Antibody Characterization Report for Charged multivesicular body protein 2b

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Charged multivesicular body protein 2b

Alternative protein name: Chromatin-modifying protein 2b, Vacuolar protein sorting-associated protein 2-2, Vps2-2

Gene name: CHMP2B

Uniprot: Q9UQN3

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 Charged multivesicular body protein 2b. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Charged multivesicular body protein 2b by immunoblot (Western blot), immunoprecipitation and immunofluorescence. U2OS was selected based on evidence of appropriate Charged multivesicular body protein 2b protein expression determined through public proteomics databases, namely PaxDB [3] and DepMap [4, 5]. U2OS was modified with CRISPR/Cas9 to knockout the corresponding *CHMP2B* 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.

Table 1: Summary of the Charged multivesicular body protein 2b antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	MAB7509*	CHEB0112101	AB_2885148	monoclonal	791521	mouse	0.50	Wb
Thermo	MA5-21591*	WA3152391	AB_2576481	monoclonal	2H6-1E6	mouse	0.50	Other application
Thermo	MA5-36184**	VL3152619	AB_2890433	recombinant-mono	JE54-35	rabbit	1.00	Wb, IF
Abcam	ab157208**	GR117930-3	AB_2885096	recombinant-mono	EPR10807(B)	rabbit	0.13	Wb, IP, IF
Cell Signaling Technology	76173*	1	AB_2799880	monoclonal	D4G3K	rabbit	not provided	Wb, IP
GeneTex	GTX118181	40625	AB_11174469	polyclonal	-	rabbit	0.59	Wb, IF
GeneTex	GTX109610	40681	AB_11163162	polyclonal	-	rabbit	1.00	Wb, IF
ABclonal	A13410	13540101	AB_2760272	polyclonal	-	rabbit	0.85	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
ATCC	HTB-96	CVCL_0042	U2OS	WT
Montreal Neurological Institute	-	CVCL_B6JX	U2OS	*CHMP2B* KO

Figure 1: Charged multivesicular body protein 2b antibody screening by immunoblot.

Lysates of U2OS (WT and *CHMP2B* KO) were prepared and 50 µg of protein were processed for immunoblot with the indicated Charged multivesicular body protein 2b antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MAB7509 at 1/400; MA5-21591 at 1/1000; MA5-36184 at 1/500; ab157208 at 1/1000; 76173 at 1/1000; GTX118181 at 1/1000; GTX109610 at 1/1000; A13410 at 1/500. Predicted band size: 24 kDa. *=monoclonal antibody; **=recombinant antibody

Figure 2: Charged multivesicular body protein 2b antibody screening by immunoprecipitation.

U2OS lysates were prepared, and immunoprecipitation was performed using 1.0 µg of the indicated Charged multivesicular body protein 2b antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Charged multivesicular body protein 2b antibody. For immunoblot, ab157208 was used at 1/2000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitated; *=monoclonal antibody; **=recombinant antibody

Figure 3: Charged multivesicular body protein 2b antibody screening by immunofluorescence.

U2OS WT and *CHMP2B* 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 Charged multivesicular body protein 2b 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: MAB7509 at 1/500; MA5-21591 at 1/500; MA5-36184 at 1/1000; ab157208 at 1/100; 76173 at 1/500; GTX118181 at 1/500; GTX109610 at 1/1000; A13410 at 1/800. Bars = 10 µm. *=monoclonal antibody; **=recombinant antibody

