Information

Description

Core Analysis Results Tutorial

Overview

A **Core Analysis** has multiple ways of helping you find biological insights by automatically using the curated information from the QIAGEN Knowledge Base to put molecules in your 'omics dataset into context. For this tutorial, we will walk through the interpretation of a transcriptomics dataset.

The **Summary** tab displays the top results for all the analyses. See Analysis Summary Page.
Canonical Pathways tab displays your molecules of interest within well-established signaling or metabolic pathways. See Canonical Pathways Overview.
Upstream Analysis tab predicts which upstream regulators (any molecule that can influence the transcription or expression of another molecule) might be activated or inhibited to explain the expression changes in your dataset. See Upstream Regulator Analysis. It also connects upstream regulators into signaling cascades called Mechanistic Networks. If you have Advanced Analytics in IPA this tab can also display Causal Networks, or hierarchical networks or regulators controlled by a master regulator.
Diseases & Functions relates molecules in your dataset to known disease states and biological functions. See Downstream Effects Analysis Tutorial.
Regulator Effects displays hypotheses for how a phenotype, function or disease is regulated in your dataset by activated or inhibited upstream regulators See Regulator Effects.
Networks displays non-directional interaction networks of molecules based on known relationships in the QIAGEN Knowledge Base to your molecules of interest. See What are Networks?

Use these tools to find insights that are most relevant to your experimental model or question. Note that this tutorial will draw on only a *subset* of these features, due to length considerations.

Scenario
You have RNA microarray data from the white blood cells drawn from children with childhood exacerbated asthma compared to the convalescent state. For this example, we will use expression data from PBMCs from PMID 19620293. You would like to know if (and how) the data supports involvement of immune/inflammatory responses in acute asthma attack. You can also find novel gene-to-disease associations that can be followed up and confirmed with future wet bench experiments.

Tasks

Open the **Core Analysis** for your microarray data.
Use the **Summary Page** to quickly identify promising directions for exploration.
Explore the results for areas of importance to your research:
View **Canonical Pathways** that contain significant numbers of genes from your dataset.
Use **MAP** (Molecule Activity Predictor) to predict effects on functional endpoints in a canonical pathway.
Overlay **Biomarkers** that identify genes in the TREM1 Signaling Pathway that are used as efficacy indicators for asthma treatments.
Explore the impact on downstream **diseases and functions**.
Use **Regulator Effects** to see a hypotheses of how activation of certain upstream regulators may lead to outcomes like asthma.

Steps
1) Start IPA
2) In the **Project Manager**, double click My Projects > Example Analyses > Analyses > Childhood exacerbated asthma GSE16032. The analysis opens in a new window displaying the **Summary** tab:

Top Canonical Pathways

Name	p-value	Overlap
TREM1 Signaling	1.46E-09	32.9 % 23/70
Inflammasome pathway	7.57E-09	57.1 % 12/21
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	5.62E-08	23.1 % 30/130
phagosome formation	4.52E-07	23.0 % 26/113
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	1.56E-06	25.6 % 20/78

1 2 3 4 5 6 7 8 9 >

Top Upstream Regulators

Upstream Regulators

Name	p-value of overlap	Predicted Activation
Immunoglobulin	4.20E-29	Inhibited
lipopolysaccharide	9.23E-27	Activated
IL13	1.34E-25	
IL4	6.09E-21	Activated
TGFB1	6.26E-20	Activated

1 2 3 4 5 6 7 8 9 >

Causal Networks

Name	p-value of overlap	Predicted Activation
TH2 Cytokine	7.11E-32	Activated
IFNA8	8.86E-32	Activated
CD300LF	1.94E-31	Inhibited
TH1 Cytokine	1.27E-30	Activated
fluticasone	1.30E-30	

1 2 3 4 5 6 7 8 9 >

Top Diseases and Bio Functions

Diseases and Disorders

Name	p-value range	# Molecules
Infectious Diseases	1.55E-06 - 9.38E-24	340
Inflammatory Response	1.60E-06 - 7.93E-23	507
Immunological Disease	1.55E-06 - 1.31E-21	623
Respiratory Disease	6.48E-07 - 1.84E-21	180
Inflammatory Disease	1.11E-06 - 1.95E-21	384

1 2 3 4 5 6 7 8 9 >

Molecular and Cellular Functions

Name	p-value range	# Molecules
Cell-To-Cell Signaling and Interaction	1.60E-06 - 1.91E-26	366
Cellular Function and Maintenance	1.52E-06 - 1.04E-22	382
Cellular Movement	1.27E-06 - 6.12E-21	377
Cellular Development	1.54E-06 - 8.44E-21	520
Cell Morphology	1.46E-06 - 2.03E-20	339

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Physiological System Development and Function

Name	p-value range	# Molecules

Hematological System Development and Function	1.60E-06 - 1.91E-26	425
Tissue Morphology	1.55E-06 - 4.65E-23	314
Immune Cell Trafficking	1.60E-06 - 7.93E-23	254
Hematopoiesis	1.54E-06 - 8.44E-21	225
Lymphoid Tissue Structure and Development	8.84E-07 - 4.10E-16	255

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Top Tox Functions

Assays: Clinical Chemistry and Hematology

Name	p-value range	# Molecules
Increased Levels of Red Blood Cells	2.35E-03 - 2.35E-03	16
Increased Levels of ALT	4.33E-01 - 3.31E-03	7
Increased Levels of Hematocrit	7.88E-03 - 7.88E-03	14
Increased Levels of Creatinine	2.99E-01 - 7.93E-02	8
Increased Levels of AST	9.00E-02 - 9.00E-02	3

1 2 3 4 5 6 7 8 9 >

Cardiotoxicity

Name	p-value range	# Molecules
Cardiac Infarction	4.42E-01 - 2.53E-09	69
Congenital Heart Anomaly	1.00E00 - 6.32E-05	49
Cardiac Pulmonary Embolism	2.09E-01 - 1.65E-03	8
Cardiac Arrythmia	1.00E00 - 3.01E-03	41
Cardiac Fibrosis	3.33E-01 - 3.65E-03	25

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Hepatotoxicity

Name	p-value range	# Molecules
Liver Inflammation/Hepatitis	5.18E-01 - 2.08E-10	64
Liver Damage	4.80E-01 - 1.68E-08	56
Liver Hyperbilirubinemia	1.50E-01 - 8.80E-06	10
Hepatocellular Carcinoma	1.00E00 - 9.40E-05	109
Liver Hyperplasia/Hyperproliferation	1.00E00 - 9.40E-05	506

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Nephrotoxicity

Name	p-value range	# Molecules
Renal Damage	4.95E-01 - 3.50E-08	38
Renal Inflammation	5.56E-01 - 4.13E-08	60
Renal Nephritis	5.56E-01 - 4.13E-08	60
Kidney Failure	4.87E-01 - 4.07E-05	47
Renal Necrosis/Cell Death	5.56E-01 - 4.10E-05	63

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Top Regulator Effect Networks

ID	Regulators	Diseases & Functions	Consistency Score
1	APOE,Ifnar,IRF3,IRF6,IRF7,MET,NOS2,TFEB,TGM2,TLR2	endocytosis by eukaryotic cells (+5 more)	35.833
2	ADAMTS12,BID,IL17R,IL17RA,TNFSF12	accumulation of granulocytes (+9 more)	31.069
3	EGR1,FN1,IL17RA,LDL,LTBP1 (+6 more)	activation of neutrophils,adhesion of granulocytes (+3 more)	28.482
4	CYP2E1,EGR1,FN1,Ifnar (+4 more)	activation of granulocytes (+7 more)	26.167
5	ADAMTS12,Alpha catenin,IL17RA,INSIG1,mir-223,TBX5 (+2 more)	accumulation of granulocytes (+11 more)	23.326

Top Networks

ID	Associated Network Functions	Score
1	Cell Cycle, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair	38
2	Hereditary Disorder, Neurological Disease, Organismal Injury and Abnormalities	36
3	Gene Expression, Embryonic Development, Lymphoid Tissue Structure and Development	34
4	Hereditary Disorder, Neurological Disease, Organismal Injury and Abnormalities	34
5	Connective Tissue Disorders, Developmental Disorder, Hematological Disease	32

Top Tox Lists

Name	p-value	Overlap
Increases Renal Nephritis	1.63E-08	36.0 % 18/50
Increases Renal Damage	2.06E-07	27.6 % 21/76
Increases Liver Hepatitis	1.33E-05	27.3 % 15/55
Increases Liver Damage	5.23E-05	19.5 % 22/113
Renal Necrosis/Cell Death	1.66E-04	12.4 % 63/509

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Top Analysis-Ready Molecules

