Supporting Information

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SI Methods

Establishment and Maintenance of Glioma Progenitor Cultures. Whole forebrain tissue was minced and digested with media containing 0.2% collagenase (C-5138; Sigma), 0.01% hyaluronidase (H3884; Sigma), and 0.002% DNaseI (DN25-1G; Sigma) at 37 °C for 30 min. The dissociated brain cells were cultured in neurosphere media, which was neurobasal media (21103-049; Life Technologies) supplemented with 1× B27 without vitamin A (12587-010; Life Technologies), 1× penicillin–streptomycin (PenStrep) (15140-122; Life Technologies), 1× GlutaMax (35050; Life Technologies), 20 ng/mL EGF (11376454001; Roche), 20 ng/mL b-FGF (GF003; Millipore), 0.2% neural survival factor-1 (NSF-1) (CC-4323; Lonza), 16 unit/mL heparin (Sigma), and 5 ng/mL LIF (LIF1010; Millipore). The *p53* status of each of the five lines (three lines of $p53^{+/-}$ and two lines of $p53^{-/-}$) was genotyped by PCR (primers: p53ko-F 5′-ACA GCG TGG TGG TAC CTT AT, p53ko-R 5′-TAT ACT CAG AGC CGG CCT, and p53neo-F 5′- AAT TAA GGG CCA GCT CAT TCC). The cultures were maintained as previously described (1) at 5% CO_2 and 3% O_2 unless otherwise indicated and passaged every 4–7 d by gentle trituration of the spheres through pipets to make a single-cell suspension. Culture appearance changed in a dynamic fashion over time after seeding; cells initially formed adherent monolayers, after which they expanded sequentially to semi-adherent monolayer, attached spheres, and suspended spheres.

Manipulation of IDH1, GLUD1, and GLUD2 in Glioma Progenitor Cultures. Human and mouse isocitrate dehydrogenase 1 (IDH1) cDNAs, human PDGF-BB cDNA, and human glutamate dehydrogenases 1 and 2 (GLUD1 and GLUD2) cDNAs were cloned by RT-PCR. The IDH1 cDNAs were mutated (R132H: CGT to CAT) by PCR-mediated mutagenesis and tagged (myc or Flag) at either 5′ or 3′ of the coding region. Mouse IDH1 shRNA sequences were obtained from The RNAi Consortium, with the DNA barcodes CCTGGGCTTAGAATGAGTCTT (TRCN0000041713), GCTGCTTGCATTAAAGGCTTA (TRCN0000041715), and GCCAAATTAGCTCAGGCCAAA (TRCN0000041717). Coding regions of the genes or DNA fragments containing the shRNA sequences were inserted at NcoI site in a Cal12Nco-derived Nco/ATG adaptor plasmid then cloned at ClaI site of a RCASBP amphotropic vector (from Memorial Sloan Kettering Cancer Center). All of the vectors were constructed using standard cloning procedures, and their sequences were verified at every step. Plasmid DNA of the replication-competent avian sarcoma leukosis virus long terminal repeat with splice acceptor (RCAS) vector expressing the interested gene was transfected by electroporation into chicken DF1 cells [CRL-12203; American Type Culture Collection (ATCC)]. The RCAS virus-producing DF1 cells were cultured in Dulbecco's modified Eagle medium supplemented with 2 mM glutamine and 10% (vol/vol) FBS (F2442; Sigma). DF1 cells at over 90% confluence were then cultured with minimum volumes of neurosphere media for 16–20 h. Supernatants of the culture were filtered and used to infect targeted cells in the presence of polybrene (TR-1003-G; Millipore) at 8 μg/mL. For murine $IDH1^{R132H}$ and IDH1 shRNA constructs, targeted cells were repeatedly infected to achieve desired levels of transgene expression or IDH1 knockdown. All of the three shRNAs resulted in significant IDH1 knockdown in mouse glioma progenitors (82–91% mRNA decrease, as quantified by Taqman). To investigate rescue of deficits induced by $IDH1^{R132H}$ or IDH1 knockdown, cultures stably expressing murine or human $IDH1^{R132H}$ or shRNA to murine IDH1 (as a result of infection with appropriate RCAS vectors) were subsequently infected with RCAS vectors encoding for $IDH1^{WT}$, GLUD1, or GLUD2.

