Supplementary Methods

Lentiviral particle generation

Short hairpins specific for human CLIC1 (5'-GATGATGAGGAGATCGAGCTC-3') and for firefly luciferase (5'-CGTACGCGGAATACTTCGA-3') mRNA were cloned into XhoI/HpaI sites of PLentiLox 3.7 lentiviral vector. PLentiLox 3.7 and packaging plasmids (vpMDLg/pRRE, pRSV-REV and pMD2G) were amplified in the E.Coli-strain Top10, purified using a QUIAGEN MAXI KIT (Quiagen, Valencia, CA), and transfected in human HEK 293T cell line by calcium phosphate according to established procedures (TronoLab). After overnight transfection, the culture medium was replaced with DMEM supplemented with 10% FBS. Viral particles were collected 48h post transfection and concentrated using PEG-it (Mountain View, CA). Transducing unit (TU) concentration was then determined by Green Fluorescent Protein (GFP) expression. Single cell suspensions derived from GBM neurospheres were infected with 10^4 TU/□l. 72 hours after infection, transduced cells were selected with 1.5 μg/ml puromycine (Sigma-Aldrich, St. Louis, MO). Interference efficiency was evaluated 72 hours post selection by western immunoblot analysis.

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

Total RNAs from cell samples was isolated by RNAeasy Mini kit (Quiagen, Valencia, CA). Total RNAs from normal brain tissues (n=20) and astrocytic tumors of different grades (n=13 WHO grade II, n=28 WHO grade III and n=20 WHO Grade IV) were a generous gift of Maria Stella Carro (University of Freiburg, Germany). RNAs from each sample (1μg) were retrotranscribed using ImProm-II Reverse

Transcriptase (Promega, Madison, WI) at the following temperature steps: 25°C for 5', 42°C for 60', 70°C for 10'. Quantitative real time PCR (qRT-PCR) analysis was then performed by 7,500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) with Syber Green PCR Master Mix (Applied Biosystems, Foster City, CA). Threshold cycle (CT) values for each gene were normalized to TATA-Box Binding Protein expression levels (TBP) for cell samples and to Hypoxanthine PhosphoRibosylTransferase expression levels (HPRT1) for FFPE samples. The sequence of primers was the following: CLIC1 fw: 5'-GTTGACACCAAAAGGCGG-3', rev: 5'-TCTCCAGATTGTCATTGAGTGC-3';TBP fw:5'-TGCACAGGAGCCAAGAGTGAA-3', rev:5'-CACATCACAGCTCCCCACCA-3' ;HPRT1 fw:5'-TGACCTTGATTTATTTTGCATACC-3',rev:5'-CGAGCAAGACGTTCAGTCCT-3'.

Western Immunoblotting

Neurosphere samples were lysated on ice in 50-100 μl of lysis buffer (50 mM Tris–HCl buffer [pH 8], 10 mM $CaCl_2$, 5mM EGTA [pH 8], 250 mM NaCl , Glycerol 10%, triton-x 100 1%) containing a cocktail of proteinase inhibitors (50 mM NAF , 10 mM NAPP ,10mM NaOrtoV , PMSF [0.1mg/ml], Leupeptin , Apoprotinin). Concentration of protein lysates was assessed by Bradford assay (Biorad, Hercules, CA). Each lysate (10 μg) was loaded onto a SDS-polyacrylamide gel electrophoresis (PAGE) under reducing conditions, and resolved proteins were transferred on to Nitrocellulose Transferring membranes (Protran ®, Indianapolis, IN) of 0.2 μm pore size. After blocking with 5% nonfat dry milk in Tris-Buffered Saline and Tween 20 (TBS-T [50mM Tris, 150mM NaCl, 0.05% Tween 20]), membranes were incubated overnight at 4°C with primary antibodies. Antibody binding was assessed by

horseradish peroxidase (HRP)-conjugated secondary antibody (1:10000, Sigma Aldrich, St. Louis, MO). Immunoreactive bands were detected with ECL western blotting reagents (GE Healthcare Bio-Sciences, Pittsburgh,PA).

Immunofluorescence analysis

Neurospheres were mechanically dissociated until single cell suspension was achieved and let adhere onto Polysine Slides (Thermo Scientific, Waltham, MA) for 40'. Cells were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). Cells were then permeabilized with 0.1% Triton-X for 10' and blocked with a 5% Bovine Seum Albumin (BSA) in PBS for 30'. Primary antibodies were used at room temperature (RT) for 60'. Fluorescein isothiocyanate (FITC)-conjugated or cyanine dye (Cy3)-coniugated secondary antibodies were applied at RT for 60'. Nuclei were counterstained with DAPI (1:5000).

The quantitative comparison between CLIC1 expression and expression of other stem/progenitors and/or differentiated markers was performed independently by two blinded operators.

