R-2-hydroxyglutarate attenuates aerobic glycolysis in leukemia by targeting the FTO/m^{6}A/*PFKP*/*LDHB* axis

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Supplemental Information:

I. Supplemental Figures

Figure S1 (related to Figure 1)

Figure S2 (related to Figure 2)

Figure S3 (related to Figure 3)

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Figure S5 (related to Figure 5)

Figure S6 (related to Figure 6)

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II. Supplemental Tables

Table S1 (related to Figure 1). Metabolite levels in NOMO-1 cells treated with PBS or R-2HG

Table S2 (related to Figure 1). Metabolite levels in NB4 cells treated with PBS or R-2HG

Table S3 (related to Figure S1). Metabolite levels in NOMO-1 IDH1^{R132H} cells treated with PBS or doxycycline

Table S4 (related to Figure 3). Metabolite levels in NOMO-1 cells stably expressing shNS or shFTO-2

Table S5 (related to Figure 4). List of the 35 glycolysis- and gluconeogenesis-related genes.

Table S6 (related to Figures 7 and S7). Information of the AML patient samples used in the study.

Table S7 (related to Star Methods). List of oligonucleotides

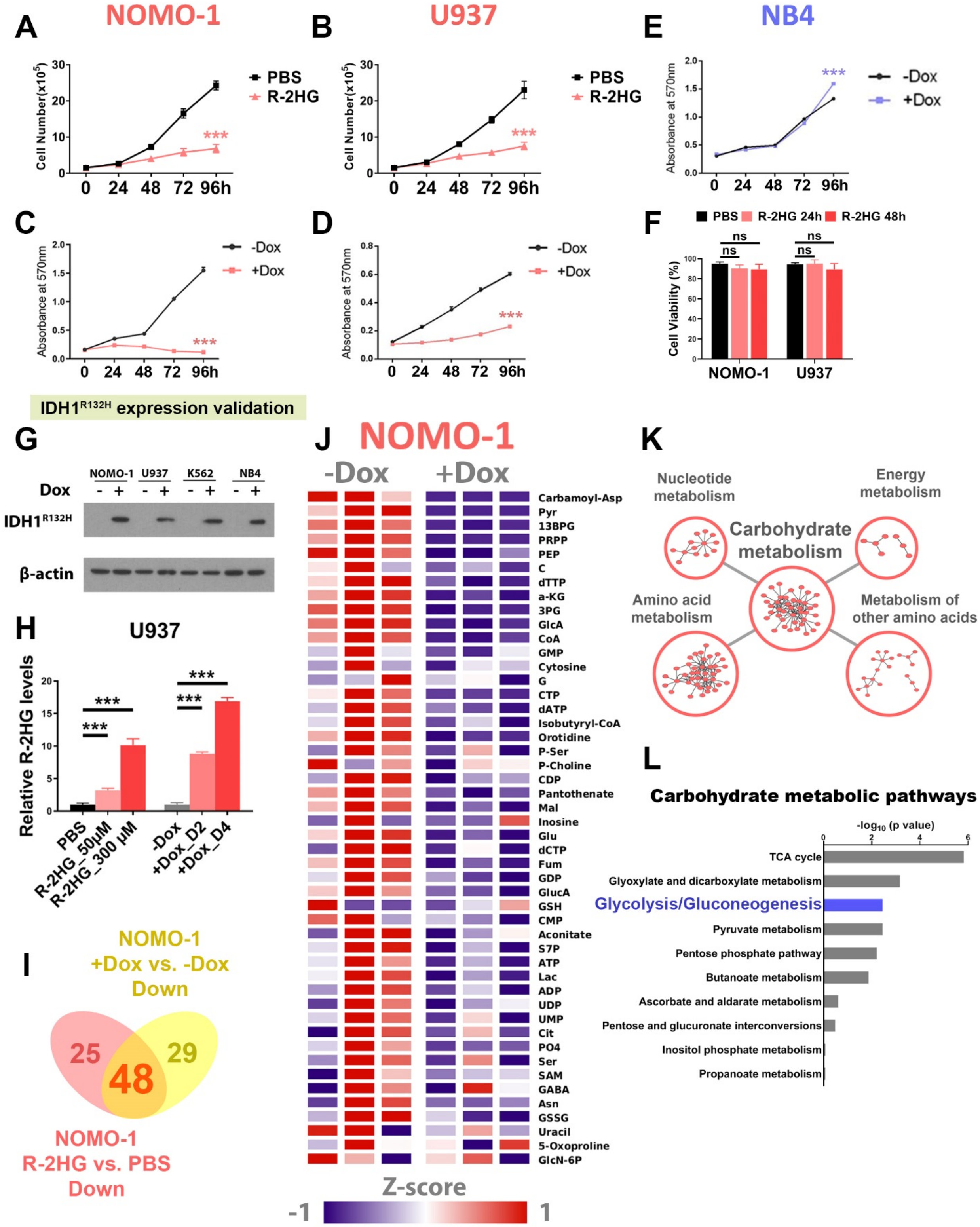
A
NOMO-1
Cell Number(x10^5)
PBS
R-2HG
***
0 24 48 72 96h
B
U937
Cell Number(x10^5)
PBS
R-2HG
***
0 24 48 72 96h
E
NB4
Absorbance at 570nm
-Dox
+Dox
***
0 24 48 72 96h
C
Absorbance at 570nm
-Dox
+Dox
***
0 24 48 72 96h
D
Absorbance at 570nm
-Dox
+Dox
***
0 24 48 72 96h
F
PBS
R-2HG 24h
R-2HG 48h
ns
Cell Viability (%)
NOMO-1
U937
IDH1R132H expression validation
G
NOMO-1
U937
K562
NB4
Dox
- + - + - + - +
IDH1R132H
β-actin
H
U937
Relative R-2HG levels
***
PBS
R-2HG_50μM
R-2HG_300 μM
-Dox
+Dox_D2
+Dox_D4
I
NOMO-1 +Dox vs. -Dox Down
25
48
29
NOMO-1 R-2HG vs. PBS Down
J
NOMO-1
-Dox
+Dox
Carbamoyl-Asp
Pyr
13BPG
PRPP
PEP
C
dTTP
a-KG
3PG
GlcA
CoA
GMP
Cytosine
G
CTP
dATP
Isobutyryl-CoA
Orotidine
P-Ser
P-Choline
CDP
Pantothenate
Mal
Inosine
Glu
dCTP
Fum
GDP
GlucA
GSH
CMP
Aconitate
S7P
ATP
Lac
ADP
UDP
UMP
Cit
PO4
Ser
SAM
GABA
Asn
GSSG
Uracil
5-Oxoproline
GlcN-6P
Z-score
-1
1
K
Nucleotide metabolism
Energy metabolism
Carbohydrate metabolism
Amino acid metabolism
Metabolism of other amino acids
L
Carbohydrate metabolic pathways
-log10 (p value)
0 2 4 6
TCA cycle
Glyoxylate and dicarboxylate metabolism
Glycolysis/Gluconeogenesis
Pyruvate metabolism
Pentose phosphate pathway
Butanoate metabolism
Ascorbate and aldarate metabolism
Pentose and glucuronate interconversions
Inositol phosphate metabolism
Propanoate metabolism

Figure S1 (related to Figure 1). Effects of exogenous and endogenous R-2HG on proliferation and cellular metabolism in leukemia cells

(A and B) Effects of exogenous R-2HG on cell proliferation/growth in R-2HG-sensitive NOMO-1 (A) and U937 (B) cells as determined by cell counting. 300 μM R-2HG were added to cell culture media at 0 h. The p values are calculated for the last time point (96 hours after cell seeding).

(C-E) Effects of endogenous R-2HG generated by doxycycline-induced IDH1^{R132H} on cell proliferation/growth in NOMO-1 (C), U937 (D), and NB4 (E) cells. Dox: doxycycline.

(F) Effects of exogenous R-2HG on cell viability in NOMO-1 and U937 cells, as determined by trypan blue staining. Cells were exposed to 300 μM R-2HG for the indicated time points.

(G) Doxycycline-induced IDH1^{R132H} expression was validated by Western blot with IDH1^{R132H}-specific antibody in NOMO-1, U937, K562, and NB4 cells. β-actin is the endogenous control for different samples.

(H) The intracellular R-2HG accumulation was confirmed after 48 hours of exogenous R-2HG treatment at indicated concentrations, and at 2 days and 4 days post doxycycline induction.

(I) Overlap analysis of the metabolites which are downregulated in NOMO-1 cells upon exogenous R-2HG (300 μM) or doxycycline-induced endogenous R-2HG treatment as detected by metabolomic assays. The 48 commonly downregulated metabolites were screened out and subject to further pathway enrichment analysis (see Figures S1J-S1L).

(J) Heatmap showing relative intracellular level changes of the 48 metabolites downregulated in both exogenous and endogenous R-2HG-treated NOMO-1 cells (see Figure S1I). Data are represented as normalized intracellular levels.

(K and L) Pathway enrichment analysis of the 48 metabolites downregulated in both exogenous and endogenous R-2HG-treated NOMO-1 cells identifies carbohydrate metabolism as one of the top enriched KEGG metabolic pathway classes (K), and glycolysis as one of the top enriched carbohydrate metabolic pathways (L).

Data are represented as mean ± SD. ns, not significant ($p \geqslant 0.05$); ***, $p < 0.001$; two-tailed student t-test.

See also Table S3.

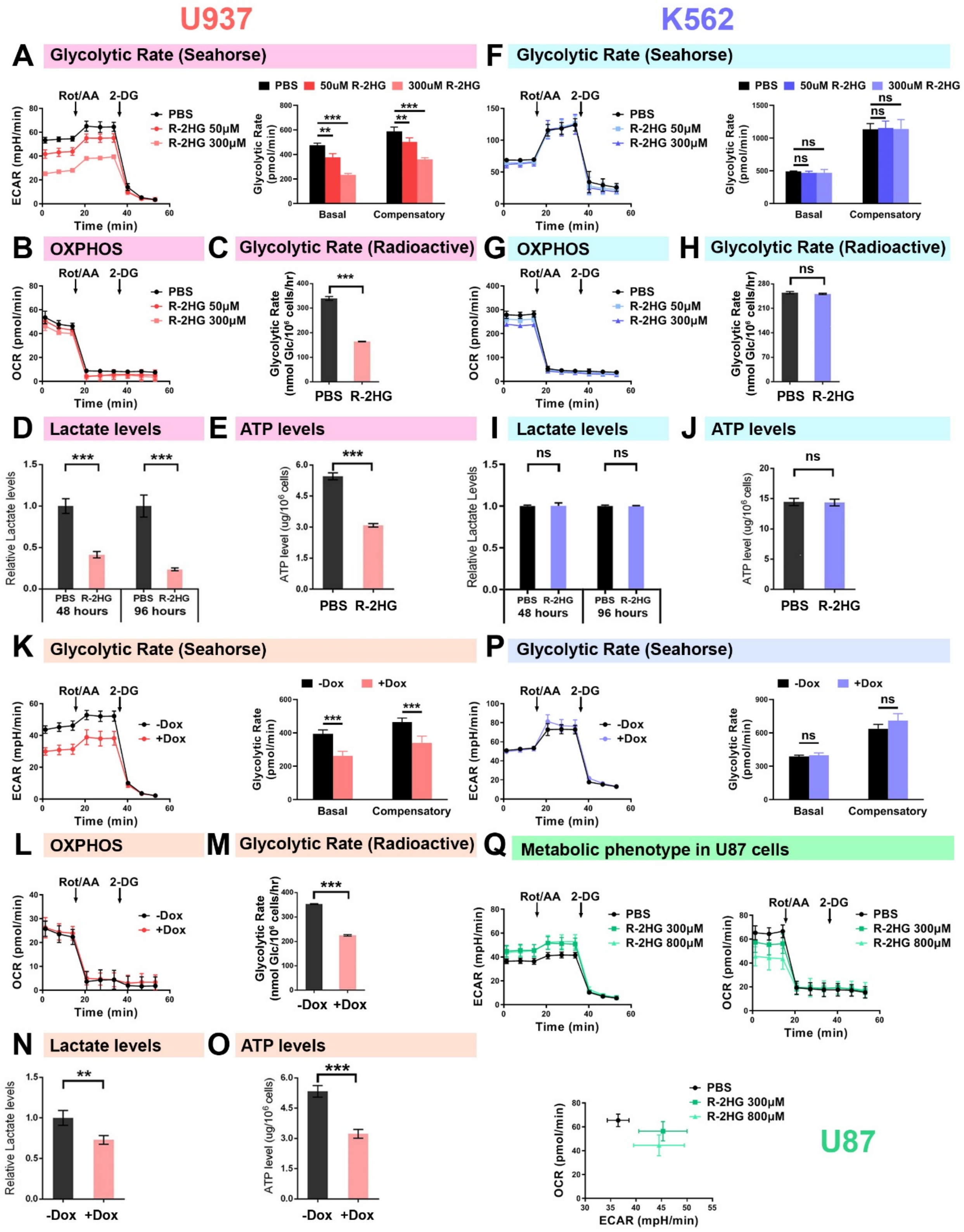
U937
K562
A Glycolytic Rate (Seahorse)
Rot/AA 2-DG
ECAR (mpH/min)
Time (min)
PBS
R-2HG 50μM
R-2HG 300μM
PBS 50uM R-2HG 300uM R-2HG
Glycolytic Rate (pmol/min)
Basal
Compensatory
** ***
F Glycolytic Rate (Seahorse)
PBS 50uM R-2HG 300uM R-2HG
ns
B OXPHOS
OCR (pmol/min)
C Glycolytic Rate (Radioactive)
Glycolytic Rate (nmol Glc/10⁶ cells/hr)
PBS R-2HG
G OXPHOS
H Glycolytic Rate (Radioactive)
D Lactate levels
Relative Lactate levels
48 hours
96 hours
E ATP levels
ATP level (ug/10⁶ cells)
I Lactate levels
Relative Lactate Levels
J ATP levels
K Glycolytic Rate (Seahorse)
-Dox
+Dox
P Glycolytic Rate (Seahorse)
L OXPHOS
M Glycolytic Rate (Radioactive)
Q Metabolic phenotype in U87 cells
PBS
R-2HG 300μM
R-2HG 800μM
N Lactate levels
O ATP levels
ECAR (mpH/min)
OCR (pmol/min)
U87

Figure S2 (related to Figure 2). Both exogenous and endogenous R-2HG target glycolysis in sensitive U937, but not resistant K562 cells, to inhibit cellular metabolism

(A) Exogenous R-2HG treatment reduced glycolytic rates in a dose-dependent manner in U937 cells, as measured by the Seahorse Glycolytic Rate Assay. U937 cells were pre-treated with PBS or R-2HG for 24 hours prior to the assay. ECAR measured over time (left panel) and the calculated basal and compensatory glycolytic rates (right panel) are shown.

