Antibody Characterization Report for Progranulin

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Progranulin

Alternative name: PGRN

Gene name: GRN

Uniprot: P28799

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. This report guides researchers to select the most appropriate antibodies for Progranulin. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Progranulin by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HEK293T was selected based on evidence of appropriate Progranulin protein expression determined through public proteomics databases, namely PaxDB [3] and DepMap [4, 5]. A HEK293T *GRN* KO line is available at Abcam.

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 Progranulin antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-techne	NBP2-61425*	MAB-03226	AB_2885160	monoclonal	CL5695	mouse	1.00	Wb
Aviva Systems Biology	ARP54642	QC72684-190222	AB_2904510	polyclonal	-	rabbit	0.50	Wb
Thermo	710191**	QK228692	AB_2532616	recombinant-poly	2HCLC	rabbit	0.50	Wb, IF
Thermo	MA5-35653**	VL3153025	AB_2849553	recombinant-mono	ARC1151	rabbit	2.00	Wb
Thermo	MA1-187*	VI307452	AB_2536878	monoclonal	2D4-2F1	mouse	1.00	Wb
Abcam	ab187070**	GR3256694-2	AB_2885106	recombinant-mono	EPR18539-59	rabbit	0.61	Wb, IP, IF
Abcam	ab208777**	GR3276244-2	AB_2885113	recombinant-mono	EPR15864	rabbit	0.22	Wb, IP
GeneTex	GTX100803	39694	AB_1240920	polyclonal	-	rabbit	0.48	Wb, IF
ABclonal	A12440	5500002505	AB_2759283	polyclonal	-	rabbit	1.72	Wb, IF

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Abcam	ab255449	CVCL_0063	HEK293T	WT
Abcam	ab266738	CVCL_B2Y9	HEK293T	*GRN* KO

Figure 1: Progranulin antibody screening by immunoblot.

A) 50 µg of total lysates were processed for immunoblot with the indicated Progranulin antibodies. The Ponceau stained transfers of each blot are shown. All antibodies were diluted at 1/5000. Progranulin predicted band size: 64 kDa. *=monoclonal antibody, **=recombinant antibody

B) Progranulin is a secreted protein. HEK293T cells (WT and *GRN* KO) were treated with Brefeldin A at 3.0 µg/ml for various time points. Lysates were prepared, and 25 µg of protein were processed for immunoblot with Progranulin (Abcam, ab208777) and Peroxiredoxin-1 (Proteintech, 15816-AP) antibodies. The Ponceau stained transfers of each blot are shown. Progranulin predicted band size: 64 kDa.

Figure 2: Progranulin antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on concentrated culture media using 1.0 µg of the indicated Progranulin antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Progranulin antibodies. For immunoblot, ab208777 was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody, **=recombinant antibody.

Figure 3: Progranulin antibody screening by immunofluorescence.

A) HEK293T WT and *GRN* 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 Progranulin 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) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: NBP2-61425 at 1/500; ARP54642 at 1/500; 710191 at 1/500; MA5-35653at 1/1000; MA1-187 at 1/1000; ab187070 at 1/600; ab205137 at 1/200; ab208777 at 1/200; ab252834 at 1/1000; GTX100803 at 1/500; A12440 at 1/500. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

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. A minimum of 20 WT and 20 KO cells from 3 different fields of view were analysed for each antibody.

