Antibody Characterization Report for Synaptotagmin-1

YCharOS Antibody Characterization Report

Author(s): Riham Ayoubi[†,1], Walaa Alshafie[†,1], Maryam Fotouhi[1], Zhipeng You[2], Thomas M. Durcan[2], Peter S. McPherson[1*] and Carl Laflamme[1*]

[†] Authors contributed equally
[1] Tanenbaum Open Science Institute, Structural Genomics Consortium, Montreal Neurological Institute, McGill University, Montreal, Canada
[2] Early Drug Discovery Unit (EDDU), Montreal Neurological Institute, McGill University, Montreal, Canada
* Corresponding authors: carl.laflamme@mcgill.ca, peter.mcpherson@mcgill.ca

Target:

Recommended protein name: Synaptotagmin-1

Alternative protein names: Synaptotagmin I, SytI, p65

Gene name: *SYT1*

Uniprot: P21579

This report guides researchers to select the most appropriate antibodies for Synaptotagmin-1. We used an antibody characterization pipeline [1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Synaptotagmin-1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HCT116 was selected based on evidence of appropriate Synaptotagmin-1 protein expression [2] determined through DepMap [3]. HCT116 was modified with CRISPR/Cas9 to knockout [4] the corresponding *SYT1* gene.

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.

1. Laflamme, C., et al., *Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72.* Elife, 2019. **8**.
2. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16.
3. *DepMap, Broad*. 2019.
4. Nicouleau, M., et al., *Knockout Cell Lines Using CRISPR-Cas9 and ddPCR Technology.* 2020.

Table 1: Summary of the Synaptotagmin-1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Synaptic System	105015	1_1	AB_2782970	polyclonal	-	guinea pig	1.00	IF
Thermo	MA1-25568	VJ3101648E	AB_795474	monoclonal	ASV30	mouse	1.00	Wb, IP, IF
GeneTex	GTX103641	39883	AB_1952125	polyclonal	-	rabbit	0.75	Wb
Bio-techne	NBP1-51925	G2E110319	AB_11023532	polyclonal	-	goat	0.50	Wb
Abcam	ab131551	GR106010-30	AB_11157546	polyclonal	-	rabbit	not provided	Wb, IF
Abcam	ab133856	GR219601-26	AB_2885088	polyclonal	-	chicken	0.40	Wb
Abcam	ab126253	GR171287-10	AB_11127713	polyclonal	-	rabbit	1.00	Wb
Proteintech	14511-1-AP	5811	AB_2199166	polyclonal	-	rabbit	0.20	Wb, IP, IF
Cell Signaling Technology	14558	1	AB_2798510	recombinant -mono	D33B7	rabbit	not provided	Wb, IP, IF

Wb=Western blot; IP= immunoprecipitation; IF=immunofluorescence

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Abcam	ab255451	CVCL_0291	HCT116	WT
Montreal Neurological Institute	-	CVCL_B3P8	HCT116	*SYT1* KO
ATCC	CRL-1573	CVCL_0045	HEK293T	WT
Montreal Neurological Institute	-	CVCL_A8JQ	HEK293T	*SYT1* KO
Abcam	ab255451	CVCL_0291	HCT 116	WT
ATCC	CCL-185	CVCL_0023	A-549	WT
-	-	CVCL_0336	Huh-7	WT
ATCC	HTB-14	CVCL_0022	U-87MG	WT
-	-	CVCL_0021	U-251MG	WT
ATCC	HTB-22	CVCL_0031	MCF-7	WT
ATCC	CRL-4000	CVCL_4388	hTERT-RPE1	WT

Figure 1: Synaptotagmin-1 antibody screening by immunoblot.

A) Lysates of HCT116 (WT and *SYT1* KO) were prepared and 50 µg of protein were processed for immunoblot with the indicated Synaptotagmin-1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: 105015 at 1/1000; MA1-25568 at 1/500; GTX103641 at 1/500; NBP1-51925 at 1/1000; ab131551 at 1/500; ab133856 at 1/500; ab126253 at 1/500; 14511-1-AP at 1/500; 14558 at 1/1000. Predicted band size: 48 kDa.