Exp Log Ratio ↑

Molecules	Value	Chart
IL1R2*	↑5.418	
MS4A4A*	↑3.333	
VSIG4	↑3.206	
VNN1*	↑3.088	
CD163*	↑2.736	
GPR34	↑2.609	
FKBP5*	↑2.550	
C3AR1	↑2.515	
CCR2*	↑2.468	
HBD	↑2.464	

Exp Log Ratio ↓

Molecules	Value	Chart
IFNG	↓-1.591	
SPTBN1*	↓-1.531	
WHAMM	↓-1.371	
DUSP8*	↓-1.298	
FGF9*	↓-1.243	
CD160	↓-1.222	
PLCL1*	↓-1.184	
ID3	↓-1.178	
LINC00282	↓-1.130	
ZNF331*	↓-1.124	

Summary Tab

The tab (shown above) lists the top results for the different types of analyses that are included in Core Analysis.
For this dataset:

Several immune-related Canonical Pathways are involved in asthma, such as the TREM1 Signaling and the Inflammasome Pathway.
Key cytokines are activated, such as IL4 and IFNA8.
Biological processes in the categories of Infectious Disease, Respiratory Disease, and Inflammatory Response are involved.
Regulator Effects networks indicate that several upstream regulators appear to drive accumulation of granulocytes and activation of neutrophils.
Interaction networks are discovered that are involved in processes such as cell cycle and organismal injury.

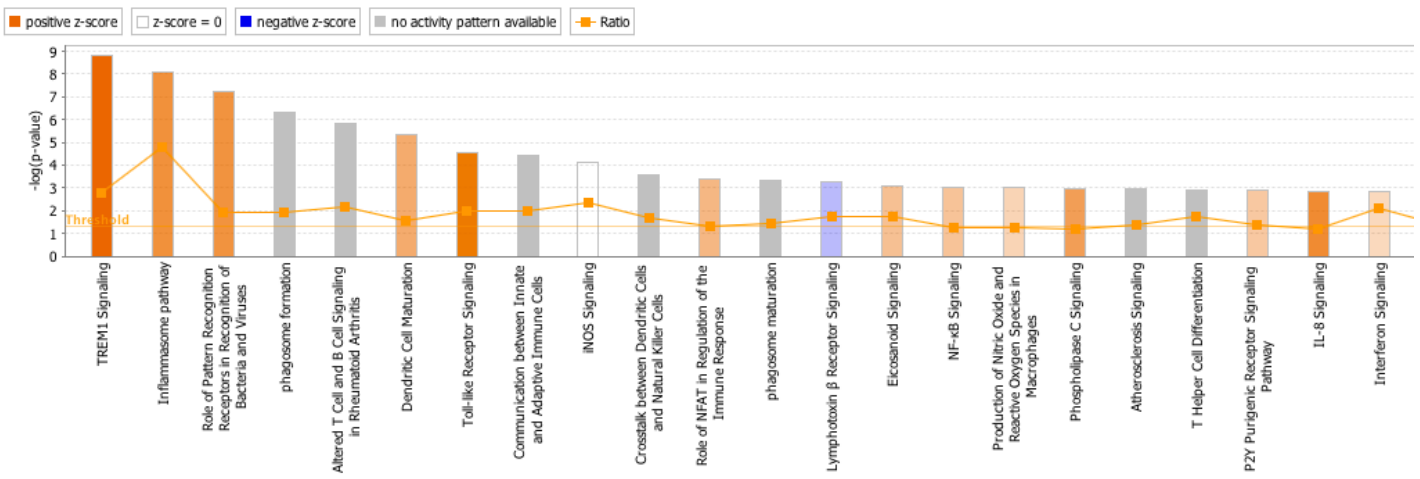
The p-values associated with the results are indicated on a scale in the Summary with red dots. Many of the results represent categories that contain many sub-categories, so a cluster of red dots is shown with each red dot indicating the p-value for each sub-category.

Note: The QIAGEN Knowledge Base is updated on a weekly basis. Details and information in these screen shots may not appear exactly the same as in the current version of this particular analysis.

Canonical Pathways tab

3) Click on the **Canonical Pathways** tab. Canonical Pathways provide information about what is known from the literature to occur on the cellular level in signaling and metabolic cascades. The TREM1 Signaling pathway is predicted to be activated (it has an orange colored bar in the bar chart) based on the expression pattern of the genes in this dataset. TREM1 is an important cell surface signaling molecule involved in the immune response. Click on the orange bar to display results from the TREM1 Signaling pathway.

The **upper pane** shows a bar chart where the height of the bars indicates the significance of the overlap of the molecules in your dataset to the pathways in the QIAGEN Knowledge Base. Significance values calculated based on the Fisher's right tailed exact test and the -log(p-value) are displayed on the y-axis of the bar chart. The taller the bar, the more significant the overlap of your dataset with the pathway. See Canonical Pathways for a Dataset to learn more about the calculations that are used. The color of the bars indicates whether the pathway is predicted to be activated (orange bars) or inhibited (blue bars), or if the pathway is ineligible for such an assessment (gray bars). See Pathway Activity Overlay for more details on the z-score calculation used to color the bars.



The lower pane:
Displays the molecules from your dataset that are members of the TREM1 pathway (which is shown because you clicked on that bar in the bar chart in the previous step). Note that some genes from the dataset may be "inside" groups or complexes that are on the pathway.

23 molecule(s) associated with **TREM1 Signaling** at **Childhood exacerbated asthma GSE16032** [Ratio: 23/70 (0.329)] [z-score: 3.962] [p-value: 1.46E-09]

VIEW REPORT | OPEN PATHWAY | ADD TO MY PATHWAY | ADD TO MY LIST | CREATE DATASET | CUSTOMIZE TABLE

Symbol	Entrez Gene Name	Identifier	Expression Value		Expected	Location	Type(s)	Biomarker Appli	Drug(s)
		Affymetrix	Exp Log Ratio	Exp p-value					
AKT2*	AKT serine/threonine	1560689_s_at	↓-0.651	9.25E-03	↑Up	Cytoplasm	kinase		triciribine,
CASP1*	caspase 1	206011_at*	↑1.137	1.19E-03	↑Up	Cytoplasm	peptidase		caspase 1
CASP5	caspase 5	207500_at	↑0.754	5.36E-03	↑Up	Cytoplasm	peptidase		
CD86*	CD86 molecule	205686_s_at*	↑0.732	5.32E-03	↑Up	Plasma	transmembrane	efficacy,	abatacept,
CXCL8*	C-X-C motif chemokine	202859_x_at*	↑0.629	2.03E-03	↑Up	Extracellular	cytokine	diagnosis,	
FCGR2B	Fc fragment of IgG	210889_s_at	↑1.052	4.19E-04	↑Up	Plasma	transmembrane		IgG
IL10	interleukin 10	207433_at	↑0.858	1.81E-04	↑Up	Extracellular	cytokine	diagnosis,	
IL18	interleukin 18	206295_at	↑0.767	2.62E-03	↑Up	Extracellular	cytokine	efficacy,	
LAT2*	linker for activation of	221581_s_at*	↑0.769	2.27E-03	↑Up	Plasma	other		
MAPK1*	mitogen-activated	1552263_at*	↑0.931	7.37E-03	↑Up	Cytoplasm	kinase	efficacy	MAP kinase1
MYD88	myeloid differentiation	209124_at	↑0.697	1.25E-03	↑Up	Plasma	other		IMO-8400
NFKB2*	nuclear factor kappa B	207535_s_at*	↓-0.602	8.29E-03	↑Up	Nucleus	transcription		
NLRC4*	NLR family CARD	1552553_a_at	↑1.810	4.16E-05	↑Up	Cytoplasm	other		
NLRP12*	NLR family pyrin domain	223944_at*	↑0.870	4.31E-03	↑Up	Cytoplasm	other		
PLCG2*	phospholipase C gamma	204613_at*	↑0.614	1.23E-04	↑Up	Cytoplasm	enzyme		
TLR1	toll like receptor 1	210176_at	↑0.731	2.07E-03	↑Up	Plasma	transmembrane		
TLR2	toll like receptor 2	204924_at	↑1.522	4.45E-05	↑Up	Plasma	transmembrane	diagnosis,	OM 174 lipid
TLR4*	toll like receptor 4	232068_s_at*	↑1.828	3.44E-05	↑Up	Plasma	transmembrane	efficacy	resatorvid, OM
TLR5	toll like receptor 5	210166_at	↑1.208	1.15E-03	↑Up	Plasma	transmembrane		
TLR6*	toll like receptor 6	239021_at*	↑0.614	8.24E-04	↑Up	Plasma	transmembrane		
TLR7*	toll like receptor 7	220146_at*	↑1.245	5.48E-03	↑Up	Plasma	transmembrane		3M-001, UC-
TLR8*	toll like receptor 8	229560_at*	↑1.903	8.66E-06	↑Up	Plasma	transmembrane		VTX-2337,
TREM1	triggering receptor	219434_at	↑1.012	7.74E-05	↑Up	Plasma	transmembrane	efficacy	

