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Supplemental Material to:

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Epigenetic deregulation in pediatric acute lymphoblastic leukemia

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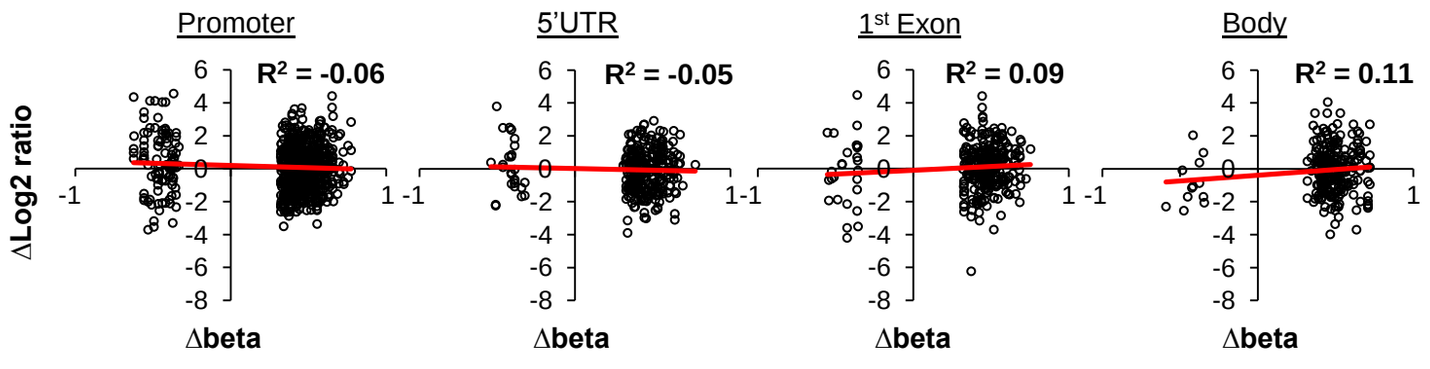
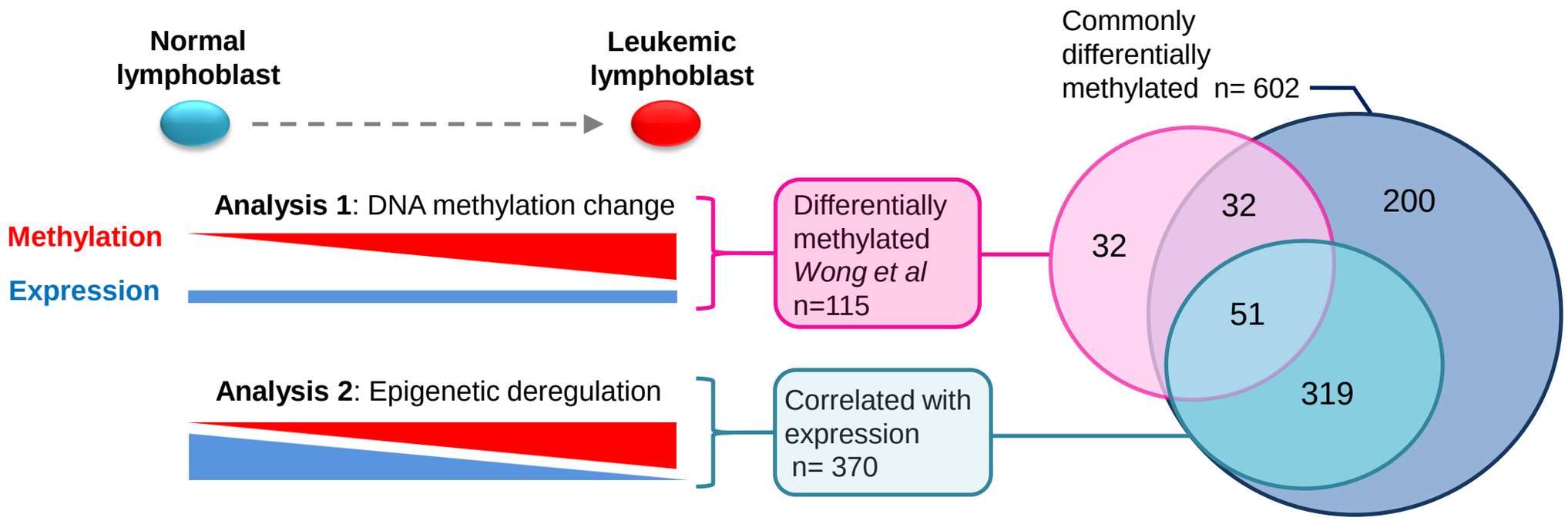
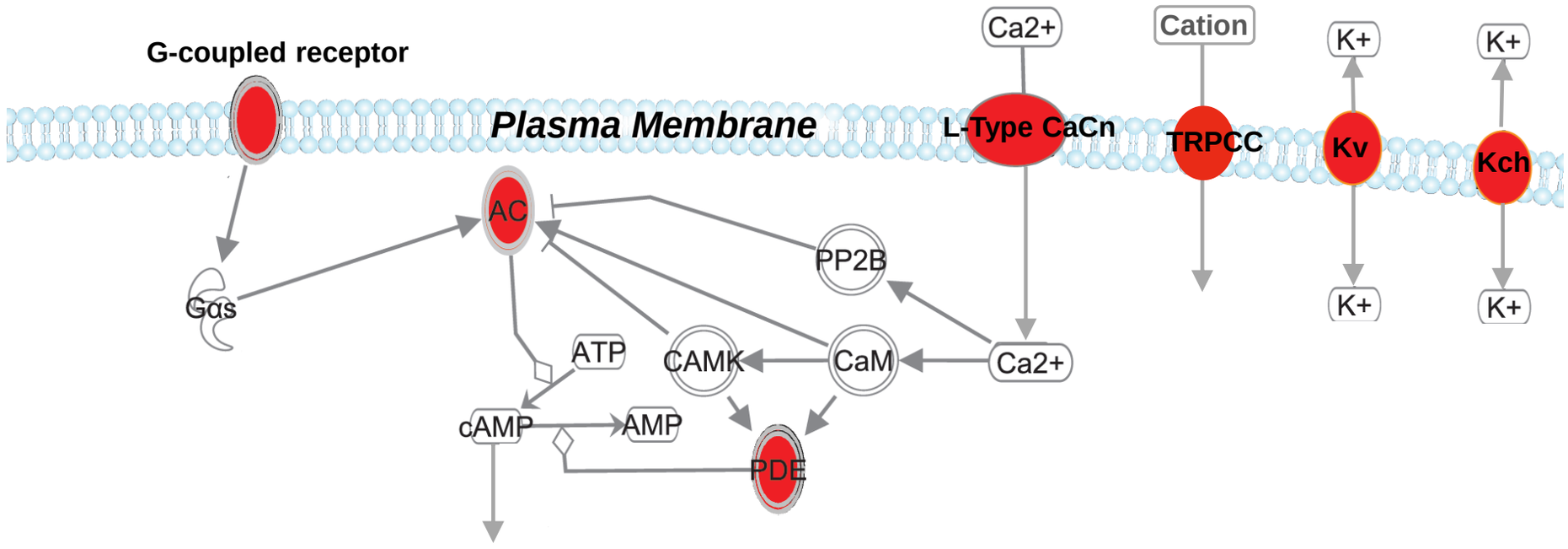


