Antibody Characterization Report for Neurosecretory protein VGF

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Neurosecretory protein VGF

Alternative protein names: VGF8a protein

Gene name: *Vgf*

Uniprot: P20156

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1, 2]. This report guides researchers to select the most appropriate antibodies for Neurosecretory protein VGF. We used an antibody characterization pipeline [3] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Neurosecretory protein VGF. Neurosecretory protein VGF is a secreted protein [4] and thus we tested antibodies by immunoblot (Western blot) on total cell lysates and on serum-free culture media, and by immunoprecipitation on serum-free culture media. U2OS was selected based on evidence of appropriate Neurosecretory protein VGF expression determined through PaxDb [5] and DepMap [6, 7]. U2OS was modified with CRISPR/Cas9 to knockout [8] the corresponding *Vgf* 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 Neurosecretory *protein VGF* antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	MAB6317	CEBE0320021	AB_10642180	monoclonal	619707	mouse	0.50	*Other application*
Thermo	PA5-63081	VJ3101906C	AB_2649426	polyclonal	-	rabbit	0.10	IF
Thermo	PA5-106693	VJ3101173A	AB_2854361	polyclonal	-	rabbit	1.00	Wb, IF
Abcam	ab69989	GR3353777-1	AB_1271455	polyclonal	-	rabbit	1.00	Wb, IF
Abcam	ab74140	GR3347547-1	AB_1524551	polyclonal	-	rabbit	1.00	Wb, IF
Abcam	ab115609	GR3208236+7	AB_10903876	polyclonal	-	rabbit	1.00	Wb
Proteintech	26781-1-AP	83050	AB_2880632	polyclonal	-	rabbit	0.50	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	HTB-96	CVCL_0042	U2OS	WT
Montreal Neurological Institute	-	CVCL_B5CQ	U2OS	*Vgf* KO

Figure 1: Inhibition of Neurosecretory protein VGF secretion by Brefeldin A.

U2OS cells (WT and *Vgf* KO) were treated with Brefeldin A (BFA) at 3.0 µg/ml for 18 hrs. Lysates of treated and non-treated U2OS cells were prepared, and 100 µg of protein were processed for immunoblot with the indicated Neurosecretory protein VGF antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: PA5-63081 at 1/500; PA5-106693 at 1/500; ab69989 at 1/2000; ab74140 at 1/500; ab115609 at 1/500; 26781-1-AP at 1/500. Predicted band size: 68 kDa.

Figure 2: Neurosecretory protein VGF antibody screening by immunoblot on culture media.

100 µg of protein from concentrated culture media were processed for immunoblot with the indicated Neurosecretory protein VGF antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MAB6317 at 1/500; PA5-63081 at 1/500; PA5-106693 at 1/500; ab69989 at 1/2000; ab74140 at 1/500; ab115609 at 1/500; 26781-1-AP at 1/500. Predicted band size: 68 kDa.

Figure 3: Neurosecretory protein VGF antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on concentrated culture media using 1.0 µg of the indicated Neurosecretory protein VGF antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Neurosecretory protein VGF antibodies. For immunoblot, ab69989 was used at 1/1000 and PA5-63081 at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

