SI APPENDIX

Figure S1

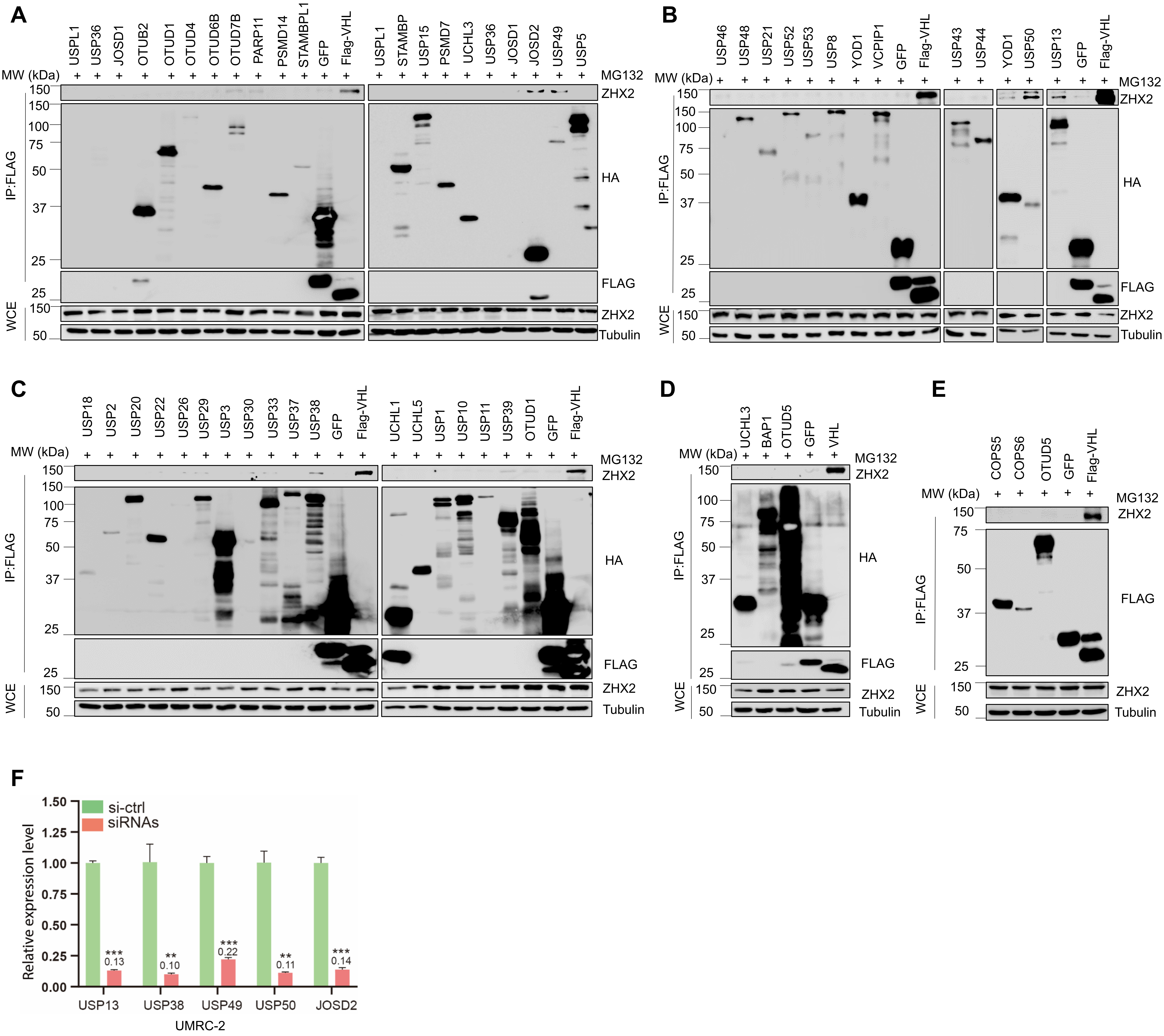

Figure S1. Screen to identify DUBs that bind with ZHX2.

A-E Immunoblots of whole cell extracts (WCE) and immunoprecipitations (IP) from 293T cells transfected with Flag-HA tagged DUB proteins and treated with 10μM MG132 as indicated. FLAG-HA-GFP is negative control, FLAG-VHL is positive control. For the blot "IP: Flag, IB: Flag", only partial gel showing focusing on GFP and VHL expression(A-D). Fig S1E only had IP:FLAG,IB:FLAG. **F.** qRT-PCR quantification of indicated genes from UMRC-2 cells transfected with corresponding siRNA pools. Error bars, SEM; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure S2

