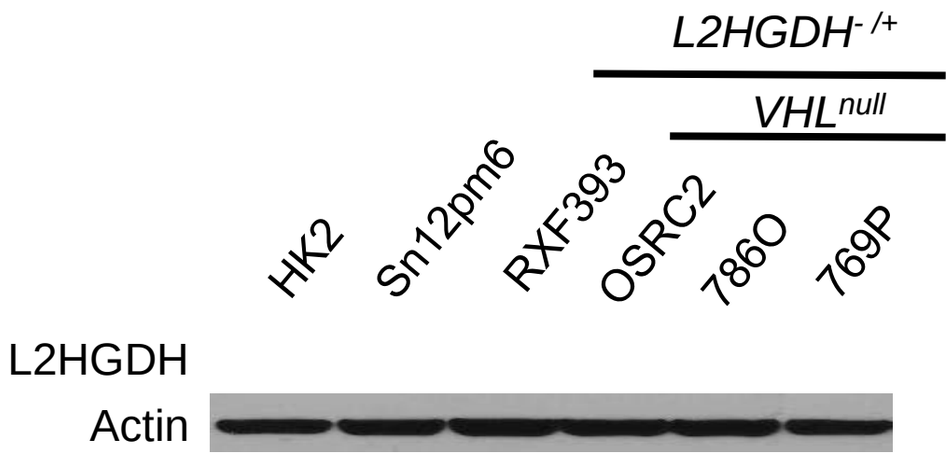
A



B

Cell line	14q-Status	VHL-status	L2HGDH-CNV	Hif1a-CNV	Dataset	Sources
HEK293T	WT	WT	0	0		(Lin et al., 2014)
HK2	WT	WT	0	0		(Brodaczewska, Szczylik, Fiedorowicz, Porta, & Czarnecka, 2016) (Tym et al., 2016)
Sn12pm6	No Data	No Data	No Data	No Data		
RXF393	No Data	WT	-1	0	NCI-60	(Reinhold et al., 2012) (Suwaki et al., 2011)
OSRC2	No Data	Mutant	-1	1	Cancer Cell Line Encyclopedia 2012	(Barretina et al., 2012) (Reinhold et al., 2012)
786O	Loss	Mutant	-1	-2	Cancer Cell Line Encyclopedia 2012	(Alimov et al., 2000) (Barretina et al., 2012) (Brodaczewska, Szczylik, Fiedorowicz, Porta, & Czarnecka, 2016) (Strefford et al., 2005)
769P	Loss	Mutant	-1	-2	Cancer Cell Line Encyclopedia 2012	(Alimov et al., 2000) (Barretina et al., 2012) (Brodaczewska, Szczylik, Fiedorowicz, Porta, & Czarnecka, 2016) (Strefford et al., 2005)
Caki1	Loss	WT	-1	-1	Cancer Cell Line Encyclopedia 2012	(Alimov et al., 2000) (Barretina et al., 2012) (Brodaczewska, Szczylik, Fiedorowicz, Porta, & Czarnecka, 2016) (Strefford et al., 2005)

Figure S1. RCC cell line description. A) Immunoblot for L2HGDH in various cell lines. Genomic *L2HGDH* and *VHL* status depicted. Sn12pm6 L2HGDH status is unknown but believed to be homozygous WT and VHL. **B)** Table characterizing Cell lines used. Dataset indicated in cbioportal (https://www.cbioportal.org/) analysis displayed in fifth column. CNV refers to copy number variants. Negative values depict copy loss of gene, and positive numbers depict copy gain of gene.

