Antibody Characterization Report for NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 (NDUFS2)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: NADH dehydrogenase [ubiquinone] iron-sulfur protein 2

Alternative protein names: Complex I-49kD, NADH-ubiquinone oxidoreductase 49 kDa subunit

Gene name: NDUFS2

Uniprot: O75306

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1, 2]. This report guides researchers to select the most appropriate antibodies for NADH dehydrogenase [ubiquinone] iron-sulfur protein 2. We used an antibody characterization pipeline [3] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HAP1 was selected based on evidence of appropriate *NDUFS2* expression [4]. A HAP1 *NDUFS2* KO line is available at Horizon discovery.

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 NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibodies used

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	NBP2-46127	W001	AB_2895017	monoclonal	OTI1D5	mouse	1.00	Wb
Thermo	MA5-26534	WI3378393	AB_2724817	monoclonal	OTI2G2	mouse	1.00	Wb
Thermo	MA5-26536	WI3378394	AB_2724816	monoclonal	OTI1D5	mouse	1.00	Wb
Thermo	PA5-84240	WI3378416B	AB_2791392	polyclonal	-	rabbit	0.10	Wb, IF
GeneTex	GTX114924	40240	AB_10624594	polyclonal	-	rabbit	0.89	Wb, IF
Aviva Sys Bio	ARP78415	QC53114-42340	AB_2895018	polyclonal	-	rabbit	0.50	Wb
Abcam	ab110249	GR3238733-3	AB_10861985	monoclonal	7A12BE5AD5	mouse	1.00	Wb, IF
Abcam	ab192022	GR217388-15	AB_2895019	recombinant-mono	EPR16266	rabbit	0.78	Wb, IP

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	HZGHC003745c010	CVCL_TA19	HAP1	*NDUFS2* KO

Figure 1: NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibody screening by immunoblot.

Lysates of HAP1 (WT and *NDUFS2* KO) were prepared and 40 µg of protein were processed for immunoblot with the indicated NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: NBP2-46127 at 1/2000; MA5-26534 at 1/2000; MA5-26536 at 1/2000; PA5-84240 at 1/500; GTX114924 at 1/1000; ARP78415 at 1/1000; ab110249 at 1/2000; ab192022 at 1/2000. Predicted band size: 52 kDa.

Figure 2: NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibody. For immunoblot, NBP2-46127 was used at 1/500, MA5-26534 and MA5-26536 at 1/2000, GTX114924 at 1/1000, and ab110249 at 1/200. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibody screening by immunofluorescence.

A) HAP1 WT and *NDUFS2* 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 NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 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: NBP2-46127 at 1/1000; MA5-26534 at 1/1000; MA5-26536 at 1/1000; PA5-84240 at 1/100; GTX114924 at 1/900; ARP78415 at 1/500; ab110249 at 1/1000; ab192022 at 1/800. 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. An antibody with a calculated ratio above 2.5-fold (dashed red line) could be considered as specific and selective for immunofluorescence. A minimum of 15 WT and 15 KO cells from 3 different fields of view were analysed for each antibody.