Supplementary Figure 1.

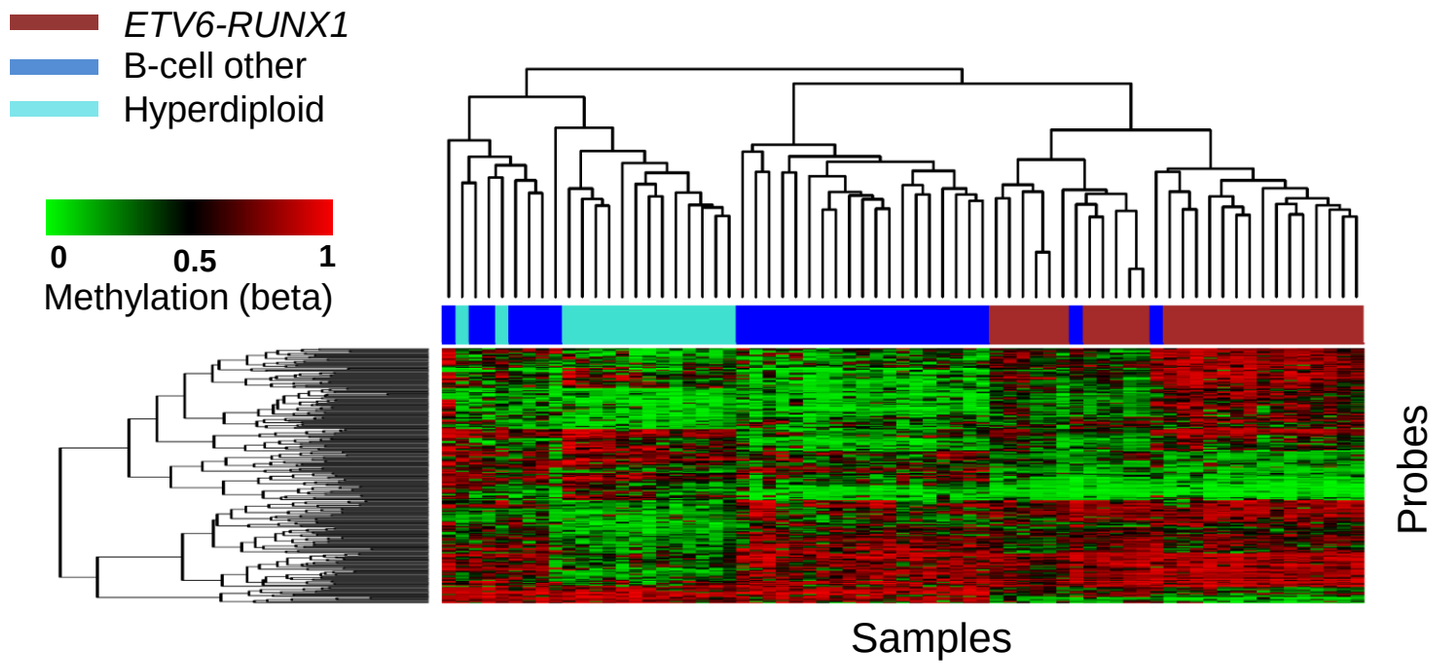


Supplementary Figure 2.

