



Antibody Characterization Report for Syntaxin-4

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Syntaxin-4

Alternative protein name: Renal carcinoma antigen NY-REN-31

Gene name: *STX4*

Uniprot: Q12846

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 Syntaxin-4. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Syntaxin-4 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HAP1 was selected based on evidence of appropriate Syntaxin-4 expression determined through DepMap [3, 4]. A HAP1 *STX4* KO line is available at Horizon discovery and was used in this study.

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 Syntaxin-4 antibodies used

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	MAB7894	CIHQ0115021	AB_10696820	monoclonal	884637	mouse	0.50	Wb, IF
Synaptic System	110 043	1-6	AB_10693907	polyclonal	-	rabbit	1.00	Wb, IP, IF
Thermo	MA5-38156	WI3377716	AB_2895069	recombinant-mono	ARC2113	rabbit	1.30	Wb, IF
Thermo	MA5-38349	WI3378343	AB_2895070	recombinant-mono	4E1	rabbit	1.30	Wb
Thermo	PA5-51560	WI3378413	AB_2648059	polyclonal	-	rabbit	0.30	Wb, IF
GeneTex	GTX114806	40233	AB_10624584	polyclonal	-	rabbit	0.70	Wb, IF
Aviva Sys Bio	ARP85503	QC62505-42956	AB_2895071	polyclonal	-	rabbit	0.50	Wb
Abcam	ab246856	GR3352220-5	AB_2895072	polyclonal	-	rabbit	0.30	Wb, IF
Cell Signaling Technology	67657	2	AB_2893256	recombinant-mono	E6W7B	rabbit	not provided	Wb, IP, IF

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence

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	HZGHC002617c009	CVCL_TR14	HAP1	STX4 KO

Figure 1: Syntaxin-4 antibody screening by immunoblot.

Lysates of HAP1 (WT and *STX4* KO) were prepared and 60 µg of protein were processed for immunoblot with the indicated Syntaxin-4 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MAB7894 at 1/2000; 110 043, MA5-38156, MA5-38349, PA5-51560, GTX114806, ARP85503, ab246856 and 67657 at 1/1000. Predicted band size: 34 kDa.

Figure 2: Syntaxin-4 antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Syntaxin-4 antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Syntaxin-4 antibody. For immunoblot, MAB7894 was used at 1/200, MA5-38156 and PA5-51560 at 1/1000, ARP85503 at 1/200, and ab246856 at 1/1000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: Syntaxin-4 antibody screening by immunofluorescence.

A) HAP1 WT and *STX4* 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 and fixed using paraformaldehyde (PFA). Cells were stained with the indicated Syntaxin-4 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 merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: MAB7894 at 1/100; 110 043 at 1/1000; MA5-38156 at 1/1300; MA5-38349 at 1/1300; PA5-51560 at 1/300; GTX114806 at 1/700; ARP85503 at 1/500; ab246856 at 1/300; 67657 at 1/100. Bars = 10 µm.

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. A minimum of 20 WT and 20 KO cells from 3 different fields of view were analysed for each antibody.

