Supplementary Figure 1. Identification of EGLN1 as a preferential cancer cell dependency.

A. Identification of cancer cells dependent on EGLN1 using CRISPR-Cas9 data from Project Achilles. Histogram shows the distribution of CERES scores (X-axis) across 436 cancer cell lines screened with CRISPR. Data shows that EGLN1 and VHL have the greatest number of dependent cell lines.

B. Identification of cancer cells dependent on EGLN1 using RNAi data from Project Achilles. Histogram shows the distribution of cancer cell lines (Y-axis) that are dependent on members of the EGLN1 pathway based on their DEMETER2 scores (X-axis). Data shows that EGLN1 has the greatest number of dependent cell lines.

C. Identification of cancer cells dependent on EGLN1 using RNAi data from Project Achilles. Histogram shows the distribution of cancer cell lines (Y-axis) that are dependent on EGLN1 based on their DEMETER2 scores (X-axis).

D. Volcano plot showing cancer dependencies associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis). Colored in red are other members of the EGLN1 pathway.

E-L. Volcano plots highlight the top features associated with EGLN1 dependency by effect size and significance for both CRISPR (left) and RNAi (right), grouped by feature type (expression, whole exome copy number, mutation). Colored in red are other members of the EGLN1 pathway. Summarized in Supplementary Tables 3 and 4.

E. Volcano plot showing gene expression associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis) in CRISPR dataset. Colored in red are other members of the EGLN1 pathway.

F. Volcano plot showing gene expression associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis) in RNAi dataset. Colored in red are other members of the EGLN1 pathway.

G. Volcano plot showing genes with copy number alterations associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis) in CRISPR dataset. Colored in red are other members of the EGLN1 pathway.

H. Volcano plot showing genes with copy number alterations associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis) in RNAi dataset. Colored in red are other members of the EGLN1 pathway.

I. Volcano plot showing genes with damaging mutations associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis) in CRISPR dataset. Colored in red are other members of the EGLN1 pathway.

J. Volcano plot showing genes with damaging mutations associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis) in RNAi dataset. Colored in red are other members of the EGLN1 pathway.

K. Volcano plot showing genes with non-damaging mutations associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis) in CRISPR dataset. Colored in red are other members of the EGLN1 pathway.

L. Volcano plot showing genes with non-damaging mutations associated with EGLN1 dependency graphed as effect size (X-axis) against p-value (-log10, Y-axis) in RNAi dataset. Colored in red are other members of the EGLN1 pathway.
M. Volcano plot showing cancer dependencies associated with HIF1A expression graphed as p-value (-\log_{10}, Y-axis) against effect size (X-axis). Colored in red are other members of the EGLN1 pathway.

N. Single-sample GSEA (ssGSEA) reveals pathways including Hypoxia as enriched in CRISPR dataset

O. Single-sample GSEA (ssGSEA) reveals pathways including Hypoxia as enriched in RNAi dataset

P. There is no strong correlation between EGLN1 expression (Y-axis) in cell lines and dependency on EGLN1 (X-axis).
Supplementary Figure 2. EGLN1 dependency is enriched in clear cell ovarian cancer and melanoma and associated with high HIF1A levels

A. Lineage enrichment analysis reveals that ovarian and melanoma lines are significantly (FDR < 0.1) and positively enriched for EGLN1-dependent lines in CRISPR.
B. Ovarian cancer cell lines exhibit strong EGLN1 dependencies in RNAi screens. DEMETER2 scores (X-axis) are stratified by lineage (Y-axis).
C. The strongest EGLN1 dependencies in RNAi are also EGLN1-dependent in CRISPR datasets. Concordance between CRISPR CERES scores and RNAi DEMETER2 scores is strong in ovarian cancer (r = 0.604).
D. RNAi EGLN1 dependencies are enriched in clear cell ovarian cancer. A two-sample t-test of clear cells vs. all other ovarian subtypes reveals that clear cells are significantly enriched for EGLN1 dependencies (p < 0.05).
E. Strong RNAi EGLN1 dependencies express high levels of HIF1A. There is a strong correlation between HIF1A expression and EGLN1 dependency in the RNAi dataset (r = -0.379).
F. ssGSEA (single sample Gene Set Enrichment Analysis) reveals that EGLN1 dependency in RNAi is associated with HIF1A-related pathways. Note that some of the strongest DEMETER2 dependencies (IGROV1 and OAW42) were not screened in CRISPR. Pearson correlations of EGLN1 dependency with each profile (Y-axis) were calculated, and z-score normalized profiles are shown.
Supplementary Figure 3. Immunoblots showing deletion of EGLN1 or VHL

