Supporting Information

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SI Experimental Procedures

RNA Interference. RNA interference was performed as previously described (68). In brief, HEK 293 T cells were transfected with 2 μg shRNA (cloned in PLKO.1) or control vector, 2 μg pREV, 2 μg pGag/Pol/PRE, and 1 μg pVSVG. Twelve hours later, cells were switched to DMEM culture medium with 10% FBS for an additional 24 h. The culture medium containing lentiviral particles was subjected to filtration through a 0.45-μm polyvinylidene difluoride filter (Millipore) and incubated with cells supplemented with 8 μg/mL polybrene (Sigma) for 12 h, followed by selection with 5 μg/mL puromycin for another 24 h. The efficiency of knockdown was assessed by Western blotting and qPCR analysis.

qPCR and RT-PCR. qPCR and RT-PCR were performed as previously described (33). In brief, total RNA was extracted using TRIzol (Ambion). One microgram of total RNA was used for cDNA synthesis with a PrimeScriptTM RT reagent kit (DRR037A; Takara) according to the instruction provided by the manufacturer. qPCR was carried out using SYBR premix EX Taq (TaKaRa) and ROX Reference Dye (ROX) and analyzed with Stratagene Mx3000p (Agilent Technologies).

Western Blot Analysis. Western blotting was performed as described before (33). Briefly, cells grown in the exponential growth phase were harvested, boiled in SDS loading buffer, and resolved on SDS/PAGE.

Luciferase Assay. To determine the effect of IDH1-AS1 on the activity of HIF-1a RE reporter construct, HeLa cells expressing either control shRNA or IDH1-AS1 shRNA were transfected with HRE-WT or HRE-MT reporter constructs plus Renilla luciferase plasmid. Twenty-four hours later, cells were harvested and the reporter activity was measured by using a luciferase assay kit (Promega) and plotted after normalizing with respect to Renilla luciferase activity. The data are represented as means ± SDs of three independent experiments. To investigate whether IDH1-AS1 is transcriptionally regulated by c-Myc, HeLa cells expressing either Flag empty or Flag-c-Myc were cotransfected with pGL3-based construct containing IDH1-AS1 promoter plus Renilla luciferase plasmid. Twenty-four hours later, cells were harvested and the reporter activity was measured by using a luciferase assay kit (Promega).

Quantitation of IDH1-AS1 Abundance in Subcellular Fractions. Cells were homogenized in the buffer containing 10 mM Tris·HCl (pH 7.5), 250 mM sucrose, and 2 mM EDTA on ice with a glass pestle douncer. After centrifugation at 600 *g* for 10 min at 4 °C, the pellet fractions contained nuclei and unbroken cells and the supernatant contained cytoplasmic and mitochondrial fractions. The pellet fractions were then resuspended in homogenization buffer [20 mM Hepes-KOH (pH 7.5)], 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM sodium EDTA, 1 mM sodium EGTA, and 1 mM DTT in the presence of 250 mM sucrose and protease inhibitor mixture (Roche Diagnostics) and incubated on ice for 5 min. Then 0.5% Nonidet P-40 was added into the homogenates for another 5 min of incubation on ice, followed by centrifuging at 500 *g* for 5 min at 4 °C. The pellet was collected as nuclei. The remaining fractions were centrifuged at 11,000 *g* for 10 min at 4 °C. The pellet was collected as mitochondria, and the supernatant was collected as cytoplasm. Total RNAs from cellular fractions were isolated using TRIzol and real-time PCR was performed to quantify IDH1-AS1 in each fraction.

In Vitro Transcription of Biotinylated IDH1-AS1. To synthesize biotinylated IDH1-AS1 transcripts, the DNA template used in the transcription system was generated by RT-PCR using forward primers containing the T7 RNA polymerase promoter sequence, allowing for subsequent transcription. PCR products were purified using DNA Gel Extraction kit (AxyPrep). In vitro transcription was then performed using MaxiScript T7 kit (Ambion) in the presence of Biotin RNA Labeling Mix (Roche) according to the instructions provided by the manufacturer.

