SUPPLEMENTARY METHODS

Culture condition. NSCs as well as glioma-derived primary cultures were cultivated in undifferentiated conditions on Matrigel-coated flasks in 1:1 DMEM:F12 medium (Irvine Scientific, Santa Ana, CA, USA), containing 1% penicillin/streptomycin (Gibco/Invitrogen, Grand Island, NY, USA), 10% BIT9500 (Stem Cell Technologies, Vancouver, BC, Canada), 292 µg/ml L-glutamine (Irvine Scientific), 40 ng/ml basic Fibroblast Growth Factor (FGF) (PeproTech, Inc., Rocky Hill, NJ, USA), 20 ng/ml Epidermal Growth Factor (EGF) (PeproTech, Inc.), 10 µg/ml Ciprofloxacin (Teva Parenteral Medicines, Inc., Irvine, CA, USA), 10 µg/ml Gentamicin (MP Biomedicals, LLC, Solon, Ohio, USA) and 2.5 µg/ml Amphotericin B (Fisher Scientific, Fair Lawn, NJ, USA). For expansion, one-half of this medium was replaced every other day, and the cultures were passaged when confluent using Non-enzymatic Cell Dissociation Solution (Sigma). All our glioma-derived primary cultures are able to form spheres when grown on non-adherent surfaces. Also, they express glial fibrillary acidic protein (GFAP) when they are grown under conditions favoring glial differentiation and βIII-tubulin when they are grown under conditions favoring neural differentiation, confirming their multipotential nature. All cells were cultured at 37°C in a humidified incubator with 5% CO_2.

In vivo 20S proteasome activity assay

Groups of male cynomolgus monkeys (3.55-7.33 kg individual body weight, 4-5 animals per group) were treated orally either weekly or twice weekly with marizomib (0.6 mg/m^2 per dose) and brain (prefrontal cortex) tissue was harvested 24 hours after the third drug treatment. Brain tissues were processed and samples were evaluated for proteolytic activities using

Proteasome-Glo™ assay kits (Promega). The CT-L, C-L and T-L activities in samples were calculated by subtracting the unspecific background activity (average RLU with proteasome inhibitor added) from the total peptidase activity (average RLU without inhibitor added).

Wound closure assay. U-251 cells were plated in 6-well plates and grew to full confluency. Similar sized wounds were then induced to monolayer cells by scraping a gap using a micropipette tip. After removing cell debris by rinsing with PBS, fresh medium was added and cells started migrating from the edge of the wound and repopulated the gap area. The time required for 'wound closure' was monitored and photographed immediately after wound incision and at indicated time points.

Antibodies for Western blotting

Antibodies used were Cleaved Caspase-3 (Asp175) (#9661, Cell Signaling), cleaved p85 PARP (Y34, NB110-57321, Novus), and β-actin (NB600-501, Novus).

ROS measurements

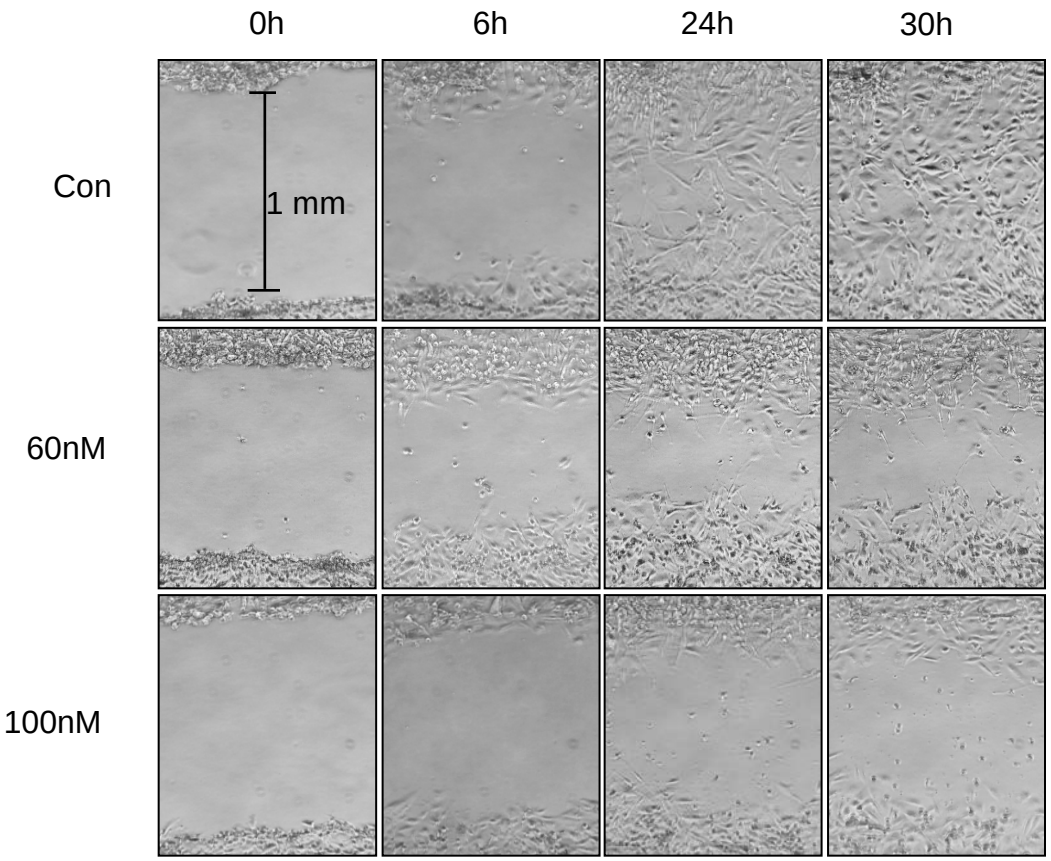
D-54 cells were treated with marizomib at 60 nM for 12 hours in the presence or absence of 10 mM of NAC. The cells were washed with cold PBS, detached and the cell number was determined. The intracellular ROS were measured by using the OxiSelect ROS Assay kit (Cell Biolabs, Inc.). The pellet was resuspended in 400 µl of 1 × 6-carboxy-2',7'-dichlorodihydrofluorescein (H2DCFDA) and incubated for 60 minutes. The samples were then plated on a 96-well plate and the fluorescence was read with a fluorometric plate reader at 480 nm/ 530 nm. The data was reported as RFU and normalized to 10,000 cells. Additionally, an

