Antibody Characterization Report for TGM2 (Protein-glutamine gamma-glutamyltransferase 2)

YCharOS Antibody Characterization Report

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Target:

Protein name used in this report: TGM2

Recommended protein name: Protein-glutamine gamma-glutamyltransferase 2

Alternative protein name: Erythrocyte transglutaminase, Protein G alpha(h), Isopeptidase TGM2, Protein-glutamine deamidase TGM2

Gene name: *TGM2*

Uniprot: P21980

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 sixteen TGM2 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. 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. An A549 *TGM2* KO line is available at Abcam and was used in this study. Expression of TGM2 protein in A549 is adequate as determined through DepMap [3, 4].

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
Abcam	ab275463	CVCL_0023	A549	WT
Abcam	ab261876	CVCL_B1I0	A549	*TGM2* KO

Table 2: Summary of the TGM2 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab109121**	1058833-3	AB_10861115	Recombinant-mono	EPR2956	rabbit	2.79	Wb
Abcam	ab109200**	1044092-1	AB_10860177	Recombinant-mono	EP2957	rabbit	0.30	Wb
Abcam	ab2386*	1051063-6	AB_2287299	Monoclonal	CUB 7402	mouse	n/a	Wb,IF
Abcam	ab310333**	1056093-5	AB_3076417	Recombinant-mono	EPR28142-86	rabbit	0.50	Wb,IF
Abcam	ab421	1034725-7	AB_304372	Polyclonal	-	rabbit	n/a	Wb,IF
ABCD	ABCD_AI748**	10/27/2023	AB_3076341	Recombinant-mono	679-14-E06	rabbit	0.12	Others
Abclonal	A21184**	3522042510	AB_3083448	Recombinant-mono	ARC52843	rabbit	1.30	Wb,IF
Aviva Systems Biology	ARP47471	QC18320-43546	AB_1107120	Polyclonal	-	rabbit	0.50	Wb
Aviva Systems Biology	ARP47472	QC16720	AB_1088480	Polyclonal	-	rabbit	0.50	Wb
Bio-Techne	AF4376	CFGU0119031	AB_10890213	Polyclonal	-	sheep	0.20	Wb
Bio-Techne	MAB4376*	CFNO0119031	AB_10971763	Monoclonal	716620	mouse	0.20	Wb
Cell Signaling Technology	3557**	3	AB_2202883	Recombinant-mono	D11A6	rabbit	0.02	Wb
GeneTex	GTX111702	44524	AB_1952227	Polyclonal	-	rabbit	1.05	Wb,IF
Proteintech	15100-1-AP	00081307	AB_2202885	Polyclonal	-	rabbit	0.60	Wb,IP,IF
Proteintech	68006-1-Ig*	10023724	AB_2918753	Monoclonal	2D4C11	mouse	1.00	Wb,IF
Thermo Fisher Scientific	MA5-32819**	YJ4089240	AB_2810095	Recombinant-mono	JU30-02	rabbit	1.00	Wb
Thermo Fisher Scientific	MA5-12739*	ZA4176225	AB_10985077	Monoclonal	CUB 7402	mouse	0.20	Wb,IP,IF

Wb=western blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody, n/a=not available

Materials and methods

Antibodies

All the TGM2 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 donkey anti-sheep is from Thermo Fisher Scientific (cat. number A16041). Alexa-555-conjugated goat anti-rabbit and anti-mouse secondary antibodies are from Thermo Fisher Scientific (cat. number A-21429 and A-21424). Alexa-555-conjugated donkey anti-sheep secondary antibody is from Thermo Fisher Scientific (cat. number A-21436).

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-glutamine (Wisent cat. number 609-065, 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 western blot on lysate

Western blots were performed as described in our standard operating procedure [5]. A549 WT and *TGM2* KO (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 P8340). 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.

Antibody screening by western blot on culture medium

A549 WT and *TGM2* KO (listed in Table 1) were washed 3x with PBS 1x 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). Culture media were supplemented with 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number P8340).

For both lysate and medium, 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 iBright™ CL1500 Imaging System from Thermo Fisher Scientific (cat. number A44240).

