Antibody Characterization Report for Ataxin-2

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Ataxin-2

Alternative protein names: Spinocerebellar ataxia type 2 protein, Trinucleotide repeat-containing gene 13 protein

Gene name: *ATXN2*

Uniprot: Q99700

This report guides researchers to select the most appropriate antibodies for Ataxin-2. We used an antibody characterization pipeline[1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Ataxin-2 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. An HAP1 *ATXN2* KO cell line is available at Horizon Discovery and was selected based on evidence of appropriate *ATXN2* expression determined using DepMap[2]. A subtle expression of *ATXN2* was detected in the KO line as shown by immunoblot with various antibodies.

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 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).

Table 1: Summary of the Ataxin-2 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
GeneTex	GTX130329	41983	AB_2885139	polyclonal	-	rabbit	0.28	Wb
GeneTex	GTX130331	42004	AB_2885140	polyclonal	-	rabbit	1.15	Wb, IF
Thermo	PA5-54183	WB3187344	AB_2638321	polyclonal	-	rabbit	0.50	-
Thermo	PA5-53775	VL3152385A	AB_2638320	polyclonal	-	rabbit	0.40	Wb, IF
Abcam	ab254362	GR3359728-1	AB_2885130	recombinant-mono	EPR23630-49	rabbit	0.52	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
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC003203c011	CVCL_SE31	HAP1	*ATXN2* KO

Figure 1: Ataxin-2 antibody screening by immunoblot.

A) Lysates of HAP1 (WT and *ATXN2* KO) were prepared and 50 µg of protein were processed for immunoblot with the indicated Ataxin-2 antibodies. For three antibodies, a shorter (left panel) and longer (right panel) exposures are presented. The Ponceau stained transfers of each blot are shown. Antibody dilution used: GTX130329 at 1/3000; GTX130331 at 1/1000, PA5-54183 at 1/2000, PA5-53775 at 1/1000, ab254362 at 1/1000. Expected band size: ~140 kDa.

B) Lysates of HAP1 (WT and *ATXN2* KO) were prepared and 150 µg of protein were processed as in A. The antibody ab254362 was used at 1/500. A 20-minute exposure is shown in the smaller cropped panel. There is a complete absence of Ataxin-2 signal in the *ATXN2* KO lysates.

Figure 2: Ataxin-2 antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 1.0 µg of the indicated Ataxin-2 antibodies pre-coupled to protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Ataxin-2 antibody. GTX130331, PA5-54183 and ab254362, all three diluted at 1/1000, were used for immunoblot. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: Ataxin-2 antibody screening by immunofluorescence.

HAP1 WT and *ATXN2* 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 Ataxin-2 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) 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 lower-right panel. Antibody dilution used: GTX130329 at 1/300; GTX130331 at 1/1000, PA5-54183 at 1/500, PA5-53775 at 1/400, ab254362 at 1/500. Bars = 10 µm.

