

# PSMA7 and PSMC5 are promising druggable targets for treating Squamous Cell Carcinoma that control activity of TP53, JUN and SMAD3 transcription factors on promoters of differentially expressed genes

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Genome Enhancer release 2.3 (TRANSFAC®, TRANSPATH® and HumanPSD™ release 2021.1)



## Abstract

In the present study we applied the software package "Genome Enhancer" to a data set that contains *transcriptomics* data. The study is done in the context of *Squamous Cell Carcinoma*. 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: TP53, JUN, NFATC2, SMAD3, SMAD2 and FOS. The subsequent network analysis suggested

- IL-1beta-p17:IL-1RI:IL-1RAcP:MyD88:tollip:IRAK-1{pS376}{pT387}:IRAK-4:IRAK-2
- PP2A
- 26S proteasome

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: Dasatinib, Bosutinib and {(2Z)-4-AMINO-2-[(4-METHOXYPHENYL)IMINO]-2,3-DIHYDRO-1,3-THIAZOL-5-YL}(4-METHOXYPHENYL)METHANONE.

## 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 devised 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
SRR349741.fastq	Transcriptomics
SRR349742.fastq	Transcriptomics
SRR349748.fastq	Transcriptomics
SRR349749.fastq	Transcriptomics

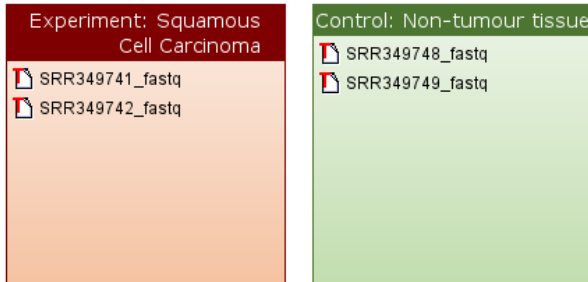


Figure 1. Annotation diagram of experimental data used in this study. With the colored boxes we show those sub-categories of the data that are compared in our analysis.

## 3. Results

We have compared the following conditions: Experiment: Squamous Cell Carcinoma *versus* Control: Non-tumour tissue.

### 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 edgeR tool (R/Bioconductor package integrated into our pipeline) and compared gene expression in the following sets: "Experiment: Squamous Cell Carcinoma" with "Control: Non-tumour tissue". edgeR 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 4994 upregulated genes (LogFC>0) out of which 1436 genes were found as significantly upregulated (p-value<0.1) and 3767 downregulated genes (LogFC<0) out of which 513 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 Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

[See full table](#) →

ID	Gene symbol	Gene description	logFC	logCPM	PValue	FDR
ENSG00000115758	ODC1	ornithine decarboxylase 1	7.17	10.32	2.21E-11	6.44E-8
ENSG00000148053	NTRK2	neurotrophic receptor tyrosine kinase 2	6.48	9.32	5.21E-11	1.14E-7
ENSG00000113140	SPARC	secreted protein acidic and cysteine rich	6.14	10.69	2.91E-9	2.03E-6
ENSG00000163359	COL6A3	collagen type VI alpha 3 chain	5.68	9.13	2.4E-8	1E-5
ENSG00000120708	TGFBI	transforming growth factor beta induced	5.24	8.77	6.25E-10	6.08E-7
ENSG00000134871	COL4A2	collagen type IV alpha 2 chain	5.14	7.97	1.36E-10	2.38E-7
ENSG00000186340	THBS2	thrombospondin 2	5.1	8.46	2.19E-7	5.04E-5
ENSG00000146648	EGFR	epidermal growth factor receptor	4.92	9.64	4.36E-6	5.44E-4
ENSG00000144824	PHLDB2	pleckstrin homology like domain family B member 2	4.9	8.29	3.7E-9	2.03E-6
ENSG00000145824	CXCL14	C-X-C motif chemokine ligand 14	4.89	8.54	1.11E-7	3.05E-5

Table 4. Top ten significant **down-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

[See full table](#) →

ID	Gene symbol	Gene description	logFC	logCPM	PValue	FDR
ENSG00000136155	SCEL	scellin	-7.36	10.74	2.01E-12	1.76E-8
ENSG00000163209	SPRR3	small proline rich protein 3	-6.39	14.08	2.27E-5	2E-3
ENSG00000143369	ECM1	extracellular matrix protein 1	-6.04	10.66	2.28E-9	1.82E-6
ENSG00000189334	S100A14	S100 calcium binding protein A14	-6	10.05	7.93E-10	6.95E-7
ENSG00000229732	AC019349.1	novel transcript	-5.88	12.56	3.53E-9	2.03E-6
ENSG00000086548	CEACAM6	CEA cell adhesion molecule 6	-5.82	9.92	2.89E-10	3.61E-7
ENSG00000171401	KRT13	keratin 13	-5.76	14.53	2.55E-8	1.02E-5
ENSG00000087128	TMPRSS11E	transmembrane serine protease 11E	-5.67	9.79	2.03E-8	8.91E-6
ENSG00000197632	SERPINB2	serpin family B member 2	-5.5	8.35	1.72E-10	2.51E-7
ENSG00000165272	AQP3	aquaporin 3 (Gill blood group)	-5.46	10.95	2.63E-6	3.78E-4

### 3.2. Regulatory regions of target genes

We mapped the uploaded Epigenomic peaks on the **target genes** and selected those peaks only that were found located in the body of the gene (in exons or introns of the genes) or in the 5000 nucleotide long flanking regions of the genes. In the tables below we demonstrate localization of such potential regulatory regions in the top up-regulated and down-regulated genes.

Table 3. Top ten **up-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue with epigenomic peaks.

[See full table](#) →

ID	Gene symbol	Gene schematic representation
ENSG00000115758	ODC1	
ENSG00000148053	NTRK2	
ENSG00000113140	SPARC	
ENSG00000163359	COL6A3	
ENSG00000120708	TGFBI	
ENSG00000134871	COL4A2	
ENSG00000186340	THBS2	
ENSG00000146648	EGFR	
ENSG00000144824	PHLDB2	
ENSG00000187134	AKR1C1	

Table 5. Top ten **down-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue with epigenomic peaks.

[See full table](#) →

ID	Gene symbol	Gene schematic representation
ENSG00000163209	SPRR3	
ENSG00000189334	S100A14	
ENSG00000136689	IL1RN	
ENSG00000134531	EMP1	
ENSG00000092295	TGM1	
ENSG00000021355	SERPINB1	
ENSG00000167757	KLK11	
ENSG00000059728	MXD1	
ENSG00000244094	SPRR2F	
ENSG00000177191	B3GNT8	

### 3.3. 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.

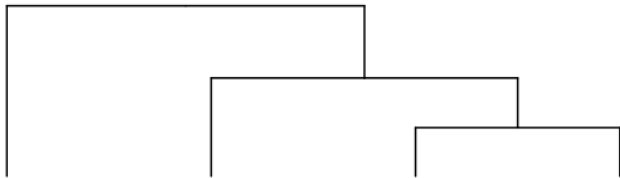
Figures 3-8 show the most significant categories.

