Antibody Characterization Report for Tuberin

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Tuberin

Alternative protein name: Tuberous sclerosis 2 protein

Gene name: TSC2

Uniprot: P49815

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 Tuberin. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Tuberin by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HAP1 was selected based on evidence of appropriate Tuberin protein expression determined using DepMap [3]. A HAP1 *TSC2* 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 Tuberin antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
GeneTex	GTX00917**	822105920	AB_2909429	recombinant-mono	GT1147	rabbit	0.80	Wb
GeneTex	GTX130510	42739	AB_2886293	polyclonal	-	rabbit	1.77	Wb
GeneTex	GTX130511	42627	AB_2886294	polyclonal	-	rabbit	0.37	Wb
GeneTex	GTX81899	822105923	AB_11167595	polyclonal	-	rabbit	0.50	Wb, IF
ABclonal	A0492	81080201	AB_2757221	polyclonal	-	rabbit	2.78	Wb, IF
Thermo	37-0500*	WC326430	AB_2533293	monoclonal	3G9D9	mouse	0.50	Wb, IF, IP
Thermo	AHO1422*	WK342352	AB_2536339	monoclonal	75R23	mouse	0.50	Wb
Thermo	MA5-32190**	WL34657	AB_2809477	recombinant-mono	SC05-59	rabbit	1.00	Wb
Bio-Techne	MAB40401*	CEJJ0220031	AB_10719268	monoclonal	614204	mouse	0.50	Wb
Bio-Techne	NBP2-67552**	H00922	AB_2909428	recombinant-mono	SC05-59	rabbit	1.00	Wb
Proteintech	24601-1-AP	60264	AB_2879633	polyclonal	-	rabbit	0.36	Wb, IP, IF
Cell Signaling Technology	4308**	6	AB_10547134	recombinant-mono	D93F12	rabbit	not provided	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	HZGHC001363c010	CVCL_TU92	HAP1	*TSC2* KO

Figure 1: Tuberin antibody screening by immunoblot.

Lysates of HAP1 (WT and *TSC2* KO) were prepared and 100 µg of protein were processed for immunoblot with the indicated Tuberin antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: GTX00917 at 1/500; GTX130510 at 1/500; GTX130511 at 1/500; GTX81899 at 1/1000; A0492 at 1/1000; 37-0500 at 1/250; AHO1422 at 1/500; MA5-32190 at 1/1000; MAB40401 at 1/500, NBP2-67552 at 1/500, 24601-1-AP at 1/1000, 4308 at 1/1000. Predicted band size: 200 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Tuberin antibody screening by immunoprecipitation.

HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Tuberin antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Tuberin antibody. A) Ability of the antibodies to capture Tuberin was first assessed by comparing the level of Tuberin from the starting material to the unbound fractions. For immunoblot, GTX130511 was used at 1/500. B) Analysis of the immunoprecipitates for antibodies that showed depletion of Tuberin in (A). For immunoblot, GTX00917 was used at 1/500, MA5-32190 at 1/1000 and NBP2-67552 at 1/500. 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: Tuberin antibody screening by immunofluorescence.

A) HAP1 WT and *TSC2* 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 Tuberin 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: GTX00917 at 1/800; GTX130510 at 1/1700; GTX130511 at 1/300; GTX81899 at 1/500; A0492 at 1/2000; 37-0500 at 1/500; AHO1422 at 1/500; MA5-32190 at 1/1000; MAB40401 at 1/500, NBP2-67552 at 1/1000, 24601-1-AP at 1/300, 4308 at 1/500. Bars = 10 µm. *=monoclonal antibody, **=recombinant antibody

B) WT and KO cells were identified and outlined by thresholding the green and far-red fluorescence dyes, respectively, using the Zen 3.4 (Zeiss) software. Evaluation of antibody performance was calculated by dividing the antibody mean fluorescence intensity measured from WT cells [F(WT)] by the antibody mean fluorescence intensity measured from KO cells [F(KO)]. The ratio of [F(WT)]/[F(KO)] for all tested antibodies is presented as a histogram. An antibody with a calculated ratio above 2.5-fold (dashed red line) could be considered as specific and selective for immunofluorescence. A minimum of 20 WT and 20 KO cells from 3 different fields of view were analysed for each antibody. *=monoclonal antibody, **=recombinant antibody

