Insights into single-cell RNA-seq via Qiagen Ingenuity Pathway analysis

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Agenda

- Introduction to QIAGEN Ingenuity Pathway Analysis
- Querying IPA's Knowledge base
  - Search in IPA
  - Custom network construction
  - Contextualization using public data
- Analyzing RNA seq using IPA
  - Data format
  - Data upload and analysis setup
  - Core analysis
- Summary

System Requirements

Operating systems

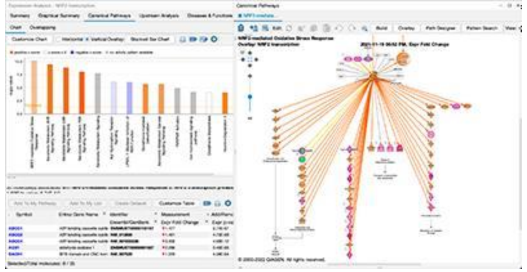
- Windows operating systems:
  - Windows 11, 10, 8
- Mac operating systems:
  - macOS Sonoma, Ventura, Monterey
- Internet browsers:
  - Firefox 91 or later*
  - Safari 16 or later*
  - Chrome 110 or later*
  - Microsoft Edge 94 or later*
- Java runtime environment (JRE; not needed if you install the IPA client):
  - JRE 8 to 10

Hardware

- CoreTM i5 processor or equivalent running at 2 GHz or higher with 64-bit OS and Java
- Minimum at least 3 GB RAM free for Java

QIAGEN – from Sample to Insight

An example: Analyzing variant data from Sample to Insight



Sample to data

NGS library prep
Sequencing

- Platform- and assay-agnostic
- Whole genome, whole exome, custom panels

Data to information

Normalization and quality control

Read mapping

Variant calling

- **QIAGEN CLC Genomics Workbench, Server and Cloud Computation**

BaseSpace ad Amazon Web Service integration

Information to knowledge

Data integration

Metadata exploration

Differential expression

- **QIAGEN OmicSoft Suite, Lands, and APIs**

Curated experiments

- **QIAGEN OmicSoft Lands: OncoLand, DiseaseLand, Single Cell Land**

Knowledge to insight

Interpretation

Pathway analysis

- **QIAGEN IPA**

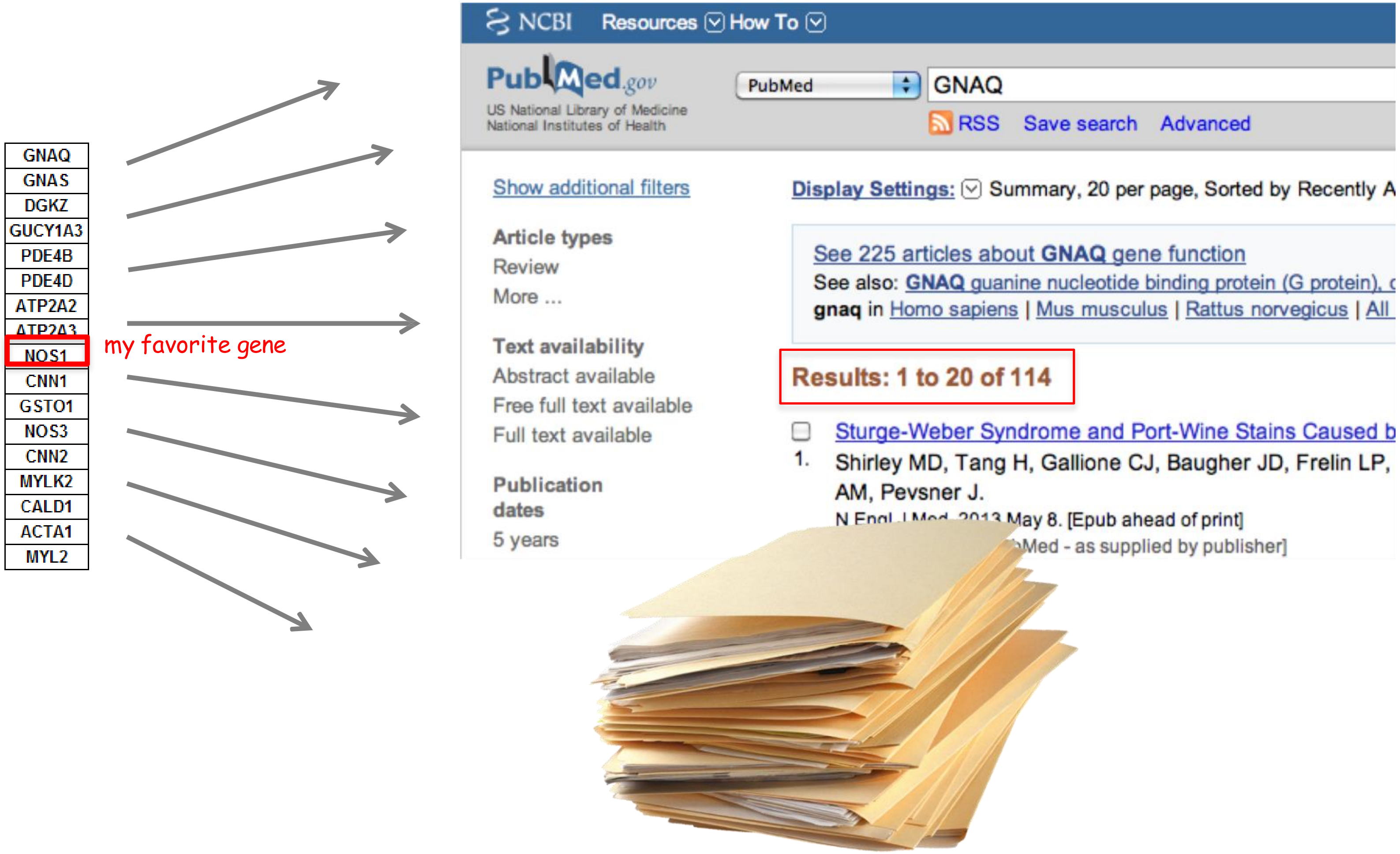
Variant interpretation

- **QCI Translational, HSMD, HGMD and COSMIC**

Portfolio designed to transition complex ′omics data into high-value actionable insights without the need for deep expertise

Pathway and Network Analysis

- Save time compared to traditional approach



GNAQ
GNAS
DGKZ
GUCY1A3
PDE4B
PDE4D
ATP2A2
ATP2A3
NOS1
CNN1
GSTO1
NOS3
CNN2
MYLK2
CALD1
ACTA1
MYL2

my favorite gene

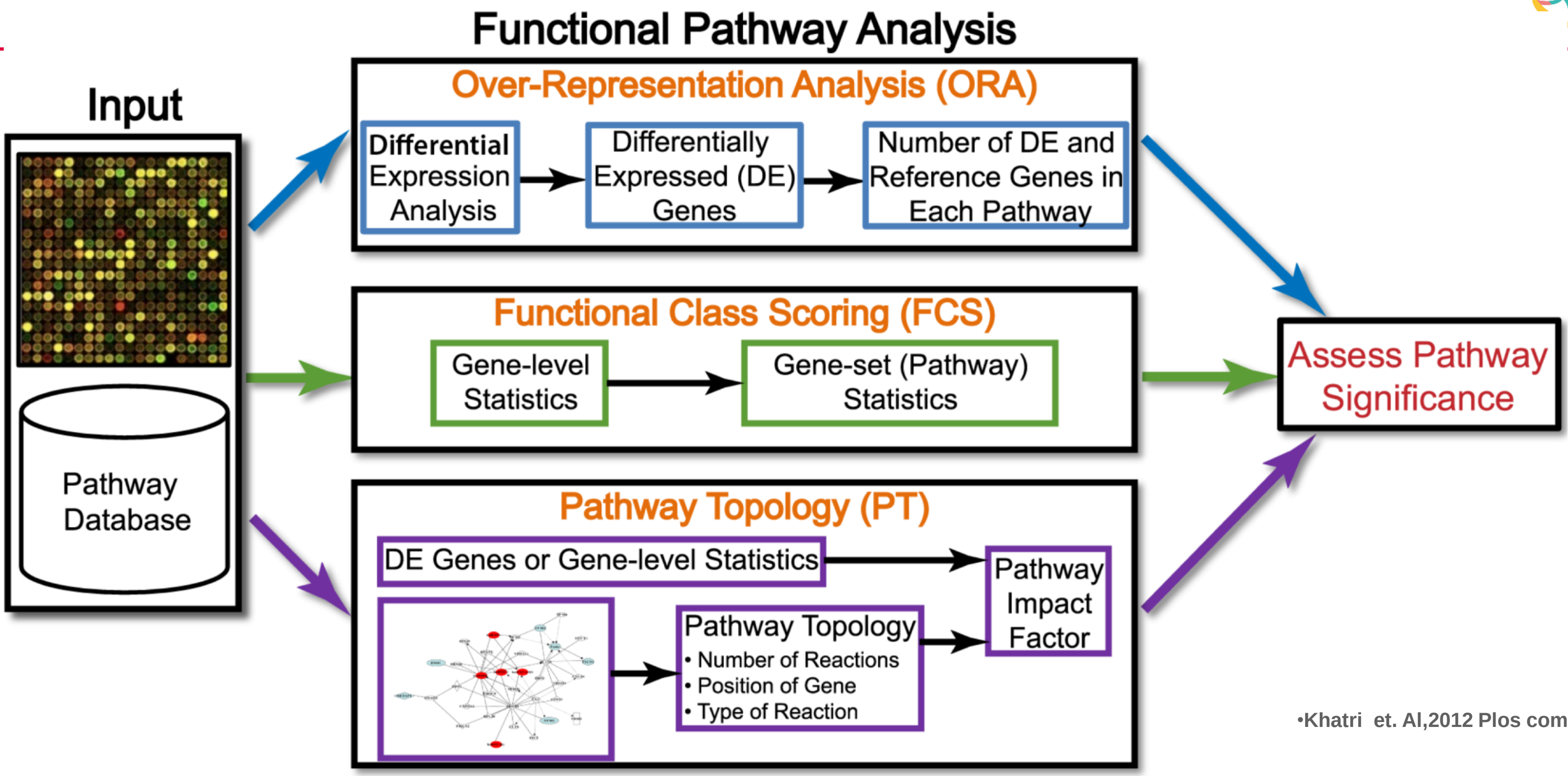
GNAQ

Display Settings: Summary, 20 per page, Sorted by Recently A

See 225 articles about GNAQ gene function
See also: GNAQ guanine nucleotide binding protein (G protein), c
gnaq in Homo sapiens | Mus musculus | Rattus norvegicus | All

Results: 1 to 20 of 114

☐ 1. Sturge-Weber Syndrome and Port-Wine Stains Caused b
Shirley MD, Tang H, Gallione CJ, Baugher JD, Frelin LP, AM, Pevsner J.
N Engl J Med. 2013 May 8. [Epub ahead of print]
bMed - as supplied by publisher]

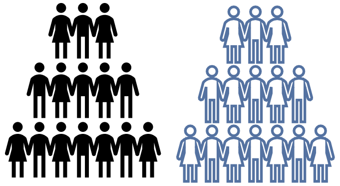


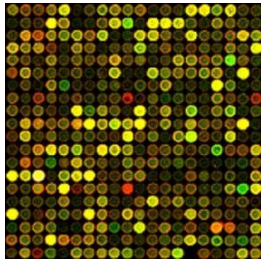
•Khatri et. Al,2012 Plos computational biology

- The data generated by an experiment using a high-throughput technology (e.g., microarray, proteomics, metabolomics), along with functional annotations (pathway database) of the corresponding genome, are input to virtually all pathway analysis methods.
- ORA methods require that the input is a list of differentially expressed genes
- FCS methods use the entire data matrix as input
- PT-based methods additionally utilize the number and type of interactions between gene products, which may or may not be a part of a pathway database.
- The result of every pathway analysis method is a list of significant pathways in the condition under study.

Why are we using Qiagen Ingenuity Pathway analysis?

Your dataset





PDE6A
SLC6A14
LPCAT1
C2
CFB
REG4
CD55
TIMP1
DPP10
PDIA4
PRKG2
NAT8B
SHISA5
LCN2
CDH3
ACAT1
NAALADL1
APOBEC3B
NMT2
KYNU
TMEM63C
S100A11
PI3
CDC25B
CNNM2
CHRNA1
LRRN2
RMDN2
CNTFR
CDC14A
C7orf31
BACE2
CXCL1
SLC36A1
WDR78
PKM

→

Public /commercial database

Drugs and chemicals

Pathway

Disease

Function

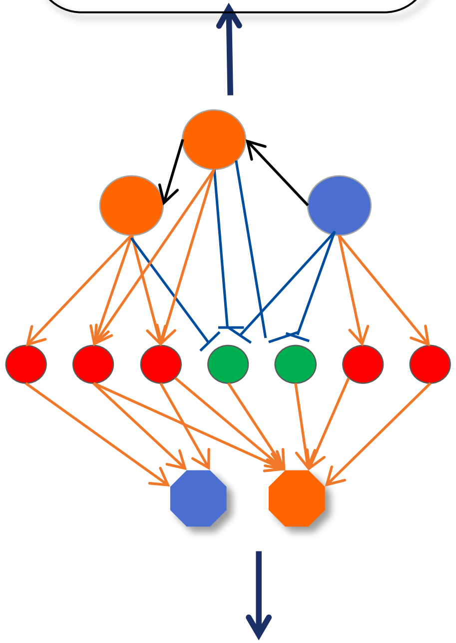
Network

ORA/FCS/Topology Pathway Analysis

Machine learning

→

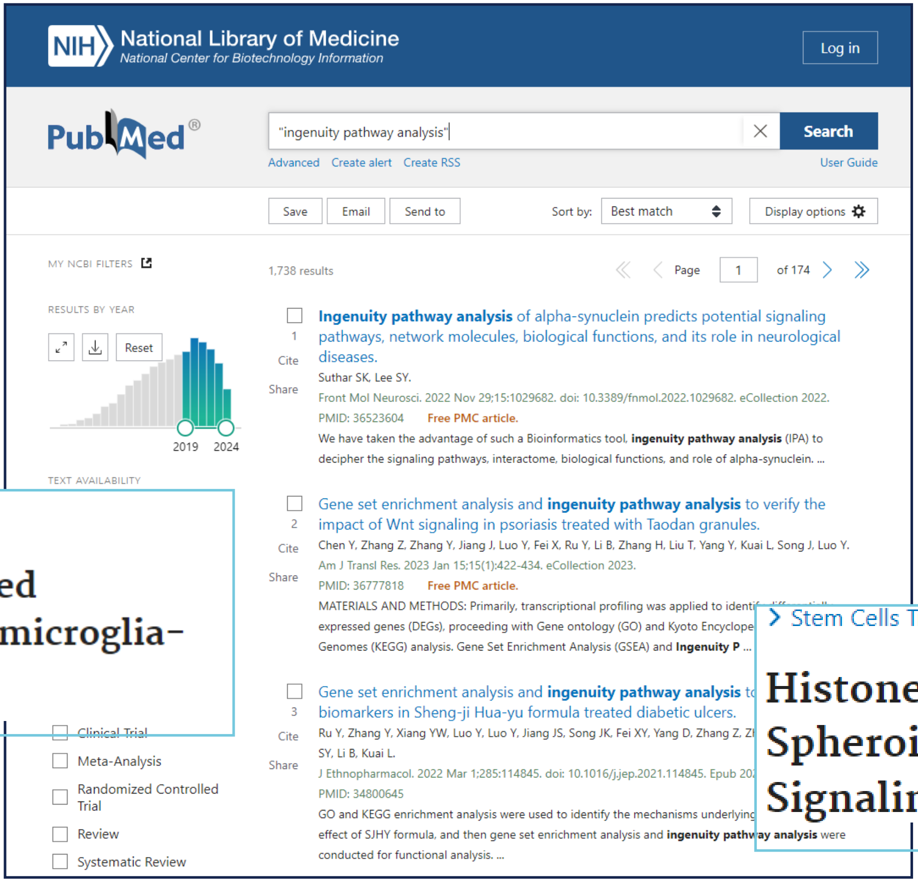
What do they relate to each other?



What are the relationship between each molecules?

Publication using Qiagen Ingenuity Pathway Analysis

From 2019-2024
1,738 literatures



"ingenuity pathway analysis"

1,738 results

☐ 1

Ingenuity pathway analysis of alpha-synuclein predicts potential signaling pathways, network molecules, biological functions, and its role in neurological diseases.
Suthar SK, Lee SY.
Front Mol Neurosci. 2022 Nov 29;15:1029682. doi: 10.3389/fnmol.2022.1029682. eCollection 2022.
PMID: 36523604 Free PMC article.
We have taken the advantage of such a Bioinformatics tool, **ingenuity pathway analysis** (IPA) to decipher the signaling pathways, interactome, biological functions, and role of alpha-synuclein. ...

☐ 2

Gene set enrichment analysis and **ingenuity pathway analysis** to verify the impact of Wnt signaling in psoriasis treated with Taodan granules.
Chen Y, Zhang Z, Zhang Y, Jiang J, Luo Y, Fei X, Ru Y, Li B, Zhang H, Liu T, Yang Y, Kuai L, Song J, Luo Y.
Am J Transl Res. 2023 Jan 15;15(1):422-434. eCollection 2023.
PMID: 36777818 Free PMC article.
MATERIALS AND METHODS: Primarily, transcriptional profiling was applied to identi
expressed genes (DEGs), proceeding with Gene ontology (GO) and Kyoto Encyclope
Genomes (KEGG) analysis. Gene Set Enrichment Analysis (GSEA) and **Ingenuity P** ...

☐ 3

Gene set enrichment analysis and **ingenuity pathway analysis** t
biomarkers in Sheng-ji Hua-yu formula treated diabetic ulcers.
Ru Y, Zhang Y, Xiang YW, Luo Y, Luo Y, Jiang JS, Song JK, Fei XY, Yang D, Zhang Z, Z
SY, Li B, Kuai L.
J Ethnopharmacol. 2022 Mar 1;285:114845. doi: 10.1016/j.jep.2021.114845. Epub 20
PMID: 34800645
GO and KEGG enrichment analysis were used to identify the mechanisms underlying
effect of SJHY formula, and then gene set enrichment analysis and **ingenuity path**way **analysis** were conducted for functional analysis. ...

☐ Clinical Trial
☐ Meta-Analysis
☐ Randomized Controlled Trial
☐ Review
☐ Systematic Review

> J Neuroinflammation. 2024 Mar 20;21(1):69. doi: 10.1186/s12974-024-03065-z.

Deletion of Slc9a1 in Cx3cr1$^+$ cells stimulated microglial subcluster CREB1 signaling and microglia-oligodendrocyte crosstalk

transcriptomic

> Stem Cells Transl Med. 2024 Mar 15;13(3):293-308. doi: 10.1093/stcltm/szad090.

Histone Trimethylations and HDAC5 Regulate Spheroid Subpopulation and Differentiation Signaling of Human Adipose-Derived Stem Cells

Single-cell RNA-seq

> J Allergy Clin Immunol. 2024 May;153(5):1268-1281. doi: 10.1016/j.jaci.2023.12.030.
Epub 2024 Mar 29.

Galectin-10 in serum extracellular vesicles reflects asthma pathophysiology

proteinomics

> Chin Med. 2022 Jun 15;17(1):71. doi: 10.1186/s13020-022-00632-5.

Serum metabolomics analysis of deficiency pattern and excess pattern in patients with rheumatoid arthritis

metabolomics

Ingenuity Pathway Analysis is Powered by QIAGEN Knowledge Base

Ingenuity Literature Findings

Ingenuity Expert Findings – manually curated Findings from the full-text with contextual details from top journals

Ingenuity ExpertAssist Findings – automated text Findings that are manually reviewed from abstracts covering a broader range of publications – comprise a small percentage of IPA's findings

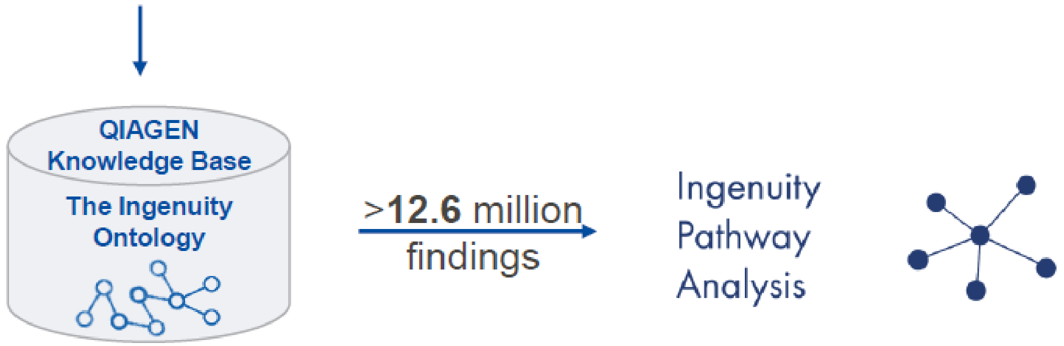
Ingenuity Modeled Knowledge

Ingenuity Expert Knowledge – content we model such as canonical pathways, toxicity lists, etc.

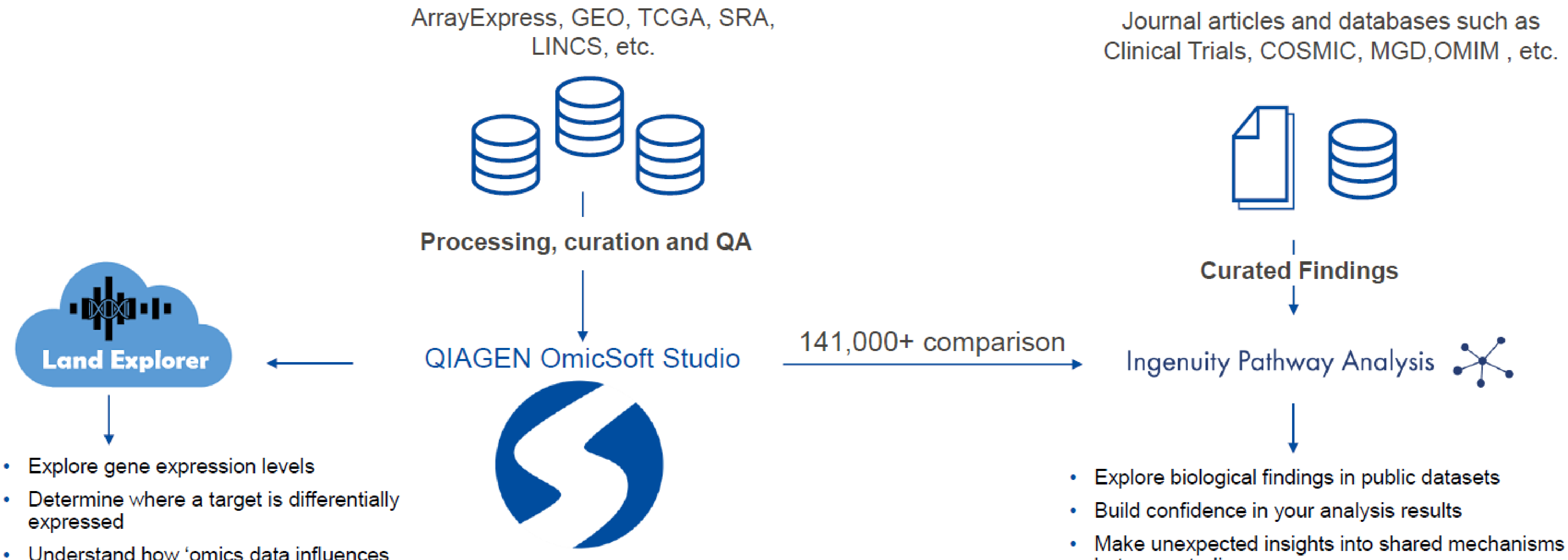
Ingenuity-Supported Third-Party Information – content areas include protein-protein, miRNA, biomarker, clinical trial information and others

Databases such as COSMIC, Clinical Trials, OMIM, TargetScan, BioGRID, MGD and HumanCyc

A massive, manually curated Knowledge Base updated weekly for the last ~20 years



QIAGEN IPA Analysis Match and Land Explorer



ArrayExpress, GEO, TCGA, SRA, LINCS, etc.

Processing, curation and QA

QIAGEN OmicSoft Studio

141,000+ comparison

Journal articles and databases such as Clinical Trials, COSMIC, MGD,OMIM , etc.

Curated Findings

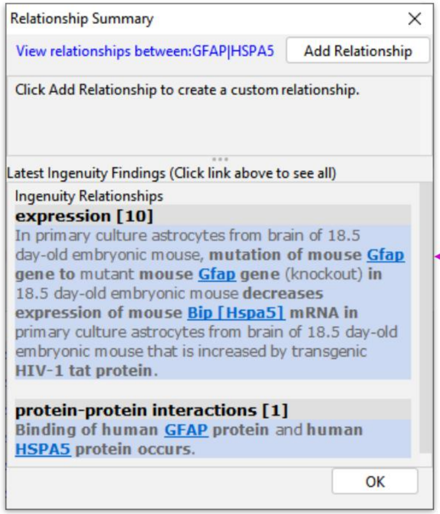
Ingenuity Pathway Analysis

Land Explorer

- Explore gene expression levels
- Determine where a target is differentially expressed
- Understand how ‘omics data influences survival
- Identify mutation status of a target

- Explore biological findings in public datasets
- Build confidence in your analysis results
- Make unexpected insights into shared mechanisms between studies
- “Anti-matches” may provide insights

Every Connection in IPA is Backed by Literature Findings



View relationships between:GFAP|HSPA5

Click Add Relationship to create a custom relationship.

Latest Ingenuity Findings (Click link above to see all)

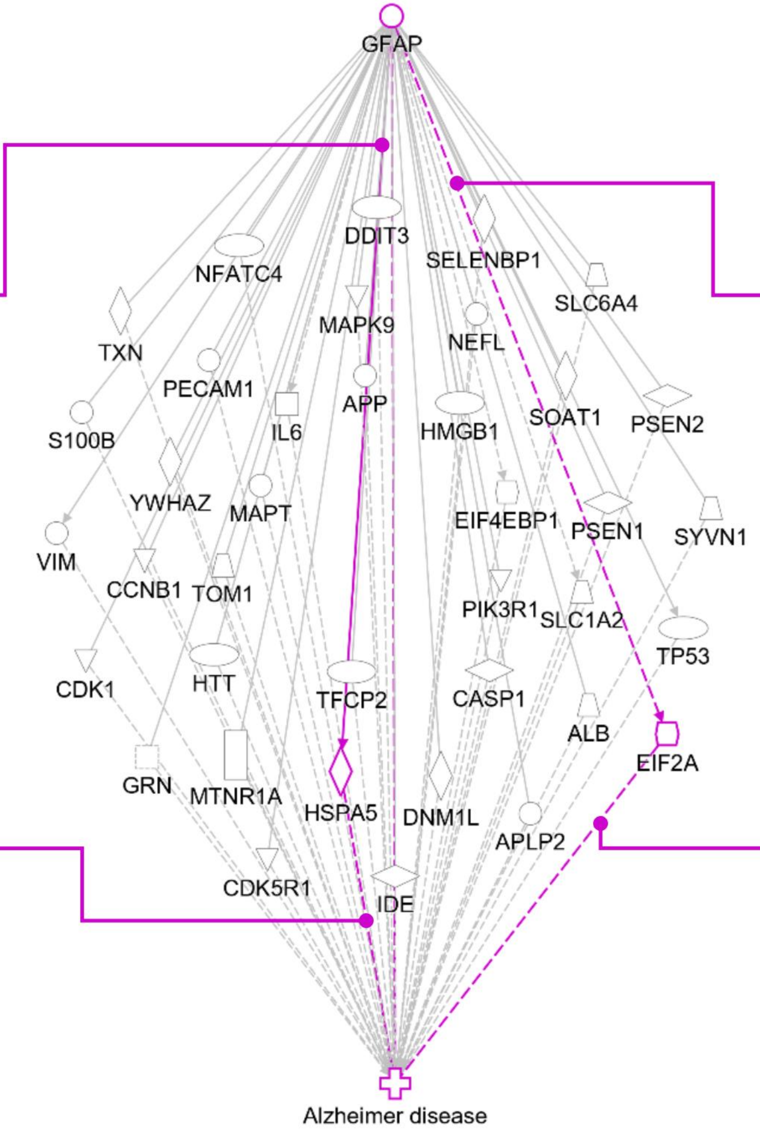
Ingenuity Relationships

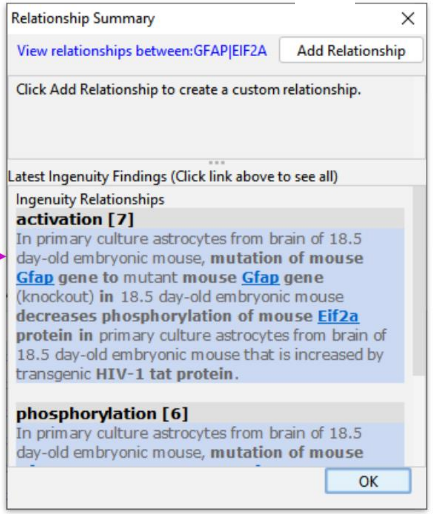
expression [10]

In primary culture astrocytes from brain of 18.5 day-old embryonic mouse, **mutation of mouse Gfap gene to mutant mouse Gfap gene** (knockout) **in** 18.5 day-old embryonic mouse **decreases expression of mouse Bip [Hspa5] mRNA in** primary culture astrocytes from brain of 18.5 day-old embryonic mouse that is increased by transgenic **HIV-1 tat protein**.

protein-protein interactions [1]

Binding of human GFAP protein and **human HSPA5 protein occurs.**





View relationships between:GFAP|EIF2A

Click Add Relationship to create a custom relationship.

Latest Ingenuity Findings (Click link above to see all)

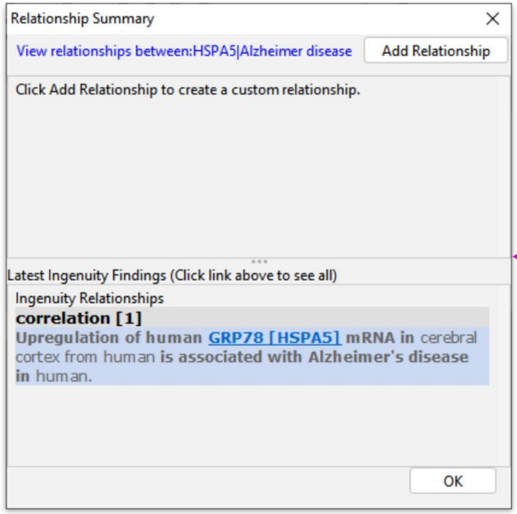
Ingenuity Relationships

activation [7]

In primary culture astrocytes from brain of 18.5 day-old embryonic mouse, **mutation of mouse Gfap gene to mutant mouse Gfap gene** (knockout) **in** 18.5 day-old embryonic mouse **decreases phosphorylation of mouse Eif2a protein in** primary culture astrocytes from brain of 18.5 day-old embryonic mouse that is increased by transgenic **HIV-1 tat protein**.

phosphorylation [6]

In primary culture astrocytes from brain of 18.5 day-old embryonic mouse, **mutation of mouse**



View relationships between:HSPA5|Alzheimer disease

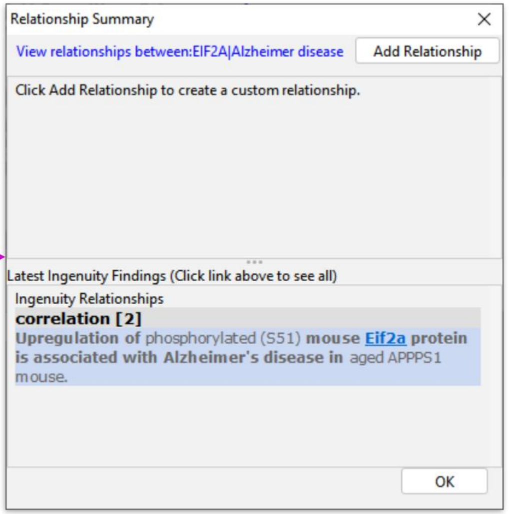
Click Add Relationship to create a custom relationship.

Latest Ingenuity Findings (Click link above to see all)

Ingenuity Relationships

correlation [1]

Upregulation of human GRP78 [HSPA5] mRNA in cerebral cortex from human **is associated with Alzheimer's disease in** human.



View relationships between:EIF2A|Alzheimer disease

Click Add Relationship to create a custom relationship.

Latest Ingenuity Findings (Click link above to see all)

Ingenuity Relationships

correlation [2]

Upregulation of phosphorylated (S51) **mouse Eif2a protein is associated with Alzheimer's disease in** aged APPPS1 mouse.

Species Support within IPA

Fully supported:

Human

Mouse

Rat

What species identifiers are accepted for analysis by IPA?

- ✓ Atlantic Salmon (*Salmo salar*)
- ✓ Thale cress (*Arabidopsis thaliana)*
- ✓ Bat (Greater horseshoe bat, *Rhinolophus ferrumequinum*)
- ✓ Brewer's yeast (*Saccharomyces cerevisiae)*
- ✓ Cat (domestic, *Felis catus*)
- ✓ Chicken (*Gallus gallus*)
- ✓ Chimpanzee (*Pan troglodytes*)
- ✓ Chinese hamster (*Cricetulus griseus*)
- ✓ Cow (*Bos taurus*)
- ✓ Crab-eating macaque (*Macaca fascicularis*)
- ✓ Dog (*Canis lupus familiaris*)
- ✓ Fission yeast (*Schizosaccharomyces pombe)*
- ✓ Fruit fly (*Drosophila melanogaster*)
- ✓ Golden hamster (*Mesocricetus auratus*)
- ✓ Guinea pig, domestic (*Cavia porcellus*)
- ✓ Horse (*Equus caballus*)
- ✓ Human (*Homo sapiens*)
- ✓ Mouse (*Mus musculus*)
- ✓ Pig (*Sus scrofa*)
- ✓ Rabbit (*Oryctolagus cuniculus*)
- ✓ Rainbow trout (*Oncorhynchus mykiss*)
- ✓ Rat (*Rattus norvegicus*)
- ✓ Rhesus Monkey (*Macaca mulatta*)
- ✓ Roundworm (*Caenorhabditis elegans)*
- ✓ Sheep (*Ovis aries*)
- ✓ Western clawed frog (*Xenopus tropicalis*)
- ✓ Zebrafish (*Danio rerio*)

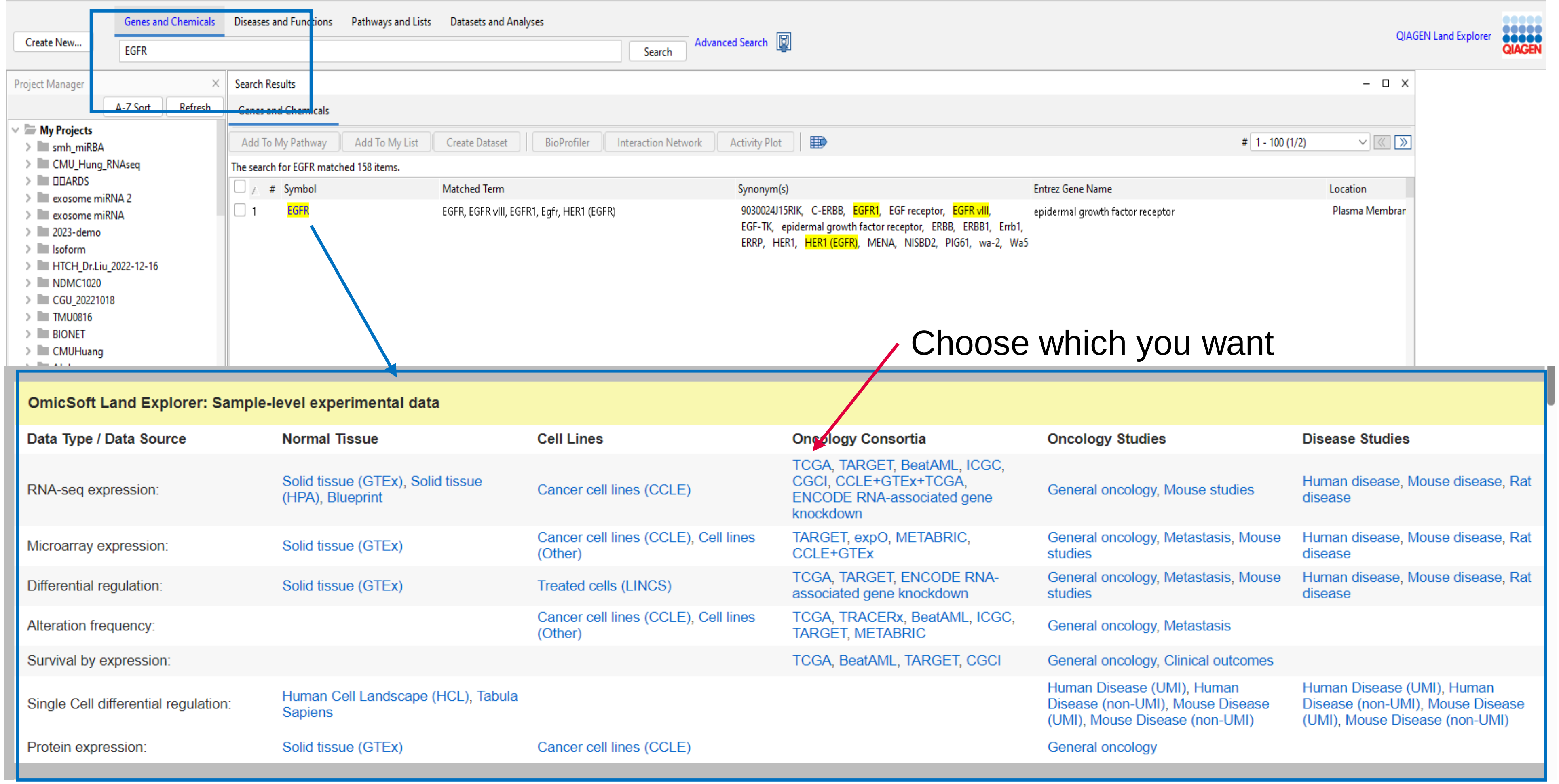
HomoloGene Release 68 (04/09/2014)
Gene2accession(24/08/2023)
Gene_orthologs(24/08/2023)
(http://www.ncbi.nlm.nih.gov/homologene/statistics/)

Supported Identifiers for Data Upload

Get more complete mapping during dataset upload!

Vendor IDs	Gene	Protein	Transcript	microRNA	SNP	Chemical
Affymetrix (na36)	Entrez Gene (2023/8)	GenPept	Ensembl (110)	miRbase (mature)	Affy SNP IDs	CAS Registry Number
Agilent	GenBank (257)	International Protein Index (IPI)	RefSeq (human、mouse)	miRBase (stemloop)	dbSNP	HMDB
Life Tech (ABI)	Symbol-human (HUGO/ HGNC, EG)	UniProt/ Swiss-Prot Accession (2022_02)	UCSC (hg18)			KEGG
Codelink	Symbol- mouse (EG)		UCSC (hg19)			PubChem CID
Illumina	Symbol- rat (EG)		UCSC (hg38)			
Ingenuity	GI Number					
	UniGene					

IPA with OmicSoft Land Explorer



The search for EGFR matched 158 items.

