Quantitative Mass Spectrometry to Discover Interactors of Parkin E3 Ubiquitin Ligase, a Protein Implicated in Early-Onset Parkinson's Disease

by

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ABSTRACT

Ubiquitylation is a major post-translational modification based on a network of about six hundred E3 ubiquitin ligases in human. It is involved in several processes such as proteolysis, vesicle trafficking and DNA damage response. Mutations in *PARK2*, which encode parkin E3 ubiquitin ligase, account for half of autosomal recessive juvenile Parkinsonism cases, an early onset form of Parkinson's disease. Multiple *PARK2* mutations underlie the RING domain, which contains ligase activity. This finding suggests an inability for substrate ubiquitylation may trigger neurodegeneration. We used a quantitative proteomics approach to seek identifying parkin substrates and interactors. We first developed and tested new methods to enrich for ubiquitylated proteins that could potentially be used to study the influence of parkin on the ubiquitin proteome. In our first approach, ubiquitin conjugates were purified from SH-SY5Y neuroblastoma expressing His_8-biotin-ubiquitin by tandem affinity purification. A second approach to purify ubiquitylated proteins was based on affinity chromatography using S5a proteasome receptor that bound to poly-ubiquitylated proteins. We determined that both approaches were not adequate for identifying low abundance parkin substrates. We then sought to identify which proteins were associated with parkin. Parkin interactors were enriched from SH-SY5Y expressing FLAG-parkin versus endogenous parkin by anti-FLAG immunoprecipitation in the context of SILAC. Proteins from the neuroendocrine chromogranin-secretogranin family were highly enriched suggesting a potential granin vesicle trafficking role for parkin. CCCP, a mitochondrial uncoupling agent was also employed to investigate parkin ligase interactors during mitochondrial stress since parkin localizes to mitochondria to promote mitophagy upon a reduction in

mitochondrial membrane potential. Several actin related proteins were enriched from FLAG-parkin cells treated with CCCP including non-muscle unconventional signaling myosin suggesting a potential role for these proteins during parkin-mediated mitophagy.

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
Arg	Arginine
ARJP	Autosomal recessive juvenile Parkinsonism
Asn	Asparagine
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
CCCP	Carbonyl cyanide m-chlorophenyl hydrazone
CDC	Cell-division cycle
cDNA	complementary DNA
Da	Dalton
DC	Detergent compatible
DCM	Dilated cardiomyopathy
ddH_2O	double distilled nanopure water
DMEM	Dulbecco's Modified Eagle's Media
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EndoLysC	Endoproteinase Lys-C
ER	Endoplasmic reticulum
ESCRT	endosomal sorting complex for transport
ESI	Electrospray ionization
ETC	Electron transport chain
FA	Formic acid
FBS	Fetal bovine serum
G418	Geneticin
GDP	Guanosine diphosphate

Gln	Glutamine
GlyGly	Glycine-glycine
GST	Glutathione S-transferase
GTP	Guanosine triphosphate
HB	His_8-biotin
HBS	HEPES buffered saline
HBU	His_8-biotin-ubiquitin
HCl	Hydrochloric acid
HDAC6	Histone deacetylase 6
His	Histidine
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
IMAC	Immobilized metal affinity chromatography
IPTG	Isopropyl β-D-1-thiogalactopyranoside
KOAc	Potassium acetate
k_{off}	Dissociation constant
LC	Liquid chromatography
LTQ	Linear trapping quadrupole
Lys	Lysine
MAIF	Mitochondrial apoptosis inducing factor
MG132	N-(benzyloxycarbonyl)leucinylleucinylleucinal
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular weight
MWCO	Molecular weight cutoff
m/z	mass over charge ratio
OD	Optical density
Pael-R	Parkin-associated endothelin-like receptor
PAGE	Polyacrylamide gel electrophoresis

PBS	Phosphate buffered saline
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PD	Parkinson's disease
PFA	Paraformaldehyde
PINK1	phosphatase and tensin homolog-induced putative kinase 1
PMSF	Phenylmethylsulphonyl fluoride
ppm	parts per million
Q-TOF	Quadrupole Time-of-Flight
RING	Really interesting new gene
RNA	Ribonucleic acid
RT	Room temperature
SDS	Sodium dodecyl sulphate
SCX	Strong cation exchange
SILAC	Stable isotope labeling with amino acids in cell culture
siRNA	Small interfering ribonucleic acid
STAGE	STop and Go Extraction
TBS	Tris buffered saline
TCA	Trichloroacetic acid
TFA	Trifluoroacetic acid
TCEP	Tris(2-carboxyethyl)phosphine
Th	Thomson
TS	Tween-salt
UBA	Ubiquitin associated
UIM	Ubiquitin interacting motif
UPS	Ubiquitin proteasome system
WT	Wild-type
YFP	Yellow fluorescent protein

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CHAPTER 1

INTRODUCTION

1.1 The ubiquitin system

Ubiquitylation is a major post-translational modification in the eukaryotic cell on par with phosphorylation[1]. Ubiquitin is a highly conserved 76-residue polypeptide that can be conjugated to target proteins via the ubiquitin C-terminal glycine carboxyl group[2]. Lysine side chains are the most common target sites within substrate proteins resulting in an amide (or isopeptide) bond between ubiquitin and substrate. A cascade of reactions is necessary to attach ubiquitin to proteins. First, the E1-activating enzyme uses ATP to form a high energy thioester bond with ubiquitin in which ubiquitin is then passed to the active cysteine of an E2-conjugating enzyme. Finally, the E3-ligase enzyme transfers ubiquitin from the E2 to specific substrates[3]. There are about six hundred E3 ubiquitin ligases in humans, reflecting how vast the ubiquitin system is[3]. Proteins can either undergo single or multiple mono-ubiquitylation or poly-ubiquitylation, in which a distal ubiquitin's C-terminus is conjugated to one of seven lysine residues of ubiquitin, forming either a Lys-6, 11, 27, 29, 33, 48, or 63 isopeptide bond[3]. The ubiquitin chain may extend up to several ubiquitin molecules. Ubiquitin C-terminal hydrolases facilitate the reverse reaction of de-ubiquitylating substrates by cleaving after the last glycine residue of ubiquitin[3].

Different types of ubiquitylation reactions can occur resulting in diverse functions including membrane protein trafficking, chromatin dynamics, DNA repair and proteasomal degradation[3]. For instance, multiple mono-ubiquitylation of membrane proteins results in endocytosis by the endosomal sorting complex for transport (ESCRT) machinery into lysosomes for destruction, an important function for proper cell signaling and metabolism[5]. Ubiquitin is also involved in gene activation in the case of histone H2A

and H2B regulation of chromatin[6-8]. Mono-ubiquitylation and poly-ubiquitylation of proliferating cell nuclear antigen (PCNA) facilitates translesion synthesis and error-free DNA-damage-tolerance pathway activation respectively, processes which are essential for DNA repair[9]. A major function of ubiquitin is to target proteins for proteasomal degradation. The ubiquitin-proteasome system (UPS) involves substrates poly-ubiquitylated through Lys-48, the most common type of ubiquitin linkage, although recently mono-ubiquitylated substrates as well as Lys-11 and Lys-29 poly-ubiquitylation were also discovered as a target for proteasomal degradation[10]. Shuttling proteins including CDC48/p97 in some cases then transport the poly-ubiquitylated substrates to the 26S proteasome, a large protein complex. After binding, the substrates are de-ubiquitylated and unfolded prior to their entry into the catalytic core where they are cleaved into peptides and finally by cytosolic peptidases into amino acids[11]. The UPS is vital for eliminating misfolded proteins and a mechanism for protecting cells from their toxic effects and also recycling amino acids. Proteasome-dependent degradation clears misfolded proteins from the cytosol, nucleus and endoplasmic reticulum (ER)[12]. Besides functioning in protein quality control, the UPS also directly regulates cell cycle progression and apoptosis; for instance, ubiquitin ligase MDM2 targets p53, implicating the UPS in cancer progression[13].

1.2 The UPS in Parkinson's disease

The presence of misfolded and aggregated proteins and inclusion bodies containing ubiquitin is a hallmark of many neurodegenerative diseases such as Alzheimer, Parkinson's and Huntington[14-15]. Understanding the UPS is therefore key for

comprehending the molecular mechanisms underlying these diseases. Parkinson's Disease (PD) is a debilitating condition characterized by resting tremor, rigidity and other features such as postural and autonomic instability[16]. Degeneration of dopaminergic neurons in the substantia nigra of the midbrain, in addition to other catecholamine and serotonin neurons in the brainstem are underlying causes of the disease[17]. Another hallmark of PD patients is the formation of Lewy bodies, which are intraneuronal inclusions enriched with ubiquitin, located in the cell body of surviving neurons[18]. One well accepted theory is that inclusion bodies are a cellular defense mechanism to sequester toxic misfolded proteins into single insoluble states, in conditions where the proteasome may be overwhelmed[19].

Inheriting forms of PD have been linked to mutations in three genes that are related to the UPS (Table 1.1): alpha-synuclein, ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) and parkin[15, 20]. Over-expression of alpha-synuclein, especially mutant forms, inhibits the UPS[21]. This effect is even more significant as triplication of the wild-type alpha-synuclein gene has been found to cause autosomal dominant PD[22]. An interesting proposed mechanism is that upon alpha-synuclein binding to the 19S proteasome complex, other substrates are not degraded due to hinderance by alpha-synuclein docking[23, 24].

Table 1.1 Genes affected in PD. Figure adapted from reference 16.

Genes affected in Parkinson's disease

Gene	Locus	Inheritance	Onset Age	Pathology
α-Synuclein	4q21	Dominant	33-59	Typical
Parkin	6q25.2-q27	Recessive	18-27	No Lewy bodies
DJ-1	1p36	Recessive	Early	Unknown
UCH-L1	4p14	Dominant	49-51	Typical
PINK-1	1p35-36	Recessive	28-48	Unknown

Another significant gene associated with inherited forms of PD is *PARK2*, coding for parkin (Table 1.1)[25]. Mutations in *PARK2* are responsible for nearly 50% of autosomal-recessive juvenile-onset parkinsonism (ARJP)[26], in which the onset of PD symptoms occur prior to the age of forty, as well as 10-15% of sporadic early onset PD. Parkin is an E3 ubiquitin ligase belonging to the RING family[27]. It contains three RING domains as well as an ubiquitin-like domain that is both structurally and sequence related to ubiquitin. Mutations causing ARJP include stop mutations, truncations and deletions that inactivate both alleles of *PARK2* (Figure 1.1)[28]. Most of the mutations associated with PD impair the ligase activity of parkin. Parkin is the first ubiquitin ligase shown to bind eight Zn^{+2} ions through conserved cysteine-rich clusters which are essential for structural maintenance, an observation that rationalizes cysteine-based ARJP mutations found throughout parkin[29].

A simple model for how loss-of-function mutations in parkin could lead to PD suggests that the accumulation of one or more parkin substrates results in selective neuronal cell death (Figure 1.2)[30]. Over-expression of parkin can rescue cultured primary neuronal cells against toxicity due to expression of mutant alpha-synuclein, a substrate of parkin,

suggesting loss of E3 ligase activity is an important contributor towards ARJP etiology[31]. *S*-nitrosylation can also reduce parkin function suggesting oxidative modification of parkin as a cause for sporadic PD[32]. Homozygous loss-of-function parkin mutations are associated with a lack of Lewy bodies postulating that parkin may be involved in Lewy body formation to protect the cell from misfolded proteins[33]. This model is further strengthened by studies showing parkin poly-ubiquitylates misfolded DJ-1 via Lys-63 chains upon proteasomal inhibition resulting in trafficking along microtubules with the histone deacetylase 6 (HDAC6) adaptor protein and delivery of mutant DJ-1 to inclusion bodies[34].

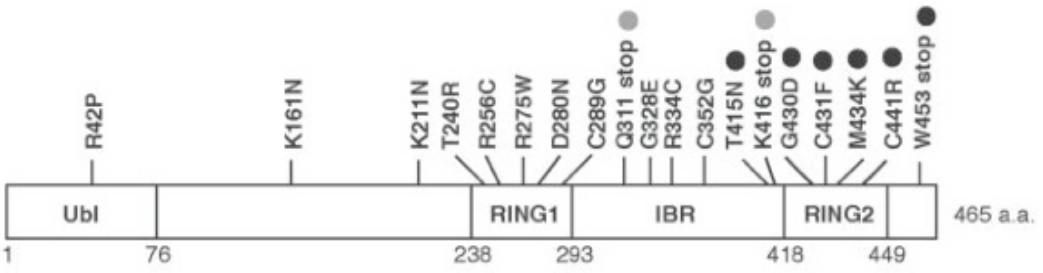


Figure 1.1 Clinical parkin mutations. The grey and black circled mutations result in inactivation of parkin ligase activity *in-vitro*. Figure adapted from reference 142.

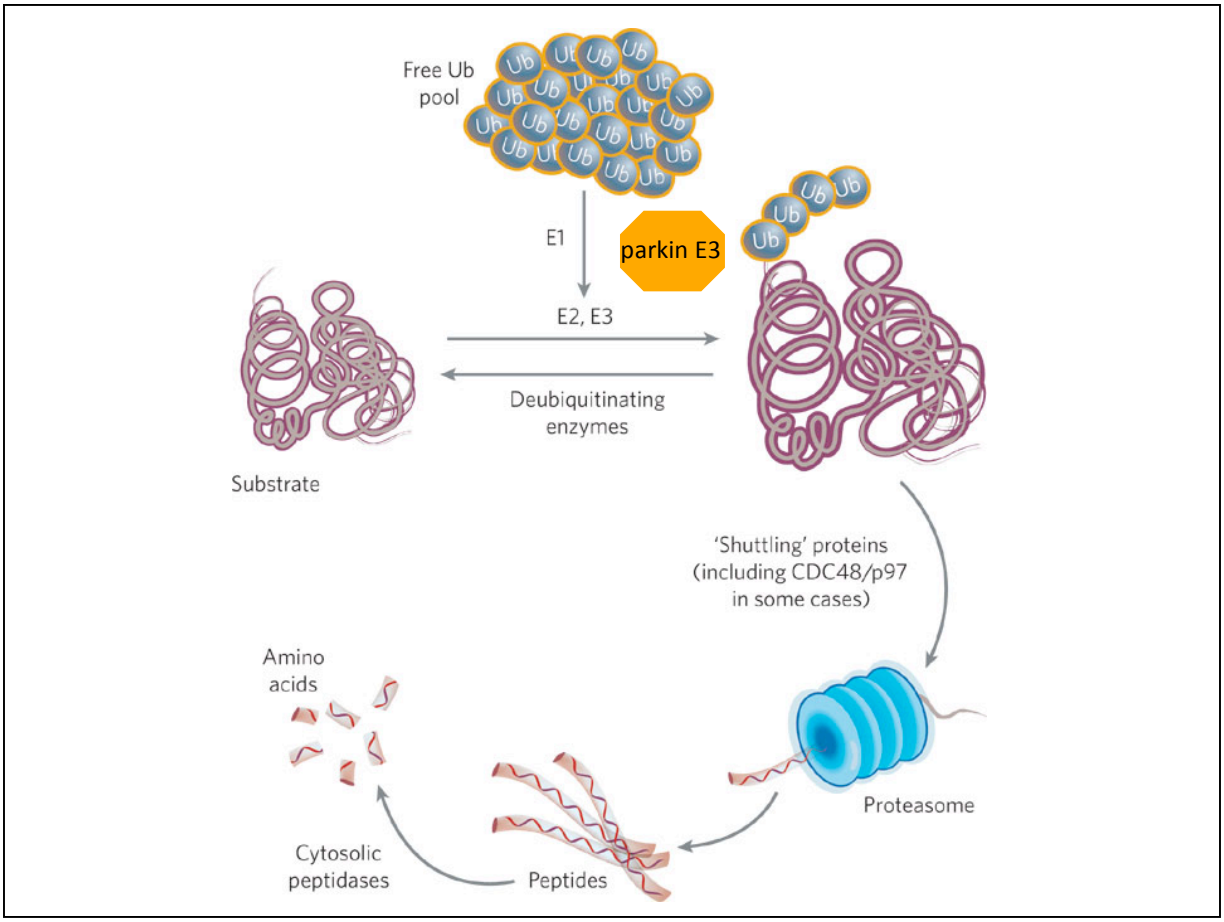


Figure 1.2 The ubiquitin-proteasome system. Parkin functions as an E3 ubiquitin ligase and conjugates ubiquitin to specific substrate proteins. Figure adapted from reference 143.

Several other parkin substrates satisfying the criteria of an authentic substrate have been identified including parkin-associated endothelin-like receptor (Pael-R), an ER membrane protein (Appendix, Table 1)[35]. Parkin is also involved in the trafficking regulation of the epidermal growth factor receptor (EGFR) through mono-ubiquitylation of the epidermal growth factor receptor substrate 15 (Eps15), thus delaying EGFR endocytosis upon EGF binding and prolonging phosphoinositide-3-kinase protein kinase B (PI3K-Akt) signaling through the activated EGFR leading to increased neuronal cell survival[36].

Parkin substrates do not have a common recognition motif so one possibility is that parkin acts globally on misfolded proteins. For example, parkin has been shown to directly ubiquitylate expanded poly-glutamine proteins *in vitro*[37] and associate with carboxyl terminus of Hsp70-interacting protein (CHIP), an E3 that targets misfolded proteins in a chaperone dependent manner[30, 38]. It will be important to determine the key substrate(s) of parkin contributing to PD upon parkin loss-of-function and how ubiquitylation may affect the substrates' activity.

1.3 Parkin and the mitochondrial connection

Mitochondrial dysfunction has also been shown to associate with PD – a deficiency in mitochondrial respiratory electron transport chain (ETC) nicotinamide adenine di-nucleotide dehydrogenase (NADH) is consistently found in PD patients[39]. Alterations in antioxidant and oxidized targets have been reported in PD linking oxidative stress with the disease[40]. This observation further links mitochondrial dysfunction to PD since the mitochondrial ETC is a source of reactive oxygen species. However, the strongest evidence of a role for mitochondria in PD is emerging from the familial PD genes *PARK2 and PINK1*. Studies in model organisms lacking parkin suggest an important role for the protein in maintenance of mitochondrial function and integrity[41, 42]. Parkin loss-of-function mutants in *Drosophila* display increased sensitivity to oxygen radical stress, dopaminergic neuron loss and degeneration of indirect flight muscles due to swollen, disordered mitochondria and fragmented cristae[43, 44]. Functional parkin is required for proper mitochondrial organization and morphology throughout spermatid development in *Drosophila*[43, 45].

Narenda *et al*. conducted an elegant study in 2008 and found parkin targets dysfunctional mitochondria for autophagic degradation[46]. They depolarized the mitochondria with the protonophore carbonyl cyanide 3-chlorophenylhydrazone (CCCP) and observed the recruitment of yellow fluorescent protein (YFP) tagged parkin to the mitochondria in several mammalian cell types resulting in mitochondrial fission and mitophagy. This study suggests parkin is an important component of mitochondria quality control.

The phosphatase and tensin homolog (PTEN)-induced putative kinase 1, PINK1, is a nuclear-expressed mitochondria-targeted kinase, in which mutations are associated with autosomal recessive PD[47]. Mitochondrial respiration is selectively impaired in the striatum of *PINK1* knock-out mice[48]. *Drosophila* expressing loss-of-function PINK1 or were PINK1 knock-out exhibited increased stress susceptibility, decreased ATP levels and mitochondrial morphological defects[49-52]. In all situations, parkin over-expression completely rescued the consequences of PINK1 deficiency whereas PINK1 over-expression did not rescue the phenotype of *PARK2* knockout *Drosophila*, and double knockouts had the same level of defect. These studies point to both proteins being involved in the same pathway to regulate mitochondria function and stability in which parkin is downstream of *PINK1*. Since parkin acts downstream, PINK1 may recruit parkin to the mitochondria since parkin is largely cytosolic[53]. One line of evidence suggesting this mechanism is in neuroblastoma cells; over-expression of parkin alone resulted in cytosolic localization, but a co-expression with PINK1 resulted in parkin translocation to mitochondria, dependent on an active PINK kinase activity[52] (PINK1 kinase domain faces the cytoplasm[54]). There is also evidence of a direct

parkin-PINK1 interaction[55]. The model of PINK1 serving as a sensor of mitochondrial stress is strengthened by the fact that two proposed PINK1 substrates are also involved in quality control: the chaperone Trap1/Hsp75[56] and the serine protease HtrA2/Omi[57]. Activation of Trap1/Hsp75, and HtrA2/Omi by PINK1 phosphorylation mediates degradation of unfolded or oxidized intermembrane space proteins, and refolding of damaged proteins and reduction of mitochondrial reactive oxygen species (ROS). A current reasonable model proposed by McBride[58] is PINK1 initiates a signaling response through phosphorylation upon mitochondrial stress resulting in the translocation of parkin to damaged mitochondria, where PINK1 and parkin promote fission of damaged mitochondria[59] (Figure 1.3). Parkin then recruits the autophagy machinery resulting in the engulfment of damaged mitochondria into autophagosomes and fusion with lysosomes. An accumulation of dysfunctional mitochondria may underlie the pathogenesis of PD upon a mutation in either gene. Ubiquitin ligase activity has been implicated in mitochondrial dynamics such as fission, fusion and trafficking[60-62]. This suggests the possibility of parkin inducing similar regulation of mitochondrial dynamics after translocation to the mitochondria. It will be pertinent to determine if parkin's ubiquitylation activity is required for the mitophagy pathway and what these mitochondrial targets are, and to determine the potential function of ubiquitylation in targeting damaged mitochondrial fragments to LC3-autophagosomes. Identification of other proteins potentially facilitating the mitophagy pathway through parkin interactions will also be an important goal.

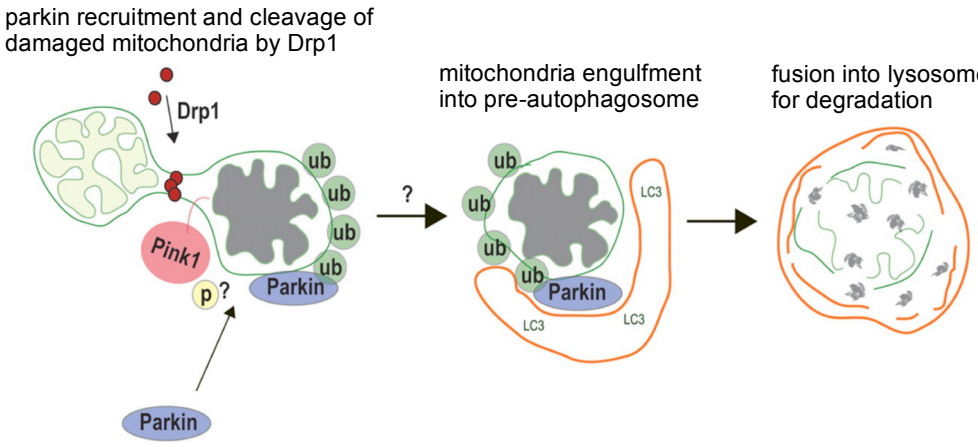


Figure 1.3 Hypothesis: parkin-mediated mitophagy. Parkin localizes to damaged mitochondria and promotes their autophagy suggesting a potential link between two genes implicated in PD, *PARK2* and *PINK1*, a mitochondrial kinase which may serve as a sensor. Drp1 is a mitochondrial fission enzyme, ub is ubiquitin and LC3 is an autophagy marker. Figure adapted from reference 58.

1.4 Methods for the purification of ubiquitylated proteins

There are likely several thousand ubiquitylated proteins *in vivo* undergoing temporal and spatial regulation, hence proteomics based methods are valuable to identify and characterize post-translationally modified proteins with ubiquitin[63, 64]. The turnover rate and labile conjugation (reversed by de-ubiquitylating enzymes) prevent the preservation of a high steady-state level of ubiquitin conjugates, which hindered initial studies[63].

The first breakthrough was achieved with peptide tags fused to ubiquitin such as hemagglutinin (HA), Myc and FLAG that are recognized by antibodies enabling affinity resin purification[65]. Biotin and poly-histidine tags have also been used[66]. Poly-histidine-tagged ubiquitin can be expressed in eukaryotic cells and enriched by immobilized metal affinity chromatography (IMAC) under denaturing conditions on nickel beads. The

advantage of purification under a stringent environment is reduction of non-specific binding to nickel resins or to ubiquitin bound on the resin[67].

1.4.1 Purification of ubiquitylated proteins from yeast

Most global strategies have been applied to the yeast system[68-71], but some studies have demonstrated feasibility in mammalian systems[72-76]. One prominent study conducted by Peng *et al*.[68] in 2003 involved knocking out all four ubiquitin genes in yeast with reintroduction of His_6-ubiquitin as the only ubiquitin source. 110 ubiquitylation sites on 72 different proteins were detected since the isopeptide bond between ubiquitin and the Lys on the substrate or another conjugated ubiquitin prevents tryptic digestion leaving a di-Gly motif. This study was the first to identify all seven Lys of ubiquitin participating in chain linkages. Tagwerker *et al*. [68] described a tandem affinity tag composed of His_6-ubiquitin and a linker region followed by a biotinylation tag (biotinylated in eukaryotic cells) *in vivo* enabling tandem IMAC, streptavidin purification. 258 proteins involved in metabolism, translation and proteolysis were identified with high confidence in their study.

1.4.2 Purification of ubiquitylated proteins from mammalian cells

Gururaja *et al*. [72] established a new approach using *in-vitro* ubiquitylation with HeLa cell extract after adding ATP along with a tagged ubiquitin. They were able to identify 22 proteasome subunits, 18 ubiquitylating enzymes, 4 ubiquitin domain proteins and 36 proteins associated with redox processes, endocytosis/vesicle trafficking, cytoskeleton, DNA damage/repair, calcium binding and mRNA splicing. Vasilescu *et al*.[75] were able to

develop a novel technique using ubiquitin antibodies coupled to protein G-agarose in order to enrich for ubiquitylated proteins from MCF-7 breast cancer cells. Over 70 proteins were identified in this screen including E3 ligases making this technique a means for analyzing endogenous ubiquitylated proteins without relying on over-expressed or tagged ubiquitin.

The 26S proteasome contains a 50 kDa integral subunit called S5a, which is capable of binding ubiquitin in chains of four or more[77]. Mammalian S5a contains two independent poly-ubiquitin binding sites of about thirty residues each, termed ubiquitin interacting motif (UIM), relying on hydrophobic interactions[78]. S5a was exploited to serve as an affinity enrichment tool for poly-ubiquitin conjugates[79]. Weekes *et al*. used S5a-affinity chromatography to purify and identify hyper-ubiquitylated proteins in dilated cardiomyopathy (DCM) and observed elevated ubiquitylation levels in explanted hearts with DCM[80]. 27 proteins in the S5a bound fractions were identified by two-dimensional gel electrophoresis only from DCM hearts.

1.5 Project Aim: Identification of novel parkin E3 ubiquitin ligase substrates and interacting proteins at the basal level and upon mitochondrial stress.

Several parkin targets and interacting proteins have been discovered (Appendix, Table 1); however, the mystery of critical parkin targets underlying PD has yet to be understood well. This project will focus on deciphering novel parkin interactors using a proteomics approach. More specifically, we will seek to identify proteins interacting with parkin upon mitochondrial stress.

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND METHODS

All chemicals were from Sigma unless otherwise stated.

2.1 Mammalian expression construct cloning

All DNA purification products were purchased from Qiagen and procedures carried out as described by the manufacturer. Oligonucleotide primers were from Integrated DNA Technologies.

2.1.1 His_8-biotin-ubiquitin

The His_8-biotin sequence tag was generated by PCR with the forward primer 5'-TTGGATCCACCATGGGACACCACCATCACCATCACCATCACCGG-3' and reverse primer 5'-TTGAATTCTTAACCACCTCTTAGTCTTAAGAC-3' against a His_6-biotin-ubiquitin Ylp yeast expression vector (ubiquitin from *S. cerevisiae*) (kind gift from Dr. Peter Kaiser, UC Irvine). PCR products were purified with the PCR purification Kit. The amplicon and pcDNA3 mammalian expression vector (kind gift from Dr. Leonard Foster, UBC) were digested with BamHI, EcoRI (New England Biolabs). Cut vector was treated with calf-intestinal phosphatase (NEB) and cut amplicon and vector were gel purified using the Gel Extraction Kit and ligated with Quick Ligase (NEB) to generate a sequence in pcDNA3. XL-1 Blue competent *E.* coli were transformed with the ligated DNA and plasmid DNA was isolated with the QIAprep Spin Miniprep Kit. To generate a human ubiquitin sequence, 5'-TATT GCTAGCGGCGGCGGCGGCGGCATGCAGATCT TCGTCAAG -3' and reverse primer 5'-TTGCTAGCTAACCACCTCTTAGTCTTAAGAC-3' was used in a PCR with ubiquitin-D77 (ubiquitin from *Homo sapiens*) pRSET and

amplicon was digested with NheI. His_6-biotin-ubiquitin pcDNA3 was digested with XbaI (compatible cohesive ends) and the fragments processed as above to generate a construct expressing His_8-biotin-ubiquitin with a human ubiquitin sequence and a five glycine residue linker between the biotin and ubiquitin. Plasmid transfection grade DNA was obtained using the QIAprep Spin Midiprep Kit.

2.1.2 His_8-ubiquitin

Yeast or human ubiquitin was amplified to generate a His_8-ubiquitin controlled under a CMV promoter. Cloning procedures were identical to Chapter 2.1.1 except the forward primer used was 5'-TTGGATCCACCATGGGAGGTAGTCATCATCACCATCATCACCA TCATGGTGGTC AGATTTTCGTCAAGACTTTG-3' and reverse primer was 5'-TTGAAT TCACCACCTCTTAGCCTTAGCAC-3'.

2.1.3 Myc-parkin

Forward primer 5'-TTGAATTCTGATAGTGTTTGTCAGGTTC-3' and reverse primer 5'-TTGCGGCCGCTACACGTCAAACCAGTG-3' was used in a PCR with FLAG-parkin pcDNA3 (parkin from *R. norvegicus*; kind gift from Dr. Edward Fon, McGill). Amplicon and N-Myc pcDNA3.1 (kind gift from Juergen Kast, UBC) were digested with EcoRI, NotI and processed as described in Chapter 2.1.1 *His_8-biotin-ubiquitin* to obtain Myc-parkin pcDNA3.1.

2.2 Cell culture techniques

All cell culture materials were from Gibco unless otherwise stated.

2.2.1 Cell maintenance

Human dopaminergic SH-SY5Y neuroblastoma were cultured in DMEM/F12 media with 10% heat-inactivated FBS and 1% penicillin/streptomycin. Cells were maintained at 37ºC in a saturated humidity atmosphere containing 95% air and 5% CO_2. Cells were passaged by aspirating media, washing once with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4), incubating cells with 1ml 0.25% trypsin/EDTA (10cm dish) for 2 min at 37ºC, adding 3ml of media to neutralize trypsin, pipet mixing cells, and diluting 1/20 into fresh media. Cells were frozen by trypsinization, centrifugation at 4ºC 1000g 3 min followed by resuspension in 4ºC 90% FBS/10% DMSO solution at $1x10^6$ cells/ml, and distribution into cryogenic tubes (Corning). Tubes were placed into the Mr. Frosty® container (Nalgene) and the container was then placed at -80ºC overnight. The next day, tubes were placed into a liquid nitrogen storage tank (Fisher Scientific).

2.2.2 DNA transfection

Cells were seeded at 40-50% confluency on a 10cm dish (BD Biosciences) one day before transfection. Media was changed three hours before transfection and a solution of 20µg plasmid DNA, 1xHBS, and 1x$CaCl_2$ at pH 7.10 (formula described by Kingston *et al*.[81]) was added to the cells for 16-20 hours. Cells were washed once with PBS and transfection media was replaced with new media and cells incubated for another 24-48 hours before further experimentation.

2.2.3 Generation of stable cell lines

Cells were transfected in 10cm plates with the FLAG-parkin and Myc-parkin constructs using calcium phosphate transfection as described above. 72 hours after transfection, cells were trypsinized and replated in serial dilutions of 1:2, 1:20, 1:200, 1:2000 in media with 500µg/ml G418 selection drug for 14 days with media exchange every 2 days containing 500µg/ml G418. Media was removed and cells were washed with PBS and 20x diluted 0.25% trypsin/EDTA in PBS was layered over plates in which cell colony diameters reached about 1mm with good spacing between each colony. Cell colonies were picked with a P1000 pipet tip and aspirated into a labeled well of a 24 multi-well dish with medium. Clones were grown to 90% confluency and then expanded to 10cm dishes with 500µg/ml G418. Clones were finally collected to assess parkin expression by western blotting and frozen stocks made according to Chapter 2.2.1. Clones with sufficient FLAG-parkin and Myc-parkin expression were further assessed by immunofluorescence to evaluate expression purity.

2.2.4 SILAC

Two cell populations were grown for six or more doublings in either SILAC DMEM/F12 media (Thermo Fisher) supplemented with 45.6 µg/ml $^{1}H_{4}$-Lys and 73.8 µg/ml $^{12}C_{6}$-Arg (light amino acids) or 45.6 µg/ml $^{2}H_{4}$-Lys and 73.8 µg/ml $^{13}C_{6}$-Arg (heavy amino acids) (Cambridge isotopes) respectively. Cells were initially seeded at 1/20 confluency on a 10cm dish, grown for one week and then expanded to at least 4x15cm dishes for a doubling of at least six times. All SILAC media was supplemented with 10% heat-

inactivated dialyzed FBS (Gibco) and 1% penicillin/streptomycin. After 6 or more doublings, cells were fully labeled and ready for further experimentation.

2.3 Preparation of biochemical purification reagents

2.3.1 GST-S5a sepharose

500 ml of BL-21 *E.* coli carrying the pGEX-5X-1-S5a sequence were grown to OD_{600} 0.8 at 30ºC and induced with 0.8mM IPTG for 4 hours and subsequently washed with ice-cold TBS 3x100 ml and pelleted by centrifugation at 4ºC 5,000 rpm in a JA-10 rotor (Beckmann). Cells were lysed on ice with buffer containing 50mM sodium phosphate pH 8.0, 300mM NaCl, 0.5% Triton X-100, 1mM EDTA, 1mM PMSF and 1xprotease inhibitor cocktail (Roche). Lysate was then incubated with 5mM $MgCl_2$ and 5µg/ml DNAse (Boehringer Mannheim) and sonicated (Misonix) with setting 3, 0.5sec ON/OFF pulses for 3x20sec with 1 min intervals on ice. Unbroken cells and debris were pelleted by centrifugation at 15,000rpm at 4ºC in a JA-20 rotor (Beckmann) and the supernatant incubated with 2ml glutathione sepharose resin (GE Healthcare) for 1 hour at 4ºC on a rotating platform (Fisher), washed with 3x10 resin volumes of lysis buffer and bound GST-S5a eluted with 1xresin volume of elution buffer (20mM glutathione in 50mM Tris pH 8.0). Glutathione sepharose resin was washed several times with lysis buffer and the binding/washing/elution steps were repeated one more time with the lysate supernatant flow-through from the first binding step to capture remaining GST-S5a. GST-S5a elution was dialyzed at RT for 1 hour three times against HEPES pH 7.5 in a 12-14,000 MWCO membrane (Spectrum) to remove free glutathione. GST-S5a was then coupled to Affigel 15 activated sepharose resin (Bio-Rad). Affigel was aliquoted

into a 10ml chromatography column (Bio-Rad) (1ml of Affigel used per 6mg of protein) and washed with ice-cold double distilled water (ddH_2O). Dialyzed GST-S5a was added to Affigel and rotated at 4ºC for 4 hours. 100mM ethanolamine pH 8.0 final concentration, was added to the protein solution-Affigel mixture and incubated for 1 hour at RT to quench unreacted N-hydroxysuccinimide esters on the Affigel. GST-S5a coupled Affigel was washed with 3x10 resin volumes of HEPES pH 7.5 and stored with one resin volume of HEPES pH 7.5 with 0.02% NaN_3 at 4ºC.

2.3.2 Anti-Myc protein G Dynabeads

1mg of 9E10 anti-Myc antibody (produced by BRC Antibody Facility, UBC) was added to 1ml of protein G Dynabeads (Invitrogen) and the coupling reaction was performed according to protein G Dynabeads manufacturer's instructions.

2.4 Protein purification techniques

2.4.1 Tandem affinity purification of ubiquitylated proteins from His_8-biotin-ubiquitin expressing cells

The purification scheme is depicted in Figure 2.1. $2x10^7$ cells were transfected with 35µg HBU pcDNA3 construct on 15cm diameter plates as described in Chapter 2.2. 41 hours after transfection, half the plates were treated with 20µM MG132 and the other half with DMSO for 7 hours. 48 hours after transfection, cells attached to plate were washed once with PBS, trypsinized and transferred into 15ml ice cold conical tubes (Sarstedt) and pelleted using a tabletop centrifuge at 1000g, 4ºC for 3 min (Eppendorf). Cells pellets were washed twice with ice cold PBS and lysed at RT with 2ml Buffer A

(8M urea, 300mM NaCl, 50mM sodium phosphate pH 8.0, 0.5% Nonidet P-40, 1mM PMSF, 10mM chloroacetamide), passaged ten times through a 27G1/2 needle attached to a 1ml syringe (Fisher Scientific) and centrifuged at 15000g at 20ºC for 30 min to clear the lysate. Protein concentrations in the two lysates were measured with the DC Protein Assay Kit (Bio-Rad) and Buffer A added to normalize total protein quantities and volume. Imidazole was added to the supernatant at 10mM and lysate was incubated overnight with MagneHis beads (Promega) at 12µl beads slurry/mg protein lysate. A magnetic rack was used to separate the magnetic beads from the liquid phase. Beads were washed with Buffer A twice and once with Buffer A containing 10mM imidazole, and bound proteins eluted with 2x120µl Buffer B (8M urea, 200mM NaCl, 50mM sodium phosphate, 2% SDS, 10mM EDTA, 100mM Tris, 500mM imidazole pH 8.0). Eluate was then incubated with Dynabeads MyOne Streptavidin (Invitrogen) overnight at 7 µl beads slurry per mg of initial protein lysate and then beads were washed twice with Buffer C (8M urea, 200mM NaCl, 2% SDS, 100mM Tris pH 8.0); once with Buffer C containing 100mM β-mercaptoethanol (to cleave E1, E2 and E3 enzymes from ubiquitin); twice with Buffer D (8M urea, 1.2M NaCl, 0.2% SDS, 100mM Tris, 10% ethanol, 10% isopropanol pH 8.0) to remove hydrophobically bound material; and three times with Buffer E (8M urea, 50mM HEPES).

