Antibody Characterization Report for Peroxiredoxin-6

YCharOS Antibody Characterization Report

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Target:

Protein name: Peroxiredoxin-6

Gene name: *PRDX6*

Uniprot: P30041

This report guides researchers to select the most appropriate antibodies for Peroxiredoxin-6. We used an antibody characterization pipeline[1] based on knockout cells to perform head-to-head comparisons of commercial antibodies for Peroxiredoxin-6 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HAP1 *PRDX6* knockout cell line is available at Horizon Discovery and was used for screening of antibodies. HeLa cell line was selected based on evidence of appropriate Peroxiredoxin-6 protein expression determined through public proteomics databases, namely PaxDB[2] and DepMap[3]. HeLa cell line was modified with CRISPR/Cas9 to knockout[4] the corresponding *PRDX6* gene.

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.

1 Laflamme, C. *et al.* Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72. *Elife* **8**, doi:10.7554/eLife.48363 (2019).

2 Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D. & von Mering, C. Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **15**, 3163-3168, doi:10.1002/pmic.201400441 (2015).

3 Nusinow, D. P. *et al.* Quantitative Proteomics of the Cancer Cell Line Encyclopedia. *Cell* **180**, 387-402 e316, doi:10.1016/j.cell.2019.12.023 (2020).

4 Nicouleau, Michael, Pimentel, Luisa, Shlaifer, IrIna & Durcan, Thomas M. Generation of Knockout Cell Lines Using CRISPR-Cas9 and ddPCR Technology. (2020). doi:10.5281/zenodo.3875777

Table 1: Summary of the Peroxiredoxin-6 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)
Proteintech	67499-1-Ig	not provided	AB_2882723	monoclonal	3C12D3	mouse	1.00
Proteintech	13585-1-AP	not provided	AB_2168637	polyclonal	-	rabbit	0.31
Abcam	ab92322	GR150831-1	AB_10562487	recombinant-mono	EPR3755	rabbit	0.90
Abcam	ab133348	GR145006-1	AB_11155931	recombinant-mono	EPR3754	rabbit	0.30
Abcam	ab73350	GR3281340-1	AB_1658907	Polyclonal	-	rabbit	1.00
Abcam	ab73683	GR71414-1	AB_2168635	Polyclonal	-	rabbit	0.40
Thermo	LF-MA0018	VJ3101922	AB_2268629	monoclonal	4A3	mouse	1.00
Thermo	MA5-34906	VJ3101165A	AB_2848812	monoclonal	7G1	mouse	2.00
Bio-Techne	MAB3490R	CKIJ0117101	AB_2890631	recombinant-mono	477068R	mouse	0.50
Bio-Techne	AF3490	130142317	AB_2168659	polyclonal	-	goat	0.20
GeneTex	GTX32779	822001166	AB_2885142	polyclonal	-	rabbit	1.12
GeneTex	GTX115262	40275	AB_10620162	polyclonal	-	rabbit	0.89

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	HZGHC006320c012	CVCL_XR82	HAP1	*PRDX6* KO
Montreal Neurological Institute	CCL-2	CVCL_0030	HeLa	WT
Montreal Neurological Institute	-	CVCL_A7AS	HeLa	*PRDX6* KO

Figure 1: Peroxiredoxin-6 antibody screening by immunoblot.

(A) Lysates of HAP1 (WT and *PRDX6* KO) were prepared and 25 µg of protein were processed for immunoblot with the indicated Peroxiredoxin-6 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 67499-1-Ig at 1/10000; 13585-1-AP at 1/2000; ab92322 at 1/1000; ab133348 at 1/1000; ab73350 at 1/400; ab73683 at 1/400; LF-MA0018 at 1/2000; MA5-34906 at 1/500; MAB3490R at 1/1000; GTX32779 at 1/5000; GTX115262 at 1/1000. Predicted band size: 25 kDa.

(B) Lysates of HAP1 (WT and *PRDX6* KO) and HeLa (WT and *PRDX6* KO) were prepared and 25 g of protein were processed for immunoblot as in (A). The Peroxiredoxin-6 antibody 67499-1-Ig was used at 1/20000.

Figure 2: Peroxiredoxin-6 antibody screening by immunoprecipitation.

HAP1 lysates were prepared and IP was performed using 2.0 µg of the indicated Peroxiredoxin-6 antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Peroxiredoxin-6 antibody. For immunoblot, the following antibodies were used: 67499-1-Ig at 1/10000, ab13348 at 1/1000, LF-MA0018 at 1/2000, MAB3490R at 1/1000 and GTX32779 at 1/5000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate;.

Figure 3: Peroxiredoxin-6 antibody screening by immunofluorescence.

