Identification of highly specific antibodies for Serine/threonine-protein kinase TBK1 for use in immunoblot, immunoprecipitation and immunofluorescence [version 2; peer review: 2 approved]

Walaa Alshafie¹, Maryam Fotouhi¹, Irina Shlaifer², Riham Ayoubi¹, Aled M. Edwards³, Thomas M. Durcan², Peter S. McPherson¹, Carl Laflamme¹

¹Department of Neurology and Neurosurgery, Structural Genomics Consortium, The Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada
²The Neuro's Early Drug Discovery Unit (EDDU), Structural Genomics Consortium, McGill University, Montreal, Quebec, Canada
³Structural Genomics Consortium, University of Toronto, Toronto, Canada

First published: 24 Aug 2022, 11:977
https://doi.org/10.12688/f1000research.124632.1

Abstract

TBK1 is a serine-threonine protein kinase that has been linked to a number of diseases including amyotrophic lateral sclerosis and frontotemporal dementia. Reproducible research on TBK1 has been hampered by the lack of well characterized antibodies. In this study, we characterized 11 commercial antibodies for TBK1 for use in immunoblot, immunofluorescence and immunoprecipitation, using an isogeneic knock-out cell line as a control. We identify antibodies that appear specific for all three applications but invite the readers to interpret the present findings based on their own scientific expertise and use this report as a guide to select the most appropriate antibody for their specific needs.

Keywords

TBK1, Uniprot# Q9UHD2, antibody characterization, antibody validation, Western blot, immunoblot, immunoprecipitation, immunofluorescence

This article is included in the Cell & Molecular Biology gateway.
Introduction

The lack of robust characterization for research antibodies contributes to the reproducibility crisis.\(^1\) Given that there are more than five million antibodies on the commercial market (CiteAb.com), we hypothesize that with appropriate characterization criteria and testing, we should be able to identify high performing antibodies for many if not most proteins in the human genome.\(^2\)

TBK1 regulates autophagy through phosphorylation of Optineurin\(^3\) and the C9ORF72/SMCR8 complex.\(^4\) Of note, mutations in both Optineurin\(^5\) and the C9ORF72/SMCR8 complex\(^6,7\) cause monogenic forms of amyotrophic lateral sclerosis and frontotemporal dementia. Moreover, TBK1 also phosphorylates LC3C, GABARAP-L2\(^8\) and AKT1\(^9\) promoting autophagy.

The endogenous localization of TBK1 under the basal state and during autophagy remains to be determined. Moreover, TBK1 protein interactomes have been determined using overexpression systems, with the exception of one study.\(^10\) TBK1 antibodies are key to address these unknowns.

To explore the availability of high-quality antibodies for human proteins, we devised an antibody characterization strategy in which we use wild-type (WT) and isogenic knockout (KO) control cells to perform head-to-head comparisons of all available commercial antibodies in immunoblot (Western blot), immunoprecipitation and immunofluorescence applications.\(^11\) Here, we apply this approach to TBK1 and identify specific antibodies for all tested applications, enabling biochemical and cellular assessment of TBK1.

Validation and discussion

To identify a cell line that expresses adequate levels of TBK1 protein to provide sufficient signal to noise, we examined the DepMap public proteomic database (depmap.org, RRID:SCR_017655). U2OS was selected as the expression of TBK1 protein level is in the average range of cancer cells analyzed,\(^12\) is easily amenable to CRISPR-Cas9 and is a rather flat cell line ideal for immunofluorescence studies. U2OS was modified with CRISPR/Cas9 to knockout the corresponding TBK1 gene (Table 1).\(^13\)

Extracts from wild-type and TBK1 KO cells were prepared and used to probe 11 commercial antibodies from 6 companies (Table 2) by immunoblot (Western blot) and immunoprecipitation. The profile of each of the antibodies is shown in Figures 1, 2 and 3.

Antibodies were screened by immunofluorescence using a mosaic strategy.\(^11\) WT cells were labelled with a green fluorescent dye, whereas the KO cells were labelled with a far-red fluorescent dye. A third channel was used to image the primary antibodies. Plating WT and KO cells together and imaging both cell type in the same field of view reduces imaging and analysis biases.

