

Supplemental Information

Supplemental Videos

See Experimental Procedures section for the details of image acquirement.

Video 1-3 (Fig. 6A, b-d)

HEK-293-GFP-LC3 cells treated with CPP for 4 hours were lysed. Representative LC3-TVS with different topological configurations were selected and imaged every 3-5 seconds for 180 seconds. Each video recorded the movement of one particular type of LC3-TVS. Also see Figure 6 legend.

Video 4 (Fig. 8)

HEK-293-GFP-LC3 cells were grown in glass bottom culture dishes, treated with CPP for 2 hours and live cell confocal imaging was then performed in a temperature controlled, humidified environmental chamber. Images were taken every 3 minutes. Video images represent the boxed area shown in panel A of Figure 8. Also see Figure 8 legend.

Supplemental Table 1

A total of 276 protein spots were detected and analyzed, among which 101 unique proteins could be clearly identified. The proteins were listed together with their location information and reported functions based on UniprotKB (protein knowledgebase, last modified February 10, 2009. Version 72). Also listed are the theoretic (theo.) and observed (Ob) *pI* and molecular weight, the priority scores and sequence coverage from mass spectrometric analysis.

Table S1. Proteins identified in LC3-TVS

Protein	Accession No.	Localization	Function	Score	Sequence coverage (%)	Theo. MW(kDa)	Ob. MW (kDa)	Theo. PI	Ob. PI
26S protease regulatory subunit S10B	P62333	Proteasome	Protein degradation	254	12.6	44	55	7.1	6.8
5'-AMP-activated protein kinase subunit gamma-1	P54619	Nucleus	Metabolism	1020	24.8	35	37	7.8	6.4
60 kDa heat shock protein, mitochondrial precursor	P10809	Mitochondrion	Stress response	7967	17.1	61	60	5.7	5
78 kDa glucose-regulated protein precursor	P11021	Endoplasmic reticulum	Stress response	2132	15.4	72	73	5.1	4.9
Actin-binding Rho-activating protein	Q8N0Z2	Cytoplasm	Signal transduction	6087	21	34	43	8.8	8.7
Acyl-coenzyme A synthetase ACSM2B, mitochondrial precursor	Q68CK6	Mitochondrion	Metabolism	10820	14.7	58	64	6.3	8.5
Adenylate kinase isoenzyme 5	Q9Y6K8	Cytoplasm	Metabolism	2620	12.1	22	15	5.4	7.8
A-kinase anchor protein 12	Q02952	Cytoplasm	Signal transduction	2212	6.5	200	191	6.2	4.4
Angiopietin-related protein 4 precursor	Q9BY76	Secreted	Angiogenesis	1593	19.5	42	45	7.2	9.1
Ankyrin repeat domain-containing protein 2	Q9GZV1	Cytoplasm	Structural constituent	20.2	15.6	37	40	5.7	5.7
Aspartyl-tRNA synthetase, cytoplasmic	P14868	Cytoplasm	Translation	4105	21.4	57	45	6.1	5.8
Atlastin-1	Q8WXF7	Golgi apparatus	Membrane trafficking	250	9	63	45	5.8	7.8
ATP synthase D chain, mitochondrial	O75947	Mitochondrion	Metabolism	325	31.1	18	22	5.2	5
ATP synthase subunit alpha, mitochondrial precursor	P25705	Mitochondrion	Metabolism	2339	22.8	58	50	9.2	8.8
ATP synthase subunit beta, mitochondrial precursor	P06576	Mitochondrion	Metabolism	1835	23.6	56	50	5.3	4.8
AT-rich interactive domain-containing protein 5A	Q03989	Nucleus	Transcription	4710	18.2	64	55	9.3	5.6
Autophagy-related protein 7	O95352	Cytoplasm	Autophagy	339	23.2	77	100	5.9	5.7
Autophagy-related protein 9A	Q7Z3C6	Golgi apparatus	Autophagy	12694	9.9	94	75	6.2	7.5
Brain development-related molecule 1	Q9ULP0	Cytoplasm	Cell differentiation	427	15.3	37	38	5	5.8

