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### **Supplemental Material**

#### **Cadmium-Associated Differential Methylation throughout the Placental Genome: Epigenome-Wide Association Study of Two U.S. Birth Cohorts**

Todd M. Everson, Tracy Punshon, Brian P. Jackson, Ke Hao, Luca Lambertini, Jia Chen, Margaret R. Karagas, and Carmen J. Marsit

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**Figure S7.** Comparison of the percent change in meta-analyses (only probes with nominally-significant associations in original analyses) effect sizes after excluding mothers that reported smoking during pregnancy – loess curve (red line) does not appreciably differ from a linear model representing no change in effect (black line, intercept = 0, slope = 1).

**Table S1.** Effect estimates at top 17 hits utilizing log-Cd as the independent variables. Ch. = Chromosome, Pos = genomic location (hg19), Reg. = Annotated Regulatory Features, TSS = Transcription Start Site, UTR = untranslated region. All models adjusted for infant sex, maternal smoking during pregnancy, highest achieved maternal education level, and PCT estimates.

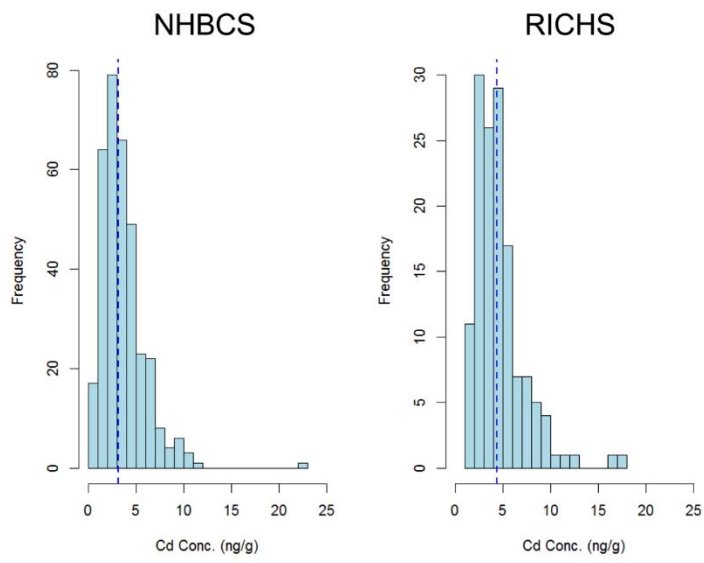
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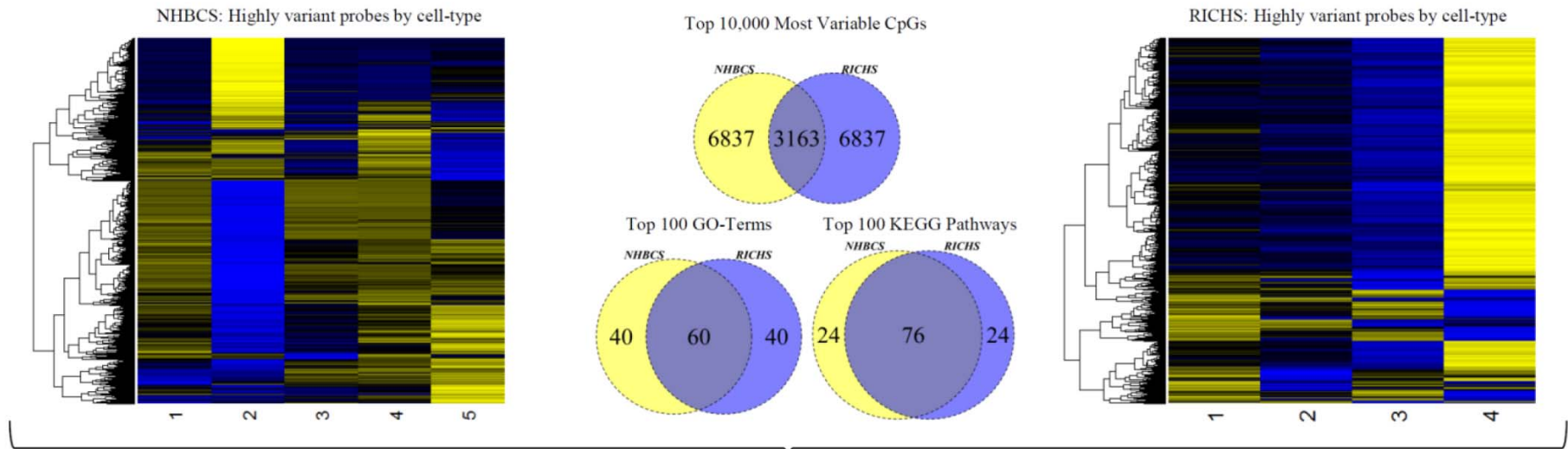
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**Additional File** – Excel Document

