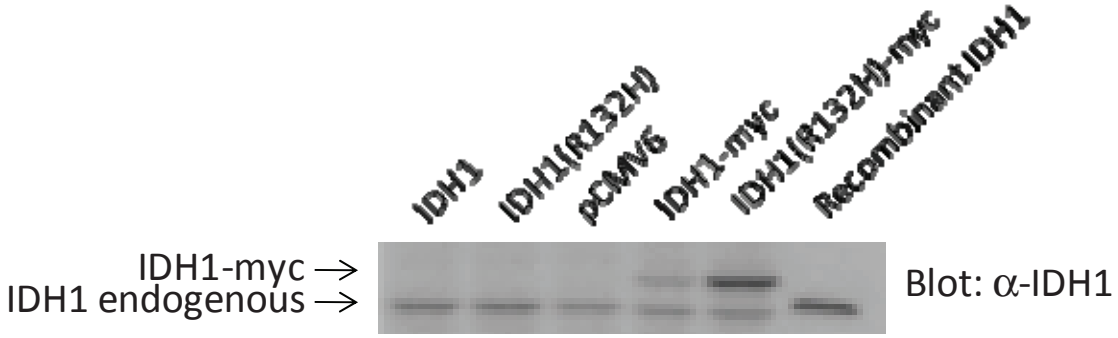
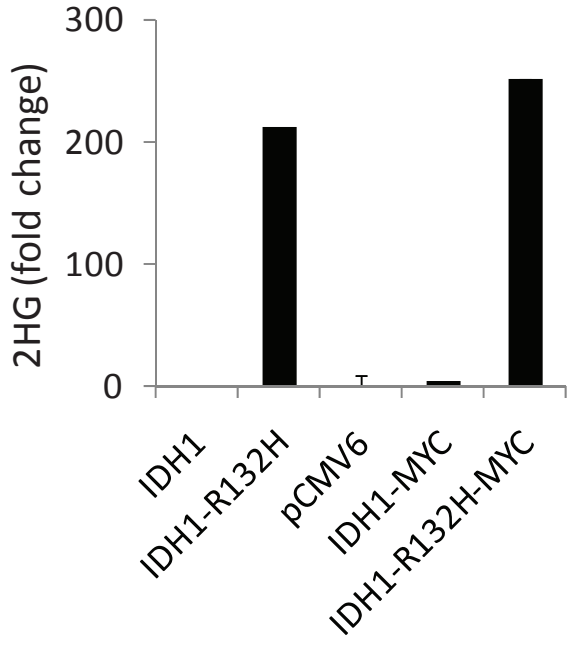
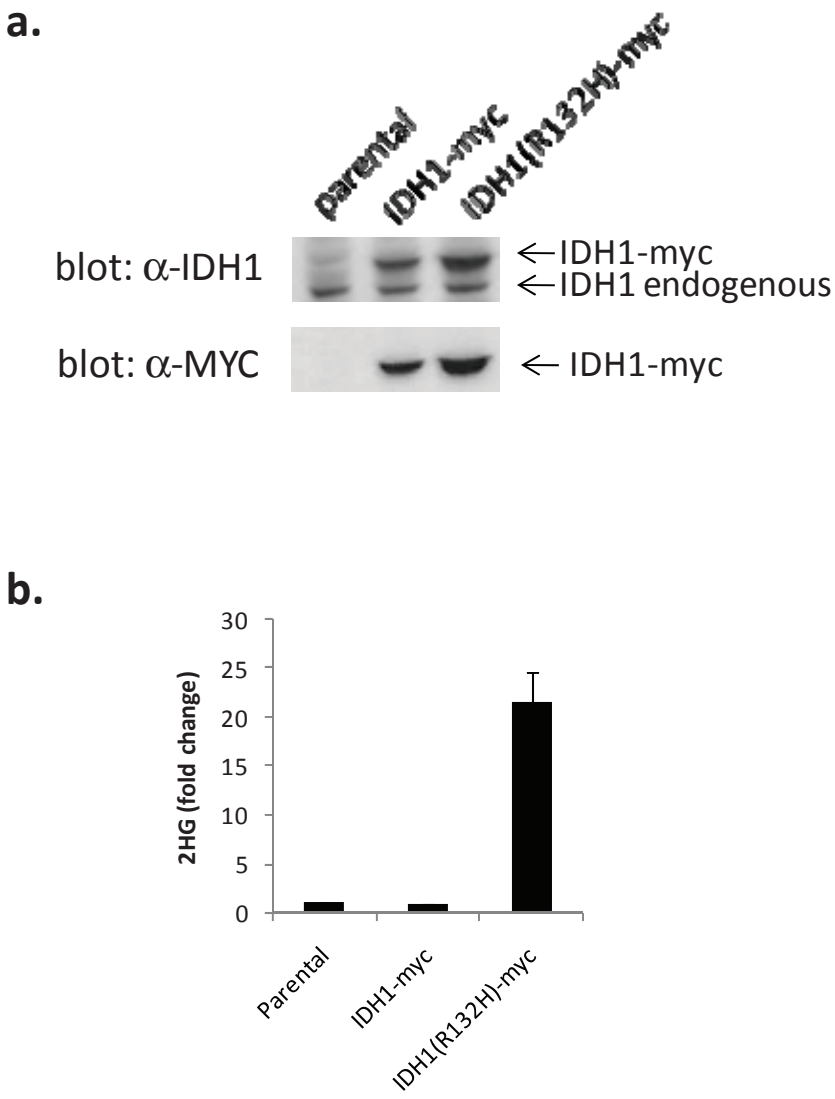
a.



