**Supplemental Material**

**Longitudinal Effects of Developmental Bisphenol A Exposure on Epigenome-Wide DNA Hydroxymethylation at Imprinted Loci in Mouse Blood**

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**Table S3.** Number of cytosine-phosphate-guanine (CpG) and cytosine-H-guanine (CHG; where H = adenine or thymine) sites at imprinted gene differentially hydroxymethylated regions (DHMRs).

**Table S4.** Enriched bisphenol A (BPA)-related differentially hydroxymethylated region (DHMR) pathways.

**Figure S1.** Exposure paradigm and blood collection time points to measure longitudinal 5-hydroxymethylcytosine (5-hmC) patterns. Two weeks prior to mate-pairing with $A^y/a$ males, six week old wild type $a/a$ dams were placed on one of two experimental diet groups: (1) Control (modified AIN-93G), (2) Control + 50 µg bisphenol A (BPA)/kg diet. Exposure continued through pregnancy and lactation, ending for offspring at postnatal day (PND) 21. Matched blood samples were collected from wildtype $a/a$ offspring at 2 months, 4 months, and 10 months of age.
**Figure S2. Distribution of bisphenol A (BPA)-related differentially hydroxymethylated regions (DHMRs) compared to random.** Using the *annotatr* R package, BPA-related DHMRs were annotated to the mm10 genome. Compared to a random distribution generated from background hydroxymethylated DNA immunoprecipitation-sequencing (HMeDIP-seq) data, BPA-related DHMRs were depleted at CpG shores, CpG shelves, promoters, 3′-UTRs, 5′-UTRs, exons, and introns, but slightly enriched at regions greater than 4,000 base pairs (bp) from a CpG island (interCGI). 3′-UTRs = 3′-untranslated regions. 5′-UTRs = 5′-untranslated regions. CpG = cytosine-phosphate-guanine.

**Figure S3. Enhanced reduced representation bisulfite sequencing (ERRBS) DNA methylation data from identified *Klf14* differentially hydroxymethylated region (DHMR).** Using the *RnBeads* R package, ERRBS data was visualized for the CpG sites contained within the identified *Klf14* DHMR. DNA methylation level is represented by intensity of color, with zero % methylation represented by white and 100% methylation represented by dark red. ERRBS data from Control mice is shown on the left, while ERRBS data from BPA exposed mice is shown on the right. Blood sample collection age (2 months, 4 months, or 10 months old) is indicated in the left column. Mean methylation across all CpGs with data is represented by line plots below the raw data. Line plots are colored according to age group. At the *Klf14* DHMR, there were no significant differentially methylated CpGs by BPA exposure, although comparisons were difficult due to limited coverage at some CpG.

**Figure S4. Additional differential imprinted gene 5-hydroxymethylcytosine (5-hmC) peaks by bisphenol A (BPA) exposure.** 5-hmC coverage was visualized at six additional imprinted loci with significant BPA-related differentially hydroxymethylated regions (DHMRs): A) *Airn*, B) *Cmah*, C) *Ppp1r9a*, D) *Kcnq1*, E) *Phactr2*, and F) *Pde4d*. 5-hmC levels are shown for matched 2 month, 4 month, and 10 month blood samples, as indicated by y-axis labels. Blue and red peaks represent forward and reverse strand 5-hmC enrichment, respectively. *Cmah*, *Ppp1r9a*, and *Kcnq1* DHMRs occurred on the forward strand. *Airn*, *Phactr2*, and *Pde4d* DHMRs occurred on the reverse strand.

**Figure S5. 5-hydroxymethylcytosine (5-hmC) peaks at the *Igf2/H19* imprinted loci.** Based on results at the *Plagl1* locus, which is a regulator of *Igf2* and *H19*, longitudinal 5-hmC patterns across the *Igf2* and *H19* imprinted genes were also visualized. 5-hmC peaks at both loci appear stable across individuals and adulthood stage in longitudinal mouse blood (2, 4, and 10 months old). Regions of non-significant bisphenol A (BPA)-related differential 5-hmC are indicated in red boxes for both genes. Despite not being detected as significant differentially hydroxymethylated regions (DHMRs) in csaw, these bisphenol A (BPA)-related changes in 5-hmC were stable at all three measured time points.