0			utorial: Analysis Results Tutorial						
iscove	er the Biology®				Sterke i				
IPA <sup>®</sup>   W	/hat is IPA?								
me Hel	p	Search		GO					
/sis Result	ts Tutorial					Printal			
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scription	Com Analysia Deculta Tytorial								
	Core Analysis Results Tutorial								
	Overview								
	A <b>Core Analysis</b> has multiple ways of helpi	ng you find biological insights by	automatically using the curated inform	ation from the QIAGEN Kno	wledge Base to put molecul	les in your 'o			
	dataset into context. For this tutorial, we will	walk through the interpretation of	of a transcriptomics dataset.						
	The <b>Summary</b> tab displays the <b>Canonical Pathways</b> tab disp	top results for all the analyses. S lays your molecules of interest w	See <u>Analysis Summary Page</u> . ithin well-established signaling or meta	bolic pathways. See Canon	ical Pathways Overview				
	Upstream Analysis tab predic	ts which upstream regulators (ar	ny molecule that can influence the tran	scription or expression of a	nother molecule) might be a	ctivated or			
	inhibited to explain the express Networks If you have Advance	ion changes in your dataset. See d Analytics in IPA this tab can als	e <u>Upstream Regulator Analysis</u> . It also so display Causal Networks, or hierard	connects upstream regulators	ors into signaling cascades c controlled by a master requ	alled <u>Mecha</u> lator			
	Diseases & Functions relates	molecules in your dataset to know	own disease states and biological func	tions. See <u>Downstream Effe</u>	cts Analysis Tutorial.				
	Regulator Effects displays hy	Regulator Effects displays hypotheses for how a phenotype, function or disease is regulated in your dataset by activated or inhibited upstream regulators See Regulator							
	Networks displays non-direction	onal interaction networks of mole	cules based on known relationships in	the QIAGEN Knowledge Ba	se to your molecules of inter	rest. See <u>Wh</u>			
	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 consideration								
	Seconario								
	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 <u>19620293</u> . 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 <b>Core Analysis</b> for your microarray data								
	Open the <b>Core Analysis</b> for your microarray data. Use the <b>Summary Page</b> to quickly identify promising directions for exploration.								
	Explore the results for areas of importance to your research: View <b>Canonical Pathways</b> that contain significant numbers of genes from your dataset								
	Use <b>MAP</b> (Molecule Activity Predictor) to predict effects on functional endpoints in a canonical pathway.								
	Overlay <b>Biomarkers</b> that ident	fy genes in the TREM1 Signaling	g Pathway that are used as efficacy inc	licators for asthma treatmer	its.				
	Use <b>Regulator Effects</b> to see	a hypotheses of how activation c	of certain upstream regulators may lea	d to outcomes like asthma.					
	Steps								
	1) Start IPA	Draiacta > Evemple Analyzaa > (	Analysis > Childhood avaparhated act	ama CSE16022 The analy	io opono in o now window di	ionloving the			
	Summary tab:	Flojecis - Example Analyses - F		ana GSE 10032. The analys	sis opens in a new window di	isplaying the			
	✓ Top Canonical Pathways								
	Name		p-value	Overlap					
	TREM1 Signaling		• 1.46E-09	32.9 % 23/70					
	Inflammasome pathway	ecognition of Ractoria and Visusas		57.1 % 12/21					
	phagosome formation	ecognition of pacteria and VIRUSES	5.02E-U8	<b>23.0</b> % 26/113					
	Altered T Cell and B Cell Signaling in Rheu	matoid Arthritis	1.56E-06	<b>25.6 %</b> 20/78					
			1 2 3 4 5 6 7 8 9 >						
	✓ Top Upstream Regulators								
	✓ Upstream Regulators								
	Name		n-value of overlap	Predicted Activation					
	Immunoglobulin		• 4.20E-29	Inhibited					
			0.225.27						

