Antibody Characterization Report for Signal transducer and activator of transcription 5B (STAT5B)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Signal transducer and activator of transcription 5B

Short protein name: STAT5B

Gene name: STAT5B

Uniprot: P51692

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 STAT5B. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for STAT5B by immunoblot (Western blot), immunoprecipitation and immunofluorescence. An HAP1 *STAT5B* KO line is available at Horizon discovery and a HeLa *STAT5B* KO line is available at Abcam. Both HAP1 and HeLa lines are expected to express adequate level of STAT5B [3] and were used in this study.

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 STAT5B antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
ABclonal	A19567**	4000000046	AB_2862672	recombinant-mono	ARC0046	rabbit	0.31	Wb,IP
GeneTex	GTX08967**	822104511	AB_2923179	recombinant-mono	GT1160	rabbit	0.31	Wb
GeneTex	GTX132113	42270	AB_2886571	polyclonal	-	rabbit	1.23	Wb,IP,IF
Aviva Systems Biology	ARP39001	QC10828	AB_1256753	polyclonal	-	rabbit	0.5	Wb
Aviva Systems Biology	ARP33379	QC2857-90812	AB_2048262	polyclonal	-	rabbit	0.5	Wb
Cell Signaling Technology	34662	1	AB_2799059	polyclonal	-	rabbit	0.057	Wb,IP
Thermo Fisher Scientific	MA5-15665*	WA3186752	AB_10985934	monoclonal	5B3	mouse	not provided	Wb,IF
Abcam	ab178941**	GR200965-24	AB_2885102	recombinant-mono	EPR16671	rabbit	0.446	Wb,IP,IF
Abcam	ab226193	GR3334520-2	AB_2893212	polyclonal	-	rabbit	0.2	Wb,IP
Bio-Techne	NBP2-67795**	HN1207	AB_2923180	recombinant-mono	SD08-08	rabbit	1	Wb,IF
Bio-Techne	MAB15841**	CJNN0117012	AB_2923181	recombinant-mono	1032E	rabbit	0.50	Wb, IF
Bio-Techne	MAB1584*	YNJ0320121	AB_2255442	monoclonal	389215	mouse	0.5	Wb
Bio-Techne	AF1584	JFQ1618031	AB_2197076	polyclonal	-	rabbit	0.2	Wb, IP, IF

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC005185c001	CVCL_TQ64	HAP1	*STAT5B* KO
Abcam	ab255928	CVCL_0030	HeLa	WT
Abcam	ab266006	CVCL_B2HL	HeLa	*STAT5B* KO

Figure 1: STAT5B antibody screening by immunoblot.

A) Lysates of HAP1 WT and *STAT5B* KO were prepared, and 20 µg of protein were processed for immunoblot with the indicated STAT5B antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: A19567** at 1/1000, GTX08967** at 1/1000, GTX132113 at 1/1000, ARP39001 at 1/1000, ARP33379 at 1/1000, 34662 at 1/1000, MA5-15665* at 1/1000, ab178941** at 1/1000, ab226193 at 1/2000, NBP2-67795** at 1/1000, AF1584 at 1/1000, MAB1584* at 1/500. Predicted band size: 90 kDa. *=monoclonal antibody, **=recombinant antibody. B) Lysates of HAP1 and HeLa (WT and *STAT5B* KO) were processed and ab178941** was used for immunoblot at 1/1000.

Figure 2: STAT5B antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated STAT5B antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated STAT5B antibody. For immunoblot, MA5-15665* and ab178941** were used at 1/1000 and 1/500, respectively. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody, **=recombinant antibody

Figure 3: STAT5B antibody screening by immunofluorescence.

HAP1 WT and *STAT5B* 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 glass bottom. Cells were stained with the indicated STAT5B 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. Antibodies were used at 1.0 µg/ml. Bars = 20 µm. *=monoclonal antibody, **=recombinant antibody

