Antibody Characterization Report for

Prolow-density lipoprotein receptor-related protein1 (LRP-1)

YCharOS Antibody Characterization Report

Author(s): Riham Ayoubi[1], Maryam Fotouhi[1], Joel Ryan[2], Sara González Bolívar[1], Donovan Worrall[1], Wolfgang Reintsch[3], Thomas M. Durcan[3], Claire M. Brown[2], Peter S. McPherson[1] and Carl Laflamme[1*]

[1] Department of Neurology and Neurosurgery, Structural Genomics Consortium, The Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada
[2] Advanced BioImaging Facility (ABIF), McGill University, Montreal, Canada
[3] The Neuro's Early Drug Discovery Unit (EDDU), Structural Genomics Consortium, McGill University, Montreal, Quebec, Canada
* Corresponding author: carl.laflamme@mcgill.ca

Target:

Recommended protein name: Prolow-density lipoprotein receptor-related protein 1

Short protein name: LRP-1

Gene name: *LRP1*

Uniprot: Q07954

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. In this study, we characterized ten LRP-1 commercial antibodies for Western Blot, immunoprecipitation, and immunofluorescence using a standardized experimental protocol [2] based on comparing read-outs in knockout cell lines and isogenic parental controls. We identified many well-performing antibodies and encourage readers to use this report as a guide to select the most appropriate antibody for their specific needs.

An HAP1 *LRP1* KO line is available at Horizon discovery and was used in this study. Expression of LRP-1 protein in HAP1 is adequate as determined through DepMap [3, 4].

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 cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC001773c001	CVCL_SV84	HAP1	*LRP1* KO

Table 2: Summary of the LRP-1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab92544**	GR259330-39	AB_2234877	recombinant-mono	EPR3724	rabbit	0.460	Wb,IP,IF
Abclonal	A0633**	4000000275	AB_2861470	recombinant-mono	ARC0275	rabbit	0.230	Wb
Bio-Techne	MAB6360*	CEDN0420111	AB_10640296	monoclonal	545503	mouse	1.000	Wb,IF
Bio-Techne	NBP2-67286**	HP0207	AB_2936898	recombinant-mono	SA0290	rabbit	1.000	Wb,IP,IF
Cell Signaling Technology	26387**	1	AB_2936897	recombinant-mono	E2Q7S	rabbit	0.014	Wb,IF
Cell Signaling Technology	64099	1	AB_2799654	polyclonal	-	rabbit	0.033	Wb,IP
DHSB	2C6*	2016-05-26	AB_528078	monoclonal	2C6	rat	0.017	Wb,IP
GeneTex	GTX64220**	822104517	AB_2888587	recombinant-mono	GT1028	rabbit	1.000	Wb,IF
Thermo Fisher Scientific	MA5-31959**	WI3376411	AB_2809253	recombinant-mono	SA0290	rabbit	0.230	Wb,IF
Thermo Fisher Scientific	MA5-35053**	WI3377711	AB_2848958	recombinant-mono	ARC0275	rabbit	0.230	Wb

DHSB= Developmental Studies Hybridoma Bank, Wb=Western Blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody

Materials and methods

Antibodies

All the LRP-1 antibodies tested are listed in Table 2. Peroxidase-conjugated goat anti-rabbit, anti-mouse, and anti-rat antibodies are from Thermo Fisher Scientific (cat. number 65-6120, 62-6520, and 31470, respectively). Alexa-555-conjugated goat anti-rabbit, anti-mouse and anti-rat secondary antibodies are from Thermo Fisher Scientific (cat. number A21429, A21424 and A-21434, respectively).

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 Western Blot

Western Blots were performed as described in our standard operating procedure [5]. HAP1 WT and *LRP1* KO (listed in Table 1) were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) from Thermo Fisher Scientific (cat. number 89901) supplemented with 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number 78429). 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 Western Blot. BLUelf prestained protein ladder (GeneDireX, cat. number PM008-0500) and the HiMark Pre-stained Protein Standard (Thermo Fisher Scientific, cat. number LC5699) were used.

