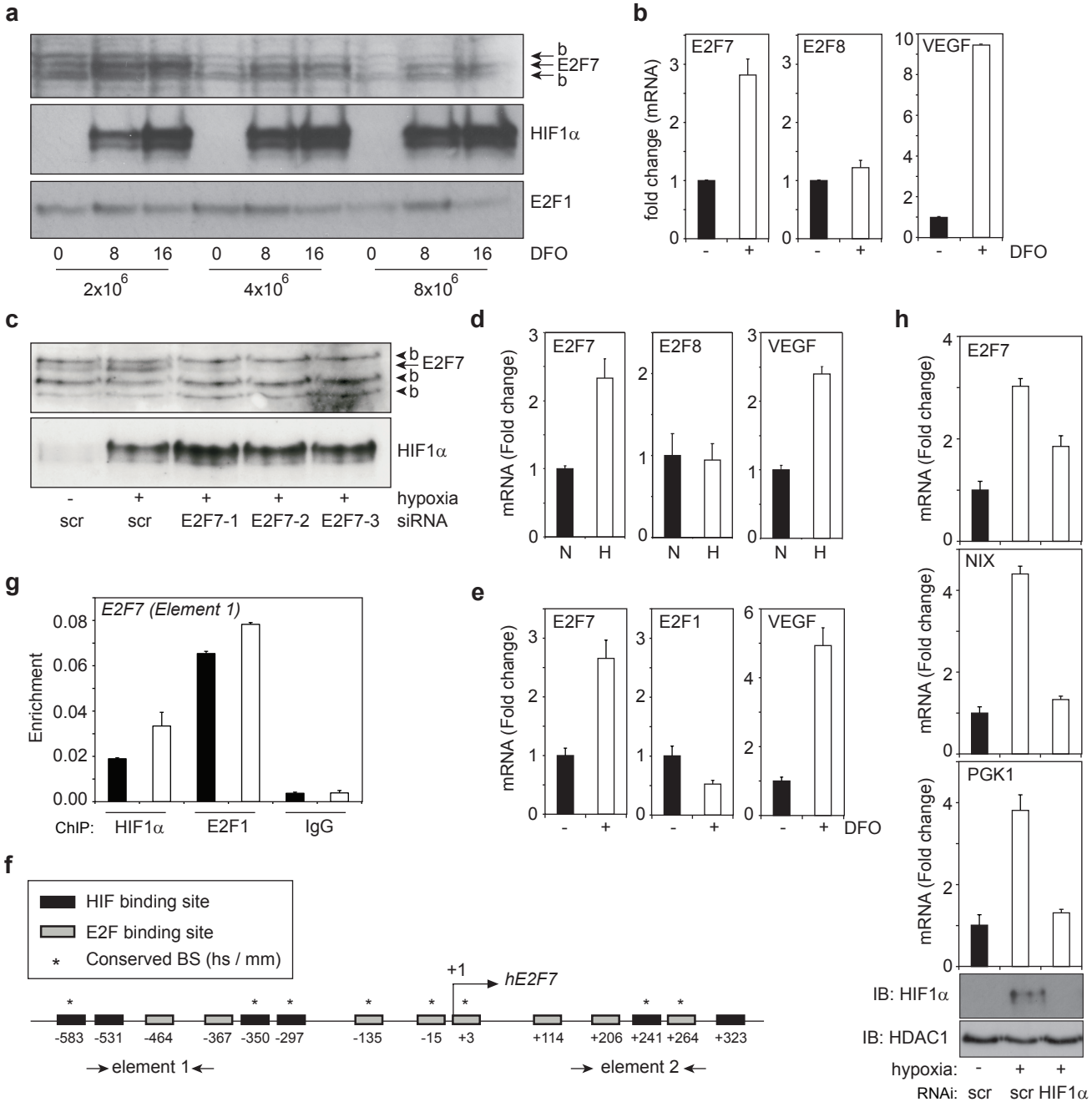
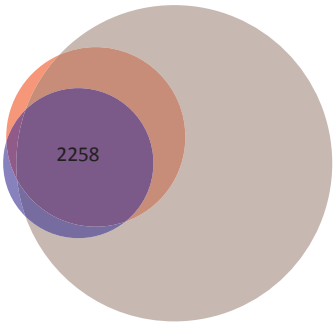
Supplemental Figure S1



Supplementary figure S2

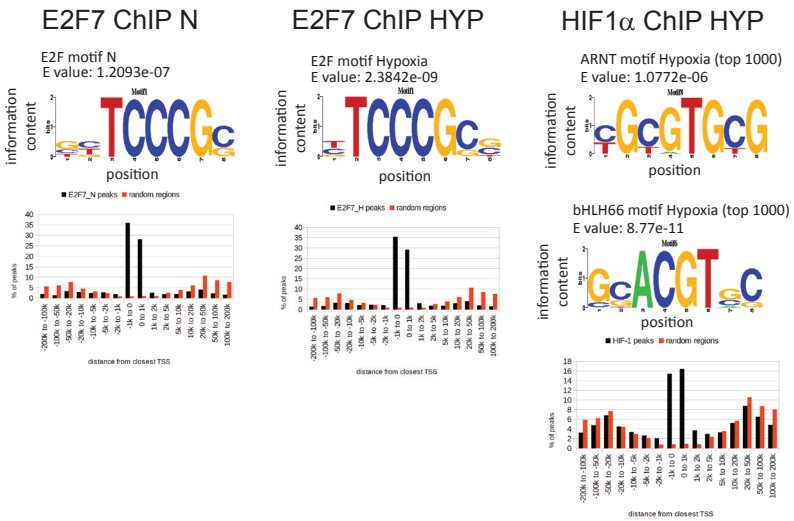
a

Commom HIF1-E2F7 targets (ChIP-sequencing analysis)