Knockdown of Glutamate Dehydrogenase in Human IDH1 Mutant Glioma Cultures. Human $IDH1^{R132H}$-glioma line BT142 (ATCC SRC-4002) was cultured in NeuroCult NS-A basal media (Stem Cell Technology) supplemented with 1× NeuroCult NS-A proliferation supplement (Stem Cell Technology), 1x PenStrep (15140-122; Life Technologies), 1× MEM Nonessential amino acids (11140-050; Life Technologies), 1 mM sodium pyruvate (11360-070; Life Technologies), 20 nM EGF (11376454001; Roche), 20 nM b-FGF (GF003; Millipore), 100 ng/mL human recombinant PDGF-AA (100-13A; PeproTech), 0.2% NSF-1 (CC-4323; Lonza), 16 units/mL heparin (H3393-1MU; Sigma), and 5 nM LIF (LIF1010; Millipore). Sequencing results confirmed that cells after passages contained homozygous $IDH1^{R132H}$.

BT142 cells were infected at a multiplicity of infection of 10 with lentiviral vectors expressing control shRNA (sc-108080; Santa Cruz Technology) or glutamate dehydrogenase 1/2 shRNAs (Sigma), which were sh589, DNA barcode CCTTCAAATATGAAAGGGATT (TRCN0000028589); sh614, DNA barcode GCTGCCTATGTCAATGCCA (TRCN0000028614); sh620, DNA barcode GCTAGCAAAGAAGGGCTTT (TRCN0000028620); sh647, DNA barcode GCAGAGTTCCAAGACAGTA (TRCN0000028647); and sh662, DNA barcode GCCTACACTCTATGAGATATT (TRCN0000028662). The infected cells were selected in culture in the presence of puromycin at 2 μg/mL. Glutamate dehydrogenase 1/-2 knockdown was verified by Western blot.

Measurement of 2-Hydroxyglutarate. Relative levels of 2-hydroxyglutarate (2-HG) were measured from supernatants of cells used for growth assays. Absolute levels of 2-HG were measured from supernatants and pellets of pooled and clonal cells expressing human or murine $IDH1^{R132H}$. The supernatants were collected 3 d after 2.5×10^5 cells were cultured in 3 mL of media in six-well plates. The uncultured media were used as negative control. The cells were precipitated and the cell numbers were counted for normalization. Samples were extracted with acetonitrile. A Thermo Accela UPLC pump was used to deliver a gradient flow to elute the analytes from the analytical column (Phenomenex Synergi polar-RP) to the mass spectrometer (API 5500 Qtrap; AB Sciex). Selected reaction monitoring transition of 2-HG was 147 → 129. Dwell time was 80 ms per channel with an interchannel delay of 5 ms. Using a standard curve, the concentrations of 2-HG in cell pellets were estimated at 0.11–0.21 mM in cell pellets of control cells and 1.9–16 mM in cells with mouse IDH1 mutation.

Cell Growth Assays. In vitro behavior of uninfected glioma progenitor culture (control) was compared with the same line infected with RCAS vector(s) engineered to express human or murine $IDH1^{R132H}$ (hMUT or mMUT), shRNA to murine IDH1 (KD), human GLUD1 (GLUD1), human GLUD2 (GLUD2), or sequential infections with human or murine $IDH1^{R132H}$ followed by a second vector, human $IDH1^{WT}$ (hMUT+hWT), murine $IDH1^{WT1}$ (mMUT+mWT), GLUD1 (MUT+GLUD1), or GLUD2 (MUT+GLUD2).

For the cloning assay, viable single cells were FACS-sorted at one cell per well into 96-well plates and cultured with 100 μL of neurosphere media under 3% oxygen and 5% CO_2 at 37 °C. To the cultures were added, once a week for up to 4 wk, 25 μL of the fresh media containing 2× of the supplements (B27 without vitamin A, EGF, bFGF, LIF, and NSF-1). Clone formation was

scored by microscopic inspection at 3–4 wk following FACS plating of single cells into standard neurosphere conditions in 3% oxygen and is reported as percentage of cells generating a viable clone.

For the CyQuant assay, viable single cells were FACS-sorted at 1,000 cells per well into 96-well plates and cultured under 3% oxygen and 5% CO_2 at 37 °C for 4 d. Cell numbers, reported as relative fluorescence units, were determined using CyQuant Cell Proliferation Assay Kit (C7026; Life Technologies) according to the manufacturer's directions.

