SUPPLEMENTAL INFORMATION.

EXTENDED EXPERIMENTAL PROCEDURES.

Western blotting.

Protein sample preparation, quantitation, and western blot analyses were carried out as described previously (Henry et al., 2012). Detection was by enhanced chemiluminescence and images were captured using an ImageQuant LAS4000 digital camera system (GE Healthcare). Antibodies used are described in Table S3–Oligonucleotides and Antibodies used in this study.

qRT-PCR analysis of gene expression in human cells.

Reverse transcription of total RNA was carried out using a qScript cDNA Synthesis Kit (Quanta Biosciences). Quantitative PCR was run with reference to a standard curve using SYBR Select Master Mix for CFX (Life Technologies) on a Viia7 Real Time PCR System (Life Technologies) and normalized to 18S rRNA signals. Primer sequences are listed in Table S3 –Oligonucleotides and Antibodies used in this study.

Statistical analysis of qRT-PCR data.

Statistical analyzes were performed using InfoStat (version 2015) or R (version 3.2.3). One-way ANOVA with Duncan or LSD Fisher post-hoc tests and Students/Welches T tests were conducted individually on normoxia and hypoxia samples.

Chromatin immunoprecipitation assays.

Quantitative ChIP analysis of the *ANKRD37* locus was performed as described previously (Galbraith et al., 2013). Real-time PCR was carried out on ChIP-enriched DNA against a standard curve of input DNA, with amplicons tiling across each locus using SYBR Select Master Mix for CFX (Life Technologies) on a Viia7 Real Time PCR System (Life Technologies). Enrichment values for each amplicon were calculated as percentage of the amplicon with maximum signal for each antibody.

Primers and Antibodies used are listed in Table S3 –Oligonucleotides and Antibodies used in this study.

HaloTag pull down assay and mass spectrometry analysis.

HEK293T (12 x 10^6 cells) were plated in a 150 mm dish and grown to 70-80% confluence (approximately 18 hr). The cells were then transfected with either 30 µg of HaloTag(HT)-HIF1A or HT alone control vector using FuGENE HD Transfection Reagent (Promega) and left to recover for 12 hr. Cells were then treated for 24 hr with 300 µM desferrioxamine (DFX) to mimic hypoxic conditions. Both HT-HIF1A and HT alone control cells were then washed in PBS, scraped from dishes, and incubated in mammalian lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate) supplemented with Protease Inhibitor cocktail (Promega) and RQ1 RNase-Free DNase (Promega) for 10 min on ice. Lysates were dounce-homogenized and centrifuged at 14,000 x g for 5 min. Clarified lysates were incubated with HaloLink Resin (Promega) pre-equilibrated in resin wash buffer (TBS and 0.05% IGEPAL CA-640 (Sigma)) for 15 min at 22^{o}C with rotation. Resin was then washed 5 times with wash buffer to reduce non-specific binding, and protein interactors were eluted with SDS elution buffer (50 mM Tris-HCl, pH 7.5, 1% SDS). Eluted complexes from two biological replicates for each sample were separated by SDS-PAGE and then stained with Coomassie and cut into fragments. Each gel piece was processed with the Progest Protein Digestion Station (Digilab). Proteins were digested with trypsin (Promega) for 4 hr and digestion was quenched with formic acid. Digested peptides were loaded on a trapping column and eluted over a 75 µm analytical column packed with Jupiter Proteo Resin (Phenomenex) at 350 nL/min. The samples were then analyzed by nano LC/MS/MS with a NanoAcquity HPLC (Waters) interfaced with an Orbitrap Velos Pro (Thermo Scientific) tandem mass spectrometer (MS Bioworks, LLC, Ann Arbor, Michigan). The mass spectrometer was operated in data-dependent mode, with MS performed in the Orbitrap at 60.000 full width at half maximum (FWHM) resolution, and MS/MS performed in the LTQ. The 15 most abundant ions were selected for MS/MS. Data were filtered using a minimum protein value of 90%, a minimum peptide value of 50% (Protein and Peptide Prophet scores), and required at least

two unique peptides per protein. Spectral counting was performed and normalized spectral abundance factors (NSAF) determined. Data were reported at less than 1% false discovery rate at the protein level based on counting the number of forward and decoy matches. Note that, although equal cell numbers were used for the control and experimental samples, NSAF values are calculated from the entire sample and HT-only controls have much lower total protein contained within them, therefore relative NSAF values can be high even with low absolute abundance. Spectral count (SpC) values have therefore been included in Figure S2A to show differences between HT control and HT-HIF1A pull downs.

NanoBRET assay.

HCT116 and HEK293 cells (8 x 10^5) were plated in each well of a 6-well plate and co-transfected with one of three acceptors: HT-Pontin, HT-Reptin, or HT-TIP60, in combination with the HIF1A-NanoLuc(NL) donor. At 20 hr post-transfection, cells were collected, washed with PBS, and exchanged into media containing phenol red-free OptiMEM in the absence (control sample) or the presence (experimental sample) of 100 nM NanoBRET 618 fluorescent ligand (Promega). Cell density was adjusted to 2 x 10^5 cells/ml and then re-plated in a 96-well assay white plate (Corning Costar #3917) and let recover for 4 hr at 37^{o}C in the presence of 5% CO_2. NanoBRET furimazine substrate (Promega) was added to both control and experimental samples at a final concentration of 10 μM. Readings were performed within 5 min using a CLARIOstar (BMG) equipped with 450/80 nm bandpass and 610 nm longpass filters with a 0.5 sec reading. A corrected BRET ratio was calculated and is defined as the ratio of the emission at 610 nm/450 nm for experimental samples (i.e. those treated with NanoBRET fluorescent ligand) subtracted by the emission at 610 nm/450 nm for control samples (not treated with NanoBRET fluorescent ligand). BRET ratios are expressed as milliBRET units (mBU), where 1 mBU corresponds to the corrected BRET ratio multiplied by 1000.

