

# MetaCore: Common nonmutational NOTCH1 activation in chronic lymphocytic leukemia

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Manager, Solution Scientists



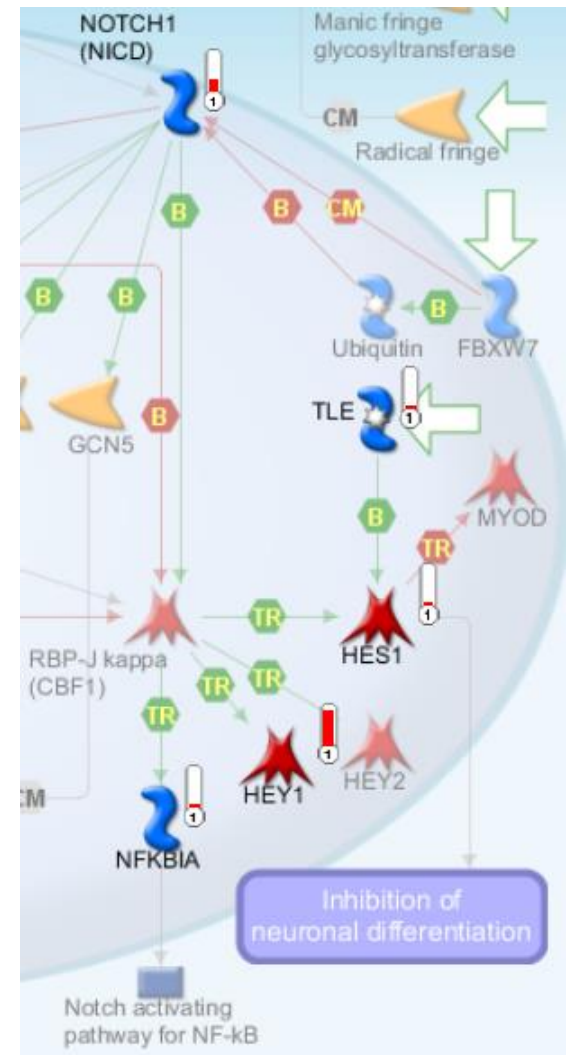
# Agenda

## Introduction to MetaCore

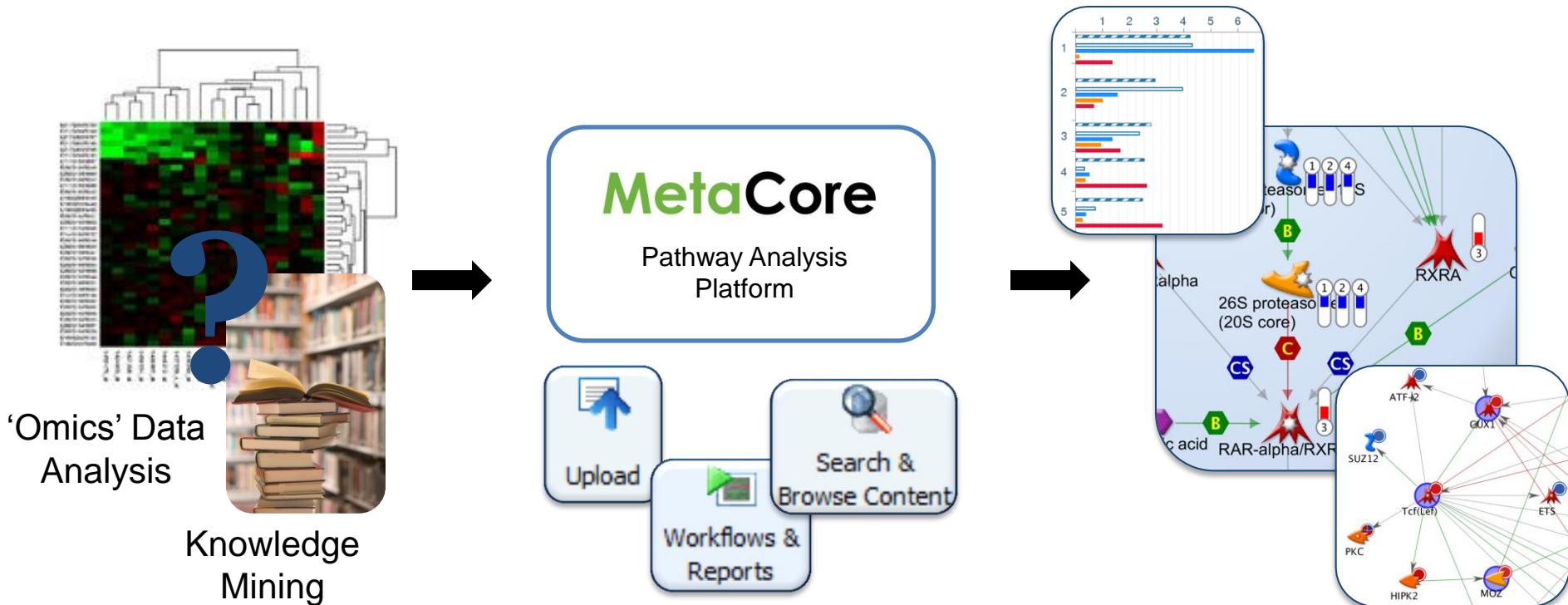
Use case: Common nonmutational NOTCH1 activation in chronic lymphocytic leukemia

- I want to know what interactions and pathways involve NOTCH1.
- What pathways are enriched by ICN1 from RNA-seq and bound to NOTCH1 in ChIP-seq experiments?
- What transcription factors could these up regulated genes regulated by?
- How does the NOTCH1 expression profile compare with RNA-seq data from chronic lymphocytic leukemia?
- What other publicly available datasets have a similar to the NOTCH1 expression profile?

## Summary



# MetaCore: Your GPS in pathway analysis

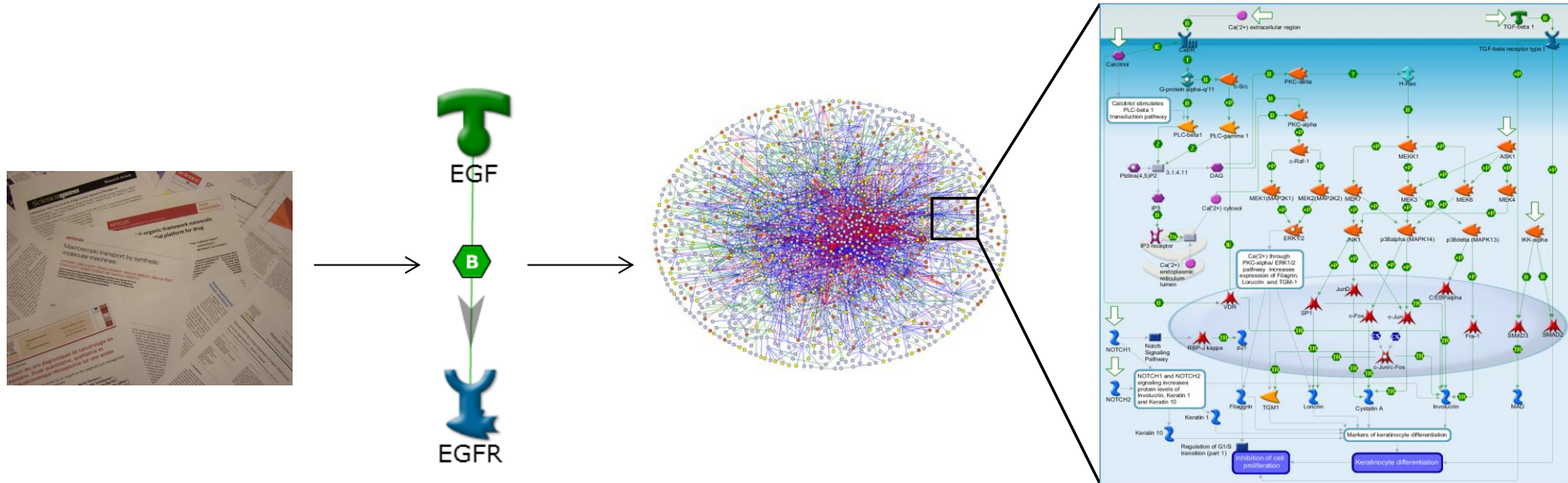


- Gain molecular understanding of disease
- Analyze and understand experimental findings (Omics data) in the context of validated biological pathways.
- Generate and confirm hypotheses for novel biomarkers, targets, mechanisms of action

# MetaBase/MetaCore content overview

• Human genes	60,119	• Human genes in network	23,665
• Human SwissProt proteins	20,115	• Mouse genes in network	20,673
• Mouse genes	68,595	• Rat genes in network	17,534
• Mouse SwissProt proteins	16,829	• Chemical compounds	351,887
• Rat genes	47,989	• Drugs	4,297
• Rat SwissProt proteins	7,968	• Endogenous compounds	3,469
• Compounds	815,453	• Metabolic reactions	33,338
• Compounds with structure	796,538	• Transport reactions	4,570
• Endogenous compounds	5,405	• Processing Reactions	1,262
• Nutritional compounds	119	• Pubmed journals	3,668
• Metabolites of xenobiotic	26,761	• Pubmed records	1,919,055
• Drugs	9,186	• Pubmed articles (unique)	254,828
		• Total amount of interactions	1,743,596
- Biologics	1,457	- Protein – Protein	764,667
- Small Molecules	7,729	- Compound – Protein	701,499
		- Compound – Compound	9,816
- Approved drugs	2,293	- Metabolic enzyme - Reaction	43,064
- Withdrawn drugs	261	- Transporter – Reaction	4,570
- Clinical trial drugs	5,055	- Substrate, Product – Reaction	85,980
- Discontinued drugs	1,189	- RNA – Protein	134,000
- Preclinical drugs	251		
- Unknown	137		
		• Pathway maps	1,632
- Drug combination regimens	8,446	- Human genes in maps	7,370
		- Mouse genes in maps	6,723
		- Rat genes in maps	6,470
		- Interactions in maps	30,494

# From peer reviewed articles to signaling pathways



**PUBLICATIONS**  
(209 for EGF-EGFR  
interaction)

**MOLECULAR  
INTERACTION**

**GLOBAL NETWORK:**  
~ 1,700,000  
molecular interactions

~ 1,600 canonical and  
disease signaling pathways

- Manual annotation from publications
- Team of PhDs, MDs
- More than 10 years



# Flexibility in data analysis tools

## 10 Different Network Building Algorithms, all with written and visual descriptions

### Choose building algorithm

Analyze network

Analyze network

Analyze network (transcription factors)

Analyze network (receptors)

Transcription regulation

Shortest paths

Trace pathways

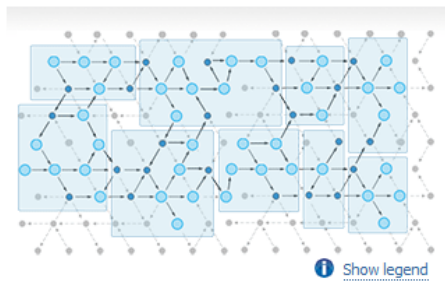
Direct interactions

Self regulation

Auto expand

Expand by one interaction

Manual expand



## Multiple automated Workflows to save, share and export



### Data Analysis Workflows

A set of simple step-by-step wizards for analysis of your data.

