Antibody Characterization Report for Amyloid-beta precursor protein

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Amyloid-beta precursor protein

Alternative protein name: Alzheimer disease amyloid A4 protein homolog, Alzheimer disease amyloid protein, Amyloid precursor protein

Gene name: *APP*

Uniprot: P05067

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 Amyloid-beta precursor protein. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Amyloid-beta precursor protein by immunoblot (Western Blot), immunoprecipitation and immunofluorescence. An HAP1 *APP* KO line is available at Horizon discovery and was used in this study. Expression of Amyloid-beta precursor protein in HAP1 is adequate as determined by searching DepMap [3].

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 Amyloid-beta precursor protein antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab126732**	GR34478901	AB_11131727	recombinant-mono	EPR5118-34	rabbit	0.114	Wb
Abcam	ab252814**	GR34013051	AB_2925224	recombinant-mono	14D6	rat	1.154	Wb
Abcam	ab252816**	GR33342983	AB_2925223	recombinant-mono	2E9	rat	0.583	Wb,IP
Aviva Systems Biology	ARP33075	QC3561-90401	AB_2044934	polyclonal	-	rabbit	0.5	Wb
Aviva Systems Biology	ARP34012	QC47046-42571	AB_2044933	polyclonal	-	rabbit	0.5	Wb
Cell Signaling Technology	29765**	1	AB_2925221	recombinant-mono	E8B3O	rabbit	n/a	Wb,IP
Cell Signaling Technology	76600**	1	AB_2925222	recombinant-mono	E4H1U	rabbit	n/a	Wb,IP
Proteintech	25524-1-AP	51555	AB_2880118	polyclonal	-	rabbit	0.55	Wb,IF
Thermo Fisher Scientific	13-0200*	XC339604	AB_2532993	monoclonal	LN27	mouse	0.5	Wb,IP,IF
Thermo Fisher Scientific	14-9749-80*	2484403	AB_2572977	monoclonal	22C11	mouse	0.5	Wb,IF
Thermo Fisher Scientific	MA5-35187**	XH3670292	AB_2849091	recombinant-mono	ARC0465	rabbit	0.93	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
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC005368c010	CVCL_SD04	HAP1	*APP* KO

Materials and methods

Antibodies

All tested Amyloid-beta precursor protein antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse, anti-rabbit and anti-rat antibodies are from Thermo Fisher Scientific (cat. number 62-6520, 65-6120 and 31470, respectively). 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).

Antibody screening by Western Blot

Western Blot were performed as described in our standard operating procedure [4]. HAP1 WT and *APP* KO (listed in Table 2) were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) 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 Blot was 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 are 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 [5]. Antibody-bead conjugates were prepared by adding 2 µg or 20 µl of antibody at an unknown concentration 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 and rat antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Tubes were rocked for ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

HAP1 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. 0.5 ml aliquots at 2.0 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs 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.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [6]. HAP1 WT and APP KO were labelled with a green and a deep red fluorescence dye, respectively. The fluorescent dyes used are from ThermoFisher 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 Amyloid-beta precursor 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/0.95 NA water objective lens and scientific CMOS camera (16-bit, 1.97mm field of view), equipped with 395, 475, 555 and 635 nm solid state LED lights (Lumencor Aura III light engine) and bandpass emission filters (432/36 nm, 520/35 nm, 600/37 nm and 692/40 nm) to excite and capture fluorescence emission for DAPI, CellTrackerTM Green, Alexa fluor 555 and CellTrackerTM Red, respectively. Images had pixel sizes of 0.68 x 0.68 microns. Exposure time was set with maximal (relevant) pixel intensity ~80% of dynamic range and verified on multiple wells before acquisition. Since the IF staining varied depending on the primary antibody used, the exposure time was set using the most intensely stained well as reference. Frequently, the focal plane varied slightly within a single field of view. To remedy this issue, a stack of three images per channel was acquired at a z-interval of 4 microns per field and best focus projections were generated during the acquisition (MetaExpress v6.7.1, Molecular Devices). Segmentation was carried out on the projections of CellTrackerTM channels using CellPose v1.0 on green (WT) and far-red (KO) channels, using as parameters the 'cyto' model to detect whole cells, and using an estimated diameter tested for each cell type, between 15 and 20 microns (26). Masks were used to generate cell outlines for intensity quantification. Figures were assembled with Adobe Photoshop (version 24.1.2) to adjust contrast then assembled with Adobe Illustrator (version 27.3.1).

