# Discover hidden relationships in your toxicological studies

Discovery Team, QIAGEN Digital Insights

# Legal disclaimer

QIAGEN products shown here are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.
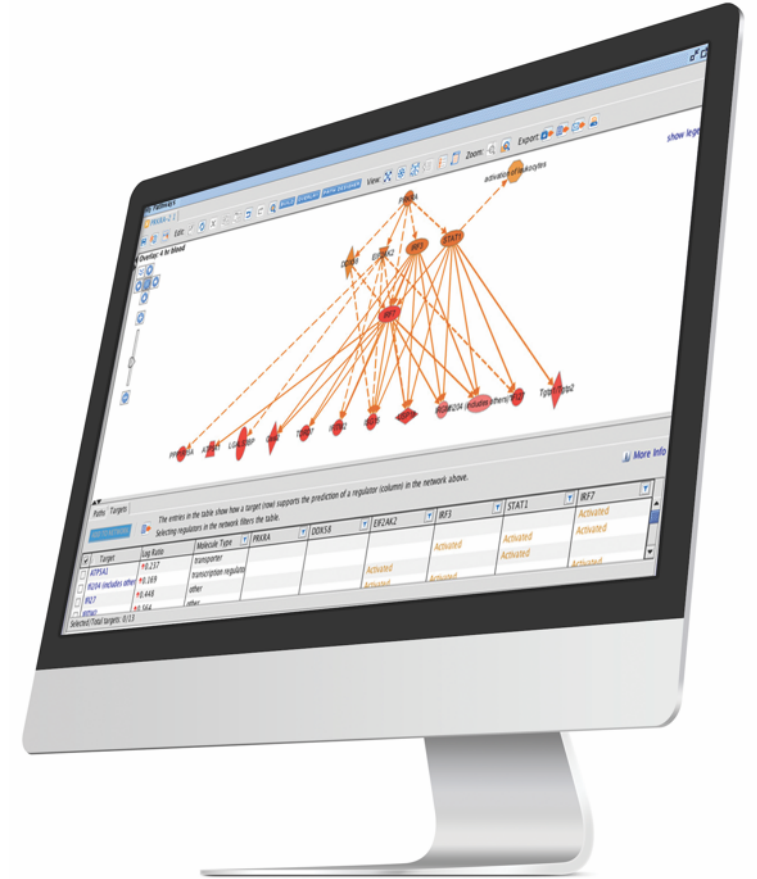
# Agenda

Analysis Match

What can you learn from analyses that are similar or different than yours?

Case study: Biological effects of gemfibrozil in the liver (of rat)

QIAGEN OmicSoft Land Explorer for IPA

Conclusions

# Analysis Match makes it easy to find insights

Discover which analyses resemble yours, to uncover insights from mechanistic similarities and differences



Match against your own analyses and

>73,000 other curated analyses
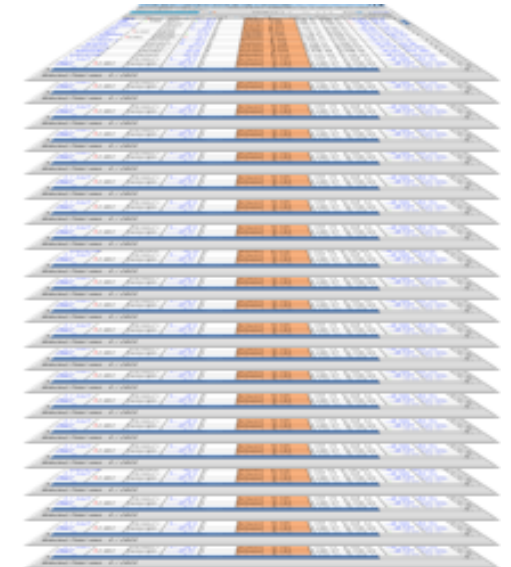
Which analyses have similar Upstream Regulators, Canonical Pathways, Diseases & Functions, etc.?

# Unprecedented discovery with Analysis Match

## Build confidence in your results

- Identify shared biology across disparate diseases, tissues, treatments and more

## Develop greater insights

- Evaluate upstream drivers, downstream phenotypes and biological pathways

## Identify key regulators/pathways

- Key in on similarly activated/inhibited entities across the groups

## Easily evaluate critical hypotheses

- Leverage an extensive collection of public data

## Applications

- Mechanism of action/toxicity
- Biomarker discovery through comparison analysis
- Target discovery/validation
- Drug repurposing

# More than 73,000 comparison datasets from QIAGEN OmicSoft Lands in QIAGEN IPA

## Lands provide expression data to QIAGEN IPA

DNA-Seq: SNPs, mutations CNVs

Gene expression: RNA-seq, microarray

>375,000 samples

RNA-seq: Fusions, exon Junctions

RNA-seq: SNPs, mutations

QIAGEN OmicSoft Land Platform

DNA methylation, protein expression

eQTL sensitivity (IC50), clinical data

Public annotation: 1000 Genomes, COSMIC, customer databases, others

GEO

SRA

ArrayExpress

Cancer Cell Line Encyclopedia

The Cancer Genome Atlas

International Cancer Genome Consortium

and others

# Analysis Match combines knowledge with data

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

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

Curation and QA

Reprocessed using the same pipeline

>7.2 million curated Findings

**QIAGEN OmicSoft Array Studio**
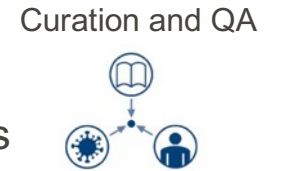
73,000+ expression comparison datasets

Built from >375,000 samples

**QIAGEN Ingenuity Pathway Analysis**

- Biological analyses of each dataset

- Compare your analysis to all QIAGEN OmicSoft analyses

Discover hidden relationships in your toxicological studies

# Case study: Biological effects of gemfibrozil in the liver (of rat)
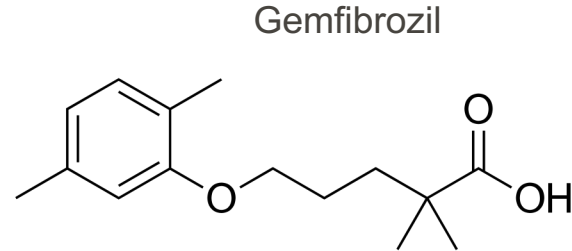


Gemfibrozil

Liver transcriptome

Lipid-lowering drug; Agonist of the Peroxisome Proliferator-Activated Receptor alpha (PPARα), a ligand-dependent transcription factor

What we expect to see:

# Using QIAGEN IPA to explore the biology of gemfibrozil in rat liver

## Analysis of liver expression of gemfibrozil-treated rats for 7 days compared to control

**Series GSE47875**                                    Query DataSets for GSE47875

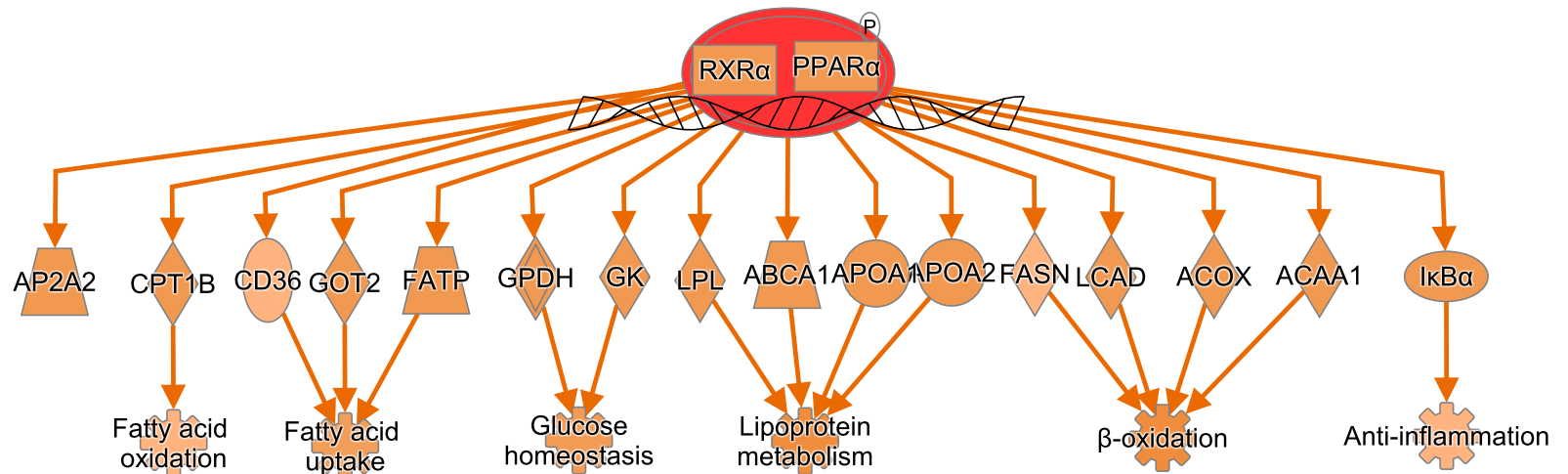| | |
|---|---|
| Status | Public on Aug 08, 2014 |
| Title | SEQC Toxicogenomics Study: microarray data set |
| Organism | Rattus norvegicus |
| Experiment type | Expression profiling by array |
| Summary | The comparative advantages of RNA-Seq and microarrays in transcriptome profiling were evaluated in the context of a comprehensive study design. Gene expression data from Illumina RNA-Seq and Affymetrix microarrays were obtained from livers of rats exposed to 27 agents that comprised of seven modes of action (MOAs); they were split into training and test sets and verified with real time PCR. |

