Benita et al. Supplementary Figures

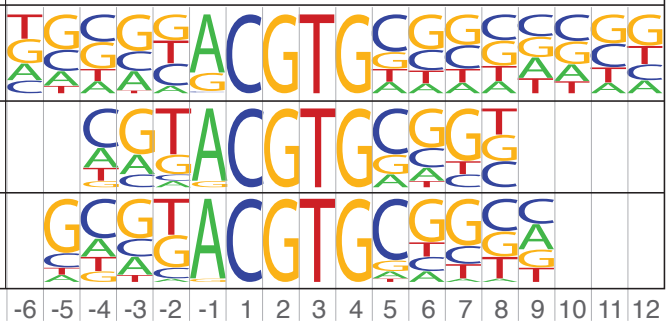
Source	Number of sites	Accession	Sequence Logo
Wenger et al.	104		
Transfac	12	M00466	
	23	M00797	

Figure S1: Position weight matrices of HIF-1. Matrices of HIF-1. The matrix at the top was compiled from 104 experimentally validated binding sites in human, mouse and rat collected by Wegner et al (Wenger, R.H., D.P. Stiehl, and G. Camenisch. 2005. Integration of oxygen signaling at the consensus HRE. Sci STKE).

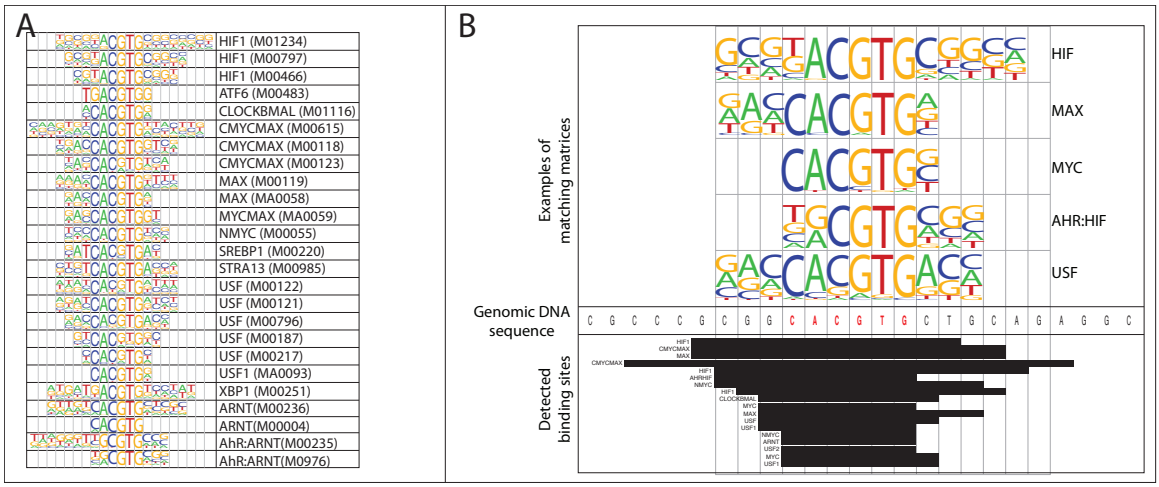


Figure S2: Transcription factors matching at the core HRE. (A) PWMs that were most commonly identified matching at the core HRE along with HIF-1. These occur most frequently when a C precedes the core HRE resulting in the E-box binding site CACGTG. (B) Example of the EBOX binding site in the proximal promoter of CITED2, a key hypoxic regulator (Yin, Z., et al. 2002. The essential role of Cited2, a negative regulator for HIF-1alpha, in heart development and neurulation. Proc Natl Acad Sci U S A 99: 10488-10493).

