Antibody Characterization Report for Syndecan-4

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Syndecan-4

Alternative protein name: Amphiglycan, Ryudocan core protein

Gene name: SDC4

Uniprot: P31431

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 Syndecan-4. We used an antibody characterization pipeline [2] based on a genetic approach to perform head-to-head comparisons of commercial antibodies for Syndecan-4 by immunoblot (Western blot) and immunoprecipitation. MCF7 was selected based on evidence of appropriate *SDC4* expression [3]. Knockdown (KD) of *SDC4* was performed using siRNA.

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 Syndecan-4 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	AF2918	VTJ0520061	AB_2183026	polyclonal	-	goat	0.20	Wb
Bio-Techne	NBP2-24630	06113333C	AB_2915988	polyclonal	-	rabbit	0.50	Wb
Bio-Techne	NBP2-50315*	CRT/16/63	AB_2915989	monoclonal	V60P2C2* F2	mouse	1.00	Wb
GeneTex	GTX87212	822104626	AB_10722468	polyclonal	-	rabbit	1.00	Wb,IF
ABclonal	A1834	9730101	AB_2763871	polyclonal	-	rabbit	2.06	Wb
Proteintech	11820-1-AP	83566	AB_2877797	polyclonal	-	rabbit	0.33	Wb
Cell Signaling Technology	12236	1	AB_2797853	polyclonal	-	rabbit	not provided	Wb
Thermo Fisher Scientific	PA1-32485	WL3464975D	AB_2285477	polyclonal	-	rabbit	0.50	Wb,IP,IF
Thermo Fisher Scientific	PA5-95950	XA3464709	AB_2807752	polyclonal	-	rabbit	2.06	Wb
Thermo Fisher Scientific	36-3100	Wb324925	AB_2533250	polyclonal	-	rabbit	0.25	Wb,IF

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

Table 2: Summary of the cell line used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
ATCC	HTB-22	CVCL_0031	MCF7	WT

Figure 1: Syndecan-4 antibody screening by immunoblot.

MCF7 WT and *SDC4* KD cells were treated with Brefeldin A at 3.0 µg/ml for 18 hrs. Lysates were prepared, and 50 µg of protein from treated and non-treated cells were processed for immunoblot with the indicated Syndecan-4 antibodies. The Ponceau stained transfers of each blot are shown. All antibodies were diluted at 1/500. Predicted band size: 21 kDa. *=monoclonal antibody

Figure 2: Syndecan-4 antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on concentrated culture media using 2.0 µg of the indicated Syndecan-4 antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Syndecan-4 antibody. For immunoblot, AF2918 was used at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; LC= antibody light chain. *=monoclonal antibody

