

Benita et al. Supplementary Figures

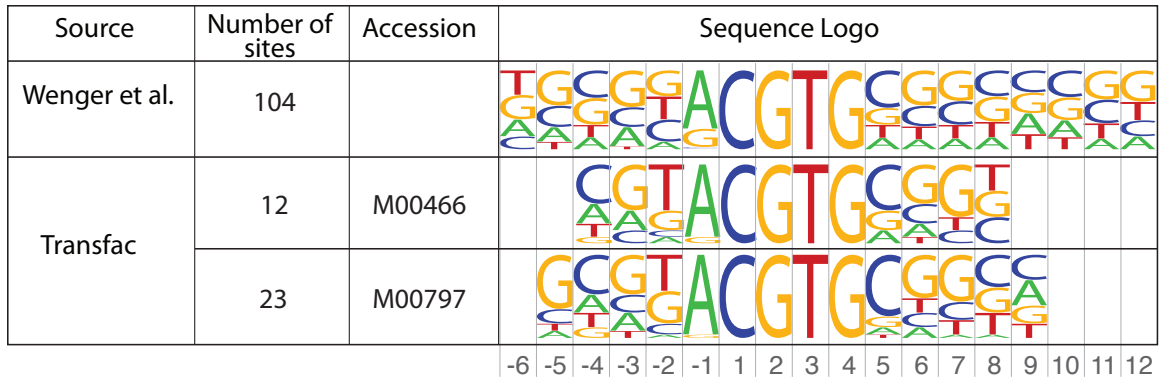


Figure S1: Position weight matrices of HIF-1. Matrices of HIF-1. The matrix at the top was compiled from 104 experimentally validated binding sites in human, mouse and rat collected by Wegner et al (Wenger, R.H., D.P. Stiehl, and G. Camenisch. 2005. Integration of oxygen signaling at the consensus HIF-1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

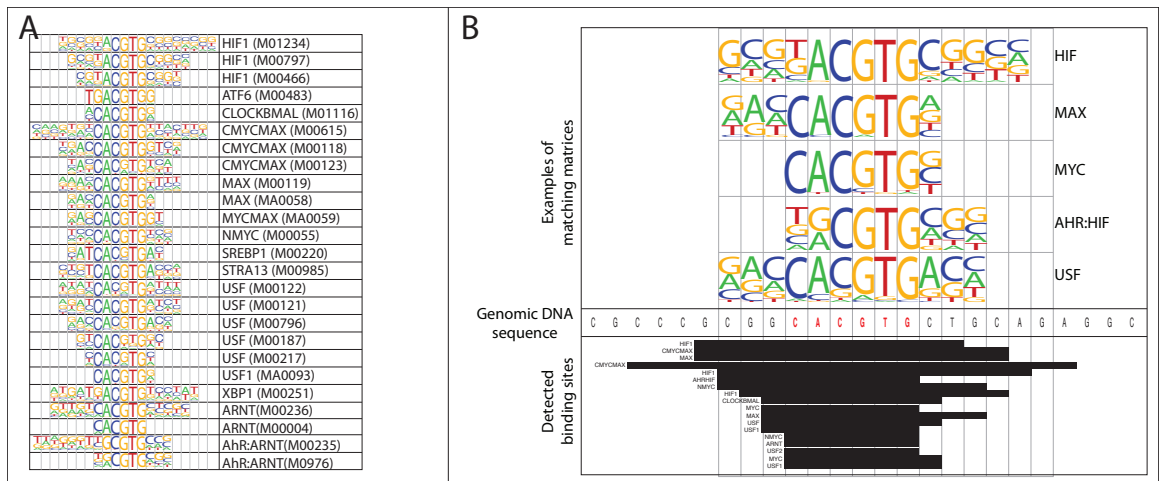


Figure S2: Transcription factors matching at the core HIF-1 binding site. (A) PWMs that were most commonly identified matching at the core HIF-1 binding site along with HIF-1. These occur most frequently when a C precedes the core HIF-1 binding site resulting in the E-box binding site CACGTG. (B) Example 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).

