Antibody Characterization Report for RNA-binding protein FUS

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: RNA-binding protein FUS

Short protein name: FUS

Gene name: FUS

Uniprot: P35637

This report guides researchers to select the most appropriate antibodies for FUS. We used an antibody characterization pipeline [1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for FUS by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HeLa was selected based on evidence of appropriate FUS protein expression determined through public proteomics databases, namely PaxDB [2] and DepMap [3]. HeLa was modified with CRISPR/Cas9 to knockout the corresponding *FUS* gene [4].

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 FUS antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-techne	NBP2-52874	MAB-03520	AB_2885157	monoclonal	CL0190	mouse	1.00	Wb, IF
GeneTex	GTX101810	40366	AB_2036972	polyclonal	-	rabbit	0.70	Wb
GeneTex	GTX01039	822100287	AB_2888934	monoclonal	JJ09-31	rabbit	1.00	Wb, IF
Proteintech	60160-1-Ig	10017695	AB_10666169	monoclonal	3A10B5	mouse	2.36	Wb, IP, IF
Proteintech	11570-1-AP	00086256	AB_2247082	polyclonal	-	rabbit	0.90	Wb, IP, IF
Thermo	MA3-089	vVB301448	AB_2633334	monoclonal	1FU-1D2	mouse	not provided	Wb, IF
Thermo	MA5-32483	VL3152611	AB_2809760	recombinant-mono	JJ09-31	rabbit	1.00	Wb, IF
Abcam	ab124923	GR85761-9	AB_10972861	recombinant-mono	EPR5812	rabbit	0.15	Wb, IF
Abcam	ab154141	GR3368481-1	AB_2885092	monoclonal	CL0190	mouse	1.00	Wb, IF
Abcam	ab243880	GR3376392-2	AB_2885123	recombinant-mono	BLR023E	rabbit	not provided	Wb, IP, IF

Wb=Western blot IP= immunoprecipitationIF=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_A8VH	HeLa	*FUS* KO

Figure 1: FUS antibody screening by immunoblot.

Lysates of HeLa (WT and *FUS* KO) were prepared and 30 µg of protein were processed for immunoblot with the indicated FUS antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used was 1/5000 for all tested antibodies. Predicted band size: 53 kDa. Observed specific band size: ~70 kDa.

Figure 2: FUS antibody screening by immunoprecipitation.

HeLa lysates were prepared and immunoprecipitation was performed using 1.0 µg of the indicated FUS antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated FUS antibody. For immunoblot, ab243880 and NBP2-52874 were used at a dilution of 1/2000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: FUS antibody screening by immunofluorescence.

HeLa WT and *FUS* 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 FUS 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. Dilution used: NBP2-52874 at 1/1000; GTX101810 at 1/700; GTX01039 at 1/1000; 60160-1-Ig at 1/2000; 11570-1-AP at 1/1000; MA3-089 at 1/1000; MA5-32483 at 1/1000; ab124923 at 1/1000; ab154141 at 1/1000; ab243880 at 1/500. Bars = 10 µm.

