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 proteolysis 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 F₁β (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.
## 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           | Isoform 39 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             |
| IPI000020510                       | CISD1              | CDGSH iron sulfur domain-containing protein 1                                                    | 0.26                      | 8.74E-18             |
| IPI00001638                        | PRELID1            | PRELI domain-containing protein 1, mitochondrial [Pyrurate dehydrogenase [acetyl-transferring]]-phosphatase 2, mitochondrial | 0.28                      | 3.07E-07             |
| IPI00002251                        | PDP2               | cDNA FLJ56039, highly similar to 5-aminolevulinate synthase, nonspecific, mitochondrial         | 0.30                      | 2.09E-08             |
| IPI00007266                        | ALAS1              | Protein; Isoform 1 of Phosphatidylserine decarboxylase proenzyme; Phosphatidylserine decarboxylase | 0.30                      | 6.95E-08             |
| IPI00916131;IPI001470              | PISD;C22orf30      | Isoform 39 of Solute carrier family 25 member 39; Isoform 2 of Solute carrier family 25 member 39 | 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;IPI0047849;IPI00910479 | SLC25A46 (Fragment)| Solute carrier family 25 member 46;cDNA FLJ60882                                               | 0.37                      | 2.20E-06             |
| IPI00028198;IPI0030528             | 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, coiled-coil-helix-coiled-helix domain containing 7 isoform b; coiled-coil-helix-coiled-helix domain containing 7 isoform d; Coiled-coil-helix-coiled-helix domain-containing protein 7 | 0.39                      | 1.84E-07             |
| IPI00552793;IPI00604730;IPI00013279 | CHCHD7            | Glycerol-3-phosphate acyltransferase, mitochondrial; Glycerol 3-phosphate acyltransferase, mitochondrial | 0.41                      | 1.35E-05             |
| IPI00844249;IPI00640749            | GPAM               | 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             |
| Accession  | Symbol  | Description                                                                 | Value 1 | Value 2 |
|-----------|---------|------------------------------------------------------------------------------|---------|---------|
| IPI00465059 | RHOT2   | Isoform 1 of Mitochondrial Rho GTPase 2, mitochondrial                       | 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;IPI0052131;IPI00045660;IPI00646750 | C1orf31;C1orf31;C1orf31;C1orf31 | Protein import inner membrane translocase subunit alpha isoform 1;Isoform 1 of Uncharacterized protein;Isoform 3 of Uncharacterized protein | 0.47    | 2.63E-05 |
| IPI00653722 | FAM136A | Protein FAM136A, Isoform Mitochondrial of Polyplolyglutamate synthase, mitochondrial;Isoform Cytoplasmic of cDNA FLJ61576, highly similar to Mitochondrial fission regulator 1;Mitochondrial fission regulator 2 | 0.51    | 1.01E-05 |
| IPI00910782;IPI00164745;IPI00759584 | FPGS    | Polypolyglutamate synthase, mitochondrial;Isoform Cytoplasmic of cDNA FLJ61576, highly similar to Mitochondrial fission regulator 1;Mitochondrial fission regulator 2 | 0.53    | 4.50E-03 |
| IPI00091082;IPI00164745;IPI00759584 | MTFR1;MTFR1 | Mitochondrial fission 1 protein;Mitochondrial fission 2, mitochondrial precursor;Peptidyl-tRNA hydrolase 2, mitochondrial precursor | 0.54    | 1.02E-03 |
| IPI0025344 | NDUF6   | NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial         | 0.55    | 1.33E-03 |
| IPI00171459 | HSDL1   | Hydroxysteroid dehydrogenase-like protein 1, mitochondrial                  | 0.55    | 5.84E-04 |
| IPI00555597;IPI0032903 | PTHR2 | Peptidyl-tRNA hydrolase 2, mitochondrial                                     | 0.57    | 3.50E-03 |
| IPI00478450;IPI0072058 | FAM82B | Regulator of microtubule dynamics protein 1, Isoform 2 of NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial;Neuronal protein | 0.57    | 1.20E-03 |
| IPI00215901;IPI0018988 | AK2     | Isoform 1 of Adenylate kinase isoenzyme 2, mitochondrial;Isoform 2 of Adenylate kinase isoenzyme 2, mitochondrial | 0.57    | 2.40E-04 |
| IPI00065063;IPI00008418;IPI00219865 | DHRS1;DIABLO | Mitochondrial import inner membrane translocase subunit 2 of Diablo homolog, mitochondrial precursor;Isoform 2 of Diablo homolog, mitochondrial | 0.57    | 1.31E-03 |
| IPI00031204 | RDH13   | Retinol dehydrogenase 13, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4 | 0.60    | 2.61E-03 |
| IPI00011770 | NDUF4A4 | Uncharacterized protein C10orf58;25 kDa protein;Putative uncharacterized protein C10orf58 1-acyl-sn-glycerol-3-phosphate acyltransferase epsilon | 0.62    | 5.47E-03 |
| IPI00028491 | AGPAT5  | Mitochondrial import inner membrane translocase subunit Tim13              | 0.63    | 6.38E-03 |
| IPI00001589 | TIMM13  | Mitochondrial import inner membrane translocase subunit Tim13              | 0.63    | 6.85E-03 |
| IPI00013459;IPI00791036;IPI00556190 | NDUF5;NDUF5 | Mitochondrial import inner membrane translocase subunit Tim8 A;Mitochondrial import inner membrane translocase subunit Tim8 A | 0.64    | 2.31E-03 |
| IPI00028376 | TIMM8A | Mitochondrial import inner membrane translocase subunit Tim8 A             | 0.65    | 8.53E-03 |
| IPI00419266 | NDUF6  | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa              | 0.65    | 3.23E-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;   | NME2:NME1-        | Nucleoside diphosphate kinase;NME1-NME2 protein | 2.36                     | 1.20E-03              |
| IPI00795292    | NME2;NME2         | Isoform 1 of L-lactate dehydrogenase A chain | 2.10                     | 4.33E-03              |
| IPI00217966    | LDHA               | L-lactate dehydrogenase B chain | 1.96                     | 8.75E-03              |
| IPI00219217    | LDHB               |                             |                          |                       |

