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DATA NOTE

REVISED Identification of high-performing antibodies for Moesin for use in Western Blot, immunoprecipitation, and immunofluorescence [version 3; peer review: 1 approved, 2 approved with reservations]

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Abstract

Moesin is a cytoskeletal adaptor protein, involved in the modification of the actin cytoskeleton, with relevance to Alzheimer's Disease. Well characterized anti-Moesin antibodies would benefit the scientific community. In this study, we have characterized ten Moesin commercial antibodies in Western Blot, immunoprecipitation, and immunofluorescence using a standardized experimental protocol based on comparing read-outs in knockout cell lines and isogenic parental controls. These studies are part of a larger, collaborative initiative seeking to address antibody reproducibility by characterizing commercially available antibodies for human proteins and publishing the results openly as a resource for the scientific community. While use of antibodies and protocols vary between laboratories, we encourage readers to use this report as a guide to select the most appropriate antibodies for their specific needs.

Keywords

Uniprot ID P26038, MSN, Moesin, antibody characterization, antibody validation, Western Blot, immunoprecipitation, immunofluorescence

Open Peer Review

Approval Status ? ? ✓

	1	2	3
version 3 (revision) 01 Dec 2023			✓ view
version 2 (revision) 08 Aug 2023			? view
version 1 13 Feb 2023	? view	? view	

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3. **Emi Hibino**, Nagoya University, Aichi, Japan
Yosuke Senju, Okayama University, Okayama, Japan

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the YCharOS (Antibody Characterization through Open Science) gateway.

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Author roles: Alshafie W: Investigation, Methodology; **Ayoubi R**: Investigation, Methodology, Visualization, Writing – Review & Editing; **Fotouhi M**: Investigation; **Southern K**: Writing – Original Draft Preparation, Writing – Review & Editing; **Laflamme C**: Conceptualization, Data Curation, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing;

Competing interests: For this project, the laboratory of Peter McPherson developed partnerships with high-quality antibody manufacturers and knockout cell line providers. The partners provide antibodies and knockout cell lines to the McPherson laboratory at no cost. These partners include: - Abcam -Aviva Systems Biology -Bio Techne -Cell Signalling Technology -Developmental Studies Hybridoma Bank -Genetex – Horizon Discovery – Proteintech – Synaptic Systems -Thermo Fisher Scientific.

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REVISED **Amendments from Version 2**

This version of the article includes a clarification of how this Data Note it is part of a larger collaborative initiative seeking to address the antibody liability crisis by characterizing commercial antibodies for human proteins and making the corresponding data open and accessible.

Furthermore, errors in figure legends 1 and 3 minor errors by a reviewer were addressed, including the removal of the second star for monoclonal antibody ab193380.

Any further responses from the reviewers can be found at the end of the article

Introduction

Moesin is a cytoskeletal adaptor protein that belongs to the Ezrin-Radixin-Moesin (ERM) family of proteins which connects the actin cytoskeleton to the plasma membrane, regulating the structure and function of specific domains of the cell cortex.[1,2] Moesin plays a pertinent role in immunity, acting on T and B-cell homeostasis and self-tolerance.[3,4] As such, specific mutations in this ERM protein have implications in immunodeficiency.[5,6] Studies have demonstrated that Moesin overexpression is associated with various cancer-related processes and can act as a prognostic marker.[7–11]

Proteomic and protein co-expression network analysis of Alzheimer's Disease (AD) brain has revealed a module that is enriched in inflammation-related proteins.[12] Moesin, along with CD44 antigen, have emerged as key drivers in this inflammation module. Disrupting the Moesin-CD44 pathway is a current focus in AD research.[13] Mechanistic studies would be greatly facilitated with the availability of high-quality antibodies.

Here, we compared the performance of a range of commercially available antibodies for Moesin in Western Blot, immunoprecipitation and immunofluorescence using a knockout based approach. This article serves as a guide to help researchers select high-quality antibodies for their specific needs, facilitating the biochemical and cellular assessment of Moesin properties and function.

Table 1. Summary of the cell lines used.