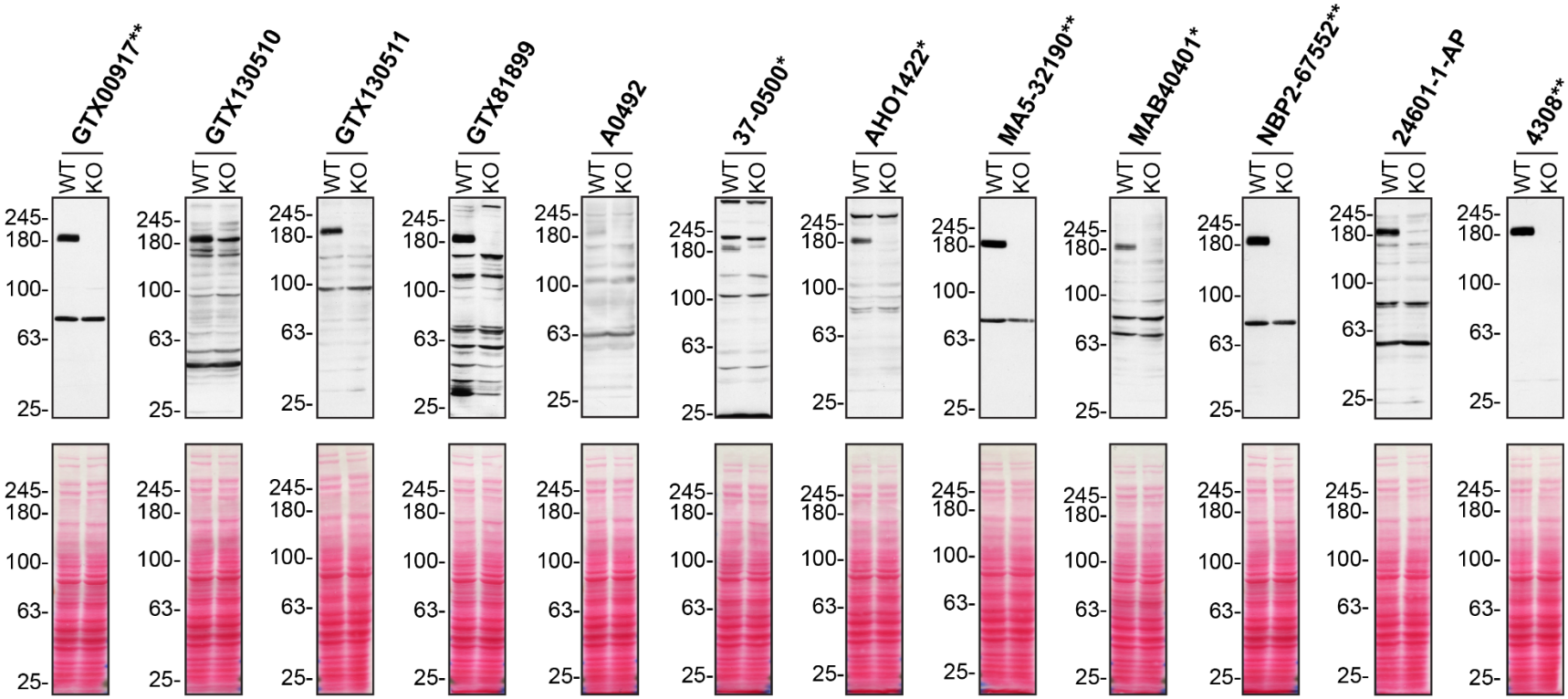

Figure 1: Tuberin antibody screening by immunoblot

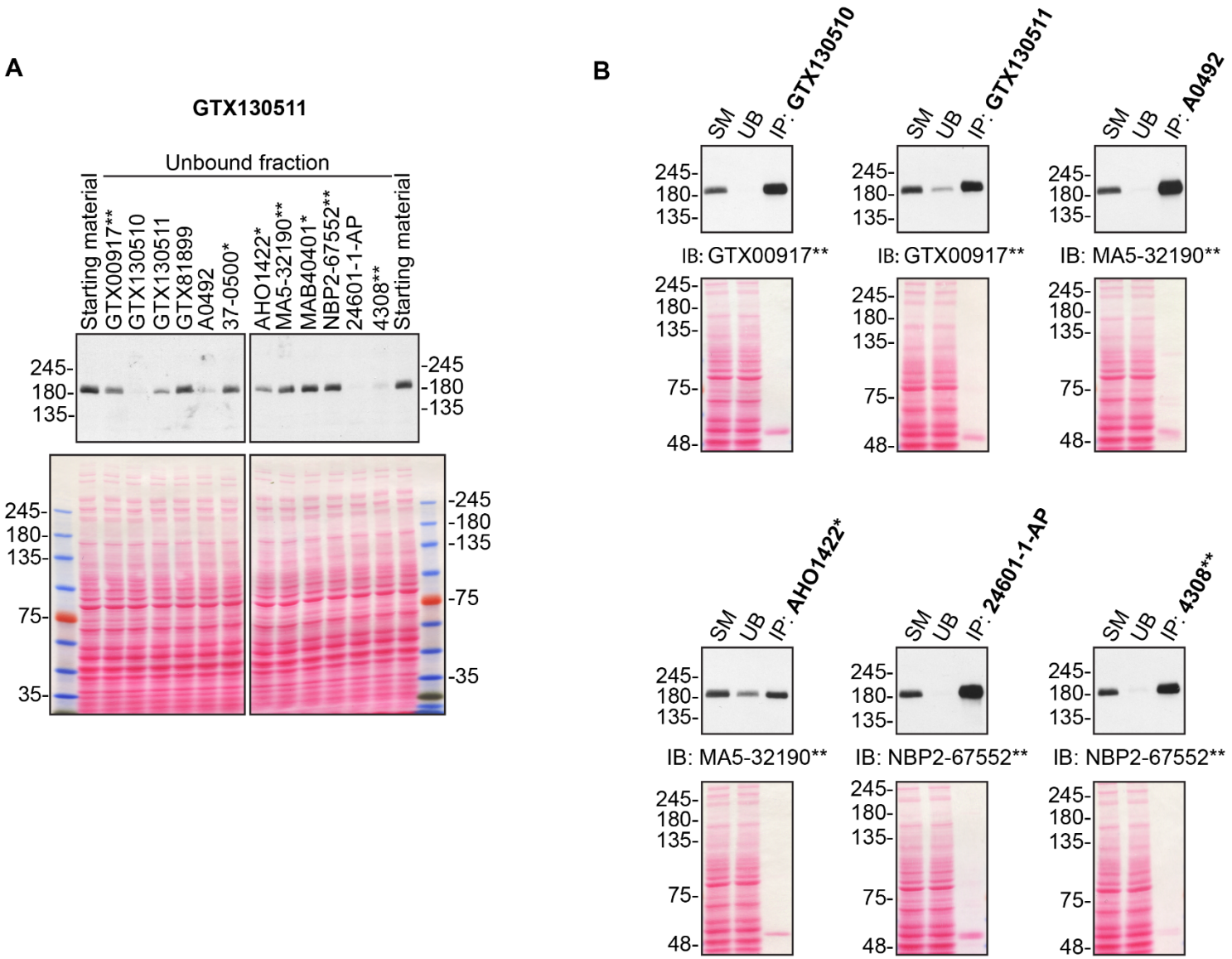

Figure 2: Tuberin antibody screening by immunoprecipitation

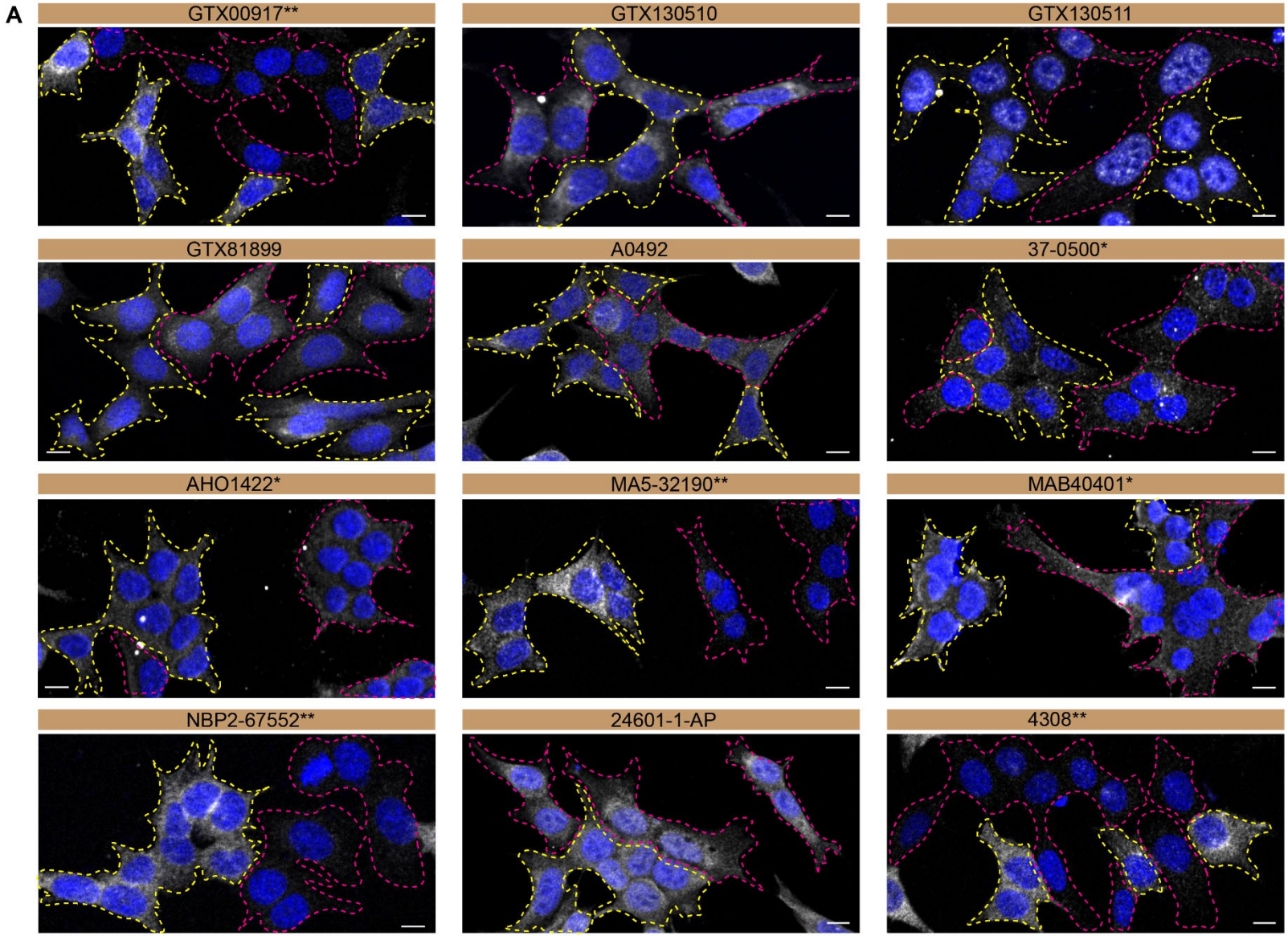

Figure 3 : Tuberin antibody screening by immunofluorescence (1/2)

