Antibody Characterization Report for Pro-cathepsin H

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Pro-cathepsin H

Gene name: *CTSH*

Uniprot: P09668

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 Pro-cathepsin H. We used an antibody characterization pipeline [3] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Pro-cathepsin H by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HEK293T was selected based on evidence of appropriate Pro-cathepsin H protein expression determined through public proteomics databases, namely PaxDB [4]. A HEK293T *CTSH* KO line is available at Abcam and was used in this study.

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 Pro-cathepsin H antibodies used

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	AF7516	CHRH0118021	AB_2895290	polyclonal	-	sheep	0.20	Wb
Abcam	ab88960	GR50443-9	AB_2040719	monoclonal	AF14D7	mouse	not provided	Wb
Abcam	ab185935	GR315948-14	AB_2895291	polyclonal	-	rabbit	1.22	Wb
Thermo	MA5-17253	WK3417172	AB_2538721	monoclonal	AF14D7	mouse	not provided	Wb
Thermo	PA5-51951	WK3417134	AB_2639314	polyclonal	-	rabbit	0.10	IF
GeneTex	GTX33065	822104511	AB_2895292	polyclonal	-	rabbit	1.22	Wb

Wb=Western blot, IF=immunofluorescence

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Abcam	ab255449	CVCL_0063	HEK293T	WT
Abcam	ab266245	CVCL_B2VE	HEK293T	*CTSH* KO
Horizon discovery	C631	CVCL_Y019	HAP1	WT
ATCC	CRL-1687	CVCL_0186	BxPC-3	WT
ATCC	CRL-1837	CVCL_3881	SU.86.86	WT
ATCC	TIB-202	CVCL_0006	THP-1	WT
ATCC	CRL-1682	CVCL_0152	AsPC-1	WT
ATCC	HTB-43	CVCL_1218	FaDu	WT

Figure 1: Pro-cathepsin H antibody screening by immunoblot.

A) Lysates of HEK293T (WT and *CTSH* KO) were prepared and 100 µg of protein were processed for immunoblot with the indicated Pro-cathepsin H antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: AF7516 at 1/1000; ab88960 at 1/1000; ab185935 at 1/1000; MA5-17253 at 1/1000; PA5-51951 at 1/500; GTX33065 at 1/1000. Predicted band size: 37 kDa.

B) Comparison of Pro-cathepsin H protein level in different cell lines. Lysates were prepared from HEK293T (WT and *CTSH* KO), HAP1, BxPC-3, SU.86.86, THP-1, AsPC-1 and FaDu. Expression (RNA 21Q3 Public; log2(TPM+1); red) and proteomics (P21579; relative protein abundance, blue) values for each cell line were taken from DepMap (depmap.org). n/a=not available. exp.=exposure.

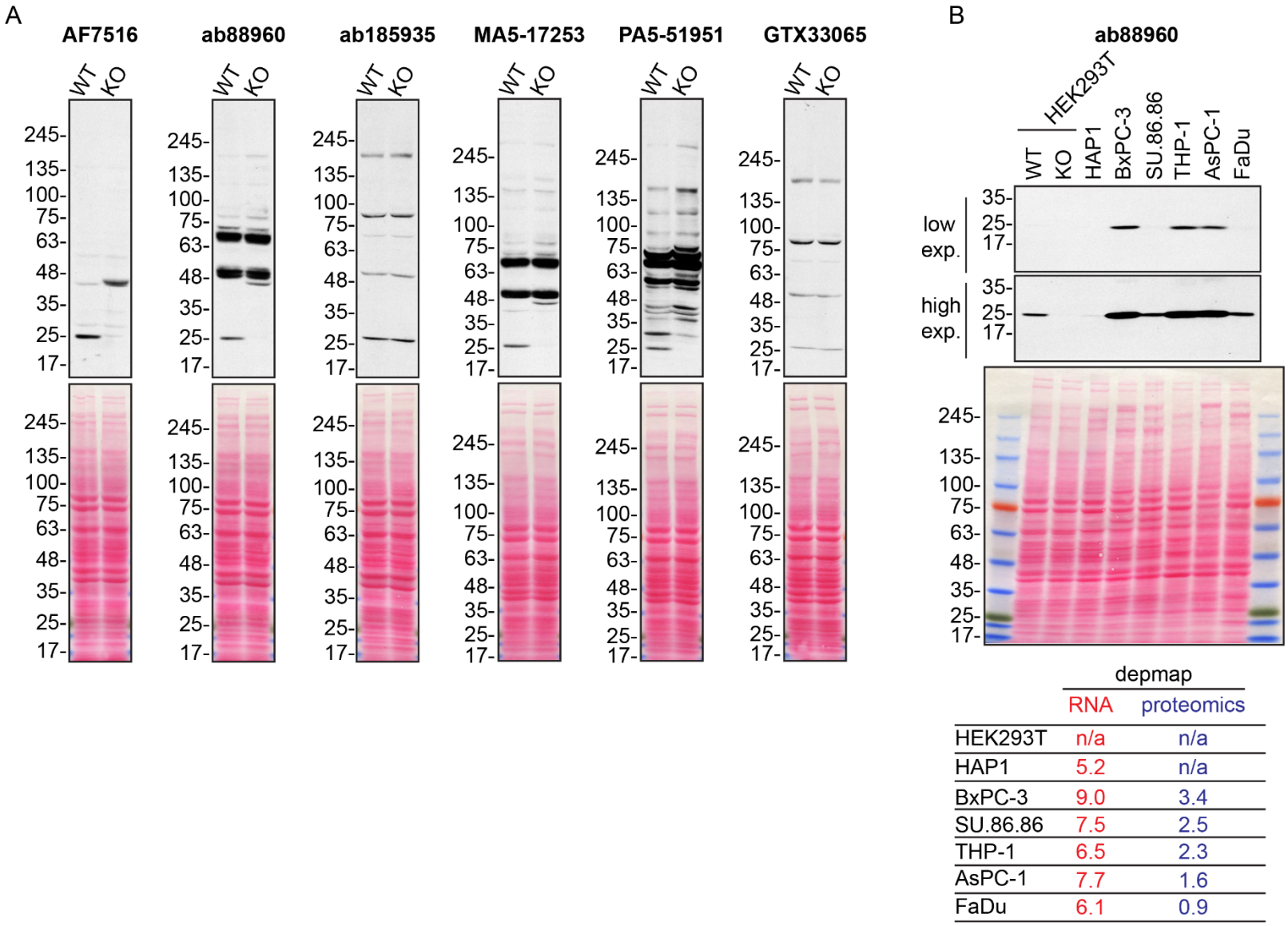
Figure 2: Pro-cathepsin H antibody screening by immunoprecipitation.