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Abcam	ab255448	CVCL_0030	HeLa	WT
Abcam	ab265020	CVCL_B9VN	HeLa	*MSN* KO

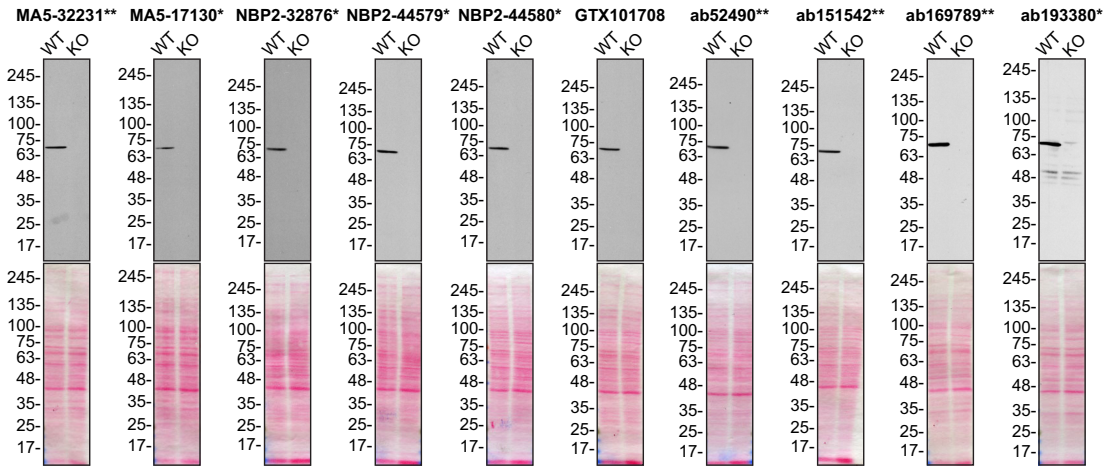

Figure 1. Moesin antibody screening by Western Blot. Lysates of HeLa (WT and *MSN* KO) were prepared, and 25 µg of protein were processed for Western Blot with the indicated Moesin antibodies. The Ponceau stained transfers of each blot are presented to show equal loading of WT and KO lysates and protein transfer efficiency from the acrylamide gels to the nitrocellulose membrane. Antibody dilutions were chosen based on the recommendations provided by suppliers with exceptions for antibodies MA5-32231**, MA5-17130* and GTX101708, which were titrated as the signal received was too strong following the supplier's recommendations. The antibody dilutions were as follows: MA5-32231** at 1/5000, MA5-17130* at 1/5000, NBP2-32876* at 1/5000, NBP2-44579* at 1/5000, NBP2-44580* at 1/5000, GTX101708 at 1/5000, ab52490** at 1/1000, ab151542** at 1/1000, ab169789** at 1/10000 and ab193380* at 1/400. Predicted band size: 68 kDa. *Monoclonal antibody, **Recombinant antibody.

Results and discussion

Our standard protocol involves comparing readouts from wild-type and knockout cells.[14,15] The first step is to identify a cell line(s) that expresses sufficient levels of a given protein to generate a measurable signal. To this end, we examined the DepMap transcriptomics database to identify all cell lines that express the target at levels greater than 2.5 $\log_2$ (transcripts per million "TPM" +1), which we have found to be a suitable cut-off (Cancer Dependency Map Portal, RRID: SCR_017655). Commercially available HeLa cells expressed the Moesin transcript at RNA levels above the average range of cancer cells analyzed. Parental and *MSN* knockout HeLa cells were obtained from Abcam (Table 1).

For Western Blot, we resolved proteins from wild-type and *MSN* KO cell extracts and probed them side-by-side with all antibodies in parallel (Figure 1).

For immunoprecipitation, we used the antibodies to immunopurify Moesin from HeLa cell extracts. The performance of each antibody was evaluated by detecting the Moesin protein in extracts, in the immunodepleted extracts and in the immunoprecipitates (Figure 2).

For immunofluorescence, as described previously, antibodies were screened using a mosaic strategy.[16] First, HeLA WT and MSN KO cell lines were labelled with different coloured fluorescent dyes to distinguish the two cell lines, and then the ten Moesin antibodies were evaluated. Cells were imaged in the same field of view to reduce staining, imaging and image analysis bias (Figure 3).