### Heatmap of differentially expressed genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue

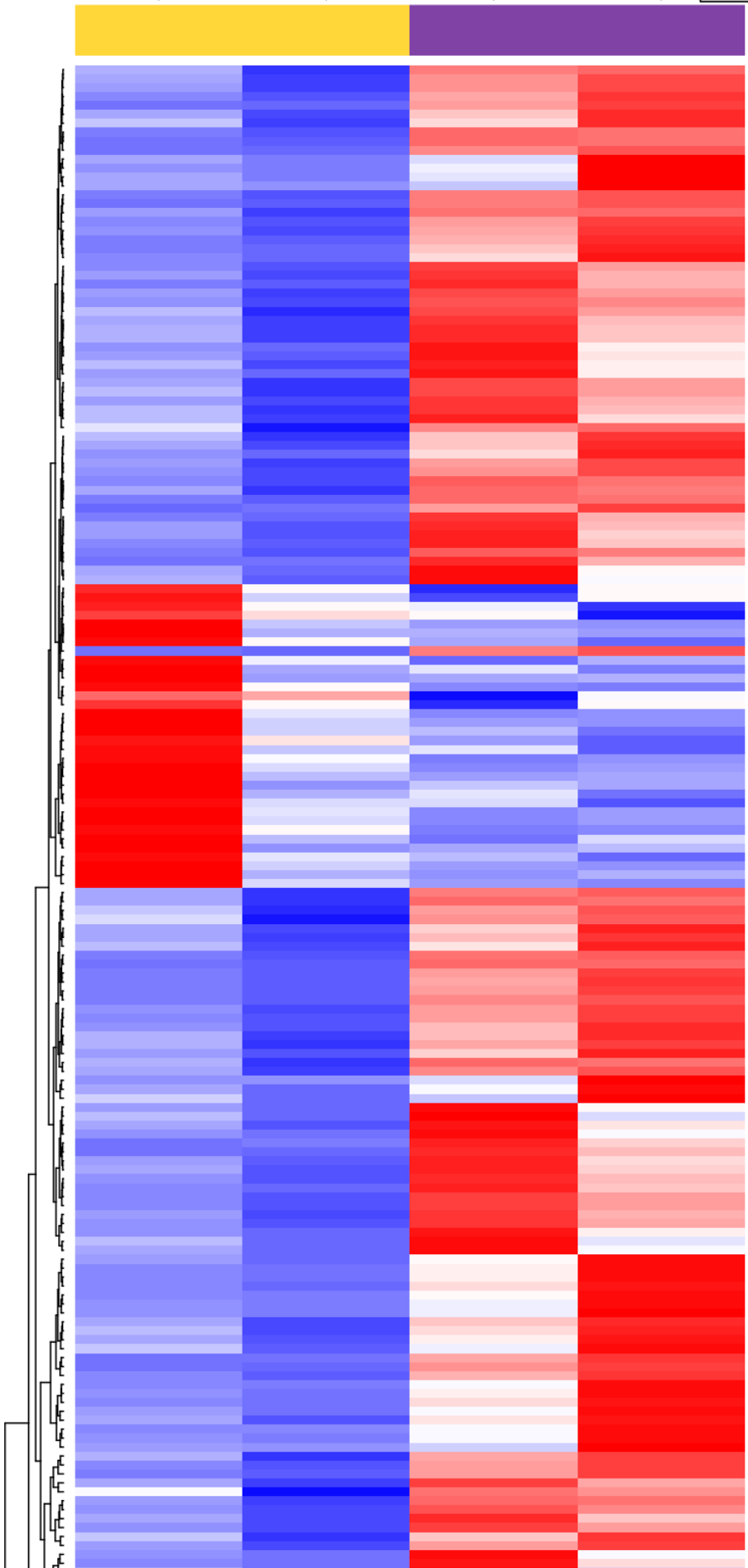
A heatmap of all differentially expressed genes playing a potential regulatory role in the system (enriched in TRANSPATH® pathways) is presented in Figure 2.



Gene Expression Normalized by rows



Control: Non-tumour tissue  
Experiment: Squamous Cell Carcinoma



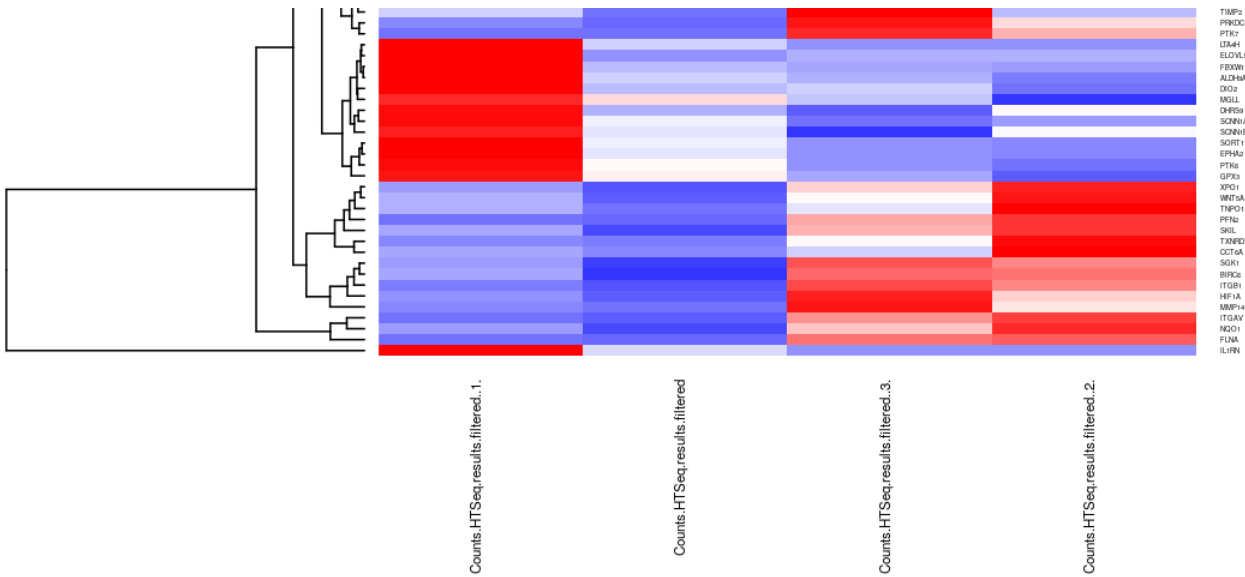


Figure 2. Heatmap of genes enriched in Transpath categories. The colored bar at the top shows the types of the samples according to the legend in the upper right corner.

[See full diagram](#) →

### Up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue:

1436 significant up-regulated genes were taken for the mapping.

#### GO (biological process)



Figure 3. Enriched GO (biological process) of up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

[Full classification](#) →

## TRANSPATH® Pathways (2021.1)

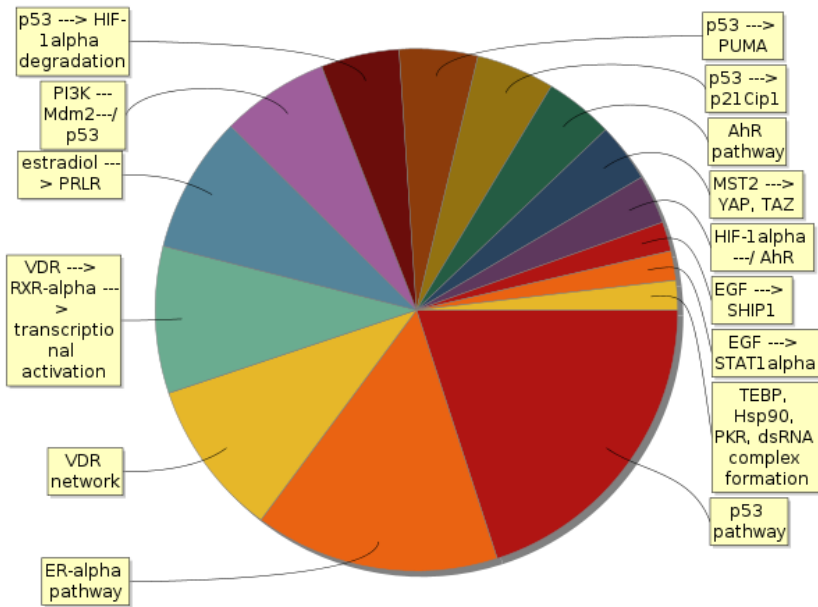


Figure 4. Enriched TRANSPATH® Pathways (2021.1) of up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. [Full classification](#) →

## HumanPSD(TM) disease (2021.1)

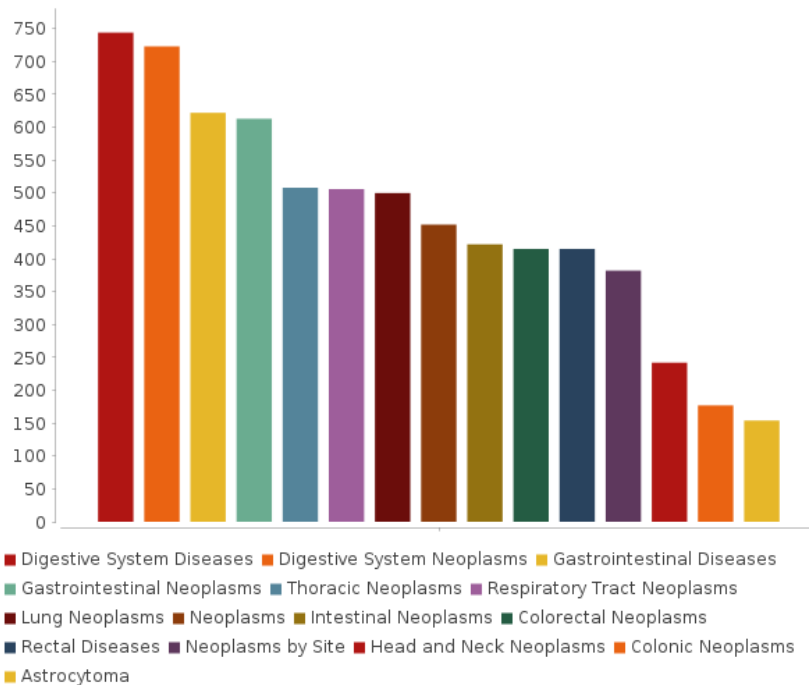


Figure 5. Enriched HumanPSD(TM) disease (2021.1) of up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. The size of the bars correspond to the number of bio-markers of the given disease found among the input set. [Full classification](#) →

