TET1-Mediated Hydroxymethylation Facilitates Hypoxic Gene Induction In Neuroblastoma

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Figure S1, Related to Figure 1

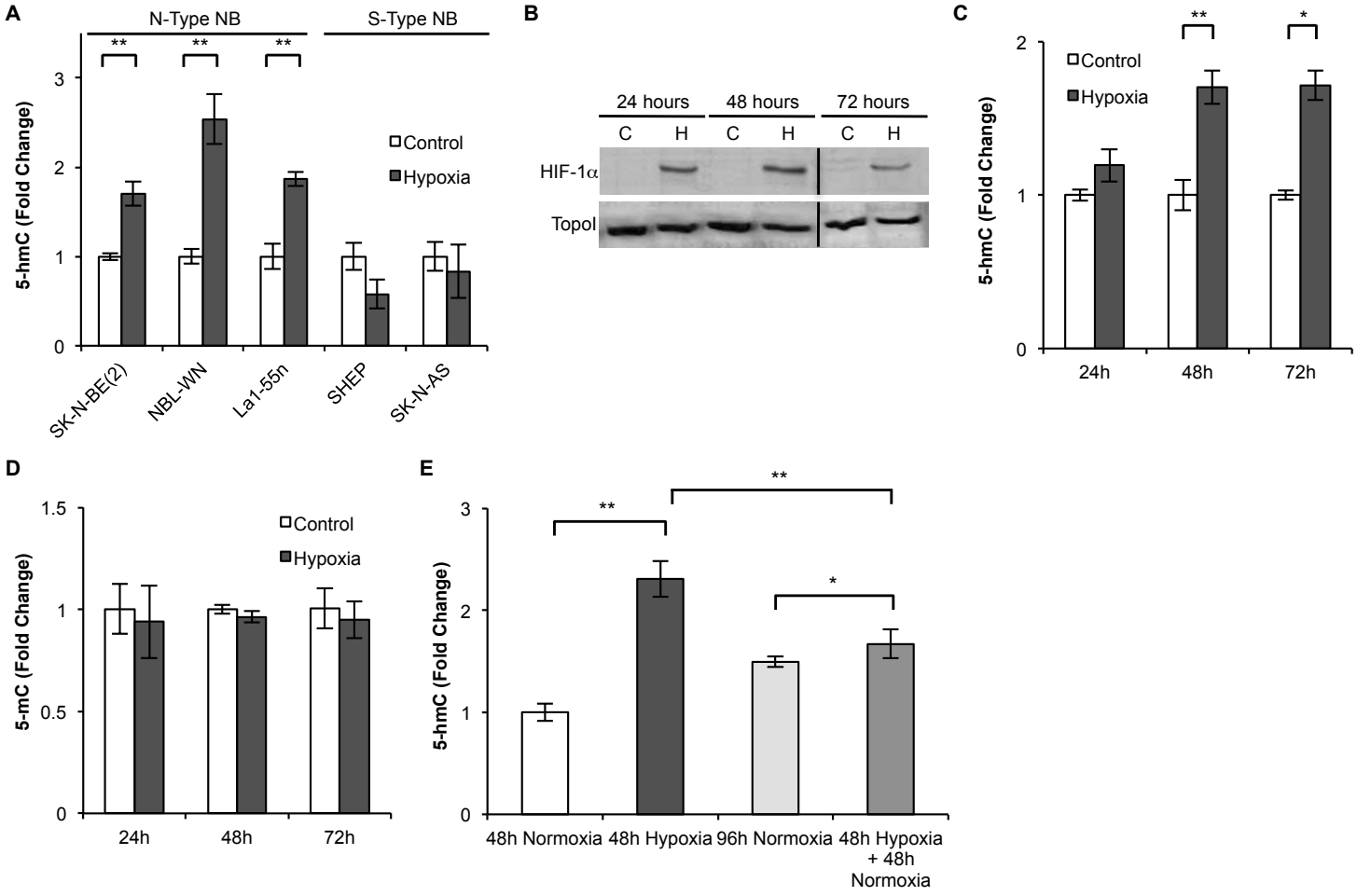


