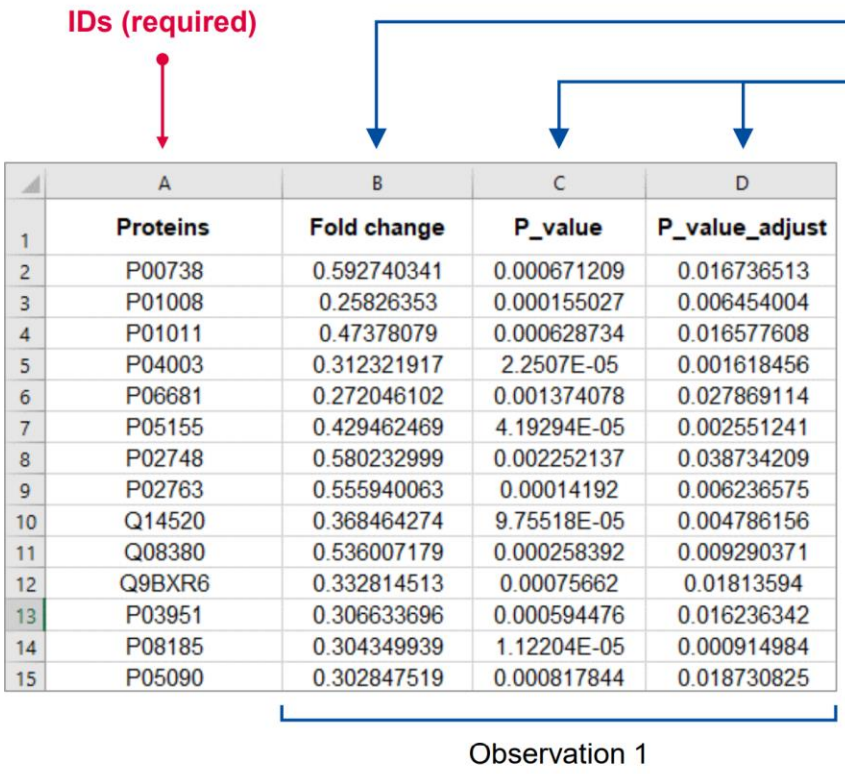
Accepted file formats

- .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

Formatting proteomics data before uploading to IPA



	A	B	C	D
1	**Proteins**	**Fold change**	**P_value**	**P_value_adjust**
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

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**

Formatting metabolomics data before uploading to IPA

Multiple ID columns

Ratio, fold change, etc. (recommended)

(optional)

Significance (optional)

	A	B	C	D	E	F	G	H
1	**Pubchem**	**Kegg**	**HMDB**	**CAS**	**Metabolites**	**Fold change**	**P_value**	**P_value_adjust**
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://biodbnet-abcc.ncifcrf.gov/db/db2db.php
- https://cts.fiehnlab.ucdavis.edu/batch
- http://csbg.cnb.csic.es/mbrole2/conversion.php

Formatting omics data before uploading to IPA

Observation 1

Observation 2

	A	B	C	D	E
1	**geneid**	**UCvsNormal.Log2FoldChange**	**UCvsNormal.pval**	**52wksVedolizumabvsBaseline.Log2FoldChange**	**52wksVedolizumabvsBaseline.pval**
2	DDX11L1	-0.1067	0.2878	0.1183	0.1624
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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

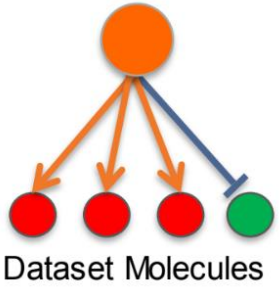
What do colors mean in IPA?

Pathway or gene activity predicted by IPA

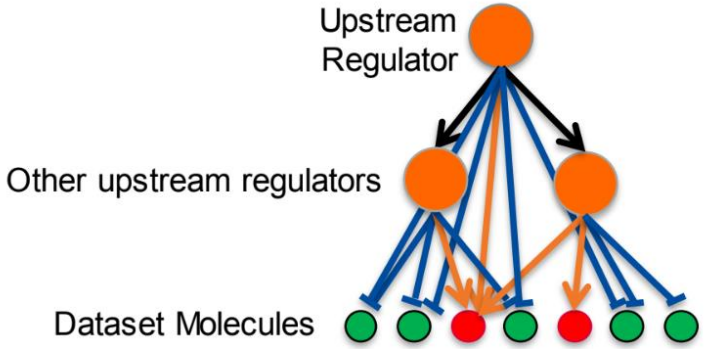
Actual measurement of gene expression in your dataset

Network types in IPA Core Analysis

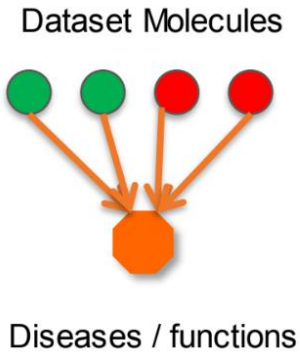
Upstream Analysis



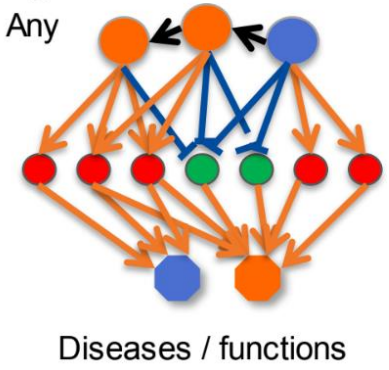
Mechanistic Network of Upstream Regulators



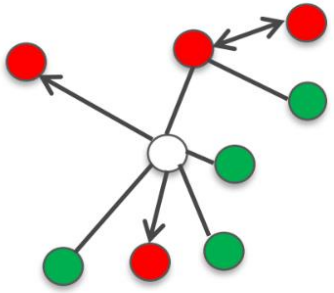
Function Analysis



Regulator Effect Network

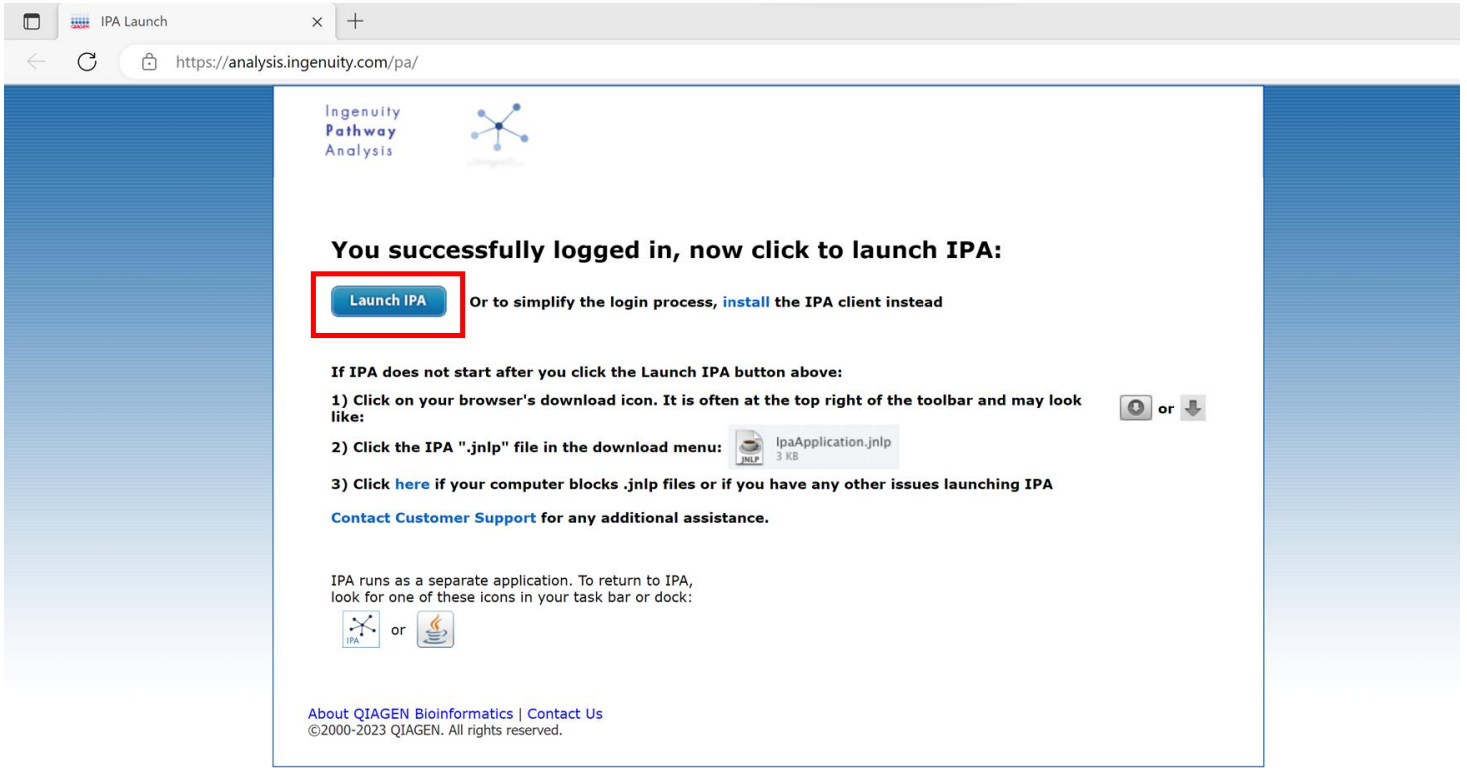


Interaction Network



Dataset: https://reurl.cc/9V2Ogj

use the browser version of IPA: (如果您有登入錯誤或是網路連線問題)
https://analysis.ingenuity.com/pa/



Live Demo

Data Upload

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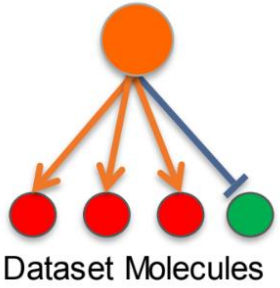
Network types in IPA Core Analysis

Upstream Analysis



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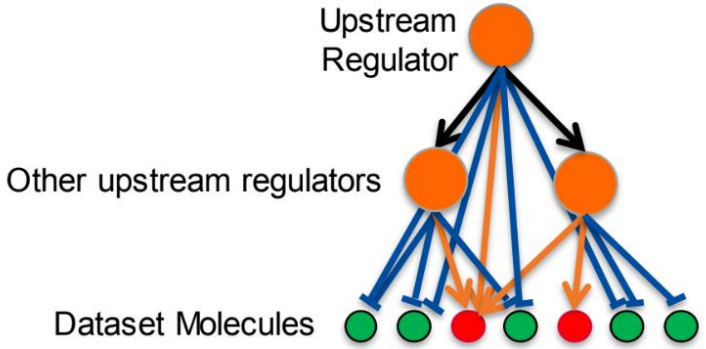

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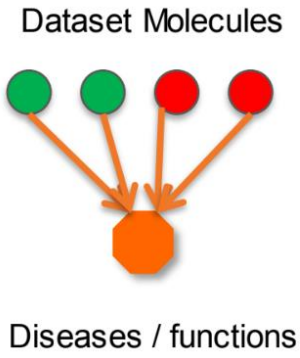
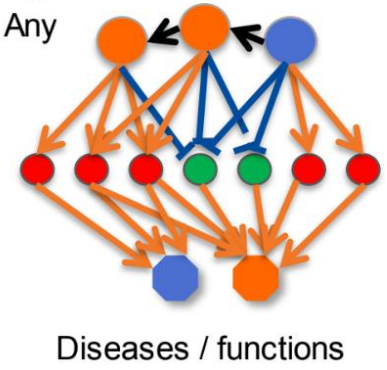


Regulator Effect Network



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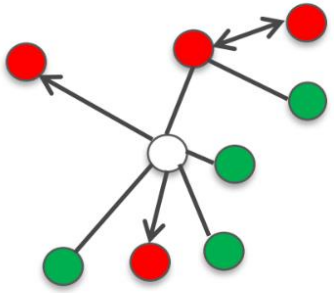


IPA analysis result

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.

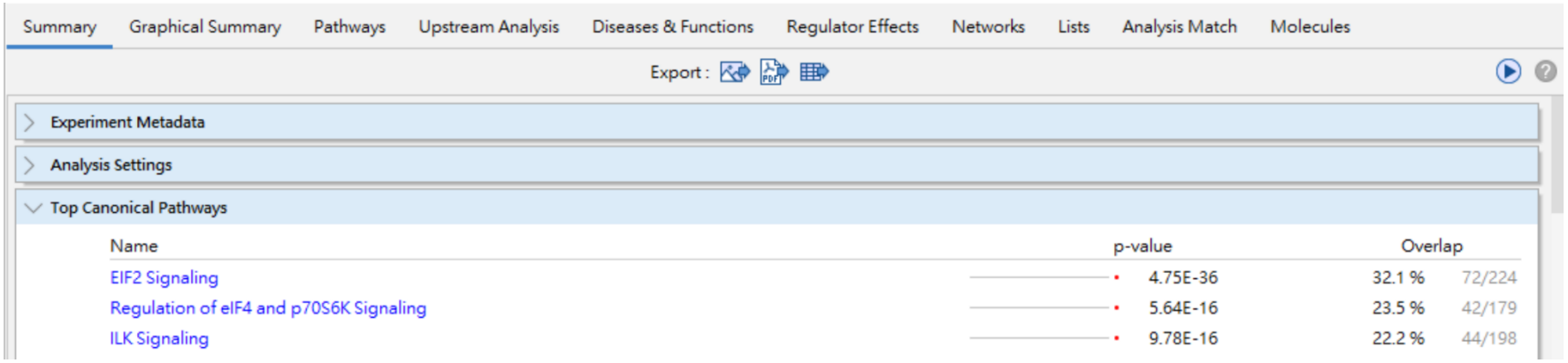
Pathways: List the Signaling Pathway and Metabolic Pathway affected by the experiment

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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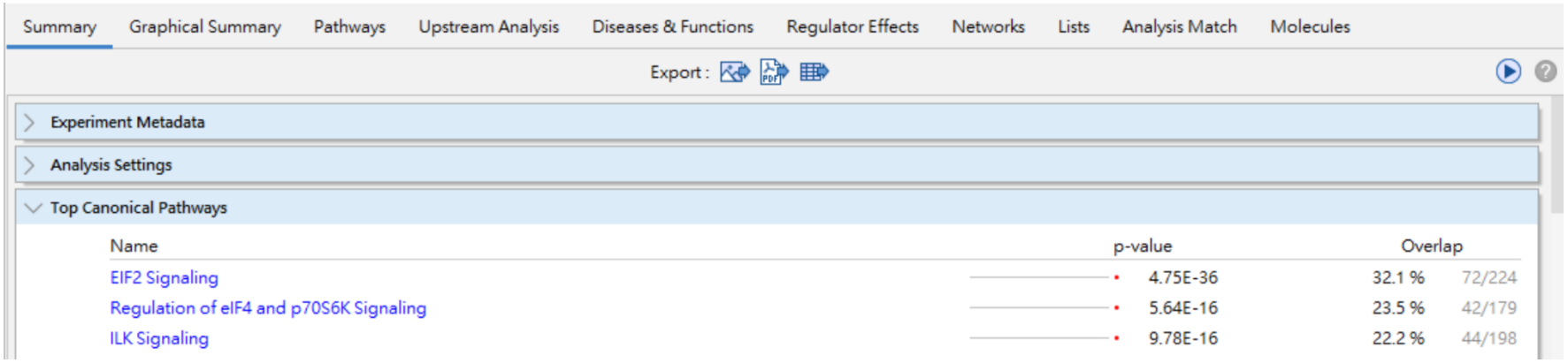
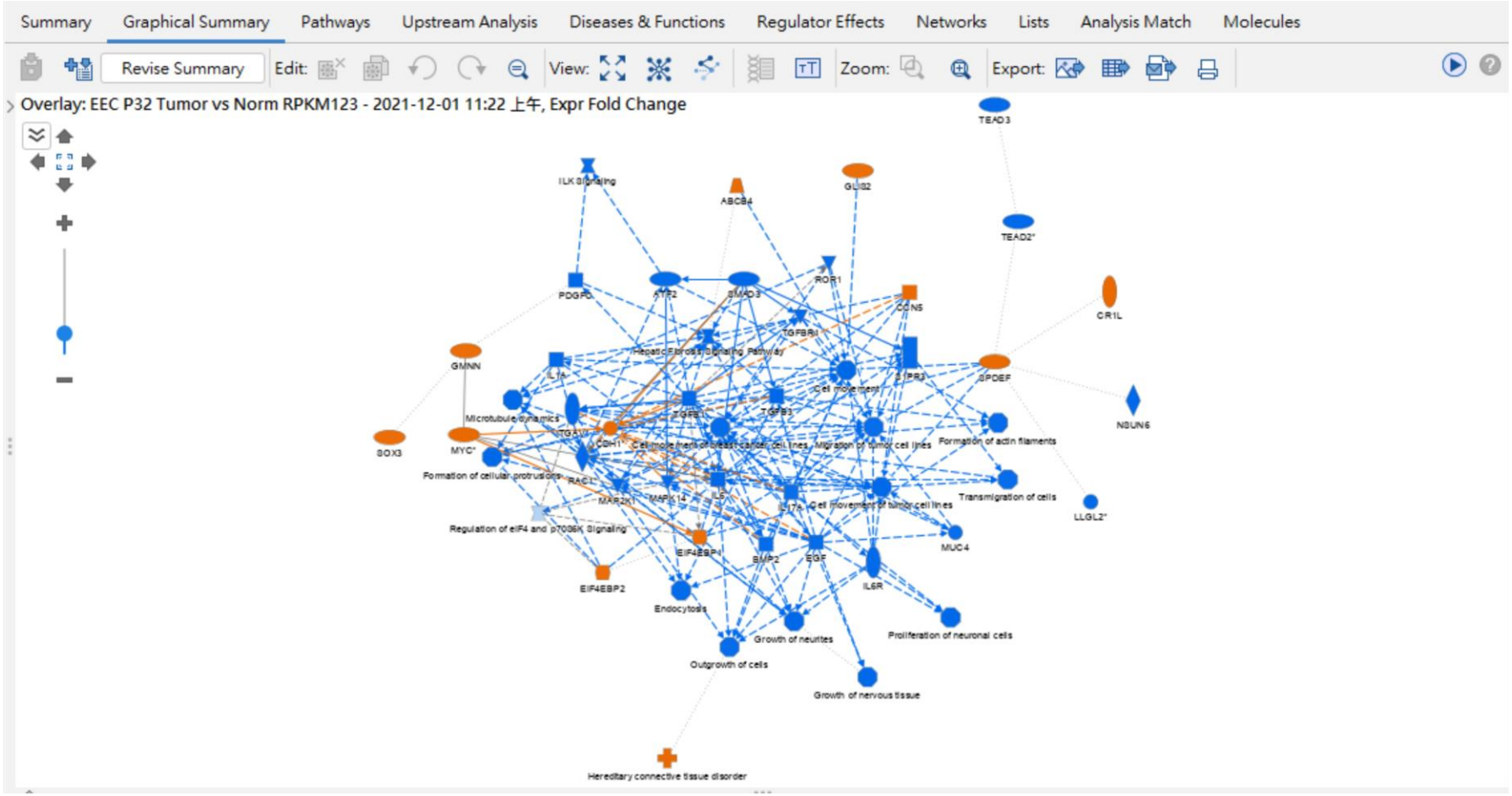


