Antibody Characterization Report for Dystroglycan 1

YCharOS Antibody Characterization Report

Author(s): Riham Ayoubi[1], Walaa Alshafie[1], Lynda Dekakra-Bellili[1], Peter S. McPherson[1*] and Carl Laflamme[1*]

[1] Tanenbaum Open Science Institute, Structural Genomics Consortium, Montreal Neurological Institute, McGill University, Montreal, Canada
* Corresponding authors: carl.laflamme@mcgill.ca, peter.mcpherson@mcgill.ca

Target:

Recommended protein name: Dystroglycan 1

Alternative protein name: Dystrophin-associated glycoprotein 1

Gene name: *DAG1*

Uniprot: Q14118

Dystroglycan 1 is cleaved into the following 2 chains:

- Alpha-dystroglycan
- Beta-dystroglycan

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 Dystroglycan 1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Dystroglycan 1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. An A431 *DAG1* KO cell line and an HAP1 *DAG1* KO cell line are available at Abcam and at Horizon, respectively. Both A431 and HAP1 are expected to express adequate level of *DAG1* [3] and both cell lines were 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 Dystroglycan 1 antibodies tested

Company	Specificity	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
ABclonal	Beta-Dystroglycan	A10076	8160101	AB_2757599	polyclonal	-	rabbit	4.87	Wb,IF
Developmental Studies Hybridoma Bank	Alpha-Dystroglycan	DAG-6F4*	3/12/2020	AB_2753232	monoclonal	DAG-6F4	mouse	0.06	Wb,IP,IF
Developmental Studies Hybridoma Bank	Beta-Dystroglycan	MANDAG1 (7A11)*	7/12/2018	AB_2753233	monoclonal	MANDAG1 (7A11)	mouse	0.07	Wb
Developmental Studies Hybridoma Bank	Beta-Dystroglycan	MANDAG2(7D11)*	4/16/2020	AB_2618140	monoclonal	MANDAG2(7D11)	mouse	0.05	other
Developmental Studies Hybridoma Bank	Alpha-Dystroglycan	IIH6 C4*	-	AB_2753232	monoclonal	IIH6 C4	mouse, IgM	0.03	Wb,IP,IF
Abcam	Beta-Dystroglycan	ab43125	GR3190436-2	AB_955822	polyclonal	-	rabbit	1.00	Wb
Abcam	Beta-Dystroglycan	ab62373**	GR3380024-5	AB_941239	recombinant-mono	EP2200Y	rabbit	0.53	Wb
Abcam	Alpha-Dystroglycan	ab234587**	GR3391410-2	AB_2910177	recombinant-mono	IIH6C4	mouse, IgM	1.03	Wb
Thermo Fisher Scientific	Beta-Dystroglycan	MA5-35931*	WI3378228A	AB_2866548	monoclonal	GT835	mouse	1.00	Wb,IF
Thermo Fisher Scientific	Alpha-Dystroglycan Beta-Dystroglycan	PA5-28179	XB3501486E	AB_2545655	polyclonal	-	rabbit	2.54	Wb,IF
Thermo Fisher Scientific	Beta-Dystroglycan	PA5-34908	XB3501482A	AB_2552259	polyclonal	-	rabbit	0.60	Wb,IF
Proteintech	Beta-Dystroglycan	11017-1-AP	39620	AB_2090032	polyclonal	-	rabbit	0.24	Wb,IP,IF
Proteintech	Beta-Dystroglycan	66735-1*	10006172	AB_2882085	monoclonal	2B1G12	mouse	1.00	Wb
GeneTex	Beta-Dystroglycan	GTX105038	43502	AB_2036733	polyclonal	-	rabbit	1.06	Wb,IF

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
Abcam	ab263975	CVCL_0037	A431	WT
Abcam	ab261906	-	A431	*DAG1* KO
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC000120c013	CVCL_SK29	HAP1	*DAG1* KO

Figure 1: Dystroglycan 1 antibody screening by immunoblot.

Lysates of A431 (WT and *DAG1* KO) were prepared and 90 µg of protein were processed for immunoblot with the indicated Dystroglycan 1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: A10076 at 1/1000; MANDAG1 (7A11) at 1/700; MANDAG2(7D11) at 1/500; ab43125 at 1/500; ab62373 at 1/1000; ab234587 at 1/500; MA5-35931at 1/1000; PA5-28179 at 1/2000; PA5-34908 at 1/500; 11017-1-AP at 1/500; 66735-1 at 1/5000; GTX105038 at 1/500. Predicted band size: 97 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Alpha-dystroglycan antibody screening by immunoblot on culture media.

