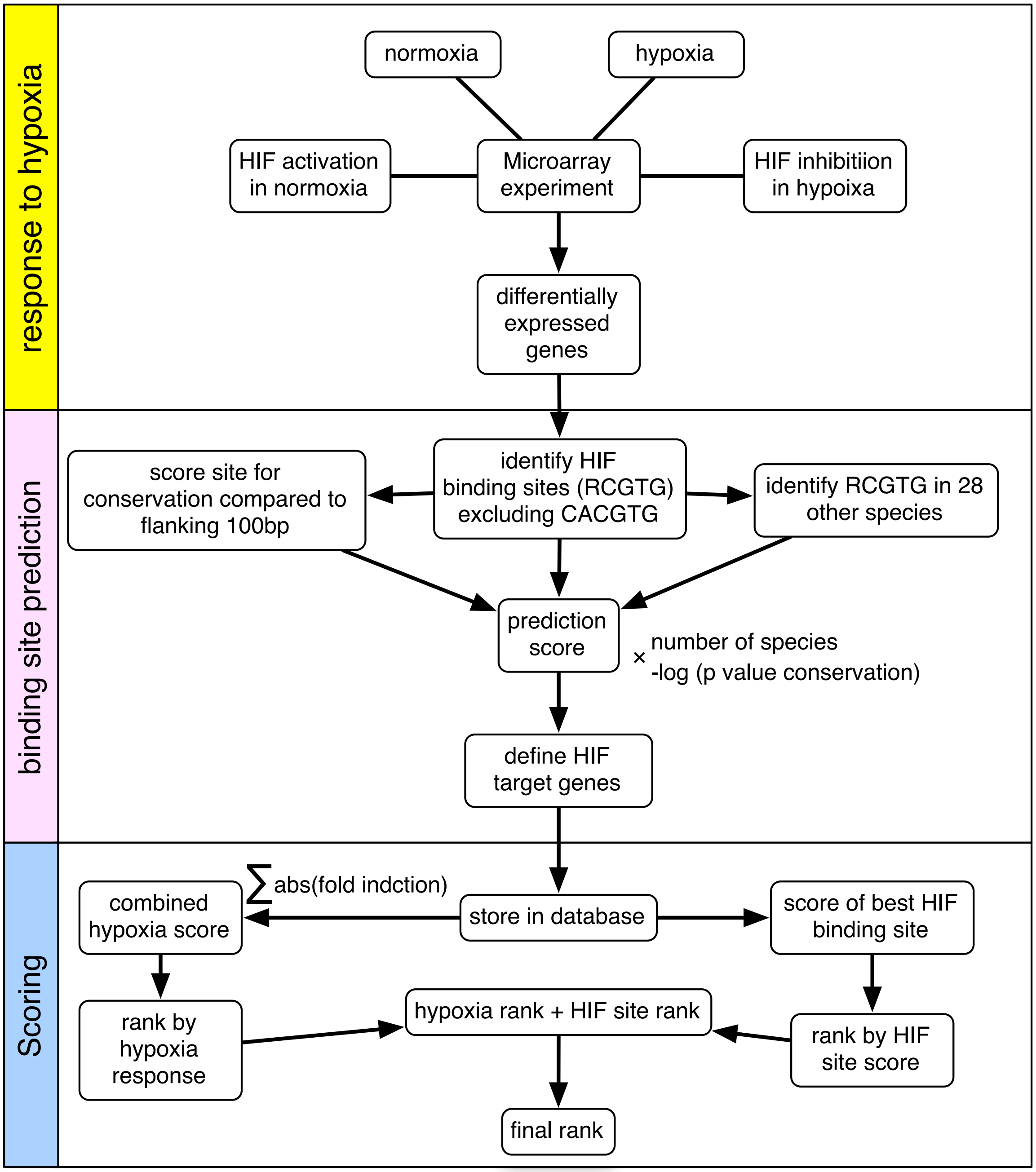