Western Blots were performed with precast midi 4-20% Tris-Glycine polyacrylamide gels from Thermo Fisher Scientific (cat. number WXP42012BOX) ran with Tris/Glycine/SDS buffer from Bio-Rad (cat. number 1610772), loaded in Laemmli loading sample buffer from Thermo Fisher Scientific (cat. number AAJ61337AD) and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau S staining (Thermo Fisher Scientific, cat. number BP103-10) 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% milk in TBS with 0,1% Tween 20 (TBST) from Cell Signaling (cat. number 9997). 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 were incubated with Pierce ECL from Thermo Fisher Scientific (cat. number 32106) or Clarity Western ECL Substrate from Bio-Rad (cat. number 1705061) prior to detection with the iBright™ CL1500 Imaging System from Thermo Fisher Scientific (cat. number A44240).

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure [6]. Antibody-beads conjugates were prepared by adding 2 µg of antibody or 10 µl of the antibodies 26387** and 64099 to 500 µl of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with with 30µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse and rat antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Tubes were rocked for ~1 hr at 4°C followed by two washes to remove unbound antibodies.

HAP1 WT were collected in Pierce IP buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) supplemented with protease inhibitor. Lysates were rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. 0.5 ml aliquots at 0.5 mg/ml of lysate were incubated with an antibody-bead conjugate for ~1 hr at 4°C. The unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of IP lysis buffer and processed for SDS-PAGE and Western Blot on precast midi 4-20% Tris-Glycine polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a concentration of 0.3 µg/ml.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [7]. HAP1 WT and *LRP1* 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 in a 96-well plate with optically clear flat-bottom. (Perkin Elmer, cat. number 6055300) 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 LRP-1 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.

Images were acquired on an ImageXpress micro confocal high-content microscopy system (Molecular Devices), using a 20x NA 0.95 water immersion objective and scientific CMOS cameras, equipped with 395, 475, 555 and 635 nm solid state LED lights (lumencor Aura III light engine) and bandpass filters to excite DAPI, Cellmask Green, Alexa-555 and Cellmask Red, respectively. Images had pixel sizes of 0.68 x 0.68 microns, and a z-interval of 4 microns. For analysis and visualization, shading correction (shade only) was carried out for all images. Then, maximum intensity projections were generated using 3 z-slices. Segmentation was carried out separately on maximum intensity projections of Cellmask channels using CellPose 1.0, and masks were used to generate outlines and for intensity quantification. Figures were assembled with Adobe Illustrator.