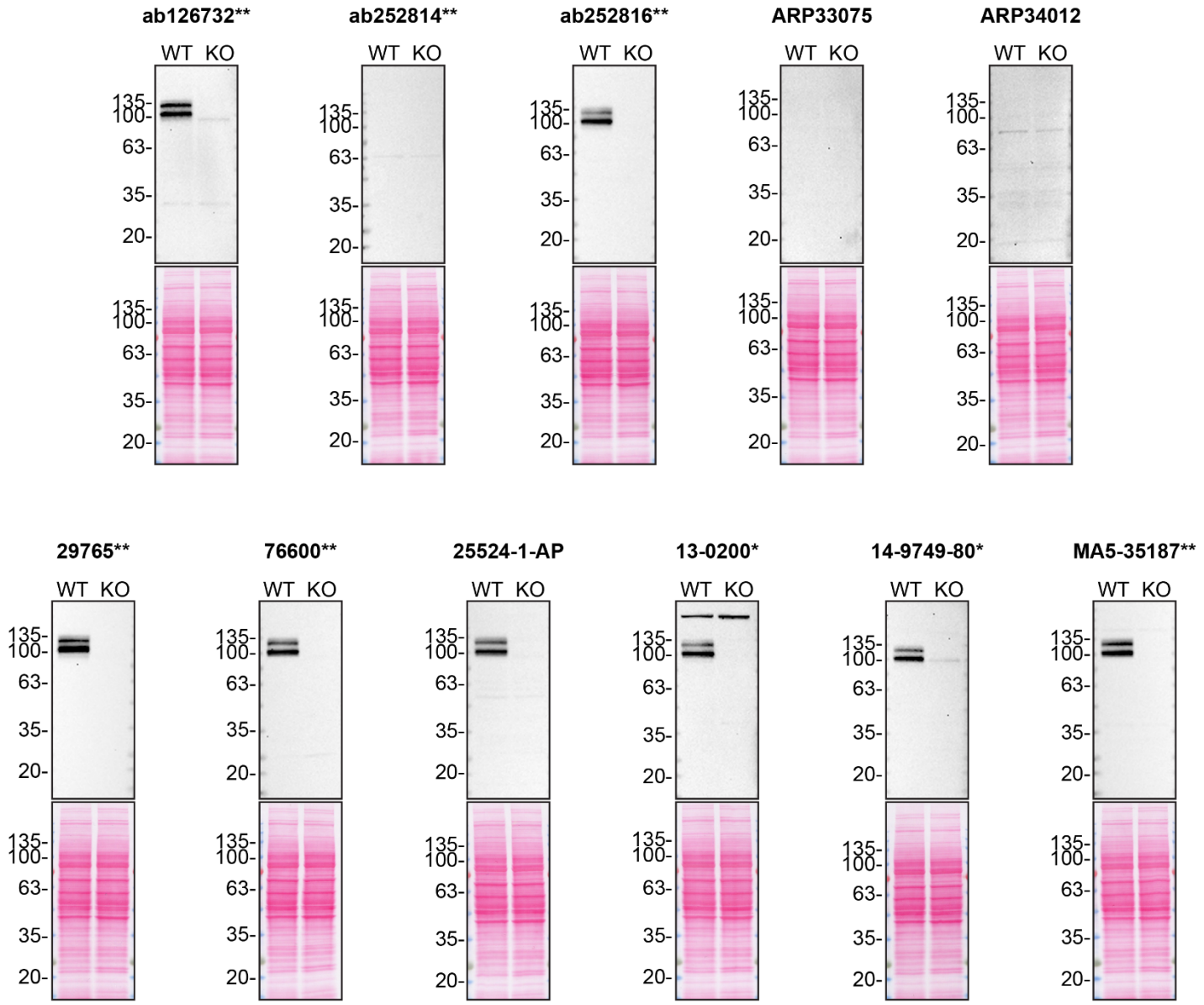

Figure 1: Amyloid-beta precursor protein antibody screening by Western Blot

