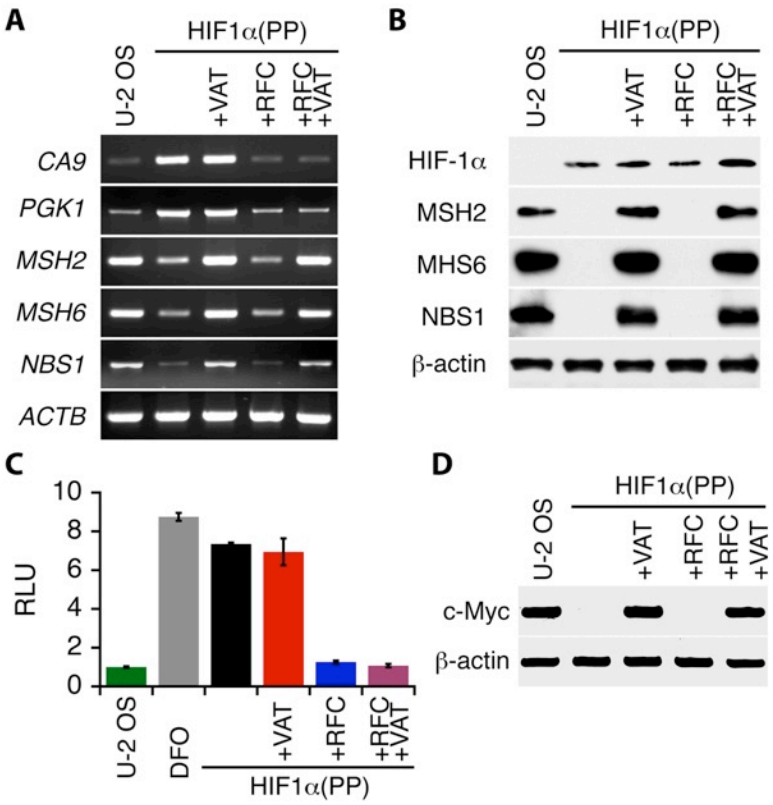
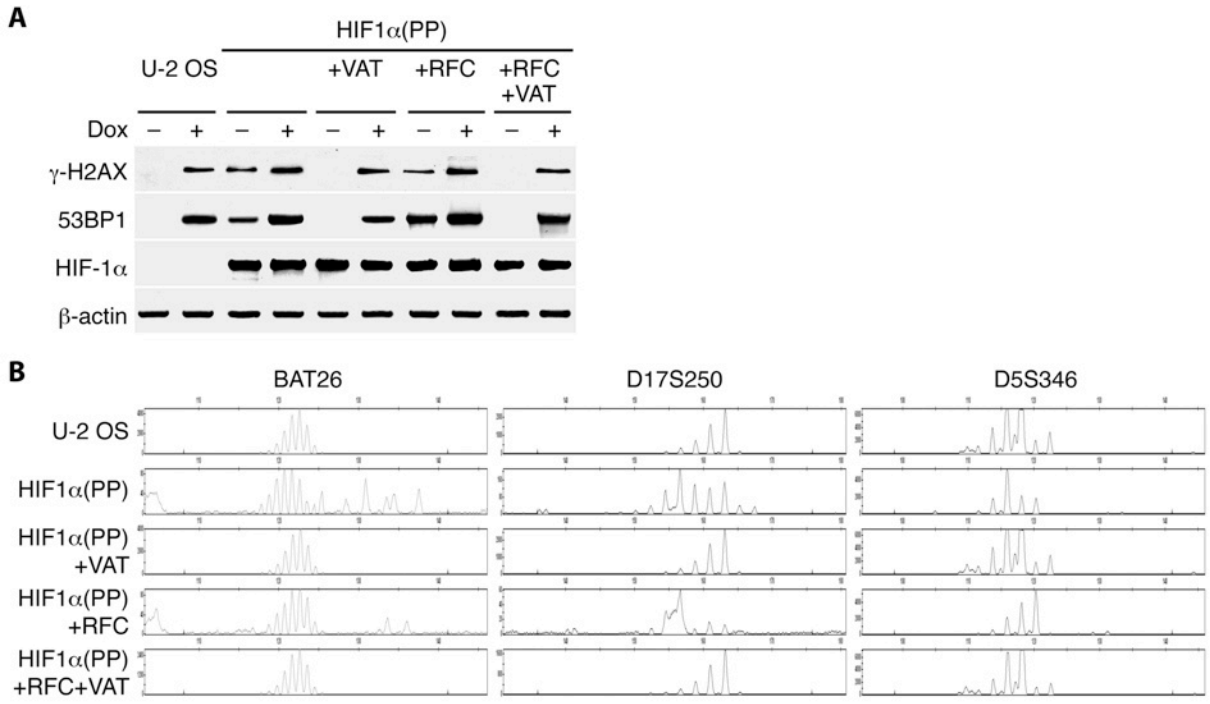
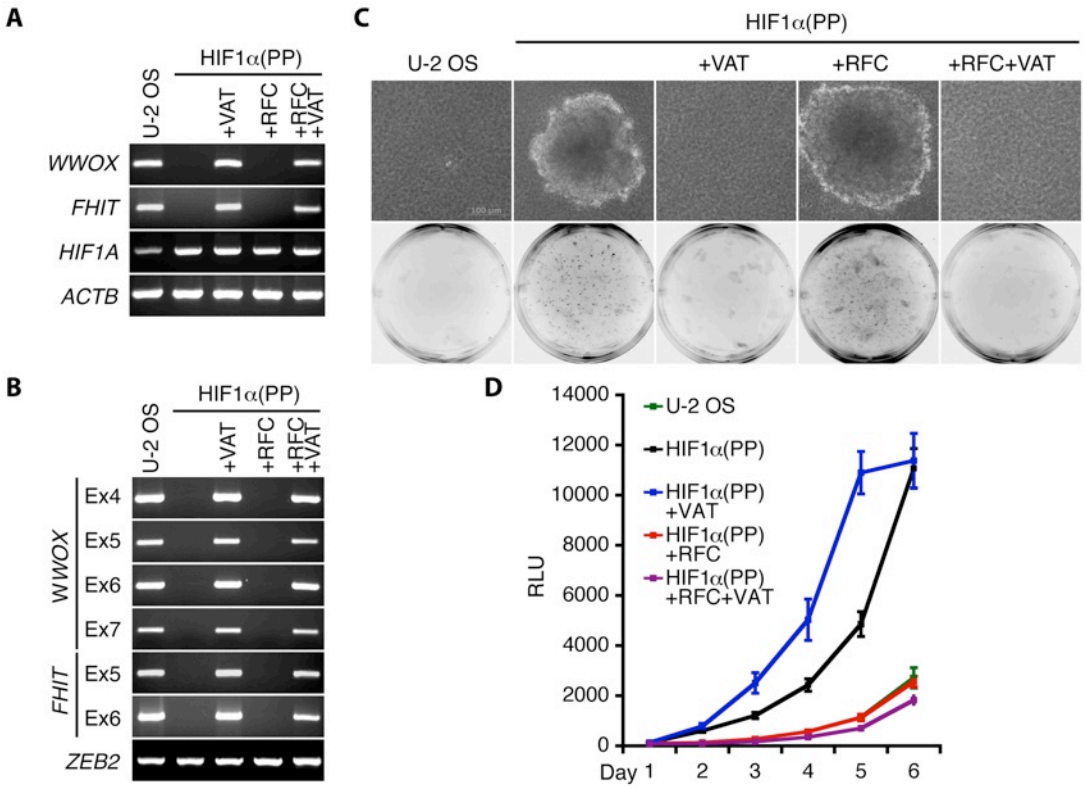
Supplementary Materials