(B) Effect of exogenous R-2HG treatment on mitochondrial respiration in U937 cells.

(C) Effect of exogenous R-2HG on glycolysis as determined by radioactive glycolysis assay in U937 cells.

(D) Effects of exogenous R-2HG on lactate levels in U937 cells. The cells were exposed to 300 μM R-2HG for 48 hours.

(E) Effects of exogenous R-2HG on ATP levels in U937 cells. The cells were exposed to 300μM R-2HG for 48 hours.

(F) Effects of exogenous R-2HG on glycolytic rates as determined by the Seahorse Glycolytic Rate Assay in K562 cells. The cells were treated with PBS or R-2HG at the indicated concentrations for 24 hours prior to measurement. ECAR over time (left panel) and the calculated glycolytic rates (right panel) are shown.

(G) Effects of exogenous R-2HG on OCR in K562 cells. The cells were treated with R-2HG for 24 hours.

(H) Effects of exogenous R-2HG on glycolytic rates as detected by radioactive glycolysis assay in K562 cells. The cells were treated with PBS or 300 μM R-2HG for 48 hours prior to measurement.

(I) Effects of exogenous R-2HG on lactate levels in K562 cells. The cells were exposed to 300 μM R-2HG for 48 hours.

(J) Effects of exogenous R-2HG on ATP levels in K562 cells. The cells were exposed to 300 μM R-2HG for 48 hours.

(K) Effects of endogenous R-2HG produced by IDH1^{R132H} on glycolytic rates in U937 cells, as determined by the Seahorse Glycolytic Rate Assay. The cells were treated with PBS or doxycycline for 48 hours to induce IDH1^{R132H} expression prior to measurement. ECAR over time (left panel) and the calculated glycolytic rates (right panel) are shown.

(L-O) Effects of endogenous R-2HG on mitochondrial respiration (L), glycolytic rates (M, as detected by radioactive glycolysis assay), lactate levels (N), and ATP levels (O) in U937 cells.

(P) Effects of endogenous R-2HG generated by IDH1^{R132H} on glycolytic rates as determined by the Seahorse Glycolytic Rate Assay in K562 cells. The cells were treated with PBS or doxycycline for 48 hours to induce IDH1^{R132H} expression prior to measurement. ECAR over time (left panel) and the calculated glycolytic rates (right panel) are shown.

(Q) Effects of exogenous R-2HG treatment on glycolytic rates (upper left panel) and mitochondrial respiration (upper right panel) in the GBM cell line U87. ECAR and OCR data are plotted on the energy map (lower panel) to reveal the overall basal metabolic profiles of cells with or without R-

2HG treatment. U87 cells were pre-treated with PBS or R-2HG (at indicated concentrations) for 1 hour prior to the assay.

Data are represented as mean ± SD. ns, not si+gnificant ($p \geqslant 0.05$); **, $p < 0.01$; ***, $p < 0.001$; two-tailed student t-test.

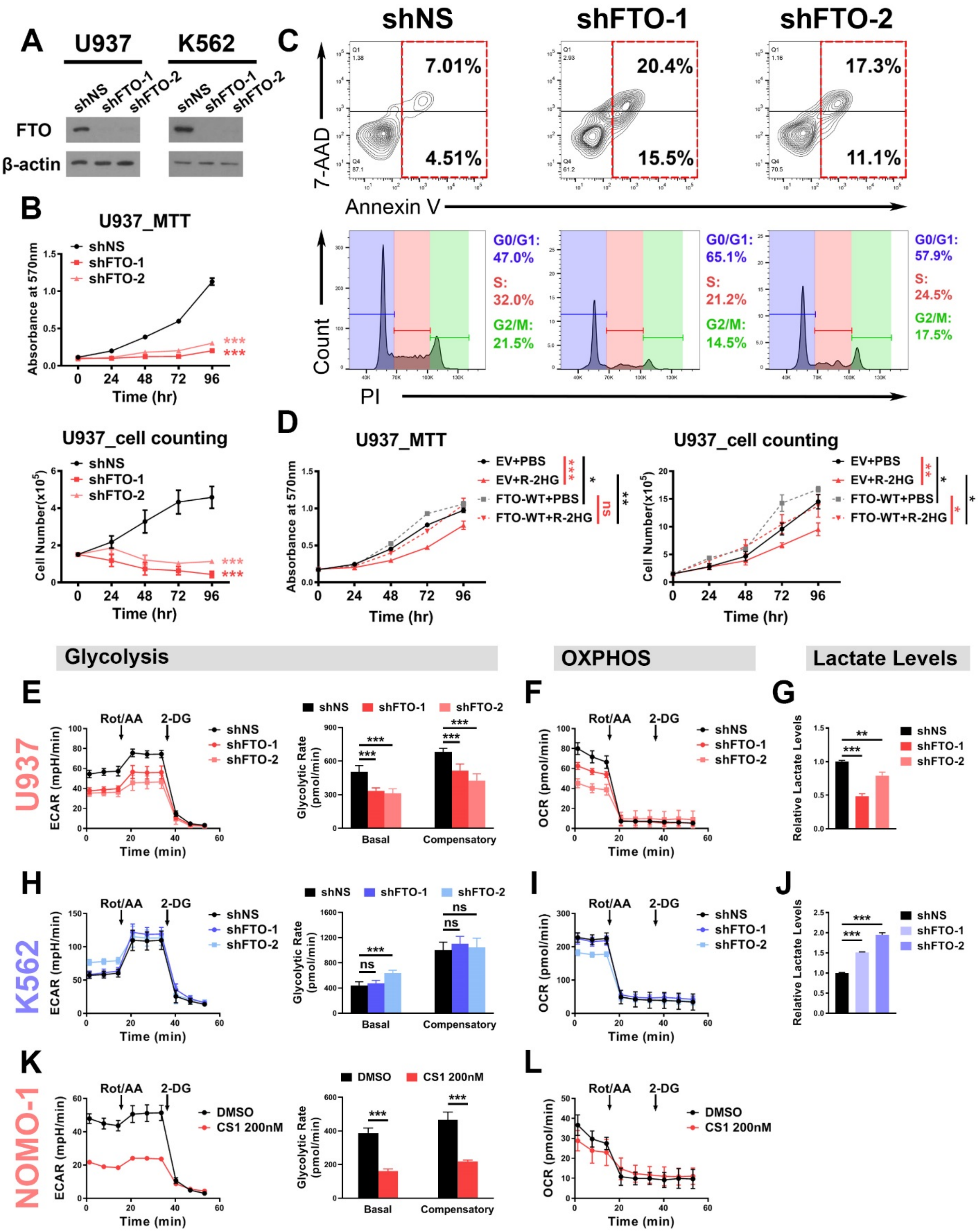
A
U937
K562
shNS
shFTO-1
shFTO-2
FTO
β-actin
B
U937_MTT
Absorbance at 570nm
Time (hr)
***
U937_cell counting
Cell Number(x10^5)
C
shNS
shFTO-1
shFTO-2
7.01%
4.51%
20.4%
15.5%
17.3%
11.1%
7-AAD
Annexin V
G0/G1: 47.0%
S: 32.0%
G2/M: 21.5%
G0/G1: 65.1%
S: 21.2%
G2/M: 14.5%
G0/G1: 57.9%
S: 24.5%
G2/M: 17.5%
Count
PI
D
U937_MTT
U937_cell counting
EV+PBS
EV+R-2HG
FTO-WT+PBS
FTO-WT+R-2HG
ns
Glycolysis
OXPHOS
Lactate Levels
E
U937
Rot/AA
2-DG
ECAR (mpH/min)
Time (min)
Glycolytic Rate (pmol/min)
Basal
Compensatory
F
OCR (pmol/min)
G
Relative Lactate Levels
H
K562
I
J
K
NOMO-1
DMSO
CS1 200nM
L

Figure S3 (related to Figure 3). FTO is required to maintain cell proliferation/survival and aerobic glycolysis in sensitive leukemia cells

(A) *FTO* knockdown efficiency is confirmed by Western blots in U937 and K562 cells.

(B) Effects of *FTO* knockdown on cell proliferation/growth in U937 cells, as determined by MTT (upper panel) and cell counting assays (lower panel). The p values are calculated for the last time point (96 hours after cell seeding).

(C) Effects of *FTO* knockdown on apoptosis (upper row) and cell-cycle (lower row) in U937 cells, as determined by flow cytometry.

(D) Overexpression of wild-type FTO rescued the growth inhibition induced by R-2HG (100 μM) in U937 cells. Cell proliferation/growth was evaluated by both MTT assay (left panel) and cell counting (right panel).

(E-G) Effects of *FTO* knockdown on basal and compensatory glycolysis (E), mitochondrial respiration (F), and lactate levels (G) in U937 cells.

(H-J) Effects of *FTO* knockdown on basal and compensatory glycolysis (H), mitochondrial respiration (I), and lactate levels (J) in K562 cells.

(K and L) Effects of the FTO inhibitor, CS1, on basal and compensatory glycolysis (K) and mitochondrial respiration (L).

Data are represented as mean ± SD. ns, not significant ($p \geqslant 0.05$); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; two-tailed student t-test.

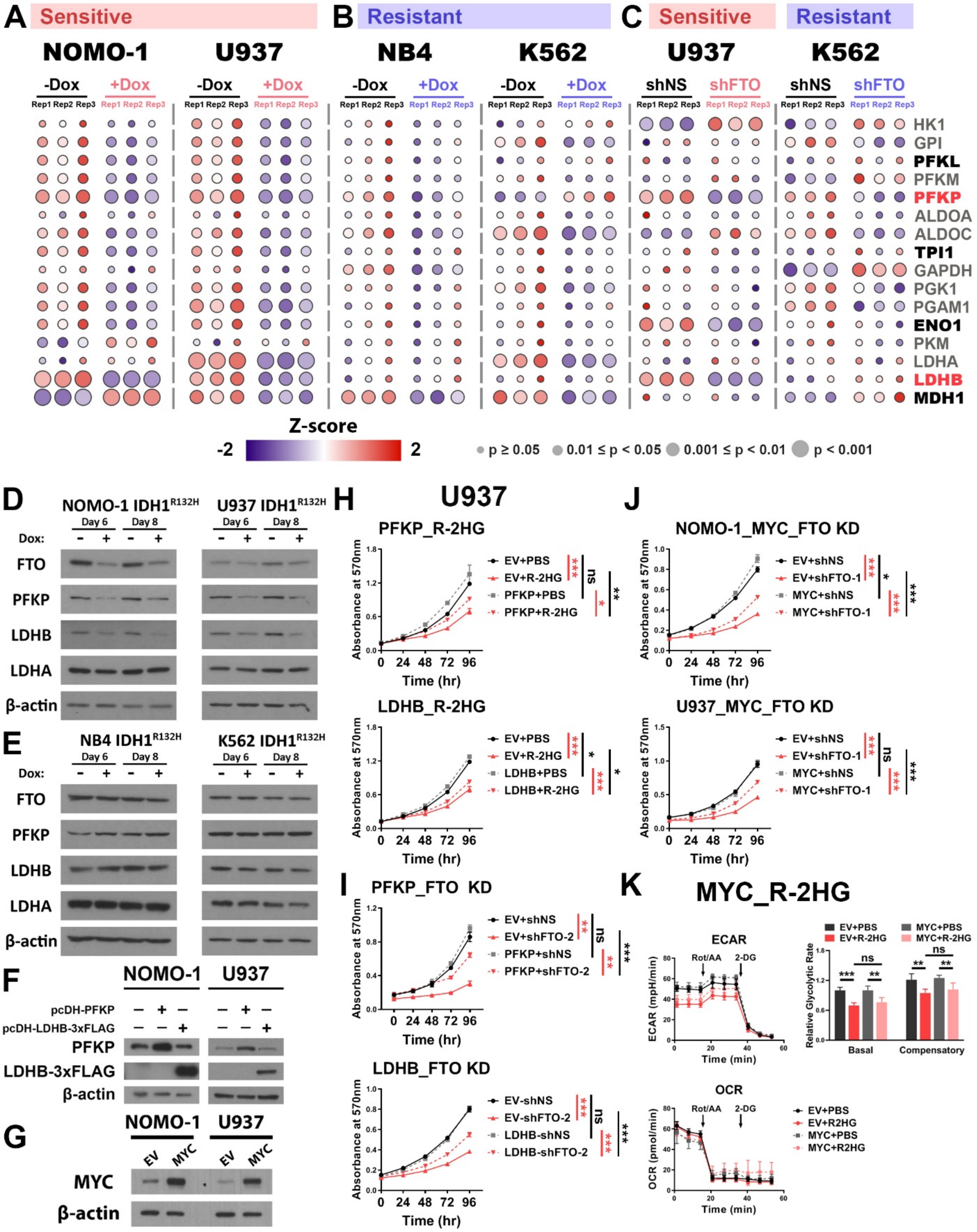
A Sensitive
NOMO-1
U937
-Dox
+Dox
Rep1 Rep2 Rep3
B Resistant
NB4
K562
C Sensitive
Resistant
U937
K562
shNS
shFTO
HK1
GPI
PFKL
PFKM
PFKP
ALDOA
ALDOC
TPI1
GAPDH
PGK1
PGAM1
ENO1
PKM
LDHA
LDHB
MDH1
Z-score
-2
2
p ≥ 0.05
0.01 ≤ p < 0.05
0.001 ≤ p < 0.01
p < 0.001
D
NOMO-1 IDH1^{R132H}
U937 IDH1^{R132H}
Day 6
Day 8
Dox:
FTO
PFKP
LDHB
LDHA
β-actin
E
NB4 IDH1^{R132H}
K562 IDH1^{R132H}
F
NOMO-1
U937
pcDH-PFKP
pcDH-LDHB-3xFLAG
PFKP
LDHB-3xFLAG
β-actin
G
NOMO-1
U937
EV
MYC
MYC
β-actin
H
U937
PFKP_R-2HG
Absorbance at 570nm
Time (hr)
EV+PBS
EV+R-2HG
PFKP+PBS
PFKP+R-2HG
ns
LDHB_R-2HG
LDHB+PBS
LDHB+R-2HG
I
PFKP_FTO KD
EV+shNS
EV+shFTO-2
PFKP+shNS
PFKP+shFTO-2
LDHB_FTO KD
EV-shNS
EV-shFTO-2
LDHB-shNS
LDHB-shFTO-2
J
NOMO-1_MYC_FTO KD
EV+shNS
EV+shFTO-1
MYC+shNS
MYC+shFTO-1
U937_MYC_FTO KD
K
MYC_R-2HG
ECAR
ECAR (mpH/min)
Rot/AA
2-DG
Time (min)
EV+PBS
EV+R-2HG
MYC+PBS
MYC+R-2HG
Relative Glycolytic Rate
Basal
Compensatory
OCR
OCR (pmol/min)
EV+PBS
EV+R2HG
MYC+PBS
MYC+R2HG