- Enrichment Analysis
- Analyze Single Experiment
- Compare Experiments
- Compare Compounds
- Toxicity Analysis
- Biomarker Assessment
- Interactome Analysis

## Data Annotation & Processing Tool calculate differential expression and upload

Data Import 1

**Data Processing:** Select a single text, SOFT or excel data file. Or select multiple CEL files.

Supported file types: Excel spreadsheets (xls,xlsx), delimited text (txt, csv), GEO SOFT and Affymetrix CEL files.

Data file(s)

Select file(s): C:\Users\j6020105\Documents\CUSTOMER DEMOS\posters and presentation 2017\GSE41620\_family.soft.gz

Files of type: GEO SOFT file

Data preview

Array: GPL6105

Samples	Title
GSM1020250	PMN-MDSC naive blood, repl. 1
GSM1020251	PMN-MDSC naive blood, repl. 2
GSM1020252	PMN-MDSC naive blood, repl. 3

Total number of array platforms in GEO SOFT file: 1

GEO ID: GSE41620

## Pathway Map Creator customize and build maps



# Getting support

Technical Support & questions:  
[sbsupport@thomsonreuters.com](mailto:sbsupport@thomsonreuters.com)

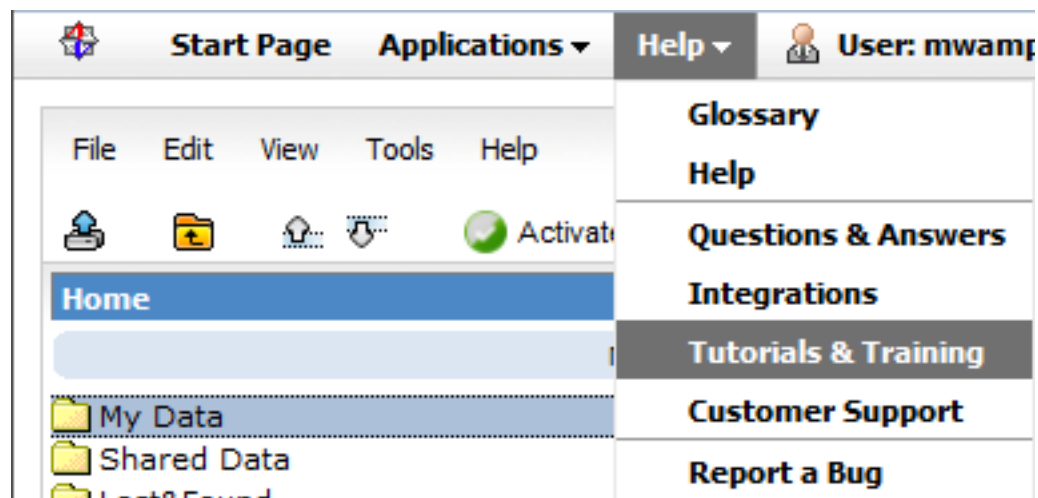
Training webinar recordings available at:  
[lsresearch.thomsonreuters.com](http://lsresearch.thomsonreuters.com)  
(in the Knowledge section)



Help

Technical Support: <http://ip-science.thomsonreuters.com/techsupport/>

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- [Recent Feature Updates](#)



## Questions & Answers

- + [FAQ](#)
- + [System Requirements](#)

## Tutorials and Training

- + [MetaCore](#)
- + [MetaDrug](#)
- + [Data Annotation & Processing Tool](#)
- + [Recent Feature Updates](#)

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TOMORROW'S NEWS -  
**EVERY DAY.**

The issues influencing the future of biopharma. Delivered to you every business day.

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“Something that I do with MetaCore™ in one afternoon now, would have taken a week before.”

DR. CHARLES LECCELLIER,  
PRINCIPAL INVESTIGATOR,  
IGMM

## THOMSON REUTERS SYSTEMS BIOLOGY SOLUTIONS

### YOUR GPS IN PATHWAY ANALYSIS

Whether you want to reduce the risk in your OMICs analysis, realize the potential of your biomarkers, or establish a target's mechanism of action, Thomson Reuters has the right **solution** for you.

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A series of industry-academy partnerships on systems biology of common human diseases and stem cells, led by Thomson Reuters.

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A leading systems pharmacology solution that incorporates extensive manually curated information on biological effects of small molecule compounds.

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# Use EZ search to look up NOTCH1

Start Page Applications ▾ Help ▾ User: mwampole ▾

NOTCH1 Search [Advanced Search](#)

File Edit View Tools Help

Activate/Deactivate

Home ▸ My Data ▸ EXPERIMENTS

Genomic Analysis Most Popular Questions Upload Workflows & Reports One-click Analysis Build Network



## EZ Search

Name  Search






### Objects Found

[Genes \(7\)](#)  
[Gene Aberrations \(860\)](#)  
[Proteins \(54\)](#)  
[RNA \(48\)](#)  
[Network Objects \(6\)](#)  
[Interactions \(3796\)](#)  
[Drugs \(1\)](#)  
[Biologics \(1\)](#)  
[Maps \(58\)](#)  
[Networks \(16\)](#)  
[Reactions \(9\)](#)  
[Articles \(489\)](#)

### Results

<input type="checkbox"/>	Network Object Details
<input type="checkbox"/>	 <b>NOTCH1 (NICD)</b> Proteins Proteins: <a href="#">NOTCH1 (NICD)</a> ( <i>Mus musculus</i> ), <a href="#">NOTCH1 (NICD)</a> ( <i>Homo sapiens</i> ) Genes: <a href="#">Notch1</a> ( <i>Mus musculus</i> ), <a href="#">NOTCH1</a> ( <i>Homo sapiens</i> ) Thomson Reuters Integrity: <a href="#">Notch1</a> ( <i>Mus musculus</i> ), <a href="#">Notch1</a> ( <i>Rattus norvegicus</i> )
<input type="checkbox"/>	 <b>NOTCH1 (NEXT)</b> Proteins Proteins: <a href="#">NOTCH1 (NEXT)</a> ( <i>Homo sapiens</i> ), <a href="#">NOTCH1 (NEXT)</a> ( <i>Mus musculus</i> ) Genes: <a href="#">Notch1</a> ( <i>Mus musculus</i> ), <a href="#">NOTCH1</a> ( <i>Homo sapiens</i> ) Thomson Reuters Integrity: <a href="#">Notch1</a> ( <i>Mus musculus</i> ), <a href="#">Notch1</a> ( <i>Rattus norvegicus</i> )

### Network Object Details

-  **NOTCH1 (NICD)** Proteins  
Proteins: [NOTCH1 \(NICD\)](#) (*Mus musculus*), [NOTCH1 \(NICD\)](#) (*Homo sapiens*)  
Genes: [Notch1](#) (*Mus musculus*), [NOTCH1](#) (*Homo sapiens*)  
Thomson Reuters Integrity: [Notch1](#) (*Mus musculus*), [Notch1](#) (*Rattus norvegicus*)
-  **NOTCH1 (NEXT)** Proteins  
Proteins: [NOTCH1 \(NEXT\)](#) (*Homo sapiens*), [NOTCH1 \(NEXT\)](#) (*Mus musculus*)  
Genes: [Notch1](#) (*Mus musculus*), [NOTCH1](#) (*Homo sapiens*)  
Thomson Reuters Integrity: [Notch1](#) (*Mus musculus*), [Notch1](#) (*Rattus norvegicus*)
-  **NOTCH1 receptor** Proteins  
Proteins: [NOTCH1 receptor](#) (*Homo sapiens*), [NOTCH1 receptor](#) (*Rattus norvegicus*)  
Genes: [Notch1](#) (*Mus musculus*), [NOTCH1](#) (*Homo sapiens*)  
Thomson Reuters Integrity: [Notch1](#) (*Mus musculus*), [Notch1](#) (*Rattus norvegicus*)
-  **NOTCH1 precursor** Proteins  
Proteins: [NOTCH1 precursor](#) (*Homo sapiens*), [NOTCH1 precursor](#) (*Rattus norvegicus*)  
Genes: [Notch1](#) (*Mus musculus*), [NOTCH1](#) (*Homo sapiens*)  
Thomson Reuters Integrity: [Notch1](#) (*Mus musculus*), [Notch1](#) (*Rattus norvegicus*)
-  **Notch** Protein groups  
Protein Groups: [Notch](#) (*Homo sapiens*), [Notch](#) (*Mus musculus*)  
Genes: [Notch1](#) (*Mus musculus*), [NOTCH1](#) (*Homo sapiens*)  
Thomson Reuters Integrity: [Notch1](#) (*Mus musculus*), [Notch1](#) (*Rattus norvegicus*)

