Antibody Characterization Report for Hamartin

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Hamartin

Alternative protein name: Tuberous sclerosis 1 protein

Gene name: TSC1

Uniprot: Q92574

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 Hamartin. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Hamartin by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HAP1 was selected based on evidence of appropriate Hamartin protein expression determined using DepMap [3]. A HAP1 *TSC1* KO line is available at Horizon discovery and was 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 Hamartin antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Bio-Techne	NBP2-46234*	W001	AB_2905617	monoclonal	OTI3A2	mouse	1.00	Wb
Thermo	37-0400*	WC313182	AB_2533292	monoclonal	5C8A12	mouse	0.50	Wb, IP, IF
Thermo	PA5-116079	WL3464271A	AB_2900713	polyclonal	-	rabbit	1.00	Wb, IF
GeneTex	GTX130062	42375	AB_2886167	polyclonal	-	rabbit	1.38	Wb
Cell Signaling Technology	6935**	4	AB_10860420	recombinant-mono	D43E2	rabbit	not provided	Wb, IP

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

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	HZGHC001290c011	CVCL_TU90	HAP1	*TSC1* KO

Figure 1: Hamartin antibody screening by immunoblot.

Lysates of HAP1 (WT and *TSC1* KO) were prepared and 100 µg of protein were processed for immunoblot with the indicated Hamartin antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: NBP2-46234 at 1/200; 37-0400 at 1/500; PA5-116079 at 1/500; GTX130062 at 1/500; 6935 at 1/500. Predicted band size: 130 kDa. *=monoclonal antibody, **=recombinant

Figure 2: Hamartin antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg (10 µl was used for 6935 as the concentration is not provided) for of the indicated Hamartin antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Hamartin antibody. For immunoblot, PA5-116079 was used at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; *=monoclonal antibody, **=recombinant

Figure 3: Hamartin antibody screening by immunofluorescence.

HAP1 WT and *TSC1* 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 Hamartin 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: NBP2-46234 at 1/1000; 37-0400 at 1/500; PA5-116079 at 1/1000; GTX130062 at 1/1300; 6935 at 1/500. Bars = 10 µm. *=monoclonal antibody, **=recombinant

