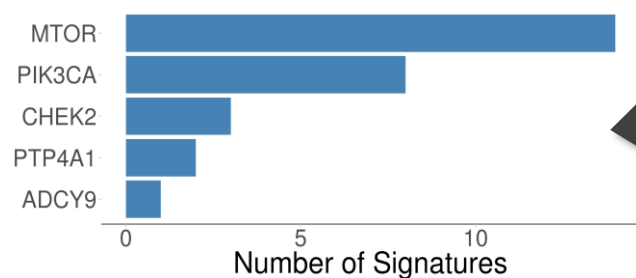


Use case 1: Identifying chemical perturbagens emulating genetic perturbation of MTOR protein

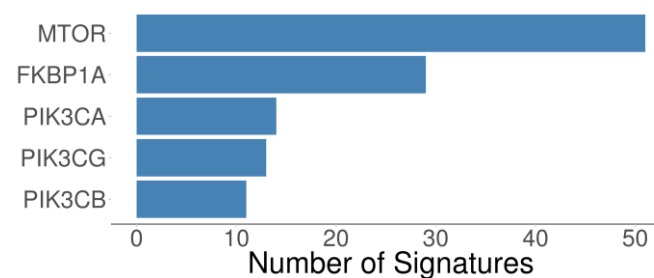
First, we search through iLINCS libraries for Consensus Genes Signatures (CGSes) of MTOR knock-down and use the CRISPR CGS in MCF-7 cell line as the query signature. The connectivity analysis identifies 258 LINCS CGSes and 831 CP Signatures with statistically significant correlation with the query signature. Top 100 most connected CGSes are dominated by the signatures of genetic perturbations of MTOR and PIK3CA genes (Fig 2A), whereas all top 5 most frequent inhibition targets of CPs among top 100 most connected CP signatures are MTOR and PIK3 proteins (Fig 2B). Results clearly indicate that the query MTOR CGS is highly specific and sensitive to perturbation of the mTOR pathway and effectively identifies chemical perturbagens capable of inhibiting mTOR signaling. The connected CP signatures also include several chemical perturbagens with highly connected signatures that have not been known to target mTOR signaling providing additional candidate inhibitors.

A) Connected CGSes



Cell Line: MCF7.101
Time: 96 h
Target Gene: MTOR

B) Targets of connected CPs



The screenshot shows the iLINCS website interface. At the top, there is a navigation bar with the iLINCS logo and tabs for '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 Category	Number of signatures
Signature Library	
✓ LINC consensus gene (CGS) knockdown signatures	37275
✓ LINC gene overexpression signatures	9291
✓ LINC chemical perturbagen signatures	143374
✓ LINC 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	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

- LINC consensus gene (CGS) knockdown signatures
- LINC gene overexpression signatures
- LINC chemical perturbation signatures
- LINC targeted proteomics signatures
- Disease related signatures
- ENCODE transcription factor binding signatures
- Connectivity Map signatures
- DrugMatrix signatures
- Transcriptional signatures from EBI Expression Atlas
- Cancer therapeutics response signatures
- Pharmacogenomics transcriptional signatures

37275
9291
143374
1178
9097
454
519
5288
5646
9901
5262

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

2. Perform MTOR knockdown signature search within LINC Consensus Gene (CGS) knockdown signature library and select the CRISPR MTOR knockdown in MCF-7 cell line as the query signature.

Signatures filtered by keyword: **MTOR**

Found 36 of LINC consensus gene (CGS) knockdown signatures

Signature Id	Target gene	CGS ID	Cell Line	Time	
<input checked="" type="checkbox"/> LINCSDK_33763	MTOR		MCF7		All
<input type="checkbox"/> LINCSDK_21637	LAMTOR3	CGS001-8649	MCF7	144 h	
<input type="checkbox"/> LINCSDK_23272	LAMTOR3	CGS001-8649	MCF7	96 h	
<input type="checkbox"/> LINCSDK_23448	MTOR	CGS001-2475	MCF7	96 h	
<input type="checkbox"/> LINCSDK_33816	MTOR		MCF7.101	96 h	
			MCF7.311	96 h	