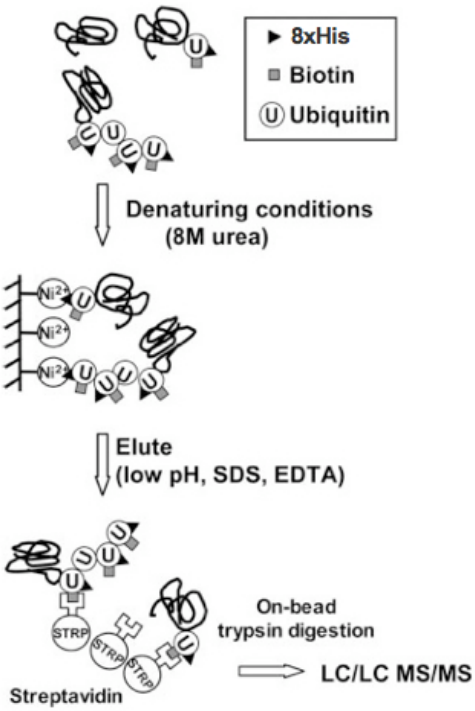


Figure 2.1 Tandem affinity purification of His_8-biotin-ubiquitin. Figure adapted from reference 23.

2.4.2 Purification of SILAC labeled ubiquitylated proteins using GST-S5a sepharose

Two populations of light and heavy labeled cells (prepared as described in Chapter 2.2.4 were treated with DMSO and 20μM MG132 for 7 hours respectively ($2x10^7$ cells per condition). Cells were collected as described in Chapter 2.4.1 and lysed on ice, each with 2ml lysis buffer (50mM Tris pH 8.0, 150mM NaCl, 0.5% Triton X-100, 25mM chloroacetamide, 1mM 1,10-phenanthroline, 1mM PMSF and 1xprotease inhibitor cocktail mix), needle passaged and centrifuged at 16200g for 15 min at 4ºC in a microcentrifuge. Proteins concentrations were measured, normalized and cell lysates from the two cell populations were mixed together. Lysate supernatant was pre-cleared with 100μl ethanolamine quenched Affigel 15 sepharose (pre-washed with lysis buffer)

for 1 hour at 4ºC and then incubated with 50 µg GST-S5a sepharose per mg of lysate for 4 hours at 4ºC. Resin was washed with 3x10 resin volumes of lysis buffer and bound proteins eluted with twice with 1 resin volume of 8M urea, 50mM HEPES pH 8.0.

2.4.3 Purification of FLAG-parkin and its interactors from SILAC cells expressing FLAG-parkin versus control

Two populations of cells, in which only one stably expressed FLAG-parkin and the other did not were labeled with light and heavy amino acids respectively. Cells were collected on ice and lysed separately with 2ml of lysis buffer (50mM HEPES pH 7.5, 0.5% Triton X-100, 10% glycerol, 70 mM KOAc, 0.2mM EDTA, 5mM chloroacetamide, 1mM PMSF, and 1xprotease inhibitor cocktail mix). Lysates were passaged, centrifuged and protein concentrations normalized. 6µl of anti-FLAG M2 affinity resin were used per mg of protein lysate and the mixture was incubated at 4ºC for 4 hours on a rocking platform. M2 resin was pelleted by centrifugation at 5000g for 2 min after completed incubation and pooled together. Resin was washed sequentially with 10 resin volumes of lysis buffer, twice with 10 resin volumes of 50mM Tris pH 7.5, 150mM NaCl, 1% Tween (potentially releasing weak interacting proteins) and twice with 10 resin volumes of 50mM Tris pH 7.5, 500mM NaCl (also weak interactor elution). FLAG-parkin and more tightly associated proteins were eluted twice with 1 resin volume of sample buffer containing 50mM Tris pH 6.8, 2% SDS, 10% glycerol (strong interactor elution).
For the CCCP experiment, two SILAC cell populations expressing FLAG-parkin were treated with DMSO or with 10µM CCCP for 3 hours prior to cell collection. All other procedures are identical to the non-CCCP SILAC experiment.

2.4.4 Formaldehyde cross-linking optimization

Paraformaldehyde was dissolved in PBS at 0.00%, 0.20%, 0.40%, 0.60%, 0.80% with gentle heating. SH-SY5Y stably expressing Myc-parkin were grown on 6cm dishes to 90% confluency, washed with PBS and various concentrations of formaldehyde solution were added and plates rocked on a shaker (Fisher Scientific) for 10 min. Cross-linking reaction was quenched with addition of 1.25M glycine pH 7.4 to a final concentration of 125mM for a 5 min incubation period. Cells were washed twice with PBS, trypsinized, collected in ice-cold tubes and pelleted. Cells were lysed with Buffer F (50mM Tris pH 7.5, 150mM NaCl, 10% glycerol, 1% Nonidet P-40, 5mM EDTA, 1mM PMSF, 1xprotease inhibitors). Protein loading was normalized for SDS-PAGE and parkin western blotting to determine the optimum concentration of formaldehyde treatment.

2.4.5 Purification of cross-linked Myc-parkin

Two cell populations ($6x10^6$ cells per population) where one stably expressed Myc-parkin, were treated with 0.40% formaldehyde solution, the optimal determined concentration (Figure 3.8A). Cells were lysed with Buffer F and protein concentrations normalized. Lysates were added to pre-equilibrated 9E10-coupled Dynabeads at 50µl beads slurry per mg of protein and incubated for 2 hours at 4ºC. Beads were washed three times with 10 bead volumes of Buffer F and bound proteins eluted with 2 resin volumes of sample buffer (2% SDS, 50mM Tris pH 6.8, 10% glycerol). Formaldehyde cross-links were reversed by heating the protein mixture at 95ºC for 10 min. Parkin immunoblotting revealed much of the Myc-parkin eluted in the 0.5% SDS fraction

(Figure 3.8B), thus the material was precipitated using chloroform and methanol as previously described[82] and processed further.

2.5 Protein detection techniques

2.5.1 Coomassie and silver staining

Cell lysates prepared as described in Chapter 2.4 were reconstituted in 3x Laemmli sample buffer (150mM Tris pH 6.8, 6% SDS, 30% glycerol, 6% β-mercaptoethanol, bromophenol blue) and loaded on pre-cast 4-20% gradient polyacrylamide gels (Pierce). SDS-PAGE was performed at 150 volts for 45 min on a Novex electrophoresis device (Invitrogen, Bio-Rad).

For coomassie staining, gels were incubated in 0.2% coomassie blue R350 solution with 40% methanol and destained with 40% methanol, 10% acetic acid solution for visualization of proteins in gels.

For silver staining, gels were fixed with 30% ethanol, 10% acetic acid for 3x20 min and incubated in 0.1% sodium thiosulphate dissolved in solution A (30% ethanol, 10mM sodium acetate, pH 6 with acetic acid) for 1 hour followed by 3x20 min ddH_2O washes. Gels were then incubated in 0.1% silver nitrate, 0.01% formaldehyde solution for 1 hour, washed and developed with 2.5% sodium carbonate 0.02% formaldehyde solution for 5 min and quenched with 1% acetic acid.

2.5.2 Western blotting

After SDS-PAGE, proteins were transferred onto 0.5µm nitrocellulose membranes (Bio-Rad) using the wet-transfer technique in Towbin buffer (25mM Tris, 192mM glycine,

20% methanol, pH 8.3) and a transfer device set at constant 0.50 ampere for 2 hours at 4ºC (Hoefer). For ubiquitin immunoblotting, membranes were microwaved in ddH_2O for 15 min prior to blocking to unfold mono-ubiquitin. Membranes were blocked with blocking buffer (3% skim milk powder dissolved in TBS, 0.1% Tween-20) for 30 min followed by primary antibody incubation dissolved in blocking buffer (P4G7, ubiquitin antibody 1:1000 (Santa Cruz) or PRK8, parkin antibody 1:1000 (Santa Cruz)) for 1 hour at RT. Membranes were washed 2x5 min with blocking buffer and then incubated with Goat anti-mouse HRP 1:3000 (Bio-Rad) for 45 min. Membranes were washed 5 min successively with blocking buffer, blocking buffer without milk, and TBS followed by chemiluminescence substrate reagent incubation (Perkin Elmer) and development on Biomax film (Kodak). ExtrAvidin® peroxidase was used to detect biotin according to manufacturer's instructions.

2.5.3 Immunofluorescence

Cells were seeded at 25% confluency on coverslips pre-treated with 1M HCl (Fisher Scientific). Media was removed 48 hours later and cells were gently washed once with PBS before incubation with 3% PFA in PBS for 15 min at RT. Cells were permeabilized with 0.5% Triton X-100 for 2 min, PBS washed, and blocked with 1% BSA (Roche) in PBS for 10 min. PRK8 antibody was diluted in 3% BSA at 1:1000 and incubated for 45 min with coverslips followed by 3x PBS washes. Alexa Fluor 488 Goat anti-mouse secondary antibody (Invitrogen) diluted 1:2000 in 3% BSA was then added for 45min. Coverslips were mounted on glass slides (Fisher scientific) with ProLong Gold Antifade reagent (Invitrogen). For mitochondria staining, cells were incubated with 400nM

MitoTracker Orange CMTMRos (kind gift from Dr. Leonard Foster, UBC) for 45 min prior to PFA fixation. Slides were analyzed on a Carl Zeiss Observer.Z1 microscope with a Colibri 62HE filter set and Axiovision Rel. 4.7 software. Forty z-stack images separated by 0.2µm on the vertical axis were captured per image panel and inverse filter deconvolution was performed to increase image quality.

2.6 Protein preparation for mass spectrometry

2.6.1 Trichloroacetic acid protein precipitation

Protein mixtures from the purified fractions described in Chapter 2.4.1, 2.4.5 were subjected to precipitation in order to remove detergents and concentrate proteins for mass spectrometry compatibility. TCA at a final concentration of 30% was added to protein mixtures and incubated overnight at 4ºC. Precipitants were pelleted by centrifugation at 4ºC for 15 min and washed twice with 100% ice cold acetone. Pellets were resuspended with 40µl 50mM HEPES pH 8.0 and 8M urea and the pH adjusted to 8.0.

2.6.2 On-bead trypsin digestion of His_8-biotin-ubiquitin conjugates

His_8-biotin-ubiquitin conjugates bound to streptavidin beads following tandem affinity purification as described in Chapter 2.4.1 were resuspended in 125µl 8M urea, 50mM HEPES. Proteins were reduced with 3µM TCEP for 20 min and alkylated with 55mM chloroacetamide for 30 min successively in the dark at 1400rpm on a Thermomixer set at 25ºC (Eppendorf). 0.1µg of endo-LysC (Roche) was added and digestion proceeded for 3 hours at 37ºC with intermittent 5 sec ON/25 sec OFF on a Thermomixer. Protein

solution was diluted 4 fold with dropwise addition of 100mM Tris pH 8.5 and addition of 1mM $CaCl_2$. 1.5µg sequencing grade trypsin (Roche) was then added and digestion proceeded similarly for 16 hours until digestion termination and peptide acidification with the addition of 10% FA. Residual peptides on the beads were extracted with 3x40 µl 0.1% FA, 25% ACN and the three extracts were pooled and concentrated with a vacuum centrifuge until ~20µl (2 hours) (Eppendorf). Peptides were purified and concentrated on a STAGE tip[83-85] with one C_{18} column and eluted with 80% ACN, 0.5% acetic acid into 96 well plates; dried in vacuum concentrator; and resuspended in sample buffer containing 1% TFA, 0.5% acetic acid and 3% ACN.

2.6.3 In-gel digestion of GST-S5a bound proteins

TCA precipitant derived from Chapter 2.4.2 was resuspended with 8M urea, 50mM HEPES pH 8.0 and reconstituted with 3xLaemmli buffer and loaded onto a 4-20% gradient polyacrylamide gel. Bromophenol blue marker dye was run for halfway down the gel (150 volts, 20 min). Gel bands were excised with a scapel corresponding to molecular weight ranges of >200, 100-200, 50-100, <50 kDa and pieces were further minced into approximately $1mm^3$ cubes to enable improved reagent exchange. Gel samples were subjected to in-gel digestion as previously described[86] without gel staining to increase peptide identification[87]. Peptides from the four gel slices were purified and concentrated on a STAGE tip with one C_{18} column each and eluted with 80% ACN, 0.5% acetic acid into 96 well plates; dried in a vacuum concentrator (Eppendorf); and resuspended in sample buffer containing 1% TFA, 0.5% acetic acid and 3% ACN.

2.6.4 In-solution digestion of FLAG-parkin immunoprecipitate

Weak and strong interactor TCA precipitants derived from Chapter 2.4.3 were resuspended with 40µl 8M urea, 50mM HEPES and pH adjusted to 8.0. Proteins were reduced, alkylated and digested as in Chapter 2.6.2, with the exception of endo-LysC. After 10% FA addition to quench the digestion, tryptic peptides were concentrated on STAGE tips containing a C_{18} column on top of an SCX column. Peptides were separated into 4 fractions with increasing concentrations of NH_4OAc at 100mM, 350mM, 500mM and 1M to elute the peptides from the SCX column. Purified fractions were subjected to another C_{18} STAGE tip processing each and eluted with 80% ACN, 0.5% acetic acid into 96 well plates; dried in vacuum concentrator (Eppendorf); and resuspended in sample buffer containing 1% TFA, 0.5% acetic acid and 3% ACN.

2.6.5 In-solution digestion of Myc-parkin immunoprecipitant

Chloroform-methanol precipitated proteins from Chapter 2.4.5 were processed as described in Chapter 2.6.2. After 10% FA addition to quench the digestion, tryptic peptides were purified and concentrated on STAGE tips containing a C_{18} column; eluted with 80% ACN, 0.5% acetic acid into 96 well plates; dried in vacuum concentrator (Eppendorf); and resuspended in sample buffer containing 1% TFA, 0.5% acetic acid and 3% ACN.

2.7 Liquid chromatography and tandem mass spectrometry

Peptide samples were analyzed on an LTQ-Orbitrap (Thermo Electron). The LTQ-Orbitrap system was on-line coupled to Agilent 1100 Series nanoflow HPLC instruments

using nanospray ionization sources (Proxeon Biosystems) containing columns packed into 15cm long, 75μm inner diameter fused silica emitters (8μm diameter opening, pulled on a P-2000 laser puller from Sutter instruments) using 3μm diameter ReproSil Pur C_{18}-AQ beads (Dr. Maisch). Buffer A (0.5% acetic acid) and Buffer B (0.5% acetic acid, 80% ACN). Gradients were run from 6% B to 30% B complemented with Buffer A over 54 min, then 30% to 80% B over the next 5 min, held at 80% B for 10 min, and then dropped to 6% B for another 10min to recondition the column. The HPLC system included Agilent 1100 series Degaser, Nano-flow pump, Autosampler and Thermostat. The termostat temperature was set at 6°C. The LTQ-Orbitrap was programmed to capture a full-range scan at 60,000 resolution from 350 to 1500 m/z in the Orbitrap and to simultaneously fragment the top five peptide ions in each cycle in the LTQ (minimum intensity 1000 counts, MS/MS starting after 20 min). Parent ions were then excluded from MS/MS for the next 30 sec. Singly charged ions were excluded since in ESI mode peptides usually carry multiple charges. An exclusion peptide list for anti-FLAG immunoglobulin and GST-S5a was programmed for anti-FLAG immunoprecipitated proteins and GST-S5a purified proteins respectively (Appendix, Table 2). The Orbitrap was continuously recalibrated using lock-mass function[88].

2.8 Analysis of mass spectrometry data

Raw MS spectra files were processed to Mascot generic format (mgf) using DTA Supercharge v.1.37 in order to correct spectrum file monoisotopic peak and charge states. Peak lists were searched against the International Protein Index Human 3.60 database (also Rat 3.60 database for parkin) compiled by the European Bioinformatics

Institute using Mascot. All searches included the following settings: trypsin/P cleavage specificity with up to two missed cleavages, cysteine carbamidomethyl fixed modification, ±20-ppm peptide tolerance, ±0.6-Da MS/MS tolerance, ESI-TRAP scoring scheme, up to two ^{13}C, and peptide charges of +1, +2 and +3. Each sample had additional variable modifications (Table 2.1). Assigned peak lists from SCX fractions were pooled using Proteus software (Genologics). After comparing the number of identified peptides from the decoy database (protein sequences in database reversed) to the real database generated by Mascot for all samples, the minimum peptide threshold score for a false positive rate ≤1% was 25. MSQuant version 1.5 was used for parsing Mascot files and iterative mass recalibration. Peptides originating from keratin were manually removed from all data sets. Peptides originating from immunoglobulin chains were manually removed from FLAG-parkin immunoprecipitate and peptides from GST-S5a were removed from GST-S5a resin purified material.

Table 2.1 Mascot variable modification settings for pull-down mascot generic format files.

Mascot variable modification	Immunoprecipitated Sample		
	His_8-biotin-ubiquitin/Myc-parkin	S5a	FLAG-parkin
protein N-terminal acetylation	yes	no	yes
Asn/Gln deamidation	yes	no	yes
Met oxidation	yes	yes	yes
Lys-GlyGly isopeptide linkage	yes	yes	no
Lys ($^2H_4$)-GlyGly	no	yes	no
Lys ($^2H_4$)	no	yes	yes
Arg ($^{13}C_6$)	no	yes	yes

2.8.1 Ingenuity pathway analysis

Protein SILAC ratio data was subjected to signaling pathway analysis with Ingenuity Pathway Analysis software v.8 (Ingenuity). The threshold for enrichment or de-enrichment significance was set at a $\log_2$(heavy/light) absolute value = 0.57 for the S5a SILAC experiment.

CHAPTER 3

RESULTS

3.1 Use of His_8-biotin-ubiquitin as a tag-based approach for isolating ubiquitin conjugates.

We first sought to determine whether we could identify parkin substrates by enriching for ubiquitylated proteins. The rationale is parkin substrates should be purified to a greater extent from cells over-expressing parkin relative to cells with lower parkin expression due to increased parkin-mediated ubiquitylation. SH-SY5Y human neuroblastoma cells were chosen because they are brain cells with endogenous parkin expression. In addition, dopamine-producing cells are typically affected in Parkinson's disease, making them relevant for studying PD etiology[89]. We chose the His_6-biotin-ubiquitin tandem purification approach described by Tagwerker *et al*.[69], because they identified the largest number of proteins by mass spectrometry at 258 compared to other studies (see Chapter 1.4). Another advantage of their method is that it enables purification under denaturing conditions resulting in lower background. The only caveat is the His_6-biotin-ubiquitin system had only been developed in yeast when we initiated the project, and required some modification to be used in mammalian cells.

We cloned a His_8-biotin-ubiquitin, termed HBU, in the pcDNA3 mammalian expression vector. The construct contained a human ubiquitin sequence preceded by an octa-histidine tag enabling a first step IMAC purification with urea and SDS to reduce non-specific binding. The second tag is a 75 amino acid long sequence which is biotinylated *in vivo* by endogenous biotin ligase enabling a second affinity capture step with streptavidin beads[90]. The extension of His_6 to His_8 has no effect on ubiquitin conjugation (Mayor, T.; personal communication). Five glycine residues were inserted between the biotin sequence and ubiquitin to serve as a linker region. A control construct as a

byproduct of the cloning of HBU was His_8-biotin, termed HB, in which a stop codon was in frame after the biotinylation sequence tag. After cellular transfection with constructs, free HBU was detectable with the P4G7 anti-ubiquitin antibody in lysates of cells transfected with HBU in contrast to HB transfected cells (Figure 3.1A). The expression level of free HBU relative to free ubiquitin was about a 2:3 ratio. We confirmed that HBU was conjugated to substrate by using horseradish peroxidase coupled to avidin to blot for biotin, albeit not with the same efficiency as wild-type ubiquitin (Figure 3.1B, compare ratio of mono-ubiquitin to poly-ubiquitin versus mono-HBU and poly-HBU in 3.1A). In contrast, the product of the control HB construct was not conjugated.

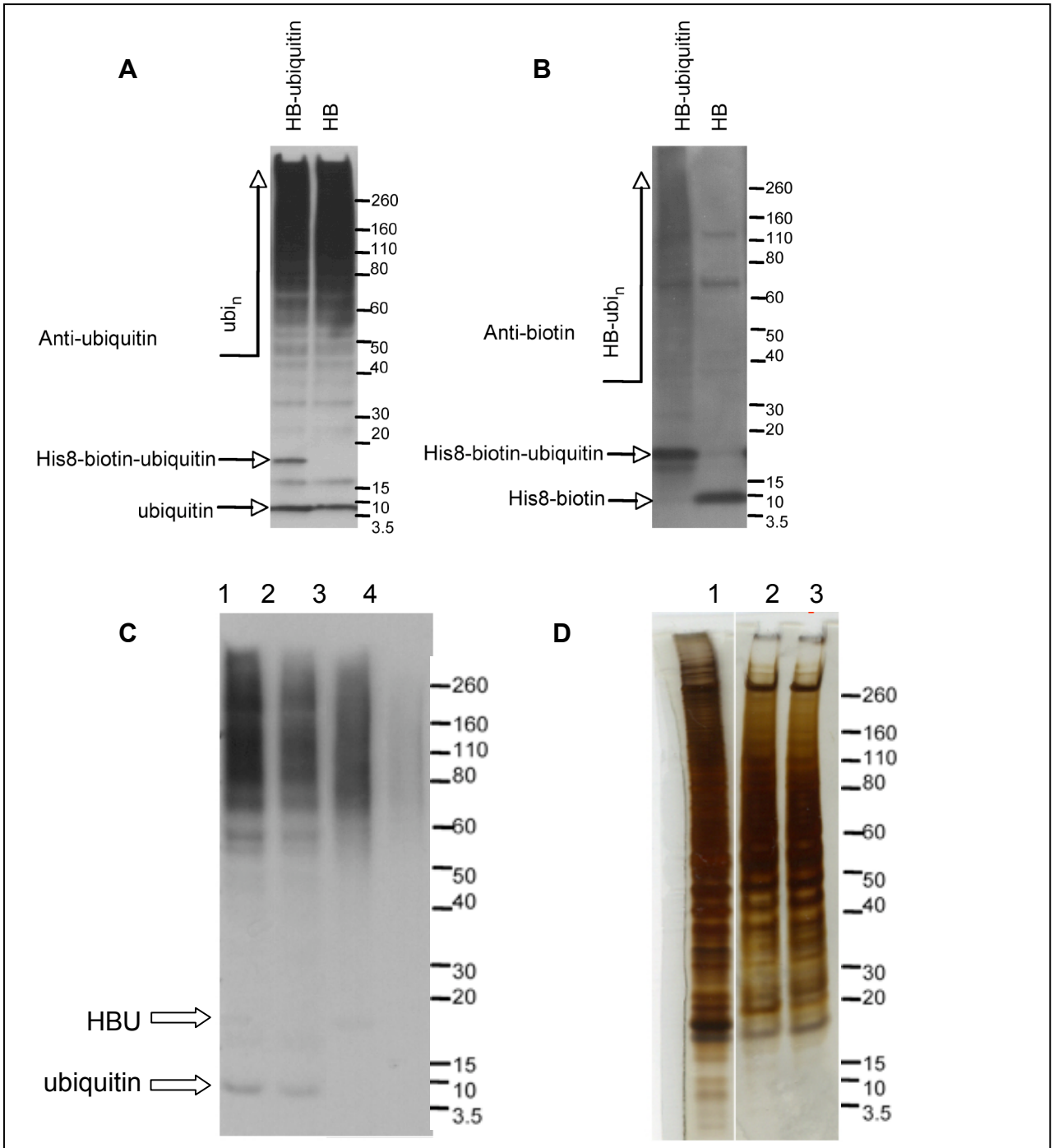


Figure 3.1 Tandem affinity purification of His_8-biotin-ubiquitin conjugates. Cells transfected with HBU or HB control were lysed and subjected to SDS-PAGE and immunoblotting for ubiquitin (A) and biotin (B) using a P4G7 anti-ubiquitin antibody and ExtrAvidin respectively. Marker positions in kDa are indicated. Aliquots at different stages of the tandem purification scheme were subjected to SDS-PAGE and immunoblotting with P4G7 (C) or silver staining (D). In C, the corresponding aliquots were assessed: lane 1 (1x) input, lane 2 (1x) Ni^{2+} resin unbound, lane 3 (3x) Ni^{2+} resin elution, lane 4 (3x) streptavidin beads unbound. In D, the corresponding aliquots were assessed: lane 1 (0.1x) input, lane 2 (10x) Ni^{2+} resin elution, lane 3 (10x) streptavidin beads unbound. There is no streptavidin elution lane because the interaction between biotin and streptavidin is nearly the strength of a covalent bond and no bound material was recovered (data not shown).

3.2 Identification of ubiquitylated proteins using the HBU system in neuronal cells.

We next sought to purify HBU conjugates to assess whether ubiquitylated substrates and known parkin targets could be identified by MS using this approach. We performed a tandem purification to enrich for ubiquitylated proteins from cells transfected with HBU. Cells were lysed in 8M urea with SDS and incubated with Ni^{2+} beads. After elution, ubiquitylated proteins were further enriched using streptavidin beads. Stringent washes with detergent and alcohol were performed to remove non-specific hydrophobically bound proteins. The Ni^{2+} pull-down efficiency of ubiquitylated conjugates was about 20% (Figure 3.1C compare ubiquitin signal from elution to input lane). The depletion of ubiquitin signals in the streptavidin-unbound lane suggested that almost all of the HBU conjugates in the Ni^{2+} eluate were bound to the streptavidin beads (Figure 3.1, lane 4). This second purification step using streptavidin was important as revealed by the amount of non-specific proteins present in the streptavidin-unbound lane (Figure 3.1D, lane 3).

Based on these encouraging results, we decided to identify by MS which proteins were purified by our method. We compared two samples ($1x10^7$ cells each) that were transfected with HBU and were either proteasome inhibited with MG132 or treated with the DMSO control solvent. Whether the HBU approach would be sufficient for the study of parkin ligase substrates depends on the number of proteins identified. Proteins were digested by sequencing grade trypsin directly on streptavidin beads and the whole peptide mixture was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using an LTQ-Orbitrap as previously described[91]. Spectra were then

processed and analyzed by Mascot. We identified 20 proteins in the absence of proteasome inhibition and a larger number of proteins upon MG132 addition.

We identified a total of 43 proteins by combining both samples with a minimum number of 2 peptides when the MS data list was searched with Mascot (Table 3.1). The top protein hit identified was ubiquitin suggesting the method is specific for enriching ubiquitin and its conjugates. The relatively low number of proteins identified compared to other studies[69, 72-76] was not encouraging. In addition, most proteins were very abundant in the cells (e.g. ribosomes[92]) indicating the method was unlikely to pick low abundant proteins such as potential parkin substrates. In order to scale up, we generated a stable cell population expressing HBU by geneticin selection. We successfully established a stable population expressing HBU; however, the expression level was much lower compared to transiently transfected cells and purification of conjugated proteins was not efficient (data not shown). We also became aware that the HB tag was preventing ubiquitylation of some substrates (Deshaies, R.J.; personal communication) and therefore, we decided to test an alternative approach.

Table 3.1 HBU enriched proteins – number of unique peptides. A subset of the 41 proteins with a minimum of 2 peptides are shown. # beside the protein name represents a known parkin ubiquitin ligase substrate[93].

Proteins identified from HBU pull-down in the presence of DMSO or MG132	unique peptides (DMSO)	unique peptides (MG132)
ubiquitin	7	10
ribosomal protein L19	2	6
ribosomal protein L3	2	4
tubulin α-1A chain#	3	4
heat shock protein 71	3	4
heat shock protein 90	5	4
ribosomal protein L15	0	3
S5a proteasome receptor	0	3
serine/threonine-protein kinase PRP4	0	3
actin	0	3
ribosomal protein L13	0	2
sequestrome-1	0	2
putative RNA-binding protein Luc7-like 1	0	2
NEDD8	0	2
catenin beta-1	0	2
neutral amino acid transporter	0	2
N-Myc proto-oncogene protein	0	2
DNA-directed RNA polymerase II subunit RPB2	0	2
XLas-1 guanine nucleotide-binding protein	0	2
hypoxia-inducible factor 1 alpha	0	2
14-3-3 zeta/delta protein	0	2
ADP/ATP translocase	0	2
3-hydroxy-3-methylglutaryl-coenzyme A reductase	0	2
DNA-damage-inducible transcript 4 protein	0	2
tubulin β chain#	0	2
histone H2A	4	2

3.3 Preparation of an alternative ubiquitin conjugate purification strategy using S5a.

We generated recombinant GST-S5a coupled to sepharose for S5a affinity chromatography as an alternative means to enrich for ubiquitin conjugates. S5a is a proteasome subunit that can bind to poly-ubiquitin and was previously used to enrich for ubiquitylated substrates[79]. GST-S5a was expressed in BL21 bacteria after IPTG induction (Figure 3.2B, lane 2). We performed a sequential pull-down until full depletion of recombinant GST-S5a (Figure 3.2A, lane 4). Pure GST-S5a was eluted from the glutathione sepharose resin (Figure 3.2A, lanes 5 to 8). We then dialyzed the GST-S5a solution to remove glutathione and successfully coupled the protein to activated sepharose resin with an efficiency of about 90% (Figure 3.2A, lanes 13 and 12; the flow-through signal was less than the 25% input signal).

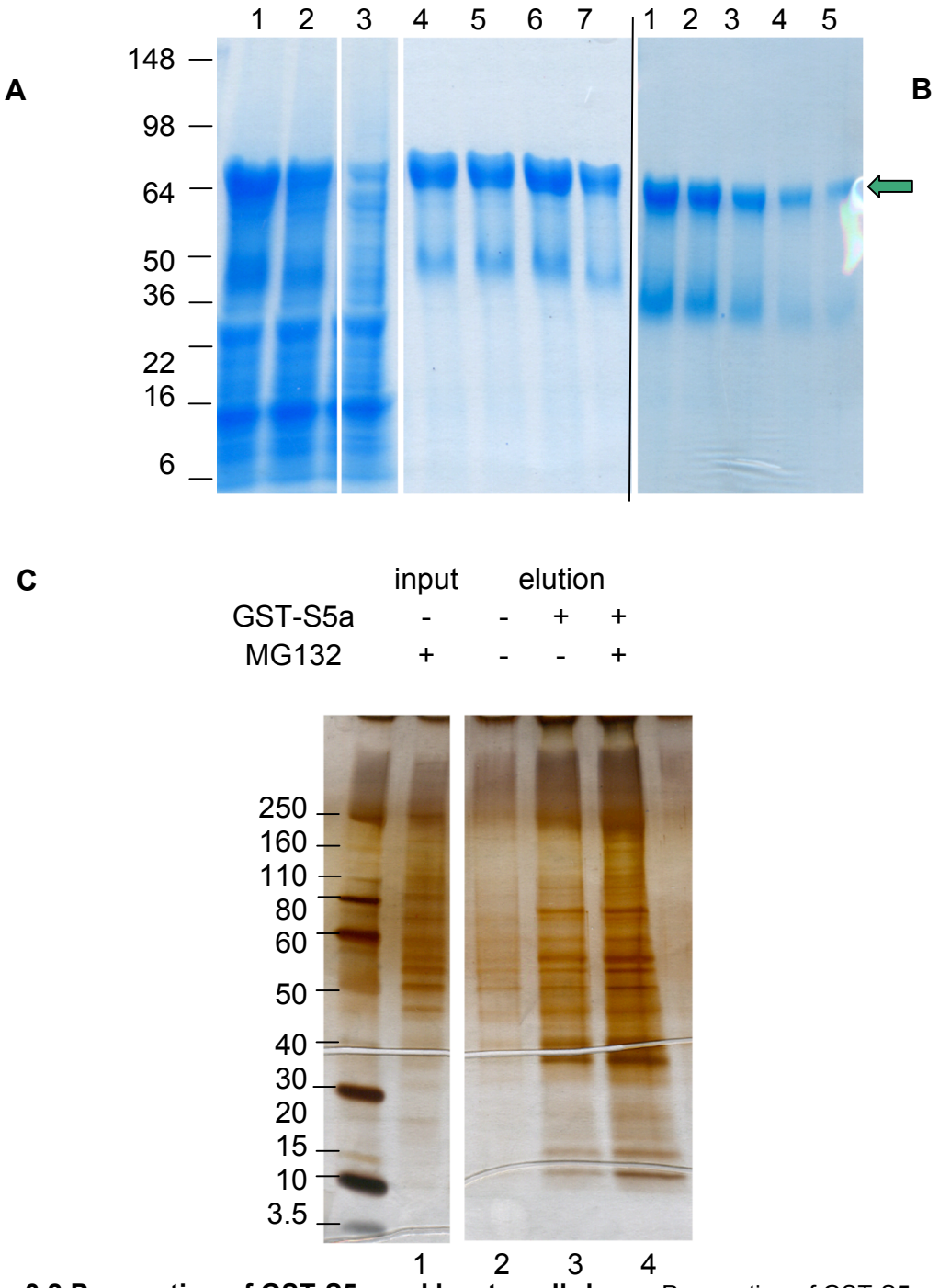


Figure 3.2 Preparation of GST-S5a and lysate pull-down. Preparation of GST-S5a sepharose (A). Expression and purification of GST-S5a was assessed by SDS-PAGE followed by coomassie staining: Protein ladder in kDa is indicated on the left, lane 1 input, lane 2 first flow-through, lane 3 second flow-through, lane 4 and 5 first two elutions, lane 6 and 7 last two elutions. Coupling of GST-S5a: lane 9 input 1/2400, lane 10 input 0.75/2400, lane 11 input 0.50/2400, lane 12 input 0.25/2400, lane 13 affigel flow-through 1/2400. The arrow marks GST-S5a at 67 kDa. GST-S5a lysate pull-down was assessed by SDS-PAGE followed by silver staining (C). SH-SY5Y were treated with DMSO or MG132, lysed and subjected to pull-down with GST-S5a resin. Quenched Affigel 15 was used as resin to assess background binding in lane 3. Molecular weight marker in kDa are indicated on the left, lane 1 is lysate input, lanes 2-4 are purified protein fractions from the chromatography resin derived from cells treated as indicated.

3.4 Quantitative proteomic analysis of S5a affinity purified proteins

We assessed the amount of coverage for the ubiquitin proteome after S5a purification was used to enrich for ubiquitin conjugates. We incubated cell lysate with the GST-S5a sepharose followed by high salt wash buffer (1 M NaCl), resulting in low background with the control resin (Figure 3.2B, lane 3). We then applied MG132 and noticed an overall increase of the bands associated to S5a. Ubiquitin immunoblotting revealed a pull-down efficiency of ubiquitin conjugates of about 50% (data not shown). Free ubiquitin was not enriched since S5a has poor affinity for monomeric ubiquitin.

In order to determine whether this approach was suitable for ubiquitin proteome studies, we then performed stable isotope labeling with amino acids in cell culture[94] (SILAC) to identify ubiquitylated protein accumulation upon proteasome inhibition. S5a had been previously used, but without a careful control[79]. Light and heavy labeled cells were treated with DMSO and MG132 respectively. After the enrichment with S5a, the protein mixture was separated by SDS-PAGE and four gel pieces corresponding to >200 kDa, 100-200 kDa, 50-100 kDa and <50 kDa were excised and subjected to in-gel trypsin digestion[86] followed by LC-MS/MS. After pooling the four fractions using Proteus, we identified 310 proteins (false positive rate ≤1%) by Mascot. About 20% of the identified quantified by MSQuant were specifically enriched from MG132 treated cells (Figure 3.3, $\log_2$(heavy/light) ≥0.57; Table 3.2). We chose to use 0.57 as the cut-off for enrichment upon proteasomal inhibition because it is the point where ratios start to deviate higher than the linear trend relationship of $\log_2$ratio versus protein number as observed between proteins 75 and 290 (Figure 3.3). MCM3, an initiator of genome replication, was the last protein meeting the cut-off and it is a known proteasome substrate[95].

Ubiquitin was enriched above the cut-off at a $\log_2$ ratio of 0.69. Proteins not enriched ($\log_2$(heavy/light) ~ 0) may also be ubiquitylated but not affected by proteasome inhibition. Some examples of known proteasome substrates enriched include proliferating cell nuclear antigen (PCNA)[96, 97] and acetyl CoA-carboxylase α (ACACA isoform 1)[98, 99]. 30% (21 proteins) of the proteins meeting the ratio cut-off were proteasome subunits reflecting the ubiquitin affinity housed within the proteasome[100]. Ingenuity pathway analysis software revealed the entire proteasome subunit network being enriched upon proteasome inhibition (data not shown). Since this purification is non-denaturing, some enriched proteins upon MG132 may actually be indirectly interacting with ubiquitylated proteins; therefore, this method was not specifically enriching ubiquitylated proteins.