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^ |
|--------------|--------------------|-----------------------------|--------------------------|-----------------------|
| IPI0000525   | PARK2              | Isoform 1 of E3 ubiquitin-protein ligase parkin | 13.5                     | 4.42E-20              |
| 4            | UBB:RPS27 A:UBC    | ubiquitin and ribosomal protein neighbor of BRCA1 gene | 8.9                      | 9.58E-15              |
| IPI0017933   | NBR1;LOC7 27732;LOC10 | 1;Isoform 1 of Next to BRCA1 gene 1 protein;69 kDa protein;hypothetical protein isoform 2 | 8.3                     | 2.90E-06              |
| IPI0078385   | 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              |
| 5;IPI008719  | SQSTM1             | Isoform 1 of Sequestosome-1 Microtubule-associated proteins 1A/1B light chain 3 beta | 5.8                     | 1.75E-06              |
| 41;IPI002999 | MAP1LC3B2;MAP1LC3B | 2;Microtubule-associated proteins 1A/1B light chain 3B TRAF-type zinc finger domain-containing protein 1 | 5.4                     | 3.70E-06              |
| 920;IPI0088  | TRAFD1             | Proteasome subunit alpha type-2 | 4.2                     | 8.08E-05              |
| 7954         | PSMA2              | Proteasome subunit beta type-5 | 4.2                     | 4.21E-04              |
| IPI0017947   | PSMB5              | Proteasome subunit beta type-5 | 4.2                     | 4.21E-04              |
| IPI002204    | TPP2               | Tripeptidyl peptidase II;Tripeptidyl-peptidase 2 Isoform Long of Proteasome subunit alpha type-1;Isoform Short of Proteasome subunit alpha type-1;Proteasome subunit alpha type | 4.1                     | 2.18E-03              |
| 2;IPI0021962 | PSMA1              | Proteasome subunit alpha type | 4.0                     | 5.38E-04              |
| 82           | PSMB3              | Proteasome subunit beta type-3 | 4.0                     | 6.20E-04              |
| IPI000914    | PSMB6              | Proteasome subunit beta type-6 | 3.9                     | 2.30E-04              |
| 6            | KIAA1618           | Isoform 1 of Protein ALO17 cDNA FLJ60299, highly similar to Rab GDP dissociation inhibitor beta | 3.9                     | 1.38E-03              |
| 16           | RAD23B             | RAD23 homolog B;cDNA | 3.7                     | 4.55E-03              |
| IPI0000822   |                    |                             |                          |                       |
| 3;IPI006425  |                    |                             |                          |                       |
| Gene ID          | Description                                                                 |
|-----------------|----------------------------------------------------------------------------|
| IPI0002962      | FLJ56531, highly similar to UV excision repair protein RAD23 homolog B     |
| IPI0055595      | PSMA6, Proteasome subunit alpha type-6                                    |
| IPI0002417      | PSMB4, Proteasome subunit beta type-4                                     |
| IPI0029915      | PSMA7, Isoform 1 of Proteasome subunit alpha type-7                        |
| IPI007902       | PSMA4, Proteasome subunit alpha type-4; 27 kDa protein                   |
| IPI0041924      | PSMA3, Proteasome subunit alpha type-3                                    |
| IPI0002417      | SMA4, Proteasome subunit alpha type-4; 27 kDa protein; ataxin 3 isoform   |
| IPI0029915      | SMA3, Isoform 1 of Proteasome subunit alpha type-3; Isoform 2 of          |
| IPI0002635      | SMA2, Small glutamine-rich tetratricopeptide repeat-containing protein alpha; cDNA |
| IPI0021902      | SGTA, Prostate stem cell antigen                                           |
| IPI0021902      | GOT1, Aspartate aminotransferase, cytoplasmic                             |
| IPI0022635      | GABARAPL2, Gamma-aminobutyric acid receptor-associated protein-like       |
| IPI0002183      | PRKAR1A, cAMP-dependent protein kinase type I-alpha regulatory subunit    |
| IPI0002501      | PSMB1, Proteasome subunit beta type-1                                      |
| IPI002409       | ANXA3, Annexin A                                                           |
| IPI000781       | ATP6V1B2, V-type proton ATPase subunit B, brain isoform                    |
| IPI0087346      | HPRT1, Putative uncharacterized protein HPRT1; Hypoxanthine-guanine      |
| IPI002184       | IMPDH1, Proline dehydrogenase 1 isoform a; inosine monophosphate dehydrogenase |
| IPI0002744      | AARS, Cytosolic inosine monophosphate dehydrogenase 1 isoform a;          |
| IPI0037552      | IMPDH1, Proline dehydrogenase 1 isoform c; inosine monophosphate dehydrogenase |