Graphical Summary

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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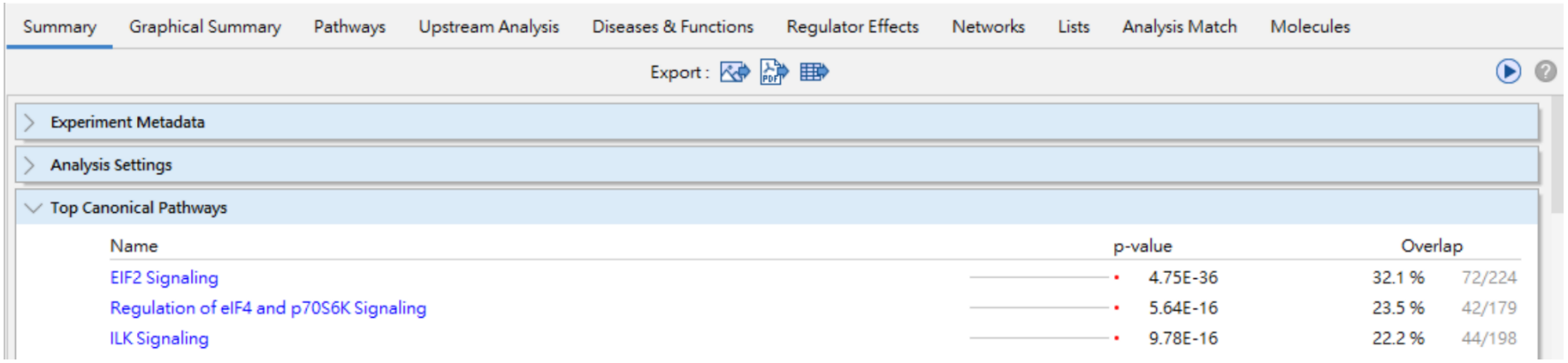
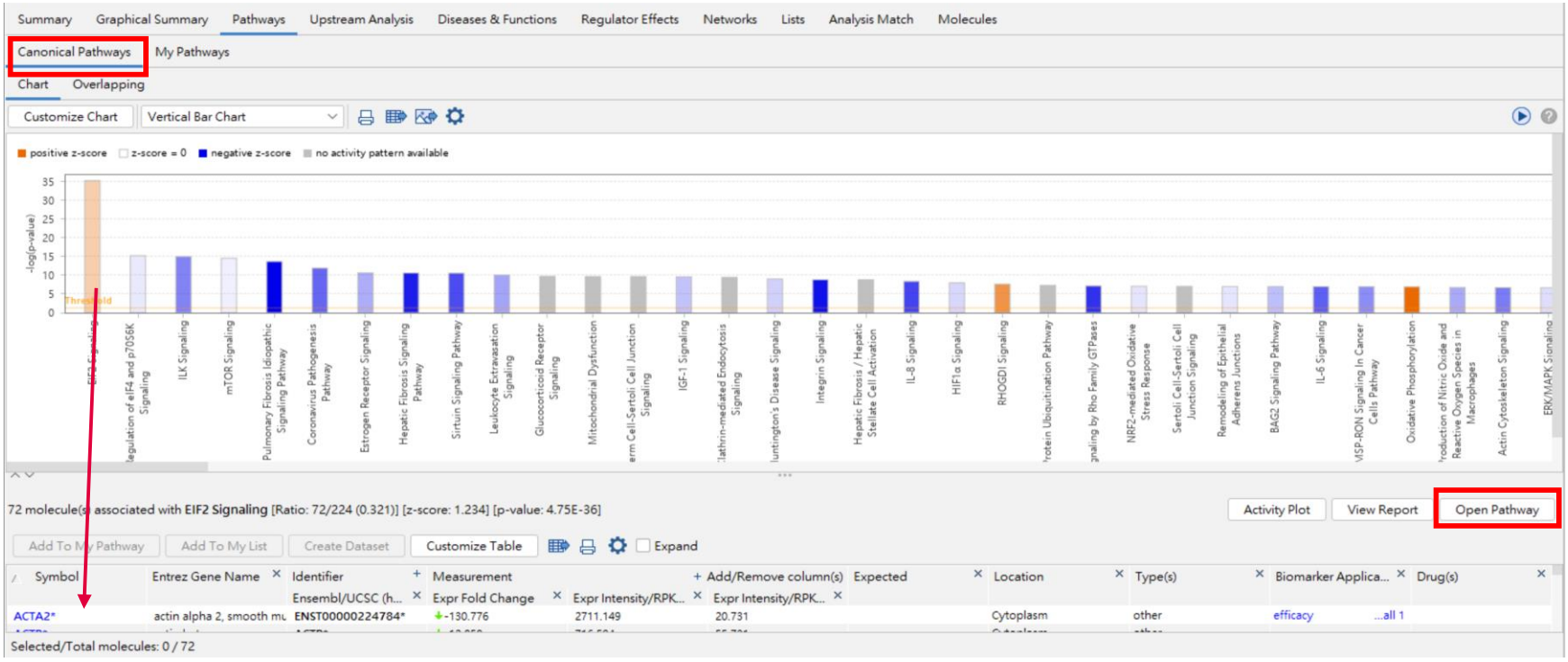
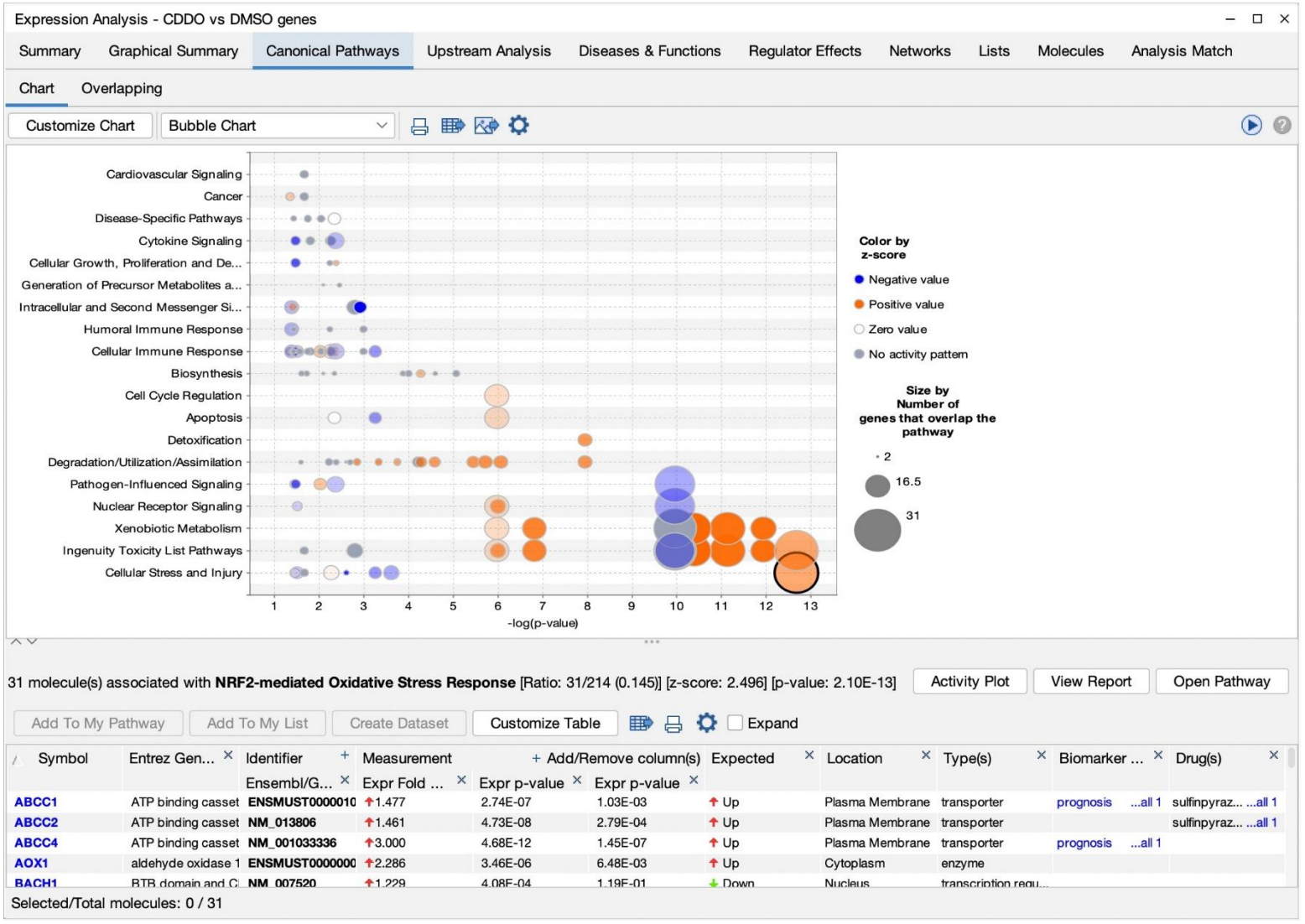


Canonical Pathway Analysis

- 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.



Bubble Charts



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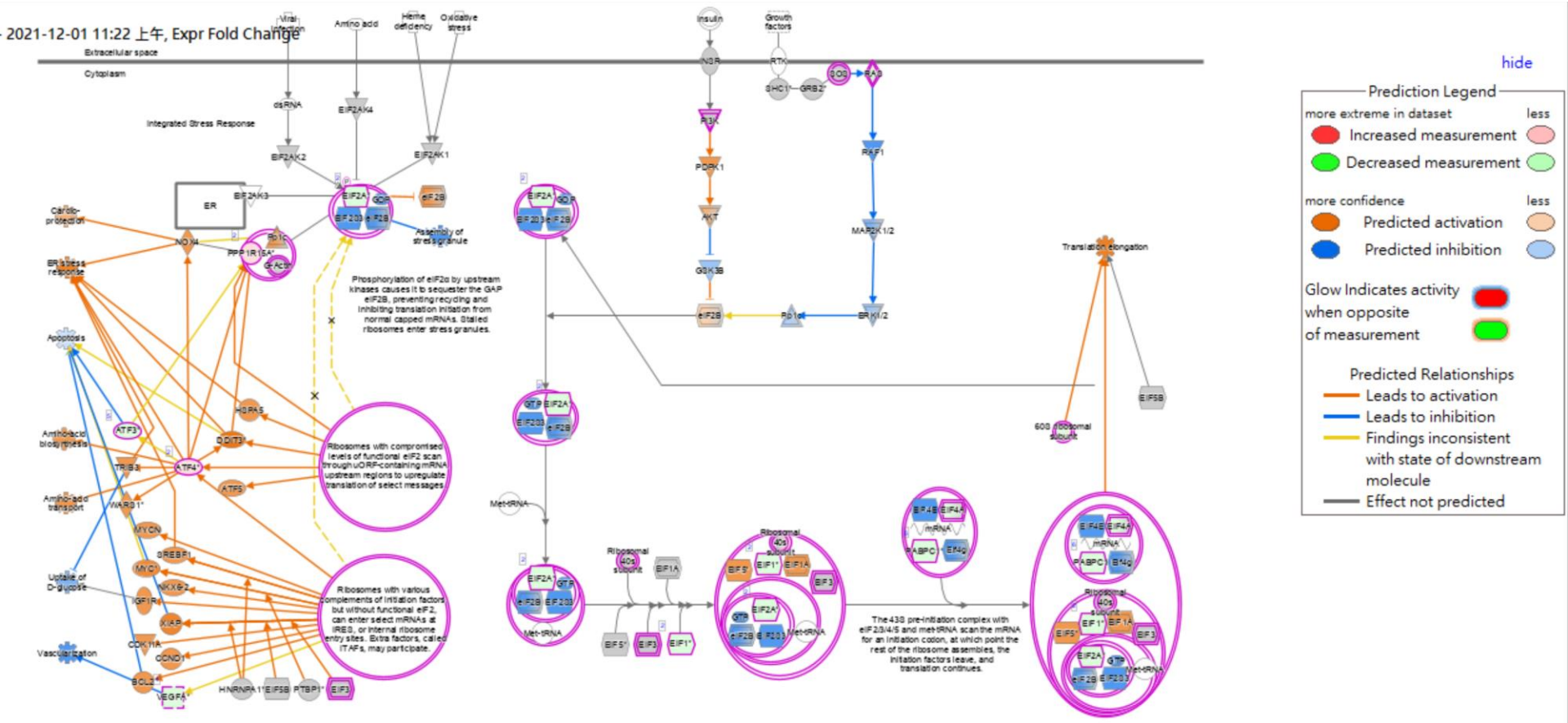
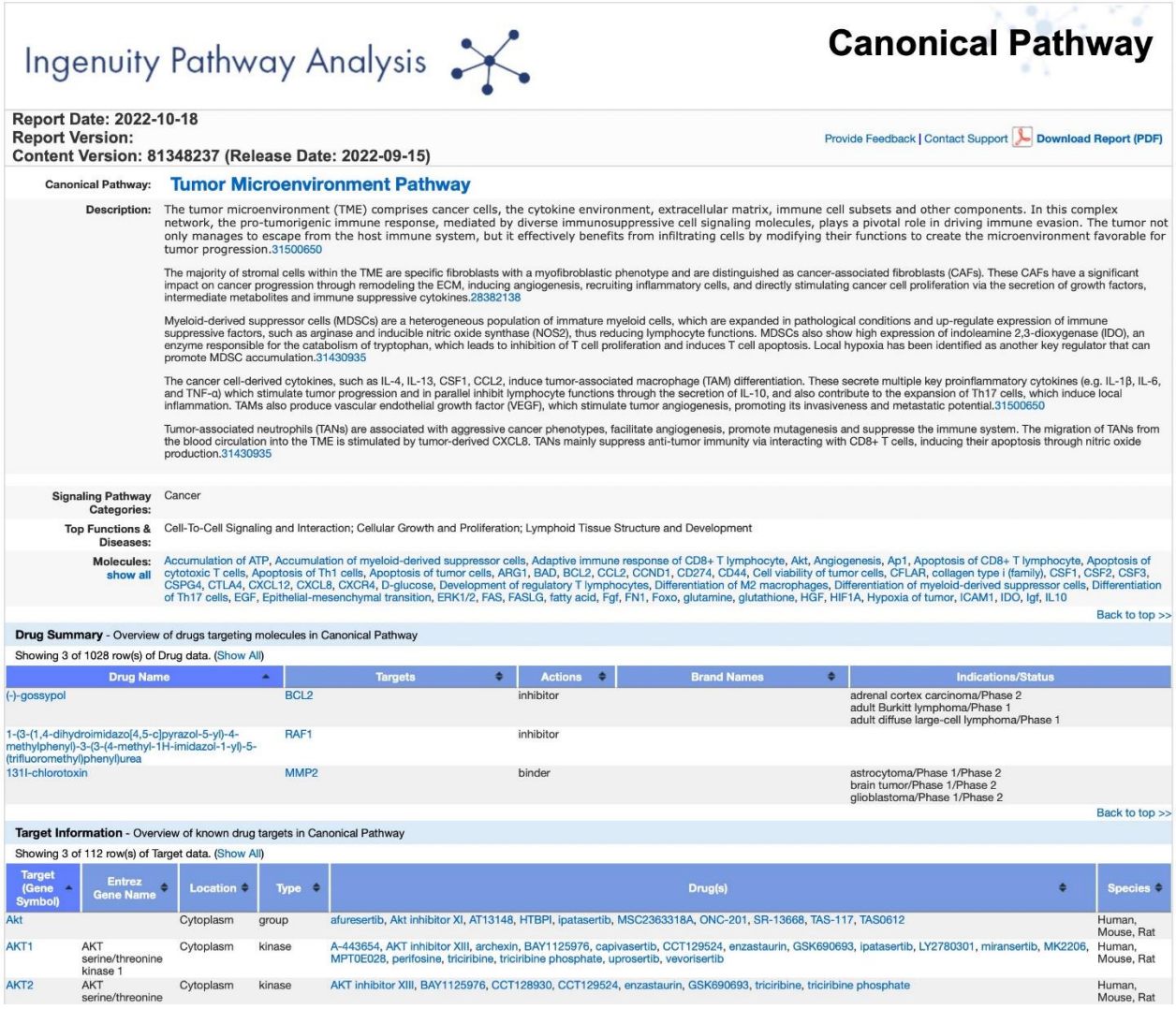


Click on the **View Report** button to display the Canonical Pathway report.



