

Figure S1. Representative western blot showing molecular standards used for normalization of individual proteins among all the gels run for all the tumors or kidneys. A sample running in the linear range of standards (rectangle) was used for normalization. In addition, all samples were normalized to the levels of GAPDH.

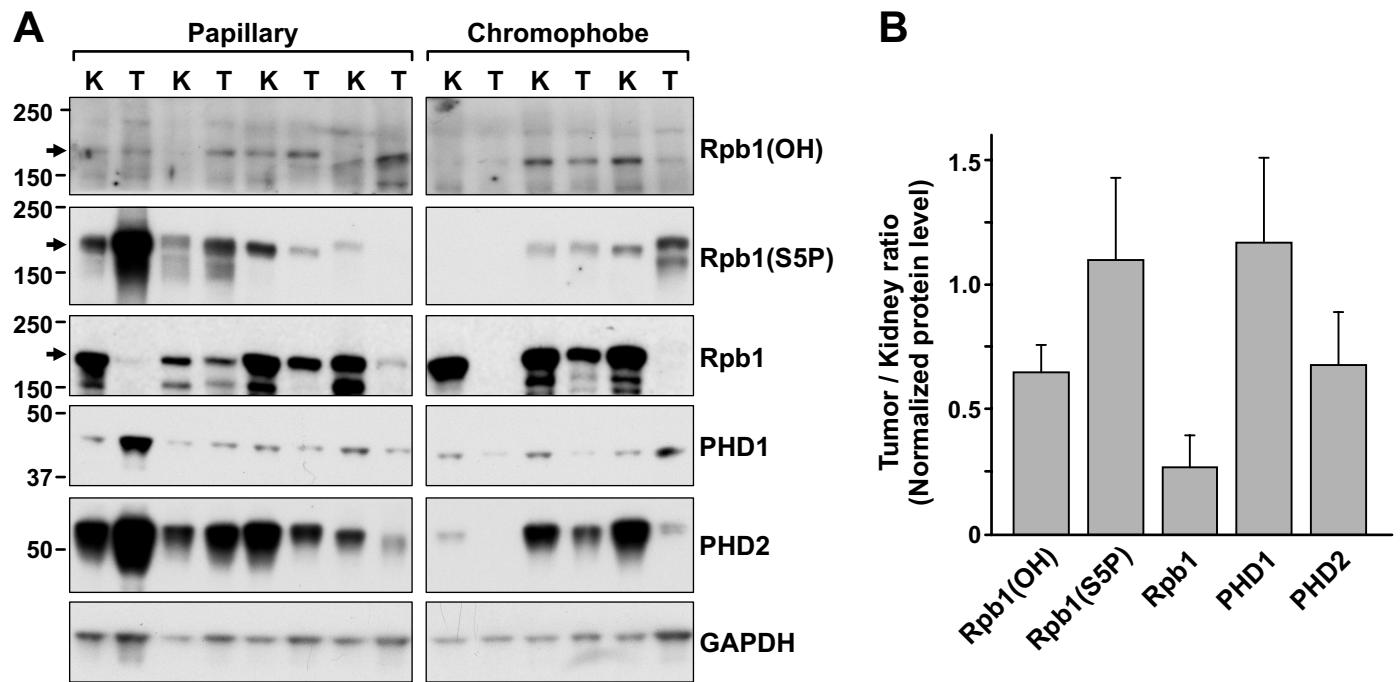


Figure S2. Western blots of extracts from four papillary and three chromophobe renal tumors and matched normal kidneys probed with the indicated antibodies (**A**). (**B**) Quantification of the tumor/kidney ratio was performed by first normalizing the optical density of relevant bands to the optical density of GAPDH bands and then calculating the T/K ratio. The analysis to date, although on a very small sample, does not indicate increases in Rpb(OH), PHD1 or PHD2 in these tumors as compared to matched kidneys.

