Antibody Characterization Report for Coiled-coil-helix-coiled-coil-helix domain-containing protein 10, mitochondrial (CHCHD10)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Coiled-coil-helix-coiled-coil-helix domain-containing protein 10, mitochondrial

Protein name (short): CHCHD10

Alternative protein name: Protein N27C7-4

Gene name: *CHCHD10*

Uniprot: Q8WYQ3

This report guides researchers to select the most appropriate antibodies for CHCHD10. We used an antibody characterization pipeline [1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for CHCHD10 by immunoblot (Western blot), immunoprecipitation and immunofluorescence.

Two cell lines were used to screen CHCHD10 antibodies: *i)* human fibroblasts were immortalized and modified with CRISPR/Cas9 to knockout the corresponding *CHCHD10* gene [2] and *ii)* a HAP1 *CHCHD10* KO line available at Horizon Discovery. HAP1 parental line expresses higher level of CHCHD10 protein compared to the human fibroblast line (as evaluated by immunoblot).

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. Straub, I.R., et al., *Loss of CHCHD10-CHCHD2 complexes required for respiration underlies the pathogenicity of a CHCHD10 mutation in ALS.* Hum Mol Genet, 2018. **27**(1): p. 178-189.

Table 1: Summary of the CHCHD10 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Proteintech	25671-1-AP	53318	AB_2880187	polyclonal	-	rabbit	0.30	Wb, IP, IF
Thermo	MA5-27532	VL3152361A	AB_2724131	monoclonal	OTI2B6	mouse	1.00	Wb
Thermo	MA5-27535	VL3152362A	AB_2724132	monoclonal	OTI3B8	mouse	1.00	Wb
Thermo	MA5-27531	VL3152369	AB_2724133	monoclonal	OTI4C12	mouse	1.00	Wb

Wb=Western blot IP= immunoprecipitation IF=immunofluorescence

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Montreal Neurological Institute	-	-	Human fibroblast	WT
Montreal Neurological Institute	-	-	Human fibroblast	*CHCHD10* KO
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC005043c003	CVCL_SI77	HAP1	*CHCHD10* KO

Figure 1: CHCHD10 antibody screening by immunoblot.

A) Lysates of HAP1 (WT and *CHCHD10* KO) were prepared and processed for immunoblot with the indicated CHCHD10 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 25671-1-AP at 1/1000, MA5-27532 at 1/500, MA5-27535 at 1/500, MA5-27531 at 1/500. Predicted band size: 14 kDa.

B) Lysates of human fibroblast (WT and *CHCHD10* KO), HAP1 (WT and *CHCHD10* KO) and HCT116 were prepared as in A). MA5-27531 was used at 1/500.

Figure 2: CHCHD10 antibody screening by immunoprecipitation.

HCT116 lysates were prepared and immunoprecipitation was performed using 1.0 µg of the indicated CHCHD10 antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated CHCHD10 antibody. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: CHCHD10 antibody screening by immunofluorescence.

A) HAP1 WT and *CHCHD10* 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 CHCHD10 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: 25671-1-AP at 1/300, MA5-27532 at 1/100, MA5-27535 at 1/1000, MA5-27531 at 1/100. Bars = 10 µm.

B) Human fibroblast WT and *CHCHD10* KO cells were processed as in A).