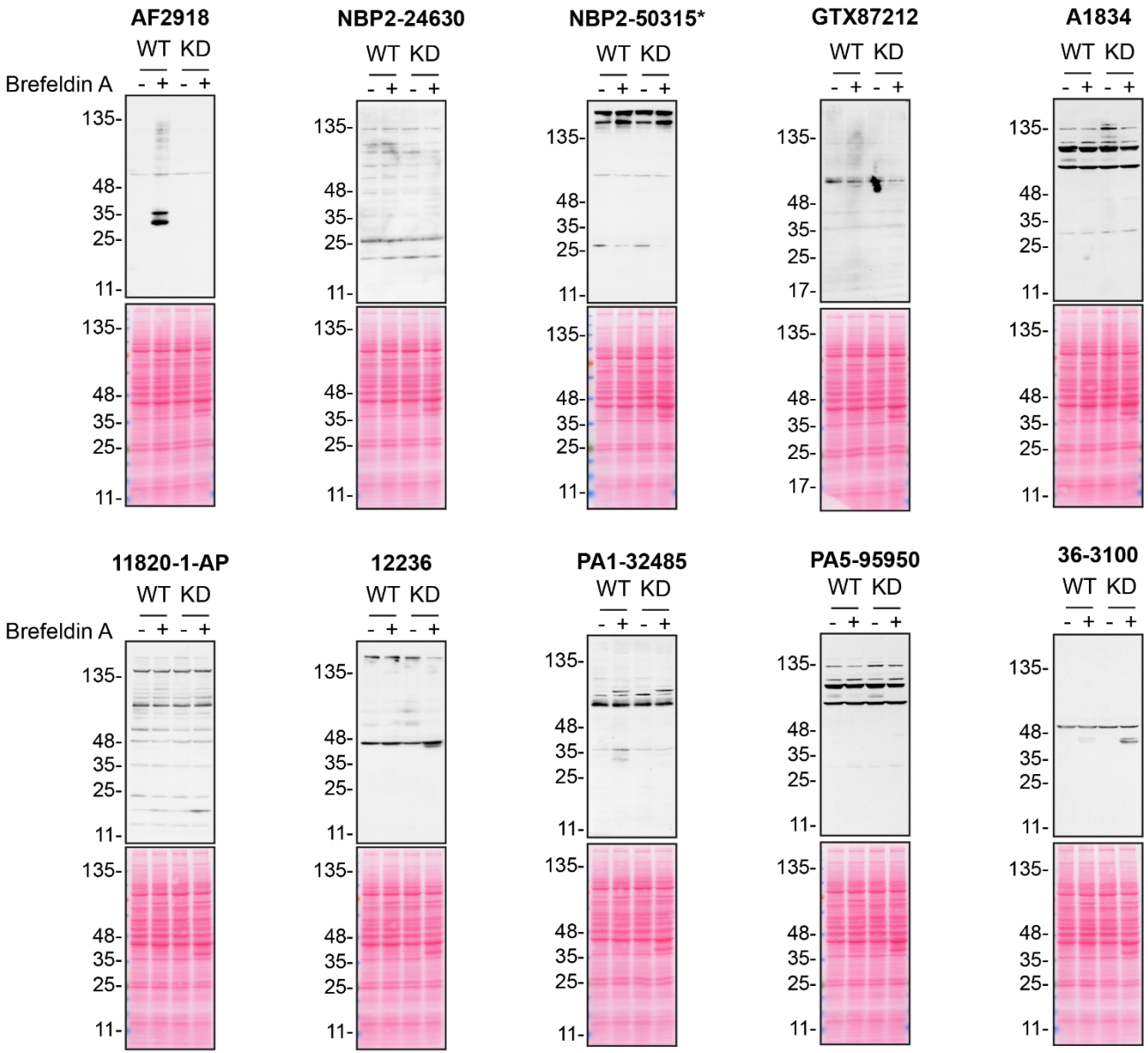

Figure 1: Syndecan-4 antibody screening by immunoblot

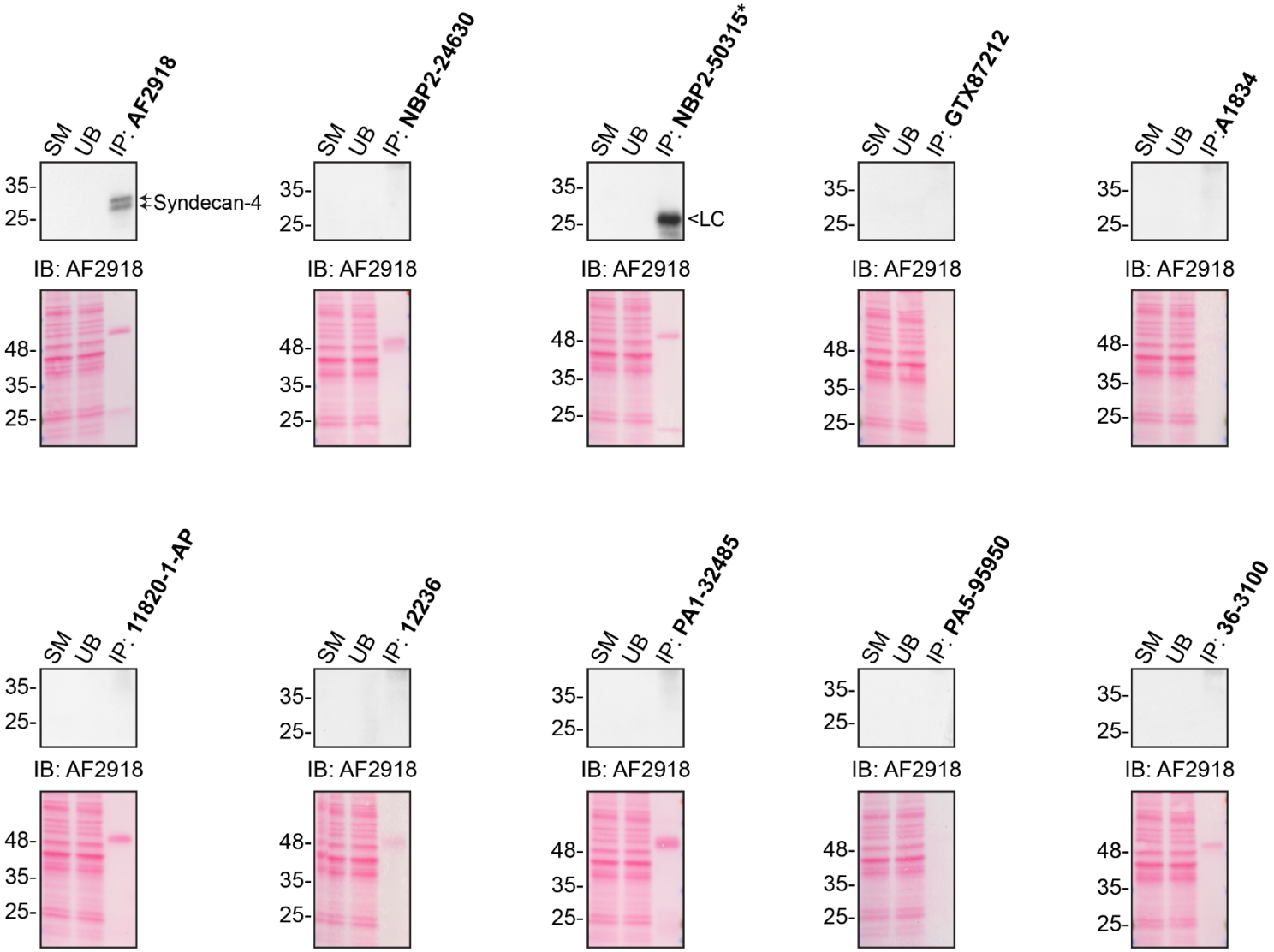

Figure 2: Syndecan-4 antibody screening by immunoprecipitation on culture media

Materials and methods

Antibodies

All Syndecan-4 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 62-6520 and 65-6120).

Knockdown strategy

MCF7 were treated with SDC4 siRNA smartpool from Horizon Discovery (cat # L-003706-00-0005). Lipofectamine RNAiMAX from Thermo (cat# 13778030) was used to transfect the siRNA following the manufacturer's protocol.

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.

Lysates from Brefeldin A-treated cells

Immunoblots were performed as described in our standard operating procedure [4]. MCF7 WT and *SDC4* KO, treated or non-treated, were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% 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.

Antibody screening by immunoblot

Immunoblots were performed with large 8-16% 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).

Collection of culture media

MCF7 WT cells 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 30min using Amicon Ultra-15 Centrifugal Filter Units with a membrane NMWL of 10kDa (MilliporeSigma cat. number UFC901024).

Antibody screening by immunoprecipitation on culture media

Immunoprecipitation was performed as described in our standard operating procedure [5]. Antibody-bead conjugates were prepared by adding 2 µg or 10µl of an antibody at an unknown concentration 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 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 MCF7 WT culture media were concentrated as described above. 1ml aliquots at 0.6 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.

References

1. Laflamme, C., et al., *Opinion: Independent third-party entities as a model for validation of commercial antibodies.* N Biotechnol, 2021. **65**: p. 1-8 DOI: 10.1016/j.nbt.2021.07.001.
2. 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.
3. Ghandi, M., et al., *Next-generation characterization of the Cancer Cell Line Encyclopedia.* Nature, 2019. **569**(7757): p. 503-508 DOI: 10.1038/s41586-019-1186-3.
4. Ayoubi, R., P.S. McPherson, and C. Laflamme, *Antibody Screening by Immunoblot.* 2021 DOI: https://doi.org/10.5281/zenodo.5717510.
5. Ayoubi, R., et al., *Antibody screening by Immunoprecitation.* 2021 DOI: https://doi.org/10.5281/zenodo.5717516.