#	Symbol	Matched Term	Synonym(s)	Entrez Gene Name	Location
1	EGFR	EGFR, EGFR vIII, EGFR1, Egfr, HER1 (EGFR)	9030024J15RIK, C-ERBB, EGFR1, EGF receptor, EGFR vIII, EGF-TK, epidermal growth factor receptor, ERBB, ERBB1, Errb1, ERRP, HER1, HER1 (EGFR), MENA, NISBD2, PIG61, wa-2, Wa5	epidermal growth factor receptor	Plasma Membrar

Choose which you want

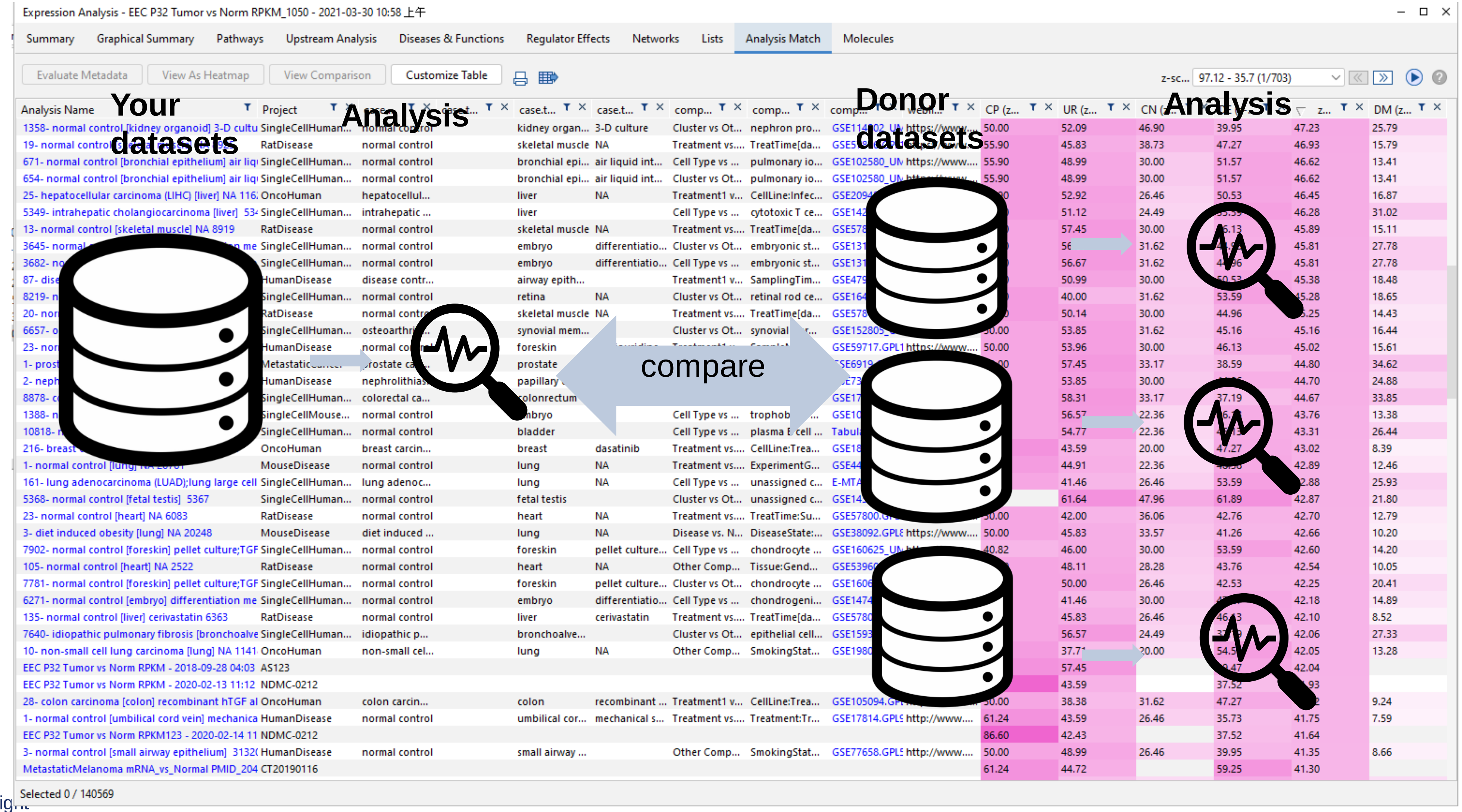
OmicSoft Land Explorer: Sample-level experimental data

Data Type / Data Source	Normal Tissue	Cell Lines	Oncology Consortia	Oncology Studies	Disease Studies
RNA-seq expression:	Solid tissue (GTEx), Solid tissue (HPA), Blueprint	Cancer cell lines (CCLE)	TCGA, TARGET, BeatAML, ICGC, CGCI, CCLE+GTEx+TCGA, ENCODE RNA-associated gene knockdown	General oncology, Mouse studies	Human disease, Mouse disease, Rat disease
Microarray expression:	Solid tissue (GTEx)	Cancer cell lines (CCLE), Cell lines (Other)	TARGET, expO, METABRIC, CCLE+GTEx	General oncology, Metastasis, Mouse studies	Human disease, Mouse disease, Rat disease
Differential regulation:	Solid tissue (GTEx)	Treated cells (LINCS)	TCGA, TARGET, ENCODE RNA-associated gene knockdown	General oncology, Metastasis, Mouse studies	Human disease, Mouse disease, Rat disease
Alteration frequency:		Cancer cell lines (CCLE), Cell lines (Other)	TCGA, TRACERx, BeatAML, ICGC, TARGET, METABRIC	General oncology, Metastasis	
Survival by expression:			TCGA, BeatAML, TARGET, CGCI	General oncology, Clinical outcomes	
Single Cell differential regulation:	Human Cell Landscape (HCL), Tabula Sapiens			Human Disease (UMI), Human Disease (non-UMI), Mouse Disease (UMI), Mouse Disease (non-UMI)	Human Disease (UMI), Human Disease (non-UMI), Mouse Disease (UMI), Mouse Disease (non-UMI)
Protein expression:	Solid tissue (GTEx)	Cancer cell lines (CCLE)		General oncology	

IPA Gene View :OmicSoft Land Explorer

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



Analysis Name	Project	case...	case.t...	case.t...	case.t...	comp...	comp...	comp...	webl...	CP (z...	UR (z...	CN (z...	DE (z...	z...	DM (z...
1358- normal control [kidney organoid] 3-D cultu	SingleCellHuman...	normal control		kidney organ...	3-D culture	Cluster vs Ot...	nephron pro...	GSE114 02_UN	https://www....	50.00	52.09	46.90	39.95	47.23	25.79
19- normal control [s	RatDisease	normal control		skeletal muscle	NA	Treatment vs....	TreatTime[da...	GSE5		55.90	45.83	38.73	47.27	46.93	15.79
671- normal control [bronchial epithelium] air liq	SingleCellHuman...	normal control		bronchial epi...	air liquid int...	Cell Type vs ...	pulmonary io...	GSE102580_UN	https://www....	55.90	48.99	30.00	51.57	46.62	13.41
654- normal control [bronchial epithelium] air liq	SingleCellHuman...	normal control		bronchial epi...	air liquid int...	Cluster vs Ot...	pulmonary io...	GSE102580_UN	https://www....	55.90	48.99	30.00	51.57	46.62	13.41
25- hepatocellular carcinoma (LIHC) [liver] NA 116	OncoHuman	hepatocellul...		liver	NA	Treatment1 v...	CellLine:Infec...	GSE2094			52.92	26.46	50.53	46.45	16.87
5349- intrahepatic cholangiocarcinoma [liver] 53	SingleCellHuman...	intrahepatic ...		liver		Cell Type vs ...	cytotoxic T ce...	GSE142			51.12	24.49		46.28	31.02
13- normal control [skeletal muscle] NA 8919	RatDisease	normal control		skeletal muscle	NA	Treatment vs....	TreatTime[da...	GSE578			57.45	30.00	6.13	45.89	15.11
3645- normal	SingleCellHuman...	normal control		embryo	differentiatio...	Cluster vs Ot...	embryonic st...	GSE131			56	31.62		45.81	27.78
3682- no	SingleCellHuman...	normal control		embryo	differentiatio...	Cell Type vs ...	embryonic st...	GSE131			56.67	31.62	44.96	45.81	27.78
87- dise	HumanDisease	disease contr...		airway epith...		Treatment1 v...	SamplingTim...	GSE479			50.99	30.00	50.53	45.38	18.48
8219- n	SingleCellHuman...	normal control		retina	NA	Cluster vs Ot...	retinal rod ce...	GSE164			40.00	31.62	53.59	45.28	18.65
20- nor	RatDisease	normal contr		skeletal muscle	NA	Treatment vs....	TreatTime[da...	GSE578			50.14	30.00	44.96	5.25	14.43
6657- o	SingleCellHuman...	osteoarthri		synovial mem...		Cluster vs Ot...	synovial	GSE152805_		0.00	53.85	31.62	45.16	45.16	16.44
23- nor	HumanDisease	normal co		foreskin				GSE59717.GPL1	https://www....	50.00	53.96	30.00	46.13	45.02	15.61
1- prost	Metastatic	rostate ca		prostate				SE6919		00	57.45	33.17	38.59	44.80	34.62
2- neph	HumanDisease	nephrolithias		papillary				E73			53.85	30.00		44.70	24.88
8878- c	SingleCellHuman...	colorectal ca...		olonrectum				GSE17			58.31	33.17	37.19	44.67	33.85
1388- n	SingleCellMouse...	normal control		mbryo		Cell Type vs ...	trophob	GSE10			56.57	22.36		43.76	13.38
10818-	SingleCellHuman...	normal control		bladder		Cell Type vs ...	plasma cell ...	Tabula			54.77	22.36		43.31	26.44
216- breast	OncoHuman	breast carcin...		breast	dasatinib	Treatment vs....	CellLine:Trea...	GSE18			43.59	20.00	47.27	43.02	8.39
1- normal control [lung]	MouseDisease	normal control		lung	NA	Treatment vs....	ExperimentG...	GSE44			44.91	22.36		42.89	12.46
161- lung adenocarcinoma (LUAD);lung large cell	SingleCellHuman...	lung adenoc...		lung	NA	Cell Type vs ...	unassigned c...	E-MTA			41.46	26.46	53.59	2.88	25.93
5368- normal control [fetal testis] 5367	SingleCellHuman...	normal control		fetal testis		Cluster vs Ot...	unassigned c...	GSE14			61.64	47.96	61.89	42.87	21.80
23- normal control [heart] NA 6083	RatDisease	normal control		heart	NA	Treatment vs....	TreatTime:Su...	GSE57800.G		0.00	42.00	36.06	42.76	42.70	12.79
3- diet induced obesity [lung] NA 20248	MouseDisease	diet induced ...		lung	NA	Disease vs. N...	DiseaseState:...	GSE38092.GPL8	https://www....	50.00	45.83	33.57	41.26	42.66	10.20
7902- normal control [foreskin] pellet culture;TGF	SingleCellHuman...	normal control		foreskin	pellet culture...	Cell Type vs ...	chondrocyte ...	GSE160625_UN		40.82	46.00	30.00	53.59	42.60	14.20
105- normal control [heart] NA 2522	RatDisease	normal control		heart	NA	Other Comp...	Tissue:Gend...	GSE5396			48.11	28.28	43.76	42.54	10.05
7781- normal control [foreskin] pellet culture;TGF	SingleCellHuman...	normal control		foreskin	pellet culture...	Cluster vs Ot...	chondrocyte ...	GSE1606			50.00	26.46	42.53	42.25	20.41
6271- normal control [embryo] differentiation me	SingleCellHuman...	normal control		embryo	differentiatio...	Cell Type vs ...	chondrogeni...	GSE1474			41.46	30.00		42.18	14.89
135- normal control [liver] cerivastatin 6363	RatDisease	normal control		liver	cerivastatin	Treatment vs....	TreatTime[da...	GSE5780			45.83	26.46	46.13	42.10	8.52
7640- idiopathic pulmonary fibrosis [bronchoalve	SingleCellHuman...	idiopathic p...		bronchoalve...		Cluster vs Ot...	epithelial cell...	GSE1593			56.57	24.49		42.06	27.33
10- non-small cell lung carcinoma [lung] NA 1141	OncoHuman	non-small cel...		lung	NA	Other Comp...	SmokingStat...	GSE1980			37.7	20.00	54.5	42.05	13.28
EEC P32 Tumor vs Norm RPKM - 2018-09-28 04:03	AS123										57.45		.47	42.04	
EEC P32 Tumor vs Norm RPKM - 2020-02-13 11:12	NDMC-0212										43.59		37.52	.93	
28- colon carcinoma [colon] recombinant hTGF al	OncoHuman	colon carcin...		colon	recombinant ...	Treatment1 v...	CellLine:Trea...	GSE105094.GP		0.00	38.38	31.62	47.27		9.24
1- normal control [umbilical cord vein] mechanica	HumanDisease	normal control		umbilical cor...	mechanical s...	Treatment vs....	Treatment:Tr...	GSE17814.GPL9	http://www....	61.24	43.59	26.46	35.73	41.75	7.59
EEC P32 Tumor vs Norm RPKM123 - 2020-02-14 11	NDMC-0212									86.60	42.43		37.52	41.64	
3- normal control [small airway epithelium] 3132(	HumanDisease	normal control		small airway ...		Other Comp...	SmokingStat...	GSE77658.GPL5	http://www....	50.00	48.99	26.46	39.95	41.35	8.66
MetastaticMelanoma mRNA_vs_Normal PMID_204	CT20190116									61.24	44.72		59.25	41.30	

Analysis Match

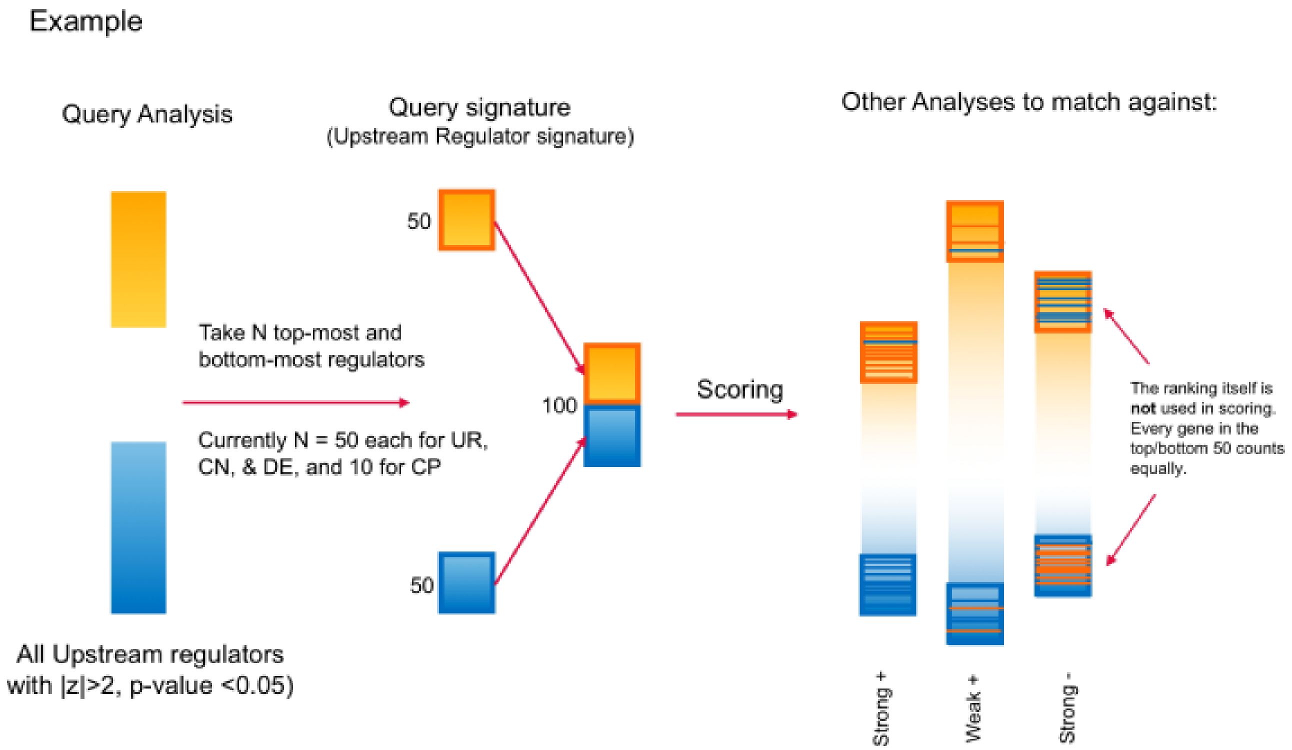
How signatures are created and compared

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

Query Analysis

Query signature
(Upstream Regulator signature)

Other Analyses to match against:

Take N top-most and bottom-most regulators

Currently N = 50 each for UR, CN, & DE, and 10 for CP

All Upstream regulators with |z|>2, p-value <0.05)

Scoring

The ranking itself is **not** used in scoring. Every gene in the top/bottom 50 counts equally.

Analysis Match

Mapping Your Results to OmicSoft Datasets by IPA Analysis Match

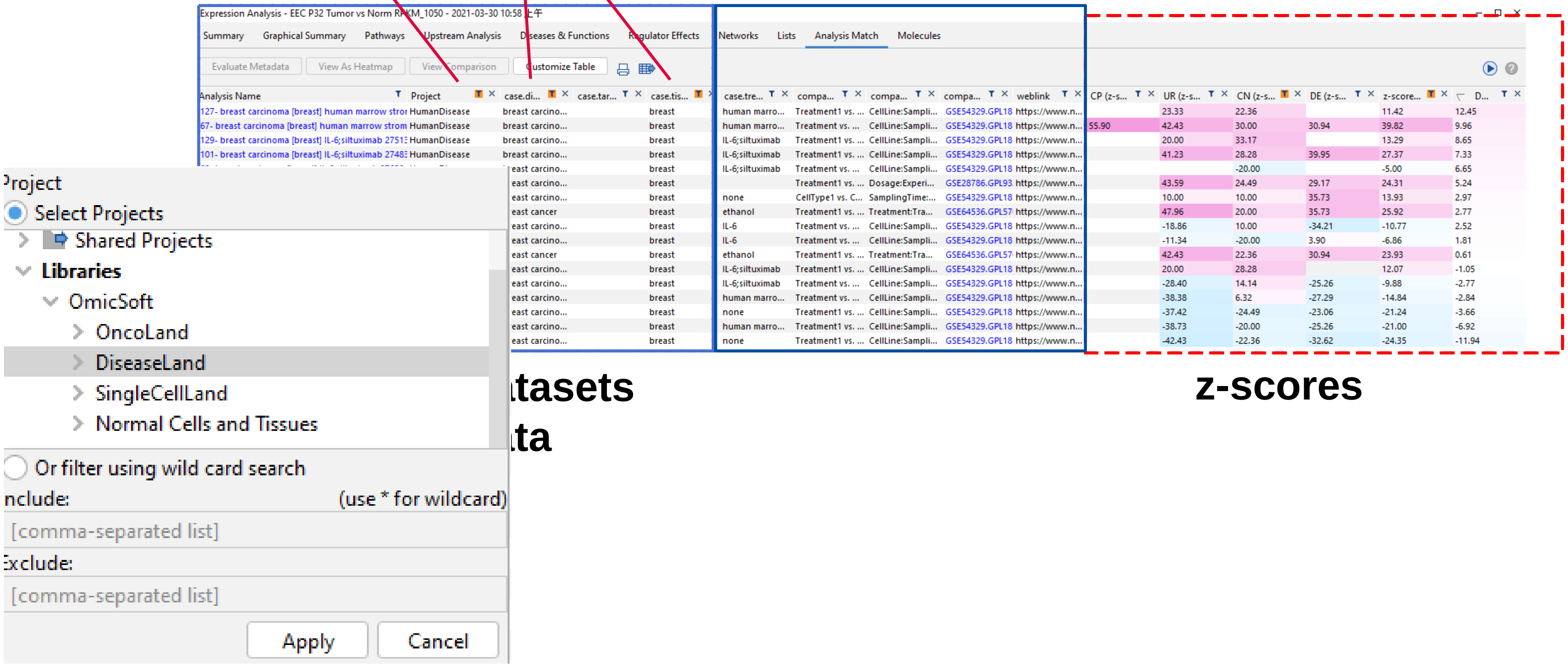
Project

Cell & Tissue

Datasets information

similar

opposite



Expression Analysis - EEC P32 Tumor vs Norm RPKM_1050 - 2021-03-30 10:58 上午

Analysis Name	Project	case.di...	case.tar...	case.tis...	case.tre...	compa...	compa...	compa...	weblink	CP (z-s...	UR (z-s...	CN (z-s...	DE (z-s...	z-score...	D...
127- breast carcinoma [breast] human marrow stror	HumanDisease	breast carcino...		breast	human marro...	Treatment1 vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		23.33	22.36		11.42	12.45
67- breast carcinoma [breast] human marrow strom	HumanDisease	breast carcino...		breast	human marro...	Treatment vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...	55.90	42.43	30.00	30.94	39.82	9.96
129- breast carcinoma [breast] IL-6;siltuximab 2751:	HumanDisease	breast carcino...		breast	IL-6;siltuximab	Treatment1 vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		20.00	33.17		13.29	8.65
101- breast carcinoma [breast] IL-6;siltuximab 2748:	HumanDisease	breast carcino...		breast	IL-6;siltuximab	Treatment1 vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		41.23	28.28	39.95	27.37	7.33
		east carcino...		breast	IL-6;siltuximab	Treatment vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...			-20.00		-5.00	6.65
		east carcino...		breast		Treatment1 vs. ...	Dosage:Experi...	GSE28786.GPL93	https://www.n...		43.59	24.49	29.17	24.31	5.24
		east carcino...		breast	none	CellType1 vs. C...	SamplingTime:...	GSE54329.GPL18	https://www.n...		10.00	10.00	35.73	13.93	2.97
		east cancer		breast	ethanol	Treatment1 vs. ...	Treatment:Tra...	GSE64536.GPL57	https://www.n...		47.96	20.00	35.73	25.92	2.77
		east carcino...		breast	IL-6	Treatment vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		-18.86	10.00	-34.21	-10.77	2.52
		east carcino...		breast	IL-6	Treatment vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		-11.34	-20.00	3.90	-6.86	1.81
		east cancer		breast	ethanol	Treatment1 vs. ...	Treatment:Tra...	GSE64536.GPL57	https://www.n...		42.43	22.36	30.94	23.93	0.61
		east carcino...		breast	IL-6;siltuximab	Treatment1 vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		20.00	28.28		12.07	-1.05
		east carcino...		breast	IL-6;siltuximab	Treatment vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		-28.40	14.14	-25.26	-9.88	-2.77
		east carcino...		breast	human marro...	Treatment vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		-38.38	6.32	-27.29	-14.84	-2.84
		east carcino...		breast	none	Treatment1 vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		-37.42	-24.49	-23.06	-21.24	-3.66
		east carcino...		breast	human marro...	Treatment1 vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		-38.73	-20.00	-25.26	-21.00	-6.92
		east carcino...		breast	none	Treatment1 vs. ...	CellLine:Sampli...	GSE54329.GPL18	https://www.n...		-42.43	-22.36	-32.62	-24.35	-11.94

roject

● Select Projects

○ Or filter using wild card search

nclude: (use * for wildcard)

[comma-separated list]

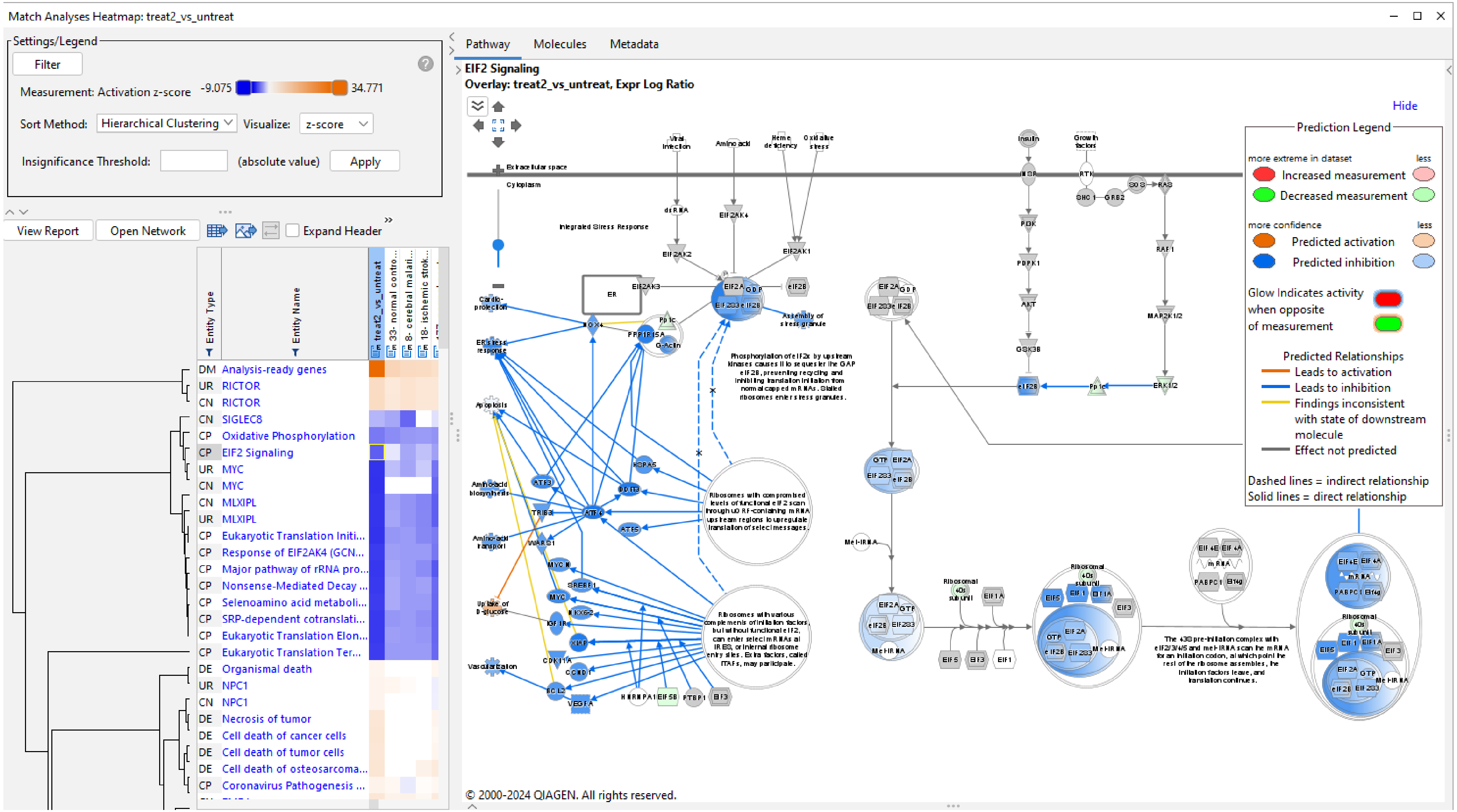
xclude:

[comma-separated list]

tasets
ta

z-scores

Analysis Match



Match Analyses Heatmap: treat2_vs_untreat

Settings/Legend

Measurement: Activation z-score -9.075 34.771

Sort Method: Hierarchical Clustering Visualize: z-score

Insignificance Threshold: (absolute value) Apply

Entity Type	Entity Name
DM	Analysis-ready genes
UR	RICTOR
CN	RICTOR
CN	SIGLEC8
CP	Oxidative Phosphorylation
CP	EIF2 Signaling
UR	MYC
CN	MYC
CN	MLXIPL
UR	MLXIPL
CP	Eukaryotic Translation Initi...
CP	Response of EIF2AK4 (GCN...
CP	Major pathway of rRNA pro...
CP	Nonsense-Mediated Decay ...
CP	Selenoamino acid metaboli...
CP	SRP-dependent cotranslati...
CP	Eukaryotic Translation Elon...
CP	Eukaryotic Translation Ter...
DE	Organismal death
UR	NPC1
CN	NPC1
DE	Necrosis of tumor
DE	Cell death of cancer cells
DE	Cell death of tumor cells
DE	Cell death of osteosarcoma...
CP	Coronavirus Pathogenesis ...

Heatmap columns: treat2_vs_untreat; 33- normal contro...; 8- cerebral malari...; 18- ischemic strok...

EIF2 Signaling
Overlay: treat2_vs_untreat, Expr Log Ratio

Phosphorylation of eIF2α by upstream kinases causes it to sequester the GAP eIF2B, preventing recycling and inhibiting translation initiation from normal capped mRNAs. Stalled ribosomes enter stress granules.

Ribosomes with compromised levels of functional eIF2 scan through uORF-containing mRNA upstream regions to upregulate translation of select messages.

Ribosomes with various complements of initiation factors, but without functional eIF2, can enter select mRNAs at IRESs, or internal ribosome entry sites. Extra factors, called ITAFs, may participate.

The 43S pre-initiation complex with eIF2/3/4/5 and met-tRNA scan the mRNA for an initiation codon, at which point the rest of the ribosome assembles, the initiation factors leave, and translation continues.

Prediction Legend

more extreme in dataset		less
🟥	Increased measurement	🟥
🟩	Decreased measurement	🟩

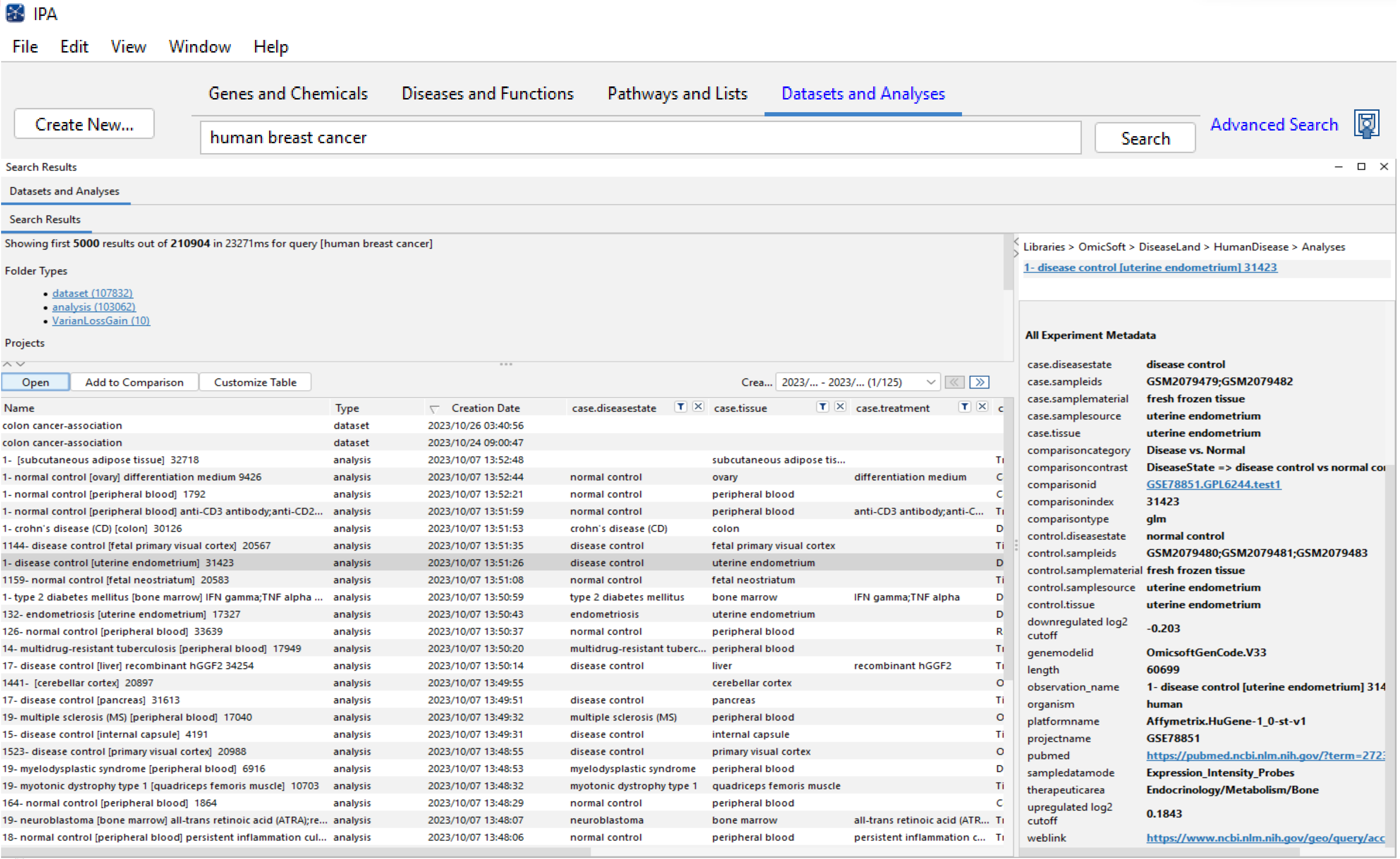
more confidence		less
●	Predicted activation	●
●	Predicted inhibition	●

Glow Indicates activity when opposite of measurement

Predicted Relationships
- Leads to activation
- Leads to inhibition
- Findings inconsistent with state of downstream molecule
- Effect not predicted

Dashed lines = indirect relationship
Solid lines = direct relationship

Disease and Analysis Search



human breast cancer

Showing first **5000** results out of **210904** in 23271ms for query [human breast cancer]

Folder Types

- dataset (107832)
- analysis (103062)
- VarianLossGain (10)

Projects

Crea... 2023/... - 2023/... (1/125)

Name	Type	Creation Date	case.diseasestate	case.tissue	case.treatment	c
colon cancer-association	dataset	2023/10/26 03:40:56				
colon cancer-association	dataset	2023/10/24 09:00:47				
1- [subcutaneous adipose tissue] 32718	analysis	2023/10/07 13:52:48		subcutaneous adipose tis...		Ti
1- normal control [ovary] differentiation medium 9426	analysis	2023/10/07 13:52:44	normal control	ovary	differentiation medium	C
1- normal control [peripheral blood] 1792	analysis	2023/10/07 13:52:21	normal control	peripheral blood		C
1- normal control [peripheral blood] anti-CD3 antibody;anti-CD2...	analysis	2023/10/07 13:51:59	normal control	peripheral blood	anti-CD3 antibody;anti-C...	Ti
1- crohn's disease (CD) [colon] 30126	analysis	2023/10/07 13:51:53	crohn's disease (CD)	colon		D
1144- disease control [fetal primary visual cortex] 20567	analysis	2023/10/07 13:51:35	disease control	fetal primary visual cortex		Ti
1- disease control [uterine endometrium] 31423	analysis	2023/10/07 13:51:26	disease control	uterine endometrium		D
1159- normal control [fetal neostriatum] 20583	analysis	2023/10/07 13:51:08	normal control	fetal neostriatum		Ti
1- type 2 diabetes mellitus [bone marrow] IFN gamma;TNF alpha ...	analysis	2023/10/07 13:50:59	type 2 diabetes mellitus	bone marrow	IFN gamma;TNF alpha	D
132- endometriosis [uterine endometrium] 17327	analysis	2023/10/07 13:50:43	endometriosis	uterine endometrium		D
126- normal control [peripheral blood] 33639	analysis	2023/10/07 13:50:37	normal control	peripheral blood		R
14- multidrug-resistant tuberculosis [peripheral blood] 17949	analysis	2023/10/07 13:50:20	multidrug-resistant tuberc...	peripheral blood		Ti
17- disease control [liver] recombinant hGGF2 34254	analysis	2023/10/07 13:50:14	disease control	liver	recombinant hGGF2	Ti
1441- [cerebellar cortex] 20897	analysis	2023/10/07 13:49:55		cerebellar cortex		O
17- disease control [pancreas] 31613	analysis	2023/10/07 13:49:51	disease control	pancreas		Ti
19- multiple sclerosis (MS) [peripheral blood] 17040	analysis	2023/10/07 13:49:32	multiple sclerosis (MS)	peripheral blood		O
15- disease control [internal capsule] 4191	analysis	2023/10/07 13:49:31	disease control	internal capsule		Ti
1523- disease control [primary visual cortex] 20988	analysis	2023/10/07 13:48:55	disease control	primary visual cortex		O
19- myelodysplastic syndrome [peripheral blood] 6916	analysis	2023/10/07 13:48:53	myelodysplastic syndrome	peripheral blood		D
19- myotonic dystrophy type 1 [quadriceps femoris muscle] 10703	analysis	2023/10/07 13:48:32	myotonic dystrophy type 1	quadriceps femoris muscle		Ti
164- normal control [peripheral blood] 1864	analysis	2023/10/07 13:48:29	normal control	peripheral blood		C
19- neuroblastoma [bone marrow] all-trans retinoic acid (ATRA);re...	analysis	2023/10/07 13:48:07	neuroblastoma	bone marrow	all-trans retinoic acid (ATR...	Ti
18- normal control [peripheral blood] persistent inflammation cul...	analysis	2023/10/07 13:48:06	normal control	peripheral blood	persistent inflammation c...	Ti

Libraries > OmicSoft > DiseaseLand > HumanDisease > Analyses

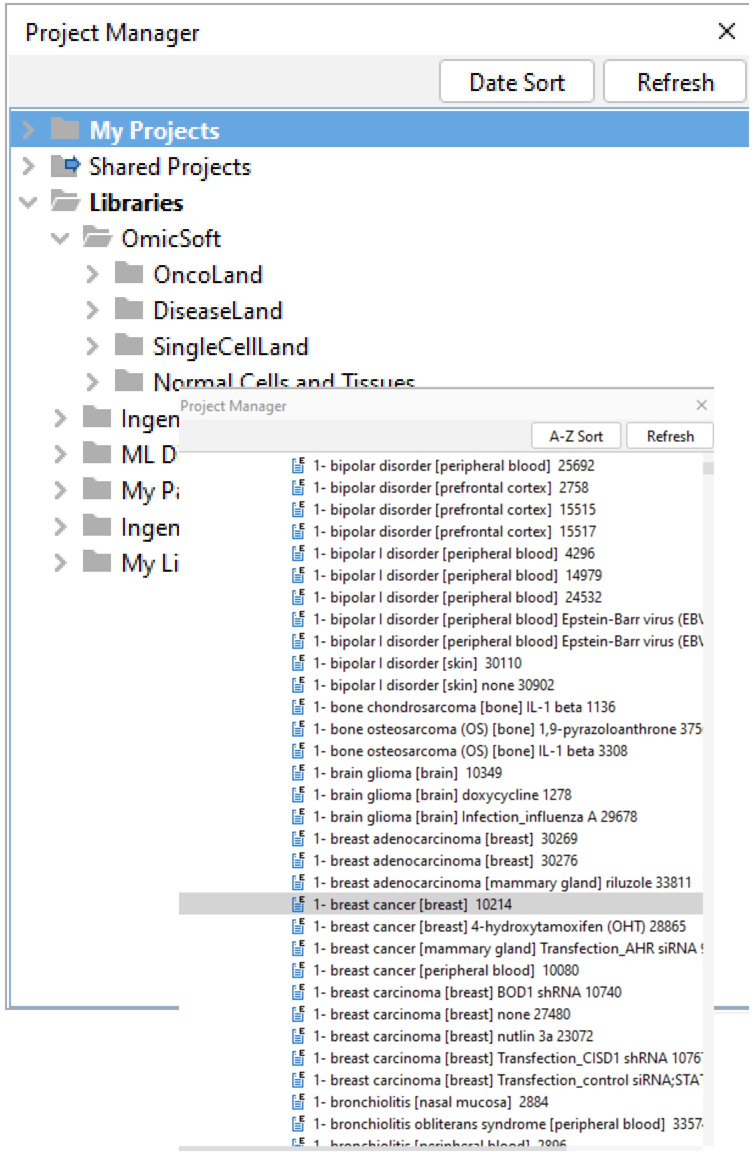
1- disease control [uterine endometrium] 31423

All Experiment Metadata

case.diseasestate	**disease control**
case.sampleids	**GSM2079479;GSM2079482**
case.samplematerial	**fresh frozen tissue**
case.samplesource	**uterine endometrium**
case.tissue	**uterine endometrium**
comparisoncategory	**Disease vs. Normal**
comparisoncontrast	**DiseaseState => disease control vs normal co**
comparisonid	GSE78851.GPL6244.test1
comparisonindex	**31423**
comparisontype	**glm**
control.diseasestate	**normal control**
control.sampleids	**GSM2079480;GSM2079481;GSM2079483**
control.samplematerial	**fresh frozen tissue**
control.samplesource	**uterine endometrium**
control.tissue	**uterine endometrium**
downregulated log2 cutoff	**-0.203**
genemodelid	**OmicsoftGenCode.V33**
length	**60699**
observation_name	**1- disease control [uterine endometrium] 314**
organism	**human**
platformname	**Affymetrix.HuGene-1_0-st-v1**
projectname	**GSE78851**
pubmed	https://pubmed.ncbi.nlm.nih.gov/?term=272
sampledatamode	**Expression_Intensity_Probes**
therapeuticarea	**Endocrinology/Metabolism/Bone**
upregulated log2 cutoff	**0.1843**
weblink	https://www.ncbi.nlm.nih.gov/geo/query/acc

OmicSoft Data Storage and Analysis in IPA

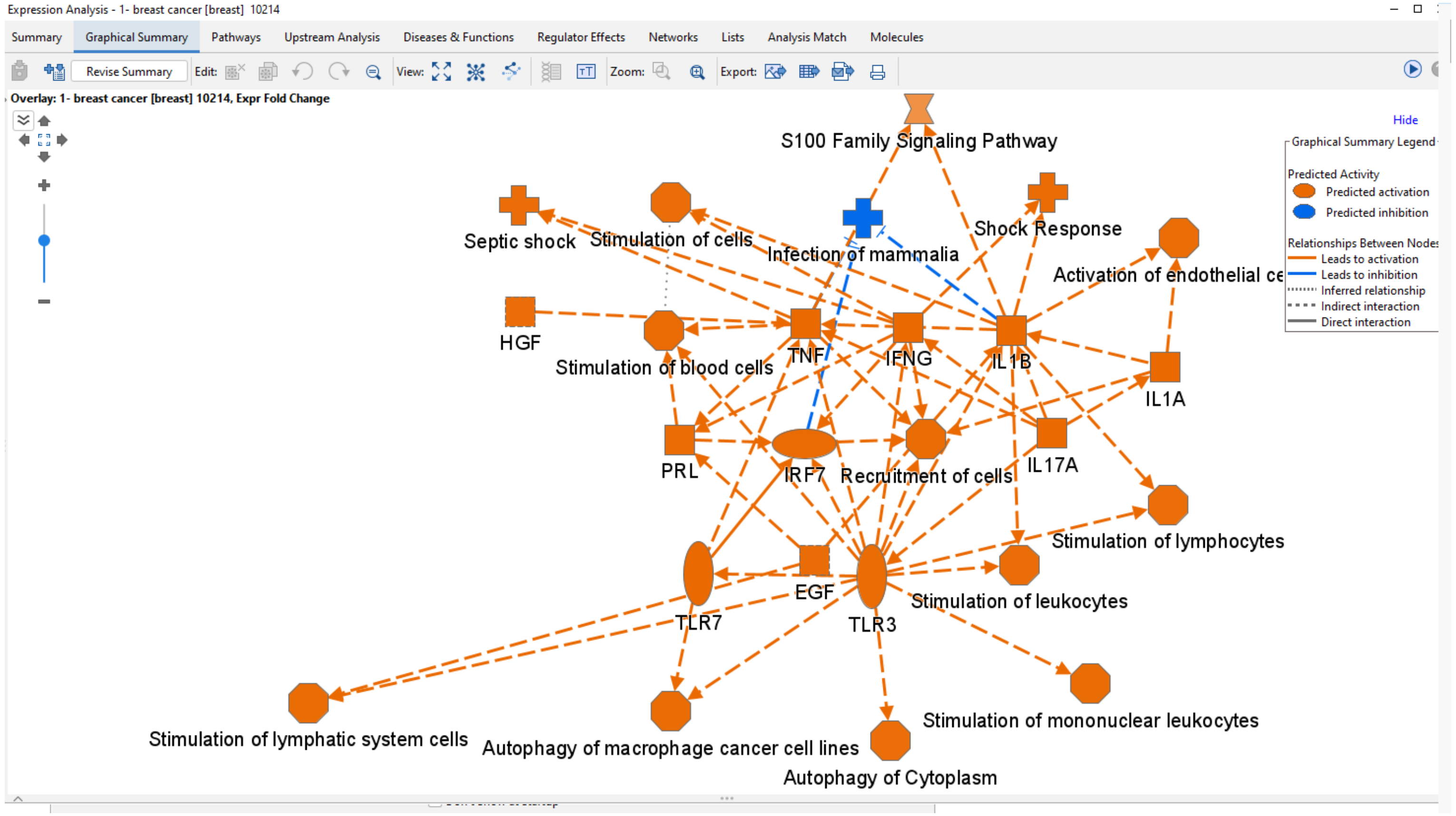
You can also use the repository without your own analysis, just by searching for available analyses of interest.