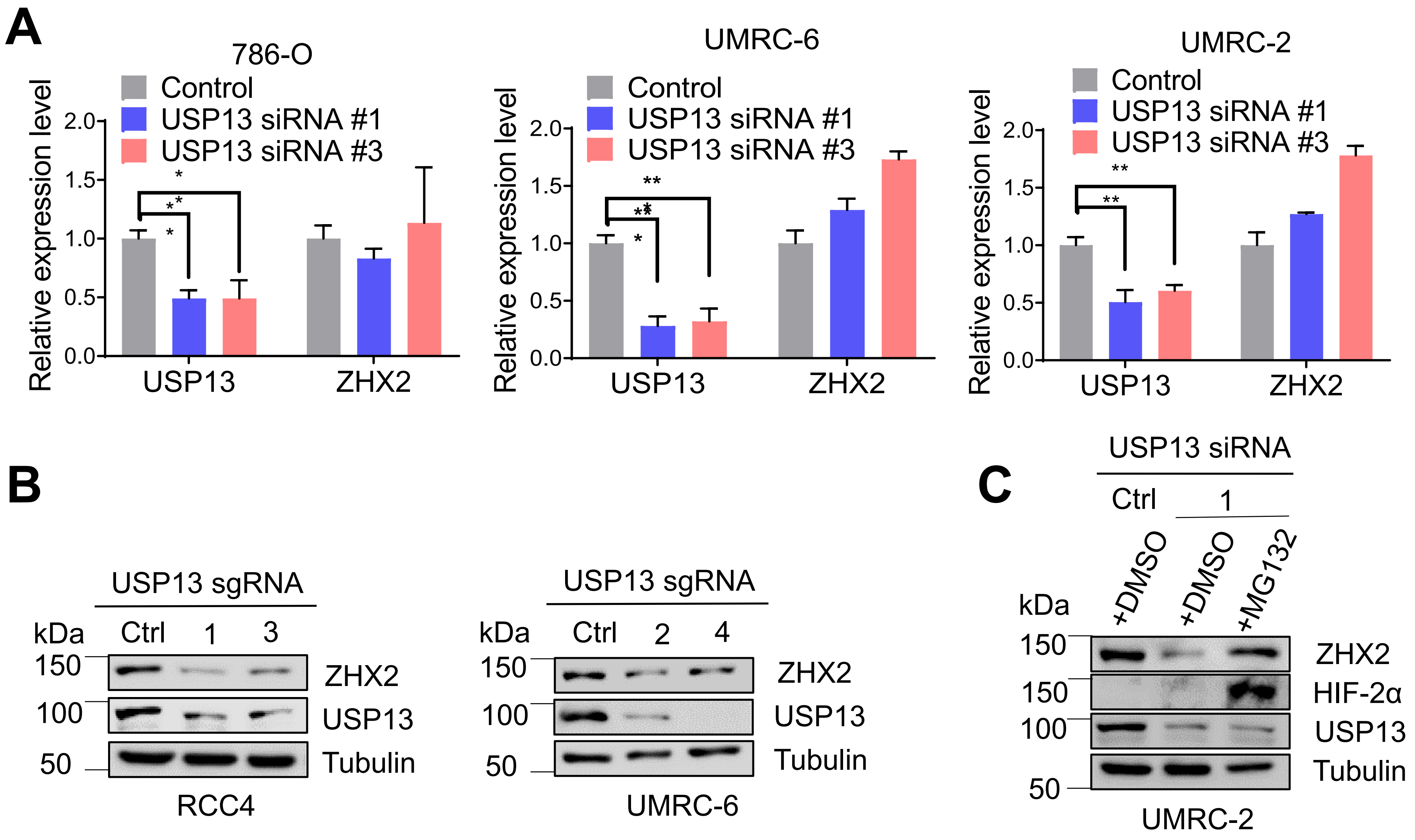

Figure S2. USP13 depletion leads to decreased ZHX2 by promoting ZHX2 degradation.

A. USP13-KD does not affect the transcription of ZHX2. The mRNA level of ZHX2 and USP13 in ccRCC cell lines (786-O, UMRC-6 and UMRC-2) transfected with siUSP13 (#1 and #3). **B.** USP13-KD affect the protein level of ZHX2. The protein level of ZHX2 and USP13 in ccRCC cell lines (786-O, UMRC-6 and UMRC-2) infected with sgUSP13. **C.** USP13 depletion leads to decreased ZHX2 by promoting ZHX2 degradation. Immunoblots of lysates from UMRC-2 infected with USP13 siRNA, and then treated with DMSO or MG132 as indicated. Error bars, SEM; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure S3

