Supplemental Figure Legends

Figure S1. Parkin-mediated proteolysis of mitochondrial outer membrane proteins is dependent on the ubiquitin ligase activity of Parkin and is induced by disruption of the electrical potential of mitochondria.

(A) Lack of Parkin expression in parental HeLa S3 cells. Total cell lysates were isolated from HeLa S3 and HeLa S3 cells expressing exogenous Parkin. Immunoblots were probed with antibodies against Parkin and actin (loading control). (B) Less efficient proteolysis by the Parkin mutant R275W. Clonal HeLa S3 cells exogenously expressing wild-type Parkin or the ubiquitin ligase mutant R275W were treated with 20 μM CCCP for the indicated time. Total cell lysates were isolated, and immunoblot analysis was used to detect the indicated proteins. (C) Outer membrane protein proteolyis induced by valinomycin but not rotenone. Clonal HeLa S3 cells expressing Parkin or the parental HeLa S3 cells were treated with DMSO (vehicle), 2 μM valinomycin, or 200 nM rotenone for the indicated times. Total cell lysates were isolated, and the indicated proteins were detected by immunoblot analysis.

Figure S2. Overexpression of the K48R ubiquitin mutant partially suppresses mitophagy in Parkin-expressing HeLa cells.

Clonal HeLa cells expressing Parkin were transiently transfected with GFP-tagged wild-type or K48R ubiquitin. 48 hours post-transfection, cells were treated with a 100 min pulse of 20 μM CCCP. 24 hours after the CCCP pulse, cells were fixed and immunostained for Hsp60. Cells that showed complete mitophagy were identified as

GFP-positive cells that were negative for Hsp60. Error bars represent standard errors from 3 independent experiments; 500 cells were analyzed per experiment. The p-value was calculated by a 2-tail student *t* test.

Figure S3. Parkin-dependent degradation of Tom20 and dispersion of mitochondria. (A) Parental HeLa cells do not show CCCP-induced loss of Tom20. HeLa cells not expressing Parkin were treated with DMSO (control) or 20 μM CCCP for 4 hours. Fixed cells were stained for Tom20 (red), Hsp60 (green), and nuclei (DAPI, blue). Insets show enlarged views of the boxed area. Note that, without exogenous Parkin expression, HeLa cells do not show accumulation of Tom20-negative mitochondria or mitochondrial aggregation in response to CCCP. (B) Degradation of Tom20 in Parkin-expressing cells induced by CCCP. Parkin-expressing HeLa cells were treated with 20 μM CCCP for 4 hours (upper panel) or 24 hours (lower panel). Fixed cells were stained for Tom20 (red), the matrix protein TRAP-1 (green), and nuclei (DAPI, blue). In the bottom panel, the mitochondrial aggregate is Tom20-negative but Hsp60-positive. (C) Same as (B), except a different matrix protein $F_1\beta$ (green) was detected. Note the presence of Tom20-negative/Hsp60-positive mitochondria. (D) Temporal dynamics of Tom20-negative/Hsp60-positive mitochondria. Parkin-expressing HeLa cells were pretreated with DMSO (vehicle) or the proteasome inhibitor epoxomicin (2 μM) for 2 hours prior to treatment with CCCP (20 μM) for the indicated times. Fixed cells were stained for Tom20 (red), Hsp60 (green), and nuclei (DAPI, blue). Scale bars equal 10 μm.

Figure S4. Inhibition of the 26S proteasome does not inhibit Parkin translocation to mitochondria, but inhibits mitophagy in HeLa, SH-SY5Y, and MEF cells.