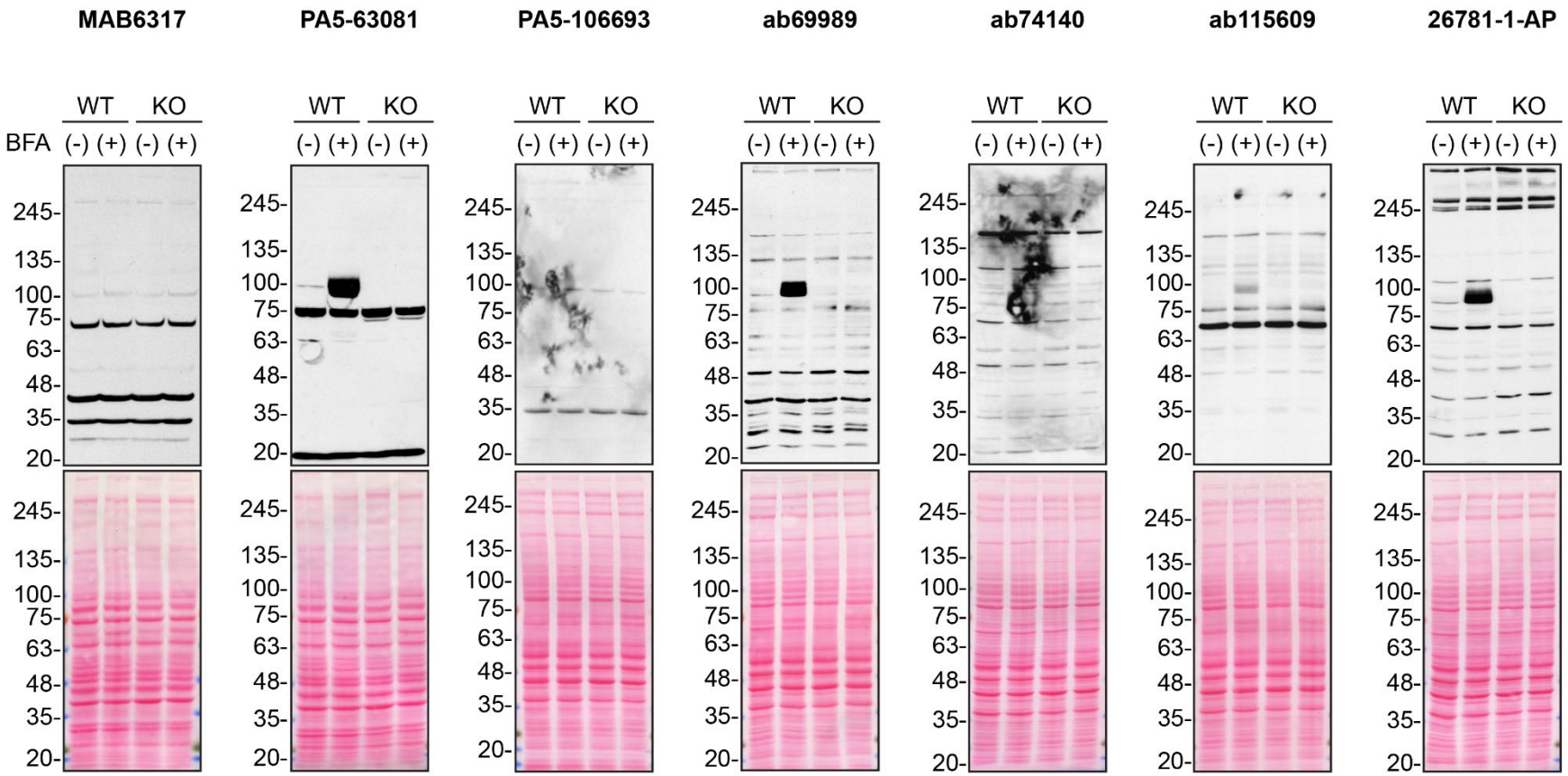

Figure 1: Inhibition of Neurosecretory protein VGF secretion by Brefeldin A