Figure S2, Related to Figure 2

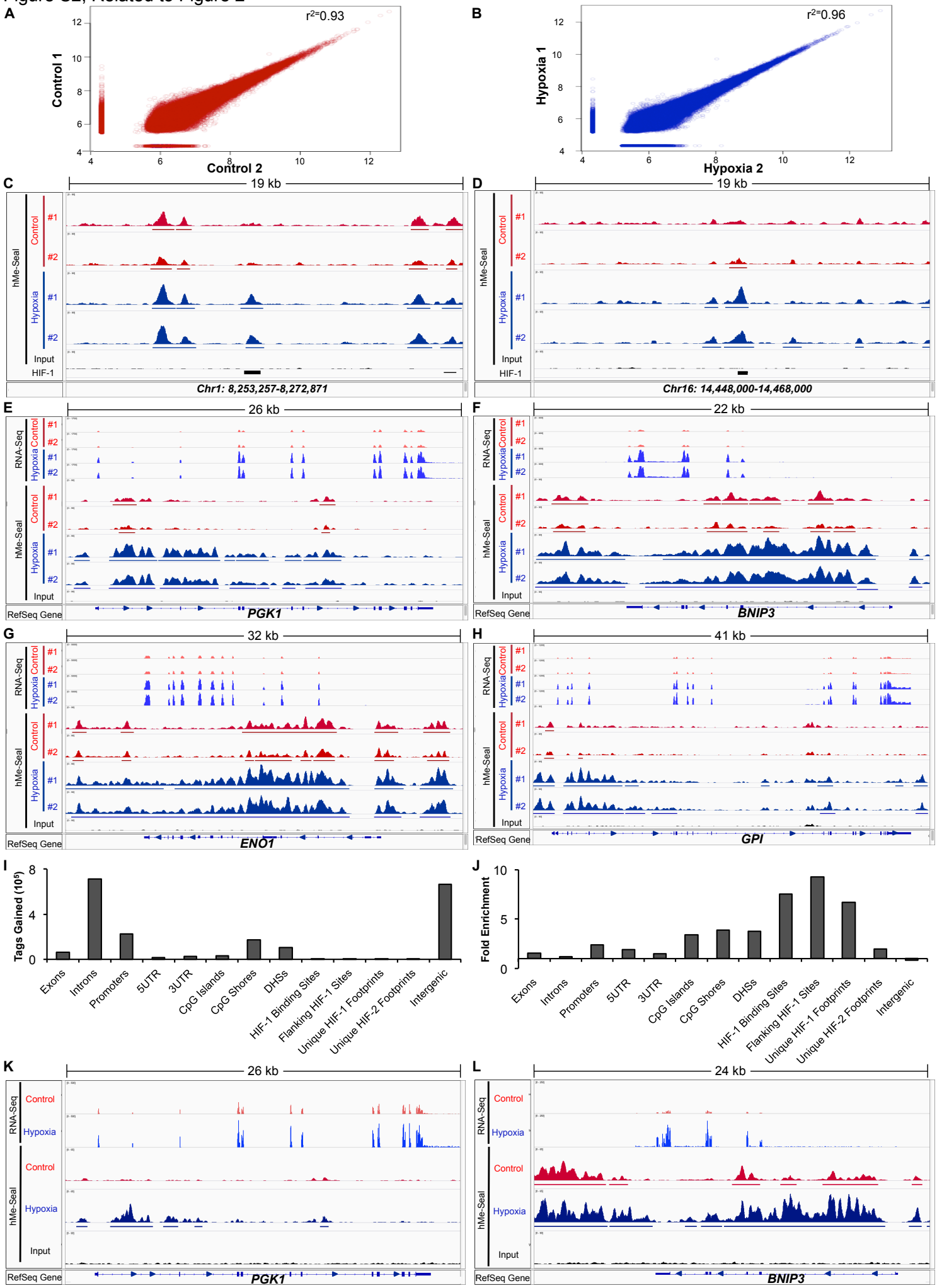


