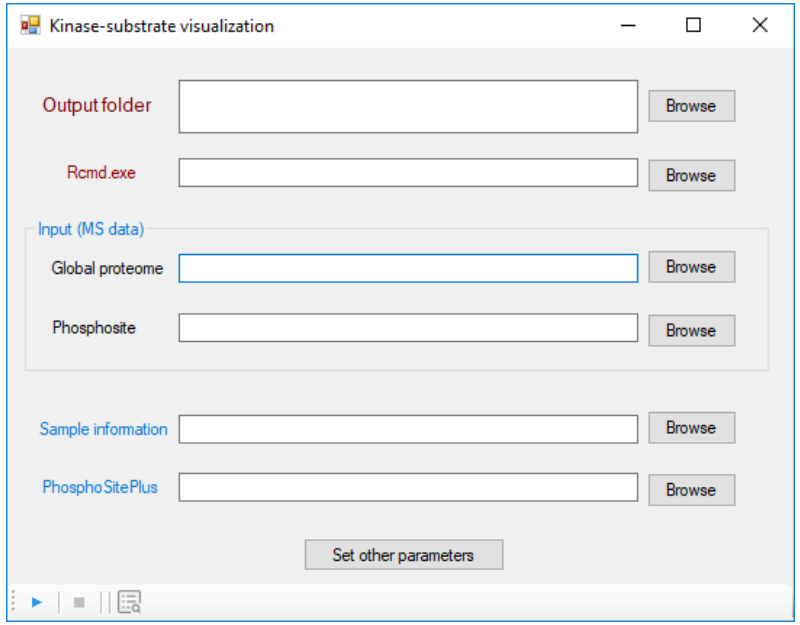
Omic-Sig

User Guide

Input parameters



Outputfolder
Specify an output directory for exporting results.

Rcmd.exe
Specify the location of Rcmd.exe file since the data visualization is done in R.
Rcmd.exe file is usually located in "..\bin\i386" of R.
Example: C:\Program Files\R\R-3.5.3\bin\i386

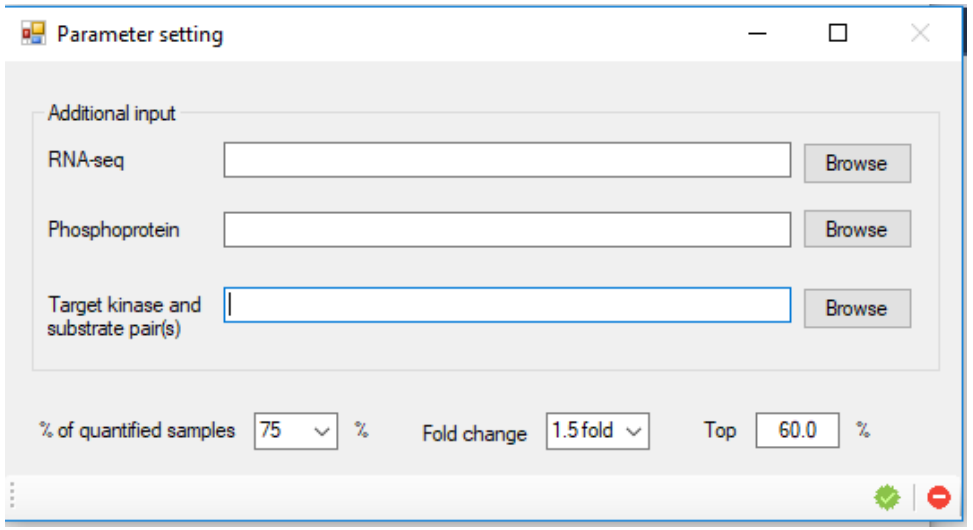
Global proteome
Expression matrix of global proteins (normalized and log-transformed).

Phosphosite
Expression matrix of phosphosites (normalized and log-transformed).

Sample information
(1) Sample.ID: sample IDs that are corresponding to the sample IDs in uploaded expression matrices.
(2) Pair.Tag: a paired sample (e.g., tumor and its normal adjacent tissue) must be given the same identification tag.
(3) Tissue: type of sample (e.g., Tumor or NAT).

PhosphoSitePlus
Kinase_Substrate_Dataset file download from PhosphoSitePlus.

Start the process
Cancel the process
Show results



RNA-Seq (optional)
Expression matrix of mRNA (normalized and log-transformed).

Phosphoprotein (optional)
Expression matrix of phosphoproteins (normalized and log-transformed).

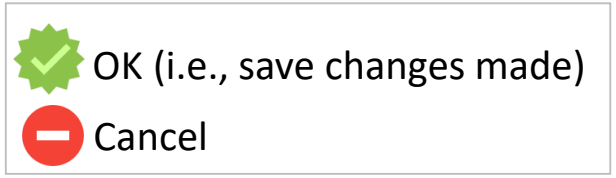
Target kinase and substrate pair(s)
If you would like to use your own list of kinases and substrates instead of the ones from PhosphoSitePlus.