# I want to know what interactions and pathways involve NOTCH1

**NOTCH1 (NICD)**

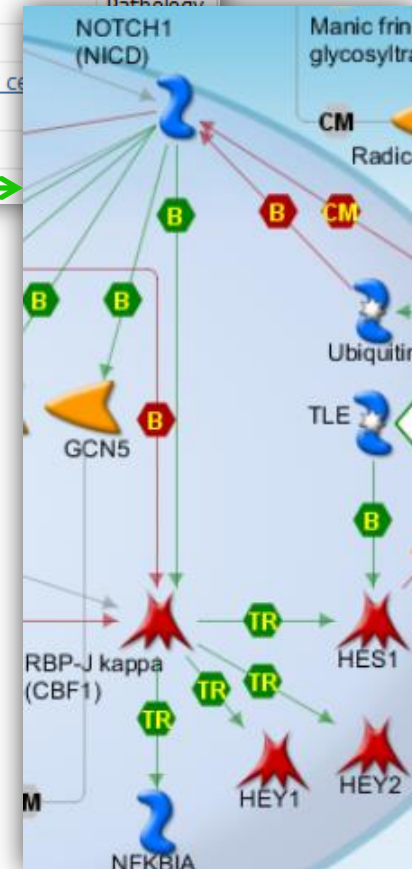
Network object | Build Network

**Table of Contents**

- General
  - Gene Details
  - Protein Details
  - Thomson Reuters Integrity
  - External Databases
  - Vendors
  - Groups/Variants
- Pathways and Processes
- Diseases
- Reactions
- Interactions

#	Name	Map Type
1	<a href="#">Aberrant B-Raf signaling in melanoma progression</a>	Pathology
2	<a href="#">Aberrant production of IL-2 and IL-17 in SLE T cells</a>	Pathology
3	<a href="#">Activation of Notch signaling in breast cancer</a>	Pathology
4	<a href="#">Canonical Notch signaling pathway in colorectal cancer</a>	Pathology
5	<a href="#">Development Epigenetic and transcriptional regulation of oligodendrocyte precursor cell differentiation and myelination</a>	Pathology
6	<a href="#">Development Keratinocyte differentiation</a>	Pathology
7	<a href="#">Development Notch Signaling Pathway</a>	Pathology

- NOTCH1 (NICD) represents cleaved intracellular portion of NOTCH1 after ligand binding.
- This region is important in the regulation of a number of genes.
- Aberrations in NOTCH1 have been associated with many diseases including chronic lymphocytic leukemia



#	From		To	Direction	Effect	Mechanism	Link info
1	NOTCH1 (NICD)	TR	c-IAP2	Outgoing	Activation	Transcription regulation	
2	NOTCH1 (NICD)	TR	CD133	Outgoing	Activation	Transcription regulation	
3	NOTCH1 (NICD)	TR	CDK4	Outgoing	Activation	Transcription regulation	
4	NOTCH1 (NICD)	TR	CDK6	Outgoing	Activation	Transcription regulation	



## Common nonmutational *NOTCH1* activation in chronic lymphocytic leukemia

Giulia Fabbri<sup>a</sup>, Antony B. Holmes<sup>a</sup>, Mara Viganotti<sup>a</sup>, Claudio Scuoppo<sup>a</sup>, Laura Belver<sup>a</sup>, Daniel Herranz<sup>a</sup>,  
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Adolfo A. Ferrando<sup>a,f,g</sup>, and Riccardo Dalla-Favera<sup>a,f,h,i,1</sup>

Author Affiliations 

Contributed by Riccardo Dalla-Favera, February 15, 2017 (sent for review December 20, 2016; reviewed by Carlo M. Croce and Louis M. Staudt)

GSE92626 – Identification of genes induced by NOTCH1 in a chronic lymphocytic leukemia (CLL) cell line and tracking of these genes in primary CLL patients

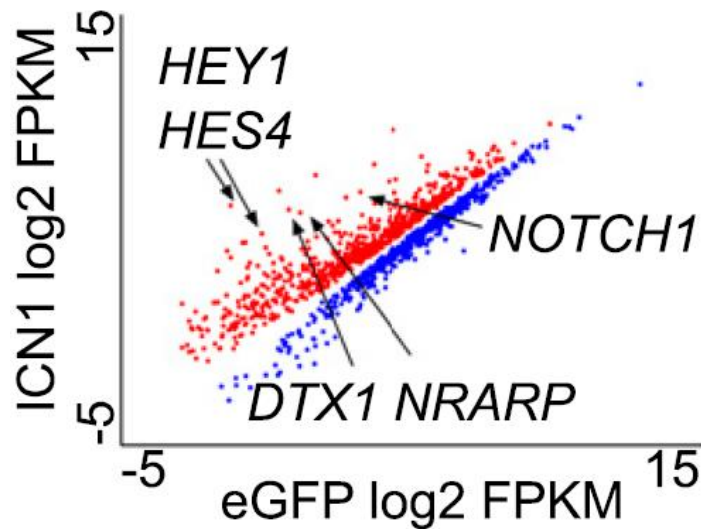
- Illumina HiSeq 2000 – RNA-seq expression analysis

GSE92701 – Identification of NOTCH1 bound genes in chronic lymphocytic leukaemia (CLL) cells

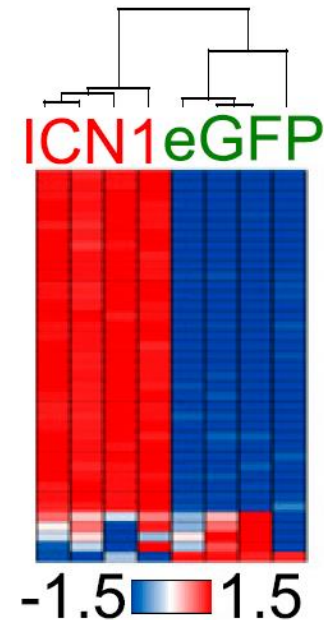
- Illumina HiSeq 2000 – ChIP-seq expression analysis

# RNA-seq data

- CLL cell line (MO1043) was used for NOTCH1 signaling induction
- MO1043 was either induced with a lentiviral system expressing either:
  - NOTCH1 intracellular region (ICN1)
  - Control eGFP
- Over 700 up-regulated transcripts were identified
  - Median fold change 1.7
  - False Discover Rate < 0.001



Scatter plot of log2-transformed RNA-Seq FPKM values of differentially expressed genes between MO1043 cells induced with ICN1 and -eGFP control CLL cells (FDR < 0.001).

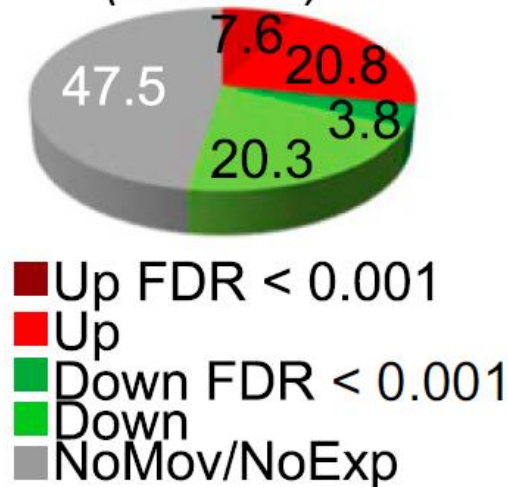


Hierarchical clustering of RNA-Seq profiles of MO1043 cells induced with NOTCH1 (ICN1) or eGFP

# ChIP-seq data and intersection with RNA-seq data

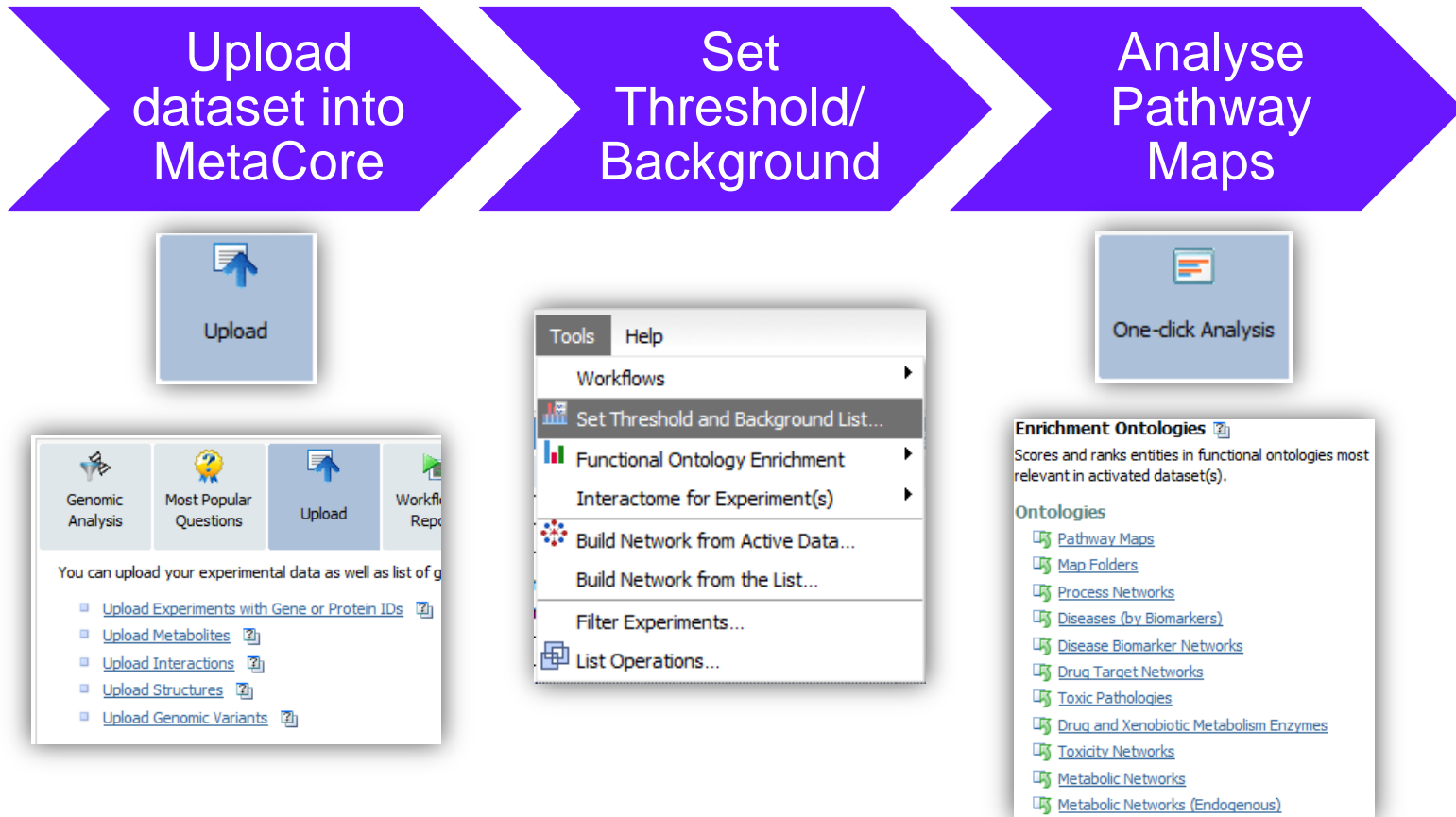
- ChIP-seq identified 4,737 NOTCH1 binding sites.
- Intersection of RNA-seq and ChIP-seq data was used to create ICN1 induced signature
  - Genes bound by NOTCH1 in ChIP-seq data
  - Genes upregulated via ICN1 induction with a FDR < 0.001