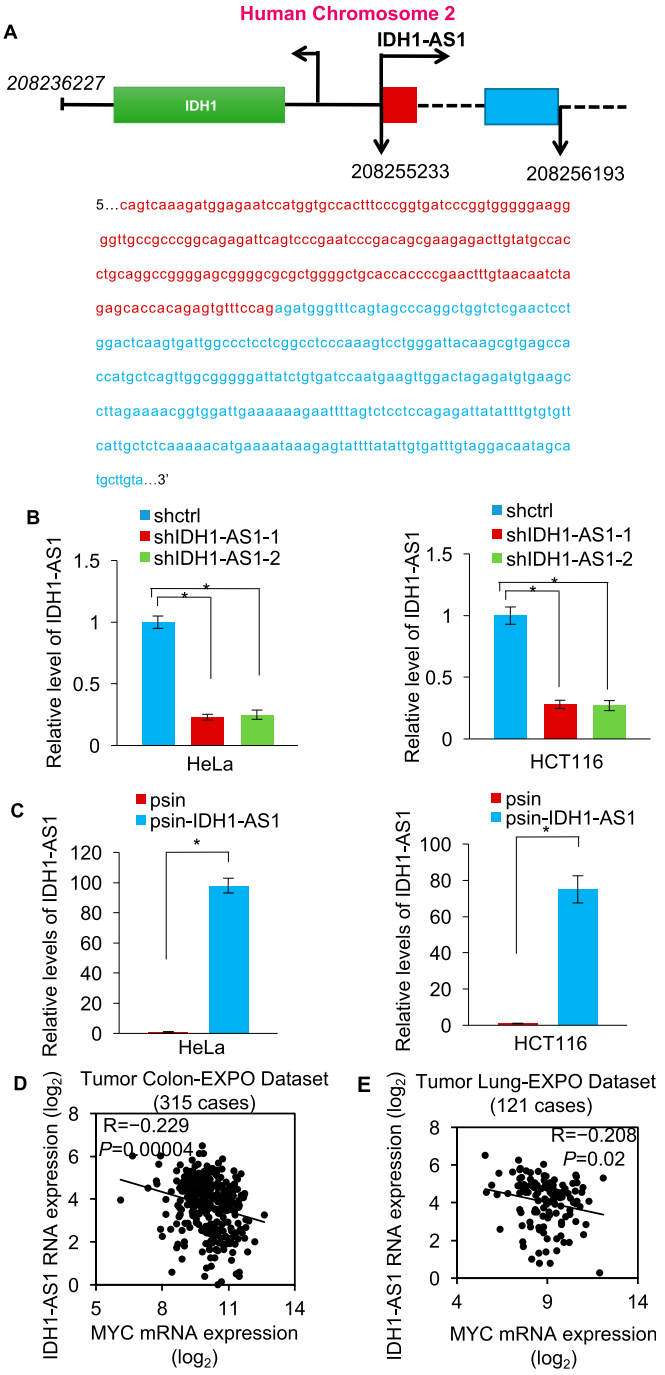


Fig. S1. Related to Fig. 1. Properties of IDH1-AS1. (*A*) Schematic illustration of genomic locations of IDH1 and IDH1-AS1 on human chromosome 2. Nucleotide sequences of IDH1-AS1 corresponding to the two exons of IDH1-AS1 are listed. (*B*) Silencing of IDH-AS1 by shRNA in HeLa and HCT116 cells. Cells were transduced with shctrl, shIDH1-AS1-1, or shIDH1-AS1-1. Forty-eight hours later, total RNA was subjected to qPCR analysis of IDH1-AS1 expression. The relative abundance of IDH-AS1 in cells transduced with shctrl was arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's t test). (*C*) Overexpression of IDH1-AS1 in HeLa and HCT116 cells. Cells were transduced with the psin vertor alone or psin-IDH1-AS1. Forty-eight hours later, total RNA was subjected to qPCR analysis of IDH1-AS1 expression. The relative abundance of IDH-AS1 in cells transduced with the psin vertor alone was arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's t test). (*D*) Analysis of a publicly available gene expression dataset showed that IDH1-AS1 expression in colon cancer tissues was negatively correlated with the expression of the *MYC* gene (Expression Project for Oncology, https://hgserver1.amc.nl/cgi-bin/r2/main.cgi). (*E*) Analysis of a publicly available gene expression dataset showed that IDH1-AS1 expression in lung cancer tissues is negatively correlated with the expression of the *MYC* gene (Expression Project for Oncology, https://hgserver1.amc.nl/cgi-bin/r2/main.cgi). shctrl, control shRNA.