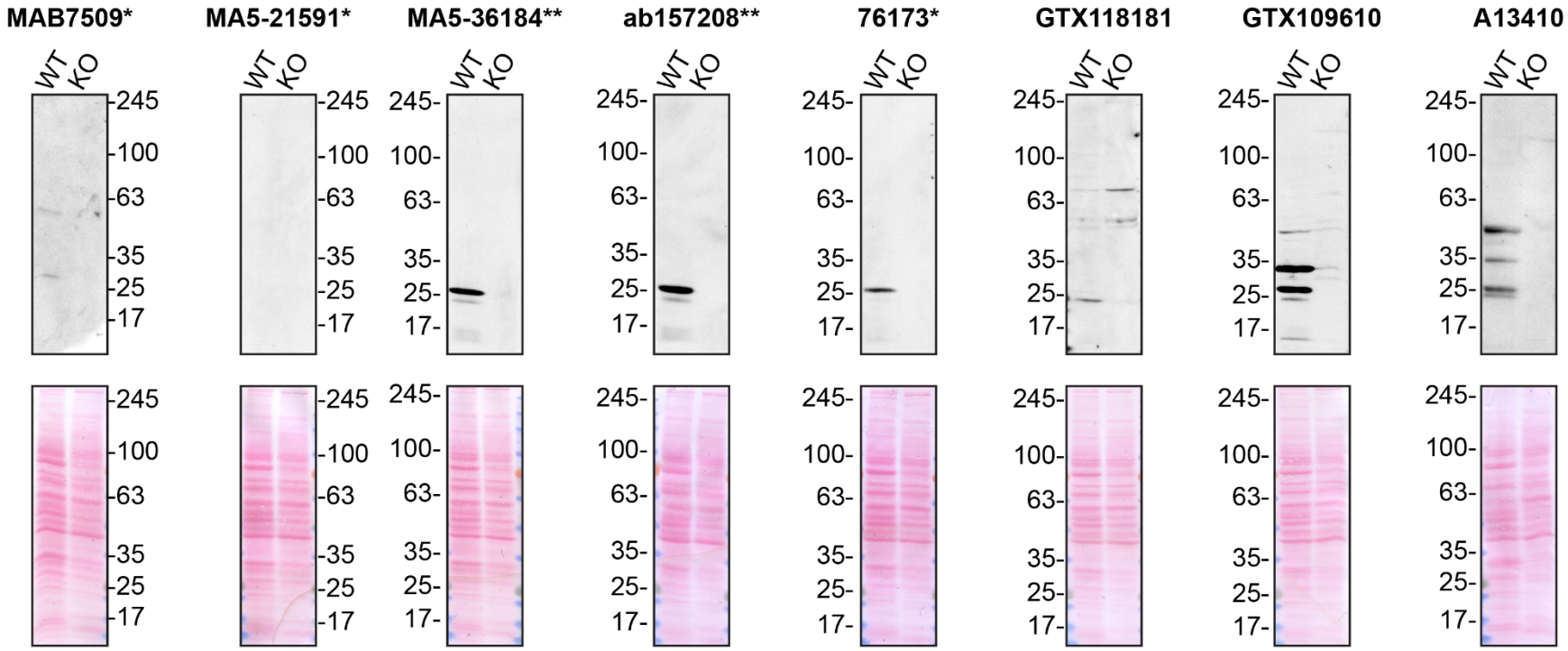

Figure 1: Charged multivesicular body protein 2b antibody screening by immunoblot

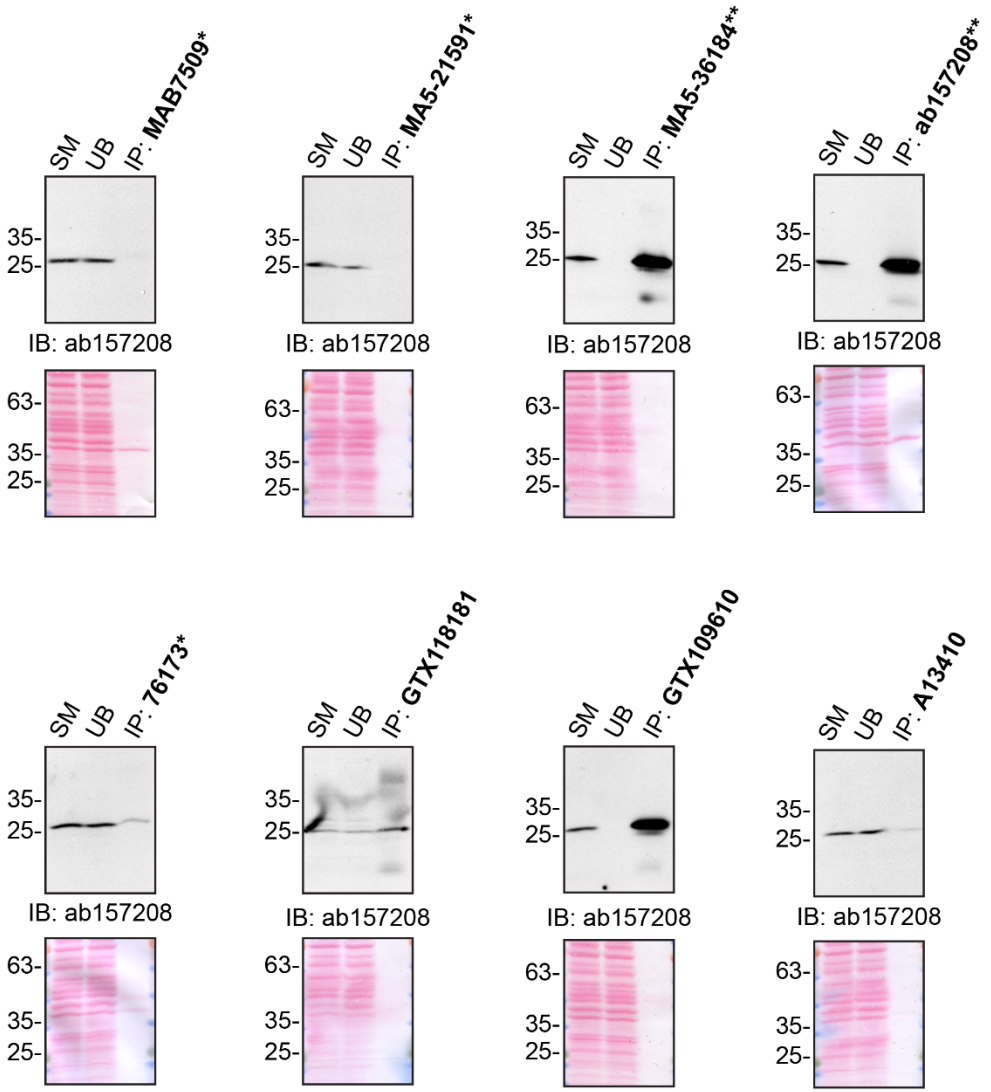

Figure 2: Charged multivesicular body protein 2b antibody screening by immunoprecipitation

