Supplementary Information

Chemical Proteomics Reveals Bolinaquinone as Clathrin-Mediated Endocytosis Inhibitor

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HPLC runs of modified BLQ immobilization onto CDI agarose beads

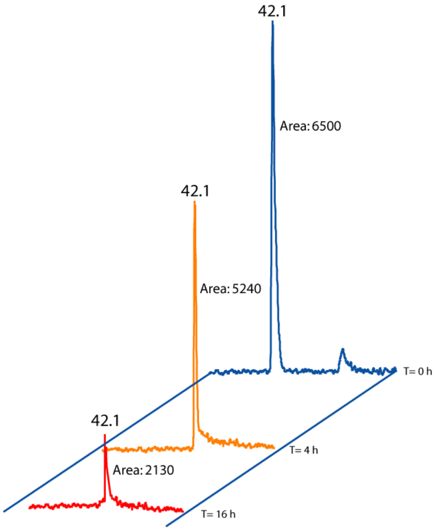
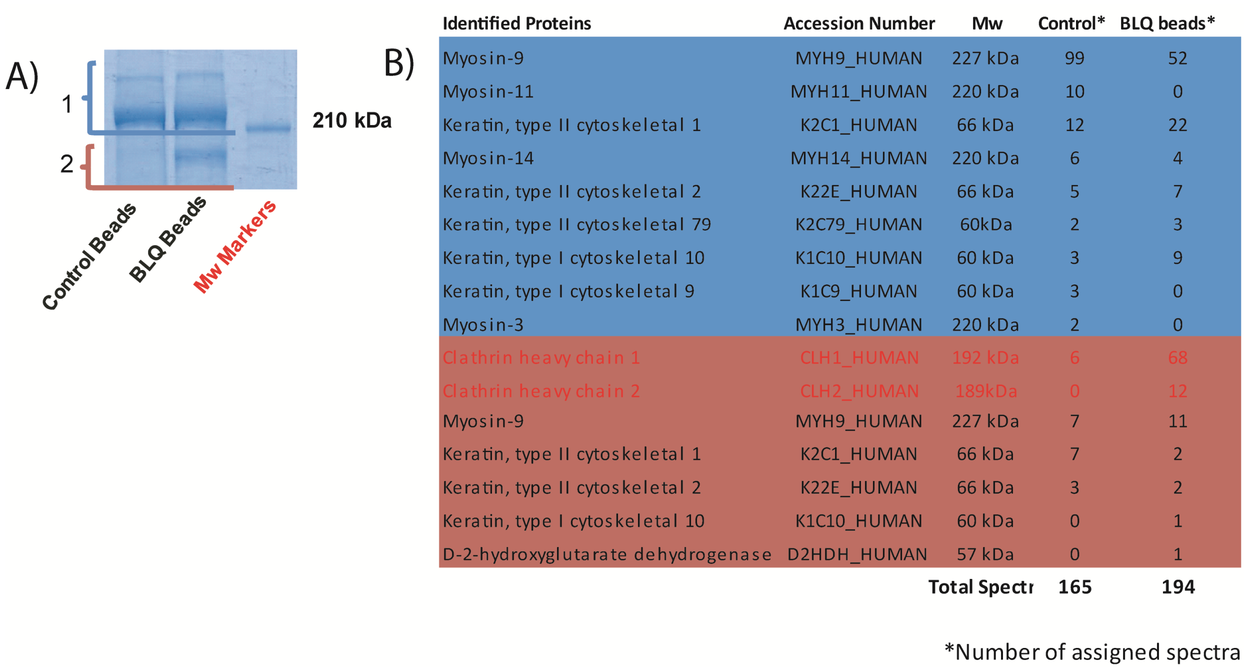


Figure S1. Panels show the peak of free BLQ-NH-PEG-NH_2 in 50 µl of reaction buffer with CDI agarose matrix after 0, 2 and 16 h incubation. The strong reduction of the peak area demonstrates that BLQ-NH-PEG-NH_2 has been anchored to the CDI beads.

Spectral count table.



