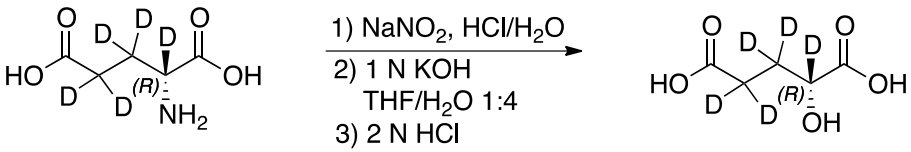
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

General methods: ^{13}C NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer. Chemical shifts are reported in ppm, referenced to tetramethylsilane. Low resolution mass spectral analyses were performed using electrospray ionization with quadrupole analyzer, and LC/MS analyses with a Waters Acquity instrument ELSD and MS detectors. EtOAc, and MeOH were purchased from SIGMA-ALDRICH and D-Glutamic-2, 3, 3, 4, 4-d_5 acid from CDN Isotopes Inc. (Quebec, Canada). Tetrahydrofuran (THF) was obtained from a dry solvent system (activated alumina column, positive pressure of argon).

Synthesis of D-2-hydroxyglutaric-2, 3, 3, 4, 4-d_5 acid



2, 3, 3, 4, 4-Pentadeutero-(R)-2-hydroxyglutaric acid was synthesized from D-2, 3, 3, 4, 4-pentadeutero glutamic acid, following experimental conditions which were reported for L-glutamic acid (Zhang, 2009). D-2, 3, 3, 4, 4-pentadeutero Glutamic acid (48 mg, 0.32 mmol) was suspended in H_20 (160 µl) and concentrated HCl (66 µl, 0.79 mmol, 2.5 eq) was added. The mixture was then cooled to 0^oC and a solution of $NaNO_2$ (48 mg, 0.695 mmol, 2.2 eq) in H_20 (160 µl) was added dropwise. The reaction mixture was allowed to warm up to ambient temperature, and it was stirred overnight. After extraction with EtOAc, the combined extracts were briefly dried over $MgSO_4$, filtered over celite and the filtrate was evaporated under reduced pressure. This produces the corresponding 5-membered lactone which was taken up in THF/ H_20 1:4 (v/v, 100 µl), cooled to 0^o C and treated with 1.0 N KOH (460 µl) and allowed to stir for 2 hours at room temperature. The organic layer was then decanted, and the aqueous phase was carefully acidified to pH 4.0 using 2.0 N HCl and it was extracted with EtOAc/MeOH (v/v, 2:1). The combined extracts were evaporated under reduced pressure to give 2, 3, 3, 4, 4-pentadeutero-(R)-2-hydroxyglutaric acid (14 mg, 29%). LRMS (ESI) calc. for $C_5H_3D_5O_5$ 153.0686, found $[M-H]^-$ 152.2; LC-MS (Waters using X Bridge HILIC column (4.6 mm x 150 mm 5 µm , using the gradient gradient 95 to 50 % 10.0 mM ammonium acetate in acetonitrile over 20 min, t_R =7.48 min; ^{13}C NMR (D_2O) δ 177.62 (COOH), 177.20 (COOH), 68.57 (t, C-2), 28.65 (m, C-3), 27.54 (m, C-4). Purity is assessed based on ^{13}C NMR and was called at 97+%.

Zhang D.-W., Luo Z., Liu G.-J., Weng L.-H., *Tetrahedron* 65, 9997 (2009).

SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURE S1. **Stem/progenitor marker expression is induced more by IDH2 R172 mutation than by IDH R140 mutation in murine primary bone marrow.** C57BL/6 murine bone marrow cells were transduced with MIGR1 retroviral vectors containing empty vector, IDH2 WT, IDH2 R140Q, or IDH2 R172K. Forty-eight hours post-transduction, cells were sorted for GFP expression and confirmed to have nearly equivalent IDH2 expression levels in IDH2 WT, R140Q, and R172K expressing cells by RT-qPCR (data not shown). Cells were then liquid cultured for 5 days in myeloid growth conditions with interleukin-3 (IL-3), IL-6, FMS-like tyrosine kinase 3(Flt-3) ligand, thrombopoietin, stem cell factor, and granulocyte macrophage colony stimulating factor. Stem/progenitor marker C-kit expression was subsequently assessed by flow cytometry of GFP-expressing cells. These data delineate a phenotypic difference between IDH2 R140Q and IDH2 R172K-expressing bone marrow cells and represent an extension of previously published work that reported the ability of the R140Q mutant to impair primary bone marrow differentiation (16). Bone marrow cells from heterozygous IDH1 R132H lineage-specific knock-in mice have been independently reported to also demonstrate increased C-kit expression (42).

SUPPLEMENTAL FIGURE S2. **Co-expression of wild-type IDH1 results in greater 2HG accumulation from mutant IDH1 expression.** *A*, Un-tagged IDH1 WT or R132H constructs, or empty vector, were transfected at the indicated combinations into 293T cells. Forty-eight hours post-transfection cells were harvested and assessed for 2HG accumulation by GC-MS (top), depicted as the 2HG peak signal intensity normalized to the intrasample glutamate signal, or protein expression by Western blot (bottom). *B*, Myc-tagged IDH1 R132H, FLAG-tagged IDH1 WT, or empty vector were transfected into 293T cells separately or in combination. (2X) indicates twice the dose of IDH1 R132H-Myc as that transfected into cells depicted in the right two lanes. Data for (*A*-*B*) are from a representative of 3 independent experiments. *C*, Myc-tagged IDH1 R132H was transfected at a fixed dose along with various doses of FLAG-tagged IDH1 WT, IDH1 A134D which has been previously reported to be catalytically inactive (14), IDH1 R132H, or empty vector. Data are from a representative of 2 independent experiments. *D*, Comparable levels of cell accumulation at the time of harvest of the transfection experiment depicted in Fig. 3*D* were confirmed by hemacytometer count of cells in parallel-transfected plates.

SUPPLEMENTAL TABLE 1. **Summary of the occurrence of IDH1 R132, IDH2 R172, and IDH2 R140 mutations in cancers to date**. Compilation of previously reported IDH mutation incidence in references 1-3, 7-10, 12, and 20-22 cited in main text. MDS, myelodysplastic syndrome. MPN, myeloproliferative neoplasm.