For IncuCyte, viable single cells were FACS-sorted at 1,000 cells per well into 96-well plate, cultured under room air and 5% CO_2 at 37 °C for 7 d, and imaged using the IncuCyte ZOOM system (Essen BioScience). The cultures grew as adherent monolayers for the first 120 h after plating. Phase contrast images were automatically acquired and registered, and percentages of confluence of the cells were averaged by the IncuCyte software system at 2-h intervals from three separate regions per well.

Lipid Synthesis from Glucose, Glutamine, or Glutamate. Lipid synthesized from glucose, glutamine, or glutamate was assessed in glioma progenitor cells grown in 20%, 3%, or 1% oxygen. Total labeled carbons from glucose and glutamine in cells under 3% oxygen are reported in Fig. 3*C* and those from glucose, glutamine, or glutamate in the cells under 20%, 3%, or 1% oxygen in Fig. S4.

For measuring ^{14}C-labeled lipids derived from glucose, glutamine, or glutamate to lipids, cells were seeded in 12-well plates at 10E+5 cells per well, cultured for 2 d under 1% O_2, 3% O_2, or room air in the neurosphere media containing 25 mM glucose and 2 mM glutamine then incubated for 5 h with L-[^{14}C(U)]glutamine (NEC451050UC; PerkinElmer) at final concentration of 4.0 μM, or D-[U-^{14}C]glucose (NEC042V250UC; PerkinElmer) at final concentration of 8.6 μM, or L-[^{14}C(U)]glutamic acid (NEC290E050UC; PerkinElmer) at final concentration of 2.0 μM. Specific activities of all tracers were equivalent. Cells were harvested and resuspended in 100 μL PBS buffer, of which 5 μL were used for determining cell numbers by CyQuant assay and 90 μL for lipid extraction. Total lipids were extracted with methanol/chloroform/HCl. All extractions were performed either in siliconized tubes or in glass tubes. Radioactivities of the extracts were counted using liquid scintillation analyzer (Tri-Carb 2810TR; PerkinElmer). For glutamate, unadjusted cpm are reported. For glucose and glutamine, cpm measured were adjusted to reflect the ratio of tracer to unlabeled metabolite in media.

Analysis for labeled lipids derived from each metabolite is based on cpm per thousand cells analyzed on a logarithmic scale, separately for each oxygen level, with a linear mixed model estimating a mean response for each metabolite + genotype combination, and including assay ID as a random intercept. Total flux was estimated by anti-logging the estimated responses from the mixed model for each combination then adding or subtracting the estimated cpm counts appropriately. SEs were estimated by the method of propagation of errors. Computations were performed in R version 3.0.2, with mixed models (2) and panel plots created using the packages nlme (3) and lattice (4), respectively.

For mass isotopologue distribution analysis using ^{13}C stable isotopes, cells were cultured for 24 h in each well of a six-well plate with 2 mL neurosphere culture media containing 1 mM glutamine and 1 mM [^{13}C-U5]glutamine. One well was used for quantification of the cell numbers; five wells of the cells and the supernatants were harvested and processed for mass spectrometry. Citrate, α-KG, and 2-HG isotopologues were measured by liquid chromatography–triple quadrupole TOF mass spectrometer (TripleTOF 5600; AB Sciex). Cells were washed once with PBS, lysed, and scraped in cold lysis buffer composed of acetonitrile:methanol:H_2O (4.5:4.5:1) on ice, sonicated in a cold water bath for 30 s, then spun at 14,000 × *g* at 4 °C for 10 min. Supernatants were collected for MS analysis. Ten microliters of each sample were injected onto a 3- × 150-mm StableBond column (Zorbax Aq 5 μm; Agilent) and eluted isocratically in water with 0.1% formic acid by volume at a flow rate of 500 μL/min. Data were collected with TOF MS scan function at a 400-ms scan time and 4.5-min run time. Monoisotopic *m/z* with a 10-mDa window was used for data integration of each analyte. Retention time for each analyte was confirmed with purified standards. MultiQuant Software (AB Sciex) was used for data analysis.

