Antibody Characterization Report for E3 ubiquitin-protein ligase parkin (Parkin)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: E3 ubiquitin-protein ligase parkin

Recommended protein name (short): Parkin

Gene name: PRKN

Uniprot: O60260

This report guides researchers to select the most appropriate antibodies for Parkin. We used an antibody characterization pipeline [1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Parkin by immunoblot (Western blot), immunoprecipitation and immunofluorescence. SH-SY5Y and HEK293T were selected based on evidence of appropriate Parkin protein expression determined through public proteomics databases, namely PaxDB [2] and DepMap [3]. An SH-SY5Y *PRKN* KO line is commercially available at Abcam. HEK293T cells were modified with CRISPR/Cas9 [4] to knockout the corresponding *PRKN* gene.

The authors do not provide an assessment of the quality of the tested antibodies as their respective performances are limited to our finite experimental conditions. The readers should interpret the present findings based on their own scientific expertise. The authors acknowledge that an antibody that demonstrates specificity in the stated test conditions can be suboptimal in a different experimental format or in cell lines that differ from those directly tested here.

1. Laflamme, C., et al., *Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72.* Elife, 2019. **8** DOI: 10.7554/eLife.48363.
2. Wang, M., et al., *Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines.* Proteomics, 2015. **15**(18): p. 3163-8 DOI: 10.1002/pmic.201400441.
3. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16 DOI: 10.1016/j.cell.2019.12.023.
4. Ran, F.A., et al., *Genome engineering using the CRISPR-Cas9 system.* Nat Protoc, 2013. **8**(11): p. 2281-2308 DOI: 10.1038/nprot.2013.143.

Table 1: Summary of the Parkin antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo	702785	2176161	AB_2724937	recombinant-mono	21H24L9	rabbit	0.50	Wb
Thermo	711820	2268810	AB_2716914	recombinant-poly	21HCLC	rabbit	0.50	Wb
Thermo	39-0900	VJ316032	AB_2533396	monoclonal	PRK109	mouse	0.50	Wb, IP, IF
Thermo	MA5-41212	WI3383822B	AB_2894713	recombinant-mono	JF82-09	rabbit	1.00	Wb, IF
Abcam	ab77924	GR3258735-1	AB_1566559	monoclonal	PRK8	mouse	1.00	Wb
Abcam	ab15954	GR243589-1	AB_443270	polyclonal	-	rabbit	1.00	other
Bio-Techne	MAB1438	XBL016021	AB_2236703	monoclonal	323302	mouse	0.50	Wb
Bio-Techne	MAB14381	YO10115111	AB_2159941	monoclonal	328122	mouse	0.50	Wb
Proteintech	14060-1-AP	85533	AB_2878005	polyclonal	-	rabbit	0.80	Wb, IF
GeneTex	GTX39745	822004579	AB_11170090	monoclonal	PRK8	mouse	2.00	Wb, IF
GeneTex	GTX65811	822103801	AB_2888609	polyclonal	-	rabbit	1.24	Wb, IF
BioLegend	865602	B285563	AB_2810778	monoclonal	5C3/Parkin	mouse	0.50	Wb
BioLegend	870402	B300502	AB_2820193	monoclonal	A15165E	mouse	0.50	other
BioLegend	870502	B300468	AB_2820195	monoclonal	A15165D	mouse	0.50	Wb
ABclonal	A0968	5500007365	AB_2757487	polyclonal	-	rabbit	0.10	Wb, IF
ABclonal	A11172	1320101	AB_2758446	polyclonal	-	rabbit	1.00	Wb, IF

Wb=Western blot; IP= immunoprecipitation; IF=immunofluorescence

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
ATCC	-	CVCL_0063	HEK293T	WT
Montreal Neurological Institute	-	CVCL_B0N8	HEK293T	*PRKN* KO
Abcam	ab275475	CVCL_0019	SH-SY5Y	WT
Abcam	ab280042	CVCL_B0N9	SH-SY5Y	*PRKN* KO
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
ATCC	CCL-2	CVCL_0030	HeLa	WT
ATCC	HTB-96	CVCL_0042	U2OS	WT
Queen's University	-	-	MDA-MB231 pWPLXd (GFP-positive)	WT
Abcam	ab255451	CVCL_0291	HCT116	WT

Figure 1: Parkin antibody screening by immunoblot.