Figure S4 (related to Figure 4). PFKP and LDHB are critical downstream metabolic targets of the R-2HG/FTO axis in sensitive leukemia cells

(A and B) Relative expression of the screened out genes (see Figure 4A) along with other glycolytic enzymes in sensitive cells (A) and resistant cells (B) with or without doxycycline-induced endogenous R-2HG production, as determined by RT-qPCR. The color represents normalized expression level of a given gene; the diameter indicates significance of the difference between PBS- and doxycycline-treated samples. The screened out genes are marked in bold, with the identified two targets PFKP and LDHB highlighted in red. Among all tested genes, HK1, PFKL, PFKM, PFKP, PKM, LDHA, and LDHB are glycolysis-related, while MDH1 is gluconeogenesis-related; all other genes are related to both glycolysis and gluconeogenesis.

(C) Relative expression of the screened out genes (see Figure 4A) along with other glycolytic enzymes in sensitive U937 cells and resistant K562 cells with or without *FTO* knockdown, as determined by RT-qPCR. The screened out genes are marked in bold, with the identified two targets PFKP and LDHB highlighted in red. Among all tested genes, HK1, PFKL, PFKM, PFKP, PKM, LDHA, and LDHB are glycolysis-related, while MDH1 is gluconeogenesis-related; all other genes are related to both glycolysis and gluconeogenesis.

(D and E) Western blot validation of the protein levels of FTO, PFKP, LDHB, and LDHA in sensitive cells (D) and resistant cells (E) in the presence or absence of endogenous R-2HG. Samples were collected at two time points: 6 days and 8 days after addition of PBS or doxycycline to the cell culture medium.

(F) Confirmation of overexpression efficiency of *PFKP* and *LDHB* by Western blots in NOMO-1 and U937 cells.

(G) Confirmation of overexpression efficiency of *MYC* by Western blots in NOMO-1 and U937 cells. EV: empty vector.

(H) Cell proliferation/growth was evaluated by MTT assays in PBS- and 100 μM R-2HG-treated U937 cells, with or without forced expression of *PFKP* (upper panel) or *LDHB* (lower panel). The same control groups (EV+PBS and EV+R-2HG) were used for the analysis.

(I) Cell proliferation/growth was evaluated in control and *FTO*-knockdown U937 cells with or without forced expression of *PFKP* (upper panel) or *LDHB* (lower panel).

(J) Cell proliferation/growth was evaluated in control and *FTO*-knockdown NOMO-1 (upper panel) and U937 (lower panel) cells, with or without forced expression of *MYC*.

(K) Effects of MYC overexpression on basal/compensatory glycolytic rates (upper panel) and mitochondrial respiration (lower panel) in 50 μM R-2HG-treated U937 cells.

Data are represented as mean ± SD. ns, not significant ($p \geqslant 0.05$); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; two-tailed student t-test.

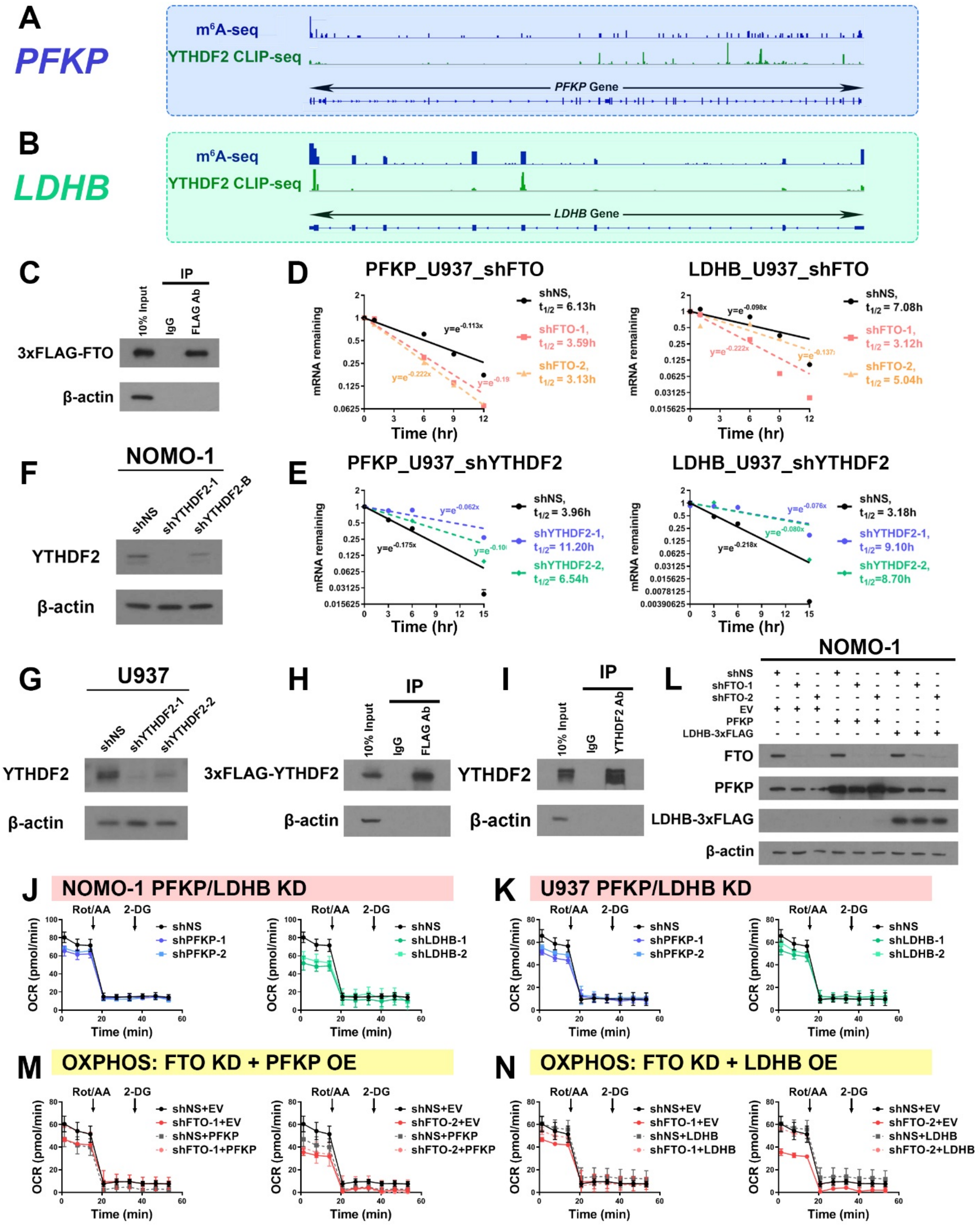
A
PFKP
m6A-seq
YTHDF2 CLIP-seq
PFKP Gene
B
LDHB
m6A-seq
YTHDF2 CLIP-seq
LDHB Gene
C
IP
10% Input
IgG
FLAG Ab
3xFLAG-FTO
β-actin
D
PFKP_U937_shFTO
mRNA remaining
Time (hr)
y=e-0.113x
y=e-0.222x
y=e-0.19
shNS, t1/2 = 6.13h
shFTO-1, t1/2 = 3.59h
shFTO-2, t1/2 = 3.13h
LDHB_U937_shFTO
y=e-0.098x
y=e-0.222x
y=e-0.137
shNS, t1/2 = 7.08h
shFTO-1, t1/2 = 3.12h
shFTO-2, t1/2 = 5.04h
E
PFKP_U937_shYTHDF2
y=e-0.062x
y=e-0.175x
y=e-0.10
shNS, t1/2 = 3.96h
shYTHDF2-1, t1/2= 11.20h
shYTHDF2-2, t1/2= 6.54h
LDHB_U937_shYTHDF2
y=e-0.076x
y=e-0.080x
y=e-0.218x
shNS, t1/2 = 3.18h
shYTHDF2-1, t1/2= 9.10h
shYTHDF2-2, t1/2=8.70h
F
NOMO-1
shNS
shYTHDF2-1
shYTHDF2-B
YTHDF2
β-actin
G
U937
shNS
shYTHDF2-1
shYTHDF2-2
YTHDF2
β-actin
H
IP
10% Input
IgG
FLAG Ab
3xFLAG-YTHDF2
β-actin
I
IP
10% Input
IgG
YTHDF2 Ab
YTHDF2
β-actin
L
NOMO-1
shNS
shFTO-1
shFTO-2
EV
PFKP
LDHB-3xFLAG
FTO
PFKP
LDHB-3xFLAG
β-actin
J
NOMO-1 PFKP/LDHB KD
Rot/AA
2-DG
OCR (pmol/min)
Time (min)
shNS
shPFKP-1
shPFKP-2
shLDHB-1
shLDHB-2
K
U937 PFKP/LDHB KD
M
OXPHOS: FTO KD + PFKP OE
shNS+EV
shFTO-1+EV
shNS+PFKP
shFTO-1+PFKP
shFTO-2+EV
shFTO-2+PFKP
N
OXPHOS: FTO KD + LDHB OE
shNS+LDHB
shFTO-1+LDHB
shFTO-2+LDHB

Figure S5 (related to Figure 5). The FTO/m^{6}A/YTHDF2 axis regulates *PFKP* and *LDHB* expression

(A and B) Genome sequencing tracks show the m^{6}A peaks (upper panel) and YTHDF2 binding to the m^{6}A-modified regions (lower panel) in *PFKP* (A) and *LDHB* (B) transcripts.

(C) Confirmation of immunoprecipitation (IP) efficiency and specificity of 3×FLAG-FTO with FLAG antibody.

(D) RNA stability of *PFKP* (left panel) and *LDHB* (right panel) transcripts in U937 cells upon *FTO* knockdown.

(E) RNA stability of *PFKP* (left panel) and *LDHB* (right panel) transcripts in U937 cells upon *YTHDF2* knockdown.

(F and G) Validation of *YTHDF2* knockdown efficiency in NOMO-1 (F) and U937 (G) cells via Western blots.

(H) Confirmation of immunoprecipitation (IP) efficiency and specificity of 3×FLAG-YTHDF2 with FLAG antibody in HEK-293T cells.

(I) Confirmation of immunoprecipitation (IP) efficiency and specificity of endogenous YTHDF2 with YTHDF2 antibody in NOMO-1 cells.

(J) Mitochondrial respiration in NOMO-1 cells upon *PFKP* (left panel) or *LDHB* (right panel) knockdown. The same control shNS group was used for the analysis.

(K) Mitochondrial respiration in U937 cells upon *PFKP* (left panel) or *LDHB* (right panel) knockdown. The same control shNS group was used for the analysis.

(L) Knockdown of *FTO* and forced expression of *PFKP* and LDHB-3xFLAG in NOMO-1 cells was confirmed by Western blots.

(M) Effects of *FTO* knockdown with or without *PFKP* overexpression on mitochondrial respiration in NOMO-1 cells. The same control groups (shNS+EV and shNS+PFKP) were used for the analysis.

(N) Effects of *FTO* knockdown with or without *LDHB* overexpression on mitochondrial respiration in NOMO-1 cells. The same control groups (shNS+EV and shNS+LDHB) were used for the analysis.

Data are represented as mean ± SD.