• 1.34E-25 

p-value of overlap

• 7.11E-32

------ 8.86E-32 • 1.94E-31

- 1.27E-30

• 1.30E-30

p-value range

123456789 >

Activated

Activated

Predicted Activation

Activated Activated

Inhibited

Activated

#### $\sim$ Top Diseases and Bio Functions arphi Diseases and Disorders p-value range # Molecules Name 1.55E-06 - 9.38E-24 340 1.60E-06 - 7.93E-23 507 Infectious Diseases Inflammatory Response Immunological Disease 6.48E-07 - 1.84E-21 180 1.11E-06 - 1.95E-21 384 **Respiratory Disease** Inflammatory Disease 123456789 > arphi Molecular and Cellular Functions p-value range # Molecules Name Cell-To-Cell Signaling and Interaction 1.60E-06 - 1.91E-26 366 Cellular Function and Maintenance 1.52E-06 - 1.04E-22 382 1.27E-06 - 6.12E-21 377 Cellular Movement • • 1.54E-06 - 8.44E-21 520 • • 1.46E-06 - 2.03E-20 339 Cellular Development Cell Morphology 123456789 > arphi Physiological System Development and Function # Molecules

Name http://ingenuity.force.com/ipa/articles/Tutorial/analysis-results-tutorial

IL13

IL4

TGFB1

Name TH2 Cytokine

IFNA8

CD300LF

TH1 Cytokine

fluticasone

 $\sim$  Causal Networks

# Tutorial: Analysis Results Tutorial

•			
276625874	1.60E-06 - 1.91E-26	425	
	1.55E-06 - 4.65E-23	314	
1776-4-2-4	1.60E-06 - 7.93E-23	254	
	1.54E-06 - 8.44E-21	225	
	8.84E-07 - 4.10E-16	255	
123456789 >			
		1.60E-06 - 1.91E-26         1.55E-06 - 4.65E-23         1.60E-06 - 7.93E-23         1.54E-06 - 8.44E-21         8.84E-07 - 4.10E-16	1.60E-06 - 1.91E-26       425         1.55E-06 - 4.65E-23       314         1.55E-06 - 7.93E-23       254         1.54E-06 - 8.44E-21       225         8.84E-07 - 4.10E-16       255

# $\sim$ Top Tox Functions

Name	p-value range	# Molecules
Increased Levels of Red Blood Cells	2.35E-03 - 2.35E-0	16
Increased Levels of ALT		7
Increased Levels of Hematocrit	7.88E-03 - 7.88E-0	14
Increased Levels of Creatinine		8
Increased Levels of AST	9.00E-02 - 9.00E-0	3
	123456789 >	

# ${}^{\bigvee}$ Cardiotoxicity

Name	p-value range	# Molecules
Cardiac Infarction	4.42E-01 - 2.53E-	09 69
Congenital Heart Anomaly	••••••••••••••••••••••••••••••••••••••	95 49
Cardiac Pulmonary Embolism	2.09E-01 - 1.65E-	03 8
Cardiac Arrythmia		3 41
Cardiac Fibrosis	3.33E-01 - 3.65E-	03 25

## ${}^{\bigvee}$ 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
✓ Nephrotoxicity		
Name	p-value range	# Molecules
Renal Damage	4.95E-01 - 3.50E-08	38

		3.30E-01 - 4.13E-08	60
Renal Nephritis	- MA 10	5.56E-01 - 4.13E-08	60
Kidney Failure		4.87E-01 - 4.07E-05	47
Renal Necrosis/Cell Death	<del>₽••<mark>]•</mark>*•</del>	5.56E-01 - 4.10E-05	63

$\sim$	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

7 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	<b>36.0 %</b> 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	<b>12.4 %</b> 63/509

Top Analysis-Ready Molecules		
∨ Exp Log Ratio ↑		
Molecules	Value	Chart
IL1R2*	↑ 5.418	
MS4A4A*	<b>↑</b> 3.333	
VSIG4	↑ 3.206	
VNN1*	<b>↑</b> 3.088	
CD163*	↑2.736	
GPR34	<b>↑</b> 2.609	
FKBP5*	↑2.550	
C3AR1	↑2.515	
CCR2*	↑2.468	
HBD	<b>↑</b> 2.464	
✓ Exp Log Ratio +		
Molecules	Value	Chart
IFNG	<b>↓</b> -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	

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Summary Tab

#### Tutorial: Analysis Results Tutorial

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

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**

For this dataset:

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 <u>Canonical Pathways for a Dataset</u> 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 <u>Pathway Activity Overlay</u> 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.