Antibody screening by immunoprecipitation on lysate

Immunoprecipitation was performed as described in our standard operating procedure [6]. Antibody-beads conjugates were prepared by adding 2 µg of antibody (except for 10 µl of antibodies ab2386** and ab421, and 20 µl of antibody 3557**) to 500 µl of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with 30µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse and sheep antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Tubes were rocked for ~1 hr at 4°C followed by two washes to remove unbound antibodies.

A549 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 20,000xg for 1 h at 4°C. 0.5 ml aliquots at 2.0 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. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a concentration of 2.0 µg/ml.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [7]. A549 WT and *TGM2* 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 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 TGM2 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 confocal 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.
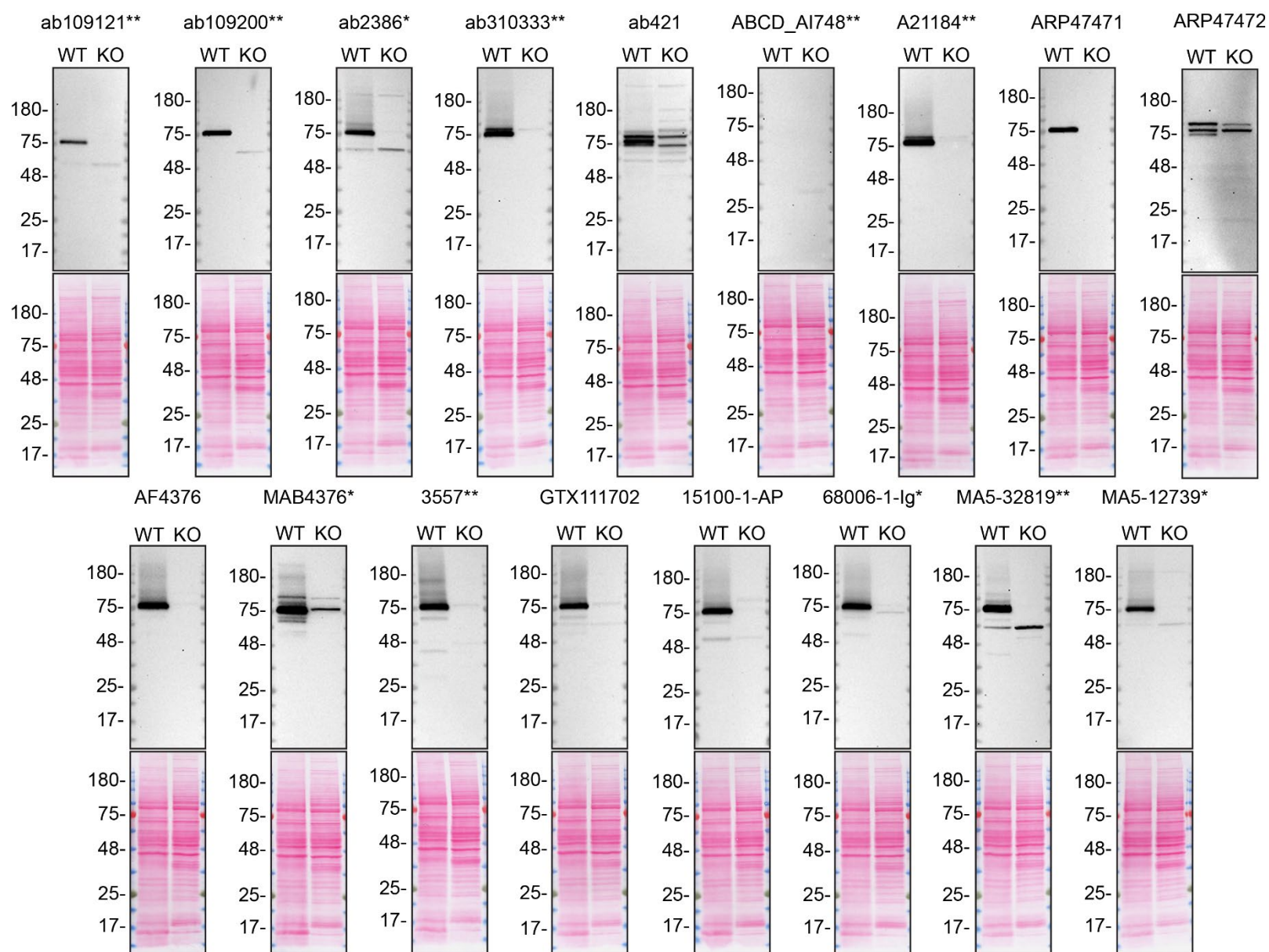
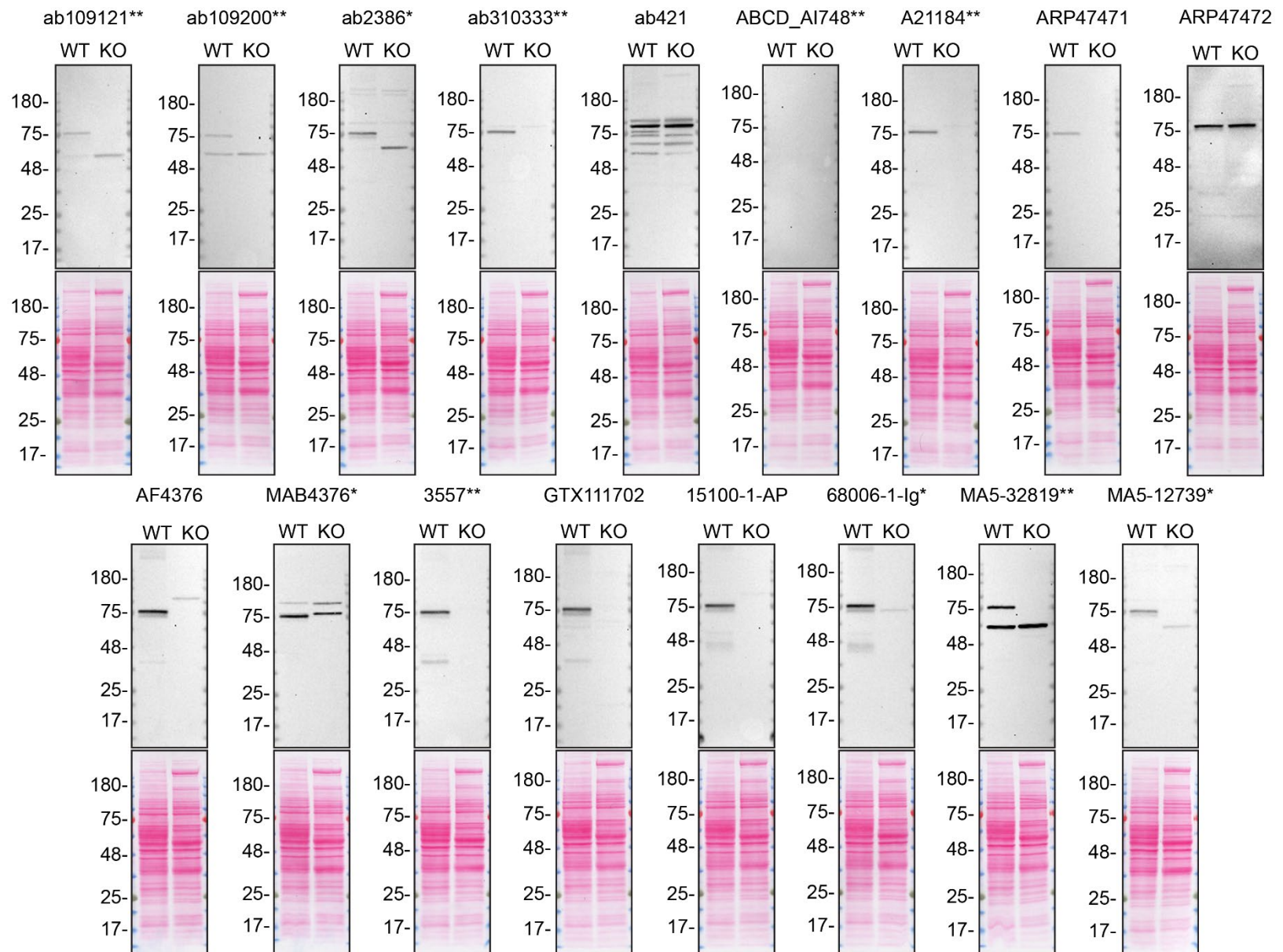**Figure 1: TGM2 antibody screening by western blot on lysate**

