Supplementary Materials for

An oncostatin M receptor and chloride intracellular channel 1 crosstalk drives key oncogenic pathways in glioblastoma

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This file includes:

Supplementary Material and methods

1. BTSC culture

Prior to use in experiments, BTSC lines were thawed from a cryopreserved state (10% dimethyl sulfoxide, DMSO) and placed in BTSC media containing serum-free NeuroCult NS-A medium (StemCell Technologies, Inc., Cat. #05750), supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin (Sigma Aldrich, P4333), heparin (2 µg/ml, StemCell Technologies, Inc., #07980), human EGF (20 ng/ml, Miltenyi Biotec, Cat. #130-093-825), and human FGF (10 ng/ml, Miltenyi Biotec, Cat. #130-093-838). BTSCs were cultured in T-25 or T-75 flasks for suspension cells (Sarstedt, Cat. #83.3910.502, #83.3911.502) to facilitate sphere formation in a non-adherent environment. All cell lines were subject to analysis for the presence of mycoplasma using the PCR method. Passaging of BTSC spheres was done every 3–7 days or once spheres reached 200–250 µm in size. This was accomplished by collecting media containing BTSC spheres followed by centrifugation at 1000 × *g* for 10 min. Cells were dissociated into a single cell suspension by incubating with 0.2 ml Accumax dissociation solution (Innovative Cell Technologies, Cat. #AM105) for 10 min at 37°C followed by the addition of 0.8 ml BTSC media. Utilizing trypan blue stain 0.4% (Gibco, Cat. #15250-061), cells were counted and subsequently transferred onto low-attachment flasks for culturing in BTSC suspension media. Cell plating densities were determined based on the size of the plating vessel (T-75 or T-25 flask) and the duration of the desired incubation (3–7 days).

2. Cell population growth assay

BTSC spheres were processed into a single-cell suspension using Accumax dissociation solution. Single cells were plated at a density of 30000 or 50000 cells per well (6-well plate) in 2 ml BTSC media for CRISPR/Cas9 clones and siRNA experiments, respectively. 1 day (24 h), 3 days (72 h), and 7 days (168 h) following plating, cells were collected, and live cells were assessed using trypan blue exclusion dye. Cell counts were performed using an automated cell counter (Countess II FL Automated Cell Counter).

3. Sphere size assessment

Spheroid diameter was measured to assess size differences between BTSC WT spheres and those generated from either transient siRNA-mediated knockdown or stable CRISPR/Cas9-mediated knockout. BTSC spheres were collected and processed into a single cell suspension using Accumax dissociation solution. Cells were plated as follows: 3 to 6 wells containing 500, 250, and

125 cells respectively in 100 µl BTSC media in a 96-well plate. The purpose of doing this was to ensure that the plating density did not impact the size of the spheres. By maintaining consistent plating densities, potential confounding factors affecting sphere size could be mitigated, allowing for a more accurate and reliable assessment of experimental results. 72 h following plating and incubation, sphere size was assessed, and visualizations captured using a scanning microscope (Olympus LS, IXplore Pro Automated Microscope system) equipped with the adjoining imaging software (Olympus LS, cellSens Version 1.12) using the 'Measure' feature at 10–20X objective magnification.

4. Cell fractionation

The plasma membrane and cytoplasm fractions were separated using the Thermofisher Subcellular Protein Fractionation Kit (Thermofisher, Cat. #78840), following the manufacturer's protocol. Briefly, 5×10^7 BTSCs were rinsed once with 1 ml of 1X PBS containing 0.3% BSA and then with 1 ml of 0.9% sodium chloride solution. For cytoplasm extraction, cells were treated with 500 µl of cytoplasmic extraction buffer (CEB) and shaken for approximately 5 min at 4°C. After centrifugation at 500 × *g* for 5 min, the supernatant was carefully collected. For plasma membrane extraction, the pellet was incubated with 500 µl of membrane extraction buffer and shaken for approximately 5 min at 4 °C. Subsequently, the cells were centrifuged at 3000 × *g* for 5 min, and the supernatant was collected.[1] Protein concentration of cytosolic, and plasma membrane lysates was determined using the Bradford assay (Bio-Rad, Cat. #5000006) and 10 mg of protein was loaded in a 10% SDS PAGE gel.

5. Electroporation of BTSC

BTSC electroporation was conducted in accordance with our established protocols.[2] Briefly, BTSCs were transfected with 100 nM siRNA via electroporation using an Amaxa Nucleofector 2b (Lonza), following the manufacturer's optimized protocols (Lonza, Cat. #AAB1001). Electroporated cells were plated into T-75 suspension culture flasks containing BTSC media and incubated at 37 °C and 5% CO_2. Plating for experiments took place 24 h after electroporation while collection of cells for gene expression analysis by RT-qPCR and protein expression by Immunoblotting were done 72 h after electroporation.

6. Transcriptomic database analysis

Six processed glioblastoma datasets, referred to as Ducray[3], Gravendeel[4], Kamoun[5], LeeY[6], Rembrandt[7], and TCGA_GBM[8], were downloaded from the GlioVis database[9], version

0.20. Datasets were analyzed in R version 4.4.1.[10] Boxplot *P*-values were calculated using Mann-Whitney U tests. Survival analysis log-rank *P*-values were calculated using the survival package version 3.7-0, in R.[11,12]

7. Gene expression analysis

RNA was extracted from cells using the TRIzol method. In this method, pelleted cells are incubated with 1 ml TRIzol digestive reagent (Invitrogen, Cat. #15596026) at RT for 5 min. Next, 0.2 ml chloroform (Sigma Aldrich, Cat. #288306) was added to the samples and shaken vigorously for 20 sec followed by a 2–3 min incubation at RT (22–25 °C). Samples were then centrifuged at 12000 × *g* for 15 min at 4 °C. Subsequently, 0.5 ml of the upper aqueous phase was transferred to a clean tube containing 0.5 ml of isopropanol. Samples were incubated at RT for 15 min, followed by centrifugation at 12000 × *g* for 10 min, at 4 °C. The resultant supernatant was removed, and the RNA pellet was washed sequentially with 75% Ethanol followed by 100% Ethanol. Each wash step was followed by centrifugation at 7500 × *g* for 5 min at 4 °C. After the final supernatant removal, the RNA pellets were air-dried for 10 min at RT within a sterile biosafety cabinet. The RNA pellet was dissolved in 20–50 µl RNase-free water and incubated at 55–60 °C for 10–15 min to ensure RNA solubilization. cDNA was synthesized via reverse transcription using the 5X All-In-One RT MasterMix cDNA synthesis system (abm, Cat. #G592). Relative gene expression was determined by RT-qPCR using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, Cat. #1725271). Each reaction mixture contained 1 µL of each primer (5 µM; sequences provided in Supplementary Table 5) and 5 µL of the SYBR Green master mix. This mix was used to generate a final plated mix of 3 µl of 30 ng cDNA and 7 µl PCR mix that was run on the QuantStudioTM 7 Flex Real-Time PCR System (Applied Biosystems). mRNA expression levels were normalized to one of two housekeeping genes: beta-glucuronidase (*GUSB*) and beta-actin (*ACTB*).

8. Lipid raft disruption

Lipid raft disruption with Methyl-β-cyclodextrin (MβCD) began by preparing a 5 mM MβCD solution (Selleckchem, Cat. # S6827) in serum-free cell culture media and warming it to 37 °C. Next, the medium was removed, and the cells were washed gently with 1X PBS. The pre-warmed 5 mM MβCD solution was added to the cells, followed by incubation at 37 °C for 1 h to deplete cholesterol from the cell membrane and disrupt lipid rafts. After the incubation period, the MβCD solution was removed, and the cells were washed with 1X PBS to eliminate any remaining MβCD. Fresh, complete cell culture medium was then added to the cells.

9. Immunoblotting and antibodies

Protein expression was analyzed by SDS-PAGE as previously described.[2] Total protein from samples was harvested from a minimum of 5×10^5 using 1X RIPA Buffer (Thermo Fisher Scientific, Cat. #89900) supplemented with protease and phosphatase inhibitors (Thermo Fisher Scientific, Cat. #A32959). Following cell lysis, protein concentration was assessed using Bradford Assay (Bio-Rad), after which samples were run on a 10–15% SDS-PAGE gel and transferred on a Nitrocellulose Membrane 0.45 µm (Bio-Rad, Cat. #1620115) for 1 h and 30 min at 100 volts. Membranes were blocked for non-specific binding using 5% bovine serum albumin (BSA) in 1X TBST for 1–2 h. Subsequently, membranes were incubated with primary antibodies at 4 °C overnight. Following three washes, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies diluted in 5 % bovine serum albumin (BSA) blocking solution. Proteins of interest were visualised by ClarityTM Western ECL Substrate (BioRad, Cat. #170-5060) using ChemiDoc Imaging System (Biorad). The antibodies used for immunoblotting include anti-OSMR (1:100, Santa Cruz Biotechnology, Cat. #sc-271695, mouse), anti-CLIC1 (1:200, Santa Cruz Biotechnology, Cat. #sc-81873, mouse), α-Tubulin (1:5000, Abcam, Cat. #ab4074, mouse), HRP-conjugated secondary antibody (1:5000, BioRad, Cat. #1706516, mouse).

10. Extreme limiting dilution assay (ELDA)

A single cell suspension was prepared and diluted using BTSC complete media to generate a final plated density of 25, 12, 6, 3 and 1 cell per well. 100 µl of the diluted single-cell suspensions was plated in a 96-well plate as follows: 12 wells plated for dilution 25, 12, 6, and 3 cells per well and 48 wells (4 replicates of 12 wells) plated for 1 cell per well dilution.[13] This was done for 1 cell per well plating to achieve comparable results as many times single cell death can occur following plating. Plates were incubated at 37 °C and 5% CO_2 for 7 days after which sphere formation was assessed. Analysis of the responding wells as a function of the plated wells was undertaken and data was input into an online software available at https://bioinf.wehi.edu.au/software/elda/. Using this data provided by the software, the number of cells required to generate a positive well (having a sphere) was determined for each condition. Stem cell frequency (SCF) or the probability of finding a stem cell in the bulk sample of cells was also given as a value of 1/stem cell frequency. To determine percent SCF 1/stem cell frequency was multiplied by 100.