## Down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue:

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

### GO (biological process)

biological\_process Gene Ontology treemap



Figure 6. Enriched GO (biological process) of down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

Full classification →

TRANSPATH® Pathways (2021.1)

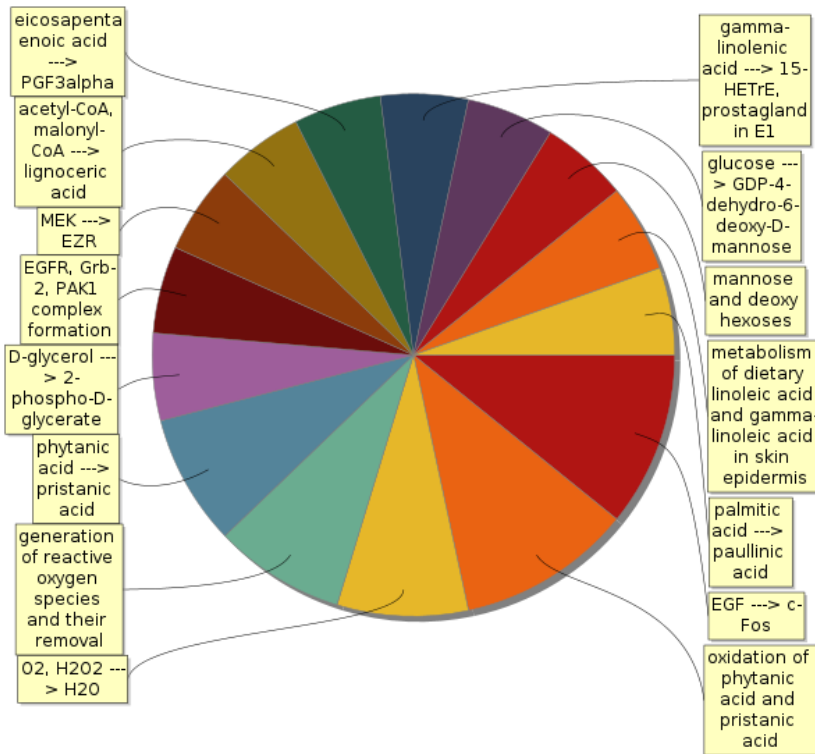


Figure 7. Enriched TRANSPATH® Pathways (2021.1) of down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

Full classification →

HumanPSD(TM) disease (2021.1)

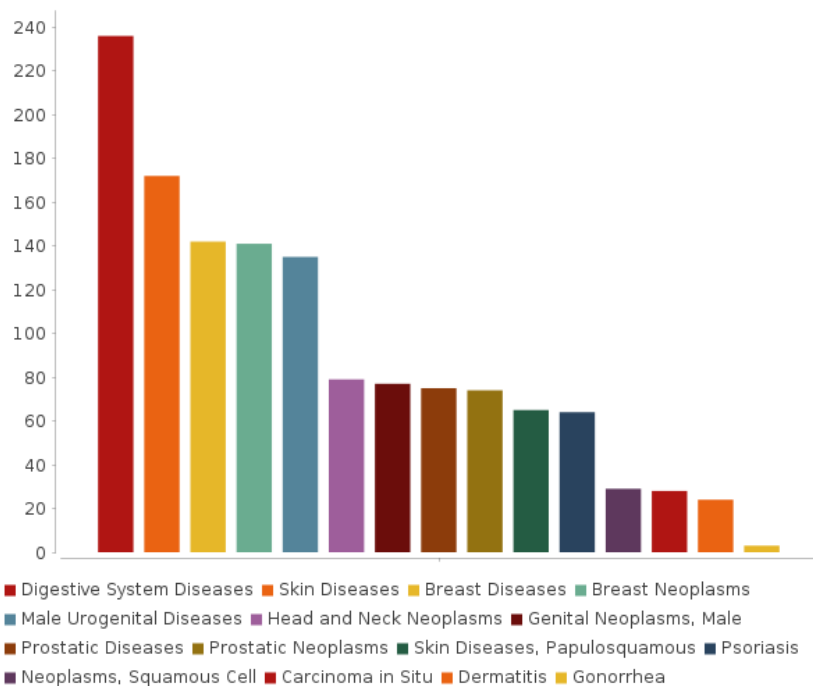
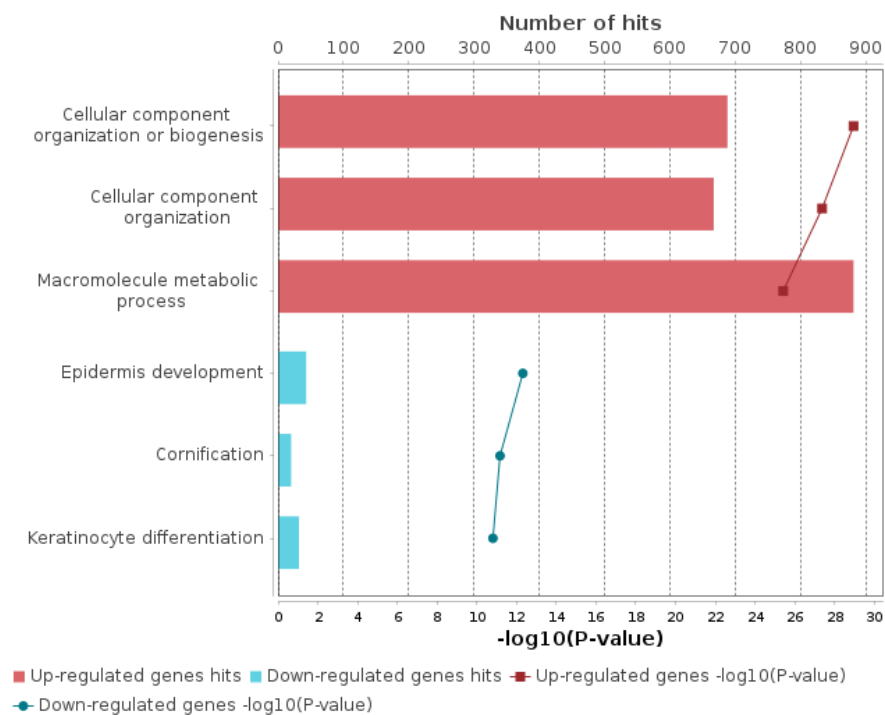


Figure 8. Enriched HumanPSD(TM) disease (2021.1) of down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. The size of the bars correspond to the number of bio-markers of the given disease found among the input set.

[Full classification](#) →

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):



### 3.4. 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].

In the current work, we use the Genomics data from the "Yes VCF track" track to predict positions of potential **enhancers** where the observed sequence variations may influence the gene expression in the pathology under study. We scan 5kb flanking regions and the body of all genes carrying the variations, with a sliding window of 1100bp size and find the position of the window with the maximal sum of the mutation weights, where we then perform the search for potential condition-specific enhancers (CMA model search).



We analyzed mutations that were revealed in the potential enhancers located upstream, downstream or inside the **target genes** (see Table 6). We identified 650 mutations potentially affecting gene regulation. Table 7 shows the following lists of PWMs whose sites were lost or gained due to these mutations. These PWMs were put in focus of the CMA algorithm that constructs the model of the enhancers by specifying combinations of TF motifs (see more details of the algorithm in the Method section).

