Antibody Characterization Report for Serine/threonine-protein kinase TBK1

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Serine/threonine-protein kinase TBK1

Short protein name: TBK1

Alternative protein names: NF-kappa-B-activating kinase, T2K, TANK-binding kinase 1, NAK

Gene name: *TBK1*

Uniprot: Q9UHD2

This report guides researchers to select the most appropriate antibodies for TBK1. We used an antibody characterization pipeline[1] based on knockout cells to perform head-to-head comparisons of commercial antibodies for TBK1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. U2OS was selected based on evidence of appropriate TBK1 protein expression determined through public proteomics databases, namely PaxDB[2] and DepMap[3]. U2OS was modified with CRISPR/Cas9 to knockout[4] the corresponding *TBK1*.

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.

1 Laflamme, C. *et al.* Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72. *Elife* **8**, doi:10.7554/eLife.48363 (2019).

2 Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D. & von Mering, C. Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **15**, 3163-3168, doi:10.1002/pmic.201400441 (2015).

3 Nusinow, D. P. *et al.* Quantitative Proteomics of the Cancer Cell Line Encyclopedia. *Cell* **180**, 387-402 e316, doi:10.1016/j.cell.2019.12.023 (2020).

4 Shlaifer, I. *et al. Generation of Knockout Cell Lines Using CRISPR-Cas9 Technology*, <https://zenodo.org/record/3738361#.YIyeDu2SlaR> (February 24, 2020).

Table 1: Summary of the TBK1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	NB100-56705	B-1	AB_838420	monoclonal	108A429	mouse	1.00	Wb, IF
Proteintech	28397-1-AP	00076443	AB_2881132	polyclonal	-	rabbit	0.43	Wb
Proteintech	67211-1-Ig	10013180	AB_2882504	monoclonal	2D7B1	mouse	1.00	Wb, IF
Thermo	PA5-17478	VL3152289A	AB_10981817	polyclonal	-	rabbit	not provided	Wb, IF, IP
Thermo	703154	2274494	AB_2848223	recombinant-mono	JM42-11	rabbit	0.50	Wb, IF
Abcam	ab12116	GR3334526-1	AB_298856	monoclonal	108A429	mouse	1.00	Wb
Abcam	ab40676	GR3275777-2	AB_776632	recombinant-mono	EP611Y	rabbit	1.48	Wb, IF
Abcam	ab109735	GR3263881-3	AB_10863562	recombinant-mono	EPR2867(2)-19	rabbit	0.50	Wb, IP
GeneTex	GTX113057	43481	AB_11174793	polyclonal	-	rabbit	0.70	Wb, IF

Wb= Western blot; IF= immunofluorescence; IP=immunoprecipitation

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Montreal Neurological Institute	-	CVCL_0042	U2OS	WT
Montreal Neurological Institute	-	CVCL_A6LQ	U2OS	*TBK1* KO

Figure 1: TBK1 antibody screening by immunoblot.

Lysates of U2OS (WT and *TBK1* KO) were prepared and 50 µg of protein were processed for immunoblot with the indicated TBK1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used was 1/5000 for all tested antibodies. Predicted band size: ~83 kDa.

Figure 2: TBK1 antibody screening by immunoprecipitation.

U2OS lysates were prepared and IP was performed using 1.0 µg of the indicated TBK1 antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated TBK1 antibody. For immunoblot, ab40676 at 1/15000 and ab12116 at 1/2000 were used. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: TBK1 antibody screening by immunofluorescence.

U2OS WT and *TBK1* KO cells were labelled with a green or a far red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated TBK1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (WT), red (antibody staining) and far-red (KO) channels was performed. Representative images of the merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Antibody dilution used: NB100-56705 at 1/1000; 28397-1-AP at 1/500; 67211-1-Ig at 1/1000; PA5-17478 at 1/1000; 703154 at 1/500; ab12116 at 1/1000; ab40676 at 1/1500; ab109735 at 1/500; GTX113057 at 1/700. Bars = 10 µm.