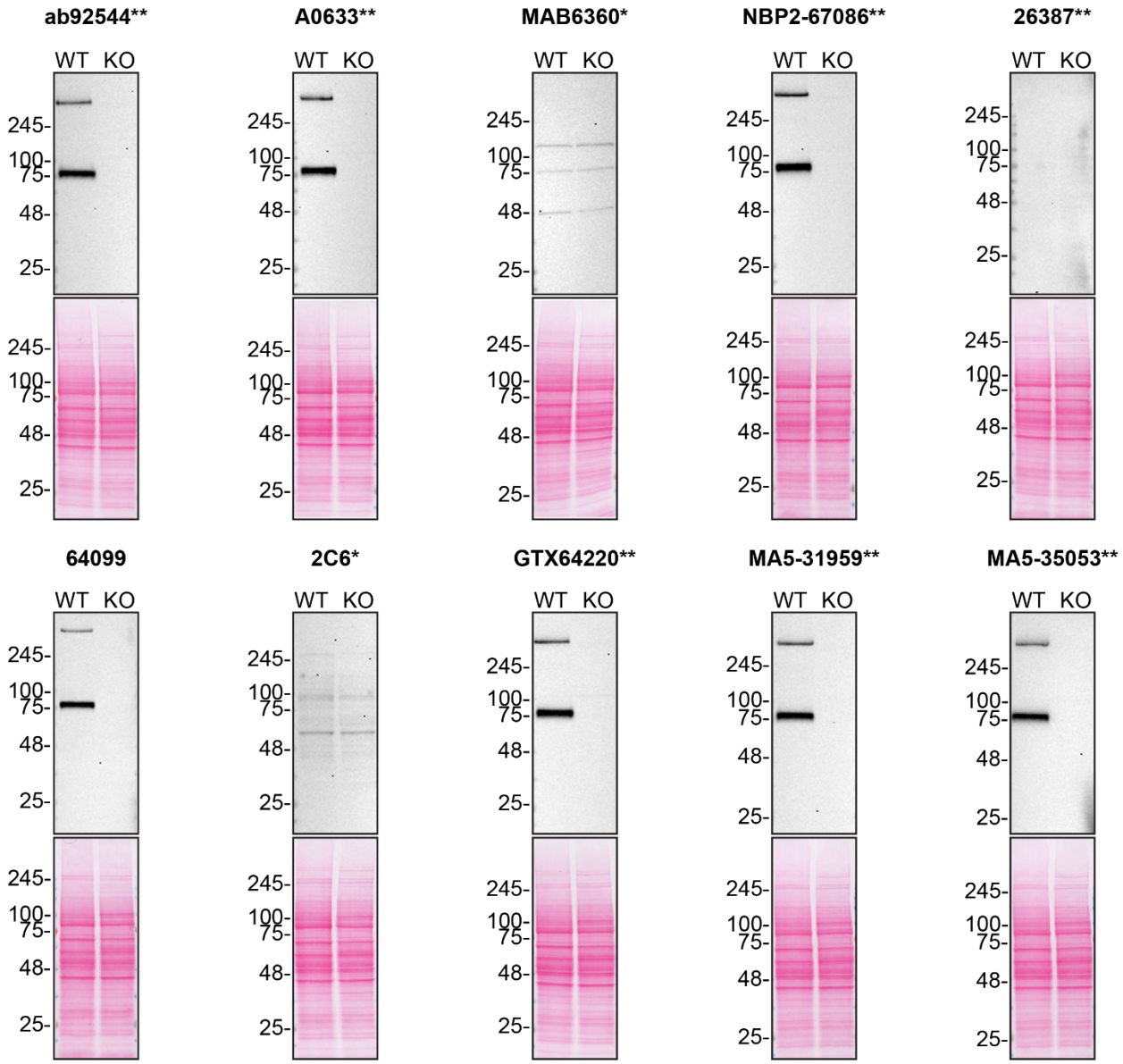

Figure 1: LRP-1 antibody screening by Western Blot

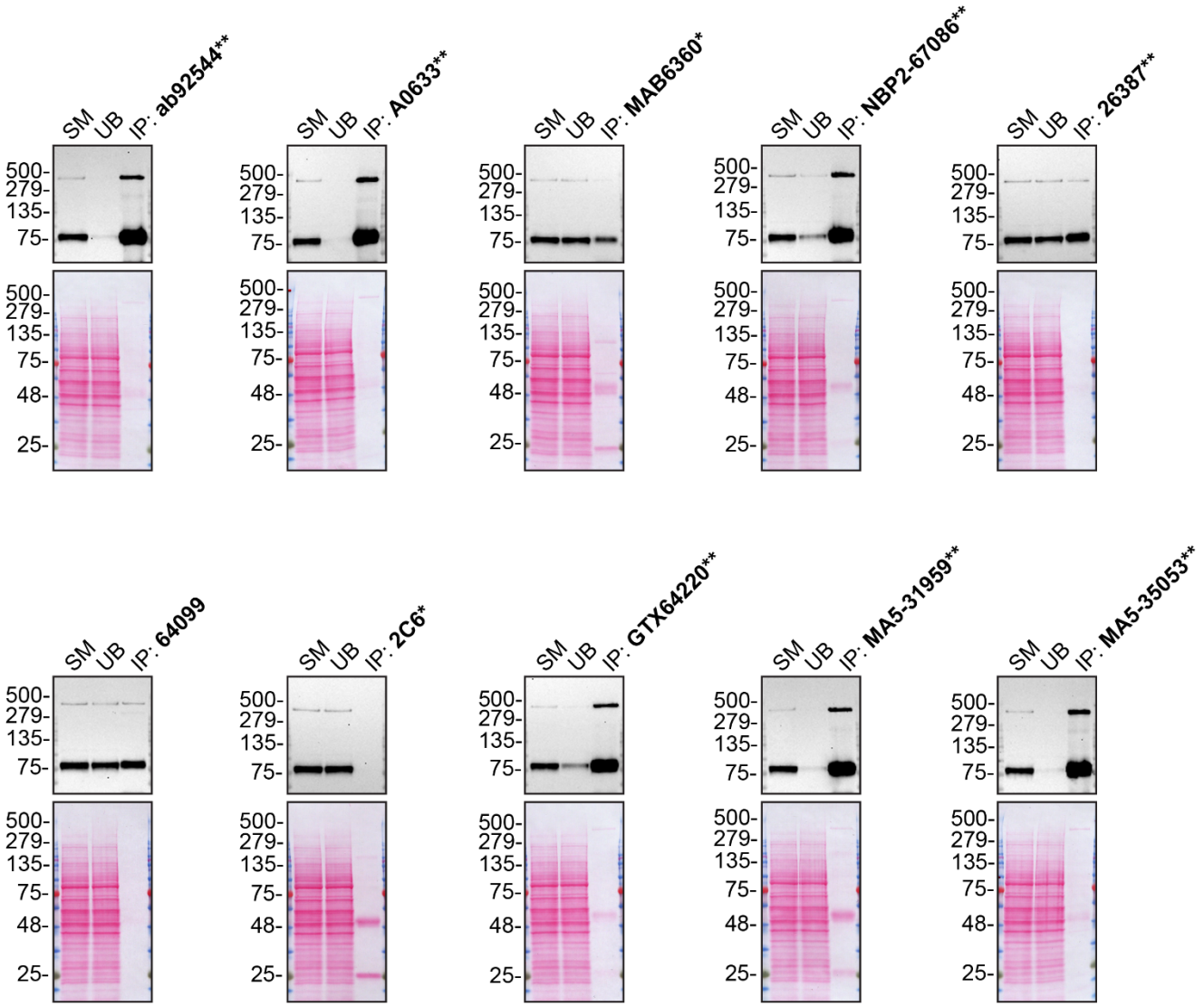

Figure 2: LRP-1 antibody screening by immunoprecipitation

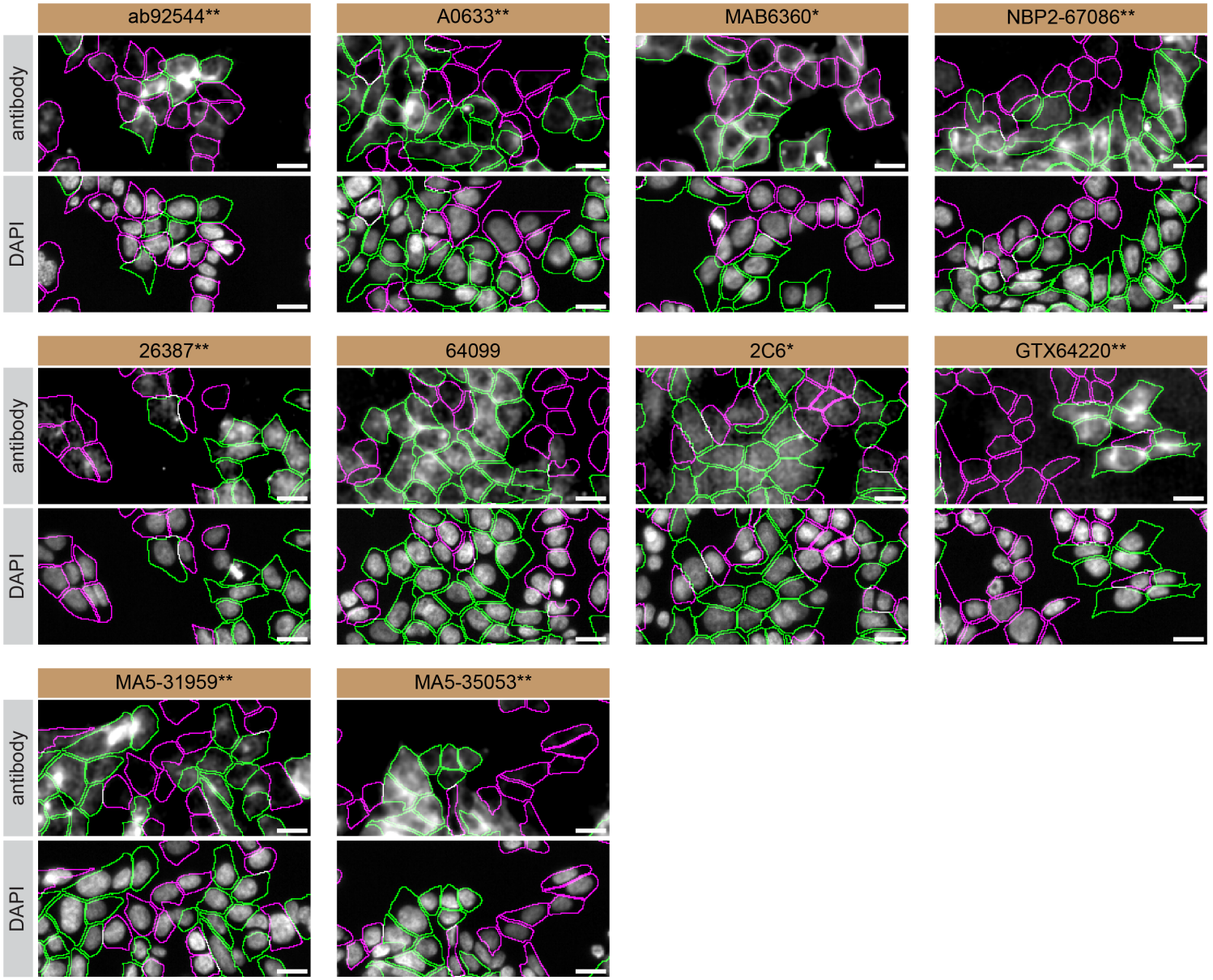

Figure 3: LRP-1 antibody screening by immunofluorescence

Figure 1: LRP-1 antibody screening by Western Blot.

Lysates of HAP1 WT and *LRP1* KO were prepared, and 30 µg of protein were processed for Western Blot with the indicated LRP-1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab92544 at 1/5000; A0633 at 1/2000; MAB6360 at 1/200; NBP2-67286 at 1/2000; 26387 at 1/200; 64099 at 1/1000; 2C6 at 1/4; GTX64220 at 1/1000; MA5-31959 at 1/2000; MA5-35053 at 1/2000. Predicted band size: 515 kDa alpha subunit and 85 kDa beta subunit. *=monoclonal antibody, **=recombinant antibody