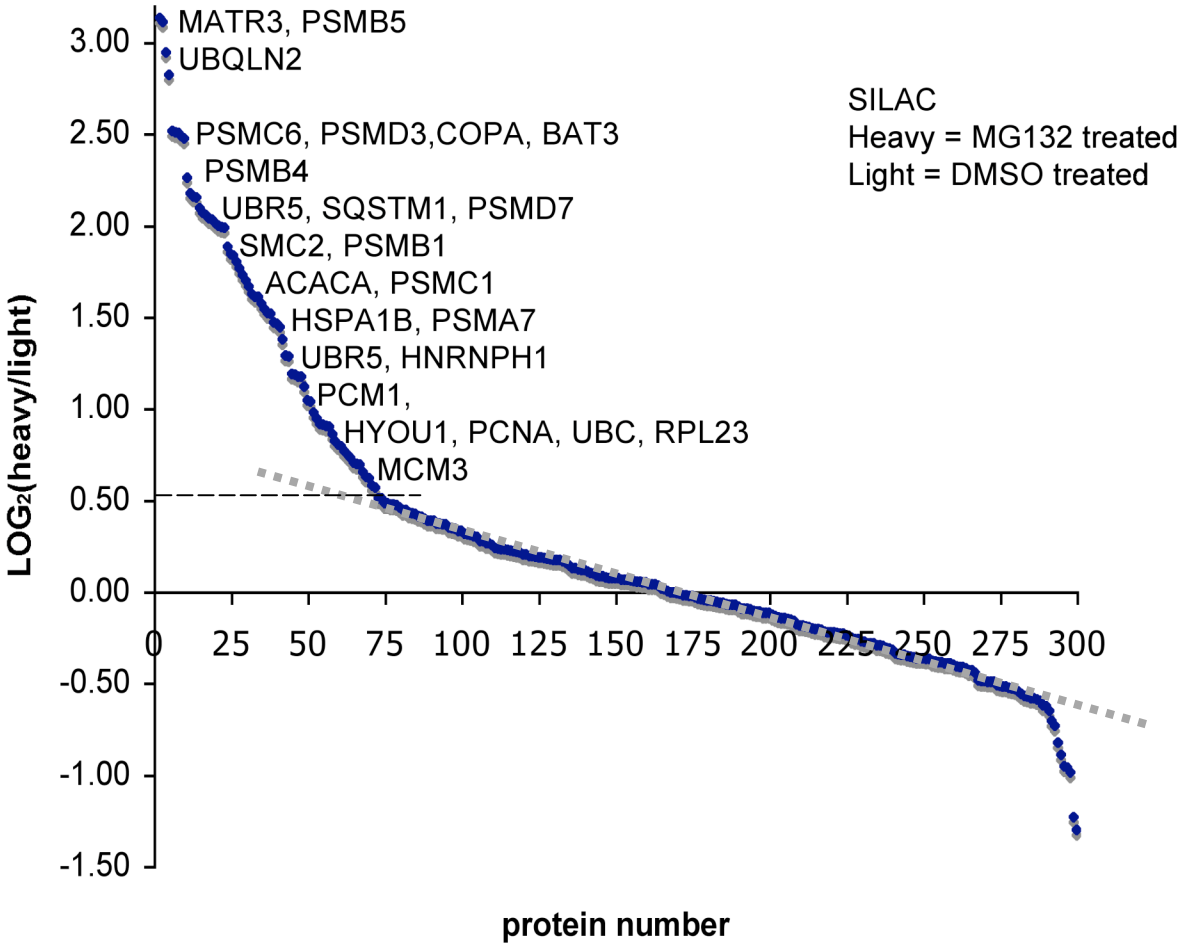


Figure 3.3 GST-S5a SILAC enrichment graph. Cells labeled with light amino acids were treated with DMSO and cells labeled with heavy amino acids were proteasome inhibited. Graph represents the enrichment ratio on a $\log_2$ scale versus the corresponding protein number. A subset of the proteins are highlighted. Proteins listed have a minimum of two peptides identified by Mascot. A trend-line for ratio versus protein number between 75 and 290 is depicted; deviation above the trend-line starts below protein number 73.

Table 3.2 GST-S5a SILAC ratios of significantly enriched proteins. MSQuant was used to calculate the enrichment ratio of heavy versus light peptide. Cells treated with MG132 and DMSO were labeled with heavy and light amino acids respectively. The cut-off ratio used for enrichment was 0.57.

Proteins from GST-S5a pull-down enriched upon proteasomal inhibition	Number of peptides	Protein Mascot score	Log_2(heavy/ light)
ANKRD5 Ankyrin repeat domain-containing protein 5	1	40	9.97
MATR3 Matrin-3	2	85	3.13
PSMB5 29 kDa protein	4	220	3.11
LOC652826 similar to 26S proteasome ATPase subunit	4	242	2.94
UBQLN2 Ubiquilin-2	3	121	2.82
PSMC6 26S protease regulatory subunit S10B	4	125	2.51
PSMD3 26S proteasome non-ATPase regulatory subunit 3	3	150	2.51
COPA Coatomer subunit alpha	2	91	2.50
MAGED1 Isoform 1 of Melanoma-associated antigen D1	2	165	2.49
BAT3 Isoform 1 of Large proline-rich protein BAT3	29	1698	2.47
PSMB4 Proteasome subunit beta type-4	3	151	2.26
PSMD2 26S proteasome non-ATPase regulatory subunit 2	22	1105	2.17
PSMA5 Proteasome subunit alpha type-5	1	79	2.15
UBR4 Isoform 3 of E3 ubiquitin-protein ligase UBR4	51	2539	2.15
PSMB6 Proteasome subunit beta type-6	2	114	2.09
SQSTM1 Isoform 1 of Sequestosome-1	2	85	2.06
PSMD7 26S proteasome non-ATPase regulatory subunit 7	1	65	2.05
PSMC5 26S protease regulatory subunit 8	7	298	2.04
GNB2 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	1	49	2.03
PSMD14 26S proteasome non-ATPase regulatory subunit 14	2	76	2.01
DBN1 Isoform 1 of Drebrin	3	272	1.99
PSMD1 Isoform 2 of 26S proteasome non-ATPase regulatory subunit 1	13	734	1.99
PSMC2 26S protease regulatory subunit 7	7	367	1.98
PSMD6 26S proteasome non-ATPase regulatory subunit 6	3	140	1.88
RGNEF RGNEF protein	3	101	1.84
SMC2 structural maintenance of chromosomes 2-like 1	8	332	1.83
PSMB1 Proteasome subunit beta type-1	3	181	1.80
ACACA Isoform 1 of Acetyl-CoA carboxylase 1	4	182	1.76
PSMC1 26S protease regulatory subunit 4	6	245	1.73
PSMD5 26S proteasome non-ATPase regulatory subunit 5	2	73	1.70
PSMD13 proteasome 26S non-ATPase subunit 13 isoform 2	2	137	1.66
PSMB3 Proteasome subunit beta type-3	2	100	1.62
BIRC6 baculoviral IAP repeat-containing 6	1	41	1.61
HUWE1 482 kDa protein	27	1558	1.61
ATXN2L Isoform 1 of Ataxin-2-like protein	1	60	1.57
C12orf51 chromosome 12 open reading frame 51	10	515	1.54
USP7 Ubiquitin carboxyl-terminal hydrolase 7	12	545	1.52
HNRNPF Heterogeneous nuclear ribonucleoprotein F	1	49	1.51
IPO5 importin 5	1	59	1.47
PSMC3 26S protease regulatory subunit 6A	6	240	1.46
PRKDC Isoform 2 of DNA-dependent protein kinase catalytic subunit	14	759	1.44
HSPA1B;HSPA1A Heat shock 70 kDa protein 1	9	552	1.38
PSMA7 Isoform 1 of Proteasome subunit alpha type-7	1	40	1.28
UBR5 E3 ubiquitin-protein ligase UBR5	11	498	1.28
C6orf174 Uncharacterized protein C6orf174	1	51	1.19
AARS2 Probable alanyl-tRNA synthetase, mitochondrial	1	43	1.18
NEFM neurofilament, medium polypeptide 150kDa isoform 1	12	690	1.17
HNRNPH1 Heterogeneous nuclear ribonucleoprotein H	3	209	1.17
- 69 kDa protein	2	93	1.12
NEFH Neurofilament heavy polypeptide	1	62	1.04
PCM1 Isoform 3 of Pericentriolar material 1 protein	10	480	1.03
KIAA1618 Isoform 3 of Protein ALO17	1	71	0.98
EIF4A1 Eukaryotic initiation factor 4A-I	5	288	0.94
HERC2 Probable E3 ubiquitin-protein ligase HERC2	11	475	0.91
HDAC2 histone deacetylase 2	1	35	0.91
NPLOC4 Isoform 2 of Nuclear protein localization protein 4 homolog	4	189	0.90
SMC3 Structural maintenance of chromosomes protein 3	9	446	0.90
HYOU1 Hypoxia up-regulated protein 1	3	113	0.86
KIF21A Isoform 1 of Kinesin-like protein KIF21A	2	82	0.82
PARC Novel protein	1	64	0.80
FAM115A Isoform 1 of Protein FAM115A	5	195	0.79
EXOC4 Exocyst complex component 4	2	158	0.77
VCP Transitional endoplasmic reticulum ATPase	30	1723	0.75
PCNA Proliferating cell nuclear antigen	1	60	0.73
LOC442497;SLC3A2 solute carrier family 3	2	73	0.70
UBC;UBB;RPS27A Ubiquitin C splice variant	13	873	0.69
RPL23 60S ribosomal protein L23	1	47	0.69
IKBKAP inhibitor of kappa light polypeptide gene enhancer in B-cells	3	111	0.65
EIF3CL;EIF3C Eukaryotic translation initiation factor 3 subunit C	7	485	0.63
ATP5B ATP synthase subunit beta, mitochondrial	15	740	0.62
PPP2R1A Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A	2	55	0.58
MCM3 DNA replication licensing factor MCM3	2	148	0.57

3.5 Direct immunoprecipitation of parkin binding partners

We have carried out two ubiquitin purification procedures and analyzed the number of proteins by MS. The HBU approach revealed a bias towards highly abundant proteins along with a low number of total proteins. S5a affinity purification lead to the identification of many ubiquitin binding proteins, including the entire proteasome complex, suggesting a difficulty to unambiguously identify proteins that are ubiquitylated themselves.

In the face of these non-ideal situations, we decided to employ direct parkin immunoprecipitation to capture interactors and identify them by MS, which had not been done before. Several parkin ligase substrates were first discovered by an initial positive co-immunoprecipitation and later confirmed by subsequent ubiquitylation assays[35, 101-103], indicating that this approach may be suitable.

We used parkin N-terminally (amino-) tagged with the short amino acid FLAG sequence that is recognized by antibodies enabling single-step affinity purification[65]. We generated a stable cell line that genomically integrated the plasmid containing FLAG-parkin and the geneticin resistance gene in order to obtain reliable and constant expression levels of the ectopic FLAG-parkin. By clonal dilution, we obtained ten pure populations of SH-SY5Y cells resistant to geneticin (G418). We were able to readily detect parkin in three clones (Figure 3.4A). Endogenous parkin was not detected due to very low levels of expression. We confirmed by immunofluorescence that expression of FLAG-parkin was homogenous in these three clonal populations (data not shown). We concluded that both the neomycin resistance cassette and the FLAG-parkin were integrated into euchromatin in these cells. Clone 4 was selected for subsequent

experiments due to the intermediate expression level of the ectopic protein. We reasoned that a large molar excess of the bait over its interacting partners (e.g. Clone 3) may lead to a higher rate of false negative identification by MS[103].

We performed FLAG-parkin immunoprecipitation using anti-FLAG sepharose, and captured about 50% of the ectopic protein (Figure 3.4B). We verified that the western blotting signal was specific to FLAG-parkin since anti-FLAG sepharose treated with SDS did not produce any signal (data not shown). Negligible amounts of FLAG-parkin were present in the lysis buffer washes (data not shown).

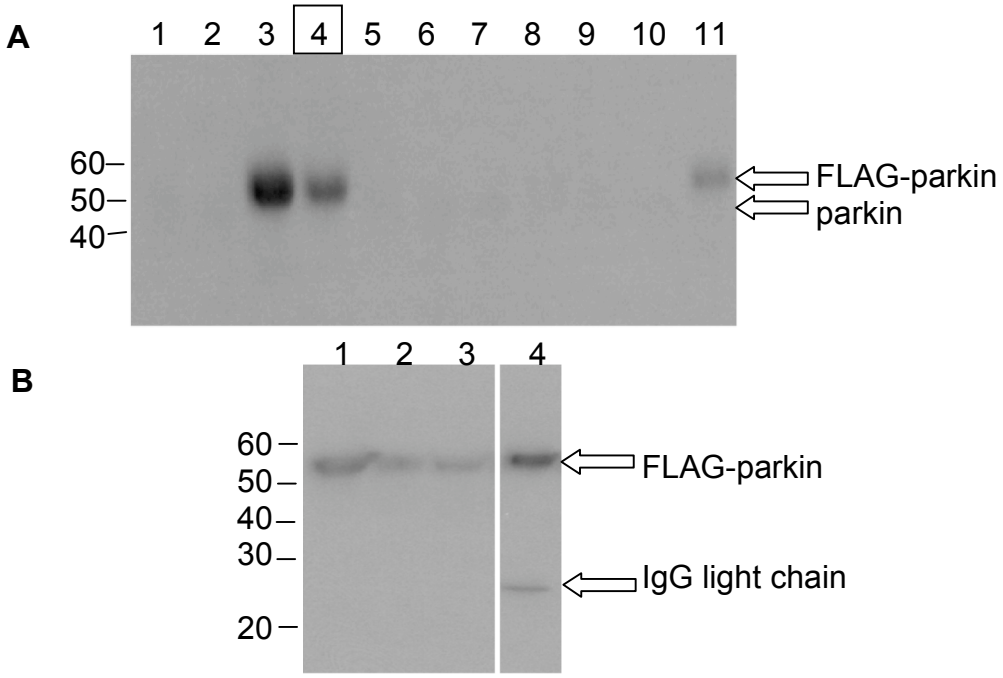


Figure 3.4 Purification of FLAG-parkin. Cells cloned after integration of the FLAG-parkin plasmid were lysed and subjected to SDS-PAGE and immunoblotting with anti-parkin PRK8 antibody (A). Lane 1 untransfected, lanes 2-11 FLAG-parkin clones. The arrows indicate where FLAG-parkin and endogenous parkin would be expected to migrate. Clone 4 was chosen for subsequent experiments. Aliquots at different stages of the purification scheme were subjected to SDS-PAGE and immunoblotting with PRK8 antibody (B). Lane 1 (1x) input, lane 2 (0.33x) input, lane 3 (1x) unbound, lane 4 (5x) elution. Molecular weights in kDa are indicated to the right of each blot.

We used SILAC to differentiate between specific parkin interactors and non-specific background proteins (Figure 3.5). Two cell populations ($2x10^7$ cells each), one stably expressing FLAG-parkin and the other not, were labeled with light and heavy amino acids, respectively. Cells were lysed and processed separately by incubating each lysate with a batch of anti-FLAG sepharose to initially capture FLAG-parkin and then the beads were pooled during the first wash with lysis buffer. The rationale for not mixing the lysates during the immunoprecipitation is that dynamic interactors of parkin could display a high exchange rate resulting in a heavy labeled dynamic interactor exchanging with a light labeled dynamic interactor originally bound to FLAG-parkin derived from light labeled cells[105]. This rapid exchange would result to relative equal quantities of light and heavy labeled dynamic interactors bound to FLAG-parkin. In order to increase the number of proteins identified by MS, we first eluted the bound proteins into a weak interactor fraction by incubating the beads with detergent (1% Tween) and salt (500mM NaCl), and then eluted the remaining proteins into a strong interactor fraction by applying denaturing SDS detergent. The two protein mixtures were trypsin digested into peptides, then subjected to strong cation exchange chromatography into four fractions and analyzed by LC-MS/MS as previously described[91]. Assigned peak lists from each sample were pooled and ratios quantified by MSQuant.

We identified 295 and 110 proteins from the weak interactor and strong interactor fractions respectively (Figure 3.6, Appendix Tables 3 and 4). FLAG-parkin was the most SILAC enriched protein and with a confident number of eight peptides identified. Parkin does not have a reported $\log_2$(heavy/light) ratio due to undetectable amounts of heavy peptides from the normal cell population relative to light peptides from FLAG-parkin

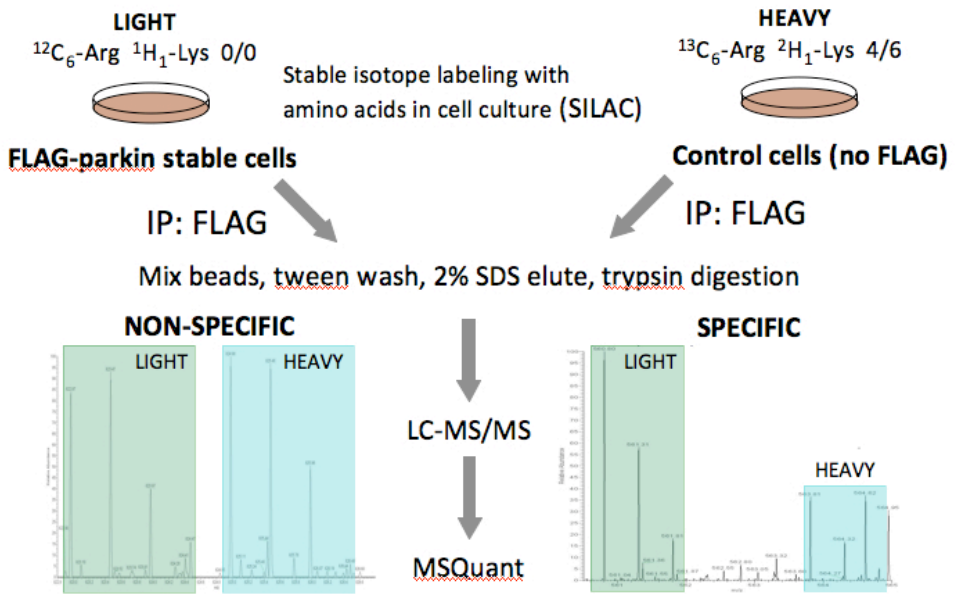


Figure 3.5 Quantitative mass spectrometry to determine parkin interactors. Schematic representation of the SILAC approach. SILAC was employed to differentiate between specific and background proteins. Specific interactors would have a heavy/light ratio < 1, non-specific having a ratio ~1.

over-expressing cells hence a heavy/light ratio of zero which cannot have a logarithmic value (Figure 3.7A). We used parkin derived from *Rattus norvegicus* cDNA which has unique peptides compared to human parkin peptides, hence each MS spectrum file was also searched against the rat database to identify and quantify these peptides. Based on a cutoff score of $\log_2$(heavy/light) = 1.00, we identified 24 potential parkin interactors (Appendix, Table 3). Candidate interactors included neuroendocrine chromogranin-secretogranin family proteins that are synthesized in the ER and stored in secretory granules in the cell[106] (Table 3.3; see also Figure 3.7B for representative spectrum of one peptide). The neuroendocrine candidate interactors were weakly interacting because they were absent in the second elution. DJ-1, a known parkin interactor, was identified in the final elution confirming the validity of the technique[55].

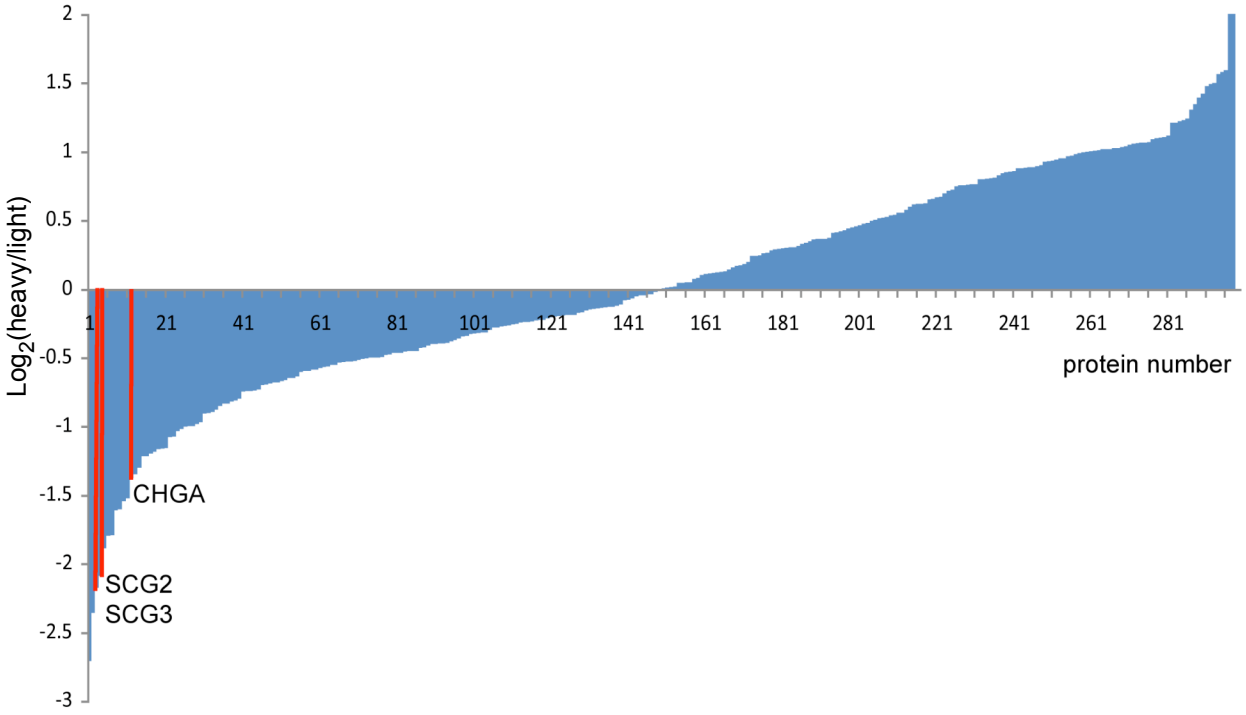
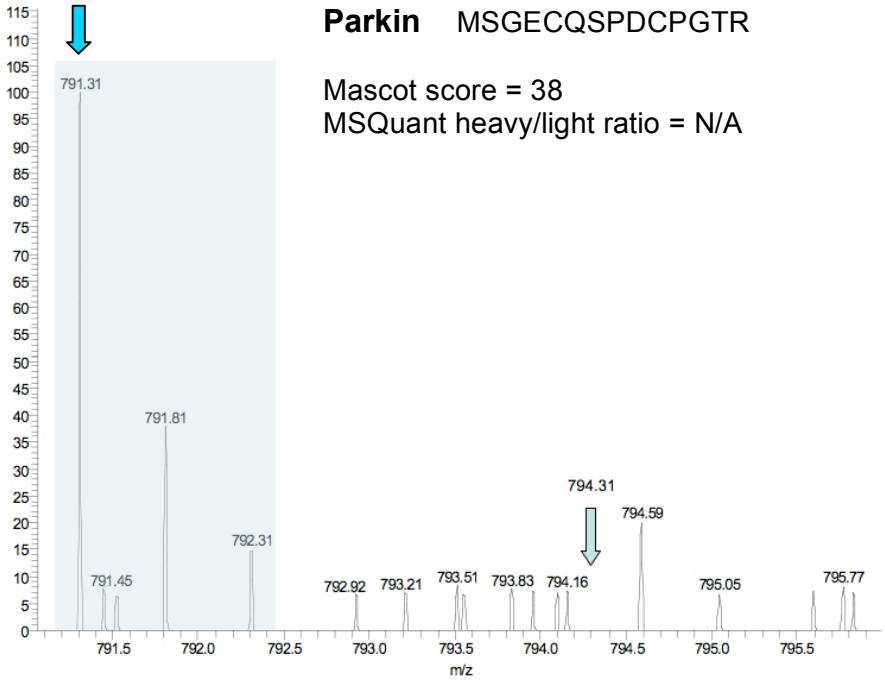


Figure 3.6 FLAG-parkin SILAC enrichment graph. Cells labeled with light amino acids were stably expressing FLAG-parkin and cells labeled with heavy amino acids were not expressing FLAG-parkin. Graph represents the enrichment ratio on a $\log_2$ scale of heavy/light SILAC labeling versus the corresponding protein number. A subset of the enriched proteins from the weak interactor fraction are highlighted.

A



B

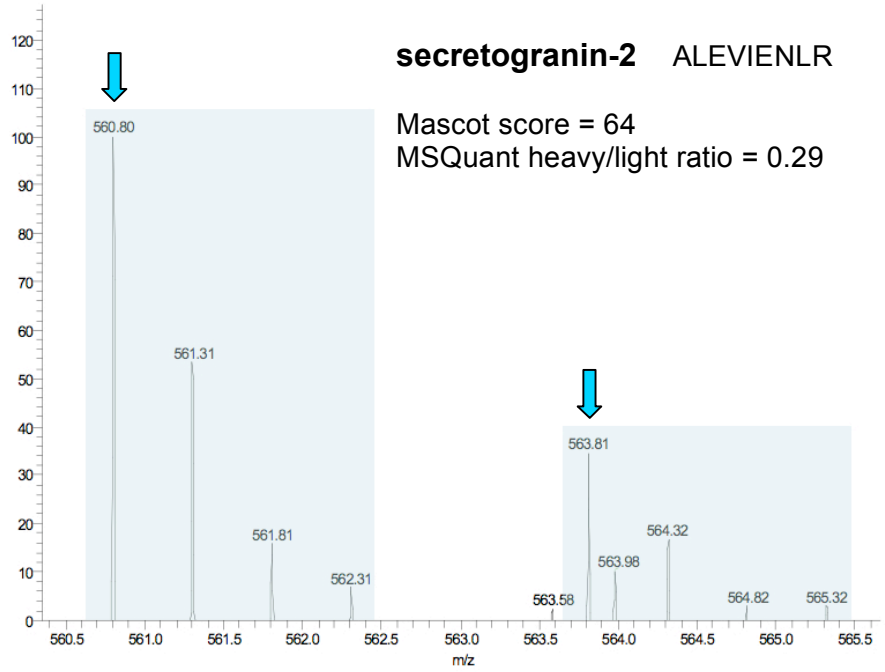


Figure 3.7 Relative intensity versus m/z of parkin and secretogranin-2 (A). Highlighted are peptide signatures belonging to parkin (A) and secretogranin-2 (B) from light amino acid labeled cells over-expressing FLAG-parkin. The peptide sequence for parkin is MSGECQSPDCPGTR with a monoisotopic mass of 791.31 Th. The peptide charge is +2 due to a spacing of 0.50 Th between isotopic peaks. The expected heavy labeled parkin peptide monoisotopic peak position is shown by the arrow at 794.31 Th as well as other indicated light/heavy monoisotopic peaks. Other spectra peaks are background signals that have the same retention time. The peptide sequence for secretogranin-2 is ALEVIENLR with a monoisotopic mass of 560.805 Th for the light peptide. The peptide charge is +2 due to a spacing of 0.50 Th between isotopic peaks.

Table 3.3 Enriched protein ratios from FLAG-parkin pull-down. n/a = not applicable. MSQuant manually corrected ratios are in fourth column. Proteins were either present in the weak interactor fraction or strong interactor fraction.

Enriched proteins from FLAG-parkin	Protein Mascot Score	Number of peptides	$\log_2$(heavy/ light)	$\log_2$(heavy/light) manual correction	Fraction
DJ-1	52	1	-0.83	-0.83	strong
secretogranin-3	175	22	-1.80	-2.12	weak
chromogranin-A	696	21	-1.36	-1.36	weak
secretogranin-2	1688	41	-2.07	-2.07	weak
parkin	379	8	n/a	n/a	strong

3.6 Capturing parkin interactors with cross-linking prior to immunoprecipitation

We were concerned that parkin was interacting with unspecific proteins after lysis because the major interactors were granin family proteins, which typically reside in granules and has no domain in the cytoplasm. Moreover, only one peptide was identified for DJ-1, a known interactor, indicating that true interactors could rapidly dissociate. In order to reduce potential background binders, we exploited the approach developed by Vasilescu *et al*.[107], in which formaldehyde was utilized to trap protein interactions *in vivo*. Formaldehyde is a small membrane permeable molecule, which can form cross-links between amine groups in close proximity such as between interacting proteins. The advantage of this technique is that transient interactors with a high k_{off} rate can be preserved intact during the immunoprecipitation procedure enabling extensive washing to remove non-specific proteins bound to the target protein or resin[107]. Formaldehyde cross-links are reversible enabling proper identification of peptides by Mascot.

We established a cell line that expresses Myc-parkin since cross-linking masked the FLAG epitope. Higher molecular weight parkin signals were detected by SDS-PAGE and parkin immunoblotting from Myc-parkin expressing cells treated with formaldehyde suggesting entrapment of parkin in a covalent bond with binding partners (Figure 3.8A). This increase in complex preservation reached a maximum at 0.40% formaldehyde treatment so this concentration was used for subsequent experiments. Excessive cross-linking is not desirable because there would potentially be more non-specific proteins reacting with real parkin complexes.

We next immunoprecipitated Myc-parkin complexes with anti-Myc 9E10 protein G magnetic beads. The pull-down efficiency of Myc-parkin was about 30% and was not affected by cross-linking (Figure 3.8B approximately equal parkin signals in the 3% eluate versus 1% input lane). The higher molecular weight parkin species disappeared along with an increase in uncross-linked parkin after incubating in high temperature reflecting the reversible nature of the formaldehyde cross-links and proteomics compatibility (Figure 3.8, lane 9).

We purified Myc-parkin complexes from formaldehyde treated cells versus cells not expressing Myc-parkin ($6x10^6$ cells each condition), reversed the cross-links and subjected the protein mixture to LC-MS/MS. We identified only four parkin peptides, which was less than the typical FLAG immunoprecipitation. The low number of parkin peptides was not encouraging because it suggested many of the identified proteins may have cross-linked non-specifically or were contaminants still binding to the beads. We were also not able to identify any known parkin interactors; therefore, we decided not to pursue this approach.

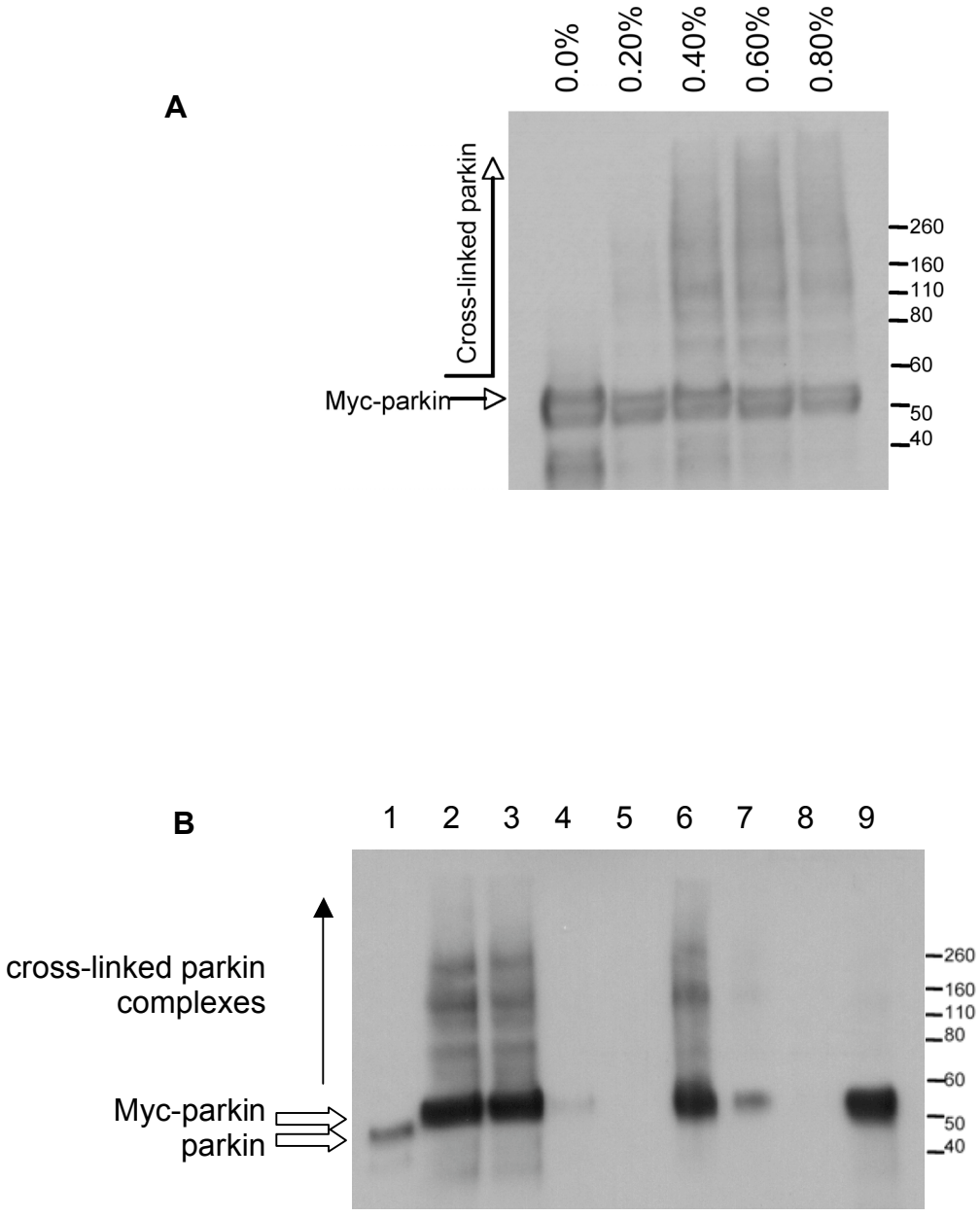


Figure 3.8 Purification of Myc-parkin from cross-linked lysate. Optimization of formaldehyde concentration in cross-linking experiments (A). % formaldehyde is indicated above lanes. Marker positions in kDa are indicated on the right. Aliquots at different stages of the purification scheme were subjected to SDS-PAGE and immunoblotting with PRK8 antibody (B). Lane 1 (1x) untransfected input, lane 2 (1x) Myc-parkin cells input, lane 3 (1x) unbound, lane 4 wash, lane 5 wash, lane 6 (3x) 0.5% SDS elution, lane 7 (3x) 2% SDS elution, lane 8 (3x) 6% SDS elution, lane 9 (3x) 0.5% boiled elution.

3.7 Quantitative proteomic analysis of parkin interactors upon mitochondrial stress

Parkin was shown to localize to the mitochondria after mitochondrial depolarization to promote mitophagy[46], which is a significant leap in further establishing a connection between mitochondrial dysfunction and PD etiology. An important task will be deciphering the proteins mediating the parkin-mitophagy pathway and whether parkin's ubiquitin ligase activity is required for downstream initiation of autophagy. We first assessed whether FLAG-parkin was able to act similarly to parkin, and indeed found that FLAG-parkin co-localized with the mitochondria upon 10μM CCCP treatment for 3 hours, but not DMSO treatment (Figure 3.9). A cross-section of the cytoplasm displayed overlap of the anti-parkin stain (green) and mitotracker stain (red) in cells treated with CCCP.

We investigated parkin interactors upon mitochondrial membrane depolarization by performing co-immunoprecipitation with SILAC labeled cell lysate using the same procedure as described in Chapter 3.5. In this experiment, we compared light and heavy labeled cells treated with DMSO and CCCP, respectively (each expressing FLAG-parkin). We identified 1180 and 450 proteins in the weak and strong interactor fractions respectively (false positive rate ≤1%) that were quantified by MSQuant (Figure 3.10, Table 3.4, Appendix Tables 5 and 6). We identified 30 proteins enriched in CCCP treated cells including several actin related proteins (Table 3.6). Myosin regulatory light chain and myosin IX, a non-muscle unconventional myosin involved in signaling[108] were both found enriched in CCCP treated cells and were readily identified with 13 and 32 peptides, respectively. Ion intensities of selected peptides for each protein in the full

scan (i.e. unfragmented) confirmed the proteins were enriched from heavy labeled cells (Figure 3.11). Mitochondrial apoptosis inducting factor (MAIF) was also enriched upon CCCP treatment suggesting specific stress on the mitochondria causing potential downstream signaling events. Only one peptide was identified for MAIF but the Mascot score was above 50, which was very high and strongly suggested the protein was present. The enriched proteins were all present in the final elution suggesting a strong binding interaction with FLAG-parkin.

Parkin was identified with 23 peptides but with a de-enriched ratio from CCCP treated cells (not absent though). We confirmed the de-enrichment was based on loss of parkin solubility (Figure 3.12). This outcome may be due to parkin being recruited to autophagosomes at mitochondria upon mitochondrial membrane depolarization resulting in a higher sedimentation coefficient and pelleting during lysate clearance (Figure 3.9). Thus, several proteins interacting with parkin due to CCCP may have a similar de-enrichment.

Cytochalasin D was used to validate the significance of the actin cytoskeleton in the parkin-mediated mitophagy pathway since the fungal metabolite inhibits actin polymerization in eukaryotic cells[109]. Immunofluorescence co-localization studies revealed that cytochalasin D prevented the full recruitment of parkin to mitochondria (Figure 3.13). In addition, several parkin punctuates were not observed to be co-localized with mitochondria and the parkin signal was much more diffuse.

