“Walking pathways” and how promoters can help to find new drugs.

(Practical guide to multi-omics and multi-scale data integration)

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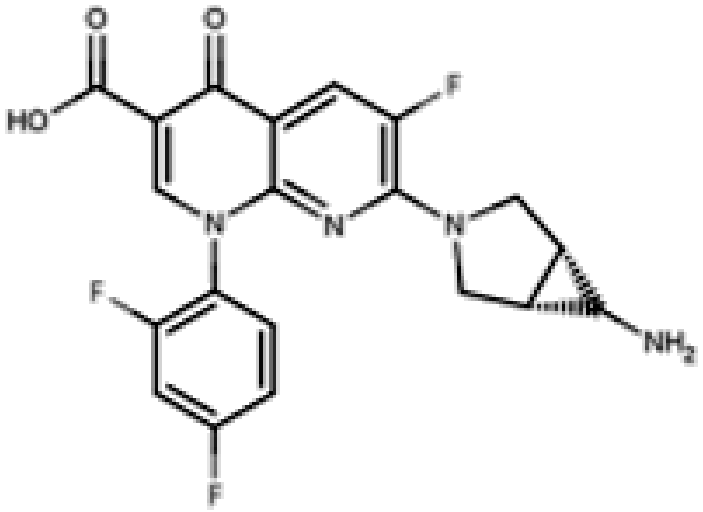
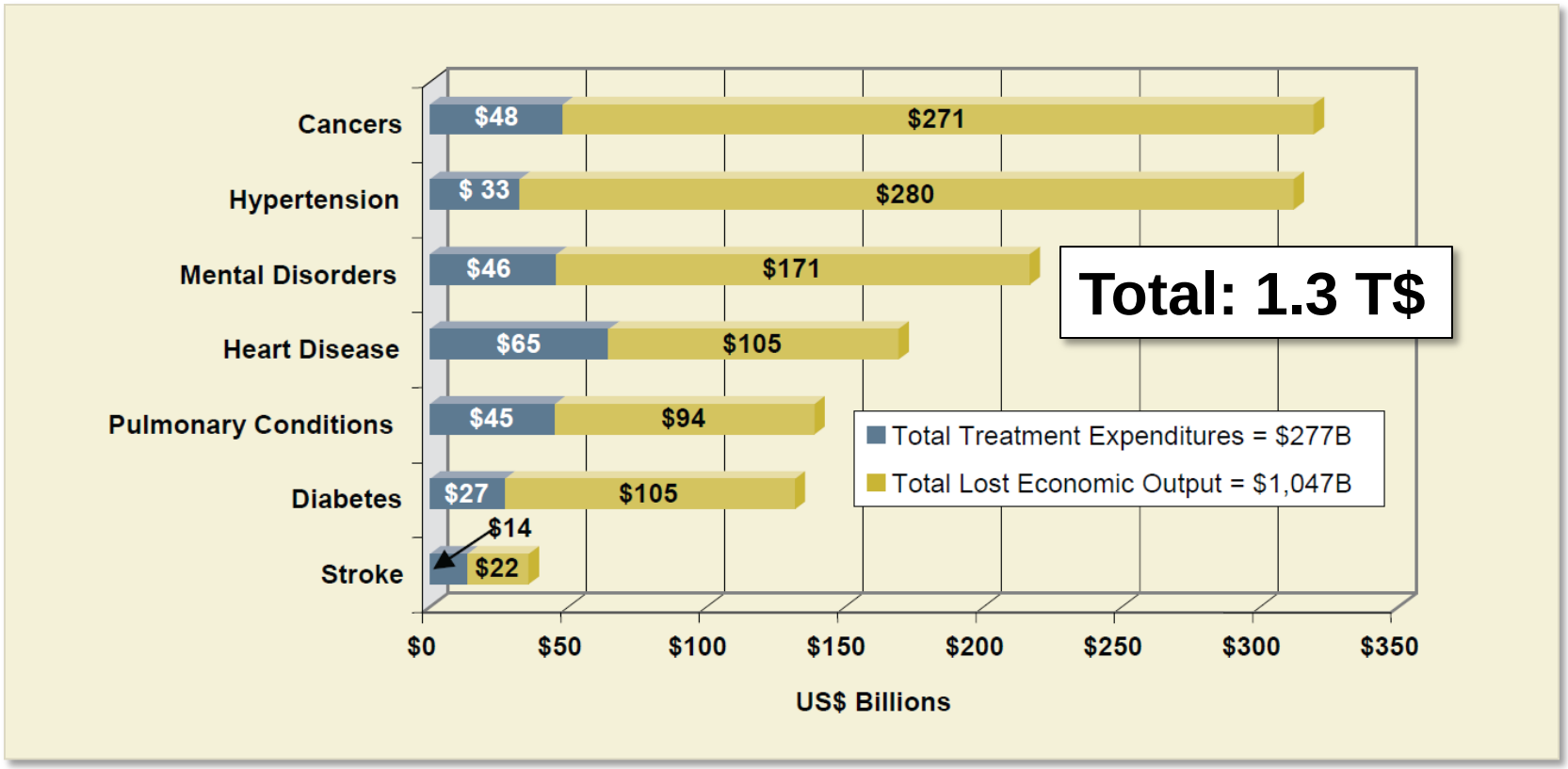
Trovafloxacin - antibiotic



Withdrawn from market due to risk of idiosyncratic hepatotoxicity in 2001.

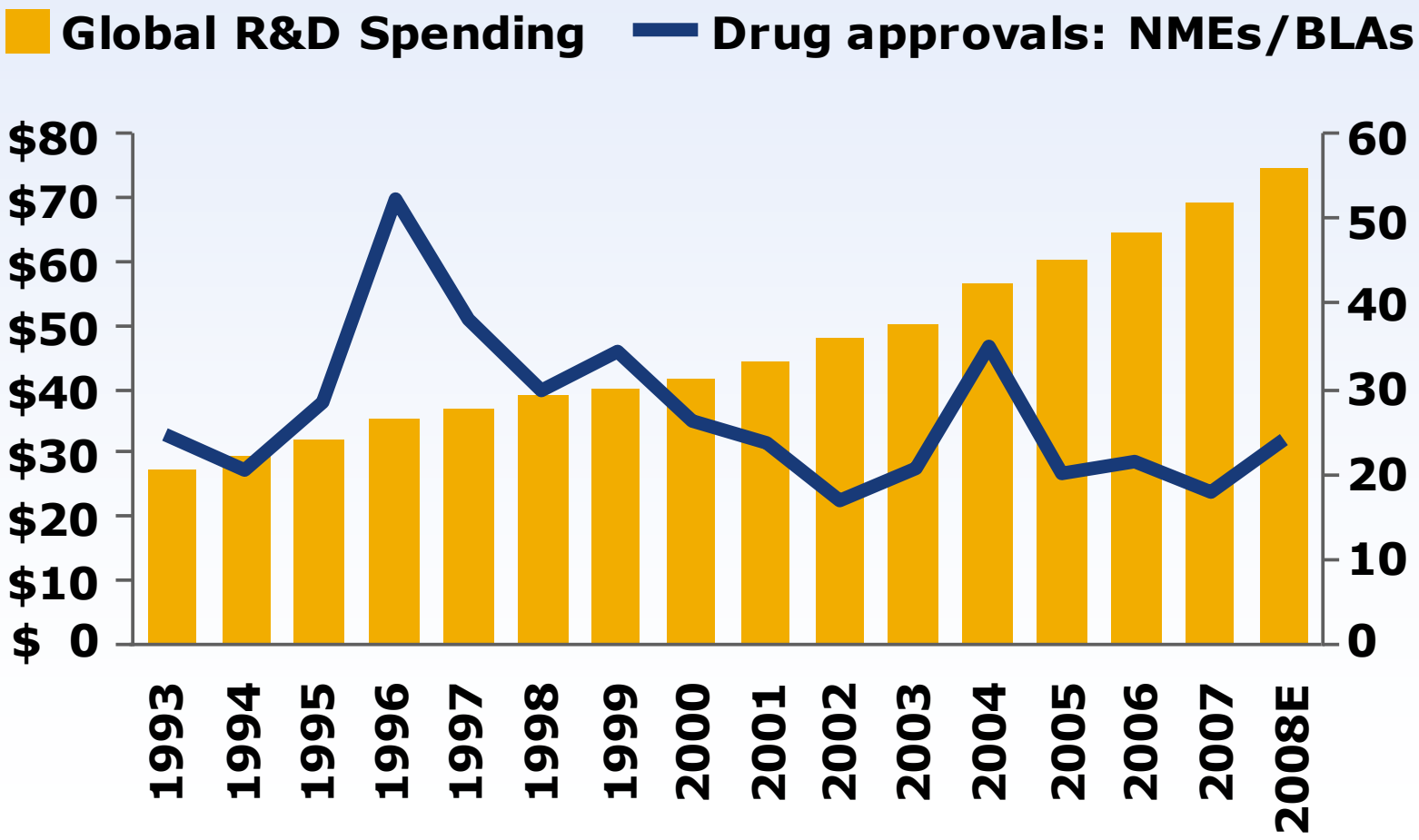
Failure Affects National Economies: Medicines & Equitable Distribution



Combined treatment and productivity costs for US in 2007

Milken Institute 2008

R&D Pipeline

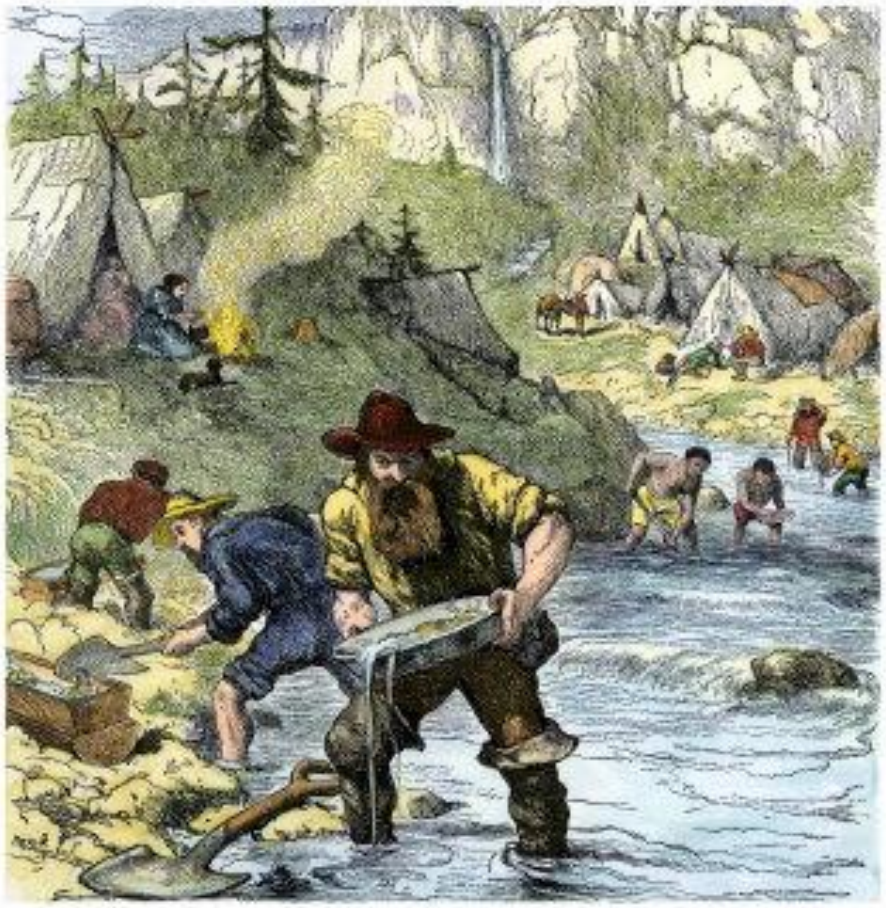

Global R&D Spending
Drug approvals: NMEs/BLAs
$80
$70
$60
$50
$40
$30
$20
$10
$ 0
60
50
40
30
20
10
0
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008E

One of the main causes of high death rate for such diseases is the unsatisfactory quality of treatment, which in the first place is brought by low efficiency and insufficient safety of today's drugs and therapies.

About 50% of prescribed medicine doesn't have any therapeutic effect at all. Moreover, 125 thousand deaths annually (in USA) are caused by the drugs' side effects.

It becomes more and more obvious that the main cause of this crisis is **the insufficient understanding of deep biological mechanisms** of initiation and flowing of pathological conditions and toxicity mechanisms used in drugs.

Drug discovery – the Gold Rush



Drug discovery – should become a technology

Disease

Therapy

Patient

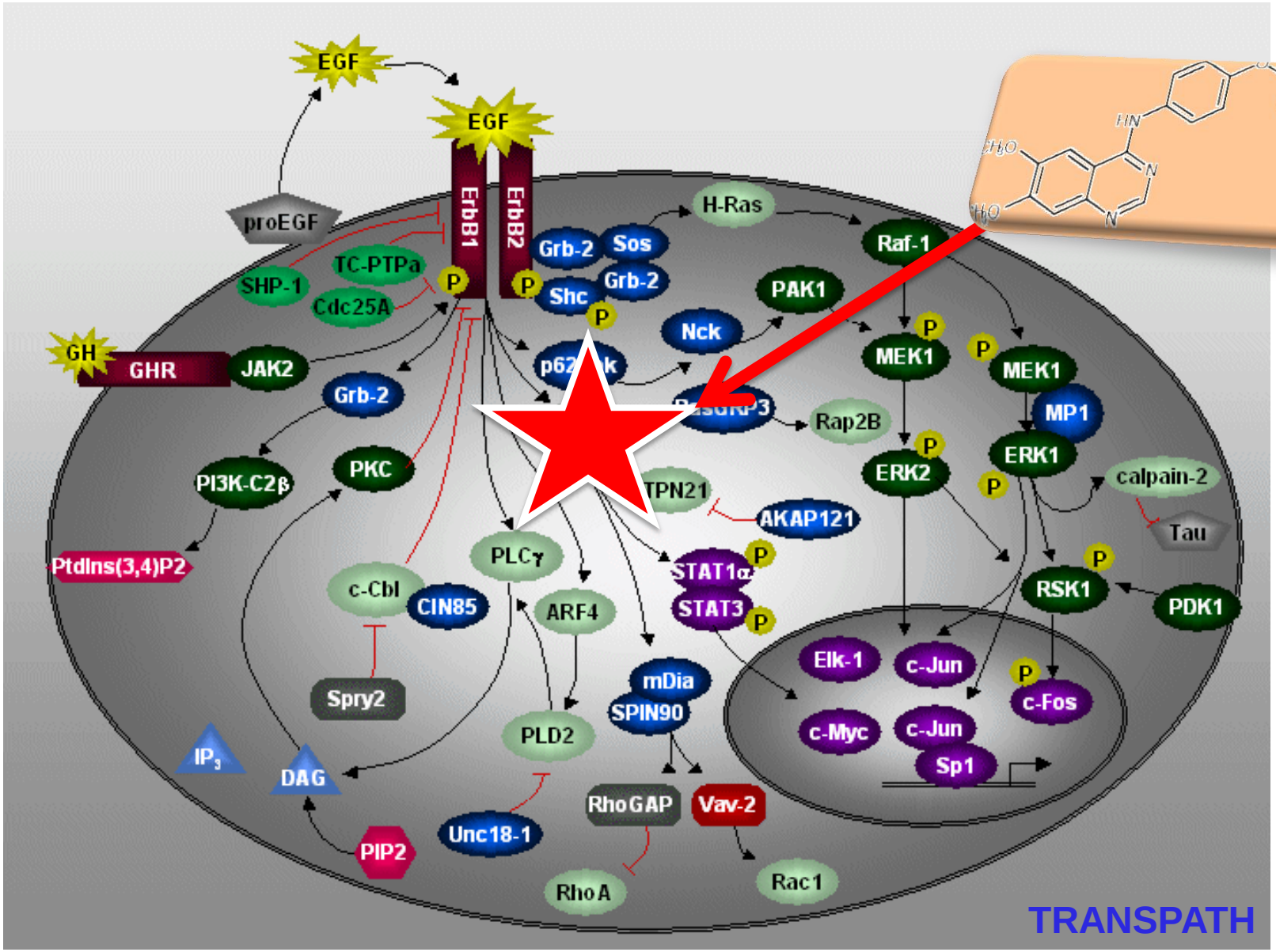
Systems medicine

Systems approaches will transform the way drugs are developed … that will target multiple components of networks and pathways perturbed in diseases.

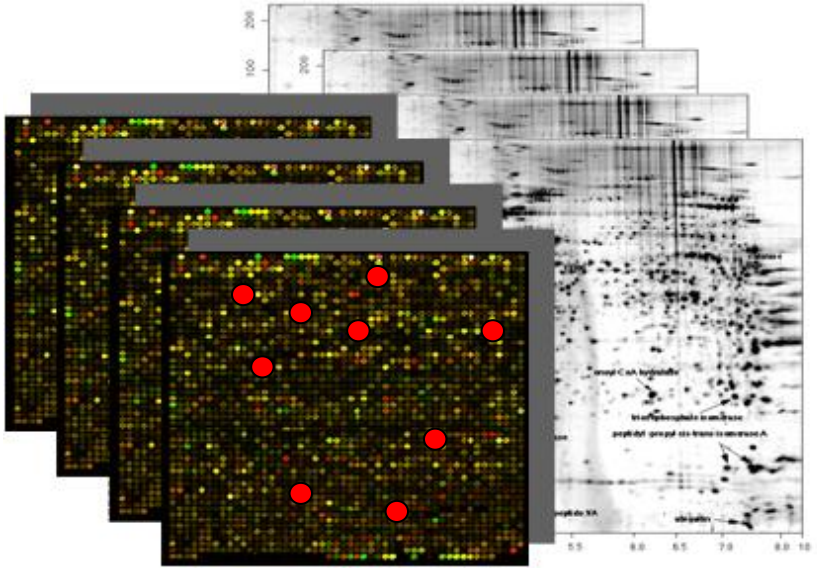
They will enable medicine to become predictive, personalized, preventive and participatory

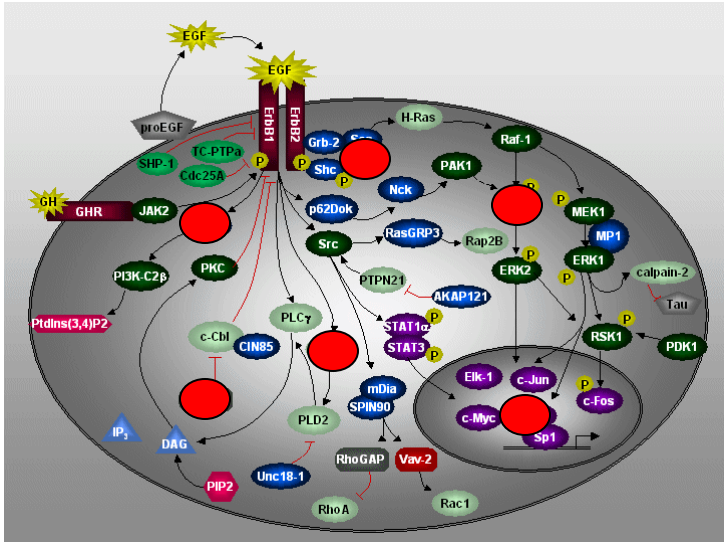
Systems medicine: the future of medical genomics and healthcare
Charles Auffray[1*], **Zhu Chen**[2] and **Leroy Hood**[3]
Genome Med 2009, **1**:2

We should find a key pathway of a disease, select a good target and inhibit it.



Pathway mapping





Differentially expressed genes/proteins

Mapping on pathways

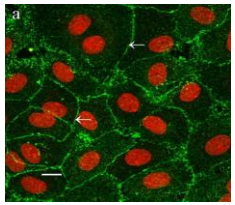
Cause of disease ??

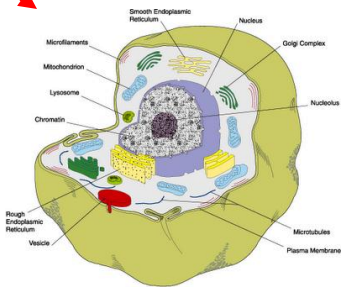
Transcriptional profiling of IKK2/NF-κB– and p38 MAP kinase–dependent gene expression in TNF-α–stimulated primary human endothelial cells

Dorothee Viemann, Matthias Goebeler, Sybille Schmid, Kerstin Klimmek, Clemens Sorg, Stephan Ludwig, and Johannes Roth

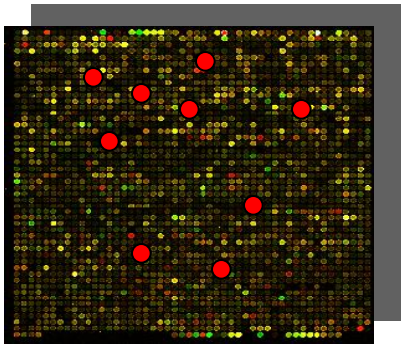
Inflammatory stimulation of endothelial cells by tumor necrosis factor α (TNF-α) involves activation of nuclear factor κB (NF-κB) and p38 mitogen-activated protein (MAP) kinase signaling pathways. A reliable analysis of the gene expression program elicited by TNF-α and its assignment to distinct signaling pathways is not available. A sophisticated analysis of oligonucleotide microarrays covering more than 13 000 genes allowed definition of the TNF-α–regulated endothelial gene expression profile and novel TNF-α–induced genes. Virtually all TNF-α–inducible genes were dependent on IκB kinase 2 (IKK2)/NF-κB activation, whereas a minor number was additionally modulated by p38. Furthermore, genes suppressed by IKK2/NF-κB were newly identified. Real-time reverse transcriptase–polymerase chain reaction (RT-PCR) and flow cytometry confirmed reliability of data. Thus, these results define a list of primary candidates for targeted modulation of endothelial functions during inflammation. (Blood. 2004;103:3365-3373)

TNF-α



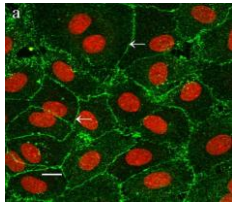


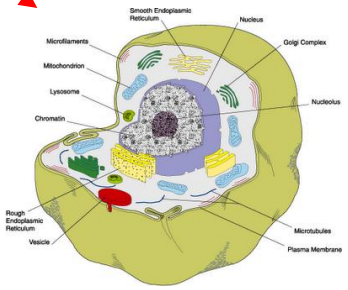
117 differentially expressed genes



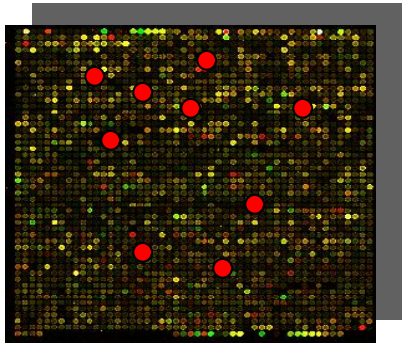
Can we predict TNF pathway?

?

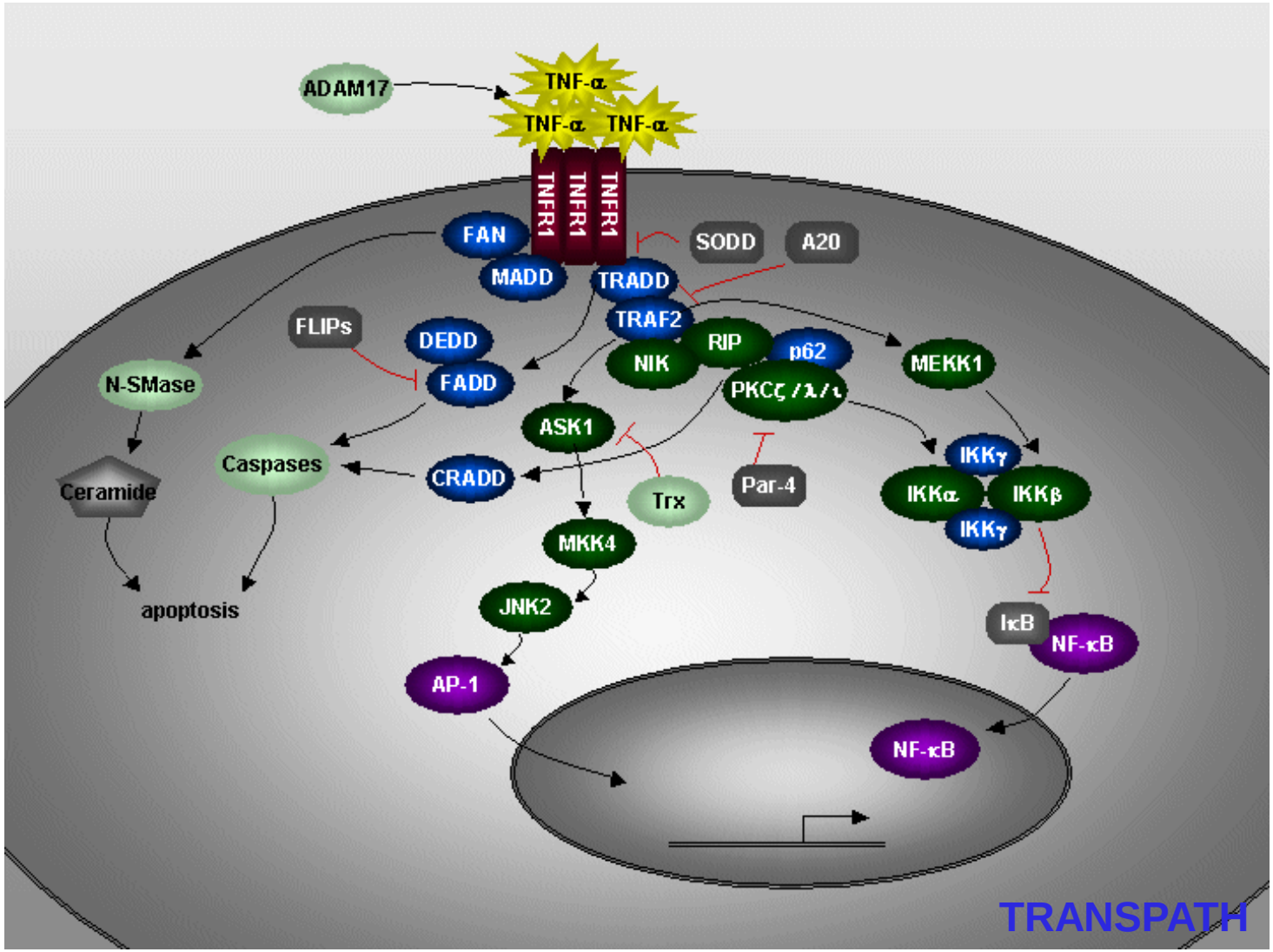




117 differentially expressed genes

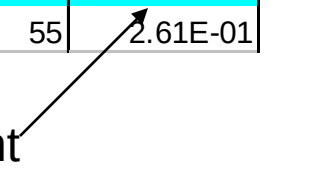


Canonical TNF pathway



Lets do mapping the differentially expressed genes on canonical pathways.

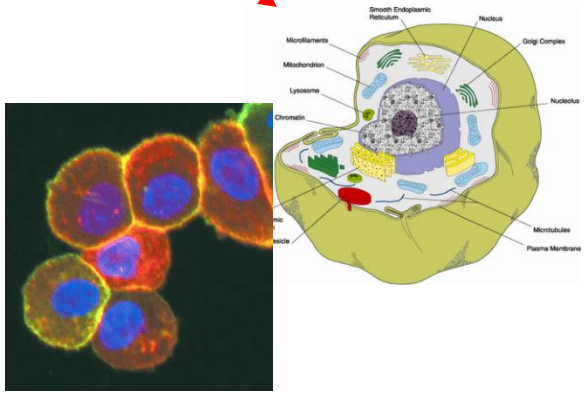
Pathway name	Hits	Pathway_id	Hit names	Pathway siz	p-value
M-CSF ---> c-Ets-2	2	CH000000060	ETS2; CSF1	5	3.07E-03
IFNalpha, IFNbeta, IFNgamma ---> Rap1	3	CH000000595	IFNGR1; TYK2; IFNGR2	19	4.34E-03
Epo ---Lyn---> STAT5A	2	CH000000524	STAT5A; LYN	6	4.56E-03
activin A ---> Smad3	2	CH000000680	INHBA; SMAD3	10	1.31E-02
IFN pathway	3	CH000000740	IFNGR1; TYK2; IFNGR2	29	1.44E-02
Sonic Hedgehog pathway	2	CH000001022	MTSS1; PTCH	19	4.48E-02
hypoxia pathways	2	CH000000987	CDKN1B; NRIP1	21	5.38E-02
EDAR pathway	2	CH000000759	NFKBIA; CYLD	27	8.40E-02
Epo pathway	2	CH000000741	STAT5A; LYN	32	1.12E-01
TGFbeta pathway	3	CH000000711	BMP2; INHBA; SMAD3	72	1.39E-01
IL-22 pathway	1	CH000000762	TYK2	9	1.51E-01
IL-10 pathway	1	CH000000761	TYK2	9	1.51E-01
VEGF-A pathway	2	CH000000723	NOS3; VEGFA	42	1.75E-01
TLR3 pathway	2	CH000000820	TANK; IKBKE	44	1.88E-01
IL-8 pathway	2	CH000000786	CXCL1; IL8	46	2.01E-01
TNF-alpha pathway	2	CH000000772	NFKBIA; OSIL	53	2.48E-01
p38 pathway	2	CH000000849	MAP2K3; DUSP8	55	2.61E-01



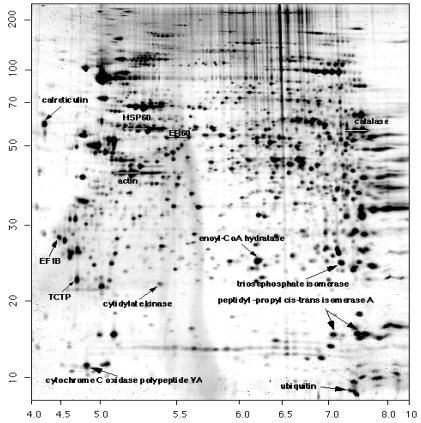
TNF pathway can not be found by direct maping on canonical pathways....

