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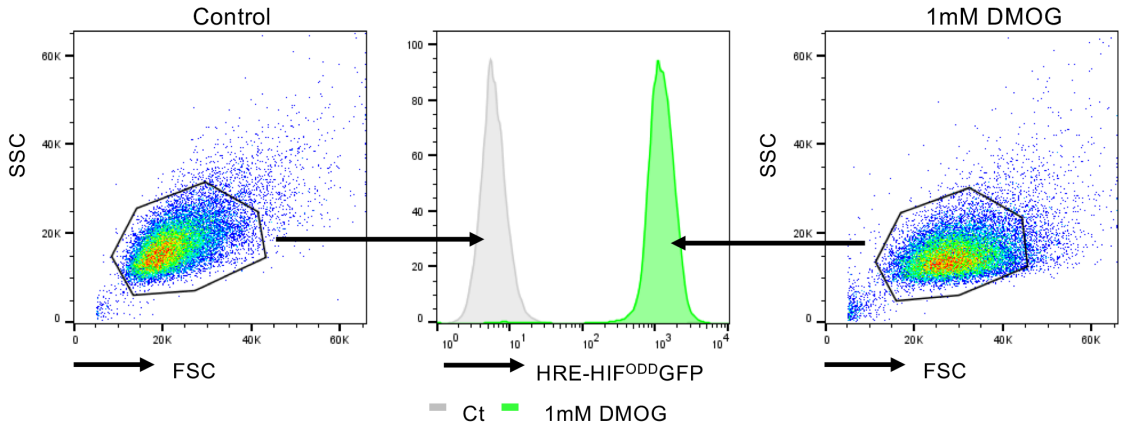
Supplementary information

The HIF complex recruits the histone methyltransferase SET1B to activate specific hypoxia-inducible genes

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Supplementary Information

Supplementary Figure 1: Gating strategy for flow cytometry



Representative gating strategy for HeLa HRE-HIF^{ODD}GFP reporter cells with or without 1 mM DMOG treatment. Cells were selected on side scatter (SCC) and forward scatter (FSC) and flow cytometry results displayed by histograms.

Supplementary Table 1: Top ranked genes enriched in the screen.

Gene Name	-log10 p value	Function	Validated with alternate sgRNA
ARNT (HIF1β)	6.55	HIF complex	Yes
ABHD2	4.52	Esterase/lipase	No
EPAS1 (HIF-2α)	4.47	HIF complex	Yes
SETD1B (SET1B)	4.19	H3K4me3 methyltransferase	Yes
UBE2A	4.04	E2 conjugating enzyme	Yes
SMTNL1	3.89	Myosin contraction	ND
PPP4C	3.59	Phosphatase	Yes
GLI3	3.51	Zinc finger, DNA binding, Hedgehog signalling	ND
ZNF319	3.44	Zinc finger, DNA binding	ND
KRCC1	3.23	Putative DNA binding	ND
CSNK1A1	3.21	LIM/Zn finger motif. Unknown function	ND
ATCAY	3.15	NBIP-2 homology, kinesin binding, putative involvement in apoptosis.	ND
GNB3	3.14	G protein, cell signalling	ND
SPRED2	3.13	Sprouty family, cell signalling	ND
CYFIP2	3.04	Signalling, involved in apoptosis	ND
CSRP1	3.04	LIM domain, Unknown function	ND
NRSN1	3.03	Neurensin 1, putative involvement in transport	ND

Genes with enriched sgRNA in the CRISPR/Cas9 mutagenesis screen above the threshold > 3 (-log10(p value)). ABHD2 did not validate with sgRNA in non-reporter cell lines. *ND* = not done.