Ingenuity Pathway Analysis

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 suppresse 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, CCND1, CD274, CD44, Cell viability of tumor cells, CFLAR, 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

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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		
131I-chlorotoxin	MMP2	binder		astrocytoma/Phase 1/Phase 2 brain tumor/Phase 1/Phase 2 glioblastoma/Phase 1/Phase 2

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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, triciribine, triciribine phosphate, uprosertib, vevorisertib	Human, Mouse, Rat
AKT2	AKT serine/threonine	Cytoplasm	kinase	AKT inhibitor XIII, BAY1125976, CCT128930, CCT129524, enzastaurin, GSK690693, triciribine, triciribine phosphate	Human, Mouse, Rat

IPA analysis result

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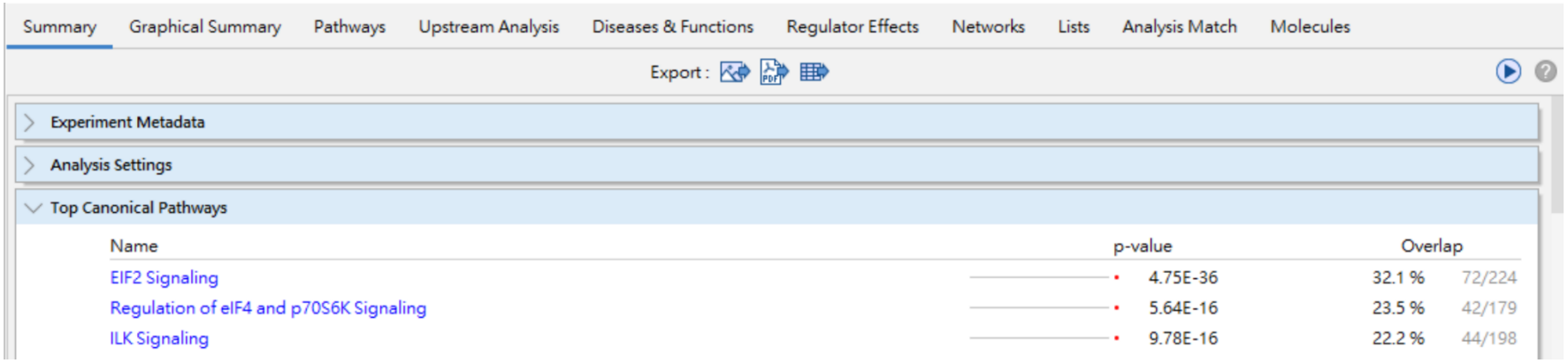


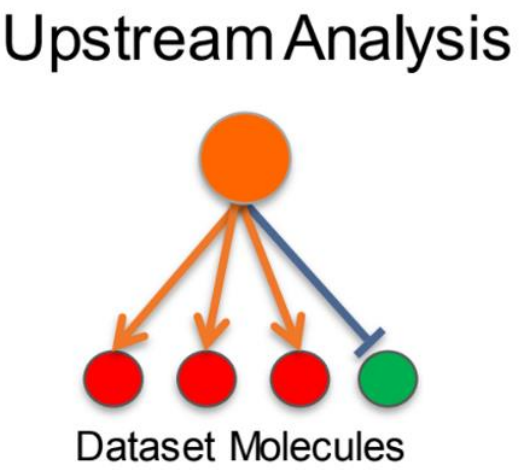
Regulator Analysis: How does it work?

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



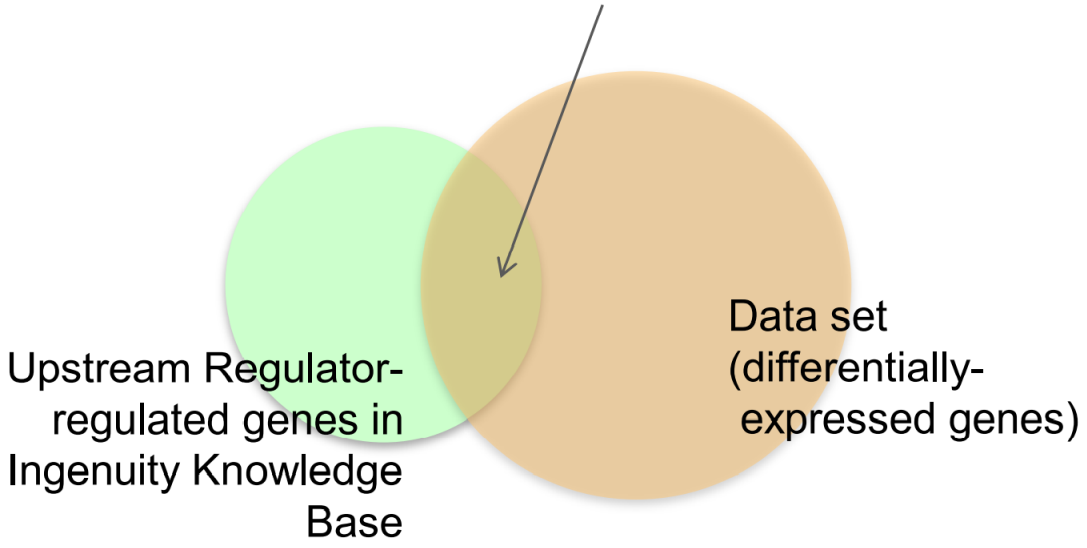
Upstream Regulator Analysis: How does it work?

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



Statistical Analyses Used in IPA

p-value of overlap

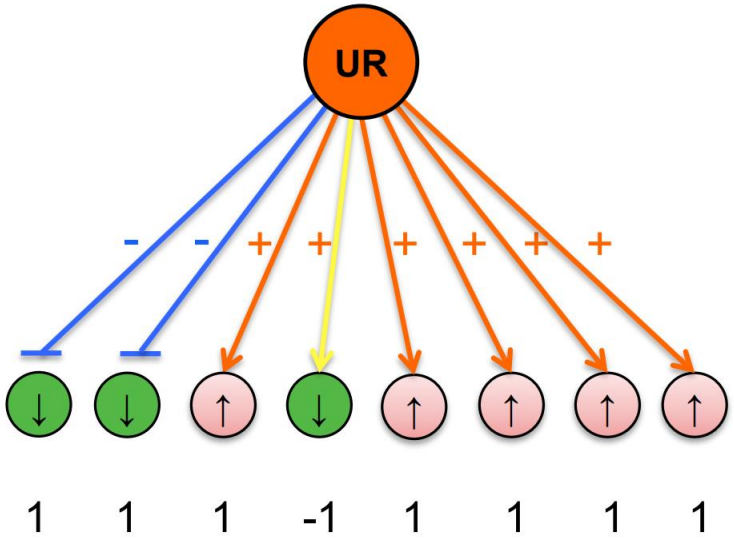
- Null hypothesis: No overlap between molecules from dataset and disease/function/upstream regulator/pathway.
- Calculate using the right-tailed Fisher's Exact Test.
- Significant p-value ≤ 0.05

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

Predicting upstream regulators of a dataset



← 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

$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}}$$

$= (7\text{-}1)/\sqrt{8} = 2.12$ (=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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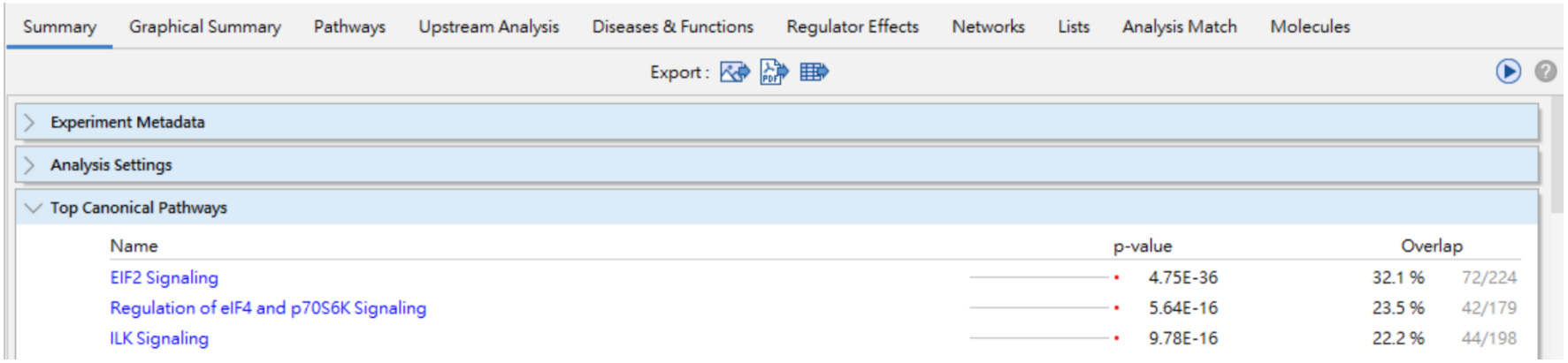
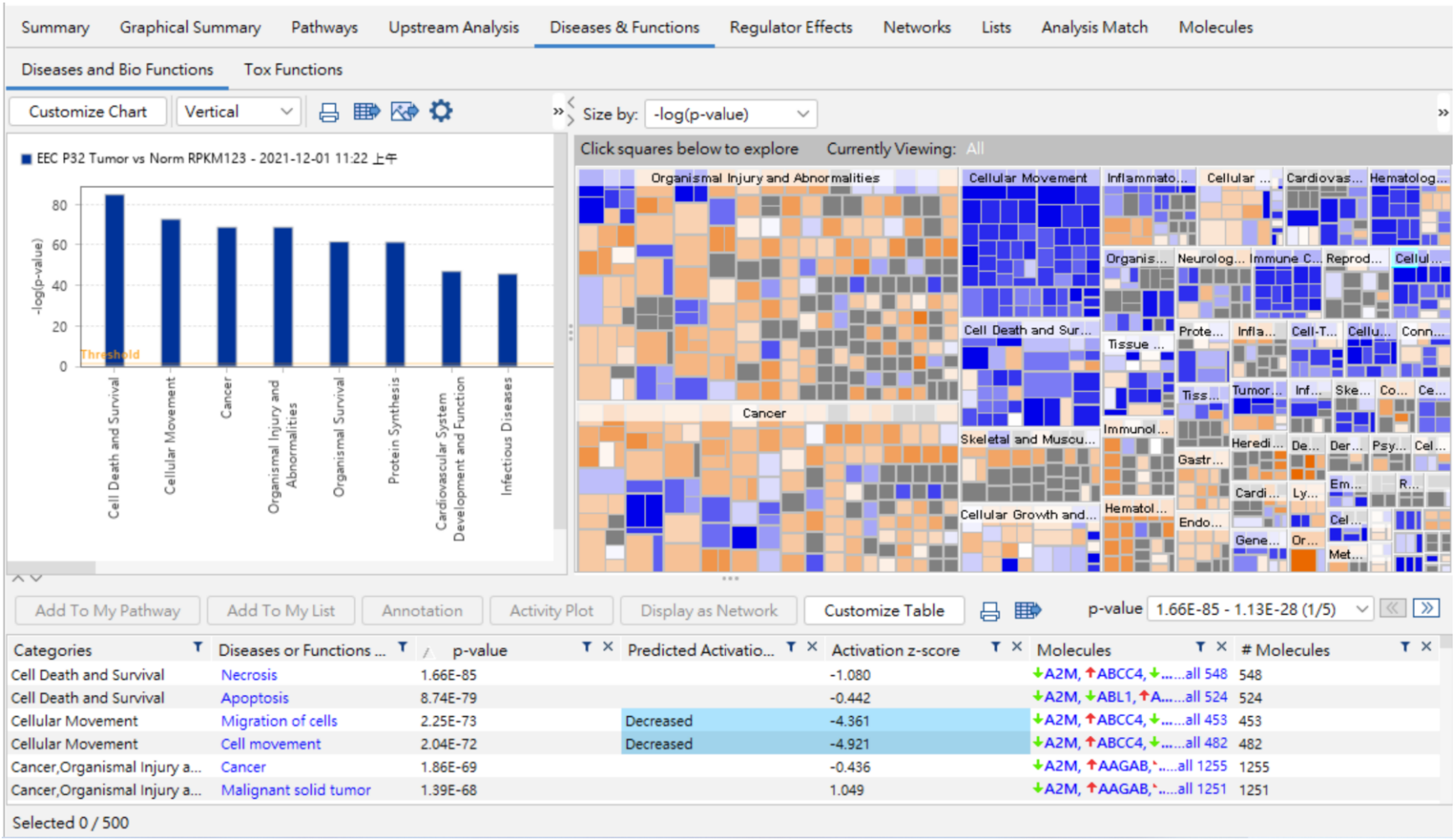


Interpret Downstream Biological Functions



Categories	Diseases or Functions ...	p-value	Predicted Activatio...	Activation z-score	Molecules	# Molecules
Cell Death and Survival	Necrosis	1.66E-85		-1.080	↓A2M, ↑ABCC4, ↓....all 548	548
Cell Death and Survival	Apoptosis	8.74E-79		-0.442	↓A2M, ↓ABL1, ↑A....all 524	524
Cellular Movement	Migration of cells	2.25E-73	Decreased	-4.361	↓A2M, ↑ABCC4, ↓....all 453	453
Cellular Movement	Cell movement	2.04E-72	Decreased	-4.921	↓A2M, ↑ABCC4, ↓....all 482	482
Cancer,Organismal Injury a...	Cancer	1.86E-69		-0.436	↓A2M, ↑AAGAB,*....all 1255	1255
Cancer,Organismal Injury a...	Malignant solid tumor	1.39E-68		1.049	↓A2M, ↑AAGAB,*....all 1251	1251

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

Diseases & Function

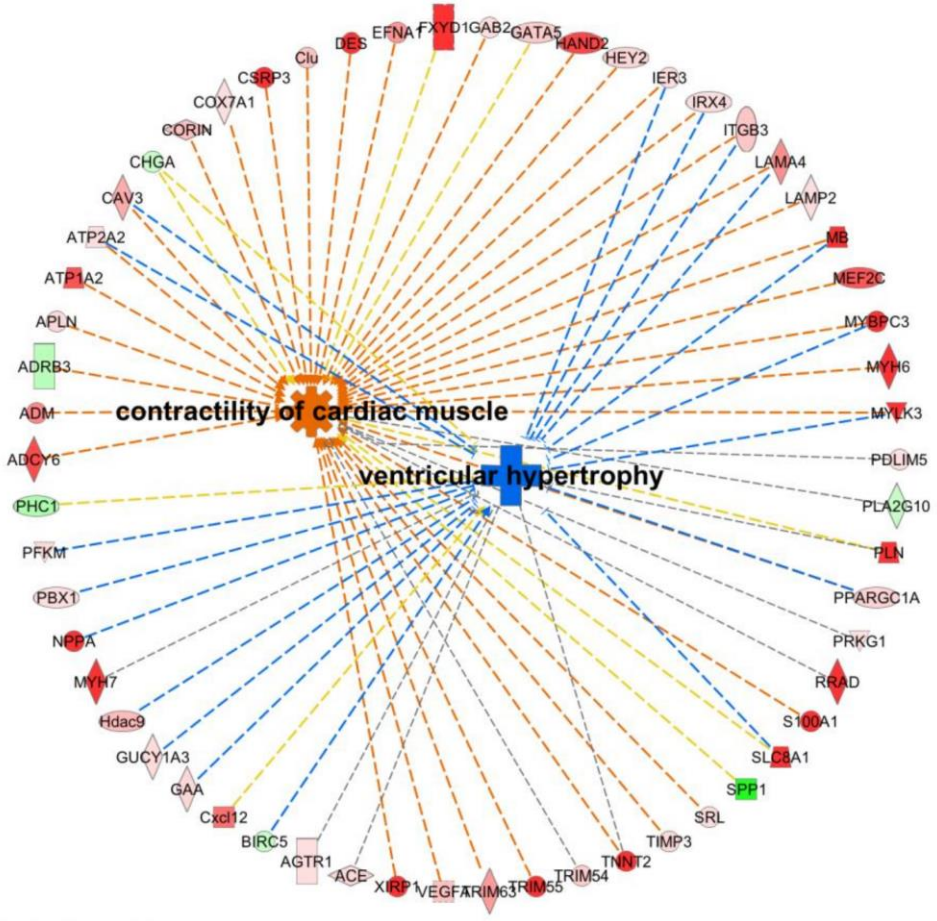
方塊代表受實驗影響的生物功能與疾病，顏色可 以用[Color by]指定是z-score, -log (p-value), 或是 # of genes上色。如果是用z-score上色的 話，藍色區塊是預測被減低的功能，橘色則是此 功能會增加。是根據實驗資料做出的演算。

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

Disease and Molecules relationships

- 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.