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

Signature LINCSD_33763

4. Signature landing page for the selected signature "LINCSD_33763".

Signature Analysis

Modify the list of selected genes >

Other analyses with selected genes >

Signature Info

Signature Id: LINCSD_33763
Library Name: LINC consensus gene (CGS) knockdown signatures
Cell Line: MCF7.101
Time: 96 h
Target Gene: MTOR 
Platform: L1000
LINC signature ID: CGS002_MCF7.101_96H:MTOR

Complete signature (978) Selected

5. 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 Perturb

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: LINCSD_33763
Library Name: LINC consensus gene (CGS) knockdown signatures
Cell Line: MCF7.101
Time: 96 h
Target Gene: MTOR 
Platform: L1000
LINC signature ID: CGS002_MCF7.101_96H:MTOR

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)

- ▶ 258 of LINC consensus gene (CGS) knockdown signatures
- ▶ 17 of LINC gene overexpression signatures
- ▶ 831 of LINC chemical perturbagen signatures
- ▶ 9 of Disease related signatures
- ▶ 10 of ENCODE transcription factor binding signatures
- ▶ 7 of Connectivity Map signatures
- 0 of DrugMatrix signatures
- ▶ 9 of Transcriptional signatures from EBI Expression Atlas
- ▶ 3 of Cancer therapeutics response signatures
- ▶ 5 of Pharmacogenomics transcriptional signatures

6. Click “258 of LINC consensus gene (CGS) knockdown signatures” and/or “831 of LINC chemical perturbagen signatures” to expand the list of connected signatures within those signature libraries.

▼ 258 of LINCS consensus gene (CGS) knockdown signatures



Signature Id	Target gene	CGS ID	Cell Line	Time	Concordance	pValue	nGenes
<input checked="" type="checkbox"/> LINCSDK_33768	PIK3CA				All		
<input checked="" type="checkbox"/> LINCSDK_33816	MTOR				All		
<input checked="" type="checkbox"/> LINCSDK_33710	MTOR				All		
<input checked="" type="checkbox"/> LINCSDK_33710	PIK3CA				All		
<input checked="" type="checkbox"/> LINCSDK_33710	MTOR				All		

Legend: top 0.5% positive (dark red), top 0.5% negative (dark blue), top 1.0% positive (red), top 1.0% negative (blue).

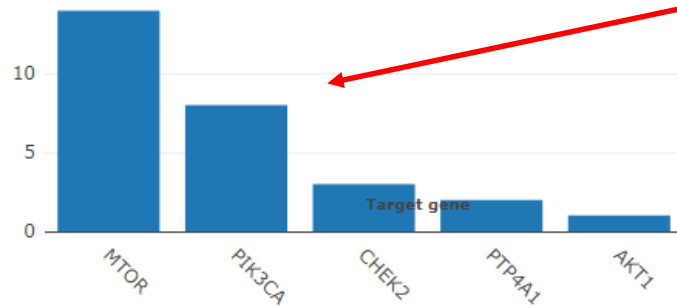
Page controls: 5, 25, 50, 100 (highlighted as 7A), First, « 1 2 3 4

7. To display statistics for top 100 connected signatures within CGS knockdown signature library, you must:

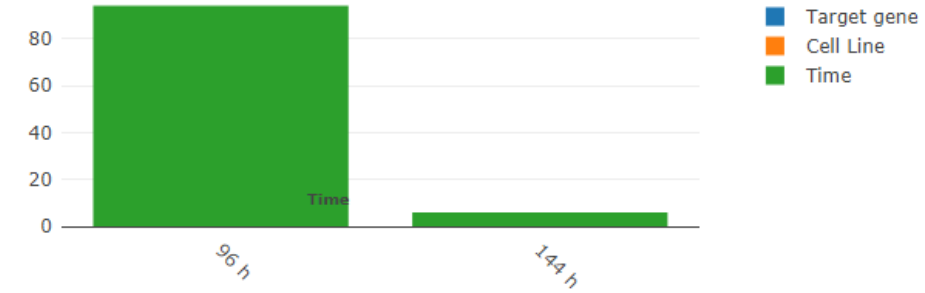
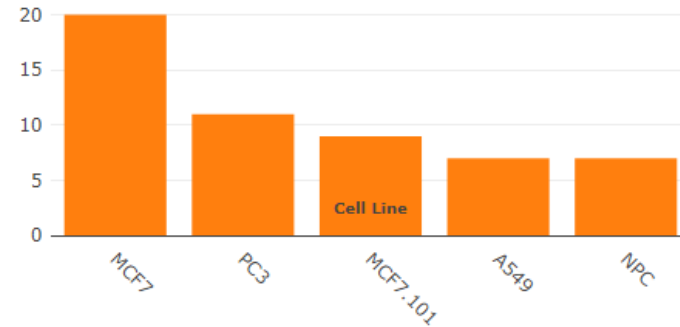
- >7A. Choose to display 100 signatures;
- >7B. Select visible signatures under the “Selection”;
- >7C. Instruct iLINCS to refresh statistics.

