Antibody Characterization Report for Plectin

YCharOS Antibody Characterization Report

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Target:

Protein name: Plectin

Alternative protein names: Hemidesmosomal protein 1, HD1, Plectin-1

Uniprot: Q15149

Gene name: *PLEC*

This report guides researchers to select the most appropriate antibodies for Plectin. We used an antibody characterization pipeline[1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Plectin by immunoblot (Western blot), immunoprecipitation and immunofluorescence. U2OS was selected based on evidence of appropriate Plectin protein expression determined through public proteomics databases, namely PaxDB[2] and DepMap[3]. U2OS was modified with CRISPR/Cas9 to knockout[4] the corresponding *PLEC* 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* **8**, doi:10.7554/eLife.48363 (2019).

2 Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D. & von Mering, C. Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **15**, 3163-3168, doi:10.1002/pmic.201400441 (2015).

3 Nusinow, D. P. *et al.* Quantitative Proteomics of the Cancer Cell Line Encyclopedia. *Cell* **180**, 387-402 e316, doi:10.1016/j.cell.2019.12.023 (2020).

4 Nicouleau, Michael, Pimentel, Luisa, Shlaifer, Irlna & Durcan, Thomas M. Generation of Knockout Cell Lines Using CRISPR-Cas9 and ddPCR Technology. (2020). doi:10.5281/zenodo.3875777

Table 1: Summary of the Plectin antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)
Abcam	ab32528	GR99479-28	AB_777339	recombinant-mono	E398P	rabbit	0.2
Abcam	ab229476	GR3225502-7	AB_2890128	polyclonal		rabbit	0.41
Thermo	MA5-32102	VG3041224	AB_2809395	recombinant-mono	SY29-04	rabbit	1.0
Thermo	PA5-56292	VJ3098854B	AB_2645646	polyclonal	-	rabbit	0.3
Bio-Techne	NBP2-67452	HJ0727	AB_2885049	recombinant-mono	SY29-04	rabbit	1.0
GeneTex	GTX130764	42095	AB_2885050	polyclonal	-	rabbit	0.41
Abnova	H00005339-M03	09328-4C3	AB_10562998	monoclonal	4C3	mouse	1.0
CST	2863	1	AB_1031213	polyclonal	-	rabbit	not provided

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_A4GM	U2OS	*PLEC* KO

Figure 1: Analysis of Plectin antibodies by immunoblot.

Lysates of U2OS (WT or *PLEC* KO) were prepared and 10 µg (ab32528, MA5-32102, PA5-56292, NBP2-67452, GTX130764) or 100 µg (H00005339-M03, 2863) of protein were processed for immunoblot with the indicated Plectin antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab32528 at 1/5000; ab229476 at 1/5000, MA5-32102 at 1/5000; PA5-56292 at 1/10000; NBP2-67452 at 1/1000; GTX130764 at 1/1000; H00005339-M03 at 1/1000; 2863 at 1/1000. Predicted band sizes: 518 and 531 kDa.

Figure 2: Analysis of Plectin antibodies by immunoprecipitation.

Lysates were prepared and IP was performed using 1.0 µg of the indicated Plectin antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Plectin antibody. For immunoblot, ab32528 and NBP2-67452 were used at 1/5000 and 1/1000, respectively. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain; LC= antibody light chain.

Figure 3: Analysis of Plectin antibodies by immunofluorescence.

Parental and *PLEC* KO cells were transfected with a GFP or mCherry plasmid, respectively. Parental and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Plectin antibodies and with the corresponding Alexa-fluor 647 coupled secondary antibody. Acquisition of the green (parental), red (KO) and far-red (antibody staining) channels was performed and representative images are shown. Parental and KO cells are outlined with green and red dashed line, respectively. Grayscale images of the far-red channel are shown. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Antibody dilution used: ab32528 at 1/1000; MA5-32102 at 1/1000, PA5-56292 at 1/500; NBP2-67452 at 1/1000; GTX130764 at 1/500; H00005339-M03 at 1/1000 and 2863 at 1/1000. Bars = 40 µm.