Identified Proteins	Accession Number	Mw	Control*	BLQ beads*
Myosin-9	MYH9_HUMAN	227 kDa	99	52
Myosin-11	MYH11_HUMAN	220 kDa	10	0
Keratin, type II cytoskeletal 1	K2C1_HUMAN	66 kDa	12	22
Myosin-14	MYH14_HUMAN	220 kDa	6	4
Keratin, type II cytoskeletal 2	K22E_HUMAN	66 kDa	5	7
Keratin, type II cytoskeletal 79	K2C79_HUMAN	60kDa	2	3
Keratin, type I cytoskeletal 10	K1C10_HUMAN	60 kDa	3	9
Keratin, type I cytoskeletal 9	K1C9_HUMAN	60 kDa	3	0
Myosin-3	MYH3_HUMAN	220 kDa	2	0
Clathrin heavy chain 1	CLH1_HUMAN	192 kDa	6	68
Clathrin heavy chain 2	CLH2_HUMAN	189kDa	0	12
Myosin-9	MYH9_HUMAN	227 kDa	7	11
Keratin, type II cytoskeletal 1	K2C1_HUMAN	66 kDa	7	2
Keratin, type II cytoskeletal 2	K22E_HUMAN	66 kDa	3	2
Keratin, type I cytoskeletal 10	K1C10_HUMAN	60 kDa	0	1
D-2-hydroxyglutarate dehydrogenase	D2HDH_HUMAN	57 kDa	0	1
		Total Spectr	**165**	**194**

*Number of assigned spectra

Figure S2. A) Zoom in the 8% of polyacrylamide gel region around 210 kDa. The upper bands (# 1 in blue) from control and BLQ beads were separately cut and analyzed as well as the lower bands (# 2 in purple). B) Identification of proteins eluted in the upper (in blue) and lower (in purple) bands from control and BLQ beads. Number of assigned spectra for identified protein are reported. The table represents one of three different experiments.

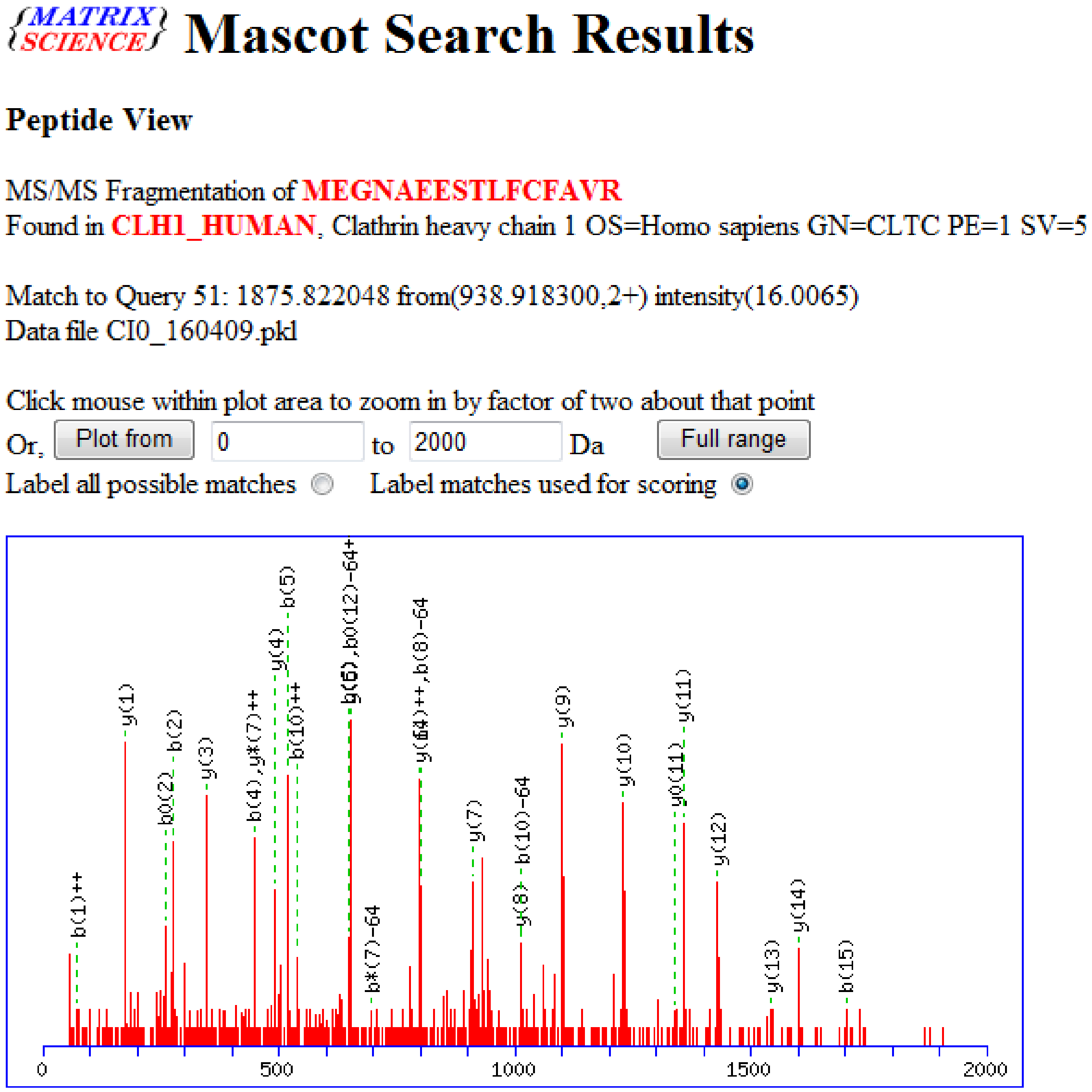
Mascot report for clathrin heavy chain I.