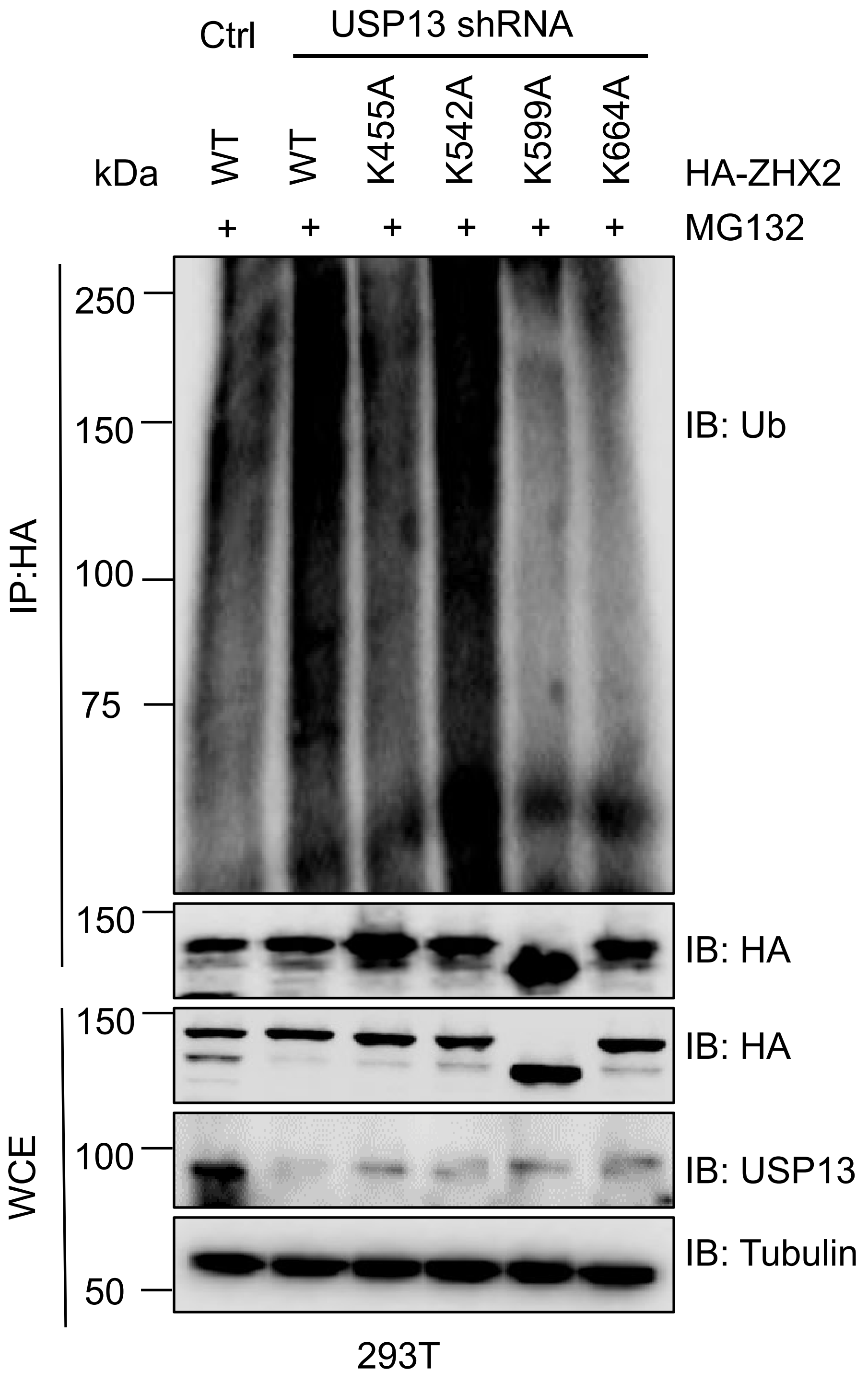

Figure S3. Potential ubiquitination site(s) of ZHX2 regulated by USP13.

Immunoblots of WCE or IP from 293T cell transfected with USP13 shRNA, Ctrl or HA-ZHX2-WT, HA-ZHX2 mutant (K455A, K542A, K599A or K664A) and then treated with 10 μM MG132 as indicated.

Figure S4

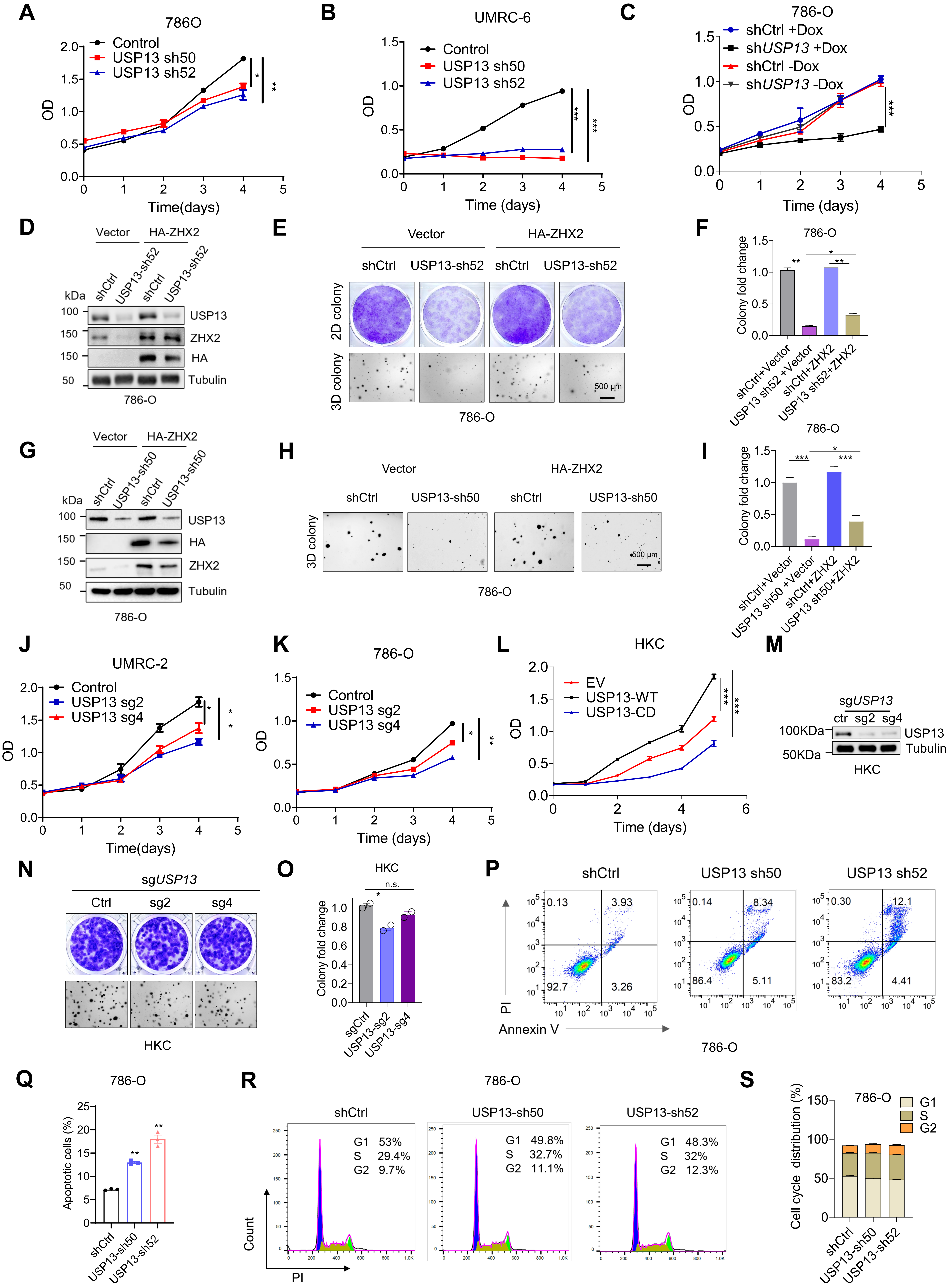

Figure S4. Loss of USP13 suppresses ccRCC cell growth.

