

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:

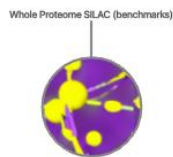
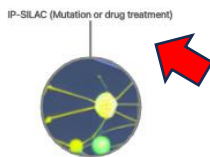
1. The choice of search engine (COMET for now)
2. The choice of analysis type (MAGMA Limma only possible due to pairing of quantifications)

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

Analysis Type

Use COMET Search results:

Magma (Limma)



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.](#)

1 Upload the 'Annotation file'

For more information: ?

	A	B	C	D
1	File	Channel	Label	Control
2	REV	Heavy	WT	FALSE
3	REV	Light	EV	TRUE
4	FWD	Heavy	EV	TRUE
5	FWD	Light	WT	FALSE

Select a file: No file chosen

[Sample file](#)

This file is to assign labels to channels according to experimental setup. Columns needed in this file are - File, Channel, Label, and Control. File is to specify the file-type (reverse vs forward SILAC). Possible values are REV or FWD. Channel are to specify heavy, light and medium (?) per file. Also need to specify which channels should be considered as "Controls". Important to choose the conditions to be compared.

NOTE: File name should not have spaces and should be ".csv" format.

NOTE(2): The "Label" column should NOT have "-" character.

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).

2 Select the Comparison You Would Like to Perform

Choose a comparison with control

('WT', 'EV')

OR Choose a comparison NOT with control

('', '')

Choose the direction for comparison:

(Condition_1/Condition_2)

(Condition_2/Condition_1)

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](#)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
	probability	spectrum	expect	ions	peptide	protein	num_prots	calc_neutral	light_area	heavy_area	express	index	start_scan	assumed_chi	precursor_ncMzratio	protein_desc pl	retention_tin	comp	
1	0.9886	230117_WJC	0.0397	14-Jun	K.TVDGPSK	sp P04406	2	759.3763	6.62E+06	2.36E+06	2.81	6	2863	2	759.375773	390.8954	Glyceraldesh	5.5	1267.8
2	0.9741	230117_WJC	0.359	12-Jun	K.AVEDEKL	sp P11142	6	790.3709	3.39E+06	8.59E+05	3.95	12	3033	2	790.369647	396.1927	Heat shock c	4.14	1298.5
3	0.9261	230117_WJC	2.43	12-May	R.TFEEAK	[13 sp P62701	1	794.4265	9.04E+06	1.08E+07	0.84	30	4041	2	794.427057	398.2205	40S ribosom	4.53	1478.5
4	0.9982	230117_WJC	0.0166	12-Aug	K.TSQVPVK	[1 sp Q9NXV2	1	765.4476	5.07E+05	1.69E+07	0.03	34	4127	2	765.448391	383.7131	8TBPOZ don	8.41	1493.4
5	0.9992	230117_WJC	0.0183	12-Jul	R.NTIPTK	Q sp P0DMV8	6	759.4127	2.67E+07	3.00E+06	8.88	35	4132	2	759.413336	380.7136	Heat shock 7	8.75	1494.2
6	0.9994	230117_WJC	0.00293	18-Nov	R.IAGQVAAN	sp P39019	2	941.5294	1.40E+07	9.35E+06	1.49	38	4157	2	941.52961	471.772	40S ribosom	8.75	1498.4
7	0.9976	230117_WJC	0.11	12-Jul	R.FTEEESR	[1 sp Q9NYF8	7	892.3802	2.17E+07	2.94E+07	0.74	39	4161	2	892.380063	447.1974	Bcl-2-associ	4.14	1499
8	0.9986	230117_WJC	0.00443	14-Jul	R.NTEEEGLK	sp Q9NYF8	6	926.4436	3.57E+07	2.60E+07	1.37	42	4224	2	926.442776	464.2291	Bcl-2-associ	4.25	1510.1
9	0.9378	230117_WJC	0.158	16-May	K.VLENAEGAI	sp P38646	7	957.488	5.66E+06	4.91E+05	11.53	58	4510	2	957.485104	479.7513	Stress-70 pr	4.53	1559.2
10	0.9486	230117_WJC	5.24	14-May	K.SVLADQGGK	sp Q9NYF8	6	816.4341	3.42E+07	7.90E+07	0.43	60	4532	2	816.434259	409.2243	Bcl-2-associ	5.55	1562.8
11	0.9996	230117_WJC	0.000285	Oct-36	R.KQDSGHLI	sp Q86U44	4	1167.5996	2.88E+07	7.56E+06	3.8	62	4631	3	1167.59944	390.2072	N6-adenosin	6.75	1580.4
12	0.9618	230117_WJC	2.44	12-Jun	K.LVEESVK	[1 sp P52732	2	810.4578	1.19E+07	2.62E+07	0.45	72	5171	2	810.455183	406.2362	Kinesin-like p	4.53	1677.5
13	0.9278	230117_WJC	1.29	12-May	K.MYEEELK	sp P84090	2	956.4517	9.48E+06	1.12E+07	0.85	85	5422	2	956.451564	479.2331	Enhancer of r	5.38	1721
14	0.9965	230117_WJC	2.2	12-Jul	K.IITINDK	G sp P54652	14	803.4389	1.39E+08	1.04E+07	13.36	91	5539	2	803.440247	402.7267	Heat shock-r	5.84	1742.2
15	0.9942	230117_WJC	3.54	12-Apr	K.IITINDK	[13 sp P54652	14	811.4531	1.39E+08	1.04E+07	13.34	92	5549	2	811.454169	406.7338	Heat shock-r	5.84	1744
16	0.9983	230117_WJC	0.0126	14-Sep	K.EHC	[160.0 sp Q86U44	1	940.48	8.37E+07	1.29E+06	64.95	93	5569	2	940.483267	471.2473	N6-adenosin	6.83	1747.6
17	0.971	230117_WJC	9.41	12-Jul	K.NADELVK	C sp P17987	5	787.4076	3.24E+07	3.27E+06	9.91	98	5801	2	787.4085	394.7111	T-complex pr	4.37	1789.5
18	0.9986	230117_WJC	0.079	14-Jul	K.LLEGEESR	sp P08670	11	931.4611	8.18E+06	4.36E+06	1.88	99	5803	2	931.462933	466.7378	Vimentin OS	4.25	1789.7
19	0.9953	230117_WJC	0.396	12-May	K.LAEPPYGL	sp P43243	7	776.4069	1.85E+07	1.39E+07	1.34	104	5932	2	776.407792	389.2107	Matrin-3 OS	6	1812.6
20	0.9944	230117_WJC	0.00237	18-Aug	K.GSATGPV	sp P62829	2	899.5076	1.61E+07	6.36E+06	2.53	107	6086	2	899.50518	450.7611	60S ribosom	8.75	1839.4
21	0.9441	230117_WJC	4.18	14-Apr	R.STEPLVK	sp P48741	6	901.4757	6.73E+07	6.04E+06	11.14	109	6136	2	901.476716	451.7451	Putative heat	4.53	1848.2
22	0.9995	230117_WJC	0.00286	16-Sep	R.VTDALNAT	sp P10809	4	969.5119	8.82E+07	4.12E+07	2.14	113	6274	2	969.512914	485.7632	60 kDa heat s	5.81	1873.9
23	0.911	230117_WJC	3.3	16-Apr	K.LLDALQAEAF	sp P84098	4	985.5193	3.32E+06	3.59E+06	0.93	122	6498	2	985.519216	493.7669	60S ribosom	4.37	1913.4
24	0.9841	230117_WJC	1.64	14-Jun	K.VISELNGK	sp P05387	2	866.4953	1.92E+07	1.67E+07	1.14	130	6690	2	866.496011	434.2549	60S acidic rit	5.97	1948.2