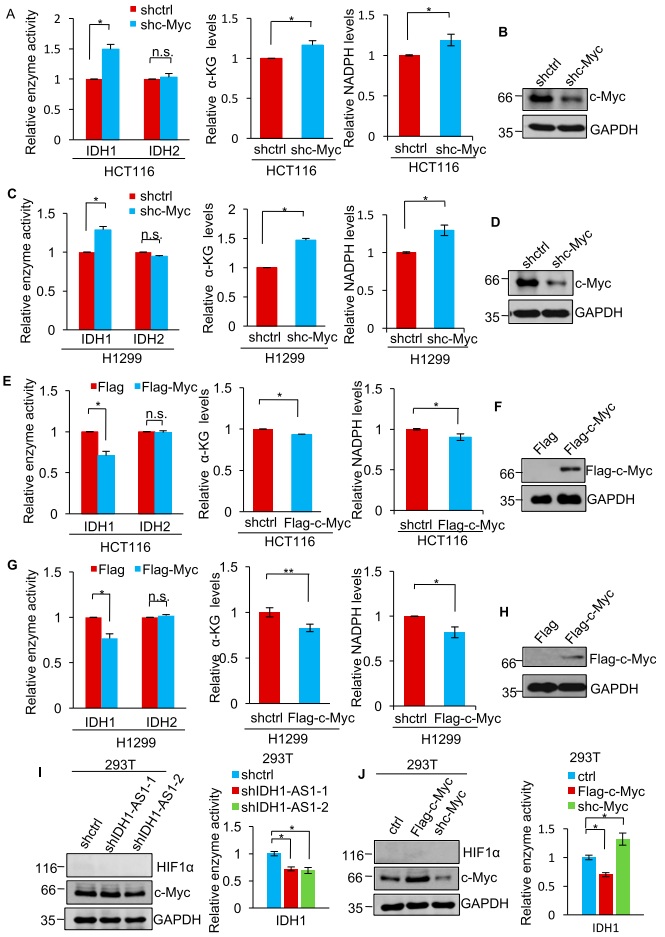


Fig. S2. Related to Fig. 2. c-Myc inhibits IDH1 enzyme activity. (*A*) c-Myc silencing enhanced IDH1 but not IDH2 activity and increased α-KG production as well as the relative NADPH/NADP+ ratio in HCT116 cells. HCT116 cells were transduced with the control (shctrl) or c-Myc shRNA (shc-Myc). Forty-eight hours later, whole cell lysates were subjected to measurement of IDH1 and IDH2 enzyme activity (*Left*), α-KG levels (*Middle*), and NADPH levels (*Right*). The relative IDH1 and IDH2 activity and α-KG abundance and NADPH/NADP+ ratio in cells transduced with shctrl were arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P <$ 0.05, two-tailed paired Student's *t* test). (*B*) c-Myc silencing by shRNA in HCT116 cells. HCT116 cells were transduced with the control (shctrl) or c-Myc shRNA (shc-Myc). Forty-eight hours later, whole cell lysates were subjected to Western blot analysis of the expression of c-Myc and GAPDH (as a loading control). $n = 3$. (*C*) c-Myc silencing enhanced IDH1 but not IDH2 activity and increased α-KG production and the relative NADPH/NADP+ ratio in H1299 cells. H1299 cells were transduced with the control (shctrl) or c-Myc shRNA (shc-Myc). Forty-eight hours later, whole cell lysates were subjected to measurement of IDH1 and IDH2 enzyme activity (*Left*), α-KG levels (*Middle*), and NADPH levels (*Right*). The relative IDH1 and IDH2 activity and α-KG abundance and NADPH/NADP+ ratio in cells transduced with shctrl were arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). (*D*) c-Myc silencing by shRNA in H1299 cells. H1299 cells were transduced with the control (shctrl) or c-Myc shRNA (shc-Myc). Forty-eight hours later, whole cell lysates were subjected to Western blot analysis of the expression of c-Myc and GAPDH (as a loading control). $n = 3$. (*E*) c-Myc overexpression reduced IDH1 but not IDH2 activity and decreased α-KG production and the relative NADPH/NADP+ ratio in HCT116 cells. HCT116 cells were transduced with Flag-vector or Flag-tagged c-Myc. Forty-eight hours later, whole cell lysates were subjected to measurement of IDH1 and IDH2 enzyme activity (*Left*), α-KG levels (*Middle*), and NADPH levels (*Right*). The relative IDH1 and IDH2 activity and α-KG abundance and NADPH/NADP+ ratio in cells transduced with shctrl were arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). (*F*) c-Myc overexpression in HCT116 cells. HCT116 cells were transduced with Flag-vector or Flag-tagged c-Myc. Forty-eight hours later, whole cell lysates were subjected to Western blot analysis of the