Expression Profiling. Three sets of expression profiling data from previously published studies were used to compare $IDH1^{R132H}$ vs. $IDH1^{WT}$ human high-grade glioma. Mean fold change and mean *P* value for three separate comparisons of $IDH1^{R132H}$ vs. $IDH1^{WT}$ tumors are reported. Dataset 1 consisted of AffyU133A&B profiles of primary newly diagnosed glioblastomas (GBMs) (n = 18 $IDH1^{R132H}$ and 63 $IDH1^{WT)}$, and dataset 2 contained Affy U133P data from n = 12 $IDH1^{R132H}$ and 88 $IDH1^{WT}$ primary newly diagnosed GBMs. Dataset 3 contained Affy U133 A&B profiles from n = 16 $IDH1^{R132H}$ and 12 $IDH1^{WT}$ newly diagnosed untreated grade III astrocytomas. To identify genes overexpressed in *IDH1* mutant tumors, AffyMassig5 values for all probesets on the microarray were compared $IDH1^{R132H}$ vs. $IDH1^{WT}$ tumors. All probesets for which mean expression showed twofold or greater difference with a *t* test *P* value less than 1×10^{-4} in all three datasets are reported in Fig. 2*A*.

RNA sequencing (RNAseq) data for GLUD1 and GLUD2 in GBM tumor samples collected by The Cancer Genome Atlas (TCGA) (5) was looked at as a function of IDH1 mutation status. RNAseq data were obtained from the Cancer Genomics Hub at the University of California, Santa Cruz and preprocessed and aligned with HTSeqGenie (6). IDH1 somatic mutation calls obtained from Illumina GA exome-seq data and processed by the Broad Institute were downloaded from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/). Analysis was restricted to 150 GBM tumor samples with both RNAseq and Exome-seq data available, of which 9 were mutant for $IDH1^{R132}$ and 141 WT. Nonparametric statistical analysis was used to compare reads for GLUD1 and GLUD2 in $IDH1^{R132H}$ vs. $IDH1^{WT}$ samples.

Western Blots. Total cell lysates were used for Western blots. Primary antibodies used all recognize both mouse and human proteins and were as follows: rabbit anti-IDH1 polyclonal antibody (3997S; Cell Signaling), mouse anti-human IDHR132H monoclonal antibody (clone H09, Dia-H09; Dianova), mouse anti-GLUD monoclonal antibody (Clone 3C2, H000027746-M01; Abnova), rabbit GLUD polyclonal antibody (LS-B2525; LifeSpan BioSciences), and mouse anti-actin monoclonal antibody (612656; BD Transduction Laboratories). Secondary antibodies were donkey anti-rabbit IgG–HRP (NA934V; GE Healthcare) and sheep anti-mouse IgG–HRP (NA931V; GE Healthcare). ECL (PRN2109; GE Healthcare) was used for protein detection.

In Vivo Glioma Growth. Incidence of glioma formation was tested in immunocompromised mice implanted with *p53* $^{+/-}$ Nestin-tva or *p53* $^{-/-}$ Nestin-tva neural stem cultures engineered to express PDGF-B or human $IDH1^{R132H}$ using RCAS vector. Effects of shRNA to GLUD1/2 was tested in immunocompromised mice implanted with $IDH1^{R132H}$ human glioma line BT142 engineered to express control shRNA (shCtr) or shRNAs to human GLUD1/2 (sh647, sh662). Orthotopic growth of grafts were assessed by T2-weighted MRI 3 mo postimplantation.

Stereotactic implantation of cells into the right striatum was conducted under isoflurane-induced anesthesia using aseptic technique. Body weights were monitored every day during the first week after the surgery and twice per week thereafter. For survival studies, mice were killed when they lost 20% of their body weight or became moribund.

Magnetic Resonance Imaging to Determine Tumor Volumes. The animals were anesthetized using 3% isoflurane in air and placed in a custom-built headholder for motion suppression. During imaging, anesthesia was maintained using 1.5–2% isoflurane in air, keeping the respiration rate at approximate 80 breaths per minute. The rectal temperature was maintained at 36.5–37.5 °C using a feedback system with warm air (SA Instruments). Experiments were performed on a 4.7T horizontal imaging system (Agilent Technologies) using a 30-mm i.d. quadrature volume coil. T2-weighted images were acquired using a 2D fast spin echo experiment with field-of-view 20 × 20 mm^2, 20 coronal slices of 0.75-mm thickness, matrix size 128 × 128 (zero-filled to 256 × 256), repetition time 4,000 ms, echo train length 8, echo spacing 10 ms, effective echo time 40 ms, and 12 averages, resulting in a total scan time of about 13 min.

For determination of tumor volumes, the area of hyperintensity on the T2-weighted images was outlined semiautomatically using the Auto Trace tool in Analyze 11.0 (AnalyzeDirect), with subsequent manual editing to exclude ventricles. A series of three samples per experimental condition were subjected to histological examination to verify that the area of T2 abnormality corresponded closely to the area of brain occupied by tumor cells.