Disease or Function View

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 leucocytes

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-ethylbenzylidine)-2-thioxothiazolidin-4-one, (±)-4-DOI, 1,1-diethyl-2-hydroxy-2-nitrosohydrazine, 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 lipoxygenase, 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-acetoxyphenyl)-2-chloro-N-methylethylamine, 2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole, 2-arachidonoylglycerol, 2-mercaptoacetate, 2610528A11Rik, 27-hydroxycholesterol, 3'-O-(4-benzoyl)benzoyladenosine 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

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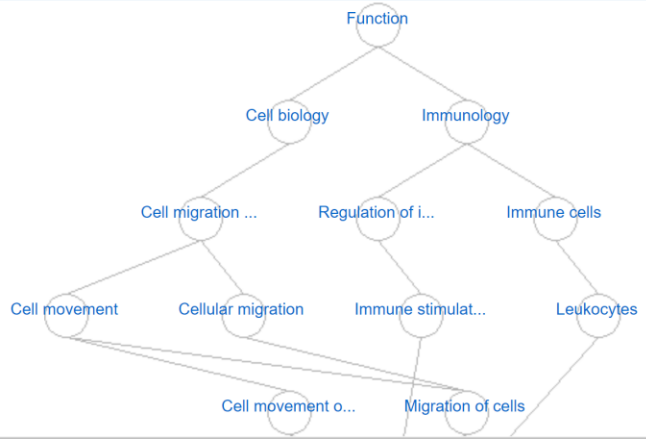
Ontology



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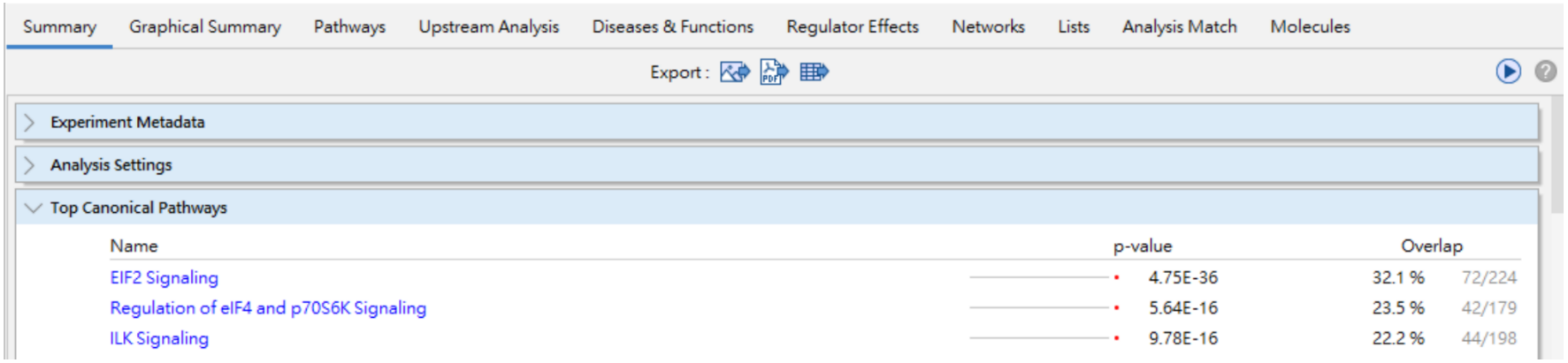
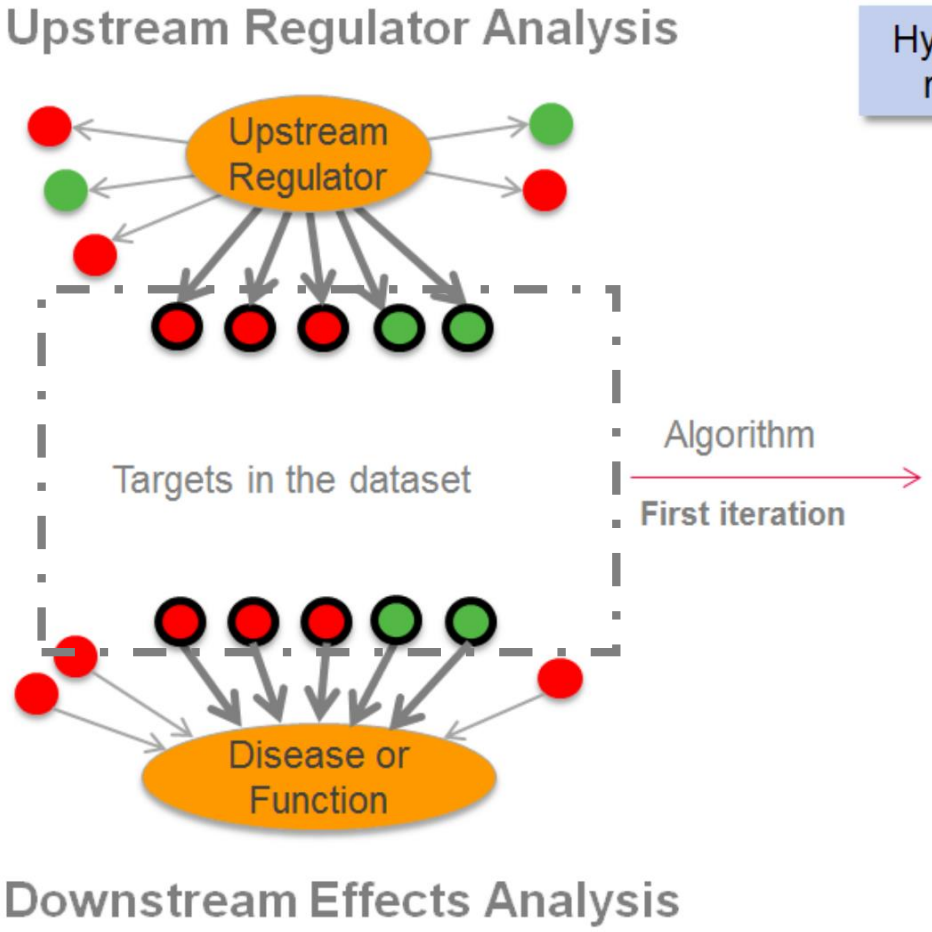
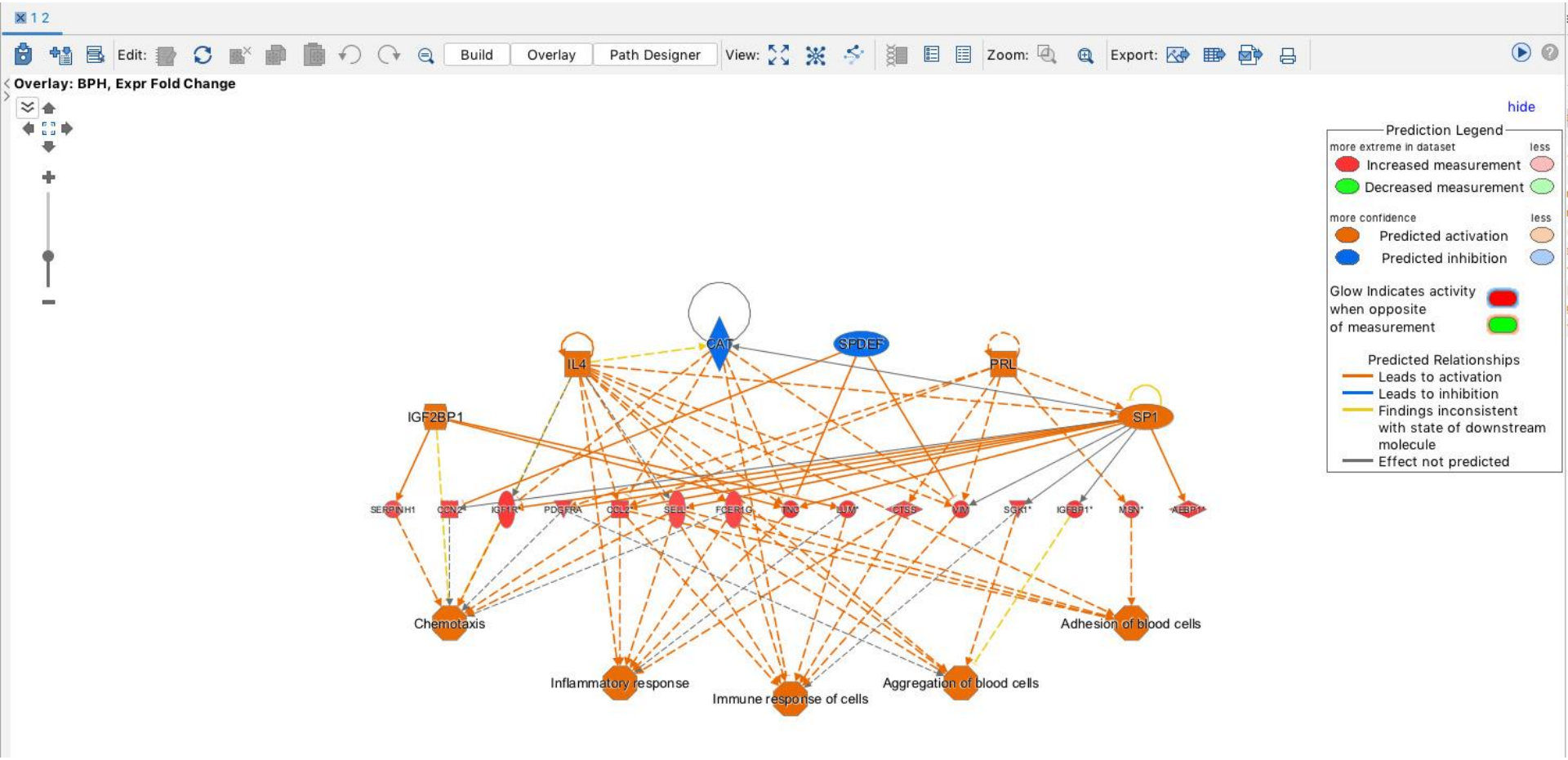


How does the Regulator Effects algorithm work?



Regulator Effects

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



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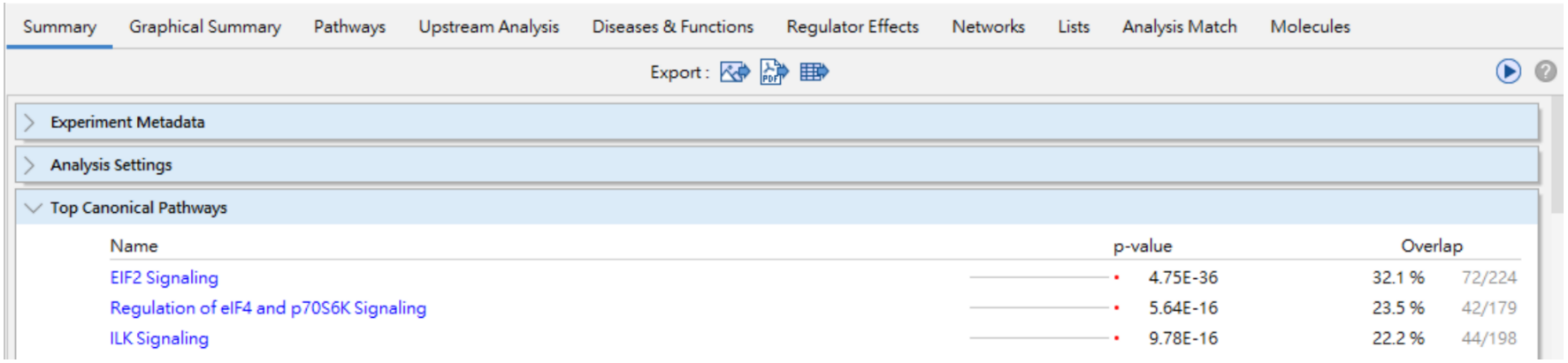
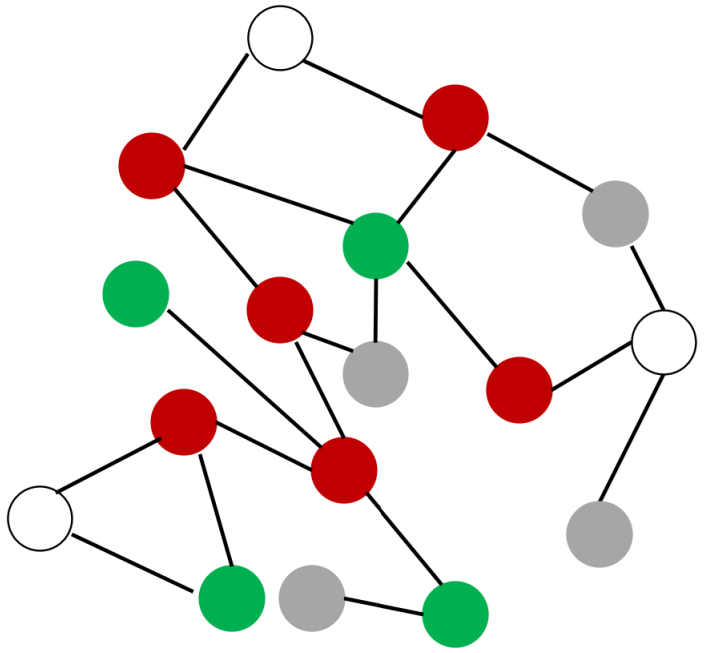


How Networks Are Generated

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

Networks in IPA

Purpose:

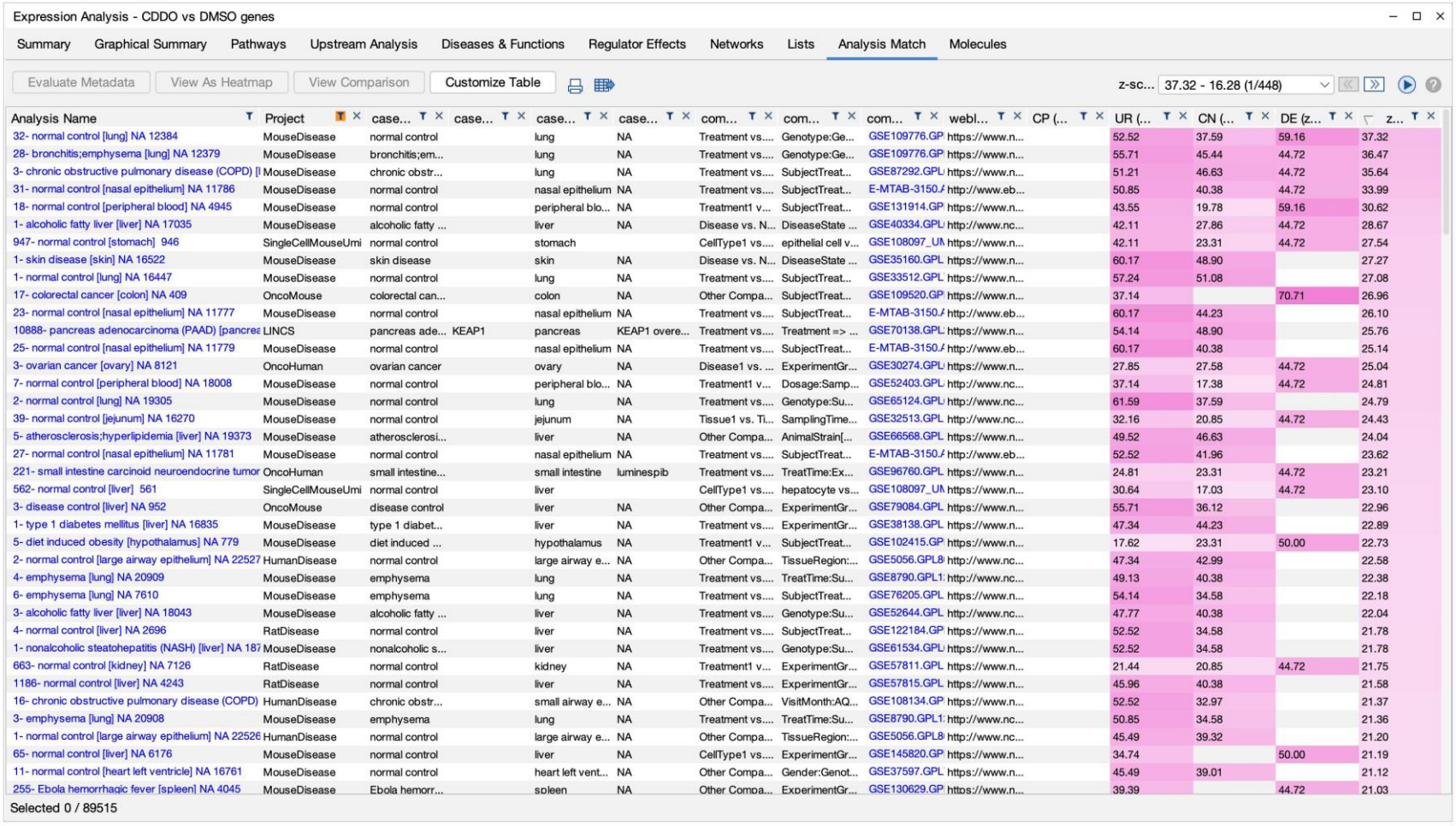
- To show as many interactions between user-specified molecules in a given dataset and how they might work together at the molecular level

Why are Ingenuity networks biologically interesting?