Table 6. Mutations revealed in Experiment: Squamous Cell Carcinoma versus Control: Non-tumour tissue

[See full table](#) →

ID	Gene symbol	Gene schematic representation	Number of variations
ENSG00000146648	EGFR		21
ENSG00000083857	FAT1		16
ENSG00000134871	COL4A2		13
ENSG00000186340	THBS2		10
ENSG00000226445	BX322234.1		9
ENSG00000145012	LPP		8
ENSG00000114999	TTL		7
ENSG00000142173	COL6A2		7
ENSG00000152291	TGOLN2		7
ENSG00000157214	STEAP2		7

Table 7. PWMs whose sites were lost or gained due to mutations in Experiment: Squamous Cell Carcinoma and Control: Non-tumour tissue

[See full table](#) →

ID	P-value (gains)	P-value (losses)	yesCount (gains)	yesCount (losses)
V\$MAFG_01	3.03E-2	1.06E-10	49	2666
V\$MAFG_02	8.5E-3	6.37E-9	87	2107
V\$TFCP2_04	5.64E-4	9.68E-19	41	2766
V\$CPBP_03	7.99E-5	2.15E-8	38	2747
V\$MAFK_12	4.61E-5	9.77E-10	788	1438
V\$RUNX3_02	3.43E-7	1.91E-20	107	1532
V\$MIZ1_01	1.4E-9	1.57E-8	215	2921
V\$E2F2_06	1.27E-29		452	null
V\$E2F_Q4_01	4.37E-30		1497	null
V\$E2F_Q4_02	1.81E-30		1476	null
V\$E2F4_Q3	5.35E-31		1362	null
V\$E2F3_05	4.76E-31		871	null
V\$ZBTB33_05	9.54E-34		689	null
V\$WT1_Q6_01	3.16E-35		457	null
V\$E2F_Q3_01	2.78E-39		1727	null
V\$WT1_Q6_02	2.21E-39		566	null
V\$ZBTB33_07	1.54E-54		1188	null
V\$ETV5CLOCK_01		1.11E-14	null	1897
V\$MYBL1MAX_02		5.43E-10	null	2272
V\$MYBL1_08		6.59E-16	null	1727

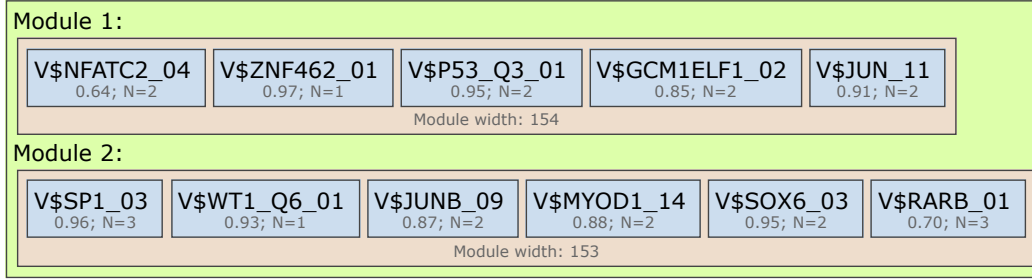
We applied the Composite Module Analyst (CMA) [8] method to detect such potential enhancers, as targets of multiple TFs bound in a cooperative manner to the regulatory regions of the genes of interest. CMA applies a genetic algorithm to construct a generalized model of the enhancers by specifying combinations 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 Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue).

To build the most specific composite modules we choose top 300 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.

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.



**Model score (-p\*log10(pval)):** 13.04

**Wilcoxon p-value (pval):** 1.72e-27

**Penalty (p):** 0.487

**Average yes-set score:** 5.15

**Average no-set score:** 3.08

**AUC:** 0.86

**Middle-point:** 4.37

**False-positive:** 19.67%

**False-negative:** 18.48%

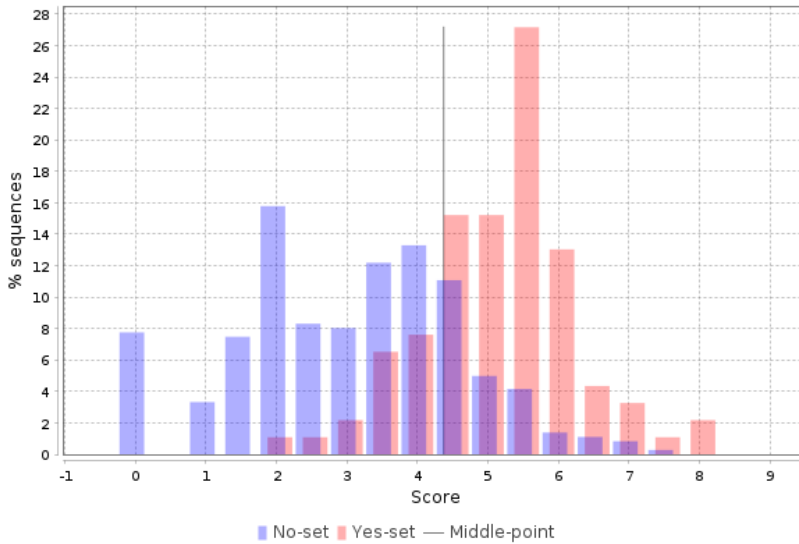


Table 8. List of top ten up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue with identified enhancers in their regulatory regions. **CMA score** - the score of the CMA model of the enhancer identified in the regulatory region.

[See full table →](#)

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000261150	EPPK1	epiplakin 1	9.17	MyoD(h), p53(h), NFATc2(h), Elf-1(h),GCMa(h), Sp1(h), WT1(h), JunB(h)...
ENSG00000147140	NONO	non-POU domain containing octamer binding	8.76	JunB(h), WT1(h), Sp1(h), Sox-6(h), MyoD(h), NFATc2(h), Elf-1(h),GCMa(h)...
ENSG00000114270	COL7A1	collagen type VII alpha 1 chain	8.55	Sp1(h), JunB(h), c-Jun(h), WT1(h), p53(h), MyoD(h), ZNF462(h)...
ENSG00000005175	RPAP3	RNA polymerase II associated protein 3	8.45	JunB(h), c-Jun(h), Sp1(h), p53(h), WT1(h), Sox-6(h), NFATc2(h)
ENSG00000085978	ATG16L1	autophagy related 16 like 1	8.43	Sp1(h), WT1(h), ZNF462(h), Elf-1(h),GCMa(h), JunB(h), c-Jun(h), NFATc2(h)
ENSG00000064726	BTBD1	BTB domain containing 1	8.29	JunB(h), c-Jun(h), WT1(h), Sox-6(h), p53(h), NFATc2(h), Elf-1(h),GCMa(h)...
ENSG00000075240	GRAMD4	GRAM domain containing 4	8.23	p53(h), JunB(h), Sox-6(h), c-Jun(h), WT1(h), Sp1(h), MyoD(h)...
ENSG00000116062	MSH6	mutS homolog 6	8.08	NR1B2(h), Sox-6(h), WT1(h), c-Jun(h), NFATc2(h), MyoD(h), p53(h)
ENSG00000138162	TACC2	transforming acidic coiled-coil containing protein 2	7.94	MyoD(h), NR1B2(h), p53(h), JunB(h), c-Jun(h), NFATc2(h), ZNF462(h)...
ENSG00000241878	PISD	phosphatidylserine decarboxylase	7.92	p53(h), ZNF462(h), NFATc2(h), c-Jun(h), JunB(h), Sox-6(h), NR1B2(h)...

### Enhancer model potentially involved in regulation of target genes (down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue).

To build the most specific composite modules we choose top 300 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.