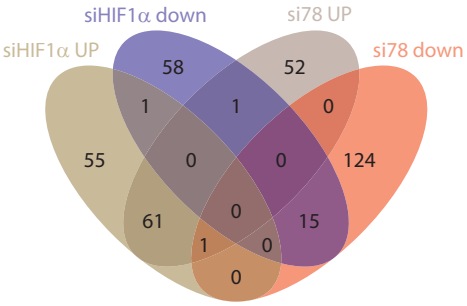
Overlap genes	H HIF1 (11666)
N E2F7 (3761)	3571
H E2F7 (2682)	2535
N&H E2F7 (2381)	2258

b



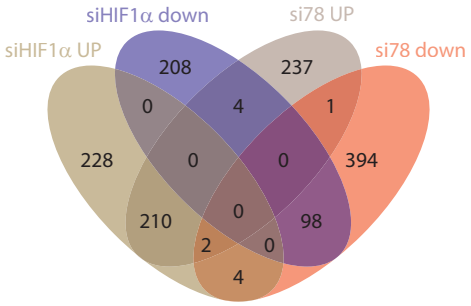
c

Commom HIF1-E2F7 Regulated targets (78) (microarray analysis: ≥ 2 fc, p<0.05)



d

Commom HIF1-E2F7 Regulated targets (318) (microarray analysis: ≥1.5 fc, p<0.05)



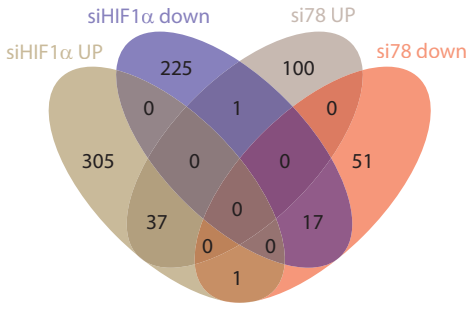
e

	Microarray selection criteria: *≥1.5 fc, p<0.05*								
(all conditions in hypoxia)	si7/8 vs scr	UP	DOWN	si7 vs scr	UP	DOWN	si1α vs scr	UP	DOWN
	951	455	496	980	438	542	754	444	310
7 ChIP-seq	207 (22%)	138	69	165 (17%)	84	80			
1α ChIP-seq							586 (78%)	343	243

56

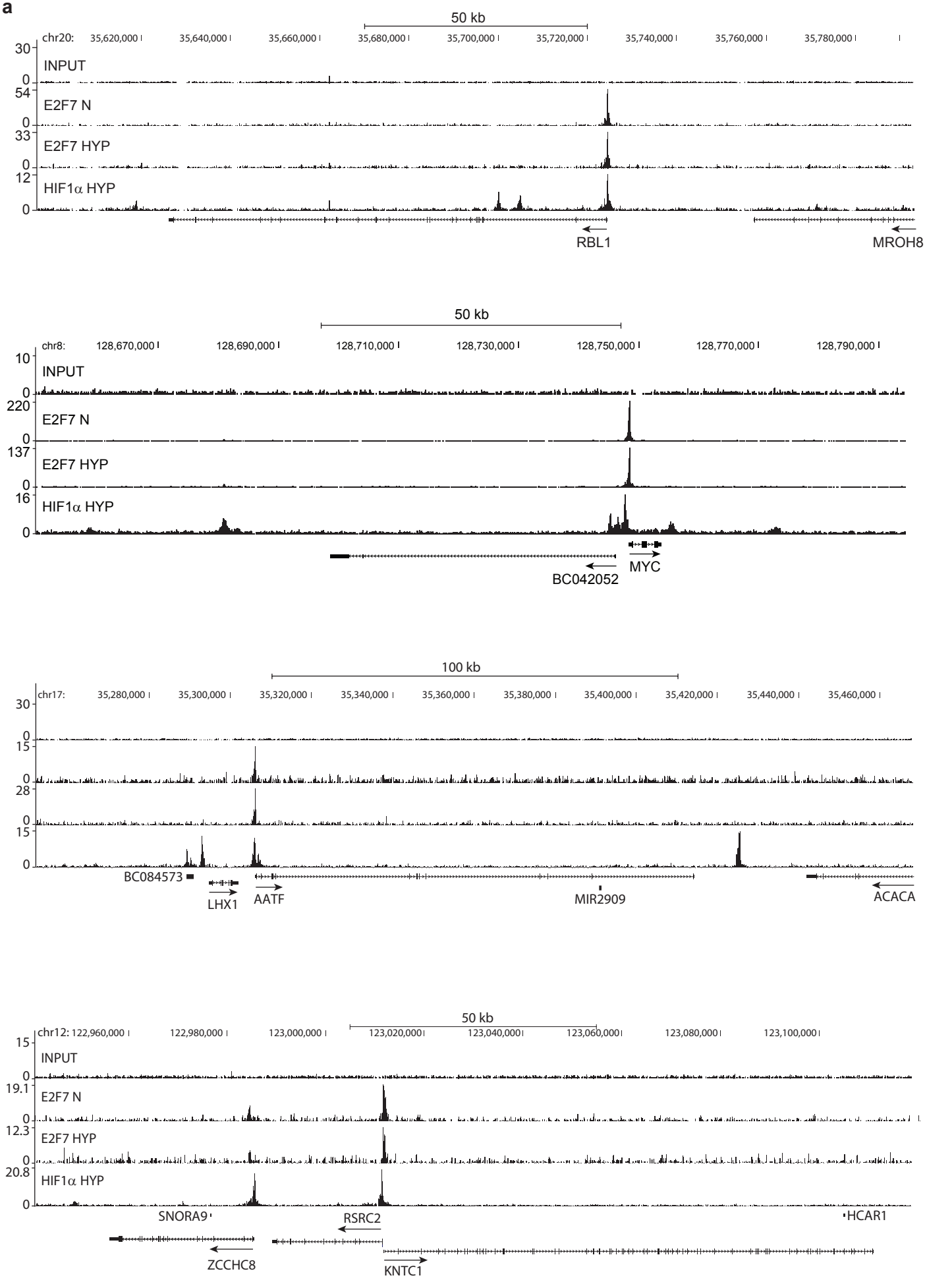
Common Directly Regulated HIF1-E2F7 targets

