Antibody Characterization Report for hnRNP A1 (Heterogeneous nuclear ribonucleoprotein A1)

YCharOS Antibody Characterization Report

Author(s): Vera Ruíz Moleón[1], Maryam Fotouhi[1], Charles Alende[†,1], Joel Ryan [†,2], Riham Ayoubi[1], Wolfgang Reintsch[3], Thomas M. Durcan[3], Claire M. Brown[2], Peter S. McPherson[1] and Carl Laflamme[1*]

[†] Authors contributed equally
[1] Department of Neurology and Neurosurgery, Structural Genomics Consortium, The Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada
[2] Advanced BioImaging Facility (ABIF), McGill University, Montreal, Canada
[3] The Neuro's Early Drug Discovery Unit (EDDU), Structural Genomics Consortium, McGill University, Montreal, Quebec, Canada
* Corresponding author: carl.laflamme@mcgill.ca

Target:
Recommended protein name: Heterogeneous nuclear ribonucleoprotein A1

Recommended short protein name: hnRNP A1

Alternative protein names: Helix-destabilizing protein, Single-strand RNA-binding protein, hnRNP core protein A1

Gene name: *HNRNPA1*

Uniprot: P09651

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. In this study, we characterized six hnRNP A1 commercial antibodies for Western blot, immunoprecipitation, and immunofluorescence using a standardized experimental protocol [2] based on comparing read-outs in knockout cell lines and isogenic parental controls. We identified many well-performing antibodies and encourage readers to use this report as a guide to select the most appropriate antibody for their specific needs. HEK 293T was selected based on evidence of appropriate hnRNP A1 protein expression [3]. An HEK 293T *HNRNPA1* KO line is available at Abcam 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 cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype
Abcam	ab255449	CVCL_0063	HEK 293T	WT
Abcam	ab266193	CVCL_B2YX	HEK 293T	*HNRNPA1* KO

Table 2: Summary of the hnRNP A1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	NB100-672*	B7	AB_10003087	monoclonal	4B10	mouse	1.00	Wb,IP,IF
Cell Signaling Technology	8443**	4	AB_10828725	recombinant-mono	D21H11	rabbit	0.44	Wb,IP,IF
GeneTex	GTX106208	39764	AB_1950504	polyclonal	-	rabbit	0.46	Wb,IF
Proteintech	67844-1-Ig*	10020585	AB_2918606	monoclonal	1A5A4	mouse	0.50	Wb
Thermo Fisher Scientific	MA5-32692**	YE3913384A	AB_2809969	recombinant-mono	JA39-21	rabbit	1.00	Wb,IF
Thermo Fisher Scientific	MA5-35132**	YE3913562	AB_2849037	recombinant-mono	ARC0633	rabbit	1.30	Wb,IP,IF

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

Materials and methods

Antibodies

All the hnRNP A1 antibodies tested are listed in Table 2. Peroxidase-conjugated goat anti-rabbit and anti-mouse are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520). Alexa-555-conjugated goat anti-rabbit and anti-mouse secondary antibodies are from Thermo Fisher Scientific (cat. number A21429 and A21424).

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-glutamine (Wisent cat. number 609-065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Antibody screening by Western blot

Western blots were performed as described in our standard operating procedure [6]. HEK 293T WT and *HNRNPA1* KO (listed in Table 1) were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) from Thermo Fisher Scientific (cat. number 89901) supplemented with 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number P8340). 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 Western blot. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

Western blots were performed with precast midi 4-20% Tris-Glycine polyacrylamide gels from Thermo Fisher Scientific (cat. number WXP42012BOX) ran with Tris/Glycine/SDS buffer from Bio-Rad (cat. number 1610772), loaded in Laemmli loading sample buffer from Thermo Fisher Scientific (cat. number AAJ61337AD) and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau S staining (Thermo Fisher Scientific, cat. number BP103-10) which is scanned to show together with individual Western blot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% milk in TBS with 0,1% Tween 20 (TBST) from Cell Signaling (cat. number 9997). 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 Pierce ECL from Thermo Fisher Scientific (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-beads conjugates were prepared by adding 2 µg to 500 µl 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). Tubes were rocked for ~1 hr at 4°C followed by two washes to remove unbound antibodies.