- My Projects
- Shared Projects
- Libraries
  - OmicSoft
    - OncoLand
    - DiseaseLand
    - SingleCellLand
    - Normal Cells and Tissues
  - Ingen
  - ML D
  - My P
  - Ingen
  - My Li

1- bipolar disorder [peripheral blood] 25692
1- bipolar disorder [prefrontal cortex] 2758
1- bipolar disorder [prefrontal cortex] 15515
1- bipolar disorder [prefrontal cortex] 15517
1- bipolar I disorder [peripheral blood] 4296
1- bipolar I disorder [peripheral blood] 14979
1- bipolar I disorder [peripheral blood] 24532
1- bipolar I disorder [peripheral blood] Epstein-Barr virus (EB
1- bipolar I disorder [peripheral blood] Epstein-Barr virus (EB
1- bipolar I disorder [skin] 30110
1- bipolar I disorder [skin] none 30902
1- bone chondrosarcoma [bone] IL-1 beta 1136
1- bone osteosarcoma (OS) [bone] 1,9-pyrazoloanthrone 375
1- bone osteosarcoma (OS) [bone] IL-1 beta 3308
1- brain glioma [brain] 10349
1- brain glioma [brain] doxycycline 1278
1- brain glioma [brain] Infection_influenza A 29678
1- breast adenocarcinoma [breast] 30269
1- breast adenocarcinoma [breast] 30276
1- breast adenocarcinoma [mammary gland] riluzole 33811
1- breast cancer [breast] 10214
1- breast cancer [breast] 4-hydroxytamoxifen (OHT) 28865
1- breast cancer [mammary gland] Transfection_AHR siRNA
1- breast cancer [peripheral blood] 10080
1- breast carcinoma [breast] BOD1 shRNA 10740
1- breast carcinoma [breast] none 27480
1- breast carcinoma [breast] nutlin 3a 23072
1- breast carcinoma [breast] Transfection_CISD1 shRNA 1076
1- breast carcinoma [breast] Transfection_control siRNA;STA
1- bronchiolitis [nasal mucosa] 2884
1- bronchiolitis obliterans syndrome [peripheral blood] 3357
1- bronchiolitis [peripheral blood] 2806

Graphical summary


Expression Analysis - 1- breast cancer [breast] 10214

Overlay: 1- breast cancer [breast] 10214, Expr Fold Change

What's new in the QIAGEN® Ingenuity Pathway Analysis Summer Release (2024)

Figure 1. View Canonical Pathways with the new Bubble Chart (Volcano) option. Simply click on the drop-down menu in the Canonical Pathways tab in your Core Analysis to easily view pathways as a function of z-scores vs -log p-values. This example was generated from gene expression data collected from TGF-b2-treated equine bone marrow-derived mesenchymal stem cell vs untreated samples (GSE207394). FASTQ files were reprocessed using QIAGEN RNA-Seq Portal. Note: Pathway names were added to this figure using PowerPoint.

Other software improvements

- Updated several links from Gene View to Land Explorer
- Fixed shapes and coloring for groups and complexes in Path Designer
- Fixed an issue where changing pages in the Molecules tab in Core Analyses could freeze the software
- Fixed an issue involving column titles in exported Comparison Analysis Causal Network heatmaps

QIAGEN IPA – A Dual Approach to Data Analysis

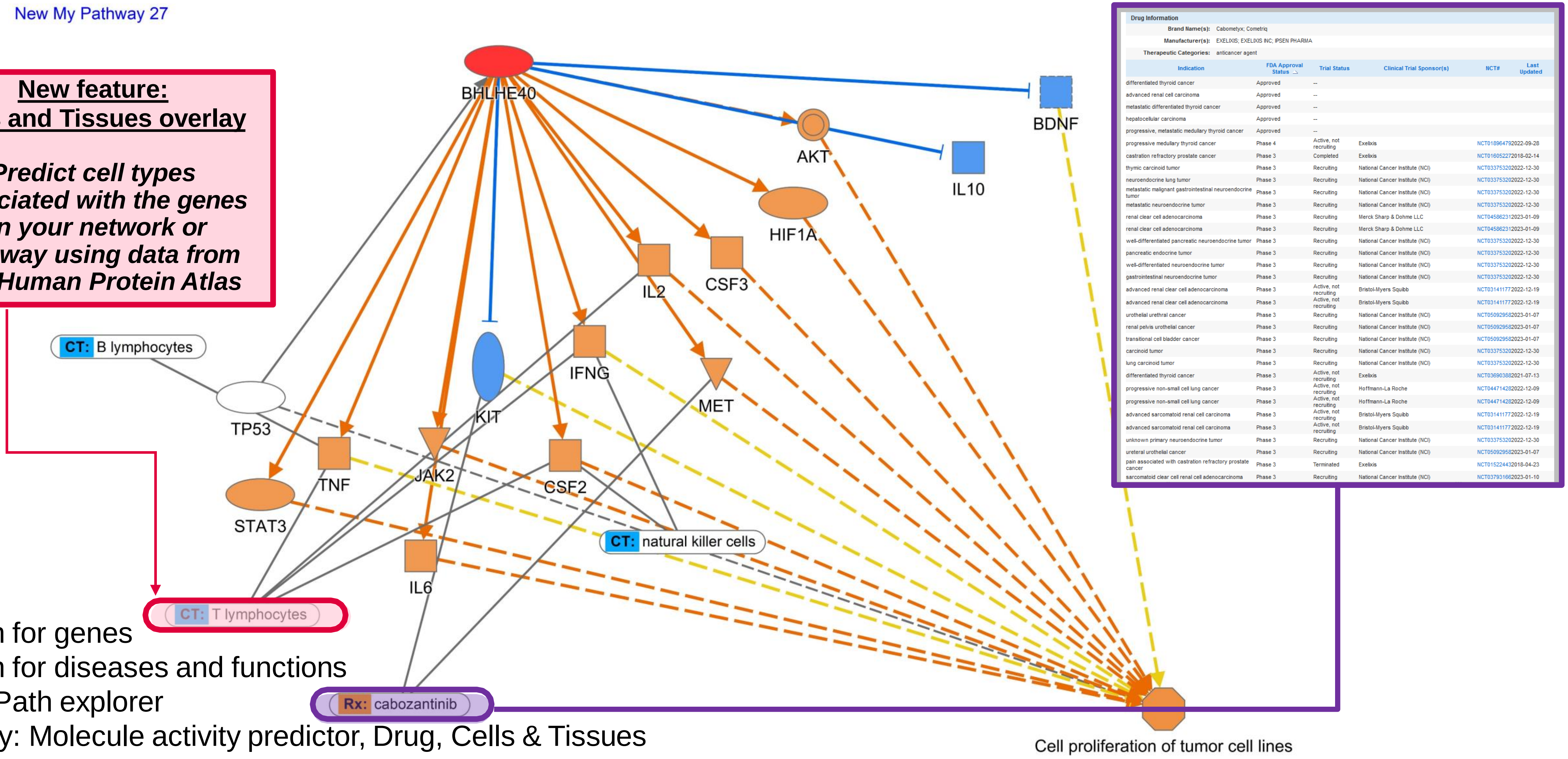
With dataset	Without dataset
• Find connections in your data	• Search and explore the QIAGEN Knowledge Base
• Identify novel biomarkers	• Test hypothesis in silico
• Uncover key targets and regulators	• Identify degree of novelty in a hypothesis
• Discover novel disease mechanisms	
• Compare across experiments	

Create Networks from Scratch and Test Activity in Silico

New My Pathway 27

<u>New feature:</u>
<u>Cells and Tissues overlay</u>

Predict cell types associated with the genes on your network or pathway using data from The Human Protein Atlas



Drug Information

Brand Name(s): Cabometyx; Cometriq

Manufacturer(s): EXELIXIS; EXELIXIS INC; IPSEN PHARMA

Therapeutic Categories: anticancer agent

Indication	FDA Approval Status △	Trial Status	Clinical Trial Sponsor(s)	NCT#	Last Updated
differentiated thyroid cancer	Approved	--			
advanced renal cell carcinoma	Approved	--			
metastatic differentiated thyroid cancer	Approved	--			
hepatocellular carcinoma	Approved	--			
progressive, metastatic medullary thyroid cancer	Approved	--			
progressive medullary thyroid cancer	Phase 4	Active, not recruiting	Exelixis	NCT01896479	2022-09-28
castration refractory prostate cancer	Phase 3	Completed	Exelixis	NCT01605227	2018-02-14
thymic carcinoid tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
neuroendocrine lung tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
metastatic malignant gastrointestinal neuroendocrine tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
metastatic neuroendocrine tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
renal clear cell adenocarcinoma	Phase 3	Recruiting	Merck Sharp & Dohme LLC	NCT04586231	2023-01-09
renal clear cell adenocarcinoma	Phase 3	Recruiting	Merck Sharp & Dohme LLC	NCT04586231	2023-01-09
well-differentiated pancreatic neuroendocrine tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
pancreatic endocrine tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
well-differentiated neuroendocrine tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
gastrointestinal neuroendocrine tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
advanced renal clear cell adenocarcinoma	Phase 3	Active, not recruiting	Bristol-Myers Squibb	NCT03141177	2022-12-19
advanced renal clear cell adenocarcinoma	Phase 3	Active, not recruiting	Bristol-Myers Squibb	NCT03141177	2022-12-19
urothelial urethral cancer	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT05092958	2023-01-07
renal pelvis urothelial cancer	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT05092958	2023-01-07
transitional cell bladder cancer	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT05092958	2023-01-07
carcinoid tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
lung carcinoid tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
differentiated thyroid cancer	Phase 3	Active, not recruiting	Exelixis	NCT03690388	2021-07-13
progressive non-small cell lung cancer	Phase 3	Active, not recruiting	Hoffmann-La Roche	NCT04471428	2022-12-09
progressive non-small cell lung cancer	Phase 3	Active, not recruiting	Hoffmann-La Roche	NCT04471428	2022-12-09
advanced sarcomatoid renal cell carcinoma	Phase 3	Active, not recruiting	Bristol-Myers Squibb	NCT03141177	2022-12-19
advanced sarcomatoid renal cell carcinoma	Phase 3	Active, not recruiting	Bristol-Myers Squibb	NCT03141177	2022-12-19
unknown primary neuroendocrine tumor	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03375320	2022-12-30
ureteral urothelial cancer	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT05092958	2023-01-07
pain associated with castration refractory prostate cancer	Phase 3	Terminated	Exelixis	NCT01522443	2018-04-23
sarcomatoid clear cell renal cell adenocarcinoma	Phase 3	Recruiting	National Cancer Institute (NCI)	NCT03793166	2023-01-10

- Search for genes
- Search for diseases and functions
- Build: Path explorer
- Overlay: Molecule activity predictor, Drug, Cells & Tissues
- Drug: IPA Chem View

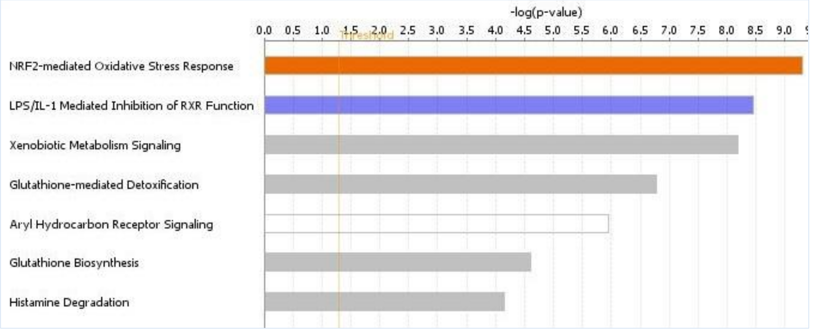
<u>Step by step slides 59-65</u>

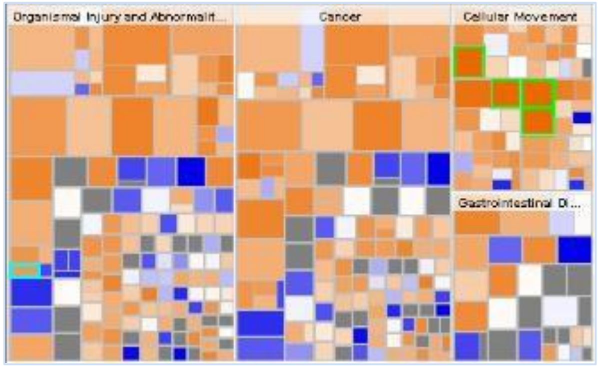
Omics data analysis using QIAGEN IPA

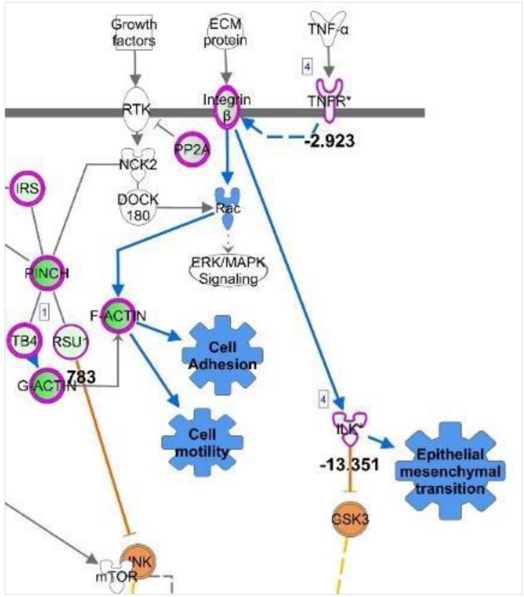
Omics data type

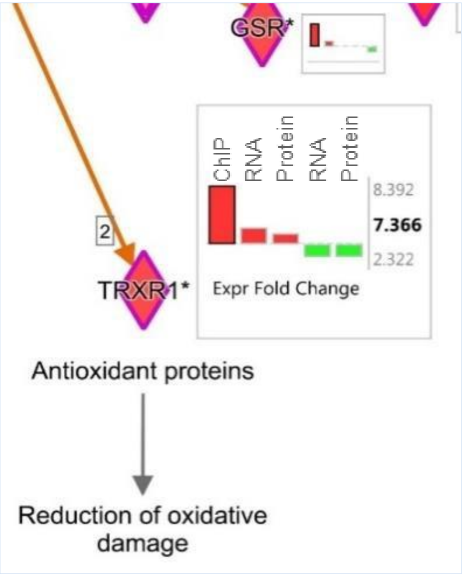
- RNA-seq
- scRNA-seq
- Microarray
- Nanostring
- qPCR
- ChIP-seq
- Proteomics
- Metabolomics
- RNAi
- CRISPR
- WGS/WES etc.

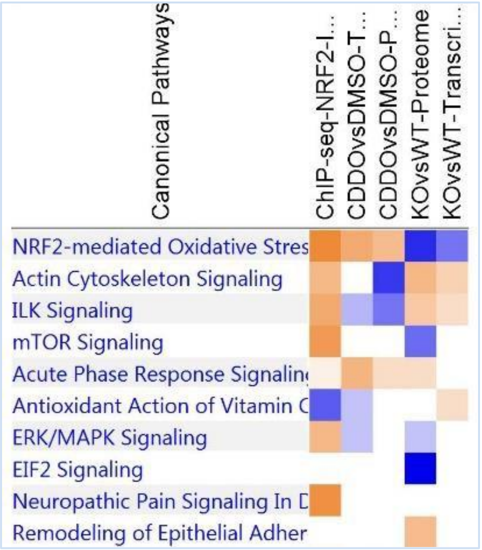
QIAGEN IPA



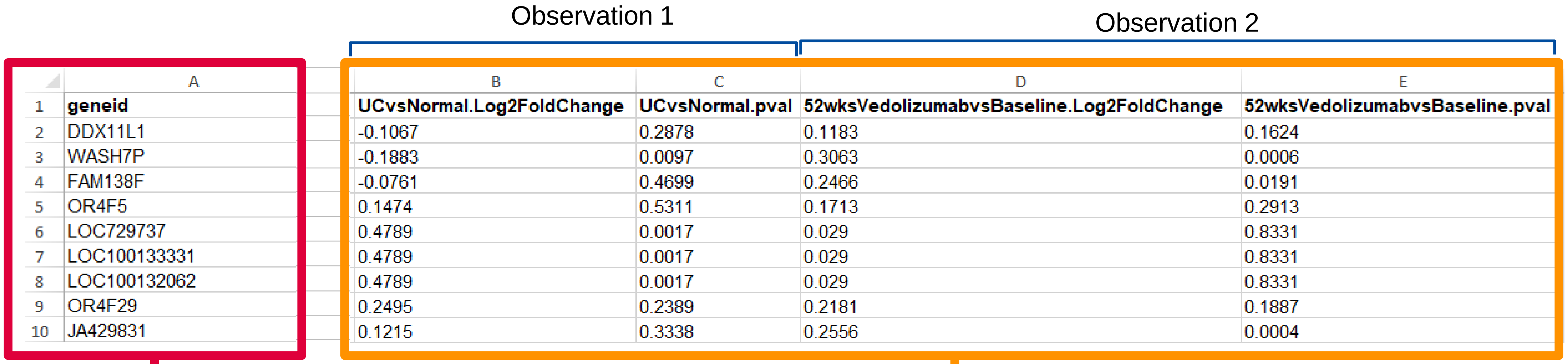








Formatting ‘omics data before uploading to IPA



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
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

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.

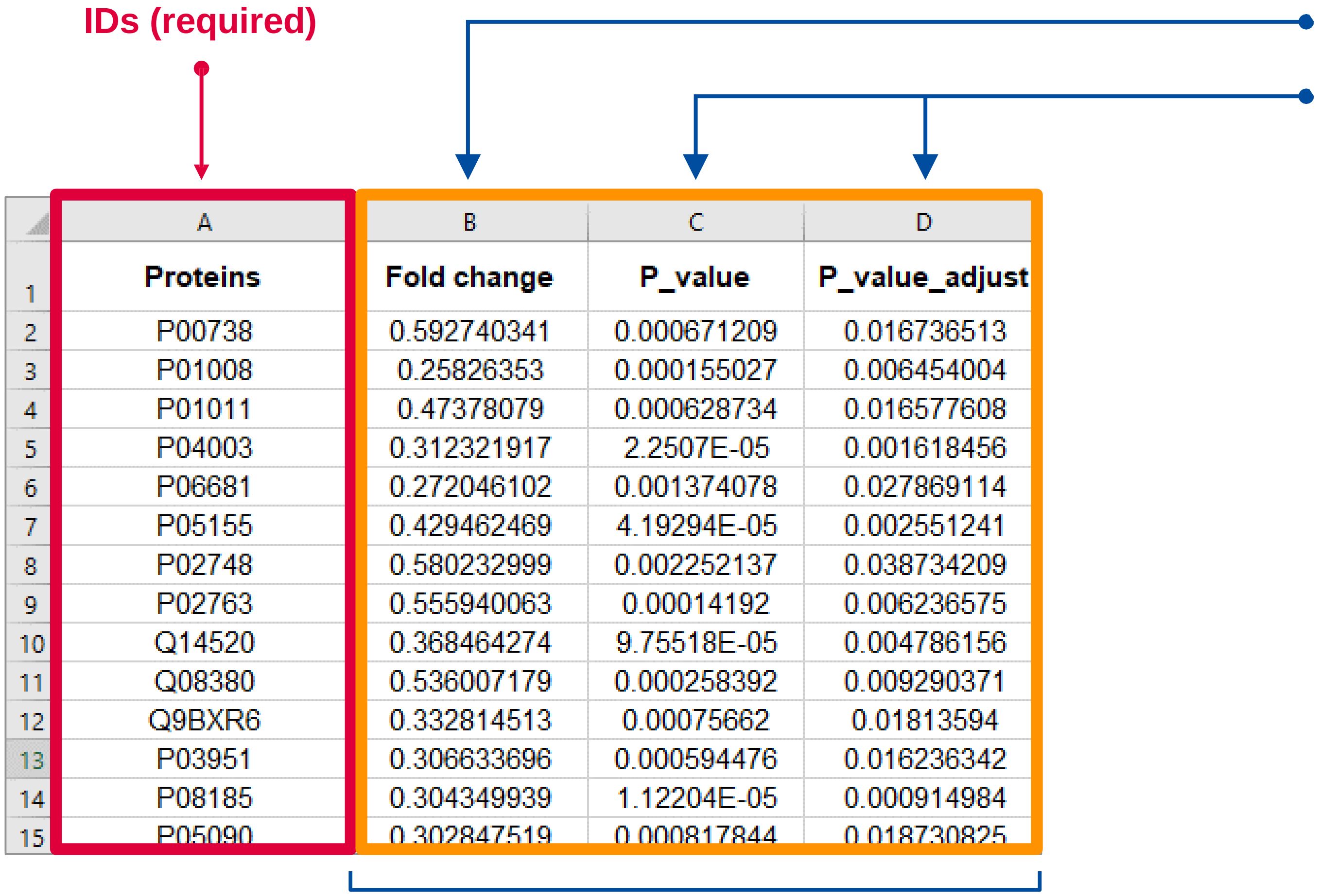
Significance values: P-values **optional but recommended to enable filtering for significance*

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

Formatting proteomics data before uploading to IPA



IDs (required)

Ratio, fold change, etc. (recommended)

Significance (optional)

	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:

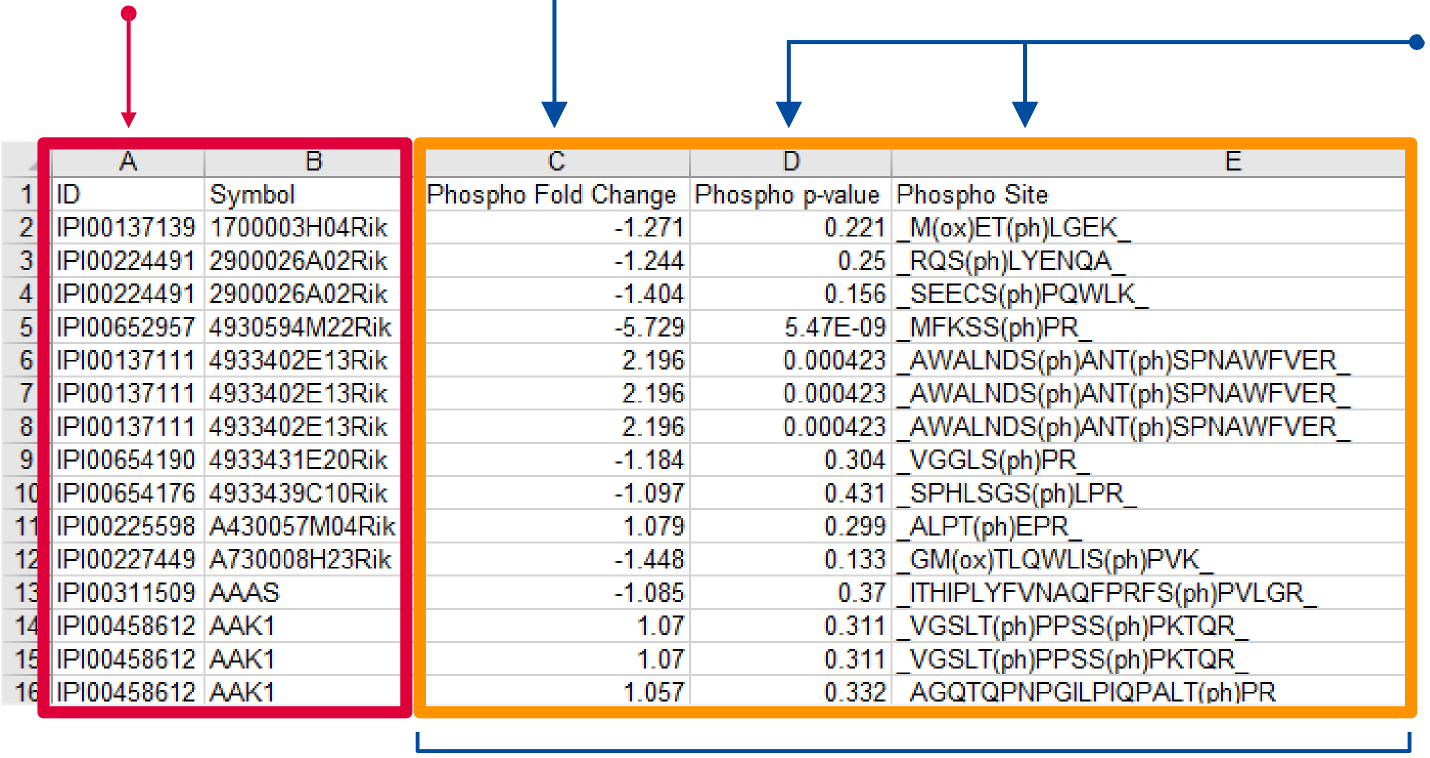
- https://www.uniprot.org/mapping/

Formatting proteomics data before uploading to IPA

IDs (required)

Ratio, fold change, etc. (recommended)

Significance (optional)



	A	B	C	D	E
1	ID	Symbol	Phospho Fold Change	Phospho p-value	Phospho Site
2	IPI00137139	1700003H04Rik	-1.271	0.221	_M(ox)ET(ph)LGEK_
3	IPI00224491	2900026A02Rik	-1.244	0.25	_RQS(ph)LYENQA_
4	IPI00224491	2900026A02Rik	-1.404	0.156	_SEECS(ph)PQWLK_
5	IPI00652957	4930594M22Rik	-5.729	5.47E-09	_MFKSS(ph)PR_
6	IPI00137111	4933402E13Rik	2.196	0.000423	_AWALNDS(ph)ANT(ph)SPNAWFVER_
7	IPI00137111	4933402E13Rik	2.196	0.000423	_AWALNDS(ph)ANT(ph)SPNAWFVER_
8	IPI00137111	4933402E13Rik	2.196	0.000423	_AWALNDS(ph)ANT(ph)SPNAWFVER_
9	IPI00654190	4933431E20Rik	-1.184	0.304	_VGGLS(ph)PR_
10	IPI00654176	4933439C10Rik	-1.097	0.431	_SPHLSGS(ph)LPR_
11	IPI00225598	A430057M04Rik	1.079	0.299	_ALPT(ph)EPR_
12	IPI00227449	A730008H23Rik	-1.448	0.133	_GM(ox)TLQWLIS(ph)PVK_
13	IPI00311509	AAAS	-1.085	0.37	_ITHIPLYFVNAQFPRFS(ph)PVLGR_
14	IPI00458612	AAK1	1.07	0.311	_VGSLT(ph)PPSS(ph)PKTQR_
15	IPI00458612	AAK1	1.07	0.311	_VGSLT(ph)PPSS(ph)PKTQR_
16	IPI00458612	AAK1	1.057	0.332	_AGQTQPNPGILPIQPALT(ph)PR_

Observation 1

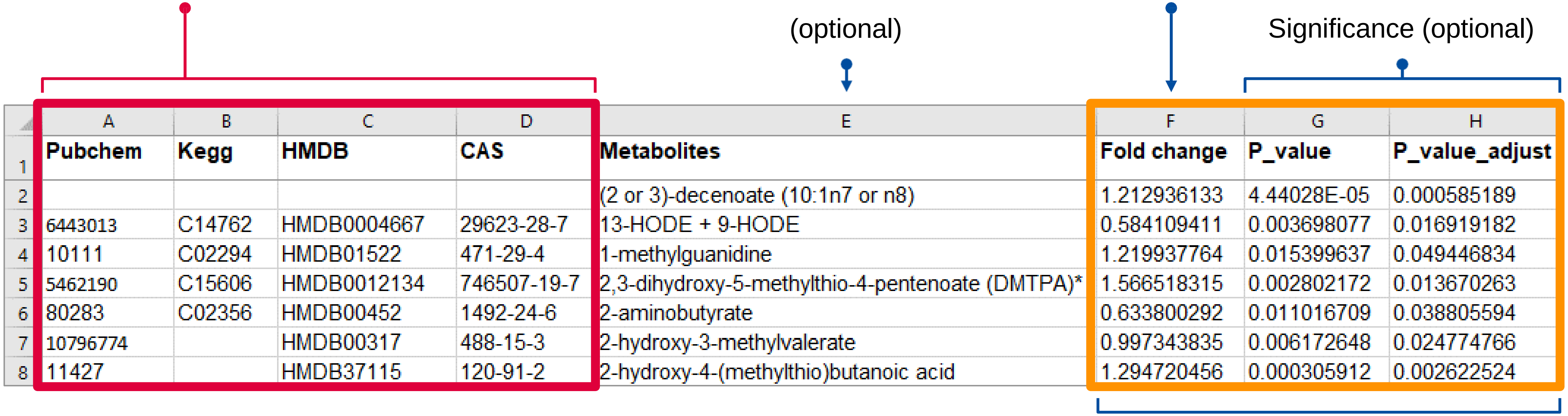
Common protein IDs

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

UniProt ID conversion tool:

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

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

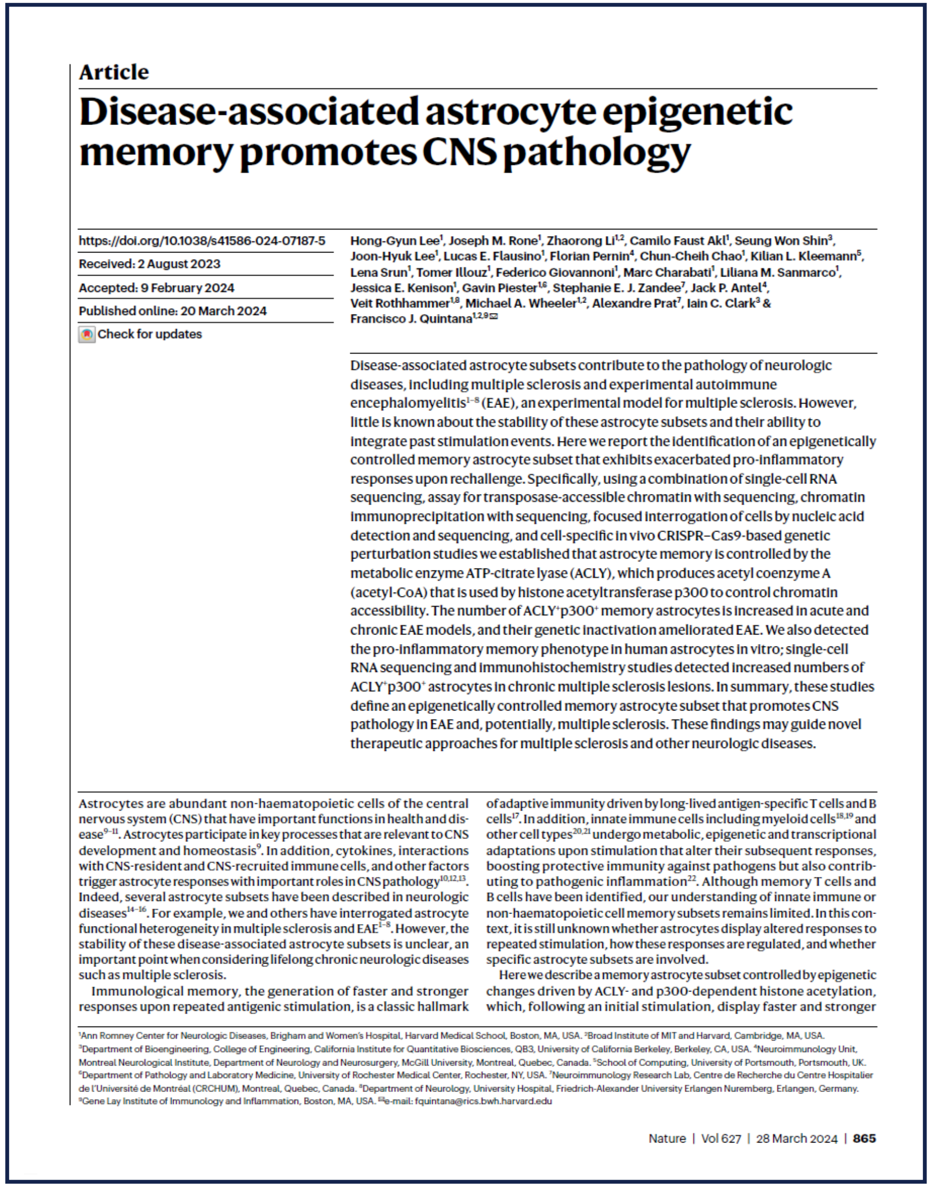
- CAS registry number
- Human Metabolome Database
- KEGG
- PubChem CID

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

Case study

Bulk RNA-seq



Article

Disease-associated astrocyte epigenetic memory promotes CNS pathology

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Hong-Gyun Lee[1], Joseph M. Rone[1], Zhaorong Li[1,2], Camilo Faust Akl[1], Seung Won Shin[3], Joon-Hyuk Lee[1], Lucas E. Flausino[1], Florian Pernin[4], Chun-Cheih Chao[1], Kilian L. Kleemann[5], Lena Srun[1], Tomer Illouz[1], Federico Giovannoni[1], Marc Charabati[1], Liliana M. Sanmarco[1], Jessica E. Kenison[1], Gavin Piester[1,6], Stephanie E. J. Zandee[7], Jack P. Antel[4], Veit Rothhammer[1,8], Michael A. Wheeler[1,2], Alexandre Prat[7], Iain C. Clark[3] & Francisco J. Quintana[1,2,9] ✉

Disease-associated astrocyte subsets contribute to the pathology of neurologic diseases, including multiple sclerosis and experimental autoimmune encephalomyelitis[1–8] (EAE), an experimental model for multiple sclerosis. However, little is known about the stability of these astrocyte subsets and their ability to integrate past stimulation events. Here we report the identification of an epigenetically controlled memory astrocyte subset that exhibits exacerbated pro-inflammatory responses upon rechallenge. Specifically, using a combination of single-cell RNA sequencing, assay for transposase-accessible chromatin with sequencing, chromatin immunoprecipitation with sequencing, focused interrogation of cells by nucleic acid detection and sequencing, and cell-specific in vivo CRISPR–Cas9-based genetic perturbation studies we established that astrocyte memory is controlled by the metabolic enzyme ATP-citrate lyase (ACLY), which produces acetyl coenzyme A (acetyl-CoA) that is used by histone acetyltransferase p300 to control chromatin accessibility. The number of ACLY$^+$p300$^+$ memory astrocytes is increased in acute and chronic EAE models, and their genetic inactivation ameliorated EAE. We also detected the pro-inflammatory memory phenotype in human astrocytes in vitro; single-cell RNA sequencing and immunohistochemistry studies detected increased numbers of ACLY$^+$p300$^+$ astrocytes in chronic multiple sclerosis lesions. In summary, these studies define an epigenetically controlled memory astrocyte subset that promotes CNS pathology in EAE and, potentially, multiple sclerosis. These findings may guide novel therapeutic approaches for multiple sclerosis and other neurologic diseases.

Astrocytes are abundant non-haematopoietic cells of the central nervous system (CNS) that have important functions in health and disease[9–11]. Astrocytes participate in key processes that are relevant to CNS development and homeostasis[9]. In addition, cytokines, interactions with CNS-resident and CNS-recruited immune cells, and other factors trigger astrocyte responses with important roles in CNS pathology[10,12,13]. Indeed, several astrocyte subsets have been described in neurologic diseases[14–16]. For example, we and others have interrogated astrocyte functional heterogeneity in multiple sclerosis and EAE[1–8]. However, the stability of these disease-associated astrocyte subsets is unclear, an important point when considering lifelong chronic neurologic diseases such as multiple sclerosis.

Immunological memory, the generation of faster and stronger responses upon repeated antigenic stimulation, is a classic hallmark of adaptive immunity driven by long-lived antigen-specific T cells and B cells[17]. In addition, innate immune cells including myeloid cells[18,19] and other cell types[20,21] undergo metabolic, epigenetic and transcriptional adaptations upon stimulation that alter their subsequent responses, boosting protective immunity against pathogens but also contributing to pathogenic inflammation[22]. Although memory T cells and B cells have been identified, our understanding of innate immune or non-haematopoietic cell memory subsets remains limited. In this context, it is still unknown whether astrocytes display altered responses to repeated stimulation, how these responses are regulated, and whether specific astrocyte subsets are involved.