Human epidermoid carcinoma A431 cells treated by epidermal growth factor (EGF)

EGF



320 differntially expressed proteins



Mapping differentially expressed proteins to canonical signal transduction pathways

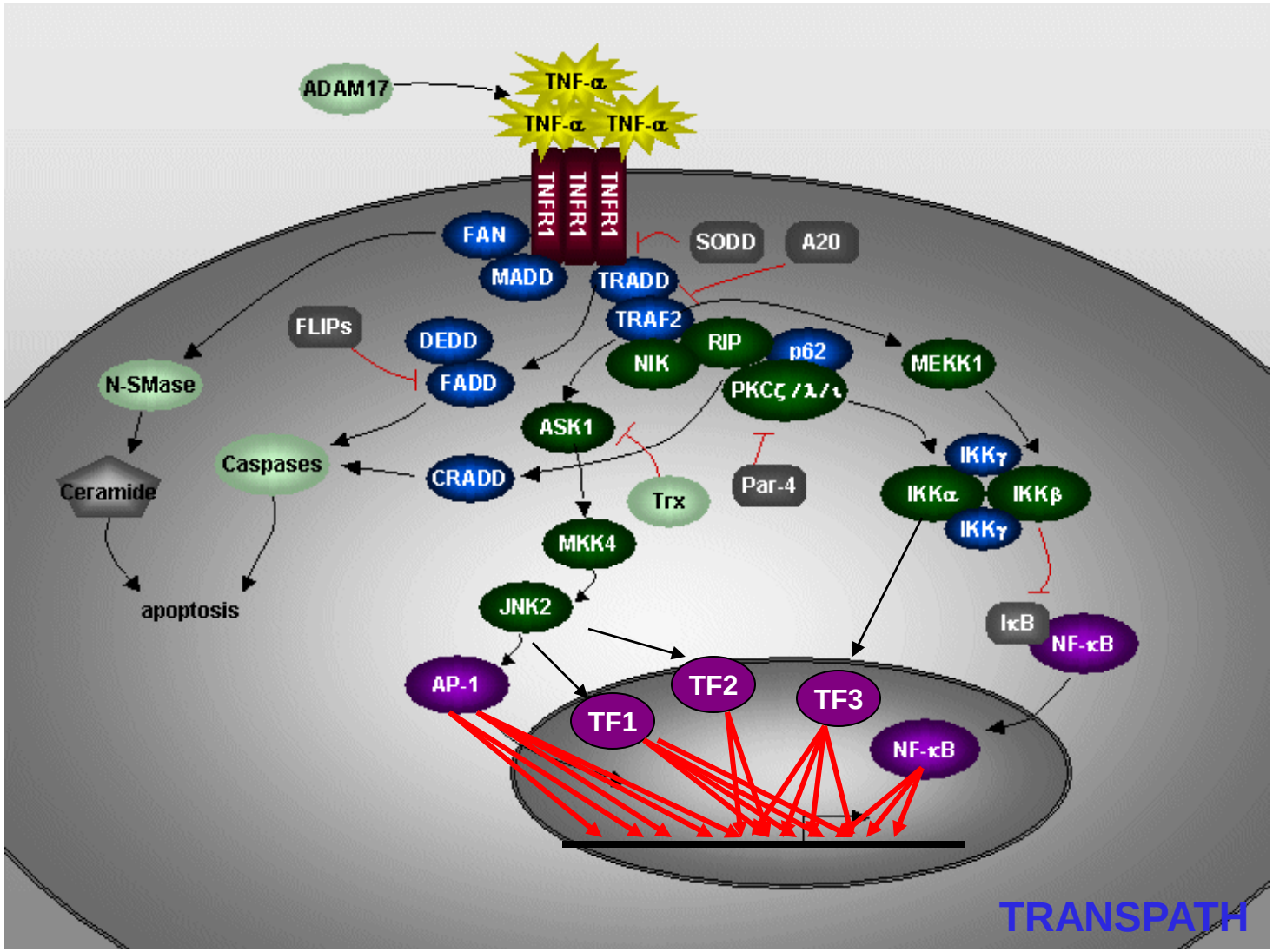
Pathway name	#Hits in group	Hit names	Group size	p-value
Caspase network	6	K18; E1; Cytochrome C; Hsp10; Ku70; Cdc42	104	0.00201348
CHIP ---/ Pael-R	2	E1; Hsc70	12	0.01177937
p53 pathway	4	E1; L23; Cytochrome C; Ku70	79	0.02072214
beta-catenin ---/ KAI1	1	Reptin52	5	0.06701759
Aurora-A cell cycle regulation	2	Ubc5B; E1	34	0.07924485
JNK pathway	3	E1; 14-3-3zeta; Trx1	75	0.0813304
parkin associated pathways	2	E1; Hsc70	40	0.10447487
beta-catenin:E-cadherin complex phosphorylation and dissociation	1	alpha-catenin	9	0.11739049
stress-associated pathways	3	E1; 14-3-3zeta; Trx1	100	0.15476
hypoxia pathways	1	Trx1	24	0.2849595
TNF-alpha pathway	1	Trx1	36	0.39594524
EGF pathway	1	E1	103	0.57615756

Mapping on pathways does not work
(even in such a simple cases)

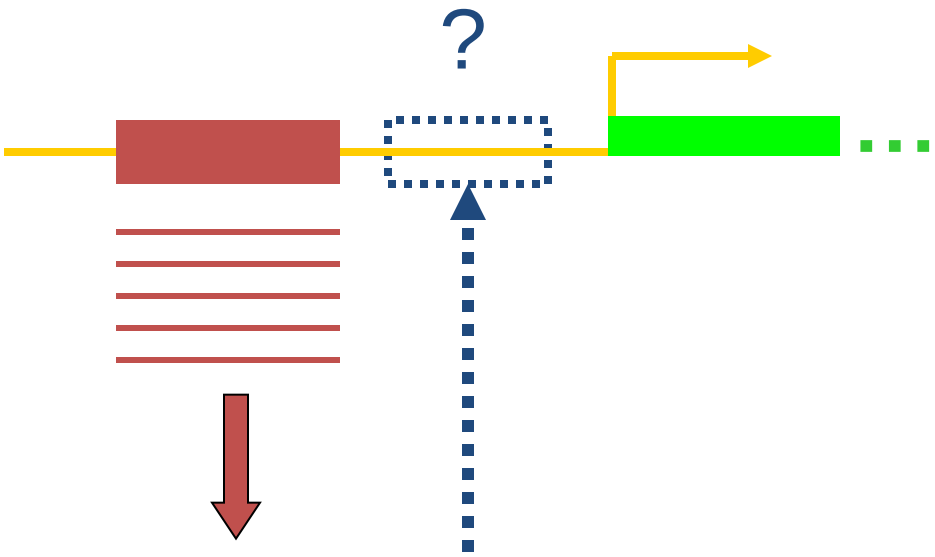
Why ?

Pathways are
far from being
fully undersood.

BIG gap of knowledge on interactions between TF and their target sites in DNA



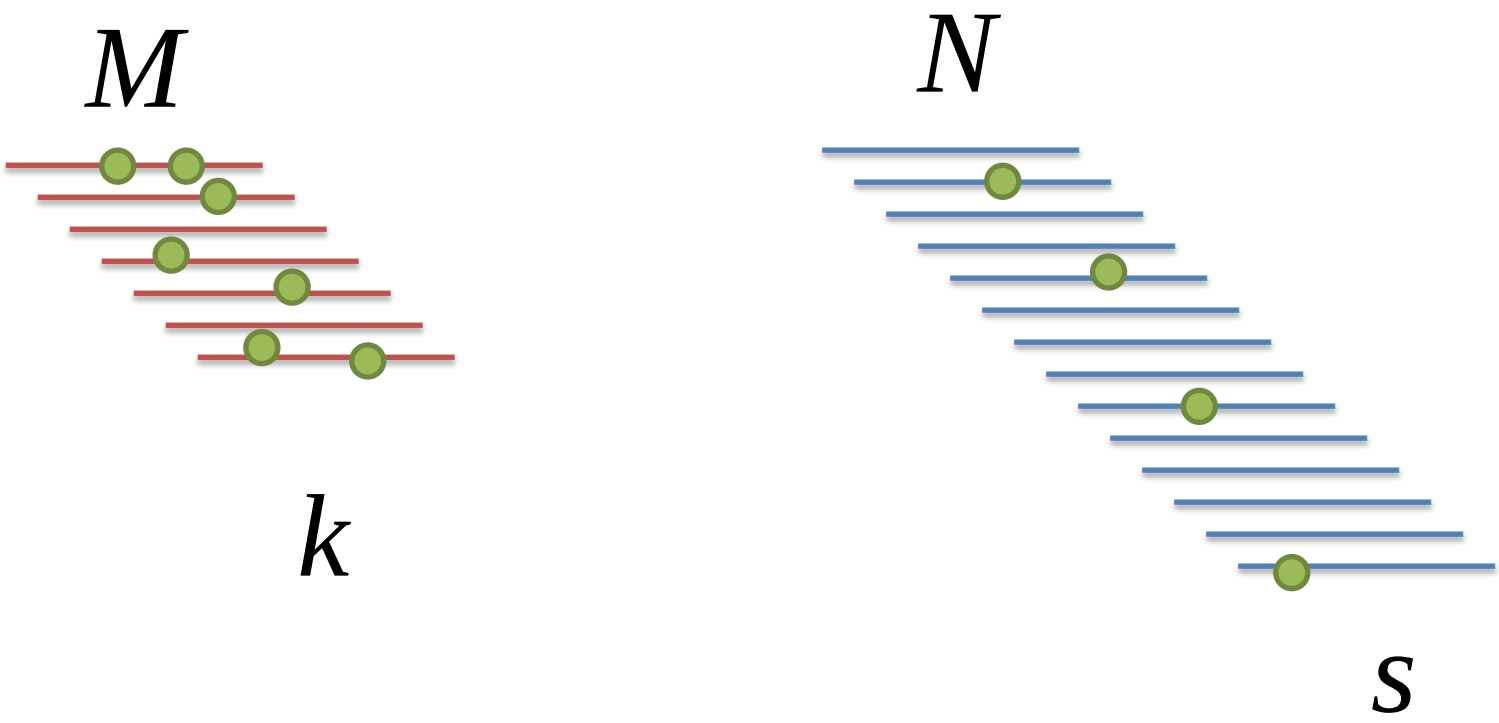
Search for new TF binding sites with PWMs (Match algorithm)



A	9	2	1	0	1	0	0	0	0	1	**15**	**13**	**13**	7
C	8	3	1	1	**13**	3	**29**	0	**22**	**8**	**9**	1	4	8
G	4	2	2	2	**15**	**26**	0	**29**	7	**17**	3	**7**	**9**	8
T	8	**22**	**25**	**26**	0	0	0	0	0	3	2	**8**	3	6
	N	**T**	**T**	**T**	**S**	**G**	**C**	**G**	**C**	**S**	**M**	**D**	**R**	**N**

$$q = \frac{\sum_{i=1}^{l} I(i) f(b_i, i) - \sum_{i=1}^{l} I(i) f^{\min}(i)}{\sum_{i=1}^{l} I(i) f^{\max}(i)} \qquad (1)$$

$$I(i) = \sum_{b \in \{A,T,G,C\}} f(b,i) \ln(4 f(b,i)) \qquad (2)$$



$p = M/N$

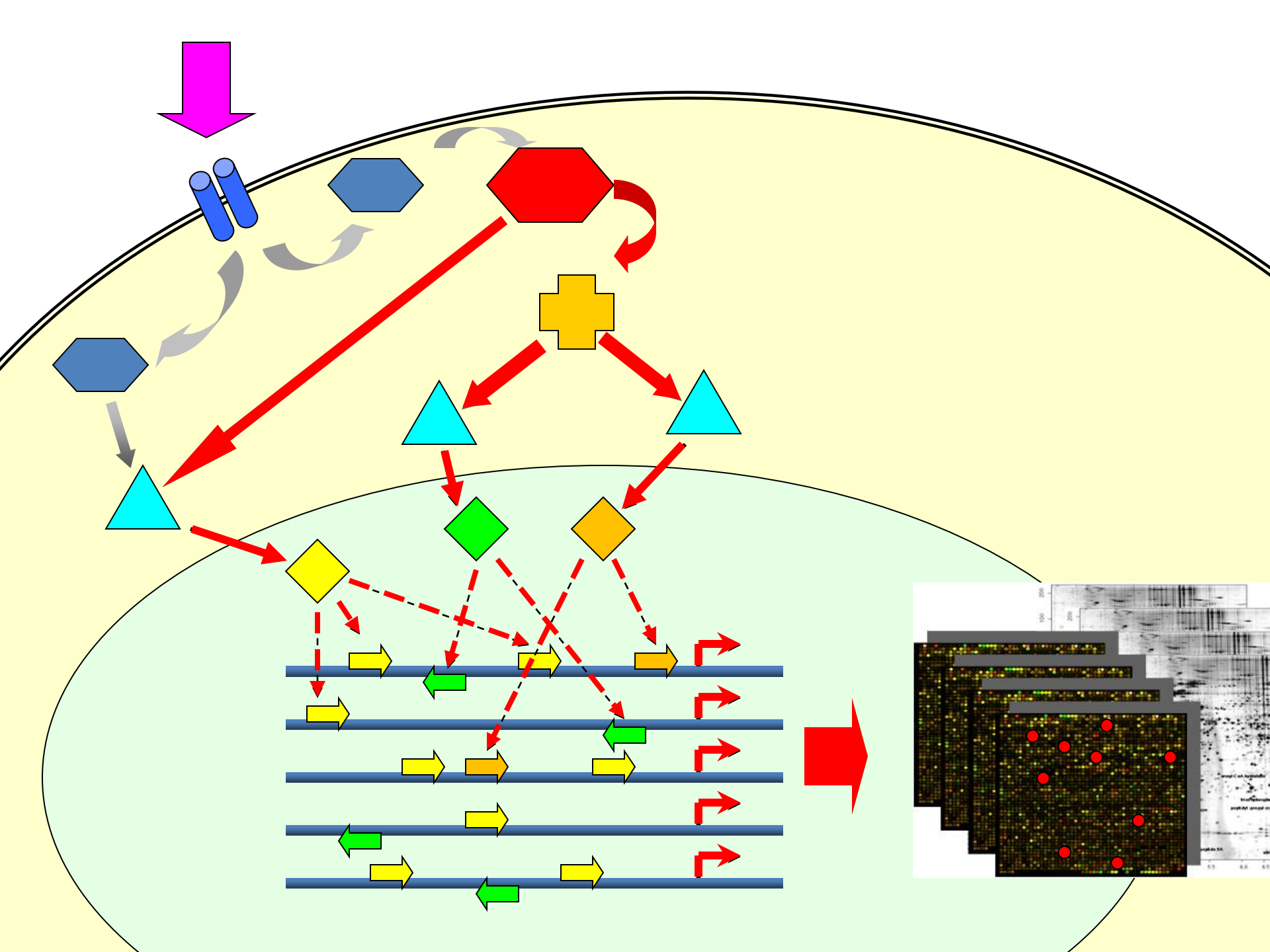
$n = k + s$

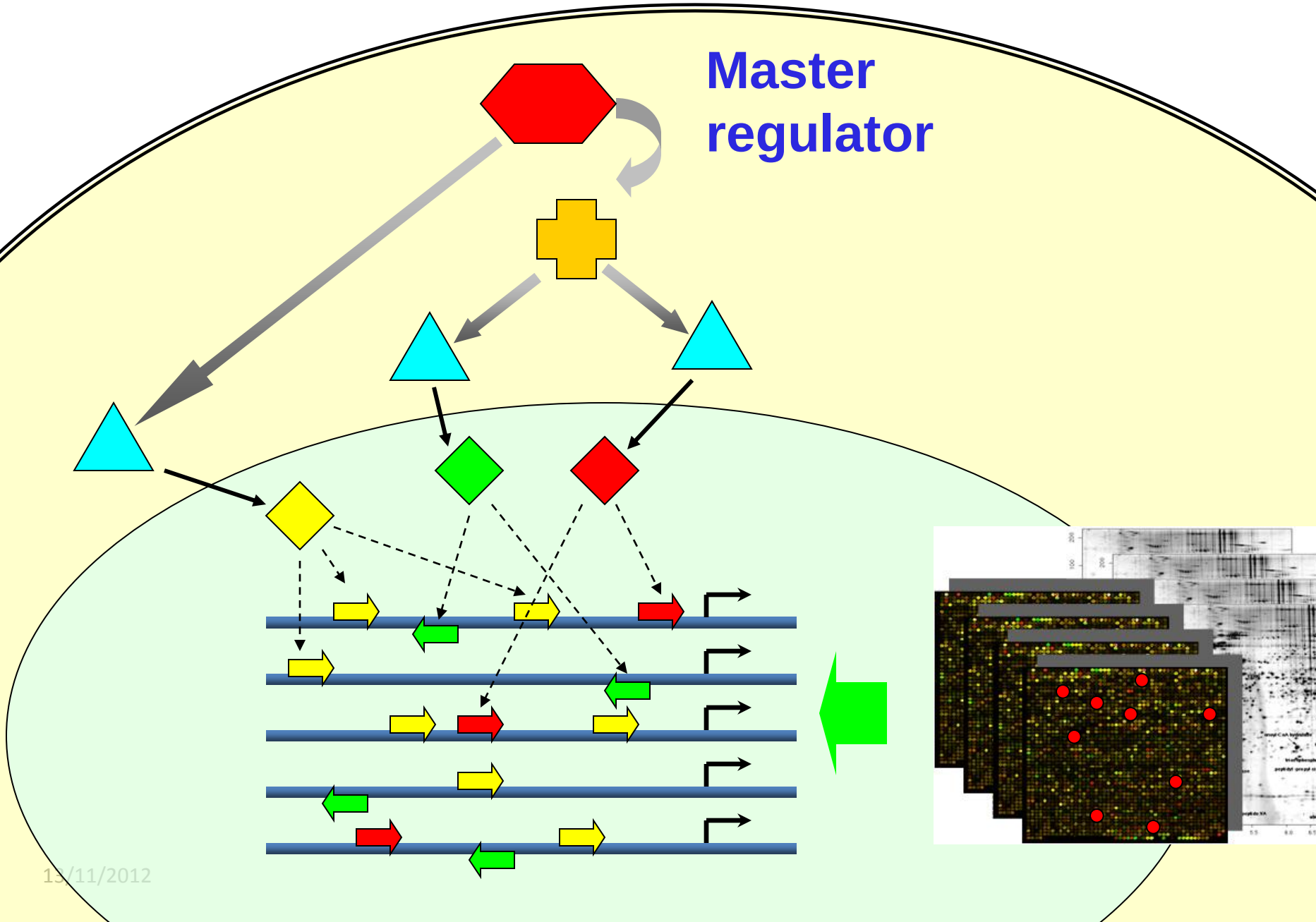
$$\text{p} - \text{value} = \sum_{i=k}^{n} \binom{n}{i} p^i (1 - \text{p})^{n-i}$$

Overrepresented TFs in TNF-alpha regulated promoters

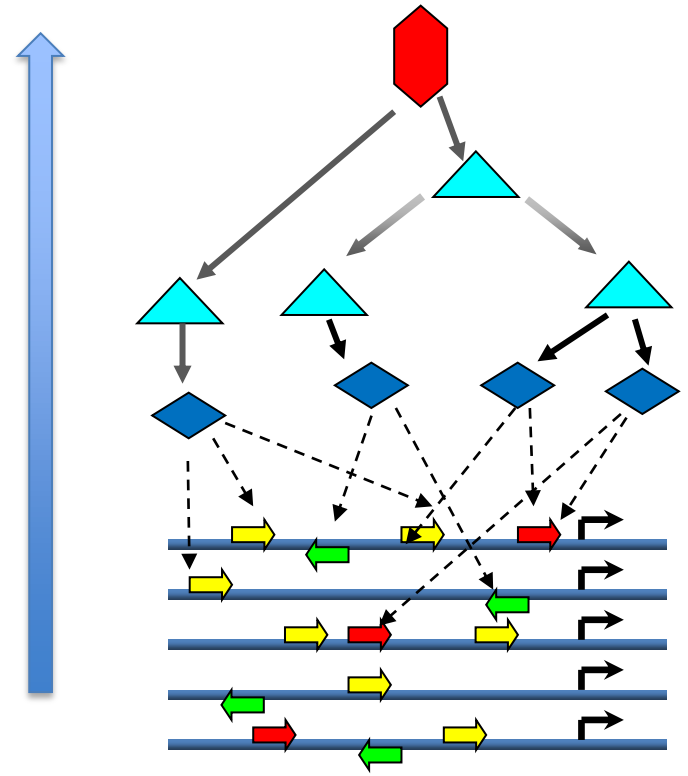
First Previous Page 1 of 1 Next Last Showing 1 to 27 of 27 entries Show 50 entries

ID	Yes density per 1000bp	No density per 1000bp	Yes-no ratio	Matrix cutoff	P-value
V$NFKB_Q6_01	0.30962	0.06917	4.47619	0.9337	1.3743E-5
V$PPARG_01	0.36891	0.12846	2.87179	0.7339	1.4549E-4
V$NKX3A_01	0.38208	0.13834	2.7619	0.9106	1.6526E-4
V$PAX4_03	0.55336	0.2668	2.07407	0.9848	3.7636E-4
V$XVENT1_01	0.14493	0.01976	7.33333	0.9328	6.6179E-4
V$CP2_02	0.39526	0.16798	2.35294	0.9243	6.855E-4
V$ZF5_B	0.21739	0.05929	3.66667	0.9211	8.5009E-4
V$NKX22_01	0.47431	0.22727	2.08696	0.8995	8.9216E-4
V$OCT1_07	0.49407	0.24704	2	0.8372	0.00119
V$COREBINDINGFACTOR_Q6	0.11199	0.00988	11.33333	1	0.00132
V$CEBPDELTA_Q6	0.21739	0.06917	3.14286	0.9615	0.00205
V$IRF2_01	0.1054	0.00988	10.66667	0.909	0.00209
V$POU3F2_02	0.16469	0.03953	4.16667	0.8875	0.0022
V$PAX_Q6	0.19104	0.05929	3.22222	0.8706	0.0034
V$AREB6_03	0.34256	0.16798	2.03922	0.9617	0.00546
V$POU1F1_Q6	0.2108	0.07905	2.66667	0.9594	0.00606
V$IRF_Q6	0.18445	0.06917	2.66667	0.9707	0.01017
V$AP2_Q6	0.07246	0.00988	7.33333	0.9678	0.01959
V$PBX_Q3	0.20422	0.09881	2.06667	0.9151	0.02736
V$DMRT3_01	0.06588	0.00988	6.66667	0.9238	0.03023
V$TTF1_Q6	0.1581	0.06917	2.28571	0.9881	0.03299
V$AR_Q2	0.07905	0.01976	4	0.8671	0.03979
V$HAND1E47_01	0.07905	0.01976	4	0.9652	0.03979
V$AHR_Q5	0.05929	0.00988	6	0.9959	0.04636
V$HOXA7_01	0.13175	0.05929	2.22222	1	0.05588



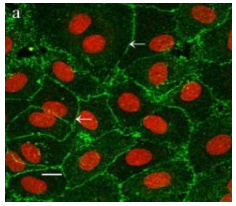

Master
regulator

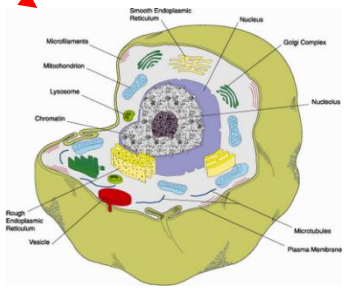
Search for the reason by the analysis of the ripples



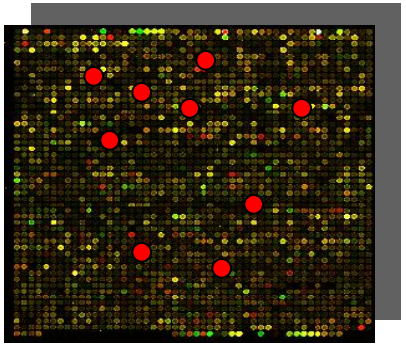
Can we predict TNF pathway?

?

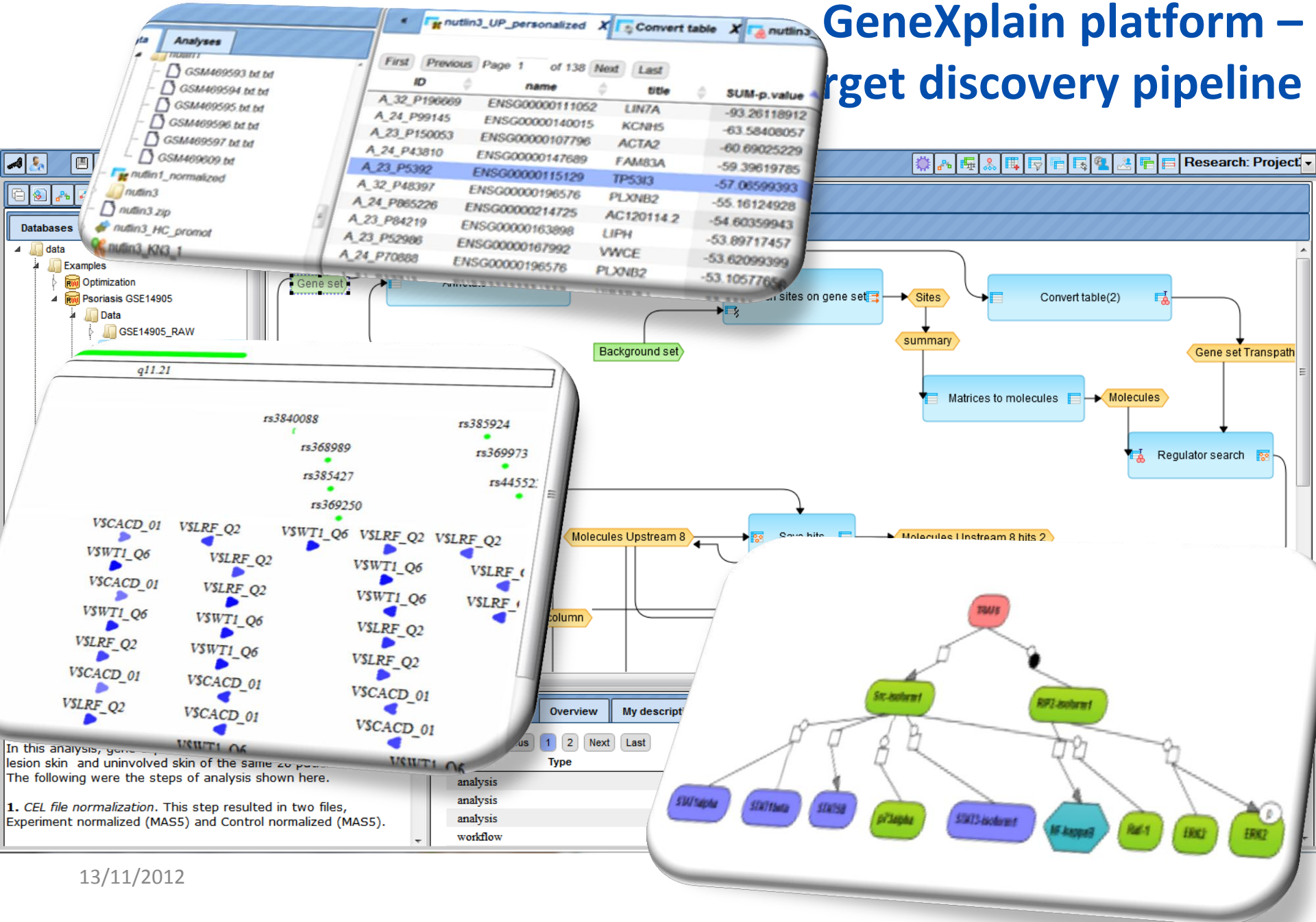




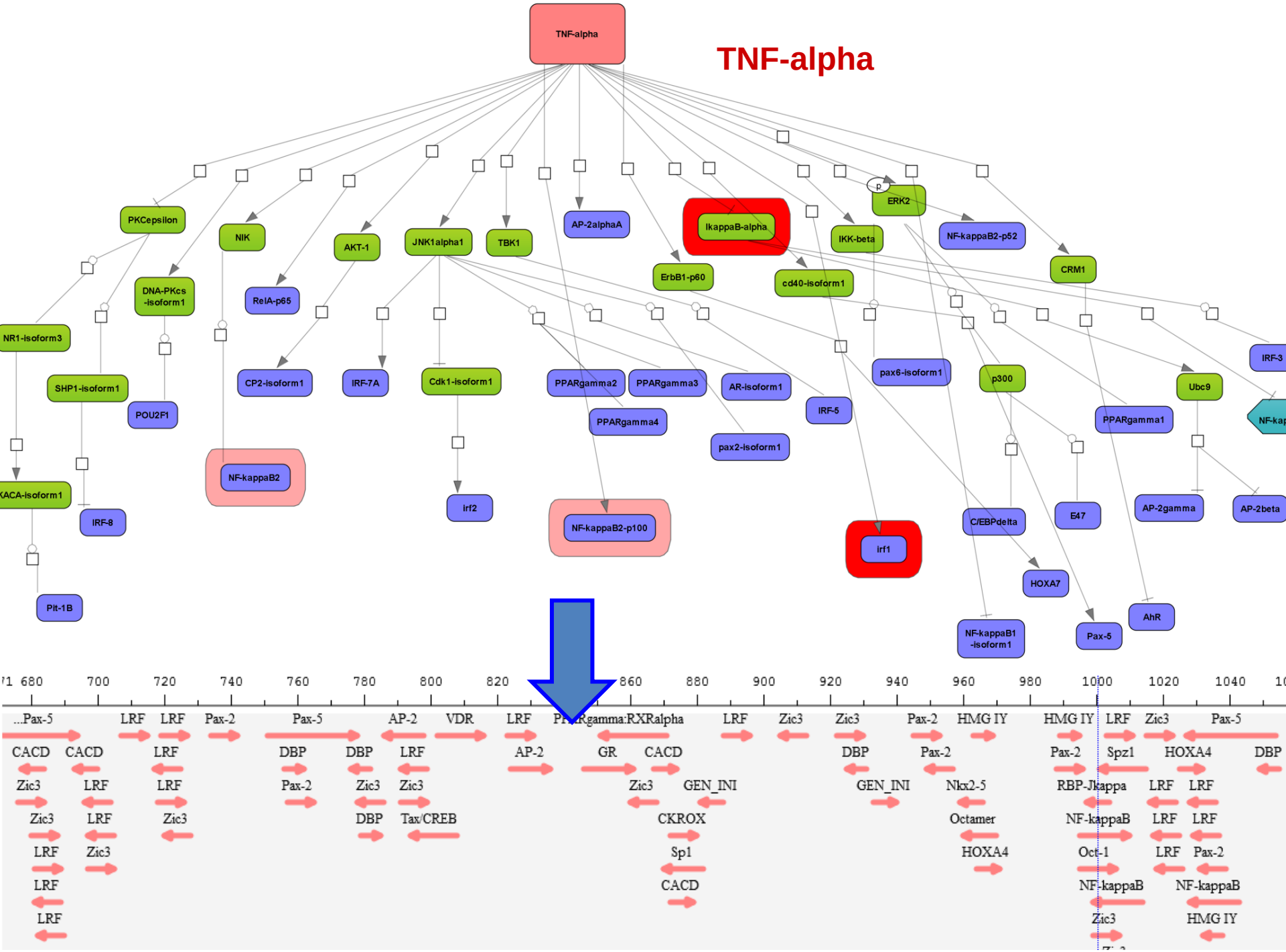
117 differntially expressed genes



GeneXplain platform – rget discovery pipeline



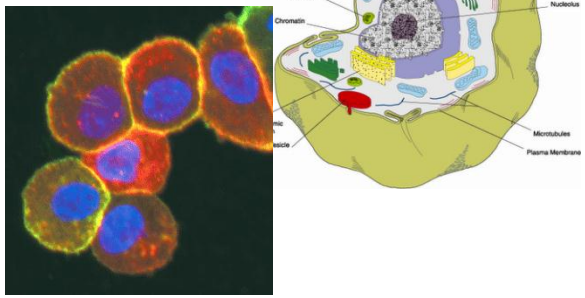
TNF-alpha

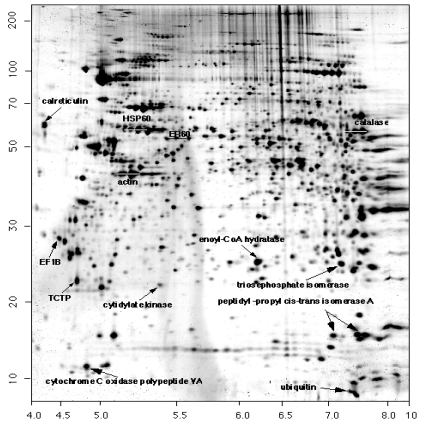

TNF-alpha
PKCepsilon
NIK
AKT-1
JNK1alpha1
TBK1
AP-2alphaA
IkappaB-alpha
IKK-beta
ERK2
NF-kappaB2-p52
CRM1
DNA-PKcs -isoform1
RelA-p65
ErbB1-p60
cd40-isoform1
NR1-isoform3
SHP1-isoform1
POU2F1
CP2-isoform1
IRF-7A
Cdk1-isoform1
PPARgamma2
PPARgamma3
AR-isoform1
pax6-isoform1
p300
IRF-3
Ubc9
PPARgamma4
IRF-5
PPARgamma1
pax2-isoform1
NF-kappaB2
irf2
NF-kappaB2-p100
irf1
C/EBPdelta
E47
AP-2gamma
AP-2beta
IRF-8
HOXA7
Pit-1B
AhR
NF-kappaB1 -isoform1
Pax-5
680
700
720
740
760
780
800
820
860
880
900
920
940
960
980
1000
1020
1040
...Pax-5
LRF
LRF
Pax-2
Pax-5
AP-2
VDR
LRF
PPARgamma:RXRalpha
LRF
Zic3
Zic3
Pax-2
HMG IY
HMG IY
LRF
Zic3
Pax-5
CACD
CACD
LRF
DBP
DBP
LRF
AP-2
GR
CACD
DBP
Pax-2
Pax-2
Spz1
HOXA4
DBP
Zic3
LRF
LRF
Pax-2
Zic3
Zic3
Zic3
GEN_INI
GEN_INI
Nkx2-5
RBP-Jkappa
LRF
LRF
Zic3
LRF
Zic3
DBP
Tax/CREB
CKROX
Octamer
NF-kappaB
LRF
LRF
LRF
Zic3
Sp1
HOXA4
Oct-1
LRF
Pax-2
LRF
CACD
NF-kappaB
NF-kappaB
LRF
Zic3
HMG IY

Human epidermoid carcinoma A431 cells treated by epidermal growth factor (EGF)

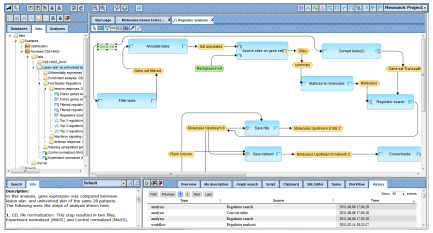
EGF ?