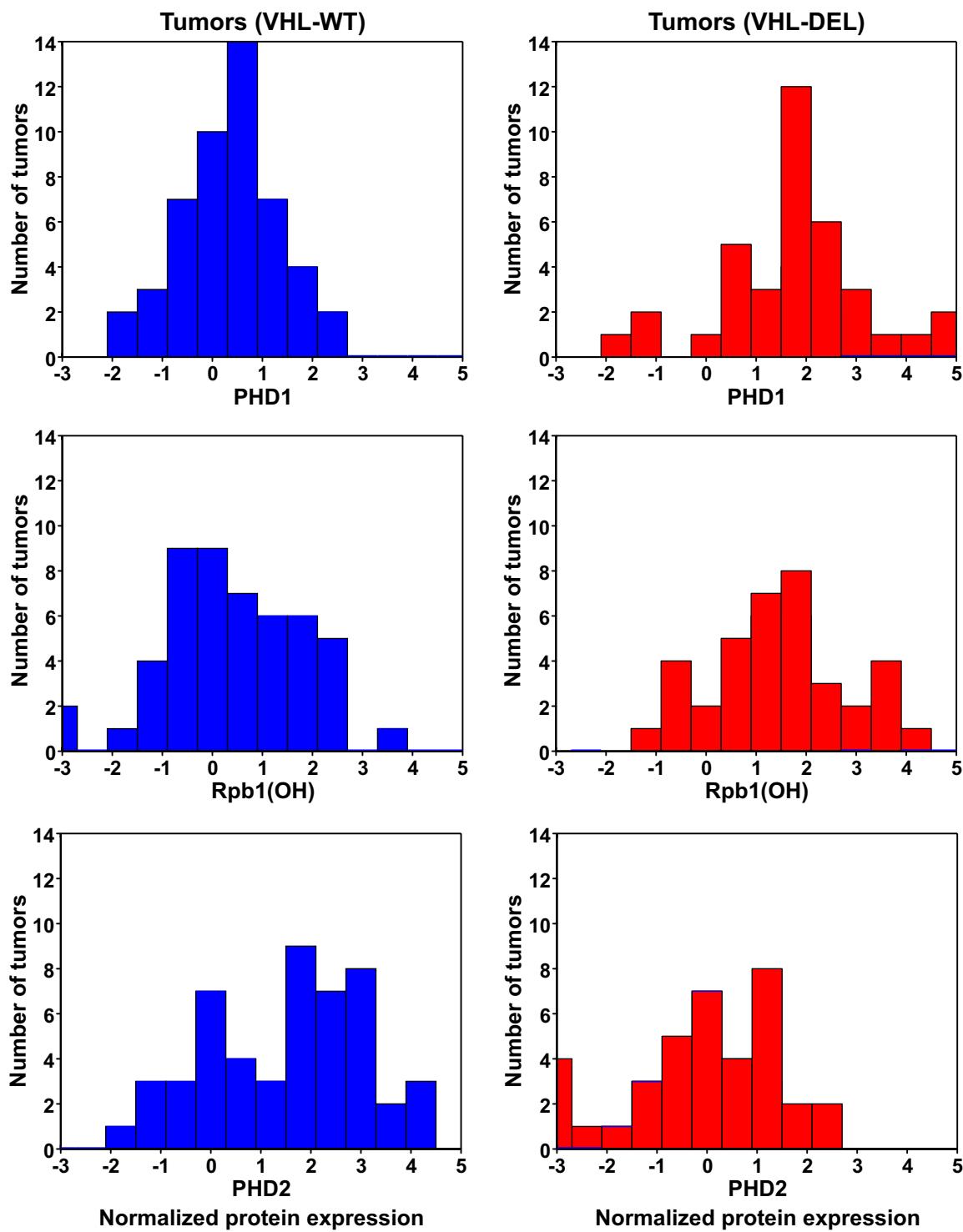


Figure S3. Histograms of the distributions of PHD1, Rpb1(OH), and PHD2 levels in VHL-WT (red bars) and -DEL (blue bars) tumors. The normalized expression levels for each protein are shown on the x-axis, and the number of tumors at a given expression level are shown on the y-axis. For example, there are 10 VHL-DEL and 1 VHL-WT tumors with PHD1 expression levels in the interval around 0 (bar #4 in the top panels). The bars at either end of the histogram account for all cases with values below -3 or above 5. The separation between distributions for VHL-WT and -DEL cases is most pronounced for PHD1, which is also reflected in multivariate decision tree-based analysis Fig. 3A).