Swiss Prot Code	Protein name	Mw (Da)	Seq. Coverage	Number oh hit	Unique	Protein Score
CLH1_HUMAN	Clathrin heavy chain 1	193260	50%	68	44	1574

Observed (mz)	Expected (mr)	z	Calculated (mr)	Pep_delta	Pep_score	Pep_sequence	Pep_modification
472.2634	942.5122	2	942.4923	0.02	30.68	K.HELIEFR.R	
528.3018	1054.589	2	1054.5699	0.0192	5.34	K.YIEIYVQK.V	
536.321	1070.6274	2	1070.6124	0.015	35.03	K.HDVVFLITK.Y	
563.8038	1125.593	2	1125.5818	0.0112	35.53	K.VANVELYYR.A	
616.8329	1231.6512	2	1231.6408	0.0104	32.67	K.VDKLDASESLR.K	
626.8367	1251.6588	2	1251.6533	0.0056	38.39	K.TLQIFNIEMK.S	Oxidation (M)
648.8458	1295.677	2	1295.6622	0.0148	41.31	K.LLYNNVSNFGR.L	
652.8394	1303.6642	2	1303.652	0.0122	44.57	R.NNLAGAEELFAR.K	
667.8295	1333.6444	2	1333.6262	0.0182	62.66	K.IYIDSNNNPER.F	
677.3546	1352.6946	2	1352.6758	0.0188	48.1	R.VVGAMQLYSVDR.K	Oxidation (M)
677.4312	1352.8478	2	1352.8391	0.0088	56.73	R.NLQNLLILTAIK.A	
694.3778	1386.741	2	1386.7289	0.0121	26.08	R.QNLQICVQVASK.Y	
701.8491	1401.6836	2	1401.6711	0.0126	53.38	R.CNEPAVWSQLAK.A	
717.4111	1432.8076	2	1432.7926	0.0151	55.77	K.SVDPTLALSVYLR.A	
732.8824	1463.7502	2	1463.7296	0.0206	30.5	R.ALEHFTDLYDIK.R	
734.3408	1466.667	2	1466.65	0.017	40.19	K.AHTMTDDVTFWK.W	Oxidation (M)
740.4077	1478.8008	2	1478.7922	0.0087	64.68	K.VGYTPDWIFLLR.R	
750.4244	1498.8342	2	1498.8256	0.0087	37.64	K.WLLLTGISAQQNR.V	
750.4249	1498.8352	2	1498.8256	0.0097	68.58	K.WLLLTGISAQQNR.V	
754.3906	1506.7666	2	1506.7501	0.0166	83.72	K.VIQCFAETGQVQK.I	
776.3873	1550.76	2	1550.7464	0.0136	61.74	R.GQFSTDELVAEVEK.R	
776.3881	1550.7616	2	1550.7464	0.0152	81.57	R.GQFSTDELVAEVEK.R	
778.434	1554.8534	2	1554.844	0.0095	36.67	K.LTDQLPLIIVCDR.F	
787.4117	1572.8088	2	1572.793	0.0159	16.54	R.RPISADSAIMNPASK.V	Oxidation (M)
806.9324	1611.8502	2	1611.8396	0.0107	54.76	R.ESYVETELIFALAK.T	
854.4385	1706.8624	2	1706.8475	0.0149	8.51	R.GQFSTDELVAEVEKR.N	
858.9445	1715.8744	2	1715.8631	0.0113	7.51	K.VSQPIEGHAASFAQFK.M	
879.9474	1757.8802	2	1757.8736	0.0066	9.46	R.KFNALFAQGNYSEAAK.V	
938.9183	1875.822	2	1875.8131	0.0089	76.1	K.MEGNAEESTLFCFAVR.G	Oxidation (M)
938.9239	1875.8332	2	1875.8131	0.0201	70.19	K.MEGNAEESTLFCFAVR.G	Oxidation (M)
971.9663	1941.918	2	1941.9068	0.0112	86.72	R.TSIDAYDNFDNISLAQR.L	
971.9672	1941.9198	2	1941.9068	0.013	90	R.TSIDAYDNFDNISLAQR.L	
974.0134	1946.0122	2	1946.007	0.0052	22.18	K.AFMTADLPNELIELLEK.I	
982.012	1962.0094	2	1962.0019	0.0075	74.26	K.AFMTADLPNELIELLEK.I	Oxidation (M)
657.6826	1970.026	3	1970.0221	0.0038	49.98	R.LASTLVHLGEYQAAVDGAR.K	
986.0242	1970.0338	2	1970.0221	0.0117	2.16	R.LASTLVHLGEYQAAVDGAR.K	