Immunohystochemistry (IHC)

All sections were counterstained with Mayer's haematoxylin and visualized using a bright field microscope. Tissue slices were incubated overnight at 4°C with the following primary antibodies: CLIC1 (mouse monoclonal, 1:1000, clone 356.1, Santa Cruz Biotechnology, Santa Cruz, CA), anti-Nuclei (mouse monoclonal, 1:1000, clone 3E1.3, Millipore, Billerica, MA), GFP (rabbit polyclonal, 1:1000, sc8334, Santa Cruz Biotechnology, Santa Cruz, CA)**.**

Electrophysiology

Cells from dissociated normal human progenitor cells (NPC), human GBM neurospheres (hGBM#7 and hGBM#10), normal (mNSCs) and tumoral murine

neurospheres (GL261) were voltage-clamped using an Axopatch 200 B amplifier (Molecular Devices, Sunnyvale, CA) in the perforated patch configuration. Ionic currents were digitized at 5 kHz and filtered at 1 kHz. The bath solution (mM) contained: 130 NMDG-Cl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose, 5 TEA-Cl (pH=7.4). The pipette solution was (mM): 145 KCl, 1 MgCl2, 10 HEPES (pH=7.25); prior experiments, 2.5 µg/ml Gramicidin (Sigma Aldrich, St. Louis, MO) was added to the solution. Gramicidin is an antibiotics capable to insert into plasma membranes and form pores permeable only to monovalent cations, allowing to gain electrical access to the cell as well as preserve the $[Cl^-]i$ and the cytoplasm content. The voltage protocol consisted of 800 ms pulses from -80 mV to +80 mV (20 mV voltage steps); the holding potential was set according to the resting potential of the cell (between -40 and -80 mV). CLIC1-mediated Cl^- currents were isolated from the other ionic currents in the cells by perfusing a specific inhibitor (Indanyloxyacetic acid 94, IAA94 100 µM) dissolved in the bath solution using a Rapid Solution Changer (RSC-200, BioLogic). Other Cl^- currents were isolated using the aspecific inhibitor DIDS 200 µM (Disodium 4,4′-diisothiocyanatostilbene-2,2′-disulfonate). Offline analysis was performed using Clampfit 9.0 (Molecular Devices), OriginPro 8.5 and Excel routines. IAA94-sensitive (I_{IAA94}) and DIDS-sensitive (I_{DIDS}) currents were estimated by analytical subtraction of ionic currents after addition of either inhibitor from the total current (I_{TOT}) of the cell at each membrane potential tested. Current/Voltage relationships (or I/V curves) were constructed by plotting the averaged ionic current data points in the last 100 ms of the pulse against the corresponding membrane potential. IAA94-sensitive and DIDS-sensitive currents were normalized to the total ionic current of the cell (I_{IAA94}/I_{TOT} and I_{DIDS}/I_{TOT}, respectively); I_{IAA94}/I_{TOT}% and I_{DIDS}/I_{TOT}% from the same cell types were averaged and plotted against the membrane

potential. In the experiments assessing the effect of CLIC1 antibody (mouse monoclonal; Santa Cruz Biotechnology, Santa Cruz, CA) on the ionic currents from hGBM#10 cells, the voltage protocol consisted of 750 ms pulses to +50 mV every 5 sec (solutions, configuration and holding potential as above). We monitored the ionic current during perfusion of bath solution, with CLIC1 antibody (5 μg/ml) or IAA94 (100 μM). As negative control, we treated cells with a mouse isotype antibody (IgG) (5 μg/ml, Sigma Aldrich, St. Louis, MO). The Current/Time relationship was constructed by plotting the average ionic current data points in the last 100 ms of the pulse against the corresponding time. Each experiment was normalized to the ionic current in control condition (bath solution). We used the last 10 points of the three different experimental conditions (untreated, antibody and IAA94) to perform the statistical analysis.

MTT Assay

GBM neurospheres were mechanically dissociated and seeded in 96-well plates at the density of 3000 cells per well. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, 50mg/ml) was added and, after incubation for 4 hours, crystals were dissolved in DMSO. Cell viability was evaluated by CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI). Three independent replicates were considered for each experiment.

Flow Cytometry

GBM neurospheres were mechanically dissociated and fixed in 70% ethanol for 3 h at 4°C. Cells were then stained with Propidium Iodide (PI, 50 mg/ml) containing RNase (250 μg/ml) and fluorescence was measured using FACS Vantage SE FACSCantoII flow cytometer (BD Biosciences, Franklin Lakes, NJ). The instrument was equipped with a bandpass 530/30-nm optical filter for GFP

fluorescence detection and a 670-nm longpass optical filter for the PI fluorescence detection. Cells doublets were discriminated by pulsed processor analysis, according to the comparison of peak and area electronic signals from the PI fluorescence. At least 20,000 events were acquired, and analysis was performed using DIVA 6.1.1 software (Becton Dickinson) and ModFit LT 3.1 software (Verity Software House, Topsham, ME).

Antibody Treatment of GBM-derived neurospheres

GBM derived neurospheres were mechanically dissociated and single cells were seeded in 96-wells at the density of 10^4 cells/cm^2. Growing concentrations (1-5-10 μg/ml) of CLIC1 antibody (mouse monoclonal clone 356.1, Santa Cruz Biotechnology, Santa Cruz, CA) were added to each well; same concentrations of a isotype control antibody (IgG_1) were used as control. After 72 h incubation, cell viability was assessed by MTT analysis (see "MTT assay" paragraph).