**Note:** The table entries include gene identifiers, protein descriptions, and expression values (3.7, 3.5, etc.) with statistical significance (1.18E-03, 3.54E-03, etc.).
| Gene ID       | Description                                                                 |
|--------------|-----------------------------------------------------------------------------|
| IPI0015464   | Isoform 1 of TBC1 domain family member                                      |
| IPI007946    | Isoform 2 of TBC1 domain family member                                      |
| IPI0032963   | Putative uncharacterized protein DKFZp68610422, highly similar to Rho-GTPase-|
|              | activating protein 1                                                        |
| IPI0000173   | V-type proton ATPase subunit E cDNA FLJ56437, highly similar to Phosphos |-  |
|              | serine aminotransferase                                                     |
| IPI0000781   | V-type proton ATPase subunit C cDNA FLJ56065, highly similar to Pyruvate k |   |
|              | kinase isoyme M1;Isoform M1 of Pyruvate kinase isozymes M1/M2 Inosine-5'-| |
|              | monophosphate dehydrogenase 2                                              |
| IPI0015668   | Synaptic vesicle membrane protein VAT-1 homolog                            |
| IPI0001415   | Protein S100-A6 26S proteasome non-ATPase regulatory subunit 6             |
| IPI0029151   | Alpha-actinin-4                                                             |
| IPI0046534   | Putative heat shock protein HSP 90-beta 4                                    |
| IPI0055556   | Synaptic vesicle membrane protein VAT-1 homolog                            |
| IPI0015668   | 45 kDa protein;Fructose-5-phosphate dehydrogenase 1, isoform CRA_d;inosine|   |
|              | monophosphate dehydrogenase 1, isoform f;Isoform 2 of Inosine-5'-monophos-|
|              | phosphate dehydrogenase 1                                                  |
| IPI0000385   | V-type proton ATPase catalytic subunit A                                    |
| IPI0000768   | PUTATIVE uncharacterized protein DKFZp68610422, highly similar to Rho-GTPas| |
| IPI0002744   | Isoform 1 of TBC1 domain family member                                      |
| IPI0002056   | Isoform 2 of TBC1 domain family member                                      |
| IPI0000781   | Isoform 1 of Triosephosphate isomerase                                       |
| IPI0000781   | Isoform 1 of Triosephosphate isomerase                                       |
| IPI0000385   | V-type proton ATPase subunit C cDNA FLJ56065, highly similar to Pyruvate k |   |
| IPI004718    | Isoform 1 of Triosephosphate isomerase                                       |
| IPI002206    | Isoform 1 of Triosephosphate isomerase                                       |
| IPI00001380  | Isoform 1 of Triosephosphate isomerase                                       |
| IPI0001415   | Isoform 1 of Triosephosphate isomerase                                       |
| IPI0000781   | Isoform 1 of Triosephosphate isomerase                                       |
| IPI0000781   | Isoform 1 of Triosephosphate isomerase                                       |
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| IPI0000781   | Isoform 1 of Triosephosphate isomerase                                       |
| IPI0000781   | Isoform 1 of Triosephosphate isomerase                                       |
| IPI0000781   | Isoform 1 of Triosephosphate isomera |
| Accession | Name                                      | Description                                                                 | Combined Ratio | MaxQuant Ratio |
|-----------|-------------------------------------------|------------------------------------------------------------------------------|----------------|----------------|
| IPI004654 | bisphosphate aldolase A                   |                                                                              |                |                |
| IPI0078409| T-complex protein 1 subunit theta          |                                                                              | 2.6            | 6.45E-03       |
| IPI0029056| T-complex protein 1 subunit alpha          |                                                                              | 2.5            | 7.02E-03       |
| IPI001226 | 26S proteasome non-ATPase regulatory subunit 2 | Annexin A5;Putative uncharacterized protein ANXA5 (Fragment)              | 2.5            | 6.38E-04       |
| IPI032980 | TCP1                                      | GTP-binding protein SAR1a Glyceraldehyde-3-phosphate dehydrogenase         | 2.5            | 7.87E-03       |
| IPI0001595| ANXA5                                     | GTP-binding protein SAR1a Glyceraldehyde-3-phosphate dehydrogenase         | 2.5            | 7.84E-03       |
| IPI001767 | SAR1A                                     | GTP-binding protein SAR1a Glyceraldehyde-3-phosphate dehydrogenase         | 2.5            | 8.41E-03       |
| IPI0021901| GAPDH                                     | Isoform alpha-enolase of Alpha-enolase                                       | 2.3            | 1.49E-03       |
| IPI046524 | Isoform alpha-enolase of Alpha-enolase    |                                                                              |                |                |
| IPI0038247| NP                                        | Purine nucleoside phosphorylase                                             | 2.5            | 8.15E-03       |
| IPI0046524| HSP90AA1                                  | heat shock protein 90kDa alpha (cytosolic), class A member 1 isoform 1;Isoform 1 of Heat shock protein HSP 90-alpha | 2.2            | 2.64E-03       |
| IPI0046524| HSP90AB1                                  | Heat shock protein HSP 90-beta                                               | 2.2            | 2.62E-03       |
| IPI0000007| LDLR                                      | Low-density lipoprotein receptor                                             | 2.1            | 3.77E-03       |
| IPI001201 | CFL1                                      | Cofilin-1 cDNA FLJ56285, highly similar to ADP-ribosylation factor-like protein 8B;cDNA FLJ61158, highly similar to ADP-ribosylation factor-like protein | 2.0            | 7.35E-03       |
| IPI001122 | ARL8B;                               |                                                                                | 2.0            | 7.90E-03       |
| CTSD      | Cathepsin D                               |                                                                              | 2.0            | 7.93E-03       |