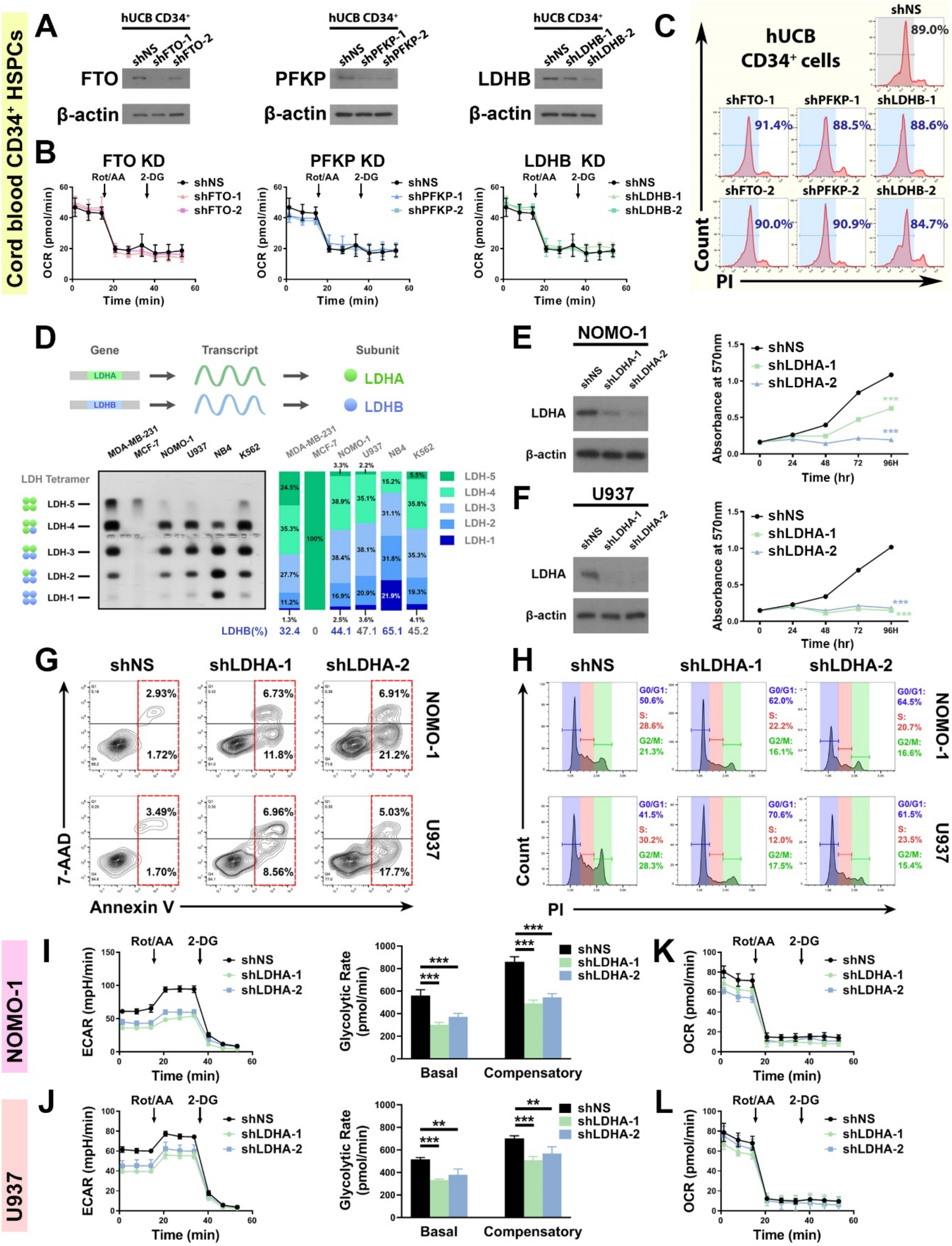
Cord blood CD34⁺ HSPCs
A
hUCB CD34⁺
shNS
shFTO-1
shFTO-2
FTO
β-actin
shPFKP-1
shPFKP-2
PFKP
shLDHB-1
shLDHB-2
LDHB
B
FTO KD
PFKP KD
LDHB KD
Rot/AA
2-DG
OCR (pmol/min)
Time (min)
C
hUCB CD34⁺ cells
89.0%
91.4%
88.5%
88.6%
90.0%
90.9%
84.7%
Count
PI
D
Gene
Transcript
Subunit
LDHA
LDHB
MDA-MB-231
MCF-7
NOMO-1
U937
NB4
K562
LDH Tetramer
LDH-5
LDH-4
LDH-3
LDH-2
LDH-1
LDHB(%) 32.4 0 44.1 47.1 65.1 45.2
E
NOMO-1
shLDHA-1
shLDHA-2
LDHA
Absorbance at 570nm
Time (hr)
96H
F
U937
G
2.93%
1.72%
6.73%
11.8%
6.91%
21.2%
3.49%
1.70%
6.96%
8.56%
5.03%
17.7%
7-AAD
Annexin V
H
G0/G1: 50.6%
S: 28.6%
G2/M: 21.3%
G0/G1: 62.0%
S: 22.2%
G2/M: 16.1%
G0/G1: 64.5%
S: 20.7%
G2/M: 16.6%
G0/G1: 41.5%
S: 30.2%
G2/M: 28.3%
G0/G1: 70.6%
S: 12.0%
G2/M: 17.5%
G0/G1: 61.5%
S: 23.5%
G2/M: 15.4%
I
J
K
L
ECAR (mpH/min)
Glycolytic Rate (pmol/min)
Basal
Compensatory
***
**

Figure S6 (related to Figure 6). ***FTO/PFKP/LDHB*** **knockdown has minimal effects in normal CD34$^+$ HSPCs and** ***LDHA*** **knockdown impairs cell metabolism and survival in leukemia cells**

(A) Validation of knockdown efficiency of *FTO* (left panel), *PFKP* (middle panel), and *LDHB* (right panel) in human cord blood CD34$^+$ HSPCs via Western blots.

(B) Effects of knockdown of *FTO* (left panel), *PFKP* (middle panel), and *LDHB* (right panel) on mitochondrial respiration in human cord blood CD34$^+$ HSPCs. The same control shNS group was used for the analysis.

(C) Effects of knockdown of *FTO*, *PFKP*, and *LDHB* on cell viability in human cord blood CD34$^+$ HSPCs, as determined by PI staining. The percentage of viable cells in each group is shown.

(D) Distribution of the five LDH isoenzymes in the leukemia cell lines used in this study (NOMO-1, U937, NB4, and K562), as determined by zymography. Two breast cancer cell lines, MDA-MB-231 and MCF-7 were included as technical controls for the zymography.

(E and F) Effects of *LDHA* knockdown on cell proliferation/growth in NOMO-1 (E) and U937 (F) cells.

(G and H) Effects of *LDHA* knockdown on apoptosis (G) and cell cycle (H) in NOMO-1 and U937 cells.

(I and J) Effects of *LDHA* knockdown on basal and compensatory glycolytic rates in NOMO-1 (I) and U937 (J) cells.

(K and L) Effects of *LDHA* knockdown on mitochondrial respiration in NOMO-1 (K) and U937 (L) cells.

Data are represented as mean ± SD. **, $p < 0.01$; ***, $p < 0.001$; two-tailed student t-test.

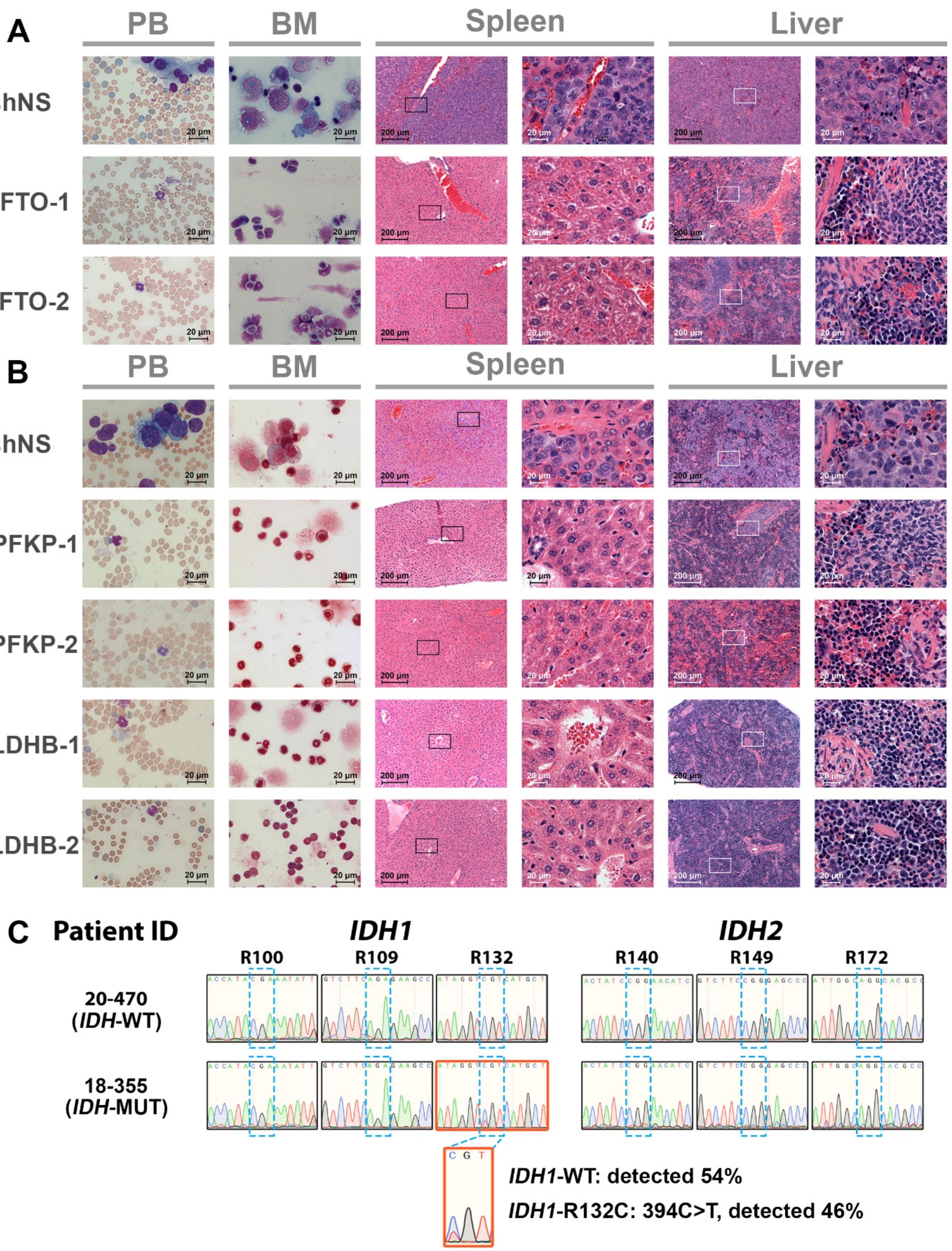
A
PB
BM
Spleen
Liver
shNS
shFTO-1
shFTO-2
20 µm
200 µm
B
PB
BM
Spleen
Liver
shNS
shPFKP-1
shPFKP-2
shLDHB-1
shLDHB-2
C
Patient ID
IDH1
IDH2
R100
R109
R132
R140
R149
R172
20-470 (IDH-WT)
18-355 (IDH-MUT)
C G T
IDH1-WT: detected 54%
IDH1-R132C: 394C>T, detected 46%

Figure S7 (related to Figure 7). Histopathology analysis of representative NRGS mice with *FTO, PFKP*, or *LDHB* knockdown and confirmation of *IDH* mutation status in primary patient-derived cells

(A) Wright-Giemsa stained peripheral blood (PB) and bone marrow (BM) smears, and hematoxylin and eosin (H&E) stained spleen and liver specimens from NRGS mice transplanted with control or *FTO*-knockdown MONO-MAC-6 cells. Specimens were collected at the same time point. The fourth and sixth columns are corresponding magnifications of the area inside the frames in the third and fifth columns. Many leukemia cells with large irregular nuclei can be observed in control but not in *FTO*-knockdown groups. The bar represents 20 µm in the first, second, fourth, and sixth columns; while the bar represents 200 µm in the third and fifth columns.

(B) Wright-Giemsa stained PB and BM smears, and H&E stained spleen and liver specimens from NRGS mice transplanted with control, *PFKP*- or *LDHB*-knockdown MONO-MAC-6 cells. Specimens were collected at the same time point. The fourth and sixth columns are corresponding magnifications of the area inside the frames in the third and fifth columns. The bar represents 20 µm in the first, second, fourth, and sixth columns; while the bar represents 200 µm in the third and fifth columns.

(C) Sanger sequencing confirmation of the *IDH* mutation status in AML patient-derived cells used in this study. Patient 20-470 is wild-type for both *IDH1* and *IDH2*, whereas 46% cells from patient 18-355 are reported to harbor $IDH1^{R132C}$ mutation. The chromatograms for six mutation hotspots are displayed.