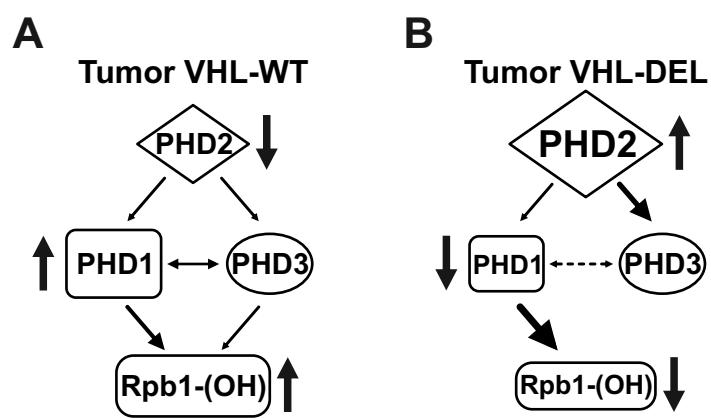


Figure S4. Proposed model for the regulation of Rpb1(OH) in RCC VHL-WT (A) and VHL-DEL (B) tumors. In VHL-WT tumors, Rpb1 is hydroxylated by PHD1 and PHD3, and relatively lower levels of PHD2 do not inhibit this hydroxylation, or inhibit it to a lesser degree. In VHL-DEL tumors, higher levels of PHD2 titrate PHD3 away from Rpb1, leaving PHD1 as the only hydroxylase and resulting in an overall lower, but primarily PHD1-mediated, Rpb1 hydroxylation.

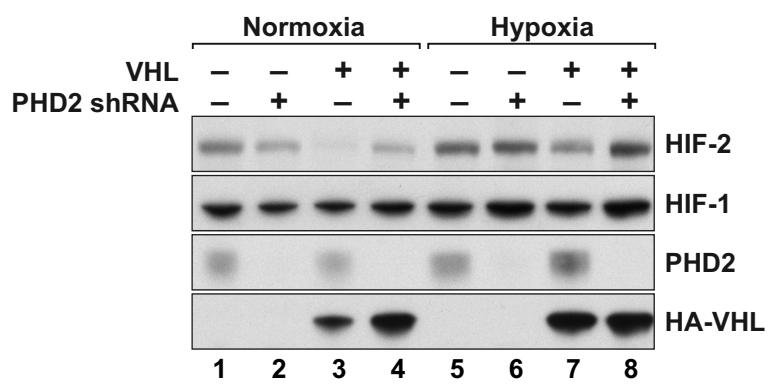


Figure S5. Knockdown of PHD2 in 786-O VHL cells induces accumulation of HIF-2 to levels similar to those measured in VHL(-) cells under both normoxic and hypoxic conditions. Western blot analysis of the indicated proteins. The control samples were treated with scramble shRNA.

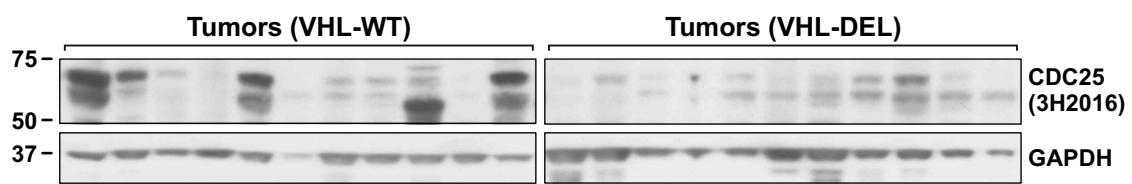


Figure S6. Western blot of CDC25A in a representative set of VHL-WT and VHL-DEL RCC tumors. Santa Cruz monoclonal antibody 3H2016 was used for immunoblotting. In human RCC tumors, the antibody detects two bands between 50 and 75 kD markers. These bands most likely correspond to different phosphorylation forms or splice variants of CDC25A.