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### 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;IPI0008732             | TRABD              | 43 kDa protein; Isoform 1 of TraB domain-containing protein                                      | 0.19                         | 3.64E-13                  |
| IPI00432065                        | C11orf83           | C11orf83                                                                                       | 0.21                         | 5.04E-12                  |
| IPI00855998                        | CENPF LOC10013180  | Centromere protein F                                                                           | 0.21                         | 6.20E-09                  |
| IPI00640118                        |                    | similar to hCG2036585 Chromosome 20 open reading frame 149; Isoform 1 of UPF0362 protein       | 0.21                         | 9.37E-09                  |
| IPI00607900;IPI00013278            | C20orf149          | 45 kDa calcium-binding protein; stromal cell derived factor 4 precursor; Stromal cell derived factor 4 | 0.23                         | 2.26E-08                  |
| IPI00106646;IPI0009794;IPI005147    | SDF4               | Uncharacterized protein                                                                         | 0.28                         | 9.88E-09                  |
| IPI000400948;IPI00916639           | C2orf64            | Isoform 1 of Protein FAM73A; Isoform 2 of Protein FAM73A                                       | 0.29                         | 1.57E-06                  |
| IPI00168047;IPI00845421            | FAM73A             | 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 | 0.30                         | 2.57E-06                  |
| IPI00009817;IPI00878815            | CHCHD8             | Isoform 8 cDNA FLJ61052, highly similar to homo sapiens myosin head domain containing 1 (MYOHID1), transcript variant 2, mRNA; Isoform 1 of Myosin-XIX | 0.32                         | 5.94E-10                  |
| IPI00555820;IPI00894163            | MYO19              | Isoform 1 of Sarcolemmal membrane-associated protein; Isoform 3 of Sarcolemmal membrane-associated protein | 0.34                         | 3.43E-07                  |
| IPI00791574;IPI0030531;IPI007945    | SLMAP              | Isoform 1 of Protein FAM54A; Isoform 2 of Protein FAM54A; Ganglioside-induced differentiation-associated protein 1 isoform a; Ganglioside-induced differentiation-associated protein 1 | 0.35                         | 3.78E-05                  |
| IPI00017979;IPI00478216            | FAM54A             | Isoform 1 of Protein FAM54A; Isoform 2 of Protein FAM54A; Ganglioside-induced differentiation-associated protein 1 isoform a; Ganglioside-induced differentiation-associated protein 1 | 0.40                         | 1.85E-04                  |
| IPI00877014;IPI00290544            | GDAP1              | Isoform 1 of Striatin; Isoform 2 | 0.35                         | 3.78E-05                  |
| IPI00014456;IPI00STRN              |                    | Isoform 1 of Striatin; Isoform 2 | 0.43                         | 4.48E-04                  |
| Accession     | Description                                                                 | Ratio | P-value     |
|--------------|------------------------------------------------------------------------------|-------|-------------|
| IPI00303753  | C1orf163, Hcp beta-lactamase-like protein                                     | 0.45  | 7.83E-06    |
| IPI00514622  | RANBP6, cDNA FLJ58573, highly similar to Exonuclease 3'-5' domain-like-containing protein 2 | 0.48  | 4.08E-04    |
| IPI00465113;IPI00915852 | EXDL2, cDNA FLJ58573, highly similar to 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, FLJ52898, highly similar to Nucleobindin-1 | 0.55  | 2.54E-03    |
| IPI00465410  | C12orf73, uncharacterized protein                                            | 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

