Antibody Characterization Report for SMOC-1

YCharOS Antibody Characterization Report

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Target:

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

Short name: SMOC-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]. In this study, we characterized seven SMOC-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. HeLa was selected based on evidence of appropriate SMOC-1 protein expression determined through DepMap [3, 4]. HeLa was modified with CRISPR/Cas9 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 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

Table 2: Summary of the SMOC-1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab313569**	3101091230	AB_2941846	recombinant-mono	EPR26922-29	rabbit	0.50	Wb, IP, IF
Abcam	ab313571**	3101065175	AB_2941847	recombinant-mono	EPR26922-31	rabbit	0.50	WB, IP
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, n/a=not available, **=recombinant antibody

Materials and methods

Antibodies

All the SMOC-1 antibodies tested are listed in Table 2. Peroxidase-conjugated goat anti-rabbit is from Thermo Fisher Scientific (cat. number 65-6120).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 1. HeLa *SMOC1* KO clone was generated with low passage cells using an open-access protocol available on Zenodo.org: https://zenodo.org/record/3875777#.X9uE11VKjIX. The guide RNA used to knockout the *SMOC1* gene 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.

Antibody screening by Western Blot on culture media

HeLa WT and *SMOC1* 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- (rabbit antibodies) from Thermo Fisher Scientific (cat. number 10002D) Tubes were rocked for ~1 hr at 4°C followed by two washes to remove unbound antibodies.

Starved HeLa WT media were concentrated as described above and supplemented with protease inhibitor. 0.3 ml aliquots at 1.6 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 0.3 µg/ml.