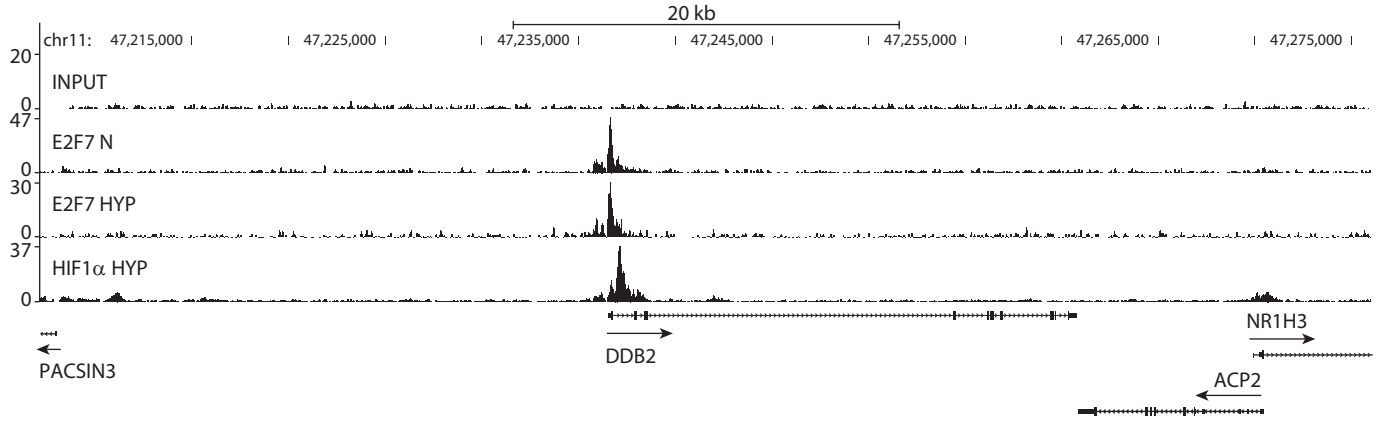
f



Supplemental Figure S3

a

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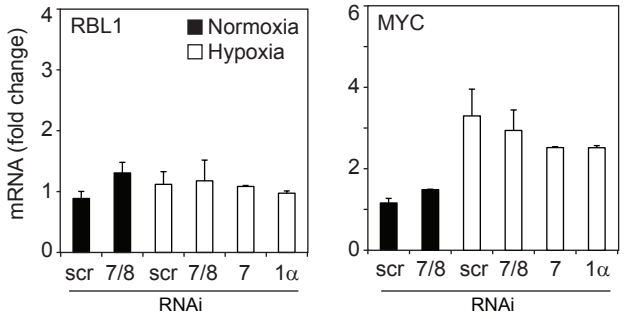
HIF1α HYP
PACSIN3
DDB2
NR1H3
ACP2

b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Normoxia
Hypoxia
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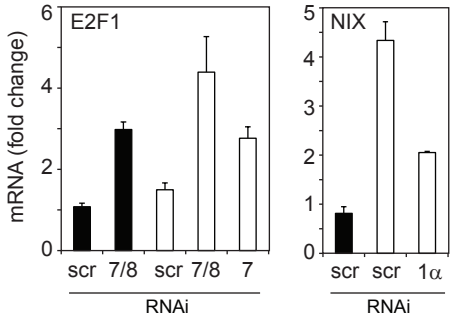
scr 7/8 scr 7/8 7 1α
RNAi
MYC
scr 7/8 scr 7/8 7 1α
RNAi

c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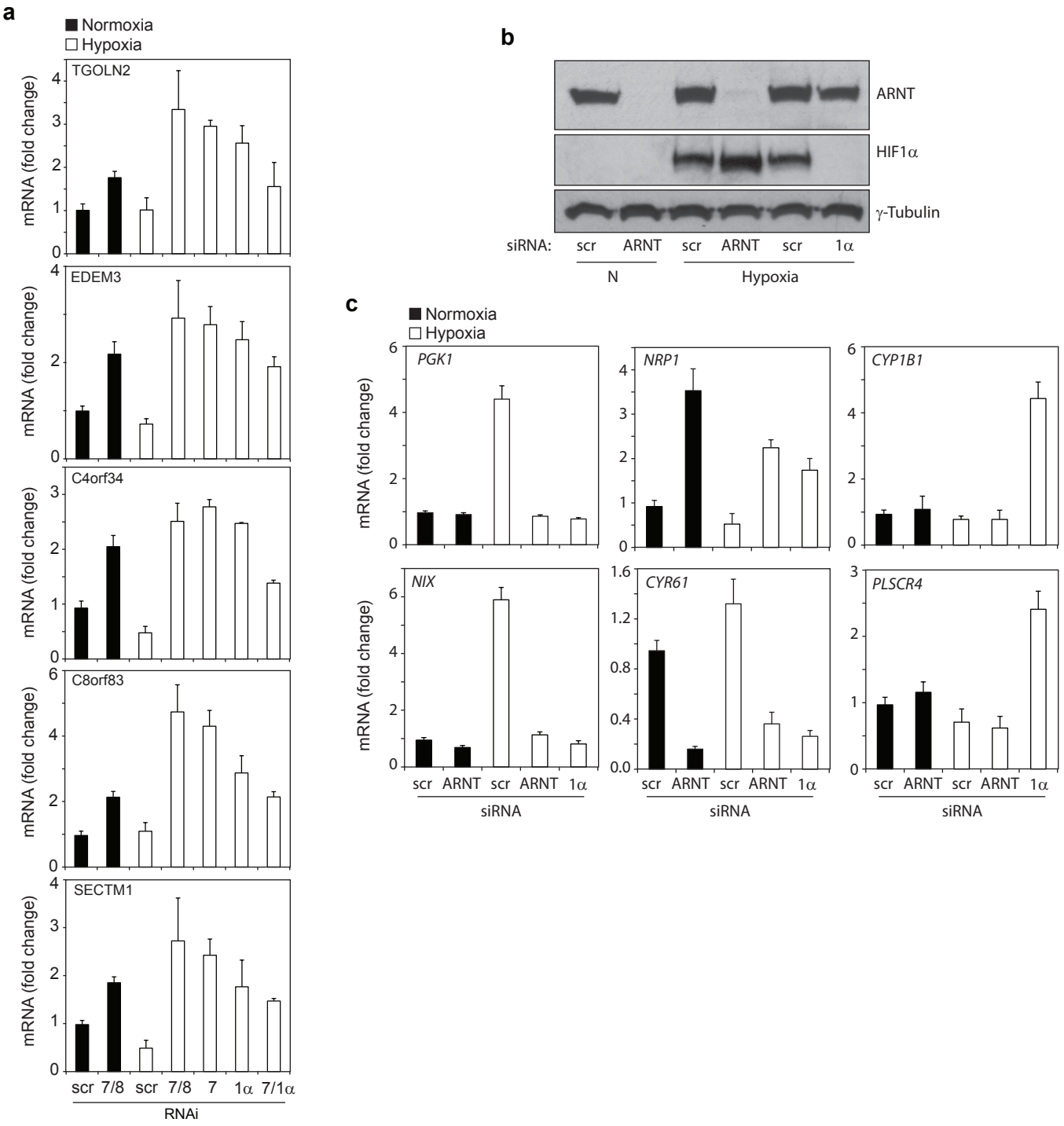
NIX
scr scr 1α
RNAi