HaloTag-NanoLuc pull down assay.

HCT116 and HEK293T cells (12 x 10^6 cells) were plated in a 150 mm dish and grown to 70-80% confluence (approximately 18 hr). The cells were then transfected with HIF1A-NL and either HT-TIP60, HT-Pontin, HT-Reptin, or HT control fusion vectors, using FuGENE HD Transfection Reagent (Promega). 24 hr post-transfection, cells were harvested and pull downs were then performed as described for HaloTag pull downs to the point of capture and washing. Elution of interacting partners was achieved by cleaving a linker region between the HaloTag and TIP60, Pontin or Reptin with TEV protease. The resulting supernatant was assayed for NanoLuciferase activity using NanoGlo detection (Promega).

RNA-seq library preparation and sequencing.

HCT116 cells were plated and treated as described under experimental procedures, followed by harvesting in cold PBS. Total RNA was extracted from cell pellets using TRI Reagent (Sigma), according to the manufacturer's instructions. Total RNA quality was assessed using Bioanalyzer RNA Pico chips (Agilent). PolyA RNA was purified from 15 µg total RNA using the Dynabeads mRNA Direct micro kit (Life Technologies) according to the manufacturer's instructions, and ribosomal RNA contamination was assessed using Bioanalyzer RNA Pico chips. Libraries for Ion Torrent sequencing were prepared using the Ion Total RNAseq v2 kit (Life Technologies), according to the manufacturer's instructions. Yield and insert-size distribution were measured using Bioanalyzer High Sensitivity DNA chips (Agilent). The final Ion Torrent RNAseq libraries were subjected to an additional size-selection for 120-200 bp on a Blue Pippin (Sage Science) using a 2% dye-free gel with marker V1. Accounting for ~90 bp of Ion adapter sequences, this ensured a library insert size range of ~30-110 bp. Ion Torrent template preparation was carried out using the Ion PI Template OT2 200 Kit v2 and library sequencing was carried out on an Ion Torrent Proton sequencer using the Ion PI Sequencing 200 Kit v2 (Life Technologies), according to the manufacturer's instructions.

RNA-seq data analysis.

Signal processing, base-calling and removal of barcode and adapter sequences was carried out automatically by the Torrent Suite Software (Life Technologies). Data quality was assessed using custom scripts and the Fastx toolkit (version 0.0.13.2, http://hannonlab.cshl.edu/fastx_toolkit/index.html). To simplify analysis, reads shorter than 30nt were removed and long reads were trimmed to 150nt using the Fastx toolkit. Alignment to the Human genome (hg19/GRCh37) was carried out using GSNAP (version 2013-10-28, (Wu and Nacu, 2010)) with a mismatch setting of 3%. Gene-level counts were obtained using htseq-count (version 0.5.4p5, (Anders et al., 2015)) with a custom modified GTF annotation file based on an hg19 RefSeq gene list obtained from UCSC table browser (Karolchik et al., 2004). Differential gene expression was assessed using DESeq (version 1.14.0) in R (version 3.0.3) with cutoffs as described in text and figure legends. The heat maps, bubble plots and volcano plots were made using the Python plotting library "matplotlib" (http://matplotlib.org/).

Soft agar colony formation assay.

HCT116 cells were counted and resuspended in 1 mL 0.5x McCoy's 5A with 0.4% w/v Noble agar (BD Difco), and plated in 6-well plates at 1 x 10^4 cells per well on top of a solidified layer of 2 mL 0.5x McCoy's 5A with 0.8% w/v Noble agar. After solidification for 10 min at room temperature, 2 mL of McCoy's 5A, supplemented with 10% fetal bovine serum and antibiotic-antimycotic mixture, was added to each well. Normoxia plates were incubated 14 days under 5% CO_2 at 37°C, and hypoxia plates incubated in hypoxia chambers (flushed twice with 120 L of a mixture of 1% O_2/ 5% CO_2/ 94% N_2) for 14 days at 37° C. At completion of the assay, media was removed and colonies were stained with 0.05% w/v Crystal Violet in 2% v/v methanol followed by destaining in 2% v/v methanol. Images of each plate were captured using an ImageQuant LAS4000 digital camera system (GE Healthcare) and colonies counted using ImageJ software (Schneider et al., 2012).

SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Related to Figure 1.

