Antibody Characterization Report for Tyrosine-protein kinase SYK

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Tyrosine-protein kinase SYK

Alternative protein names: Spleen tyrosine kinase, p72-Syk

Gene name: SYK

Uniprot: P43405

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 Tyrosine-protein kinase SYK. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Tyrosine-protein kinase SYK by immunoblot (Western blot), immunoprecipitation and immunofluorescence. THP-1 was selected based on evidence of appropriate Tyrosine-protein kinase SYK protein expression determined through public proteomics databases, namely and DepMap [3, 4]. A custom made THP-1 KO line for the corresponding *SYK* gene was generated using CRISPR/Cas9.

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 Tyrosine-protein kinase SYK antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo Fisher Scientific	MA1-19332*	Wb3186395	AB_2197214	monoclonal	SYK-01	mouse	1.00	Wb,IP,IF
Cell Signaling Technology	13198**	9	AB_2687924	recombinant-mono	D3Z1E	rabbit	not provided	Wb,IP
Cell Signaling Technology	80460*	1	AB_2799953	monoclonal	4D10	mouse	not provided	Wb,IP,IF
Cell Signaling Technology	12358**	1	AB_2687923	recombinant-mono	D1I5Q	rabbit	not provided	Wb,IP
GeneTex	GTX633910*	43314	AB_2888388	monoclonal	GT351	mouse	2.83	Wb,IF
Bio-Techne	MAB7166*	CFUQ0117021	AB_10972948	monoclonal	720402	mouse	0.50	Wb
Bio-Techne	NBP1-03250*	199549	AB_1522471	monoclonal	SYK-01	mouse	0.50	Wb,IP
Proteintech	66721-1-Ig*	10006710	AB_2910243	monoclonal	4C4A12	mouse	1.00	Wb,IF
Developmental Studies Hybridoma Bank	AFFN-SYK-5A10*	4/28/2016	AB_2617957	monoclonal	AFFN-SYK-5A10	mouse	0.062	other
Abcam	ab3993*	GR3203808-21	AB_304217	monoclonal	SYK-01	mouse	1.00	Wb,IF
Abcam	ab40781**	GR3273231-3	AB_778196	recombinant-mono	EP573Y	rabbit	0.71	Wb,IF
Abcam	ab244701**	GR3273514-3	AB_2910244	recombinant-mono	EPR19414-176	rabbit	1.01	other
Abcam	ab244968**	GR3273515-3	AB_2910245	recombinant-mono	EPR573-69	rabbit	0.99	other

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
Abcam	ab271147	CVCL_0006	THP-1	WT
Abcam	ab288700	-	THP-1	*SYK* KO

Figure 1: Tyrosine-protein kinase SYK antibody screening by immunoblot.

Lysates of THP-1 (WT and *SYK* KO) were prepared and 30 µg of protein were processed for immunoblot with the indicated Tyrosine-protein kinase SYK antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: MA1-19332* at 1/1000, 13198** at 1/1000, 80460* at 1/1000, 12358** at 1/1000, GTX633910* at 1/250, MAB7166* at 1/250, NBP1-03250* at 1/500, 66721-1-Ig* at 1/2000, AFFN-SYK-5A10* at 1/200, ab3993* at 1/500, ab40781** at 1/1000, ab244701** at 1/1000, ab244968** at 1/1000. Predicted band size: 72 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Tyrosine-protein kinase SYK antibody screening by immunoprecipitation.

A) THP-1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Tyrosine-protein kinase SYK antibodies pre-coupled to Dynabeads protein G or protein A. Ability of the antibodies to capture Tyrosine-protein kinase SYK was first assessed by comparing the levels of Tyrosine-protein kinase SYK from the starting material to the unbound fractions. **B)** Analysis of the immunoprecipitate for the antibody that showed depletion of Tyrosine-protein kinase SYK in (A). For immunoblot, 80460* and ab244968** were used at 1/3000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitated. *=monoclonal antibody, **=recombinant antibody

Figure 3: Tyrosine-protein kinase SYK antibody screening by immunofluorescence.