1021.0594	2040.1042	2	2040.0813	0.0229	81.63	R.LPVVIGGLLDVDCSEDVIK.N	
1036.0273	2070.04	2	2070.0343	0.0057	51.84	R.GYFEELITMLEAALGLER.A	Oxidation (M)
1036.0303	2070.046	2	2070.0343	0.0117	58.52	R.GYFEELITMLEAALGLER.A	Oxidation (M)
1061.0251	2120.0356	2	2120.0313	0.0043	83.81	K.DTELAEELLQWFLQEEK.R	
1061.0267	2120.0388	2	2120.0313	0.0075	79.46	K.DTELAEELLQWFLQEEK.R	
1077.0118	2152.009	2	2151.9929	0.0162	68.39	R.GQCDLELINVCNENSLFK.S	
1106.064	2210.1134	2	2210.1001	0.0133	31.92	K.VGEQAQVVIIDMNDPSNPIR.R	Oxidation (M)
747.3946	2239.162	3	2239.1597	0.0023	34.52	K.FDVNTSAVQVLIEHIGNLDR.A	
785.087	2352.2392	3	2352.2438	-0.0046	43.97	R.ISGETIFVTAPHEATAGIIGVNR.K	
785.087	2352.2392	3	2352.2438	-0.0046	68.63	R.ISGETIFVTAPHEATAGIIGVNR.K	
1178.0859	2354.1572	2	2354.139	0.0183	28.85	K.SVNESLNNLFITEEDYQALR.T	
1178.0867	2354.1588	2	2354.139	0.0199	62.85	K.SVNESLNNLFITEEDYQALR.T	
790.0921	2367.2545	3	2367.2547	-0.0002	18.26	R.KFDVNTSAVQVLIEHIGNLDR.A	
800.036	2397.0862	3	2397.0769	0.0092	43.48	K.YGYIHLYDLETGTCIYMNR.I	Oxidation (M)
822.0825	2463.2257	3	2463.2142	0.0115	35.97	R.LAELEEFINGPNNAHIQQVGDR.C	
822.0837	2463.2293	3	2463.2142	0.0151	43.99	R.LAELEEFINGPNNAHIQQVGDR.C	
823.4224	2467.2454	3	2467.2377	0.0077	29.72	R.EKVGEQAQVVIIDMNDPSNPIR.R	Oxidation (M)
823.4258	2467.2556	3	2467.2377	0.0179	21.7	R.EKVGEQAQVVIIDMNDPSNPIR.R	Oxidation (M)
908.4526	2722.336	3	2722.3313	0.0047	16.52	K.KAVDVFFPPEAQNDFPVAMQISEK.H	Oxidation (M)
919.4144	2755.2214	3	2755.2283	-0.0069	20.27	K.ADDPSSYMEVVQAANTSGNWEELVK.Y	Oxidation (M)
1378.6316	2755.2486	2	2755.2283	0.0204	29.78	K.ADDPSSYMEVVQAANTSGNWEELVK.Y	Oxidation (M)
961.1807	2880.5203	3	2880.508	0.0122	83.43	R.RPLIDQVVQTALSETQDPEEVSVTVK.A	
961.1813	2880.5221	3	2880.508	0.014	81.54	R.RPLIDQVVQTALSETQDPEEVSVTVK.A	
1024.5024	3070.4854	3	3070.4923	-0.007	84.1	R.LDNYDAPDIANIAISNELFEEAFAIFR.K	
1054.5215	3160.5427	3	3160.5499	-0.0072	3.3	R.FQEHLQLQNLGINPANIGFSTLTMESDK.F	Oxidation (M)
794.8784	3175.4845	4	3175.4889	-0.0044	29.62	R.LLEMNLMHAPQVADAILGNQMFTHYDR.A	3 Oxidation (M)
1063.2257	3186.6553	3	3186.6714	-0.0161	43.55	R.FQSVPAQPGQTSPLLQYFGILLDQGQLNK.Y	
1063.228	3186.6622	3	3186.6714	-0.0092	36.52	R.FQSVPAQPGQTSPLLQYFGILLDQGQLNK.Y	
1064.1884	3189.5434	3	3189.5441	-0.0007	8.61	K.WISLNTVALVTDNAVYHWSMEGESQPVK.M	Oxidation (M)
1064.1895	3189.5467	3	3189.5441	0.0026	38.13	K.WISLNTVALVTDNAVYHWSMEGESQPVK.M	Oxidation (M)
1083.1678	3246.4816	3	3246.4914	-0.0098	33.27	K.DAMQYASESKDTELAEELLQWFLQEEK.R	Oxidation (M)
1390.4233	5557.6641	4	5557.6983	-0.0342	3.09	R.ISPDQGQQFAQMLVQDEEPLADITQIVDVFMEYNLIQQCTAFLLDALK.N	2 Oxidation (M)