(A) Knockdown efficiency, as assessed by qRT-PCR, of *Tip60*, *sima*, *pontin*, *reptin*, *domino*, and *Ino80* mRNAs in *Drosophila* S2 cells transfected with the indicated dsRNAs. Expression values were normalized to Rpl29 RNA and are expressed relative to control. Data are represented as mean ± SEM from at least three independent replicates. Asterisks indicate p-values ≤0.05 by T-test. (B) TIP60 mRNA levels assessed by qRT-PCR in normoxia or hypoxia (1% O_2 24 hr) for HCT116 cells stably expressing one of three different TIP60 shRNAs (shTIP60 #1, #2, or #3) or a non-targeting control shRNA (NT). Expression values are normalized to 18S rRNA and are expressed relative to normoxic control (NT). Data are represented as mean ± SEM. Asterisks indicate p-values ≤0.05 by one-way ANOVA. (C) Western blots showing levels of HIF1A, TIP60 and tubulin for control (NT) and shTIP60 HCT116 cells in normoxia and after 24 hr hypoxia. Blots are representative of at least three independent biological replicates. (D) Relative expression of a panel of known HIF1A target genes as measured by qRT-PCR in $HIF1A^{+/+}$ (gray) or $HIF1A^{-/-}$ (teal) HCT116 cells in hypoxia. Expression values were normalized to 18S ribosomal RNA (rRNA) and are expressed relative to the $HIF1A^{+/+}$ normoxia value. Data are represented as mean ± SD from three independent biological replicates. (E) Western blots showing levels of HIF1A and tubulin for control ($HIF1A^{+/+}$) and $HIF1A^{-/-}$ HCT116 cells in normoxia and after 24 hr hypoxia. Blots are representative of at least three independent biological replicates. (F) Relative mRNA levels for HIF1A target genes as assessed by qRT-PCR for HCT116 cells stably expressing shRNAs targeting TIP60 and subjected to normoxia or hypoxia (1% O_2 24 hr). Expression values were normalized to 18S ribosomal RNA (rRNA) and are expressed relative to the control normoxia value. Data are represented as mean ± SEM from at least three independent replicates. None of the comparisons across normoxia or hypoxia values gave p-values ≤0.05 by one-way ANOVA. (G) Relative expression of TIP60, ANKRD37, DUSP1, ALDOC, HK2, and JMJD1A mRNAs as measured by qRT-PCR for SW480 cells stably expressing a TIP60 shRNA or a non-targeting control shRNA (NT) in normoxia and hypoxia. Expression values are normalized to 18S

rRNA and are expressed relative to normoxic control (NT). Data are represented as mean ± SEM from three independent replicates. Asterisks indicate p-values ≤0.05 by T-test.

Figure S2. Related to Figure 3.

Identification of HaloTag (HT)-HIF1A interactors by pull down and LC-MS/MS analysis. (A) Spectral counts with corresponding normalized spectral abundance factors (NSAF) for proteins isolated in complex with HT-HIF1A from DFX-treated HEK293T cells from two biological replicates. Known HIF1A interactors are shown in blue and TIP60 complex subunits are shown in red. (B) NanoBRET assays measuring proximity of HIF1A to TIP60, Pontin, and Reptin. HEK293 cells were co-transfected with HIF1A-NL (donor) and either HT-TIP60, HT-Pontin, or HT-Reptin (acceptors). Graph shows corrected milliBRET units for each acceptor fusion protein when combined with the HIF1A-NL donor. Higher values indicate closer proximity. (C) Donor saturation assay showing specificity for the detection of the interaction of HIF1A with TIP60. HEK293 cells were transfected with a constant amount of HIF1A-NanoLuc(NL) donor DNA and increasing amounts of HT-TIP60 acceptor DNA to generate increasing Acceptor to Donor (A/D) ratios (blue). The negative control is un-fused HaloTag protein used as a mock acceptor in place of the HT-TIP60 fusion (gray). (D) HaloTag pull down from HEK293 cells co-expressing HT control or HT-TIP60 with HIF1A-NL. Pull down of HIF1A-NL by physical association with HT-TIP60, or HT control, was measured by detection of luciferase activity.

Figure S3. Related to Figure 4.

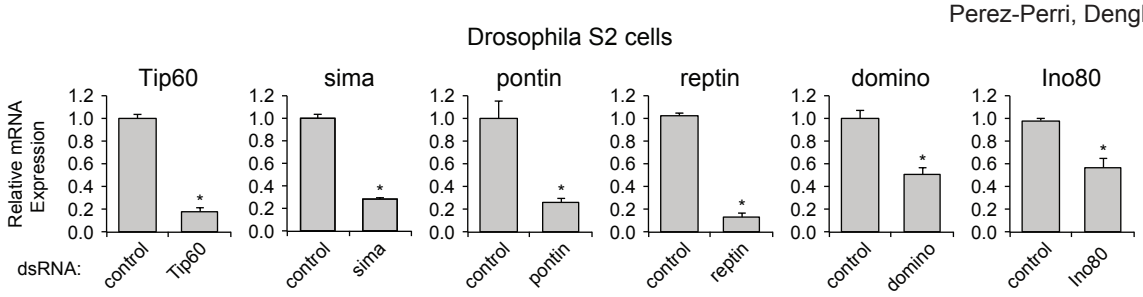
(A) Relative mRNA levels for genes induced by hypoxia, as assessed by qRT-PCR, for shNT control, shTIP60, or HIF1A$^{-/-}$ HCT116 cells subjected to normoxia or hypoxia (1% O_2 24 hr). Expression values were normalized to 18S ribosomal RNA (rRNA) and are expressed relative to the control normoxia value. Data are represented as mean ± SEM from at least three independent replicates. Asterisks indicate p-values <0.05 for one-way ANOVA. (B, C) Quantitative ChIP analysis of total RNAPII, serine-5-phosphorylated RNAPII CTD (S5P), TIP60, and pan histone H4 acetylation (H4ac) at the the indicated loci in shNT control versus shTIP60 cells (panel B) and shNT control versus HIF1A$^{-/-}$ cells

(panel C) subjected to normoxia or hypoxia (1% O_2 24 hr). To represent profiles across the locus, values are plotted as percentage of maximum signal for each epitope. Data are represented as mean ± SEM from three independent replicates.