Figure S3, Related to Figure 3

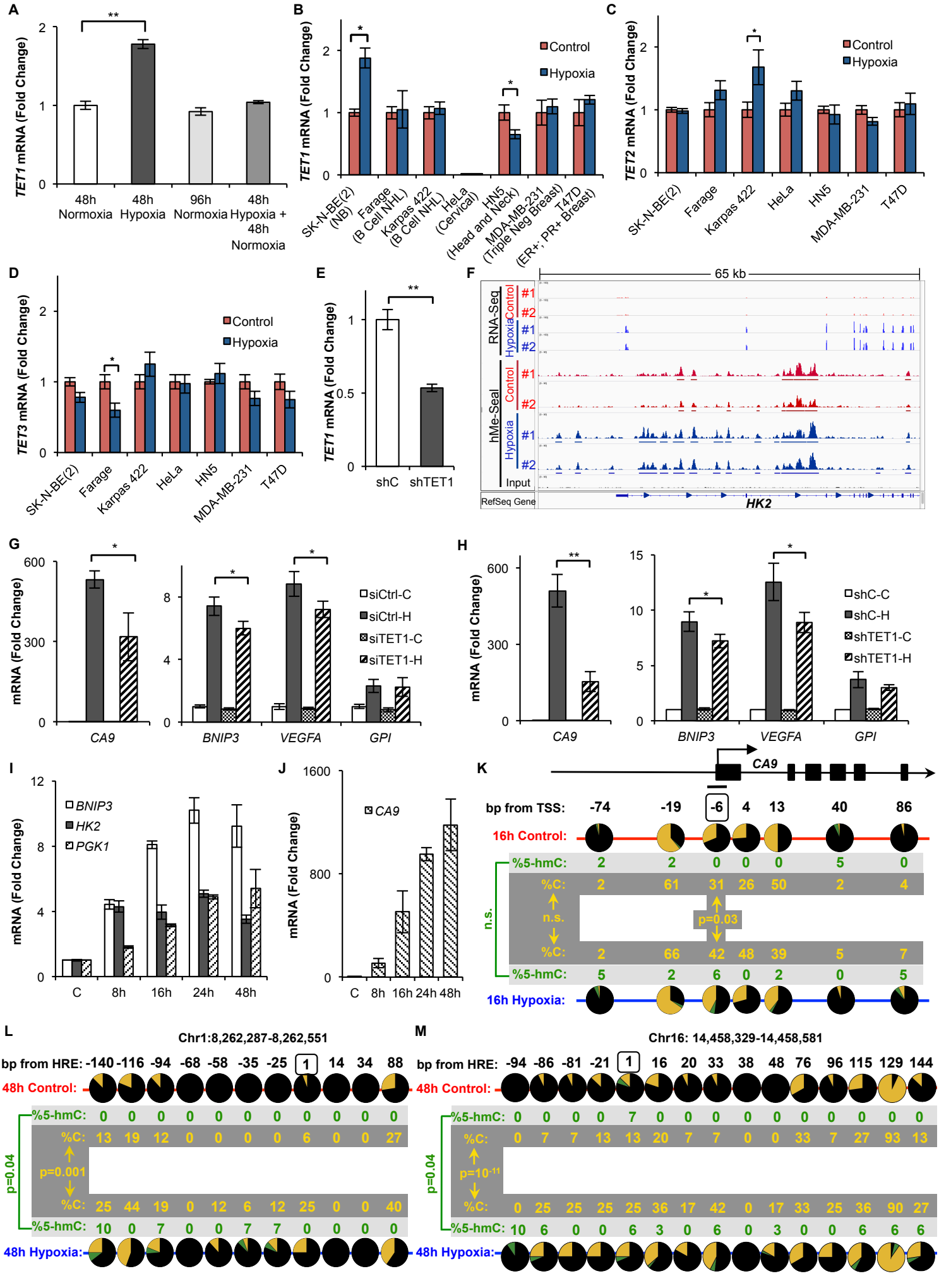
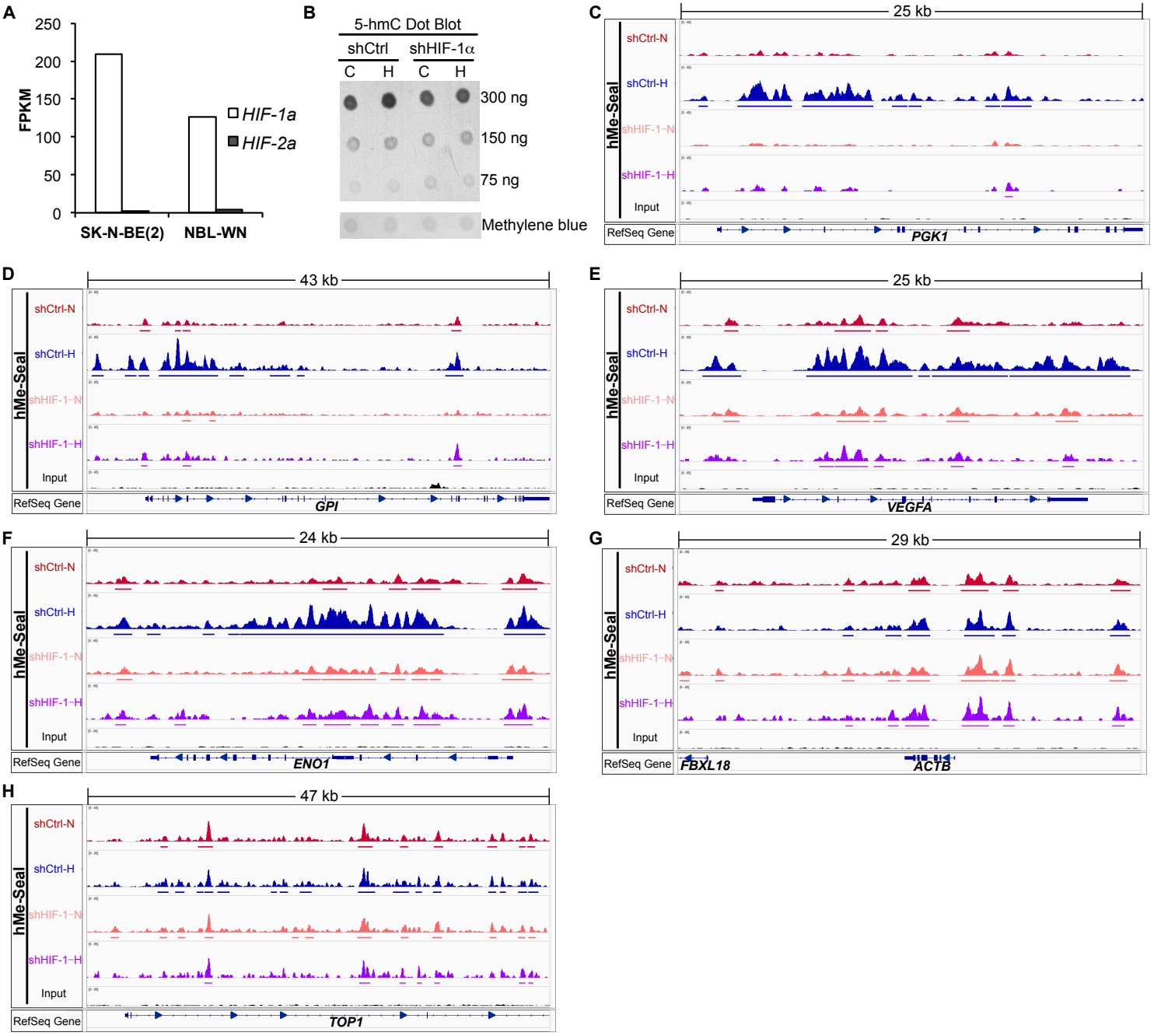