NOTCH1-bound genes  
( $n=3845$ )



Intersection between RNA-Seq  
and ChIP-Seq data obtained in ICN1  
induced MO1043 CLL cells

# Upload data and run a pathway enrichment



Question:

- What pathways are enriched by ICN1 from RNA-seq and bound to NOTCH1 in ChIP-seq experiments?



# Upload RNA-seq expression data into MetaCore

**1**

Genomic Analysis Most Popular Questions **Upload** Workflows & Reports One-click Analysis

You can upload your experimental data as well as list of genes/proteins/metabolites.

- ☒ Upload Experiments with Gene or Protein IDs
- ☐ Upload Metabolites
- ☐ Upload Interactions
- ☐ Upload Structures
- ☐ Upload Genomic Variants

**2**

Data Analysis Wizard (General parser)

**Step 1**

Click "browse" to select file(s) to upload:

Browse... GSE92626-ICN1-HA vs GFP.txt

**Data format**

**Warning:** do not mix IDs in the same column.  
Excel or plain text with tab separated fields formats are supported.  
**Warning:** Currently, Excel 2007 files are not supported older Excel version.  
The file has to be in the following format:

	A	B	C	D
1	Gene Sym	Fold Change ICN1-HA vs GFP		
2	HES4	170.4		
3	P2RY2	86.1		
4	CR2	60.3		
5	CHI3L2	40.2		
6	DTX1	37.5		
7	TEKT4	36.3		
8	HEY1	32.3		
9	ALDH1A1	18.9		

**3**

Data Analysis Wizard (General parser)

**Step 2**

Only first 10 lines of your file are shown. Use horizontal scrolling if needed.  
Use checkboxes against each row to specify table header lines

Specify the column types in your file:

**File data**

Experiments name prefix: GSE92626-ICN1-HA vs GFP

Type: Gene symbol (official only) Intensity

Name	
<input checked="" type="checkbox"/> Gene Symbol	Fold Change ICN1-HA vs GFP
<input type="checkbox"/> HES4	170.4
<input type="checkbox"/> P2RY2	86.1
<input type="checkbox"/> CR2	60.3

**4**

Data Analysis Wizard (General parser)

**Step 3**

**Species**

Choose species: Homo sapiens

Next >>

Analytics

Note: use OFFICIAL gene symbols

# Create background list for RNA-seq

[Advanced Search](#)

Conditional Search. Choose a query and click "Search".

Find Genes (Human) that ... (condition is not defined yet)

Find:  that     that

add condition

Gene Filter

General Filter




[Tissues/Fluids](#)

[Gene Orthologs](#)

Species:

- ☒ Human  
☐ Mouse  
☐ Rat

Function/Icon:

- ☐  Receptor ligands  
☒  Generic receptors  
☐  Transcription factors

Localization:

- ☐ Secreted peptides/proteins  
☐ Plasma membrane peptides/...  
☐ Cytoplasm peptides/proteins  
☐ Nucleus peptides/proteins

Grouping:

- ☐ Individual objects  
☐ Protein/peptide groups  
☐ Protein/peptide complexes  
☐ RNA groups

Find Genes (Human) that ... (condition is not defined yet)

[Export](#) [Build network](#)

#	Entrez Gene ID	Symbol	Full Name
1	553192	MYP8	myop...
2	378011	CDY9P	chrom...
3	100421273	LOC100421273	F-box...
4	728858	C12orf71	chrom...

Name:

To:  [Show lists](#)

- ☒ Genes of  
☐ Network objects  
☒ Human  
☐ Mouse  
☐ Rat

☐ Through:

[Hide additional options](#)

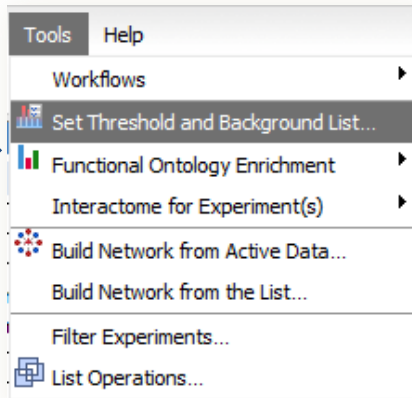
Rows:

- ☒ All rows  
☐ Current page  
☐ Selected rows

☐ With active data only

# Set background list and run pathway map enrichment

1



2

A screenshot of a dialog box titled 'Background List and Threshold'. It has two sections: 'Threshold' and 'Background List'. The 'Threshold' section has two rows: 'General' and 'Metabolic', each with a 'Threshold' input field (both set to 0) and a 'P Threshold' input field (both set to 1). There are 'Statistics' buttons next to each row. The 'Background List' section has a 'List Source' section with three radio buttons: 'Array' (unselected), 'Gene list' (selected), and 'Network object list' (unselected). Below it is a 'List Name' dropdown menu showing 'all genes in MetaCore'. At the bottom are 'Cancel' and 'OK' buttons. A blue arrow points to the 'List Name' dropdown from the number '2'.

3

A screenshot of a table titled 'Active Data'. The table has columns: 'Name', 'Type', and a third column. The first row is a header with 'Name' and 'Type'. The second row is a sub-header with 'Active Data'. The third row contains the text 'GSE92626-ICN1-HA vs GFP', a green checkmark, 'GX', and '04'.

4

A screenshot of a software interface showing a 'One-click Analysis' button highlighted with a blue box. Below it are two sections: 'Enrichment Ontologies' and 'Interactome'. The 'Enrichment Ontologies' section has a list of links: 'Pathway Maps', 'Map Folders', 'Process Networks', 'Diseases (by Biomarkers)', 'Disease Biomarker Networks', 'Drug Target Networks', 'Toxic Pathologies', 'Drug and Xenobiotic Metabolism Enzymes', 'Toxicity Networks', 'Metabolic Networks', and 'Metabolic Networks (Endogenous)'. The 'Interactome' section has a list of links: 'Interactions', 'Transcription', 'Significant In', 'Interactome', 'Enrichment b', 'Interactions', 'Interactions', and 'Drug Lookup'. At the bottom right, there is a 'Microarray Rep' section with links for 'Similarity sea' and 'Similarity sea'.

# What pathways are enriched by NOTCH1 induced up-regulation signature?

Ratio of differentially expressed genes from dataset (**GREEN**) over all network objects in folder (**RED**)