expression of c-Myc and GAPDH (as a loading control). $n = 3$. (*G*) c-Myc overexpression reduced IDH1 but not IDH2 activity and decreased α-KG production and the relative NADPH/NADP+ ratio in H1299 cells. H1299 cells were transduced with Flag-vector or Flag-tagged c-Myc. Forty-eight hours later, whole cell lysates were subjected to measurement of IDH1 and IDH2 enzyme activity (*Left*), α-KG levels (*Middle*), and NADPH levels (*Right*). The relative IDH1 and IDH2 activity and α-KG abundance and NADPH/NADP+ ratio in cells transduced with shctrl were arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). (*H*) c-Myc overexpression in H1299 cells. H1299 cells were transduced with Flag-vector or Flag-tagged c-Myc. Forty-eight hours later, whole cell lysates were subjected to Western blot analysis of the expression of c-Myc and GAPDH (as a loading control). $n = 3$. (*I*) IDH1-AS1 silencing reduced IDH1 activity in 293T cells. 293T cells were transduced with shctrl, shIDH1-AS1-1 or shIDH1-AS1-2. Forty-eight hours later, whole cell lysates were subjected to Western blot analysis (*Left*) and analysis of IDH1 enzymatic activity (*Right*). The activity of IDH1 in cells transduced with shctrl was arbitrarily designated as 1. Mean ± SEM; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). (*J*) c-Myc repressed IDH1 activity in 293T cells. 293T cells were transduced with ctrl, Flag-tagged c-Myc, or shc-Myc. Forty-eight hours later, whole cell lysates were subjected to Western blot analysis (*Left*) and analysis of IDH1 enzymatic activity (*Right*). The activity of IDH1 in cells transduced with ctrl was arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). ctrl, control; n.s., not significant; shctrl, control shRNA.

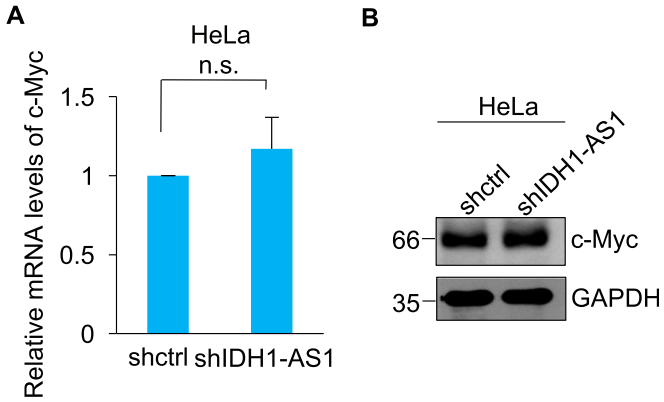


Fig. S3. Related to Fig. 3. IDH1-AS1 does not regulate c-Myc expression. (*A*) IDH1-AS1 silencing did not significantly alter c-Myc mRNA expression in HeLa cells. HeLa cells were transduced with shctrl or shIDH1-AS1-1. Forty-eight hours later, total RNA was subjected to qPCR analysis of the expression of c-Myc mRNA. The relative abundance of c-Myc mRNA in cells transduced with shctrl was arbitrarily designated as 1. Means ± SEMs; $n = 3$ (two-tailed paired Student's *t* test). (*B*) IDH1-AS1 silencing did not alter c-Myc protein expression in HeLa cells. HeLa cells were transduced with shctrl or shIDH1-AS1-1. Forty-eight hours later, whole cell lysates were subjected to Western blot analysis of the expression of c-Myc and GAPDH (as a loading control). $n = 3$. n.s., not significant; shctrl, control shRNA.