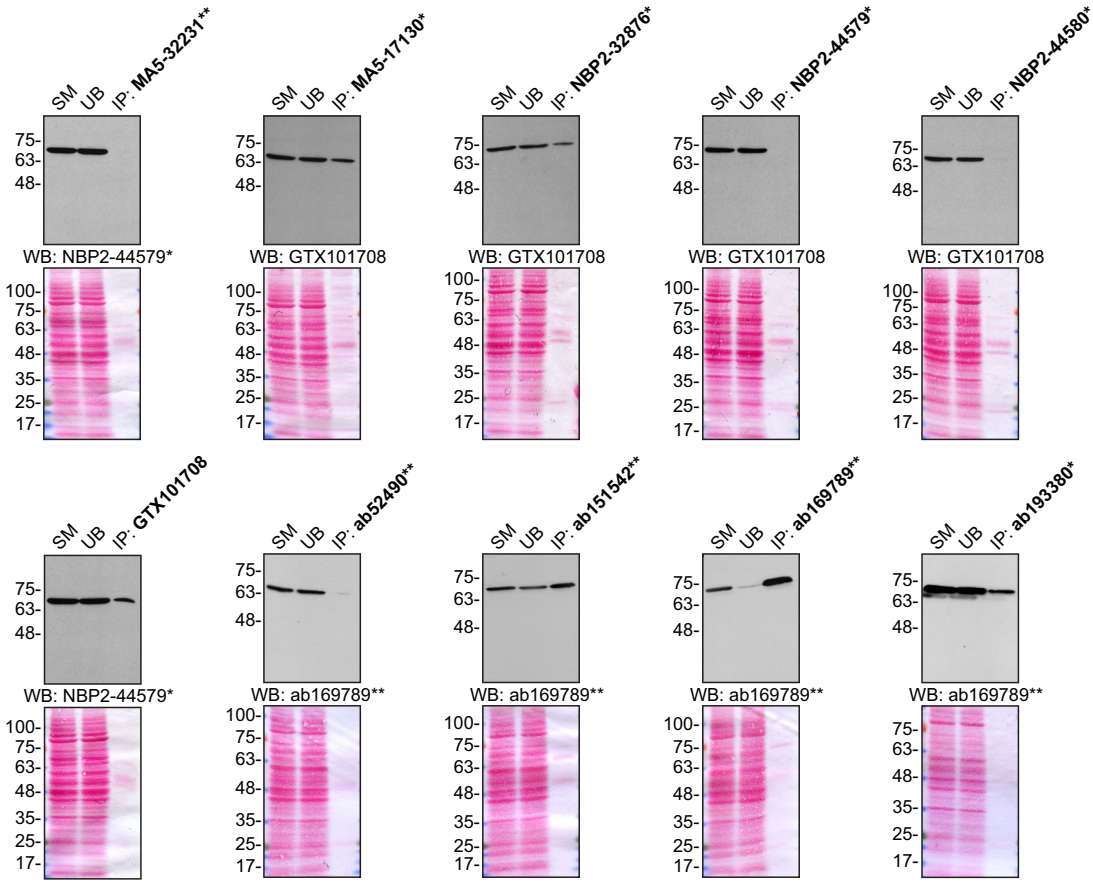

Figure 2. Moesin antibody screening by immunoprecipitation. HeLa lysates were prepared, and IP was performed using 1.0 µg of the indicated Moesin antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for Western Blot with the indicated Moesin antibody. For immunoblot, NBP2-44579*, GTX101708 and ab169789** were used at 1/20000, 1/20000 and 1/10000, respectively. The Ponceau stained transfers of each blot are shown for similar reasons as in Figure 1. SM = 10% starting material; UB = 10% unbound fraction; IP = immunoprecipitate. *Monoclonal antibody, **Recombinant antibody.