(A) Inhibition of the 26S proteasome does not affect Parkin translocation to mitochondria. Parkin-expressing HeLa cells were pre-treated with DMSO (vehicle) or 20 μM MG132 for 2 hours, followed by incubation in the presence or absence of CCCP (20 μM) for 4 hours. Formalin-fixed cells were stained for Hsp60 (green), Parkin (red), and nuclei (DAPI, blue). Scale bar equals 10 μm. (B) Quantitation of the experiment in (A). Parkin translocation to mitochondria was scored by co-localization with Hsp60. Results represent the mean from 2 independent experiments; 100 cells were scored per experiment. (C) The proteasome inhibitor epoxomicin prevents degradation of Tom20. Parkin-expressing HeLa cells were pretreated with DMSO (vehicle) or epoxomicin (2 μM) for 2 hours, followed by treatment with CCCP (20 μM) for 4 hours (4 h), or pulse treatment for 100 min followed by incubation without CCCP for 12 hours (12 h). Fixed cells were stained for Hsp60 (green), Tom20 (red), and nuclei (DAPI, blue). Insets show enlarged views of the boxed area. (D) Quantitation of the 4 h time point in (C). Each cell was scored into one of the 5 indicated bins, depending on the number of Tom20-negative/Hsp60-positive mitochondria found. Error bars represent standard deviations from 3 independent experiments; 100 cells were scored per experiment. (E) Quantitation of the 12 h time point in (C). Each cell was scored as having fragmented mitochondria, tubular mitochondria, or no mitochondria. Error bars represent standard deviation from 3 independent experiments; 100 cells were analyzed per experiment. (F) Epoxomicin prevents Tom20 degradation and mitophagy in SH-SY5Y cells. SH-SY5Y cells with exogenous Parkin were pretreated with DMSO (vehicle) or epoxomicin (2 μM) for 2 hours, followed by treatment with CCCP (20 μM) for 4 hours (4 h), or pulse treatment for 100 min followed by incubation without CCCP for 24 hours (24 h). Immunostaining was performed as in (C). In the top panel, insets show enlarged views of the boxed area. Arrowheads mark an example of a dispersed mitochondrion positive for Hsp60, but negative for Tom20. Scale bars in A, C, F equal 10 μm. (G) MG132 abrogates CCCP-induced mitophagy in MEFs. Wild-type MEFs expressing EGFP-Parkin were pretreated with DMSO (vehicle) or MG132 (5 μM), followed by treatment with CCCP (20 μM) for 24 hours. Cells were fixed and stained for Hsp60 and nuclei (DAPI). Cells that underwent complete loss of mitochondria were identified as EGFP- and DAPI-positive cells with no Hsp60 signal. Error bars represents standard deviations from 3 independent experiments; 200 cells were analyzed per experiment.

Supplementary Table 1 │ MitoCarta proteins with decreased abundance in mitochondria of CCCP-treated cells

Leading IPIs	Leading Gene Names	Leading Protein Descriptions	Overall Normalized Ratio*	Overall Significance^
IPI00412274	MFN1	Isoform 3 of Mitofusin-1	0.09	6.94E-18
IPI00293073	MFN1	Mitochondrial transmembrane GTPase FZO-2	0.10	1.14E-23
IPI00642329	MFN2	Isoform 1 of Mitofusin-2	0.10	2.51E-35
IPI00015602	TOMM70A	Mitochondrial import receptor subunit TOM70	0.13	3.78E-40
IPI00807412	NT5C3	Isoform 2 of Cytosolic 5'-nucleotidase 3	0.15	1.03E-24
IPI00432452	RHOT1	Isoform 1 of Mitochondrial Rho GTPase 1	0.16	4.29E-15
IPI00219778	SLMO2	Protein slowmo homolog 2	0.17	6.01E-15
IPI00877136;IPI00019477;IPI00743132	SLC25A39	solute carrier family 25, member 39 isoform a;Isoform 1 of Solute carrier family 25 member 39;Isoform 2 of Solute carrier family 25 member 39	0.18	3.17E-10
IPI00032038	CPT1A	Isoform 1 of Carnitine O-palmitoyltransferase 1, liver isoform	0.23	1.62E-21
IPI00218144	COX17	Cytochrome c oxidase copper chaperone	0.23	6.06E-11
IPI00329552	MOSC2	Isoform 1 of MOSC domain-containing protein 2, mitochondrial	0.26	6.67E-10
IPI00020510	CISD1	CDGSH iron sulfur domain-containing protein 1	0.26	8.74E-18
IPI00001638	PRELID1	PRELI domain-containing protein 1, mitochondrial	0.28	3.07E-07
IPI00002251	PDP2	[Pyruvate dehydrogenase [acetyl-transferring]]-phosphatase 2, mitochondrial	0.30	2.09E-08
IPI00007266	ALAS1	cDNA FLJ56039, highly similar to 5-aminolevulinate synthase, nonspecific, mitochondrial	0.30	6.95E-08
IPI00916131;IPI00447419;IPI00514470	PISD;C22orf30	Protein;Isoform 1 of Phosphatidylserine decarboxylase proenzyme;Phosphatidylserine decarboxylase	0.32	2.28E-07
IPI00013829	TRIAP1	TP53-regulated inhibitor of apoptosis 1	0.34	4.56E-07
IPI00446798	MTCP1	Protein p8 MTCP-1	0.34	1.03E-06
IPI00328383;IPI00478497;IPI00910479	SLC25A46	Putative uncharacterized protein SLC25A46 (Fragment);Solute carrier family 25 member 46;cDNA FLJ60882	0.37	2.20E-06
IPI00828198;IPI00305258	MOSC1	Isoform 2 of MOSC domain-containing protein 1, mitochondrial;Isoform 1 of MOSC domain-containing protein 1, mitochondrial	0.39	1.39E-07
IPI00171445	ATAD1	ATPase family AAA domain-containing protein 1	0.39	1.84E-07
IPI00552793;IPI00604730;IPI00013279	CHCHD7	coiled-coil-helix-coiled-coil-helix domain containing 7 isoform b;coiled-coil-helix-coiled-coil-helix domain containing 7 isoform d;Coiled-coil-helix-coiled-coil-helix domain-containing protein 7	0.41	1.35E-05
IPI00844249;IPI00640749	GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial;Glycerol 3-phosphate acyltransferase, mitochondrial	0.42	3.39E-04
IPI00031118	ALKBH7	Alkylated DNA repair protein alkB homolog 7	0.42	1.45E-04
IPI00328161;IPI00640341	FKBP8	Isoform 2 of FK506-binding protein 8;Isoform 1 of FK506-binding protein 8	0.43	2.81E-06

