Expanded View Figures

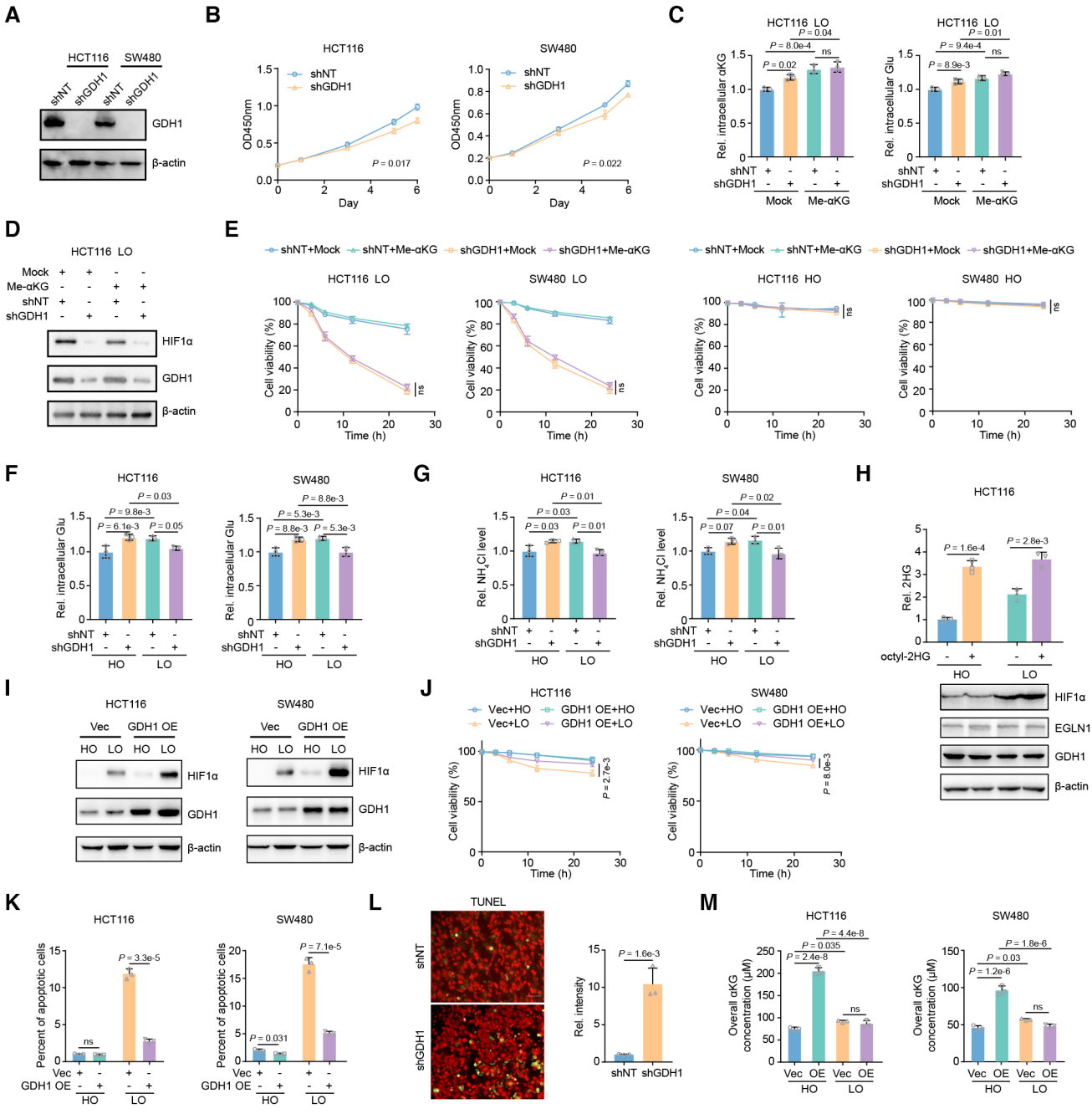

Figure EV1.

Figure EV1. GDH1 depletion sensitizes CRC cells to hypoxia.

