Table S4. TFBS matrices that are over-represented at HIF-immunoprecipitating gene loci. The table gives the raw p-value for comparison of the frequency of each motif at HIF binding loci, versus similarly sited control promoter regions that do not bind HIF. Motifs that showed very strong enrichment that remained significant after correction for multiple comparisons are indicated in bold type.

Transcription factor	TRANSFAC Matrix	p-value
AHR	V_AHR_01	0.008641
AHRARNT	V_AHRARNT_02	0.001599
ARNT	V_ARNT_01	0.004226
AHRHIF	**V_AHRHIF_Q6**	**4.87E-11**
HIF1	**V_HIF1_Q5**	**6.87E-12**
HIF1	**V_HIF1_Q3**	**1.03E-06**
AP1	V_AP1_Q4	0.001314
AP1	V_AP1_Q2	0.01684
AP1	V_AP1_Q6	0.03855
AP2	V_AP2_Q6	0.0108
ATF	V_ATF_01	0.000182
ATF	V_ATF_B	0.002934
ATF1	V_ATF1_Q6	0.0001149
ATF6	V_ATF6_01	0.007882
BCL6	V_BCL6_Q3	0.03286
CIZ	V_CIZ_01	0.00677
CLOCKBMAL	V_CLOCKBMAL_Q6	0.006379
CREB	V_CREB_Q2_01	0.0007594
CREB	V_CREB_Q4	0.001179
CREB	V_CREB_Q3	0.001345
CREB	V_CREB_01	0.00167
CREB	V_CREB_02	0.003965
CREB	V_CREB_Q4_01	0.006948
CREBATF	V_CREBATF_Q6	0.003571
CREBP1	V_CREBP1_Q2	0.003714
DR4	V_DR4_Q2	0.008326
E2F	**V_E2F_Q2**	**8.10E-05**
E2F	V_E2F_Q4_01	0.0004922
E2F	V_E2F_Q6_01	0.001131
E2F	V_E2F_Q3_01	0.002045
E2F	V_E2F_02	0.007408
E2F	V_E2F_01	0.03058
E2F	V_E2F_Q6	0.03248
E2F	V_E2F_Q3	0.03896
E2F1	V_E2F1_Q4	9.70E-05
E2F1	V_E2F1_Q6	0.0003085
E2F1	V_E2F1_Q4_01	0.005919
E2F1	V_E2F1_Q3	0.008204
E2F1	V_E2F1_Q3_01	0.01068
E2F1DP1	V_E2F1DP1_01	0.0003577
E2F1DP1RB	V_E2F1DP1RB_01	0.02379
E2F4DP1	V_E2F4DP1_01	0.01637
E2F4DP2	V_E2F4DP2_01	6.51E-05
EGR	V_EGR_Q6	0.02669

Transcription factor	TRANSFAC Matrix	p-value
ETF	V_ETF_Q6	0.01612
ETS	V_ETS_Q6	0.00672
GATA4	V_GATA4_Q3	0.03814
GR	V_GR_01	0.04885
HMGIY	V_HMGIY_Q6	0.002761
HMGIY	V_HMGIY_Q3	0.04821
HSF1	V_HSF1_01	0.008644
HSF2	V_HSF2_01	0.02112
HTF	V_HTF_01	9.76E-05
IRF	V_IRF_Q6_01	0.02656
IRF2	V_IRF2_01	0.001255
KROX	V_KROX_Q6	0.03809
MAF	V_MAF_Q6	0.001962
MAX	V_MAX_01	0.01351
MEF2	V_MEF2_Q6_01	0.04077
MIF1	**V_MIF1_01**	**1.89E-05**
MYB	V_MYB_Q6	0.0004821
MYB	V_MYB_Q5_01	0.00336
MYCMAX	V_MYCMAX_B	0.003563
MZF1	V_MZF1_02	0.02065
NFAT	V_NFAT_Q6	0.03558
NFKB	V_NFKB_C	0.04746
NKX25	V_NKX25_Q5	0.006206
NRF1	**V_NRF1_Q6**	**5.87E-07**
POLY	V_POLY_C	0.00299
PU1	V_PU1_Q6	0.006268
RBPJK	V_RBPJK_01	0.001952
RORA	V_RORA_Q4	0.04623
SP1	V_SP1_Q6	0.02178
SP1	V_SP1_Q6_01	0.02209
STAT	V_STAT_01	0.003794
STAT1	V_STAT1_03	0.0003586
STAT1	V_STAT1_02	0.02091
STAT3	V_STAT3_02	0.00134
STAT4	V_STAT4_01	0.00243
USF	V_USF_Q6	0.02282
USF	V_USF_01	0.0265
USF	V_USF_Q6_01	0.03955
XFD1	V_XFD1_01	0.02582
ZF5	V_ZF5_01	0.001589
ZF5	V_ZF5_B	0.01457