A

B

C

| Time (h) | DMSO | valino. | roten. | valino. | roten. |
|---------|------|---------|--------|---------|--------|
| 0       | 0    | 0       | 0      | 0       | 0      |
| 1       | 0    | 0       | 0      | 0       | 0      |
| 2       | 0    | 0       | 0      | 0       | 0      |
| 3       | 0    | 0       | 0      | 0       | 0      |
| 4       | 0    | 0       | 0      | 0       | 0      |
| 6       | 0    | 0       | 0      | 0       | 0      |

Mfn1, Mfn2, VDAC, Fis1, Tom70, Tom20, Sod2, Actin

50kDa extended exposure
Chan et al., Supplemental Figure S2

% of cells with no mitochondria

- Wild-type ubiquitin
- K48R ubiquitin

$p = 0.02$
Chan et al., Supplemental Figure S3

A) DAPI Hsp60 Tom20 merged

B) CCCP 4 h

C) CCCP 24 h

D) 0 h 1 h 2 h 3 h 4 h

DMSO + CCCP

epoxomicin + CCCP
Chan et al., Supplemental Figure S4

A Hsp60 Parkin merged

B % of cells with Parkin/Hsp60 colocalization

C Hsp60 Tom20 DAPI merged

D % of cells with no mitochondria

E % of cells with 1-10 11-20 21-30 >30 number of Tom20-negative, Hsp60-positive mitochondria per cell

F Hsp60 Tom20 DAPI merged

G % of cells with no mitochondria

MG132 -CCCP +MG132 -CCCP +MG132 +CCCP

DMSO epoxomicin DMSO + CCCP epoxomicin + CCCP DMSO + CCCP epoxomicin + CCCP

4 h 12 h 4 h 24 h