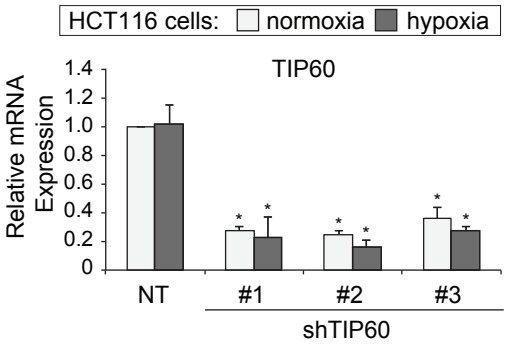
Figure S4. Related to Figure 4.

(A) Venn diagram comparing numbers of genes expressed under normoxic conditions in HCT116 cells to those affected by TIP60 or CDK8 depletion. Dysregulation was defined as FDR adjusted p-value <10% in cells expressing shTIP60 or shCDK8 relative to cells expressing a non-targeting shRNA. (B) The top 5 enriched upstream regulators for the various gene sets indicated were identified using Ingenuity Pathway Analysis (IPA). (C) Predicted upstream regulators HIF1A, HIF2A, SP3, STAT6, EGR1, and CREB, and their putative target genes, for each of the four classes of HIF1A target genes. (D) Schematic representation of the transcriptional response to hypoxia, highlighting diverse enriched canonical pathways controlled by HIF1A and its coactivators, as predicted by IPA.

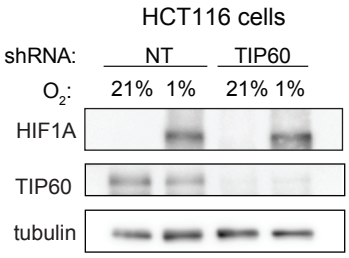
A


Drosophila S2 cells
Tip60
sima
pontin
reptin
domino
Ino80
Relative mRNA Expression
dsRNA:
control
Tip60
sima
pontin
reptin
domino
Ino80

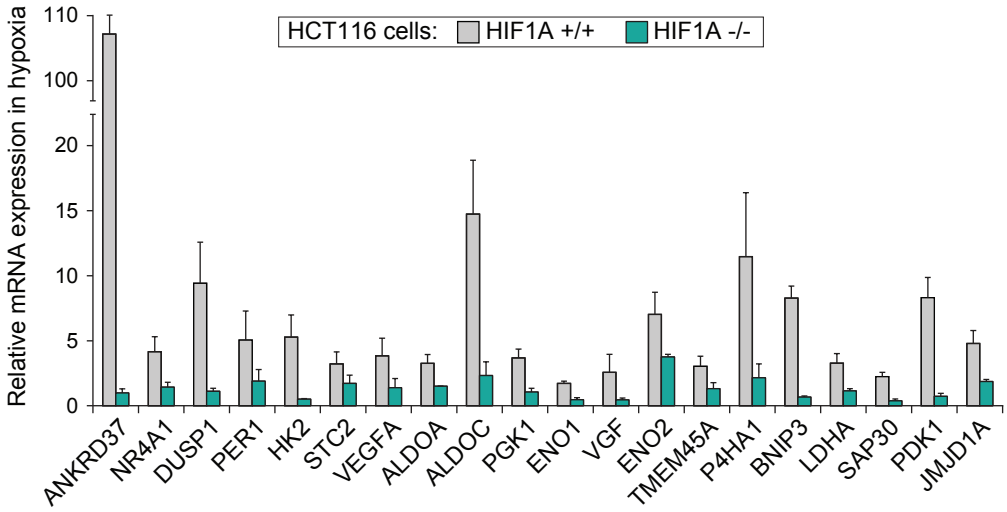
B


HCT116 cells: normoxia hypoxia
TIP60
Relative mRNA Expression
NT
#1
#2
#3
shTIP60