320 differntially expressed proteins





Master regulator analysis



EGF was still not in the list !

Pathways are far .....far....far from being fully undersood!

Combinatorial regulation

„Fuzzy puzzle“

AP-1

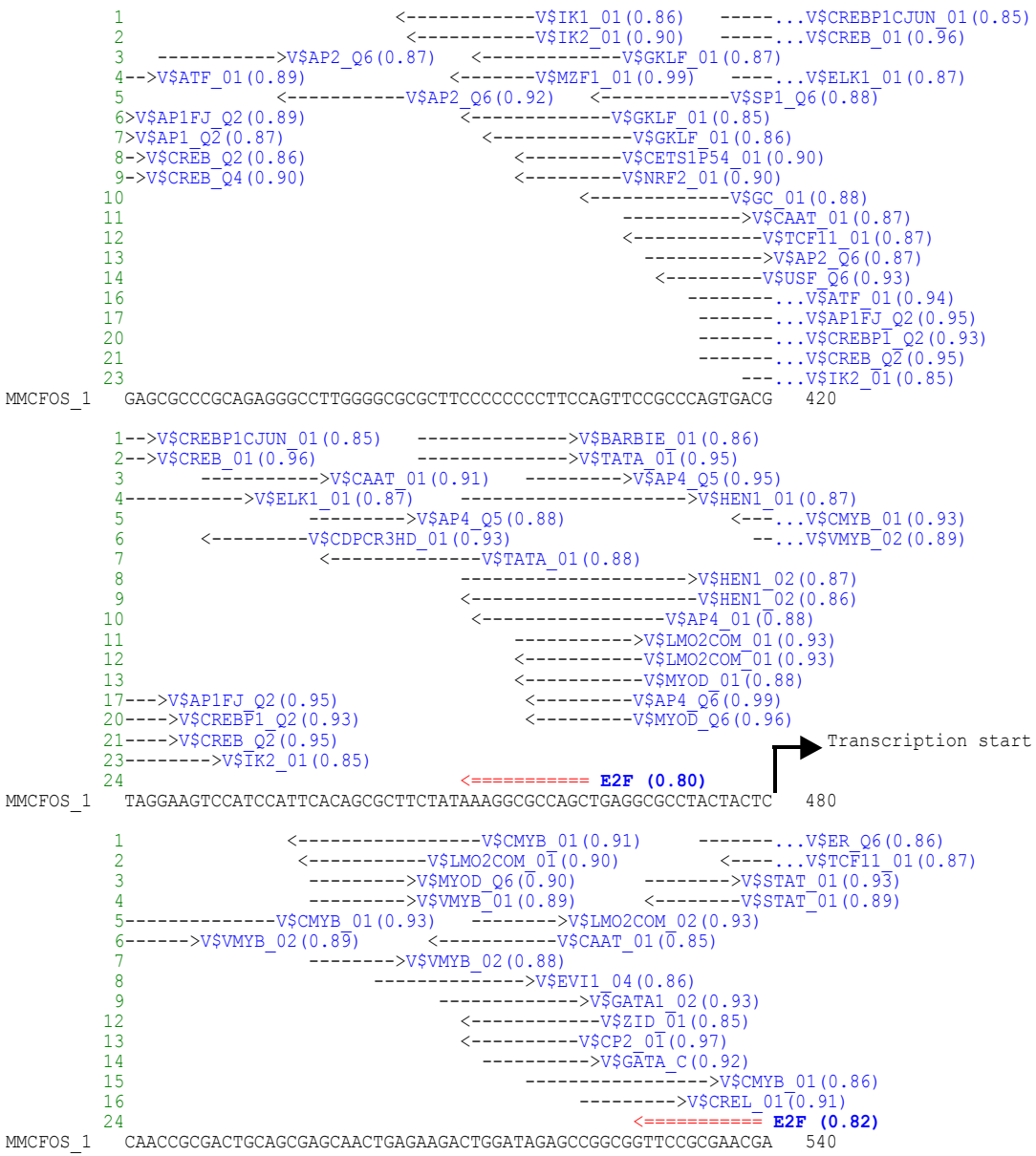
Consensus: TGAgTCA

Human collagenase (-2013) TGAGTCA

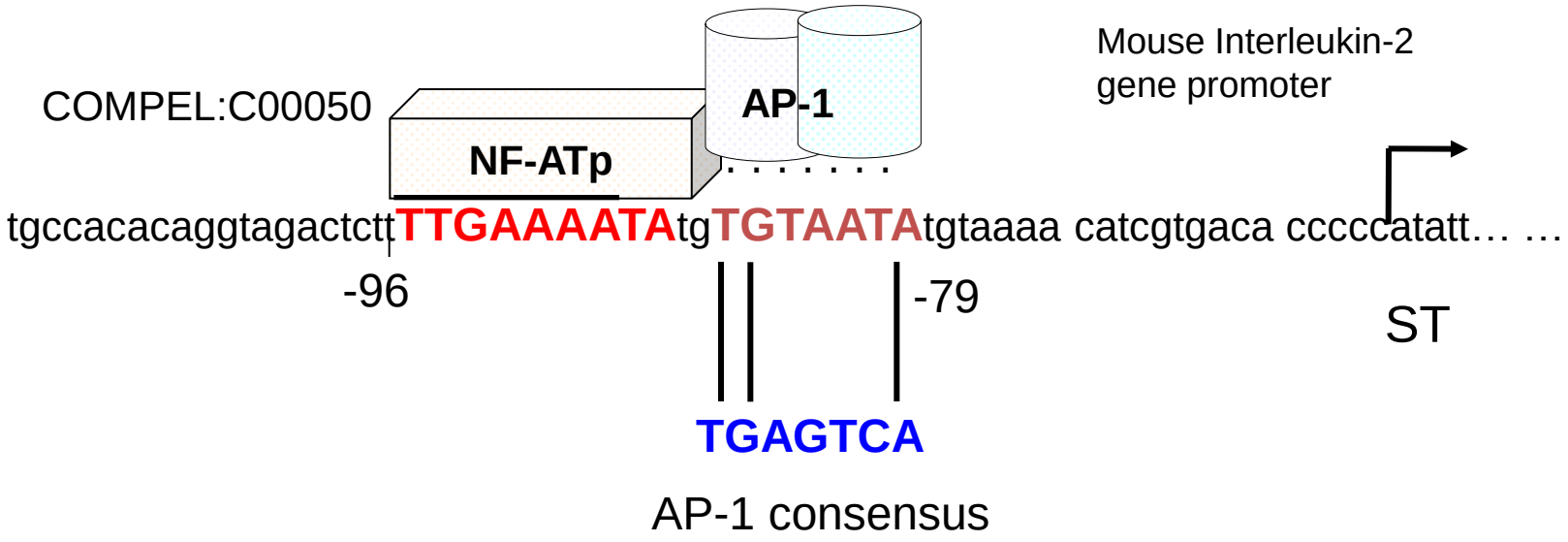
Mouse IL-2 (-143) TGTGTAA

Mouse IL-2 (-82) TGTAATA

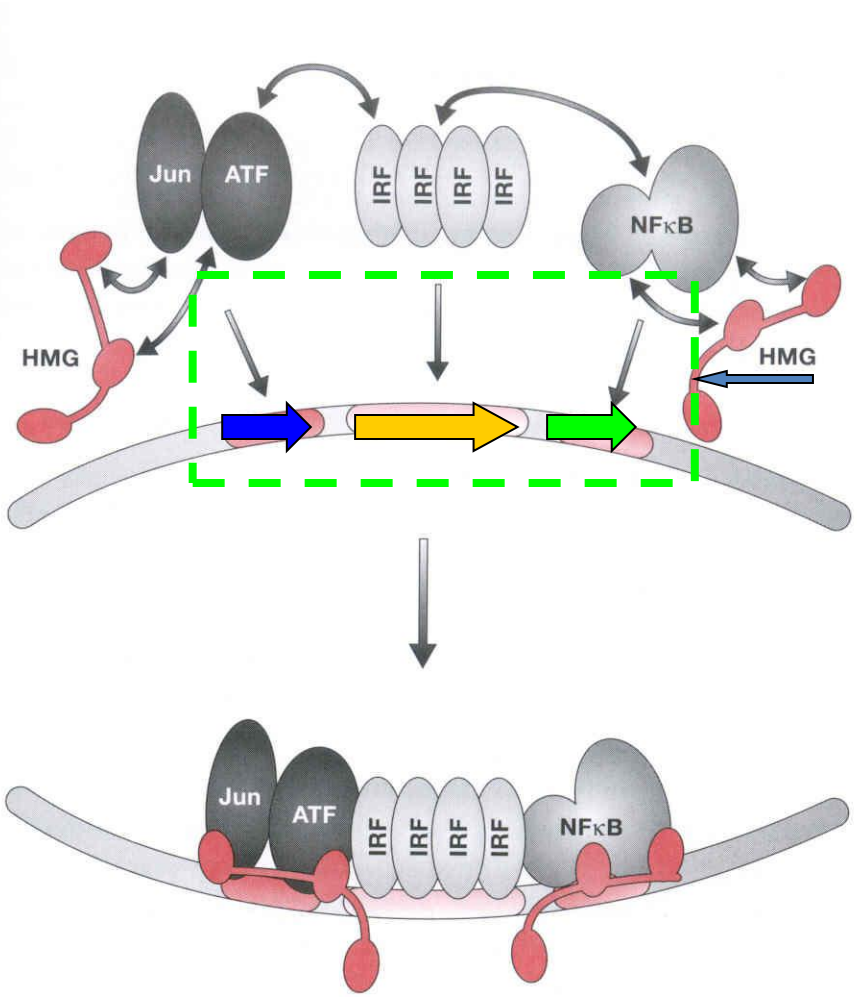
Mouse c-fos promoter
(Matrix search for TF binding sites)



One of the TF binding sites in a composite elements can be rather weak.
Weak DNA-protein interactions are stabilized by protein-protein interactions.



Composite Modules (CM)

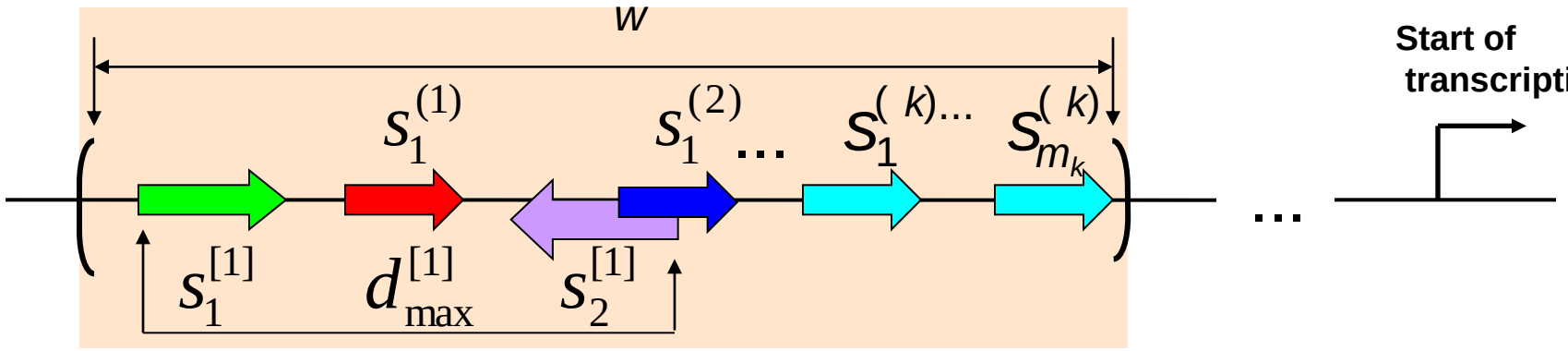


Composite Module (CM)

FIGURE 3.3. The human interferon-β enhanceosome. HMG represents HMGI/Y, a ubiquitous protein that binds cooperatively with the three activators. HMGI/Y both bends the DNA and contacts the activators. Each of the transcription factors shown is a member of a family of related activators.

(Mark Ptashne, Alexander Gann Genes and Signals, 2002)

Composite Modules (CM)



$$
\begin{array}{cccc}
d_{\max}^{[1]} & d_{\max}^{[1]} & \cdots & d_{\max}^{[R]} \\
q_{cut-off}^{(1)} & q_{cut-off}^{(2)} & \cdots & q_{cut-off}^{(k)} \\
\phi^{(1)} & \phi^{(2)} & \cdots & \phi^{(k)}
\end{array}
$$

Parameters of the model to be estimated by GA

We created a genetic algorithm to find site combinations

Composite Modules (CM)

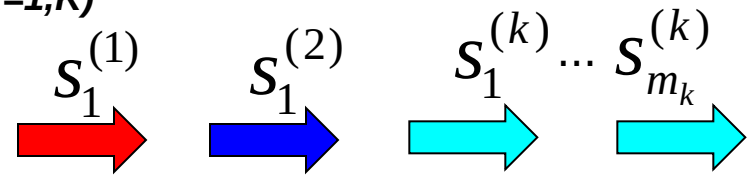
Composite Module Score (*cms*)

K, the number of individual PWMs in the module, ***(k=1,K)***

Matrix cut-off values: $q_{cut-off}^{(k)}$

Relative impact values: $\phi^{(k)}$

Maximal number of best matches: m_k

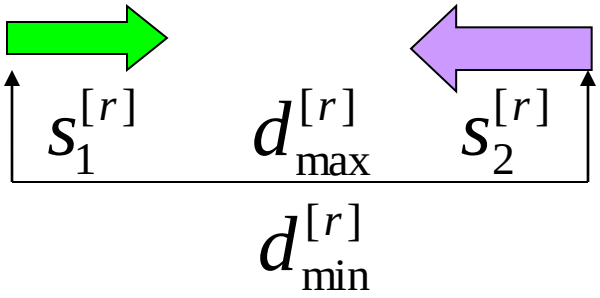


R, the number of pairs of PWMs ***(r=1,R)***

Matrix cut-off values: $q_{1,cut-off}^{[r]}$ $q_{2,cut-off}^{[r]}$

Relative impact values: $\phi^{[r]}$

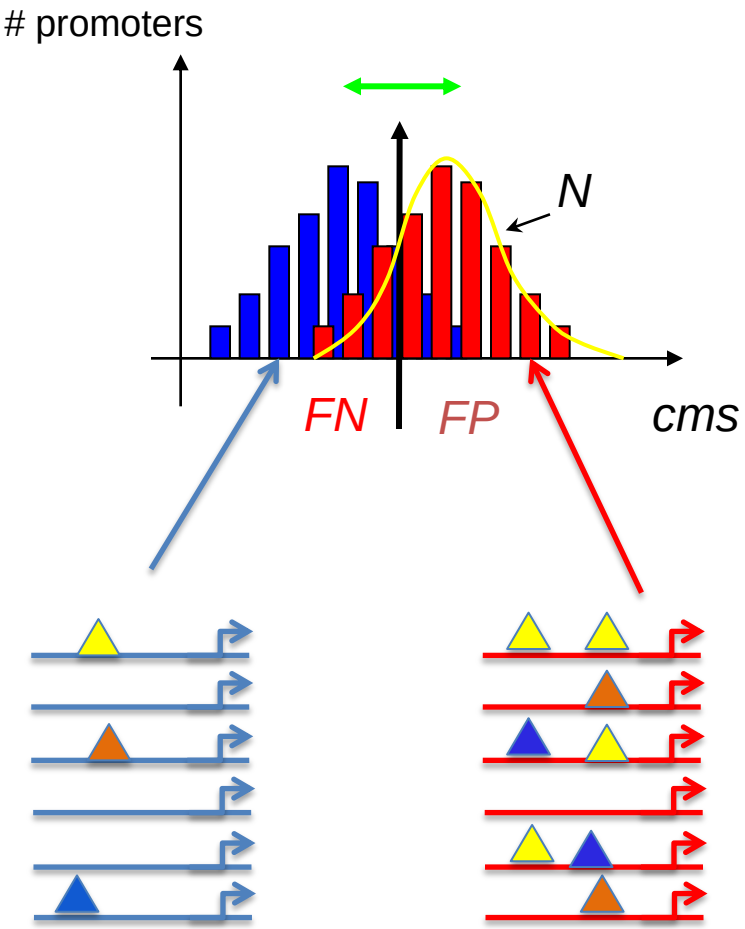
Maximal and minimal distances: $d_{\max}^{[r]}$ $d_{\min}^{[r]}$



$$cms = \sum_{k=1,K} \phi^{(k)} \times \sum_{i=1}^{m_k} q_i^{(k)} + \sum_{r=1,R} \phi^{[r]} \times (q_1^{[r]} + q_2^{[r]}) / MAX$$

Fitness function of the Genetic-Regression Algorithm (GRA)

$$F = \alpha \cdot R + \beta \cdot (1 - FN) + (1 - \beta) \cdot (1 - FP) + \gamma \cdot T + \delta \cdot N - \mu \cdot k$$



R – linear regression

FN – false negatives

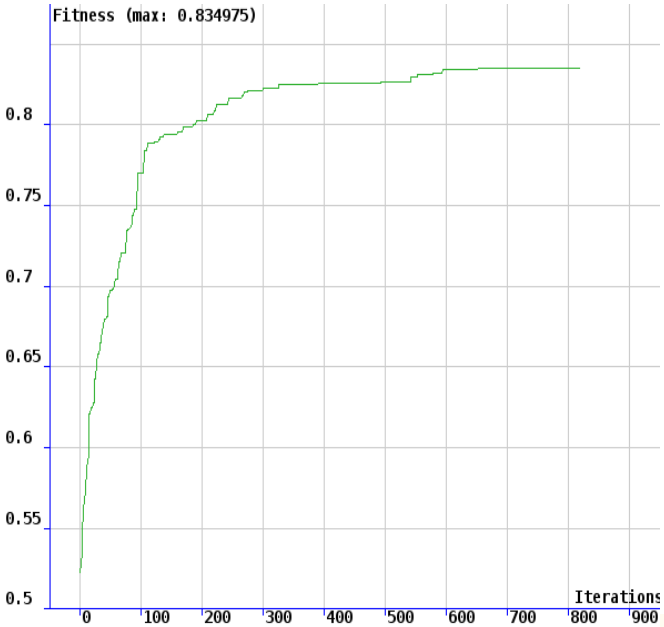
FP – false positives

T – T-test (difference between mean values)

N – normal likeness

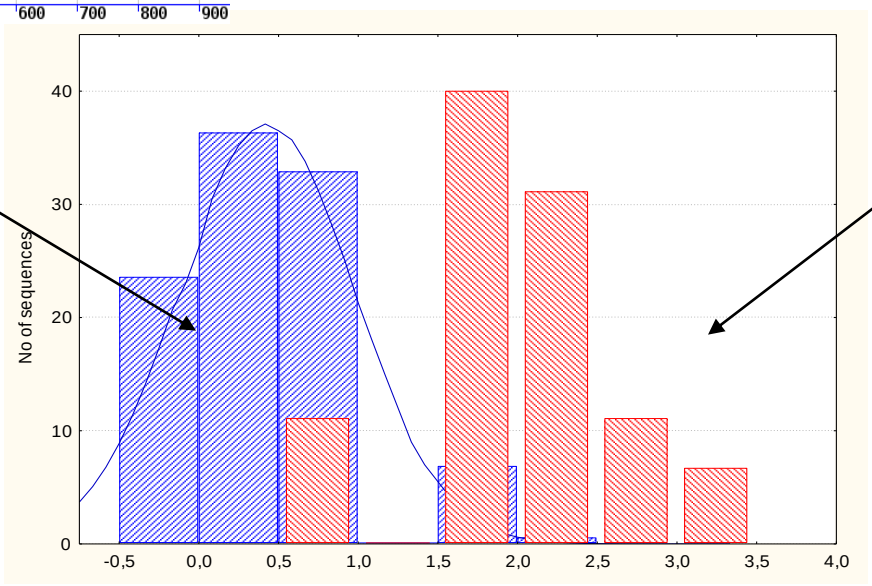
k – number of free parameters

Composite module in promoters of cell cycle-related genes



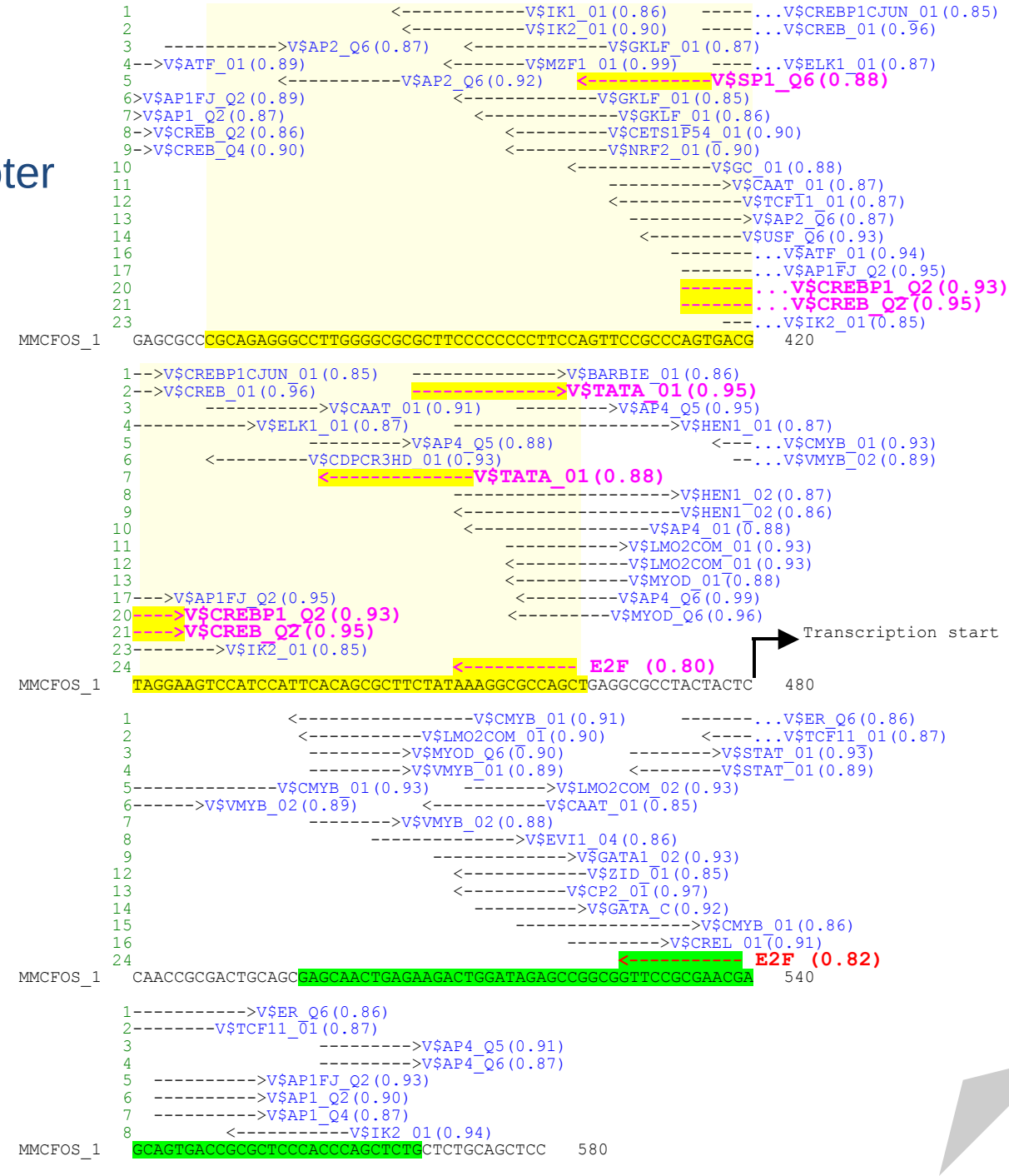
Weight: ϕ	$q_{cut-off}$	TF matrix
1.000000	0.840072	V$E2F_19
0.954483	0.737637	V$TATA_01
0.888064	0.939687	V$CREB_01
0.816179	0.941583	V$SP1_Q6
0.039746	0.839702	V$TAL1BETAE47_01