Selected/Total molecules: 0/23

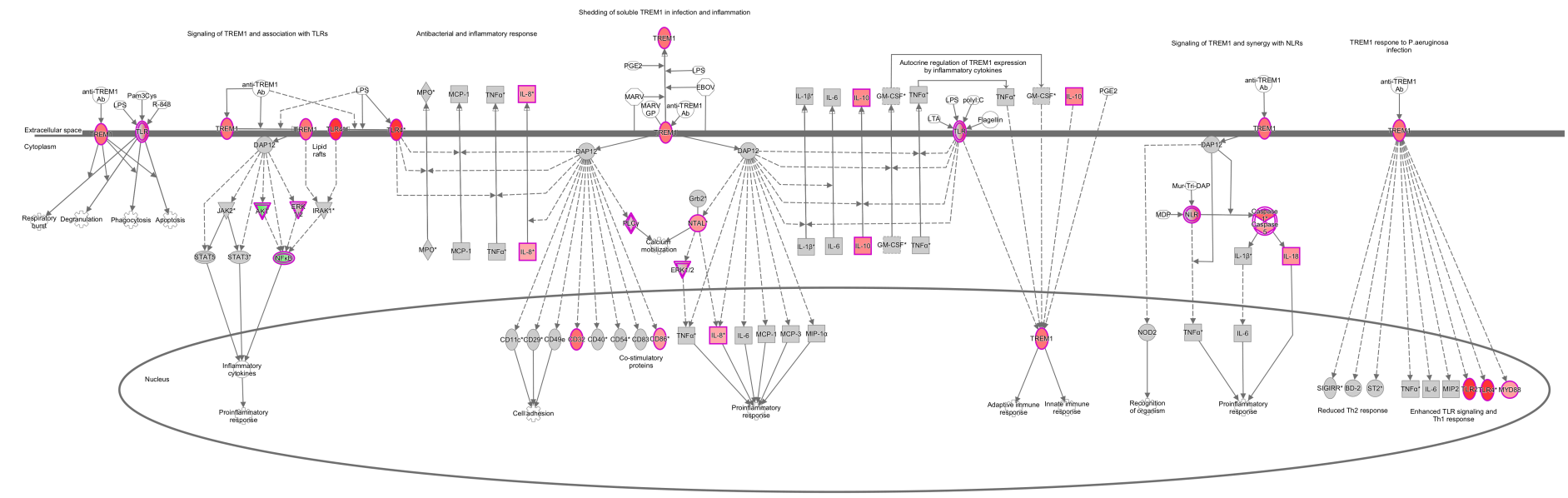
The table conveys the following information that can be useful in interpreting the results of your experiment:

The Gene Symbol and Entrez Gene name, which identifies specific molecules in the pathway that are affected.
The observed expression changes in the dataset, in this example, log ratio and p-value.
The identifier uploaded from the dataset.
The expected "direction" (up or down regulated) for the molecule in the pathway, if the pathway were activated. This column is available only for pathways that are eligible for Pathway Activity Analysis.
The cellular location of the molecule and the molecule type.
If the molecule has been identified as a biomarker it will indicate the type of biomarker (diagnosis, efficacy, etc.), which is helpful in determining if the molecule has been studied in a particular disease state that is relevant to the one your are studying.
If there are any drugs that target a given molecule, information on how and in what biological context the molecule has been targeted.

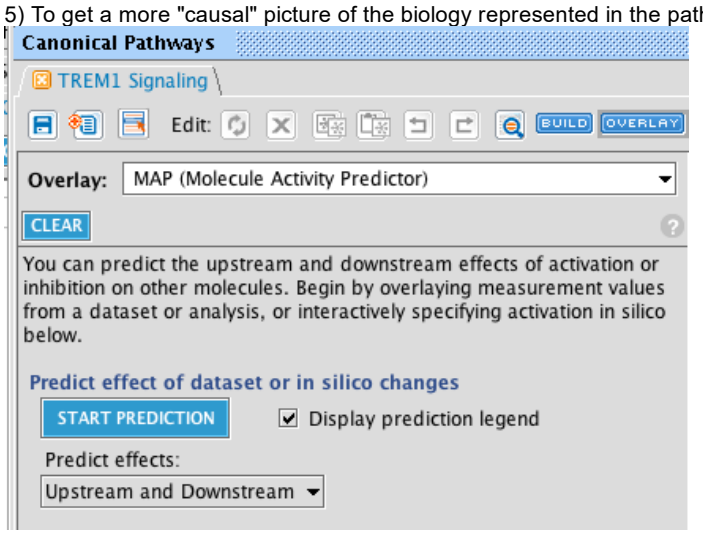
If the pane is too small, you can drag the vertical partition bar up to adjust it.

4) Click the **Open Pathway** button at the top right of the lower pane. A new window displays the canonical pathway diagram.

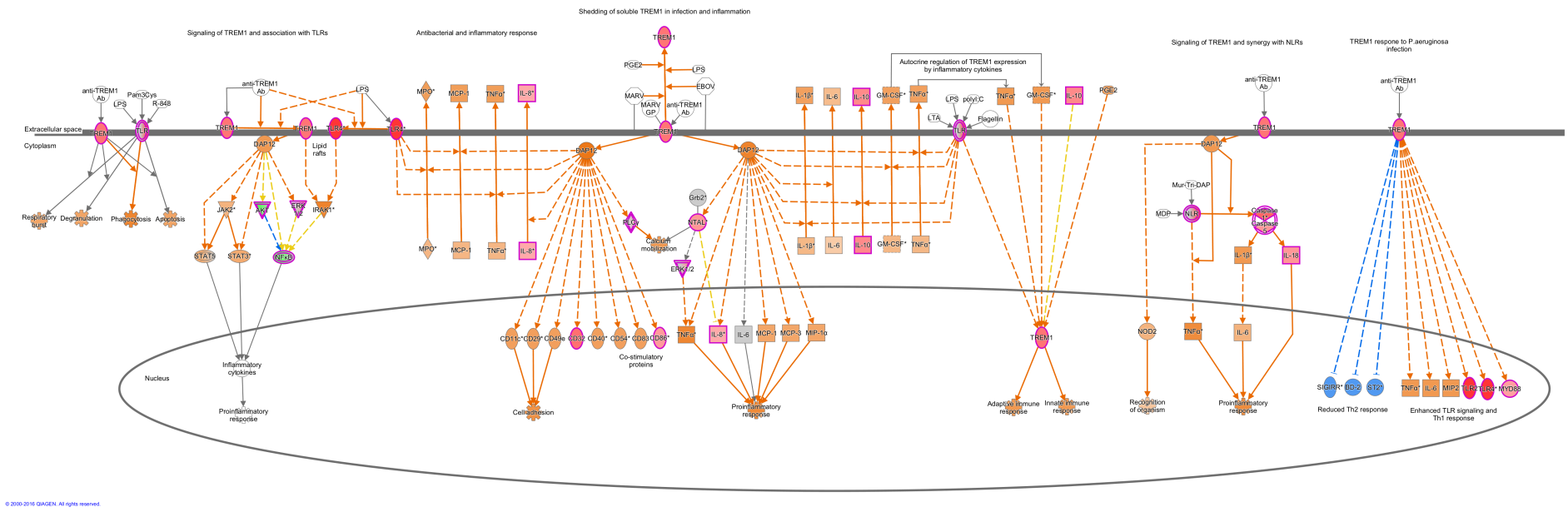
Canonical pathways are usually directional, following the biological information flow in the cell. Arrows and top to bottom flow indicate upstream and downstream location, respectively.
Dataset molecules that meet the filters and data value cutoff criteria for up- and down-regulation are shaded red and green, respectively.
Dataset molecules that are below the cutoff or do not meet filter criteria are shaded grey in the pathway.
Pathway molecules that are not in your dataset are white.
The shapes and positions of the molecules in the pathway define gene type and cellular location, respectively.
Double outlined shapes represent groups of molecules (generally protein families). Groups can be multicolored to indicate they include both up- and down-regulated molecules as their members. Right click and choose "Show Members/Membership" to expand groups to see their individual members.
The pathway shows the biological picture of the activation of the TREM1 pathway, indicating that not only are several of the key receptors up-regulated in asthma, so are a number of downstream effectors.



