Antibody Characterization Report for Spastin

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Spastin

Gene name: *SPAST*

Uniprot: Q9UBP0

This report guides researchers to select the most appropriate antibodies for Spastin. We used an antibody characterization pipeline [1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Spastin by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HEK293T and HAP1 cell lines were selected based on evidence of appropriate Spastin protein expression determined through public proteomics databases, namely PaxDB [2] and DepMap [3].

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** DOI: 10.7554/eLife.48363.
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 DOI: 10.1002/pmic.201400441.
3. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16 DOI: 10.1016/j.cell.2019.12.023.

Table 1: Summary of the Spastin antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo	PA5-53581	VL3152382B	AB_2647785	polyclonal	-	rabbit	0.20	Wb, IF
Thermo	MA5-27980	VL3152304	AB_2744995	recombinant-mono	RM346	rabbit	0.5	Wb
Abcam	ab31850	GR108838-2	AB_778036	monoclonal	Sp 3G11/1	mouse	1.00	Wb
Abcam	ab77144	GR141935-15	AB_1524436	monoclonal	Sp 6C6	mouse	1.00	Wb
Abcam	ab238311	GR3367830-4	AB_2885120	recombinant-mono	RM346	rabbit	not provided	Wb

Wb=Western blot IP= immunoprecipitation IF=immunofluorescence

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
ATCC	CRL-1573	CVCL_0045	HEK293T	WT
Abcam	ab267238	-	HEK293T	*SPAST* KO
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC007766c012	-	HAP1	*SPAST* KO

Figure 1: Spastin antibody screening by immunoblot.

Lysates of HEK293T and HAP1 (WT and *SPAST* KO) were prepared and 80 µg of protein were processed for immunoblot with the indicated Spastin antibodies. Middle panels show longer exposures. The Ponceau stained transfers of each blot are shown. Dilution used was 1/500 for all tested antibodies. Predicted band size: 67 kDa.

Figure 2: Spastin antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 1.0 µg of the indicated Spastin antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Spastin antibody. For immunoblot, ab77144 and ab31850 were used at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; v.= variant; HC=antibody heavy chain.

Figure 3: Spastin antibody screening by immunofluorescence.

HAP1 (top panels) and HEK293T (bottom panels) WT and *SPAST* KO were used for screening antibodies. WT and *SPAST* 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 Spastin 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: PA5-53581 at 1/200; MA5-27980 at 1/500; MA5-27980 at 1/1000; ab77144 at 1/1000; ab238311 at 1/500. Bars = 10 µm.