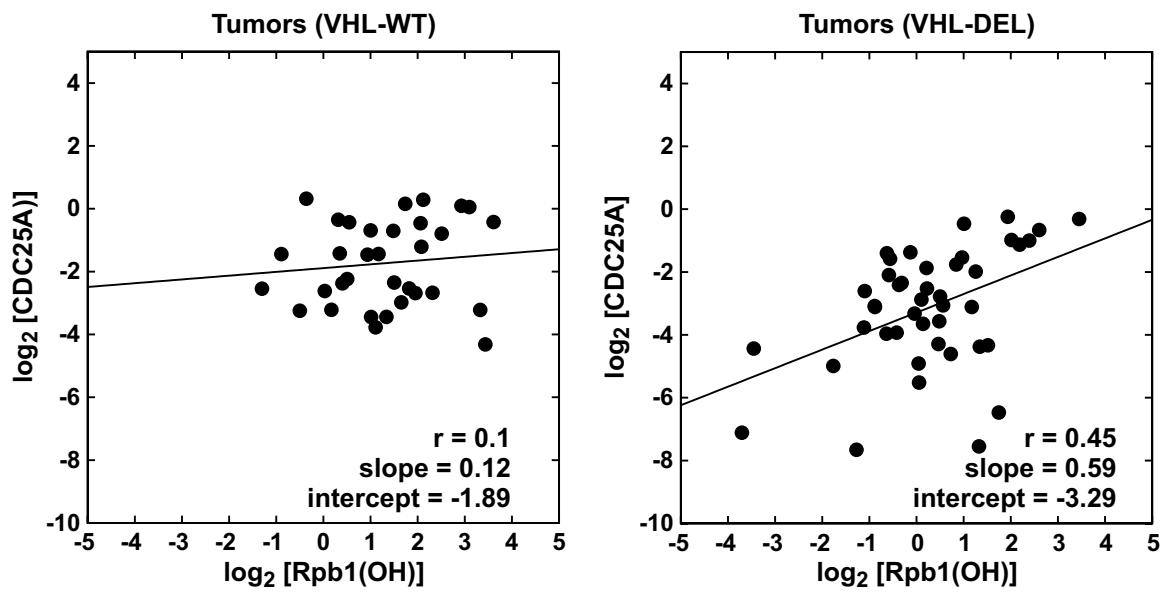


Figure S7. Regression analysis of CDC25A levels as function of Rpb1(OH) levels in tumor samples stratified by VHL gene status.
 Each dot represents a sample and the solid line represents the linear regression fit, with the Pearson correlation coefficients (r) as well as the slope and intercept of the fitted line shown in the lower right corner of each box. ** $p < 0.01$

Table S1. Sequence of forward and reverse primers used for RT-PCR of the indicated human mRNAs.

hVEGF-A	F	5' TTTCTGCTGTCTGGGTGCATTGG 3'	hVEGFb	5' CTCGAAGGTGACACATGGCTTT
hVEGF-A	F	5' TTTCTGCTGTCTGGGTGCATTGG 3'	hVEGFb	3' GCAAGTCACCCTGCTGAGTCT
GLUT-1	F	5' CCGGCGGAATTCAATGCTGATGAT 3'	hVEGFc	5' CCAGAAATCAACCCCTAAATCCTGGAAAA
hGLUT-1	F	5' CCGGCGGAATTCAATGCTGATGAT 3'	hVEGFc	3' CCATAATAGAAAATCGATGAAGTGGAAAA
GLUT-1	B	5' TCAGCATCTCAAAGGACTTGCCC 3'		

Table S2. Summary of general information on all human RCC tumors used for analysis

total # of kidneys	114
total # of tumors	128
# of tumors with known grade	114
G1	10
G2	64
G3	32
G4	8
unknown	14
tumor stage:	
0	1
1	6
1a	25
1b	20
2	11
3	3
3a	11
3b	11
3c	1
unknown	39
VHL gene status:	
wild type	46
missense point mutations	17
hypermethylation	13
frameshift, nonsense mutations	52

Table S3. Status of the *VHL* gene sequence and promoter hypermethylation in all investigated RCC tumors. (WT - wild-type; PM/MS - missense point mutations; HypMe - hypermethylated; DEL - frameshift (FS), nonsense (Non), in-frame-deletion (IFD) mutations; nd - not determined)