IPI00465059	RHOT2	Isoform 1 of Mitochondrial Rho GTPase 2	0.44	4.94E-06
IPI00061229	OMA1	Isoform 1 of Metalloendopeptidase OMA1, mitochondrial	0.45	1.17E-04
IPI00290614	ENDOG	Endonuclease G, mitochondrial	0.45	1.56E-04
IPI00830136;IPI00552131;IPI00045660;IPI00646750	C1orf31	Isoform 2 of Uncharacterized protein C1orf31;cDNA FLJ60021, moderately similar to Protein transport protein Sec61 subunit alpha isoform 1;Isoform 1 of Uncharacterized protein C1orf31;Isoform 3 of Uncharacterized protein C1orf31	0.47	2.63E-05
IPI00303722	FAM136A	Protein FAM136A	0.51	1.01E-05
IPI00016745;IPI00759584	FPGS	Isoform Mitochondrial of Folylpolyglutamate synthase, mitochondrial;Isoform Cytoplasmic of Folylpolyglutamate synthase, mitochondrial	0.53	4.50E-03
IPI00910782;IPI00016699	MTFR1;-	cDNA FLJ61576, highly similar to Mitochondrial fission regulator 1;Mitochondrial fission regulator 1	0.54	1.02E-03
IPI00046828	CCDC58	Coiled-coil domain-containing protein 58	0.54	4.57E-05
IPI00007052	FIS1	Mitochondrial fission 1 protein	0.55	1.33E-03
IPI00025344	NDUFS6	NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial	0.55	5.84E-04
IPI00171459	HSDL1	Hydroxysteroid dehydrogenase-like protein 1	0.57	3.50E-03
IPI00555597;IPI00032903	PTRH2	Peptidyl-tRNA hydrolase 2, mitochondrial precursor;Peptidyl-tRNA hydrolase 2, mitochondrial	0.57	2.04E-04
IPI00329696	FAM82B	Regulator of microtubule dynamics protein 1	0.57	1.20E-03
IPI00478450;IPI00472058	NDUFB11	Isoform 2 of NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial;Neuronal protein	0.57	1.31E-03
IPI00215901;IPI00218988	AK2	Isoform 1 of Adenylate kinase isoenzyme 2, mitochondrial;Isoform 2 of Adenylate kinase isoenzyme 2, mitochondrial	0.57	2.40E-04
IPI00065063	DHRS1	Dehydrogenase/reductase SDR family member 1	0.58	4.74E-03
IPI00008418;IPI00219865	DIABLO	Diablo homolog, mitochondrial precursor;Isoform 2 of Diablo homolog, mitochondrial	0.59	5.12E-04
IPI00301204	RDH13	Retinol dehydrogenase 13	0.60	2.61E-03
IPI00011770	NDUFA4	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4	0.62	5.47E-03
IPI00296190;IPI00646289;IPI00646889	C10orf58	Uncharacterized protein C10orf58;25 kDa protein;Putative uncharacterized protein C10orf58	0.63	6.38E-03
IPI00028491	AGPAT5	1-acyl-sn-glycerol-3-phosphate acyltransferase epsilon	0.63	6.85E-03
IPI00001589	TIMM13	Mitochondrial import inner membrane translocase subunit Tim13	0.64	2.31E-03
IPI00013459;IPI00791036;IPI00556190	NDUFB5	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5, mitochondrial;22 kDa protein;NDUFB5 protein	0.64	8.53E-03
IPI00028376	TIMM8A	Mitochondrial import inner membrane translocase subunit Tim8 A	0.65	3.23E-03
IPI00419266	NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa	0.65	3.51E-03
IPI00020332	ARG2	Arginase-2, mitochondrial	0.68	7.41E-03

Mitochondria from Parkin-expressing HeLa S3 cells with and without CCCP (2 hours) were compared.