HEK 293T 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 were rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. 0.5 ml aliquots at 2.0 mg/ml of lysate were incubated with an antibody-bead conjugate for ~1 hr at 4°C. 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 Western blot on precast midi 4-20% Tris-Glycine polyacrylamide gels. Anti-mouse IgG for IP:HRP (Abcam, cat. number ab131368) was used as a secondary detection system at a concentration of 0.3 µg/ml.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [8]. HEK 293T WT and *HNRNPA1* 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 in a 96-well plate with optically clear flat-bottom. (Perkin Elmer, cat. number 6055300) 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 hnRNP A1 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, Alexa-555 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. Figures were assembled with Adobe Illustrator.

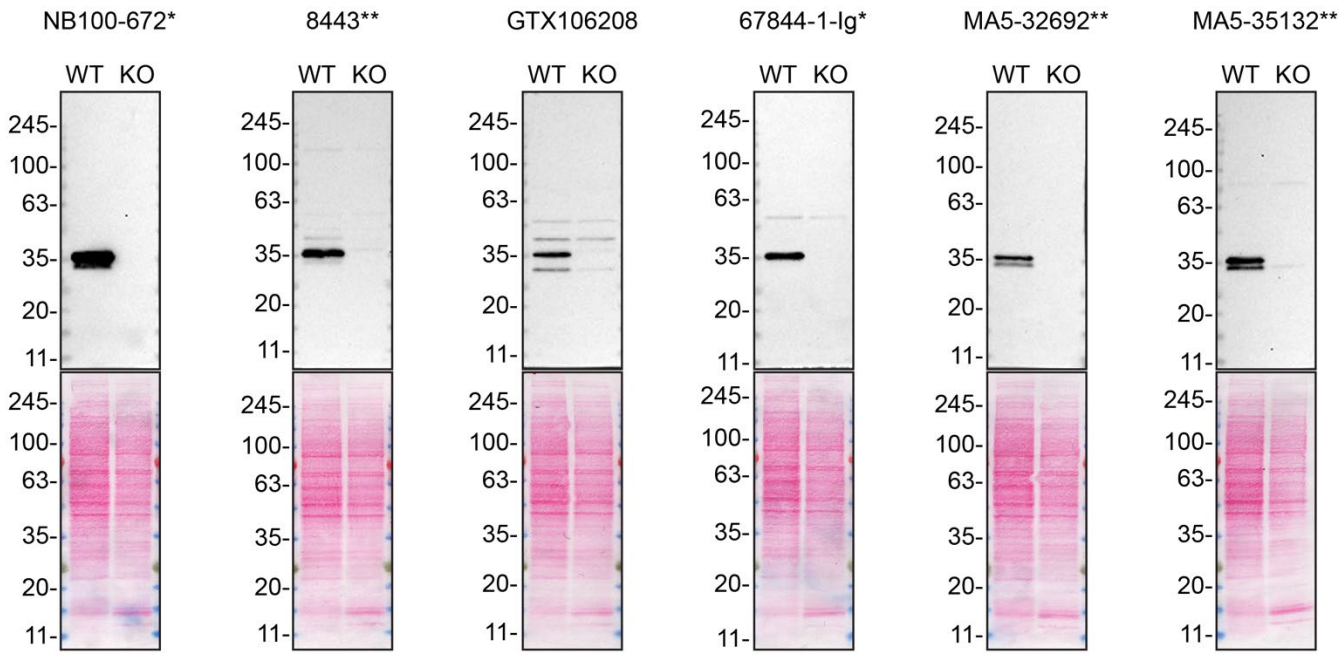

Figure 1: hnRNP A1 antibody screening by Western blot

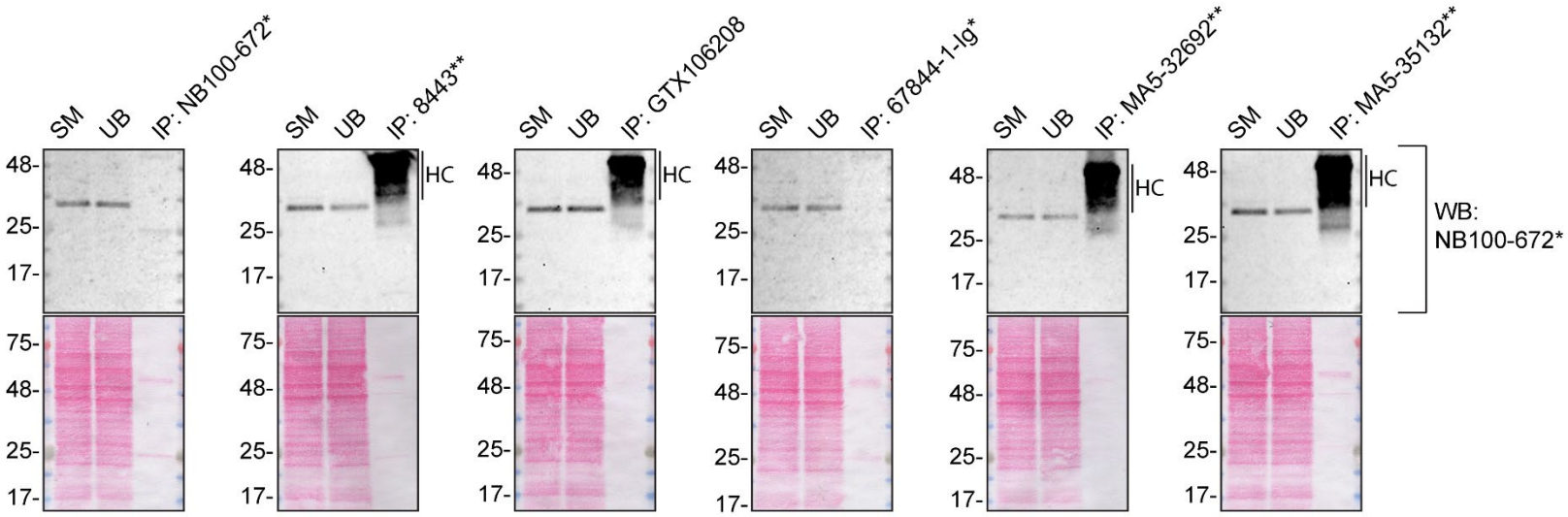

Figure 2: hnRNP A1 antibody screening by immunoprecipitation

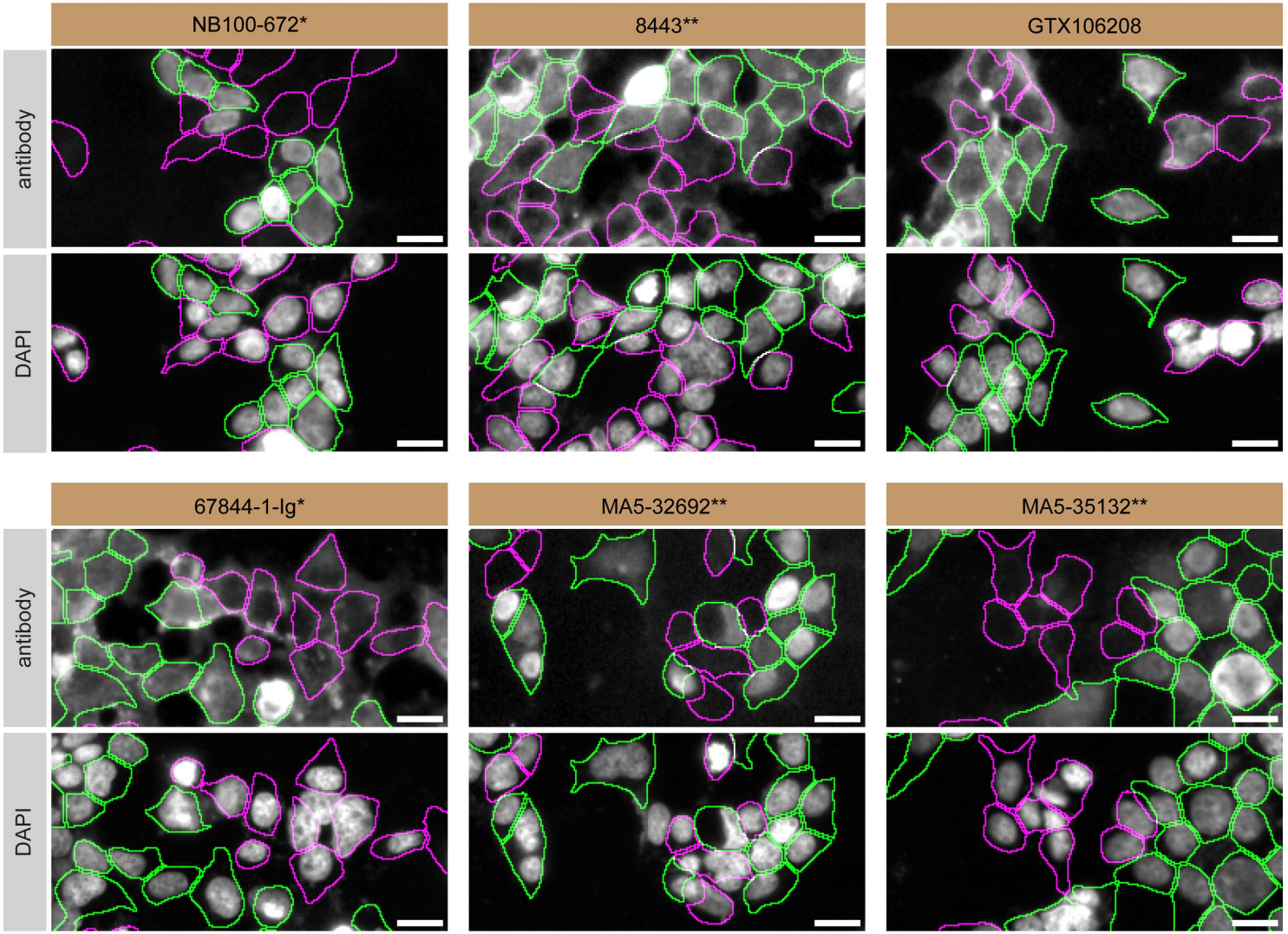

