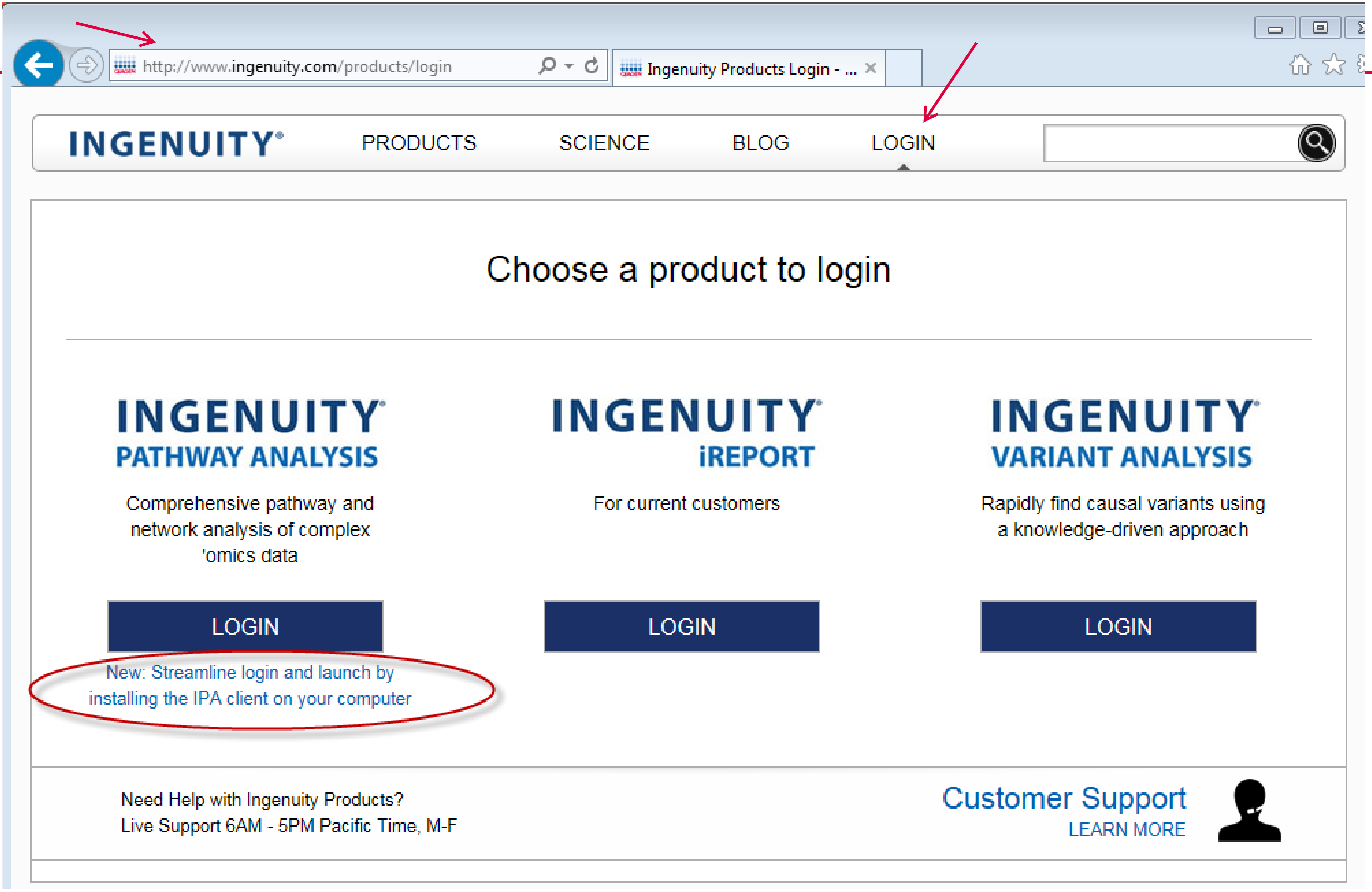

Choose a product to login

INGENUITY PATHWAY ANALYSIS

Comprehensive pathway and network analysis of complex 'omics data

LOGIN

New: Streamline login and launch by installing the IPA client on your computer

INGENUITY iREPORT

For current customers

LOGIN

INGENUITY VARIANT ANALYSIS

Rapidly find causal variants using a knowledge-driven approach

LOGIN

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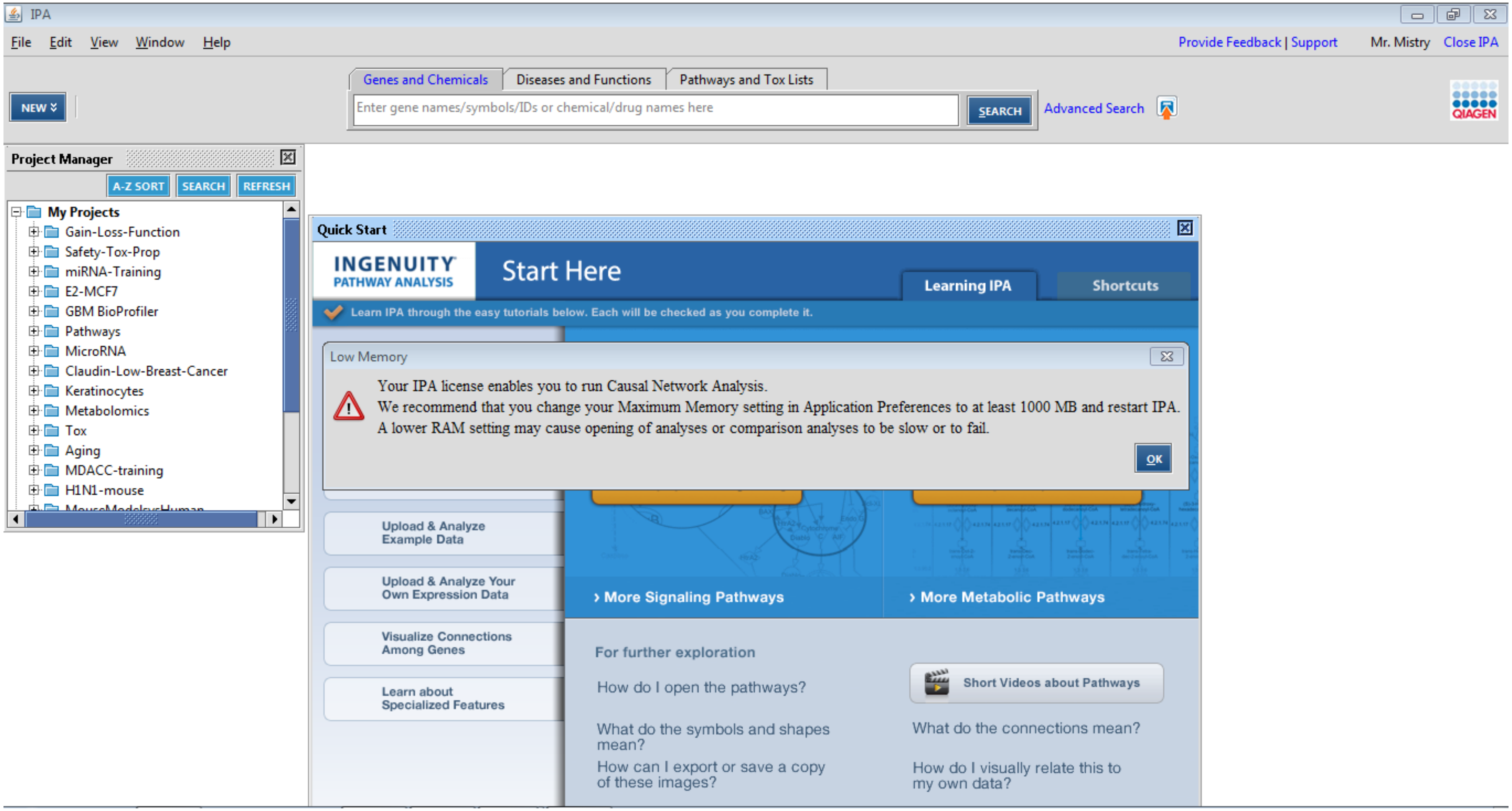
Live Support 6AM - 5PM Pacific Time, M-F

Customer Support
LEARN MORE



Learn IPA through the easy tutorials below. Each will be checked as you complete it.

Your IPA license enables you to run Causal Network Analysis.
We recommend that you change your Maximum Memory setting in Application Preferences to at least 1000 MB and restart IPA.
A lower RAM setting may cause opening of analyses or comparison analyses to be slow or to fail.

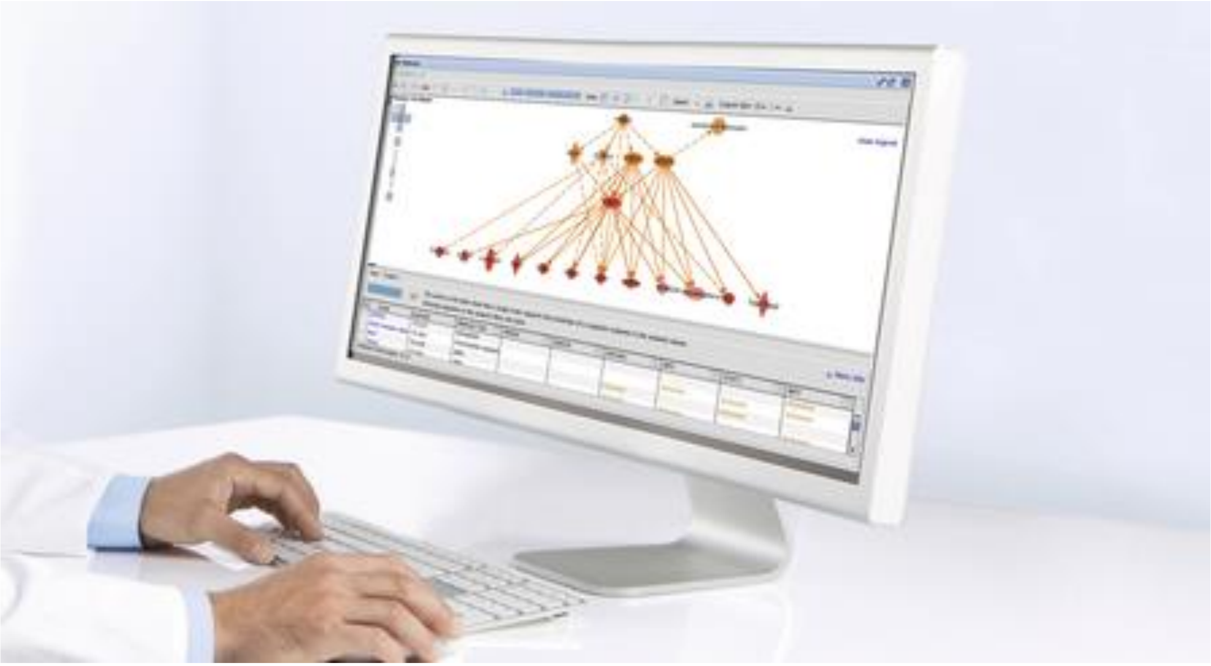
How do I open the pathways?

What do the symbols and shapes mean?

How can I export or save a copy of these images?

What do the connections mean?

How do I visually relate this to my own data?



Ingenuity Pathway Analysis (IPA) : Maximizing the Biological Interpretation of Gene, Transcript & Protein Expression Data with IPA

Ted King
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Dev Mistry, Ph.D.
Field Applications Scientist
Qiagen Advanced Genomics
Devendra.Mistry@qiagen.com

Overview

- Introduction to IPA
- Search and Explore
  - Growing a network out of a molecule
  - Bioprofiler (Advanced Analytics)

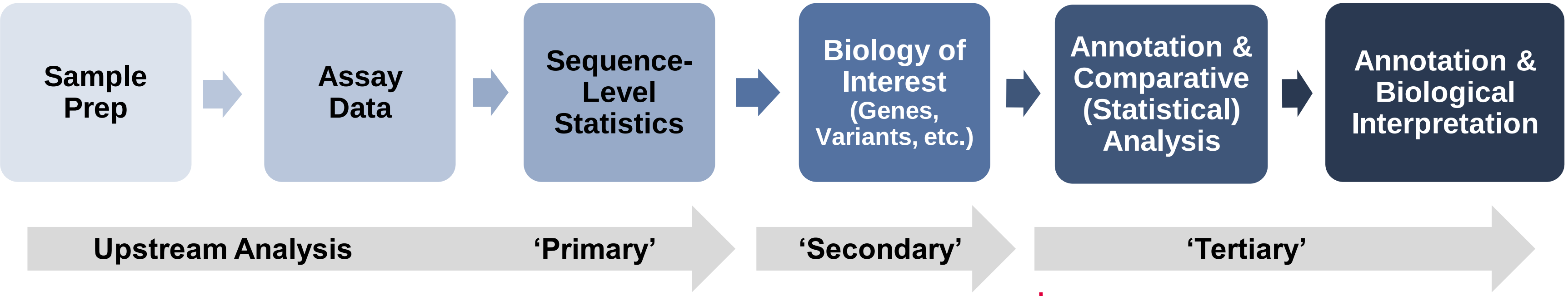
- Large Dataset Analysis
  - Uploading your dataset(s) and starting a core analysis
  - Core Analysis
    - Canonical Pathways
    - Upstream Regulators
      - Causal Network (Advanced Analytics)
    - Diseases and Functions
    - Regulator Effect
    - Networks
  - Comparison Analysis

- Questions/Answer

Introduction

When do you use IPA?

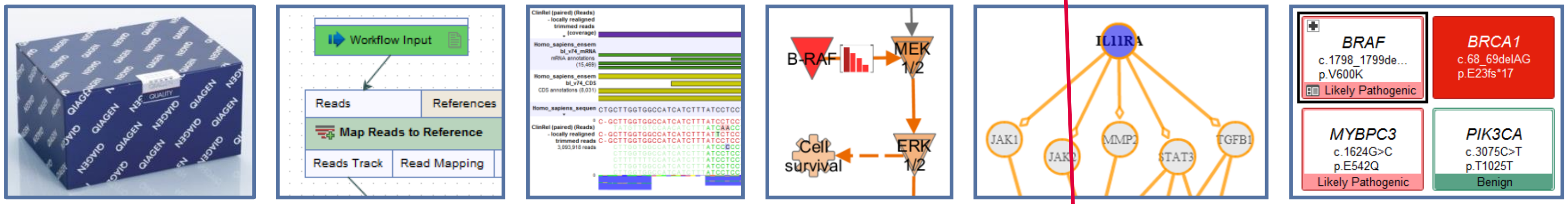
QIAGEN Sample to Insight



Sample Prep → **Assay Data** → **Sequence-Level Statistics** → **Biology of Interest (Genes, Variants, etc.)** → **Annotation & Comparative (Statistical) Analysis** → **Annotation & Biological Interpretation**

Upstream Analysis **'Primary'** → **'Secondary'** → **'Tertiary'**

Sample

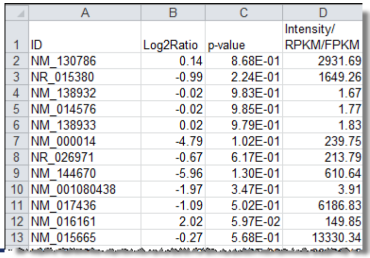


Insight

INGENUITY® VARIANT ANALYSIS

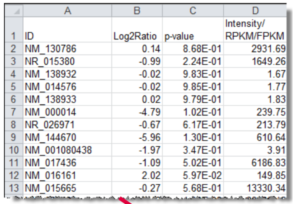
INGENUITY® CLINICAL

INGENUITY® PATHWAY ANALYSIS



	A	B	C	D
1	ID	Log2Ratio	p-value	Intensity/ RPKM/FPKM
2	NM_130786	0.14	8.68E-01	2931.69
3	NR_015380	-0.99	2.24E-01	1649.26
4	NM_138932	-0.02	9.83E-01	1.67
5	NM_014576	-0.02	9.85E-01	1.77
6	NM_138933	0.02	9.79E-01	1.83
7	NM_000014	-4.79	1.02E-01	239.75
8	NR_026971	-0.67	6.17E-01	213.79
9	NM_144670	-5.96	1.30E-01	610.64
10	NM_001080438	-1.97	3.47E-01	3.91
11	NM_017436	-1.09	5.02E-01	6186.83
12	NM_016161	2.02	5.97E-02	149.85
13	NM_015665	-0.27	5.68E-01	13330.34

What can IPA do?