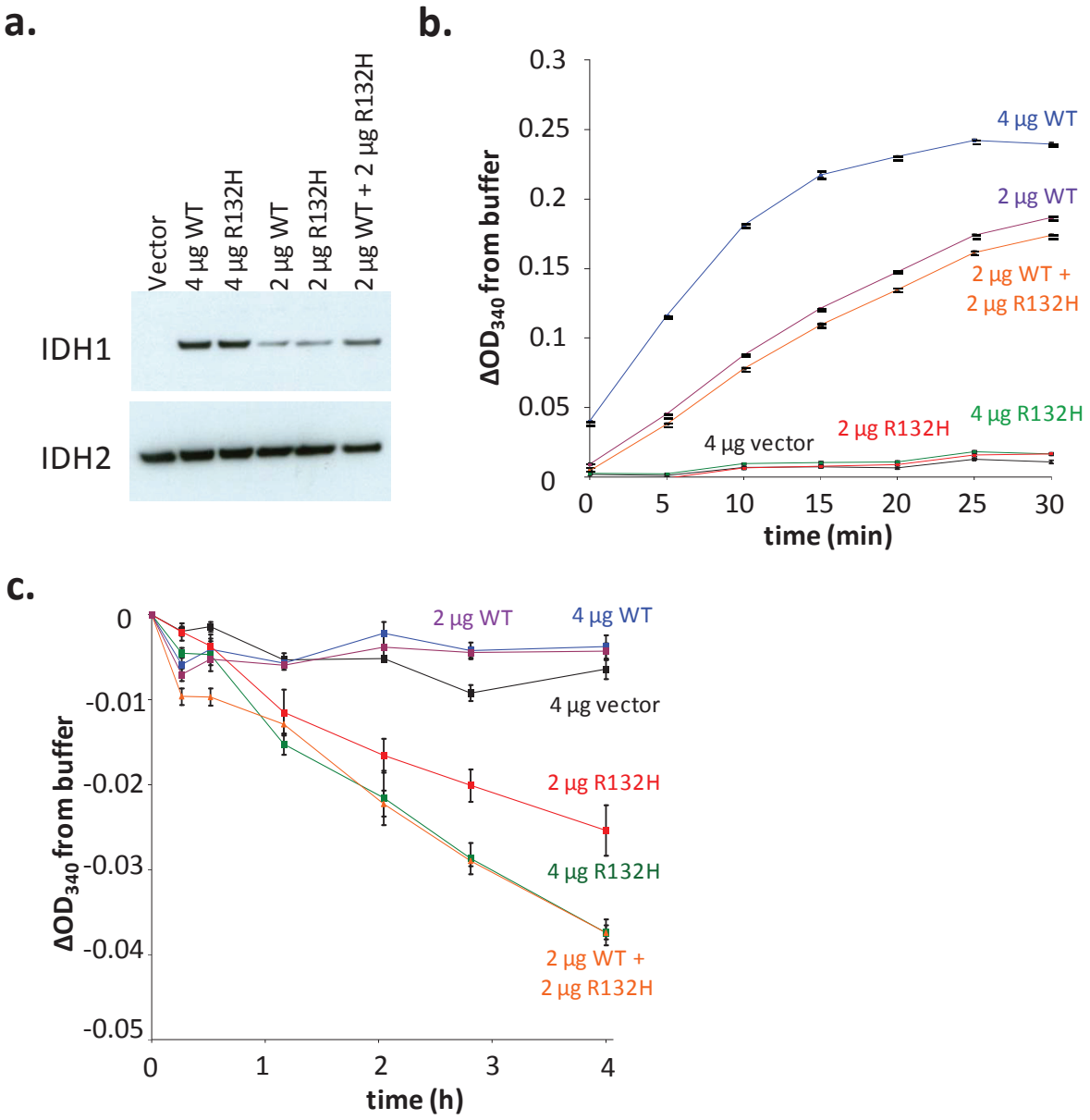
b.