Tumor ID	VHL Gene	Grade and pTNM	HypMe	Mutation				
				Genomic change	mRNA change	Exon	Codon and predicted amino acid change	Mutation type
1	WT	G2 T1a	No	—	—	—	—	—
2	WT	G2 T1a	No	—	—	—	—	—
3	WT	G2 T1a	No	—	—	—	—	—
4	WT	G2 T1a	No	—	—	—	—	—
5	WT	G2 T1a	No	—	—	—	—	—
6	WT	G2 T1a	No	—	—	—	—	—
7	WT	G2 T1a	No	—	—	—	—	—
8	WT	G2 T1a	No	—	—	—	—	—
9	WT	G2 T1a	No	—	—	—	—	—
10	WT	G2 T1a	No	—	—	—	—	—
11	WT	G2 T1b	No	—	—	—	—	—
12	WT	G2 T1b	No	—	—	—	—	—
13	WT	G2 T1b	No	—	—	—	—	—
14	WT	G2 T1b	No	—	—	—	—	—
15	WT	G2 T1b	No	—	—	—	—	—
16	WT	G2 T1b	No	—	—	—	—	—
17	WT	G2 T1b	No	—	—	—	—	—
18	WT	G2 T2	No	—	—	—	—	—
19	WT	G2 T3a	No	—	—	—	—	—
20	WT	G2 T3b	No	—	—	—	—	—
21	WT	G2 T3b	No	—	—	—	—	—
22	WT	G2 T3b	No	—	—	—	—	—
23	WT	G2 T3b	No	—	—	—	—	—
24	WT	G2 T3b	No	—	—	—	—	—
25	WT	G2	No	—	—	—	—	—
26	WT	G2	No	—	—	—	—	—
27	WT	G2	No	—	—	—	—	—
28	WT	G2	No	—	—	—	—	—
29	WT	G3 T1a	No	—	—	—	—	—
30	WT	G3 T1b	No	—	—	—	—	—
31	WT	G3 T1b	No	—	—	—	—	—
32	WT	G3 T2	No	—	—	—	—	—
33	WT	G3 T3a	No	—	—	—	—	—
34	WT	G3 T3b	No	—	—	—	—	—
35	WT	G3	No	—	—	—	—	—
36	WT	G3	No	—	—	—	—	—
37	WT	G3	No	—	—	—	—	—
38	WT	G3	No	—	—	—	—	—
39	WT	G4 T3a	No	—	—	—	—	—
40	WT	G4 T3a	No	—	—	—	—	—
41	WT	G4 T3b	No	—	—	—	—	—
42	WT	G4	No	—	—	—	—	—
43	WT	nd	No	—	—	—	—	—
44	WT	nd	No	—	—	—	—	—
45	WT	nd	No	—	—	—	—	—
46	WT	nd	No	—	—	—	—	—
47	PM	G1 T1b	No	445 A>C	445 A>C	1	N78H	MS
48	PM	G1 T1b	No	434 T>A	434 T>A	1	V74D	MS
49	PM	G1 T3	No	470 C>T	470 C>T	1	P86L	MS
50	PM	G2 T1a	No	446 A>T	446 A>T	1	N78I	MS
51	PM	G2 T1a	No	458 G>C	458 G>C	1	R82P	MS
52	PM	G2 T1a	No	4928 T>A	602 T>A	2	V130D	MS
53	PM	G2 T1a	No	4922 T>A	596 T>A	2	L128H	MS
54	PM	G2 T1b	No	8188 C>G	712 C>G	3	R167G	MS
55	PM	G2	No	4900 G>T	574 G>T	2	D121Y	MS
56	PM	G3 T1a	No	407 C>T	407 C>T	1	S65L	MS
57	PM	G3 T1b	No	8180 A>T	704 A>T	3	Q164L	MS
58	PM	G3 T3b	No	4991T>A	665 T>A	2	I151N	MS
59	PM	G3 T3c	No	4889 G>C	563 G>C	2	W117S	MS
60	PM	G3	No	4997 T>G	671 T>G	2	L153R	MS
61	PM	G3	No	4889 G>C	563 G>C	2	W117S	MS
62	PM	G3	No	4882 C>A	556 C>A	2	H115N	MS
63	PM	nd	No	445 A>G	445 A>G	1	N78D	MS
64	HypMe	G1 T1b	Yes	-	-	-	-	-
65	HypMe	G2 T1	Yes	-	-	-	-	-
66	HypMe	G2 T2	Yes	-	-	-	-	-
67	HypMe	G2	Yes	-	-	-	-	-
68	HypMe	G3 T2	Yes	-	-	-	-	-
69	HypMe	G3 T2	Yes	-	-	-	-	-
70	HypMe	G3 T3a	Yes	-	-	-	-	-
71	HypMe	G3	Yes	-	-	-	-	-
72	HypMe	nd	Yes	-	-	-	-	-
73	HypMe	nd	Yes	-	-	-	-	-
74	HypMe	nd	Yes	-	-	-	-	-
75	HypMe	nd	Yes	-	-	-	-	-
76	HypMe	nd	Yes	-	-	-	-	-
77	DEL	G1 T1	No	440 del T	440 del T	1	F76fsX158	FS
78	DEL	G1 T1	No	8161-8162 insT	685-686 insT	3	L158fsX173	FS
79	DEL	G1 T1a	No	512 del C	512 del C	1	T100fsX158	FS
80	DEL	G1 T1a	No	5004 T>C	IVS2+2 T>C	IVS2+2	Splicing effect	Spl
81	DEL	G1 T1b	No	421 G>T	421 G>T	1	E70X	Non
82	DEL	G1	No	374-375 insG	374-375 insG	1	m54fsX131	FS
83	DEL	G2 T1	No	4937 ins2	611 ins2	2	T133fsX159	FS
