Supplemental Information

Supplemental Materials and Methods

PCR for determination of mutant IDH1

Genomic DNA was extracted from parental Ovcar3 and Ovcar10 cells (Qiagen). Primers were designed to flank the R132 mutation of IDH1 (**Table S1**). OneTaq® (New England Bioscience) was used to PCR the IDH1 mutation region from Ovcar3 and Ovcar10. The following PCR cycling was used: 94°C for 30 seconds, 94°C for 30 seconds, 62°C for 1 min, 68°C for 1 min, repeated steps 2-4 for 30 cycles, 68°C for 5 min, 4°C hold. The products were run on a 1.5% agarose gel and visualized to confirm amplification. The band was gel extracted (Zymo), and amplicons were sent for sequencing (GeneWiz) with the forward primer.

YSI Metabolite Measurement

Lactate production was measured using a YSI 2950 Bioanalyzer (Yellow Springs, OH). Briefly, the same number of cells were seeded in 12-well plates and media was changed 24-hours later. The next day, fresh media was harvested and cells were counted to normalize for proliferation.

NADPH/NADP+ quantification

The NADP/NADPH-Glo™ Assay kit (Promega) was used to quantify the NADPH/NADP+ ratio. Cells were seeded in white-lined 96-well plates (Corning) at 12,000 cells per well. The next day, media was removed and 50μL of PBS was added to cells. Cells were treated with 50μL of 0.2N NaOH with 1% DTAB (dodecyltrimethylammonium bromide, Sigma) solution. Plate was briefly mixed. 50μL of solution was removed and added to another well. These well were treated with 25μL of 0.4 HCl (termed acid cells). The plate was covered and incubated at 60°C for 15 min. The plate was removed and left to sit at room temperature for 10 min. 25μL of 0.5M Tris base was added to acid cells. 50μL for Tris-HCl was added to remaining wells. The glo reagent was prepared as recommended by the manufacturer. An equal amount of glo-reagent was added to each well and

incubated for 30 mins. The plate was read using a luminometer and NADPH/NADP+ ratio was calculated. 500μM exogenous NADPH (Sigma Aldrich) was added to control wells 30 mins prior to start of experiment.

Flow Cytometry

7AAD: Cells were trypsinized and washed once with 1X PBS. Cells were then resuspended and 5μL of 7-aminoactinomycin D (7AAD) (Tonbo Biosciences) was added. Live cells were run through a 10-color FACSCanto flow cytometer. Cells harvested from the dish with a scraper were used as a positive control. DHE: HGSC cells were incubated for 30 min with 10μM Dihydroethidium (DHE Abcam). Cells were then washed and run through a 10-color FACSCanto flow cytometer. Positive control cells were incubated with 100μM H_2O_2 prior to DHE treatment. Data was analyzed using FlowJo software (Ashland, OR).

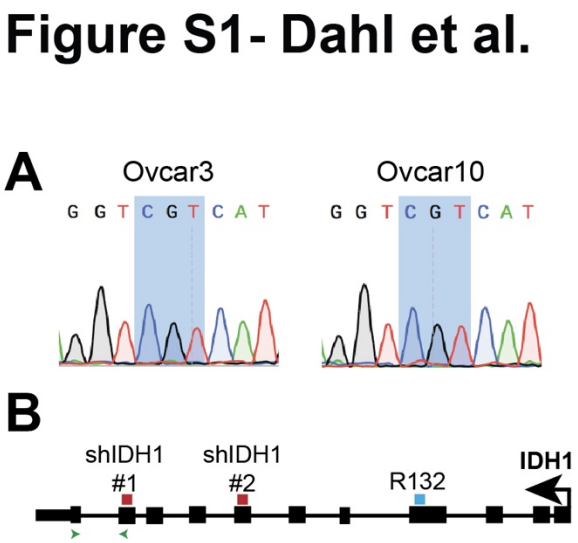
Citrate supplementation

Cells were treated with 1 mM triethyl citrate (Sigma Aldrich) where indicated.