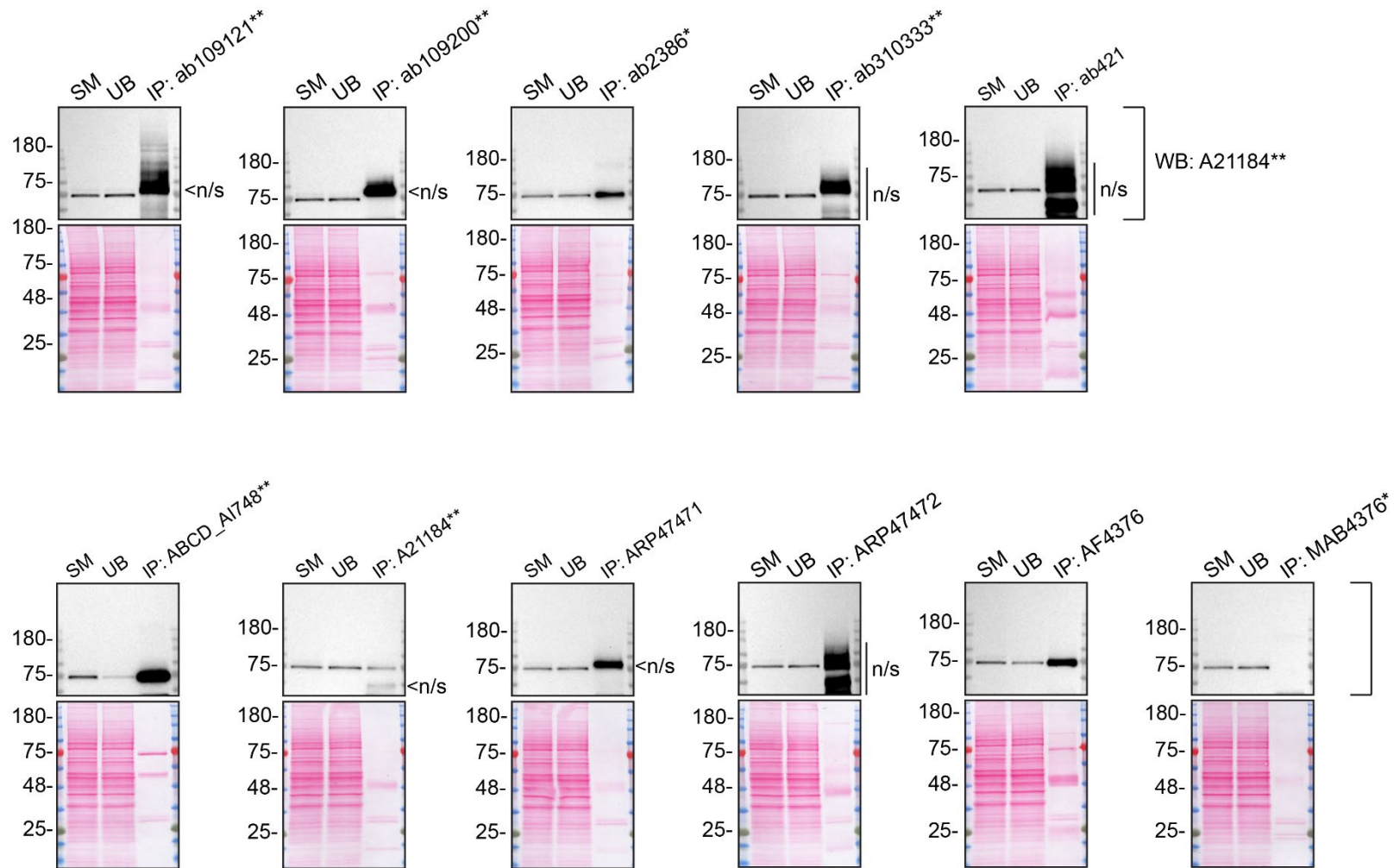Figure 2: TGM2 antibody screening by western blot on culture medium

