Cellular and Molecular Biotechnology Research Institute
(CMB), AIST

Phosprof

User Guide

Version 1.1
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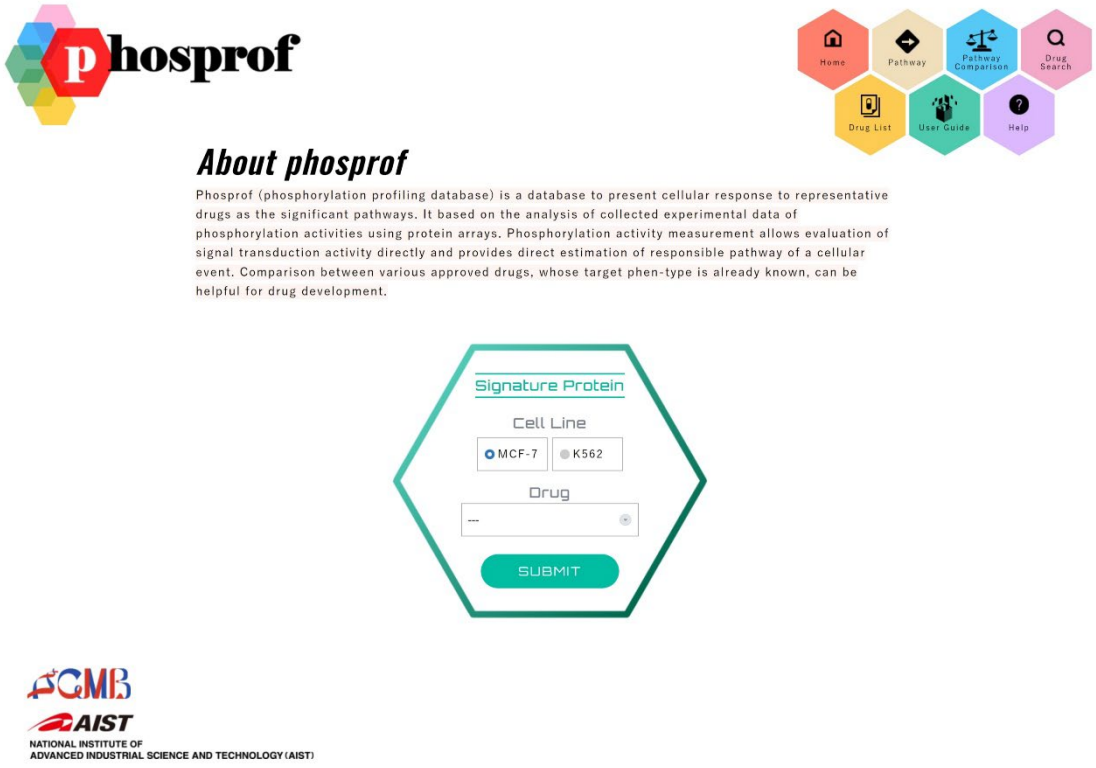
Contents

1 Introduction

Phosprof (phosphorylation profiling DB) is a database that presents cellular responses to representative drugs as significant pathways. It is based on the analysis of collected experimental data on the phosphorylation activities using protein arrays. Measurement of the phosphorylation activity allows direct evaluation of the signal transduction activity and estimation of the pathways responsible for a particular cellular event. A comparison between the various approved drugs with known target phenotypes can be helpful for further investigation of the drug functions and for drug development.

Phosprof : http://phosprof.medals.jp/

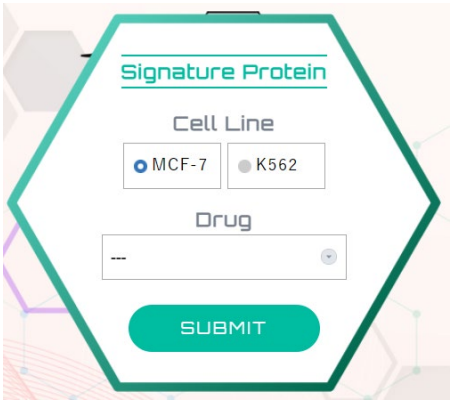


2 Signature Protein

Phosphorylation reactions in the protein array allow measurement of the kinase activity of the specimen. We summarize the resultant 'phosphorylation profiles' of lysates of cells treated with 94 drugs by highlighting the proteins showing significant phosphorylation level changes induced by the drug treatment, on the selected pathway map.

