Antibody Characterization Report for SPARC-related modular calcium-binding protein 1 (SMOC1)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: SPARC-related modular calcium-binding protein 1

Short name: SMOC1

Alternative name: Secreted modular calcium-binding protein 1

Gene name: *SMOC1*

Uniprot: Q9H4F8

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 SMOC1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for SMOC1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HeLa was selected based on evidence of appropriate SMOC1 gene expression determined using DepMap [3]. HeLa was modified with CRISPR/Cas9 [4] to knockout the corresponding *SMOC1* gene.

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 SMOC1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab200219	GR3370372-1	AB_2833001	polyclonal	-	rabbit	0.50	Wb
GeneTex	GTX119208	40331	AB_10618293	polyclonal	-	rabbit	0.90	Wb
Thermo Fisher Scientific	PA5-31392	130141931	AB_2548866	polyclonal	-	rabbit	0.90	Wb
Thermo Fisher Scientific	PA5-113408	WL3463969	AB_2868141	polyclonal	-	rabbit	3.50	Wb
ABclonal	A20482	125410101	AB_2909795	polyclonal	-	rabbit	2.65	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	CCL-2	CVCL_0030	HeLa	WT
Montreal Neurological Institute	-	CVCL_B7DT	HeLa	*SMOC1* KO

Figure 1: SMOC1 antibody screening by immunoblot on culture media.

80 µg of protein from concentrated culture media were processed for immunoblot with the indicated SMOC1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab200219 at 1/500; GTX119208 at 1/500; PA5-31392 at 1/500; PA5-113408 at 1/1000; A20482 at 1/1000. SMOC1 predicted band size: 48 kDa.

Figure 2: SMOC1 antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on concentrated culture media using 2.0 µg of the indicated SMOC1 antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated SMOC1 antibodies. For immunoblot, GTX119208 was used at 1/500, PA5-31392 at 1/500 and PA5-113408 at 1/1000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. LC= light chain **=recombinant antibody.