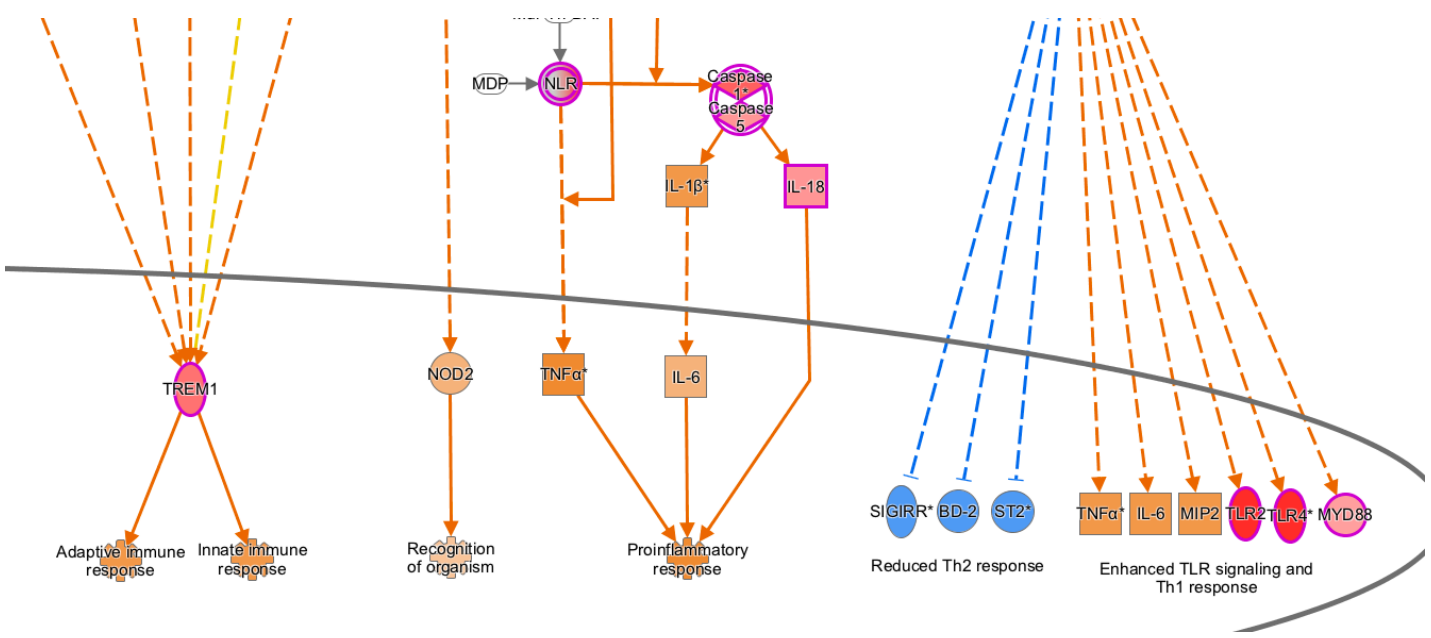
5) To get a more "causal" picture of the biology represented in the pathway, go to **Overlay > MAP (Molecule Activity Predictor)** and click the Start Prediction button.



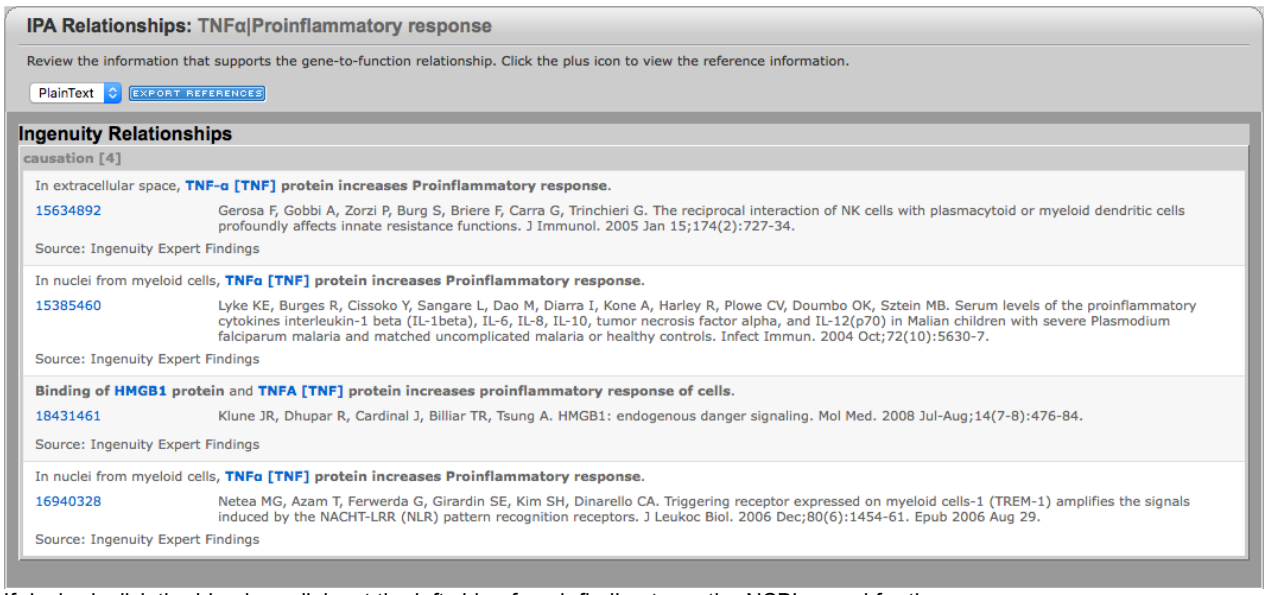
This predicts the activity of nodes on the pathway that are not part of your dataset-- i.e. it colors as many gray or white nodes in the pathway orange or blue as possible based on the expected influence of the molecules in the dataset that are up or down regulated (i.e. the red and green molecules on the pathway). For example, if a gene is upregulated in your dataset, and the literature states that it activates a downstream gene that it is connected to in the pathway diagram, then that downstream gene is predicted to be activated. Orange nodes indicate predictions of activation, and blue nodes indicate prediction of inhibition.



Zooming in on the bottom of the pathway, you can see that several biological functions related to immunity are predicted to be increased in the pathway. For example, both adaptive and immune responses as well as proinflammatory response are predicted to be increased.

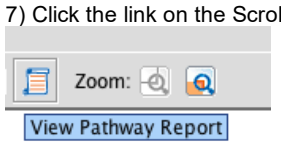


6) Double click on any relationship on the pathway to view the curated findings and the literature support for it. Clicking on the line between TNFa and the biological function "Proinflammatory response" brings up this summary:



If desired, click the blue hyperlinks at the left side of each finding to go the NCBI record for the paper.

7) Click the link on the Scroll icon in the pathway tool bar to read the curated report about this pathway.



The pathway report discusses the role of the TREM1 pathway in immune function, and shows that several of the proteins on the pathway are targets in drug trials for asthma:

INGENUITY PATHWAY ANALYSIS

Canonical Pathway

Report Date: 2016-10-12
Report Version: 401642
Content Version: 28820210 (Release Date: 2016-09-24)

Provide Feedback | Live Support | Download Report (PDF)

Canonical Pathway: **TREM1 Signaling**

Description: The triggering receptor expressed on myeloid cells 1(TREM1) belongs to the Immunoglobulin (Ig) family of cell surface receptors and is selectively expressed on blood neutrophils, monocytes and macrophages. TREM-1 lacks known signaling motifs in the cytoplasmic domain and thus activation by TREM1 is mediated by a transmembrane adaptor molecule DNAX-activating protein 12 (DAP12), leading to proinflammatory immune responses. The natural ligand for TREM1 is however, unknown.

TREM1 activation triggers the Janus kinase 2 (JAK2), protein kinase B (PKB/AKT) and extracellular signal related kinase (ERK1/2) pathways leading to the phosphorylation of signal transducers of activation of transcription (STAT3, 5) and NF kappa B (NF-κB). These transcription factors upregulate the expression of genes involved in the inflammatory response. Stimulation of TREM by its ligand or toll like receptor (TLR) by lipopolysaccharide (LPS) can lead to an association of TREM1 and TLR. This association leads to the activation of interleukin-1 receptor-associated kinase 1(IRAK1), which in turn triggers NF-κB and the proinflammatory response. Engagement and activation of TREM-1 triggers expression and secretion of chemokines and cytokines like monocyte chemotactic protein 1(MCP-1) macrophage inflammatory protein-1alpha (MIP-1α), interleukins (IL-6,-8) and tumor necrosis factor (TNF).Many of these effects are potentiated by LPS. Cytokines like TNF and Granulocyte macrophage colony stimulating factor (GM-CSF) in turn upregulate the expression of TREM1 in an autocrine fashion. The synergy between TLR and TREM1 leads to neutrophil degranulation, phagocytosis and the respiratory burst in addition to the production of proinflammatory cytokines. TREM1 activation also results in the upregulation of cell surface proteins like CD11, CD29 and CD40, CD83 that are involved in cell adhesion and costimulation respectively, as well as phospholipase gamma (PLCγ) mediated Ca2+ release. Thus TREM1 activation is involved in diverse aspects of innate and adaptive immune response.