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

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:

15/12/2016

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IPA Relationships: 1	NFα Proinflammatory response
Review the information that	supports the gene-to-function relationship. Click the plus icon to view the reference information.
PlainText ᅌ EXPORT REF	ERENCES
Ingenuity Relationsh	ips
causation [4]	
In extracellular space, TN	F-a [TNF] protein increases Proinflammatory response.
15634892	Gerosa F, Gobbi A, Zorzi P, Burg S, Briere F, Carra G, Trinchieri G. The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. J Immunol. 2005 Jan 15;174(2):727-34.
Source: Ingenuity Expert	Findings
In nuclei from myeloid cel	s, TNFa [TNF] protein increases Proinflammatory response.
15385460	Lyke KE, Burges R, Cissoko Y, Sangare L, Dao M, Diarra I, Kone A, Harley R, Plowe CV, Doumbo OK, Sztein MB. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe Plasmodium falciparum malaria and matched uncomplicated malaria or healthy controls. Infect Immun. 2004 Oct;72(10):5530-7.
Source: Ingenuity Expert	Findings
Binding of HMGB1 prot	ain and TNFA [TNF] protein increases proinflammatory response of cells.
18431461	Klune JR, Dhupar R, Cardinal J, Billiar TR, Tsung A. HMGB1: endogenous danger signaling. Mol Med. 2008 Jul-Aug;14(7-8):476-84.
Source: Ingenuity Expert	Findings
In nuclei from myeloid cel	s, TNFa [TNF] protein increases Proinflammatory response.
16940328	Netea MG, Azam T, Ferwerda G, Girardin SE, Kim SH, Dinarello CA. Triggering receptor expressed on myeloid cells-1 (TREM-1) amplifies the signals induced by the NACHT-LRR (NLR) pattern recognition receptors. J Leukoc Biol. 2006 Dec;80(6):1454-61. Epub 2006 Aug 29.
Source: Ingenuity Expert	Findings

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:

INGEN PATHWAY AN	<b>JITY</b> NALYSIS						Canonical Pat	hway
Report Date: 2016-10 Report Version: 40164 Content Version: 2882	-12 42 20210 (Release Date: 201	6-09-24)					Provide Feedback   Live Support 🍌 Downlo	ad Report (PDF)
Canonical Pathway	TREM1 Sign	aling						
Description	The triggering receptor neutrophils, monocytes adaptor molecule DNA	expressed on r and macropha cactivating pro	nyeloid cells 1(TREM1) be ges. TREM-1 lacks known tein 12 (DAP12), leading t	longs to the In signaling motil to proinflamma	nmunoglobulin (Ig) fami is in the cytoplasmic do tory immune responses	ily of cell main and . The nat	surface receptors and is selectively expressed o thus activation by TREM1 is mediated by a tran rural ligand for TREM1 is however, unknown.	n blood smembrane
	TREM1 activation trigg signal transducers of a response. Stimulation activation of interleuki expression and secreti- tumor necrosis factor ( the expression of TREM the production of proin adhesion and costimuli adaptive immune resp	ers the Janus ki ctivation of tran of TREM by its I I-1 receptor-as: on of chemokin TNF).Many of th 11 in an autocri flammatory cyt ation respective onse.	nase 2 (JAK2), protein kir scription (STAT3, 5) and f igand or toll like receptor sociated kinase 1 (IRAK1), as and cytokines like mon- nese effects are potentiate e fashion. The synergy b okines. TREM1 activation - ly, as well as phospholipas	hase B (PKB/AK NF kappa B (NF (TLR) by lipopo which in turn t ocyte chemotac ad by LPS. Cyto etween TLR ann also results in t se gamma (PLC	T) and extracellular sign -kB). These transcription lysaccharide (LPS) can riggers NF-kB and the p tic protein 1(MCP-1) m kines like TNF and Grand d TREMI leads to neutro he upregulation of cell s y) mediated Ca2+ relea	nal relate on factors lead to a proinflam acrophag nulocyte i ophil deg surface p ase. Thus	d kinase (ERK1/2) pathways leading to the phos- supregulate the expression of genes involved in n association of TREM1 and TLR. This association matory response. Engagement and activation of le inflammatory protein-1alpha (MIP-10), interle macrophage colony stimulating factor (GM-CSF) ranulation, phagocytosis and the respiratory bur roteins like CD11, CD29 and CD40, CD83 that a s TREM1 activation is involved in diverse aspects	phorylation of the inflammatory n leads to the TREM-1 triggers ukins (IL-6,-8) an in turn upregulate st in addition to re involved in cell of innate and
	In addition to TLRs, TR microorganisms. The T pathway. Thus TREM-1 This pathway highlight	EM1 also syner REM-1/NLR syn acts to amplify s the important	gizes with a second major ergism results in the prod signals from both major p components of TREM1 sig	class of patter luction of proint pathways of pa gnaling.	n recognition receptors flammatory cytokines lii ttern recognition- extrad	-the NAC ke TNF, II cellular T	CHT-LRR receptors (NLR), which recognize intrac L-1β, IL-6 and IL-18- the latter three via a caspa LR receptors and the intracellular NLR proteins.	ellular se -1 dependent
Signaling Pathway Categories	Cellular Immune Respo	onse; Cytokine S	Signaling					
Top Functions 8	Cell-To-Cell Signaling a	nd Interaction;	Hematological System De	evelopment and	Function; Immune Cell	l Trafficki	ng	
Molecules show al	<ul> <li>adaptive immune respine</li> <li>DEFB4A/DEFB4B, degring</li> <li>Ala-y-D-Glu-meso-diarisoglutamine, NFkB (construction)</li> </ul>	onse, Akt, anti- anulation, EBOV ninopimelinic ad mplex), NLR, N	FREM1 Ab, apoptosis, CAS /, ERK1/2, FCGR2B, Flage cid, LAT2, lipopolysacchari OD2, Pam3-Cys, phagocy	SP1, Casp1-Cas Ilin, GRB2, ICA de, lipoteichoic tosis	p5, CASP5, CCL2, CCL3 M1, IL10, IL18, IL1B, IL acid, MARV, MARV GP,	, CCL7, C .1RL1, IL mobiliza	D40, CD83, CD86, cell adhesion, CSF2, CXCL3, 6, innate immune response, IRAK1, ITGA5, ITG iion of Ca2+, MPO, MYD88, N-acetylmuramyl-L-	CXCL8, AX, ITGB1, JAK2, alanyl-D- Back to top >
<b>Prug Summary</b> - Ov	erview of drugs targeting	molecules in Ca	nonical Pathway					
howing 3 of 111 row(s	) of Drug data. (Show All	)						
Drug Name	•	Targets	Actions	\$	Brand Names	÷	Indications/Status	
-001	TLR7		agonist				acute lymphocytic leukemia/Phase 2 acute myeloid leukemia/Phase 2 Barrett's syndrome/Unspecified phase	
luorouracil/imiquimod	TLR7		stimulator					
atacept	CD86		binder	Orencia			allergic asthma/Phase 2 alopecia areata/Phase 2 ankylosing spondylitis/Phase 2	
								Back to top >
arget information	- Overview of known dru	g targets in Can	ionical Pathway					
nowing 3 of 29 row(s)	or larget data. (Show All	)						
(Gene A Entro Symbol)	ame + Location	<b>\$ Туре \$</b>			Drug	ı(s)	•	Species
	Cytoplasm	group	afuresertib, AT13148, ip	atasertib, MSC	2363318A, ONC-201, S	R-13668		Human, Mouse Pat
T1 AKT seri kinase 1	ne/threonine Cytoplasm	kinase	archexin, ARQ 092, AZD perifosine, triciribine, tri	5363, BAY112 iciribine phosph	5976, enzastaurin, GSK ate	2141795	, ipatasertib, LY2780301, MK2206, MPT0E028,	Human, Mouse, Rat
T2 AKT serii kinase 2	ne/threonine Cytoplasm	kinase	BAY1125976, enzastaur	in, triciribine, t	riciribine phosphate			Human, Mouse, Rat Back to top >
upporting Referen	ces (Show details) - Refe	rences from wh	hich the Canonical Pathwa	v was derived				
				,				Back to top 1
								buen to top