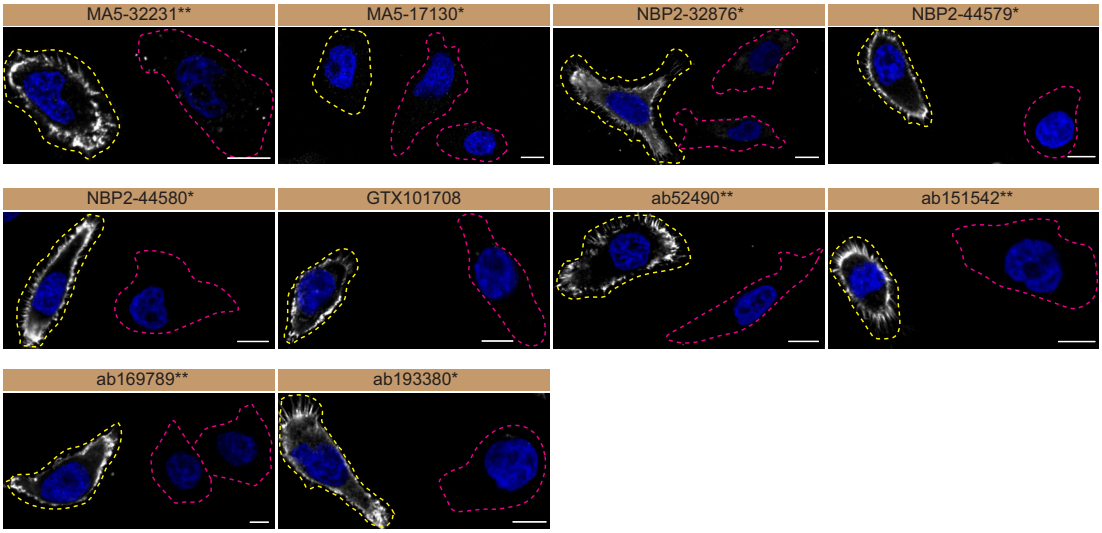

Figure 3. Moesin antibody screening by immunofluorescence. HeLa WT and *MSN* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Moesin antibodies and with the corresponding 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 lines, respectively. Antibody dilutions were chosen based on supplier recommendations, except for MA5-32231** which was titrated to 1/1000 as the signal was too strong. When concentrations were not provided by the supplier, antibodies were tested at 1/1000, which was the case for MA5-17130*. Antibody dilutions used; MA5-32231** at 1/1000; MA5-17130* at 1/1000; NBP2-32876* at 1/200; NBP2-44579* at 1/200; NBP2-44580* at 1/200; GTX101708 at 1/200; ab52490** at 1/200, ab151542** at 1/200, ab169789** at 1/100, ab193380* at 1/200. Bars = 10 µm. *Monoclonal antibody, **Recombinant antibody.

In conclusion, we have screened Moesin commercial antibodies by Western Blot, immunoprecipitation and immunofluorescence, and identified several high-quality antibodies under our standardized experimental conditions.

Methods

Antibodies

All Moesin antibodies are listed in Table 2. Peroxidase-conjugated goat anti-rabbit and anti-mouse antibodies are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520). Alexa-555-conjugated goat anti-rabbit and anti-mouse secondary antibodies are from Thermo Fisher Scientific (cat. number A21429 and A21424).

Cell culture

HeLa WT and *MSN* KO cells used are listed in Table 1. Cells were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) containing 10% fetal bovine serum (GE Healthcare cat. Number SH30072.03), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Antibody screening by Western Blot

Western Blots were performed as described in our standard operating procedure.[17] HeLa WT and *MSN* KO cells 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 ~110,000 × g for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and Western Blot.

Western Blots were performed 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 Western Blot. 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).

Table 2. Summary of Moesin antibodies tested.

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo Fisher Scientific	MA5-32231**	VJ3101165	AB_2809517	recombinant-mono	SC69-01	rabbit	1.00	Wb, IF
Thermo Fisher Scientific	MA5-17130*	VJ3101185	AB_2538601	monoclonal	2C12	mouse	1.00	Wb
Novus Biologicals (a Bio-Techne brand)	NBP2-32876*	4478-1XP160531	AB_2885048	monoclonal	SPM562	mouse	0.20	Wb, IF
Novus Biologicals (a Bio-Techne brand)	NBP2-44579*	44578-2P190315	AB_2885047	monoclonal	MSN/492	mouse	0.20	Wb, IF
Novus Biologicals (a Bio-Techne brand)	NBP2-44580*	4478-3P190605	AB_2885046	monoclonal	MSN/493	mouse	0.20	Wb, IF
GeneTex	GTX101708	40198	AB_10618789	polyclonal	-	rabbit	0.21	Wb, IP, IF
Abcam	ab52490**	GR3207377-11	AB_881245	recombinant-mono	EP1863Y	rabbit	0.20	Wb, IP, IF
Abcam	ab151542**	GR112662-8	AB_2893185	recombinant-mono	EPR2428(2)	rabbit	0.09	Wb, IF
Abcam	ab169789**	GR121830-3	AB_2885098	recombinant-mono	EPR2429(2)	rabbit	0.07	Wb, IP, IF
Abcam	ab193380*	GR3373113-1	AB_2885109	monoclonal	MSN/491	mouse	0.20	Wb, IP

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

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure.[18] Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 µl 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 overnight at 4°C followed by two washes to remove unbound antibodies.