84	DEL	G2 T1	No	8155 ins2	678 ins2	3	Y156fsX159	FS
85	DEL	G2 T1a	No	499-509 del11 ins3	499-509 del11 ins3	1	Q96fsX128	FS
86	DEL	G2 T1a	No	287 C>T, 8219-8221 del2	287 C>T, 743-744 del2	1, 3	P25L, R177fsX94	MS, FS
87	DEL	G2 T1a	No	8168 del A ins6	692 del A ins6	3	E160fsX171	FS
88	DEL	G2 T1a	No	8214 C>A	738 C>A	3	Y175X	Non
89	DEL	G2 T1a	No	8191-8195 del5	715-719 del5	3	S168fsX171	FS
90	DEL	G2 T1b	No	4943 delT	617 delT	2	L135fsX158	FS
91	DEL	G2 T1b	No	5005 A>C	IVS2+3 A>C	IVS2+3	Splicing effect	Spl
92	DEL	G2 T1b	No	8279-8285 del7	803-809 del7	3	D197fsX199	FS
93	DEL	G2 T2	No	520- Deleted	520- Deleted	1	P103truncated	truncated
94	DEL	G2 T2	No	553-556 del4	553+3 del4	1	Splicing effect	Spl/FS
95	DEL	G2 T2	No	482-488 del 7	482-488 del 7	1	N90fsX156	FS
96	DEL	G2 T2	No	4939-4942 del4	613-616 del4	2	Y134fsX157	FS
97	DEL	G2 T3	No	4885-4890 del 6	559 del 6	2	L116IFD	IFD
98	DEL	G2 T3a	No	447 del T	447 del T	1	N78fxX158	FS
99	DEL	G2 T3a	No	500-520 del21	500-521 del21	1	Q96IFD	IFD
100	DEL	G2 T3b	No	4983 del T	657 del T	2	F148fsX157	FS
101	DEL	G2 T3b	No	8160 del T	684 del T	3	T157fxX158	FS
102	DEL	G2	No	544 del A	544 del A	1	S111fsX158	FS
103	DEL	G2	No	4989-4991 del3 ins2	663-665 del3 ins2	2	N150fsX158	FS
104	DEL	G2	No	4884 ins C	558 ins C	2	H115fs131	FS
105	DEL	G2	No	8187 del C	711 del C	3	V166fsX169	FS
106	DEL	G2	No	8179 C>T	703 C>T	3	Q164X	Non
107	DEL	G2	No	8275 A>T	799 A>T	3	K196X	Non
108	DEL	G2	No	8176-8194 del 19 insG	700-718 del 19 insG	3	L163IFD	IFD
109	DEL	G3 T1	Yes	446 ins C	446 ins C	1	N78fsX131	FS
110	DEL	G3 T1a	No	523 del G ins 3	523 del G ins 3	1	G104fsX159	FS
111	DEL	G3 T1a	No	384-409 del26	384-409 del26	1	G57fsX122	FS
112	DEL	G3 T1b	No	416-422 del7 insT	416-422 del7 insT	1	S68IFD	IFD
113	DEL	G3 T1b	No	485 delT	485 delT	1	F91fsX158	FS
114	DEL	G3 T2	No	544-557 del14, 8227 A>G	544-553+4 del14, 751 A>G	1, 3	S111fsX115, I180V	FS/Spl, MS
115	DEL	G3 T2	No	4965 delT	638 delT	2	V142fsX158	FS
116	DEL	G3 T3a	No	424-427 del4	424-427 del4	1	P71fsX157	FS
117	DEL	G3 T3a	No	4889 G>A	563 G>A	2	W117X	Non
118	DEL	G3 T3a	No	8146-8160 del15	S2-(7-1) del7, 677-684 de	IVS2, 3	Splicing effect	Spl/FS
119	DEL	G3 T3b	No	384-409 del26	384-409 del26	1	G57fsX122	FS
120	DEL	G3	No	375 ins2	375 ins2	1	M54fsX67	FS
121	DEL	G4 T0	No	8163-8165 del3 insC	687-689 del 3 insC	3	I158fsX165	FS
122	DEL	G4 T2	No	4944-4946 del3	618-620 del3	2	L135IFD	IFD
123	DEL	G4 T3	No	555 T>A	553+2 T>A	IVS1+1	Splicing effect	Spl
124	DEL	G4 T3a	No	8229 del C	753 del C	3	I180fsX201	FS
125	DEL	G4	No	482 del A	482 del A	1	N90fsX158	FS
126	DEL	nd	No	512-514 del3 insA	512-514 del3 insA	1	T100fsX130	FS
127	DEL	nd	No	4966 del G	640 del G	2	D143fsX158	FS
128	DEL	nd	No	8202 del G	726 del G	3	K171fsX201	FS