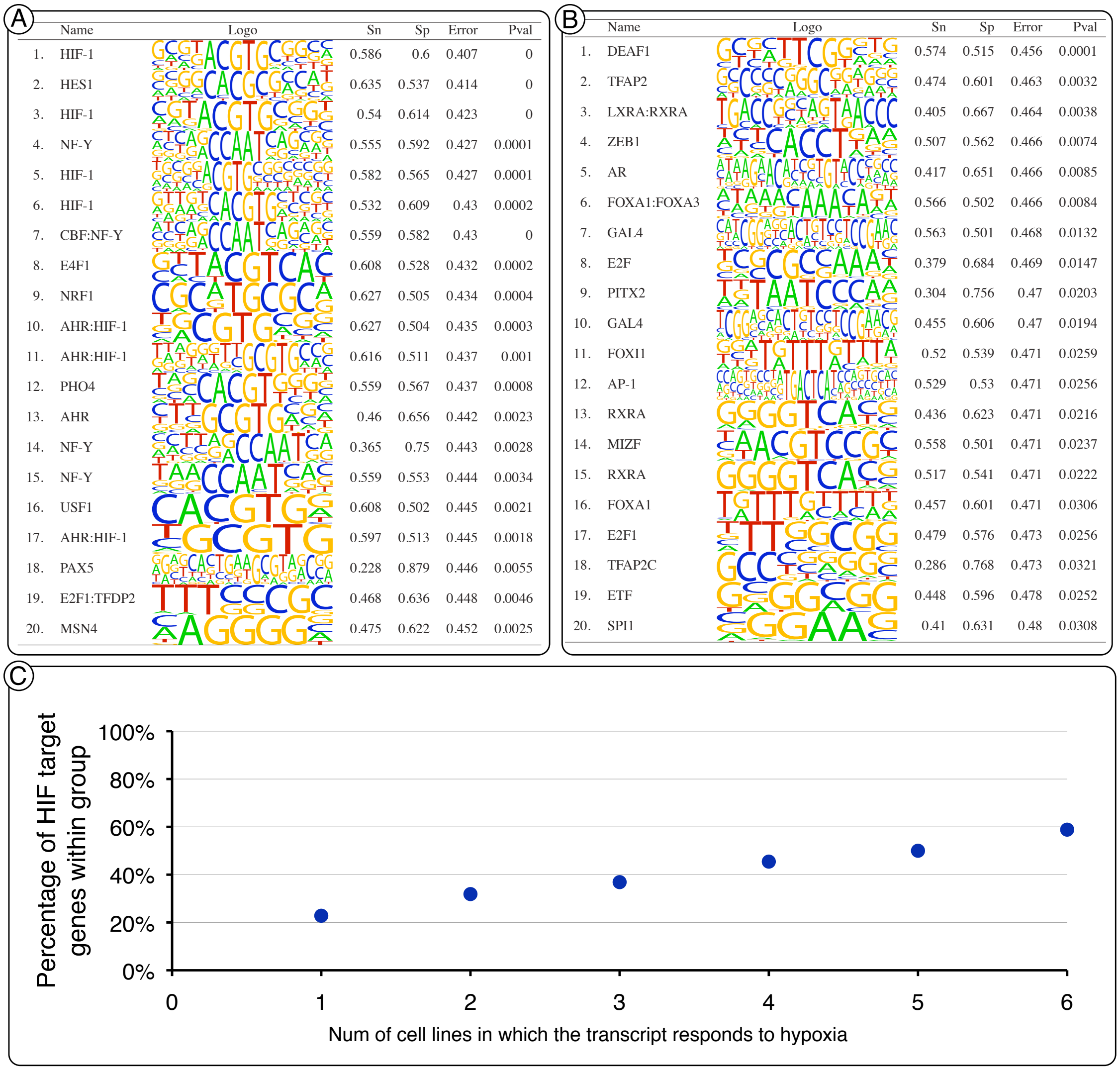