Figure S4, Related to Figure 4



Supplemental Figure Legends

Supplemental Figure S1. Hypoxia Induces 5-hmC Increases That Are Reversible Following Re-Oxygenation, Related to Figure 1

(A) Quantification of 5-hmC in neuroblastoma cells exposed to 48 hours of normoxia or hypoxia. 5-hmC levels are calculated relative to normoxic controls for each cell line ($n \geq 3$).

(B) HIF-1α immunoblot of SK-N-BE(2) cells exposed to 24, 48, or 72 hours of hypoxia or normoxia.

(C and D) Quantitation of 5-hmC and 5-mC by HPLC-MS/MS in SK-N-BE(2) cells exposed to 24, 48, or 72 hours of hypoxia. Levels of 5-mC and 5-hmC are calculated relative to normoxic controls for each time point ($n \geq 3$).

(E) Global 5-hmC levels in SK-N-BE(2) cells quantified by HPLC-MS/MS after: 48 hours of normoxia, 48 hours of hypoxia, 96 hours of normoxia, and 48 hours of hypoxia followed by 48 hours of normoxia. Bar graphs represent means ± SEM of three experiments. P-values calculated by Student's t-test, *$p<0.05$, **$p<0.01$.

Supplemental Figure S2. 5-hmC Gains at Hypoxia Induced Genes and HIF-1 Binding Sites Are Highly Reproducible Between Biological Replicates and Neuroblastoma Cell Lines, Related to Figure 2

(A and B) The number of hMe-Seal sequencing tags mapping to each genomic position from two biological replicates of experiments performed with SK-N-BE(2) cells is plotted for control (A) and hypoxia (B) samples. R^2 values represent results from linear regression analysis.

(C and D) Examples of HIF-1 ChIP-Seq peaks that gain 5-hmC in hypoxia. hMe-Seal sequencing data represent two biological replicates of experiments using SK-N-BE(2) cells. The

HIF-1 track represents the positions of previously published ChIP-Seq peaks, which are displayed as black bars (Schödel et al., 2011).

(E-H) RNA-Seq and hMe-Seal sequencing data at hypoxia induced genes. Data represent two biological replicates of experiments using SK-N-BE(2) cells.

(I and J) Absolute 5-hmC gains (I) and enrichment of 5-hmC gains (J) at genomic elements in NBL-WN cells.

(K and L) RNA-Seq and hMe-Seal sequencing data at hypoxia-induced genes in NBL-WN cells.

Supplemental Figure S3. *TET1* is Up-Regulated in Hypoxia and is Required for Full Hypoxic Gene Induction, Related to Figure 3

(A) *TET1* mRNA quantified by qPCR after: 48 hours of normoxia, 48 hours of hypoxia, 96 hours of normoxia, or 48 hours of hypoxia followed by 48 hours of normoxia. Experiments performed using SK-N-BE(2) cells. Data represent means ± SEM of three experiments. P-value calculated by Student's t-test, **p<0.01.

(B-D) *TET* expression measured by qPCR after 48 hours of hypoxia using: Farage (B-cell non-Hodgkin lymphoma), Karpas 422 (diffuse large B-cell lymphoma with t(11;14)), HeLa (cervical cancer), HN5 (head and neck cancer), MDA-MB-231 (triple negative breast cancer), and T47D (ER+ PR+ breast cancer) cell lines. Data represent means ± SEM of three experiments. P-values calculated by Student's t-test, *p<0.05.

(E) *TET1* expression in shTET1 and shCtrl transduced cells measured by qPCR. Data represent means ± SEM of three experiments. P-value calculated by Student's t-test, **p<0.01.

(F) hMe-Seal and RNA-Seq data at *HK2* from SK-N-BE(2) cells exposed to 48 hours of hypoxia. Data represent two biological replicates.

(G and H) Gene expression after 16 hours of hypoxia in cells depleted of *TET1* by siRNA (G) or shRNA (H). Data represent means ± SEM of three experiments. P-value calculated by Student's t-test, *p<0.05, **p<0.01.

(I and J) Induction kinetics of hypoxia-induced genes in SK-N-BE(2) cells exposed to 8-48 hours of hypoxia. mRNA levels quantified by qPCR. Data represent means ± SEM from three experiments.

(K) TAB-Seq of the *CA9* TSS after 16 hours of hypoxia. The boxed CpG represents the CpG within the ACGTG consensus sequence. P-values were calculated by Chi-squared tests using data from all CpGs within the amplicon or the CpG within the HRE only. Black, green, and gold represent 5-mC, 5-hmC, and unmodified cytosine, respectively. Data represent pooled sequencing data from three independent experiments.

(L and M) TAB-Seq of HREs gaining 5-hmC by hMe-Seal experiments after 48 hours of hypoxia or normoxia. Data represent pooled sequencing data from three independent experiments. P-values calculated by Chi-squared tests.

Supplemental Figure S4. Genome-Wide and Site-Specific 5-hmC Gains are HIF-1-Dependent, Related to Figure 4

(A) *HIF-1α* and *HIF-2α* expression in SK-N-BE(2) and NBL-WN cells as measured by RNA-Seq.

(B) Dot blot quantification of global 5-hmC levels in shCtrl or shHIF-1α transduced SK-N-BE(2) cells exposed to 48 hours of hypoxia.

(C-F) hMe-Seal sequencing data at hypoxia-induced genes obtained from shCtrl or shHIF-1α transduced cells exposed to 48 hours of hypoxia or normoxia.

(G and H) hMe-Seal sequencing data acquired from shCtrl or shHIF-1α transduced cells at regions not regulated by hypoxia.