Graphical representation of  $-\log(\text{pValue})$

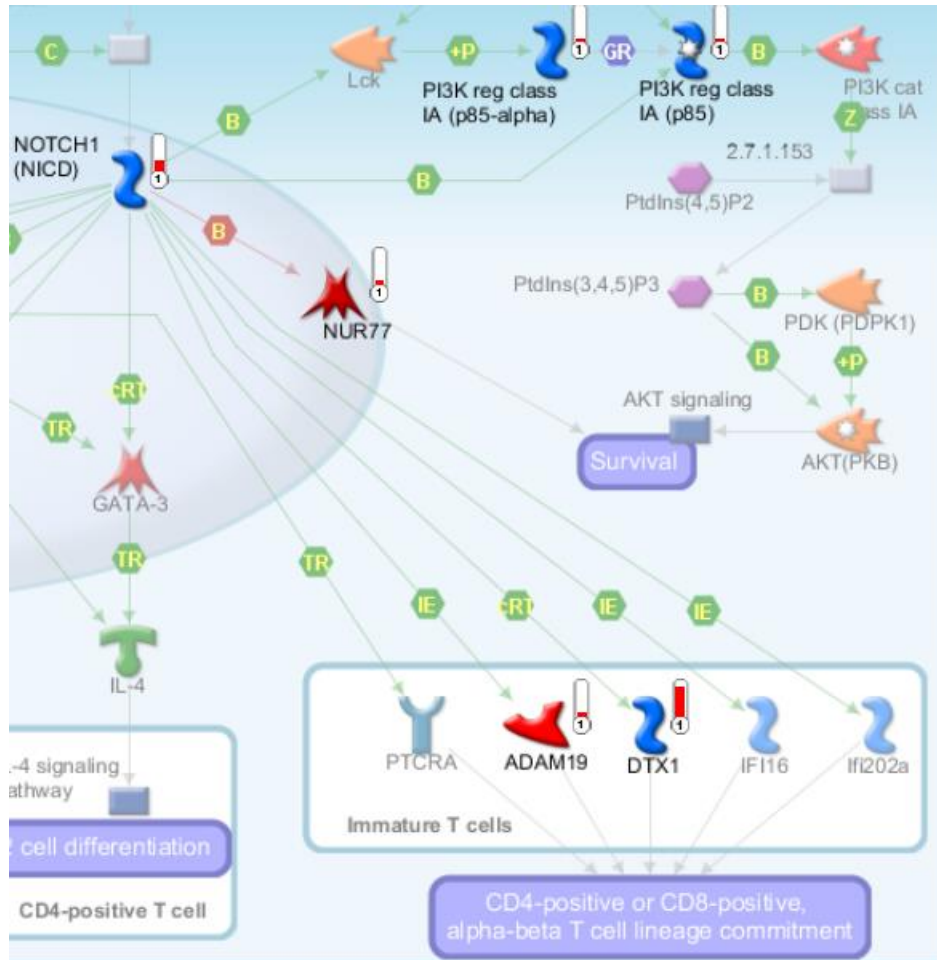
#	Maps	0	1	2	3	4	5	$-\log(\text{pValue})$	pValue ↑	FDR	Ratio
1	<a href="#">Regulation of immune cell differentiation by Notch signaling</a>	████████	████████	████████	████████	████████	████████	2.902e-7	1.788e-4		8/30
2	<a href="#">Notch signaling in oligodendrocyte precursor cell differentiation in multiple sclerosis</a>	████████	████████	████████	████████	████████	████████	4.358e-7	1.788e-4		7/22
3	<a href="#">Stem cells NOTCH in inhibition of WNT/Beta-catenin-induced osteogenesis</a>	████████	████████	████████	████████	████████	████████	6.485e-7	1.788e-4		8/33
4	<a href="#">Development Notch Signaling Pathway</a>	████████	████████	████████	████████	████████	████████	1.057e-6	2.184e-4		8/35
5	<a href="#">Role of SHH and Notch in SCLC</a>	████████	████████	████████	████████	████████	████████	1.553e-6	2.444e-4		7/26
6	<a href="#">Immune response Generation of memory CD4+ T cells</a>	████████	████████	████████	████████	████████	████████	2.056e-6	2.444e-4		7/27

Map name

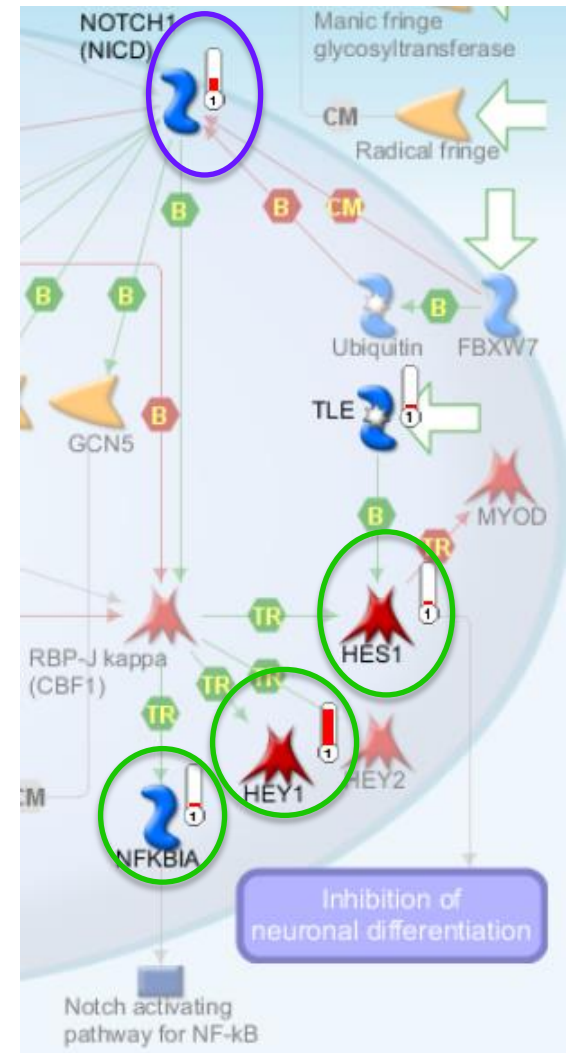
Significance of overlap of differentially expressed genes in the folder

- Notch related signaling pathways are disrupted by this signature
- Could impact many processes related to immune cell processes in diseases ranging from multiple sclerosis to cancer.

# How could this signature be impacting cellular processes?



Regulation of immune cell differentiation by Notch signaling

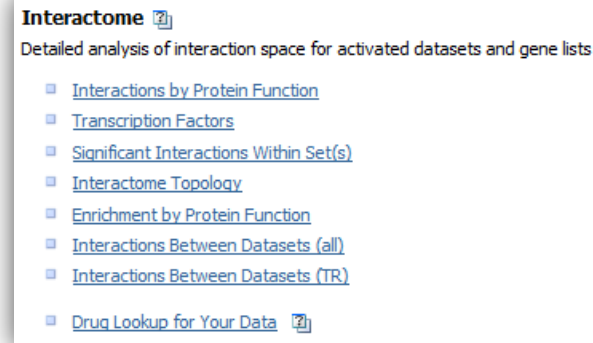
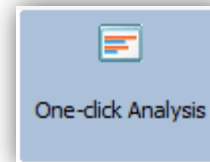
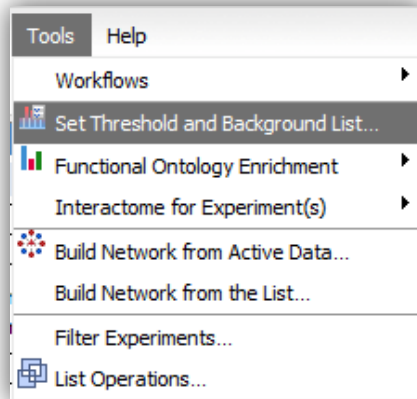


Development Notch Signaling Pathway

# How to find transcription factors potentially regulating expression data

Set Threshold/  
Background

Interactome Analysis  
(Transcription Factors)

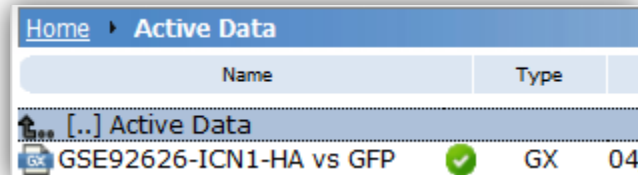
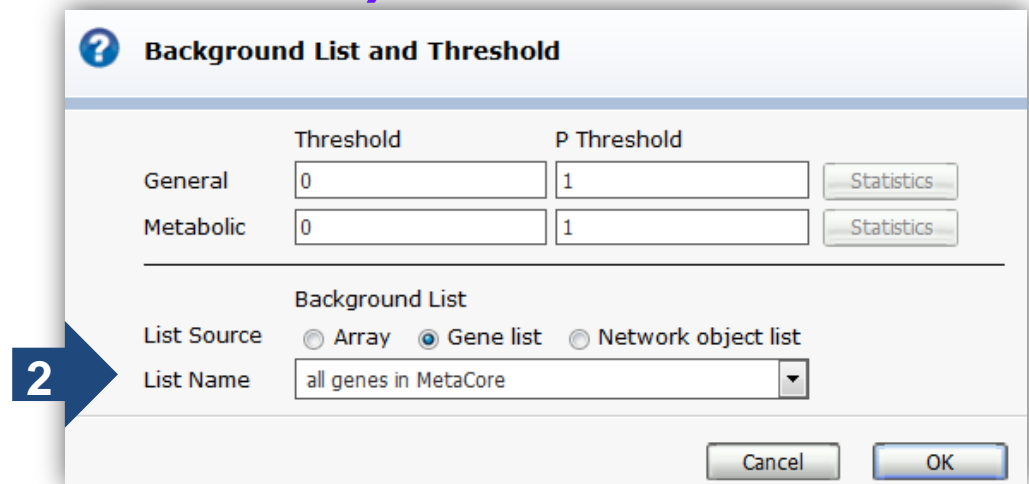
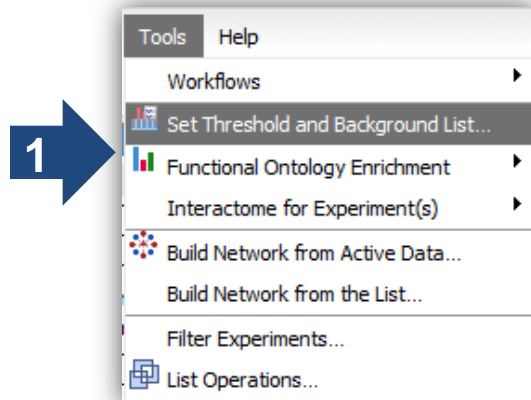


Question:

- What transcription factors could these up regulated genes regulated by?