Table S1 (related to Figure 1). Metabolite levels in NOMO-1 cells treated with PBS or R-2HG

Name	KEGG.ID	PBS#1	PBS#2	PBS#3	R-2HG#1	R-2HG#2	R-2HG#3	p value
13BPG	C00236	5.29	5.24	4.64	0.00	0.00	0.00	0.00
2-HG	C02630	93.95	86.36	80.56	7768.93	6736.89	6655.30	0.00
2-Oxoadipate	C00322	0.75	0.10	0.29	0.63	0.47	0.16	0.87
2-Oxosuccinamate	C02362	0.00	0.00	0.00	0.91	0.18	0.05	0.23
3PG	C00197	19.98	12.54	14.88	2.34	3.46	5.59	0.01
5-Oxoproline	C01879	942.82	917.63	806.09	629.34	430.32	565.94	0.01
5M-adenosine	C00170	12.80	16.99	8.27	22.58	9.36	27.18	0.30
A	C00212	0.00	0.00	0.00	0.00	0.03	0.05	0.15
a-KG	C00026	550.32	540.10	470.82	47.45	42.67	34.58	0.00
Ac-carnitine	C02571	298.47	298.46	259.54	38.28	29.32	25.45	0.00
Ac-choline	C01996	30.94	27.82	25.79	12.56	8.78	9.46	0.00
Acetyl-CoA	C00024	0.43	0.43	0.21	0.94	0.50	0.39	0.24
Aconitate	C00417	16.22	15.54	12.93	3.69	0.70	0.59	0.00
Adenine	C00147	0.00	0.00	0.00	0.00	0.10	0.05	0.15
ADP	C00008	33.78	40.06	43.37	14.60	14.65	17.01	0.00
ADP/ATP	NA	0.17	0.22	0.30	0.24	0.34	0.36	0.19
Ala	C00041	51.72	44.94	43.30	11.92	4.18	4.17	0.00
AMP	C00020	10.88	13.96	21.59	4.53	3.28	6.69	0.03
AMP/ATP	NA	0.05	0.08	0.15	0.07	0.08	0.14	0.91
Arg	C00062	1091.45	1149.60	972.52	952.66	1059.78	1319.84	0.76
Arg-Succ	C03406	1.57	1.25	1.05	0.54	0.31	0.40	0.01
Asn	C00152	436.20	408.60	368.06	285.94	224.88	263.42	0.01
Asp	C00049	184.68	169.98	159.61	222.48	187.17	214.08	0.05
ATP	C00002	200.51	182.68	145.49	61.66	43.40	46.78	0.00
C	D07769	7.99	6.26	3.24	1.21	0.37	0.57	0.02
Carbamoyl-Asp	C00438	2.59	1.68	1.40	0.00	0.00	0.00	0.01
Carnitine	C00318	608.79	556.05	521.76	273.98	219.45	230.46	0.00
CDP	C00112	4.13	4.47	5.31	0.60	0.56	0.93	0.00
CDP-choline	C00307	2.29	2.27	2.35	6.78	5.62	5.36	0.00
Choline	C00114	62.08	70.49	67.76	486.55	260.32	334.45	0.01
Cit	C00158	987.39	909.21	866.46	215.18	106.32	79.37	0.00
Citrulline	C00327	20.13	6.13	5.57	49.41	3.85	2.33	0.65
CMP	C00055	0.52	0.70	1.07	0.08	0.03	0.16	0.02
CoA	C00010	2.37	2.05	1.81	0.91	0.75	0.81	0.00
Creatine	C00300	1116.61	1063.38	957.88	144.84	119.62	109.33	0.00
Creatine/P-Creatine	NA	60.64	62.14	68.68	449.51	131.87	123.02	0.19
Creatinine	C00791	5.71	10.99	5.05	34.44	4.00	4.89	0.52
CTP	C00063	14.28	13.42	9.30	2.75	1.65	1.34	0.00
Cys	C00097	0.00	0.00	0.00	1.57	2.12	2.50	0.00
Cys Sulfinic Acid	C00606	0.00	0.00	0.00	0.69	0.05	0.11	0.24
Cytosine	C00380	0.84	0.56	0.06	0.19	0.03	0.03	0.16
dATP	C00131	1.75	1.85	1.13	0.00	0.00	0.00	0.00
dCDP	C00705	0.00	0.04	0.07	0.00	0.00	0.00	0.15

dCTP	C00458	0.42	0.31	0.07	0.04	0.00	0.04	0.08
Dihydroorotate	C00337	0.00	0.00	0.00	0.05	0.04	0.00	0.12
DMG	C01026	0.14	0.05	0.11	10.33	0.33	0.62	0.33
dTTP	C00459	0.73	0.66	0.54	0.00	0.00	0.00	0.00
F16BP	C00354	1.08	1.18	1.07	3.21	1.76	1.18	0.20
Folate	C00504	0.90	1.05	0.52	0.81	0.98	0.85	0.76
Fru	C00095	40.40	24.30	33.79	26.04	16.89	22.07	0.11
Fum	C00122	73.23	65.68	59.44	9.90	6.96	6.43	0.00
G	C00387	0.49	0.33	0.20	0.00	0.00	0.00	0.02
g-GluCys	C00669	1.00	0.80	0.93	0.31	0.13	0.33	0.00
G6P-F6P	C00085	31.52	26.16	23.37	50.41	28.95	17.46	0.63
GABA	C00334	47.79	41.41	39.82	4.43	2.50	3.73	0.00
GDP	C00035	2.23	3.50	3.75	1.18	1.26	1.23	0.01
Glc	C00031	102.27	104.77	90.96	140.80	118.47	136.84	0.02
GlcA	C00257	636.79	621.84	477.98	229.51	69.56	72.35	0.00
GlcN	C00329	2.95	3.36	4.09	9.92	5.93	2.86	0.25
GlcN-6P	C00352	0.05	0.00	0.00	0.00	0.00	0.00	0.37
GlcNAc	C00140	111.06	82.04	99.76	7.43	8.57	10.33	0.00
GlcNAc-6P	C00357	0.66	0.63	0.60	0.85	0.57	0.35	0.79
Gln	C00064	2018.58	2024.36	1848.30	1930.36	1587.59	1780.93	0.16
Glu	C00025	2575.32	2056.11	2035.48	257.23	226.41	240.58	0.00
GlucA	C00191	43.71	36.44	38.65	14.64	12.23	10.45	0.00
Gly	C00037	21.35	16.16	14.60	93.89	13.93	13.92	0.43
Glycerol	C00116	140.11	135.56	136.66	162.63	144.75	137.22	0.23
Glycerol-3-P	C00093	8.16	3.51	3.34	20.40	13.75	13.14	0.02
GMP	C00144	0.37	0.46	0.72	0.03	0.00	0.19	0.02
GSH	C00051	1695.27	1393.29	1290.39	417.42	352.52	304.85	0.00
GSH/GSSG	NA	11.39	9.55	10.30	10.37	11.72	8.25	0.81
GSSG	C00127	148.82	145.93	125.30	40.27	30.08	36.94	0.00
His	C00135	239.86	252.86	205.19	250.87	193.74	268.14	0.86
HS2O3	C00320	1.65	1.70	1.49	12.26	2.30	2.42	0.29
Hypoxanthine	C00262	1.03	0.19	0.42	0.22	0.00	0.14	0.17
IMP	C00130	0.09	0.16	0.35	0.96	0.16	0.16	0.46
Inosine	C00294	3.15	1.03	2.21	0.29	0.09	0.25	0.04
Inositol	C00137	401.92	374.27	370.75	650.51	540.67	508.85	0.01
Isobutyryl-CoA	C00630	0.00	0.00	0.00	0.00	0.00	0.00	0.18
Lac	C00186	369.72	355.40	303.52	42.17	40.24	44.40	0.00
Leu/Ile	C00123	2007.25	1898.61	1575.79	2386.03	1921.95	2256.90	0.13
Lys	C00047	33.85	33.84	28.97	41.43	38.75	47.62	0.03
Mal	C00149	1192.06	1059.18	927.84	115.85	87.24	96.75	0.00
Met	C00073	336.73	356.28	315.18	434.80	437.55	530.33	0.02
NAD+	C00003	4.86	3.85	2.87	17.05	12.94	12.49	0.00
NADH	C00004	0.06	0.07	0.00	0.00	0.17	0.51	0.28
Nicotinamide	C00153	133.61	156.33	216.68	24.08	33.64	59.92	0.01
Ornithine	C00077	2.00	2.05	1.85	2.79	1.41	1.93	0.86
Orotidine	X00006	82.83	72.86	64.81	7.52	5.85	5.62	0.00
P-Choline	C00588	7031.18	6827.85	5504.64	362.47	312.32	336.06	0.00
P-Creatine	C02305	18.41	17.11	13.95	0.32	0.91	0.89	0.00

P-EtA	C00346	63.28	53.67	47.65	30.25	24.29	27.48	0.00
P-Ser	C01005	0.39	0.43	0.20	0.03	0.00	0.04	0.01
Palmitate	C00249	3006.99	2969.85	3488.52	8967.64	2758.37	2832.57	0.46
Pantothenate	C00864	176.22	150.80	128.61	37.10	26.44	27.43	0.00
PC(34:2)	X00001	0.10	0.00	0.07	0.00	0.08	0.05	0.72
PEP	C00074	5.99	3.34	3.74	0.44	0.62	1.49	0.02
Phe	C00079	754.84	780.83	642.01	731.41	664.87	828.99	0.82
Pipecolate	C00408	3.34	3.31	2.88	3.65	3.68	4.42	0.06
PO4	C00009	43.79	44.30	32.86	29.40	23.88	36.54	0.12
PPi	C00013	0.19	0.25	0.05	2.44	0.06	0.09	0.43
Pro	C00148	2011.15	1991.75	1701.31	661.12	408.37	493.02	0.00
Pro-OH	C01157	364.07	349.40	318.16	229.19	182.64	214.97	0.00
Propanoyl-CoA	C00100	0.00	0.00	0.00	0.01	0.00	0.00	0.14
PRPP	C00119	3.99	3.04	1.41	1.41	0.71	1.10	0.09
Pyr	C00022	0.95	0.46	0.62	0.24	0.30	0.79	0.37
R5P	C00117	7.12	5.93	6.82	1.48	1.74	2.53	0.00
Rib	C00121	0.53	6.60	15.92	10.94	6.34	10.53	0.75
S7P	C05382	16.64	14.75	11.62	10.78	7.11	6.56	0.04
SAM	C00019	81.46	72.36	64.36	64.84	47.76	54.58	0.07
Sarc	C00213	3.72	3.46	2.81	4.05	1.76	1.50	0.36
Ser	C00065	15.43	14.82	11.43	11.18	7.81	8.63	0.04
Sorbitol	C00794	124179.59	122310.41	121639.19	134611.37	135776.79	132844.00	0.00
Succ	C00042	16.82	15.90	13.30	31.14	69.94	20.33	0.17
Thr	C00188	75.33	69.04	65.11	63.95	42.08	50.84	0.07
Trp	C00078	135.40	141.26	124.10	169.83	149.28	181.26	0.04
Tyr	C00082	299.54	304.97	258.65	315.63	263.54	319.82	0.63
Tyramine	C00483	3.98	3.46	3.34	3.56	2.77	2.86	0.17
UDP	C00015	16.57	19.67	20.45	2.79	2.91	3.84	0.00
UDP-Glc	C00029	0.68	0.49	0.18	0.97	0.83	0.69	0.08
UDP-GlcNAc	C00043	0.00	0.00	0.00	1.09	0.53	0.51	0.02
UMP	C00105	3.10	4.11	5.88	1.03	0.46	0.98	0.01
Uracil	C00106	12.00	39.24	16.70	13.68	3.83	20.30	0.36
Uric Acid	C00366	0.40	0.86	0.38	1.63	0.26	0.12	0.82
Val	C00183	432.94	511.05	412.53	399.86	440.24	481.00	0.77
Vit-C	C00072	0.82	0.80	0.60	1.50	1.24	0.65	0.21
Xanthine	C00385	0.08	0.00	0.19	1.95	0.11	0.04	0.39

Table S2 (related to Figure 1). Metabolite levels in NB4 cells treated with PBS or R-2HG

Name	KEGG.ID	PBS#1	PBS#2	PBS#3	R-2HG#1	R-2HG#2	R-2HG#3	p value
13BPG	C00236	37.31	39.65	27.28	13.42	11.40	10.52	0.00
2-AB	C02356	52.27	47.64	15.21	78.49	58.44	6.01	0.72
2-HG	C02630	64.51	59.06	54.49	10201.33	9073.37	9478.78	0.00
2-KB	C00109	0.05	0.18	0.00	0.11	0.06	0.17	0.61
2-Oxoadipate	C00322	0.66	0.66	0.35	2.45	0.00	0.36	0.65
2-Oxosuccinamate	C02362	0.05	0.07	0.00	0.09	0.05	0.00	0.85
3PG	C00197	19.48	16.21	13.86	14.26	12.16	14.72	0.20
4-OH-PheLac	C03672	11.18	0.00	6.14	0.00	10.79	13.38	0.68
5M-adenosine	C00170	932.22	1036.71	785.89	1088.84	818.14	215.62	0.48
5-Oxoproline	C01879	604.97	746.33	632.00	916.52	614.92	749.90	0.37
A	C00212	0.54	3.24	1.14	2.06	0.52	0.13	0.51
Ac-carnitine	C02571	195.55	1077.23	103.14	1130.00	121.81	2.54	0.94
Ac-choline	C01996	96.46	148.26	7.33	151.00	55.08	1.55	0.82
Acetyl-CoA	C00024	0.00	0.01	0.00	0.00	0.00	0.00	0.32
Aconitate	C00417	28.59	30.06	22.87	21.77	22.40	27.70	0.33
Adenine	C00147	1.17	3.98	0.56	1.94	0.82	0.11	0.47
ADP	C00008	157.22	151.60	152.57	159.64	156.62	147.98	0.82
ADP/ATP	NA	0.33	0.34	0.42	0.41	0.50	0.40	0.16
a-KG	C00026	114.33	97.99	67.44	40.70	53.77	47.02	0.03
Ala	C00041	33.42	1141.29	0.17	736.85	8.73	0.00	0.77
AMP	C00020	1.39	4.38	0.00	8.56	1.36	1.02	0.57
AMP/ATP	NA	0.00	0.01	0.00	0.02	0.00	0.00	0.46
Arg	C00062	1287.11	2576.70	756.42	2863.55	169.60	0.00	0.65
Arg-Succ	C03406	8.42	9.21	7.76	6.77	4.57	6.27	0.03
Asn	C00152	1647.88	1815.07	1575.91	1789.90	1390.37	1599.81	0.56
Asp	C00049	2074.48	1992.78	1721.86	1107.35	1095.17	945.02	0.00
ATP	C00002	476.82	450.93	361.48	386.13	310.96	370.81	0.15
C	D07769	45.65	75.27	30.62	36.15	10.13	2.60	0.11
Carbamoyl-Asp	C00438	0.63	0.47	0.00	0.57	0.30	0.26	0.97
Carnitine	C00318	738.75	767.50	450.59	893.59	522.31	8.80	0.56
CDP	C00112	21.41	19.19	20.78	17.53	15.00	15.49	0.01
CDP-choline	C00307	2.55	2.64	0.19	5.42	1.96	1.15	0.53
CDP-EtA	C00570	6.36	7.46	6.07	7.15	6.63	7.33	0.44
Choline	C00114	1599.60	1330.39	652.00	4295.20	2692.16	0.18	0.43
Cit	C00158	1865.58	1525.75	1538.69	1071.58	1306.96	1305.19	0.04
Citrulline	C00327	0.00	0.00	0.00	1.59	0.95	0.00	0.14
CMP	C00055	2.56	1.86	3.62	2.54	2.59	3.24	0.85
CoA	C00010	6.12	7.71	6.71	11.42	9.05	11.62	0.02
Creatine	C00300	188.86	567.42	0.64	716.66	192.64	1.58	0.86
Creatine/P-Creatine	NA	63.28	47.79	0.00	97.09	219.12	0.00	0.36
Creatinine	C00791	5.98	15.19	3.28	17.95	8.36	0.13	0.92
CTP	C00063	38.89	40.89	30.71	29.16	26.91	26.98	0.05
Cystathionine	C02291	8.31	11.38	1.07	11.47	3.07	1.28	0.73