Figure S3. Mascot report of all matched peptides of clathrin heavy chain I.

MSMS spectra of Clathrin heavy chain 1



Mascot Search Results

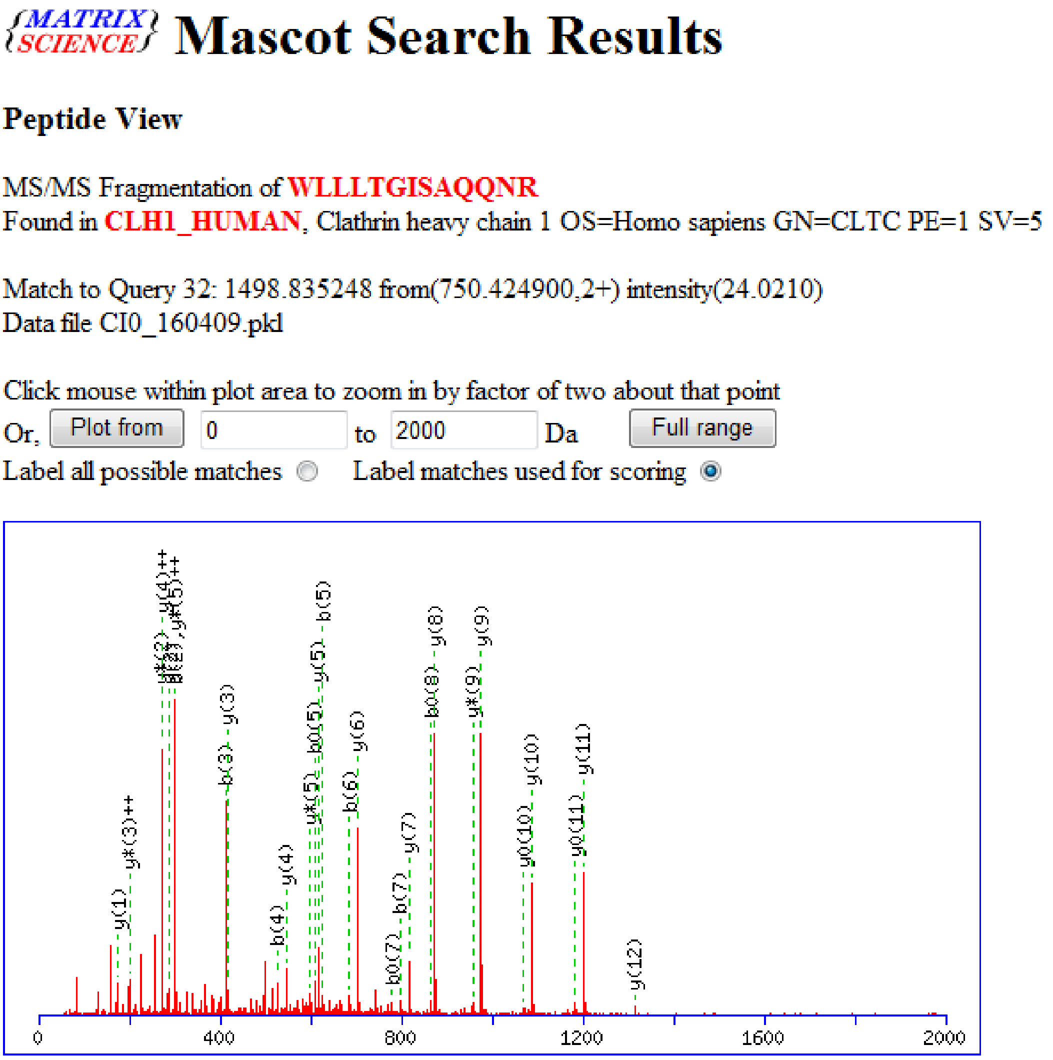
Peptide View

MS/MS Fragmentation of **MEGNAEESTLFCFAVR**
Found in **CLH1_HUMAN**, Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5

Match to Query 51: 1875.822048 from(938.918300,2+) intensity(16.0065)
Data file CI0_160409.pkl

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 0 to 2000 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Figure S4. Tandem mass spectrum of the Clathrin peptide (MEGNAEESTLFCFAVR)