Supplementary Figure S4



Supplementary Figure S5

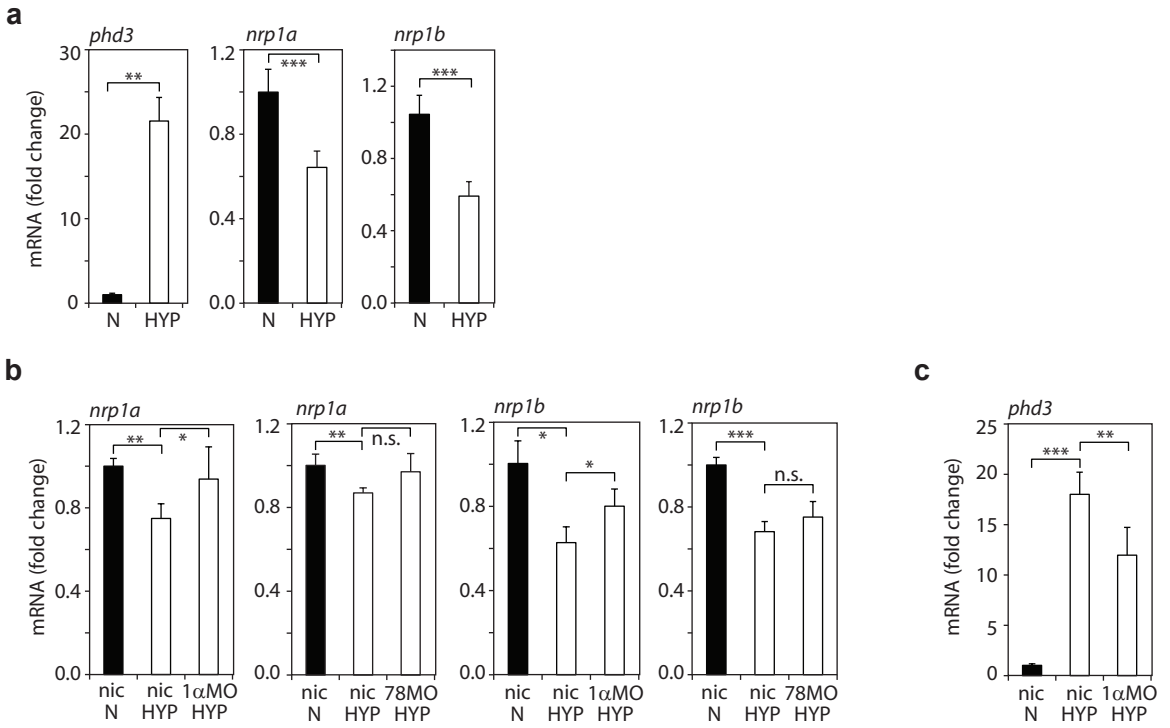
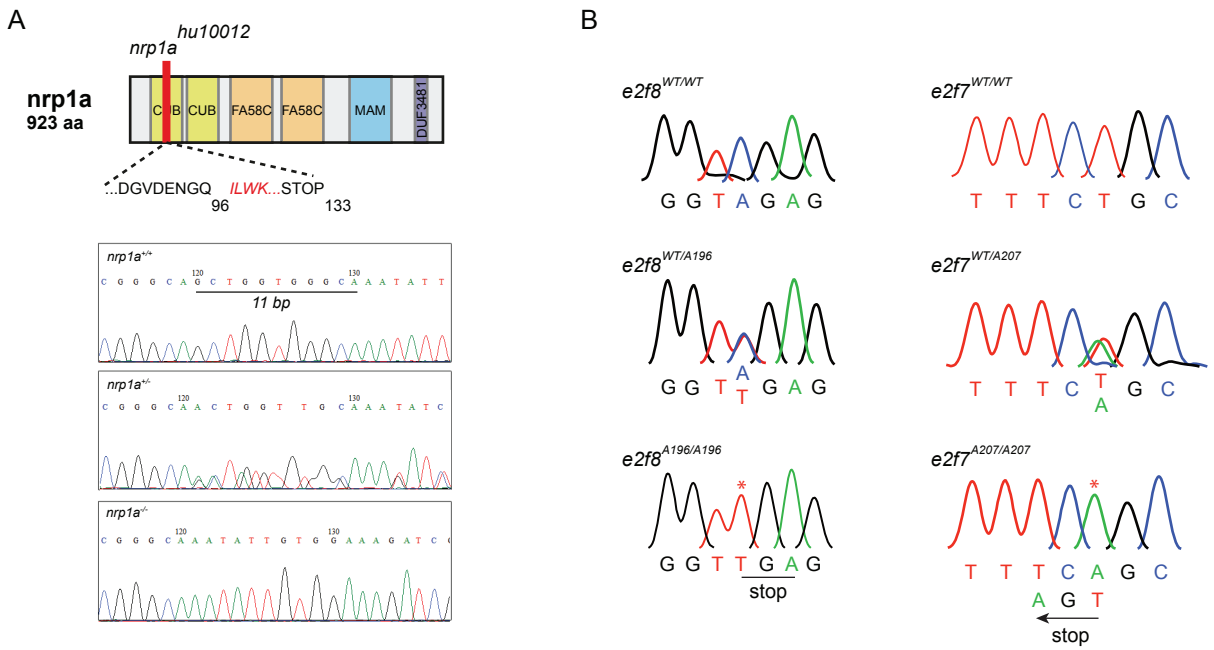


