Antibody Characterization Report for Sigma non-opioid intracellular receptor 1

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Sigma non-opioid intracellular receptor 1

Alternative protein name: Aging-associated gene 8 protein, SR31747-binding protein, SR-BP, SIG-1R, Sigma1-receptor, Sigma1R, hSigmaR1.

Gene name: *SIGMAR1*

Uniprot: Q99720

This report guides researchers to select the most appropriate antibodies for Sigma non-opioid intracellular receptor 1. We used an antibody characterization pipeline [1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Sigma non-opioid intracellular receptor 1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HEK293T and HeLa were selected based on evidence of appropriate Sigma non-opioid intracellular receptor 1 protein expression determined through public proteomics databases, namely PaxDB [2] and DepMap [3]. An HEK293T *SIGMAR1* KO line is available at Abcam. HeLa was modified with CRISPR/Cas9 [4] to knockout the corresponding *SIGMAR1* 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** DOI: 10.7554/eLife.48363.
2. Wang, M., et al., *Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines.* Proteomics, 2015. **15**(18): p. 3163-8 DOI: 10.1002/pmic.201400441.
3. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16 DOI: 10.1016/j.cell.2019.12.023.
4. Nicouleau, M., et al., *Generation of Knockout Cell Lines Using CRISPR-Cas9 and ddPCR Technology.* 2020 DOI: 10.5281/zenodo.3875777.

Table 1: Summary of the Sigma non-opioid intracellular receptor 1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo	42-3300	VI309559	AB_2533521	polyclonal	-	rabbit	0.25	Wb
Abcam	ab253192	GR3341811-1	AB_2885128	recombinant-mono	EPR23266-69	rabbit	0.62	Wb
GeneTex	GTX115389	40302	AB_10623075	polyclonal	-	rabbit	1.00	Wb
Cell Signaling Technology	61994	1	AB_2799617	recombinant-mono	D4J2E	rabbit	not provided	Wb, IP
Cell Signaling Technology	74807	1	AB_2799860	recombinant-mono	D7L1M	rabbit	not provided	Wb

Wb=Western blot; IP= immunoprecipitation

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Abcam	ab255449	CVCL_0063	HEK293T	WT
Abcam	ab266619	CVCL_B0XT	HEK293T	*SIGMAR1* KO
Montreal Neurological Institute	-	CVCL_0030	HeLa	WT
Montreal Neurological Institute	-	CVCL_B0XU	HeLa	*SIGMAR1* KO

Figure 1: Sigma non-opioid intracellular receptor 1 antibody screening by immunoblot.

Lysates of HEK293T and HeLa (WT and *SIGMAR1* KO) were prepared and 30 µg of protein were processed for immunoblot with the indicated Sigma non-opioid intracellular receptor 1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used was 1/5000. Predicted band size: 25 kDa

Figure 2: Sigma non-opioid intracellular receptor 1 antibody screening by immunoprecipitation.

HEK293T lysates were prepared, and immunoprecipitation was performed using 1.0 µg of the indicated Sigma non-opioid intracellular receptor 1 antibodies pre-coupled to either protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Sigma non-opioid intracellular receptor 1 antibody. For immunoblot, 61994 was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain.

Figure 3: Sigma non-opioid intracellular receptor 1 antibody screening by immunofluorescence.

A) HEK293T (WT and *SIGMAR1* KO) and **B)** HeLa (WT and *SIGMAR1* 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 Sigma non-opioid intracellular receptor 1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody. Acquisition of the green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: 42-3300 at 1/250; ab253192 at 1/600; GTX115389 at 1/1000; 61994 at 1/500; 74807 at 1/500. Bars = 10 µm.

