# HSPD1 and TXN are promising druggable targets for treating Neoplasm Metastasis and Osteosarcoma that control activity of RXRA, VDR and SMAD2 transcription factors on promoters of differentially expressed genes

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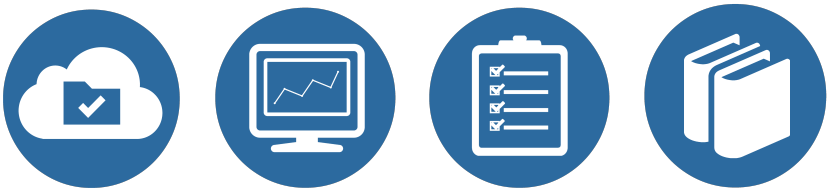
Data received on 07/09/2019 ; Run on 28/06/2024 ; Report generated on 28/06/2024

Genome Enhancer release 3.4 (TRANSFAC®, TRANSPATH® and HumanPSD™ release 2024.1)



## Abstract

In the present study we applied the software package "Genome Enhancer" to a multiomics data set that contains *transcriptomics and proteomics* data. The study is done in the context of *Neoplasm Metastasis and Osteosarcoma*. The goal of this pipeline is to identify potential drug targets in the molecular network that governs the studied pathological process. In the first step of analysis pipeline discovers transcription factors (TFs) that regulate genes activities in the pathological state. The activities of these TFs are controlled by so-called master regulators, which are identified in the second step of analysis. After a subsequent druggability checkup, the most promising master regulators are chosen as potential drug targets for the analyzed pathology. At the end the pipeline comes up with (a) a list of known drugs and (b) investigational active chemical compounds with the potential to interact with selected drug targets.

From the data set analyzed in this study, we found the following TFs to be potentially involved in the regulation of the differentially expressed genes: RXRA, VDR, TCF3, SMAD2, SMAD3 and SMAD1. The subsequent network analysis suggested

- NCL
- proCaspase-3(h):Hsp10(h):Hsp60
- HSPA4
- Trx1

as the most promising molecular targets for further research, drug development and drug repurposing initiatives on the basis of identified molecular mechanism of the studied pathology. Having checked the actual druggability potential of the full list of identified targets, both, via information available in medical literature and via cheminformatics analysis of drug compounds, we have identified the following drugs as the most promising treatment candidates for the studied pathology: Temsirolimus, perillyl alcohol, (CHLOROACETYL)CARBAMIC ACID (3R,4S,5S,5R)-5-METHOXY-4-[(2R,3R)-2-METHYL-3-(3-METHYL-2-BUTENYL)OXIRANYL]-1-OXASPIRO[2.5]OCT-6-YL ESTER and Myo-Inositol.

## 1. Introduction

Recording "-omics" data to measure gene activities, protein expression or metabolic events is becoming a standard approach to characterize the pathological state of an affected organism or tissue. Increasingly, several of these methods are applied in a combined approach leading to large "multiomics" datasets. Still the challenge remains how to reveal the underlying molecular mechanisms that render a given pathological state different from the norm. The disease-causing mechanism can be described by a re-wiring of the cellular regulatory network, for instance as a result of a genetic or epigenetic alterations influencing the activity of relevant genes. Reconstruction of the disease-specific regulatory networks can help identify potential master regulators of the respective pathological process. Knowledge about these master regulators can point to ways how to block a pathological regulatory cascade. Suppression of certain molecular targets as components of these cascades may stop the pathological process and cure the disease.

Conventional approaches of statistical "-omics" data analysis provide only very limited information about the causes of the observed phenomena and therefore contribute little to the understanding of the pathological molecular mechanism. In contrast, the "upstream analysis" method [1-4] applied here has been deviced to provide a casual interpretation of the data obtained for a pathology state. This approach comprises two major steps: (1) analysing promoters and enhancers of differentially expressed genes for the transcription factors (TFs) involved in their regulation and, thus, important for the process under study; (2) re-constructing the signaling pathways that activate these TFs and identifying master regulators at the top of such pathways. For the first step, the database TRANSFAC® [6] is employed together with the TF binding site identification algorithms Match [7] and CMA [8]. The second step involves the signal transduction database TRANSPATH® [9] and special graph search algorithms [10] implemented in the software "Genome Enhancer".

The "upstream analysis" approach has now been extended by a third step that reveals known drugs suitable to inhibit (or activate) the identified molecular targets in the context of the disease under study. This step is performed by using information from HumanPSD™ database [5]. In addition, some known drugs and investigational active chemical compounds are subsequently predicted as potential ligands for the revealed molecular targets. They are predicted using a pre-computed database of spectra of biological activities of chemical compounds of a library of 2245 known drugs and investigational chemical compounds from HumanPSD™ database. The spectra of biological activities for these compounds are computed using the program PASS on the basis of a (Q)SAR approach [11-13]. These predictions can be used for the research purposes - for further drug development and drug repurposing initiatives.

# 2. Data

For this study the following experimental data was used:

*Table 1. Experimental datasets used in the study*

| File name | Data type |
|---|---|
| Proteomics | Proteomics |
| RNAseq | Transcriptomics |

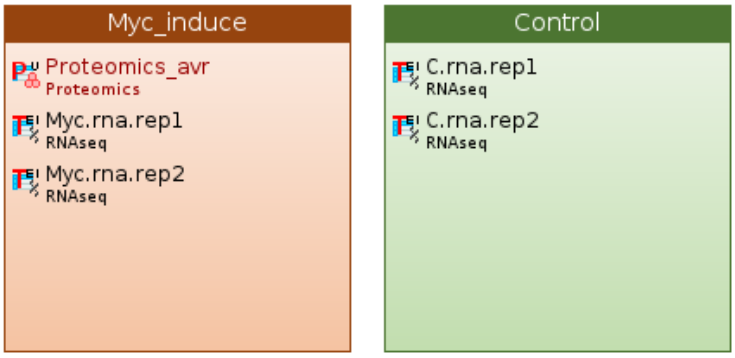



*Figure 1. Annotation diagram of experimental data used in this study. With the colored boxes we show those sub-categories of the data that are compared in our analysis.*

# 3. Results

We have compared the following conditions: Myc_induce *versus* Control.

## *3.1. Identification of target genes*

In the first step of the analysis ***target genes*** were identified from the uploaded experimental data. We applied the Limma tool (R/Bioconductor package integrated into our pipeline) and compared gene expression in the following sets: "Myc_induce" with "Control". Limma calculated the LogFC (the logarithm to the base 2 of the fold change between different conditions), the p-value and the adjusted p-value (corrected for multiple testing) of the observed fold change. As a result, we detected 408 upregulated genes (LogFC>0.1) out of which 97 genes were found as significantly upregulated (p-value<0.1) and 389 downregulated genes (LogFC<-0.1) out of which 99 genes were significantly downregulated (p-value<0.1). See tables below for the top significantly up- and downregulated genes. Below we call **target genes** the full list of up- and downregulated genes revealed in our analysis (see tables in Supplementary section).

*Table 2. Top ten significant **up-regulated** genes in Myc_induce vs. Control.*
**See full table →**

| ID | Gene symbol | Gene description | logFC | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| ENSG00000136997 | MYC | MYC proto-oncogene, bHLH transcription factor | 5.96 | 9.49E-6 | 9.85E-3 |
| ENSG00000120738 | EGR1 | early growth response 1 | 3.51 | 6.94E-4 | 0.17 |
| ENSG00000198576 | ARC | activity regulated cytoskeleton associated protein | 2.14 | 1.44E-3 | 0.17 |
| ENSG00000114767 | RRP9 | ribosomal RNA processing 9, U3 small nucleolar RNA binding protein | 1.29 | 8.38E-3 | 0.28 |
| ENSG00000176170 | SPHK1 | sphingosine kinase 1 | 1.2 | 9.2E-3 | 0.28 |
| ENSG00000052749 | RRP12 | ribosomal RNA processing 12 homolog | 1.19 | 5.07E-2 | 0.44 |
| ENSG00000178053 | MLF1 | myeloid leukemia factor 1 | 1.12 | 6.73E-2 | 0.44 |
| ENSG00000198805 | PNP | purine nucleoside phosphorylase | 1.11 | 2.69E-3 | 0.2 |
| ENSG00000168003 | SLC3A2 | solute carrier family 3 member 2 | 1.11 | 2.45E-3 | 0.2 |
| ENSG00000132768 | DPH2 | diphthamide biosynthesis 2 | 1.01 | 3.37E-3 | 0.2 |

*Table 3. Top ten significant **down-regulated** genes in Myc_induce vs. Control.*
**See full table →**

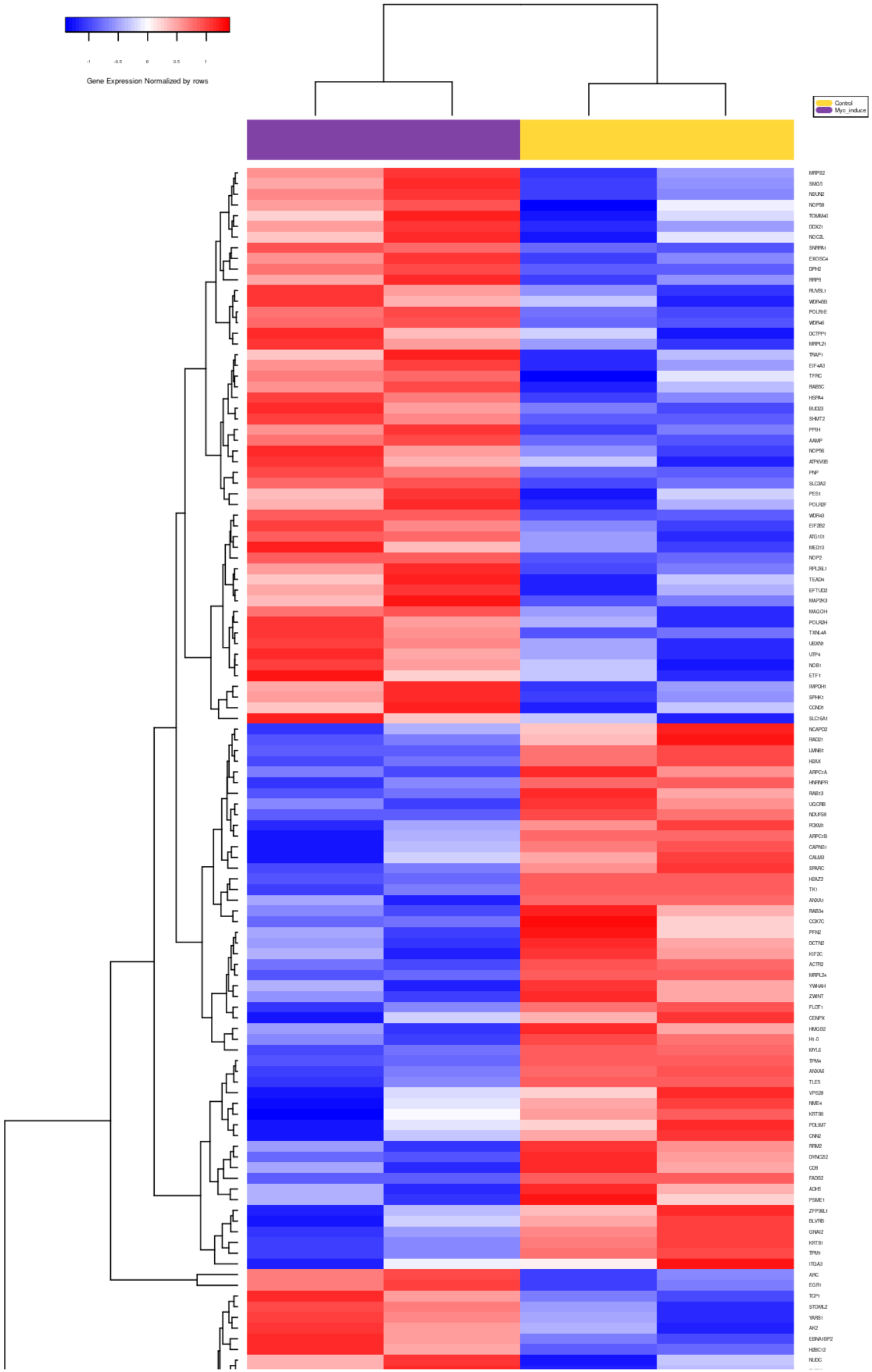
| ID | Gene symbol | Gene description | logFC | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| ENSG00000205542 | TMSB4X | thymosin beta 4 X-linked | -2.58 | 2.38E-4 | 0.12 |
| ENSG00000140416 | TPM1 | tropomyosin 1 | -1.94 | 1.16E-3 | 0.17 |
| ENSG00000197747 | S100A10 | S100 calcium binding protein A10 | -1.75 | 9.6E-4 | 0.17 |
| ENSG00000205426 | KRT81 | keratin 81 | -1.67 | 2.21E-3 | 0.2 |
| ENSG00000092841 | MYL6 | myosin light chain 6 | -1.58 | 7.88E-4 | 0.17 |
| ENSG00000100097 | LGALS1 | galectin 1 | -1.55 | 1.8E-3 | 0.19 |
| ENSG00000120708 | TGFBI | transforming growth factor beta induced | -1.52 | 1.51E-2 | 0.35 |
| ENSG00000114353 | GNAI2 | G protein subunit alpha i2 | -1.47 | 5.43E-3 | 0.25 |
| ENSG00000005884 | ITGA3 | integrin subunit alpha 3 | -1.41 | 9.61E-2 | 0.52 |
| ENSG00000134824 | FADS2 | fatty acid desaturase 2 | -1.4 | 8.3E-4 | 0.17 |

## *3.2. Functional classification of genes*

A functional analysis of differentially expressed genes was done by mapping the significant up-regulated and significant down-regulated genes to several known ontologies, such as Gene Ontology (GO), disease ontology (based on HumanPSD™ database) and the ontology of signal transduction and metabolic pathways from the TRANSPATH® database. Statistical significance was computed using a binomial test.
Figures 3-8 show the most significant categories.

## Heatmap of differentially expressed genes in Myc_induce vs. Control

A heatmap of all differentially expressed genes playing a potential regulatory role in the system (enriched in TRANSPATH® pathways) is presented in Figure 2.


-1
-0.5
0
0.5
1
Gene Expression Normalized by rows
Control
Myc_induce
MRPS2
SMG5
NSUN2
NOP58
TOMM40
DDX21
NOC3L
SNRPA1
EXOSC4
DPH2
RPP9
RUVBL1
WDR49B
POLR1E
WDR46
DCTPP1
MRPL21
TRAP1
EIF4A3
TFRC
RAB5C
HSPA4
BUD23
SHMT2
PPIH
AAMP
NOP56
ATP6V0B
PNP
SLC3A2
PES1
POLR2F
WDR43
EIF2B2
ATG101
MED10
NOP2
RPL26L1
TEAD4
EFTUD2
MAP2K3
MAGOH
POLR2H
TXNL4A
UBXN1
UTP4
NOB1
ETF1
IMPDH1
SPHK1
CCND1
SLC16A1
NCAPD2
RAD21
LMNB1
H2AX
ARPC1A
HNRNPR
RAB13
UQCRB
NDUFS8
FOXM1
ARPC1B
CAPNS1
CALM3
SPARC
H2AZ2
TK1
ANXA1
RAB34
CDK7C
PFN2
DCTN2
KIF2C
ACTR2
MRPL24
YWHAH
ZWINT
FLOT1
CENPX
HMGB2
H1-0
MYL6
TPM4
ANXA6
TLE5
VPS28
NME4
KRT80
PDLIM7
CNN2
RRM2
DYNC2I2
CD9
FADS2
ADH5
PSME1
ZFP36L1
BLVRB
GNAI2
KRT81
TPM1
ITGA3
ARC
EGR1
TCP1
STOML2
YARS1
AK2
EBNA1BP2
H2BC12
NUDC

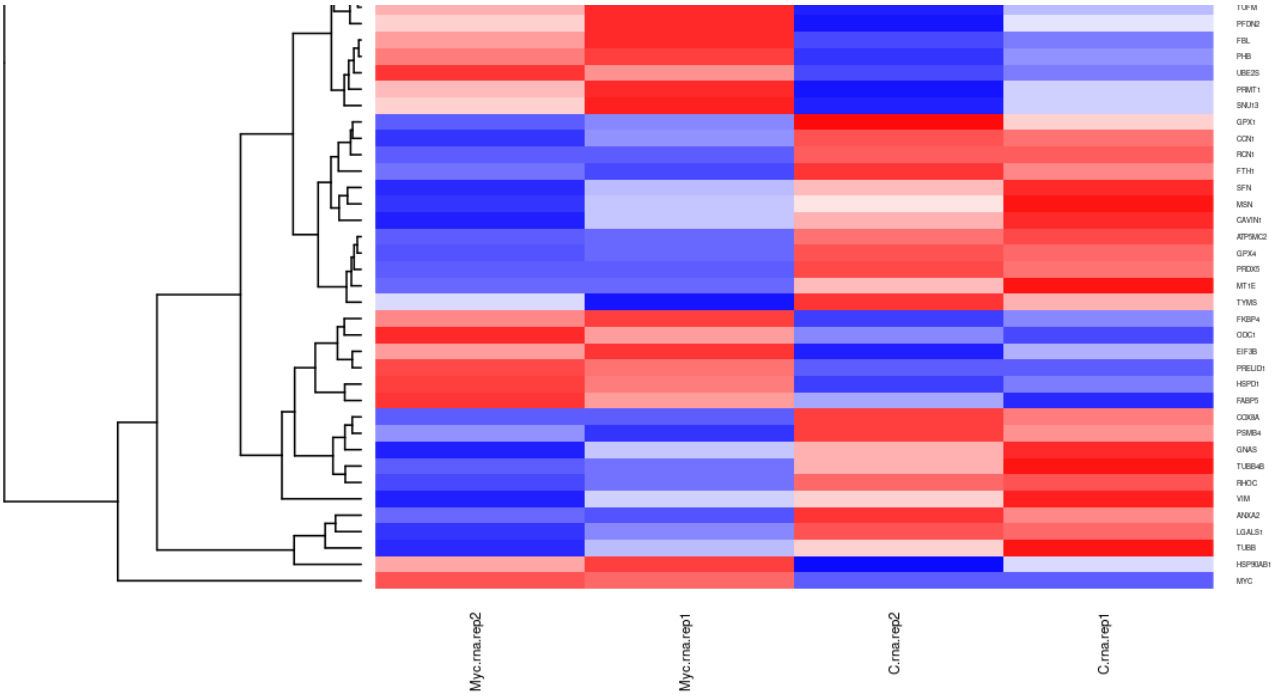



*Figure 2. Heatmap of genes enriched in Transpath categories. The colored bar at the top shows the types of the samples according to the legend in the upper right corner.*

**See full diagram →**

## Up-regulated genes in Myc_induce vs. Control:

97 significant up-regulated genes were taken for the mapping.