contributor: DrugMatrix, National Toxicology program (NTP)          GSE47875

- Treatment group: 3 rats with 700 mg/kg gemfibrozil for 7 days
- Control group: 6 rats with corn oil for 7 days
- Illumina HiScanSQ FASTQ processed in QIAGEN OmicSoft Array Studio
- Analysis cutoffs in QIAGEN IPA:
  - Fold change: <-1.5 or >1.5
  - Adjusted p-value: <0.01
  - Average expression in experiment or control samples: >10 FPKM
- Analyzed 503 down-regulated and 461 up-regulated genes



chemical => GEMFIBROZIL vs Vehicle
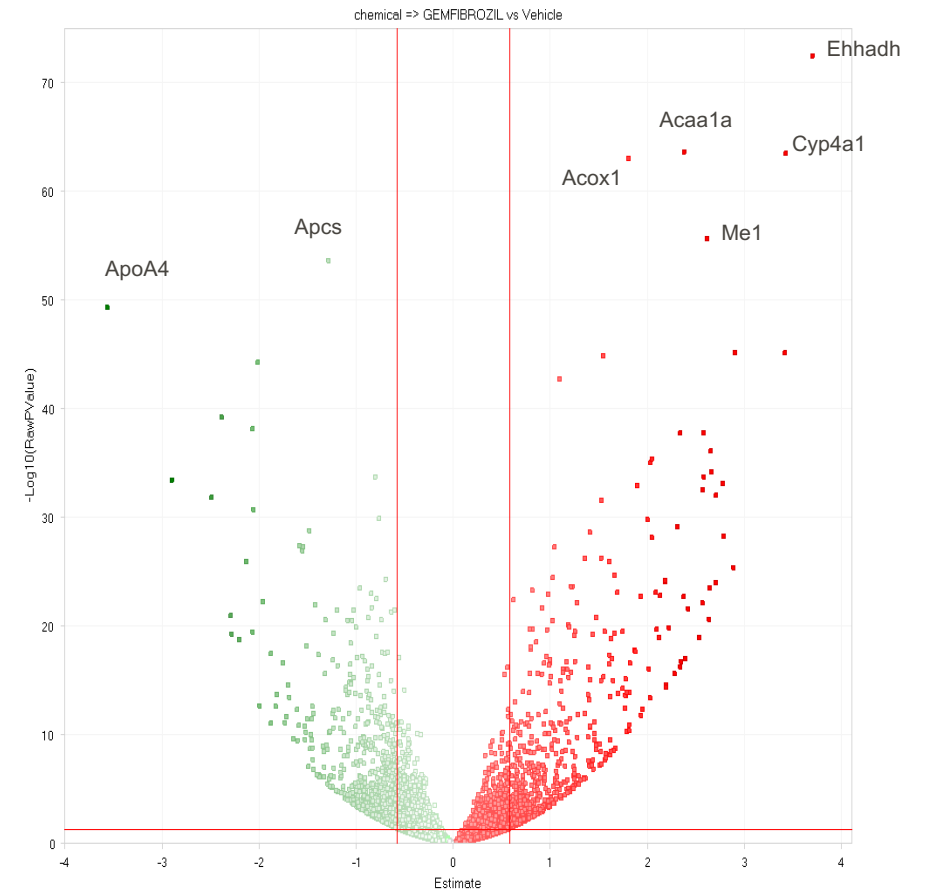
# Summary of IPA core analysis

## Effects on regulators and pathways (IPA Core Analysis)

### Activated regulators and pathways
- PPARα upstream regulator
- Cholesterol biosynthesis
- Fatty acid β-oxidation
- Ketogenesis

### Inhibited pathways
- LXR/RXR pathway
- Cholesterol transport
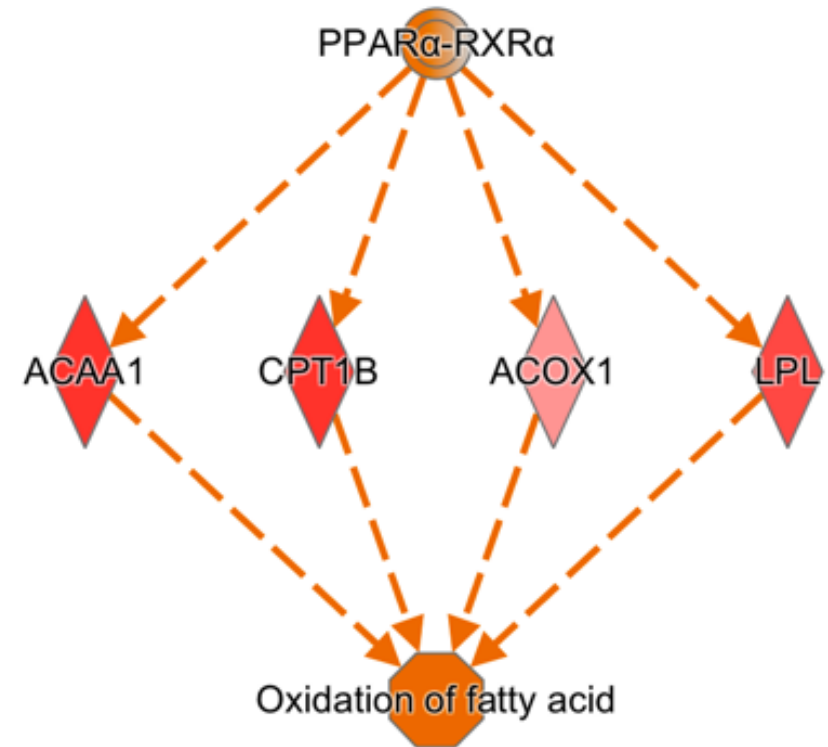
# Confirm known biology – we see the expected target activation

## Activation of RXRα/PPARα by gemfibrozil (predicted from the gemfibrozil RNA-seq data)



Note that PPARA itself is not significantly upregulated in the dataset

(zoomed in to show details)