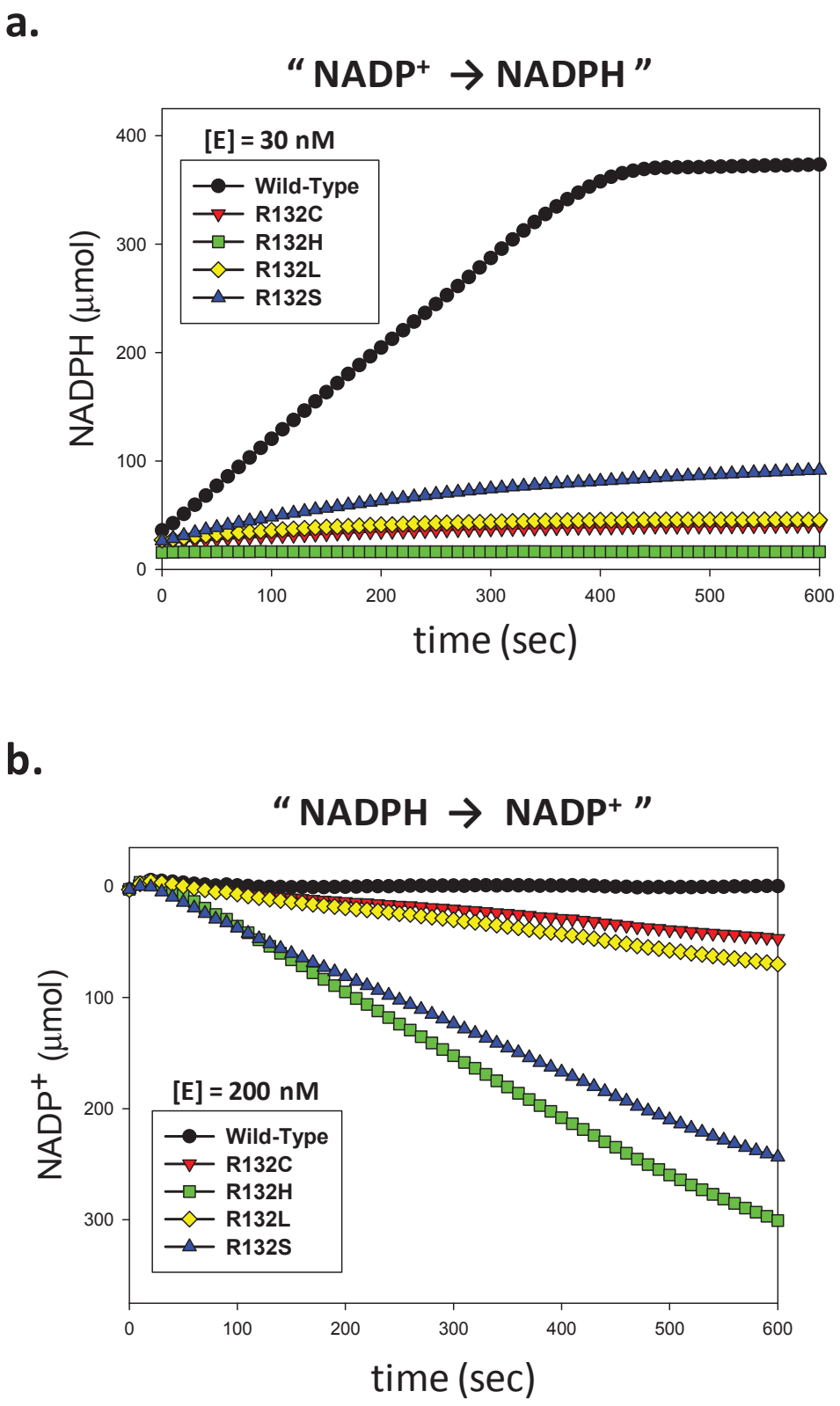
Supplementary Figure 1. Untagged and Myc-tagged IDH1(R132H) produce similar levels of 2-hydroxyglutarate. **(a)** Myc-tagged and untagged IDH1 constructs were transiently expressed in 293T cells. A Western blot to assess expression is shown. **(b)** 2HG levels in cell extracts were measured by LC-MS. Data were normalized by cell number and expressed as fold increase in 2HG relative to 293T cells transfected with vector alone. Error bars depict one s.d. from the mean of 3 independent experiments.