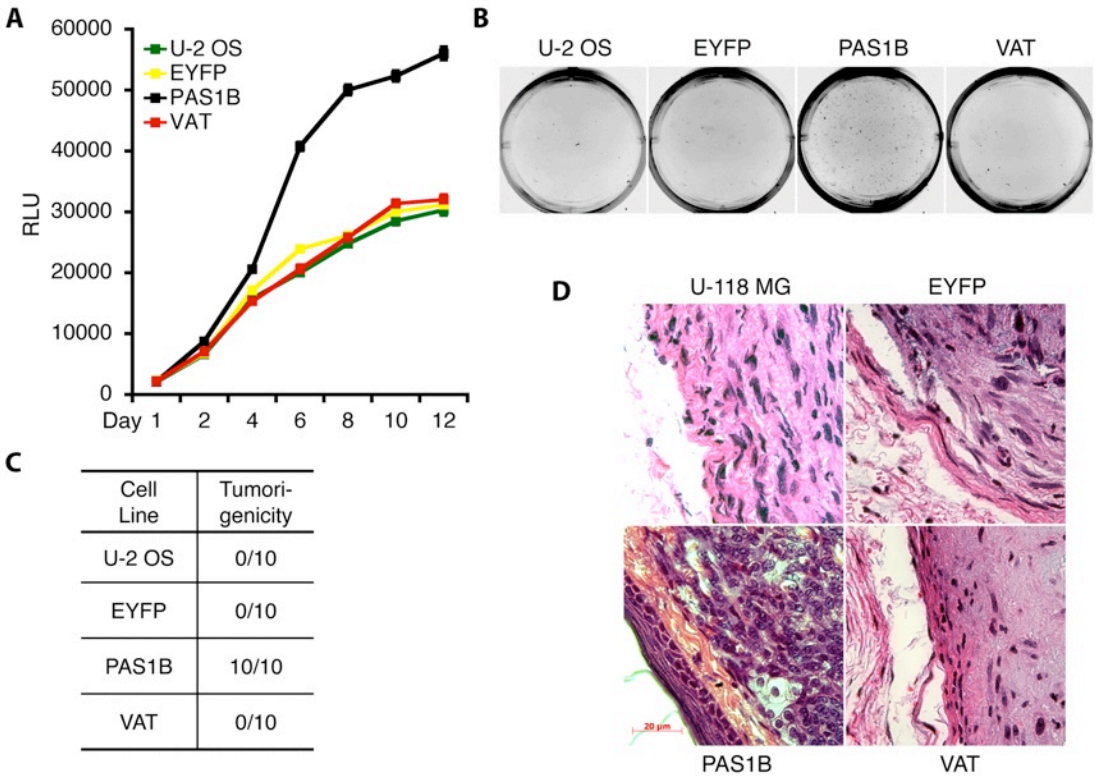
Supplementary Figure 1. Functional independence of the HIF-1α–ARNT pathway and the HIF-1α–c-Myc pathway. *A* and *B*, U-2 OS cells and those transduced with HIF-1α variants were assayed for target gene expression of the HIF-1α–ARNT pathway (*CA9* and *PGK1*) and of the HIF-1α–c-Myc pathway (*MSH2*, *MSH6*, and *NBS1*) by conventional RT-PCR (*A*) and by immunoblotting with antibodies against specified proteins as indicated (*B*). *C*, these cells were also assayed for activity of the HIF-1α–ARNT pathway with an erythropoietin reporter plasmid pEpoE-luc (1). Desferrioxamine (DFO, 100 μM overnight) served as a positive control of hypoxic induction. Relative luciferase units (RLU) were measured in triplicates and plotted in mean ± SEM. *D*, c-Myc protein levels of these cells were determined by Western blot.



