# Antibody Characterization Report for Moesin

**YCharOS Antibody Characterization Report**

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**Target:**

**Protein name**: Moesin

**Gene name**: *MSN*

**Uniprot**: P26038

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**Table 1: Summary of the Moesin antibodies tested**

| Company | Catalog number | Lot number | RRID | Clonality | Clone ID | Host | Concentration (µg/µl) |
|---|---|---|---|---|---|---|---|
| Thermo | MA5-32231 | VJ3101165 | AB_2809517 | recombinant-mono | SC69-01 | rabbit | 1.00 |
| Thermo | MA5-17130 | VJ3101185 | AB_2538601 | monoclonal | 2C12 | mouse | 1.00 |
| Bio-Techne | NBP2-32876 | 4478-1XP160531 | AB_2885048 | monoclonal | SPM562 | mouse | 0.20 |
| Bio-Techne | NBP2-44579 | 44578-2P190315 | AB_2885047 | monoclonal | MSN/492 | mouse | 0.20 |
| Bio-Techne | NBP2-44580 | 4478-3P190605 | AB_2885046 | monoclonal | MSN/493 | mouse | 0.20 |
| GeneTex | GTX101708 | 40198 | AB_10618789 | polyclonal | - | rabbit | 0.21 |
| Abcam | ab52490 | GR3207377-11 | AB_881245 | recombinant-mono | EP1863Y | rabbit | 0.20 |
| Abcam | ab151542 | GR112662-8 | AB_151542 | recombinant-mono | EPR2428(2) | rabbit | 0.09 |
| Abcam | ab169789 | GR121830-3 | AB_2885098 | recombinant-mono | EPR2429(2) | rabbit | 0.07 |
| Abcam | ab193380 | GR3373113-1 | AB_2885109 | monoclonal | MSN/491 | mouse | 0.20 |

**Table 2: Summary of the cell lines used**

| Company | Catalog number | RRID (Cellosaurus) | Cell line | genotype |
|---|---|---|---|---|
| Abcam | ab255448 | - | HeLa | WT |
| Abcam | ab265020 | - | HeLa | *MSN* KO |

**Figure 1: Analysis of Moesin antibodies by immunoblot.**

Lysates of HeLa (WT or *MSN* KO) were prepared and 25 µg of protein were processed for immunoblot with the indicated Moesin antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 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 1/1000, ab193380 at 1/400.

**Figure 2: Analysis of Moesin antibodies by immunoprecipitation.**

Lysates were prepared and immunoprecipitation 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 immunoblot with the indicated Moesin antibody. For immunoblot, NBP2-44579 and GTX101708 were used both at 1/20000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

**Figure 3: Analysis of Moesin antibodies by immunofluorescence.**

WT and *MSN* KO cells were labelled with a green or a far red 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. Acquisition of the green (WT), red (antibody staining) and far-red (KO) channels was performed. Representative grayscale images of the red channel are shown and 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: 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. Bar = 10 µm.

