Antibody Characterization Report for Dynamin-1

YCharOS Antibody Characterization Report

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Target:

Protein name: Dynamin-1

Gene name: *DNM1*

Uniprot: Q05193

This report guides researchers to select the most appropriate antibodies for Dynamin-1. We used an antibody characterization pipeline[1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Dynamin-1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. U2OS was selected based on evidence of appropriate Dynamin-1 protein expression determined through public proteomics databases, namely PaxDB[2] and DepMap[3]. U2OS was modified with CRISPR/Cas9 to knockout[4] the corresponding *DNM1* 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 Dynamin-1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)
GeneTex	GTX110379	40478	AB_10726661	polyclonal	-	rabbit	1.00
Bio-Techne	NB110-60491	A-1	AB_921194	monoclonal	3G4B6	mouse	not provided
Thermo	MA5-34957	VJ3101166A	AB_2848863	monoclonal	A1A12	mouse	2.00
Thermo	MA5-15285	VJ3101181	AB_10981827	monoclonal	3G4B6	mouse	not provided
Sigma	MABT188	3314355	AB_11203312	monoclonal	Hudy-1	mouse	1.00
Abcam	ab13251	GR3327541	AB_299794	monoclonal	D5	mouse	1.00
Abcam	ab52852	GR103077-4	AB_869530	recombinant-mono	EP772Y	rabbit	0.04
Abcam	ab52611	GR3339071-1	AB_869531	recombinant-mono	EP801Y	rabbit	0.31

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_A4NZ	U2OS	*DNM1* KO

Figure 1: Analysis of Dynamin-1 antibodies by immunoblot.

Lysates of U2OS (WT or *DNM1* KO) were prepared and 130 µg of protein were processed for immunoblot with the indicated Dynamin-1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used is 1/1000. Predicted band size: 97 kDa.

Figure 2: Analysis of Dynamin-1 antibodies by immunoprecipitation.

Lysates were prepared and immunoprecipitation was performed using 1.0 µg* of the indicated Dynamin-1 antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Dynamin-1 antibody. For immunoblot, NB110-60491 was used at 1/10000, ab52611 at 1/5000 and MA5-15285 at 1/1000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

* Concentration of NB110-60491 and MA5-15285 is not provided and 5 µl was used for each immunoprecipitation experiment.

Figure 3: Analysis of Dynamin-1 antibodies by immunofluorescence.

U2OS WT and *DNM1* KO cells were labelled with a green or a far red fluorescence dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Dynamin-1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody. Acquisition of the green (WT), red (antibody staining) and far-red (KO) channels was performed. Representative grayscale images of the red channel 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-right panel. Antibody dilution used: GTX110379 at 1/1000; NB110-60491 at 1/1000; MA5-34957 at 1/2000; MA5-15285 at 1/1000; MABT188 at 1/1000; ab13251 at 1/1000; ab52852 at 1/200; ab52611 at 1/500. Bars = 20 µm.