A-B. Proliferation of indicated cells infected with lentivirus containing either Ctrl shRNA or shRNA targeting USP13. **C.** Proliferation of 786-O infected with either inducible Ctrl shRNA or inducible shRNA targeting USP13 (sh50) and then treated with doxycycline as indicated. **D-I.** Immunoblots of lysates (D or G), crystal violet staining (E), representative 3D soft agar growth pictures (E or H) and quantification of colony numbers (triplicate wells) (F or I) of 786-O cells infected with lentivirus encoding Ctrl, USP13 sh52(D-F) or sh50(G-I) or pLenti6-vec, pLenti6-HA-ZHX2 as indicated. **J-K.** Proliferation of indicated cells infected with lentivirus containing either Ctrl sgRNA or sgRNA targeting USP13. **L**. Proliferation of HKC infected with lentivirus containing empty vector, USP13-WT or USP13- CD. **M-O.** Immunoblots of lysates (M), crystal violet staining (N), representative 3D soft agar growth pictures (N) and quantification of colony numbers (triplicate wells) (O) of HKC cells infected with lentivirus encoding Ctrl, USP13 sg2 or sg4 as indicated. **P-Q.** Apoptotic rates (P) and quantification of apoptosis cells (Q) of 786-O cells infected with lentivirus encoding Ctrl, USP13 sh50 or sh52 as indicated. **R-S.** The proportion of cell cycle (R) and quantification of each cell cycle (S) of 786-O cells infected with lentivirus encoding Ctrl, USP13 sh50 or sh52 as indicated. Error bars, SEM; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure S5

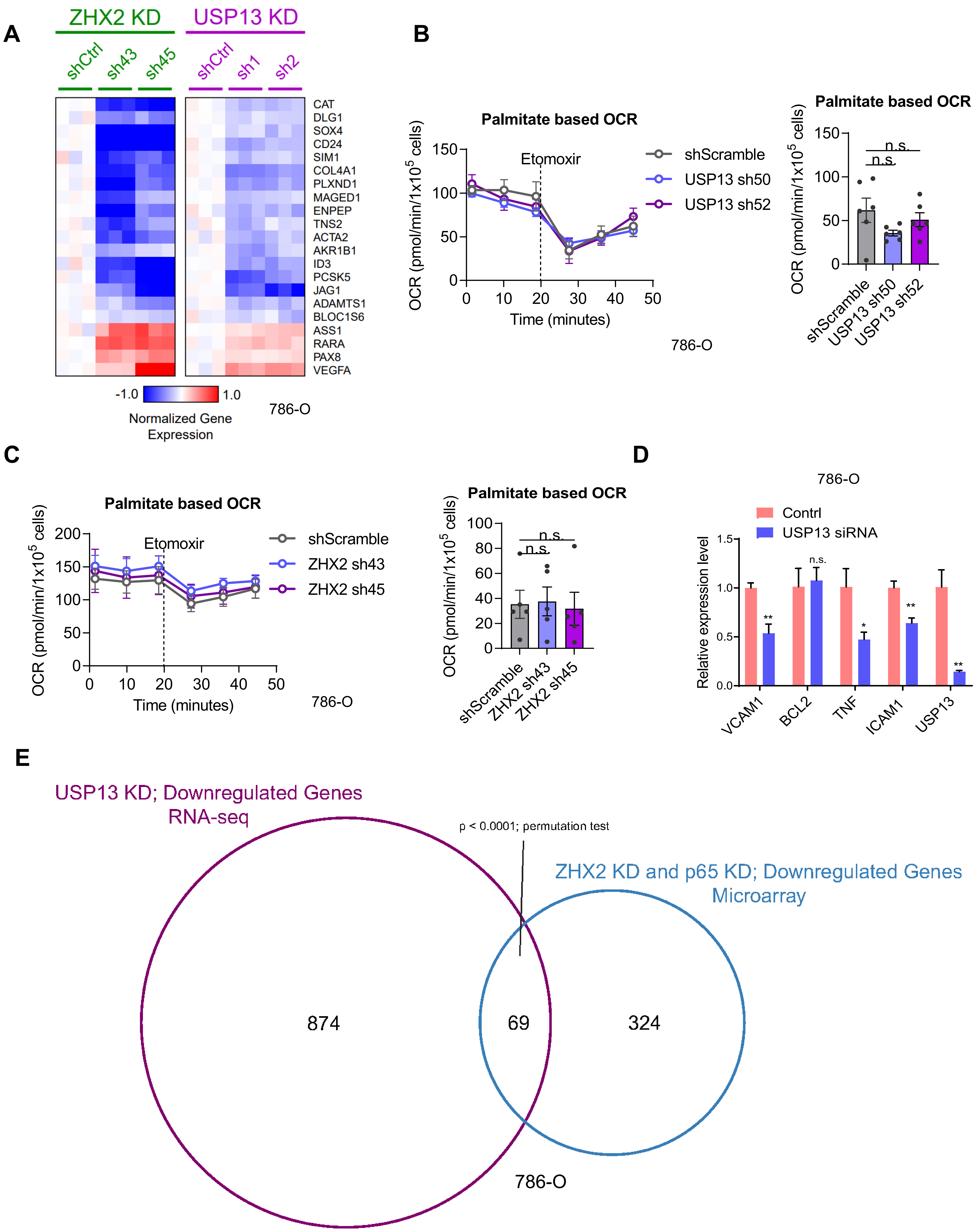

Figure S5. USP13 regulates gene expression involved in ZHX2 signaling in ccRCC.

