# Antibody Characterization Report for Annexin A11

**YCharOS Antibody Characterization Report**

Author(s): Walaa Alshafie[†,1], Riham Ayoubi[†,1], Michael Nicouleau[2], Thomas M. Durcan[2], Peter S. McPherson[1*] and Carl Laflamme[1*]

[†] Authors contributed equally
[1] Tanenbaum Open Science Institute, Structural Genomics Consortium, Montreal Neurological Institute, McGill University, Montreal, Canada
[2] Early Drug Discovery Unit (EDDU), Montreal Neurological Institute, McGill University, Montreal, Canada
* Corresponding authors: carl.laflamme@mcgill.ca, peter.mcpherson@mcgill.ca

**Target**:

**Recommended protein name:** Annexin A11

**Alternative protein names:** 56 kDa autoantigen, Annexin XI, Annexin-11, Calcyclin-associated annexin 50

**Gene name:** *ANXA11*

**Uniprot:** P50995

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. This report guides researchers to select the most appropriate antibodies for Annexin A11. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Annexin A11 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. A synthetic human single-chain fragment variable (scFv) was tested and a protocol on how to properly use scFv for research purposes is available [3]. HeLa was selected based on evidence of appropriate Annexin A11 protein expression determined through public proteomics databases, namely PAXdb [4] and DepMap [5]. HeLa was modified with CRISPR/Cas9 to knockout the corresponding *ANXA11* gene [6].

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 Annexin A11 antibodies tested**

| Company | Catalog number | Lot number | RRID (Antibody Registry) | Clonality | Clone ID | Host | Concentration (µg/µl) | Vendors recommended applications |
|---|---|---|---|---|---|---|---|---|
| Thermo | MA5-25052 | VL3152361 | AB_2723879 | monoclonal | OTI1C6 | mouse | 1.00 | Wb |
| Thermo | PA5-96670 | VL3153028A | AB_2808472 | polyclonal | - | rabbit | 6.91 | Wb, IP, IF |
| Thermo | PA5-82983 | VL3152388C | AB_2790139 | polyclonal | - | rabbit | 0.30 | Wb, IF |
| Abcam | ab236599 | GR3361696-3 | AB_2893216 | polyclonal | - | rabbit | 0.37 | Wb, IF |
| Abcam | ab137424 | GR111186-6 | AB_2893183 | polyclonal | - | rabbit | 0.35 | Wb, IF |
| Aviva Sys Bio | ARP36586_T100 | QC5533-42684 | AB_841984 | polyclonal | - | rabbit | 1.00 | Wb |
| Aviva Sys Bio | ARP85286_P050 | QC62296-42950 | AB_2894926 | polyclonal | - | rabbit | 0.50 | Wb |
| Aviva Sys Bio | ARP87950_P050 | QC63496-42991 | AB_2894925 | polyclonal | - | rabbit | 0.50 | Wb |
| GeneTex | GTX114255 | 40156 | AB_10620672 | polyclonal | - | rabbit | 0.35 | Wb |
| GeneTex | GTX100047 | 39399 | AB_1080933 | polyclonal | - | rabbit | 1.00 | Wb |
| GeneTex | GTX114256 | 40156 | AB_10619201 | polyclonal | - | rabbit | 0.50 | Wb |
| GeneTex | GTX84884 | 822104503 | AB_10730690 | monoclonal | 1C6 | mouse | 1.00 | Wb, IF |
| GeneTex | GTX33010 | 822104511 | AB_2887692 | polyclonal | - | rabbit | 0.08 | Wb, IF, IP |
| Structural Genomics Consortium | AC-ANXA11-1* | YSANXA11A-c001 | - | recombinant-mono (single chain-Fv) | YSANXA11A-c001 | human | 0.40 | IP |
| Proteintech | 10479-2-AP | 16633 | AB_2057171 | polyclonal | - | rabbit | 0.30 | WB, IP, IF |

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

***Sequence of AC-ANXA11-1single chain-Fv**:
EVQLLESGGGLVQPGGSLRLSCAASGFTFGYSYMSWVRQAPGKGLEWVSSIGGGGSYTSYADSVKGRFTISRDNSKNTLY
LQMNSLRAEDTAVYYCARYYFSYLDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQS
ISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYYYALSTFGQGTKLEIK
**Tag on the single chain-Fv**: 3xFLAG, His6