THP-1 WT and *SYK* 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 Tyrosine-protein kinase SYK 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: MA1-19332* at 1/1000, 13198** at 1/400, 80460* at 1/400, 12358** at 1/400, GTX633910* at 1/2000, MAB7166* at 1/500, NBP1-03250* at 1/500, 66721-1-Ig* at 1/1000, AFFN-SYK-5A10* at 1/60, ab3993* at 1/1000, ab40781** at 1/700, ab244701** at 1/1000, ab244968** at 1/1000. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

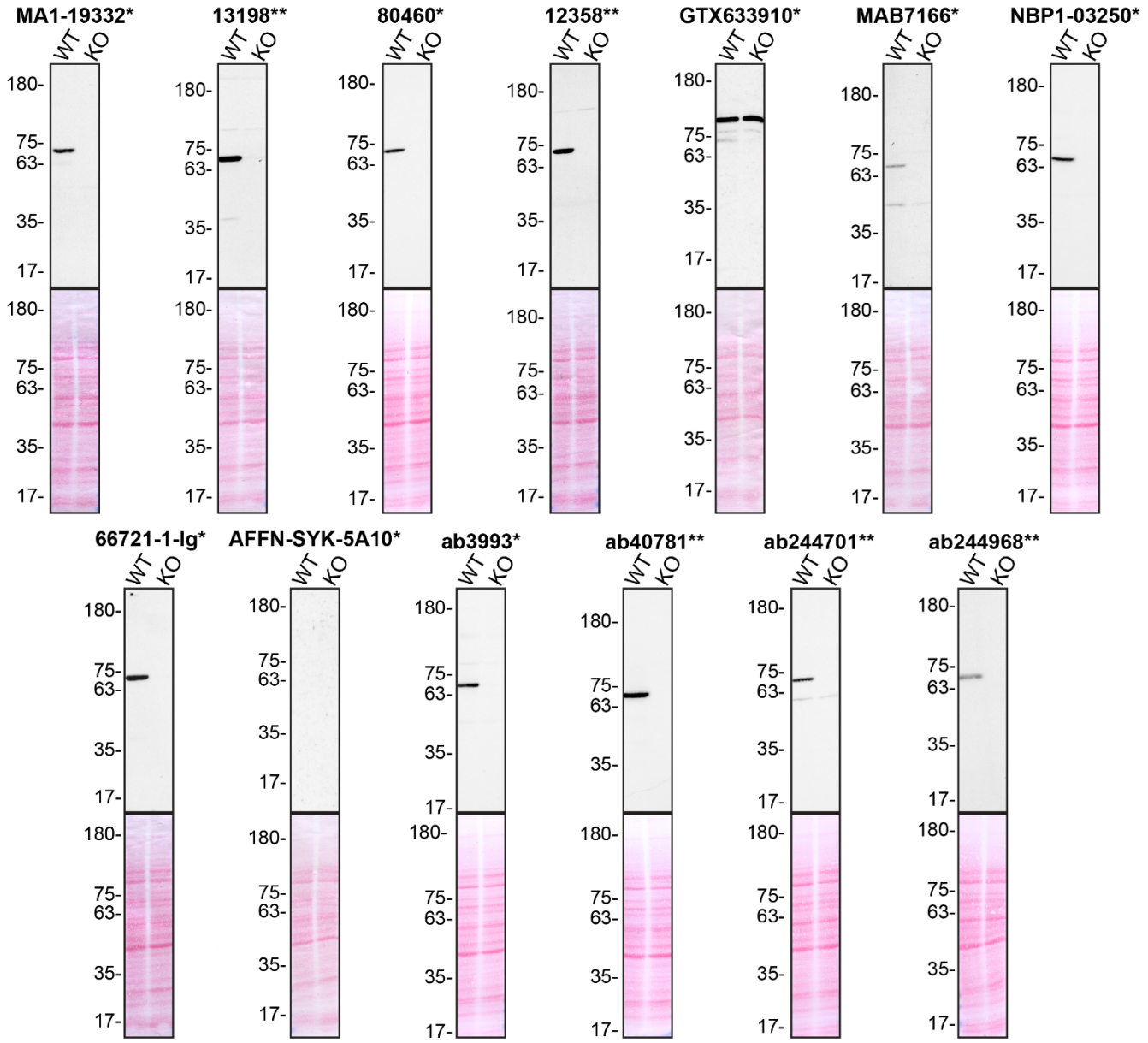

Figure1: Tyrosine-protein kinase SYK antibody screening by immunoblot

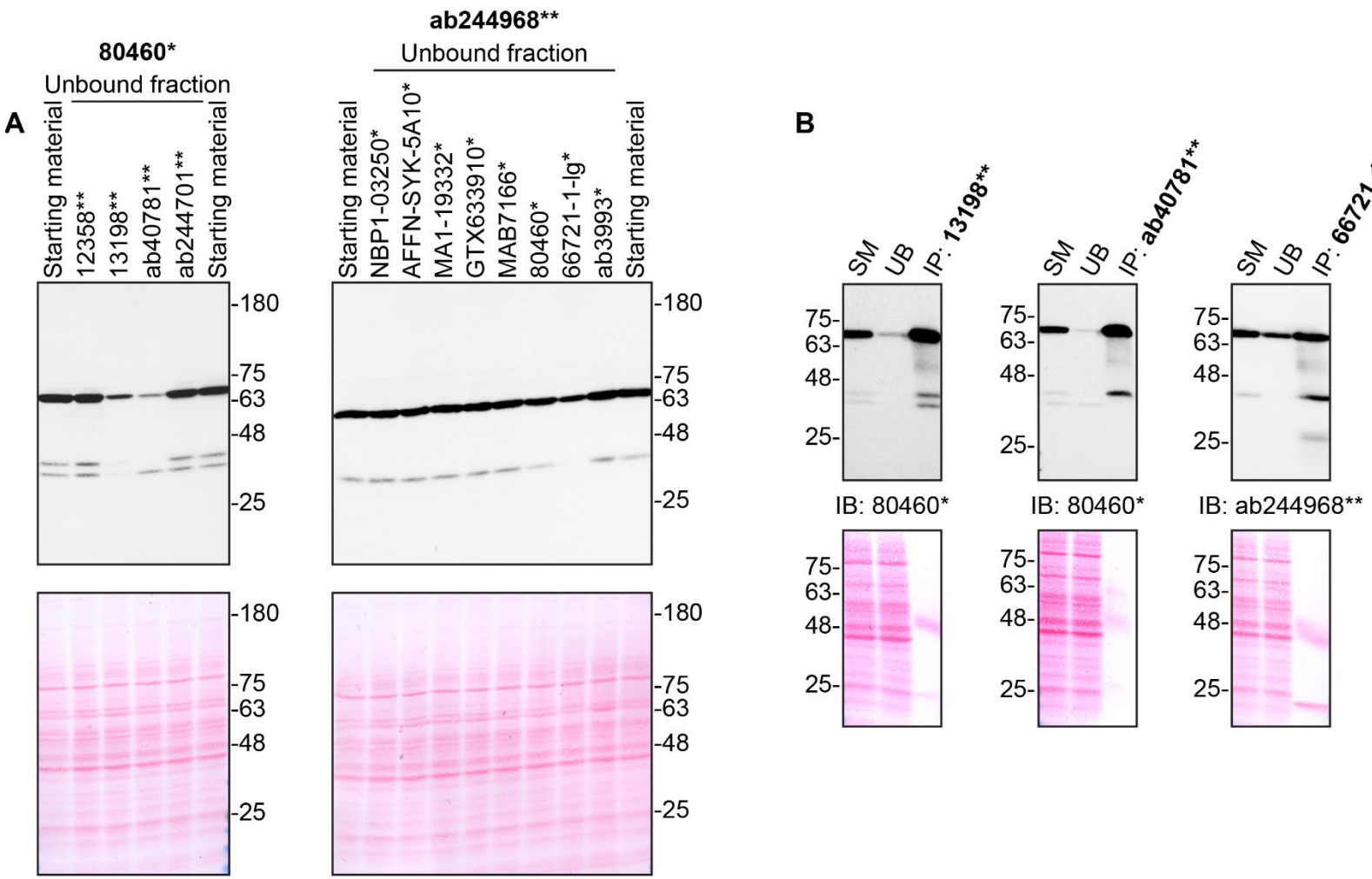

Figure 2: Tyrosine-protein kinase SYK antibody screening by immunoprecipitation

