Antibody Characterization Report for Retinoic acid receptor RXR-alpha

YCharOS Antibody Characterization Report

Author(s): Riham Ayoubi[1], Walaa Alshafie[1], Maryam Fotouhi[1], Peter S. McPherson[1*] and Carl Laflamme[1*]

[1] Tanenbaum Open Science Institute, Structural Genomics Consortium, Montreal Neurological Institute, McGill University, Montreal, Canada
* Corresponding authors: carl.laflamme@mcgill.ca, peter.mcpherson@mcgill.ca

Target:

Recommended protein name: Retinoic acid receptor RXR-alpha

Alternative protein name: Nuclear receptor subfamily 2 group B member 1

Gene name: RXRA

Uniprot: P19793

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 Retinoic acid receptor RXR-alpha. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Retinoic acid receptor RXR-alpha by immunoblot (Western blot), immunoprecipitation and immunofluorescence. An HCT116 *RXRA* KO line is available at Abcam and was used in this study. HCT116 is expected to express adequate Retinoic acid receptor RXR-alpha expression determined through DepMap [3-5].

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 Retinoic acid receptor RXR-alpha antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Cell Signaling Technology	3085**	4	AB_11140620	recombinant-mono	D6H10	rabbit	0.1	Wb,IP
Proteintech	21218-1-AP	94775	AB_10693633	polyclonal	-	rabbit	0.55	Wb,IP,IF
ABclonal	A19105**	4000000468	AB_2862598	recombinant-mono	ARC0468	rabbit	0.43	Wb,IP,IF
Bio-Techne	NBP2-75653**	HN0831	AB_2910612	recombinant-mono	JG99-38	rabbit	1	Wb,IP,IF
Abcam	ab125001**	GR3197430-9	AB_10975632	recombinant-mono	EPR7106	rabbit	0.12	Wb,IP,IF
Abcam	ab227292	GR3257159-9	AB_2893215	polyclonal	-	rabbit	1	IF
Thermo Fisher Scientific	433900*	WG3325111	AB_2532208	recombinant-mono	K8508	mouse	1	Wb,IF
Thermo Fisher Scientific	MA5-34806**	WJ3417749C	AB_2848714	recombinant-mono	JG99-38	rabbit	1	Wb,IF
Thermo Fisher Scientific	MA5-35265**	WJ3417793B	AB_2849167	recombinant-mono	ARC0468	rabbit	0.43	Wb,IF
DSHB	PCRP-RXRA-2A8*	5/5/2016	AB_2619055	monoclonal	PCRP-RXRA-2A8	mouse	0.05	IP

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
Abcam	ab255451	CVCL_0291	HCT116	WT
Abcam	ab273708	CVCL_B1E4	HCT116	*RXRA* KO

Figure 1: Retinoic acid receptor RXR-alpha antibody screening by immunoblot.

Lysates of HCT116 WT and *RXRA* KO were prepared, and 100 µg of protein were processed for immunoblot with the indicated Retinoic acid receptor RXR-alpha antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 3085** at 1/1000, 21218-1-AP at 1/500, A19105** at 1/500, NBP2-75653** at 1/500, ab125001** at 1/500, ab227292 at 1/500, 433900* at 1/500, MA5-34806** at 1/500, MA5-35265** at 1/500, PCRP-RXRA-2A8* at 1/160. Predicted band size: 50 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Retinoic acid receptor RXR-alpha antibody screening by immunoprecipitation.

HCT116 lysates were prepared, and immunoprecipitation was performed using 1.0 µg of the indicated Retinoic acid receptor RXR-alpha antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Retinoic acid receptor RXR-alpha antibody. For immunoblot, 3085** was used at 1/200, and NBP2-75653**, A19105**, ab125001**, 433900*, MA5-35265** were used at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody, **=recombinant antibody

Figure 3: Retinoic acid receptor RXR-alpha antibody screening by immunofluorescence.