Here we describe a memory astrocyte subset controlled by epigenetic changes driven by ACLY- and p300-dependent histone acetylation, which, following an initial stimulation, display faster and stronger

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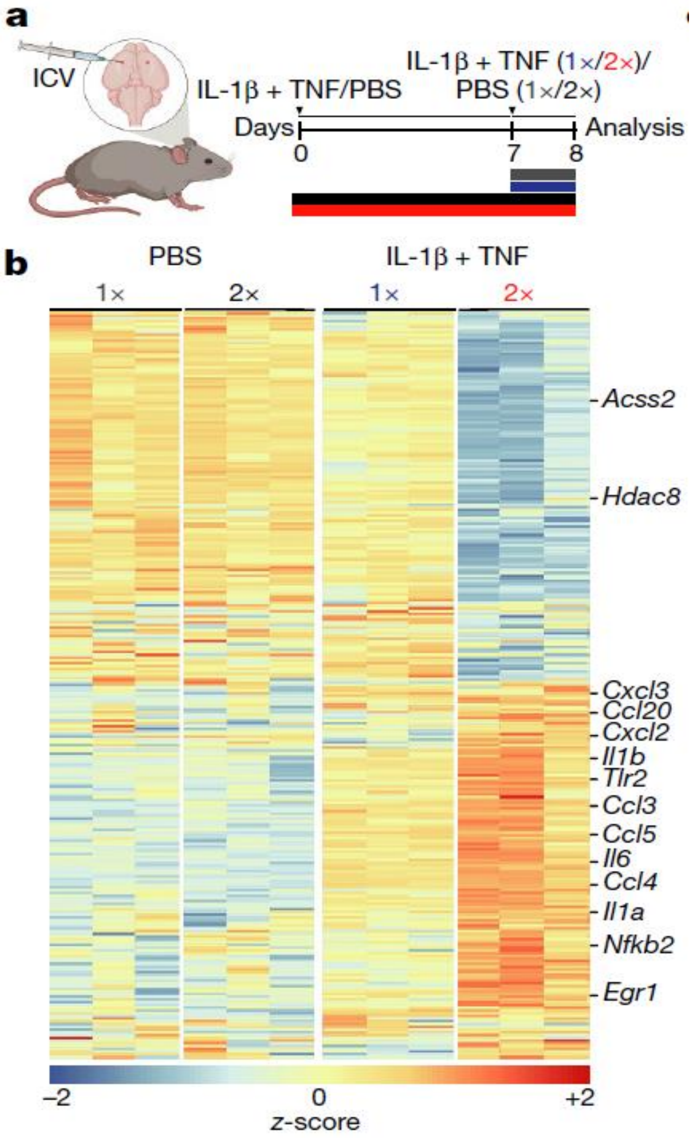
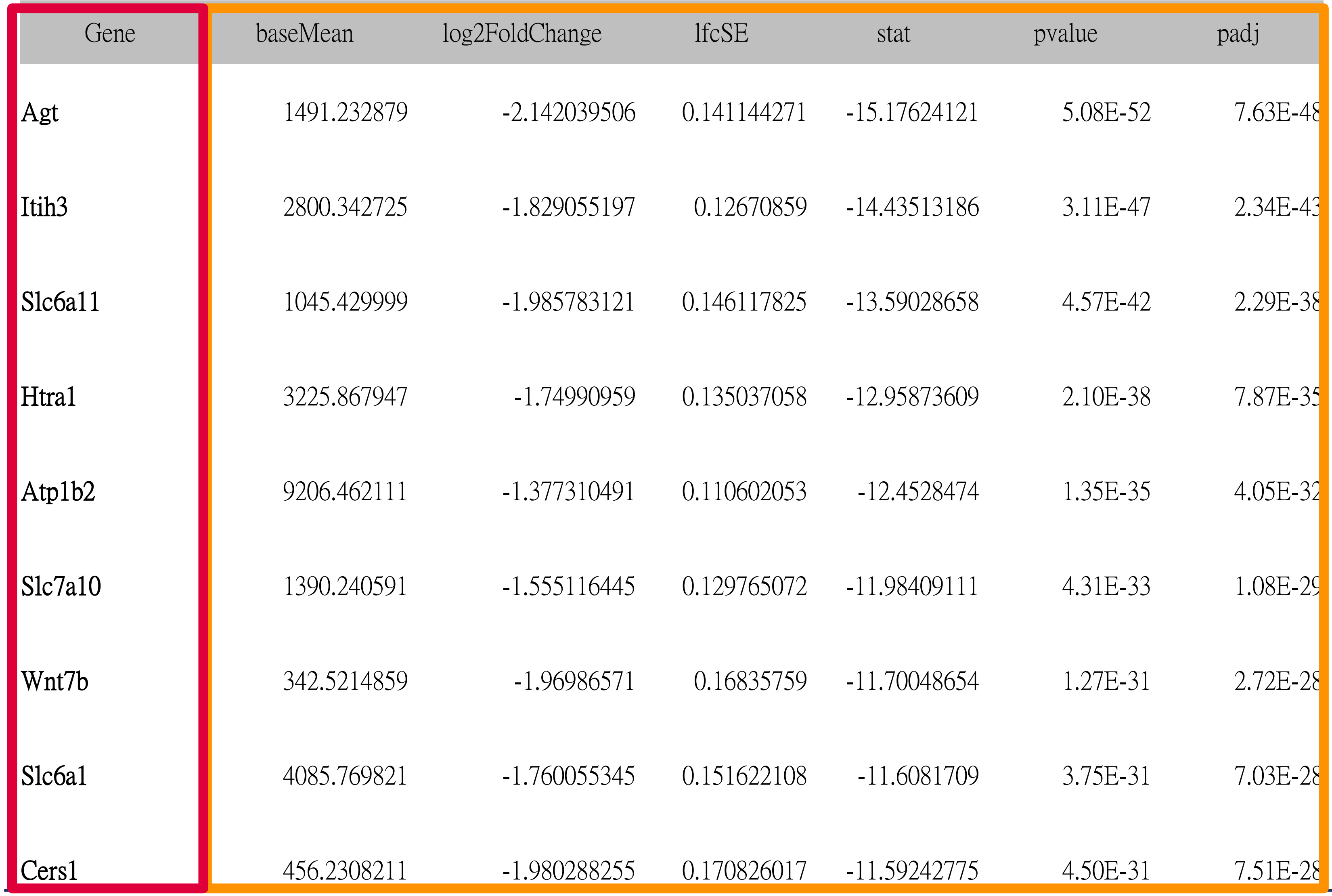


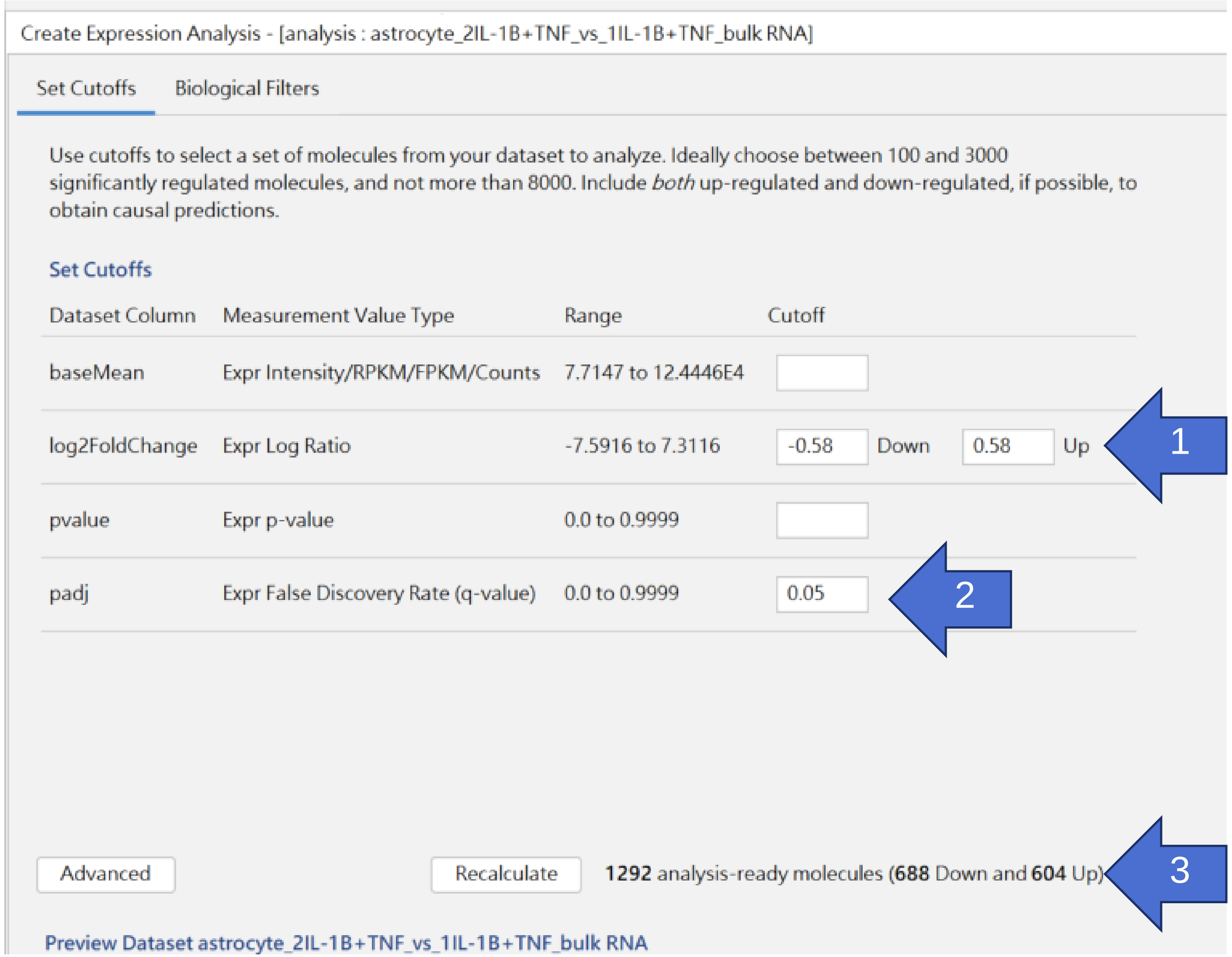
Upload Dataset

IDs (required)



Gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Agt	1491.232879	-2.142039506	0.141144271	-15.17624121	5.08E-52	7.63E-48
Itih3	2800.342725	-1.829055197	0.12670859	-14.43513186	3.11E-47	2.34E-43
Slc6a11	1045.429999	-1.985783121	0.146117825	-13.59028658	4.57E-42	2.29E-38
Htra1	3225.867947	-1.74990959	0.135037058	-12.95873609	2.10E-38	7.87E-35
Atp1b2	9206.462111	-1.377310491	0.110602053	-12.4528474	1.35E-35	4.05E-32
Slc7a10	1390.240591	-1.555116445	0.129765072	-11.98409111	4.31E-33	1.08E-29
Wnt7b	342.5214859	-1.96986571	0.16835759	-11.70048654	1.27E-31	2.72E-28
Slc6a1	4085.769821	-1.760055345	0.151622108	-11.6081709	3.75E-31	7.03E-28
Cers1	456.2308211	-1.980288255	0.170826017	-11.59242775	4.50E-31	7.51E-28

Volcano Plot



Create Expression Analysis - [analysis : astrocyte_2IL-1B+TNF_vs_1IL-1B+TNF_bulk RNA]

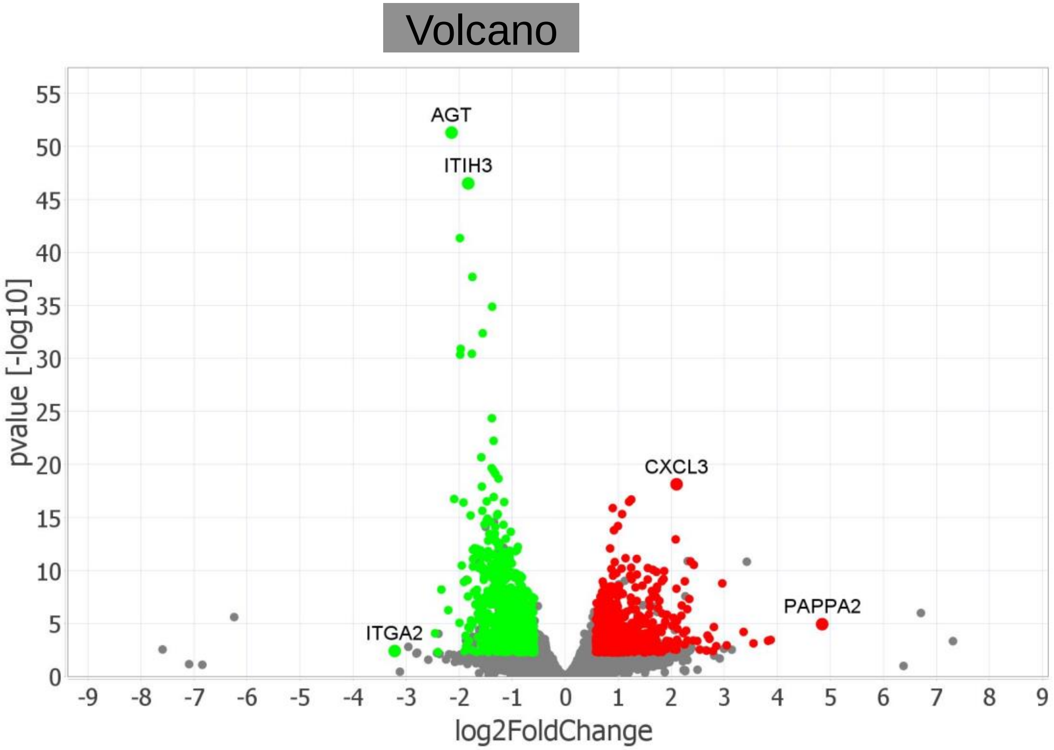
Use cutoffs to select a set of molecules from your dataset to analyze. Ideally choose between 100 and 3000 significantly regulated molecules, and not more than 8000. Include *both* up-regulated and down-regulated, if possible, to obtain causal predictions.

Set Cutoffs

Dataset Column	Measurement Value Type	Range	Cutoff
baseMean	Expr Intensity/RPKM/FPKM/Counts	7.7147 to 12.4446E4	
log2FoldChange	Expr Log Ratio	-7.5916 to 7.3116	-0.58 Down 0.58 Up
pvalue	Expr p-value	0.0 to 0.9999	
padj	Expr False Discovery Rate (q-value)	0.0 to 0.9999	0.05

1292 analysis-ready molecules (**688** Down and **604** Up)

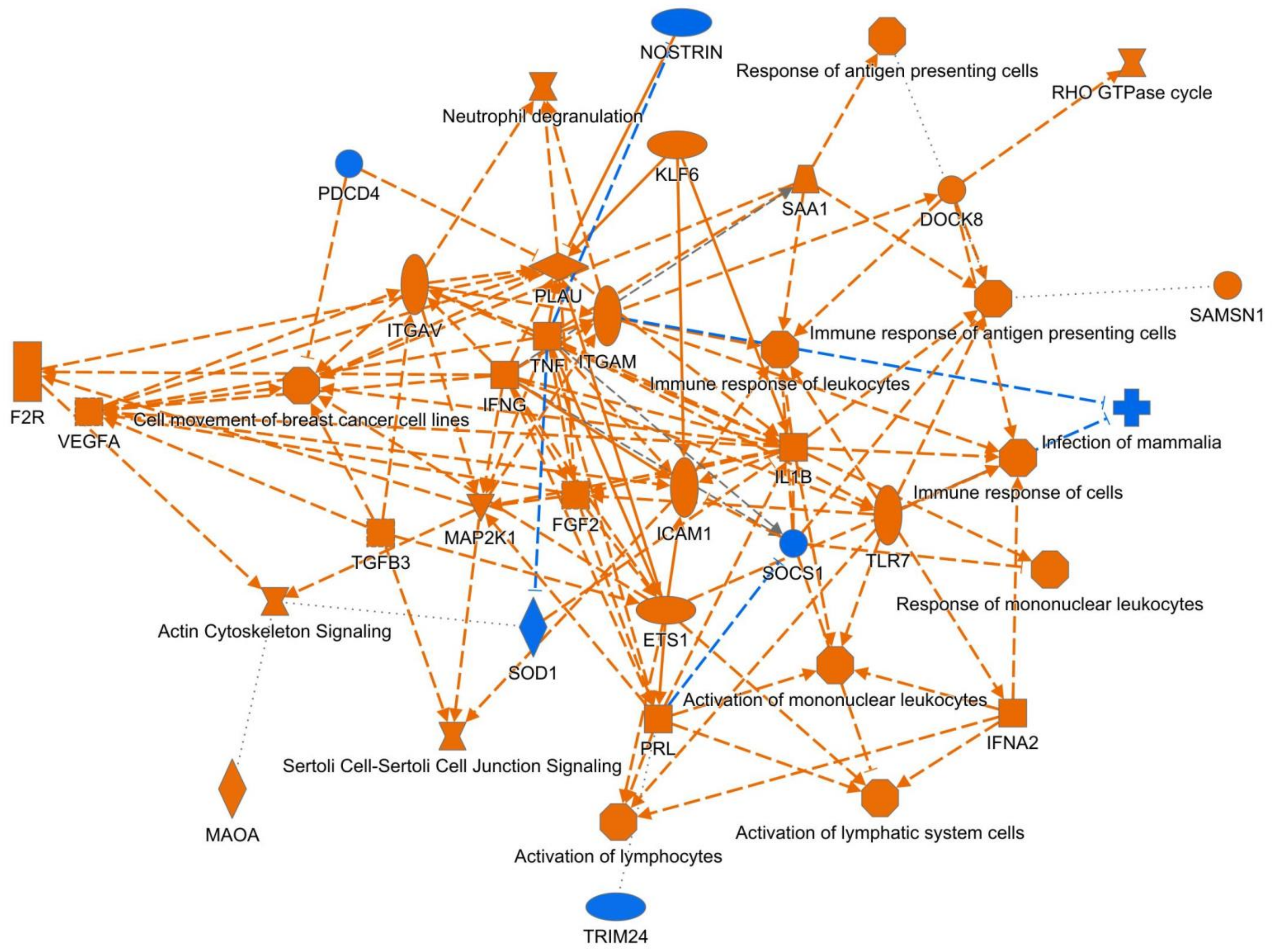
Preview Dataset astrocyte_2IL-1B+TNF_vs_1IL-1B+TNF_bulk RNA



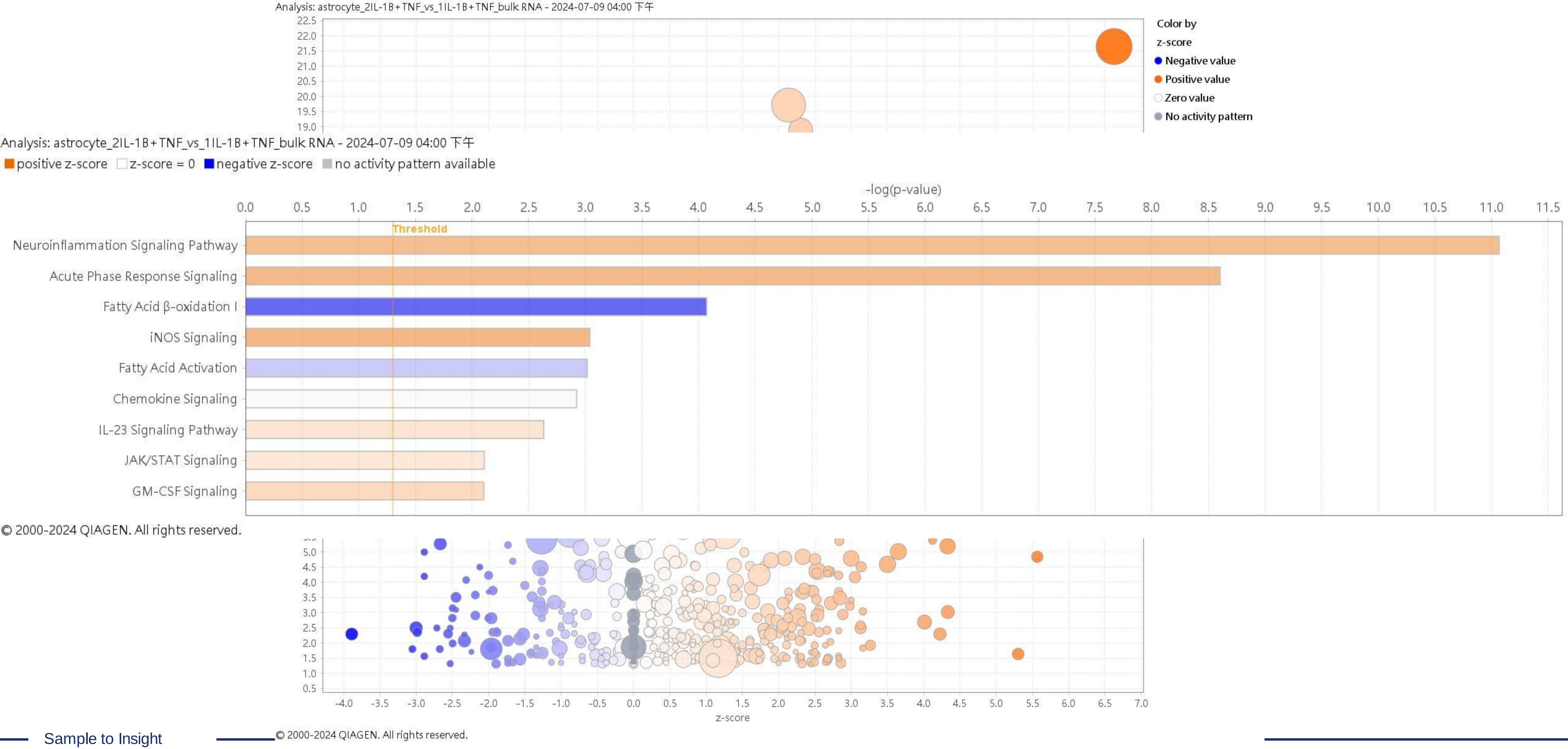
You can click on the dot and it could be labeled gene name on the plot

Core analysis –Graphical Summary

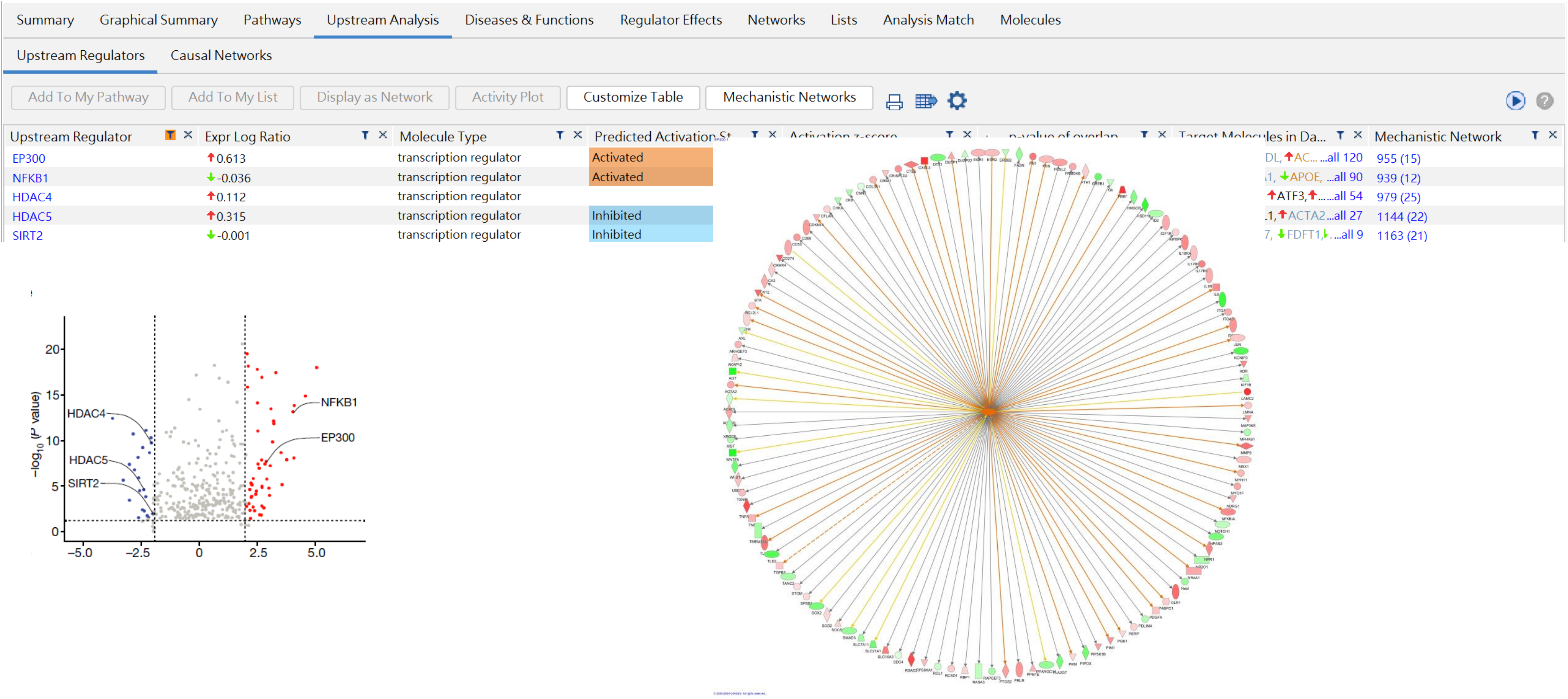
astrocyte_2IL-1B+TNF_vs_1IL-1B+TNF_bulk RNA - 2024-07-09 04:00 □□ Summary Graph



Canonical pathway

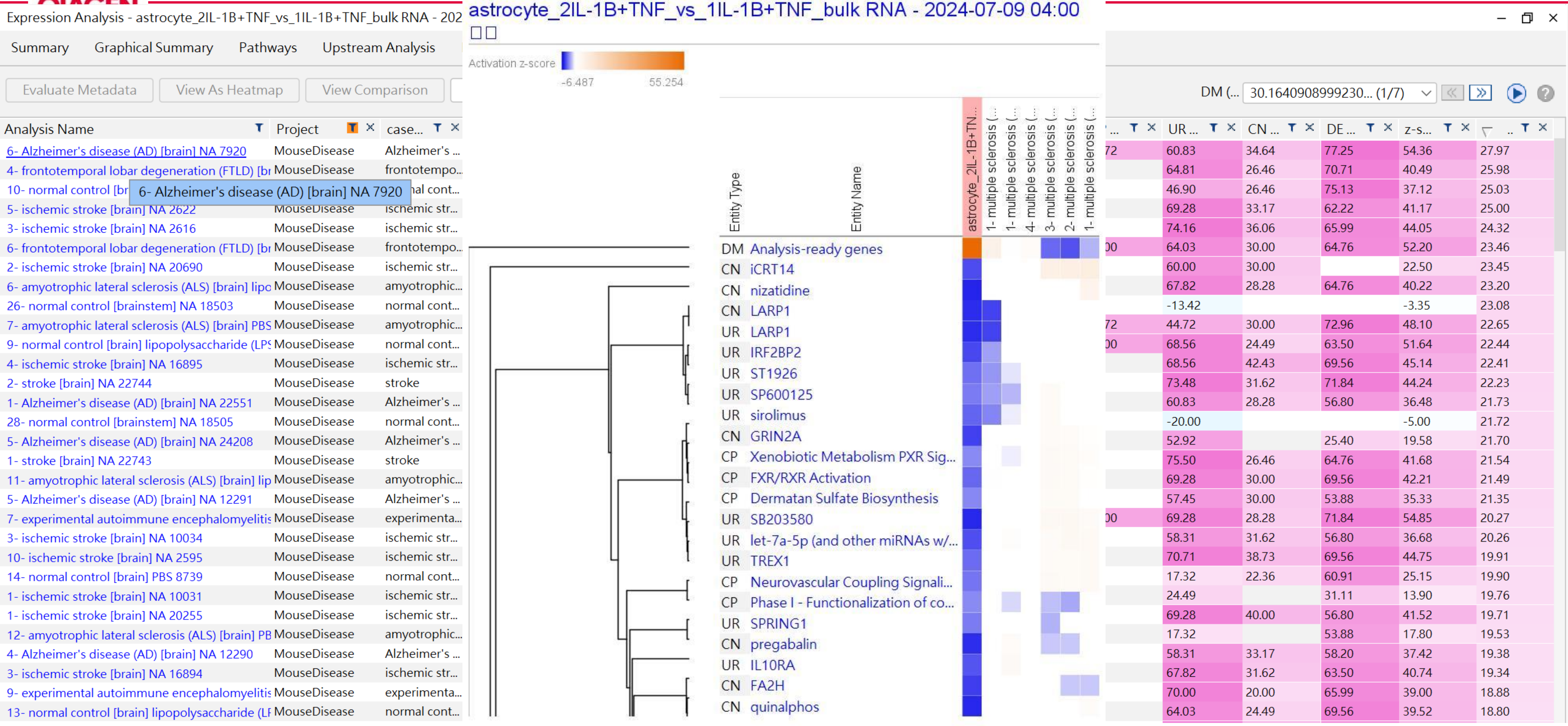


Upstream Analysis



Upstream Regulator	Expr Log Ratio	Molecule Type	Predicted Activation St	Activation z-score	p-value of overlap	Target Molecules in Da...	Mechanistic Network
EP300	↑0.613	transcription regulator	Activated			DL, ↑AC... ...all 120	955 (15)
NFKB1	↓-0.036	transcription regulator	Activated			1, ↓APOE, ...all 90	939 (12)
HDAC4	↑0.112	transcription regulator				↑ATF3,↑... ...all 54	979 (25)
HDAC5	↑0.315	transcription regulator	Inhibited			1,↑ACTA2...all 27	1144 (22)
SIRT2	↓-0.001	transcription regulator	Inhibited			7, ↓FDFT1,↓. ...all 9	1163 (21)

Analysis Match



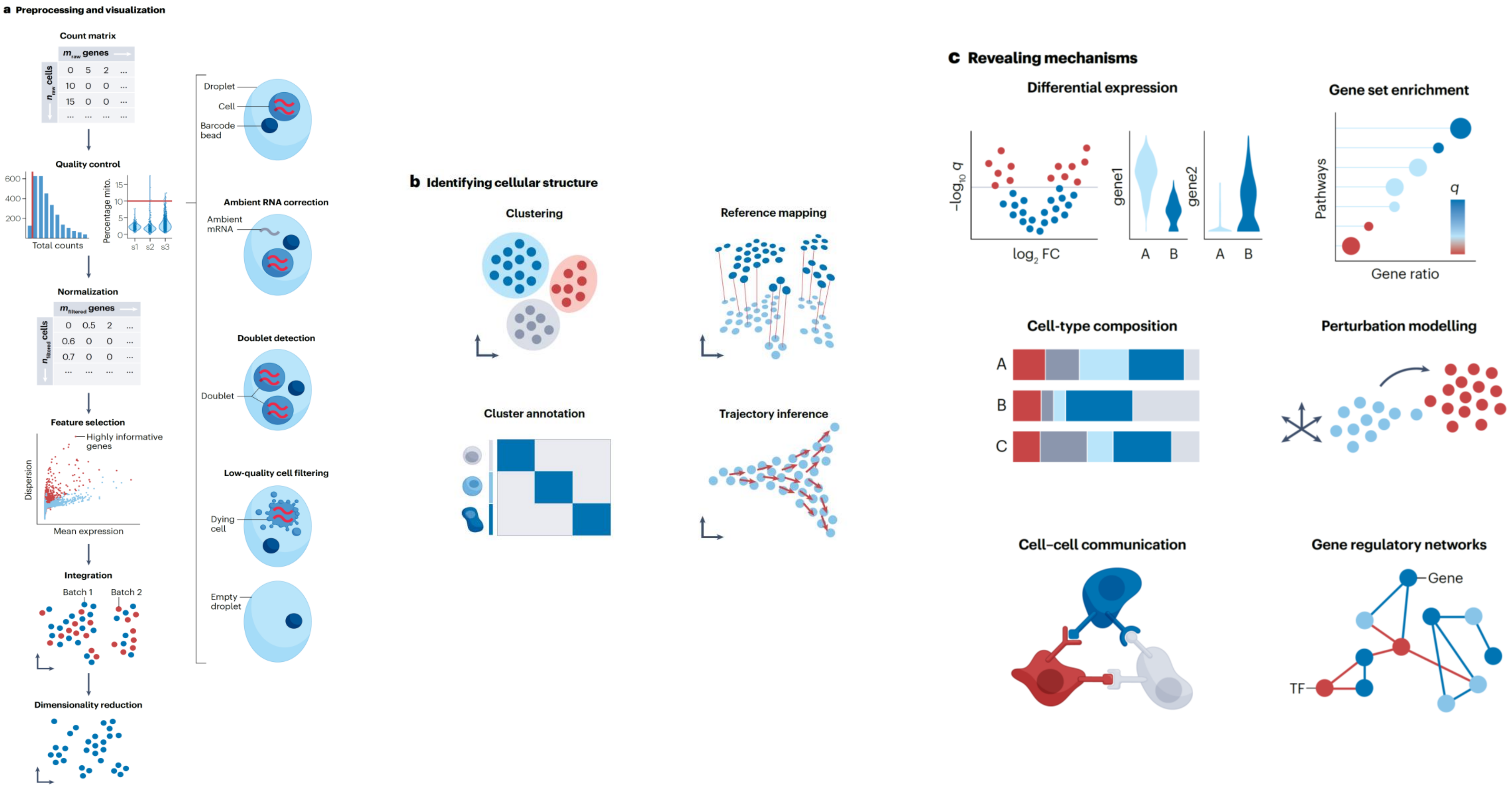
Expression Analysis - astrocyte_2IL-1B+TNF_vs_1IL-1B+TNF_bulk RNA - 202

astrocyte_2IL-1B+TNF_vs_1IL-1B+TNF_bulk RNA - 2024-07-09 04:00

Entity Type	Entity Name
DM	Analysis-ready genes
CN	iCRT14
CN	nizatidine
CN	LARP1
UR	LARP1
UR	IRF2BP2
UR	ST1926
UR	SP600125
UR	sirolimus
CN	GRIN2A
CP	Xenobiotic Metabolism PXR Sig...
CP	FXR/RXR Activation
CP	Dermatan Sulfate Biosynthesis
UR	SB203580
UR	let-7a-5p (and other miRNAs w/...
UR	TREX1
CP	Neurovascular Coupling Signali...
CP	Phase I - Functionalization of co...
UR	SPRING1
CN	pregabalin
UR	IL10RA
CN	FA2H
CN	quinalphos

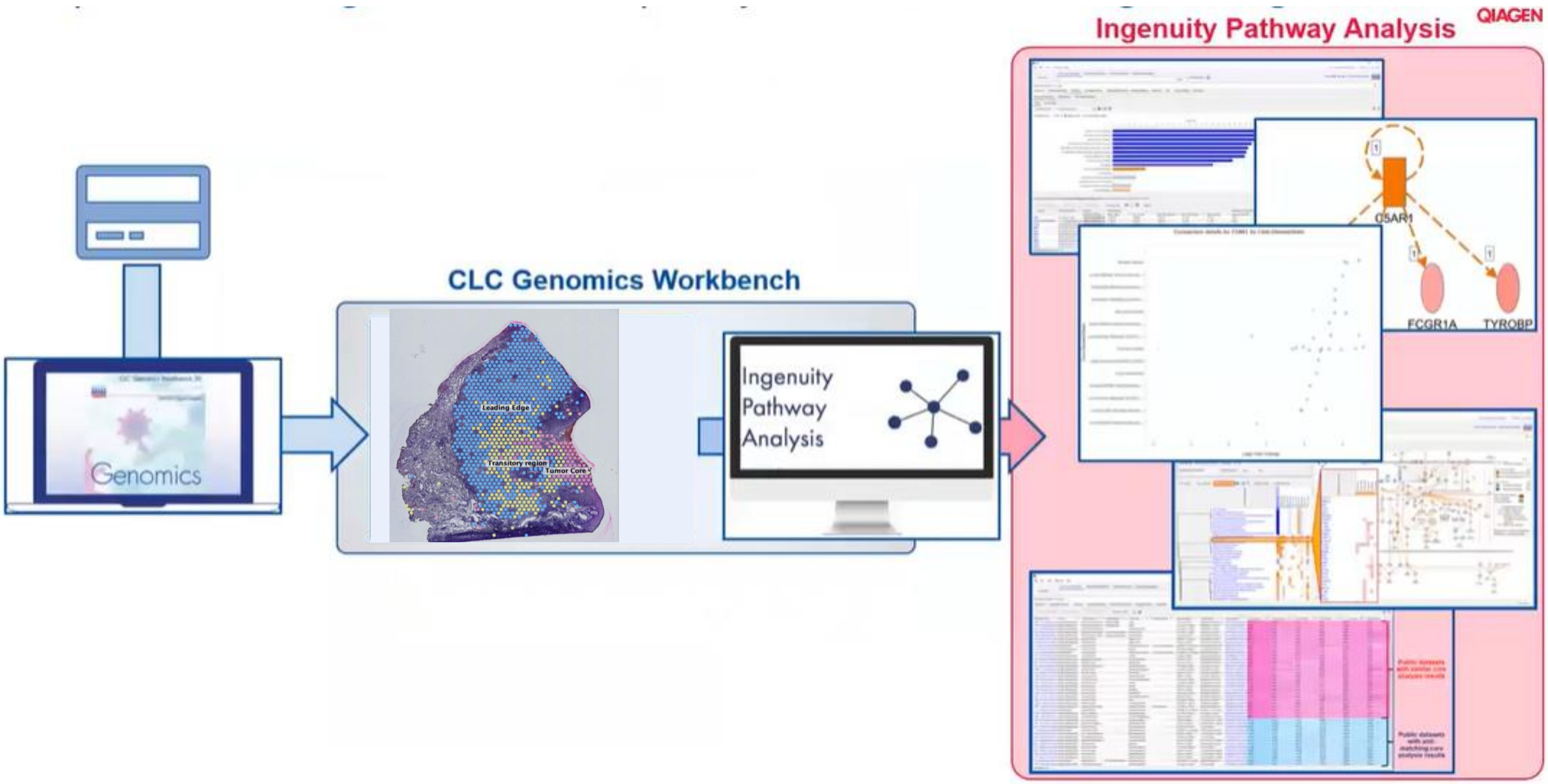
Analysis Name	Project	case...	...	UR ...	CN ...	DE ...	z-s...	..
6- Alzheimer's disease (AD) [brain] NA 7920	MouseDisease	Alzheimer's ...	72	60.83	34.64	77.25	54.36	27.97
4- frontotemporal lobar degeneration (FTLD) [br	MouseDisease	frontotempo...		64.81	26.46	70.71	40.49	25.98
10- normal control [br	6- Alzheimer's disease (AD) [brain] NA 7920	al cont...		46.90	26.46	75.13	37.12	25.03
5- ischemic stroke [brain] NA 2622	MouseDisease	ischemic str...		69.28	33.17	62.22	41.17	25.00
3- ischemic stroke [brain] NA 2616	MouseDisease	ischemic str...		74.16	36.06	65.99	44.05	24.32
6- frontotemporal lobar degeneration (FTLD) [br	MouseDisease	frontotempo...	00	64.03	30.00	64.76	52.20	23.46
2- ischemic stroke [brain] NA 20690	MouseDisease	ischemic str...		60.00	30.00		22.50	23.45
6- amyotrophic lateral sclerosis (ALS) [brain] lipo	MouseDisease	amyotrophic...		67.82	28.28	64.76	40.22	23.20
26- normal control [brainstem] NA 18503	MouseDisease	normal cont...		-13.42			-3.35	23.08
7- amyotrophic lateral sclerosis (ALS) [brain] PBS	MouseDisease	amyotrophic...	72	44.72	30.00	72.96	48.10	22.65
9- normal control [brain] lipopolysaccharide (LPS	MouseDisease	normal cont...	00	68.56	24.49	63.50	51.64	22.44
4- ischemic stroke [brain] NA 16895	MouseDisease	ischemic str...		68.56	42.43	69.56	45.14	22.41
2- stroke [brain] NA 22744	MouseDisease	stroke		73.48	31.62	71.84	44.24	22.23
1- Alzheimer's disease (AD) [brain] NA 22551	MouseDisease	Alzheimer's ...		60.83	28.28	56.80	36.48	21.73
28- normal control [brainstem] NA 18505	MouseDisease	normal cont...		-20.00			-5.00	21.72
5- Alzheimer's disease (AD) [brain] NA 24208	MouseDisease	Alzheimer's ...		52.92		25.40	19.58	21.70
1- stroke [brain] NA 22743	MouseDisease	stroke		75.50	26.46	64.76	41.68	21.54
11- amyotrophic lateral sclerosis (ALS) [brain] lip	MouseDisease	amyotrophic...		69.28	30.00	69.56	42.21	21.49
5- Alzheimer's disease (AD) [brain] NA 12291	MouseDisease	Alzheimer's ...		57.45	30.00	53.88	35.33	21.35
7- experimental autoimmune encephalomyelitis	MouseDisease	experimenta...	00	69.28	28.28	71.84	54.85	20.27
3- ischemic stroke [brain] NA 10034	MouseDisease	ischemic str...		58.31	31.62	56.80	36.68	20.26
10- ischemic stroke [brain] NA 2595	MouseDisease	ischemic str...		70.71	38.73	69.56	44.75	19.91
14- normal control [brain] PBS 8739	MouseDisease	normal cont...		17.32	22.36	60.91	25.15	19.90
1- ischemic stroke [brain] NA 10031	MouseDisease	ischemic str...		24.49		31.11	13.90	19.76
1- ischemic stroke [brain] NA 20255	MouseDisease	ischemic str...		69.28	40.00	56.80	41.52	19.71
12- amyotrophic lateral sclerosis (ALS) [brain] PB	MouseDisease	amyotrophic...		17.32		53.88	17.80	19.53
4- Alzheimer's disease (AD) [brain] NA 12290	MouseDisease	Alzheimer's ...		58.31	33.17	58.20	37.42	19.38
3- ischemic stroke [brain] NA 16894	MouseDisease	ischemic str...		67.82	31.62	63.50	40.74	19.34
9- experimental autoimmune encephalomyelitis	MouseDisease	experimenta...		70.00	20.00	65.99	39.00	18.88
13- normal control [brain] lipopolysaccharide (L	MouseDisease	normal cont...		64.03	24.49	69.56	39.52	18.80

Overview of unimodal analysis steps for scRNA-seq

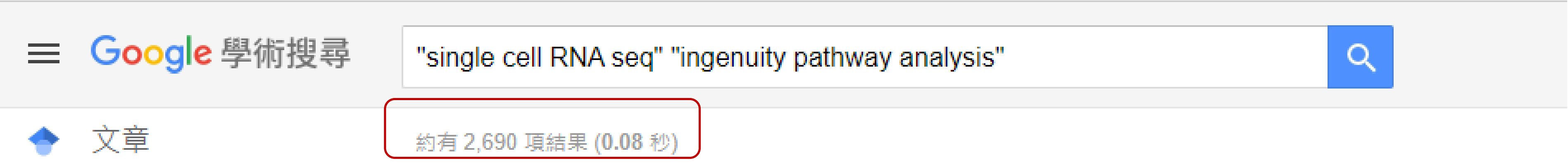


Heumos, L., Schaar, A.C., Lance, C. *et al.* Best practices for single-cell analysis across modalities. *Nat Rev Genet* **24**, 550–572 (2023). https://doi.org/10.1038/s41576-023-00586-w

Comprehensive single-cell RNA-seq analysis with Qiagen Digital Insights



Publication of Single-cell RNA Seq and Qiagen IPA



"single cell RNA seq" "ingenuity pathway analysis"

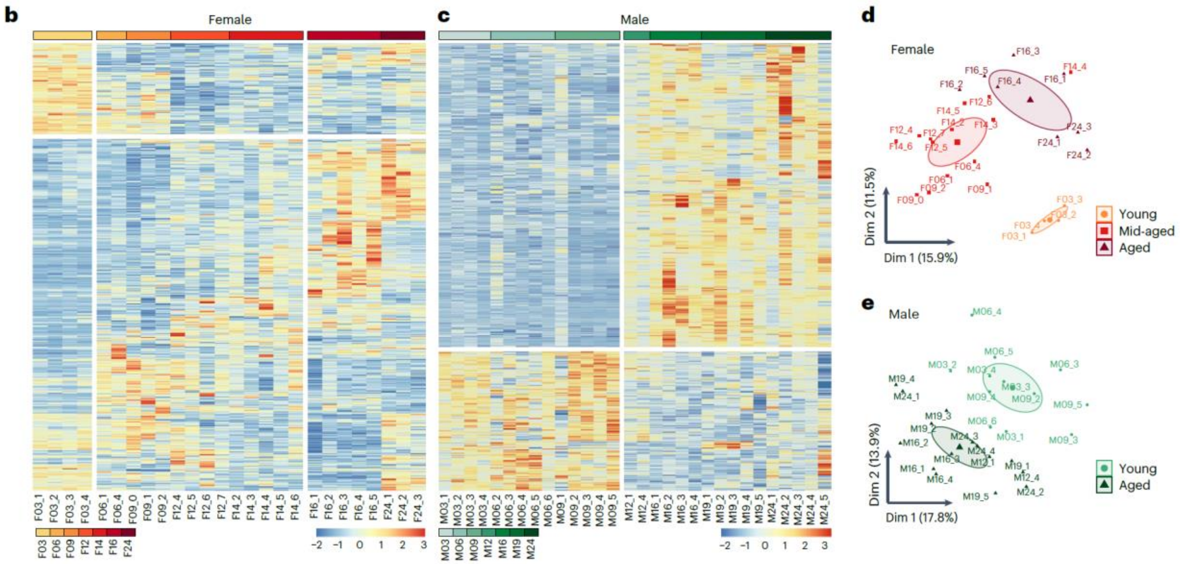
約有 2,690 項結果 (0.08 秒)

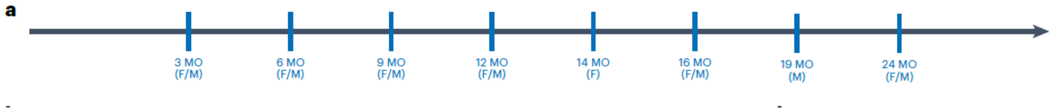

nature aging

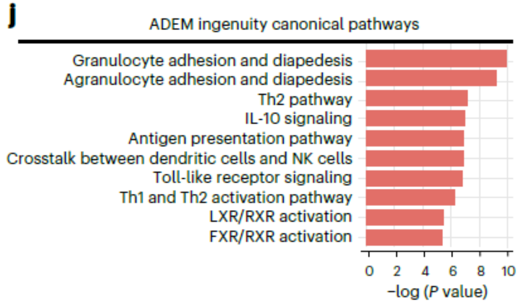
Resource

https://doi.org/10.1038/s43587-023-00479-x

Transcriptional and epigenetic decoding of the microglial aging process



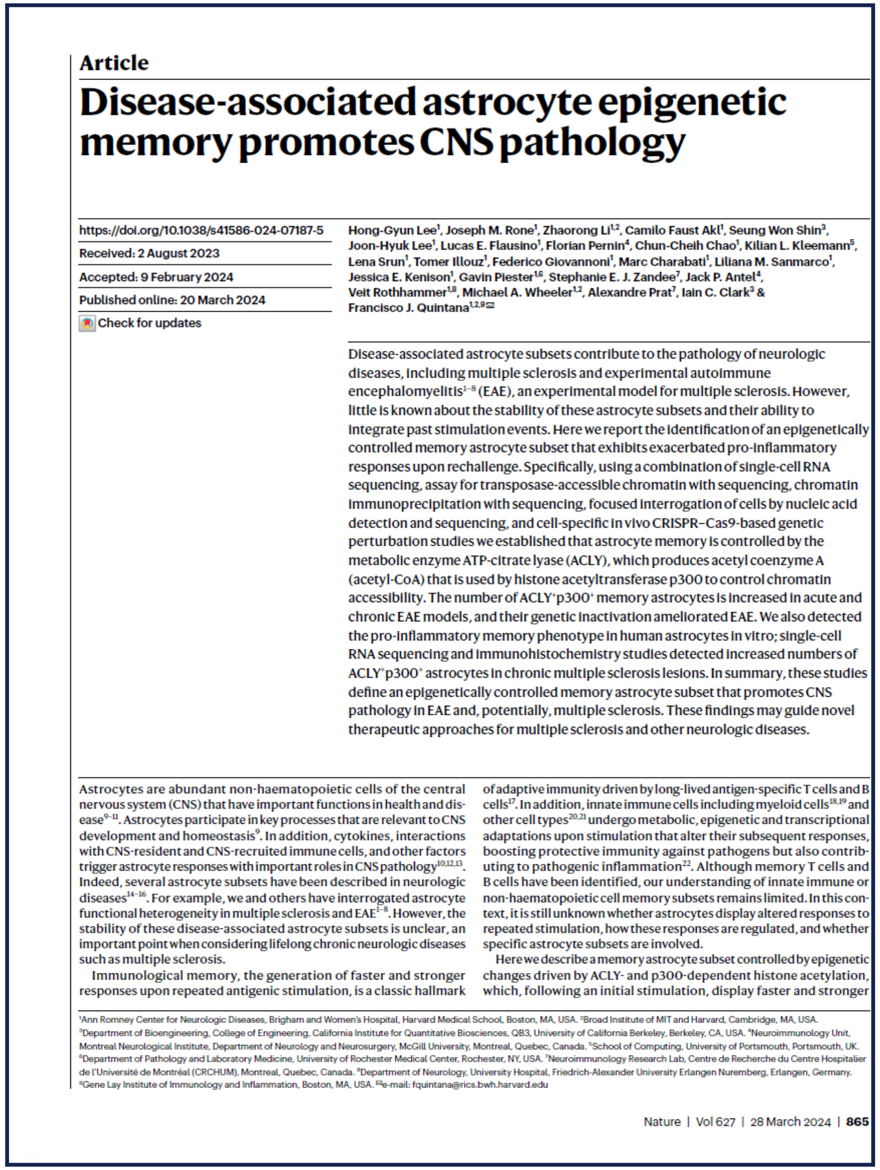




UCLA Health Jonsson Comprehensive Cancer center also use Qiagen IPA to interpreted spatial transcriptome

https://www.uclahealth.org/cancer/researchers/shared-resources/genomics

Use case



Article

Disease-associated astrocyte epigenetic memory promotes CNS pathology

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Hong-Gyun Lee[1], Joseph M. Rone[1], Zhaorong Li[1,2], Camilo Faust Akl[1], Seung Won Shin[3], Joon-Hyuk Lee[1], Lucas E. Flausino[1], Florian Pernin[4], Chun-Cheih Chao[1], Kilian L. Kleemann[5], Lena Srun[1], Tomer Illouz[1], Federico Giovannoni[1], Marc Charabati[1], Liliana M. Sanmarco[1], Jessica E. Kenison[1], Gavin Piester[1,6], Stephanie E. J. Zandee[7], Jack P. Antel[4], Veit Rothhammer[1,8], Michael A. Wheeler[1,2], Alexandre Prat[7], Iain C. Clark[3] & Francisco J. Quintana[1,2,9] ✉

Disease-associated astrocyte subsets contribute to the pathology of neurologic diseases, including multiple sclerosis and experimental autoimmune encephalomyelitis[1–8] (EAE), an experimental model for multiple sclerosis. However, little is known about the stability of these astrocyte subsets and their ability to integrate past stimulation events. Here we report the identification of an epigenetically controlled memory astrocyte subset that exhibits exacerbated pro-inflammatory responses upon rechallenge. Specifically, using a combination of single-cell RNA sequencing, assay for transposase-accessible chromatin with sequencing, chromatin immunoprecipitation with sequencing, focused interrogation of cells by nucleic acid detection and sequencing, and cell-specific in vivo CRISPR–Cas9-based genetic perturbation studies we established that astrocyte memory is controlled by the metabolic enzyme ATP-citrate lyase (ACLY), which produces acetyl coenzyme A (acetyl-CoA) that is used by histone acetyltransferase p300 to control chromatin accessibility. The number of ACLY$^+$p300$^+$ memory astrocytes is increased in acute and chronic EAE models, and their genetic inactivation ameliorated EAE. We also detected the pro-inflammatory memory phenotype in human astrocytes in vitro; single-cell RNA sequencing and immunohistochemistry studies detected increased numbers of ACLY$^+$p300$^+$ astrocytes in chronic multiple sclerosis lesions. In summary, these studies define an epigenetically controlled memory astrocyte subset that promotes CNS pathology in EAE and, potentially, multiple sclerosis. These findings may guide novel therapeutic approaches for multiple sclerosis and other neurologic diseases.

