

PFKFB4 interacts with FBXO28 to promote HIF-1 α signaling in glioblastoma

Emma Phillips¹, Jörg Balss¹, Frederic Bethke¹, Stefan Pusch^{2,3}, Stefan Christen^{4,5}, Thomas Hielscher⁶, Martina Schnölzer⁷, Michael N. C. Fletcher⁸, Antje Habel², Claudia Tessmer⁹, Lisa-Marie Brenner¹, Mona Göttmann¹, David Capper^{2,3,10}, Christel Herold-Mende¹¹, Andreas von Deimling^{2,3}, Sarah-Maria Fendt^{4,5}, and Violaine Goidts¹

Supplementary Tables

Table S1: Gene Set Enrichment Analysis of the transcriptome of GSCs upon *PFKFB4* silencing using the gene sets that represent expression signatures of genetic and chemical perturbations. Signatures with a false discovery rate of less than 25% are shown.

Rank	NAME	SIZE ^a	ES	NES	NOM p-val	FDR q-val ^b
1	VERHAAK GLIOBLASTOMA PRONEURAL	174	-0.573	-2.011	0	0.005
2	HOELZEL NF1 TARGETS DN	106	-0.602	-1.992	0	0.005
3	CONCANNON APOPTOSIS BY EPOXOMICIN DN	164	-0.524	-1.827	0	0.050
4	MARTORIATI MDM4 TARGETS NEUROEPITHELIUM DN	154	-0.500	-1.748	0	0.107
5	RICKMAN TUMOR DIFFERENTIATED WELL VS MODERATELY UP	102	-0.526	-1.743	0	0.109
6	RICKMAN TUMOR DIFFERENTIATED WELL VS POORLY UP	227	-0.484	-1.736	0	0.118
7	NIKOLSKY BREAST CANCER 17Q11 Q21 AMPLICON	125	-0.505	-1.724	0	0.124
8	BASAKI YBX1 TARGETS DN	365	-0.448	-1.678	0	0.163
9	ELVIDGE HIF1A AND HIF2A TARGETS DN	102	-0.511	-1.684	0	0.169
10	QI HYPOXIA	134	-0.484	-1.657	0.003	0.182
11	DEBIASI APOPTOSIS BY REOVIRUS INFECTION DN	282	-0.456	-1.659	0	0.186
12	NIKOLSKY BREAST CANCER 20Q12 Q13 AMPLICON	134	-0.471	-1.617	0	0.224
13	SENESE HDAC1 TARGETS DN	237	-0.435	-1.580	0.002	0.224
14	WANG CLIM2 TARGETS UP	240	-0.438	-1.578	0	0.225
15	HOOI ST7 TARGETS DN	114	-0.480	-1.604	0.004	0.227
16	ONDER CDH1 TARGETS 1 UP	136	-0.460	-1.580	0	0.228
17	REACTOME NEURONAL SYSTEM	275	-0.433	-1.580	0	0.229
18	MORI EMU MYC LYMPHOMA BY ONSET TIME UP	102	-0.480	-1.593	0.003	0.231
19	RODRIGUES DCC TARGETS DN	120	-0.473	-1.600	0	0.232
20	BLUM RESPONSE TO SALIRASIB DN	340	-0.424	-1.581	0	0.233
21	BERENJENO TRANSFORMED BY RHOA DN	384	-0.418	-1.566	0	0.238
22	XU GH1 AUTOCRINE TARGETS DN	133	-0.456	-1.553	0.001	0.239
23	KEGG SYSTEMIC LUPUS ERYTHEMATOSUS	124	-0.459	-1.564	0.004	0.239
24	REACTOME TRANSMISSION ACROSS CHEMICAL SYNAPSES	183	-0.440	-1.560	0	0.240
25	PECE MAMMARY STEM CELL DN	134	-0.457	-1.567	0	0.240
26	MARKEY RB1 CHRONIC LOF UP	112	-0.468	-1.550	0.003	0.242
27	NIKOLSKY BREAST CANCER 16P13 AMPLICON	108	-0.460	-1.541	0.008	0.248

^aGene sets of more than 100 genes were selected for the analysis. ^bThe data are sorted according to the FDR.

Table S2: Interaction partners of PFKFB4 in NCH421k GSCs. PFKFB4 was immunoprecipitated from NCH421k lysate and subjected to mass spectrometry. Proteins were only considered if three or more unique peptide sequences were identified.

Gene Symbol	Mass [kDa]	Replicate	Mascot Score	# significant peptides	Coverage [%]	
PFKFB4	54.7	1	1413	20	57	
		2	978	15	44	
		3	1210	21	56	
		Ctr 1	not detected			
		Ctr 2	not detected			
PFKFB2	59	1	751	12	29	
		2	686	10	30	
		3	893	14	37	
		Ctr 1	not detected			
		Ctr 2	not detected			
FBXO28	41.4	1	423	5	18	
		2	634	10	30	
		3	636	9	30	
		Ctr 1	not detected			
		Ctr 2	not detected			
BDH2	27	1	350	5	30	
		2	434	6	34	
		3	563	8	43	
		Ctr 1	not detected			
		Ctr 2	not detected			
PFKFB3	60.4	1	469	7	15	
		2	349	5	15	
		3	419	7	20	
		Ctr 1	not detected			
		Ctr 2	not detected			

Table S3: Information about patient-derived glioblastoma stem-like cell lines (PFS = progression free survival, OS = overall survival).