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\$NR1H4_02 0.77; N=2	V\$MIZ1_01 0.88; N=2	V\$ETS2_05 0.68; N=2	V\$FOS_03 0.84; N=2	V\$RELBP50_Q3 0.86; N=3	V\$IRF4_10 0.81; N=2	V\$SMAD_Q6_01 0.97; N=2
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Module width: 104

**Module 2:**

V\$MIZ1_01 0.86; N=3	V\$JUNDFOS_01 0.87; N=2	V\$SLUG_07 0.91; N=1	V\$NKX25_08 0.98; N=2	V\$CEBPE_Q6_01 0.99; N=3	V\$MEF2_03 0.79; N=2	V\$ESR2_02 0.83; N=3
V\$TWIST1_01 0.91; N=3						

Module width: 130

**Model score (-p\*log10(pval)):** 12.03

**Wilcoxon p-value (pval):** 7.69e-28

**Penalty (p):** 0.444

**Average yes-set score:** 6.75

**Average no-set score:** 5.23

**AUC:** 0.77

**Middle-point:** 5.98

**False-positive:** 29.33%

**False-negative:** 30.30%

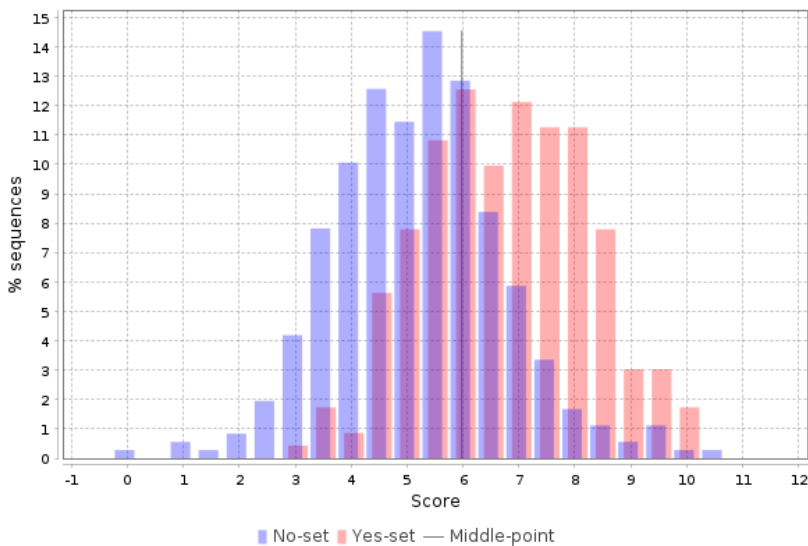


Table 9. List of top ten down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue with identified enhancers in their regulatory regions. **CMA score** - the score of the CMA model of the enhancer identified in the regulatory region.

[See full table →](#)

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000089723	OTUB2	OTU deubiquitinase, ubiquitin aldehyde binding 2	12.92	C/EBPepsilon(h), FXR(h), c-Ets-2(h), TWIST(h), c-Fos(h), Smad1(h),Smad2(h),Smad3(h),Smad4(h),Smad5(h),Smad6(h),Smad7(h),Smad8(h), RelB(h)...
ENSG00000126368	NR1D1	nuclear receptor subfamily 1 group D member 1	12.84	RelB(h), Smad1(h),Smad2(h),Smad3(h),Smad4(h),Smad5(h),Smad6(h),Smad7(h),Smad8(h), FXR(h), IRF-4(h), ER-beta(h), c-Ets-2(h), CSX(h)...
ENSG00000214248	AC010336.1	novel transcript, antisense to LRR8E	12.63	Miz-1(h), CSX(h), ER-beta(h), FXR(h), c-Ets-2(h), Smad1(h),Smad2(h),Smad3(h),Smad4(h),Smad5(h),Smad6(h),Smad7(h),Smad8(h), RelB(h)
ENSG00000204257	HLA-DMA	major histocompatibility complex, class II, DM alpha	11.82	slug(h), ER-beta(h), FXR(h), IRF-4(h), CSX(h), Miz-1(h), c-Ets-2(h)...
ENSG00000138050	THUMP2	THUMP domain containing 2	11.49	ER-beta(h), Miz-1(h), CSX(h), TWIST(h), slug(h), JunD(h),c-Fos(h), Mef-2a(h)...
ENSG00000267551	AC005264.1	novel transcript, antisense to GNA15	11.4	c-Ets-2(h), RelB(h), c-Fos(h), FXR(h), Miz-1(h), JunD(h),c-Fos(h), TWIST(h)...
ENSG00000157600	TMEM164	transmembrane protein 164	11.34	RelB(h), c-Fos(h), c-Ets-2(h), Miz-1(h), CSX(h), FXR(h), ER-beta(h)...
ENSG00000166024	R3HCC1L	R3H domain and coiled-coil containing 1 like	10.98	Miz-1(h), C/EBPepsilon(h), ER-beta(h), CSX(h), slug(h), RelB(h), Mef-2a(h)...
ENSG00000183486	MX2	MX dynamin like GTPase 2	10.95	TWIST(h), slug(h), Miz-1(h), CSX(h), c-Ets-2(h), c-Fos(h), JunD(h),c-Fos(h)...
ENSG00000204421	LY6G6C	lymphocyte antigen 6 family member G6C	10.88	c-Fos(h), Miz-1(h), CSX(h), slug(h), FXR(h), ER-beta(h), c-Ets-2(h)...

On the basis of the enhancer models we identified transcription factors potentially regulating the **target genes** of our interest. We found 12 and 21 transcription factors controlling expression of up- and down-regulated genes respectively (see Tables 10-11).

Table 10. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue). **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 →](#)

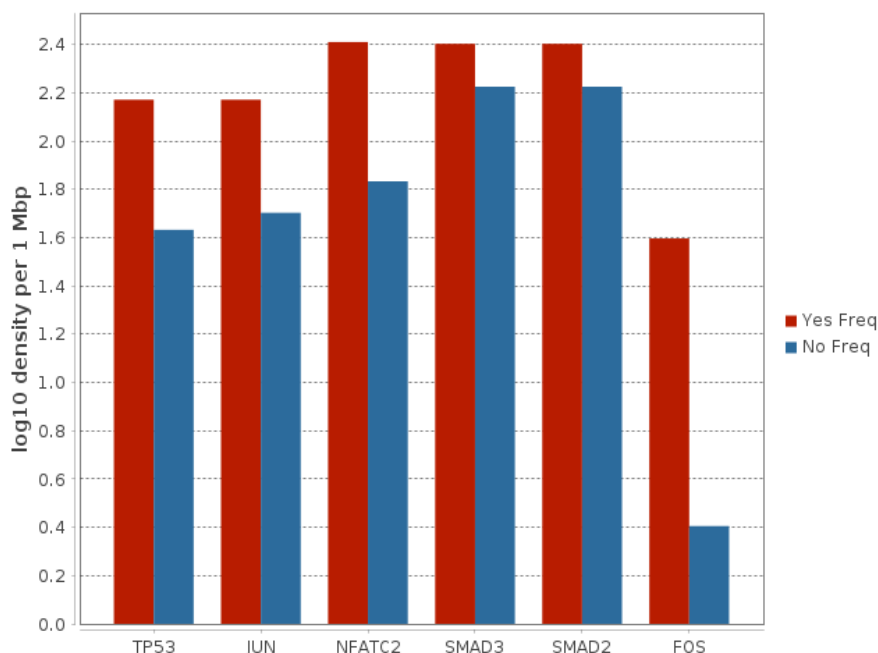
ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
MO000019548	TP53	tumor protein p53	4.85	3.46
MO000019469	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	4.55	2.94
MO000026044	NFATC2	nuclear factor of activated T cells 2	3.23	3.78
MO000026306	GCM1	glial cells missing transcription factor 1	3	9.8
MO000033308	SP1	Sp1 transcription factor	2.64	3.14
MO000092587	ZNF462	zinc finger protein 462	2.6	1.8
MO000019612	MYOD1	myogenic differentiation 1	2.3	5.23
MO000007830	JUNB	JunB proto-oncogene, AP-1 transcription factor subunit	2.13	2.94
MO000025410	ELF1	E74 like ETS transcription factor 1	2.03	1.61
MO000019618	RARB	retinoic acid receptor beta	1.71	1.54

Table 11. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue). **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
MO000057832	SMAD3	SMAD family member 3	2.37	1.51
MO000057829	SMAD2	SMAD family member 2	2.3	1.51
MO000018137	FOS	Fos proto-oncogene, AP-1 transcription factor subunit	2.23	15.53
MO000028695	TWIST1	twist family bHLH transcription factor 1	2.19	5.69
MO000056529	ETS2	ETS proto-oncogene 2, transcription factor	2.17	1.42
MO000019609	SMAD1	SMAD family member 1	2.07	1.51
MO000007834	JUND	JunD proto-oncogene, AP-1 transcription factor subunit	2.05	5.43
MO000028767	SNAI2	snail family transcriptional repressor 2	2	1.46
MO000059335	ESR2	estrogen receptor 2	1.93	5.05
MO000020402	SMAD4	SMAD family member 4	1.86	1.51

The following diagram represents the key transcription factors, which were predicted to be potentially regulating differentially expressed genes in the analyzed pathology: TP53, JUN, NFATC2, SMAD3, SMAD2 and FOS.