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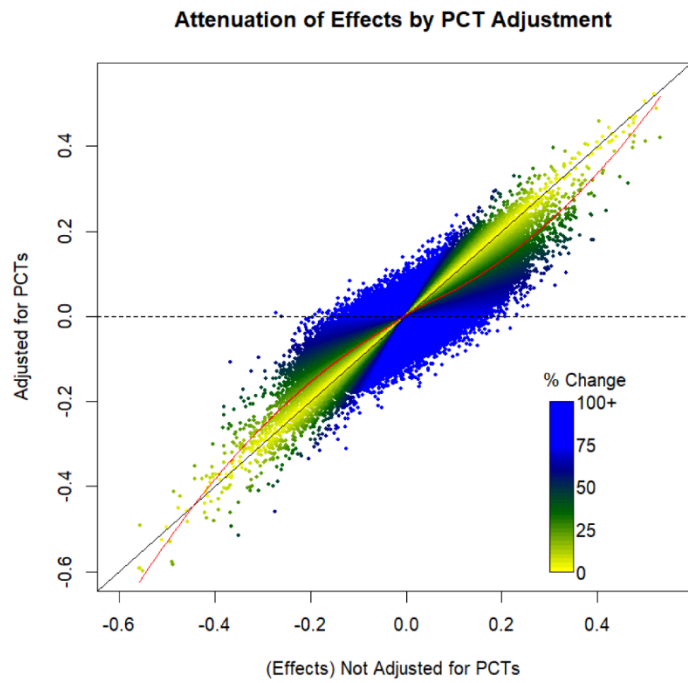
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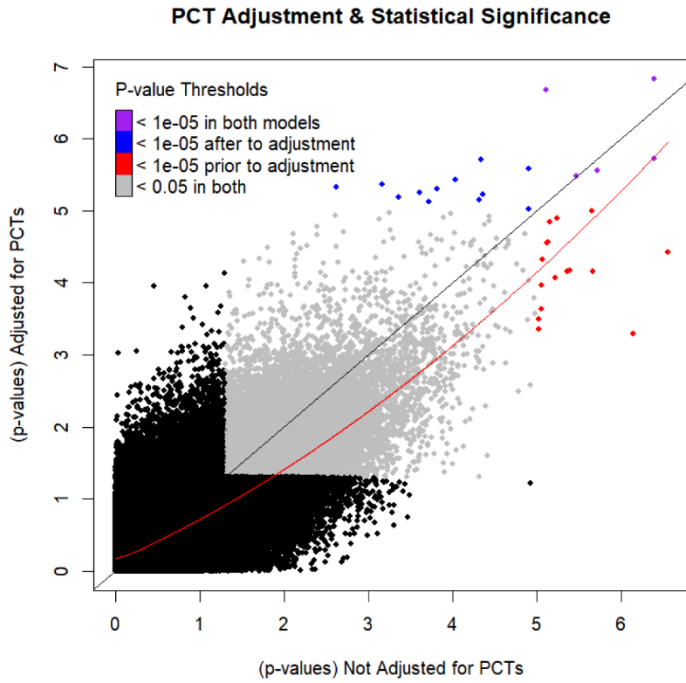
Top 10 KEGG Pathways from NHBCS	N	NHBCS		RICHs	
		DE	P-value	DE	P-Value
Metabolic pathways	1191	181	8.61E-61	102	1.61E-24
Neuroactive ligand-receptor interaction	257	66	3.46E-35	68	2.96E-45
Axon guidance	168	58	6.33E-30	36	3.03E-17
Calcium signaling pathway	172	51	5.58E-26	36	1.15E-18
Pathways in cancer	384	75	8.03E-25	48	3.82E-15
Rap1 signaling pathway	206	53	1.62E-23	30	1.32E-11
Olfactory transduction	348	51	4.39E-23	110	1.80E-78
Cell adhesion molecules (CAMs)	133	40	1.68E-22	35	2.53E-22
cAMP signaling pathway	192	48	4.58E-22	39	2.91E-20
PI3K-Akt signaling pathway	318	61	5.01E-21	45	1.39E-16