N-acetyl-L-cysteine supplementation

Cells were treated with 500 μM n-acetyl cysteine (NAC Sigma Aldrich) where indicated.

Supplemental Figures and Figure Legends

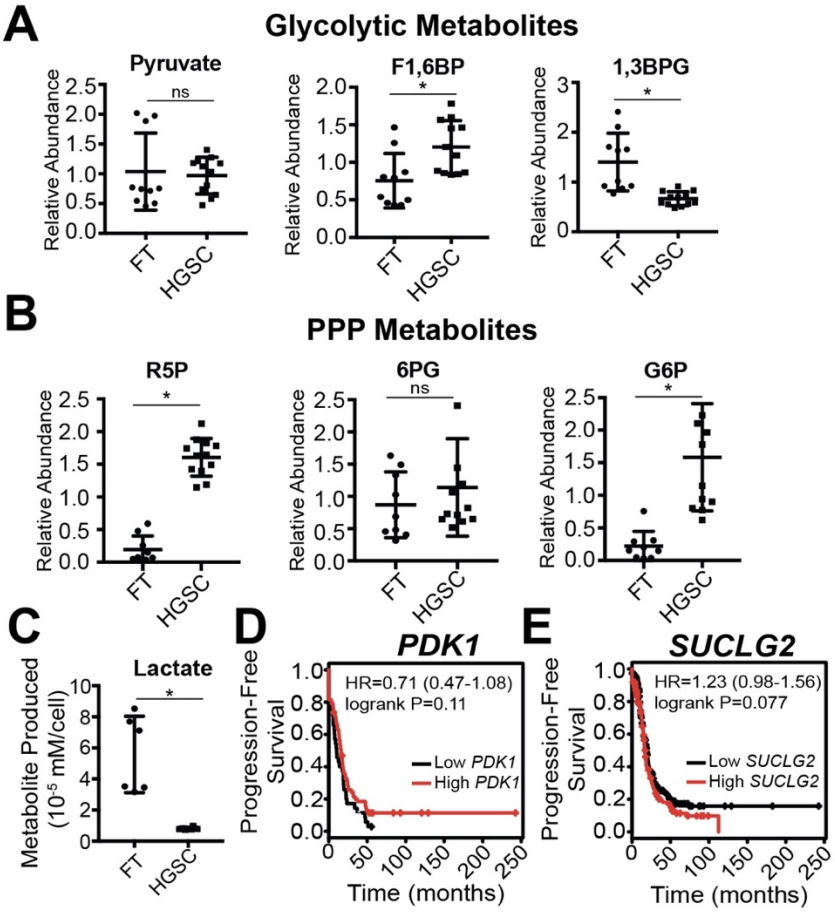


Supplemental Figure 1: Ovcar3 and Ovcar10 cells are wildtype for IDH1; RT-qPCR primers and shRNA binding sites.

(A) Sequencing results of R132 in Ovcar3 and Ovcar10 cells.

(B) Schematic representing the location of the shRNAs targeting IDH1 (#1 and #2 in red), the primer set used to amplify IDH1 in RT-qPCR (green arrows), and the IDH1 mutation location (R132 in blue).

Figure S2- Dahl et al.



Supplemental Figure 2: Glycolytic and PPP metabolites in HGSC cells compared to FT cells; increased expression of TCA cycle enzymes *PDK1* and *SUCLG2* do not decrease progression-free survival of HGSC patients. Related to Figure 1.

(A) LC/MS comparison of FT cell lines (FT282 and FT4-Tag) and HGSC cell lines (Ovcar3 and Ovcar10). Relative abundance of indicated glycolytic metabolites is shown. F1,6BP (fructose 1,6-bisphosphate) and 1,3BPG (1,3-bisphosphoglyceric acid). Data represent replicates. Mean ± SD is shown. *p<0.009. ns=not significant.