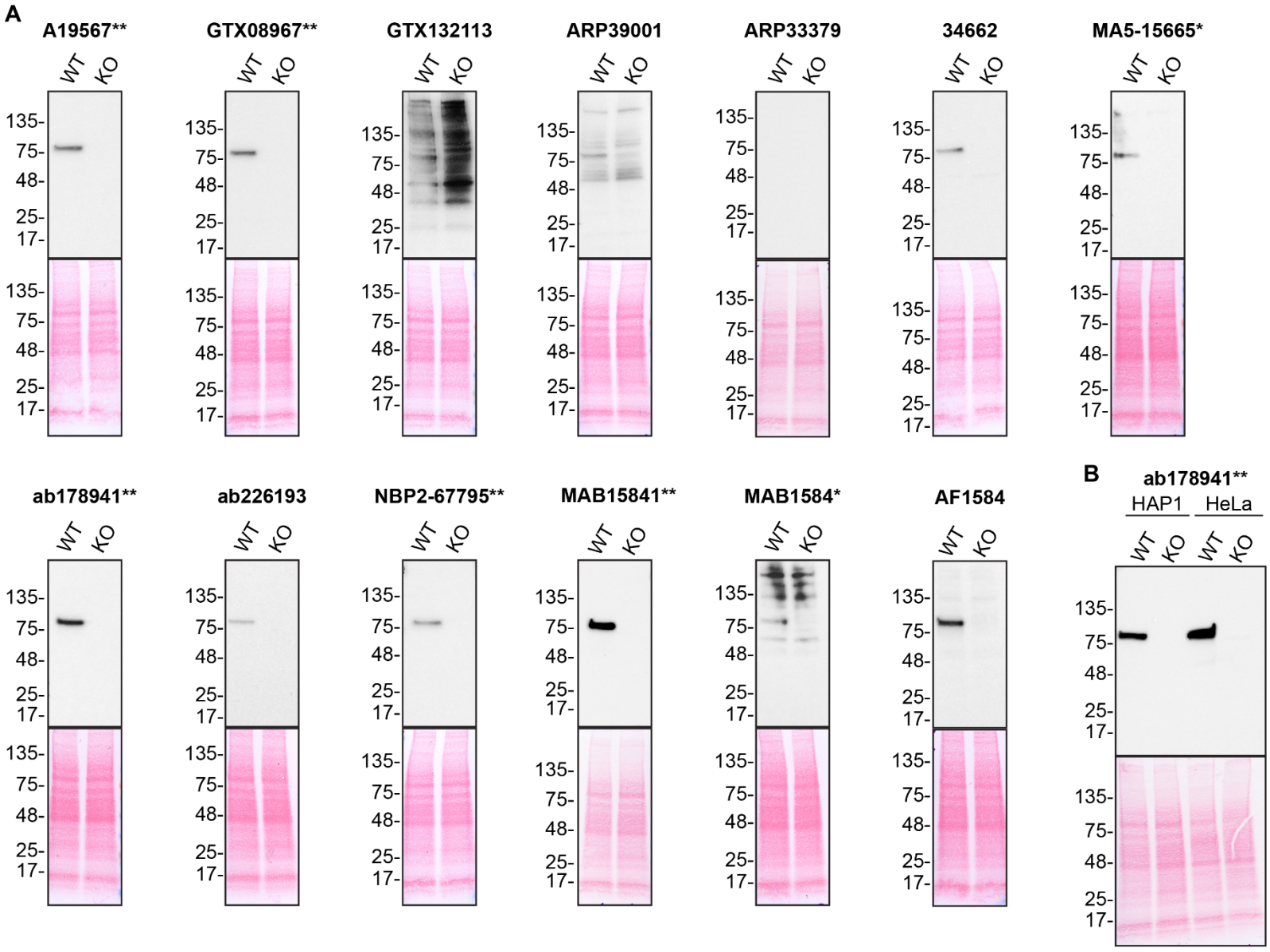

Figure 1: STAT5B antibody screening by immunoblot