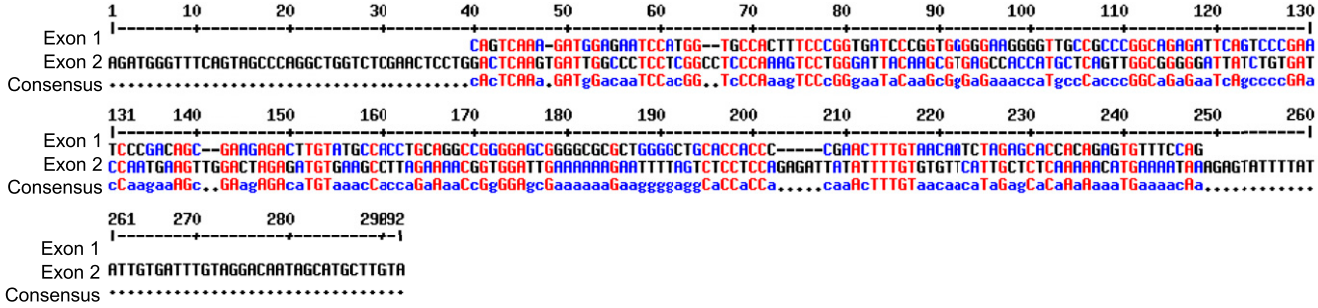


Fig. S4. Related to Fig. 4. Nucleotide sequences of the two exons of IDH1-AS1 were aligned using BLAST.