(B) LC/MS comparison of fallopian tube cell lines (FT282 and FT4-Tag) and HGSC cell lines (Ovcar3 and Ovcar10). Relative abundance of indicated pentose phosphate pathway (PPP) metabolites is shown. R5P (ribose 5-phosphate), 6PG

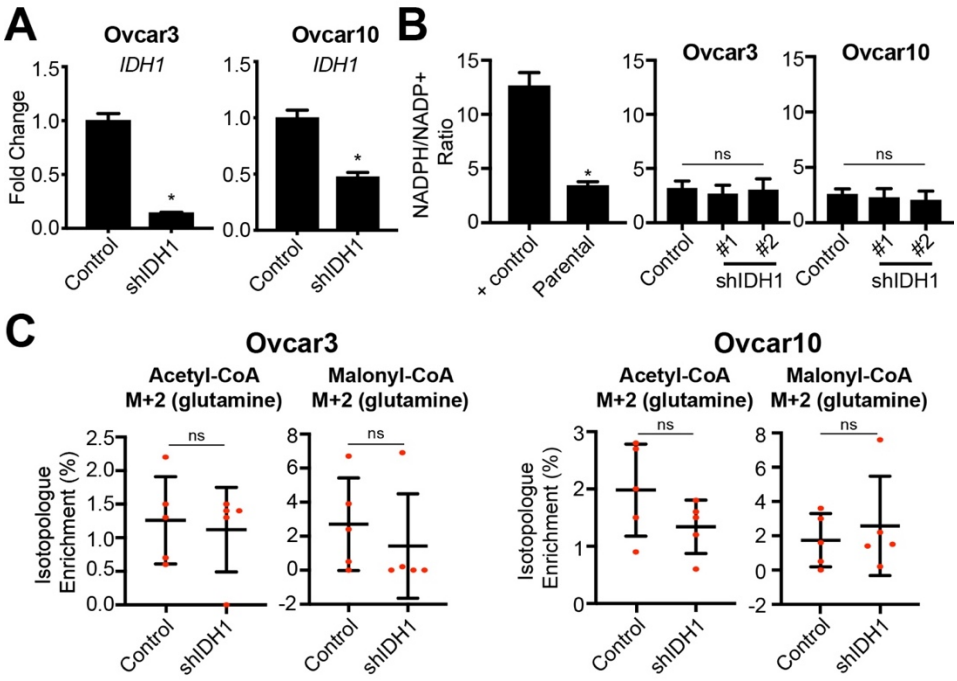
(6-phosphogluconic acid) and G6P (glucose 6-phosphate). Data represent replicates. Mean ± SD is shown. *p<0.0001. ns=not significant.

(C) Lactate production of FT and HGSC cells utilizing the YSI bioanalyzer. Data was normalized to control media and cell number. Data represent replicates. Mean ± SD is shown. *p<0.0008.

(D) Kaplan-Meier curve for progression-free survival of ovarian cancer patients stratified according to *PDK1* expression level. Ovarian cancer patients were filtered for a serous histosubtype and *TP53* mutation. Hazard ratio (HR) and logrank p-values are indicated.

(E) Kaplan-Meier curve for progression-free survival of ovarian cancer patients stratified according to *SUCLG2* expression level. Ovarian cancer patients were filtered for a serous histosubtype and *TP53* mutation. Hazard ratio (HR) and logrank p-values are indicated.

Figure S3- Dahl et al



Supplemental Figure 3: Knockdown of IDH1 does not alter the NADPH/NADP+ ratio or lipid biosynthesis of HGSC cells. Related to Figure 2.

