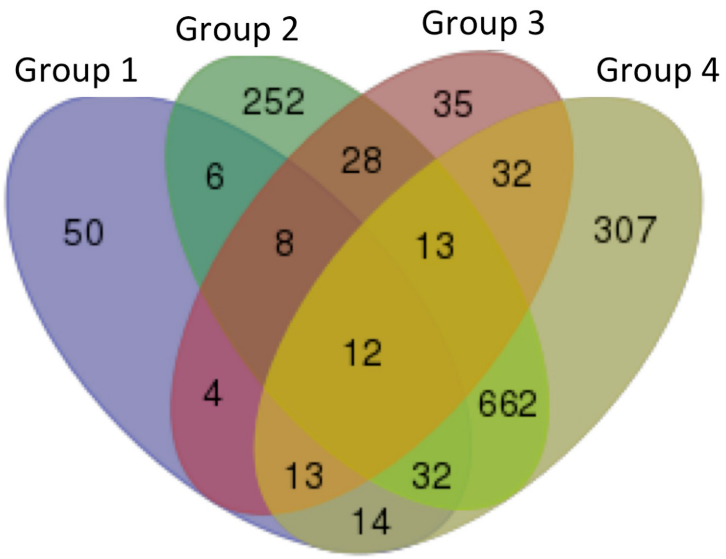
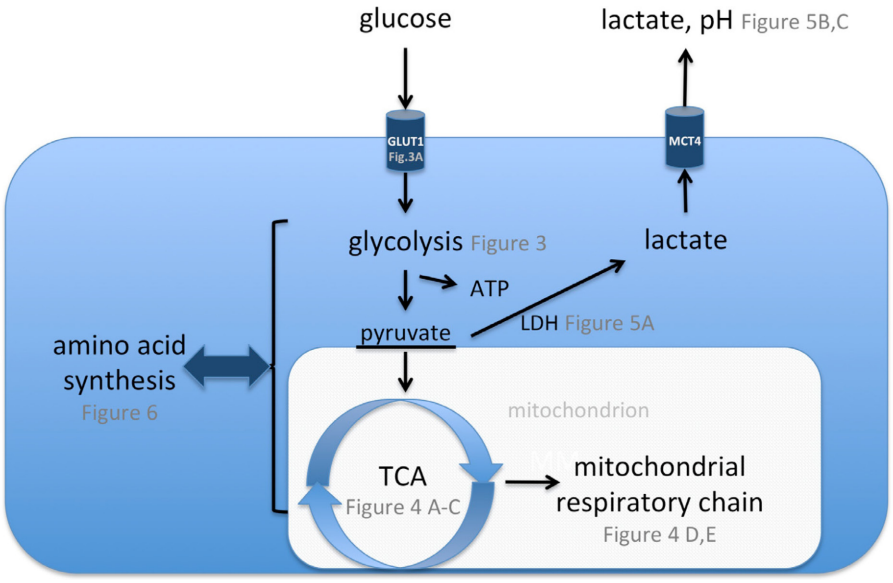
SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1: Number of overlapping genes of the comparison of 786-O VHL$^-$ and VHL$^+$ cells during normoxia and hypoxia. The venn diagram shows the overlapping differentially expressed genes identified via cDNA microarray as described in materials and methods. Group 1: 786-O hypoxia vs. 786-O normoxia; group 2: VHL normoxia vs. 786-O normoxia; group 3: VHL hypoxia vs. VHL normoxia; group 4: VHL hypoxia vs. 786-O normoxia;



Supplementary Figure S2: Altered metabolism in VHL-deficient RCC cells. The scheme presents a summary of the metabolic changes observed in VHL-deficient RCC cells. Changes in glucose uptake, expression of glycolysis and TCA enzymes, intracellular ATP production, amino acid content, activity of mitochondrial dehydrogenase and lactate dehydrogenase, lactate secretion, and extracellular pH were measured in three independent RCC VHL cell models.