Alimov, A., Kost-Alimova, M., Liu, J., Li, C., Bergerheim, U., Imreh, S., . . . Zabarovsky, E. R. (2000). Combined LOH/CGH analysis proves the existence of interstitial 3p deletions in renal cell carcinoma. *Oncogene, 19*(11), 1392-1399. doi:10.1038/sj.onc.1203449

Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A. A., Kim, S., . . . Garraway, L. A. (2012). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature, 483*(7391), 603-607. doi:10.1038/nature11003

Brodaczewska, K. K., Szczylik, C., Fiedorowicz, M., Porta, C., & Czarnecka, A. M. (2016). Choosing the right cell line for renal cell cancer research. *Mol Cancer, 15*(1), 83. doi:10.1186/s12943-016-0565-8

Lin, Y. C., Boone, M., Meuris, L., Lemmens, I., Van Roy, N., Soete, A., . . . Callewaert, N. (2014). Genome dynamics of the human embryonic kidney 293 lineage in response to cell biology manipulations. *Nat Commun, 5*, 4767. doi:10.1038/ncomms5767

Reinhold, W. C., Sunshine, M., Liu, H., Varma, S., Kohn, K. W., Morris, J., . . . Pommier, Y. (2012). CellMiner: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the NCI-60 cell line set. *Cancer Res, 72*(14), 3499-3511. doi:10.1158/0008-5472.CAN-12-1370

Strefford, J. C., Stasevich, I., Lane, T. M., Lu, Y. J., Oliver, T., & Young, B. D. (2005). A combination of molecular cytogenetic analyses reveals complex genetic alterations in conventional renal cell carcinoma. *Cancer Genet Cytogenet, 159*(1), 1-9. doi:10.1016/j.cancergencyto.2004.09.020

Suwaki, N., Vanhecke, E., Atkins, K. M., Graf, M., Swabey, K., Huang, P., . . . Thomas, G. V. (2011). A HIF-regulated VHL-PTP1B-Src signaling axis identifies a therapeutic target in renal cell carcinoma. *Sci Transl Med, 3*(85), 85ra47. doi:10.1126/scitranslmed.3002004

Tym, J. E., Mitsopoulos, C., Coker, E. A., Razaz, P., Schierz, A. C., Antolin, A. A., & Al-Lazikani, B. (2016). canSAR: an updated cancer research and drug discovery knowledgebase. *Nucleic Acids Res, 44*(D1), D938-943. doi:10.1093/nar/gkv1030

A

	Kidney	Kidney	Liver	Liver	Muscle	Muscle
	L2HG nM/g tissue	D2HG nM/g tissue	L2HG nM/g tissue	D2HG nM/g tissue	L2HG nM/g tissue	D2HG nM/g tissue
WT	10.82170153	33.57294421	1.205087373	2.88015882	24.06087393	28.84996874
WT	3.015182025	6.083730104	1.066566642	1.947983574	17.46666102	27.4105522
WT	7.594435277	21.12633813	2.147866761	7.041052875	12.3863429	21.06340664
WT	12.12458138	43.60550698	3.323879612	10.40413194	18.63936778	27.07146272
WT	12.77616118	25.94254448	5.738375694	14.30179788	21.18718248	28.76073958
HET	17.61376121	17.4397504	2.762264261	7.512958099	34.3930556	20.5975885
KO	4995.348645	129.6887608	227.2257509	23.27037387	376.7103529	31.45932202
KO	2935.883314	127.9893919	42.69314487	4.649831253	201.1203439	34.01738755
KO	1323.109679	75.91966485	106.9425919	12.63449326	382.2049692	34.3988449
KO	4135.785813	175.3279618	101.261952	7.433704276	388.2907435	35.97909948
KO	1873.173888	80.71231653	120.4738564	12.04866266	423.2089644	49.10728638
KO	2303.892806	91.60806449	79.00750239	9.937049517	422.4779693	31.41682567

Figure S2. L-2HG measurement values from KO mice and littermate controls. A) L-2HG measurements by GC/MS. Mouse Genotype depicted as *L2hgdh*$^{+/+}$ (WT), *L2hgdh*$^{+/-}$ (Het), and *L2hgdh*$^{-/-}$ (KO). As described in methods, mice were fasted for 12 hours prior to metabolite extraction. Het mice demonstrated similar L-2HG measurements as WT in all tissues. Values color coordinated based on value with red = higher and blue = lesser values of 2HG.

