Antibody Characterization Report for Mitogen-activated protein kinase 1 (MAPK1)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Mitogen-activated protein kinase 1

Alternative protein name: MAP kinase 1, MAPK 1, ERK-2, MAP kinase isoform p42, p42-MAPK

Gene name: MAPK1

Uniprot: P28482

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 Mitogen-activated protein kinase 1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Mitogen-activated protein kinase 1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. A HeLa *MAPK1* KO line is available at Abcam and was used in this study. HeLa is expected to express appropriate level of Mitogen-activated protein kinase 1 determined through public expression and proteomic databases, namely PaxDB [3] and DepMap [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 Mitogen-activated protein kinase 1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo Fisher Scientific	MA1099**	UJ297429	AB_2536743	recombinant-mono	6F8	mouse	1.00	Wb,IF
Thermo Fisher Scientific	MA5-32025**	WJ3417742B	AB_2809319	recombinant-mono	SZ25-01	rabbit	1.00	Wb,IF
Thermo Fisher Scientific	PA5-17710	WJ3417028A	AB_10982125	polyclonal	-	rabbit	1.00	Wb,IF
Bio-Techne	MAB1230*	HSH025011	AB_2141131	monoclonal	191801	mouse	0.50	Wb
Bio-Techne	NBP2-67360**	HM0114	AB_2917961	recombinant-mono	SZ25-01	rabbit	1.00	Wb,IP,IF
Abcam	ab32081**	GR240605	AB_732210	recombinant-mono	6F8	rabbit	0.45	Wb,IF
ABclonal	A11186	5500001966	AB_2814870	polyclonal	-	rabbit	2.16	Wb,IF
GeneTex	GTX113094	40436	AB_2036872	polyclonal	-	rabbit	1.00	Wb,IP
GeneTex	GTX104613	39624	AB_1240776	polyclonal	-	rabbit	1.00	Wb
GeneTex	GTX134457	43194	AB_2887286	polyclonal	-	rabbit	0.20	Wb
Developmental Studies Hybridoma Bank	AFFN-MAPK1-1E5*	11/29/2018	AB_2617723	monoclonal	AFFN-MAPK1-1E5	mouse	0.01	other
Developmental Studies Hybridoma Bank	PCRP-MAPK1-1D1*	9/2/2021	AB_2722234	monoclonal	PCRP-MAPK1-1D1	mouse	0.01	Wb,IP
Proteintech	16443-1-AP	92057	AB_10603369	polyclonal	-	rabbit	0.34	Wb,IF
Proteintech	51068-1-AP	4476	AB_2250380	polyclonal	-	rabbit	0.47	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
Abcam	ab255448	CVCL_0030	HeLa	WT
Abcam	ab265052	CVCL_B1WP	HeLa	*MAPK1* KO

Figure 1: Mitogen-activated protein kinase 1 antibody screening by immunoblot.

Lysates of HeLa WT and *MAPK1* KO were prepared, and 30 µg of protein were processed for immunoblot with the indicated Mitogen-activated protein kinase 1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MA1099** at 1/1000, MA5-32025** at 1/1000, PA5-17710 at 1/1000, MAB1230* at 1/1000, NBP2-67360** at 1/1000, ab32081** at 1/1000, A11186 at 1/1000, GTX113094 at 1/1000, GTX104613 at 1/1000, GTX134457 at 1/1000, AFFN-MAPK1-1E5* at 1/70, PCRP-MAPK1-1D1* at 1/50, 16443-1-AP at 1/1000, 51068-1-AP at 1/1000. Predicted band size: 41 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Mitogen-activated protein kinase 1 antibody screening by immunoprecipitation.

HeLa lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Mitogen-activated protein kinase 1 antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Mitogen-activated protein kinase 1 antibody. For immunoblot, GTX134457 was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=2% starting material; UB=2% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain. *=monoclonal antibody, **=recombinant antibody

Figure 3: Mitogen-activated protein kinase 1 antibody screening by immunofluorescence.