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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:

http://ingenuity.force.com/ipa/articles/Tutorial/analysis-results-tutorial

## Tutorial: Analysis Results Tutorial

TREM1 Signaling \ TREM1 Signaling \ Edit: • X  Interpret to the second s	i i i cuid overay	PATH DESIGNE	🛚 View: 🔀 🔣 🍝 🌾	🖹 🧮 Zoom: 🧑 👩 Export: 🍘 📾 🔤 📟
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erlay: Biomarkers		PATH DESIGNE	🚽 View: 🎦 📰 🔛 🤉 🔛	= Zoom: 🕘 🙋 Export: 🔤 = 🔤 🗠 =
erlay: Biomarkers				
ct Biomarker labels from tab			_	TREM1 Signaling
ct Biomarker labels from tab				• Overlay: Childhood exacerbated asthma GSE16032, Exp Log Ratio
	ole to be displayed on pathway.		•	Shedding of soluble TREM1 in infection and inflammation show legend
Application	Disease	# Molecules 🗸	Molecule(s)	
efficacy	asthma	28	CD54*, IL-6(5), IL-10(3),	TREM1 BILL of Cacy - asthma
efficacy	multiple myeloma	27	AKT, GM-CSF*(3), IL-6(5	
efficacy	Alzheimer's disease	26	IL-6(5), IL-10(3), IL-1β*(	
efficacy	chronic obstructive pulmonary disease	24	IL-6(5), IL-10(3), IL-1β*(	PGE2 CPS
prognosis	colon cancer	23	AKT, IL-6(5), IL-10(3), IL	
efficacy	cystic fibrosis	21	IL-6(5), IL-1B*(3), IL-8*(	EBOY
efficacy	breast cancer	21	AKT, ERK 1/2, ERK1/2, I	
response to therapy	rheumatoid arthritis	21	IL-6(5), IL-10(3), MCP-1	
efficacy	ovarian cancer	21	AKT, IL-6(5), IL-10(3), IL	
prognosis	pancreatic cancer	20	AKT, IL-6(5), IL-10(3), IL	
prognosis	acute respiratory distress syndrome	20	CD54*, IL-6(5), IL-10(3)	
efficacy	atherosclerosis	19	CD54*, IL-6(5), MCP-1(3	
efficacy	psoriatic arthritis	19	IL-6(5), IL-10(3), IL-8*(3	
efficacy	non-insulin-dependent diabetes melli	19	IL-6(5), IL-1B*(3), MCP	
efficacy	rheumatoid arthritis	19	IL-6(5), IL-1B*(3), IL-8*(	inflammatori of King (1)
diagnosis	Parkinson's disease	19	IL-6(5), IL-10(3), IL-1B*(	Groz Groz
efficacy	chronic b-cell leukemia	19	CD40*, CD86*, IL-6(5), I	activation of leukocytes
efficacy	sepsis	19	IL-6(5), IL-10(3), IL-8*(3	
diagnosis	breast cancer	18	AKT, IL-6(5), MCP-1(3),	
efficacy	lymphoma	17	IL-6(5), IL-10(3), NFKB,	
efficacy	acute myeloid leukemia	17	IL-6(5), IL-18*(3), IAK2*	NFat Las / / / / mobilization / /// / Las Las Las Las Internation
efficacy	myelodysplastic syndrome	17	IL-6(5), IL-1B*(3), NFKB,	
efficacy	non-Hodgkin lymphoma	16	IL-6(5), IL-10(3), TNFa*(8)	
efficacy	cirrhosis	16	IL-6(5), IL-10(3), TNFα*(8)	
efficacy	congenital heart disease	16	IL-6(5), IL-8*(3), TNFa*(8)	
efficacy	atrial fibrillation	16	IL-6(5), MCP-1(3), TNFq	
efficacy	pain	16	IL-6(5), IL-1β*(3), TNFα*	$1 \neq 1 \neq 1 + 1 + X + M = M = 1 + L + L$
diagnosis	ovarian cancer	16	IL-6(5), MCP-1(3), TNFα	▏ <mark>ᡒ</mark> ᡒᡒᠼᡵᢩᢂᢘᢐ᠉᠋ᢁᢙᢩᠲᢆᡨᢛᢛ
efficacy	myeloproliferative syndrome	16	IL-6(5), IL-1β*(3), TNFα*	
prognosis	non-Hodgkin lymphoma	16	IL-6(5), IL-10(3), TNFα*(8)	$  \langle \langle \rangle \rangle = \langle \rangle $
efficacy	acute lung injury	16	IL-6(5), IL-8*(3), TNFα*(8)	Co-stimulatory
efficacy	hypertension	14	CD54*, MCP-1(3), MPO*(	proteins
efficacy	primary sclerosing cholangitis	14	IL-10(3), IL-8*(3), TNFα*	
efficacy	marginal zone cell lymphoma	14	IL-10(3), IL-1β*(3), TNF	
diagnosis	non-insulin-dependent diabetes melli	13	IL-6(5), TNFα*(8)	Broken Broken Broken Broken
efficacy	hypercholesterolemia	13	IL-6(5), TNFα*(8)	Celladhesion response
diagnosis	diabetic retinopathy	13	IL-6(5), TNFα*(8)	
safety	chronic heart failure	13	IL-6(5), TNFα*(8)	1
diagnosis	hypertension	13	IL-6(5), TNFα*(8)	1
efficacy	heart failure	13	IL-6(5), TNFα*(8)	1
efficacy	liver failure	13	IL-6(5), TNFα*(8)	1
diagnosis	sarcopenia	13	IL-6(5), TNFα*(8)	
safety	HIV infection	13	IL-6(5), TNFα*(8)	
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	Mode Label 🔻 Interactive 🕻	OFF 🔻		