## GO (biological process)

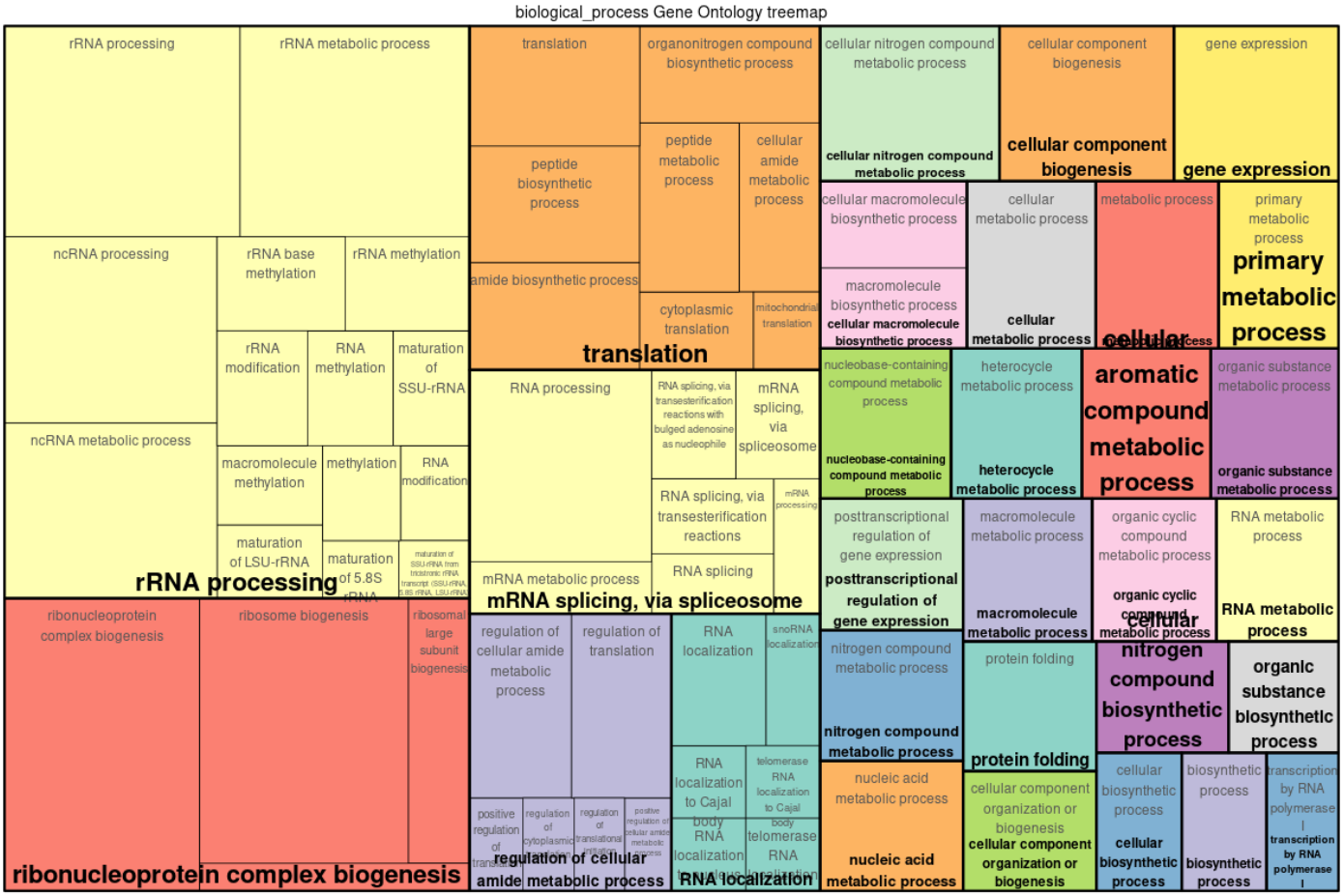



*Figure 3. Enriched GO (biological process) of up-regulated genes in Myc_induce vs. Control.*

**Full classification →**

**TRANSPATH® Pathways (2024.1)**

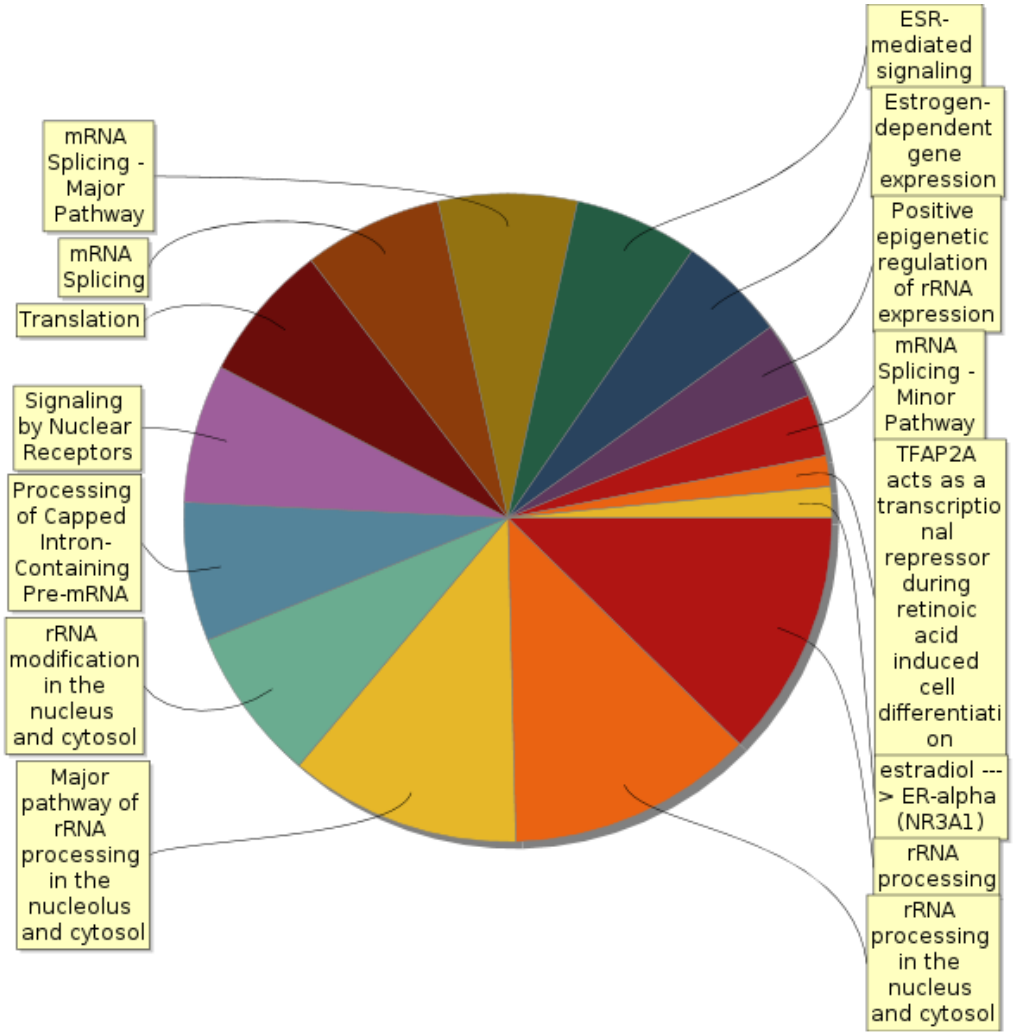



*Figure 4. Enriched TRANSPATH® Pathways (2024.1) of up-regulated genes in Myc_induce vs. Control.*
**Full classification →**

**HumanPSD(TM) disease (2024.1)**

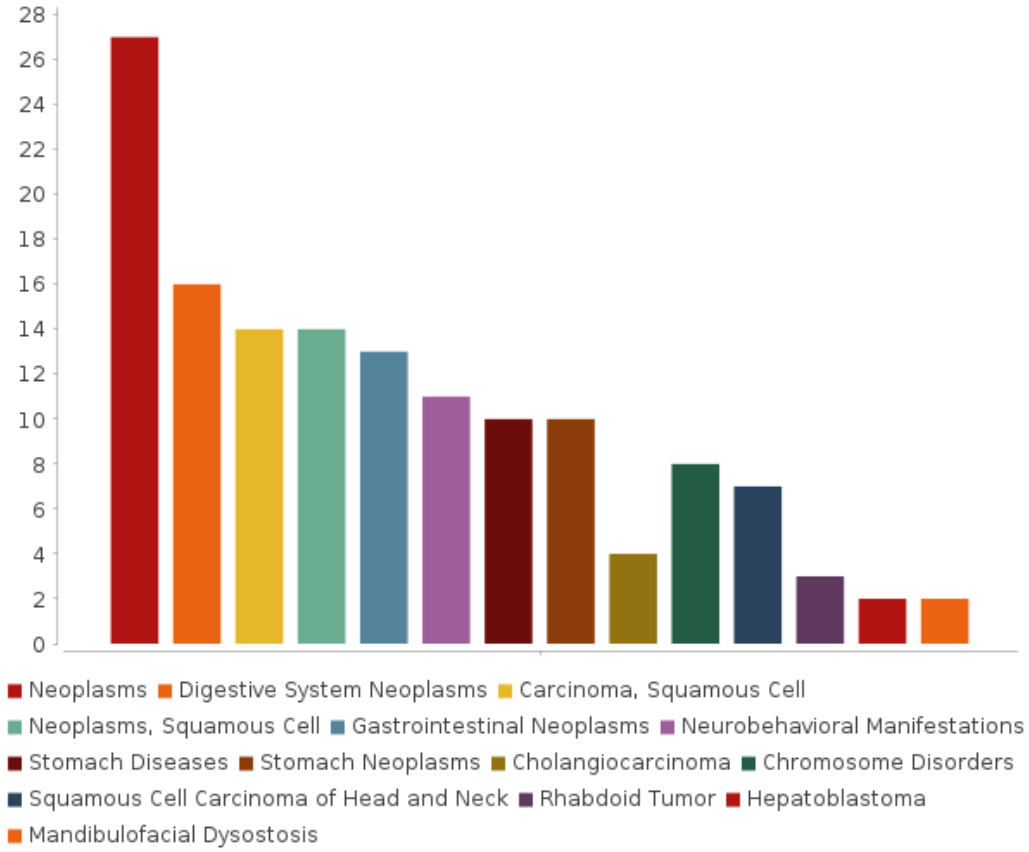



*Figure 5. Enriched HumanPSD(TM) disease (2024.1) of up-regulated genes in Myc_induce vs. Control. The size of the bars correspond to the number of biomarkers of the given disease found among the input set.*

**Full classification →**

## Down-regulated genes in Myc_induce vs. Control:

99 significant down-regulated genes were taken for the mapping.

## GO (biological process)

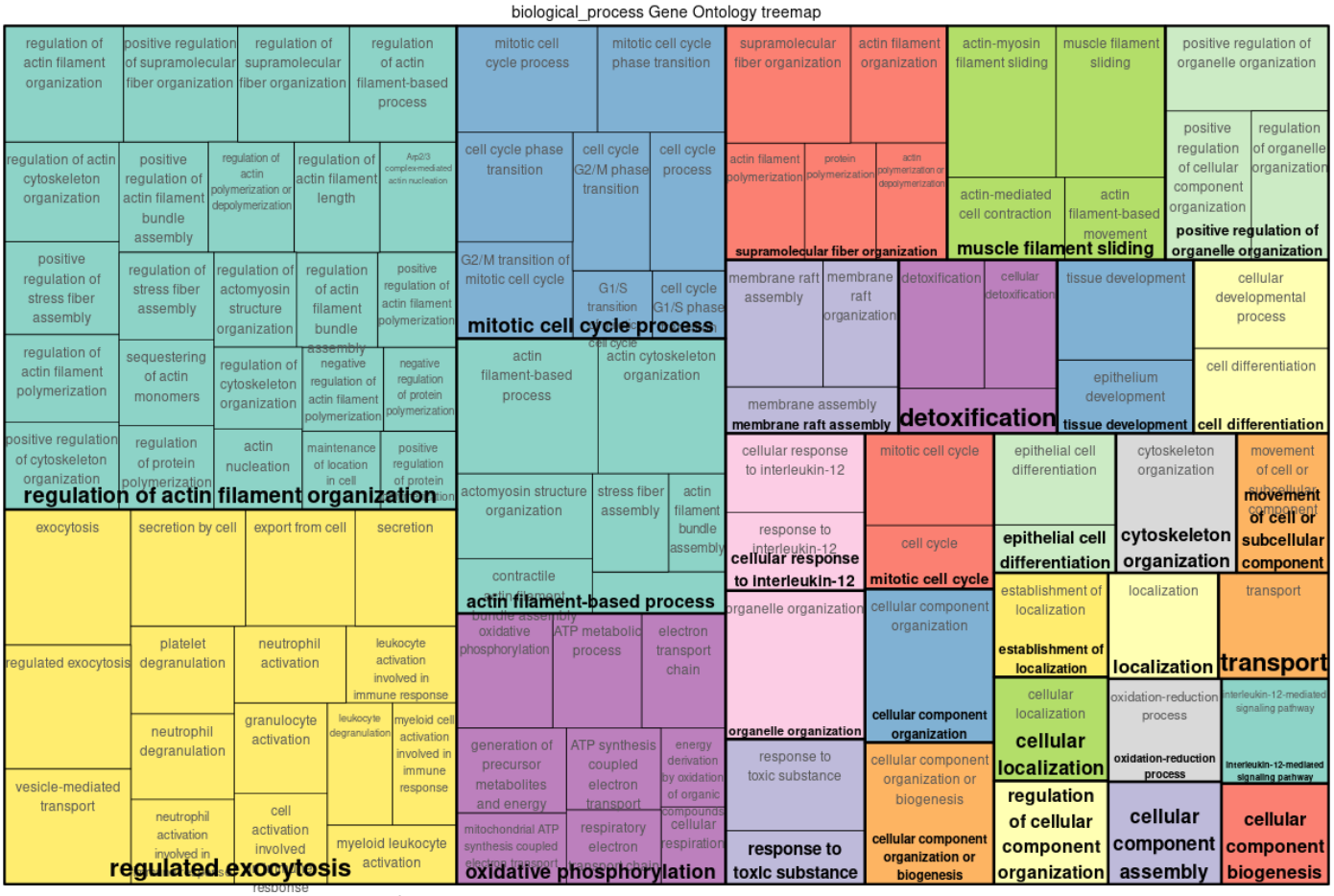



*Figure 6. Enriched GO (biological process) of down-regulated genes in Myc_induce vs. Control.*

**Full classification →**

**TRANSPATH® Pathways (2024.1)**

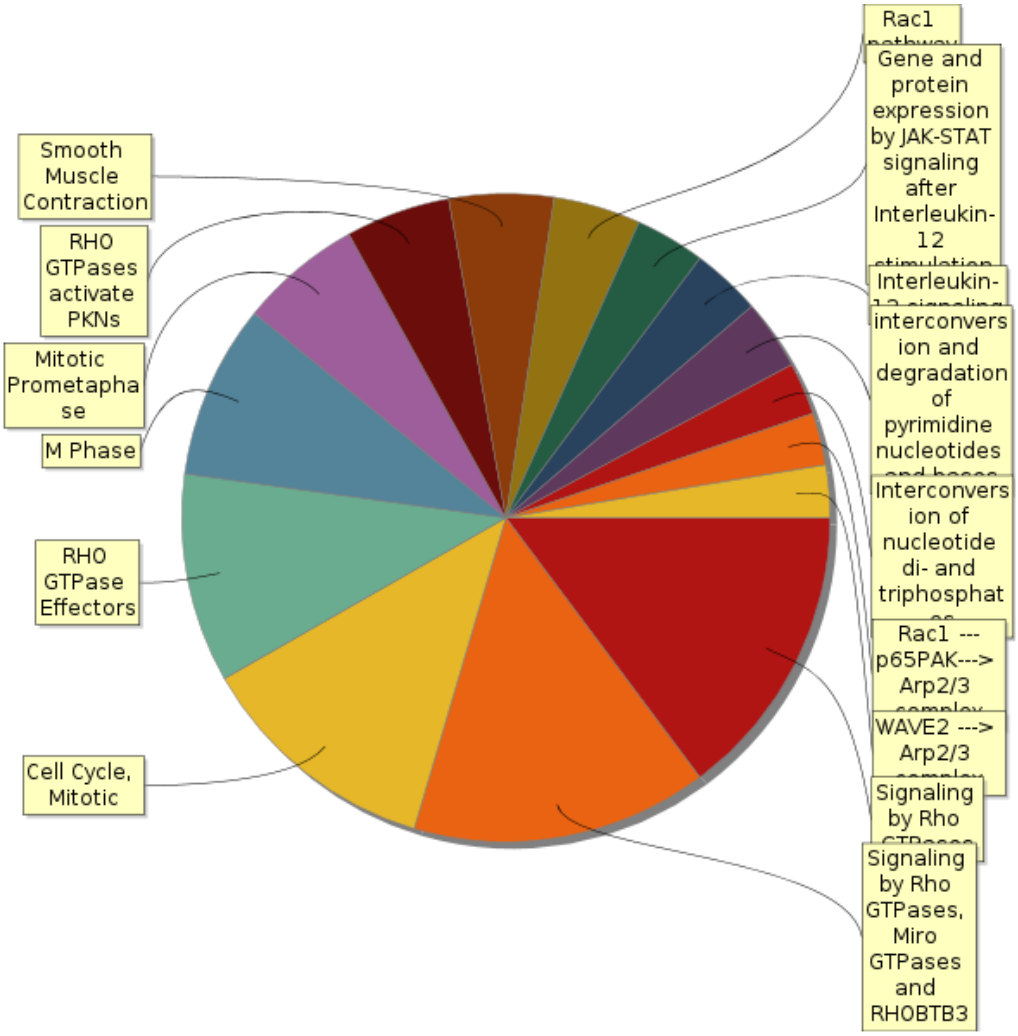



*Figure 7. Enriched TRANSPATH® Pathways (2024.1) of down-regulated genes in Myc_induce vs. Control.*
**Full classification →**

**HumanPSD(TM) disease (2024.1)**

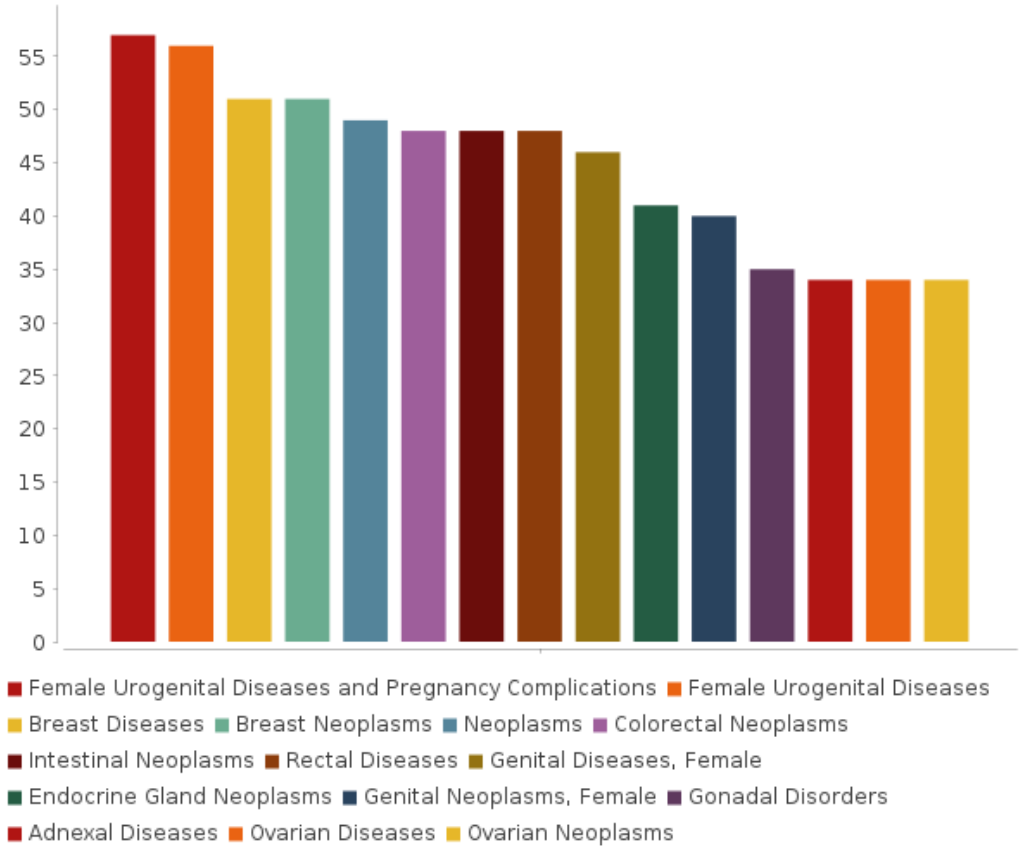



*Figure 8. Enriched HumanPSD(TM) disease (2024.1) of down-regulated genes in Myc_induce vs. Control. The size of the bars correspond to the number of biomarkers of the given disease found among the input set.*

**Full classification →**

## *3.3. Identification of proteins*

In the first step of the proteome data analysis target proteins were identified from the uploaded experimental data (the list of 4665 proteins) and were converted to corresponding genes. These genes were used in the further steps of analysis.