HeLa cells 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 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 Western Blot on a 4-15% acrylamide gel.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure.[16] HeLa cells (WT and MSN KO) were labelled with a green dye and with a deep red fluorescent dye from Abcam (cat. number ab176735 and ab176736), respectively. 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. Coverslips were incubated face down on a 50 µl drop (on paraffin film in a moist chamber) with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Moesin antibodies O/N at 4°C. Cells were washed 3 times for 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies, including DAPI, in IF buffer at a dilution of 1.0 µg/ml for 1 hr at room temperature. Cells were washed 3 times for 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). Analysis was done using Image J. All cell images represent a single focal plane. Figures were prepared using Adobe Photoshop to adjust contrast, apply 1 pixel Gaussian blur and then assembled with Adobe Illustrator.

Data availability

Underlying data

Zenodo: Antibody Characterization Report for Moesin, DOI: https://doi.org/10.5281/zenodo.4724169.[19]

Zenodo: Dataset for the Moesin antibody screening study, DOI: https://doi.org/10.5281/zenodo.7566164.[20]

This project contains the following data:

- Head to head comparison of available commercial antibodies against Moesin by immunoblot (Western blot), immunoprecipitation and immunofluorescence.

- This project contains the following underlying data included in a study aiming at characterizing antibodies for the Moesin protein. The study is available on Zenodo (https://doi.org/10.5281/zenodo.4724169).[19]

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

Acknowledgment

We'd like to thank the NeuroSGC/YCharOS collaborative group for their important contributions to the creation of an open scientific ecosystem of antibody manufacturers and knockout cell line suppliers as well as the development of community-agreed protocols. Members of the group can be found below.

NeuroSGC/YCharOS collaborative group: Riham Ayoubi, Aled M. Edwards, Carl Laflamme, Peter S. McPherson, Chetan Raina and Kathleen Southern.

An earlier version of this of this article can be found on Zenodo (DOI: 10.5281/zenodo.4724169).[19]

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Publisher Full Text

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Publisher Full Text

Open Peer Review

Current Peer Review Status: ? ? ✓

Version 3

Reviewer Report 15 December 2023

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Research Institute for Interdisciplinary Science (RIIS), Okayama University, Okayama, Japan
Emi Hibino
Department of Basic Medicinal Sciences Graduate School of Pharmaceutical Sciences, Nagoya University, Aichi, Japan

The authors have addressed the concerns.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: biophysics, synthetic biology

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

Version 2

Reviewer Report 15 November 2023

https://doi.org/10.5256/f1000research.154210.r220232

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? **Emi Hibino**
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Yosuke Senju
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Moesin is vital for linking the actin cytoskeleton to the plasma membrane to stabilize the cell cortex, and is related to several diseases. Thus, it is essential to characterize moesin-specific antibodies. In this study, the authors characterized moesin-specific commercially available antibodies for western blotting, immunoprecipitation, and immunofluorescence using *MSN* knockout cell lines. This study will help readers to select the appropriate antibodies according to their applications.

To make the article scientifically sounds, the following points should be addressed.

Major comments:

Figure 1: Regarding the ab52490 antibody, the author loaded 20 ug of protein for western blot in the company's webpage, while they described 25 ug in this manuscript.

Figures 1 and 3: The ab193380 antibody is described as a monoclonal antibody (*) in Table 2, while it is described as a recombinant antibody (**) in Figures 1 and 3.

Figures 1 and 3: Regarding the NBP2-32876, NBP2-44579, NBP2-44580 antibodies (Bio-Techne), the authors used U2OS cells for the same western blot and immunofluorescence images in the company's webpage, while they described using HeLa cells in this manuscript.

Figure 2: Is there any reason why the vendor recommends IP for ab52490, and does not recommend for MA5-17130 and NBP2-32876 in Table 2?

Figure 3: Is there any reason why the vendor does not recommend IF for ab193380 in Table 2?

Minor comments:

Figure 3: yellow and magenta dashed "line" should be yellow and magenta dashed "lines".

