Antibody Characterization Report for TDP-43

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: TAR DNA-binding protein 43

Recommended short protein name: TDP-43

Gene name: TARDBP

Uniprot: Q13148

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. This report guides researchers to select the most appropriate antibodies for TDP-43. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for TDP-43 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HAP1 was selected based on evidence of appropriate TDP-43 protein expression determined by searching DepMap [4, 5]. A HAP1 *TARDBP* KO line is available at Horizon discovery and was used in this study.

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.

Table 1: Summary of the TDP-43 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Proteintech	10782-2-AP	97534	AB_615042	polyclonal	-	rabbit		Wb,IF
Proteintech	12892-1-AP	94163	AB_2200505	polyclonal	-	rabbit		Wb,IP,IF
Proteintech	80001-1-RR**	23000002	AB_2882933	recombinant-mono	11N20	rabbit	0.25	Wb
Proteintech	80002-1-RR**	23000003	AB_2882934	recombinant-mono	16A22	rabbit	0.25	IF
Bio-Techne	MAB7778*	CHGW0121061	AB_292057	monoclonal	671834	mouse	0.50	Wb
Bio-Techne	NBP1-92695*	122117	AB_11005586	monoclonal	3H8	mouse	1.00	Wb,IF
Thermo Fisher Scientific	711051**	2352341	AB_2633110	recombinant-poly	1HCLC	rabbit	0.50	Wb,IP,IF
Thermo Fisher Scientific	MA5-27828*	XB3501489	AB_2735390	monoclonal	GT733	mouse	1.00	Wb,IF
Thermo Fisher Scientific	MA5-32627**	XB3501035	AB_2809904	recombinant-mono	JM51-10	rabbit	1.00	Wb,IP,IF
ABclonal	A19123**	4000000492	AB_2862616	recombinant-mono	ARC0492	rabbit	0.93	Wb,IF
Cell Signaling Technology	89789**	1	AB_2800143	recombinant-mono	D9R3L	rabbit	0.003	Wb,IF
Cell Signaling Technology	89718**	1	AB_292057	recombinant-mono	E2G6G	rabbit	0.014	Wb
GeneTex	GTX630196*	41505	AB_2888198	monoclonal	GT225	mouse	1.00	Wb,IF
GeneTex	GTX630197*	41505	AB_2888199	monoclonal	GT733	mouse	1.00	Wb,IF
Abcam	ab109535**	GR3324454-4	AB_10859634	recombinant-mono	EPR5810	rabbit	0.03	Wb,IF-Methanol
Abcam	ab133547**	GR3345629-4	AB_2920621	recombinant-mono	EPR5811	rabbit	0.67	Wb,IF
Abcam	ab190963**	GR233962-6	AB_2920622	recombinant-mono	EPR18554	rabbit	0.79	Wb,IP,IF
Abcam	ab254166**	GR3316998-3	AB_2920620	recombinant-mono	DB9	mouse	0.47	Wb

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody

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	HZGHC003730c001	CVCL_TR64	HAP1	*TARDBP* KO

Figure 1: TDP-43 antibody screening by immunoblot.

Lysates of HAP1 (WT and *TARDBP* KO) were prepared, and 50 µg of protein were processed for immunoblot with the indicated TDP-43 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 10782-2-AP at 1/5000, 12892-1-AP at 1/1000, 800001-1-RR** at 1/1000, 80002-1-RR** at 1/1000, MAB7778* at 1/500, NBP1-92695* at 1/1000, , 711051** at 1/1000, , MA5-27828** at 1/1000, MA5-32627** at 1/1000, A19123** at 1/1000, GTX630196* at 1/500, GTX630197* at 1/500, 89789** at 1/1000, 89718** at 1/1000, ab109535** at 1/2000, ab133547** at 1/1000, ab190963** at a1/1000, ab254166** at a1/1000. Predicted band size: 45 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: TDP-43 antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated TDP-43 antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated TDP-43 antibody. For immunoblot, 80002-1-RR** was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody, **=recombinant antibody

Figure 3: TDP-43 antibody screening by immunofluorescence.