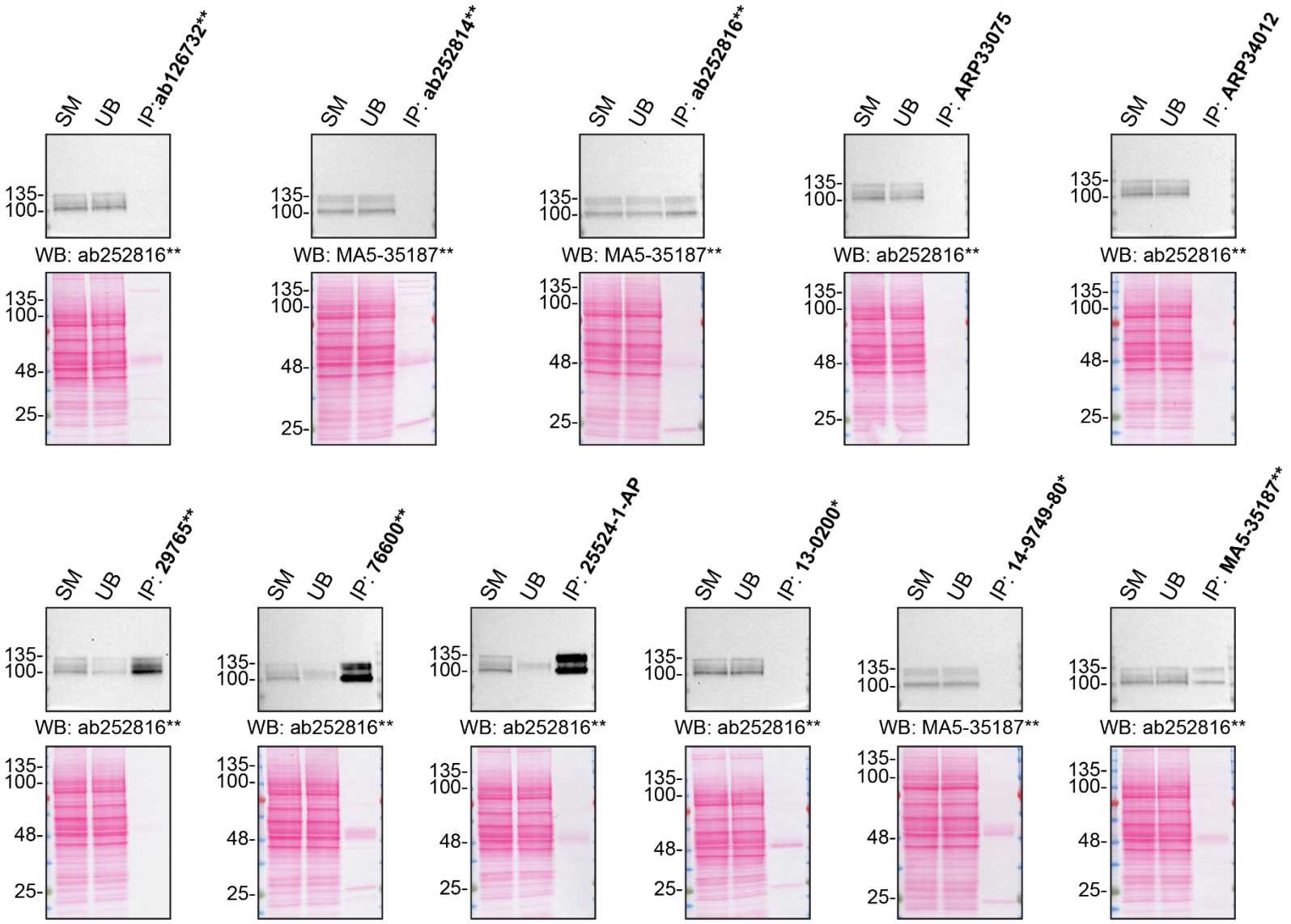

Figure 2: Amyloid-beta precursor protein antibody screening by immunoprecipitation