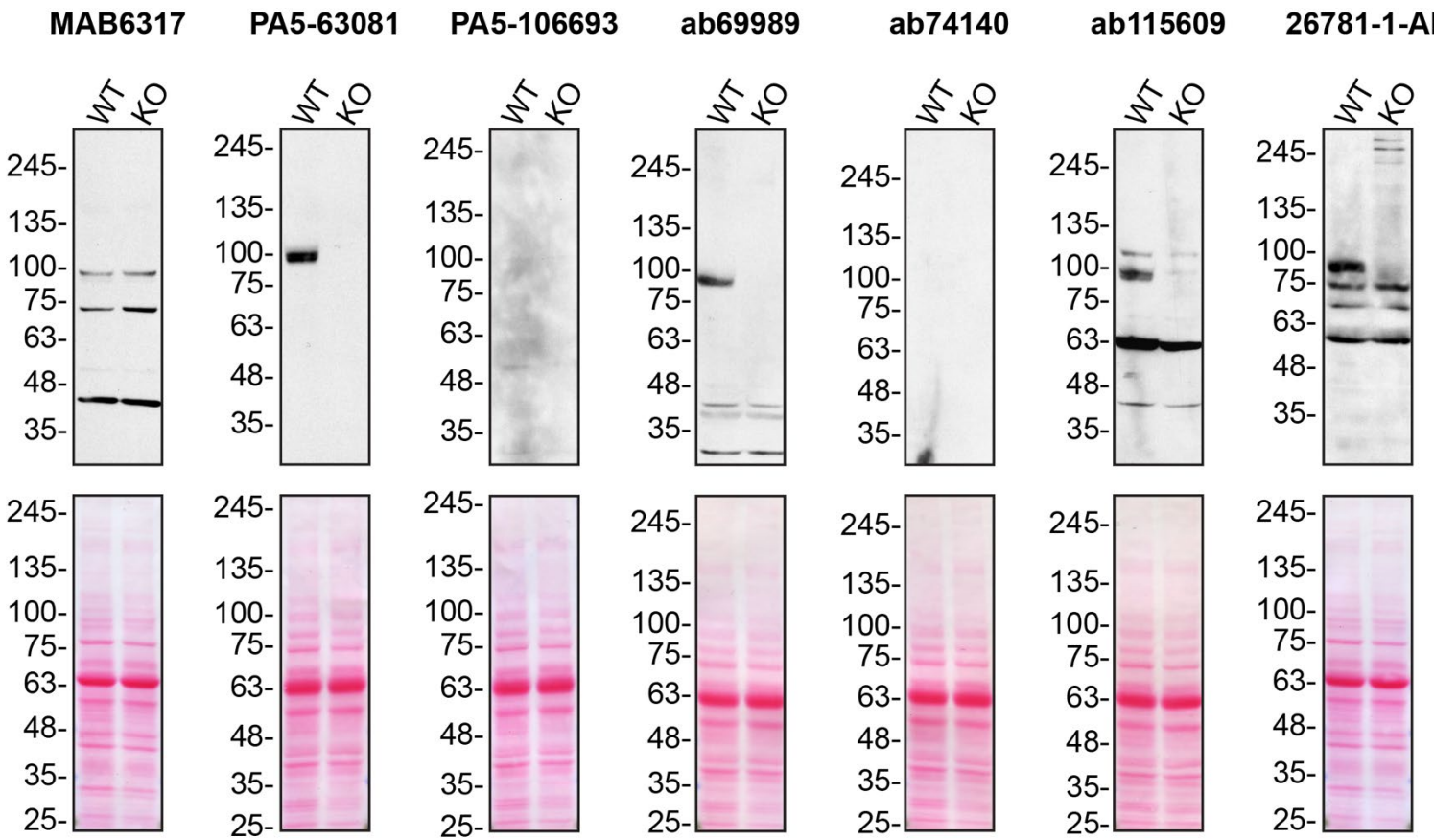

Figure 2: Neurosecretory protein VGF antibody screening by immunoblot on culture media