In conclusion, we have screened TBK1 commercial antibodies by immunoblot, immunoprecipitation and immunofluorescence. The data provided can be used as a guide to purchase the most appropriate antibody for a researcher's needs.

| Table 1. Summary of the cell lines used. |
|------------------------------------------|
| **Institution** | RRID (Cellosaurus) | **Cell line** | genotype |
| Montreal Neurological Institute | CVCL_0042 | U2OS | WT |
| Montreal Neurological Institute | CVCL_A6LQ | U2OS | TBK1 KO |
| 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-lg     | 10013180   | AB_2882504               | monoclonal| 2D7B1   | mouse| 1.00                  | Wb, IF                            |
| Thermo Fisher Scientific     | PA5-17478      | VL3152289A | AB_10981817              | polyclonal| -        | rabbit| not provided          | Wb, IF, IP                        |
| Thermo Fisher Scientific     | 703154         | 2274494    | AB_2848223               | recombinant-mono| 12H60L39 | 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                            |
| Cell Signaling Technology    | 38066          | 1          | AB_2827657               | recombinant-mono| E8I3G   | rabbit| not provided          | Wb, IP, IF                        |
| Cell Signaling Technology    | 3504           | -          | AB_2255663               | recombinant-mono| D1B4    | rabbit| not provided          | Wb, IP                            |

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

Antibodies

All TBK1 antibodies are listed in Table 2. 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).

Figure 1. Serine/threonine-protein kinase 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 presented to show equal loading of WT and KO lysates and protein transfer efficiency from the acrylamide gels to the nitrocellulose membrane. Antibody dilutions were chosen according to the recommendations of the antibody supplier, except for antibodies 28397-1-AP, PA5-17478, 703154, ab40676, and ab109735, which were titrated at 1/10000 because the signal was too strong following the supplier's recommendations. Antibody dilution used: NB100-56705 at 1/500; 28397-1-AP at 1/10000; 67211-1-Ig at 1/1000; PA5-17478 at 1/10000; 703154 at 1/10000; ab12116 at 1/600; ab40676 at 1/10000; ab109735 at 1/10000; GTX113057 at 1/2000, 38066 at 1/1000, 3504 at 1/1000. Predicted band size: ~83 kDa.
CRISPR/Cas9 genome editing
Cell lines used are listed in Table 1. U2OS TBK1 KO clone was generated using an open-access protocol with an inducible Cas9 U2OS line. Two guide RNAs (purchased at Synthego) were used to introduce a STOP codon in the TBK1 gene (sequence guide 1: UUUGAACAUCCACUGGACGA, sequence guide 2: CAAAUUAUUUGCUAUAAG).

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
Immunoblot were performed as described in our standard operating procedure. Lysates were sonicated briefly and incubated 30 min on ice. Lysates were spun at ~110,000×g 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 at 300 dpi using a regular flatbed scanner to show together with individual immunoblot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N 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 immunoprecipitation
Immunoprecipitation were performed as described in our standard operating procedure. 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...
30 μl 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 110,000 × g 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

Immunofluorescence was performed as described in our standard operating procedure. U2OS WT and TBK1 KO were labelled with a green and a deep 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. Antibodies were tested at 1.0 μg/ml. When the concentration was not indicated by the supplier, we tested the antibodies at 1/500 or 1/1000. At this concentration, the signal from each antibody was in the range of detection of the microscope used. Antibody dilution used: NB100-56705 at 1/1000; 28397-1-AP at 1/500; 67211-1-lg at 1/1000; PA5-17478 at 1/1000; 703154 at 1/500; ab12116 at 1/1000; ab40676 at 1/1500; 703154 at 1/500; GTX113057 at 1/700, 38066 at 1/500, 3504 at 1/500. Bars = 10 μm.

Figure 3. Serine/threonine-protein kinase 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. Antibodies were tested at 1.0 μg/ml. When the concentration was not indicated by the supplier, we tested the antibodies at 1/500 or 1/1000. At this concentration, the signal from each antibody was in the range of detection of the microscope used. Antibody dilution used: NB100-56705 at 1/1000; 28397-1-AP at 1/500; 67211-1-lg at 1/1000; PA5-17478 at 1/1000; 703154 at 1/500; ab12116 at 1/1000; ab40676 at 1/1500; 703154 at 1/500; GTX113057 at 1/700, 38066 at 1/500, 3504 at 1/500. Bars = 10 μm.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure. U2OS WT and TBK1 KO were labelled with a green and a deep red fluorescent 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 40× oil objective (NA = 1.40). Resulting images were cropped and adjusted for brightness and contrast using the Zen navigation software (Zeiss, Zen blue 3.4.91.00000). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator (version 26.3.1).