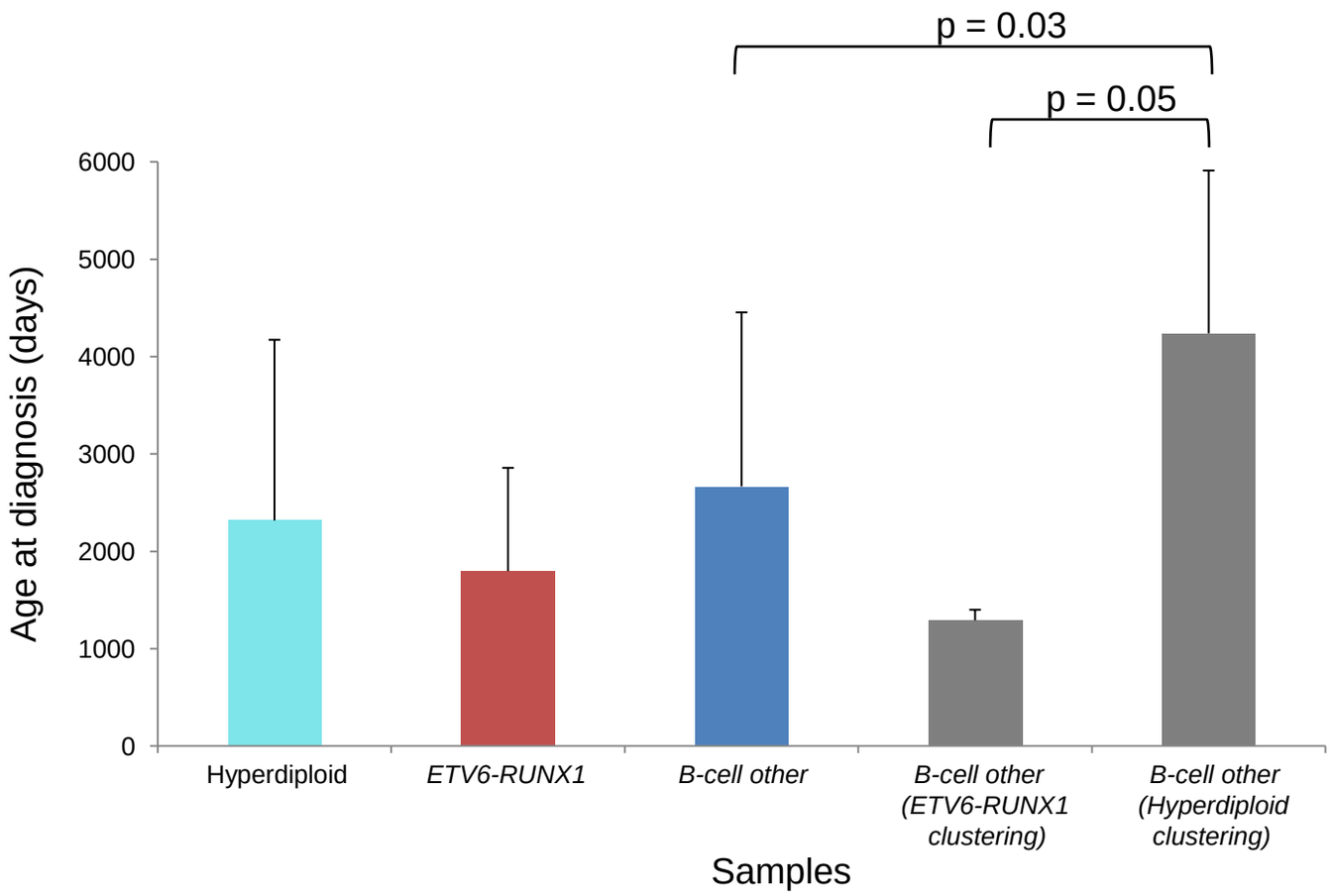


Supplementary