HEK293T lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Pro-cathepsin H antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Pro-cathepsin H antibody. For immunoblot, ab88960 was used at 1/1000 and AF7516 at 1/1000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; LC= antibody light chain.

Figure 3: Pro-cathepsin H antibody screening by.

A) HEK293T WT and *CTSH* 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 Pro-cathepsin H 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: AF7516 at 1/200; ab88960 at 1/500; ab185935 at 1/1000; MA5-17253 at 1/500; PA5-51951 at 1/100; GTX33065 at 1/1000. 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.



	depmap	
	RNA	proteomics
HEK293T	n/a	n/a
HAP1	5.2	n/a
BxPC-3	9.0	3.4
SU.86.86	7.5	2.5
THP-1	6.5	2.3
AsPC-1	7.7	1.6
FaDu	6.1	0.9

Figure 1: Pro-cathepsin H antibody screening by immunoblot

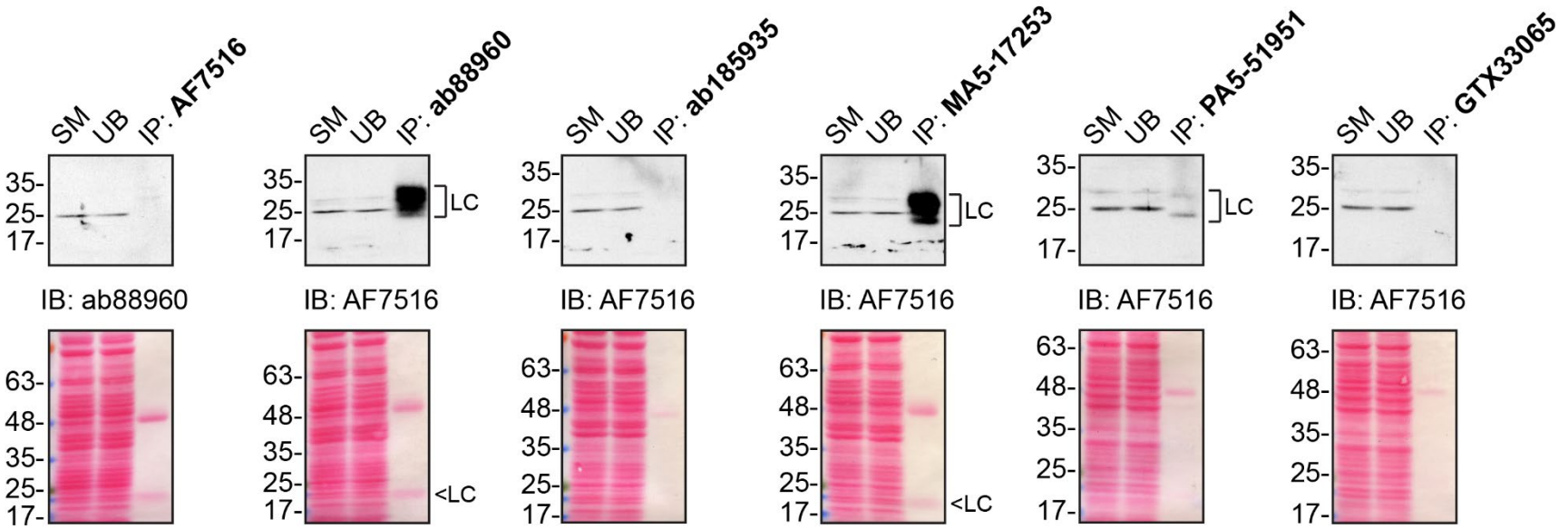