Background sequences

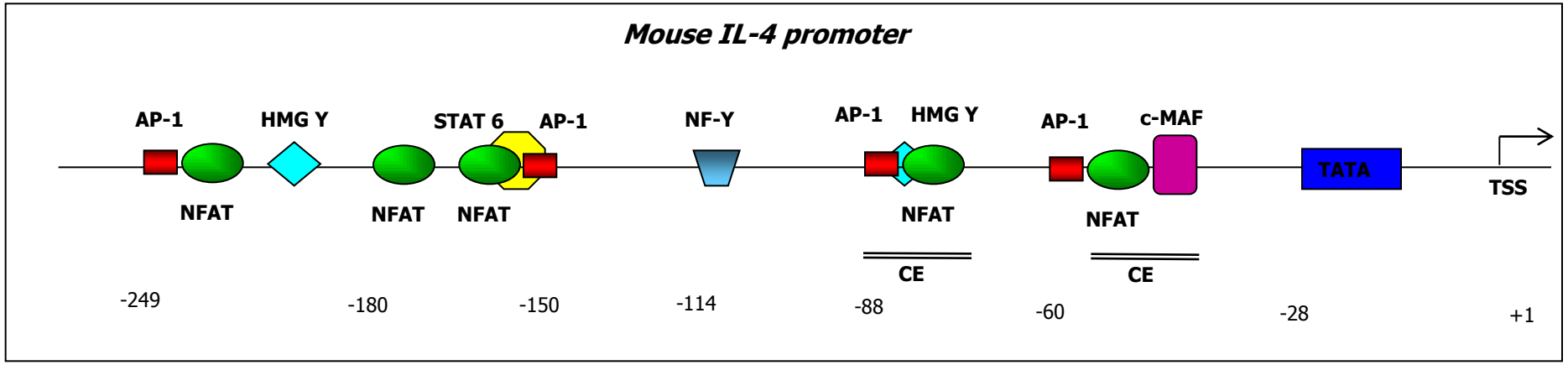


Cell cycle-related promoters

Mouse c-fos promoter



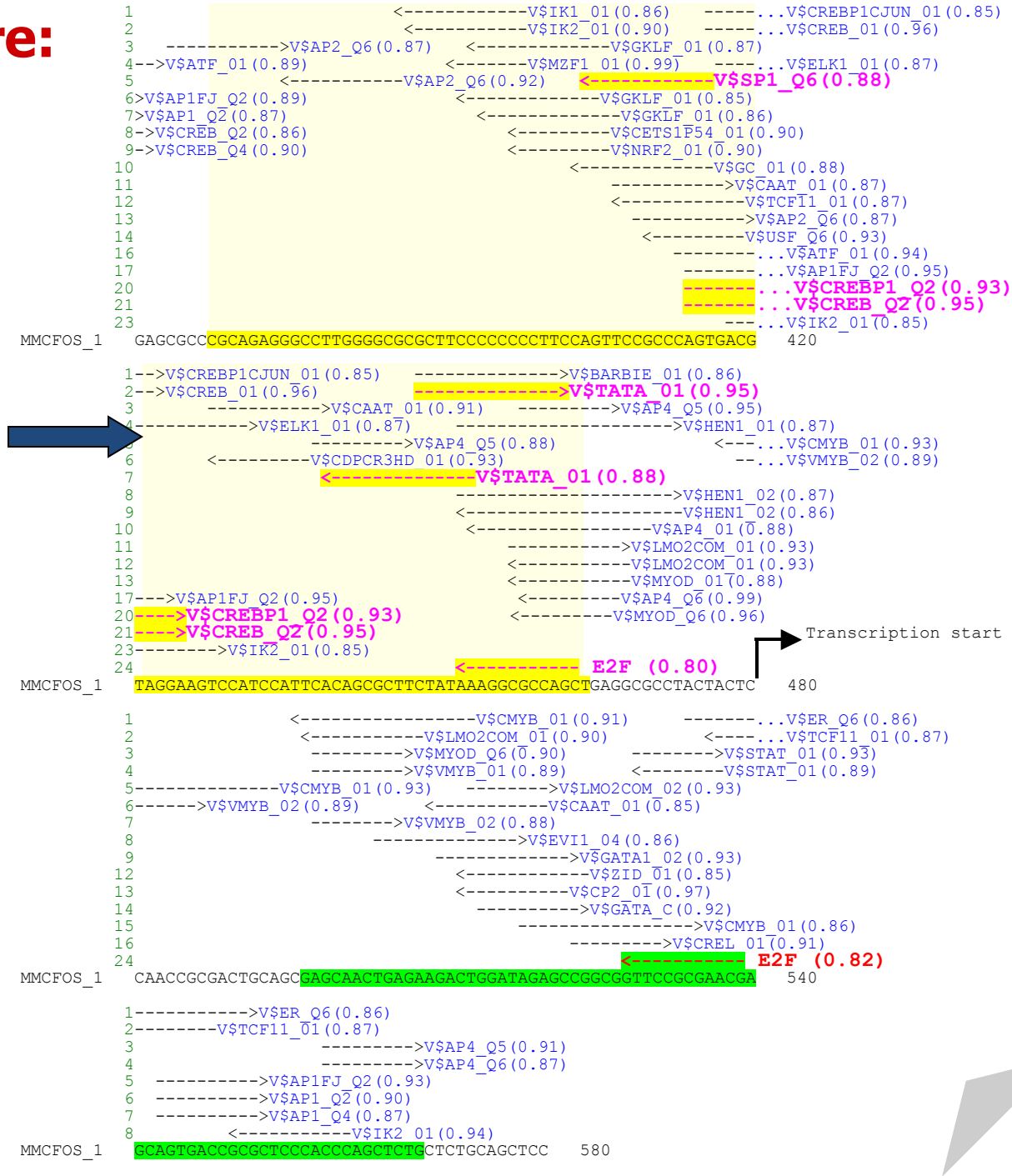
Promoter structure: curret paradigm



Promoter is a parking place

Promoter structure: reality

Mouse c-fos promoter



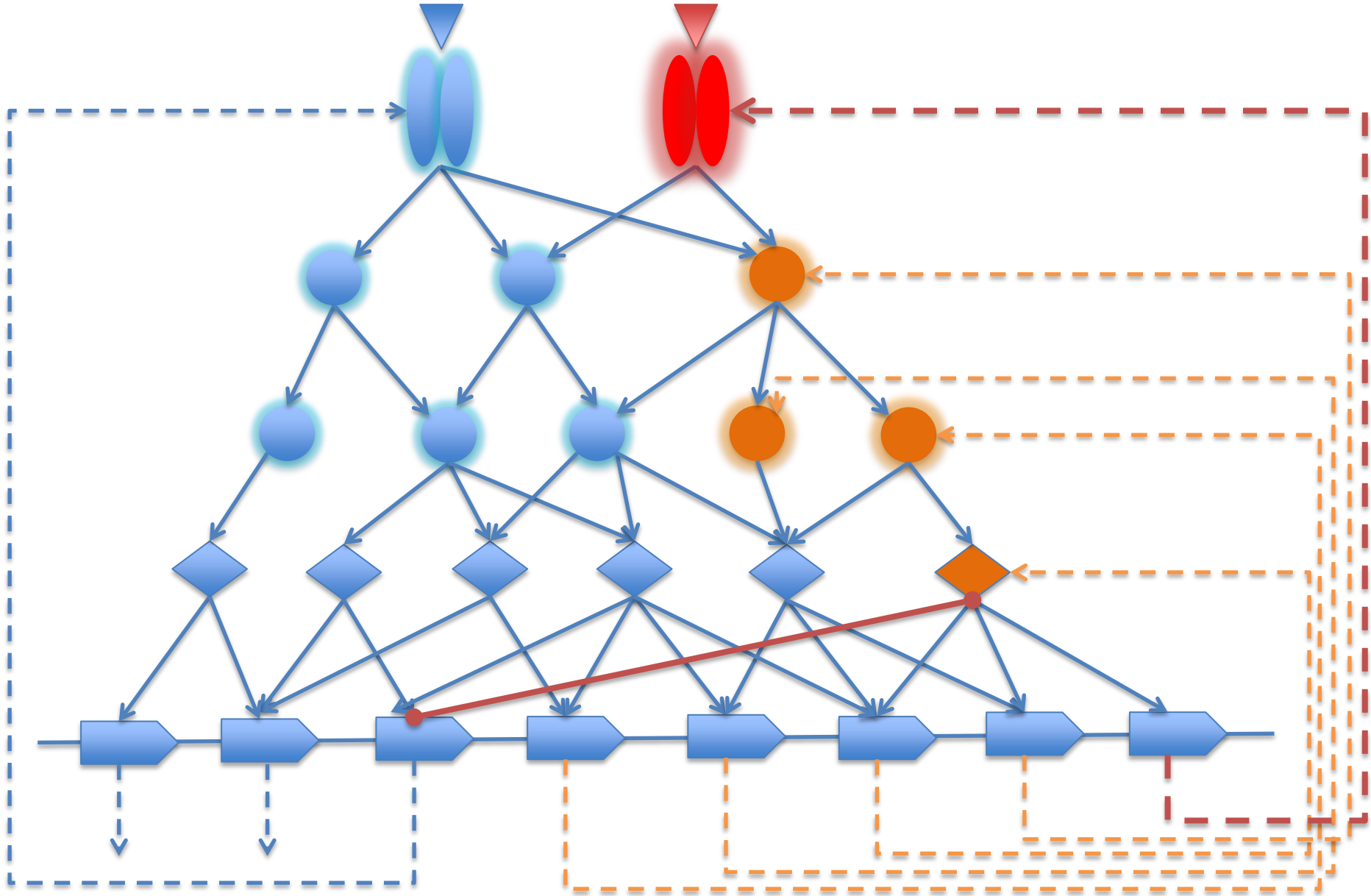
Parking in Italy

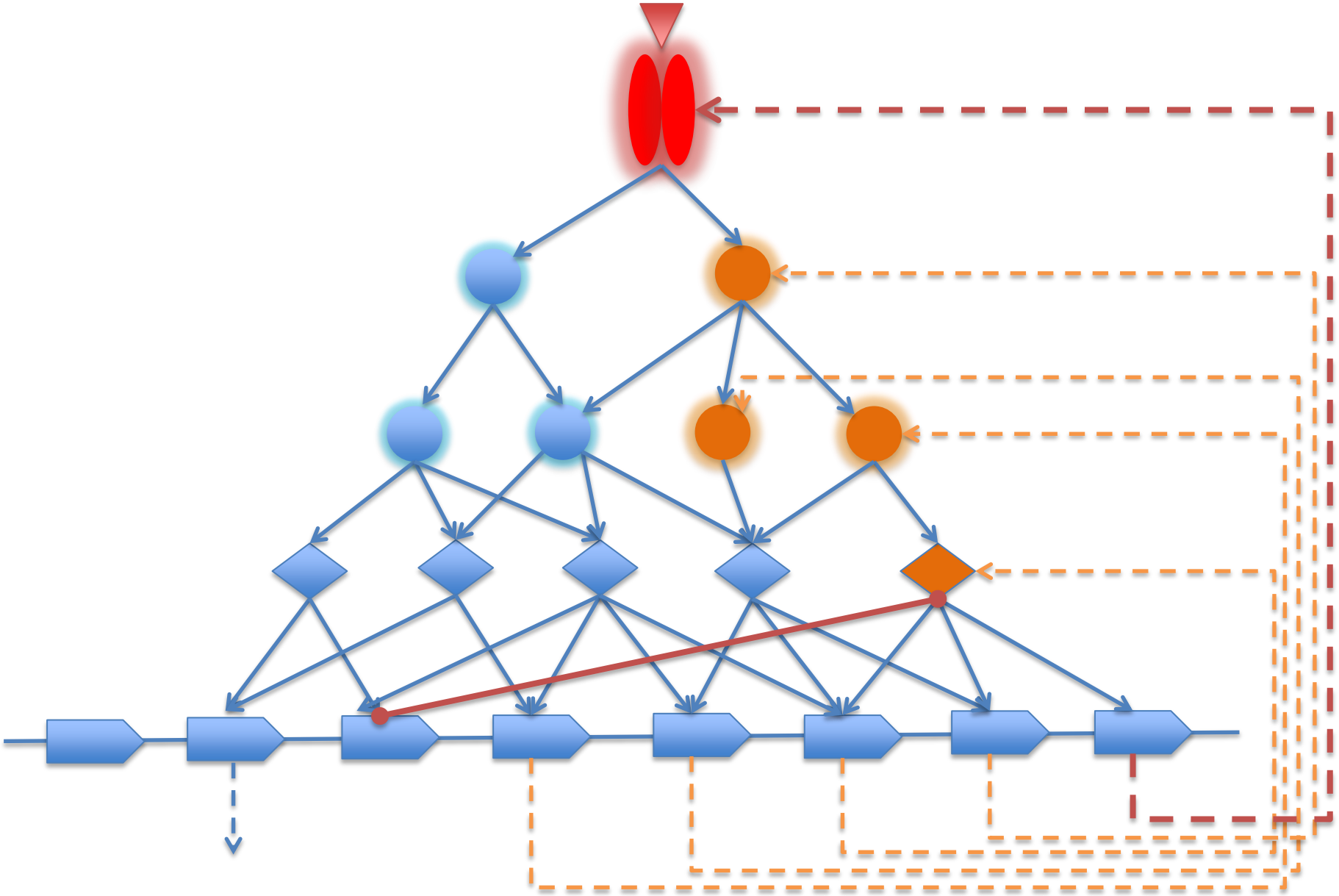
ChIP-seq data: EPS-FLI1

Ewing sarcoma transcription factor – gene fusion

Network plasticity

„Walking pathways“



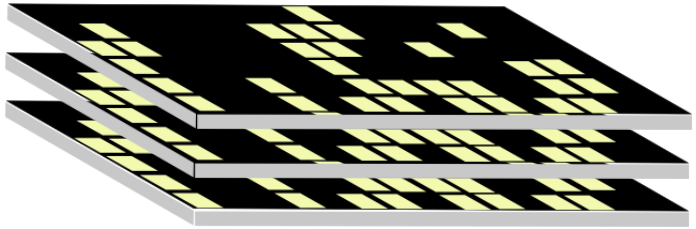


Epidermal Growth Factor induced Carcinogenicity

Philip Stegmaier[1], Alexander Kel[1], Edgar Wingender[1,2], and Jürgen Borlak[3]

Hepatocellular transcriptome data of IgEGF-overexpressing mice

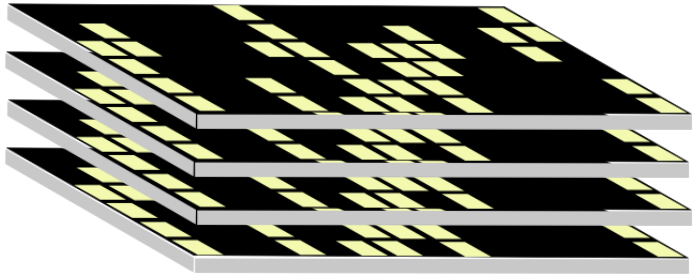
transgenic



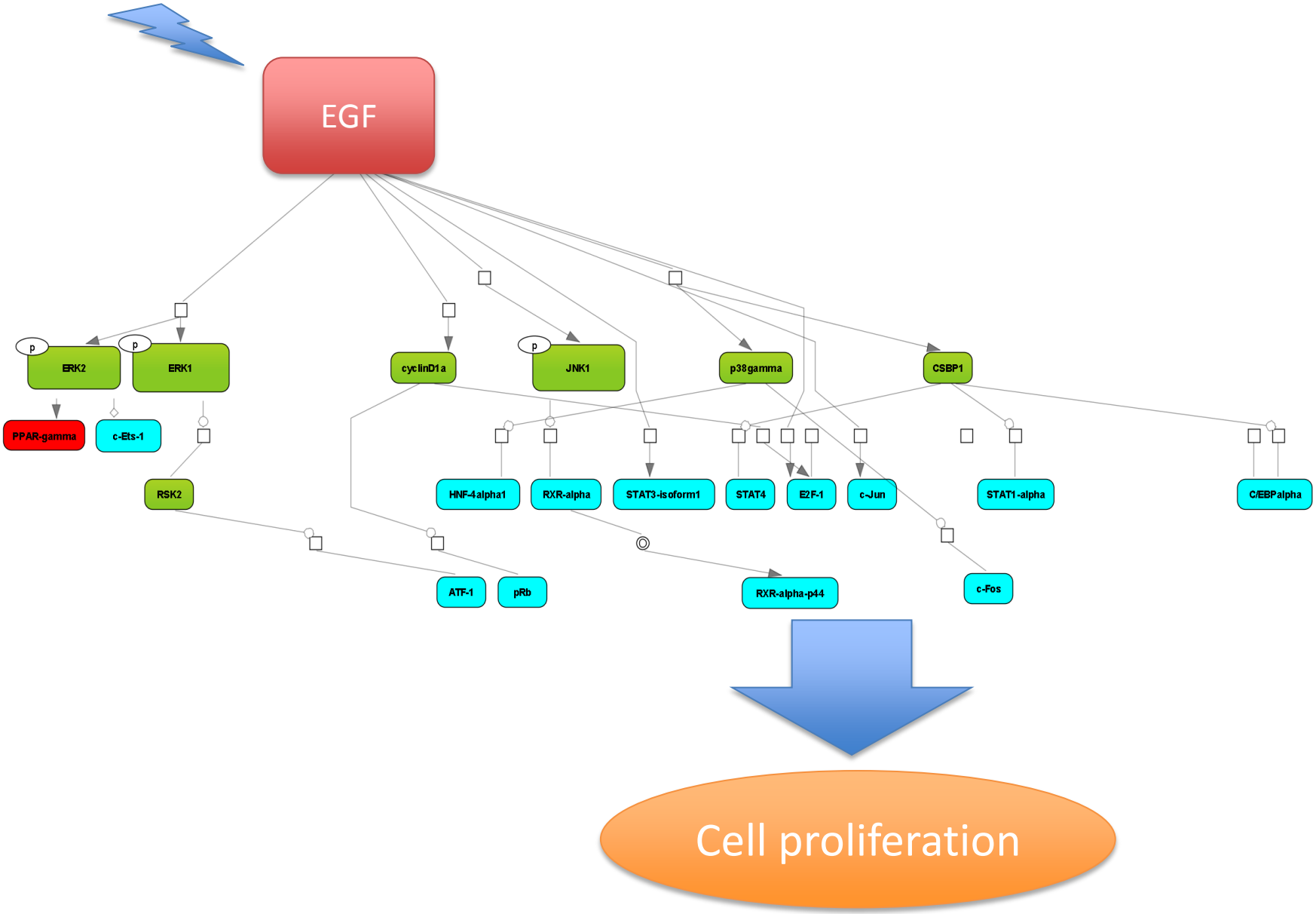
transgenic/normal

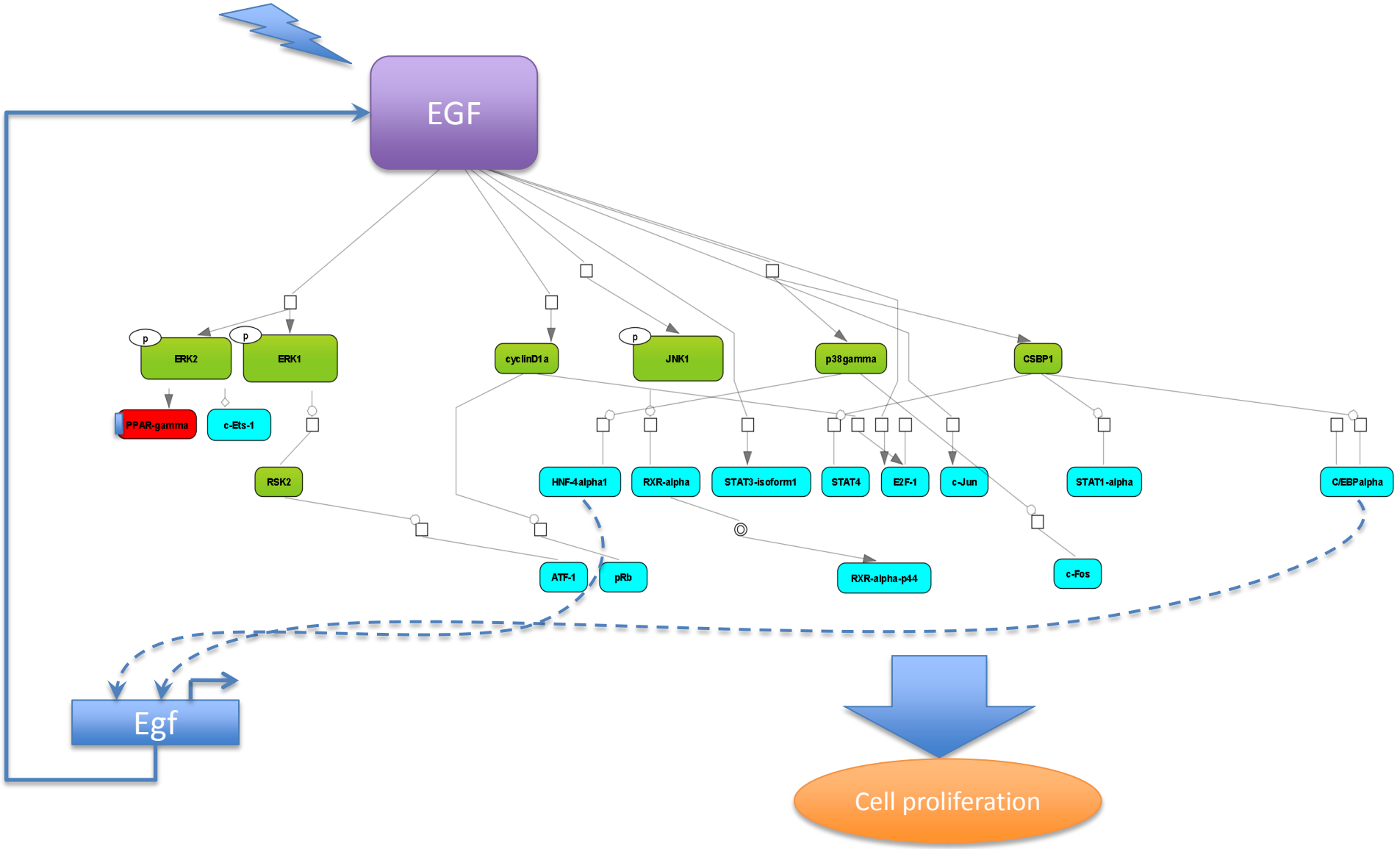
Tumoregenic switch ?

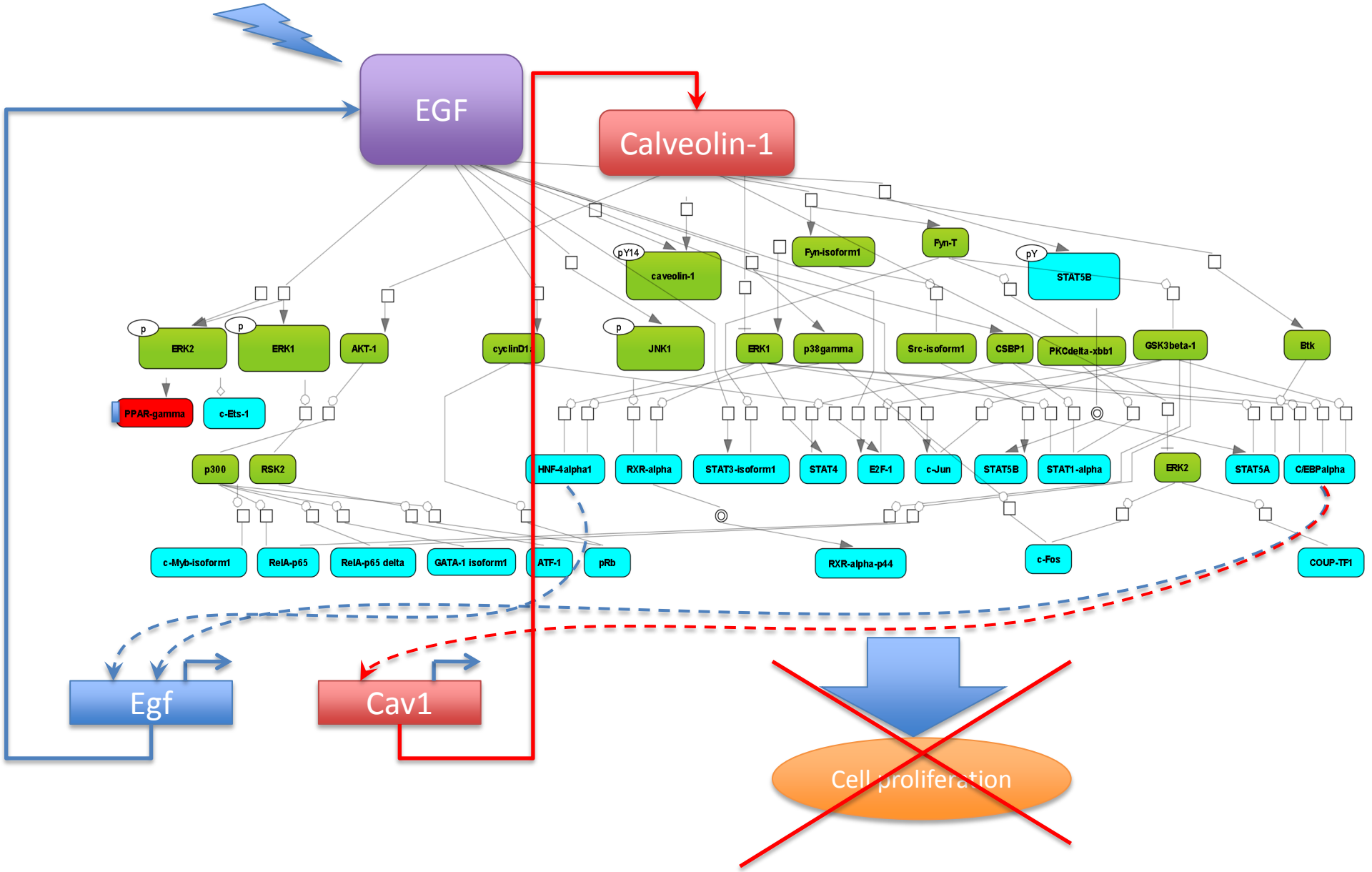
small tumor

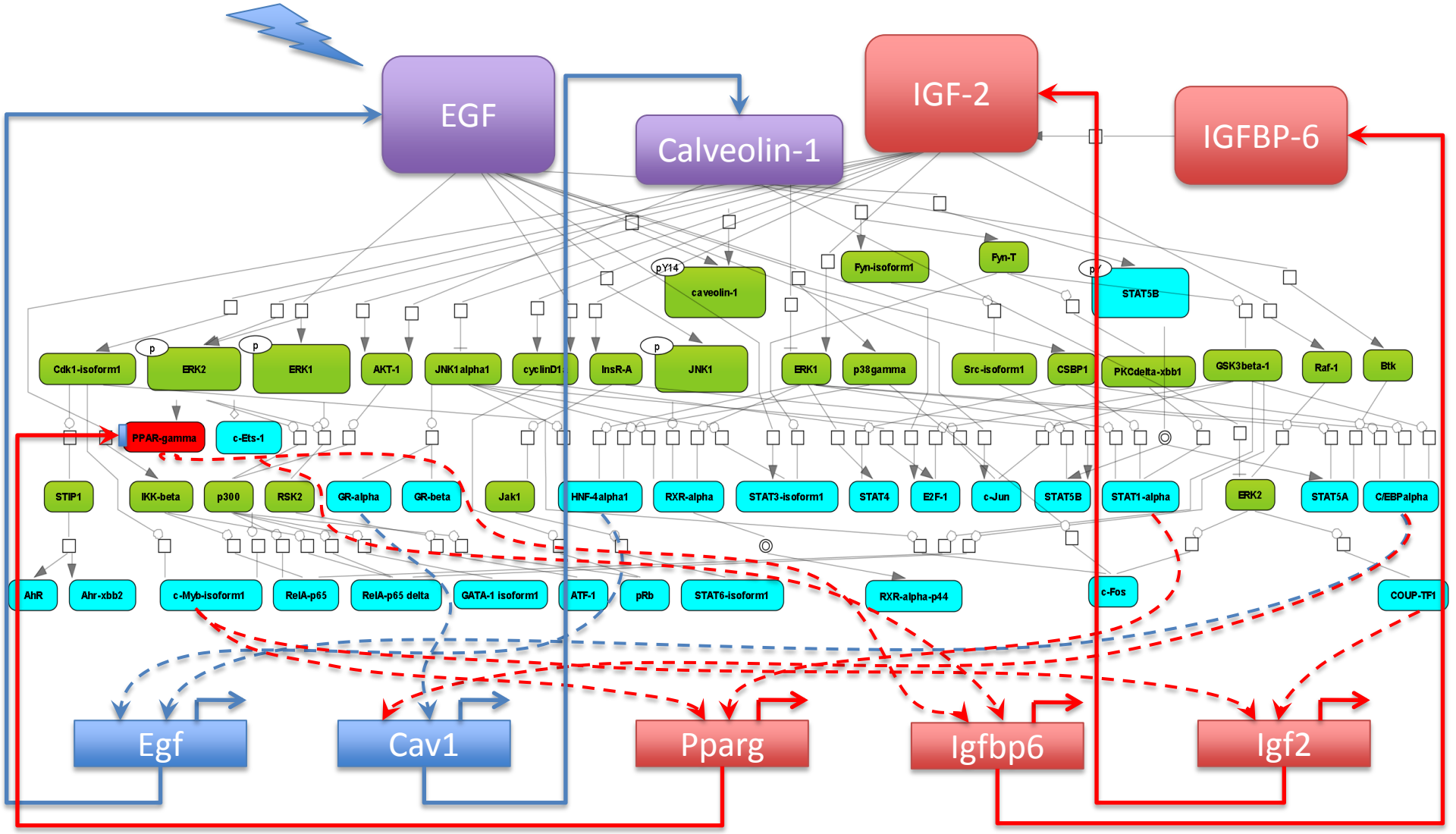


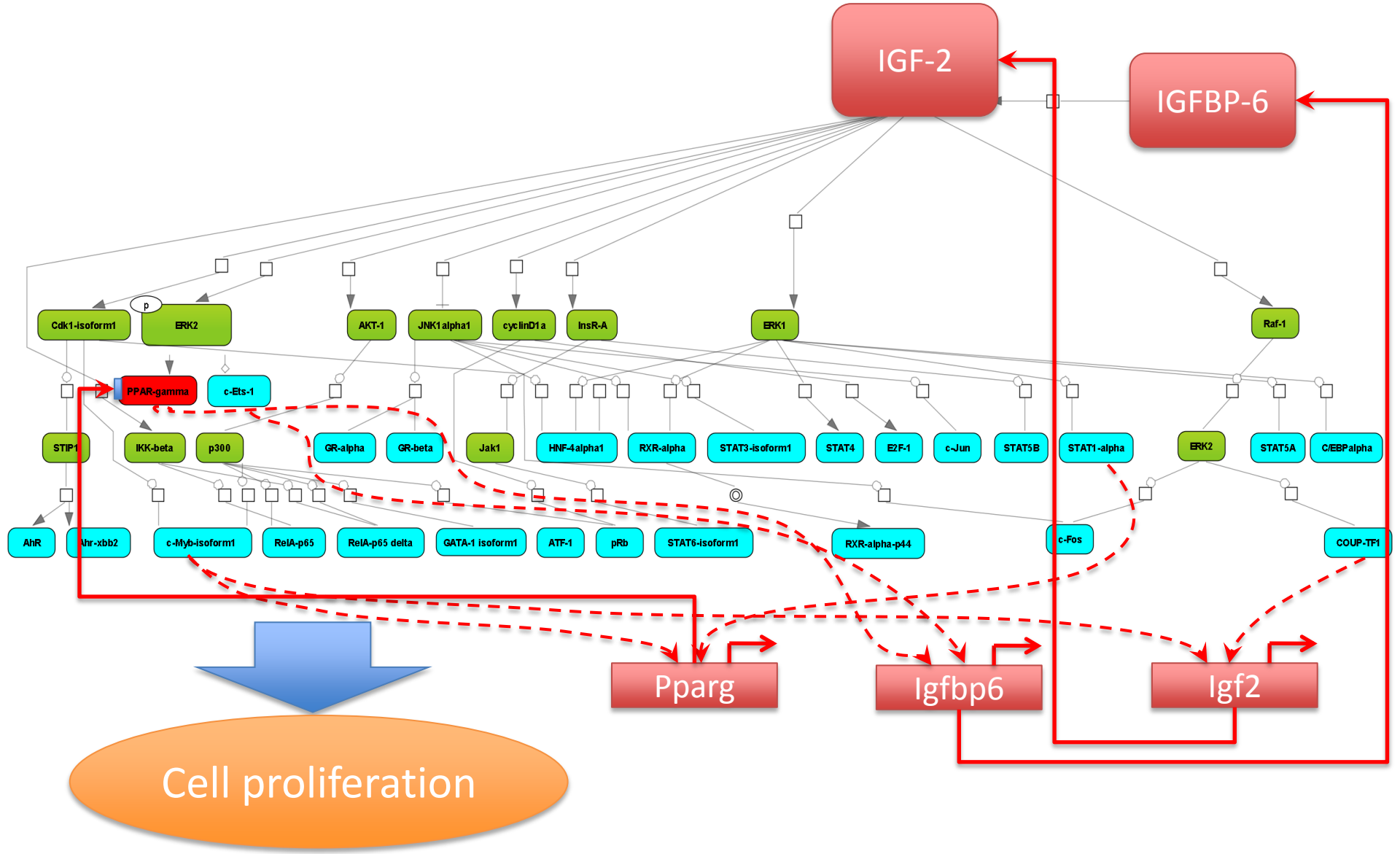
small tumor/normal


EGF
p
ERK2
p
ERK1
cyclinD1a
p
JNK1
p38gamma
CSBP1
PPAR-gamma
c-Ets-1
RSK2
HNF-4alpha1
RXR-alpha
STAT3-isoform1
STAT4
E2F-1
c-Jun
STAT1-alpha
C/EBPalpha
ATF-1
pRb
RXR-alpha-p44
c-Fos
Cell proliferation


EGF
p
ERK2
p
ERK1
cyclinD1a
p
JNK1
p38gamma
CSBP1
PPAR-gamma
c-Ets-1
RSK2
HNF-4alpha1
RXR-alpha
STAT3-isoform1
STAT4
E2F-1
c-Jun
STAT1-alpha
C/EBPalpha
ATF-1
pRb
RXR-alpha-p44
c-Fos
Egf
Cell proliferation


EGF
Calveolin-1
pY14
caveolin-1
Fyn-isoform1
Fyn-T
pY
STAT5B
p
ERK2
p
ERK1
AKT-1
cyclinD1
p
JNK1
ERK1
p38gamma
Src-isoform1
CSBP1
PKCdelta-xbb1
GSK3beta-1
Btk
PPAR-gamma
c-Ets-1
p300
RSK2
HNF-4alpha1
RXR-alpha
STAT3-isoform1
STAT4
E2F-1
c-Jun
STAT5B
STAT1-alpha
ERK2
STAT5A
C/EBPalpha
c-Myb-isoform1
RelA-p65
RelA-p65 delta
GATA-1 isoform1
ATF-1
pRb
RXR-alpha-p44
c-Fos
COUP-TF1
Egf
Cav1
Cell proliferation

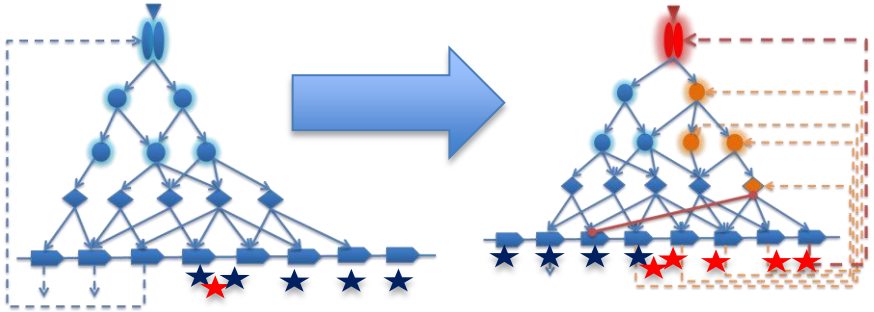

EGF
Calveolin-1
IGF-2
IGFBP-6
pY14
caveolin-1
Fyn-isoform1
Fyn-T
p
STAT5B
Cdk1-isoform1
p
ERK2
p
ERK1
AKT-1
JNK1 alpha1
cyclinD1
InsR-A
p
JNK1
ERK1
p38gamma
Src-isoform1
CSBP1
PKCdelta-xbb1
GSK3beta-1
Raf-1
Btk
PPAR-gamma
c-Ets-1
STIP1
IKK-beta
p300
RSK2
GR-alpha
GR-beta
Jak1
HNF-4alpha1
RXR-alpha
STAT3-isoform1
STAT4
E2F-1
c-Jun
STAT5B
STAT1-alpha
ERK2
STAT5A
C/EBPalpha
AhR
Ahr-xbb2
c-Myb-isoform1
RelA-p65
RelA-p65 delta
GATA-1 isoform1
ATF-1
pRb
STAT6-isoform1
RXR-alpha-p44
c-Fos
COUP-TF1
Egf
Cav1
Pparg
Igfbp6
Igf2


IGF-2
IGFBP-6
Cdk1-isoform1
p
ERK2
AKT-1
JNK1 alpha1
cyclinD1a
InsR-A
ERK1
Raf-1
PPAR-gamma
c-Ets-1
STIP1
IKK-beta
p300
GR-alpha
GR-beta
Jak1
HNF-4alpha1
RXR-alpha
STAT3-isoform1
STAT4
E2F-1
c-Jun
STAT5B
STAT1-alpha
ERK2
STAT5A
C/EBPalpha
AhR
Ahr-xbb2
c-Myb-isoform1
RelA-p65
RelA-p65 delta
GATA-1 isoform1
ATF-1
pRb
STAT6-isoform1
RXR-alpha-p44
c-Fos
COUP-TF1
Pparg
Igfbp6
Igf2
Cell proliferation

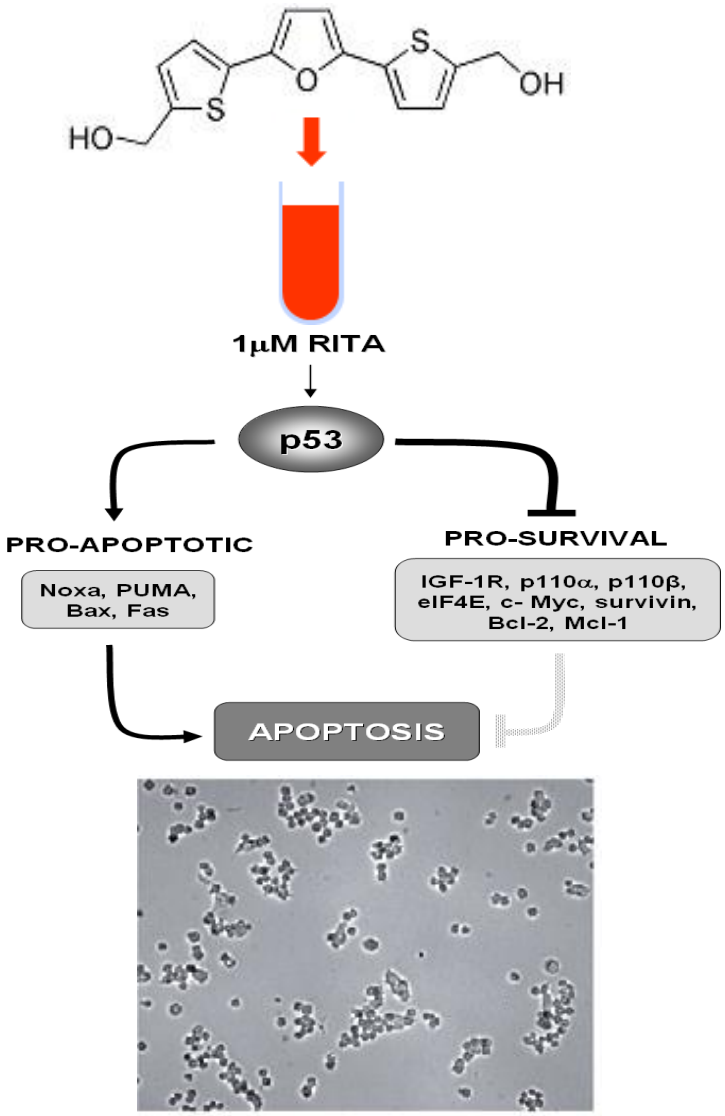
DNA is an active component of biochemical networks.