Supplementary Figure 2. Induction of DNA damage and microsatellite instability via the HIF-1α–c-Myc pathway. *A*, transduced cells as indicated were assayed for the expression levels of γ-H2AX and 53BP1 in the absence and presence of 0.5-*μ*M doxorubicin (+Dox) for 24 h. *B*, microsatellite instability was analyzed with the genomic DNA isolated from the transduced cells using indicated markers.



Supplementary Figure 3. Loss of tumor-suppressing activity and gain of malignant properties arising from the HIF-1α–c-Myc pathway. *A*, U-2 OS cells transduced with HIF-1α variants as indicated were assayed for mRNA levels of *FHIT* and *WWOX* by conventional RT-PCR. *HIF1A* and *ACTB* genes served as controls. *B*, these cells were also assayed for the exon regions of *FHIT* and *WWOX* as specified by PCR amplification of genomic DNA. *ZEB2* genomic DNA served as control. *C*, these cells were seeded in soft agar for anchorage-independent growth. Individual colonies (top) and the entire wells (bottom) were photographed and presented. *D*, the proliferative potential of these cells was determined by a cell viability assay. Relative luciferase units (RLU) were measured in 6 replicates and plotted as mean ± SEM.

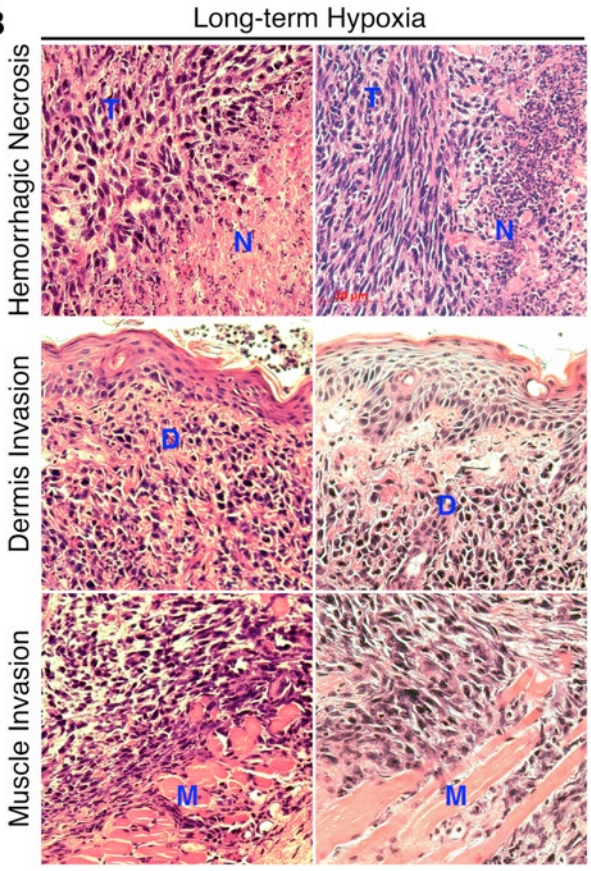


Cell Line	Tumori-genicity
U-2 OS	0/10
EYFP	0/10
PAS1B	10/10
VAT	0/10

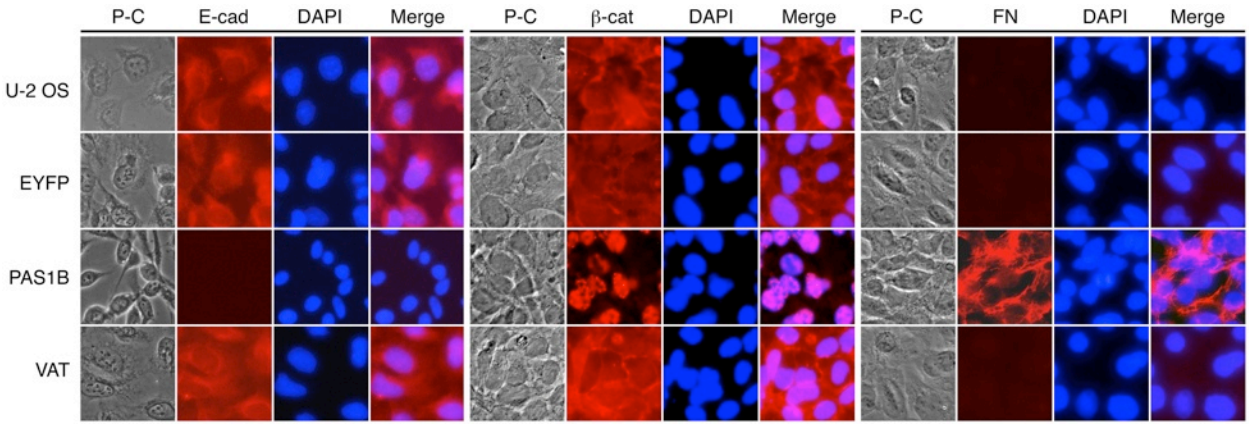
Supplementary Figure 4. Gain of malignant traits in tumor cells expressing HIF-1α PAS-B. *A*, transduced U-2 OS cells as indicated were assayed for proliferation in 6 replicates and presented as mean ± SEM. *B*, transduced cells as above were assayed for anchorage-independent growth. Images of colonies are shown. *C*, tumorigenicity of transduced U-2 OS cells was determined in 10 CD-1 mice per group that were subjected to bilateral, subcutaneous injections. Ten out of 10 mice injected with U-2 OS cells expressing PAS1B developed tumors. *D*, U-118 MG cells expressing PAS1B developed fast growing tumors that invaded dermal layers in xenografts. By contrast, the parental U-118 cells and those expressing EYFP or PAS1B mutant (VAT) formed tiny, circumscribed tumors. Images are presented in hematoxylin-eosin staining with 400 × magnification.

A

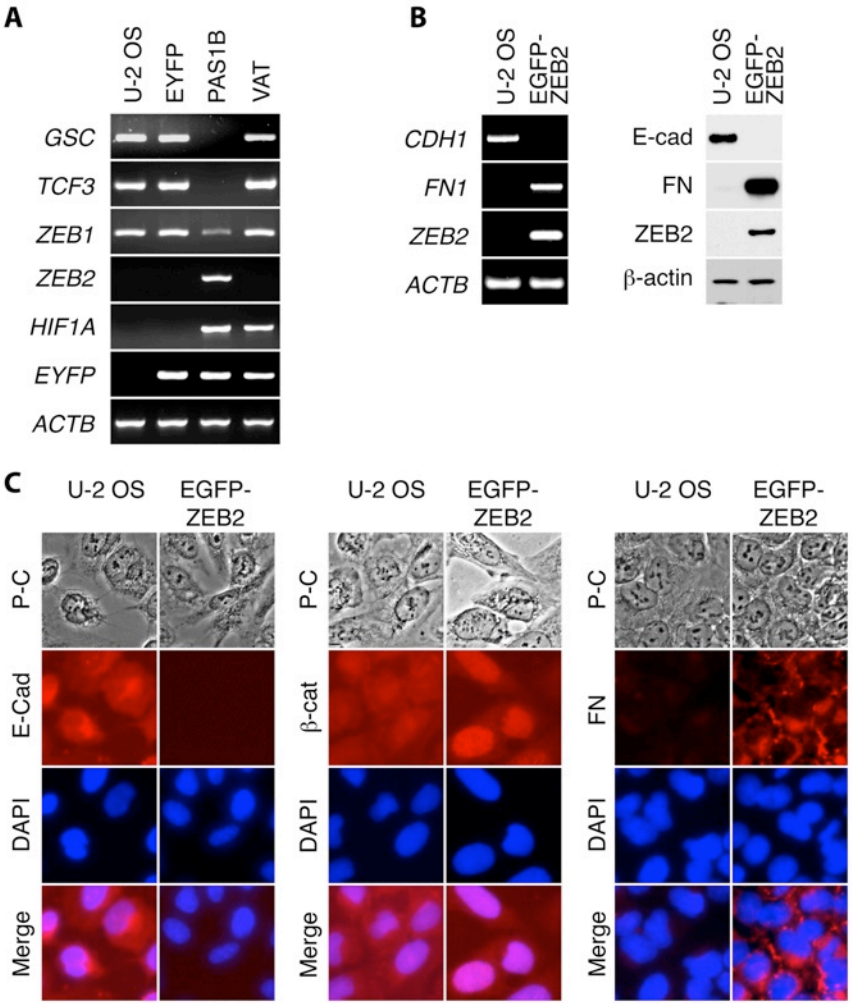
Cell Line	Tumori-genicity
HIF1α(PP)	5/5
LT Hyp	5/5
sh*HIF1A* + LT Hyp	0/5
sh*HIF1A*	0/5
ST Hyp	0/5
ST Hyp + Rec	0/5