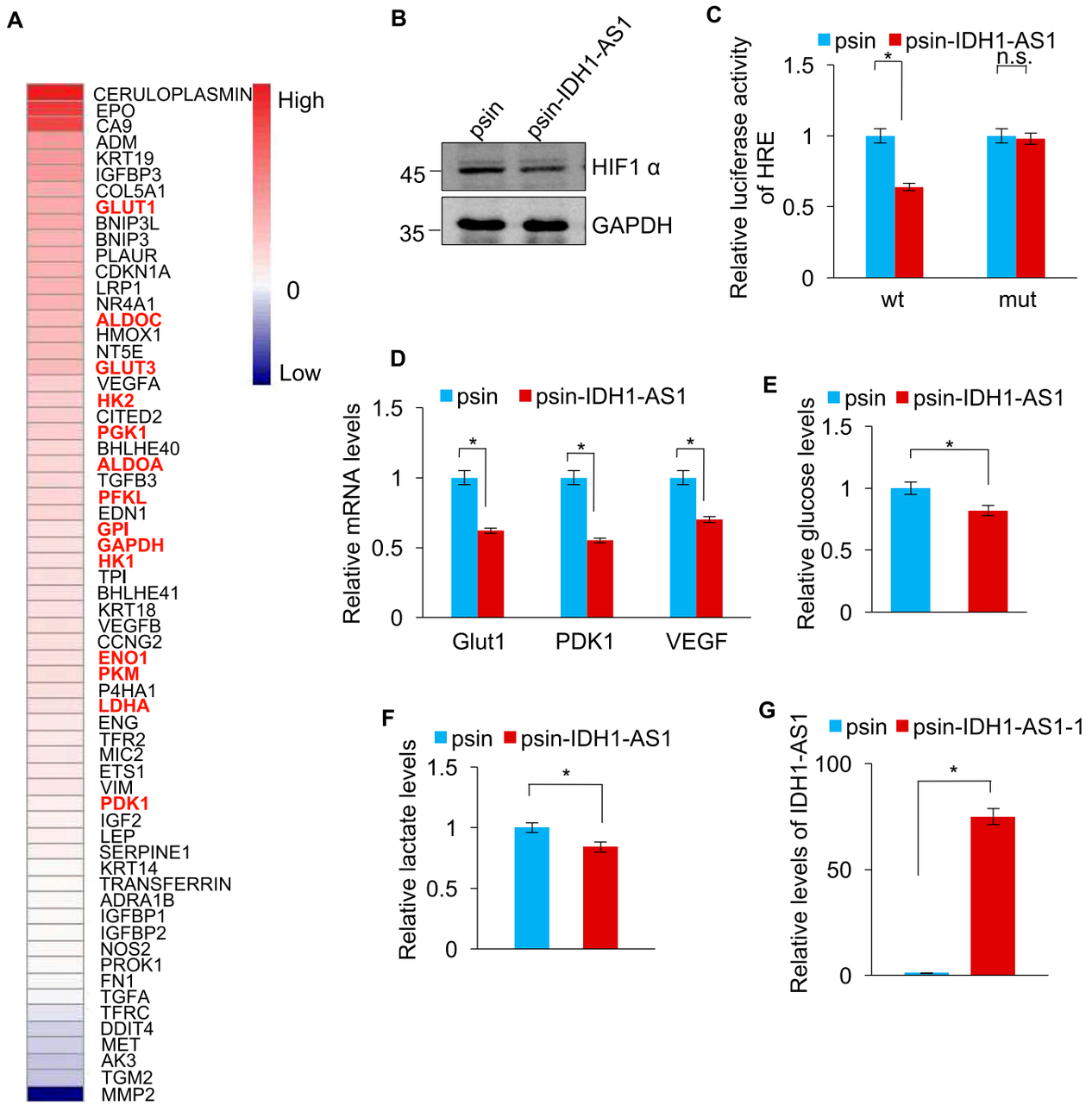
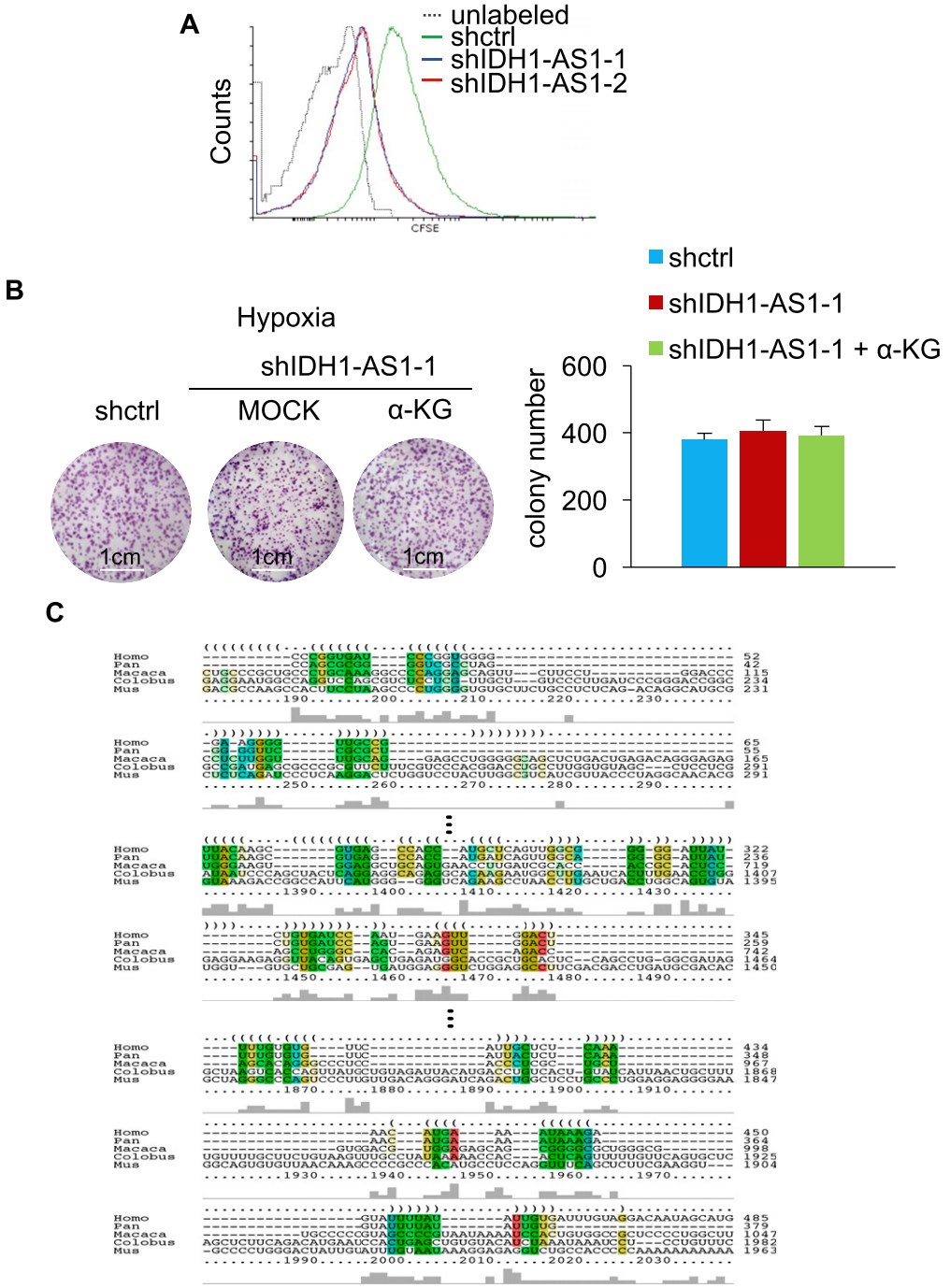