▼ 258 of LINC consensus gene (CGS) knockdown signatures

Analyze Selection My list Download Clear filters Stats Top 5 Selected Sign

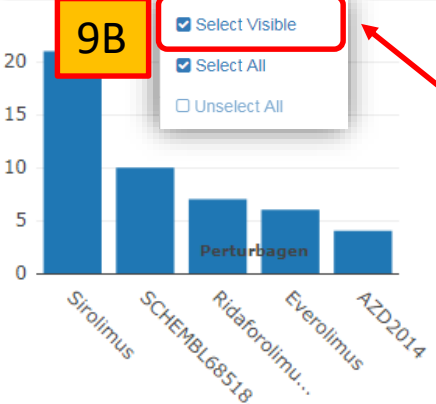


8. Top 100 most connected CGS knockdown signatures are enriched for the genetic perturbations of MTOR and PIK3CA genes.



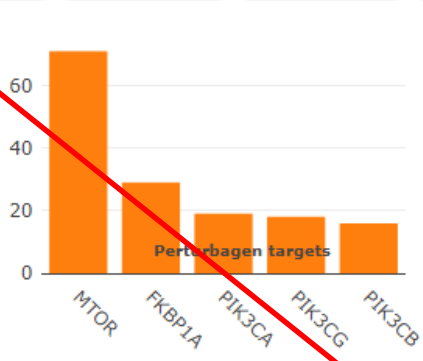
▼ 831 of LINC chemical perturbation signatures

Analyze ▾ / □ Selection ▾ ★ My list ▾ ⬇ Download ▾ ✍ Clear filters ⚙ Stats ▾



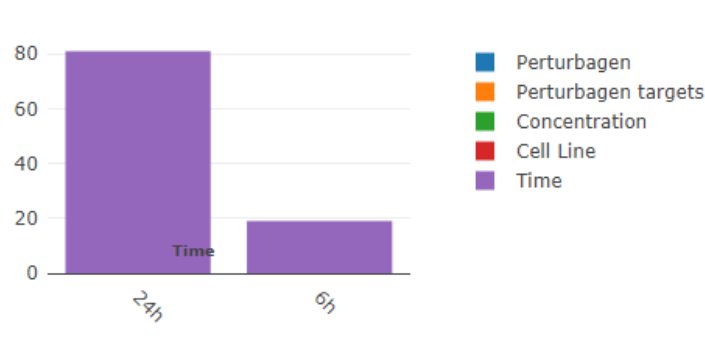
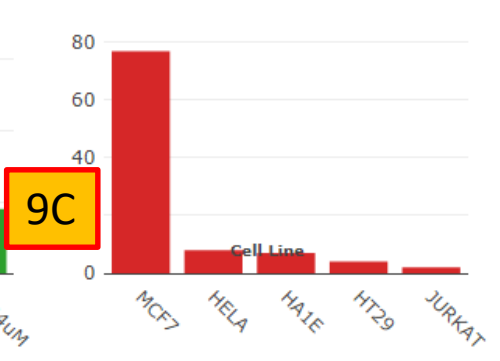
9B

Select Visible
 Select All
 Unselect All



Top 5 Selected Signatures ▾

- All Signatures
- Top 5 All Signatures
- Top 10 All Signatures
- Selected Signatures
- Top 5 Selected Signatures**
- Top 10 Selected Signatures



