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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Flow cytometry: BD FACSDiva 8.3 QPCR: Quantstudio 7 Plate reader: CLARIOstar 5.60 R2 Seahorse: Wave 2.3 Immunofluorescence: Images taken on Axioimager Z2 Upright microscope (Zeiss) using Zen Blue Pro 2012 software. (version 1.1.2.0; Zeiss) Tumour Vasculature: Images taken on Aperio AT2 digital pathology slide scanner
Data analysis	Flow cytometry: FlowJo v10 (BD) CRISPR screen: Reads trimmed with FASTX-toolkit (0.0.14), and aligned using Bowtie (1.2.2). Analysed with MAGeCK (0.5.5) Immunoblot quantification: ImageJ (1.52d) RNA-Seq: Reads trimmed with Trimmimatic v0.36 and aligned to reference genome on ENSEMBL using STAR aligner v.2.5.2b. Unique gene counts were calculated using Subread package v.1.5.2. Differential expression was calculated using DESeq2. Chromatin Immunoprecipitation: Illumina adaptor sequences were trimmed using Trimgalore (0.3.3) and reads were aligned to Genome Reference Consortium GRCh37. Encode black list regions (http://hgwdev.cse.ucsc.edu/cgi-bin/hgFileUi?db=hg19&g=wgEncodeMapability) were excluded using BEDTools (2.17.0). Normalised read counts for each peak were determined using samtools c0.1.19. Duplicate reads were marked for exclusion using Picard tools (1.106). ChIP-seq peaks were identified using T-PIC (Tree shape Peak Identification for ChIP-Seq)[27] and MACS (Model-based analysis of ChIP-Seq). Peaks detected by both peak callers were filtered quantitatively using the total count under the peak to include only peaks that were above the 99.99th percentile of random background regions selected from the ENCODE DNASE II cluster (p-value < 0.0001). H3K4me3 peaks were visualized using IGV. Image quantification of tumour sections: For all analyses at least two independent sections were analysed per tumour and analyses were performed using ImageJ software (version 1.52a) Microsoft Excel for Mac version 16 Graphpad Prism version 8

Venn diagrams and heat maps were generated in R version 3.6.3 and ggplot2 3.3.0.
Qupath (v0.2.0) and Angioquant software (v1.33) : Analysis of vasculature images.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

SgRNA read counts tables from CRISPR/Cas9 genetic screen are shown in Supplementary Dataset 1. RNA-seq data and ChIP-seq have been deposited at Gene (GSE159128, GSE169040, and together under SuperSeries GSE169041) data deposited at Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/).
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE159128
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE169040
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE169041

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	All experiments were performed are representative of a minimum of three biologically independent experiments. For mouse experiments sample size was determined by the Resource Equation which ensures the minimal number of mice were used to achieve statistical significance. The Resource Equation (Mead R. 1988. The Design of Experiments. Cambridge: Cambridge University Press) was used to calculate sample size for the following reasons. First, we could not estimate the standard deviation in advance since the parameters following these genetic manipulations had not been previously measured. Second, in many studies, numerous parameters were being quantified, without a priori assignment that one was of prime importance. Third, other power analyses are very challenging to apply to complex experiments involving comparison of multiple different groups.
Data exclusions	No data were excluded from the study
Replication	Western blotting data were confirmed with more than 3 independent samples (specific details provided in the methods under Statistics and Reproducibility). Flow cytometry, Seahorse bioenergetics, qPCR, cell fractionation and chromatin immunoprecipitation experiments were performed with at least two biologically independent experiments. Animal experiments were performed with enough animals to be statistical according to Resource Equation and according to guidance in methods section. RNA-Seq and ChIP-Seq experiments were performed in duplicate. All replication was sucessful for each experiment where there was no technical problems.
Randomization	Flow cytometry experiments were analysed in random order. Other experiments were allocated into experimental groups in order of presentation, as systematic bias was not felt to affect these experiments. Mice were randomly assigned into groups (using a randomization function =RAND(); excel).
Blinding	Samples for RNA-Seq and ChIP-Seq were submitted as coded samples and results analysed independently of primary research lab. All mouse experiments were randomised and group blinded. The CRISPR screen was analysed blinded and is unbias. Other samples, such as immunoblotting and qPCR were not blinded as it was practical and were prepared in an order that allowed effective presentation). All mouse experiments were blinded to the investigators carrying out tumour volume measurements and tumour image analysis. Investigators were blinded to group allocation/genetic status during tumour volume measurements and data collection by assigning mice an ID number and withholding genetic/group information.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Details of primary and secondary antibodies, and the dilutions used for immunoblot, flow cytometry, immunoprecipitation and chromatin immunoprecipitation are described in Supplementary Table 1

