Antibody Characterization Report for Superoxide dismutase [Cu-Zn] (SOD1)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Superoxide dismutase [Cu-Zn]

Alternative protein name: Superoxide dismutase 1

Gene name: *SOD1*

Uniprot: P00441

This report guides researchers to select the most appropriate antibodies for Superoxide dismutase 1. We used an antibody characterization pipeline[1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Superoxide dismutase 1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HeLa was selected based on evidence of appropriate Superoxide dismutase 1 protein expression determined through public proteomics databases, namely PaxDB [2] and DepMap [3]. HeLa was modified with CRISPR/Cas9 to knockout [4] the corresponding *SOD1* 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, 2019. **8**.
2. Wang, M., et al., *Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines.* Proteomics, 2015. **15**(18): p. 3163-8.
3. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16.
4. Ran, F.A., et al., *Genome engineering using the CRISPR-Cas9 system.* Nat Protoc, 2013. **8**(11): p. 2281-2308.

Table 1: Summary of the Superoxide dismutase 1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	MAB3418	XJQ0216121	AB_2193899	monoclonal	348808	mouse	0.50	Wb, IF
Proteintech	67480-1-Ig	10014544	AB_2882707	monoclonal	2F10G1	mouse	0.50	Wb
Proteintech	10269-1-AP	00069112	AB_2193750	polyclonal	-	rabbit	0.43	Wb, IP, IF
Thermo	MA1-105	VL315171	AB_2536811	monoclonal	8B10	mouse	1.00	Wb, IF
Thermo	711818	SH256097	AB_2688303	recombinant-poly	not provided	rabbit	0.50	Wb, IF
Thermo	702783	2107589	AB_2716893	recombinant-mono	11H3L1	rabbit	0.50	Wb, IF
Abcam	ab51254	GR3231443-1	AB_882757	recombinant-mono	EP1727Y	rabbit	0.15	Wb, IF
Abcam	ab79390	GR221266-10	AB_1603741	recombinant-mono	EPR1726	rabbit	0.18	Wb
Abcam	ab252426	GR3334282-1	AB_2885125	recombinant-mono	EPR23549-163	rabbit	0.48	Wb, IP, IF
GeneTex	GTX100554	43222	AB_10618670	polyclonal	-	rabbit	0.15	Wb, IF
GeneTex	GTX100659	41822	AB_1951972	polyclonal	-	rabbit	0.47	Wb, IF
Enzo Life Sciences	ADI-SOD-100	5021645	AB_10616253	polyclonal	-	rabbit	1.00	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
ATCC	CCL-2	CVCL_0030	HeLa	WT
Montreal Neurological Institute	-	CVCL_A8PZ	HeLa	*SOD1* KO

Figure 1: Superoxide dismutase 1 antibody screening by immunoblot.

Lysates of HeLa (WT and *SOD1* KO) were prepared and 20 µg of protein were processed for immunoblot with the indicated Superoxide dismutase 1 antibodies. A longer exposure for most antibodies is showed in the cropped middle panel. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MAB3418 at 1/1000, 67480-1-Ig at 1/10000, 10269-1-AP at 1/1000, MA1-105 at 1/1000, 711818 at 1/200, 702783 at 1/200, ab51254 at 1/15000, ab79390 at 1/10000, ab252426 at 1/1000, GTX100554 at 1/1000, GTX100659 at 1/1000, ADI-SOD-100 at 1/1000. Predicted band size: 16 kDa.

Figure 2: Superoxide dismutase 1 antibody screening by immunoprecipitation.

HeLa lysates were prepared and immunoprecipitation was performed using 2.0 µg of the indicated Superoxide dismutase 1 antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Superoxide dismutase 1 antibody. For immunoblot, the following Superoxide dismutase 1 antibodies were used: MAB3418 at 1/1000, 67480-1-Ig at 1/1000, 10269-1-AP at 1/2000, MA1-105 at 1/2000, ab79390 at 1/15000 and GTX100554 at 1/2000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: Superoxide dismutase 1 antibody screening by immunofluorescence.

HeLa WT and *SOD1* 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 Superoxide dismutase 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) channel are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: MAB3418 at 1/500, 67480-1-Ig at 1/500, 10269-1-AP at 1/500, MA1-105 at 1/1000, 711818 at 1/500, 702783 at 1/500, ab51254 at 1/200, ab79390 at 1/200, ab252426 at 1/500, GTX100554 at 1/200, GTX100659 at 1/500, ADI-SOD-100 at 1/500. Bars = 10 µm.