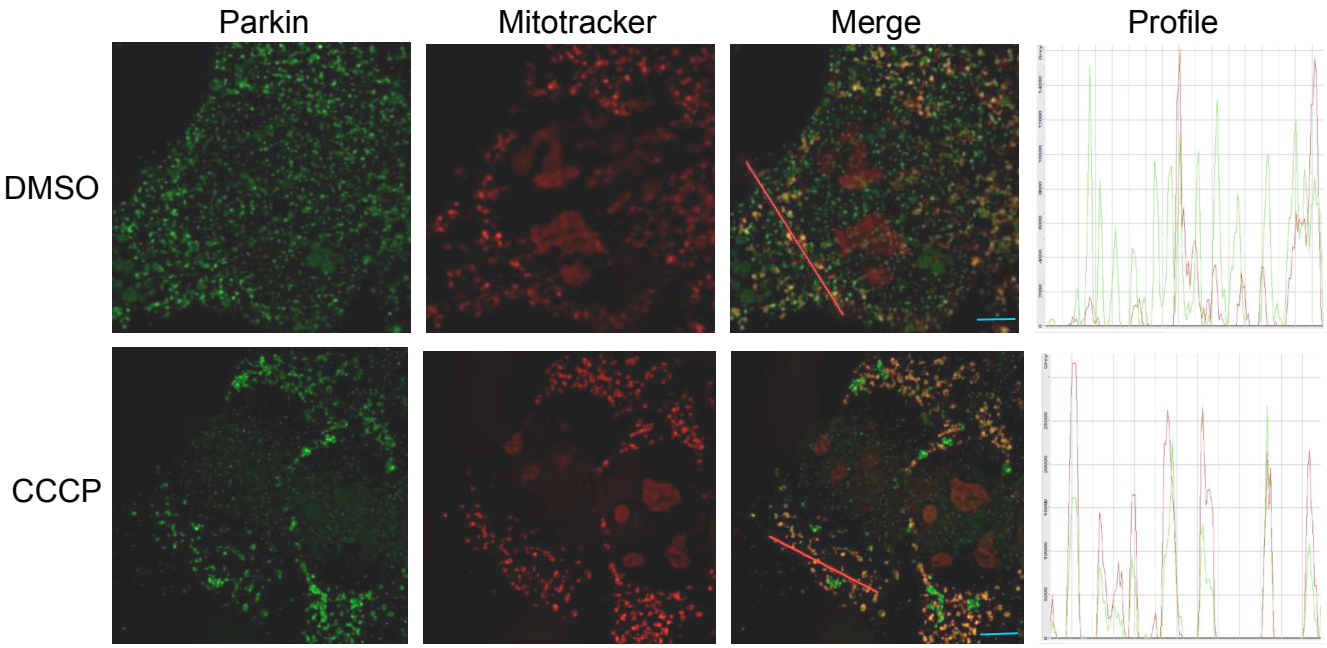


Figure 3.9 Parkin recruitment to the mitochondria upon CCCP treatment. Mitotracker orange was used to stain mitochondria and PRK8 used to stain parkin. Profile graphs show relative signal intensities of parkin and mitochondria based on the selected profile depicted by the red line. Magnification is 63X and the blue scale bar represents 10µm.

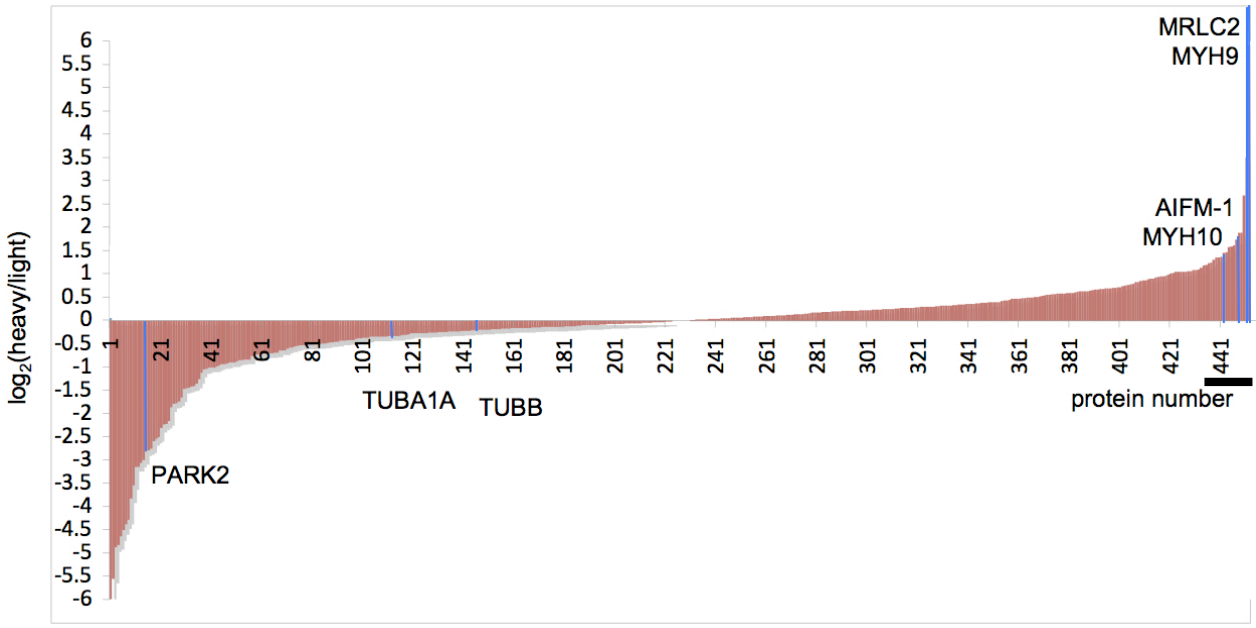


Figure 3.10 FLAG-parkin CCCP SILAC enrichment graph. FLAG-parkin stable cells treated with DMSO or CCCP were labeled with light and heavy amino acids, respectively. Graph represents the enrichment ratio on a $\log_2$ scale of the heavy/light SILAC ratio versus the corresponding protein number. A subset of the enriched proteins from the strong interactor fraction are highlighted. Proteins listed have a minimum of two peptides identified by Mascot. A subset of proteins in the region underlined had ratios manually validated using MSQuant. MRLC2 and MYH9 actually have much greater ratios than 6, the graph maximum.

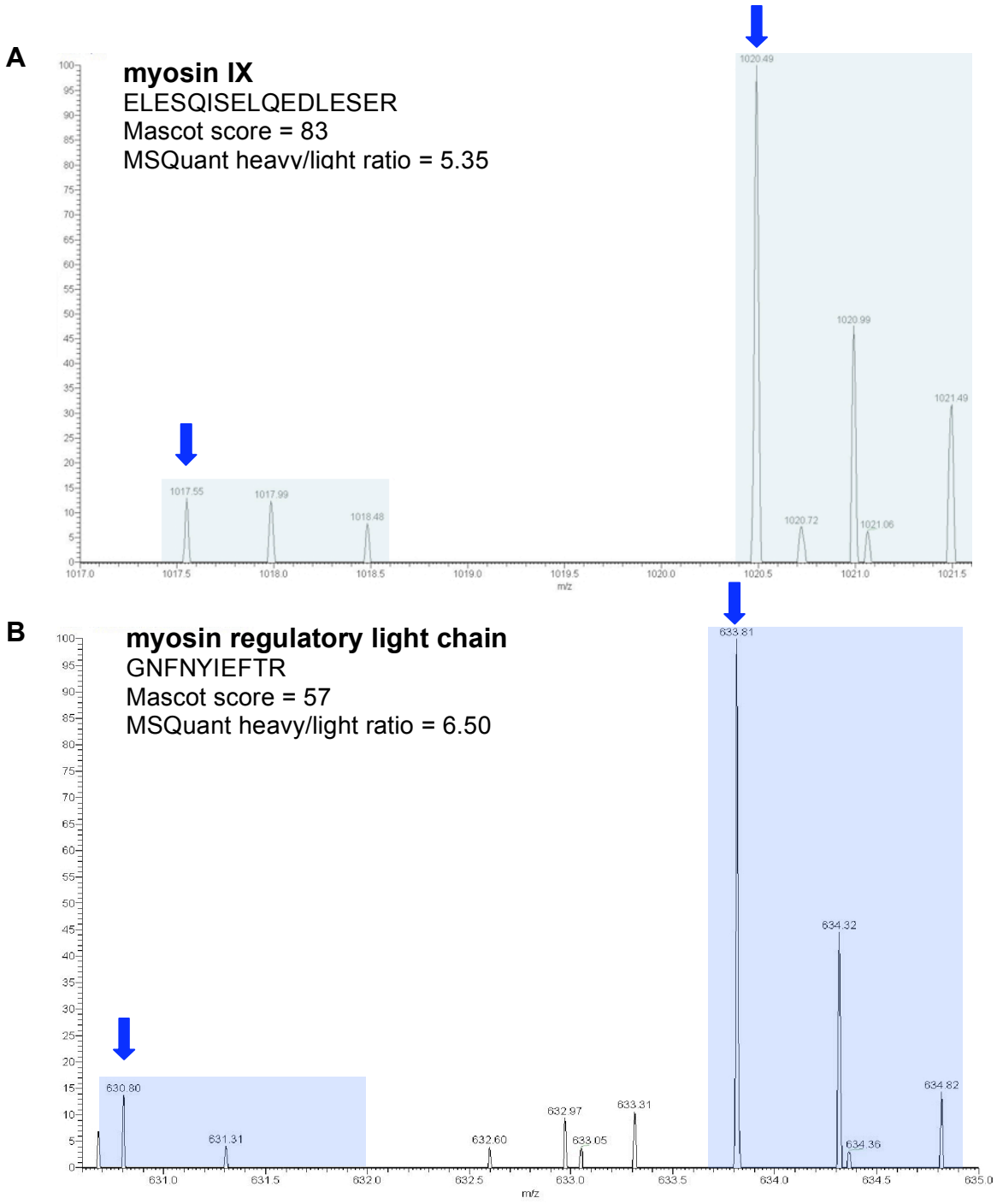


Figure 3.11 Relative intensity versus m/z showing myosin-IX and myosin regulatory light chain peptides. Highlighted are peptide signatures belonging to myosin-IX (A) and myosin regulatory light chain (B) from light amino acid labeled cells treated with DMSO and heavy amino acid labeled cells treated with CCCP. The peptide sequence for myosin IX is ELESQISELQEDLESER with a monoisotopic mass of 1020.493 Th for the heavy peptide. The peptide charge is +2 due to a spacing of 0.50 Th between isotopic peaks. The peptide sequence for myosin regulatory light chain is GNFNYIEFTR with a monoisotopic mass of 634.316 Th for the heavy peptide. The peptide charge is +2 due to a spacing of 0.50 Th between isotopic peaks. Arrows indicate monoisotopic peaks for light/heavy peptides.

Table 3.4 Enriched proteins from CCCP treated FLAG-parkin lysate. MSQuant manually corrected ratios are in fourth column. Proteins were all present in the strong interactor fraction.

Proteins enriched with CCCP	Protein Mascot Score	Number of peptides	log_2(heavy /light)	log_2(heavy/light) manual correct	Fraction
myosin regulatory light chain	700	13	3.54	19.14	strong
myosin IX	1722	32	2.03	16.95	strong
MAIF	52	1	1.86	1.88	strong
myosin X	995	19	1.39	1.57	strong
drebrin	2024	33	1.09	1.09	strong
parkin	1534	23	-2.86	-2.86	strong

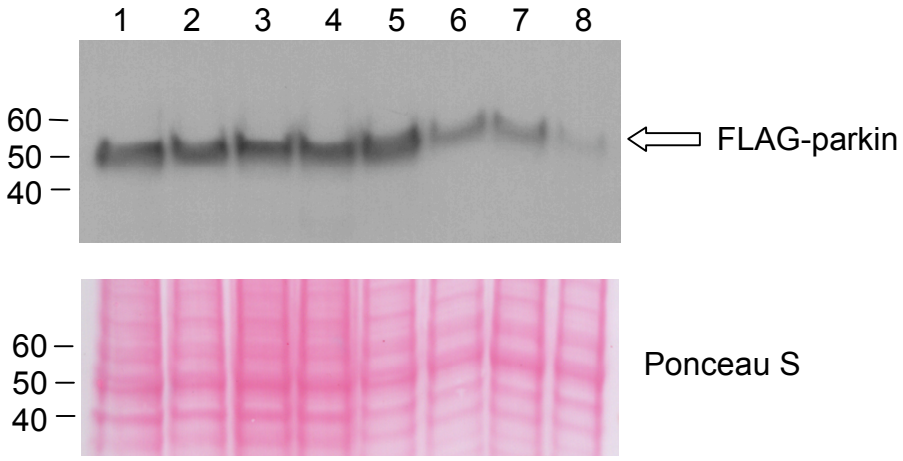


Figure 3.12 Parkin solubility is reduced upon CCCP treatment. Cell were lysed as in Chapter 2.4.3 and debris pelleted at 4ºC, 16200g 10 min. Lane 1 to 4 represent cell lysates that have not be pelleted, lane 1 is 6 hours DMSO, lane 2 is 3 hours CCCP, lane 3 is 4.5 hours CCCP, lane 4 is 6 hours CCCP; lanes 5-8 represent cleared cell lysate after centrifugation, lane 5 is 6 hours DMSO, lane 6 is 3 hours CCCP, lane 7 is 4.5 hours CCCP and lane 8 is 6 hours CCCP. CCCP was used at 10µM. Membranes were incubated with Ponceau S total protein stain after transfer for 2 min, which shows relatively equal protein loading.

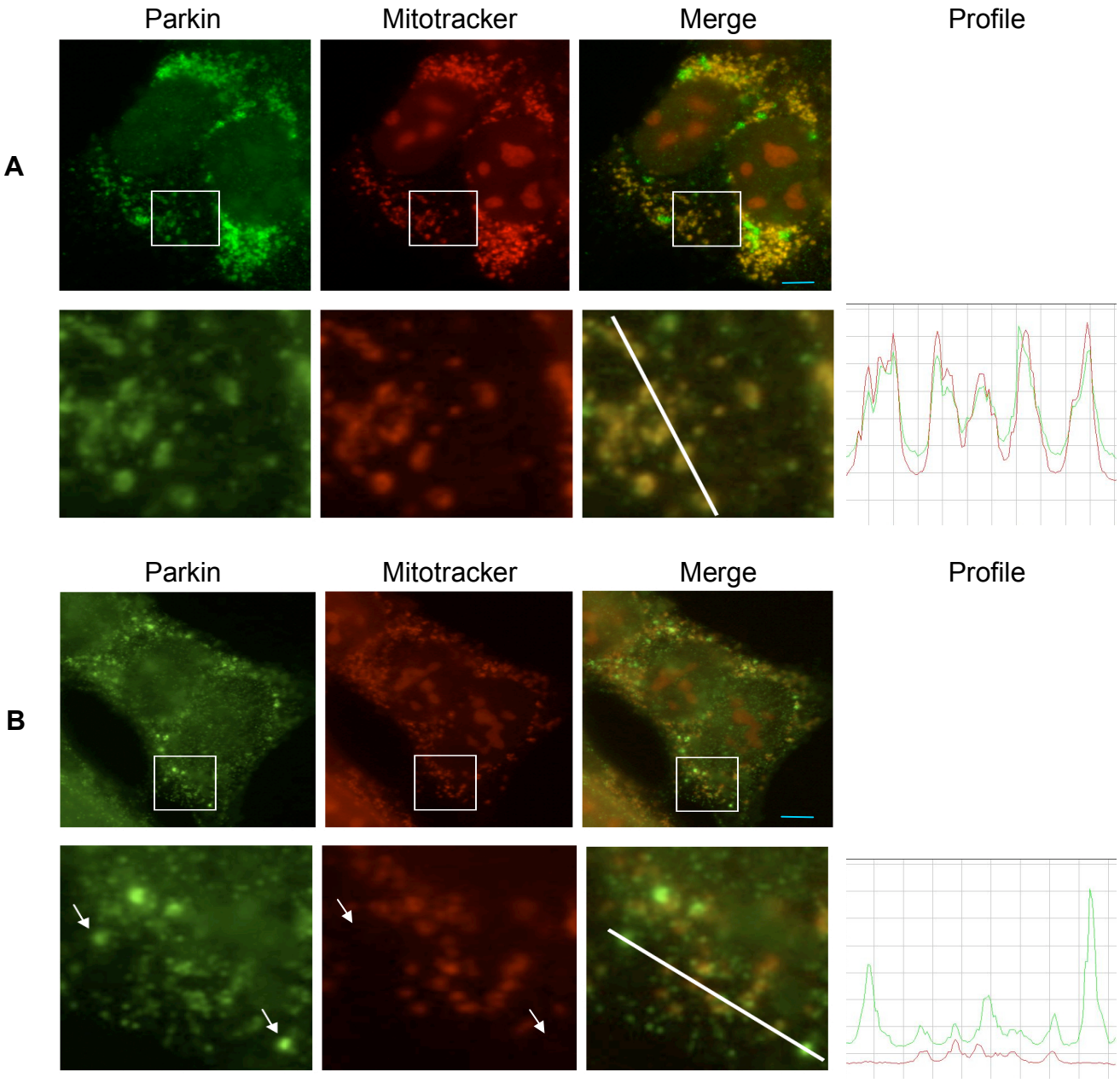


Figure 3.13 Cytochalasin D hampers parkin localization to mitochondria. FLAG-parkin cells were treated with either CCCP alone (A) or CCCP and cytochalasin D (B) and stained with PRK8 antibodies and mitotracker orange. A zoomed in box panel provides a detailed raw image of a profile graph. On the right, profile graphs show intensities of the parkin (green) and mitochondria (red) based on the selected profile in the zoomed panel of the merged images. There is no significant overlap between the red and green channels in cells with cytochalasin D treatment (B) relative to CCCP only (A). Magnification is 63X. Arrows represent stained parkin in which the corresponding mitochondrial signal is absent. Blue scale bar is 10μm.

CHAPTER 4

DISCUSSION

4.1 Ubiquitin purification methods for mammalian cells

We tested different strategies to investigate parkin E3 ubiquitin ligase function, a protein implicated in juvenile Parkinsonism. We first sought to determine whether the enrichment of ubiquitylated proteins could allow us to identify proteins affected by parkin. Provided a good enrichment for low abundant poly-ubiquitylated proteins, ectopic parkin expression could reveal the presence of new ubiquitylated proteins that could correspond to its substrates due to increased parkin-mediated ubiquitylation and purification. The two methods used to purify the ubiquitin proteome were tandem purification of HBU conjugates from cells transfected with the HBU construct and purification of ubiquitin conjugates using S5a affinity chromatography. The coverage of a variety of low-abundance ubiquitylated proteins was essential when assessing putative substrates of parkin or any other E3 ligase because ligase substrates are often low in abundance such as ubiquitylated cell cycle proteins and parkin-associated endothelin-like receptor (Pael-R)[35].

Although the expression level of HBU was adequate relative to endogenous ubiquitin in a 2:3 ratio upon transfection, we found the incorporation of HBU into polyubiquitin chains was hindered leading to a low amount of purified ubiquitin conjugates. The molecular weight of the His_8-biotinylation signal tag itself was relatively the same as ubiquitin; therefore, steric hinderance may have prevented ubiquitin conjugation enzyme machineries from properly recognizing and incorporating HBU. Furthermore, SILAC experiments with this approach[110] in Dr. P. Kaiser's laboratory (UC Irvine) yielded no specific enrichment of ubiquitin conjugates in cells with p97 impairment (which normally leads to an increase of ubiquitin conjugates; R. Deshaies, personal communication).

The low yield of ubiquitin conjugates was evident in the low number of peptides identified when subjecting the tandem affinity purified material for MS.

There existed an unfavourable bias for enrichment of highly abundant ubiquitylated proteins such as ribosome subunits[92] and histones[111]. α/β-tubulin were the only known parkin substrates identified, but these are also highly abundant proteins and would not serve as the ideal reference parkin substrates to be potentially enriched in a parkin over-expression experiment. Proteasome inhibition increased the number of identified proteins as expected, although 41 total proteins was not representative enough of the entire ubiquitin landscape. This result was drastically different than the ubiquitin pull-downs under denaturing conditions performed with yeast lysate, in which several hundred ubiquitylated proteins were identified including low-abundance cell cycle proteins like CDC5[69]. We initially hoped the yield of ubiquitin conjugates from the transfected mammalian cells would be similar to the His_6-biotin-ubiquitin expressing yeast described by Tagwerker *et al*.[69], but due to unforeseen circumstances of impaired conjugation, this approach is not likely feasible.

We also tested His_8-ubiquitin but were not able to obtain a high purification yield due to lower expression levels. We are currently testing a construct harboring four copies of His_8-ubiquitin in tandem (His_8-ubiquitin-His_8-ubiquitin-His_8-ubiquitin-His_8-ubiquitin) in mammalian cells. We hope this construct will offer higher expression and improved conjugation.

We were the first to employ GST-S5a affinity chromatography in the context of SILAC to determine ubiquitylated proteins specifically enriched upon proteasomal inhibition. The

GST-S5a chromatography experiment enabled purification of a greater number of proteins than the HBU approach but the data was confounded by a large quantity of ubiquitin binding proteins due to protein-protein interactions under non-denaturing conditions. Many proteasome subunits were enriched due to the ubiquitin binding domains of the proteasome regulatory cap, which may interact with ubiquitylated proteins bound to the resin under native conditions during the pull-down incubation. Proteins with ubiquitin-associated (UBA) domains such as ubiquilin-2[112], sequestosome-1[113] and HUWE1[114] were three of the top enriched proteins upon proteasomal inhibition reflecting the nature of secondary interactions with the S5a resin. We identified a total of 310 proteins, much more than the HBU approach; however, only 20% of the total were enriched upon proteasomal inhibition (with a low cutoff of 0.57) suggesting the actual proportion of ubiquitylated proteins in the pool was smaller. Detergents cannot be added to the pull-down procedure to reduce background since hydrophobic interactions between S5a UIM domains and ubiquitin would be eliminated under such conditions (T. Mayor; personal communication). There may also be enriched ubiquitylated proteins that are not proteasome substrates and these cannot be excluded. We identified α/β-tubulin again as the only known parkin substrate. We estimated that it would be difficult to employ this method as a tool for sifting parkin substrates upon parkin over-expression based on a highly-abundant protein.

4.2 Searching for parkin interactors by direct immunoprecipitation

We sought to identify parkin interactors by parkin immunoprecipitation and mass spectrometry after the HBU and S5a purifications gave a shallow coverage of the ubiquitin proteome. We employed direct FLAG-parkin immunoprecipitation as well as formaldehyde cross-linking to enrich for parkin interactors and determined that the former was more appropriate. Using this approach, we then assessed parkin's function in the mitochondrial autophagosome pathway upon mitochondrial dysfunction by enriching for potential novel interactors.

We successfully purified Myc-parkin formaldehyde cross-linked complexes. However, a high number of peptides from naturally abundant proteins such as actin and tubulin comprised most of the top identified proteins (data not shown). Identification of highly abundant proteins may be due to the nature of the formaldehyde cross-link as parkin is known to bind to actin and tubulin resulting in the pull-down of large cytoskeletal components under non-denaturing conditions. Other identified proteins in the immunoprecipitant that are naturally abundant such as heat shock proteins and neuronal cell specific proteins may be indicators of non-specific proteins either sticking to the beads or non-specific cross-linking into large complexes containing parkin. The overshadowing of parkin peptides by a significant number of other proteins will hamper the identification of potential novel interactors because such interactors should at most be as abundant as the bait parkin. There were only four parkin peptides identified which was a concern during candidate interactor screening of the Mascot data list because the bait was not present at a high enough level to provide enough confidence that other

proteins are true interactors. We were also not able to identify any known parkin interactors.

We identified many more bait parkin peptides using the FLAG-parkin direct immunoprecipitation method enabling more confident screening of other enriched candidate interactors. We were able to detect DJ-1, a known parkin interactor in the strong interactor fraction, which supported the validity of the parkin pull-down method. Many enriched proteins in the immunoprecipited fractions however, were represented by only one peptide which is not a confident indicator of whether the protein is really present. Analysis of the weak interactor fraction revealed several interesting parkin interactor candidates from the neuroendocrine chromogranin-secretogranin family including secretogranin-2, secretogranin-3 and chromogranin A. These proteins are acid, soluble secretory proteins in vesicles of the neuroendocrine system[106]. They contain an N-terminal signal peptide for translocation into the ER lumen and possess both intracellular and extracellular functions. Intracellular functions include contribution to secretory granule formation upon immature vesicle budding from the trans-Golgi network and extracellular functions are due to granin-derived peptide functioning[106]. Peptides identified by Mascot do not include the transmembrane signal peptide region suggesting possible interaction with parkin after ER translocation. Parkin is not known to localize within the ER, thus the interaction may be induced non-physiologically after cell lysis. However, a clinical connection between chromogranin A and PD also occurs in which chromogranin A accumulates in Lewy bodies of the substantia nigra and is diminished from the cerebrospinal fluid from PD patients[115]. We are currently

investigating whether this result is physiologically relevant by co-localization and co-immunoprecipitation methods.

We identified several actin related proteins from FLAG-parkin cells upon CCCP treatment and validated the significance of the actin cytoskeleton in the parkin-mitophagy process with co-localization studies. The MS enriched hits included proteins from the myosin family and actin binding protein known as drebrin. Myosin proteins have a motor domain and bind actin in an ATP sensitive manner and generate force through ATP hydrolysis. The amino-terminal motor domain is linked to a carboxyl-terminal tail via a neck domain that serves as the binding site for myosin light chains enabling the neck region to swing like a lever arm[108]. Expression of at least a dozen "unconventional" non-muscle myosins have been documented suggesting a wide range of functions for actin-based motors in the cell[116]. Myosins may participate in signal transduction such as myosin IX, one of the highest enriched proteins from the final elution fraction with CCCP treatment. Myosin IX appears to be cytoplasmic and partly associated with membranes and the actin cytoskeleton (Figure 4.1). Myosin IX may participate in signal transduction as the tail domain has 30% sequence identity with GTPase activating proteins of the Rho subfamily of small G-proteins, which act like molecular switches being active in their GTP-bound conformation and inactive in their GDP-bound conformation[108]. The Rho family of GTP-binding proteins has been implicated in the regulation of actin-based motile processes[116]. Myosin IX may serve to inactivate Rho in order to enable actin remodeling to occur. The role of myosin IX in the recruitment of parkin to the mitochondria may be due to the redistribution of parkin from the actin filaments to the mitochondria upon myosin IX actin remodeling[117]. Myosin IX

may also influence the trafficking of damaged mitochondria along microtubules by regulating the mitochondrial outer-membrane resident Miro GTPase, a protein regulating mitochondria transport, to make the mitochondria more accessible by parkin[118]. Interestingly, PINK1 was recently found to form a complex with Miro suggesting it may also play a role in mitochondrial transport[119]. The actin cytoskeleton has also been reported to mediate selective autophagy in *S. cerevisiae*, confirming our observations[120].

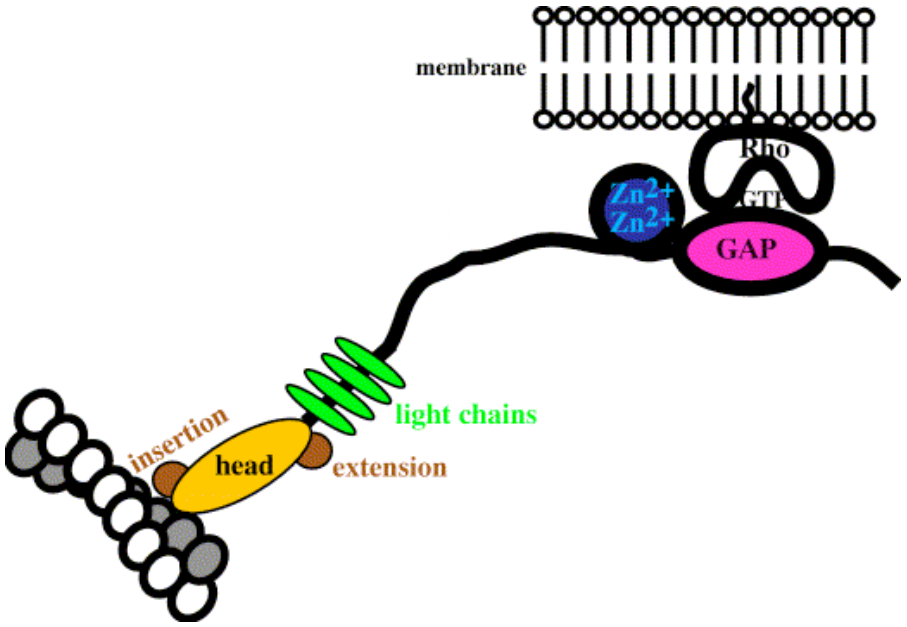


Figure 4.1 Myosin IX domains. The head domain interacts with actin and the GTPase-activating protein domain GAP stimulates the GTPase activity of GTP-bound Rho. Figure was adapted from reference 108.

4.3 Concluding remarks

We were able to specifically enrich for ubiquitylated proteins using tandem affinity purification of HBU conjugates and S5a affinity chromatography. The depth of coverage of the ubiquitin proteome based on these two approaches was still quite shallow resulting in an inadequacy to screen for parkin ubiquitin E3 ligase substrates. Future experiments to improve the enrichment of ubiquitin conjugates could combine the usage of a tetra-His_8-ubiquitin with GST-S5a affinity chromatography. MRM may also be used to detect low abundance known parkin substrate peptides in the ubiquitin purified material as a control for observing enrichment upon parkin over-expression (Kast, J; personal communication).

We were able to identify candidate parkin interactors from the chromogranin-secretogranin neuroendocrine family of proteins using direct FLAG-parkin immunoprecipitation. We were also able to identify non-muscle unconventional signaling myosin participating in the process of parkin-mediated mitophagy. Future experiments would be to validate these MS hits by co-immunoprecipitation and/or co-localization immunofluorescence to show a direct parkin interaction.

CHAPTER 5

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APPENDIX

Table 1. Known parkin substrates (A) and interactors that are not ligase targets (B). References are provided for each protein.

A

Known parkin substrate	Reference
CDCrel-1	121
synphilin-1	101
glycosylated alpha-synuclein	122
Pael-R	12
alpha and beta tubulin	93
synaptotagmin XI	102
aminoacyl-tRNA synthetase cofactor	123
cyclin E	124
S5a	125
programmed cell death-2 isoform 1	125
phospholipase C-gamma1	103
PICK1	127
far upstream element-binding protein-1	128
LIM kinase 1	129
Eps15	36
Ataxin-2	130
RanBP2	131

B

Known parkin non-substrate interactor	Reference
proteasome subunit alpha4	132
Nrdp1/FLRF	133
SEPT5_v2	134
SCF-like ubiquitin ligase complex	135
CHIP	136
CASK/LIN-2	137
PINK1	138
SUMO-1	139
microtubules	140
DJ-1	55
HDAC6	141

A

647.4006	LIFAGK
729.4095	LPEMLK
918.4559	NGDFLPTR
919.4399	NGDFLPTR
962.5225	SPILGYWK
966.4957	ITFCTGIR
1031.5797	LTQSMAIIR
1032.5637	LTQSMAIIR
1038.5094	EGIPPDQQR
1043.6352	VAHLALKHR
1047.5746	LTQSMAIIR
1066.6135	ESTLHLVLR
1090.5692	LTQSMAIIR
1093.563	MSPILGYWK
1109.5579	MSPILGYWK
1176.6503	LTAFVNTLNGK
1177.5761	NAMGSLASQATK
1177.6343	LTAFVNTLNGK
1178.5601	NAMGSLASQATK
1181.6768	RIEAIPQIDK
1193.5710	NAMGSLASQATK
1193.5710	NAMGSLASQATK
1194.5550	NAMGSLASQATK
1195.5656	MTISQQEFGR
1211.5605	MTISQQEFGR
1236.5656	NAMGSLASQATK
1350.737	MSPILGYWKIK
1418.7843	VDFLSKLPEMLK
1429.818	IEAIPQIDKYLK
1434.7792	VDFLSKLPEMLK
1440.75	DFETLKVDFLSK
1459.7783	LIFAGKQLEDGR
1460.7623	LIFAGKQLEDGR
1477.7195	NAMGSLASQATKDGK
1493.7144	NAMGSLASQATKDGK
1515.7966	AEISMLEGAVLDIR
1520.7140	NAMGSLASQATKDGK
1522.7740	IQDKEGIPPDQQR
1527.778	VGSPVEDNEKDLVK
1528.7621	VGSPVEDNEKDLVK
1531.7916	AEISMLEGAVLDIR
1536.7090	NAMGSLASQATKDGK
1565.7685	IQDKEGIPPDQQR
1602.7646	YIADKHNMLGGCPK
1603.7487	YIADKHNMLGGCPK
1618.7596	YIADKHNMLGGCPK
1709.8519	LQAQQDAVNIVCHSK
1710.8359	LQAQQDAVNIVCHSK
1752.8465	LQAQQDAVNIVCHSK
1753.8305	LQAQQDAVNIVCHSK
1764.9006	AKIQDKEGIPPDQQR
1786.9200	TITLEVEPSDTIENVK
1800.9403	ERAEISMLEGAVLDIR
1828.9306	TITLEVEPSDTIENVK
1947.9425	VNVDIINFGEEEVNTEK
1948.9265	VNVDIINFGEEEVNTEK
1949.9106	VNVDIINFGEEEVNTEK
1972.0517	IIAFVGSPVEDNEKDLVK
2129.1480	TLSDYNIQKESTLHLVLR
2130.1321	TLSDYNIQKESTLHLVLR
2205.0801	EKVNVDIINFGEEEVNTEK
2207.0481	EKVNVDIINFGEEEVNTEK
2248.0746	EKVNVDIINFGEEEVNTEK
2268.1313	LLLEYLEEKYEEHLYER
2288.1999	TLTGKTITLEVEPSDTIENVK
2319.1077	AAAASAAEAGIATTGTEDSDDALLK
2325.1331	YIAWPLQGWQATFGGGDHPPK
2329.2264	TLTGKTITLEVEPSDTIENVK
2356.1991	KFELGLEFPNLPYYIDGDVK
2357.1831	KFELGLEFPNLPYYIDGDVK
2399.1936	KFELGLEFPNLPYYIDGDVK
2401.2588	TLTGKTITLEVEPSDTIENVK
2486.3479	TLTGKTITLEVEPSDTIENVKAK
2827.4828	QLEDGRTLSDYNIQKESTLHLVLR
3013.4662	SNPENNVGLITLANDCEVLTTLTPDTGR
3015.4343	SNPENNVGLITLANDCEVLTTLTPDTGR
3107.5663	VNVDIINFGEEEVNTEKLTAFVNTLNGK
3270.615	TRSNPENNVGLITLANDCEVLTTLTPDTGR
3654.9178	HLTMQIFVKTLTGKTITLEVEPSDTIENVK

B

776.3817	FSGVPDR
1104.6655	LLIYKVSNR
1242.6608	VNSAAFPAPIEK
1302.6092	FSGSGSGTDFTLK
1591.7114	QNGVLNSWTDQDSK
1620.709	SLTSEDSAVYYCAR
1980.8775	NTQPIMNTNGSYFVYSK
2241.9947	DSTYSMSSTLTLTKDEYER

Table 2. Exclusion list for anti-FLAG immunoglobulin chain peptides (A) and GST-S5a peptides (B). The left column is the m/z of the corresponding excluded peptide. Different variable modifications assigned to peptides may result in more than one m/z per peptide sequence such as a regular versus Met oxidized peptide.