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

| Institution | Catalog number | RRID (Cellosaurus) | Cell line | genotype |
|---|---|---|---|---|
| ATCC | CCL-2 | CVCL_0030 | HeLa | WT |
| Montreal Neurological Institute | - | CVCL_B3SH | HeLa | *ANXA11* KO |
| ATCC | CRL-1573 | CVCL_0045 | HEK293T | WT |
| Horizon Discovery | C631 | CVCL_Y019 | HAP1 | WT |
| Queen's University | - | - | MDA-MB-231 pWPLXd (GFP positive) | WT |
| Abcam | ab255451 | CVCL_0291 | HCT 116 | WT |
| ATCC | CRL-4000 | CVCL_4388 | hTERT-RPE1 | WT |

**Figure 1: Annexin A11 antibody screening by immunoblot.**

A) Lysates of HeLa (WT and *ANXA11* KO) were prepared and 50 μg of protein were processed for immunoblot with the indicated Annexin A11 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MA5-25052 at 1/200, PA5-96670 at 1/1000, PA5-82983 at 1/1000, ab236599 at 1/1000, ab137424 at 1/1000, ARP36586_T100 at 1/1000, ARP85286_P050 at 1/1000, ARP87950_P050 at 1/1000, GTX114255 at 1/1000, GTX100047 at 1/1000, GTX114256 at 1/1000, GTX84884 at 1/200, GTX33010 at 1/1000, AC-ANXA11-1* at 1/1000, 10479-2-AP at 1/2000. Predicted band size: 54 kDa.

B) Comparison of Annexin A11 protein level in different cell lines. Lysates were prepared from the indicated cell lines and processed as in A). ab236599 was used at 1/1000.

**Figure 2: Annexin A11 antibody screening by immunoprecipitation.**

HeLa lysates were prepared, and immunoprecipitation was performed using 1.0 μg of the indicated Annexin A11 antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Annexin A11 antibody. For immunoblot, ab137424, MA5-25052 and GTX114255 were used at 1/1000, 1/200 and 1/1000, respectively. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain.

**Figure 3: Annexin A11 antibody screening by immunofluorescence using PFA for fixation.**

HeLa WT and *ANXA11* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed, plated to a 1:1 ratio on coverslips and fixed using paraformaldehyde (PFA). Cells were stained with the indicated Annexin A11 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) 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. Antibody dilution used: MA5-25052 at 1/1000, PA5-96670 at 1/700, PA5-82983 at 1/300, ab236599 at 1/400, ab137424 at 1/400, ARP36586_T100 at 1/400, ARP85286_P050 at 1/400, ARP87950_P050 at 1/400, GTX114255 at 1/400, GTX100047 at 1/400, GTX114256 at 1/400, GTX84884 at 1/400, GTX33010 at 1/800, AC-ANXA11-1* at 1/400, 10479-2-AP at 1/200. Bars = 10 μm.

**Figure 4: Annexin A11 antibody screening by immunofluorescence using methanol for fixation.**

HeLa WT and *ANXA11* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed, plated to a 1:1 ratio on coverslips and fixed using methanol. Cells were processed for immunofluorescence as in Figure 3.