Supplementary Table 2: Reagents, antibodies, and primer sequences

Reagents and Antibodies	**Source**	**Identifier**
Antibodies		
Mouse monoclonal anti-β actin Immunoblot: 1:20,000	Sigma	Cat #A2228;
Mouse monoclonal anti-CA9 (M75) Immunoblot: 1:5000 Flow cytometry: 1:100	Absolute Antibody	Cat: Ab00414-1.1
Rabbit monoclonal anti-CFP1 Immunoblot: 1:1000 Chromatin Immunoprecipitation: 1:100	Cell Signalling	Cat: #40672
Rabbit monoclonal anti-H3 Immunoblot: 1:2000	Cell Signalling	Cat: #4499
Rabbit monoclonal anti-H3K4me3 Immunoblot: 1:1000 Chromatin immunoprecipitation: 1:50	Cell Signalling	Cat: #9751
Rabbit monoclonal anti-Acetyl-Histone H3 (Lys27) Chromatin immunoprecipitation: 1:100	Cell Signalling	Cat: #8173
Rat monoclonal anti-HA Immunoblot: 1:2000	Roche	Cat: 11867423001
Mouse monoclonal anti-HIF-1α Immunoblot: 1:1000	BD Biosciences	Cat: #610959
Rabbit monoclonal anti-HIF-1α Immunoprecipitation: 1:50 Chromatin immunoprecipitation: 1:50	Cell Signalling	Cat: #36169
Rabbit polyclonal anti-HIF-1α (PM14) PMID: 30429208	Generated by David Mole	
Rabbit monoclonal anti-HIF-2α Immunoblot: 1:1000	Cell Signalling	Cat: #7096
Rabbit polyclonal anti-HIF-2α (PM9) PMID: 30429208	Generated by David Mole	
Rabbit monoclonal anti-HIF1β Immunoblot: 1:1000 Chromatin immunoprecipitation: 1:50	Cell Signalling	Cat: #5537
Rabbit polyclonal anti-HIF-1β Chromatin immunoprecipitation: 1:100	Novus Biologicals	Cat: NB100-110
Rabbit monoclonal anti-Hydroxy-HIF-1α Immunoblot: 1:1000	Cell Signalling	Cat: #3434
Rabbit polyclonal anti-PARP1 Immunoblot: 1:1000	Cell Signalling	Cat: #9542

Rabbit monoclonal anti-SET1A Immunoblot 1:1000	Cell Signalling	Cat: 61702
Rabbit monoclonal anti-SET1B Immunoblot: 1:1000 Immunoprecipitation: 1:100 Chromatin immunoprepitation: 1:100	Cell Signalling	Cat: 44922
Rabbit monoclonal anti-SUZ12 Immunoblot: 1:1000	Cell Signaling	Cat: 3737
Mouse monoclonal anti-alpha Tubulin Immunoblot: 1:2000	eBioscience	Cat: 14-4502
Rabbit monoclonal anti-WDR82 Immunoblot: 1:1000	Cell Signalling	Cat: #99715
Alexa-Fluor 488 Goat Anti-Mouse IgG Flow cytometry secondary: 1:1000	Thermo	**Cat: #**A32723
Peroxidase-AffiniPure Goat Anti-Mouse IgG Immunoblot secondary: 1:20,000	Jackson	Cat: 115-035-146
Peroxidase-AffiniPure Goat Anti-Rabbit IgG Immunoblot secondary: 1:20,000	Jackson	Cat: 111-035-045
Peroxidase-AffiniPure Goat Anti-Rat IgG Immunoblot secondary: 1:20,000	Jackson	Cat: 112-035-167
Reagents		
Dimethyloxalylglycine (DMOG)	Cayman Chemical	Cat: #71210
MG132	Sigma	Cat: 474790
VH298	A gift from Alessio Ciulli	
Roxadustat (FG-4592)	Cayman Chemicals	Cat:15294
Daprodustat	Cayman Chemical	Cat:19915

CRISPR sgRNA oligonucleotide sequences:	
CFP1 sgRNA 1	GGTGTGACAACTGCAATGAG
HIF-1α sgRNA 1	CCTCACACGCAAATAGCTGA
HIF1β sgRNA 1	CAGTCCTCCGTCTCCTCACC
HIF-2α sgRNA 1	GCTGATTGCCAGTCGCATGA
PPP4C sgRNA 1	CAGGTCGCTGATCTCCGCCA
PPP4C sgRNA 2	CCTTAAGTGCCAGCAGCAGG
RNF20 sgRNA 1	GCTAGGAGGTGTCTCTTCAA
RNF20 sgRNA 2	GGTGTCTCTTCAACGGTATG
RNF20 sgRNA 3	GTTCTGGTTCAGGCACAACA