Top 10 KEGG Pathways from RICHs	N	NHBCS		RICHs	
		DE	P-value	DE	P-Value
Olfactory transduction	348	51	4.39E-23	110	1.80E-78
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cAMP signaling pathway	192	48	4.58E-22	39	2.91E-20
Glutamatergic synapse	113	33	4.89E-16	32	8.61E-20
Calcium signaling pathway	172	51	5.58E-26	36	1.15E-18
Morphine addiction	88	34	3.01E-20	28	1.18E-18
Axon guidance	168	58	6.33E-30	36	3.03E-17
Nicotine addiction	36	14	1.24E-09	18	4.12E-17

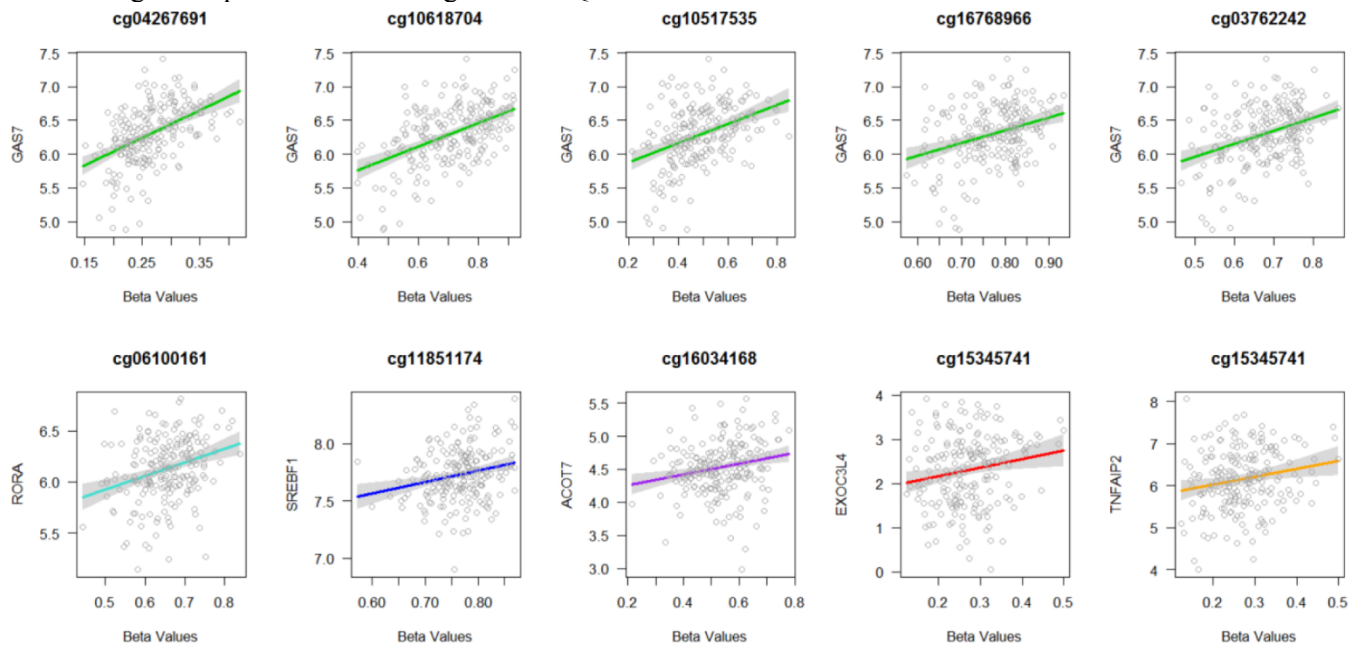
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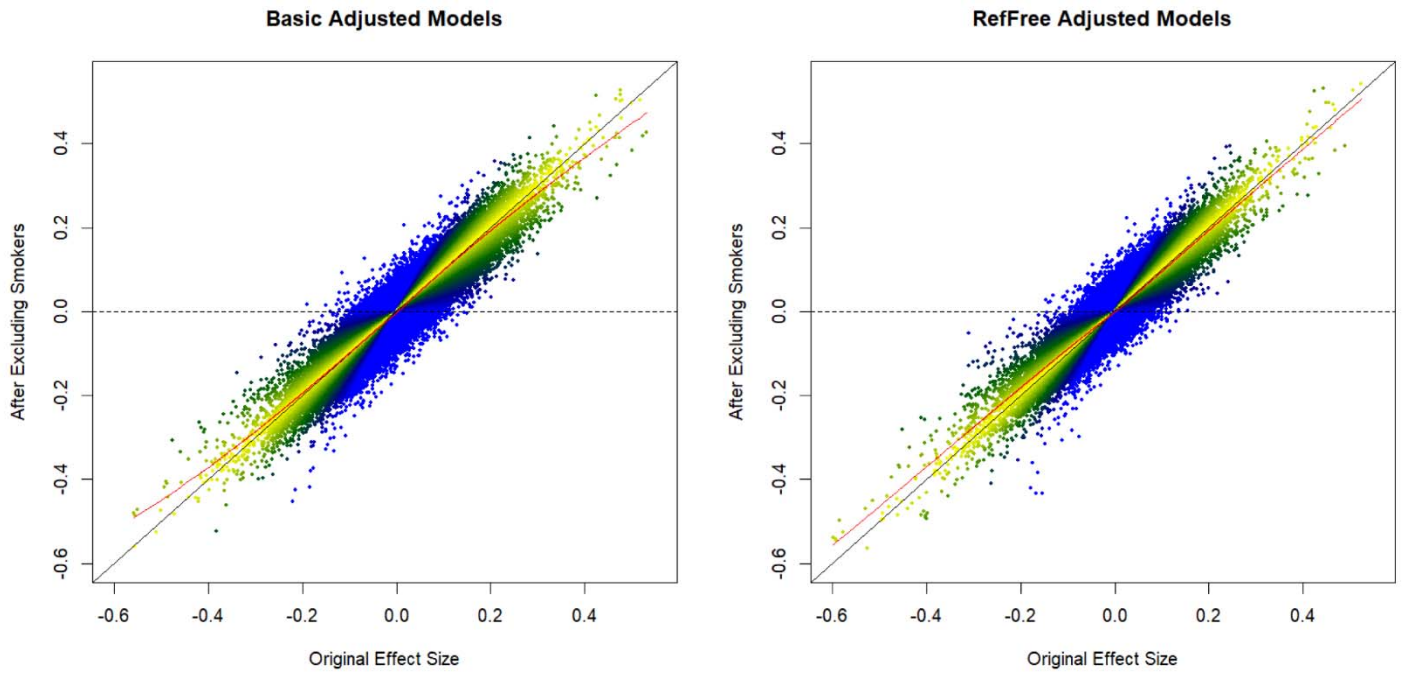
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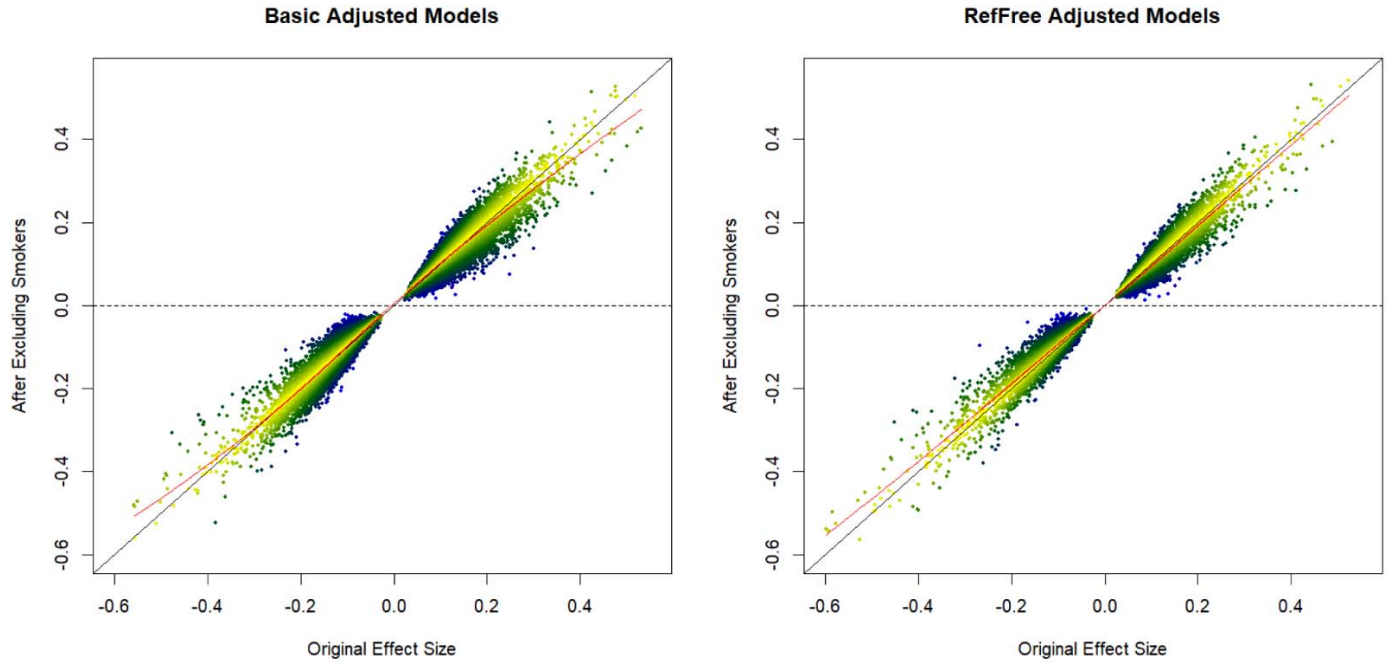


