Materials and methods

Patients and biopsies. Human glioma biopsies were obtained from patients (median age 53.8; range, 18-65) hospitalized in the Department of Neurosurgery, Xijing Hospital, Fourth Military Medical University between March 2014 and December 2016, who were diagnosed with the glioblastoma by pathological analysis following surgery. The use of human samples was approved by the Ethics Committee of Xijing Hospital (Xi'an, China) and procedures were performed in accordance with the Declaration of Helsinki (1). Written informed consent was obtained from all patients involved.

Immunofluorescence. Immunofluorescence staining was used to evaluate protein expression within glioma stem-like cells (GSCs). A total of $5x10^4$ cells/well were plated on cover slips and fixed in 4% paraformaldehyde at room temperature for 20 min. After washing with PBS, samples were blocked with PBS containing 1% BSA and 0.3% Triton X-100 for 2 h at room temperature. Subsequently, slides were incubated with one of the primary antibodies (Table SI) diluted in PBS containing 1% BSA and 0.05% Triton X-100 overnight at 4˚C. The following day, samples were incubated with a secondary antibody in PBS for 1 h at 37˚C. Cells were counterstained with Hoechst 33258 (Sigma-Aldrich; Merck KGaA) for 10 min at room temperature. Between each of the steps described above, the slides were washed three times with PBS for 10 min each time. Immunohistochemical staining with the samples was examined by laser scanning confocal microscopy at a magnification of x40 or x80 (FV-1000, Olympus Corporation).

Measurement of antioxidant enzyme levels. Cells were lysed in isolation buffers provided in the respective assay kits for measuring the activity of superoxide dismutase (cat. no. A001-3-2) and catalase (cat. no. A007-1-1) (both form Nanjing Jiancheng Bioengineering Institute), and the levels of each were measured according to the manufacturer's protocol. For each enzyme, total enzyme activity was normalized to the concentration of the respective protein in the cell lysates of the respective control group. In addition, glutathione production by GSCs, including reduced glutathione and oxidized glutathione, was determined using a glutathione assay kit (cat. no. A061-1-2; Nanjing Jiancheng Bioengineering Institute).

References

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C. and Abate D *et al*: Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: A systematic analysis for the global burden of disease study. JAMA Oncol e192996: 2996, 2019.

Figure S1. Characterization of NCH421k cells and primary GSCs obtained from patients Y1 and Y2 (Table SII). Tumor sphere derived from (A) NCH421k cells and (B) primary cells obtained from patients Y1 and Y2. Cells were stained with anti-Nestin and counterstained with Hoechst. (C) NCH421k cells and (D) primary cells obtained from patients Y1 and Y2 cells were stained with anti-CD133 and analyzed by FACS. (D) Positively stained cells from the patients were quantified. GSC, glioma stem-like cell; Ab, antibody; PE, phycoerythrin.