Figure S6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11 bp
nrp1a+/-
nrp1a-/-
B
e2f8WT/WT
G G T A G A G
e2f7WT/WT
T T T C T G C
e2f8WT/A196
G G T A T G A G
e2f7WT/A207
T T T C T A G C
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stop
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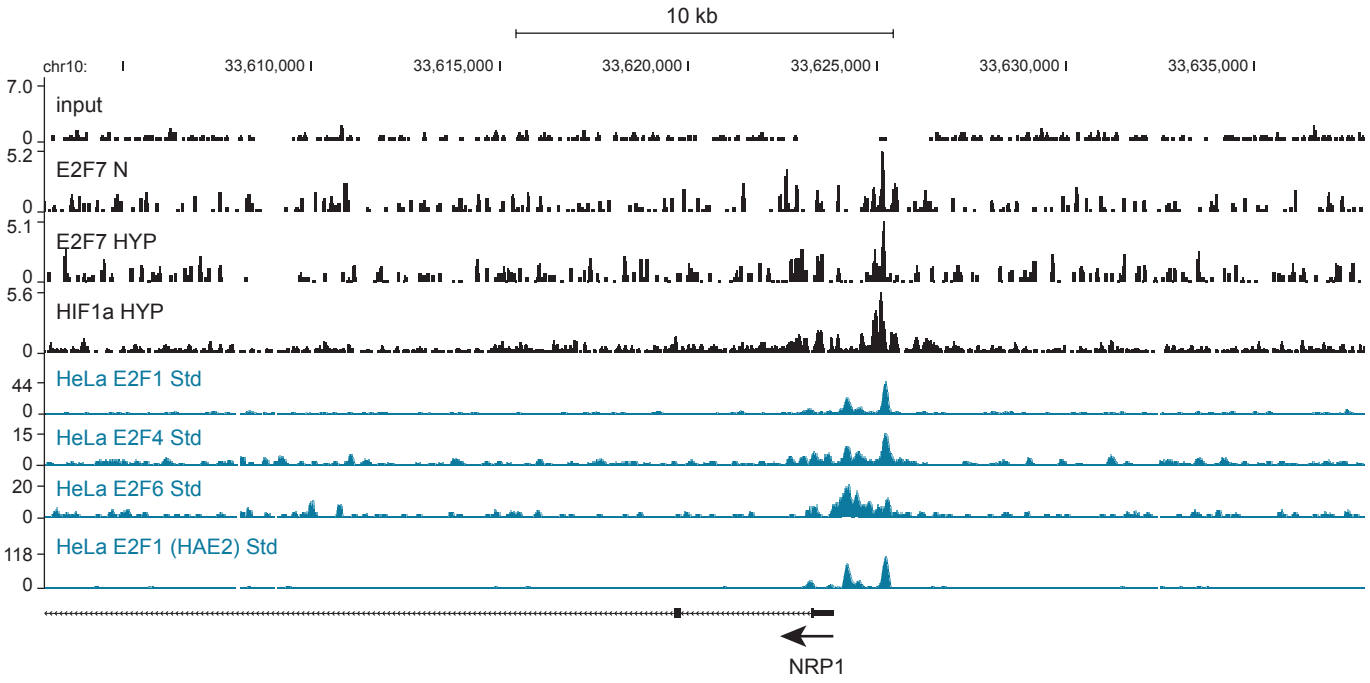
Figure S7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HeLa E2F4 Std
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HeLa E2F6 Std
0
118
HeLa E2F1 (HAE2) Std
0
NRP1

***Supplementary Table S1**. Oligonucleotide sequences*

Oligonucleotide sequences are annotated from 5' to 3'. *Homo sapiens* (Hs), *Danio rerio* (Dr).