HAP1 WT and *PRDX6* KO cells were labelled with a green or a far red fluorescence dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Peroxiredoxin-6 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody, including DAPI. Acquisition of the blue (nucleus-DAPI), green (WT), red (antibody staining) and far-red (KO) 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: 67499-1-Ig at 1/1000; 13585-1-AP at 1/300; ab92322 at 1/1000; ab133348 at 1/300; ab73350 at 1/1000; ab73683 at 1/500; LF-MA0018 at 1/1000; MA5-34906 at 1/2000; MAB3490R at 1/500, GTX32779 at 1/1000, GTX115262 at 1/1000. Bars = 10 µm.

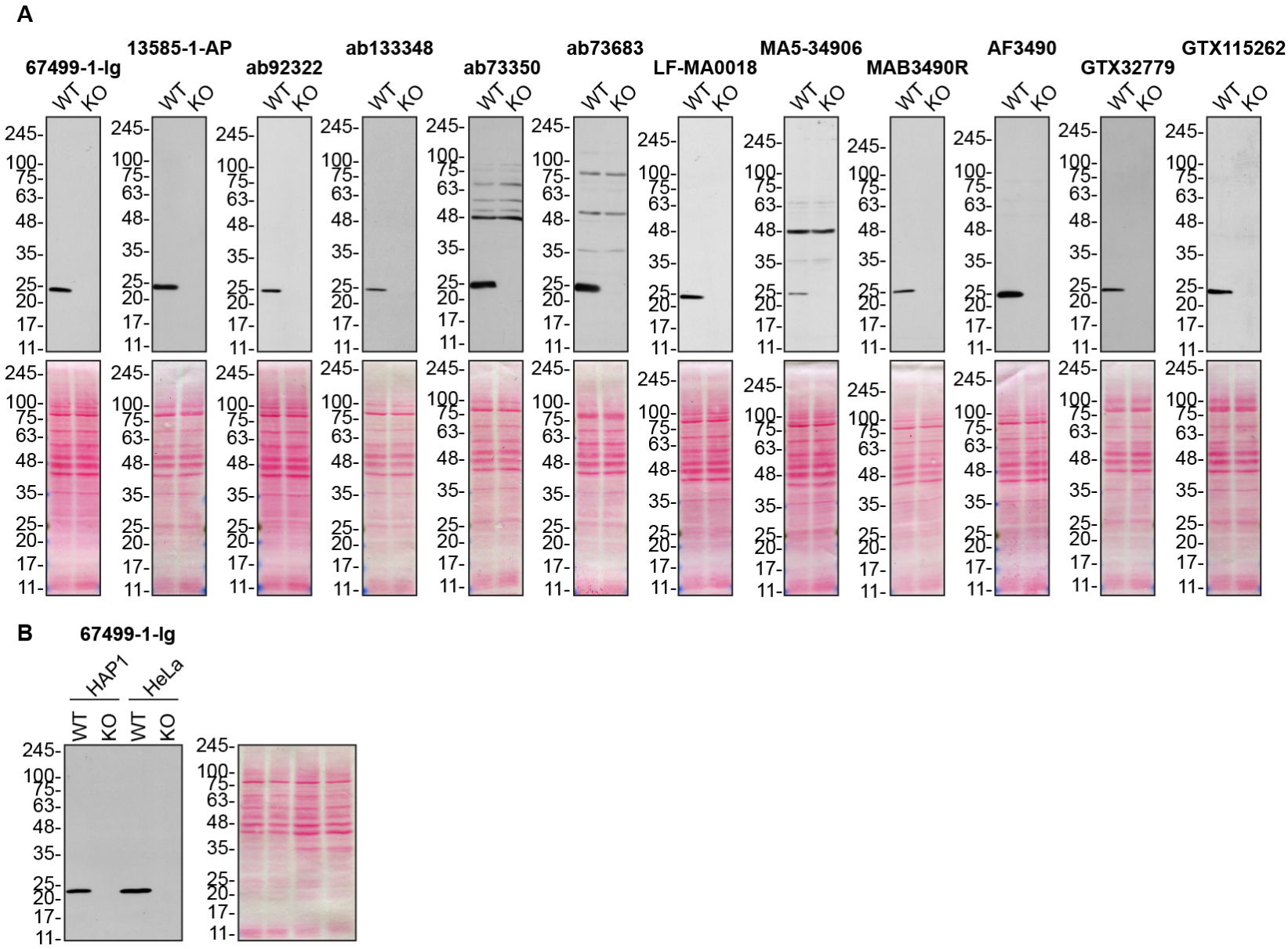

Figure 1 : Peroxiredoxin-6 antibody screening by immunoblot

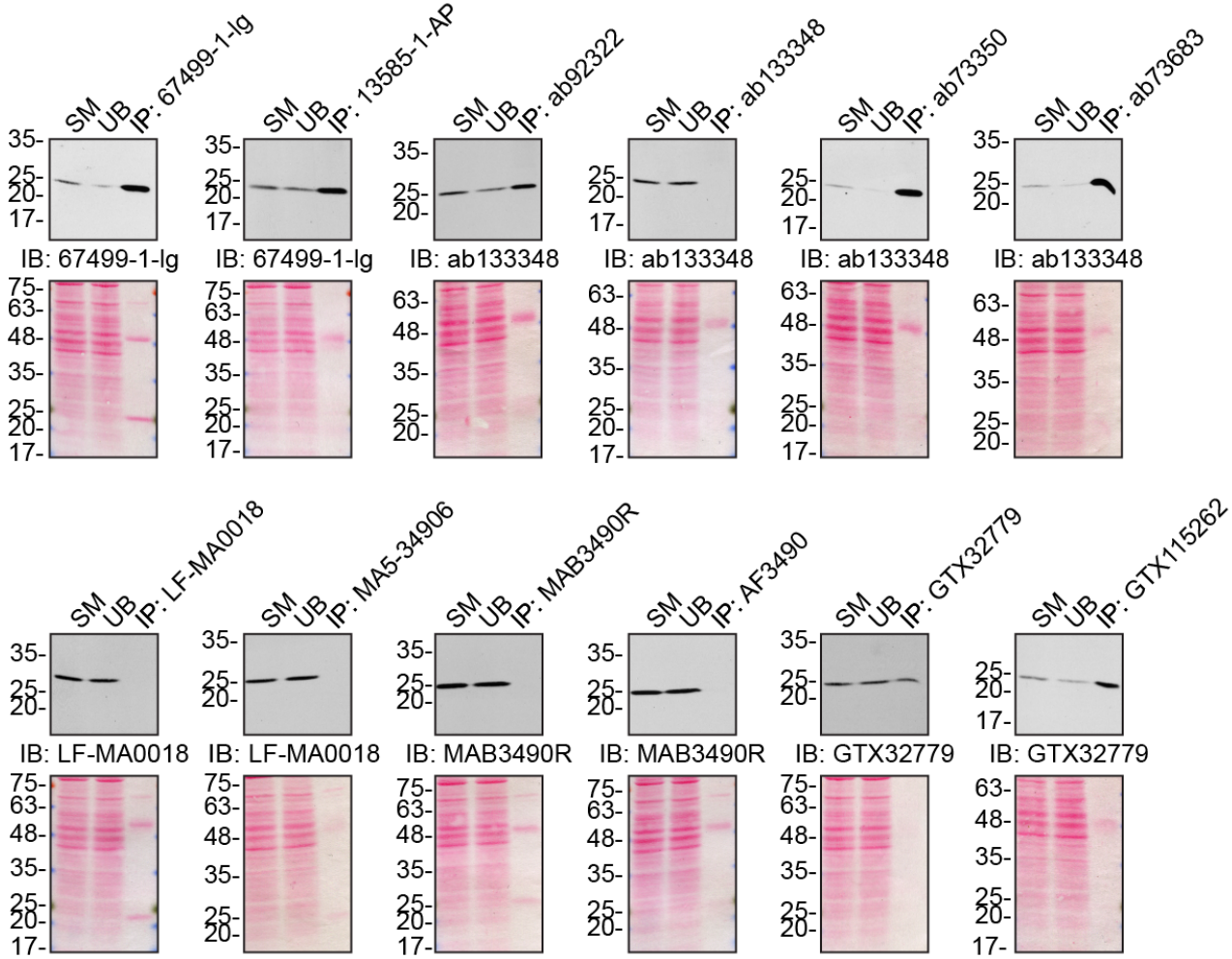

Figure 2 : Peroxiredoxin-6 antibody screening by immunoprecipitation

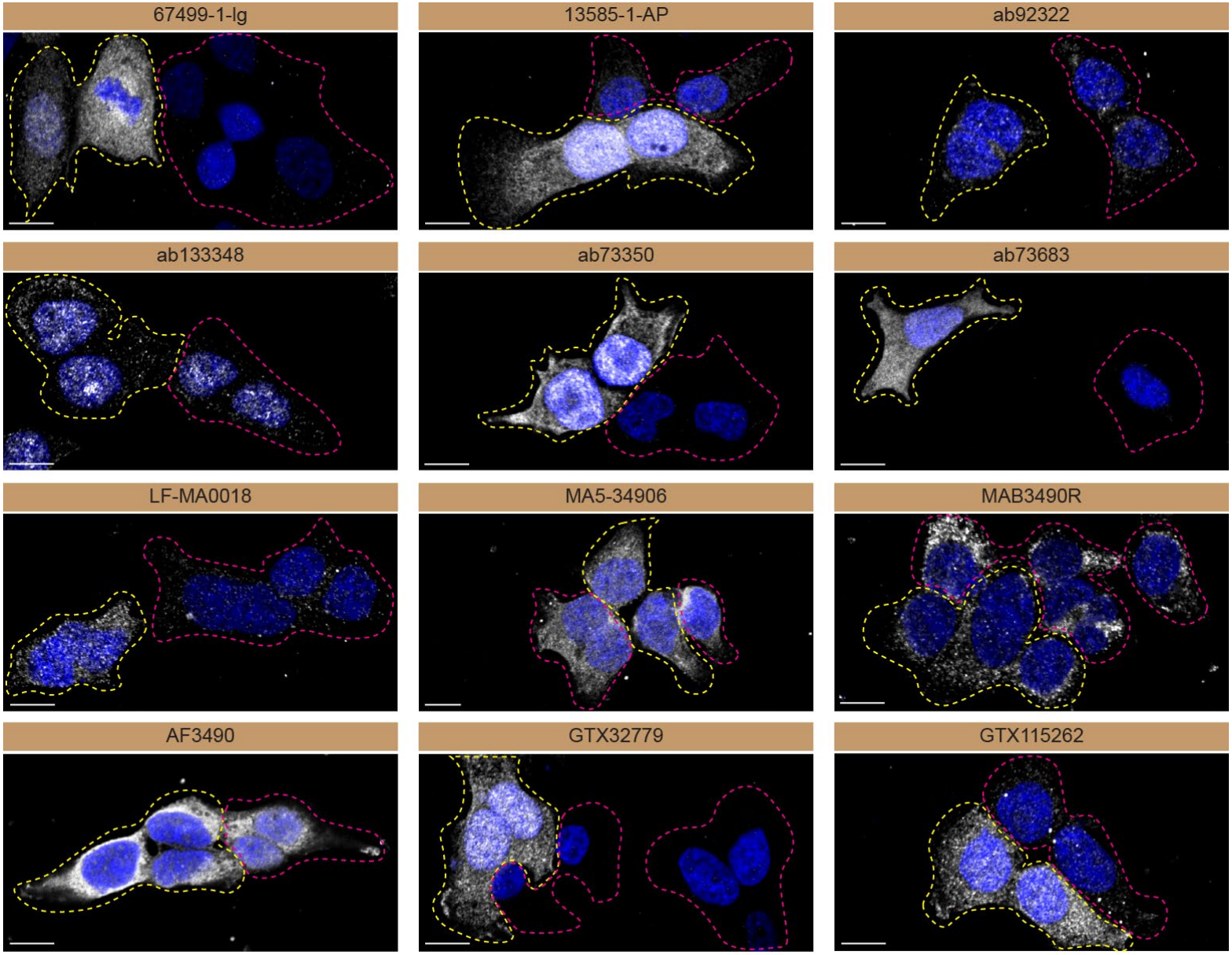

Figure 3 : Peroxiredoxin-6 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Peroxiredoxin-6 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) whereas the donkey