Please carefully check citations, figure labels, format, language, and readability once more.

I would like to thank the authors for their efforts to promote open science.

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: biophysics

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 28 Nov 2023

Kathleen Southern

Thank you Emi Hibino and Yosuke Senju for your peer-review report on this guide to selecting high-performing antibodies for Moesin in three common applications.

The following changes have been made in response to your comments regarding Figure 1 and 3:

1. ab193380 is in fact a monoclonal antibody and thus the second star has been removed

2. “yellow and magenta dashed line” has been changed to “yellow and magenta dashed lines”

Thank you for being observant and catching these errors! A new version of this publication has been submitted to F1000 which include the changes above.

Please see our responses below regarding your major comments:

Figure 1: *Regarding the ab52490 antibody, the author loaded 20 ug of protein for western blot in the company's webpage, while they described 25 ug in this manuscript.*

When performing the Western Blot, each lane of the gel is loaded with equal amounts of protein. That is why we couldn’t make an exception for this specific antibody as we have to have equal amounts of protein in order to correctly compare the antibodies performance side by side.

Figures 1 and 3: *Regarding the NBP2-32876, NBP2-44579, NBP2-44580 antibodies (Bio-Techne), the authors used U2OS cells for the same western blot and immunofluorescence images in the company's webpage, while they described using HeLa cells in this manuscript.*

As described in our results & discussion section, we select cell lines from based on public transcriptomic database, DepMap. The cell lines chosen are based on transcript level cut-off of the protein of interest. Based on the expression datasheet for Moesin, available on DepMap, HeLa cells expressed higher RNA levels of MSN compared to U2OS, which is why HeLa WT and MSN KO cells were obtained.

Figure 2: *Is there any reason why the vendor recommends IP for ab52490, and does not*

recommend for MA5-17130 and NBP2-32876 in Table 2?

The vendors recommend applications column is prior to validation by YCharOS. Once the validation data for Wb, IP and IF is collected, it is shared with the antibody manufacturer partners. Based on the results, it is then up the discretion of each companies whether or not they change the vendor recommended applications on their website. If you were to visit the vendor's website for antibodies MA5-17130 and NBP2-32876, you'd see that they have included IP in their recommended applications based on the characterization data provided by YCharOS. Antibody ab52490, although was not successful in IP was not removed by the supplier.

Figure 3: *Is there any reason why the vendor does not recommend IF for ab193380 in Table 2?*

As mentioned previously, the list of vendor recommended applications in Table 2 is based on the vendors website, prior to the availability of validation data provided by YCharOS. The vendors did not know prior to YCharOS testing that this antibody was successful in IF. Regardless, the vendors have decided to discontinue this specific antibody from their catalog.

Thank you again for this thorough and detailed report. We hope we addressed all of your concerns and that the article meets your standards to be approved.

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 02 August 2023

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Sungsoo Na
Indiana University Purdue University Indianapolis, Indianapolis, Indiana, USA

This manuscript compared the commercially available antibodies through various assays, including Western blot, immunoprecipation, and immunofluorescence. All the experimental methods and data are well presented. However, this manuscript lacks descriptions on the comparison, characterization, and identification of the antibodies in the results section although the authors claimed that this manuscript is a "guide to select the most appropriate antibody for their specific needs".

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?
Partly

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

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: live cell imaging, cell signaling analysis

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, however I have significant reservations, as outlined above.

Author Response 03 Aug 2023

Kathleen Southern

Thank you Sungsoo Na for reviewing this article.

As a third-party entity, YCharOS refers to remain unbiased which is why the authors do not compare the performance of antibodies based on the displayed results. After presenting the YCharOS initiative on several occasions we have found that, for the most part, scientists interested in our reports have the expertise to interpret the results and decipher which antibodies are appropriate for their research needs. Furthermore, these antibodies are tested under one specific condition and scoring the antibodies would only be valid under this setup and cell line used. We believe that the results from Western Blot, immunoprecipitation and immunofluorescence are telling and will help viewers in finding high-quality antibodies for Moesin. For those that have trouble interpreting the results, we have a clear description on what a “successful” antibody looks like in each application on our FAQ page of the YCharOS gateway (https://f1000research.com/gateways/ycharos/faqs).