Table 3. FLAG-parkin pull-down proteins with SILAC ratios – weak interactor fraction

Proteins eluted with mild detergent and salt (weak parkin interactors)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
NPY Neuropeptide Y	52	1	-3.20
HMGB3 High mobility group protein B3	31	1	-2.71
C9orf86 Isoform 2 of Putative GTP-binding protein Parf	1688	2	-2.17
TPM1 Tropomyosin isoform	90	2	-2.09
SCG2 Secretogranin-2	48	41	-2.07
HNRNPUL1 Isoform 1 of Heterogeneous nuclear ribonucleoprotein U-like protein 1	108	3	-1.89
SCG3 Secretogranin-3	175	5	-1.80
C1orf144 Isoform 4 of UPF0485 protein C1orf144	74	2	-1.79
NPDC1 Neural proliferation differentiation and control protein 1	71	1	-1.61
BAG4 BAG family molecular chaperone regulator 4	50	1	-1.61
FAM3C Protein FAM3C	163	2	-1.54
EIF4EBP1 Eukaryotic translation initiation factor 4E-binding protein 1	696	8	-1.38
CHGA Chromogranin-A	343	21	-1.36
HN1L Isoform 1 of Hematological and neurological expressed 1-like protein	201	6	-1.35
PPP1R14B Protein phosphatase 1 regulatory subunit 14B	47	1	-1.30
BASP1 Brain acid soluble protein 1	240	5	-1.22
PTTG1IP Uncharacterized protein PTTG1IP	93	3	-1.22
COX7A2 Uncharacterized protein COX7A2	72	1	-1.20
C19orf43 Uncharacterized protein C19orf43	121	3	-1.19
RPL12 Isoform 2 of 60S ribosomal protein L12	48	1	-1.17
VGF VGF nerve growth factor inducible precursor	874	22	-1.16
LOC389842 similar to RanBP1	26	1	-1.16
SSNA1 Sjoegren syndrome nuclear autoantigen 1	104	2	-1.08
PIN4 protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4	99	2	-1.07
TMSB10 Thymosin beta-10	253	6	-1.03
TTYH3 Isoform 1 of Protein tweety homolog 3	61	1	-1.02
MRPS36 28S ribosomal protein S36, mitochondrial	179	5	-1.00
C14orf166 UPF0568 protein C14orf166	127	2	-1.00
HNRNPU Isoform Long of Heterogeneous nuclear ribonucleoprotein U	99	2	-1.00
C17orf49 20 kDa protein	78	1	-0.98
MYCBP C-Myc-binding protein	49	1	-0.97
LOC348262 hypothetical protein LOC348262	132	4	-0.91
CRIP2 Cysteine-rich protein 2	518	6	-0.90
FAM107B cDNA FLJ45505 fis, clone BRTHA2020642, weakly similar to DRR1 protein	99	2	-0.90
LSM4 U6 snRNA-associated Sm-like protein LSm4	97	2	-0.88
EIF4H Similar to mKIAA0038 protein	48	1	-0.85
TAGLN2 Transgelin-2	356	9	-0.83
RPL36 60S ribosomal protein L36	74	1	-0.83
ANP32E Acidic leucine-rich nuclear phosphoprotein 32 family member E	52	1	-0.82
KIAA0515 hypothetical protein LOC84726	48	1	-0.81
NDUFA5 Putative uncharacterized protein DKFZp781K1356	210	4	-0.80
GTF2A1 Isoform 42 kDa of Transcription initiation factor IIA subunit 1	160	3	-0.75
XTP3TPA XTP3-transactivated gene A protein	434	10	-0.74
HDGF Hepatoma-derived growth factor	420	8	-0.74
RPLP2 60S acidic ribosomal protein P2	1721	32	-0.74
HN1 Isoform 1 of Hematological and neurological expressed 1 protein	448	9	-0.73
PFDN4 Prefoldin subunit 4	404	10	-0.70
PABPC1 Isoform 1 of Polyadenylate-binding protein 1	289	6	-0.69
- cDNA FLJ44434 fis, clone UTERU2019491, moderately similar to Homo sapiens 41-kDa phosphoribosylpyrophosphate synthetase-associated protein	434	7	-0.69
CARHSP1 Calcium-regulated heat stable protein 1	216	4	-0.68
PGRMC2 Membrane-associated progesterone receptor component 2	66	1	-0.68
FAM162A UPF0389 protein FAM162A	79	1	-0.67
BCAP31 B-cell receptor-associated protein 31	99	2	-0.66
MAPT Isoform Tau-A of Microtubule-associated protein tau	675	17	-0.65
RPL38 8 kDa protein	47	1	-0.65
SCAMP3 secretory carrier membrane protein 3 isoform 1	201	5	-0.63
RPL8 60S ribosomal protein L8	69	2	-0.60
GADD45GIP1 Growth arrest and DNA-damage-inducible proteins-interacting protein 1	51	1	-0.60
PSME3 Isoform 1 of Proteasome activator complex subunit 3	112	2	-0.59
PDAP1 28 kDa heat- and acid-stable phosphoprotein	624	15	-0.59
ATP5J ATP synthase-coupling factor 6, mitochondrial	145	5	-0.59
SF3B2 splicing factor 3B subunit 2	87	2	-0.57
ST13 Hsc70-interacting protein	670	17	-0.57
MRPL12 39S ribosomal protein L12, mitochondrial	202	6	-0.56
RPS25 40S ribosomal protein S25	465	12	-0.55
FAU 40S ribosomal protein S30	287	10	-0.55
EIF3J Eukaryotic translation initiation factor 3 subunit J	343	9	-0.54
LYRM7 LYR motif-containing protein 7	76	2	-0.53
VAMP3 Vesicle-associated membrane protein 3	218	4	-0.53
CPLX2 Complexin-2	57	1	-0.53
UBE2L3 Ubiquitin-conjugating enzyme E2 L3	254	6	-0.52
RPS10 40S ribosomal protein S10	451	10	-0.51
PEBP1 Phosphatidylethanolamine-binding protein 1	1423	29	-0.51
PRKCSH Glucosidase 2 subunit beta	69	2	-0.50
TPM4 Isoform 1 of Tropomyosin alpha-4 chain	961	24	-0.50
RPE Isoform 1 of Ribulose-phosphate 3-epimerase	180	2	-0.50
EDF1 Isoform 1 of Endothelial differentiation-related factor 1	129	3	-0.50
- 78 kDa protein	92	2	-0.49
SFRS3 Splicing factor, arginine/serine-rich 3	661	18	-0.48
SFRS7 Isoform 1 of Splicing factor, arginine/serine-rich 7	82	3	-0.48
SRP14 Signal recognition particle 14 kDa protein	288	4	-0.47

Proteins eluted with mild detergent and salt (weak parkin interactors)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
PFDN6 Prefoldin subunit 6	429	9	-0.46
NUCB1 Nucleobindin-1	195	6	-0.46
UBAP2L Isoform 2 of Ubiquitin-associated protein 2-like	253	5	-0.46
HMGB1 High mobility group protein B1	1645	44	-0.45
RPS19 40S ribosomal protein S19	625	17	-0.45
RPL23A;hCG_16001 60S ribosomal protein L23a	141	4	-0.45
CHCHD8 Isoform 2 of Coiled-coil-helix-coiled-coil-helix domain-containing protein 8	194	4	-0.43
LRRC59 Leucine-rich repeat-containing protein 59	763	16	-0.42
STMN1 Stathmin	1295	34	-0.41
RPS13 40S ribosomal protein S13	320	7	-0.40
PNPO 19 kDa protein	258	6	-0.40
RANBP1 Ran-specific GTPase-activating protein	340	9	-0.40
MMAB Cob(I)yrinic acid a,c-diamide adenosyltransferase, mitochondrial	54	1	-0.40
NOL3 Isoform 1 of Nucleolar protein 3	241	2	-0.39
RPLP1 60S acidic ribosomal protein P1	146	2	-0.38
TPM3 tropomyosin 3 isoform 4	2107	55	-0.37
EBNA1BP2 EBNA1 binding protein 2	68	1	-0.36
HIST1H2AH Histone H2A type 1-H	1110	32	-0.34
hCG_22804 hypothetical protein LOC645441	327	8	-0.34
NUCKS1 Isoform 1 of Nuclear ubiquitous casein and cyclin-dependent kinases substrate	421	7	-0.33
IGBP1 Immunoglobulin-binding protein 1	60	1	-0.33
ATPIF1 Putative uncharacterized protein DKFZp564G0422	286	6	-0.32
PCNP Isoform 1 of PEST proteolytic signal-containing nuclear protein	112	3	-0.32
RPS15 40S ribosomal protein S15	130	3	-0.32
- Similar to 60S ribosomal protein L35	189	4	-0.30
H2AFV Histone H2A.V	210	6	-0.28
CCDC124 Coiled-coil domain-containing protein 124	165	5	-0.28
RPS5 40S ribosomal protein S5	130	2	-0.27
BTF3 Isoform 1 of Transcription factor BTF3	243	5	-0.27
H1FX Histone H1x	155	3	-0.26
CNBP cDNA FLJ77718	167	4	-0.26
YBX1 Nuclease-sensitive element-binding protein 1	1354	28	-0.25
NUPL1 Isoform 1 of Nucleoporin p58/p45	49	1	-0.25
MRPS33 MRPS33 protein (Fragment)	57	1	-0.24
RPL31 60S ribosomal protein L31	230	5	-0.24
FUS Isoform Short of RNA-binding protein FUS	169	3	-0.24
S100A4 Protein S100-A4	115	3	-0.23
PDIA3 14 kDa protein	50	1	-0.22
HINT1 Histidine triad nucleotide-binding protein 1	288	7	-0.22
EEF1A2 Elongation factor 1-alpha 2	83	3	-0.21
MRPL23 39S ribosomal protein L23, mitochondrial	100	2	-0.20
RPS26 Ribosomal protein 26 (RPS26) pseudogene	82	2	-0.20
PRPS1 Ribose-phosphate pyrophosphokinase 1	163	3	-0.20
MIF;LOC284889 Macrophage migration inhibitory factor	735	15	-0.19
PIN1 Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	450	10	-0.19
EIF4B eukaryotic translation initiation factor 4B	311	7	-0.19
ENAH Isoform 2 of Protein enabled homolog	82	2	-0.19
CHMP4B Charged multivesicular body protein 4b	91	1	-0.17
UBQLN4 Ubiquilin-4	77	2	-0.17
CNPY2 Isoform 1 of Protein canopy homolog 2	247	5	-0.16
PGRMC1 Membrane-associated progesterone receptor component 1	223	6	-0.15
DBI Isoform a 1 of Acyl-CoA-binding protein	337	10	-0.14
TPD52L2 Isoform 2 of Tumor protein D54	115	1	-0.14
HIST1H1C Histone H1.2	362	5	-0.14
DRAP1 Isoform 1 of Dr1-associated corepressor	144	2	-0.13
TBCA Tubulin-specific chaperone A	846	21	-0.13
TIMM13 Mitochondrial import inner membrane translocase subunit Tim13	111	2	-0.13
RPL18 60S ribosomal protein L18	125	2	-0.12
TPD52 N8 protein long isoform (Fragment)	479	10	-0.11
MRPL49 39S ribosomal protein L49, mitochondrial	98	2	-0.08
UBE2N Ubiquitin-conjugating enzyme E2 N	143	4	-0.08
C14orf156 SRA stem-loop-interacting RNA-binding protein, mitochondrial	514	12	-0.07
FTL Ferritin light chain	154	3	-0.05
NDUFB10 NDUFB10 protein	56	1	-0.04
- 4 kDa protein	221	6	-0.04
HMGB2 High mobility group protein B2	684	18	-0.04
SFRS2 Splicing factor, arginine/serine-rich 2	258	8	-0.04
NAP1L4 Nucleosome assembly protein 1-like 4	69	1	-0.02
KHDRBS1 Isoform 1 of KH domain-containing, RNA-binding, signal transduction-associated	177	4	0.00
PODXL2 Isoform 2 of Podocalyxin-like protein 2	46	1	0.01
PSME1 Proteasome activator complex subunit 1	581	12	0.01
HNRNPK Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	484	12	0.01
PSME2 Uncharacterized protein PSME2	275	8	0.02
WHSC2 Isoform 1 of Negative elongation factor A	70	1	0.04
EEF1E1 Eukaryotic translation elongation factor 1 epsilon-1	48	1	0.04
FKBP3 FK506-binding protein 3	126	3	0.05
RPS20 40S ribosomal protein S20	178	6	0.05
NOLA3 H/ACA ribonucleoprotein complex subunit 3	174	3	0.08
DDT D-dopachrome decarboxylase	91	2	0.08
NAP1L1 Nucleosome assembly protein 1-like 1	459	10	0.10
RPS24 Isoform 2 of 40S ribosomal protein S24	249	5	0.11
CRMP1 collapsin response mediator protein 1 isoform 1	88	2	0.12
RPS29 40S ribosomal protein S29	47	1	0.12
BID Isoform 1 of BH3-interacting domain death agonist	174	4	0.12

Proteins eluted with mild detergent and salt (weak parkin interactors)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
RBP1 21 kDa protein	416	8	0.13
SET Isoform 1 of Protein SET	500	11	0.13
UBA52 ubiquitin and ribosomal protein L40 precursor	103	3	0.14
S100A11 Protein S100-A11	190	3	0.16
PFDN2 Prefoldin subunit 2	464	13	0.17
KIAA1704 Isoform 3 of Uncharacterized protein KIAA1704	101	3	0.18
C11orf59 UPF0404 protein C11orf59	157	2	0.18
STX12 Syntaxin-12	180	5	0.20
TUBB3 Tubulin beta-3 chain	296	7	0.24
LOC100128936 similar to ribosomal protein L10a	58	1	0.24
PAFAH1B3 Platelet-activating factor acetylhydrolase IB subunit gamma	51	1	0.25
MYO15A Myosin-XV	51	3	0.26
SOD1 Superoxide dismutase [Cu-Zn]	1774	38	0.27
NEDD8 Uncharacterized protein NEDD8 (Fragment)	239	6	0.28
MRLC2 Myosin regulatory light chain	79	2	0.29
NENF Neudesin	78	1	0.29
PEF1 Peflin	163	2	0.30
C19orf53 Leydig cell tumor 10 kDa protein homolog	76	2	0.30
RBMX Heterogeneous nuclear ribonucleoprotein G	156	3	0.30
RPL24 60S ribosomal protein L24	74	1	0.30
RPS17 40S ribosomal protein S17	450	11	0.31
- 28 kDa protein	257	5	0.33
ATP6V1E1 Vacuolar proton pump subunit E 1	472	7	0.34
HIST2H2BE Histone H2B type 2-E	78	2	0.35
HIST2H4B;HIST1H4F;HIST1H4J;HIST1H4C;HIST1H4B;HIST1H4K;HIST1H4E;HIST2H4A;HIST1H4H;HIST1H4L;HIST1H4A;HIST4H4;HIST1H4D;HIST1H4I Histone H4	778	16	0.36
SNRPD2 Small nuclear ribonucleoprotein Sm D2	63	1	0.36
ANP32A Acidic leucine-rich nuclear phosphoprotein 32 family member A	964	24	0.37
TFRC Transferrin receptor protein 1	55	1	0.37
SNRPG Small nuclear ribonucleoprotein G	110	2	0.37
SCYE1 Multisynthetase complex auxiliary component p43	401	11	0.41
LOC653314 similar to ribosomal protein L19	51	1	0.41
EEF1B2 Elongation factor 1-beta	80	1	0.42
CCT5 T-complex protein 1 subunit epsilon	168	4	0.43
ATP5I ATP synthase, H+ transporting, mitochondrial F0 complex, subunit E	69	2	0.44
TUBB2B Tubulin beta-2B chain	182	2	0.45
PTBP1 Isoform 1 of Polypyrimidine tract-binding protein 1	71	1	0.46
SNRPD1 Small nuclear ribonucleoprotein Sm D1	352	7	0.46
- Uncharacterized protein ENSP00000348237	247	7	0.48
AIP AH receptor-interacting protein	94	2	0.48
STIP1 cDNA FLJ76863, highly similar to Homo sapiens stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein) (STIP1), mRNA	351	9	0.50
NPM1 nucleophosmin 1 isoform 3	1768	30	0.51
PRDX6 Peroxiredoxin-6	287	5	0.52
HSPE1 10 kDa heat shock protein, mitochondrial	1436	30	0.52
DUT deoxyuridine triphosphatase isoform 1 precursor	120	2	0.52
HNRNPA2B1 Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1	124	3	0.54
FKBP4 FK506-binding protein 4	58	1	0.54
GNB1 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	48	1	0.56
TUBA1A Tubulin alpha-1A chain	1210	26	0.56
- Uncharacterized protein ENSP00000375523	180	3	0.58
HIST1H2BD Histone H2B type 1-D	790	19	0.60
SORD Sorbitol dehydrogenase	76	2	0.62
RPS21 40S ribosomal protein S21	137	4	0.62
SYNCRIP Isoform 1 of Heterogeneous nuclear ribonucleoprotein Q	84	2	0.62
SDHB Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	132	3	0.63
YWHAE 14-3-3 protein epsilon	650	16	0.65
GOT2 Aspartate aminotransferase, mitochondrial	62	2	0.66
GLO1 Lactoylglutathione lyase	63	1	0.67
KHSRP Isoform 1 of Far upstream element-binding protein 2	257	6	0.67
ACAT1 Acetyl-CoA acetyltransferase, mitochondrial	531	14	0.70
SOD2 Superoxide dismutase [Mn], mitochondrial	111	2	0.72
PDCD5 Programmed cell death protein 5	535	11	0.72
SEC22B Vesicle-trafficking protein SEC22b	73	1	0.75
CALM3;CALM2;CALM1 Calmodulin	2025	43	0.75
CKB Creatine kinase B-type	1382	27	0.76
PSMC1 26S protease regulatory subunit 4	193	4	0.76
NME2 Nucleoside diphosphate kinase	314	10	0.76
PGAM2 Phosphoglycerate mutase 2	103	2	0.76
GSTP1 Glutathione S-transferase P	260	4	0.80
TUBB Tubulin beta chain	1950	42	0.80
HSP90AB1 Heat shock protein HSP 90-beta	186	5	0.80
HSPA9 Stress-70 protein, mitochondrial	707	15	0.81
HMGA1 Isoform HMG-Y of High mobility group protein HMG-I/HMG-Y	57	1	0.81
TPI1 Isoform 1 of Triosephosphate isomerase	836	18	0.83
- Calnexin (Fragment)	167	2	0.84
HSPA5 HSPA5 protein	159	3	0.85
PPIA Peptidyl-prolyl cis-trans isomerase A	2425	52	0.86
PRDX2 Peroxiredoxin-2	222	5	0.86
CALU Calumenin precursor	126	3	0.88
TUBB4 Tubulin beta-4 chain	63	1	0.88
CSTB Cystatin-B	61	1	0.88
PRDX1 19 kDa protein	197	5	0.89
RPS16 40S ribosomal protein S16	60	1	0.89

Proteins eluted with mild detergent and salt (weak parkin interactors)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
EEF1AL3;EEF1A1 Putative elongation factor 1-alpha-like 3	331	11	0.89
PFN1 Profilin-1	891	18	0.90
TOMM34 Mitochondrial import receptor subunit TOM34	49	1	0.93
- Peptidyl-prolyl cis-trans isomerase	31	1	0.93
NASP Isoform 1 of Nuclear autoantigenic sperm protein	57	1	0.94
YWHAG 14-3-3 protein gamma	207	3	0.95
CCT2 T-complex protein 1 subunit beta	110	3	0.95
ACTB Actin, cytoplasmic 1	1085	25	0.95
EEF2 Elongation factor 2	151	3	0.97
PDIA3 Protein disulfide-isomerase A3	349	9	0.97
HSPD1 60 kDa heat shock protein, mitochondrial	1780	43	0.99
EFTUD2 116 kDa U5 small nuclear ribonucleoprotein component	48	1	0.99
HSPA8 Isoform 1 of Heat shock cognate 71 kDa protein	179	5	0.99
PSMC5 26S protease regulatory subunit 8	54	1	1.00
KPNB1 Importin subunit beta-1	264	4	1.00
PDIA6 Isoform 2 of Protein disulfide-isomerase A6	334	6	1.01
GPI Glucose-6-phosphate isomerase	200	4	1.01
CAPZA1 F-actin-capping protein subunit alpha-1	52	1	1.02
PCMT1 Isoform 1 of Protein-L-isoaspartate(D-aspartate) O-methyltransferase	238	8	1.02
CCT6A T-complex protein 1 subunit zeta	81	2	1.02
PRDX3 Thioredoxin-dependent peroxide reductase, mitochondrial	319	6	1.03
hCG_1984468 hypothetical protein LOC389672	51	1	1.03
YWHAB Isoform Long of 14-3-3 protein beta/alpha	83	1	1.04
MDH2 Malate dehydrogenase, mitochondrial	644	11	1.04
HSPH1 Heat shock 105kDa/110kDa protein 1	54	1	1.05
CFL1 Cofilin-1	296	8	1.06
ENO1 Isoform alpha-enolase of Alpha-enolase	2995	56	1.06
GAPDH Glyceraldehyde-3-phosphate dehydrogenase	1378	32	1.07
ALCAM Isoform 1 of CD166 antigen	53	1	1.07
ATP5B ATP synthase subunit beta, mitochondrial	238	6	1.07
CLIC1 Chloride intracellular channel protein 1	83	2	1.09
AK1 Adenylate kinase isoenzyme 1	472	11	1.10
FARSA Phenylalanyl-tRNA synthetase alpha chain	99	2	1.10
SSB Lupus La protein	320	6	1.11
DCI Isoform 1 of 3,2-trans-enoyl-CoA isomerase, mitochondrial	55	1	1.12
SNRPD3 Small nuclear ribonucleoprotein Sm D3	48	1	1.21
NCL Isoform 1 of Nucleolin	328	8	1.21
- Uncharacterized protein ENSP00000382131 (Fragment)	22	1	1.22
MSI2 Isoform 1 of RNA-binding protein Musashi homolog 2	73	2	1.23
PSMA5 Proteasome subunit alpha type-5	134	2	1.24
PIR Pirin	133	2	1.31
ENO2 Gamma-enolase	199	3	1.34
PKM2 Isoform M2 of Pyruvate kinase isozymes M1/M2	1001	22	1.39
PGK1 Phosphoglycerate kinase 1	711	13	1.42
NP CDNA FLJ25678 fis, clone TST04067, highly similar to PURINE NUCLEOSIDE PHOSPHORYLASE	50	1	1.48
ANXA5 Annexin A5	174	2	1.50
PYCR1 pyrroline-5-carboxylate reductase 1 isoform 2	89	2	1.50
LOC439992 similar to v-fos transformation effector protein isoform 2	86	1	1.56
ATP5A1 ATP synthase subunit alpha, mitochondrial	274	5	1.58
ALDOA Fructose-bisphosphate aldolase A	59	1	1.59
NCAM1 Isoform 3 of Neural cell adhesion molecule 1	48	1	2.01
SNX13 Isoform 2 of Sorting nexin-13	46	1	4.44

Table 4. FLAG-parkin pull-down proteins with SILAC ratios – strong interactor fraction

Proteins eluted with SDS sample buffer (strong parkin interactors)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
SKP1 Isoform 2 of S-phase kinase-associated protein 1	72	1	-1.42
IVNS1ABP Influenza virus NS1A-binding protein	231	4	-1.25
NAPA Alpha-soluble NSF attachment protein	92	3	-1.20
PPA1 Inorganic pyrophosphatase	187	4	-1.13
CDC37 Hsp90 co-chaperone Cdc37	55	1	-1.04
FBXO21 FBXO21 protein	54	1	-0.89
PARK7 Protein DJ-1	52	1	-0.83
HNRNPD Isoform 1 of Heterogeneous nuclear ribonucleoprotein D0	45	1	-0.83
DUT Isoform DUT-M of Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial	395	5	-0.76
ENO3 Beta-enolase	68	1	-0.71
ENO2 Gamma-enolase	172	3	-0.61
ATP6V1G2 Vacuolar proton pump subunit G 2	148	3	-0.60
PDIA3 14 kDa protein	69	1	-0.57
- 28 kDa protein	303	5	-0.56
NEFH Neurofilament heavy polypeptide	97	2	-0.54
SPIN3 Isoform 1 of Spindlin-3	51	1	-0.51
PRDX1 Peroxiredoxin-1	552	13	-0.50
KRT9 Keratin, type I cytoskeletal 9	351	6	-0.49
HNRNPA1 Isoform A1-B of Heterogeneous nuclear ribonucleoprotein A1	287	4	-0.48
CALR Calreticulin	142	3	-0.48
PSMC4 Isoform 1 of 26S protease regulatory subunit 6B	69	1	-0.44
TUBA1B Tubulin alpha-1B chain	58	1	-0.44
UBA52 ubiquitin and ribosomal protein L40 precursor	134	3	-0.44
LOC646817 similar to template acyivating factor-I alpha	298	5	-0.42
PDIA3 Protein disulfide-isomerase A3	401	9	-0.34
EIF3F Eukaryotic translation initiation factor 3 subunit 5	274	2	-0.31
KIAA0515 hypothetical protein LOC84726	45	1	-0.31
TUBA1A Tubulin alpha-1A chain	2949	51	-0.30
PRDX2 Peroxiredoxin-2	148	4	-0.30
ACTA2 Actin, aortic smooth muscle	21	1	-0.29
HSPA5 HSPA5 protein	287	6	-0.28
CRKL Crk-like protein	101	2	-0.28
STIP1 Stress-induced-phosphoprotein 1	181	2	-0.28
ARHGDIA Rho GDP-dissociation inhibitor 1	425	8	-0.28
TPM3 Putative uncharacterized protein DKFZp686J1372	357	7	-0.27
TAGLN2 Transgelin-2	86	2	-0.27
RBP1 Retinol-binding protein I, cellular	363	7	-0.27
TUBB2A Tubulin beta-2A chain	332	4	-0.25
GTF2I Isoform 1 of General transcription factor II-I	58	1	-0.23
HSP90AB1 Heat shock protein HSP 90-beta	1324	36	-0.22
UCHL1 Ubiquitin carboxyl-terminal hydrolase isozyme L1	491	14	-0.20
PEBP1 Phosphatidylethanolamine-binding protein 1	608	10	-0.20
- RcNSEP1 (Fragment)	121	2	-0.17
YWHAE 14-3-3 protein epsilon	541	11	-0.17
YWHAQ 14-3-3 protein theta	183	3	-0.17
BOLA2;BOLA2B Isoform 2 of BolA-like protein 2	57	1	-0.11
ACTB Actin, cytoplasmic 1	338	5	-0.10
HNRNPK Heterogeneous nuclear ribonucleoprotein K	493	12	-0.10
PRDX4 Peroxiredoxin-4	208	4	-0.10
TUBB Tubulin beta chain	3864	69	-0.09
HSPA8 Isoform 1 of Heat shock cognate 71 kDa protein	1891	40	-0.09
PFDN5 Prefoldin subunit 5	96	2	-0.08
CFL1 Cofilin-1	1091	22	-0.08
NCL cDNA FLJ45706 fis, clone FEBRA2028457, highly similar to Nucleolin	586	10	-0.08
GNB1 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	63	1	-0.07
YWHAG 14-3-3 protein gamma	613	10	-0.06
HSP90AA1 Isoform 1 of Heat shock protein HSP 90-alpha	337	8	-0.06
ATP5B ATP synthase subunit beta, mitochondrial	306	5	-0.05
NPM1 Isoform 1 of Nucleophosmin	187	4	-0.05
ATP5A1 ATP synthase subunit alpha, mitochondrial	48	1	-0.04
PPIA Peptidyl-prolyl cis-trans isomerase A	1985	38	-0.03
HSPD1 60 kDa heat shock protein, mitochondrial	2143	43	-0.03
CANX Calnexin	131	2	-0.03
RCN2 Reticulocalbin-2	149	3	0.01
CALU Calumenin precursor	282	8	0.01
TUBB2C Tubulin beta-2C chain	78	3	0.02
ACTG1 Actin, cytoplasmic 2	1178	21	0.02
HSPA9 Stress-70 protein, mitochondrial	685	16	0.05
EIF5A2 Eukaryotic translation initiation factor 5A-2	138	2	0.06
TCEB2 Transcription elongation factor B polypeptide 2	107	2	0.06
UCHL3 Ubiquitin carboxyl-terminal hydrolase isozyme L3	72	2	0.15
PFN1 Profilin-1	932	18	0.17
MAP3K7IP1 Mitogen-activated protein kinase kinase kinase 7-interacting protein 1	53	1	0.17
CCT2 T-complex protein 1 subunit beta	148	3	0.17
EEF1B2 Elongation factor 1-beta	66	1	0.21
HNRNPL heterogeneous nuclear ribonucleoprotein L isoform a	46	1	0.22
WDR77 Methylosome protein 50	518	8	0.24
CSTB Cystatin-B	280	8	0.27
PGK1 Phosphoglycerate kinase 1	50	1	0.28
LOC100133841 similar to Peptidase D	74	1	0.29

Proteins eluted with SDS sample buffer (strong parkin interactors)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
CKB Creatine kinase B-type	683	14	0.29
HNRNPA2B1 Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1	144	4	0.30
PRDX5 Isoform Mitochondrial of Peroxiredoxin-5, mitochondrial	49	1	0.30
GSTP1 Glutathione S-transferase P	329	4	0.32
HSPE1 10 kDa heat shock protein, mitochondrial	243	3	0.34
YWHAB Isoform Long of 14-3-3 protein beta/alpha	235	4	0.38
PFN2 Isoform IIb of Profilin-2	120	2	0.39
HNRNPH1 Heterogeneous nuclear ribonucleoprotein H	104	3	0.40
PA2G4 20 kDa protein	76	2	0.42
ECH1 Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	127	2	0.45
ENO1 Isoform alpha-enolase of Alpha-enolase	1159	21	0.46
TUBB3 Tubulin beta-3 chain	430	7	0.48
TPI1 Isoform 1 of Triosephosphate isomerase	239	5	0.53
MIF;LOC284889 Macrophage migration inhibitory factor	174	3	0.55
ATAD4 Proteasome 26S ATPase subunit 5 variant (Fragment)	88	1	0.56
NP CDNA FLJ25678 fis, clone TST04067, highly similar to PURINE NUCLEOSIDE PHOSPHORYLASE	54	1	0.58
PDIA6 Isoform 2 of Protein disulfide-isomerase A6	53	1	0.59
DPYSL2 Dihydropyrimidinase-related protein 2	110	2	0.59
PCMT1 Isoform 1 of Protein-L-isoaspartate(D-aspartate) O-methyltransferase	580	11	0.59
STRAP Serine-threonine kinase receptor-associated protein	119	2	0.60
MDH2 Malate dehydrogenase, mitochondrial	83	2	0.61
PRDX6 Peroxiredoxin-6	259	6	0.62
NME1;NME2 Nucleoside diphosphate kinase A	107	3	0.72
CCT8 59 kDa protein	60	1	0.86
PRMT5 protein arginine methyltransferase 5 isoform b	124	3	0.88
PAFAH1B3 Platelet-activating factor acetylhydrolase IB subunit gamma	53	1	0.92
FKBP4 FK506-binding protein 4	57	1	0.98
ACAT1 Acetyl-CoA acetyltransferase, mitochondrial	194	5	1.02
ALDOA Fructose-bisphosphate aldolase A	112	3	1.15
PKM2 Isoform M1 of Pyruvate kinase isozymes M1/M2	124	2	1.33
GAPDH Glyceraldehyde-3-phosphate dehydrogenase	448	10	1.44

Table 5. FLAG-parkin pull-down proteins under CCCP with SILAC ratios – strong interactor fraction

Proteins eluted with SDS sample buffer (strong parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
KIAA1618 Isoform 3 of Protein ALO17	33	1	6.29
SH3YL1 isoform 5 of SH3 domain-containing YSC84-like protein 1	28	1	3.60
MRLC2 Myosin regulatory light chain	700	13	19.14
TMEM64 transmembrane protein 64	26	1	3.49
PSMC6 26S protease regulatory subunit S10B	40	1	2.68
MYH9 Myosin-9	1722	32	16.95
EIF4A1 Eukaryotic initiation factor 4A-I	101	3	1.88
AIFM1 Isoform 1 of Apoptosis-inducing factor 1, mitochondrial	52	1	1.88
RAD50 Isoform 1 of DNA repair protein RAD50	105	3	1.73
MYL6;MYL6B Isoform Non-muscle of Myosin light polypeptide 6	187	4	1.61
HYOU1 Hypoxia up-regulated protein 1	133	3	1.56
RPS24 Isoform 1 of 40S ribosomal protein S24	254	4	1.46
CALU Isoform 2 of Calumenin	287	7	1.44
MYH10 Isoform 1 of Myosin-10	995	19	1.57
AKR1C1 Aldo-keto reductase family 1 member C1	25	2	1.36
UCHL5 Isoform 2 of Ubiquitin carboxyl-terminal hydrolase isozyme L5	57	1	1.35
SF1 Isoform 2 of Splicing factor 1	188	1	1.35
GTF2F2 General transcription factor IIF subunit 2	30	1	1.30
LOC728453 similar to 40S ribosomal protein S28	32	1	1.24
EIF3E Eukaryotic translation initiation factor 3 subunit E	32	1	1.23
CALR Calreticulin	28	4	1.19
DBN1 Isoform 1 of Drebrin	26	33	1.17
TMOD2 Tropomodulin-2	35	3	1.13
CAPZB Capping protein	223	9	1.09
LIMA1 Isoform Beta of LIM domain and actin-binding protein 1	2024	1	1.08
PDIA3 Protein disulfide-isomerase A3	34	17	1.07
RPS28 40S ribosomal protein S28	345	2	1.06
FOLH1 Isoform PSMA-1 of Glutamate carboxypeptidase 2	182	1	1.05
ATAD3B TOB3	429	1	1.05
ACTB Actin, cytoplasmic 1	79	14	1.05
ABLIM1 Isoform 1 of Actin-binding LIM protein 1	767	3	1.04
CAPZA1 F-actin-capping protein subunit alpha-1	66	7	1.04
CCNT1 Cyclin-T1	27	2	1.04
DHX15 Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	29	4	1.01
ACTG1 Actin, cytoplasmic 2	1019	178	1.00
PFKFB3 Fructose-6-phosphate,2-kinase/fructose-2, 6-bisphosphatase (Fragment)	146	1	0.99
L1TD1 LINE-1 type transposase domain-containing protein 1	281	1	0.96
SVIL Isoform 2 of Supervillin	50	2	0.95
SLC9A5 Solute carrier family 9 (Sodium/hydrogen exchanger), isoform 5 variant	163	1	0.95
ACTA2 Actin, aortic smooth muscle	12531	5	0.93
TPM4 Isoform 1 of Tropomyosin alpha-4 chain	33	6	0.92
HDX Isoform 2 of Highly divergent homeobox	26	7	0.90
IDH2 Isocitrate dehydrogenase [NADP], mitochondrial	66	1	0.89
CHGB Secretogranin-1	42	2	0.88
EIF3I Eukaryotic translation initiation factor 3 subunit I	222	8	0.86
EXOSC8 Exosome complex exonuclease RRP43	327	9	0.85
HSPA5 HSPA5 protein	442	26	0.85
KLKB1 Plasma kallikrein	41	1	0.82
EIF3F Eukaryotic translation initiation factor 3 subunit 5	79	3	0.81
SEPT11 Septin-11	378	2	0.79
SUMO3 Small ubiquitin-related modifier 3	510	2	0.77
RPL8 60S ribosomal protein L8	1507	8	0.76
CCDC39 Coiled-coil domain-containing protein 39	25	1	0.75
GPR78 Probable G-protein coupled receptor 78	330	1	0.74
NBN Nibrin	64	1	0.71
ACTR1A Alpha-centractin	93	3	0.71
EIF4B eukaryotic translation initiation factor 4B	374	11	0.70
UBA52 ubiquitin and ribosomal protein L40 precursor	26	11	0.70
RPL4 60S ribosomal protein L4	26	1	0.69
RBM17 Splicing factor 45	28	2	0.69
TPM3 Putative uncharacterized protein DKFZp686J1372	144	5	0.69
SIP1 Isoform 1 of Survival of motor neuron protein-interacting protein 1	452	1	0.67
RPL29P4;RPL29 Novel protein similar to ribosomal protein L29 RPL29	665	1	0.67
LSM14A Isoform 2 of LSM14 protein homolog A	29	3	0.66
CEP170 Isoform 1 of Centrosomal protein of 170 kDa	90	1	0.66
TBR1 T-brain-1 protein	430	1	0.64
EPB41L3 Isoform A of Band 4.1-like protein 3	44	1	0.64
RPL13A 60S ribosomal protein L13a	59	1	0.62
RPS3 40S ribosomal protein S3	180	2	0.62
S100A6 Protein S100-A6	78	1	0.62
RPS20 40S ribosomal protein S20	41	3	0.62
VCP Transitional endoplasmic reticulum ATPase	88	4	0.62

Proteins eluted with SDS sample buffer (strong parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
PPP2R1A Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	31	3	0.59
KCTD5 BTB/POZ domain-containing protein KCTD5	110	1	0.59
HSP90AA1 Isoform 1 of Heat shock protein HSP 90-alpha	37	8	0.59
CSTB Cystatin-B	121	1	0.58
RPL9 60S ribosomal protein L9	281	1	0.58
IMMT Isoform 1 of Mitochondrial inner membrane protein	154	2	0.57
FUBP1 Isoform 1 of Far upstream element-binding protein 1	73	2	0.57
PSMC3 26S protease regulatory subunit 6A	449	3	0.57
PSMD11 Proteasome 26S non-ATPase subunit 11 variant (Fragment)	46	5	0.57
PPIB peptidylprolyl isomerase B precursor	55	3	0.56
CALD1 Isoform 3 of Caldesmon	106	4	0.55
LOC643677 similar to hCG2011852	58	1	0.55
FSCN1 Fascin	171	3	0.53
VPS72 Vacuolar protein sorting-associated protein 72 homolog	289	1	0.52
HSP90AB1 Heat shock protein HSP 90-beta	162	17	0.52
HSPA1L Heat shock 70 kDa protein 1L	212	3	0.50
GSR Isoform Mitochondrial of Glutathione reductase, mitochondrial	25	3	0.49
CCAR1 Cell division cycle and apoptosis regulator protein 1	100	1	0.49
VGF Neurosecretory protein VGF	28	4	0.49
TUFM Tu translation elongation factor, mitochondrial precursor	1015	8	0.49
TXN Thioredoxin	120	1	0.48
RPL14 60S ribosomal protein L14	106	1	0.48
CLASP2 CLASP2 protein	40	1	0.46
CTTN Src substrate cortactin	189	1	0.46
PDCD6 Programmed cell death protein 6	478	3	0.46
RPS14 40S ribosomal protein S14	83	3	0.46
PFDN2 Prefoldin subunit 2	47	3	0.43
CRMP1 Dihydropyrimidinase-related protein 1	33	1	0.43
UBE2N Ubiquitin-conjugating enzyme E2 N	31	3	0.43
ATP5B ATP synthase subunit beta, mitochondrial	92	14	0.41
MTDH Protein LYRIC	181	1	0.39
RPL36AL 60S ribosomal protein L36a-like	107	1	0.39
PSMD4 Isoform Rpn10A of 26S proteasome non-ATPase regulatory subunit 4	32	29	0.39
DUT Isoform DUT-M of Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial	151	6	0.39
DCTN2 6 kDa protein	733	1	0.38
YWHAQ 14-3-3 protein theta	32	8	0.38
C1orf57 Probable UPF0334 kinase-like protein C1orf57	25	1	0.38
PHB2 Prohibitin-2	1852	1	0.37
HSPD1 60 kDa heat shock protein, mitochondrial	257	24	0.36
PPP1R9B protein phosphatase 1, regulatory subunit 9B	56	4	0.36
THOC4 THO complex subunit 4	541	7	0.36
RPS8 40S ribosomal protein S8	30	4	0.35
HNRNPH3 Isoform 1 of Heterogeneous nuclear ribonucleoprotein H3	60	2	0.35
PDIA6 Isoform 2 of Protein disulfide-isomerase A6	1675	1	0.35
ZNF415 Isoform 1 of Zinc finger protein 415	183	2	0.34
STARD13 Isoform 2 of StAR-related lipid transfer protein 13	428	1	0.34
HIGD1B 10 kDa protein	187	1	0.33
LRRFIP1 Isoform 2 of Leucine-rich repeat flightless-interacting protein 1	97	2	0.33
MAP1S Microtubule-associated protein 1S	84	1	0.32
RBM14 Isoform 1 of RNA-binding protein 14	64	3	0.32
PPP1CC Isoform Gamma-1 of Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	26	1	0.32
RPS19 40S ribosomal protein S19	27	3	0.32
LOC284064 similar to ribosomal protein L29	79	1	0.31
VGF VGF nerve growth factor inducible precursor	32	1	0.31
EEF1G Elongation factor 1-gamma	148	3	0.30
RPL7 60S ribosomal protein L7	44	2	0.30
SCG2 Secretogranin-2	134	1	0.29
RPL35A 60S ribosomal protein L35a	28	2	0.29
SGTA Small glutamine-rich tetratricopeptide repeat-containing protein alpha	26	2	0.29
PSMC1 26S protease regulatory subunit 4	180	6	0.29
SCYE1 Multisynthetase complex auxiliary component p43	56	4	0.29
PFN2 Isoform IIb of Profilin-2	47	2	0.29
CCT5 T-complex protein 1 subunit epsilon	70	2	0.28
SNRPG Small nuclear ribonucleoprotein G	85	1	0.28
HSPA8 Isoform 1 of Heat shock cognate 71 kDa protein	338	57	0.27
CORO1C Coronin-1C_i3 protein	165	3	0.27
RPSA 33 kDa protein	130	16	0.27
EEF1D Elongation factor 1-delta	102	3	0.27
- 15 kDa protein	34	1	0.26
RPLP0 60S acidic ribosomal protein P0	3605	11	0.26
HAGH hydroxyacyl glutathione hydrolase isoform 1	115	1	0.25
KPNB1 Importin subunit beta-1	1335	1	0.25
PSMC2 26S protease regulatory subunit 7	225	7	0.25
SLC25A5 ADP/ATP translocase 2	42	8	0.24
RPS3A 40S ribosomal protein S3a	570	2	0.24
EMID1 Isoform 1 of EMI domain-containing protein 1	32	1	0.24
CCT8 59 kDa protein	39	4	0.24
FAM40A Isoform 3 of Protein FAM40A	365	1	0.23
NEFL Neurofilament light polypeptide	465	12	0.23
DARS Aspartyl-tRNA synthetase, cytoplasmic	92	3	0.23
ACAT1 Acetyl-CoA acetyltransferase, mitochondrial	27	26	0.22