*Table 4. Top ten the list of genes provided as input in Myc_induce.*

**See full table →**

| ID | Gene description | Gene symbol | Proteomics_avr |
|---|---|---|---|
| ENSG00000173598 | nudix hydrolase 4 | NUDT4 | 4.36 |
| ENSG00000100335 | mitochondrial elongation factor 1 | MIEF1 | 3.8 |
| ENSG00000115884 | syndecan 1 | SDC1 | 3.62 |
| ENSG00000102910 | lon peptidase 2, peroxisomal | LONP2 | 3.3 |
| ENSG00000179046 | tripartite motif family like 2 | TRIML2 | 2.87 |
| ENSG00000114648 | kelch like family member 18 | KLHL18 | 2.76 |
| ENSG00000170525 | 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 | PFKFB3 | 2.69 |
| ENSG00000120949 | TNF receptor superfamily member 8 | TNFRSF8 | 2.46 |
| ENSG00000188158 | NHS actin remodeling regulator | NHS | 2.46 |
| ENSG00000119599 | DDB1 and CUL4 associated factor 4 | DCAF4 | 2.42 |

## *3.4. Functional classification of expressed proteins*

A functional analysis of expressed proteins was done by mapping the protein IDs to several known ontologies, such as Gene Ontology (GO), disease ontology (based on HumanPSD™ database) and the ontology of signal transduction and metabolic pathways from the TRANSPATH® database. Statistical significance was computed using a binomial test.
Figures 9-11 show the most significant categories.

## The list of proteins provided as input in Myc_induce:

4660 the list of genes provided as input in Myc_induce genes were taken for the mapping.

### GO (biological process)

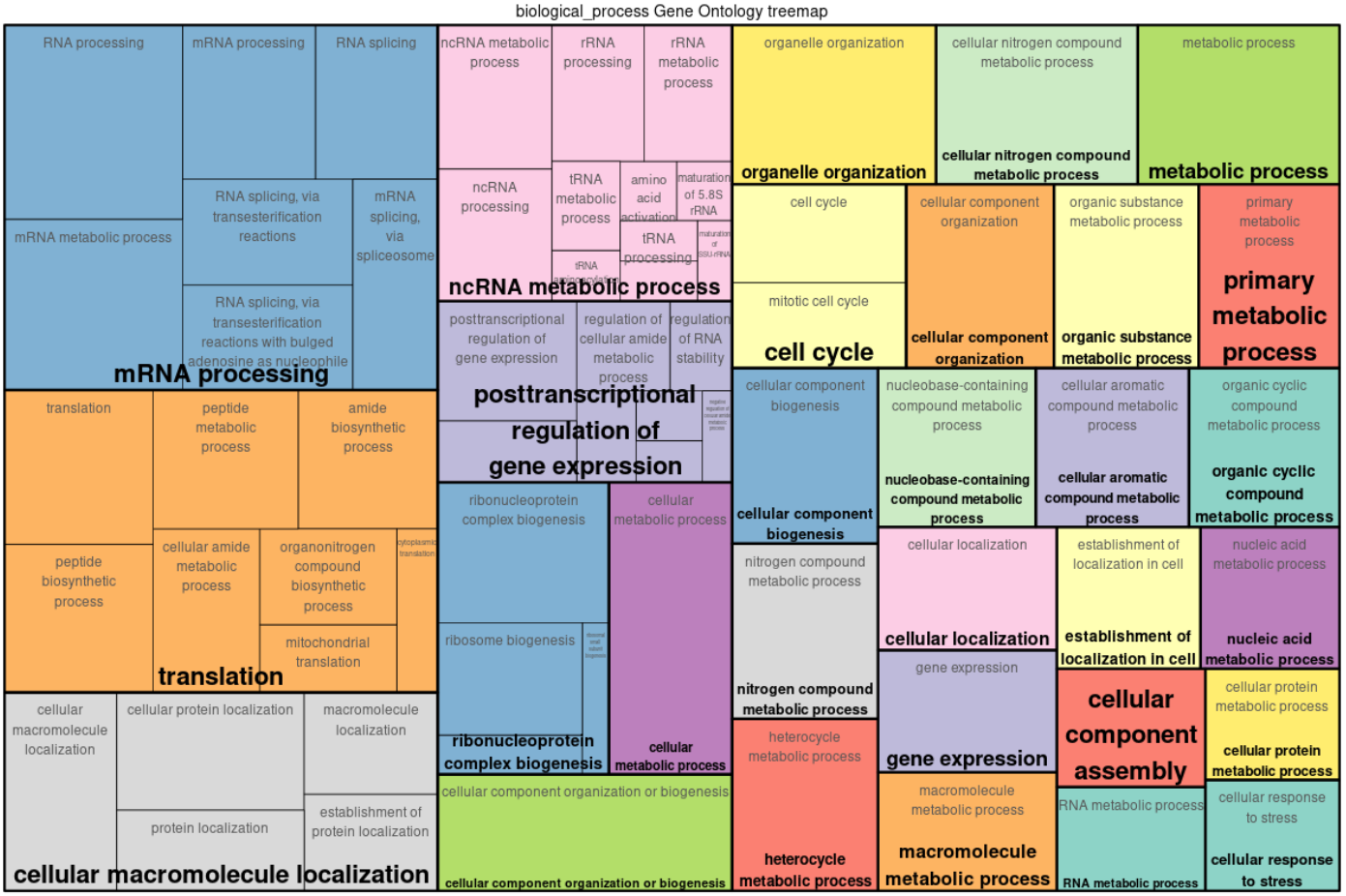



*Figure 9. Enriched GO (biological process) of the list of proteins provided as input in Myc_induce.*
**Full classification →**

**TRANSPATH® Pathways (2024.1)**

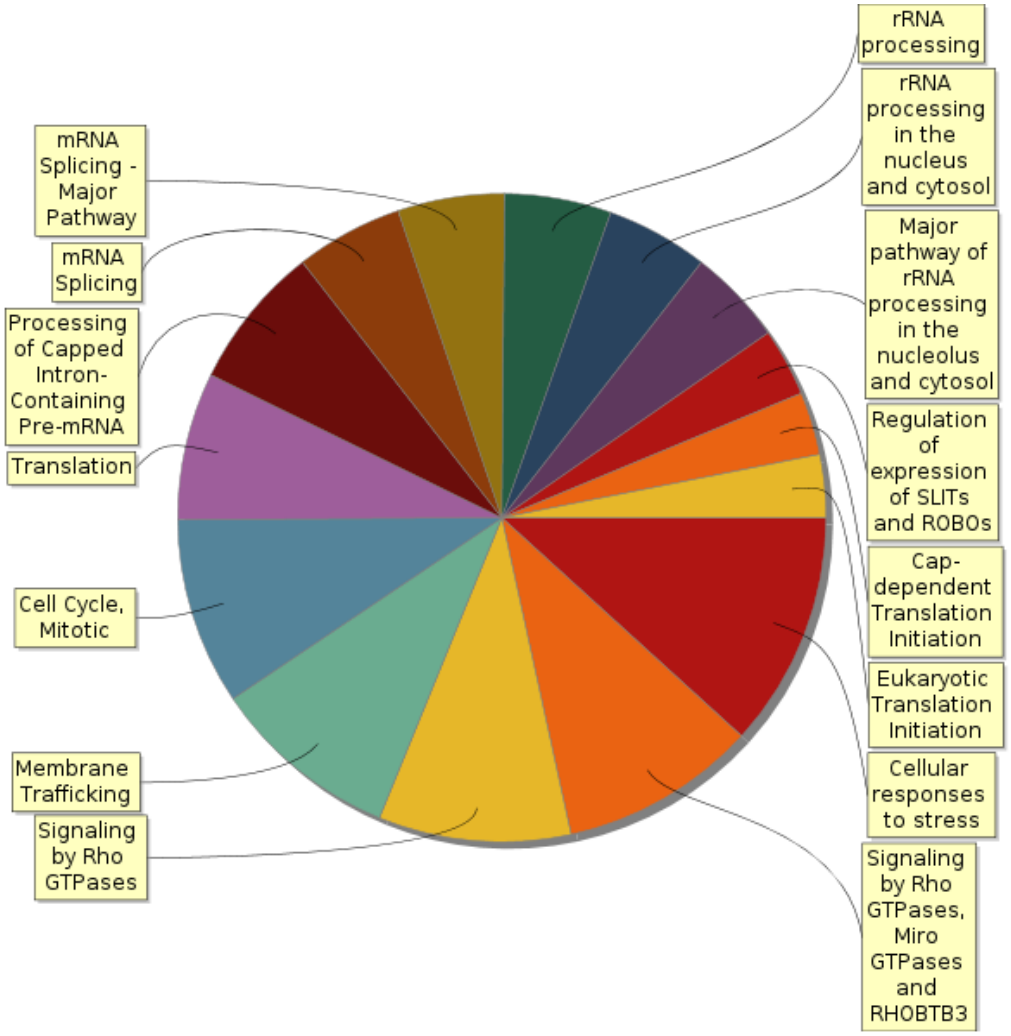



*Figure 10. Enriched TRANSPATH® Pathways (2024.1) of the list of proteins provided as input in Myc_induce.*
**Full classification →**

**HumanPSD(TM) disease (2024.1)**

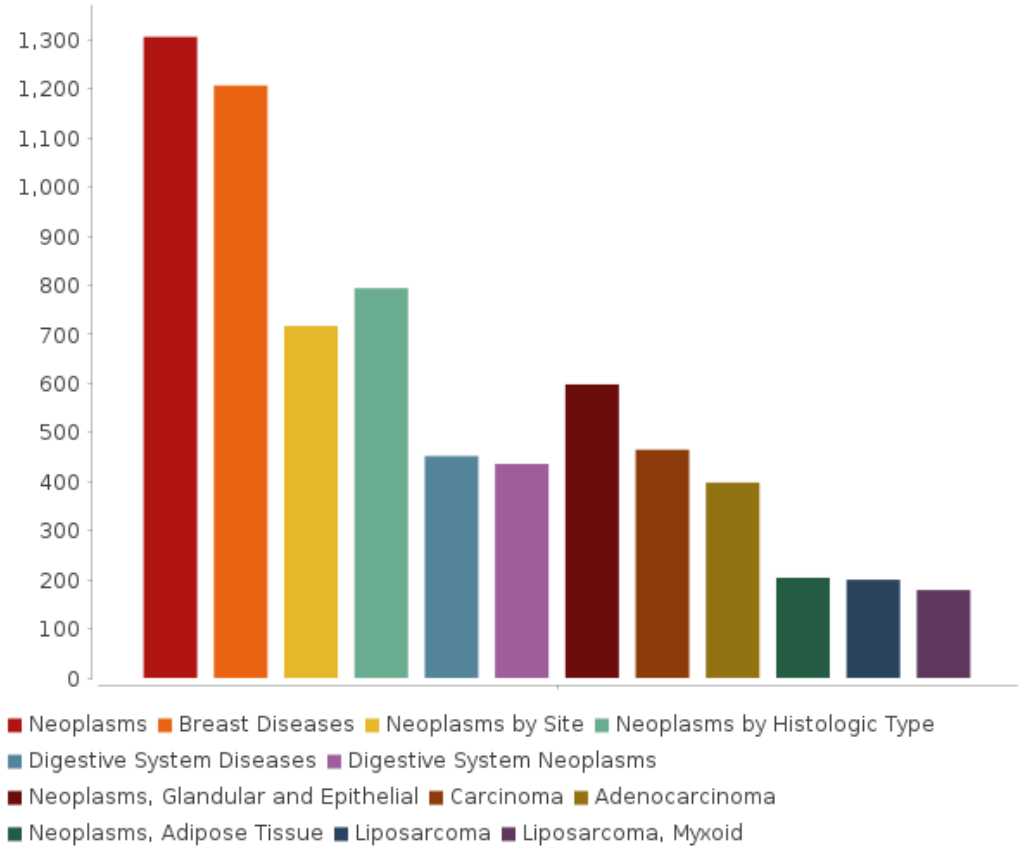



*Figure 11. Enriched HumanPSD(TM) disease (2024.1) of the list of proteins provided as input in Myc_induce. The size of the bars correspond to the number of biomarkers of the given disease found among the input set.*

**Full classification →**

## *3.5. Comparison plot of transcriptome and proteome*

After the analysis of transcriptome and proteome data they were compared with each other. Below we plot 1037 genes and 4660 proteins.

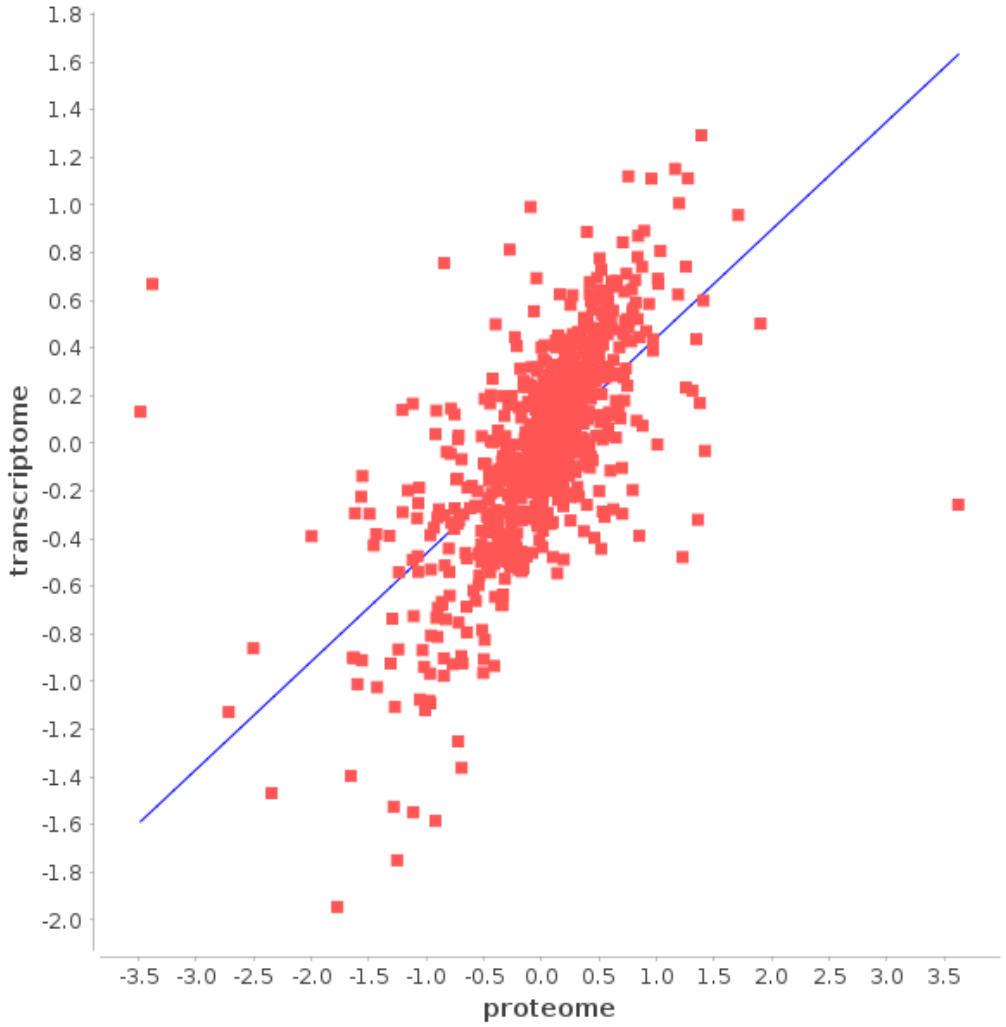



*Figure 12. Comparison plot of comparison proteome vs transcriptome. X axis: protein expression value - Proteomics_avr. Y axis: LogFC of differential gene expression.*