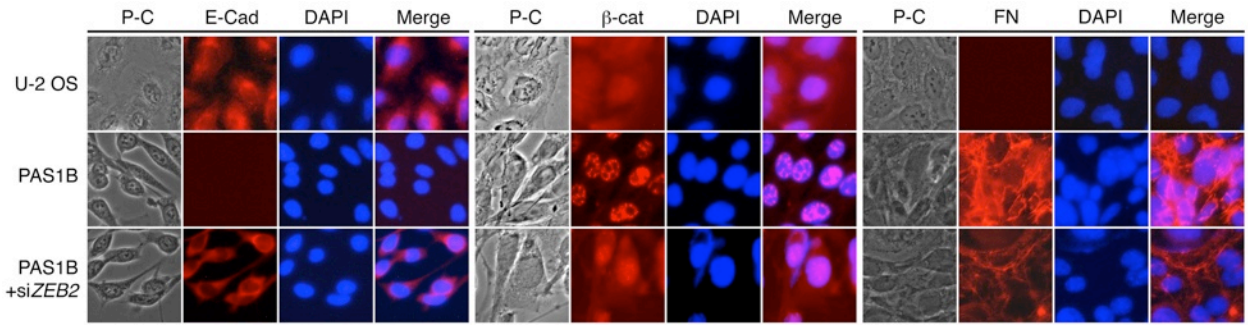
Supplementary Figure 4. Requirement of HIF-1α for the gain of tumorigenicity with long-term hypoxia. *A*, 5 CD-1 nude mice per group were injected subcutaneously with HIF1α(PP) cells or U-2 OS cells that had been subjected to long-term hypoxia (LT Hyp) or HIF-1α knockdown prior to the treatment (sh*HIF1A*+LT Hyp), HIF-1α knockdown alone, short-term hypoxia (ST Hyp), or short-term hypoxia plus days of recovery in normoxia (ST Hyp+Rec). Gain of tumorigenicity is expressed in a ratio. *B*, H-E staining of tumor specimens (T) shows hemorrhagic necrosis (N) and invasion of dermal layers (D) and skeletal muscles (M). Two representative images with 200 × magnification are shown.



Supplementary Figure 6. Induction of epithelial–mesenchymal transition by HIF-1α PAS-B. Transduced U-2 OS cells as indicated were subjected to immunofluorescent staining with antibodies against E-cadherin (E-cad), β-catenin (β-cat), and fibronectin (FN). Cell nuclei were visualized with DAPI staining. P-C, phase-contrast microscopy.



Supplementary Figure 7. Induction of ZEB2 by HIF-1α PAS-B for epithelial–mesenchymal transition. *A*, *ZEB2* expression was upregulated at mRNA levels as determined by conventional RT-PCR in U-2 OS cells transduced with HIF-1α PAS-B. Other known *CDH1* transcriptional repressors Goosecoid (GSC), TCF3, and ZEB1 were downregulated. *B* and *C*, U-2 OS cells were transfected stably with EGFP-ZEB2 fusion and assayed for the expression of E-cadherin and fibronectin at mRNA (left) and protein (right) levels (*B*), and were subjected to immunofluorescent staining for the detection of E-cadherin, β-catenin, and fibronectin in (*C*).