*Combined ratio from three independent mass spectrometry experiments consisting of two independent biological samples, and one technical replicate. The ratio represents the protein level in mitochondria of CCCP-treated cells divided by the level in untreated cells.

^Corresponds to the significance in MaxQuant

Supplementary Table 2 | MitoCarta proteins enriched in mitochondria of CCCP-treated cells

Leading IPIs	Leading Gene Names	Leading Protein Descriptions	Overall Normalized Ratio*	Overall Significance^
IPI00026781	FASN	Fatty acid synthase	4.08	4.03E-07
IPI00216298	TXN	Thioredoxin	3.20	2.12E-05
IPI00220301	PRDX6	Peroxiredoxin-6	3.19	3.71E-03
IPI00604590; IPI00795292	NME2:NME1- NME2;NME2	Nucleoside diphosphate kinase;NME1-NME2 protein	2.36	1.20E-03
IPI00217966	LDHA	Isoform 1 of L-lactate dehydrogenase A chain	2.10	4.33E-03
IPI00219217	LDHB	L-lactate dehydrogenase B chain	1.96	8.75E-03

Mitochondria from Parkin-expressing HeLa S3 cells with and without CCCP (2 hours) were compared.

*Combined ratio from three independent mass spectrometry experiments consisting of two independent samples and one technical replicate. The ratio represents the protein level in mitochondria of CCCP-treated cells divided by the level in untreated cells.

^Corresponds to significance in MaxQuant

Supplementary Table 3 | Non-MitoCarta proteins enriched in mitochondria of CCCP-treated cells

Leading IPIs	Leading Gene Names	Leading Protein Descriptions	Overall Normalized Ratio*	Overall Significance^
IPI00005254	PARK2	Isoform 1 of E3 ubiquitin-protein ligase parkin	13.5	4.42E-20
IPI00179330	UBB:RPS27A:UBC	ubiquitin and ribosomal protein S27a precursor	8.9	9.58E-15
IPI00783855;IPI00871941;IPI00299920;IPI00887954	NBR1;LOC727732;LOC100133166	neighbor of BRCA1 gene 1;Isoform 1 of Next to BRCA1 gene 1 protein;69 kDa protein;hypothetical protein isoform 2	8.3	2.90E-06
IPI00235412;IPI00871742;IPI00146935;IPI00555883;IPI00037283;IPI00473085	DNM1L	cDNA FLJ56381, highly similar to Dynamin-1-like protein;cDNA FLJ55044, highly similar to Dynamin-1-like protein;Isoform 1 of Dynamin-1-like protein;Isoform 2 of Dynamin-1-like protein;Isoform 5 of Dynamin-1-like protein;Isoform 4 of Dynamin-1-like protein	6.3	4.78E-07
IPI00179473	SQSTM1	Isoform 1 of Sequestosome-1	5.8	1.75E-06
IPI00151462;IPI00220482	MAP1LC3B2;MAP1LC3B	Microtubule-associated proteins 1A/1B light chain 3 beta 2;Microtubule-associated proteins 1A/1B light chain 3B	5.4	3.70E-06
IPI00009146	TRAFD1	TRAF-type zinc finger domain-containing protein 1	4.9	1.48E-04
IPI00219622	PSMA2	Proteasome subunit alpha type-2	4.2	8.08E-05
IPI00479306	PSMB5	Proteasome subunit beta type-5	4.2	4.21E-04
IPI00640197;IPI00020416	TPP2	Tripeptidyl peptidase II;Tripeptidyl-peptidase 2	4.1	2.18E-03
IPI00472442;IPI00016832;IPI00871889	PSMA1	Isoform Long of Proteasome subunit alpha type-1;Isoform Short of Proteasome subunit alpha type-1;Proteasome subunit alpha type	4.0	5.38E-04
IPI00028004	PSMB3	Proteasome subunit beta type-3	4.0	6.20E-04
IPI00000811	PSMB6	Proteasome subunit beta type-6	3.9	2.30E-04
IPI00642126	KIAA1618	Isoform 1 of Protein ALO17	3.9	1.38E-03
IPI00031461	GDI2	cDNA FLJ60299, highly similar to Rab GDP dissociation inhibitor beta	3.8	1.76E-04
IPI00008223;IPI006425	RAD23B	UV excision repair protein RAD23 homolog B;cDNA	3.7	4.55E-03