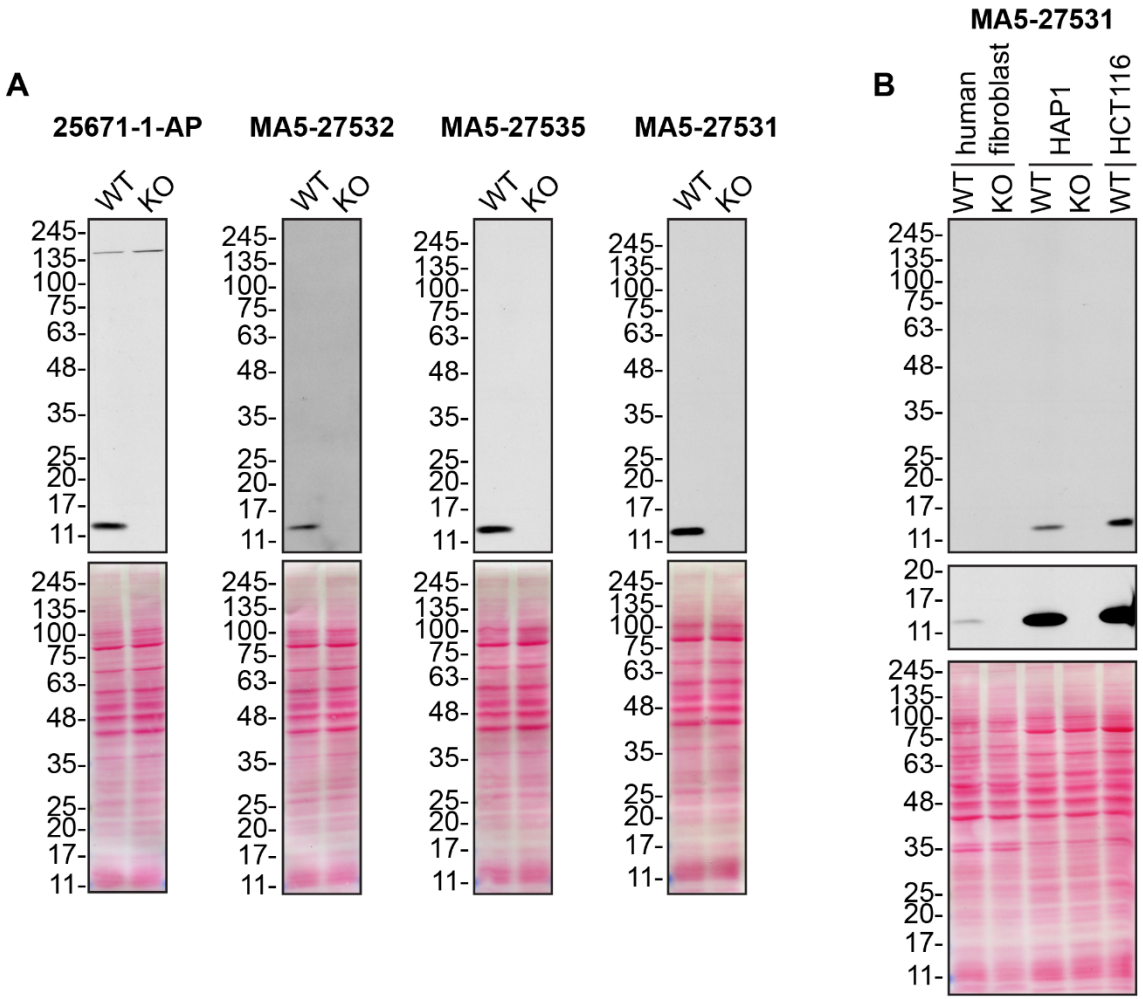

Figure 1: CHCHD10 antibody screening by immunoblot

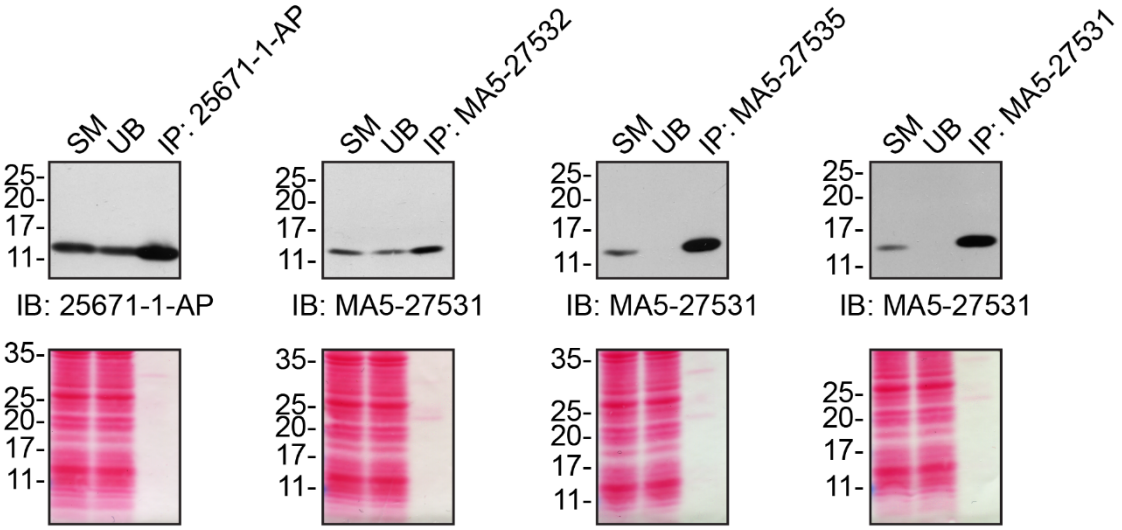

Figure 2: CHCHD10 antibody screening by immunoprecipitation

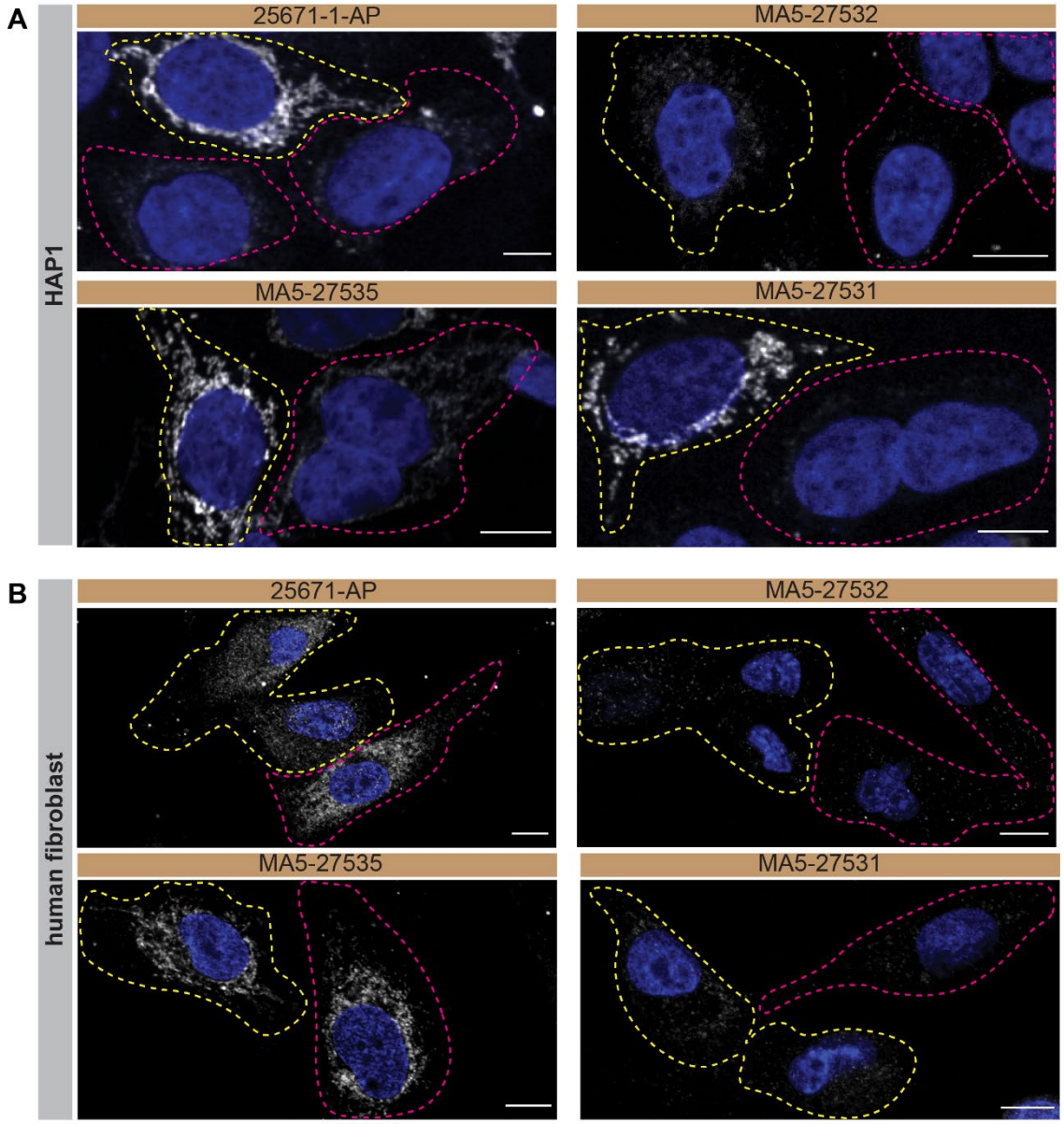

Figure 3 : CHCHD10 antibody screening by immunofluorescence

Materials and methods

Antibodies

All CHCHD10 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.

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

Cells 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 (50 µg/lane in Figure 1A, 80 µg/lane in Figure 1B). 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 1.0 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.

HCT116 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 1.0 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. As secondary detections systems, the Veriblot for immunoprecipitation detection reagent and the anti-mouse IgG for immunoprecipitation (HRP) from Abcam (cat.# ab131366 and ab131368) were used.

Antibody screening by immunofluorescence

HAP1 (WT and *CHCHD10* KO) and human fibroblast (WT and *CHCHD10* 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 CHCHD10 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 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.