In addition to TLRs, TREM1 also synergizes with a second major class of pattern recognition receptors -the NACHT-LRR receptors (NLR), which recognize intracellular microorganisms. The TREM-1/NLR synergism results in the production of proinflammatory cytokines like TNF, IL-1β, IL-6 and IL-18- the latter three via a caspase -1 dependent pathway. Thus TREM-1 acts to amplify signals from both major pathways of pattern recognition- extracellular TLR receptors and the intracellular NLR proteins.

This pathway highlights the important components of TREM1 signaling.

Signaling Pathway Categories: Cellular Immune Response; Cytokine Signaling

Top Functions & Diseases: Cell-To-Cell Signaling and Interaction; Hematological System Development and Function; Immune Cell Trafficking

Molecules: **show all** adaptive immune response, Akt, anti-TREM1 Ab, apoptosis, CASP1, Casp1-Casp5, CASP5, CCL2, CCL3, CCL7, CD40, CD83, CD86, cell adhesion, CSF2, CXCL3, CXCL8, DEFB4A/DEFB4B, degranulation, EBOV, ERK1/2, FCGR2B, Flagellin, GRB2, ICAM1, IL10, IL18, IL1B, IL1RL1, IL6, innate immune response, IRAK1, ITGA5, ITGAX, ITGB1, JAK2, L-Ala-γ-D-Glu-meso-diaminopimelinic acid, LAT2, lipopolysaccharide, lipoteichoic acid, MARV, MARV GP, mobilization of Ca2+, MPO, MYD88, N-acetylmuramyl-L-alanyl-D-isoglutamine, NFkB (complex), NLR, NOD2, Pam3-Cys, phagocytosis

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Drug Summary - Overview of drugs targeting molecules in Canonical Pathway

Showing 3 of 111 row(s) of Drug data. (Show All)

Drug Name	Targets	Actions	Brand Names	Indications/Status
3M-001	TLR7	agonist		acute lymphocytic leukemia/Phase 2 acute myeloid leukemia/Phase 2 Barrett's syndrome/Unspecified phase
5-fluorouracil/imiquimod [imiquimod]	TLR7	stimulator		
abatacept	CD86	binder	Orencia	allergic asthma/Phase 2 alopecia areata/Phase 2 ankylosing spondylitis/Phase 2

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Target Information - Overview of known drug targets in Canonical Pathway

Showing 3 of 29 row(s) of Target data. (Show All)

Target (Gene Symbol)	Entrez Gene Name	Location	Type	Drug(s)	Species
Akt		Cytoplasm	group	afuresertib, AT13148, ipatasertib, MSC2363318A, ONC-201, SR-13668	Human, Mouse, Rat
AKT1	AKT serine/threonine kinase 1	Cytoplasm	kinase	archexin, ARQ 092, AZD5363, BAY1125976, enzastaurin, GSK2141795, ipatasertib, LY2780301, MK2206, MPT0E028, perifosine, triciribine, triciribine phosphate	Human, Mouse, Rat
AKT2	AKT serine/threonine kinase 2	Cytoplasm	kinase	BAY1125976, enzastaurin, triciribine, triciribine phosphate	Human, Mouse, Rat

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Supporting References (Show details) - References from which the Canonical Pathway was derived

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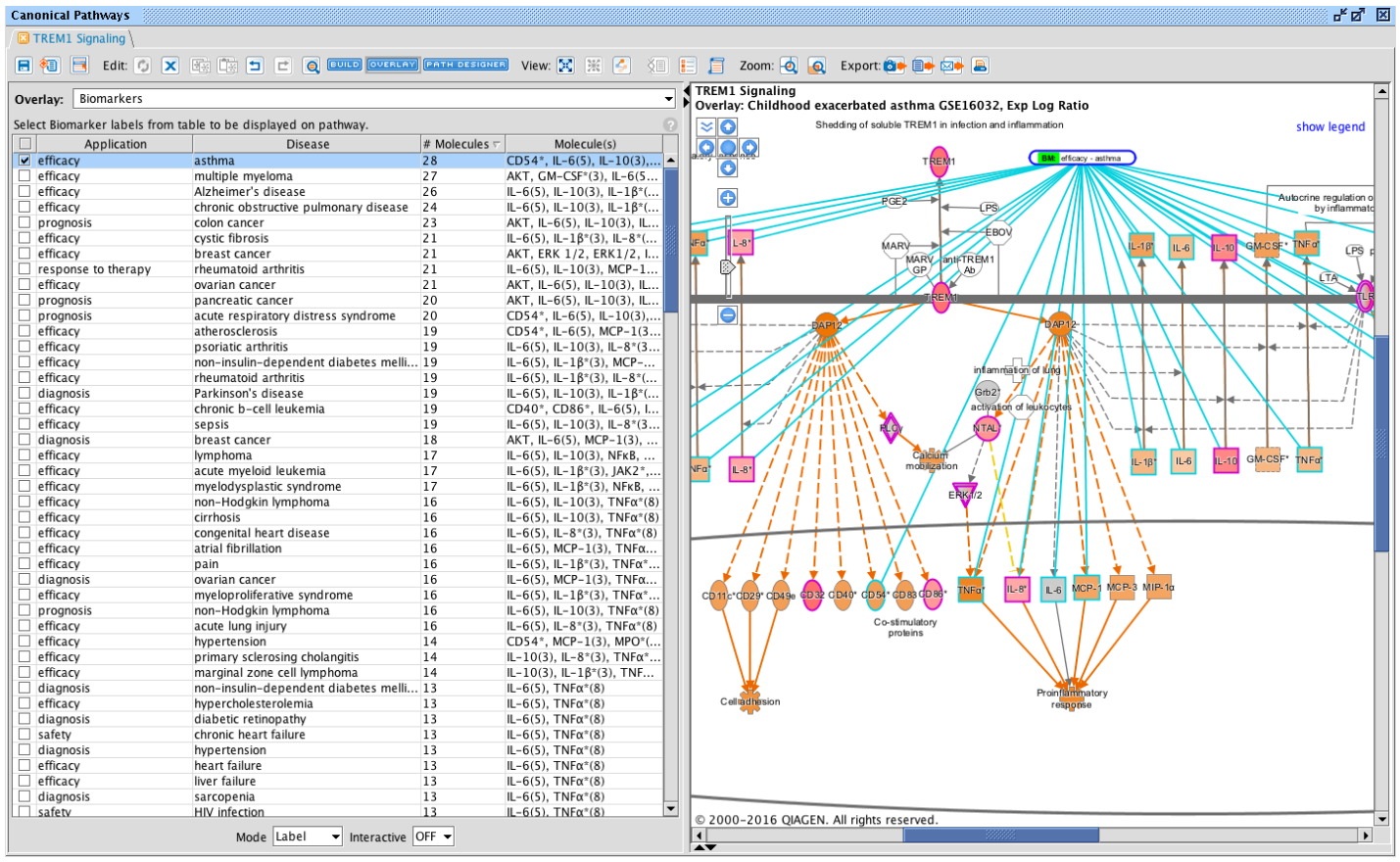
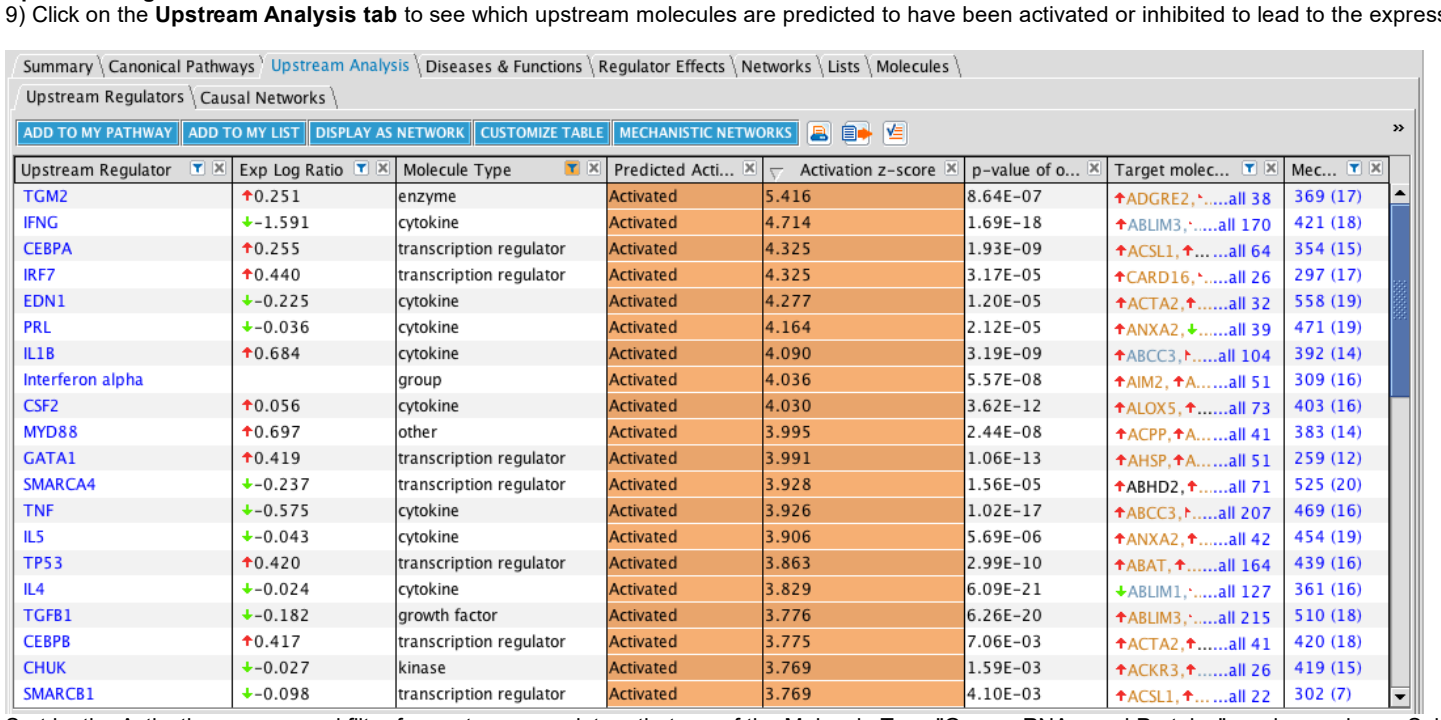
8) **Overlay biomarkers** to determine if genes in the TREM1 Signaling pathway are known asthma biomarkers. Go to Overlay > Biomarkers. Notice that these known biomarkers are ranked simply based on the number of biomarker molecules found in the given pathway. Clearly the TREM1 pathway is replete with asthma markers, as 28 molecules on the pathway are known biomarkers for asthma efficacy:



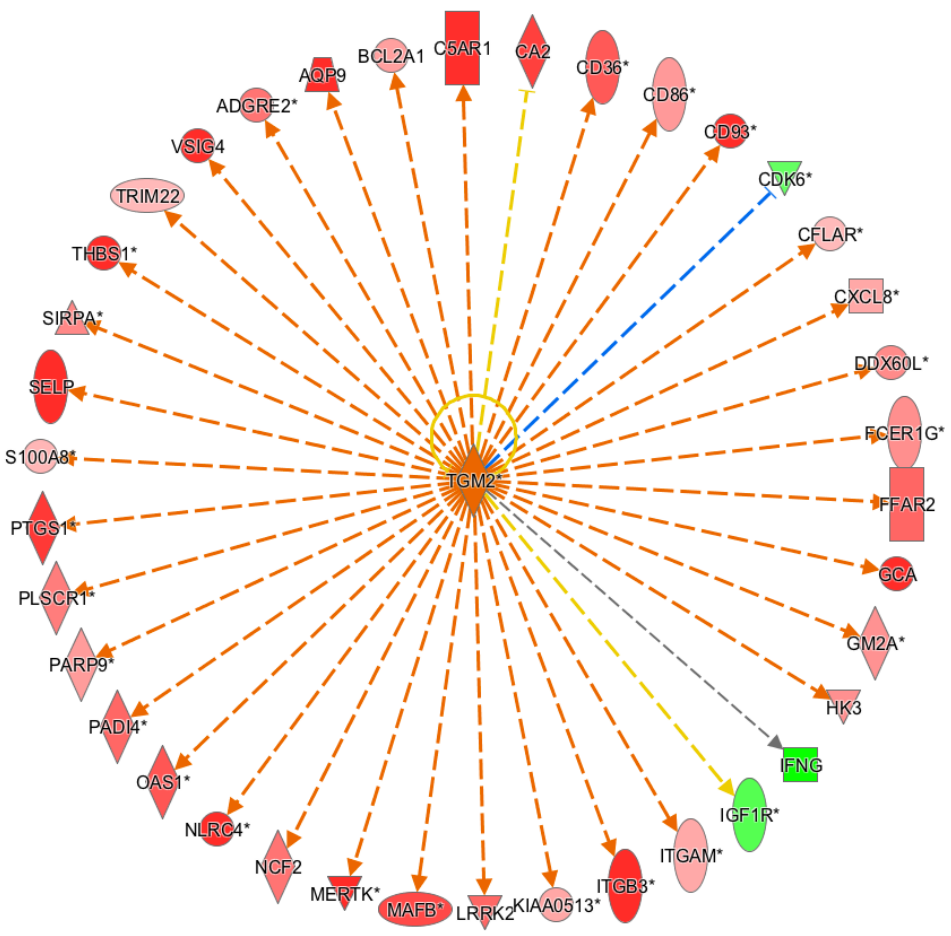
Click the checkbox next to Efficacy - Asthma as shown to add the biomarker tag to the canonical pathway. It will show lines connecting it to the associated genes. Double-clicking a biomarker tag or any of the connecting lines will open the supporting findings.

Upstream Regulators

9) Click on the **Upstream Analysis tab** to see which upstream molecules are predicted to have been activated or inhibited to lead to the expression patterns in your dataset.

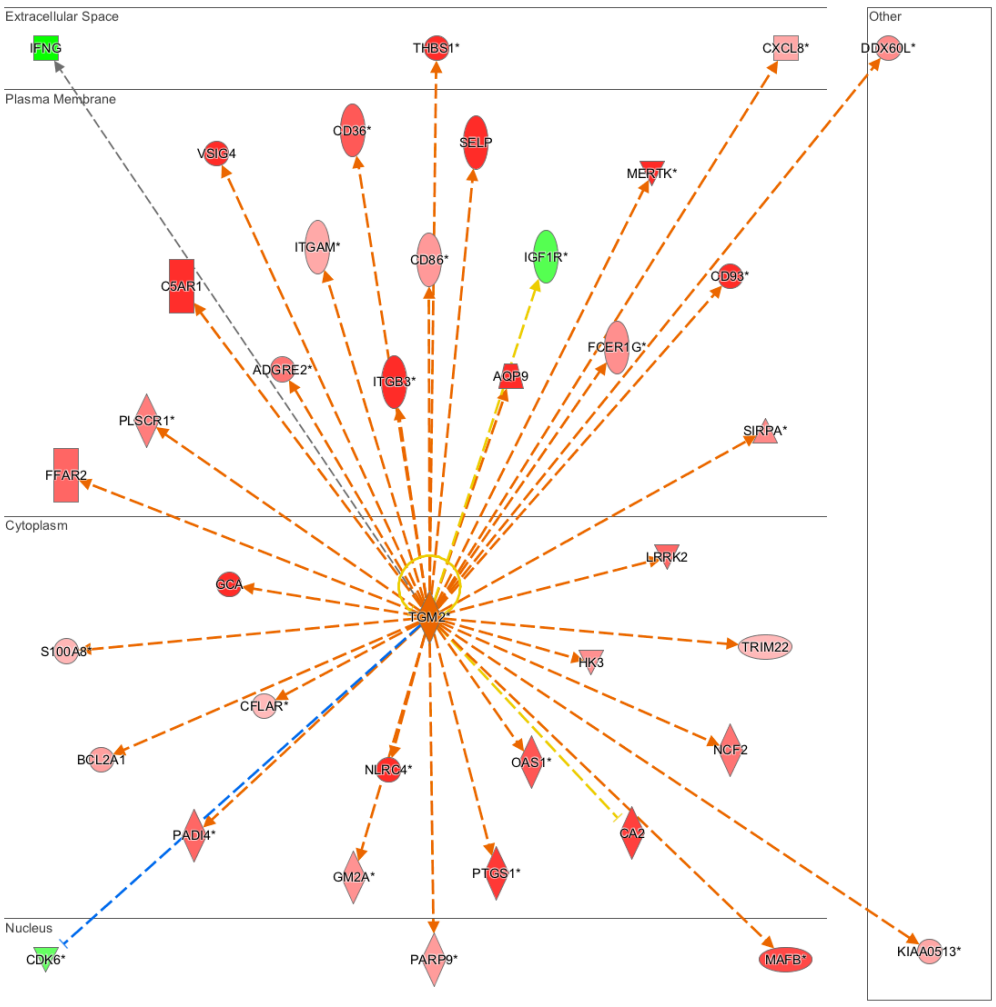


Sort by the Activation z-score and filter for upstream regulators that are of the Molecule Type "Genes, RNAs, and Proteins" as shown above. Select the first upstream regulator row (TGM2) and then click the Display as Network button.