49		FLJ56531, highly similar to UV excision repair protein RAD23 homolog B		
IPI00029623	PSMA6	Proteasome subunit alpha type-6	3.7	1.18E-03
IPI00555956	PSMB4	Proteasome subunit beta type-4	3.7	1.96E-03
IPI00024175	PSMA7	Isoform 1 of Proteasome subunit alpha type-7	3.7	1.29E-03
IPI00299155;IPI00790207	PSMA4	Proteasome subunit alpha type-4;27 kDa protein	3.6	4.70E-04
IPI00419249;IPI00171199	PSMA3	Isoform 1 of Proteasome subunit alpha type-3;Isoform 2 of Proteasome subunit alpha type-3	3.6	4.85E-04
IPI00005495;IPI00219863;IPI00607670;IPI00872048;IPI00895831;IPI00895820	ATXN3	Isoform 1 of Ataxin-3;Isoform 2 of Ataxin-3;ATXN3 protein;42 kDa protein;ataxin 3 isoform 3;ataxin 3 isoform 4	3.6	6.08E-03
IPI00013446	PSCA	Prostate stem cell antigen	3.6	2.63E-03
IPI00013949;IPI00908410	SGTA	Small glutamine-rich tetratricopeptide repeat-containing protein alpha;cDNA FLJ56566, highly similar to Small glutamine-rich tetratricopeptiderepeat-containing protein A	3.5	3.01E-03
IPI00219029	GOT1	Aspartate aminotransferase, cytoplasmic	3.5	7.23E-04
IPI00026358	GABARAPL2	Gamma-aminobutyric acid receptor-associated protein-like 2	3.4	7.88E-04
IPI00021831	PRKAR1A	cAMP-dependent protein kinase type I-alpha regulatory subunit	3.3	4.16E-03
IPI00025019	PSMB1	Proteasome subunit beta type-1	3.3	2.66E-03
IPI00024095	ANXA3	Annexin A3	3.3	4.85E-03
IPI00007812	ATP6V1B2	V-type proton ATPase subunit B, brain isoform	3.3	1.64E-05
IPI00873466;IPI00218493	HPRT1	Putative uncharacterized protein HPRT1;Hypoxanthine-guanine phosphoribosyltransferase	3.2	5.49E-03
IPI00291922	PSMA5	Proteasome subunit alpha type-5	3.2	9.68E-04
IPI00027442	AARS	Alanyl-tRNA synthetase, cytoplasmic	3.2	3.54E-03
IPI00375527;IPI00856084;IPI00442268;IPI00644730;IPI009	IMPDH1	inosine monophosphate dehydrogenase 1 isoform a;inosine monophosphate dehydrogenase 1 isoform c;inosine monophosphate	3.2	1.70E-03

16697;IPI00916378;IPI00647813		dehydrogenase 1 isoform d;Isoform 3 of Inosine-5'-monophosphate dehydrogenase 1;IMP (Inosine monophosphate) dehydrogenase 1, isoform CRA_d;inosine monophosphate dehydrogenase 1 isoform f;Isoform 2 of Inosine-5'-monophosphate dehydrogenase 1		
IPI00154645;IPI00794613	TBC1D15	Isoform 1 of TBC1 domain family member 15;Isoform 2 of TBC1 domain family member 15	3.2	6.18E-03
IPI00903119;IPI00005102;IPI00642393	SMS	45 kDa protein;Isoform 1 of Spermine synthase;Isoform 2 of Spermine synthase	3.2	6.36E-03
IPI00329633	TARS	Threonyl-tRNA synthetase, cytoplasmic	3.1	4.26E-03
IPI00027444	SERPINB1	Leukocyte elastase inhibitor	3.1	1.96E-03
IPI00020567	ARHGAP1	cDNA FLJ60782, highly similar to Rho-GTPase-activating protein 1	3.1	7.38E-03
IPI00003856	ATP6V1E1	V-type proton ATPase subunit E 1	3.1	4.77E-03
IPI00001734	PSAT1	cDNA FLJ56437, highly similar to Phosphoserine aminotransferase	2.9	6.62E-03
IPI00465028	TPI1	Isoform 1 of Triosephosphate isomerase	2.9	7.60E-05
IPI00007814	ATP6V1C1	V-type proton ATPase subunit C 1	2.9	7.24E-03
IPI00007682	ATP6V1A	V-type proton ATPase catalytic subunit A	2.9	2.45E-03
IPI00413451;IPI00749398	SERPINB6	Putative uncharacterized protein DKFZp686I04222;Serpin B6	2.8	4.43E-03
IPI00479186;IPI00220644	PKM2	cDNA FLJ56065, highly similar to Pyruvate kinase isozyme M1;Isoform M1 of Pyruvate kinase isozymes M1/M2	2.8	1.25E-04
IPI00291510	IMPDH2	Inosine-5'-monophosphate dehydrogenase 2	2.8	2.96E-03
IPI00027463	S100A6	Protein S100-A6	2.8	9.70E-03
IPI00014151	PSMD6	26S proteasome non-ATPase regulatory subunit 6	2.7	6.22E-03
IPI00013808	ACTN4	Alpha-actinin-4	2.6	5.46E-03
IPI00555565	HSP90AB4P	Putative heat shock protein HSP 90-beta 4	2.6	9.08E-03
IPI00156689	VAT1	Synaptic vesicle membrane protein VAT-1 homolog	2.6	5.89E-03
IPI0079633	ALDOA	45 kDa protein;Fructose-	2.6	4.26E-04