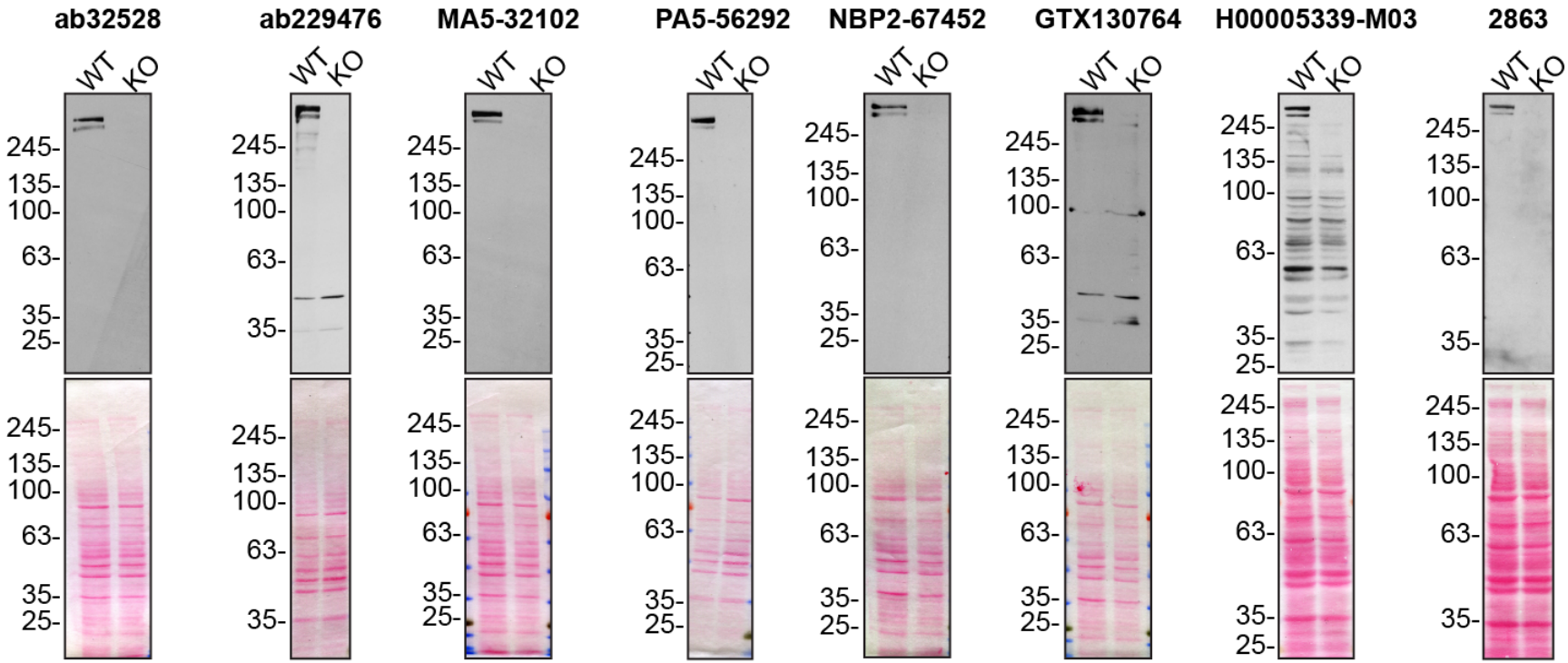

Figure 1: Plectin antibody screening by immunoblot

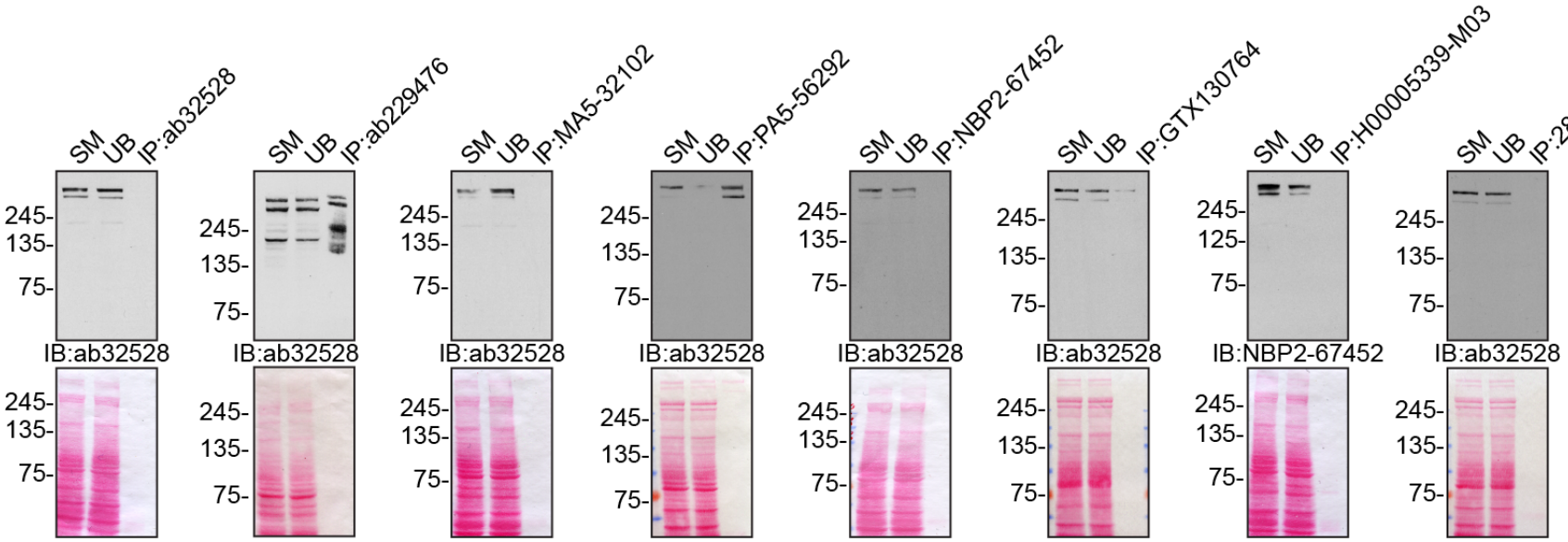

Figure 2: Plectin antibody screening by immunoprecipitation

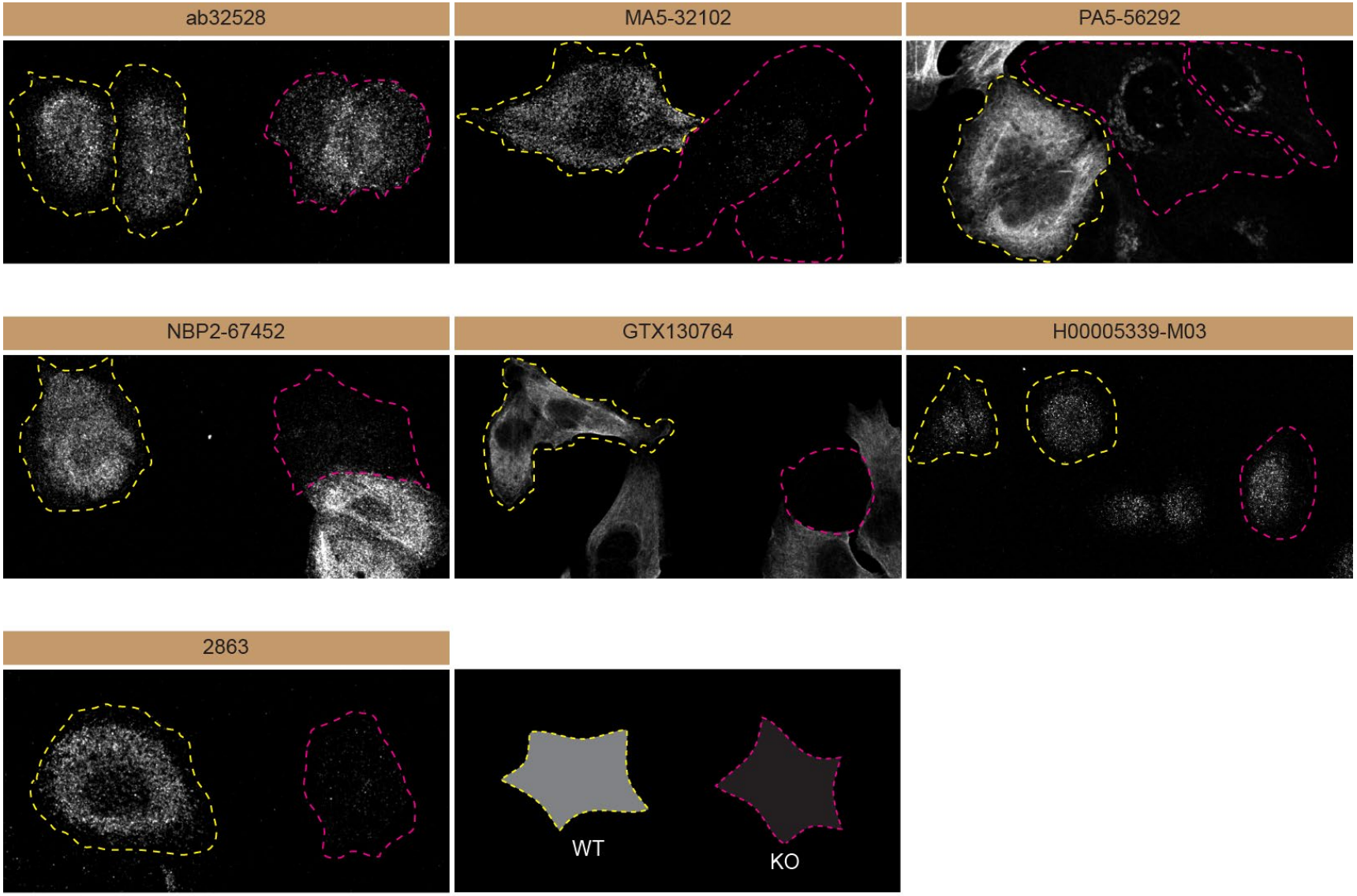

Figure 3: Plectin antibody screening by immunofluorescence

Materials and methods

Antibodies

All Plectin 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-647-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21236 and A21245).

Cell lines

U2OS *PLEC* KO clone was generated with low passage cells using an open-access protocol available on Zenodo.org: https://zenodo.org/record/3875777#.X9uE11VKjIX. The gRNA sequence used to introduce a STOP codon in the *PLEC* gene is: cttgttgacccacttggtga.

U2OS WT and KO lines were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) containing 10% bovine calf serum (GE Healthcare cat. number SH30072.03), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Immunoblot

U2OS parental and *PLEC* KO cells 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 ~240,000xg for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot.

Immunoblots were performed with large 4-15% 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 ECL from Pierce (cat. number 32106) prior to detection with HyBlot CL autoradiography films from Denville (cat. number 1159T41).

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) Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

U2OS 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. Prot-A:HRP was used as a secondary detection system (MilliporeSigma, cat. number P8651) at a dilution of 0.1 µg/ml.

Immunofluorescence

U2OS cells (parental and *PLEC* KO) were transfected with GFP and mCherry respectively. At 48 hrs post transfection, both cell lines 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 Plectin antibodies O/N at 4°C. Cells were washed 3 × 10 min with IF buffer and incubated with corresponding Alexa Fluor 647-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 Leica SP8 laser scanning confocal microscope equipped with a 40x oil objective (NA = 1.30) and HyD detectors. Acquisition was performed using Leica Application Suite X software (version 3.1.5.16308). Analysis was done using Image J. All cell images represent a single focal plane. Figures were prepared for publication using Adobe Photoshop to adjust contrast, apply 1 pixel Gaussian blur and then assembled with Adobe Illustrator.