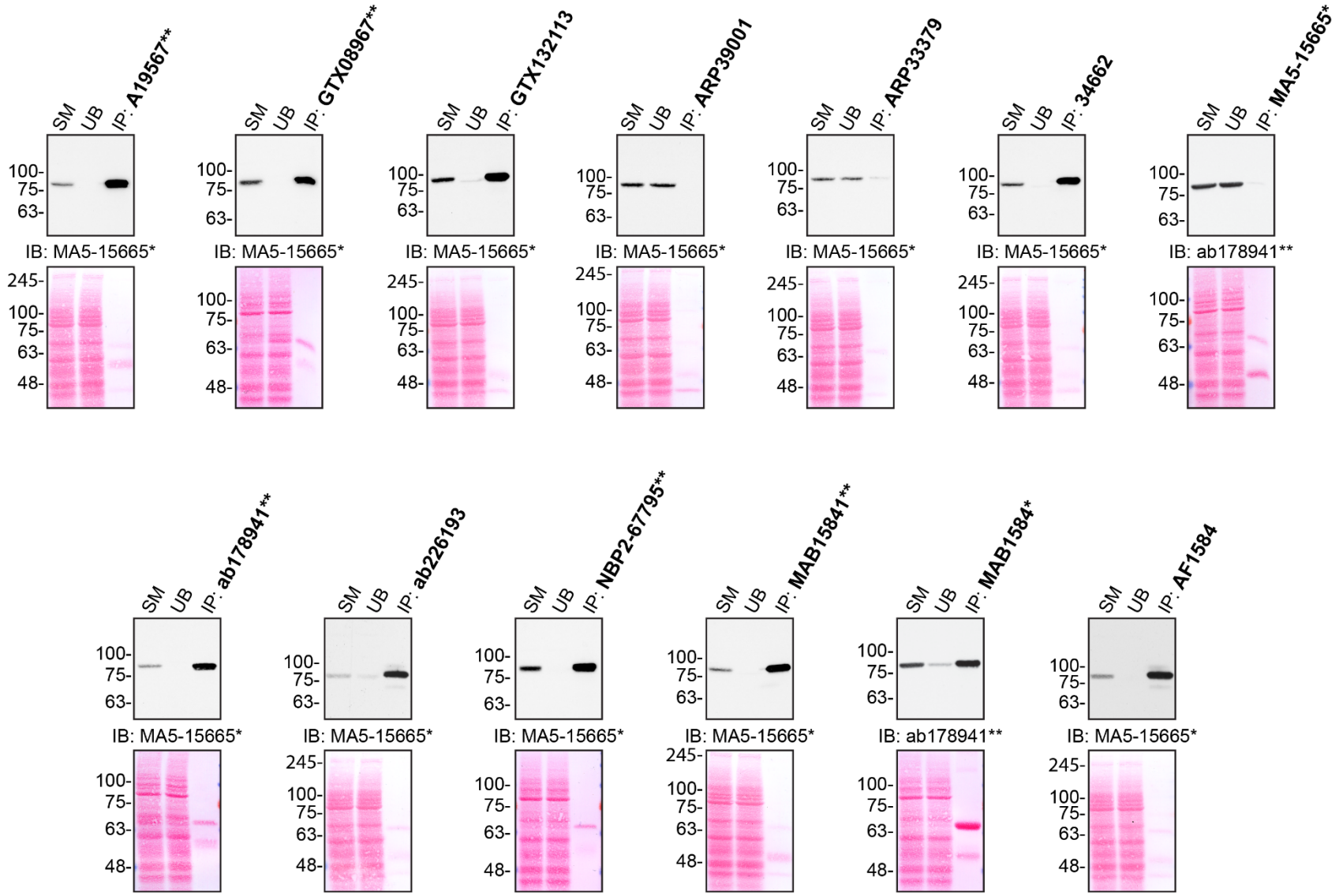

Figure 2: STAT5B antibody screening by immunoprecipitation