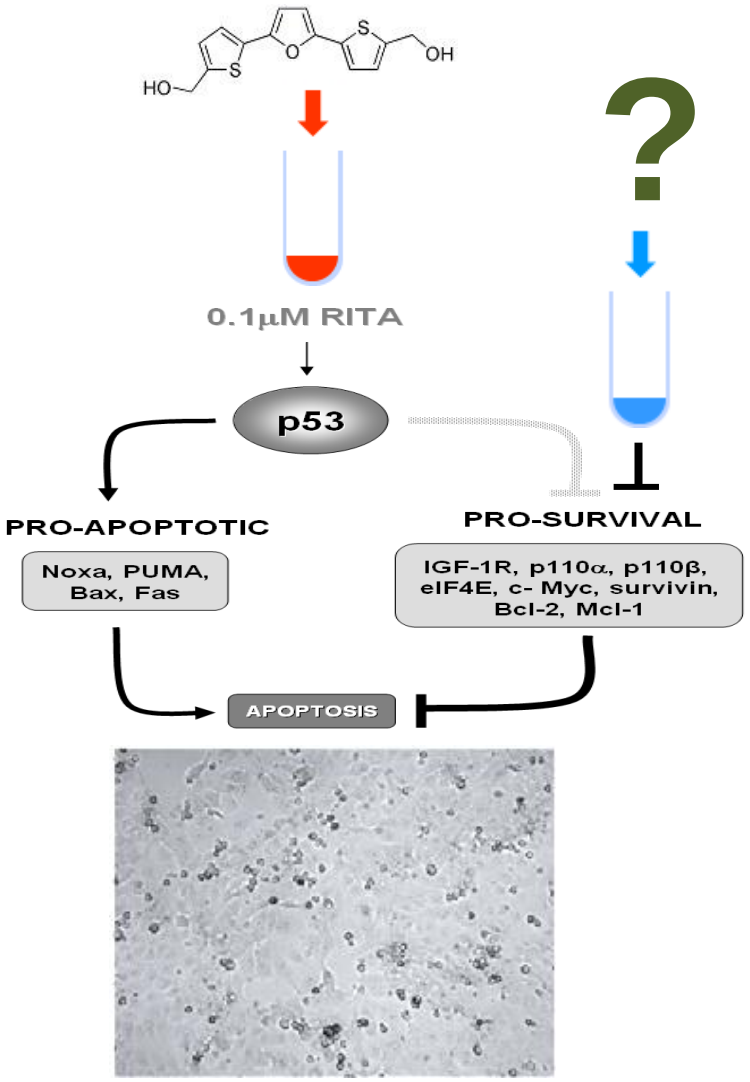
Network plasticity is a result of epigenetic evolution in the cells.

Cancer is cracking the combinatorial regulatory code

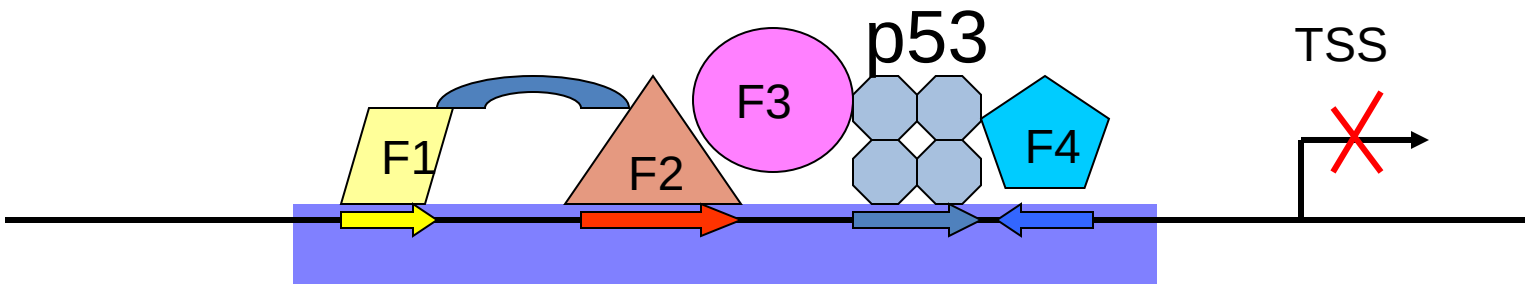


Net2Drug


HO
S
O
S
OH
1μM RITA
p53
PRO-APOPTOTIC
Noxa, PUMA, Bax, Fas
PRO-SURVIVAL
IGF-1R, p110α, p110β, eIF4E, c- Myc, survivin, Bcl-2, Mcl-1
APOPTOSIS

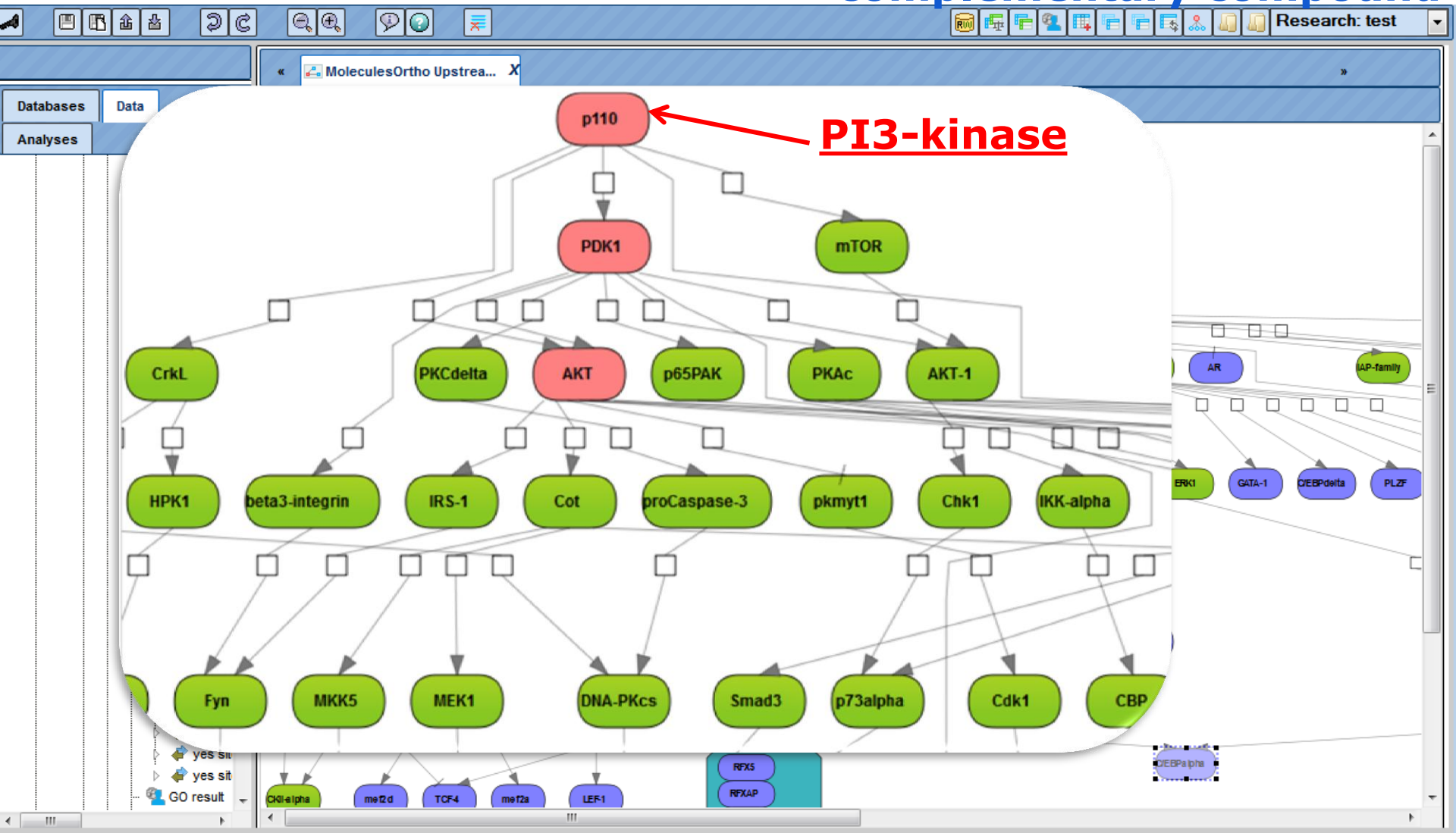

HO
S
O
S
OH
?
0.1μM RITA
p53
PRO-APOPTOTIC
Noxa, PUMA, Bax, Fas
PRO-SURVIVAL
IGF-1R, p110α, p110β, eIF4E, c- Myc, survivin, Bcl-2, Mcl-1
APOPTOSIS

Enhanceosome binding aria

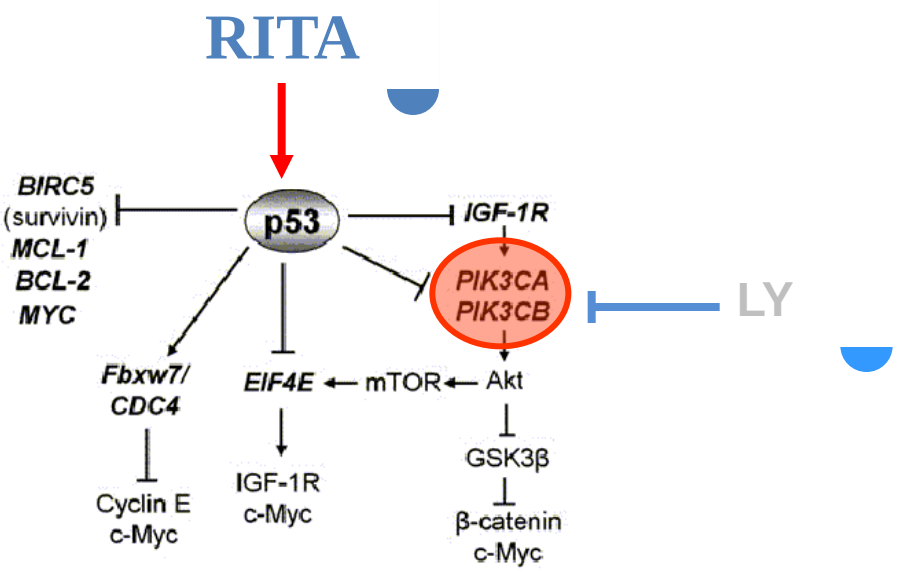


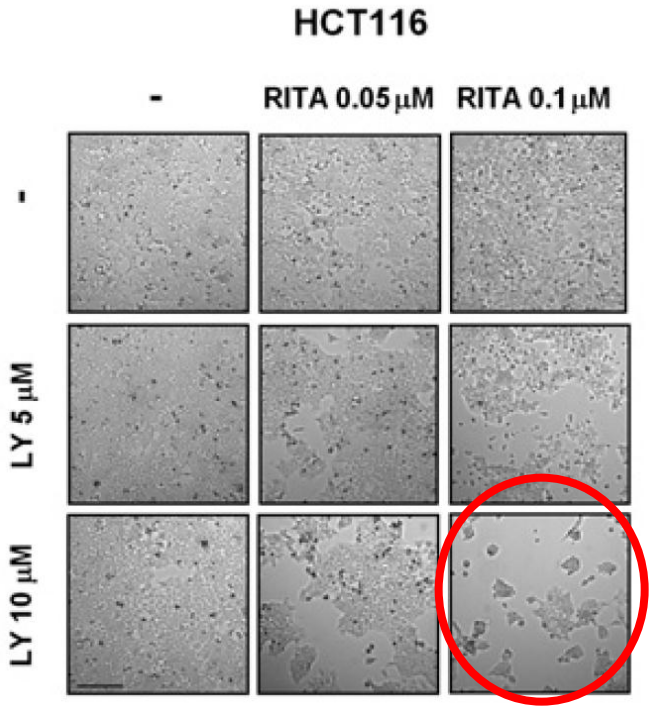
Repression of genes

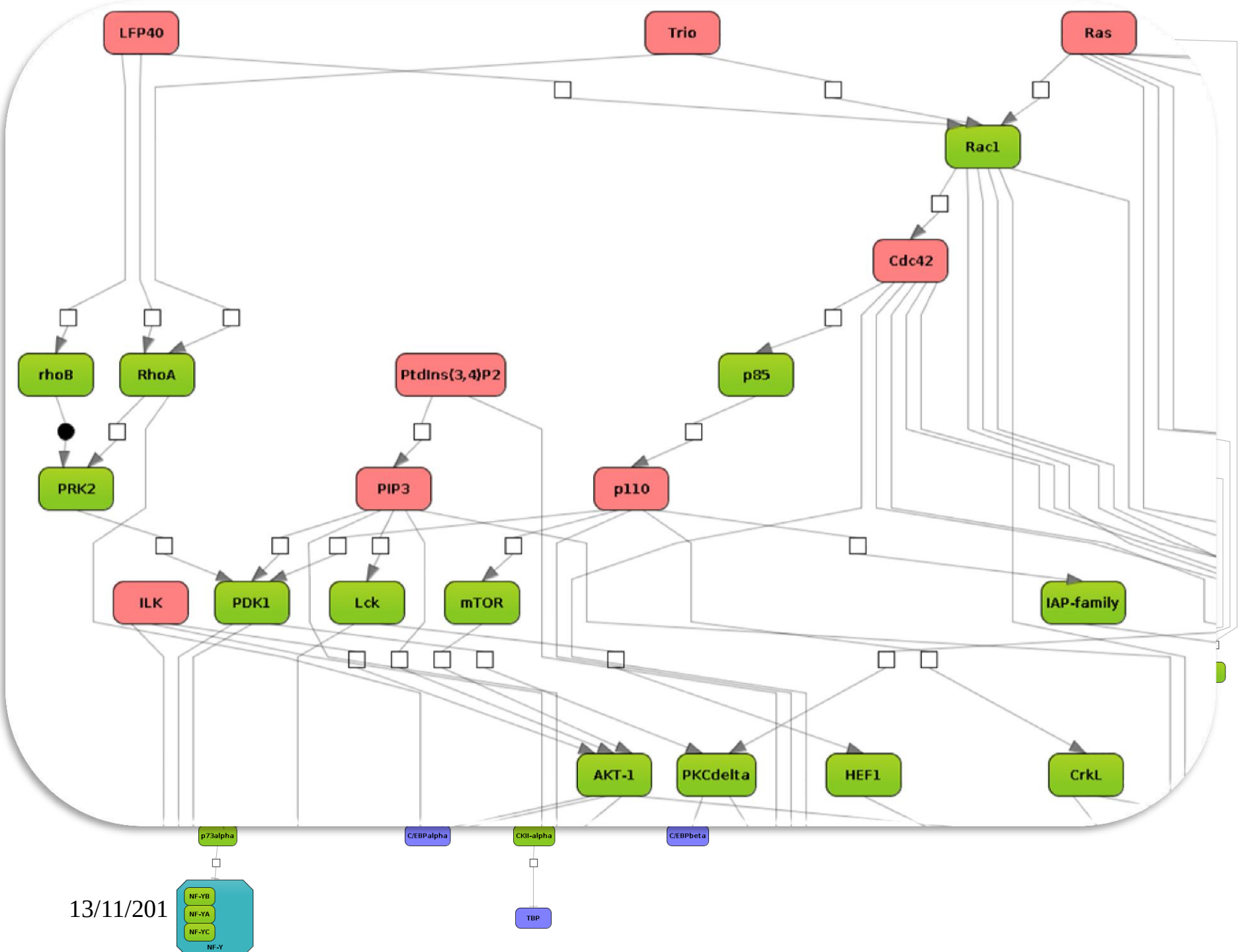
Survival mechanisms of cancer cells upon RITA treatment and potential target proteins for a complementary compound



Death of Cancer cells treated with 0.1 μM RITA and PI3-kinase inhibitor LY294002






LFP40
Trio
Ras
Rac1
Cdc42
rhoB
RhoA
PtdIns(3,4)P2
p85
PRK2
PIP3
p110
ILK
PDK1
Lck
mTOR
IAP-family
AKT-1
PKCdelta
HEF1
CrkL
p73alpha
C/EBPalpha
CKII-alpha
C/EBPbeta
NF-YB
NF-YA
NF-YC
NF-Y
TBP

Systems Medicine

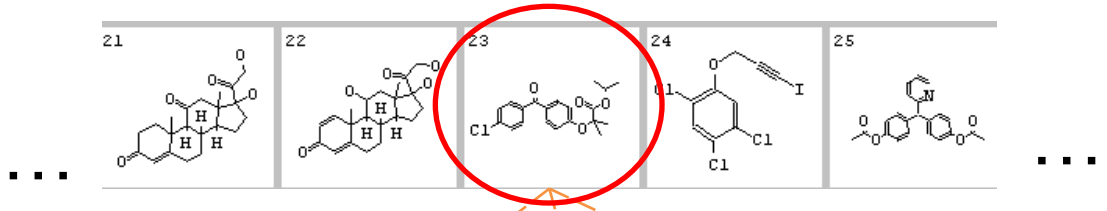
Identified

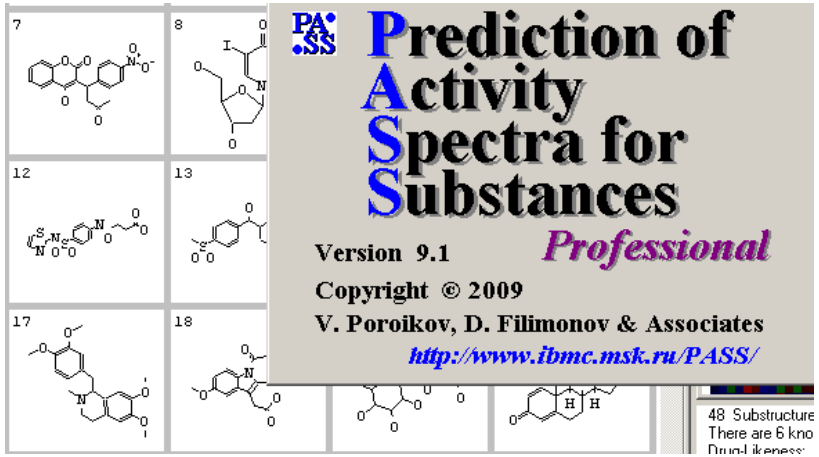
16 novel componds

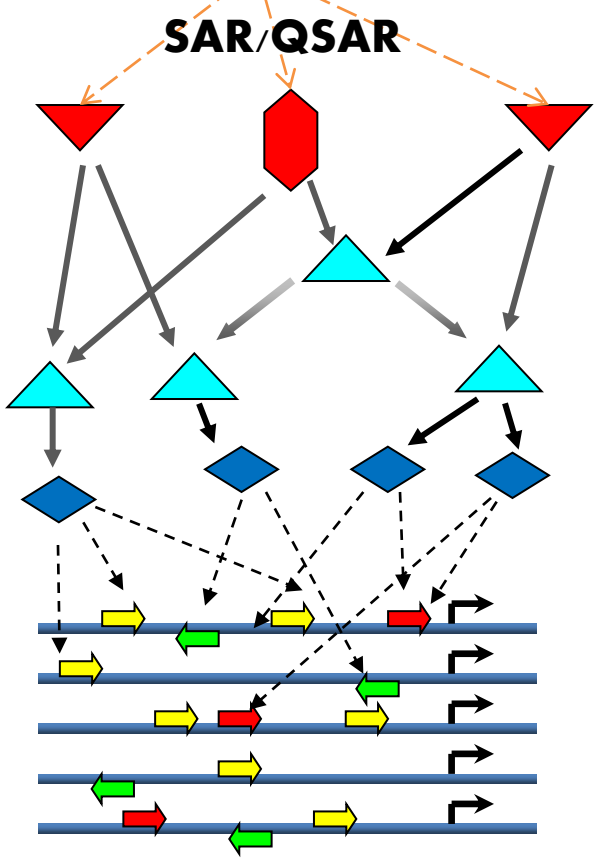
ChemNavigator Library

24 million

compounds







Tested 16 compounds in a panel of several cancer cell lines.

Found active: Compound N15

Hypoxia inducible factor 1 alpha inhibitor	**Phosphatidylinositol 3-kinase beta inhibitor**

Targets

Showed growth suppression in 3 different breast cancer cell lines. The effect appears to be p53-independent (kills p53-null colon cancer cells) and it does not affect the growth of non-transformed mammary epithelial cells

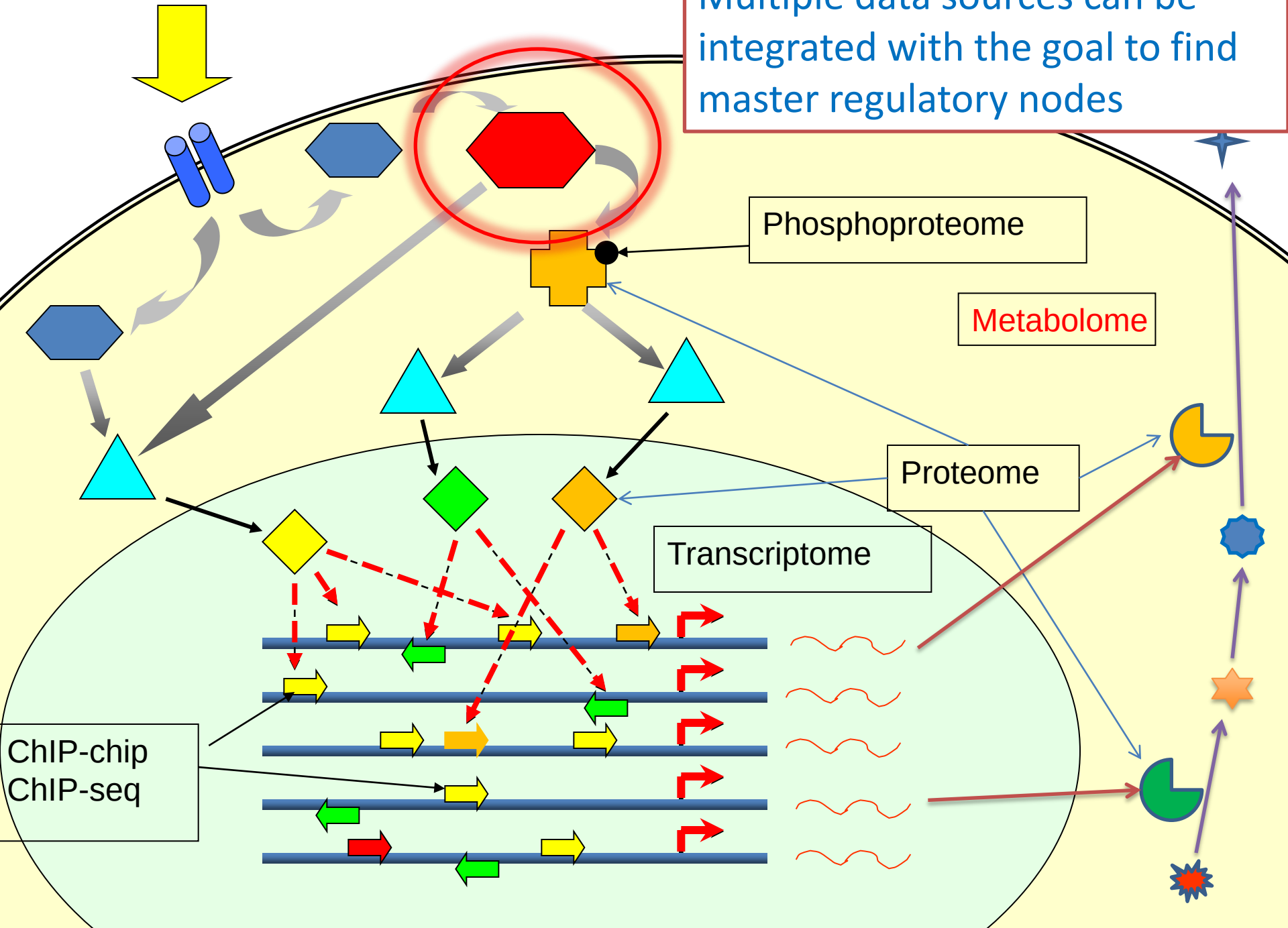
Found active: Compound N6

Cyclin-dependent kinase 2 inhibitor	**Myc inhibitor**

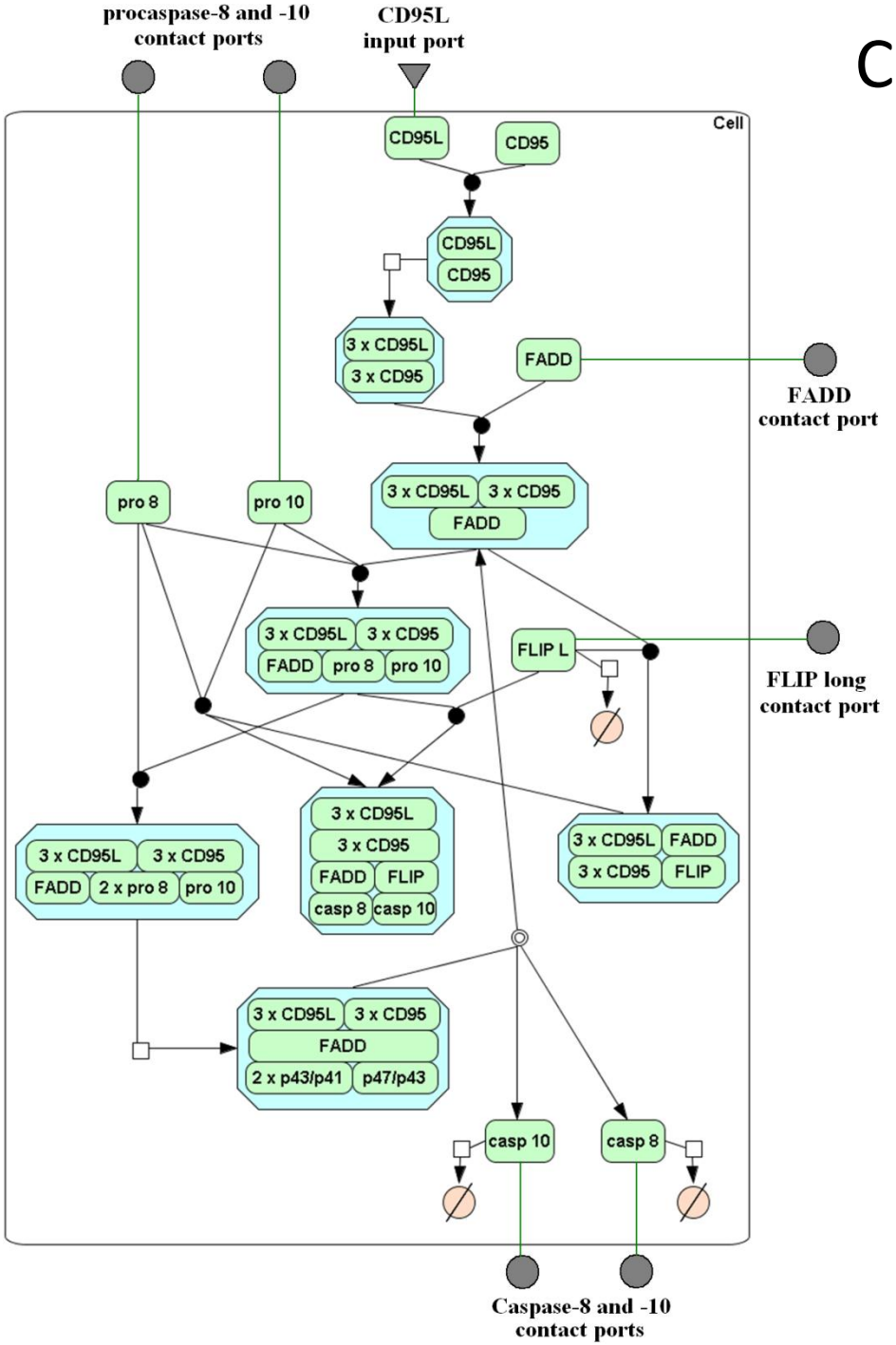
Targets

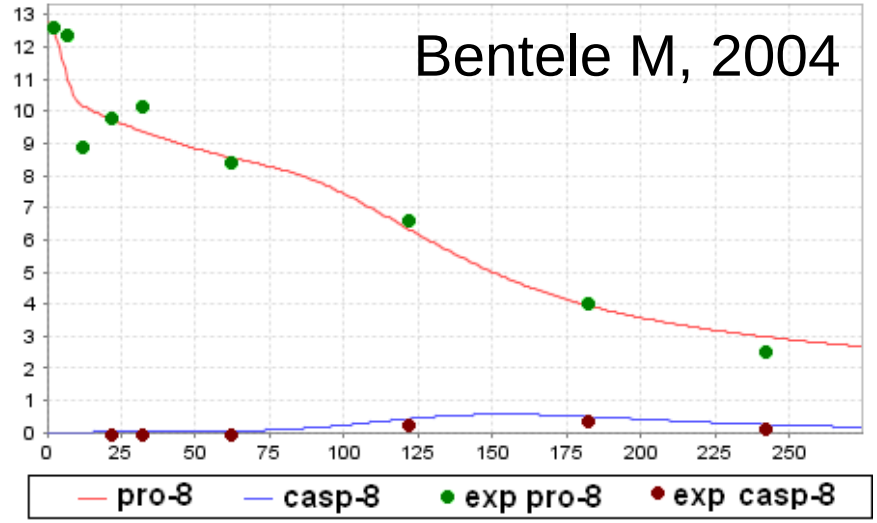
Out of panel of 7 different cancer lines it killed only melanoma cells without any effects in other cell lines and on control non-transformed mammary epithelial cells.