RNF40 sgRNA 1	GCGATTGACGATGAGGAGTG
RNF40 sgRNA 2	GGGCTCGATAAGAGTCGTGG
RNF40 sgRNA 3	GGACTCCTCTCCCAGAGCCG
SET1A sgRNA 1	CCGGAGCCTCCAGAACCTGG
SET1B sgRNA 1	GATGTCCAGCAACCGCCC
SET1B sgRNA 2	CATGTGCAAGAAGTATGGGG
SET1B sgRNA 3	GTATGGGGAGGTGGAGGAGG
UBE2A sgRNA 1	GTCTTCCCGAAGGTTGCAGG
UBE2A sgRNA 2	CAACATAATGGTGTGGAACG
UBE2A sgRNA 3	ATGGAAGACACATCATAGGT
WDR82 sgRNA 1	TTTCAGCCCCAACGGCGAGA
Custom screen Primer (TKO)	
TKO Inner PCR forward	AATGATACGGCGACCACCGAGATCTACA CTCTCTTGTGGAAAGGACGAGGTACCG
TKO Custom Sequencing Primer	ACACTCTCTTGTGGAAAGGACGAGGTACCG
NEBuilder PCR primer to clone SET1B into pHRSIN lentiviral vector	
Forward	CAGTCCTCCGACAGACTGAGTCGCCCGGGGGGGATCCGCCACCATGGAGAACAGTCA CCCCCCCCACCAC
Reverse	CTTGCATGCCTGCAGGTCGACTCTAGAGTCGCGGCCGCTCAGTTGAGGGTCCCCCGG CAGTTCTCG
NEBuilder PCR primer to clone HIF-1α truncation mutants into pHRSIN lentiviral vector	
Fw_2	GCTTATCCTTACGACGTGCCTGACTACGCCGGATCCGAGGGCGCCGGCGGCGCG
Fw_78	GCTTATCCTTACGACGTGCCTGACTACGCCGGATCCTTGGATATTGAAGATGACAT GAAAGCACAGATGAATTGCTTTTATTTGAAAGC
Fw_208	GCTTATCCTTACGACGTGCCTGACTACGCCGGATCCCCTCAGTGTGGGTATAAGAA ACCACCTATGACCTG
Fw_396	GCTTATCCTTACGACGTGCCTGACTACGCCGGATCCGCTTTAACTTTGCTGGCCCCA GCCGCTG
Fw_648	GCTTATCCTTACGACGTGCCTGACTACGCCGGATCCCATAAAGAAACTACTAGTGC CACATCATCACCATATAGAGATACTC
Rv_78	CTTGCATGCCTGCAGGTCGACTCTAGAGTCGCGGCCGCTCACAAATCACCAGCATC CAGAAGTTTCCTCACACGC
Rv_208	CTTGCATGCCTGCAGGTCGACTCTAGAGTCGCGGCCGCTCAAGGTTGGTTACTGTT GGTATCATATACGTGAATGTGGCC
Rv_396	CTTGCATGCCTGCAGGTCGACTCTAGAGTCGCGGCCGCTCAAGCATCAGGTTCCTT CTTAAGTTTGTCAAAGAGGCTACTTG
Rv_648	CTTGCATGCCTGCAGGTCGACTCTAGAGTCGCGGCCGCTCAATGTATGTGGGTAG GAGATGGAGATGCAATCAATATTTTAATGTCTTC
Rv_826	CTTGCATGCCTGCAGGTCGACTCTAGAGTCGCGGCCGCTCAGTTAACTTGATCCAA AGCTCTGAGTAATTCTTCACCCTGC

qPCR Primers:	
Actin Forward	CTGGGAGTGGGTGGAGGC
Actin Reverse	TCAACTGGTCTCAAGTCAGTG
ANGPTL4 Forward	TCCGTACCCTTCTCCACTTG
ANGPTL4 Reverse	AGTACTGGCCGTTGAGGTTG
BAP1 Forward	GGAGGTAGAGAAGAGGAAGAA
BAP1 Reverse	GAGCCAGCATGGAGATAAAG
CA9 Forward	GCCGCCTTTCTGGAGGA
CA9 Reverse	TCTTCCAAGCGAGACAGCAA
GLUT1 Forward	CCAGGGTAGCTGCTGGAGC
GLUT1 Reverse	TGGCATGGCGGGTTGT
HIF-1α Forward	CCAGTTACGTTCCTTCGATCAGT
HIF-1α Reverse	TTTGAGGACTTGACTTGCGCTTTCA
LDHA Forward	CCATGATTAAGGGTCTTTACGG
LDHA Reverse	GGTCTGAGATTCCATTCTGTCC
PHD3 Forward	TCCTGCGGATATTTCCAGAGG
PHD3 Reverse	GGTTCCTACGATCTGACCAGAA
SERPINE1 Forward	ACCGCAACGTGGTTTTCTCA
SERPINE1 Reverse	TTGAATCCCATAGCTGCTTGAAT
SET1A Forward	CAGTGGCGGAACTACAAGCTC
SET1A Reverse	CATAGCGGTACACCTTCTGAGA
SET1B Forward	AGGGGCATCATAAACTGTACCG
SET1B Reverse	GGGGATCTTCGACAATTTCCAC
VEGF Forward	TACCTCCACCATGCCAAGTG
VEGF Reverse	ATGATTCTGCCCTCCTCCTTC
ChIP qPCR Primers:	
BAP1 Forward	TCTGAAGGTCGTAGATCTCCTC
BAP1 Reverse	CTGTCCTTCCCTACTGCTT
CA9 Forward	GACAAACCTGTGAGACTTTGGCTCC
CA9 Reverse	AGTGACAGCAGCAGTTGCACAGTG
PHD3 Forward	TGCGGGATCAAGATTCTATTC
PHD3 Reverse	GCCTTTCAGTGGCTTCTT
VEGF Forward	CCTTTGGGTTTTGCCAGA
VEGF Reverse	CCAAGTTTGTGGAGCTGA