Antibody Characterization Report for Vesicle-associated membrane protein-associated protein B/C (VAPB)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Vesicle-associated membrane protein-associated protein B/C

Short protein name: VAMP-associated protein B/C

Gene name: VAPB

Uniprot: O95292

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 VAMP-associated protein B/C. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for VAMP-associated protein B/C by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HeLa was selected based on evidence of appropriate VAMP-associated protein B/C protein expression determined through public proteomics databases, namely PaxDB [3] and DepMap [4, 5]. HeLa was modified with CRISPR/Cas9 [6] to knockout the corresponding *VAPB* 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 VAMP-associated protein B/C antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo	PA5-53023	Wb3187343	AB_2649391	polyclonal	-	rabbit	0.30	Wb, IF
Thermo	MA5-29639**	WA3152374	AB_2785468	recombinant-mono	208	rabbit	1.00	Wb
GeneTex	GTX131631	43103	AB_2886511	polyclonal	-	rabbit	0.47	Wb, IF
ABclonal	A5363	12860201	AB_2766173	polyclonal	-	rabbit	1.41	Wb, IF

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

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
ATCC	CCL-2	CVCL_0030	HeLa	WT
Montreal Neurological Institute	-	CVCL_B6RU	HeLa	*VAPB* KO

Figure 1: VAMP-associated protein B/C antibody screening by immunoblot.

Lysates of HeLa (WT and *VAPB* KO) were prepared and 50 µg of protein were processed for immunoblot with the indicated VAMP-associated protein B/C antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: PA5-53023 at 1/250, MA5-29639** at 1/500, GTX131631 at 1/1000, A5363 at 1/1000. Predicted band size: 28 kDa. **=recombinant antibody

Figure 2: VAMP-associated protein B/C antibody screening by immunoprecipitation.

HeLa lysates were prepared, and immunoprecipitation was performed using 1.0 µg of the indicated VAMP-associated protein B/C antibodies pre-coupled to Dynabeads protein A. Samples were washed and processed for immunoblot with the indicated VAMP-associated protein B/C antibody. For immunoblot, GTX131631 was used at 1/2000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. **=recombinant antibody

Figure 3: VAMP-associated protein B/C antibody screening by immunofluorescence.

A) HeLa WT and *VAPB* 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 VAMP-associated protein B/C antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody. Acquisition of the green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: PA5-53023 at 1/300, MA5-29639** at 1/1000, GTX131631 at 1/400, A5363 at 1/1400. Bars = 10 µm. **=recombinant antibody

B) WT and KO cells were identified and outlined by thresholding the green and far-red fluorescence dyes, respectively, using the Zen 3.4 (Zeiss) software. Evaluation of antibody performance was calculated by dividing the antibody mean fluorescence intensity measured from WT cells [F(WT)] by the antibody mean fluorescence intensity measured from KO cells [F(KO)]. The ratio of [F(WT)]/[F(KO)] for all tested antibodies is presented as a histogram. An antibody with a calculated ratio above 2.5-fold (dashed red line) could be considered as specific and selective for immunofluorescence. A minimum of 20 WT and 20 KO cells from 3 different fields of view were analysed for each antibody. **=recombinant antibody