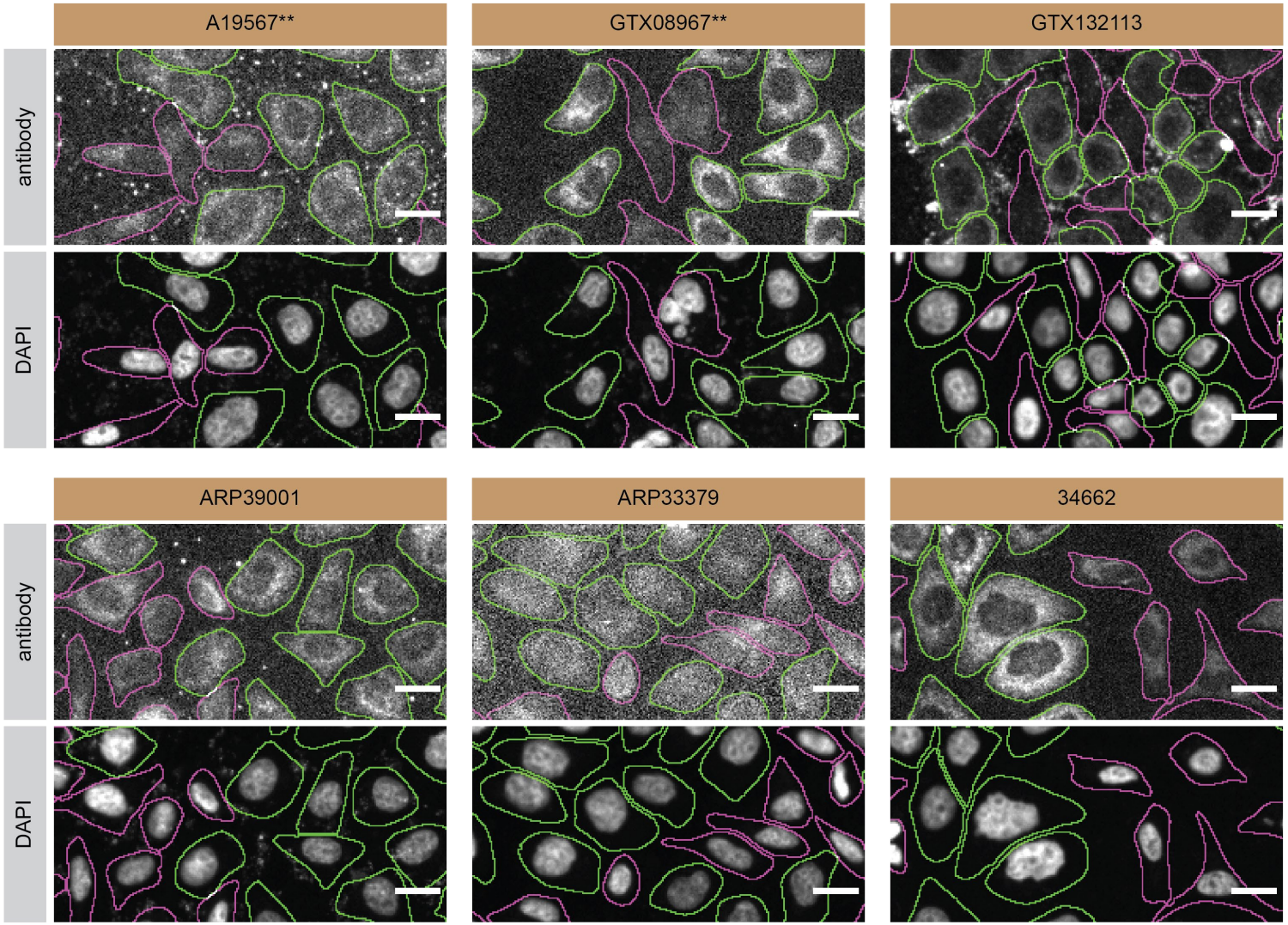

Figure 3: STAT5B antibody screening by immunofluorescence (1/3)