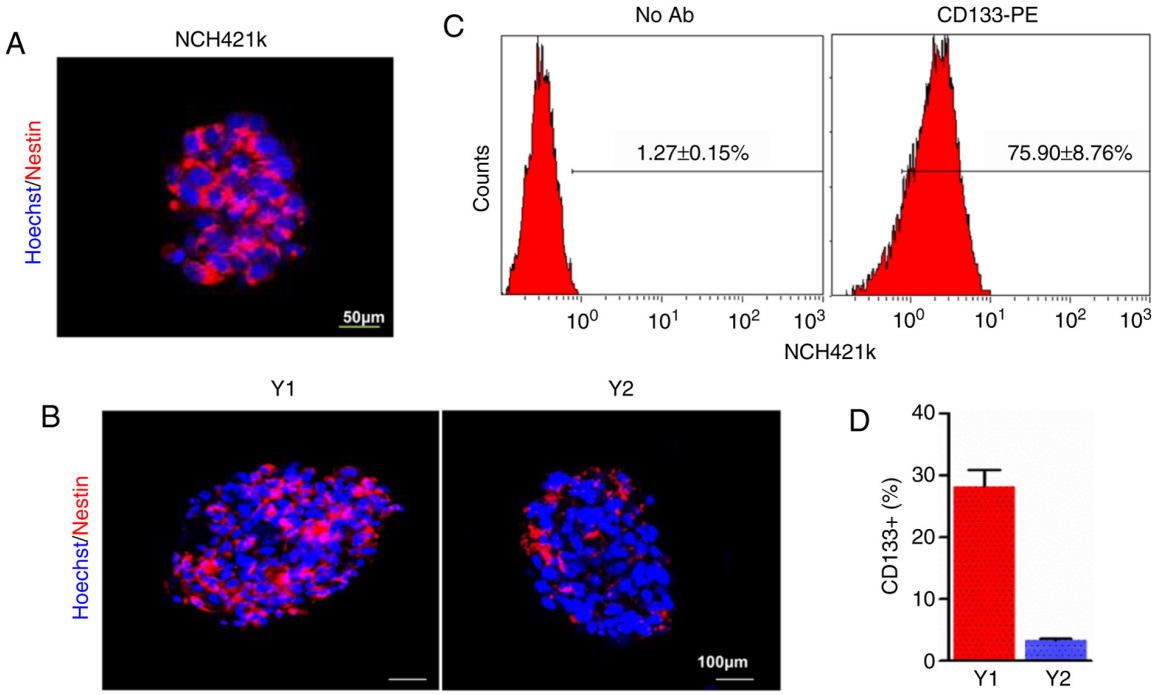

Figure S2. BHB inhibits the growth of primary GSCs. (A-C) Primary GSCs obtained from patients Y1 and Y2 (Table SII) were cultured in Ctrl, G^{low} or 10 mM BHB-G^{low} medium for 7 days. (A) Tumor spheres were imaged and the (B) number and (C) size of tumor spheres were quantitatively compared. Scale bar, 200 μm. (D) Hoechst/Edu^{+} staining and (E) analysis of proliferation of primary GSCs obtained from patient Y1. Scale bar, 200 μm. (F) Schematic of orthotopic implantation. Data are presented as the mean ± standard deviation. *P<0.05, **P<0.01 vs. Ctrl; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. Glow. n.s., not significant; BHB, β-hydroxybutyrate; Ctrl, control medium with 25 mM glucose; G^{low}, medium with 2.5 mM glucose; BHB-G^{low}, G^{low} + BHB; Edu, EdU, 5-ethynyl-2'-deoxyuridine.