Fig. S5. Related to Fig. 5. IDH1-AS1 regulates the Warburg effect via HIF1α. (*A*) RNA-seq analysis of HIF-1a target gene expression in HeLa cells with or without knockdown of IDH1-AS1. Log2-transformed fold gene expression changes were subject to hierarchical clustering and are displayed in thumbnail-dendogram format (down-regulated and up-regulated genes are shown in blue and red tiles, respectively). Genes marked in red are those associated with glycolysis. (*B*) IDH1-AS1 overexpression reduced the expression of HIF-1α in HeLa cells. HeLa cells were transduced with the psin vector or psin-IDH1-AS1. Forty-eight hours later, whole cell lysates were subjected to Western blot analysis of the expression of HIF-1α and GAPDH (as a loading control). $n = 3$. (*C*) IDH1-AS1 overexpression reduced the activity of a luciferase reporter containing the HIF-1α RE (HRE), which was reversed when the HRE was mutated in HeLa cells. HeLa cells transduced with the psin vector or psin-IDH1-AS1 were cotransfected with reporter constructs containing the WT HRE or mutant HRE and Renilla luciferase plasmids. Twenty-four hours later, transcriptional activity was determined by luciferase assays. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). (*D*) IDH1-AS1 overexpression reduced the expression of HIF-1α downstream transcriptional targets in HeLa cells. HeLa cells were transduced with the psin vector or psin-IDH1-AS1. Forty-eight hours later, total RNA was subjected to qPCR analysis of the expression of GULT1, PDK1, and VEGF mRNA. The relative abundance of GULT1, PDK1, and VEGF mRNA in cells transduced with the psin vector was arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). (*E*) IDH1-AS1 overexpression reduced glucose uptake in HeLa cells. HeLa cells were transduced with the psin vector or psin-IDH1-AS1. Forty-eight hours later, whole cell lysates were subjected to measurement of glucose levels. The relative abundance of glucose in cells transduced with the psin vector alone was arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). (*F*) IDH1-AS1 overexpression reduced lactate production in HeLa cells. HeLa cells were transduced with the psin vector or psin-IDH1-AS1. Forty-eight hours later, whole cell lysates were subjected to measurement of lactate levels. The relative abundance of lactate in cells transduced with the psin vector alone was arbitrarily designated as 1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). (*G*) Overexpression of IDH1-AS1 in HeLa cells. HeLa cells were transduced with the psin vector or psin-IDH1-AS1. Forty-eight hours later, total RNA was subjected to qPCR analysis of the expression of IDH1-AS1. Means ± SEMs; $n = 3$ (*$P < 0.05$, two-tailed paired Student's *t* test). n.s., not significant.



Species	Homo	Pan	Macaca	Colobus	Mus
Alignment Score	886	459	129	243	37.4
Cover (%)	100	55	22	22	5

Fig. S6. Related to Fig. 7. IDH1-AS1 alter HeLa cell proliferation under normoxic conditions but not hypoxic conditions. (*A*) Representative flow cytometry histograms of analysis of proliferation of HeLa cell transduced with shctrl or shIDH1-AS1-1 using CFSE proliferation assays. $n = 3$. (*B*) Silencing of IDH1-AS1 did not significantly alter proliferation of HeLa cells under hypoxic conditions. HeLa cells transduced with shctrl or shIDH1-AS1-1 were seeded onto six-well plates (1×10^3 cells per well) in the presence or absence of Octyl-α-KG (1 mM). Two weeks later, cells were fixed, stained with crystal violet, and photographed. The colony number was counted. Means ± SEMs; $n = 3$. (*C*) Transcripts that shared moderate sequence similarity with human IDH1-AS1 were identified in *Pan troglodytes*, *Macaca fascicularis*, and *Colobus angolensis* palliates, but not in *Mus musculus*. shctrl, control shRNA. (Scale bars, 1 cm.)