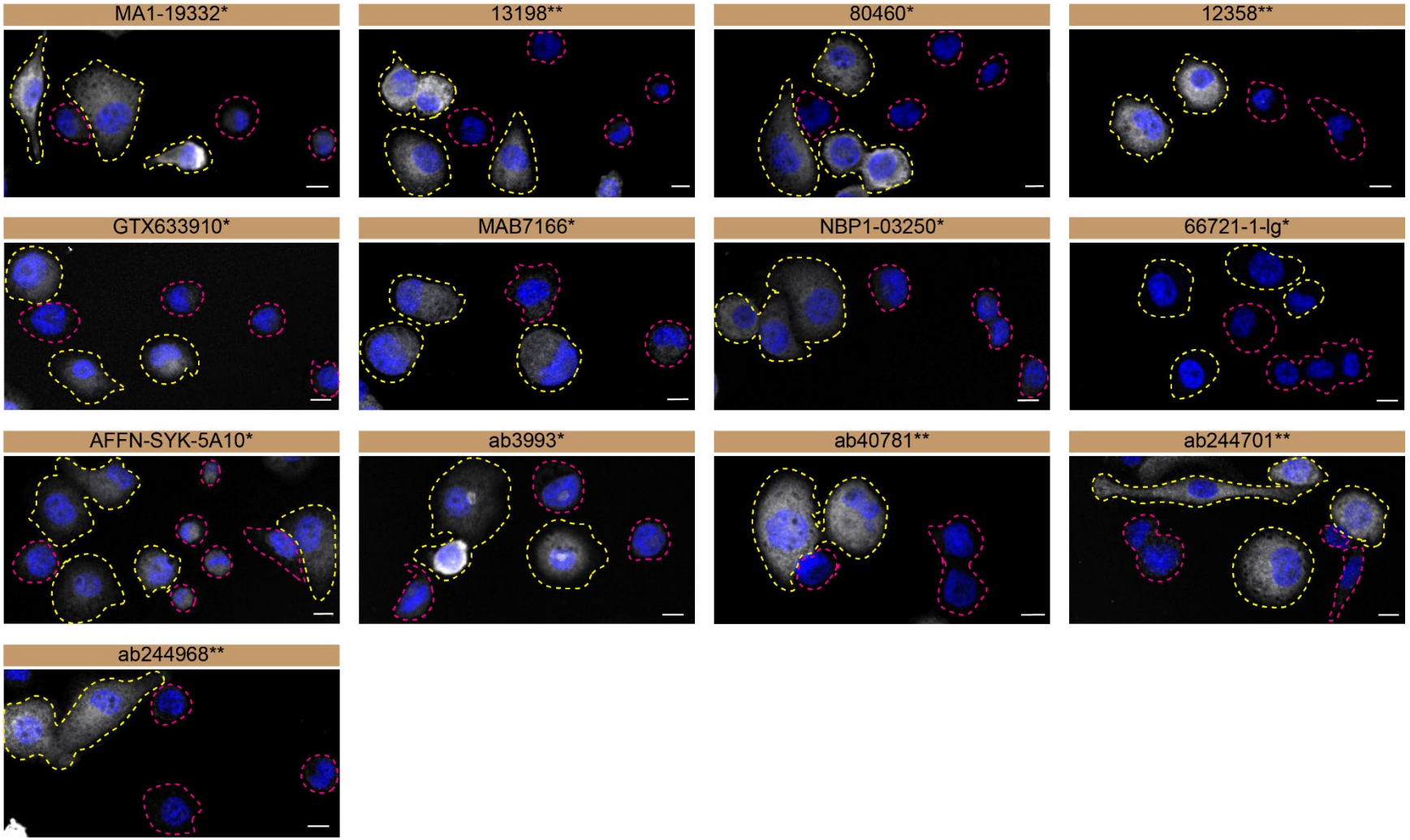

Figure 3 :Tyrosine-protein kinase SYK antibody screening by immunofluorescence

Materials and methods

Antibodies

All Tyrosine-protein kinase SYK 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).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. Two guide RNAs were used to knockout *SYK* in THP-1 using the CRISPR-Cas9 technology (sequence guide 1: TTTCGGCAACATCACCCGGG, sequence guide 2: GCTCCCGCTCGATGGTGTAG).

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 [5]. THP-1 WT and *SYK* 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 large 5-16% 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 [6]. Antibody-bead conjugates were prepared by adding 2 µg or 10 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.

THP-1 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 5-16% polyacrylamide gels.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [7]. THP-1 WT and *SYK* 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 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 Tyrosine-protein kinase SYK 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. 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 20x air objective (NA = 0.8). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.

References

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