Supplementary Figure 8. Transduced cells expressing PAS1B were transfected with small-interfering RNA targeting *ZEB2* (si*ZEB2*) and then subjected to immunofluorescent staining with antibodies against E-cadherin (E-cad), β-catenin (β-cat), and fibronectin (FN). Cell nuclei were visualized with DAPI staining. P-C, phase-contrast microscopy.

Table S1

Symbol	Unigene	GenBank	U-2 OS	HIF1α(PP)	HIF1α(PP) +VAT	HIF1α(PP) +RFC
AKT1	Hs.525622	NM_005163	1.00	0.01	1.26	0.00
ANGPT1	Hs.369675	NM_001146	1.00	5.06	0.77	7.87
ANGPT2	Hs.583870	NM_001147	1.00	0.08	0.84	0.04
APAF1	Hs.708112	NM_001160	1.00	0.34	0.93	0.19
ATM	Hs.367437	NM_000051	1.00	0.05	1.10	0.04
BAD	Hs.370254	NM_004322	1.00	0.14	1.62	0.05
BAX	Hs.631546	NM_004324	1.00	42.82	1.35	0.65
BCL2	Hs.150749	NM_000633	1.00	12.99	1.82	6.82
BCL2L1	Hs.516966	NM_138578	1.00	0.00	1.29	0.96
BRCA1	Hs.194143	NM_007294	1.00	0.01	1.28	0.00
CASP8	Hs.655983	NM_001228	1.00	0.03	0.99	0.02
CCNE1	Hs.244723	NM_001238	1.00	0.01	0.98	0.00
CDC25A	Hs.437705	NM_001789	1.00	0.03	1.09	0.01
CDK2	Hs.19192	NM_001798	1.06	0.00	0.90	0.00
CDK4	Hs.95577	NM_000075	1.00	0.36	1.24	0.17
CDKN1A	Hs.370771	NM_000389	1.00	0.00	0.97	0.00
CDKN2A	Hs.512599	NM_000077	1.00	56.48	0.93	14.29
CFLAR	Hs.390736	NM_003879	1.00	0.00	1.00	0.00
CHEK2	Hs.291363	NM_007194	1.00	0.02	1.82	0.00
COL18A1	Hs.517356	NM_030582	1.00	0.01	1.54	0.01
E2F1	Hs.654393	NM_005225	1.00	0.68	1.59	0.33
EPDR1	Hs.563491	NM_017549	1.00	0.01	3.18	0.01
ERBB2	Hs.446352	NM_004448	1.00	0.01	1.28	0.01
ETS2	Hs.644231	NM_005239	1.00	0.01	1.22	0.03
FAS	Hs.244139	NM_000043	1.00	0.01	0.71	0.00
FGFR2	Hs.533683	NM_000141	1.00	0.04	1.20	0.02
FOS	Hs.25647	NM_005252	1.00	0.53	1.04	1.01

Table S1

GZMA	Hs.90708	NM_006144	1.00	0.00	1.03	0.00
HPRT1	Hs.412707	NM_000194	1.00	1.00	1.00	1.00
HTATIP2	Hs.90753	NM_006410	1.00	0.01	0.95	0.00
IFNA1	Hs.37026	NM_024013	1.00	32.39	1.64	4.67
IFNB1	Hs.93177	NM_002176	1.00	2.43	1.81	2.82
IGF1	Hs.160562	NM_000618	1.00	0.06	0.97	0.02
IL8	Hs.624	NM_000584	1.00	0.00	0.96	0.00
ITGA1	Hs.696076	NM_181501	1.00	0.01	1.00	0.01
ITGA2	Hs.482077	NM_002203	1.00	0.00	0.88	0.00
ITGA3	Hs.265829	NM_002204	1.00	0.00	1.16	0.00
ITGA4	Hs.694732	NM_000885	1.00	0.45	1.40	0.06
ITGAV	Hs.436873	NM_002210	1.00	0.00	1.73	0.00
ITGB1	Hs.707987	NM_002211	1.00	0.00	1.18	0.00
ITGB3	Hs.218040	NM_000212	1.00	0.03	1.02	0.00
ITGB5	Hs.536663	NM_002213	1.00	0.00	1.19	0.00
JUN	Hs.525704	NM_002228	1.00	0.01	1.55	0.00
MAP2K1	Hs.145442	NM_002755	1.00	0.01	1.69	0.00
MCAM	Hs.599039	NM_006500	1.00	0.00	1.89	0.00
MDM2	Hs.567303	NM_002392	1.00	0.00	1.38	0.00
MET	Hs.132966	NM_000245	1.00	0.00	1.30	0.00
MMP1	Hs.83169	NM_002421	1.00	0.06	0.46	0.03
MMP2	Hs.513617	NM_004530	1.00	0.02	1.08	0.02
MMP9	Hs.297413	NM_004994	1.00	0.00	1.22	0.00
MTA1	Hs.525629	NM_004689	1.00	1.76	1.68	0.65
MTA2	Hs.173043	NM_004739	1.00	0.00	1.32	0.00
MTSS1	Hs.336994	NM_014751	1.00	1.98	1.86	0.77
MYC	Hs.202453	NM_002467	1.00	0.01	0.71	0.00
NFKB1	Hs.654408	NM_003998	1.00	0.00	0.78	0.00
NFKBIA	Hs.81328	NM_020529	1.00	0.00	1.18	0.00
NME1	Hs.118638	NM_000269	1.00	1.74	1.44	0.47