response to hypoxia
normoxia
hypoxia
HIF activation in normoxia
Microarray experiment
HIF inhibitiion in hypoixa
differentially expressed genes
binding site prediction
score site for conservation compared to flanking 100bp
identify HIF binding sites (RCGTG) excluding CACGTG
identify RCGTG in 28 other species
prediction score
× number of species
-log (p value conservation)
define HIF target genes
Scoring
∑ abs(fold indction)
combined hypoxia score
store in database
score of best HIF binding site
rank by hypoxia response
hypoxia rank + HIF site rank
rank by HIF site score
final rank

Figure 1



A

	Name	Logo	Sn	Sp	Error	Pval
1.	HIF-1		0.586	0.6	0.407	0
2.	HES1		0.635	0.537	0.414	0
3.	HIF-1		0.54	0.614	0.423	0
4.	NF-Y		0.555	0.592	0.427	0.0001
5.	HIF-1		0.582	0.565	0.427	0.0001
6.	HIF-1		0.532	0.609	0.43	0.0002
7.	CBF:NF-Y		0.559	0.582	0.43	0
8.	E4F1		0.608	0.528	0.432	0.0002
9.	NRF1		0.627	0.505	0.434	0.0004
10.	AHR:HIF-1		0.627	0.504	0.435	0.0003
11.	AHR:HIF-1		0.616	0.511	0.437	0.001
12.	PHO4		0.559	0.567	0.437	0.0008
13.	AHR		0.46	0.656	0.442	0.0023
14.	NF-Y		0.365	0.75	0.443	0.0028
15.	NF-Y		0.559	0.553	0.444	0.0034
16.	USF1		0.608	0.502	0.445	0.0021
17.	AHR:HIF-1		0.597	0.513	0.445	0.0018
18.	PAX5		0.228	0.879	0.446	0.0055
19.	E2F1:TFDP2		0.468	0.636	0.448	0.0046
20.	MSN4		0.475	0.622	0.452	0.0025

B

	Name	Logo	Sn	Sp	Error	Pval
1.	DEAF1		0.574	0.515	0.456	0.0001
2.	TFAP2		0.474	0.601	0.463	0.0032
3.	LXRA:RXRA		0.405	0.667	0.464	0.0038
4.	ZEB1		0.507	0.562	0.466	0.0074
5.	AR		0.417	0.651	0.466	0.0085
6.	FOXA1:FOXA3		0.566	0.502	0.466	0.0084
7.	GAL4		0.563	0.501	0.468	0.0132
8.	E2F		0.379	0.684	0.469	0.0147
9.	PITX2		0.304	0.756	0.47	0.0203
10.	GAL4		0.455	0.606	0.47	0.0194
11.	FOXI1		0.52	0.539	0.471	0.0259
12.	AP-1		0.529	0.53	0.471	0.0256
13.	RXRA		0.436	0.623	0.471	0.0216
14.	MIZF		0.558	0.501	0.471	0.0237
15.	RXRA		0.517	0.541	0.471	0.0222
16.	FOXA1		0.457	0.601	0.471	0.0306
17.	E2F1		0.479	0.576	0.473	0.0256
18.	TFAP2C		0.286	0.768	0.473	0.0321
19.	ETF		0.448	0.596	0.478	0.0252
20.	SPI1		0.41	0.631	0.48	0.0308

