Antibody Characterization Report for Optineurin

YCharOS Antibody Characterization Report

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Target:

Protein name: Optineurin

Alternative protein names: E3-14.7K-interacting protein, FIP-2, Huntingtin yeast partner L, Huntingtin-interacting protein 7, HIP-7, Huntingtin-interacting protein L, NEMO-related protein, Optic neuropathy-inducing protein, Transcription factor IIIA-interacting protein, TFIIIA-IntP

Gene name: *OPTN*

Uniprot: Q96CV9

Table 1: Summary of the Optineurin antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)
Thermo	711879	2226080	AB_2723433	recombinant-poly		rabbit	0.50
Thermo	702766	2248017	AB_2723432	recombinant-mono	22H12L20	rabbit	0.50
GeneTex	GTX132575	42508	AB_2885141	polyclonal	-	rabbit	0.28
Proteintech	60293-1-Ig	not provided	AB_2881408	monoclonal	6C1H4	mouse	1.70
Proteintech	10837-1-AP	not provided	AB_2156665	polyclonal	-	rabbit	0.45
Abcam	ab213556	GR3244089-4	AB_2890221	recombinant-mono	EPR20654	rabbit	0.50

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Montreal Neurological Institute	-	CVCL_0042	U2OS	WT
Montreal Neurological Institute	-	CVCL_A6LN	U2OS	*OPTN* KO

Figure 1: Optineurin antibody screening by immunoblot.

Lysates of U2OS (WT or *OPTN* KO) were prepared and 30 µg of protein were processed for immunoblot with the indicated Optineurin antibodies. Longer exposure of the ~48-75 kDa area is depicted in the smaller cropped panel. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 711879 at 1/5000; 702766 at 1/200; GTX132575 at 1/10000; 60293-1-Ig at 1/5000; 10837-1-AP at 1/5000; ab213556 at 1/5000. Predicted band size of the canonical isoform: 66 kDa.

Figure 2: Optineurin antibody screening by immunoprecipitation.

Lysates of U2OS were prepared and IP was performed using 1.0 µg of the indicated Optineurin antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Optineurin antibody. For immunoblot, 10837-1-AP at 1/5000, 60293-1-Ig at 1/5000 and ab213556 at 1/5000 were used. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: Optineurin antibody screening by immunofluorescence.

U2OS WT and *OPTN* KO cells were labelled with a green or a deep 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 Optineurin antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody, together with DAPI. Acquisition of the green (WT), red (antibody staining) and far-red (KO) channels was performed. Representative images of the merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-left panel. Antibody dilution used: 711879 at 1/500; 702766 at 1/500; GTX132575 at 1/300; 60293-1-Ig at 1/2000; 10837-1-AP at 1/500; ab213556 at 1/500. Bars = 10 µm.