Lysates of SH-SY5Y (WT and *PRKN* KO) were prepared and 50 µg of protein were processed for immunoblot with the indicated Parkin antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 702785 at 1/200; 711820 at 1/200; 39-0900 at 1/500; ab77924 at 1/200; ab15954 at 1/500; 14060-1-AP at 1/1000; MAB1438 at 1/250; MAB14381 at 1/500; GTX39745 at 1/1000; GTX65811 at 1/1000. Predicted band size: 52 kDa

Figure 2: Analysis of Parkin protein expression in various cell lines.

Lysates of HEK293T (WT and *PRKN* KO), SH-SY5Y (WT and *PRKN* KO), HAP1, HeLa, U2OS, MDA-MB231, A549 and HCT116 were prepared and 100 µg of protein were processed for immunoblot using the Parkin antibody 702785 at 1/200. The Ponceau stained transfer is shown as a loading control.

Figure 3: Parkin antibody screening by immunoprecipitation.

SH-SY5Y lysates were prepared, and immunoprecipitation was performed using 1.0 µg of the indicated Parkin antibodies. **A)** Ability of the antibodies to capture Parkin was first assessed by comparing the level of Parkin available in the starting material to its level remaining in the unbound fraction. Antibody 702785 was used for the immunoblot. **B)** Analysis of the immunoprecipitate for antibodies that showed specificity for Parkin in A). For immunoblot, 39-0900 and 702785 were both used at 1/200. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain.

Figure 4: Parkin antibody screening by immunofluorescence.

SH-SY5Y WT and *PRKN* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Parkin antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody. Acquisition of the green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: 702785 at 1/500; 711820 at 1/500; 39-0900 at 1/500; ab77924 at 1/1000; MAB1438 at 1/500; MAB14381 at 1/500; 14060-1-AP at 1/800; GTX39745 at 1/2000; GTX65811at 1/1200, 865602 at 1/500, 870402 at 1/500, 870502 at 1/500, A0968 at 1/100, A11172 at 1/1000. Bars = 10 µm.