Gene name	Detects	Species	Forward primer sequence	Reversed primer sequence
mRNA-qPCR primers				
β-actin	mRNA	Dr	CGTCTGGATCTAGCTGGTCGTGA	CAATTTCTCTTTCGGCTGTGGTG
tbp	mRNA	Dr	TCACCCCTATGACGCCTATC	CAAGTTTGCACCCCAAGTTT
phd3	mRNA	Dr	GGTTAATGGGACCGAGAGG	TTCCAGGATAACAAGCCACC
nrp1a	mRNA	Dr	CGATCAGGGAGATGAGCACC	CCACTGGACTGAATCTGCCAC
nrp1b	mRNA	Dr	CGAGAGATTCTGATCCGCTTG	TCCAGGGAGATGTAGCCAGAG
e2f1	mRNA	Dr	GCTTCAAATGGACCCACACC	CCAGAAAGCGTTTGGTGGTC
epo	mRNA	Dr	GCATCAGACAAGTGCTGCG	AGACAGGTGCATTGGCGAG
E2F7	mRNA	Hs	CTCCTGTGCCAGAAGTTTC	CATAGATGCGTCTCCTTTCC
E2F8	mRNA	Hs	AATATCGTGTTGGCAGAGATCC	AGGTTGGCTGTCGGTGTC
VEGFA	mRNA	Hs	ACCTCCACCATGCCAAGTG	TCTCGATTGGATGGCAGTAG
ACTB	mRNA	Hs	GATCGGCGGCTCCATCCTG	GACTCGTCATACTCCTGCTTGC
RSP18	mRNA	Hs	AGTTCCAGCATATTTTGCGAG	CTCTTGGTGAGGTCAATGTC
PLSCR4	mRNA	Hs	AGGAATGCCTATCGGACAC	ACAGAAGCAACAGCAGGTG
MTAP	mRNA	Hs	GGAAGCAGTTTCGGTGGAC	TCTGACCATTCTGTGGACCC
SETD7	mRNA	Hs	TCCAGGGCACGTATGTAGAC	TCTCTCCAGTCATCTCCCCA
IMPA2	mRNA	Hs	TTTGGTAACTGCTACGGACC	ATGATCCATGTGGGGTTG
LBR	mRNA	Hs	ATTCTGAGCCACGACAGC	AACTGGAAGTTGAGCCACC
TMX1	mRNA	Hs	GCCGTACCCATACCCTTCAA	AATGATGGACCCAGAGAGCG
CYP1B1	mRNA	Hs	AAGTTCTTGAGGCACTGCG	CGTTCTCCAAATCCAGCC
SYPL1	mRNA	Hs	TTGGTGAGCACTTCAGCCTG	ATCCCATACTGGTCACAGAGCC
CYR61	mRNA	Hs	GACTGTGAAGATGCGGTTCC	CTGTAGAAGGGAAACGCTGC
DDAH1	mRNA	Hs	TGTTCAGGAAGCGATCACCT	AGTGCCGCATTGTTCTTTGT
ATF3	mRNA	Hs	GCCTTAACACACTGGCCATT	TGAATCCTAACGGTGGGCAT
E2F1	mRNA	Hs	GACCACCTGATGAATATCTG	TGCTACGAAGGTCCTGAC
NIX	mRNA	Hs	CGTACCATCCTCATCCTCCA	TGTGGCGAAGGGCTGTCAC
PGK1	mRNA	Hs	GTTGACCGAATCACCGACCTC	CTCATAACGACCCGCTTCCC
RBL1	mRNA	Hs	TGAACCTGGACGAGGGGAG	GGGAATAATGCTTTTGCGGC
MYC	mRNA	Hs	GTCTGGATCACCTTCTGCTGG	TGTGCTGATGTGTGGAGACG
C4orf34	mRNA	Hs	TTTGGGGTTGTCTGGGATTG	TCCAAGAGCTACCACAGTCC
C8orf83	mRNA	Hs	GCCAAACATCTGAGGGCAAA	TATTCCCTTGCCACCCACTT
SECTM1	mRNA	Hs	GAGAACACCGTCATGTCCTGC	AGAAGTAGCCTGGAGCCACCTC
EDEM3	mRNA	Hs	GATAGTGGAGTTGGAGCAGG	CAAGTAGAAGAGGTGGCTGG
TGOLN2	mRNA	Hs	GACCATTCCAAGCCCATC	AGGTCAGTTTCCTCCCCAG
ChIP-qPCR primers				
E2F7 el1	DNA	Hs	GGTTCATGTGTCACACCAGCG	AGGACAGGAAAGCAGATGGGG
E2F7 el2	DNA	Hs	TCGCTCTCCCTTCCCGATGC	CAATTCCCGCCTCCCCACTG
E2F1	DNA	Hs	AGGAACCGCCGCCGTTGTTCCCGT	CTGCCTGCAAAGTCCCGGCCACTT
E2F1 ctrl	DNA	Hs	CGCCCAGACGCCACTTCATC	TTCATTCCCTCACTCATTCAACAA
BNIP3L	DNA	Hs	ATTTGCTCGTCTAGGGTTGGC	AATCTTGGGTGGTTCAGGAGG
E2F3	DNA	Hs	TTGTACTTTACCCCTATCCAGGC	TAGACAGAAGCATGTGGGCAG
MCM2	DNA	Hs	GTTCCGTAGGGCTCTTCCCG	CAGTACCACGATCCTCTCCGC
MTAP	DNA	Hs	CTGTAAAGGGCTCGTGGTG	TTAACCCGTTCTACGCCG
IMPA2	DNA	Hs	TGCCGTTTGATTGTCCTGG	TCCTGAACTCTGAGTCCGTGG
CYP1B1	DNA	Hs	CAATTCCCATGCCCTTGC	TGCCCTCCTTCTACCCAGTC
DDAH1	DNA	Hs	GATTGGAAACTTGAGTCCGTC	AACACTGGTAAGAATCAGGGTG
CYR61	DNA	Hs	CCGTTTCTTTAAGCACTCTCCC	AGCCTCTGATCTCGTCCAGC
PLSCR4	DNA	Hs	GAAACTGCTCGCTCCCAAAC	CAGGTGGTTGCAGAAGTTCC
NRP1	DNA	Hs	GAAGAAACAGGTTGCGGTCAC	TCAGGCTGTACTTTGGCTGTG
SYPL1	DNA	Hs	CAAGACAAAATCGGCTTCCC	GAAAACTTCCTGCTGCGGTC
TMX1	DNA	Hs	TTGCTCTGTTCCTTCCCC	AGTGAGTGGTGAAGTCGGG
SETD7	DNA	Hs	CCAGCCTGAATTCCTCCAGA	GCCAAAGAACCTCAGAGTGC