A Construction of GDH1 depleted cell lines.
B Cell proliferation assay was performed using cell lines constructed in Fig S1A (n = 3).
C, D HCT116 and SW480 cells with or without GDH1 were supplemented with cell permeable methyl-αKG under hypoxia. Then, the intracellular level of αKG was tested by αKG determination kit (C) (n = 3). Cells were collected and indicated antibodies were applied to test HIF1α, EGLN1 and GDH1 proteins (D).
E HCT116 and SW480 cells with or without GDH1 were supplemented with cell permeable methyl-αKG under normoxia and hypoxia. Then, cell viability was counted by trypan blue staining (n = 3).
F, G HCT116 and SW480 cells with or without GDH1 were cultured under normoxia or hypoxia. Cells were collected for determining glutamate (Glu, F) and ammonia (NH_4Cl, G) (n = 3).
H HCT116 were incubated with or without cell permeable octyl-2HG under normoxia or hypoxia. Indicated antibodies were applied to test HIF1α, EGLN1 and GDH1 proteins (n = 3).
I HIF1α and GDH1 were tested by western blot in HCT116 or SW480 cells with Vec or GDH1 overexpression under normoxia and hypoxia.
J HCT116 and SW480 cells with or without GDH1 overexpression were incubated under normoxia and hypoxia, respectively. Then, cell viability was counted by trypan blue staining (n = 3).
K The apoptotic percent of cells in Fig S1D was counted by PI/Annexing V double staining (n = 3).
L TUNEL analysis of GDH1 knockdown HCT116 cells under hypoxia. The left was the representative microscopic data, the right was the calculated relative intensity data (n = 3).
M The overall αKG level in HCT116 or SW480 cells with or without GDH1 overexpression under normoxia and hypoxia (n = 3).

Data information: Data are mean ± SD from the biological replicates (B, C, E–H, J–M). Statistics: two-way ANOVA with Tukey's HSD *post hoc* test (B, E, J); one-way ANOVA with Tukey's HSD *post hoc* test (C, F–H, M); unpaired two-tailed Student's *t*-test (K, L).
Source data are available online for this figure.

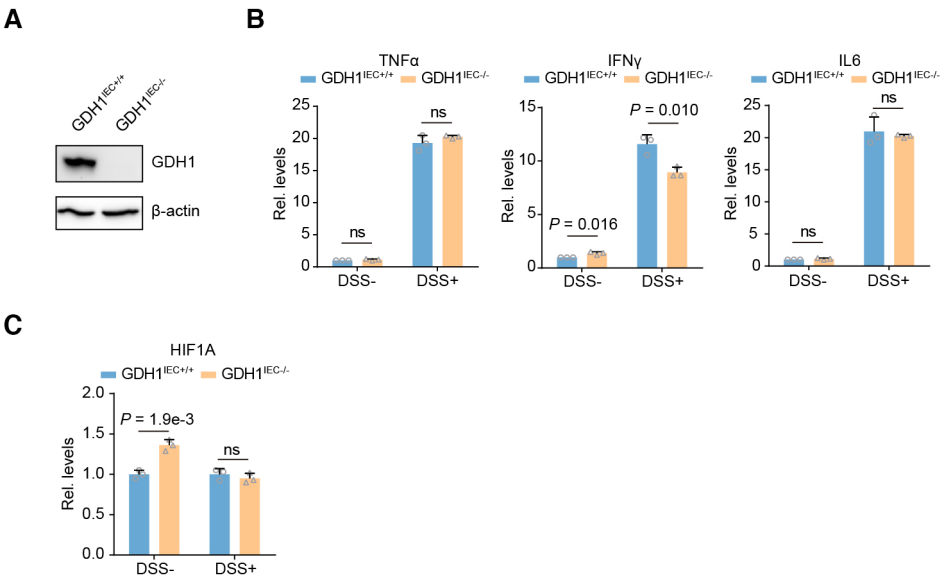

Figure EV2. GDH1 depletion did not significantly affect cytokine and HIF1A expression.