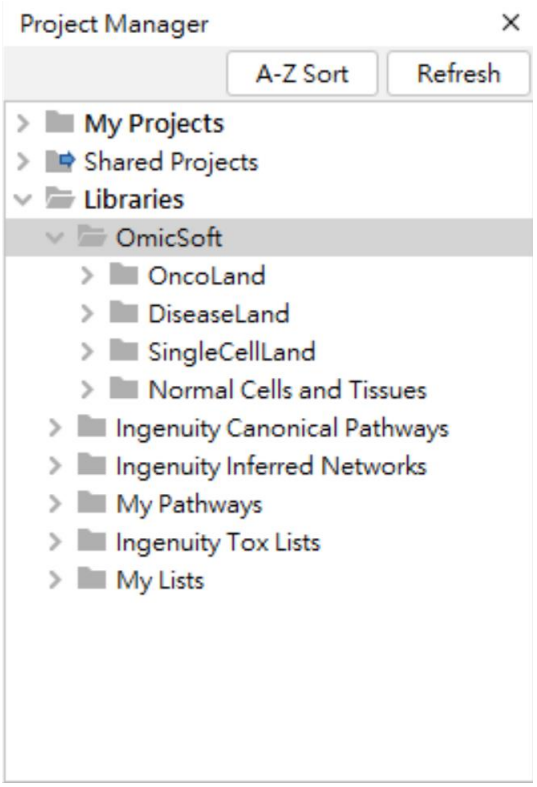
- Highly-interconnected networks are likely to represent significant biological function
- Networks involve molecules you don’t see in your data set. This allows genes you have assayed to be linked to metabolites and chemicals that you could not have assayed for, to imply a regulation network that is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

Analysis Match

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.



OmicSoft Datasets Stored and Analyzed in IPA



DiseaseLandHumanDisease
- •MouseDisease
- •RatDisease
- •LINCS

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

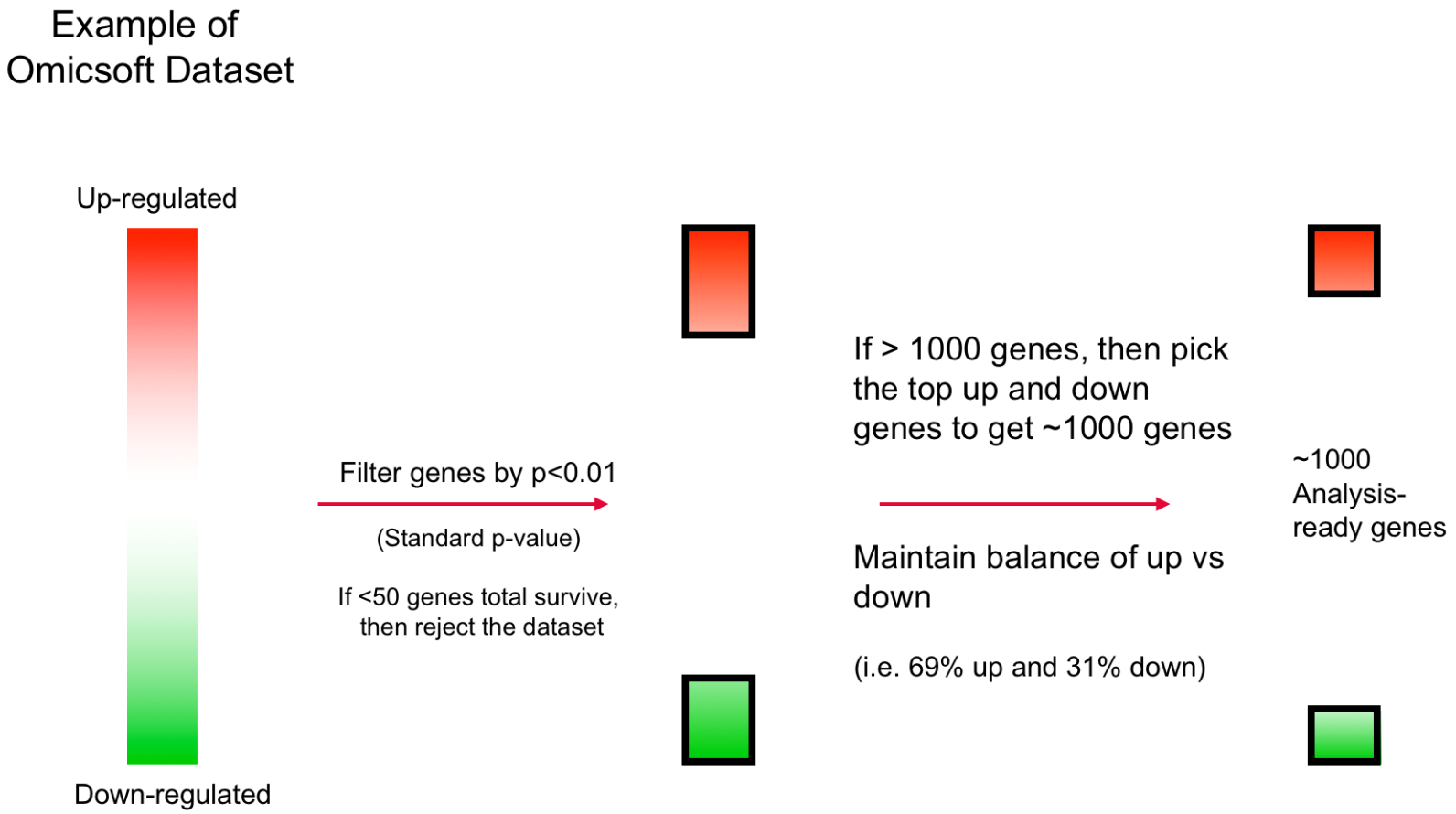
SingleCellLand
- •SingleCellHuman
- •SingleCellHumanUmi
- •SingleCellHumanUmiLite
- •SingleCellHumanHCL
- •SingleCellMouse
- •SingleCellMouseUmi
- •SingleCellMouseUmiLite

Normal Cells and Tissues
- •Human Tissues (GTEx)

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

Analysis Match

How OmicSoft datasets were analyzed in IPA



Analysis Match

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

- 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

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

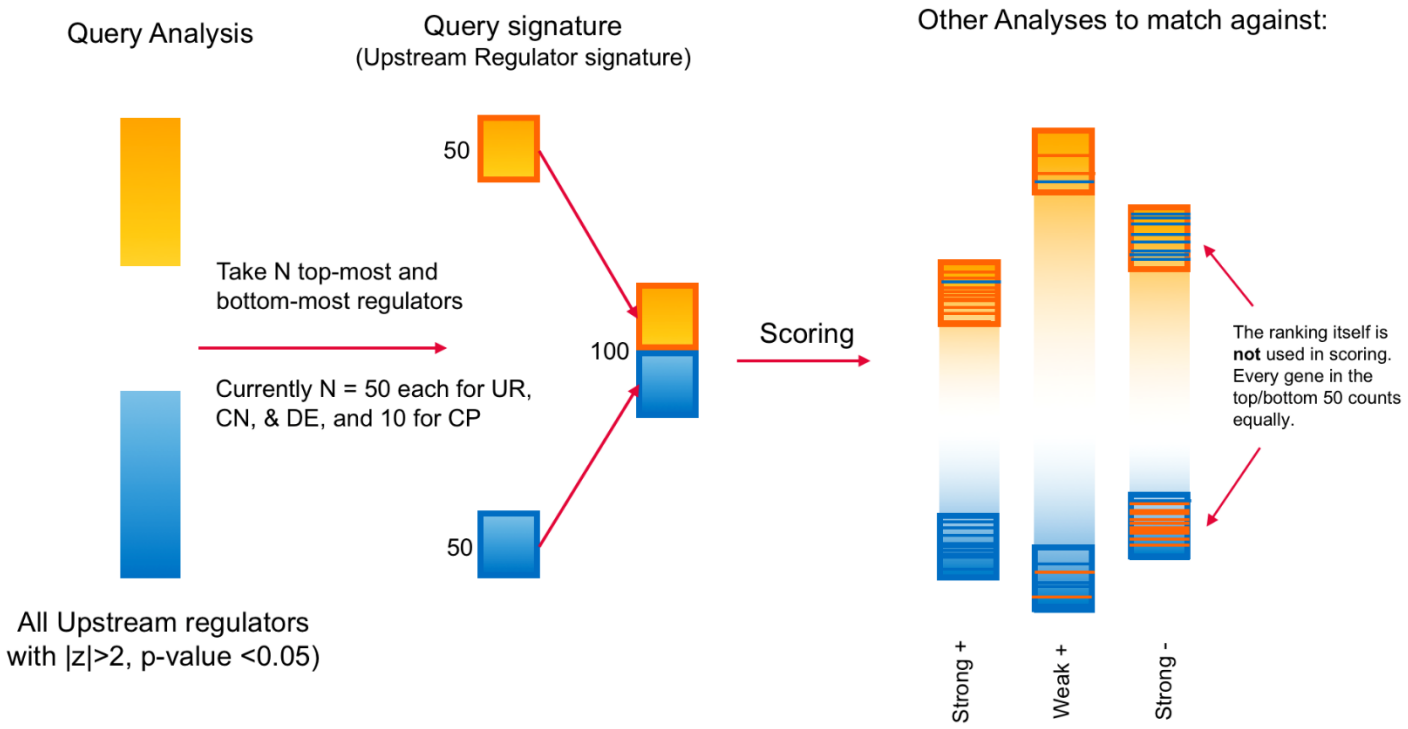
Yes, it matches (because z>2)