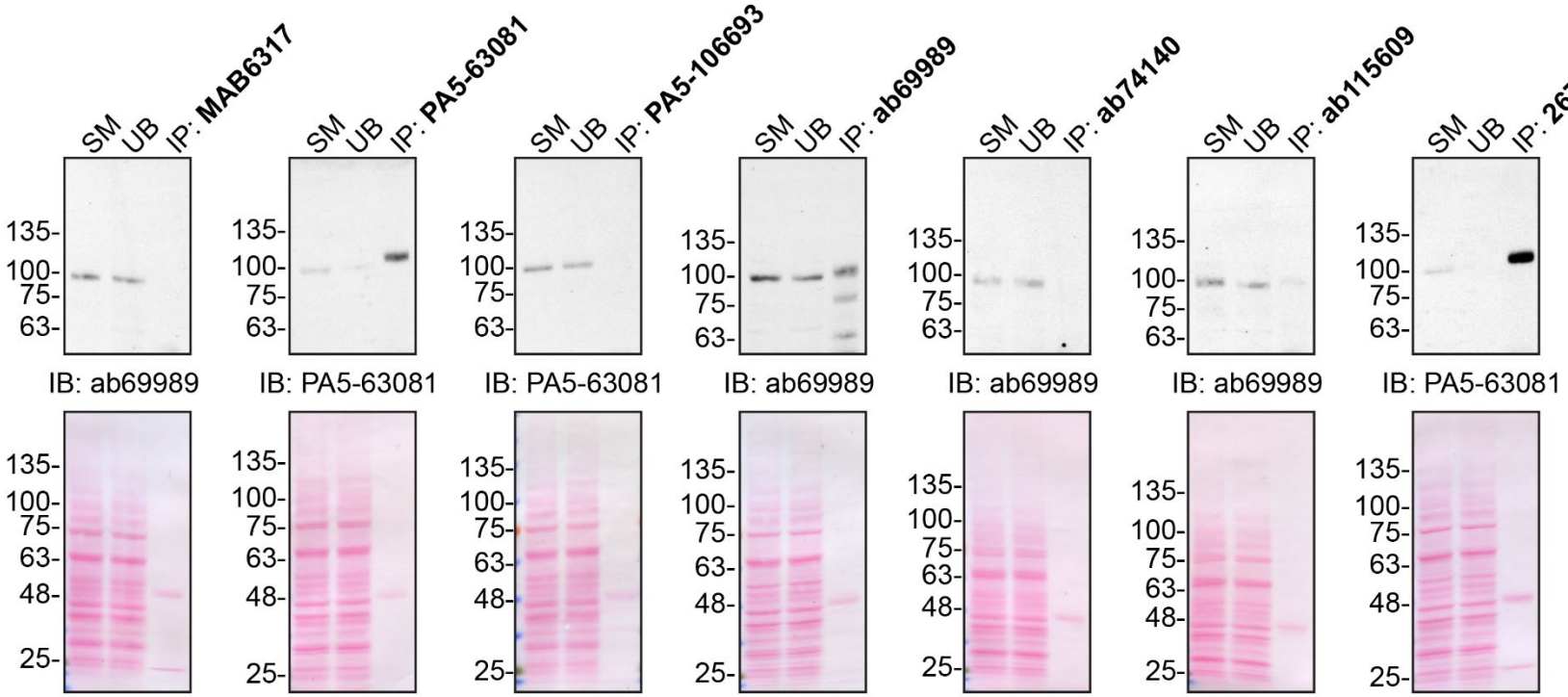

Figure 3: Neurosecretory protein VGF antibody screening by immunoprecipitation on culture media

Materials and methods

Antibodies

All Neurosecretory protein VGF 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. U2OS *Vgf* KO clone was generated with low passage cells. The guide RNA used to knockout the *Vgf* gene is UGGGAGCCGCUUGGUGCCGG.

Cell culture

U2OS 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

U2OS were treated with 0.3 µg/ml of Brefeldin A from Thermo Fisher Scientific (cat. number 00-4506-51). U2OS (WT and *Vgf* KO) treated and 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.

Collection of culture media

U2OS cells (WT and *Vgf* KO) 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 50kDa (MilliporeSigma cat. number UFC905024). Immunoblots were performed as described above.

Screening of antibodies by immunoblots

Immunoblots were performed as described in our standard operating procedure (SOP) [9] using total cell lysates (Figure 1) or concentrated culture media (Figure 2). We performed immunoblots with large 4-15% 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% Tween20 (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 [10]. Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 µl of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together 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 100x Protease Inhibitor Cocktail from MilliporeSigma (cat. number P8340) at a final concentration of 1x. Tubes were rocked for ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

Starved U2OS WT culture media were concentrated as described above. 1ml aliquots at 0.85 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 of IP Lysis Buffer and processed for SDS-PAGE and immunoblot on 4-15% polyacrylamide gels. Prot-A: HRP was used as a secondary detection system (MilliporeSigma, cat. number P8651) at a dilution of 0.4 µg/ml.

References

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