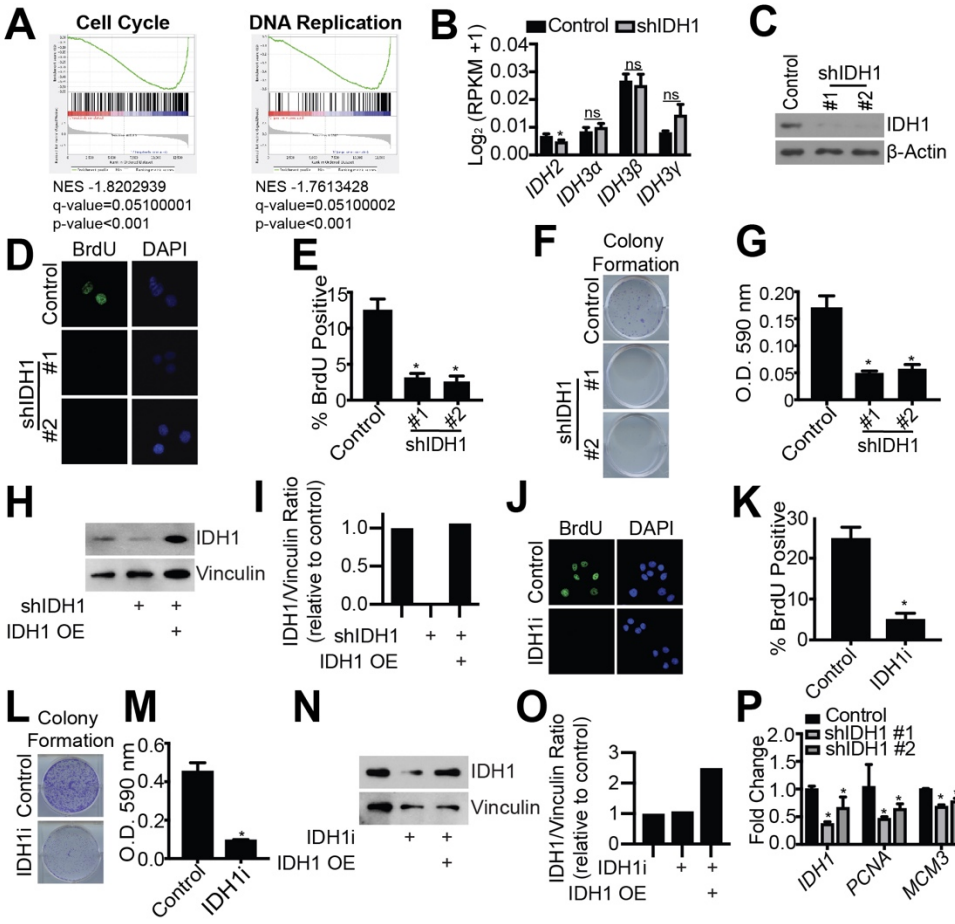
(A-D) Ovcar3 or Ovcar10 cells were infected with lentivirus expressing short hairpin RNAs (shRNAs) targeting IDH1 (shIDH1). Empty vector was used as a control.

(A) RT-qPCR analysis of *IDH1*. *B2M* was used as a reference gene. One of three experiments is shown. *p<0.0003

(B) NADPH/NADP+ ratio of Ovcar3 and Ovcar10 shIDH1 cells. Addition of exogenous NADPH was used as a positive control. One of three experiments is shown. *p<0.0001. ns=not significant

(C) Cells were incubated with $^{13}C_5$ glutamine for 1h. M+2 isotopologue enrichment of acetyl-CoA and malonyl-CoA was determined by LC-MS. Data represent replicates. Mean ± SD is shown. ns=not significant

Figure S4- Dahl et al.



Supplemental Figure 4: Knockdown of IDH1 decreases expression of genes related to cell cycle and DNA replication but does not lead to compensation of other IDH family members; knockdown or pharmacological inhibition of IDH1 suppresses proliferation of Ovcar10 HGSC cells. Related to Figure 3.

(A) Cell cycle and DNA replication GSEA Reactome enrichment analysis in shIDH1 vs. control.

(B) Analysis of *IDH2, IDH3α, IDH3β,* and *IDH3γ* expression of Ovcar3 shIDH1 from RNA-sequencing data. Data represent mean ± SD. *p<0.02. ns=not significant

(C-G) Ovcar10 cells were infected with lentivirus expressing two independent short hairpin RNAs (shRNAs) targeting IDH1 (shIDH1). Empty vector was used as a control.

(C) Immunoblot analysis of IDH1. β-Actin was used as a loading control. One of 5 experiments is shown.