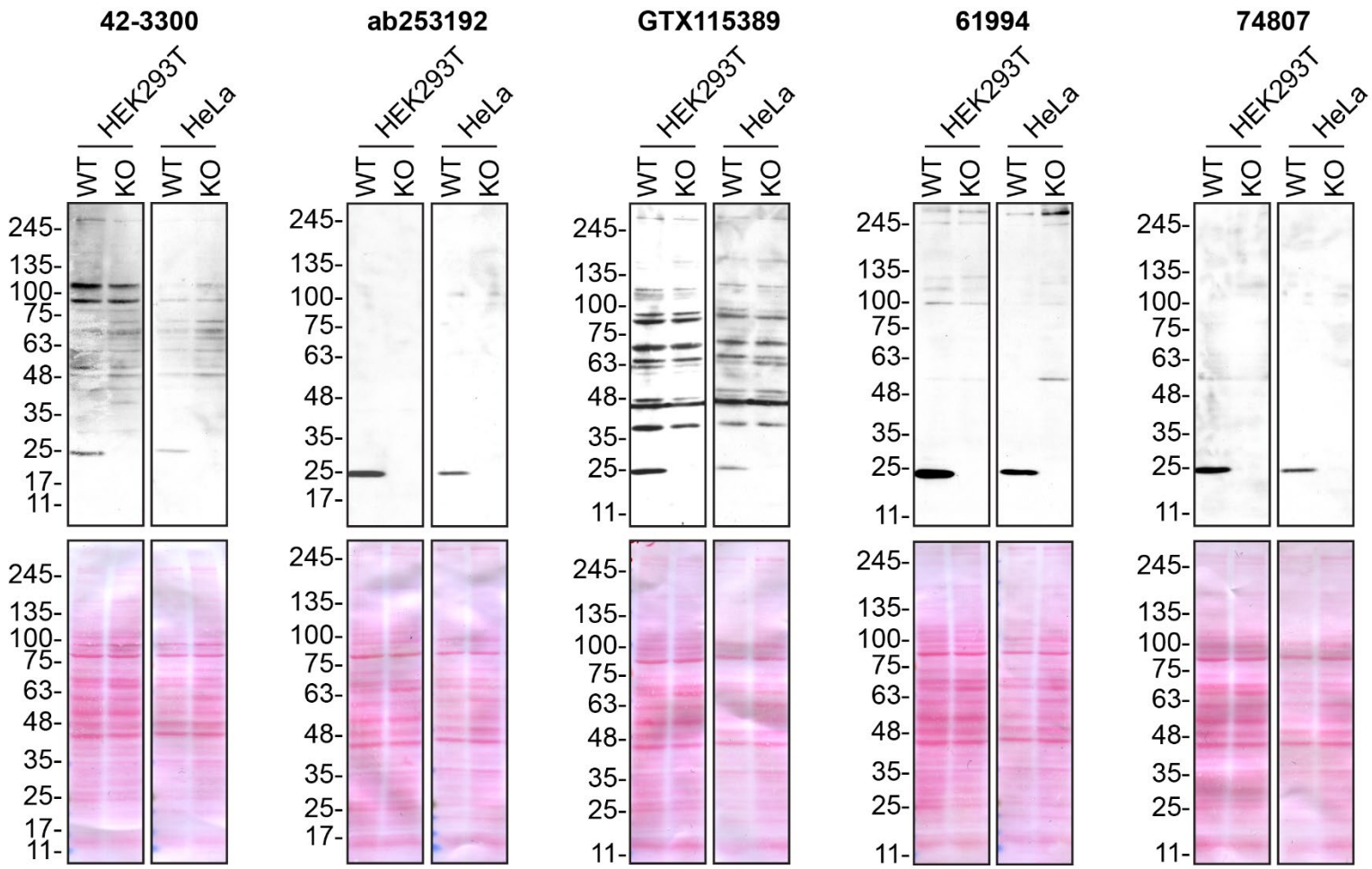

Figure 1: Sigma non-opioid intracellular receptor 1 antibody screening by immunoblot