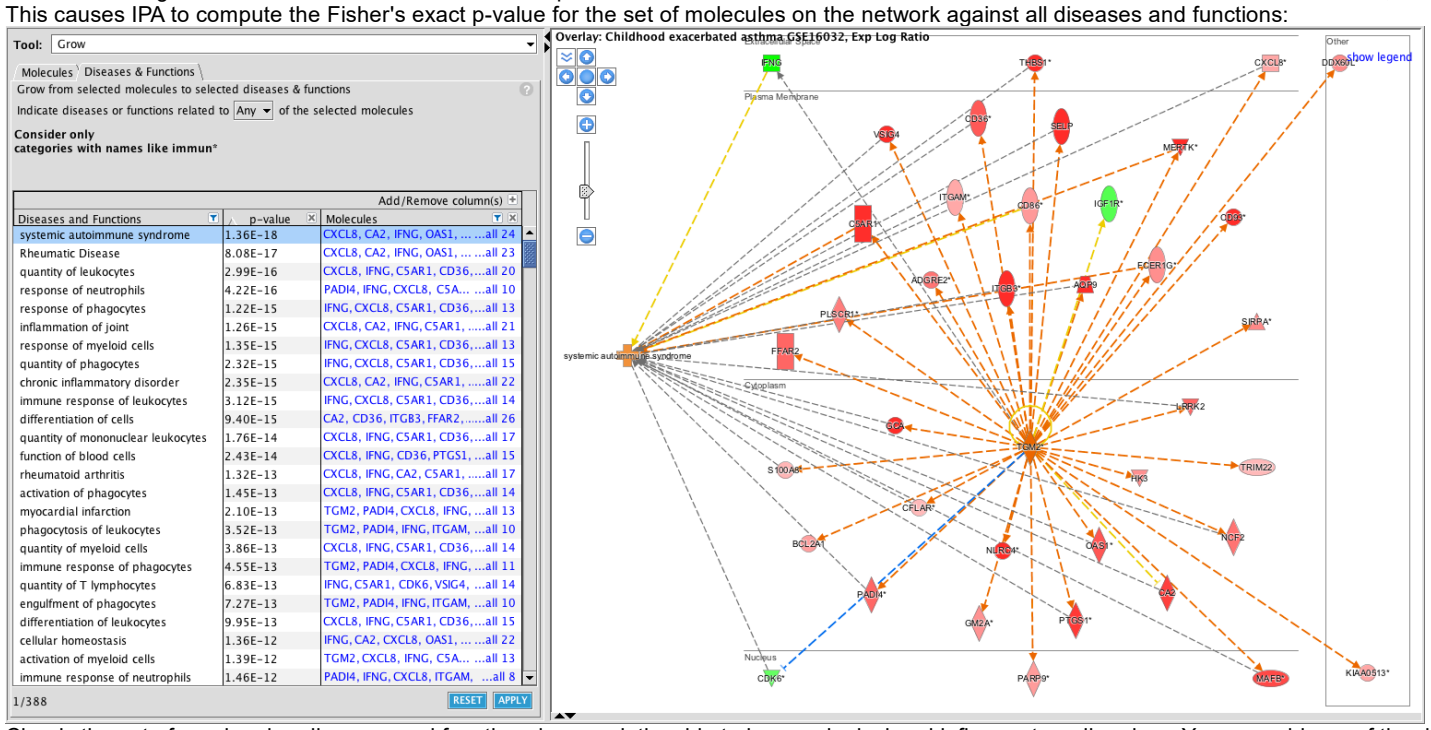


This displays the upstream regulator with its targets from the dataset in a circle surrounding it. The expression changes of the molecules in the perimeter are what led to the prediction of the activation of TGM2 as an upstream regulator.

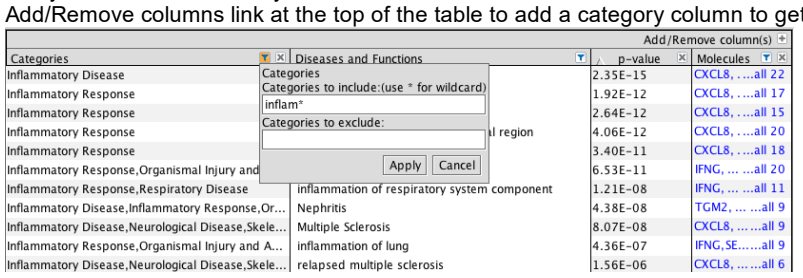
You can change the layout of the network using this button  in the toolbar. Choosing Subcellular layout is helpful, because you can see that TGM2 is a cytoplasmic protein, but leads to expression changes in proteins that are secreted out of the cell, are found in the plasma membrane etc.



You can interrogate this network to see what relationship the molecules have to diseases and function. Click the Build button, choose Grow, then click the Diseases & Function tab. This causes IPA to compute the Fisher's exact p-value for the set of molecules on the network against all diseases and functions:



Clearly the set of overlapping diseases and functions has a relationship to immunological and inflammatory disorders. You can add any of the diseases or functions to the network as shown for systemic autoimmune syndrome, which indicates that the disorder is exacerbated by the expression of these genes (the added disease node is orange). You can also use the Add/Remove columns link at the top of the table to add a category column to get a more global picture of the associated functions. You can filter the table well:

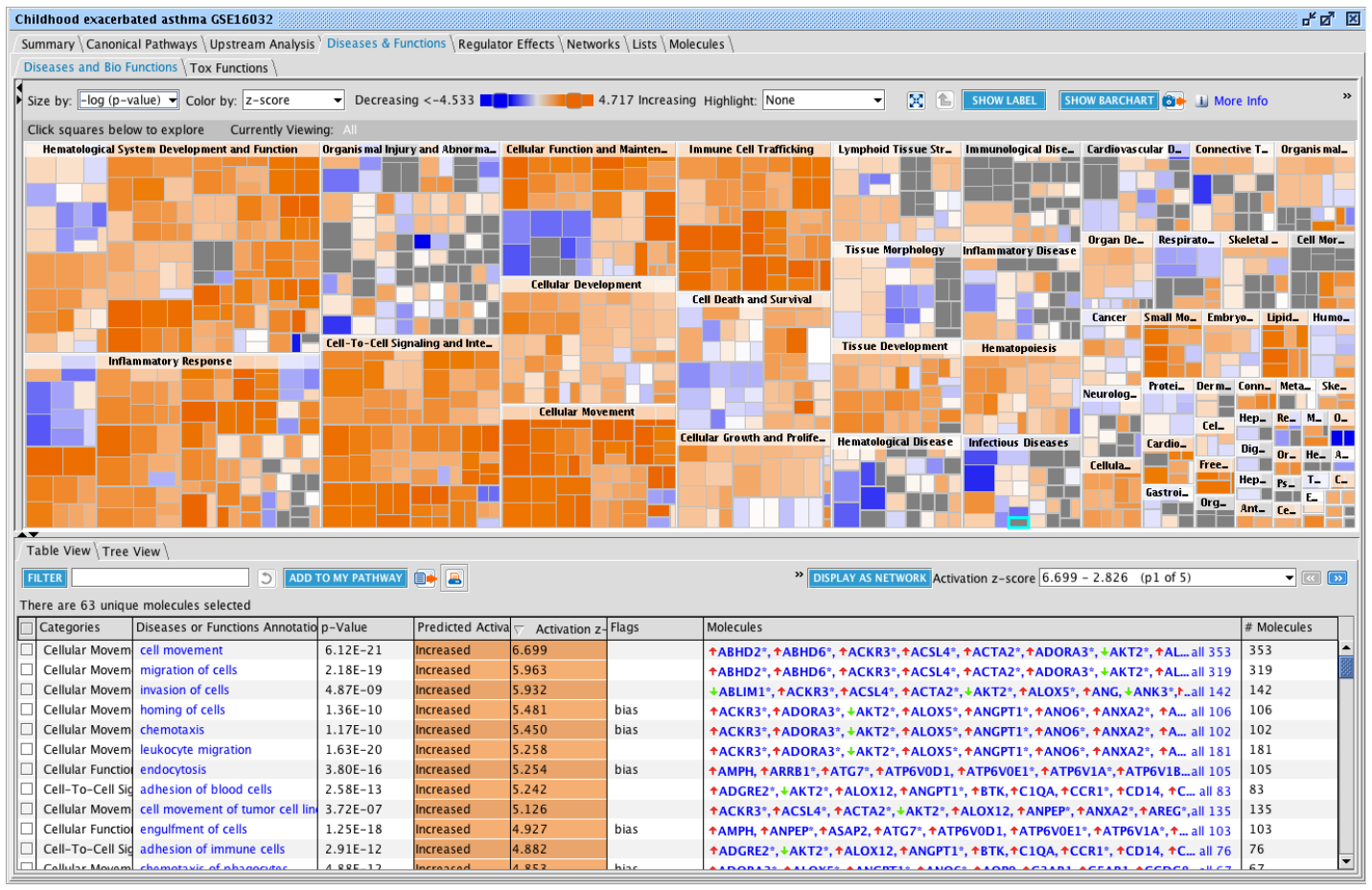


A number of the other upstream regulators can be found to have direct associations to asthma, such as NR3C1 and IL4. But there are a number of other regulators which have not yet been connected to asthma, and await validation at the bench. See Regulator Effects analysis below as well.