Supplementary Table S1: Primer sequences for qPCR

Primer name	Primer sequence	Reference sequence
VEGFAfwd	AACCATGAACTTTCTGCTGTCTTGG	NM_001171630
VEGFArev	ATCAGGGTACTCCTGGAAGATGTCC	
GLUT1fwd	CTTCACTGTCGTGTCGCTGTTTGT	NM_006516
GLUT1rev	AAATTTGAGGTCCAGTTGGAGAAGC	
ACTBfwd	GGACTTCGAGCAAGAGATGG	NM_001101
ACTBrev	AGCACTGTGTTGGCGTACAG	
VHLfwd	CATCCGTTGATGTGCAATG	NM_198156
VHLrev	GAAAGAGCGATGCCTCCA	
SDHAfwd	GATTACTCCAAGCCCATCCA	NM_004168
SDHArev	CACAGTCAGCCTCGTTCAAA	
ECHS1fwd	CAGTCATCGCTGCTGTCAAT	NM_004092
ECHS1rev	AGTGAGGACCATCTCCATCG	
ACO1fwd	CTTTCCTGCTGGGAATCAAA	NM_002197
ACO1rev	TCCAGCTTGACCTGGACTTT	
FAHfwd	CGAGCCCTACACATTTGACA	NM_000137
FAHrev	CATGGAGCCGAAGTTTTCTG	
ALDOAfwd	GTGCTGGCTGCTGTCTACAA	NM_000034
ALDOArev	TCCAGACAGGAAGGTGATCC	
TPI1fwd	CCCTGGCATGATCAAAGACT	NM_000365
TPI1rev	TCTGCGATGACCTTTGTCTG	

Supplementary Table S2: cDNA microarray data of 786-O VHL$^-$ and VHL$^+$ RCC cells incubated under different oxygen conditions

Please see Supplementary File 2

Supplementary Table S3: Number of VHL- and/or hypoxia-regulated genes and proteins

sample	786-O hypoxia vs. 786-O normoxia	VHL normoxia vs. 786-O normoxia	VHL hypoxia vs. VHL normoxia	VHL hypoxia vs. 786-O normoxia
microarray total number	194	1202	186	1292
Up-regelated	106	538	102	606
Down-regulated	88	664	84	686
2DE total number	9	28	2	39
Up-regulated	9	15	2	28
Down-regulated	0	13	0	11

Differentially expressed cDNAs and proteins of the comparison of VHL$^-$/VHL$^+$ 786-O cells during normoxia and hypoxia incubation. Transcriptome and proteome analysis were determined as outlined in materials and methods. Differentially expressed genes were defined by one-way ANOVA with a p value <0.005 and proteins found to be at least two-fold regulated (factor ≥ 2.0 or ≤ 0.50; $p \leq 0.05$).

Supplementary Table S4: Overlap of different expressed genes of the comparison of 786-O VHL$^-$ and VHL$^+$ cells during normoxia and hypoxia

Please see Supplementary File 4

Supplementary Table S5: Differentially expressed proteins identified by 2DE followed by peptide mass fingerprint

Setting	Gene Symbol	Ratio	Fold-Change
VHL^+ vs. VHL^- during normoxia			
	KRT8	0.13	–7.69
	SOD2	0.25	–4
	ENO2	0.27	–3.70
	EZR	0.31	–3.23
	SEPT11	0.35	–2.86
	PDCD6IP	0.41	–2.44
	PPP1CC	0.42	–2.38
	TPI1	0.45	–2.22
	GMPS	0.46	–2.17
	ENO1	0.47	–2.13
	FAH	0.47	–2.13
	AKR1B1	0.47	–2.13
	TPI1	0.48	–2.08
	PKM2	0.49	–2.04
	UQCRC1	2.00	2
	PGAM1	2.02	2.02
	G6PD	2.02	2.02
	HSPA4	2.16	2.16
	SAHH	2.75	2.75
	ANXA4	2.78	2.78
	VDAC1	2.89	2.89
	PRDX3	2.92	2.92
	APRT	3.17	3.17
	PRDX2	3.56	3.56
	GSTP1	4.55	4.55
	QPRT	4.68	4.68
	TXN	6.08	6.08
	UCHL1	6.28	6.28
VHL^- hypoxia vs. normoxia			
	MSN	2.0	2
	XRCC5	2.1	2.1

