

Supplementary Figure 4

Due to the size limit for data upload, we have uploaded the annotated MS/MS spectra on the website:

http://ms.iis.sinica.edu.tw/chem/Supplementary_Figure_4.html

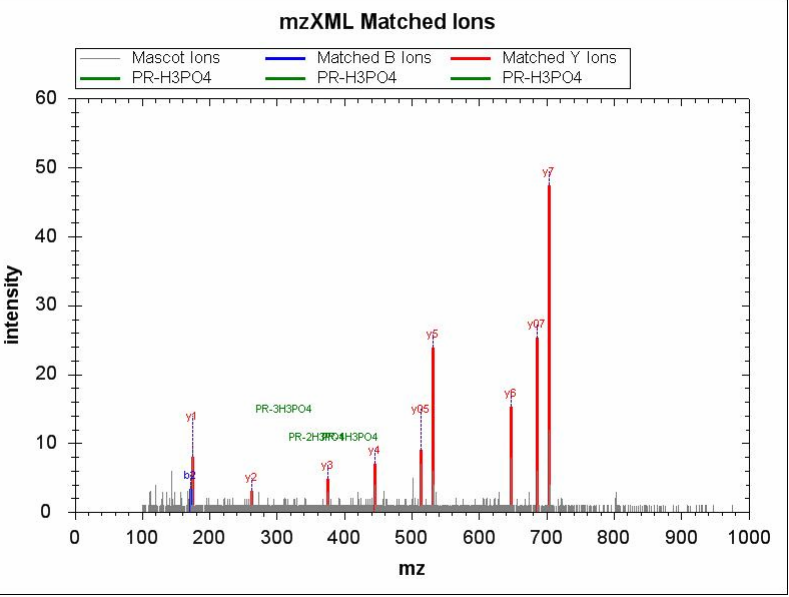
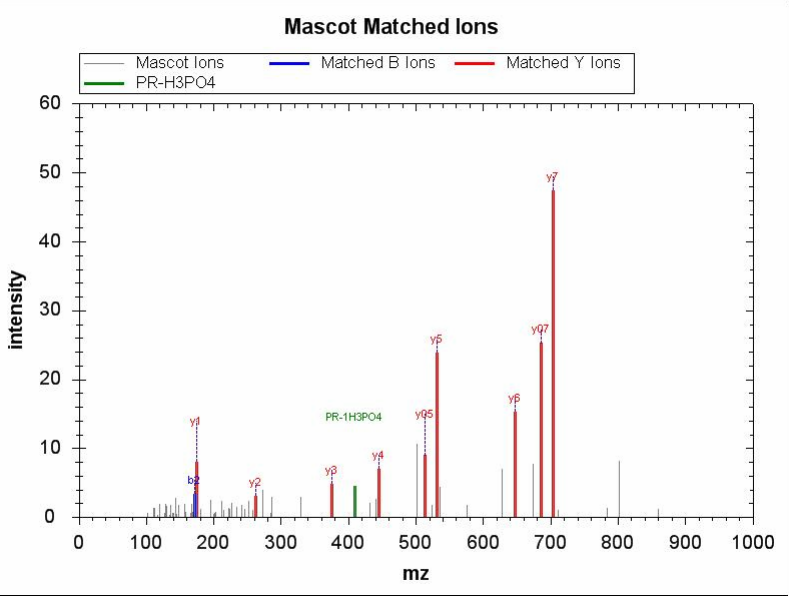
This website contains the MS/MS spectra and assignment on the identified peptides in the section of "Large Scale Phosphoproteome Profiling of Non-small-cell Lung Cancer Cell (H1299)".

There are a total of six experiments listed below:

Name of Sheet	Experiment
SDS-PAGE	4 mg H1299 cell lysate with SDS-PAGE fractionation
LC-80	single LC-MS/MS analysis of 550 μ g H1299 cell lysate using a 80 mins gradient
LC-120	single LC-MS/MS analysis of 550 μ g H1299 cell lysate using a 120 mins gradient
LC-180	single LC-MS/MS analysis of 550 μ g H1299 cell lysate using a 180 mins gradient
LC-210	single LC-MS/MS analysis of 550 μ g H1299 cell lysate using a 210 mins gradient
LC-270	single LC-MS/MS analysis of 550 μ g H1299 cell lysate using a 270 mins gradient

An example of annotated spectrum can be seen in the following page. For better assessment of our identification confidence, it is noted that the format was extensively revised and prepared by home-built software to include the annotated MS/MS spectra (upper Figure in "**Mascot Matched Ions**") and raw spectra (lower Figure in "**mzXML Matched Ions**"). In addition to y- and b-ions, the precursor ions (designated as "**PR**") and neutral loss ions were also annotated.

Query 89 Hit 1
MS/MS Fragmentation of **LGDSSLR**
Found in **IPI00029012**, Tax_Id=9606 Gene_Symbol=EIF3S10 Eukaryotic translation initiation factor 3 subunit 10
Match to Query 89: 913.3428from(457.6786,2+)
Title: 985: Scan 3760 (rt=4268.19, f=3, i=311) [Z:\ET\imac_paper_gradient\4mg_gra_120.raw]
Data File:4mg_gra_120.mgf
Monoisotopic mass of neutral peptide Mr(calc): 913.3428
Variable modifications:
S5 :Phospho (ST), with neutral losses 97.9769
Ions Score: 64.8 Expect: 0.000



No	b	b++	b0	b0++	Seq	y	y++	y*	y*++	y0	y0++	RevNo
1	114.09	57.55			L							8
2	171.11	86.06			G	703.34	352.17	686.31	343.66	685.33	343.17	7
3	286.14	143.57	268.13	134.57	D	646.32	323.66	629.29	315.15	628.30	314.66	6
4	373.17	187.09	355.16	178.08	S	531.29	266.15	514.26	257.63	513.28	257.14	5
5	442.19	221.60	424.18	212.59	S	444.26	222.63	427.23	214.12	426.25	213.63	4
6	555.28	278.14	537.27	269.14	L	375.24	188.12	358.21	179.61	357.22	179.12	3
7	642.31	321.66	624.30	312.65	S	262.15	131.58	245.12	123.07	244.14	122.57	2
8					R	175.12	88.06	158.09	79.55			1