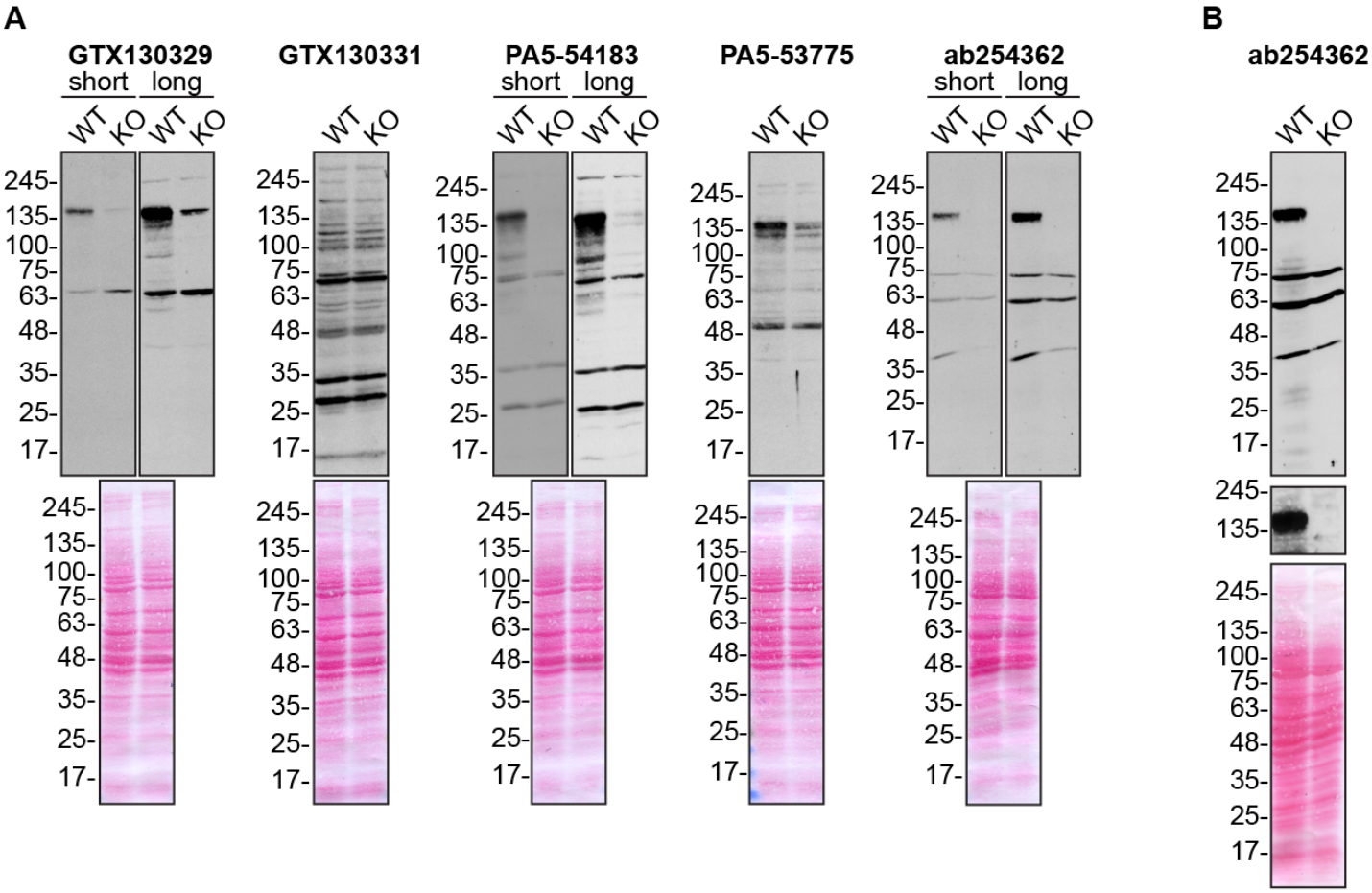

Figure 1: Ataxin-2 antibody screening by immunoblot

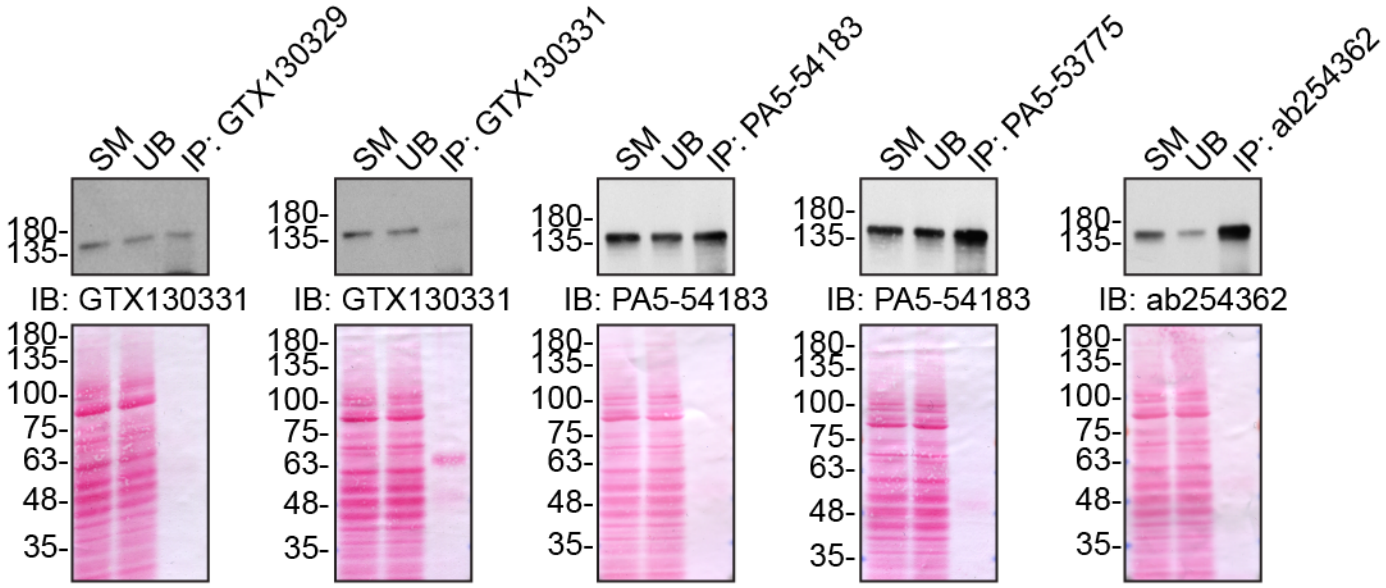

Figure 2: Ataxin-2 antibody screening by immunoprecipitation

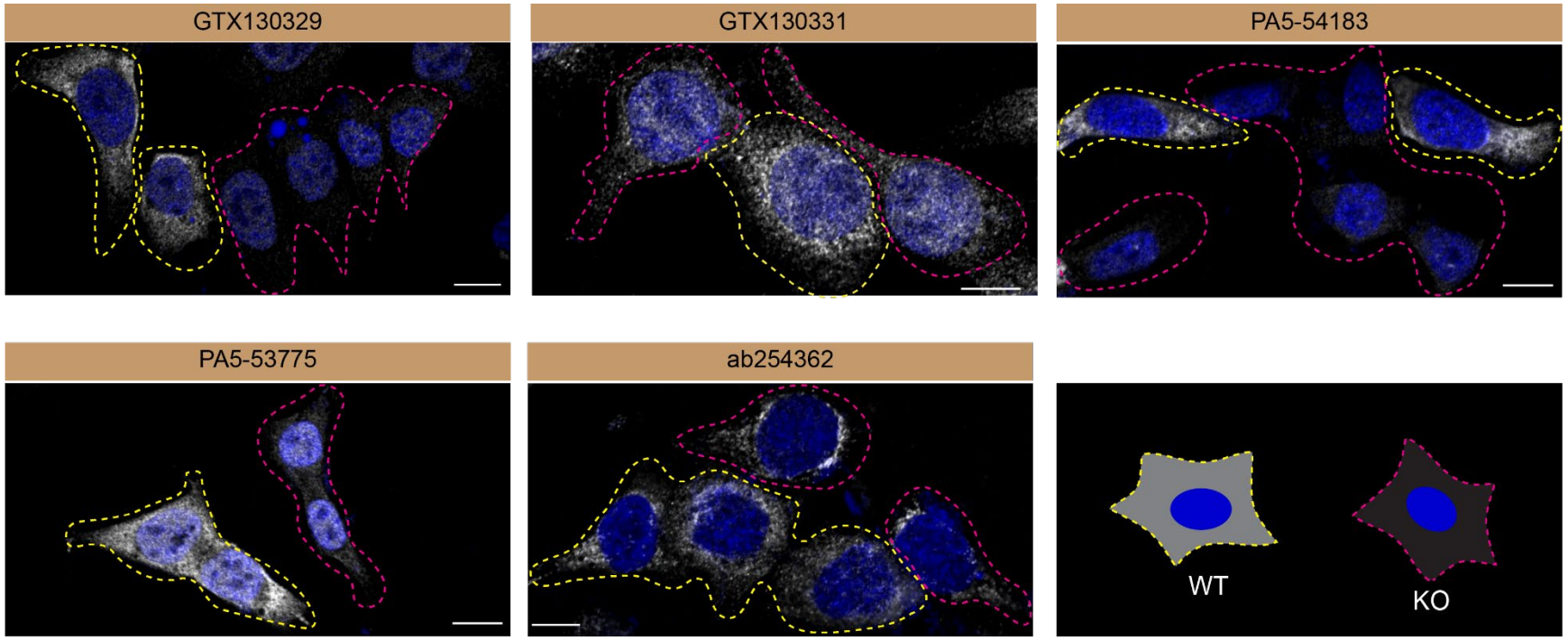

Figure 3: Ataxin-2 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Ataxin-2 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-rabbit antibody is from Thermo Fisher Scientific (cat. number 65-6120). Alexa-555-conjugated goat anti-rabbit secondary antibody is from Thermo Fisher Scientific (cat. number A21429).

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

HAP1 (WT and *ATXN2* 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 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- Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

HAP-1 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 5-16% acrylamide gel.

Antibody screening by immunofluorescence

HAP1 WT and *ATXN2* 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 Ataxin-2 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 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 the Zen navigation software (Zeiss). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.