	A	B	C	D
1	ID	Log2Ratio	p-value	Intensity/ RPKM/FPKM
2	NM_130786	0.14	8.68E-01	2931.69
3	NR_015380	-0.99	2.24E-01	1649.26
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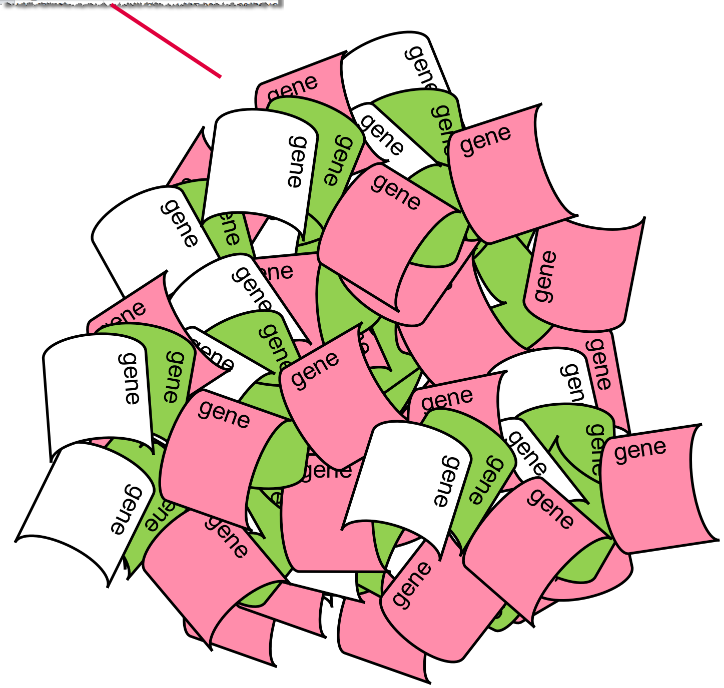
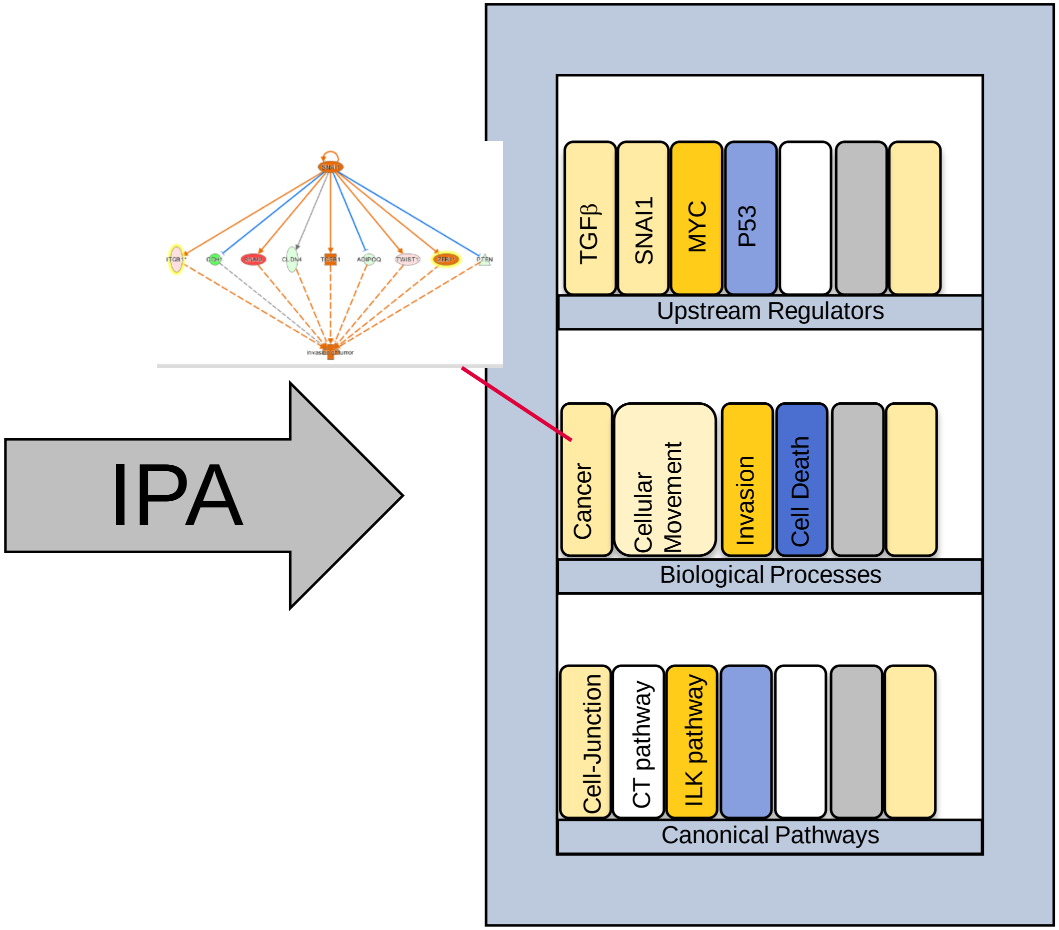
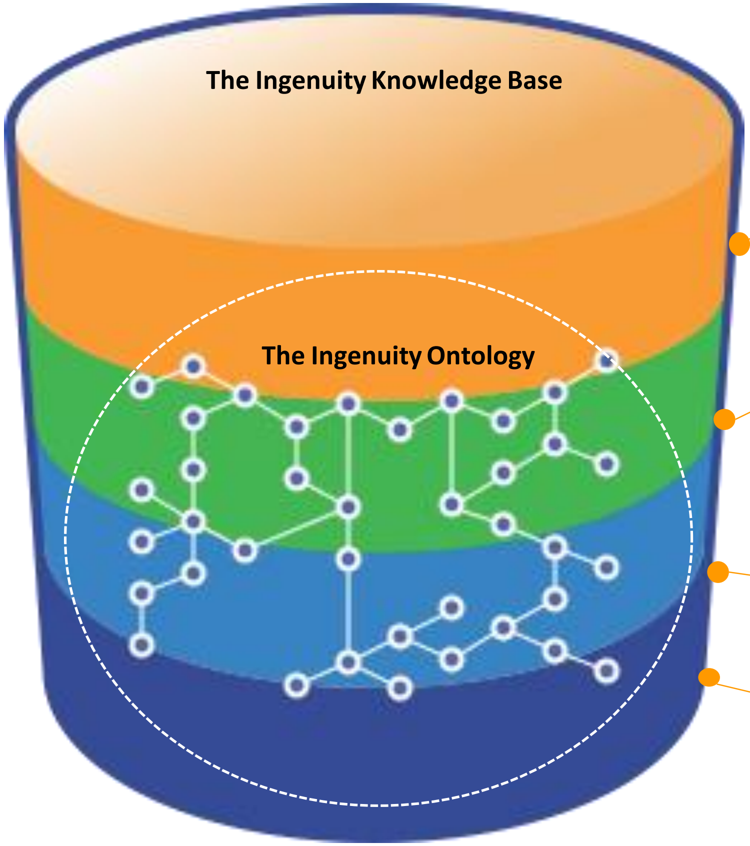


Large RNA seq dataset in form of a huge pile of papers



Methodical analysis by IPA in form of organized binders on a bookshelf

Foundation behind all of Ingenuity's products



Ingenuity Content

Ingenuity Findings

Ingenuity® Expert Findings – Manually curated Findings that are reviewed, from the full-text, rich with contextual details, and are derived from top journals.

Ingenuity® ExpertAssist Findings – Automated text Findings that are reviewed, from abstracts, timely, and cover a broad range of publications.

Ingenuity Modeled Knowledge

Ingenuity® Expert Knowledge – Content we model such as pathways, toxicity lists, etc.

Ingenuity® Supported Third Party Information – Content areas include Protein-Protein, miRNA, biomarker, clinical trial information, and others

Species: human, mouse and rat
Data from other species can be mapped to human, mouse and rat orthologues

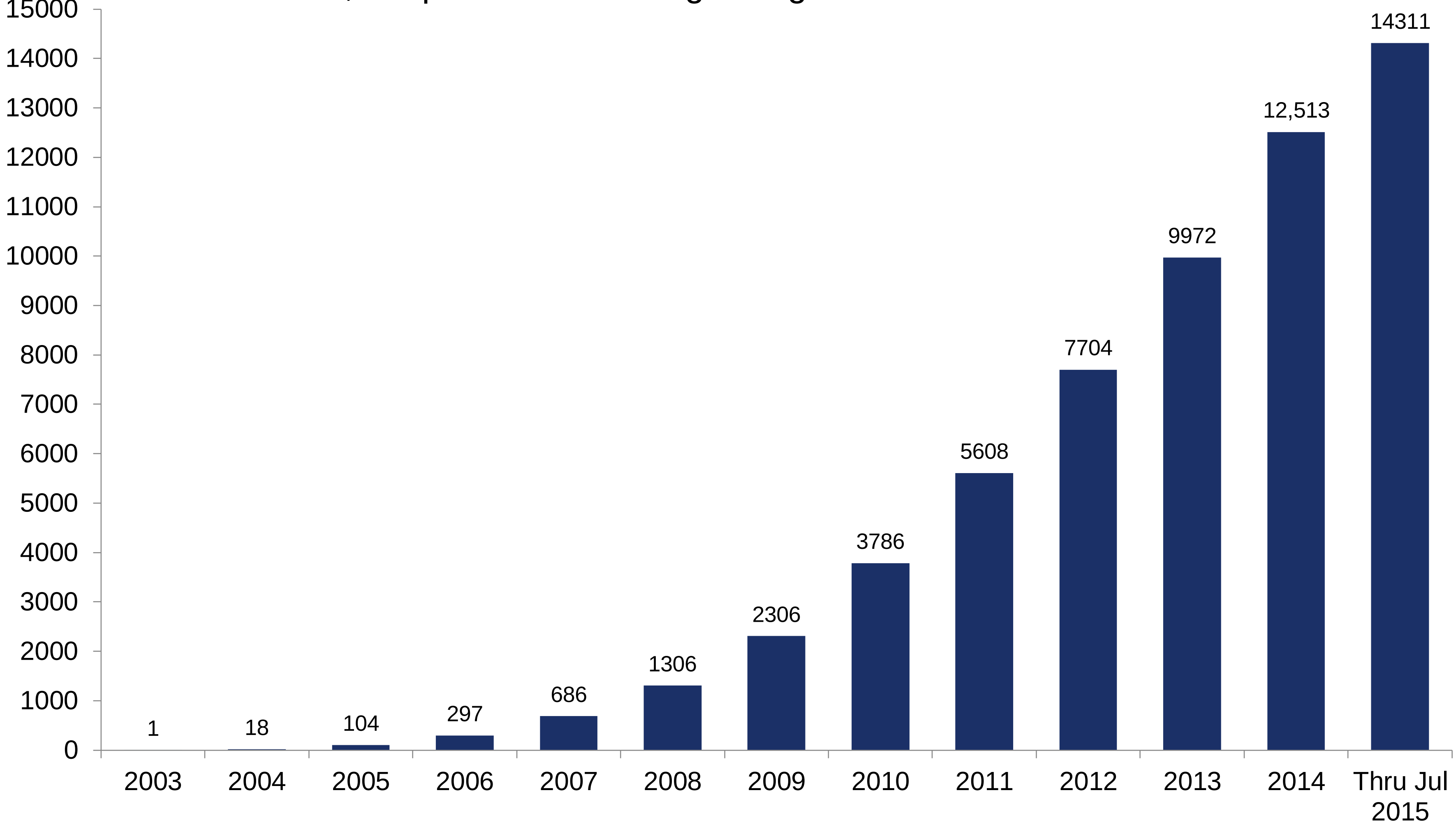
Species Supported

Species Supported

- **Human, Mouse, Rat in full content**
- **IPA uses HomoloGene to map other identifiers to human/mouse/rat orthologs** (though supporting content for the additional species will be specific to human, mouse, and rat)
  - Arabidopsis thaliana
  - Bos taurus (bovine)
  - Caenorhabditis elegans
  - Gallus gallus (chicken)
  - Pan troglodytes (chimpanzee)
  - Danio rerio (zebrafish)
  - Canis lupus familiaris (canine)
  - Drosophila melanogaster
  - Macaca mulatta (Rhesus Monkey)
  - Saccharomyces cerevisiae
  - Schizosaccharomyces pombe

Peer-reviewed publications citing QIAGEN’s Ingenuity products

14,311 publications and growing!



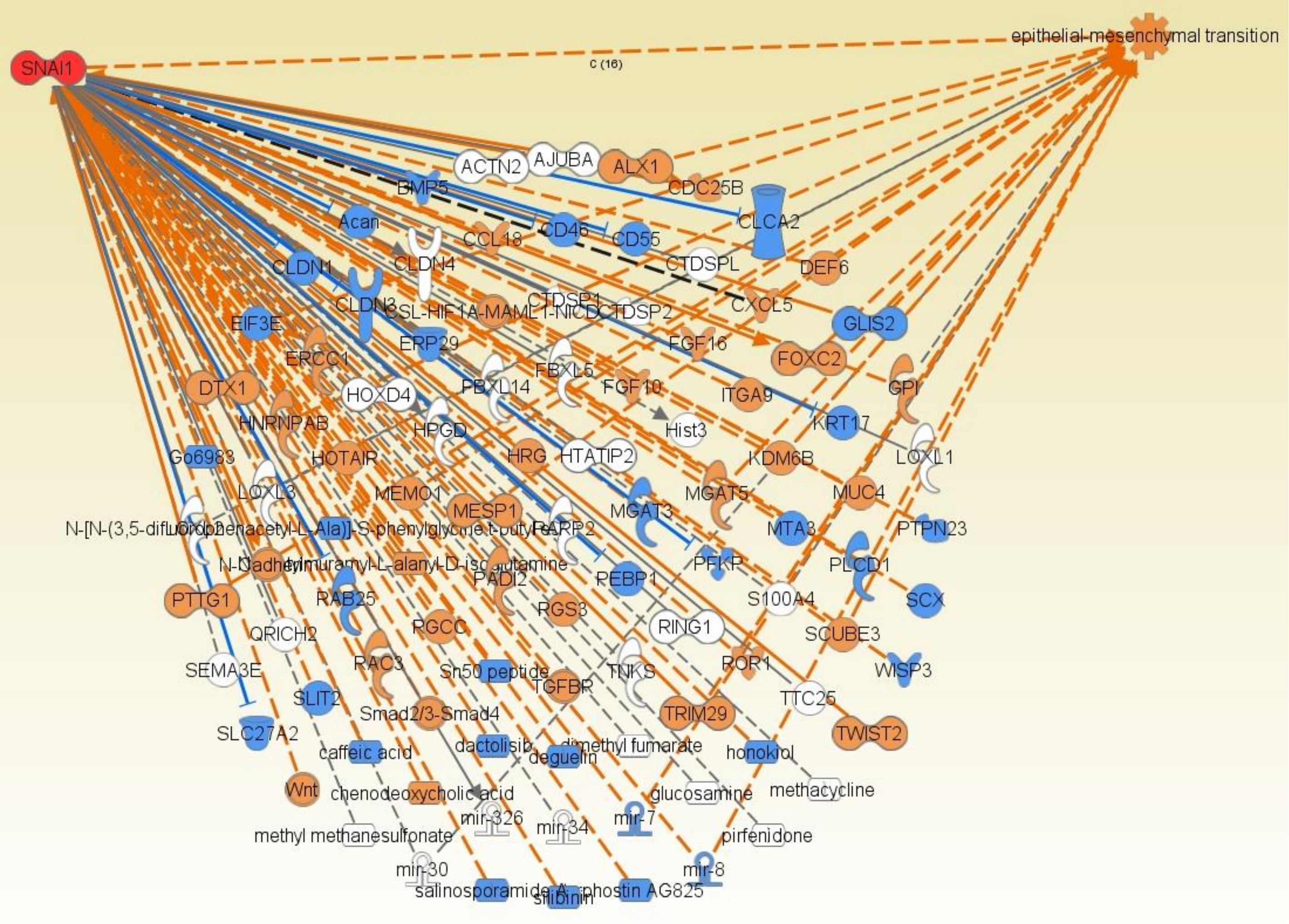
Two different types of analyses by IPA

- Deep pathway understanding of a single gene/protein
- Biological understanding of large data sets

How can IPA help you?

Deep pathway understanding of a <u>single</u> gene/protein

- ☐ Drug/therapeutic target discovery



How can IPA help you?