Figure 2

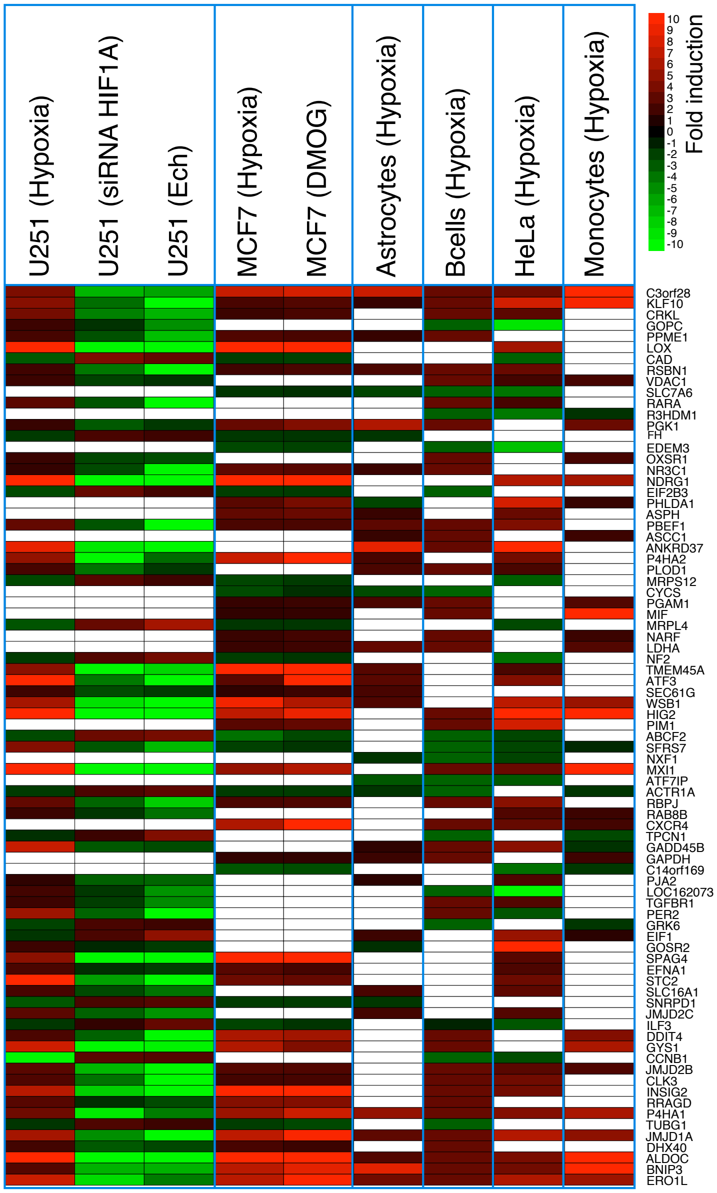

U251 (Hypoxia)
U251 (siRNA HIF1A)
U251 (Ech)
MCF7 (Hypoxia)
MCF7 (DMOG)
Astrocytes (Hypoxia)
Bcells (Hypoxia)
HeLa (Hypoxia)
Monocytes (Hypoxia)
Fold induction
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
C3orf28
KLF10
CRKL
GOPC
PPME1
LOX
CAD
RSBN1
VDAC1
SLC7A6
RARA
R3HDM1
PGK1
FH
EDEM3
OXSR1
NR3C1
NDRG1
EIF2B3
PHLDA1
ASPH
PBEF1
ASCC1
ANKRD37
P4HA2
PLOD1
MRPS12
CYCS
PGAM1
MIF
MRPL4
NARF
LDHA
NF2
TMEM45A
ATF3
SEC61G
WSB1
HIG2
PIM1
ABCF2
SFRS7
NXF1
MXI1
ATF7IP
ACTR1A
RBPJ
RAB8B
CXCR4
TPCN1
GADD45B
GAPDH
C14orf169
PJA2
LOC162073
TGFBR1
PER2
GRK6
EIF1
GOSR2
SPAG4
EFNA1
STC2
SLC16A1
SNRPD1
JMJD2C
ILF3
DDIT4
GYS1
CCNB1
JMJD2B
CLK3
INSIG2
RRAGD
P4HA1
TUBG1
JMJD1A
DHX40
ALDOC
BNIP3
ERO1L