HAP1 (WT and *DAG1* KO) were grown in culture, starved for 24 hrs and 100 µg of protein from concentrated culture media were processed for immunoblot with the indicated Alpha-dytroglycan antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: DAG-6F4 at 1/240; IIH6 C4 at 1/120; ab234587 at 1/500; PA5-28179 at 1/2000. Alpha-dystroglycan was detected in HAP1 cell line but was not detected in A431 (data not shown). *=monoclonal antibody

Figure 3: Dystroglycan 1 antibody screening by immunoprecipitation.

A) A431 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Dystroglycan 1 antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Dystroglycan 1 antibody. Ability of the antibodies to capture Dystroglycan 1 was first assessed by comparing the level of Dystroglycan 1 from the starting material to the unbound fractions. For immunoblot, MA5-35931 was used at 1/1000. **B)** Analysis of the immunoprecipitate for the antibody that showed depletion of Dystroglycan 1 in (A). **C)** HAP1 lysates were prepared, and immunoprecipitation using Alpha-dystroglycan antibodies was performed as described in (A). For immunoblot, ab234587 was used at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody, **=recombinant antibody

Figure 4: Dystroglycan 1 antibody screening by immunofluorescence.

A431 WT and *DAG1* 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 Dystroglycan 1 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. Antibody dilution used: A10076 at 1/4000; MANDAG1 (7A11) at 1/70; MANDAG2(7D11) at 1/50; ab43125 at 1/1000; ab62373 at 1/500; MA5-35931 at 1/1000; PA5-28179 at 1/2000; PA5-34908 at 1/600; 11017-1-AP at 1/200; 66735-1 at 1/1000; GTX105038 at 1/1000. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