### 3.5. Finding master regulators in networks

In the second step of the upstream analysis common regulators of the revealed TFs were identified. We identified 9 signaling proteins whose structure and function is highly damaged by the mutations (see Table 12).

Table 12. Signaling proteins whose structure and function is damaged by the mutations in Experiment: Squamous Cell Carcinoma and Control: Non-tumour tissue

[See full table →](#)

ID	Title	Mutation count	Consequence	Codons
MO000189841	ZSWIM1(h)	2	stop_gained	tGg/tAg
MO000208420	GJB3(h)	2	stop_gained	tGg/tAg
MO000109306	PSMA4(h)	1	stop_lost	Tga/Cga
MO000144222	APT2(h)	1	stop_lost	Tag/Cag
MO000172130	c3orf1(h)	1	NMD_transcript_variant,stop_lost	tGa/tCa
MO000175986	oas2(h)	1	stop_lost	tAg/tGg
MO000212738	EMC10(h)	1	stop_lost	taG/taT
MO000219203	PSMG1(h)	1	NMD_transcript_variant,stop_lost	Taa/Caa
MO000222634	TCP11L1(h)	1	NMD_transcript_variant,stop_gained	Cag/Tag

Top 9 mutated proteins for Experiment: Squamous Cell Carcinoma and Control: Non-tumour tissue were used in the algorithm of master regulator search as a list of nodes of the signal transduction network that are removed from the network during the search of master regulators (see more details about the algorithm in the Method section). 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 13-14.

Table 13. Master regulators that may govern the regulation of **up-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics data.

[See full table →](#)

ID	Master molecule name	Gene symbol	Gene description	logFC	Total rank
MO000020249	26S proteasome(h)	PSMA7, PSMC2, PSMC3, PSMC5, PSMD4, PSMD5	proteasome 20S subunit alpha 7, proteasome 26S subunit, ATPase 2, proteasome 26S subunit, ATPase 3, ...	1.71	86
MO000018003	PP2A(h)	PPP2CA, PPP2R3A, PPP2R3B, PPP2R5A, PPP2R5B, PPP2R5C, PPP2R5D	protein phosphatase 2 catalytic subunit alpha, protein phosphatase 2 regulatory subunit B"alpha, pr...	1.93	212
MO000031006	ATM(h)	ATM	ATM serine/threonine kinase	2.15	223
MO000018901	CKII-alpha(h):CKII-alpha2(h):(CKII-beta(h))2	CSNK2A1, CSNK2A2, CSNK2B	casein kinase 2 alpha 1, casein kinase 2 alpha 2, casein kinase 2 beta	1.46	238
MO000020449	Caspase-2(h)	CASP2	caspase 2	1.43	276
MO000157536	CKII-alpha(h):CKII-alpha2(h):CKII-beta(h)	CSNK2A1, CSNK2A2, CSNK2B	casein kinase 2 alpha 1, casein kinase 2 alpha 2, casein kinase 2 beta	1.46	277
MO000019548	p53(h)	TP53	tumor protein p53	1.43	282
MO000090791	RPTPzeta-L(h)	PTPRZ1	protein tyrosine phosphatase receptor type Z1	3.37	295
MO000019674	p110alpha(h)	PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	2.32	309
MO000033243	huntingtin(h)	HTT	huntingtin	1.35	361

Table 14. Master regulators that may govern the regulation of **down-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. **Total rank** is the sum of the ranks of the master molecules sorted by keynote score, CMA score, transcriptomics data.

See full table →

ID	Master molecule name	Gene symbol	Gene description	logFC	Total rank
MO000033396	DUSP5(h)	DUSP5	dual specificity phosphatase 5	-4.43	45
MO000137304	DUSP5(h)	DUSP5	dual specificity phosphatase 5	-4.43	51
MO000022222	MKP-1(h)	DUSP1	dual specificity phosphatase 1	-2.29	81
MO000102190	PTK6-isoform1(h)	PTK6	protein tyrosine kinase 6	-3.89	104
MO000176198	JKAP(h)	DUSP22	dual specificity phosphatase 22	-0.99	107
MO000031101	plk3(h)	PLK3	polo like kinase 3	-2.46	128
MO000083769	MKP-1(h)	DUSP1	dual specificity phosphatase 1	-2.29	133
MO000056491	p/CAF(h)	KAT2B	lysine acetyltransferase 2B	-2.74	139
MO000033299	pim1(h)	PIM1	Pim-1 proto-oncogene, serine/threonine kinase	-2.6	143
MO000018962	ErbB2(h)	ERBB2	erb-b2 receptor tyrosine kinase 2	-1.16	147

The intracellular regulatory pathways controlled by the above-mentioned master regulators are depicted in Figures 9 and 10. 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.

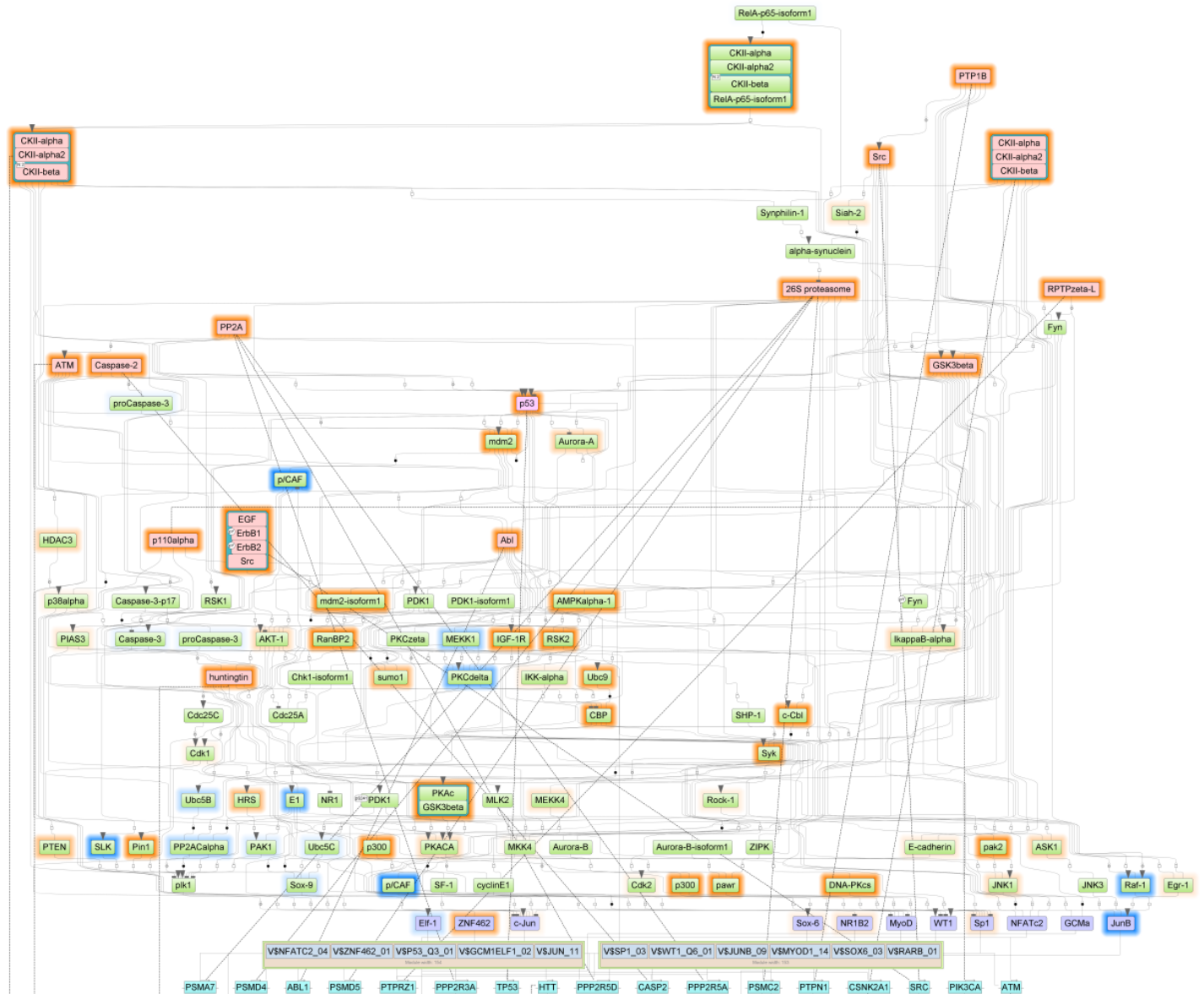


Figure 9. Diagram of intracellular regulatory signal transduction pathways of up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, 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.

