Antibody Characterization Report for Valosin-containing protein VCP

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Valosin-containing protein

Alternative protein name: Transitional endoplasmic reticulum ATPase, TER ATPase, 15S Mg(2+)-ATPase p97 subunit

Gene name: *VCP*

Uniprot: P55072

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 16 Valosin-containing protein 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. The VCP gene is considered essential, and a knockdown (KD) approach was used to deplete the corresponding mRNA using siRNA. Moreover, VCP is ubiquitously expressed, and U2OS were selected for this study. 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.

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
ATCC	HTB-96	CVCL_0042	U2OS	WT

Table 2: Summary of the Valosin-containing protein antibodies tested

Compagnie	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab109198**	GR107429-5	AB_10859334	recombinant-mono	EPR3308	rabbit	0.821	Wb
Abcam	ab109240**	GR3176974-8	AB_10862588	recombinant-mono	EPR3307(2)	rabbit	0.350	Wb,IP,IF
Abcam	ab110308*	GR82826-3	AB_10861815	monoclonal	3E8DC11	mouse	1.000	IP,IF
Abcam	ab36047	GR226632-1	AB_2288422	polyclonal	-	rabbit	0.300	Wb
ABclonal	A13368	81090201	AB_2760226	polyclonal	-	rabbit	1.770	Wb,IF
Aviva Systems Biology	ARP74898	QC54391-42557	AB_2936880	polyclonal	-	rabbit	0.500	Wb
Cell Signaling Technology	2648	1	AB_2214632	polyclonal	-	rabbit	0.009	Wb
Cell Signaling Technology	2649**	2	AB_2214629	recombinant-mono	7F3	rabbit	0.034	Wb
GeneTex	GTX101089	41801	AB_1952544	polyclonal	-	rabbit	1.000	Wb,IP,IF
GeneTex	GTX113030	43187	AB_1952542	polyclonal	-	rabbit	0.300	Wb,IP,IF
GeneTex	GTX113099	40457	AB_10731852	polyclonal	-	rabbit	1.000	Wb,IF
Proteintech	10736-1-AP	83755	AB_2214635	polyclonal	-	rabbit	0.267	Wb,IP,IF
Proteintech	60316-1-Ig*	10001981	AB_2881427	monoclonal	2A4B10	mouse	0.667	Wb,IF
Structural Genomics Consortium	Z-VCP-10**,A	YSVCPA-c001	n/a	recombinant-mono	Z-VCP-10	human	1.234	IP
Thermo Fisher Scientific	MA3-004*	VG292219	AB_2214638	monoclonal	5	mouse	1.000	Wb,IP,IF
Thermo Fisher Scientific	MA5-32612**	VL3152612	AB_2809889	recombinant-mono	JM11-15	rabbit	1.000	Wb,IF

Wb=Western Blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody,
A= Z-VCP-10 was a gift from Susanne Gräslund (Addgene plasmid # 166559 ; http://n2t.net/addgene:166559 ; RRID:Addgene_166559),

Materials and methods

Antibodies

All the Valosin-containing protein 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). Peroxidase-conjugated monoclonal anti-Flag M2 used as a secondary for Z-VCP-10 is from MilliporeSigma (cat. number A8592). Alexa-555-conjugated goat anti-rabbit and anti-mouse secondary antibodies are from Thermo Fisher Scientific (cat. number A21429 and A21424). Cy3-conjugated monoclonal anti-Flag M2 is from MilliporeSigma (cat. number A9594).

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).

Knockdown strategy

U2OS were treated with VCP smart pool siRNA (Horizon Discovery, cat # L-008727-00-0005) for three days. Lipofectamine RNAiMAX (Thermo, cat# 13778030) was used to transfect the siRNA following the manufacturer's protocol.

Antibody screening by Western Blot

Western Blots were performed as described in our standard operating procedure [3]. U2OS WT and *VCP* KD (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 78429). 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 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 [4]. 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) or 30uL anti-FLAG M2 magnetic beads from MilliporeSigma (cat. number M8823). Tubes were rocked for ~1 hr at 4°C followed by two 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 were rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. 0.5 ml aliquots at 1.0 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 [5]. U2OS WT and *VCP* KD 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 KD 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 Valosin-containing protein 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 widefield 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.