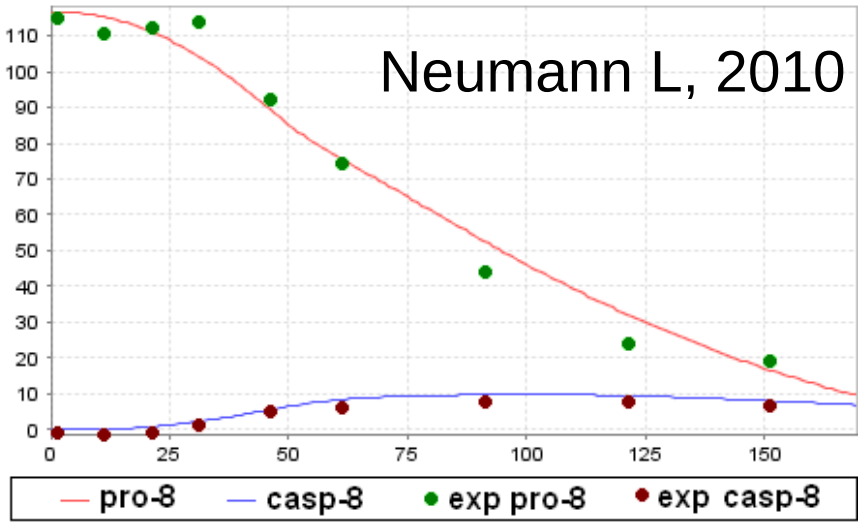
Multiple data sources can be integrated with the goal to find master regulatory nodes



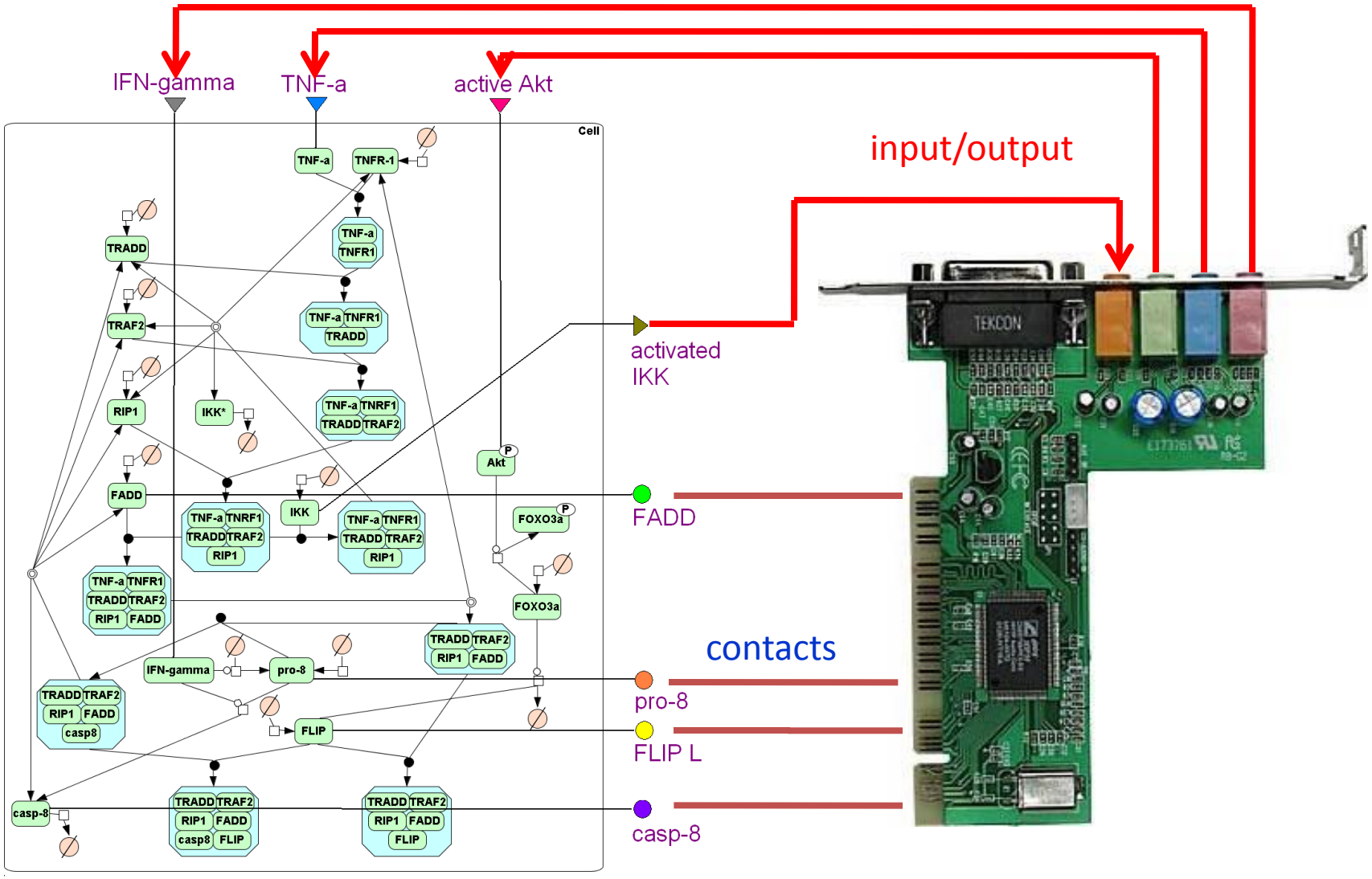
CD95L module and results of fitting its dynamics to experimental data





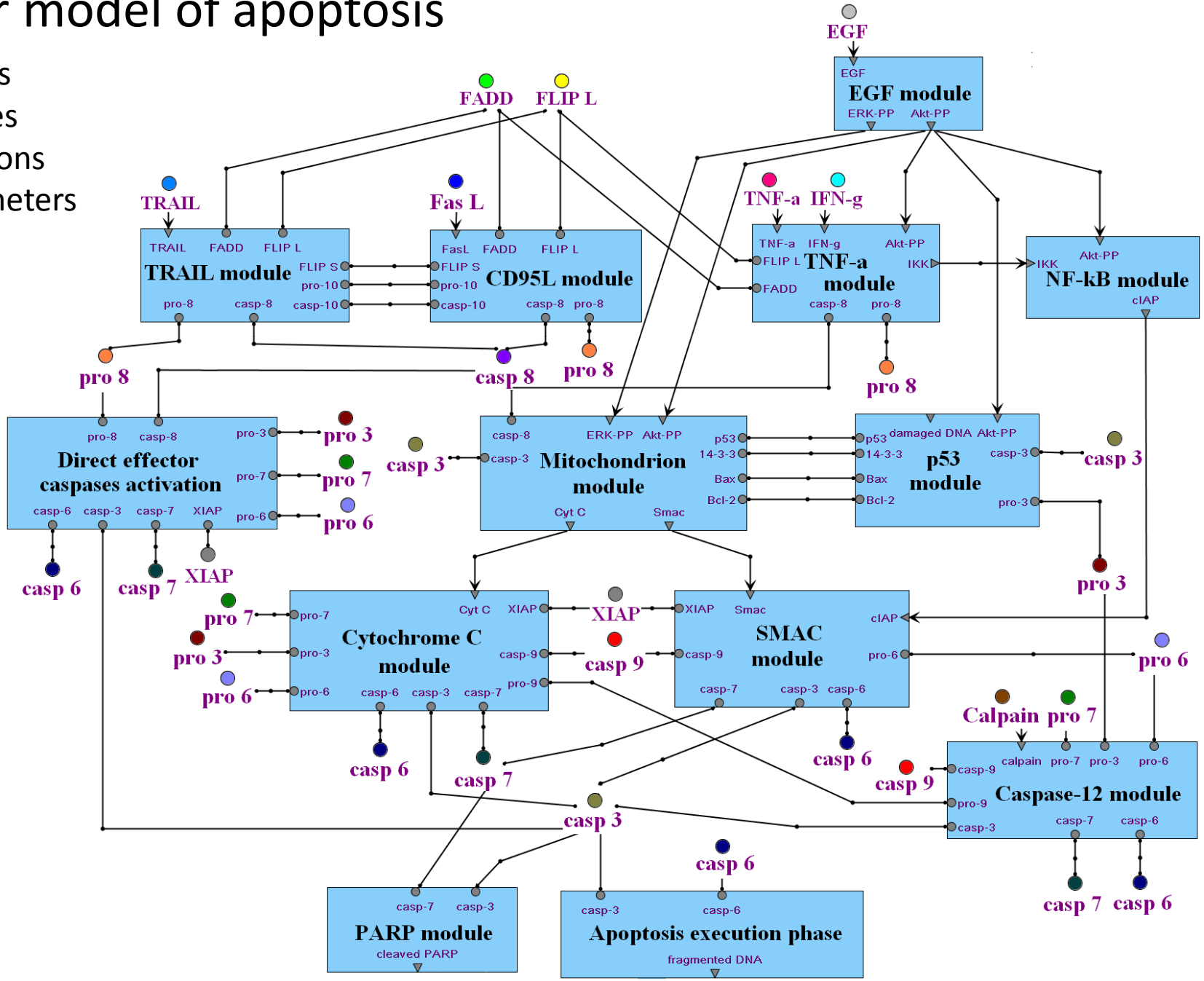


Modules:
clear specification of interfaces



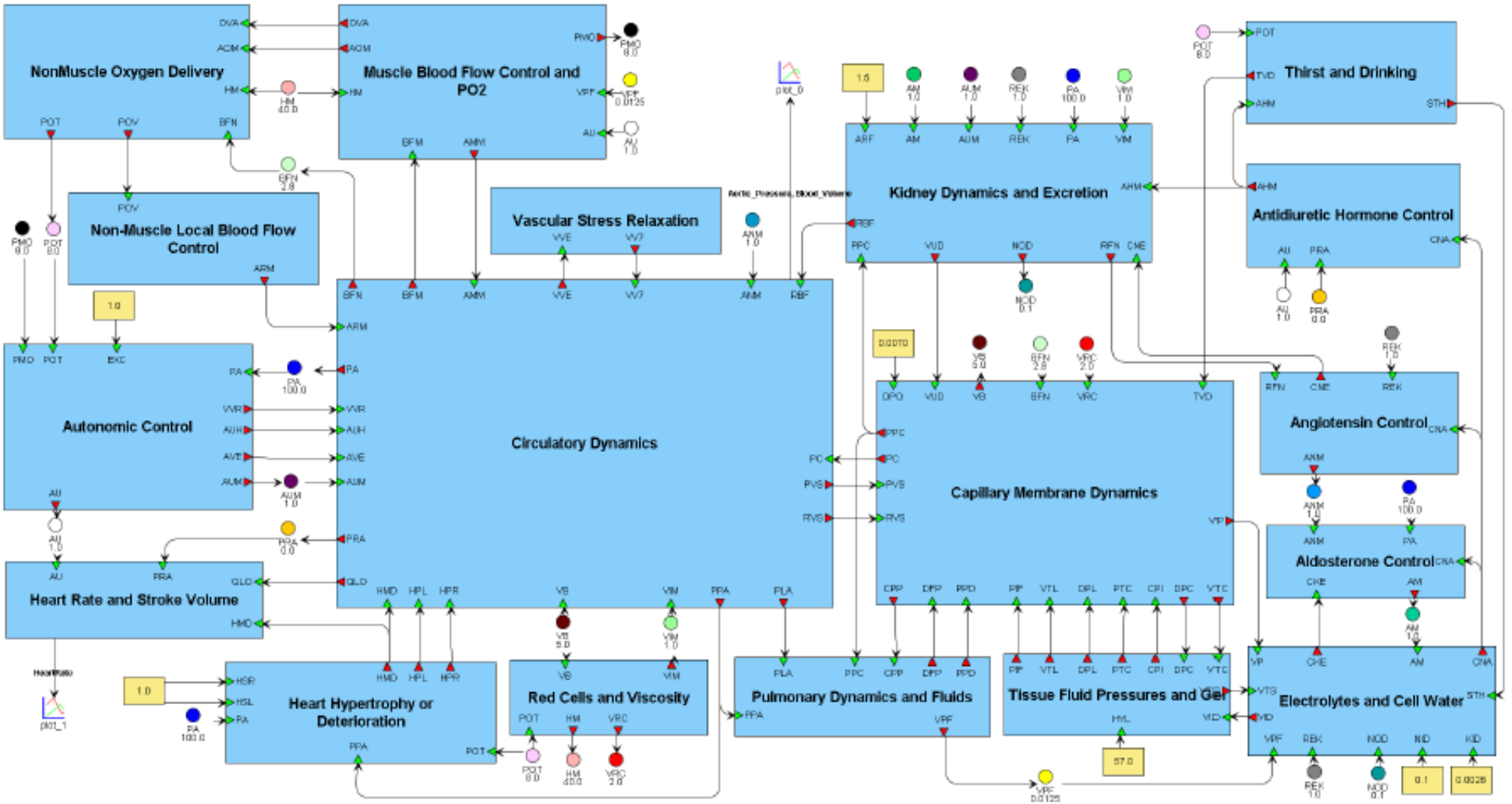
Modular model of apoptosis

- 13 modules
- 286 species
- 684 reactions
- 719 parameters



Modular Overall Circulation model

18 modules
234 parameters
39 ordinary differential equations
172 assignments



From virtual cell to virtual human

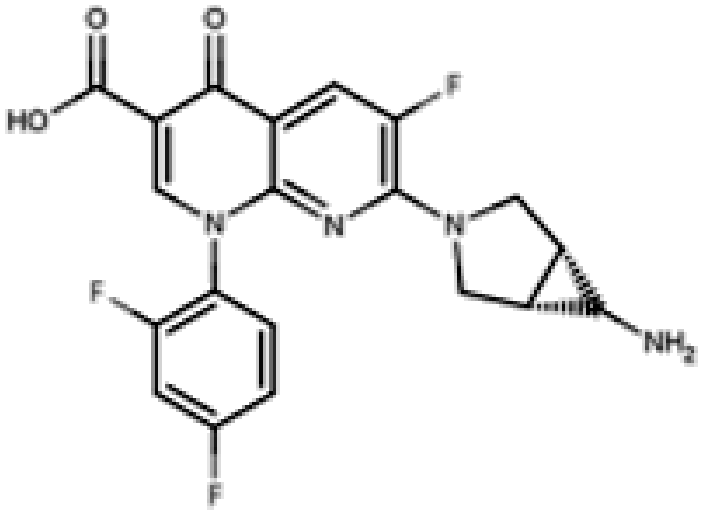
www.genexplain.com

www.biouml.org

Build virtual human to find novel drugs

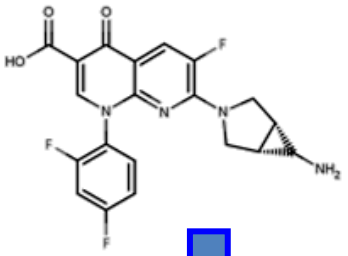
www.genexplain.com

Trovafloxacin - antibiotic

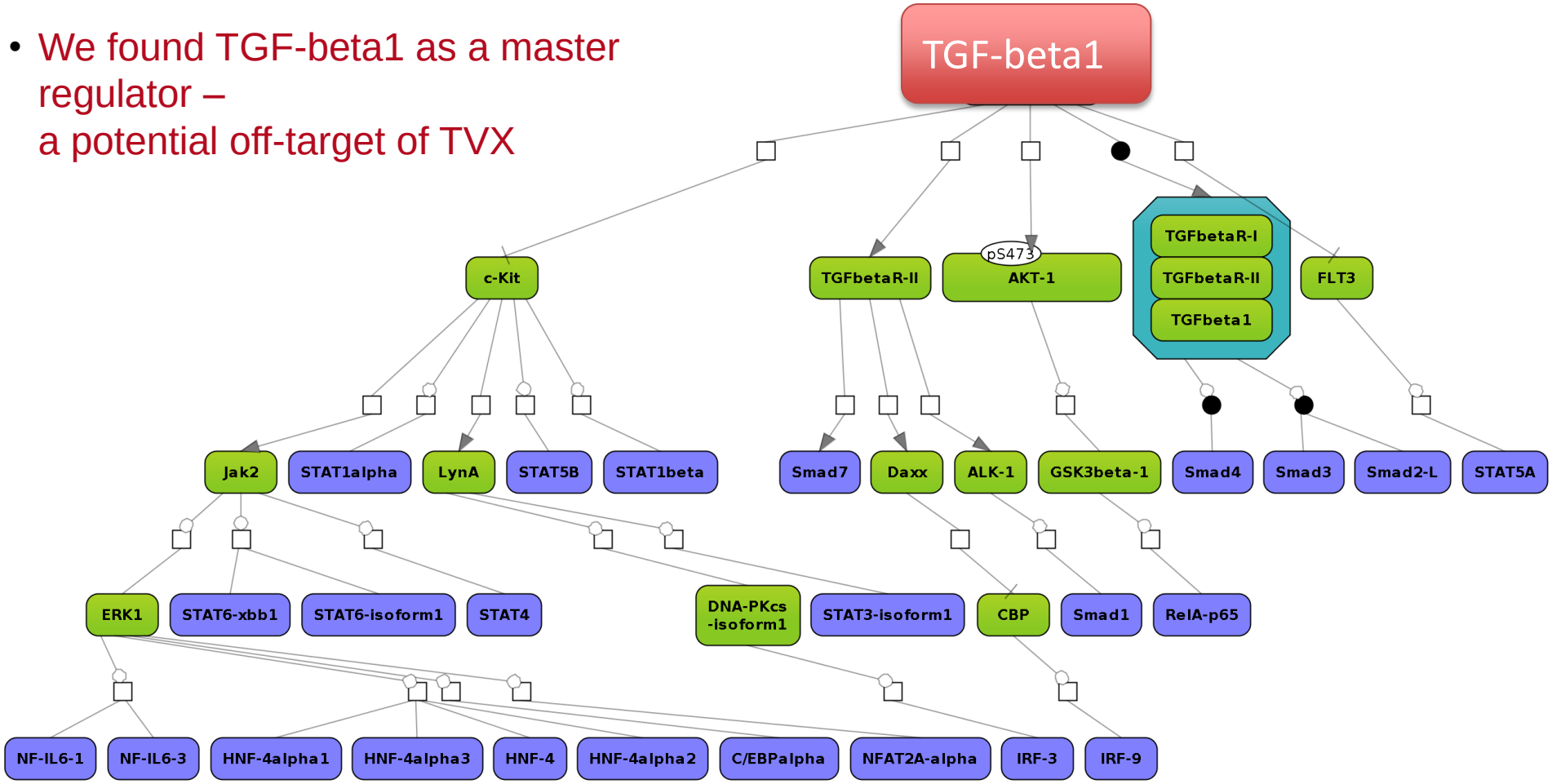


Withdrawn from market due to risk of idiosyncratic hepatotoxicity in 2001.

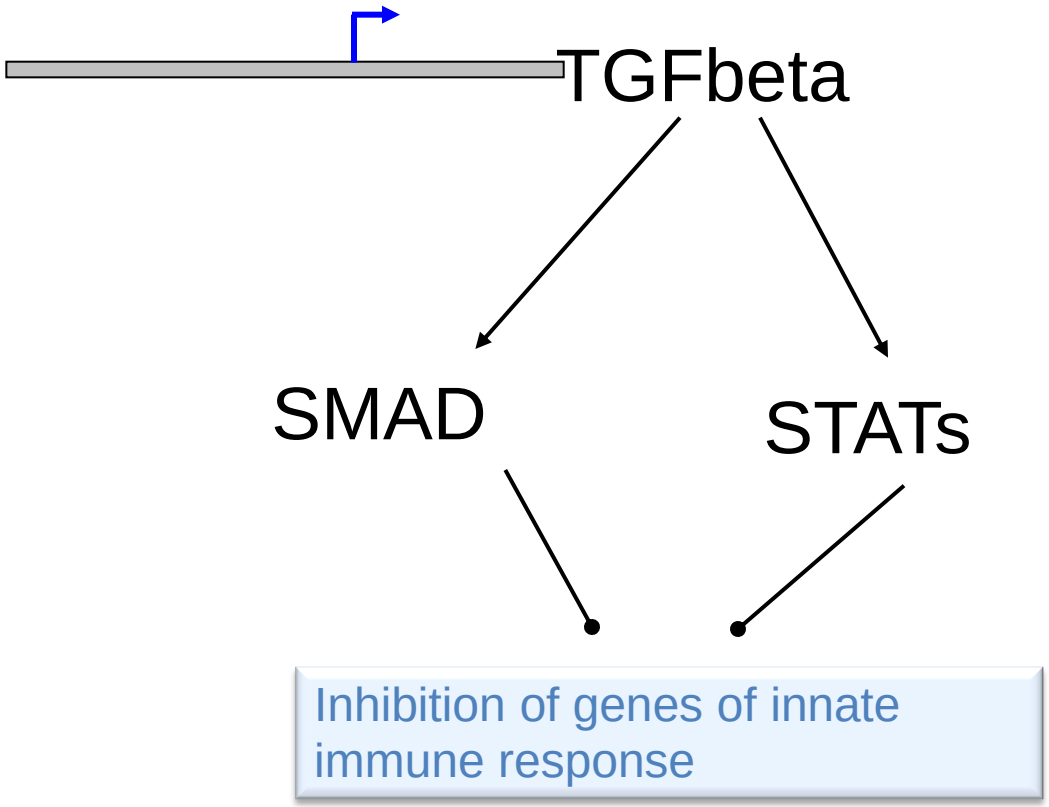
Trovafloxacin (TVX)



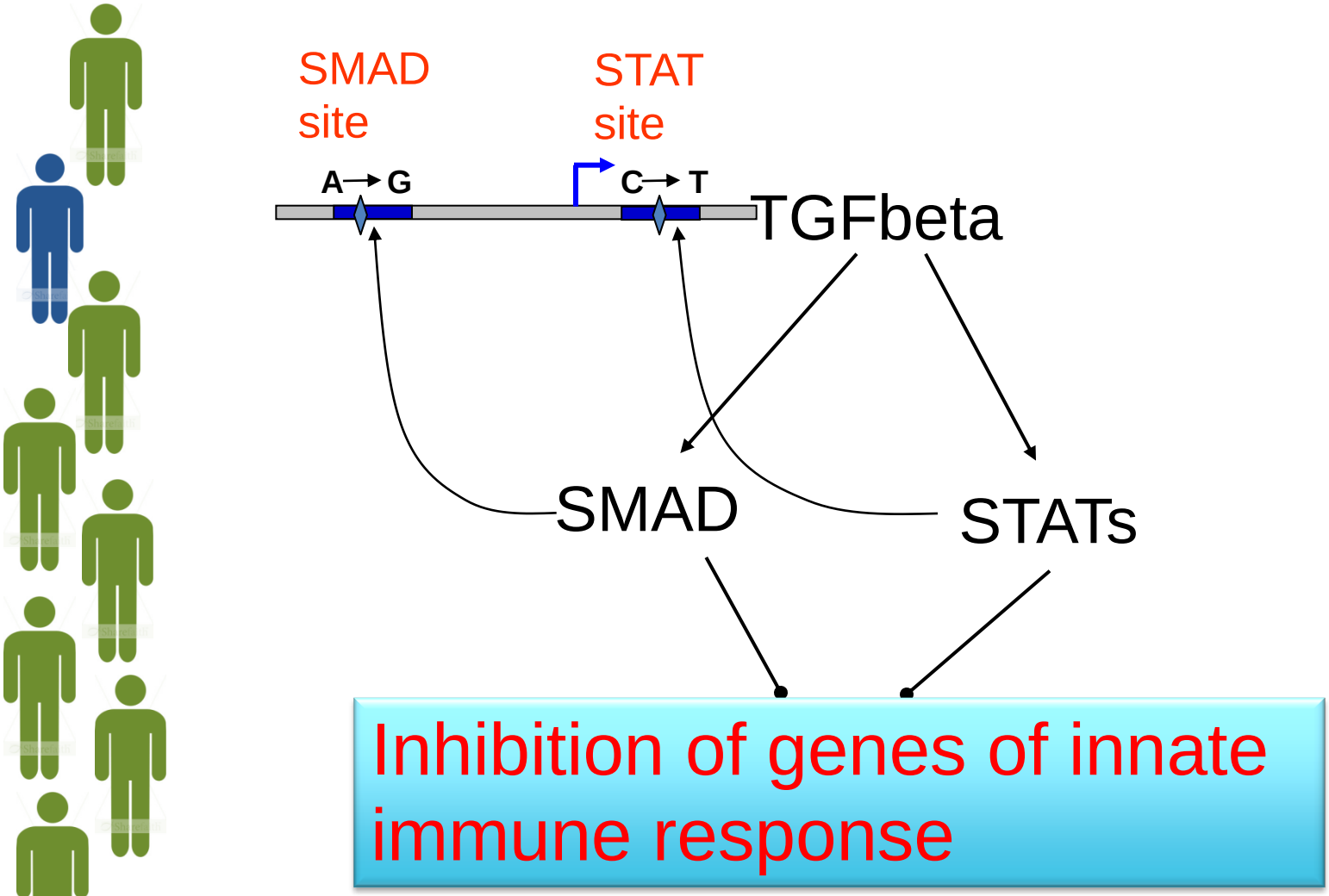
- We found TGF-beta1 as a master regulator –
  a potential off-target of TVX



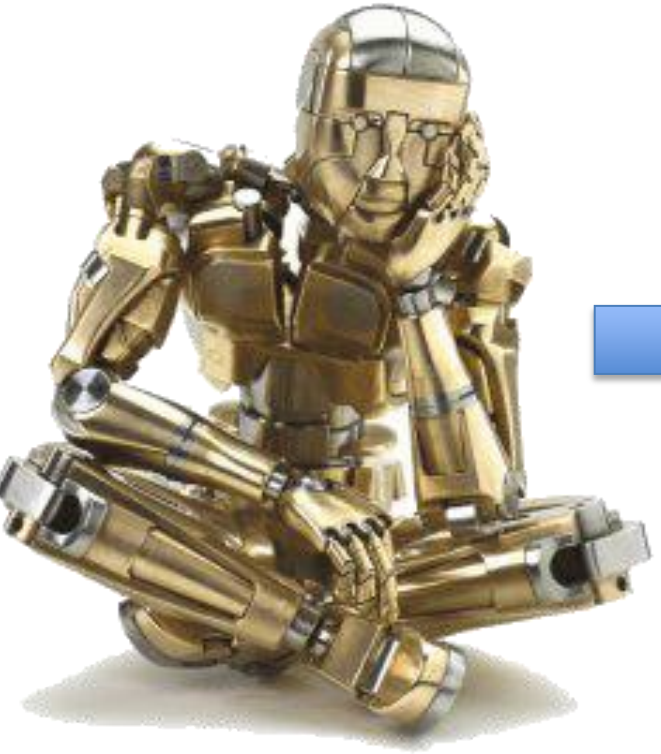
TGF-beta dependent positive feedback



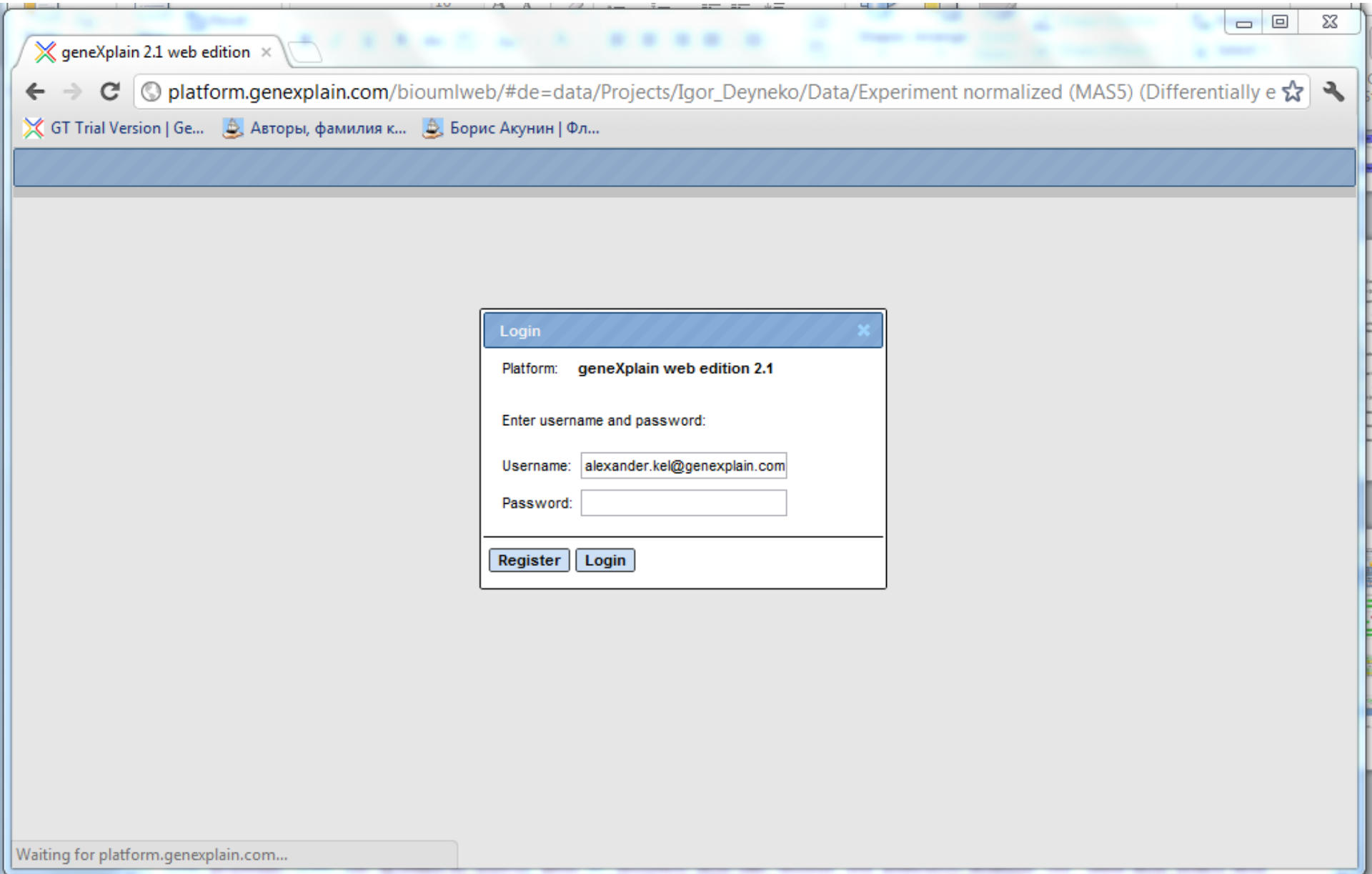
TGF-beta dependent positive feedback

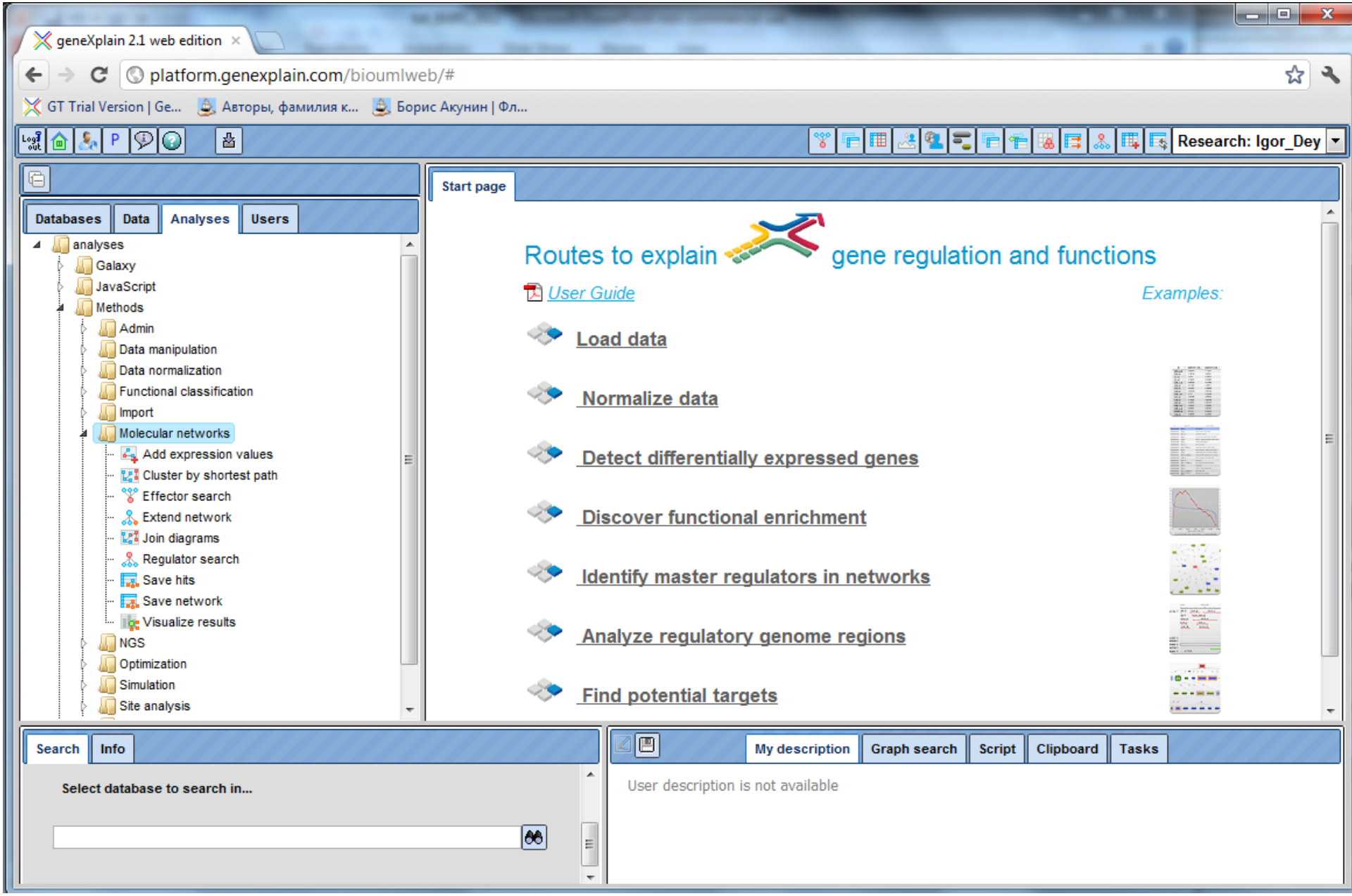


From virtual human to virtual patients



geneXplain Platform

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Борис Акунин | Фл...
Research: Igor_Dey
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Data manipulation
Data normalization
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Molecular networks
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Extend network
Join diagrams
Regulator search
Save hits
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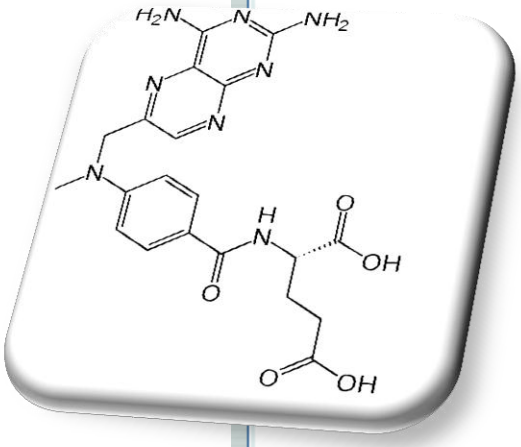
Visualize results
NGS
Optimization
Simulation
Site analysis
Start page
Routes to explain gene regulation and functions
User Guide
Examples:
Load data
Normalize data
Detect differentially expressed genes
Discover functional enrichment
Identify master regulators in networks
Analyze regulatory genome regions
Find potential targets
Search
Info
Select database to search in...
My description
Graph search
Script
Clipboard
Tasks
User description is not available