A The identification of GDH1 knock out in the intestine by western blot.
B qRT-PCR analysis of whole colon homogenates to assess cytokines production after AOM/DSS treatment (n = 3).
C qRT-PCR analysis of whole colon homogenates to assess HIF1A expression after AOM/DSS treatment (n = 3).

Data information: Data are mean ± SD from the biological replicates (B, C). Statistics: unpaired two-tailed Student's *t*-test (B, C).
Source data are available online for this figure.

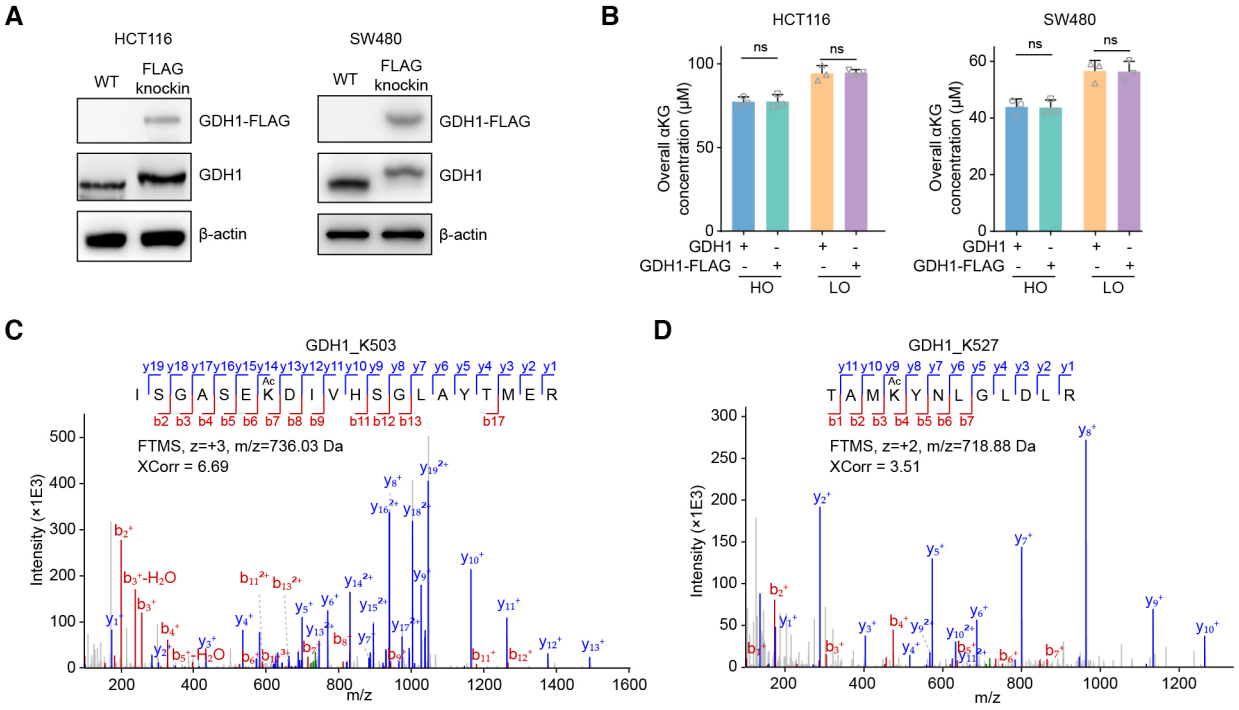

Figure EV3. The enrichment of GDH1 for acetylation identification.

A The successful knockin of FLAG into C terminal of GDH1 in HCT116 or SW480 cells was tested by western blot.

B The overall αKG level in HCT116 or SW480 cells with or without FLAG tagging endogenous GDH1 C terminal under normoxia and hypoxia (n = 3).

C, D MS/MS spectra identified K503 and K527 acetylation of GDH1 purified from HCT116 cells.

Data information: Data are mean ± SD from the biological replicates (B). Statistics: unpaired two-tailed Student's t-test (B).
Source data are available online for this figure.