Figure S1

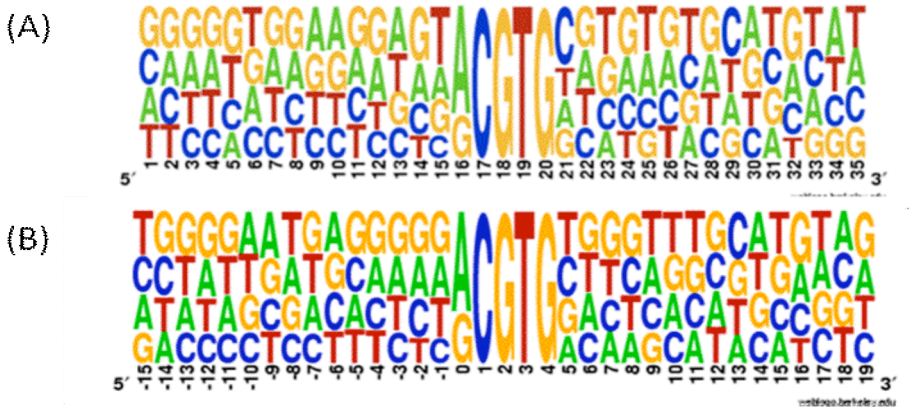

Figure S1. Sequences surrounding core RCGTG motifs. All RCGTG motifs within HIF-1α and HIF-2α immunoprecipitating sequences were identified. The regions 15 bp 5' and 3' from this motif were then analyzed for evidence of over-representation. (A) HIF-1α. (B) HIF-2α. No significant over-representation of bases outside the RCGTG motif was observed.