Figure 3.

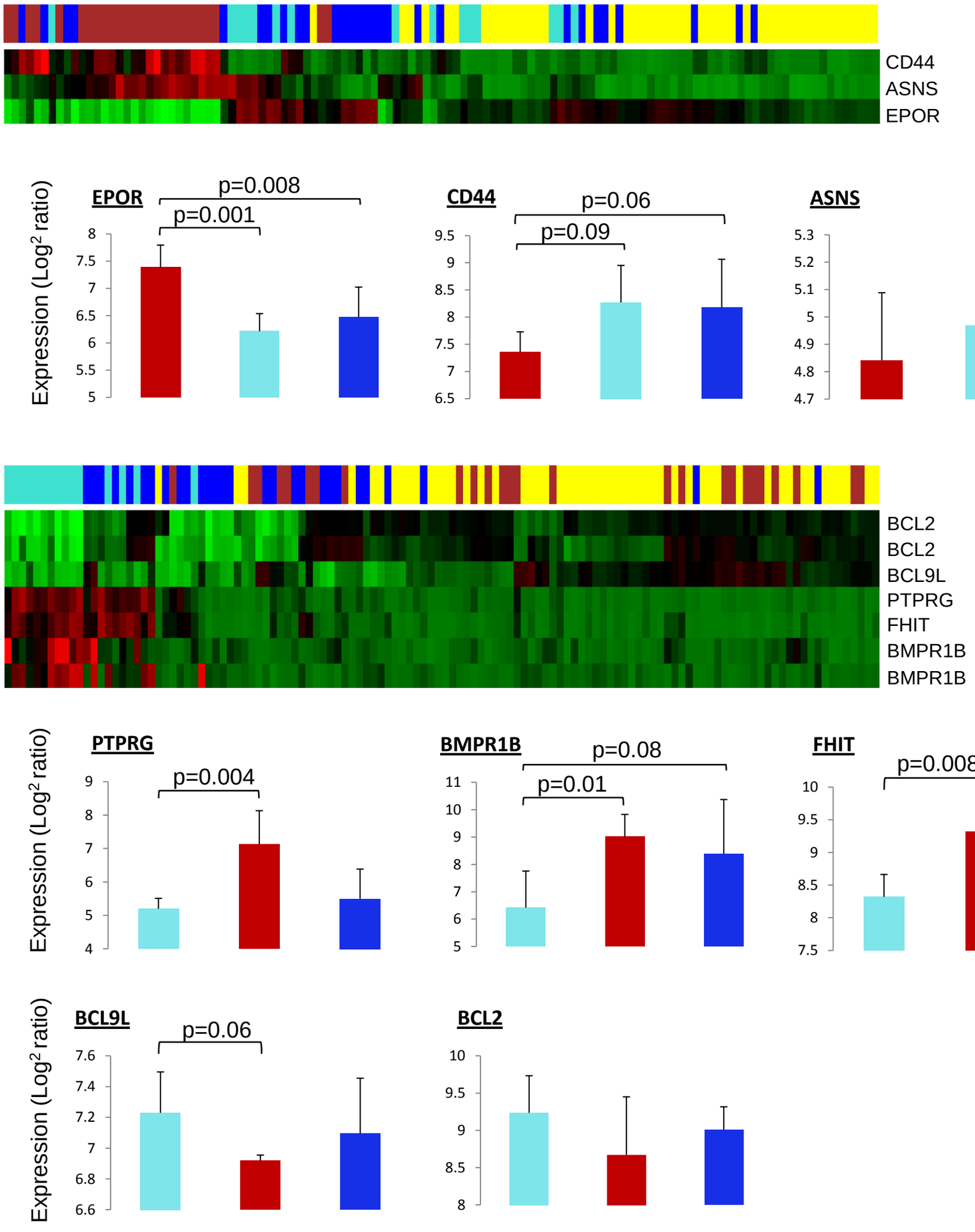
A.