Figure 3

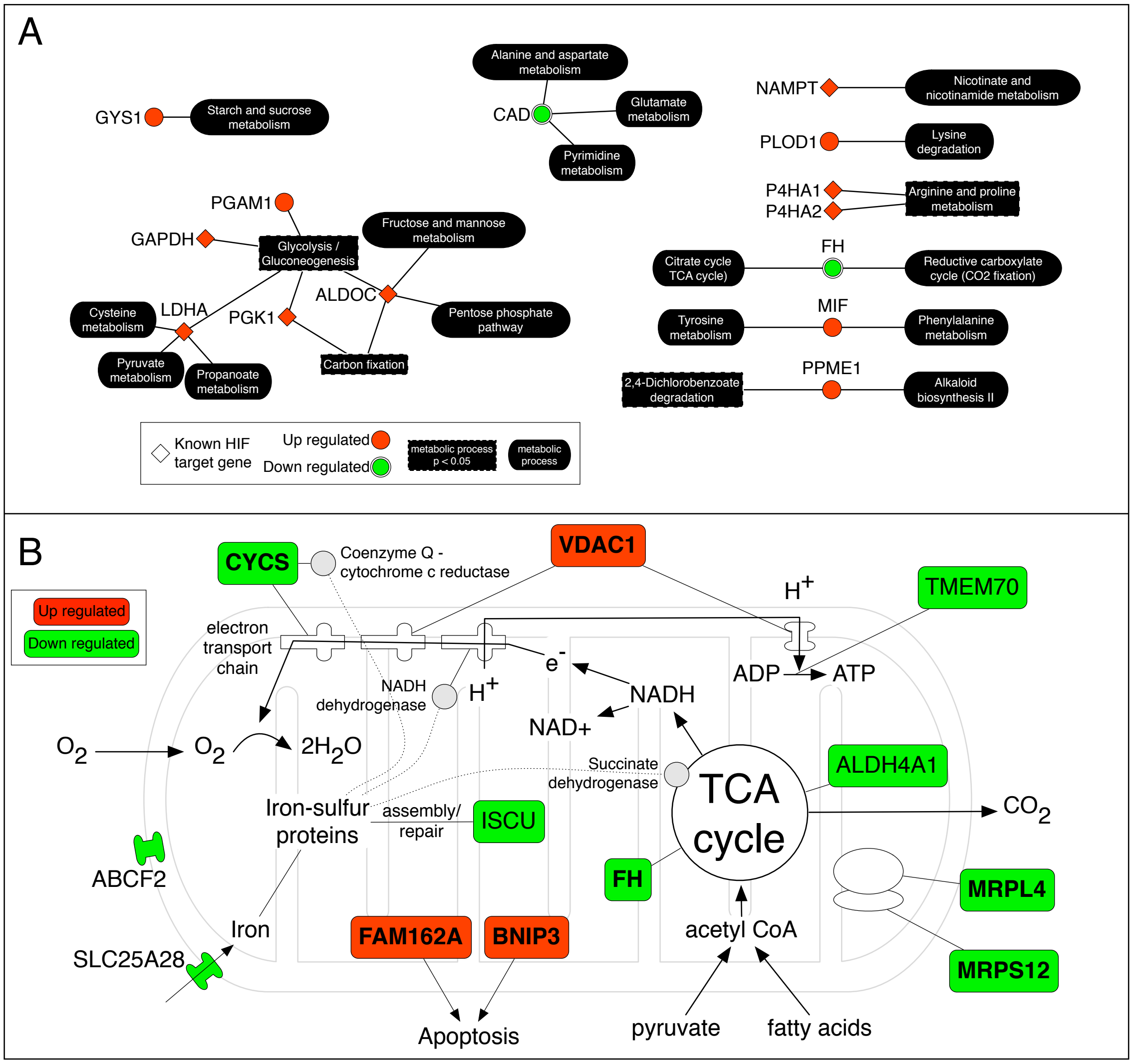

A
GYS1
Starch and sucrose metabolism
Alanine and aspartate metabolism
CAD
Glutamate metabolism
Pyrimidine metabolism
NAMPT
Nicotinate and nicotinamide metabolism
PLOD1
Lysine degradation
P4HA1
P4HA2
Arginine and proline metabolism
PGAM1
GAPDH
Glycolysis / Gluconeogenesis
Fructose and mannose metabolism
ALDOC
Pentose phosphate pathway
Cysteine metabolism
LDHA
PGK1
Pyruvate metabolism
Propanoate metabolism
Carbon fixation
FH
Citrate cycle TCA cycle)
Reductive carboxylate cycle (CO2 fixation)
MIF
Tyrosine metabolism
Phenylalanine metabolism
PPME1
2,4-Dichlorobenzoate degradation
Alkaloid biosynthesis II
Known HIF target gene
Up regulated