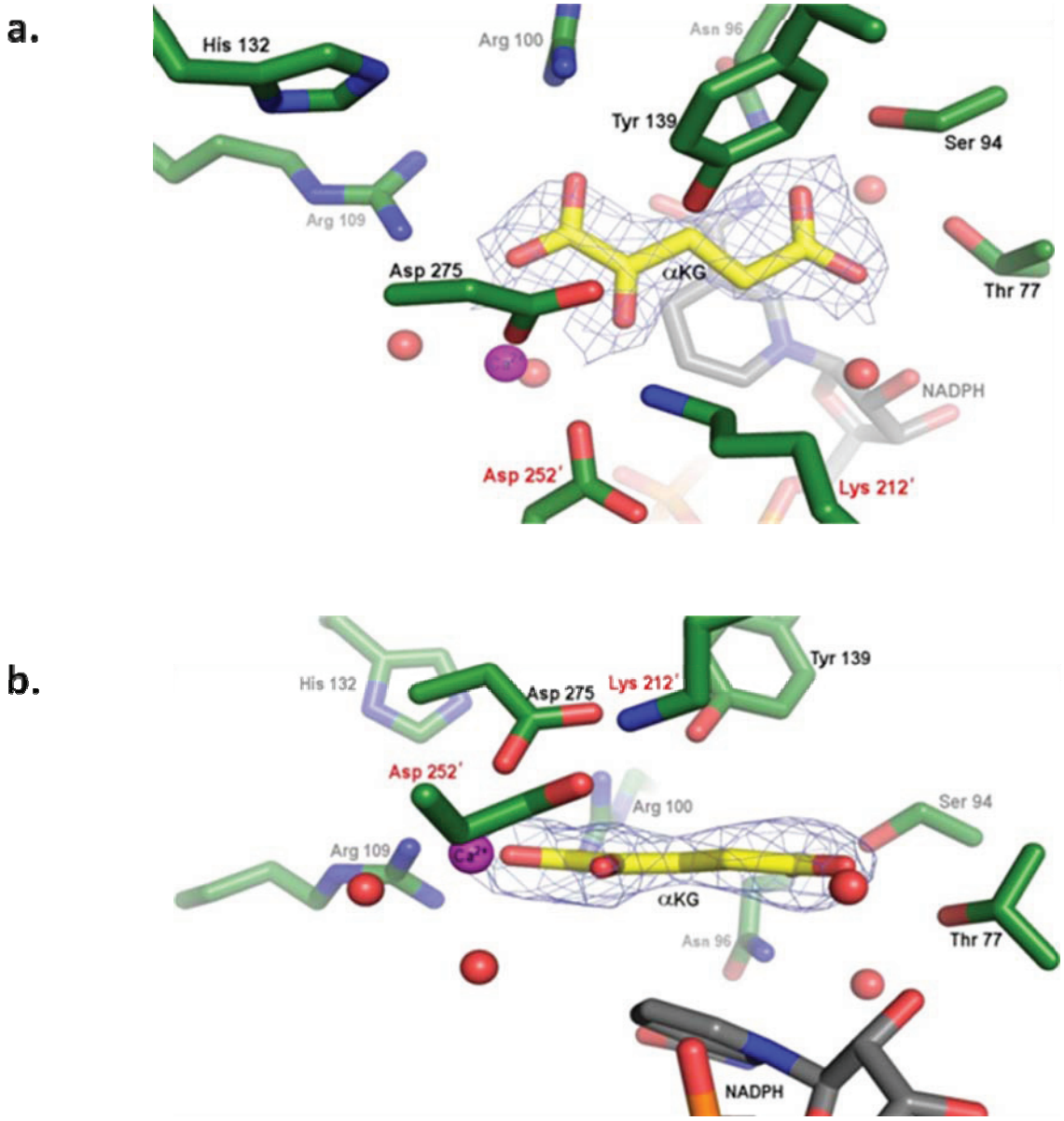
Supplementary Figure 2. LN-18 cells expressing human R132H IDH1 also contain elevated levels of 2HG. (a) Myc-tagged human isocitrate dehydrogenase 1 (IDH1-myc) or R132H mutant (R132H-myc) were transfected into LN-18 human glioblastoma cells. Expression was confirmed by Western blot of cell lysates using antibodies recognizing either human IDH1 or the carboxy-terminal Myc epitope tag. **(b)** Extracts from cells expressing R132H mutant IDH1 (IDH1 (R132H)-myc) contain elevated levels of 2HG compared with cells expressing wild-type IDH1 (IDH1-myc). Data were normalized by cell number and expressed as fold difference relative to parental values. Error bars depict one s.d. from the mean of 3 independent experiments.