Series GSE11440

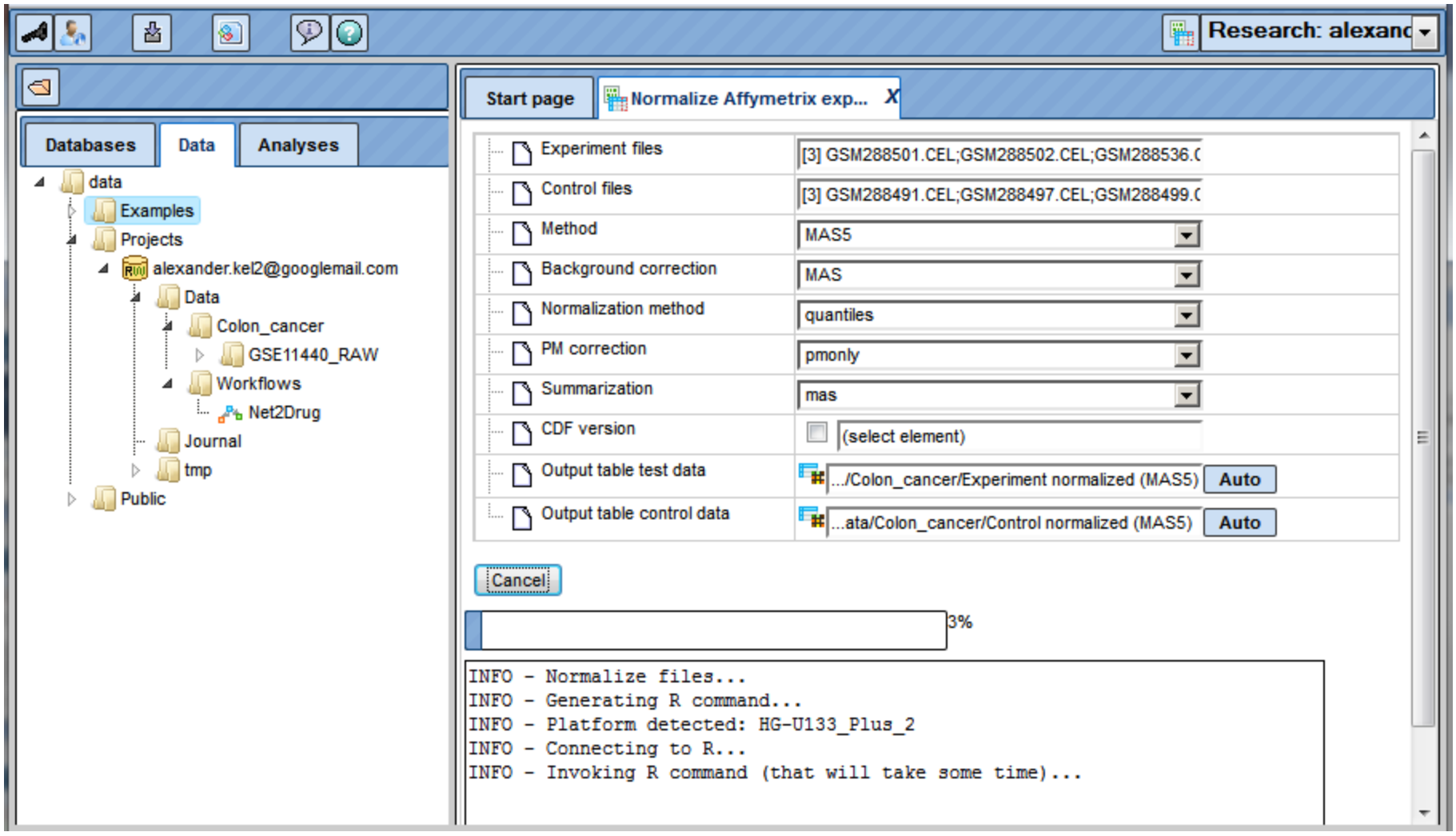
Query DataSets for GSE11440

Status	Public on Sep 08, 2008
Title	Role of Caveolin 1, E-Cadherin, Enolase 2 and PKCa on resistance to methotrexate in human HT29 colon cancer cells
Organism	Homo sapiens
Experiment type	Expression profiling by array
Summary	A summary of the work associated to these microarrays is the following:



Methotrexate (MTX) is one of the earliest cytotoxic drugs used in cancer therapy, and despite the isolation of multiple other folate antagonists, methotrexate maintains its significant role as a treatment for different types of cancer and other disorders. The usefulness of treatment with methotrexate is limited by the development of drug resistance, which may be acquired through different ways. To get insights into the mechanisms associated with drug resistance and sensitization we have performed a functional analysis of genes deregulated in methotrexate resistant cells, either due to its co-amplification with the DHFR gene or as a result of a transcriptome screening using microarrays. Genes adjacent to dhfr locus and included in the 5q14 amplicon were overexpressed in HT29 MTX-resistant cells. Treatment with siRNAs against those genes caused a slight reduction in cell viability in both HT29 sensitive and resistant cells. On the other hand, microarray analysis of HT29 and HT29 MTX resistant cells unveiled overexpression of caveolin 1, enolase 2 and PKCa genes in treated cells without concomitant copy number gain. siRNAs against these three genes effectively reduced cell viability and caused a decreased MTX resistance capacity. Moreover, overexpression of E-cadherin, which was found underexpressed in MTX-resistant cells, also sensitized the cells toward the chemotherapeutic agent. We provide functional evidences indicating that caveolin 1 and E-cadherin may play a critical role in cell survival and may constitute potential targets for coadjuvant therapy.
Keywords: DHFR, Methotrexate, drug resistance

Overall design	Two cell lines are compared in the study, which are HT29 colon cancer cells sensitive to methotrexate and HT29 cells resistant to 10e-5M MTX. Six


Research: alexanc
Databases
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Examples
Projects
alexander.kel2@googlemail.com
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[3] GSM288491.CEL;GSM288497.CEL;GSM288499.C
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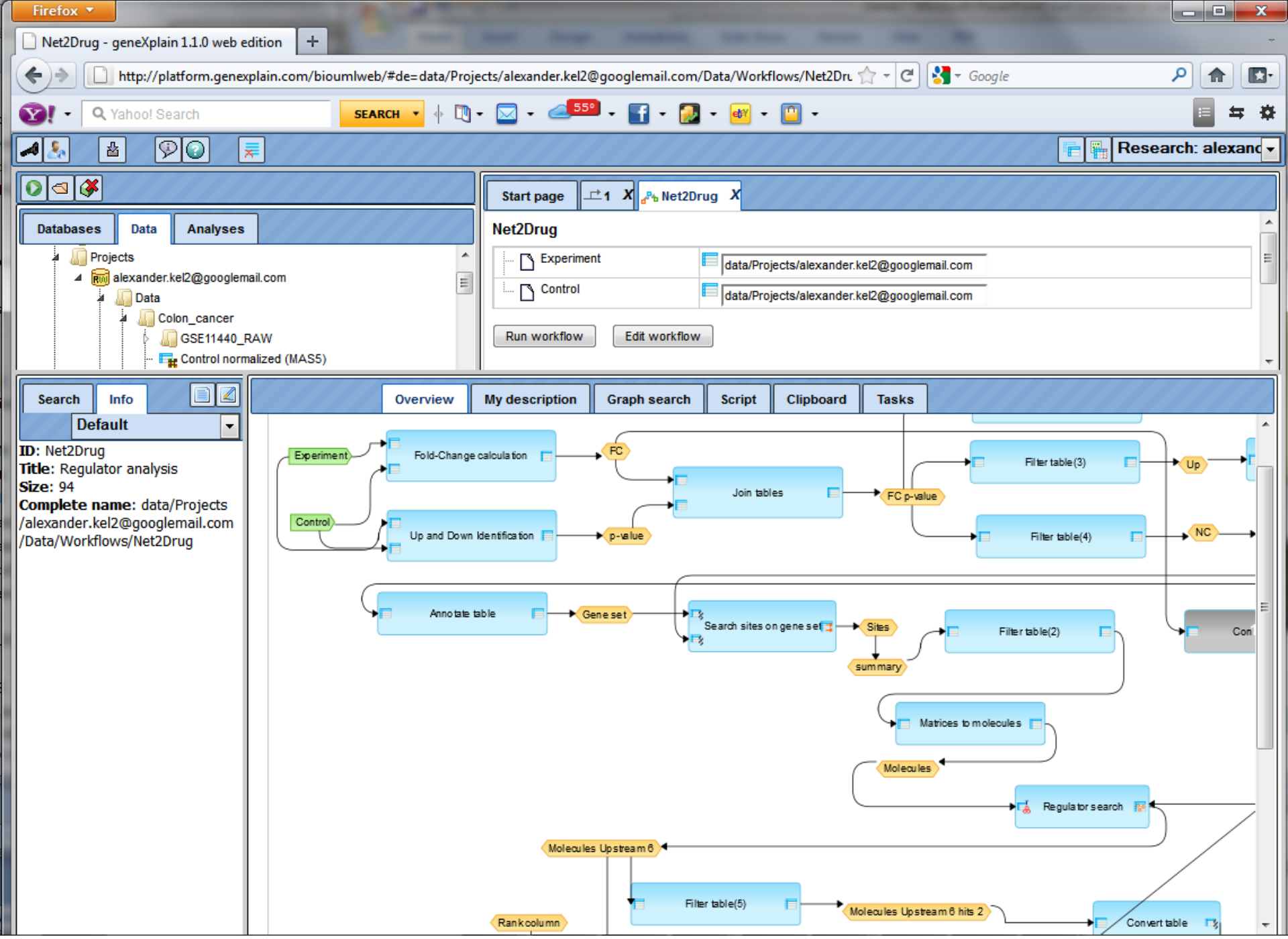
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Run workflow
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Size: 94
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/Data/Workflows/Net2Drug
Overview
My description
Graph search
Script
Clipboard
Tasks
Experiment
Control
Fold-Change calculation
Up and Down Identification
FC
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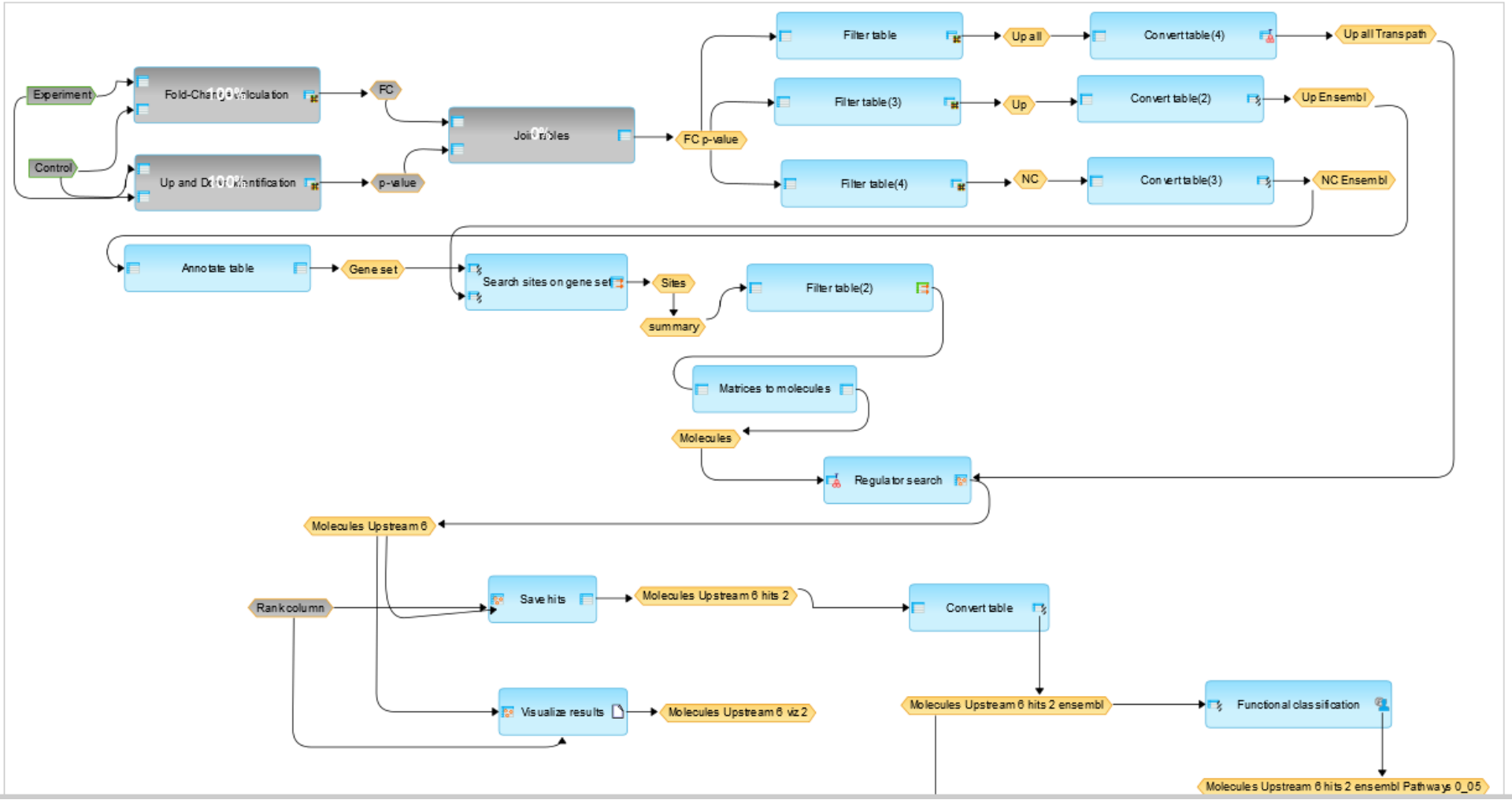
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Molecules Upstream 6 hits 2 ensembl
Functional classification
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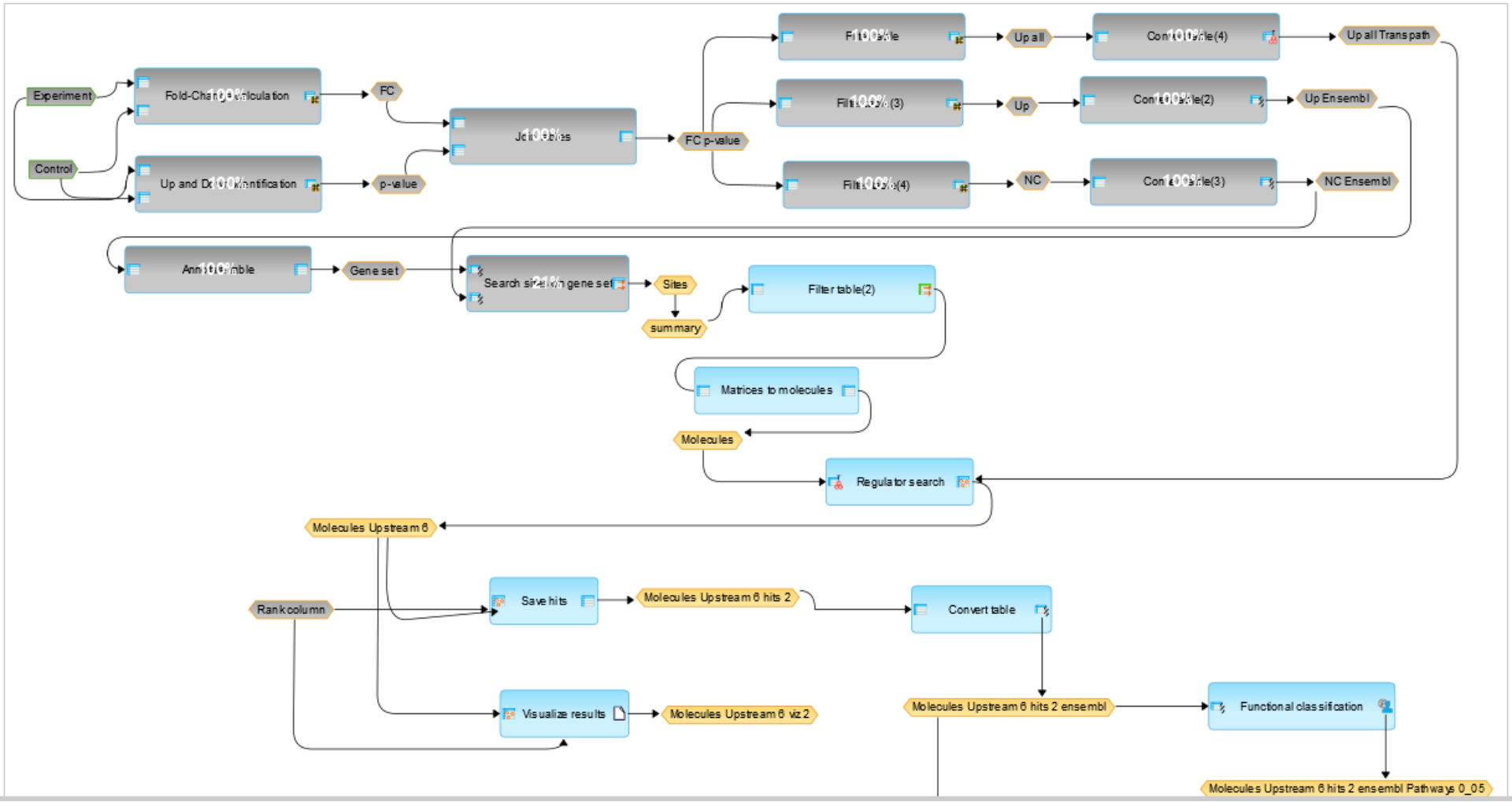
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Save hits
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Visualize results
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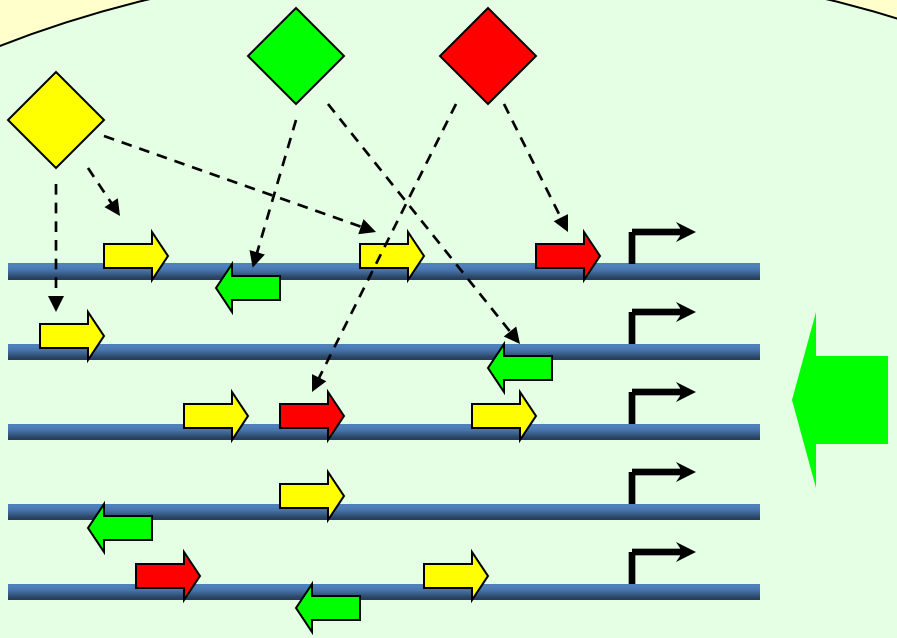
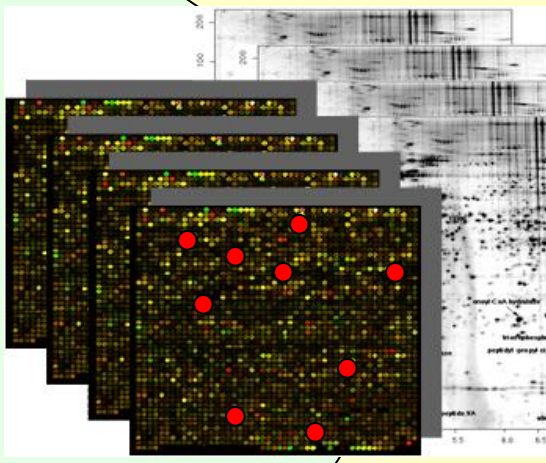
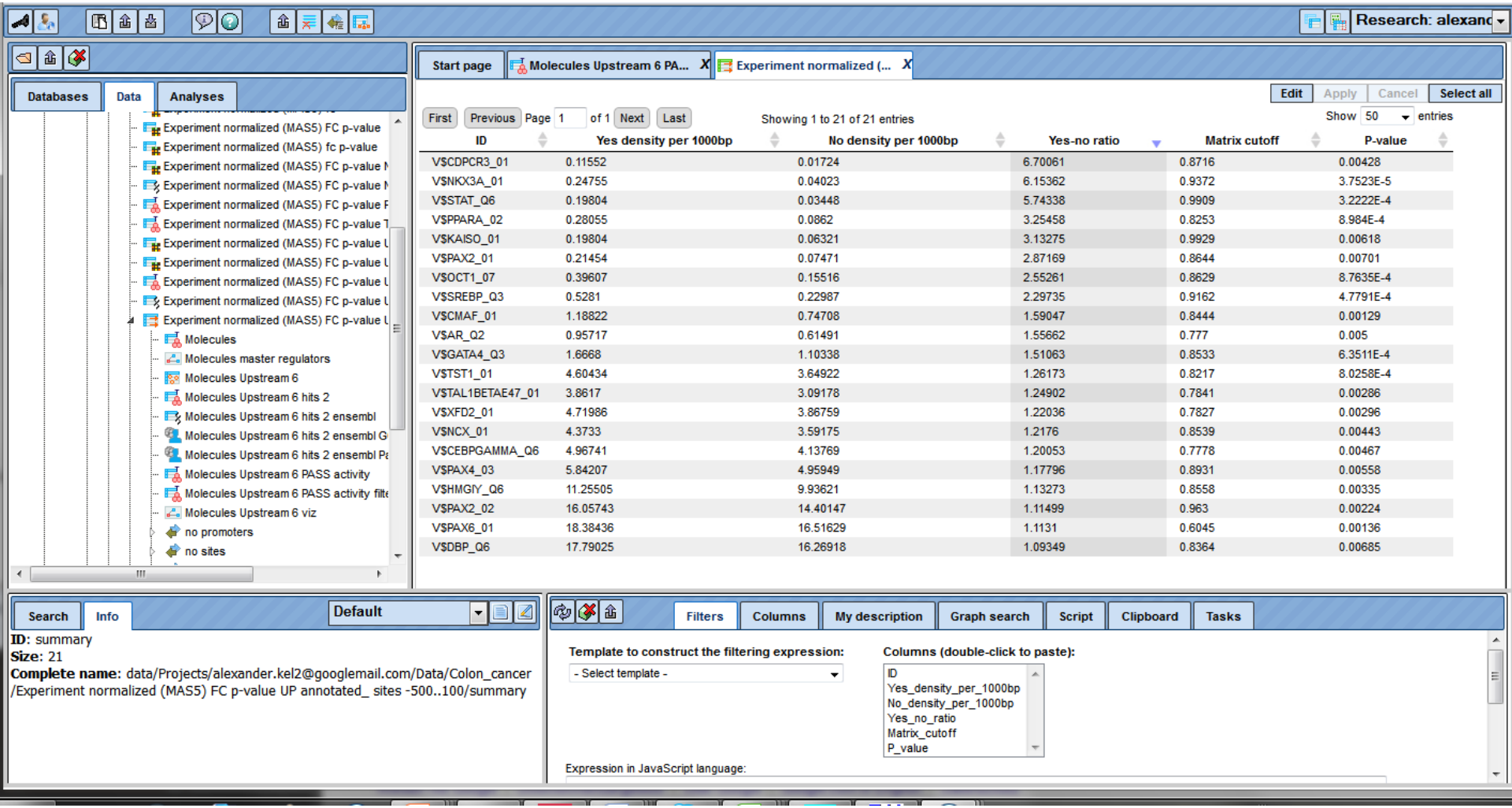
Functional classification
Molecules Upstream 6 hits 2 ensembl Pathways 0_05

On the first step the workflow identified the differentially expressed genes in the resistant versus sensitive patients and identified transcription factors involved.

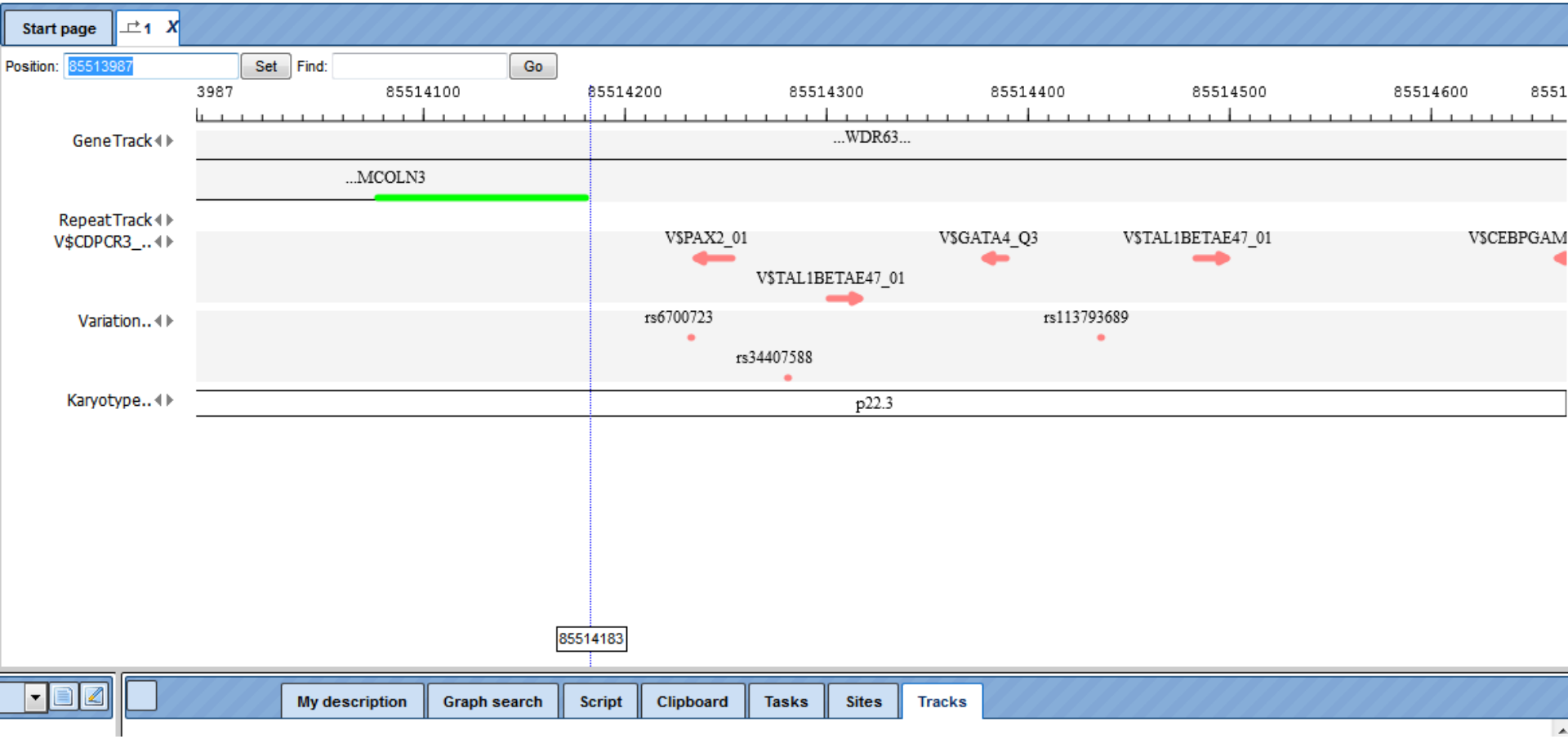


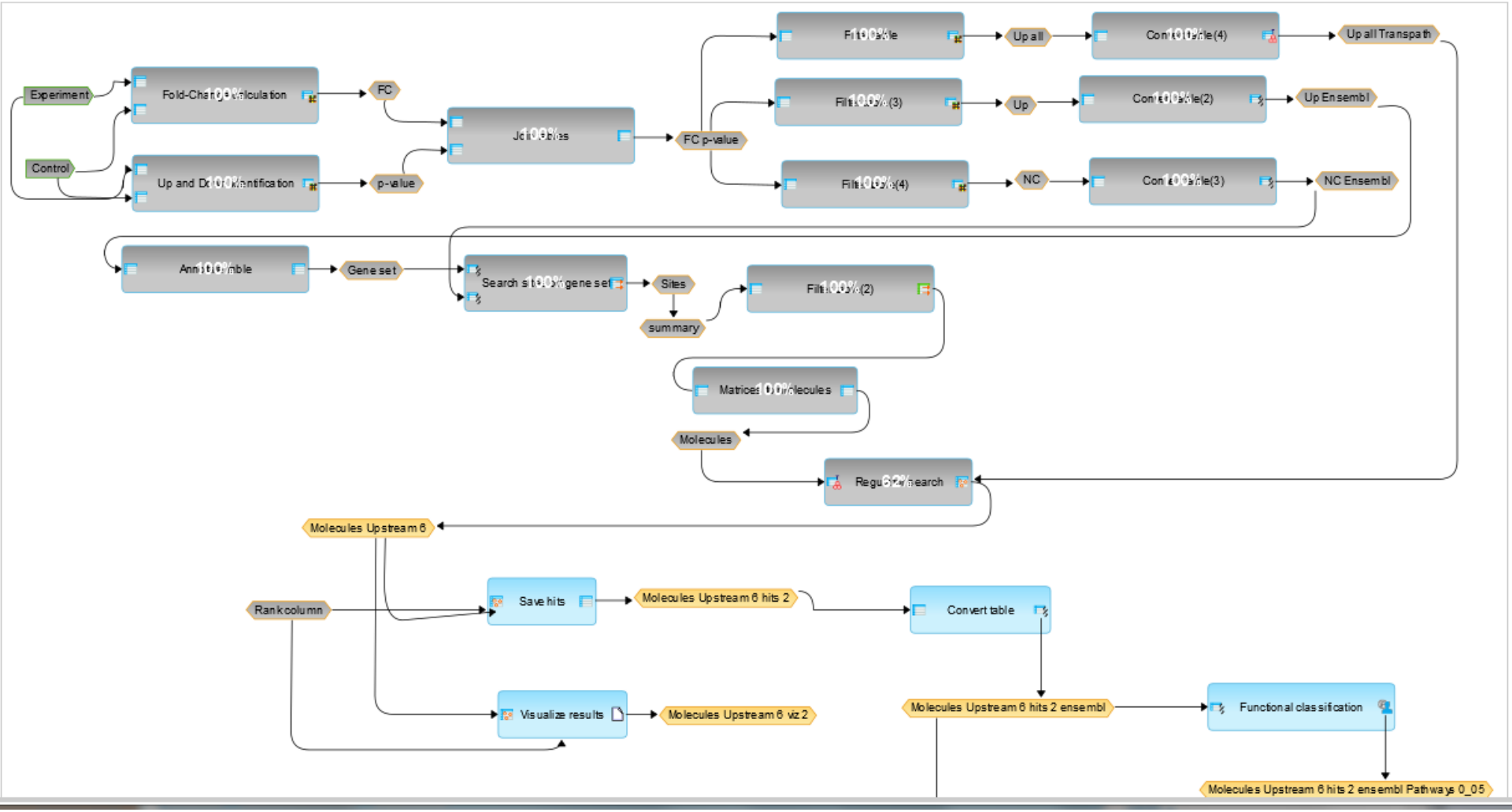


Here is the list of transcription factors predicted to be involved



Here are the predicted sites for these transcription factors.

