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Supplemental Material

Associations between Maternal Tobacco Smoke Exposure and the Cord Blood $CD4^+$ DNA Methylome

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Figure S1. Dot and boxplots for the 10 CpGs with the smallest p-values and absolute %methylation differences between 10-11%. The CpG position is shown on the x-axis and the %methylation level is shown on the y-axis. Horizontal lines within each boxplot indicate the median %methylation level for each CpG. Interquartile ranges are represented by the upper and lower boundaries of the boxplots. The vertical lines ("whiskers") at the top and bottom of the boxplots indicate the boundaries of 1.5 times the interquartile range. Points beyond these whiskers are outliers. Newborns who were exposed to maternal tobacco smoke *in utero* are indicated in blue, while unexposed newborns are indicated in pink.

Figure S2. Circos plot showing the 10,381 differentially methylated CpG sites and the 557 differentially methylated regions identified in cord blood $CD4^+$ samples from the Maternal and Child Health Study (n=10 exposed, n=10 unexposed to any maternal tobacco smoke during pregnancy) by chromosome and genomic location. The outermost ring is comprised of chromosome ideograms, which show the relative size of each chromosome, in megabases (MB), and its banding patterns (darker black and gray bands indicate heterochromatin, white bands indicates euchromatin, red bands indicate centromeres, and blue bands indicate stalks for acrocentric chromosomes). The middle ring shows the differentially methylated CpG sites and the innermost ring shows the differentially methylated regions. CpGs and regions that were hypermethylated in the maternal tobacco smoke exposed, compared with unexposed, group are shown in dark blue and dark red, respectively. CpGs and regions that were hypomethylated in the maternal tobacco smoke exposed, compared with unexposed, group are shown in light blue and pink, respectively. The height of each bar indicates the %methylation difference between groups.

Figure S3. Histograms showing distributions for the **A)** %methylation differences within differentially methylated regions (DMRs), **B)** base pair lengths of DMRs, and **C)** number of differentially methylated CpG sites (raw and false discovery rate-adjusted p<0.05) within DMRs.

Figure S4. (A) Proportion of differentially methylated regions (DMRs) that were hypermethylated (black) and hypomethylated (gray) in the maternal tobacco smoke exposed, compared with unexposed, group by genomic region, **(B)** corresponding enrichment tests (Fisher's exact test), comparing the number of DMRs overlapping each genomic region with a set of similar-sized regions randomly selected from the genome, and **(C)** median (range) %methylation differences by genomic region.

Figure S5. Dot and boxplots for the 33 CpGs that were identified as differentially methylated in MACHS **(A)**, which replicated (%methylation difference in the same direction and raw p-value<0.05) in the WakeMed SMKE EPIC array study of cord blood $CD4^+$ cells **(B)**. The name of the EPIC array CpG is shown on the x-axis, and the %methylation level is shown on the y-axis. Horizontal lines within each boxplot indicate the median %methylation value for each CpG. The interquartile range is represented by the upper and lower boundaries of the boxplot. The vertical lines ("whiskers") at the top and bottom of each boxplot indicate the boundaries of 1.5 times the interquartile range. Points beyond these whiskers are outliers. Newborns who were exposed to maternal tobacco smoke *in utero* are indicated in blue, while unexposed newborns are indicated in pink.

References

Additional File- Excel Document