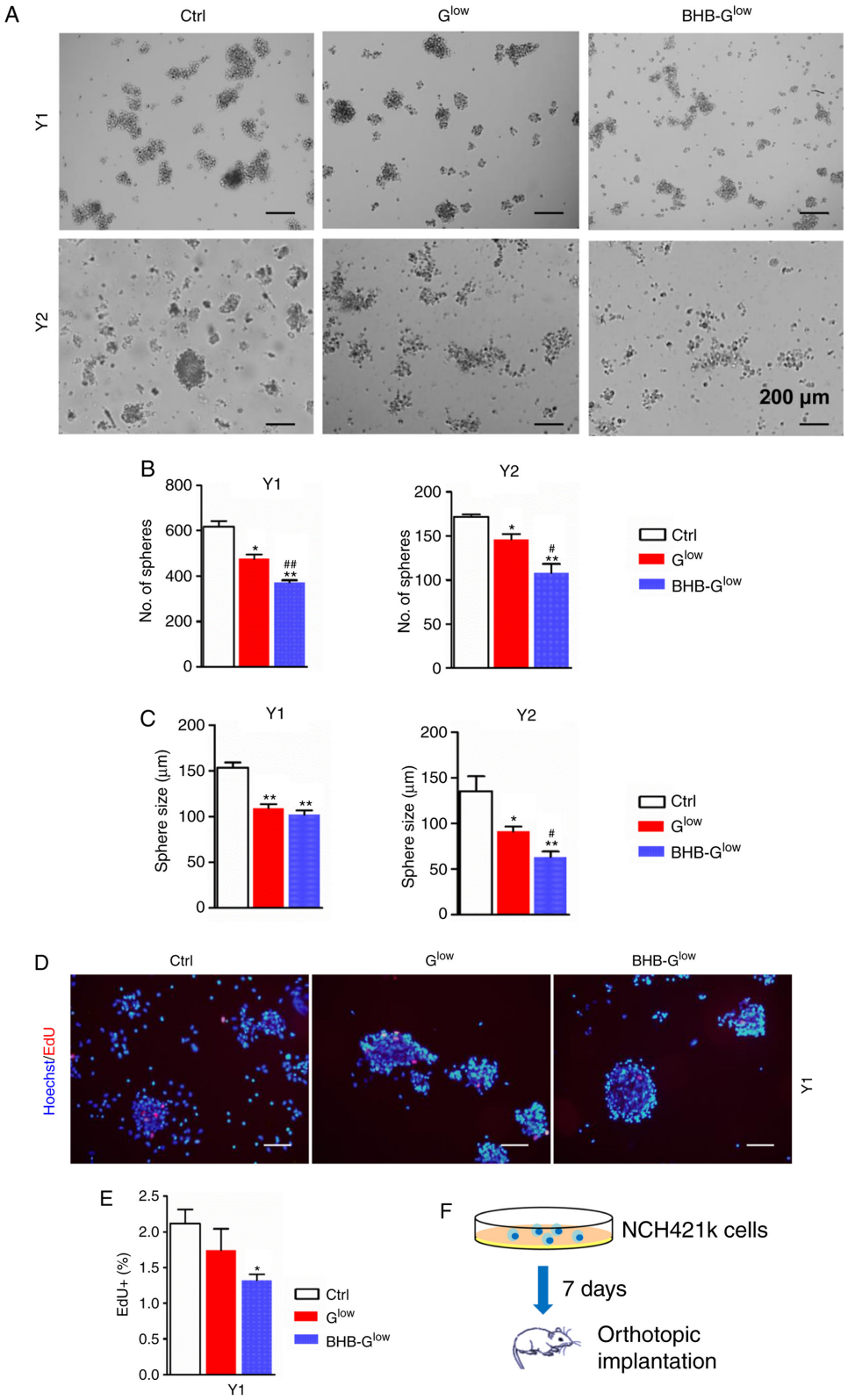

Figure S3. Expression of glycolysis-associated enzymes in glioma tissues from patients. Expression of glycolysis-associated enzymes in glioma tissues and surrounding brain tissues from patients with glioma was determined by (A) western blotting and (B) quantitatively compared. *P<0.05, **P<0.01. (C) NCH421k cells were treated with different concentrations of NAC and tumor sphere formation was quantified. **P<0.01 vs. PBS. Data are presented as the mean ± standard deviation. n.s., not significant; Glut1, glucose transport 1; HK1/2, hexokinase1/2; PK, pyruvate kinase; PKM1/2, M1/2 isoform of pyruvate kinase; PFKP, phosphofructokinase platelet-type; LDHA, lactate dehydrogenase A; PDH, pyruvate dehydrogenase; NAC, N-acetylcysteine.