B) Comparison of Synaptotagmin-1 protein level in different tissue/cell lines. Lysates were prepared from rat whole brain, HEK293T (WT and *SYT1* KO), A-549, Huh-7, U-87MG, U-251MG, HCT 116, MCF-7, hTERT-RPE-1. Expression (21Q3 Public; log2(TPM+1); blue) and Proteomics (P21579; relative protein abundance, red) values for each line were taken from DepMap (depmap.org). n/a=not available.

Figure 2: Synaptotagmin-1 antibody screening by immunoprecipitation.

HCT116 lysates were prepared, and immunoprecipitation was performed using 1.0 µg of the indicated Synaptotagmin-1 antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Synaptotagmin-1 antibody. For immunoblot, ab126253 and 14511-1-AP were used at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitated.

Figure 3: Synaptotagmin-1 antibody screening by immunofluorescence.

HCT116 WT and *SYT1* 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 Synaptotagmin-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 merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: 105015 at 1/1000; MA1-25568 at 1/1000; GTX103641 at 1/750; NBP1-51925 at 1/500; ab131551 at 1/500; ab133856 at 1/400; ab126253 at 1/1000; 14511-1-AP at 1/200; 14558 at 1/500. Bars = 10 µm.

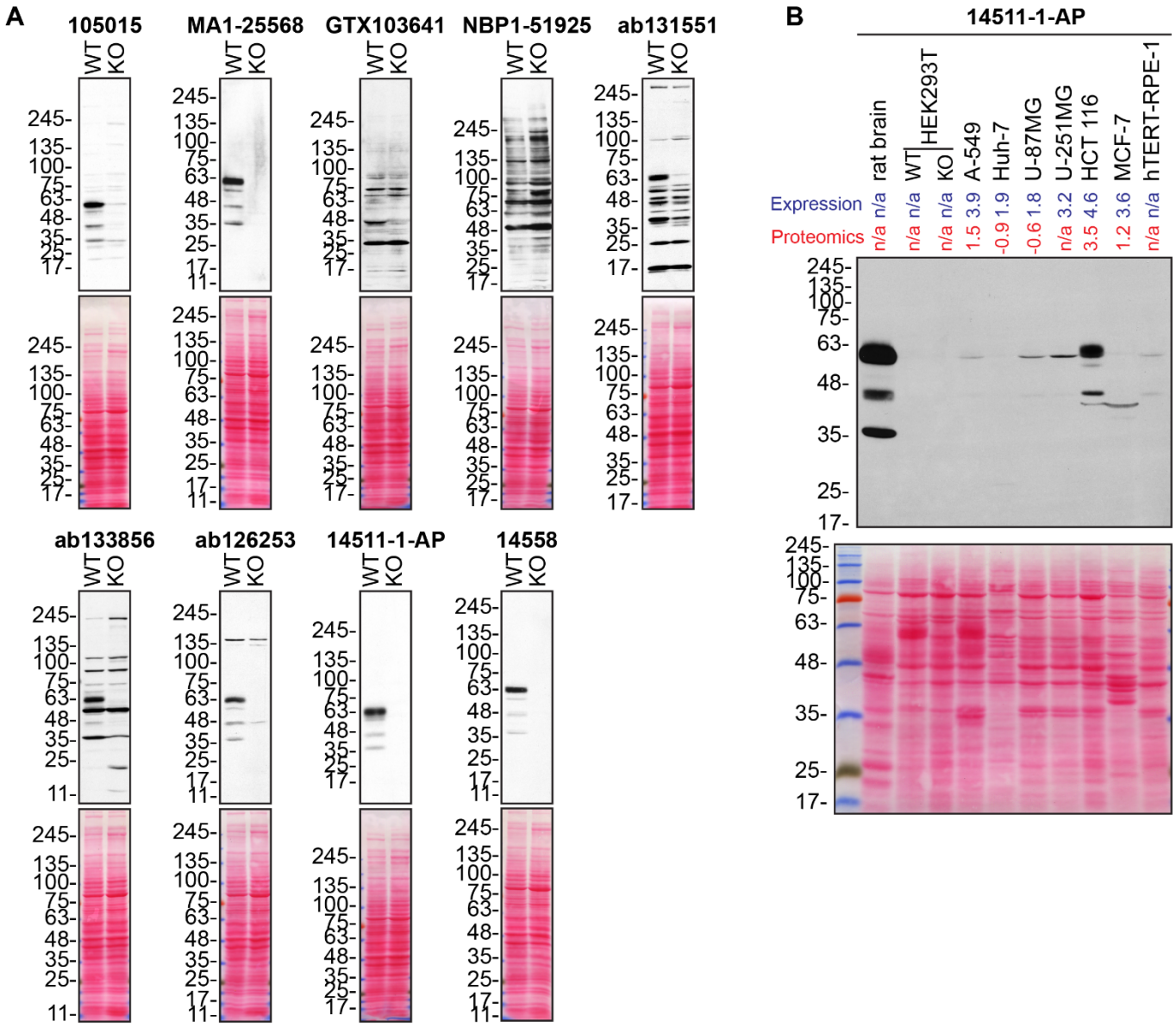

Figure 1: Synaptotagmin-1 antibody screening by immunoblot

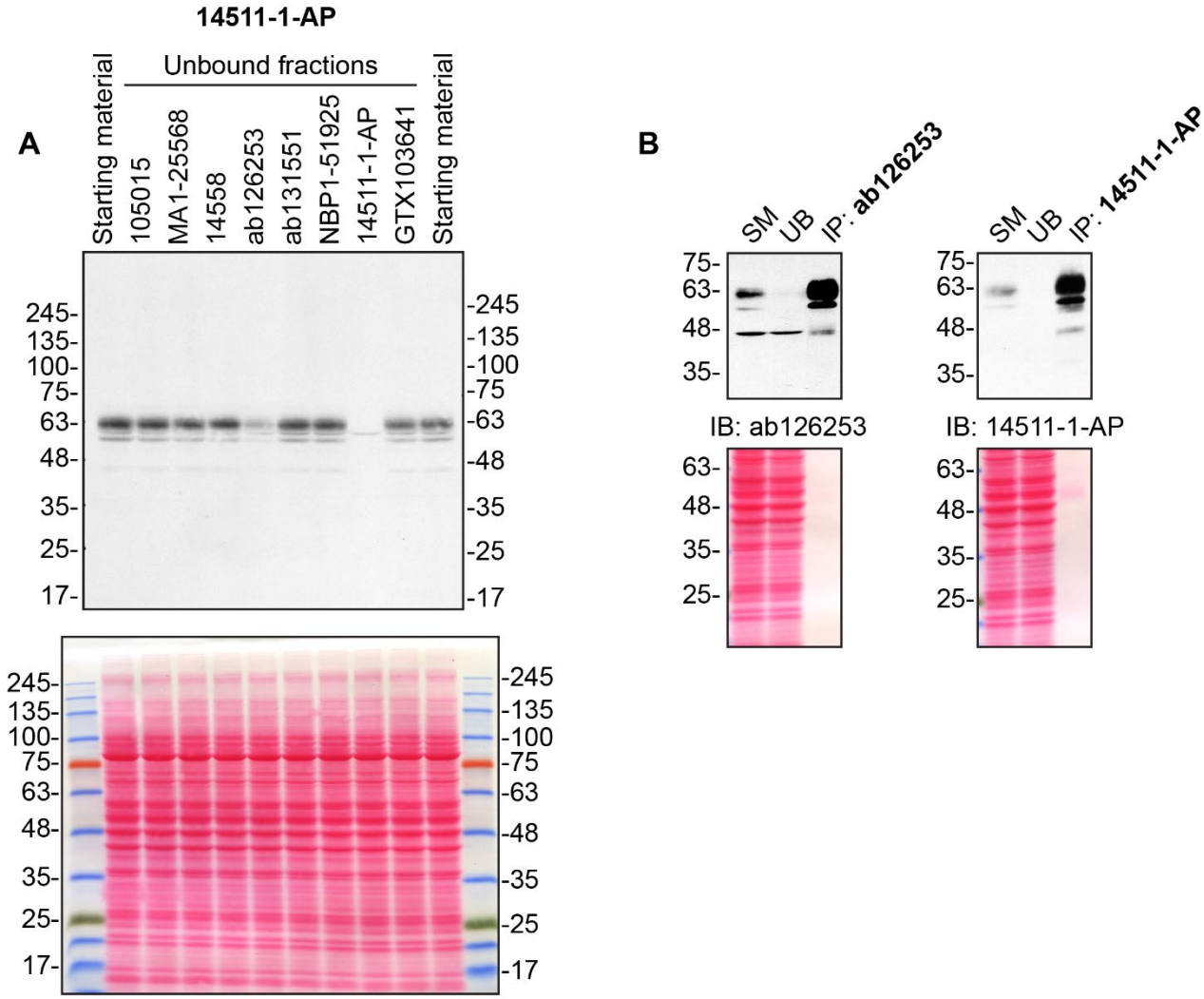

Figure 2: Synaptotagmin-1 antibody screening by immunoprecipitation

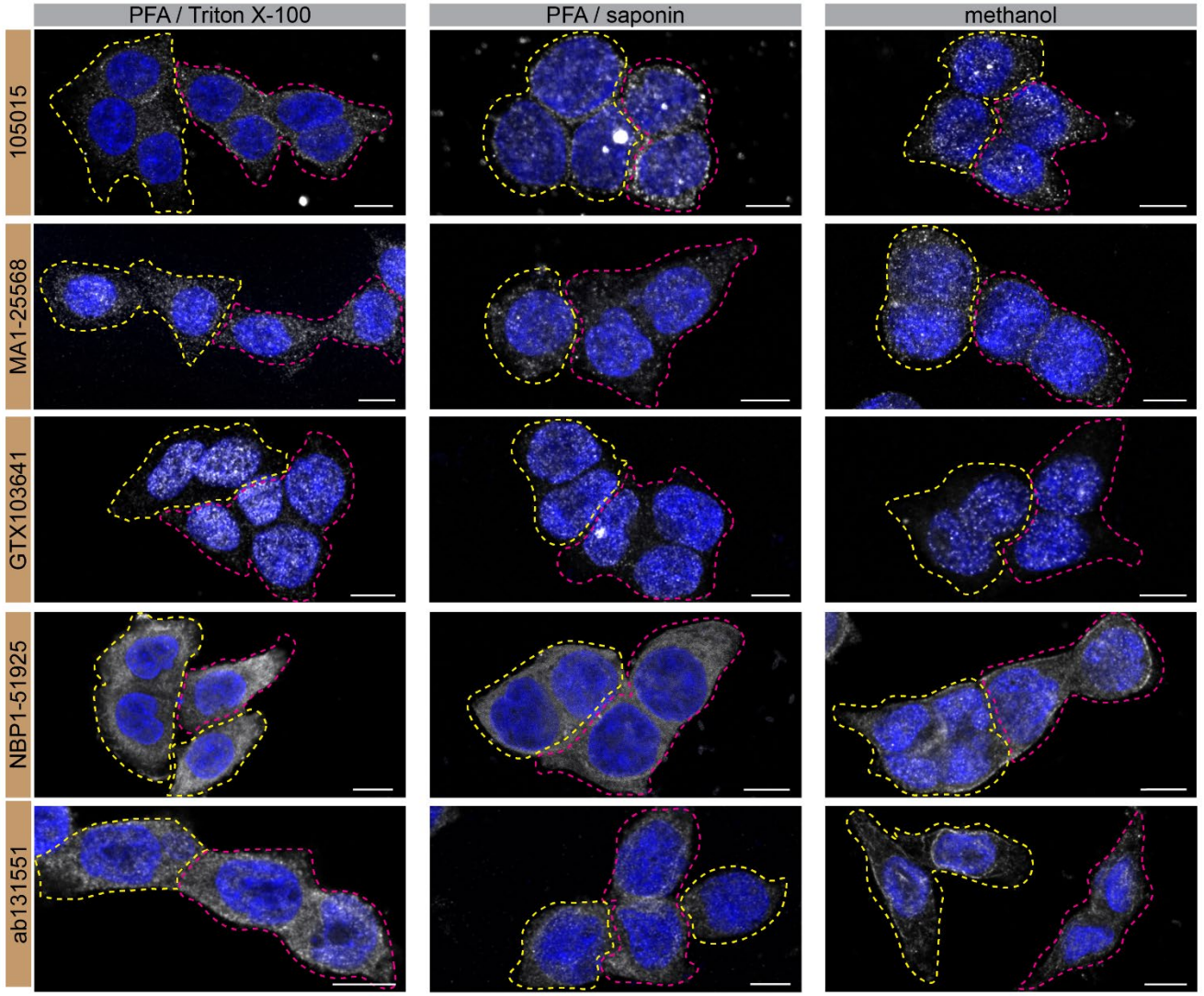

Figure 3: Synaptotagmin-1 antibody screening by immunofluorescence (1/2)