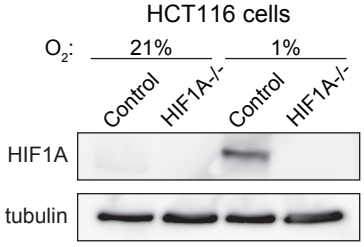
C


HCT116 cells
shRNA:
NT
TIP60
O2:
21% 1% 21% 1%
HIF1A
TIP60
tubulin

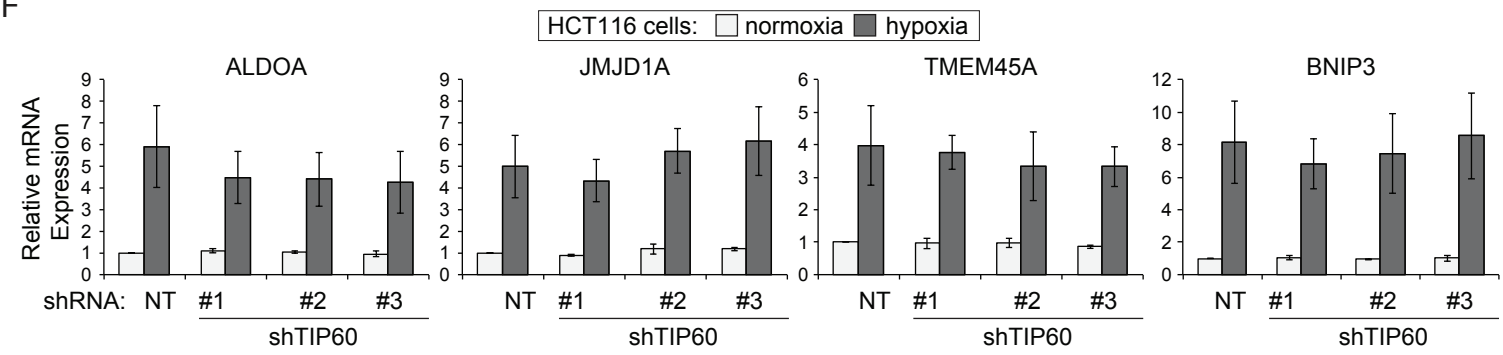
D


HCT116 cells: HIF1A +/+ HIF1A -/-
Relative mRNA expression in hypoxia
ANKRD37
NR4A1
DUSP1
PER1
HK2
STC2
VEGFA
ALDOA
ALDOC
PGK1
ENO1
VGF
ENO2
TMEM45A
P4HA1
BNIP3
LDHA
SAP30
PDK1
JMJD1A

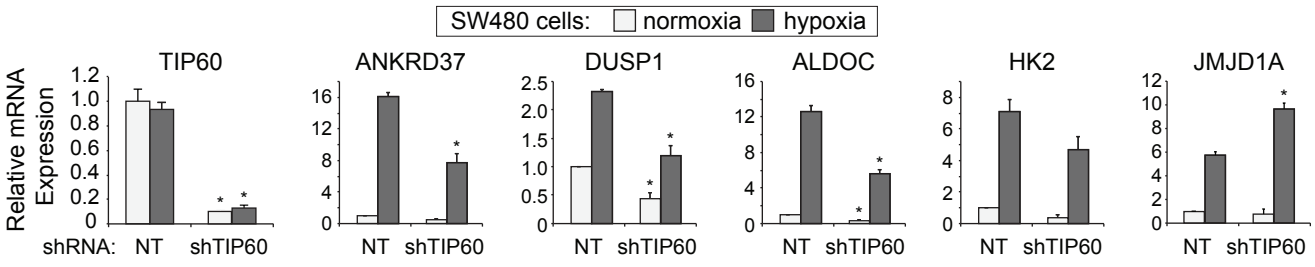
E


HCT116 cells
O2:
21%
1%
Control
HIF1A-/-
Control
HIF1A-/-
HIF1A
tubulin

F


HCT116 cells: normoxia hypoxia
ALDOA
JMJD1A
TMEM45A
BNIP3
Relative mRNA Expression
shRNA:
NT
#1
#2
#3
shTIP60

G


SW480 cells: normoxia hypoxia
TIP60
ANKRD37
DUSP1
ALDOC
HK2
JMJD1A
Relative mRNA Expression
shRNA:
NT
shTIP60

A

HEK293 cells: Mass Spectrometry

HT-HIF1A or HT Ctrl → HaloTag Pull Down → LC-MS/MS → Identify HIF1A-interacting factors

abundance in HIF1A-interacting complexes (+ / -)

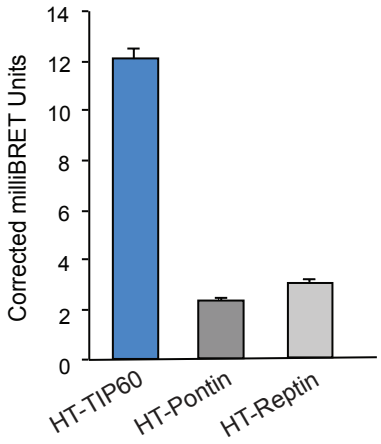
	Spectral Counts				NSAF			
	Ctrl		HIF1A		Ctrl		HIF1A	
Replicate	1	2	1	2	1	2	1	2
Reptin	4	3	26	25	1.8 E-03	1.4 E-03	2.9 E-03	2.9 E-03
Pontin	8	7	24	22	3.7 E-03	3.3 E-03	2.7 E-03	2.6 E-03
HIF1B	0	0	35	40	0.0 E+00	0.0 E+00	2.3 E-03	2.7 E+03
VHL	0	0	10	8	0.0 E+00	0.0 E+00	1.4 E-03	2.0 E+03
BAF53A	0	0	2	3	0.0 E+00	0.0 E+00	2.4 E-04	2.1 E-04
TRRAP	0	0	5	6	0.0 E+00	0.0 E+00	6.5 E-05	8.0 E-05
p300	0	0	7	0	0.0 E+00	0.0 E+00	8.6 E-05	0.0 E+00
p400	0	0	2	0	0.0 E+00	0.0 E+00	1.9 E-05	0.0 E+00