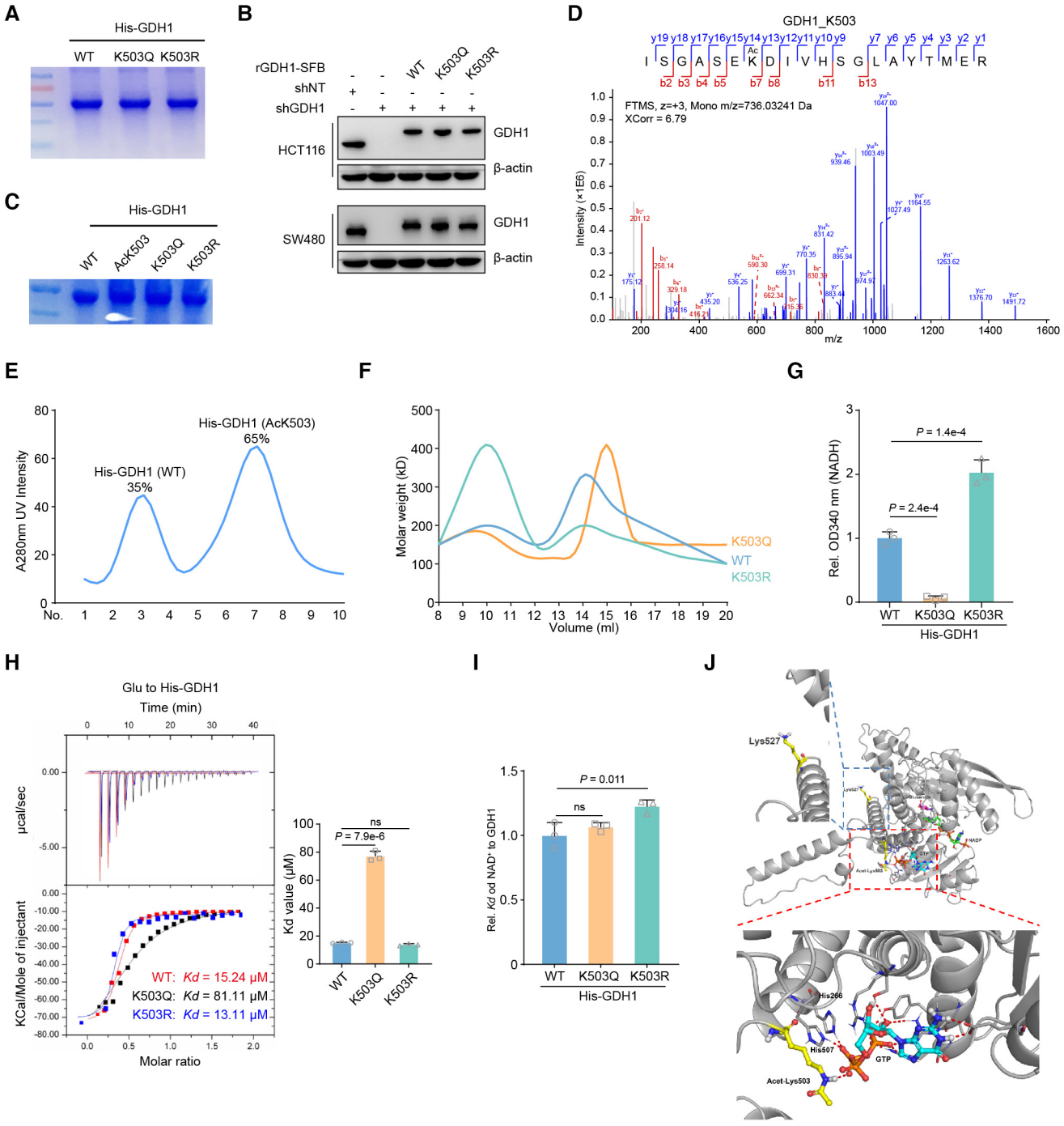

Figure EV4.

Figure EV4. GDH1-K503 acetylation modulates its enzyme activity.