A. Heatmap with USP13 shRNA and ZHX2 shRNA data for those 21 commonly regulated genes in the kidney, renal and urogenital system development. **B.** Palmitate-based oxygen consumption rate (OCR) indicating the fatty acid utilization in USP13 depleted 786-O cells. Etomoxir was added at indicated time to block the fatty acid oxidation (FAO) in mitochondria. And the quantified FAO rates were calculated between the OCR value before or after Etomoxir treatment. **C.** OCR indicating the fatty acid utilization in ZHX2 depleted 786-O cells. And the quantified FAO rates were calculated between the OCR value before or after Etomoxir treatment. D. qRT-PCR quantification of indicated genes from 786-O cells transfected with Control or USP13 siRNAs. E. Venn-Diagram of overlap between the 390 coordinately downregulated ZHX2 and p65 genes[1] and the 943 downregulated USP13 KD Genes. Error bars, SEM; *, P < 0.05; **, P < 0.01; ***, P < 0.001. “n.s.” represent not significant.

Figure S6

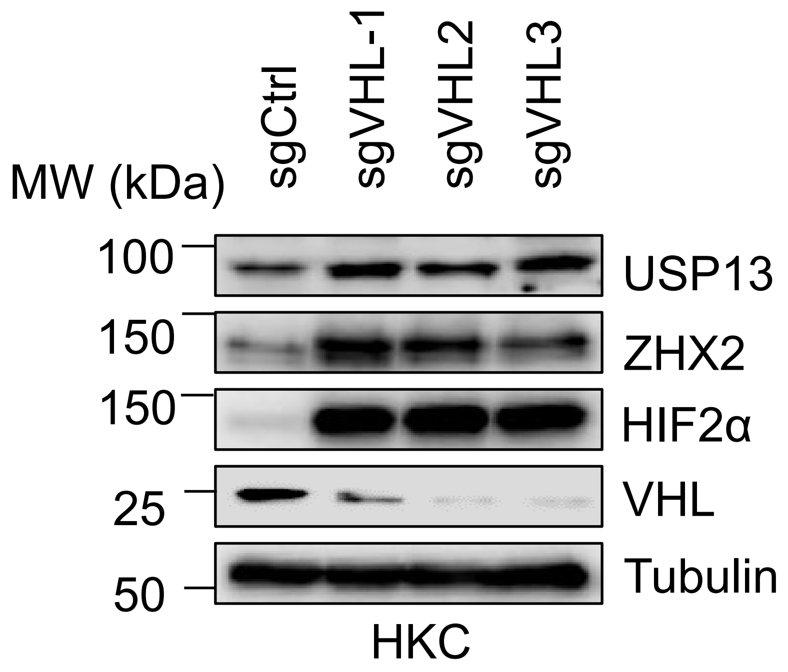

Figure S6.Validation of the potential correlation between USP13 and pVHL status in HKC cells

Immunoblots of the lysates from HKC cells transfected with sgVHL-1, sgVHL-2, sgVHL-3 or sgCtrl as indicated.