See full diagram →

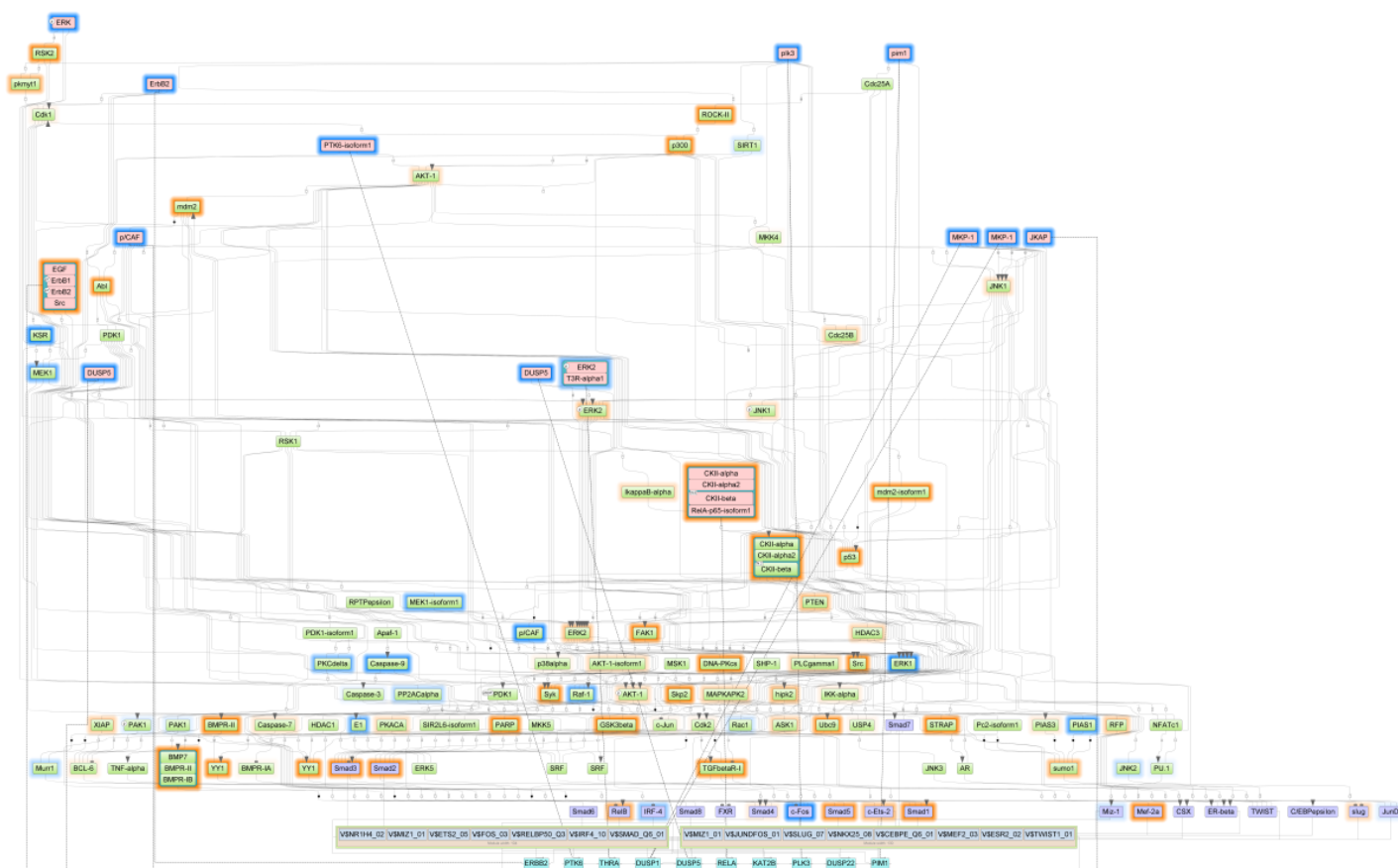


Figure 10. Diagram of intracellular regulatory signal transduction pathways of down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate 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.

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 Method 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 15. 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	logFC	Total rank
PSMA7	proteasome 20S subunit alpha 7	3	1.71	86
IL1R1	interleukin 1 receptor type 1	3	1.93	563
CREBBP	CREB binding protein	1	1.63	606
ROCK2	Rho associated coiled-coil containing protein kinase 2	2	2.61	691
LYN	LYN proto-oncogene, Src family tyrosine kinase	4	1.2	719
CSNK2A1	casein kinase 2 alpha 1	24	1.46	732

**Table 16.** 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.

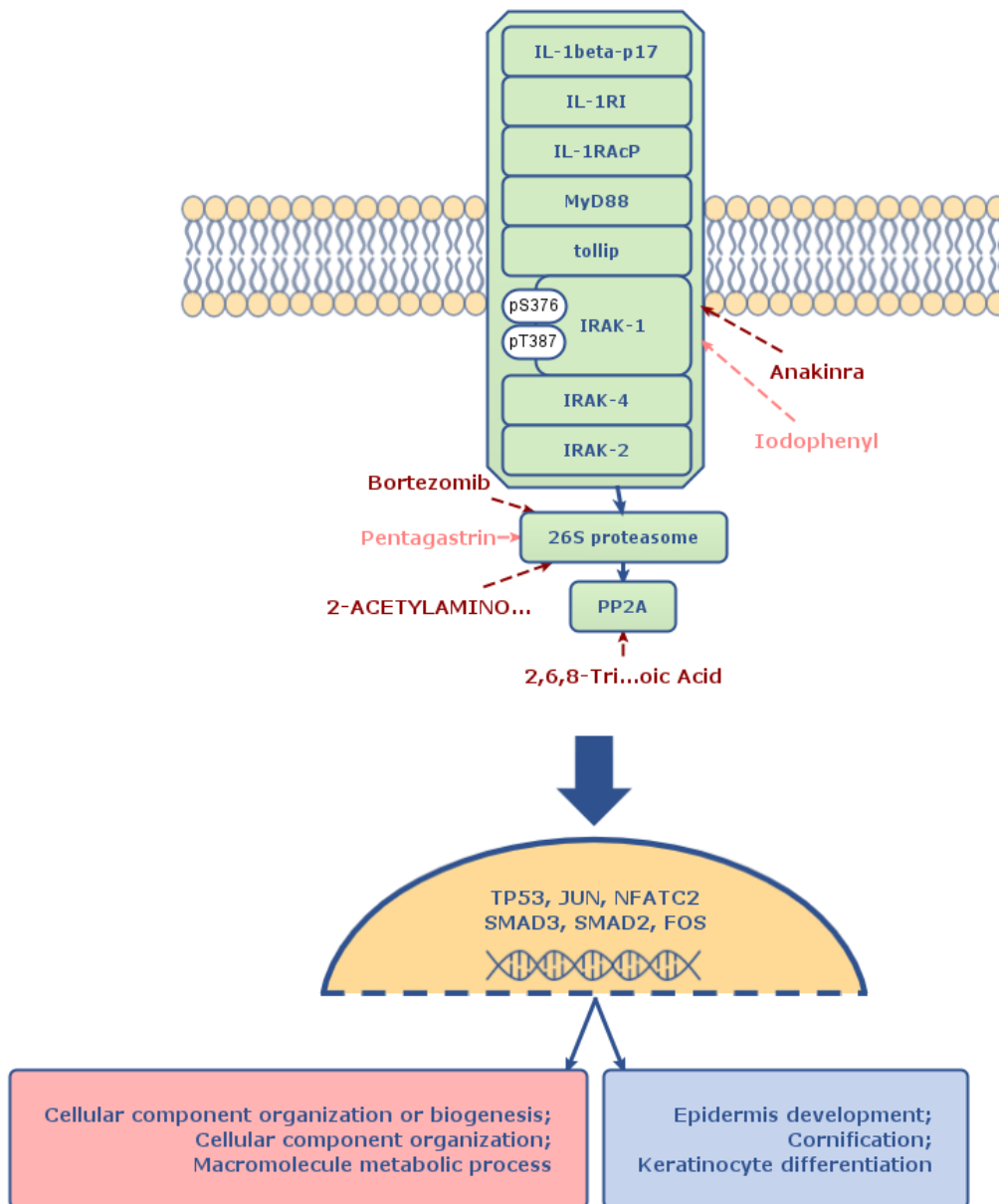
See full table →

Gene symbol	Gene Description	Druggability score	logFC	Total rank
PSMC5	proteasome 26S subunit, ATPase 5	3.43	1.71	86
PSMD5	proteasome 26S subunit, non-ATPase 5	3.43	1.71	86
PSMA7	proteasome 20S subunit alpha 7	9.25	1.71	86
PSMD4	proteasome 26S subunit, non-ATPase 4	3.43	1.71	86
PSMC2	proteasome 26S subunit, ATPase 2	3.43	1.71	86
PTPRZ1	protein tyrosine phosphatase receptor type Z1	17.53	3.37	295