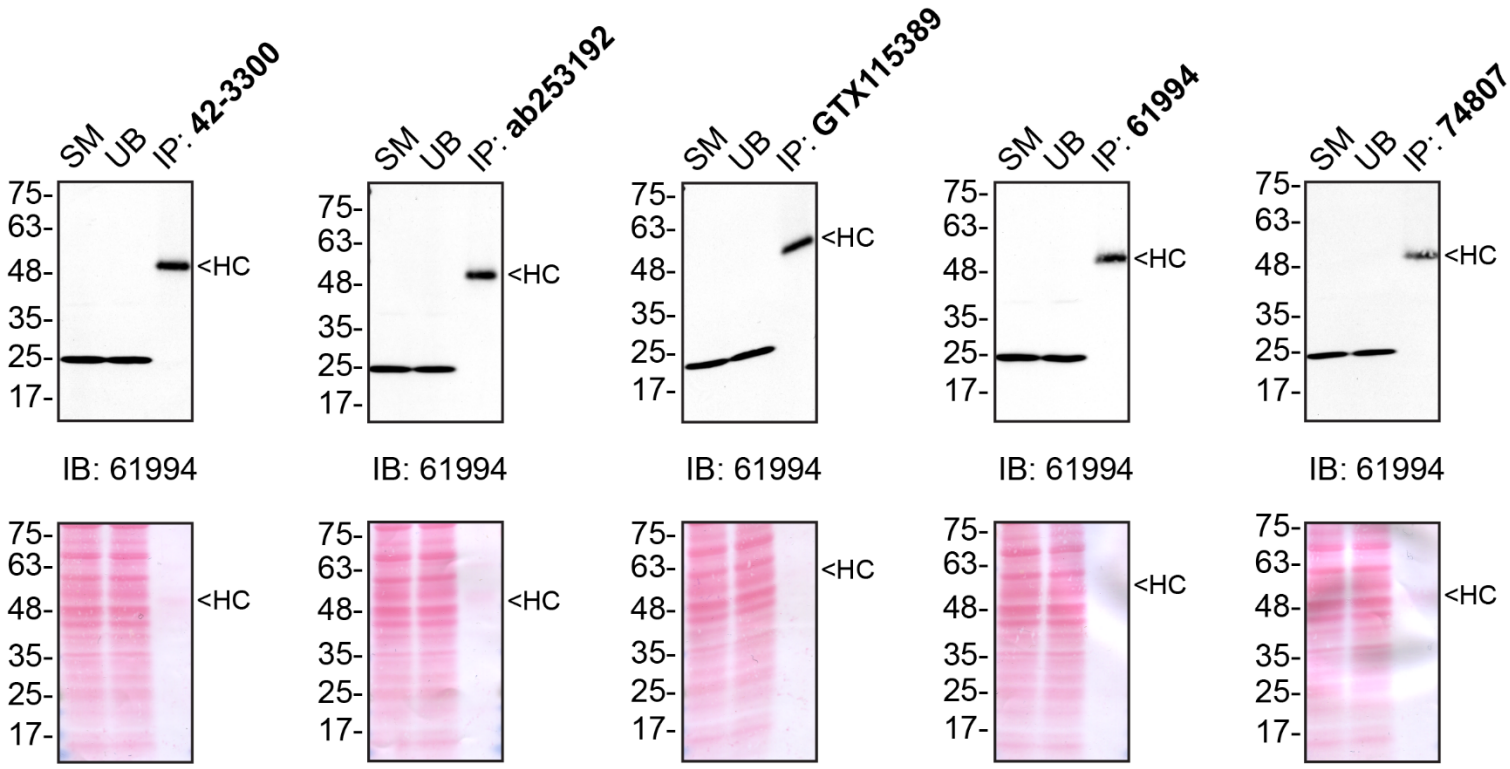

Figure 2: Sigma non-opioid intracellular receptor 1 antibody screening by immunoprecipitation