HCT116 WT and *RXRA* 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 Retinoic acid receptor RXR-alpha 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 blue (blue color) and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: 3085** at 1/100, 21218-1-AP at 1/500, A19105** at 1/400, NBP2-75653** at 1/1000, ab125001** at 1/100, ab227292 at 1/1000, 433900* at 1/1000, MA5-34806** at 1/1000, MA5-35265** at 1/400, PCRP-RXRA-2A8* at 1/50. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

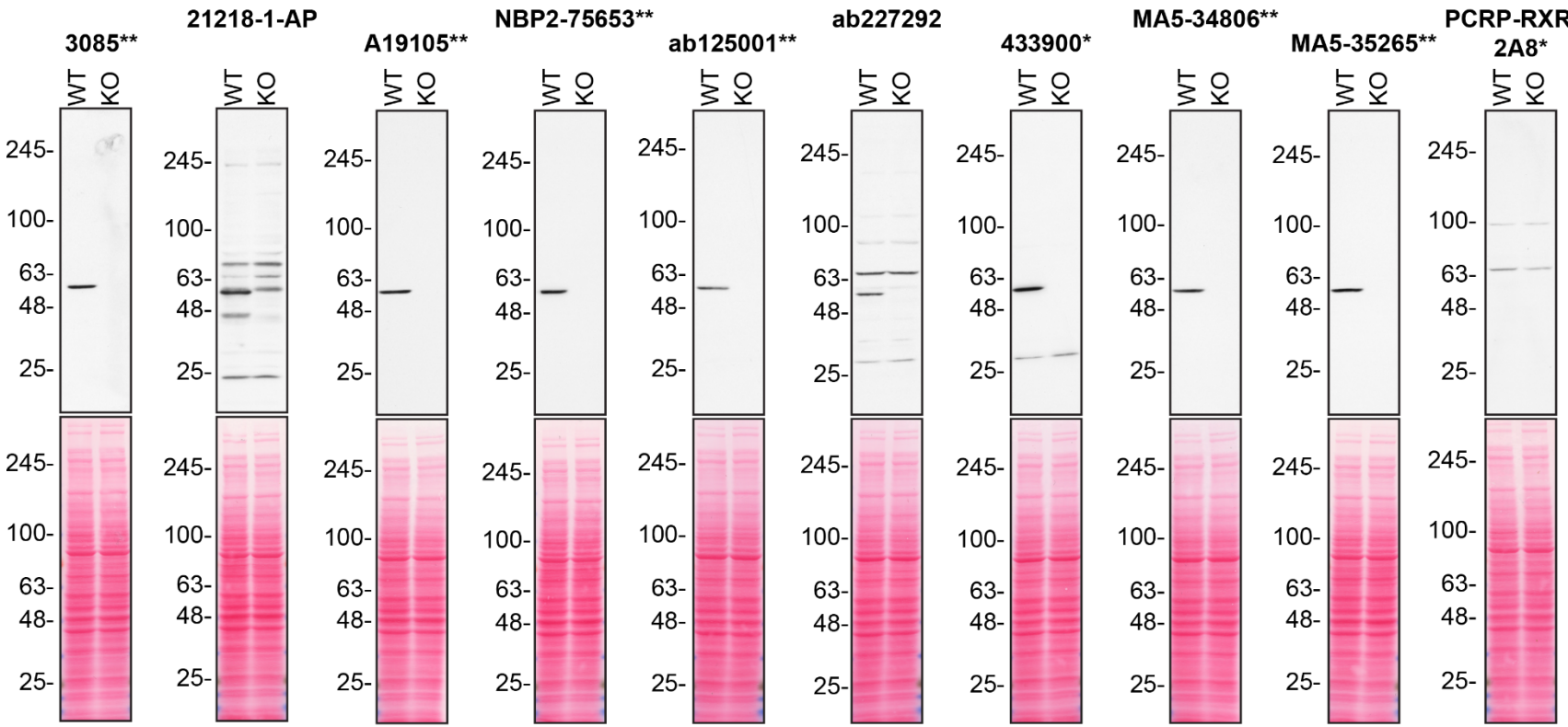

Figure 1: Retinoic acid receptor RXR-alpha antibody screening by immunoblot

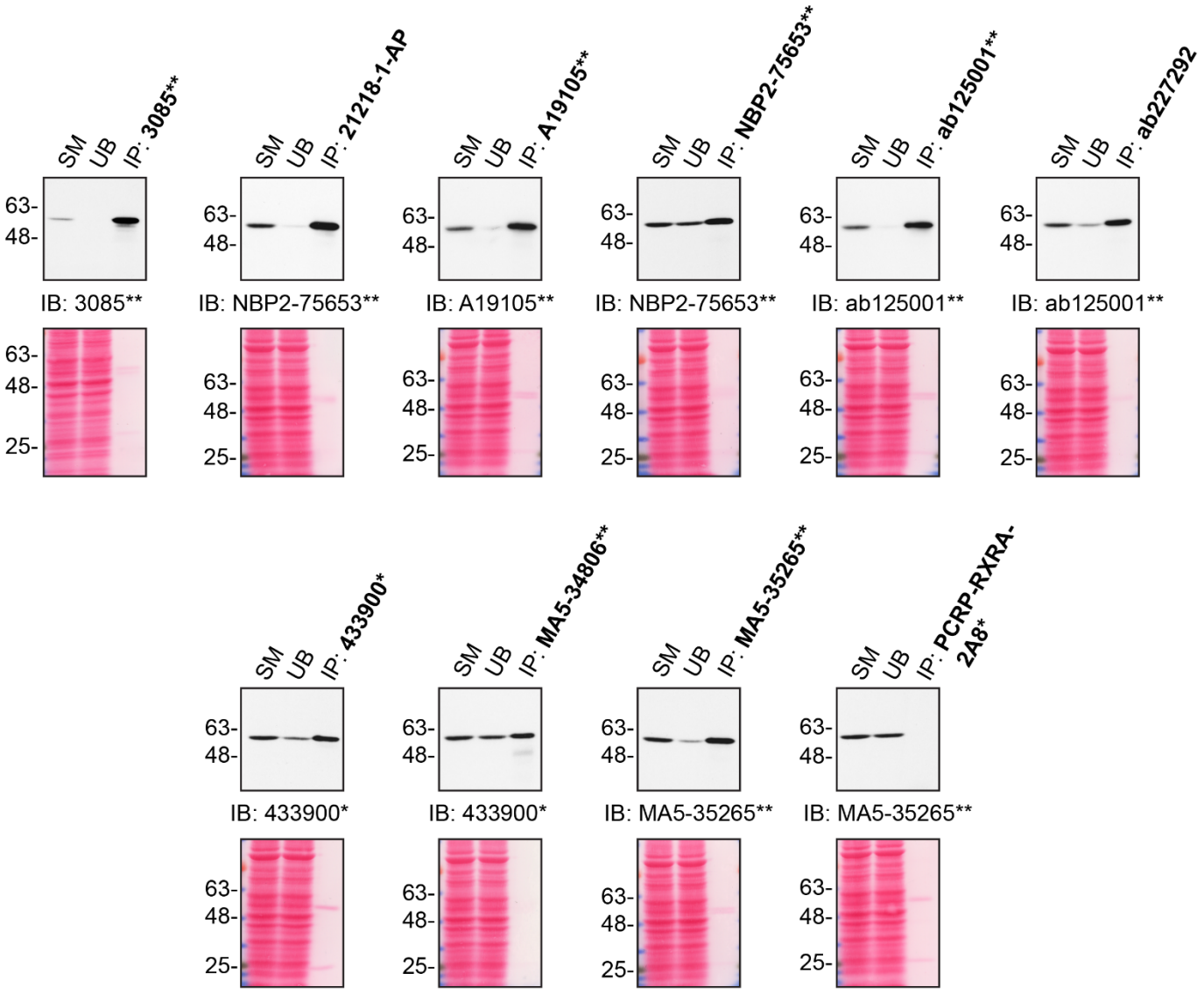

Figure 2: Retinoic acid receptor RXR-alpha antibody screening by immunoprecipitation