Down regulated
metabolic process p < 0.05
metabolic process
B
Up regulated
Down regulated
CYCS
Coenzyme Q - cytochrome c reductase
VDAC1
H+
TMEM70
electron transport chain
e-
NADH dehydrogenase
H+
NADH
ADP
ATP
NAD+
O2
O2
2H2O
Succinate dehydrogenase
ALDH4A1
TCA cycle
CO2
Iron-sulfur proteins
assembly/ repair
ISCU
ABCF2
FH
MRPL4
Iron
SLC25A28
FAM162A
BNIP3
acetyl CoA
MRPS12
Apoptosis
pyruvate
fatty acids

Figure 4

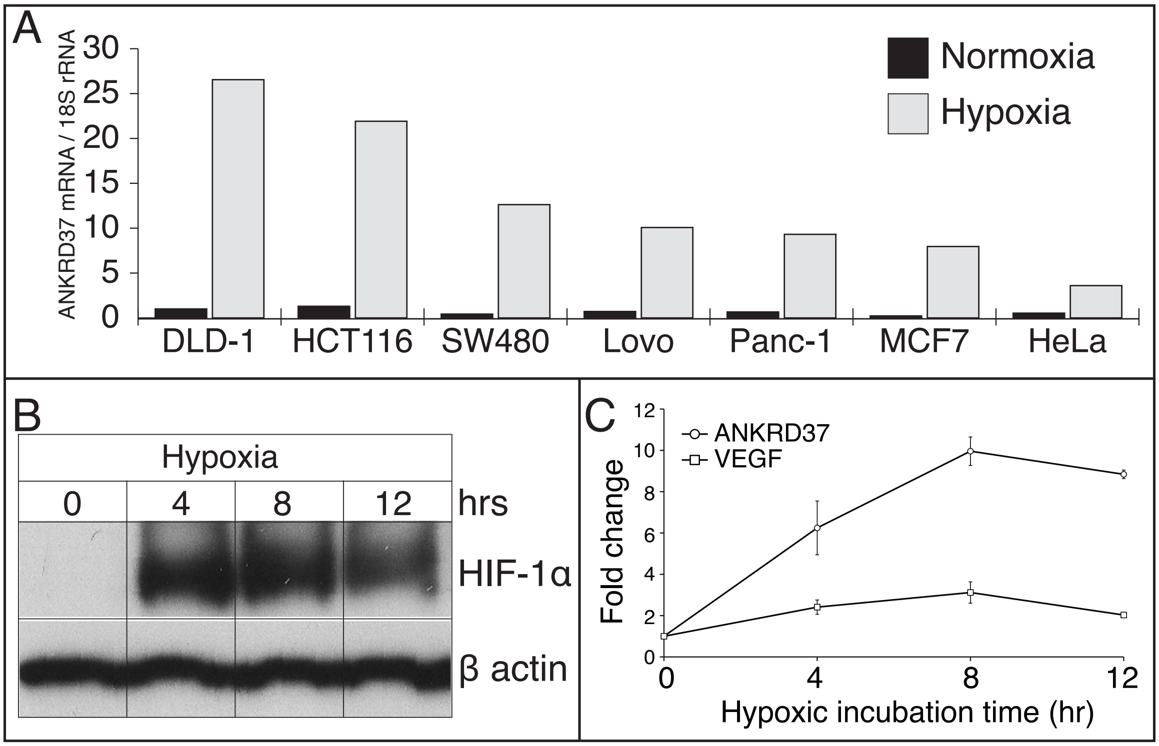

A
ANKRD37 mRNA / 18S rRNA
30
25
20
15
10
5
0
Normoxia
Hypoxia
DLD-1
HCT116
SW480
Lovo
Panc-1
MCF7
HeLa
B
Hypoxia
0
4
8
12
hrs
HIF-1α
β actin
C
ANKRD37
VEGF
Fold change
12
10
8
6
4
2
0
0
4
8
12
Hypoxic incubation time (hr)