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Filter file(3)
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Annotate table
Gene set
Search sub gene set
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summary
Filter file(2)
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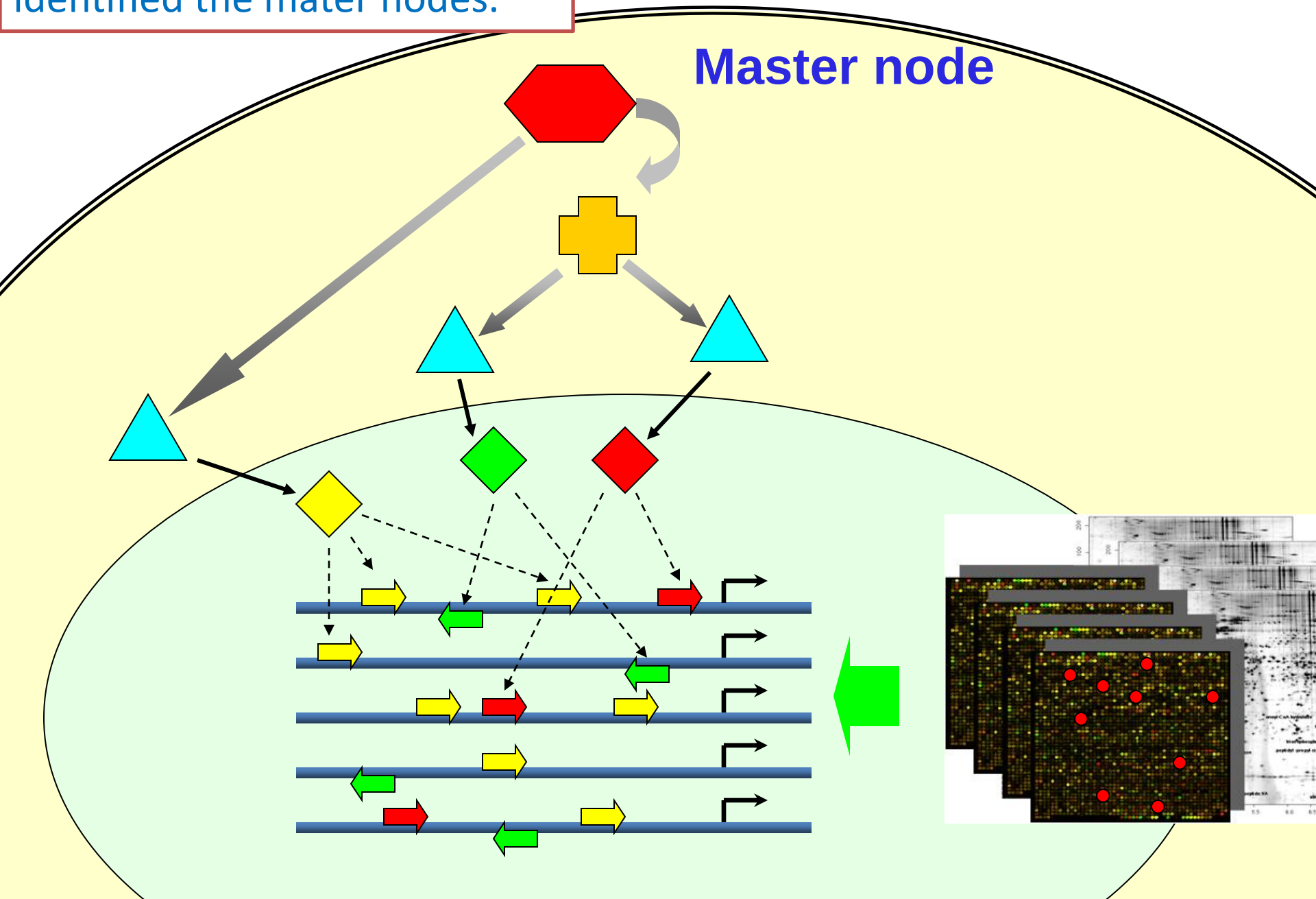
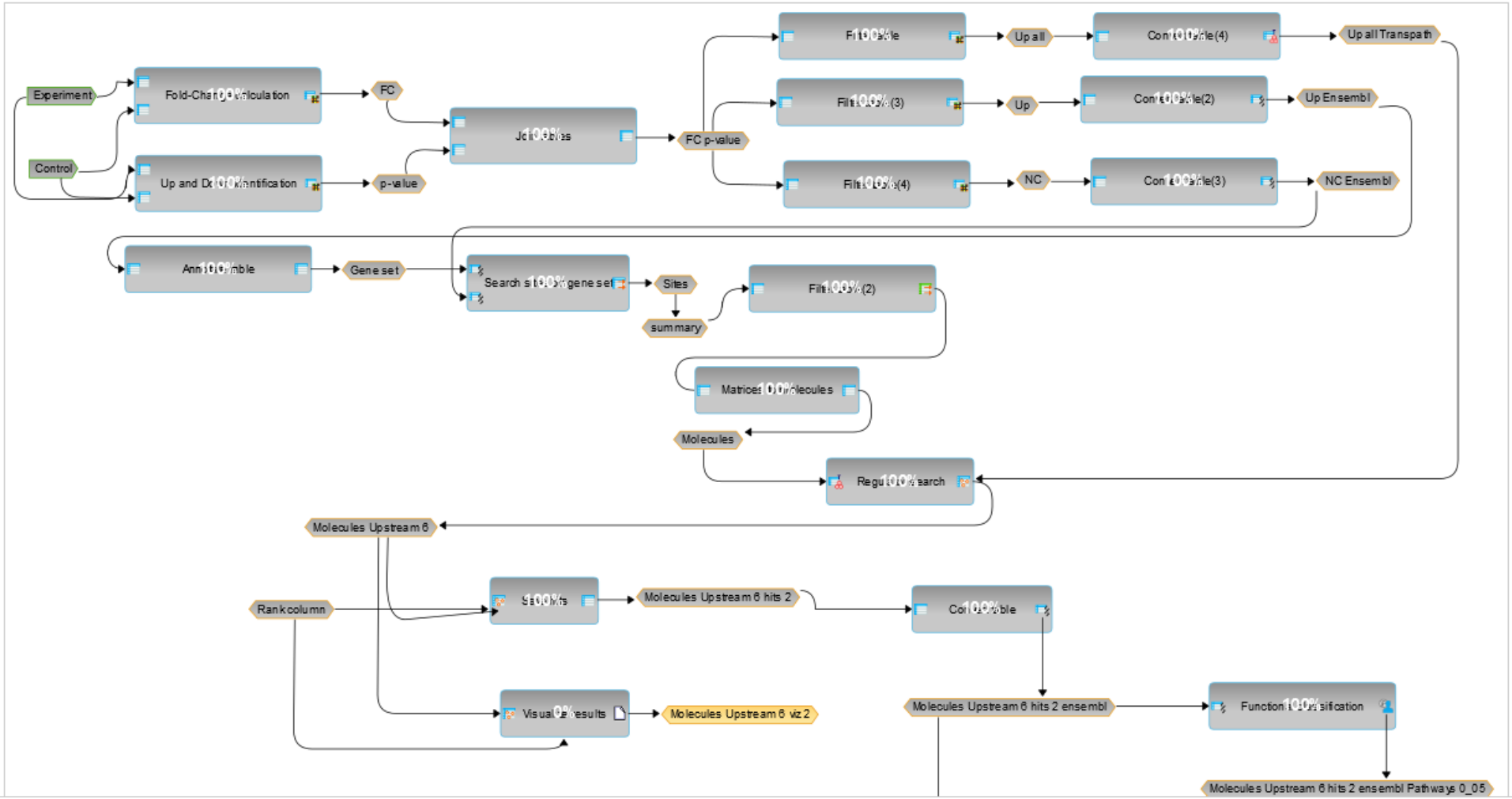
Molecules
Regulator search
Molecules Upstream 6
Rank column
Save hits
Molecules Upstream 6 hits 2
Convert table
Visualize results
Molecules Upstream 6 viz 2
Molecules Upstream 6 hits 2 ensembl
Functional classification
Molecules Upstream 6 hits 2 ensembl Pathways 0_05

On the next step the workflow identified the mater nodes.

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Convert file(2)
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Annotate table
Gene set
Search sites in gene set
Sites
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Filter file(2)
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Functional classification
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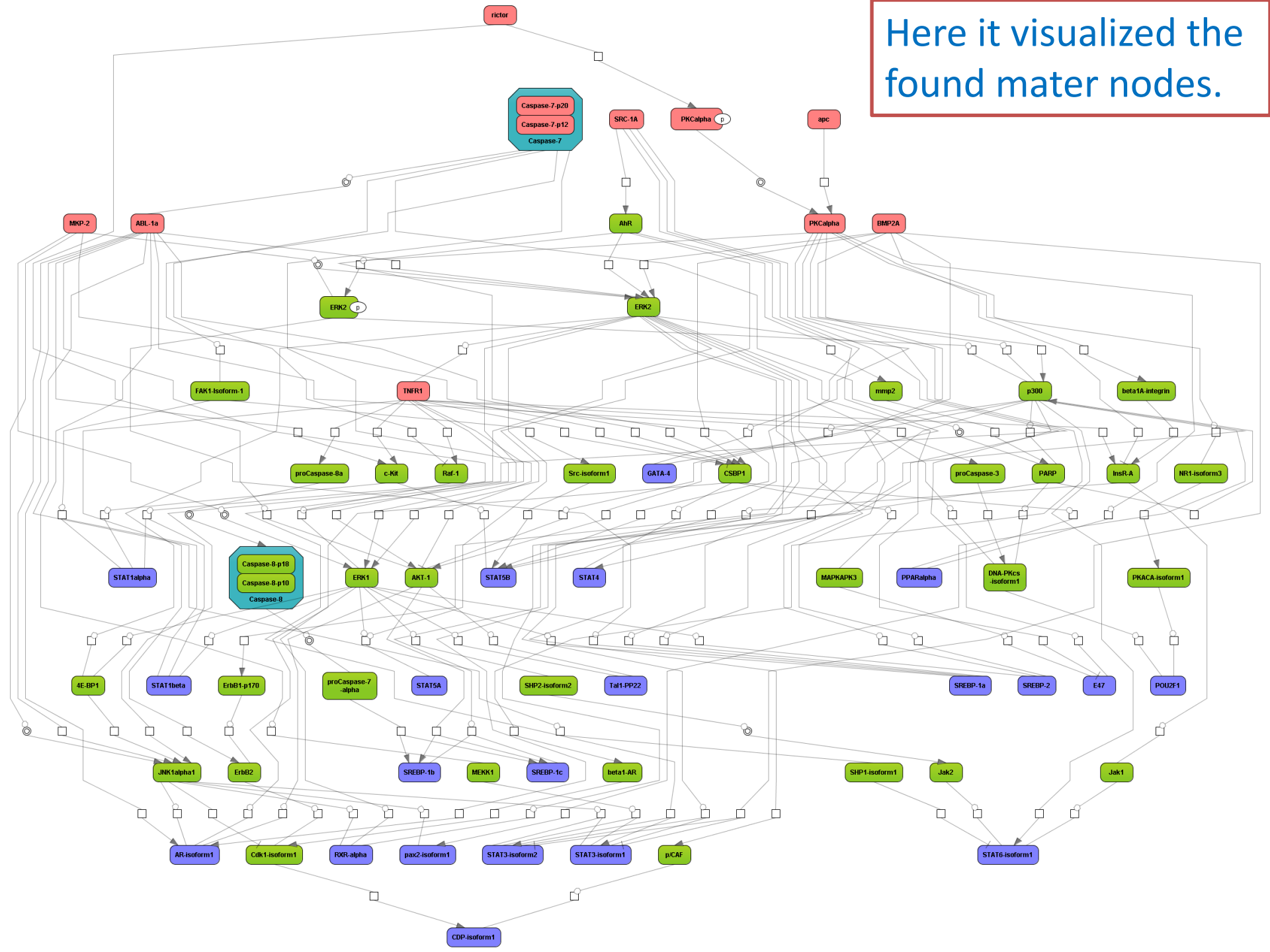
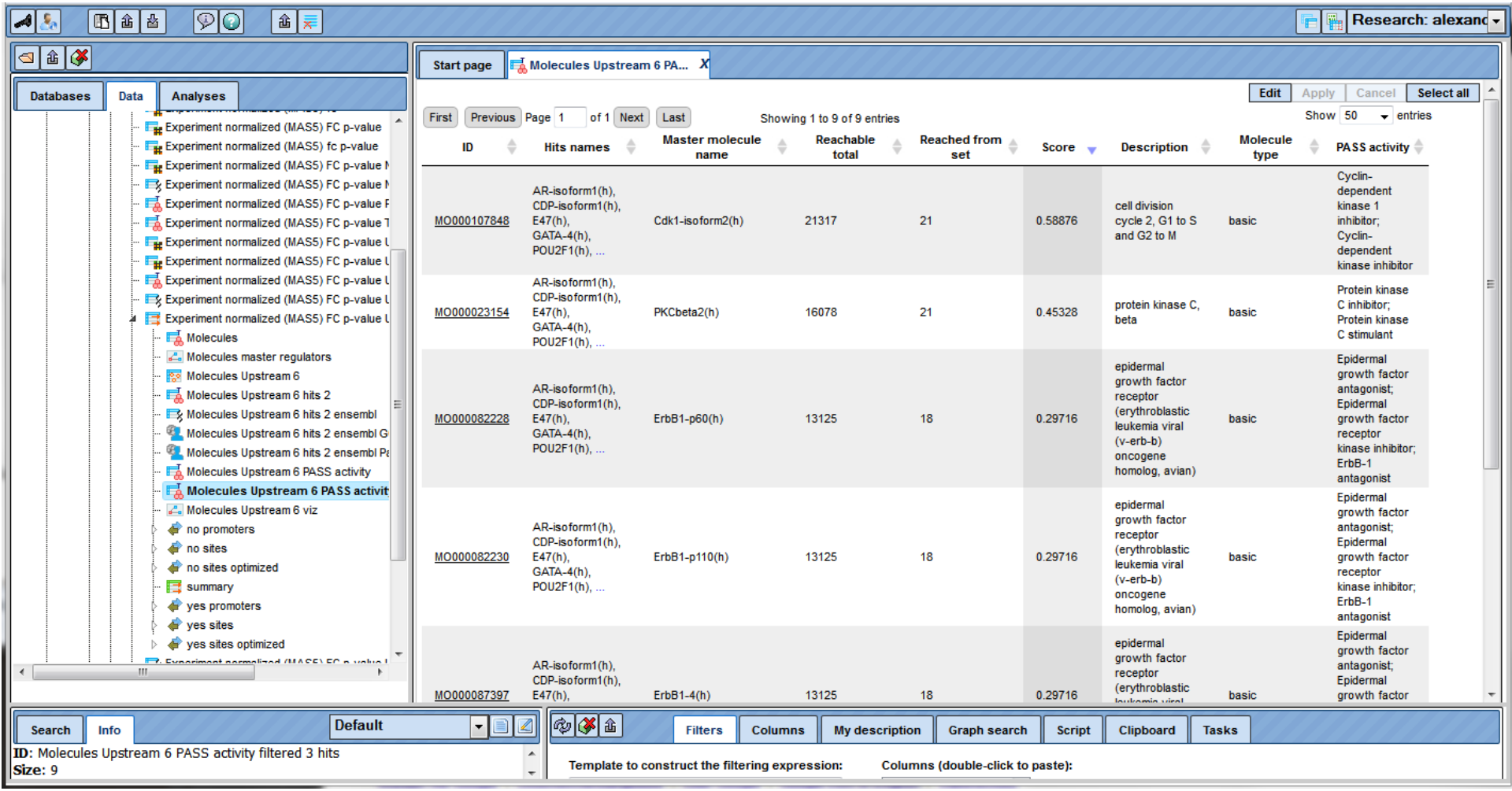
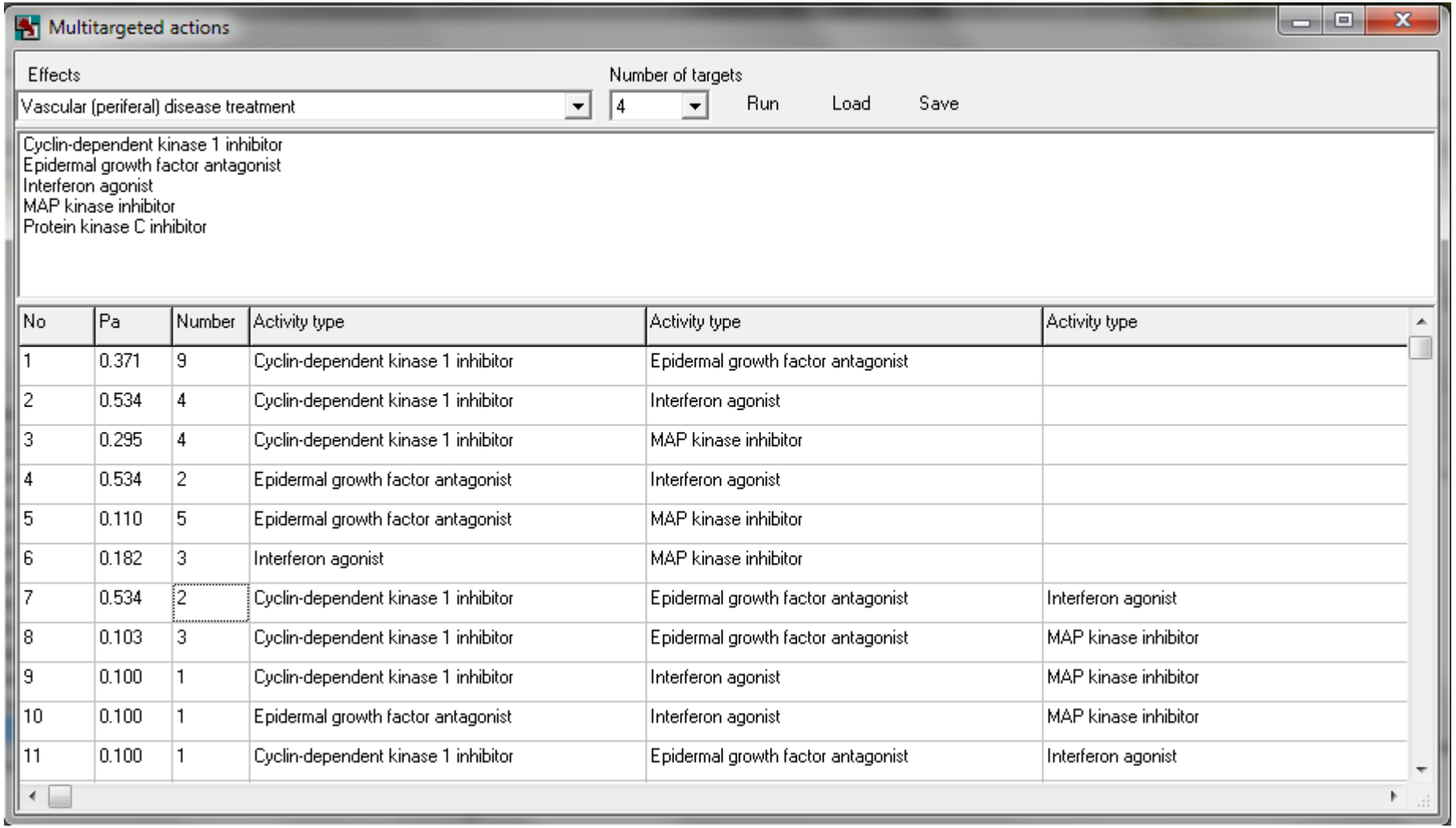

Here it visualized the found mater nodes.
rictor
Caspase-7-p20
Caspase-7-p12
Caspase-7
SRC-1A
PKCalpha
p
apc
MKP-2
ABL-1a
AhR
PKCalpha
BMP2A
ERK2
p
ERK2
FAK1-isoform-1
TNFR1
mmp2
p300
beta1A-integrin
proCaspase-8a
c-Kit
Raf-1
Src-isoform1
GATA-4
CSBP1
proCaspase-3
PARP
InsR-A
NR1-isoform3
STAT1alpha
Caspase-8-p18
Caspase-8-p10
Caspase-8
ERK1
AKT-1
STAT5B
STAT4
MAPKAPK3
PPARalpha
DNA-PKcs -isoform1
PKACA-isoform1
4E-BP1
STAT1beta
ErbB1-p170
proCaspase-7 -alpha
STAT5A
SHP2-isoform2
Tal1-PP22
SREBP-1a
SREBP-2
E47
POU2F1
JNK1alpha1
ErbB2
SREBP-1b
MEKK1
SREBP-1c
beta1-AR
SHP1-isoform1
Jak2
Jak1
AR-isoform1
Cdk1-isoform1
RXR-alpha
pax2-isoform1
STAT3-isoform2
STAT3-isoform1
p/CAF
STAT6-isoform1
CDP-isoform1

Some of the master nodes have information in PASS about inhibitors or agonists



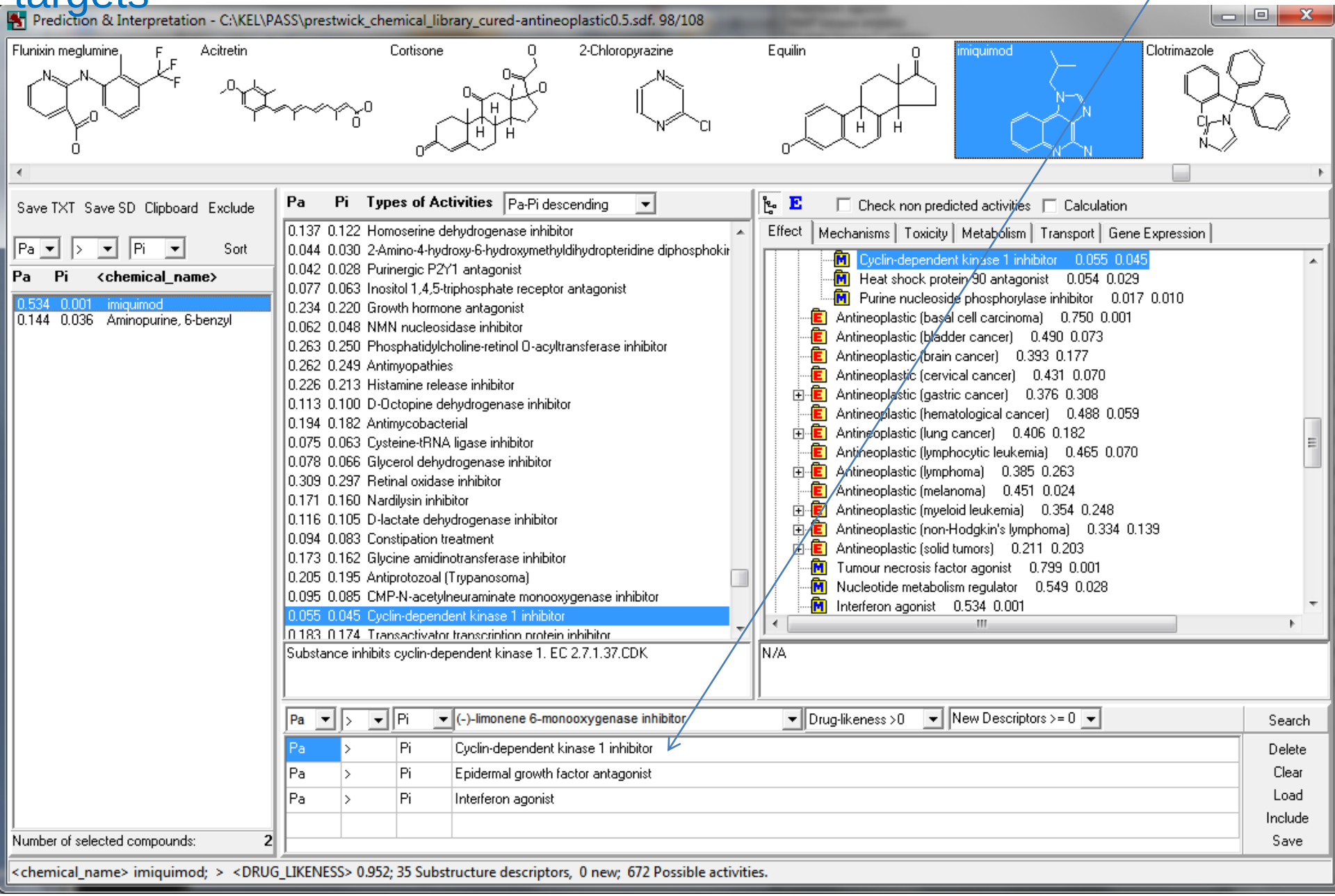
PASS / PharmaExpert multi-target search

Searching in a library of known drugs for compounds
with potential of multi-target activity against selected targets



No	Pa	Number	Activity type	Activity type	Activity type
1	0.371	9	Cyclin-dependent kinase 1 inhibitor	Epidermal growth factor antagonist	
2	0.534	4	Cyclin-dependent kinase 1 inhibitor	Interferon agonist	
3	0.295	4	Cyclin-dependent kinase 1 inhibitor	MAP kinase inhibitor	
4	0.534	2	Epidermal growth factor antagonist	Interferon agonist	
5	0.110	5	Epidermal growth factor antagonist	MAP kinase inhibitor	
6	0.182	3	Interferon agonist	MAP kinase inhibitor	
7	0.534	2	Cyclin-dependent kinase 1 inhibitor	Epidermal growth factor antagonist	Interferon agonist
8	0.103	3	Cyclin-dependent kinase 1 inhibitor	Epidermal growth factor antagonist	MAP kinase inhibitor
9	0.100	1	Cyclin-dependent kinase 1 inhibitor	Interferon agonist	MAP kinase inhibitor
10	0.100	1	Epidermal growth factor antagonist	Interferon agonist	MAP kinase inhibitor
11	0.100	1	Cyclin-dependent kinase 1 inhibitor	Epidermal growth factor antagonist	Interferon agonist

Found a drug– imiquimod, with potential of activity for three targets



We predict that Imiquimod can be used as a second drug to overcome the resitance to methotrexate

Imiquimod

From Wikipedia, the free encyclopedia

Imiquimod (INN) is a prescription medication that acts as an immune response modifier. It is marketed by MEDA AB, Graceway Pharmaceuticals and iNova Pharmaceuticals under the trade names **Aldara** and **Zyclara**, and by Mochida as **Beselna**.

Contents [hide]

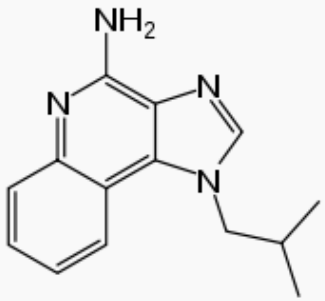
History [edit]

The original FDA approval was on February 27, 1997, FDA Application No. (NDA) 020723, by 3M. Imiquimod is approved to treat actinic keratosis, superficial basal cell carcinoma, and external genital warts. Adverse side effects have been reported, in some cases serious and systemic, resulting in the revision of warning labels.

Uses [edit]

Imiquimod is a patient-applied cream used to treat certain diseases of the skin, including skin cancers (basal cell carcinoma, Bowen's disease,[1] superficial squamous cell carcinoma, some superficial malignant melanomas, and actinic keratosis) as well as genital warts (*condylomata acuminata*). It has also been tested for treatment of molluscum contagiosum, vulvar intraepithelial neoplasia, common warts that have proven difficult to treat,[2] and vaginal intraepithelial neoplasia.[3] Outstanding cosmetic result has resulted from the treatment of both

Imiquimod



Systematic (IUPAC) name	
3-(2-methylpropyl)-3,5,8-triazatricyclo[7.4.0.0^{2,6}]trideca-1(9),2(6),4,7,10,12-hexaen-7-amine	
Clinical data	
Licence data	EMA:Link, US FDA:link
Pregnancy cat.	B1(AU) C(US)
Legal status	POM (UK) ℞-only (US)
Routes	Topical
Pharmacokinetic data	
Half-life	30 hours (topical dose), 2 hours (subcutaneous dose)
Identifiers	
CAS number	99011-02-6
ATC code	D06BB10
PubChem	CID 57469
DrugBank	APRD01030

Net 2 Drug

geneXplain

BIOBASE
BIOLOGICAL DATABASES

0100100010011101
Institute of Systems Biology

PharmaExpert
RESEARCH LABORATORY

Fraunhofer
ITEM

Karolinska Institutet

UNIVERSITY OF HELSINKI

UNIVERSITÄTSMEDIZIN GÖTTINGEN : UMG

Consiglio Nazionale delle Ricerche