TIP60 subunits

Known HIF1A-binding factors

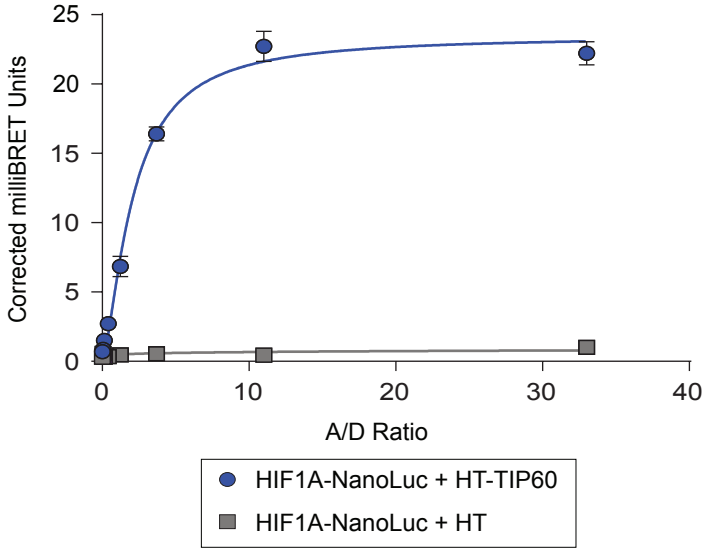
B

HEK293 cells: NanoBRET



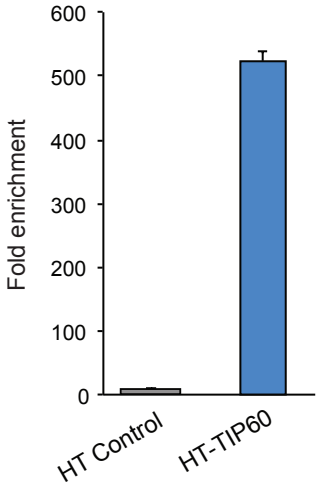
C

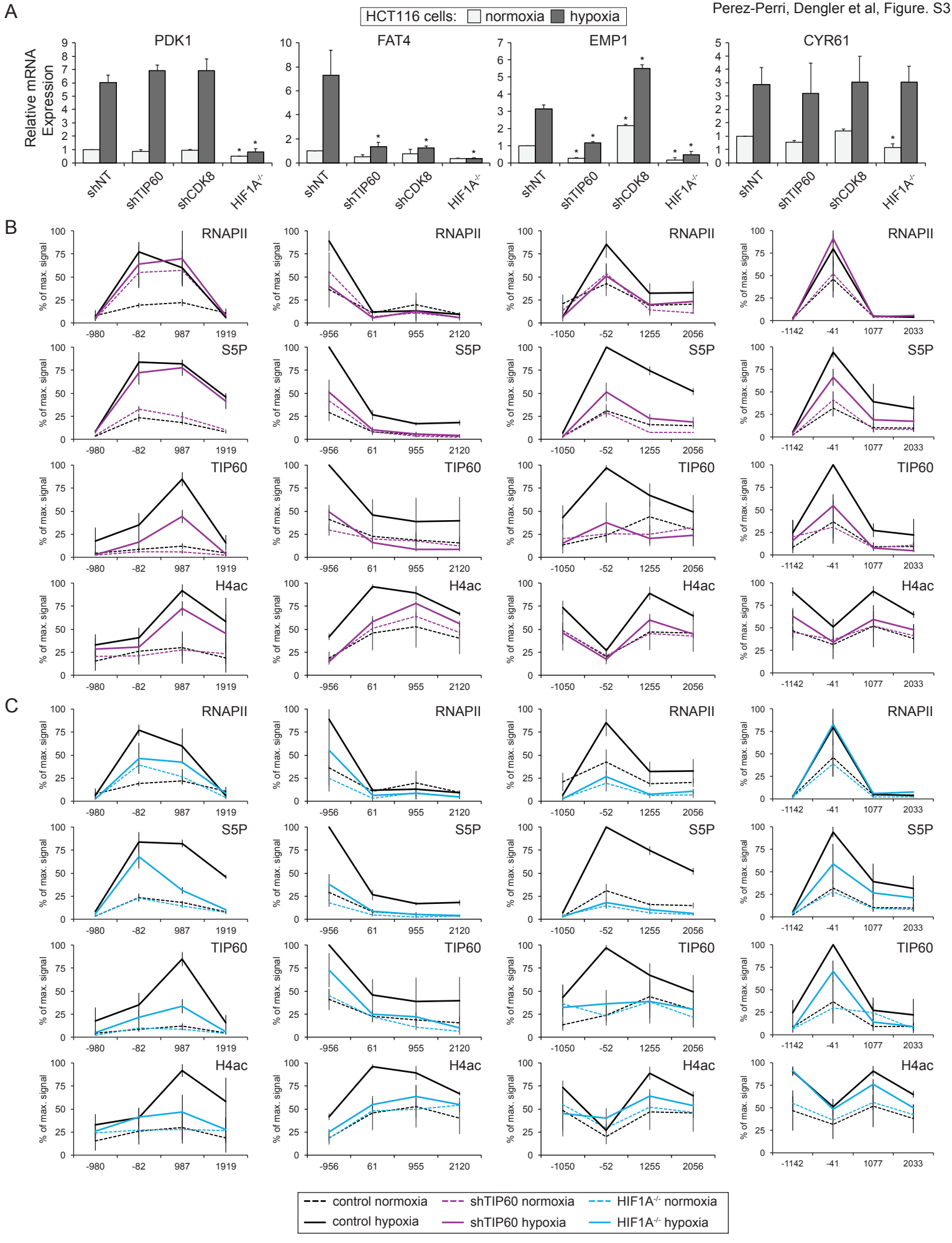
HEK293 cells: nanoBRET donor saturation assay