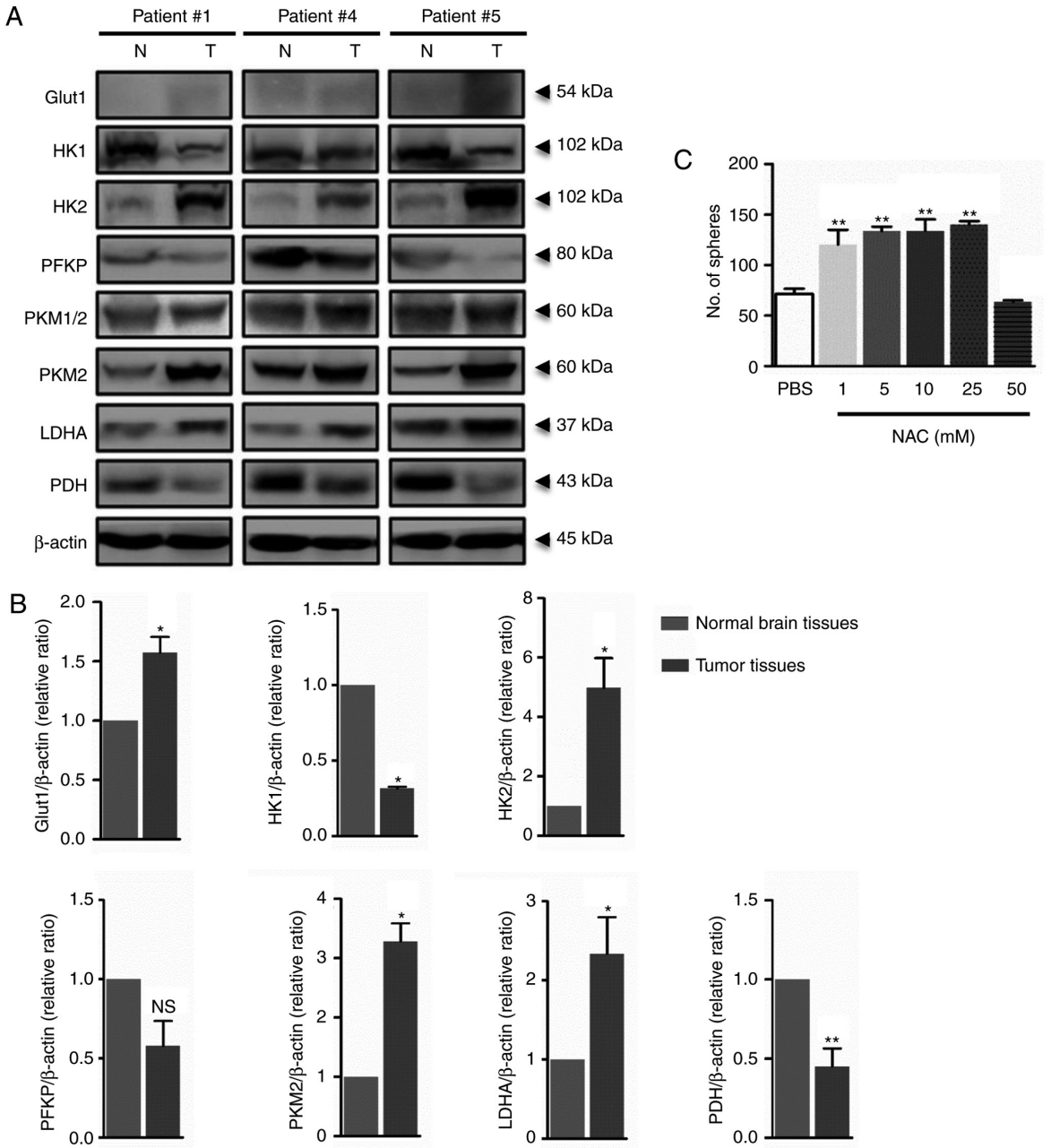

Figure S4. Examination of anti-oxidative signaling pathway. (A) SOD and CAT activity, and GSH and GSSG concentration were determined in NCH421k cultured in Ctrl, G^{low} or 10 mM BHB-G^{low} medium. $^{*}P<0.05$, $^{**}P<0.01$ vs. Ctrl; $^{\#\#}P<0.01$ vs. G^{low}. (B) SOD and CAT activity, and GSH and GSSG concentration were determined after ROS scavenging using NAC. $^{*}P<0.05$, $^{**}P<0.01$. Data are presented as the mean ± standard deviation of 4 repeats. SOD, superoxide dismutase; CAT, catalase; GSH, reduced-glutathione; GSSG, oxidized glutathione; NAC, N-acetylcysteine.