Biological understanding of <u>large data sets</u>

- Differential gene expression, array and RNA-seq (transcriptomics)
- Differential protein expression (proteomics)
- Metabolomics
- miRNA expression
- Gene List
  - Chip-seq
  - siRNA screening
- Methylation
- Protein phosphorylation

Transcriptomics

IPA can help with almost any transcriptomics-related question or application

Biomarker Discovery

Identifies the most promising and relevant biomarker candidates within experimental datasets

microRNA Research

Combines filtering tools and microRNA-mRNA content to provide insight into the biological effects of microRNAs

Toxicogenomics

Delivers a focused toxicity and safety assessment of candidate compounds, and provides a more complete understanding of pharmacological response, drug mechanism of action, and mechanism of toxicity

Metabolomics

Overcomes the metabolomics data analysis challenge by providing the critical context necessary to gain biological insight into cell physiology and metabolism from metabolite data

Drug Repositioning

Expression profiling of approved drugs and comparison to profiles of diseased tissue can lead to discovery of new uses for these already approved entities

Proteomics

Perform a comprehensive analysis of your proteomics for a deep understanding of proteins and related biological processes

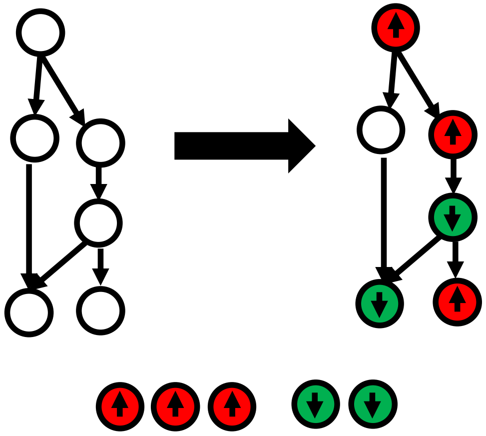
Target Discovery

Genes that are shown to be activated in a pathological condition may serve as promising targets for therapeutic development efforts

Gene/Protein Expression Analysis: IPA Core Analysis

Canonical Pathways

- Check whether molecules from your dataset belong to pre-defined pathways
- Predict the pathway activation/inhibition

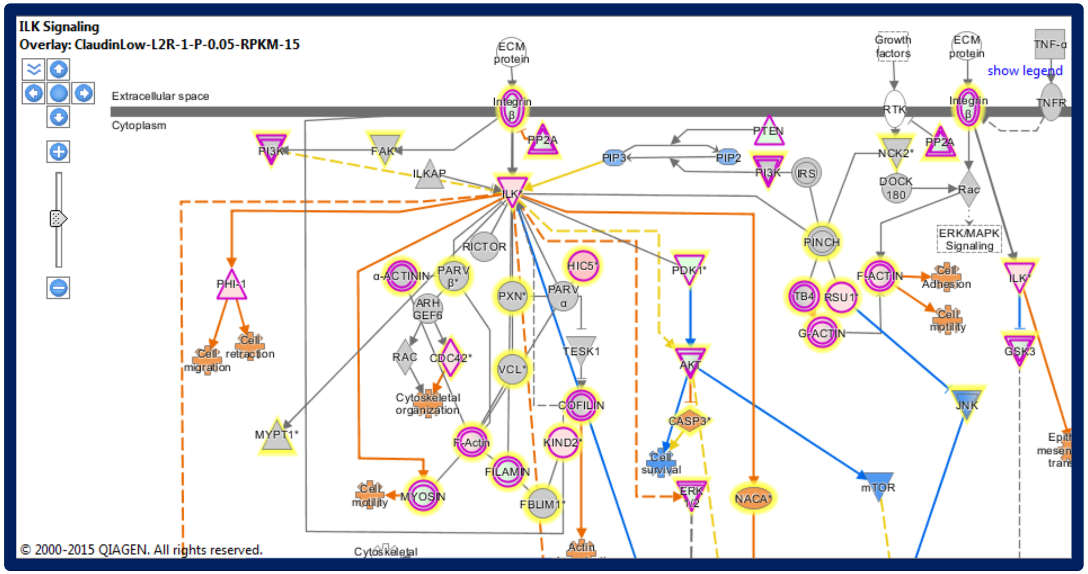


Upstream Analysis

Diseases and Functions

Regulator Effect

Networks



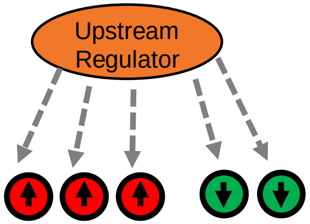
ILK Signaling
Overlay: ClaudinLow-L2R-1-P-0.05-RPKM-15

Gene/Protein Expression Analysis: IPA Core Analysis

Canonical Pathways

Upstream Analysis

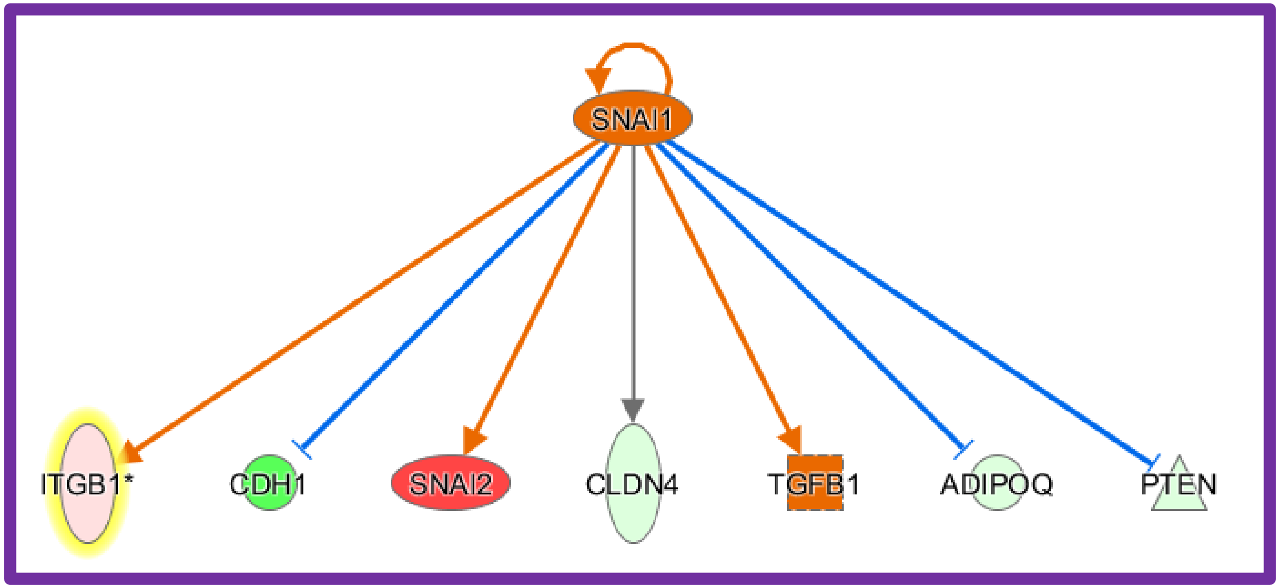
- Predict what regulators caused changes in gene expression
- Predicts directional state of regulator



Diseases and Functions

Regulator Effect

Networks



Gene/Protein Expression Analysis: IPA Core Analysis

Canonical Pathways

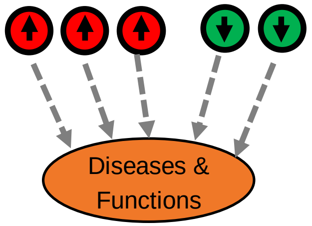
Upstream Analysis

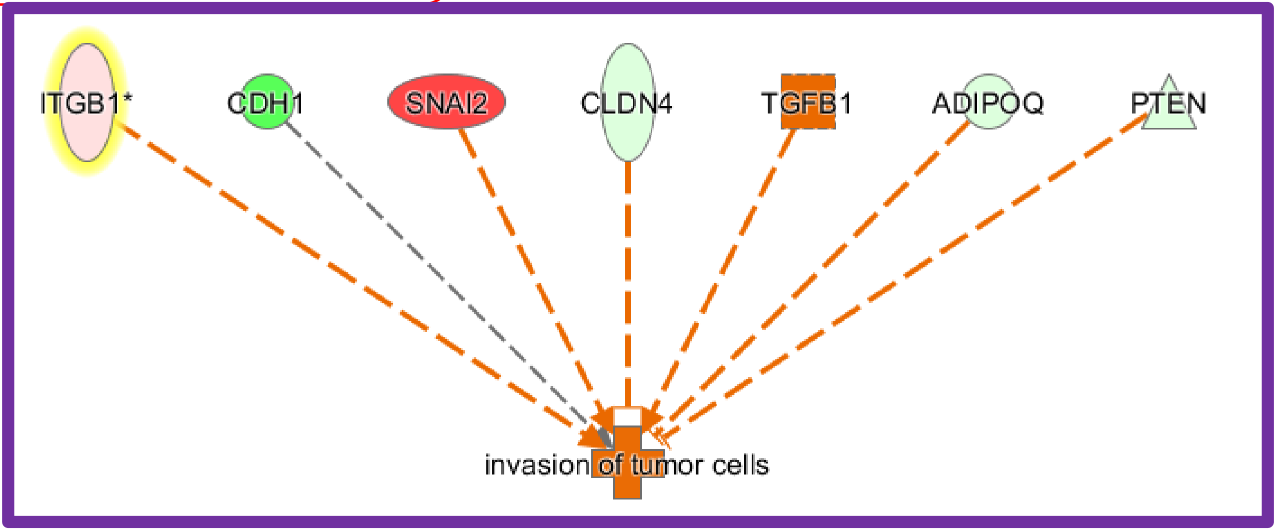
Diseases and Functions

- Predicts effected biology based on gene expression and predicts directional change on that effect
  - “Increase in EMT”
  - “Decrease in proliferation”

Regulator Effect

Networks





Gene/Protein Expression Analysis: IPA Core Analysis

Canonical Pathways

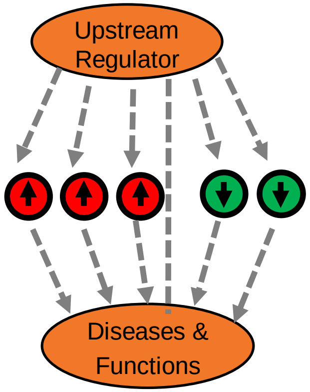
Upstream Analysis

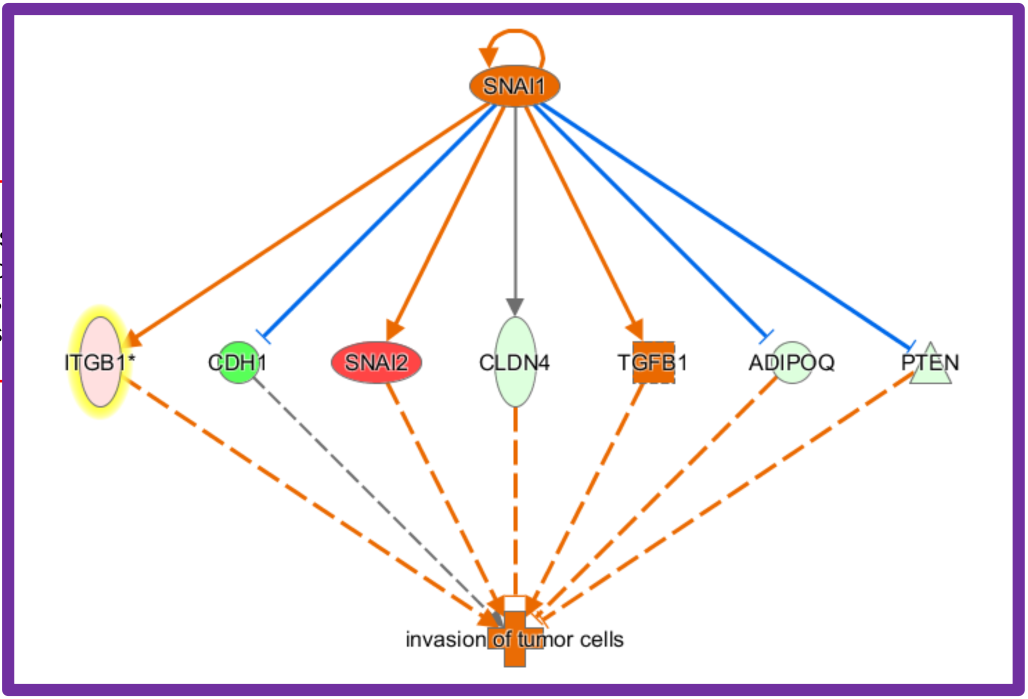
Diseases and Functions

Regulator Effect

- Models pathway interaction predicted upstream regulato through differentially expres genes, to biological process

Networks





Gene/Protein Expression Analysis: IPA Core Analysis

Canonical Pathways

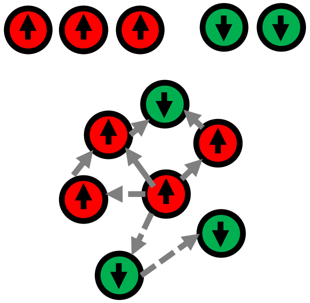
Upstream Analysis

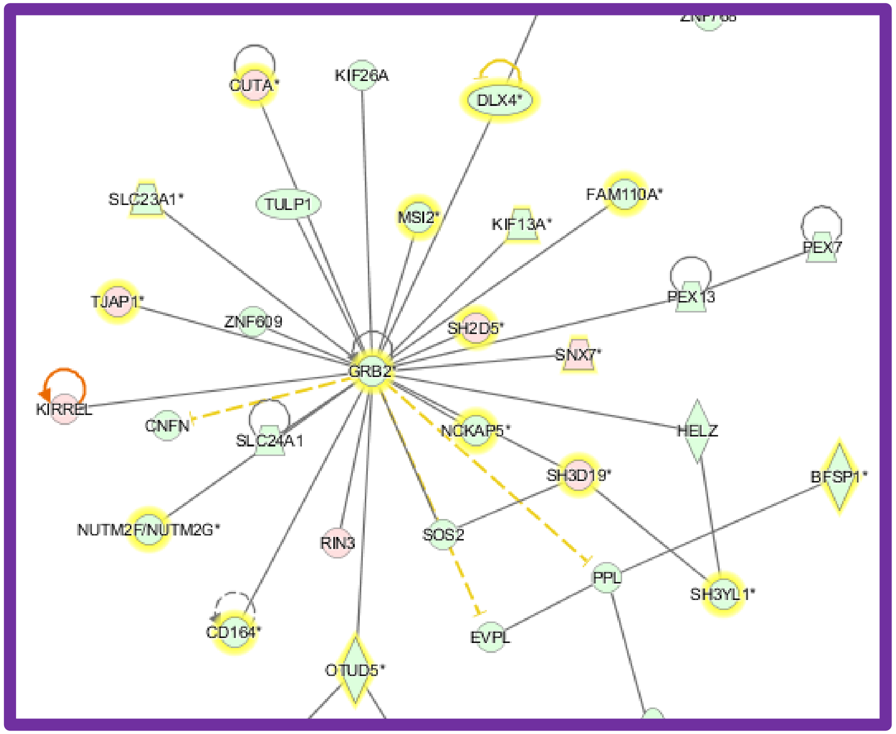
Diseases and Functions

Regulator Effect

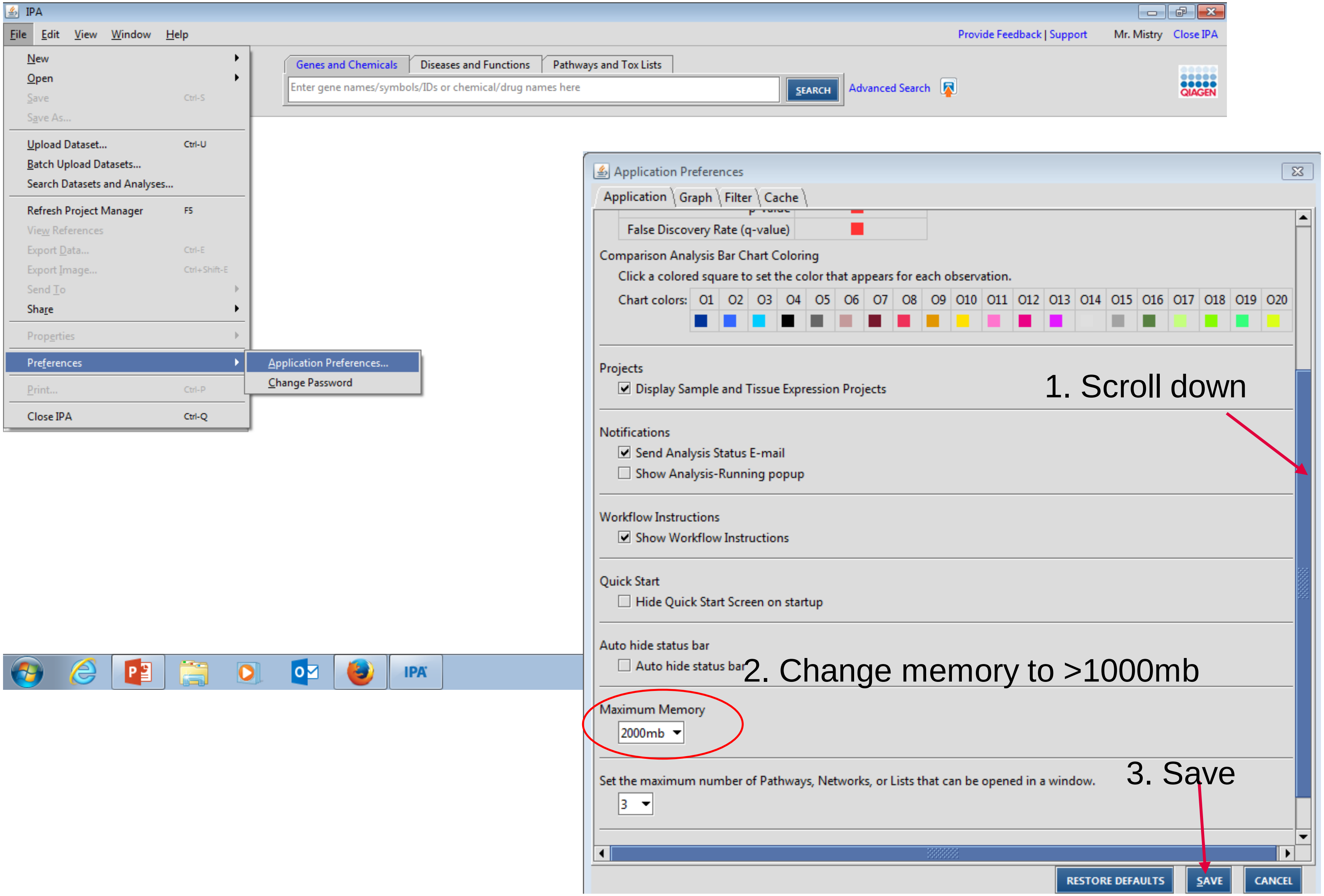
Networks

☐ Predicts non-directional gene interaction map





Change Preferences



False Discovery Rate (q-value) 🟥

Comparison Analysis Bar Chart Coloring

Click a colored square to set the color that appears for each observation.