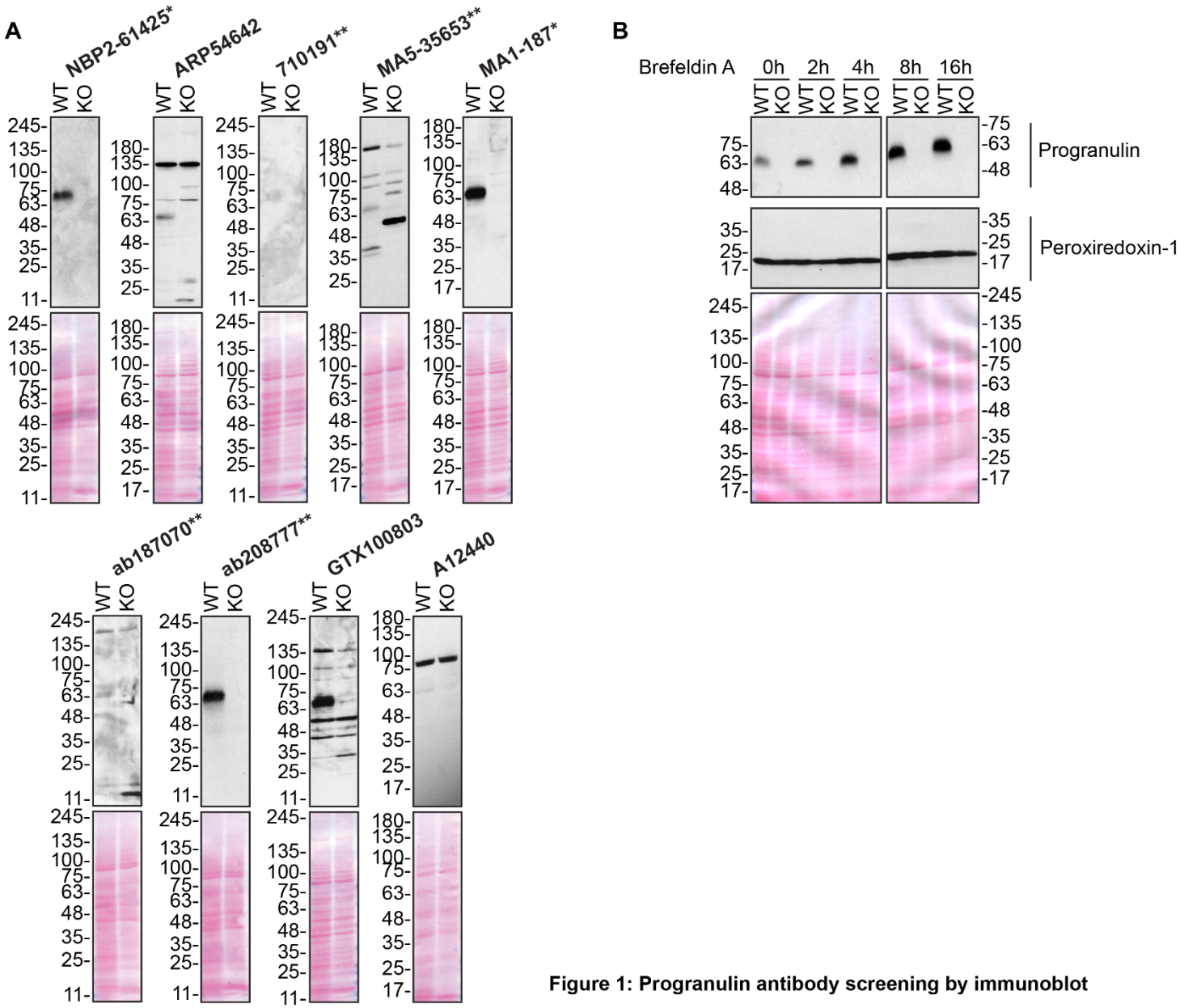

Figure 1: Progranulin antibody screening by immunoblot

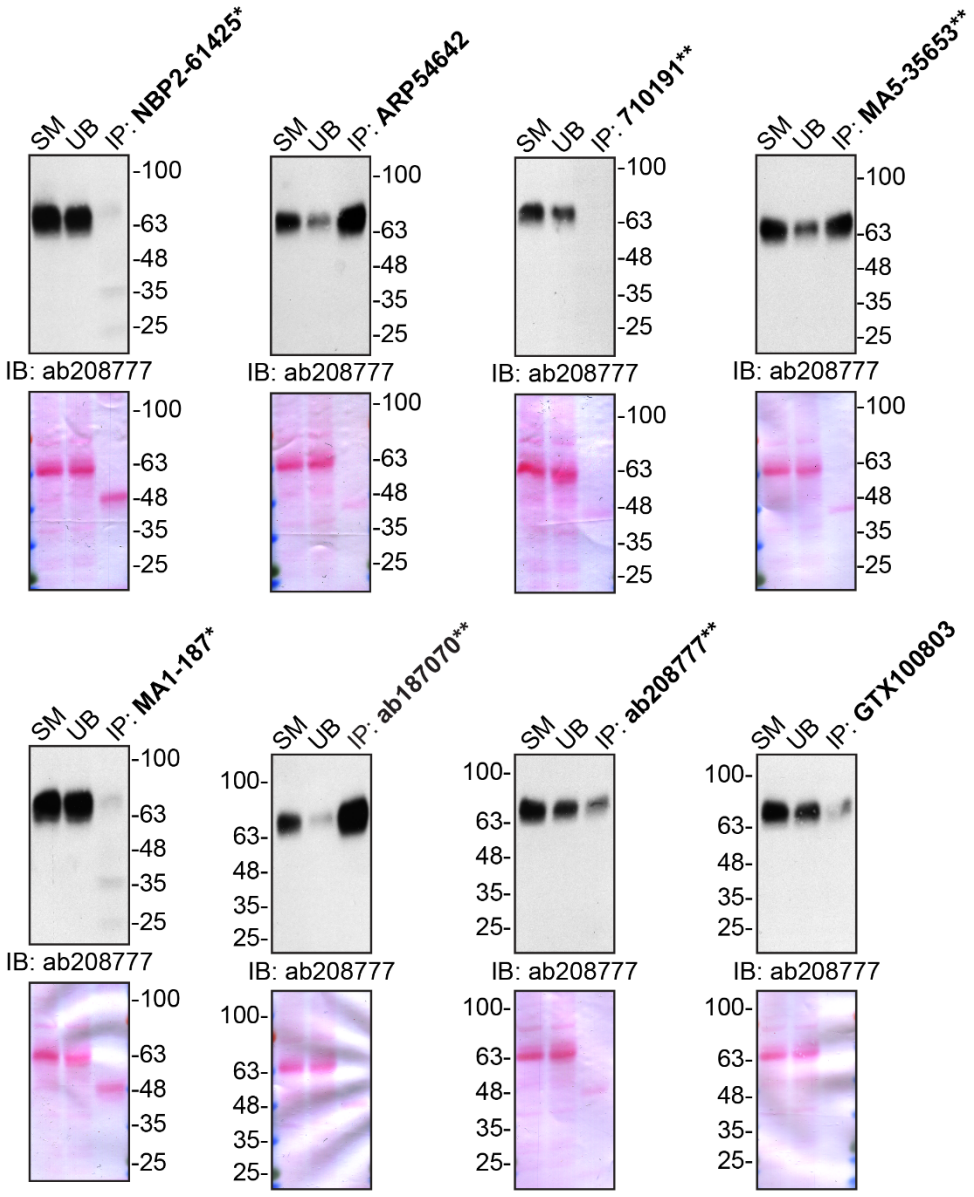

Figure 2: Progranulin antibody screening by immunoprecipitation on culture media

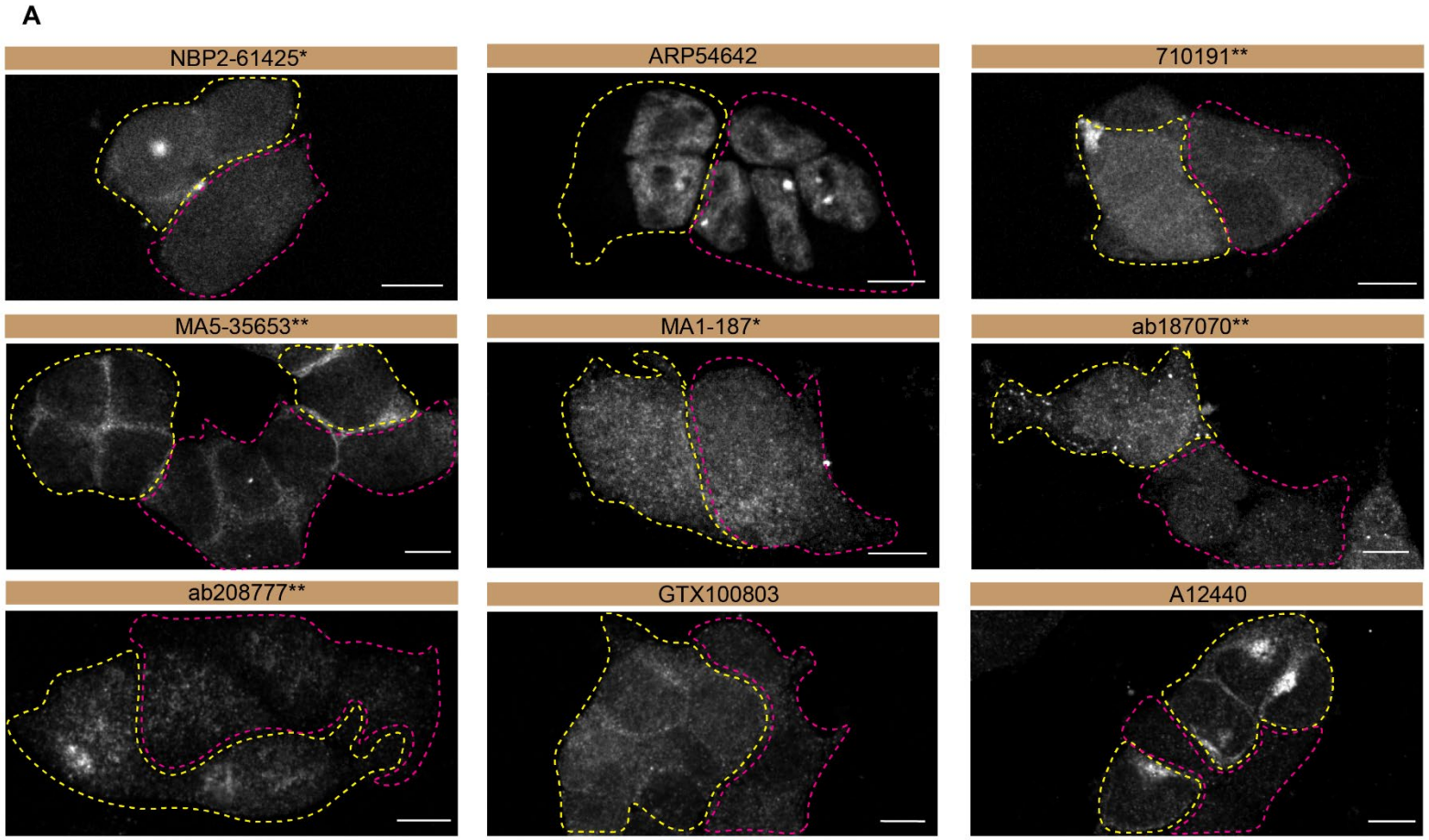

Figure 3: Progranulin antibody screening by immunofluorescence (1/2)

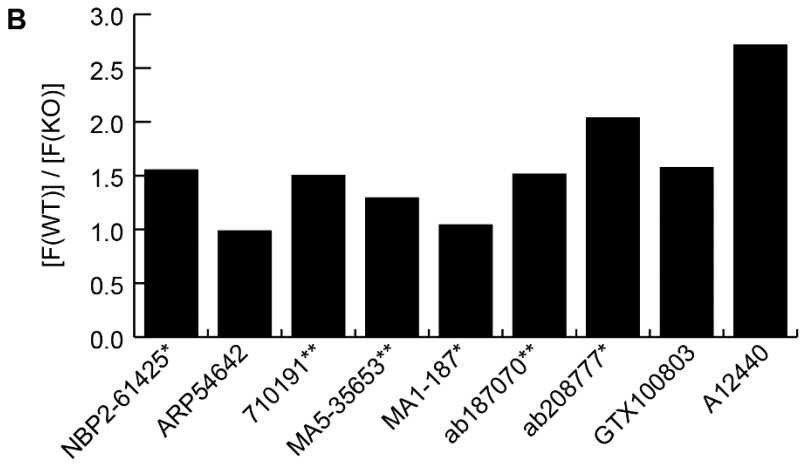

Figure 3: Progranulin antibody screening by immunofluorescence (2/2)

Materials and methods

Antibodies

All Progranulin 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). Cells were starved in DMEM high glucose containing L-glutamate and penicillin/ streptomycin.

Collection of culture media

HEK293T cells (WT and *GRN* KO) were washed 3x with PBS and starved for ~18 hrs. Culture media were collected and centrifuged for 10 min at 500 x g to eliminate cells and larger contaminants, then for 10 min at 4500 x g to eliminate smaller contaminants. Culture media were concentrated by centrifuging at 4000 x g for 10min using Amicon Ultra-15 Centrifugal Filter Units with a membrane NMWL of 50kDa (MilliporeSigma cat. number UFC905024). Immunoblots were performed as described above.

Antibody screening by immunoblot

Immunoblots were performed as described in our standard operating procedure [6]. HEK293T (WT and *GRN* 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 5-16% 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 µg or 2 µl of antibody at an unknown concentration to 500 ul of Pierce IP 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 (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) 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.

Starved HEK293T WT culture media were concentrated as described above. 1ml aliquots at 0.3 mg/ml of protein 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 IP Lysis Buffer and processed for SDS-PAGE and immunoblot on 5-16% 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.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [8]. HEK293T WT and *GRN* 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 Progranulin 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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