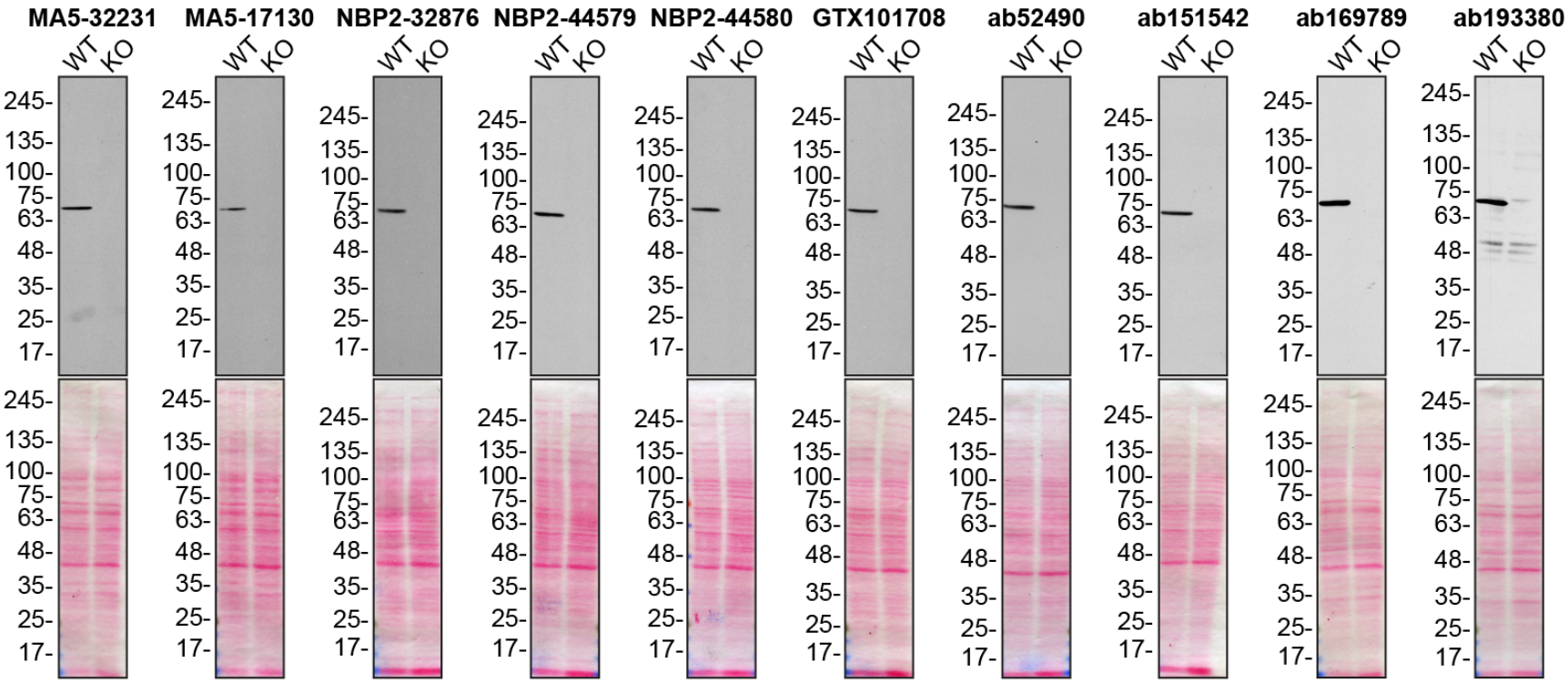


**Figure 1: Moesin antibody screening by immunoblot**

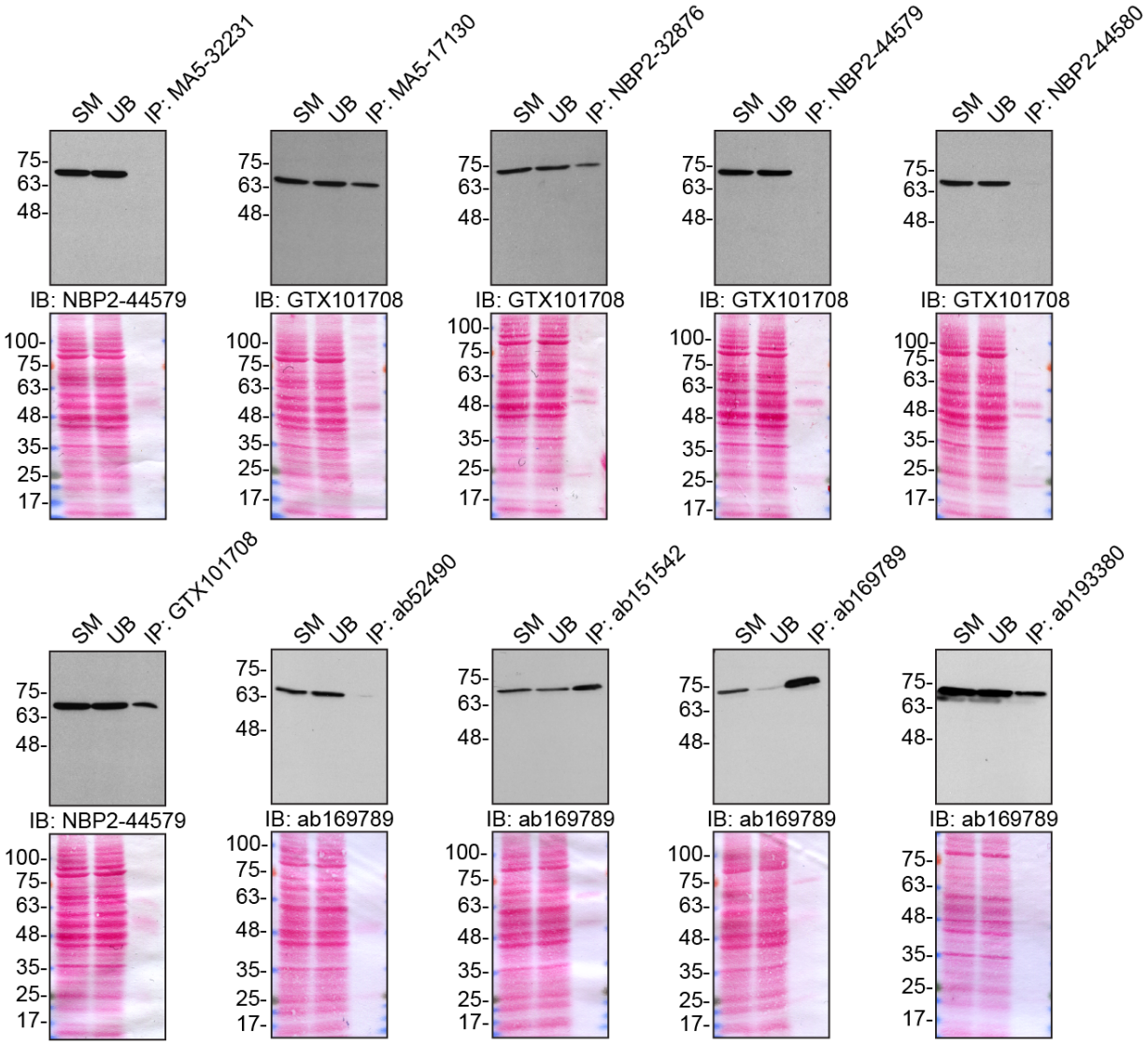


**Figure 2: Moesin antibody screening by immunoprecipitation**

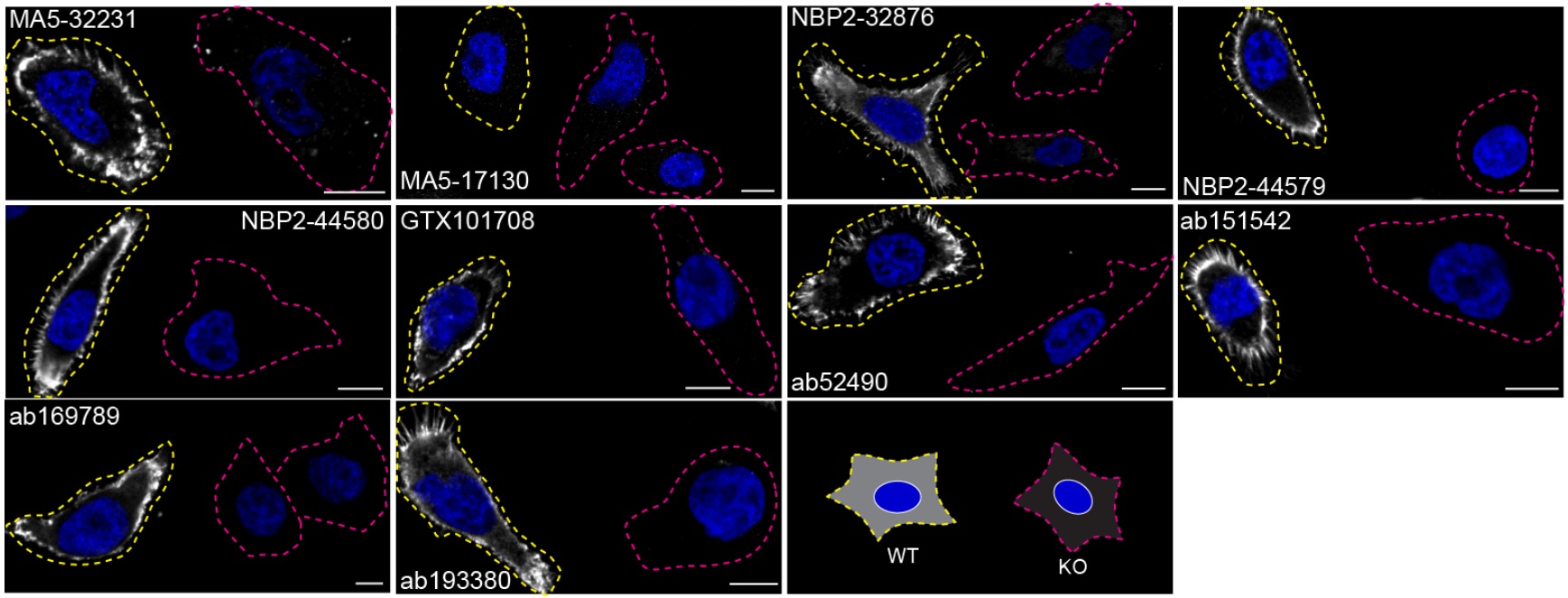


**Figure 3: Moesin antibody screening by immunofluorescence**

## Materials and methods

### Antibodies

All Moesin 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 A2A21429).

### Cell lines

HeLa WT and *MSN* KO cell lines used are listed in Table 2. Cells were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) containing 10% bovine calf 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).

### Immunoblot

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 ~110,000xg for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot.

Immunoblots 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 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).

### 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.

HeLa 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. Prot-A:HRP was used as a secondary detection system (MilliporeSigma, cat. number P8651) at a dilution of 0.1 µg/ml when a rabbit antibody was used by immunoblot to detect the immunoprecipitate fraction performed with a rabbit antibody.

**Immunofluorescence**

HeLa cells (WT and *MSN* KO) were labelled with a green dye and with a deep red 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 647-conjugated secondary antibodies 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 40x 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.