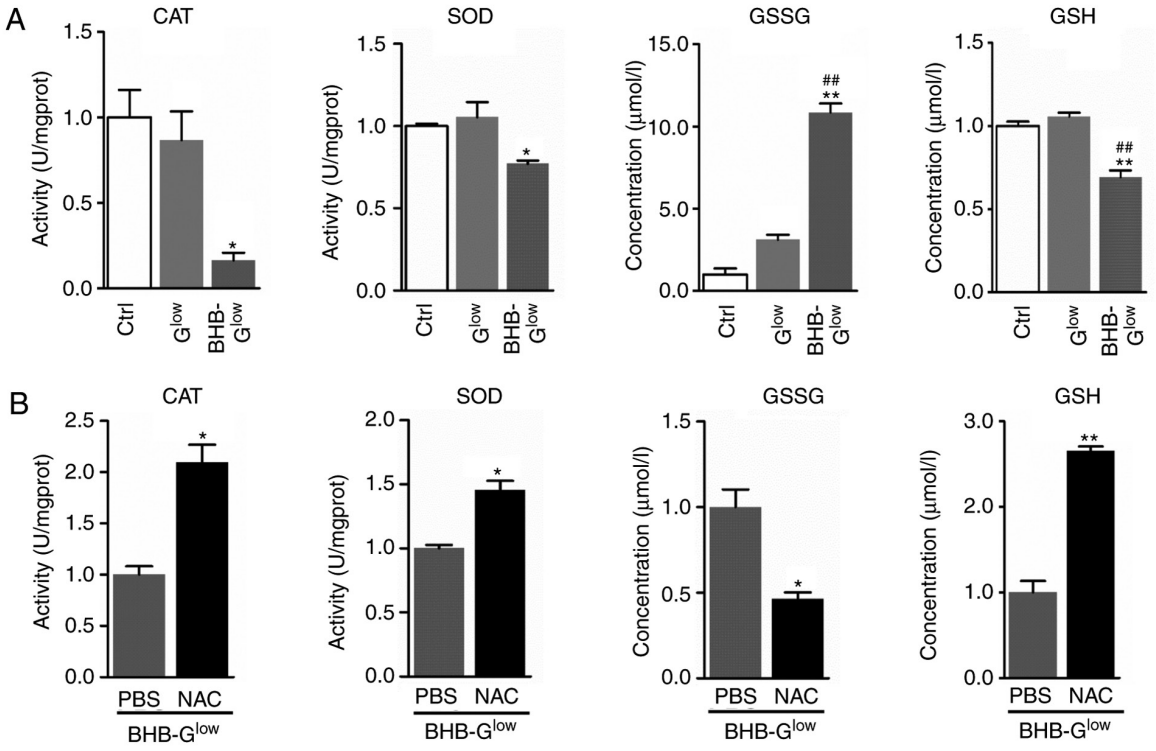

Table SI. Antibodies used in the present study.

Antibody	Supplier	Cat. no.	Dilution	Purpose
Glut1	Abcam	115730	1:2,000	WB
HK1	Cell Signaling Technology, Inc.	2024	1:1,000	WB
HK2	Cell Signaling Technology, Inc.	2867	1:1,000	WB
PFKP	Cell Signaling Technology, Inc.	8164	1:1,000	WB
PKM1/2	Cell Signaling Technology, Inc.	3190	1:1,000	WB
PKM2	Cell Signaling Technology, Inc.	4053	1:1,000	WB
LDHA	Cell Signaling Technology, Inc.	3582	1:1,000	WB
PDH	Cell Signaling Technology, Inc.	3205	1:1,000	WB
β-actin	Cell Signaling Technology, Inc.	3700	1:2,000	WB
Caspase 3	Cell Signaling Technology, Inc.	9962	1:1,000	WB
Sox2	Abcam	137385	1:200	WB
mTOR	Cell Signaling Technology, Inc.	2983	1:1,000	WB
p-mTOR	Cell Signaling Technology, Inc.	2974	1:1,000	WB
Hif-1a	Santa Cruz Biotechnology, Inc.	Sc-10790	1:800	WB
Bcl2	Cell Signaling Technology, Inc.	2872	1:1,000	WB
CD133	Miltenyi Biotec GmbH	130-098-897	1:11	FACS
CD133	Invitrogen; Thermo Fisher Scientific, Inc.	PA5-38014	1:200	IF
Nestin	Santa Cruz Biotechnology, Inc.	Sc-20978	1:200	IF
Goat Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch, Inc.	111-005-144	1:5000	WB
Goat Anti-Mouse IgG (H+L)	Jackson ImmunoResearch, Inc.	115-005-166	1:5000	WB
Alexa Fluor® 680 Goat Anti-Rabbit IgG	Jackson ImmunoResearch, Inc.	111-627-008	1:500	IF

WB, western blotting; IF, immunofluorescence; mTOR, mammalian target of rapamycin; Glut1, glucose transport 1; HK1/2, hexokinase1/2; PKM1/2, M1/2 isoform of pyruvate kinase; PFKP, phosphofructokinase platelet-type; LDHA, lactate dehydrogenase A; PDH, pyruvate dehydrogenase; HIF-1α, hypoxia inducible factor 1α; Bcl2, B cell lymphoma 2; p-, phospho; IgG, immunoglobulin G.

Table SII. Characteristics of the patients with GBM.

Patient	Age, years/sex	Pathology	Tumor location	WHO grade	Primary	Recurrence
1	58/M	GBM	Right side of temporal and parietal lobe	IV	Yes	Yes
4	60/M	GBM	Left frontotemporal lobe	IV	Yes	No
5	55/F	GBM	Left temporal lobe	IV	Yes	No
Y1	46/M	GBM	Left frontal lobe	IV	Yes	No
Y2	50/M	GBM	Right side of parietal lobe	IV	Yes	Np

GBM, glioblastoma multiforme; M, male; F, female; WHO, world health organization.