Brain-specific angiogenesis inhibitor 1-associated protein 2	Q9UQB8	Cytoplasmic membrane	Cell projection	163	15.6	60	55	9	6.3
BSD domain-containing protein 1	Q9NW68	Unknown	Unknown	465	13.5	47	38	4.4	8
cAMP-specific 3',5'-cyclic phosphodiesterase 4A	P27815	Cytoplasmic membrane	Metabolism	1006	14.3	98	110	5.1	5.9
Carbonic anhydrase 4 precursor	P22748	Cytoplasmic membrane	Metabolism	1407	12.8	35	42	7.7	8.9
Carboxypeptidase A5 precursor	Q8WXQ8	Secreted	Protein degradation	578	17.4	49	55	6.2	6.8
Carboxypeptidase E precursor	P16870	Secreted	Protein degradation	154	13.2	53	75	5	6.2
Cartilage matrix protein precursor	P21941	Secreted	Structural constituent	2930	22.4	50	53	7.4	7.8
Claudin-20	P56880	Cytoplasmic membrane	Cell adhesion	590	31.1	23	14	7	4.5
Coiled-coil domain-containing protein 109A	Q8NE86	Membrane	Unknown	1380	21.9	39	24	8.8	7.2
Coiled-coil domain-containing protein 136	Q96JN2	Membrane	Unknown	98.3	6.8	134	110	4.6	4
Coiled-coil domain-containing protein 76	Q9NUP7	Nucleus	Metabolism	180	15.4	53	54	6.5	8.4
Collagenase 3 precursor [MMP13]	P45452	Secreted	Protein degradation	2356	19.1	53	55	5.3	5.7
Cytochrome b-c1 complex subunit 2, mitochondrial	P22695	Mitochondrion	Respiratory chain	16336	21.9	48	42	8.7	8.8
Dual specificity protein phosphatase 9	Q99956	Endoplasmic reticulum	Cell differentiation	1569	15.9	41	64	5.8	5.2
EF-hand domain-containing protein 1	Q5JVL4	Cytoplasm	Cell death	5357	24.2	73	62	5.8	6
Eukaryotic translation initiation factor 2 subunit 3	P41091	Cytoplasm	Translation	128	12.9	51	39	8.7	6.2
Forkhead box protein L2	P58012	Nucleus	Transcription	974	6.4	38	45	9.3	9.5
FtsJ methyltransferase domain containing 2	Q8N1G2	Cytoplasm	Metabolism	404	10.3	95	100	6.6	6.3
Galectin-12	Q96DT0	Nucleus	Cell death	544	23.5	37	30	9.3	6.2
Gamma-tubulin complex component 2	Q9BSJ2	Centrosome	Microtubule nucleation	347	12.1	102	100	6.4	5
Glutamate carboxypeptidase 2	Q04609	Cytoplasmic membrane	Metabolism	5707	17.5	80	84	4.3	6.5

G-rich sequence factor 1	Q12849	Cytoplasm	RNA binding	342	21.5	60	50	5	5.7
Growth-inhibiting protein 26	Q9HBA9	Cytoplasm	Protein degradation	107	9.7	40	50	6	6.5
Guanine nucleotide-binding protein G(k) subunit alpha [G(i)alpha3]	P08754	Membrane	Signal transduction	1284	20.3	40	50	5.5	5
Guanine nucleotide-binding protein G(q) subunit alpha	P50148	Cytoplasmic membrane	Signal transduction	1897	15	41	40	5.6	5.3
Huntingtin-interacting protein 1	O00291	Cytoplasmic membrane	Membrane trafficking	132	5.8	115	100	5.2	5.8
Ig mu heavy chain disease protein	P04220	Cytoplasmic membrane	Immune response	2669	16.1	32	43	4.2	5.1
Importin-13	O94829	Cytoplasm	Transport	3372	6	108	110	5.1	4.6
Inositol-trisphosphate 3-kinase A	P23677	Unknown	Signal transduction	1295	16.7	51	50	7.6	6.6
Kelch-like protein 30	Q0D2K2	Unknown	Unknown	2620	17.6	64	63	5.6	5.7
Keratin, type II cytoskeletal 8	P05787	Cytoplasm	Structural constituent	401	15.7	53	50	5.5	4.8
LC3B [MAP 1A/1B light chain 3B]	Q9GZQ8	Cytoplasm	Autophagy	1063	39.2	16	14	9.1	8.9
Lymphoid-specific helicase	Q9NRZ9	Nucleus	Cell cycle	5748	10.7	97	90	8.1	6
MAP3K12-binding inhibitory protein 1	Q9NS73	Cytoplasm	Signal transduction	143	22.4	39	32	6.6	6.4
Microtubule-associated protein 9	Q49MG5	Microtubule	Cell cycle	62.9	8.7	74	55	7.6	6.2
NACHT, LRR and PYD domains-containing protein 13	Q86W25	Cytoplasm	Immune response	1331	9.2	118	150	5.4	6.2
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial precursor	O95299	Mitochondrion	Metabolism	1482	14.6	40	27	8.7	7.2
Neuroplastin precursor	Q9Y639	Cytoplasmic membrane	Cell adhesion	213	20.9	31	17	6.4	5.9
Nuclear protein localization protein 4 homolog	Q8TAT6	Endoplasmic reticulum	Protein degradation	2806	10.4	68	49	5.9	6.4
Pancreatic secretory granule membrane major glycoprotein GP2	P55259	Membrane	Unknown	241	13.7	58	69	5	4.5
Peroxiredoxin-4	Q13162	Cytoplasm	Redox regulation	35315	32.5	30	26	5.9	6.2
Peroxiredoxin-6	P30041	Cytoplasm	Redox regulation	391	28.1	34	25	7.2	6