Chart colors:	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12	O13	O14	O15	O16	O17	O18	O19	O20
	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Projects

☑ Display Sample and Tissue Expression Projects

Notifications

☑ Send Analysis Status E-mail

☐ Show Analysis-Running popup

Workflow Instructions

☑ Show Workflow Instructions

Quick Start

☐ Hide Quick Start Screen on startup

Auto hide status bar

☐ Auto hide status bar

Maximum Memory

2000mb

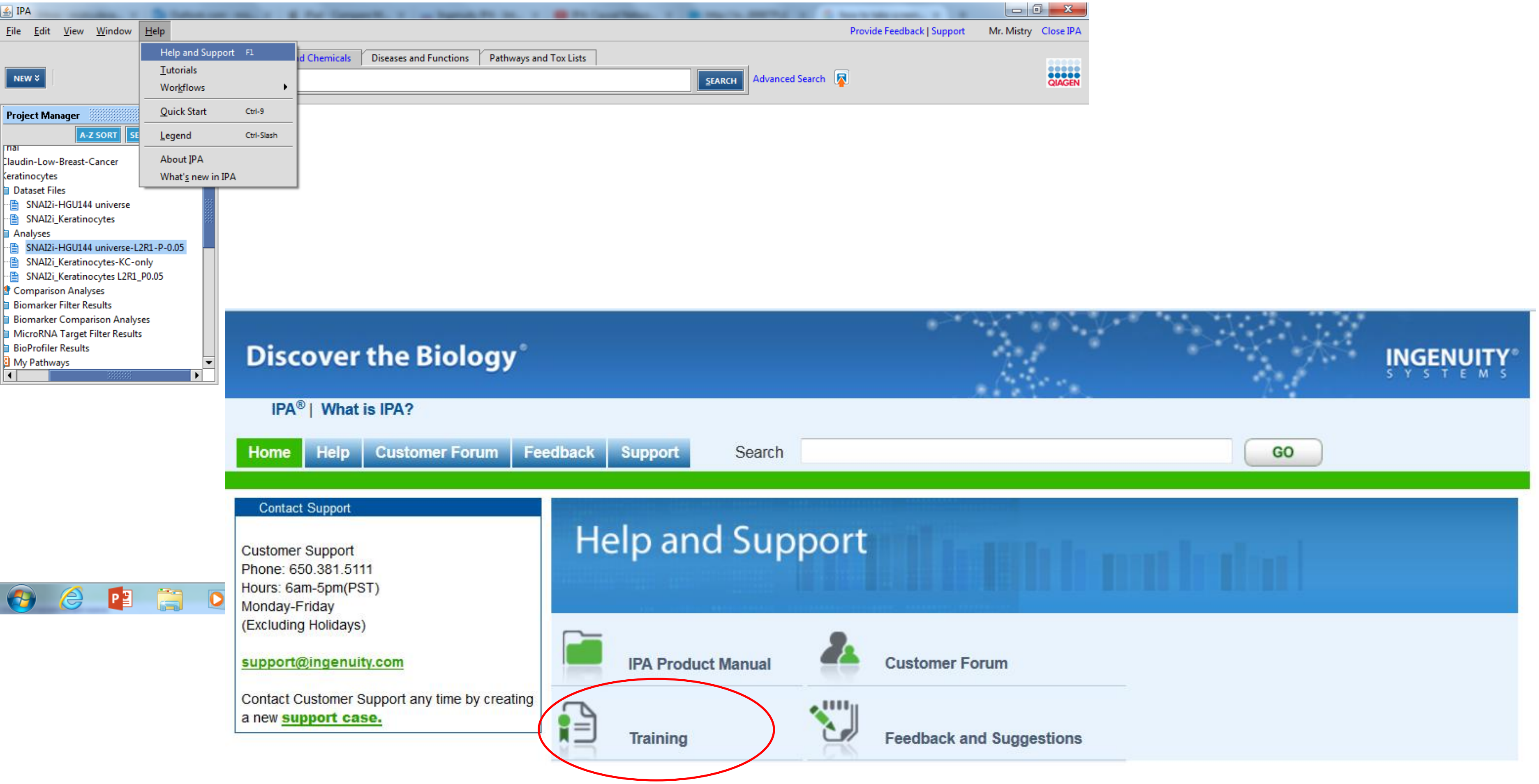
Set the maximum number of Pathways, Networks, or Lists that can be opened in a window.

3

1. Scroll down

2. Change memory to >1000mb

3. Save



Contact Support

Customer Support
Phone: 650.381.5111
Hours: 6am-5pm(PST)
Monday-Friday
(Excluding Holidays)

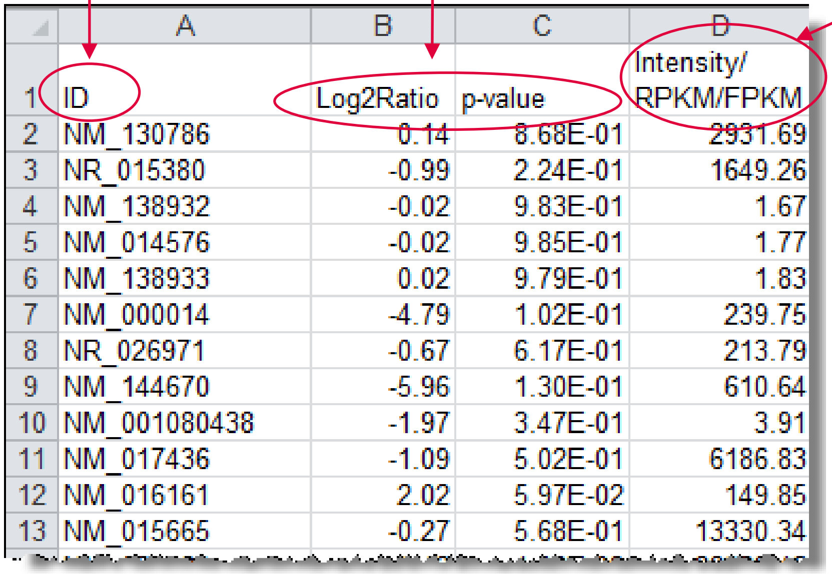
support@ingenuity.com

Contact Customer Support any time by creating a new support case.

Don’t worry too much about notes or if you fall behind during the point and click training. We have manuals/videos for everything.

Uploading your dataset

Suggested Format for uploading RNA-seq data



Required

Recommended

Max RPKM recommended for RNA seq

	A	B	C	D
1	ID	Log2Ratio	p-value	Intensity/ RPKM/FPKM
2	NM_130786	0.14	8.68E-01	2931.69
3	NR_015380	-0.99	2.24E-01	1649.26
4	NM_138932	-0.02	9.83E-01	1.67
5	NM_014576	-0.02	9.85E-01	1.77
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11	NM_017436	-1.09	5.02E-01	6186.83
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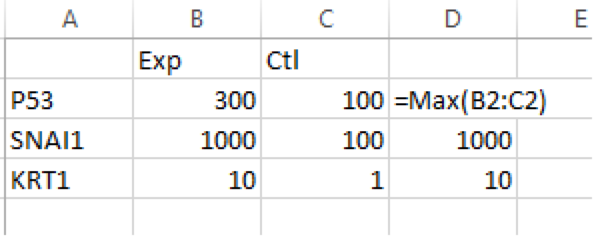


A	B	C	D	E
	Exp	Ctl		
P53	300	100	=Max(B2:C2)	
SNAI1	1000	100	1000	
KRT1	10	1	10	

Verify the differential expression calculation

- Recommend Log_2(ratio) differential expression

$$\text{Log}_2\left(\frac{Experimental\ Condition\ Exp.}{Control\ Exp}\right)$$

- Ratio differential expression

$$\left(\frac{Experimental\ Condition\ Exp.}{Control\ Exp}\right)$$

- Fold Change
  - If increased differential expression

$$\left(\frac{Experimental\ Condition\ Exp.}{Control\ Exp}\right)$$

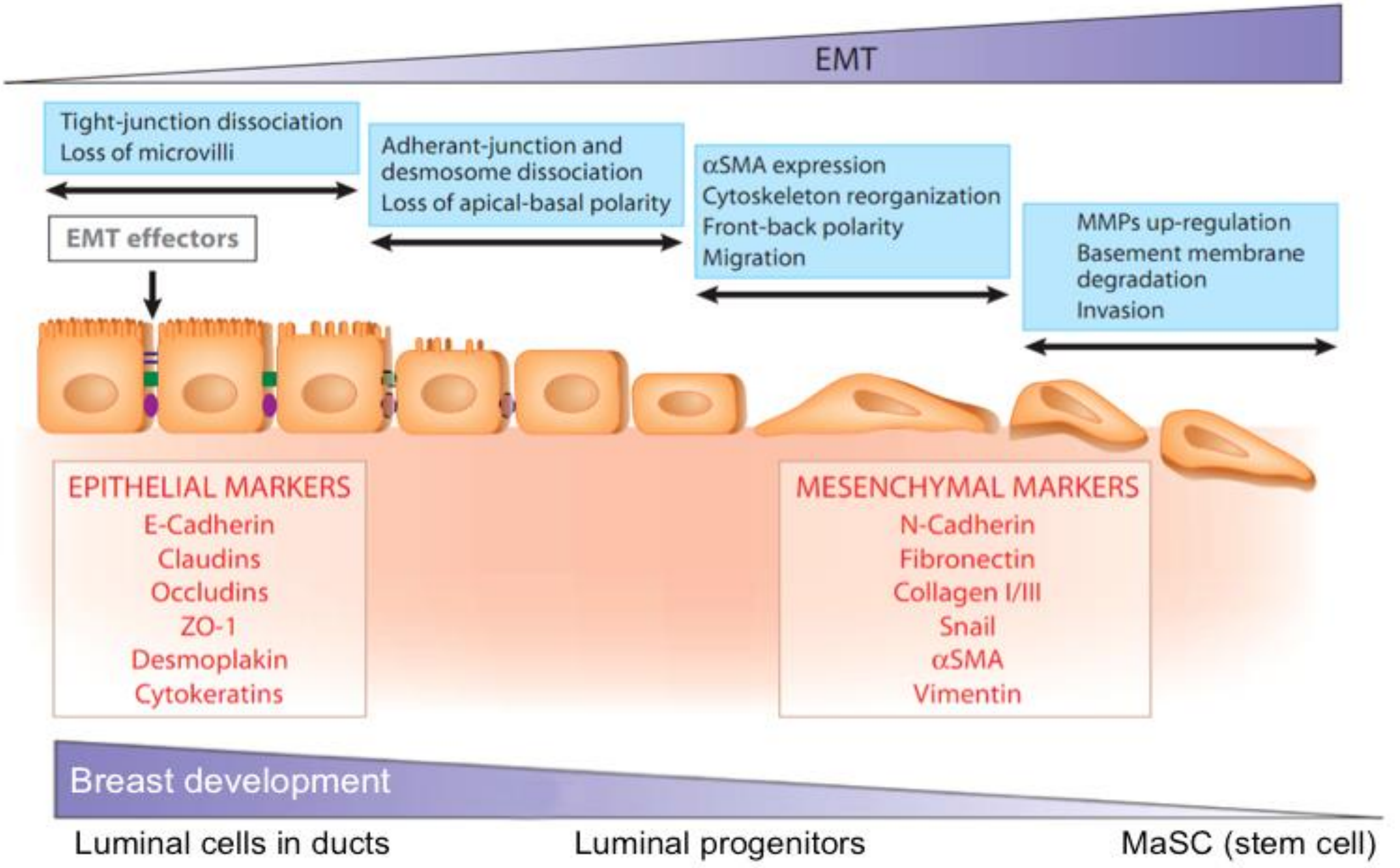
  - If decreased differential expression

$$-1\left(\frac{Control\ Exp.}{Experimental\ Condition\ Exp.}\right)$$

Fold change will never have values between 1 and -1

Case Study
RNA Seq: Claudin Low vs Luminal Breast cancer cell lines

Epithelial to Mesenchymal Transition



EMT

Tight-junction dissociation
Loss of microvilli

Adherant-junction and desmosome dissociation
Loss of apical-basal polarity

αSMA expression
Cytoskeleton reorganization
Front-back polarity
Migration

MMPs up-regulation
Basement membrane degradation
Invasion

EMT effectors

EPITHELIAL MARKERS
E-Cadherin
Claudins
Occludins
ZO-1
Desmoplakin
Cytokeratins

MESENCHYMAL MARKERS
N-Cadherin
Fibronectin
Collagen I/III
Snail
αSMA
Vimentin

Breast development

Luminal cells in ducts
Luminal progenitors
MaSC (stem cell)

Adapted from Aroeira et al. J Am Soc Nephrol 18: 2004 –2013, 2007

Luminal Breast cancer ↓ **Luminal cell lines**

HER2-enriched

Basal

Mesenchymal / stem cell-like breast cancer ↓ **Claudin-low cell lines**

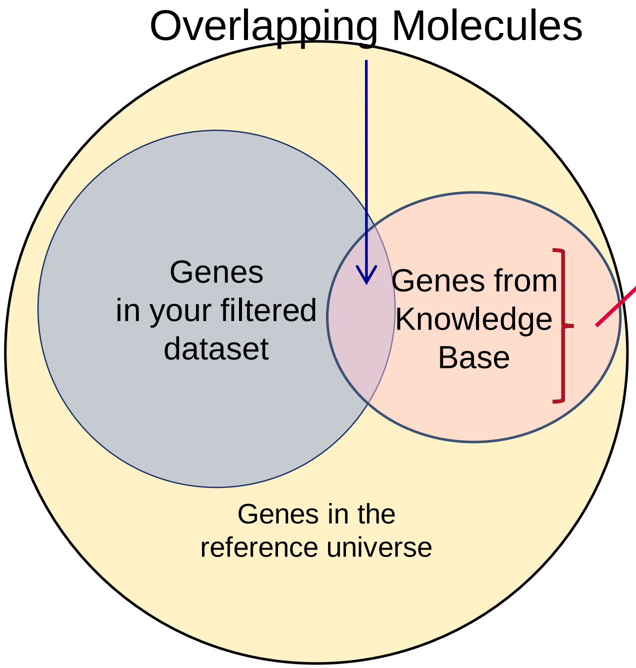
Ratio Claudin-low to Luminal
5 vs 5 cell lines, RNA-Seq data

IPA Analysis

- Verify the biology
  - Can IPA identify cancer and EMT related pathways and biological functions in this dataset?
  - What are some of the relevant pathways?
  - What are some of the relevant biological functions?
- Identification of transcriptional regulators
  - What are the transcriptional regulators that are causing the gene expression changes in this dataset?
  - Are they activated or inhibited?
- Hypothesis generation
  - Are the predicted upstream regulators increasing or decreasing downstream biological functions?