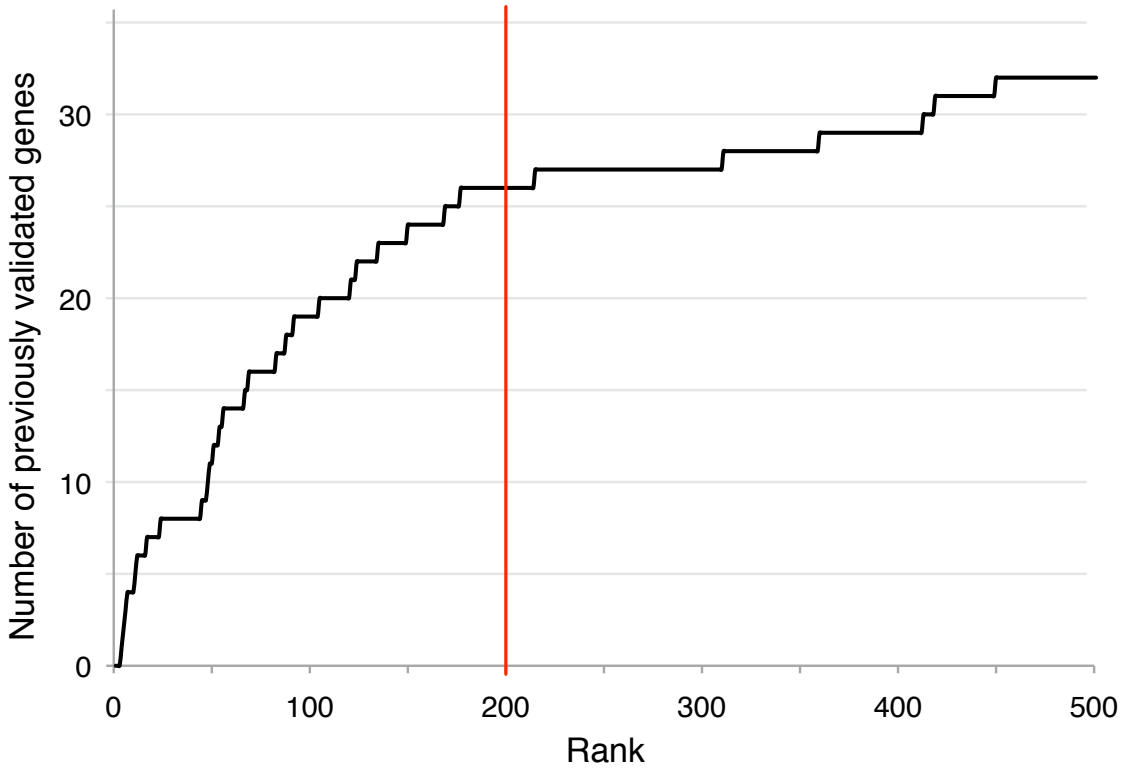


Figure S3: Cumulative distribution of previously validated HIF-1 target genes as a function of gene rank. The number of validated targets raises exponentially for genes ranked below 200. Therefore, the 200th rank was used as a cutoff of high confidence, indicated by the red line. HIF-1 validated targets, references and ranks are listed in supplementary Table S4.