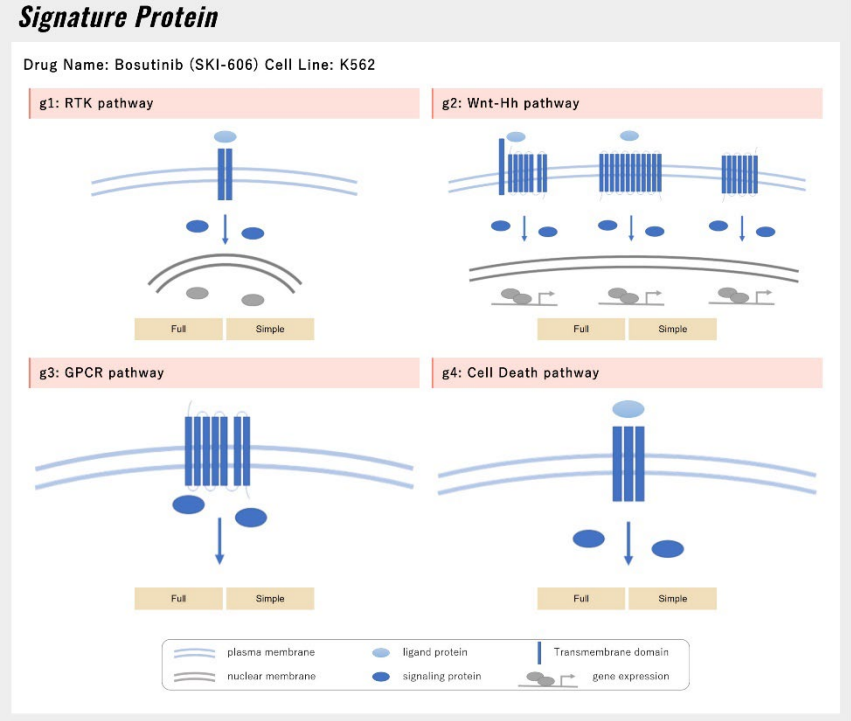
2.1 Preparation

Signature Proteins can be browsed on the top page of Phosprof by selecting the cell line tested (click button) and the drug of interest (select from a drop-down list). The tested cell lines are MCF-7 (human breast cancer cell line) and K562 (immortalized human myelogenous leukemia cell line). The 94 tested drugs are from the FDA-approved Drug Library (Selleck Pfizer Licensed Library, L2400 Selleck chem).



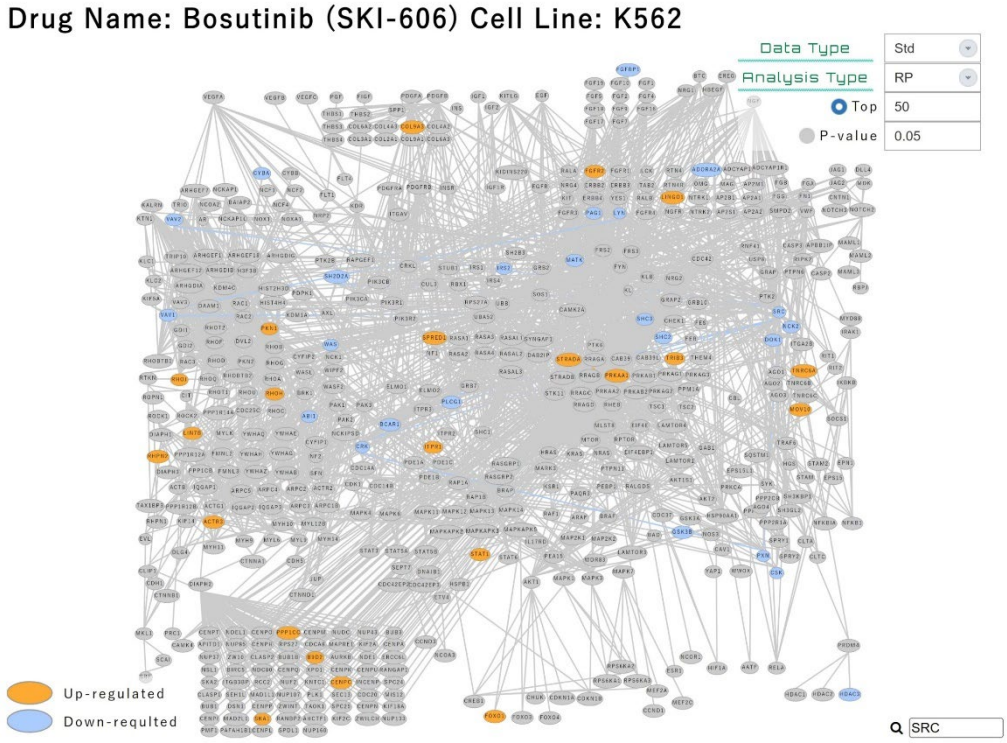
2.2 Pathway Selection

On the next page, pathway maps from the four pathway groups are selected to visualize significant proteins colored on the selected pathway map. For legibility, we provide two types of pathway maps: full pathways containing all the 1,376 proteins as pathway nodes, and simple pathways with integrated nodes of complex proteins or protein groups that are not directly connected by main binaries along the membrane-to-nucleotide direction. A full or simple map according to is chosen acc as required.

