SILAC (IP-MS)

Step 1: Click on the SILAC tab on the home page to access all the SILAC workflows (as shown in figure below with the grey arrow).

Step 2: Choose the type of analysis to run. You can hover over the question mark to get more details on the different choices and recommended settings (as shown by the grey arrow below). The choices to be made here include:
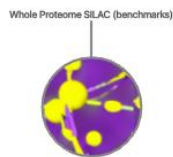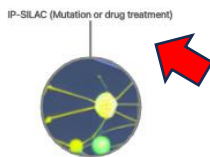
1. The choice of search engine (COMET for now)
2. The choice of analysis type (MAGMa Limma only possible due to pairing of quantifications)

Click on the IP-SILAC arm (highlighted with red arrow in the figure below) to access to workflow.

Step 3: Upload the annotation file. This is made using the experimental setup.

An example can be seen below taken from a METTL3 IP-MS SILAC (forward and reverse, two channel) experiment. Here each SILAC isotope label corresponds to a different condition, where forward run labels are switched around in the reverse run. Control column here lets the underlying tool know whether the label is a control in your experimental setup or not. You can also hover over question mark for more details. Click on the "Sample File" in blue to download this example annotation file.

Step 4: Choose the conditions you want to compare in a pairwise fashion. And choose the direction of comparison. So, choosing comparison ('WT','EV') and direction (Condition_1/Condition_2) means the following comparison will run – (WT/EV).

Step 5: Upload the input files (PSM list) for quantification.

For COMET search after Peptide-Prophet and Libra, upload a tab separated file of PSM (filtered at 0.9 probability or the probability associated with FDR cutoff choice) and associated light, heavy and (medium?) MS1 precursor intensity. Make sure the files (one for forward and one for reverse SILAC) have the following columns –
"spectrum","precursor_intensity","peptide","assumed_charge","retention_time_sec".

As before hover over question mark for more information. An example of the file (WT/EV) is also below. Click on the "Sample File" in blue to download these example PSM files.

3

Upload a tab separated PSM file (Forward SILAC) with light and heavy AUC.

For more information: **?**

Select a file:

- This field is required.

Choose File | No file chosen

Reset

Sample file

4

Upload a tab separated PSM file (Reverse SILAC) with light and heavy AUC.

For more information: **?**

- This field is required.

Choose File | No file chosen

Reset

Sample file

Step 6: You can modify settings (type of column normalization) for your specific analysis. Recommendation is to use the default settings. Hover over question mark for more information.

Step 7: Enter the Uniprot-ID of the bait that is pulled down in your IP-MS experiment. This is especially important for the non-control comparisons to normalize by bait for Condition 2-vs-

Condition 1 type of comparisons (for example, drug treatments vs non-drug treatment). If you want to switch off bait normalization for non-control comparison type in "NA" in this field.

Make sure the Uniprot-ID is in the column 'protein' in COMET search files (input in step 5). The example below of "**Q86U44**" corresponds to METTL3 bait. As before hover over question mark to get more information.

Step 8: Choose whether to do imputation on your data or not. Recommendation is to not to not introduce biases.

A successful start of run with look like this –

The output page (given a successful run) looks like the screenshots below. You can download the protein level differential expression analysis results as well as the pdf of resulting volcano plot (set at 5 PSM cutoff). You can highlight specific proteins on the volcano plot by entering the associated Uniprot IDs. You can dynamically set the thresholds and download the resulting file, as well as subset the result file with user-defined thresholds. –
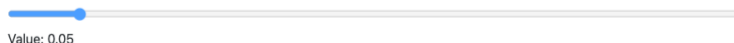
Success!

Click Here to Download the Result File
Click Here to Download the baseline Volcano plot (PDF)

FC cutoff choice: Drag the slider to display the current value.
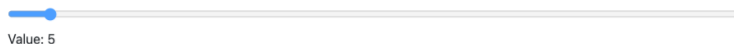
Value: 2

Adjusted P-value cutoff choice: Drag the slider to display the current value.