Figure 5

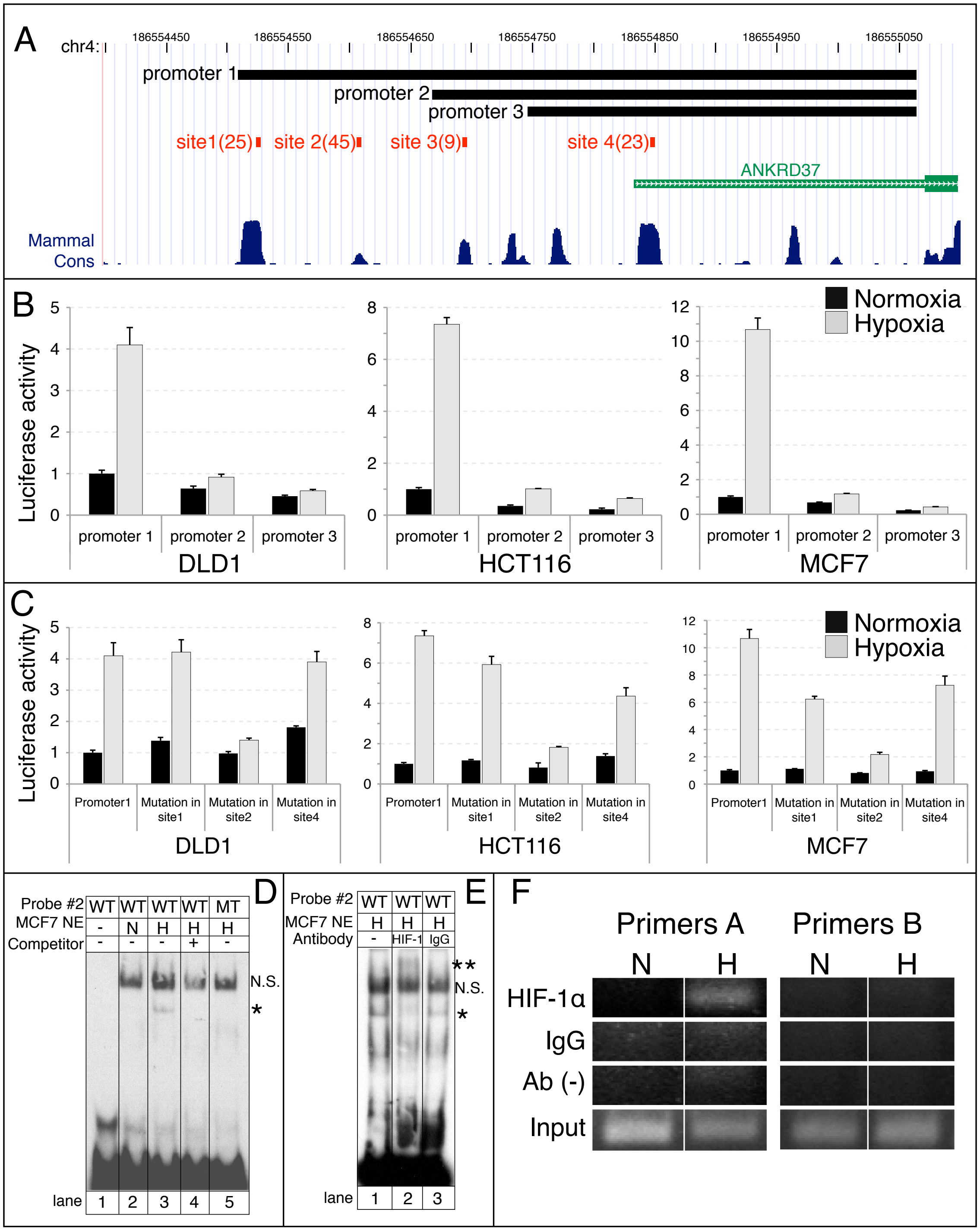

A
chr4:
186554450
186554550
186554650
186554750
186554850
186554950
186555050
promoter 1
promoter 2
promoter 3
site1(25)
site 2(45)
site 3(9)
site 4(23)
ANKRD37
Mammal Cons
B
Luciferase activity
5
4
3
2
1
0
promoter 1
promoter 2
promoter 3
DLD1
8
6
4
2
0
promoter 1
promoter 2
promoter 3
HCT116
12
10
8
6
4
2
0
promoter 1
promoter 2
promoter 3
MCF7
Normoxia
Hypoxia
C
Luciferase activity
5
4
3
2
1
0
Promoter1
Mutation in site1
Mutation in site2
Mutation in site4
DLD1
8
6
4
2
0
Promoter1
Mutation in site1
Mutation in site2
Mutation in site4
HCT116
12
10
8
6
4
2
0
Promoter1
Mutation in site1
Mutation in site2
Mutation in site4
MCF7
Normoxia
Hypoxia
D
Probe #2 WT WT WT WT MT
MCF7 NE - N H H H
Competitor - - - + -
N.S.
*
lane 1 2 3 4 5
E
Probe #2 WT WT WT
MCF7 NE H H H
Antibody - HIF-1 IgG
**
N.S.
*
lane 1 2 3
F
Primers A
Primers B
N
H
N
H
HIF-1α
IgG
Ab (-)
Input

Figure 6

Tables:

	MCF7	U251	Astrocytes	Monocytes	B cells	HeLA
Genes that respond to hypoxia	830	1702	1371	486	1920	2119
Genes that respond to hypoxia in which a HIF binding site was identified	278 (33%)	546 (32%)	380 (28%)	159 (33%)	534 (28%)	555 (26%)
Known target genes that respond to hypoxia	35	31	20	22	32	27
Known target genes recovery	19 (54%)	17 (55%)	8 (40%)	11 (50%)	13 (41%)	10 (37%)