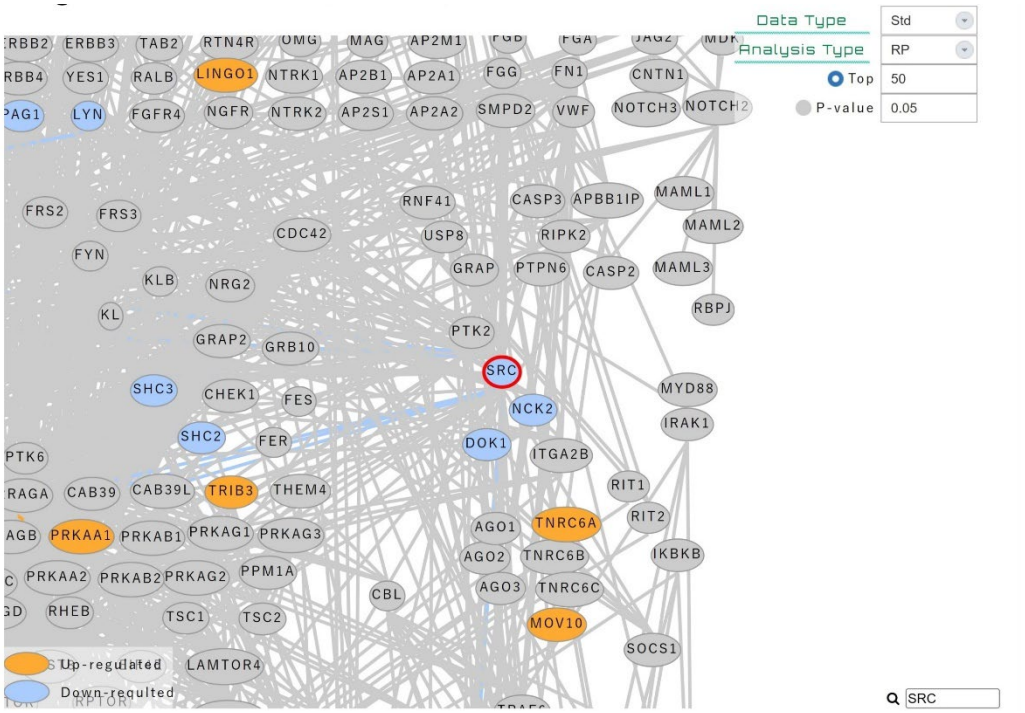


2.3 Visualization

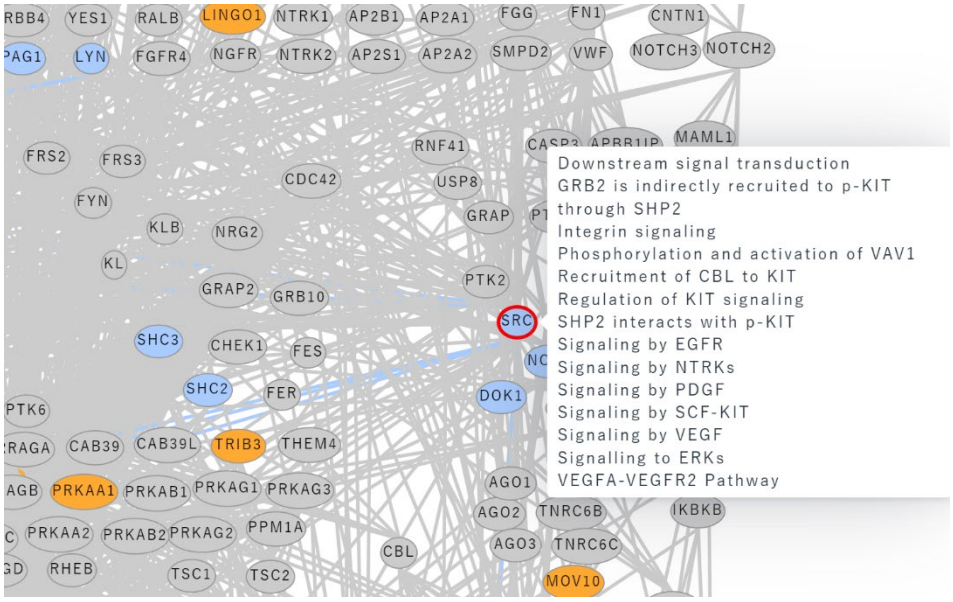
Proteins with significant changes are shown in the pathway map. Proteins with increased (Up) or decreased (Down) tyrosine phosphorylation levels are shown as orange and blue nodes, respectively. The proteins of the pathway are aligned in the membrane (top)-to-nucleus (bottom) direction, referring to the Reactome database. The protein nodes of the pathway maps are connected one-by-one with edges, if the interactions between two proteins are known. The data type (Std [standardized] or Raw [raw-data]), analysis type (RP [rank product] or Pearson), and threshold (by rank (enter number in the blank space left of 'Top') or P-value) can be selected using the boxes in the upper right corner.



The protein of interest can be searched on the map using the box in the bottom-right corner of the screen.