(D) BrdU incorporation. One of three experiments shown.

(E) Quantification of (D). Data represent mean ± SD. *p<0.0001

(F) Colony formation. One of three experiments is shown.

(G) Quantification of (F). Data represent mean ± SD. *p<0.0001

(H-I) Ovcar3 cells were infected with lentivirus expressing a short hairpin RNAs (shRNA) targeting IDH1 (shIDH1) with or without transient overexpressing of IDH1 cDNA (IDH1 OE).

(H) Immunoblot analysis of IDH1. Vinculin was used as a loading control. One of 3 experiments is shown.

(I) IDH1 protein expression from (H) normalized to vinculin and quantified using ImageJ.

(J-M) Ovcar10 cells were treated with either DMSO or 15μM GSK864 (IDH1i) for 7 days.

(J) BrdU incorporation. One of three experiments shown.

(K) Quantification of (J). Data represent mean ± SD. *p<0.0001

(L) Colony formation. One of three experiments is shown.

(M) Quantification of (L). Data represent mean ± SD. *p<0.0001

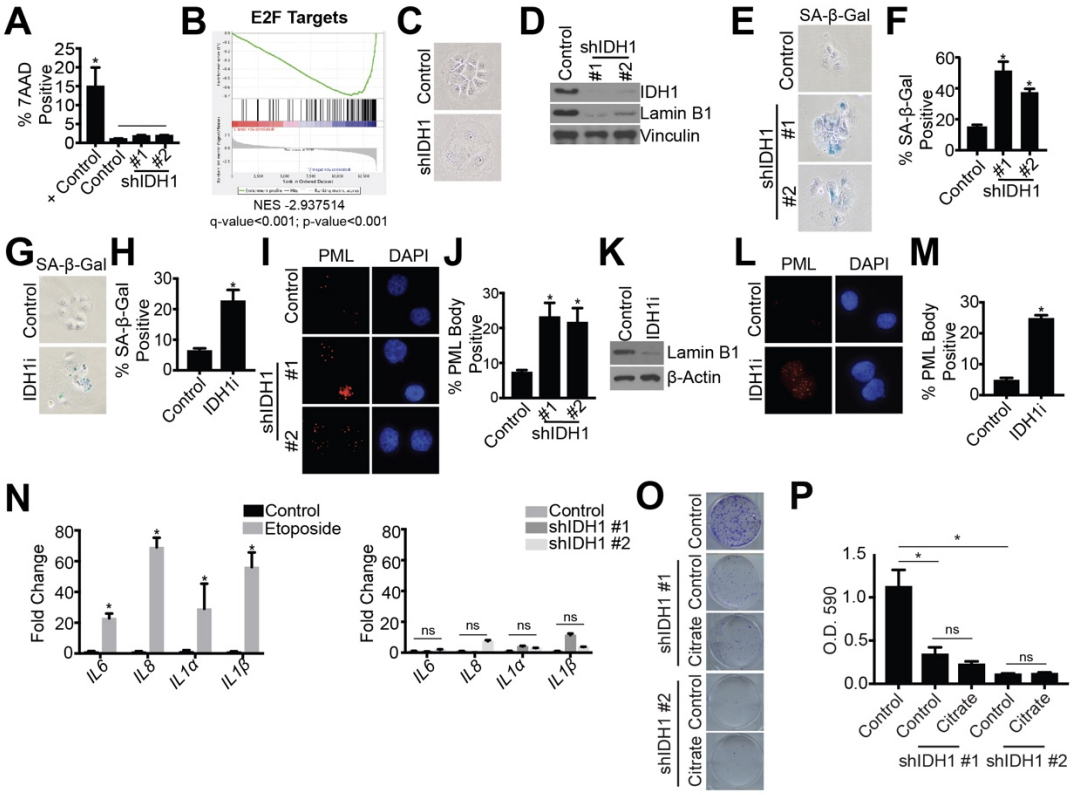
(N-O) Ovcar3 cells were treated with either DMSO or 15μM GSK864 (IDH1i) for 7 days with or without transient overexpressing of IDH1 cDNA (IDH1 OE).