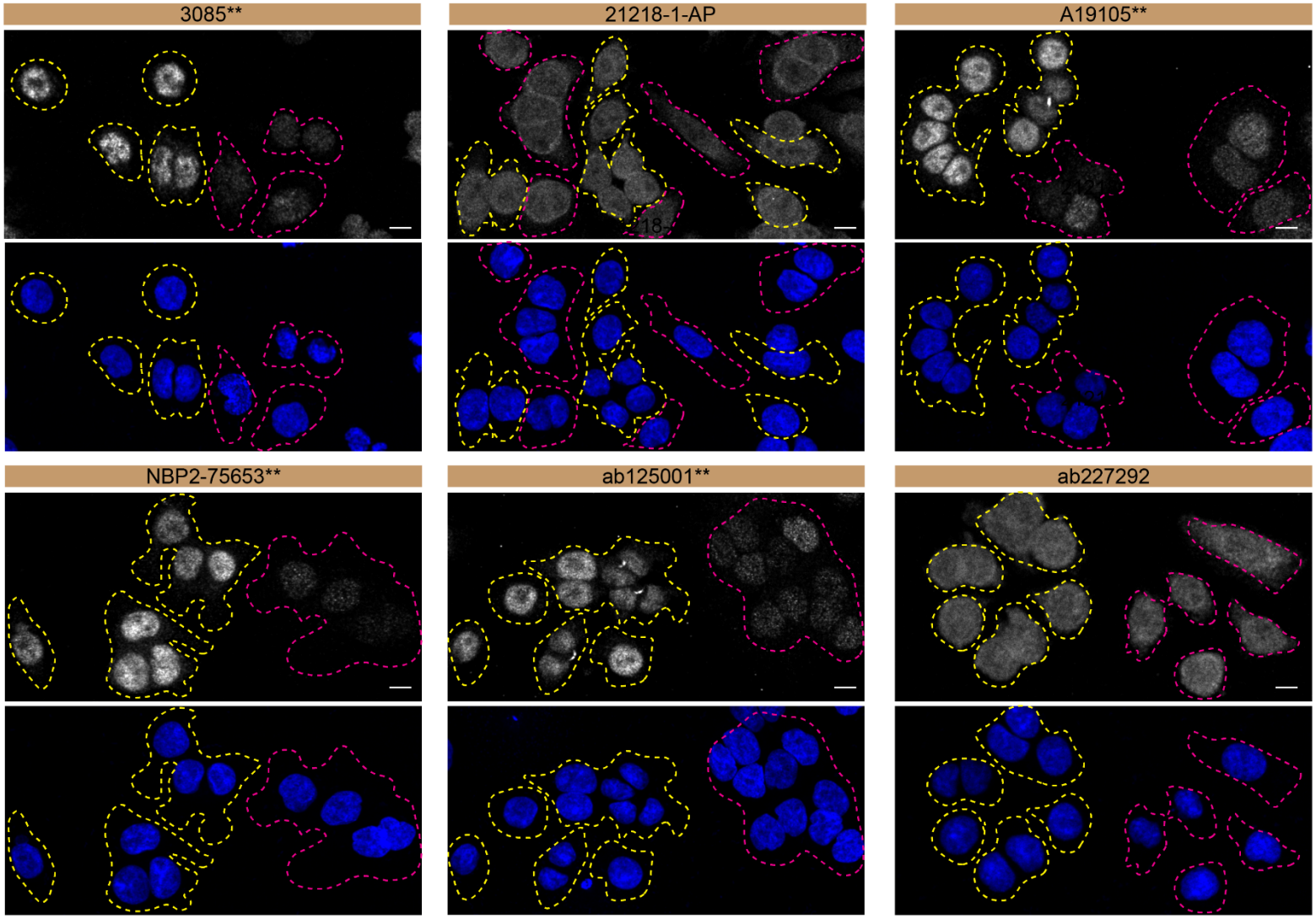

Figure 3 : Retinoic acid receptor RXR-alpha antibody screening by immunofluorescence (1/2)