Analysis Match

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

Agenda

1. What's New in the IPA Winter Release (December 2022)

2. Data Upload and How to Run a Core Analysis
   Upload experiment data

3. Functional Interpretation in IPA
   Introduction for Analysis Tools

4. Comparison Analyses

5. Q&A

Single Experiment

- Time Course
- Dose Response

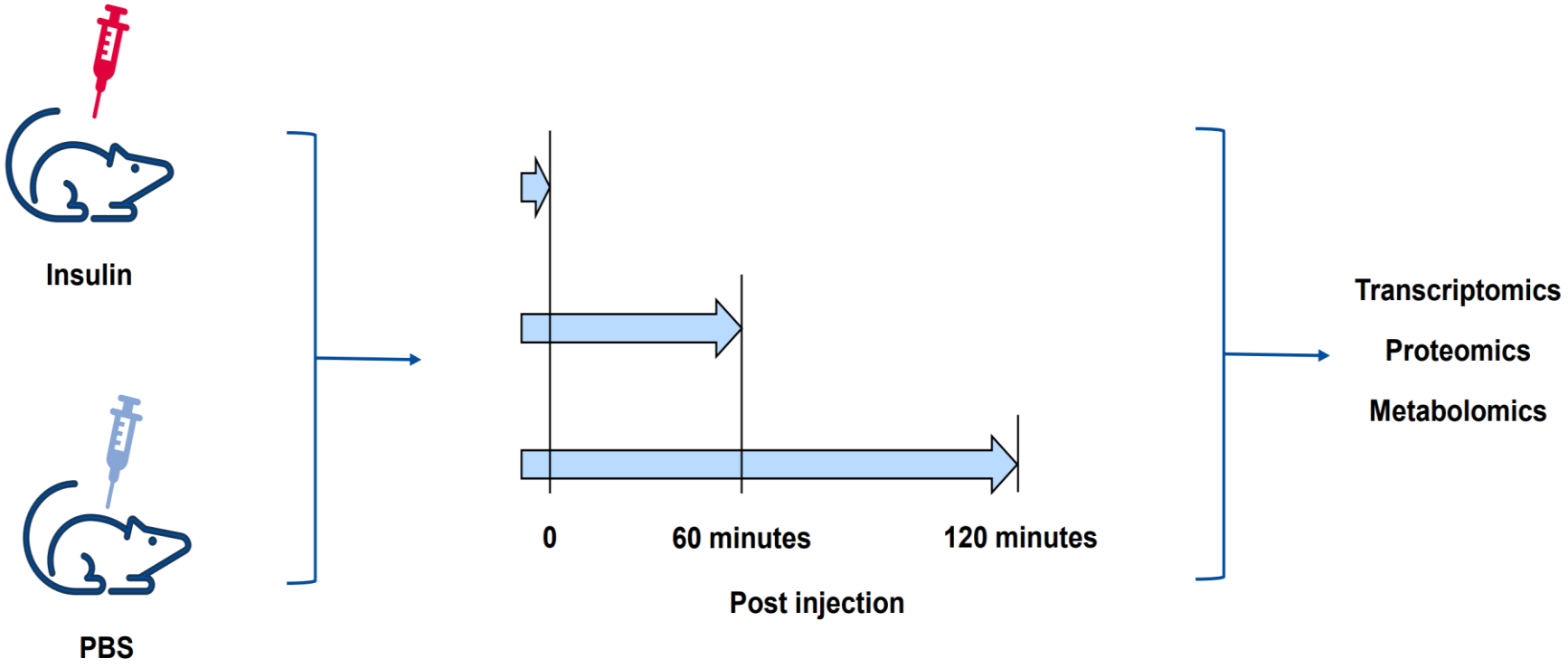
Multi Experiment

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

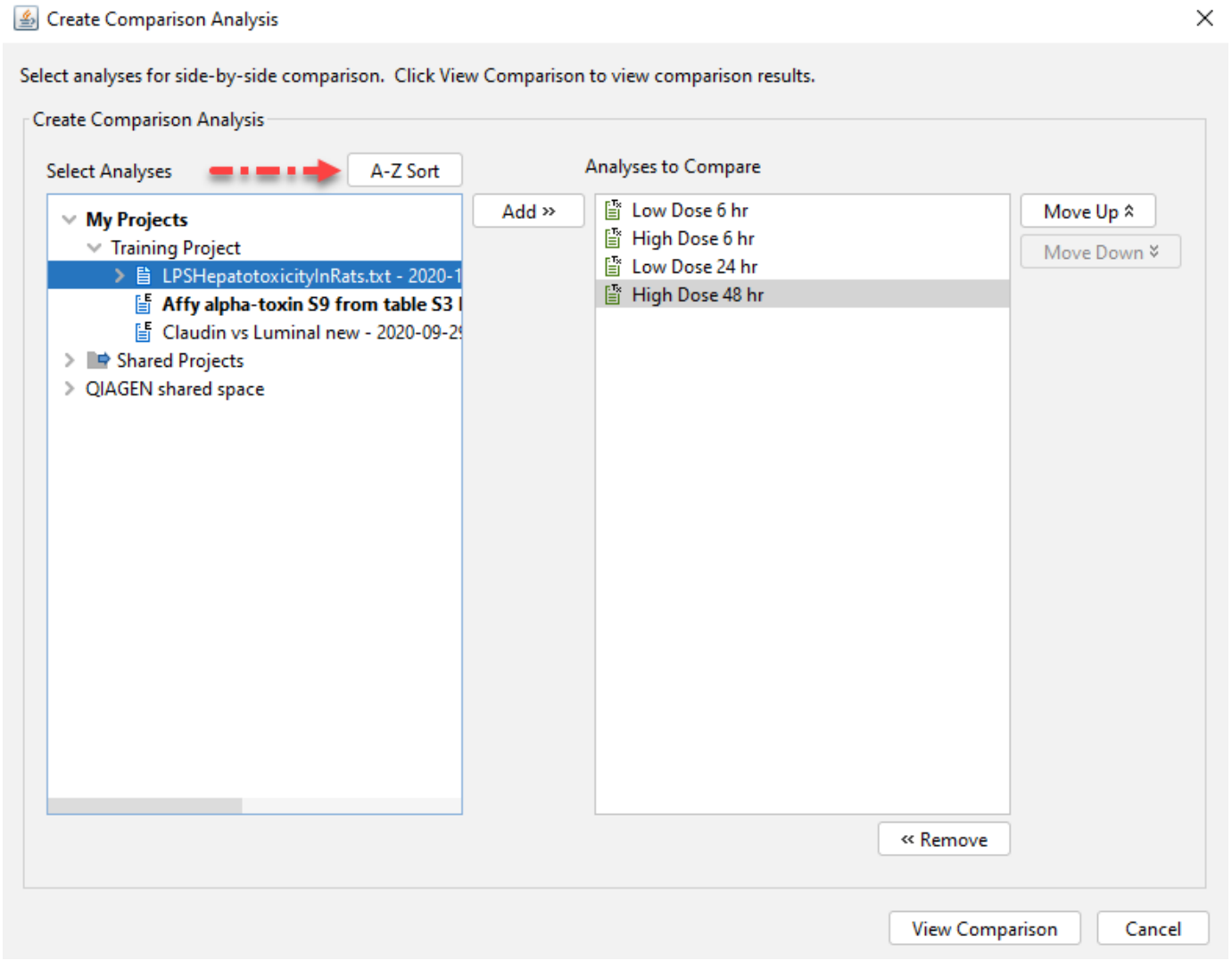
Set Analysis

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

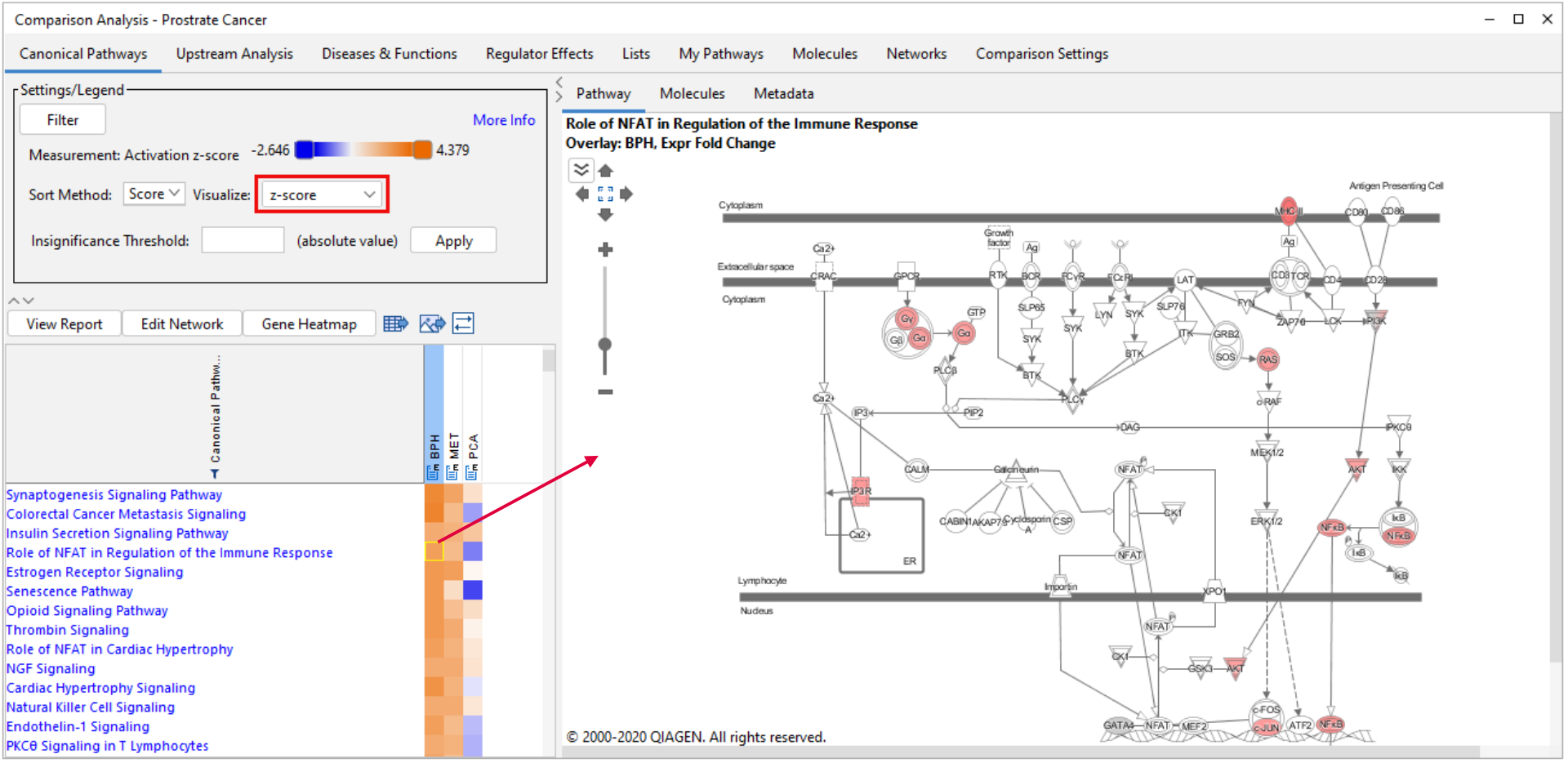
Multi-omics analysis of the liver in response to insulin



To start a Comparison Analysis



Comparison analysis heat map for canonical pathways



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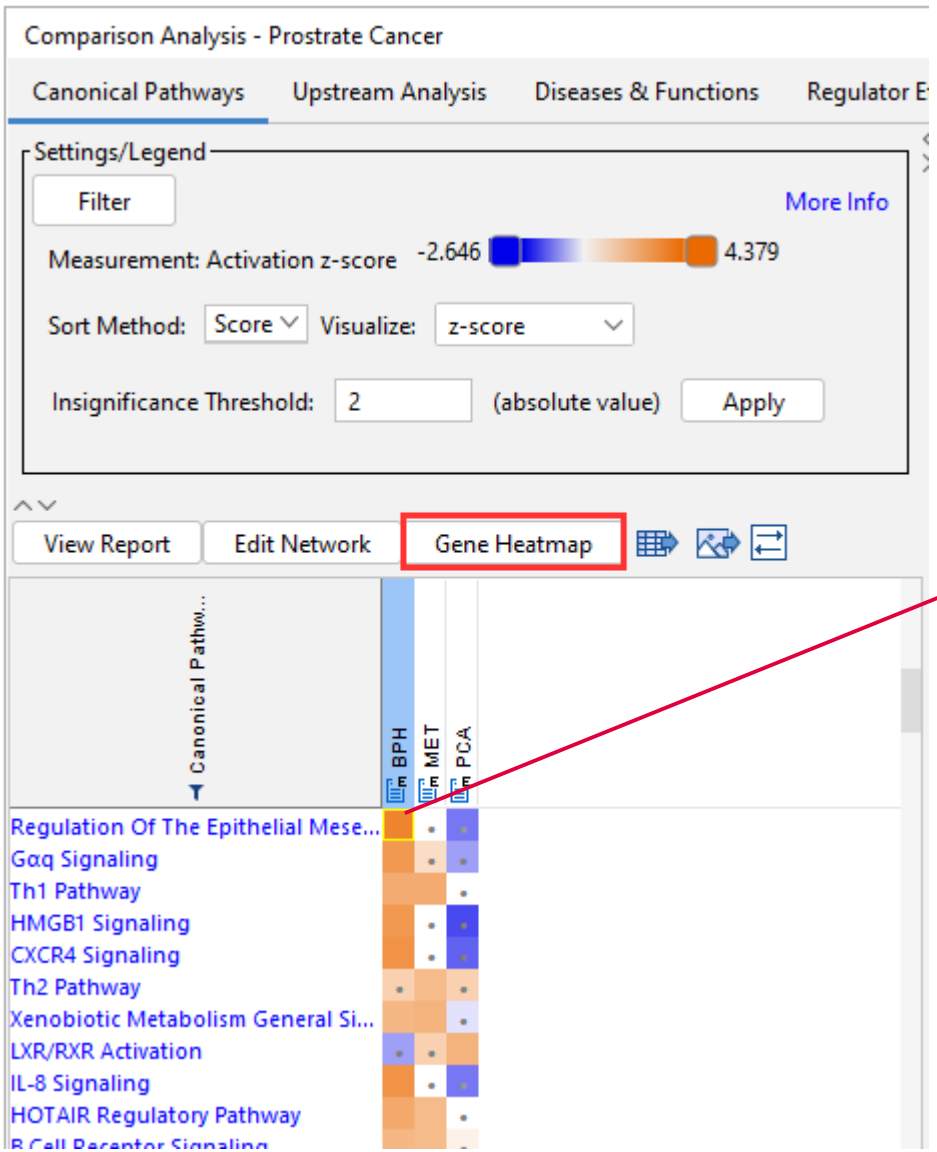
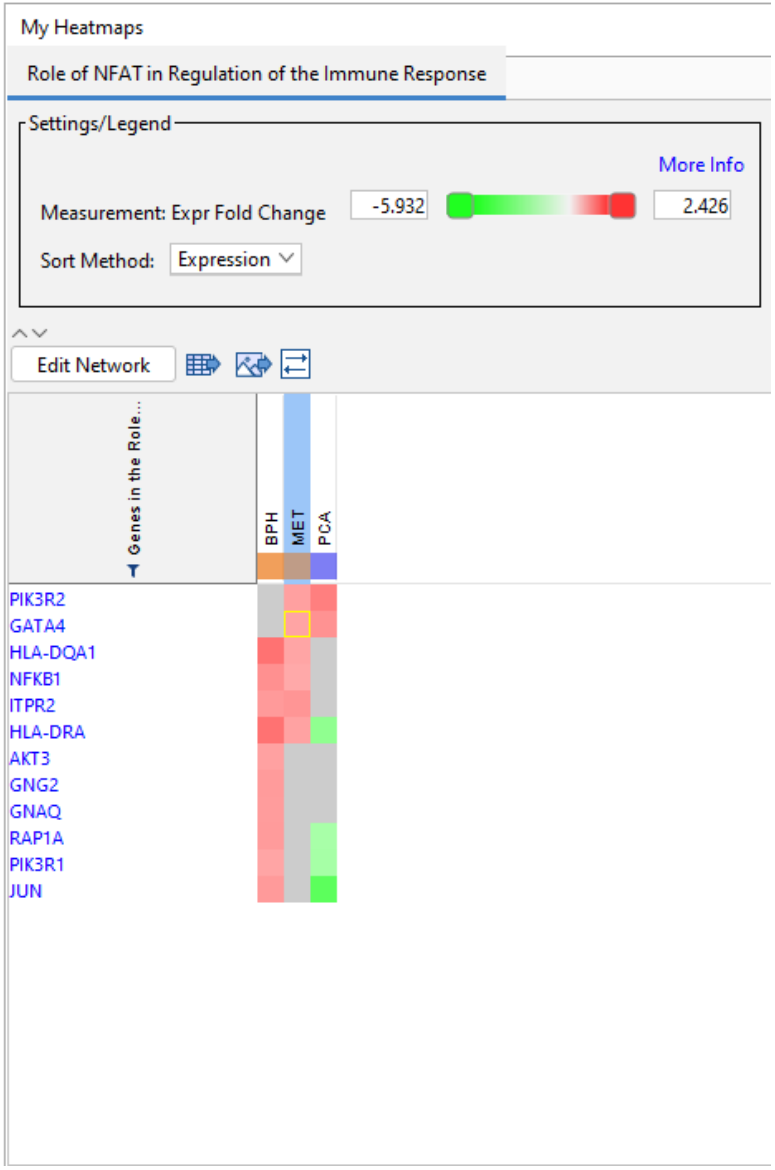
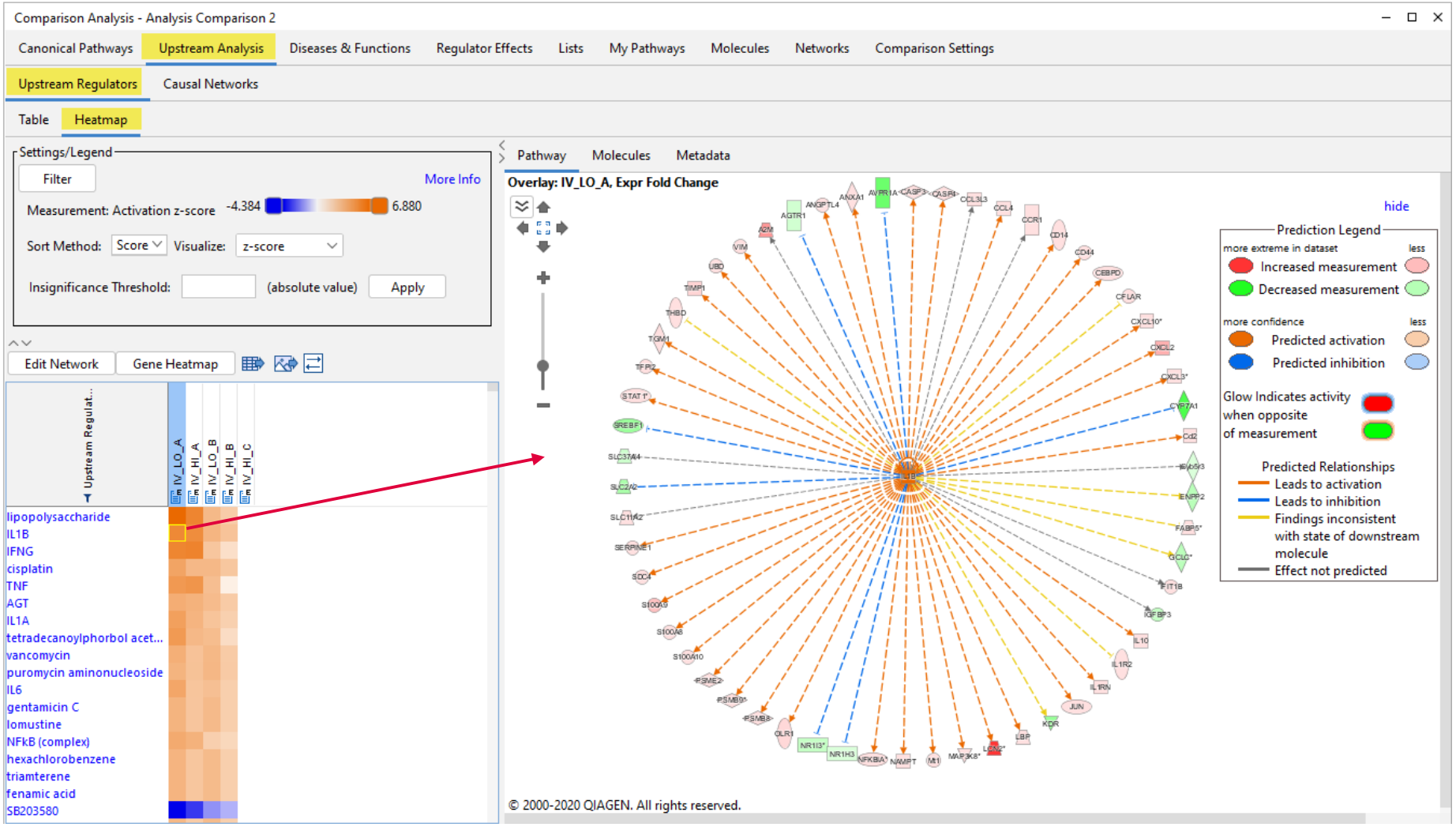
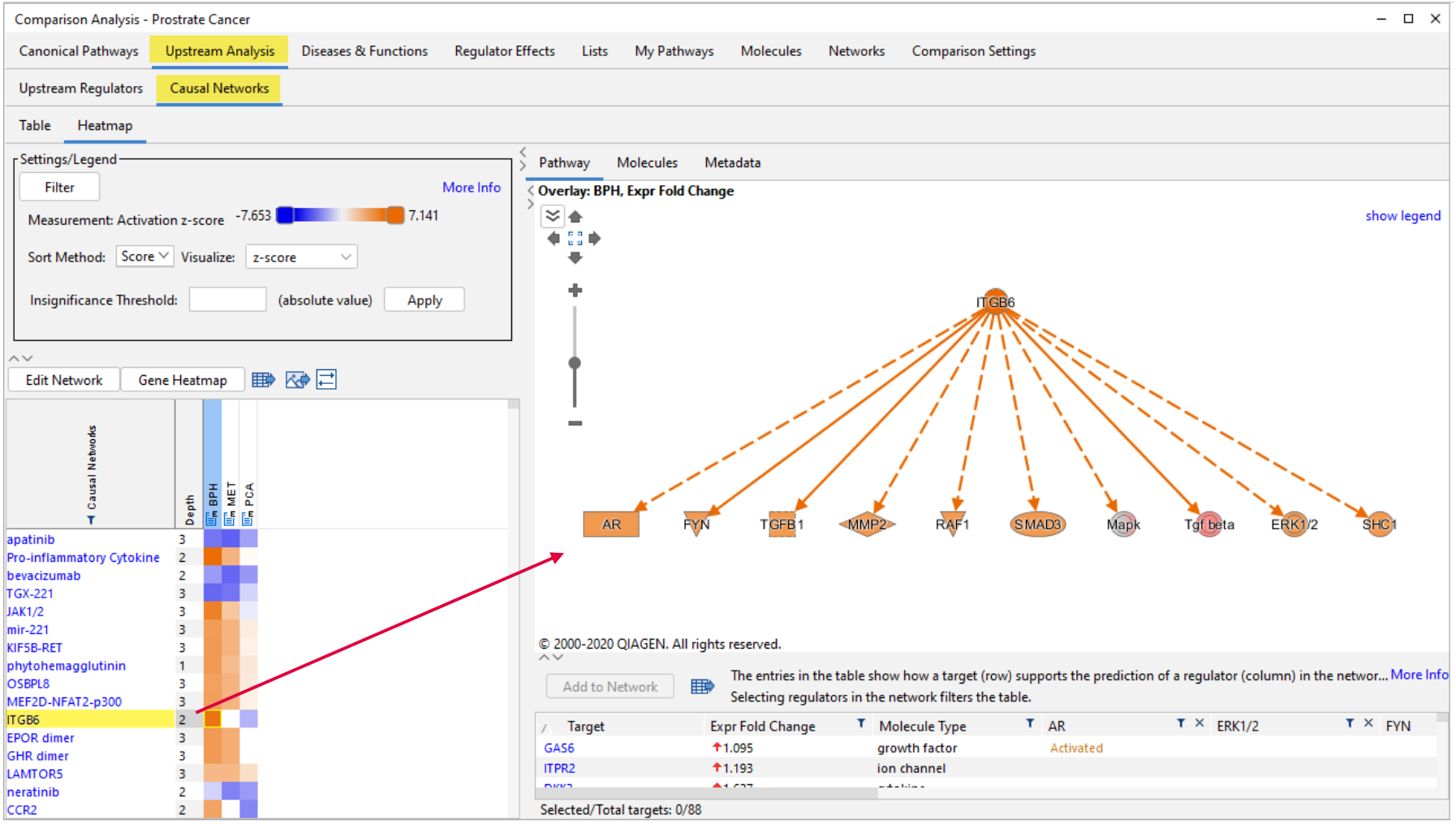