1. Chen R, et al. (2010) A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. *Cancer Cell* 17(4):362–375.
2. R Develoment Core Team (2013) *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna).
3. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2013) *nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-113* (R Foundation for Statistical Computing, Vienna).
4. Sarkar D (2008) *Lattice: Multivariate Data Visualization with R* (Springer, New York).
5. Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455(7216): 1061–1068.
6. Pau G, et al. (2013) *HTSeqGenie: A Software Package to Analyse High-Throughput Sequencing Experiments* (R Foundation for Statistical Computing, Vienna).

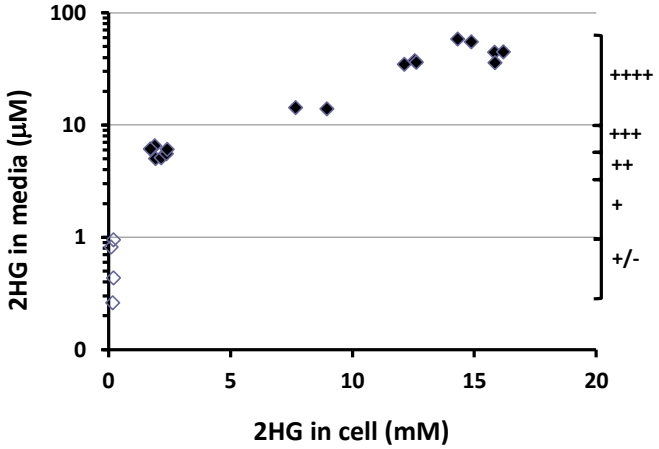

Fig. S1. Correlation of intracellular levels of 2-HG and concentrations of 2-HG in culture supernatant. Relative and absolute amount of 2-HG were measured from pellets of cells with or without *IDH1* mutation and from supernatants of the cultured cells. ♦, *IDH1* mutant cells (a variety of clonal and bulk cultures); ◇, *IDH1* WT cells. +/–, basal levels (<1 μM in the supernatant); +, one- to twofold (1–2 μM); ++, three- to fivefold (3–5 μM); +++, 5- to 10-fold (5–10 μM); ++++, 10- to 50-fold (10–50 μM).

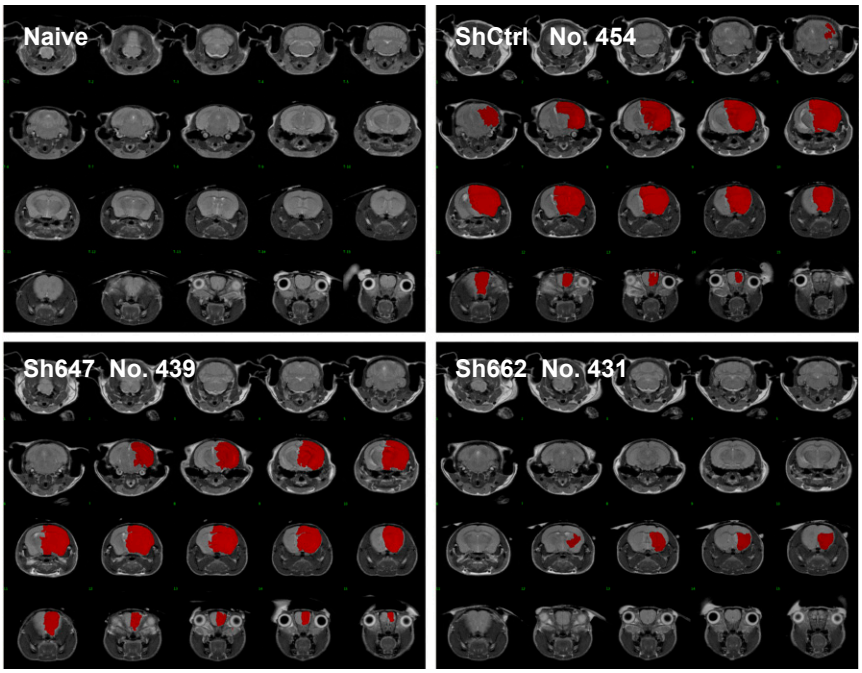

Fig. S2. Knockdown of GLUD1/2 inhibits orthotopic growth of an *IDH1*^{R132H} glioma line. Entire T2-MRI series of a representative mouse from naïve, shCtrl, sh647, and sh662 groups, respectively. Colored area represents region quantified as tumor.