Data availability

Underlying data

Zenodo: Antibody Characterization Report for Serine/threonine-protein kinase TBK1, https://doi.org/10.5281/zenodo.6402968.17

Zenodo: Dataset for the TBK1 antibody screening study, https://doi.org/10.5281/zenodo.6914815.18

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgments

We thank Chetan Raina (YCharOS Inc.) for his important contribution to the creation of an open scientific ecosystem of antibody manufacturers and knockout cell line suppliers.

A previous version of this article was published on bioRxiv: https://doi.org/10.1101/2022.06.03.494699.

References

1. Baker M: When antibodies mislead: the quest for validation. Nature. 2020; 585(7824): 313–314. PubMed Abstract | Publisher Full Text
2. Laflamme C, et al.: Opinion: Independent third-party entities as a model for validation of commercial antibodies. New Biotechnol. 2021; 65: 1–8. PubMed Abstract | Publisher Full Text
3. Wild P, et al.: Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. Science. 2011; 333(6039): 228–233. PubMed Abstract | Publisher Full Text
4. Sellier C, et al.: Loss of C9ORF72 impairs autophagy and synergizes with polyQ Ataxin-2 to induce motor neuron dysfunction and cell death. EMBO J. 2016; 35(12): 1276–1297. PubMed Abstract | Publisher Full Text
5. Maruyama H, et al.: Mutations of optineurin in amyotrophic lateral sclerosis. Nature. 2010; 465(7295): 223–226. PubMed Full Text
6. Renton AE, et al.: A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. 2011; 72(2): 257–268.
7. DeJesus-Hernandez M, et al.: Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011; 72(2): 245–256. PubMed Abstract | Publisher Full Text
8. Herhaus L, et al.: TBK1-mediated phosphorylation of LC3C and GABARAP-L2 controls autophagosome shedding by ATG4 protease. EMBO Rep. 2020; 21(1): e48317. PubMed Abstract | Publisher Full Text
9. Xie X, et al.: IκB kinase epsilon and TANK-binding kinase 1 activate AKT by direct phosphorylation. Proc. Natl. Acad. Sci. U. S. A. 2011; 108(16): 6474–6479. PubMed Abstract | Publisher Full Text
10. Shang j, et al.: Quantitative Proteomics Identified TTC4 as a TBK1 Interactor and a Positive Regulator of SeV-Induced Innate Immunity. Proteomics. 2018; 18(2). PubMed Full Text
11. Laflamme C, et al.: Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72. elife. 2019; 8. PubMed Abstract | Publisher Full Text
12. Nusinow DP, et al.: Quantitative Proteomics of the Cancer Cell Line Encyclopedia. Cell. 2020; 180(2): 387–402.e16. PubMed Abstract | Publisher Full Text
13. Shlaifer I, et al.: Generation of Knockout Cell Lines Using CRISPR-Cas9 Technology. February 24, 2020. Reference Source
14. Ayoubi R, McPherson PS, Laflamme C: Antibody Screening by Immunoblot. 2021. Publisher Full Text
15. Ayoubi R, et al.: Antibody screening by Immunoprecipitation. 2021. Publisher Full Text
16. Alshafie W, McPherson P, Laflamme C: Antibody Screening by Immunoblot. 2021. Publisher Full Text
17. Alshafie W, Fotouhi M, Shlaifer I, et al.: Antibody Characterization Report for Serine/threonine-protein kinase TBK1. 2021. Publisher Full Text
18. Laflamme C: Dataset for the TBK1 antibody screening study [Data set]. Zenodo. 2022. Publisher Full Text
Nicolas Charlet
Institut de Genetique et de Biologie Moleculaire et Cellulaire (IGBMC), INSERM, CNRS, Université of Strasbourg, Illkirch, France

The authors adequately addressed my comments!

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Simon L. Goodman
The University of Turku, Turku, Finland

The researchers examine a set of 11 commercially available antibodies marketed as recognizing TBK1, a protein linked to several human neurological conditions, for reactivity in Western blot, immunoprecipitation, and fixed cell Immunofluorescence.
The specificity of the antibodies in the various techniques is shown beautifully, but not commented upon, which I find extremely strange. The YCharOs team follow a publication strategy of letting the results "speak for themselves".