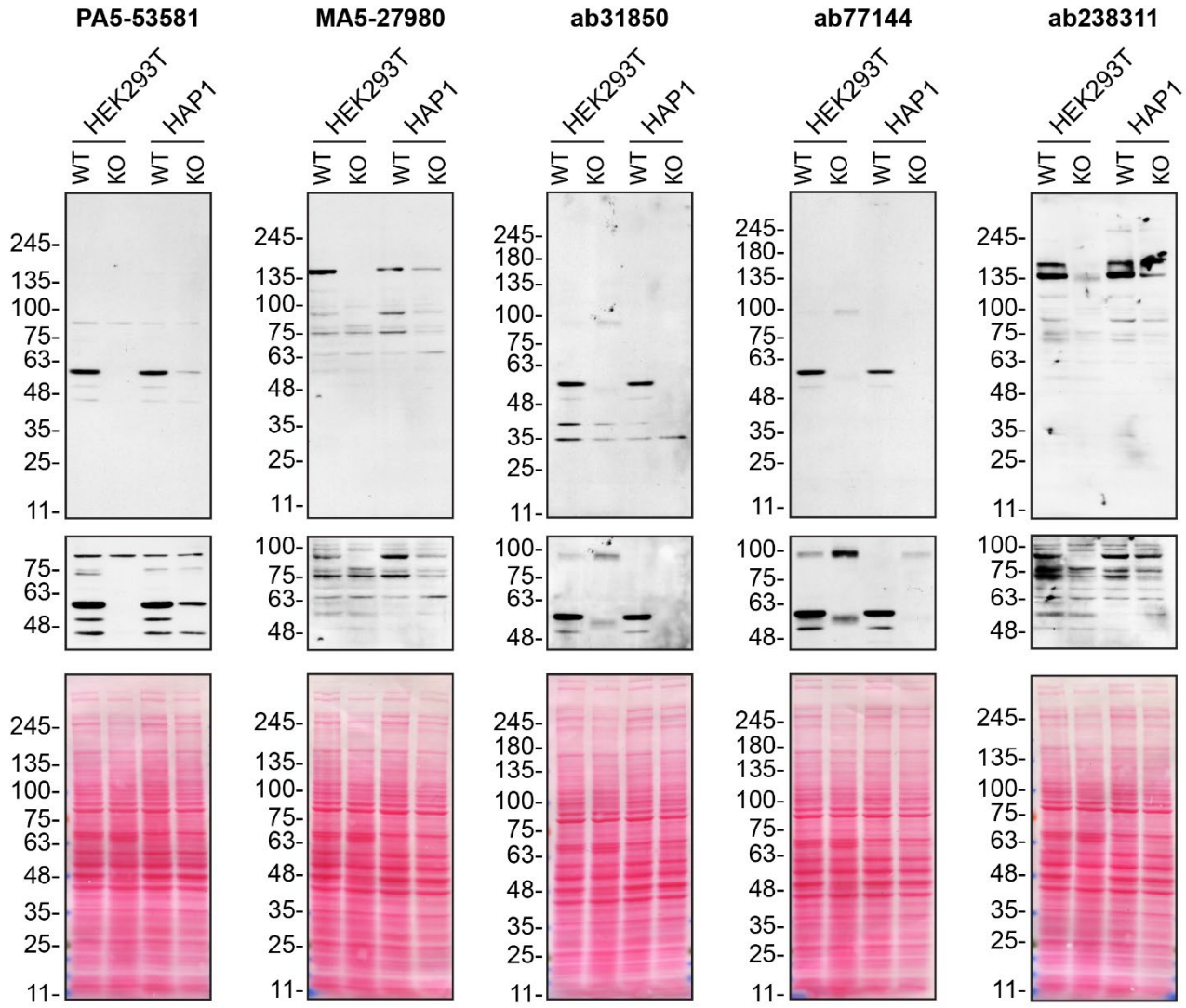

Figure 1: Spastin antibody screening by immunoblot

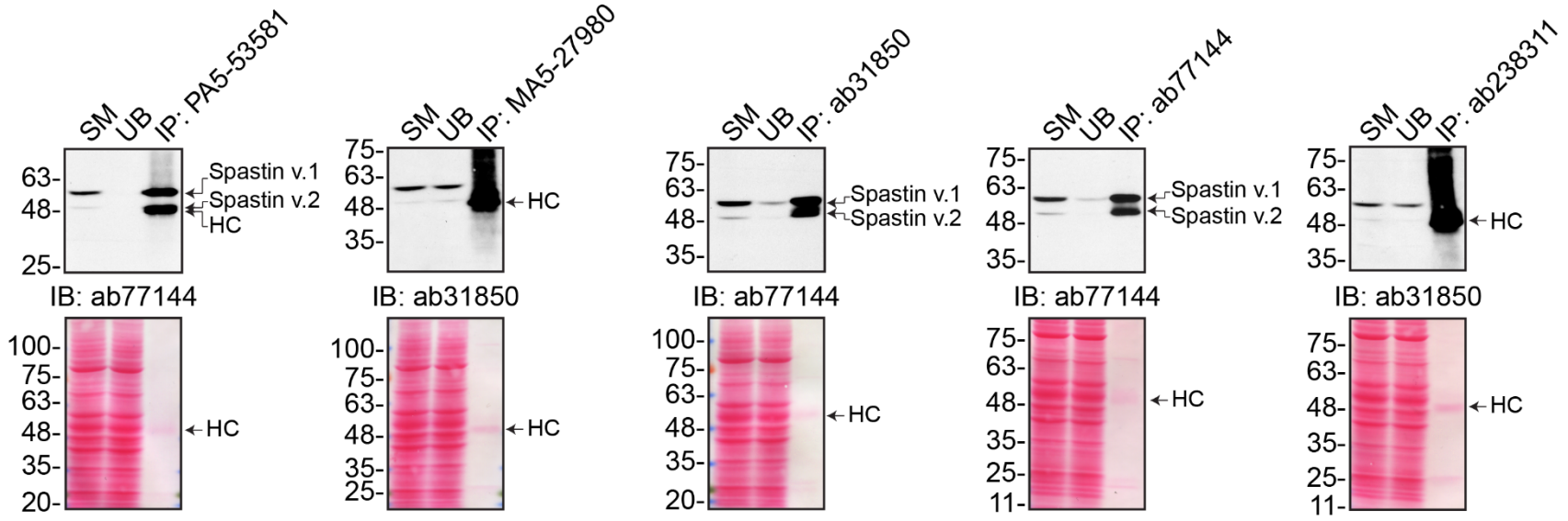

Figure 2 : Spastin antibody screening by immunoprecipitation

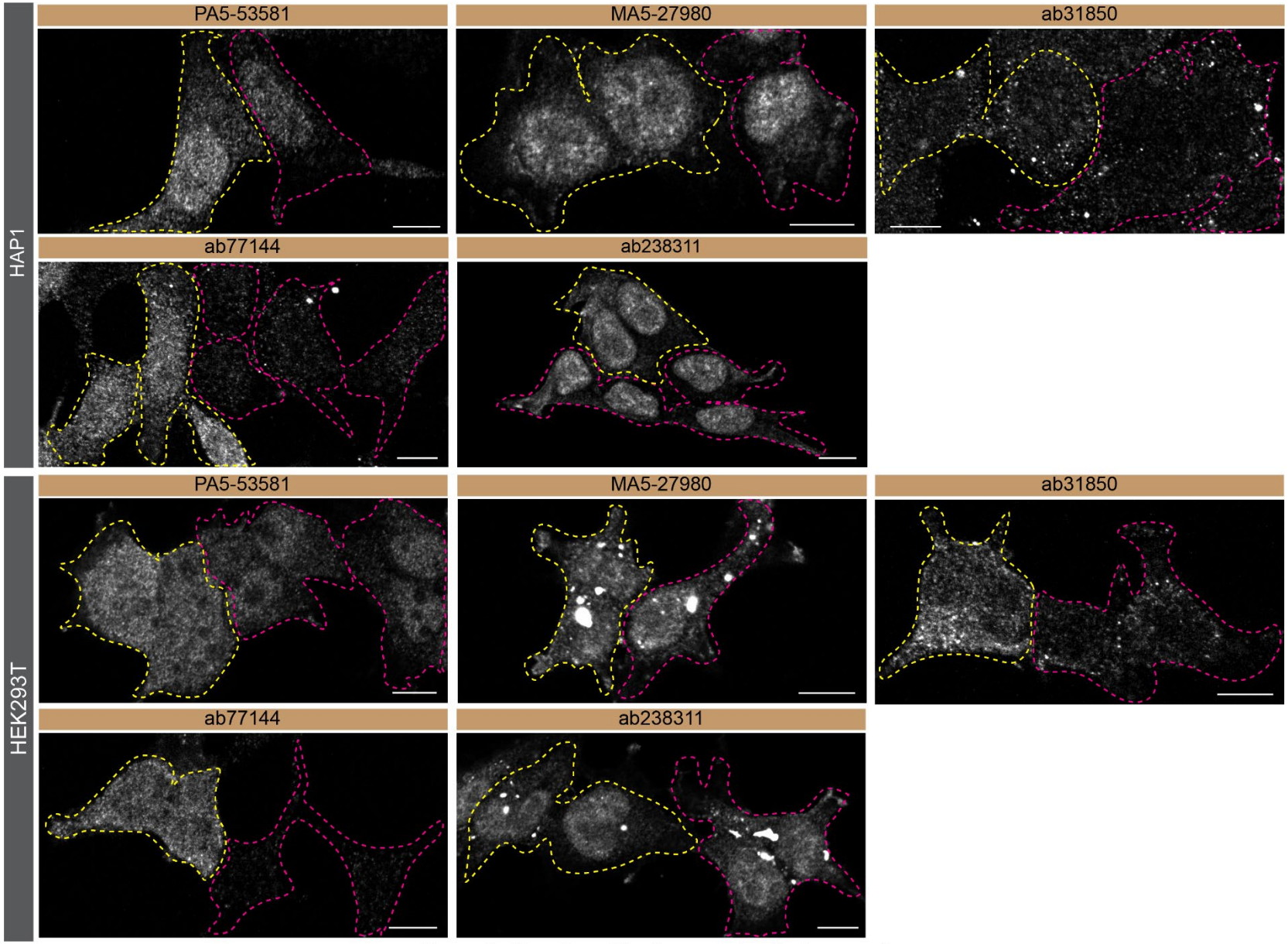

Figure 3 : Spastin antibody screening by immunofluorescence

Materials and methods

Antibodies

All Spastin 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

HEK293T and HAP1 (WT and *SPAST* 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 (Figure 1) 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.

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. Anti-mouse IgG for IP was used as a secondary detection system (Abcam, cat. number ab131368) at a dilution of 0.3 µg/ml for an experiment where a mouse antibody was used for both immunoprecipitation and its corresponding immunoblot.

Antibody screening by immunofluorescence

HAP1 and HEK293T WT and *SPAST* 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 Spastin 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