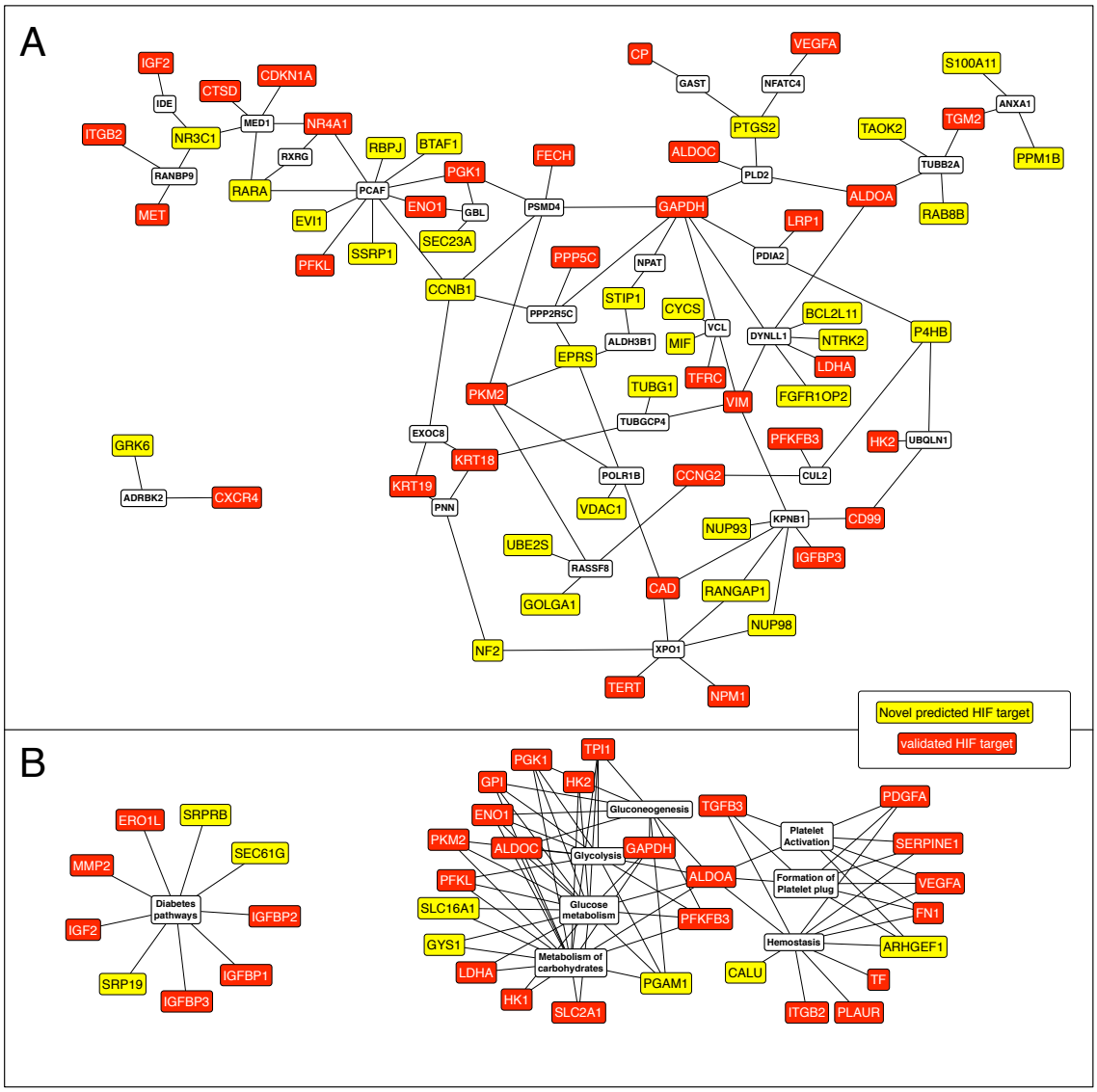


Figure S4. Graphical representation of protein-protein interaction (A) and Reactome pathways (B) enrichment for previously validated HIF-1 targets (red) and novel predicted targets (yellow). Only proteins/pathways that were significantly enriched ($p<0.05$) are shown. See supplementary Table S6 for analysis details.