Figure 2: Pro-cathepsin H antibody screening by immunoprecipitation

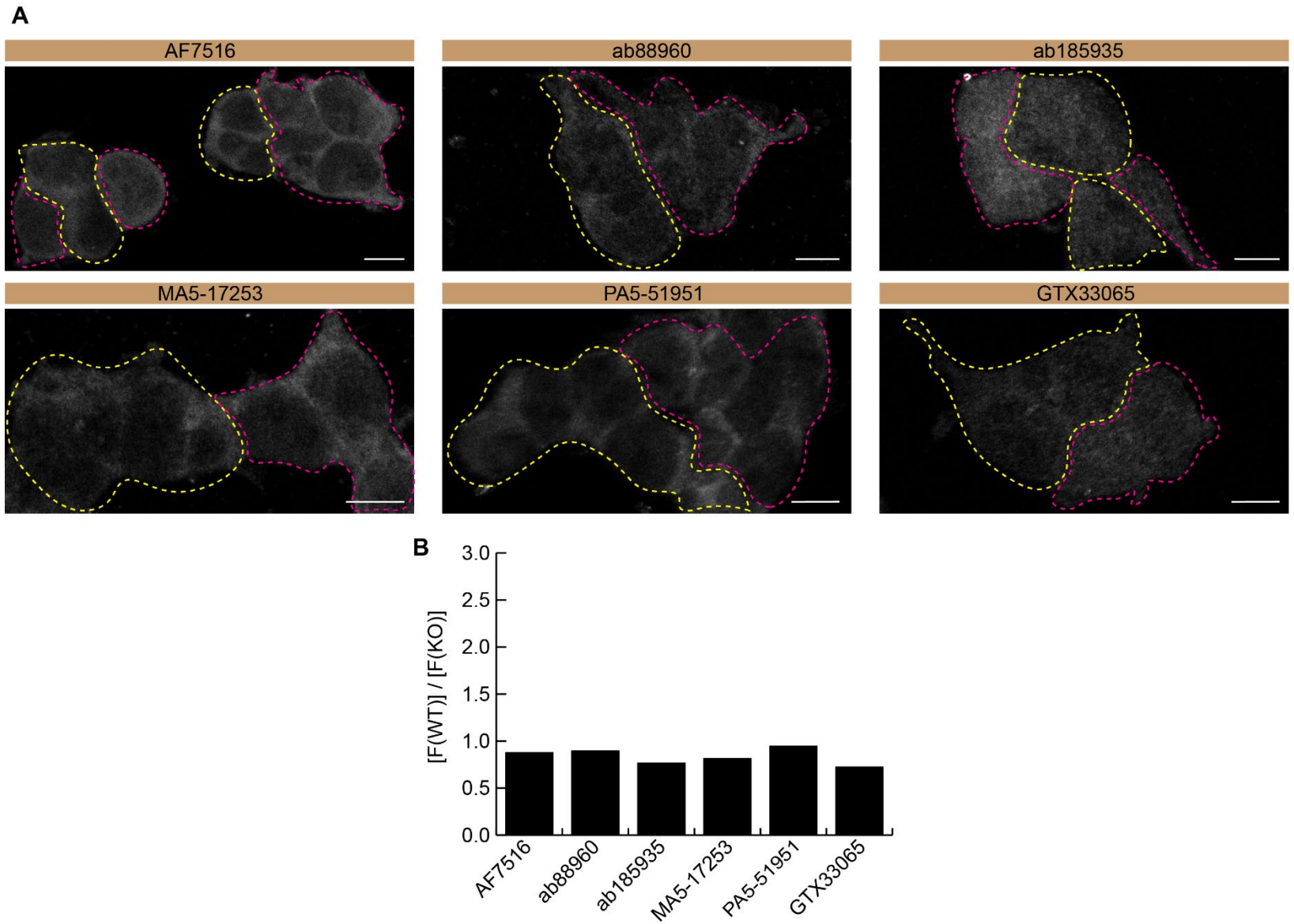


Figure 3 : Pro-cathepsin H antibody screening by immunofluorescence

Materials and methods

Antibodies

All Pro-cathepsin H 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]. HEK293T (WT and *CTSH* 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 µg or 10 µl of antibody at an unknown

concentration 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 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.

HEK293T 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 1 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.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [7]. HEK293T WT and *CTSH* 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 Pro-cathepsin H 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 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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