Figure 2: LRP-1 antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated LRP-1 antibodies (as an exception, 10 µl of the antibodies 26387** and 64099) pre-coupled to Dynabeads protein A or protein G. Samples were washed and processed for Western Blot with the indicated LRP-1 antibody. For Western Blot, NBP2-67286 was used at 1/2000. The Ponceau stained transfers of each blot are shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody, **=recombinant antibody

Figure 3: LRP-1 antibody screening by immunofluorescence.

HAP1 WT and *LRP1* 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 in a 96-well plate with optically clear flat-bottom. Cells were stained with the indicated LRP-1 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 blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Antibody dilution used: ab92544 at 1/500; A0633 at 1/200; MAB6360 at 1/1000; NBP2-67286 at 1/1000; 26387 at 1/50; 64099 at 1/100; 2C6 at 1/10; GTX64220 at 1/1000; MA5-31959 at 1/200; MA5-35053 at 1/200. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

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. 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.
4. *DepMap, Broad*. 2019.
5. Ayoubi, R., P.S. McPherson, and C. Laflamme, *Antibody Screening by Immunoblot.* 2021 DOI: https://doi.org/10.5281/zenodo.5717510.
6. Ayoubi, R., et al., *Antibody screening by Immunoprecitation.* 2021 DOI: https://doi.org/10.5281/zenodo.5717516.
7. Alshafie, W., P. McPherson, and C. Laflamme, *Antibody screening by Immunofluorescence.* 2021 DOI: https://doi.org/10.5281/zenodo.5717498.