Table S1

NME4	Hs.9235	NM_005009	1.00	0.01	1.31	0.00
PDGFA	Hs.707991	NM_002607	1.00	0.00	0.79	0.00
PDGFB	Hs.1976	NM_002608	1.00	0.03	1.01	0.00
PIK3R1	Hs.132225	NM_181504	1.00	0.01	1.62	0.01
PLAU	Hs.77274	NM_002658	1.00	0.00	0.77	0.00
PLAUR	Hs.466871	NM_002659	1.00	0.00	1.30	0.00
PNN	Hs.409965	NM_002687	1.00	1.98	1.24	0.43
RAF1	Hs.159130	NM_002880	1.00	0.00	1.27	0.00
RB1	Hs.408528	NM_000321	1.00	0.02	1.17	0.00
S100A4	Hs.654444	NM_002961	1.00	0.00	1.40	0.01
SERPINB5	Hs.55279	NM_002639	1.00	4.28	1.63	0.18
SERPINE1	Hs.414795	NM_000602	1.00	0.00	1.26	0.00
SNCG	Hs.349470	NM_003087	1.00	0.01	1.43	0.01
SYK	Hs.371720	NM_003177	1.00	3.75	1.23	3.75
TEK	Hs.89640	NM_000459	1.00	0.09	0.89	0.04
TERT	Hs.492203	NM_198253	1.00	46.21	8.24	9.23
TGFB1	Hs.645227	NM_000660	1.00	0.00	0.99	0.00
TGFBR1	Hs.494622	NM_004612	1.00	0.12	1.18	0.05
THBS1	Hs.164226	NM_003246	1.00	0.01	1.00	0.00
TIMP1	Hs.522632	NM_003254	1.00	0.00	0.93	0.00
TIMP3	Hs.701968	NM_000362	1.00	0.66	1.97	0.66
TNF	Hs.241570	NM_000594	1.00	2.98	2.34	4.50
TNFRSF10B	Hs.521456	NM_003842	1.00	0.00	1.06	0.00
TNFRSF1A	Hs.279594	NM_001065	1.00	0.03	1.36	0.01
TNFRSF25	Hs.462529	NM_003790	1.00	0.14	1.59	0.03
TP53	Hs.654481	NM_000546	1.00	0.00	1.02	0.00
TWIST1	Hs.66744	NM_000474	1.00	971.74	1.04	269.04
VEGFA	Hs.73793	NM_003376	1.00	0.00	0.38	0.00

Table S2

Mutagenesis					
			sequences		
HIF-1α (F99A)	forward	5'	GCCTTGGATGGGCTTGTTATGGTTCTCACAGATGATGC	3'	
	reverse	5'	CCATCATCTGTGAGAACCATAACAAGCCCATCCAAGGC	3'	
Conventional RT-PCR					
gene			**sequences**		**product size**
HIF1A	forward	5'	CCGGAATTCTCAACCACAGTGCATTG	3'	914 bp
	reverse	5'	CGGGGATCCATACGGTCTTTTGTCACTG	3'	
MSH2	forward	5'	TCTGACTTCTCCAAGTTTCAGG	3'	390 bp
	reverse	5'	CTGGGCTTCTTCATATTCTGTTT	3'	
MSH6	forward	5'	CACGCCATCCTTGCATTACG	3'	405 bp
	reverse	5'	TTGCTATTGCCGTCCCATCA	3'	
NBS1	forward	5'	TCTGTCAGGACGGCAGGAAAGAAA	3'	584 bp
	reverse	5'	ACTCCTTTACAGTGGGTGCATCTT	3'	
CA9	forward	5'	AGTGCCTATGAGCAGTTGCTGTCT	3'	305 bp
	reverse	5'	GCCTCAATCACTCGCCCATTCAAA	3'	
PGK1	forward	5'	TTGGACAATGGAGCCAAGTCGGTA	3'	854 bp
	reverse	5'	ACAATCTGCTTAGCCCGAGTGACA	3'	
CDH1	forward	5'	TTCCCTCGACACCCGATTCAAAGT	3'	382 bp
	reverse	5'	TCCTTGGCCAGTGATGCTGTAGAA	3'	
CTNNB1	forward	5'	TGGCCATCTTTAAGTCTGGAGGCA	3'	727 bp
	reverse	5'	AGATGACGAAGAGCACAGATGGCA	3'	
FN1	forward	5'	AACTGTACATGCTTCGGTCAGGGT	3'	583 bp
	reverse	5'	AGCTACTGGCTGTGATTTCGGTCA	3'	
SNAI1	forward	5'	TACAGCGAGCTGCAGGACTCTAAT	3'	447 bp
	reverse	5'	ACCCAGGCTGAGGTATTCCTTGTT	3'	
SNAI2	forward	5'	AGCCAAACTACAGCGAACTGGACA	3'	511 bp
	reverse	5'	ACACAAGGTAATGTGTGGGTCCGA	3'	
GSC	forward	5'	CCAGCATGTTCAGCATCGACAACA	3'	696 bp
	reverse	5'	CCAGCATGTTCAGCATCGACAACA	3'	
TCF3	forward	5'	ACAGCAGCCTCTCTTCATCCACAT	3'	806 bp
	reverse	5'	AGGGCTGGACGAGAAGTTATTGCT	3'	
ZEB1	forward	5'	ATGCACAACCAAGTGCAGAAGAGC	3'	512 bp
	reverse	5'	TGCGCAAGACAAGTTCAAGGGTTC	3'	
ZEB2	forward	5'	AAGCTTGCCTCCAGAGCTTGACTA	3'	562 bp