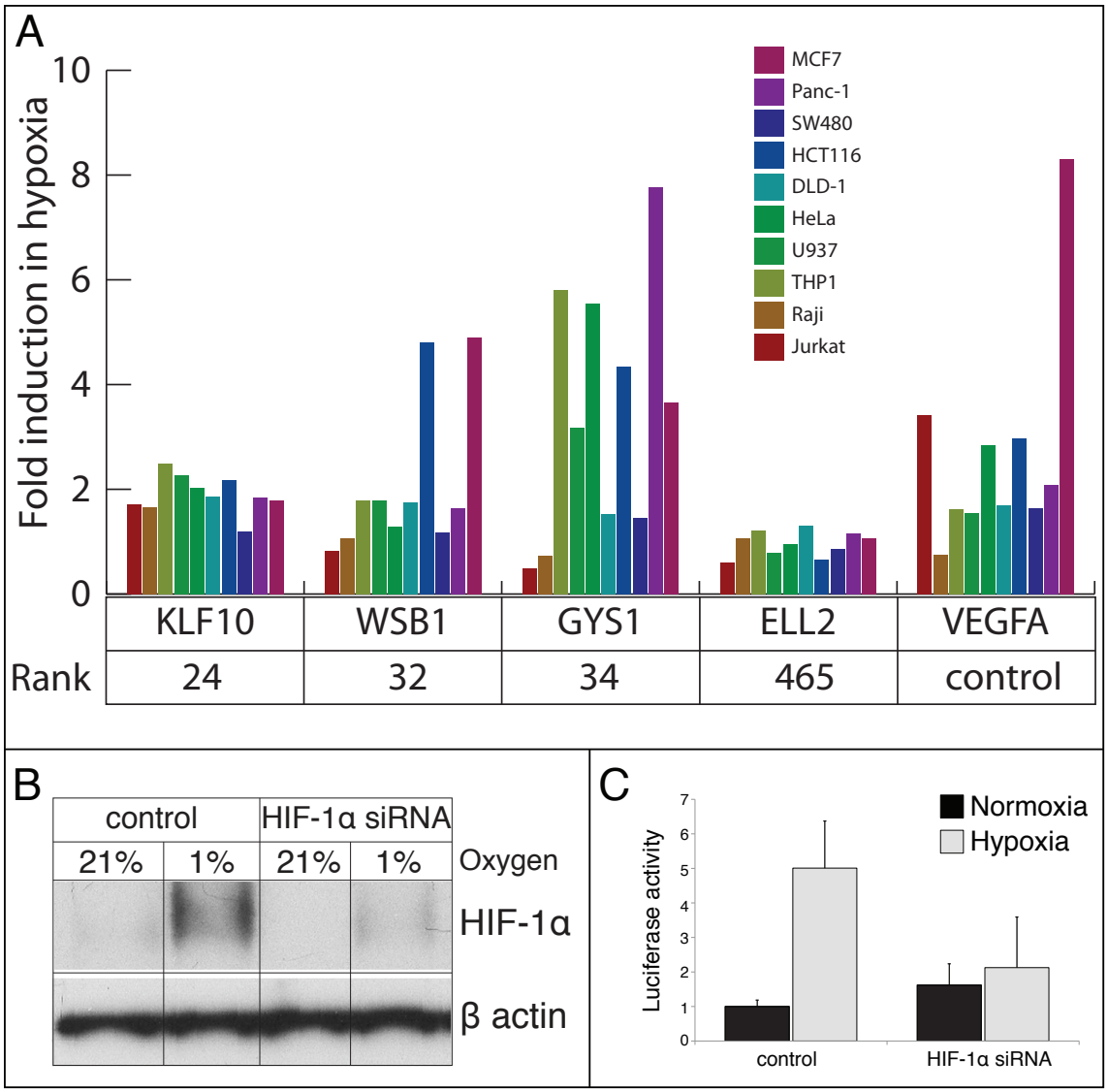


Figure S5: (A) Hypoxia response determined by qPCR for 3 predicted HIF targets within the top 50 genes and one within the top 500. VEGFA was used as a positive control. (B) HIF-1α protein levles as visualized by western blot using a HIF-1α antibody in normoxia (21%) and hypoxia (1%) in the absence (control) and presence of the HIF-1α siRNA. (C) Luciferase activity of ANKRD37 promoter1 in normoxia and hypoxia in the presence and absence of HIF-1α siRNA.

Supplementary Tables:

	Cell type	MCF7	U251	Astrocytes	Monocytes	B cells	HeLA
Microarray description	Available samples	normoxia	no treatment	Normoxia	Normoxia	Normoxia	Normoxia
		hypoxia	Hypoxia-mimetic				
		HIF activation in normoxia	HIF-1 siRNA in presence of hypoxia-mimetic	Hypoxia	Hypoxia	Hypoxia	Hypoxia
			Blocking HIF binding site in presence of hypoxia-mimetic				
	Replication	Triplicates	Triplicates	Duplicates	Duplicates	Duplicates	Duplicates
	Platform	Affymetrix U133A	Affymetrix U133 Plus 2.0	Affymetrix U133 Plus 2.0	Affymetrix U133A	Affymetrix U133 Plus 2.0	Affymetrix U133 Plus 2.0
	Reference	16565084	17708671	16507782	16849508	16517405	16507782

Table S1. Microarray data sets used for identifying genes that respond to hypoxia.

Table S2 (see Excel table). HIF-1 target genes. A list of known and validated HIF-1 target genes compiled from the scientific literature.

Table S3 (see Excel table): Top 5 PWMs enriched in each cell type in genes that respond to hypoxia but do not have a detectable HIF binding site.

Table S4 (see Excel table). Top 500 predicted HIF target genes.

Table S5 (see Excel table). KEGG analysis enrichment analysis for a set of 97 HIF target genes that ranked in the top 300 and responded to hypoxia in at least 3 cell types.

Table S6 (see Excel table). Functional enrichment of 101 previously validated HIF-1 targets and mapping of novel targets to those significantly enriched ($p<0.05$). The functional enrichment was also performed for novel targets alone (all predicted targets excluded all validated) and for a combined list of all validated and predicted targets (combined p-value).

Table S7. The HIF transcriptional network.

Table S8. HIF PWMs matching de-novo predicted motifs in the promoters of 97 HIF target genes that ranked in the top 300 and responded to hypoxia in at least 3 cell types. DME2 was employed to identify motifs 5-12 bases long and the score and rank shown are relevant for a that motif length only. The divergence from the HIF matrices is shown in parenthesis for each matrix. A divergence of 0 is a perfect match.

Table S9. De-novo identified motifs in the range of 5-12 bases which match a known PWM. This analysis was performed on a set of 97 HIF target genes that ranked in the top 300 and responded to hypoxia in at least 3 cell types. TF divergence was calculated using

MATCOMPARE (0=perfect match between motif and PWM, 1=no match between motif and PWM). Only motifs with a divergence below 0.25 are shown.