Supplementary Figure 3. Expression of R132H mutant IDH1 results in αKG-dependent NADPH consumption. **(a)** 293T cells were transfected with the indicated amount of wild-type (WT) or R132H mutant IDH1. The expression level of IDH1 was confirmed by Western blot as shown. Re-probing of the same blot with antibody against IDH2 is also shown as a control. **(b)** 293T protein lysates, from cells transfected with the listed amounts of wild-type or R132H mutant IDH1 cDNA, or empty vector, were assayed for their ability to generate NADPH from $NADP^+$ in the presence of 0.1 mM isocitrate. **(c)** The same cell lysates described in (b) were assessed for their ability to consume NADPH in the presence of 0.6 mM αKG. The data in the figure are representative of 4 independent experiments. In both (b) and (c) error bars represent the standard error of the mean from 3 independent measurements at the time point indicated.



Supplementary Figure 4. R132H mutation alters the enzymatic properties of IDH1. (a) Recombinant human wild-type (WT) and various R132 mutant IDH1 enzymes were assessed for oxidative decarboxylation of isocitrate to αKG with $NADP^+$ as cofactor. **(b)** WT and various R132 mutant IDH1 enzymes were assessed for reduction of αKG with NADPH as cofactor. Different concentrations of enzyme were used to generate the curves presented in (a) and (b).



Supplementary Figure 5. The OMIT map for the substrate-binding region. αKG substrate was omitted from the final model and 2Fo-Fc map was calculated using the last set of structure factors. The omitted ligand αKG is displayed in the resultant electron density map contoured at 1 σ viewed face-on in **(a)** and edge-on in **(b)**.

Crystallography data and refinement statistics

	IDH1 R132H
Data collection	
Space group	*C* 2 2 2_1
Cell dimensions	
a, *b*, *c* (Å)	96.1, 274.7, 116.5
α, β, γ (°)	90, 90, 90
Resolution (Å)	25 - 2.10 (2.18 - 2.10)*
R_{merge}	0.080 (0.305)
I/σ_I	20.3 (2.4)
Completeness (%)	92.4 (58.8)
Redundancy	8.6 (2.1)
Refinement	
Resolution (Å)	25-2.10
No. reflections	83109
R_{work}/R_{free}	0.220/0.262
No. atoms	
Protein	9706
Ligand/ion	198
Water	301
B-factors	
Protein	33.2
Ligand/ion	30.9
Water	38.7
r.m.s deviations	
Bond lengths (Å)	0.014
Bond angles (º)	1.1

Structure R132H IDH1 was refined using data from a single crystal.
**Highest resolution shell is shown in parenthesis.*

Supplementary Table 1: Crystallography data and refinement statistics. X-ray diffraction data processing and refinement statistics. Values in parentheses are for the highest resolution bin of diffraction data. $R_{work} = \Sigma_{hkl} |F_o - F_c| / \Sigma_{hkl} F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively, for all reflections hkl used in refinement. R_{free} is calculated for the 5% of the data that were not used in refinement.