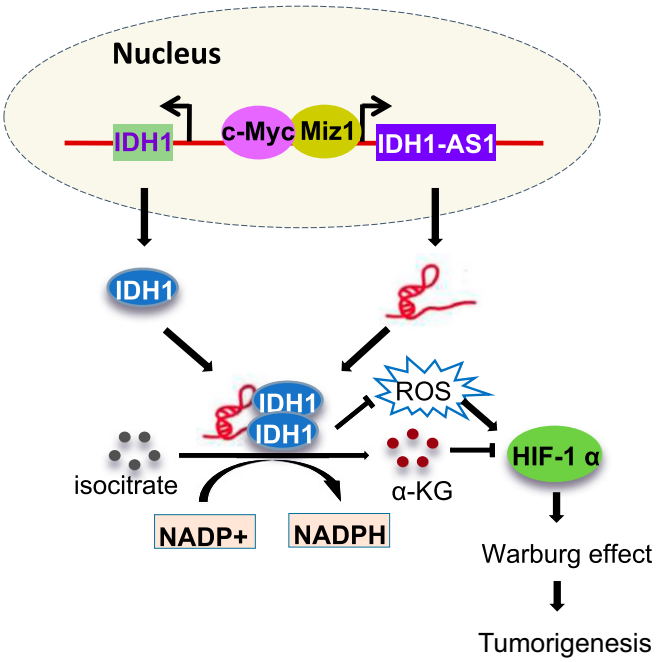


Fig. S7. Related to *Discussion*. Schematic illustration of a c-Myc-IDH1-AS1-IDH1-α-KG/ROS-HIF-1a signal axis whereby the lncRNA IDH1-AS1 links c-Myc and HIF1α via WT IDH1 to cooperatively regulate the Warburg effect.

Table S1. List of primer, probe, and shRNA sequences

Name	Sequence
Primers used for qRT-PCR	
IDH1-AS1	FW: TCCATGGTGCCACTTTCCCG
	RV: GGCATACAAGTCTCTTCGCT
β-actin	FW: CTGTCCCTGTATGCCTCTG
	RV: ATGTCACGCACGATTTCC
IDH1	FW: TGGTGACTTGGTCGTTGG
	RV: AGTGGCGGTTCTGTGGTA
IDH2	FW: GAAGGTGTGCGTGGAGAC
	RV: CCGTGGTGTTCAGGAAGT
c-Myc	FW: AGCGACTCTGAGGAGGAAC
	RV: TGTGAGGAGGTTTGCTGTG
Glut1	FW: AACTCTTCAGCCAGGGTCCAC
	RV: CACAGTGAAGATGATGAAGAC
PDK1	FW: ATCCCAAGTTACTGAGTTGTGTTGGAAG
	RV: GTATGCTATACGAAGTTATAGCTTCAGGAAG
VEGF	FW: AAATGCTTTCTCCGCTCTGA
	RV: CCCACTGAGGAGTCCAACAT
Primers used for ChIP	
−400 to −200	FW: CCCAAGATCTGCGCTTTTTTCC
	RV: TCCGGTTTGGGATTGCCAG
−200 to −1	FW: ACTCCCAGTGCCTCCGCTTC
	RV: TTGACTGTGGCCACGCCCCT
+1 to +200	FW: CAGTCAAAGATGGAGAATCCAT
	RV: CCTGGAAACACTCTGTGGTGCT
β-Actin	FW: TCGATATCCACGTGACATCCA
	RV: GCAGCATTTTTTTACCCCCTC
Oligonucleotide sequence of shRNAs	
shIDH1-AS1-1	GAACTTTGTAACAATCTAGAG
shIDH1-AS1-2	AAGAGACTTGTATGCCACCTG
shc-Myc-1	AACTATGACCTCGACTACGA
shc-Myc-2	CCTGAGACAGATCAGCAACAA
shMiz1	GTGTTCACTTTAAGGCTCATA
shHIF1α-1	CCGCTGGAGACACAATCATAT
shHIF1α-2	CCAGTTATGATTGTGAAGTTA
Oligonucleotide sequence of DNA probes for biotin-pulldown	
Sense	CGGTGATCCCGGTGGGGGAAG
Anti-sense	CTTCCCCCACCGGGATCACCG

FW, forward; RV, reverse.