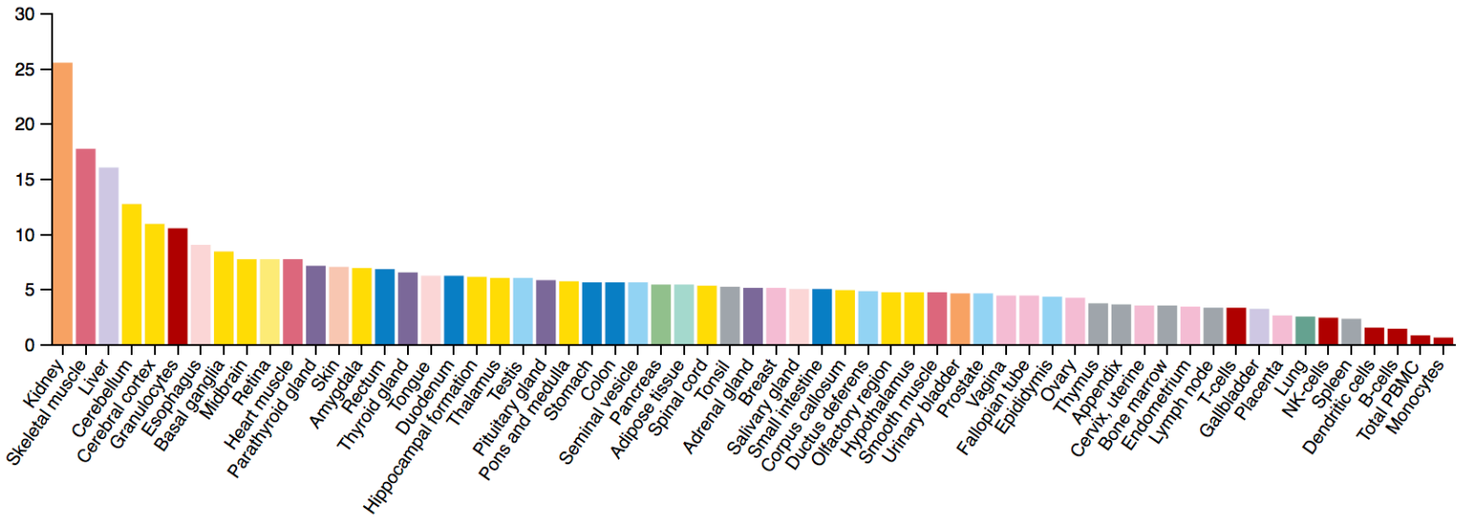


Figure S3. Consensus tissue expression of L2HGDH from three transcriptomic data sets (Human Protein Atlas RNAseq data, GTEx, and CAGE data from the FANTOM5 project)(https://www.proteinatlas.org/ENSG00000087299-L2HGDH/tissue).

Uhlen, M., Fagerberg, L., Hallstrom, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., . . . Ponten, F. (2015). Proteomics. Tissue-based map of the human proteome. *Science, 347*(6220), 1260419. doi:10.1126/science.1260419