HeLa WT and *MAPK1* 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 Mitogen-activated protein kinase 1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody. Acquisition of the green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: MA1099** at 1/400, MA5-32025** at 1/1000, PA5-17710 at 1/400, MAB1230* at 1/500, NBP2-67360** at 1/1000, ab32081** at 1/400, A11186 at 1/2000, GTX113094 at 1/1000, GTX104613 at 1/1000, GTX134457 at 1/200, AFFN-MAPK1-1E5* at 1/20, PCRP-MAPK1-1D1* at 1/10, 16443-1-AP at 1/300, 51068-1-AP at 1/400. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

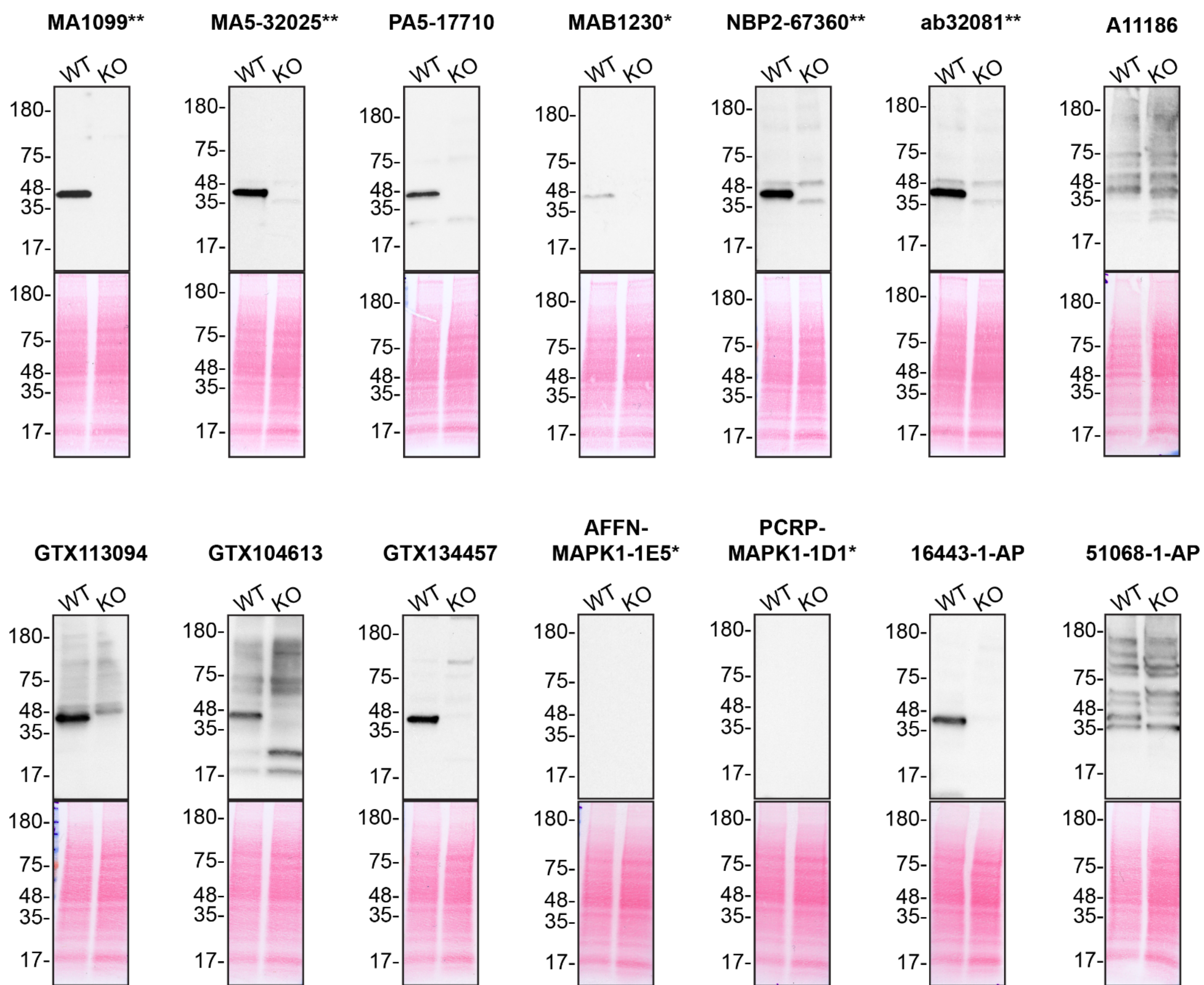

Figure 1: Mitogen-activated protein kinase 1 antibody screening by immunoblot

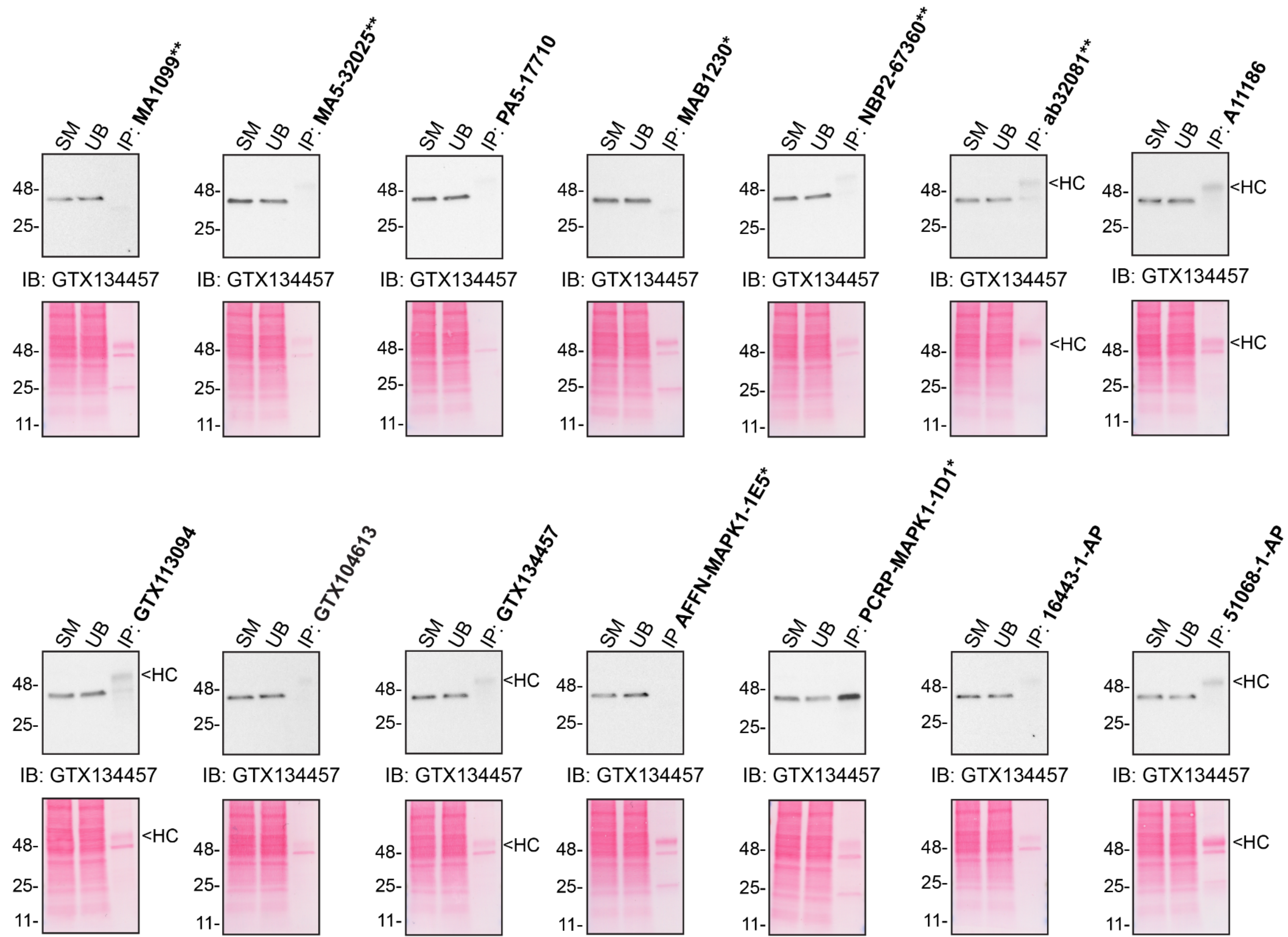

Figure 2: Mitogen-activated protein kinase 1 protein antibody screening by immunoprecipitation

