INVENTORY OF SUPPLEMENTAL INFORMATION

Supplemental Data

Supplemental Experimental Procedures

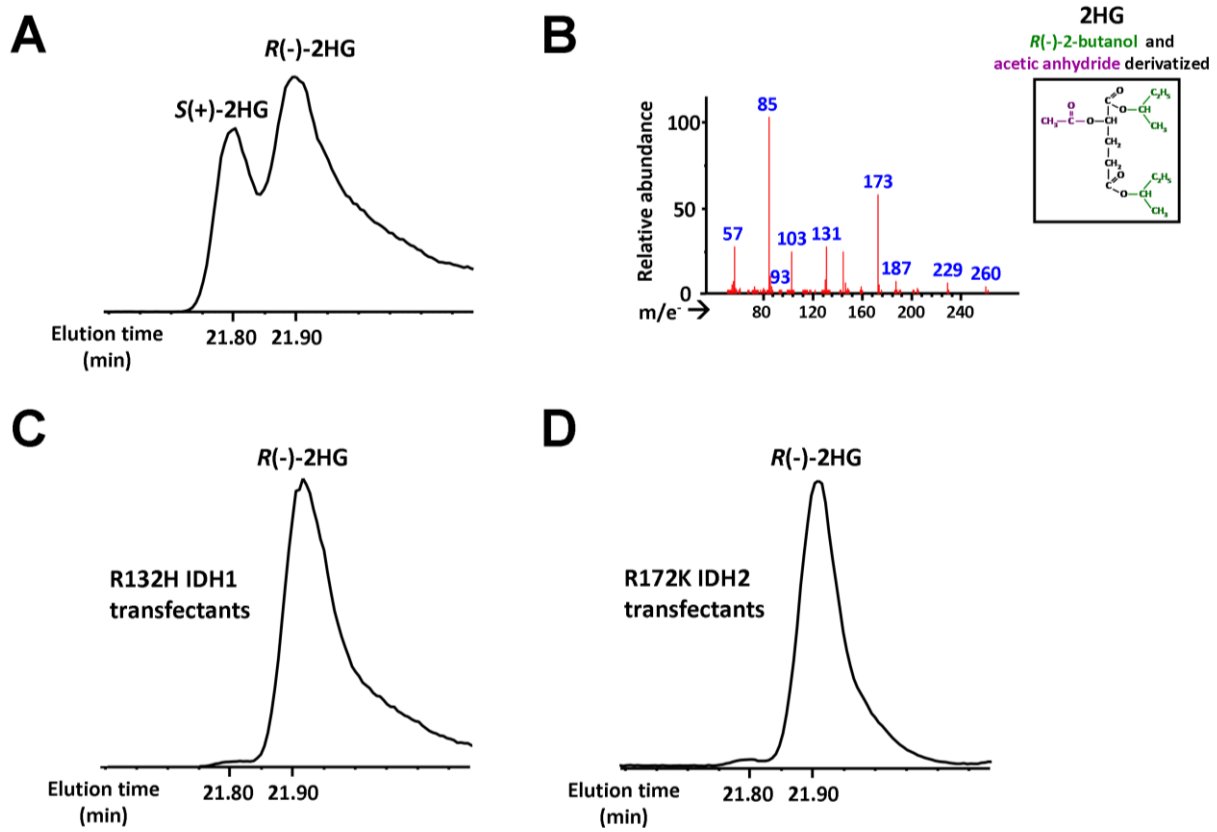


Figure S1. **Relates to Figure 3. Expression of R172K mutant IDH2 specifically produces the (*R*) enantiomer of 2HG.** (**A**) Separation of the (*R*) and (*S*) enantiomers of 2HG as the O-acetylated di-(-)-2-butyl esters was demonstrated using a two step derivatization method with *R*(-)-2-butanolic HCl followed by acetic anhydride on commercial *S*(+)-2HG and *R*(-)-2HG standards. (**B**) Representative mass spectrum of the metabolites eluting at 21.8 and 21.9 minutes, confirming their identities as the derivatized 2HG that is structurally depicted in the inset. Esterified butyl groups added during the first derivatization step with *R*(-)-2-butanolic HCl are shown in green, and the acetyl group added to the 2-hydroxyl during the second derivatization step with acetic anhydride is shown in violet. (**C**) and (**D**) Cells expressing R132H mutant IDH1 (C) or R172K mutant IDH2 (D) were extracted for analysis of intracellular metabolites. After purification of organic acids, followed by the two step derivatization method described in (A), samples were analyzed by GC-MS to determine the chirality of the 2HG produced.