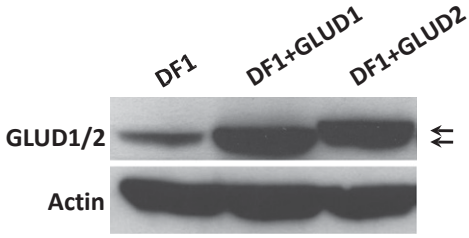

Fig. S3. Both human GLUD1 and GLUD2 are recognized by anti-GLUD antibody. Total cell lysates from chicken fibroblast DF1 cells transduced with RCAS vectors expressing human GLUD1 or GLUD2 were run on a long 8% Tris-glycine gel. Western blotting was performed using monoclonal antibody to GLUD (Clone 3C2, H00002746-M01; Abnova). The arrows point to positions of the bands representing GLUD1 and GLUD2.

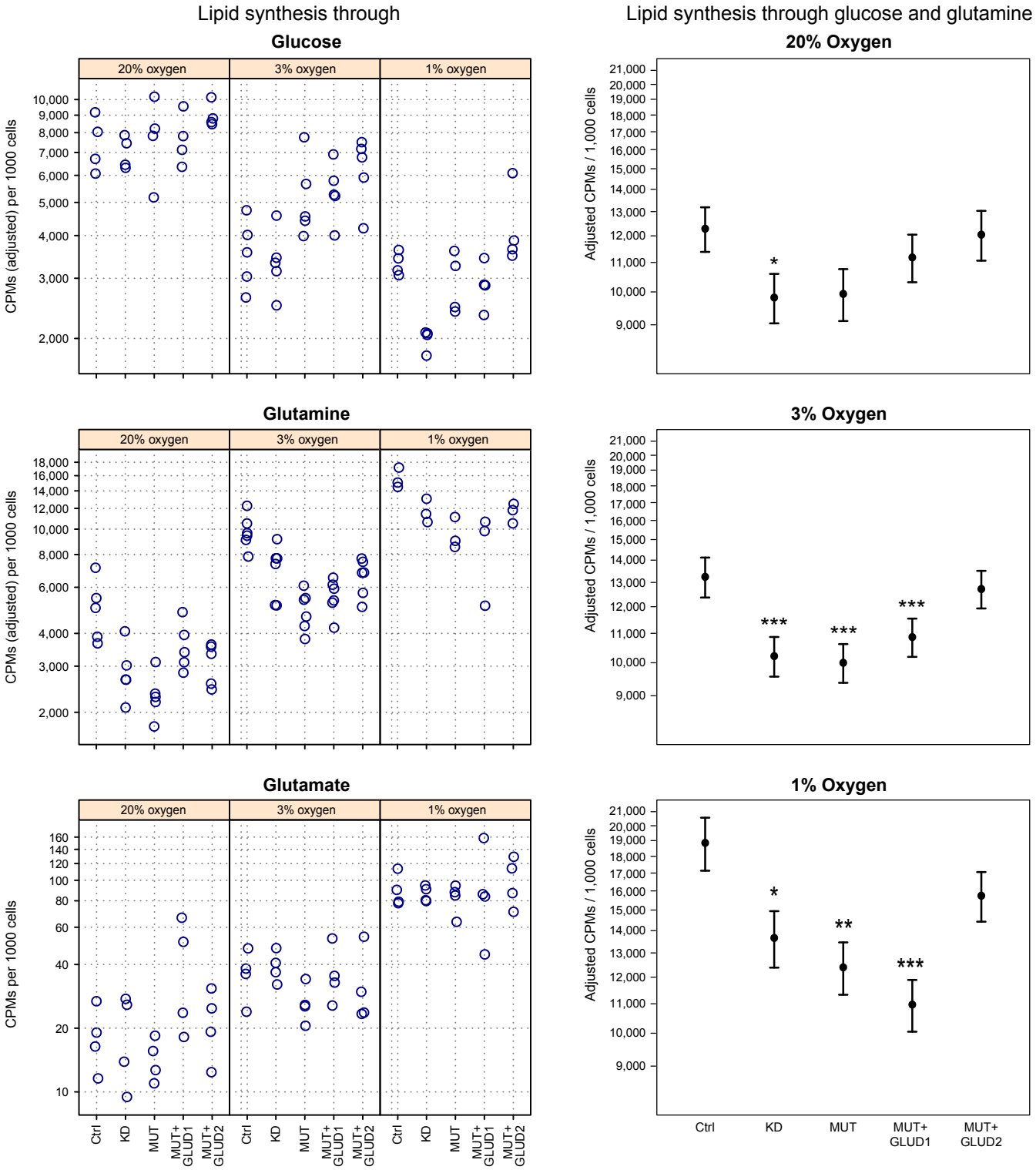

Fig. S4. Effects of IDH1 status, GLUD1/2, and oxygen levels on metabolite flux to lipid in IDH1^{R132H} glioma progenitors. Cells were grown in 20, 3, or 1% oxygen in standard tissue culture media with [^{14}C]glutamine, [^{14}C]glucose, or [^{14}C]glutamate tracer. A minimum of three experiments was conducted for each line of the cells under each of the oxygen conditions. (*Left*) Each circle represents data from one experiment. (*Right*) Summary plots of total flux from glucose and glutamine. *$P < 0.05$, **$P < 0.01$, *** for $P < 0.001$ for comparison with control, mixed linear effects test.