11. Duolink proximity ligation assay (PLA)

Single cell BTSC suspension was plated on Nunc Lab-Tek, II CC2 Chamber Slide System (Thermo Scientific, Cat. #154941) that was coated overnight with a Poly-D-Lysine (PDL) coating. The working PDL solution (10 µg/ml) was made from a 2 mg/ml stock (Fisher Scientific, Cat. # CB-40210) using sterile water and incubated on the chamber slide overnight at 37 °C and washed thoroughly (3 times) with sterile water prior to addition of cell suspension. Following Accumax-mediated dissociation, the single cell suspension was plated at a density of ~2.5×10^4 cells in media supplemented with 10% FBS. Cells were incubated for 1–6 h until distinct cellular projections were visible. Following incubation, cells were quickly washed with 1X PBS and fixed using 4% paraformaldehyde for 15 min at RT. Next, samples were washed with 1X PBS and permeabilized using 0.5% Triton X-100 for 25 min and subsequently blocked for non-specific binding for 1 h using the Duolink Blocking Solution provided in the starter kit at 37 °C. Samples were then incubated overnight at 4 °C in a humid chamber with primary antibodies against the protein of interest: anti-OSMR (1:400, Abnova, Cat. #H00009180-D01P, rabbit) and anti-CLIC1 (1:400, Santa Cruz Biotechnology, Cat. #sc-81873, mouse). Following overnight incubation, samples were washed with Wash Buffer A (available in the Kit) and incubated with the PLUS and MINUS oligonucleotide probes conjugated to secondary antibodies for 1 h at 37 °C in a humid chamber. Samples were subsequently washed with Wash Buffer A and incubated with Ligation Buffer with Ligase (available in the Starter Kit) for 30 min at 37 °C in a humid chamber. Rolling Circle Amplification (RCA) was achieved by washing samples with Wash Buffer A and incubating with polymerase and amplification solution (available in the Starter Kit) containing nucleotides for 100 min at 37 °C in a humid chamber and protected from light. Following RCA, samples were washed with Wash Buffer B (available in the Kit) and mounted with Duolink In Situ Mounting Medium (Sigma, Cat. #DUO82040). PLA signals were visualized and captured by a laser scanning microscope (Olympus LS, IXplore Pro Automated Microscope system) equipped with the adjoining imaging software (Olympus LS, cellSens Version 1.12) at 40–60X objective magnification.

12. Generation and characterization of tmCLIC1omab

12.1. Growth curve

Cells (2×10^4) were plated and counted over time using Trypan Blue exclusion and an automated counter in the presence or absence of 100 µM of IAA94 tmCLIC1 inhibitor used as control or 3 µg/ml of tmCLIC1omab antibody. Triplicates were used per condition (Fig. 6l, m).

12.2. Dose-response

Cells were seeded in 96-well plates at a density of 2×10^4/well and allowed to adhere overnight. The following day, cells were treated with increasing concentrations of tmCLIC1omab ranging from 0.1 to 3 µM. After 72 h of treatment, cell viability was assessed using the MTT assay (Fig. 6n).

12.3. 3D culture

Human glioblastoma cells Negative Control (NC), knockout for *CLIC1* (*CLIC1*$^{-/-}$), and rescued with *CLIC1* WT were cultured in three-dimensional spheroids using the hanging drop method. To generate spheroids, 20–30 µL droplets containing (500–2000 cells/drop) were pipetted onto the inner surface of a sterile Petri dish lid. The lid was then inverted and placed over a dish containing sterile PBS or distilled water to maintain humidity and prevent evaporation. Plates were incubated at 37 °C with 5% CO_2 for 24 h, allowing spheroids to form through gravity-driven cell aggregation. After formation, spheroids were harvested by gently flushing the drops into a sterile conical tube using a pipette and transferred into low-attachment plates covered by 1.5% (w/v) Agarose for further analysis or treatment. Spheroids were transferred in complete media and /or to tmCLIC1omab-containing media. Images were captured at 0 and 72 h, and the spheroid area was quantified using ImageJ, and the area obtained after 72 h of incubation was normalized to the initial spheroid's area (Supplementary Fig. 9a, b).

12.4. tmCLIC1omab irreversible binding

These experiments were performed as a growth curve analysis of human primary glioblastoma cell lines in which tmCLIC1omab was added for 3, 5, and 24 h respectively and then washed out and substituted with fresh medium. Proliferation was evaluated after 72 h (Fig. 6o).

12.5. tmCLIC1omab internalization

pHrodo™ (Life Technologies, Cat. #P35372) was prepared according to the manufacturer's instructions, labeling the dye with tmCLIC1omab. Labeled particles were resuspended in warm assay buffer then added to the cells. Cells were incubated at 37 °C for 30 min to allow uptake. In control wells, parallel incubations were done in the presence of antibody isotype to assess non-specific or blocked uptake. After incubation, cells were washed gently with PBS to remove excess particles and fluorescence microscopy was performed using a Spinning disk confocal microscope (Ex 560 nm/Em 585 nm). Quantification of fluorescence intensity was performed using ImageJ/Fiji. Increased fluorescence intensity indicates successful internalization

and acidification of pHrodo-labeled particles within endosomes or lysosomes, as pHrodo becomes highly fluorescent in acidic environments but remains non-fluorescent at neutral pH (Supplementary. Fig. 9c, d).

12.6. Antibody sequences of tmCLIC1omab

12.6.1. Heavy chain: DNA sequence (411 bp)

Signal sequence-*FR1*-CDR1-**FR2**-*CDR2*-FR3-**CDR3**-*FR4*

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAG
TGT*GAAGTGATGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAA*
*ACTCTCCTGTGCAGCCTCTGGATTTGCTTTCAGT*AATTATGCCATGTCC**TGGATTCGCC**
AGATTCCGGAGAAGAGGCTGGAGTGGGTCGCA*ACCATTAGTACTGGTGGTAGTTCC*
*ACCTTCTATCCAGACAGTGTGAAGGGG*CGATTCACCATCTCCAGAGACAATGCCAAGA
ATACCCTATACCTGCAAATGAGCAGTCTGAGGTCTGAGGACACGGCCATGTATTACT
GTACAAGA**CATGCCTACGAGGCCTGGTTTGCTTAC***TGGGGCCAAGGGACTCTGGTC*
ACTGTCTCTGCA

12.6.2. Heavy chain: Amino acid sequence (137 aa)

Signal peptide-*FR1*-CDR1-**FR2**-*CDR2*-FR3-**CDR3**-*FR4*

MNFGLSLIFLVLVLKGVQC*EVMLVESGGGLVKPGGSLKLSCAASGFAFS*NYAMS**WIRQI**
PEKRLEWVA*TISTGGSSTFYPDSVKG*RFTISRDNAKNTLYLQMSSLRSEDTAMYYCTR**H**
AYEAWFAY*WGQGTLVTVSA*

12.6.3. Light chain: DNA sequence (384 bp)

Signal sequence-*FR1*-CDR1-**FR2**-*CDR2*-FR3-**CDR3**-*FR4*

ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATGAGTGCCTCAGTCATA
ATGTCCAGGGGA*CAAATTGTTCTCACCCAGTCTCCAGCACTCATGTCTGCATCTCCAGG*
*GGAGAAGGTCACCATGACCTGC*AGTGCCAGCTCAAGTGTAAGTTACATGTAC**TGGTA**
CCAACAGAGGCCAAGATCCTCCCCCAAACCCTGGATTTAT*CTCACATCCACCCTG*
*GCTTCT*GGAGTCCCTACTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCA
CAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGC**CAGCAGTGGAAT**
AGTCACCCACTCACG*TTCGGCTCGGGGACAAAGTTGGAAATAAAA*

12.6.4. Light chain: Amino acid sequence (128 aa)

Signal peptide-*FR1*-CDR1-**FR2**-*CDR2*-FR3-**CDR3**-*FR4*

MDFQVQIFSFLLMSASVIMSRG*QIVLTQSPALMSASPGEKVTMTC*SASSSVSYMY**WYQQ RPRSSPKPWIY***LTSTLAS*GVPTRFSGSGSGTSYSLTISSMEAEDAATYYC**QQWNSHPLT***F GSGTKLEIK*

12.7. Project specifications

Sequence	Clones sequenced	Clones with >99% sequence identity
V_H	5	5
V_L	5	5

12.8. International ImMunoGeneTics information system (IMGT) analysis of V(D)J junctions

Sequence	V-GENE and allele	Functionality	V-REGION identity % (nt)	J-GENE and allele	D-GENE and allele	AA Junction	Junction frame
V_H	Musmus IGHV5-9-1*01 F	productive	95.83% (276/288 nt)	Musmus IGHJ3*01 F	Musmus IGHD1-2*01 F	CTRHAYEAWFAYW	in-frame
V_L	Musmus IGKV4-68*01 F	productive	98.19% (271/276 nt)	Musmus IGKJ4*01 F	-	CQQWNSHPLTF	in-frame

Total RNA was isolated from the hybridoma cells following the technical manual of RNeasy Plus Micro Kit (QIAGEN, Cat. #74034). Total RNA was then reverse-transcribed into cDNA using either isotype-specific anti-sense primers or universal primers following the technical manual of SMARTScribe Reverse Transcriptase (TaKaRa, Cat. #639536). Antibody fragments of heavy chain and light chain were amplified according to the standard operating procedure (SOP) of rapid amplification of cDNA ends (RACE) of GenScript. Amplified antibody fragments were cloned into a standard cloning vector separately. Colony PCR was performed to screen for clones with inserts of correct sizes.

12.9. Animals and ethical approval

C57BL/6 mice, aged 8 weeks, were obtained from Envigo s.r.l. and housed in a specific pathogen-free (SPF) facility under controlled conditions (22 ± 2°C, 55 ± 10% humidity, 12 h

light/dark cycle). All animal procedures were approved by the Ministero della Salute (26/2014) conducted in accordance with institutional and national guidelines.