Cytosine	C00380	23.83	17.92	5.01	5.68	6.09	0.53	0.12
dATP	C00131	0.00	0.00	0.00	0.10	0.15	0.00	0.13
dC	C00881	1.55	0.26	0.47	0.22	0.13	0.48	0.31
dCTP	C00458	0.00	0.00	0.00	0.05	0.00	0.07	0.12
dG	C00330	0.14	0.76	0.05	0.06	0.00	0.05	0.28
DMG	C01026	11.42	12.78	1.95	20.27	3.94	2.73	0.97
dTMP	C00364	0.00	0.00	0.00	0.00	0.12	0.19	0.14
dTTP	C00459	0.00	0.28	0.00	0.17	0.16	0.00	0.91
Folate	C00504	0.23	0.28	0.58	0.23	0.48	0.13	0.64
Fru	C00095	6.91	10.46	10.14	17.18	10.56	15.93	0.08
Fum	C00122	131.60	120.30	115.08	89.30	65.60	74.66	0.01
G	C00387	4.04	0.40	5.21	0.21	4.65	5.37	0.93
G3P	C00118	11.02	11.26	8.07	9.02	9.73	11.03	0.88
G6P-F6P	C00085	143.80	179.94	106.27	174.74	152.82	171.10	0.36
GABA	C00334	52.27	47.63	15.21	78.49	58.43	6.01	0.72
GDP	C00035	15.11	13.56	16.37	15.23	14.35	14.82	0.82
g-GluCys	C00669	0.13	0.00	0.22	0.15	0.11	0.13	0.83
Glc	C00031	135.75	197.33	167.31	208.49	117.58	138.44	0.73
GlcA	C00257	2287.06	2627.71	2266.79	2392.15	1938.71	2214.08	0.30
GlcN	C00329	0.82	8.27	2.87	11.77	1.84	0.74	0.86
GlcN-6P	C00352	0.09	0.09	0.00	0.06	0.12	0.00	0.97
GlcNAc	C00140	1.90	6.18	0.90	4.95	1.40	1.03	0.81
GlcNAc-6P	C00357	1.74	1.70	0.69	2.75	3.64	3.10	0.01
Gln	C00064	6825.73	7555.00	6646.44	8175.22	6667.81	6918.17	0.68
Glu	C00025	7453.11	7529.96	6104.27	3516.80	3091.03	3218.72	0.00
GlucA	C00191	30.96	35.09	27.05	32.12	25.89	29.29	0.55
Gly	C00037	7.14	371.84	5.10	396.22	5.40	0.21	0.98
Glycerol	C00116	289.23	144.99	165.60	150.63	199.61	160.78	0.57
Glycerol-3-P	C00093	1177.72	1155.75	958.16	533.11	491.27	489.89	0.00
GMP	C00144	4.03	6.01	4.66	6.32	4.25	5.06	0.73
GSH	C00051	0.27	316.06	0.93	0.29	0.13	0.00	0.37
GSH/GSSG	NA	0.00	3.73	0.01	0.00	0.00	0.00	0.37
GSSG	C00127	122.95	84.83	89.09	87.96	75.54	78.44	0.22
His	C00135	82.12	720.77	17.47	945.85	31.45	0.00	0.90
Homocysteine	C00155	2.11	29.07	0.35	5.93	1.67	0.00	0.45
HS2O3	C00320	9.50	7.44	7.59	8.14	8.78	7.18	0.87
Hypoxanthine	C00262	1.71	58.39	0.89	10.88	0.48	0.00	0.44
IMP	C00130	4.04	2.97	1.48	5.18	2.46	3.33	0.49
Inosine	C00294	0.78	3.69	0.88	0.69	0.13	0.00	0.20
Inositol	C00137	1231.75	1163.62	991.76	1617.08	1229.24	1438.72	0.09
Isobutyryl-CoA	C00630	0.11	0.07	0.09	0.07	0.08	0.08	0.41
Lac	C00186	617.08	899.33	503.29	842.31	517.73	646.30	0.98
Leu/Ile	C00123	6901.06	8000.01	4512.72	10745.08	6104.91	247.09	0.82
Lys	C00047	52.08	80.19	0.35	88.50	42.95	0.00	0.99
Mal	C00149	2005.38	1804.23	1726.39	1272.69	989.49	1069.35	0.00
Met	C00073	638.80	890.22	35.12	1245.19	109.82	3.03	0.89
NAD+	C00003	1.86	3.72	1.86	8.35	5.31	4.52	0.05
NADP+	C00006	0.00	0.00	0.00	0.00	0.05	0.14	0.20

Nicotinamide	C00153	380.51	317.54	210.40	537.57	471.08	0.17	0.86
Ornithine	C00077	9.13	16.68	0.36	19.42	5.12	0.60	0.96
Orotate	C00295	7.14	18.02	9.73	8.20	3.82	3.44	0.15
Orotidine	X00006	40.41	43.00	32.94	44.62	32.20	38.25	0.93
Palmitate	C00249	6888.02	6838.06	7138.03	7628.37	7192.50	7258.02	0.07
Pantothenate	C00864	769.13	978.59	798.50	558.20	397.23	516.62	0.01
P-Choline	C00588	5139.74	9362.07	8.50	5610.70	917.33	40.86	0.46
P-Creatine	C02305	2.98	11.87	0.00	7.38	0.88	0.00	0.63
PEP	C00074	3.43	3.11	2.29	1.64	0.93	2.54	0.10
P-EtA	C00346	23.70	26.83	21.87	47.41	38.58	44.49	0.00
Phe	C00079	831.69	1876.92	0.10	2621.69	790.36	0.13	0.82
Pipecolate	C00408	7.15	11.96	2.21	12.01	2.79	0.00	0.66
PO4	C00009	100.90	101.38	93.15	102.92	87.04	91.74	0.45
PPi	C00013	10.08	11.34	7.03	7.14	5.94	6.72	0.10
Pro	C00148	11293.95	11603.59	5911.16	12686.20	8274.32	1077.14	0.59
Pro-OH	C01157	243.89	3182.28	28.57	3035.16	86.18	1.31	0.94
Propanoyl-CoA	C00100	0.00	0.00	0.00	0.00	0.00	0.00	0.12
PRPP	C00119	0.50	0.53	0.51	0.43	0.90	1.10	0.21
P-Ser	C01005	15.65	5.35	7.39	3.17	6.62	3.58	0.21
R5P	C00117	6.51	6.03	5.36	6.83	6.39	6.39	0.19
Rib	C00121	18.76	14.02	17.22	21.22	18.80	19.28	0.12
S7P	C05382	27.41	26.87	17.80	47.57	34.95	39.86	0.03
SAM	C00019	158.12	185.69	138.32	224.42	194.52	156.94	0.26
Sarc	C00213	3.77	67.71	0.74	2.55	0.91	0.00	0.35
Ser	C00065	93.39	76.84	71.20	86.12	78.05	81.34	0.86
Sorbitol	C00794	182805.95	159637.33	151888.97	152098.24	154423.19	139828.31	0.20
Succ	C00042	37.39	36.50	33.02	20.47	12.92	14.17	0.00
T	C00214	0.16	0.42	0.23	0.05	0.05	0.07	0.05
Thr	C00188	543.10	757.93	39.88	806.00	60.83	0.00	0.66
Thymine	C00178	0.72	0.55	0.70	0.75	0.13	0.75	0.62
Trp	C00078	211.34	350.70	38.90	523.62	60.85	1.86	0.98
Tyr	C00082	14.15	778.23	5.47	1014.63	4.79	1.66	0.87
Tyramine	C00483	3.71	4.14	0.63	4.63	3.70	0.57	0.94
U	C00299	0.16	0.07	0.06	0.00	0.00	0.00	0.04
UDP	C00015	60.89	56.24	65.18	60.93	48.96	56.10	0.28
UDP-Glc	C00029	2.14	1.68	2.23	2.62	2.38	2.47	0.06
UDP-GlcNAc	C00043	21.93	1.51	48.46	1.57	32.12	54.56	0.80
UMP	C00105	34.13	21.47	26.11	27.71	23.11	27.33	0.78
Uracil	C00106	14.42	17.28	14.28	8.85	3.52	5.00	0.01
Val	C00183	1786.44	1577.50	210.03	2034.13	1281.08	0.00	0.92
Vit-C	C00072	0.00	0.00	0.00	0.33	0.19	0.00	0.14
Xanthine	C00385	0.00	0.00	0.00	0.35	0.12	0.06	0.12

Table S3 (related to Figure S1). Metabolite levels in NOMO-1 IDH1^{R132H} cells treated with PBS or doxycycline

Name	KEGG.ID	-Dox#1	-Dox#2	-Dox#3	+Dox#1	+Dox#2	+Dox#3	p value
13BPG	C00236	12.33	19.39	12.46	1.18	0.50	1.22	0.00
2-AB	C02356	0.00	113.79	88.69	1.51	110.99	58.09	0.83
2-HG	C02630	196.37	222.58	196.79	3476.87	3979.21	3520.09	0.00
2-Oxoadipate	C00322	0.59	0.09	0.25	0.88	0.29	1.64	0.21
2-Oxosuccinamate	C02362	0.05	0.00	0.09	0.05	0.11	0.15	0.24
3PG	C00197	19.52	23.51	20.63	6.13	7.05	7.24	0.00
5-Oxoproline	C01879	545.25	653.36	568.32	572.93	429.35	628.56	0.54
5M-adenosine	C00170	74.40	270.25	205.52	100.22	186.08	140.20	0.55
A	C00212	0.61	0.39	0.76	0.23	1.34	1.68	0.33
a-KG	C00026	200.01	305.42	247.41	70.41	89.26	65.74	0.00
Ac-carnitine	C02571	0.22	2.60	1.44	0.08	49.92	70.30	0.14
Ac-choline	C01996	0.59	21.55	2.85	0.55	28.27	26.31	0.42
Acetoacetyl-CoA	C00332	0.03	0.08	0.04	0.02	0.03	0.01	0.11
Acetyl-CoA	C00024	0.12	0.09	0.07	0.02	0.02	0.01	0.00
Aconitate	C00417	0.51	1.02	0.93	0.35	0.58	0.69	0.20
Adenine	C00147	0.00	0.27	0.10	0.12	1.79	2.81	0.14
ADP	C00008	43.44	67.82	57.46	39.83	46.19	35.68	0.11
ADP/ATP	NA	0.24	0.24	0.28	0.29	0.27	0.27	0.17
Ala	C00041	0.08	4.69	0.28	0.09	148.92	94.86	0.14
AMP	C00020	0.20	0.11	0.00	0.00	0.25	8.93	0.37
AMP/ATP	NA	0.00	0.00	0.00	0.00	0.00	0.07	0.37
Arg	C00062	800.71	243.31	4.00	6.84	777.35	964.42	0.57
Arg-Succ	C03406	2.25	2.69	2.48	2.96	2.92	2.50	0.18
Asn	C00152	442.92	626.27	555.31	455.49	450.60	462.66	0.19
Asp	C00049	191.89	254.01	217.76	189.48	231.64	173.46	0.41
ATP	C00002	178.63	278.26	207.64	139.60	168.29	134.00	0.08
C	D07769	0.74	2.10	0.44	0.19	0.44	0.08	0.18
Carbamoyl-Asp	C00438	3.17	3.81	1.87	0.00	0.00	0.00	0.01
Carnitine	C00318	4.95	324.50	105.52	0.00	261.18	208.45	0.93
CDP	C00112	2.20	3.98	3.49	1.32	2.18	2.20	0.09
CDP-choline	C00307	1.02	0.00	0.73	0.00	0.44	1.07	0.87
CDP-EtA	C00570	10.99	13.86	12.40	7.13	8.46	6.93	0.01
Choline	C00114	29.39	50.43	56.07	23.88	244.72	155.25	0.21
Cit	C00158	48.55	108.82	90.58	58.10	80.65	57.16	0.42
Citrulline	C00327	0.00	0.00	0.00	0.00	0.16	0.12	0.12
CMP	C00055	0.33	0.42	0.24	0.25	0.23	0.16	0.12
CoA	C00010	4.93	7.19	5.53	2.27	2.12	2.54	0.01
Creatine	C00300	0.66	59.62	16.37	0.53	1756.59	1044.35	0.15
Creatine/P-Creatine	NA	0.02	4.29	19.80	0.00	58.71	25.36	0.33
Creatinine	C00791	0.00	0.11	0.04	0.38	11.85	0.00	0.36
CTP	C00063	14.40	24.37	17.68	9.13	9.20	8.60	0.03
Cytosine	C00380	0.04	0.25	0.05	0.00	0.07	0.06	0.37