P value and Z Score

Overlapping P-value

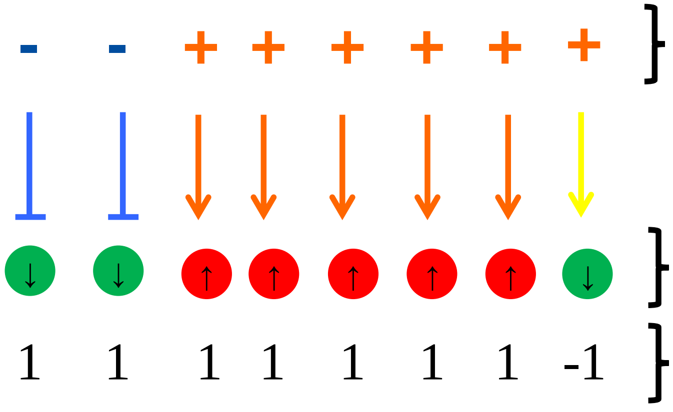


Genes from previous literature that belong to

- A canonical pathway OR
- Downstream of an upstream regulator OR
- Upstream of a disease or function

- Different from the "Expression P-value" uploaded with your dataset
- Calculated using **Fisher's exact test**
- The statistical test looks for an unexpectedly large overlap given the number of molecules in each category
- p-values should be insignificant (<0.05) for random datasets
- Gene expression direction is **not** taken into account for this calculation

Z-score: Activation Prediction



Gene expression from Knowledge Base (literature)

Gene expression in your dataset

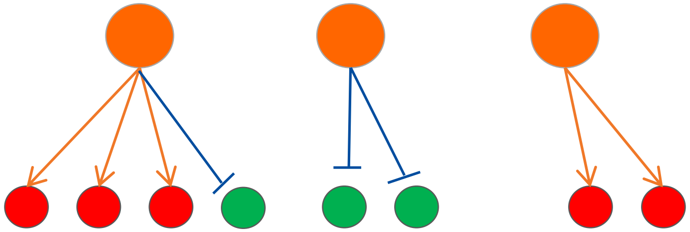
+1 score for the consistent and -1 for the inconsistent relationships

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

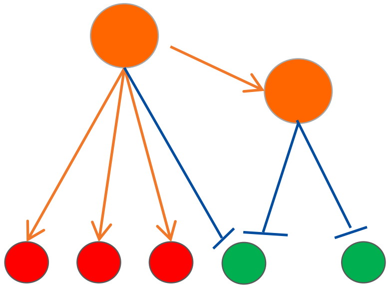
- z-score is a statistical measure of the match between expected relationship direction and observed gene expression
- z-score > 2 or < -2 is considered significant
- Note that the actual z-score is weighted by the underlying findings, the relationship bias, and dataset bias

Upstream Regulators, Mechanistic Networks and Causal Networks

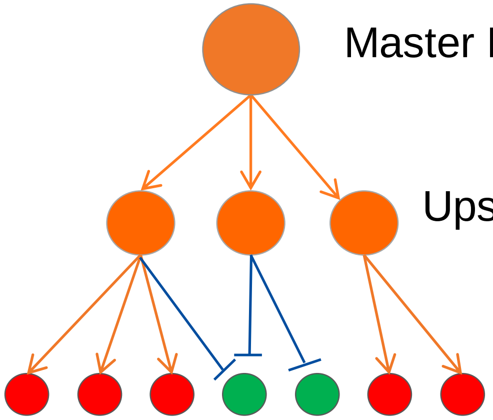
Upstream Regulators



Mechanistic Networks



Advanced Analytics: Causal Network Analysis

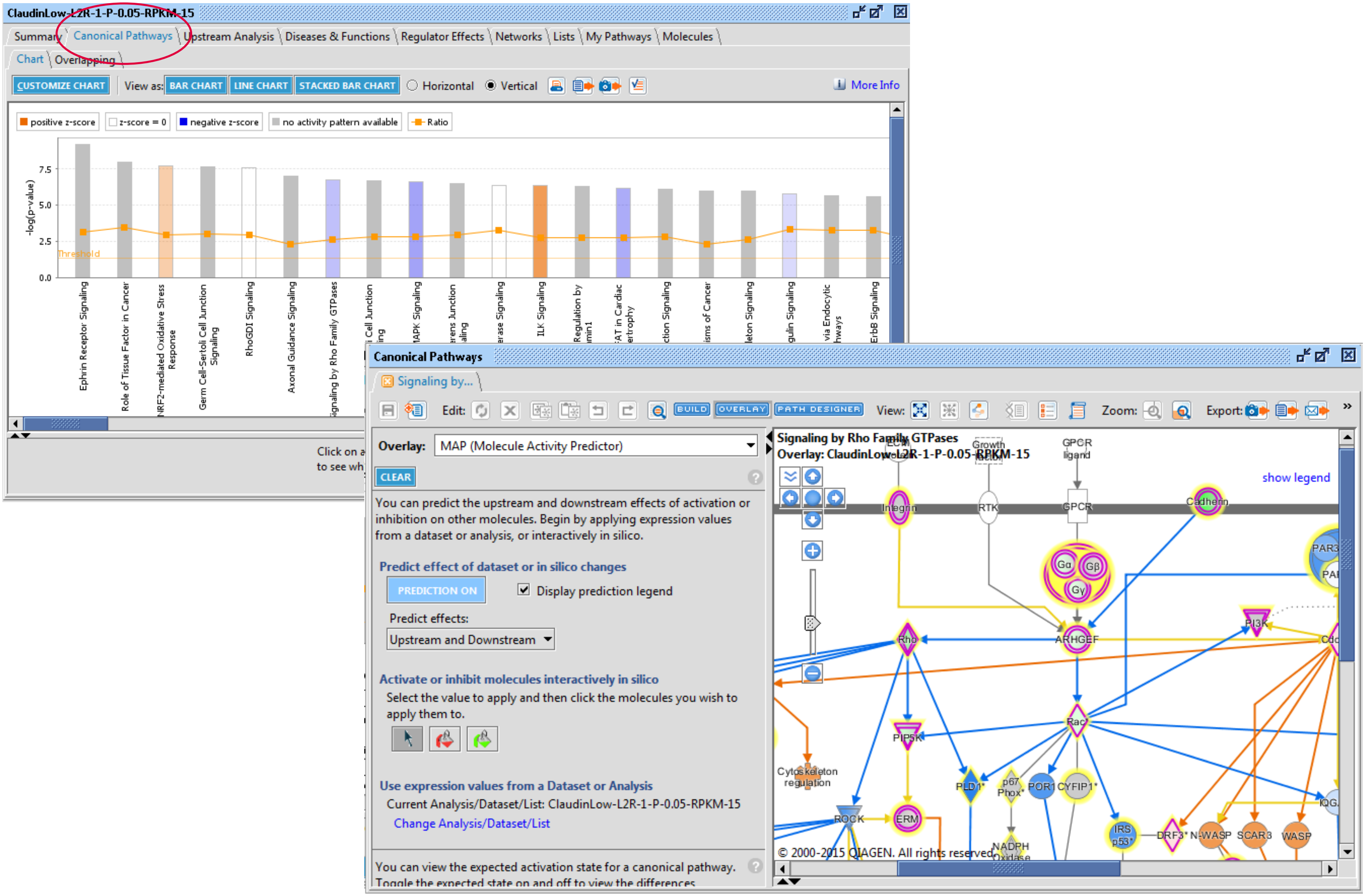


Master Regulators

Upstream Regulators

Your Dataset Genes/Molecules

Canonical Pathways



Overlay: MAP (Molecule Activity Predictor)

CLEAR

You can predict the upstream and downstream effects of activation or inhibition on other molecules. Begin by applying expression values from a dataset or analysis, or interactively in silico.

Predict effect of dataset or in silico changes

PREDICTION ON ☑ Display prediction legend

Predict effects: Upstream and Downstream

Activate or inhibit molecules interactively in silico

Select the value to apply and then click the molecules you wish to apply them to.

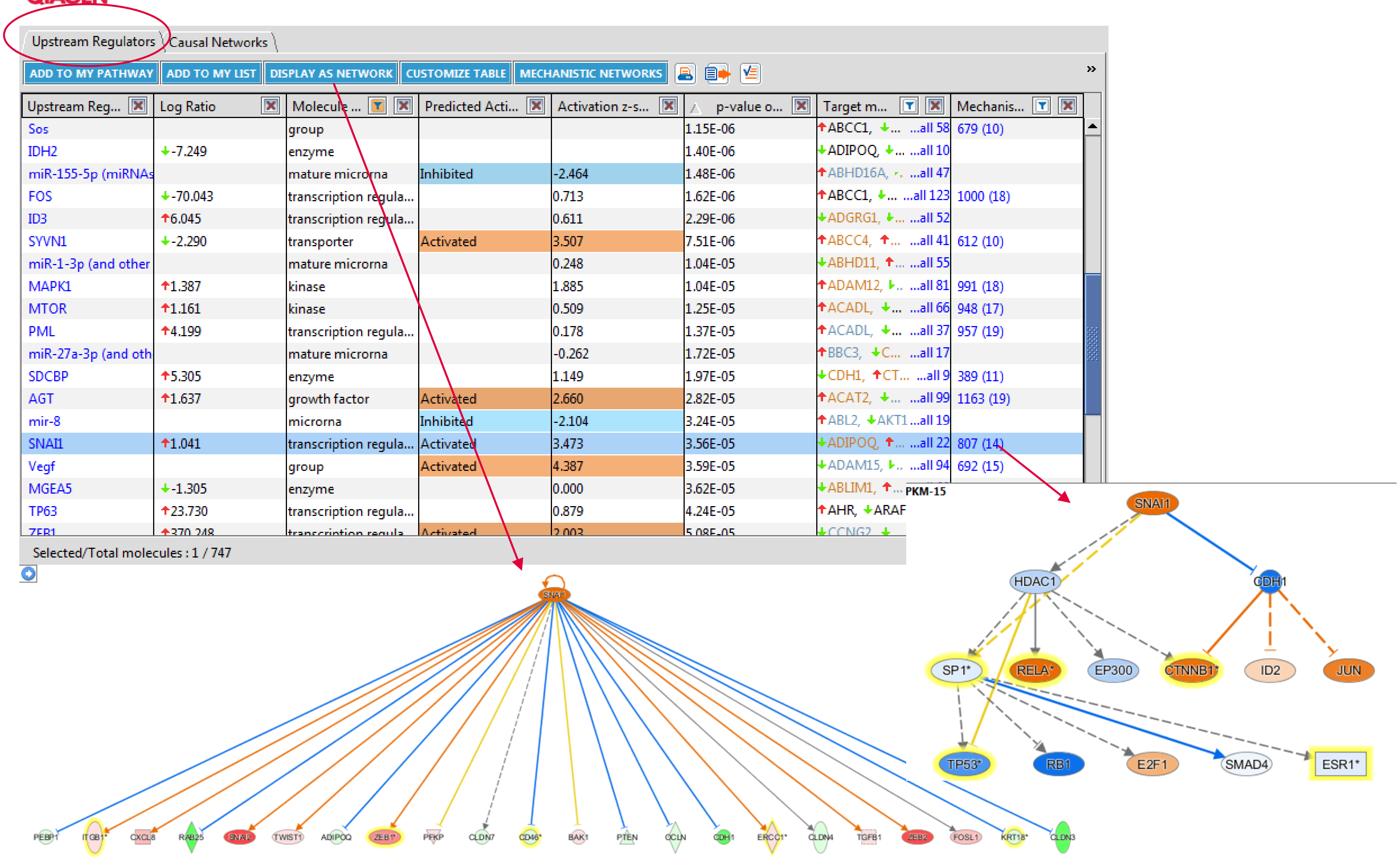
Use expression values from a Dataset or Analysis

Current Analysis/Dataset/List: ClaudinLow-L2R-1-P-0.05-RPKM-15

Change Analysis/Dataset/List

You can view the expected activation state for a canonical pathway. Toggle the expected state on and off to view the differences

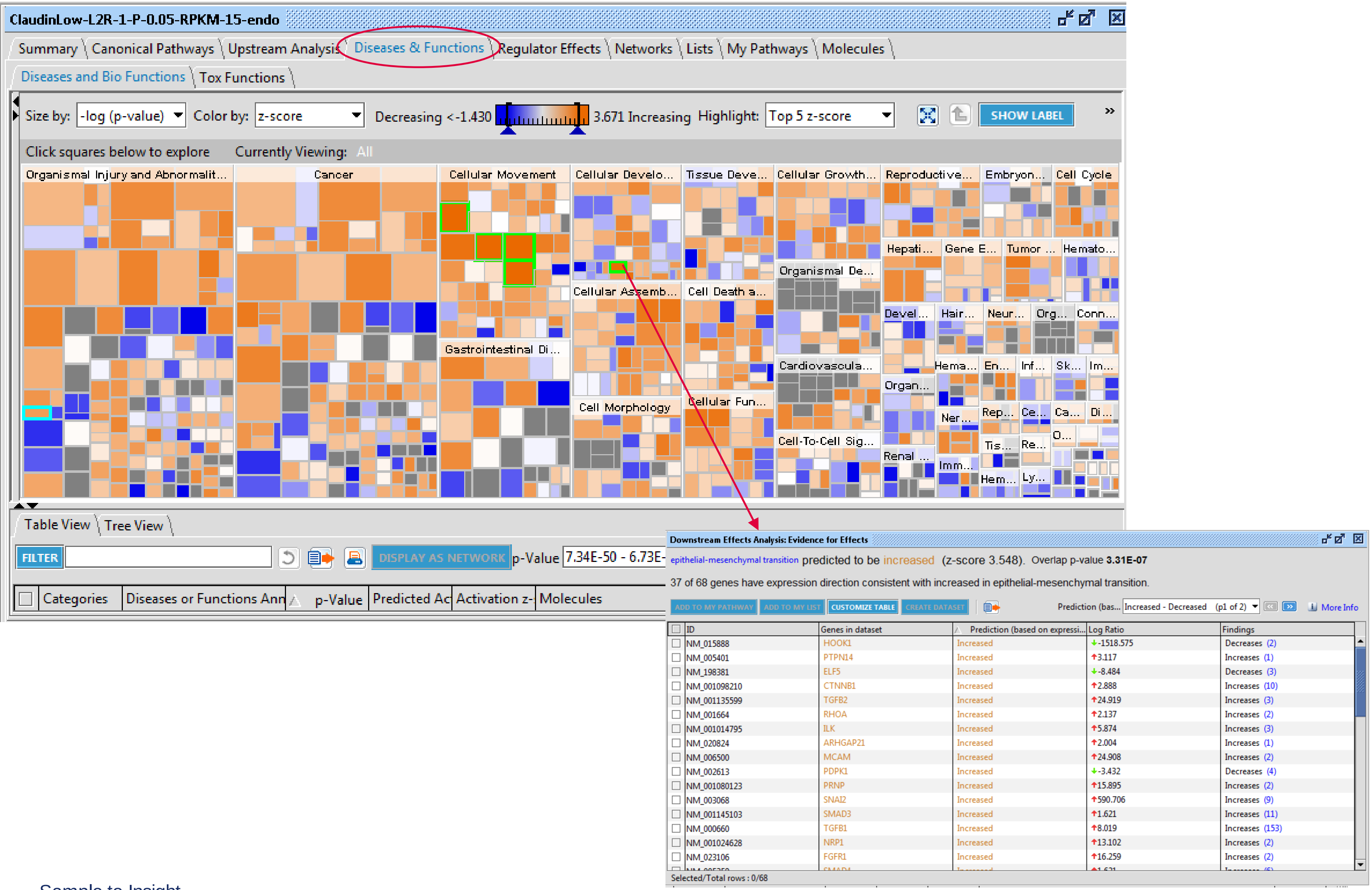
Upstream Analysis



Upstream Reg...	Log Ratio	Molecule ...	Predicted Acti...	Activation z-s...	p-value o...	Target m...	Mechanis...
Sos		group			1.15E-06	↑ABCC1, ↓... ...all 58	679 (10)
IDH2	↓-7.249	enzyme			1.40E-06	↓ADIPOQ, ↓... ...all 10	
miR-155-5p (miRNAs		mature microrna	Inhibited	-2.464	1.48E-06	↑ABHD16A, ↑. ...all 47	
FOS	↓-70.043	transcription regula...		0.713	1.62E-06	↑ABCC1, ↓... ...all 123	1000 (18)
ID3	↑6.045	transcription regula...		0.611	2.29E-06	↓ADGRG1, ↓... ...all 52	
SYVN1	↓-2.290	transporter	Activated	3.507	7.51E-06	↑ABCC4, ↑... ...all 41	612 (10)
miR-1-3p (and other		mature microrna		0.248	1.04E-05	↓ABHD11, ↑... ...all 55	
MAPK1	↑1.387	kinase		1.885	1.04E-05	↑ADAM12, ↓.. ...all 81	991 (18)
MTOR	↑1.161	kinase		0.509	1.25E-05	↑ACADL, ↓... ...all 66	948 (17)
PML	↑4.199	transcription regula...		0.178	1.37E-05	↑ACADL, ↓... ...all 37	957 (19)
miR-27a-3p (and oth		mature microrna		-0.262	1.72E-05	↑BBC3, ↓C... ...all 17	
SDCBP	↑5.305	enzyme		1.149	1.97E-05	↓CDH1, ↑CT... ...all 9	389 (11)
AGT	↑1.637	growth factor	Activated	2.660	2.82E-05	↑ACAT2, ↓... ...all 99	1163 (19)
mir-8		microrna	Inhibited	-2.104	3.24E-05	↑ABL2, ↓AKT1 ...all 19	
SNAI1	↑1.041	transcription regula...	Activated	3.473	3.56E-05	↓ADIPOQ, ↑... ...all 22	807 (14)
Vegf		group	Activated	4.387	3.59E-05	↓ADAM15, ↓.. ...all 94	692 (15)
MGEA5	↓-1.305	enzyme		0.000	3.62E-05	↓ABLIM1, ↑...	
TP63	↑23.730	transcription regula...		0.879	4.24E-05	↑AHR, ↓ARAF	
ZEB1	↑370.248	transcription regula...	Activated	2.003	5.08E-05	↓CCNG2, ↓	