We hope you understand our reasoning for our wish to remain unbiased. If there are any other changes you believe would be appropriate to enhance this manuscript that do not involve comparing or scoring the antibodies based on performance, please let us know and we would be happy to do so.

Thank you again for your feedback.

Competing Interests: No competing interests were disclosed.

Reviewer Report 02 August 2023

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Lyndsay Avery
Biology, Saint Michael's College, Colchester, Vermont, UK

In this manuscript, the authors aim to validate 10 commercially available moesin antibodies for western blot, immunoprecipitation, and immunofluorescence. This work makes evident that there is great variability in both what each antibody is suited for and the conditions in which it can be used. In most cases, the vendor has not included some potential uses for the antibody as validated by the authors. But in some, the vendors recommendations don't work well for the tested application.

Suggestions for improvement:

- In introducing the work, the authors point out that moesin is implicated in inflammation seen in Alzheimer's Disease. However, they neglect to introduce its role in other important diseases such as immunodeficiency and cancer, among many others. A more thorough introduction with a more recent literature search would make this paper searchable for many scientists who are interested in these data.

- It's unclear how the antibodies were chosen for these assays. There are many commercially available moesin antibodies. It's important to include more cited antibodies in the analysis such as those from Cell Signaling Technologies (CST) and Developmental Studies Hybridoma Bank (DSHB) as these are significantly cheaper than the antibodies currently assessed. Nonetheless, this analysis is informative for research scientists across many fields.

- It should be noted that the Biotechne antibodies are available through Novus Biologicals. While Novus is a Biotechne company, the antibodies are not searchable on their site.

- For immunoprecipitation data, a negative control for non-specific binding is important. The antibodies that positively IP in WT HeLa cells should be assessed in the MKO HeLa cells.

- Flow cytometry is a vendor recommended application for many of these antibodies. If possible, adding this to the analysis would strengthen the results.

- One concern is that one moesin antibody from Abcam (registry # AB_2885109) has since been discontinued by the manufacturer as they found that it was not specific to moesin. This antibody should be removed from analysis or addressed as nonspecific in the discussion.

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

Partly

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: Immunology, T cell biology, migration, cytoskeleton, hematopoiesis

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, however I have significant reservations, as outlined above.

Author Response 03 Aug 2023

Kathleen Southern

Thank you, Lyndsay Avery, for your response, your feedback is much appreciated.

We will be submitting a new version of the manuscript with your following suggestions included. In the introduction, we have included more recent information on Moesin's implications in disease. As for Table 2, we have made it clear that the Bio-Techne antibodies are available from Novus Biologicals, which is a Bio-Techne brand.

As for your point regarding how the antibodies were chosen for the assays, the antibodies to be tested are donated by YCharOS partners (https://ycharos.com/partners/), who cover nearly 28% of all antibodies listed on the Antibody registry (antibodyregistry.org). When the industry partners are informed of upcoming targets, they donate the antibodies they have the capacity to supply at the time the study commences. At the time this study for Moesin was performed, we did not yet establish partnerships with CST nor DHSB, which is why their antibodies were not tested. It would be a great to characterize these antibodies as a follow-up study.

To respond to your comment regarding a negative control for immunoprecipitation, we too agree that negative controls to demonstrate any non-specific binding are important when validating antibodies by immunoprecipitation. That being said, under our protocol, all antibodies were tested under the same conditions within the same experiment, any antibody that did not show an enrichment in the immunoprecipitate acts as the negative control.

As majority of researchers seem to be more interested in protein localization rather than quantifying the presence of a protein in a cell, we have prioritized testing the antibodies

under immunofluorescence and have included it in our standard operating procedure. Nonetheless, flow cytometry is a very useful application that will be considered as a follow-up study in the future.

To respond to your last point, Abcam made the decision to remove antibody AB_2885109 based on the result of this study and was discontinued thereafter as to provide their customers with highest quality standards of products. For more information as to why the antibody was discontinued, you can visit their website (https://www.abcam.com/products/primary-antibodies/moesin-antibody-msn491-ab193380.html). We believe it's important to demonstrate that our industry partners trust our validation procedure and use it as an opportunity to re-evaluate the quality of their products, which is why we kept antibody AB_2885109 in the results.

We hope you understand our reasoning for not making all of your suggested changes and are happy to receive further feedback!

Competing Interests: No competing interests were disclosed.

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