D

HEK293 cells: HaloTag Pull Down

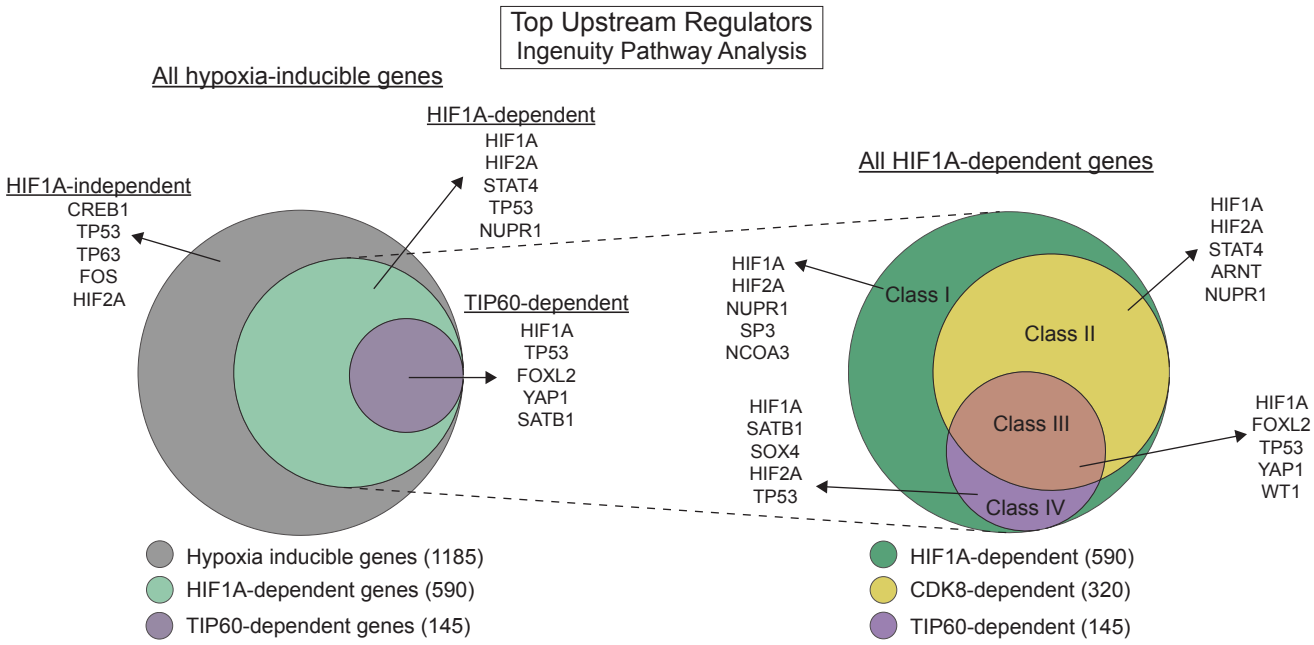



A
HCT116 cells: normoxia hypoxia
Relative mRNA Expression
PDK1
FAT4
EMP1
CYR61
shNT
shTIP60
shCDK8
HIF1A-/-
B
C
RNAPII
S5P
TIP60
H4ac
% of max. signal
-980
-82
987
1919
-956
61
955
2120
-1050
-52
1255
2056
-1142
-41
1077
2033
control normoxia
shTIP60 normoxia
HIF1A-/- normoxia
control hypoxia
shTIP60 hypoxia
HIF1A-/- hypoxia

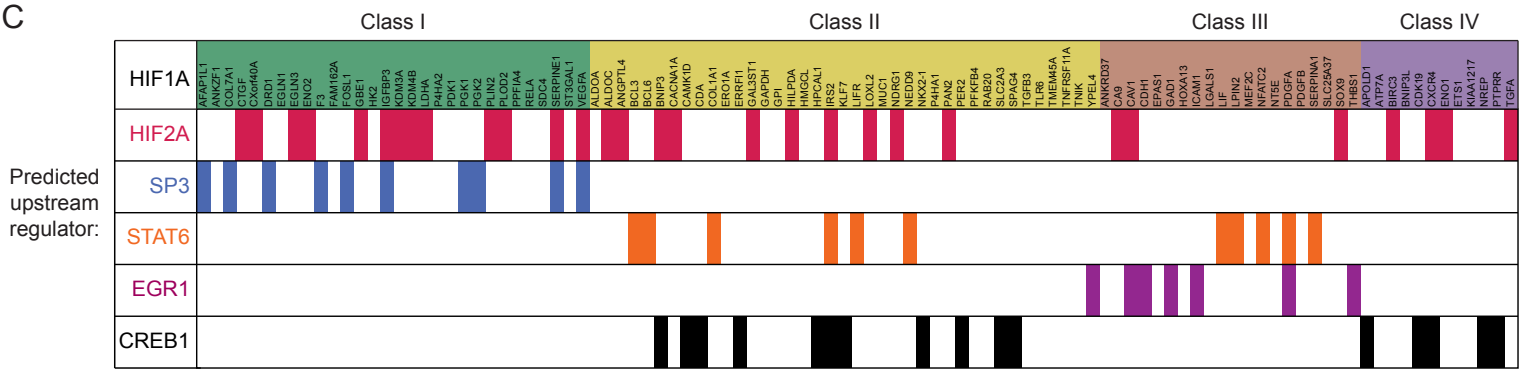
A


Expression in normoxia
Expressed in normoxia (14,070)
Dysregulated in shCDK8 (1793)
Dysregulated in shTIP60 (4691)