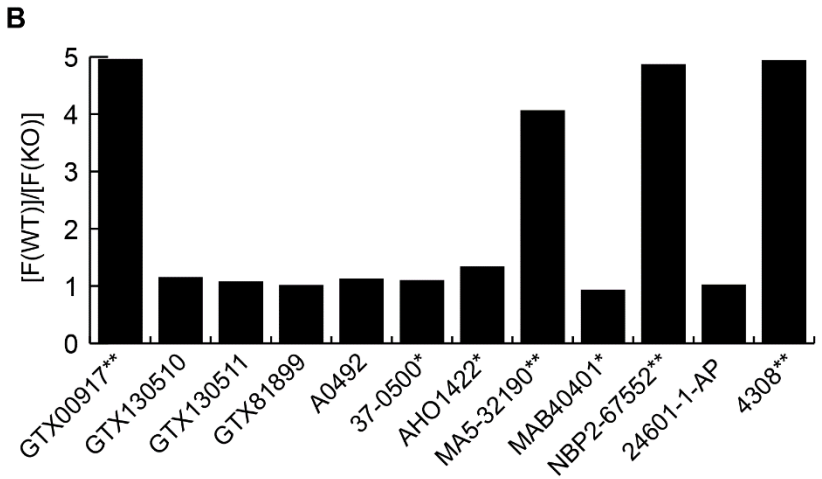

Figure 3 : Tuberin antibody screening by immunofluorescence (2/2)

Materials and methods

Antibodies

All Tuberin 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 *TSC2* 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 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.

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 0.5 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 *TSC2* 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 Tuberin 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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