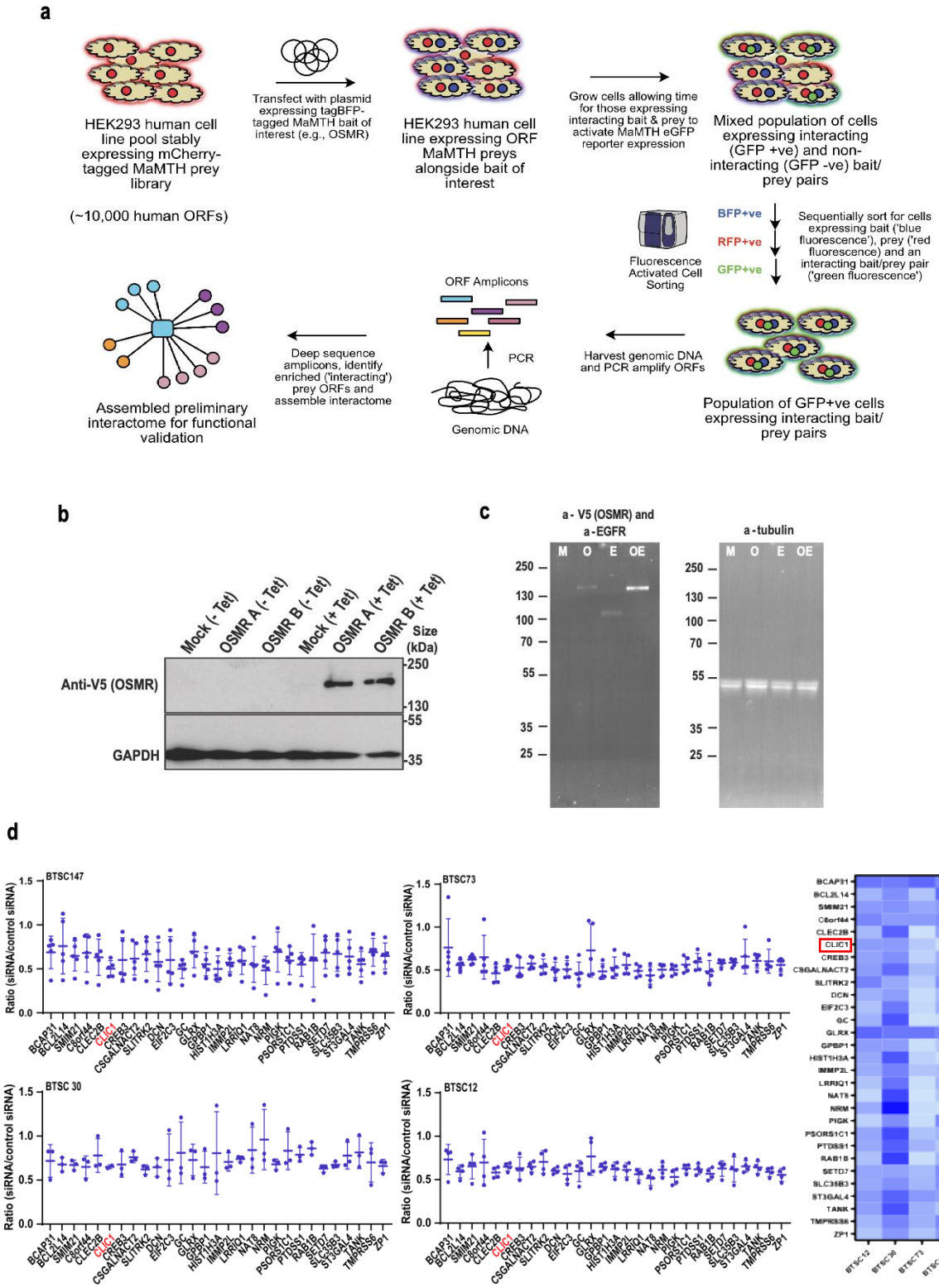
12.10. Monoclonal antibody administration

Mice were randomly assigned to treatment and control groups (n = 8 per group). The treatment group received tmCLIC1omab at a dose of 10 mg/kg, administered via intravenous (I.V.) injection twice per week for a total of 3 weeks. Control animals received equivalent volumes of vehicle (isotype control antibody) on the same schedule.

12.11. Body weight monitoring

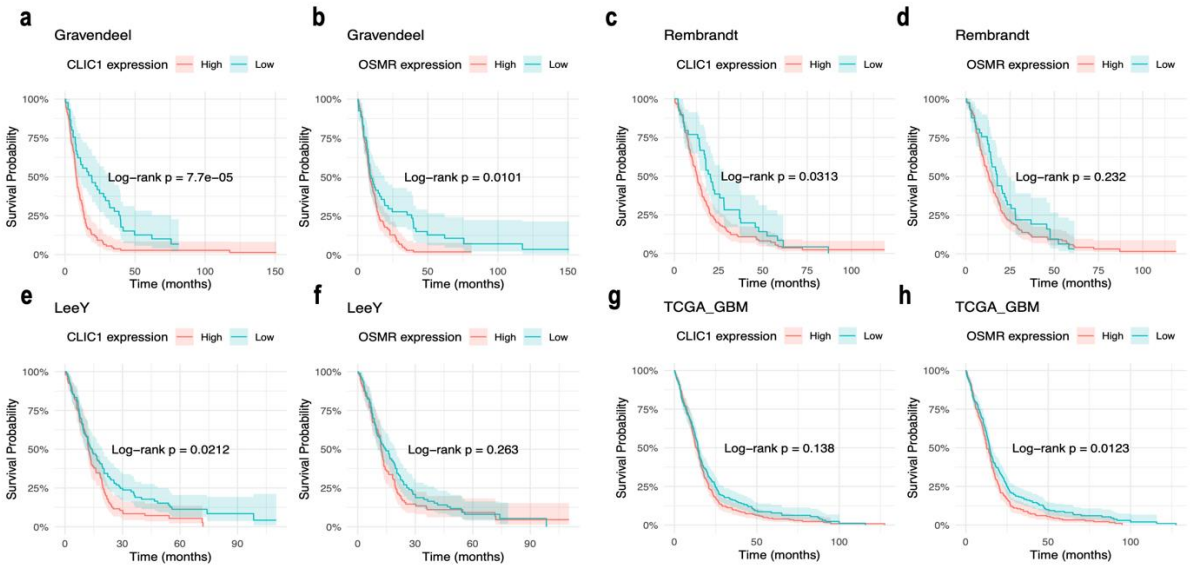
Body weight was measured twice weekly, prior to each antibody injection, using a calibrated digital scale. Baseline weight was recorded on Day 0 before the first treatment. Body weights were monitored throughout the dosing period and expressed as absolute weight (grams). Animals were observed daily for signs of stress, discomfort, or adverse effects. Any animal exhibiting a weight loss of > 20% from baseline, severe behavioral changes, or other signs of distress was removed from the study and humanely euthanized according to ethical guidelines (Fig. 6p).

Figure S1.


a
HEK293 human cell line pool stably expressing mCherry-tagged MaMTH prey library
(~10,000 human ORFs)
Transfect with plasmid expressing tagBFP-tagged MaMTH bait of interest (e.g., OSMR)
HEK293 human cell line expressing ORF MaMTH preys alongside bait of interest
Grow cells allowing time for those expressing interacting bait & prey to activate MaMTH eGFP reporter expression
Mixed population of cells expressing interacting (GFP +ve) and non-interacting (GFP -ve) bait/ prey pairs
Fluorescence Activated Cell Sorting
BFP+ve
RFP+ve
GFP+ve
Sequentially sort for cells expressing bait ('blue fluorescence'), prey ('red fluorescence) and an interacting bait/prey pair ('green fluorescence')
Population of GFP+ve cells expressing interacting bait/ prey pairs
Harvest genomic DNA and PCR amplify ORFs
Genomic DNA
PCR
ORF Amplicons
Deep sequence amplicons, identify enriched ('interacting') prey ORFs and assemble interactome
Assembled preliminary interactome for functional validation
b
Mock (- Tet)
OSMR A (- Tet)
OSMR B (- Tet)
Mock (+ Tet)
OSMR A (+ Tet)
OSMR B (+ Tet)
Size (kDa)
Anti-V5 (OSMR)
GAPDH
250
130
55
35
c
a - V5 (OSMR) and a-EGFR
a-tubulin
M O E OE
250
130
100
70
55
35
25
d
BTSC147
BTSC73
BTSC 30
BTSC12
Ratio (siRNA/control siRNA)
1.5
1.0
0.5
0.0
BCAP31
BCL2L14
SMIM21
C9orf44
CLEC2B
CLIC1
CREB3
CSGALNACT2
SLITRK2
DCN
EIF2C3
GC
GLRX
GPBP1
HIST1H3A
IMMP2L
LRRIQ1
NAT8
NRM
PIGK
PSORS1C1
PTDSS1
RAB1B
SETD7
SLC35B3
ST3GAL4
TANK
TMPRSS6
ZP1