**Full comparison →**

### Comparison of up-regulated genes (transcriptome data) and the list of proteins provided as input (proteome data)

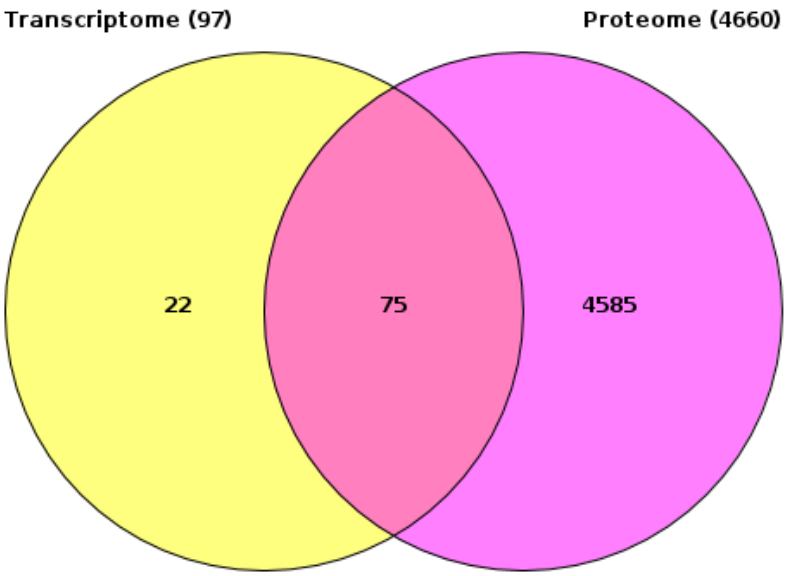



*Figure 13. Intersection of up-regulated genes and the list of proteins provided as input*

**See full diagram →**

**Comparison of down-regulated genes (transcriptome data) and the list of proteins provided as input (proteome data)**

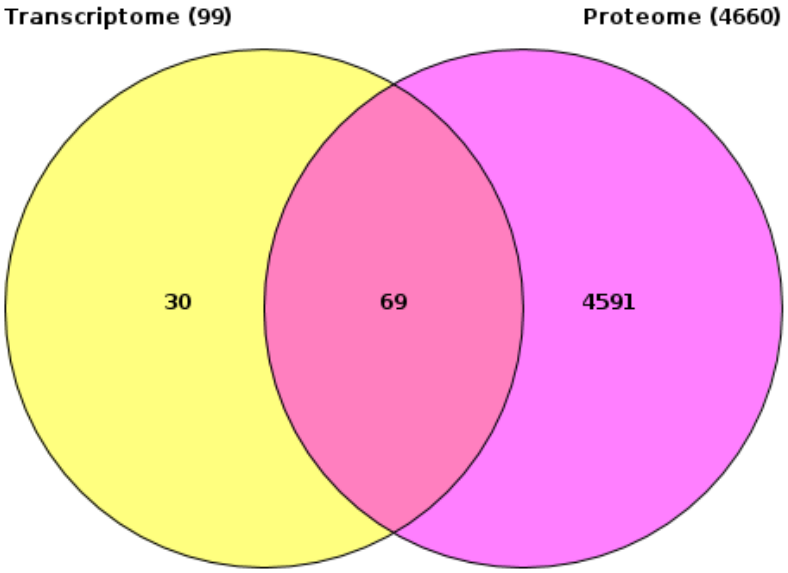
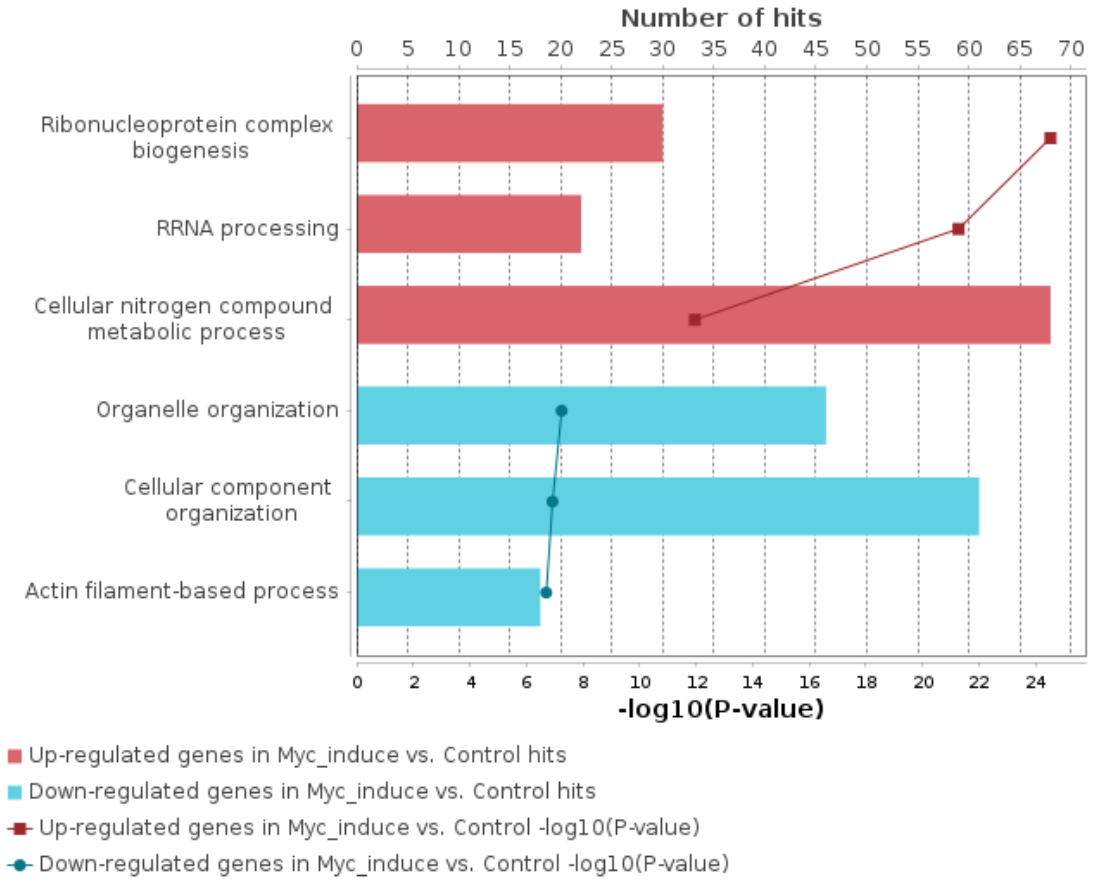



*Figure 14. Intersection of down-regulated genes and the list of proteins provided as input*
**See full diagram →**

The result of overall Gene Ontology (GO) analysis of the differentially expressed genes of the studied pathology can be summarized by the following diagram, revealing the most significant functional categories overrepresented among the observed (differentially expressed genes):




## *3.6. Analysis of enriched transcription factor binding sites and composite modules*

In the next step a search for transcription factors binding sites (TFBS) was performed in the regulatory regions of the ***target genes*** by using the TF binding motif library of the TRANSFAC® database. We searched for so called **composite modules** that act as potential condition-specific **enhancers** of the ***target genes*** in their upstream regulatory regions (-1000 bp upstream of transcription start site (TSS)) and identify transcription factors regulating activity of the genes through such **enhancers**.

Classically, **enhancers** are defined as regions in the genome that increase transcription of one or several genes when inserted in either orientation at various distances upstream or downstream of the gene [8]. Enhancers typically have a length of several hundreds of nucleotides and are bound by multiple transcription factors in a cooperative manner [9].

We applied the Composite Module Analyst (CMA) [8] method to detect such potential enhancers, as targets of multiple TFs bound in a cooperative manner to the regulatory regions of the genes of interest. CMA applies a genetic algorithm to construct a generalized model of the enhancers by specifying combinations of TF motifs (from TRANSFAC®) whose sites are most frequently clustered together in the regulatory regions of the studied genes. CMA identifies the transcription factors that through their cooperation provide a synergistic effect and thus have a great influence on the gene regulation process.

### Enhancer model potentially involved in regulation of target genes (up-regulated genes in Myc_induce vs. Control).

To build the most specific composite modules we choose all significant up-regulated genes as the input of CMA algorithm. The obtained CMA model is then applied to compute CMA score for all up-regulated genes in Myc_induce vs. Control.

The model consists of 2 module(s). Below, for each module the following information is shown:
- PWMs producing matches,
- number of individual matches for each PWM,
- score of the best match.

Module 1:

| V$SP3_01 | V$LYL1_01 | V$RUNX2_03 | V$VDRRXRA_01 |
|---|---|---|---|
| 0.89; N=1 | 0.84; N=2 | 0.92; N=2 | 0.84; N=2 |

Module width: 130

Module 2:

| V$P73_02 | V$PR_03 | V$TCF3_09 | V$HOXA1_03 | V$NR2F1_14 | V$ESRRA_15 |
|---|---|---|---|---|---|
| 0.84; N=3 | 0.81; N=3 | 1.00; N=3 | 1.00; N=2 | 0.70; N=2 | 0.99; N=3 |

Module width: 127

**Model score (-p*log10(pval)):** 10.68
**Wilcoxon p-value (pval):** 4.94e-22
**Penalty (p):** 0.501
**Average yes-set score:** 4.92
**Average no-set score:** 3.22
**AUC:** 0.84
**Separation point:** 4.09
**False-positive:** 24.88%
**False-negative:** 20.62%

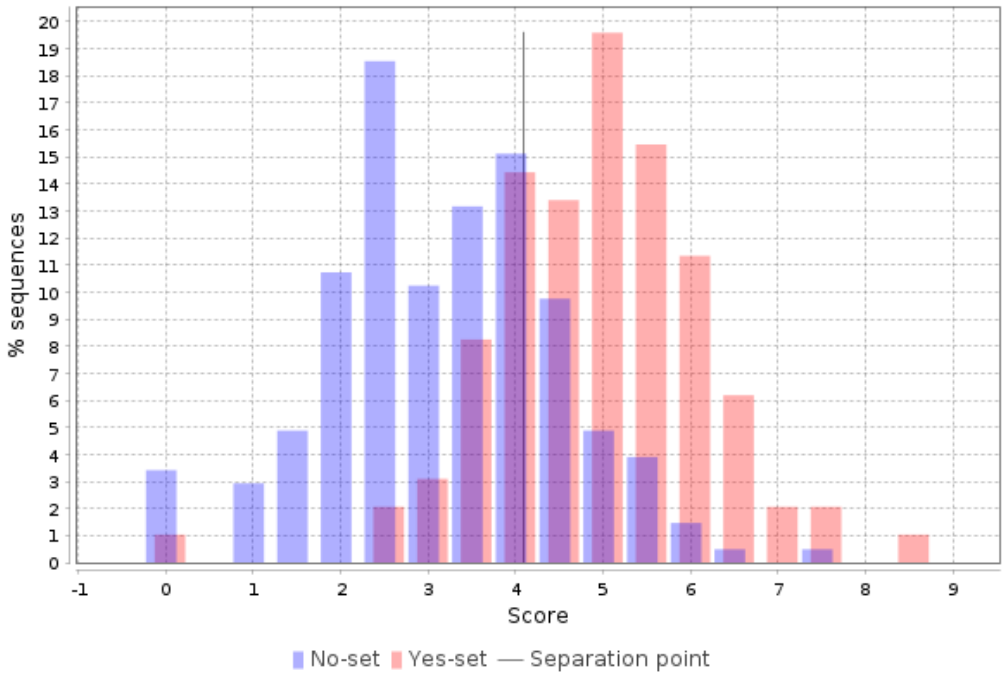


*Table 5. List of top ten up-regulated genes in Myc_induce vs. Control with identified enhancers in their regulatory regions.* ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

**See full table →**

| Ensembl IDs | Gene symbol | Gene description | CMA score | Factor names |
|---|---|---|---|---|
| ENSG00000141580 | WDR45B | WD repeat domain 45B | 8.28 | PR(h), COUP-TFI(h), Lyl-1(h), RXRalpha(h),VDR(h), Sp3(h) |
| ENSG00000159720 | ATP6V0D1 | ATPase H+ transporting V0 subunit d1 | 7.86 | p73(h), RXRalpha(h),VDR(h), ERR1(h), COUP-TFI(h), Hox-A1(h), Runx2(h), Sp3(h)... |
| ENSG00000185803 | SLC52A2 | solute carrier family 52 member 2 | 7.83 | PR(h), p73(h), E2A(h), COUP-TFI(h), RXRalpha(h),VDR(h), Lyl-1(h), Sp3(h) |
| ENSG00000136997 | MYC | MYC proto-oncogene, bHLH transcription factor | 7.46 | COUP-TFI(h), Hox-A1(h), Runx2(h), Lyl-1(h), RXRalpha(h),VDR(h), Sp3(h) |
| ENSG00000110651 | CD81 | CD81 molecule | 7.42 | p73(h), E2A(h), RXRalpha(h),VDR(h), COUP-TFI(h), PR(h), Sp3(h), Lyl-1(h) |
| ENSG00000115307 | AUP1 | AUP1 lipid droplet regulating VLDL assembly factor | 7.32 | PR(h), p73(h), COUP-TFI(h), RXRalpha(h),VDR(h), Lyl-1(h), Sp3(h), E2A(h) |
| ENSG00000084090 | STARD7 | StAR related lipid transfer domain containing 7 | 7.18 | COUP-TFI(h), p73(h), Lyl-1(h), RXRalpha(h),VDR(h), ERR1(h), Sp3(h) |
| ENSG00000198517 | MAFK | MAF bZIP transcription factor K | 7.12 | RXRalpha(h),VDR(h), Lyl-1(h), p73(h), Sp3(h), E2A(h), COUP-TFI(h), PR(h) |
| ENSG00000117395 | EBNA1BP2 | EBNA1 binding protein 2 | 6.97 | p73(h), Lyl-1(h), Sp3(h), COUP-TFI(h), PR(h) |
| ENSG00000163597 | SNHG16 | small nucleolar RNA host gene 16 | 6.94 | Lyl-1(h), Sp3(h), PR(h), COUP-TFI(h), p73(h) |

**Enhancer model potentially involved in regulation of target genes (down-regulated genes in Myc_induce vs. Control).**

To build the most specific composite modules we choose all significant down-regulated genes as the input of CMA algorithm. The obtained CMA model is then applied to compute CMA score for all down-regulated genes in Myc_induce vs. Control.

The model consists of 2 module(s). Below, for each module the following information is shown:
- PWMs producing matches,
- number of individual matches for each PWM,
- score of the best match.

Module 1:

| V$NKX25_10 | V$SMAD_Q4 | V$AP2GAMMA_Q4 | V$NFE2_01 | V$NR3C1_03 |
|---|---|---|---|---|
| 0.93; N=1 | 0.96; N=2 | 0.90; N=2 | 0.88; N=2 | 0.71; N=2 |

Module width: 130

Module 2:

| V$SP1_07 | V$ZNF462_01 | V$NFKB1_04 | V$SNAI2_01 | V$SMAD2_02 | V$SP3_Q3 | V$GATA3_02 |
|---|---|---|---|---|---|---|
| 0.92; N=1 | 0.93; N=3 | 0.93; N=2 | 0.94; N=3 | 0.91; N=3 | 0.82; N=2 | 0.94; N=2 |

Module width: 134

**Model score (-p*log10(pval)):** 12.19
**Wilcoxon p-value (pval):** 2.05e-26
**Penalty (p):** 0.475
**Average yes-set score:** 7.51
**Average no-set score:** 5.40
**AUC:** 0.87
**Separation point:** 6.32
**False-positive:** 25.37%
**False-negative:** 11.11%

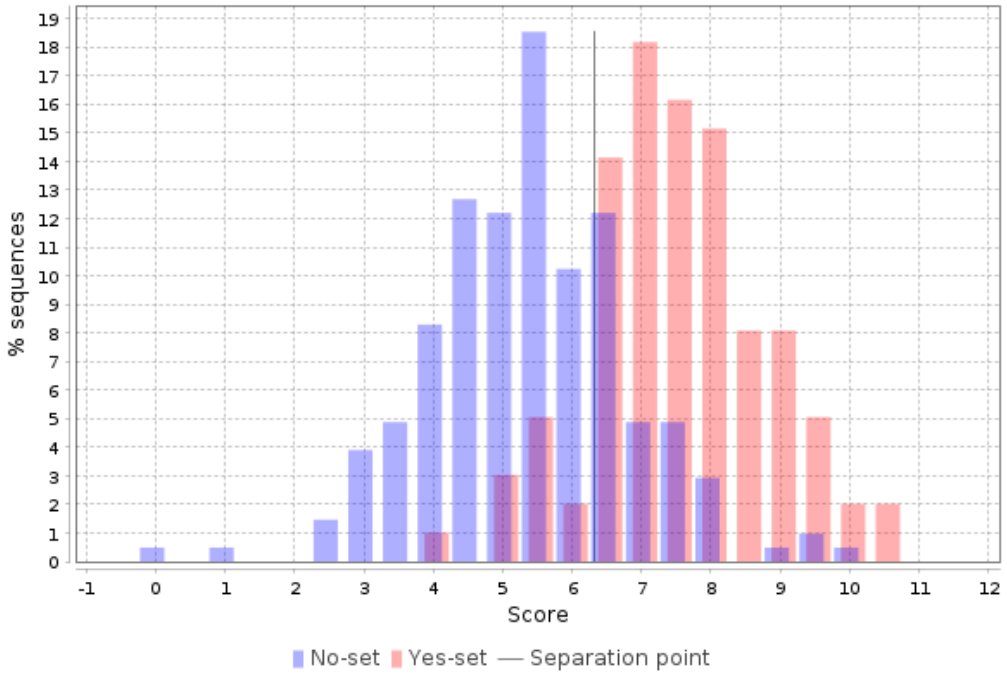


*Table 6. List of top ten down-regulated genes in Myc_induce vs. Control with identified enhancers in their regulatory regions.* ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

**See full table →**

| Ensembl IDs | Gene symbol | Gene description | CMA score | Factor names |
|---|---|---|---|---|
| ENSG00000204525 | HLA-C | major histocompatibility complex, class I, C | 11.36 | SNAI2(h), SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h), ZNF462(h), GR(h), NF-kappaB-p105(h), AP-2gamma(h), Sp3(h)... |
| ENSG00000140988 | RPS2 | ribosomal protein S2 | 11.08 | AP-2gamma(h), SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h), SMAD2(h), Sp3(h), GR(h), NF-kappaB-p105(h), ZNF462(h)... |
| ENSG00000105048 | TNNT1 | troponin T1, slow skeletal type | 10.58 | AP-2gamma(h), GR(h), SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h), SMAD2(h), ZNF462(h), Sp3(h), Sp1(h)... |
| ENSG00000167767 | KRT80 | keratin 80 | 10.49 | ZNF462(h), Sp3(h), SNAI2(h), NF-E2 p45(h), AP-2gamma(h), NKX-2.5(h), GR(h) |
| ENSG00000213719 | CLIC1 | chloride intracellular channel 1 | 10.48 | GR(h), AP-2gamma(h), SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h), SNAI2(h), Sp3(h), ZNF462(h), Sp1(h)... |
| ENSG00000125868 | DSTN | destrin, actin depolymerizing factor | 10.36 | GATA-3(h), SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h), GR(h), NF-kappaB-p105(h), ZNF462(h), Sp3(h), SNAI2(h)... |
| ENSG00000177156 | TALDO1 | transaldolase 1 | 10.17 | SNAI2(h), NF-E2 p45(h), GR(h), SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h), AP-2gamma(h), ZNF462(h), NF-kappaB-p105(h)... |
| ENSG00000120885 | CLU | clusterin | 10.02 | ZNF462(h), Sp1(h), SNAI2(h), AP-2gamma(h), Sp3(h), GR(h), SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h)... |
| ENSG00000152795 | HNRNPDL | heterogeneous nuclear ribonucleoprotein D like | 9.89 | SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h), GR(h), AP-2gamma(h), Sp1(h), ZNF462(h), SMAD2(h), Sp3(h)... |
| ENSG00000135390 | ATP5MC2 | ATP synthase membrane subunit c locus 2 | 9.85 | GATA-3(h), Sp1(h), ZNF462(h), SMAD2(h), SNAI2(h), NKX-2.5(h), SMAD1(h),SMAD2(h),SMAD3(h),SMAD4(h),SMAD5(h),SMAD6(h),SMAD9(h)... |

On the basis of the enhancer models we identified transcription factors potentially regulating the ***target genes*** of our interest. We found 11 and 17 transcription factors controlling expression of up- and down-regulated genes respectively (see Tables 7-8).