Proteins eluted with SDS sample buffer (strong parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
RPL3 60S ribosomal protein L3	215	3	0.22
YBX1 Nuclease-sensitive element-binding protein 1	25	10	0.22
HNRNPA1 Isoform A1-B of Heterogeneous nuclear ribonucleoprotein A1	755	5	0.22
TUBB2B Tubulin beta-2B chain	143	7	0.21
RPL12 Isoform 1 of 60S ribosomal protein L12	1757	8	0.21
LOC388344 similar to RPL13 protein	197	1	0.21
- Calnexin (Fragment)	795	1	0.21
PPM1B Isoform Beta-1 of Protein phosphatase 1B	257	19	0.21
CGNL1 Isoform 1 of Cingulin-like protein 1	432	1	0.21
RPL28 60S ribosomal protein L28	648	2	0.21
MATR3 Matrin-3	75	2	0.20
FLJ12529 Isoform 1 of Cleavage and polyadenylation specificity factor subunit 7	28	2	0.20
SLC25A3 Isoform A of Phosphate carrier protein, mitochondrial	1241	2	0.20
FHL1 Four and a half LIM domains 1 variant	33	1	0.20
MAP1B Microtubule-associated protein 1B	80	148	0.20
PSMC5 26S protease regulatory subunit 8	84	2	0.19
PSMC4 Isoform 1 of 26S protease regulatory subunit 6B	93	13	0.18
NME2 Nucleoside diphosphate kinase	100	1	0.18
TUBB1 Tubulin beta-1 chain	28	2	0.18
TMPO Isoform Gamma of Lamina-associated polypeptide 2, isoforms beta/gamma	9042	5	0.17
YWHAH 14-3-3 protein eta	79	1	0.17
ATP5A1 ATP synthase subunit alpha, mitochondrial	620	19	0.17
IFT74 Intraflagellar transport protein 74 homolog	25	1	0.17
CFL1 Cofilin-1	85	10	0.16
PKM2 Isoform M2 of Pyruvate kinase isozymes M1/M2	239	26	0.15
PRDX3 Thioredoxin-dependent peroxide reductase, mitochondrial	38	4	0.14
YWHAG 14-3-3 protein gamma	1260	11	0.14
STMN2 Stathmin-2	44	2	0.13
PCBP2 poly(rC) binding protein 2 isoform b	750	1	0.13
VIM Vimentin	1752	28	0.12
C22orf28 UPF0027 protein C22orf28	370	2	0.12
SERBP1 Isoform 1 of Plasminogen activator inhibitor 1 RNA-binding protein	647	1	0.12
ACTL8 Actin-like protein 8	97	1	0.12
EWSR1 cDNA FLJ31747 fis, clone NT2RI2007377, highly similar to RNA-BINDING PROTEIN	65	14	0.11
CLTC Isoform 1 of Clathrin heavy chain 1	1479	8	0.11
MRPL12 39S ribosomal protein L12, mitochondrial	64	2	0.10
RPL5 60S ribosomal protein L5	31	3	0.10
MUTED;TXNDC5 Thioredoxin domain-containing protein 5	34	2	0.10
EEF1D eukaryotic translation elongation factor 1 delta isoform 1	880	1	0.10
HNRNPK Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	418	22	0.10
EEF1B2 Elongation factor 1-beta	79	4	0.09
IGF2BP1 Insulin-like growth factor 2 mRNA-binding protein 1	125	3	0.09
SEPT7 Isoform 1 of Septin-7	129	3	0.09
SPTAN1 Isoform 2 of Spectrin alpha chain, brain	45	25	0.08
ILF2 Interleukin enhancer-binding factor 2	1334	1	0.08
WDR77 Methylosome protein 50	449	52	0.08
PCMT1 Isoform 1 of Protein-L-isoaspartate(D-aspartate) O-methyltransferase	178	16	0.07
SLAIN2 SLAIN motif-containing protein 2	102	1	0.07
TUBB2C Tubulin beta-2C chain	1075	18	0.07
PSMD2 26S proteasome non-ATPase regulatory subunit 2	56	9	0.07
DNAJA2 DnaJ homolog subfamily A member 2	3106	4	0.06
ARHGDIA Rho GDP-dissociation inhibitor 1	898	6	0.06
MAP2 Isoform 1 of Microtubule-associated protein 2	60	4	0.06
HIST2H4B;HIST1H4F;HIST1H4J;HIST1H4C;HIST1H4B;HIST1H4K;HIST1H4E;HIST2H4A;HIST1H4H;HIST1H4L;HIST1H4A;HIST4H4;HIST1H4D;HIST1H4I Histone H4	1285	3	0.06
PHB Prohibitin	487	2	0.05
ARGLU1 Isoform 1 of Arginine and glutamate-rich protein 1	137	5	0.05
ACTN4 Alpha-actinin-4	262	2	0.05
POLR2E DNA-directed RNA polymerases I, II, and III subunit RPABC1	179	1	0.04
HDGF Hepatoma-derived growth factor	138	1	0.04
PRDX4 Peroxiredoxin-4	98	1	0.04
YWHAE 14-3-3 protein epsilon	168	3	0.03
HIST1H1C Histone H1.2	83	1	0.03
KLC1 kinesin light chain 1 isoform 2	54	2	0.03
NUDT21 Cleavage and polyadenylation specificity factor subunit 5	39	3	0.03
SUB1 Activated RNA polymerase II transcriptional coactivator p15	58	1	0.03
RPL23A;hCG_16001 60S ribosomal protein L23a	198	1	0.02
HNRNPR Heterogeneous nuclear ribonucleoprotein R	48	2	0.02
INA Alpha-internexin	75	25	0.02
CCT2 T-complex protein 1 subunit beta	150	8	0.01
DYNC1I2 Isoform 2D of Cytoplasmic dynein 1 intermediate chain 2	29	4	0.01
CCT7 T-complex protein 1 subunit eta	50	3	0.01
SF3A3 Splicing factor 3A subunit 3	88	1	0.00
RPL11 Isoform 1 of 60S ribosomal protein L11	1468	4	0.00
PRMT5 Protein arginine N-methyltransferase 5	412	64	0.00
HDLBP cDNA FLJ45936 fis, clone PLACE7004103, highly similar to Vigilin	323	1	0.00
TCP1 T-complex protein 1 subunit alpha	145	10	-0.01
SNRPC U1 small nuclear ribonucleoprotein C	64	1	-0.01
SF3B3 Isoform 1 of Splicing factor 3B subunit 3	152	3	-0.01
NME1;NME2 Nucleoside diphosphate kinase A	3221	6	-0.01
GNB1 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	40	3	-0.01

Proteins eluted with SDS sample buffer (strong parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
SFRS2B Isoform 1 of Splicing factor, arginine/serine-rich 2B	551	1	-0.02
HSPA1B;HSPA1A Heat shock 70 kDa protein 1	33	1	-0.02
NEFM Neurofilament medium polypeptide	169	6	-0.03
FBXO21 Isoform 1 of F-box only protein 21	257	4	-0.03
LARS Leucyl-tRNA synthetase, cytoplasmic	138	3	-0.03
SFPQ Isoform Long of Splicing factor, proline- and glutamine-rich	48	73	-0.04
HSPA9 Stress-70 protein, mitochondrial	28	27	-0.04
EEF1AL3;EEF1A1 Putative elongation factor 1-alpha-like 3	305	25	-0.04
SNRPD2 Small nuclear ribonucleoprotein Sm D2	228	3	-0.04
RPS4X 40S ribosomal protein S4, X isoform	117	1	-0.04
EPRS Bifunctional aminoacyl-tRNA synthetase	3759	7	-0.04
PDHA1 Mitochondrial PDHA1	2084	1	-0.05
PSMD13 proteasome 26S non-ATPase subunit 13 isoform 2	1160	1	-0.05
DDX5 Probable ATP-dependent RNA helicase DDX5	102	1	-0.05
HIST1H1B Histone H1.5	41	1	-0.05
QPCT Glutaminyl-peptide cyclotransferase	297	5	-0.06
CCT6A T-complex protein 1 subunit zeta	31	7	-0.06
TKT 37 kDa protein	26	2	-0.06
TMOD1 Tropomodulin-1	66	2	-0.06
DDX3X ATP-dependent RNA helicase DDX3X	47	1	-0.06
SNRPD3 Small nuclear ribonucleoprotein Sm D3	186	2	-0.07
FUS Isoform Short of RNA-binding protein FUS	456	4	-0.07
AHCY Adenosylhomocysteinase	122	3	-0.07
RBMX cDNA FLJ38696 fis, clone KIDNE2001931, highly similar to HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN G	73	3	-0.07
FASN Fatty acid synthase	39	1	-0.07
IMPDH1 inosine monophosphate dehydrogenase 1 isoform a	62	2	-0.07
NUDC Nuclear migration protein nudC	170	2	-0.08
RPL23 60S ribosomal protein L23	153	1	-0.08
DLAT Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	168	7	-0.09
NUDT13 Isoform 2 of Nucleoside diphosphate-linked moiety X motif 13	62	1	-0.09
EMD Emerin	97	1	-0.09
MDH2 Malate dehydrogenase, mitochondrial	96	1	-0.09
PIM2 Serine/threonine-protein kinase Pim-2	40	1	-0.09
NPM1 Isoform 2 of Nucleophosmin	361	16	-0.10
PABPC1 Isoform 1 of Polyadenylate-binding protein 1	29	16	-0.10
GMPS GMP synthase [glutamine-hydrolyzing]	30	1	-0.10
SLC25A11 Mitochondrial 2-oxoglutarate/malate carrier protein	94	1	-0.11
MAP3K7IP2 Isoform 1 of Mitogen-activated protein kinase kinase kinase 7-interacting protein 2	28	3	-0.11
SYNCRIP Isoform 1 of Heterogeneous nuclear ribonucleoprotein Q	999	3	-0.12
PSMD3 26S proteasome non-ATPase regulatory subunit 3	928	1	-0.12
NCL Isoform 2 of Nucleolin	31	14	-0.12
VDAC2 Voltage-dependent anion-selective channel protein 2	76	1	-0.12
YWHAB Isoform Long of 14-3-3 protein beta/alpha	153	2	-0.12
ZYX Zyxin	184	22	-0.13
PPIA Peptidyl-prolyl cis-trans isomerase A	34	35	-0.13
PRPF31 Isoform 1 of U4/U6 small nuclear ribonucleoprotein Prp31	634	2	-0.13
PRDX1 Peroxiredoxin-1	46	39	-0.13
GAPDH Glyceraldehyde-3-phosphate dehydrogenase	81	11	-0.13
PSPC1 Isoform 2 of Paraspeckle component 1	1296	6	-0.13
RUVBL1 Isoform 1 of RuvB-like 1	1679	12	-0.13
RPL24 60S ribosomal protein L24	105	1	-0.13
HADHA Trifunctional enzyme subunit alpha, mitochondrial	1824	1	-0.15
MYOM2 Myomesin-2	696	1	-0.15
SPIN3 Isoform 1 of Spindlin-3	374	1	-0.15
EIF5A Isoform 2 of Eukaryotic translation initiation factor 5A-1	462	2	-0.15
PHGDH D-3-phosphoglycerate dehydrogenase	62	4	-0.15
PRPH Isoform 1 of Peripherin	29	16	-0.15
VDAC3 Isoform 1 of Voltage-dependent anion-selective channel protein 3	26	1	-0.15
SF3A1 Splicing factor 3 subunit 1	56	2	-0.15
YWHAZ 14-3-3 protein zeta/delta	64	6	-0.15
ROD1 ROD1 regulator of differentiation 1	129	2	-0.16
RALY RNA binding protein, autoantigenic	740	1	-0.16
ALDOA Fructose-bisphosphate aldolase A	51	12	-0.16
ARID1B Novel protein (Fragment)	56	1	-0.16
CCT3 chaperonin containing TCP1, subunit 3 isoform b	419	1	-0.17
SF3B4 Splicing factor 3B subunit 4	93	3	-0.17
LDHB L-lactate dehydrogenase B chain	39	6	-0.17
XRCC5 ATP-dependent DNA helicase 2 subunit 2	478	5	-0.17
RAN GTP-binding nuclear protein Ran	28	4	-0.17
NUFIP2 Nuclear fragile X mental retardation-interacting protein 2	38	1	-0.18
HNRNPH1 Heterogeneous nuclear ribonucleoprotein H	245	10	-0.18
CPSF6 Isoform 1 of Cleavage and polyadenylation specificity factor subunit 6	393	9	-0.19
TUBB3 Tubulin beta-3 chain	239	7	-0.19
PABPC4 Isoform 1 of Polyadenylate-binding protein 4	158	4	-0.19
TUBB Tubulin beta chain	26	87	-0.19
C7orf24 C7orf24 protein	660	1	-0.20
KTN1 Isoform 2 of Kinectin	635	2	-0.20
SSBP1 Single-stranded DNA-binding protein, mitochondrial	585	3	-0.20
TRAP1 57 kDa protein	141	1	-0.20

Proteins eluted with SDS sample buffer (strong parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
FKBP4 FK506-binding protein 4	5058	2	-0.21
HNRNPU Isoform Short of Heterogeneous nuclear ribonucleoprotein U	32	14	-0.22
KIAA0562 Protein	73	1	-0.22
SKP1 Isoform 1 of S-phase kinase-associated protein 1	98	2	-0.22
SPTBN1 Isoform Long of Spectrin beta chain, brain 1	36	5	-0.22
RPL26L1 60S ribosomal protein L26-like 1	62	1	-0.22
CLNS1A Methylosome subunit pICln	1021	7	-0.22
GSTP1 Glutathione S-transferase P	39	9	-0.22
RPL30 60S ribosomal protein L30	95	3	-0.23
CCBL2 kynurenine aminotransferase III isoform 3	173	1	-0.24
HIST1H2BL Histone H2B type 1-L	33	12	-0.24
PRDX2 Peroxiredoxin-2	350	6	-0.24
PRDX6 Peroxiredoxin-6	747	11	-0.25
FLNA filamin A, alpha isoform 1	199	57	-0.25
EXOSC6 Exosome complex exonuclease MTR3	26	7	-0.25
MAP3K7IP1 Mitogen-activated protein kinase kinase kinase 7-interacting protein 1	821	44	-0.26
HPRT1 Hypoxanthine-guanine phosphoribosyltransferase	246	1	-0.26
XRCC6 ATP-dependent DNA helicase 2 subunit 1	629	1	-0.26
RPN1 Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit precursor	3077	1	-0.26
MTHFD1 C-1-tetrahydrofolate synthase, cytoplasmic	327	1	-0.26
RBM10 Putative uncharacterized protein DKFZp686E2459	2478	22	-0.27
PRPS1L1 Ribose-phosphate pyrophosphokinase 3	38	1	-0.27
NAP1L1 Nucleosome assembly protein 1-like 1	29	2	-0.27
HIST2H3D;HIST2H3C;HIST2H3A Histone H3.2	43	7	-0.27
TUBA1A Tubulin alpha-1A chain	58	50	-0.29
IARS IARS protein	1055	1	-0.30
SF3B14 Pre-mRNA branch site protein p14	31	1	-0.30
HIST1H2AE;HIST1H2AB Histone H2A type 1-B	66	1	-0.32
EEF2 Elongation factor 2	210	6	-0.33
RBBP4 Histone-binding protein RBBP4	2843	1	-0.33
ARCN1 Putative uncharacterized protein DKFZp686M09245	36	4	-0.33
SFRS3 Splicing factor, arginine/serine-rich 3	25	2	-0.33
CDK3 Cell division protein kinase 3	28	1	-0.33
RARS Isoform Complexed of Arginyl-tRNA synthetase, cytoplasmic	218	3	-0.33
BAT2 Isoform 1 of Large proline-rich protein BAT2	42	1	-0.33
CKB Creatine kinase B-type	213	6	-0.34
NONO Non-POU domain-containing octamer-binding protein	99	161	-0.34
TAGLN2 Transgelin-2	63	1	-0.34
LMNA Progerin	118	23	-0.34
HNRNPAB Isoform 4 of Heterogeneous nuclear ribonucleoprotein A/B	31	1	-0.34
HSPA6 Heat shock 70 kDa protein 6	308	1	-0.36
UCHL1 Uncharacterized protein UCHL1	9113	3	-0.36
TCEB2 Transcription elongation factor B polypeptide 2	29	3	-0.36
DDX1 ATP-dependent RNA helicase DDX1	1208	2	-0.37
NCBP1 Nuclear cap-binding protein subunit 1	69	1	-0.38
EPHB6 Ephrin type-B receptor 6	33	1	-0.39
RPLP2 60S acidic ribosomal protein P2	155	1	-0.41
IMPDH2 Inosine-5'-monophosphate dehydrogenase 2	147	7	-0.41
HSPE1 10 kDa heat shock protein, mitochondrial	59	2	-0.41
ENO1 Isoform alpha-enolase of Alpha-enolase	40	14	-0.42
HNRNPL heterogeneous nuclear ribonucleoprotein L isoform a	29	5	-0.43
CLIC1 Chloride intracellular channel protein 1	44	1	-0.43
PRMT1 HMT1 hnRNP methyltransferase-like 2 isoform 1	297	4	-0.45
PEBP1 Phosphatidylethanolamine-binding protein 1	107	3	-0.46
RUVBL2 RuvB-like 2	719	7	-0.47
CTSB Cathepsin B	209	1	-0.47
PPP1CA protein phosphatase 1, catalytic subunit, alpha isoform 2	35	1	-0.48
SET Isoform 1 of Protein SET	165	4	-0.48
SPIN1 Spindlin-1	118	23	-0.49
RIOK1 Serine/threonine-protein kinase RIO1	401	8	-0.50
HNRNPA2B1 Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1	32	5	-0.50
MAP3K7 Isoform 1A of Mitogen-activated protein kinase kinase kinase 7	45	11	-0.50
ACTN1 Alpha-actinin-1	310	1	-0.51
ACTL6A Isoform 1 of Actin-like protein 6A	1233	1	-0.51
HADHB Trifunctional enzyme subunit beta, mitochondrial	433	2	-0.51
DNAJB11 DnaJ homolog subfamily B member 11	305	2	-0.51
PSMA4 20 kDa protein	648	1	-0.52
HSD17B10 Isoform 1 of 3-hydroxyacyl-CoA dehydrogenase type-2	28	1	-0.53
U2AF2 Splicing factor U2AF 65 kDa subunit	26	2	-0.54
ASS1 Argininosuccinate synthase	84	1	-0.55
TUBB2A Tubulin beta-2A chain	73	1	-0.57
UQCRC1 Cytochrome b-c1 complex subunit 1, mitochondrial	35	2	-0.58
RPL21;LOC729402 60S ribosomal protein L21	39	1	-0.60
RCN2 Reticulocalbin-2	84	1	-0.63
IGF2BP3 Isoform 2 of Insulin-like growth factor 2 mRNA-binding protein 3	32	1	-0.63
RBBP7 Histone-binding protein RBBP7	27	1	-0.63
WARS Tryptophanyl-tRNA synthetase, cytoplasmic	119	1	-0.68
LOC144097 Uncharacterized protein LOC144097	53	8	-0.69
U2AF1L4 Isoform 2 of Splicing factor U2AF 26 kDa subunit	30	2	-0.69
SFRS1 Isoform ASF-1 of Splicing factor, arginine/serine-rich 1	26	2	-0.69
SDF2L1 Dihydropyrimidinase-like 2	39	1	-0.70

Proteins eluted with SDS sample buffer (strong parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
CDY1;CDY1B Isoform 1 of Testis-specific chromodomain protein Y 1	94	1	-0.70
HNRNPM Isoform 1 of Heterogeneous nuclear ribonucleoprotein M	367	10	-0.71
PFN1 Profilin-1	82	4	-0.72
STRAP Serine-threonine kinase receptor-associated protein	71	1	-0.72
PGK1 Phosphoglycerate kinase 1	67	2	-0.75
OTUD4 Isoform 1 of OTU domain-containing protein 4	29	3	-0.76
DHX9 ATP-dependent RNA helicase A	582	2	-0.83
TAF4 Transcription initiation factor TFIID subunit 4	223	1	-0.83
PDHB Isoform 1 of Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	55	1	-0.83
LUC7L Isoform 1 of Putative RNA-binding protein Luc7-like 1	59	2	-0.84
SEPT9 Isoform 3 of Septin-9	114	1	-0.85
KIF11 Kinesin-like protein KIF11	70	70	-0.86
LMNB1 Lamin-B1	47	5	-0.89
CDK5RAP1 Isoform 4 of CDK5 regulatory subunit-associated protein 1	35	1	-0.89
BOLA2;BOLA2B BolA-like protein 2	135	1	-0.89
HNRNPC Isoform C1 of Heterogeneous nuclear ribonucleoproteins C1/C2	50	11	-0.92
LOC729708;LOC388642 Triosephosphate isomerase (Fragment)	4027	2	-0.93
HDAC2 histone deacetylase 2	267	2	-0.93
STK38L Serine/threonine-protein kinase 38-like	25	5	-0.95
STK38 Serine/threonine-protein kinase 38	69	9	-0.97
CRKL Crk-like protein	771	1	-1.00
SMC4 Isoform 2 of Structural maintenance of chromosomes protein 4	105	2	-1.01
ERH Enhancer of rudimentary homolog	69	3	-1.01
EIF6 Eukaryotic translation initiation factor 6	177	5	-1.03
USP29 Ubiquitin carboxyl-terminal hydrolase 29	431	2	-1.05
PCNA Proliferating cell nuclear antigen	64	1	-1.12
MTHFD1L Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	59	1	-1.26
TSC22D4 TSC22 domain family protein 4	147	1	-1.35
COX15 Isoform 1 of Cytochrome c oxidase assembly protein COX15 homolog	252	1	-1.40
CTNNB1 Isoform 1 of Catenin beta-1	56	1	-1.41
SF3B1 Splicing factor 3B subunit 1	67	2	-1.43
ARHGEF11 Rho guanine nucleotide exchange factor 11	28	1	-1.45
ACTR2 Actin-related protein 2	25	1	-1.46
RNF7 Isoform 1 of RING-box protein 2	26	1	-1.64
A26C1B ANKRD26-like family C member 1B	25	1	-1.74
NUMA1 Isoform 2 of Nuclear mitotic apparatus protein 1	117	2	-1.77
SLFN13 Isoform 1 of Schlafen family member 13	26	1	-1.79
DHRSX 29 kDa protein	25	1	-1.86
LOC550643 Putative uncharacterized protein LOC550643	35	1	-2.16
GOLGB1 Golgin subfamily B member 1	37	2	-2.21
FUT3 Galactoside 3(4)-L-fucosyltransferase	59	1	-2.22
CCT4 T-complex protein 1 subunit delta	28	1	-2.29
C1orf65 Uncharacterized protein C1orf65	28	1	-2.50
SNRPB Isoform SM-B' of Small nuclear ribonucleoprotein-associated proteins B and B'	25	1	-2.54
ZNF681 Zinc finger protein 681	57	4	-2.58
PARK2 Isoform 1 of E3 ubiquitin-protein ligase parkin	25	4	-2.74
LOC646730 similar to hCG2036631	33	1	-2.78
CDADC1 Cytidine and dCMP deaminase domain containing 1	27	1	-2.81
LOC388720 similar to ubiquitin	26	1	-3.00
- Uncharacterized protein ENSP00000379605	110	1	-3.06
Uncharacterized protein ENSP00000381458 (Fragment)	228	2	-3.13
TCFL5 Isoform 1 of Transcription factor-like 5 protein	40	1	-3.13
METTL8 methyltransferase like 8	56	1	-3.54
HPX-2 similar to facioscapulohumeral muscular dystrophy	33	1	-3.82
PCM1 Isoform 3 of Pericentriolar material 1 protein	25	6	-4.27
LOC100130931 cDNA FLJ46693 fis, clone TRACH3012864	60	1	-4.38
PDZD3 Isoform 2 of PDZ domain-containing protein 3	28	1	-4.50

Table 5. FLAG-parkin pull-down proteins under CCCP with SILAC ratios – weak interactor fraction

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
DNAJC28 J domain-containing protein C21orf55	52	2	3.14
WDR82 WD repeat-containing protein 82	65	2	1.89
LOC654340 similar to KIAA1839 protein	57	2	1.59
VDAC3 Isoform 2 of Voltage-dependent anion-selective channel protein 3	190	4	1.46
TOP1 DNA topoisomerase 1	71	2	1.27
HNRNPM Isoform 1 of Heterogeneous nuclear ribonucleoprotein M	208	5	1.15
MCCC2 Isoform 1 of Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	90	2	1.12
FKBP1A Peptidyl-prolyl cis-trans isomerase	205	3	1.07
MAN2B2 Isoform 1 of Epididymis-specific alpha-mannosidase	54	2	0.98
STK38 Serine/threonine-protein kinase 38	31	1	0.98
KCTD5 BTB/POZ domain-containing protein KCTD5	45	1	0.98
SDF4 stromal cell derived factor 4 precursor	64	1	0.97
SYCP1 Synaptonemal complex protein 1	27	1	0.95
PPM1B Isoform Beta-1 of Protein phosphatase 1B	86	2	0.94
ITGBL1 Integrin beta-like protein 1	25	1	0.91
STK32C Protein kinase	30	1	0.91
DHX38 Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16	34	1	0.85
DDX50 ATP-dependent RNA helicase DDX50	39	1	0.85
CASK Isoform 3 of Peripheral plasma membrane protein CASK	32	1	0.85
PKN3 Serine/threonine-protein kinase N3	29	1	0.84
DDX60L DEAD (Asp-Glu-Ala-Asp) box polypeptide 60-like	26	1	0.84
OSGEP Probable O-sialoglycoprotein endopeptidase	45	1	0.82
LOC729176 LOC729176 protein (Fragment)	32	1	0.82
RPL32 60S ribosomal protein L32	168	3	0.80
CBFA2T2 Isoform 1 of Protein CBFA2T2	27	1	0.79
ITPR3 Inositol 1,4,5-trisphosphate receptor type 3	27	1	0.77
LOC203510 similar to hCG1644442	32	1	0.74
ASCC3L1 Isoform 1 of U5 small nuclear ribonucleoprotein 200 kDa helicase	121	4	0.73
C21orf114 Putative transposase element C21orf114	58	2	0.72
DDB1 DNA damage-binding protein 1	56	1	0.72
FBXL21 F-box/LRR-repeat protein 21	34	1	0.71
LAMB1 Laminin subunit beta-1	106	3	0.70
PARP1 Poly [ADP-ribose] polymerase 1	459	7	0.63
PSMA1 Isoform Short of Proteasome subunit alpha type-1	114	3	0.61
PSMA2 Proteasome subunit alpha type-2	189	3	0.61
TMED9 transmembrane emp24 protein transport domain containing 9	86	1	0.60
DCI Isoform 1 of 3,2-trans-enoyl-CoA isomerase, mitochondrial	58	1	0.55
EIF3D Eukaryotic translation initiation factor 3 subunit D	254	5	0.54
CIRBP Cold-inducible RNA-binding protein	26	1	0.52
MYH9 Myosin-9	967	18	0.52
WDR77 Methylosome protein 50	170	2	0.51
PPME1 Isoform 1 of Protein phosphatase methylesterase 1	26	1	0.51
SMC3 Structural maintenance of chromosomes protein 3	29	1	0.51
OSBPL8 Oxysterol-binding protein	40	1	0.50
ATP5D ATP synthase subunit delta, mitochondrial	94	1	0.50
PSMA7 Isoform 1 of Proteasome subunit alpha type-7	118	2	0.50
HP1BP3 Isoform 5 of Heterochromatin protein 1-binding protein 3	64	1	0.48
NARS Asparaginyl-tRNA synthetase, cytoplasmic	155	3	0.48
PRIC285 Isoform 1 of Peroxisomal proliferator-activated receptor A-interacting complex 285 kDa	25	1	0.48
EIF3A Eukaryotic translation initiation factor 3 subunit A	542	14	0.47
BCCIP Isoform 1 of BRCA2 and CDKN1A-interacting protein	47	1	0.47
SDHB Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	133	4	0.47
SART3 Isoform 1 of Squamous cell carcinoma antigen recognized by T-cells 3	42	1	0.47
FTH1 Ferritin heavy chain	226	5	0.46
EIF3EIP Eukaryotic translation initiation factor 3, subunit E interacting protein	134	2	0.45
DYNC1H1 Cytoplasmic dynein 1 heavy chain 1	574	11	0.44
EIF4B Eukaryotic translation initiation factor 4B	439	9	0.44
HNRNPR heterogeneous nuclear ribonucleoprotein R isoform 3	120	3	0.44
CHGB Secretogranin-1	475	8	0.44
ICT1 Immature colon carcinoma transcript 1 protein	57	1	0.43
EIF3G Eukaryotic translation initiation factor 3 subunit G	46	1	0.42
TMOD3 Tropomodulin-3	107	2	0.42
LMNB1 Lamin-B1	32	1	0.41
SPIN1 Spindlin-1	37	1	0.40
MAP3K7IP1 Mitogen-activated protein kinase kinase kinase 7-interacting protein 1	325	7	0.39
PSMA5 Proteasome subunit alpha type-5	118	3	0.39
THOC3 THO complex subunit 3	48	1	0.38
DPYSL5 Dihydropyrimidinase-related protein 5	401	9	0.38
YARS2 Tyrosyl-tRNA synthetase, mitochondrial	32	1	0.37
UBE2O Ubiquitin-conjugating enzyme E2 O	67	1	0.36
LSM14A Isoform 2 of LSM14 protein homolog A	62	1	0.36
RP11-631M21.2 Tubulin beta-8 chain	31	1	0.35
RUVBL2 RuvB-like 2	233	4	0.35
NUP153 Nuclear pore complex protein Nup153	31	1	0.34
RBM25 RNA binding motif protein 25	26	1	0.34
HDGF2 Isoform 2 of Hepatoma-derived growth factor-related protein 2	118	2	0.33

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
TTYH3 Isoform 1 of Protein tweety homolog 3	70	1	0.33
HGS Hepatocyte growth factor-regulated tyrosine kinase substrate	39	1	0.33
TLN1 Talin-1	110	3	0.32
LAMA1 Laminin subunit alpha-1	28	1	0.32
PDCD4 programmed cell death 4 isoform 2	85	3	0.32
ABLIM1 Isoform 1 of Actin-binding LIM protein 1	250	5	0.31
GALNT2 Polypeptide N-acetylgalactosaminyltransferase 2	80	2	0.30
MARCKSL1 MARCKS-related protein	97	2	0.30
SEC22B Vesicle-trafficking protein SEC22b	36	1	0.30
POP1 Ribonucleases P/MRP protein subunit POP1	27	1	0.29
GNRHR Isoform 1 of Gonadotropin-releasing hormone receptor	26	1	0.29
IMMT Isoform 1 of Mitochondrial inner membrane protein	84	3	0.28
MRLC2 Myosin regulatory light chain	210	5	0.28
ACTN1 Alpha-actinin-1	561	12	0.28
PTGIS Prostacyclin synthase	25	1	0.28
CKAP4 Isoform 1 of Cytoskeleton-associated protein 4	288	4	0.27
MSI2 Isoform 1 of RNA-binding protein Musashi homolog 2	87	2	0.26
RPS13 40S ribosomal protein S13	83	1	0.26
RPS26 Ribosomal protein 26 (RPS26) pseudogene	203	6	0.25
CKMT1B;CKMT1A;LOC100133623 Creatine kinase, ubiquitous mitochondrial	423	9	0.25
PRMT5 Protein arginine N-methyltransferase 5	1270	27	0.25
PSMC3 26S protease regulatory subunit 6A	397	6	0.25
EIF3J Eukaryotic translation initiation factor 3 subunit J	250	3	0.25
RPL28 60S ribosomal protein L28	77	2	0.25
EIF2S1 Eukaryotic translation initiation factor 2 subunit 1	252	6	0.24
CNPY2 Isoform 2 of Protein canopy homolog 2	26	1	0.24
PSMD11 Proteasome 26S non-ATPase subunit 11 variant (Fragment)	30	1	0.23
HGSNAT Heparan-alpha-glucosaminide N-acetyltransferase	28	1	0.23
RPS18;LOC100130553 40S ribosomal protein S18	330	8	0.22
RCC2 Protein RCC2	156	4	0.22
ARPC1B Actin-related protein 2/3 complex subunit 1B	38	1	0.21
NUCKS1 Isoform 1 of Nuclear ubiquitous casein and cyclin-dependent kinases substrate	298	3	0.20
H1FX Histone H1x	186	3	0.20
RBM10 Putative uncharacterized protein DKFZp686E2459	35	1	0.20
ATP2A2 Isoform SERCA2A of Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	210	4	0.19
XRCC5 ATP-dependent DNA helicase 2 subunit 2	1542	25	0.19
HIST1H1C Histone H1.2	524	10	0.19
BAX Isoform Zeta of Apoptosis regulator BAX	31	1	0.18
CTSD 20 kDa protein	44	1	0.18
EIF3F Eukaryotic translation initiation factor 3 subunit 5	658	9	0.18
CYB5R3 Isoform 1 of NADH-cytochrome b5 reductase 3	241	5	0.18
RPL31 60S ribosomal protein L31	195	4	0.18
NDUFV2 NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	126	2	0.17
HMGA1 Isoform HMG-Y of High mobility group protein HMG-I/HMG-Y	66	1	0.17
TOR1AIP1 Isoform 1 of Torsin-1A-interacting protein 1	29	1	0.17
XRCC6 ATP-dependent DNA helicase 2 subunit 1	34	1	0.17
HDAC1 Histone deacetylase 1	30	1	0.17
EIF4A2 BM-010	29	1	0.16
PDAP1 28 kDa heat- and acid-stable phosphoprotein	132	2	0.16
RPS10 40S ribosomal protein S10	490	8	0.15
EIF2S2 Eukaryotic translation initiation factor 2 subunit 2	69	2	0.15
CALD1 Isoform 1 of Caldesmon	70	2	0.14
MYL6;MYL6B Isoform Non-muscle of Myosin light polypeptide 6	187	5	0.14
ILF3 Isoform 5 of Interleukin enhancer-binding factor 3	623	13	0.12
CCT6A T-complex protein 1 subunit zeta	550	12	0.12
RPS17 40S ribosomal protein S17	292	6	0.12
MAP1LC3A Isoform 2 of Microtubule-associated proteins 1A/1B light chain 3A	39	1	0.12
NDUFV1 Isoform 1 of NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial	86	3	0.12
PFKM Isoform 2 of 6-phosphofructokinase, muscle type	486	11	0.11
EIF6 Eukaryotic translation initiation factor 6	60	1	0.11
- 46 kDa protein	54	2	0.11
CABC1 Isoform 1 of Chaperone activity of bc1 complex-like, mitochondrial	26	1	0.10
HSPD1 60 kDa heat shock protein, mitochondrial	6486	120	0.10
HSPD1 60 kDa heat shock protein, mitochondrial	6486	120	0.10
IQGAP3 Ras GTPase-activating-like protein IQGAP3	31	1	0.10
RPS19 40S ribosomal protein S19	435	10	0.10
SATB1 DNA-binding protein SATB1	37	1	0.09
SLC25A6 ADP/ATP translocase 3	492	11	0.09
MYH10 Isoform 1 of Myosin-10	514	10	0.08
H2AFV Histone H2A.V	54	1	0.08
TIMM13 Mitochondrial import inner membrane translocase subunit Tim13	156	2	0.08
RPS15 40S ribosomal protein S15	849	13	0.08
ADNP Activity-dependent neuroprotector homeobox protein	29	1	0.08
HSPA5 HSPA5 protein	3297	56	0.08
HSPA5 HSPA5 protein	3297	56	0.08
RPS3 40S ribosomal protein S3	1476	32	0.08
ACTN4 Alpha-actinin-4	1355	29	0.08
ENSA Isoform 2 of Alpha-endosulfine	50	1	0.08
STXBP1 Isoform 2 of Syntaxin-binding protein 1	75	1	0.08
RPS4X 40S ribosomal protein S4, X isoform	1244	24	0.07
PSPC1 Isoform 2 of Paraspeckle component 1	281	7	0.07

