Antibody Characterization Report for Spondin-1

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Spondin-1

Alternative protein name: F-spondin, Vascular smooth muscle cell growth-promoting factor

Gene name: *SPON1*

Uniprot: Q9HCB6

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. In this study, we characterized five Spondin-1 commercial antibodies for Western blot and immunoprecipitation, using a standardized experimental protocol [2] based on comparing read-outs in knockout cell lines and isogenic parental controls. We identified many well-performing antibodies and encourage readers to use this report as a guide to select the most appropriate antibody for their specific needs. An OVCAR3 *SPON1* custom KO line was generated at Abcam and used in this study. Expression of Spondin-1 protein in OVCAR3 was determined through DepMap [3, 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.

Table 1: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype
ATCC	HTB-161	CVCL_0465	OVCAR3	WT
Abcam	-	-	OVCAR3	*SPON1* KO

Table 2: Summary of the Spondin-1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	AF3135	YSE032311	AB_2194692	polyclonal	-	goat	0.2	Wb
Bio-Techne	NBP2-69044	R111751	AB_3068003	polyclonal	-	rabbit	0.2	other
MilliporeSigma	WH0010418M1*	H4131-3F4	AB_1843742	monoclonal	3F4	mouse	0.5	other
Thermo Fisher Scientific	PA5-90241	XH3670294C	AB_2805976	polyclonal	-	rabbit	2.24	Wb
Thermo Fisher Scientific	PA5-116176	XH3670303	AB_2900810	polyclonal	-	rabbit	0.5	Wb

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

Materials and methods

Antibodies

All the Spondin-1 antibodies tested are listed in Table 2. Peroxidase-conjugated goat anti-rabbit and anti-mouse, and donkey anti-goat antibodies are from Thermo Fisher Scientific (cat. number 65-6120, 62-6520, and A15999, respectively).

Cell culture

Cells were cultured in RPMI 1640 (ATCC modification) (Gibco, cat. number A1049101) containing 20% fetal bovine serum (Wisent, cat. number 080450), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin, 100 µg/ml streptomycin (Wisent cat. number 450201) and 0.01mg/ml bovine insulin (MilliporeSigma, cat. number I6634. Cells were starved in RPMI 1640 (ATCC modification) medium containing L-glutamate and penicillin/ streptomycin.

Antibody screening by Western blot on culture media

OVCAR3 WT and *SPON1* KO (listed in Table 1) were washed 3x with PBS 1x 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 30min using Amicon Ultra-15 Centrifugal Filter Units with a membrane NMWL of 10kDa (MilliporeSigma cat. number UFC901024). Culture media were supplemented with 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number P8340).

Western blots were performed as described in our standard operating procedure [5]. Western blots were performed with precast midi 4-20% Tris-Glycine polyacrylamide gels from Thermo Fisher Scientific (cat. number WXP42012BOX) ran with Tris/Glycine/SDS buffer from bio-Rad (cat. number 1610772), loaded in Laemmli loading sample buffer from Thermo Fisher Scientific (cat. number AAJ61337AD) and transferred on nitrocellulose membranes. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used. Proteins on the blots were visualized with Ponceau S staining (Thermo Fisher Scientific, cat. number BP103-10) which is scanned to show together with individual Western blot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% milk in TBS with 0,1% Tween 20 (TBST) from Cell Signaling (cat. number 9997). 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 were incubated with Pierce ECL from Thermo Fisher Scientific (cat. number 32106) prior to detection with the iBright™ CL1500 Imaging System from Thermo Fisher Scientific (cat. number A44240).

Antibody screening by immunoprecipitation on culture media

Immunoprecipitation was performed as described in our standard operating procedure [6]. Antibody-bead conjugates were prepared by adding 2 µg to 500 µl of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with with 30µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse and goatantibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Tubes were rocked for ~1 hr at 4°C followed by two washes to remove unbound antibodies.