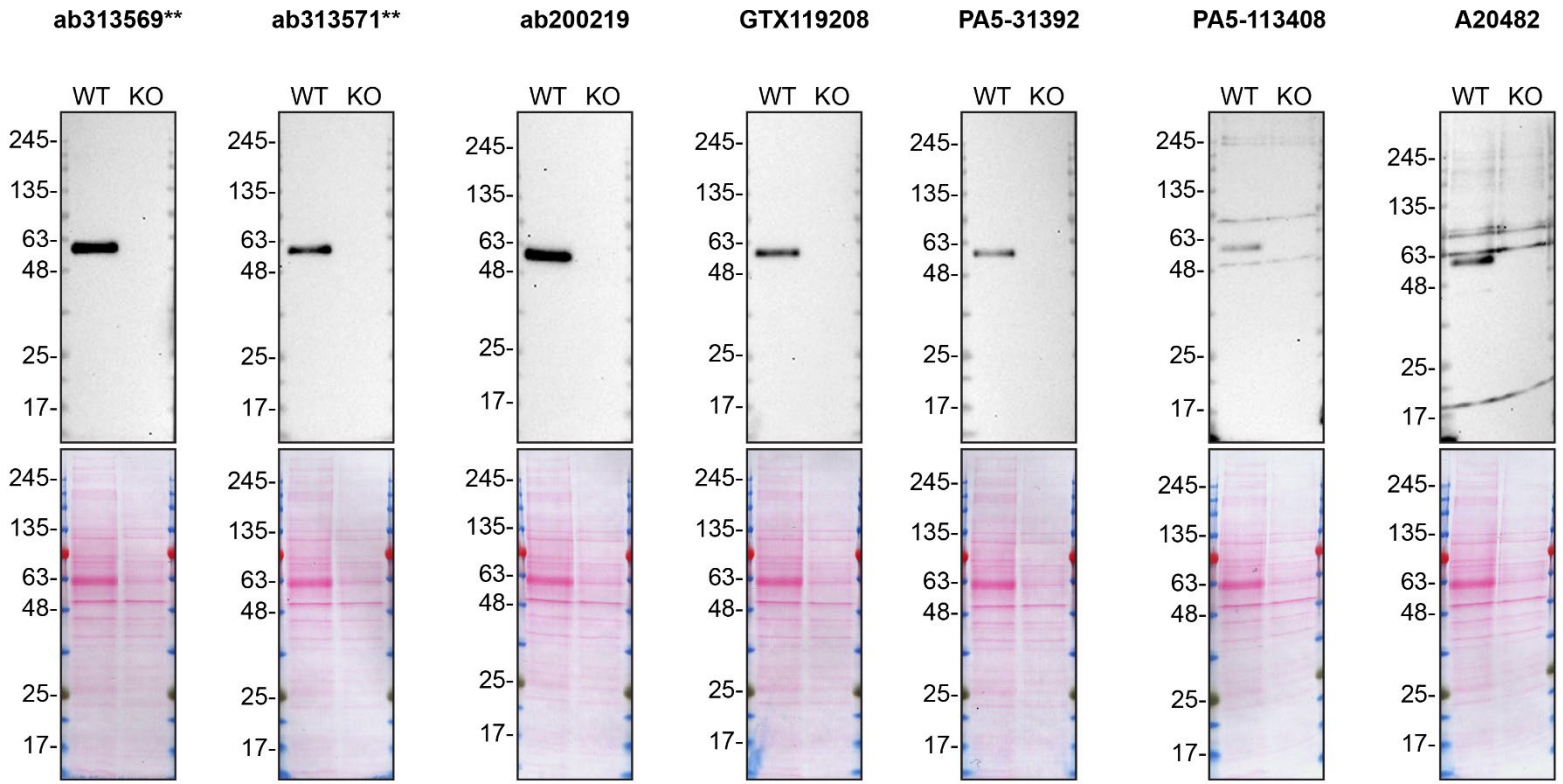

Figure 1: SMOC-1 antibody screening by Western Blot on culture media

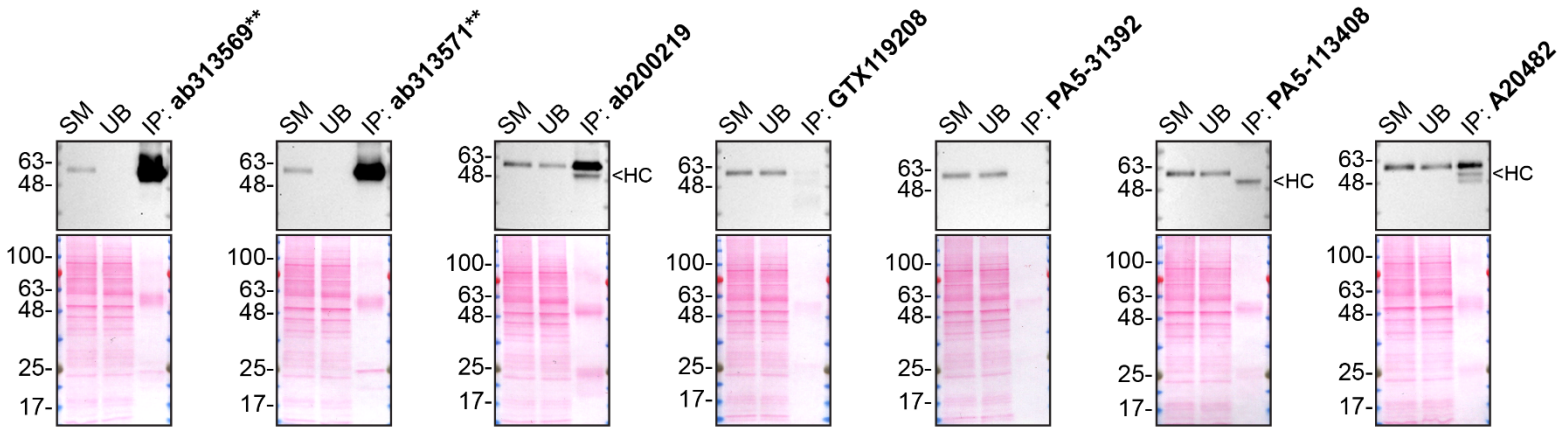

Figure 2: SMOC-1 antibody screening by immunoprecipitation on culture media

Figure 1: SMOC-1 antibody screening by Western Blot on culture media.

HeLa WT and *SMOC1* KO were cultured in serum free media, and 30 µg of protein from concentrated culture media were processed for Western Blot with the indicated SMOC-1 antibodies. The Ponceau stained transfers of each blot are shown. All antibpdies were used at 1/2000. Predicted band size: 48 kDa. **=recombinant antibody

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

Immunoprecipitation was performed on concentrate culture media from HeLa WT, and using 2.0 µg of the indicated SMOC-1 antibodies pre-coupled to Dynabeads protein A. Samples were washed and processed for Western Blot with the indicated SMOC-1 antibody. For Western Blot, ab131569** was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=8% starting material; UB=8% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain. **=recombinant antibody

References

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