Figure S1

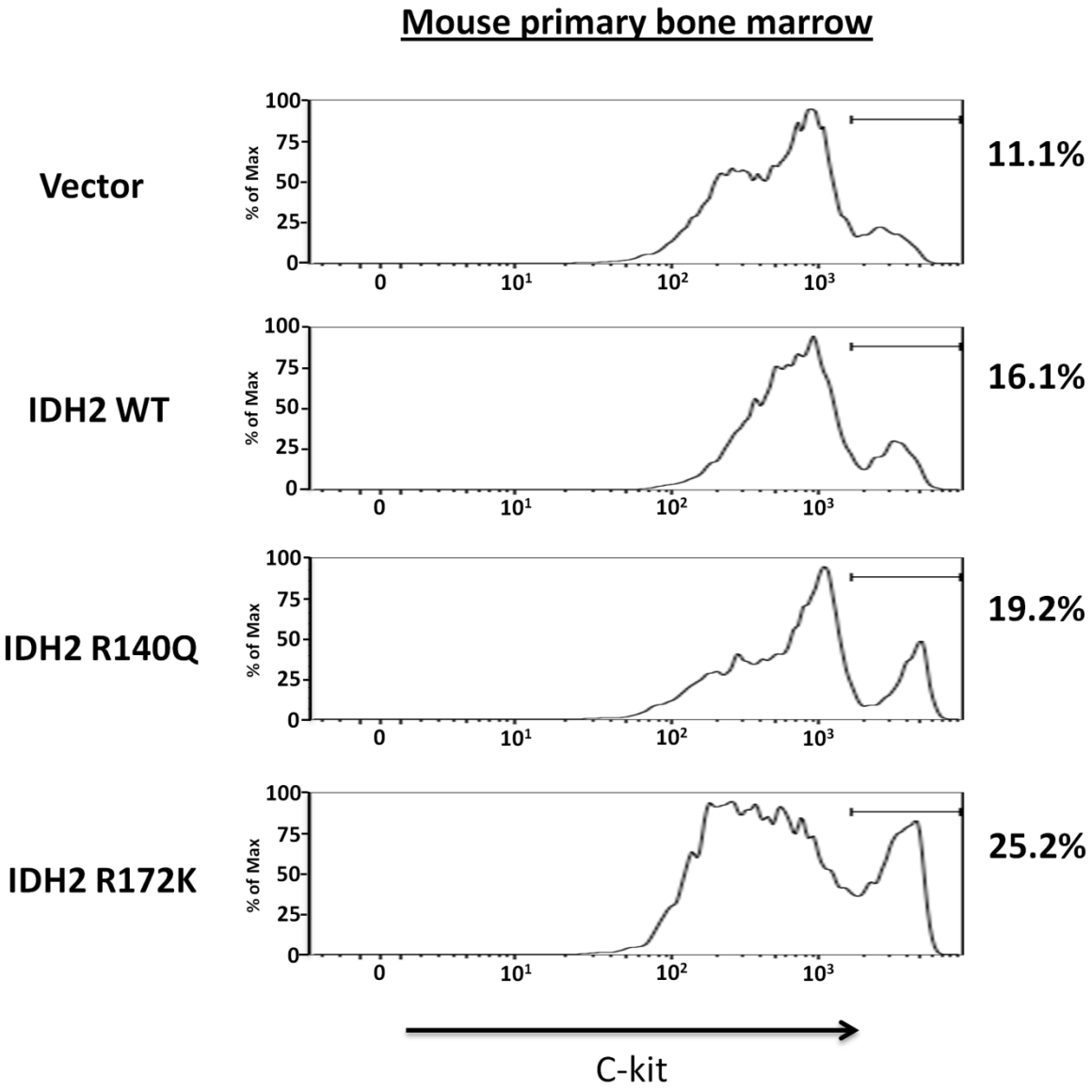

Mouse primary bone marrow
Vector
IDH2 WT
IDH2 R140Q
IDH2 R172K
% of Max
100
75
50
25
0
0
10¹
10²
10³
11.1%
16.1%
19.2%
25.2%
C-kit