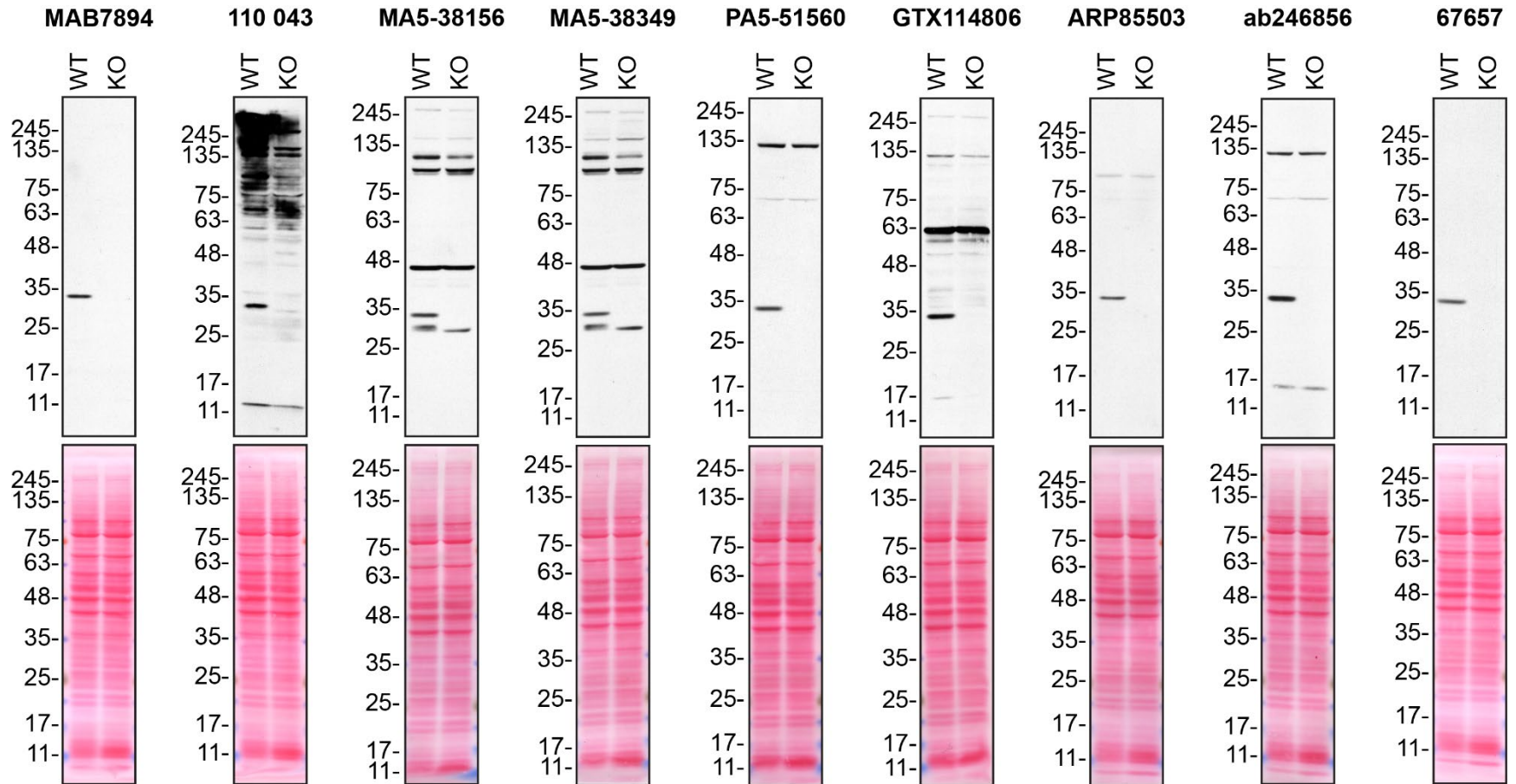


Figure 1: Syntaxin-4 antibody screening by immunoblot

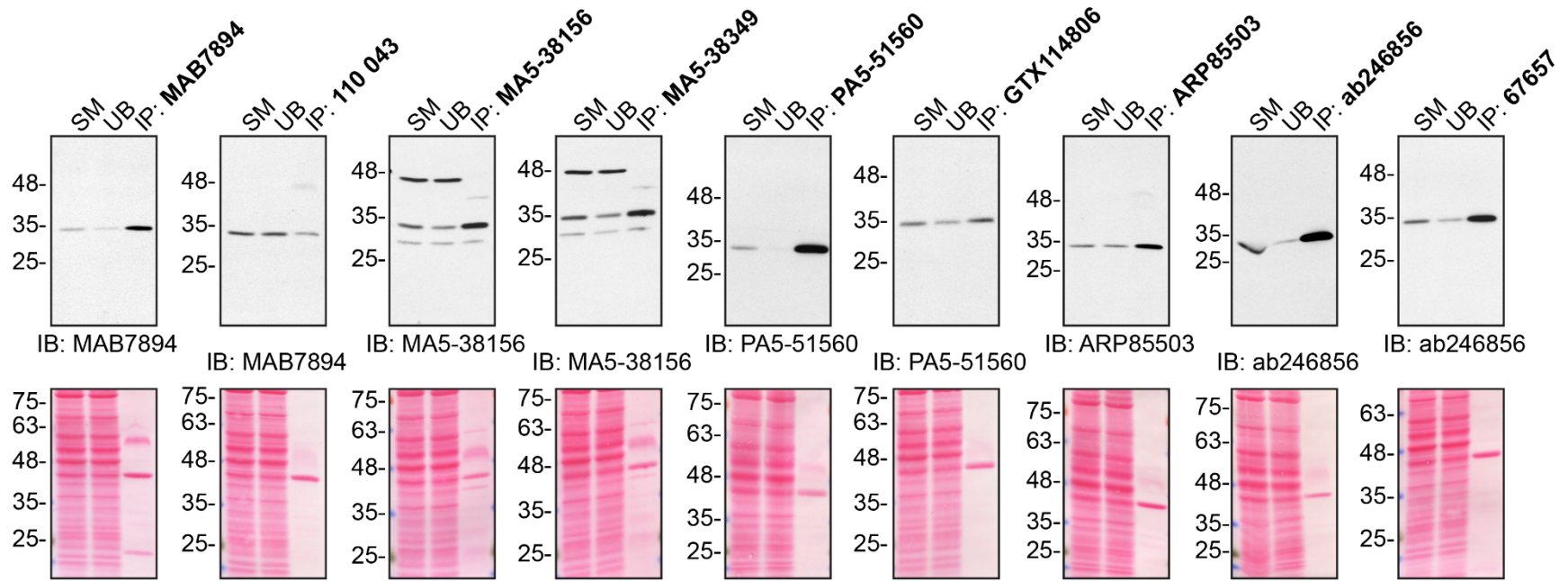


Figure 2: Syntaxin-4 antibody screening by immunoprecipitation

A

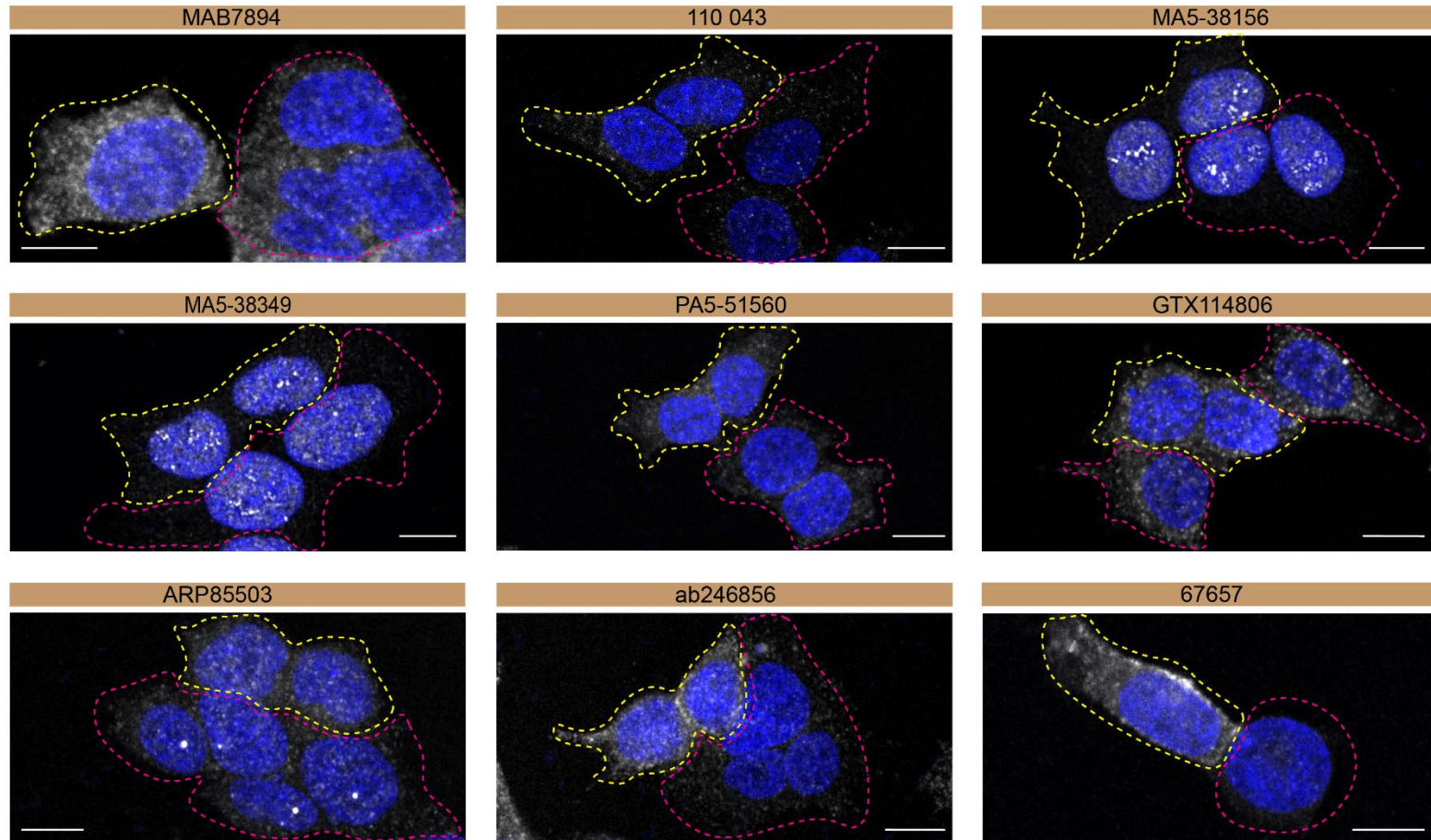


Figure 3 : Syntaxin-4 antibody screening by immunofluorescence (1/2)

B

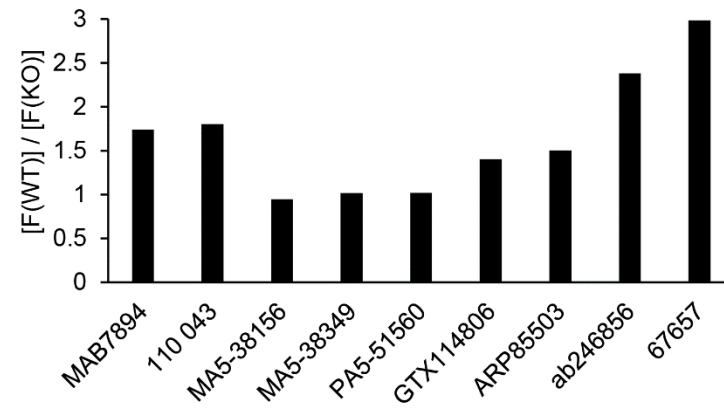


Figure 3 : Syntaxin-4 antibody screening by immunofluorescence (2/2)

Materials and methods

Antibodies

All Syntaxin-4 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-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 [5]. HAP1 (WT and *STX4* 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 [6]. Antibody-bead conjugates were prepared by adding 2.0 µg or 5 µl of antibody at an unknown

concentration to 500 μ l of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with 30 μ l 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.

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. One ml aliquots at 0.5 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 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 μ g/ml for an experiment where a rabbit antibody was used for both immunoprecipitation and its corresponding immunoblot. Anti-mouse IgG for IP:HRP (Abcam, cat. number ab131368) was used as a secondary detection system at a dilution of 0.3 μ g/ml for an experiment where a mouse antibody was used for both immunoprecipitation and its corresponding immunoblot.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [7]. HAP1 WT and *STX4* 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 Syntaxin-4 antibodies O/N at 4°C. Cells were then washed 3 \times 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 μ g/ml for 1 hr at room temperature with DAPI. Cells were washed 3 \times 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 700 laser scanning confocal microscope equipped with a Plan-Apo 63x oil objective (NA = 1.40). 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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