dATP	C00131	2.05	4.29	3.19	1.77	1.52	1.25	0.07
dC	C00881	0.31	0.88	0.00	0.00	0.00	0.00	0.20
dCMP	C00239	0.08	0.00	0.05	0.00	0.00	0.00	0.13
dCTP	C00458	0.07	0.14	0.15	0.06	0.10	0.05	0.16
dG	C00330	0.00	0.00	0.00	0.00	0.06	0.35	0.27
dGDP	C00361	185.97	265.70	213.14	149.49	165.68	138.48	0.05
DHAP	C00111	1.22	2.89	2.24	1.09	1.87	1.10	0.24
Dihydroorotate	C00337	0.65	0.77	0.62	0.00	0.00	0.00	0.00
DMG	C01026	1.94	6.97	72.48	1.84	21.60	7.38	0.51
dTMP	C00364	0.00	0.05	0.21	0.00	0.00	0.00	0.24
dTTP	C00459	0.54	0.95	0.83	0.26	0.12	0.17	0.01
F16BP	C00354	82.81	104.97	105.48	64.36	57.63	56.30	0.01
Folate	C00504	0.15	0.13	0.24	0.36	0.11	0.35	0.31
Fru	C00095	5.42	5.83	3.72	7.18	3.28	8.40	0.48
Fum	C00122	84.20	112.35	94.89	56.99	64.36	57.68	0.01
G	C00387	1.99	2.57	9.45	2.69	3.47	0.10	0.38
G3P	C00118	8.64	13.98	12.58	6.07	7.85	5.61	0.04
G6P-F6P	C00085	73.37	108.01	88.15	57.91	62.23	64.93	0.05
GABA	C00334	1.27	121.47	90.33	0.00	113.03	62.34	0.81
GDP	C00035	3.11	4.97	4.01	2.86	2.46	2.15	0.06
Glc	C00031	77.57	103.69	97.46	122.11	105.72	137.58	0.08
GlcA	C00257	624.76	798.18	707.63	265.30	240.06	282.01	0.00
GlcN	C00329	0.47	0.87	0.67	0.33	4.56	3.33	0.18
GlcN-6P	C00352	0.17	0.12	0.04	0.11	0.14	0.05	0.85
GlcNAc	C00140	0.68	2.27	1.45	0.34	1.31	3.88	0.76
GlcNAc-6P	C00357	0.20	0.66	0.74	1.75	1.35	1.17	0.02
Gln	C00064	1195.90	1435.24	1423.49	2310.27	2084.45	2181.26	0.00
Glu	C00025	2282.74	3117.34	2710.01	1575.78	1769.12	1522.85	0.01
GlucA	C00191	27.39	38.08	29.01	21.50	20.86	18.12	0.03
Gly	C00037	0.00	0.83	1.48	0.00	48.94	90.09	0.16
Glycerol	C00116	149.16	212.65	150.03	149.60	408.84	140.53	0.53
Glycerol-3-P	C00093	7.42	8.40	6.52	11.42	11.33	11.90	0.00
GMP	C00144	0.19	0.62	0.22	0.11	0.07	0.22	0.22
GSH	C00051	119.34	2.10	2.19	1.88	27.59	51.28	0.75
GSH/GSSG	NA	1.17	0.02	0.02	0.02	0.29	0.55	0.79
GSSG	C00127	101.91	119.07	118.97	103.23	95.59	92.74	0.07
His	C00135	79.51	6.94	0.00	0.00	29.71	190.63	0.53
HMG-CoA	C00356	0.04	0.01	0.02	0.01	0.00	0.00	0.10
HS2O3	C00320	5.98	7.11	11.26	6.41	7.73	5.84	0.44
IMP	C00130	0.40	0.79	0.69	0.42	0.37	0.64	0.35
Inosine	C00294	0.00	0.24	0.00	0.00	0.00	0.13	0.71
Inositol	C00137	367.10	484.68	448.17	624.29	750.31	632.72	0.01
Isobutyryl-CoA	C00630	0.06	0.11	0.08	0.04	0.06	0.02	0.07
Lac	C00186	615.32	867.71	738.36	473.30	566.69	489.07	0.04
Leu/Ile	C00123	77.04	1273.75	630.87	276.10	2062.53	2662.13	0.27
Lys	C00047	23.97	8.09	6.48	0.70	27.22	37.60	0.51
Mal	C00149	1360.69	1820.07	1519.05	897.98	1027.99	878.79	0.01
Met	C00073	0.00	25.68	16.67	1.81	329.67	347.43	0.13

NAD+	C00003	1.97	3.61	3.22	3.32	5.61	2.63	0.42
NADP+	C00006	0.00	0.09	0.06	0.00	0.07	0.07	0.93
Nicotinamide	C00153	0.00	82.37	39.27	21.02	107.74	78.83	0.46
Ornithine	C00077	5.38	2.16	3.35	1.19	9.10	6.17	0.50
Orotate	C00295	0.87	2.54	3.05	0.00	0.00	0.00	0.03
Orotidine	X00006	58.33	76.76	67.82	35.38	39.08	37.99	0.01
P-Choline	C00588	6073.31	2338.98	4052.02	1.22	3618.23	3347.61	0.31
P-Creatine	C02305	27.89	13.91	0.83	0.00	29.92	41.18	0.55
P-EtA	C00346	13.78	16.56	15.54	132.33	139.69	130.31	0.00
P-Ser	C01005	2.55	6.56	4.47	2.12	4.14	1.29	0.23
Palmitate	C00249	6281.93	6054.96	10686.16	6590.43	6591.04	8286.18	0.76
Pantothenate	C00864	145.96	190.09	149.66	103.35	83.88	102.58	0.01
PEP	C00074	3.00	3.17	2.51	0.21	0.36	1.07	0.00
Phe	C00079	0.00	25.11	1.49	0.95	487.59	578.31	0.13
Pipecolate	C00408	3.34	1.37	0.54	0.00	3.82	5.05	0.52
PO4	C00009	59.76	78.11	64.78	54.47	55.67	55.50	0.09
PPi	C00013	7.11	9.77	6.32	4.18	0.00	2.07	0.02
Pro	C00148	0.25	3788.22	1350.19	66.31	3565.70	2355.08	0.86
Pro-OH	C01157	0.87	247.98	6.75	0.14	746.99	544.35	0.22
PRPP	C00119	6.42	11.13	7.93	1.09	1.27	1.28	0.01
Pyr	C00022	0.28	0.56	0.50	0.00	0.00	0.00	0.01
R5P	C00117	0.80	2.08	0.84	1.74	1.42	1.98	0.35
Rib	C00121	13.06	18.94	17.84	20.14	20.34	14.14	0.59
S7P	C05382	18.80	27.02	24.43	14.28	18.06	14.30	0.04
SAM	C00019	22.60	54.23	37.22	28.94	32.03	32.77	0.50
Sarc	C00213	0.00	0.69	0.42	0.00	8.87	16.35	0.16
Ser	C00065	27.53	37.90	33.64	25.56	32.83	22.98	0.23
Sorbitol	C00794	52292.39	113190.28	103857.63	92246.39	126376.02	100401.26	0.49
Succ	C00042	14.12	17.23	16.60	5.73	6.85	5.73	0.00
T	C00214	0.22	0.09	0.00	0.19	0.08	0.07	0.90
Thr	C00188	1.96	9.11	3.84	0.00	203.77	157.43	0.13
Thymine	C00178	0.11	0.21	0.08	0.08	0.21	0.10	0.91
Trp	C00078	0.00	18.71	3.11	0.87	98.59	113.04	0.15
Tyr	C00082	0.10	1.04	1.61	0.24	97.84	224.13	0.18
Tyramine	C00483	0.00	1.58	3.47	0.23	2.96	2.98	0.80
U	C00299	55.55	32.88	21.81	20.20	24.58	12.94	0.17
UDP	C00015	8.67	15.96	12.55	7.82	9.76	10.74	0.26
UDP-Glc	C00029	48.67	78.88	63.15	44.56	54.74	34.47	0.15
UDP-GlcNAc	C00043	0.58	5.23	19.81	14.48	5.49	1.84	0.86
UMP	C00105	11.16	14.79	12.85	9.83	11.88	8.72	0.12
Uracil	C00106	0.18	0.19	0.00	0.10	0.15	0.09	0.87
Uric Acid	C00366	0.00	0.00	0.00	0.10	0.00	0.15	0.13
Val	C00183	7.51	584.15	488.88	0.00	1317.64	944.11	0.41

Dox: doxycycline.

Table S4 (related to Figure 3). Metabolite levels in NOMO-1 cells stably expressing shNS or shFTO-2

Name	KEGG.ID	shNS#1	shNS#2	shNS#3	shFTO-2#1	shFTO-2#2	shFTO-2#3	p value
13BPG	C00236	0.29	0.68	1.63	0.11	0.00	0.06	0.11
2-AB	C02356	3.47	4.97	4.33	5.00	3.43	6.26	0.53
2-HG	C02630	139.35	137.22	146.61	153.43	172.89	161.74	0.03
2-Oxoadipate	C00322	3.84	4.21	3.47	3.75	3.45	4.11	0.82
2-Oxosuccinamate	C02362	0.00	0.13	0.07	0.07	0.08	0.15	0.49
3PG	C00197	9.54	7.98	10.54	5.66	6.80	9.55	0.22
4-OH-PheLac	C03672	0.00	0.00	0.00	0.00	4.19	1.22	0.22
5-Oxoproline	C01879	1046.51	1052.32	1377.71	970.79	997.67	1037.68	0.23
5M-adenosine	C00170	57.47	39.49	63.27	48.21	52.13	58.09	0.94
A	C00212	0.14	0.00	0.00	0.13	0.00	1.43	0.36
a-KG	C00026	70.36	52.55	72.62	53.76	53.27	61.51	0.26
Ac-carnitine	C02571	50.08	48.28	67.31	173.59	187.43	167.17	0.00
Ac-choline	C01996	28.56	26.23	36.06	16.69	19.69	20.38	0.02
Acetyl-CoA	C00024	0.11	0.07	0.15	0.05	0.07	0.05	0.09
Aconitate	C00417	3.49	2.83	5.49	3.19	3.45	3.93	0.64
Adenine	C00147	3.23	0.92	4.09	3.15	2.83	1.73	0.87
ADP	C00008	26.89	30.80	29.64	29.06	28.50	33.71	0.55
ADP/ATP	NA	0.28	0.34	0.21	0.37	0.33	0.38	0.09
Ala	C00041	264.54	249.41	367.94	108.56	168.87	147.01	0.02
AMP	C00020	4.17	5.86	10.79	4.44	4.27	3.88	0.24
AMP/ATP	NA	0.04	0.06	0.08	0.06	0.05	0.04	0.34
Arg	C00062	653.92	1021.04	1045.45	721.95	952.82	916.42	0.78
Arg-Succ	C03406	2.06	2.02	3.37	1.80	2.53	2.34	0.63
Asn	C00152	347.85	344.00	442.00	330.80	371.47	370.50	0.59
Asp	C00049	121.46	125.60	150.40	90.41	102.91	105.14	0.03
ATP	C00002	96.75	91.36	138.84	78.65	85.32	89.47	0.19
C	D07769	2.23	3.17	1.71	2.50	2.28	1.20	0.56
Carbamoyl-Asp	C00438	1.87	0.32	3.51	0.99	1.29	1.00	0.43
Carnitine	C00318	435.25	388.14	534.83	229.45	251.13	266.14	0.01
CDP	C00112	0.70	1.07	1.14	1.52	1.15	2.78	0.18
CDP-EtA	C00570	11.94	9.52	14.40	15.05	15.94	16.47	0.06
Choline	C00114	37.44	56.13	39.75	62.18	80.01	67.90	0.03
Cit	C00158	56.37	44.15	78.02	56.75	66.65	68.37	0.70
Citrulline	C00327	2.39	3.33	2.20	3.29	3.82	2.65	0.28
CMP	C00055	0.81	1.46	1.48	0.58	0.04	0.46	0.03
CoA	C00010	0.35	0.29	0.52	0.18	0.19	0.21	0.05
Creatine	C00300	579.11	526.08	710.82	752.36	912.48	826.06	0.04
Creatine/P-Creatine	NA	20.36	17.75	20.41	22.48	23.28	19.40	0.21
Creatinine	C00791	20.19	24.43	22.13	23.40	29.43	23.95	0.22
CTP	C00063	8.79	9.45	14.19	6.82	6.92	7.62	0.10
Cytosine	C00380	0.08	0.48	0.09	0.68	0.25	0.12	0.58
dA	C00559	1.85	2.19	0.94	2.44	2.75	1.93	0.18