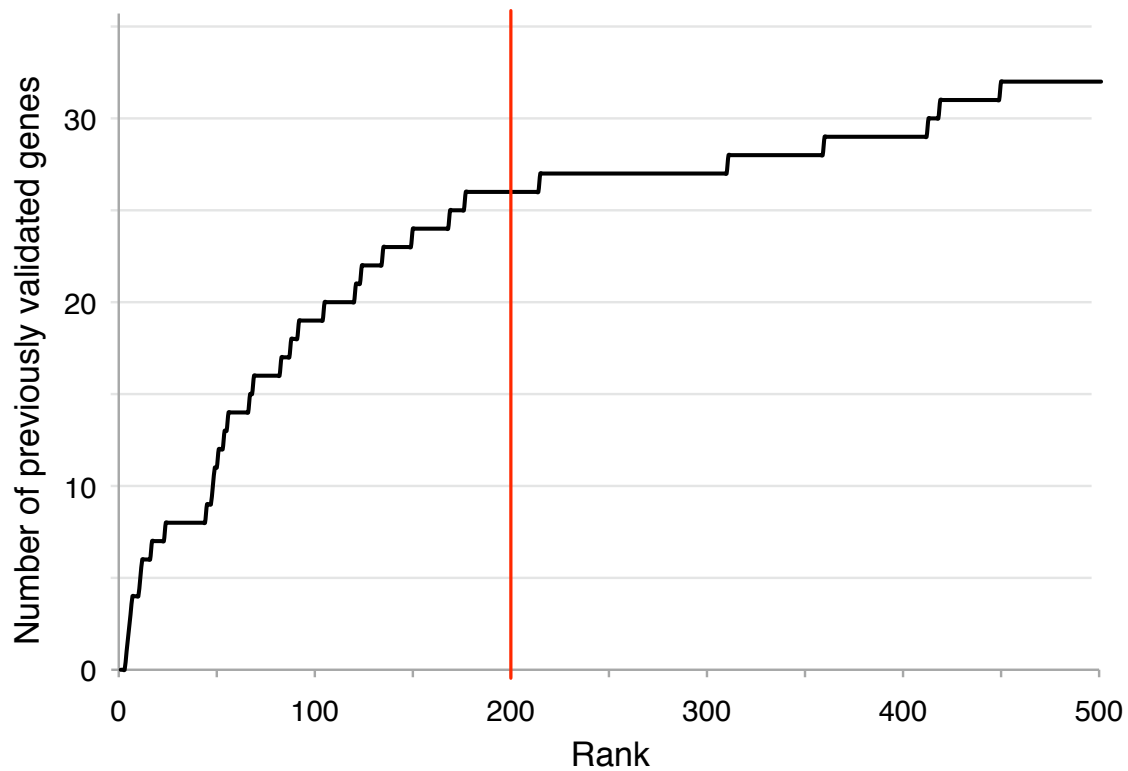


Figure S3: Cumulative distribution of previously validated HIF-1 target genes as a function of gene rank. The number of validated targets raises exponentially for genes ranked below 200. Therefore, the 200th rank was used as a cutoff of high confidence, indicated by the red line. HIF-1 validated targets,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