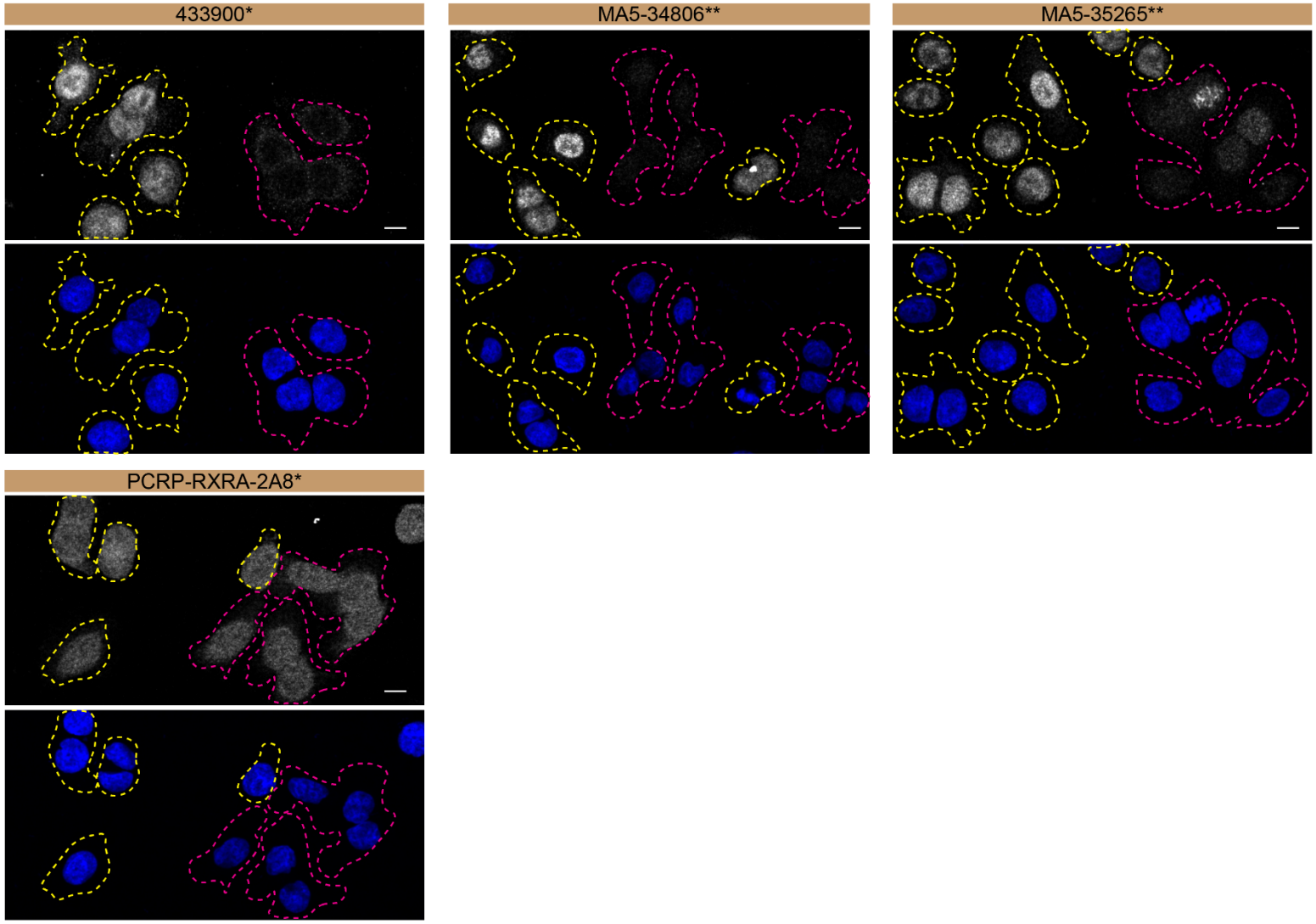

Figure 3 : Retinoic acid receptor RXR-alpha antibody screening by immunofluorescence (2/2)

Materials and methods

Antibodies

All Retinoic acid receptor RXR-alpha antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 62-6520 and 65-6120). 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 immunoblot

Immunoblots were performed as described in our standard operating procedure [6]. HCT116 (WT and *RXRA* 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 4-15% 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 [7]. Antibody-bead conjugates were prepared by adding 1 µg or 10 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.

HCT116 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 4-15% 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 [8]. HCT116 WT and *RXRA* 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 Retinoic acid receptor RXR-alpha 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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