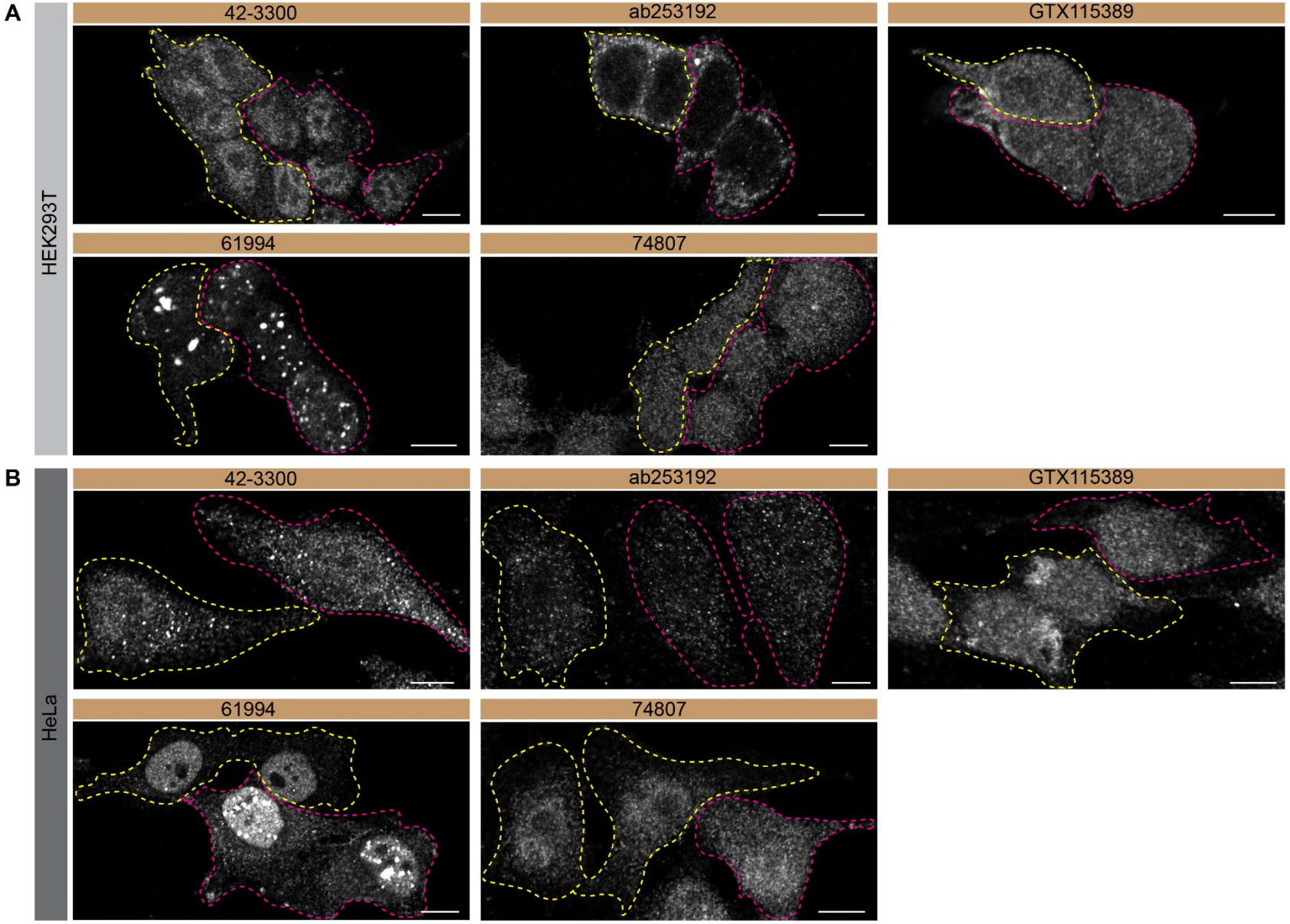

Figure 3: Sigma non-opioid intracellular receptor 1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Sigma non-opioid intracellular receptor 1 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-rabbit antibodies is from Thermo Fisher Scientific (cat. number 62-6520). Alexa-555-conjugated goat anti-rabbit secondary antibodies is from Thermo Fisher Scientific (cat. number A21429).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. HeLa *SIGMAR1* KO clone was generated with low passage cells using an open-access protocol available on Zenodo.org: https://zenodo.org/record/3875777#.YUs6a7hKg2y. The sequence of the guide RNA used is GCGCUACUGGGCUGAGAUCU.

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

HEK293T and HeLa (WT and *SIGMAR1* 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 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

Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 ul of PBS with 0.01% triton X-100 in a microcentrifuge tube, together with 30µl of protein A-Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

HEK293T WT were collected in HEPES buffer (20 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, pH 7.4) 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 HEPES lysis buffer and processed for SDS-PAGE and immunoblot on 5-16% 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

HEK293T and HeLa (WT and *SIGMAR1* 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 Sigma non-opioid intracellular receptor 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. 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.