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:

- IL-1beta-p17:IL-1RI:IL-1RAcP:MyD88:tollip:IRAK-1{pS376}{pT387}:IRAK-4:IRAK-2
- PP2A
- 26S proteasome

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: Pentagastrin, Bortezomib, 2,6,8-Trimethyl-3-Amino-9-Benzyl-9-Methoxynonanoic Acid, Iodophenyl, 2-ACETYLAMINO-4-METHYL-PENTANOIC ACID [1-(1-FORMYL-PENTYL-CARBAMOYL)-3-METHYL-BUTYL]-AMIDE and Anakinra, 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 two 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)).

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


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

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

 Table 17. 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 rank	Disease activity score	Phase 4	Status (provided by Drugbank)
Dasatinib	SRC, ABL1, YES1, ABL2	18	4	Leukemia, Leukemia, Lymphoid, Leukemia, Myelogenous, Chronic, BCR-ABL Positive, Leukemia, Myeloid, Precursor Cell Lymphoblastic Leukemia-Lymphoma	small molecule, approved, investigational
Nintedanib	FGFR3, SRC, LYN	37	2	Idiopathic Pulmonary Fibrosis, Pulmonary Fibrosis	small molecule, approved
Palbociclib	CDK6, CDK4	159	3	Breast Neoplasms, Neoplasms	small molecule, approved
Arsenic trioxide	CCND1, MAPK1, AKT1, JUN	160	2	Leukemia, Leukemia, Myeloid, Leukemia, Promyelocytic, Acute	small molecule, approved, investigational
Vandetanib	VEGFA, EGFR	225	2	Neoplasms, Thyroid Neoplasms	small molecule, approved

### **Repurposing drugs**

 Table 18. 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 rank	Phase 4	Status (provided by Drugbank)
Bosutinib	CAMK2G, SRC, ABL1, HCK, LYN, CDK2	40	Leukemia, Myeloid	small molecule, approved
Anakinra	IL1R1	53	Arthritis, Arthritis, Rheumatoid, Diabetes Mellitus, Diabetes Mellitus, Type 2, Knee Injuries, Myocarditis, Pericarditis...	biotech, approved
Ponatinib	FGFR3, SRC, ABL1, LYN	125	This drug was not tested on Phase 4 clinical trials yet. See full table for more details.	small molecule, approved
Flavopiridol	EGFR, CDK6, CDK1, CDK2, CDK4	155	This drug was not tested on Phase 4 clinical trials yet. See full table for more details.	small molecule, experimental, investigational
XL228	SRC, ABL1, ABL2, IGF1R	172	This drug was not tested on Phase 4 clinical trials yet. See full table for more details.	small molecule, investigational


 No prospective drugs were found, which would be predicted by PASS software to be active against the identified drug targets and would be predicted to have biological activity against the studied disease(s).



Table 19. 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 rank	Target activity score
{(2Z)-4-AMINO-2-[(4-METHOXYPHENYL)IMINO]-2,3-DIHYDRO-1,3-THIAZOL-5-YL}(4-METHOXYPHENYL)METHANONE	CCND1, CDK6, CCNH, CCND3, CCNB1, CLK1, CCNA2...	2	8.72
Iodophenyl	RPS6KA3, ROCK2, MAP4K4, MARK3, NEK7, PAK2, GSK3B...	3	7.4
3-Bromo-7-Nitroindazole	RPS6KA3, CDK6, GSK3A, CCND3, CCNB1, GSK3B, CDK1...	4	7.3
6-CYCLOHEXYLMETHYLOXY-5-NITROSO-PYRIMIDINE-2,4-DIAMINE	CCND1, CDK6, GSK3A, MTOR, CCNH, CCND3, CCNB1...	5	7.2
2-(2-HYDROXYETHYLAMINO)-6-(3-CHLOROANILINO)-9-ISOPROPYLPURINE	CDK6, SRC, CCND3, CCNB1, CDK5R1, CDK1, CDK4...	6	6.75

As the result of drug search we propose the following drugs as most promising candidates for treating the pathology under study: Dasatinib, Bosutinib and {(2Z)-4-AMINO-2-[(4-METHOXYPHENYL)IMINO]-2,3-DIHYDRO-1,3-THIAZOL-5-YL}(4-METHOXYPHENYL)METHANONE. These drugs were selected for acting on the following targets: SRC, LYN and CCNB2, 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.

## 6. Conclusion

We applied the software package "Genome Enhancer" to a data set that contains *transcriptomics* data. The study is done in the context of *Squamous Cell Carcinoma*. 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:

 **Dasatinib, Bosutinib and {(2Z)-4-AMINO-2-[(4-METHOXYPHENYL)IMINO]-2,3-DIHYDRO-1,3-THIAZOL-5-YL}(4-METHOXYPHENYL)METHANONE**

These drugs were selected for acting on the following targets: SRC, LYN and CCNB2, 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:

 **IL-1beta-p17:IL-1RI:IL-1RAcP:MyD88:tollip:IRAK-1{pS376}{pT387}:IRAK-4:IRAK-2, PP2A and 26S proteasome**

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: Pentagastrin, Bortezomib, 2,6,8-Trimethyl-3-Amino-9-Benzyl-9-Methoxynonanoic Acid, Iodophenyl, 2-ACETYLAMINO-4-METHYL-PENTANOIC ACID [1-(1-FORMYL-PENTYL CARBAMOYL)-3-METHYL-BUTYL]-AMIDE and Anakinra. 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.

- IL-1beta-p17:IL-1RI:IL-1RAcP:MyD88:tollip:IRAK-1{pS376}{pT387}:IRAK-4:IRAK-2
- PP2A
- 26S proteasome

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®](https://genexplain.com/transfac) library, release 2021.1 (geneXplain GmbH, Wolfenbüttel, Germany) (<https://genexplain.com/transfac>).

The master regulator search uses the [TRANSPATH®](https://genexplain.com/transpath) database (BIOBASE), release 2021.1 (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®](https://genexplain.com/transpath).

The information about drugs corresponding to identified drug targets and clinical trials references were extracted from [HumanPSD™](https://genexplain.com/humanpsd) database, release 2021.1 (<https://genexplain.com/humanpsd>).

The Ensembl database release Human100.38 (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 sum of two other ranks:

1. ranking by "Target activity score" ( $T\text{-score}_{PSD}$ ),
2. ranking by "Disease activity score" ( $D\text{-score}_{PSD}$ ).

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

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

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,  $\text{rank}(t)$  is rank of given target,  $\text{maxRank}(T)$  equals  $\text{max}(\text{rank}(t))$  for all targets  $t$  in  $T$ .

We use following formula to calculate "Disease activity score" ( $D\text{-score}_{PSD}$ ):

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

where  $D$  is the set of selected diseases, and if  $D$  is empty set,  $D\text{-score}_{PSD} = 0$ .  $P$  is a set of all known phases for each disease,  $\text{phase}(p, d)$  equals to the phase number if there are known clinical trials for the selected disease on this phase and zero otherwise.

### Method for prediction of pharmaceutical compounds

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 ( $P_a$ ).

We selected compounds that satisfied the following conditions:

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

The maximum  $P_a$  value for all toxicities corresponding to the given compound is selected as the "Toxicity score". The maximum  $P_a$  value for all activities corresponding to the selected diseases for the given compound is used as the "Disease activity score". "Target activity score" ( $T\text{-score}$ ) 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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## 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 \(up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue\).](#)
4. [Supplementary table 4 - Detailed report. Composite modules and master regulators \(down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue\).](#)
5. [Supplementary table 5 - Detailed report. Pharmaceutical compounds and drug targets.](#)

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