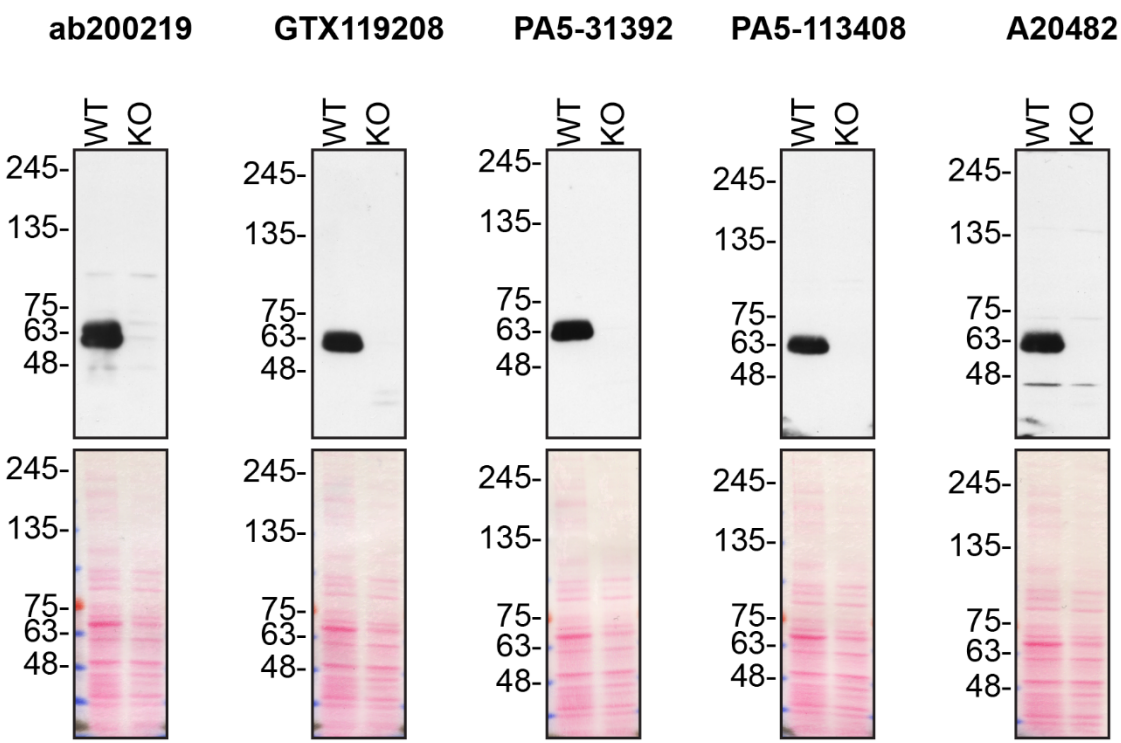

Figure 1: SMOC1 antibody screening by immunoblot

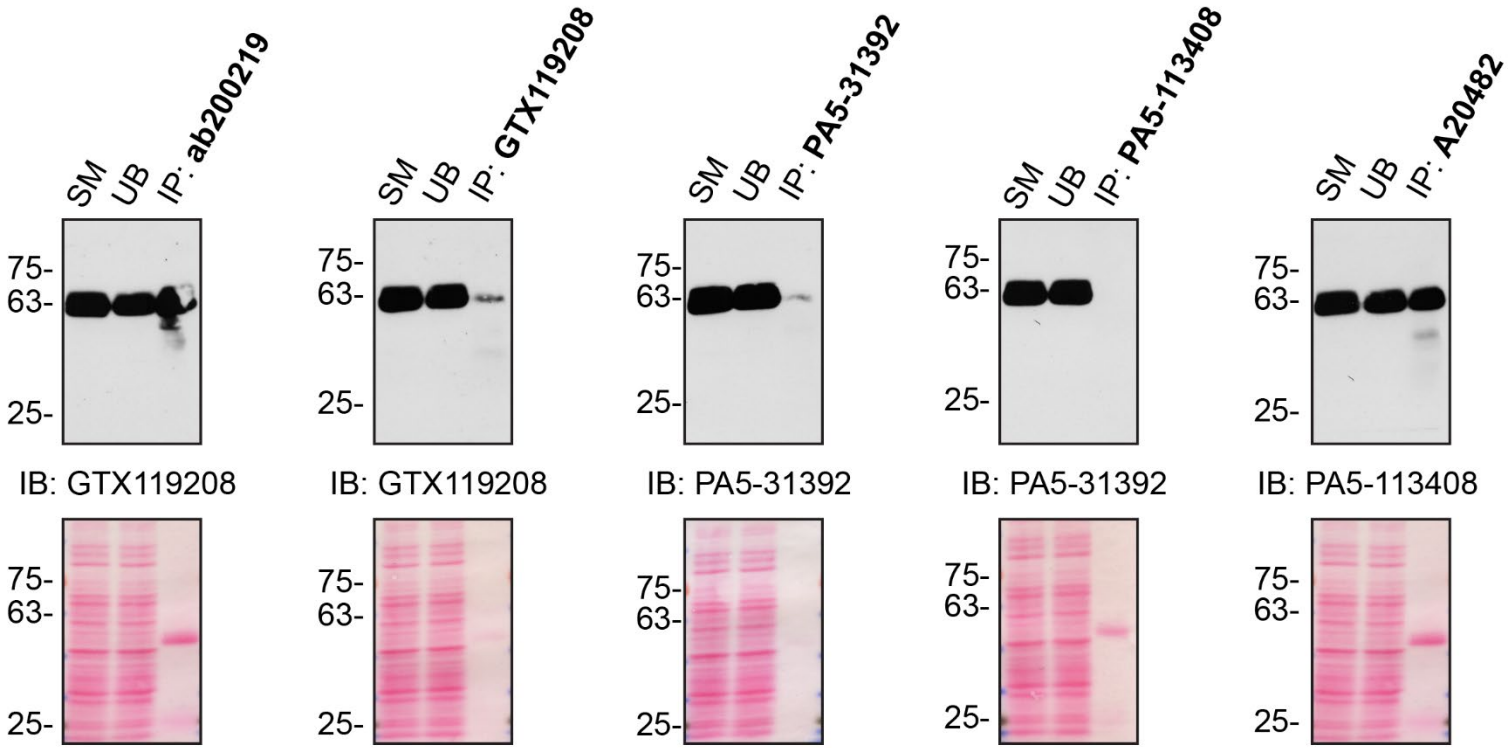

Figure 2: SMOC1 antibody screening by immunoprecipitation

Materials and methods

Antibodies

All SMOC1 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).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. HeLa *SMOC1* KO clone was generated with low passage cells. The sequence of the guide RNA used to KO *SMOC1* is CUCGUAGGACCUGCCAUCAG.

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

HeLa WT and *SMOC1* KO cells 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 10kDa (MilliporeSigma cat. number UFC901024).
Immunoblots were performed

Antibody screening by immunoblot

Immunoblots were performed as described in our standard operating procedure [5]. Large 3-12% gradient polyacrylamide gels were used 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 on culture media

Immunoprecipitation was performed as described in our SOP for immunoprecipitation [6]. Antibody-bead conjugates were prepared by adding 2 µg of antibody 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) 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 HeLa WT culture media were concentrated as described above. 1ml aliquots at 0.5 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 8-16% polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a dilution of 0.4 µg/ml.

References

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