Supplemental Methods

Tumors
All tumors were obtained in accordance with Institutional Review Board policies. Tumor samples were acquired from two biorepositories: 1) Genitourinary tissue bank at the University of Texas Health Science Center and 2) Cooperative Human Tissue Network. All tumors were histologically confirmed to be clear cell histology by a certified pathologist.

Tissue Metabolomics
100 µl of tissue sample was thawed on ice and extracted using an automated MicroLab STAR® system (Hamilton Company, Salt Lake City, UT) in 400 µl of methanol, containing recovery standards with vigorous shaking for 2 minutes (Glen Mills Genogrinder 2000). The resulting extract was divided into four fractions: one for analysis by UHLC/MS/MS2 optimized for basic species, one for analysis by UHLC/MS/MS2 optimized for acidic species, one for analysis by GC-MS, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. Each sample was then frozen and dried under vacuum, then prepared for the appropriate instrument. The samples were placed on a TurboVap (Zymark) to evaporate the organic solvent and dried under vacuum. Each sample was analyzed on the GC/MS Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer and LC/MS Waters ACQUITY UPLC and a Thermo-Finnigan LTQ mass spectrometer.

Synthesis of L-2HG-octyl ester
L-2HG-octyl ester was synthesized following a reported procedure (1) with slight modifications. Briefly, commercial γ-benzyl-L-glutamate was diazotized using NaNO2
and acetic acid in the presence of water to obtain the intermediate alcohol. The product was alkylation with octyl iodide in the presence of NaHCO₃ in anhydrous DMF to obtain the dibenzyl ester. Debenzylation of the compound using H₂ gas in the presence of Pd/C in EtOAc afforded L-2HG octyl ester.

**VHL Mutation Analysis**

Genomic DNA for VHL mutational analysis was isolated from 25 mg of tissue using the PureLink Genomic DNA Mini Kit (Life Technologies). Four previously reported PCR primer sets that cover the VHL gene 5'-UTR and the three exons were used for this analysis(2). Fifty nanograms of the genomic DNA was amplified by PCR in 25 l reactions containing 10 M primers and Taq 2X Master Mix (New England Biolabs). PCR reaction conditions were as follows: 30 second hot start (95 °C) followed by 30 cycles of 95 °C for 30 seconds, annealing at the appropriate temperature for 1 minute, extension for 1 minute at 68 °C with a final extension of 5 minutes at 68 °C. The annealing temperature for the 5'-UTR, exon 1 and exon 2 amplicons was 60 °C and the annealing temperature for the exon 3 amplicon was 54 °C. The PCR amplicons were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). The amplicons were sequenced in both directions using the BigDye Terminator cycle sequencing kit (Life Technologies) and analyzed on an Applied Biosystems 3130XL Genetic Analyzer. The mutations identified were compared with the VHL mutations database (http://www.umd.be/VHL/gene.shtml).

**Histone extraction and Western Blotting**

For histone acid extraction, cell pellets were resuspended in Triton Extraction Buffer (TEB;PBS containing 0.5% Triton X100, 2mM PMSF, .02% NaN₃) followed Abcam Histone extraction protocol. After centrifugation, cell pellets was resuspended in 0.2N
HCl and incubated for overnig at 4C. Histone extracts were collected by centrifugation and protein amount was determined using Bradford assay. For western blots, cell lysates were extracted with IP lysis buffer (Thermo) and quantified by BCA protein assay (Pierce). Samples were resolved on SDS polyacrylamide gel (Bio-Rad) and transferred to nitrocellulose membrane by semidry transfer. Samples were analyzed by immunoblotting with antibodies for FLAG, beta-actin (Sigma), L2HGDH (Proteintech), H3K9me3(Abcam), H3K27me3(Millipore) and H3 (CST).

TCGA Data Analysis
Level 3 data on TCGA samples was extracted through the cBIO Genomics Portal. Tumors with both copy number analysis (as determined by GISTIC) and mRNA expression data (RNAseq) were analyzed. A comparative analysis of RNAseq z-scores was determined between tumors with copy loss (i.e. loss of heterozygosity) and tumors without copy number alterations (diploid). Two sample t-test was used to compare the differences between two groups.

Immunohistochemistry
The paraffin sections were cleared in the series of xylene/alcohol and were treated in citrate buffer and 3% H2O2. The sections were blocked with 10% normal goat serum (vector) for 1 hour at room temperature (RT) and incubated with anti-L2HGH for overnight. After washing, the sections were treated anti-Rabbit Ig(Vector). The sections were developed with DAB substrate (INNOVEX) at RT and counterstained with haematoxylin.

Proliferation assay
Cells were counted and plated in 25, 000 per 6 well-plate each in triplicates. Cells were incubated at 37C, 5% CO2 and counted in every 2 days.

Colony-forming Assay
Cells were counted and plated in 1,500 cells in 100mm dishes (duplicates) and incubated at 37°C, 5% CO2 for 10-14 days. Colonies were fixed with 10% (v/v) methanol for 15 min and stained with Giemsa (Sigma) for 20 min for colony visualization.

References