Selected/Total molecules : 1 / 747

Diseases and Functions



Size by: -log (p-value)   Color by: z-score   Decreasing <-1.430   3.671 Increasing   Highlight: Top 5 z-score

Currently Viewing: All

p-Value 7.34E-50 - 6.73E-

epithelial-mesenchymal transition predicted to be increased (z-score 3.548). Overlap p-value **3.31E-07**

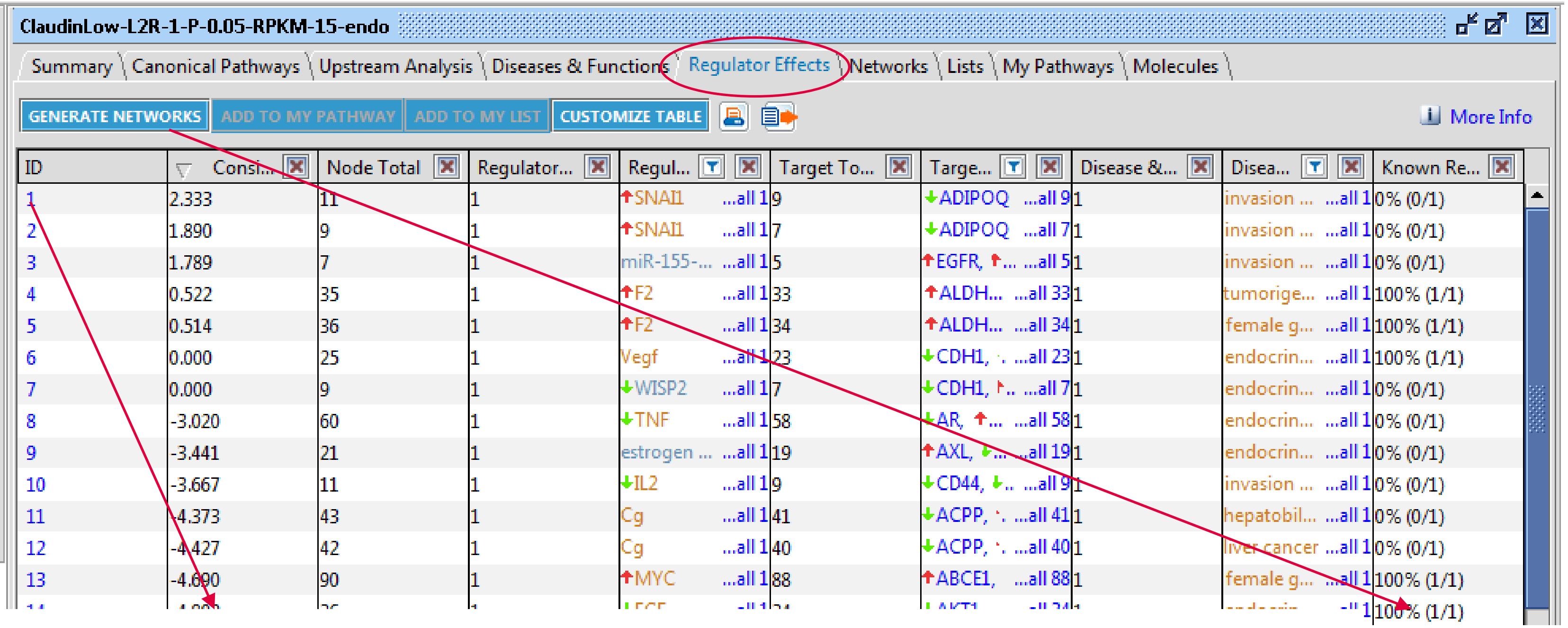
37 of 68 genes have expression direction consistent with increased in epithelial-mesenchymal transition.

Prediction (bas... Increased - Decreased (p1 of 2)

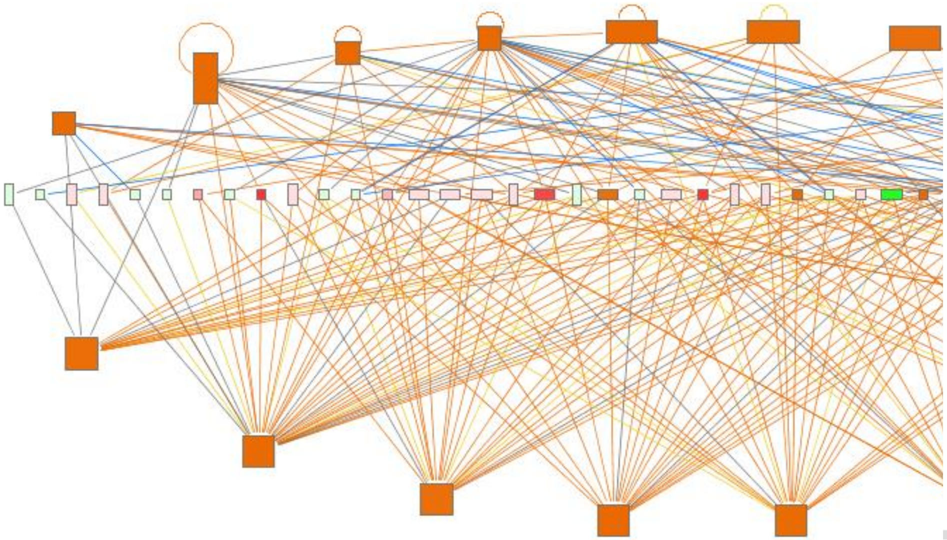
☐	ID	Genes in dataset	Prediction (based on expressi...	Log Ratio	Findings
☐	NM_015888	HOOK1	Increased	↓-1518.575	Decreases (2)
☐	NM_005401	PTPN14	Increased	↑3.117	Increases (1)
☐	NM_198381	ELF5	Increased	↓-8.484	Decreases (3)
☐	NM_001098210	CTNNB1	Increased	↑2.888	Increases (10)
☐	NM_001135599	TGFB2	Increased	↑24.919	Increases (3)
☐	NM_001664	RHOA	Increased	↑2.137	Increases (2)
☐	NM_001014795	ILK	Increased	↑5.874	Increases (3)
☐	NM_020824	ARHGAP21	Increased	↑2.004	Increases (1)
☐	NM_006500	MCAM	Increased	↑24.908	Increases (2)
☐	NM_002613	PDPK1	Increased	↓-3.432	Decreases (4)
☐	NM_001080123	PRNP	Increased	↑15.895	Increases (2)
☐	NM_003068	SNAI2	Increased	↑590.706	Increases (9)
☐	NM_001145103	SMAD3	Increased	↑1.621	Increases (11)
☐	NM_000660	TGFB1	Increased	↑8.019	Increases (153)
☐	NM_001024628	NRP1	Increased	↑13.102	Increases (2)
☐	NM_023106	FGFR1	Increased	↑16.259	Increases (2)

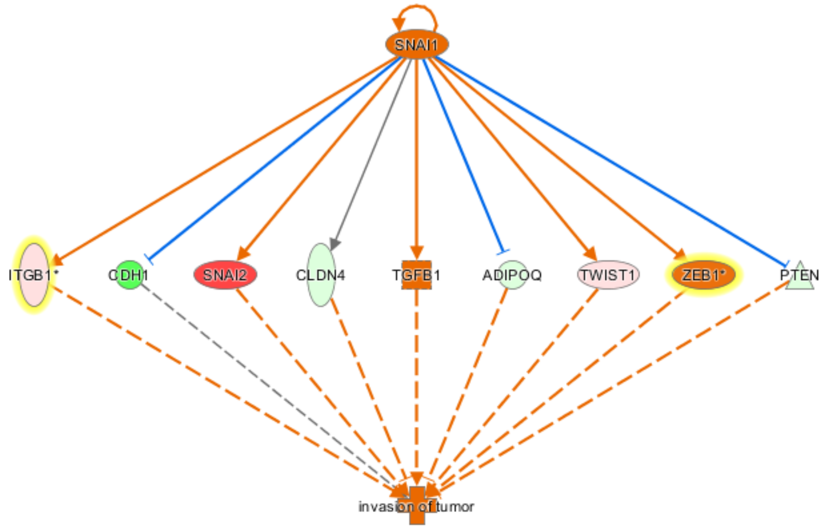
Selected/Total rows : 0/68

Regulator Effects

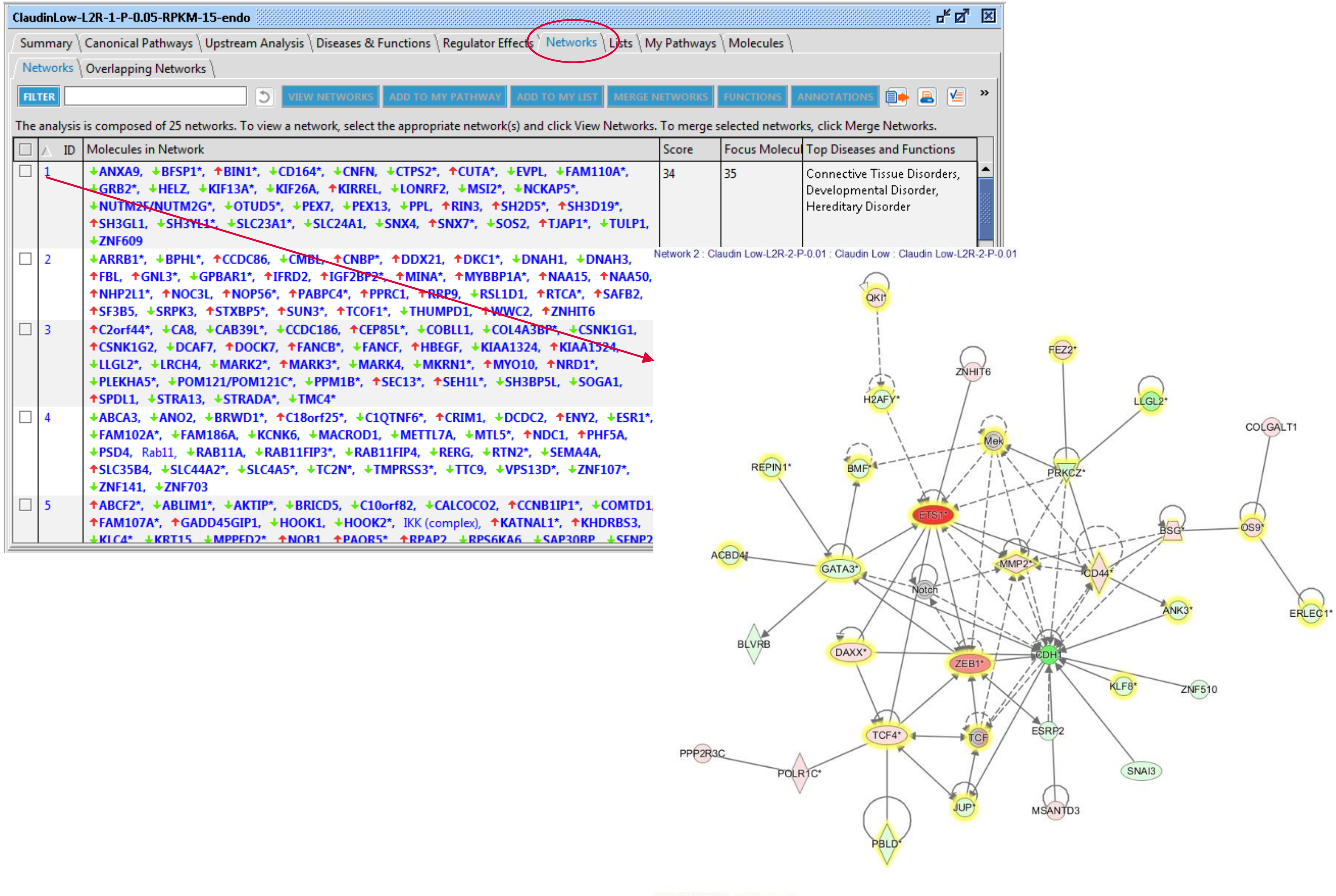


ID	Consi...	Node Total	Regulator...	Regul...	Target To...	Targe...	Disease &...	Disea...	Known Re...
1	2.333	11	1	↑SNAI1 ...all 1	9	↓ADIPOQ ...all 9	1	invasion ... ...all 1	0% (0/1)
2	1.890	9	1	↑SNAI1 ...all 1	7	↓ADIPOQ ...all 7	1	invasion ... ...all 1	0% (0/1)
3	1.789	7	1	miR-155-... ...all 1	5	↑EGFR, ↑... ...all 5	1	invasion ... ...all 1	0% (0/1)
4	0.522	35	1	↑F2 ...all 1	33	↑ALDH... ...all 33	1	tumorige... ...all 1	100% (1/1)
5	0.514	36	1	↑F2 ...all 1	34	↑ALDH... ...all 34	1	female g... ...all 1	100% (1/1)
6	0.000	25	1	Vegf ...all 1	23	↓CDH1, ...all 23	1	endocrin... ...all 1	100% (1/1)
7	0.000	9	1	↓WISP2 ...all 1	7	↓CDH1, ↑.. ...all 7	1	endocrin... ...all 1	0% (0/1)
8	-3.020	60	1	↓TNF ...all 1	58	↓AR, ↑... ...all 58	1	endocrin... ...all 1	0% (0/1)
9	-3.441	21	1	estrogen ... ...all 1	19	↑AXL, ↓... ...all 19	1	endocrin... ...all 1	0% (0/1)
10	-3.667	11	1	↓IL2 ...all 1	9	↓CD44, ↓.. ...all 9	1	invasion ... ...all 1	0% (0/1)
11	-4.373	43	1	Cg ...all 1	41	↓ACPP, ...all 41	1	hepatobil... ...all 1	0% (0/1)
12	-4.427	42	1	Cg ...all 1	40	↓ACPP, ...all 40	1	liver cancer ...all 1	0% (0/1)
13	-4.690	90	1	↑MYC ...all 1	88	↑ABCE1, ...all 88	1	female g... ...all 1	100% (1/1)
									100% (1/1)