B.



Supplementary Figure 4.



Supplementary Figure 5.

Supplementary Figure Legends

Supplementary Figure 1. Plot of gene expression (ΔLog_2 Ratio) and methylation (Δ beta) change between leukemic and non-leukemic control samples based on probe location.

Supplementary Figure 2. EWAS-like study designs (analysis 1) correlate DNA methylation status of aberrantly methylated loci to gene expression within the malignant tissue. The association does not describe correlated changes in DNA methylation and gene expression (epigenetic deregulation). Therefore, we integrate DNA methylation to gene expression change data to identify epigenetic deregulation (analysis 2). Anti-correlated genes are selected based on prior knowledge of the inverse relationship between DNA methylation and gene expression. The 602 genes we identified as displaying aberrant DNA methylation in pediatric B-cell ALL were compared to genes selected in *Wong et al, 2012*, based on Infinium HM27 analysis of *ETV6-RUNX1* pediatric ALL. The overlay shows that less than half of the differentially methylated genes identified in *Wong et al, 2012* show concordant expression changes.

Supplementary Figure 2. Ontology analysis of the 370 genes found epigenetically deregulated in pediatric B-cell ALL revealed the over-representation of subunits involved in G-protein signaling, cAMP mediated signaling and cation transport, red indicates hypermethylation and repression. The repression of plasma membrane bound G-coupled receptor subunits (22 molecules), transient receptor potential cation channel subunits (TRPCC) (3 molecules), Ca^{2+} (2 molecules) and K^{+} (7 molecules, voltage gated, Kv and pH- dependent, Kch) indicate a reduced potential for cell-microenvironment interactions in ALL cells. The repression of these plasma membrane bound subunits and downstream cAMP-mediated signaling molecules also indicate a reduced apoptotic potential of the ALL cells.

Supplementary Figure 3. A. Unsupervised hierarchical clustering of 250 probes displaying subtype-specific methylation patterns. **B**. Mean age at diagnosis for each subtype and B-cell other samples that clustered unexpectedly with Hyperdiploid and *ETV6-RUNX1* samples. There is a significant difference in the age of B-cell other samples that cluster with Hyperdiploid samples compared to the

B-cell other samples that cluster with *ETV6-RUNX1* and the remaining B-cell other samples. Error bars indicate SD.

Supplementary Figure 4. Heatmaps display unsupervised hierarchical clustering of DNA methylation for genes (discussed in text) displaying subtype-specific DNA methylation profiles. Bar graphs below show the mean expression of each subtype for the genes displayed in the heatmaps, with error bars indicating standard deviation.

Supplementary Table Legends

Supplementary Table 1: Sample phenotype table

Supplementary Table 2: Gene list of common aberrant methylation in pediatric ALL (n=795)

Supplementary Table 3: Table of 370 epigenetically deregulated genes, found inversely-correlated for DNA methylation and expression between leukemic and non-leukemic controls.

Supplementary Table 4: *ETV6-RUNX1* subtype specific gene list

Supplementary Table 5: Hyperdiploid subtype specific gene list

Supplementary Table 6: B-cell other subtype specific gene list