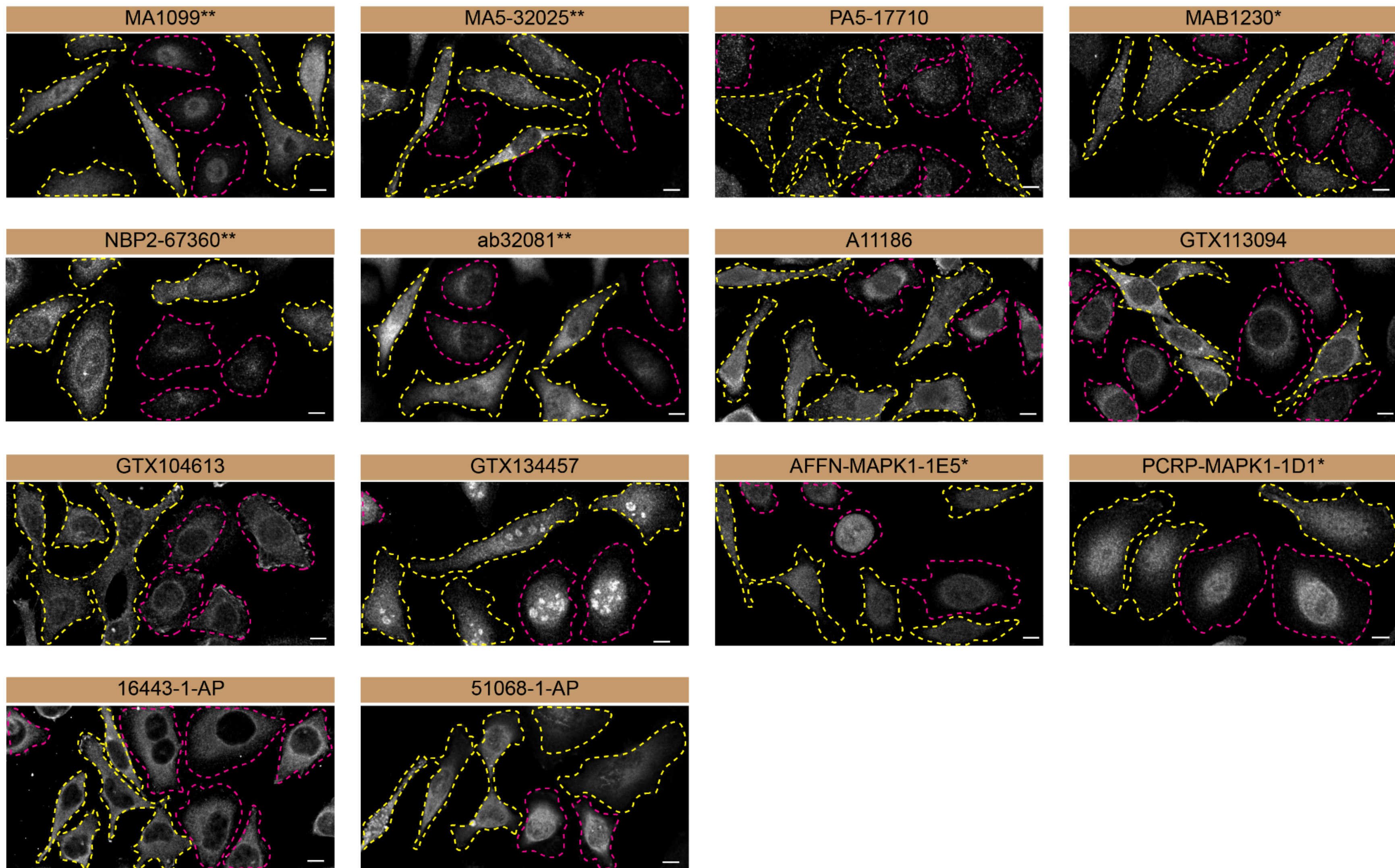

Figure 3 : Mitogen-activated protein kinase 1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Mitogen-activated protein kinase 1 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 [5]. HeLa WT and *MAPK1* 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 pre-cast mini 4-15% gradient polyacrylamide gels (Bio-Rad cat. number 4561084) 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 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). 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.

HeLa 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 immunoblot on mini 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.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [7]. HeLa WT and *MAPK1* 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 Mitogen-activated protein kinase 1 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. 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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