Antibody Characterization Report for QPRTase (Nicotinate-nucleotide pyrophosphorylase [carboxylating])

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Nicotinate-nucleotide pyrophosphorylase [carboxylating]

Alternative protein name: QPRTase

Gene name: *QPRT*

Uniprot: Q15274

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

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Proteintech	25174-1-AP	00021997	AB_2879941	polyclonal	-	rabbit	0.750	Wb,IF
Abcam	ab171944**	GR1300793	AB_2924834	recombinant-mono	EPR11941(B)	rabbit	0.214	Wb
Thermo Fisher Scientific	MA5-25198*	XH3669601	AB_2723540	monoclonal	OTI4E5	mouse	0.630	Wb,IF
Thermo Fisher Scientific	MA5-25199*	XH3669602	AB_2723541	monoclonal	OTI4E6	mouse	0.830	Wb

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	HZGHC003815c010	CVCL_TH90	HAP1	*QPRT* KO

Figure 1: QPRTase antibody screening by immunoblot.

A) Lysates of HAP1 WT and *QPRT* KO were prepared, and 30 µg of protein were processed for immunoblot with the indicated QPRTase antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 25174-1-AP at 1/500, ab171944** at 1/500, MA5-25198* at 1/500, MA5-25199* at 1/500.

B) HAP1 WT and *QPRT* KO were cultured in serum-free media. Media was collected and concentrated. 30 µg of protein from concentrated culture media were then processed for immunoblot. Antibody dilution used is the same as in A).

Predicted band size: 30 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: QPRTase antibody screening by immunoprecipitation.

A) Immunoprecipitation was performed using 1.0 mg of HAP1 lysates and 2.0 µg of the indicated QPRTase antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated QPRTase antibody. For immunoblot, ab171944** was used at 1/500.

B) Immunoprecipitation was performed using 0.6 mg of HAP1 media and 2.0 µg of the indicated QPRTase antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot as indicated in A).

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: QPRTase antibody screening by immunofluorescence.

HAP1 WT and *QPRT* 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 glass bottom. Cells were stained with the indicated QPRTase 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: 25174-1-AP at 1/800, ab171944** at 1/200, MA5-25198* at 1/600, MA5-25199* at 1/800. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

