Supporting Information

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SI Materials and Methods

Identification of ChIP-chip Peaks. The model-based analysis of tiling-array (*MAT*) algorithm (11) was used to identify regions enriched by ChIP-chip ("hits") on Affymetrix whole-genome or promoter tiling arrays. *MAT* was used to identify probe intensity peaks comparing triplicate hypoxic ChIP vs. hypoxic input ("Hypoxic") and normoxic ChIP vs. normoxic input ("Normoxic"). *MAT* was run with the same parameters for each: bandwidth = 200, maximum gap = 400, minimum probes = 10, and P value cutoff = 1×10^{-5}. Hits appearing solely in the hypoxic samples were retained as hypoxia-unique. In cases where hypoxic and normoxic hits overlapped, hits for which the *MAT* score of hypoxic subtracted by that of normoxic samples were above the cutoff were also retained. The *MAT* library and mapping files were based on the March 2006 Human Genome Assembly (HG18). Hits flagged by *MAT* as mapping to repeat regions were excluded from consideration.

Linking ChIP Hits to RefSeq Genes. ChIP hits were associated with RefSeq genes from the University of California Santa Cruz (UCSC) RefGene table for HG18 based on chromosomal position. Specifically, hits falling within a window of 10 kb upstream and 2.5 kb downstream of a given RefSeq TSS were annotated as being in the "promoter" region of that gene. This definition of promoter is used as shorthand for "promoter and proximal enhancer region." Hits falling between 2.5 kb downstream of the TSS and the end of the transcribed region were annotated as being intragenic to the associated gene. Hits within 3 kb downstream of the transcriptional end position were termed "3-prime" to the gene. Hits within 50 kb of a gene but excluded from the above 3 categories were labeled "within 50 kb" and tentatively assigned to that gene. Any hit falling >50 kb of any gene was tagged as "long range" and not associated with any gene.

De Novo Motif Analysis. For de novo motif scanning, we used *Weeder* (12) at the www.pesolelab.it/modtools/ web site, and *MEME* (13) on the http://meme.sdsc.edu/meme4/cgi-bin/meme.cgi web site.

shRNA Knockdown. shRNA plasmids (pLKO.1) for HIF-1α and 2α, ARNT, JARID1B, and GFP (as a control) were obtained from the RNAi Consortium. Lentivirus was packaged according to RNAi Consortium protocols.

qPCR Validation of ChIP Hits (ChIP-qPCR). qPCR primers were designed against regions of interest and also 2 negative control regions (5 and 10 kb upstream of the VEGF gene). All primer sequences are specified in Table S1. Fold enrichment was assessed by performing qPCR for the target region on samples taken before (Input) and after ChIP (ChIP) and calculated from the critical threshold cycles (Ct) as: fold enrichment = target region ratio[2$\hat{\Delta}$Ct (Ct ChIP − Ct Input)]/control region ratio[2$\hat{\Delta}$Ct (Ct ChIP − Ct Input)]. HIF-1-specific binding was defined as a >2-fold enrichment comparing HIF-1 ChIP with matched input samples and a >2-fold greater binding in hypoxic samples relative to either normoxic cells or hypoxic HIF-1α knockdown (sh-HIF1α) cells.

mRNA Expression Profiling. HepG2, U87, and MDA-MB231 breast cancer cells were collected under normoxic conditions (0 h) and after 4, 8, and 12 h of hypoxia treatment (0.5% O_2). For each cell line, 3 replicates of total RNA at each time point were prepared by using TRIzol, labeled, and hybridized to Affymetrix HG-U133Plus2 oligonucleotide arrays. We used GCRMA module on the GenePattern web site (http://genepattern.broad.mit.edu) to normalize the microarrays and identify probe sets that changed significantly comparing hypoxic samples with normoxic samples. For analysis of human tumor material, a dataset for Grade IV glioblastoma multiforme (14) was downloaded from the NCBI.

Immunofluorescence Microscopy. HepG2 or HepG2-shARNT cells were transiently transfected with a JARID1B expression plasmid pCS2 + 3HA-JARID1B, a gift from Yang Shi, Harvard Medical School. After 24 h, cells were plated on coverslips in 6-well plates. Cells were then incubated for another 24 h under either normoxic or hypoxic (0.5%O_2) conditions. Cells were then fixed with 4% paraformaldehyde, permeablized, and blocked with 3% milk-PBS. The coverslips were incubated with primary antibodies at RT for 1 h with anti-HA mAb (12CA5) and H3K4me3 pAb (ab8580, 1:4000; Abcam). After washes, the coverslips were incubated with Alexa Fluor 594-conjugated goat-anti-mouse and Alexa Fluor 488-conjugated goat-anti-rabbit secondary antibodies (1:500; Molecular Probes) for another half hour. The coverslips were then washed and mounted with Vectashield (Vector Laboratories) containing DAPI.

