

Use case 2: Mechanism of action analysis via connection to genetic perturbation signatures

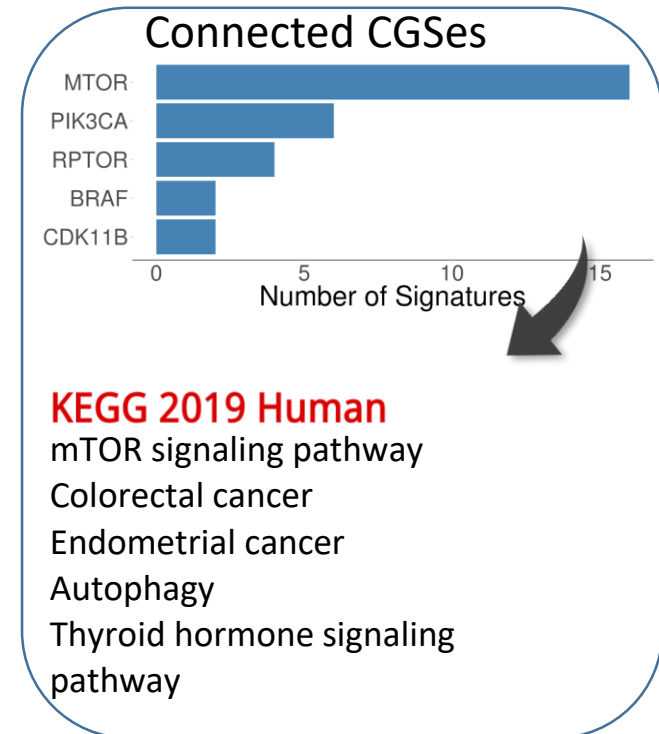
Transcriptional signature of the chemical perturbagens often carry only an echo of such effects since the proteins directly targeted by the chemical and associated signaling proteins are not transcriptionally changed. iLINCS offers the solution for this problem by connecting the CP signatures to LINCS CGSes and follow-up systems biology analysis of genes whose CGSes are highly correlated with the CP signature. This is demonstrated by the analysis of one of the CP signatures of the 24 hour, 0.04 μ M treatment of the MCF-7 cell line with the mTOR inhibitor Everolimus (Fig 2CDE). Traditional pathway enrichment analysis of the transcriptional signatures via iLINCS connection to Enrichr (Fig 2C) fails to identify the mTOR pathway as being affected. In the next step, we first connect the CP signature to LINCS CGSes and then perform pathway enrichment analysis of genes with correlated CGSes. This analysis correctly identifies mTOR signaling pathway as the top most affected pathway (Fig 2D). Similarly, connectivity analysis with other CP signatures followed by the enrichment analysis of protein targets of connected CPs again identifies the Pi3k-Akt signaling pathway as one of the most enriched (Fig 2E).

C) KEGG 2019 Human

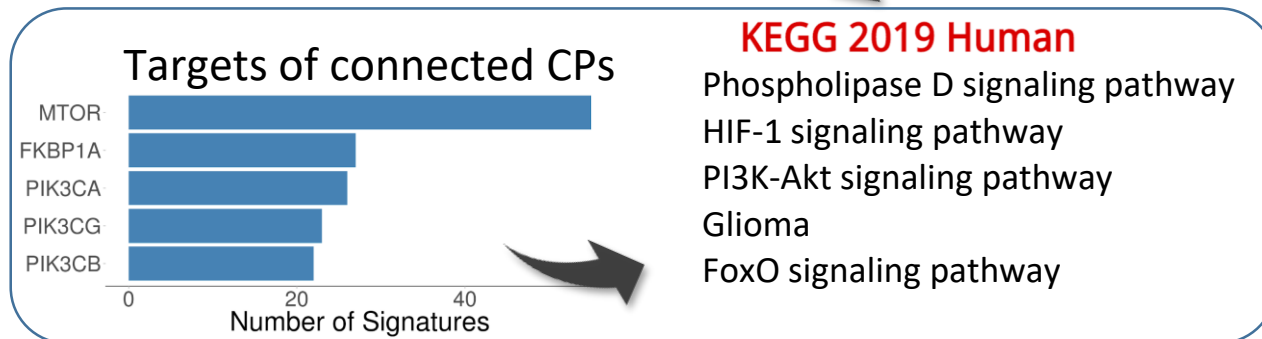
Colorectal cancer
Valine, leucine and isoleucine degradation
Terpenoid backbone biosynthesis
Endometrial cancer
Cell cycle