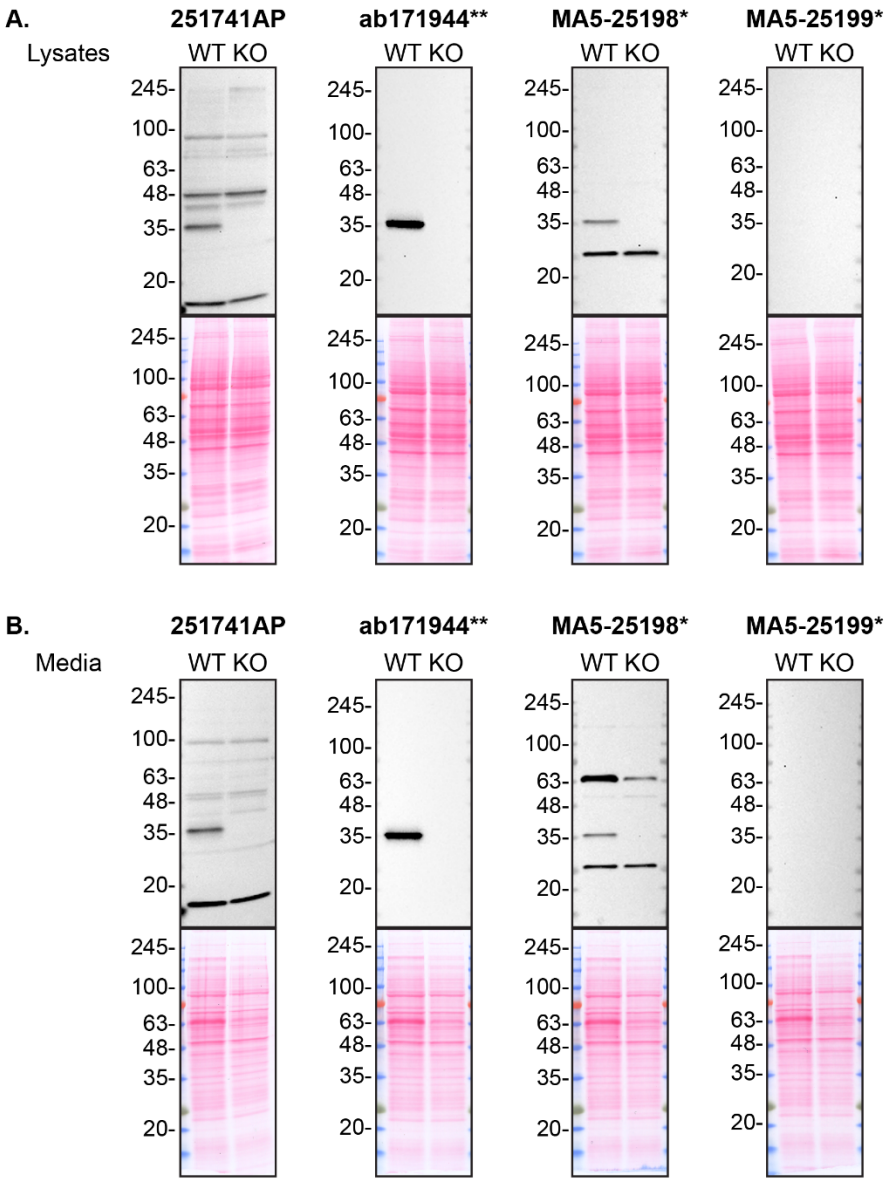

Figure 1: QPRTase antibody screening by immunoblot

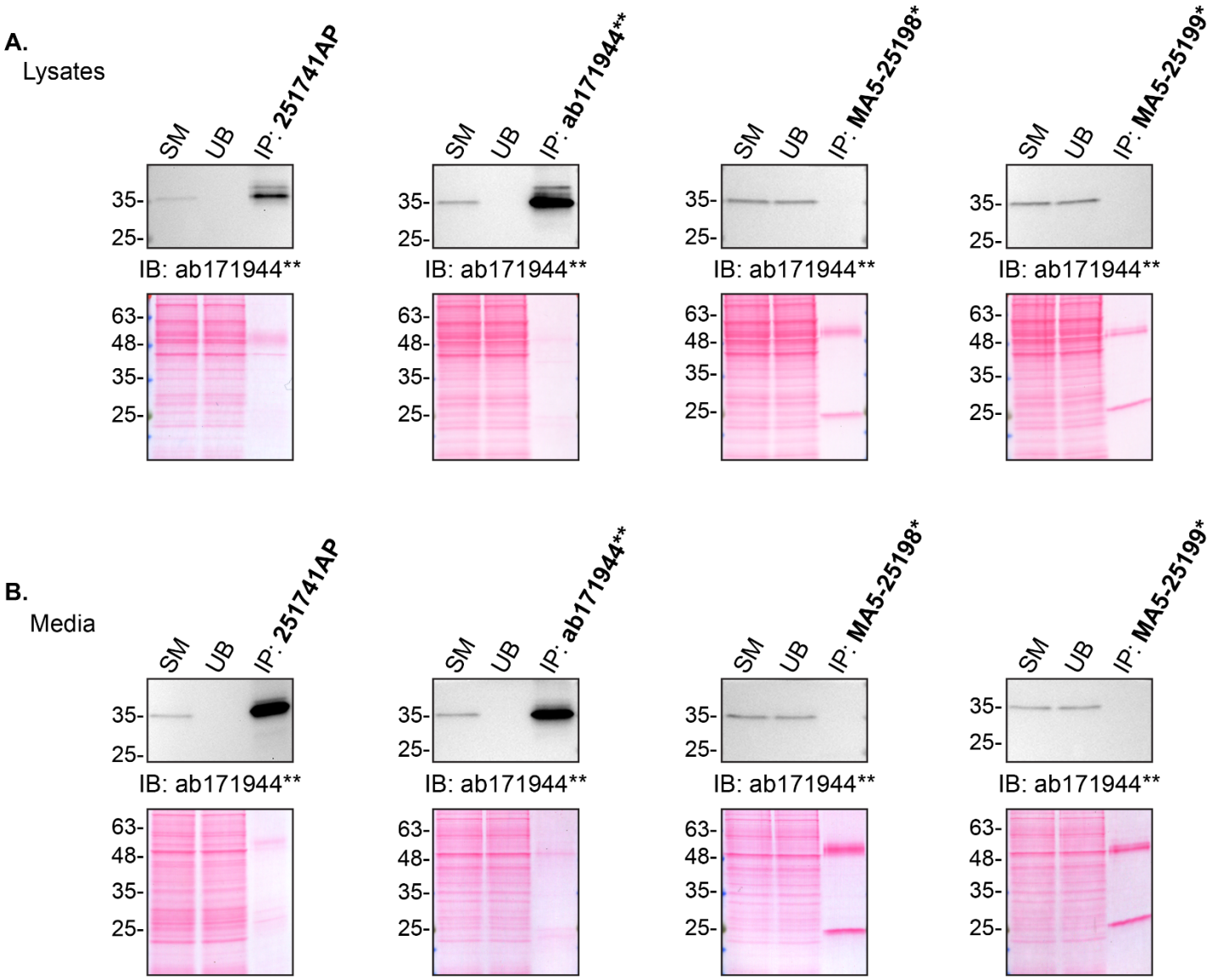

Figure 2: QPRTase antibody screening by immunoprecipitation

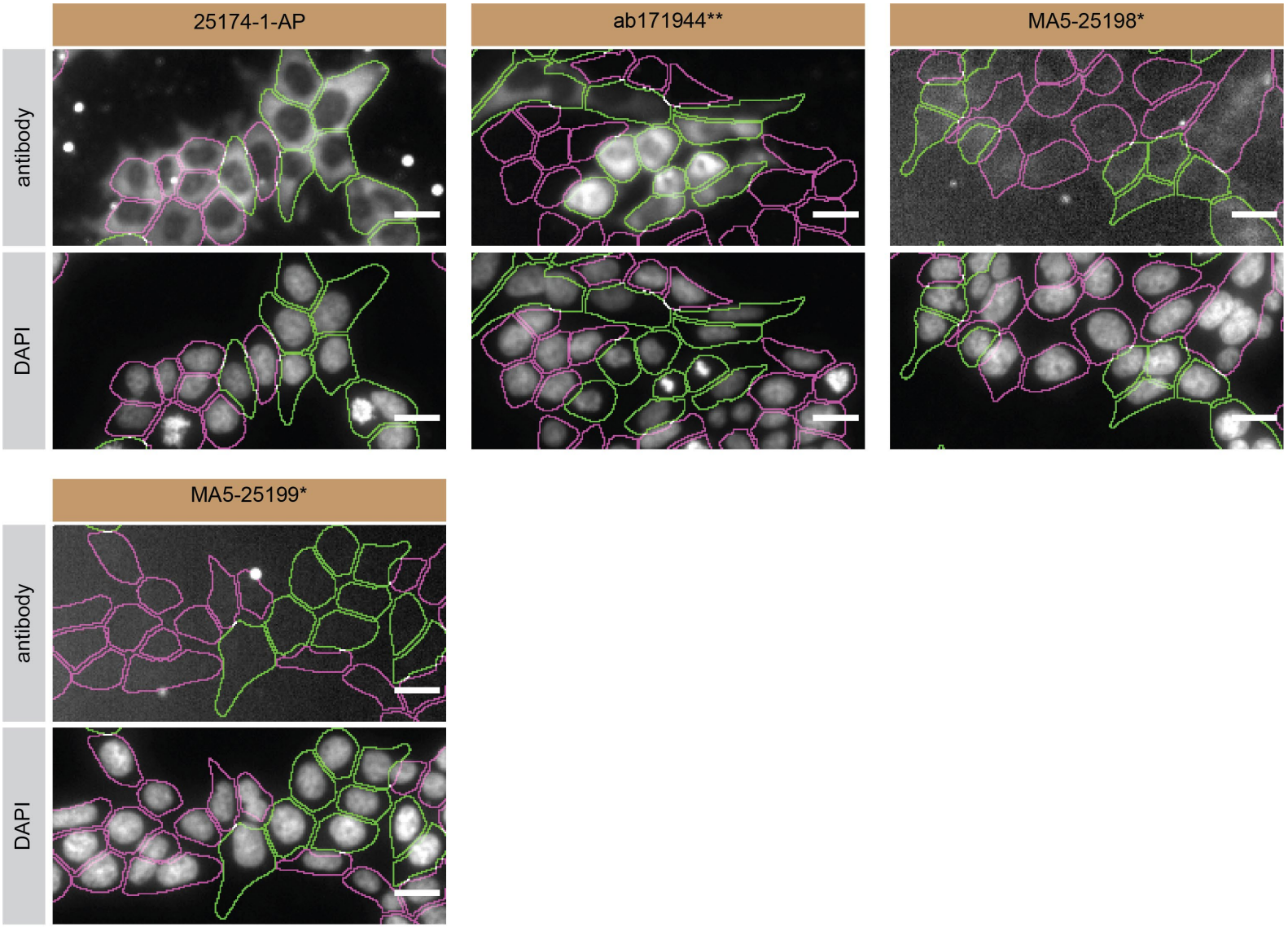

Figure 3: QPRTase antibody screening by immunofluorescence

Materials and methods

Antibodies

All tested QPRTase 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). Cells were starved in DMEM high glucose containing L-glutamate and penicillin/ streptomycin.

Antibody screening by immunoblot

Lysates: immunoblots were performed as described in our standard operating procedure [5]. HAP1 WT and *QPRT* 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 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 immunoblot.

Collection of culture media: HAP1 WT and *QPRT* KO were washed 3x with PBS 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).

For both lysates and media, immunoblots 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. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used. 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) 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 [6]. Antibody-bead conjugates were prepared by adding 2 µg 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). Tubes were rocked for ~1 hr at 4°C followed by two washes to remove unbound antibodies.

Lysates: 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 were 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 ~1 hr at 4°C.

Culture media: media from HAP1 WT was concentrated as described previously. 1 ml aliquots at 0.6 mg/ml of lysate were incubated with an antibody-bead conjugate for ~1 hr at 4°C.

For both lysates and media immunoprecipitations, 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 precast midi 4-20% Tris-Glycine polyacrylamide gels from Thermo Fisher Scientific (cat. number WXP42012BOX). Prot-A:HRP (MilliporeSigma, cat. number P8651) 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 [7]. HAP1 WT and *QPRT* 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 96 well glass plates (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 QPRTase 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 0.45 air immersion objective and scientific CMOS camera, 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, Alexa568 and Cellmask Red, respectively. Images had pixel sizes of 0.68 x 0.68 microns. Three images per field were acquired at a z-interval of 4 microns. Then, best focus intensity projections were generated from the z-stack. 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.

References

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