Downstream Effects

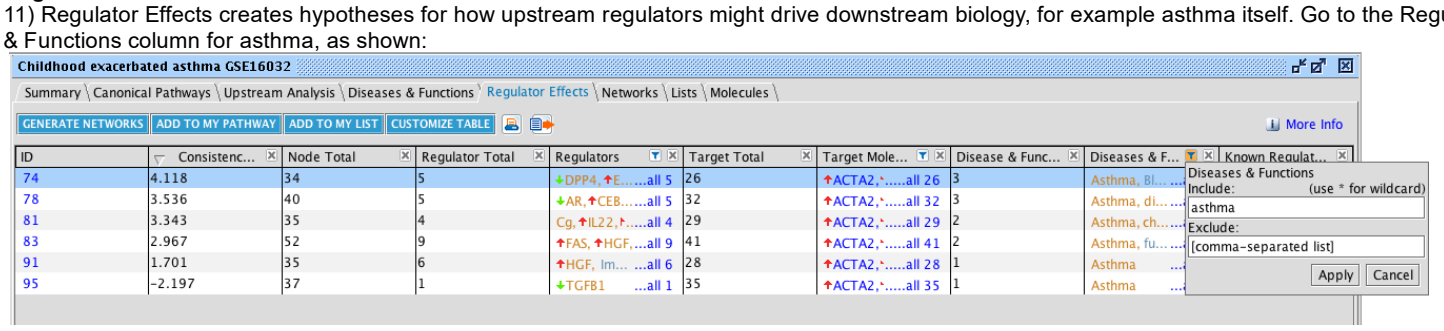
10) Click on the **Diseases and Functions tab** to explore the diseases and biological processes which are predicted to be increasing or decreasing based on the pattern of differentially expressed genes in the dataset.
The large labeled boxes in the treemap shown below are major functional categories in IPA. In this view, it is easy to see the overall directionality of the effects, because orange functions within the categories are predicted to be increasing, and blue decreasing.

From this visualization, many of the individual functions categorized under Immune Cell Trafficking, Inflammatory Response and several others are predicted to be increased, as you would expect if the cells were involved in an immune response involved in acute asthma.

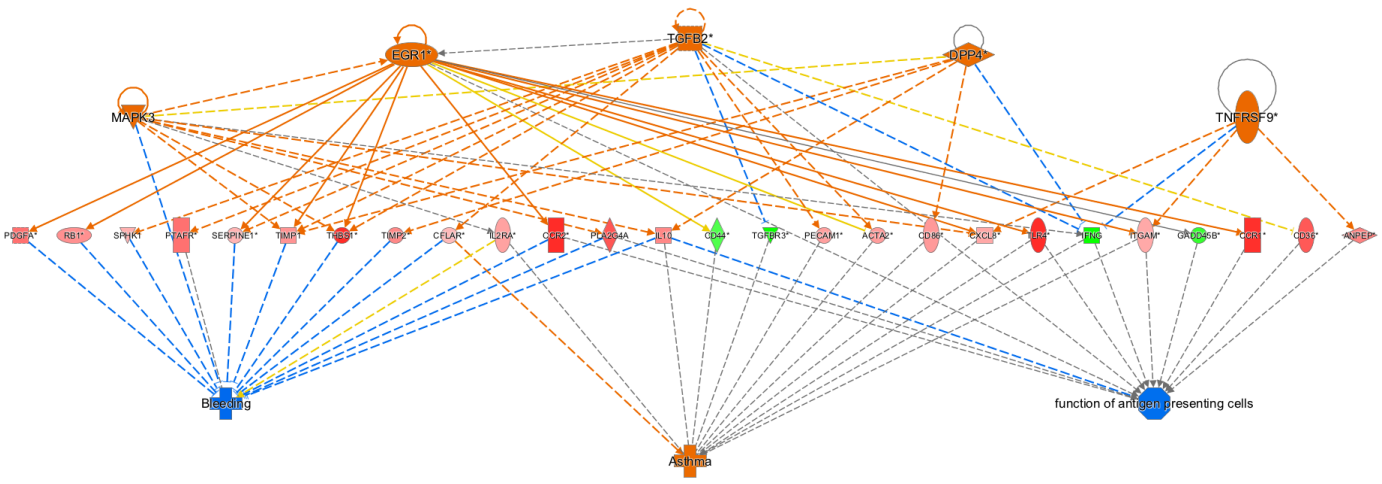


On your own you can explore further, for example by displaying the genes connected to the functions as networks, or drilling onto individual functions to examine how the literature supports these predictions.

Regulator Effects
11) Regulator Effects creates hypotheses for how upstream regulators might drive downstream biology, for example asthma itself. Go to the Regulator Effects tab and filter in the Diseases & Functions column for asthma, as shown:



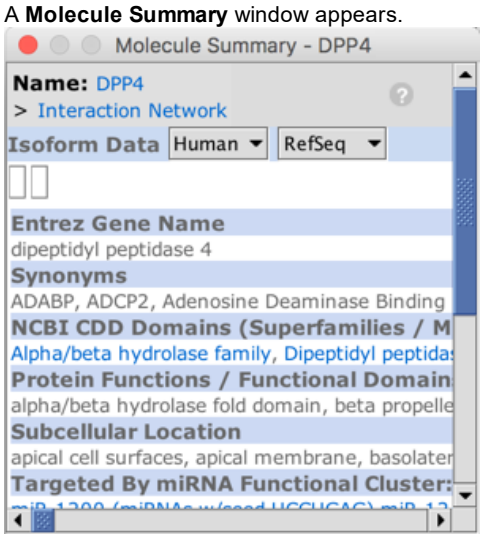
Now click the hyperlinked # in the left most column to display the network:



In this hypothesis, five upstream regulators at the top of the network are predicted to be activated and in turn drive expression changes in the dataset genes shown in the middle tier, which in part may lead to an increase in asthma as shown at the bottom of the network. Interestingly, none of the upstream regulators are known from the curated literature to be directly associated with asthma, otherwise there would be a line connecting the upstream regulator to the asthma node. To delve into more detail about any gene on the network, double click the node-- DPP4 for example.

12) Access the more detailed information **Gene View** on a particular gene. Double click DPP4, an upstream regulator in the network above.

A **Molecule Summary** window appears.



Click the DPP4 hyperlink at the top of the **Molecule Summary** window.
The **Gene View** window appears with detailed information about the DPP4 gene, including a diagram showing the splicing patterns of its encoded transcripts and links to the supporting findings and references. Inspection of the Gene View reveals the involvement of DPP4 in some immune cell functions. Interestingly, looking at less well known literature outside of IPA, there is some evidence that DPP4 is a biomarker in human airway inflammation in asthma. So although this particular study was done in PBMC's, IPA was able to infer a possible role for DPP4 in asthma in the respiratory system.

In Conclusion:

The **Summary Page** showed strong indications that the affected molecules from your dataset are involved with signaling and inflammation.
Canonical Pathways displayed the affected genes from your asthma study in context of the TREM1 pathway
Overlays indicated that many of the TREM1 pathway genes are themselves biomarkers for dugs to treat asthma.
Downstream Effects reveal a very pronounced increase in activation of immune cells and their functions such as cell-cell signaling and cellular trafficking and movement.
Regulator Effects provided a hypothesis of how several upstream regulators might lead to increase in asthma. This included an upstream regulator that had not been directly connected to asthma yet in the Knowledge Base.

Attachment

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