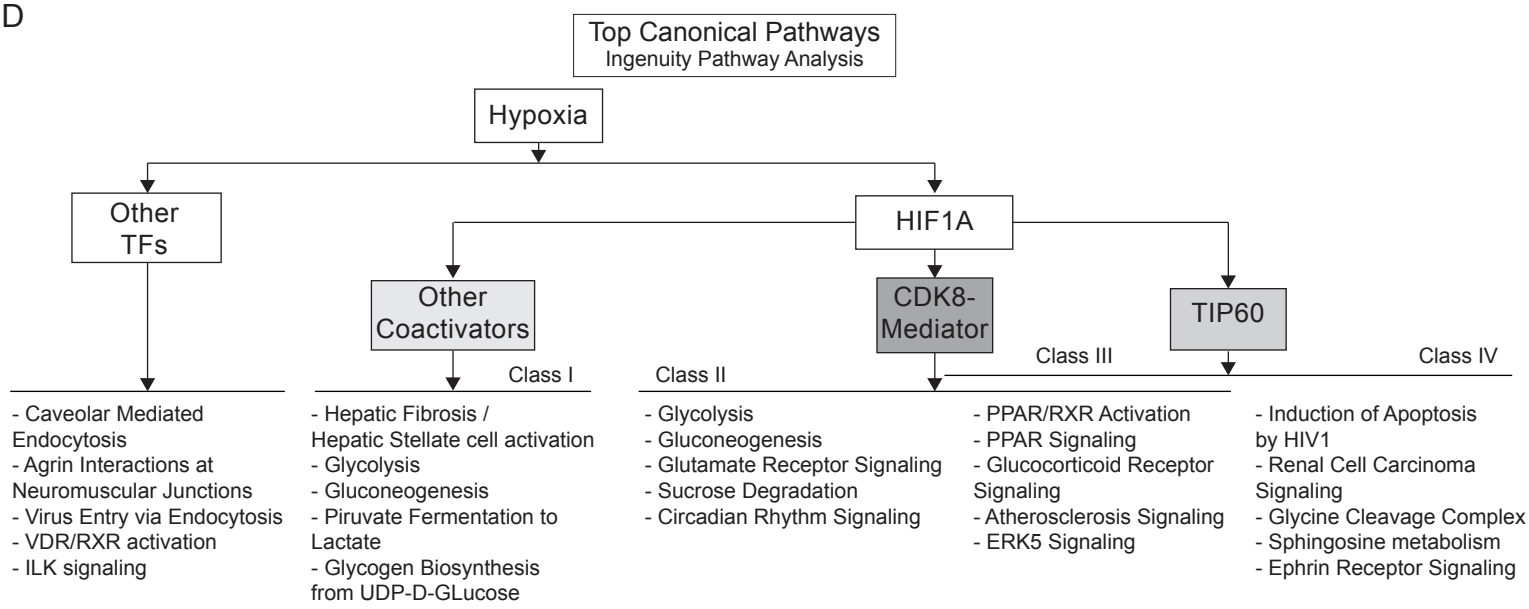
B


Top Upstream Regulators
Ingenuity Pathway Analysis
All hypoxia-inducible genes
HIF1A-dependent
HIF1A
HIF2A
STAT4
TP53
NUPR1
HIF1A-independent
CREB1
TP53
TP63
FOS
HIF2A
TIP60-dependent
HIF1A
TP53
FOXL2
YAP1
SATB1
All HIF1A-dependent genes
HIF1A
HIF2A
NUPR1
SP3
NCOA3
Class I
Class II
Class III
Class IV
HIF1A
HIF2A
STAT4
ARNT
NUPR1
HIF1A
SATB1
SOX4
HIF2A
TP53
HIF1A
FOXL2
TP53
YAP1
WT1
Hypoxia inducible genes (1185)
HIF1A-dependent genes (590)
TIP60-dependent genes (145)
HIF1A-dependent (590)
CDK8-dependent (320)
TIP60-dependent (145)

C


Class I
Class II
Class III
Class IV
HIF1A
HIF2A
SP3
STAT6
EGR1
CREB1
Predicted upstream regulator:

D


Top Canonical Pathways
Ingenuity Pathway Analysis
Hypoxia
Other TFs
HIF1A
Other Coactivators
CDK8-Mediator
TIP60
Class I
Class II
Class III
Class IV
- Caveolar Mediated Endocytosis
- Agrin Interactions at Neuromuscular Junctions
- Virus Entry via Endocytosis
- VDR/RXR activation
- ILK signaling
- Hepatic Fibrosis / Hepatic Stellate cell activation
- Glycolysis
- Gluconeogenesis
- Piruvate Fermentation to Lactate
- Glycogen Biosynthesis from UDP-D-GLucose
- Glycolysis
- Gluconeogenesis
- Glutamate Receptor Signaling
- Sucrose Degradation
- Circadian Rhythm Signaling
- PPAR/RXR Activation
- PPAR Signaling
- Glucocorticoid Receptor Signaling
- Atherosclerosis Signaling
- ERK5 Signaling
- Induction of Apoptosis by HIV1
- Renal Cell Carcinoma Signaling
- Glycine Cleavage Complex
- Sphingosine metabolism
- Ephrin Receptor Signaling