Table S4. Normalized values of indicated protein levels in all samples of kidneys (left) and RCC tumors (right) where meaningful values were obtained for all proteins of interest. The same ID number is used to indicate samples where kidneys and tumors are from the same patient, and correspond to the ID numbers in Table S3. Each value is an average of at least two independent runs and quantification procedures. While tumor stage is known for some tumors, it is not shown in the table because we did not detect any meaningful correlation between the levels of the investigated proteins and tumor stage. Note that for some statistical analyses in Figures 1, 2, and 3 a log₂ transformation of these normalized values was used (data not shown).

Kidney ID	Rpb1(OH)	Rpb1(S5P)	Rpb1	PHD1	PHD2	PHD3	VHL	Tumor ID	VHL Gene	Tumor Grade	Rpb1(OH)	Rpb1(S5P)	Rpb1	PHD1	PHD2	PHD3
1	0.779134	0.330303874	0.675807	0.351504	0.602262	0.734235	0.149277	1	WT	G2	7.610721	2.609797488	2.166894	7.549522	3.209464	2.30729
2	0.852668	1.403032937	0.600851	1.176604	0.540309	0.99428	1.032996	2	WT	G2	4.221295	2.928816489	0.934721	2.853641	2.044011	1.090349
4	1.399742	1.542422989	0.918707	0.459954	1.172805	0.95796	0.643101	4	WT	G2	2.83374	0.912482714	2.851552	5.752435	0.066321	2.53494
5	2.086502	2.9323137	0.178467	0.117408	0.185177	0.319598	0.404281	5	WT	G2	10.04669	0.433463901	1.961252	4.26345	1.94647	4.800583
7	1.73543	0.660043959	1.701935	0.703419	1.002106	1.813295	1.034266	6	WT	G2	4.17295	1.671309893	1.394931	5.853612	2.256066	1.822048
8	1.529465	2.392883249	1.142019	0.718516	0.978171	1.620433	1.233934	7	WT	G2	12.2131	0.858607543	1.716053	6.195441	0.170077	1.621158
10	1.238125	2.140309109	1.283523	1.362834	1.650705	1.138483	0.167285	8	WT	G2	3.144421	0.840287077	1.32382	2.855549	1.234943	1.331657
11	0.439318	0.617201666	1.01269	1.192838	1.261866	1.210608	0.462562	10	WT	G2	3.532139	0.412943931	1.261965	3.622387	1.141059	1.519409
12	1.525342	0.368016167	0.846772	0.829452	0.679163	0.889435	1.471188	11	WT	G2	1.460306	1.626966231	1.256497	3.978753	0.757928	2.723771
13	0.711689	1.265966774	0.29769	0.974965	0.047785	0.719737	0.564606	12	WT	G2	3.87533	2.061466465	1.826423	2.693131	2.347028	0.306002
14	0.634307	0.254380638	0.64452	0.237525	0.070996	0.70618	0.353888	13	WT	G2	4.342426	3.087447961	2.732057	14.88925	5.96958	2.418347
15	1.032809	0.521782931	0.746261	0.424122	1.932919	0.877271	2.518942	14	WT	G2	8.545708	1.41363241	1.23494	9.104568	3.781291	0.673659
16	0.848916	0.684038133	0.279306	0.656426	0.471584	0.857188	1.514478	15	WT	G2	2.527555	0.615177313	0.212113	1.868718	1.725931	0.245523
17	0.977678	0.377061674	0.754522	1.41923	1.767182	1.828016	0.619576	16	WT	G2	0.706526	0.974716435	0.639101	3.679075	0.083415	1.788212
18	1.830559	0.16488071	0.722557	0.91794	1.15639	3.403458	0.983551	17	WT	G2	3.126437	0.999783556	1.713818	3.771628	2.157587	0.362602
19	1.014508	0.050713271	0.329183	0.174989	0.116834	1.399744	0.30872	18	WT	G2	5.698271	1.910628554	0.742363	1.955665	1.797571	0.27312