% of samples quantified
Only consider substrates and kinases quantified in 75% (by default) of samples.

Fold change
Either 1.5 fold or 2 fold changes currently.

Top *k* %

Only phospho-substrates (based on phosphosite data) have more than *k*% (*k*=60 by default) of tumors with > 1.5 fold changes (by default) are considered for analysis.



Input file format

Basic format for phospho, global, and mRNA expression matrices

Index	Sample 1	Sample 2	…	Sample n-1	Sample n

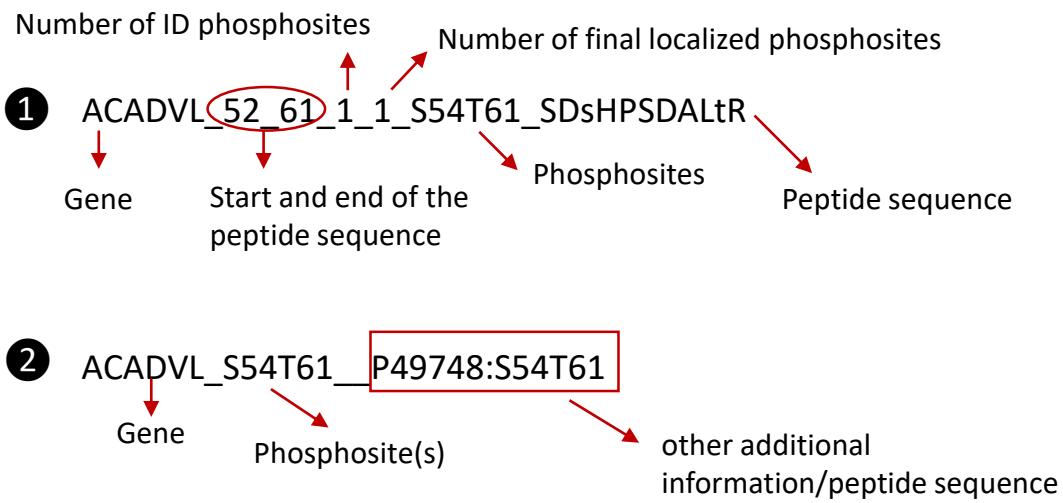
Row = phosphosites/proteins/genes
Columns = samples

- **Global/RNA/Phosphoprotein**

The index should be the official gene symbols/gene names, such as SYNM, CD44, and COL6A1.

- **Phosphosites**

Format of the index can be one of the following:



- **Sample information**

(1) Sample.ID: sample IDs that are corresponding to the sample IDs in uploaded expression matrices.
(2) Pair.Tag: a paired sample (e.g., tumor and its normal adjacent tissue) must be given the same identification tag.
(3) Tissue: type of sample (e.g., Tumor or NAT).

Example:

Sample.ID	Pair.Tag	Tissue
Sample 1	1	NAT
Sample 2	1	Tumor
Sample 3	S3	NAT
Sample 4	S3	Tumor

This is a pair

This is another pair

- **Target kinase and substrate pairs**

Kinase	Substrate	Substrate.Site
PRKCD	GSK3A	S21
EIF2AK1	EIF2S1	S52

One line per kinase-substrate pair

Output

Generate plots of selected kinases and substrates

Kinase table (RNA, global, and phosphoprotein)

Phospho-substrate table (RNA, global, and phosphosite)

Kinase-substrate table

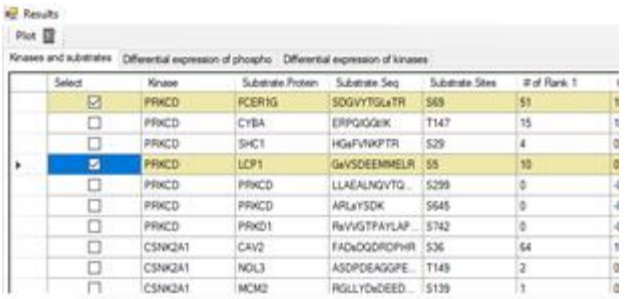
Plot

Kinases and substrates | Differential expression of phospho | Differential expression of kinases