Starved OVCAR3 WT media were concentrated as described above and supplemented with protease inhibitor. 0.35 ml aliquots at 1.5 mg/ml of protein were incubated with an antibody-bead conjugate for ~1 hr at 4°C. The unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of IP lysis buffer and processed for SDS-PAGE and Western blot on precast midi 4-20% Tris-Glycine polyacrylamide gels. VeriBlot for IP Detection Reagent:HRP (Abcam, cat. number ab131366) was used as a secondary detection system at a concentration of 0.1 µg/ml.
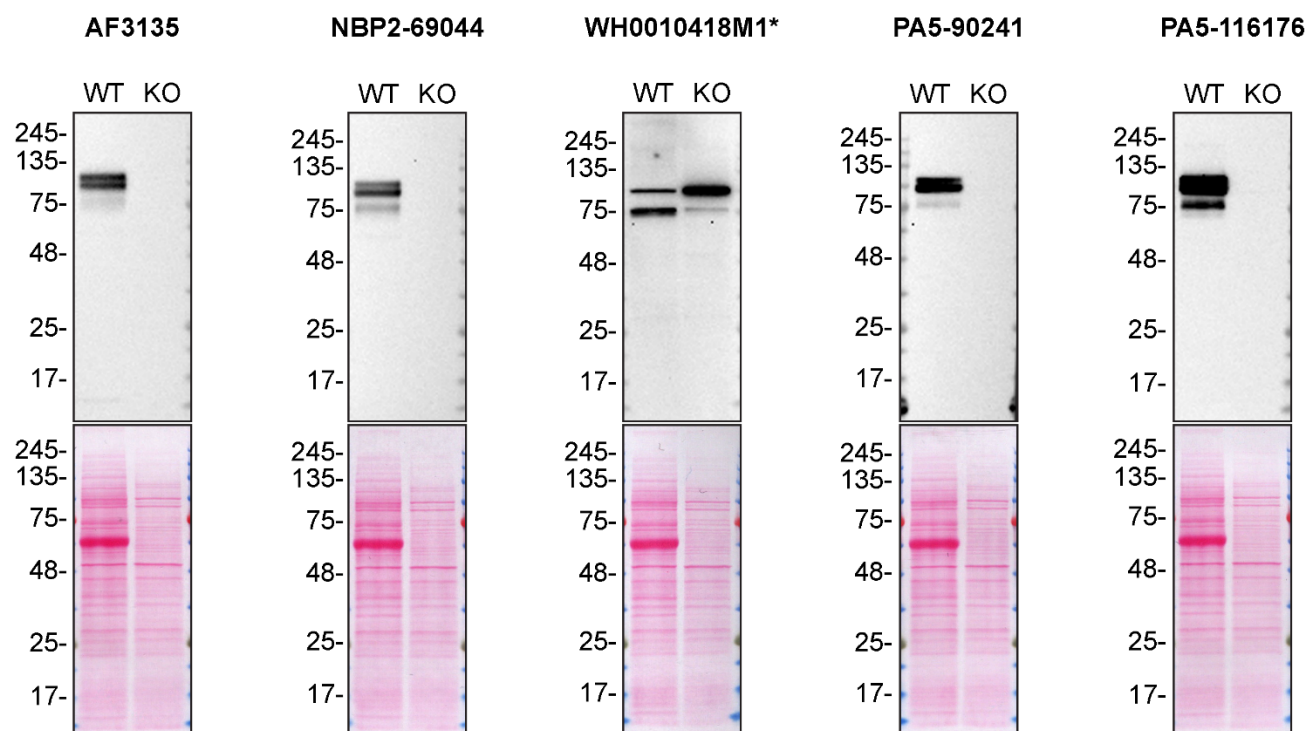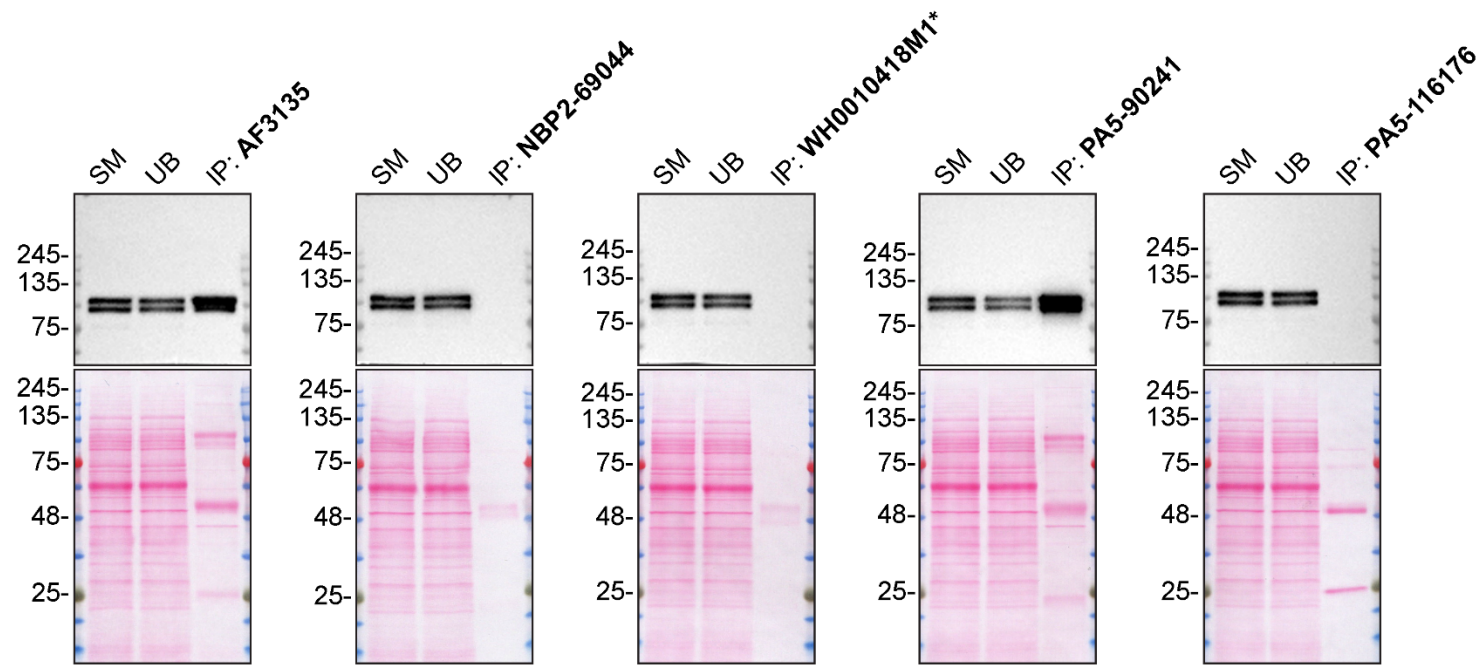
Figure 1: Spondin-1 antibody screening by Western blot on culture media

All immunobots were performed with PA5-116176

Figure 2: Spondin-1 antibody screening by immunoprecipitation on culture media

Figure 1: Spondin-1 antibody screening by Western blot on culture media.

OVCAR3 WT and *SPON1* KO were cultured in serum free media, and 30 µg of protein from concentrated culture media were processed for Western blot with the indicated Spondin-1 antibodies. The Ponceau stained transfers of each blot are shown. All antibodies were tested at 1/500. Predicted band size: 91 kDa. *=monoclonal antibody

Figure 2: Spondin-1 antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on concentrate culture media from OVCAR3 WT and using 2.0 µg of the indicated Spondin-1 antibodies pre-coupled to Dynabeads protein A or protein G Samples were washed and processed for Western blot with the indicated Spondin-1 antibody. For Western blot, PA5-116176 was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=7% starting material; UB=7% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody

References

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