20	1.104317	0.6800587	0.426616	0.625111	0.884925	0.902793	0.639178	19	WT	G2	2.249171	1.807538702	0.730466	4.593919	0.96836	0.657196
21	1.224991	0.243276298	0.702925	0.974546	0.337203	0.870618	0.153836	20	WT	G2	2.01575	0.619447536	0.177615	6.155768	1.334911	0.287366
23	1.234885	0.767624921	0.891906	1.071071	1.076205	1.204561	2.479155	21	WT	G2	15.90326	2.356500303	1.559703	26.54102	4.652444	6.758368
24	0.484898	3.025389807	0.883321	0.947498	1.385623	1.2104	0.207341	23	WT	G2	0.639568	0.63622467	0.527932	1.801526	0.820324	0.075701
25	0.855548	1.209648434	0.824362	0.753569	0.332653	1.299855	2.649292	24	WT	G2	10.82134	2.066354159	0.301164	3.480229	0.928283	1.146996
26	1.021742	0.822667153	0.786866	1.343752	1.40269	1.156253	0.131166	26	WT	G2	0.405188	0.751005193	0.300873	3.601277	1.794225	0.122568
27	0.363271	0.649626853	0.435652	0.386448	0.053826	1.209378	3.158267	28	WT	G2	3.331607	0.779156723	1.386426	6.177442	2.459399	1.208647
29	2.048585	0.422187099	0.638977	0.710106	0.935307	1.522553	2.451929	29	WT	G3	4.977022	4.99826329	1.144375	2.80426	0.854764	2.453674
31	2.234053	1.023556791	0.129195	0.490583	3.879793	1.194947	0.113855	31	WT	G3	2.154387	0.253352712	0.644823	3.273449	0.0895	1.12008
32	0.505768	0.474709321	0.995073	0.95836	1.560713	1.153602	0.884373	32	WT	G3	2.0051	1.129993914	1.858506	8.307124	2.769522	1.475267
33	1.304056	2.718241413	0.966777	1.090319	1.497746	0.892243	0.493036	33	WT	G3	1.418504	0.824179367	0.397504	0.51321	0.728931	0.491974
34	0.171719	0.997167073	0.198455	0.096584	0.074189	1.80349	0.116223	34	WT	G3	0.53923	0.653988007	1.732395	0.289925	0.392766	1.219036
35	0.495588	0.574924406	0.50145	0.898915	0.125229	3.188219	1.73294	35	WT	G3	1.91411	1.169328133	1.453236	1.460506	1.040441	0.087264
41	0.482683	0.096620516	0.506842	1.149068	0.043023	0.996746	0.743588	36	WT	G3	1.124474	0.864471544	0.433973	0.490456	0.34083	0.495563
47	1.511765	0.257129455	1.131149	0.837439	0.548138	0.772244	0.221933	37	WT	G3	1.273205	0.630082856	1.609168	1.344365	0.761081	1.168957
48	1.360669	0.293700695	0.571919	0.390878	0.133033	1.279247	1.171562	38	WT	G3	1.322905	0.134880849	1.733695	1.678658	0.072821	2.093644
49	2.862962	0.247613326	0.760236	1.052349	0.860655	1.277219	1.145268	41	WT	G4	1.020683	0.158251636	2.040644	1.04664	0.423803	0.482065
50	1.601027	0.489600067	0.329988	1.625675	0.706196	8.591856	1.773688	42	WT	G4	1.245149	10.56668652	1.893975	1.334592	0.449199	2.113997
51	2.040814	0.81862637	1.218039	2.295907	1.119129	9.206772	1.278299	43	WT	n/a	2.79529	0.443972722	1.371594	4.091988	0.802756	0.84924
52	2.810401	1.022906864	0.715666	1.569299	0.892665	9.001847	1.63274	44	WT	n/a	14.62169	1.097825758	2.328005	17.94265	2.603325	1.991075
53	3.710821	0.461967208	0.843589	1.797918	0.690993	1.620706	0.066228	46	WT	n/a	0.777681	0.809551446	2.919566	3.66551	0.643682	1.074374
55	2.559682	2.430764061	0.988361	2.353551	1.135427	5.345315	1.523431	47	PM	G1	0.413175	0.709353049	0.585986	0.129344	1.142549	0.700506
59	1.765817	0.481772374	0.631826	1.595579	0.196468	1.492636	0.327719	48	PM	G1	4.064763	0.79218263	1.274302	0.135973	0.974876	