aliquot of cells was placed on a slide and the image captured using light microscopy and fluorescent microscopy.

Supplementary Table 1. IC50 of primary cultures

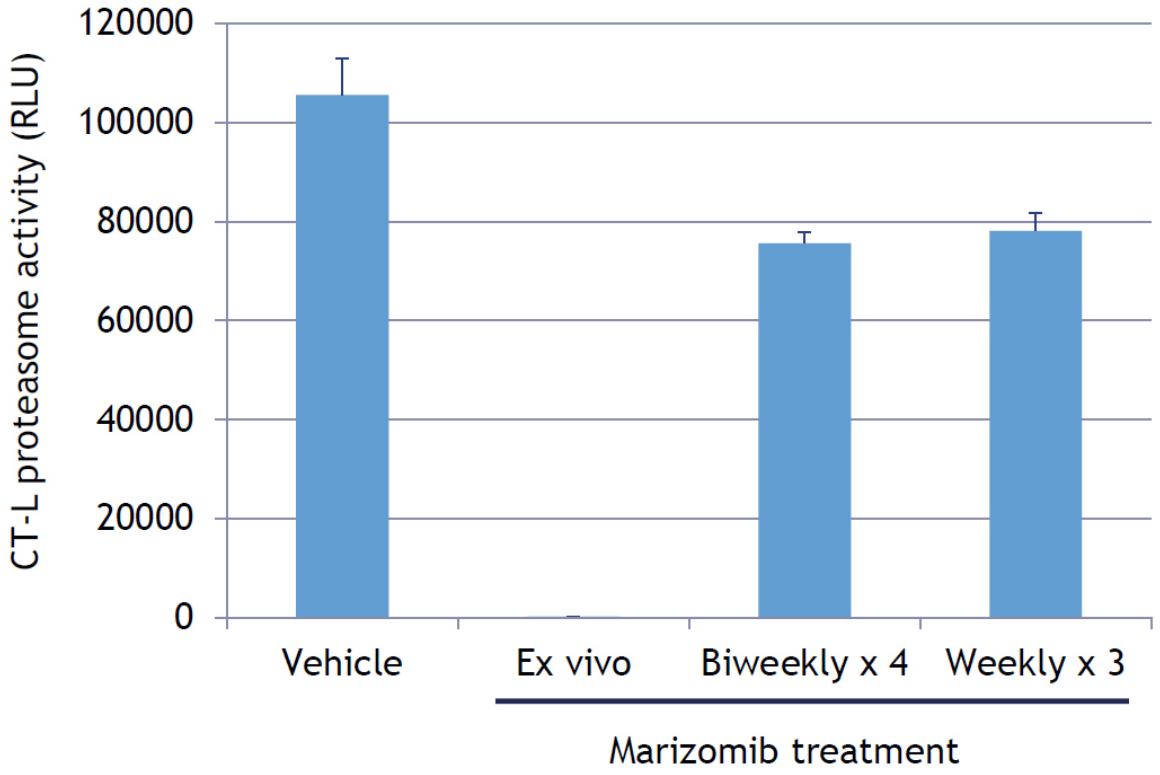
Cell type	IC50 (nM)
NSC-SC23	89.44
HG-GSC-DB17	51.06
HG-GSC-DB26	23.93
HG-GSC-DB32	26.44
HG-GSC-DB33	9.323
HG-GSC-HuTuP01	17.23

Supplementary Fig 1.



Notes: Marizomib inhibits the migration of malignant glioma cells. Wound closure assay was performed using U-251 MG cells with or without 60 nM or 100 nM marizomib treatment. The time required for cell migration was monitored and photographed at indicated time points.

Supplementary Fig 2.



Notes: Cynomologus monkeys (n = 3 to 5) received vehicle or oral marizomib either biweekly (0.55 mg/m^2) on Days 1, 4, 8, and 11 or weekly (0.64 mg/m^2) on Days 1, 8, and 15, and tissues were harvested and frozen 24 hours after the final dose. Brain samples were thawed and lysed under mild conditions, and chymotrypsin-like proteasome activity was assayed using fluorogenic substrates. To determine the proportion of the observed enzymatic activity that was proteasomally derived, duplicate samples from vehicle-treated animals were treated ex vivo with an excess of marizomib (1 µM) to ensure full inhibition.