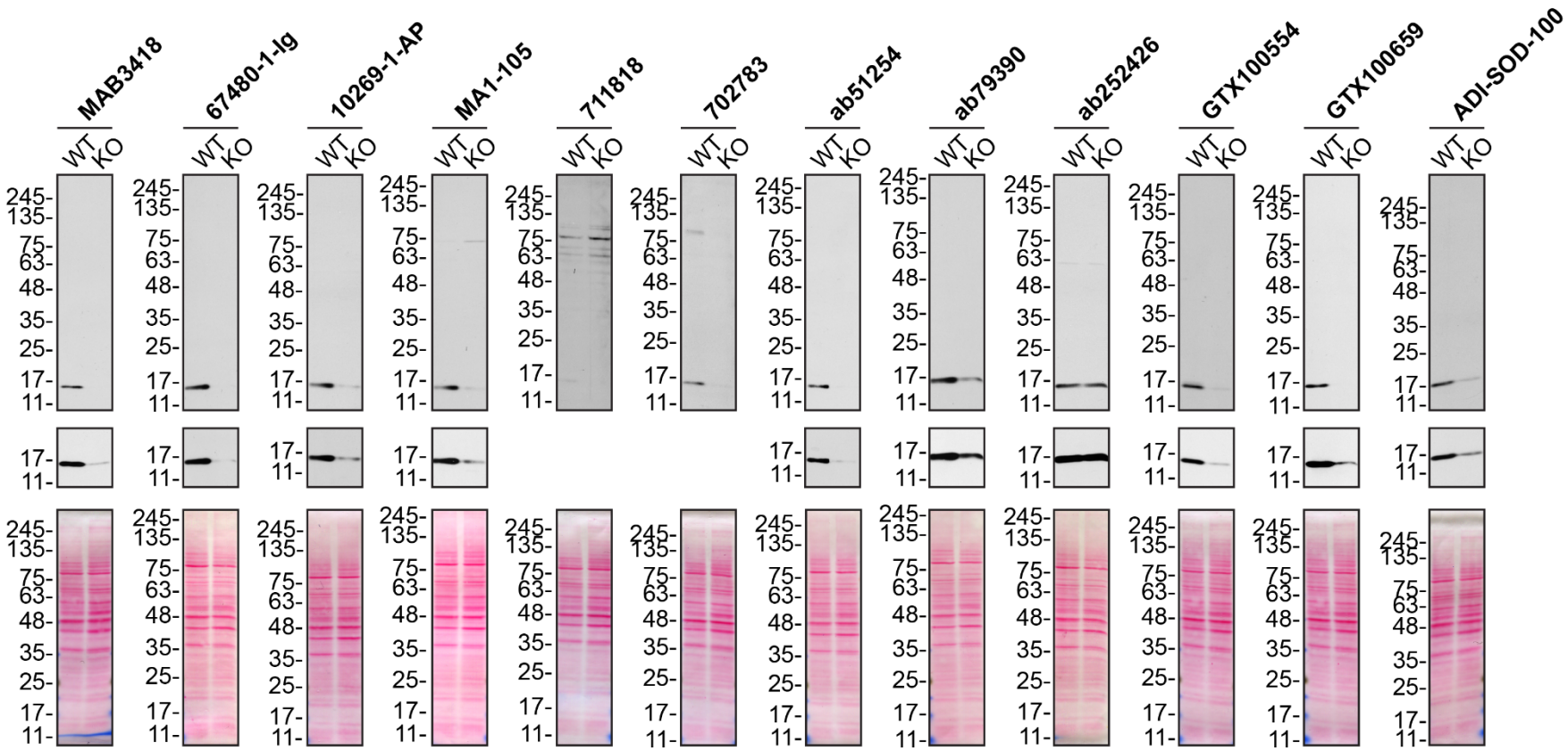

Figure 1: SOD1 antibody screening by immunoblot

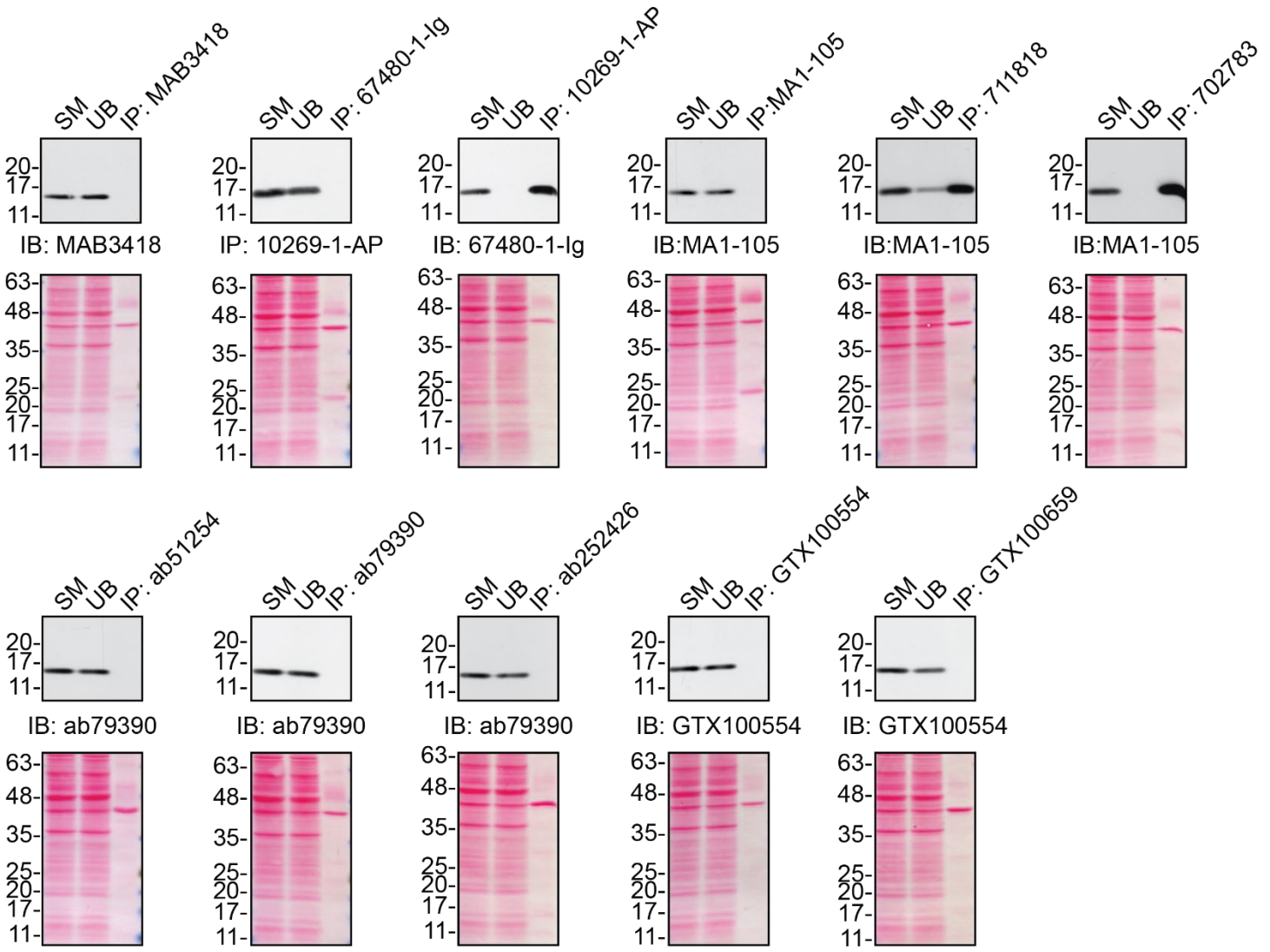

Figure 2: SOD1 antibody screening by immunoprecipitation

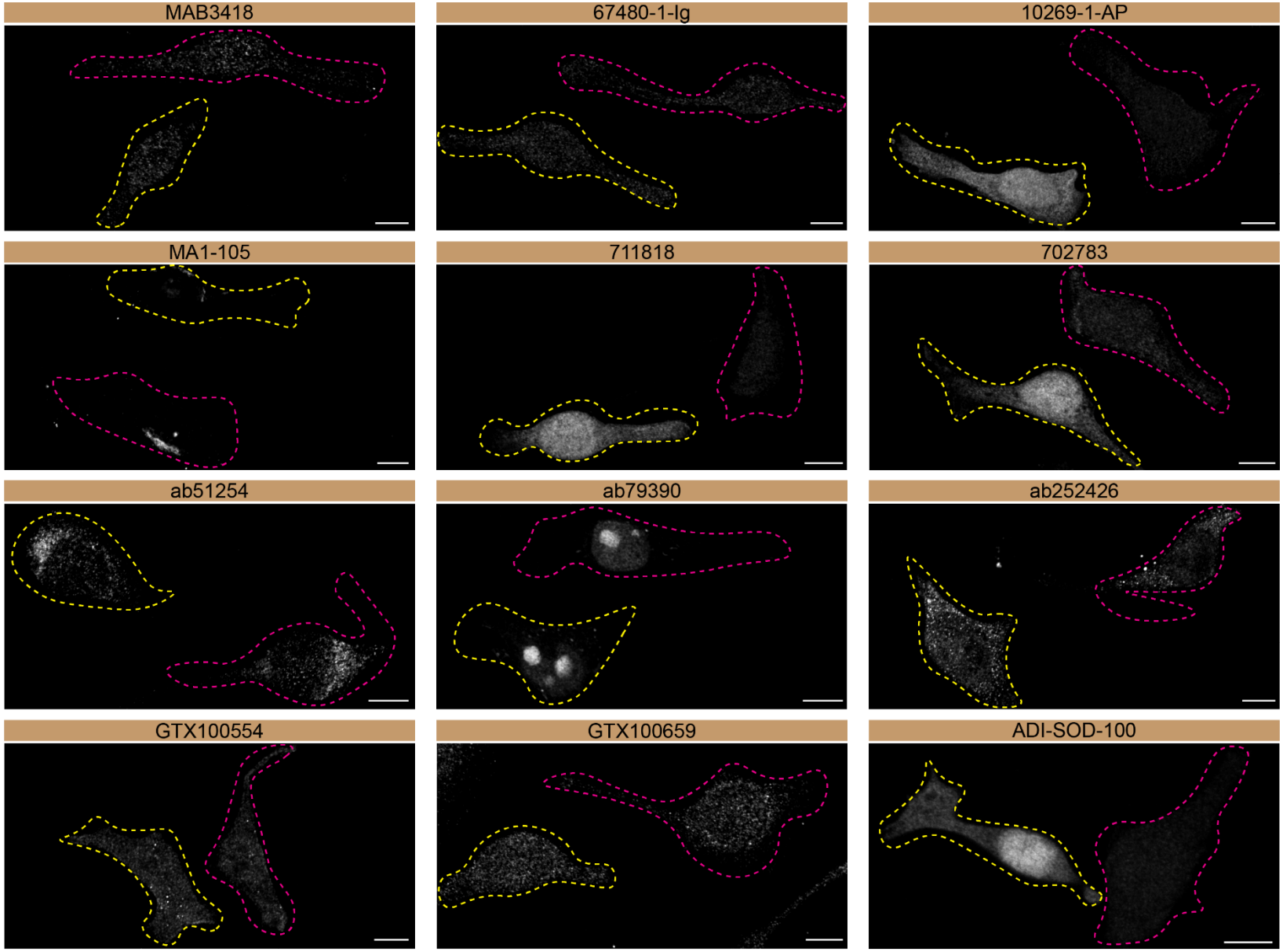

Figure 3 : SOD1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Superoxide dismutase 1 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).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. HeLa *SOD1* KO clone was generated with low passage cells. Two guide RNAs were used to KO the *SOD1* gene (sequence guide 1: CCGTTGCAGTCCTCGGAACC, sequence guide 2: GCGCGGGGGGACGAGCGGGT).

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

HeLa (WT and *SOD1* 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.

HeLa 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 8-16% polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) and VeriBlot for IP Detection Reagent HRP (Abcam, cat. number ab131366) were used as secondary detection systems for an experiment where a rabbit antibody was used for both immunoprecipitation and its corresponding immunoblot. Similarly, anti-mouse IgG for IP HRP (Abcam, cat. number ab131368) was used for experiment which involved mouse antibodies for the immunoprecipitation and the corresponding immunoblot.

Antibody screening by immunofluorescence

HeLa WT and *SOD1* 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 Superoxide dismutase 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 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.