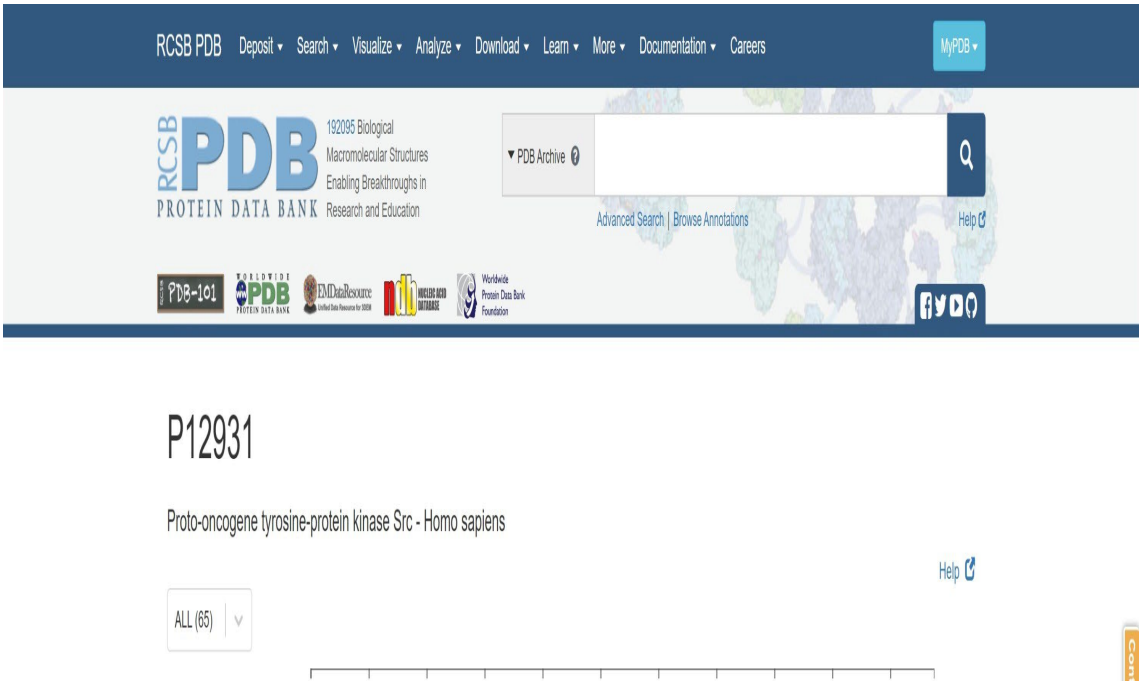
A list of significant pathways that include each protein can be visualized by hovering the cursor over each node.



2.4 Detailed information

Detailed information on the protein of interest can be visualized by double-clicking on the node on the pathway map. The 'Detail' page of the selected protein provides their gene symbol, gene ID, and amino acid sequence. The disorder score is plotted along the amino acid sequence as the red line on the graph titled 'Sequential information.' The green vertical lines in the graph correspond to the tyrosine residues of the protein. The tyrosine residues that are annotated to be 'Phospho-tyrosine' in UNIPROT are indicated by light-blue circles.

'Structural Information' provides links to the PDB site of the protein via UNIPROT.



The PDB ID is shown if available and linked to the 3D image. The PDB ENTITY with the longest sequence was selected. In the 3D image, tyrosine residues are illustrated as a ball-and-stick model.