3;IPI00465439		bisphosphate aldolase A		
IPI00784090	CCT8	T-complex protein 1 subunit theta	2.6	6.45E-03
IPI00290566	TCP1	T-complex protein 1 subunit alpha	2.5	7.02E-03
IPI00012268	PSMD2	26S proteasome non-ATPase regulatory subunit 2	2.5	6.38E-04
IPI00329801;IPI00872379	ANXA5	Annexin A5;Putative uncharacterized protein ANXA5 (Fragment)	2.5	7.84E-03
IPI00015954	SAR1A	GTP-binding protein SAR1a	2.5	7.87E-03
IPI00219018	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	2.5	6.83E-04
IPI00017672;IPI00871140	NP	cDNA FLJ25678 fis, clone TST04067, highly similar to PURINE NUCLEOSIDE PHOSPHORYLASE;Purine nucleoside phosphorylase	2.5	8.41E-03
IPI00465248	ENO1	Isoform alpha-enolase of Alpha-enolase	2.3	1.49E-03
IPI00382470;IPI00784295	HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1 isoform 1;Isoform 1 of Heat shock protein HSP 90-alpha	2.2	2.46E-03
IPI00414676	HSP90AB1	Heat shock protein HSP 90-beta	2.2	2.62E-03
IPI00000070	LDLR	Low-density lipoprotein receptor	2.1	3.77E-03
IPI00012011	CFL1	Cofilin-1	2.0	7.35E-03
IPI00018871;IPI00789069	ARL8B;-	cDNA FLJ56285, highly similar to ADP-ribosylation factor-like protein 8B;cDNA FLJ61158, highly similar to ADP-ribosylation factor-like protein 8B	2.0	7.90E-03
IPI00011229	CTSD	Cathepsin D	2.0	7.93E-03

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^Corresponds to significance in MaxQuant

Supplementary Table 4 | Non-MitoCarta proteins with decreased abundance in mitochondria of CCCP-treated cells

Leading IPIs	Leading Gene Names	Leading Protein Descriptions	Overall H/L Normalized Ratio*	Overall H/L Significance^
IPI00852851;IPI00008732	TRABD	43 kDa protein;Isoform 1 of TraB domain-containing protein	0.19	3.64E-13
IPI00432065	C11orf83	Uncharacterized protein C11orf83	0.21	5.04E-12
IPI00855998	CENPF	Centromere protein F	0.21	6.20E-09
IPI00640118	LOC100131801	similar to hCG2036585	0.21	9.37E-09
IPI00607900;IPI00013278	C20orf149	Chromosome 20 open reading frame 149;Isoform 1 of UPF0362 protein C20orf149	0.23	2.26E-08
IPI00106646;IPI00009794;IPI00514755	SDF4	45 kDa calcium-binding protein;stromal cell derived factor 4 precursor;Stromal cell derived factor 4	0.28	9.88E-09
IPI00400948;IPI00916639	C2orf64	Uncharacterized protein C2orf64;8 kDa protein	0.29	1.57E-06
IPI00168047;IPI00845421	FAM73A	Isoform 1 of Protein FAM73A;Isoform 2 of Protein FAM73A	0.30	2.57E-06
IPI00009817;IPI00878815	CHCHD8	Isoform 2 of Coiled-coil-helix-coiled-coil-helix domain-containing protein 8;Isoform 1 of Coiled-coil-helix-coiled-coil-helix domain-containing protein 8	0.32	5.94E-10
IPI00555820;IPI00894163	MYO19	cDNA FLJ61052, highly similar to Homo sapiens myosin head domain containing 1 (MYOHD1), transcript variant 2, mRNA;Isoform 1 of Myosin-XIX	0.34	3.43E-07
IPI00791574;IPI00030531;IPI00794566	SLMAP	Isoform 1 of Sarcolemmal membrane-associated protein;Isoform 3 of Sarcolemmal membrane-associated protein;Isoform 2 of Sarcolemmal membrane-associated protein	0.34	5.55E-07
IPI00017979;IPI00478216	FAM54A	Isoform 1 of Protein FAM54A;Isoform 2 of Protein FAM54A	0.35	3.78E-05
IPI00877014;IPI00290544	GDAP1	ganglioside-induced differentiation-associated protein 1 isoform a;Ganglioside-induced differentiation-associated protein 1	0.40	1.85E-04
IPI00014456;IPI00	STRN	Isoform 1 of Striatin;Isoform 2	0.43	4.48E-04