Cell Line: MCF7 
Time: 24h
Concentration: 0.04 μ M
Perturbagen: Everolimus

D)



E)



The screenshot shows the iLINCS website interface. At the top, there is a navigation bar with the iLINCS logo and three tabs: "Signatures", "Datasets", and "Genes". A red arrow points from a yellow callout box to the "Signatures" tab. The callout box contains the text: "1. Click 'Signatures' tab on the top of iLINCS homepage to open signatures pipeline consisting of over 200,000 pre-computed signatures." Below the navigation bar, there is a search bar with the text "Search for signatures". The main content area is titled "Signatures" and includes buttons for "Search", "Submit", and "Maps". Below this is a section titled "Find signatures to analyze" which contains a list of signature categories with their respective counts, a search input field, and a "Find Signatures with Pharmacological Actions" button.

| Signature Library | Number of signatures |
|--|----------------------|
| <input checked="" type="checkbox"/> LINCS consensus gene (CGS) knockdown signatures | 37275 |
| <input checked="" type="checkbox"/> LINCS gene overexpression signatures | 9291 |
| <input checked="" type="checkbox"/> LINCS chemical perturbagen signatures | 143374 |
| <input checked="" type="checkbox"/> LINCS targeted proteomics signatures | 1178 |
| <input checked="" type="checkbox"/> Disease related signatures | 9097 |
| <input checked="" type="checkbox"/> ENCODE transcription factor binding signatures | 494 |
| <input checked="" type="checkbox"/> Connectivity Map signatures | 519 |
| <input checked="" type="checkbox"/> DrugMatrix signatures | 5288 |
| <input checked="" type="checkbox"/> Transcriptional signatures from EBI Expression Atlas | 2802 |

Search for keyword... Search

Example keywords : sirolimus, MCF7, vorinostat, MTOR, RAF inhibitor

OR

Find Signatures with Pharmacological Actions

Search Submit Maps

Find signatures to analyze

- Signature Library Number of signatures
- LINCS consensus gene (CGS) knockdown signatures 37275
- LINCS gene overexpression signatures 9291
- LINCS chemical perturbagen signatures 143374
- LINCS targeted proteomics signatures 1178
- Disease related signatures 9097
- ENCODE transcription factor binding signatures 494
- Connectivity Map signatures 519
- DrugMatrix signatures 5288
- Transcriptional signatures from EBI Expression Atlas 5646
- Cancer therapeutics response signatures 9901
- Pharmacogenomics transcriptional signatures 5262

Everolimus

Example keywords : sirolimus, MCF7, vorinostat, MTOR, RAF inhibitor

2. Search for a signature of 24 hour, 0.04μM treatment of the MCF-7 cell line with the mTOR inhibitor Everolimus within LINCS chemical perturbagen signatures library.

Signatures filtered by keyword: Everolimus

Found 187 of LINCS chemical perturbagen signatures

Analyze Selection My list Download Clear filters Stats

| Signature Id | Perturbagen | Perturbagen targets | Concentration | Cell Line | Time |
|--|---------------|---------------------|---------------|-----------|------|
| <input type="checkbox"/> LINCSCP_133495 | BRD-A25736793 | | 0.04 | MCF7 | |
| <input checked="" type="checkbox"/> LINCSCP_137891 | Everolimus | MTOR | 0.04uM | MCF7 | 24h |
| | | FKBP1A MTOR | 0.04uM | MCF7 | 24h |

3. Click on the signature ID to open the signature landing page.

Signature LINCSCP_137891

4. Signature landing page for the selected signature

“LINCSCP_137891”.

Signature Id: LINCSCP_137891
Library Name: LINC chemical perturbation signatures
Cell Line: MCF7
Time: 24h
Concentration: 0.04uM
Perturbagen: Everolimus
Perturbagen ID: BRD-K13154216
Mechanism Of Action: FK506-binding protein 1A inhibitor
Perturbagen targets:
Platform:
PubChem:
Stitch:
LINC perturbation ID:
LINC signature ID:
Matching pharmacological actions:
[Complete signature \(978\)](#) Selected

5. Click “Enrichment Analysis” to send the list of selected genes to the Enrichr to perform computational analysis checking whether an input set of genes (i.e. selected genes from LINCS signature) significantly overlaps with annotated gene sets.