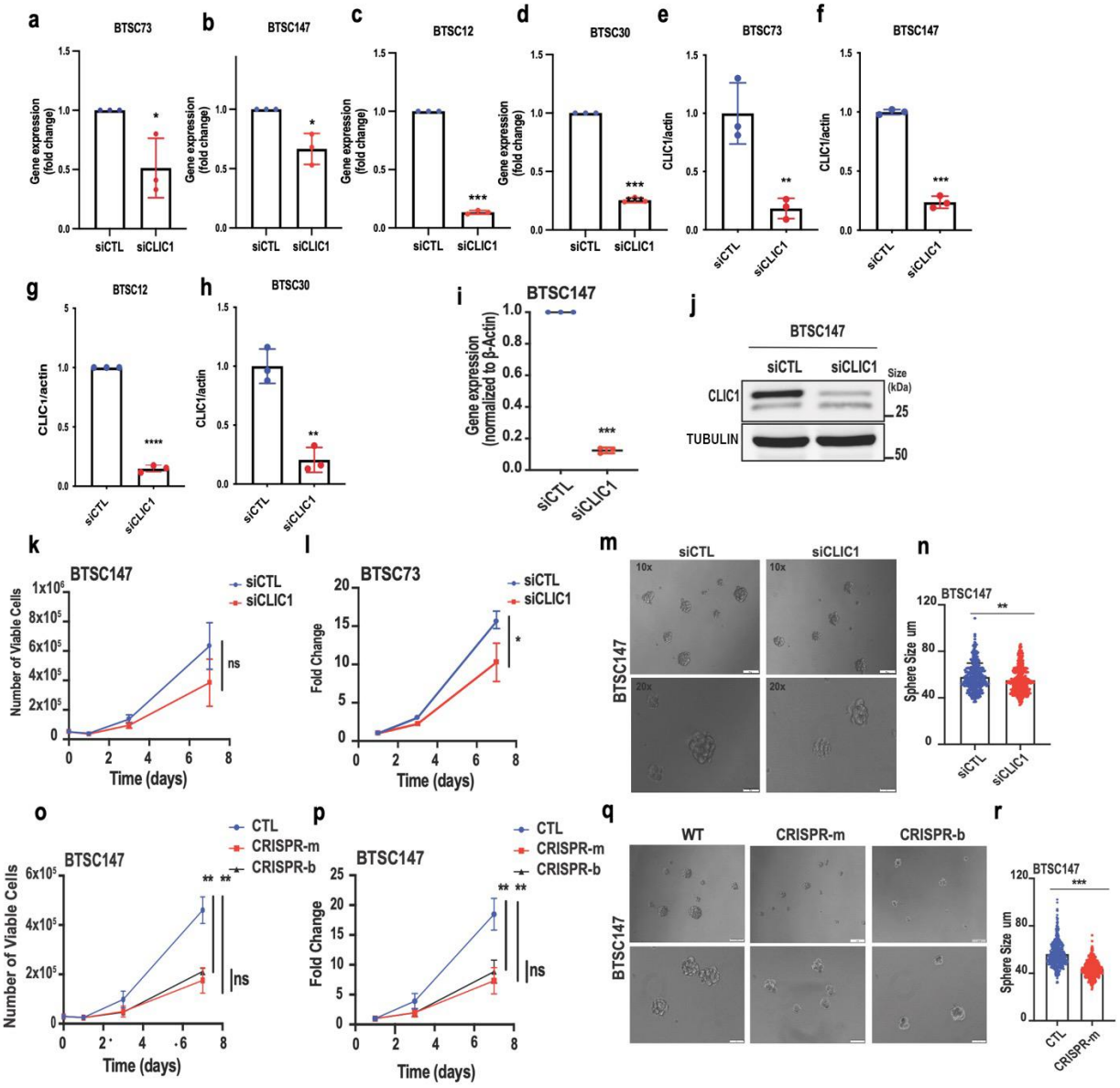
Supplementary Fig. 1 Schematic outline of the MaMTH-HTS screening workflow and mapping global binding partners of OSMR and Characterization of OSMR bait induction in MaMTH HEK293T reporter cells. **a** Schematic of the MaMTH system: expressed bait and prey are indicated by blue circles (corresponding to tagBFP tag) and red circles (corresponding to mCherry tag), respectively. Green circles (corresponding to expressed GFP reporter) are indicative of bait/prey interaction. **b** Western blot analysis of HEK293T reporter cells transfected with *OSMR* MaMTH bait construct verifying that tagged OSMR is properly expressed in response to induction with 0.5 µg/ml tetracycline, with no detectable leakage in the absence of inducer. 'OSMR A' and 'OSMR B' correspond to MaMTH bait constructs tagged at their C-terminus with Cub-GAL4TF-V5 or Cub-GAL4TF-V5-P2A-tagBFP, respectively. GAPDH was used as a loading control. **c** OSMR and EGFRvIII proof of expression in the bait. **d** Cell viability was assessed in four different patient-derived BTSCs (#12, #30, #73, and #147), transfected with siRNAs targeting different genes encoding candidate binding proteins. Each graph is normalized to the control group transfected with a non-targeting siRNA. Data are presented as means ± SEM, n = 3 biological replicates in a-d with Heatmap plot comparing the differences across four BTSC cell lines. The color gradient, represented by column Z-scores, illustrates relative cell viability assessed by PrestoBlue analysis. **M**: Mock transfected; **O**: OSMR Bait transfecte; **E**: EGFRvIII transfected; **OE**: OSMR Bait and EGFRvIII co-transfected.

Figure S2.



Supplementary Fig. 2 Clinical relevance of *CLIC1* and *OSMR* expression to GB patient survival across public datasets. **a-h** Kaplan-Meier survival curves for glioblastoma patients stratified by median gene expression of *CLIC1* and *OSMR* in Gravendeel (**a**, **b**), Rembrandt (**c**, **d**), LeeY (**e**, **f**), and TCGA_GBM (**g**, **h**) datasets. Rose lines represent patients with high gene expression (> median), while turquoise lines indicate low expression (< median). Across the Gravendeel, Rembrandt, and LeeY datasets, higher *CLIC1* expression is associated with reduced survival probability over time, whereas the impact of *OSMR* expression on survival is less consistent.

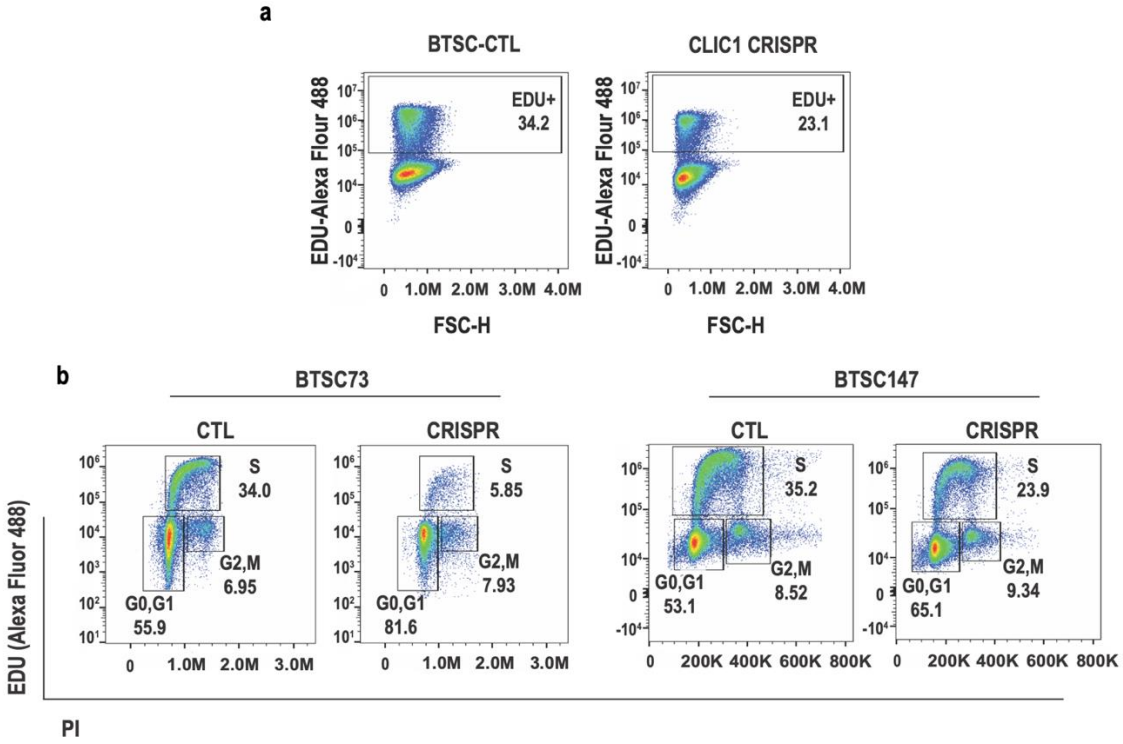
Figure S3.



Supplementary Fig. 3 Effects of *CLIC1* gene modification on BTSC viability and sphere size. **a-d** BTSC73, BTSC147, BTSC12, and BTSC30 cells were transfected with an siRNA pool targeting *CLIC1* (*siCLIC1*) or non-targeting siRNA control (siCTL) and mRNA expression of *CLIC1* in each BTSC line was assessed by RT-qPCR. Data was normalized to the housekeeping gene, *GUSB*. **e-h** BTSCs were subjected to immunoblotting analysis using an anti-CLIC1 antibody. CLIC1 protein levels were normalized to ACTIN (n = 3 biological replicates). Statistical analysis was performed using Student's t-test. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. **i** BTSC147 was transfected with an siRNA targeting *CLIC1* (*siCLIC1*) or non-targeting siRNA control (siCTL)