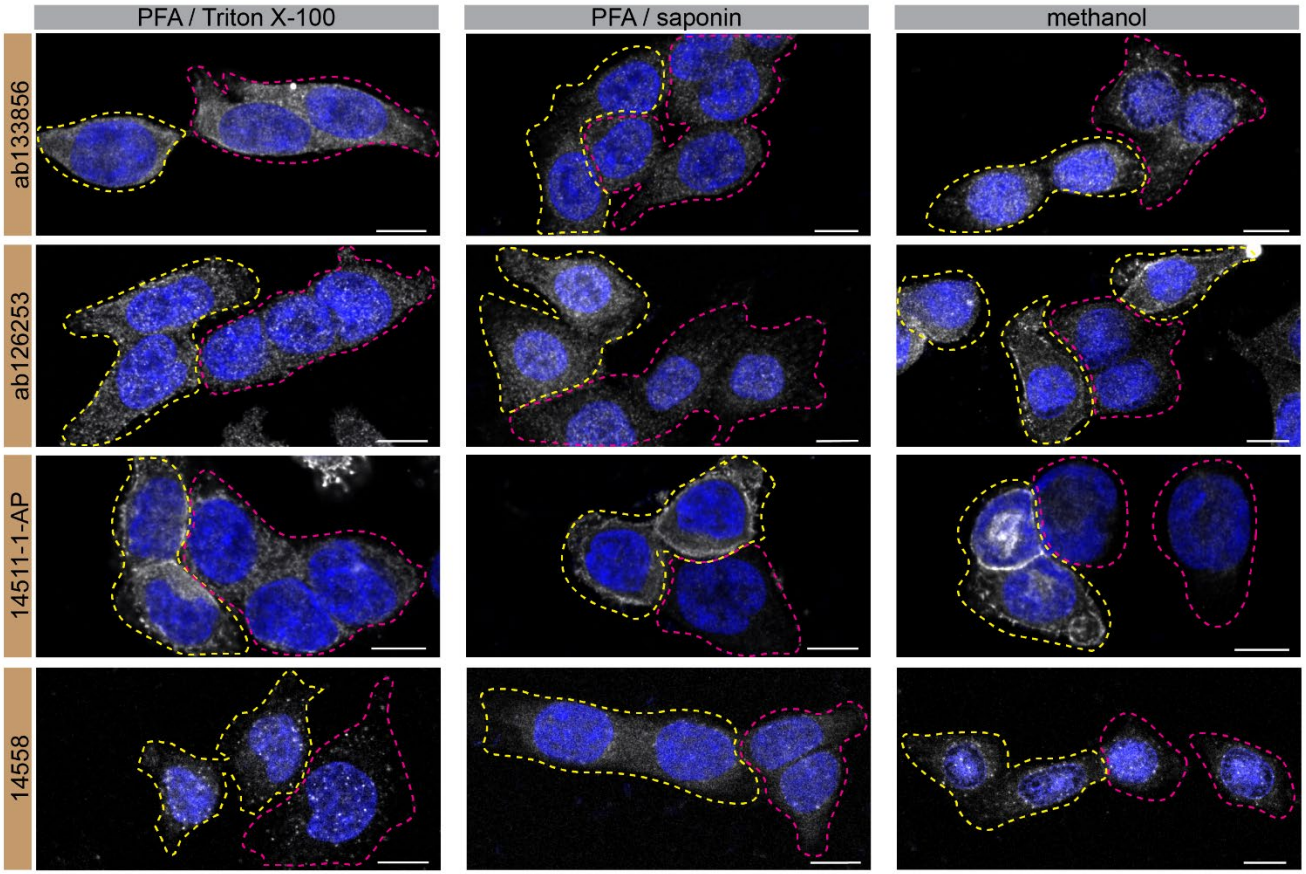

Figure 3: Synaptotagmin-1 antibody screening by immunofluorescence (2/2)

Materials and methods

Antibodies

All Synaptotagmin-1 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse, anti-rabbit, anti-guineapig, and anti-chicken secondary antibodies are from Thermo Fisher Scientific (cat. number 65-6120, 62-6520, A16054 and A18769, respectively). Alexa-555-conjugated goat anti-mouse, anti-rabbit, anti-guinea pig, and anti-chicken antibodies are from Thermo Fisher Scientific (cat. number A21424, A21429, A21435, and A21437, respectively).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. HCT116 and HEK293T *SYT1* KO clone was generated with low passage cells. The guide RNA used to introduce knockout the *SYT1* gene is AGATACAAAAGCAGCAGGTC.

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

HCT116 (WT and *SYT1* KO) were collected in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1.0 mM EDTA, 1% Triton X-100, 0.5% 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 4-15% 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

Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 ul of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together 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). Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