HAP1 WT and *TARDBP* 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 in a 96-well plate with glass bottom. Cells were stained with the indicated TDP-43 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 blue and red (grayscale) channels are shown. WT and KO cells are outlined on both channels with green and magenta dashed line, respectively. Antibody dilution used: 10782-2-AP at 1/400, 12892-1-AP at 1/400, 800001-1-RR** at 1/400, 80002-1-RR** at 1/200, MAB7778* at 1/500, NBP1-92695* at 1/1000, 711051** at 1/500, MA5-27828** at 1/1000, MA5-32627** at 1/1000, A19123** at 1/900, GTX630196* at 1/1000, GTX630197* at 1/1000, 89789** at 1/30, 89718** at 1/10, ab109535** at 1/1000, ab133547** at 1/1000, ab190963** at a1/1000, ab254166** at a1/1000. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

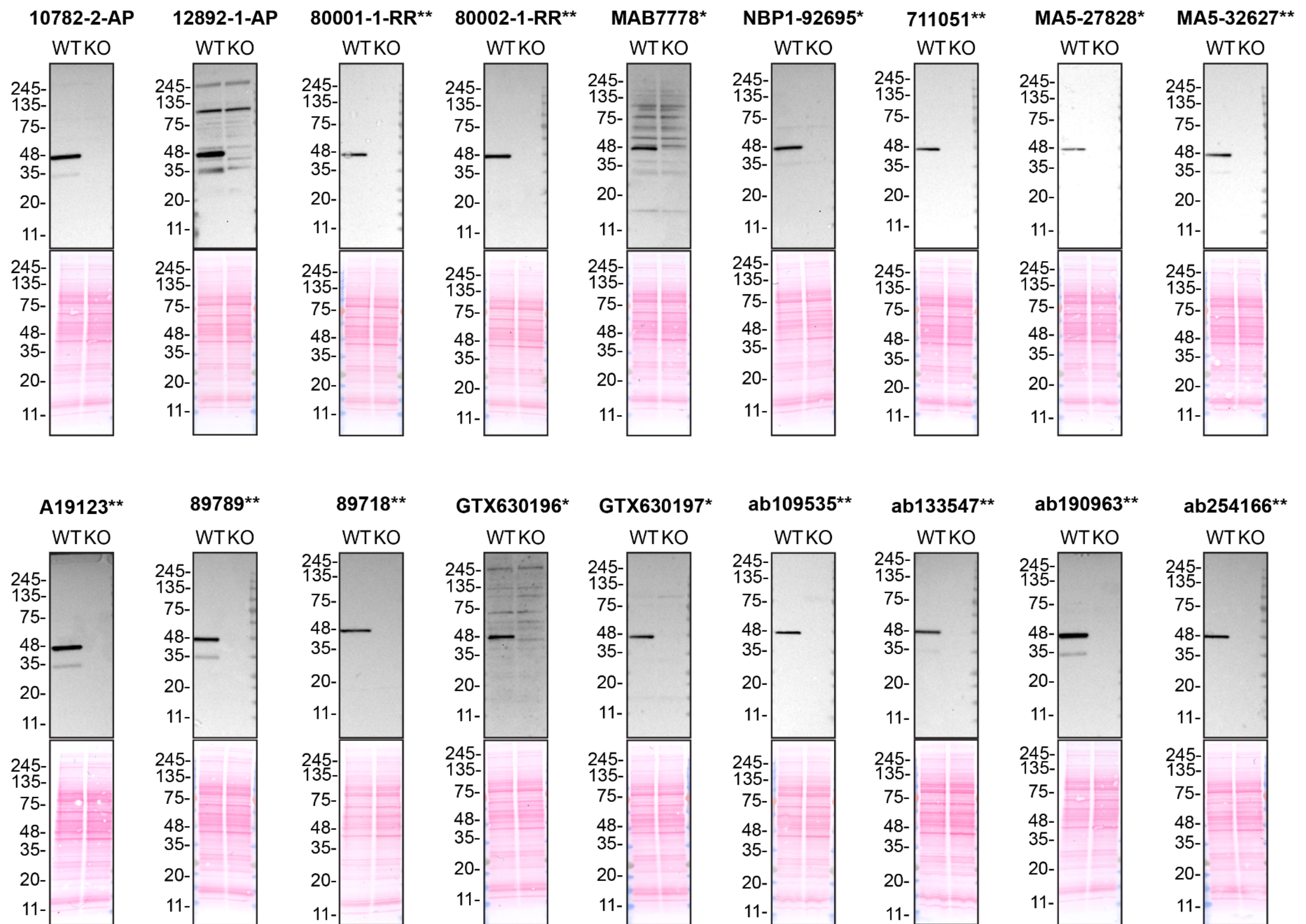

Figure 1: TDP-43 antibody screening by immunoblot

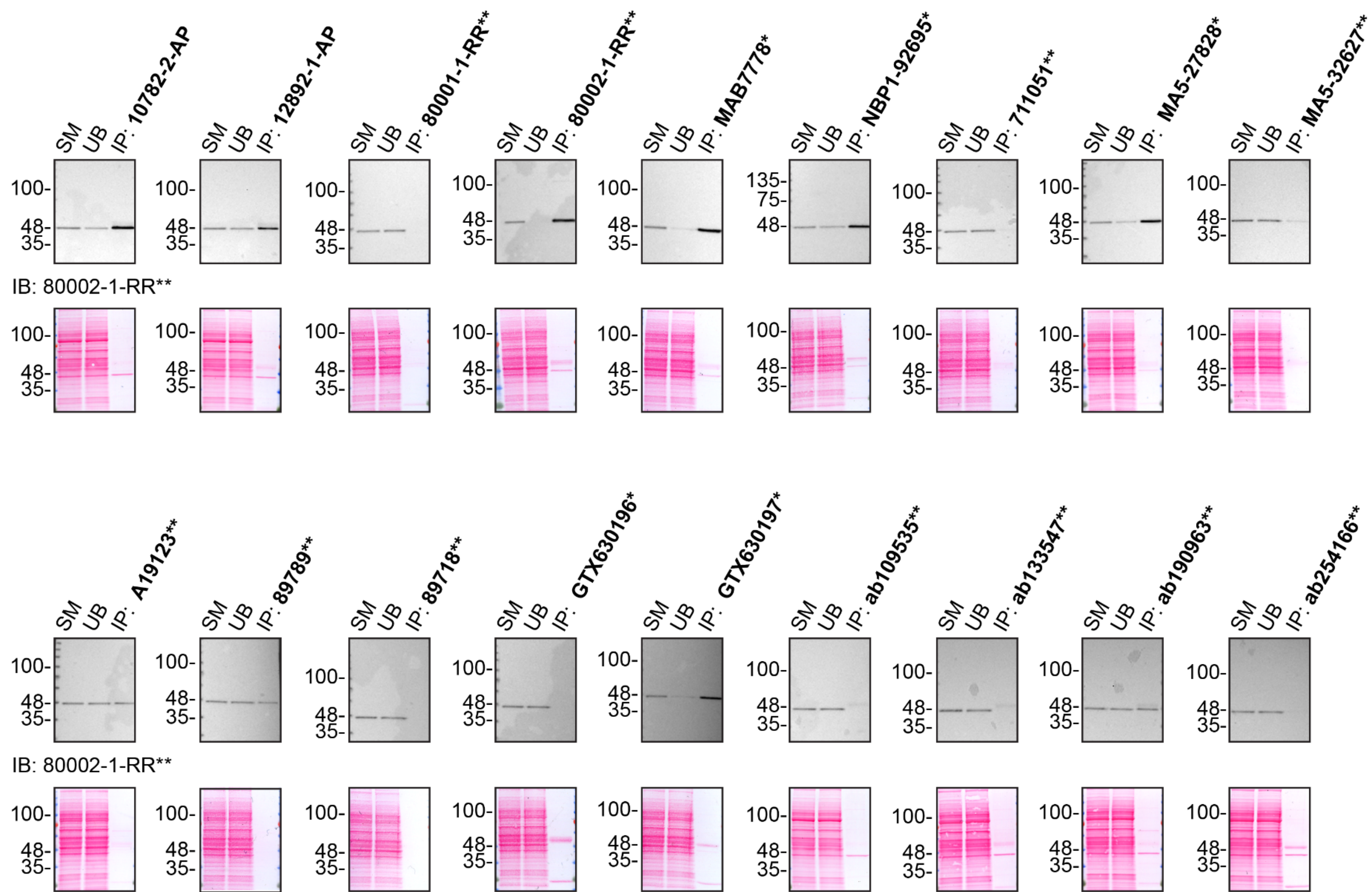

Figure 2: TDP-43 antibody screening by immunoprecipitation

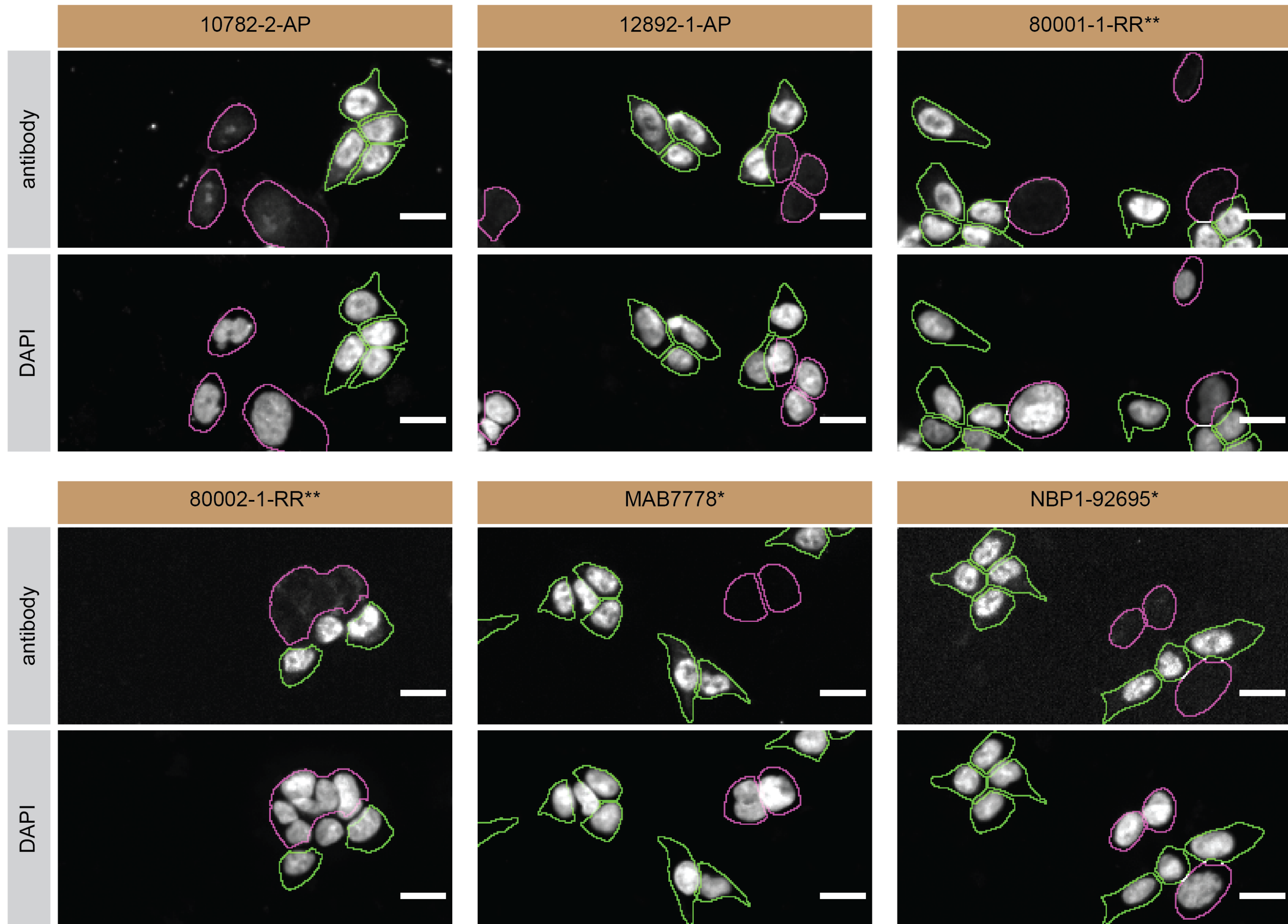

Figure 3: TDP-43 antibody screening by immunofluorescence (1/3)