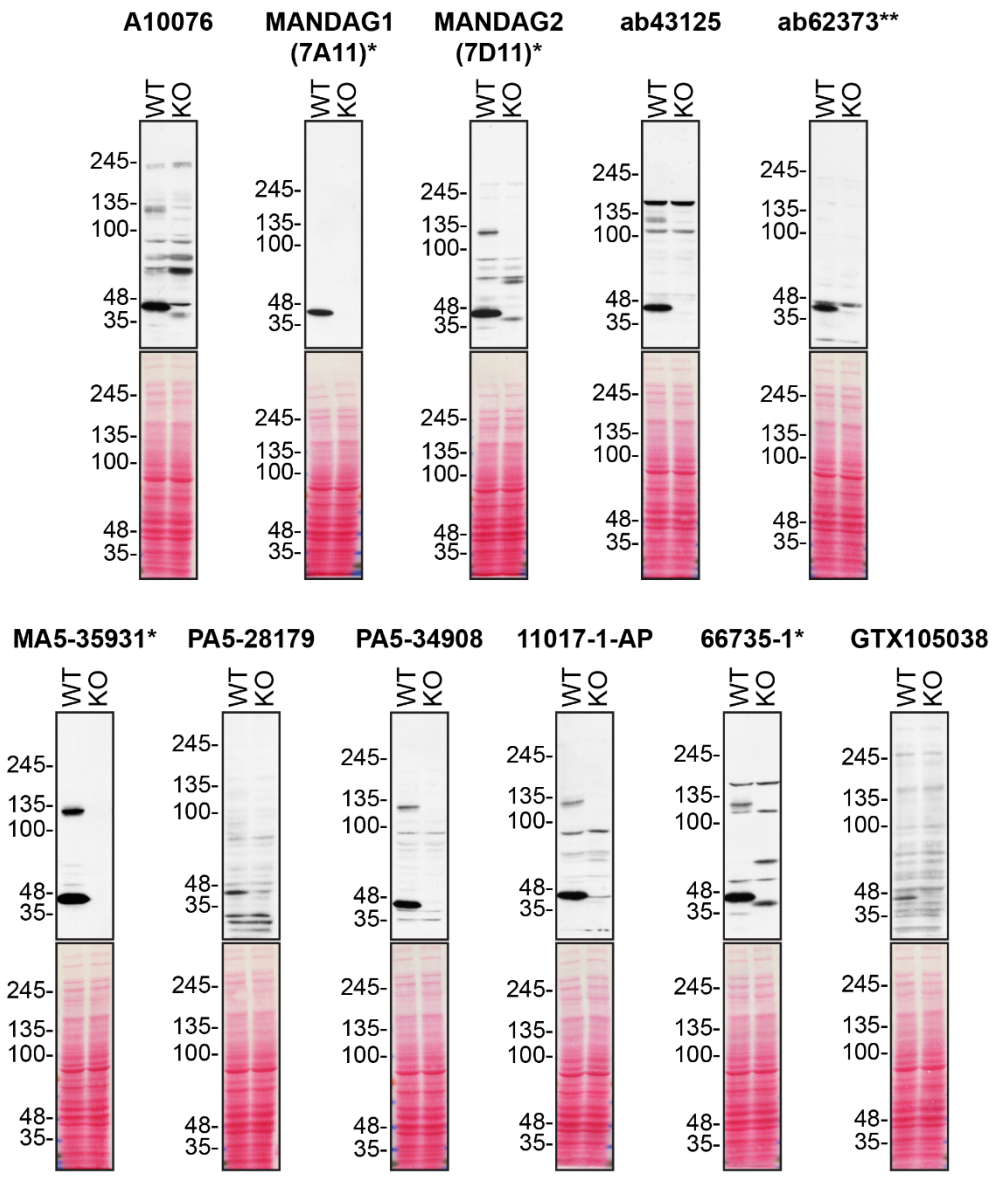

Figure 1: Beta-dystroglycan antibody screening by immunoblot in A431

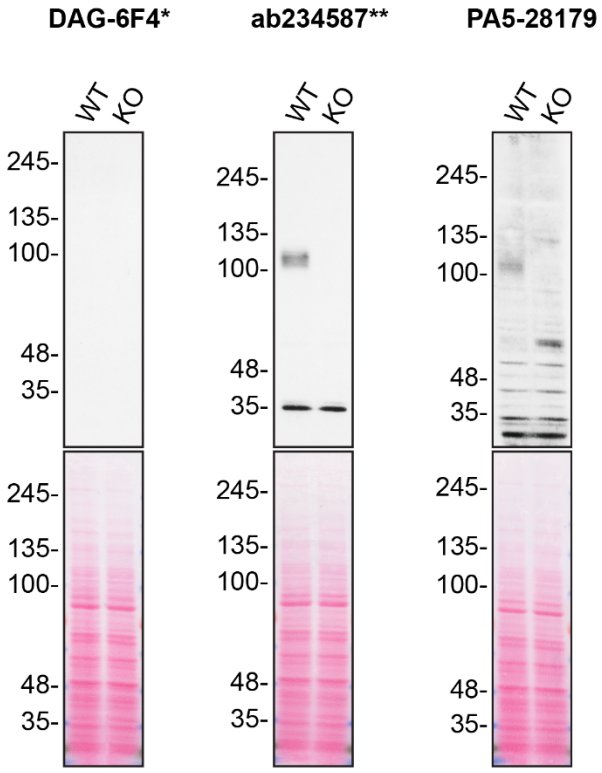

Figure 2: Alpha-dystroglycan antibody screening by immunoblot on HAP1 culture media