# Confirm known biology – expected drug target, pathways and functional effects



## Examples of pathway impacts

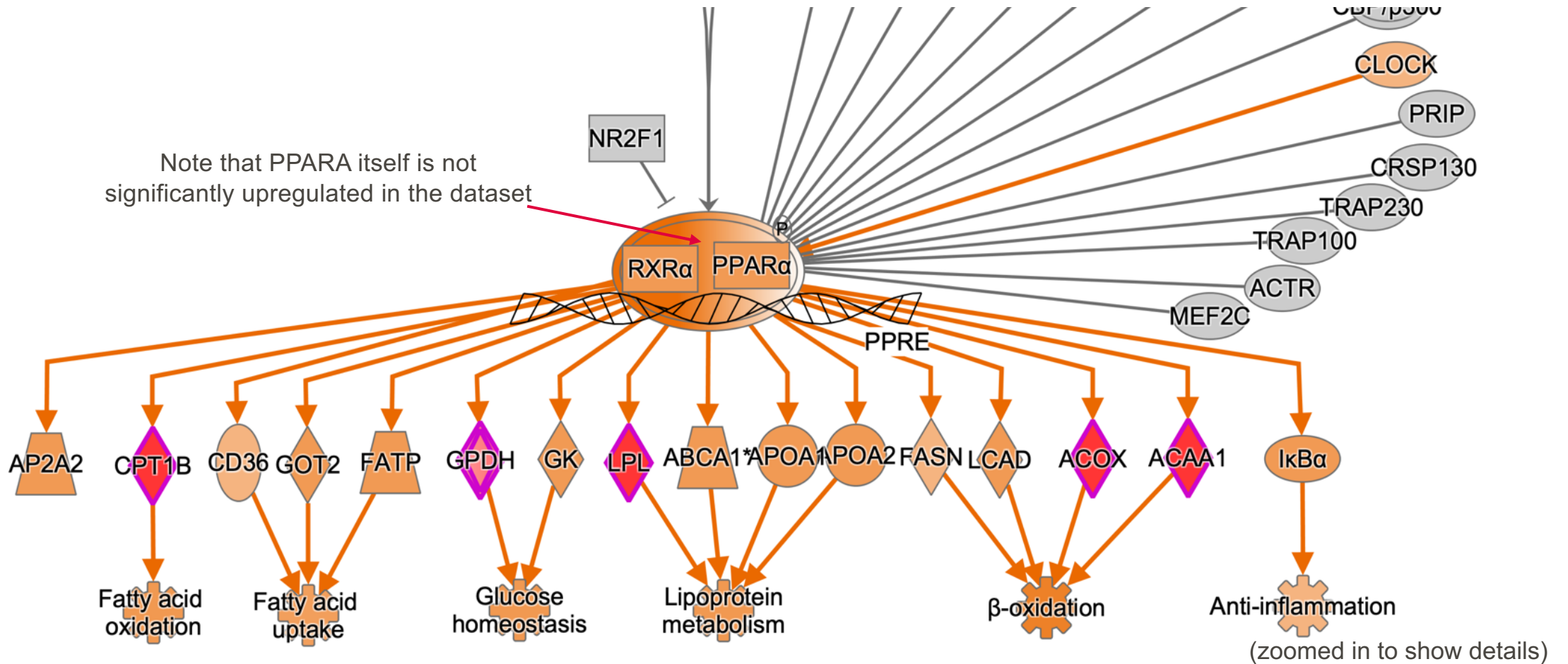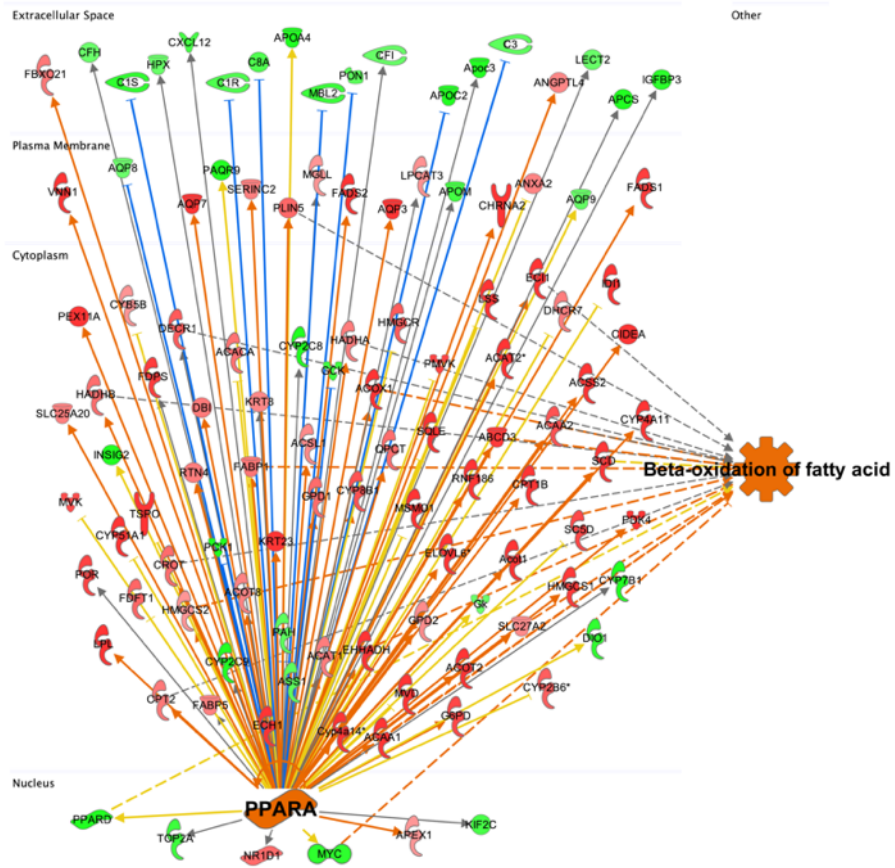| Canonical pathway | B-H p-value | z-score |
|---|---|---|
| Super pathway of cholesterol biosynthesis | 3.14E-14 | 4.8 |
| Ketogenesis | 7.05E-08 | 2.6 |
| Acyl-coA hydrolysis | 3.33E-06 | 2.4 |
| Fatty acid beta oxidation I | 4.84E-06 | 3 |
| Isoleucine degradation I | 8.01E-04 | 2.4 |
| LXR/RXR activation | 2.47E-11 | –3.7 |

## Examples of functional impacts

| Disease or function | B-H p-value | z-score |
|---|---|---|
| Oxidation of fatty acid | 2.30E-11 | 2.6 |
| Synthesis of cholesterol | 1.05E-17 | 2.1 |
| Vascularization | 3.50E-04 | –2.1 |
| Invasion of cells | 3.43E-04 | –2.2 |
| Cholesterol transport | 9.87E-05 | –3.2 |

■ Activated    ■ Inhibited

● What other conditions have predictions similar to these?

# How can we find matches to other analyses?

Conceptually, create signatures of the predicted 'entities' for every analysis and compare them

Query signature

| | Upstream regulator | Predicted activation |
|---|---|---|
| | PPARA | Activated |
| | ACSL3 | Activated |
| | INSR | Activated |
| | RPE65 | Activated |
| | SREBF1 | Activated |
| | SCAP | Activated |
| | SREBF2 | Activated |
| | ZNF423 | Activated |
| | PPARG | Activated |
| | POR | Inhibited |
| | ASXL1 | Inhibited |
| | NR1D2 | Inhibited |
| | ST3GAL5 | Inhibited |
| | CREB3L3 | Inhibited |
| | ACOX1 | Inhibited |
| | GRB14 | Inhibited |
| | PDE8A | Inhibited |

Compare →

Signature from another analysis

| Does it match? | Upstream regulator | Predicted activation |
|---|---|---|
| YES | PPARA | Activated |
| | ABDH5 | Activated |
| | ASXL2 | Activated |
| YES | RPE65 | Activated |
| YES | SREBF1 | Activated |
| | KLF15 | Activated |
| YES | SREBF2 | Activated |
| | BTN2A2 | Activated |
| | ACSBG1 | Activated |
| | NR1I3 | Inhibited |
| | CR1 | Inhibited |
| | ASXL1 | Inhibited |
| | DUT | Inhibited |
| YES | ACOX1 | Inhibited |
| | NR1I3 | Inhibited |
| YES | GRB14 | Inhibited |
| YES | PDE8A | Inhibited |

Create and score signatures for:
- Upstream Regulators
- Causal Networks
- Canonical Pathways
- Diseases & Functions

The **sign** of the entity (activated or inhibited) is important, but not its **order** in the signature