A His-GDH1 (including WT, K503Q and K503R mutants) were purified from *E. coli* and separated by SDS-PAGE. The coomassie blue staining was shown.
B Construction of GDH1-depleted cell lines, which were rescued with expression of WT GDH1 or K503Q, or K503R mutant GDH1.
C His-GDH1 (including WT, AcK503, K503Q and K503R mutants) were purified from *E. coli* and separated by SDS-PAGE. The coomassie blue staining was shown.
D The acetyl modification of AcK503 His-GDH1 from *E. coli* was confirmed by LC–MS/MS.
E His-GDH1 (including WT and AcK503) purified from *E. coli* were separated and analyzed by FPLC.
F His-GDH1 (including WT and AcK503) purified from *E. coli* were analyzed by dynamic light scattering.
G Measure the NADH level by the OD340 absorbance value, respectively ($n = 3$).
H His-GDH1 proteins (including WT, K503Q or K503R) were purified from *E. coli* to determine Kd value. ITC assays were performed with precipitated His-GDH1 proteins and αKG ($n = 3$)
I ITC assays were performed with precipitated His-GDH1 proteins and NAD^+ ($n = 3$)
J The complex of GDH1 (PDB code: 4ED5) with GTP is superimposed on the GDH1-GTP complex. The protein and metabolites are shown as cartoon and are colored gray and orange or yellow, respectively. Acetyl-K503, K527 and GTP are shown as sticks.

Data information: Data are mean ± SD from the biological replicates (G–I). Statistics: one-way ANOVA with Tukey's HSD *post hoc* test (G–I).
Source data are available online for this figure.