Mascot Search Results

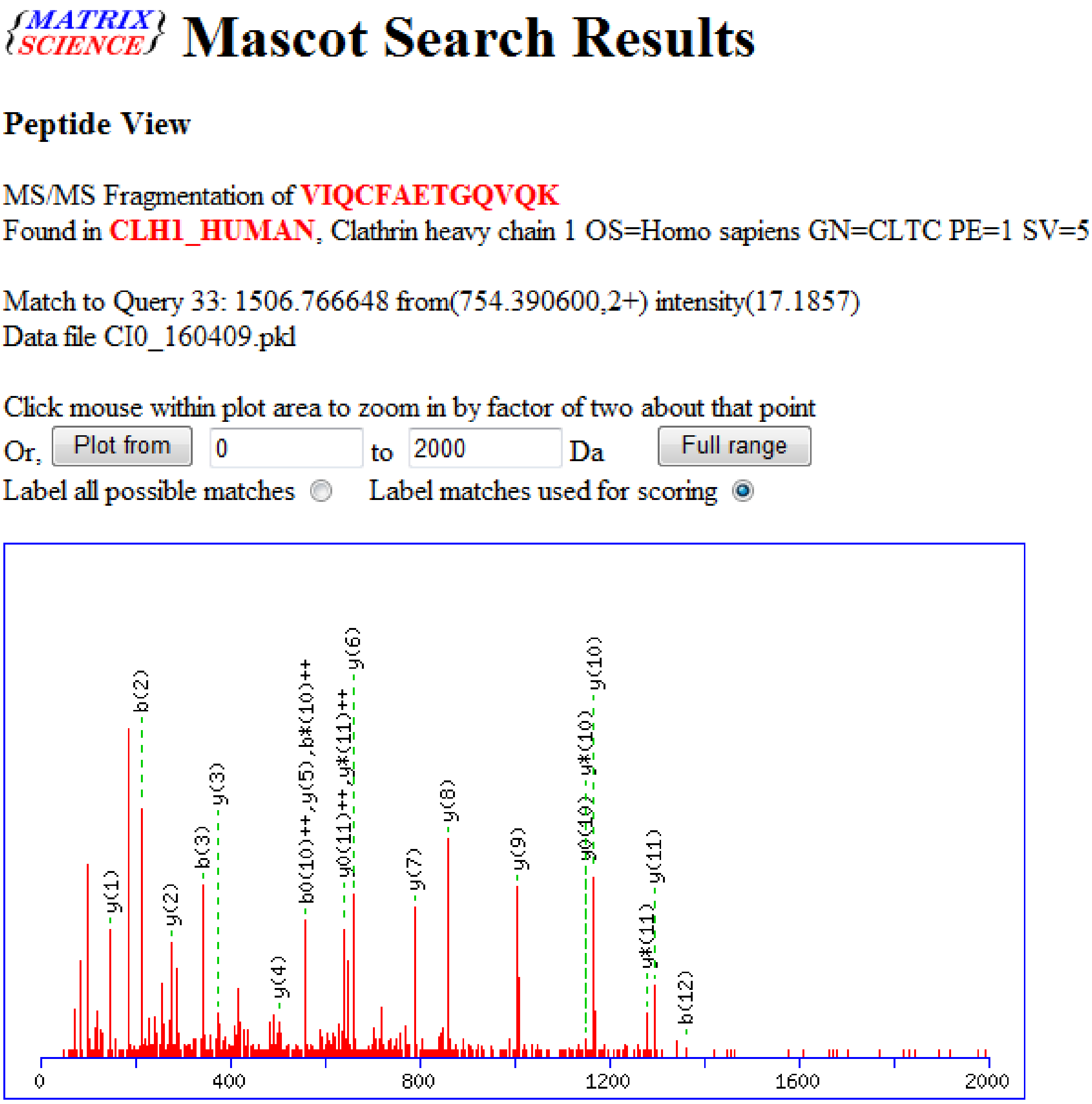
Peptide View

MS/MS Fragmentation of **WLLLTGISAQQNR**
Found in **CLH1_HUMAN**, Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5

Match to Query 32: 1498.835248 from(750.424900,2+) intensity(24.0210)
Data file CI0_160409.pkl

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 0 to 2000 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Figure S5. Tandem mass spectrum of the Clathrin peptide (WLLLTGISAQQNR)



Mascot Search Results

Peptide View

MS/MS Fragmentation of VIQCFAETGQVQK
Found in CLH1_HUMAN, Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5

Match to Query 33: 1506.766648 from(754.390600,2+) intensity(17.1857)
Data file CI0_160409.pkl

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 0 to 2000 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Figure S6. Tandem mass spectrum of the Clathrin peptide (VIQCFAETGQVQK)