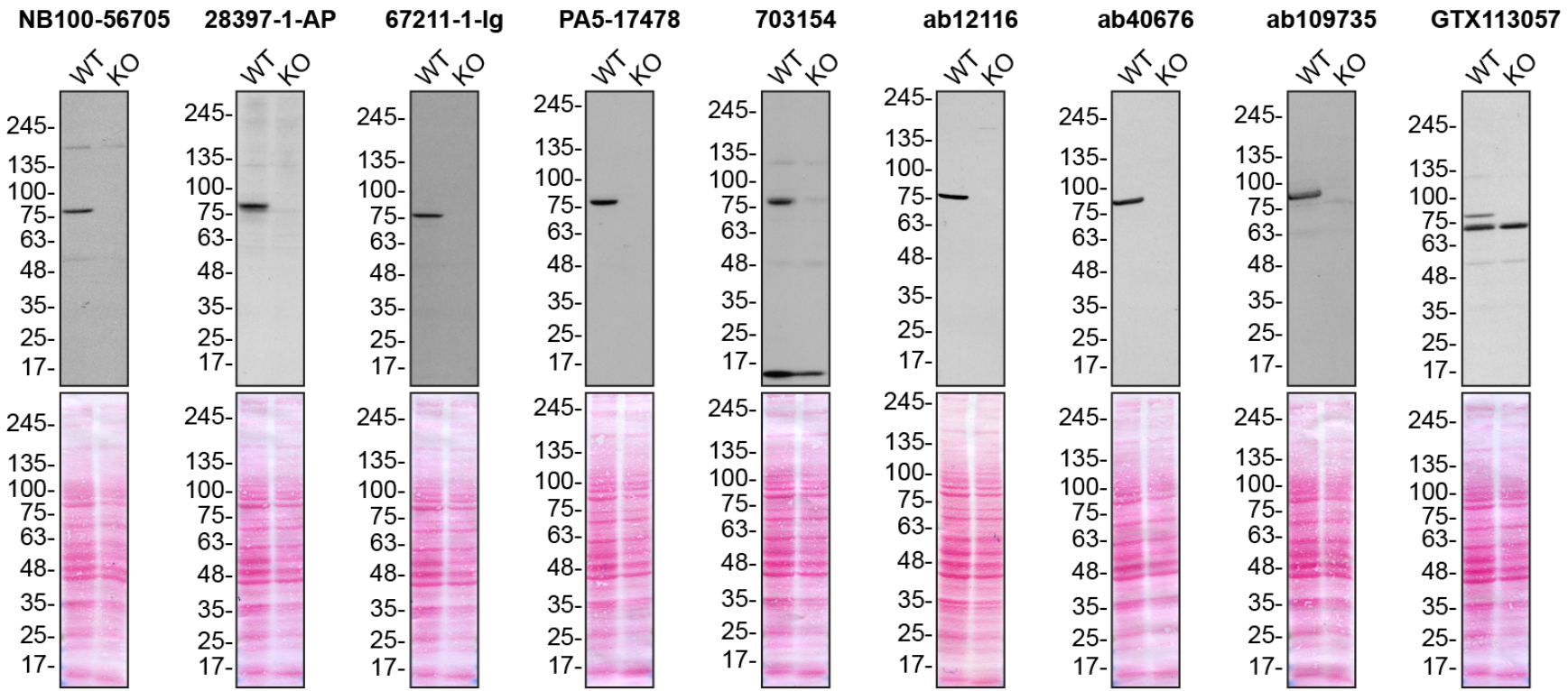

Figure 1: TBK1 antibody screening by immunoblot

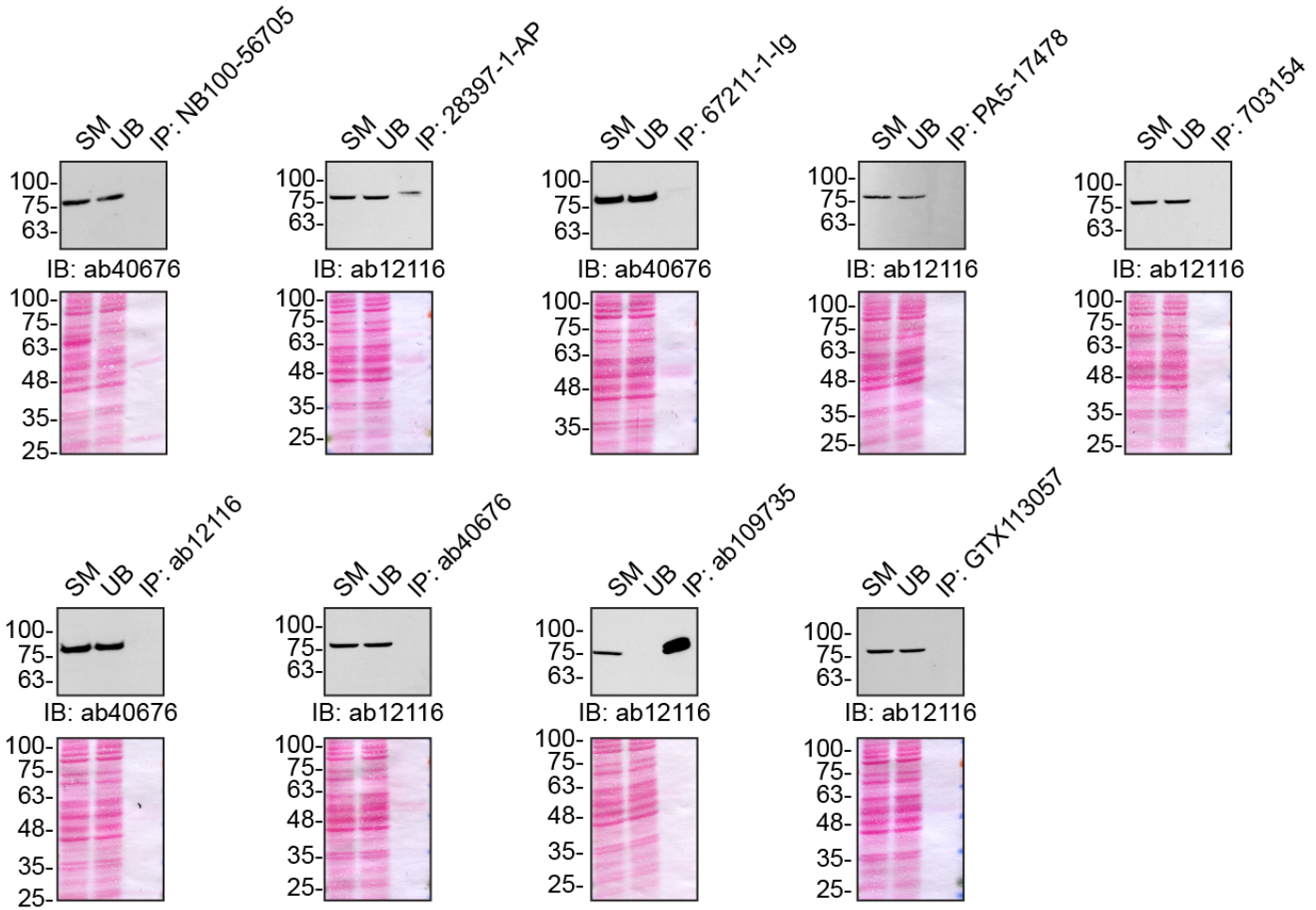

Figure 2: TBK1 antibody screening by immunoprecipitation

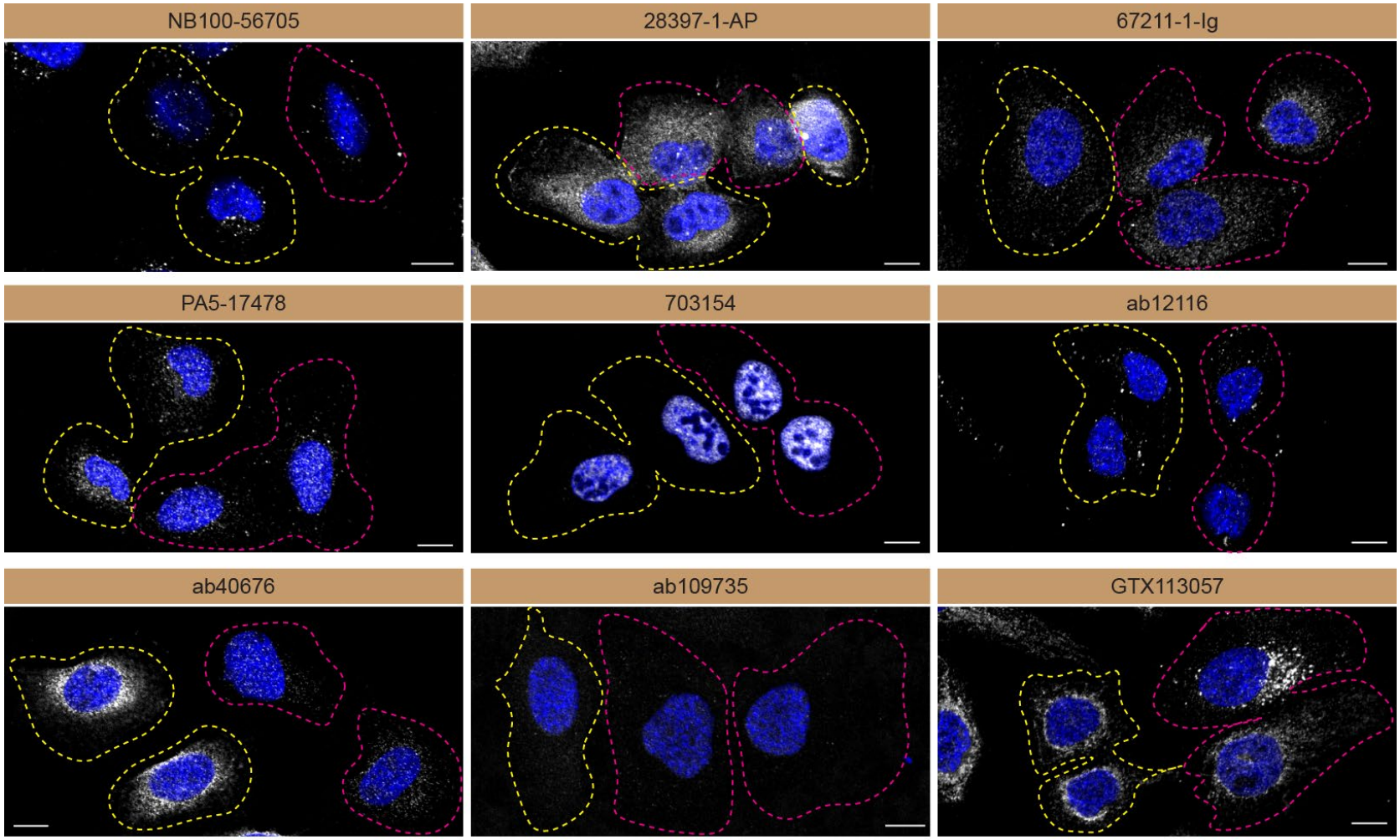

Figure 3: TBK1 antibody screening by immunoblot

Materials and methods

Antibodies

All TBK1 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). Alexa-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and A21429).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. U2OS *TBK1* KO clone was generated with low passage cells using an open-access protocol available on Zenodo.org: https://zenodo.org/record/3738361#.YIyeDu2SlaR. Two guide RNAs were used to introduce a STOP codon in the *TBK1* gene (sequence guide 1: UUUGAACAUCCACUGGACGA, sequence guide 2: CAAAUUAUUUGCUAUUGAAG).

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).

Antibody screening by immunoblot