Astrocytes are abundant non-haematopoietic cells of the central nervous system (CNS) that have important functions in health and disease[9–11]. Astrocytes participate in key processes that are relevant to CNS development and homeostasis[9]. In addition, cytokines, interactions with CNS-resident and CNS-recruited immune cells, and other factors trigger astrocyte responses with important roles in CNS pathology[10,12,13]. Indeed, several astrocyte subsets have been described in neurologic diseases[14–16]. For example, we and others have interrogated astrocyte functional heterogeneity in multiple sclerosis and EAE[1–8]. However, the stability of these disease-associated astrocyte subsets is unclear, an important point when considering lifelong chronic neurologic diseases such as multiple sclerosis.

Immunological memory, the generation of faster and stronger responses upon repeated antigenic stimulation, is a classic hallmark of adaptive immunity driven by long-lived antigen-specific T cells and B cells[17]. In addition, innate immune cells including myeloid cells[18,19] and other cell types[20,21] undergo metabolic, epigenetic and transcriptional adaptations upon stimulation that alter their subsequent responses, boosting protective immunity against pathogens but also contributing to pathogenic inflammation[22]. Although memory T cells and B cells have been identified, our understanding of innate immune or non-haematopoietic cell memory subsets remains limited. In this context, it is still unknown whether astrocytes display altered responses to repeated stimulation, how these responses are regulated, and whether specific astrocyte subsets are involved.

Here we describe a memory astrocyte subset controlled by epigenetic changes driven by ACLY- and p300-dependent histone acetylation, which, following an initial stimulation, display faster and stronger

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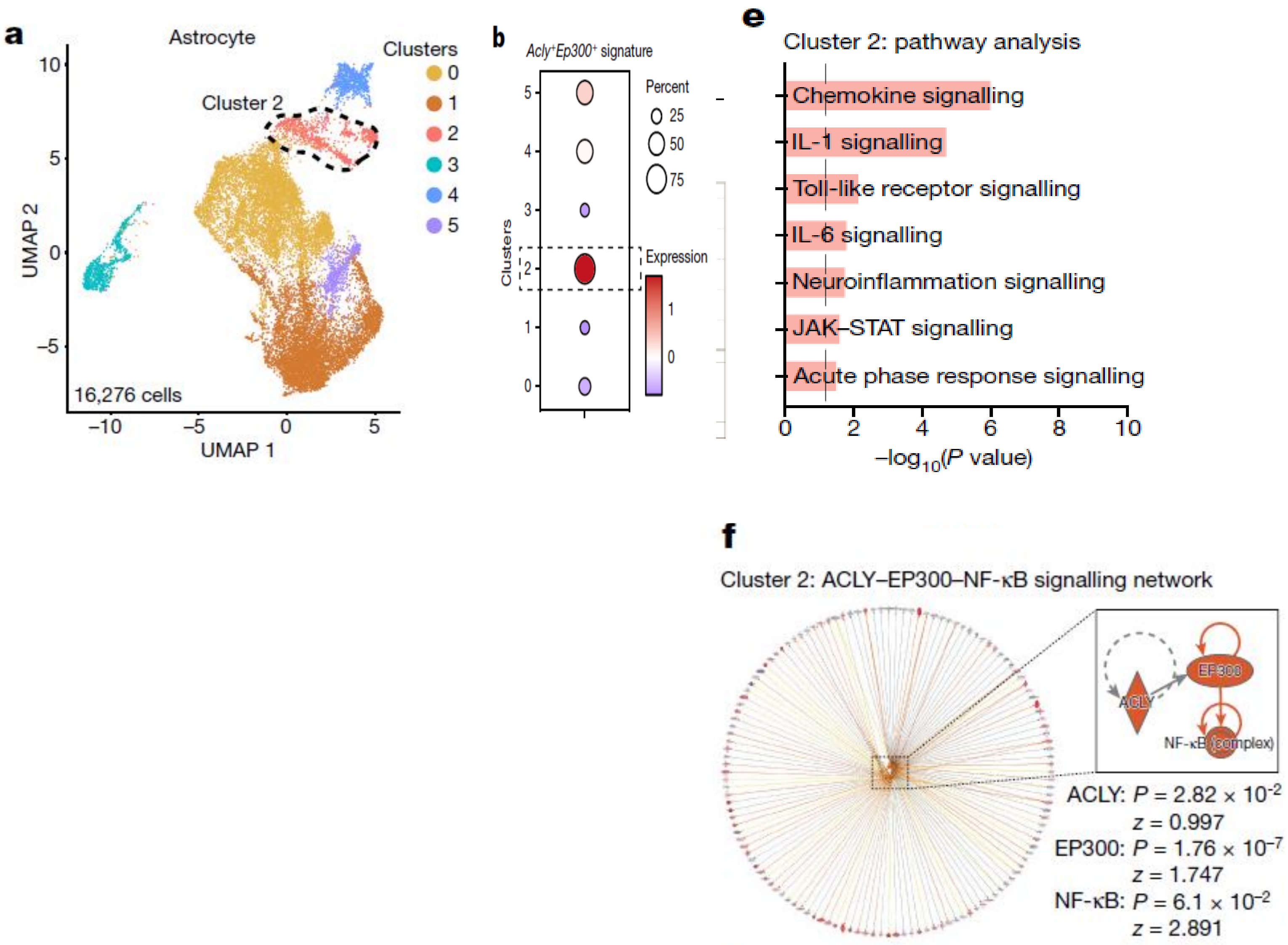
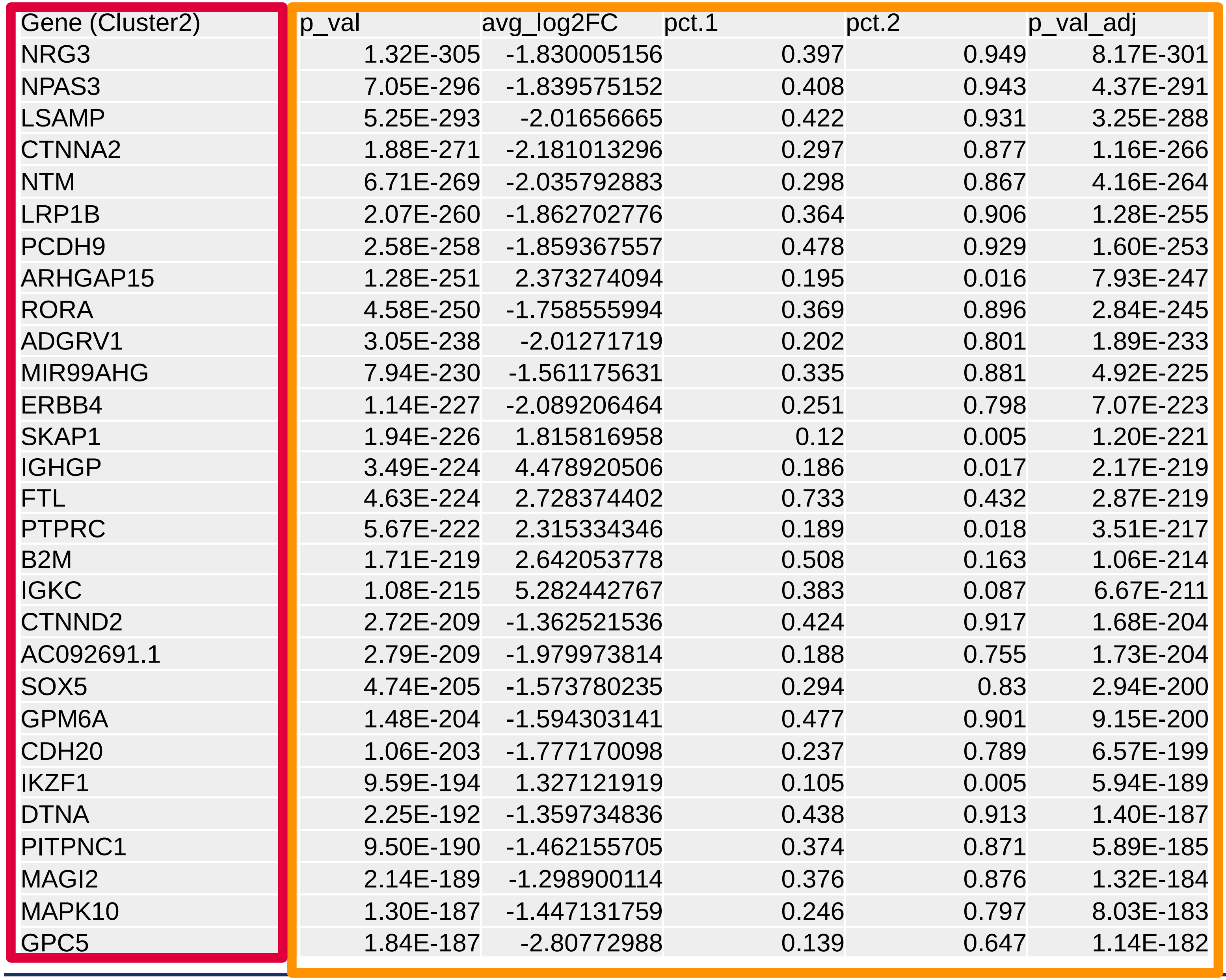


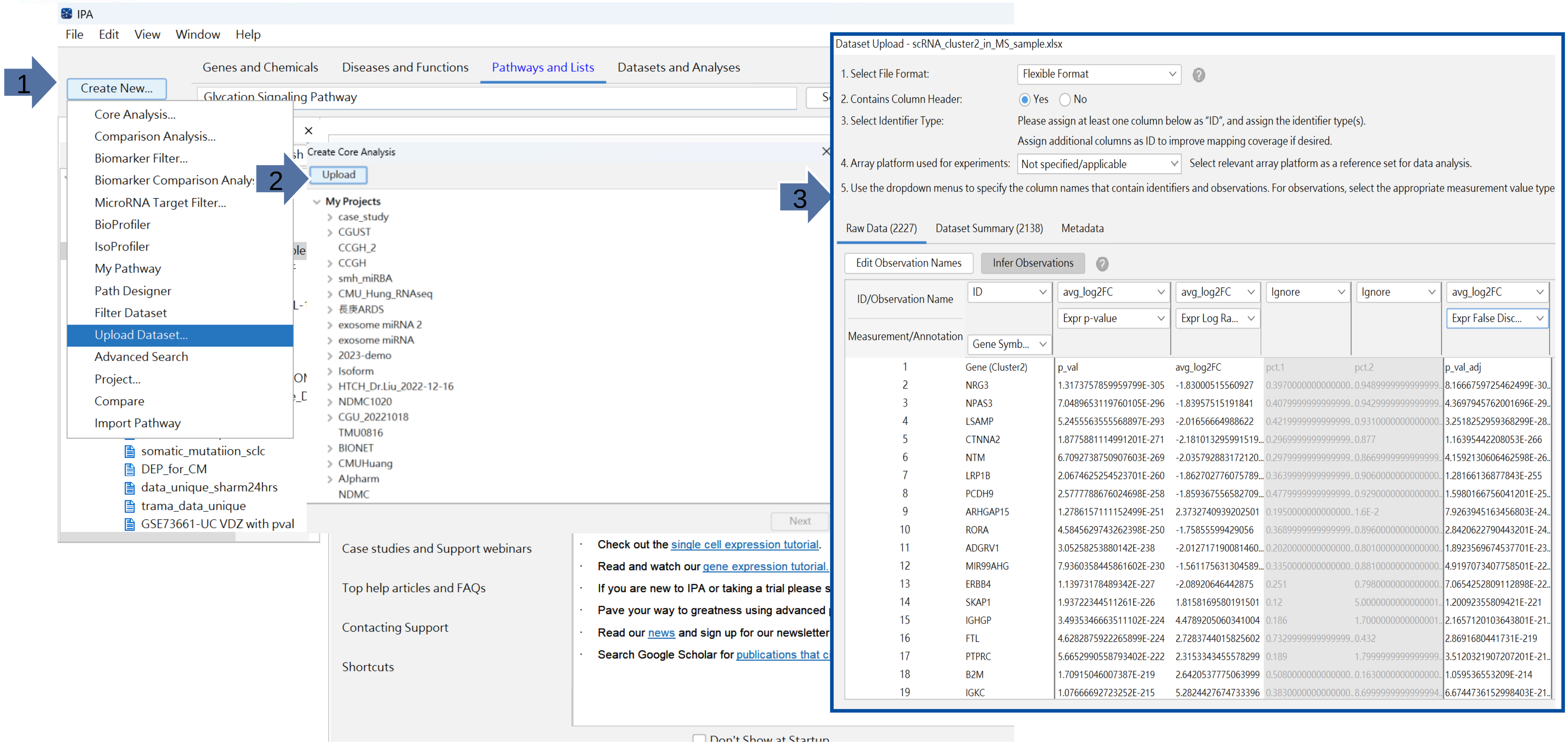
ACLY: $P = 2.82 \times 10^{-2}$
$z = 0.997$
EP300: $P = 1.76 \times 10^{-7}$
$z = 1.747$
NF-κB: $P = 6.1 \times 10^{-2}$
$z = 2.891$

Upload dataset



Gene (Cluster2)	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
NRG3	1.32E-305	-1.830005156	0.397	0.949	8.17E-301
NPAS3	7.05E-296	-1.839575152	0.408	0.943	4.37E-291
LSAMP	5.25E-293	-2.01656665	0.422	0.931	3.25E-288
CTNNA2	1.88E-271	-2.181013296	0.297	0.877	1.16E-266
NTM	6.71E-269	-2.035792883	0.298	0.867	4.16E-264
LRP1B	2.07E-260	-1.862702776	0.364	0.906	1.28E-255
PCDH9	2.58E-258	-1.859367557	0.478	0.929	1.60E-253
ARHGAP15	1.28E-251	2.373274094	0.195	0.016	7.93E-247
RORA	4.58E-250	-1.758555994	0.369	0.896	2.84E-245
ADGRV1	3.05E-238	-2.01271719	0.202	0.801	1.89E-233
MIR99AHG	7.94E-230	-1.561175631	0.335	0.881	4.92E-225
ERBB4	1.14E-227	-2.089206464	0.251	0.798	7.07E-223
SKAP1	1.94E-226	1.815816958	0.12	0.005	1.20E-221
IGHGP	3.49E-224	4.478920506	0.186	0.017	2.17E-219
FTL	4.63E-224	2.728374402	0.733	0.432	2.87E-219
PTPRC	5.67E-222	2.315334346	0.189	0.018	3.51E-217
B2M	1.71E-219	2.642053778	0.508	0.163	1.06E-214
IGKC	1.08E-215	5.282442767	0.383	0.087	6.67E-211
CTNND2	2.72E-209	-1.362521536	0.424	0.917	1.68E-204
AC092691.1	2.79E-209	-1.979973814	0.188	0.755	1.73E-204
SOX5	4.74E-205	-1.573780235	0.294	0.83	2.94E-200
GPM6A	1.48E-204	-1.594303141	0.477	0.901	9.15E-200
CDH20	1.06E-203	-1.777170098	0.237	0.789	6.57E-199
IKZF1	9.59E-194	1.327121919	0.105	0.005	5.94E-189
DTNA	2.25E-192	-1.359734836	0.438	0.913	1.40E-187
PITPNC1	9.50E-190	-1.462155705	0.374	0.871	5.89E-185
MAGI2	2.14E-189	-1.298900114	0.376	0.876	1.32E-184
MAPK10	1.30E-187	-1.447131759	0.246	0.797	8.03E-183
GPC5	1.84E-187	-2.80772988	0.139	0.647	1.14E-182

Upload dataset



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- Read and watch our gene expression tutorial.
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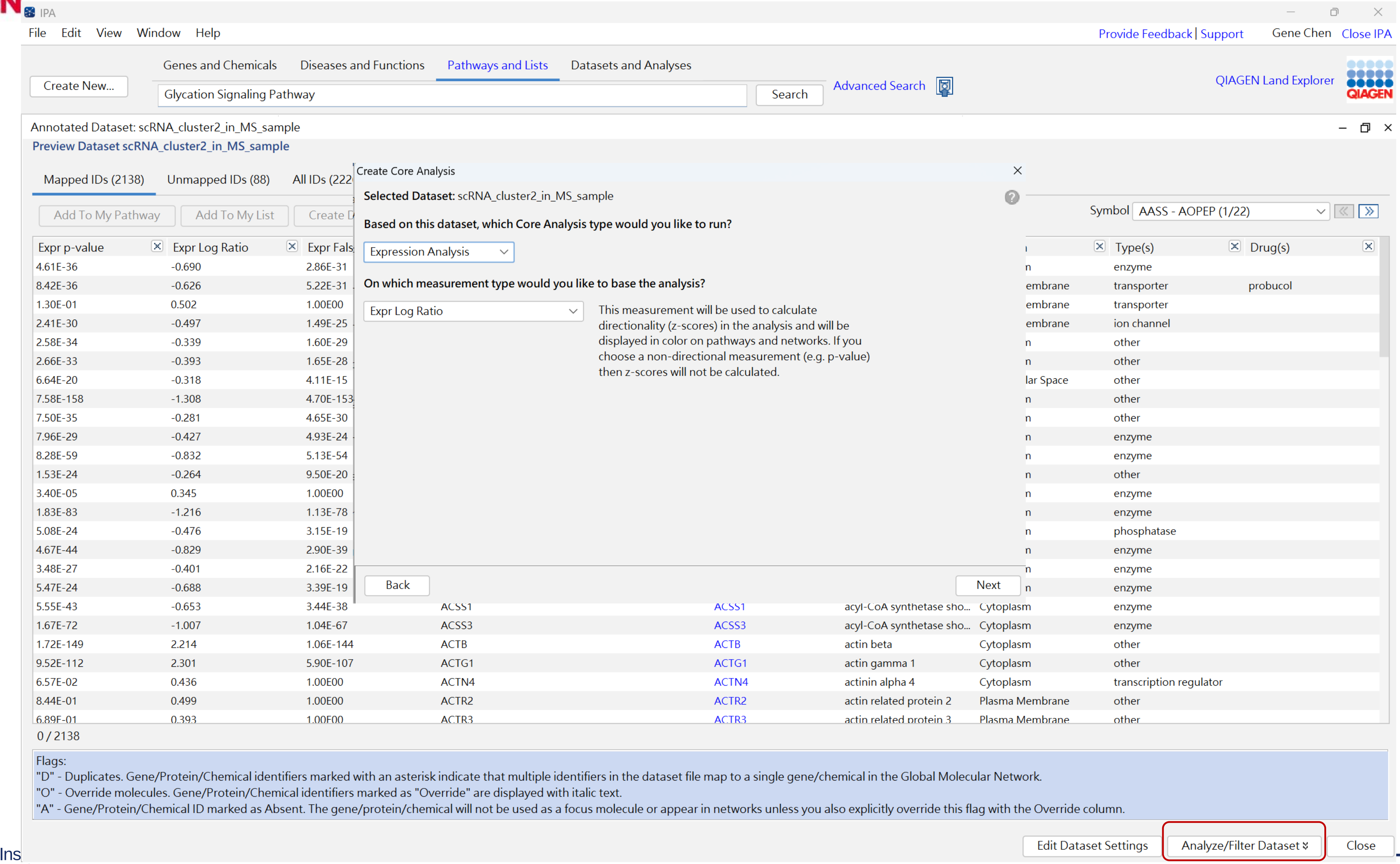
- Read our news and sign up for our newsletter
- Search Google Scholar for publications that c

Dataset Upload - scRNA_cluster2_in_MS_sample.xlsx

1. Select File Format: Flexible Format
2. Contains Column Header: ● Yes ○ No
3. Select Identifier Type: Please assign at least one column below as "ID", and assign the identifier type(s). Assign additional columns as ID to improve mapping coverage if desired.
4. Array platform used for experiments: Not specified/applicable — Select relevant array platform as a reference set for data analysis.
5. Use the dropdown menus to specify the column names that contain identifiers and observations. For observations, select the appropriate measurement value type

ID/Observation Name	ID	avg_log2FC	avg_log2FC	Ignore	Ignore	avg_log2FC
Measurement/Annotation	Gene Symb...	Expr p-value	Expr Log Ra...			Expr False Disc...
1	Gene (Cluster2)	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
2	NRG3	1.3173757859959799E-305	-1.83000515560927	0.3970000000000000..	0.9489999999999999..	8.1666759725462499E-30..
3	NPAS3	7.0489653119760105E-296	-1.83957515191841	0.4079999999999999..	0.9429999999999999..	4.3697945762001696E-29..
4	LSAMP	5.2455563555568897E-293	-2.01656664988622	0.4219999999999999..	0.9310000000000000..	3.2518252959368299E-28..
5	CTNNA2	1.8775881114991201E-271	-2.181013295991519...	0.2969999999999999..	0.877	1.16395442208053E-266
6	NTM	6.7092738750907603E-269	-2.035792883172120...	0.2979999999999999..	0.8669999999999999..	4.1592130606462598E-26..
7	LRP1B	2.0674625254523701E-260	-1.862702776075789...	0.3639999999999999..	0.9060000000000000..	1.28166136877843E-255
8	PCDH9	2.5777788676024698E-258	-1.859367556582709...	0.4779999999999999..	0.9290000000000000..	1.5980166756041201E-25..
9	ARHGAP15	1.2786157111152499E-251	2.3732740939202501	0.1950000000000000..	1.6E-2	7.9263945163456803E-24..
10	RORA	4.5845629743262398E-250	-1.75855599429056	0.3689999999999999..	0.8960000000000000..	2.8420622790443201E-24..
11	ADGRV1	3.05258253880142E-238	-2.012717190081460...	0.2020000000000000..	0.8010000000000000..	1.8923569674537701E-23..
12	MIR99AHG	7.9360358445861602E-230	-1.561175631304589...	0.3350000000000000..	0.8810000000000000..	4.9197073407758501E-22..
13	ERBB4	1.13973178489342E-227	-2.08920646442875	0.251	0.7980000000000000..	7.0654252809112898E-22..
14	SKAP1	1.93722344511261E-226	1.8158169580191501	0.12	5.0000000000000001..	1.20092355809421E-221
15	IGHGP	3.4935346663511102E-224	4.4789205060341004	0.186	1.7000000000000001..	2.1657120103643801E-21..
16	FTL	4.6282875922265899E-224	2.7283744015825602	0.7329999999999999..	0.432	2.8691680441731E-219
17	PTPRC	5.6652990558793402E-222	2.3153343455578299	0.189	1.7999999999999999..	3.5120321907207201E-21..
18	B2M	1.70915046007387E-219	2.6420537775063999	0.5080000000000000..	0.1630000000000000..	1.059536553209E-214
19	IGKC	1.07666692723252E-215	5.2824427674733396	0.3830000000000000..	8.6999999999999994..	6.6744736152998403E-21..

Core Analysis



IPA

Glycation Signaling Pathway

Annotated Dataset: scRNA_cluster2_in_MS_sample

Preview Dataset scRNA_cluster2_in_MS_sample

Symbol AASS - AOPEP (1/22)

Expr p-value	Expr Log Ratio	Expr Fals					Type(s)	Drug(s)
4.61E-36	-0.690	2.86E-31				n	enzyme	
8.42E-36	-0.626	5.22E-31				embrane	transporter	probucol
1.30E-01	0.502	1.00E00				embrane	transporter	
2.41E-30	-0.497	1.49E-25				embrane	ion channel	
2.58E-34	-0.339	1.60E-29				n	other	
2.66E-33	-0.393	1.65E-28				n	other	
6.64E-20	-0.318	4.11E-15				lar Space	other	
7.58E-158	-1.308	4.70E-153				n	other	
7.50E-35	-0.281	4.65E-30				n	other	
7.96E-29	-0.427	4.93E-24				n	enzyme	
8.28E-59	-0.832	5.13E-54				n	enzyme	
1.53E-24	-0.264	9.50E-20				n	other	
3.40E-05	0.345	1.00E00				n	enzyme	
1.83E-83	-1.216	1.13E-78				n	enzyme	
5.08E-24	-0.476	3.15E-19				n	phosphatase	
4.67E-44	-0.829	2.90E-39				n	enzyme	
3.48E-27	-0.401	2.16E-22				n	enzyme	
5.47E-24	-0.688	3.39E-19				n	enzyme	
5.55E-43	-0.653	3.44E-38	ACSS1	ACSS1	acyl-CoA synthetase sho...	Cytoplasm	enzyme	
1.67E-72	-1.007	1.04E-67	ACSS3	ACSS3	acyl-CoA synthetase sho...	Cytoplasm	enzyme	
1.72E-149	2.214	1.06E-144	ACTB	ACTB	actin beta	Cytoplasm	other	
9.52E-112	2.301	5.90E-107	ACTG1	ACTG1	actin gamma 1	Cytoplasm	other	
6.57E-02	0.436	1.00E00	ACTN4	ACTN4	actinin alpha 4	Cytoplasm	transcription regulator	
8.44E-01	0.499	1.00E00	ACTR2	ACTR2	actin related protein 2	Plasma Membrane	other	
6.89E-01	0.393	1.00E00	ACTR3	ACTR3	actin related protein 3	Plasma Membrane	other	

0 / 2138

Create Core Analysis

Selected Dataset: scRNA_cluster2_in_MS_sample

Based on this dataset, which Core Analysis type would you like to run?

Expression Analysis

On which measurement type would you like to base the analysis?

Expr Log Ratio

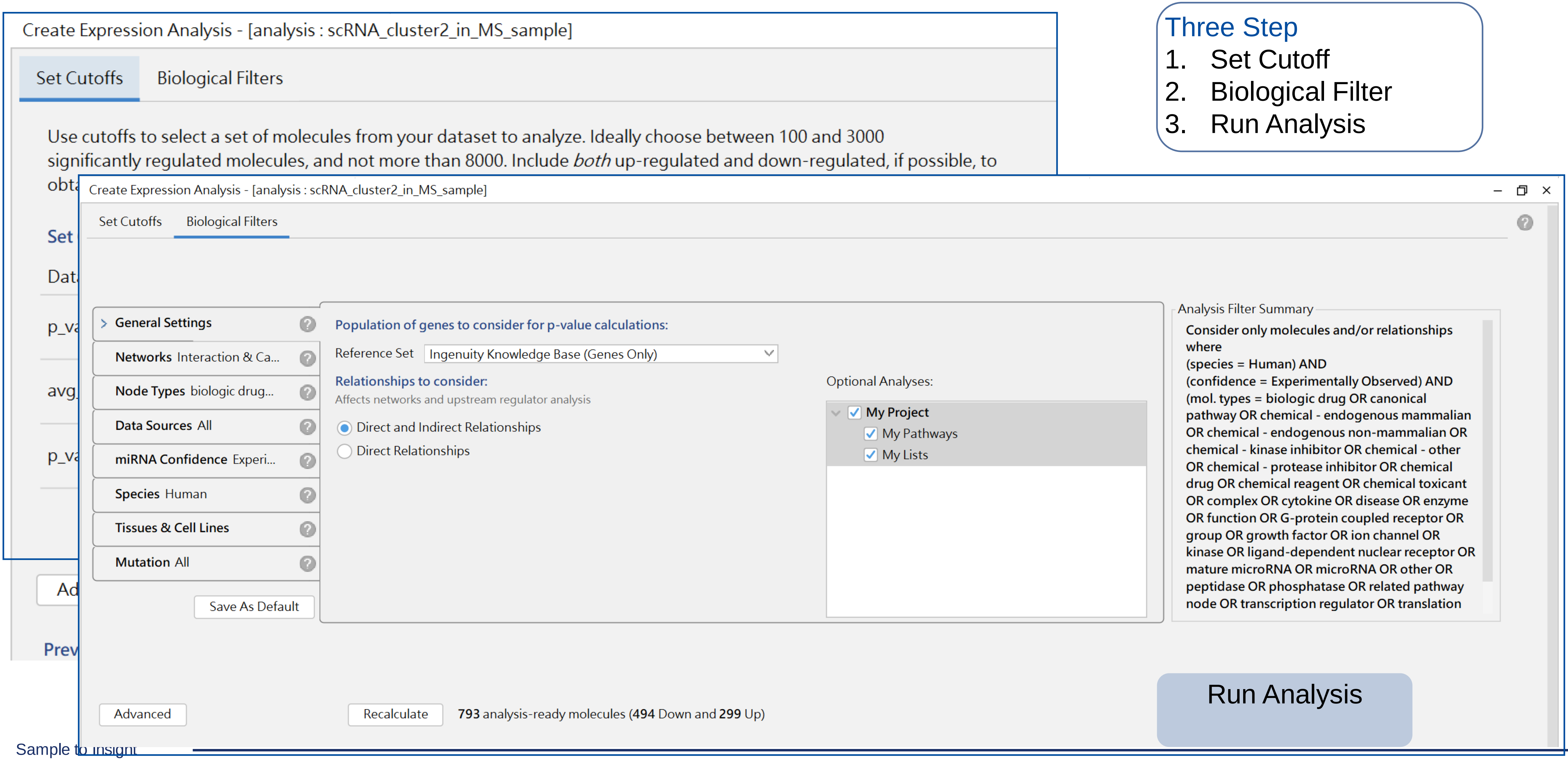
This measurement will be used to calculate directionality (z-scores) in the analysis and will be displayed in color on pathways and networks. If you choose a non-directional measurement (e.g. p-value) then z-scores will not be calculated.

Flags:
"D" - Duplicates. Gene/Protein/Chemical identifiers marked with an asterisk indicate that multiple identifiers in the dataset file map to a single gene/chemical in the Global Molecular Network.
"O" - Override molecules. Gene/Protein/Chemical identifiers marked as "Override" are displayed with italic text.
"A" - Gene/Protein/Chemical ID marked as Absent. The gene/protein/chemical will not be used as a focus molecule or appear in networks unless you also explicitly override this flag with the Override column.

