Supplementary Methods

DNA constructs

The IDH2 wildtype and R172V constructs were cloned by standard site-directed mutagenesis (Thermo) and Gibson Assembly (New England Biolabs) into pCDNA3.1 (Thermo) and verified by Sanger sequencing. For transient transfection experiments, cells were transfected using polyethylenimine. Two days after transfection, cells were treated with DMSO, ivosidenib (50 μmol/L), or ivosidenib (50 μmol/L) plus enasidenib (50 μmol/L) and then harvested for protein and metabolite analysis one day later. The IDH1 wildtype and R132H constructs were cloned by standard site-directed mutagenesis (Thermo) and Gibson Assembly (New England Biolabs) into a modified pTRE-Tight retroviral vector (Clontech) and verified by Sanger sequencing. For retroviral infection experiments, supernatant from 293T cells transfected with helper virus and plasmids was collected after 2 days, filtered and applied to CS-1 cells. Two days after infection, cells were subjected to antibiotic selection with hygromycin (250 μg/ml) for four days or until an uninfected control plate was completely dead. Stably transduced CS-1 were treated for 24 hours with doxycycline (500 ng/ml) to induce expression of the cDNAs, then treated with treated with DMSO, ivosidenib (50 μmol/L), or ivosidenib (50 μmol/L) plus enasidenib (50 μmol/L) and harvested for protein and metabolite analysis one day later.

Gel electrophoresis and western blotting

For denatured gel electrophoresis, cells were harvested in 1x RIPA buffer (Cell Signaling), centrifuged at 21,000 g at 4° C, and supernatants were collected. Cleared cell lysates were quantified by BCA assay (ThermoFisher) and normalized for total protein concentration. Samples were separated by SDS-PAGE, transferred to nitrocellulose membranes (Life Technologies), blocked in 5% milk prepared in Tris buffered saline with 0.1% Tween 20 (TBST), incubated with primary antibodies overnight at 4° C then horseradish peroxidase (HRP)-conjugated secondary

antibodies (GE Healthcare; anti-mouse, NA931V, sheep, 1:5000; anti-rabbit, NA934V, donkey, 1:5000). After incubation with ECL (ThermoFisher or GE Healthcare), imaging was performed using the Amersham Imager 600 (GE Healthcare). Primary antibodies used included: anti-caspase 3 (Cell Signaling Technology, 9662S; rabbit; 1:1000), anti-IDH1 (Proteintech, 12332-1-AP; rabbit; 1:1000), anti-IDH2 (Abcam, ab55271; mouse; 1:1000), and anti-vinculin (Sigma, V4505; mouse; 1:1000).

Metabolite extraction and analysis

Metabolites were extracted from plasma (100 μl) or cell cultures (6-well plates) with ice-cold 80:20 methanol:water containing 2 μmol/L deuterated 2-hydroxyglutarate (D-2-hydroxyglutaric-2,3,3,4,4-d_5 acid; deuterated-2HG) as an internal standard. After overnight incubation at –80° C, plasma or cell extracts were sonicated and centrifuged at 21,000 g for 20 min at 4° C to precipitate protein. Extracts were then dried in an evaporator (Genevac EZ-2 Elite). Metabolites were resuspended by addition of 50 μl of methoxyamine hydrochloride (40 mg/ml in pyridine) and incubated at 30° C for 90 min with agitation. Metabolites were further derivatized by addition of 80 μl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) + 1% 2,2,2-trifluoro-N-methyl-N-(trimethylsilyl)-acetamide, chlorotrimethylsilane (TCMS; Thermo Scientific) and 70 μl of ethyl acetate (Sigma) and incubated at 37° C for 30 min. Samples were diluted 1:2 with 200 μl of ethyl acetate, then analyzed using an Agilent 7890A GC coupled to Agilent 5975C mass selective detector. The GC was operated in splitless mode with constant helium carrier gas flow of 1 ml/min and with a HP-5MS column (Agilent Technologies). The injection volume was 1 μl and the GC oven temperature was ramped from 60° C to 290° C over 25 min. Peaks representing compounds of interest were extracted and integrated using MassHunter vB.08.00 (Agilent Technologies) and then normalized to both the internal standard (deuterated-2HG) peak area and plasma volume or protein content as applicable. Ions used for quantification of metabolite levels were 2HG *m/z* 247

(confirmatory ion *m/z* 349) and deuterated-2HG *m/z* 252 (confirmatory ion *m/z* 354). Peaks were manually inspected and verified relative to known spectra for each metabolite. Absolute metabolite quantitation was performed using an external calibration curve with deuterated-2HG internal standard and the resulting concentrations corrected for the total plasma volume extracted. For the patient with IDH1-mutant intrahepatic cholangiocarcinoma, plasma and tumor 2HG was measured using a qualified liquid chromatography–tandem mass spectrometry (LC-MS/MS) method with a lower limit of quantitation of 30 ng/ml for plasma or 100 ng/g for tumor.