# Set threshold/background and run interactome analysis (Transcription Factors)



# How does interactome analysis work?

Export		<input type="checkbox"/> Active data set only <input checked="" type="checkbox"/> Show all columns								
<input checked="" type="checkbox"/> IDs in active data set	Object name	Actual	n	R	N	Expected	Ratio	p-value	z-score	
<input checked="" type="checkbox"/> 202531_at; 238725_at	IRF1	45	371	199	19137	3.858	11.66	4.462e-35	21.26	

**N** – Total number of genes in the database (or in array background list)

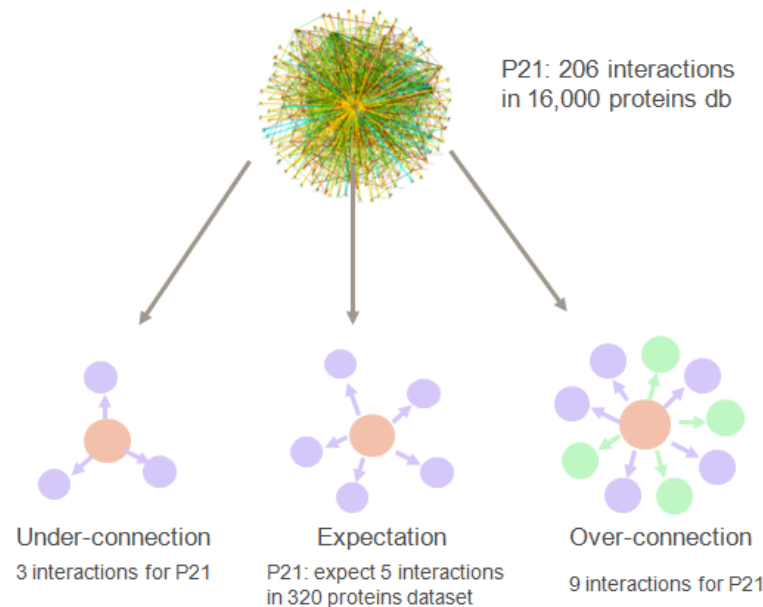
**R** – Total number of genes being regulated by the TRANSCRIPTION FACTOR

**n** – Total number of genes in the dataset

**ACTUAL** – genes in dataset being regulated by this transcription factor

**EXPECTED** –  $(R / N) * n$

**RATIO** – **ACTUAL** / **EXPECTED**

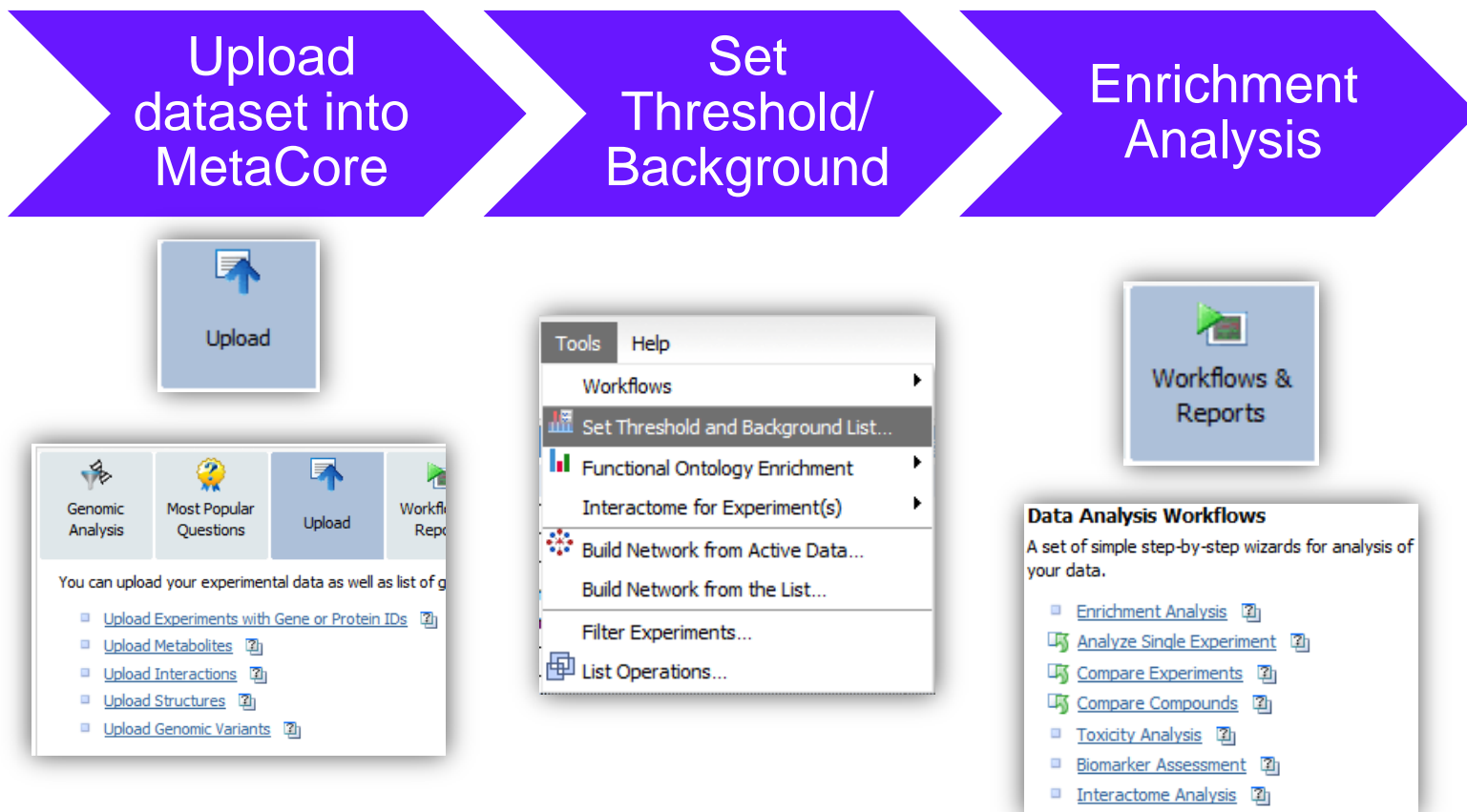


# More then just RBP-J are affect by NOTCH1

IDs in active data set	Object name	Actual	n	R	N	Expected	Ratio	p-value	†	z-score
	<a href="#">CREB1</a>	111	289	5126	38450	38.53	2.881	8.74e-27		12.59
	<a href="#">c-Myc</a>	72	289	2522	38450	18.96	3.798	2.85e-23		12.65
	<a href="#">RelA (p65 NF-kB subunit)</a>	42	289	939	38450	7.058	5.951	1.829e-20		13.37
	<a href="#">p53</a>	47	289	1283	38450	9.643	4.874	2.506e-19		12.28
	<a href="#">ESR1 (nuclear)</a>	40	289	1289	38450	9.688	4.129	3.671e-14		9.943
	<a href="#">SP1</a>	45	289	1648	38450	12.39	3.633	7.311e-14		9.507
IRF8	<a href="#">STAT3</a>	29	289	708	38450	5.322	5.45	1.918e-13		10.4
	<a href="#">IRF8</a>	18	289	258	38450	1.939	9.282	1.497e-12		11.62
	<a href="#">HIF1A</a>	28	289	726	38450	5.457	5.131	2.149e-12		9.78
	<a href="#">IRF4</a>	21	289	434	38450	3.262	6.438	2.188e-11		9.914
	<a href="#">PU.1</a>	20	289	399	38450	2.999	6.669	3.551e-11		9.906
	<a href="#">FOXP3</a>	17	289	282	38450	2.12	8.02	6.348e-11		10.3
	<a href="#">EBF</a>	12	289	126	38450	0.947	12.67	2.352e-10		11.42
	<a href="#">SRF</a>	19	289	413	38450	3.104	6.121	4.692e-10		9.105
	<a href="#">AHR</a>	16	289	286	38450	2.15	7.443	6.964e-10		9.518
	<a href="#">PAX5</a>	12	289	145	38450	1.09	11.01	1.203e-09		10.51
	<a href="#">c-Jun</a>	26	289	835	38450	6.276	4.143	1.338e-09		7.99
	<a href="#">Ikaros</a>	9	289	71	38450	0.5337	16.86	3.349e-09		11.64
	<a href="#">NF-kB1 (p50)</a>	19	289	473	38450	3.555	5.344	4.358e-09		8.273
	<a href="#">EGR1</a>	18	289	454	38450	3.412	5.275	1.371e-08		7.974
	<a href="#">RBP-J kappa (CBF1)</a>	11	289	145	38450	1.09	10.09	1.484e-08		9.547
	<a href="#">STAT1</a>	16	289	361	38450	2.713	5.897	1.914e-08		8.135
NR3C1	<a href="#">SMAD3</a>	14	289	268	38450	2.014	6.95	1.955e-08		8.506
	<a href="#">GCR</a>	18	289	488	38450	3.668	4.907	4.103e-08		7.56
	<a href="#">p63</a>	17	289	445	38450	3.345	5.083	5.955e-08		7.538

Highlights of top 3 Transcription Factors the authors found via Transfac enrichment

# Upload data and run enrichment analysis

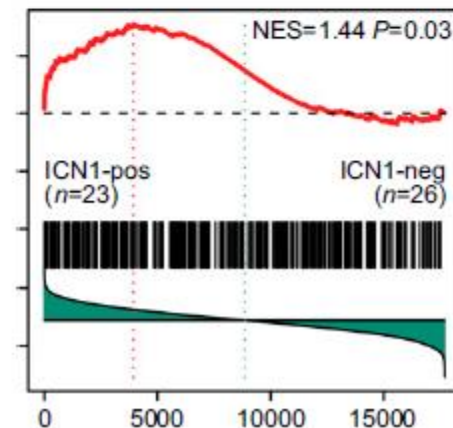
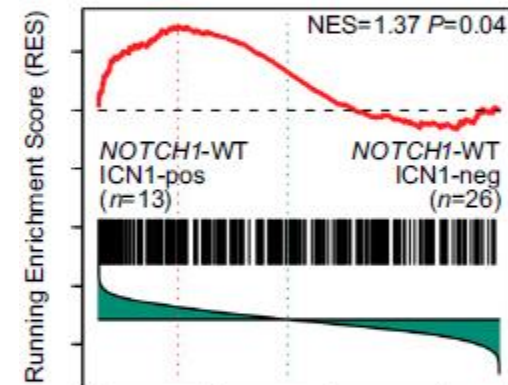
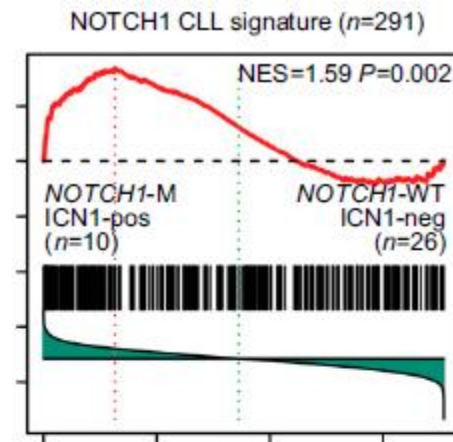


Question:

- How does the NOTCH1 expression profile compare with RNA-seq data from chronic lymphocytic leukemia?