Signature Id	Perturbagen	Perturbagen targets	Concentration	Cell Line	Time	Concordance	pValue	nGenes
<input checked="" type="checkbox"/> LINCSCP_137891	Everolimus	FKBP1A MTOR				All ▾		
<input checked="" type="checkbox"/> LINCSCP_141783	SCHEMBL6851809	MTOR				All ▾		
<input checked="" type="checkbox"/> LINCSCP_137889	Everolimus	FKBP1A MTOR				All ▾		
<input checked="" type="checkbox"/> LINCSCP_141783	SCHEMBL6851809	MTOR				All ▾		
<input checked="" type="checkbox"/> LINCSCP_38...	Sirolimus	FKBP1A MTOR				All ▾		

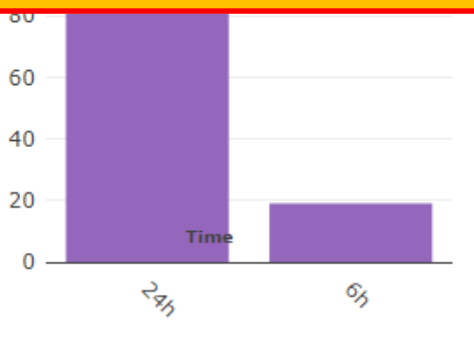
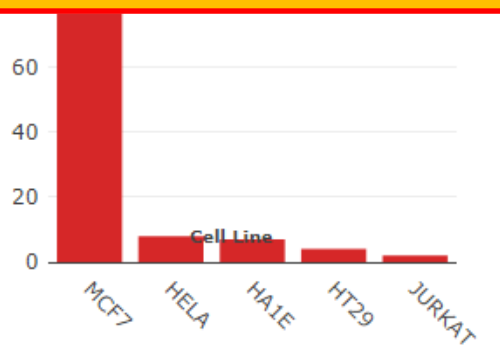
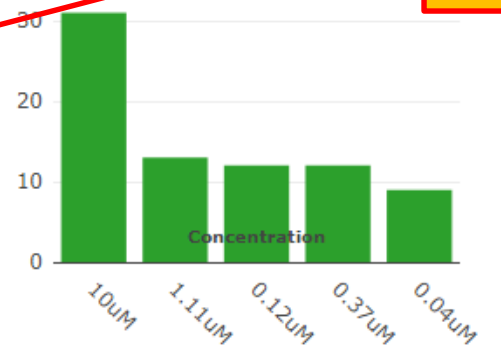
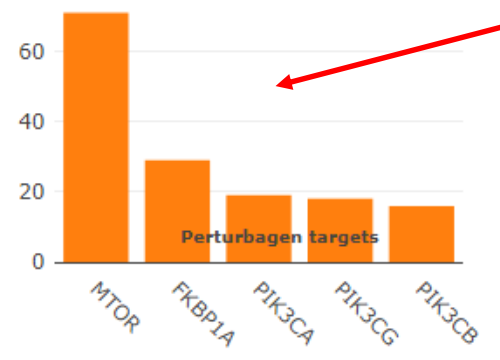
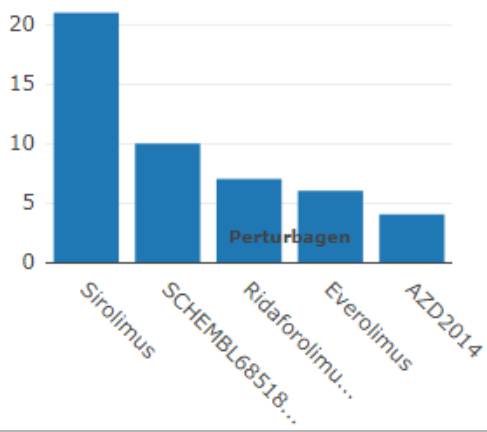
5 25 50 **100** First « 1 2 3 4

9. To display statistics for top 100 connected signatures within chemical perturbation signature library, you must:

- >9A. Choose to display 100 signatures;
- >9B. Select visible signatures under the "Selection";
- >9C. Instruct iLINCS to refresh statistics.

▼ 831 of LINCS chemical perturbation signatures

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



10. Top 5 inhibition targets of chemical perturbagens among most connected chemical perturbation signatures are MTOR and PIK3 proteins.