Figure 1: Annexin A11 antibody screening by immunoblot

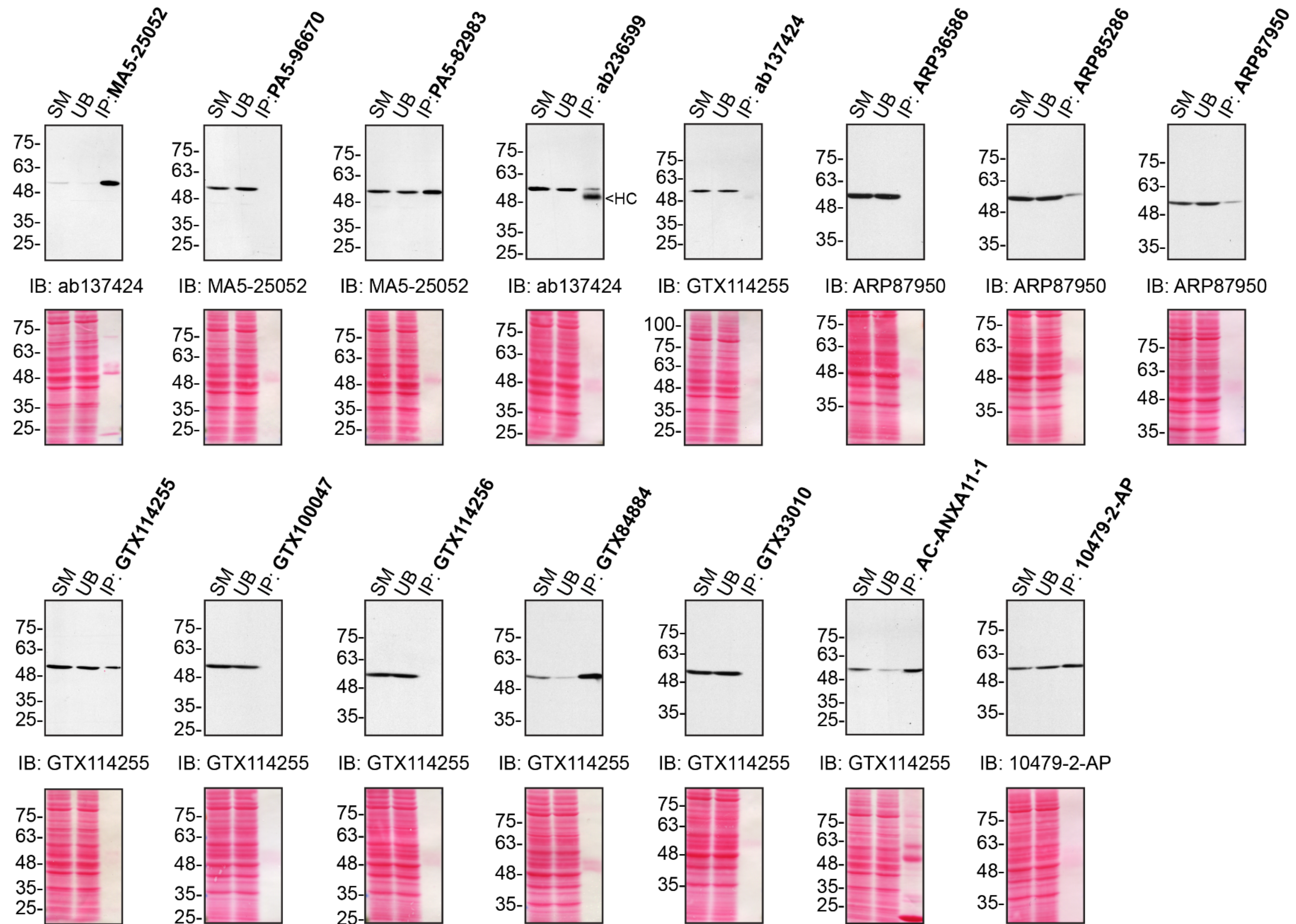


**Figure 2: Annexin A11 antibody screening by immunoprecipitation**

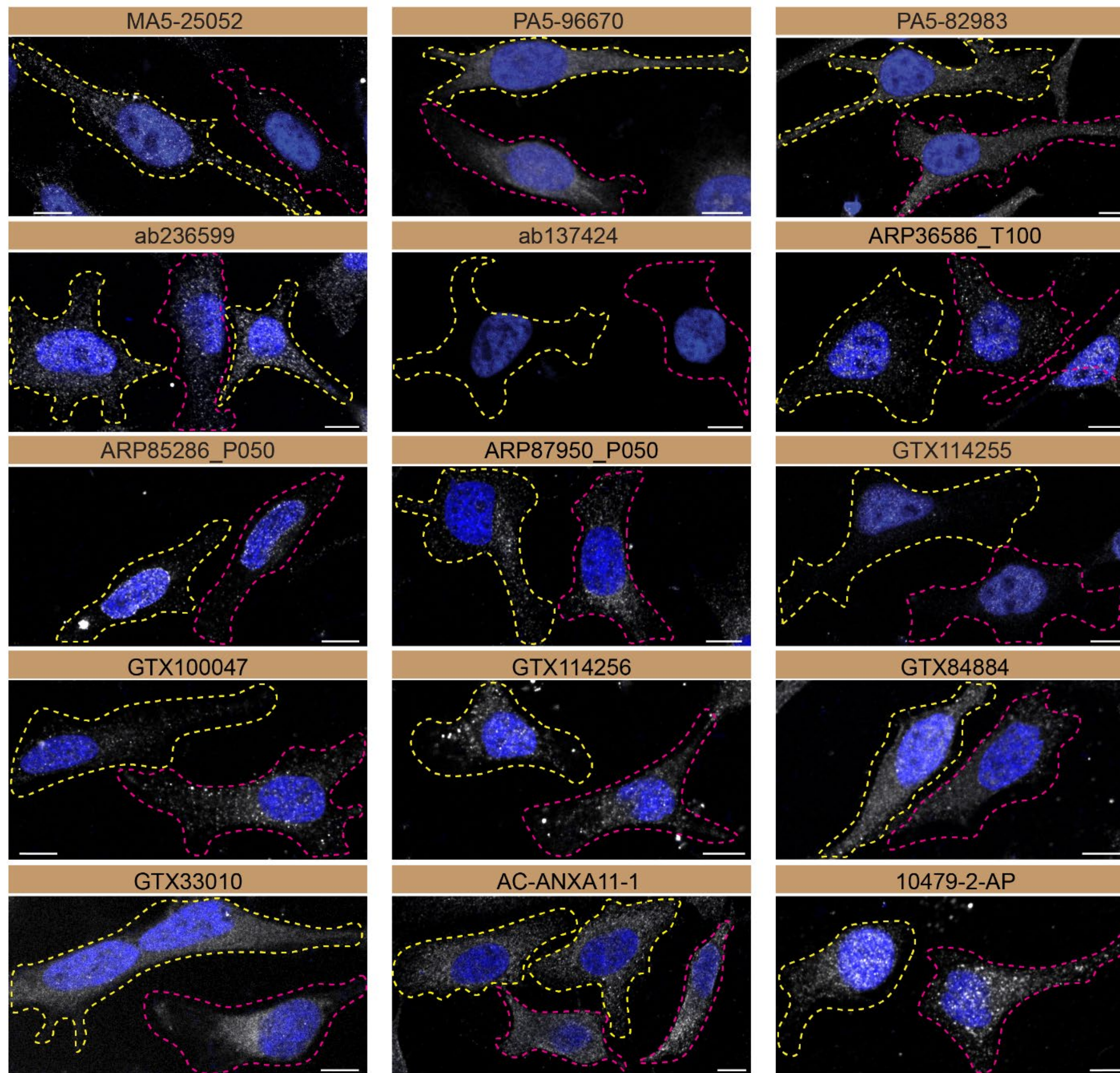


**Figure 3 : Annexin A11 antibody screening by immunofluorescence using PFA for fixation**

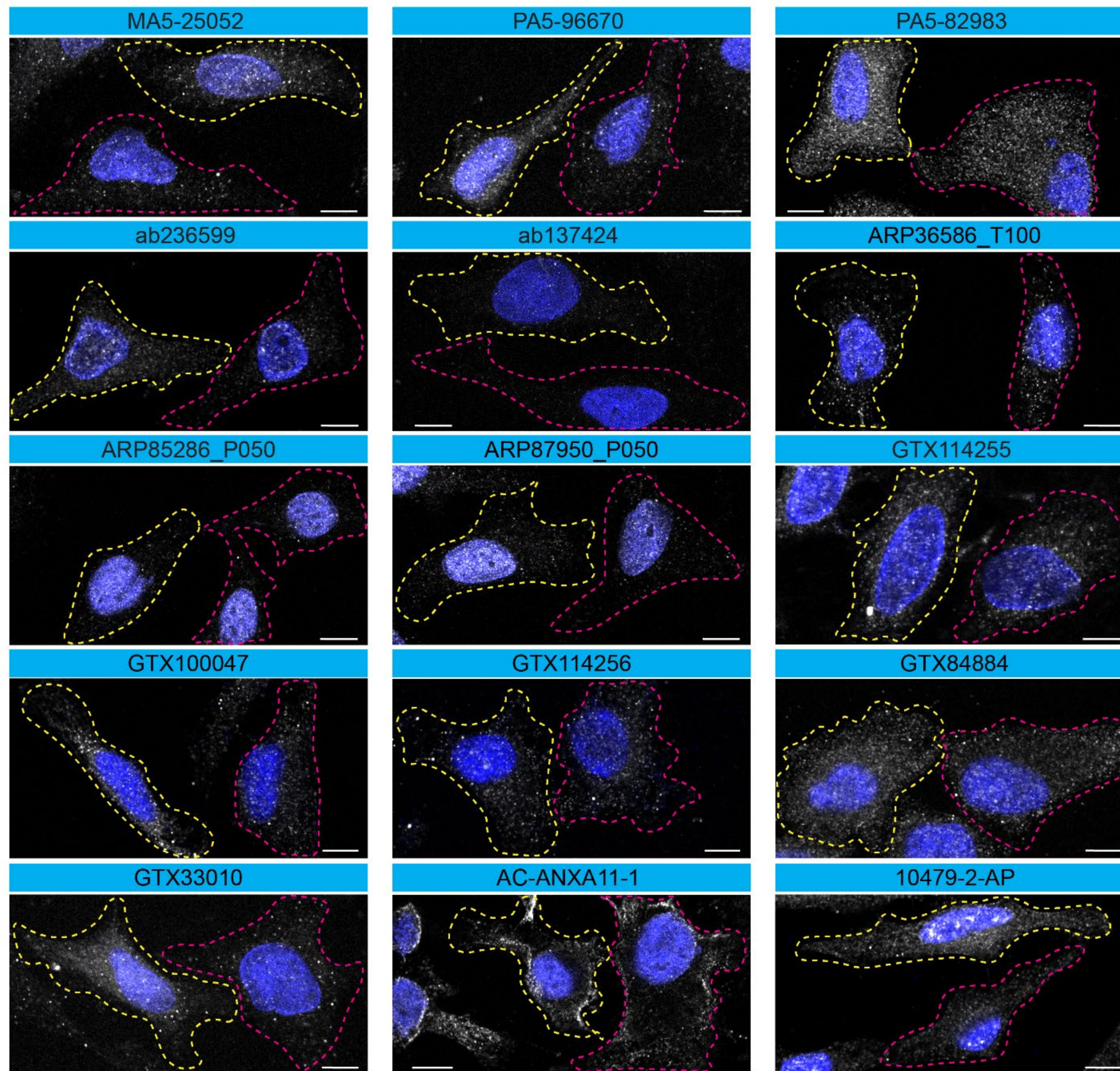


**Figure 4 : Annexin A11 antibody screening by immunofluorescence using Methanol for fixation**

## Materials and methods

### Antibodies

All Annexin A11 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. A single guide RNA was used to knockout the *ANXA11* gene in HeLa cells (sequence of the guide: ACAUGCCCAACCUGUACCCU).

### 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

Immunoblots were performed as described in our standard operating procedure [7]. HeLa (WT and *ANXA11* 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 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).

**Antibody screening by immunoprecipitation**

Immunoprecipitation was performed as described in our standard operating procedure [8]. 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 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 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 for an experiment where a rabbit antibody was used for both immunoprecipitation and its corresponding immunoblot.