LBR	DNA	Hs	TGCCCTCCTTAGCGTGTTAA	GTTCGCTTCTACGCAGGTTC
ATF3	DNA	Hs	GGACTCCGATCTTTTCACGC	CGAAACTAGGGCGAGTGGTA

LEGENDS SUPPLEMENTARY FIGURES AND TABLES

Supplementary Figure S1. *E2F7* expression is induced in response to hypoxia/DFO by HIF1 in HeLa and U2OS cells. (**A**) Western blot analysis showing E2F7, HIF1α or E2F1 (loading) protein levels in lysates from HeLa cells grown under different density (as indicated) for 0, 8 or 16 hour in the presence of 100μM DFO. (**B**) Graphs showing *E2F7*, *E2F8* or *VEGFA* mRNA levels determined by qPCR, and presented as fold change compared to non-treated cells. RNA was isolated from HeLa cells grown in the absence or presence of DFO (100μM, 16 hour). (**C**) Western blot analysis of E2F7 and HIF1α expression in lysates from U2OS cells transfected with control (scr) or one of three different E2F7-specific siRNAs, and cultured in normoxia (-) or hypoxia (+) as indicated. Background bands ('b') in the E2F7 blot serve as loading control. (**D**) Graphs showing *E2F7, E2F8* or *VEGFA* mRNA levels as determined by qPCR, and presented as fold change in hypoxia compared to normoxia. RNA was isolated from U2OS cells grown in normoxia (N) or hypoxia (H). (**E**) Similar as in (D) but now for U2OS cells treated with 100μM DFO for 16 hour, and showing *E2F7, E2F1* or *VEGFA* mRNA levels. (**F**) Putative HIF- and E2F-binding sites (BS) and their location (calculated from the transcriptional start point (+1)) in the human *E2F7* promoter. Arrows indicate sites amplified by qPCR after ChIP. Asterisks present conserved binding sites between the mouse and human *E2F7* promoter. (**G**) Graph shows enrichment as determined by ChIP-qPCR of HIF1α and E2F1 (positive control) to element 1 of the *E2F7* promoter. Non-specific IgG serve as a negative control. (**H**) Graphs show *E2F7*, *NIX* or *PGK1* mRNA levels as determined by qPCR. RNA was isolated from U2OS cells grown in normoxia (-) or hypoxia (+), transfected with control (scr) or HIF1α siRNA as indicated. Lower panels are western blots for HIF1α and HDAC1 (loading) under these conditions. All quantified data present the average ± S.D. compared to the indicated controls in at least three independent experiments.

Supplementary Figure S2. Genome-wide analysis of HIF1α-E2F7 targets by ChIP-seq and microarray analysis. (**A**) Proportional Venn diagram showing the overlap between E2F7 bound genes in normoxia and hypoxia (2381), and the overlap between those and the HIF1α bound genes (2258) from the ChIP-seq data. (**B**) Position weight matrix of E2F and HIF motifs identified within E2F7 (normoxia and hypoxia) or HIF1α peaks, respectively. For the E2F7 ChIP-seq all peaks were analyzed, for HIF1α the top 1,000. Graphs present positioning of all peaks in an individual ChIP-seq

experiment (black) with respect to the closest transcriptional start site (TSS) compared with random DNA regions (red). (**C**) Venn diagram of microarray analysis showing the overlap (78) between E2F7/8 and HIF1 regulated genes (cut-off ≥ 2 fc, $p<0.05$) in hypoxia. Regulated genes are divided in up or down regulated genes. Microarray analysis was performed using mRNA isolated from HeLa cell transfected with either HIF1α, E2F7/8 or control (scr) siRNAs and cultured in hypoxia. Fold change was calculated compared control transfected cells. (**D**) Similar as in (C) but now using a cut-off fold regulation of ≥ 1.5 (and $p<0.05$), revealing 318 common genes. (**E**) Similar as in (2B). Table summarizes the overlap between the microarray (cut-off ≥ 1.5 fc, and $p<0.05$) and ChIP-seq data. Now also including the siE2F7 microarray data. (**F**) Venn diagram showing the overlap (56) between the E2F7/8 (207) and HIF1 (586) direct & regulated targets identified in (S2E).

Supplementary Figure S3. Examples of novel overlapping E2F7 and HIF1α target genes. (**A**) Shown are overlapping E2F7 (N/HYP) and HIF1α (HYP) binding peaks on the *RBL1*, *MYC*, *AATF1*, *RSRC2* and *DDB2* genes (y-axis presents peak height). Input DNA serves as a control. Lines underneath the graphs indicate annotated genes, boxes present exons, and line with arrows present introns. Arrows indicate direction of transcription. (**B**) Graphs showing mRNA levels (presented as fold change comparing to scr normoxia of *RBL1* and *MYC*, examples of direct targets that are not regulated by HIF1α-E2F7 under the tested conditions. Messenger RNA levels (measured by qPCR) were analyzed in HeLa cells transfected with control (scr), E2F7 & E2F8 (7/8), E2F7 (7) or HIF1α (1α) siRNAs, and grown in normoxia or hypoxia, as indicated. (**C**) Similar as in (B), but now showing mRNA levels of the E2F7/8 target *E2F1*, and the HIF1 target *NIX*, positive controls for functional siRNA-depletion of E2F7/8 of HIF1α. All quantified data present the average ± S.D. compared to the indicated controls in at least three independent experiments.

Supplementary Figure S4. Validation of mRNA regulation of targets by HIF1α-E2F7 and ARNT. (**A**) Validation of mRNA regulation of the novel common repressed HIF1α-E2F7 targets *TGOLN2, EDEM3, C4orf34, C8orf83 and SECTM1*. Graphs show mRNA levels (presented as fold change comparing to scr normoxia) of the HIF1α-E2F7 common repressed targets *TGOLN2, EDEM3, C4orf34, C8orf83 and SECTM1*. Messenger RNA levels (as measured by qPCR) were analyzed in lysates isolated from HeLa cells transfected with control (scr), E2F7 & E2F8 (7/8), E2F7 (7), HIF1α

(1α) or E2F7 & HIF1α (7/1α) siRNAs and grown under normoxic or hypoxic conditions, as indicated. (**B**) Western blot analysis to confirm protein knockdown of ARNT (HIF1β) and HIF1α in HeLa lysates, 48 hour after transfection with the indicated siRNA. The hypoxic cells were grown the last 16 (of the 48) hour at 1% O_2, as described in Materials and Methods. Normoxic lysates are indicated with N. γ-TUBULIN staining served as a loading control. (**C**) In parallel with the protein analysis described in (B), also samples were harvested for mRNA analysis (measured by qPCR). Graphs show mRNA levels (presented as fold change compared to scr normoxia) for the HIF1 targets *PGK1* and *NIX*, and for the HIF1α-E2F7 targets *NRP1*, *CYR61*, *CYP1B1* and *PLSCR4*. Messenger RNA levels were analyzed in lysates isolated from HeLa cells transfected with control (scr), ARNT, or HIF1α (1α) siRNA as described in (B). All quantified data present the average ± S.D. compared to the indicated controls in at least three independent experiments.