Figure S2

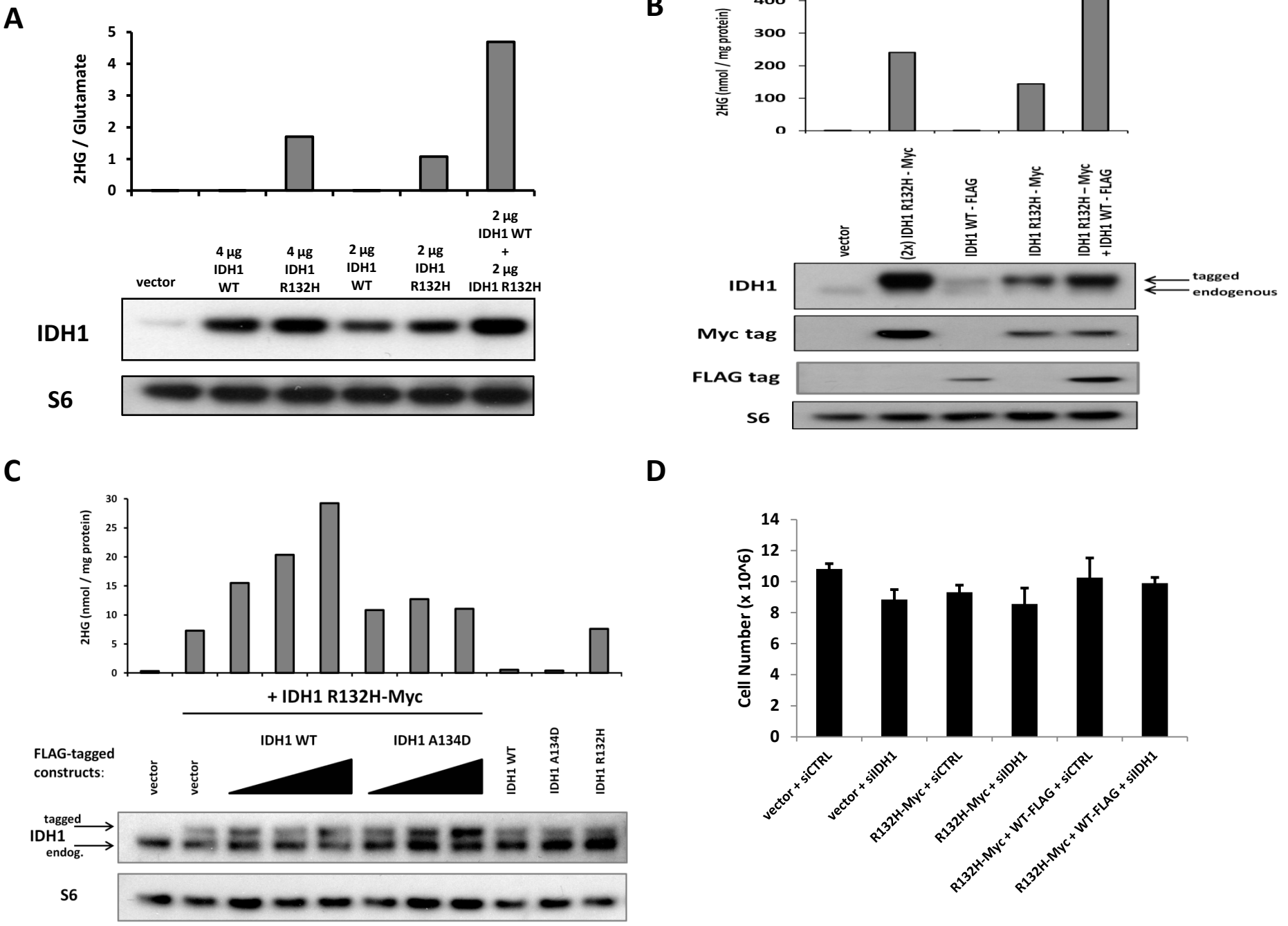

A
2HG / Glutamate
vector
4 µg IDH1 WT
4 µg IDH1 R132H
2 µg IDH1 WT
2 µg IDH1 R132H
2 µg IDH1 WT + 2 µg IDH1 R132H
IDH1
S6
B
2HG (nmol / mg protein)
vector
(2x) IDH1 R132H - Myc
IDH1 WT - FLAG
IDH1 R132H - Myc
IDH1 R132H – Myc + IDH1 WT - FLAG
IDH1
tagged
endogenous
Myc tag
FLAG tag
S6
C
2HG (nmol / mg protein)
+ IDH1 R132H-Myc
FLAG-tagged constructs:
vector
vector
IDH1 WT
IDH1 A134D
IDH1 WT
IDH1 A134D
IDH1 R132H
tagged IDH1
endog.
S6
D
Cell Number (x 10^6)
vector + siCTRL
vector + siIDH1
R132H-Myc + siCTRL
R132H-Myc + siIDH1
R132H-Myc + WT-FLAG + siCTRL
R132H-Myc + WT-FLAG + siIDH1

Table S1

	Cytosolic IDH1 mutation	**Analogous** Mitochondrial IDH2 mutation		**Non-analogous** Mitochondrial IDH2 mutation
	IDH1 R132	IDH2 R172		IDH2 R140
AML/MDS/MPN	Yes-poor AML prognosis	Yes-poor AML prognosis		Yes-good AML prognosis
Glioma	Yes	Yes		No
Chondrosarcoma	Yes	Yes		No
Cholangiocarinoma	Yes	Yes		No
T-cell angioimmunoblastic lymphoma	No	Yes		rare