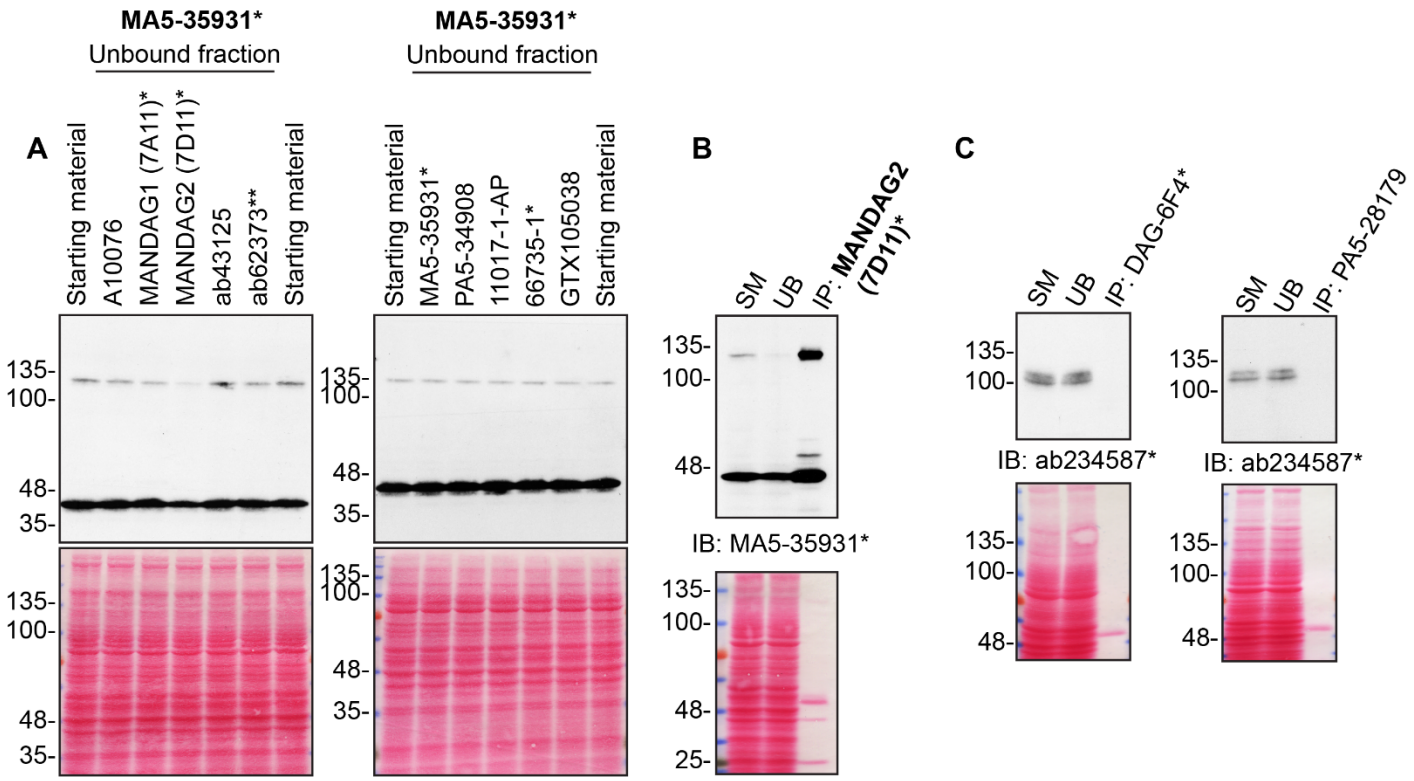

Figure 3: Dystroglycan 1 antibody screening by immunoprecipitation

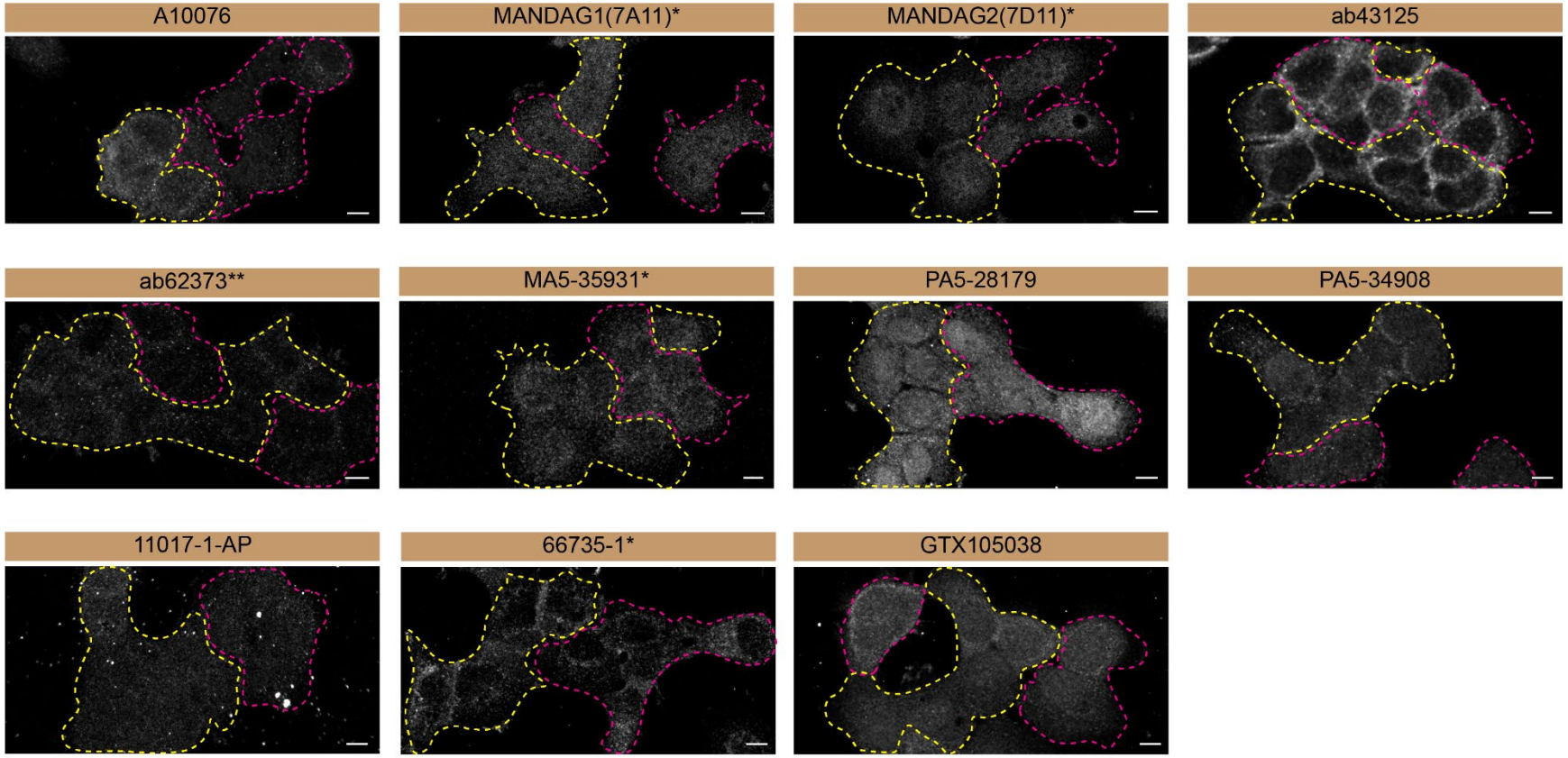

Figure 4: Beta-dystroglycan antibody screening by immunofluorescence on A431

Materials and methods

Antibodies

All Dystroglycan 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 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). Cells were starved in DMEM high glucose containing L-glutamate and penicillin/ streptomycin.

Antibody screening by immunoblot

Immunoblots were performed as described in our standard operating procedure [4]. A431 (WT and *DAG1* 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 with large 3-12% 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).

Collection of culture media

HAP1 WT and *DAG1* KO cells were washed 3x with PBS and starved for ~18 hrs. Culture media were collected and centrifuged for 10 min at 500 x g to eliminate cells and larger contaminants, then for 10 min at 4500 x g to eliminate smaller contaminants. Culture media were concentrated by centrifuging at 4000 x g for 30min using Amicon Ultra-15 Centrifugal Filter Units with a membrane NMWL of 10kDa (MilliporeSigma cat. number UFC901024). Immunoblots were performed as described above.

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure [5]. 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.

A431 (A-B) or HAP1 (C) 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 3-12% polyacrylamide gels.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [6]. A431 WT and *DAG1* 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 Dystroglycan 1 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 20x air objective (NA = 0.8). Analysis was done using the Zen navigation software (Zeiss). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.

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. Ghandi, M., et al., *Next-generation characterization of the Cancer Cell Line Encyclopedia.* Nature, 2019. **569**(7757): p. 503-508 DOI: 10.1038/s41586-019-1186-3.
4. Ayoubi, R., P.S. McPherson, and C. Laflamme, *Antibody Screening by Immunoblot.* 2021 DOI: https://doi.org/10.5281/zenodo.5717510.
5. Ayoubi, R., et al., *Antibody screening by Immunoprecitation.* 2021 DOI: https://doi.org/10.5281/zenodo.5717516.
6. Alshafie, W., P. McPherson, and C. Laflamme, *Antibody screening by Immunofluorescence.* 2021 DOI: https://doi.org/10.5281/zenodo.5717498.