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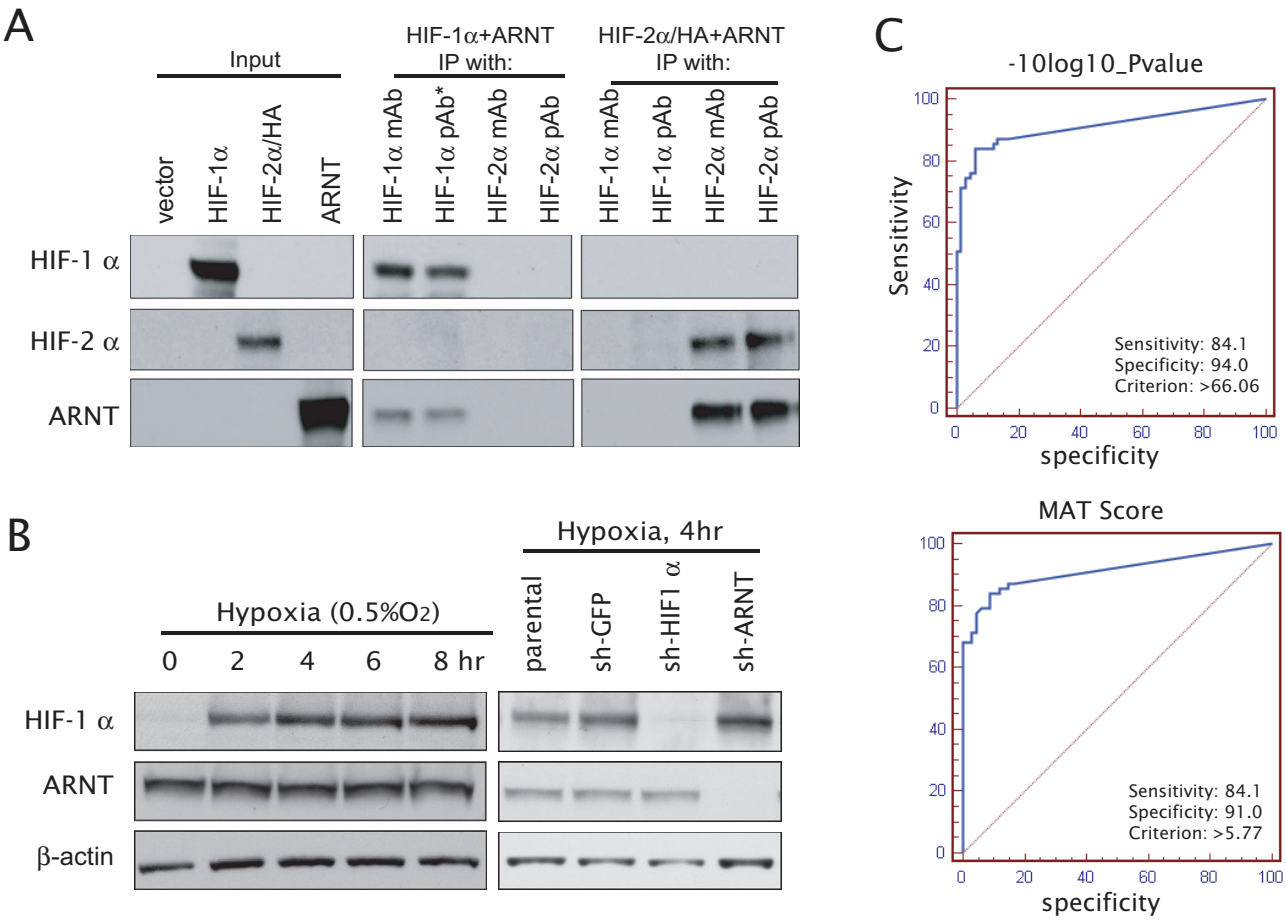


D

All loci tested		total	ChIP-chip Hits Yes	No
		130	55	75
ChIP-qPCR enriched?	Yes	63	52	11
	No	67	3	64
Sensitivity =TP/(TP+FN)			82.5% (52/63)	
Specificity =TN/(TN+FP)				95.5% (64/67)