3 Pathway

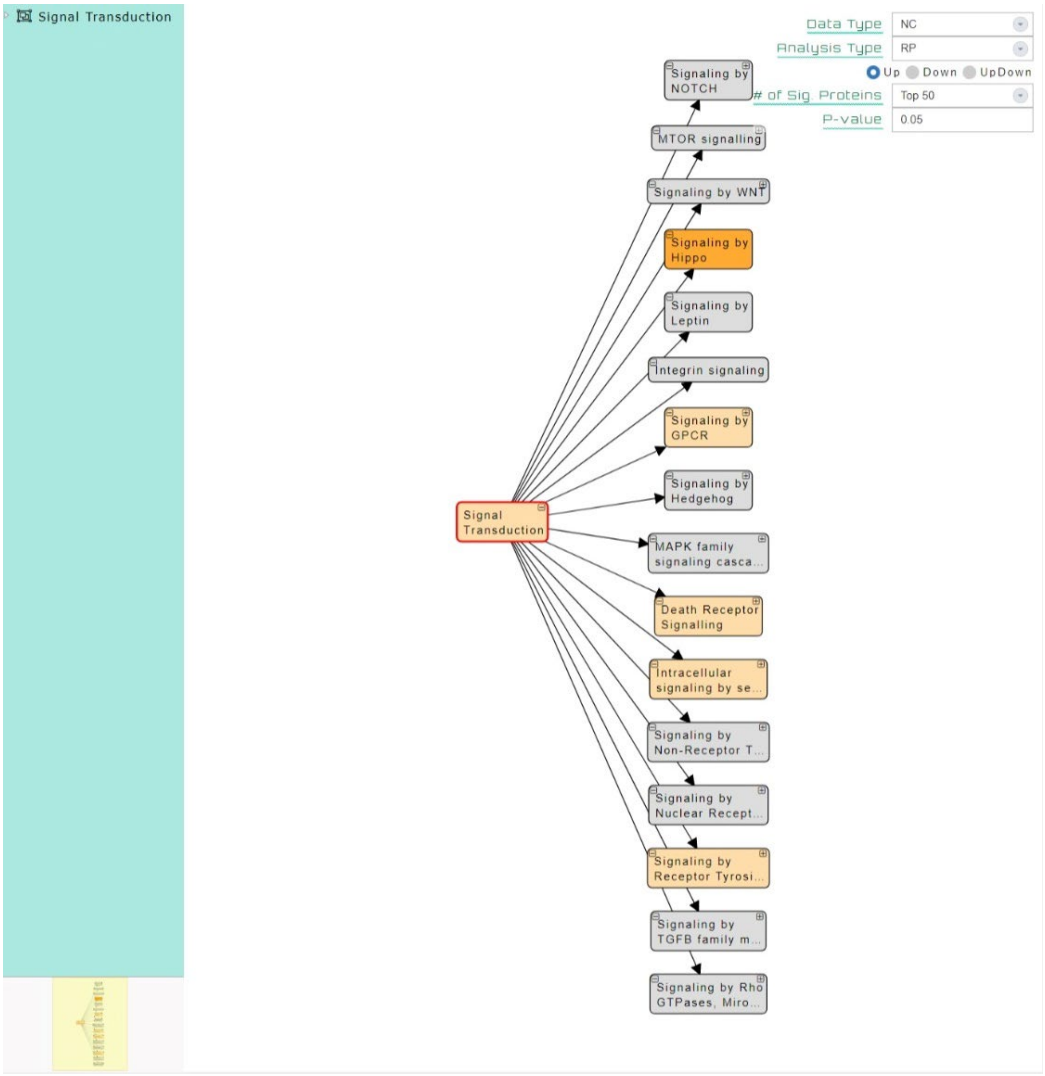
Based on the distribution of the significant proteins, a pathway analysis is performed to estimate the significance of the 376 pathways. To systematically review the significant pathways in the interconnected pathway network, they are highlighted in the hierarchical tree in the 'Pathway' section. The pathway hierarchy is constructed according to the Reactome database, and every pathway node is linked to the original Reactome pathway site that provides more detailed information.

3.1 Preparation

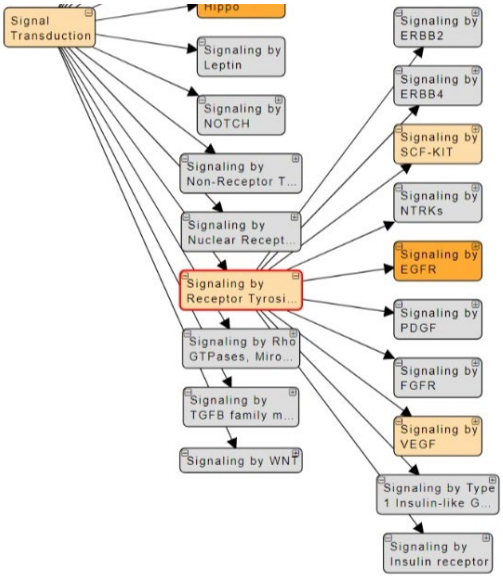
On the 'Pathway' section, select the tested cell line and drug of interest, and then click 'SUBMIT'.

3.2 Visualization

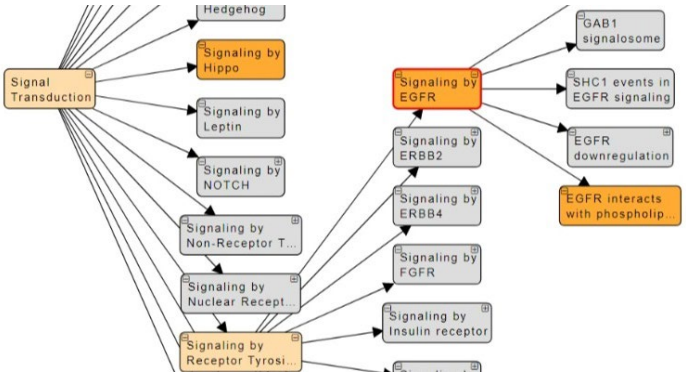
Pathways are displayed in a tree form, and each pathway node is connected to the Reactome database, which provides more detailed information. Significant pathways with up- or downregulated signature proteins are shown as orange- or blue-colored nodes, respectively. The data type, analysis type, thresholds of signature proteins (number of signature proteins), and pathway analysis (p-value) can also be selected using the boxes in the upper right corner.