Signature Analysis Tools

Signature data

Connected Signatures

Connected Perturbations

Pathway Analysis



Enrichment Analysis



DAVID



ToppFun



GeneMANIA



Reactome



PINET



L1000CDS2



L1000FWD



X2K



Morpheus Heatmap



SigNetA



Description No description available (100 genes)**WikiPathways 2019 Human****WikiPathways 2019**

6. Pathway enrichment analysis of the selected genes from LINCS CP transcriptional signature for Everolimus fails to identify the mTOR pathway.

KEGG 2019 Human**Bar Graph**[Table](#)[Clustergram](#)

Click the bars to sort. Now sorted by p-value ranking.

[SVG](#) [PNG](#) [JPG](#)

Signature Analysis

Modify the list of selected genes >

Other analyses with selected genes >

Signature Info

Signature Id: LINCSCP_137891
Library Name: LINC chemical perturbation signatures
Cell Line: MCF7
Time: 24h
Concentration: 0.04uM
Perturbagen: Everolimus
Perturbagen ID: BRD-K13154216
Mechanism Of Action: FK506-binding protein 1A inhibitor

Complete signature (878) Selected genes (100) Download Add to list

7. Click "Connected Signatures" tab to instruct iLINCS to perform connectivity analysis to identify pre-computed genome-wide signatures that correlate (positively or negatively) with the selected signature.

Signature Analysis Tools

Signature data **Connected Signatures** Connected Perturbations

Pathway Analysis Enrichment Analysis DAVID ToppFun GeneMANIA Reactome

PINET L1000CDS2 L1000FWD X2K Morpheus Heatmap SigNetA

Signature Analysis

Modify the list of selected genes >

Other analyses with selected genes >

Signature Info

Signature Id: LINCSCP_137891
 Library Name: LINC chemical perturbation signatures
 Cell Line: MCF7 ⚡
 Time: 24h
 Concentration: 0.04uM
 Perturbagen: Everolimus
 Perturbagen ID: BRD-K13154216 ⚡🌈
 Mechanism Of Action: FK506-binding protein 1A inhibitor | mTOR inhibitor
 Perturbagen targets: FKBP1A | MTOR
 Platform: L1000
 PubChem: 6442177 📄🌐
 Stitch: CIDs06442177 ⚡
 LINC perturbation ID: LSM-43172 🌈
 LINC signature ID: REPA010_MCF7_24H:H06 ⚡
 Matching pharmacological actions: Antineoplastic Agents, Chemical Actions and Uses, Immunologic Factors, Immunosuppressive A... [More](#)

Complete signature (978) Selected genes (100)

[Download](#) [Add to list](#)

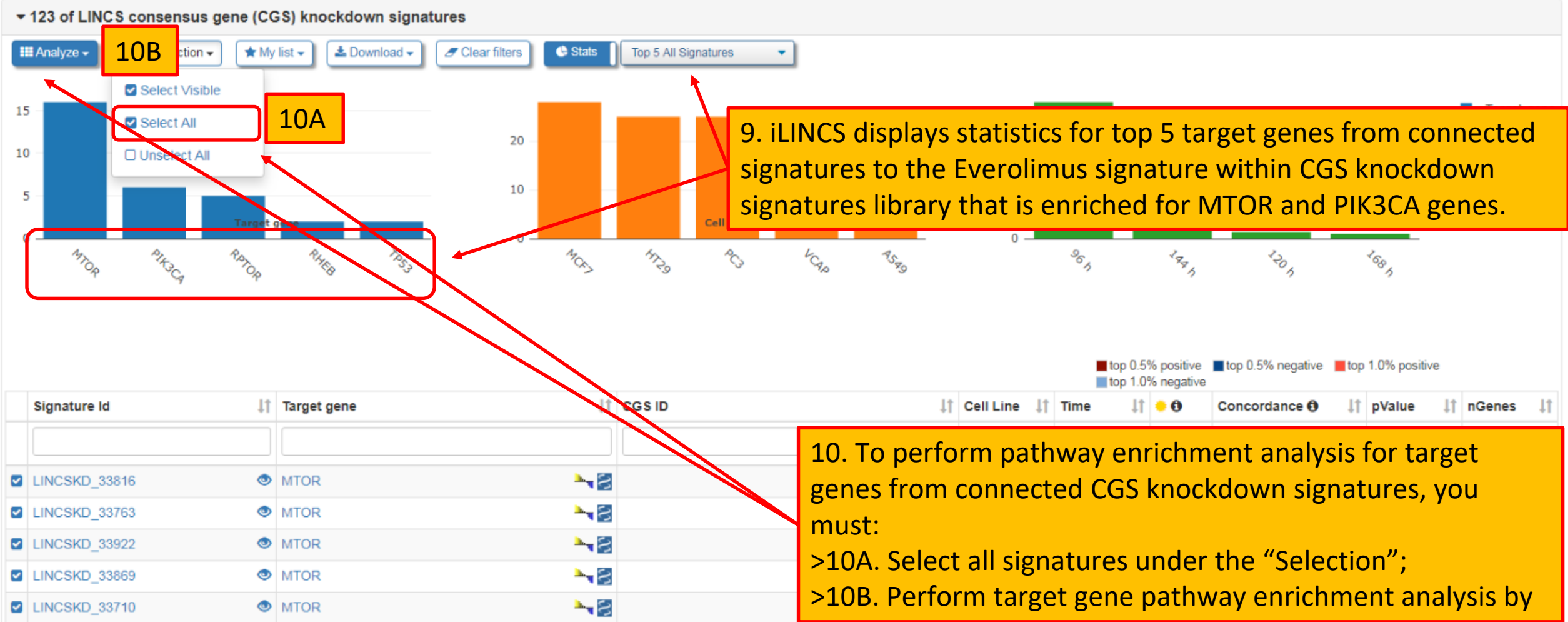
- Signature Analysis Tools
- Signature data
- Connected Signatures**
- Connected Perturbations