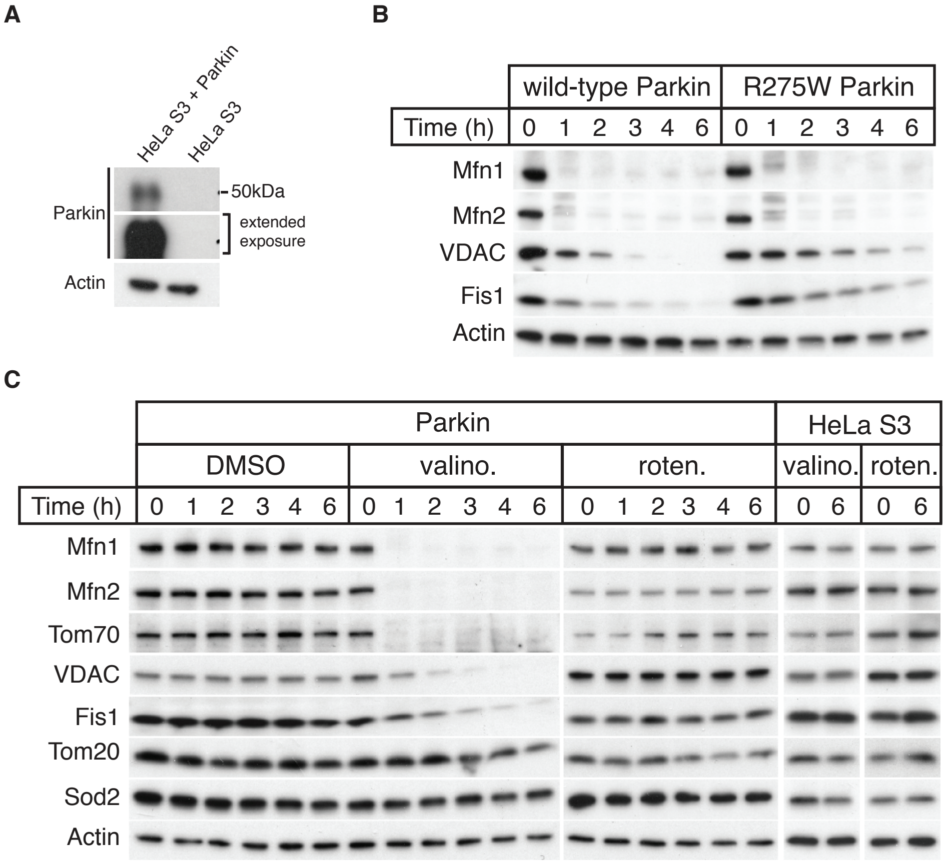
654594		of Striatin		
IPI00303753	C1orf163	Hcp beta-lactamase-like protein C1orf163	0.45	7.83E-06
IPI00514622	RANBP6	Ran-binding protein 6	0.48	4.08E-04
IPI00465113;IPI00915852	EXDL2	cDNA FLJ58573, highly similar to Exonuclease 3'-5' domain-like-containing protein 2;Exonuclease 3'-5' domain-like-containing protein 2	0.50	4.19E-04
IPI00015890	CCRN4L	Nocturnin	0.52	3.47E-03
IPI00413436	COX19	Cytochrome c oxidase assembly protein COX19	0.53	1.36E-03
IPI00295542;IPI00893190	NUCB1	Nucleobindin-1;cDNA FLJ52898, highly similar to Nucleobindin-1	0.55	2.54E-03
IPI00465410	C12orf73	Uncharacterized protein C12orf73	0.55	2.76E-03
IPI00853224	STARD7	StAR-related lipid transfer protein 7, mitochondrial	0.56	1.76E-03
IPI00305552;IPI00395372	CHCHD5	cDNA FLJ39671 fis, clone SMINT2008917;Coiled-coil-helix-coiled-coil-helix domain-containing protein 5	0.57	9.07E-03
IPI00024619	C16orf61	UPF0287 protein C16orf61	0.62	5.03E-03

Mitochondria from Parkin-expressing HeLa S3 cells with and without CCCP (2 hours) were compared.