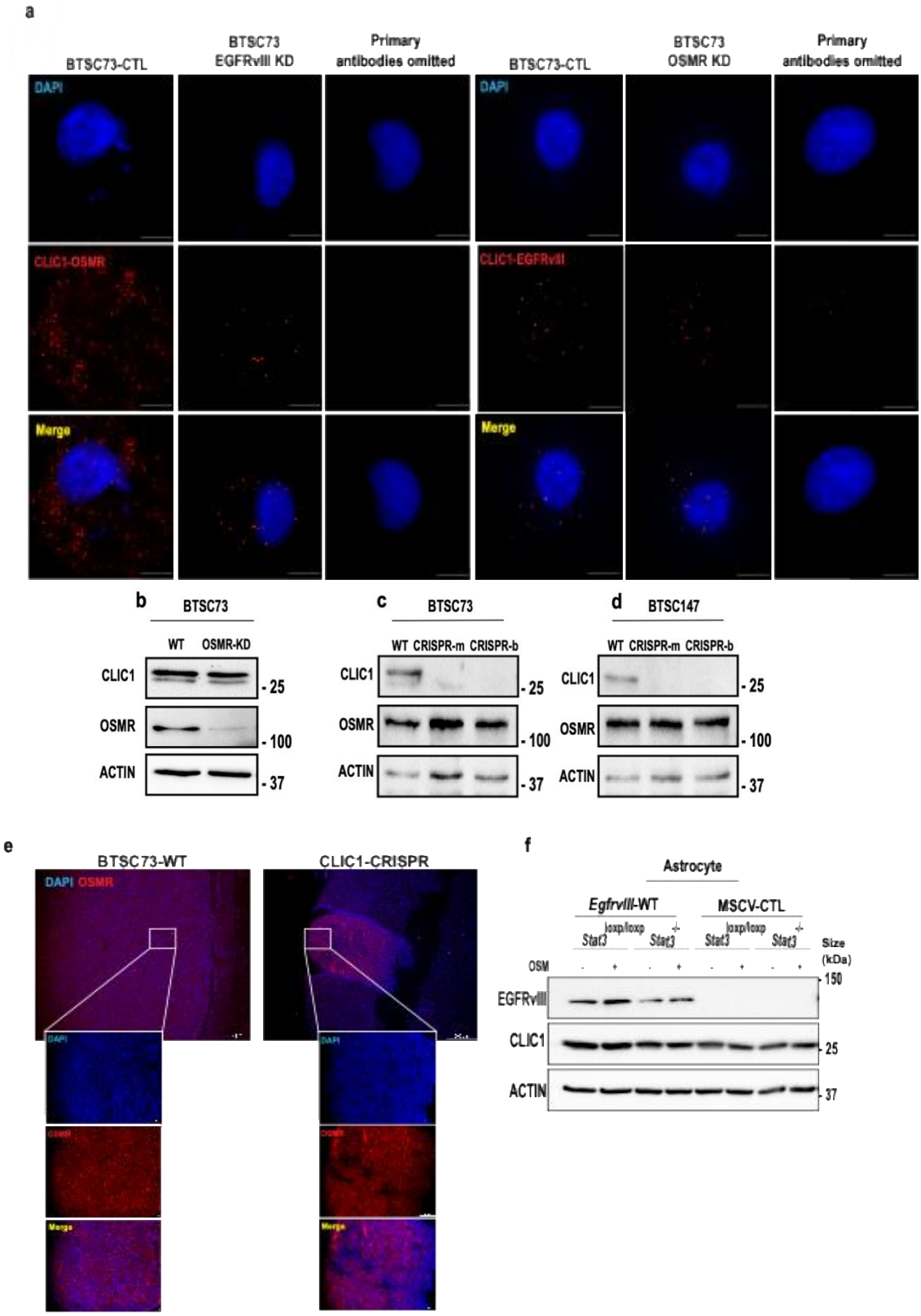
and mRNA expression of *CLIC1* was assessed by RT-qPCR. Data was normalized to the housekeeping gene, *GUSB*. **j** The protein expression of CLIC1 was assessed by immunoblotting using an anti-CLIC1 antibody. TUBULIN was used as a loading control. **k, l** Proliferation ability plotted based on the number of viable cells counted using trypan blue exclusion dye (**k**). Cell viability was plotted as fold change based on the initial count on day 1 for each condition (**l**). A Student's t-test was used to compare two conditions at day 7. Data are presented as mean ± SD, n = 3 biological replicates, non-significant (ns), $*P < 0.05$. **m** Representative images of *siCLIC1* and siCTL sphere sizes following 3 days of incubation at 37 °C and 5% CO_2. Sphere images were taken using 10X (top panel, scale bar 100 μm) and 20X (bottom panel, scale bar 100 μm) objectives. **n** Sphere size was quantified (minimum of 100 spheres per replicate) and plotted as mean ± SD. A Student's t-test was used to identify significance, $**P < 0.01$. **o-r** Two different transgenic *CLIC1* CRISPR lines were generated for each of BTSC147 and BTSC73. **o** Proliferation ability plotted based on the number of viable cells counted using trypan blue exclusion dye. **p** Proliferation capability plotted as fold change based on the initial count on day 1 for each condition. A student's t-test was used to compare two conditions at day 7. Data are presented as mean ± SD, n = 3 biological replicates, non-significant (ns), $**P < 0.01$. **q** Representative images of *CLIC1* CRISPR cells and control BTSC147 sphere sizes following 3 days of incubation at 37 °C and 5% CO_2. Sphere images were taken using 10X (top panel, scale bar 100 μm) and 20X (bottom panel, scale bar 100 μm) objectives. **r** Sphere sizes in *CLIC1* CRISPR and control BTSC147 were quantified. A minimum of 100 spheres was assessed from each biological replicate and data was plotted as a mean ± SD. A Student's t-test was used to determine significance, $***P < 0.001$.

Figure S4.



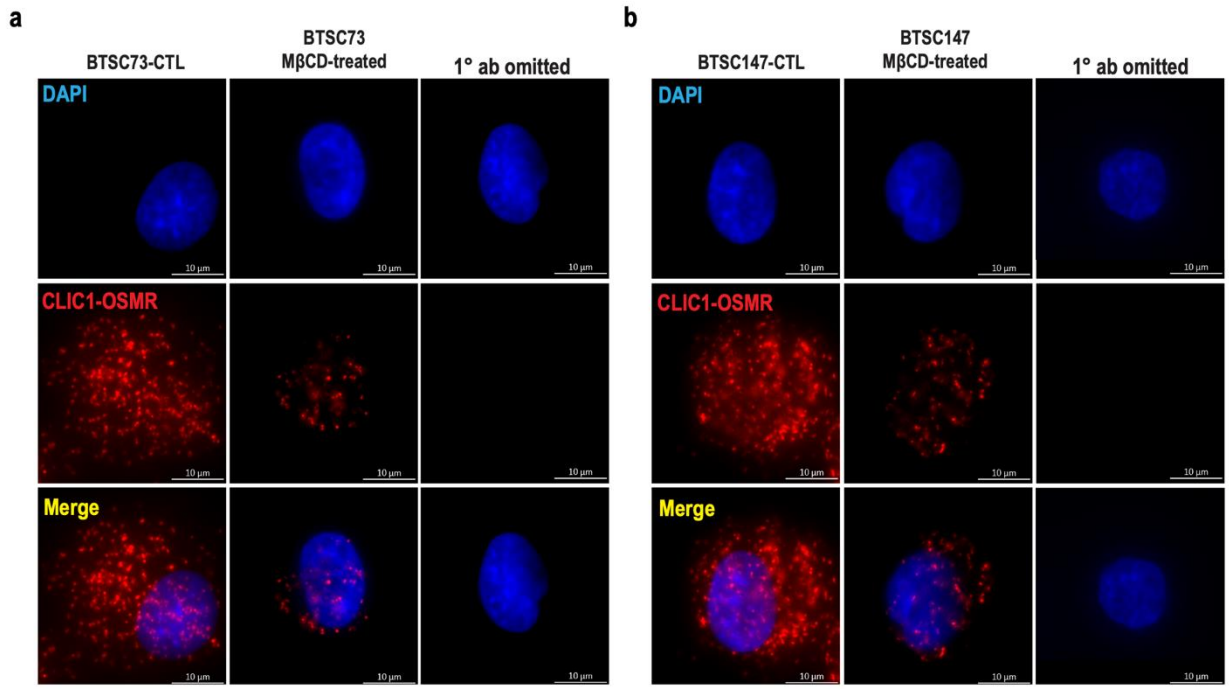
Supplementary Fig. 4 Cell cycle analysis of EdU-labeled cells. BTSC147 cells and their corresponding CLIC1-knockout lines were labeled with the Click-iT EdU-Alexa 488 kit and analyzed by flow cytometry. **b** BTSC73 and BTSC147 cells, along with their corresponding *CLIC1* CRISPR lines, were simultaneously stained with EdU and propidium iodide (PI). The resulting dual-parameter plots, which combine DNA content and EdU incorporation data, show three distinct cell populations. This analysis allows for the direct quantification of cells in the S-phase, as well as the identification of the G0/G1 and G2/M populations.

Figure S5.


a
BTSC73-CTL
BTSC73 EGFRvIII KD
Primary antibodies omitted
BTSC73-CTL
BTSC73 OSMR KD
Primary antibodies omitted
DAPI
CLIC1-OSMR
CLIC1-EGFRvIII
Merge
b
BTSC73
WT
OSMR-KD
CLIC1
OSMR
ACTIN
25
100
37
c
BTSC73
WT CRISPR-m CRISPR-b
d
BTSC147
e
BTSC73-WT
CLIC1-CRISPR
DAPI OSMR
f
Astrocyte
Egfrviii-WT
MSCV-CTL
loxp/loxp
Stat3
OSM
EGFRvIII
Size (kDa)
150

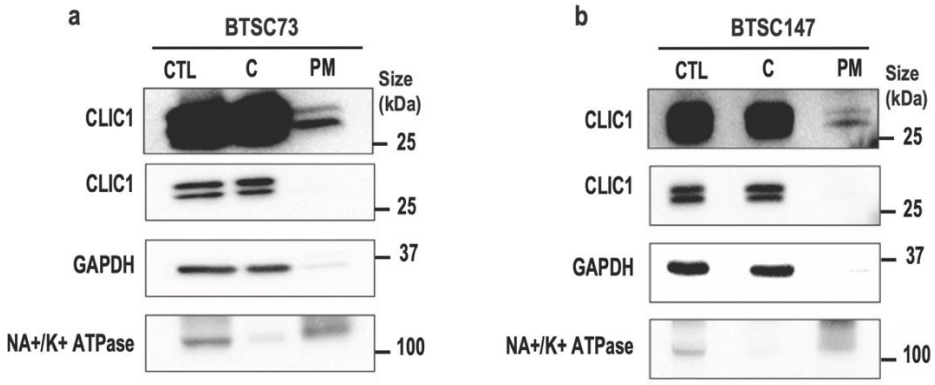
Supplementary Fig. 5 Proximity ligation assay (PLA) and correlative protein analysis following *OSMR* and *CLIC1* expression alteration. **a** BTSC73-CTL, BTSC73 *EGFRvIII*-KD, and BTSC73 *OSMR*-KD were subjected to PLA analysis using antibodies targeting CLIC1/OSMR, and CLIC1/EGFRvIII. Each red punctum represents a PLA signal. Primary antibodies were omitted for control group. Nuclei were stained with DAPI. Scale bar = 10 μm. **b** BTSC73 cells were subjected to *OSMR* knockdown using siRNA, and the protein expression of CLIC1 was assessed by immunoblotting with an anti-CLIC1 antibody. ACTIN was used as a loading control. **c** BTSC73 and **d** BTSC147 cells, along with their corresponding *CLIC1* CRISPR lines, were subjected to Western blotting using anti-CLIC1 and anti-OSMR antibodies. ACTIN was used as a loading control. **e** Brain tissues from the BTSC73 WT and *CLIC1* CRISPR groups were immunostained with an anti-OSMR antibody to assess OSMR localization and expression levels. Nuclei were counterstained with DAPI. Scale bar = 200 μm. Inset scale bar = 50 μm. **f** *EgfrvIII* expressing $Stat3^{loxp/loxp}$, $Stat3^{-/-}$ and murine stem cell virus (MSCV)-CTL (non-*EgfrvIII* expressing) $Stat3^{loxp/loxp}$, $Stat3^{-/-}$ astrocytes[14], were treated with 10 ng/mL OSM for 5 min and subjected to immunoblotting using antibodies against EGFRvIII and CLIC1. ACTIN was used as a loading control.