Figure S2

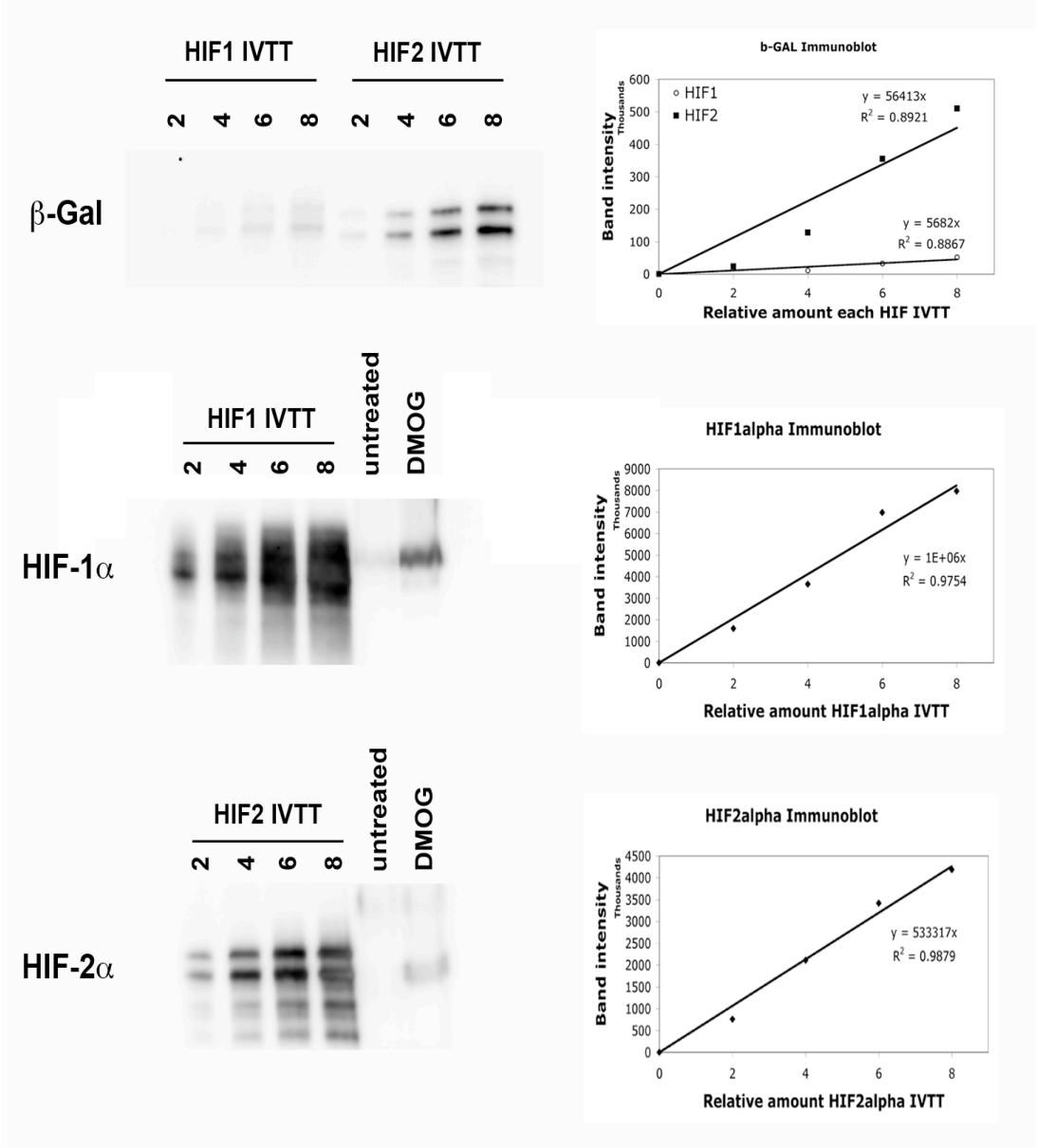

Figure S2. (A) Fusion proteins of Gal4 (amino acids 1-147) linked to full-length HIF-1α and HIF-2α were prepared by in vitro transcription and translation (IVTT) in rabbit reticulocyte lysate and blotted using anti-Gal antibody. (B & C) Each Gal-tagged IVTT preparation was then blotted with anti-HIF-1α and anti-HIF-2α antibodies and compared with lysates from DMOG treated MCF-7 cells to determine relative amounts of each isoform.

Figure S3

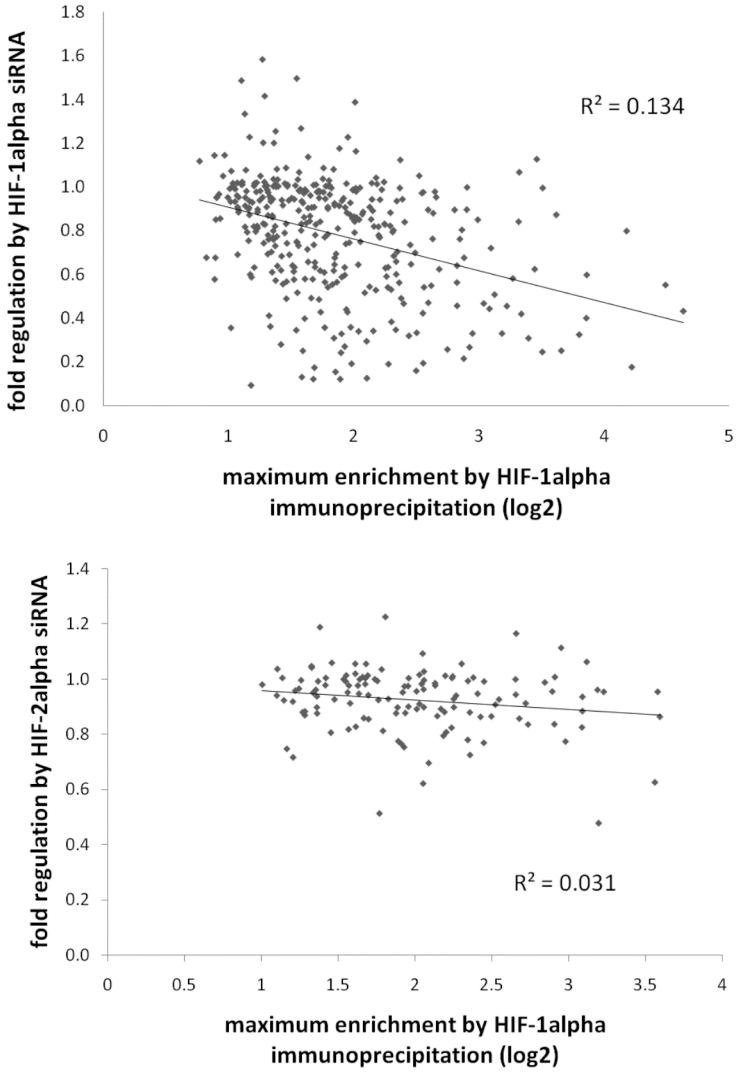

Figure S3. Fold regulation of gene expression by siRNA vs maximum enrichment by ChIP for each HIFα isoform. For each immunoprecipitated gene locus the fold regulation by HIF-1α or HIF-2α siRNA at that locus was plotted against the maximum fold enrichment for the immunoprecipitation as an indicator of strength of binding.