(N) Immunoblot analysis of IDH1. Vinculin was used as a loading control. One of 3 experiments is shown.

(O) IDH1 protein expression from (N) normalized to vinculin and quantified using ImageJ.

(P) RT-qPCR analysis of *IDH1, PCNA,* and *MCM3* of shIDH1 Ovcar10 cells cultured in ULA conditions. *B2M* was used as a reference gene. RNA was collected four days after infection. One of three experiments is shown. Data represent mean ± SD. *p<0.04

Figure S5- Dahl et al.



Supplemental Figure 5: Knockdown of IDH1 induces senescence in Ovcar10 cells but does not increase SASP gene expression. Related to Figure 4.

(A) 7AAD flow cytometry analysis of Ovcar10 shIDH1 cells. Scraped cells were used as a positive control. One of three experiments is shown. *p<0.0005

(B) E2F GSEA Hallmarks enrichment analysis in shIDH1 vs. control.

(C) Brightfield image of cell morphology of Ovcar10 control and shIDH1 cells.

(D) Immunoblot analysis of IDH1 and lamin B1. Vinculin was used as a loading control. One of three experiments is shown.

(E) SA-β-Gal activity. One of three experiments is shown.

(F) Quantification of (E). Data represent mean ± SD. *p<0.0001

(G) SA-β-Gal activity. One of three experiments is shown.

(H) Quantification of (G). Data represent mean ± SD. *p<0.002.

(I) PML body foci. One of three experiments is shown.

(J) Quantification of (I). Data represent mean ± SD. *p<0.004.

(K) Immunoblot analysis of lamin B1. β-Actin was used as a loading control. One of two experiments is shown.

(L) PML body foci. One of three experiments is shown.

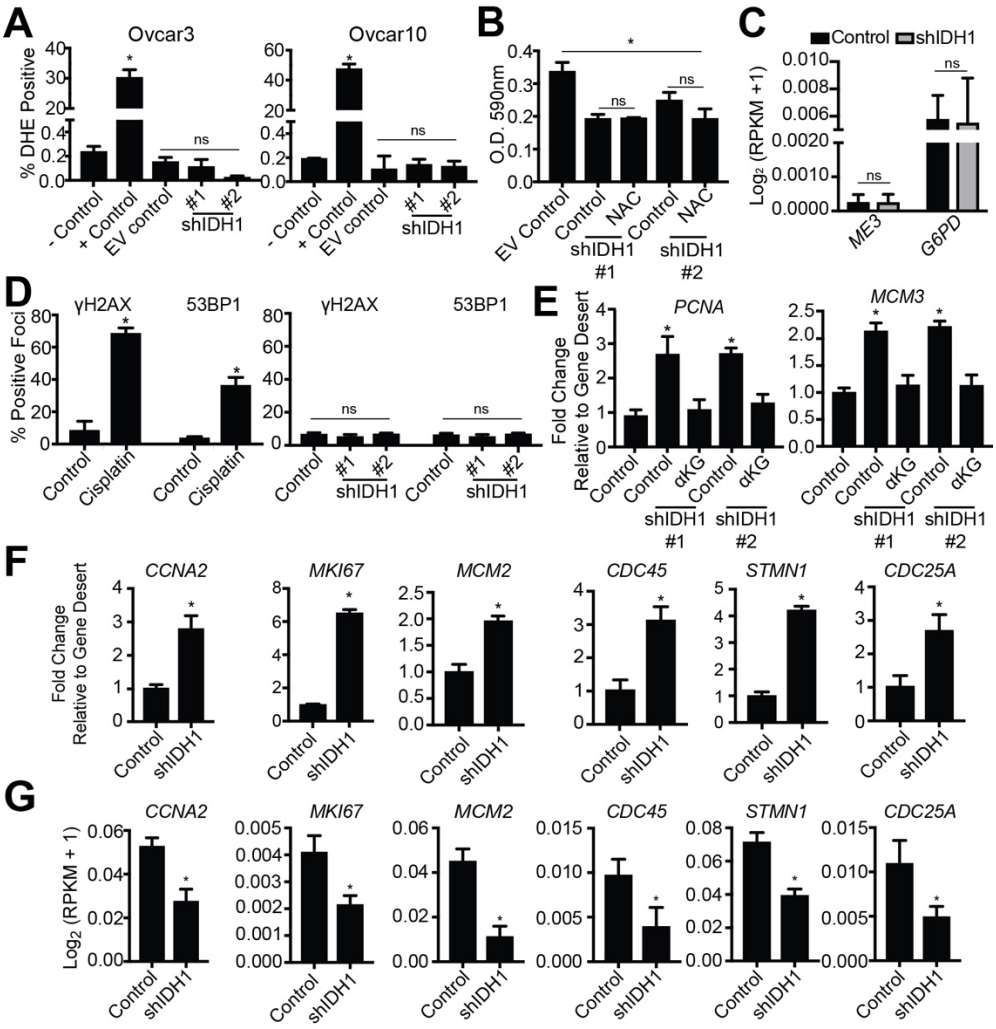
(M) Quantification of (L). Data represent mean ± SD. *p<0.0001