Figure S6.



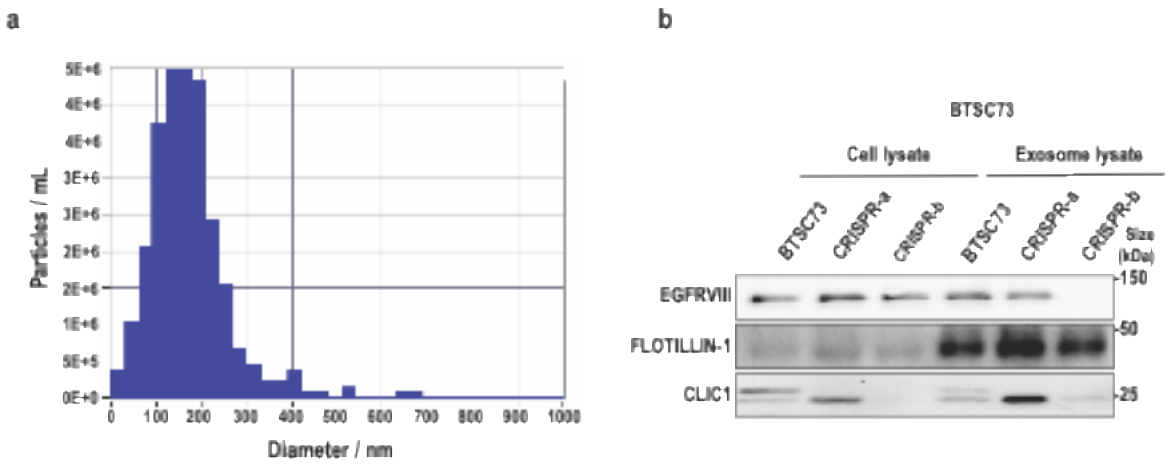
Supplementary Fig. 6 Lipid raft disruption reduces the proximity of OSMR and CLIC1 in BTSCs. **a** BTSC73 and **b** BTSC147 cells were subjected to lipid raft disruption using 5 mM Methyl-β-cyclodextrin (MβCD) for 1 h at 37°C. Subsequently, a PLA was performed using anti-CLIC1 and anti-OSMR antibodies. Each red punctum indicates a PLA signal. Primary antibodies were omitted for the control group. Cell nuclei were stained with DAPI. Scale bar = 10 μm.

Figure S7.



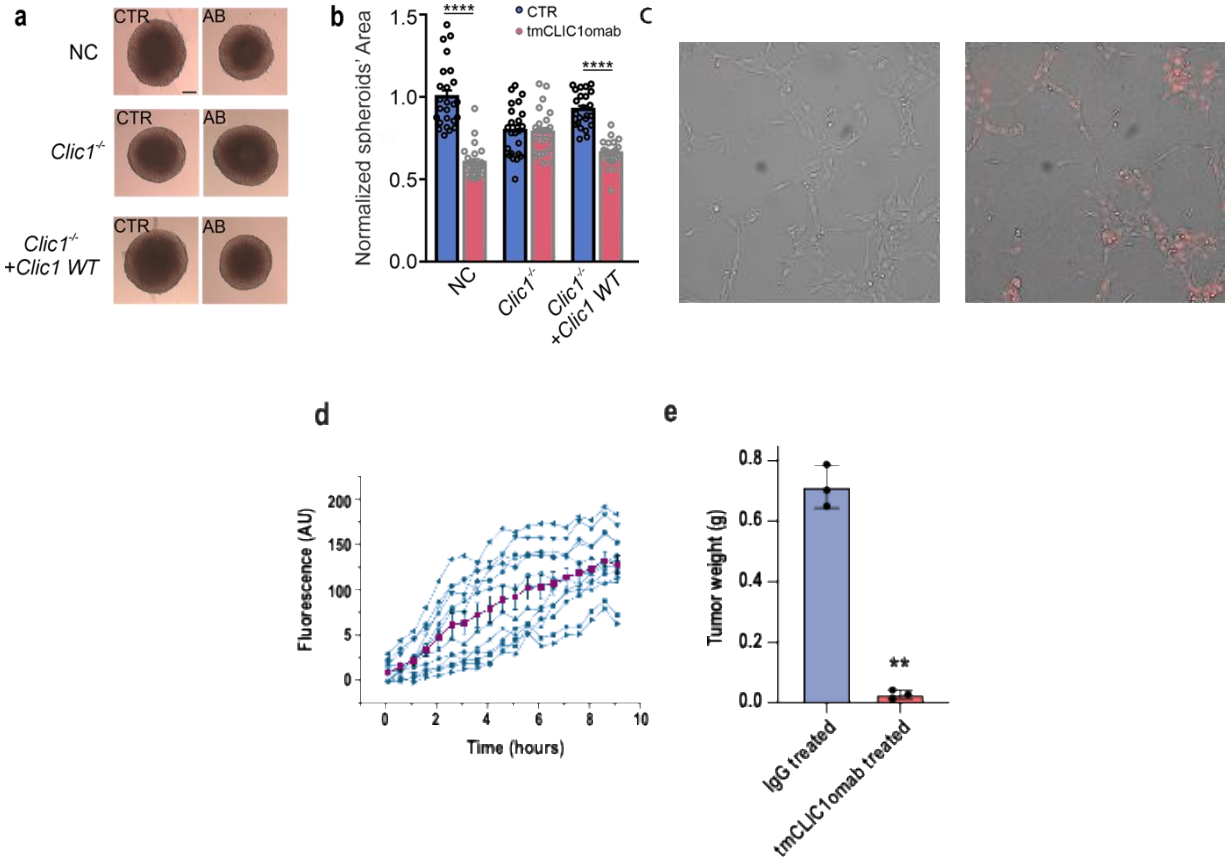
Supplementary Fig. 7 Subcellular localization of CLIC1 in BTSCs. **a** BTSC73 and **b** BTSC147 were subjected to subcellular fractionation and cytoplasmic (C), and plasma membrane (PM), fractions were analyzed by immunoblotting using the indicated antibodies. Whole cell lysates were loaded on the same gel. Both high exposure and low exposure images are shown for CLIC1 levels. Na^+/K^+ ATPase and GAPDH were used as PM and cytoplasmic markers, respectively.

Figure S8.



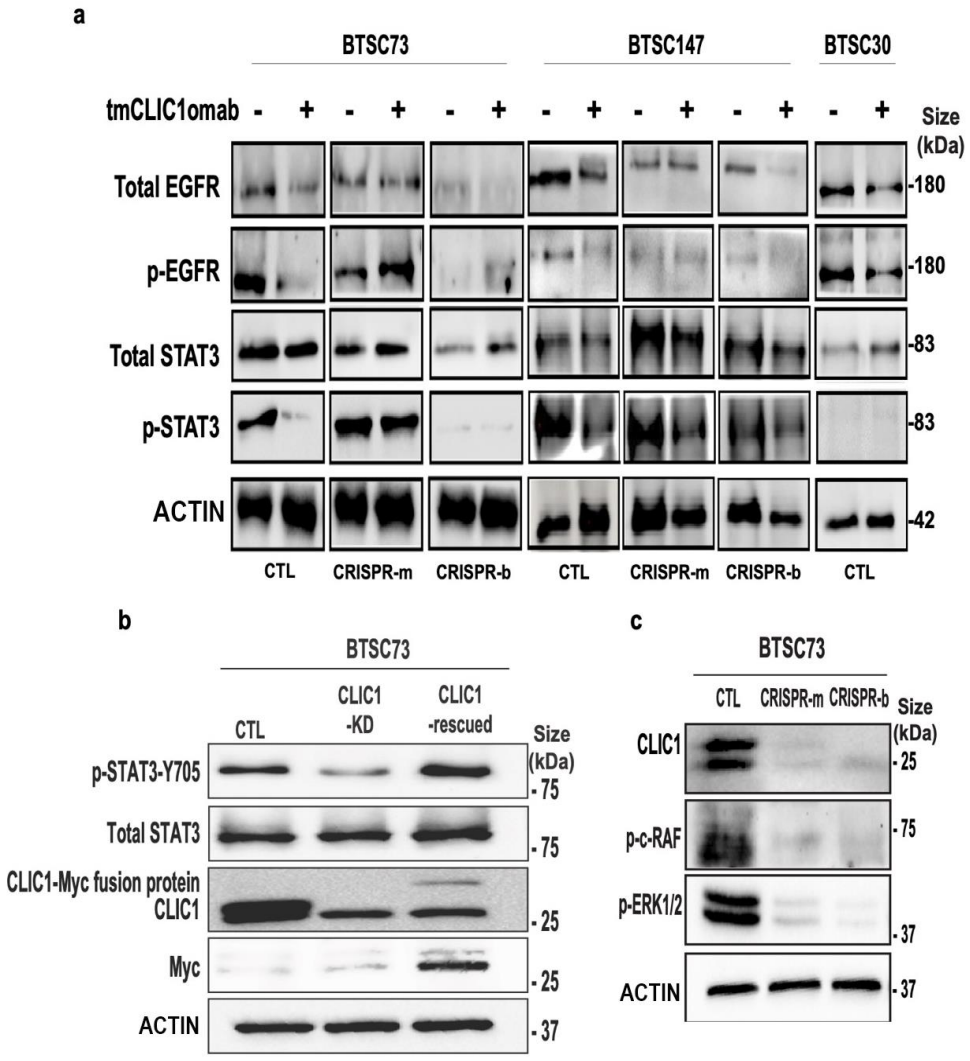
Supplementary Fig. 8 Extracellular vesicle (EV) isolation and characterization. BTSC73 cell supernatants were sequentially centrifuged as described the Materials and methods. **a** Particle size distribution (PSD) for BTSC73 EVs was analyzed in the range of 0–1000 nm using ZetaView. **b** EV and whole-cell lysate fractions from control and *CLIC1* CRISPR BTSC73 cells were analyzed by immunoblotting using the indicated antibodies.

Figure S9.