Figure 3: hnRNP A1 antibody screening by immunofluorescence

Figure 1: hnRNP A1 antibody screening by Western blot.
Lysates of HEK 293T WT and *HNRNPA1* KO were prepared, and 30 µg of protein were processed for Western blot with the indicated hnRNP A1 antibodies. The Ponceau stained transfers of each blot are shown. All antibodies were tested at 1/1000. Predicted band size: 38.7 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: hnRNP A1 antibody screening by immunoprecipitation.
HEK 293T lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated hnRNP A1 antibodies pre-coupled to Dynabeads protein A or protein G. Samples were washed and processed for Western blot with the indicated hnRNP A1 antibody. For Western blot, NB100-672* was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain. *=monoclonal antibody, **=recombinant antibody

Figure 3: hnRNP A1 antibody screening by immunofluorescence.
HEK 293T WT and *HNRNPA1* 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 optically clear flat-bottom. Cells were stained with the indicated hnRNP A1 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 with green and magenta dashed line, respectively. Antibody dilution used: NB100-672* at 1/1000, 8443** at 1/500, GTX106208 at 1/500, 67844-1-Ig* at 1/800, MA5-32692** at 1/1000, MA5-35132** at 1/1300. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

References

1. Laflamme, C., et al., *Opinion: Independent third-party entities as a model for validation of commercial antibodies.* N Biotechnol, 2021. **65**: p. 1-8 DOI: 10.1016/j.nbt.2021.07.001.
2. Laflamme, C., et al., *Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72.* Elife, 2019. **8** DOI: 10.7554/eLife.48363.
3. Wang, M., et al., *Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines.* Proteomics, 2015. **15**(18): p. 3163-8 DOI: 10.1002/pmic.201400441.
4. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16 DOI: 10.1016/j.cell.2019.12.023.
5. *DepMap, Broad*. 2019.
6. Ayoubi, R., P.S. McPherson, and C. Laflamme, *Antibody Screening by Immunoblot.* 2021 DOI: https://doi.org/10.5281/zenodo.5717510.
7. Ayoubi, R., et al., *Antibody screening by Immunoprecitation.* 2021 DOI: https://doi.org/10.5281/zenodo.5717516.
8. Alshafie, W., P. McPherson, and C. Laflamme, *Antibody screening by Immunofluorescence.* 2021 DOI: https://doi.org/10.5281/zenodo.5717498.