# Analysis Match results for gemfibrozil in rat liver

## Filtered to show top matching (>45% match) or top anti-matching (< -45% match)

Percent matching or anti-matching

Upstream Regulators

Diseases & Functions

Canonical Pathways

Master Regulators

Overall average

Filtered to exclude matches to own analyses

Examples of available metadata

Brief description of the comparison

| Analysis Name | Project | case.diseasestate | case.tissue | case.treatment | comparisoncate... | comparisoncontrast | CP (... | UR (... | CN... | DE (... | z... |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6- normal control [liver] NA 8236 | MouseDisease | normal control | liver | NA | Treatment vs. Control | Tissue:Compound => liver -> tesaglitazar vs s... | 84.52 | 64.81 | 63.32 | 33.33 | 61.49 |
| 5- normal control [liver] NA 8235 | MouseDisease | normal control | liver | NA | Treatment vs. Control | Tissue:Compound => liver -> fenofibrate vs sa... | 70.71 | 64.03 | 64.87 | 33.33 | 58.24 |
| 4- small intestine carcinoid neuro | OncoGEO | small intestine carcinoid neuroe... | small intestine | imatinib | Treatment vs. Control | Treatment:TreatTime[hours] => 24 -> imatinib... | 65.47 | 63.25 | 40.00 | 33.33 | 50.51 |
| 672- normal control [fetal testis] | HumanDisease | normal control | fetal testis | NA | Other Comparisons | ExperimentGroup => testis 10 weeks gestation... | 65.47 | 55.78 | 43.59 | 36.00 | 50.21 |
| 7- normal control [embryo] DMSO | MouseDisease | normal control | embryo | DMSO | Other Comparisons | Treatment:CultureCondition => DMSO -> N2B... | 65.47 | 60.00 | 47.96 | 27.22 | 50.16 |
| 1- normal control [liver] amiodaro | MouseDisease | normal control | liver | amiodarone | Treatment vs. Control | Treatment => amiodarone vs DMSO | 59.76 | 60.00 | 44.72 | 36.00 | 50.12 |
| 8- normal control [embryo] tazem | MouseDisease | normal control | embryo | tazemetostat | Other Comparisons | Treatment:CultureCondition => tazemetostat -... | 65.47 | 58.40 | 45.83 | 30.43 | 50.03 |
| 3- obesity [subcutaneous adipos | HumanDisease | obesity | subcutaneous adip... | differentiation medium | Treatment1 vs. Treatment2 | Tissue:Treatment => subcutaneous adipose tis... | 70.71 | 56.57 | 48.99 | 22.68 | 49.74 |
| 8- lung adenocarcinoma (LUAD) | HumanDisease | lung adenocarcinoma (LUAD) | lung | adipocyte exosome | Treatment vs. Control | Dosage => 10.56 ug/ml vs 0 ug/ml | 65.47 | 55.68 | 41.23 | 36.00 | 49.59 |
| 1- obesity [mesenteric adipose ti | HumanDisease | obesity | mesenteric adipose... | differentiation medium | Treatment1 vs. Treatment2 | Tissue:Treatment => mesenteric adipose tissu... | 65.47 | 64.03 | 67.08 | | 49.14 |
| 2- normal control [white adipose | MouseDisease | normal control | white adipose tissue | rosiglitazone | Treatment vs. Control | Transfection:Treatment => PRDM16 shRNA -> ... | 59.76 | 51.96 | 40.00 | 43.03 | 48.69 |
| 3- normal control [liver] NA 379 | MouseDisease | normal control | liver | NA | Treatment1 vs. Treatment2 | Genotype:CircadianTime => NOCT-/- -> Z18 v... | 70.71 | 64.81 | 58.31 | | 48.46 |
| 1- NA [epididymal white adipose | RatDisease | NA | epididymal white a... | NA | Treatment vs. Control | Tissue:SubjectTreatment => epididymal white... | 53.45 | 51.12 | 44.72 | 43.03 | 48.08 |
| 42- hypercholesterolemia [viscer | MouseDisease | hypercholesterolemia | visceral adipose tis... | NA | Treatment1 vs. Treatment2 | Tissue:ExperimentGroup => visceral adipose ti... | 70.71 | 59.16 | 61.64 | | 47.88 |
| 8- normal control [subcutaneous | HumanDisease | normal control | subcutaneous adip... | differentiation medium | Treatment vs. Control | Treatment:TreatTime[days] => 14 -> differenti... | 65.47 | 43.59 | 46.90 | 39.28 | 47.38 |
| 1- normal control [liver] NA 4109 | MouseDisease | normal control | liver | NA | Treatment vs. Control | SubjectTreatment:Gender:AnimalStrain => 129... | -70.71 | -44.91 | -31.62 | -43.03 | -47.57 |
| 3- normal control [white adipose | MouseDisease | normal control | white adipose tissue | none | Treatment vs. Control | Transfection:Treatment => none -> PRDM16 s... | -65.47 | -47.96 | -36.06 | -43.03 | -48.13 |
| 2- psoriasis [skin] NA 11962 | HumanDisease | psoriasis | skin | NA | Disease vs. Normal | SamplePathology => non-lesional vs normal | -80.18 | -60.00 | -52.92 | | -48.27 |
| 7- liver carcinoma [liver] phenol | OncoGEO | liver carcinoma | liver | phenol | Treatment vs. Control | Treatment => phenol vs none | -70.71 | -54.77 | -37.42 | -33.33 | -49.06 |
| 2- atopic dermatitis [skin] NA 39 | HumanDisease | atopic dermatitis | skin | NA | Disease vs. Normal | DiseaseState => atopic dermatitis vs normal c... | -75.59 | -69.34 | -51.96 | | -49.22 |
| 6- normal control [epididymal wh | MouseDisease | normal control | epididymal white a... | NA | Tissue1 vs. Tissue2 | Tissue:SubjectTreatment => low fat diet -> epi... | -59.76 | -53.85 | -48.99 | -36.00 | -49.65 |

Selected 0 / 21

● But what are the details behind the matching?