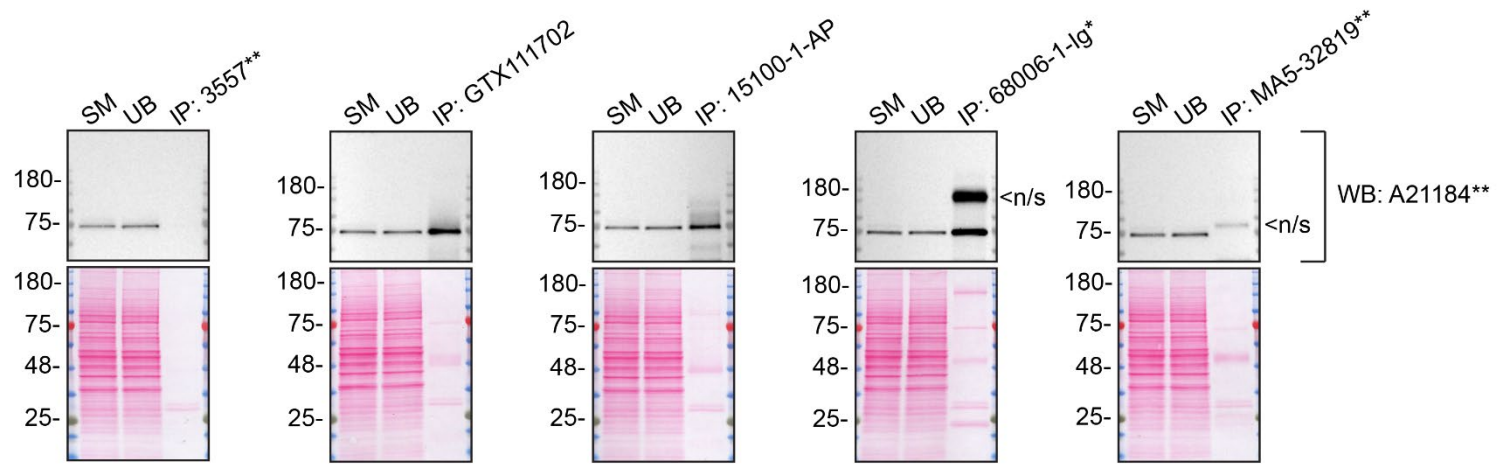**Figure 3: TGM2 antibody screening by immunoprecipitation on lysate (1/2)**

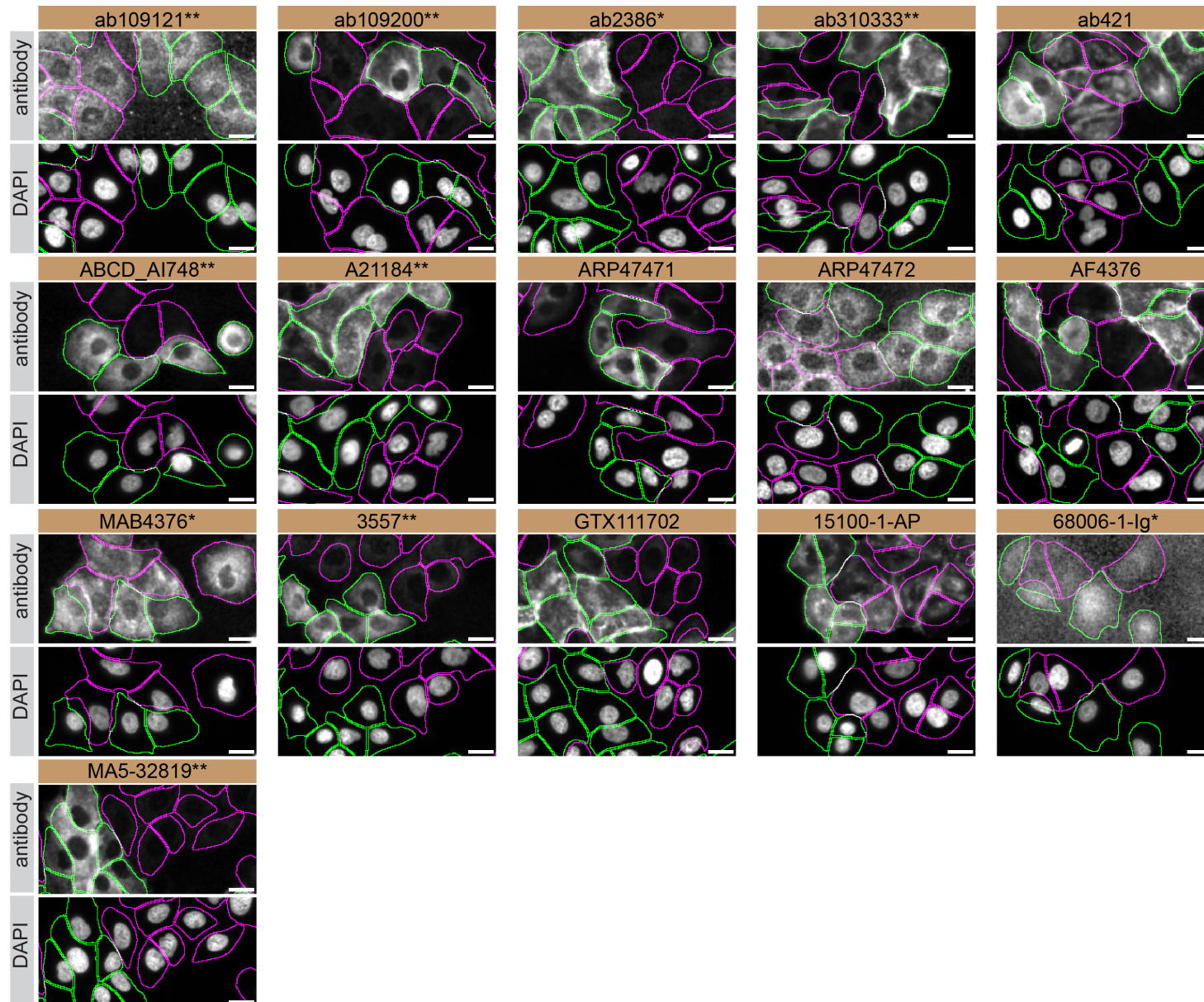**Figure 3: TGM2 antibody screening by immunoprecipitation on lysate (2/2)**