# RNA-seq expression from peripheral blood of chronic lymphocytic leukemia patients

- RNA-seq expression data from 49 samples characterized with NOTCH1 mutational status and ICN1 expression
  - 10 cases mutated NOTCH1 with ICN1 expression
  - 13 cases wildtype NOTCH1 with ICN1 expression
  - 26 cases wildtype NOTCH1 without ICN1 expression
- GSEA analysis found significant enrichment of NOTCH1 CLL signature in both NOTCH1 mutated and NOTCH1 wildtype expression ICN1 as compared to NOTCH1 wildtype not expressing ICN1.
- Data uploaded into MetaCore are the 90 leading edge genes for each group with mean FMPK values



# Upload RNA-seq expression data into MetaCore

**1**



Genomic Analysis Most Popular Questions **Upload** Workflows & Reports One-click Analysis

You can upload your experimental data as well as list of genes/proteins/metabolites.

- Upload Experiments with Gene or Protein IDs**
- Upload Metabolites
- Upload Interactions
- Upload Structures
- Upload Genomic Variants

**2** **Step 1**

Click "browse" to select file(s) to upload:

Browse... CLL patient PB samples.txt

Data format

	A	B	C	D
1	Gene ID	NOTCH1-M / ICN1 pos	NOTCH1-WT / ICN1 pos	NOTCH1-WT / ICN1 neg
2	ZNF318	38.75	42.51	22.09
3	STRADB	5.28	5.95	3.43
4	PLAC8	87.51	62.11	28.82
5	MALT1	27.54	30.74	21.17

**3**

**File data**

Experiments name prefix: CLL patient PB samples Expand

Type	Gene symbol (official only)	Intensity	Intensity	Intensity
Name	Gene ID	NOTCH1-M / ICN1 pos	NOTCH1-WT / ICN1 pos	NOTCH1-WT / ICN1 neg
<input checked="" type="checkbox"/>	Gene ID	NOTCH1-M / ICN1 pos	NOTCH1-WT / ICN1 pos	NOTCH1-WT / ICN1 neg
<input type="checkbox"/>	ZNF318	38.75	42.51	22.09
<input type="checkbox"/>	STRADB	5.28	5.95	3.43
<input type="checkbox"/>	PLAC8	87.51	62.11	28.82

Note: use OFFICIAL gene symbols

**4** **Step 3**

Data Analysis Wizard (General parser)

Species

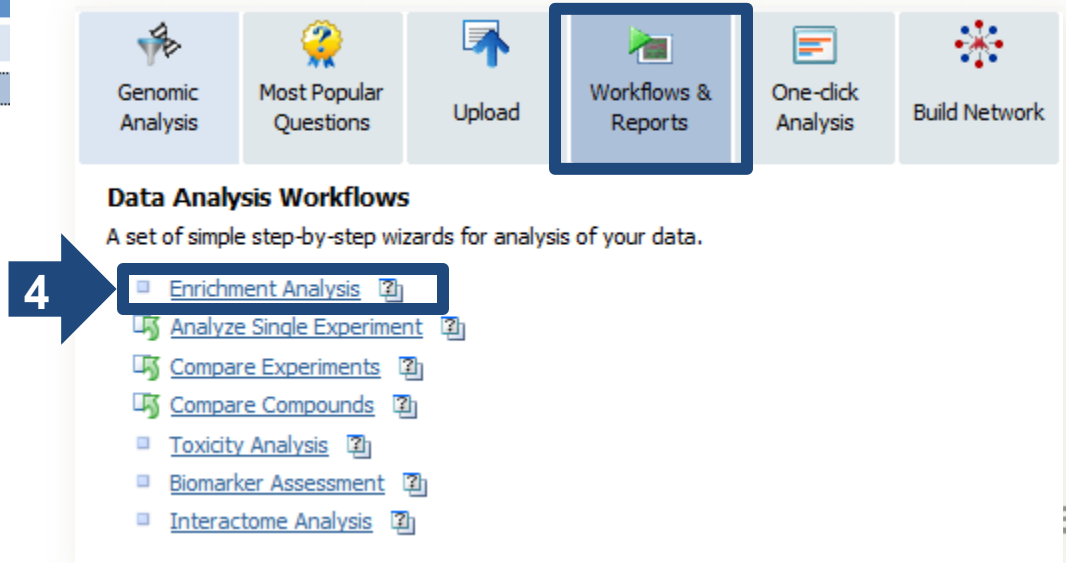
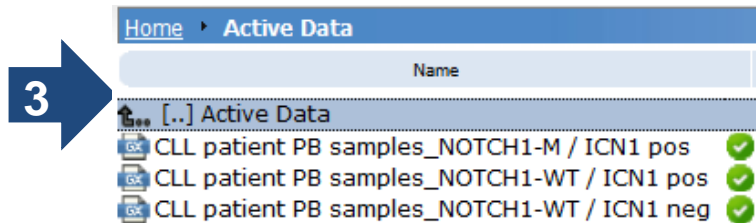
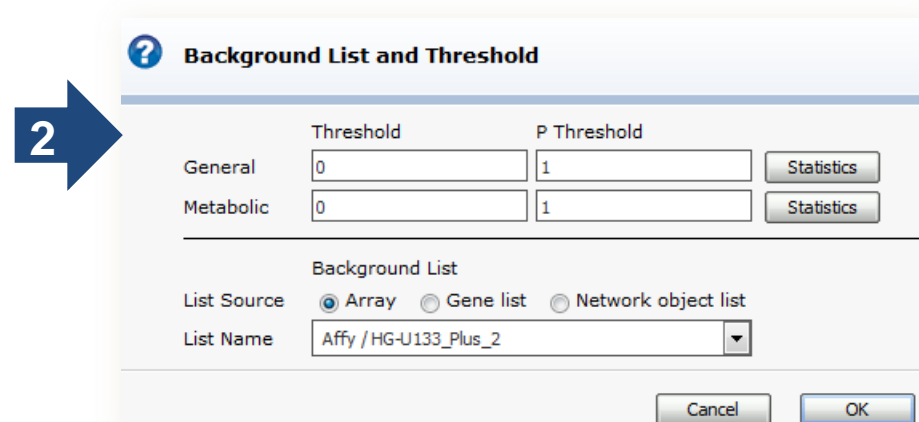
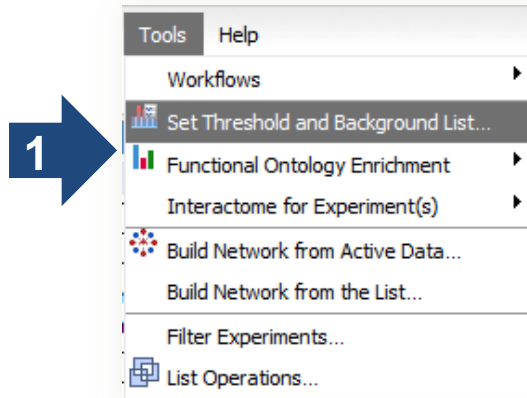
Choose species Homo sapiens

<< Back

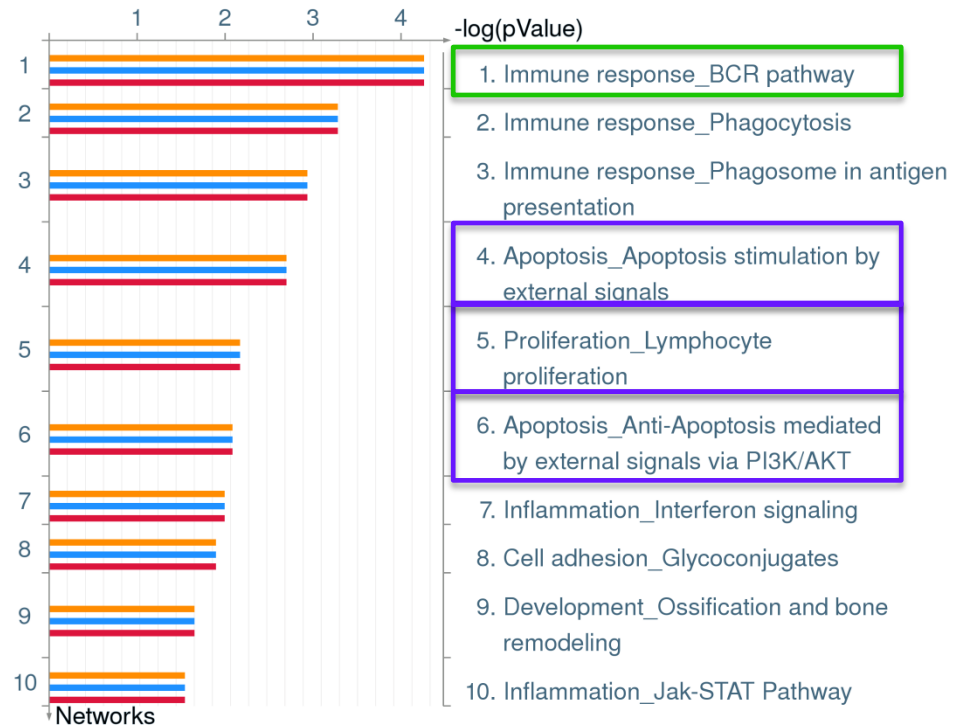
Next >>



# Set threshold/background and run 'Compare Experiments' workflow



# What pathways are affected by genes unique to patients with a low count of infiltrating T-cells?



The leading edge genes pull a number of maps and processes related to **B cell signaling** and **apoptotic** processes

# Use similarity search by genes to find datasets with similar gene signatures

Similarity Search by  
Genes

Export Similar  
Comparisons



One-click Analysis

## Interactome

Detailed analysis of interaction space for activated datasets and gene lists

- [Interactions by Protein Function](#)
- [Transcription Factors](#)
- [Significant Interactions Within Set\(s\)](#)
- [Interactome Topology](#)
- [Enrichment by Protein Function](#)
- [Interactions Between Datasets \(all\)](#)
- [Interactions Between Datasets \(TR\)](#)
- [Drug Lookup for Your Data !\[\]\(67ff022fd78f943b679992c2874bbfd1\_img.jpg\)](#)

## Microarray Repository

- [Similarity search by Genes !\[\]\(2a133ebb0337313d16cc068f19494aa2\_img.jpg\)](#)
- [Similarity search by Functional Descriptors !\[\]\(e5831951c2bb646a242d812c288ddabc\_img.jpg\)](#)

## ▼ Similar comparisons to experiments

### Export

<input type="checkbox"/> Case Group	Control Group
<input type="checkbox"/> Ulcerative Colitis Colonic Mucosa, without Response to Infliximab Treatment, before Treatment	Normal Colonic Mucosa
<input type="checkbox"/> Ulcerative Colitis, Infliximab Treated, Resistant to Treatment	Normal Colon
<input type="checkbox"/> Active Ulcerative Colitis, Azathioprine Treatment	Normal Colon
<input type="checkbox"/> Crohn Disease Colonic Mucosa, without Response to Infliximab Treatment, before Treatment	Normal Colonic Mucosa

Question:

- What other publicly available datasets have a similar to the NOTCH1 expression profile?