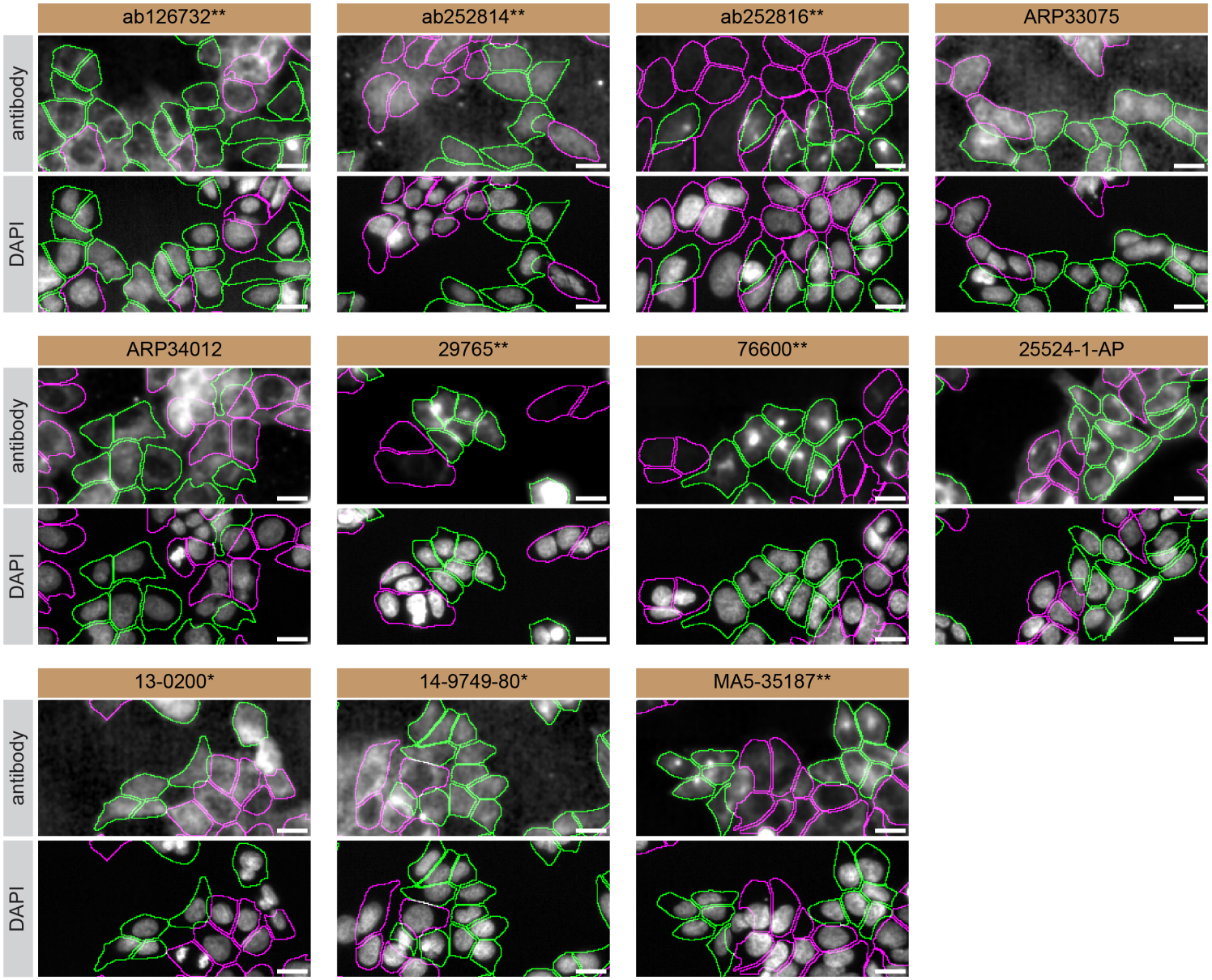

Figure 3: Amyloid-beta precursor protein antibody screening by immunofluorescence

Figure 1: Amyloid-beta precursor protein antibody screening by Western Blot.

Lysates of HAP1 WT and *APP* KO were prepared, and 50 µg of protein were processed for Western Blot with the indicated Amyloid-beta precursor protein antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab126732** at 1/500, ab252814** at 1/500, ab252816** at 1/500, ARP33075 at 1/500, ARP34012 at 1/500, 29765** at 1/500, 76600** at 1/500, 25524-1-AP at 1/500, 13-0200* at 1/200, 14-9749-80* at 1/500, MA5-35187** at 1/500. Predicted band size: 87 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Amyloid-beta precursor protein antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Amyloid-beta precursor protein antibodies pre-coupled to Dynabeads protein A or protein G. Samples were washed and processed for Western Blot with the indicated Amyloid-beta precursor protein antibody. For Western Blot, MA5-35187** and ab252816** were used at 1/500. The Ponceau stained transfers of each blot are shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate; *=monoclonal antibody, **=recombinant antibody

Figure 3: Protein antibody screening by immunofluorescence.

HAP1 WT and Gene 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 Amyloid-beta precursor 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 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: ab126732** at 1/500, ab252814** at 1/500, ab252816** at 1/500, ARP33075 at 1/500, ARP34012 at 1/500, 29765** at 1/100, 76600** at 1/50, 25524-1-AP at 1/500, 13-0200* at 1/500, 14-9749-80* at 1/500, MA5-35187** at 1/1000. *=monoclonal antibody, **=recombinant antibody, Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

References

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