- Use complete signature (978) Use selected genes (100)
- ▶ 123 of LINC consensus gene (CGS) knockdown signatures
 - ▶ 21 of LINC gene overexpression signatures
 - ▶ 3602 of LINC chemical perturbation signatures
 - ▶ 28 of Disease related signatures
 - ▶ 1 of ENCODE transcription factor binding signatures
 - ▶ 43 of Connectivity Map signatures
 - ▶ 2 of DrugMatrix signatures

8. Expand the list of LINC consensus gene (CGS) knockdown signatures that connect to the selected Everolimus chemical perturbation transcriptional signature.

Use complete signature (978)

Use selected genes (100)



9. iLINCS displays statistics for top 5 target genes from connected signatures to the Everolimus signature within CGS knockdown signatures library that is enriched for MTOR and PIK3CA genes.

10. To perform pathway enrichment analysis for target genes from connected CGS knockdown signatures, you must:
 >10A. Select all signatures under the "Selection";
 >10B. Perform target gene pathway enrichment analysis by clicking "Analyze" and selecting "Enrich".

Description No description available (87 genes)

WikiPathways 2019 Human

WikiPathways 201

KEGG 2019 Human

11. Pathway enrichment analysis of the target genes from correlated LINCS CGS knockdown transcriptional signatures correctly identifies mTOR signaling pathway as the most affected pathway.

Click the bars to sort. Now sorted by p-value ranking.

mTOR signaling pathway

Colorectal cancer

Endometrial cancer

Autophagy

Thyroid hormone signaling pathway

Hepatocellular carcinoma

Insulin signaling pathway

Pathways in cancer

Central carbon metabolism in cancer

Breast cancer

Signature Analysis

Modify the list of selected genes >

Other analyses with selected genes >

Signature Info

Signature Id: LINCSCP_137891
 Library Name: LINC chemical perturbagen signatures
 Cell Line: MCF7 ⭐
 Time: 24h
 Concentration: 0.04uM
 Perturbagen: Everolimus
 Perturbagen ID: BRD-K13154216 ⭐🌈
 Mechanism Of Action: FK506-binding protein 1A inhibitor | mTOR inhibitor
 Perturbagen targets: FKBP1A | MTOR
 Platform: L1000
 PubChem: 6442177 🧪🌐
 Stitch: CIDs06442177 ⚙️
 LINC perturbation ID: LSM-43172 🌈
 LINC signature ID: REP.A010_MCF7_24H:H06 ⭐
 Matching pharmacological actions: Antineoplastic Agents, Chemical Actions and Uses, Immunologic Factors, Immunosuppressive A... [More](#)

Complete signature (978) Selected genes (100)

Download ▾ Add to list

Signature Analysis Tools Signature data Connected Signatures Connected Perturbations

Use complete signature (978) Use selected genes (100)

- ▶ 123 of LINC consensus gene (CGS) knockdown signatures
- ▶ 21 of LINC gene overexpression signatures
- ▶ 3602 of LINC chemical perturbagen signatures
- ▶ 28 of Disease related signatures
- ▶ 1 of ENCODE transcription factor binding signatures
- ▶ 43 of Connectivity Map signatures
- ▶ 2 of DrugMatrix signatures

12. Click "3602 of LINC chemical perturbagen signatures" to expand the list of signatures that connect to the selected Everolimus chemical perturbagen transcriptional signature.