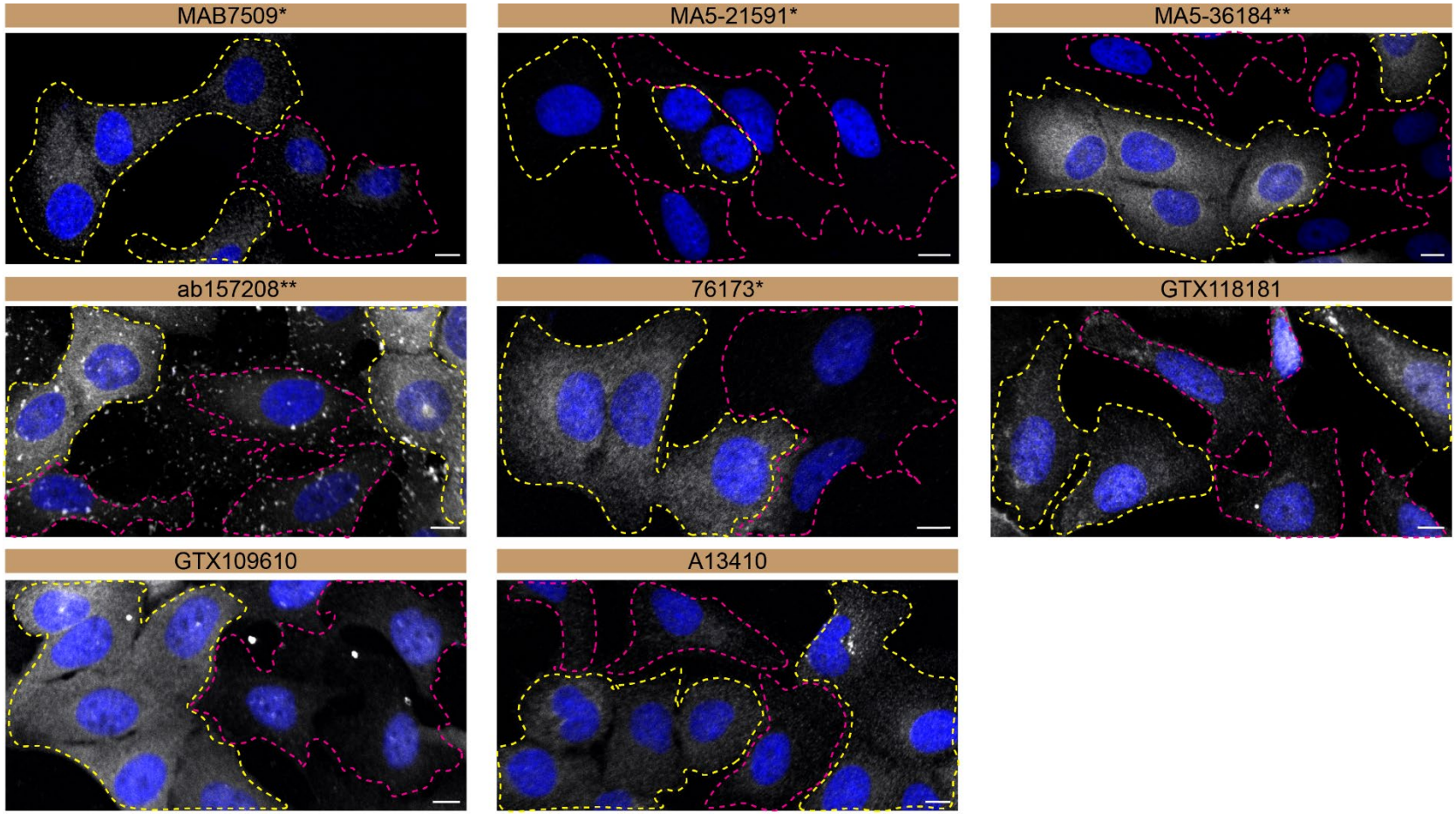

Figure 3 : Charged multivesicular body protein 2b antibody screening by immunofluorescence

Materials and methods

Antibodies

All Charged multivesicular body protein 2b 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).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. U2OS *CHMP2B* KO clone was generated [6] with low passage cells. The sequence of the guide RNA is the following: CCAAACAACUUGUGCAUCUA.

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 [7]. U2OS (WT and *CHMP2B* 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 4-20% 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

Immunoprecipitation was performed as described in our standard operating procedure [8]. Antibody-bead conjugates were prepared by adding 1 µg or 2 µl of antibody at an unknown concentration 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.

U2OS 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 4-20% 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 [9]. U2OS WT and *CHMP2B* 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 Charged multivesicular body protein 2b 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 63x 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.

References

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