*Table 7. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (up-regulated genes in Myc_induce vs. Control).* ***Yes-No ratio*** *is the ratio between frequencies of the sites in Yes sequences versus No sequences. It describes the level of the enrichment of binding sites for the indicated TF in the regulatory target regions.* ***Regulatory score*** *is the measure of involvement of the given TF in the controlling of expression of genes that encode master regulators presented below (through positive feedback loops).*

**See full table →**

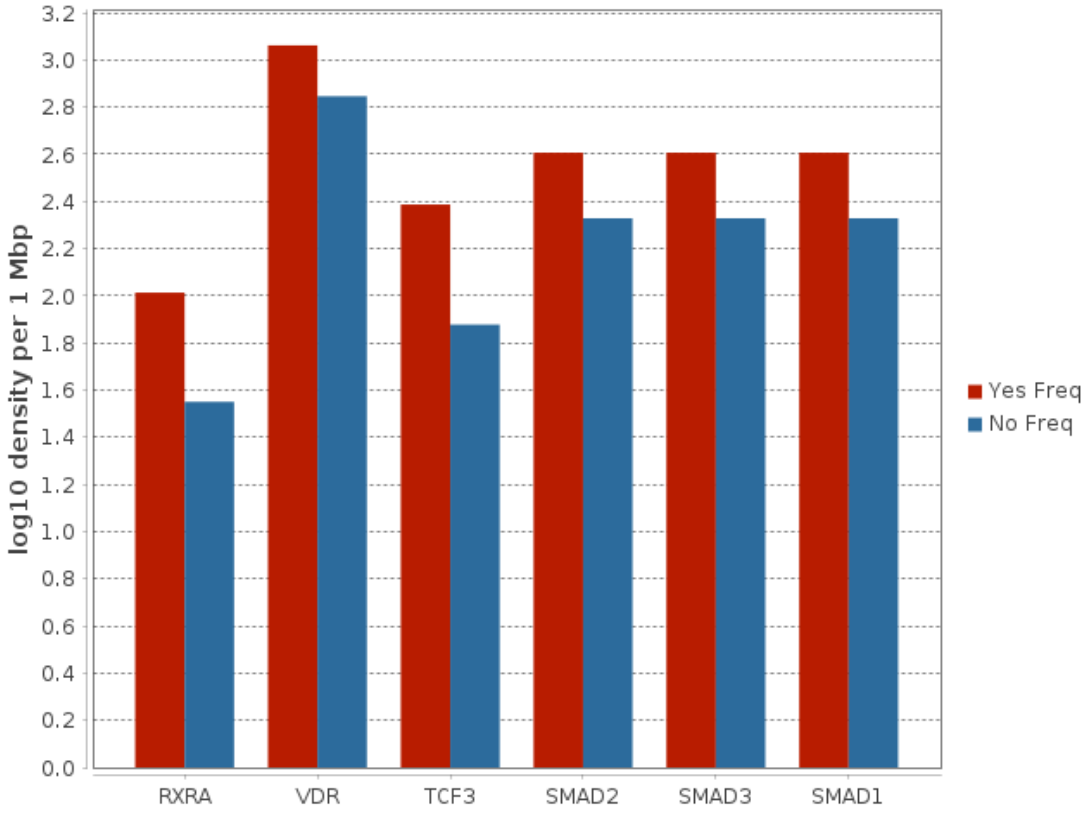
| ID | Gene symbol | Gene description | Regulatory score | Yes-No ratio |
|---|---|---|---|---|
| MO000019619 | RXRA | retinoid X receptor alpha | 2.37 | 2.9 |
| MO000021495 | VDR | vitamin D receptor | 2.29 | 1.64 |
| MO000032492 | TCF3 | transcription factor 3 | 2.1 | 3.23 |
| MO000054297 | PGR | progesterone receptor | 2.1 | 2.11 |
| MO000028707 | TP73 | tumor protein p73 | 1.98 | 1.48 |
| MO000026285 | RUNX2 | RUNX family transcription factor 2 | 1.96 | 2.11 |
| MO000025819 | LYL1 | LYL1 basic helix-loop-helix family member | 1.84 | 1.31 |
| MO000046079 | SP3 | Sp3 transcription factor | 1.8 | 1.42 |
| MO000026738 | ESRRA | estrogen related receptor alpha | 1.64 | 9.5 |
| MO000024736 | NR2F1 | nuclear receptor subfamily 2 group F member 1 | 1.63 | 3.38 |

*Table 8. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (down-regulated genes in Myc_induce vs. Control). **Yes-No ratio** is the ratio between frequencies of the sites in Yes sequences versus No sequences. It describes the level of the enrichment of binding sites for the indicated TF in the regulatory target regions. **Regulatory score** is the measure of involvement of the given TF in the controlling of expression of genes that encode master regulators presented below (through positive feedback loops).*

**See full table →**

| ID | Gene symbol | Gene description | Regulatory score | Yes-No ratio |
|---|---|---|---|---|
| MO000057829 | SMAD2 | SMAD family member 2 | 2.45 | 1.9 |
| MO000057832 | SMAD3 | SMAD family member 3 | 2.28 | 1.9 |
| MO000019609 | SMAD1 | SMAD family member 1 | 2.22 | 1.9 |
| MO000020635 | SMAD5 | SMAD family member 5 | 2.12 | 1.9 |
| MO000031266 | NR3C1 | nuclear receptor subfamily 3 group C member 1 | 2.1 | 1.84 |
| MO000033308 | SP1 | Sp1 transcription factor | 2.08 | 1.69 |
| MO000020402 | SMAD4 | SMAD family member 4 | 2.01 | 1.9 |
| MO000019359 | NFKB1 | nuclear factor kappa B subunit 1 | 1.99 | 5.17 |
| MO000028767 | SNAI2 | snail family transcriptional repressor 2 | 1.93 | 2.12 |
| MO000021185 | SMAD9 | SMAD family member 9 | 1.9 | 1.9 |

The following diagram represents the key transcription factors, which were predicted to be potentially regulating differentially expressed genes in the analyzed pathology: RXRA, VDR, TCF3, SMAD2, SMAD3 and SMAD1.




## *3.7. Finding master regulators in networks*

In the second step of the upstream analysis common regulators of the revealed TFs were identified. Using proteomics data we selected differentially expressed proteins that are involved in signal transduction pathways and used these proteins as "context set" [4] in the algorithm of identification of master regulators. These master regulators appear to be the key candidates for therapeutic targets as they have a master effect on regulation of intracellular pathways that activate the pathological process of our study. The identified master regulators are shown in Tables 9-10.

*Table 9. Master regulators that may govern the regulation of **up-regulated** genes in Myc_induce vs. Control. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics and proteomics data.*

**See full table →**

| ID | Master molecule name | Gene symbol | Gene description | Contained in proteome set | Total rank | logFC (transcriptome) |
|---|---|---|---|---|---|---|
| MO000031061 | NCL(h) | NCL | nucleolin | 1 | 175 | 0.46 |
| MO000032096 | proCaspase-3(h):Hsp10(h):Hsp60(h) | CASP3, HSPD1, HSPE1 | caspase 3, heat shock protein family D (Hsp60) member 1, heat shock protein family E (Hsp10) member ... | 1 | 219 | 0.53 |
| MO000023504 | cyclinD1(h):Cdk6(h) | CCND1, CDK6 | cyclin D1, cyclin dependent kinase 6 | 1 | 240 | 0.68 |
| MO000023505 | cyclinD1(h):Cdk6(h){pT177} | CCND1, CDK6 | cyclin D1, cyclin dependent kinase 6 | 1 | 241 | 0.68 |
| MO000119221 | E2-EPF(h) | UBE2S | ubiquitin conjugating enzyme E2 S | 1 | 241 | 0.5 |
| MO001082012 | Apoptosome(h) | APAF1, CASP9, CYCS | apoptotic peptidase activating factor 1, caspase 9, cytochrome c, somatic | 1 | 253 | 0.41 |
| MO000083025 | HSPA4(h) | HSPA4 | heat shock protein family A (Hsp70) member 4 | 1 | 254 | 0.38 |
| MO000031196 | cyclinD1(h):Cdk4(h) | CCND1, CDK4 | cyclin D1, cyclin dependent kinase 4 | 1 | 255 | 0.68 |
| MO001082013 | Apoptosome | APAF1, CASP9, CYCS | apoptotic peptidase activating factor 1, caspase 9, cytochrome c, somatic | 1 | 256 | 0.41 |
| MO000021112 | Cytochrome C:(Apaf-1)2:dATP:(Caspase-9)2 | APAF1, CASP9, CYCS | apoptotic peptidase activating factor 1, caspase 9, cytochrome c, somatic | 1 | 257 | 0.41 |

*Table 10. Master regulators that may govern the regulation of **down-regulated** genes in Myc_induce vs. Control. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics and proteomics data.*

**See full table →**

| ID | Master molecule name | Gene symbol | Gene description | Contained in proteome set | Total rank | logFC (transcriptome) |
|---|---|---|---|---|---|---|
| MO000032629 | AXL(h) | AXL | AXL receptor tyrosine kinase | 1 | 125 | -1.13 |
| MO000030879 | 14-3-3sigma(h) | SFN | stratifin | 1 | 153 | -0.97 |
| MO000032571 | RhoC(h) | RHOC | ras homolog family member C | 1 | 160 | -0.86 |
| MO000021293 | 14-3-3eta(h) | YWHAH | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein eta | 1 | 170 | -0.57 |
| MO000017291 | integrins | ITGA1, ITGA2B, ITGA3, ITGA4, ITGA5, ITGA6, ITGA8, ITGA9, ITGAL, ITGAV, ITGB1, ITGB2, ITGB3, ITGB4, I... | integrin subunit alpha 1, integrin subunit alpha 2b, integrin subunit alpha 3, integrin subunit alph... | 1 | 226 | -1.41 |
| MO000258012 | AXL-Short(h) | AXL | AXL receptor tyrosine kinase | 1 | 239 | -1.13 |
| MO000019345 | IRAK-1(h) | IRAK1 | interleukin 1 receptor associated kinase 1 | 1 | 259 | -0.33 |
| MO000144818 | osteonectin(h) | SPARC | secreted protein acidic and cysteine rich | 1 | 265 | -1.01 |
| MO000022158 | IRAK-1(h){p} | IRAK1 | interleukin 1 receptor associated kinase 1 | 1 | 269 | -0.33 |
| MO000089580 | IRAK-1c(h) | IRAK1 | interleukin 1 receptor associated kinase 1 | 1 | 270 | -0.33 |

The intracellular regulatory pathways controlled by the above-mentioned master regulators are depicted in Figures 15 and 16. These diagrams display the connections between identified transcription factors, which play important roles in the regulation of differentially expressed genes, and selected master regulators, which are responsible for the regulation of these TFs.

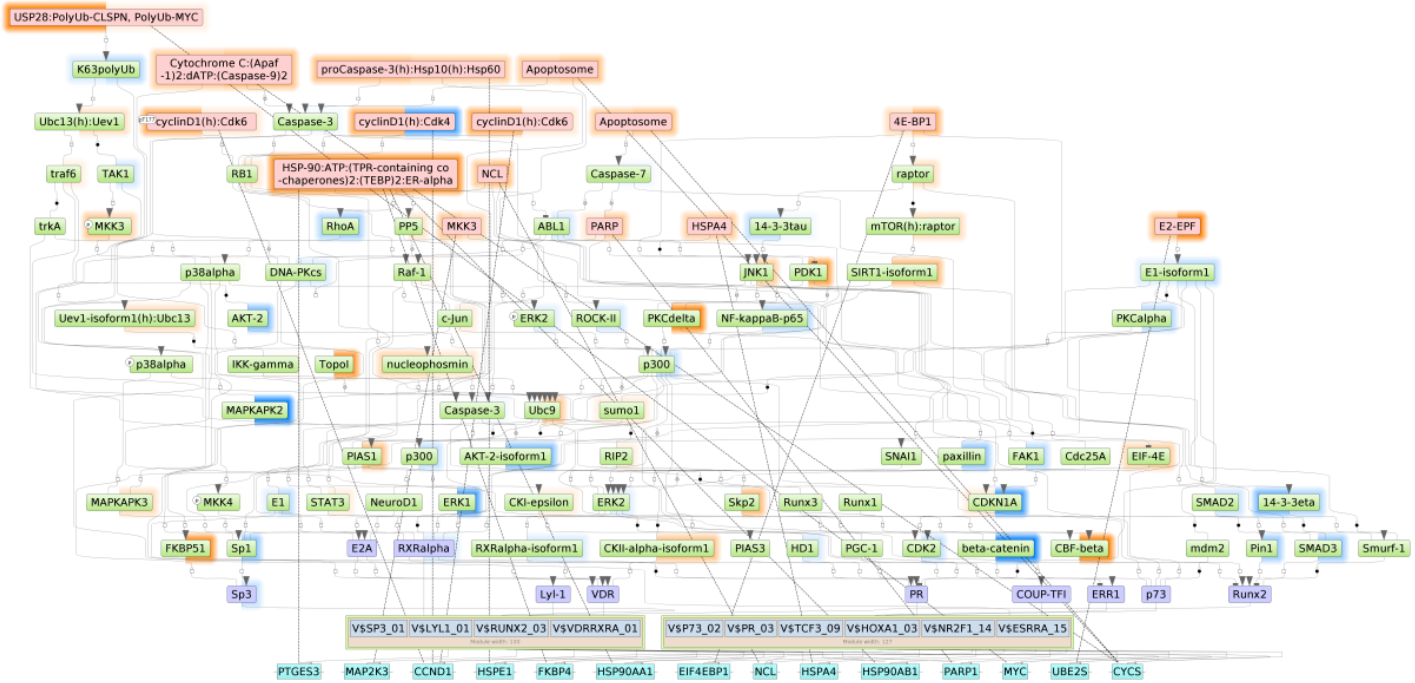



*Figure 15. Diagram of intracellular regulatory signal transduction pathways of up-regulated genes in Myc_induce vs. Control. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network during the search for master regulators from selected TFs. Orange and blue frames highlight molecules that are encoded by up- and downregulated genes, resp. The left half of a highlighting frame corresponds to transcriptomic data, the right one to proteomic data.*

**See full diagram →**

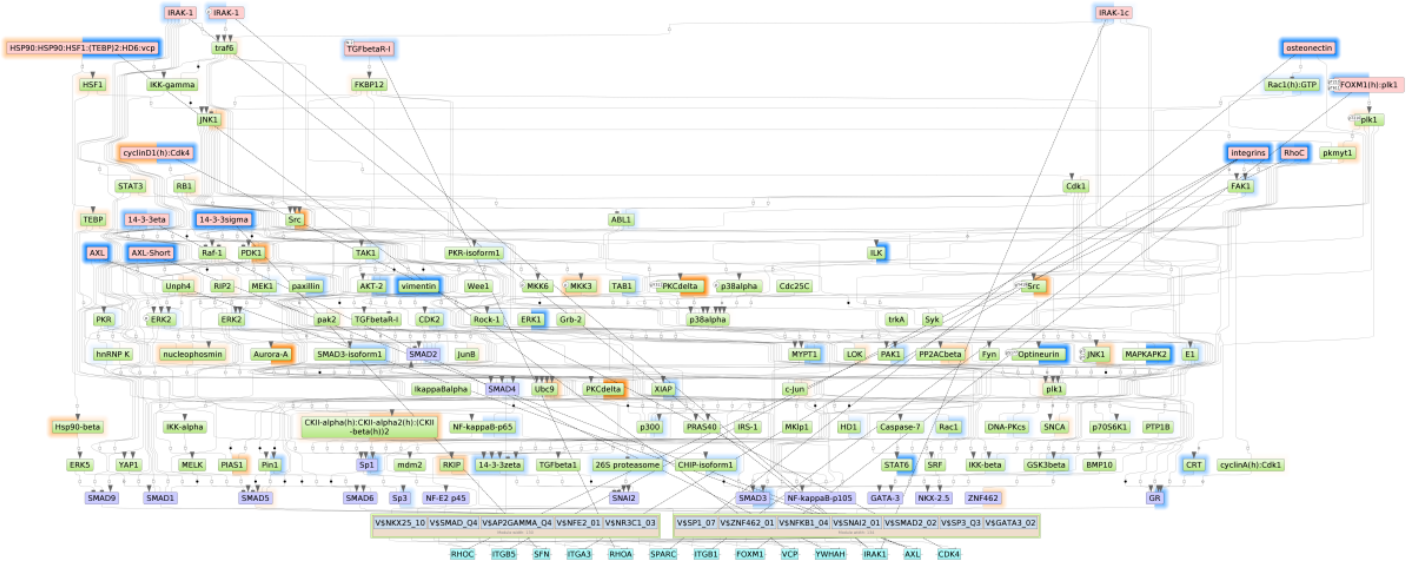



*Figure 16. Diagram of intracellular regulatory signal transduction pathways of down-regulated genes in Myc_induce vs. Control. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network during the search for master regulators from selected TFs. Orange and blue frames highlight molecules that are encoded by up- and downregulated genes, resp. The left half of a highlighting frame corresponds to transcriptomic data, the right one to proteomic data.*

**See full diagram →**

## 4. Finding prospective drug targets

The identified master regulators that may govern pathology associated genes were checked for druggability potential using HumanPSD™ [5] database of gene-disease-drug assignments and PASS [11-13] software for prediction of biological activities of chemical compounds on the basis of a (Q)SAR approach. Respectively, for each master regulator protein we have computed two Druggability scores: HumanPSD Druggability score and PASS Druggability score. Where Druggability score represents the number of drugs that are potentially suitable for inhibition (or activation) of the corresponding target either according to the information extracted from medical literature (from HumanPSD™ database) or according to cheminformatics predictions of compounds activity against the examined target (from PASS software).