Core Analysis –Set Up

Three Step

1. Set Cutoff
2. Biological Filter
3. Run Analysis



Create Expression Analysis - [analysis : scRNA_cluster2_in_MS_sample]

Set Cutoffs | Biological Filters

Use cutoffs to select a set of molecules from your dataset to analyze. Ideally choose between 100 and 3000 significantly regulated molecules, and not more than 8000. Include *both* up-regulated and down-regulated, if possible, to obta

Create Expression Analysis - [analysis : scRNA_cluster2_in_MS_sample]

Set Cutoffs | Biological Filters

- General Settings
- Networks Interaction & Ca...
- Node Types biologic drug...
- Data Sources All
- miRNA Confidence Experi...
- Species Human
- Tissues & Cell Lines
- Mutation All

Population of genes to consider for p-value calculations:

Reference Set: Ingenuity Knowledge Base (Genes Only)

Relationships to consider:

Affects networks and upstream regulator analysis

● Direct and Indirect Relationships

○ Direct Relationships

Optional Analyses:

- ☑ My Project
  - ☑ My Pathways
  - ☑ My Lists

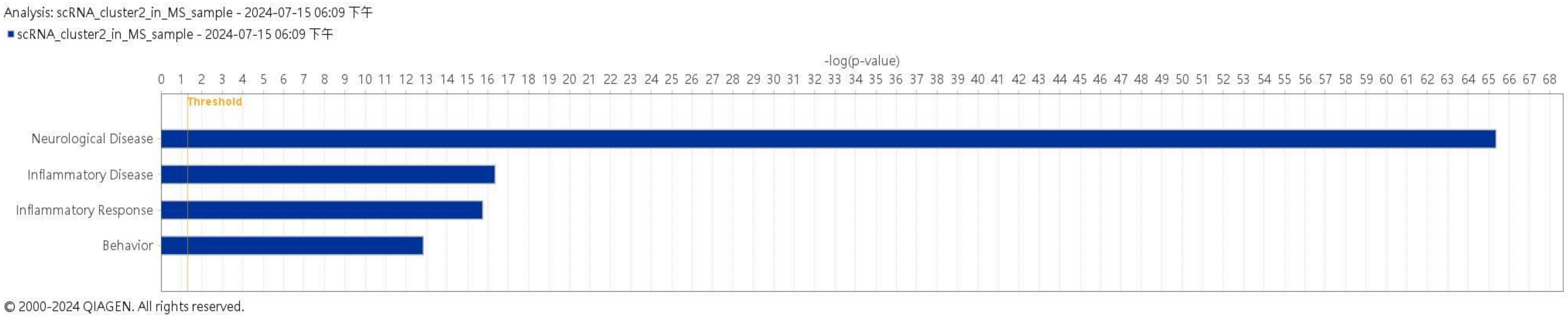
Analysis Filter Summary

Consider only molecules and/or relationships where
(species = Human) AND
(confidence = Experimentally Observed) AND
(mol. types = biologic drug OR canonical pathway OR chemical - endogenous mammalian OR chemical - endogenous non-mammalian OR chemical - kinase inhibitor OR chemical - other OR chemical - protease inhibitor OR chemical drug OR chemical reagent OR chemical toxicant OR complex OR cytokine OR disease OR enzyme OR function OR G-protein coupled receptor OR group OR growth factor OR ion channel OR kinase OR ligand-dependent nuclear receptor OR mature microRNA OR microRNA OR other OR peptidase OR phosphatase OR related pathway node OR transcription regulator OR translation

793 analysis-ready molecules (**494** Down and **299** Up)

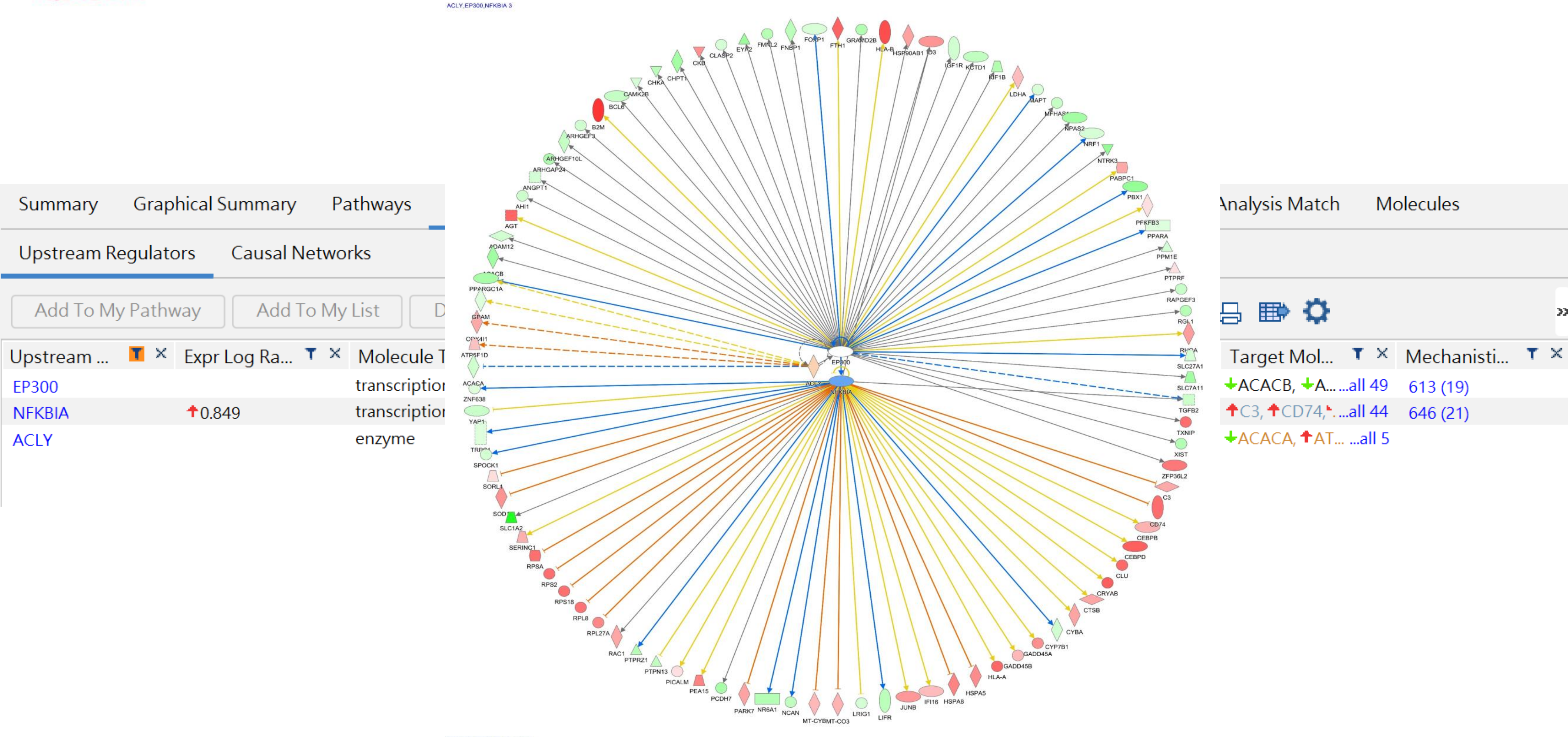
Core analysis –Canonical pathway

Core analysis – Disease and function



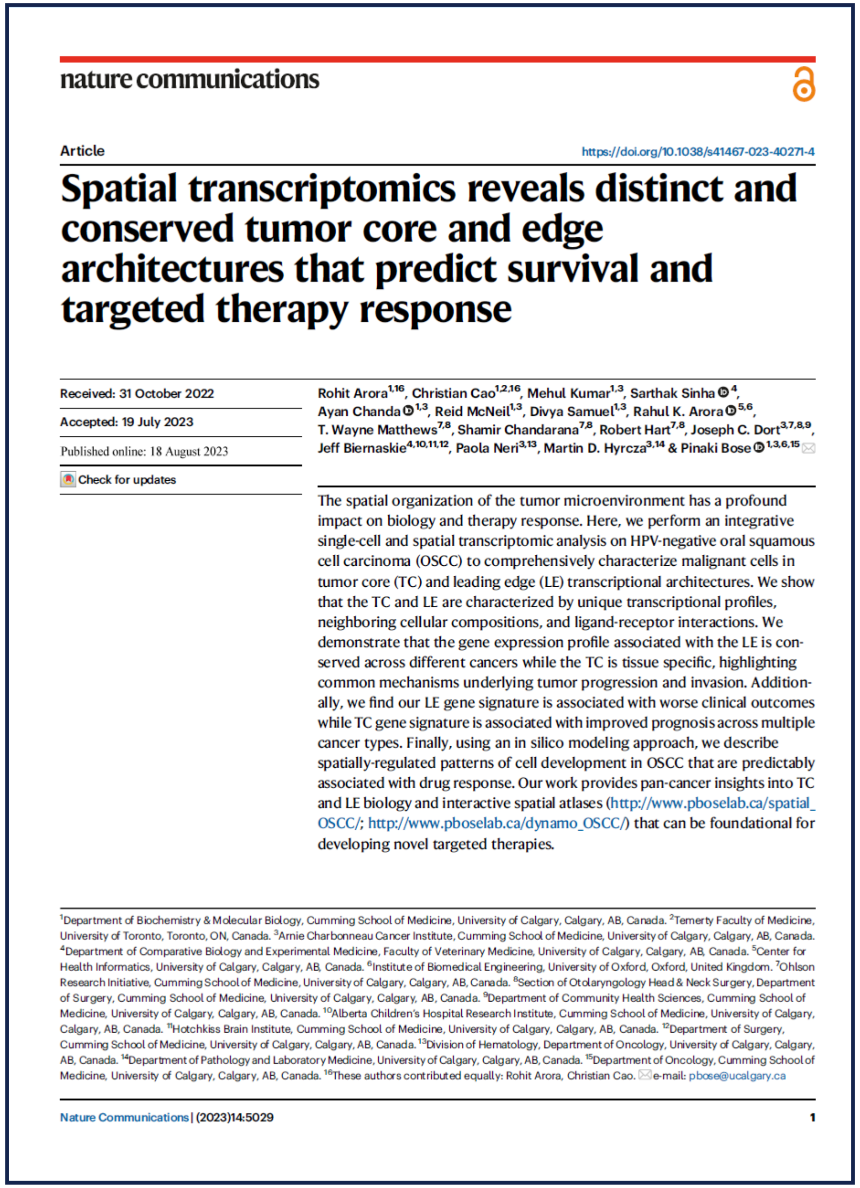
Analysis: scRNA_cluster2_in_MS_sample - 2024-07-15 06:09 下午

Upstream results



Upstream ...	Expr Log Ra...	Molecule T	Target Mol...	Mechanisti...
EP300		transcriptio	↓ACACB, ↓A... ...all 49	613 (19)
NFKBIA	↑0.849	transcriptio	↑C3, ↑CD74, ...all 44	646 (21)
ACLY		enzyme	↓ACACA, ↑AT... ...all 5	

Use Case



nature communications

Article

https://doi.org/10.1038/s41467-023-40271-4

Spatial transcriptomics reveals distinct and conserved tumor core and edge architectures that predict survival and targeted therapy response

Received: 31 October 2022

Accepted: 19 July 2023

Rohit Arora[1,16], Christian Cao[1,2,16], Mehul Kumar[1,3], Sarthak Sinha[4], Ayan Chanda[1,3], Reid McNeil[1,3], Divya Samuel[1,3], Rahul K. Arora[5,6], T. Wayne Matthews[7,8], Shamir Chandarana[7,8], Robert Hart[7,8], Joseph C. Dort[3,7,8,9], Jeff Biernaskie[4,10,11,12], Paola Neri[3,13], Martin D. Hyrcza[3,14] & Pinaki Bose[1,3,6,15] ✉

The spatial organization of the tumor microenvironment has a profound impact on biology and therapy response. Here, we perform an integrative single-cell and spatial transcriptomic analysis on HPV-negative oral squamous cell carcinoma (OSCC) to comprehensively characterize malignant cells in tumor core (TC) and leading edge (LE) transcriptional architectures. We show that the TC and LE are characterized by unique transcriptional profiles, neighboring cellular compositions, and ligand-receptor interactions. We demonstrate that the gene expression profile associated with the LE is conserved across different cancers while the TC is tissue specific, highlighting common mechanisms underlying tumor progression and invasion. Additionally, we find our LE gene signature is associated with worse clinical outcomes while TC gene signature is associated with improved prognosis across multiple cancer types. Finally, using an in silico modeling approach, we describe spatially-regulated patterns of cell development in OSCC that are predictably associated with drug response. Our work provides pan-cancer insights into TC and LE biology and interactive spatial atlases (http://www.pboselab.ca/spatial_OSCC/; http://www.pboselab.ca/dynamo_OSCC/) that can be foundational for developing novel targeted therapies.

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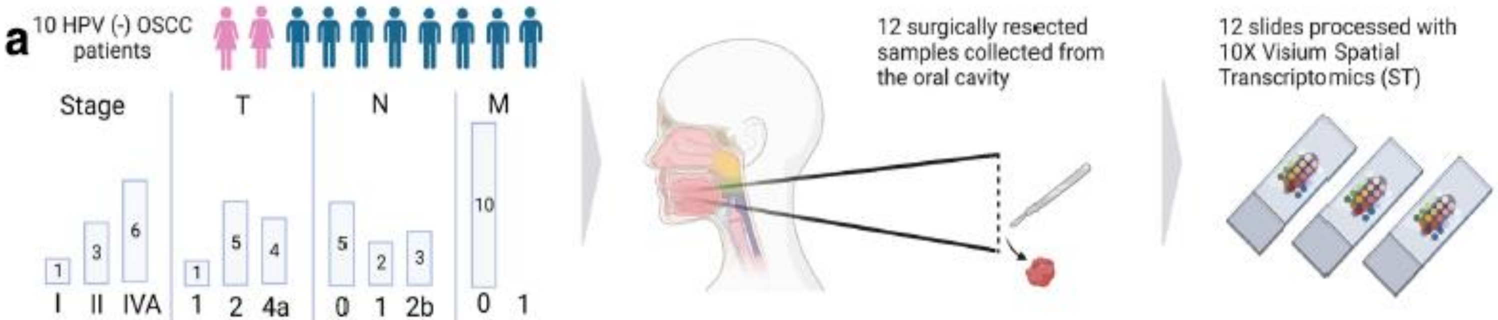
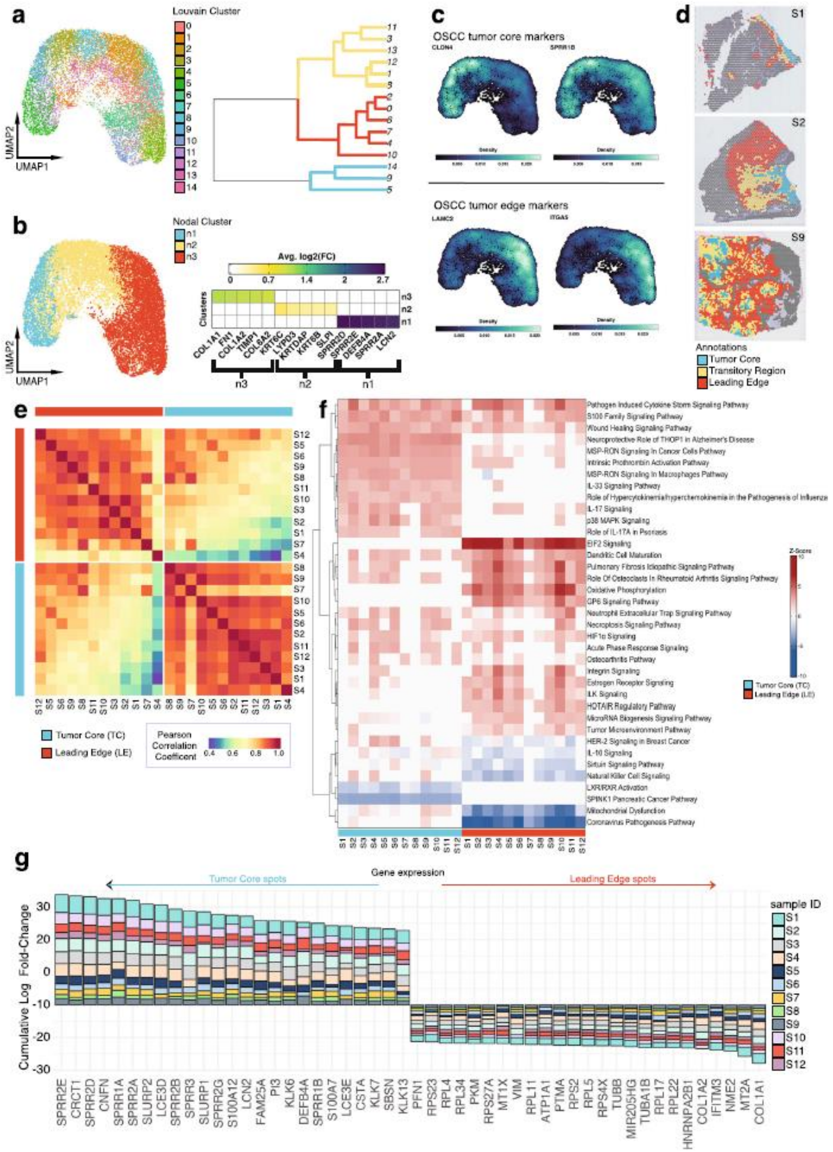
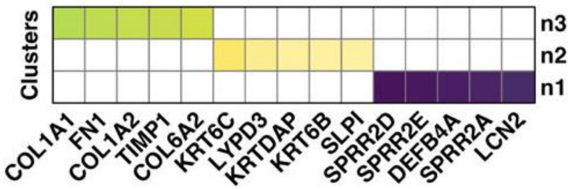
Nature Communications | (2023)14:5029 1



Data Format

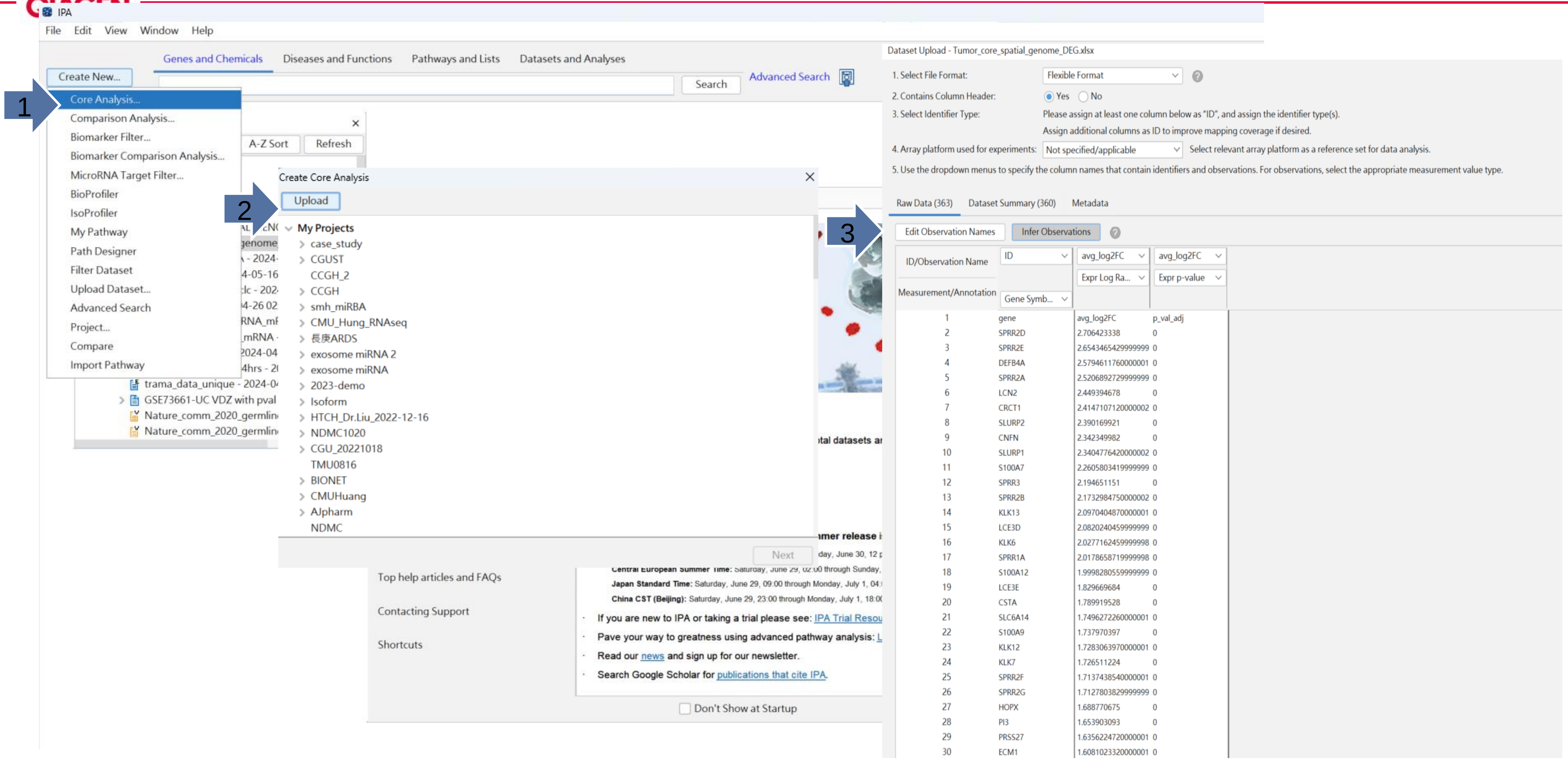




gene	avg_log2FC	p_val_adj
SPRR2D	2.706423338	0
SPRR2E	2.654346543	0
DEFB4A	2.579461176	0
SPRR2A	2.520689273	0
LCN2	2.449394678	0
CRCT1	2.414710712	0
SLURP2	2.390169921	0
CNFN	2.342349982	0
SLURP1	2.340477642	0
S100A7	2.260580342	0
SPRR3	2.194651151	0
SPRR2B	2.173298475	0
KLK13	2.097040487	0
LCE3D	2.082024046	0
KLK6	2.027716246	0
SPRR1A	2.017865872	0

gene	avg_log2FC	p_val_adj
COL1A1	1.119188557	0
FN1	1.077456695	0
COL1A2	1.047688083	0
TIMP1	1.040462139	0
COL6A2	0.98234798	0
COL3A1	0.916656628	0
SPARC	0.902377431	0
VIM	0.87526439	0
HMGN2	0.791542974	0
TNC	0.778830913	0
IGFBP7	0.716862936	0
FTL	0.653061976	0
MT2A	0.612219081	0
PFN1	0.546409497	0
FBLN2	0.472353201	0
LGALS1	0.668868409	9.25E-299

Upload dataset



Central European Summer Time: Saturday, June 29, 02:00 through Sunday,

Japan Standard Time: Saturday, June 29, 09:00 through Monday, July 1, 04:

China CST (Beijing): Saturday, June 29, 23:00 through Monday, July 1, 18:0

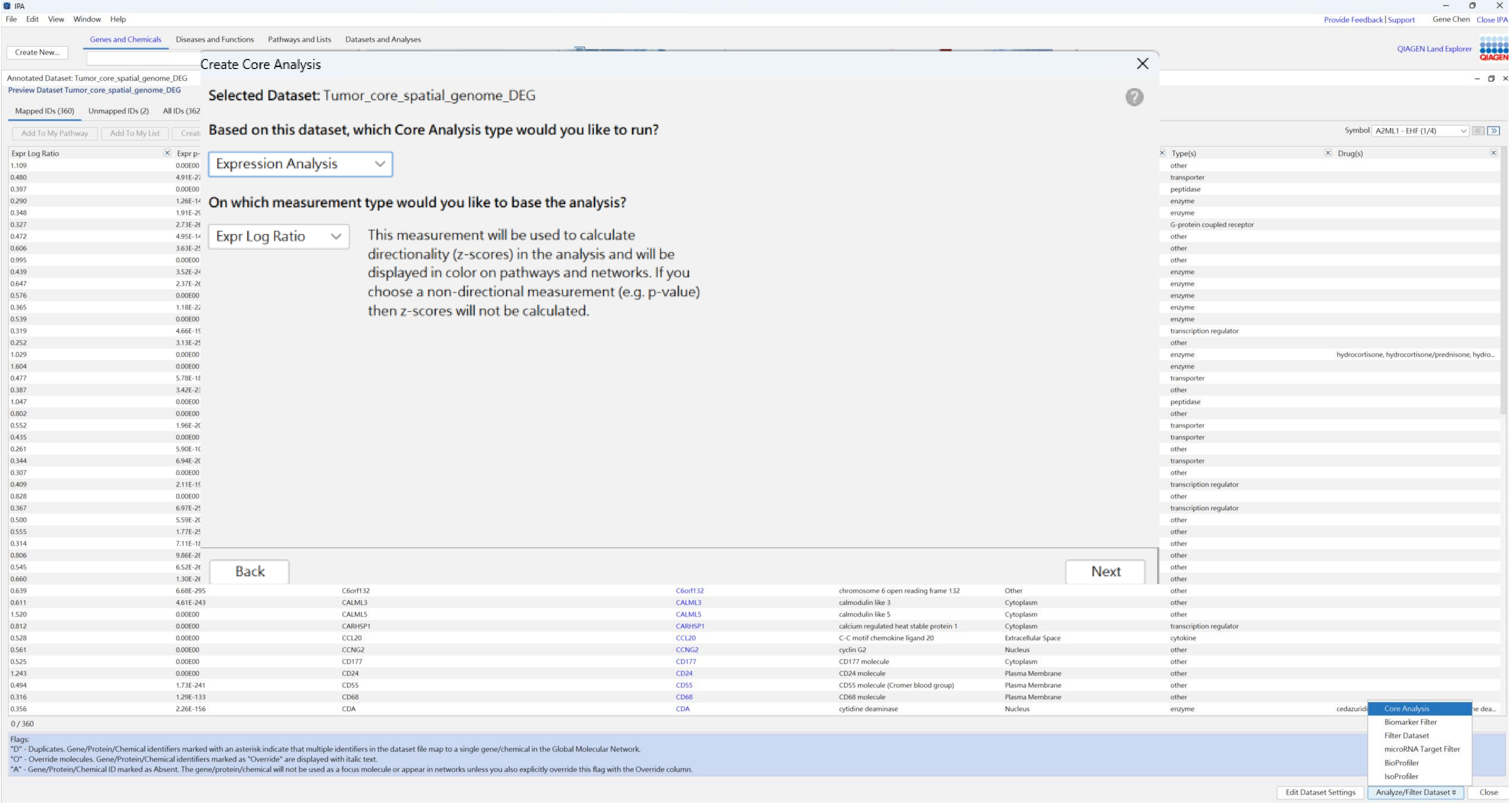
- If you are new to IPA or taking a trial please see: IPA Trial Resou
- Pave your way to greatness using advanced pathway analysis: L
- Read our news and sign up for our newsletter.
- Search Google Scholar for publications that cite IPA.

Dataset Upload - Tumor_core_spatial_genome_DEG.xlsx

1. Select File Format: Flexible Format
2. Contains Column Header: ● Yes ○ No
3. Select Identifier Type: Please assign at least one column below as "ID", and assign the identifier type(s). Assign additional columns as ID to improve mapping coverage if desired.
4. Array platform used for experiments: Not specified/applicable — Select relevant array platform as a reference set for data analysis.
5. Use the dropdown menus to specify the column names that contain identifiers and observations. For observations, select the appropriate measurement value type.

ID/Observation Name	ID	avg_log2FC	avg_log2FC
Measurement/Annotation	Gene Symb...	Expr Log Ra...	Expr p-value
1	gene	avg_log2FC	p_val_adj
2	SPRR2D	2.706423338	0
3	SPRR2E	2.6543465429999999	0
4	DEFB4A	2.5794611760000001	0
5	SPRR2A	2.5206892729999999	0
6	LCN2	2.449394678	0
7	CRCT1	2.4147107120000002	0
8	SLURP2	2.390169921	0
9	CNFN	2.342349982	0
10	SLURP1	2.3404776420000002	0
11	S100A7	2.2605803419999999	0
12	SPRR3	2.194651151	0
13	SPRR2B	2.1732984750000002	0
14	KLK13	2.0970404870000001	0
15	LCE3D	2.0820240459999999	0
16	KLK6	2.0277162459999998	0
17	SPRR1A	2.0178658719999998	0
18	S100A12	1.9998280559999999	0
19	LCE3E	1.829669684	0
20	CSTA	1.789919528	0
21	SLC6A14	1.7496272260000001	0
22	S100A9	1.737970397	0
23	KLK12	1.7283063970000001	0
24	KLK7	1.726511224	0
25	SPRR2F	1.7137438540000001	0
26	SPRR2G	1.7127803829999999	0
27	HOPX	1.688770675	0
28	PI3	1.653903093	0
29	PRSS27	1.6356224720000001	0
30	ECM1	1.6081023320000001	0

Core Analysis



IPA

Annotated Dataset: Tumor_core_spatial_genome_DEG

Preview Dataset Tumor_core_spatial_genome_DEG

Create Core Analysis

Selected Dataset: Tumor_core_spatial_genome_DEG

Based on this dataset, which Core Analysis type would you like to run?

Expression Analysis

On which measurement type would you like to base the analysis?

Expr Log Ratio

This measurement will be used to calculate directionality (z-scores) in the analysis and will be displayed in color on pathways and networks. If you choose a non-directional measurement (e.g. p-value) then z-scores will not be calculated.

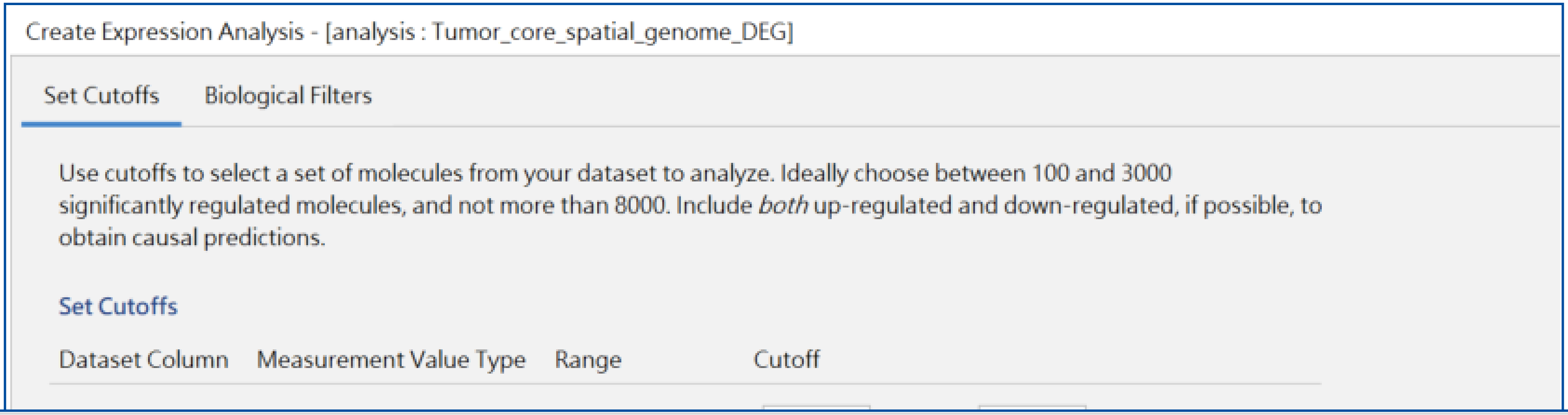
Symbol A2ML1 - EHF (1/4)

Expr Log Ratio	Expr p-					Type(s)	Drug(s)
1.109	0.00E00					other	
0.480	4.91E-2					transporter	
0.397	0.00E00					peptidase	
0.290	1.26E-1					enzyme	
0.348	1.91E-2					enzyme	
0.327	2.73E-2					G-protein coupled receptor	
0.472	4.95E-1					other	
0.606	3.63E-2					other	
0.995	0.00E00					other	
0.439	3.52E-2					enzyme	
0.647	2.37E-2					enzyme	
0.576	0.00E00					enzyme	
0.365	1.18E-2					enzyme	
0.539	0.00E00					enzyme	
0.319	4.66E-1					transcription regulator	
0.252	3.13E-2					other	
1.029	0.00E00					enzyme	hydrocortisone, hydrocortisone/prednisone, hydro...
1.604	0.00E00					enzyme	
0.477	5.78E-1					transporter	
0.387	3.42E-2					other	
1.047	0.00E00					peptidase	
0.802	0.00E00					other	
0.552	1.96E-2					transporter	
0.435	0.00E00					transporter	
0.261	5.90E-1					other	
0.344	6.94E-2					transporter	
0.307	0.00E00					other	
0.409	2.11E-1					transcription regulator	
0.828	0.00E00					other	
0.367	6.97E-2					transcription regulator	
0.500	5.59E-2					other	
0.555	1.77E-2					other	
0.314	7.11E-1					other	
0.806	9.86E-2					other	
0.545	6.52E-2					other	
0.660	1.30E-2					other	
0.639	6.68E-295	C6orf132	C6orf132	chromosome 6 open reading frame 132	Other	other	
0.611	4.61E-243	CALML3	CALML3	calmodulin like 3	Cytoplasm	other	
1.520	0.00E00	CALML5	CALML5	calmodulin like 5	Cytoplasm	other	
0.812	0.00E00	CARHSP1	CARHSP1	calcium regulated heat stable protein 1	Cytoplasm	transcription regulator	
0.528	0.00E00	CCL20	CCL20	C-C motif chemokine ligand 20	Extracellular Space	cytokine	
0.561	0.00E00	CCNG2	CCNG2	cyclin G2	Nucleus	other	
0.525	0.00E00	CD177	CD177	CD177 molecule	Cytoplasm	other	
1.243	0.00E00	CD24	CD24	CD24 molecule	Plasma Membrane	other	
0.494	1.73E-241	CD55	CD55	CD55 molecule (Cromer blood group)	Plasma Membrane	other	
0.316	1.29E-133	CD68	CD68	CD68 molecule	Plasma Membrane	other	
0.356	2.26E-156	CDA	CDA	cytidine deaminase	Nucleus	enzyme	cedazuridi

0 / 360

Flags:
"D" - Duplicates. Gene/Protein/Chemical identifiers marked with an asterisk indicate that multiple identifiers in the dataset file map to a single gene/chemical in the Global Molecular Network.
"O" - Override molecules. Gene/Protein/Chemical identifiers marked as "Override" are displayed with italic text.
"A" - Gene/Protein/Chemical ID marked as Absent. The gene/protein/chemical will not be used as a focus molecule or appear in networks unless you also explicitly override this flag with the Override column.

Core Analysis –Set Up

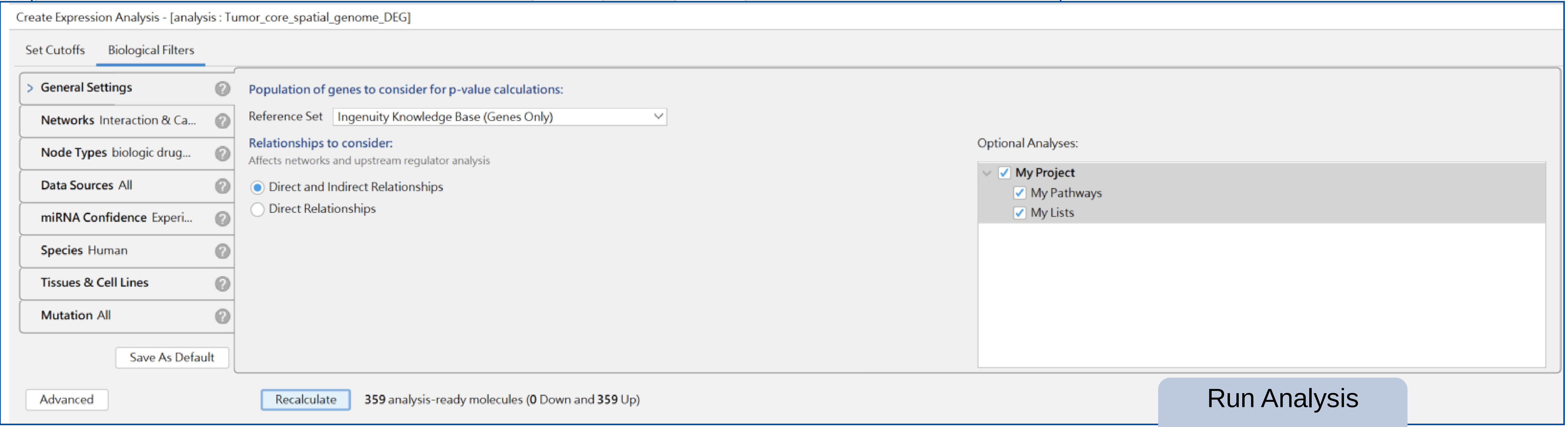


Create Expression Analysis - [analysis : Tumor_core_spatial_genome_DEG]

Use cutoffs to select a set of molecules from your dataset to analyze. Ideally choose between 100 and 3000 significantly regulated molecules, and not more than 8000. Include *both* up-regulated and down-regulated, if possible, to obtain causal predictions.

Set Cutoffs

Dataset Column	Measurement Value Type	Range	Cutoff



Create Expression Analysis - [analysis : Tumor_core_spatial_genome_DEG]

- **General Settings**
- **Networks** Interaction & Ca...
- **Node Types** biologic drug...
- **Data Sources** All
- **miRNA Confidence** Experi...
- **Species** Human
- **Tissues & Cell Lines**
- **Mutation** All

Population of genes to consider for p-value calculations:

Reference Set: Ingenuity Knowledge Base (Genes Only)

Relationships to consider:

Affects networks and upstream regulator analysis

● Direct and Indirect Relationships
○ Direct Relationships

Optional Analyses:

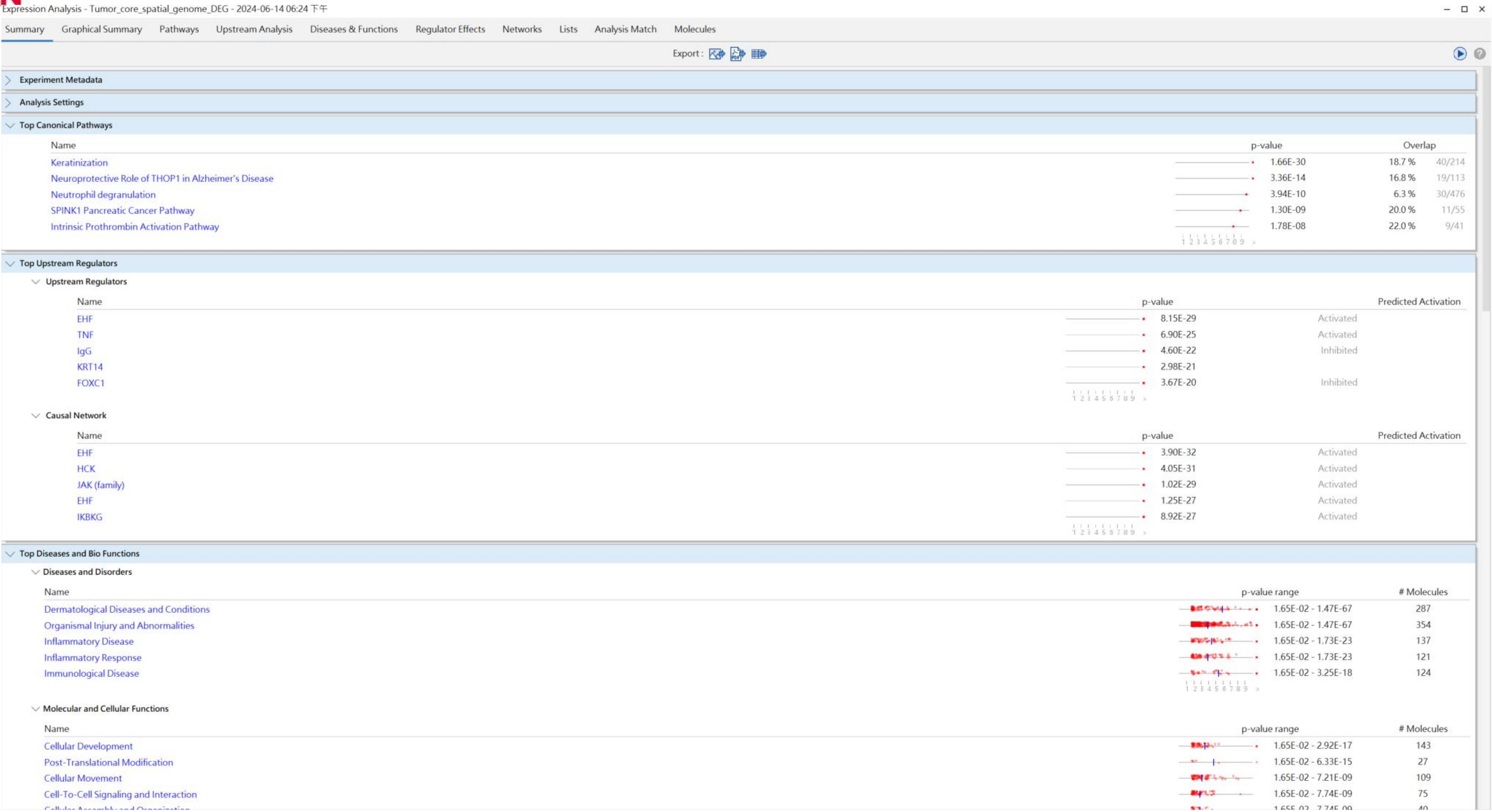
- ☑ My Project
  - ☑ My Pathways
  - ☑ My Lists

359 analysis-ready molecules (0 Down and 359 Up)

Three Step
1. Set Cutoff
2. Biological Filter
3. Run Analysis

Run Analysis

Tumor Core Analysis Result Overview



Experiment Metadata

Analysis Settings

Top Canonical Pathways

Name	p-value	Overlap	
Keratinization	1.66E-30	18.7 %	40/214
Neuroprotective Role of THOP1 in Alzheimer's Disease	3.36E-14	16.8 %	19/113
Neutrophil degranulation	3.94E-10	6.3 %	30/476
SPINK1 Pancreatic Cancer Pathway	1.30E-09	20.0 %	11/55
Intrinsic Prothrombin Activation Pathway	1.78E-08	22.0 %	9/41

Top Upstream Regulators

Upstream Regulators

Name	p-value	Predicted Activation
EHF	8.15E-29	Activated
TNF	6.90E-25	Activated
IgG	4.60E-22	Inhibited
KRT14	2.98E-21	
FOXC1	3.67E-20	Inhibited

Causal Network

Name	p-value	Predicted Activation
EHF	3.90E-32	Activated
HCK	4.05E-31	Activated
JAK (family)	1.02E-29	Activated
EHF	1.25E-27	Activated
IKBKG	8.92E-27	Activated

Top Diseases and Bio Functions

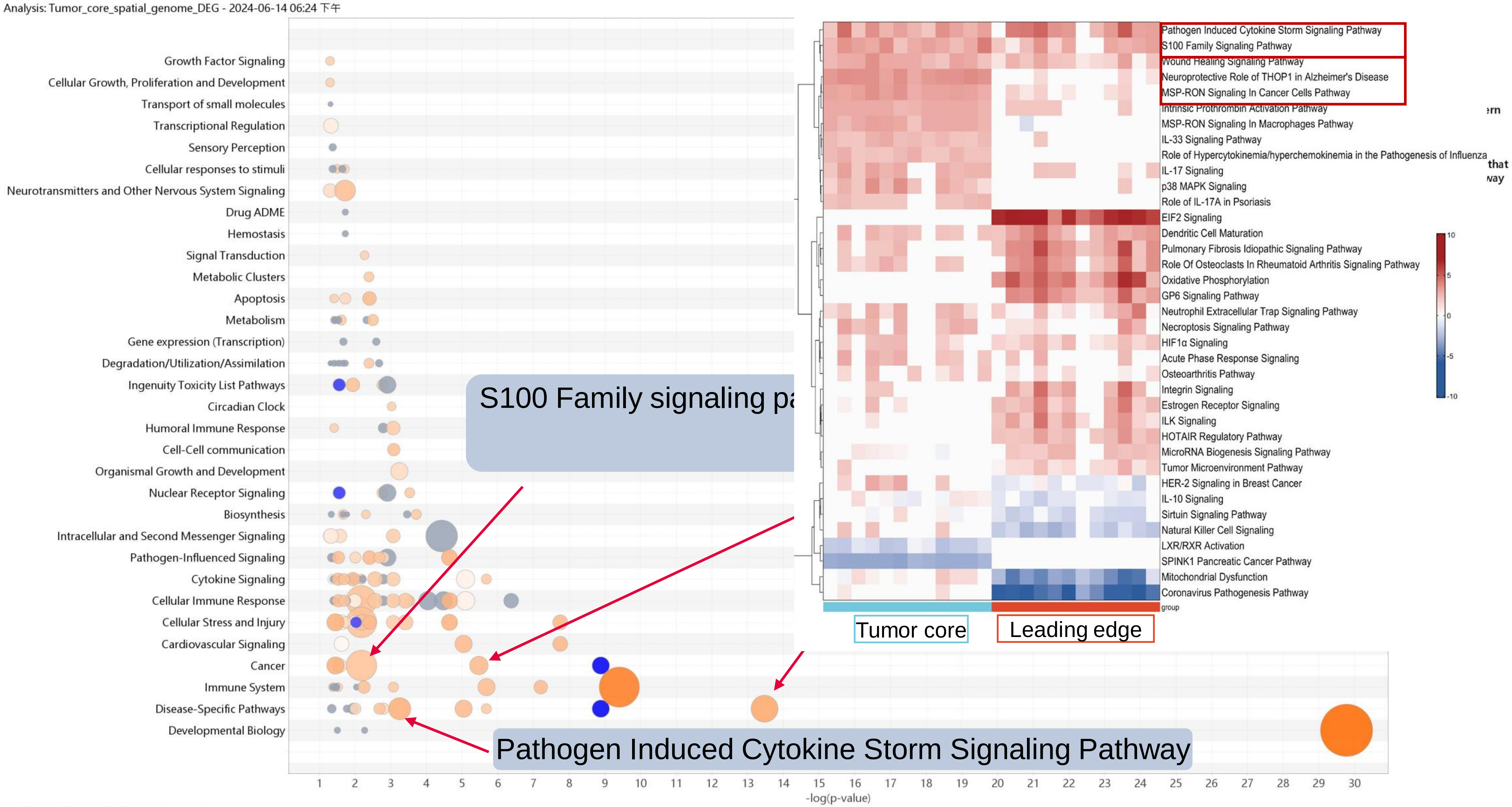
Diseases and Disorders

Name	p-value range	# Molecules
Dermatological Diseases and Conditions	1.65E-02 - 1.47E-67	287
Organismal Injury and Abnormalities	1.65E-02 - 1.47E-67	354
Inflammatory Disease	1.65E-02 - 1.73E-23	137
Inflammatory Response	1.65E-02 - 1.73E-23	121
Immunological Disease	1.65E-02 - 3.25E-18	124

Molecular and Cellular Functions

Name	p-value range	# Molecules
Cellular Development	1.65E-02 - 2.92E-17	143
Post-Translational Modification	1.65E-02 - 6.33E-15	27
Cellular Movement	1.65E-02 - 7.21E-09	109
Cell-To-Cell Signaling and Interaction	1.65E-02 - 7.74E-09	75
Cellular Assembly and Organization	1.65E-02 - 7.74E-09	40