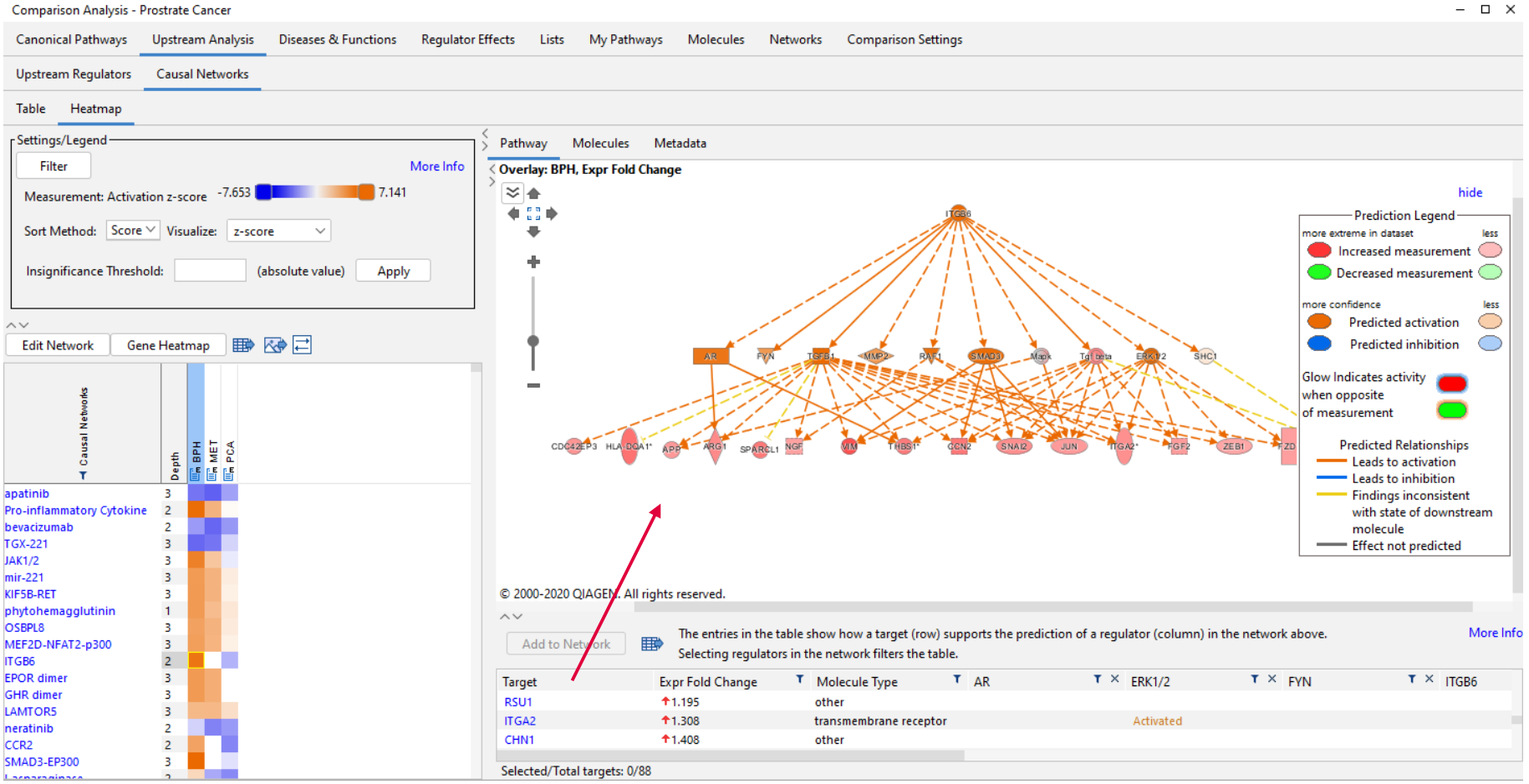
Comparison analysis heat map for upstream analysis



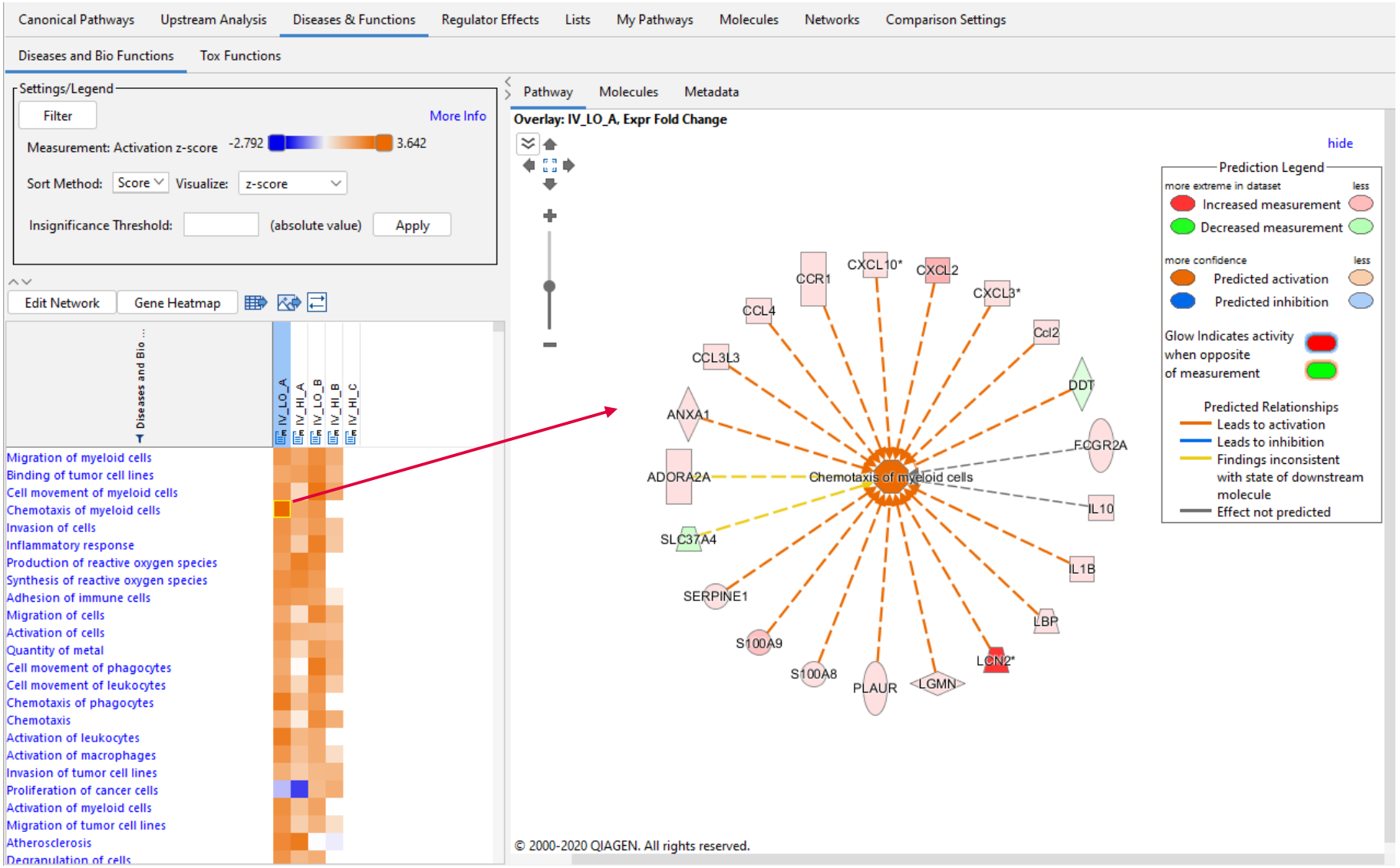
Causal Networks-Comparison Analysis Heat Map



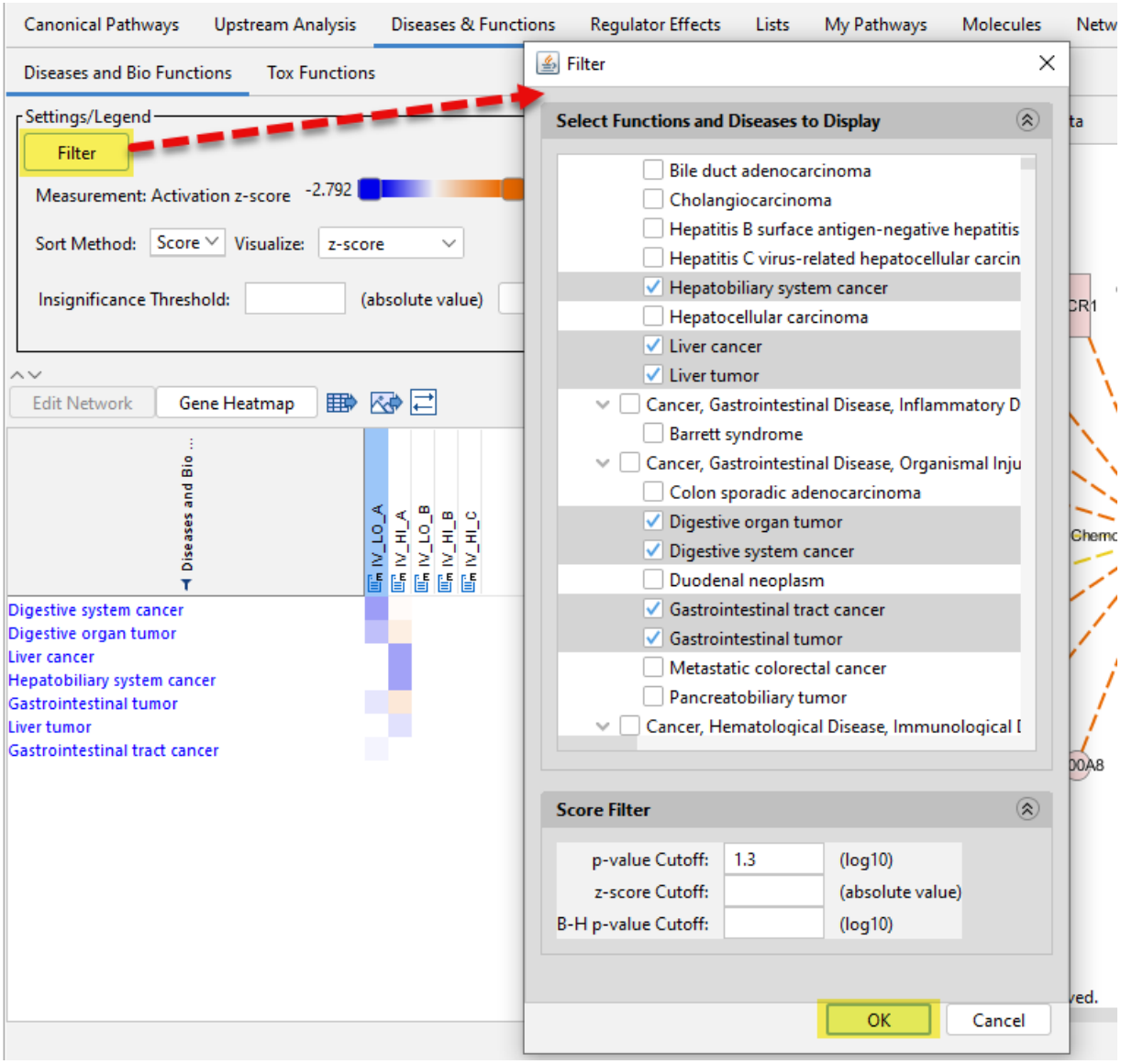
Causal Networks-Comparison Analysis Heat Map