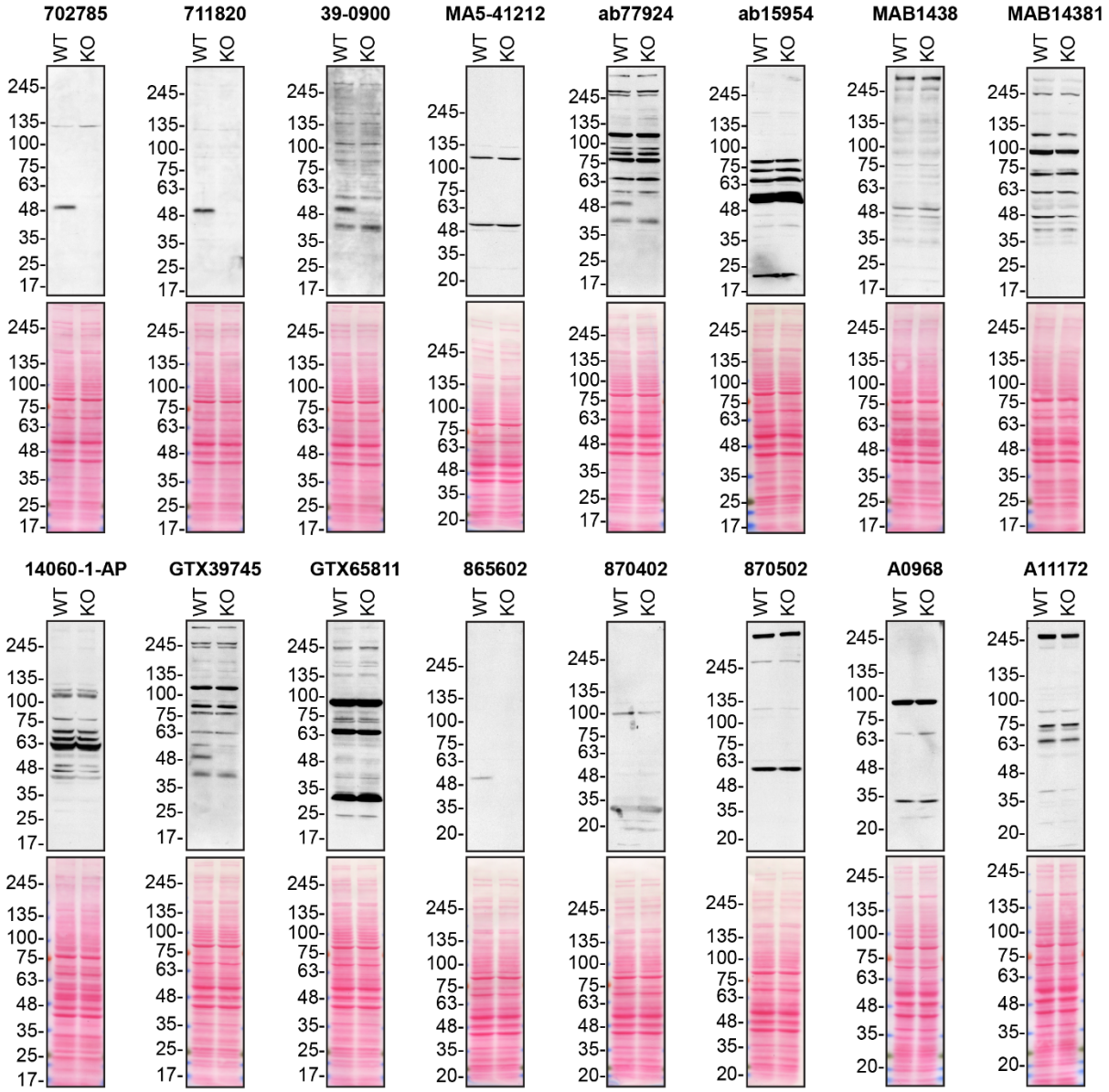

Figure 1: Parkin antibody screening by immunoblot

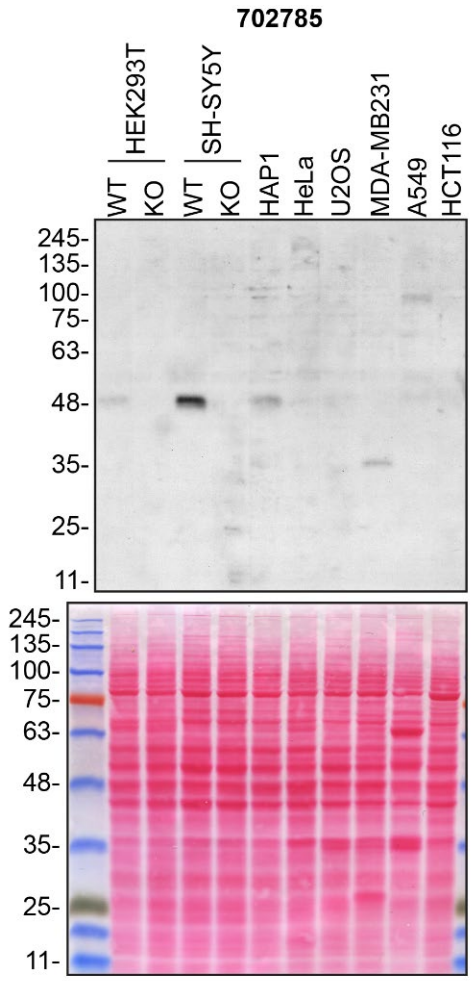

Figure 2: Analysis of Parkin protein expression in various cell lines

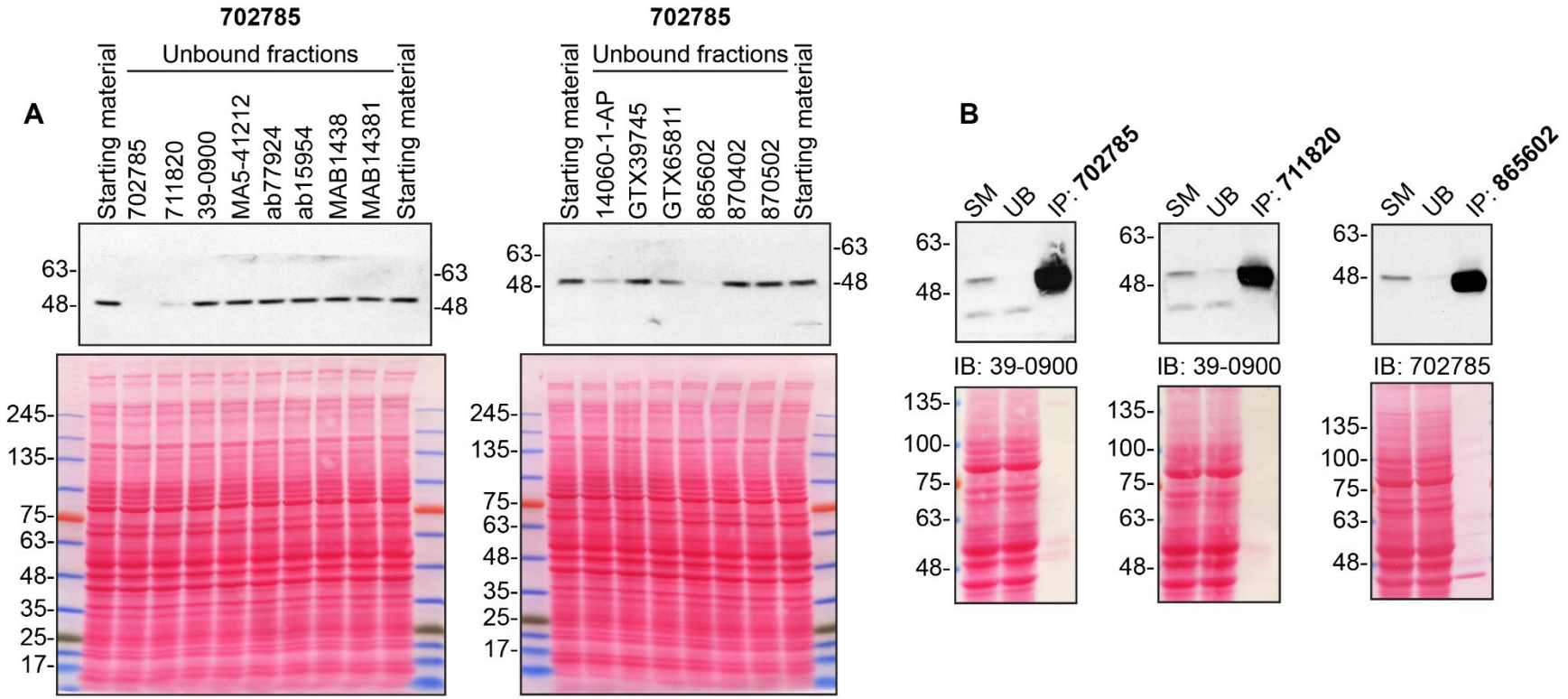

Figure 3: Parkin antibody screening by immunoprecipitation

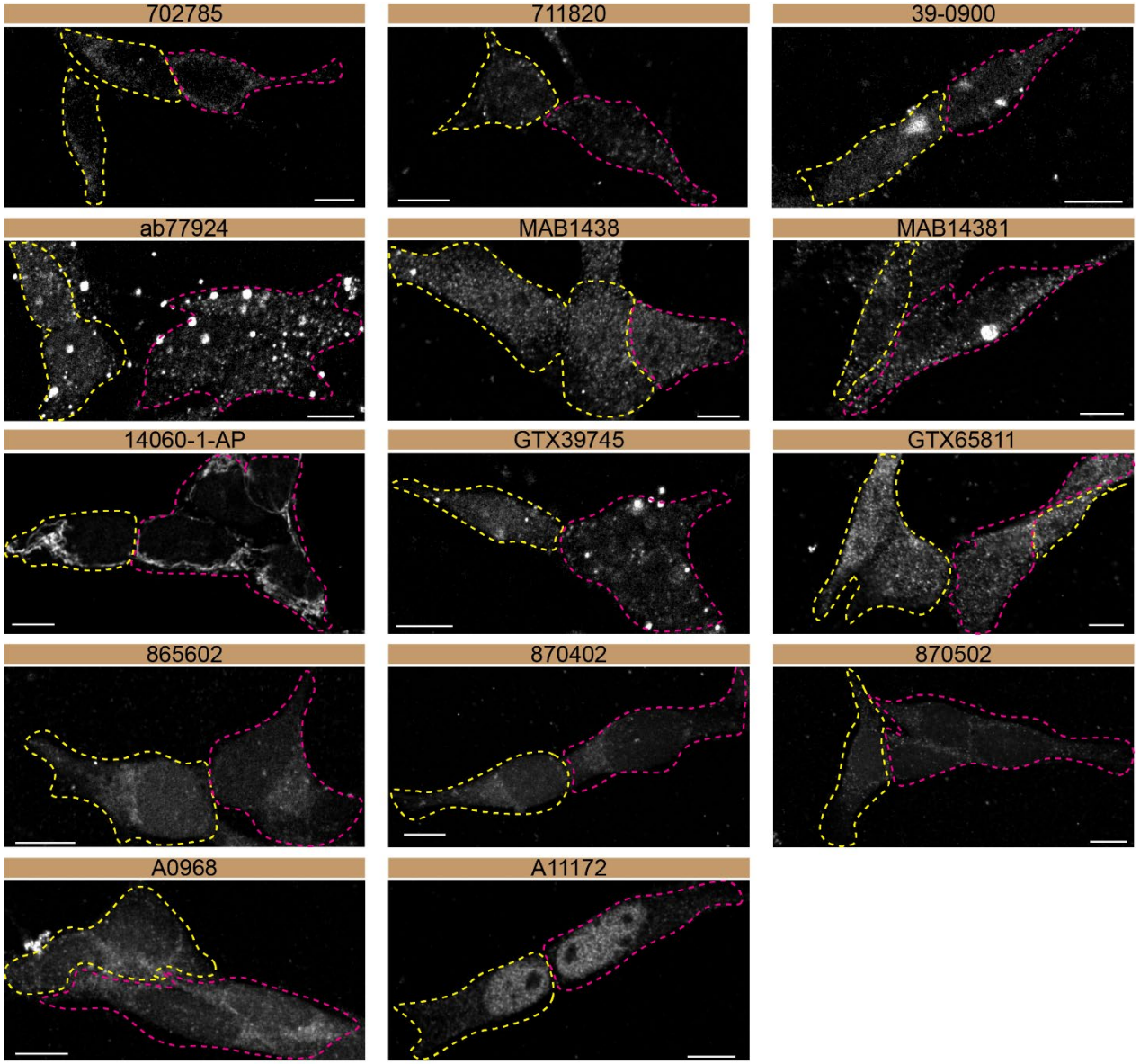

Figure 4 : Parkin antibody screening by immunofluorescence

Materials and methods

Antibodies

All Parkin antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. Number 65-6120 and 62-6520). Alexa-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and A21429).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. Two guide RNAs were used to knockout *PRKN* in HEK293T (sequence guide 1: CUCCAGCCAUGGUUUCCCAG, sequence guide 2: CUGCGAAAAUCACACGCAAC).

Cell culture

SH-SY5Y cells were cultured in DMEM/F-12 (Thermo Fisher Scientific cat. number 11320033) containing 10% fetal bovine serum (Wisent, cat. number 080450), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Antibody screening by immunoblot

SH-SY5Y (WT and *PRKN* KO) were collected in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1.0 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor. Lysates were sonicated briefly and incubated 30 min on ice. Lysates were spun at ~110,000xg for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