Table 1. Transcriptional response to hypoxia across six different cell types as determined by microarrays.

Source	Description	Number of genes	Previously Validated HIF-1 Targets		Novel HIF-1 Targets
			p-value	Gene symbols	
Protein domains	IPR005123; 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase	6/19	2.E-07	P4HA2, EGLN3, EGLN1, P4HA1, PH-4	PLOD1
GSEA	Poor prognosis marker genes in Breast Cancer	13/104	1.E-07	CA9, CP, PGK1, EGLN1, TFRC, VEGFA, NDRG1, ADM, BNIP3	TMEFF1, IVNS1ABP, TMEM45A, RRAGD
KEGG	Arginine and proline metabolism	6/35	4.E-05	P4HA2, NOS3, P4HA1, NOS2A	EPRS, ALDH4A1
Reactome	Platelet Activation	7/86	1.E-02	PDGFA, TGFB3, FN1, VEGFA, ALDOA, SERPINE1	ARHGEF1
TargetScan	miR-1/206	23/583	3.E-03	PDGFA, NAMPT, HSP90B1, MET, ETS1, VEGFA, EDN1, CITED2, STC2	RNF165, GRK6, SLC7A6, PTPLAD1, ASPH, MYLK, RSBN1, SLC31A1, EVI1, PGAM1, SOX6, BTAF1, MXD1, GLCCI1
Protein interactions	PCAF	10/106	9.E-05	ENO1, NR4A1, PFKL, PGK1	RARA, CCNB1, SSRP1, RBPJ, EVI1, BTAF1

Table 2. Selected examples of functional enrichment of 101 previously validated HIF-1 targets and mapping of novel targets to these categories. The number of genes reflect the total number of known and predicted targets within the functional group. P-values for enrichment of validated HIF-1 targets were obtained using the hypergeometric distribution as described in Materials and Methods.