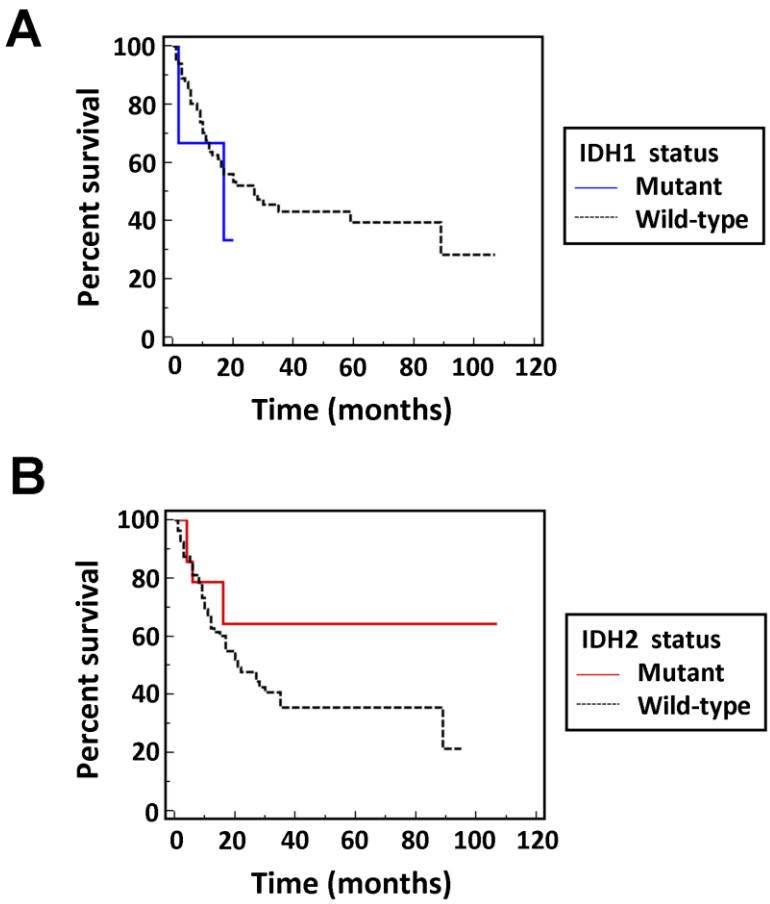


Figure S2. Relates to Table 2. Effect of IDH1 and IDH2 mutations on survival after diagnosis of acute myeloid leukemia. (**A**) Kaplan-Meier survival curves for patients with IDH1 mutations and for patients wild-type for IDH1. IDH1 mutant and wild-type patients did not differ in overall survival (p=0.37). (**B**) Kaplan-Meier survival curves for patients with IDH2 mutations and for patients wild-type for IDH2. There was a trend for improved overall survival in patients with IDH2 mutations compared to IDH2 wild-type patients (p=0.08).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Determination of 2HG chirality

For distinguishing the (*R*) and (*S*) enantiomers of 2HG, a variant of a previously described extended derivatization procedure was used (Kamerling et al., 1981). Dried HCl eluates from organic acid purification were resuspended in 1M HCl in *R*(-)-2-butanol, heated for 3 h at 95 ºC, and dried under nitrogen gas. Samples were then redissolved in 1:1 pyridine:acetic anhydride, heated for 30 min at 100 ºC, dried, and redissolved in acetonitrile before analysis on an Agilent 7890A GC with an HP-5MS capillary column as described previously. Identification of *S*(+)-2HG and *R*(-)-2HG was confirmed using standards obtained from Sigma.

Kaplan-Meier survival analysis

Survival was calculated for each patient from the time of diagnosis, according to last follow-up or according to death as noted in the medical record. Statistical analysis was performed using MedCalc (MedCalc, Belgium). All p-values were two-tailed. Survival curves for patients with and without IDH1 and IDH2 mutations were constructed by Kaplan-Meier method taking the interval from the date of diagnosis to death and compared using the log-rank test.