▶ 123 of LINCS consensus gene (CGS) knockdown signatures

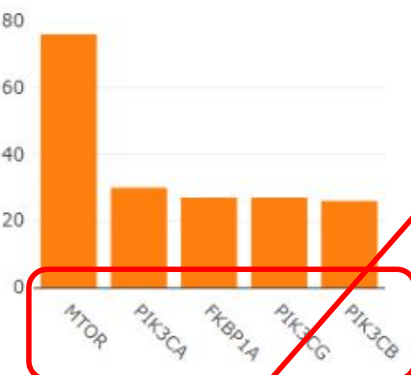
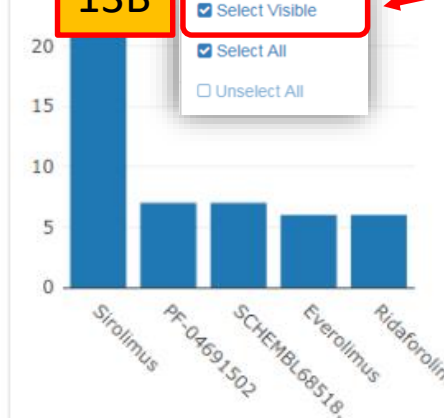
▶ 21 of LINCS gene overexpression signatures

▼ 3602 of LINCS chemical perturbagen signatures

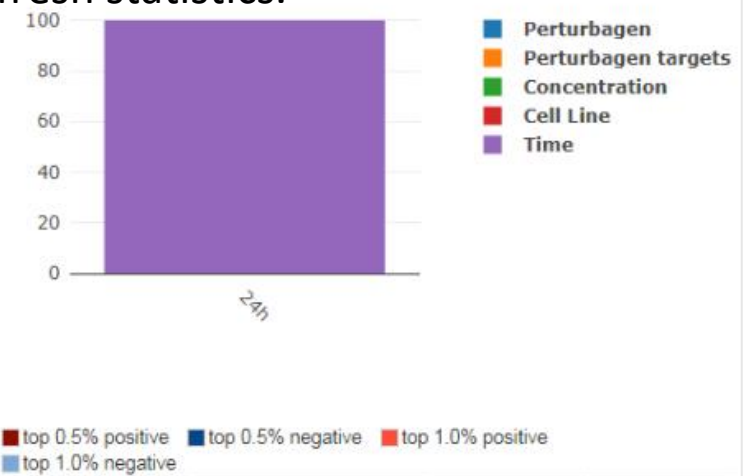
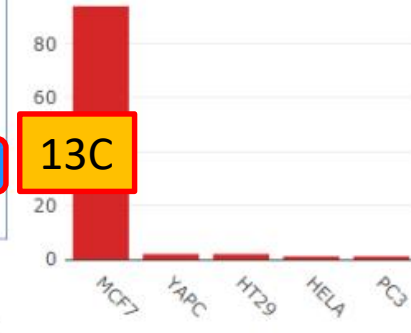
Analyze Selection My list Download Clear filters Stats

13B

Select Visible
Select All
Unselect All



13C



| Signature Id | Perturbagen | Perturbagen targets | Concentration | Cell Line | Time | Concordance | pValue | nGenes |
|----------------|-------------|---------------------|---------------|-----------|------|-------------|--------|--------|
| LINCSCP_137889 | Everolimus | FKBP1A MTO | | | | | | |
| LINCSCP_137888 | Everolimus | FKBP1A MTO | | | | | | |
| LINCSCP_137886 | Everolimus | FKBP1A MTO | | | | | | |
| LINCSCP_14... | Sirolimus | FKBP1A MTO | | | | | | |
| LINCSCP_14... | Sirolimus | FKBP1A MTO | | | | | | |

13A

100

14. After instructing iLINCS to refresh statistics in step 13 (above), top 5 inhibition targets of chemical perturbagens among top 100 most connected chemical perturbagen signatures are for MTOR and PIK3 proteins.