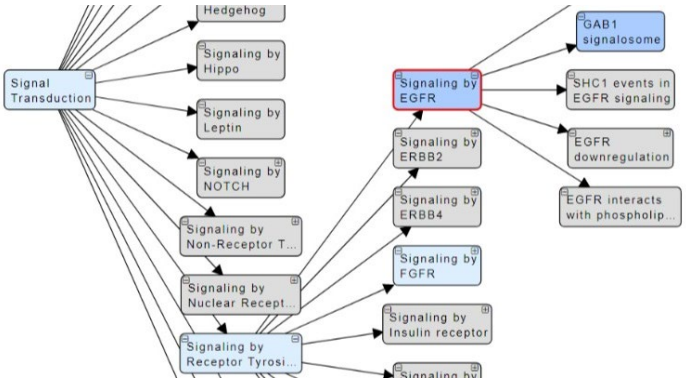
The pathways are shown in a tree form as they form a hierarchical network. The top stratum pathway is signal transduction, since all the 376 pathways examined in Phosprof belong to this pathway group. Significant pathways are shown as colored boxes, and the pathways of the higher stratum are also shown in the same colors, but in their paler shades.



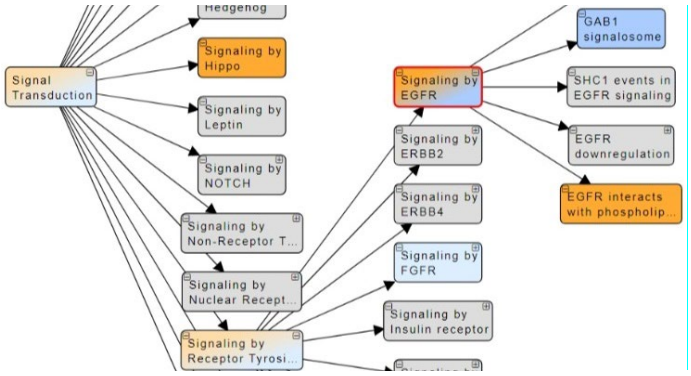
On clicking the 'Up' button, significant pathways with upregulated signature proteins are visualized as orange boxes.



On clicking the 'Down' button, significant pathways with downregulated signature proteins are visualized as blue boxes.



On clicking the UpDown button, significant pathways with both up- and downregulated signature proteins are visualized as orange and blue boxes, respectively.