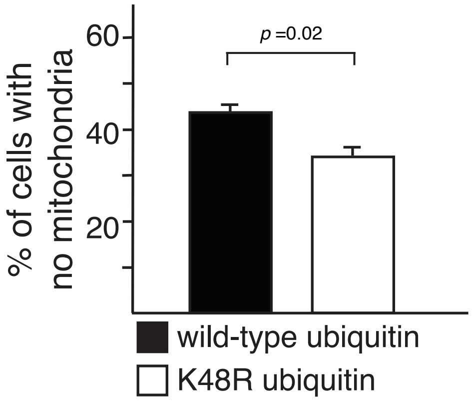
*The protein level in the mitochondria of CCCP-treated cells divided by the level in untreated cells.

^Corresponds to the significance in MaxQuant

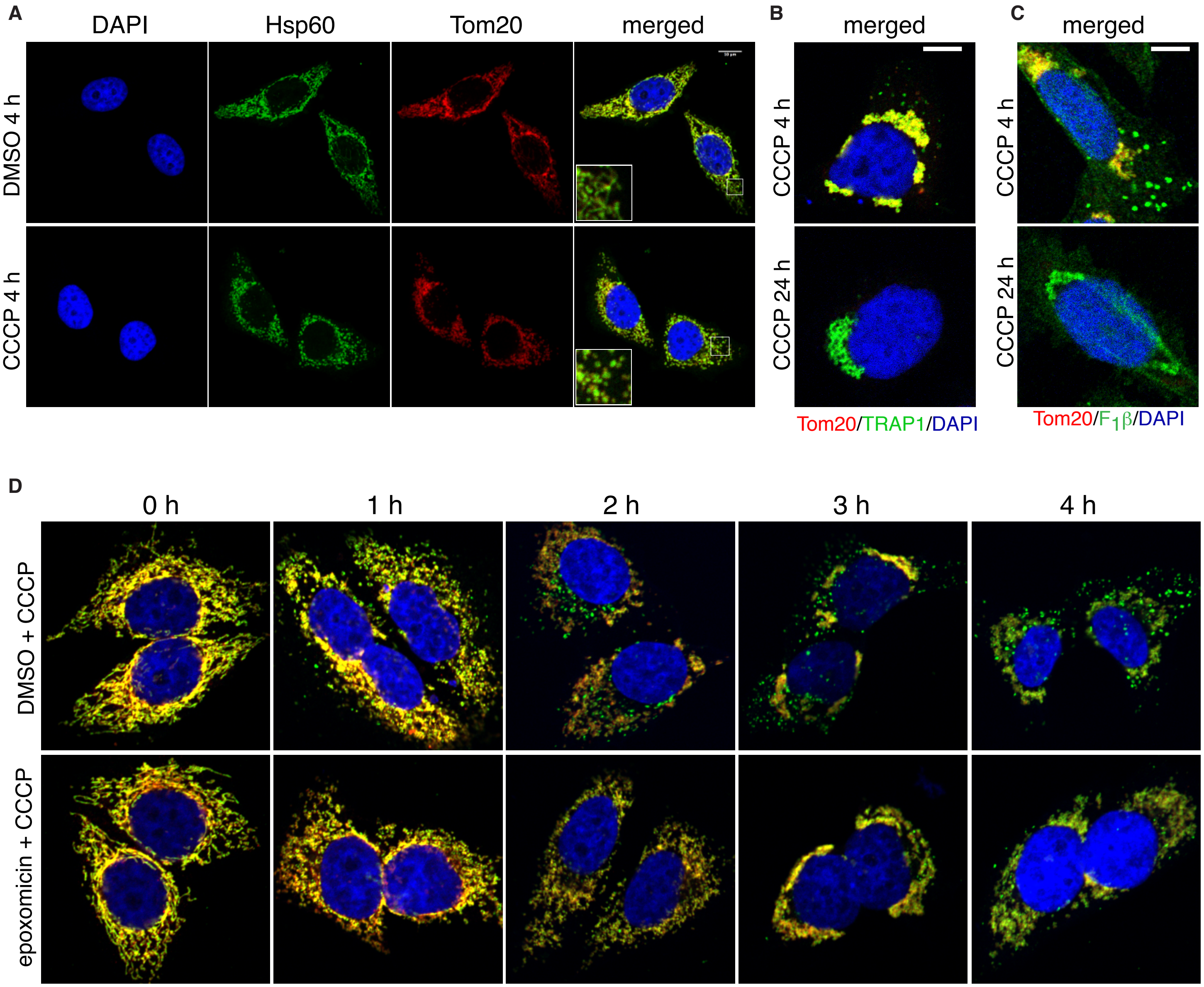
Chan *et al.*, Supplemental Figure S1



Chan *et al.*, Supplemental Figure S2



Chan *et al.*, Supplemental Figure S3



Chan *et al.*, Supplemental Figure S4