# Matching and anti-matching analyses fall into three distinct biological clusters

Part of upstream regulator signature:
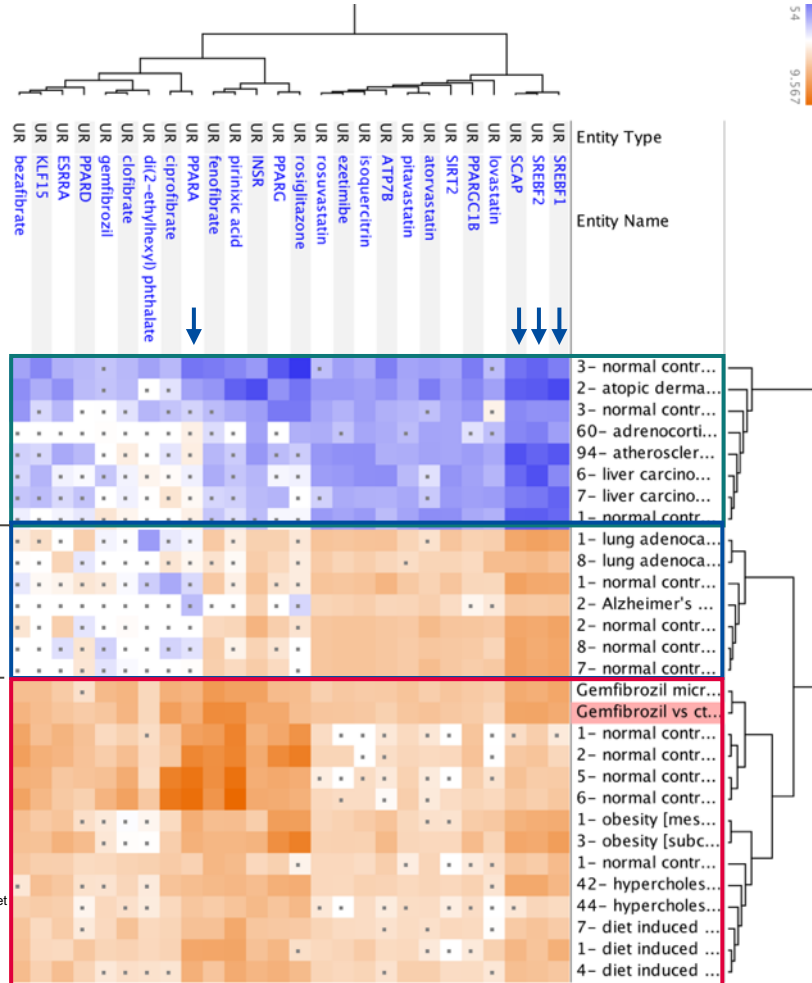


| species | case.tissue | Notes: | comparisoncontrast |
|---|---|---|---|
| mouse | white adipose | Knockdown of PPAR regulator | Transfection:Treatment => none -> PRDM16 shRNA vs control shRNA |
| human | skin | Disease state | DiseaseState => atopic dermatitis vs normal control |
| mouse | embryo | | GeneticModification:Treatment => none -> knockdown IRF3 vs control |
| human | adrenal gland | | NOTCH2_Somatic_Mutation_[DNASeq]_Status => MUT vs WT |
| mouse | liver | | AnimalStrain[maternal] => SM/J vs C57BL/6J |
| human | liver | | Treatment => N-nitrosodimethylamine (DMN) vs none |
| human | liver | | Treatment => phenol vs none |
| mouse | fetal neostriatum | | Treatment => 3-nitropropionic acid vs none |
| human | lung | Flu infection | SamplingTime => 24 hpi vs 0 hpi |
| human | lung | Adipocyte exosomes | Dosage => 10.56 ug/ml vs 0 ug/ml |
| mouse | liver | Caloric restriction | SubjectTreatment => calorie restriction vs normal diet |
| mouse | hippocampus | | DiseaseState => Alzheimer's disease (AD) vs normal control |
| mouse | embryo | | Treatment:TreatTime[hours] => 12 -> miconazole vs DMSO |
| mouse | embryo | Differentiation | Treatment:CultureCondition => tazemetostat -> N2B27 medium vs serum |
| mouse | embryo | Differentiation | Treatment:CultureCondition => DMSO -> N2B27 medium vs serum |
| rat | liver | PPAR alpha | Gemfibrozil vs corn oil (microarray) |
| rat | liver | PPAR alpha | Gemfibrozil vs corn oil (RNA-seq) |
| mouse | white adipose | PPAR gamma | Transfection:Treatment => control shRNA -> rosiglitazone vs none |
| mouse | white adipose | PPAR gamma | Transfection:Treatment => PRDM16 shRNA -> rosiglitazone vs none |
| mouse | liver | PPAR alpha | Tissue:Compound => liver -> fenofibrate vs saline |
| mouse | liver | PPAR gamma | Tissue:Compound => liver -> tesaglitazar vs saline |
| human | mesenteric adipose | Adipose differentiation | Tissue:Treatment => mesenteric adipose -> differentiation medium vs … |
| human | subcutaneous adipose | Adipose differentiation | Tissue:Treatment => subcutaneous adipose -> differentiation medium … |
| mouse | liver | PPAR gamma | Treatment => amiodarone vs DMSO |
| mouse | visceral adipose | High fat | Tissue:ExperimentGroup => visceral adipose -> high fat diet 9 weeks … |
| mouse | gonadal adipose | High fat | Tissue:SubjectTreatment => gonadal adipose -> high fat diet; pioglitazone vs chow diet |
| mouse | inguinal adipose | High fat, PPAR gamma | SubjectTreatment => high fat diet;rosiglitazone vs low fat diet |
| mouse | liver | High fat | AnimalStrain[maternal] => BFMI vs C57BL/6N |
| human | subcutaneous adipose | PPAR alpha | Treatment => amorfrutin 4 vs DMSO |

Clusters:

SCAP / SREBP / **PPARA**

SCAP / SREBP / **PPARA**

SCAP / SREBP / **PPARA**

# PRDM16 knockdown leads to down-regulation of several relevant genes

PRDM16 is a regulator of PPAR activity in adipose tissue and master transcriptional co-regulator in brown adipocytes, promoting expression of brown fat-selective genes and repression of white-selective genes
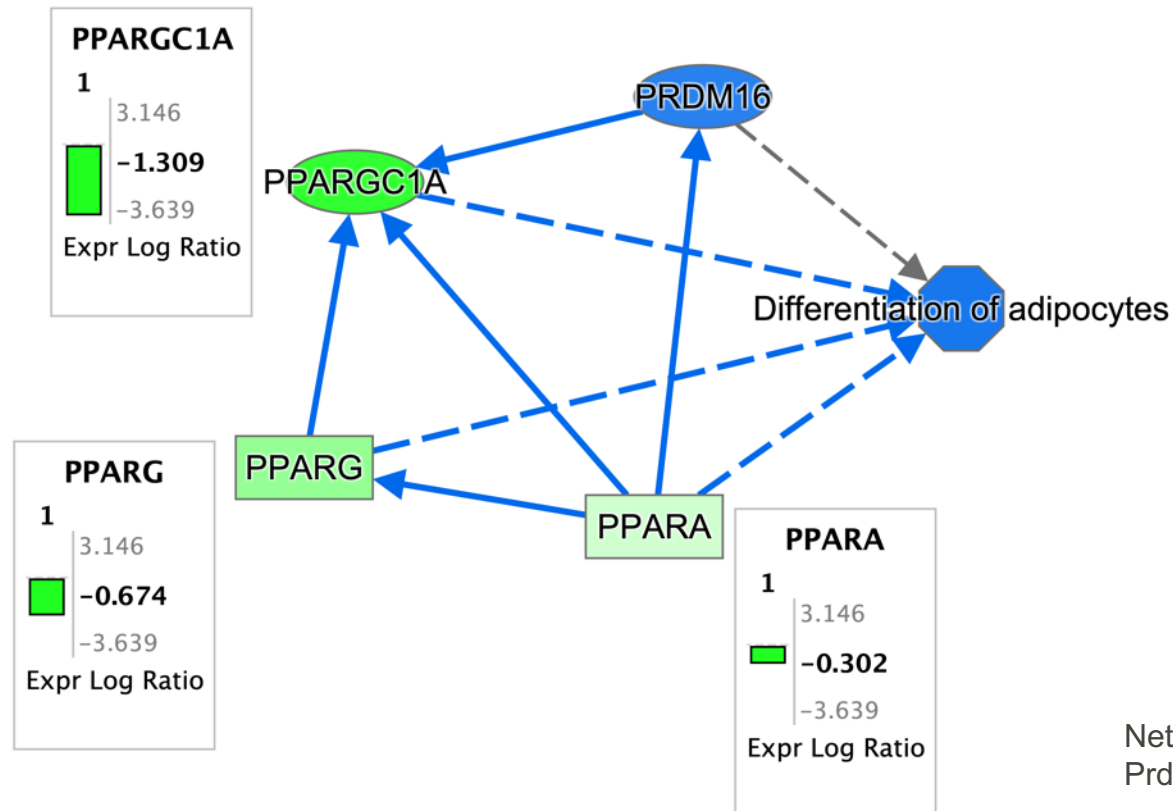


Network overlaid with expression profile of Prdm16 shRNA vs. scrambled control

# Atopic dermatitis "anti-matches" the gemfibrozil treatment

## PPARα is predicted to be inhibited in this condition; Application of PPARα agonists may treat it
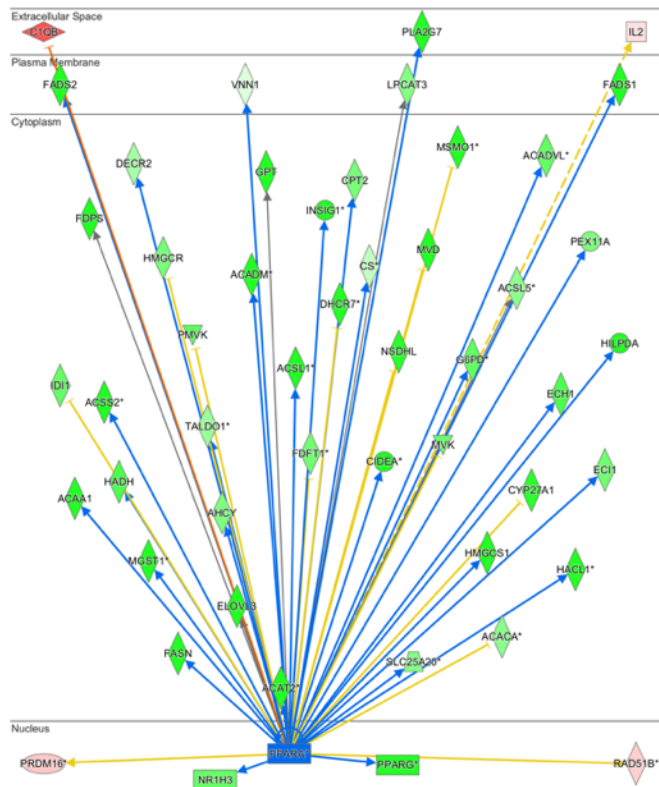


PPARA inhibited

Staumont-Sallé, D. et. Al.(2008) Peroxisome proliferator-activated receptor α regulates skin inflammation and humoral response in atopic dermatitis. Journal of Allergy and Clinical Immunology, 121, 962–968.