Supplementary Figure S5. Hypoxia represses *nrp1a* and *nrp1b* expression in zebrafish embryos. (**A**) Zebrafish embryos were dechorionated at 24hpf and next incubated for 5 hour at 1% O_2 at 28.5 ^{0}C, after which they were anesthetized with tricaine mesylate (MS222) and snap frozen for later RNA isolation (~30hpf). Graphs show mRNA levels (as determined by qPCR) of *nrp1a*, *nrp1b* and *phd3*, presented as fold change compared to normoxia. All black bars in this figure present normoxia (N), all white bars hypoxia (HYP). Messenger RNA levels of *phd3* served as a hypoxia marker. For each sample ~20 embryos were harvested. We used a short hypoxic exposure as prolonged hypoxia delays zebrafish development (1, 2), making it impossible to compare hypoxic mRNA levels to normoxia. (**B**) Zebrafish embryos were either non-injected (nic: non-injected control), or injected with 5+5ng e2f7/8 (78MO), or 5ng hif1ab (1αMO) morpholinos (MO), and were harvested similarly as described in (A). Graphs show mRNA levels (as determined by qPCR) of *nrp1a*, *nrp1b* and *phd3* presented as fold change compared to nic normoxia. Messenger RNA levels of *nrp1a* and *nrp1b* were significantly derepressed in hypoxia upon MO-depletion of hif1ab. 78MO also derepressed hypoxic mRNA *nrp1a* and *nrp1b* expression, although to a non-significant level. (**C**) Similar as in (B), but now *phd3* mRNA levels are shown to show functional MO-inhibition of hif1ab under hypoxia. All quantified data present the average ± S.D. compared to the indicated controls in at least three independent experiments. *p<0.05, **p<0.001, ***p<0.0001.

Supplementary Figure S6. Description of the nrp1a^{hu10012} mutant. (**A**) Schematic Figure of nrp1a domain structure is shown in top, indicating position of *nrp1a*^{hu10012} (red bar); CUB, CUB domain; FA58C, coagulation factor 5/8 C-terminal domain; MAM, MAM domain; and DUF3481, domain of unknown function. Similar to the published *nrp1a*^{hu9963} mutant, the *nrp1a*^{hu10012} mutant also has a frameshift mutation at amino acid 96, caused by an 11 bp deletion, resulting in a stop codon at amino acid 133. Lower three sequences show genotyping (by sequencing) of wildtype, heterozygous and *nrp1a*^{hu10012} mutant zebrafish. The 11 bp deletion is shown underlined in wildtype zebrafish. (**B**) Examples of sequencing reads of *e2f7* and *e2f8* wildtype, heterozygous and mutant zebrafish embryos as indicated. For e2f7 sequencing a forward primer was used, for e2f8 a reversed primer. Mutated nucleotides are indicated with an asterix.

Supplementary Figure S7. ChIP-seq analysis reveals binding of multiple E2Fs to the *NRP1* promoter. ChIP-seq signal of E2F7 (N & HYP) and HIF1α (HYP) on the *NRP1* promoter are shown (as presented in Figure 4A), as well as publically available ChIP-seq signals for E2F1 (2 experiments), E2F4 and E2F6 as also performed in HeLa cells (shown in blue). Lines underneath the graphs indicate annotated genes, boxes present exons, and lines with arrows represent introns. E2F1 (GEO sample accession: GSM935484, GSM935366), E2F4 (GSM935365) and E2F6 (GSM935476) tracks are publically available at the UCSC Genome Browser.

Supplementary Dataset S1. E2F7 and HIF1α Targets identified by ChIP-seq. This dataset (excel file) lists the peaks and target genes of the E2F7 (N and HYP), and HIF1α (HYP) ChIP-seq experiments.

Supplementary Dataset S2. Motif analysis of the E2F7 (N and HYP) and HIF1α binding regions. This dataset (excel file) lists the binding motifs of transcription factors significantly enriched in E2F7 (N and HYP) and HIF1α (HYP) binding regions. Their fold enrichment over random regions is also given.

Supplementary Dataset S3. Gene Ontology analysis of the ChIP-seq and microarray data. This dataset (excel file) lists the gene ontology analysis that was performed using DAVID and PANTHER gene ontology tools. Results are shown for the genes identified in the E2F7 (N and HYP) and HIF1α (HYP) ChIP-seq experiments, as well as for genes identified in the microarrays: siE2F7/8 *versus* scr

(HYP), siHIF1α *versus* scr (HYP). The GO analysis for the microarrays were performed both on ≥2 fc, p<0.05; and ≥1.5 fc, p<0.05 selected targets.

Supplementary Dataset S4. Overlap of regulated targets in the E2F7 and E2F7/8 microarrays. This dataset (excel file) lists the number of significantly deregulated transcripts (≥1.5 fc, p<0.05) in the individual siE2F7 vs scr (HYP) and siEF7/8 vs scr (HYP) microarrays, and also lists the number of targets that are regulated under both conditions.

SUPPLEMENTARY REFERENCES

1. Padilla,P.A. and Roth,M.B. (2001) Oxygen deprivation causes suspended animation in the zebrafish embryo. *Proc. Natl. Acad. Sci. U. S. A.,* **98,** 7331-7335.

2. Kajimura,S., Aida,K. and Duan,C. (2005) Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation. *Proc. Natl. Acad. Sci. U. S. A.,* **102,** 1240-1245.