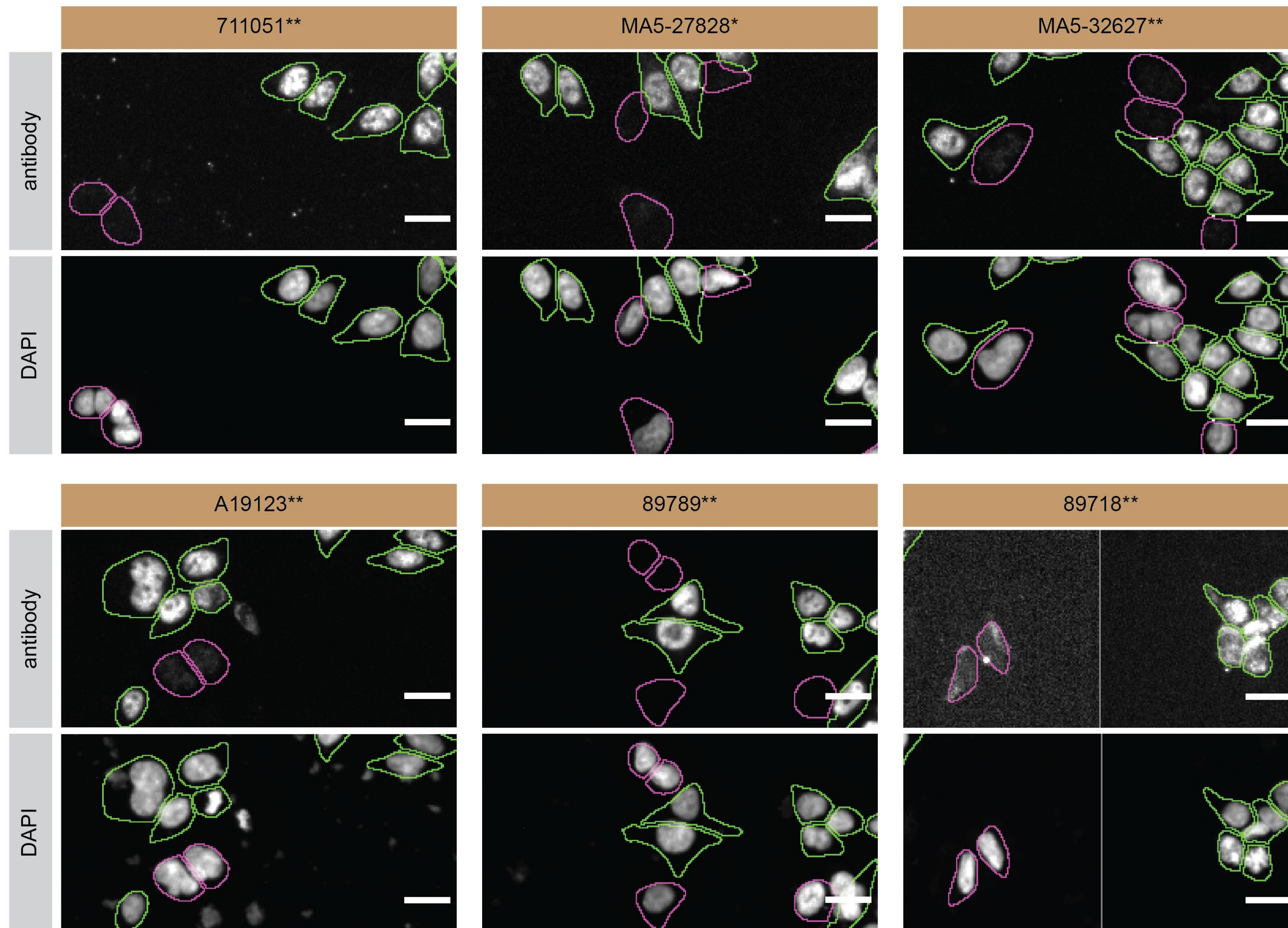

Figure 3: TDP-43 antibody screening by immunofluorescence (2/3)