Supplementary Fig. 9 tmCLIC1omab validation and effectiveness. Human glioblastoma primary cell line 3D culture in presence of tmCLIC1omab is shown. **a** Representative image showing the development of GBM1 3D structure (spheroid) after 72 h of incubation in the absence or presence of tmCLIC1omab, as indicated in each panel. **b** Quantification of spheroid area in the absence (black circles) or presence (grey circles) of tmCLIC1omab. NC: CT, n = 25; tmCLIC1omab n = 23; ****$P < 0.0001$; *CLIC1*$^{-/-}$: CT n = 25; tmCLIC1omab n = 23; *CLIC1*$^{-/-}$ + *CLIC1* WT: CT, n = 23; tmCLIC1omab n = 23; ***$P < 0.0001$. Data are presented as the mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Scale bar 100 µm. **c** Representative image of control cells or cells incubated with tmCLIC1omab labeled with pHrodo red dye. **d** Quantification of fluorescence deriving from labeled particles with tmCLIC1omab and the dye. The average level of fluorescence is represented by purple line. Blue points represent the single well. **e**, Tumor weight comparisons between IgG and tmCLIC1omab-treated groups are presented, n = 3; **$P = 0.0025$.

Figure S10.



Supplementary Fig. 10 Inhibition of CLIC1 attenuates the phosphorylation of STAT3, EGFR, and ERK. **a** BTSC73, BTSC147, and BTSC30, along with their corresponding *CLIC1* CRISPR lines, were treated with the tmCLIC1omab or IgG control. Whole cell lysates were subjected to immunoblotting analysis using antibodies indicated on the blots. ACTIN was used as a loading control. **b** *CLIC1* rescue in BTSC73 cells was achieved using a Myc-tagged plasmid. pSTAT3 phosphorylation/total STAT3 ratio was assessed using Western blotting. **c** Western blot analysis of BTSC73 cells and their *CLIC1* CRISPR counterparts revealed a significant decrease in ERK1/2 and c-RAF phosphorylation levels in the *CLIC1* CRISPR-m and -b cells, indicating disruption of the MAPK/ERK signaling pathway.

Table S1.

Supplementary Table 1 High-confidence binding partners identified as unique to OSMR or shared between OSMR and EGFRvIII, independent of the cellular genetic background, were selected for follow-up analysis. The predicted cellular localization for each candidate is indicated.

#	Common candidate binding partners	Detected by MaMTH	Novel MaMTH PPI	localization
1	Argonaute 3 (AGO3)	high conf	y	cytoplasm
2	B-cell receptor-associated protein 31 (BCAP31)	high conf	y	plasma membrane
3	BCL2 Like 14 (BCL2L14)	high conf	y	cytoplasm
4	Chromosome 8 open reading frame 44 (C8orf44)	high conf	y	nucleus
5	C-type lectin domain family 2 member B (CLEC2B)	high conf	y	plasma membrane
6	Chloride Intracellular Channel 1 (CLIC1)	high conf	y	plasma membrane
7	Cyclic AMP-responsive element-binding protein 3 (CREB3)	high conf	y	Golgi apparatus
8	Chondroitin sulfate N-acetylgalactosaminyltransferase 2 (CSGALNACT2)	high conf	y	Golgi apparatus
9	Chromosome X open reading frame 1 (CXorf1)	high conf	y	cytoplasm
10	Decorin (DCN)	high conf	y	Golgi apparatus
11	Guanylate cyclase (GC)	high conf	y	cytoplasm
12	Glutaredoxin (GLRX)	high conf	y	nucleus
13	GC-rich promoter binding protein 1 (GPBP1)	high conf	y	plasma membrane
14	Histone H3, clustered gene 1 (H3C1)	high conf	y	nucleus
15	Inner Mitochondrial Membrane Peptidase Subunit 2 (IMMP2L)	high conf	y	mitochondrion
16	Leucine Rich Repeats and IQ Motif Containing 1 (LRRIQ1)	high conf	y	Cytoplasm and plasma membrane
17	Microtubule-Associated Protein 4 (MAP4)	high conf	y	plasma membrane
18	N-acetyltransferase 8 (NAT8)	high conf	y	endoplasmic reticulum
19	Nurim (NRM)	high conf	y	nucleus
20	phosphatidylinositol glycan anchor biosynthesis class K (PIGK)	high conf	y	endoplasmic reticulum
21	Psoriasis susceptibility 1 candidate 1 (PSORS1C1)	high conf	y	
22	Phosphatidylserine Synthase 1 (PTDSS1)	high conf	y	endoplasmic reticulum
23	Ras-related protein Rab-1B (RAB1B)	high conf	y	Golgi apparatus
24	SET domain-containing 7 histone lysine methyltransferase (SETD7)	high conf	y	nucleus
25	Solute Carrier Family 35 Member B3 (SLC35B3)	high conf	y	Golgi apparatus
26	Small Integral Membrane Protein 21 (SMIM21)	high conf	y	plasma membrane
27	ST3 beta-galactoside alpha-2,3-sialyltransferase 4 (ST3GAL4)	high conf	y	Golgi apparatus
28	TRAF family member associated NFKB activator (TANK)	high conf	y	cytoplasm
29	Transmembrane protease, serine 6 (TMPRSS6)	high conf	y	plasma membrane
30	Zona Pellucida Glycoprotein 1 (ZP1)	high conf	y	plasma membrane

Table S2.

Supplementary Table 2 Four different patient-derived BTSC lines were employed in this study. The gender and age of the glioblastoma patients, along with the characterization of key genetic mutations are shown.

Cell line	Sex	Age	*EGFR*	*TP53*	*PTEN*	*IDH1*	*NF1*	*CDK2A*
BTSC12	M	59Y	wt	mut	mut	wt	NA	NA
BTSC30	M	67Y	wt	wt	mut	wt	NA	NA
BTSC73	M	52Y	vIII	mut	mut	wt	NA	homo del
BTSC147	M	55Y	vIII	mut	mut	wt	wt	homo del

M, male; **wt**, wild type; **mut**, mutant; **vIII**, EGFR variant III; **homo del**, homozygous deletion; **NA**, not available.

Table S3.

Supplementary Table 3 Pools of siRNAs were designed to knockdown the genes encoding the common high-confidence binding partners of OSMR. The sequences for the sense and antisense siRNA strands for each target gene are provided.

Target Gene Name	Sense siRNA Sequence	Antisense siRNA Sequence
B-cell receptor-associated protein 31	UGGUGACUCUCAUUUCGCAtt	UGCGAAAUGAGAGUCACCAgg
B-cell receptor-associated protein 31	GCAAGUUGGAUGUCGGGAAtt	UUCCCGACAUCCAACUUGCct
B-cell receptor-associated protein 31	GCACUAAGCAAAAACUAGAtt	UCUAGUUUUUGCUUAGUGCtg
BCL2-like 14 (apoptosis facilitator)	CCGAGUAGCUGAAAUUGUUtt	AACAAUUUCAGCUACUCGGtt
BCL2-like 14 (apoptosis facilitator)	CCAUAGAAUUCAAAAUCCUtt	AGGAUUUUGAAUUCUAUGGtg
BCL2-like 14 (apoptosis facilitator)	AAGAGAUUUUUGUAACUGAtt	UCAGUUACAAAAAUCUCUUtg
small integral membrane protein 21	GCAUAUUUCGAAACUUUCUtt	AGAAAGUUUCGAAAUAUGCtg
small integral membrane protein 21	GCUGGGAACAUUUAAACAAtt	UUGUUUAAAUGUUCCCAGCtg
small integral membrane protein 21	CACCAUAUUCGUUUCUUCAtt	UGAAGAAACGAAUAUGGUGtt
chromosome 8 open reading frame 44	CAGAUGUACCGAGUUUGAAtt	UUCAAACUCGGUACAUCUGgt
chromosome 8 open reading frame 44	CCGAGUUUGAAAUCCCAGAtt	UCUGGGAUUUCAAACUCGGta
chromosome 8 open reading frame 44	GUAUUAGAAUUAUUUCUGAtt	UCAGAAAUAAUUCUAAUACct
C-type lectin domain family 2, member B	GAAUUUUCUUAGGCGGUAUtt	AUACCGCCUAAGAAAAUUCat
C-type lectin domain family 2, member B	AUACAACUGUUCCACUCAAtt	UUGAGUGGAACAGUUGUAUtt
C-type lectin domain family 2, member B	CAGCAACAGCUAGAUGUUAtt	UAACAUCUAGCUGUUGCUGca
chloride intracellular channel 1	GGUUUUAGACAAUUACUUAtt	UAAGUAAUUGUCUAAAACCtt
chloride intracellular channel 1	CAGCUGGGCUGGACAUAUUtt	AAUAUGUCCAGCCCAGCUGtg
chloride intracellular channel 1	GUUUUAGACAAUUACUUAAtt	UUAAGUAAUUGUCUAAAACct
cAMP responsive element binding protein 3	CUGUCUCUAUGGAUCUAGAtt	UCUAGAUCCAUAGAGACAGtt
cAMP responsive element binding protein 3	GGCUAGUACUGACAGAUGAtt	UCAUCUGUCAGUACUAGCCta
cAMP responsive element binding protein 3	GAGUGAGAGCUGUAGAAAAtt	UUUUCUACAGCUCUCACUCtc
chondroitin sulfate N-acetylgalactosaminyltransferase 2	GGUCAUUAAUAAUCCUGAUtt	AUCAGGAUUAUUAAUGACCtc
chondroitin sulfate N-acetylgalactosaminyltransferase 2	CUAGUGAUCUUUUAGAGUUtt	AACUCUAAAAGAUCACUAGgt
chondroitin sulfate N-acetylgalactosaminyltransferase 2	GGACCUCUCAUGAAAGUGAtt	UCACUUUCAUGAGAGGUCCaa
SLIT and NTRK-like family, member 2	CCAGUAGCCUAUUACCGAAtt	UUCGGUAAUAGGCUACUGGgt
SLIT and NTRK-like family, member 2	GGAACCGUCUUGUCAAUGAtt	UCAUUGACAAGACGGUUCCat
SLIT and NTRK-like family, member 2	GACUGUAUCCAAACGAAUUtt	AAUUCGUUUGGAUACAGUCtt
decorin	GCUGGACCGUUUCAACAGAtt	UCUGUUGAAACGGUCCAGCcc
decorin	AGAGGCUUAUUUGACUUUAtt	UAAAGUCAAAUAAGCCUCUct
decorin	GCUAGAUACUGGAAACCUAtt	UAGGUUUCCAGUAUCUAGCtt
eukaryotic translation initiation factor 2C, 3	GAAGAGACAUCACACUCGAtt	UCGAGUGUGAUGUCUCUUCtg
eukaryotic translation initiation factor 2C, 3	CAGUCGUCCUUCACACUAUtt	AUAGUGUGAAGGACGACUGgt
eukaryotic translation initiation factor 2C, 3	CCAUAUGAGUUCGAUUUUUtt	AAAAAUCGAACUCAUAUGGgt