The cheminformatics druggability check is done using a pre-computed database of spectra of biological activities of chemical compounds from a library of all small molecular drugs from HumanPSD™ database, 2507 pharmaceutically active known chemical compounds in total. The spectra of biological activities has been computed using the program PASS [11-13] on the basis of a (Q)SAR approach.

If both Druggability scores were below defined thresholds (see Methods section for the details) such master regulator proteins were not used in further analysis of drug prediction.

As a result we created the following two tables of prospective drug targets (top targets are shown here):

*Table 11. Prospective drug targets selected from full list of identified master regulators filtered by Druggability score from HumanPSD™ database. **Druggability score** contains the number of drugs that are potentially suitable for inhibition (or activation) of the target. The drug targets are sorted according to the **Total rank** which is the sum of three ranks computed on the basis of the three scores: keynode score, CMA score and expression change score (logFC, if present). See Methods section for details.*

**See full table →**

| Gene symbol | Gene Description | Druggability score | Contained in proteome set | Total rank | logFC (transcriptome) |
|---|---|---|---|---|---|
| HSPD1 | heat shock protein family D (Hsp60) member 1 | 2 | 1 | 219 | 0.53 |
| HSPA4 | heat shock protein family A (Hsp70) member 4 | 31 | 1 | 356 | 0.38 |
| CYCS | cytochrome c, somatic | 4 | 1 | 459 | 0.41 |
| TXN | thioredoxin | 2 | 1 | 465 | 0.22 |
| PARP1 | poly(ADP-ribose) polymerase 1 | 77 | 1 | 530 | 0.19 |
| MAP2K3 | mitogen-activated protein kinase kinase 3 | 30 | 1 | 565 | 0.45 |

*Table 12. Prospective drug targets selected from full list of identified master regulators filtered by Druggability score predicted by PASS software. Here, the **Druggability score** for master regulator proteins is computed as a sum of PASS calculated probabilities to be active as a target for various small molecular compounds. The drug targets are sorted according to the **Total rank** which is the sum of three ranks computed on the basis of the three scores: keynode score, CMA score and expression change score (logFC, if present). See Methods section for details.*

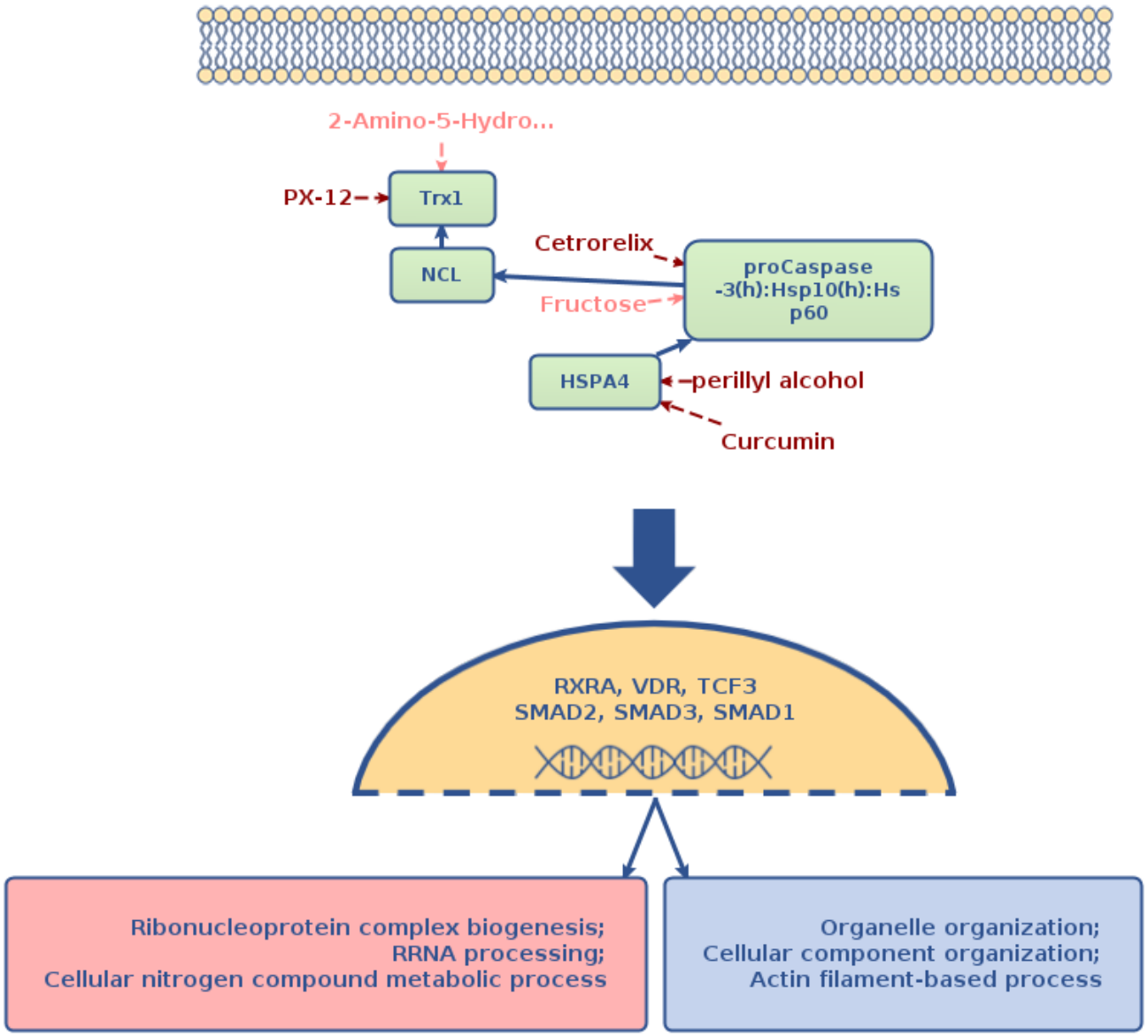
**See full table →**

| Gene symbol | Gene Description | Druggability score | Contained in proteome set | Total rank | logFC (transcriptome) |
|---|---|---|---|---|---|
| HSPD1 | heat shock protein family D (Hsp60) member 1 | 48.91 | 1 | 219 | 0.53 |
| TXN | thioredoxin | 0.87 | 1 | 465 | 0.22 |
| PARP1 | poly(ADP-ribose) polymerase 1 | 2.49 | 1 | 530 | 0.19 |
| MAP2K3 | mitogen-activated protein kinase kinase 3 | 6.15 | 1 | 565 | 0.45 |
| PRMT1 | protein arginine methyltransferase 1 | 1.34 | 1 | 617 | 0.56 |
| POLR2B | RNA polymerase II subunit B | 17.08 | 1 | 619 | 0.15 |

Below we represent schematically the main mechanism of the studied pathology. In the schema we considered the top two drug targets of each of the two categories computed above. In addition we have added two top identified master regulators for which no drugs may be identified yet, but that are playing the crucial role in the molecular mechanism of the studied pathology. Thus the molecular mechanism of the studied pathology was predicted to be mainly based on the following key master regulators:

- NCL
- proCaspase-3(h):Hsp10(h):Hsp60
- HSPA4
- Trx1

This result allows us to suggest the following schema of affecting the molecular mechanism of the studied pathology:




*Drugs which are shown on this schema: 2-Amino-5-Hydroxy-Benzimidazole, PX-12, perillyl alcohol, Curcumin, Cetrorelix and Fructose, should be considered as a prospective research initiative for further drug repurposing and drug development. These drugs were selected as top matching treatments to the most prospective drug targets of the studied pathology, however, these results should be considered with special caution and are to be used for research purposes only, as there is not enough clinical information for adapting these results towards immediate treatment of patients.*
*The drugs given in dark red color on the schema are FDA approved drugs or drugs which have gone through various phases of clinical trials as active treatments against the selected targets.*
*The drugs given in pink color on the schema are drugs, which were cheminformatically predicted to be active against the selected targets.*

## 5. Identification of potential drugs

In the last step of the analysis we strived to identify known activities as well as drugs with cheminformatically predicted activities that are potentially suitable for inhibition (or activation) of the identified molecular targets in the context of specified human diseases(s).

Proposed drugs are top ranked drug candidates, that were found to be active on the identified targets and were selected from 4 categories:

1. FDA approved drugs or used in clinical trials drugs for the studied pathology;
2. Repurposing drugs used in clinical trials for other pathologies;
3. Drugs, predicted by PASS to be active against identified drug targets and against the studied pathology;
4. Drugs, predicted by PASS to be active against identified drug targets but for other pathologies.

Proposed drugs were selected on the basis of Drug rank which was computed from the ranks sum based on the individual ranks of the following scores:

- Target activity score (depends on ranks of all targets that were found for the selected drug);
- Disease activity score (weighted sum of number of clinical trials on disease(s) under study where the selected drug is known to be applied or PASS Disease activity score - cheminformatically predicted property of the compound to be active against the studied disease(s));

- Clinical validity score (applicable only for drugs predicted on the basis of literature curation in HumanPSD™ database (Tables 13 and 14), reflects the number of the highest clinical trials phase on which the drug was tested for any pathology).

You can refer to the Methods section for more details on drug ranking procedure.

Based on the Drug rank, a numerical value of Drug score was calculated, which reflects the potential activity of the respective drug on the overall molecular mechanism of the studied pathology. Drug score values belong to the range from 1 to 100 and are calculated as a quotient of maximum drug rank and the drug rank of the given drug multiplied by 100.

Top drugs of each category are given in the tables below:

## ***Drugs approved in clinical trials***

*Table 13. FDA approved drugs or drugs used in clinical trials for the studied pathology (most promising treatment candidates selected for the identified drug targets on the basis of literature curation in HumanPSD™ database)*
**See full table →**

| Name | Target names | Drug score | Disease activity score | Disease trial phase |
|---|---|---|---|---|
| Temsirolimus | CCND1, EIF4EBP1, EIF4E | 90 | 8 | Phase 3: Neoplasm Metastasis, Arteriovenous Fistula, Breast Neoplasms, Carcinoma, Renal Cell, Constriction, Pathologic, Coronary Artery Disease, Coronary Disease, Coronary Occlusion, Coronary Stenosis, Fistula, Hypertension, Kidney Diseases, Kidney Failure, Chronic, Lymphoma, Lymphoma, Mantle-Cell, Myosarcoma, Neoplasms, Renal Insufficiency, Renal Insufficiency, Chronic, Rhabdomyosarcoma, Rhabdomyosarcoma, Alveolar, Rhabdomyosarcoma, Embryonal, Sarcoma |
| Curcumin | CCND1, PARP1, HSPA4, ADAM15, EIF4EBP1, EIF2S1, EGR1, TFRC, MYC, CDKN1A | 87 | 3 | Phase 2: Osteosarcoma, Aberrant Crypt Foci, Acute Kidney Injury, Adenocarcinoma, Amyotrophic Lateral Sclerosis, Aneurysm, Anorexia, Aortic Aneurysm, Aortic Aneurysm, Abdominal, Asthma, Bipolar Disorder, Bites and Stings, Brain Abscess, Cachexia, Cognitive Dysfunction, Cytopenia, Diabetes Mellitus, Diabetes Mellitus, Type 2, Diabetic Nephropathies, Diverticulitis, Dyslipidemias, Fibrosis, Head and Neck Neoplasms, Hyperemia, Hyperlipidemias, Inflammation, Insulin Resistance, Kidney Diseases, Leukemia, Lung Diseases, Lung Neoplasms, Lupus Nephritis, Mental Disorders, Monoclonal Gammopathy of Undetermined Significance, Motor Neuron Disease, Mucositis, Multiple Myeloma, Multiple Sclerosis, Myelodysplastic Syndromes, Myeloproliferative Disorders, Neoplasms, Neoplasms, Plasma Cell, Nephritis, Oral Submucous Fibrosis, Pain, Paraproteinemias, Polycythemia, Polycythemia Vera, Preleukemia, Prostatic Neoplasms, Proteinuria, Psychotic Disorders, Renal Insufficiency, Renal Insufficiency, Chronic, Sarcoma, Schizophrenia, Sclerosis, Smoldering Multiple Myeloma, Syndrome, Thrombocythemia, Essential, Thrombocytosis, Tic Disorders, Tobacco Use, Tobacco Use Disorder, Wasting Syndrome, Wounds and Injuries |
| pu-h71 | HSP90AB1, HSP90AA1 | 86 | 1 | Phase 1: Neoplasm Metastasis, Lymphoma, Myeloproliferative Disorders, Polycythemia, Polycythemia Vera, Primary Myelofibrosis, Thrombocythemia, Essential, Thrombocytosis |
| erdafitinib | PARP1, MCM2, PRDX4, MYC | 86 | 2 | Phase 2: Osteosarcoma, Anger, Brain Abscess, Breast Neoplasms, Carcinoma, Carcinoma, Hepatocellular, Carcinoma, Ovarian Epithelial, Carcinoma, Renal Cell, Central Nervous System Neoplasms, Colonic Neoplasms, Colorectal Neoplasms, Endometrial Neoplasms, Ependymoma, Esophageal Neoplasms, Glioma, Granuloma, Hepatoblastoma, Histiocytic Sarcoma, Histiocytosis, Histiocytosis, Langerhans-Cell, Kidney Neoplasms, Lung Neoplasms, Lymphoma, Lymphoma, Non-Hodgkin, Medulloblastoma, Melanoma, Multiple Myeloma, Myosarcoma, Neoplasms, Neoplasms, Germ Cell and Embryonal, Neoplasms, Plasma Cell, Nervous System Neoplasms, Neuroblastoma, Neuroectodermal Tumors, Neuroectodermal Tumors, Primitive, Neuroectodermal Tumors, Primitive, Peripheral, Non-Muscle Invasive Bladder Neoplasms, Ovarian Neoplasms, Pancreatic Neoplasms, Prostatic Neoplasms, Rectal Neoplasms, Recurrence, Rhabdoid Tumor, Rhabdomyosarcoma, Sarcoma, Sarcoma, Ewing, Skin Neoplasms, Stomach Neoplasms, Thyroid Diseases, Thyroid Neoplasms, Urinary Bladder Neoplasms, Uterine Neoplasms, Wilms Tumor, Xanthogranuloma, Juvenile |
| Ifosfamide | HSPA4 | 85 | 12 | Phase 3: Neoplasm Metastasis, Osteosarcoma, Carcinoma, Non-Small-Cell Lung, Carcinosarcoma, Chondrosarcoma, Chondrosarcoma, Mesenchymal, Choriocarcinoma, Endodermal Sinus Tumor, Endometrial Neoplasms, Fever, Fibrosarcoma, Germinoma, Glomus Tumor, Granular Cell Tumor, Histiocytoma, Histiocytoma, Benign Fibrous, Histiocytoma, Malignant Fibrous, Hodgkin Disease, Hyperthermia, Kidney Neoplasms, Leiomyosarcoma, Leukemia, Leukemia, Lymphoid, Liposarcoma, Lymphoma, Lymphoma, B-Cell, Lymphoma, Large-Cell, Anaplastic, Lymphoma, Non-Hodgkin, Myosarcoma, Neoplasms, Neoplasms, Germ Cell and Embryonal, Nerve Sheath Neoplasms, Neuroblastoma, Neuroectodermal Tumors, Neurofibrosarcoma, Ovarian Neoplasms, Pheochromocytoma, Precursor Cell Lymphoblastic Leukemia-Lymphoma, Ranula, Rhabdomyosarcoma, Sarcoma, Sarcoma, Alveolar Soft Part, Sarcoma, Clear Cell, Sarcoma, Ewing, Sarcoma, Synovial, Seminoma, Spina Bifida Occulta, Teratoma, Testicular Neoplasms |

The ***Disease trial phase*** column reflects the maximum clinical trials phase in which the drug was studied for the analyzed pathology.

## ***Repurposing drugs***

*Table 14. Repurposed drugs used in clinical trials for other pathologies (prospective drugs against the identified drug targets on the basis of literature curation in HumanPSD™ database)*
**See full table →**

| Name | Target names | Drug score | Maximum trial phase |
|---|---|---|---|
| perillyl alcohol | HSPA4, MYC | 83 | Phase 1: Lymphoma |
| alvespimycin | HSP90AB1, HSP90AA1 | 83 | Phase 1: Burkitt Lymphoma, Hodgkin Disease, Intestinal Neoplasms, Intraocular Lymphoma, Leukemia, Lymphocytic, Chronic, B-Cell, Leukemia-Lymphoma, Adult T-Cell, Lymphoma, Lymphoma, B-Cell, Marginal Zone, Lymphoma, Follicular, Lymphoma, Large B-Cell, Diffuse, Lymphoma, Large-Cell, Anaplastic, Lymphoma, Mantle-Cell, Lymphoma, Non-Hodgkin, Lymphoma, T-Cell, Lymphoma, T-Cell, Cutaneous, Mycosis Fungoides, Sezary Syndrome, Waldenstrom Macroglobulinemia |
| onalespib | HSP90AB1, HSP90AA1 | 83 | N/A |
| retaspimycin | HSP90AB1, HSP90AA1 | 83 | Phase 2: Neoplasms, Prostatic Neoplasms |
| tas-116 | PARP1, HSPA4, HSP90AB1, HSP90AA1 | 82 | Phase 1: Gastrointestinal Stromal Tumors, Neoplasms |

The ***Maximum trial phase*** column reflects the maximum clinical trials phase in which the drug was studied for any pathology.