Figure S7

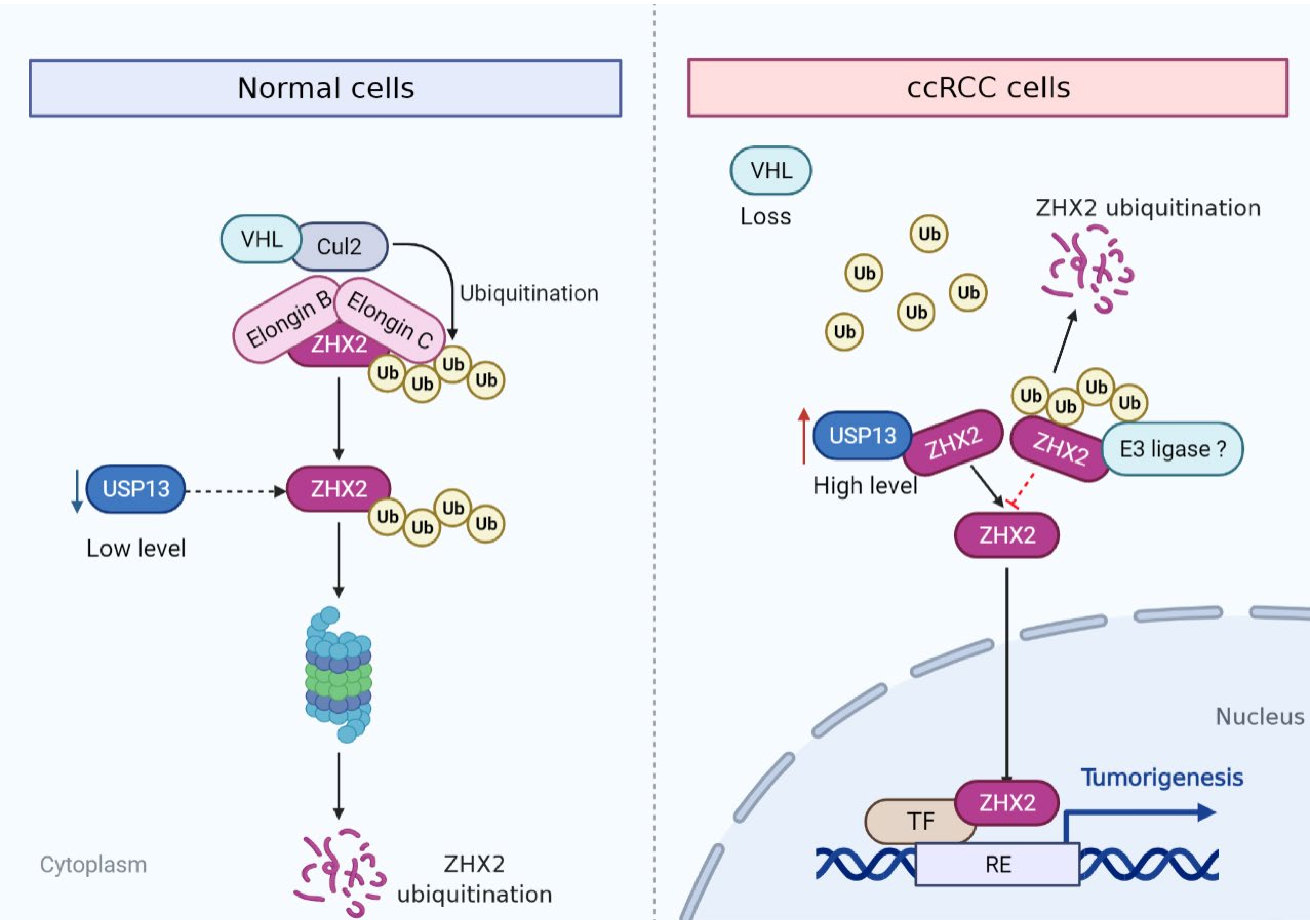

Figure S7. Proposed model depicts the molecular mechanism that USP13 regulates ZHX2 ubiquitination and ccRCC progression.

Ub, ubiquitin. TF, transcriptional factor, RE, response elements.

Table S1. USP13 and ZHX2 commonly regulated gene in 786-O cells.

Common upregulated DEGs	Common downregulate DEGs	Common upregulated DEGs	Common downregulate DEGs	Common upregulated DEGs	Common downregulate DEGs
ANGPTL4	ESPN	MAFK	MAP2K6	CASKIN2	GLUD1
FOSL1	SFRP4	USP40	SKAP2	DMPK	NFYA
PINLYP	UGT2A3	FANCA	KLHL4	TCERG1	LAPTM4B
WDR62	BTG2	WASH3P	SYT11	SNHG3	DLG1
TFPI2	ZHX2	RTEL1-TNFRSF6B	LMCD1	RPL5	CDKN1A
MAFF	VAV3	DAZAP1	ENPEP	NASP	DYNC2LI1
SLC2A3	TMC4	CCT6A	ERV3-1	UBE2G2	ERBIN
MYEOV	LGALS9	DDX55	ACTA2	MORF4L2	MAGED1
MAPK13	ALDH1A1	LOC729218	GBE1	TRAP1	MLEC
PPP1R15A	WDR72	DDX11	TLR3	PTPN23	TMCO3
ITPKA	GMNC	RPL36A	TMEM141	MYO9B	LDLR
RIN1	FLRT3	MRPL41	SLC7A7	COL6A2	SGCB
KLF2	C1orf116	SH3BP5-AS1	TOM1L1	SPG7	KDELC1
TRIB2	AMACR	WASH7P	DAB2	ANXA5	RTN4
NOV	CLEC18A	HAGHL	SLC24A1		FDFT1
LOC100287497	HOGA1	MRPS6	SH3D19		EPB41
NCEH1	C1QL3	IDH3A	WBP1L		STARD4
DOK3	FILIP1L	RPL38	TIMD4		FAM107B
C5orf66	MMP7	CAD	AIG1		SAMD9L
ZNF90	SCD	GDI1	SLC41A2		NAPEPLD
SH2D5	SULT1A1	POU5F1	SLC44A1		ARL6IP5
P2RY11	SOX4	SAFB	COL4A1		PIK3CB
MROH6	SLC12A3	RPL35	HOXA5		MEX3D
SLC16A3	DDC	KATNAL1	UBE2H		NDFIP2
TTN	FGFR3	MPP3	DCAF6		H3F3AP4
LOC728392	ELF3	PSMG4	ALDH4A1		AIM1
CRIPAK	C1QTNF3-AMACR	MSANTD2	TM7SF3		SKP2
HERC2P9	ACE2	SNU13	TNFAIP6		CD164
SMG1P7	CD24	SNX15	JAG1		NUMB
ASS1	MAP7D2	ALG3	ADGRL2		ATPAF1
AMIGO2	PTER	ZNF621	RPRD2		SECISBP2L
RNR2	LRG1	NXPH4	MIA2		FUT8
CHTF18	PRRG4	ADM	CCDC85C		DHCR7
SRM	PLXDC2	SLC25A16	S100A2		TPST1
EMC3-AS1	HS6ST1	RPS28	FARP1		IFT46
TMEM265	FSTL1	HNRNPH3	PNPLA3		WASHC4
ZNF276	ANXA4	AKT1S1	LSS		KIAA1191
CLDN15	HGD	ANAPC7	NEFL		DSG2
FAM50A	LOC102724593	PELP1	LOC339803		SKP1
VEGFA	SIM1	POLE	KCTD20		RIN2
DYNLL2	LYNX1	RABGGTB	TMEM169		SLC1A1
ZNF432	SLC6A12	FAAP20	DGCR5		VIM-AS1
BAIAP2L2	S100A4	CHAF1A	PGAP3		SLFN5
CYSTM1	ARSD	BTG3	RTN2		ADAMTS1
GOLGA6L9	CDH6	NEURL4	CPEB2		KIAA1522
NOP2	COL8A1	SS18	CTNND1		GLB1
TRMT1	B4GALT5	CALCOCO2	PDLIM5		GALNS
RARA	AQP3	LCAT	LFNG		F11R
PSMC3IP	ZFHX4	SLC23A2	ARMC9		SNX5
H2AFX	LIPC	PAX8	HOXA9		LRRFIP1
GPRC5C	TRIM5	POGLUT1	ECHDC1		MARCKS
RPL22L1	TM4SF18	HMGXB3	VGLL4		CMBL
AGAP6	PCSK5	RRM2	TTYH3		PPIC
SPRYD3	USH1C	DGUOK	BMT2		MLLT11
NECAP1	ID3	MRPL4	MAGT1		UBE2G1
KLC2	PIK3R3	GEN1	GSK3B		MSMO1
ZNF141	FADS2	MAPK12	SGCE		SCP2
TIMP1	PATJ	NDUFS5	MMAB		PTTG1IP
PSRC1	CYS1	NTNG1	TNS2		EFNA5
MRTO4	ADD3	ATP5L	LEPR		RAP2A
MIER2	LRRC61	NT5DC2	DDX60		TUSC3
MGC27345	CAT	ZNF202	DSE		ARPC1B
PCOLCE2	SGK2	HAUS6	HOXA10-HOXA9		RNF10
UBE2T	MCU	DPM2	B4GALT1		TNFRSF1A
KRI1	IPP	CKAP5	HSPB8		SNAP23
HSD17B10	MAL	HOXD9	SERPINB1		CCDC71L
REXO1	CDH16	GPI	SLITRK5		DSCR3
NANS	GUCY1B3	SPATC1L	MAP3K7		SPECC1
CCNB1IP1	CTTNBP2NL	TAF1C	PTPRD		NDRG3
WDR75	IGF2BP3	DONSON	TMED5		IFNAR1
ANKRD52	ARMT1	TICRR	C9orf3		TGOLN2