Cell Line	Sex	Age (Years)	PFS (months)	OS (Months)
NCH421k	M	66	15	34
NCH441	M	75	22	31
NCH644	F	67	17	30

Table S4: shRNA nucleotide sequences

shRNA	Nucleotide Sequence
shNT	CAACAAGATGAAGAGCACCAA
<i>PFKFB4</i> -sh1	CCTGTGGCATATGGTTGTA
<i>PFKFB4</i> -sh2	GACGTGGTCAAGACCTACAAA
<i>PFKFB4</i> -sh3	GCTGATTGGCTGCCACATTC
<i>FBXO28</i>	GAGAATGTTGAATCAGGGATT
<i>HIF1A</i> _UTR	TGCTCTTTGTGGTTGGATCTA
<i>HIF1A</i>	CCGCTGGAGACACAATCATAT

Table S5: sgRNA sequences

sgRNA	Nucleotide Sequence
PFKFB4 CRISPR_1	TGTAGGTCTTGACCACGTCC
PFKFB4 CRISPR_2	TGACCACGTCCCGGCGATAC
PFKFB3 CRISPR_1	CAACGTCGGGGAGTATCGCC
PFKFB3 CRISPR_2	TCAACGTCGGGGAGTATCGC
PFKFB3 CRISPR_3	CTTCTTCGCCCCGACAATG

Table S6: Cloning primers

Primer	Nucleotide Sequence (5'-3')
FBXO28_F	GATCgaattcATGGCGGCAGCGGCG
FBXO28_R	GCGCGtctagaGGGACACCATGTTGC
PFKFB4_F	ACTGgaattcATGGCGTCCCCACGG
PFKFB4_R	ACTGtctagaTCACTGGTGAGCAGGCACC
SKP1_F	GGCCggatccATGCCTTCAATTAAGTTGCAGAG
SKP1_R	GCGCaccggtTCACTTCTCTTCACACCACTG
CUL1_F	GGCCggatccATGTCGTCAACCCGGAGC
CUL1_R	GCGCaccggtTTAAGCCAAGTAACTGTAGGTGTCC
HIF1A_F	GCTAaggcccTACCATGGCCTACCCCTA
HIF1A_R	CGATtctagaGCCGCCAGTGTGATGGATA
NanoBiT [®] PFKFB4 C-term_F	ACTGacgctaGCACCATGGCGTCCCCACGG
NanoBiT [®] PFKFB4 C-term_R	ACTGacgaatTCCCCTGGTGAGCAGGCACC
NanoBiT [®] FBXO28 C-term_F	ACTGacgctaGCACCATGGCGGCAGCGGCG
NanoBiT [®] FBXO28 C-term_R	ACTGacgaatTCCCCTTTCTATTCCGAAGACG
NanoBiT [®] PFKFB4 N-term_F	ACTGacgaatTCAGCGTCCCCACGGGAA
NanoBiT [®] PFKFB4 N-term_R	ACTGactctaGACTGGTGAGCAGGCACC
NanoBiT [®] FBXO28 N-term_F	ACTGacgaatTCAGCGGCAGCGGCGGAG
NanoBiT [®] FBXO28 N-term_R	ACTGactctaGATTACTTTCTATTCCGAAGACG
N-LgBiT_F	ACTGacttcgAAATGGTCTTCACACTCG
N-SmBiT_F	ACTGacttcgAAATGGTGACCGGCTACC
C-LgBiT_R	ACTGactctaGATTAGCTGTTGATGGTACTCGG
C-SmBiT_R	ACTGactctaGACTAGATTACAGAATCTCCTCG
PFKFB4_F	ACTGacttcgAAATGGCGTCCCCAC
FBXO28_F	ACTGacttcgAAATGGCGGCAGCGG
HaloTag_F	ACTGacttcgAAATGGCAGAAATCGGTACTGG
HaloTag_R	ACTGactctaGACGGCCTTACAGAATCTCC

Table S7: Mutagenesis primers

Primer	Nucleotide Sequence (5'-3')
Mut_Hif1a_A1595G_F	AATGAATTCAgGTTGGAATTGG
Mut_Hif1a_A1595G_R	GACCATATCACTATCCAC
Mut_Hif1a_A1613G_A1640G_F	ACAGAAGCAAgGAACCCATTTTC
Mut_Hif1a_A1613G_A1640G_F	GTCTTCAGCAAAAAGTcTTTC

Table S8: RT-PCR primers

Primer	Nucleotide Sequence
ARF1 fw	GACCACGATCCTCTACAAGC
ARF1 rev	TCCCACACAGTGAAGCTGATG
DCTN2 fw	CGCCATGGCGACCCTAAAT
DCTN2 rev	TTGTCAGCTCCTCCGCATCGAA
HIF1A fw	AACCTGATGCTTTAACTTGCTG
HIF1A rev	CTTCCTCAAGTTGCTGGTCA
HPRT fw	TGACACTGGCAAAACAATGCA
HPRT rev	GGTCCTTTTCACCAGCAAGCT
PFKFB4 fw	CCACTCGGGAGTTCAATGTT
PFKFB4 rev	TTTTCAGGCCCTCTTCATTG