U2OS (WT and *TBK1* KO) were collected in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1.0 mM EDTA, 1% Triton X-100, 0.5% 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.

Immunoblots were performed with large 5-16% 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).

Antibody screening by immunoprecipitation

Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 ul of PBS with 0,01% triton X-100 in a microcentrifuge tube, together with 30µl of protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

U2OS WT were collected in HEPES buffer (20 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, pH 7.4) supplemented with protease inhibitor. Lysates are rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. One ml aliquots at 1.0 mg/ml of lysate 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 HEPES lysis buffer and processed for SDS-PAGE and immunoblot on a 5-16% acrylamide gel.

Antibody screening by immunofluorescence

U2OS WT and *TBK1* KO were labelled with a green and a deep red fluorescence dye, respectively. The fluorescent dyes used are from Thermo Fisher Scientific (cat. number C2925 and C34565). WT and KO cells were plated on glass coverslips as a mosaic and incubated for 24 hrs in a cell culture incubator. Cells were fixed in 4% PFA (in PBS) for 15 min at room temperature and then washed 3 times with PBS. Cells were permeabilized in PBS with 0,1% Triton X-100 for 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Cells were incubated with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary TBK1 antibodies O/N at 4°C. Cells were then washed 3 × 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 µg/ml for 1 hr at room temperature with DAPI. Cells were washed 3 × 10 min with IF buffer and once with PBS. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO).

Imaging was performed using a Zeiss LSM 880 laser scanning confocal microscope equipped with a Plan-Apo 40x oil objective (NA = 1.40). Analysis was done using the Zen navigation software (Zeiss). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.