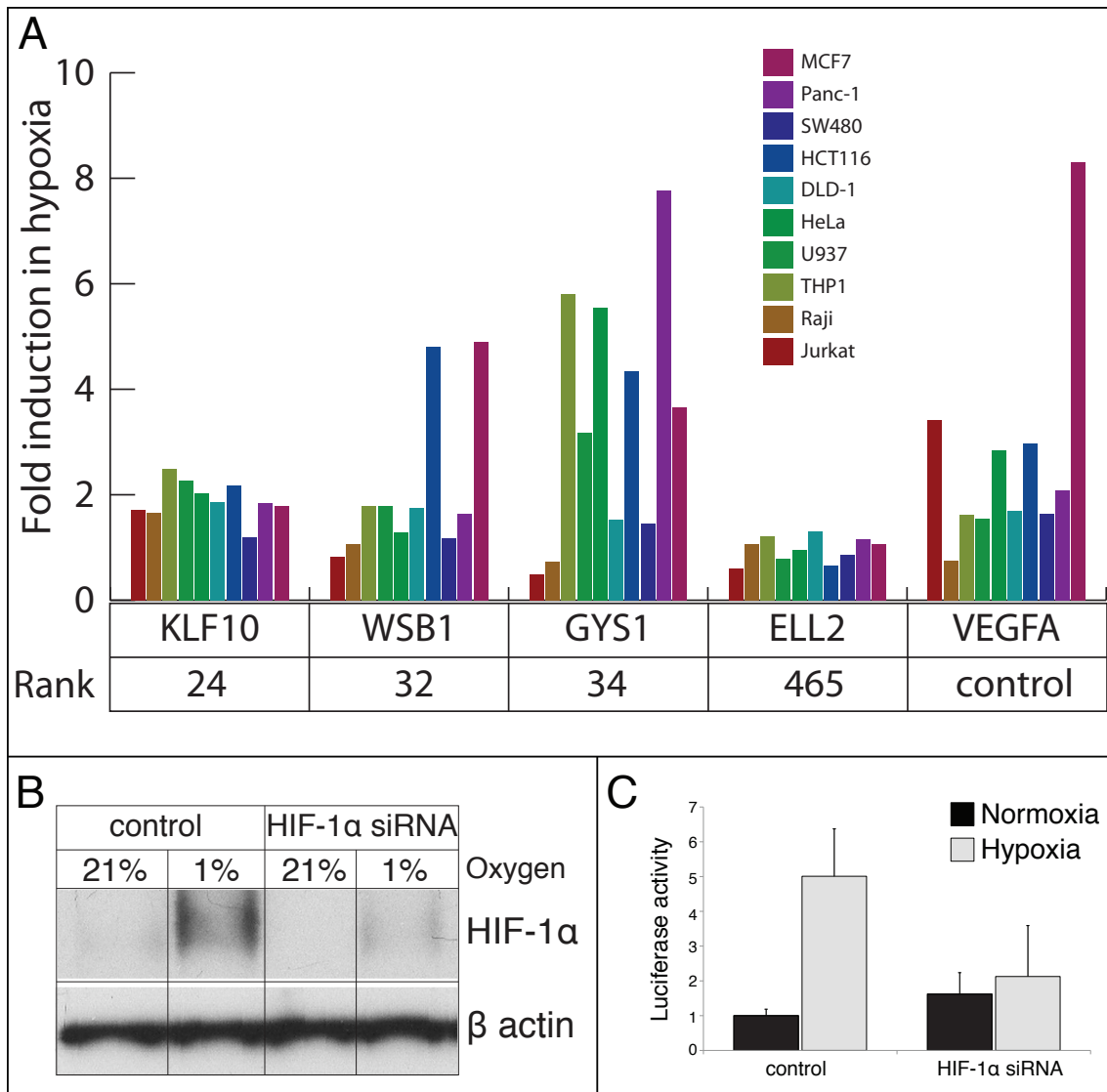


Figure S4: (A) Hypoxia response determined by qPCR for 3 predicted HIF targets within the top 50 genes and one within the top 500. VEGFA was used as a positive control. (B) HIF-1 α protein levels as visualized by western blot using a HIF-1 α antibody in normoxia (21%) and hypoxia (1%) in the absence (control) and presence of the HIF-1 α siRNA. (C) Luciferase activity of ANKRD37 promoter1 in normoxia and hypoxia in the presence and absence of HIF-1 α siRNA.

Supplementary Tables:

Cell type	MCF7	U251	Astrocytes	Monocytes	B cells	HeLA
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 (PubMed ID)	1	2	3	4	5	3

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

References for Table S1:

1. Elvidge, G.P., Glenny, L., Appelhoff, R.J., Ratcliffe, P.J., Ragoussis, J. and Gleadle, J.M. (2006) Concordant regulation of gene expression by hypoxia and 2-oxoglutarate-dependent dioxygenase inhibition: the role of HIF-1 α , HIF-2 α , and other pathways. *J Biol Chem*, **281**, 15215-15226.
2. Nickols, N.G., Jacobs, C.S., Farkas, M.E. and Dervan, P.B. (2007) Modulating hypoxia-inducible transcription by disrupting the HIF-1-DNA interface. *ACS Chem Biol*, **2**, 561-571.
3. Mense, S.M., Sengupta, A., Zhou, M., Lan, C., Bentsman, G., Volsky, D.J. and Zhang, L. (2006) Gene expression profiling reveals the profound upregulation of hypoxia-responsive genes in primary human astrocytes. *Physiol Genomics*, **25**, 435-449.
4. Bosco, M.C., Puppo, M., Santangelo, C., Anfosso, L., Pfeffer, U., Fardin, P., Battaglia, F. and Varesio, L. (2006) Hypoxia modifies the transcriptome of primary human monocytes: modulation of novel immune-related genes and identification of CC-chemokine ligand 20 as a new hypoxia-inducible gene. *J Immunol*, **177**, 1941-1955.
5. Kim, J.W., Tchernyshyov, I., Semenza, G.L. and Dang, C.V. (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab*, **3**, 177-185.

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.