A. Effects of EGLN1 deletion on HIF1A levels. Whole cell lysates of TOV112D-Cas9 with the indicated sgRNAs. Equal amounts of protein were analyzed by immunoblotting with GAPDH included as a marker of equal loading. Deletion of EGLN1 leads to increased HIF1A but not HIF1A phosphorylation. *sgRNAs used for subsequent experiments.

B. VHL deletion. Whole cell lysates of ES2-Cas9 with VHL sgRNA-1 and VHL sgRNA-2. Equal amounts of protein were analyzed by immunoblotting with GAPDH included as a marker of equal loading. Immunoblot shows addition of sgRNA results in deletion of VHL.
Supplementary Figure 4

A. TOV112D
Roxadustat µM

B. ES2
Molidustat µM

C. TOV112D
Molidustat µM

D. EGLN1 inhibitors

E. Relative Proliferation

F. Relative Apoptosis

G. JHOC5
Cell Doublings

H. TOV21G
Cell Doublings

I. 2uM FG-4592

J. Relative colony growth
Supplementary Figure 4. Inhibiting EGLN1 increases HIF1A expression and reduces proliferation in EGLN1-dependent cells.

A. Immunoblot showing that pharmaceutical inhibition (Roxadustat - FG-4592) of EGLN1 increases HIF1A levels in a dose-dependent manner in TOV112D.
B. Immunoblot showing that pharmaceutical inhibition (Molidustat – Bay 85-3934) of EGLN1 increases HIF1A levels in a dose-dependent manner in ES2.
C. Immunoblot showing that pharmaceutical inhibition (Molidustat – Bay 85-3934) of EGLN1 increases HIF1A levels in a dose-dependent manner in TOV112D.
D. Cellular viability measured by CellTiter-Glo of ES2 treated with various EGLN1 inhibitors including IOX2, FG-2216, Daprodustat (GSK1278863). IC50 was observed around 40-50uM for most of these compounds as observed with FG-4592 and Bay 85-3934.
E. Cellular proliferation assessed through flow cytometry. Increasing concentration of FG-4592 reduces cell proliferation in ES2 while increasing concentration of FG-4592 does not reduce cell proliferation in TOV112D. Proliferation was measured by CFSE stain over 48 hours.
F. Apoptosis assessed through flow cytometry. Treatment with 40uM of FG-4592 increases apoptosis in ES2, OVISE while not increasing apoptosis in TOV112D, OVCAR4. Apoptosis was measured by Annexin V stain after 48 hours of treatment.
G. EGLN1 inhibitor FG-4592 reduces long term proliferation in EGLN1-dependent cell line JHOC5 in a dose-dependent manner. Results are representative of 3 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.
H. EGLN1 inhibitor FG-4592 reduces long term proliferation in EGLN1-dependent cell line TOV21G in a dose-dependent manner. Results are representative of 3 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.
I. EGLN1 inhibitor FG-4592 reduces colony formation in EGLN1-dependent cell lines and has no effect on not EGLN1-dependent cell lines. Results are representative of 3 independent experiments.
J. Inhibition of EGLN1 reduces cell viability in hypoxia. Cells were cultured for up to 11 days in 5% hypoxia or normoxia in a colony forming assay. Inhibition of EGLN1 reduces relative colony growth over time in EGLN1-dependent cell lines OVTOKO, OVISE and ES2. Inhibition of EGLN1 in cell line TOV112D, not dependent on EGLN1, did not reduce colony growth.
Supplementary Figure 5. Inhibiting EGLN1 or VHL reduces proliferation in EGLN1-dependent cells.