Figure S4

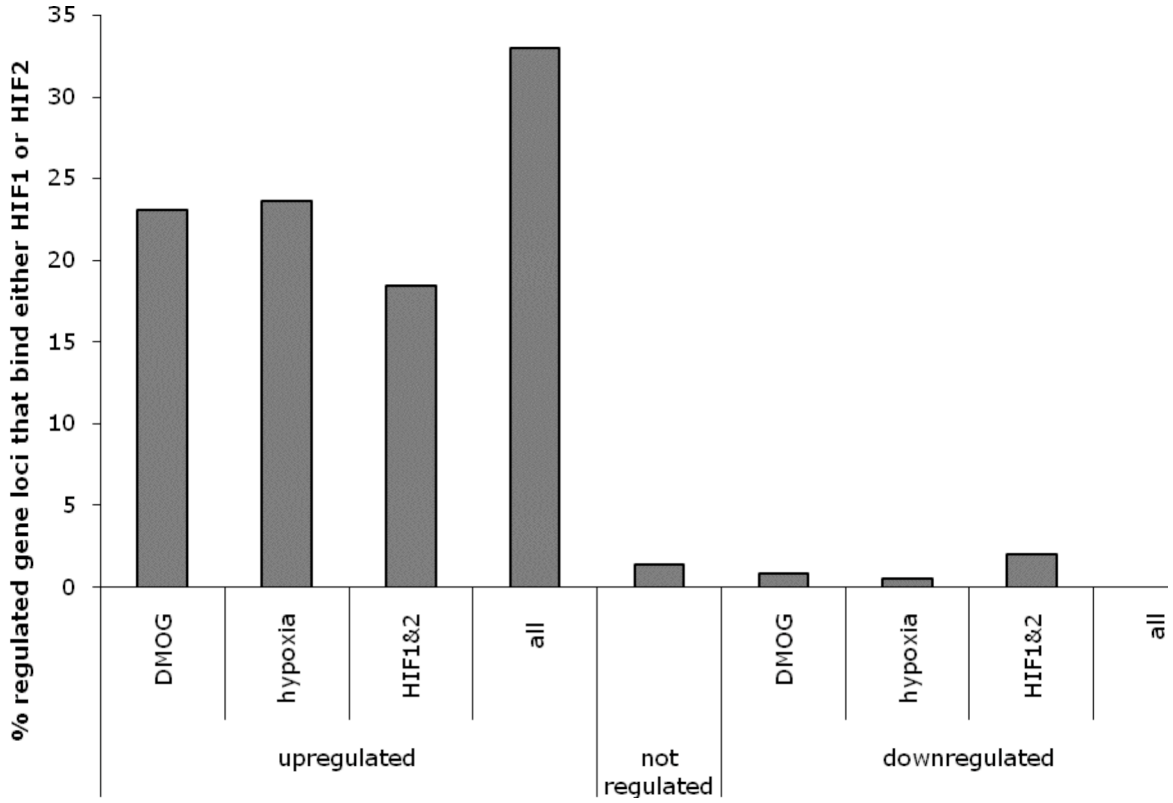

Figure S4. Relationship between direction of regulation by hypoxic stimuli and chromatin immunoprecipitation by anti-HIF-1α or anti-HIF-2α. Gene loci that were either up- or down-regulated (FDR < 0.05) by DMOG, hypoxia, or HIF, assessed by combined siRNA directed against HIF-1α and HIF-2α, (HIF1 and HIF-2), or all three stimuli (all) were defined. The proportion of gene loci captured by either of the anti-HIFα immunoprecipitations is given for each of these functional groups. A similar proportion of gene loci up regulated by each or all stimuli bound to HIF, whereas HIF binding to gene loci that were down regulated by each or all stimuli, was not different from unregulated loci (defined on the basis of an expression ratio of 0.95 to 1.05 in DMOG treated versus untreated cells).