group-specific component (vitamin D binding protein)	GCUUAAACAUUUAUCACUUtt	AAGUGAUAAAUGUUUAAGCtg
group-specific component (vitamin D binding protein)	CUACCUGUUUUAAUGCUAAtt	UUAGCAUUAAAACAGGUAGtt
group-specific component (vitamin D binding protein)	GAUCCAAAGGAAUAUGCUAtt	UAGCAUAUUCCUUUGGAUCtt
glutaredoxin (thioltransferase)	CAACCACACUAACGAGAUUtt	AAUCUCGUUAGUGUGGUUGgt
glutaredoxin (thioltransferase)	GCAGUGAUCUAGUCUCUUUtt	AAAGAGACUAGAUCACUGCat
glutaredoxin (thioltransferase)	GAGUCUUUAUUGGUAAAGAtt	UCUUUACCAAUAAAGACUCga
GC-rich promoter binding protein 1	GCAUGGACAGAGAAUCGUUtt	AACGAUUCUCUGUCCAUGCaa
GC-rich promoter binding protein 1	GGAAGUUCCCGUUCUCGUAtt	UACGAGAACGGGAACUUCCac
GC-rich promoter binding protein 1	GAAAGGGAUAUAAACCGAAtt	UUCGGUUUAUAUCCCUUUCtt
histone cluster 1, H3a	CUGAACUGCUUAUUCGUAAtt	UUACGAAUAAGCAGUUCAGtg
histone cluster 1, H3a	GUAGGGCUAUUUGAGGACAtt	UGUCCUCAAAUAGCCCUACca
histone cluster 1, H3a	GCAAACAGUUGGCCACUAAtt	UUAGUGGCCAACUGUUUGCgt
IMP2 inner mitochondrial membrane peptidase-like (S. cerevisiae)	GUGGUGACAUUGUAUCAUUtt	AAUGAUACAAUGUCACCACgg
IMP2 inner mitochondrial membrane peptidase-like (S. cerevisiae)	GGGUUGAAGGUGAUCAUCAtt	UGAUGAUCACCUUCAACCCag
IMP2 inner mitochondrial membrane peptidase-like (S. cerevisiae)	AGAGAGUGAUUGCUCUUGAtt	UCAAGAGCAAUCACUCUCUta
leucine-rich repeats and IQ motif containing 1	GGUCUUUGUGAUACACCUAtt	UAGGUGUAUCACAAAGACCtt
leucine-rich repeats and IQ motif containing 1	CAGCUUGACUAAAAUCGUAtt	UACGAUUUUAGUCAAGCUGtt
leucine-rich repeats and IQ motif containing 1	CUACCAUUGUAUACCUAGAtt	UCUAGGUAUACAAUGGUAGgt
N-acetyltransferase 8 (GCN5-related, putative)	CAGUCUUUUUGAUUCCCAUtt	AUGGGAAUCAAAAAGACUGaa
N-acetyltransferase 8 (GCN5-related, putative)	GCUUAUUGUCCAUUCACAUtt	AUGUGAAUGGACAAUAAGCtt
N-acetyltransferase 8 (GCN5-related, putative)	CAACCUUUCACUGCAAUGAtt	UCAUUGCAGUGAAAGGUUGgg
nurim (nuclear envelope membrane protein)	CCUUCUCGUCUUUGACUAUtt	AUAGUCAAAGACGAGAAGGat
nurim (nuclear envelope membrane protein)	CGGGCCCAGCUACAAAGAAtt	UUCUUUGUAGCUGGGCCCGga
nurim (nuclear envelope membrane protein)	GCCUCAAACAGGUAUACUAtt	UAGUAUACCUGUUUGAGGCcc
phosphatidylinositol glycan anchor biosynthesis, class K	GGACAUCGCACUGAUCUUUtt	AAAGAUCAGUGCGAUGUCCag
phosphatidylinositol glycan anchor biosynthesis, class K	GGCUCUAGCUAGUAGUCAAtt	UUGACUACUAGCUAGAGCCat
phosphatidylinositol glycan anchor biosynthesis, class K	CCAACAUAGAACUCGCGGAtt	UCCGCGAGUUCUAUGUUGGta
psoriasis susceptibility 1 candidate 1	AGAAGUAACAUGUCCAAAAtt	UUUUGGACAUGUUACUUCUga
psoriasis susceptibility 1 candidate 1	UCCAAGCAAUGAUAUCCAAtt	UUGGAUAUCAUUGCUUGGAgg
psoriasis susceptibility 1 candidate 1	GCAAUGAUAUCCAAGGAAUtt	AUUCCUUGGAUAUCAUUGCtt
phosphatidylserine synthase 1	GGAUGAUGUGAACUACAAAtt	UUUGUAGUUCACAUCAUCCtt
phosphatidylserine synthase 1	GAGUGUACCUUUUCAUGAUtt	AUCAUGAAAAGGUACACUCca
phosphatidylserine synthase 1	CCAUCGUCAGCCUCAUGUAtt	UACAUGAGGCUGACGAUGGtg
RAB1B, member RAS oncogene family	GCUGAAAUCAAAAAGCGGAtt	UCCGCUUUUUGAUUUCAGCag
RAB1B, member RAS oncogene family	GCACCAGCCUUAACCCUCAtt	UGAGGGUUAAGGCUGGUGCcc
RAB1B, member RAS oncogene family	AGAGCGACCUCACCACCAAtt	UUGGUGGUGAGGUCGCUCUtg

SET domain containing (lysine methyltransferase) 7	GGACCGCACUUUAUGGGAAtt	UUCCCAUAAAGUGCGGUCCtc
SET domain containing (lysine methyltransferase) 7	GCACGUAUGUAGACGGAGAtt	UCUCCGUCUACAUACGUGCcc
SET domain containing (lysine methyltransferase) 7	CCUGGACGAUGACGGAUUAtt	UAAUCCGUCAUCGUCCAGGtg
solute carrier family 35, member B3	CCUACAUGAUAAUAGCUUUtt	AAAGCUAUUAUCAUGUAGGtt
solute carrier family 35, member B3	CUGUAUGAUUUGAUAAACAtt	UGUUUAUCAAAUCAUACAGtg
solute carrier family 35, member B3	GGACCUAUGGUUAUGCGUUtt	AACGCAUAACCAUAGGUCCga
ST3 beta-galactoside alpha-2,3-sialyltransferase 4	GCAGACCAUUCACUACUAUtt	AUAGUAGUGAAUGGUCUGCtt
ST3 beta-galactoside alpha-2,3-sialyltransferase 4	UCUGGGAUGUCAAUCCUAAtt	UUAGGAUUGACAUCCCAGAtg
ST3 beta-galactoside alpha-2,3-sialyltransferase 4	GGAAGACAGUUUUUAUUUUtt	AAAAUAAAAACUGUCUUCCcg
TRAF family member-associated NFKB activator	CACUCAAGAUAACAAUUAUtt	AUAAUUGUUAUCUUGAGUGga
TRAF family member-associated NFKB activator	GAACUAUGAGCAGAGAAUAtt	UAUUCUCUGCUCAUAGUUCtc
TRAF family member-associated NFKB activator	GAGAUUCUGCAGUAAAAGAtt	UCUUUUACUGCAGAAUCUCta
transmembrane protease, serine 6	GGAACUUACUACAACUCCAtt	UGGAGUUGUAGUAAGUUCCca
transmembrane protease, serine 6	GCCUGUGAAGUGAACCUGAtt	UCAGGUUCACUUCACAGGCct
transmembrane protease, serine 6	GCUGACCGCUGGGUGAUAAtt	UUAUCACCCAGCGGUCAGCga
zona pellucida glycoprotein 1 (sperm receptor)	CAGUCUUCUCGGCCGAUUAtt	UAAUCGGCCGAGAAGACUGca
zona pellucida glycoprotein 1 (sperm receptor)	ACACUGGGAUGUGAACAAAtt	UUUGUUCACAUCCCAGUGUtc
zona pellucida glycoprotein 1 (sperm receptor)	CACCCACUGUGGAACCACAtt	UGUGGUUCCACAGUGGGUGag

Table S4.

Supplementary Table 4 Two different gRNAs were employed in this study to generate monoallelic and biallelic *CLIC1* deletions in BTSC73 and BTSC147 cells. The sequences for the gRNAs and the primers employed to screen each clone are listed.

gRNA1-*CLIC1*-Fwd	caccGTCAACGGTGGTAACATTGA
gRNA1-*CLIC1*-RC	aaacTCAATGTTACCACCGTTGAC
gRNA2-*CLIC1*-Fwd	caccGTACCGATGCACTCCCCGGA
gRNA2-*CLIC1*-RC	aaacTCCGGGGAGTGCATCGGTAC
CLIC1 Internal-Fwd	TAGCTGAGGTTCCTCCCAGG
CLIC1 Internal-Rev	TATTCCTCCCAGGACCCAGG
CLIC1 External-Fwd	AGGGACTGGCCTAGGGATG
CLIC1 External-Rev	AAAATGGAGGGGGTTGAGGG

Table S5.

Supplementary Table 5 Primer sequences for *OSMR*, *CLIC1*, and the housekeeping genes *GUSB* and *ACTB* are provided.

Gene	**Forward Primer Sequence**	**Reverse Primer sequence**
GUSB	GCGTTCCTTTTGCGAGGAGA	GGTGGTATCAGTCTTGCTCAA
ACTB	CAGCAGATGTGGATCAGCAAG	GCATTTGCGGTGGACGAT
OSMR	ACTGGAACCTGCCACAGAGT	TCCAAGCTCACAATTCTCCA
CLIC1	ACCGCAGGTCGAATTCTTC	ACGGTGGTAACATTGAAGGTG

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