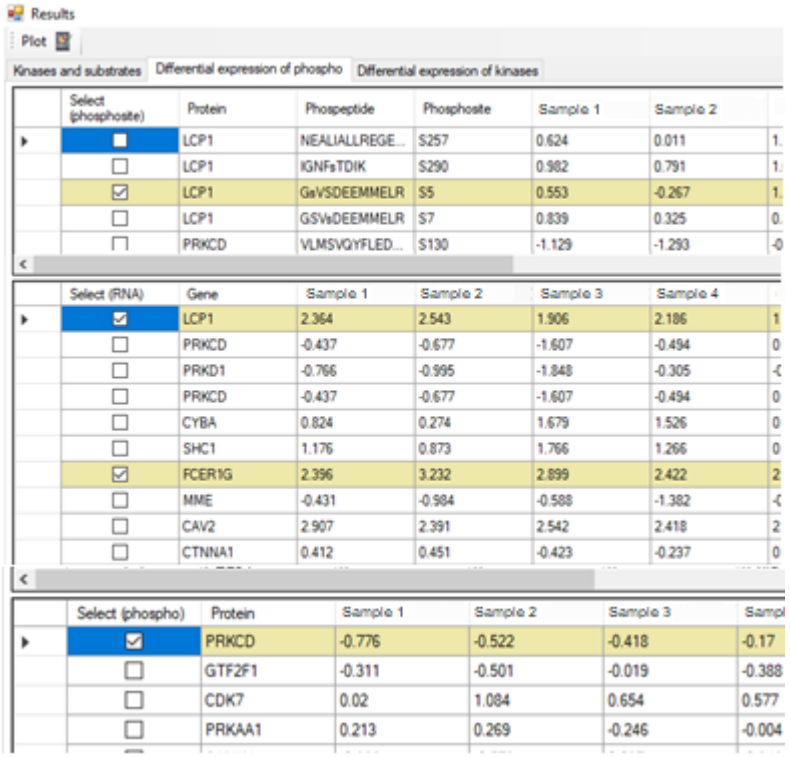
Select	Kinase	Substrate.Protein	Substrate.Seq	Substrate.Sites	# of Rank 1	Sa
☐	AKT1	PKM	TATEsFASDPIL...	S97	14	0.8
		FAF1	TPsGEFLER	S582	0	0.8
		BCL3	RPLRAPsPEPAA...	S41	6	0.8
		HSPB1	QLsSGVSEIR	S82	10	0.8
☐	AKT1	FLNC	ERLGsFGSITR	S2233	5	0.7
☐	AKT1	FAM129A	RAsAILPGVLGS...	S602	5	0.6
☐	AKT1	PKM	GADFLVTEVEN...	S202	0	0.6
☐	AKT1	PPP1CA	YGQFSGLNPGG...	T320	4	0.5
☐	AKT1	FOXO3	RAVsMDNSNK	S253	5	0.4
☐	AKT1	PTPN1	YRDVsPFDHSR	S50	13	0.3
☐	AKT1	AKT1S1	LNtSDFQK			
☐	AKT1	ATG4B	KYsIFTEK			
☐	AKT1	PFKFB2	NYsVGSRPLKPL...	S483	0	-0.
☐	AKT2	FLNC	ERLGsFGSITR	S2233	80	0.7

*** All computational results of phospho/global/mRNA are automatically exported to the user-specified output directory as csv files upon completion of data analysis.**

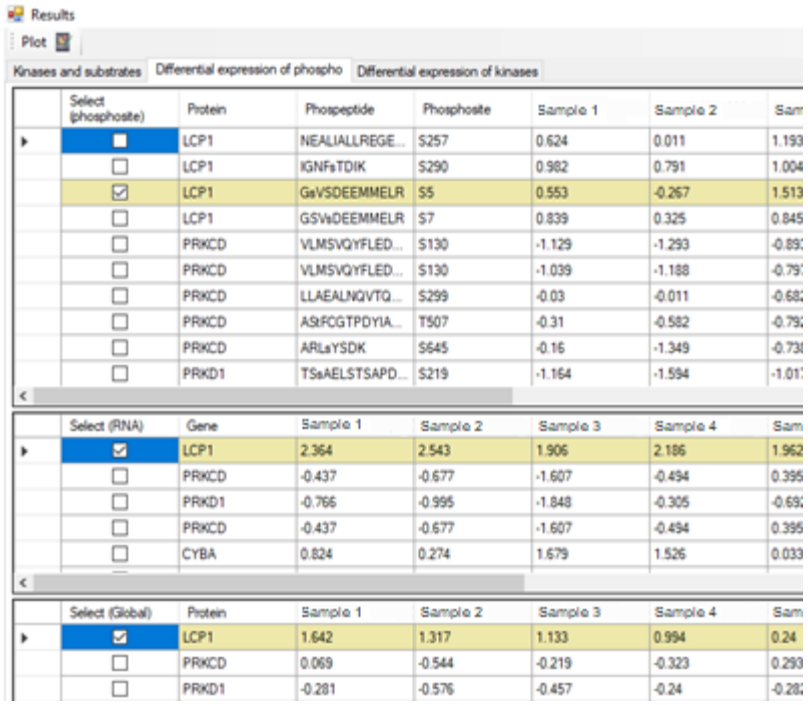
Kinase-substrate table



Phospho-substrate able



Kinase able



By selecting the kinases and substrates (highlighted rows) in the kinase-substrate table, the same group of substrates and kinases in the phospho-substrate and kinase tables will also be automatically selected.