Chiba, T. et. Al.(2012) Topical application of PPARα (not β/δ or γ) suppresses atopic dermatitis in NC/Nga mice. Allergy, 67, 936–942.

# Clustering provides insight into the signature entities as well

Key regulators of cholesterol biosynthesis cluster with the enzymatic pathway, but are not members of the pathway
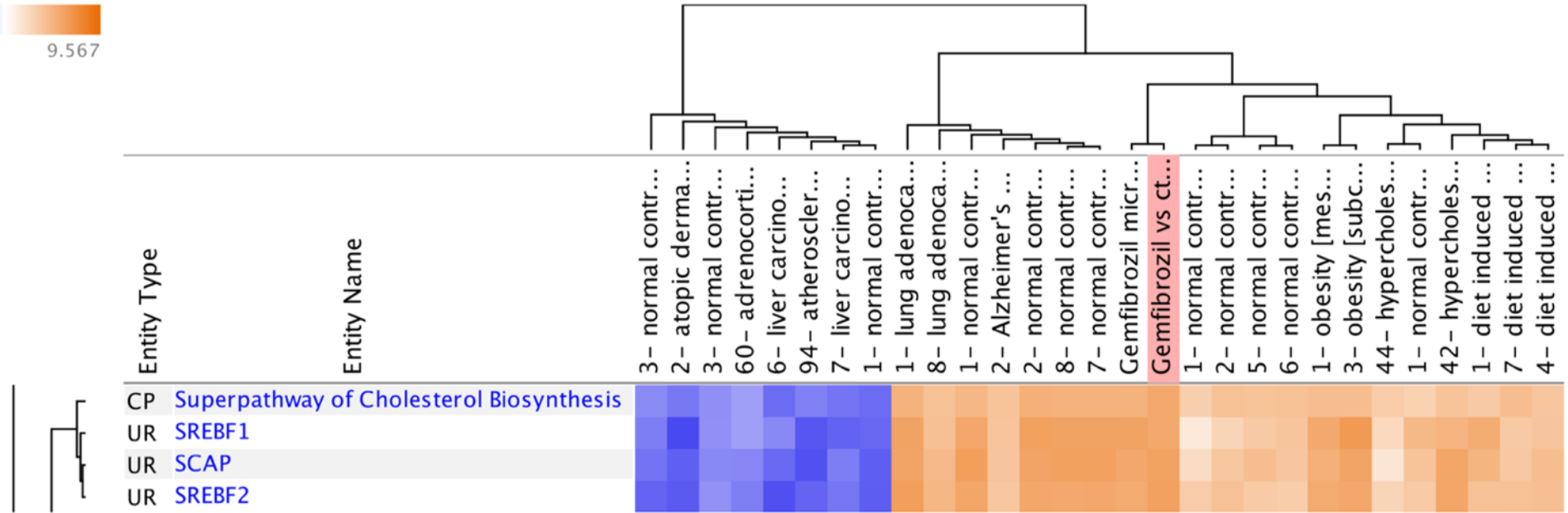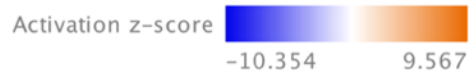


Heatmap rotated 90° from previous views

# All three regulators activate the rate-limiting step in cholesterol synthesis

## HMGCR is upregulated by gemfibrozil, consistent with the activation of the other regulators
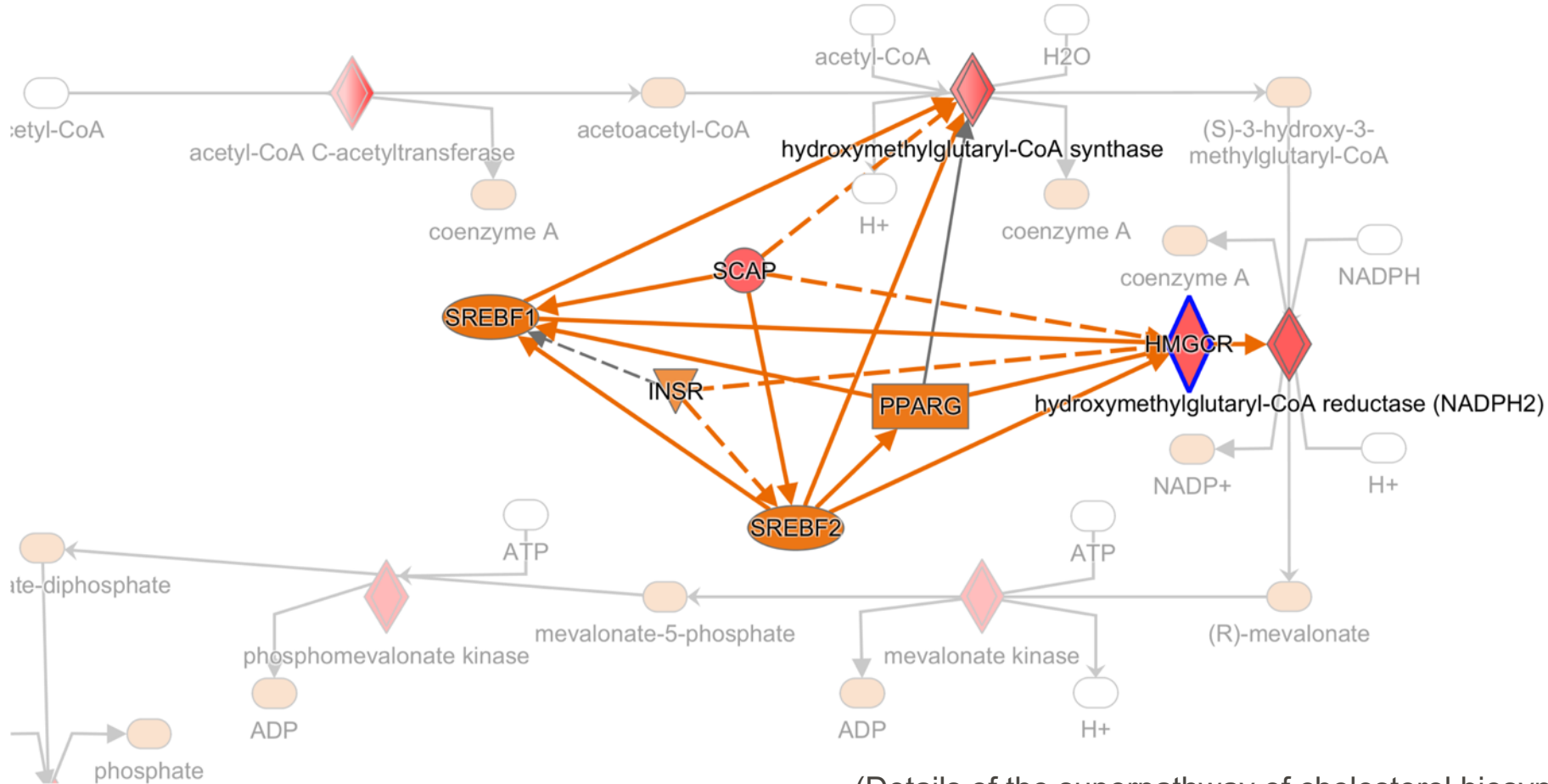


(Details of the superpathway of cholesterol biosynthesis pathway)