E

Known HREs tested		total	ChIP-chip Hits Yes	No
		40	15	25
ChIP-qPCR enriched?	Yes	20	15	5
	No	20	0	20
Sensitivity =TP/(TP+FN)			75% (15/20)	
Specificity =TN/(TN+FP)				100% (20/20)

Fig. S1. HIF-1 ChIP. (*A*) HIF-1α, HIF-2α, and ARNT (HIF-1β) were produced by in vitro-coupled transcription/translation. HIF-1 and HIF-2 heterodimers were immunoprecipitated and detected with the indicated antibodies. (*B*) HepG2 cells were exposed to hypoxia (0.5% O_2) for the specified periods of time, and the abundance of HIF-1α, ARNT/HIF-1β, and β-actin were determined by Western blot analysis. To knock down HIF-1α or ARNT, HepG2 cells were infected with the lentivirus-expressing shRNAs targeting HIF-1α (sh-HIF1α) or ARNT (sh-ARNT), in comparison with an shRNA targeting EGFP (sh-GFP) as a control. (*C*) The ChIP-qPCR results from Table S1 were used as benchmarks to generate receiver operating characteristic (ROC) curves for ChIP-chip vs. ChIP-qPCR results. Based on the results, we choose the cutoff as MAT score >5.77 and $-10\log10$ *P* value >66.05, which equals a *P* value $<2.5 \times 10^{-7}$. (*D*) Sensitivity and specificity of our analysis pipeline based on analysis of 130 loci. (*E*) Sensitivity and specificity of our analysis pipeline based on analysis of a subset of 40 well-characterized HIF-1 target loci.