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.

6 Enter the Uniprot ID of your bait below:

For more information: ?

- This field is required.

Q86U44	SILAC_METTL3_IP_annc
Reset	

Run the Analysis

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 –

Run the Analysis

Your job has been successfully submitted, you will be redirected to the result page once the analysis is complete

⌂ Loading...

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](#)

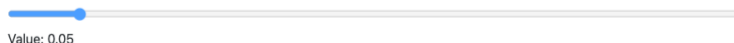
[Click Here to Download the baseline Volcano plot \(PDF\)](#)

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



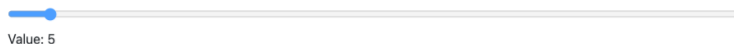
Value: 2

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

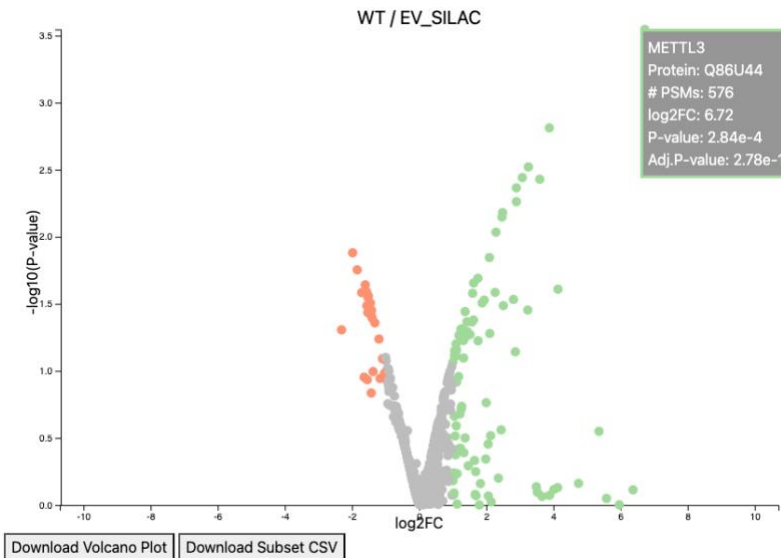
Size of scatter point: Drag the slider to display the current value.



Value: 5

Enter Uniprot IDs separated by @
Example: Q08945@Q9UQE7

Update Volcano Plot



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	SEN2	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	