# Visualize and explore the activity of a single IPA entity with new Activity Plot



Evaluate metadata

Activity Plot requires an Analysis Match license

# What is the expression of a gene in normal human tissues?

## QIAGEN OmicSoft Land Explorer integration with IPA
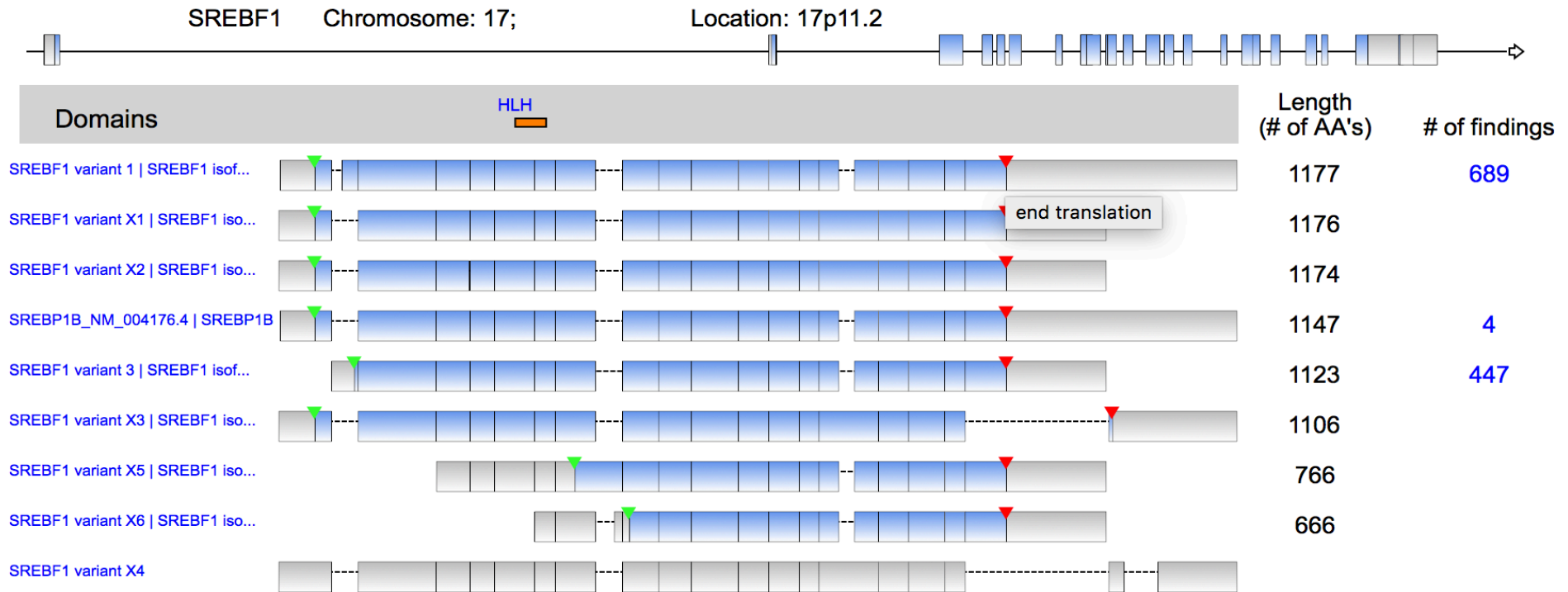


GTEx access from Isoform View free to all IPA users

# QIAGEN OmicSoft Land Explorer for IPA
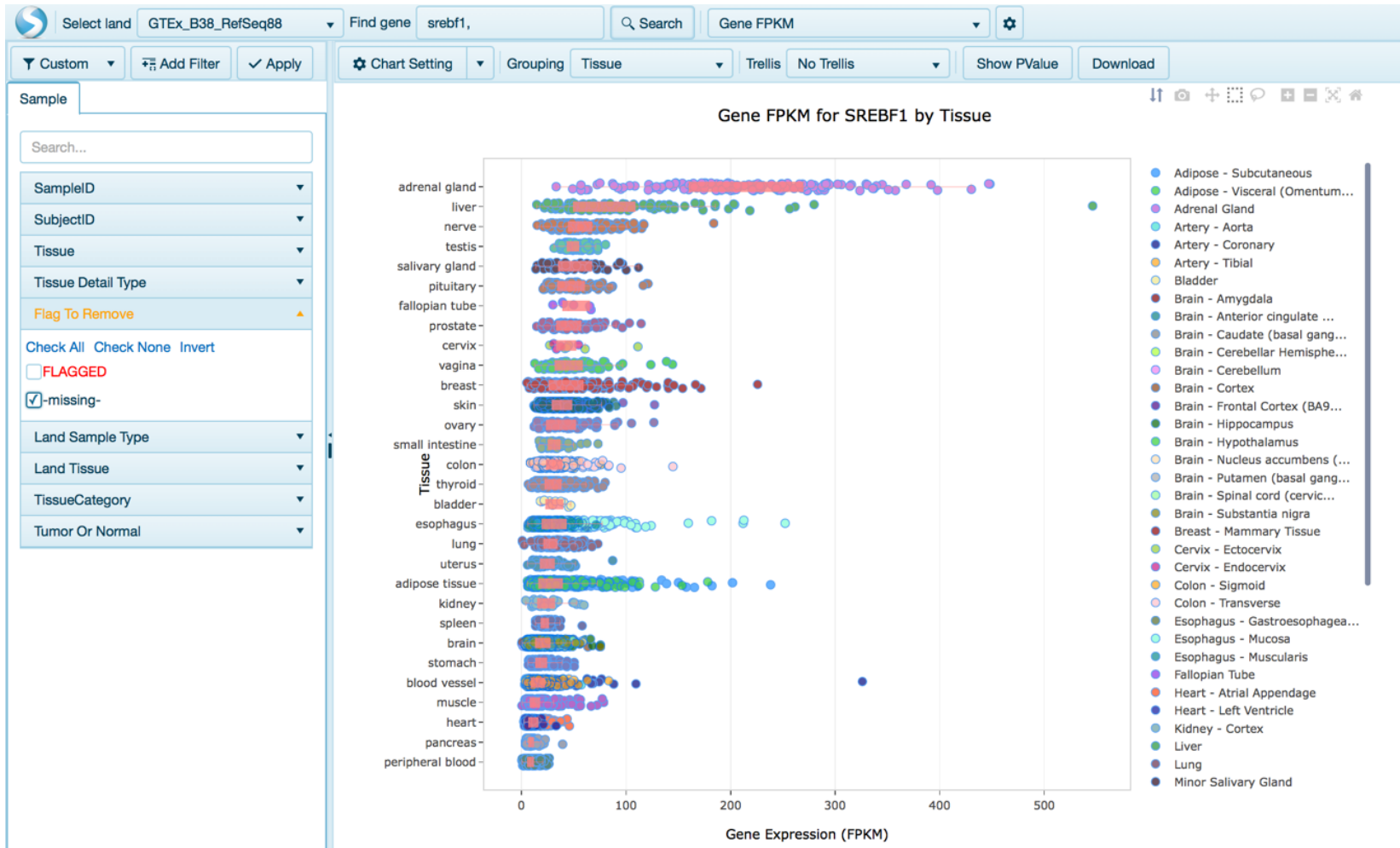


Display the GTEx data sample-level data for a gene or for individual splice variants in 51 human tissues