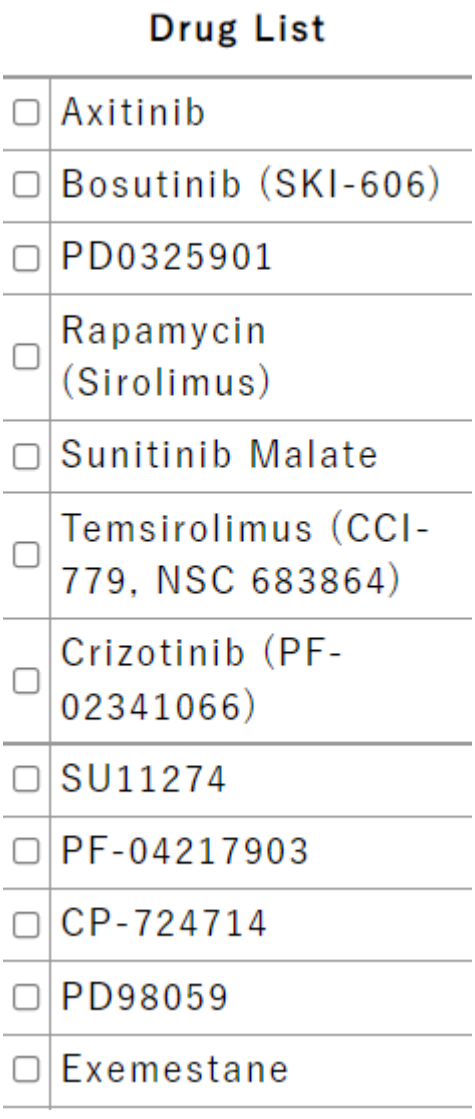


4 Pathway comparison

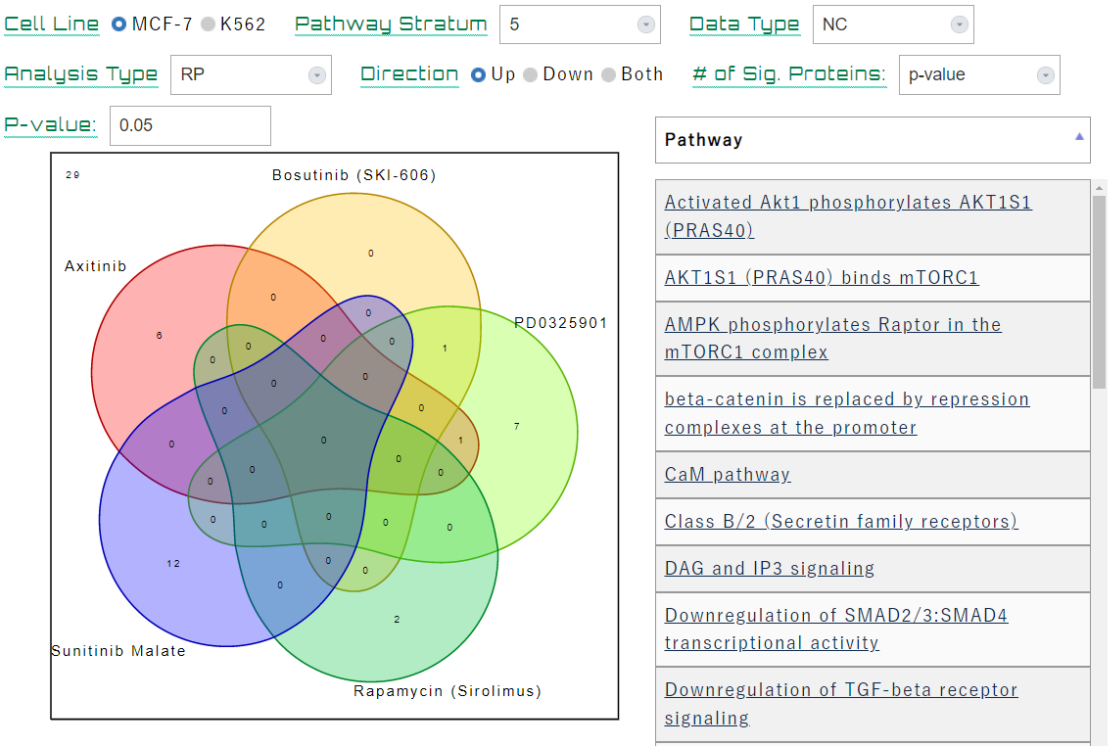
To examine the similarities and differences in functions among drugs, the significant pathways for each drug are compared and displayed in a Venn's diagram in the Compare Pathway section. The Compare Pathway tool enables comparison of significant pathways among five or fewer drugs of interest, with variable thresholds. The numbers on the Venn's diagram can be clicked on to visualize the pathway list.

4.1 Preparation

A drug can be selected to compare it with the drugs on the drug list on the left by checking the boxes.



4.2 Compared Pathways



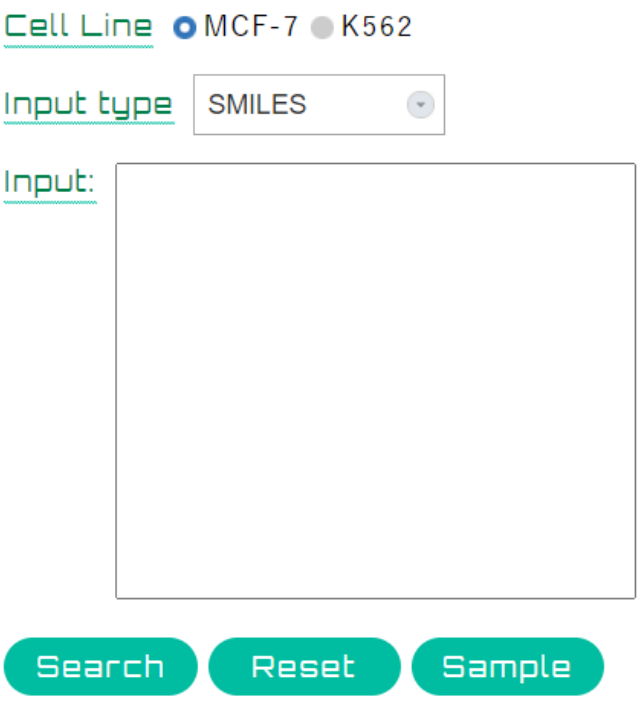
Common and uncommon pathways among the selected drugs are shown in a Venn's diagram. As the pathways form a structural hierarchy network, they can be compared at various depths of the pathway stratum by selecting a pathway stratum of 1–5 to search for common features. All the analyzed proteins belonged to signal transduction, which is the top stratum (pathway stratum = 1). On clicking on an area of the Venn's diagram, the corresponding pathway list is shown on the right.

5 Drug Search

The phosphorylation profile data of compounds not analyzed in Phosprof can also be searched for based on similarity with the drugs that were analyzed. On entering the SMILES or SDF of the drug of interest in the Drug Search section, the similarity with drugs in Phosprof is represented as the Tanimoto coefficient score.