Network Analysis



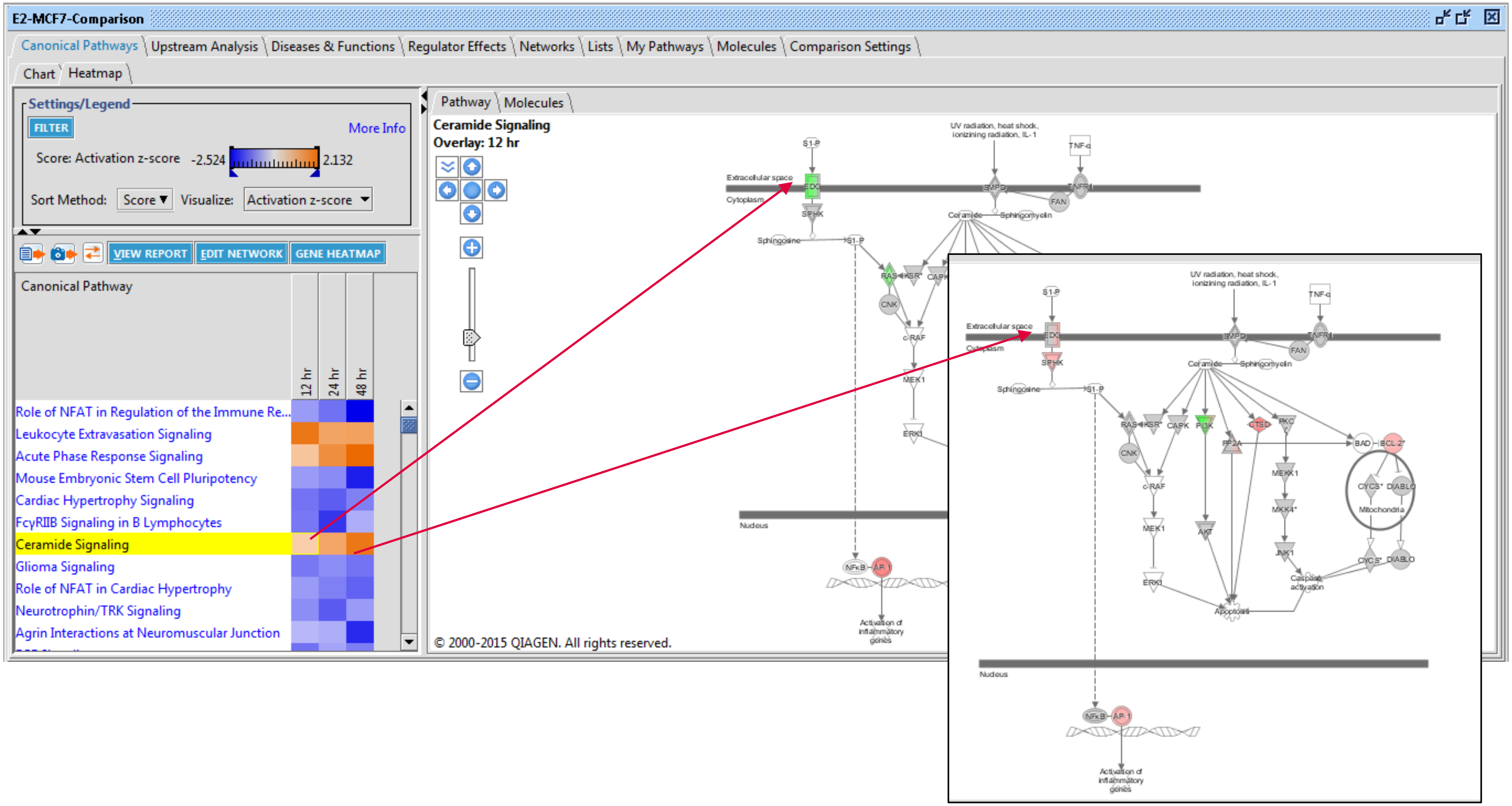
The analysis is composed of 25 networks. To view a network, select the appropriate network(s) and click View Networks. To merge selected networks, click Merge Networks.

☐	ID	Molecules in Network	Score	Focus Molecul	Top Diseases and Functions
☐	1	↓ANXA9, ↓BFSP1*, ↑BIN1*, ↓CD164*, ↓CNFN, ↓CTPS2*, ↑CUTA*, ↓EVPL, ↓FAM110A*, ↓GRB2*, ↓HELZ, ↓KIF13A*, ↓KIF26A, ↑KIRREL, ↓LONRF2, ↓MSI2*, ↓NCKAP5*, ↓NUTM2F/NUTM2G*, ↓OTUD5*, ↓PEX7, ↓PEX13, ↓PPL, ↑RIN3, ↑SH2D5*, ↑SH3D19*, ↑SH3GL1, ↓SH3YL1*, ↓SLC23A1*, ↓SLC24A1, ↓SNX4, ↑SNX7*, ↓SOS2, ↑TJAP1*, ↓TULP1, ↓ZNF609	34	35	Connective Tissue Disorders, Developmental Disorder, Hereditary Disorder
☐	2	↓ARRB1*, ↓BPHL*, ↑CCDC86, ↓CMBL, ↑CNBP*, ↑DDX21, ↑DKC1*, ↓DNAH1, ↓DNAH3, ↑FBL, ↑GNL3*, ↓GPBAR1*, ↑IFRD2, ↑IGF2BP2*, ↑MINA*, ↑MYBBP1A*, ↑NAA15, ↑NAA50, ↑NHP2L1*, ↑NOC3L, ↑NOP56*, ↑PABPC4*, ↑PPRC1, ↑RRP9, ↓RSL1D1, ↑RTCA*, ↑SAFB2, ↑SF3B5, ↓SRPK3, ↑STXBP5*, ↑SUN3*, ↑TCOF1*, ↓THUMPD1, ↑WWC2, ↑ZNHIT6			
☐	3	↑C2orf44*, ↓CA8, ↓CAB39L*, ↓CCDC186, ↑CEP85L*, ↓COBLL1, ↓COL4A3BP*, ↓CSNK1G1, ↑CSNK1G2, ↓DCAF7, ↑DOCK7, ↑FANCB*, ↓FANCF, ↑HBEGF, ↓KIAA1324, ↑KIAA1524, ↓LLGL2*, ↓LRCH4, ↓MARK2*, ↑MARK3*, ↓MARK4, ↓MKRN1*, ↑MYO10, ↑NRD1*, ↓PLEKHA5*, ↓POM121/POM121C*, ↓PPM1B*, ↑SEC13*, ↑SEH1L*, ↓SH3BP5L, ↓SOGA1, ↑SPDL1, ↓STRA13, ↓STRADA*, ↓TMC4*			
☐	4	↓ABCA3, ↓ANO2, ↓BRWD1*, ↑C18orf25*, ↓C1QTNF6*, ↑CRIM1, ↓DCDC2, ↑ENY2, ↓ESR1*, ↓FAM102A*, ↓FAM186A, ↓KCNK6, ↓MACROD1, ↓METTL7A, ↓MTL5*, ↑NDC1, ↑PHF5A, ↓PSD4, Rab11, ↓RAB11A, ↓RAB11FIP3*, ↓RAB11FIP4, ↓RERG, ↓RTN2*, ↓SEMA4A, ↑SLC35B4, ↓SLC44A2*, ↓SLC4A5*, ↓TC2N*, ↓TMPRSS3*, ↓TTC9, ↓VPS13D*, ↓ZNF107*, ↓ZNF141, ↓ZNF703			
☐	5	↑ABCF2*, ↓ABLIM1*, ↓AKTIP*, ↓BRICD5, ↓C10orf82, ↓CALCOCO2, ↑CCNB1IP1*, ↓COMTD1, ↑FAM107A*, ↑GADD45GIP1, ↓HOOK1, ↓HOOK2*, IKK (complex), ↑KATNAL1*, ↑KHDRBS3, ↓KLC4*, ↓KRT15, ↓MPPED2*, ↑NOB1, ↑PAQR5*, ↑RPAP2, ↓RPS6KA6, ↓SAP30BP, ↓SENP2			

Network 2 : Claudin Low-L2R-2-P-0.01 : Claudin Low : Claudin Low-L2R-2-P-0.01

Comparison Analysis:
Comparing Multiple Observations (Experimental Groups)

Comparison Analysis: Gene Expression Comparison on the Pathway Chart



Score: Activation z-score -2.524 … 2.132

Sort Method: Score

Visualize: Activation z-score

Canonical Pathway	12 hr	24 hr	48 hr
Role of NFAT in Regulation of the Immune Re...			
Leukocyte Extravasation Signaling			
Acute Phase Response Signaling			
Mouse Embryonic Stem Cell Pluripotency			
Cardiac Hypertrophy Signaling			
FcγRIIB Signaling in B Lymphocytes			
Ceramide Signaling			
Glioma Signaling			
Role of NFAT in Cardiac Hypertrophy			
Neurotrophin/TRK Signaling			
Agrin Interactions at Neuromuscular Junction			

Ceramide Signaling
Overlay: 12 hr

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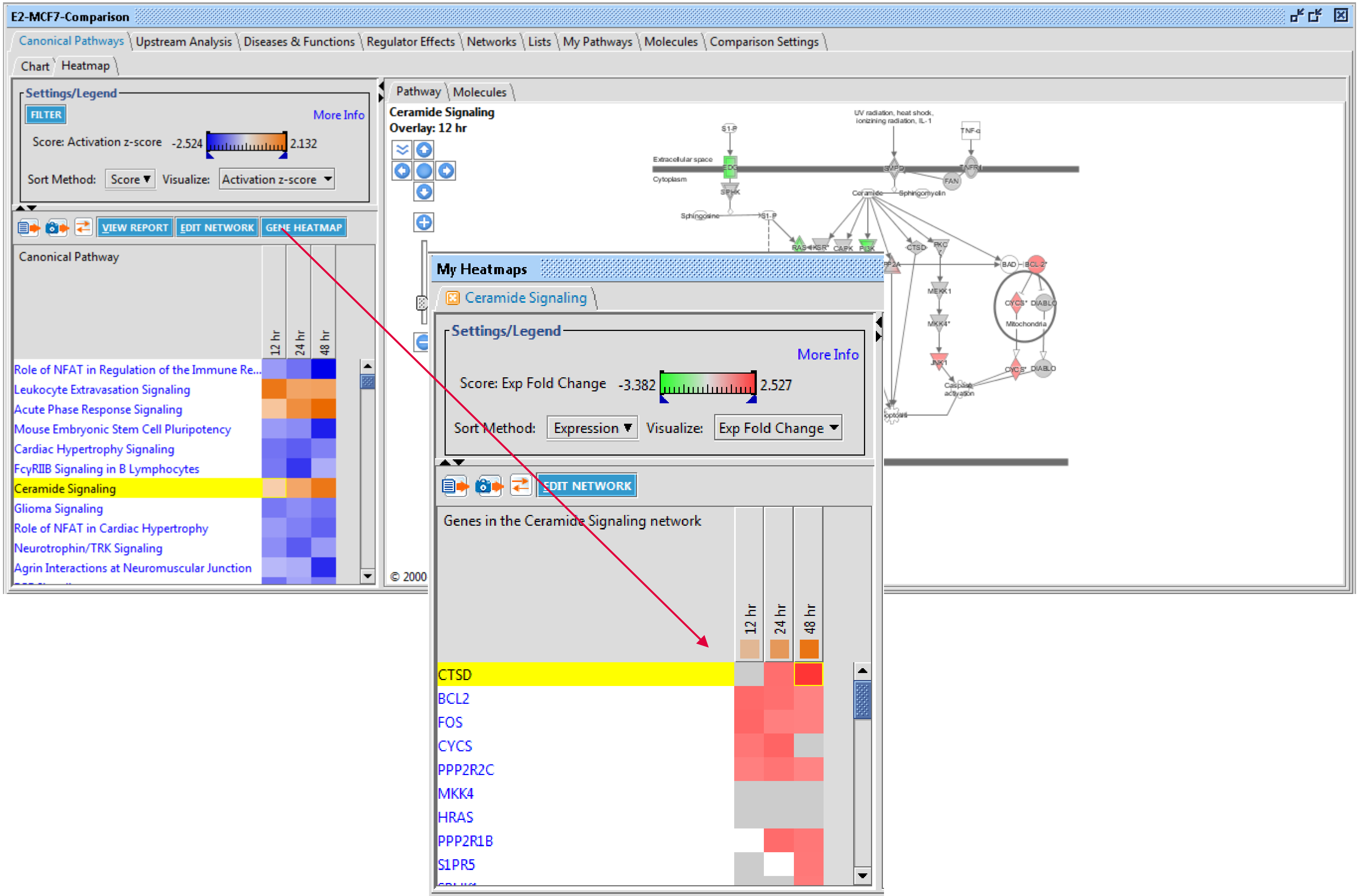
Comparison Analysis: Gene Expression Comparison through Heatmap



Settings/Legend

Score: Activation z-score -2.524 2.132

Sort Method: Score ▼ Visualize: Activation z-score ▼

Canonical Pathway

	12 hr	24 hr	48 hr
Role of NFAT in Regulation of the Immune Re...			
Leukocyte Extravasation Signaling			
Acute Phase Response Signaling			
Mouse Embryonic Stem Cell Pluripotency			
Cardiac Hypertrophy Signaling			
FcγRIIB Signaling in B Lymphocytes			
Ceramide Signaling			
Glioma Signaling			
Role of NFAT in Cardiac Hypertrophy			
Neurotrophin/TRK Signaling			
Agrin Interactions at Neuromuscular Junction			

Ceramide Signaling
Overlay: 12 hr

Settings/Legend

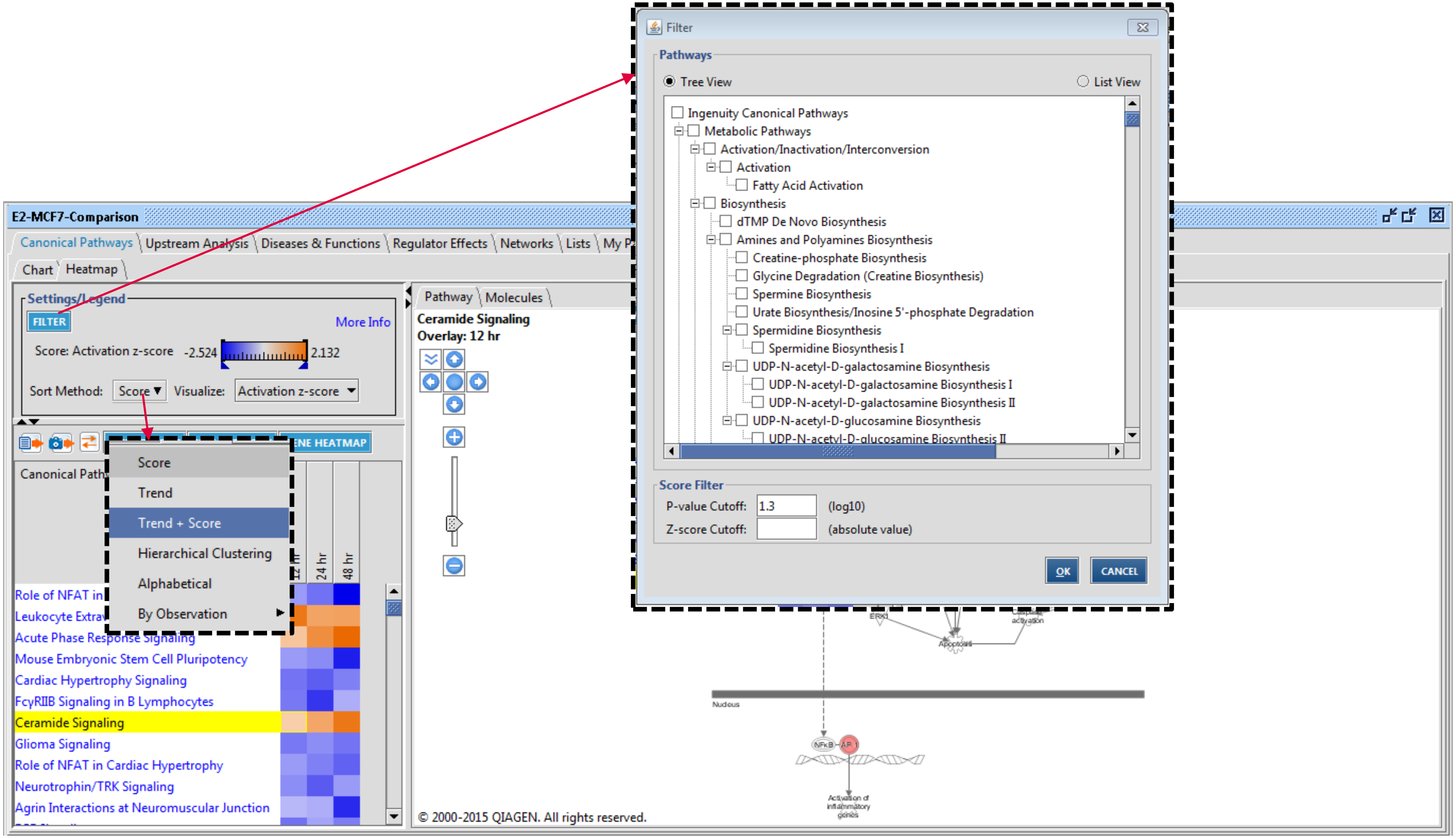
Score: Exp Fold Change -3.382 2.527

Sort Method: Expression ▼ Visualize: Exp Fold Change ▼

Genes in the Ceramide Signaling network

	12 hr	24 hr	48 hr
CTSD			
BCL2			
FOS			
CYCS			
PPP2R2C			
MKK4			
HRAS			
PPP2R1B			
S1PR5			

Comparison Analysis: Filtering and Sorting the Heatmap



Score: Activation z-score -2.524 2.132

Sort Method: Score

Visualize: Activation z-score

Ceramide Signaling

Overlay: 12 hr

P-value Cutoff: 1.3 (log10)

Z-score Cutoff: (absolute value)