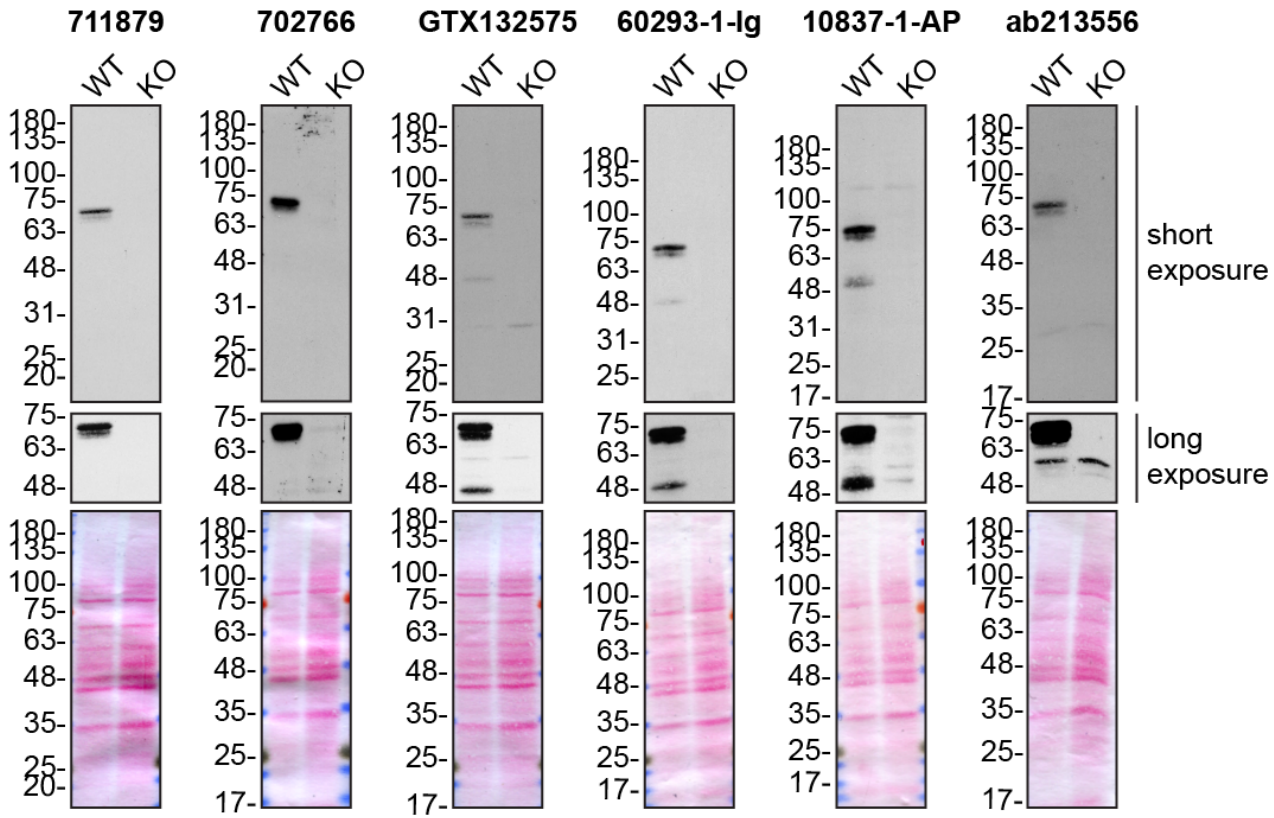

Figure 1: Optineurin antibody screening by immunoblot

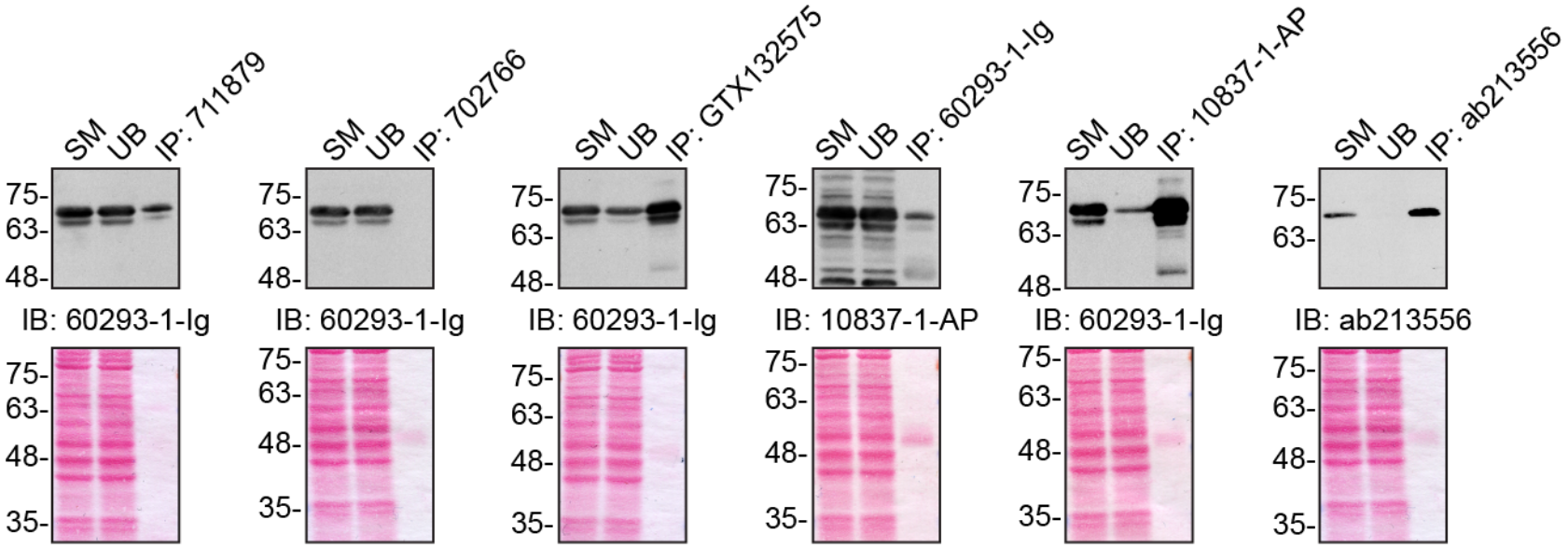

Figure 2: Optineurin antibody screening by immunoprecipitation

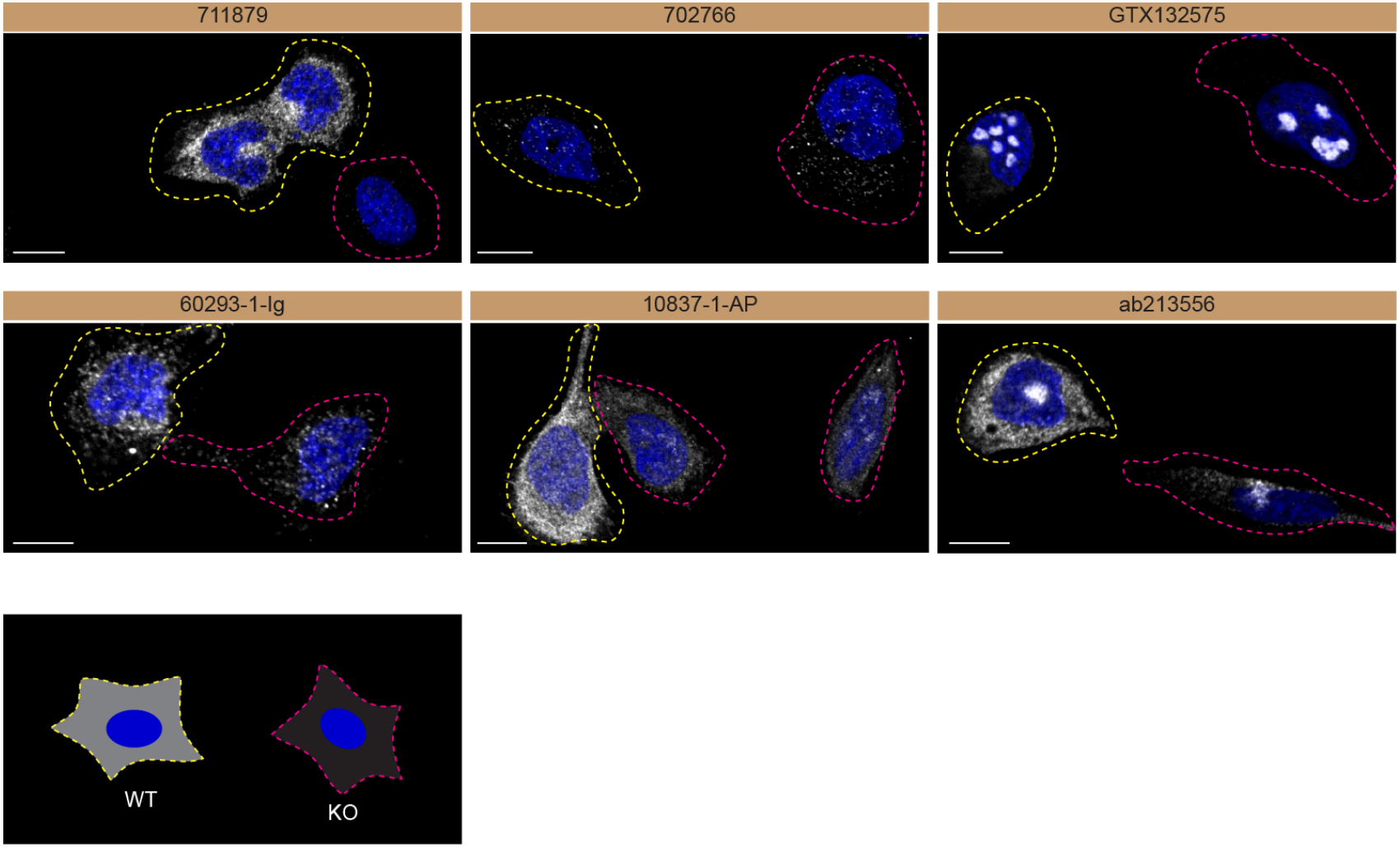

Figure 3: Optineurin antibody screening by immunofluorescence

Materials and methods

Antibodies

All Optineurin 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. U2OS *OPTN* KO clone was generated with low passage cells using an open-access protocol available on Zenodo.org: https://zenodo.org/record/3875777#.X9uE11VKjIX. Two guide RNAs were used to introduce a STOP codon in the *OPTN* gene (sequence guide 1: CUAAAUAAUCAAGCCAUGAA, sequence guide 2: GAGAAAUUGAAGGAAGAGCU).

Cell culture

Cells were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) 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

U2OS (WT and *OPTN* 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 5-16% gradient 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 regular ECL

(cat. number 32106) or with super signal West Femto (cat. number 34096) from Pierce 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 30 µl of protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

U2OS WT were collected in HEPES buffer (20 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, pH 7.4) supplemented with protease inhibitor. Lysates are rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. One ml aliquots at 1.0 mg/ml 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 HEPES lysis buffer and processed for SDS-PAGE and immunoblot on a 10% acrylamide gel. Prot-A:HRP was used as a secondary detection system (MilliporeSigma, cat. number P8651) at a dilution of 0.1 µg/ml for the ab213556 testing.

Antibody screening by immunofluorescence

U2OS WT and *OPTN* KO were labelled with a green and a deep 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. Coverslips were incubated face down on a 50 µl drop (on paraffin film in a moist chamber) with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Optineurin antibodies O/N at 4°C. Cells were 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 with DAPI. 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 40x oil objective (NA = 1.40). Analysis was done using Image J. All cell images

represent a single focal plane. Figures were prepared using Adobe Photoshop to adjust contrast, apply 1 pixel Gaussian blur and then assembled with Adobe Illustrator.