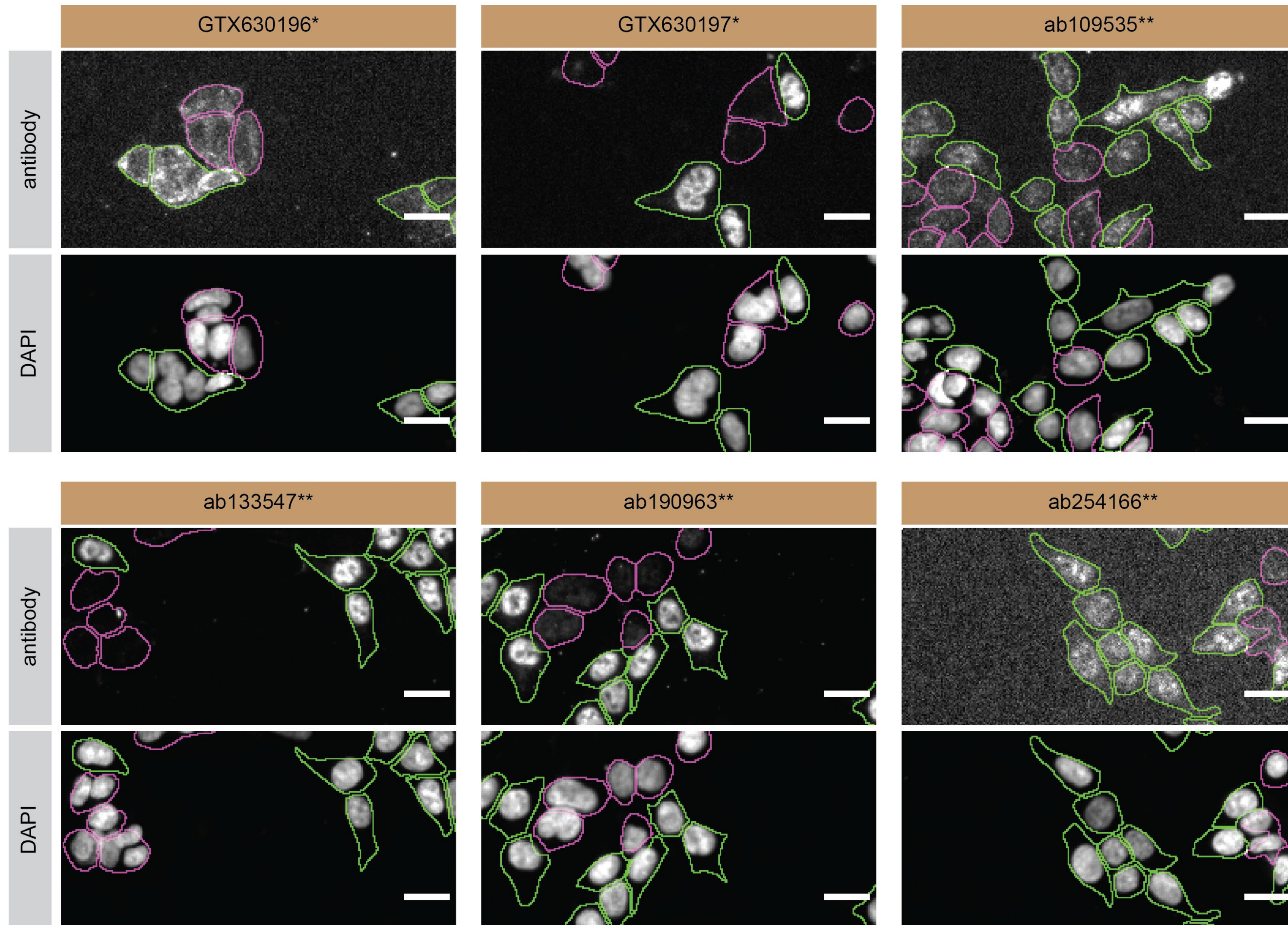

Figure 3: TDP-43 antibody screening by immunofluorescence (3/3)

Materials and methods

Antibodies

All TDP-43 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 62-6520 and 65-6120). Alexa-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and 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

Immunoblots were performed as described in our standard operating procedure [6]. HAP1 WT and *TARDBP* KO were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% 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 midi precast 4-20% gradient polyacrylamide gels from Thermo Fisher Scientific (cat. Number WXP42012BOX) 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 were incubated with ECL from Pierce (cat. number 32106) prior to detection with the iBright™ CL1500 Imaging System from Thermo Fisher Scientific (cat. number A44240).

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure [7]. Antibody-bead conjugates were prepared by adding 2 µg to 500 ul of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with with 30µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Pierce IP Lysis Buffer was supplemented with the Halt Protease Inhibitor Cocktail 100X from Thermo Fisher Scientific (cat. number 78446) at a final concentration of 1x. Tubes were rocked for ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

HAP1 WT were collected in Pierce IP buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) 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 IP lysis buffer and processed for SDS-PAGE and immunoblot on 5-16% polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a dilution of 0.4 µg/ml for an experiment where a rabbit antibody was used for both immunoprecipitation and its corresponding immunoblot.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [8]. HAP1 WT and *TARDBP* 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 TDP-43 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.

Images were acquired on an ImageXpress micro confocal high-content microscopy system (Molecular Devices), using a 20x NA 0.95 water immersion objective and scientific CMOS cameras, equipped with 395, 475, 555 and 635 nm solid state LED lights (lumencor Aura III light engine) and bandpass filters to excite DAPI, Cellmask Green, Alexa568 and Cellmask Red, respectively. Images had pixel sizes of 0.68 x 0.68 microns, and a z-interval of 4 microns. For analysis and visualization, shading correction (shade only) was carried out for all images. Then, maximum intensity projections were generated using 3 z-slices. Segmentation was carried out separately on maximum intensity projections of Cellmask channels using CellPose 1.0, and masks were used to generate outlines and for intensity quantification.

References

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