Table S2

	reverse	5'	TTTGTGGGAGGGTTACTGTTGGGA	3'	
WWOX	forward	5'	CGGGATTTCACTGGCAAAGTGGTT	3'	382 bp
	reverse	5'	AAACATCCTGGAGGAGCTGGACAA	3'	
FHIT	forward	5'	TTTGGCCAACATCTCATCAAGCCC	3'	402 bp
	reverse	5'	TTCTGCTGCCATTTCCTCCTCTGA	3'	
EYFP	forward	5'	TGACCCTGAAGTTCATCTGCACCA	3'	384 bp
	reverse	5'	TGTGGCGGATCTTGAAGTTCACCT	3'	
ACTB	forward	5'	GTGGGGCGCCCCAGGCACCA	3'	539 bp
	reverse	5'	CTCCTTAATGTCACGCACGATTTC	3'	

table S3

genomic DNA-PCR						
gene	**exon**			**sequences**		**product size**
WWOX	4	forward	5'	AGGCCAGAAGATAGATTCAGTGGG	3'	524 bp
		reverse	5'	CCTACACAGGCTTCCATGACAACA	3'	
	5	forward	5'	GCTGCCCTGTTCATGGTAAGATGT	3'	423 bp
		reverse	5'	AATCTCCATATGGTTAGCCCGGCA	3'	
	6	forward	5'	AGGTTTAGCAGAATCCCAGCCTCA	3'	453 bp
		reverse	5'	ATACGGTTCACCTTAACAGGGCCA	3'	
	7	forward	5'	GCCCACTCAAAGCCTTGTGACATT	3'	511 bp
		reverse	5'	AAACATCCTGGAGGAGCTGGACAA	3'	

FHIT	5	forward	5'	TGGATTTGAGTTAAGGTGGCACCG-3	3'	488 bp
		reverse	5'	TTGGCTGGTTAGGCTCAGAAGACT-3	3'	
	6	forward	5'	TCCTGTGGGTATGAACTGCTTGGT-3	3'	461 bp
		reverse	5'	ATCCATTACTCCCACCTGCTTGGT-3	3'	

ZEB2	1	forward	5'	ACAAAGATAGGTGGCGCGTG	3'	269 bp
		reverse	5'	ATGAAGAAGCCGCGAAGTGT	3'	
	8	forward	5'	AAGCTTGCCTCCAGAGCTTGACTA	3'	562 bp
		reverse	5'	TTTGTGGGAGGGTTACTGTTGGGA	3'	

Supplementary Table 1. Alteration of gene expression profile by transduced HIF-1α.

As determined by real-time PCR arrays, fold changes of gene expression in HIF1α(PP), HIF1α(PP)+VAT, HIF1α(PP)+RFC, and HIF1α(PP)+sh*HIF1A* cells were compared in reference to the parental U-2 OS cells with *HPRT* expression for normalization. The data presented are the average of the results from two independent arrays. Upregulation is highlighted in green with fold changes > 1.5 as cutoff, whereas downregulation is shown in red with fold changes < 0.5 as cutoff.

Supplementary Table 2. Primer sequences of mutagenesis and conventional RT-PCR

Supplementary Table 3. Primer sequences of genomic PCR