Immunoblots were performed with large 4-15% gradient (Figure 1A and B) polyacrylamide gels and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau staining which is scanned to show together with individual immunoblot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% bovine serum albumin in TBS with 0,1% Tween 20 (TBST). Following three washes with TBST, the peroxidase conjugated secondary antibody was incubated at a dilution of ~0.2 µg/ml in TBST with 5% milk for 1 hr at room temperature followed by three washes with TBST. Membranes are incubated with ECL from

Pierce (cat. number 32106) prior to detection with HyBlot CL autoradiography films from Denville (cat. number 1159T41).

Antibody screening by immunoprecipitation

Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 ul of PBS with 0,01% triton X-100 in a microcentrifuge tube, together with 30µl of protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) magnetic beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

SH-SY5Y WT were collected in Pierce IP lysis buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol; cat. number 87787) supplemented with protease inhibitor. Lysates are rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. For the starting material versus unbound fraction screening, 1 ml aliquots at 1.0 mg/ml (Figure 3A) or 2 ml aliquots at 2 mg/ml (Figure 3B) of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. Following centrifugation, the unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of IP lysis buffer and processed for SDS-PAGE and immunoblot on 4-15% polyacrylamide gels.

Antibody screening by immunofluorescence

SH-SY5Y WT and *PRKN* KO were labelled with a green and a far-red fluorescence dye, respectively. The fluorescent dyes used are from Thermo Fisher Scientific (cat. number C2925 and C34565). WT and KO cells were plated on glass coverslips as a mosaic and incubated for 24 hrs in a cell culture incubator. Cells were fixed in 4% PFA (in PBS) for 15 min at room temperature and then washed 3 times with PBS. Cells were permeabilized in PBS with 0,1% Triton X-100 for 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Cells were incubated with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Parkin antibodies O/N at 4°C. Cells were then washed 3 × 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 µg/ml for 1 hr at room temperature. Cells were washed 3 × 10 min with IF buffer and once with PBS. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO).

Imaging was performed using a Zeiss LSM 880 laser scanning confocal microscope equipped with a Plan-Apo 63x oil objective (NA = 1.40). Analysis was done using the Zen navigation software (Zeiss). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.