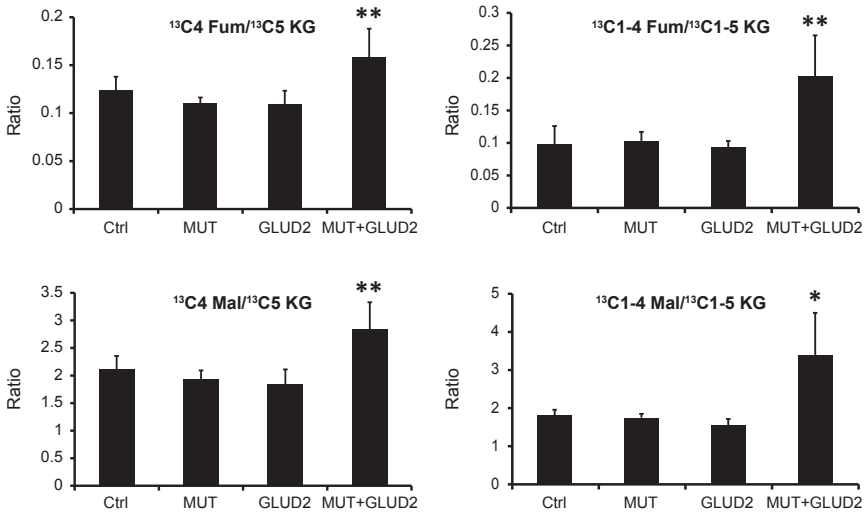

Fig. S5. Effects of GLUD2 on ratios of tricarboxylic acid cycle metabolites with incorporation of ^{13}C derived from ^{13}C5 glutamine. (*Upper Left*) Fumarate (^{13}C4-) produced from ^{13}C5–α-KG vs. ^{13}C5–α-KG. (*Upper Right*) All labeled fumarate (^{13}C1-, ^{13}C2-, ^{13}C3-, and ^{13}C4-fumarate combined) produced from all labeled α-KG (^{13}C1-, ^{13}C2-, ^{13}C3-, and ^{13}C5–α-KG combined, no ^{13}C4–α-KG detected). (*Lower Left*) Malate (^{13}C4-) produced from ^{13}C5–α-KG vs. ^{13}C5–α-KG. (*Lower Right*) All labeled malate (^{13}C1-, ^{13}C2-, ^{13}C3-, and ^{13}C4-fumarate combined) vs. all labeled α-KG. *$P < 0.05$, **$P < 0.01$, for GLUD2 effect compared with Ctrl (for GLUD2) or MUT (for MUT + GLUD2).

Table S1. Knockdown of GLUD1/2 alleviates metabolic changes in grafted *IDH1^{R132H}* tumor