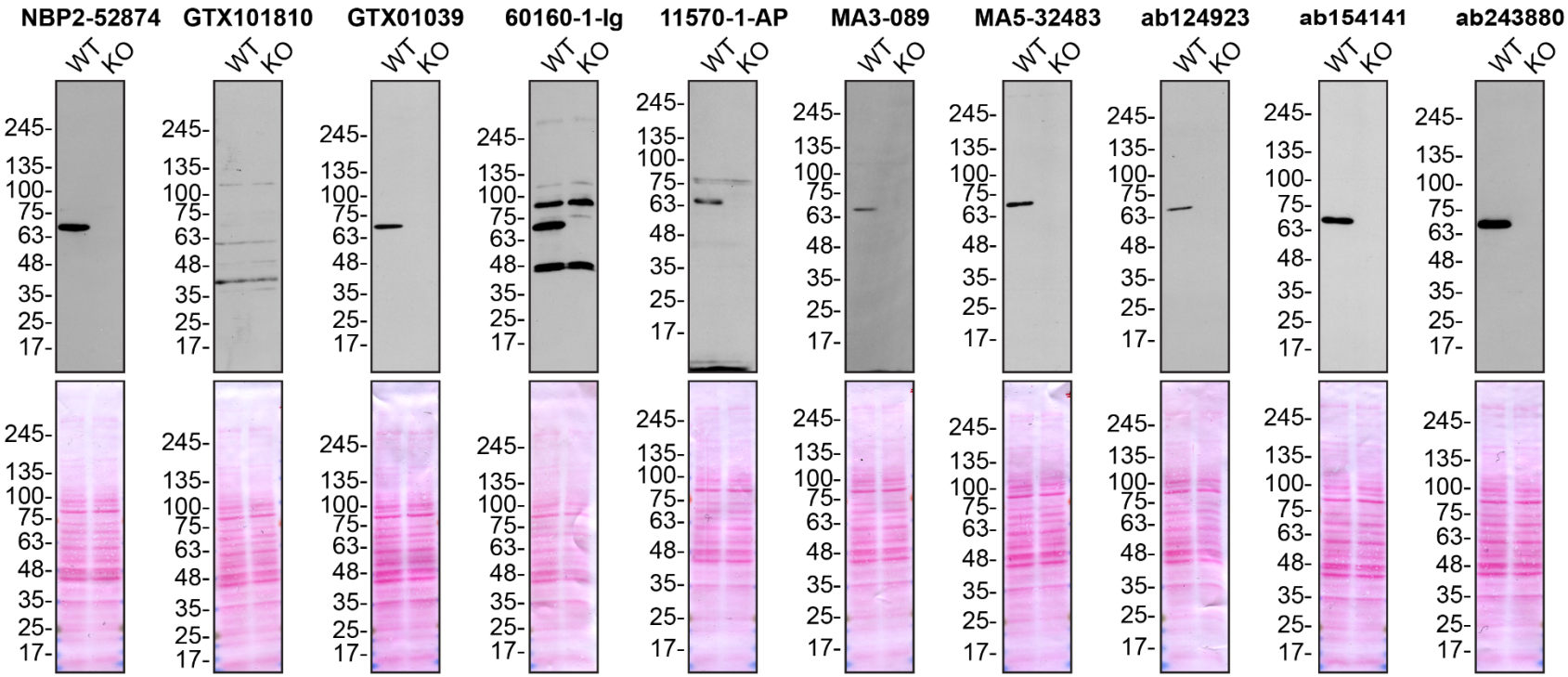

Figure 1: FUS antibody screening by immunoblot

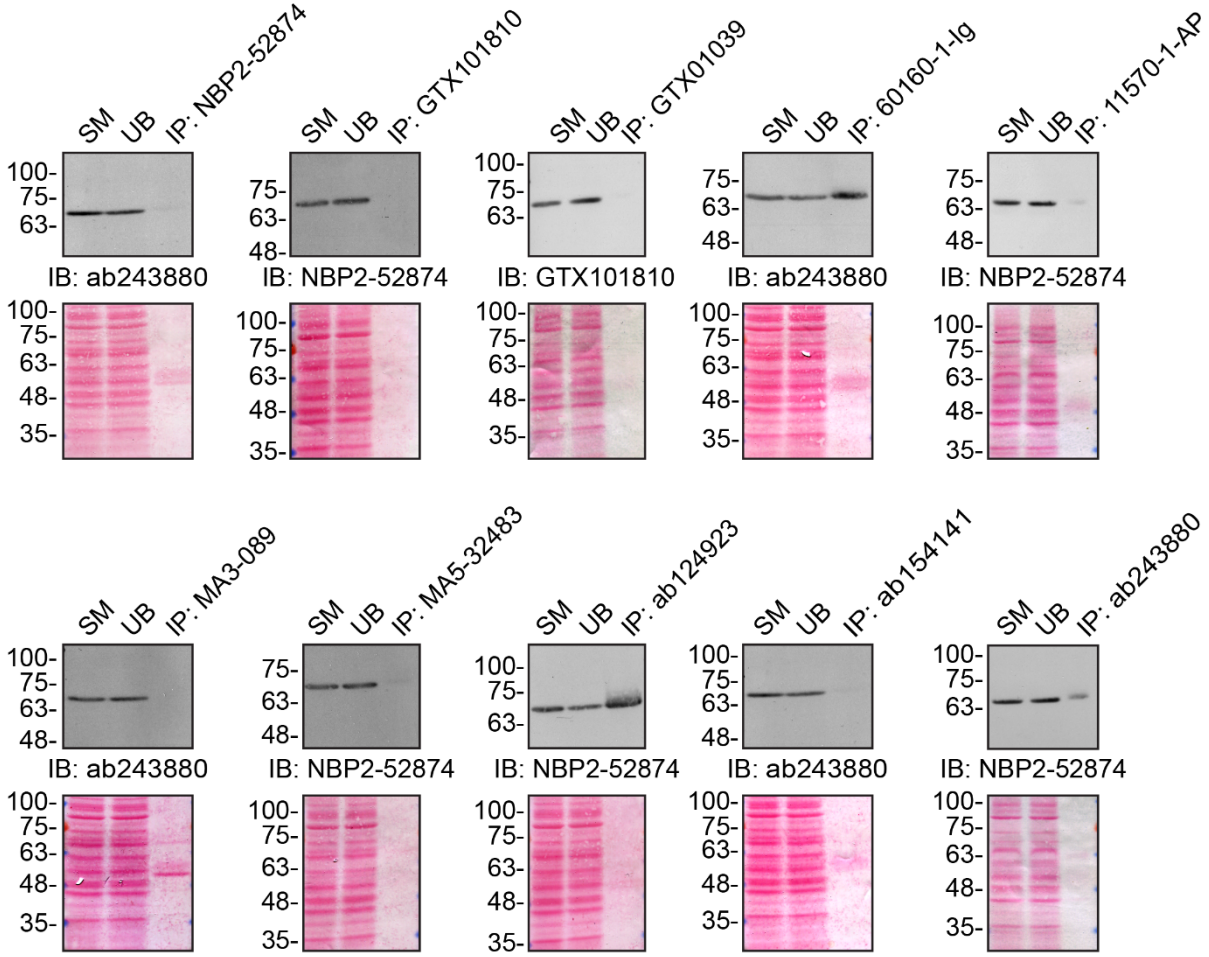

Figure 2: FUS antibody screening by immunoprecipitation

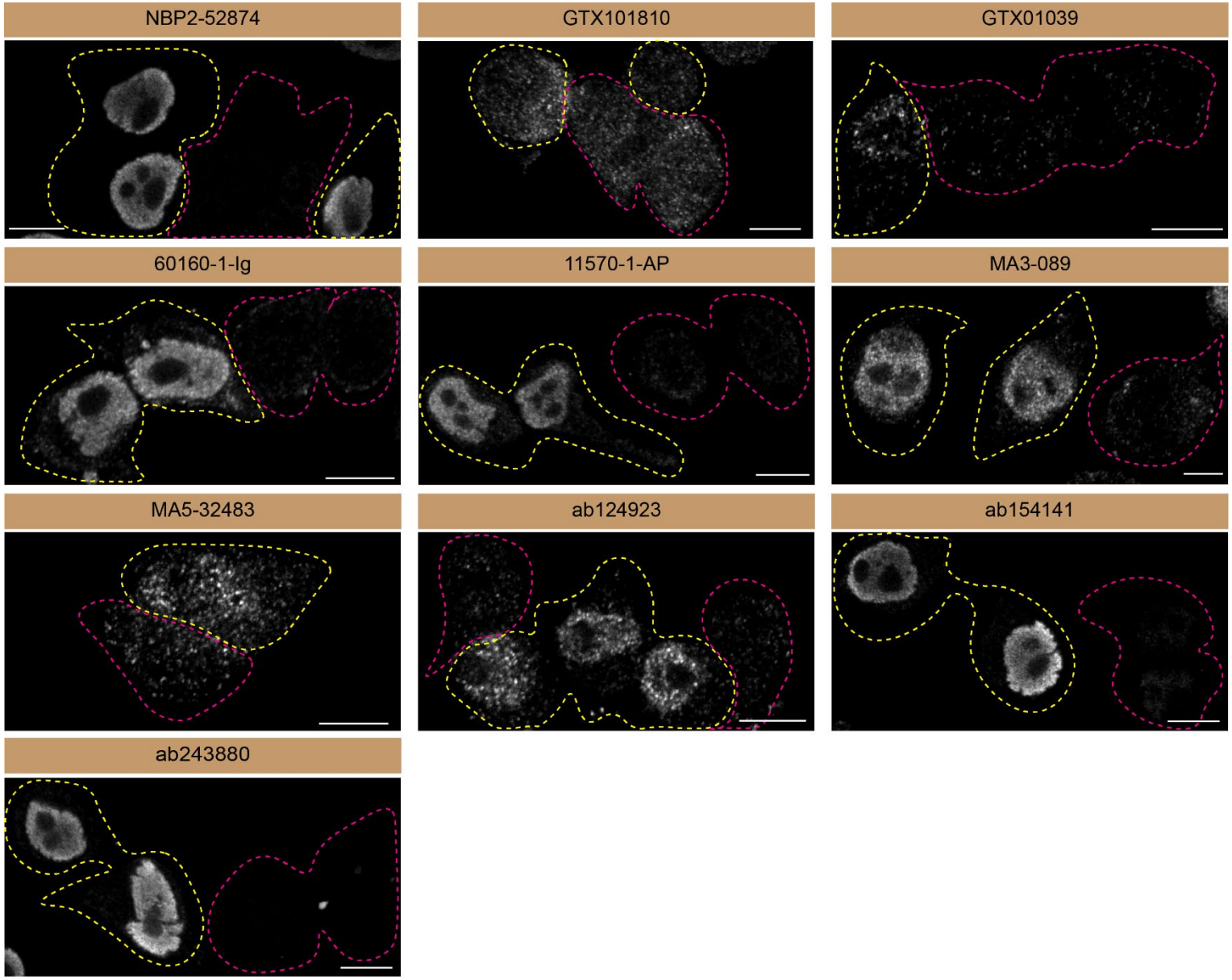

Figure 3: FUS antibody screening by immunofluorescence

Materials and methods

Antibodies

All FUS 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 *FUS* KO clone was generated with low passage cells. Two guide RNAs were used to introduce a large deletion in the *FUS* gene (sequence guide 1: AGGGAGUCACAAAAGCCACC, sequence guide 2: GGUACGGUGGUGUUGAUGUC).

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 *FUS* 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 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 1.0 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 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 5-16% polyacrylamide gels.

Antibody screening by immunofluorescence

HeLa WT and *FUS* 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 FUS 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.