*Table 15. Prospective drugs, predicted by PASS software to be active against the identified drug targets with predicted activity against the studied disease(s) (drug candidates predicted with the cheminformatics tool PASS)*
**See full table →**

| Name | Target names | Drug score | Target activity score |
|---|---|---|---|
| (CHLOROACETYL)CARBAMIC ACID (3R,4S,5S,5R)-5-METHOXY-4-[(2R,3R)-2-METHYL-3-(3-METHYL-2-BUTENYL)OXIRANYL]-1-OXASPIRO[2.5]OCT-6-YL ESTER | FKBP1A, FKBP4 | 91 | 5.63E-2 |
| 1-Anilino-8-Naphthalene Sulfonate | FASN, PRDX4 | 84 | 4.13E-2 |
| Pentosan Polysulfate | FKBP1A, FKBP4 | 52 | 1.22E-2 |

*Table 16. Prospective drugs, predicted by PASS software to be active against the identified drug targets, though without cheminformatically predicted activity against the studied disease(s) (drug candidates predicted with the cheminformatics tool PASS)*
**See full table →**

| Name | Target names | Drug score | Target activity score |
|---|---|---|---|
| Myo-Inositol | FASN, FKBP1A, PRDX4, MYC, SPHK1, FKBP4 | 99 | 0.14 |
| Gluconolactone | FKBP1A, MYC, SPHK1, FKBP4 | 99 | 0.16 |
| D-Galctopyranosyl-1-On | FKBP1A, MYC, SPHK1, FKBP4 | 99 | 0.16 |
| Methyl alpha-galactoside | FKBP1A, MYC, SPHK1, FKBP4 | 98 | 9.88E-2 |
| Methyl beta-galactoside | FKBP1A, MYC, SPHK1, FKBP4 | 98 | 9.88E-2 |

As the result of drug search we propose the following drugs as most promising candidates for treating the pathology under study: Temsirolimus, perillyl alcohol, (CHLOROACETYL)CARBAMIC ACID (3R,4S,5S,5R)-5-METHOXY-4-[(2R,3R)-2-METHYL-3-(3-METHYL-2-BUTENYL)OXIRANYL]-1-OXASPIRO[2.5]OCT-6-YL ESTER and Myo-Inositol. These drugs were selected for acting on the following targets: EIF4EBP1, HSPA4 and FKBP4, which were predicted to be active in the molecular mechanism of the studied pathology.

The selected drugs are top ranked drug candidates from each of the four categories of drugs: (1) FDA approved drugs or used in clinical trials drugs for the studied pathology; (2) repurposing drugs used in clinical trials for other pathologies; (3) drugs, predicted by PASS software to be active against the studied pathology; (4) drugs, predicted by PASS software to be repurposed from other pathologies.

## ***Supplementary drug info***

In addition to the approved and repurposed drugs proposed by Genome Enhancer, below the ***Supplementary drug info*** table is given, which contains an extended list of drugs used for treatment of neoplasms. Those drugs which were predicted by Genome Enhancer as prospective treatment candidates for the studied case (both approved and repurposed) have a respective ***Predicted Drug Score*** assigned to them. This value on a scale from 1 to 100 reflects the potential activity of the respective drug on the overall molecular mechanism of the studied pathology. The ***Predicted Drug Score*** column contains the "-" (Not Identified) value in case the drug targets of the respective treatment were not found in the molecular mechanism of the studied pathology.

*Table 17. Supplementary drug info: extended list of drugs used for treatment of neoplasms with respective drug scores predicted for the studied pathology.*

| Drug | Disease | Predicted Drug Score |
|---|---|---|
| Abarelix | Prostatic Neoplasms | - |
| Abemaciclib | Breast Neoplasms | 68 |
| Abiraterone | Prostatic Neoplasms, Castration-Resistant | - |
| Abiraterone acetate | Prostatic Neoplasms, Castration-Resistant | - |
| Acalabrutinib | Lymphoma, Mantle-Cell | - |
| Acitretin | Psoriasis | - |
| Ado-trastuzumab emtansine | Breast Neoplasms\|Neoplasms | 50 |
| Afatinib | Carcinoma, Non-Small-Cell Lung | - |
| Aflibercept | Colorectal Neoplasms\|Diabetic Retinopathy\|Edema\|Vascular Diseases\|Wet Macular Degeneration | - |
| Alectinib | Carcinoma, Non-Small-Cell Lung | - |
| Alemtuzumab | Brain Abscess\|Leukemia, Lymphocytic, Chronic, B-Cell\|Multiple Sclerosis\|Multiple Sclerosis, Relapsing-Remitting\|Sclerosis | - |
| Alitretinoin | Sarcoma, Kaposi | - |
| Alpelisib | Breast Neoplasms | - |
| Altretamine | Ovarian Neoplasms | - |
| Aminolevulinic acid | Keratosis\|Keratosis, Actinic | - |
| Anagrelide | Thrombocythemia, Essential\|Thrombocytosis | - |
| Anastrozole | Breast Neoplasms\|Hypersensitivity\|Obesity\|Obesity, Morbid\|Recurrence\|Weight Loss | - |
| Apalutamide | Prostatic Neoplasms, Castration-Resistant | - |
| Aprepitant | Nausea\|Neoplasms\|Postoperative Nausea and Vomiting | - |
| Arsenic trioxide | Leukemia, Promyelocytic, Acute | 27 |
| Atezolizumab | Carcinoma, Non-Small-Cell Lung\|Carcinoma, Transitional Cell\|Triple Negative Breast Neoplasms | - |
| Avelumab | Carcinoma, Merkel Cell\|Carcinoma, Renal Cell\|Carcinoma, Transitional Cell | - |
| Axitinib | Carcinoma, Renal Cell | - |
| Azacitidine | Anemia, Refractory\|Anemia, Refractory, with Excess of Blasts\|Leukemia, Myelomonocytic, Chronic\|Myelodysplastic Syndromes\|Preleukemia\|Syndrome | 33 |
| Belinostat | Lymphoma, T-Cell, Peripheral | 68 |
| Bendamustine | Leukemia, Lymphocytic, Chronic, B-Cell\|Leukemia, Lymphoid | - |
| Bevacizumab | Breast Neoplasms\|Colonic Neoplasms\|Colorectal Neoplasms\|Corneal Neovascularization\|Diabetic Retinopathy\|Dilatation, Pathologic\|Edema\|Epistaxis\|Glaucoma\|Hemorrhage\|Macular Degeneration\|Macular Edema\|Neoplasm Metastasis\|Neoplasms\|Neovascularization, Pathologic\|Optic Nerve Diseases\|Pterygium\|Rectal Neoplasms\|Retinal Detachment\|Retinal Diseases\|Retinal Vein Occlusion\|Telangiectasia, Hereditary Hemorrhagic\|Telangiectasis\|Vitreous Hemorrhage | - |
| Bexarotene | Lymphoma, T-Cell\|Lymphoma, T-Cell, Cutaneous | - |
| Bicalutamide | Prostatic Neoplasms | - |
| Binimetinib | Melanoma | - |
| Blinatumomab | Precursor B-Cell Lymphoblastic Leukemia-Lymphoma | - |
| Bortezomib | Brain Abscess\|Glomerulonephritis\|Glomerulonephritis, IGA\|Kidney Diseases\|Multiple Myeloma\|Neoplasms, Plasma Cell\|Nephritis\|Renal Insufficiency | 25 |
| Bosutinib | Leukemia, Myelogenous, Chronic, BCR-ABL Positive | - |
| Brentuximab vedotin | Hodgkin Disease\|Lymphoma\|Lymphoma, Large-Cell, Anaplastic\|Lymphoma, T-Cell, Peripheral | - |
| Brigatinib | Carcinoma, Non-Small-Cell Lung | 36 |
| Buserelin | Prostatic Neoplasms | - |
| Cabazitaxel | Prostatic Neoplasms, Castration-Resistant | 71 |
| Cabergoline | Drug-Related Side Effects and Adverse Reactions\|Pituitary Neoplasms | - |
| Cabozantinib | Thyroid Neoplasms | - |
| Capecitabine | Breast Neoplasms\|Colonic Neoplasms\|Colorectal Neoplasms | - |
| Carboplatin | Carcinoma, Non-Small-Cell Lung\|Lung Neoplasms\|Neoplasms\|Neuroendocrine Tumors\|Ovarian Neoplasms\|Retinoblastoma | - |
| Carfilzomib | Multiple Myeloma | 40 |
| Carmustine | Astrocytoma\|Glioblastoma\|Hodgkin Disease\|Medulloblastoma\|Multiple Myeloma\|Neoplasms | - |
| Ceritinib | Carcinoma, Non-Small-Cell Lung | 32 |
| Cetuximab | Colorectal Neoplasms | - |
| Cinacalcet | Anemia\|Calcinosis\|Cardiovascular Diseases\|Hyperparathyroidism\|Hyperparathyroidism, Secondary\|Kidney Diseases\|Kidney Failure, Chronic\|Neoplasm Metastasis\|Neoplasms\|Parathyroid Neoplasms\|Renal Insufficiency\|Vascular Calcification\|Vascular Diseases\|Vision Disorders | - |
| Cisplatin | Carcinoma, Squamous Cell\|Neoplasms\|Uterine Cervical Neoplasms\|Carcinoma, Non-Small-Cell Lung\|Esophageal Neoplasms\|Carcinoma | - |
| Cladribine | Leukemia, Hairy Cell | 30 |

| | | |
|---|---|---|
| Clofarabine | Precursor Cell Lymphoblastic Leukemia-Lymphoma | 25 |
| Cobimetinib | Melanoma | - |
| Copanlisib | Lymphoma, Follicular | 46 |
| Crizotinib | Carcinoma, Non-Small-Cell Lung | - |
| Cyproterone acetate | Prostatic Neoplasms | - |
| Dabrafenib | Melanoma | - |
| Dacomitinib | Carcinoma, Non-Small-Cell Lung | 39 |
| Daratumumab | Multiple Myeloma | - |
| Dasatinib | Leukemia, Myelogenous, Chronic, BCR-ABL Positive\|Leukemia, Myeloid, Chronic-Phase\|Precursor Cell Lymphoblastic Leukemia-Lymphoma | 6 |
| Decitabine | Anemia, Refractory\|Anemia, Refractory, with Excess of Blasts\|Leukemia, Myelomonocytic, Chronic\|Myelodysplastic Syndromes | - |
| Degarelix | Cardiovascular Diseases\|Prostatic Neoplasms\|Vascular Diseases | - |
| Denosumab | Arthritis, Rheumatoid\|Bone Diseases\|Bone Diseases, Metabolic\|Breast Neoplasms\|Hyperparathyroidism\|Hyperparathyroidism, Primary\|Metabolic Diseases\|Neoplasm Metastasis\|Neoplasms\|Osteoporosis\|Osteoporosis, Postmenopausal\|Prostatic Neoplasms | - |
| Dexrazoxane | Breast Neoplasms\|Cardiomyopathies | - |
| Dienogest | Menorrhagia | - |
| Dinutuximab | Neuroblastoma | - |
| Docetaxel | Breast Neoplasms\|Carcinoma, Non-Small-Cell Lung\|Prostatic Neoplasms\|Squamous Cell Carcinoma of Head and Neck\|Stomach Neoplasms | - |
| Doxorubicin | Neoplasms\|Multiple Myeloma\|Carcinoma, Ovarian Epithelial\|Ovarian Neoplasms\|Leukemia, Lymphoid\|Breast Neoplasms\|Lymphoma, Follicular\|Thyroid Neoplasms\|Triple Negative Breast Neoplasms\|Glioma | - |
| Durvalumab | Carcinoma, Non-Small-Cell Lung\|Carcinoma, Transitional Cell | - |
| Dutasteride | Alcoholism\|Hyperplasia\|Hypertrophy\|Neoplasms\|Prostatic Hyperplasia | - |
| Duvelisib | Leukemia, Lymphocytic, Chronic, B-Cell\|Lymphoma, Follicular | - |
| Elotuzumab | Multiple Myeloma | - |
| Enasidenib | Leukemia, Myeloid, Acute | - |
| Encorafenib | Colorectal Neoplasms\|Melanoma | - |
| Enfortumab vedotin | Carcinoma, Transitional Cell\|Neoplasms | - |
| Entrectinib | Carcinoma, Non-Small-Cell Lung | - |
| Enzalutamide | Prostatic Neoplasms\|Prostatic Neoplasms, Castration-Resistant | - |
| Epirubicin | Breast Neoplasms | - |
| Erdafitinib | Urinary Bladder Neoplasms | 86 |
| Eribulin | Breast Neoplasms\|Drug-Related Side Effects and Adverse Reactions\|Neoplasms | - |
| Erlotinib | Carcinoma, Non-Small-Cell Lung\|Neoplasms\|Pancreatic Neoplasms | 20 |
| Erlotinib hydrochloride | Carcinoma, Non-Small-Cell Lung\|Gastrointestinal Stromal Tumors | - |
| Estramustine | Prostatic Neoplasms | - |
| Ethinyl Estradiol | Acne Vulgaris\|Neoplasms | - |
| Everolimus | Angiomyolipoma\|Arthrogryposis\|Astrocytoma\|Breast Neoplasms\|Carcinoma, Renal Cell\|Cysts\|Idiopathic Pulmonary Fibrosis\|Kidney Diseases, Cystic\|Kidney Failure, Chronic\|Lipoma\|Neuroendocrine Tumors\|Primary Graft Dysfunction\|Sclerosis\|Tuberous Sclerosis | - |
| Exemestane | Breast Neoplasms | - |
| Fedratinib | Primary Myelofibrosis | - |
| Finasteride | Hyperplasia\|Neoplasms\|Prostatic Hyperplasia | - |
| Flavopiridol | Leukemia, Lymphocytic, Chronic, B-Cell | 5 |
| Fluorouracil | Skin Neoplasms\|Neoplasms, Basal Cell\|Neoplasms, Second Primary\|Neoplasms, Squamous Cell\|Neoplasms\|Colorectal Neoplasms\|Pancreatic Neoplasms | 28 |
| Fluoxymesterone | Breast Neoplasms\|Hypogonadism\|Puberty, Delayed | - |
| Flutamide | Premenstrual Dysphoric Disorder\|Premenstrual Syndrome\|Prostatic Neoplasms | 57 |
| Fulvestrant | Breast Neoplasms | - |
| Gefitinib | Carcinoma, Non-Small-Cell Lung | 16 |
| Gemcitabine | Breast Neoplasms\|Carcinoma, Non-Small-Cell Lung\|Ovarian Neoplasms\|Pancreatic Neoplasms | - |
| Gemtuzumab ozogamicin | Leukemia, Myeloid, Acute | - |
| Gilteritinib | Leukemia, Myeloid, Acute | 63 |
| Glasdegib | Leukemia, Myeloid, Acute | - |
| Goserelin | Atrophy\|Breast Neoplasms\|Bulbo-Spinal Atrophy, X-Linked\|Endometriosis\|Muscular Atrophy\|Myoma\|Prostatic Neoplasms | - |
| Histrelin | Puberty, Precocious | - |
| Homoharringtonine | Leukemia, Myelogenous, Chronic, BCR-ABL Positive | 59 |
| Ibritumomab | Lymphoma, B-Cell\|Lymphoma, Follicular | - |
| Ibrutinib | Graft vs Host Disease\|Leukemia, Lymphocytic, Chronic, B-Cell\|Lymphoma, B-Cell, Marginal Zone\|Lymphoma, Mantle-Cell\|Waldenstrom Macroglobulinemia | - |

| | | |
|---|---|---|
| Idarubicin | Leukemia, Myeloid, Acute | - |
| Idelalisib | Leukemia, Lymphocytic, Chronic, B-Cell\|Lymphoma, Follicular | - |
| Ifosfamide | Neoplasms | 85 |
| Imatinib | Leukemia, Myelogenous, Chronic, BCR-ABL Positive\|Mastocytosis, Systemic\|Neoplasms | 24 |
| Inotuzumab ozogamicin | Precursor B-Cell Lymphoblastic Leukemia-Lymphoma | - |
| Ipilimumab | Carcinoma, Renal Cell\|Melanoma | - |
| Irinotecan | Colorectal Neoplasms | 34 |
| Ivosidenib | Leukemia, Myeloid, Acute | - |
| Ixabepilone | Breast Neoplasms | - |
| Ixazomib | Multiple Myeloma | - |
| Lapatinib | Breast Neoplasms | 22 |
| Larotrectinib | Neoplasm Metastasis | - |
| Lenalidomide | Brain Abscess\|Lupus Erythematosus, Cutaneous\|Myelodysplastic Syndromes\|Neoplasms, Plasma Cell | 45 |
| Lenvatinib | Carcinoma, Hepatocellular\|Carcinoma, Renal Cell\|Thyroid Neoplasms | - |
| Letrozole | Breast Neoplasms\|Cysts\|Fibroma\|Myofibroma\|Myoma\|Ovarian Cysts\|Syndrome | - |
| Leuprolide | Hot Flashes\|Ovarian Hyperstimulation Syndrome\|Prostatic Neoplasms\|Puberty, Precocious | - |
| Levamisole | Ascariasis\|Colonic Neoplasms\|Helminthiasis | - |
| Levonorgestrel | Epilepsy\|Hyperplasia\|Menorrhagia | - |
| Lomustine | Brain Neoplasms\|Hodgkin Disease | - |
| Lonafarnib | Breast Neoplasms\|Carcinoma, Non-Small-Cell Lung\|Central Nervous System Neoplasms\|Colorectal Neoplasms\|Gliosarcoma\|Head and Neck Neoplasms\|Leukemia, Myelomonocytic, Chronic\| Liver Neoplasms\|Lymphoma\|Myelodysplastic Syndromes\|Ovarian Neoplasms\|Urethral Neoplasms\|Urinary Bladder Neoplasms | - |
| Lorlatinib | Carcinoma, Non-Small-Cell Lung | - |
| Masoprocol | Keratosis, Actinic | - |
| Medroxyprogesterone Acetate | Depression\|Depression, Postpartum\|Depressive Disorder\|Metrorrhagia\|Neoplasms\|Uterine Hemorrhage | - |
| Megestrol acetate | Acquired Immunodeficiency Syndrome\|Bites and Stings\|Breast Neoplasms\|Pain\|Wasting Syndrome | - |
| Methotrexate | Neoplasms\|Breast Neoplasms\|Head and Neck Neoplasms\|Ovarian Neoplasms\|Lymphoma, T-Cell, Peripheral\|Brain Neoplasms\|Colorectal Neoplasms\|Neuroblastoma\|Carcinoma, Squamous Cell | 30 |
| Methyltestosterone | Breast Neoplasms\|Hypogonadism\|Puberty, Delayed | - |
| Midostaurin | Leukemia, Mast-Cell\|Leukemia, Myeloid, Acute\|Mastocytosis, Systemic | 17 |
| Mitotane | Adrenocortical Carcinoma | - |
| Mitoxantrone | Autoimmune Diseases\|Autoimmune Diseases of the Nervous System\|Demyelinating Autoimmune Diseases, CNS\|Immune System Diseases\|Leukemia, Myeloid, Acute\|Multiple Sclerosis\|Myelitis\|Myelitis, Transverse\|Nervous System Diseases\|Neuromyelitis Optica\|Prostatic Neoplasms, Castration-Resistant | - |
| Mogamulizumab | Mycosis Fungoides\|Neoplasms\|Sezary Syndrome | - |
| Moxetumomab pasudotox | Leukemia, Hairy Cell\|Neoplasms | - |
| Necitumumab | Carcinoma, Non-Small-Cell Lung\|Neoplasms | - |
| Nelarabine | Precursor T-Cell Lymphoblastic Leukemia-Lymphoma | - |
| Neratinib | Breast Neoplasms | 27 |
| Nilotinib | Blast Crisis\|Leukemia, Myelogenous, Chronic, BCR-ABL Positive\|Leukemia, Myeloid, Chronic-Phase | - |
| Nilutamide | Prostatic Neoplasms | - |
| Nintedanib | Fibrosis\|Idiopathic Pulmonary Fibrosis | - |
| Niraparib | Carcinoma, Ovarian Epithelial\|Fallopian Tube Neoplasms\|Peritoneal Neoplasms | 85 |
| Nivolumab | Carcinoma, Non-Small-Cell Lung\|Kidney Neoplasms\|Neoplasms\|Lung Neoplasms\|Melanoma | - |
| Obinutuzumab | Leukemia, Lymphocytic, Chronic, B-Cell | - |
| Octreotide | Acromegaly\|Adenoma\|Ascites\|Carcinoid Tumor\|Fistula\|Pancreatic Fistula\|Pituitary Diseases\|Renal Insufficiency\|Vipoma | - |
| Ofatumumab | Leukemia, Lymphocytic, Chronic, B-Cell | - |
| Olaparib | Breast Neoplasms\|Carcinoma, Ovarian Epithelial\|Fallopian Tube Neoplasms\|Ovarian Neoplasms\|Pancreatic Neoplasms\|Peritoneal Neoplasms\|Prostatic Neoplasms, Castration-Resistant | 77 |
| Olaratumab | Sarcoma | - |
| Osimertinib | Carcinoma, Non-Small-Cell Lung | - |
| Oxaliplatin | Colonic Neoplasms\|Colorectal Neoplasms\|Neoplasms\|Rectal Neoplasms | - |
| Paclitaxel | Acute Coronary Syndrome\|Angina Pectoris\|Arteriosclerosis\|Breast Neoplasms\|Carcinoma, Non-Small-Cell Lung\|Cardiovascular Diseases\|Coronary Artery Disease\|Coronary Disease\|Coronary Stenosis\|Heart Diseases\|Myocardial Ischemia\|Ovarian Neoplasms\|Vascular Diseases | 72 |
| Palbociclib | Breast Neoplasms | - |
| Panitumumab | Colorectal Neoplasms | - |
| Panobinostat | Multiple Myeloma | - |
| Pazopanib | Carcinoma\|Carcinoma, Renal Cell\|Sarcoma | 22 |