Phosducin-like protein	Q13371	Cytoplasm	Signal transduction	112	22.6	36	34	5.2	4.7
PiggyBac transposable element-derived protein 2	Q6P3X8	Unknown	Unknown	1424	17.2	68	59	8.8	6.5
Poly(rC)-binding protein 1	Q15365	Nucleus	Transcription	281691	16.3	38	37	7.5	6.7
Polymeric-immunoglobulin receptor precursor	P01833	Cytoplasmic membrane	Transport	6138	12.7	83	87	5.6	9.5
Probable tubulin polyglutamylase	O95922	Microtubule	Protein modification	126	9	48	35	8.9	5.8
Prohibitin	P35232	Mitochondrion	DNA replication	2014	28.7	29	30	5.6	5.6
Prohibitin-2	Q99623	Nucleus	Transcription	7417	32.8	33	34	9.8	9.8
Prolyl 3-hydroxylase 2 precursor	Q8IVL5	Endoplasmic reticulum	Redox regulation	723	13	80	100	5.5	4.7
Protein disulfide-isomerase A3 precursor	P30101	Endoplasmic reticulum	Redox regulation	718935	33.9	56	57	6	5.9
Protein FAM83C	Q9BQN1	Unknown	Unknown	15529	19.3	81	85	8.3	9.1
Prune homolog 2	Q8WUY3	Cytoplasm	Cell death	147	20.5	29	39	4.9	5.7
Pyruvate dehydrogenase protein X component, mitochondrial precursor	O00330	Mitochondrion	Metabolism	792	15	54	50	8.8	6.4
Quinone oxidoreductase-like 1	O95825	Cytoplasm	Redox regulation	251	16.3	38	27	5.5	7.2
Replication factor C subunit 2	P35250	Nucleus	Cell cycle	529	17.2	39	50	6	6
Ribosome recycling factor, mitochondrial precursor	Q96E11	Mitochondrion	Translation	1560	9.9	27	29	7.8	9.8
S100 calcium-binding protein A10	P60903	Mitochondrion	Signal transduction	4.46	23.7	11	14	6.8	7.8
Septin-14	Q6ZU15	Cytoplasm	Cell cycle	140	20.8	50	48	5.9	5.9
Signal recognition particle receptor subunit beta	Q9Y5M8	Endoplasmic reticulum	Transport	1270	23.6	29	32	9.2	9
Sorting nexin-25	Q9H3E2	Endosomes	Membrane trafficking	57.7	13.4	62	62	5.5	6.5
Sorting nexin-5	Q9Y5X3	Endosomes	Membrane trafficking	264	19.6	50	46	6.7	6.3
Spindlin-2B	Q9BPZ2	Nucleus	Cell cycle	4305	33.3	29	33	6.7	7.4

SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily C member 1	Q92922	Nucleus	Transcription	86.9	5.2	122	120	5.6	6
Syntaxin-binding protein 4	Q6ZWJ1	Cytoplasm	Transport	3901	14.8	61	45	5.2	6.8
Transcription factor 25	Q9BQ70	Nucleus	Transcription	129	14.9	76	73	6	7.9
Transmembrane protein 85	Q5J8M3	Membrane	Unknown	933	20.8	20	17	8.8	9.1
Tripartite motif-containing 21	P19474	Cytoplasm	Protein degradation	1457	17.7	54	55	6	6.1
Tripartite motif-containing protein 10	Q9UDY6	Cytoplasm	Cell differentiation	2304	14.6	55	62	5.7	6.2
Tripartite motif-containing protein 11	Q96F44	Cytoplasm	Protein degradation	2827	12.8	52	150	5.5	5.7
Tyrosine-protein kinase transmembrane receptor ROR2	Q01974	Cytoplasmic membrane	Signal transduction	3188	8.4	95	104	6.4	6.1
Ubiquilin-1	Q9UMX0	Cytoplasm	Protein modification	244	8.8	45	62	5	5
Ubiquitin carboxyl-terminal hydrolase 10	Q14694	Cytoplasm	Protein degradation	4700	22.7	87	60	5.2	6.3
UMP-CMP kinase	P30085	Cytoplasm	Metabolism	1561	41.3	22	12	5.4	8.9
Uncharacterized protein C21orf63	P58658	Membrane	Unknown	990	18.4	47	49	5.4	6.5
Vacuolar fusion protein MON1 homolog B	Q7L1V2	Membrane?	Membrane trafficking?	6806	24.1	59	55	5.9	4.5
Vacuolar protein sorting-associated protein 33A	Q96AX1	Endosomes	Membrane trafficking	743	11.9	65	67	6.3	6.5
Vimentin	P08670	Cytoplasm	Structural constituent	608	30.5	55	53	5	5.1
Voltage-dependent anion-selective channel protein 1	P21796	Mitochondrion	Transport	41746	40.6	30	32	8.6	9.2
Voltage-dependent anion-selective channel protein 2	P45880	Mitochondrion	Transport	5187	34.4	31	34	7.5	8.2
WNT1-inducible-signaling pathway protein 3 precursor	O95389	Secreted	Signal transduction	379	17.8	39	45	8.9	8.9
Zinc finger and BTB domain-containing protein 9	Q96C00	Nucleus	Transcription	1788	13.1	50	43	6.3	7.7