Comparison analysis heat map for Diseases and Functions



Filter



Single Experiment

- Time Course
- Dose Response

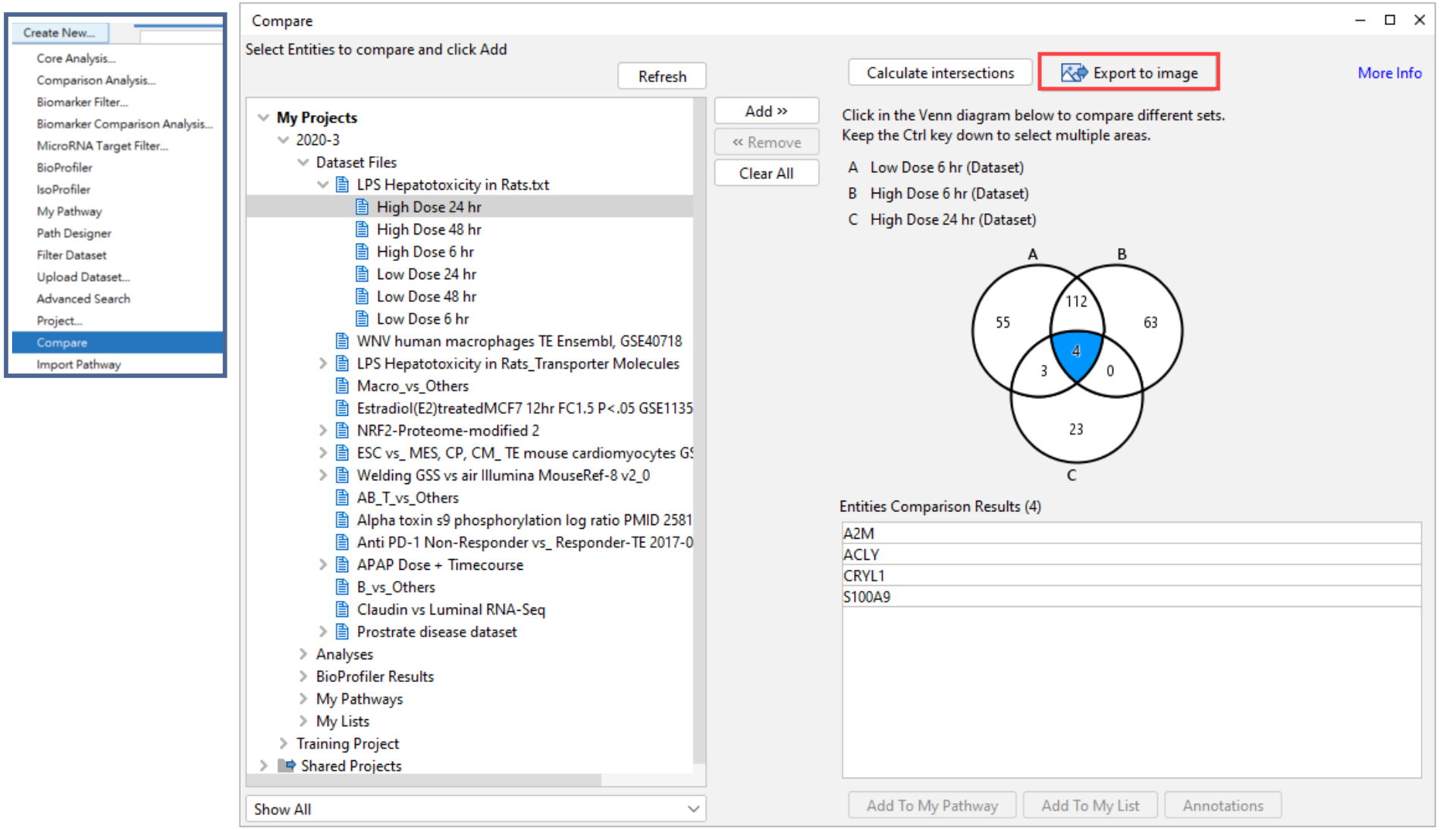
Multi Experiment

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

Set Analysis

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

Compare Data Tool with Interactive Venn Diagram

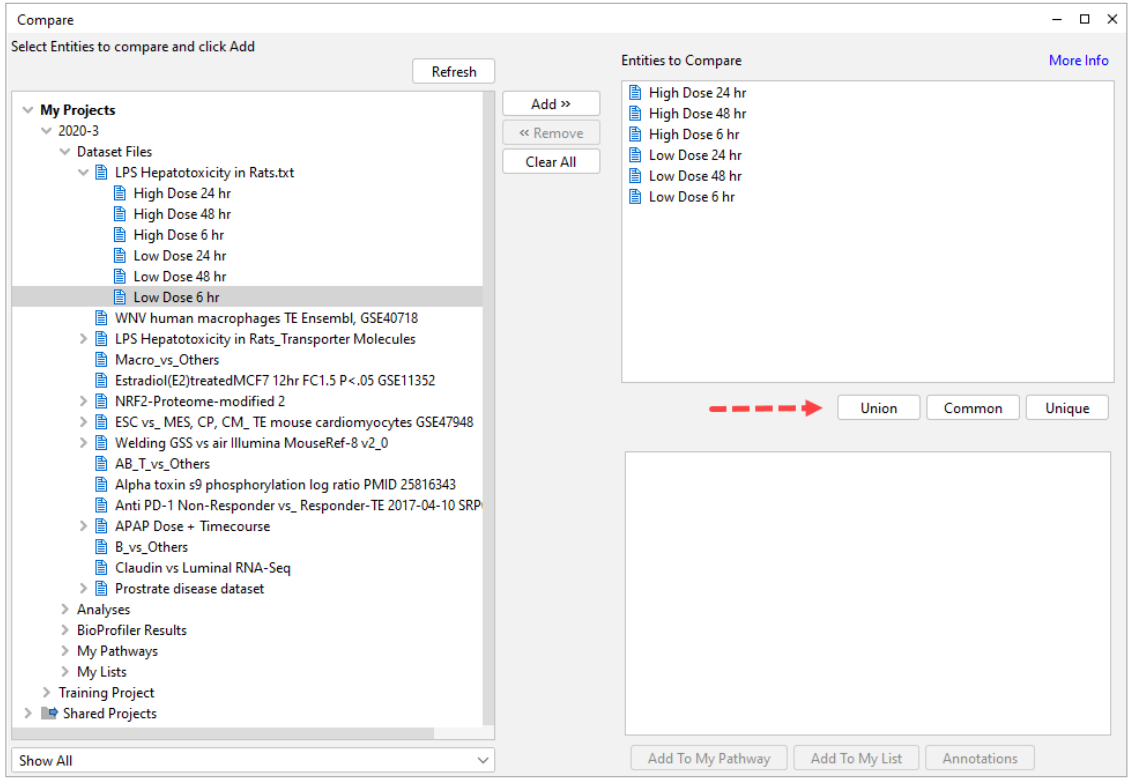


Compare Data Tool with Interactive Venn Diagram

- The **Union** operator will display a list of the total population of molecules present in all off the entities (the sum of the molecules).

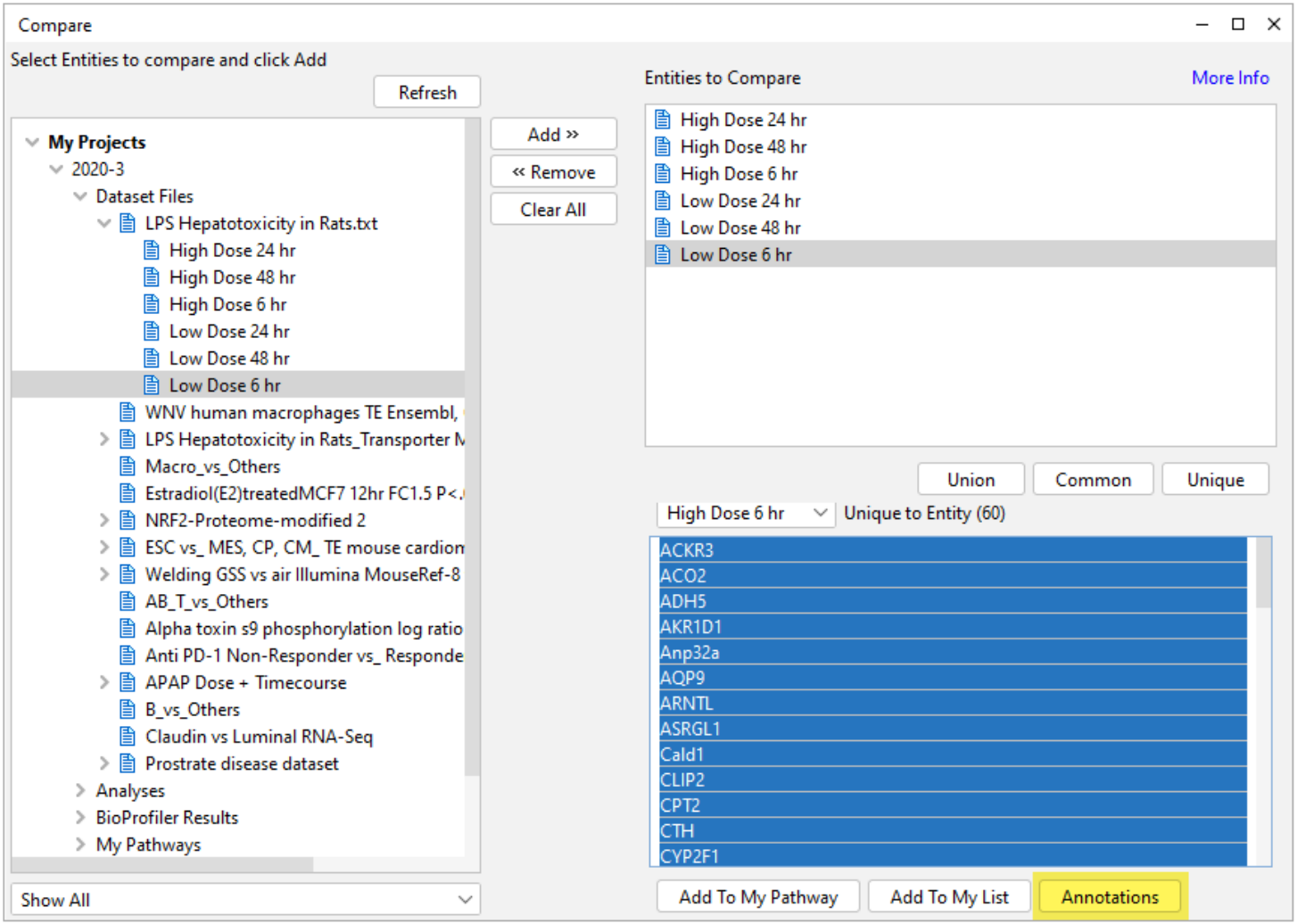
- The **Common** operator will display the intersection of molecules. In order to appear on this list, a molecule would have to be present on each of the individual entities used in the General Comparison.

- 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.



Compare Data Tool with Interactive Venn Diagram

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



Compare Data Tool with Interactive Venn Diagram

Molecule Annotations

Add To My Pathway | Add To My List | Create Dataset | Customize Table

Symbol	Expr. Chart	Entrez Gene Name	Identifier Affymetrix	Measurement Expr Fold Change	Location	Type(s)	Biomarker Applica	Drug(s)
Anp32a		acidic (leucine-rich) nucle...	1371986_at	-2.379	Nucleus	other		
ACO2		aconitase 2	1367589_at	-2.026	Cytoplasm	enzyme		
ADH5		alcohol dehydrogenase 5 ...	1371470_at	-2.747	Cytoplasm	enzyme		
AKR1D1		aldo-keto reductase famil...	1398310_at	-2.215	Cytoplasm	enzyme		azelaic acid
AQP9		aquaporin 9	1368621_at	-2.856	Plasma Membrane	transporter		
ARNTL		aryl hydrocarbon receptor...	1370510_a_at	2.653	Nucleus	transcription re...		
ASRGL1		asparaginase and isoaspa...	1387966_at	-2.527	Cytoplasm	enzyme		
ACKR3		atypical chemokine recept...	1367940_at	5.610	Plasma Membrane	G-protein coupl...		
Cald1		caldesmon 1	1371969_at	-2.208	Plasma Membrane	other		
CLIP2		CAP-Gly domain containin...	1368571_at	2.295	Cytoplasm	transcription re...		
CPT2		carnitine palmitoyltransfe...	1386927_at	-5.226	Cytoplasm	enzyme	unspecified appli	perhexiline
CTH		cystathionine gamma-lyase	1367838_at	-13.240	Cytoplasm	enzyme		
CYP2F1		cytochrome P450 family 2 ...	1368608_at	-4.498	Cytoplasm	enzyme		
DGAT2		diacylglycerol O-acyltransf...	1371615_at	-2.656	Cytoplasm	enzyme		
DDIT3		DNA damage inducible tr...	1369590_a_at	3.956	Nucleus	transcription re...		

Selected/Total molecules: 5/60

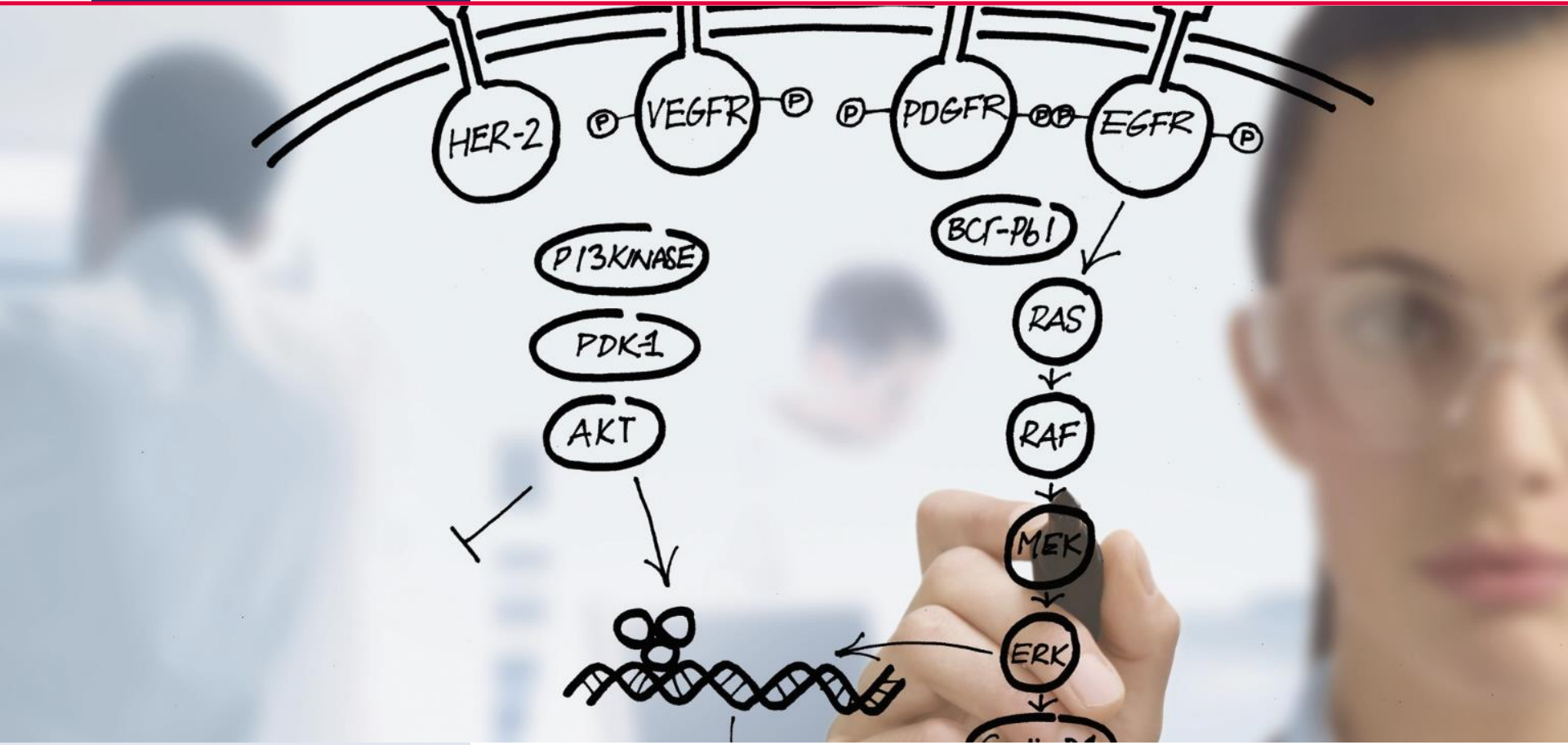
Live Demo

課程滿意度調查

創源生技 GGA

若有需要進一步的資訊或在使用軟體上遇到問題歡迎聯繫以下窗口:

周儀柔 資深業務專員 GraceChou@gga.asia 02-2795 1777 #3019
黃柔諭 產品專員 ZoeHuang@gga.asia 02-2795 1777 #3028



歡迎與我們聯絡

創源生技 GGA

Office: +886-2-2795-1777#3024

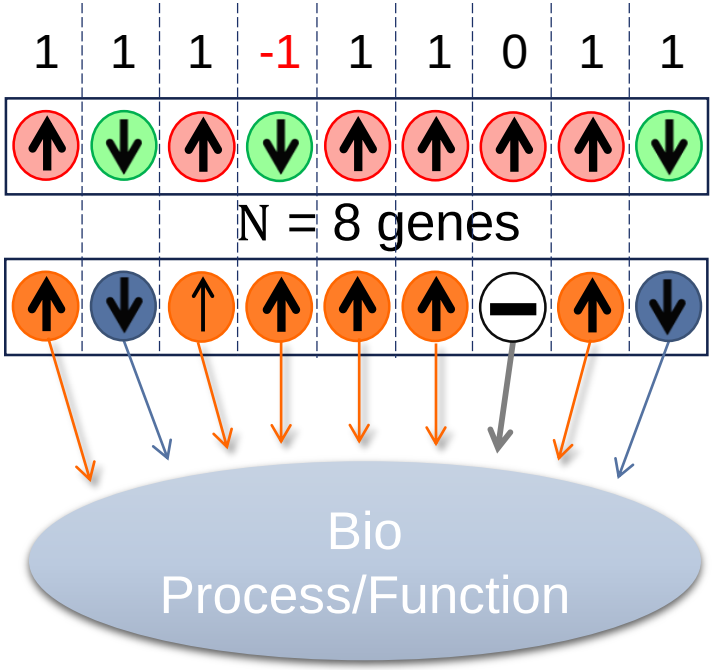
Fax: +886-2-2793-8009 EXT 1022

My E-mail: ZoeHuang@gga.asia

MSC Support: **msc-support@gga.asia**

Downstream Effect Activation z–score

Statistical measure of correlation between the relationship direction and resulting gene expression



Differential gene expression
(Uploaded Data)

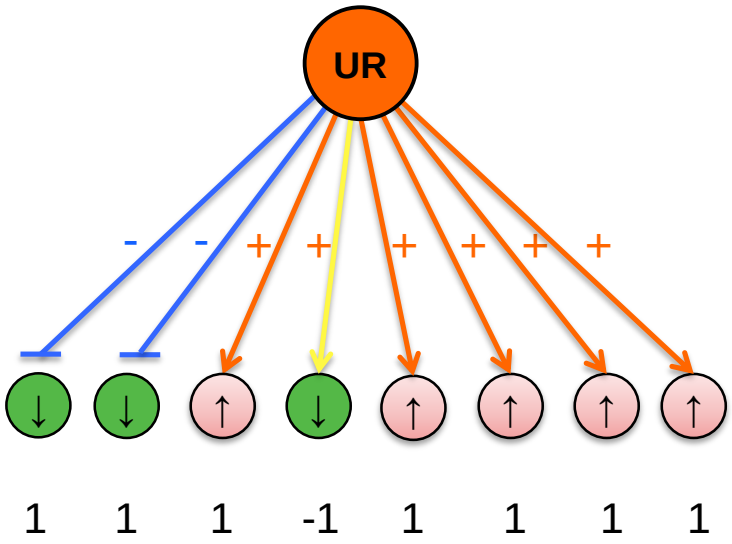
Effect genes have on a process or function
(Literature)

$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}}$$

z-score > 2 or < -2 is considered significant

Actual z-score *can* be weighted by relationship types, relationship bias, data bias

Predicting upstream regulators of a dataset



← 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

$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}}$$

$= (7\text{-}1)/\sqrt{8} = 2.12$ (=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