(*Continued*)

Setting	Gene Symbol	Ratio	Fold-Change
	ACO1	2.1	2.1
	C22orf28	2.4	2.4
	GANAB	2.7	2.7
	EIF3B	2.7	2.7
	EEF2	2.8	2.8
	EIF5A	3.7	3.7
	VCL	4.4	4.4
VHL^+ vs. VHL^- during hypoxia			
	CFL1	0.06	–16.67
	KRT19	0.22	–4.55
	PCNA	0.32	–3.13
	TGM2	0.34	–2.94
	SOD2	0.34	–2.94
	FABP5	0.36	–2.78
	PSME1	0.38	–2.63
	KRT8	0.38	–2.63
	SERPINB9	0.42	–2.38
	CLR	0.49	–2.04
	ACO1	0.49	–2.04
	MAPK1	2.01	2.01
	VDAC1	2.03	2.03
	GLRX3	2.05	2.05
	WDR1	2.06	2.06
	NAPA	2.07	2.07
	PPP2CA	2.07	2.07
	G6PD	2.08	2.08
	HSP90B1	2.09	2.09
	ENO1	2.11	2.11
	SDHA	2.16	2.16
	VDAC2	2.23	2.23
	ALDOA	2.30	2.3
	ALDH9A1	2.36	2.36
	EFHD2	2.39	2.39
	SEPT8	2.40	2.4
	PRDX1	2.66	2.66
	ERP29	2.77	2.77

(*Continued*)

Setting	Gene Symbol	Ratio	Fold-Change
	QPRT	2.94	2.94
	ECHS1	3.03	3.03
	ANXA4	3.34	3.34
	PRDX2	4.19	4.19
	LPP	4.50	4.5
	RPS12	5.35	5.35
	FHL2	8.78	8.78
	UCHL1	9.67	9.67
	GSTP1	12.3	12.3
	PFD2	22.67	22.67
	ADI1	29.66	29.66
VHL^+ hypoxia vs. normoxia			
	FTL	3.06	3.06
	NDRG1	2.03	2.03

550 µg protein lysate isolated from VHL^-/VHL^+ 786–O cells incubated under normoxic or hypoxic (1 % O_2, 48 h) conditions respectively, were loaded onto IPG strips (pH 3–10, non–linear, Amersham Biosciences) followed by an isoelectric focusing and second–dimension SDS–PAGE separation (13 %) and staining with colloidal Coomassie as described (13). The gels were analyzed using the Delta2D software package (Decodon). Proteins found to be at least two–fold regulated (factor ≥ 2.0 or ≤ 0.50; $p \leq 0.05$) were subjected to mass spectrometric identification.

Supplementary Table S6: Functional classification of differentially expressed metabolic proteins

Metabolic function	VHL-dependent	hypoxia-dependent
Glycolysis	PKM2 ↓ ENO2 ↓TPI1 ↓ PGAM 1↑	TPI1, ALDOA, ENO2 ↑
Citrate cycle/mitochondrial respiratory chain	ACO1 ↓UQCRC1, ECHS1, SDHA ↑	-
Fatty acid uptake	FABP5 ↓	-
Energy metabolism	GMPS ↓	GANAB ↑
Crosslinking of proteins	TGM 2 ↓ ANXA4 ↑	TGM2 ↑
ROS degradation	PRDX2+3, GSTP1 ↑SOD2 ↓	PRDX1+2, TXN ↑

Via 2DE-based proteome analysis followed by mass spectrometry identified proteins, as described in material and methods, were validated via qPCR and immunoblot analysis and classified in the different metabolic functions. ↑ up-regulated ↓ down-regulated.