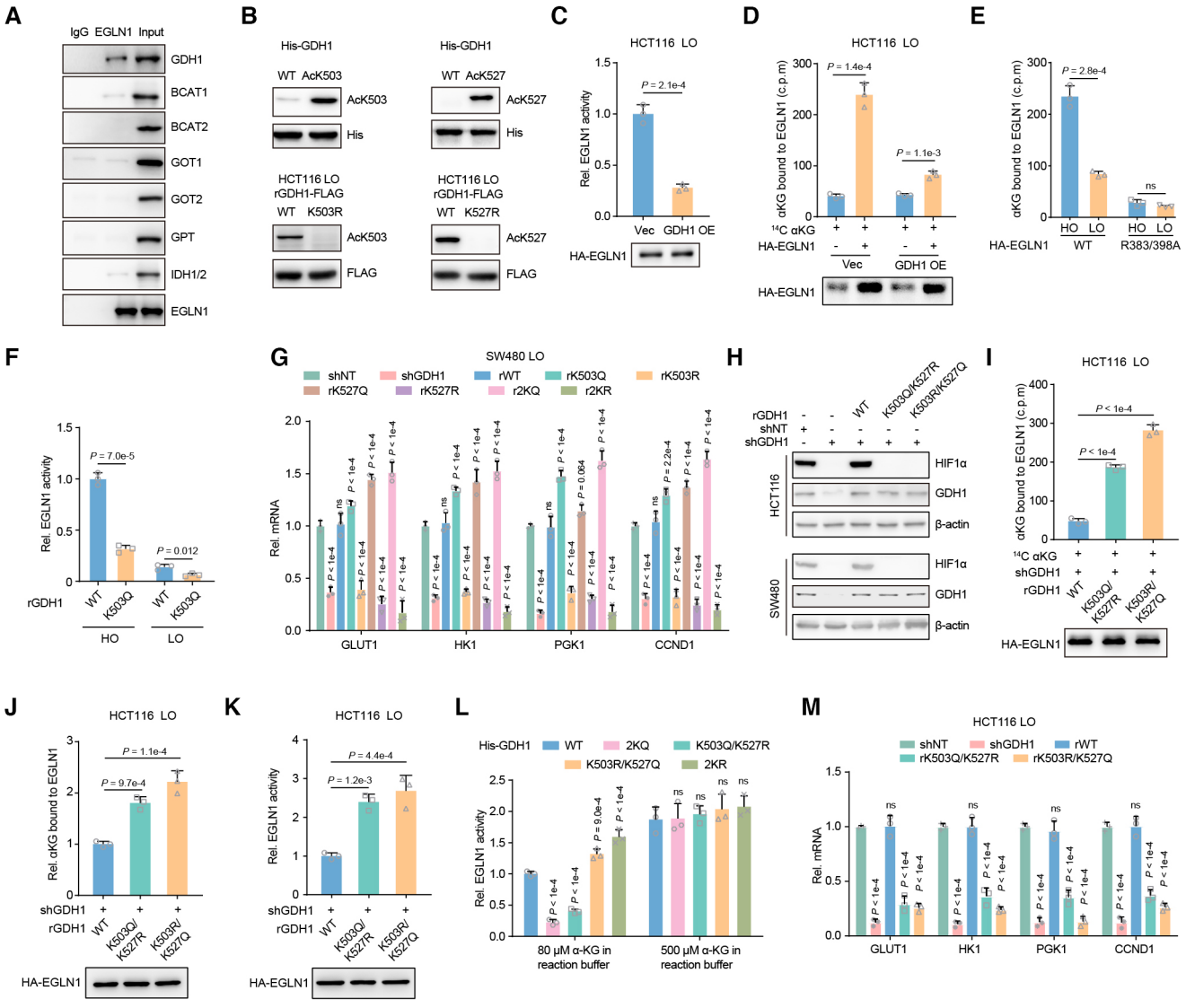

Figure EV5.

Figure EV5. Acetylation modification at both K503 and K527 regulates the survival of CRC cells under hypoxia.

A EGLN1 was enriched from hypoxia pretreated HCT116 cells and indicated antibodies were applied to test the association of EGLN1 with αKG producing or consuming metabolic enzymes.

B The specificity of antibodies against GDH1-AcK503 or -AcK527 was analyzed by western blot. His- GDH1-AcK503 or -AcK527 was prepared from *E. coli*. Restored expression of GDH1-FLAG (including WT, K503R and K527R) was enriched from HCT116 cells.

C HA-EGLN1 was enriched from HCT116 cells with or without GDH1 overexpression under hypoxia and then EGLN1 activity was tested by converting αKG to succinate ($n = 3$).

D Relative αKG in EGLN1. HA-EGLN1 was enriched from Vec or GDH1 OE cells under hypoxia. A radiometric 14C-αKG-EGLN1 binding assay was performed using HA-EGLN1 incubating with 14C-αKG ($n = 3$).

E Negative control used for testing relative αKG in EGLN1. HA-EGLN1 (including WT and R383/398A mutant) was enriched from HCT116 under normoxia and hypoxia. A radiometric 14C-αKG-EGLN1 binding assay was performed using HA-EGLN1 incubating with 14C-αKG ($n = 3$).

F Measure the relative EGNL1 activity affected by restored WT or K503Q GDH1 under normoxia or hypoxia *in vitro* ($n = 3$).

G The mRNA expression of HIF1α target genes, GLUT1, HK1, PGK1 and CCND1 was tested in indicated cell lines ($n = 3$).

H Detection of HIF1α protein stability in GDH1 depleted cell lines which were rescued with expression of WT GDH1 or K503Q/K527R or K503R/K527Q mutant GDH1.

I, J Relative αKG in EGLN1. HA-EGLN1 was enriched from indicated cells under hypoxia. A radiometric 14C-αKG-EGLN1 binding assay was performed using HA-EGLN1 incubating with 14C-αKG (H). EGLN1 was digested by trypsin and LC–MS was applied to detect αKG eluted from EGLN1 (I) ($n = 3$).

K HA-EGLN1 was enriched from indicated genetic manipulated HCT116 cells under hypoxia and then EGLN1 activity was tested by converting αKG to succinate ($n = 3$).

L HA-EGLN1 was enriched from HCT116 cells under hypoxia and then incubated with indicated His-GDH1 in the buffer containing 80 or 500 μM αKG. EGLN1 activity was tested by converting αKG to succinate ($n = 3$).

M The mRNA expression of HIF1α target genes, GLUT1, HK1, PGK1 and CCND1, in indicated cell lines ($n = 3$).

Data information: Data are mean ± SD from the biological replicates (C–G, I–M). Statistics: unpaired two-tailed Student's *t*-test (C–F); one-way ANOVA with Tukey's HSD *post hoc* test (G, I–M).
Source data are available online for this figure.