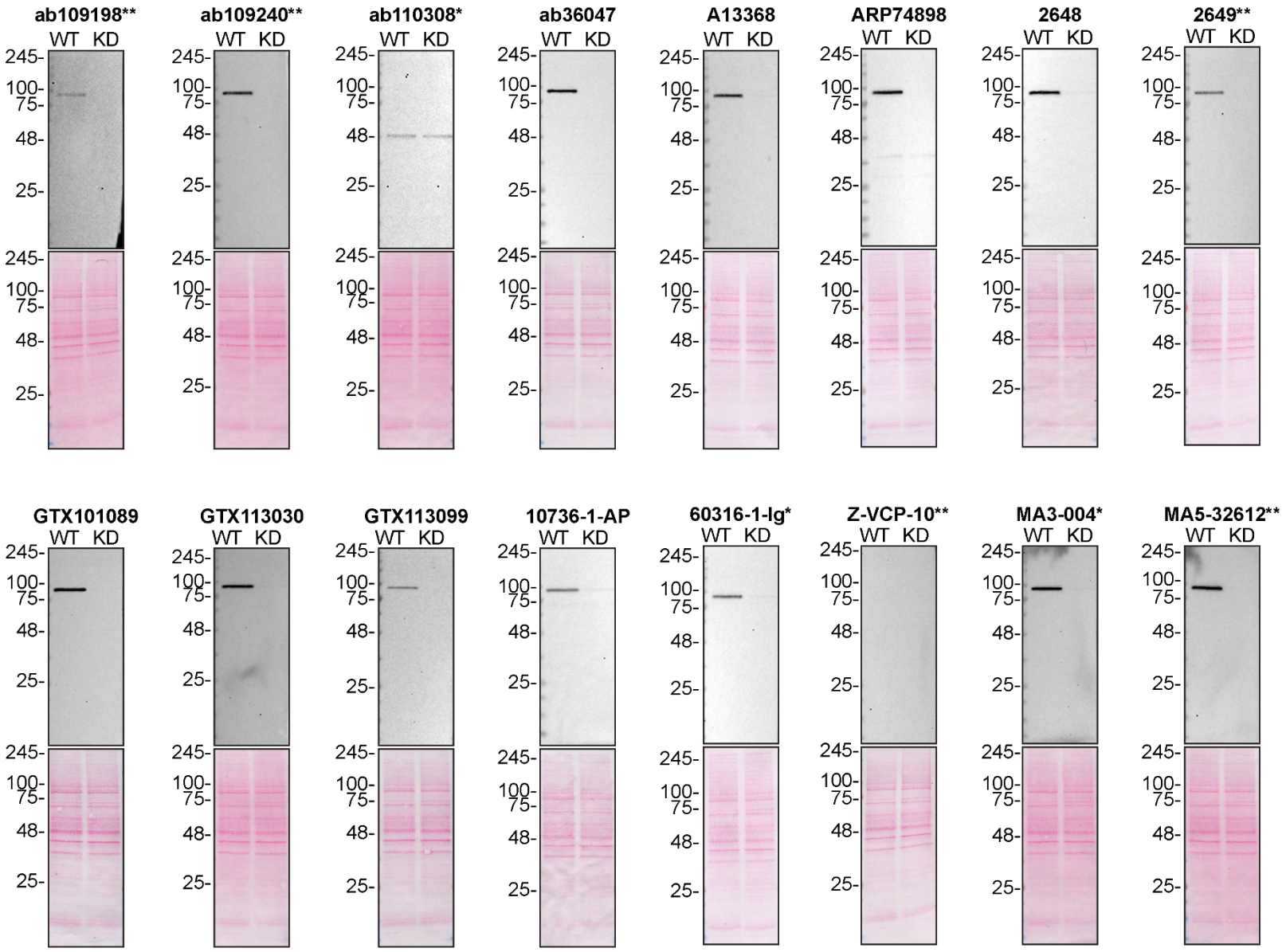

Figure 1: Valosin-containing protein antibody screening by Western Blot

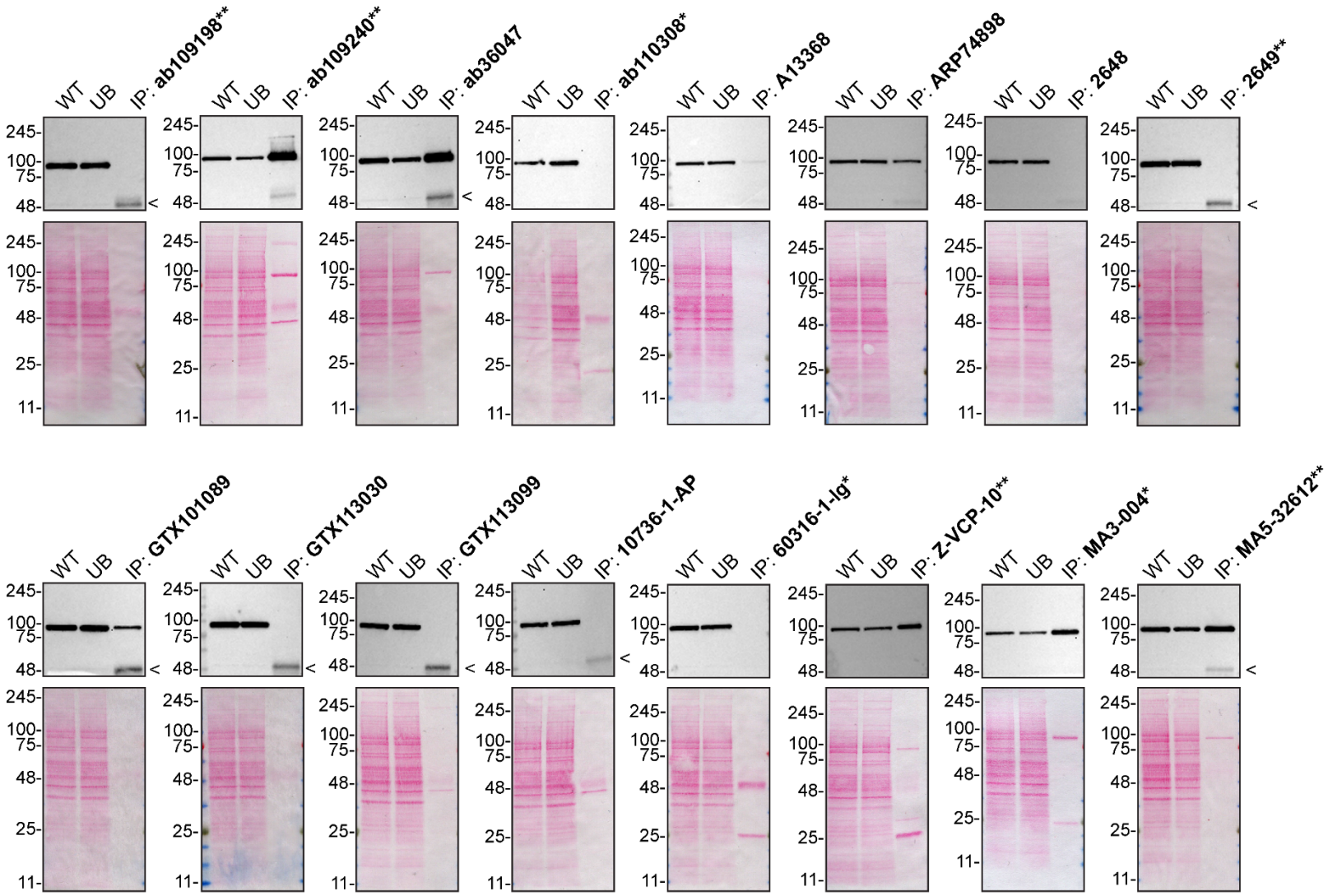

Figure 2: Valosin-containing protein antibody screening by Immunoprecipitation

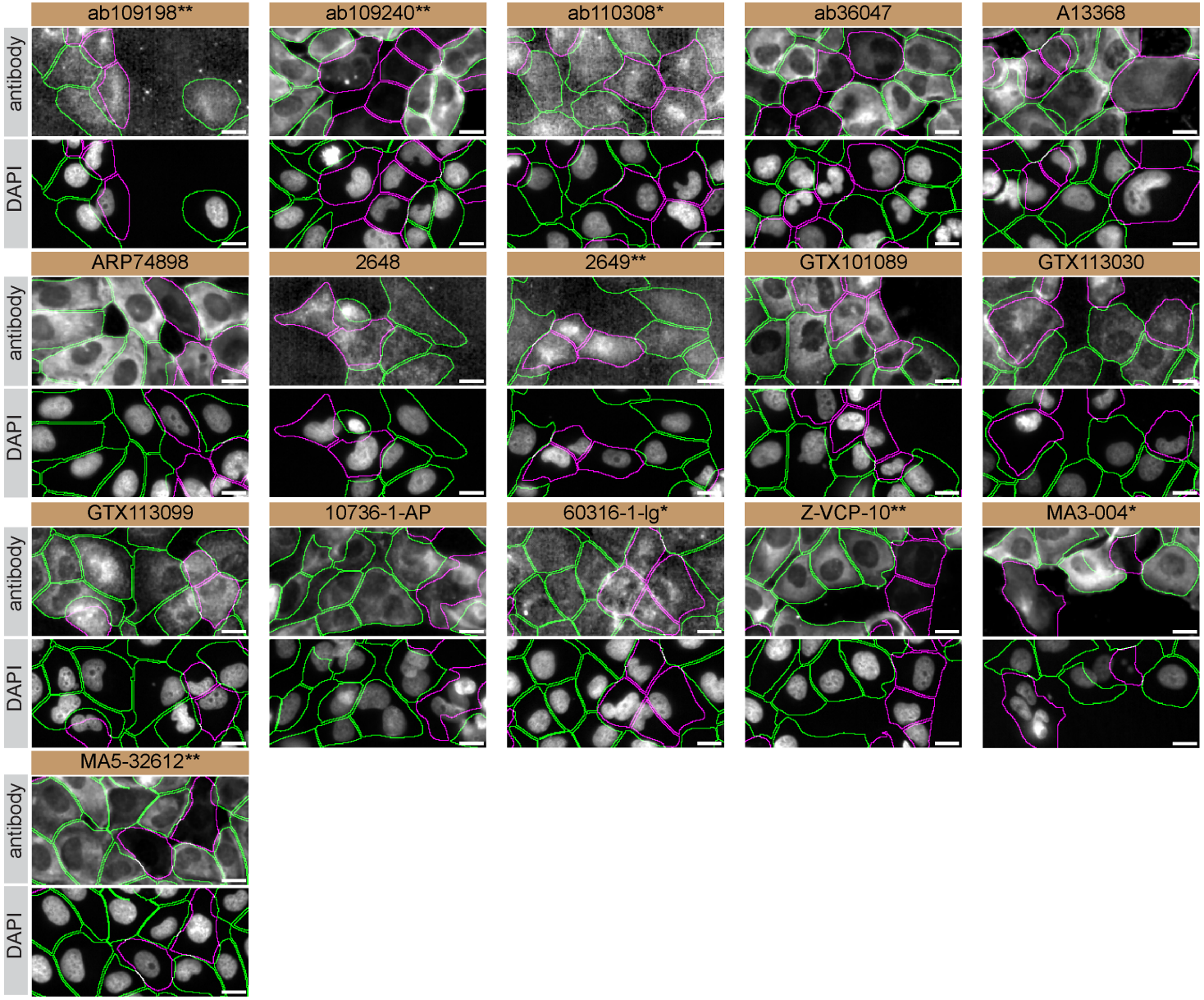

Figure 3: Valosin-containing protein antibody screening by immunofluorescence

Figure 1: Valosin-containing protein antibody screening by Western Blot.

Lysates of U2OS WT and *VCP* KD were prepared, and 10 µg of protein were processed for Western Blot with the indicated Valosin-containing protein antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab109198** at 1/10,000; ab109240** at 1/50,000; ab110308* at 1/10,000; ab36047 at 1/10,000; A13368 at 1/10,000; ARP74898 at 1/10,000; 2648 at 1/10,000; 2649** at 1/10,000; GTX101089 at 1/50,000; GTX113030 at 1/50,000; GTX113099 at 