(N) RT-qPCR analysis of SASP gene expression (*IL6*, *IL8*, *IL1*α, *IL1*β) of Ovcar10 shIDH1 cells. *B2M* was used as a reference gene. Etoposide (10μM) was used as a positive control. One of three experiments is shown. Data represent mean ± SD. *p<0.0001.

(O) Colony formation of Ovcar3 shIDH1 cells treated with 1mM citrate for 7 days. One of two experiments is shown.

(P) Quantification of (O). Data represent mean ± SD. *p<0.003.

Figure S6- Dahl et al.



Supplemental Figure 6: Senescence induced by IDH1 knockdown is independent of ROS and DNA damage; addition of exogenous αKG rescues the repressive histone methylation; knockdown of IDH1 increases repressive histone methylation at E2F target genes. Related to Figure 5.

(A) Ovcar3 and Ovcar10 cells were infected with two independent hairpins targeting IDH1. Flow cytometry analysis of ROS using DHE as a marker. H_2O_2 was used as a positive control and H_2O was used as a negative control. Empty vector (EV) was also used as a negative control. One of three experiments is shown. Data represent mean ± SD. *p<0.0001

(B) Ovcar10 cells were infected with two independent hairpins targeting IDH1. 500μM of n-acetyl cysteine (NAC) or vehicle control (water) was added to cells. Cells were seeded in 6-well plates, stained with crystal violet, and quantified. One of two experiments is shown. Data represent mean ± SD. *p<0.005

(C) Analysis of *ME3* and *G6PD* expression of Ovcar3 shIDH1 from RNA-sequencing data. Data represent mean ± SD. ns=not significant

(D) Ovcar3 cells were infected with two independent hairpins targeting IDH1. Immunofluorescence quantification of 53BP1 and γH2AX. Cisplatin (1μM) was used as a positive control. One of three experiments is shown. Data represent mean ± SD. *p<0.02.

(E) Ovcar10 cells were infected with two independent hairpins targeting IDH1 with or without 1mM αKG. H3K9me2 ChIP was performed, and H3K9me2 binding to *PCNA* and *MCM3* was determined by qPCR and is normalized to a gene desert region control. One of two experiments is shown. Data represent mean ± SD. *p<0.0001

(F) Ovcar3 cells were infected with two independent hairpins targeting IDH1. H3K9me2 ChIP was performed, and H3K9me2 binding to *CCNA2, MKI67, MCM2, CDC45, STMN1, and CDC25A* was determined by qPCR and is normalized to a gene desert region control. One of three experiments is shown. Data represent mean ± SD. *p<0.04.

(G) Analysis of *CCNA2, MKI67, MCM2, CDC45, STMN1, and CDC25A* expression of Ovcar3 shIDH1 from RNA-sequencing data. Data represent mean ± SD. *p<0.03.