**Antibody screening by immunofluorescence**

Immunofluorescence was performed as described in our standard operating procedure [9]. HeLa WT and *ANXA11* KO were labelled with a green and a far-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 or in 100% methanol (chilled at -20°C) for 5 min on ice and then washed 3 times with PBS. PFA-fixed cells were permeabilized in PBS with either 0.1% Triton X-100. Cells were incubated in PBS with 5% BSA and 5% goat serum together with 0.01% Triton X-100 for 30 min at room temperature to block unspecific binding of the antibodies. Cells were incubated with IF buffer (PBS, 5% BSA, 0.01% Triton X-100) containing the primary Annexin A1 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. Cells were washed 3 × 10 min with IF buffer and once with PBS containing DAPI. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO).

Imaging was performed using a Zeiss LSM 700 laser scanning confocal microscope equipped with a Plan-Apo 63x 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.

## References


1. Laflamme, C., et al., *Opinion: Independent third-party entities as a model for validation of commercial antibodies.* N Biotechnol, 2021. **65**: p. 1-8 DOI: 10.1016/j.nbt.2021.07.001.
2. Laflamme, C., et al., *Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72.* Elife, 2019. **8** DOI: 10.7554/eLife.48363.
3. Preger, C., et al., *Generation and validation of recombinant antibodies to study human aminoacyl-tRNA synthetases.* J Biol Chem, 2020. **295**(41): p. 13981-13993 DOI: 10.1074/jbc.RA120.012893.
4. Wang, M., et al., *Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines.* Proteomics, 2015. **15**(18): p. 3163-8 DOI: 10.1002/pmic.201400441.
5. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16 DOI: 10.1016/j.cell.2019.12.023.
6. Nicouleau, M., et al., *Knockout Cell Lines Using CRISPR-Cas9 and ddPCR Technology.* 2020 DOI: 10.5281/zenodo.3875777.
7. Ayoubi, R., P.S. McPherson, and C. Laflamme, *Antibody Screening by Immunoblot.* 2021 DOI: https://doi.org/10.5281/zenodo.5717510.
8. Ayoubi, R., et al., *Antibody screening by Immunoprecitation.* 2021 DOI: https://doi.org/10.5281/zenodo.5717516.
9. Alshafie, W., P. McPherson, and C. Laflamme, *Antibody screening by Immunofluorescence.* 2021 DOI: https://doi.org/10.5281/zenodo.5717498.