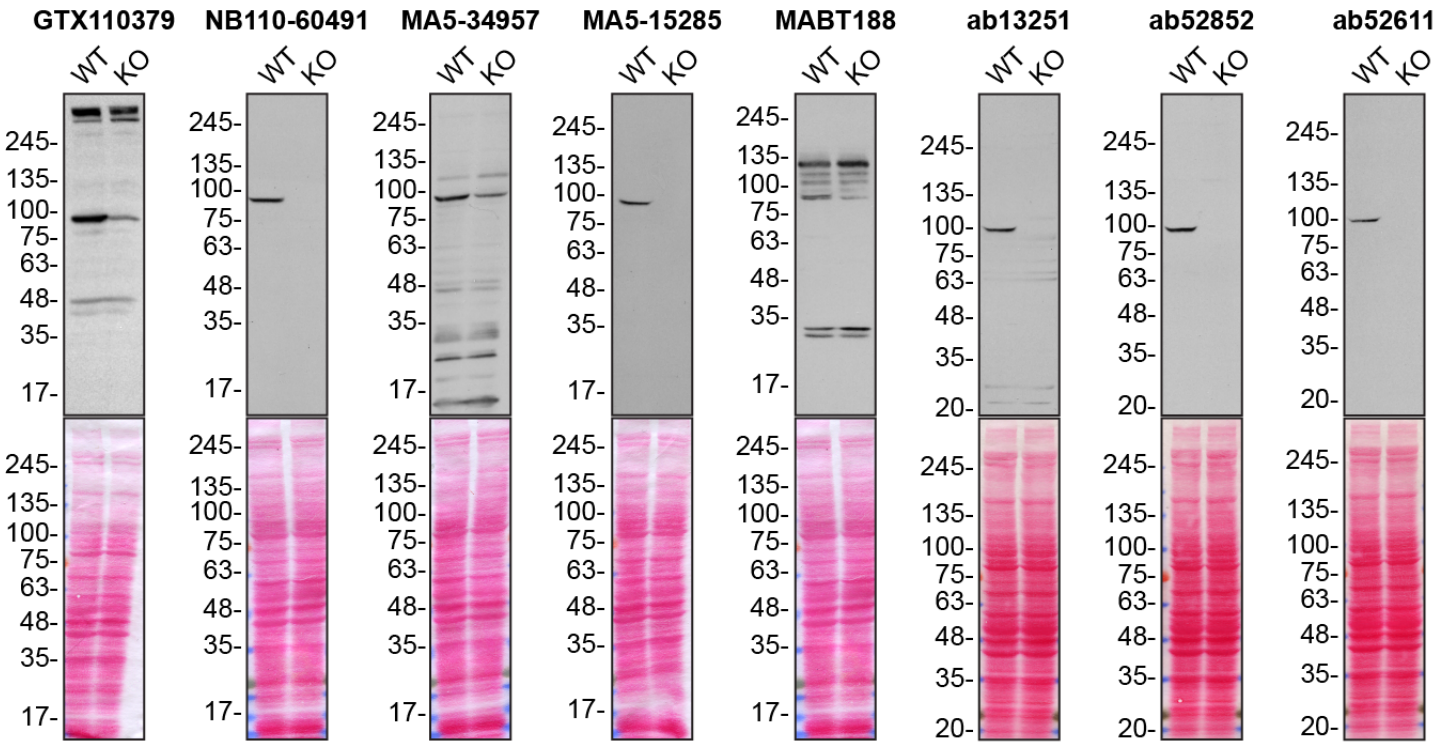

Figure 1: Dynamin-1 antibody screening by immunoblot

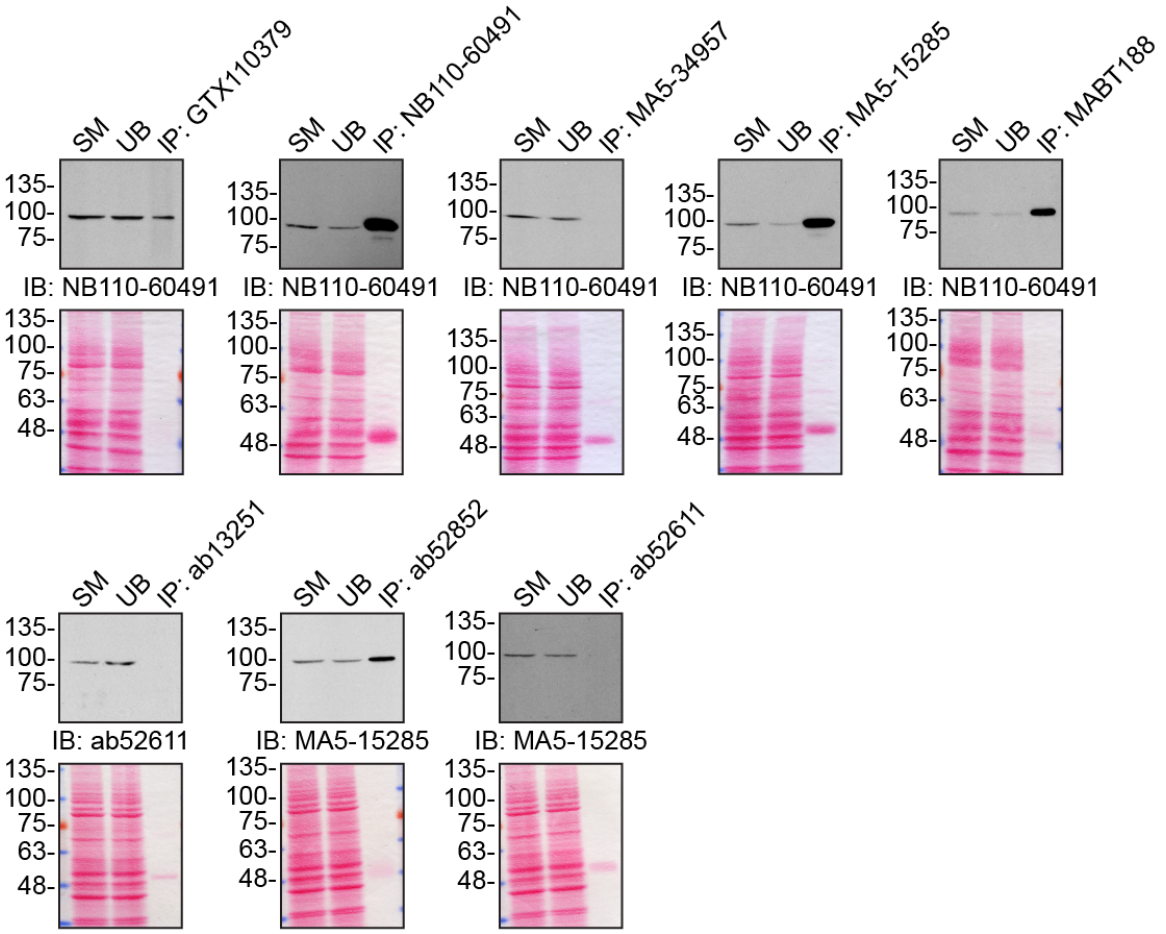

Figure 2: Dynamin-1 antibody screening by immunoprecipitation

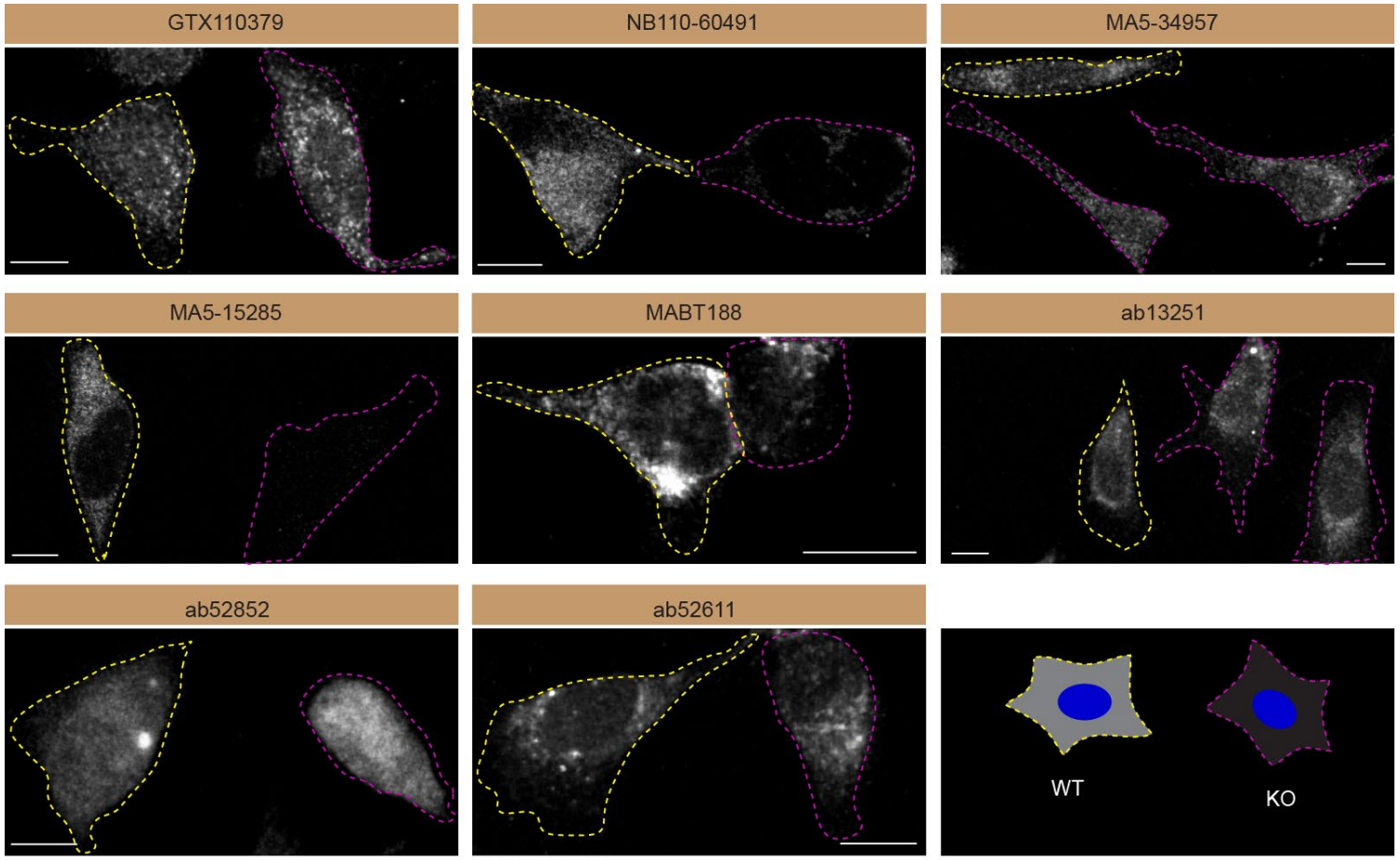

Figure 3 : Dynamin-1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Dynamin-1 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).

Cell lines

U2OS *DNM1* KO clone was generated with low passage cells following 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 *DNM1* gene is: GTGACAATGCCAGATCCTCG.

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 WT and *DNM1* 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 ~110,000xg 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 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 ml of HEPES lysis buffer and processed for SDS-PAGE and immunoblot.

Immunofluorescence

U2OS WT and *DNM1* KO were labelled with a green dye and with a deep red fluorescent dye from Abcam (cat. number ab176735 and ab176736), respectively. 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 Dynamin-1 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. 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.