Validation

B-Actin: Sigma #A2228 Immunoblot: 1:20000. Validated for immunoblot by manufacturer; references available on website: https://www.sigmaaldrich.com/catalog/product/sigma/a2228.
H3: Cell Signaling #4499 Immunoblot 1:2000. Validated for immunoblot by manufacturer; references available: https://www.cellsignal.com/products/primary-antibodies/histone-h3-d1h2-xp-rabbit-mab/4499.
H3K4me3: Cell Signaling #9751 Immunoblot 1:1000, Chromatin Immunoprecipitation 1:50 Validated for immunoblot, chromatin immunopreciptiation by manufacturer; references available: https://www.cellsignal.co.uk/products/primary-antibodies/tri-methyl-histone-h3-lys4-c42d8-rabbit-mab/9751.
HA: Roche 11867423001 Immunoblot 1:2000 Validated for immunoblot by manufacturer: https://www.sigmaaldrich.com/catalog/product/roche/roahaha
HIF-1a: BD Biosciences #610959 Immunoblot 1:1000. Validated for dot blot by manufacturer; references available on manufacturer website: https://www.bdbiosciences.com/us/applications/research/stem-cell-research/mesoderm-markers/human/purified-mouse-anti-human-hif-1-54hif-1/p/610959.
CA9: Absolute Antibody Ab00414-1.1 (M75 clone). Immunoblot 1:5000, Flow Cytometry 1:100. Validated for immunoblot and Flow cytometry by manufacturer: https://absoluteantibody.com/product/anti-human-carbonic-anhydrase-ix-m75/
CD31 (PECAM-1): Cell Signaling #77699 D8V9E. Immunohistochemistry 1:100. Validated for Immunohistochemistry by manufacturer: https://www.cellsignal.co.uk/products/primary-antibodies/cd31-pecam-1-d8v9e-xp-rabbit-mab/77699?site-search-type=Products&N=4294956287&Ntt=cd31&fromPage=plp.
CFP1: Cell Signaling #40672. Immunoblot 1:1000, Chromatin immunoprecipitation 1:100, Immunoprecipitation 1:100. Validated for immunoblot and chromatin immunoprecipitation by manufacturer and by knockout by the authors: https://www.cellsignal.co.uk/products/primary-antibodies/cxxc1-d1r5r-rabbit-mab/40672?Ntk=Products&site-search-type=Products&N=4294956287&Ntt=cfp1&fromPage=plp
H3K27ac: Cell Signaling #8173. Chromatin immunoporecipitation 1:100. Validated for chromatin immunoprecipitation by manufacturer: https://www.cellsignal.co.uk/products/primary-antibodies/acetyl-histone-h3-lys27-d5e4-xp-rabbit-mab/8173?Ntk=Products&_=1601992355588&Ntt=8173&tahead=true
HIF1a: Cell Signaling #36169. Immunoprecipitation 1:50, Chromatin immunoprecipitation 1:50. Validated for chromatin immunoprecipitation and immunoprecipitation by manufacturer: https://www.cellsignal.co.uk/products/primary-antibodies/hif-1a-d1s7w-xp-rabbit-mab/36169?Ntk=Products&_=1601992396516&Ntt=36169&tahead=true
HIF1a (PM14): Validated for chromatin immunprecipitation by Mole Lab (PMID:30429208)
HIF2a: Cell Signaling #7096. Immunoblot 1:1000. Validated for immunoblot by manufacturer: https://www.cellsignal.co.uk/products/primary-antibodies/hif-2a-d9e3-rabbitmab/7096?Ntk=Products&_=1601992623753&Ntt=7096&tahead=true
HIF2a (PM9): Validated for chromatin immunprecipitation by Mole Lab (PMID:30429208)
HIF1B: Cell Signaling #5537. Immunoblot 1:1000, Chromatin immunoporecipitation 1:50. Validated for immunoblot and chromatin immunoprecipitation by the manufacturer and by knockout by the authors: https://www.cellsignal.co.uk/products/primary-antibodies/hif-1b-arnt-d28f3-xp-rabbit-mab/5537Ntk=Products&_=1601992658418&Ntt=5537&tahead=true
HIF1B (NB100-110): Validated for chromatin immunprecipitation by Mole Lab (PMID:30429208)
Hydroxy-HIF1a: Cell Signaling #3434. Immunoblot 1:1000. Validated for immunoblot by manufacturer: https://www.cellsignal.co.uk/products/primary-antibodies/hydroxy-hif-1a-pro564-d43b5-xp-rabbit-mab/3434?Ntk=Products&_=1601992839666&Ntt=3434&tahead=true
PARP1: Cell Signaling #9542. Immunoblot 1:1000. Validated for immunoblot by manufacturer: https://www.cellsignal.co.uk/products/primary-antibodies/parp-antibody/9542?Ntk=Products&_=1601993478212&Ntt=9542&tahead=true
SET1A: Cell Signaling #61702. Immunoblot 1:1000. Validated for immunoblot by manufacturer: https://www.cellsignal.co.uk/products/primary-antibodies/set1a-d3v9s-rabbit-mab/61702?Ntk=Products&site-search-type=Products&N=4294956287&Ntt=set1a&fromPage=plp
SET1B: Cell Signaling #44922. Immunoblot 1:1000, Immunoprecipitation 1:100, Chromatin immunopreciptiation 1:100. Validated for immunoblot and immunoprecipitation by manufacturer and chromatin immunoprecipitation by authors using knockouts: https://www.cellsignal.co.uk/products/primary-antibodies/set1b-d1u5d-rabbit-mab/44922?Ntk=Products&site-search-type=Products&N=4294956287&Ntt=set1b&fromPage=plp
SUZ12: Cell Signaling #3737. Immunoblot 1:1000. Validated for immunoblot by manufacturer: https://www.cellsignal.co.uk/products/primary-antibodies/suz12-d39f6-xp-rabbit-mab/3737
Alpha Tubulin: eBioscience 14-4502 DM1A. Immunoblot 1:2000 Validated for immunoblot by manufacturer: https://www.thermofisher.com/antibody/product/alpha-Tubulin-Antibody-clone-DM1A-Monoclonal/14-4502-82
Peroxidase-AffiniPure Goat Anti-Mouse IgG: Jackson Immunoresearch 115-035-146. Immunoblot 1:20000
Peroxidase-AffiniPure Goat Anti-Rabbit IgG: Jackson Immunoresearch 111-035-045. Immunoblot 1:20000