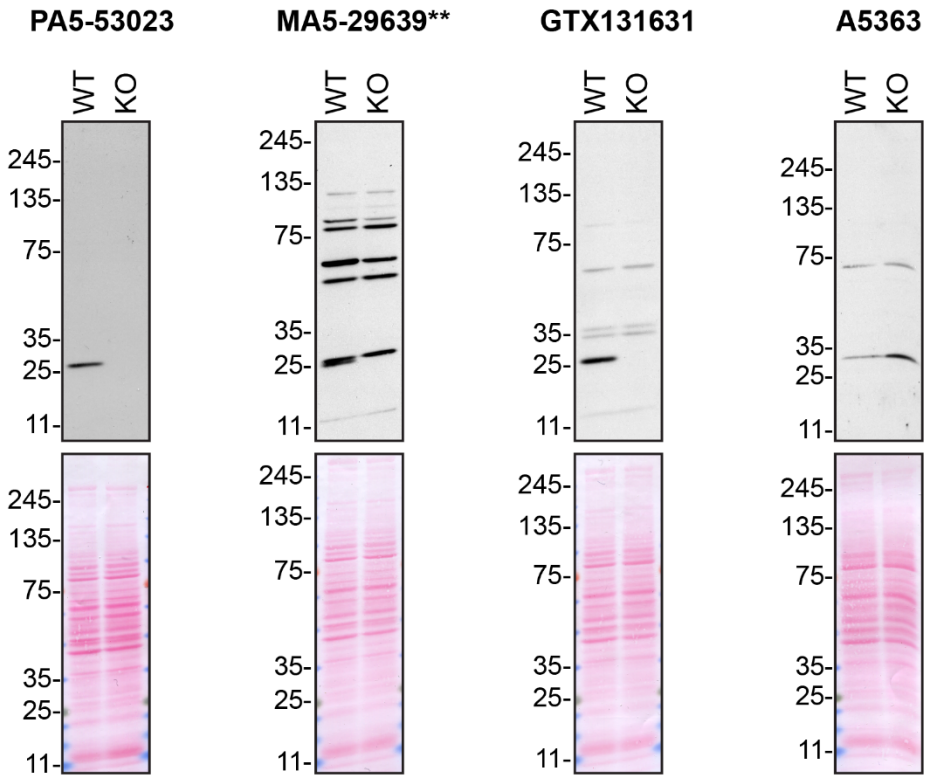

Figure 1: VAMP-associated protein B/C antibody screening by immunoblot

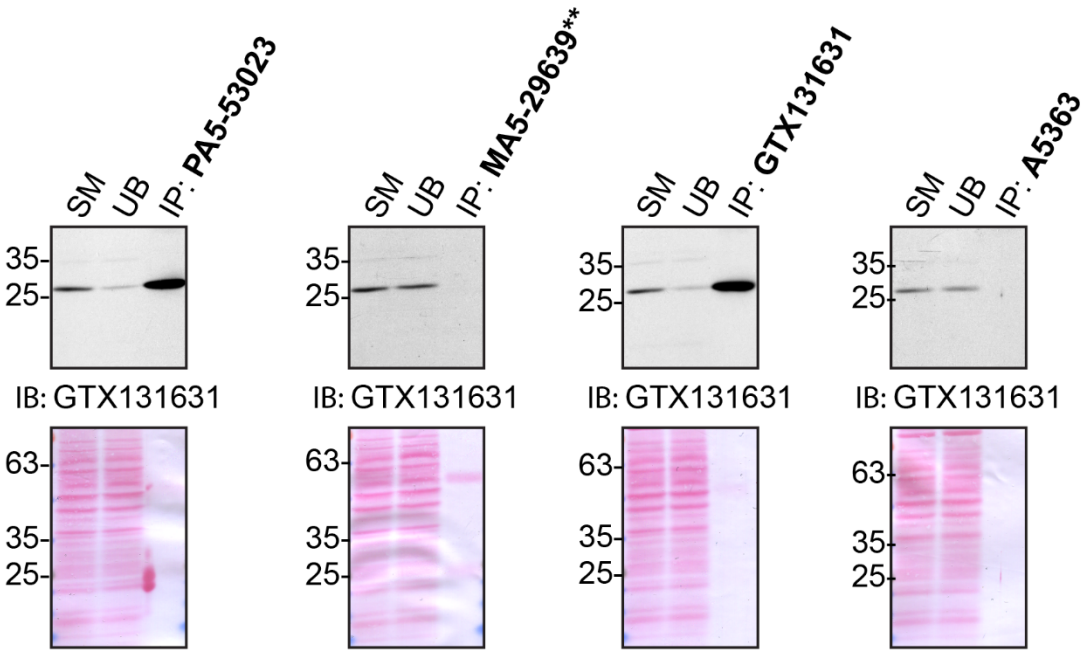

Figure 2: VAMP-associated protein B/C antibody screening by immunoprecipitation

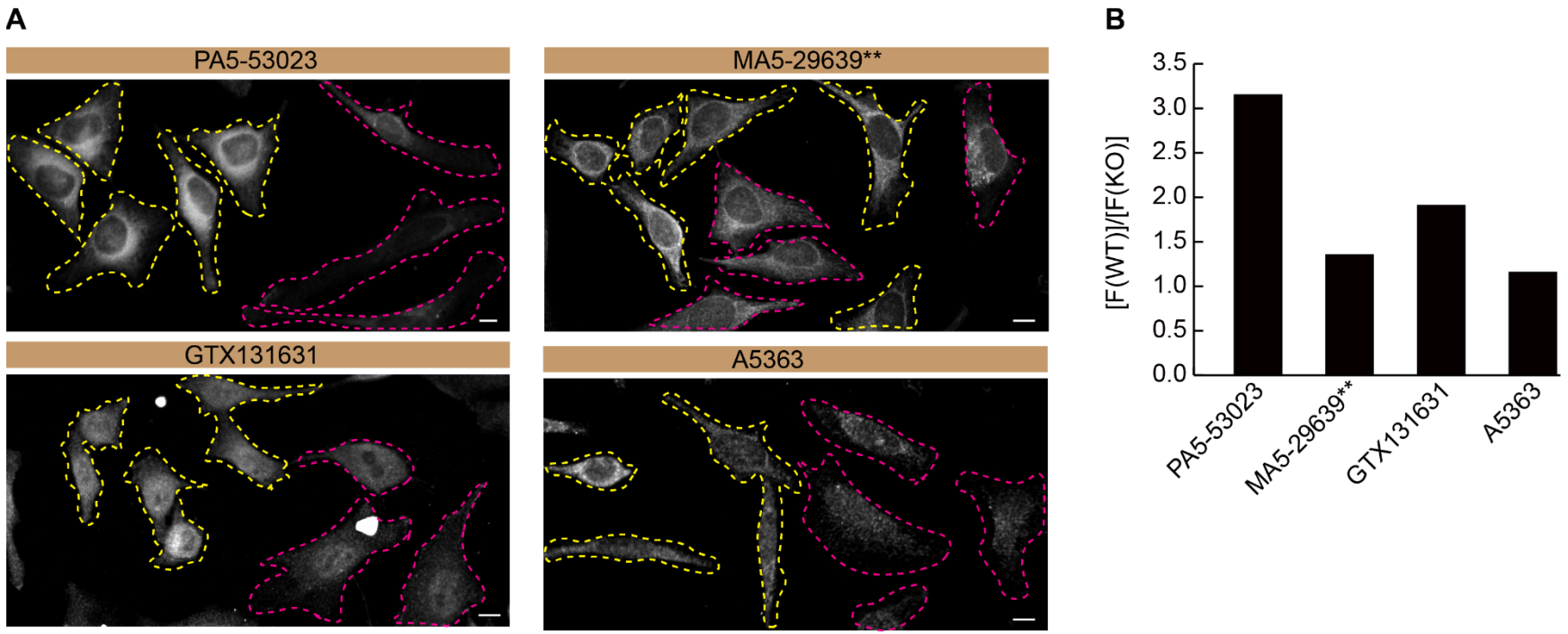

Figure 3 :VAMP-associated protein B/C antibody screening by immunofluorescence

Materials and methods

Antibodies

All VAMP-associated protein B/C 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-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. HeLa *VAPB* KO clone was generated using the a guide RNA with the following sequence: UGAAGACUACAGCACCACGU.

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]. HeLa (WT and *VAPB* 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 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

Immunoprecipitation was performed as described in our standard operating procedure [8]. Antibody-bead conjugates were prepared by adding 1.0 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.

HeLa 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. 1 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.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [9]. HeLa WT and *VAPB* 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 VAMP-associated protein B/C 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

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