dATP	C00131	1.99	1.15	2.52	0.89	0.97	1.15	0.10
dCMP	C00239	0.26	0.24	0.30	0.31	0.50	0.29	0.24
dCTP	C00458	0.14	0.08	0.12	0.00	0.05	0.13	0.31
dG	C00330	0.26	0.40	0.10	0.24	0.50	0.80	0.23
DHAP	C00111	1.43	0.94	1.67	2.21	2.05	2.87	0.04
Dihydroorotate	C00337	1.17	0.49	1.16	0.48	0.55	0.15	0.10
DMG	C01026	176.13	197.99	186.56	186.21	219.82	204.55	0.22
dTMP	C00364	0.00	0.52	0.44	0.21	0.07	0.15	0.35
dTTP	C00459	0.78	0.71	1.23	0.64	0.61	0.66	0.18
dU	C00526	0.00	0.00	0.00	0.12	0.18	0.93	0.19
F16BP	C00354	0.18	0.18	0.26	0.00	0.00	0.00	0.00
Folate	C00504	0.04	0.00	0.05	0.00	0.00	0.00	0.12
Fru	C00095	19.40	20.88	19.49	18.75	16.09	21.64	0.55
Fum	C00122	77.92	74.82	89.11	71.22	81.59	78.30	0.54
G	C00387	0.52	0.51	0.55	0.36	0.26	1.03	0.93
G3P	C00118	1.87	2.80	3.51	1.97	4.53	4.07	0.44
G6P-F6P	C00085	62.46	62.19	93.46	40.92	47.36	52.76	0.08
GABA	C00334	78.67	83.95	105.67	58.52	79.40	70.38	0.12
GDP	C00035	1.22	1.38	1.61	1.12	1.05	1.23	0.10
Glc	C00031	158.69	243.95	252.23	185.31	291.75	260.48	0.56
GlcA	C00257	644.79	642.63	596.42	640.29	644.20	641.81	0.42
GlcN	C00329	2.16	3.16	2.37	1.41	2.05	2.47	0.25
GlcN-6P	C00352	0.07	0.08	0.24	0.07	0.07	0.11	0.45
GlcNAc	C00140	10.15	8.98	9.99	6.73	12.68	11.61	0.75
GlcNAc-6P	C00357	1.91	1.38	2.49	1.56	1.68	2.15	0.74
Gln	C00064	591.31	759.16	847.59	1035.86	1250.52	1315.54	0.01
Glu	C00025	1537.25	1467.37	1909.10	1432.96	1574.35	1674.90	0.64
GlucA	C00191	21.52	18.17	24.62	15.52	14.05	16.26	0.04
Gly	C00037	64.16	89.86	84.32	59.80	80.06	71.58	0.41
Glycerol	C00116	3.49	6.35	4.98	4.91	5.65	3.48	0.82
Glycerol-3-P	C00093	6.07	5.40	7.54	7.79	7.95	9.93	0.08
GMP	C00144	0.19	0.57	0.69	0.34	0.31	0.33	0.36
GSH	C00051	61.07	465.00	141.81	686.10	1077.21	220.60	0.19
GSH/GSSG	NA	0.36	3.38	0.75	2.71	4.99	0.89	0.42
GSSG	C00127	169.35	137.71	188.91	253.60	215.84	248.84	0.02
His	C00135	67.99	90.39	105.94	66.24	87.71	84.47	0.54
HMG-CoA	C00356	0.01	0.02	0.01	0.01	0.02	0.00	0.86
Homocysteine	C00155	0.83	0.66	1.68	0.85	1.77	0.00	0.77
HS2O3	C00320	0.60	0.90	0.66	0.35	0.52	0.68	0.20
Hypoxanthine	C00262	0.21	0.68	1.29	0.65	1.69	0.00	0.93
IMP	C00130	0.49	0.23	0.49	2.75	2.85	4.46	0.01
Inosine	C00294	0.00	0.00	0.36	0.00	0.60	1.78	0.28
Inositol	C00137	287.87	236.18	310.44	379.34	447.91	469.48	0.01
Isobutyryl-CoA	C00630	0.01	0.01	0.01	0.00	0.00	0.01	0.25
Lac	C00186	489.34	527.74	734.94	332.09	435.85	478.56	0.13
Leu/Ile	C00123	1740.90	1982.48	2422.05	1652.32	2235.96	2150.72	0.90
Lys	C00047	27.02	48.55	45.11	34.78	47.65	41.86	0.88
Mal	C00149	1234.06	1169.08	1412.79	1157.79	1354.45	1258.56	0.88

Met	C00073	173.97	206.64	262.39	170.36	202.98	215.60	0.57
NAD+	C00003	2.91	2.16	1.92	1.54	2.69	1.08	0.38
Nicotinamide	C00153	43.44	53.00	55.89	30.64	44.92	72.58	0.92
Ornithine	C00077	12.58	23.20	22.99	16.35	23.00	18.25	0.93
Orotate	C00295	7.29	5.50	8.45	3.45	4.14	6.51	0.13
Orotidine	X00006	48.46	38.20	53.04	46.21	46.41	50.10	0.84
P-Choline	C00588	4563.98	4001.55	5531.76	3489.95	3877.18	3954.61	0.12
P-Creatine	C02305	28.45	29.63	34.83	33.47	39.19	42.59	0.09
P-EtA	C00346	28.46	24.25	32.37	133.34	140.45	149.87	0.00
P-Ser	C01005	2.99	4.87	4.65	4.15	6.61	6.38	0.19
Palmitate	C00249	9998.68	10433.86	9516.46	8999.89	14429.68	9764.64	0.56
Pantothenate	C00864	282.50	315.21	385.63	324.54	361.35	391.99	0.43
PEP	C00074	1.10	1.19	1.51	0.47	0.63	1.35	0.20
Phe	C00079	391.08	430.49	567.75	363.45	431.65	440.36	0.43
Pipecolate	C00408	1.55	3.94	4.55	3.40	4.29	2.71	0.91
PO4	C00009	41.04	51.67	56.78	42.64	46.04	49.12	0.48
PPi	C00013	1.09	0.97	2.03	0.56	0.49	1.00	0.14
Pro	C00148	2393.49	2406.63	3152.28	1995.25	2501.86	2375.04	0.29
Pro-OH	C01157	470.71	501.74	614.55	423.38	618.64	507.40	0.87
PRPP	C00119	1.00	1.56	3.33	0.81	0.28	0.37	0.11
Pyr	C00022	0.34	1.24	1.52	0.74	1.34	0.62	0.78
R5P	C00117	1.73	1.85	4.22	1.17	1.94	3.31	0.68
Rib	C00121	6.43	8.95	8.38	7.64	5.69	6.05	0.21
S7P	C05382	18.26	16.64	23.82	18.82	20.87	21.47	0.74
SAM	C00019	58.90	59.01	79.24	63.84	70.67	72.07	0.69
Sarc	C00213	24.03	25.12	30.88	27.56	33.71	29.21	0.28
Ser	C00065	39.54	39.53	51.55	25.92	36.27	34.39	0.09
Sorbitol	C00794	145196.60	156391.10	148580.00	150414.88	157975.23	145209.67	0.83
Succ	C00042	34.96	37.10	39.18	41.63	46.90	42.33	0.03
Succinyl-CoA	C00091	0.01	0.00	0.02	0.00	0.00	0.01	0.28
T	C00214	2.93	2.16	2.60	0.10	3.00	2.80	0.57
Thr	C00188	135.83	153.45	173.64	124.35	174.98	154.93	0.88
Thymine	C00178	0.50	0.33	0.25	4.55	0.98	6.22	0.08
Trp	C00078	69.05	73.15	98.57	64.98	75.27	83.56	0.62
Tyr	C00082	159.91	186.61	234.37	151.64	175.19	176.81	0.33
Tyramine	C00483	1.58	0.42	0.55	0.73	9.16	0.77	0.39
U	C00299	2.97	11.95	20.48	1.28	1.07	9.53	0.25
UDP	C00015	5.04	4.70	5.26	7.49	6.02	14.17	0.17
UDP-Glc	C00029	40.96	35.08	49.46	42.27	50.29	51.39	0.29
UDP-GlcNAc	C00043	2.91	2.85	1.51	2.19	1.68	2.74	0.71
UMP	C00105	9.74	10.48	14.32	8.08	13.21	9.03	0.54
Uracil	C00106	9.81	9.22	9.77	11.81	10.09	11.49	0.05
Uric Acid	C00366	0.24	0.30	0.52	0.57	0.15	0.30	0.94

Table S5 (related to Figure 4). List of the 35 glycolysis- and gluconeogenesis-related genes

Symbol	**Full Name**
ALDOA	aldolase A, fructose-bisphosphate
ALDOB	aldolase B, fructose-bisphosphate
ALDOC	aldolase C, fructose-bisphosphate
BPGM	2,3-bisphosphoglycerate mutase
ENO1	enolase 1 (alpha)
ENO2	enolase 2 (gamma, neuronal)
ENO3	enolase 3 (beta, muscle)
FBP1	fructose-1,6-bisphosphatase 1
FBP2	fructose-1,6-bisphosphatase 2
G6PC	glucose-6-phosphatase, catalytic subunit
G6PC2	glucose-6-phosphatase, catalytic, 2
G6PC3	glucose 6 phosphatase, catalytic, 3
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAPDHS	glyceraldehyde-3-phosphate dehydrogenase, spermatogenic
GCK	glucokinase (hexokinase 4)
GPI	glucose-6-phosphate isomerase
HK1	hexokinase 1
HK2	hexokinase 2
HK3	hexokinase 3 (white cell)
MDH1	malate dehydrogenase 1, NAD (soluble)
MDH2	malate dehydrogenase 2, NAD (mitochondrial)
PC	pyruvate carboxylase
PCK1	phosphoenolpyruvate carboxykinase 1 (soluble)
PFKL	phosphofructokinase, liver
PFKM	phosphofructokinase, muscle
PFKP	phosphofructokinase, platelet
PGAM1	phosphoglycerate mutase 1 (brain)
PGAM2	phosphoglycerate mutase 2 (muscle)
PGK1	phosphoglycerate kinase 1
PGK2	phosphoglycerate kinase 2
PKLR	pyruvate kinase, liver and RBC
PKM	pyruvate kinase, muscle
TPI1	triosephosphate isomerase 1
LDHA	lactate dehydrogenase A
LDHB	lactate dehydrogenase B

Table S6 (related to Figures 7 and S7). Information of the AML patient samples used in the study

Sample ID	Age	Sex	Cytogentics (Karotype)	Gene Mutation
20-470	70	F	Normal	FLT3-ITD; TET2; NPM1
18-355	71	F	Nonclonal aberration: 46, XX, inv(5)(q13q35)	IDH1 (c.394C>T, p.R132C), detected 46%

Table S7 (related to Star Methods). List of oligonucleotides

Name	Sequence (5'-3')	Note
HK1 Forward	CACATGGAGTCCGAGGTTTATG	RT-qPCR
HK1 Reverse	CGTGAATCCCACAGGTAACTTC	RT-qPCR
GPI Forward	CCGCGTCTGGTATGTCTCC	RT-qPCR
GPI Reverse	CCTGGGTAGTAAAGGTCTTGGA	RT-qPCR
PFKL Forward	GCTGGGCGGCACTATCATT	RT-qPCR
PFKL Reverse	TCAGGTGCGAGTAGGTCCG	RT-qPCR
PFKM Forward	AGCTGCCTACAACCTGGTGA	RT-qPCR
PFKM Reverse	TCCACTCAGAACGGAAGGTGT	RT-qPCR
PFKP Forward	CGCCTACCTCAACGTGGTG	RT-qPCR & RNA stability
PFKP Reverse	ACCTCCAGAACGAAGGTCCTC	RT-qPCR & RNA stability
ALDOA Forward	CAGGGACAAATGGCGAGACTA	RT-qPCR
ALDOA Reverse	GGGGTGTGTTCCCCAATCTT	RT-qPCR
ALDOC Forward	ATGCCTCACTCGTACCCAG	RT-qPCR
ALDOC Reverse	TTTCCACCCCAATTTGGCTCA	RT-qPCR
TPI1 Forward	AGTGACTAATGGGGCTTTTACTG	RT-qPCR
TPI1 Reverse	GCCCAATCAGCTCATCTGACTC	RT-qPCR
GAPDH Forward	TCAACGACCACTTTGTCAAGCTCA	RT-qPCR
GAPDH Reverse	GCTGGTGGTCCAGGGGTCTTACT	RT-qPCR
PGK1 Forward	GAACAAGGTTAAAGCCGAGCC	RT-qPCR
PGK1 Reverse	GTGGCAGATTGACTCCTACCA	RT-qPCR
PGAM1 Forward	TCAGCTACCCTCCTGTGAGAG	RT-qPCR
PGAM1 Reverse	TACACGTTTCCCCTCCTTGAT	RT-qPCR
ENO1 Forward	TGGTGTCTATCGAAGATCCCTT	RT-qPCR
ENO1 Reverse	CCTTGGCGATCCTCTTTGG	RT-qPCR
PKM Forward	ATGTCGAAGCCCCATAGTGAA	RT-qPCR
PKM Reverse	TGGGTGGTGAATCAATGTCCA	RT-qPCR
LDHA Forward	TTGACCTACGTGGCTTGGAAG	RT-qPCR
LDHA Reverse	GGTAACGGAATCGGGCTGAAT	RT-qPCR
LDHB Forward	CCTCAGATCGTCAAGTACAGTCC	RT-qPCR & RNA stability
LDHB Reverse	ATCACGCGGTGTTTGGGTAAT	RT-qPCR & RNA stability
FTO Forward	AGAATGTCTGTGACGATGTGG	RT-qPCR
FTO Reverse	GCACTTTCTGTATCGATTGCC	RT-qPCR

PFKP-IP-Forward	GAGCACAGGATTCCCAAAGA	m^{6}A IP-RT-qPCR & CLIP-RT-qPCR
PFKP-IP-Reverse	ACTGGGTCAGACACTCCA	m^{6}A IP-RT-qPCR & CLIP-RT-qPCR
LDHB-IP-Forward	AAGGATGATGAGGTTGCTCAG	m^{6}A IP-RT-qPCR & CLIP-RT-qPCR
LDHB-IP-Reverse	TTCTACAGCCTAGAGCTCACT	m^{6}A IP-RT-qPCR & CLIP-RT-qPCR
shYTHDF2-2	AGGCTTTGGTTCAGAATAT	pLKO.1-shYTHDF2-2 construct
FTO_pCDH_XbaI_Forward	CATAGAAGATTCTAGAACCACCATGGATTACAAAGATGACGACGATAAGAAGCGCACCCCGACTGCCGA	In-Fusion cloning
FTO_pCDH_NotI_Reverse	AGATCCTTCGCGGCCGCCTAGGGTTTTGCTTCCAGAAGC	In-Fusion cloning
PFKP_pCDH_XbaI_Forward	CATAGAAGATTCTAGAGCCACCATGGACGCGGACGACTCCCGGGC	In-Fusion cloning
PFKP_pCDH_NotI_Reverse	AGATCCTTCGCGGCCGCTCAGACACTCCAGGGCTGCACATGTTCC	In-Fusion cloning
LDHB_pCDH_XbaI_Forward	CATAGAAGATTCTAGAGCCACCATGGCAACTCTTAAGGAAAAACT	In-Fusion cloning
LDHB_3×FLAG_pCDH_NotI_Reverse	AGATCCTTCGCGGCCGCTCACTTGTCATCGTCATCCTTGTAATCGATGTCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCGCCGCCCAGGTCTTTTAGGTCCTTCTGGAT	In-Fusion cloning