The data is clearly presented in a standard format used by the YCharOs consortium, and detailed protocols are available online. I dislike the use of truncated gels in the IP data set (Fig2). It would improve the credibility if the whole IPs were shown, maybe as supplementary data?

Especially, the use of Table#2 including batch and RRID identifiers is an excellent example which all should follow. Would it be possible to have links in print for the RRIDs?

I was rather puzzled by the choice of working concentrations, for the various techniques, which is not explained anywhere. Were all the reagents pre-titred in the techniques? Please, could the authors clarify a little?

The appalling situation with commercial antibodies being inadequately validated is now quite widely known, so YCharOs efforts are laudable, and to be encouraged. For the naive reader, the team at YCharos have an admirable mission to correctly and independently validate commercial tool antibodies. Their model involves external funding from antibody suppliers and the Canadian government.

This may be the source of my only criticism of the presentation method: it is a little odd, because the authors do not follow scientific traditions of discussing their data. One speculates that antibody providers, or managers within providers, of less-than-perfect antibodies might decide that negative commentary on their products was not worth the funding? But this does reduce the usability and accessibility of the data set. Can't the authors add a table summarizing the data sets (+ / - format stating apparent antibody specificity in each technology would do). Even without further detailed comment. I am perfectly well aware of why the authors have not chosen to do this; however, it is a little sad.

Regardless: as a reviewer, I see that all the reagents WB well, but only 2-3 IP a band of an appropriate MW. And only 2 are some way specific in pfa-IF. Only one antibody, clone E813G, appears specific in all techniques.

This shows the power of the YCharOs approach, and the importance of their validation work - which should of course be published. This article could save the average researcher who need to find a specific TBK1 antibody for IF many thousands of dollars, and several weeks of frustrated effort, for example.

Some minor points about the antibody table #2:

108A429: this antibody has some 10 suppliers (CiteAb), but BioTechne is not one of them. Please clarify.

2D7B1 = clone name also used by the supplier for another target specificity (TFF1).

JM42-11 = anti- TFF1 from thermo fisher (and anti-TIA-1 from other companies) clone identifier and
catalog number don't align. Catalog describes clone 12H60L39.

Methods:
IPs lysates: 110000g? Typo? g max? If not, please mention centrifuge used. "Several washes". Please specify wash buffer.

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antibody validation, IHC, integrins,

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 16 Nov 2022

Carl Laflamme, The Montreal Neurological Institute, McGill University, Montreal, Canada

We thank Simon Goodman (reviewer #2) for his review of our manuscript, and for summarizing the YCharOS effort.

Dr. Goodman states “The specificity of the antibodies in the various techniques is shown beautifully, but not commented upon, which I find extremely strange. The YCharOs team follow a publication strategy of letting the results "speak for themselves".
As discusses in respond to Dr. Chartrand, we believe that scientists interested in reading our reports have the expertise to interpret the antibody characterization data.

Dr. Goodman mentions: “I dislike the use of truncated gels in the IP data set (Fig2). It would improve the credibility if the whole IPs were shown, maybe as supplementary data?". We presented truncated gels in Figure 2 for space considerations. The complete lanes are shown in a collection of all raw data available to the public through the Zenodo open science repository. This link is also indicated in the article in the "Data Availability" section.
This article was written under the "Data Notes" specification, and supplementary materials are not accepted. The posting of the supplementary materials on Zenodo was made in accordance with F1000's open data policy.

Dr. Goodman states, “Especially, the use of Table#2 including batch and RRID identifiers is an
excellent example which all should follow. Would it be possible to have links in print for the RRIDs?"

We thank Dr Goodman for his supportive comment. We have followed his recommendation and added the corresponding RRID link for each antibody to Table 2. Moreover, we confirmed that Novus (Bio-Techne) sells clone # 108A429 (cat# NB100-56705). We confirmed clone # 2D7B1 for Proteintech 67211-1-Ig. The clone number for Thermo 703154 is indeed 12H60L39 and not JM42-11 as originally indicated. This mistake was corrected in the revised version of the manuscript.