Peroxidase-AffiniPure Goat Anti-Rat IgG: Jackson Immunoresearch 112-035-167. Immunoblot 1:20000

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T, HeLa, MCF-7 cells were a gift from Paul Lehner (Cambridge Institute for Therapeutic Immunology & Infectious Disease). Cell were originally purchased from the ATCC (CCL-185). Human Skin fibroblasts were a gift from Delphine Larrieu and were taken from a young healthy individual (GM03440: 20 years old). (Cambridge Institute for Medical Research). A549 and 786-0 cells were purchased from ATCC (CCL-185).
Authentication	All cells were authenticated and were performed by Eurofins following DNA extraction in our labratory: genetic characteristics of each cell line were determined by PCR-single-locus-technology from genomic DNA. 21 independent PCR-systems Amelogenin, D3S1358, D1S1656, D6S1043, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433 and FGA were investigated (Promega, PowerPlex 21 PCR Kit). The resulting cell line profiles were compared against published reference profiles for full authentication.
Mycoplasma contamination	All cell lines tested negative for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Male and female athymic nude mice Crl: NU(NCr)-Foxn1nu (Charles River Laboratories, Inc), aged 8-9 weeks.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field collected samples were used in this study.
Ethics oversight	Procedures were ethically approved by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB) and complied with the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012, under the authority of a UK Home Office License. The ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines (https://www.nc3rs.org.uk/arriveguidelines) were used for planning, conducting and reporting of experiments.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	https://www.ncbi.nlm.nih.gov/gds/?term=GSE169041
Files in database submission	Processed data files: S01_ControlNormoxiaChIP_1_Treat_nodup_norm.bigwig bigwig S02_ControlHypoxiaChIP_1_Treat_nodup_norm.bigwig bigwig S03_HIF1BNormoxiaChIP_1_Treat_nodup_norm.bigwig bigwig S04_HIF1BHypoxiaChIP_1_Treat_nodup_norm.bigwig bigwig S05_SET1BNormoxiaChIP_1_Treat_nodup_norm.bigwig bigwig S06_SET1BHypoxiaChIP_1_Treat_nodup_norm.bigwig bigwig S07_ControlNormoxiaChIP2_1_Treat_nodup_norm.bigwig bigwig S08_ControlHypoxiaChIP2_1_Treat_nodup_norm.bigwig bigwig S09_HIF1BNormoxiaChIP2_1_Treat_nodup_norm.bigwig bigwig S10_HIF1BHypoxiaChIP2_1_Treat_nodup_norm.bigwig bigwig S11_SET1BNormoxiaChIP2_1_Treat_nodup_norm.bigwig bigwig S12_SET1BHypoxiaChIP2_1_Treat_nodup_norm.bigwig bigwig S01_ControlNormoxiaChIP_1_peaks.bed bed S02_ControlHypoxiaChIP_1_peaks.bed bed S03_HIF1BNormoxiaChIP_1_peaks.bed bed S04_HIF1BHypoxiaChIP_1_peaks.bed bed S05_SET1BNormoxiaChIP_1_peaks.bed bed S06_SET1BHypoxiaChIP_1_peaks.bed bed S07_ControlNormoxiaChIP2_1_peaks.bed bed S08_ControlHypoxiaChIP2_1_peaks.bed bed S09_HIF1BNormoxiaChIP2_1_peaks.bed bed