SREBF1 has highest expression in adrenal gland and liver in human subjects

GTEx access from Isoform View free to all IPA users

# New quick links to Full Land Explorer in IPA



Access to OmicSoft links requires Land Explorer license

# With Full Land Explorer for IPA, view gene & isoform expression data across Land samples

HMGCR is upregulated in atopic dermatitis in mouse, and psoriasis in human, as well as other conditions
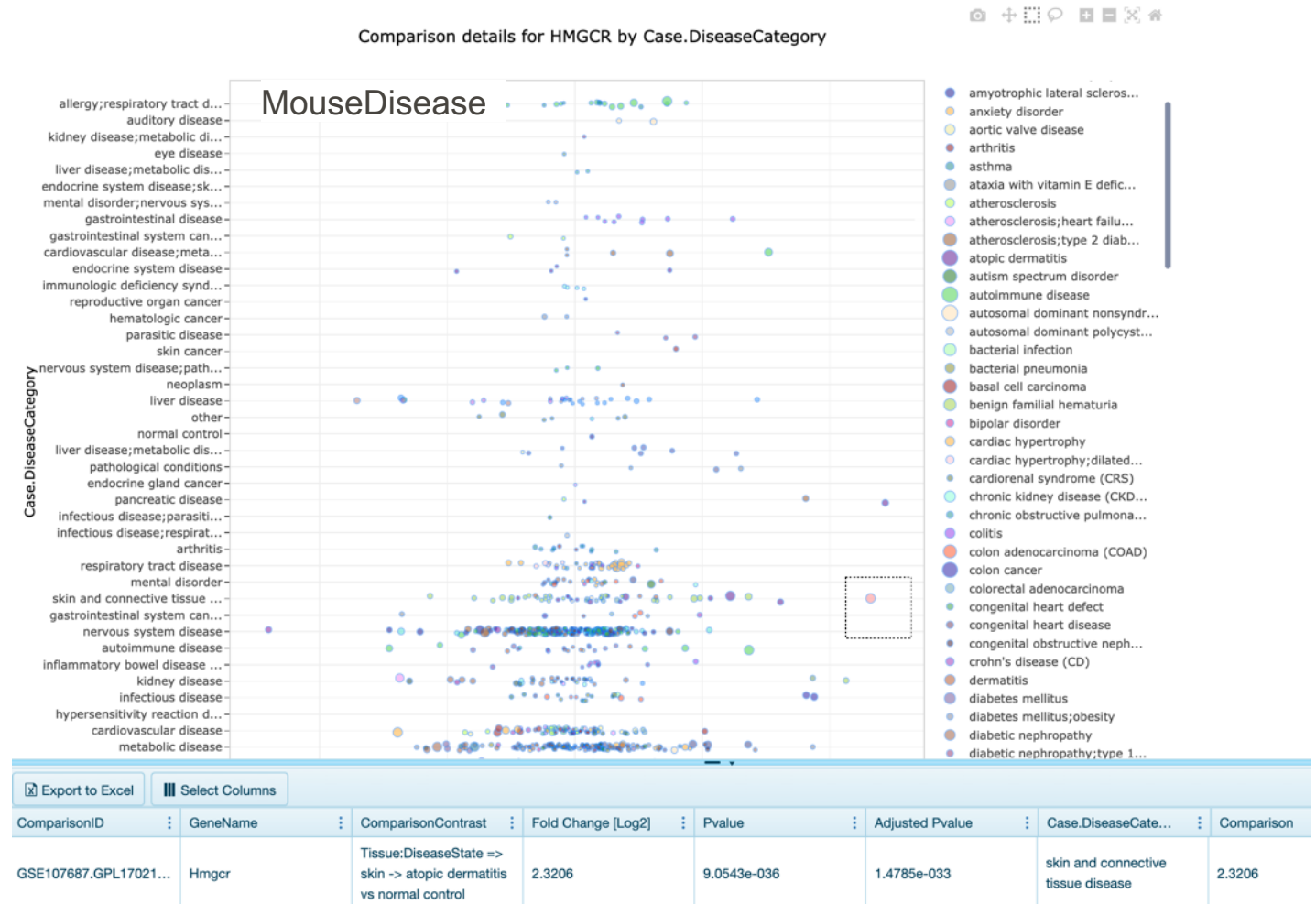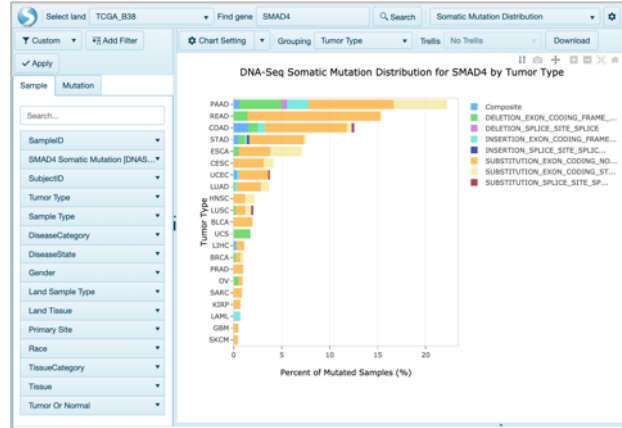
Full Land Explorer in IPA requires an additional license. Ask for a free trial!
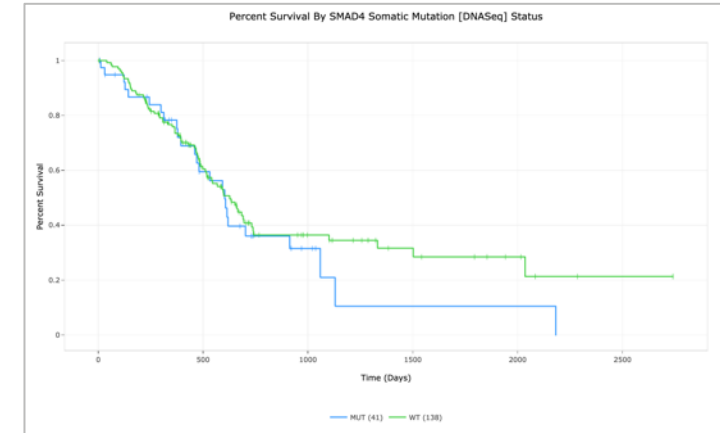
# Land Explorer can provide many insights


Expression in Rat, Mouse, and Human Disease


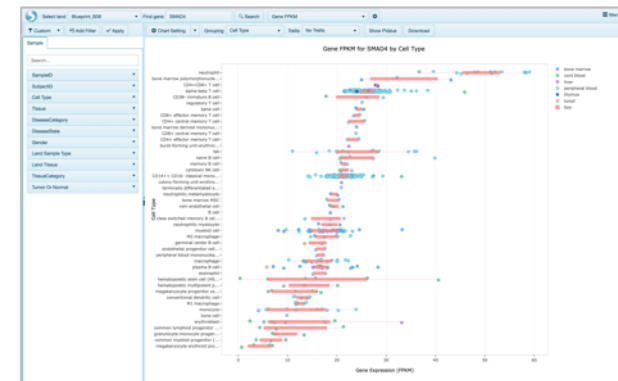Mutation frequency


Survival plots


Cell line expression


Tumor expression


Hematopoietic expression (BluePrint)

# Download the Analysis Match white paper

Literature-powered causal analytics from QIAGEN IPA combined with a massive, well-curated dataset collection provided by QIAGEN OmicSoft creates a unique opportunity for you to make biological discoveries

**https://go.qiagen.com/LP=1543**

# Resources

QIAGEN IPA

- IPA product info: https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa

- IPA Analysis Match: https://tv.qiagenbioinformatics.com/video/37242337/exploring-ipas-analysis-match-an

- Land Explorer: https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/content-exploration-and-databases/qiagen-omicsoft-land-explorer/

- Coronavirus Network Explorer: https://digitalinsights.qiagen.com/coronavirus-network-explorer/


QIAGEN  OmicSoft:

- Product Info: https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/qiagen-omicsoft/

QIAGEN CLC Genomics

- Product info: https://digitalinsights.qiagen.com/products-overview/analysis-and-visualization/qiagen-clc-genomics-workbench/

QIAGEN expands integrated coronavirus NGS and software solutions to accelerate COVID-19 research

- QIAseq SARS-CoV-2 Primer Panel converts viral RNA samples into libraries ready for sequencing

- QIAGEN Digital Insights solutions support COVID-19 drug, vaccine and epidemiology research

- For an overview of QIAGEN's coronavirus testing solutions, please visit http://www.qiagen.com/coronavirus.

- To explore QIAGEN's NGS-specific solutions for COVID-19 research, please visit https://go.qiagen.com/CoronavirusNGS

- For details of QIAGEN's SARS-CoV-2 Whole Genome Sequencing Service, please visit https://www.qiagen.com/applications/genomic-services/sars-cov-2-whole-genome-sequencing-services

# Customer support and additional resources

**Contact**

Contact us via email or telephone

**Reply**

A response within ONE business day

**Hours**

08:00 - 17:00 Pacific
08:00 - 13:00 GMT

Telephone:

- Global: +1 (650) 381-5111

- US toll free: +1 (866) 464-3684

- Denmark toll free: +45 80 82 01 67

- German toll: +49 (0)341 33975301

Email:

ts-bioinformatics@qiagen.com

Websites:

https://digitalinsights.qiagen.com/

http://tv.qiagenbioinformatics.com

# Trademarks