Cell Viability Assay

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded in 96-well microtiter plates in 100 µl of growth medium.

After 16 h incubation at 37 °C, cells were exposed to different concentration of BLQ, ranging from 0.01 µM to 100 µM, containing 1% DMSO. The same amount the of DMSO has been applied as control and the incubation was carried out for 48 h.

The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan was used to assess the possible BLQ cytotoxic effect. The experiment was carried out in triplicate and all the values were normalized to control.

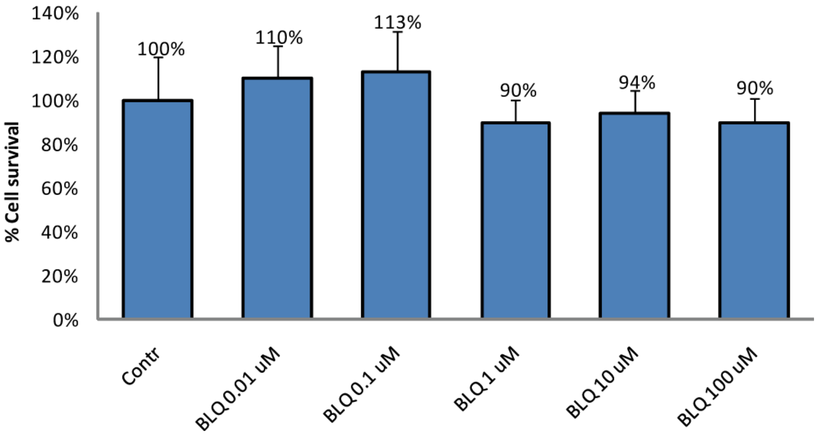


Figure S7. % cell survival of THP1-LPS induced macrophages at different concentration of BLQ after 48 h of incubation. Each value is the mean ± standard deviation of three different experiment performed in triplicate.