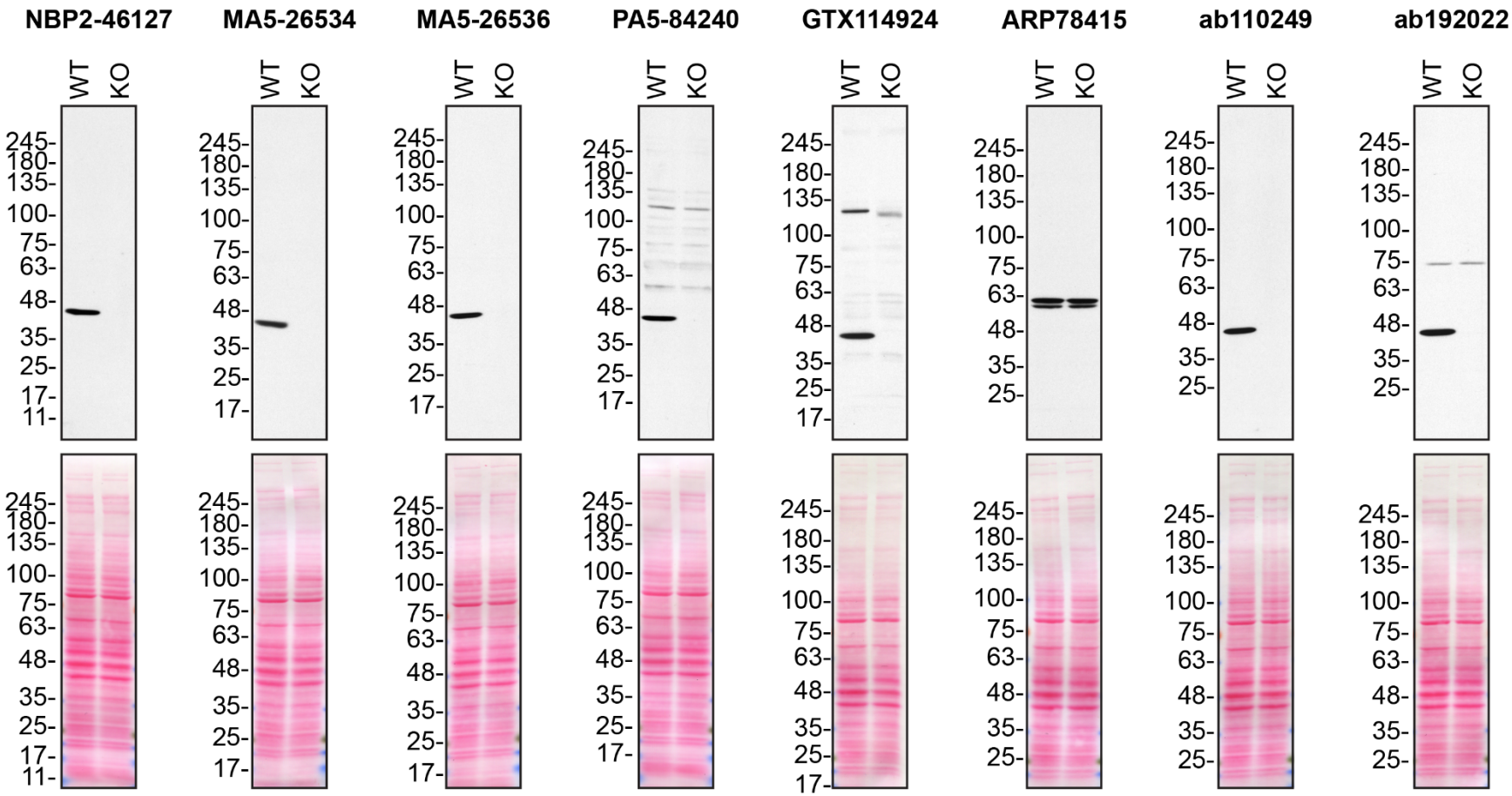

Figure 1: NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibody screening by immunoblot

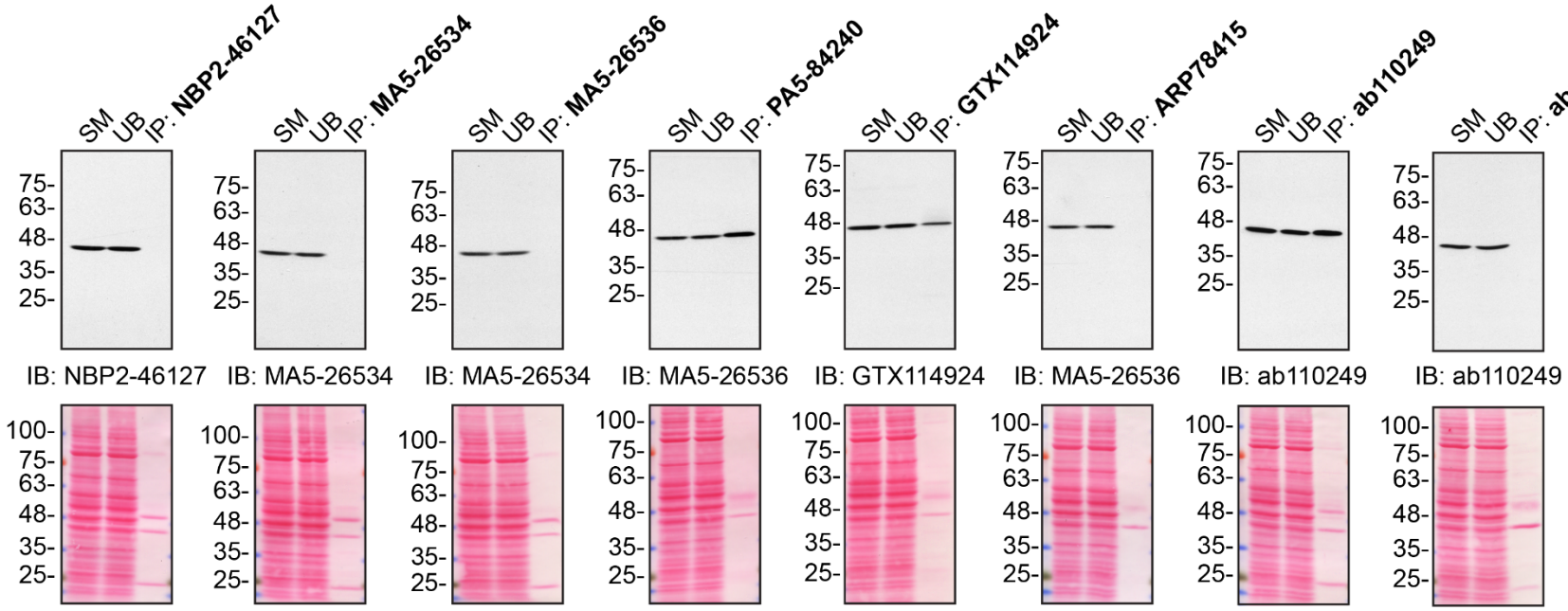

Figure 2: NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibody screening by immunoprecipitation