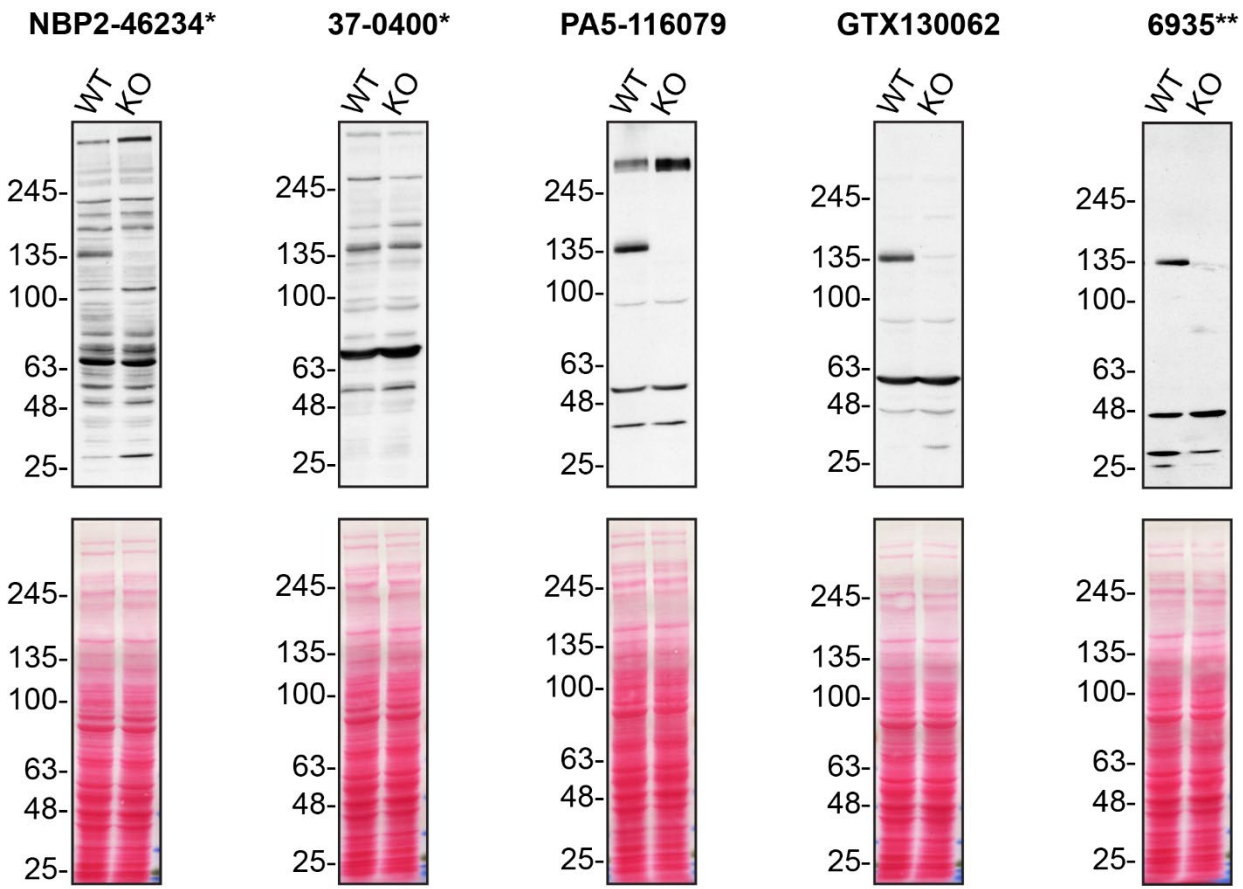

Figure 1: Hamartin antibody screening by immunoblot

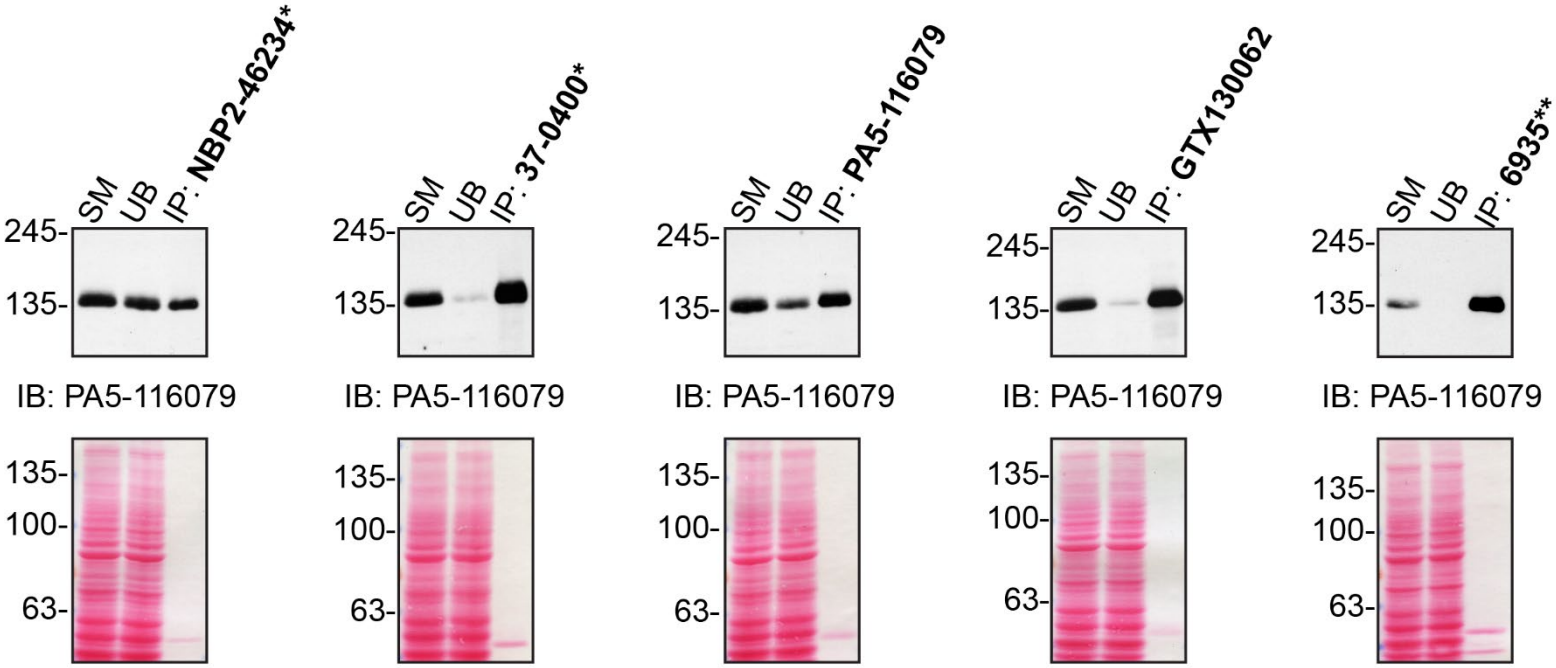

Figure 2: Hamartin antibody screening by immunoprecipitation

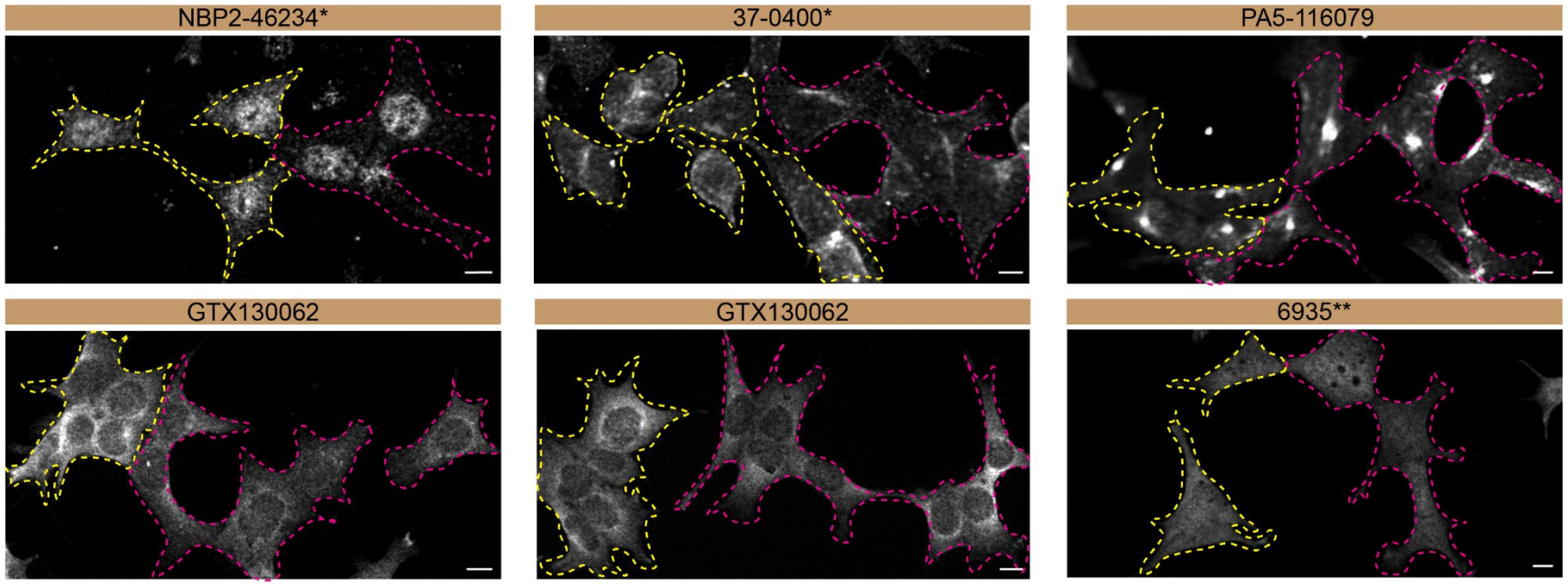

Figure 3 : Hamartin antibody screening by immunofluorescence

Materials and methods

Antibodies

All Hamartin 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).

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 (WT and *TSC1* 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 3-12% 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 [5]. Antibody-bead conjugates were prepared by adding 2 µg 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 3-12% polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a dilution of 0.4 µg/ml.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [6]. HAP1 WT and *TSC1* 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 Hamartin 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 880 laser scanning confocal microscope equipped with a Plan-Apo 20x air objective (NA = 0.8). Analysis was done using the Zen navigation software (Zeiss). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.

References

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