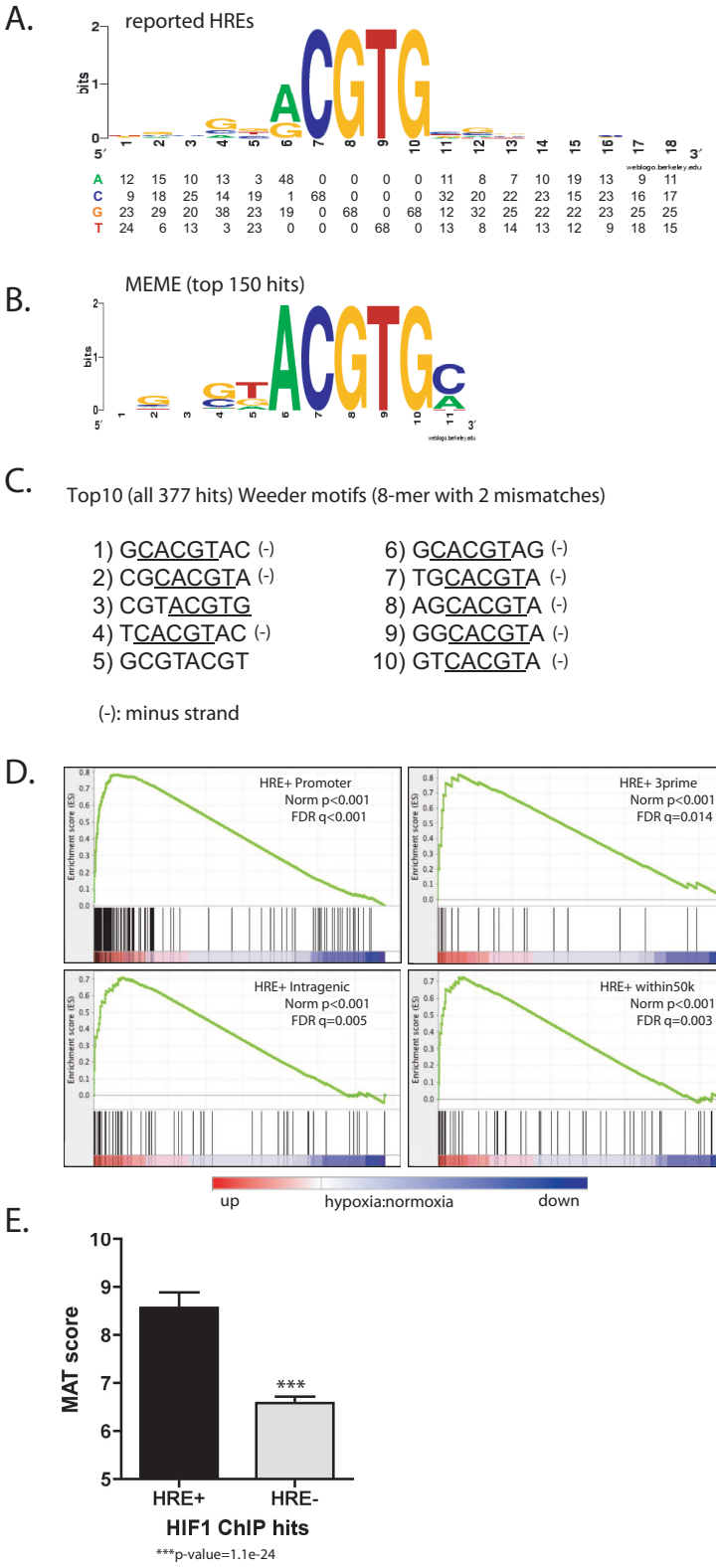
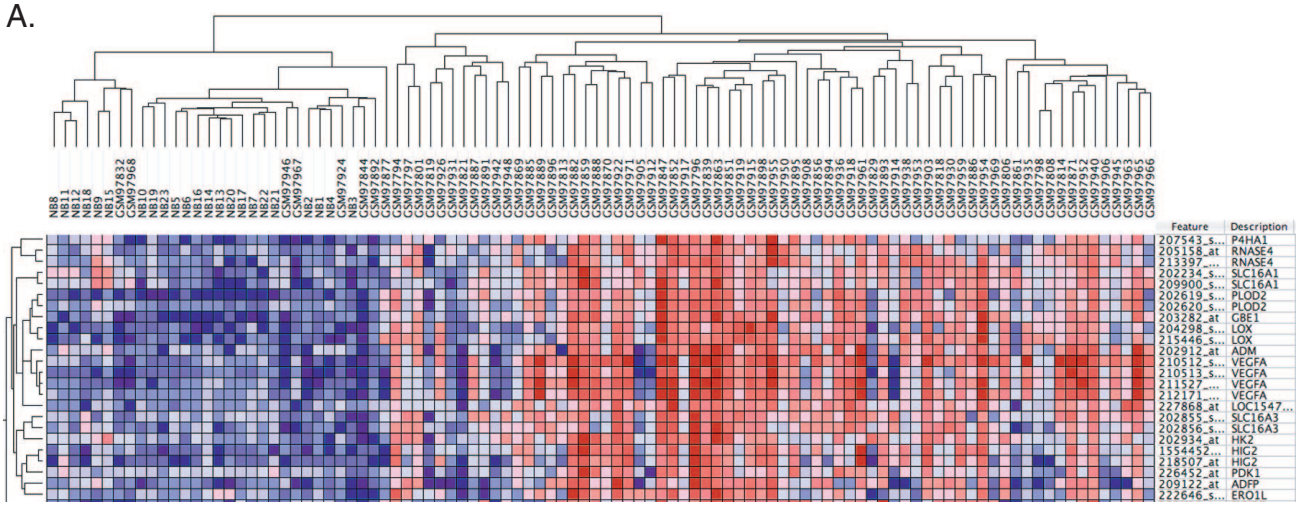


Fig. S2. Consensus and de novo HIF-1-binding motifs. (*A*) WebLogo and position-weighted matrix was derived from 68 previously well-characterized human HIF-1-bound loci (1). (*B* and *C*) Overrepresented motifs were identified by de novo motif search using *MEME* (*B*) and *Weeder* (*C*) using the top ChIP hits. (*D*) *GSEA* establishes that HIF-1 binding is positively correlated with up-regulated expression under hypoxia regardless of the binding location. (*E*) *MAT* scores of HRE+ vs. HRE− ChIP hits, represented as mean ± 95% CI.

A.



B.

HIF-1 targets	
P4HA1	SLC16A3
PLOD2	HK2
GBE1	HIG2
LOX	PDK1
ADM	ADFP
VEGFA	ERO1L

Fig. S3. Glioblastoma samples are hypoxic. (*A*) Unsupervised hierarchical clustering was used to analyze gene expression profiles from glioblastoma multiforme samples (GSM-xxxxx) and normal brain (NB-xx) from GEO dataset GSE42900. Nearly all tumor samples clustered separately from the normal brain. (*B*) One large gene cluster that drove this distinction was a collection of genes consisting of known HIF-1 target genes or previously unknown HIF-1 targets identified in the present study.

Other Supporting Information Files

Table S1 (XLS)
Table S2 (XLS)
Table S3 (XLS)
Table S4 (XLS)