Tumor Core Bubble Plot



S100 Family signaling pa

Tumor core

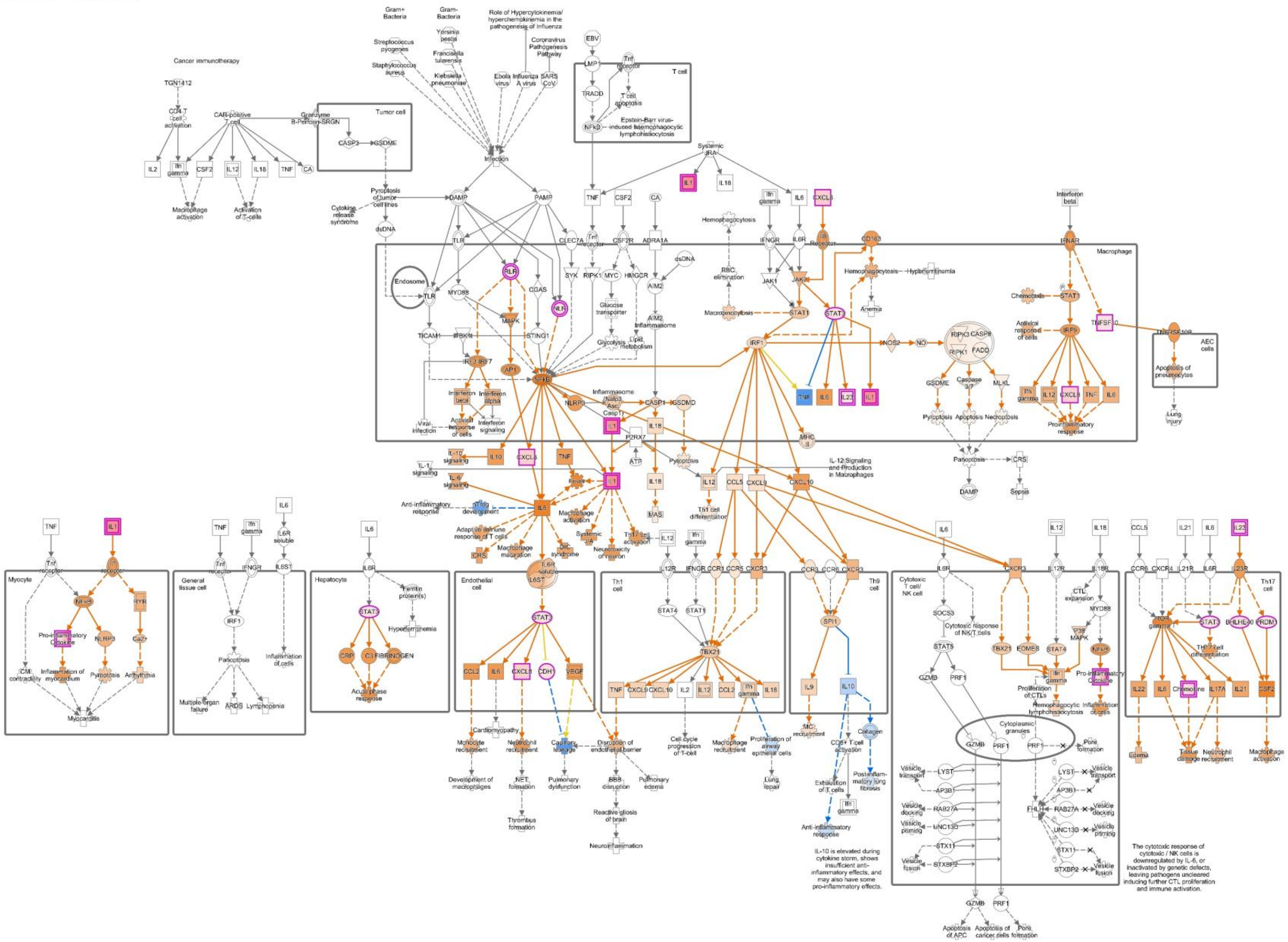
Leading edge

Pathogen Induced Cytokine Storm Signaling Pathway

Pathogen Induced Cytokine Storm Signaling Pathway

訊聯基因數位

Pathogen Induced Cytokine Storm Signaling Pathway

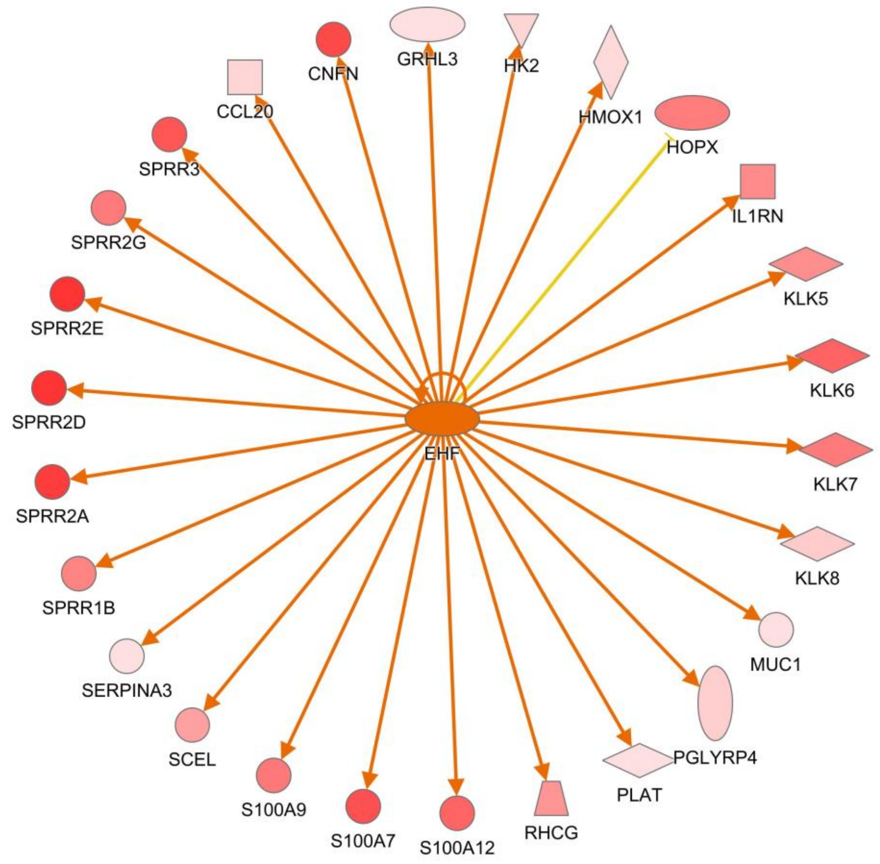


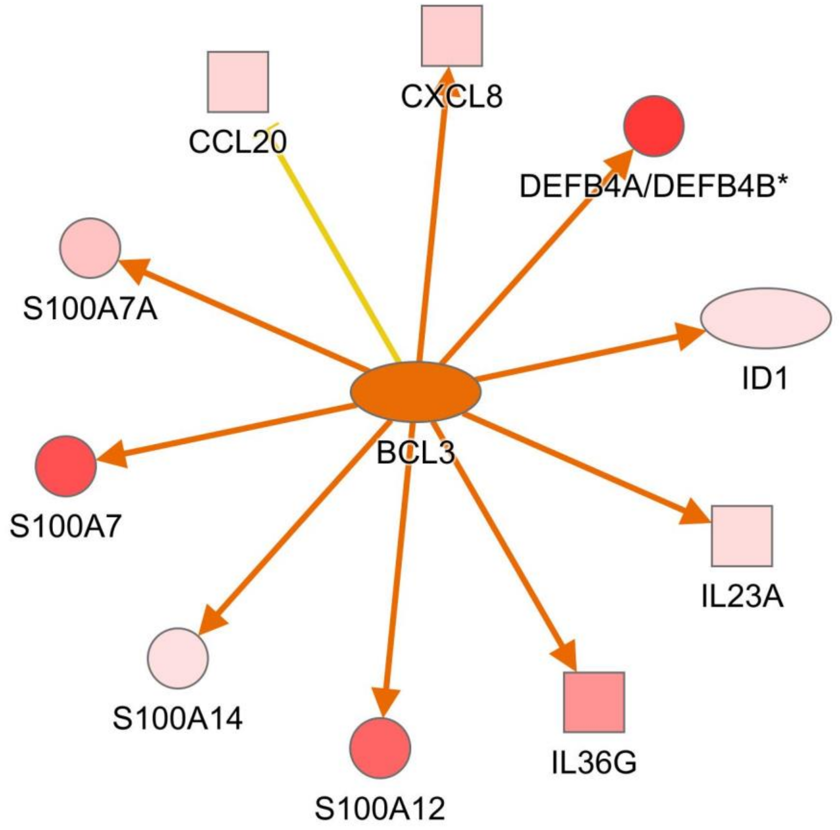
IL-10 is elevated during cytokine storm, shows insufficient anti-inflammatory effects, and may also have some pro-inflammatory effects.

The cytotoxic response of cytotoxic / NK cells is downregulated by IL-6, or inactivated by genetic defects, leaving pathogens uncleared inducing further CTL proliferation and immune activation.

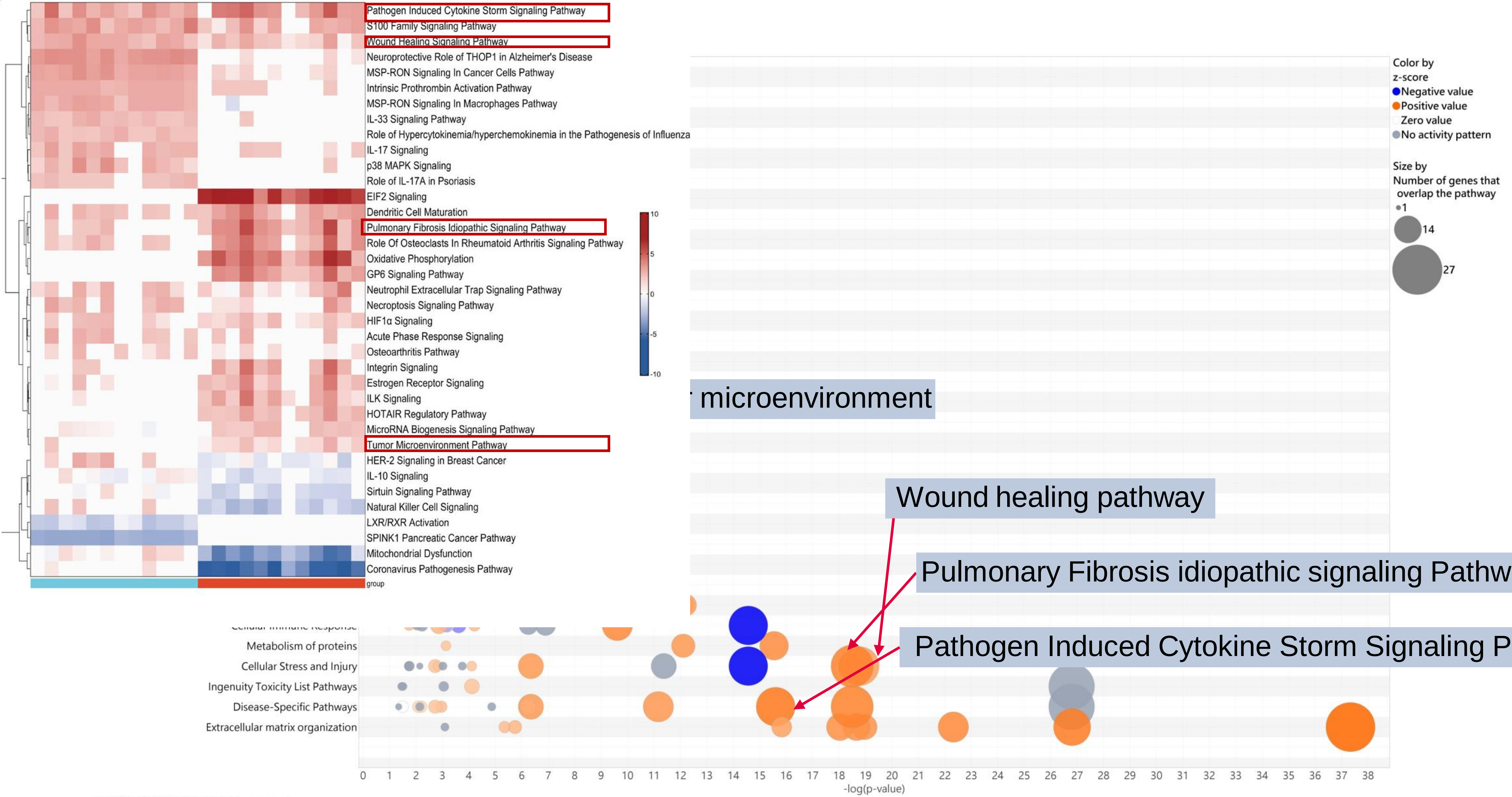
Upstream analysis in Tumor Core

Proto-Oncogene transcription factor


EHF 1


BCL3 2

Leading Edge Bubble Plot



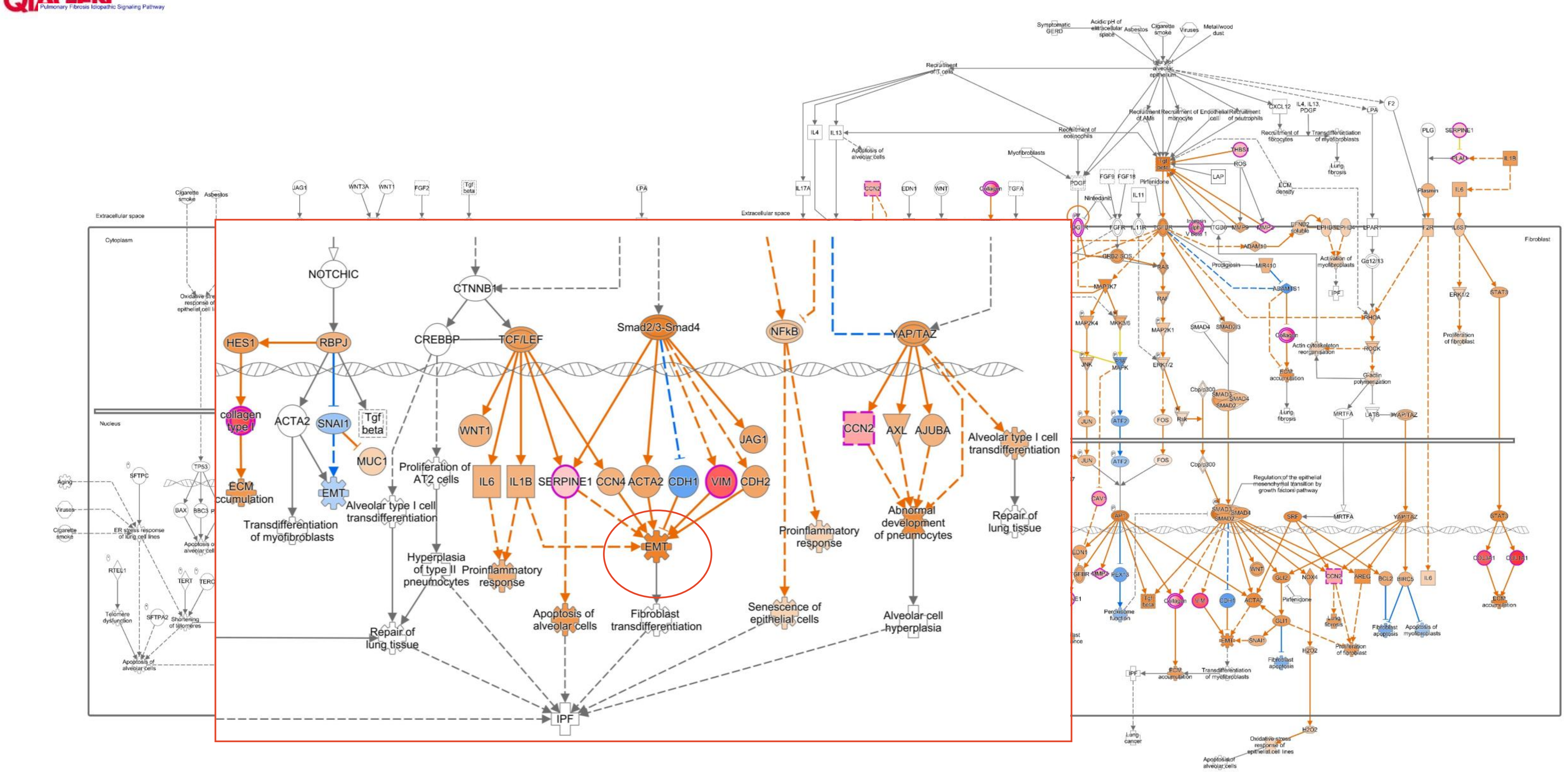
microenvironment

Wound healing pathway

Pulmonary Fibrosis idiopathic signaling Pathway

Pathogen Induced Cytokine Storm Signaling Pathway

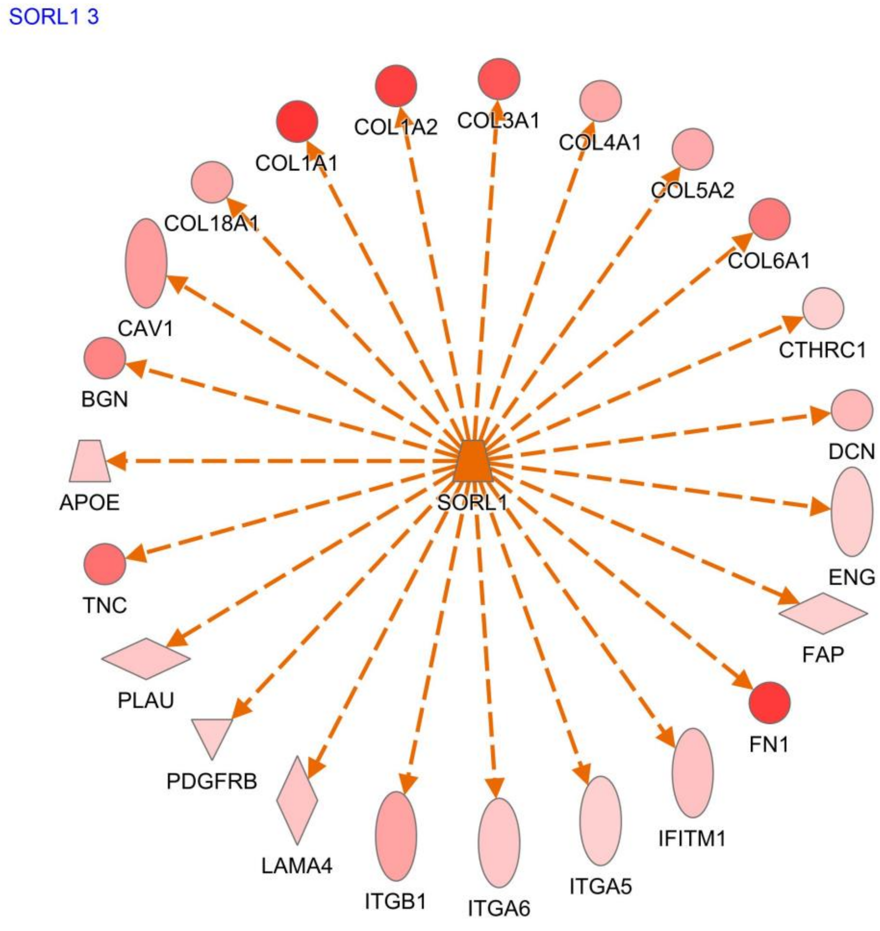
Pulmonary Fibrosis idiopathic signaling Pathway

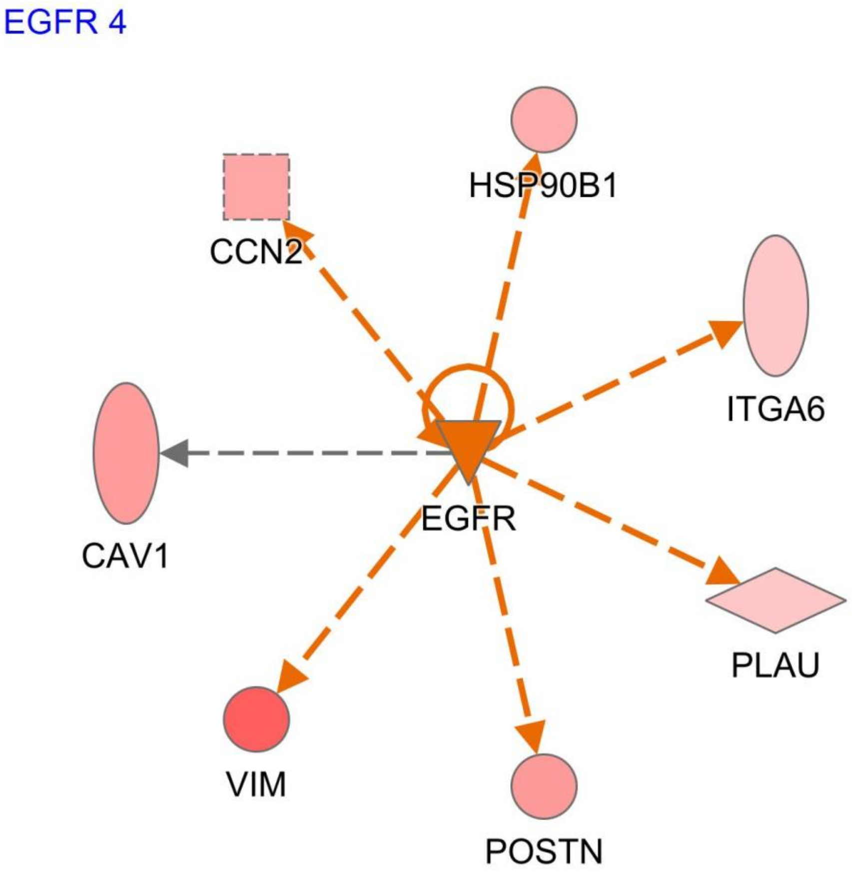


Upstream analysis in Leading Edge

訊聯基因數位

EMT regulator

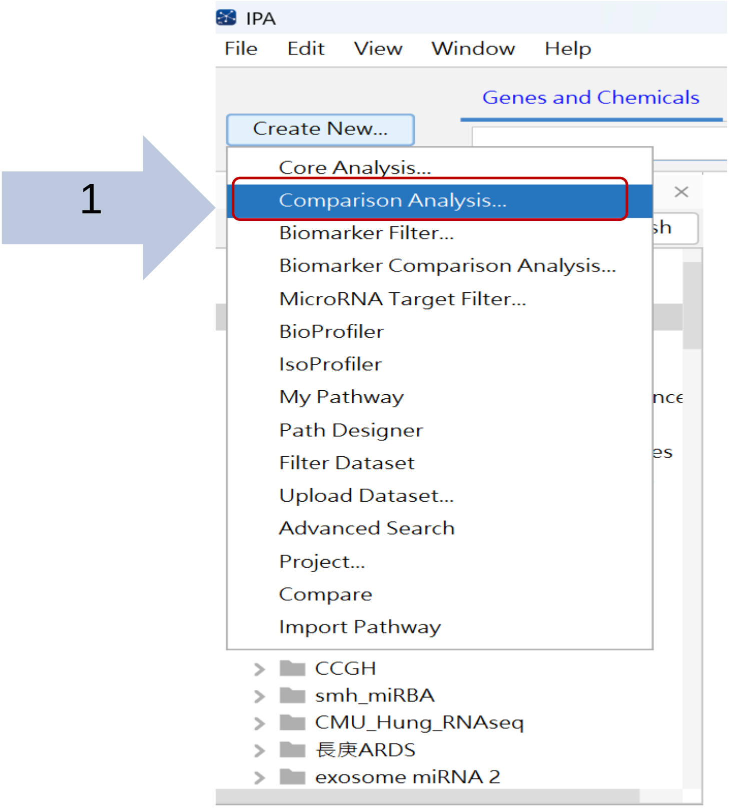


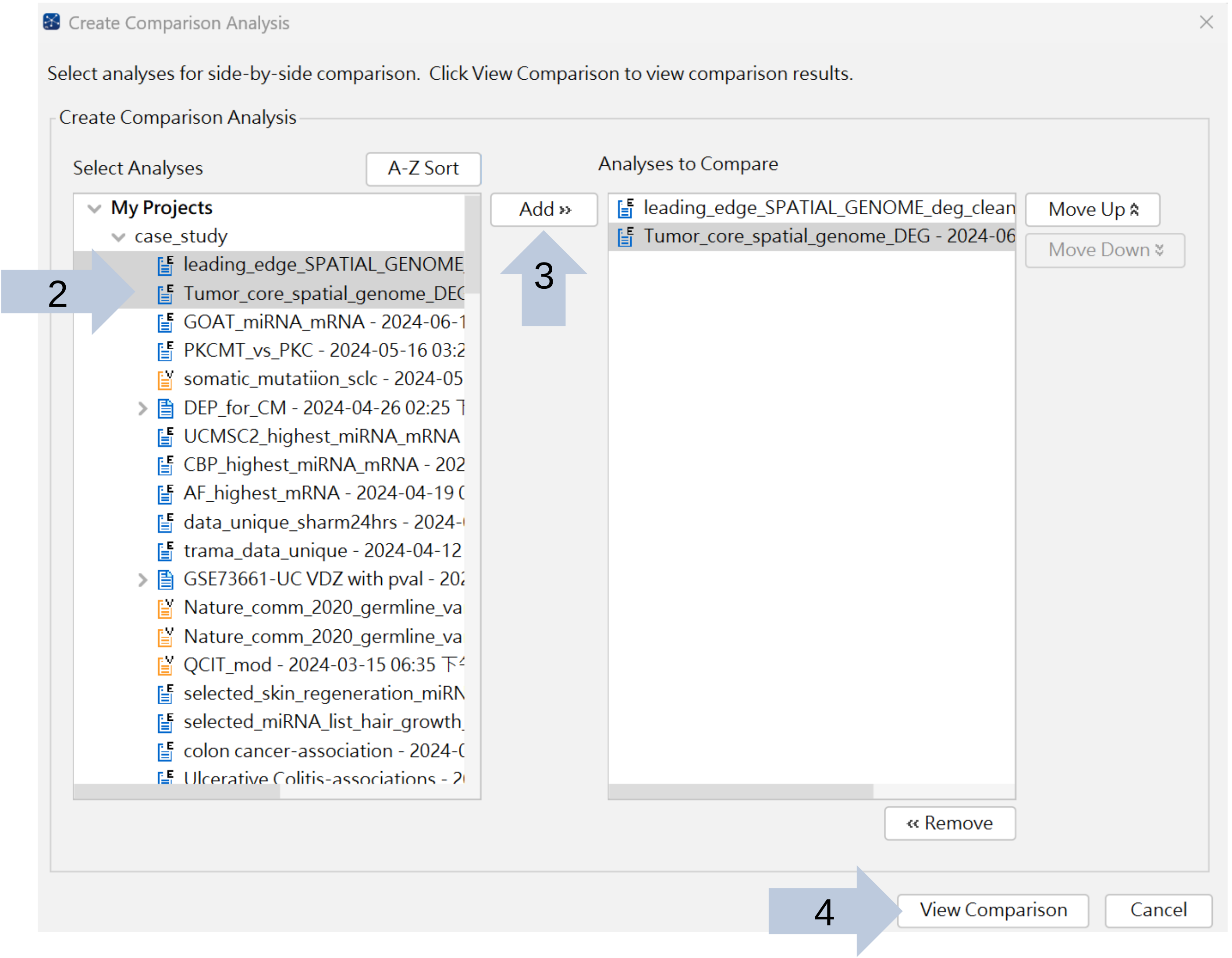


Compare Analysis

訊聯基因數位

Step for You to do your compared analysis




Create Comparison Analysis

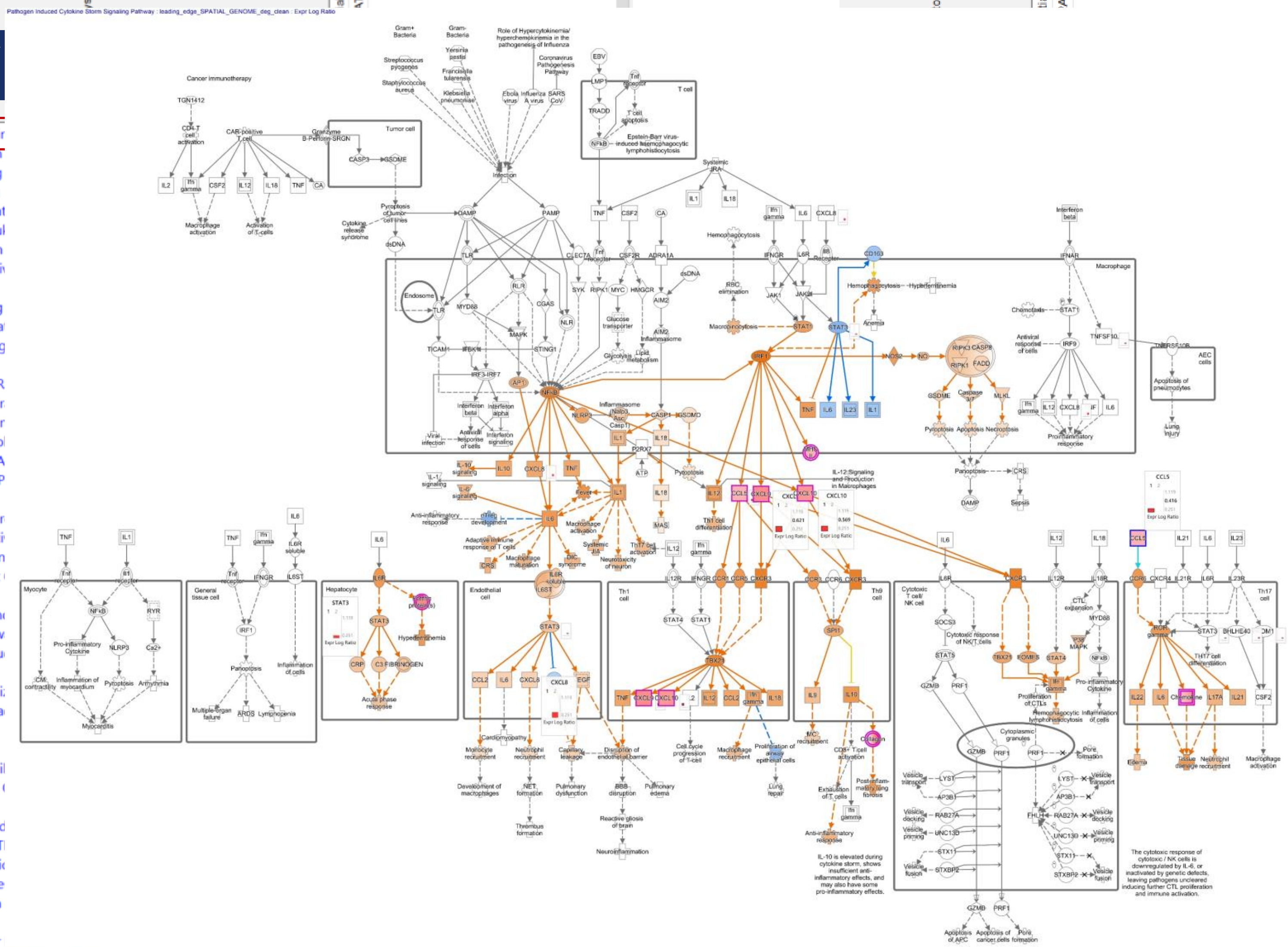
Select analyses for side-by-side comparison. Click View Comparison to view comparison results.

Compare Analysis Result

Canonical pathway

- Pathogen Induced Cytokir
- Neutrophil degranulation
- Wound Healing Signaling
- Atherosclerosis Signaling
- Cell surface interactions at
- Interleukin-4 and Interleul
- Cell junction organization
- Intrinsic Prothrombin Acti
- Dendritic Cell Maturation
- Hepatic Fibrosis Signaling
- Pulmonary Fibrosis Idiopa
- Acute Phase Response Sig
- FAK Signaling
- Role of Chondrocytes in R
- Neutrophil Extracellular Tr
- Sertoli Cell-Sertoli Cell Jur
- IL-17A Signaling in Fibrob
- Macrophage Alternative A
- Activin Inhibin Signaling P
- Osteoarthritis Pathway
- Role of Macrophages, Fibr
- Macrophage Classical Acti
- Multiple Sclerosis Signalin
- HER-2 Signaling in Breast
- ID1 Signaling Pathway
- Sertoli Cell-Germ Cell Junc
- HOTAIR Regulatory Pathw
- IL-12 Signaling and Produ
- Keratinization
- Extracellular matrix organi
- Integrin cell surface intera
- GP6 Signaling Pathway
- IL-4 Signaling
- Assembly of collagen fibril
- Regulation of Insulin-like
- Collagen degradation
- Collagen biosynthesis and
- Neuroprotective Role of T
- Collagen chain trimerizati
- Role of Osteoclasts in Rhe
- Post-translational protein
- Signaling by MET
- SPINK1 Pancreatic Cancer
- Cachexia Signaling Pathway
- Syndecan interactions
- Signaling by PDGF
- Binding and Uptake of Ligands by Scavenger Receptors
- MSP-RON Signaling in Macrophages Pathway

Pathogen Induced Cytokine Storm Signaling Pathway : leading_edge_SPATIAL_GENOME_deg_clean : Expr Log Ratio



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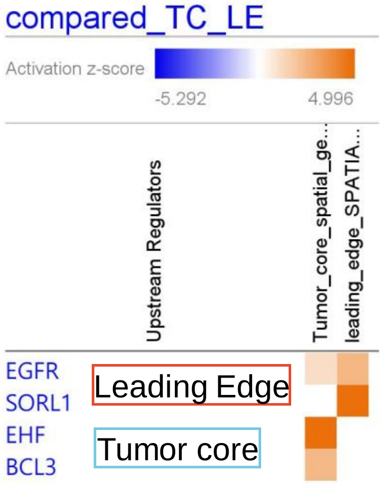
ERK
TGFB2
TNF

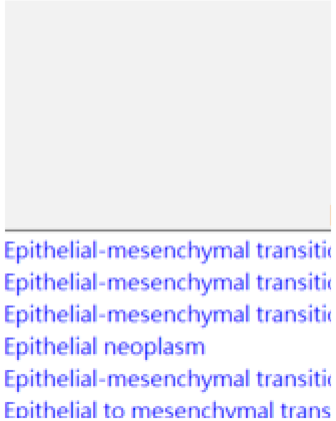


Leading Edge

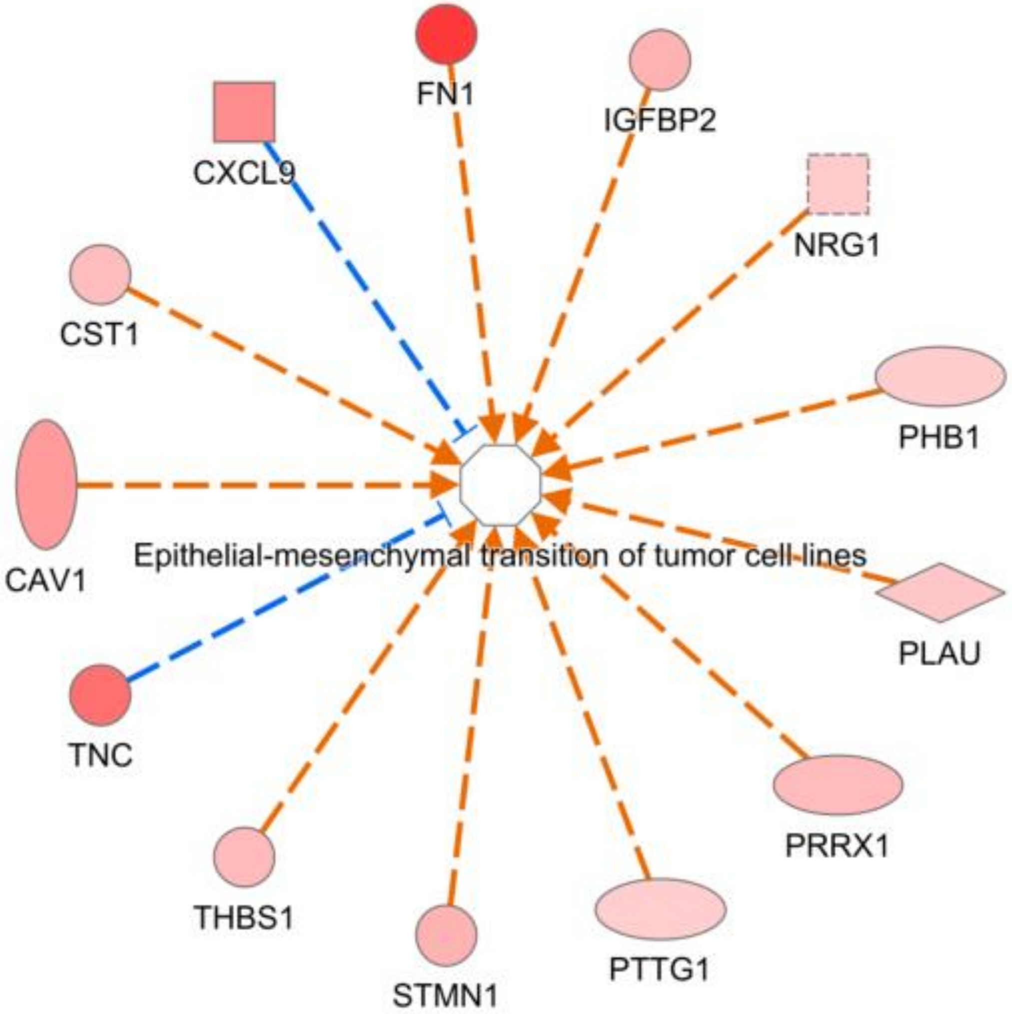
Tumor core

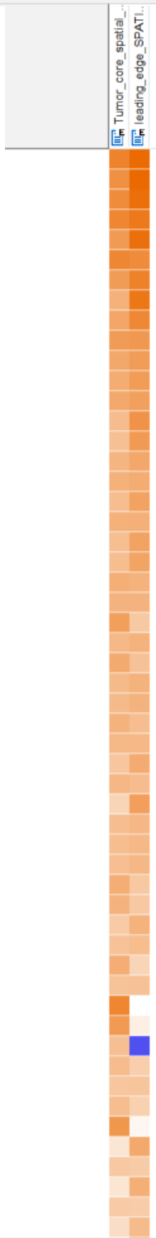
Compare Analysis Result

EMT signaling



Epithelial-mesenchymal transiti
Epithelial-mesenchymal transiti
Epithelial-mesenchymal transiti
Epithelial neoplasm
Epithelial-mesenchymal transiti
Epithelial to mesenchymal trans





Cell proliferation of breast cancer cell lines
Binding of tumor cell lines
Sphere formation of carcinoma cell lines
Cell proliferation of colorectal cancer cell lines

Summary: Evaluating your ′omics data using IPA

gene	avg_log2FC	p_val_adj
SPRR2D	2.706423338	0
SPRR2E	2.654346543	0
DEFB4A	2.579461176	0
SPRR2A	2.520689273	0
LCN2	2.449394678	0
CRCT1	2.414710712	0
SLURP2	2.390169921	0
CNFN	2.342349982	0
SLURP1	2.340477642	0
S100A7	2.260580342	0
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SPRR2B	2.173298475	0
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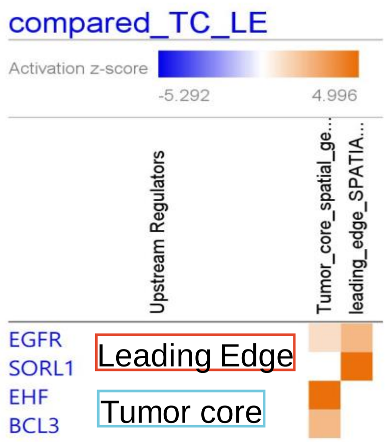
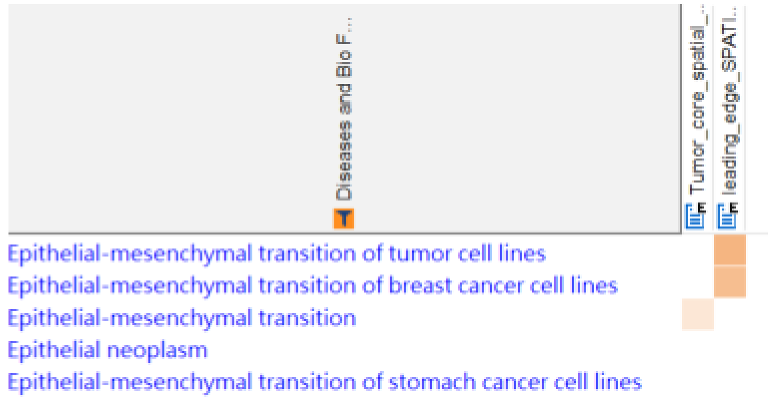
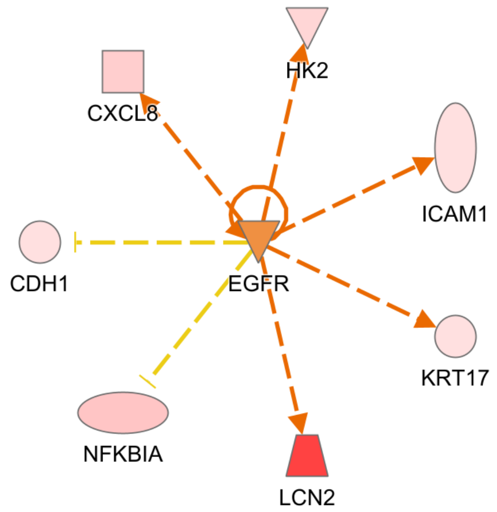
LCE3D	2.082024046	0
KLK6	2.027716246	0
SPRR1A	2.017865872	0

gene	avg_log2FC	p_val_adj
COL1A1	1.119188557	0
FN1	1.077456695	0
COL1A2	1.047688083	0
TIMP1	1.040462139	0
COL6A2	0.98234798	0
COL3A1	0.916656628	0
SPARC	0.902377431	0
VIM	0.87526439	0
HMGN2	0.791542974	0
TNC	0.778830913	0
IGFBP7	0.716862936	0
FTL	0.653061976	0
MT2A	0.612219081	0
PFN1	0.546409497	0
FBLN2	0.472353201	0
LGALS1	0.668868409	9.25E-299