Dr. Goodman mentioned to be “puzzled by the choice of working concentration”. We agree and have clarified how we selected antibody concentration. For WB, we follow the antibody manufacturer’s recommendations. Most TBK1 antibodies are recommended at 1/500 to 1/2000. For six TBK1 antibodies, the signal-to-noise ratio was satisfactory following manufacturers’ recommendations. However, the signal was too strong for 28397-1-AP, PA5-17478, 703154, ab40676 and ab109735 which we titrated at 1/10000. The figure legend has been updated in the revised version of the manuscript. For IF, we tested a dilution of 1.0 µg/ml for all antibodies. At this concentration, the signal from each antibody was in the range of detection of the microscope used.

Dr. Goodman mentioned that “it is a little odd, because the authors do not follow scientific traditions of discussing their data”. In the F1000 Data Note format, discussion is not required, and we think it is not relevant for this presentation.

Dr Goodman suggested “can't the authors add a table summarizing the data sets (+ / - format stating apparent antibody specificity in each technology would do”). We do not score/recommend antibodies because we tested the antibodies under one set of conditions, and the scoring/recommendation would be valid only under this precise experimental setup and in the cell line used. That said, YCharOS reports serve as an invaluable guide pointing scientists to appropriate antibodies for their experimental needs.

Dr. Goodman speculates that “antibody providers, or managers within providers, of less-than-perfect antibodies might decide that negative commentary on their products was not worth the funding?” We can reassure Dr. Goodman and all readers and users of the YCharOS data that participating companies do not influence data presentation beyond informed scientific feedback. Every antibody tested is presented in the report, we omit no antibodies and never have. Prior to their release on Zenodo, each antibody characterization report is shared for technical review by a group of scientific advisors from academia and from participant antibody providers. Once the technical aspects have been reviewed, the reports, without edits, are uploaded without restriction on Zenodo. To date, antibody providers have actively removed sub-performing antibodies or modified recommendations after assessing our data. One TBK1 antibody has been already removed from the market, and recommendation has been modified for two TBK1 antibodies.

**Competing Interests:** No competing interests were disclosed.
Nicolas Charlet
Institut de Genetique et de Biologie Moleculaire et Cellulaire (IGBMC), INSERM, CNRS, Université of Strasbourg, Illkirch, France

In this work, Laflamme and collaborators investigate the quality and specificity of various commercial antibodies directed against the TBK1 protein, an important kinase regulating immunity and which loss- or gain-of-function mutations cause Amyotrophic Lateral Sclerosis or Normal-Tension Glaucoma, respectively.

Importantly, this work revealed that while most of the tested antibodies are suitable for classical immunoblot, only two of them are usable for immunoprecipitation. Similarly, only two, maybe three, TBK1-antibodies are specific in a well-controlled immunofluorescence assay. This is an extremely important result as the use of an incorrect antibody will undoubtedly result in the wrong interpretation of TBK1 localization by immunofluorescence and/or identification of erroneous interactants in immunoprecipitation followed by mass spectrometry analyzes.

Overall, the text is clear and well written, experiments are well presented and technically extremely well performed and controlled, notably by the use of cells knockout for TBK1 expression, which is classical in immunoblot assays, but when mixed with WT cells is extremely well thought to test antibodies specificity in immunofluorescence. My only recommendation would be to include a table summarizing what antibody is best recommended for what usage, as the current “Invitation to the readers to interpret authors finding” will be a source of misinterpretation, especially in immunoprecipitation and immunofluorescence assays.

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.
**Reviewer Expertise:** Molecular Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

---

**Author Response 16 Nov 2022**

**Carl Laflamme**, The Montreal Neurological Institute, McGill University, Montreal, Canada

We thank Nicolas Charlet (reviewer #1) for review of our manuscript. Dr. Charlet suggests we “include a table summarizing what antibody is best recommended for what usage” to avoid misinterpretation of our antibody characterization data.

We have presented the YCharOS initiative on several occasions and have found that for the most part, scientists interested in our reports have the expertise to interpret the antibody characterization data. Moreover, we do not score/recommend antibodies because we tested the antibodies under one set of conditions, and the scoring/recommendation would be valid only under this precise experimental setup and in the cell line used. That said, YCharOS reports serve as an invaluable guide pointing scientists to appropriate antibodies for their experimental needs.

**Competing Interests:** No competing interests were disclosed.

---

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com