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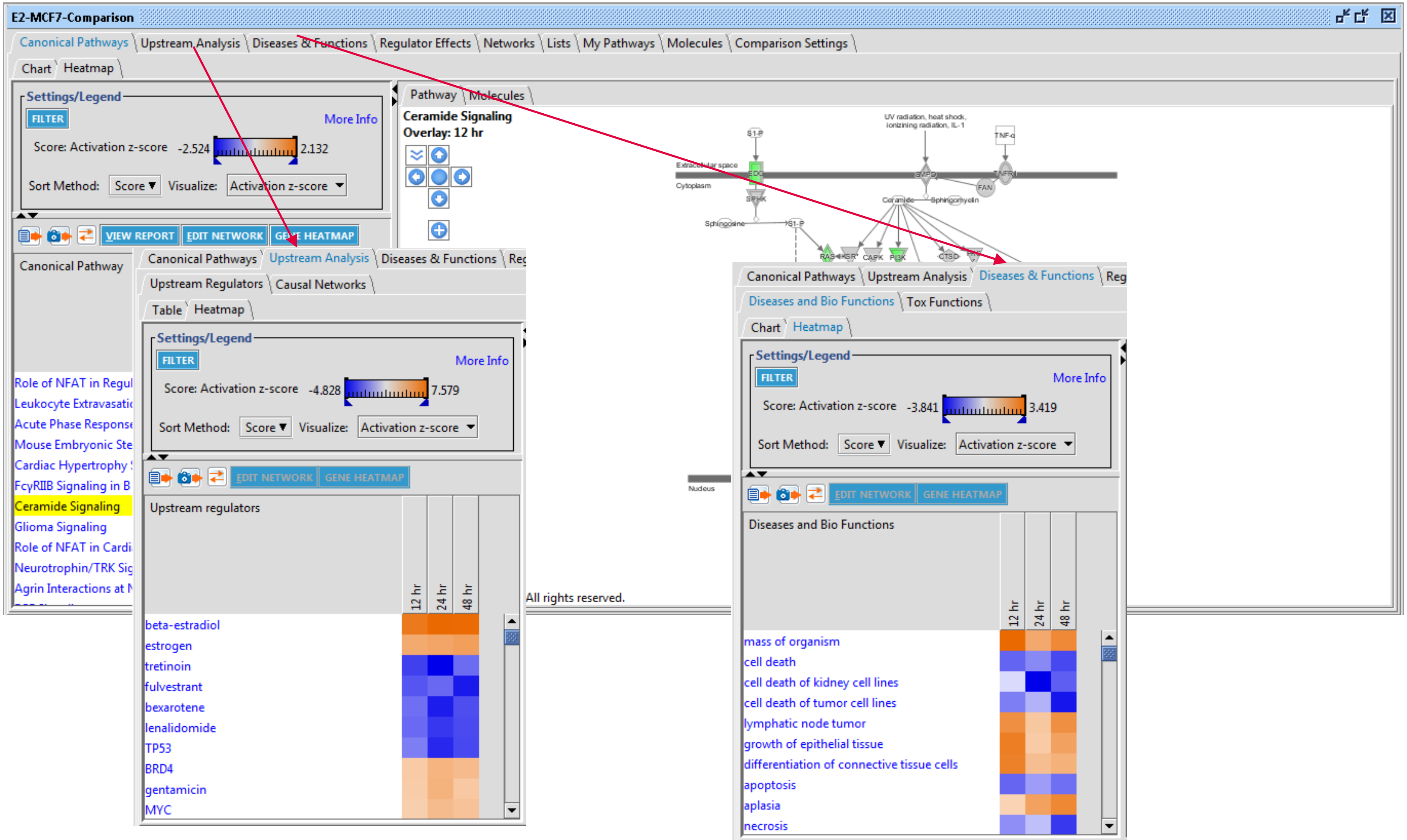
Comparison Analysis: Canonical Pathway, Upstream Analysis and Diseases and Functions



Canonical Pathways – Chart

Score: Activation z-score -2.524 – 2.132

Sort Method: Score

Visualize: Activation z-score

Canonical Pathway

- Role of NFAT in Regul
- Leukocyte Extravasatio
- Acute Phase Response
- Mouse Embryonic Ste
- Cardiac Hypertrophy S
- FcγRIIB Signaling in B
- Ceramide Signaling
- Glioma Signaling
- Role of NFAT in Cardi
- Neurotrophin/TRK Sig
- Agrin Interactions at N

Ceramide Signaling
Overlay: 12 hr

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Upstream Analysis – Upstream Regulators – Heatmap

Score: Activation z-score -4.828 – 7.579

Sort Method: Score

Visualize: Activation z-score

Upstream regulators

- beta-estradiol
- estrogen
- tretinoin
- fulvestrant
- bexarotene
- lenalidomide
- TP53
- BRD4
- gentamicin
- MYC

Diseases & Functions – Diseases and Bio Functions – Heatmap

Score: Activation z-score -3.841 – 3.419

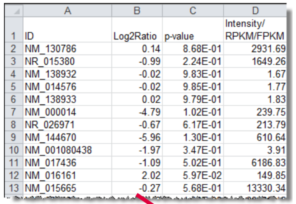
Sort Method: Score

Visualize: Activation z-score

Diseases and Bio Functions

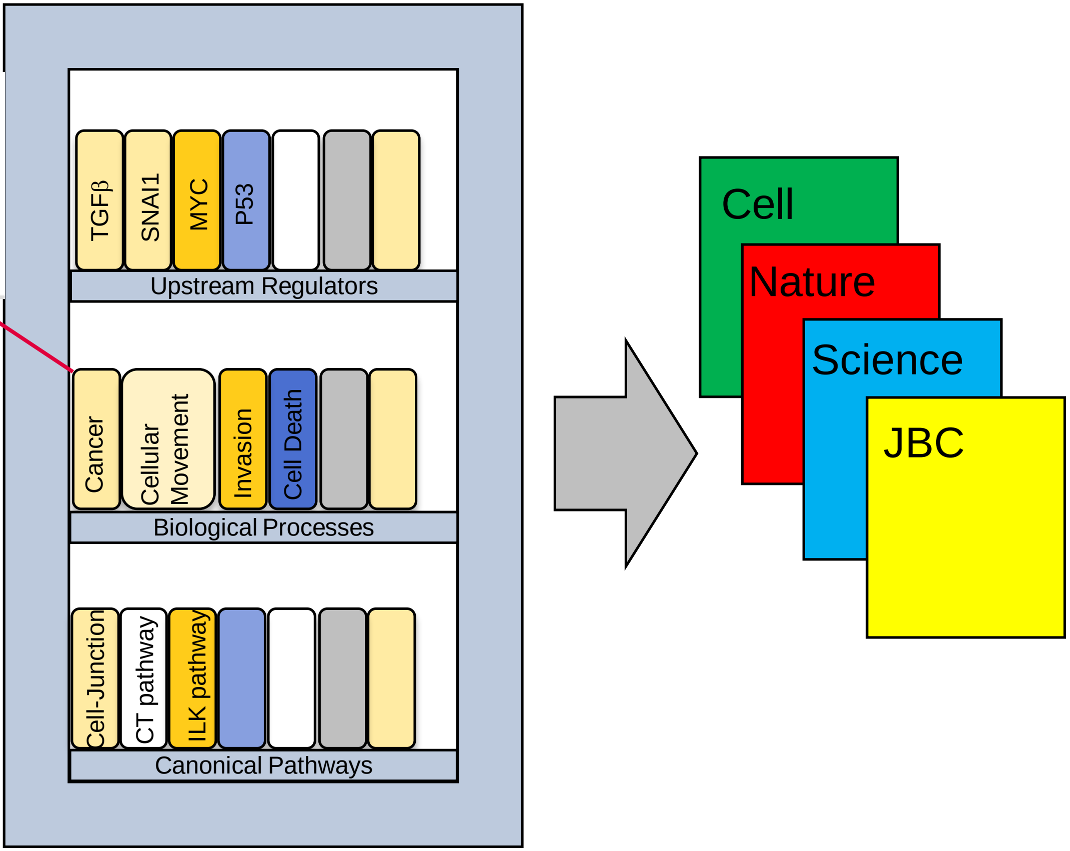
- mass of organism
- cell death
- cell death of kidney cell lines
- cell death of tumor cell lines
- lymphatic node tumor
- growth of epithelial tissue
- differentiation of connective tissue cells
- apoptosis
- aplasia
- necrosis

What can IPA do?



	A	B	C	D
1	ID	Log2Ratio	p-value	Intensity/ RPKM/FPKM
2	NM_130786	0.14	8.68E-01	2931.69
3	NR_015380	-0.99	2.24E-01	1649.26
4	NM_138932	-0.02	9.83E-01	1.67
5	NM_014576	-0.02	9.85E-01	1.77
6	NM_138933	0.02	9.79E-01	1.83
7	NM_000014	-4.79	1.02E-01	239.75
8	NR_026971	-0.67	6.17E-01	213.79
9	NM_144670	-5.96	1.30E-01	610.64
10	NM_001080438	-1.97	3.47E-01	3.91
11	NM_017436	-1.09	5.02E-01	6186.83
12	NM_016161	2.02	5.97E-02	149.85
13	NM_015665	-0.27	5.68E-01	13330.34

IPA



TGFβ
SNAI1
MYC
P53

Upstream Regulators

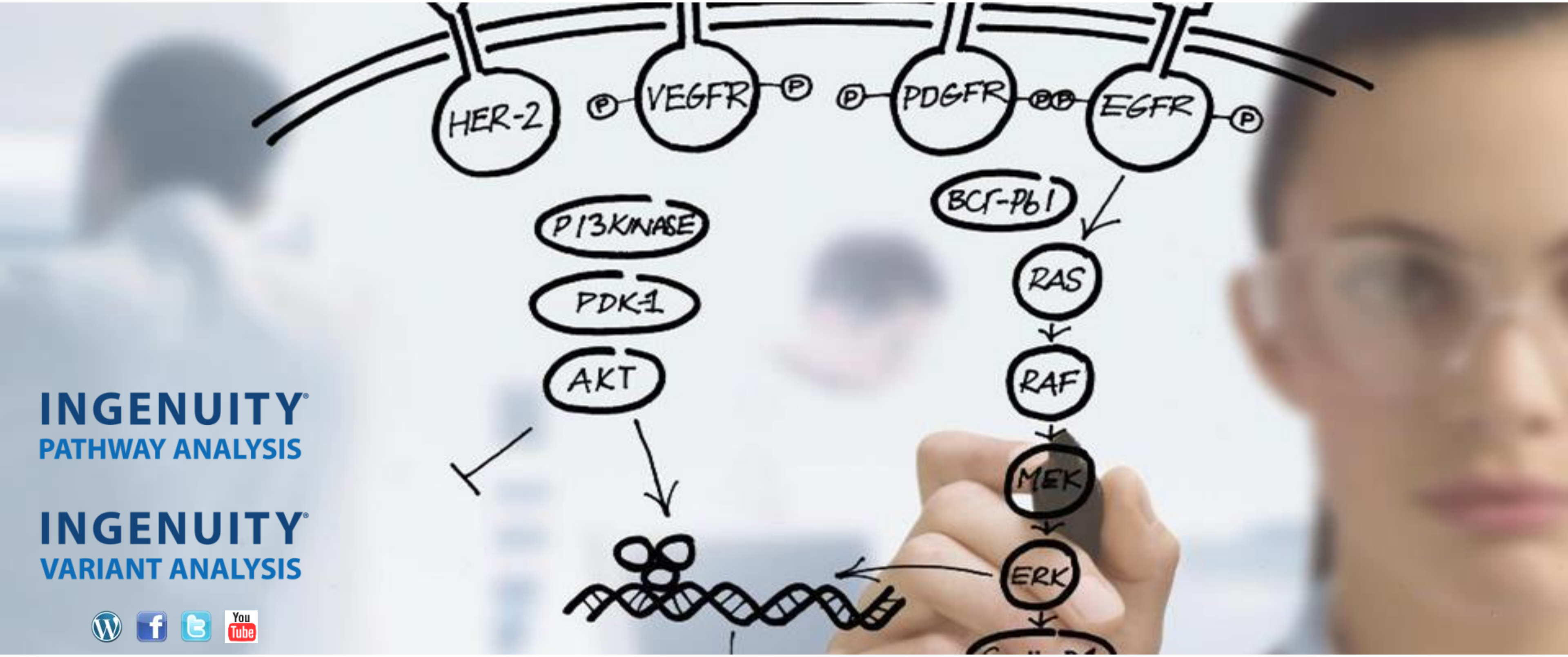
Cancer
Cellular Movement
Invasion
Cell Death

Biological Processes

Cell-Junction
CT pathway
ILK pathway

Canonical Pathways

Cell
Nature
Science
JBC



QUESTIONS?

- **CONTACTS**:
  - ☐ General: support@ingenuity.com
  - ☐ +1 650 381-5111
    6am-5pm Pacific Time (M-F)

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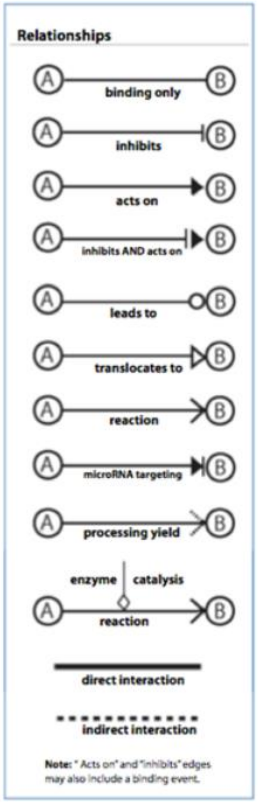
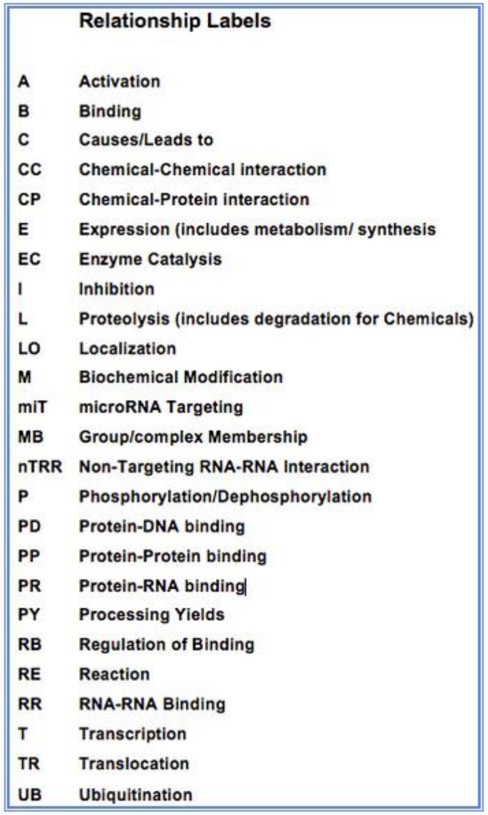
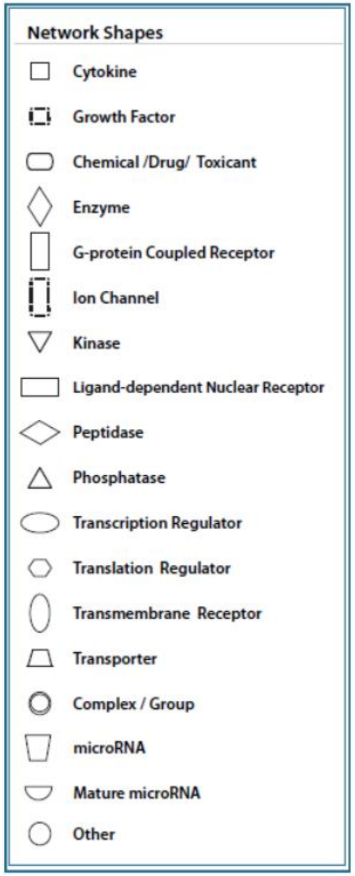


Note: "Acts on" and "inhibits" edges may also include a binding event.



Relationship Labels

A	Activation
B	Binding
C	Causes/Leads to
CC	Chemical-Chemical interaction
CP	Chemical-Protein interaction
E	Expression (includes metabolism/ synthesis
EC	Enzyme Catalysis
I	Inhibition
L	Proteolysis (includes degradation for Chemicals)
LO	Localization
M	Biochemical Modification
miT	microRNA Targeting
MB	Group/complex Membership
nTRR	Non-Targeting RNA-RNA Interaction
P	Phosphorylation/Dephosphorylation
PD	Protein-DNA binding
PP	Protein-Protein binding
PR	Protein-RNA binding
PY	Processing Yields
RB	Regulation of Binding
RE	Reaction
RR	RNA-RNA Binding
T	Transcription
TR	Translocation
UB	Ubiquitination



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