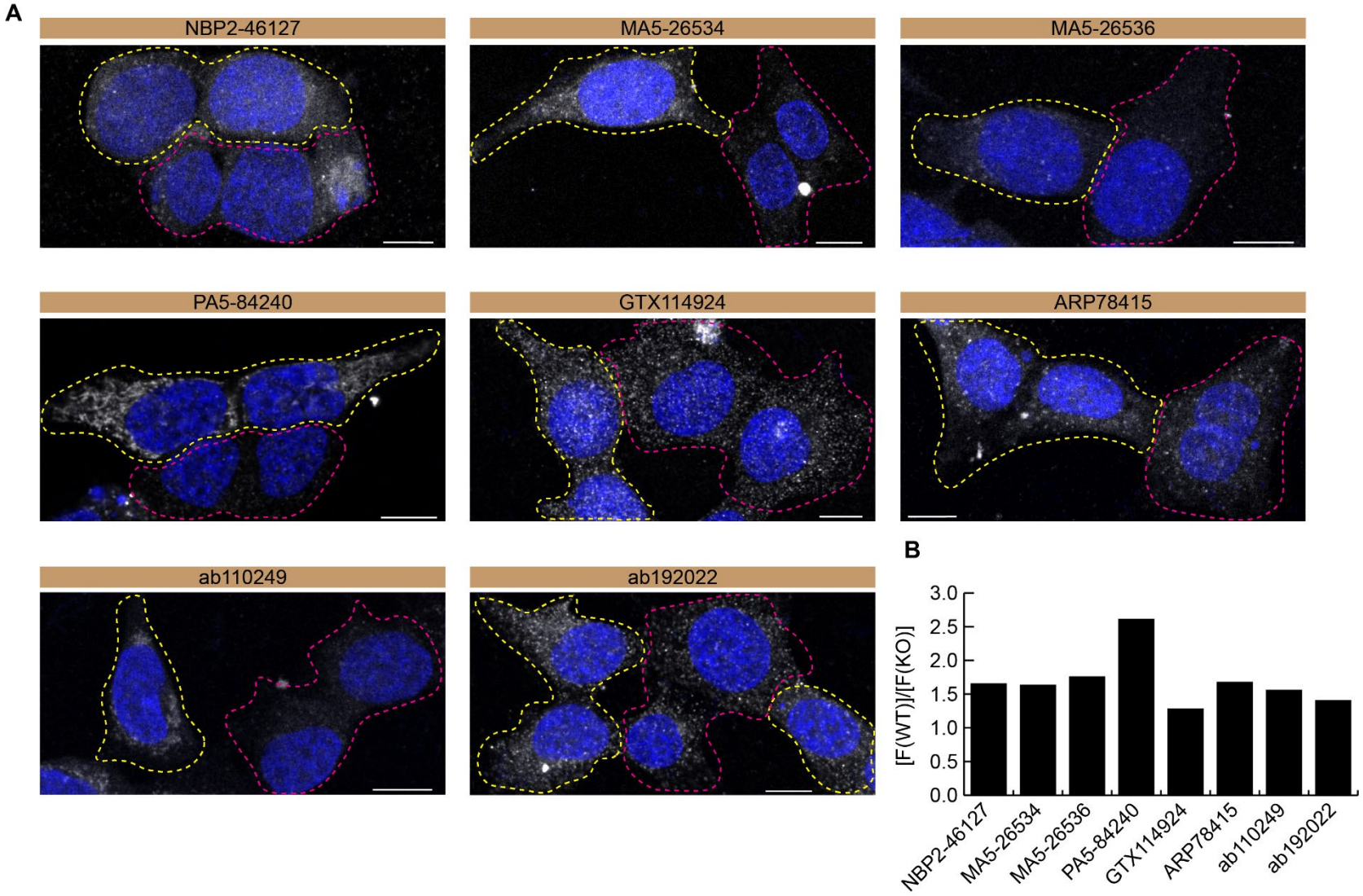

Figure 3 : NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 antibody screening by immunofluorescence

Materials and methods

Antibodies

All NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 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 (SOP) [5]. HAP1 (WT and *NDUFS2* 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 of antibody to 500 ul 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 antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Pierce IP Lysis Buffer was supplemented with the Halt Protease and Phosphatase 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 µg/ml for an experiment where a rabbit antibody was used for both immunoprecipitation and its corresponding immunoblot. Anti-mouse IgG for IP:HRP (Abcam, ab131368) was used as a secondary detection system at a dilution of 0.3 µg/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 *NDUFS2* 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 NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 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 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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