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.

Summary \ Canonical Pathways \ Upstream Analysis \ Diseases & Functions \ Regulator Effects \ Networks \ Lists \ Molecules \								
Upstream Regulators \ Cau	sal Networks \							
ADD TO MY PATHWAY ADD TO MY LIST DISPLAY AS NETWORK CUSTOMIZE TABLE MECHANISTIC NETWORKS 🔒 📑 🛀								
Upstream Regulator 🔳 🗵	Exp Log Ratio 🝸 🗵	Molecule Type 🛛 🕱 🗵	Predicted Acti 🗵		p-value of o 🗵	Target molec 🝸 🗵	Mec 🝸 🗵	
TGM2	<b>†</b> 0.251	enzyme	Activated	5.416	8.64E-07	↑ADGRE2, •all 38	369 (17)	
IFNG	+-1.591	cytokine	Activated	4.714	1.69E-18	↑ABLIM3,all 170	421 (18)	
CEBPA	<b>†</b> 0.255	transcription regulator	Activated	4.325	1.93E-09	↑ACSL1, ↑all 64	354 (15)	
IRF7	<b>†</b> 0.440	transcription regulator	Activated	4.325	3.17E-05	↑CARD16, •all 26	297 (17)	555
EDN1	+-0.225	cytokine	Activated	4.277	1.20E-05	↑ACTA2,↑all 32	558 (19)	
PRL	<b>↓</b> -0.036	cytokine	Activated	4.164	2.12E-05	↑ANXA2, ↓all 39	471 (19)	2441
IL1B	<b>†</b> 0.684	cytokine	Activated	4.090	3.19E-09	↑ABCC3,all 104	392 (14)	
Interferon alpha		group	Activated	4.036	5.57E-08	↑AIM2, ↑Aall 51	309 (16)	
CSF2	<b>†</b> 0.056	cytokine	Activated	4.030	3.62E-12	↑ALOX5, ↑all 73	403 (16)	
MYD88	<b>†</b> 0.697	other	Activated	3.995	2.44E-08	↑ACPP, ↑Aall 41	383 (14)	
GATA1	<b>†</b> 0.419	transcription regulator	Activated	3.991	1.06E-13	↑AHSP, ↑Aall 51	259 (12)	
SMARCA4	<b>↓</b> -0.237	transcription regulator	Activated	3.928	1.56E-05	↑ABHD2, ↑all 71	525 (20)	
TNF	+-0.575	cytokine	Activated	3.926	1.02E-17	↑ABCC3, 1all 207	469 (16)	
IL5	<b>↓</b> -0.043	cytokine	Activated	3.906	5.69E-06	↑ANXA2,↑all 42	454 (19)	
TP53	<b>†</b> 0.420	transcription regulator	Activated	3.863	2.99E-10	↑ABAT, ↑all 164	439 (16)	
IL4	<b>↓</b> -0.024	cytokine	Activated	3.829	6.09E-21	+ABLIM1,all 127	361 (16)	
TGFB1	+-0.182	growth factor	Activated	3.776	6.26E-20	↑ABLIM3,all 215	510 (18)	
CEBPB	<b>†</b> 0.417	transcription regulator	Activated	3.775	7.06E-03	↑ACTA2, ↑all 41	420 (18)	
СНИК	+-0.027	kinase	Activated	3.769	1.59E-03	↑ACKR3,↑all 26	419 (15)	
SMARCB1	<b>↓</b> -0.098	transcription regulator	Activated	3.769	4.10E-03	↑ACSL1, ↑all 22	302 (7)	-