1/50,000; 10736-1-AP at 1/15,000; 60316-1-Ig* at 1/10,000; Z-VCP-10** at 1/1,000; MA3-004* at 1/30,000; MA5-32612** at 1/30,000. Predicted band size: 89 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Valosin-containing protein antibody screening by immunoprecipitation.

U2OS lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Valosin-containing protein antibodies pre-coupled to Dynabeads protein G or protein A or Flag-M2 magnetic beads. Samples were washed and processed for Western Blot with the indicated Valosin-containing protein antibody. For Western Blot, MA3-004* was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=4% starting material, UB=4% unbound fraction, IP=immunoprecipitate, <=points toward the antibody heavy chain, *=monoclonal antibody, **=recombinant antibody.

Figure 3: Valosin-containing protein antibody screening by immunofluorescence.

U2OS WT and *VCP* KD cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KD 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 Valosin-containing protein 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 KD cells) channels was performed. Representative images of the blue and red (grayscale) channels are shown. WT and KD cells are outlined with green and magenta dashed line, respectively. Antibody dilution used: ab109198** at 1/800; ab109240** at 1/500; ab110308* at 1/1000; ab36047 at 1/100; A13368 at 1/100; ARP74898 at 1/100; 2648 at 1/100; 2649** at 1/100; GTX101089 at 1/500; GTX113030 at 1/500; GTX113099 at 1/1000; 10736-1-AP at 1/1000; 60316-1-Ig* at 1/50; Z-VCP-10** at 1/100; MA3-004* at 1/1,000; MA5-32612** at 1/1,000. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

References

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