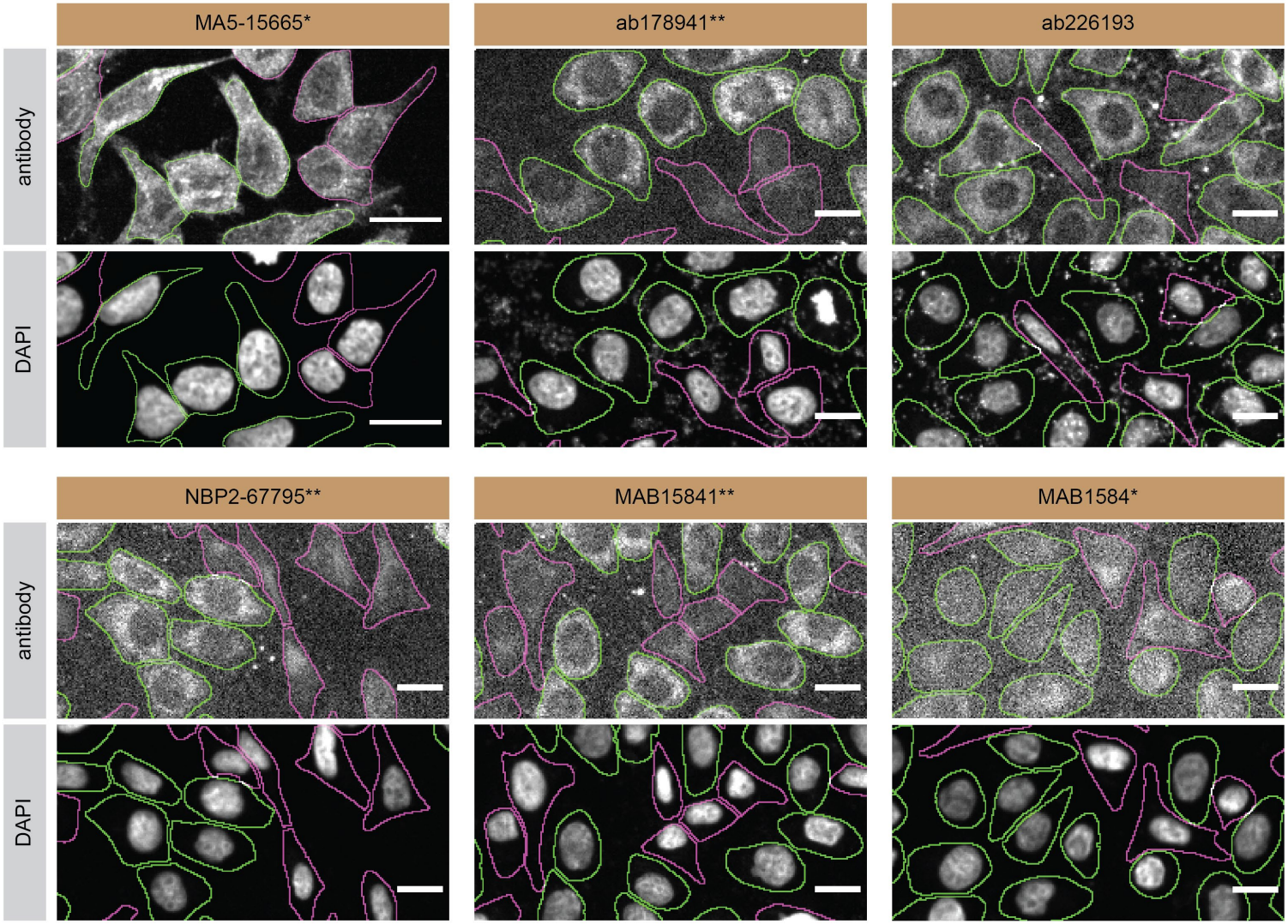

Figure 3: STAT5B antibody screening by immunofluorescence (2/3)

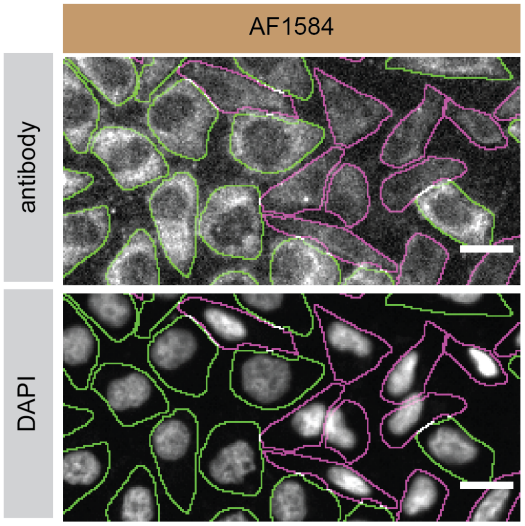

Figure 3: STAT5B antibody screening by immunofluorescence (3/3)

Materials and methods

Antibodies

All STAT5B antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 62-6520 and 65-6120). Alexa-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and A21429).

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 [4]. HAP1 and HeLa (WT and *STAT5B* KO) were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% 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 precast mini 4-15% gradient polyacrylamide gels from Bio-Rad (cat. number 4561084) 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 [5]. Antibody-bead conjugates were prepared by adding 2 µg or 5 µl of antibody at an unknown

concentration to 500 ul 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 antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Pierce IP Lysis Buffer was supplemented with the Halt Protease Inhibitor Cocktail 100X from Thermo Fisher Scientific (cat. number 78446) at a final concentration of 1x. Tubes were rocked for ~2 hrs at 4°C followed by several 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 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 IP lysis buffer and processed for SDS-PAGE and immunoblot on large 10% polyacrylamide gels.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [6]. HAP1 WT and *STAT5B* 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 96 well glass plates (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 STAT5B 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 Opera Phenix spinning disk high-content microscopy system (PerkinElmer), using a 40x NA 1.1 water immersion objective and sCMOS cameras, equipped with 425, 488, 561 and 640 nm solid state laser lines and bandpass filters to excite DAPI, Cellmask Green, Alexa568 and Cellmask Red, respectively. Images had pixel sizes of 0.3

microns, and a z-interval of 1 micron. For analysis and visualization, flat field correction was carried out using a minimum intensity projection of all images per channel to normalize raw images. Then, maximum intensity projections were generated using 8 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.

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. Ghandi, M., et al., *Next-generation characterization of the Cancer Cell Line Encyclopedia.* Nature, 2019. **569**(7757): p. 503-508 DOI: 10.1038/s41586-019-1186-3.
4. Ayoubi, R., P.S. McPherson, and C. Laflamme, *Antibody Screening by Immunoblot.* 2021 DOI: https://doi.org/10.5281/zenodo.5717510.
5. Ayoubi, R., et al., *Antibody screening by Immunoprecitation.* 2021 DOI: https://doi.org/10.5281/zenodo.5717516.
6. Alshafie, W., P. McPherson, and C. Laflamme, *Antibody screening by Immunofluorescence.* 2021 DOI: https://doi.org/10.5281/zenodo.5717498.