Table S1. USP13 and ZHX2 commonly regulated gene in 786-O cells(Supplement).

Common upregulated DEGs	Common downregulate DEGs	Common upregulated DEGs	Common downregulate DEGs	Common upregulated DEGs	Common downregulate DEGs
GRB14	FAM84B	DNMT3B	SHISA4		TSC22D3
RELL2	PHACTR4	JMJD7-PLA2G4B	TRAM1		MKRN1
RAD54L	TCF12	NDUFA6	ACBD5		CALM3
C11orf1	MPC2	ATF4	CDK15		AKR1B1
IGFN1	VCAN	RPS10	USP6NL		MAN1B1
SLC25A32	MID1	NR2C2AP	EPDR1		TMSB4X
SNHG1	SLC4A4	DDA1	SC5D		ARL8B
TMEM164	CA2	SRPK1	CREB5		MAP4K4
DUS1L	SEMA5B	IMPDH2	PHF6		ATP5B
GADD45GIP1	PLXND1	BRD9	CDKN2C		CAPN1
DSN1	SLC6A6	ATG4D	CTSV		SMARCA2
BCS1L	PTPRG	GIGYF1	CYB561D1		LUZP6
RTEL1	TRIM22	LRRC8E	CNN3		BLOC1S6
SDCCAG3	TRABD2B	ELMO2	USP25		ME1
SLC25A25-AS1	CAMK2N1	CLASRP	TNFAIP2		SRP72
TRMT61A	TTC30A	RPS15	MDFIC		KLF6
RPS26	MAN1A1	ATP5H	TPD52L1		PCBP2
DYNLRB1	AMOT	SLC25A12	C9orf172		ELOVL5
FAU	CDKL5	TONSL	FADS1		
ARFRP1	IDH1	TRMT2A	AHCYL1		

Table S2. The list and data of 69_common_genes the 390 coordinately downregulated ZHX2 and p65 genes and the 943 downregulated USP13 KD Genes.