Figure S1.

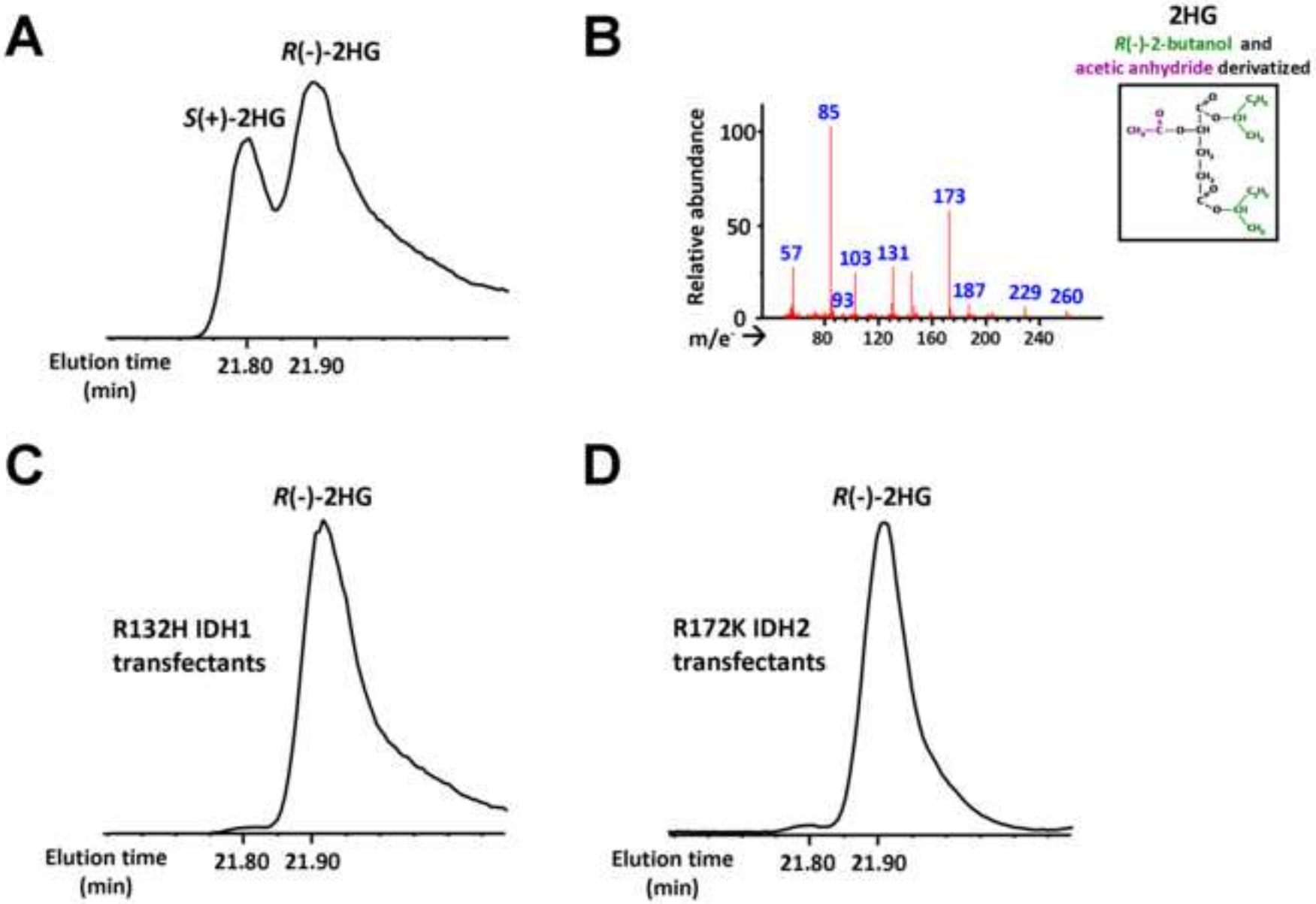
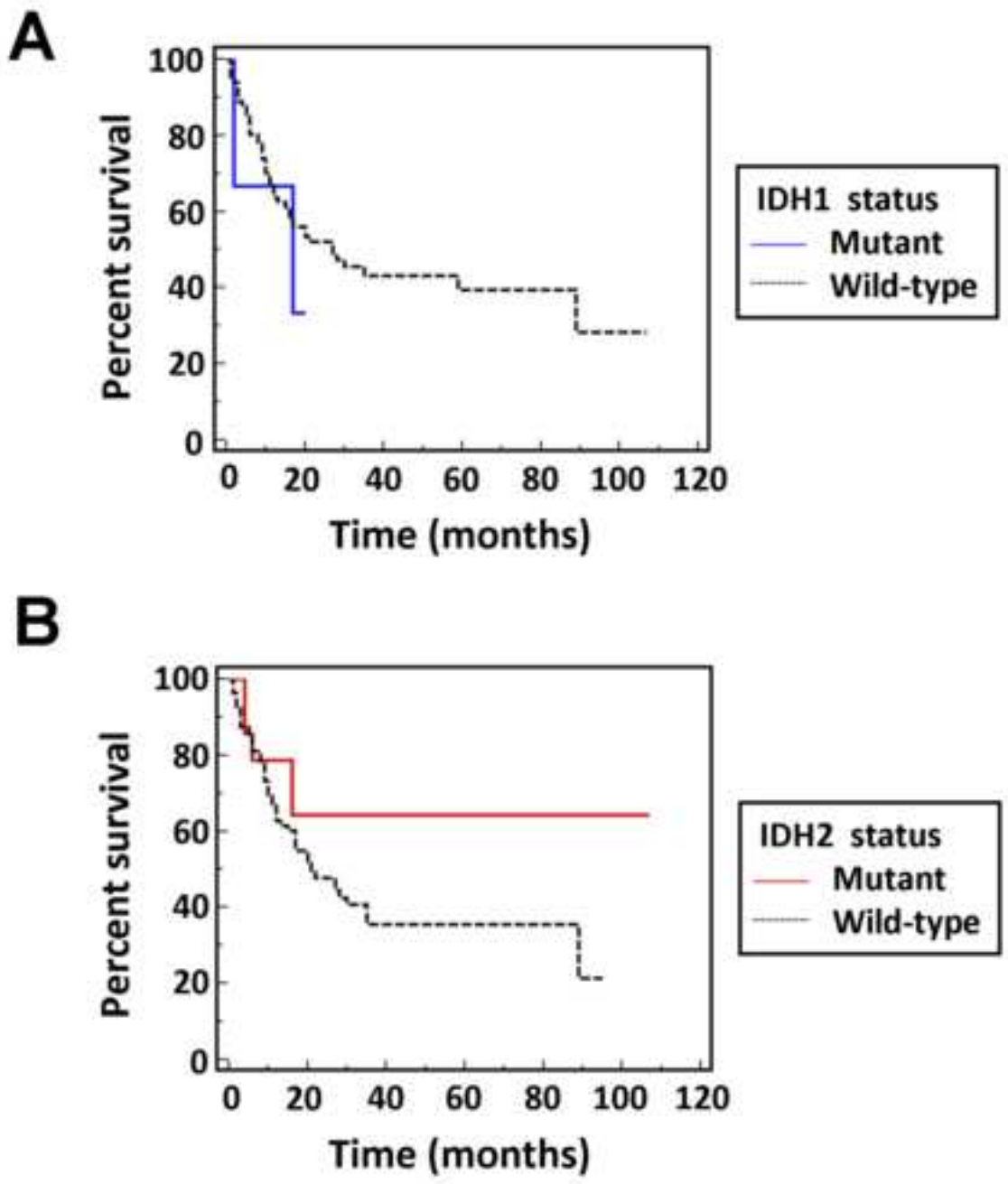

A
R(-)-2HG
S(+)-2HG
Elution time (min)
21.80 21.90
B
2HG
R(-)-2-butanol and acetic anhydride derivatized
Relative abundance
100
50
0
m/e
80 120 160 200 240
85
173
57
103
131
93
187
229
260
C
R(-)-2HG
R132H IDH1 transfectants
Elution time (min)
21.80 21.90
D
R(-)-2HG
R172K IDH2 transfectants
Elution time (min)
21.80 21.90

Figure S2.


A
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (months)
IDH1 status
Mutant
Wild-type
B
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (months)
IDH2 status
Mutant
Wild-type