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|---|---|---|
| Pembrolizumab | Carcinoma, Hepatocellular\|Carcinoma, Merkel Cell\|Carcinoma, Non-Small-Cell Lung\|Carcinoma, Renal Cell\|Carcinoma, Transitional Cell\|Hodgkin Disease\|Melanoma\|Neoplasms\|Stomach Neoplasms | - |
| Pemetrexed | Carcinoma, Non-Small-Cell Lung\|Mesothelioma | - |
| Pentostatin | Leukemia, Hairy Cell | - |
| Pertuzumab | Breast Neoplasms | - |
| Pomalidomide | Multiple Myeloma | 85 |
| Ponatinib | Leukemia, Myelogenous, Chronic, BCR-ABL Positive\|Precursor Cell Lymphoblastic Leukemia-Lymphoma | - |
| Pralatrexate | Lymphoma, T-Cell, Peripheral | - |
| Radium Ra 223 Dichloride | Prostatic Neoplasms, Castration-Resistant | - |
| Ramucirumab | Stomach Neoplasms | - |
| Rasburicase | Hyperuricemia\|Leukemia\|Lymphoma\|Neoplasms\|Syndrome\|Tumor Lysis Syndrome | - |
| Regorafenib | Colorectal Neoplasms | - |
| Relugolix | Prostatic Neoplasms | - |
| Ribociclib | Breast Neoplasms | - |
| Rituximab | Arthritis\|Arthritis, Rheumatoid\|Granulomatosis with Polyangiitis\|Leukemia\|Leukemia, Lymphoid\|Lymphoma\|Lymphoma, B-Cell\|Lymphoma, Follicular\|Lymphoma, Non-Hodgkin\|Myelitis\|Neuromyelitis Optica\|Purpura\|Purpura, Thrombocytopenic\|Purpura, Thrombocytopenic, Idiopathic\|Thrombocytopenia | - |
| Romidepsin | Lymphoma, T-Cell, Cutaneous | 34 |
| Rucaparib | Carcinoma, Ovarian Epithelial\|Fallopian Tube Neoplasms\|Peritoneal Neoplasms\|Prostatic Neoplasms, Castration-Resistant | 40 |
| Ruxolitinib | Graft vs Host Disease\|Polycythemia\|Polycythemia Vera\|Primary Myelofibrosis\|Thrombocytosis | - |
| Selinexor | Multiple Myeloma | - |
| Selumetinib | Neurofibromatosis 1 | - |
| Siltuximab | Giant Lymph Node Hyperplasia | - |
| Sirolimus | Angiomyolipoma\|Constriction, Pathologic\|Coronary Restenosis\|Eye Diseases\|Immune System Diseases\|Kidney Failure, Chronic\|Lipoma\|Tuberous Sclerosis | 70 |
| Sonidegib | Carcinoma, Basal Cell | - |
| Sorafenib | Carcinoma, Hepatocellular\|Carcinoma, Renal Cell\|Thyroid Neoplasms | 14 |
| Sunitinib | Adenoma\|Carcinoma, Renal Cell\|Digestive System Neoplasms\|Gastrointestinal Neoplasms\|Gastrointestinal Stromal Tumors\|Intestinal Neoplasms | 9 |
| Talazoparib | Breast Neoplasms | 62 |
| Tamoxifen | Breast Diseases\|Cystic Fibrosis\|Cysts\|Fibroadenoma\|Fibrocystic Breast Disease\|Hemorrhage\|Menorrhagia\|Menstruation Disturbances\|Metrorrhagia\|Neoplasms | 24 |
| Tamsulosin | Calculi\|Coronary Artery Disease\|Heart Diseases\|Hernia\|Hernia, Inguinal\|Inflammation\|Ischemia\|Lithiasis\|Lower Urinary Tract Symptoms\|Myocardial Ischemia\|Prostatic Hyperplasia\|Ureteral Calculi\|Urinary Calculi\|Urolithiasis\|Urologic Diseases | - |
| Temozolomide | Astrocytoma\|Nervous System Neoplasms | 30 |
| Temsirolimus | Carcinoma, Renal Cell | 90 |
| Teniposide | Precursor Cell Lymphoblastic Leukemia-Lymphoma | 30 |
| Thalidomide | Brain Abscess\|Immune System Diseases\|Multiple Myeloma\|Neoplasms, Plasma Cell | 20 |
| Tivozanib | Carcinoma, Renal Cell | - |
| Tocilizumab | Arthritis\|Arthritis, Juvenile\|Arthritis, Rheumatoid\|Behavior\|Cytokine Release Syndrome\|Giant Cell Arteritis\|Neurobehavioral Manifestations\|Oral Manifestations\|Psychotic Disorders\|Schizophrenia\|Tic Disorders | - |
| Topotecan | Small Cell Lung Carcinoma | - |
| Toremifene | Breast Neoplasms | - |
| Trabectedin | Leiomyosarcoma\|Liposarcoma | - |
| Trametinib | Carcinoma, Non-Small-Cell Lung\|Melanoma | 50 |
| Trastuzumab | Breast Neoplasms\|Neoplasms | - |
| Tretinoin | Lentigo | 10 |
| Triptorelin | Fatty Liver\|Hypogonadism\|Infertility, Female\|Prostatic Neoplasms | 54 |
| Tucatinib | Breast Neoplasms | - |
| Valrubicin | Urinary Bladder Neoplasms | - |
| Vandetanib | Thyroid Neoplasms | 13 |
| Vemurafenib | Melanoma | - |
| Venetoclax | Leukemia, Lymphocytic, Chronic, B-Cell\|Leukemia, Myeloid, Acute | - |
| Vinblastine | Glioma | 19 |
| Vincristine | Precursor Cell Lymphoblastic Leukemia-Lymphoma | 51 |
| Vinorelbine | Carcinoma, Non-Small-Cell Lung | - |
| Vismodegib | Carcinoma, Basal Cell | - |
| Vorinostat | Lymphoma, T-Cell, Cutaneous | 32 |

| | | |
|---|---|---|
| Zoledronate | Arthritis\|Bone Marrow Diseases\|Brain Abscess\|Chronic Kidney Disease-Mineral and Bone Disorder\|Chronic Periodontitis\|HIV Infections\|Hypersensitivity\|Infections\|Kidney Diseases\|Metabolic Diseases\|Multiple Myeloma\|Neoplasms\|Neoplasms, Plasma Cell\|Neoplasms, Second Primary\|Osteitis\|Osteoarthritis\|Periodontitis\|Pleural Effusion, Malignant\|Prostatic Neoplasms\|Renal Insufficiency, Chronic\|Thalassemia\|Wounds and Injuries | - |

# 6. Conclusion

We applied the software package "Genome Enhancer" to a multi-omics data set that contains *transcriptomics and proteomics* data. The study is done in the context of *Neoplasm Metastasis and Osteosarcoma*. The data were pre-processed, statistically analyzed and differentially expressed genes were identified. Also checked was the enrichment of GO or disease categories among the studied gene sets.

We propose the following drugs as most promising candidates for treating the pathology under study:

**Temsirolimus, perillyl alcohol, (CHLOROACETYL)CARBAMIC ACID (3R,4S,5S,5R)-5-METHOXY-4-[(2R,3R)-2-METHYL-3-(3-METHYL-2-BUTENYL)OXIRANYL]-1-OXASPIRO[2.5]OCT-6-YL ESTER and Myo-Inositol**

These drugs were selected for acting on the following targets: EIF4EBP1, HSPA4 and FKBP4, which were predicted to be involved in the molecular mechanism of the pathology under study.

The identified molecular mechanism of the studied pathology was predicted to be mainly based on the following key drug targets:

**NCL, proCaspase-3(h):Hsp10(h):Hsp60, HSPA4 and Trx1**

These potential drug targets should be considered as a prospective research initiative for further drug repurposing and drug development purposes. The following drugs were predicted as, matching those drug targets: 2-Amino-5-Hydroxy-Benzimidazole, PX-12, perillyl alcohol, Curcumin, Cetrorelix and Fructose. These drugs should be considered with special caution for research purposes only.

In this study, we came up with a detailed signal transduction network regulating differentially expressed genes in the studied pathology. In this network we have revealed the following top master regulators (signaling proteins and their complexes) that play a crucial role in the molecular mechanism of the studied pathology, which can be proposed as the most promising molecular targets for further drug repurposing and drug development initiatives.

- NCL
- proCaspase-3(h):Hsp10(h):Hsp60
- HSPA4
- Trx1

Potential drug compounds which can be affecting these targets can be found in the "Finding prospective drug targets" section.

# 7. Methods

### Databases used in the study

Transcription factor binding sites in promoters and enhancers of differentially expressed genes were analyzed using known DNA-binding motifs described in the TRANSFAC® library, release 2024.1 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transfac).
The master regulator search uses the TRANSPATH® database (BIOBASE), release 2024.1 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transpath). A comprehensive signal transduction network of human cells is built by the software on the basis of reactions annotated in TRANSPATH®.
The information about drugs corresponding to identified drug targets and clinical trials references were extracted from HumanPSD™ database, release 2024.1 (https://genexplain.com/humanpsd).
The Ensembl database release Human104.38 (hg38) (http://www.ensembl.org) was used for gene IDs representation and Gene Ontology (GO) (http://geneontology.org) was used for functional classification of the studied gene set.

## Methods for the analysis of enriched transcription factor binding sites and composite modules

Transcription factor binding sites in promoters and enhancers of differentially expressed genes were analyzed using known DNA-binding motifs. The motifs are specified using position weight matrices (PWMs) that give weights to each nucleotide in each position of the DNA binding motif for a transcription factor or a group of them.
We search for transcription factor binding sites (TFBS) that are enriched in the promoters and enhancers under study as compared to a background sequence set such as promoters of genes that were not differentially regulated under the condition of the experiment. We denote study and background sets briefly as Yes and No sets. In the current work we used a workflow considering promoter sequences of a standard length of 1100 bp (-1000 to +100). The error rate in this part of the pipeline is controlled by estimating the adjusted p-value (using the Benjamini-Hochberg procedure) in comparison to the TFBS frequency found in randomly selected regions of the human genome (adj.p-value < 0.01).
We have applied the CMA algorithm (Composite Module Analyst) for searching composite modules [7] in the promoters and enhancers of the Yes and No sets. We searched for a composite module consisting of a cluster of 10 TFs in a sliding window of 200-300 bp that statistically significantly separates sequences in the Yes and No sets (minimizing Wilcoxon p-value).

## Methods for finding master regulators in networks

We searched for master regulator molecules in signal transduction pathways upstream of the identified transcription factors. The master regulator search uses a comprehensive signal transduction network of human cells. The main algorithm of the master regulator search has been described earlier [3,4]. The goal of the algorithm is to find nodes in the global signal transduction network that may potentially regulate the activity of a set of transcription factors found at the previous step of the analysis. Such nodes are considered as most promising drug targets, since any influence on such a node may switch the transcriptional programs of hundreds of genes that are regulated by the respective TFs. In our analysis, we have run the algorithm with a maximum radius of 12 steps upstream of each TF in the input set. The error rate of this algorithm is controlled by applying it 10000 times to randomly generated sets of input transcription factors of the same set-size. Z-score and FDR value of ranks are calculated then for each potential master regulator node on the basis of such random runs (see detailed description in [9]). We control the error rate by the FDR threshold 0.05.

## Methods for analysis of pharmaceutical compounds

We seek for the optimal combination of molecular targets (key elements of the regulatory network of the cell) that potentially interact with pharmaceutical compounds from a library of known drugs and biologically active chemical compounds, using information about known drugs from HumanPSD™ and predicting potential drugs using PASS program.

### *Method for analysis of known pharmaceutical compounds*

We selected compounds from HumanPSD™ database that have at least one target. Next, we sort compounds using "*Drug rank*" that is the sum of the following ranks:

1. ranking by "Target activity score" (*T-score*$_{PSD}$),
2. ranking by "Disease activity score" (*D-score*$_{PSD}$),
3. ranking by "Clinical validity score".

"Target activity score" ( *T-score*$_{PSD}$) is calculated as follows:

$$T\text{-}score_{PSD} = -\frac{|T|}{|T| + w(|AT| - |T|))} \sum_{t \in T} log_{10}\left(\frac{rank(t)}{1 + maxRank(T)}\right),$$

where *T* is set of all targets related to the compound intersected with input list, |*T*| is number of elements in *T*, *AT* and |*AT*| are set set of all targets related to the compound and number of elements in it, *w* is weight multiplier, *rank(t)* is rank of given target, *maxRank(T)* equals *max(rank(t))* for all targets *t* in *T*.
We use following formula to calculate "Disease activity score" ( *D-score*$_{PSD}$):

$$D\text{-}score_{PSD} = \begin{cases} \sum_{d \in D} \sum_{p \in P} phase(d,p) \\ 0, \ D = \varnothing \end{cases},$$

where *D* is the set of selected diseases, and if *D* is empty set, *D-score*$_{PSD}$*=0*. *P* is a set of all known phases for each disease, *phase(p,d)* equals to the phase number if there are known clinical trials for the selected disease on this phase and zero otherwise.
The clinical validity score reflects the number of the highest clinical trials phase (from 1 to 4) on which the drug was ever tested for any pathology.

In this study, the focus was put on compounds with high pharmacological efficiency and low toxicity. For this purpose, comprehensive library of chemical compounds and drugs was subjected to a SAR/QSAR analysis. This library contains 13040 compounds along with their pre-calculated potential pharmacological activities of those substances, their possible side and toxic effects, as well as the possible mechanisms of action. All biological activities are expressed as probability values for a substance to exert this activity (*Pa*).
We selected compounds that satisfied the following conditions:

1. Toxicity below a chosen toxicity threshold (defines as *Pa*, probability to be active as toxic substance).
2. For all predicted pharmacological effects that correspond to a set of user selected disease(s) *Pa* is greater than a chosen effect threshold.
3. There are at least 2 targets (corresponding to the predicted activity-mechanisms) with predicted *Pa* greater than a chosen target threshold.

The maximum *Pa* value for all toxicities corresponding to the given compound is selected as the "Toxicity score". The maximum *Pa* value for all activities corresponding to the selected diseases for the given compound is used as the "Disease activity score". "Target activity score" (T-score) is calculated as follows:

$$T\text{-}score(s) = \frac{|T|}{|T| + w(|AT| - |T|))} \sum_{m \in M(s)} \left( pa(m) \sum_{g \in G(m)} IAP(g) optWeight(g) \right),$$

where *M(s)* is the set of activity-mechanisms for the given structure (which passed the chosen threshold for activity-mechanisms *Pa*); *G(m)* is the set of targets (converted to genes) that corresponds to the given activity-mechanism (m) for the given compound; *pa(m)* is the probability to be active of the activity-mechanism (m), *IAP(g)* is the invariant accuracy of prediction for gene from *G(m)*; *optWeight(g)* is the additional weight multiplier for gene. *T* is set of all targets related to the compound intersected with input list, |*T*| is number of elements in *T*, *AT* and |*AT*| are set set of all targets related to the compound and number of elements in it, *w* is weight multiplier.
"Druggability score" (D-score) is calculated as follows:

$$D\text{-}score(g) = IAP(g) \sum_{s \in S(g)} \sum_{m \in M(s,g)} pa(m),$$

where *S(g)* is the set of structures for which target list contains given target, *M(s,g)* is the set of activity-mechanisms (for the given structure) that corresponds to the given gene, *pa(m)* is the probability to be active of the activity-mechanism (m), *IAP(g)* is the invariant accuracy of prediction for the given gene.

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## Thank you for using the Genome Enhancer!

In case of any questions please contact us at support@genexplain.com

## Supplementary material

1. Supplementary table 1 - Up-regulated genes
2. Supplementary table 2 - Down-regulated genes
3. Supplementary table 3 - Detailed report. Composite modules and master regulators (up-regulated genes in Myc_induce vs. Control).
4. Supplementary table 4 - Detailed report. Composite modules and master regulators (down-regulated genes in Myc_induce vs. Control).
5. Supplementary table 5 - Detailed report. Pharmaceutical compounds and drug targets.

## Disclaimer

Decisions regarding care and treatment of patients should be fully made by attending doctors. The predicted chemical compounds listed in the report are given only for doctor's consideration and they cannot be treated as prescribed medication. It is the physician's responsibility to independently decide whether any, none or all of the predicted compounds can be used solely or in combination for patient treatment purposes, taking into account all applicable information regarding FDA prescribing recommendations for any therapeutic and the patient's condition, including, but not limited to, the patient's and family's medical history, physical examinations, information from various diagnostic tests, and patient preferences in accordance with the current standard of care. Whether or not a particular patient will benefit from a selected therapy is based on many factors and can vary significantly.

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