	shCtrl		sh647			sh662			Brain		
Metabolite	Mean	SEM	Mean	SEM	*P*	Mean	SEM	*P*	Mean	SEM	*P*
2-HG	2,029,969	138,096	1,846,208	156,919	3.9E-01	904,887	65,914	8.1E-05	65,459	7,167	3.3E-06
a-KG	41,935	3,653	32,199	2,136	4.4E-02	24,087	1,227	1.6E-03	9,156	1,043	1.1E-04
ADP	4,412,331	1,003,058	4,039,861	248,871	1.8E-01	5,045,420	403,233	5.2E-01	3,368,092	1,029,300	4.7E-01
Alanine	1,070,280	44,982	790,349	63,155	4.3E-03	682,141	44,163	2.7E-05	675,550	35,181	2.4E-04
Arginine	1,640,117	130,769	997,262	101,043	2.6E-03	996,188	86,287	2.7E-03	1,582,142	13,244	7.2E-01
Aspartic acid	1,354,247	98,990	1,381,786	53,888	8.2E-01	1,904,255	122,059	2.1E-02	3,078,510	197,682	2.7E-05
Carnitine	7,809,486	333,910	7,073,319	269,256	1.1E-01	7,388,665	595,440	1.5E-01	4,925,240	196,750	2.0E-04
Citrate	4,499,887	159,413	3,996,365	121,374	3.1E-02	2,865,366	119,556	9.6E-06	1,262,643	46,714	3.1E-07
Cysteine	104,988	7,527	103,939	4,156	9.0E-01	84,696	4,989	2.9E-02	100,864	14,460	7.8E-01
Fumarate	89,947	6,506	64,123	4,389	7.8E-03	63,955	4,605	1.1E-02	76,931	12,662	3.3E-01
G6P	1,043,500	33,139	924,454	15,309	8.3E-03	800,747	27,826	4.0E-04	828,521	82,000	2.1E-02
Ghyde3P	88,665	8,906	107,646	5,669	1.0E-01	91,600	5,378	9.9E-01	103,683	10,510	3.1E-01
Glucose	90,890	22,698	110,869	16,295	5.0E-01	24,592	4,459	2.8E-02	3,451	1,993	1.6E-02
Glutamate	1,3207,010	348,604	13,583,858	366,966	4.8E-01	16,003,026	411,301	1.7E-02	19,566,127	359,633	9.9E-07
Glutamine	12,301,912	349,596	14,024,908	325,176	4.4E-03	152,75,496	434,894	2.8E-03	19,271,047	393,753	1.1E-06
Gly3P	396,114	29,403	300,774	17,212	1.8E-02	285,960	26,188	1.5E-02	445,545	86,302	5.4E-01
Glycine	329,628	20,808	273,146	13,650	4.4E-02	256,977	30,609	3.5E-02	190,324	2,675	1.6E-03
GSH	13,265,389	229,192	12,764,489	230,156	4.5E-02	12,396,927	423,018	7.2E-03	12,956,936	25,121	8.2E-02
GSSG	1,043,821	24,268	973,866	29,442	9.4E-02	525,838	40,778	2.5E-06	787,790	35,057	3.4E-04
Lactate	1,854,257	104,008	1,620,928	25,201	5.0E-02	1,773,236	46,477	2.7E-01	1,702,158	34,602	2.8E-01
Leu/Ile	3,867,708	221,557	3,228,534	183,849	4.6E-02	3,790,239	108,748	4.3E-01	4,240,770	79,871	2.3E-01
Malate	1,419,868	61,022	1,184,092	71,433	2.8E-02	1,329,823	38,424	1.7E-01	1,422,542	137,541	1.0E+00
NADP	208,215	10,145	180,386	2,869	2.4E-02	156,248	2,648	1.5E-03	164,969	5,875	1.1E-02
Pento5P	318,925	11,018	344,638	14,290	1.8E-01	261,883	8,703	1.2E-03	269,443	45,196	2.2E-01
Proline	4,921,874	264,085	4,525,651	234,793	2.8E-01	3,537,537	431,094	3.0E-03	1,627,664	30,336	9.8E-06
PRPP	7,664	1,244	7,276	655	7.9E-01	9,834	1,621	3.8E-01	10,957	2,352	7.8E-01
PRV	58,266	4,122	5,3571	2,149	3.4E-01	36,545	4,407	6.0E-03	43,068	11,943	1.9E-01
Sedo7P	139,288	19,936	137,614	14,741	9.4E-01	87,360	3,714	4.0E-02	22,412	2,550	1.7E-03
Serine	908,705	26,393	800,914	36,350	3.4E-02	937,113	44,101	1.0E+00	1,311,129	44,836	1.8E-05
Succinate	663,882	42,174	573,600	1,1047	6.5E-02	534,394	39,221	1.4E-02	434,179	76,042	2.0E-02
UMP	825,359	89,489	1,155,293	130,152	6.2E-02	2,032,611	142,017	1.3E-04	1,616,719	54,810	1.7E-04

The levels of each metabolite are presented as mean of normalized areas under the curve and SEM from six tumor samples (shCtrl, sh647, or sh662) or four brain tissues of matched normal mice (same age and sex as those of tumor-bearing mice). *P* value: with comparison with shCtrl, *t* test.