anti-goat is from Santa Cruz (cat. number sc-2020). The Alexa-555-conjugated goat anti-mouse, goat anti-rabbit and donkey anti-goat secondary antibodies are from Thermo Fisher Scientific (cat. number A21424, A21429, A21432).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. HeLa *PRDX6* KO clone was generated with low passage cells using an open-access protocol available on Zenodo.org: https://zenodo.org/record/3875777#.X9uE11VKjIX. The sequence of the guide RNA used to introduce a STOP codon in the *PRDX6* gene is CCAUCAUCGAUGAUAGGAAU.

Cell culture

Cells were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) containing 10% bovine calf serum (GE Healthcare cat. number SH30072.03), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Antibody screening by immunoblot

HAP1 (WT and *PRDX6* KO) were collected in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1.0 mM EDTA, 1% Triton X-100, 0.5% 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 8-16% 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

Antibody-bead conjugates were prepared by adding 2.0 µg of antibody to 500 ul of PBS with 0,01% triton X-100 in a microcentrifuge tube, together with 30µl of protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

HAP-1 WT were collected in HEPES buffer (20 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, pH 7.4) 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 HEPES lysis buffer and processed for SDS-PAGE and immunoblot on a 8-16% acrylamide gel. Prot-A:HRP (MilliporeSigma, cat. number P8651) and HRP-conjugated anti-mouse IgG for IP (Abcam, ab131368) were used as secondary detection system at a dilution of 0.2 µg/ml.

Antibody screening by immunofluorescence

U2OS WT and *PRDX6* KO were labelled with a green and a deep 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. Coverslips were incubated with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Peroxiredoxin-6 antibodies O/N at 4°C. Cells were 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 40x 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.