# Run similarity search by gene analysis and choose filtering thresholds

1

Home ▸ Active Data			
Name	Type		
[..] Active Data			
GSE92626-ICN1-HA vs GFP	GX	04	

Similarity search by Genes

Home

Name	Type	Date
My Data		
Shared Data		
Lost&Found		

Selected Data

Name	Type	Date
GSE92626-ICN1-HA vs GFP	GX	04/10/2017 02:10:54

Searches for comparisons which are similar by gene content with your experiment/gene list. Please select a single experiment GX, or gene list GL file.

Next>>

3

2

One-click Analysis

Build Network

Custom Content

Predict Compound Activity (MetaDrug)

Interactome

Dataset(s).

Detailed analysis of interaction space for activated datasets and gene lists

Interactions by Protein Function

Transcription Factors

Significant Interactions Within Set(s)

Interactome Topology

Enrichment by Protein Function

Interactions Between Datasets (all)

Interactions Between Datasets (TR)

Drug Lookup for Your Data

Microarray Repository

Similarity search by Genes

Similarity search by Functional Descriptors

4











Settings	
Threshold	6
P-value	0.05
Signals	<div><div><input checked="" type="radio"/> up</div><div><input type="radio"/> down</div><div><input type="radio"/> both</div></div>

- ☒ Similar comparisons only

☐ Experiments and similar comparisons

Apply

# Other public data with a similar signature to the NOTCH1 induced gene signature

Case Group	Control Group	Species	Gene Overlap	p-Value ↑	View
Colorectal Cancer Leukocytes (CD45+ EpCAM-)	Colorectal Cancer Stromal Cells (CD45- EpCAM-)	Homo sapiens	52/1038	7.556e-37	
Choroideremia CD14+ Monocytes	Choroideremia Cultured Fibroblasts	Homo sapiens	39/562	5.322e-33	
Severe Asthma CD8+ T-Lymphocytes	Non-Severe Asthma CD8+ T-Lymphocytes	Homo sapiens	40/733	8.386e-30	
Severe Asthma CD4+ T-Lymphocytes	Non-Severe Asthma CD4+ T-Lymphocytes	Homo sapiens	33/521	9.224e-27	
Cultured B-Lymphocytes from B-Cell Chronic Lymphocytic Leukemia under Dimethyl Sulfoxide (DMSO) Control	Normal Cultured T-Lymphocytes under Dimethyl Sulfoxide (DMSO) Control	Homo sapiens	26/334	1.443e-23	
Normal Monocytes, 1.5 Hours IFN $\gamma$ Stimulated	Normal Monocytes without Any Incubation	Homo sapiens	23/243	6.396e-23	
Normal B-Lymphocytes	Normal Plasma Cells	Homo sapiens	23/263	3.943e-22	
Psoriasis, Normal Adjacent Skin (Non-Lesional), Interferon gamma-1b Treated	Psoriasis, Normal Adjacent Skin (Non-Lesional), Untreated	Homo sapiens	21/232	1.268e-20	
Anaplastic Thyroid Carcinoma	Follicular Thyroid Carcinoma	Homo sapiens	24/403	3.976e-19	
Cultured B-Lymphocytes from B-Cell Chronic Lymphocytic Leukemia Rolipram (20 uM) Treated	Normal Cultured T-Lymphocytes, Rolipram (20 uM) Treated	Homo sapiens	22/334	1.468e-18	

B cell related dataset come up, as well as other interesting datasets related to Asthma, colorectal cancer and interferon stimulated cells.

(interferon signaling came up in the leading edge analysis)

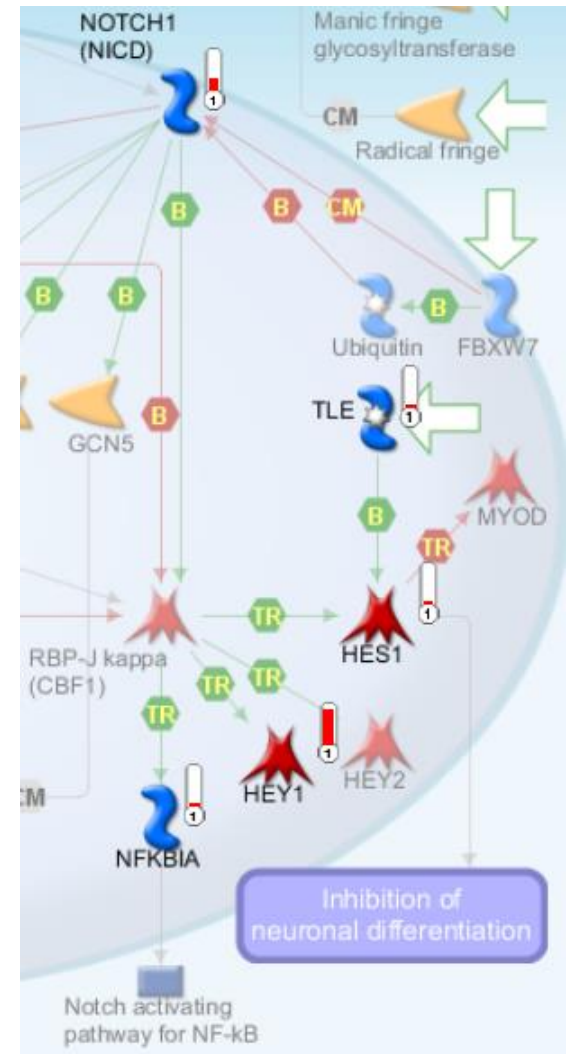
# Summary

The NOTCH1 induced gene signature can impact many different pathways.

NOTCH1 signaling could potentially regulate a wide range of transcription factors including STATs and IRFs.

The 90 leading edge genes from CLL patients that overlap with the NOTCH1 signature point towards disruptions in apoptosis and IFN signaling processes.

Comparing the NOTCH1 induced signature with against other public datasets, pulled up similar signature in other B-lymphocyte comparisons as well as similarities with IFN induced signatures, colorectal cancer, asthma, and other diseases.

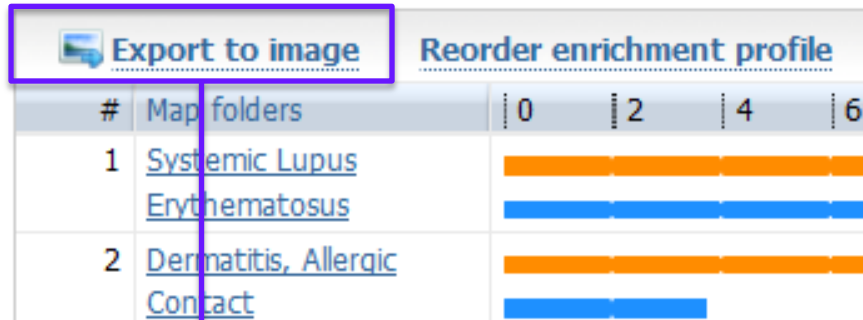




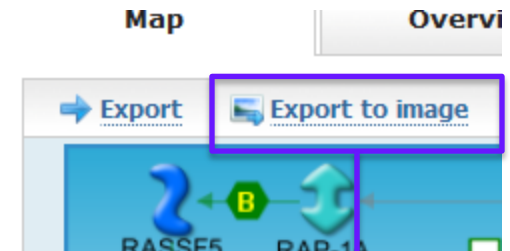
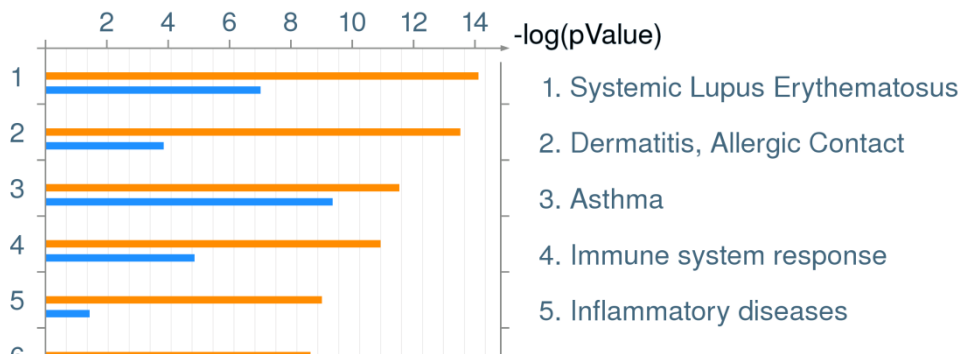
# Tips on exporting visuals and citing MetaCore

MetaCore+MetaDrug™ version 6.30 build 68780

Cite that you used MetaCore and the version number when you ran the analysis



Export to image will create a png file of your enrichment results



Can also export images of pathway maps overlaid with data