Value: 0.05

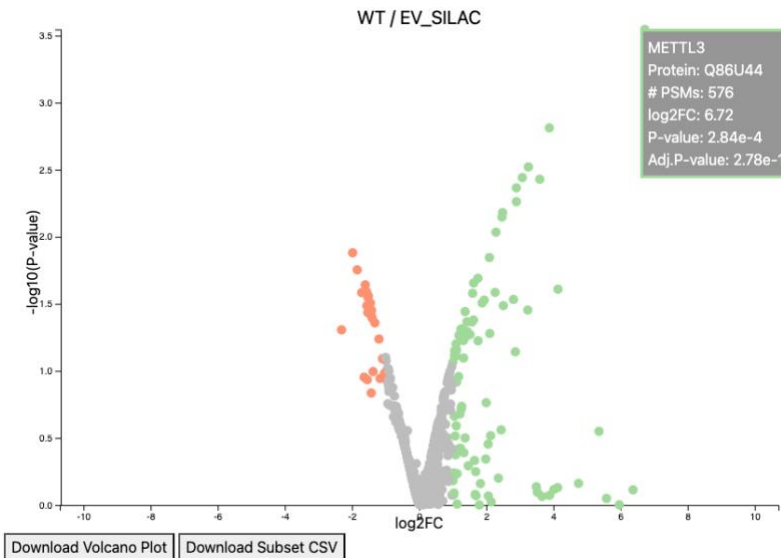PSM cutoff choice: Drag the slider to display the current value.

Value: 5

Size of scatter point: Drag the slider to display the current value.

Value: 5

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Enter Uniprot IDs separated by @
    Example: Q08945@Q9UQE7
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[Update Volcano Plot]

WT / EV_SILAC

METTL3
Protein: Q86U44
PSMs: 576
log2FC: 6.72
P-value: 2.84e-4
Adj.P-value: 2.78e-1

[Download Volcano Plot] [Download Subset CSV]

The output file (protein) looks like this –

	A	B	C	D	E	F	G	H
1	Protein	log2FC	pval	adjpval	Proteinnoiso	Gene Symbol	# PSMs	PSM Cutoff
2	P49458	0.03274533	0.00017945	0.00057287	P49458	SRP9	14	10
3	O00154	5.35767076	0.01659821	0.01737951	O00154	ACOT7	14	
4	Q8IWX8	-0.2194749	0.00017013	0.00057287	Q8IWX8	CHERP	7	
5	P51571	-0.0159671	0.00020331	0.00057287	P51571	SSR4	17	
6	Q9NQ39	-0.1280107	0.00021228	0.00057287	Q9NQ39	RPS10P5	13	
7	Q05D32	-0.2698115	0.00016725	0.00057287	Q05D32	CTDSPL2	3	
8	Q8N3Z3	0.17880555	0.00367871	0.00398096	Q8N3Z3	GTPBP8	21	
9	P50991	2.09407575	0.00039177	0.00061349	P50991	CCT4	72	
10	P22830	0.27112776	0.00019107	0.00057287	P22830	FECH	8	
11	Q9NSD9	0.96937232	0.00024277	0.00057287	Q9NSD9	FARSB	29	
12	Q8N6H7	0.30449419	0.00024538	0.00057287	Q8N6H7	ARFGAP2	5	
13	Q10469	-0.4730667	0.00015962	0.00057287	Q10469	MGAT2	19	
14	Q9P1Y5	-1.6379685	0.00379094	0.00409356	Q9P1Y5	CAMSAP3	7	
15	Q99733	-0.0403441	0.00025896	0.00057287	Q99733	NAP1L4	1	
16	Q8N1G4	0.51395537	0.000399	0.0006183	Q8N1G4	LRRC47	11	
17	Q15386	-0.2695828	0.0004829	0.00068663	Q15386	UBE3C	1	
18	P12270	-0.4438866	0.00035408	0.00059432	P12270	TPR	11	
19	O15198	0.16326143	0.00026094	0.00057287	O15198	SMAD9	14	
20	Q99714	0.26244061	0.00037855	0.00060472	Q99714	HSD17B10	20	
21	Q9HC62	-0.4786855	0.00017363	0.00057287	Q9HC62	SENP2	7	
22	Q9BQG0	0.10222169	0.00031397	0.00057864	Q9BQG0	MYBBP1A	31	
23	A0A075B6Z2	4.74954616	0.06340039	0.0648914	A0A075B6Z2		6	
24	P24928	-0.6005613	0.00034403	0.00059158	P24928	POLR2A	37	

◄ ► | WT-vs-EV_SILAC_FC_and_pval_YuLa | +