A. Inhibition of EGLN1 using small molecule inhibitor IOX2 reduces viability in a dose-dependent manner. Cells were treated with IOX2 over 5 days and viability was measured using CTG. Results are representative of 2 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.

B. Inhibition of EGLN1 using small molecule inhibitor FG-4592 reduces viability in a dose-dependent manner. Cells were treated with FG-4592 over 5 days and viability was measured using CTG. Results are representative of 3 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.

C. EGLN1 inhibitor IOX2 reduces colony formation in EGLN1-dependent cell lines and has no effect on not EGLN1-dependent cell lines. Results are representative of 3 independent experiments.

D. EGLN1 inhibitor IOX2 reduces long term proliferation in EGLN1-dependent cell line ES2 in a dose-dependent manner. Results are representative of 2 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.

E. EGLN1 inhibitor IOX2 reduces long term proliferation in EGLN1-dependent cell line OVISE in a dose-dependent manner. Results are representative of 2 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.

F. EGLN1 inhibitor IOX2 reduces long term proliferation in EGLN1-dependent cell line OVTOKO in a dose-dependent manner. Results are representative of 2 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.

G. Immunoblot showing that pharmaceutical inhibition (VH298) of VHL increases HIF1A levels in a dose-dependent manner in ES2.

H. VHL inhibitor VH298 reduces long term proliferation in EGLN1-dependent cell line OVISE in a dose-dependent manner. Results are representative of 2 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.

I. VHL inhibitor VH298 reduces long term proliferation in EGLN1-dependent cell line OVTOKO in a dose-dependent manner. Results are representative of 2 independent experiments and data is graphed as mean ± standard deviation of 3 replicates.
Supplementary Figure 6

A

EGLN1 WT KO

row max

A

B

Enrichment plot: GROSS_HIF1A_TARGETS_DN

Enrichment plot: ELVIDGE_HYPOXIA_UP

Enrichment plot: ELVIDGE_HYPOXIA_BY_DMOG_UP
Supplementary Figure 6. Inhibiting EGLN1 increases HIF1A expression and reduces proliferation in EGLN1-dependent cells.

A. RNA-seq of ES2 and OVISE cells with control (sgChr2-1, Lanes 1-4) sgRNAs or EGLN1 (sgEGLN1-9, Lanes 5-8) sgRNAs. Red = high, Blue = low
B. Gene Set Enrichment Analysis of differentially expressed genes in EGLN1 KO cells compared to EGLN1 WT cells. Several of the top scoring pathways related to HIF1A-related genes or Hypoxia pathway.
Supplementary Figure 7. Small molecule inhibition of EGLN1 or VHL in vivo increases apoptosis

Microdevice delivery of EGLN1 inhibitors and VHL inhibitors to ovarian tumor with HIF1A expression increases apoptosis. Microdevices were loaded with PEG-formulated compounds and implanted into 1cm² tumors formed from ES2 or from ES2 with HIF1A KO and grown to 1cm². At 48 hours post microdevice implantation tumors are harvested and serially sectioned and stained. The control for drug formulation (PEG Control) has no effect on the cell death of ES2 tumor cells with or without HIF1A (top). Doxorubicin treatment increases tumor cell apoptosis (top middle). Inhibition of EGLN1 with FG-4592 increases apoptosis of tumor cells, while knockout of HIF1A rescues EGLN1 inhibition (bottom middle). Inhibition of VHL with VH298 increases apoptosis of tumor cells while knockout of HIF1A rescues VHL inhibition. Figure is representative of three independent experiments. Arrow shows area where drug is released. Black line indicates 1mM.