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
BBS1;DPP3 Isoform 1 of Dipeptidyl-peptidase 3	38	1	0.07
EIF3CL;EIF3C Eukaryotic translation initiation factor 3 subunit C	499	10	0.07
LOC220429 Similar to CTAGE family, member 5 isoform 2	28	1	0.07
QDPR Dihydropteridine reductase	158	3	0.06
UQCRC1 Cytochrome b-c1 complex subunit 1, mitochondrial	160	3	0.06
GLG1 golgi apparatus protein 1	65	2	0.06
AP1B1 Isoform A of AP-1 complex subunit beta-1	63	2	0.06
TUBAL3 Isoform 1 of Tubulin alpha chain-like 3	91	1	0.06
IARS Isoleucyl-tRNA synthetase, cytoplasmic	132	2	0.06
DNMT1 Isoform 1 of DNA (cytosine-5)-methyltransferase 1	61	1	0.06
KTN1 Isoform 1 of Kinectin	32	1	0.06
ATP5A1 ATP synthase subunit alpha, mitochondrial	1748	30	0.05
PRDX2 Peroxiredoxin-2	811	18	0.05
PGRMC1 Membrane-associated progesterone receptor component 1	178	4	0.05
SNRPG Small nuclear ribonucleoprotein G	26	1	0.05
SEPT2 Septin-2	1046	21	0.05
RPL22 60S ribosomal protein L22	223	5	0.05
HEATR2 Isoform 1 of HEAT repeat-containing protein 2	55	2	0.05
RPS14 40S ribosomal protein S14	469	9	0.05
GNB2L1 Lung cancer oncogene 7	1138	18	0.04
APEX1 DNA-(apurinic or apyrimidinic site) lyase	347	7	0.04
DPP7 Dipeptidyl-peptidase 2	320	7	0.04
RPS20 40S ribosomal protein S20	145	3	0.04
EDF1 Isoform 2 of Endothelial differentiation-related factor 1	31	1	0.04
PSMD2 26S proteasome non-ATPase regulatory subunit 2	831	16	0.04
RPS23 40S ribosomal protein S23	370	8	0.04
AK2 Isoform 1 of Adenylate kinase isoenzyme 2, mitochondrial	29	1	0.04
HIST1H2BD Histone H2B type 1-D	770	14	0.03
CORO1C Coronin-1C_i3 protein	202	3	0.03
BZW2 Basic leucine zipper and W2 domain-containing protein 2	29	1	0.03
MPST 3-mercaptopyruvate sulfurtransferase	242	4	0.03
PRKAR2A PRKAR2A protein	49	1	0.03
MYBL2 Uncharacterized protein MYBL2 (Fragment)	64	2	0.03
MYO1B Isoform 1 of Myosin-Ib	146	4	0.03
FARSA Phenylalanyl-tRNA synthetase alpha chain	574	10	0.02
HIST2H4B;HIST1H4F;HIST1H4J;HIST1H4C;HIST1H4B;HIST1H4K;HIST1H4E;HIST2H4A;HIST1 H4H;HIST1H4L;HIST1H4A;HIST4H4;HIST1H4D;HIST1H4I Histone H4	405	9	0.02
ERO1L ERO1-like protein alpha	208	4	0.02
SEPT11 Septin-11	882	15	0.02
CCT5 T-complex protein 1 subunit epsilon	1239	23	0.02
RPS16 40S ribosomal protein S16	157	5	0.02
DLST Full-length cDNA 5-PRIME end of clone CS0DB006YE12 of Neuroblastoma of H. sapiens	334	4	0.01
THOC4 THO complex subunit 4	450	9	0.01
RPS9 40S ribosomal protein S9	469	13	0.01
EIF5B Eukaryotic translation initiation factor 5B	30	1	0.01
CCDC102B Isoform 1 of Coiled-coil domain-containing protein 102B	73	2	0.01
LENG8 Isoform 1 of Leukocyte receptor cluster member 8	35	1	0.01
PC Pyruvate carboxylase, mitochondrial	315	6	0.01
BUB3 Mitotic checkpoint protein BUB3	139	3	0.01
FASN Fatty acid synthase	2790	60	0.01
FASN Fatty acid synthase	2790	60	0.01
- Uncharacterized protein ENSP00000343748	1312	25	0.01
ACAT1 Acetyl-CoA acetyltransferase, mitochondrial	1971	29	0.01
PRDX4 Peroxiredoxin-4	1246	21	0.01
PGD 6-phosphogluconate dehydrogenase, decarboxylating	806	9	0.01
PPP1CC Isoform Gamma-1 of Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	155	4	0.01
EIF3E Eukaryotic translation initiation factor 3 subunit E	53	2	0.01
ACLY ATP-citrate synthase	2278	39	0.00
DBN1 Isoform 1 of Drebrin	678	11	0.00
CTSC Dipeptidyl-peptidase 1	82	2	0.00
PDCD6IP PDCD6IP protein	85	2	0.00
ATP1A3 Sodium/potassium-transporting ATPase subunit alpha-3	28	1	0.00
HSP90B1 Endoplasmin	1958	40	0.00
TUBB1 Tubulin beta-1 chain	143	4	0.00
RUVBL1 Isoform 1 of RuvB-like 1	277	7	0.00
SLC25A3 Isoform A of Phosphate carrier protein, mitochondrial	187	4	0.00
NME2 Nucleoside diphosphate kinase	1706	32	-0.01
FUBP1 Isoform 1 of Far upstream element-binding protein 1	359	8	-0.01
TH Tyrosine hydroxylase isoform D2,8,9	202	4	-0.01
PPP2CA Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	301	6	-0.01
DNPEP aspartyl aminopeptidase	46	1	-0.01
GNS N-acetylglucosamine-6-sulfatase	29	1	-0.01
UBFD1 Ubiquitin domain-containing protein UBFD1	27	1	-0.01
HIST1H1B Histone H1.5	311	7	-0.01
PPAT Amidophosphoribosyltransferase	288	6	-0.01
MAP2 Isoform 1 of Microtubule-associated protein 2	203	3	-0.01
PSMB1 Proteasome subunit beta type-1	132	2	-0.01
ME2 NAD-dependent malic enzyme, mitochondrial	37	1	-0.01
EIF3B Isoform 1 of Eukaryotic translation initiation factor 3 subunit B	613	13	-0.02
NUDT5 ADP-sugar pyrophosphatase	40	1	-0.02
DPYSL3 DPYSL3 protein	67	2	-0.02

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
SCG2 Secretogranin-2	859	15	-0.02
LBR Lamin-B receptor	407	8	-0.02
XRCC4 Isoform 1 of DNA-repair protein XRCC4	26	1	-0.02
DDX17 DEAD box polypeptide 17 isoform 1	376	8	-0.02
CALU Isoform 2 of Calumenin	330	9	-0.02
RPL36AL 60S ribosomal protein L36a-like	41	1	-0.02
APP Isoform APP770 of Amyloid beta A4 protein (Fragment)	25	1	-0.03
RPS11 40S ribosomal protein S11	597	13	-0.03
RPS15A 40S ribosomal protein S15a	323	7	-0.03
CBR1 Carbonyl reductase [NADPH] 1	222	4	-0.03
MTCH2 Mitochondrial carrier homolog 2	94	2	-0.03
TFRC 6 kDa protein	33	1	-0.03
CAPZB Isoform 1 of F-actin-capping protein subunit beta	686	16	-0.03
RPL15 22 kDa protein	192	4	-0.03
NIPSNAP1 Protein NipSnap homolog 1	58	1	-0.03
HPRT1 Hypoxanthine-guanine phosphoribosyltransferase	107	3	-0.03
PSMD4 Isoform Rpn10A of 26S proteasome non-ATPase regulatory subunit 4	88	2	-0.03
SFPQ Isoform Long of Splicing factor, proline- and glutamine-rich	1041	22	-0.04
LOC442497;SLC3A2 4F2 cell-surface antigen heavy chain	189	3	-0.04
ATP5B ATP synthase subunit beta, mitochondrial	4746	70	-0.04
ATP5B ATP synthase subunit beta, mitochondrial	4746	70	-0.04
TCP1 T-complex protein 1 subunit alpha	1533	31	-0.04
PDHB Isoform 1 of Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	65	1	-0.04
DEK Protein DEK	95	3	-0.04
RPL23A;hCG_16001 60S ribosomal protein L23a	170	4	-0.04
MAP1B Microtubule-associated protein 1B	2793	48	-0.05
PRPS1 Ribose-phosphate pyrophosphokinase 1	344	6	-0.05
P4HB Protein disulfide-isomerase	303	7	-0.05
MTHFD1L Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	58	2	-0.05
CPSF6 Isoform 1 of Cleavage and polyadenylation specificity factor subunit 6	103	2	-0.05
NME1;NME2 non-metastatic cells 1, protein (NM23A) expressed in isoform a	144	2	-0.05
TCEA1 Isoform 2 of Transcription elongation factor A protein 1	38	1	-0.05
NPEPPS Puromycin-sensitive aminopeptidase	113	3	-0.06
TARS Threonyl-tRNA synthetase, cytoplasmic	509	10	-0.06
SEC23A Protein transport protein Sec23A	66	1	-0.06
IDH3A Isoform 1 of Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	197	6	-0.06
ALDOA Fructose-bisphosphate aldolase A	4093	72	-0.06
ALDOA Fructose-bisphosphate aldolase A	4093	72	-0.06
TUBB3 Tubulin beta-3 chain	1683	28	-0.06
CFL1 Cofilin-1	1001	14	-0.06
CRIP2 Cysteine-rich protein 2	220	4	-0.06
UMODL1 Uromodulin-like 1 protein variant 6	26	1	-0.06
YWHAQ 14-3-3 protein theta	1032	13	-0.06
LMAN2 Vesicular integral-membrane protein VIP36	211	4	-0.06
PPP2R2A Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	211	4	-0.07
ATP1B1 Isoform 1 of Sodium/potassium-transporting ATPase subunit beta-1	47	1	-0.07
ACTG1 Actin, cytoplasmic 2	7343	128	-0.07
ACTG1 Actin, cytoplasmic 2	7343	128	-0.07
NLRC3 Isoform 4 of Protein NLRC3	29	1	-0.07
GTPBP1 GTP-binding protein 1	139	3	-0.07
IPO9 Importin-9	27	1	-0.07
TXNRD1 Isoform 5 of Thioredoxin reductase 1, cytoplasmic	185	3	-0.08
TOMM40 Isoform 1 of Mitochondrial import receptor subunit TOM40 homolog	100	2	-0.08
SUB1 Activated RNA polymerase II transcriptional coactivator p15	50	1	-0.08
NDUFS1 NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	69	2	-0.08
USP7 Ubiquitin carboxyl-terminal hydrolase 7	32	1	-0.08
DDX19B Isoform 1 of ATP-dependent RNA helicase DDX19B	31	1	-0.08
PHB2 Prohibitin-2	433	8	-0.09
IQGAP1 Ras GTPase-activating-like protein IQGAP1	120	3	-0.09
VCP Transitional endoplasmic reticulum ATPase	1783	29	-0.09
RPN1 Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit precursor	892	17	-0.09
NAP1L1 Nucleosome assembly protein 1-like 1	406	7	-0.09
ARCN1 Putative uncharacterized protein DKFZp686M09245	192	2	-0.09
OGDH 2-oxoglutarate dehydrogenase E1 component, mitochondrial	118	3	-0.09
MUTED;TXNDC5 Thioredoxin domain-containing protein 5	144	4	-0.09
SDHA Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	788	18	-0.09
PPP1R7 Protein	62	1	-0.09
FLNA filamin A, alpha isoform 1	5079	98	-0.09
FLNA filamin A, alpha isoform 1	5079	98	-0.09
GARS Glycyl-tRNA synthetase	1175	22	-0.09
RPS24 Isoform 1 of 40S ribosomal protein S24	578	12	-0.10
XTP3TPA XTP3-transactivated gene A protein	293	4	-0.10
HN1L Isoform 1 of Hematological and neurological expressed 1-like protein	94	2	-0.10
SRP54 Signal recognition particle 54 kDa protein	47	1	-0.10
RPS8 25 kDa protein	1074	17	-0.10
PSMC2 26S protease regulatory subunit 7	542	10	-0.10
PFDN2 Prefoldin subunit 2	27	1	-0.10
CENPN Isoform 2 of Centromere protein N	27	1	-0.10
C19orf63 Isoform 2 of UPF0510 protein C19orf63	26	1	-0.10
SKIV2L2 Superkiller viralicidic activity 2-like 2	85	2	-0.10
CANX Calnexin	735	13	-0.10

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
LOC389901 hypothetical LOC389901	510	10	-0.10
SCYE1 Multisynthetase complex auxiliary component p43	186	5	-0.11
GLUD1 GLUD1 protein	537	11	-0.11
SEC62 Translocation protein SEC62	70	1	-0.11
CDC5L Cell division cycle 5-like protein	33	1	-0.11
MRPL12 39S ribosomal protein L12, mitochondrial	33	1	-0.11
NCL Isoform 2 of Nucleolin	3694	70	-0.11
NCL Isoform 2 of Nucleolin	3694	70	-0.11
DLAT Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	283	7	-0.11
NDUFA8 NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	104	3	-0.11
VARS Valyl-tRNA synthetase	1662	26	-0.11
HIST1H3F;HIST1H3B;HIST1H3D;HIST1H3A;HIST1H3H;HIST1H3I;HIST1H3E;HIST1H3C;HIST1H3J;HIST1H3G;HIST1H2BN Histone H3.1	311	8	-0.11
ALCAM 62 kDa protein	679	11	-0.12
AKAP12 Isoform 1 of A-kinase anchor protein 12	338	6	-0.12
KCTD12 BTB/POZ domain-containing protein KCTD12	250	4	-0.12
SPAG9 Isoform 4 of C-jun-amino-terminal kinase-interacting protein 4	152	4	-0.12
RIMS1 Isoform 1 of Regulating synaptic membrane exocytosis protein 1	27	1	-0.12
PSMB5 29 kDa protein	245	5	-0.12
TPM3 tropomyosin 3 isoform 4	873	13	-0.12
EIF4H Isoform Long of Eukaryotic translation initiation factor 4H	262	5	-0.12
TPM4 Isoform 1 of Tropomyosin alpha-4 chain	1341	24	-0.13
ATP5C1 Isoform Heart of ATP synthase subunit gamma, mitochondrial	205	4	-0.13
IMPDH1 inosine monophosphate dehydrogenase 1 isoform a	91	2	-0.13
RPS25 40S ribosomal protein S25	254	5	-0.13
EEF1G Elongation factor 1-gamma	756	14	-0.13
SLC25A5 ADP/ATP translocase 2	184	3	-0.13
DPYSL2 Dihydropyrimidinase-related protein 2	3108	48	-0.13
PCMT1 Isoform 1 of Protein-L-isoaspartate(D-aspartate) O-methyltransferase	583	9	-0.13
GANAB Isoform 1 of Neutral alpha-glucosidase AB	704	15	-0.13
TUBB Tubulin beta chain	13048	217	-0.13
TUBB Tubulin beta chain	13048	217	-0.13
TUBB Tubulin beta chain	13048	217	-0.13
PUS7 Pseudouridylate synthase 7 homolog	293	6	-0.13
SEC63 Translocation protein SEC63 homolog	36	1	-0.13
DDOST dolichyl-diphosphooligosaccharide-protein glycosyltransferase precursor	255	6	-0.14
NT5DC1 5'-nucleotidase domain-containing protein 1	40	1	-0.14
RPSA 33 kDa protein	1836	30	-0.14
EIF3I Eukaryotic translation initiation factor 3 subunit I	835	15	-0.14
CNTN1 Isoform 1 of Contactin-1	87	2	-0.14
OGT Isoform 3 of UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase 110 kDa subunit	68	2	-0.14
HNRNPU Isoform Short of Heterogeneous nuclear ribonucleoprotein U	2307	44	-0.14
GPHN Isoform 1 of Gephyrin	40	1	-0.14
SMCHD1 Isoform 1 of Structural maintenance of chromosomes flexible hinge domain-containing protein 1	29	1	-0.14
GOT1 Aspartate aminotransferase, cytoplasmic	26	1	-0.15
LRPPRC Leucine-rich PPR motif-containing protein, mitochondrial	1269	23	-0.15
CCT2 T-complex protein 1 subunit beta	848	13	-0.15
C1QBP Complement component 1 Q subcomponent-binding protein, mitochondrial	902	14	-0.15
ATP1A1 Isoform Long of Sodium/potassium-transporting ATPase subunit alpha-1	642	8	-0.15
HSPA4 Heat shock 70 kDa protein 4	624	11	-0.15
BSG Isoform 2 of Basigin	279	6	-0.15
IMPDH2 Inosine-5'-monophosphate dehydrogenase 2	683	12	-0.15
C1orf115 Uncharacterized protein C1orf115	25	1	-0.15
SEPT7 Isoform 1 of Septin-7	882	13	-0.15
RPS7 40S ribosomal protein S7	530	8	-0.15
PSMA6 Similar to Prosomal P27K protein	408	5	-0.15
DIAPH1 diaphanous 1 isoform 2	47	1	-0.15
PSMD14 26S proteasome non-ATPase regulatory subunit 14	160	3	-0.15
GTF2A1 Isoform 42 kDa of Transcription initiation factor IIA subunit 1	61	1	-0.15
ZNF207 Isoform 1 of Zinc finger protein 207	29	1	-0.15
ACTA2 Actin, aortic smooth muscle	231	6	-0.16
SEPT9 Isoform 1 of Septin-9	626	15	-0.16
PHB Prohibitin	166	2	-0.16
PSMB6 Proteasome subunit beta type-6	31	1	-0.16
CCT7 T-complex protein 1 subunit eta	1145	21	-0.16
SCRN1 Secernin-1	210	4	-0.16
ENAH Isoform 2 of Protein enabled homolog	123	3	-0.16
EIF2A Eukaryotic translation initiation factor 2A	59	1	-0.16
ARPC3 Actin-related protein 2/3 complex subunit 3	43	1	-0.16
PPP2R2C Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B gamma isoform	25	1	-0.16
HNRNPK Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	2880	50	-0.16
RPS5 40S ribosomal protein S5	214	3	-0.16
RARS Isoform Complexed of Arginyl-tRNA synthetase, cytoplasmic	148	4	-0.16
MAPRE1 Microtubule-associated protein RP/EB family member 1	161	4	-0.16
VPS13D Isoform 2 of Vacuolar protein sorting-associated protein 13D	26	1	-0.16
HSPA9 Stress-70 protein, mitochondrial	3083	53	-0.16
HSPA9 Stress-70 protein, mitochondrial	3083	53	-0.16
HSPA1L Heat shock 70kDa protein 1-like variant	265	4	-0.17

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
PRKDC Isoform 1 of DNA-dependent protein kinase catalytic subunit	802	19	-0.17
DNM2 Dynamin 2 isoform 4 variant (Fragment)	83	3	-0.17
CCT4 T-complex protein 1 subunit delta	1114	21	-0.17
DUT 24 kDa protein	574	7	-0.17
VPS35 Vacuolar protein sorting-associated protein 35	102	2	-0.17
PDIA6 Isoform 2 of Protein disulfide-isomerase A6	1065	18	-0.17
HIST2H2AA4;HIST2H2AA3 Histone H2A type 2-A	661	14	-0.17
ERP29 Endoplasmic reticulum protein ERp29	116	1	-0.17
SET Isoform 1 of Protein SET	863	16	-0.17
TROVE2 Isoform Long of 60 kDa SS-A/Ro ribonucleoprotein	308	5	-0.17
GSR Isoform Mitochondrial of Glutathione reductase, mitochondrial	88	2	-0.17
JTV1 Multisynthetase complex auxiliary component p38	54	1	-0.17
NPM1 Isoform 1 of Nucleophosmin	3159	51	-0.17
NPM1 Isoform 1 of Nucleophosmin	3159	51	-0.17
PRKCSH Glucosidase 2 subunit beta	608	11	-0.18
UBE2I SUMO-conjugating enzyme UBC9	57	1	-0.18
GLO1 Lactoylglutathione lyase	294	6	-0.18
SNRPD3 Small nuclear ribonucleoprotein Sm D3	25	1	-0.18
SRPK1 Isoform 1 of Serine/threonine-protein kinase SRPK1	27	1	-0.18
SRM Spermidine synthase	37	1	-0.18
SFRS3 Splicing factor, arginine/serine-rich 3	526	11	-0.18
MARCKS Myristoylated alanine-rich C-kinase substrate	342	5	-0.18
GCN1L1 Translational activator GCN1	62	2	-0.18
PODXL2 Isoform 2 of Podocalyxin-like protein 2	27	1	-0.18
RPL23 Similar to ribosomal protein L23	944	13	-0.18
EEF1D eukaryotic translation elongation factor 1 delta isoform 1	877	17	-0.18
- Farnesyl pyrophosphate synthetase like-4 protein (Fragment)	66	2	-0.18
DCTN2 dynactin 2	345	6	-0.19
RPL18 60S ribosomal protein L18	326	6	-0.19
CCT8 59 kDa protein	837	16	-0.19
AP2M1 Isoform 1 of AP-2 complex subunit mu-1	29	1	-0.19
HSPA8 Isoform 1 of Heat shock cognate 71 kDa protein	6557	111	-0.19
HSPA8 Isoform 1 of Heat shock cognate 71 kDa protein	6557	111	-0.19
SPTAN1 Isoform 2 of Spectrin alpha chain, brain	821	14	-0.19
WBP2 WW domain-binding protein 2	29	1	-0.19
U2AF2 Splicing factor U2AF 65 kDa subunit	511	8	-0.19
OCIAD1 Isoform 2 of OCIA domain-containing protein 1	27	1	-0.19
PGM1 Isoform 1 of Phosphoglucomutase-1	26	1	-0.19
RPL27A 60S ribosomal protein L27a	276	8	-0.20
ACTR2 Actin-related protein 2	68	2	-0.20
CALR Calreticulin	1113	23	-0.20
UQCRC2 Cytochrome b-c1 complex subunit 2, mitochondrial	248	6	-0.20
SND1 Staphylococcal nuclease domain-containing protein 1	701	12	-0.20
C14orf156 SRA stem-loop-interacting RNA-binding protein, mitochondrial	166	3	-0.20
PDIA3 14 kDa protein	79	1	-0.20
CSE1L Isoform 1 of Exportin-2	352	7	-0.20
SF3A3 Splicing factor 3A subunit 3	84	2	-0.20
- Similar to Mitochondrial ribosomal protein L38	38	1	-0.20
COPS4 COP9 signalosome complex subunit 4	30	1	-0.20
RPLP2 60S acidic ribosomal protein P2	527	9	-0.20
PSMD13 proteasome 26S non-ATPase subunit 13 isoform 2	247	4	-0.20
NCBP1 Nuclear cap-binding protein subunit 1	150	4	-0.20
CIP29 18 kDa protein	35	1	-0.20
YBX1 Nuclease-sensitive element-binding protein 1	1221	18	-0.21
FKBP4 FK506-binding protein 4	611	12	-0.21
PFAS Phosphoribosylformylglycinamidine synthase	211	5	-0.21
CCDC25 5 kDa protein	57	2	-0.21
ARID4A Isoform III of AT-rich interactive domain-containing protein 4A	27	1	-0.21
- cDNA FLJ46890 fis, clone UTERU3018172	26	1	-0.21
TUBA1B Tubulin alpha-1B chain	7000	126	-0.21
TUBA1B Tubulin alpha-1B chain	7000	126	-0.21
PAFAH1B1 Uncharacterized protein PAFAH1B1 (Fragment)	103	2	-0.21
GNB1 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	411	9	-0.21
CNN3 Calponin-3	252	5	-0.21
LRRC59 Leucine-rich repeat-containing protein 59	131	3	-0.21
TLE1 Transducin-like enhancer protein 1	32	1	-0.21
VDAC1 Voltage-dependent anion-selective channel protein 1	1490	28	-0.21
SORD Sorbitol dehydrogenase	1004	20	-0.21
HN1 Isoform 1 of Hematological and neurological expressed 1 protein	265	5	-0.21
ECH1 Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	109	2	-0.21
RPS2 40S ribosomal protein S2	478	10	-0.22
SF3B5 Splicing factor 3B subunit 5	173	3	-0.22
VBP1 Von Hippel-Lindau binding protein 1	98	3	-0.22
RPS21 40S ribosomal protein S21	482	8	-0.22
PPIB peptidylprolyl isomerase B precursor	312	6	-0.22
RPS6 40S ribosomal protein S6	290	7	-0.22
NDUFS4 NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitochondrial	44	1	-0.22
SMARCC1 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1	130	4	-0.22
RNF130 Goliath homolog	25	1	-0.22
HK1 Isoform 1 of Hexokinase-1	439	8	-0.22

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
- Uncharacterized protein ENSP00000348237	847	14	-0.22
DHX15 Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	530	9	-0.22
HADH Isoform 1 of Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	331	6	-0.22
RPL3 60S ribosomal protein L3	321	6	-0.22
RPL27 60S ribosomal protein L27	153	4	-0.22
CCT3 chaperonin containing TCP1, subunit 3 isoform b	2519	44	-0.22
MDH2 Malate dehydrogenase, mitochondrial	1892	32	-0.22
GBAS Protein NipSnap homolog 2	36	1	-0.22
UBA1 Ubiquitin-like modifier-activating enzyme 1	2935	44	-0.23
SNRPB Isoform SM-B' of Small nuclear ribonucleoprotein-associated proteins B and B'	131	3	-0.23
GOT2 Aspartate aminotransferase, mitochondrial	1097	21	-0.23
GNG4 Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-4	29	1	-0.23
RPL8 60S ribosomal protein L8	795	16	-0.23
RPL30 60S ribosomal protein L30	268	5	-0.23
NUDC Nuclear migration protein nudC	205	3	-0.23
LARP1 Isoform 3 of La-related protein 1	105	3	-0.23
HNRNPAB Isoform 2 of Heterogeneous nuclear ribonucleoprotein A/B	667	11	-0.24
CAP1 Adenylyl cyclase-associated protein 1	545	10	-0.24
ALDH18A1 Isoform Long of Delta-1-pyrroline-5-carboxylate synthetase	329	7	-0.24
ATP6V1B2 Vacuolar ATP synthase subunit B, brain isoform	237	5	-0.24
NLN cDNA FLJ14696 fis, clone NT2RP2005775, highly similar to NEUROLYSIN	58	1	-0.24
TAGLN2 24 kDa protein	893	13	-0.24
SGTA Small glutamine-rich tetratricopeptide repeat-containing protein alpha	192	4	-0.24
ARD1A N-terminal acetyltransferase complex ARD1 subunit homolog A	31	1	-0.24
DLD Dihydrolipoyl dehydrogenase, mitochondrial	514	9	-0.24
SNRPD1 Small nuclear ribonucleoprotein Sm D1	399	7	-0.24
CS citrate synthase precursor, isoform b	215	6	-0.24
GLT25D1 Glycosyltransferase 25 family member 1	188	4	-0.24
MAPK1IP1L MAPK-interacting and spindle-stabilizing protein-like	67	1	-0.24
GPSN2 Isoform 1 of Synaptic glycoprotein SC2	60	1	-0.24
CDC42 Isoform 2 of Cell division control protein 42 homolog	35	1	-0.24
BPNT1 Isoform 1 of 3'(2'),5'-bisphosphate nucleotidase 1	31	1	-0.24
FARSB Phenylalanyl-tRNA synthetase beta chain	184	5	-0.24
PSMC5 26S protease regulatory subunit 8	384	7	-0.24
PLD3 Phospholipase D3	57	2	-0.24
PSMC4 Isoform 1 of 26S protease regulatory subunit 6B	115	3	-0.25
GAP43 Neuromodulin	128	4	-0.25
ETFB Isoform 1 of Electron transfer flavoprotein subunit beta	32	1	-0.25
FH Isoform Mitochondrial of Fumarate hydratase, mitochondrial	1283	21	-0.25
MAT2A S-adenosylmethionine synthetase isoform type-2	190	3	-0.25
FUS Isoform Short of RNA-binding protein FUS	272	6	-0.25
DCX Doublecortex	177	4	-0.25
MT-CO2 Cytochrome c oxidase subunit 2	27	1	-0.25
KHSRP Isoform 1 of Far upstream element-binding protein 2	591	14	-0.26
HINT1 Histidine triad nucleotide-binding protein 1	168	4	-0.26
CHGA chromogranin A precursor	247	6	-0.26
CALM3;CALM2;CALM1 Calmodulin	207	5	-0.26
RIOK1 Serine/threonine-protein kinase RIO1	58	2	-0.26
PSMD12 26S proteasome non-ATPase regulatory subunit 12	80	2	-0.26
BCKDHA 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial	66	2	-0.26
S100A6 Protein S100-A6	237	3	-0.26
HSP90AA1 Isoform 1 of Heat shock protein HSP 90-alpha	2330	46	-0.26
OLA1 Isoform 2 of Obg-like ATPase 1	37	1	-0.26
ENO1 Isoform alpha-enolase of Alpha-enolase	6222	97	-0.26
ENO1 Isoform alpha-enolase of Alpha-enolase	6222	97	-0.26
- Dihydropyrimidinase-like 2 long form (Fragment)	185	3	-0.27
ILF2 Interleukin enhancer-binding factor 2	121	3	-0.27
RBM12 RNA-binding protein 12	47	1	-0.27
PDIA3 Protein disulfide-isomerase A3	2852	52	-0.27
PDIA3 Protein disulfide-isomerase A3	2852	52	-0.27
NONO Non-POU domain-containing octamer-binding protein	2632	46	-0.27
G3BP1 Ras GTPase-activating protein-binding protein 1	315	5	-0.27
RPL7 60S ribosomal protein L7	255	7	-0.27
PRDX1 Peroxiredoxin-1	1084	27	-0.27
NANS Sialic acid synthase	439	9	-0.27
SF3B3 Isoform 1 of Splicing factor 3B subunit 3	108	3	-0.27
MTDH Protein LYRIC	85	2	-0.27
1 SNRPD2 Small nuclear ribonucleoprotein Sm D2	221	6	-0.27
MRPS22 28S ribosomal protein S22, mitochondrial	130	2	-0.27
KIF5B Kinesin-1 heavy chain	550	10	-0.28
AHCY Adenosylhomocysteinase	535	11	-0.28
SFRS2 Splicing factor, arginine/serine-rich 2	288	5	-0.28
PSMC6 26S protease regulatory subunit S10B	461	11	-0.28
TIMM23 Mitochondrial import inner membrane translocase subunit Tim23	32	1	-0.28
PDCD5 Programmed cell death protein 5	91	2	-0.28
NAP1L4 Nucleosome assembly protein 1-like 4	271	4	-0.28
PFN2 Isoform IIb of Profilin-2	59	1	-0.28
TBCA Tubulin-specific chaperone A	36	1	-0.28
BIRC6 baculoviral IAP repeat-containing 6	26	1	-0.28
SAE1 SUMO-activating enzyme subunit 1	25	1	-0.28

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
IDH2 Isocitrate dehydrogenase [NADP], mitochondrial	714	13	-0.29
PPA1 Inorganic pyrophosphatase	361	6	-0.29
EIF2S3 Eukaryotic translation initiation factor 2 subunit 3	181	3	-0.29
PTPLAD1 Protein tyrosine phosphatase-like protein PTPLAD1	166	4	-0.29
KPNB1 Importin subunit beta-1	877	19	-0.29
IDH1 Isocitrate dehydrogenase [NADP] cytoplasmic	385	7	-0.29
PSMD1 Isoform 1 of 26S proteasome non-ATPase regulatory subunit 1	158	3	-0.29
SRP9 SRP9 protein	106	3	-0.29
CRMP1 Dihydropyrimidinase-related protein 1	875	16	-0.29
RPL18A 60S ribosomal protein L18a	220	5	-0.29
BCLAF1 Isoform 1 of Bcl-2-associated transcription factor 1	31	1	-0.29
EZR Ezrin	930	15	-0.29
SNRPA U1 small nuclear ribonucleoprotein A	208	6	-0.29
IARS2 Isoleucyl-tRNA synthetase, mitochondrial	30	1	-0.29
ANP32B Isoform 1 of Acidic leucine-rich nuclear phosphoprotein 32 family member B	198	4	-0.30
PAICS Multifunctional protein ADE2	314	6	-0.30
FAM50A Protein FAM50A	66	1	-0.30
HSP90AB1 Heat shock protein HSP 90-beta	6777	132	-0.30
HSP90AB1 Heat shock protein HSP 90-beta	6777	132	-0.30
TKT Transketolase	1845	30	-0.30
ATP6V1E1 Vacuolar proton pump subunit E 1	200	3	-0.30
CTNNA1 Isoform 1 of Catenin alpha-1	120	2	-0.30
LOC729611 similar to hCG1641491 isoform 2	90	2	-0.30
RPA2 Isoform 1 of Replication protein A 32 kDa subunit	48	1	-0.30
SHMT2 Serine hydroxymethyltransferase, mitochondrial	628	11	-0.30
NACA Nascent polypeptide-associated complex subunit alpha	238	4	-0.30
FKBP10 CDNA: FLJ22221 fis, clone HRC01651	184	3	-0.30
VAPA Vesicle-associated membrane protein-associated protein A	27	1	-0.30
FRAS1 Isoform 2 of Extracellular matrix protein FRAS1	52	2	-0.30
PRDX5 Isoform Mitochondrial of Peroxiredoxin-5, mitochondrial	37	1	-0.30
ATP6V1A Vacuolar ATP synthase catalytic subunit A	380	9	-0.30
GAPDH Glyceraldehyde-3-phosphate dehydrogenase	7488	132	-0.30
GAPDH Glyceraldehyde-3-phosphate dehydrogenase	7488	132	-0.30
HSPE1 10 kDa heat shock protein, mitochondrial	990	16	-0.31
PDIA4 Protein disulfide-isomerase A4	358	9	-0.31
DCLK1 Isoform 2 of Serine/threonine-protein kinase DCLK1	29	1	-0.31
ACAA2 3-ketoacyl-CoA thiolase, mitochondrial	443	6	-0.31
TCEB1 Transcription elongation factor B polypeptide 1	61	1	-0.31
TUFM Tu translation elongation factor, mitochondrial precursor	1037	17	-0.31
ANXA2 Annexin A2	467	11	-0.31
RBBP4 Histone-binding protein RBBP4	344	7	-0.31
C20orf3 Adipocyte plasma membrane-associated protein	315	9	-0.31
EIF4EBP1 Eukaryotic translation initiation factor 4E-binding protein 1	119	3	-0.31
METAP2 Methionine aminopeptidase 2	83	2	-0.31
AHCYL2 Putative adenosylhomocysteinase 3	39	1	-0.31
PRMT1 HMT1 hnRNP methyltransferase-like 2 isoform 1	636	12	-0.31
TPP2 Tripeptidyl-peptidase 2	137	4	-0.31
THY1 Thy-1 membrane glycoprotein	39	1	-0.31
MCM3 DNA replication licensing factor MCM3	272	7	-0.32
PSMC1 26S protease regulatory subunit 4	345	8	-0.32
SUGT1 Isoform 2 of Suppressor of G2 allele of SKP1 homolog	197	4	-0.32
SLC25A1 Tricarboxylate transport protein, mitochondrial	27	1	-0.32
TUBB2C Tubulin beta-2C chain	878	12	-0.32
TMPO Isoform Gamma of Lamina-associated polypeptide 2, isoforms beta/gamma	568	11	-0.32
USP14 Ubiquitin carboxyl-terminal hydrolase 14	152	3	-0.32
LOC651249 similar to Ribosomal protein L34	219	6	-0.32
RPL11 Isoform 1 of 60S ribosomal protein L11	323	7	-0.32
COX4I1 Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	58	2	-0.32
PSMB3 Proteasome subunit beta type-3	88	3	-0.32
- PRO1446	28	1	-0.32
SCAPER Isoform 1 of S phase cyclin A-associated protein in the endoplasmic reticulum	27	1	-0.32
NNT NAD(P) transhydrogenase, mitochondrial	538	10	-0.32
HYOU1 Hypoxia up-regulated protein 1	586	11	-0.32
CPNE7 Isoform 1 of Copine-7	28	1	-0.32
YWHAE 14-3-3 protein epsilon	2113	33	-0.32
- 12 kDa protein	132	2	-0.32
PSMD5 26S proteasome non-ATPase regulatory subunit 5	92	1	-0.32
CTTN cortactin isoform b	107	2	-0.32
RPL9 60S ribosomal protein L9	189	5	-0.32
CDC45L CDC45-related protein	34	1	-0.32
PHGDH D-3-phosphoglycerate dehydrogenase	1351	25	-0.33
HYDIN2;HYDIN Uncharacterized protein HYDIN2	30	1	-0.33
CNBP Isoform 1 of Cellular nucleic acid-binding protein	255	5	-0.33
RPL14 60S ribosomal protein L14	220	5	-0.33
RPL7A 60S ribosomal protein L7a	580	10	-0.33
NP CDNA FLJ25678 fis, clone TST04067, highly similar to PURINE NUCLEOSIDE PHOSPHORYLASE	533	12	-0.33
SDHA SDHA protein	27	1	-0.33
LAP3 Isoform 1 of Cytosol aminopeptidase	110	2	-0.33
YWHAB Isoform Long of 14-3-3 protein beta/alpha	743	10	-0.33
LDHB L-lactate dehydrogenase B chain	682	17	-0.33