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:

			Add/	Remove column(s)
Categories	T 🗵 Diseases and Functions	<b>T</b>	∧ p-value	🗵 Molecules 🔳
Inflammatory Disease	Categories		2.35E-15	CXCL8,all 2
Inflammatory Response	Categories to include:(use * for wildcard)		1.92E-12	CXCL8,all 1
Inflammatory Response	Inflam^		2.64E-12	CXCL8,all 1
Inflammatory Response	Categories to exclude:	region	4.06E-12	CXCL8,all 2
Inflammatory Response			3.40E-11	CXCL8,all 1
Inflammatory Response,Organismal Injury and	Apply Cancel		6.53E-11	IFNG,all 2
Inflammatory Response, Respiratory Disease	inflammation of respiratory system o	omponent	1.21E-08	IFNG,all 1
Inflammatory Disease, Inflammatory Response,	Or Nephritis		4.38E-08	TGM2,all
Inflammatory Disease, Neurological Disease, Sk	ele Multiple Sclerosis		8.07E-08	CXCL8,all
Inflammatory Response, Organismal Injury and	A inflammation of lung		4.36E-07	IFNG, SEall
Inflammatory Disease, Neurological Disease, Sk	ele relapsed multiple sclerosis		1.56E-06	CXCL8,all

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:

Childhood exacerb	ated astnma GSE160:	52							
/ Summary \ Canonical Pathways \ Upstream Analysis \ Diseases & Functions \ Regulator Effects \ Networks \ Lists \ Molecules \									
GENERATE NETWORKS ADD TO MY PATHWAY ADD TO MY LIST CUSTOMIZE TABLE 🚇									
ID	∇ Consistenc      X	Node Total 🛛 🗶	Regulator Total 🛛 🗵	Regulators 🛛 🗶	Target Total 🛛 🗵	Target Mole 🍸 🗵	Disease & Func 🗵	Diseases & F	🔨 🗵 Known Regulat 🗵
74	4.118	34	5	+DPP4, ↑Eall 5	26	↑ACTA2,all 26	3	Asthma, Bl	Diseases & Functions
78	3.536	40	5	+AR, +CEBall 5	32	↑ACTA2,all 32	3	Asthma, di	asthma
81	3.343	35	4	Cg, ↑IL22, 1all 4	29	↑ACTA2,*all 29	2	Asthma, ch	Exclude:
83	2.967	52	9	✦FAS, ✦HGF,all 9	41	↑ACTA2, •all 41	2	Asthma, fu	[comma-separated list]
91	1.701	35	6	✦HGF, Imall 6	28	↑ACTA2, •all 28	1	Asthma	Learning coherence with
95	-2.197	37	1	+TGFB1all 1	35	+ACTA2,all 35	1	Asthma	Apply Can

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 Sum	mary wind	low appe	ars.
🔴 🔍 🔍 Moleci	ule Summa	ary - DPP4	1
Name: DPP4 > Interaction Net	twork		0
Isoform Data	Human 🔻	RefSeq	-
Enduran Come M			55

Liftlez delle Mallie
dipeptidyl peptidase 4
Synonyms
ADABP, ADCP2, Adenosine Deaminase Binding
NCBI CDD Domains (Superfamilies / M
Alpha/beta hydrolase family, Dipeptidyl peptida:
<b>Protein Functions / Functional Domain</b>
alpha/beta hydrolase fold domain, beta propelle
Subcellular Location
apical cell surfaces, apical membrane, basolater
Targeted By miRNA Functional Cluster:

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

<u>Home | Help</u>

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