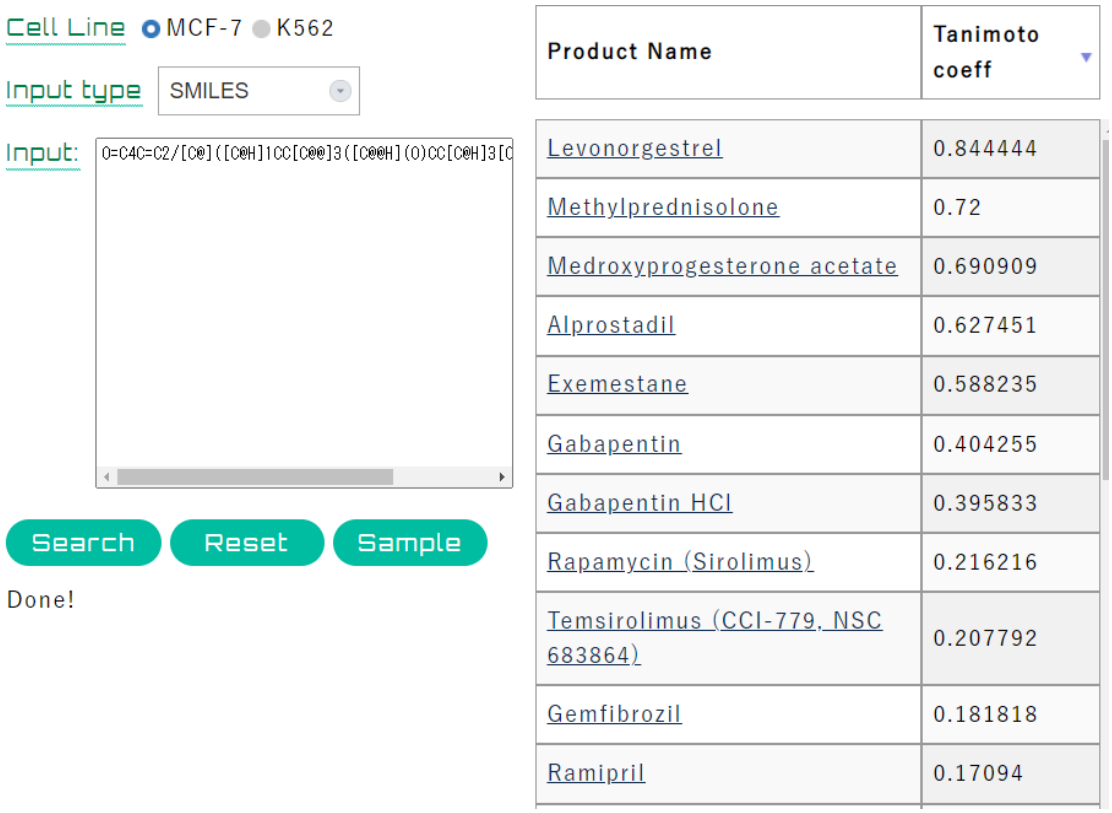
5.1 Input

The tested cell line can be selected, and the information of the drug of interest can be used as input in a SMILES or SDF form followed by clicking on 'Search.'



5.2 Search Results

When the input forms are accepted and the calculations are completed, 94 drugs are aligned in the list based on their Tanimoto coefficient scores. Drugs are linked with the signature section to check the properties of the drug in Phosprof that share analogous features with the drug of interest used as input.



6 Drug List

All the 94 drugs examined in Phosprof are listed in the drug list section with detailed information, including the formulas, CAS numbers, and SMILES, with links to PubChem.

Axitinib

Information	Target
Axitinib is a multi-target inhibitor of VEGFR1, VEGFR2, VEGFR3, PDGFRβ and c-Kit with IC50 of 0.1 nM, 0.2 nM, 0.1-0.3 nM, 1.6 nM and 1.7 nM, respectively.	c-Kit,PDGFR,VEGFR
Pathway	**Formula**
Protein Tyrosine Kinase	C22H18N4OS
CAS Number	**SMILES**
319460-85-0	CNC(=O)C1=CC=CC=C1SC1=CC2=C(C=C1)C(¥C=C¥C1=CC=CC=N1)=NN2 |c:6,8,14,16,24,26,28,t:4,12,22|

Bosutinib (SKI-606)

Information	Target
Bosutinib (SKI-606) is a novel, dual Src/Abl inhibitor with IC50 of 1.2 nM and 1 nM, respectively.	Src
Pathway	**Formula**
Angiogenesis	C26H29Cl2N5O3
CAS Number	**SMILES**
380843-75-4	COC1=C(Cl)C=C(Cl)C(NC2=C(C=NC3=CC(OCCCN4CCN(C)CC4)=C(OC)C=C23)C#N)=C1 |c:2,12,37,t:5,10,14,28,32|

PD0325901

Information	Target
PD0325901 (PD325901) is selective and non ATP-competitive MEK inhibitor with IC50 of 0.33 nM, roughly 500-fold more potent than CI-1040 on phosphorylation of ERK1 and ERK2. Phase 1/2.	MEK

7 Contact

Phosprof
URL: https://phosprof.medals.jp

If you have any questions or comments, please contact the following e-mail address.
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