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
RPS27 40S ribosomal protein S27	232	5	-0.33
EEF1A1 Elongation factor 1-alpha 1	6058	121	-0.33
EEF1A1 Elongation factor 1-alpha 1	6058	121	-0.33
GPI Glucose-6-phosphate isomerase	1964	35	-0.33
SERBP1 Isoform 1 of Plasminogen activator inhibitor 1 RNA-binding protein	120	4	-0.33
ISYNA1 58 kDa protein	36	1	-0.33
YWHAG 14-3-3 protein gamma	1277	15	-0.34
VAT1 Synaptic vesicle membrane protein VAT-1 homolog	465	8	-0.34
RPL12 Isoform 1 of 60S ribosomal protein L12	445	8	-0.34
RPLP1 60S acidic ribosomal protein P1	47	1	-0.34
YWHAZ 14-3-3 protein zeta/delta	826	11	-0.34
RPL13 60S ribosomal protein L13	322	7	-0.34
TOMM70A Mitochondrial import receptor subunit TOM70	209	6	-0.34
PKM2 Isoform M2 of Pyruvate kinase isozymes M1/M2	6290	110	-0.34
PKM2 Isoform M2 of Pyruvate kinase isozymes M1/M2	6290	110	-0.34
EIF4A1 Eukaryotic initiation factor 4A-I	1003	19	-0.34
DHX9 ATP-dependent RNA helicase A	672	15	-0.34
RPLP0 60S acidic ribosomal protein P0	467	9	-0.35
TUBB2B Tubulin beta-2B chain	518	9	-0.35
HADHB Trifunctional enzyme subunit beta, mitochondrial	270	7	-0.35
RNPEP Uncharacterized protein RNPEP	103	3	-0.35
C14orf166 UPF0568 protein C14orf166	79	2	-0.35
DNM3 Isoform 1 of Dynamin-3	115	4	-0.35
PA2G4 Proliferation-associated protein 2G4	309	8	-0.35
PFN1 Profilin-1	1218	23	-0.35
SYNCRIP Isoform 2 of Heterogeneous nuclear ribonucleoprotein Q	725	14	-0.35
PROSC Proline synthetase co-transcribed bacterial homolog protein	32	1	-0.35
UBE2M NEDD8-conjugating enzyme Ubc12	143	3	-0.36
- 8 kDa protein	27	1	-0.36
VGF VGF nerve growth factor inducible precursor	883	14	-0.36
PSMD3 26S proteasome non-ATPase regulatory subunit 3	314	7	-0.36
COPA Coatomer subunit alpha	94	3	-0.36
SF3B2 splicing factor 3B subunit 2	418	8	-0.36
MACROD1 MACRO domain-containing protein 1	112	3	-0.36
STIP1 STIP1 protein	573	12	-0.36
PTBP1 Isoform 1 of Polypyrimidine tract-binding protein 1	1571	27	-0.37
RPL6 60S ribosomal protein L6	345	9	-0.37
SLC25A11 Mitochondrial 2-oxoglutarate/malate carrier protein	154	2	-0.37
TIPRL Isoform 2 of TIP41-like protein	50	2	-0.37
VDAC2 Isoform 3 of Voltage-dependent anion-selective channel protein 2	742	15	-0.37
ACTBL2 Beta-actin-like protein 2	224	6	-0.37
SNRP70 Isoform 2 of U1 small nuclear ribonucleoprotein 70 kDa	35	1	-0.38
IPO5 importin 5	266	7	-0.38
MCTS1 Isoform 1 of Malignant T cell amplified sequence 1	58	2	-0.38
HS6ST2 Isoform 2 of Heparan-sulfate 6-O-sulfotransferase 2	29	1	-0.38
UBA2 SUMO-activating enzyme subunit 2	72	2	-0.38
PTK7 PTK7 protein tyrosine kinase 7 isoform a variant (Fragment)	28	1	-0.38
ASAH1 Acid ceramidase	30	1	-0.38
ANKRD20A1 Ankyrin repeat domain-containing protein 20A1	26	1	-0.38
ANP32A Acidic leucine-rich nuclear phosphoprotein 32 family member A	1319	22	-0.38
HSPH1 Isoform Beta of Heat shock protein 105 kDa	326	7	-0.38
DDX1 ATP-dependent RNA helicase DDX1	345	7	-0.38
CHMP4B Charged multivesicular body protein 4b	34	1	-0.38
SV2A Isoform 1 of Synaptic vesicle glycoprotein 2A	96	3	-0.38
RCC1 regulator of chromosome condensation 1 isoform a	255	4	-0.39
MTA2 Metastasis-associated protein MTA2	163	4	-0.39
NDUFS2 NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial	110	2	-0.39
TDRKH Isoform 1 of Tudor and KH domain-containing protein	28	1	-0.39
HADHA Trifunctional enzyme subunit alpha, mitochondrial	1491	27	-0.39
MTHFD1 C-1-tetrahydrofolate synthase, cytoplasmic	985	18	-0.39
RPL37A 60S ribosomal protein L37a	229	6	-0.39
ATAD3B Isoform 2 of ATPase family AAA domain-containing protein 3B	82	2	-0.39
ATP5L ATP synthase subunit g, mitochondrial	27	1	-0.39
TUBB2A Tubulin beta-2A chain	56	2	-0.39
PSMA3 Isoform 2 of Proteasome subunit alpha type-3	68	1	-0.39
EMD Emerin	36	1	-0.39
FNIP2 Isoform 1 of Folliculin-interacting protein 2	37	1	-0.39
CLTC Isoform 1 of Clathrin heavy chain 1	1773	31	-0.40
GNB2 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	140	3	-0.40
EEF1A2 Elongation factor 1-alpha 2	1121	23	-0.40
PRDX6 Peroxiredoxin-6	1157	19	-0.40
RLTPR Leucine-rich repeat-containing protein 16C	34	1	-0.40
UQCRH Cytochrome b-c1 complex subunit 6, mitochondrial	130	2	-0.40
TMED10 Transmembrane emp24 domain-containing protein 10	172	3	-0.40
TUBA1A Tubulin alpha-1A chain	236	5	-0.40
PPT1 Palmitoyl-protein thioesterase 1	321	3	-0.40
L1CAM Isoform 1 of Neural cell adhesion molecule L1	120	3	-0.40
AARSD1 Alanyl-tRNA synthetase, class IIc family protein	29	1	-0.40
ARHGDIA Rho GDP-dissociation inhibitor 1	572	8	-0.41
RPL35 60S ribosomal protein L35	233	6	-0.41
MRE11A Isoform 1 of Double-strand break repair protein MRE11A	80	2	-0.41

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
CSTB Cystatin-B	40	1	-0.41
TRAP1 Heat shock protein 75 kDa, mitochondrial	1188	20	-0.41
ZYX Zyxin	81	2	-0.41
RPL36 60S ribosomal protein L36	98	3	-0.41
RBP1 Retinol-binding protein I, cellular	33	1	-0.41
NDUFAF2 Mimitin, mitochondrial	34	1	-0.42
MDH1 Malate dehydrogenase, cytoplasmic	250	5	-0.42
ATPIF1 Putative uncharacterized protein DKFZp564G0422	94	2	-0.42
FEN1 Flap endonuclease 1	41	1	-0.42
PYCR1 Pyrroline-5-carboxylate reductase 1, mitochondrial	397	5	-0.42
SERPINH1 Serpin H1	129	2	-0.42
SCG3 Secretogranin-3	116	3	-0.43
HNRNPF Heterogeneous nuclear ribonucleoprotein F	287	4	-0.43
AKR1C1 Aldo-keto reductase family 1 member C1	175	3	-0.43
EIF4G1 eukaryotic translation initiation factor 4 gamma, 1 isoform 2	753	13	-0.43
ALDOC Fructose-bisphosphate aldolase C	148	4	-0.43
YARS Tyrosyl-tRNA synthetase, cytoplasmic	141	3	-0.43
ATIC Bifunctional purine biosynthesis protein PURH	771	12	-0.43
ALDH7A1 aldehyde dehydrogenase 7 family, member A1	35	1	-0.43
KARS Lysyl-tRNA synthetase	479	8	-0.44
SF3A1 Splicing factor 3 subunit 1	116	2	-0.44
MBD3 Isoform 1 of Methyl-CpG-binding domain protein 3	227	4	-0.44
CACYBP Isoform 1 of Calcyclin-binding protein	468	11	-0.44
MAP4 110 kDa protein	281	6	-0.44
PSMB4 Proteasome subunit beta type-4	31	1	-0.44
PGK1 Phosphoglycerate kinase 1	3350	53	-0.44
PGK1 Phosphoglycerate kinase 1	3350	53	-0.44
PPP2R1A Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	222	5	-0.45
C7orf24 Uncharacterized protein C7orf24	40	1	-0.45
CNIH4 Isoform 1 of Protein cornichon homolog 4	30	1	-0.45
UBC;UBB;RPS27A ubiquitin and ribosomal protein S27a precursor	1296	30	-0.45
CEP170 Isoform 1 of Centrosomal protein of 170 kDa	118	2	-0.45
GDI1 Rab GDP dissociation inhibitor alpha	937	15	-0.45
AARS Alanyl-tRNA synthetase, cytoplasmic	709	13	-0.45
SOD1 Superoxide dismutase [Cu-Zn]	944	19	-0.45
NOMO3;NOMO1 Nodal modulator 1	41	1	-0.45
PABPC1 Isoform 1 of Polyadenylate-binding protein 1	972	17	-0.45
ZNF217 Zinc finger protein 217	30	1	-0.45
TPI1 Isoform 1 of Triosephosphate isomerase	1339	24	-0.45
COPG Coatomer subunit gamma	82	2	-0.45
HSD17B10 Isoform 1 of 3-hydroxyacyl-CoA dehydrogenase type-2	246	4	-0.46
DARS Aspartyl-tRNA synthetase, cytoplasmic	161	4	-0.46
IKBKG 29 kDa protein	29	1	-0.46
NR2C2AP 19 kDa protein	33	1	-0.46
EEF2 Elongation factor 2	4184	74	-0.46
EEF2 Elongation factor 2	4184	74	-0.46
TUBB4 Tubulin beta-4 chain	61	1	-0.46
EWSR1 cDNA FLJ31747 fis, clone NT2RI2007377, highly similar to RNA-BINDING PROTEIN EWS	242	5	-0.46
TPT1 TPT1 protein	124	3	-0.46
DYNC1I2 Isoform 2C of Cytoplasmic dynein 1 intermediate chain 2	41	1	-0.46
AKR1B1 Aldose reductase	86	3	-0.46
SARS Seryl-tRNA synthetase, cytoplasmic	494	9	-0.46
LARS Leucyl-tRNA synthetase, cytoplasmic	1222	19	-0.46
RAD23B UV excision repair protein RAD23 homolog B	495	12	-0.46
RPS12 ribosomal protein S12	331	7	-0.46
PCNP Isoform 1 of PEST proteolytic signal-containing nuclear protein	222	6	-0.46
SPTBN1 Isoform Long of Spectrin beta chain, brain 1	154	4	-0.46
NSUN2 tRNA (cytosine-5-)-methyltransferase NSUN2	115	3	-0.46
SRP14 Signal recognition particle 14 kDa protein	43	1	-0.46
CKB Creatine kinase B-type	4063	65	-0.46
CKB Creatine kinase B-type	4063	65	-0.46
MIF;LOC284889 Macrophage migration inhibitory factor	279	6	-0.47
ACTL6A Isoform 1 of Actin-like protein 6A	27	1	-0.47
PPIA Peptidyl-prolyl cis-trans isomerase A	3804	74	-0.47
PPIA Peptidyl-prolyl cis-trans isomerase A	3804	74	-0.47
LANCL1 LanC-like protein 1	28	1	-0.47
ATP5F1 ATP synthase subunit b, mitochondrial	26	1	-0.47
HMGB1 High mobility group protein B1	1781	30	-0.47
RPL19 60S ribosomal protein L19	735	12	-0.47
TNPO1 transportin 1 isoform 1	292	5	-0.47
PEPD Xaa-Pro dipeptidase	65	2	-0.47
LOC641293 Similar to 60S ribosomal protein L21	635	9	-0.48
UCHL1 Ubiquitin carboxyl-terminal hydrolase isozyme L1	1297	24	-0.48
MCM6 DNA replication licensing factor MCM6	179	5	-0.48
XPO1 Exportin-1	48	1	-0.48
ACTR1A Alpha-centractin	28	1	-0.48
HNRNPUL1 Isoform 1 of Heterogeneous nuclear ribonucleoprotein U-like protein 1	60	2	-0.48
ENO2 Gamma-enolase	477	6	-0.48
TM9SF3 Transmembrane 9 superfamily member 3	32	1	-0.48
PEBP1 Phosphatidylethanolamine-binding protein 1	1582	24	-0.48

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
FHL1 Four and a half LIM domains 1 variant	421	10	-0.48
UBQLN1 Isoform 2 of Ubiquilin-1	138	3	-0.48
USO1 Putative uncharacterized protein DKFZp451D234	51	1	-0.48
C14orf2 6.8 kDa mitochondrial proteolipid	31	1	-0.48
ABCC8 Uncharacterized protein ABCC8	26	1	-0.48
EPRS Bifunctional aminoacyl-tRNA synthetase	791	19	-0.49
RPL10A 60S ribosomal protein L10a	149	3	-0.49
S100A11 Protein S100-A11	48	1	-0.49
CAPZA2 F-actin-capping protein subunit alpha-2	107	3	-0.49
SMC4 Isoform 2 of Structural maintenance of chromosomes protein 4	66	2	-0.49
ST13 Hsc70-interacting protein	408	9	-0.49
RPL26L1 60S ribosomal protein L26-like 1	151	3	-0.49
PARK7 Protein DJ-1	208	5	-0.49
HMGB3 High mobility group protein B3	76	1	-0.49
OXCT1 Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial	53	1	-0.49
ABCA13 Isoform 1 of ATP-binding cassette sub-family A member 13	25	1	-0.49
FSCN1 Fascin	2121	36	-0.49
HMGB2 High mobility group protein B2	806	18	-0.49
RPN2 Ribophorin II	411	6	-0.49
ACSL4 Isoform Long of Long-chain-fatty-acid--CoA ligase 4	106	1	-0.49
PPM1G Protein phosphatase 1G	135	4	-0.49
ASPM Isoform 1 of Abnormal spindle-like microcephaly-associated protein	25	1	-0.49
HDGF Hepatoma-derived growth factor	502	11	-0.50
CTSB Cathepsin B	387	5	-0.50
UBE2L3 Ubiquitin-conjugating enzyme E2 L3	170	3	-0.50
C14orf102 hypothetical protein LOC55051 isoform 2	34	1	-0.51
CLIC4 Chloride intracellular channel protein 4	140	3	-0.51
ATP5O ATP synthase subunit O, mitochondrial	59	2	-0.51
DNA2 DNA replication helicase 2 homolog	25	1	-0.51
ACOT7 Isoform 1 of Cytosolic acyl coenzyme A thioester hydrolase	473	9	-0.51
DBNL Isoform 3 of Drebrin-like protein	57	2	-0.51
HNRNPD Isoform 1 of Heterogeneous nuclear ribonucleoprotein D0	678	14	-0.52
RANGAP1 Ran GTPase-activating protein 1	56	1	-0.52
EML4 EML4 protein	409	6	-0.52
ABCF1 Isoform 2 of ATP-binding cassette sub-family F member 1	265	4	-0.52
RAD23A UV excision repair protein RAD23 homolog A	37	1	-0.52
TFG Protein TFG	94	3	-0.52
LONP1 Lon protease homolog, mitochondrial	565	12	-0.52
RPL24 19 kDa protein	246	5	-0.52
CBS Isoform 1 of Cystathionine beta-synthase	151	4	-0.52
AHSA1 Activator of 90 kDa heat shock protein ATPase homolog 1	28	1	-0.52
CSNK2B;LY6G5B Casein kinase II subunit beta	31	1	-0.52
HERC2 Putative uncharacterized protein DKFZp547P028	29	1	-0.52
NXF1 Nuclear RNA export factor 1	28	1	-0.52
CD2AP CD2-associated protein	34	1	-0.53
PAFAH1B3 Platelet-activating factor acetylhydrolase IB subunit gamma	233	5	-0.53
TPD52L2 Isoform 2 of Tumor protein D54	95	2	-0.53
SCT Secretin	26	1	-0.53
EIF5A Isoform 2 of Eukaryotic translation initiation factor 5A-1	658	17	-0.54
LOC554235 Putative L-aspartate dehydrogenase	34	1	-0.54
PRPF19 Pre-mRNA-processing factor 19	130	2	-0.54
LASP1 Isoform 1 of LIM and SH3 domain protein 1	75	2	-0.54
PSAT1 Isoform 1 of Phosphoserine aminotransferase	392	9	-0.54
SMC1A Structural maintenance of chromosomes protein 1A	126	3	-0.54
SSB Lupus La protein	183	5	-0.54
GSTP1 Glutathione S-transferase P	811	9	-0.55
SF3B1 Splicing factor 3B subunit 1	337	8	-0.55
DNAJA2 DnaJ homolog subfamily A member 2	84	2	-0.55
AIFM1 Isoform 1 of Apoptosis-inducing factor 1, mitochondrial	394	8	-0.55
MAPT Isoform Tau-B of Microtubule-associated protein tau	255	6	-0.55
NOLC1 Isoform Beta of Nucleolar phosphoprotein p130	39	1	-0.55
PRDX3 Thioredoxin-dependent peroxide reductase, mitochondrial	203	4	-0.55
FABP5;FABP5L7 Fatty acid-binding protein, epidermal	160	5	-0.56
RNMTL1 RNA methyltransferase-like protein 1	27	1	-0.56
KHDRBS1 Isoform 1 of KH domain-containing, RNA-binding, signal transduction-associated protein 1	303	8	-0.57
C22orf32 Similar to Cullin-associated NEDD8-dissociated protein 1	178	4	-0.57
TIMM44 Mitochondrial import inner membrane translocase subunit TIM44	33	1	-0.57
RAB1A Isoform 1 of Ras-related protein Rab-1A	61	1	-0.57
SMS Isoform 1 of Spermine synthase	31	1	-0.57
CTPS CTP synthase 1	117	3	-0.57
PRPF31 Isoform 2 of U4/U6 small nuclear ribonucleoprotein Prp31	44	1	-0.57
DDX39 DDX39 protein	227	4	-0.58
DDX3Y;LOC100130220 ATP-dependent RNA helicase DDX3Y	200	4	-0.58
SF3B4 Splicing factor 3B subunit 4	125	2	-0.58
HSPA1B;HSPA1A Heat shock 70 kDa protein 1	402	8	-0.58
WARS Tryptophanyl-tRNA synthetase, cytoplasmic	280	5	-0.58
PHACTR2 Isoform 1 of Phosphatase and actin regulator 2	33	1	-0.58
STMN1 Stathmin	543	14	-0.58
PMPCB Mitochondrial-processing peptidase subunit beta	36	1	-0.58
TSPYL5 Testis-specific Y-encoded-like protein 5	32	1	-0.58

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
RAN GTP-binding nuclear protein Ran	342	7	-0.59
CTSD Cathepsin D	32	1	-0.59
ASS1 Argininosuccinate synthase	770	19	-0.59
SLC25A13 Mitochondrial aspartate-glutamate carrier protein	115	2	-0.59
ASNS Asparagine synthetase [glutamine-hydrolyzing]	84	2	-0.59
CAMK4 Calcium/calmodulin-dependent protein kinase type IV	62	1	-0.60
MSH2 DNA mismatch repair protein Msh2	36	1	-0.60
NUCB1 Nucleobindin-1	28	1	-0.60
PCNA Proliferating cell nuclear antigen	802	11	-0.60
SFRS6 Isoform SRP55-1 of Splicing factor, arginine/serine-rich 6	38	1	-0.60
TBCB Tubulin folding cofactor B	184	4	-0.60
MCM4 DNA replication licensing factor MCM4	154	5	-0.60
LOC284393 similar to QM protein isoform 1	706	15	-0.61
SEC24C Protein transport protein Sec24C	44	1	-0.61
TPR nuclear pore complex-associated protein TPR	27	1	-0.61
UBE2V2 Ubiquitin-conjugating enzyme E2 variant 2	99	2	-0.62
GAB2 Isoform 1 of GRB2-associated-binding protein 2	58	2	-0.62
GSPT1 Eukaryotic peptide chain release factor GTP-binding subunit ERF3A	85	2	-0.62
PDHA1 Mitochondrial PDHA1	62	2	-0.62
ATP6V1H Isoform 1 of Vacuolar proton pump subunit H	31	1	-0.62
FRYL Isoform 2 of Protein furry homolog-like	29	1	-0.62
HNRNPA1 Isoform A1-B of Heterogeneous nuclear ribonucleoprotein A1	1312	23	-0.62
SEC31A Isoform 3 of Protein transport protein Sec31A	149	3	-0.62
HNRNPL heterogeneous nuclear ribonucleoprotein L isoform b	907	15	-0.63
DNAJC7 DnaJ homolog subfamily C member 7	121	2	-0.63
ARS2 Isoform 2 of Arsenite-resistance protein 2	93	2	-0.63
MFAP5 Microfibrillar-associated protein 5	25	1	-0.63
NUP93 Nuclear pore complex protein Nup93	141	3	-0.64
RBM8A Isoform 1 of RNA-binding protein 8A	71	2	-0.64
CORO1A Coronin-1A	33	1	-0.64
LMNA Isoform A of Lamin-A/C	1700	31	-0.64
PCK2 Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	323	5	-0.64
TCEB2 Transcription elongation factor B polypeptide 2	177	3	-0.64
SLC1A5 Neutral amino acid transporter B(0)	183	3	-0.64
SSBP1 Single-stranded DNA-binding protein, mitochondrial	115	2	-0.64
RPL5 60S ribosomal protein L5	491	11	-0.65
ATP5J ATP synthase-coupling factor 6, mitochondrial	85	2	-0.65
ITPA Inosine triphosphate pyrophosphatase	38	1	-0.65
SFRS1 Isoform ASF-1 of Splicing factor, arginine/serine-rich 1	102	3	-0.65
TOMM34 Mitochondrial import receptor subunit TOM34	29	1	-0.65
CCAR1 Cell division cycle and apoptosis regulator protein 1	40	1	-0.65
C19orf10 UPF0556 protein C19orf10	31	1	-0.66
TMED2 Transmembrane emp24 domain-containing protein 2	61	1	-0.66
ACO2 Aconitate hydratase, mitochondrial	290	5	-0.66
CDC37 Hsp90 co-chaperone Cdc37	300	3	-0.66
PSME1 Proteasome activator complex subunit 1	117	3	-0.67
PCBP2 poly(rC) binding protein 2 isoform b	445	8	-0.67
ASNA1 Arsenical pump-driving ATPase	51	1	-0.67
NASP Isoform 1 of Nuclear autoantigenic sperm protein	161	3	-0.67
USP5 Isoform Long of Ubiquitin carboxyl-terminal hydrolase 5	167	5	-0.68
PSMD7 26S proteasome non-ATPase regulatory subunit 7	77	2	-0.68
GART Isoform Short of Trifunctional purine biosynthetic protein adenosine-3	98	3	-0.68
MCM7 Isoform 2 of DNA replication licensing factor MCM7	30	1	-0.68
ATP5I ATP synthase, H+ transporting, mitochondrial F0 complex, subunit E	75	2	-0.68
ETFA Electron transfer flavoprotein subunit alpha, mitochondrial	734	6	-0.69
HDLBP Vigilin	139	3	-0.69
CPNE1 Copine I	65	2	-0.69
HNRNPA3 Isoform 1 of Heterogeneous nuclear ribonucleoprotein A3	343	3	-0.69
KIAA1967 Isoform 1 of Protein KIAA1967	195	3	-0.69
MCM2 DNA replication licensing factor MCM2	169	3	-0.69
NMT1 Isoform Short of Glycylpeptide N-tetradecanoyltransferase 1	26	1	-0.69
U2AF1 Splicing factor U2AF 35 kDa subunit	324	5	-0.70
RPL4 60S ribosomal protein L4	401	9	-0.70
C3orf60 Uncharacterized protein C3orf60	35	1	-0.70
GAPVD1 Isoform 6 of GTPase-activating protein and VPS9 domain-containing protein 1	56	2	-0.70
PACSIN2 Isoform 1 of Protein kinase C and casein kinase substrate in neurons protein 2	25	1	-0.70
RAPGEF2 Rap guanine nucleotide exchange factor 2	27	1	-0.71
PHYHIPL Isoform 1 of Phytanoyl-CoA hydroxylase-interacting protein-like	31	1	-0.71
PTRH2 Peptidyl-tRNA hydrolase 2, mitochondrial	53	1	-0.71
FKBP3 FK506-binding protein 3	42	1	-0.72
TXN Thioredoxin	76	2	-0.72
BASP1 Brain acid soluble protein 1	574	10	-0.73
CKM Creatine kinase M-type	149	3	-0.73
HNRNPA2B1 Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1	1803	23	-0.75
FERMT2 Isoform 1 of Fermitin family homolog 2	192	3	-0.75
TXNL1 Thioredoxin-like protein 1	108	3	-0.76
EHD1 EH domain-containing protein 1	26	1	-0.76
C1orf55 Isoform 1 of Uncharacterized protein C1orf55	26	1	-0.76
ACADVL Isoform 1 of Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	37	1	-0.77
PIR Pirin	80	2	-0.77
LSM14B Isoform 1 of LSM14 protein homolog B	74	2	-0.77

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
SRP72 Signal recognition particle 72 kDa protein	58	2	-0.78
PABPC4 Isoform 1 of Polyadenylate-binding protein 4	76	2	-0.78
BMP2K Isoform 3 of BMP-2-inducible protein kinase	27	1	-0.78
ATXN10 Ataxin-10	152	2	-0.79
PSMD6 17 kDa protein	47	1	-0.79
ARRB1 Isoform 1A of Beta-arrestin-1	50	1	-0.80
GDI2 Rab GDP dissociation inhibitor beta	27	1	-0.80
HNRNPH1 Heterogeneous nuclear ribonucleoprotein H	525	7	-0.81
DNAJA1 DnaJ homolog subfamily A member 1	110	2	-0.82
FKBP5 FK506-binding protein 5	55	2	-0.82
NT5E 5'-nucleotidase	225	4	-0.82
CLIC1 Chloride intracellular channel protein 1	102	3	-0.82
PRPF8 Pre-mRNA-processing-splicing factor 8	66	2	-0.83
PCBP1 Poly(rC)-binding protein 1	184	5	-0.83
MCM5 DNA replication licensing factor MCM5	184	3	-0.83
RIOK1 RIO kinase 1 isoform 2	63	1	-0.83
NLN Neurolysin, mitochondrial	29	1	-0.83
SNX6 sorting nexin 6 isoform a	32	1	-0.83
MTPN Myotrophin	118	2	-0.84
GTF2I Isoform 1 of General transcription factor II-I	95	3	-0.84
PAK3 Isoform 2 of Serine/threonine-protein kinase PAK 3	32	1	-0.84
IPO4 IPO4 protein variant (Fragment)	48	1	-0.85
VAPA 14 kDa protein	76	2	-0.85
JUND Transcription factor jun-D	26	1	-0.86
MARS Methionyl-tRNA synthetase, cytoplasmic	129	3	-0.86
TAGLN3 Transgelin-3	61	1	-0.87
PPP5C Serine/threonine-protein phosphatase 5	228	2	-0.87
NCAM1 Isoform 3 of Neural cell adhesion molecule 1	65	1	-0.87
MESDC2 Mesoderm development candidate 2	48	1	-0.87
SF3A2 SF3A2 protein (Fragment)	160	3	-0.88
CHCHD2 Coiled-coil-helix-coiled-coil-helix domain-containing protein 2, mitochondrial	477	7	-0.88
ANKRD30A Ankyrin repeat domain-containing protein 30A	54	2	-0.88
RBBP7 Histone-binding protein RBBP7	45	1	-0.89
ECHS1 Enoyl-CoA hydratase, mitochondrial	53	1	-0.89
NUDT21 Cleavage and polyadenylation specificity factor subunit 5	82	1	-0.90
TXLNA Alpha-taxilin	25	1	-0.90
CCDC47 Isoform 1 of Coiled-coil domain-containing protein 47	166	3	-0.90
ARPC5 Isoform 1 of Actin-related protein 2/3 complex subunit 5	49	1	-0.90
SRP72 CaM kinase II isoform	68	2	-0.90
CSNK2A1P;CSNK2A1 Casein kinase II alpha subunit	35	1	-0.90
CHD4 Isoform 1 of Chromodomain-helicase-DNA-binding protein 4	214	6	-0.90
SNRPC U1 small nuclear ribonucleoprotein C	130	3	-0.91
HNRPDL Isoform 1 of Heterogeneous nuclear ribonucleoprotein D-like	101	3	-0.91
CCBL2 kynurenine aminotransferase III isoform 3	28	1	-0.91
LUC7L2 Isoform 1 of Putative RNA-binding protein Luc7-like 2	76	2	-0.94
MRPL45 39S ribosomal protein L45, mitochondrial	25	1	-0.95
GTF2F2 General transcription factor IIF subunit 2	37	1	-0.95
C22orf28 UPF0027 protein C22orf28	60	2	-0.96
NEDD8 NEDD8	27	1	-0.96
ZNF781 Isoform 1 of Zinc finger protein 781	26	1	-0.97
CDIPT Isoform 1 of CDP-diacylglycerol--inositol 3-phosphatidyltransferase	29	1	-0.97
ANXA5 Annexin A5	32	1	-0.97
PTGES3 Prostaglandin E synthase 3	247	6	-0.98
CHCHD3 Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial	144	3	-0.99
EFTUD2 116 kDa U5 small nuclear ribonucleoprotein component	42	1	-0.99
TERF2IP Telomeric repeat-binding factor 2-interacting protein 1	63	2	-1.00
CSDE1 Isoform Long of Cold shock domain-containing protein E1	120	2	-1.00
DDX23 Probable ATP-dependent RNA helicase DDX23	33	1	-1.00
NAPA Alpha-soluble NSF attachment protein	36	1	-1.01
C1orf61 Protein	36	1	-1.02
MAP7D2 Isoform 2 of MAP7 domain-containing protein 2	40	1	-1.02
LOC100130999;FAM81A hypothetical protein LOC145773	29	1	-1.03
TMSB10 Thymosin beta-10	54	1	-1.04
LOC731605 hypothetical LOC731605	29	1	-1.04
RBM14 Isoform 1 of RNA-binding protein 14	152	3	-1.04
RPL13A 60S ribosomal protein L13a	35	1	-1.04
CAMKK2 Isoform 3 of Calcium/calmodulin-dependent protein kinase kinase 2	34	1	-1.05
ANP32E Acidic leucine-rich nuclear phosphoprotein 32 family member E	698	8	-1.05
RPA1 Replication protein A 70 kDa DNA-binding subunit	95	2	-1.06
PSMA4 Proteasome subunit alpha type-4	61	1	-1.06
LOC51035 Isoform 1 of SAPK substrate protein 1	33	1	-1.07
ZSWIM6 zinc finger, SWIM domain containing 6	26	1	-1.07
SMC2 Isoform 1 of Structural maintenance of chromosomes protein 2	139	3	-1.07
RORB Isoform 2 of Nuclear receptor ROR-beta	26	1	-1.07
UFD1L Isoform Short of Ubiquitin fusion degradation protein 1 homolog	79	2	-1.08
WIZ Isoform 2 of Protein Wiz	48	1	-1.10
C10orf58 Uncharacterized protein C10orf58	41	1	-1.10
SYN2 Uncharacterized protein SYN2 (Fragment)	65	2	-1.11
DDR2 Discoidin domain receptor tyrosine kinase 2	27	1	-1.11
CAD CAD protein	626	14	-1.12
EIF4A3 Eukaryotic initiation factor 4A-III	34	1	-1.13

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	log_2(heavy /light)
ARGLU1 Isoform 1 of Arginine and glutamate-rich protein 1	29	1	-1.13
FKSG30 Kappa-actin	28	1	-1.13
PELO Protein pelota homolog	26	1	-1.13
KIF15 Isoform 4 of Kinesin-like protein KIF15	25	1	-1.13
GLS Isoform GAC of Glutaminase kidney isoform, mitochondrial	185	3	-1.14
NPLOC4 Isoform 2 of Nuclear protein localization protein 4 homolog	63	2	-1.14
LSM4 U6 snRNA-associated Sm-like protein LSm4	33	1	-1.15
EIF5 Eukaryotic translation initiation factor 5	71	2	-1.15
VPS13C Isoform 1 of Vacuolar protein sorting-associated protein 13C	64	2	-1.16
DDC Aromatic-L-amino-acid decarboxylase	78	2	-1.16
MTIF3 Translation initiation factor IF-3, mitochondrial	30	1	-1.16
CDC73 Putative uncharacterized protein (Fragment)	29	1	-1.18
RANBP3 Isoform 1 of Ran-binding protein 3	188	3	-1.19
- NEFM protein	357	5	-1.21
BAT3 Isoform 1 of Large proline-rich protein BAT3	73	2	-1.24
IGF2BP3 Isoform 1 of Insulin-like growth factor 2 mRNA-binding protein 3	92	1	-1.26
CCDC49 24 kDa protein	33	1	-1.27
LUC7L Isoform 1 of Putative RNA-binding protein Luc7-like 1	171	4	-1.28
DOCK1 Dedicator of cytokinesis protein 1	25	1	-1.28
VIM Vimentin	1015	19	-1.29
THRAP3 Thyroid hormone receptor-associated protein 3	213	5	-1.29
TPT1 Tumor protein, translationally-controlled 1	31	1	-1.29
HDAC2 histone deacetylase 2	166	4	-1.30
DLG1 Isoform 2 of Disks large homolog 1	57	2	-1.32
DCTN1 Isoform p150 of Dynactin subunit 1	35	1	-1.32
INA Alpha-internexin	620	11	-1.33
HNRNPH3 Isoform 1 of Heterogeneous nuclear ribonucleoprotein H3	254	4	-1.33
- 71 kDa protein	64	2	-1.37
- SMT3 suppressor of mif two 3 homolog 2 isoform b precursor	88	1	-1.41
PHOX2A Paired mesoderm homeobox protein 2A	26	1	-1.41
TERF2 Isoform 1 of Telomeric repeat-binding factor 2	35	1	-1.44
HNRNPC Isoform C1 of Heterogeneous nuclear ribonucleoproteins C1/C2	164	4	-1.44
- Pseudogene candidate	53	1	-1.51
PRPH Isoform 1 of Peripherin	475	9	-1.52
YWHAH 14-3-3 protein eta	87	1	-1.52
ADAR Isoform 2 of Double-stranded RNA-specific adenosine deaminase	69	1	-1.53
ZNF415 Isoform 5 of Zinc finger protein 415	62	2	-1.55
STX5 Isoform 2 of Syntaxin-5	34	1	-1.63
SYNE2 Isoform 1 of Nesprin-2	26	1	-1.67
NHSL2 NHS-like protein 2	54	1	-1.68
ALDH2 Aldehyde dehydrogenase, mitochondrial	28	1	-1.68
ACTR3 Actin-related protein 3	50	1	-1.78
RAB2B cDNA FLJ14824 fis, clone OVARC1000771, moderately similar to RAS-RELATED PROTEIN RAB-2	29	1	-1.78
PHTF1 Putative homeodomain transcription factor 1	28	1	-1.78
- 3 kDa protein	25	1	-1.79
FLNB Isoform 1 of Filamin-B	45	1	-1.80
SYT1 Synaptotagmin-1	55	1	-1.85
AP3M1 AP-3 complex subunit mu-1	26	1	-1.89
KIF15 Isoform 1 of Kinesin-like protein KIF15	30	1	-1.94
RDX Radixin isoform b	49	1	-2.01
ATP5H Isoform 1 of ATP synthase subunit d, mitochondrial	33	1	-2.02
SLC38A8 Putative sodium-coupled neutral amino acid transporter 8	27	1	-2.06
LEMD3 Inner nuclear membrane protein Man1	32	1	-2.08
PABPN1 Isoform 1 of Polyadenylate-binding protein 2	27	1	-2.09
RBM39 Isoform 1 of RNA-binding protein 39	26	1	-2.20
ZSCAN21 Zinc finger and SCAN domain-containing protein 21	25	1	-2.30
CORO7;Magmas Coronin-7	178	4	-2.31
APAF1 Isoform 1 of Apoptotic protease-activating factor 1	37	1	-2.31
NEFL Putative uncharacterized protein DKFZp761K0922 (Fragment)	139	3	-2.35
C1orf87 Isoform 1 of Uncharacterized protein C1orf87	26	1	-2.51
SOX13 SRY-box 13	59	2	-2.52
RAB40A Ras-related protein Rab-40A	30	1	-2.53
FAM13A1 family with sequence similarity 13, member A1 isoform a	26	1	-2.56
ADCK1 Isoform 2 of Uncharacterized aarF domain-containing protein kinase 1	33	1	-2.58
CD320 CD320 antigen	30	1	-2.59
MAST2 MAST2 protein	25	1	-2.61
C1orf168 Isoform 1 of Uncharacterized protein C1orf168	27	1	-2.67
S100A7 Protein S100-A7	56	1	-2.71
HNRNPA0 Heterogeneous nuclear ribonucleoprotein A0	32	1	-2.75
- 12 kDa protein	25	1	-2.87
BEST1 Isoform 1 of Bestrophin-1	32	1	-2.89
PARK2 Isoform 1 of E3 ubiquitin-protein ligase parkin	41	1	-2.91
STX18 Syntaxin-18	26	1	-2.94
STRA8 Stimulated by retinoic acid gene 8 protein homolog	35	1	-3.50
KIAA0564 Isoform 3 of Uncharacterized protein KIAA0564	28	1	-3.52
AGRN Agrin	28	1	-3.52
PDE4C PDE4C-delta109	26	1	-3.79
LOC646821 similar to beta-actin	96	3	-3.84
RAB8B 10 kDa protein	26	1	-3.96
CCDC66 51 kDa protein	56	2	-4.12

Proteins eluted with mild detergent and salt (weak parkin interactors – CCCP conditions)	Protein Mascot Score	# of peptides	$\log_2$(heavy /light)
DUSP19 Isoform 1 of Dual specificity protein phosphatase 19	26	1	-4.15
VWA3A cDNA FLJ40941 fis, clone UTERU2008426	29	1	-4.26
MOS Proto-oncogene serine/threonine-protein kinase mos	26	1	-4.26
- Ig kappa chain V-III region GOL	29	1	-4.33
SLC12A2 Isoform 1 of Solute carrier family 12 member 2	37	1	-4.64
SPATA5 Isoform 1 of Spermatogenesis-associated protein 5	50	2	-4.64
CEACAM18 Carcinoembryonic antigen-related cell adhesion molecule 18	27	1	-4.79
STAB1 Isoform 2 of Stabilin-1	29	1	-5.15
LOC389765 Uncharacterized protein ENSP00000297820	52	2	-5.46
PPM1H Protein phosphatase 1H	70	2	-5.46
- Hypothetical short protein	26	1	-5.54
RALY Putative uncharacterized protein (Fragment)	86	2	-5.84
LGI3 Leucine-rich repeat LGI family member 3	28	1	-6.08
LOC121006 hypothetical protein	28	1	-6.22
CBFA2T3 Isoform 2 of Protein CBFA2T3	26	1	-6.74
NRG1 Isoform 3 of Pro-neuregulin-1, membrane-bound isoform	52	2	-7.54