Figure 4: TGM2 antibody screening by immunofluorescence

Figure 1: TGM2 antibody screening by western blot on lysate.

Lysates of A549 WT and *TGM2* KO were prepared, and 40 µg of protein were processed for Western blot with the indicated TGM2 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab109121** at 1/1000, ab109200** at 1/10000, ab2386* at 1/500, ab310333** at 1/1000, ab421 at 1/500, ABCD_AI748** at 1/10, A21184** at 1/10000, ARP47471 at 1/500, ARP47472 at 1/500, AF4376 at 1/400, MAB4376* at 1/200, 3557** at 1/500, GTX111702 at 1/500, 15100-1-AP at 1/6000, 68006-1-Ig* at 1/10000, MA5-32819** at 1/500, MA5-12739* at 1/200. Predicted band size: 77 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: TGM2 antibody screening by western blot on culture medium.

A549 WT and *TGM2* KO were cultured in serum free media, and 40 µg of protein from concentrated culture media were processed for western blot with the indicated TGM2 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab109121** at 1/1000, ab109200** at 1/10000, ab2386* at 1/500, ab310333** at 1/1000, ab421 at 1/500, ABCD_AI748** at 1/10, A21184** at 1/10000, ARP47471 at 1/500, ARP47472 at 1/500, AF4376 at 1/400, MAB4376* at 1/200, 3557** at 1/500, GTX111702 at 1/500, 15100-1-AP at 1/6000, 68006-1-Ig* at 1/10000, MA5-32819** at 1/500, MA5-12739* at 1/200. Predicted band size: 77 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 3: TGM2 antibody screening by immunoprecipitation on lysate.

A549 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated TGM2 antibodies pre-coupled to Dynabeads protein A or protein. Samples were washed and processed for western blot with the indicated TGM2 antibody. For western blot, A21184** was used at 1/10000. The Ponceau stained transfers of each blot are shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate; n/s=non-specific signal. *=monoclonal antibody, **=recombinant antibody

Figure 4: TGM2 antibody screening by immunofluorescence.

A549 WT and *TGM2* 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 TGM2 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: ab109121** at 1/1000, ab109200** at 1/300, ab2386* at 1/1000, ab310333** at 1/500, ab421 at 1/1000, ABCD_AI748** at 1/1000, A21184** at 1/1000, ARP47471 at 1/500, ARP47472 at 1/250, AF4376 at 1/100, MAB4376* at 1/100, 3557** at 1/500, GTX111702 at 1/1000, 15100-1-AP at 1/300, 68006-1-Ig* at 1/500, MA5-32819** at 1/1000. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

References

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