Use complete signature (978)

Use selected genes (100)

▶ 123 of LINCS consensus gene (CGS) knockdown signatures

▶ 21 of LINCS gene overexpression signatures

▼ 3602 of LINCS chemical perturbagen signatures

Analyze Selection My list Download Clear filters Stats Top 5 From Selected Signatures

Signature analysis
 Select Visible **15B**
 Select All
 Unselect All

Perturbagen targets analysis
iLINCS
GeneMania
Enrichr **15C**
ToppGene
DAVID
Kegg Pathways

■ Perturbagen
■ Perturbagen targets
■ Concentration
■ Cell Line
■ Time

■ top 0.5% positive ■ top 0.5% negative ■ top 1.0% positive
■ top 1.0% negative

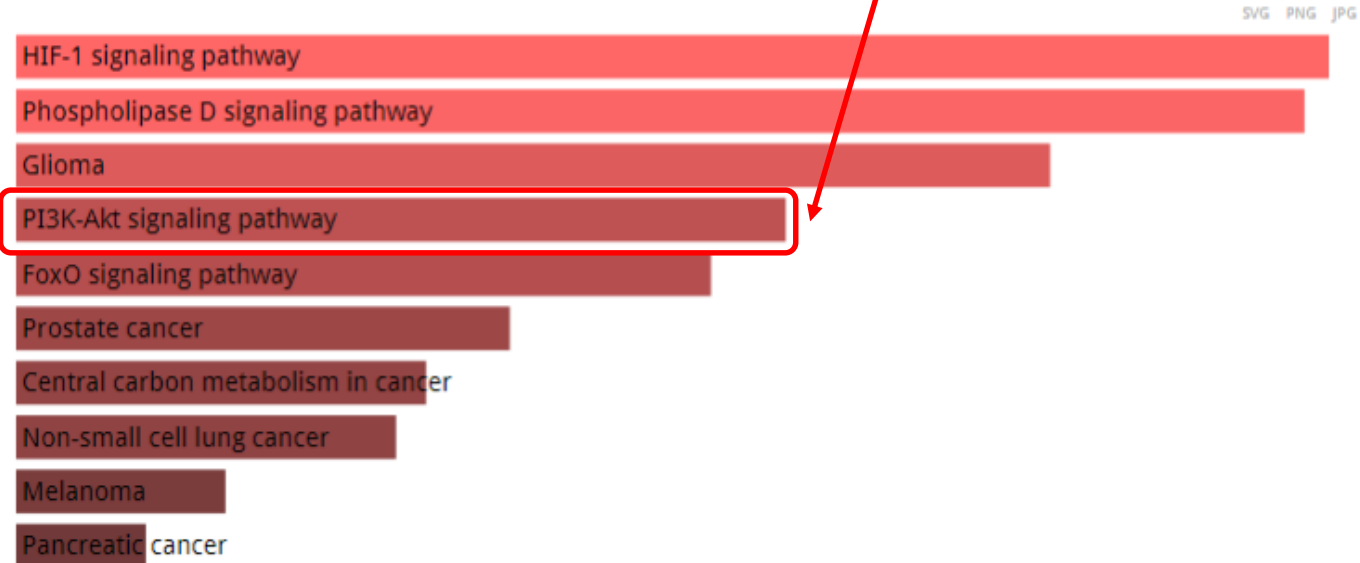
| Signature Id | Perturbagen | Perturbagen targets | Concentration | Cell Line | Time | Concordance | pValue | nGenes |
|--|-------------|---------------------|---------------|-----------|------|-------------|--------|--------|
| <input checked="" type="checkbox"/> LINCSCP_137889 | Everolimus | | | | | | | |
| <input checked="" type="checkbox"/> LINCSCP_137888 | Everolimus | | | | | | | |
| <input checked="" type="checkbox"/> LINCSCP_137886 | Everolimus | | | | | | | |
| <input checked="" type="checkbox"/> LINCSCP_14... | Sirolimus | | | | | | | |
| <input checked="" type="checkbox"/> LINCSCP_14... | Sirolimus | | | | | | | |

5 25 50 **100** **15A**

15. To perform pathway enrichment analysis for perturbagen targets from connected LINCS chemical perturbagen signatures, you must:
>15A. Choose to display 100 signatures (if not selected already in previous step);
>15B. Select visible signatures under the “Selection”;
>15C. Perform target gene pathway enrichment analysis by clicking “Analyze” and selecting “Enrichr”.

Description No description available (22 genes)**WikiPathways 2019 Human****WikiPathways 2019****KEGG 2019 Human**

Click the bars to sort. Now so



16. Pathway enrichment analysis of the perturbagen targets from correlated LINCS chemical perturbagen transcriptional signatures identifies PI3K-Akt signaling pathway as one of the most affected pathways.