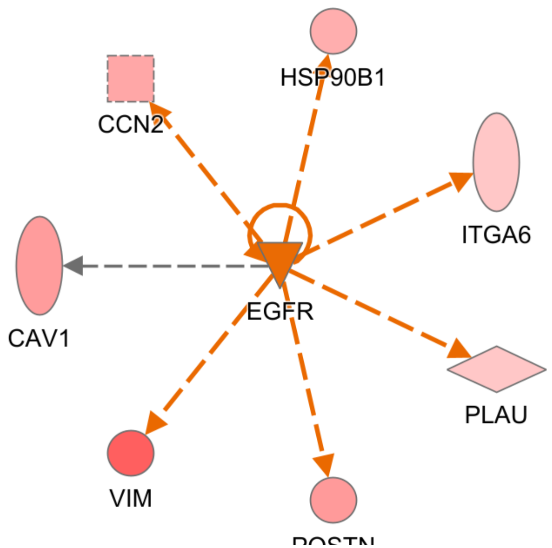




Tumor core



Leading edge



- Data upload and analysis setup
- Canonical pathways
- Upstream regulators
- Diseases and functions
- Comparison analysis

Summary

QIAGEN IPA – access to manually-curated knowledge base

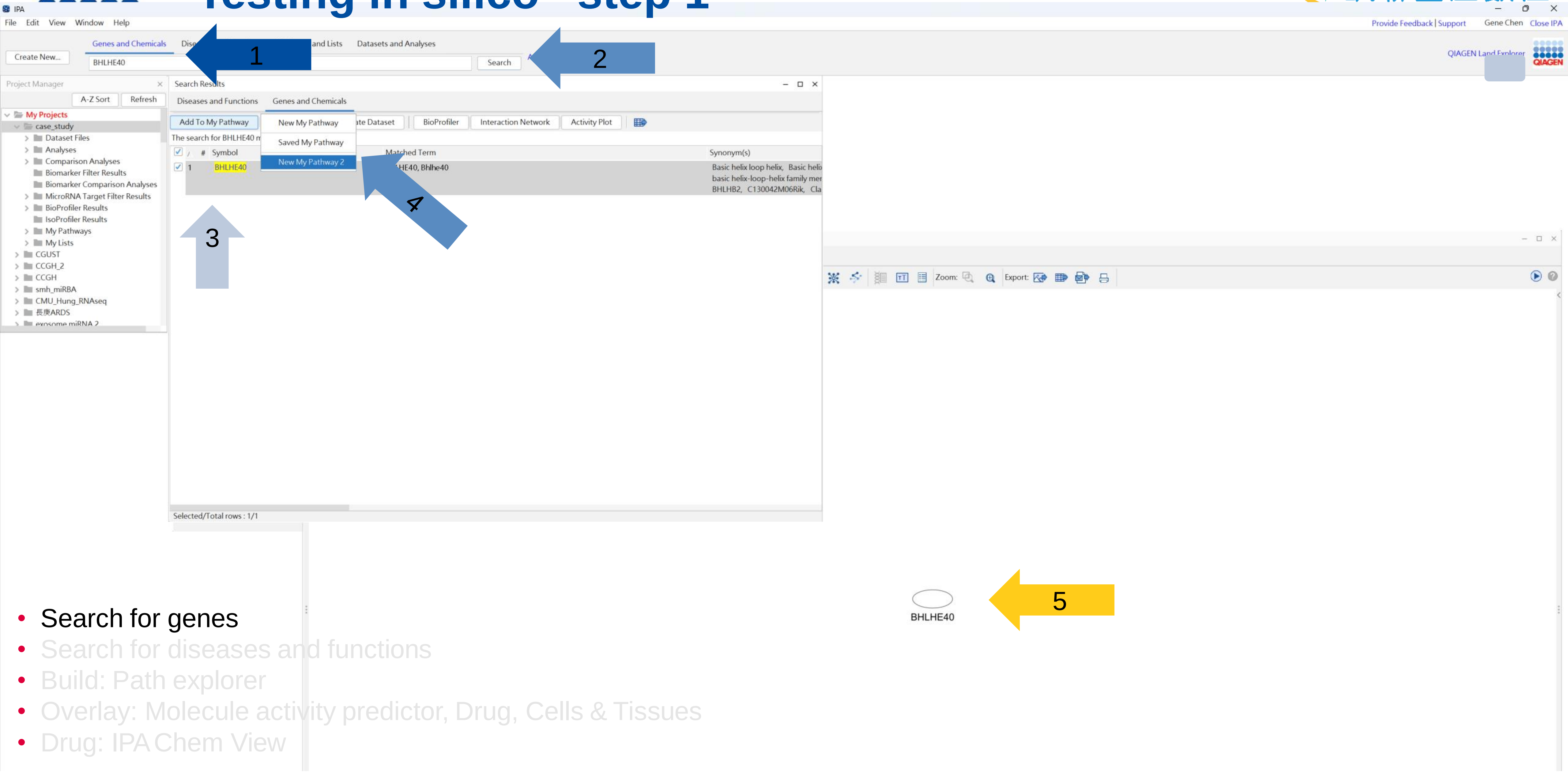
- **Perform expression analysis and compare cell clusters**
  - Discover novel biological mechanisms
  - Identify cell type-specific biomarkers and key regulators/targets

Better Care with Better Knowledge

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:
席佩妤 資深業務專員 CleoHsi@gga.asia 02-2795 1777 #3014
熊嘉妮 專案主任 ChristineHsiung@gga.asia 02-2795 1777 #3028

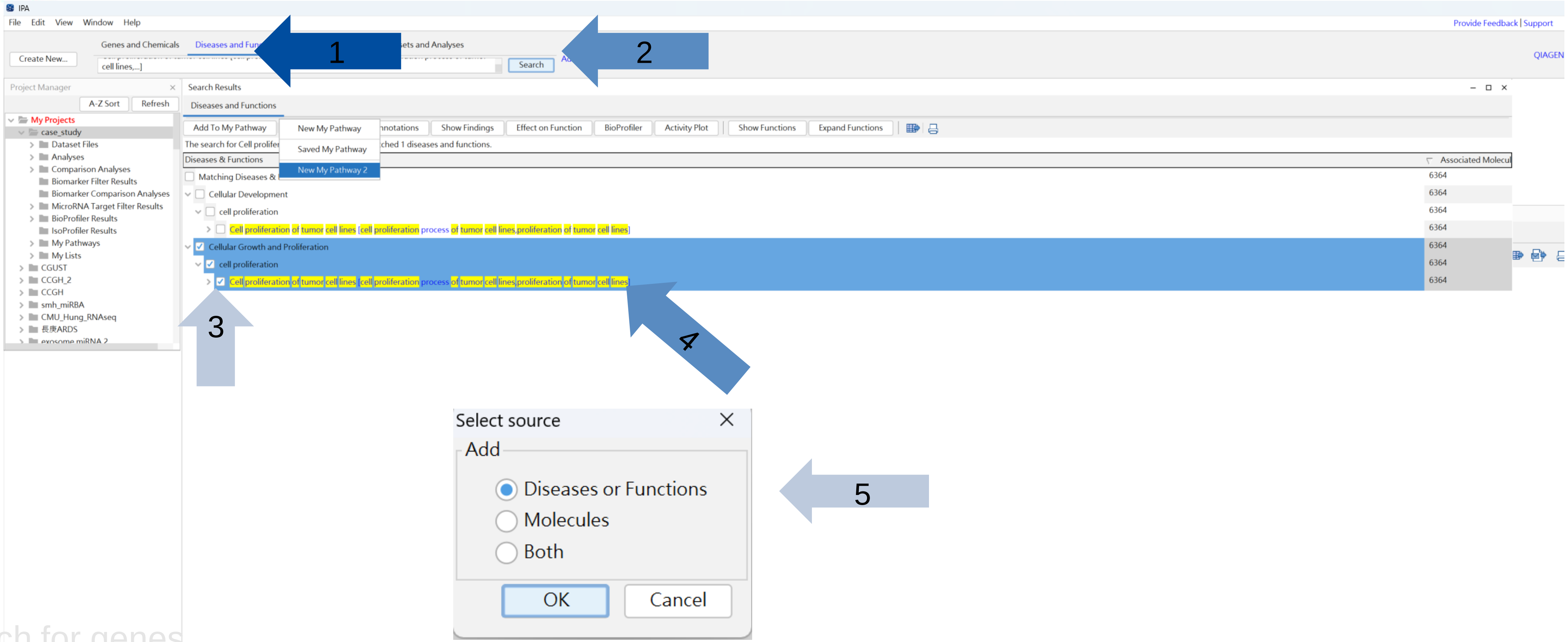
Bioinfo@GGA.ASIA

Testing in silico –step 1



- Search for genes
- Search for diseases and functions
- Build: Path explorer
- Overlay: Molecule activity predictor, Drug, Cells & Tissues
- Drug: IPA Chem View

Testing in silico –step 2



The search for Cell prolifer… ched 1 diseases and functions.

☐ Cell proliferation of tumor cell lines [cell proliferation process of tumor cell lines,proliferation of tumor cell lines]

☑ Cellular Growth and Proliferation

☑ cell proliferation

☑ Cell proliferation of tumor cell lines [cell proliferation process of tumor cell lines,proliferation of tumor cell lines]

Select source

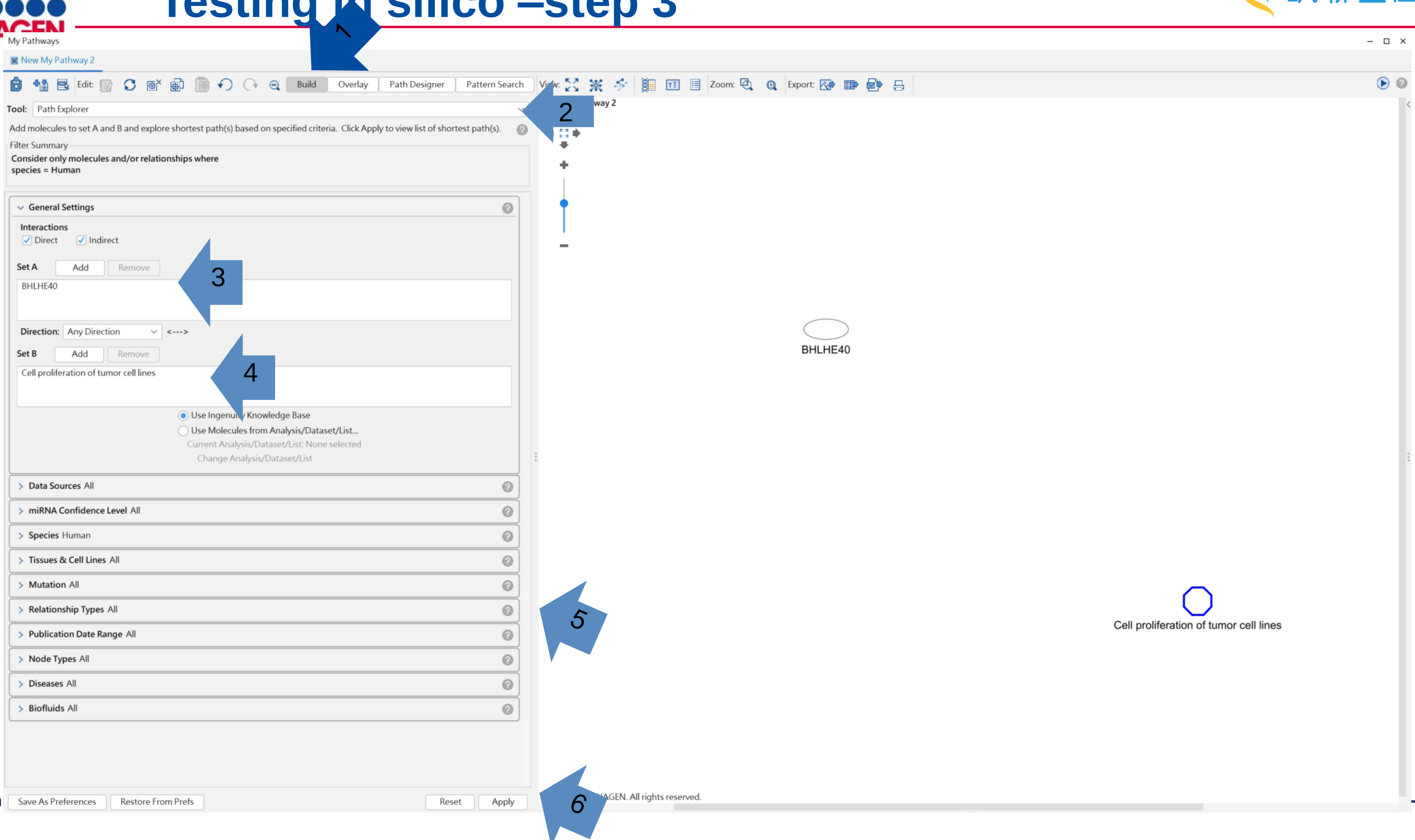
Add

● Diseases or Functions
○ Molecules
○ Both

OK Cancel

- Search for genes
- Search for diseases and functions
- Build: Path explorer
- Overlay: Molecule activity predictor, Drug, Cells & Tissues
- Drug: IPA Chem View

Testing in silico –step 3



Tool: Path Explorer

Add molecules to set A and B and explore shortest path(s) based on specified criteria. Click Apply to view list of shortest path(s).

Filter Summary

Consider only molecules and/or relationships where
species = Human

General Settings

Interactions

☑ Direct ☑ Indirect

Set A: BHLHE40

Direction: Any Direction <--->

Set B: Cell proliferation of tumor cell lines

● Use Ingenu y Knowledge Base

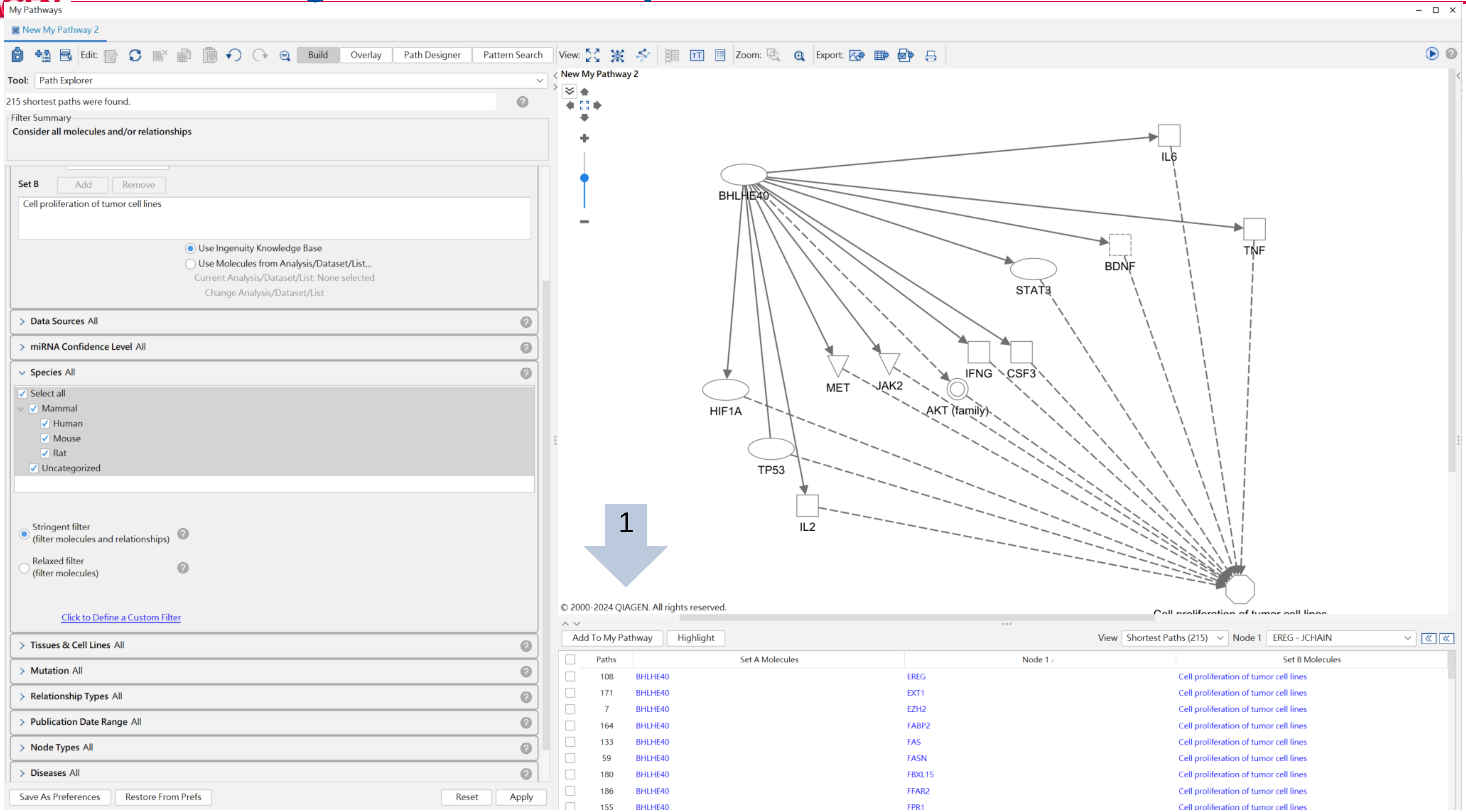
○ Use Molecules from Analysis/Dataset/List...

Current Analysis/Dataset/List: None selected

Change Analysis/Dataset/List

- Data Sources All
- miRNA Confidence Level All
- Species Human
- Tissues & Cell Lines All
- Mutation All
- Relationship Types All
- Publication Date Range All
- Node Types All
- Diseases All
- Biofluids All

Testing in silico –step 4



Tool: Path Explorer

215 shortest paths were found.

Filter Summary
Consider all molecules and/or relationships

Set B Add Remove
Cell proliferation of tumor cell lines

● Use Ingenuity Knowledge Base
○ Use Molecules from Analysis/Dataset/List...
Current Analysis/Dataset/List: None selected
Change Analysis/Dataset/List

- Data Sources All
- miRNA Confidence Level All
- Species All
  - ☑ Select all
  - ☑ Mammal
    - ☑ Human
    - ☑ Mouse
    - ☑ Rat
  - ☑ Uncategorized

● Stringent filter (filter molecules and relationships)
○ Relaxed filter (filter molecules)

Click to Define a Custom Filter

- Tissues & Cell Lines All
- Mutation All
- Relationship Types All
- Publication Date Range All
- Node Types All
- Diseases All

Save As Preferences | Restore From Prefs | Reset | Apply

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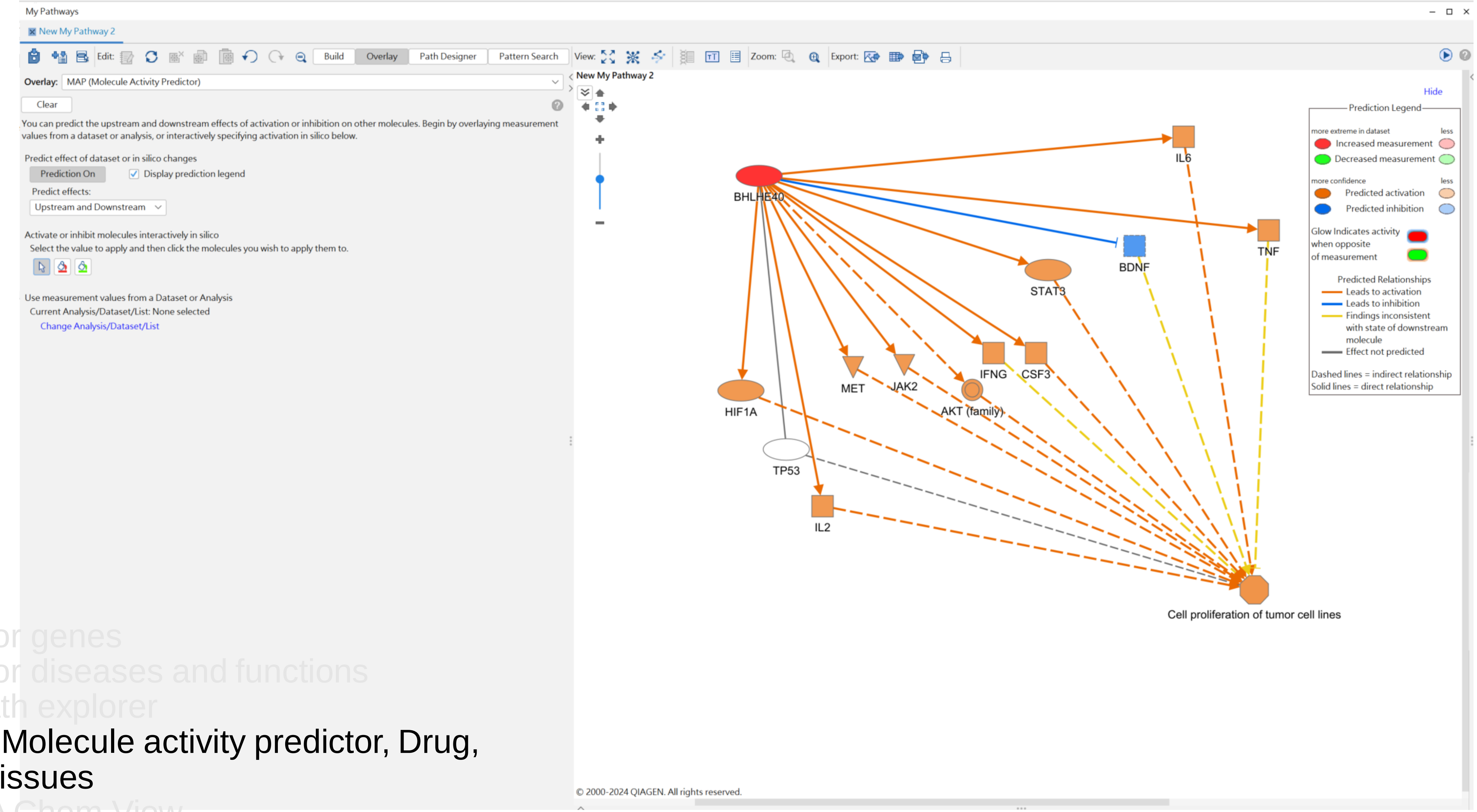
Add To My Pathway | Highlight

View: Shortest Paths (215) Node 1: EREG - JCHAIN

☐	Paths	Set A Molecules	Node 1	Set B Molecules
☐	108	BHLHE40	EREG	Cell proliferation of tumor cell lines
☐	171	BHLHE40	EXT1	Cell proliferation of tumor cell lines
☐	7	BHLHE40	EZH2	Cell proliferation of tumor cell lines
☐	164	BHLHE40	FABP2	Cell proliferation of tumor cell lines
☐	133	BHLHE40	FAS	Cell proliferation of tumor cell lines
☐	59	BHLHE40	FASN	Cell proliferation of tumor cell lines
☐	180	BHLHE40	FBXL15	Cell proliferation of tumor cell lines
☐	186	BHLHE40	FFAR2	Cell proliferation of tumor cell lines
☐	155	BHLHE40	FPR1	Cell proliferation of tumor cell lines

Testing in silico –step 5



Overlay: MAP (Molecule Activity Predictor)

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

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.

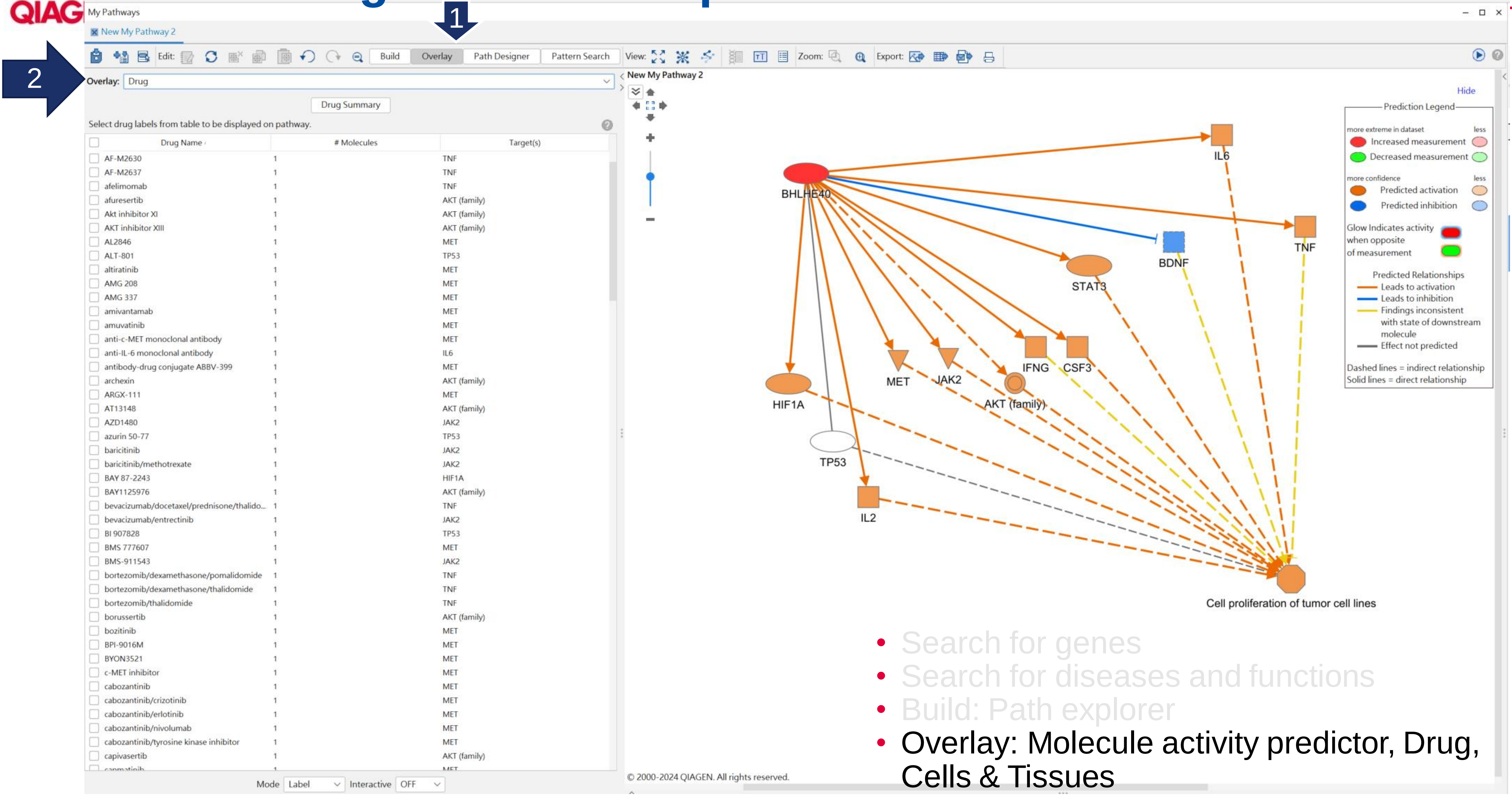
Use measurement values from a Dataset or Analysis

Current Analysis/Dataset/List: None selected

Change Analysis/Dataset/List

- Search for genes
- Search for diseases and functions
- Build: Path explorer
- Overlay: Molecule activity predictor, Drug, Cells & Tissues
- Drug: IPA Chem View

Testing in silico –step 6



Overlay: Drug

Drug Summary

Select drug labels from table to be displayed on pathway.

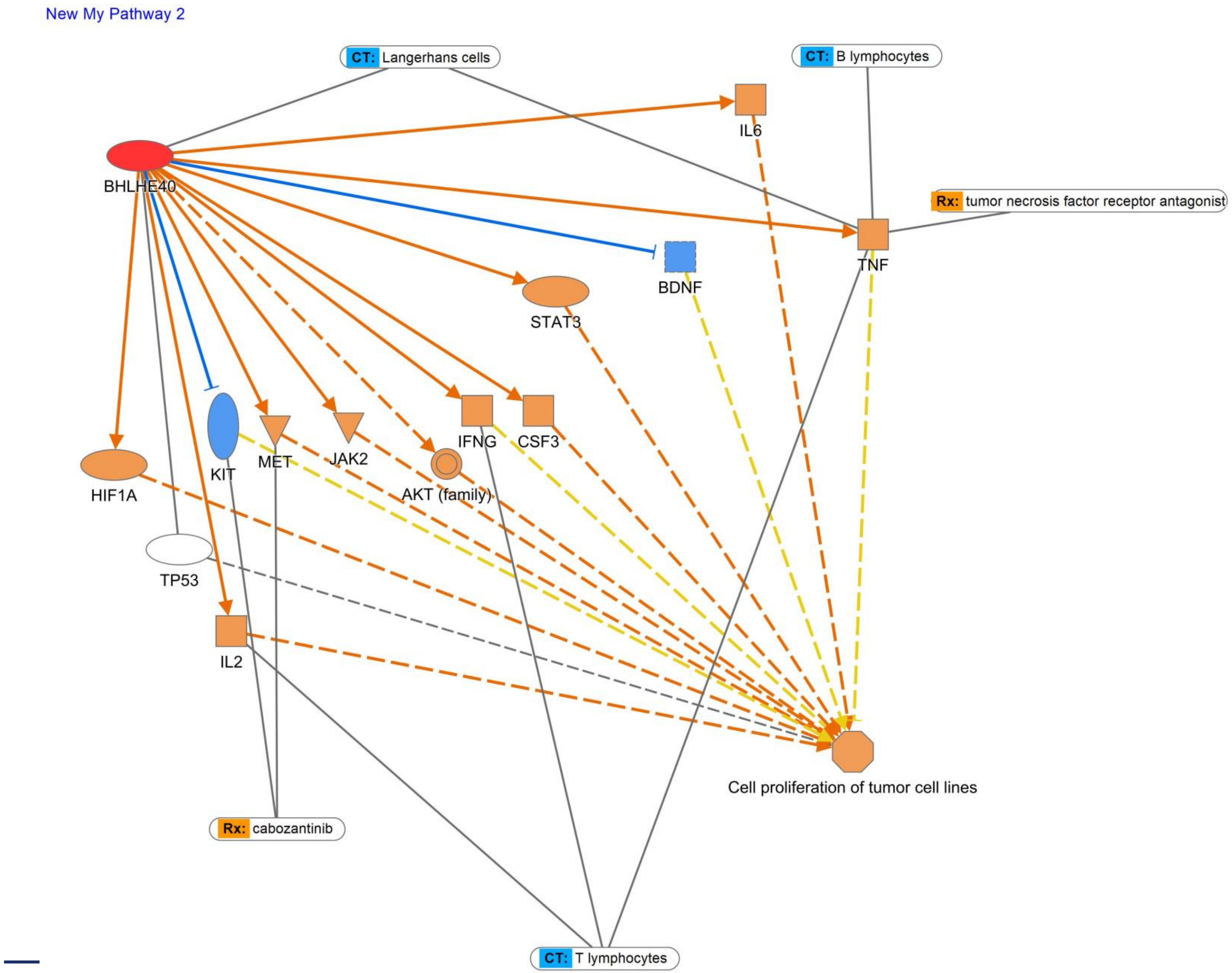
☐	Drug Name	# Molecules	Target(s)
☐	AF-M2630	1	TNF
☐	AF-M2637	1	TNF
☐	afelimomab	1	TNF
☐	afuresertib	1	AKT (family)
☐	Akt inhibitor XI	1	AKT (family)
☐	AKT inhibitor XIII	1	AKT (family)
☐	AL2846	1	MET
☐	ALT-801	1	TP53
☐	altiratinib	1	MET
☐	AMG 208	1	MET
☐	AMG 337	1	MET
☐	amivantamab	1	MET
☐	amuvatinib	1	MET
☐	anti-c-MET monoclonal antibody	1	MET
☐	anti-IL-6 monoclonal antibody	1	IL6
☐	antibody-drug conjugate ABBV-399	1	MET
☐	archexin	1	AKT (family)
☐	ARGX-111	1	MET
☐	AT13148	1	AKT (family)
☐	AZD1480	1	JAK2
☐	azurin 50-77	1	TP53
☐	baricitinib	1	JAK2
☐	baricitinib/methotrexate	1	JAK2
☐	BAY 87-2243	1	HIF1A
☐	BAY1125976	1	AKT (family)
☐	bevacizumab/docetaxel/prednisone/thalido...	1	TNF
☐	bevacizumab/entrectinib	1	JAK2
☐	BI 907828	1	TP53
☐	BMS 777607	1	MET
☐	BMS-911543	1	JAK2
☐	bortezomib/dexamethasone/pomalidomide	1	TNF
☐	bortezomib/dexamethasone/thalidomide	1	TNF
☐	bortezomib/thalidomide	1	TNF
☐	borussertib	1	AKT (family)
☐	bozitinib	1	MET
☐	BPI-9016M	1	MET
☐	BYON3521	1	MET
☐	c-MET inhibitor	1	MET
☐	cabozantinib	1	MET
☐	cabozantinib/crizotinib	1	MET
☐	cabozantinib/erlotinib	1	MET
☐	cabozantinib/nivolumab	1	MET
☐	cabozantinib/tyrosine kinase inhibitor	1	MET
☐	capivasertib	1	AKT (family)

Mode: Label

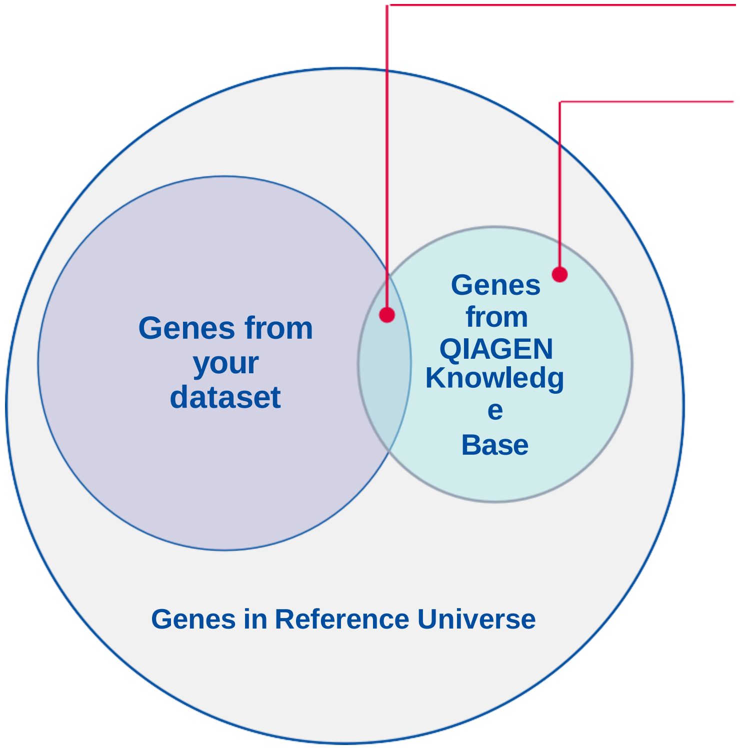
Interactive: OFF

- Search for genes
- Search for diseases and functions
- Build: Path explorer
- Overlay: Molecule activity predictor, Drug, Cells & Tissues
- Drug: IPA Chem View

Create networks from scratch and test activity in silico



p-value



Overlapping molecules

Genes from previous literature that belong to

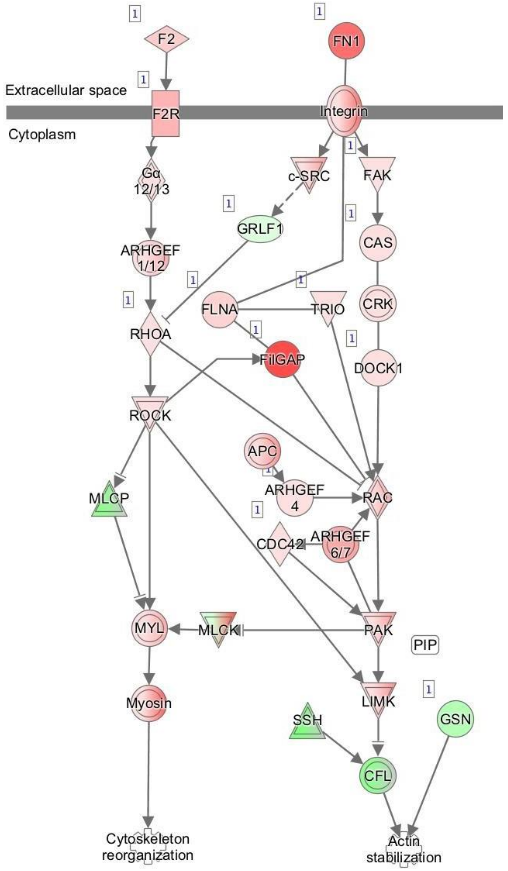
- A canonical pathway OR
- Downstream of an upstream regulator OR
- Upstream of a disease or function

What the p-value represents

- p-value is different from the "Expression p-value" uploaded with your dataset
- p-value is 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 significant (<0.05) for random datasets
- Gene expression direction is not taken into account for this calculation

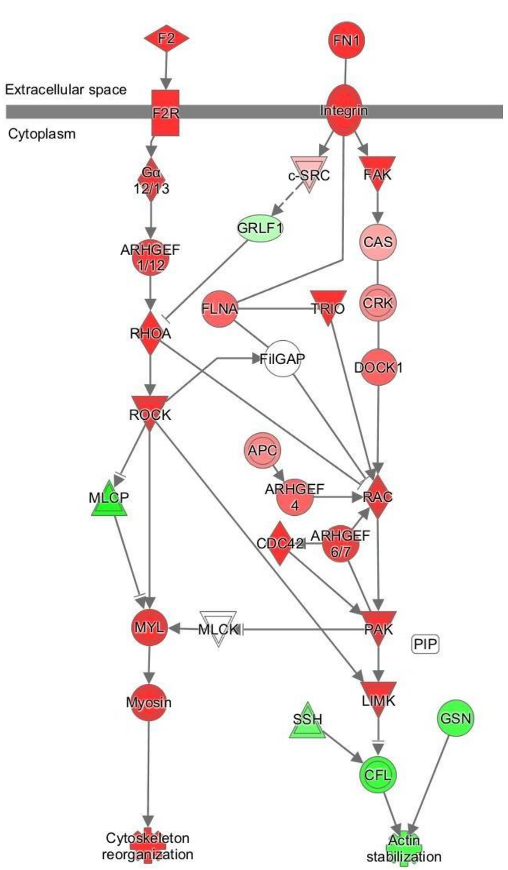
訊聯基因數位

Pathway Activity



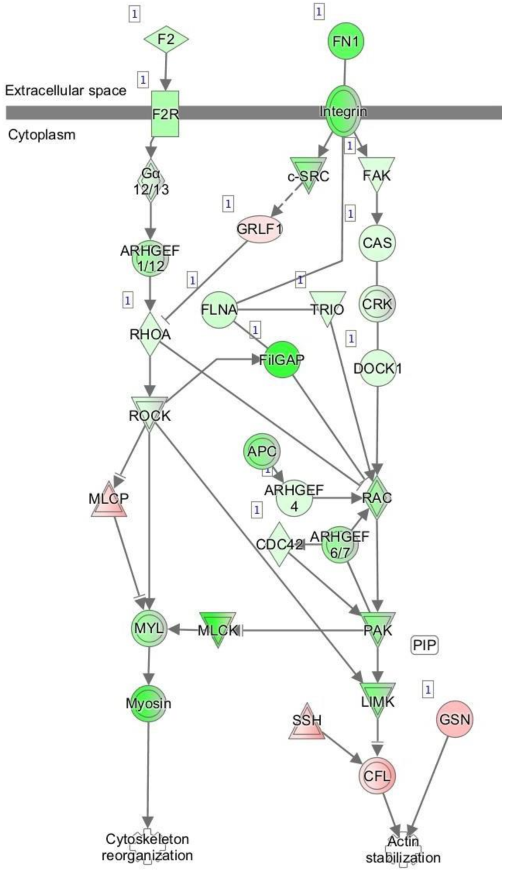
Positive z-score

(User data)



Expected activation state

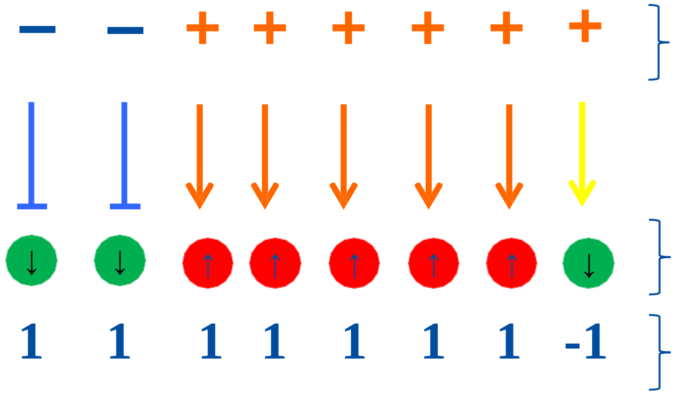
(QIAGEN Knowledge Base)



Negative z-score

(User data)

z-score: Activation prediction



Gene expression from QIAGEN Knowledge Base (literature)

Gene expression in your dataset

+1 score for consistent relationships
and –1 for 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 greater than 2 or less than –2 is considered significant
- Note that the actual z-score is weighted by the underlying findings, the relationship bias and dataset bias