HCT116 WT were collected in Pierce IP Buffer supplemented with the Halt Protease Inhibitor Cocktail 100X from Thermo Fisher Scientific (cat. number 78446) at a final concentration of 1x. 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 Pierce IP Buffer and processed for SDS-PAGE and immunoblot on 4-15% polyacrylamide gels. Prot-A:HRP was used as a secondary detection system (MilliporeSigma, cat. number P8651) at a dilution of 0.4 µg/ml.

Antibody screening by immunofluorescence

HCT116 WT and *SYT1* 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 or in 100% methanol (chilled at -20°C) for 5 min on ice and then washed 3 times with PBS. PFA-fixed cells were permeabilized in PBS with either 0.1% Triton X-100 or 0.05% saponin for 10 min at room temperature. Cells were incubated in PBS with 5% BSA, 5% goat serum or 5% donkey serum together with 0.01% Triton X-100 or 0.05% saponin for 30 min at room temperature to block unspecific binding of the antibodies. Cells were incubated with IF buffer (PBS, 5% BSA, 0.01% Triton X-100) or (PBS, 5% BSA, 0.05% saponin) containing the primary Synaptotagmin-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. Cells were washed 3 × 10 min with IF buffer and once with

PBS containing DAPI. 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 63x oil objective (NA = 1.40). Analysis was done using the Zen navigation software (Zeiss). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.