Antibody Characterization Report for Matrin-3

YCharOS Antibody Characterization Report

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Target:
Recommended protein name: Matrin-3
Gene name: *MATR3*
Uniprot: P43243

This report guides researchers to select the most appropriate antibodies for Matrin-3. We used an antibody characterization pipeline [1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Matrin-3 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. A synthetic human single-chain fragment variable (scFv) was tested and a protocol on how to properly use scFv for research purposes is available [2]. HAP1 was selected based on evidence of appropriate Matrin-3 expression determined through DepMap, a public database [3, 4]. A *MATR3* HAP1 KO line is available at Horizon Discovery.

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. Preger, C., et al., *Generation and validation of recombinant antibodies to study human aminoacyl-tRNA synthetases.* J Biol Chem, 2020. **295**(41): p. 13981-13993.
3. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16.
4. Ghandi, M., et al., *Next-generation characterization of the Cancer Cell Line Encyclopedia.* Nature, 2019. **569**(7757): p. 503-508.

Table 1: Summary of the Matrin-3 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo	PA5-57720	VL3152384D	AB_2643782	polyclonal	-	rabbit	0.10	Wb, IF
Thermo	MA5-34642	VL3152618	AB_2848550	recombinant-mono	JU93-43	rabbit	1.00	Wb, IF
Abcam	ab151714	GR292211-7	AB_2491618	recombinant-mono	EPR10635(B)	rabbit	1.49	Wb, IF
Abcam	ab151739	GR108770-5	AB_2885091	recombinant-mono	EPR10634(B)	rabbit	0.52	Wb, IF
GeneTex	GTX115291	40653	AB_11178352	polyclonal	-	rabbit	0.86	Wb, IF
Human Protein Atlas	HPA036565	B116350	AB_10673623	polyclonal	-	rabbit	0.10	Wb, IF
Bio-Techne	NB100-1761	A1	AB_2266219	polyclonal	-	rabbit	0.20	WB, IF, IP
BETHYL	A300-591A-T	A300-591A-T-1	AB_495514	polyclonal	-	rabbit	0.20	Wb, IP, IF
Structural Genomics Consortium *(available at Addgene)*	Z-MATR3-4***** *(Addgene plasmid #166558)*	YSMATR3A-c001		recombinant-mono *(single chain-Fv)*	Z-MATR3-4	human	1.04	IP

Wb=Western blot; IP= immunoprecipitation; IF=immunofluorescence

***Sequence of Z-MATR3-4 single chain-Fv**:
AEVQLLESGGGLVQPGGSLRLSCAASGFTFSSSSMYWVRQAPGKGLEWVSSIGSGGSYTGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVY
YCARPSHGSYSGVYFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAA
SSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGTAPHTFGQGTKLEIKRTDYKDHDGDYKDHDIDYKDDDDKAAAHHHHHH*
Tag on the single chain-Fv: 3xFLAG, His6

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	HZGHC007500c005	-	HAP1	Matrin-3 KO

Figure 1: Matrin-3 antibody screening by immunoblot.
Lysates of HAP1 (WT and *MATR3* KO) were prepared and 20 µg of protein were processed for immunoblot with the indicated Matrin-3 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: PA5-57720 at 1/200; MA5-34642 at 1/1000; ab151714 at 1/10000; ab151739 at 1/10000; GTX115291 at 1/500; HPA036565 at 1/2000; NB100-1761 at 1/5000; A300-591A-T at 1:1000; Z-MATR3-4 at 1:1000. Predicted band size: 95 kDa.

Figure 2: Matrin-3 antibody screening by immunoprecipitation.
HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Matrin-3 antibodies. **(A)** Ability of the antibodies to capture Matrin-3 was first assessed by comparing the level of Matrin-3 available in the starting material (SM) to its level remaining in the unbound fraction (UB). Antibody NB100-1761 was used for the immunoblot. **(B)** Analysis of the immunoprecipitates for antibodies which showed specificity for Matrin-3 in (A). Antibodies PA5-57720, ab151714 and MA5-34642 were used at 1/200, 1/2000 and 1/500, respectively. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain.