S10_HIF1BHypoxiaChIP2_1_peaks.bed bed
S11_SET1BNormoxiaChIP2_1_peaks.bed bed
S12_SET1BHypoxiaChIP2_1_peaks.bed bed

Raw data files:
WTCHG_687817_209107_1.fastq.gz fastq
WTCHG_687817_210119_1.fastq.gz fastq
WTCHG_687817_211131_1.fastq.gz fastq
WTCHG_687817_212143_1.fastq.gz fastq
WTCHG_687817_213155_1.fastq.gz fastq
WTCHG_687817_214167_1.fastq.gz fastq
WTCHG_687817_215179_1.fastq.gz fastq
WTCHG_687817_216191_1.fastq.gz fastq
WTCHG_687817_217108_1.fastq.gz fastq
WTCHG_687817_218120_1.fastq.gz fastq
WTCHG_687817_220144_1.fastq.gz fastq
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WTCHG_689380_229157_2.fastq.gz fastq

	WTCHG_689380_242123_2.fastq.gz fastq WTCHG_689380_244147_2.fastq.gz fastq
Genome browser session (e.g. UCSC)	https://www.ncbi.nlm.nih.gov/gds/?term=GSE169041

Methodology

Replicates	Hela wildtype, SET1B mixed KO cells and HIF1beta mixed KO cells. Two replicates.
Sequencing depth	75-bp paired-end sequencing. HiSeq 4000 platform (Illumina).
Antibodies	Rabbit monoclonal anti-H3K4me3 (Cell Signalling) 9751
Peak calling parameters	ChIP-seq peaks were identified using both the T-PIC (Tree shape Peak Identification for ChIP-Seq) and MACS (Model-based analysis of ChIP-Seq) and only peaks identified with both peak callers were considered.
Data quality	Differentially bound peaks (fdr < 10-5) were identified using DiffBind v2.16.0 (https://bioconductor.org/packages/release/bioc/html/DiffBind.html). Peaks detected by both peak callers were filtered quantitatively using the total count under the peak to include only peaks that were above the 99.99th percentile of random background regions selected from the ENCODE DNASE II cluster (p-value < 0.0001).
Software	Illumina adaptor sequences were trimmed using Trimgalore (0.3.3). BEDTools (2.17.0) to exclude encode black list regions (http://hgwdev.cse.ucsc.edu/cgi-bin/hgFileUi?db=hg19&g=wgEncodeMapability). Picard tools (1.106) to mark duplicate reads for exclusion . T-PIC (Tree shape Peak Identification for ChIP-Seq) and MACS (Model-based analysis of ChIP-Seq) to identify peaks. IGV Genome Browser v 2.7.2 (http://software.broadinstitute.org/software/igv/).

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For flow cytometric sorting for the CRISPR screen, HeLa cells were suspended using Trypsin-EDTA, centrifuged at 800g, washed in cold PBS, centrifuged again and resuspended in PBS containing 2% fetal calf serum and 10 mM HEPES.
Instrument	Cells were sorted using a BD Influx cell sorter
Software	BD FACS Sortware 1.2.0.142. Analysed using FlowJo v10 (BD)
Cell population abundance	As displayed in figure 1a (x axis is GFP, y axis is SSC), at the first sort approximately 1% of cells above one log10 unit of GFP fluorescence above the mode of the main population were selected. At the first sort this represented 30% of events.
Gating strategy	Live cells were selected by drawing a gate around the modal population as seen with FSC against SSC. Single cells were selected by drawing a gate around the modal population as seen with trigger pulse width against FSC

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.