	Base-Mean	log2FoldChange	lfcSE	stat	pvalue	padj
VCAM1	141.5808116	-1.435598644	0.11375138	-12.62049	1.63E-36	3.62E-33
SLC6A12	159.6749017	-0.659801224	0.11739298	-5.620449	1.90E-08	1.35E-06
NXNL2	98.34322114	-0.575711235	0.1331792	-4.322831	1.54E-05	0.000429
MSC	193.0181291	-0.571091436	0.12218968	-4.673811	2.96E-06	0.000106
HMGCS1	2542.404618	-0.549342921	0.07950173	-6.909824	4.85E-12	8.31E-10
CSF1	962.6492177	-0.536793324	0.07292707	-7.360687	1.83E-13	4.21E-11
IFI44L	462.0744148	-0.53454194	0.11193628	-4.775413	1.79E-06	6.94E-05
MSMO1	1941.183338	-0.522607756	0.06667581	-7.838041	4.58E-15	1.45E-12
CA2	4308.128046	-0.506881031	0.04666167	-10.8629	1.73E-27	2.27E-24
BAMBI	126.1246557	-0.506328803	0.13478008	-3.756703	0.0001722	0.00309
MMAA	185.828576	-0.497909892	0.10726399	-4.641911	3.45E-06	0.000121
MAF	939.0205698	-0.471997758	0.074032	-6.375591	1.82E-10	2.17E-08
ABCC2	674.140003	-0.467573575	0.09165329	-5.101547	3.37E-07	1.61E-05
PLEK	70.04356102	-0.461855419	0.13979207	-3.303874	0.0009536	0.011989
GBP2	194.5512502	-0.45809744	0.10041262	-4.56215	5.06E-06	0.00017
BIRC3	795.9863616	-0.453122061	0.14281735	-3.172738	0.0015101	0.017058
TMEM65	902.6081526	-0.452503894	0.06344556	-7.13216	9.88E-13	1.88E-10
DMRTA1	164.0443315	-0.447080977	0.11696706	-3.822281	0.0001322	0.002497
PLXND1	1423.813096	-0.445253986	0.06029873	-7.384136	1.53E-13	3.66E-11
FGFR3	1257.800948	-0.44010992	0.08044478	-5.470957	4.48E-08	2.89E-06
SQLE	3066.225956	-0.436214629	0.05716122	-7.631304	2.32E-14	6.75E-12
GMNC	110.1473753	-0.432923953	0.14447078	-2.99662	0.0027299	0.026204
PORCN	310.9369308	-0.418948967	0.11673292	-3.588953	0.000332	0.005271
TNFAIP6	95.55415304	-0.411760991	0.12415887	-3.316404	0.0009118	0.011573
TLR3	176.9916631	-0.392737512	0.12786731	-3.071446	0.0021302	0.021829
S1PR1	96.60027144	-0.392594951	0.12921241	-3.038369	0.0023786	0.023754
VAV3	1776.007644	-0.388710329	0.10280828	-3.780924	0.0001562	0.002854
TMEM141	2924.028462	-0.386323606	0.07780074	-4.965552	6.85E-07	3.00E-05
LSS	2642.084285	-0.375549585	0.06264899	-5.994503	2.04E-09	1.85E-07
DDX60	1026.026415	-0.374350517	0.1347831	-2.777429	0.0054791	0.04397
KIAA1217	1321.195369	-0.356344456	0.07201419	-4.948253	7.49E-07	3.25E-05
STARD4	1137.248496	-0.355322586	0.08073456	-4.401121	1.08E-05	0.000316
SLC44A2	1371.045995	-0.349527589	0.071199	-4.909165	9.15E-07	3.88E-05
RASSF4	678.453212	-0.348908287	0.07871996	-4.432272	9.32E-06	0.000279
SFRP4	133.3544672	-0.346388486	0.11876356	-2.916623	0.0035384	0.031729
CDKL5	645.8671249	-0.341133955	0.08799164	-3.876891	0.0001058	0.002074
OAS1	325.0215125	-0.338021197	0.11486319	-2.942816	0.0032524	0.029703
SCD	16318.27623	-0.335308671	0.07441905	-4.505683	6.62E-06	0.000211
SEMA5B	538.4854395	-0.330134333	0.11398846	-2.896208	0.003777	0.033372
MTMR11	119.2072175	-0.326721587	0.11473866	-2.847528	0.004406	0.037116
SAMD9L	582.8166025	-0.325517703	0.10605495	-3.069331	0.0021454	0.021967
PTPRD	1485.503675	-0.323516694	0.07699358	-4.201866	2.65E-05	0.000674
ADD3	1657.566825	-0.319924018	0.08526654	-3.752046	0.0001754	0.003143
MAP7D2	520.1899954	-0.31579407	0.08098805	-3.899268	9.65E-05	0.001923
SGK2	1120.771005	-0.315374364	0.06737029	-4.681209	2.85E-06	0.000103
MAN1A1	803.2465269	-0.309122132	0.08815025	-3.506764	0.0004536	0.00667
TMEM2	1703.145747	-0.302692329	0.05893593	-5.135956	2.81E-07	1.36E-05
CD24	11234.19395	-0.302142559	0.05698882	-5.301787	1.15E-07	6.41E-06
TRABD2B	443.0562852	-0.301152445	0.08497113	-3.544174	0.0003938	0.006044
TRIM5	987.6361021	-0.293133348	0.07671004	-3.821316	0.0001327	0.002502
TSC22D3	444.2108474	-0.290141327	0.09573539	-3.030659	0.0024402	0.024168
NEFL	3666.992765	-0.28473211	0.05430405	-5.243294	1.58E-07	8.39E-06
AKR1B1	14326.46676	-0.281408437	0.06843071	-4.112312	3.92E-05	0.000934
SHISA4	340.6602262	-0.280999517	0.0991879	-2.833002	0.0046113	0.038314
DSE	405.1065809	-0.279225922	0.09735976	-2.867981	0.004131	0.035517
CACHD1	590.2392219	-0.275190525	0.08835347	-3.114655	0.0018416	0.01975
HGD	951.7478393	-0.248557811	0.06052933	-4.106403	4.02E-05	0.000948
TM4SF18	2154.665972	-0.24730118	0.08278972	-2.9871	0.0028164	0.026785
SLC25A1	1792.884646	-0.246666697	0.06694975	-3.684356	0.0002293	0.0039
TRIM22	886.1560607	-0.242551044	0.08771578	-2.765193	0.0056889	0.045079
SLC4A4	2168.614851	-0.237525309	0.06588348	-3.605233	0.0003119	0.005017
ARSD	1165.289545	-0.231247578	0.07811855	-2.960214	0.0030743	0.028387
SLC6A6	8104.397606	-0.219697182	0.07997632	-2.747028	0.0060138	0.046684
MVP	9671.299488	-0.219573051	0.05670513	-3.87219	0.0001079	0.002102
H6PD	1790.096805	-0.214323552	0.06219875	-3.445785	0.0005694	0.008079
TTYH3	9793.013445	-0.207236816	0.06677896	-3.103325	0.0019136	0.02023
OPTN	2104.034445	-0.185457403	0.06404301	-2.895826	0.0037816	0.033372
ZHX2	1950.075916	-0.175832293	0.04935152	-3.562855	0.0003668	0.005682
MID1	2047.590877	-0.174407397	0.05559515	-3.137097	0.0017063	0.018629

Reference:

1.Zhang J, *et al.* (2018) VHL substrate transcription factor ZHX2 as an oncogenic driver in clear cell renal cell carcinoma. *Science* 361(6399):290-295.