Characterization of human brain tumor samples.

Sample ID	Primary Specimen Diagnosis	WHO Grade	Tumor Cells in Tumor Foci (%)	Genotype	Nucleotide change	Codon	2HG (μmole/g)	αKG (μmole/g)	Malate (μmole/g)	Fumarate (μmole/g)	Succinate (μmole/g)	Isocitrate (μmole/g)
1	Glioblastoma, residual/recurrent	IV	n/a	wild type	wild type	R132	0.18	0.161	1.182	0.923	1.075	0.041
2	Glioblastoma	IV	n/a	wild type	wild type	R132	0.16	0.079	1.708	1.186	3.156	0.100
3	Glioblastoma	IV	n/a	wild type	wild type	R132	0.13	0.028	0.140	0.170	0.891	0.017
4	Oligoastrocytoma	II	n/a	wild type	wild type	R132	0.21	0.016	0.553	1.061	1.731	0.089
5	Glioblastoma	IV	n/a	mutant	G364A	R132H	16.97	0.085	1.091	0.807	1.357	0.058
6	Glioblastoma	IV	n/a	mutant	G364A	R132H	19.42	0.023	0.462	0.590	1.966	0.073
7	Glioblastoma	IV	n/a	mutant	G364A	R132H	31.56	0.068	0.758	0.503	2.019	0.093
8	Oligodendroglioma, anaplastic	III	75	mutant	G364A	R132H	12.49	0.033	0.556	0.439	0.507	0.091
9	Oligodendroglioma, anaplastic	III	90	mutant	G364A	R132H	4.59	0.029	1.377	1.060	1.077	0.574
10	Oligoastrocytoma	II	n/a	mutant	G364A	R132H	6.80	0.038	0.403	0.503	1.561	0.065
11	Glioblastoma	IV	n/a	wild type	wild type	R132	0.686	0.011	0.113	0.990	5.085	0.007
12	Glioblastoma	IV	n/a	mutant	G364A	R132H	18.791	0.016	0.470	0.462	2.673	0.031
13	Glioblastoma	IV	n/a	mutant	G364A	R132H	4.59	0.029	1.377	1.060	1.077	0.043
14	Glioblastoma	IV	n/a	wild type	wild type	R132	0.199	0.046	0.180	0.170	0.221	0.014
15	Glioblastoma	IV	n/a	mutant	C363G	R132G	13.827	0.030	0.905	0.599	1.335	0.046
16	Glioblastoma	IV	n/a	mutant	G364A	R132H	28.364	0.068	0.535	0.488	2.105	0.054
17	Glioblastoma	IV	n/a	mutant	C363A	R132S	9.364	0.029	1.038	0.693	2.151	0.121
18	Glioblastoma	IV	n/a	wild type	wild type	R132	0.540	0.031	0.468	0.608	1.490	0.102
19	Glioma, malignant, astrocytoma	IV	80	mutant	G364A	R132H	19.000	0.050	0.654	0.391	2.197	0.171
20	Oligodendroglioma	III	80	wild type	wild type	R132	0.045	0.037	1.576	0.998	1.420	0.018
21	Glioma, malignant, astrocytoma	IV	95	wild type	wild type	R132	0.064	0.034	0.711	0.710	2.105	0.165
22	Glioblastoma	IV	70	wild type	wild type	R132	0.171	0.041	2.066	1.323	0.027	0.072

Supplementary Table 2: Characterization of human brain tumor samples. The clinical characteristics, IDH1 mutation status, as well as the levels of metabolites measurement in the clinical specimens used for this study, are listed. Clinical information for tumors where data was not available is labeled “n/a”.