Figure 3: Matrin-3 antibody screening by immunofluorescence.
HAP1 WT and *MATR3* 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 Matrin-3 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: PA5-57720 at 1/2000; MA5-34642 at 1/1000; ab151714 at 1/1500; ab151739 at 1/500; GTX115291 at 1/900; HPA036565 at 1/100; NB100-1761 at 1/200; A300-591A-T at 1:200; Z-MATR3-4 at 1:1000. Bars = 10 µm.

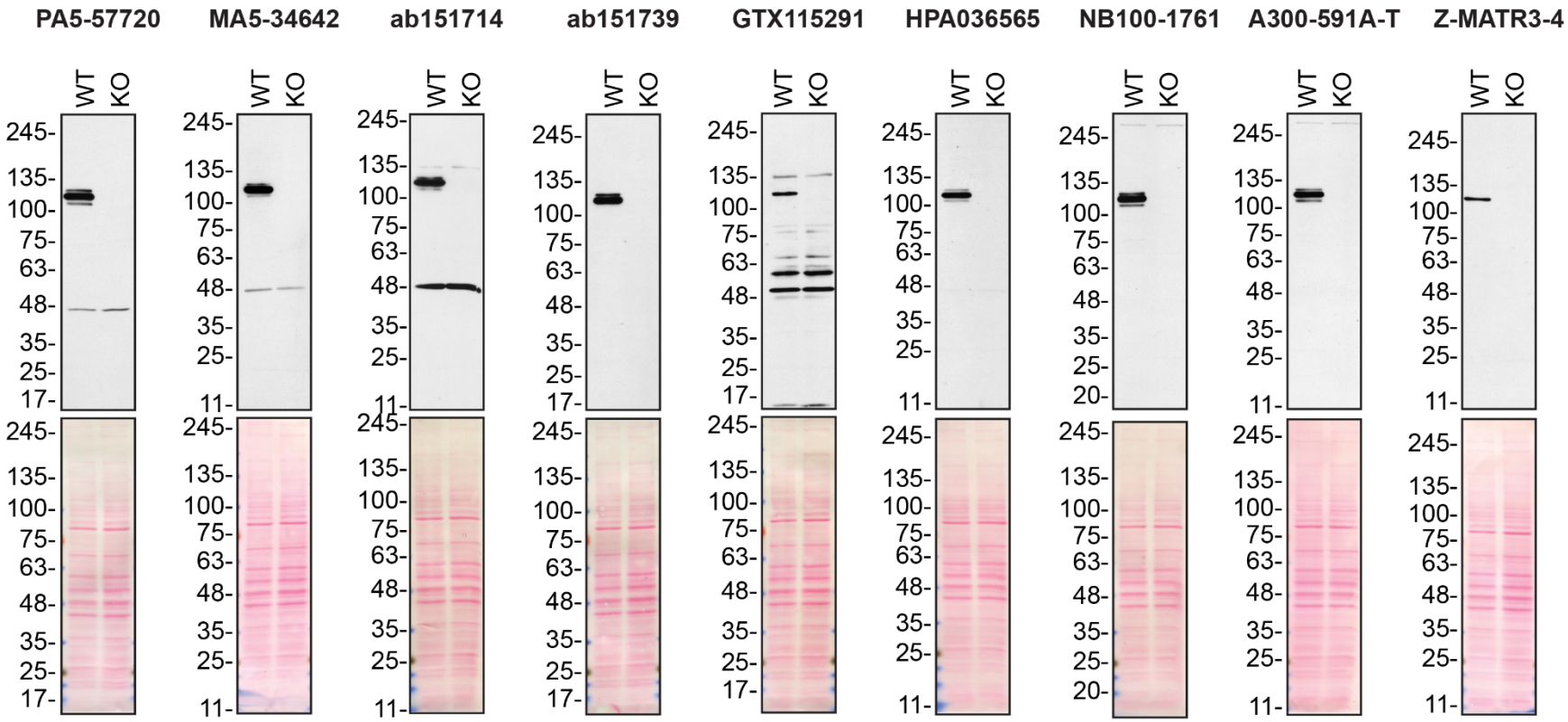

Figure 1: Matrin-3 antibody screening by immunoblot

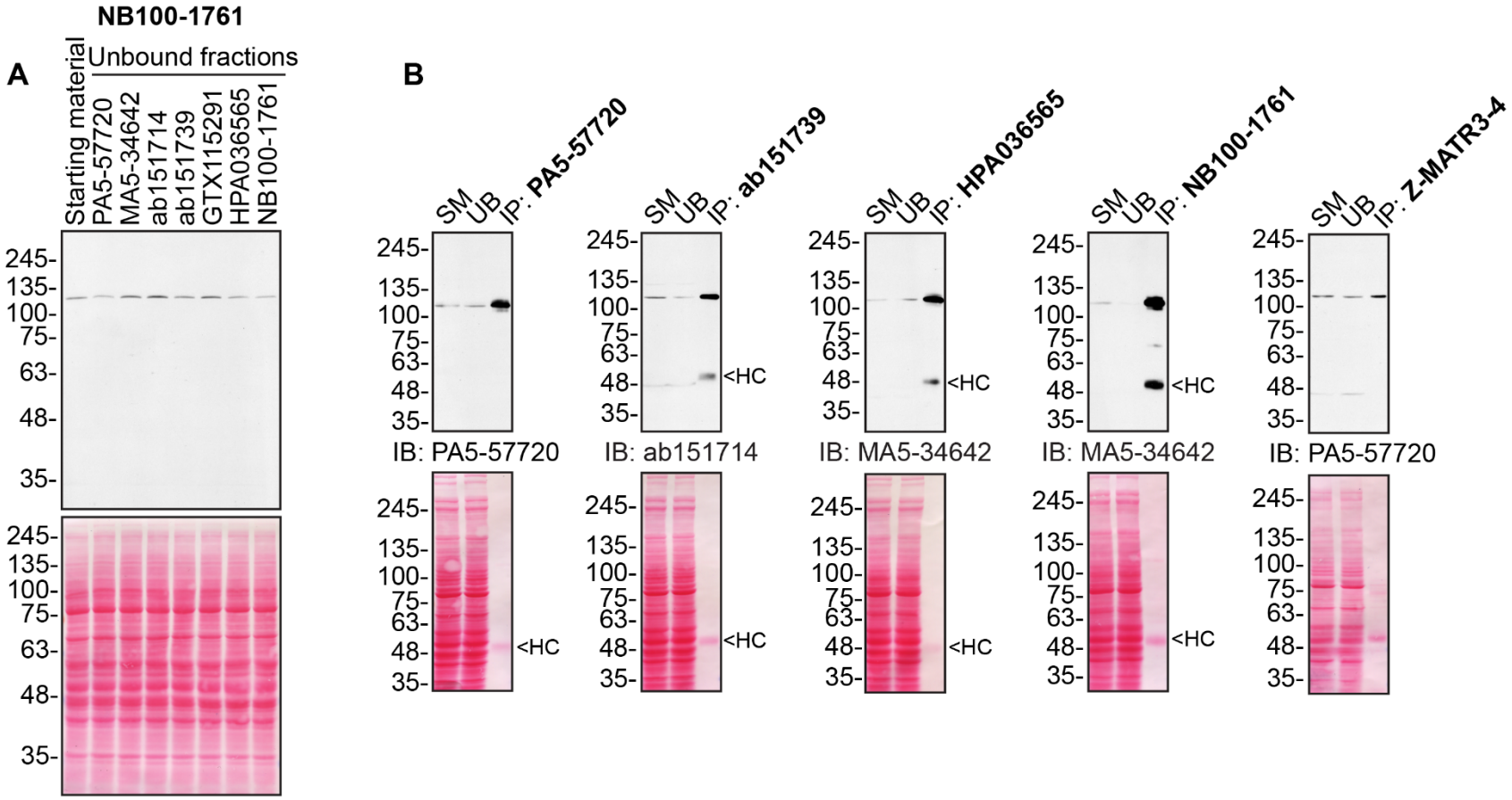

Figure 2: Matrin-3 antibody screening by immunoprecipitation

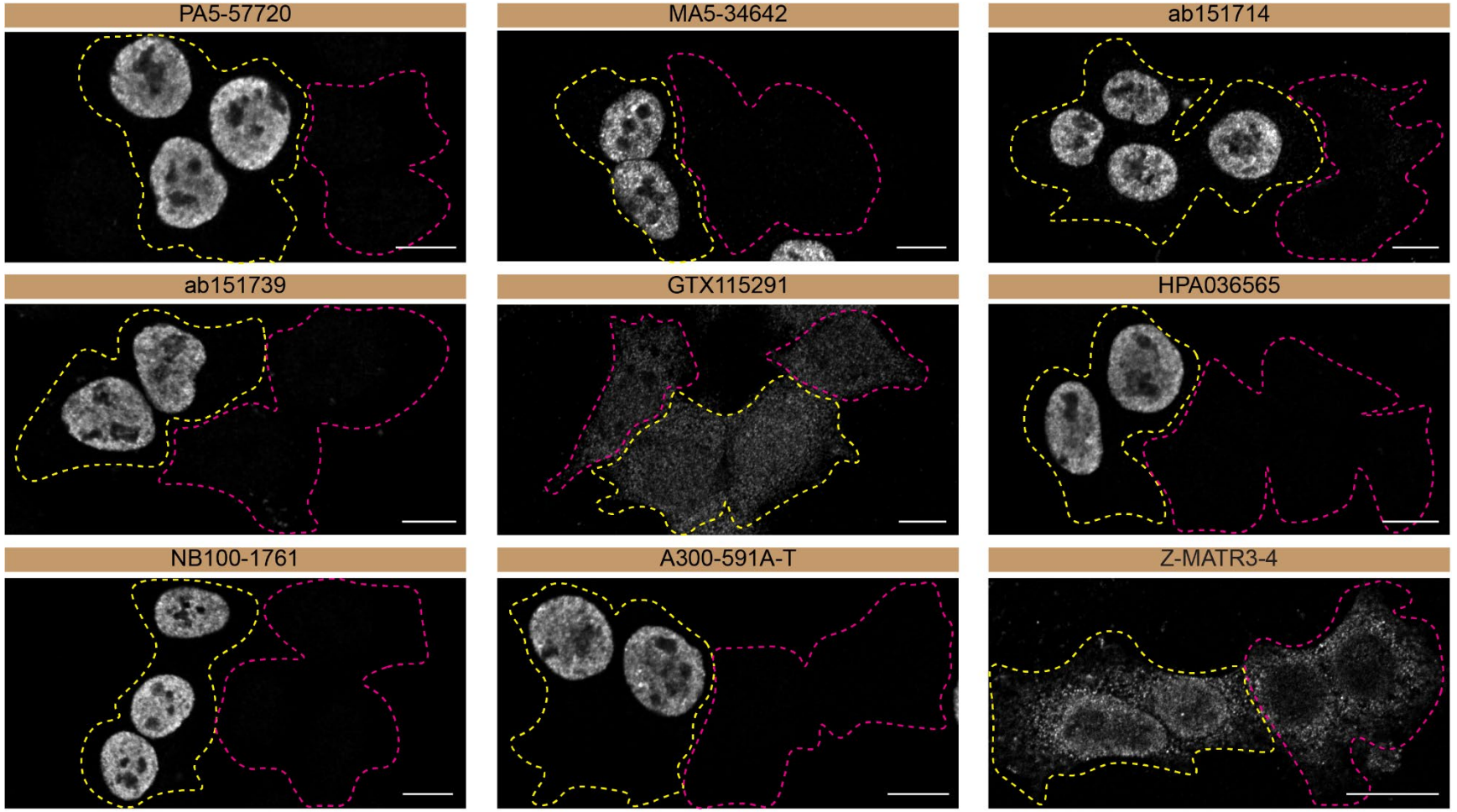

Figure 3 : Matrin-3 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Matrin-3 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-rabbit antibody is from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520). Peroxidase-conjugated mouse anti-Flag M2 antibody is from MilliporeSigma (cat. number A8592).

Alexa-555-conjugated goat anti-rabbit secondary antibody is from Thermo Fisher Scientific (cat. number A21429). Cy3-conjugated mouse anti-Flag M2 antibody is from MilliporeSigma (cat. number A9594).

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

HAP1 (WT and *MATR3* 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 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

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-Sepharose beads (for rabbit antibodies) or anti-FLAG M2 magnetic beads (for synthetic single chain Fv antibodies). Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

HAP1 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 4-15% polyacrylamide gels. Prot-A: HRP was used as a secondary detection system (MilliporeSigma, cat. number P8651) at a dilution of 0.4 µg/ml.

Antibody screening by immunofluorescence

HAP1 WT and Matrin-3 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 Matrin-3 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.