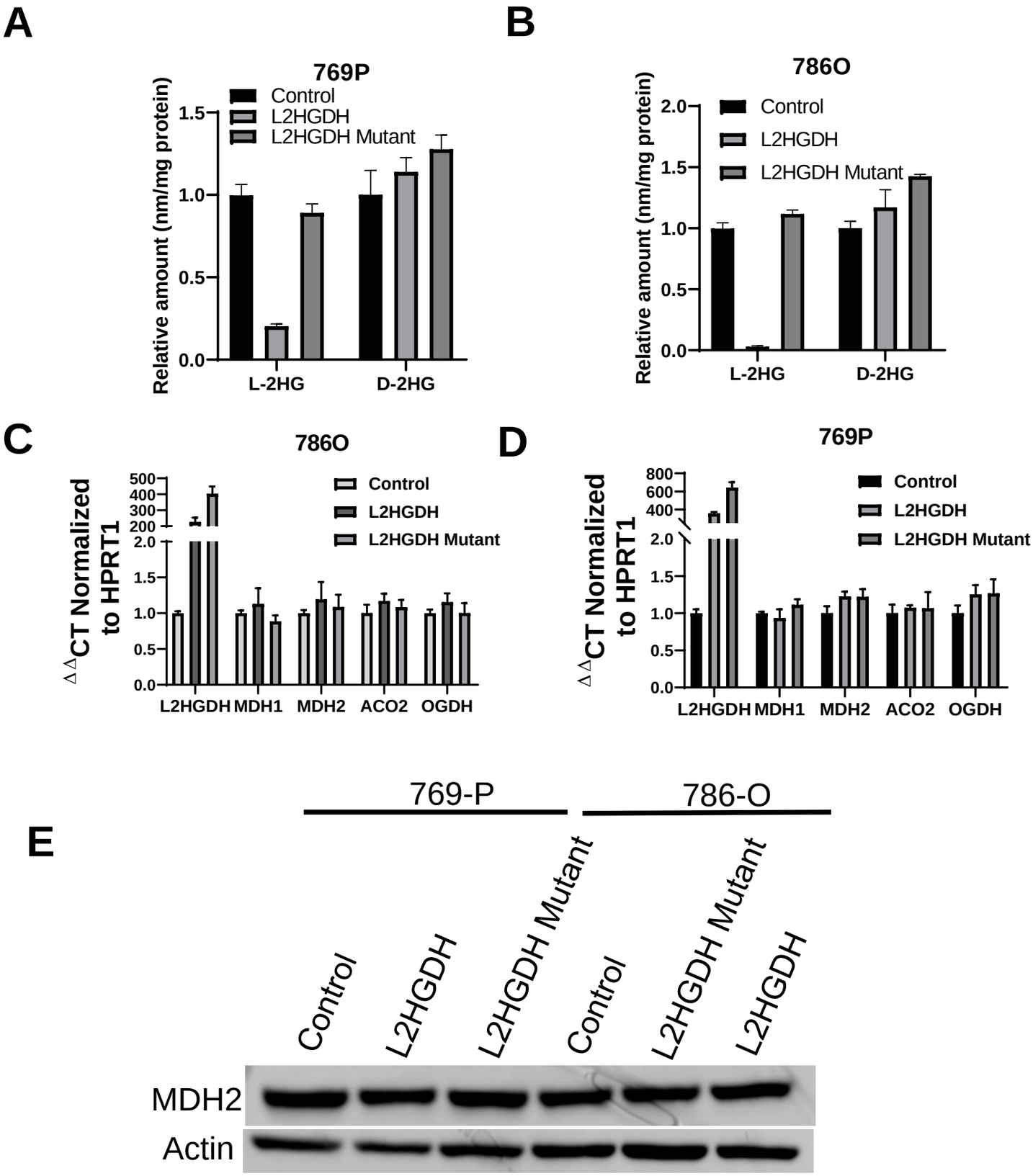


Figure S4. L-2HG does not impact TCA cycle enzyme levels. A, B) D/L- 2HG levels in 769-P and 786O RCC cells expressing control vector , WT L2HGDH, and mutant A241G L2HGDH. Cells were then analyzed for relative mRNA (panels C,D) and protein (panel E) expression of TCA cycle enzymes.

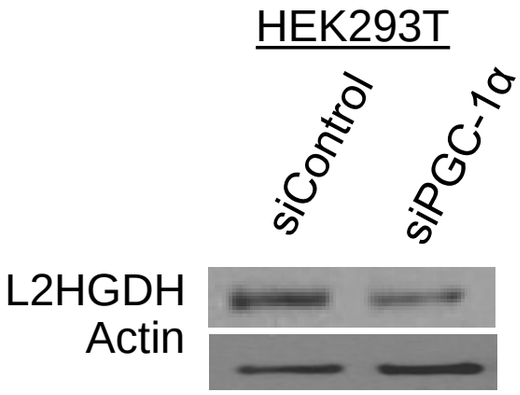


Figure S5. PGC-1α knockdown in HEK293T. Immunoblot of embryonic kidney cell line HEK293T cells with both siControl and siPGC-1α knockdown depicting L2HGDH and actin levels.

Table S1. Gas chromatography L-2HG measurements in *L2hgdh* transgenic mouse

Click here to Download Table S1

Table S2. [U-^{13}C] glucose labeling LC/MS data

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Table S3. GRACE RCC L2HGDH correlation results

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