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**Table S1.** Effect estimates at top 17 hits utilizing log-Cd as the independent variables. Ch. = Chromosome, Pos = genomic location (hg19), Reg. = Annotated Regulatory Features, TSS = Transcription Start Site, UTR = untranslated region. All models adjusted for infant sex, maternal smoking during pregnancy, highest achieved maternal education level, and PCT estimates.

CpG Annotations					Meta-Analysis		NHBCS (N=343)		RICHS (N=141)	
CpG ID	Ch.	Pos	Gene	Reg.	$\beta_1$	p-value	$\beta_1$	p-value	$\beta_1$	p-value
cg16034168	1	6336711	<i>ACOT7</i>	Body	0.1075	0.001779	0.105389915	0.007185106	0.114803274	0.110169937
cg24000528	2	177013630	<i>MIR10B</i>	TSS1500	0.0714	0.0009701	0.080765065	0.009374949	0.062549423	0.037871092
cg24696183	11	2891360	<i>KCNQ1DN</i>	Body	-0.0838	9.98E-05	-0.073234174	0.020318023	-0.092991316	0.001595027
cg23193177	11	45717613	-	-	0.1048	0.0001008	0.100391023	0.00164609	0.11570351	0.021565814
cg15345741	14	103674333	-	-	0.0899	0.0005673	0.092375843	0.002475107	0.083328327	0.097561653
cg06100161	15	60987894	<i>RORA</i>	Body	0.1007	1.87E-05	0.049991211	0.109698174	0.167084844	2.96E-06
cg03917020	16	85285139	-	-	0.0439	0.00181	0.033530654	0.032256057	0.087958584	0.006315225
cg02600679	16	88832700	<i>FAM38A</i>	Body	0.1068	0.0003185	0.084005173	0.018686187	0.157291436	0.003121685
cg04267691	17	9862752	<i>GAS7</i>	Body;5'UTR;1stExon	0.0588	0.006968	0.03359771	0.217987695	0.103342572	0.004360351
cg10517535	17	9929698	<i>GAS7</i>	Body;TSS200	0.0881	0.007682	0.023996167	0.512378622	0.369329885	1.47E-06
cg03762242	17	9940004	<i>GAS7</i>	Body;1stExon;5'UTR	0.1033	5.19E-05	0.05464858	0.104782368	0.168909222	1.57E-05
cg16768966	17	9940227	<i>GAS7</i>	Body;TSS200	0.1097	0.001365	0.045394174	0.273538299	0.247800353	4.56E-05
cg10618704	17	9940970	<i>GAS7</i>	TSS1500;Body	0.1079	0.006731	0.03869942	0.394452734	0.336065153	4.67E-05
cg11851174	17	17712609	<i>RAI1</i>	Body	0.0795	0.001596	0.077067438	0.007385044	0.08769538	0.093126582
cg19944656	17	76876040	<i>TIMP2</i>	Body	0.0642	0.005459	0.054293055	0.072429574	0.078115568	0.029265054
cg11707084	19	48345263	<i>CRX</i>	3'UTR	0.0916	8.81E-05	0.100161672	0.000183187	0.06428528	0.178764902
cg03566291	21	44831040	-	-	0.0833	0.01375	0.040312345	0.284500444	0.261089902	0.000655329

**Table S2.** Comparison of effect estimates at top 17 hits before (Original  $\beta_1$ ) and after excluding mothers that reported smoking during pregnancy (SA  $\beta_1$ ). Ch = Chromosome, Pos = genomic location (hg19), SA = Sensitivity Analysis, %  $\Delta$  =  $((\text{Original } \beta_1 - \text{SA } \beta_1)/(\text{Original } \beta_1)) * 100$ . All models adjusted for infant sex, maternal smoking during pregnancy, highest achieved maternal education level, and PCT estimates.

CpG Annotations			Meta Analysis					NHBCS					RICHs				
CpG ID	Ch	Pos	Original $\beta_1$	SA $\beta_1$	% $\Delta$	Original p-value	SA p-value	Original $\beta_1$	SA $\beta_1$	% $\Delta$	Original p-value	SA p-value	Original $\beta_1$	SA $\beta_1$	% $\Delta$	Original p-value	SA p-value
cg16034168	1	6336711	0.3197	0.3076	3.78	2.80E-06	7.50E-06	0.3202	0.3196	0.17	3.11E-04	2.95E-04	0.3191	0.2892	9.35	8.13E-03	2.78E-03
cg24000528	2	177013630	0.1648	0.1657	-0.55	7.59E-06	4.45E-05	0.1778	0.2002	-12.60	1.52E-03	5.78E-04	0.1550	0.1330	14.20	1.89E-02	1.50E-03
cg24696183	11	2891360	-0.1620	-0.1563	3.52	4.31E-06	3.70E-05	-0.1965	-0.2013	-2.48	5.67E-04	4.89E-04	-0.1407	-0.1222	13.12	1.49E-02	1.71E-03
cg23193177	11	45717613	0.2402	0.2138	10.99	4.68E-06	1.18E-04	0.2244	0.2316	-3.23	4.94E-04	5.13E-04	0.2712	0.1733	36.10	8.39E-02	2.70E-03
cg15345741	14	103674333	0.2455	0.1955	20.37	1.49E-07	5.56E-05	0.2533	0.1899	25.02	9.69E-05	3.46E-03	0.2372	0.2025	14.60	5.48E-03	4.22E-04
cg06100161	15	60987894	0.2042	0.1656	18.90	1.97E-06	3.07E-04	0.1178	0.1028	12.74	6.28E-02	1.27E-01	0.2776	0.2198	20.82	4.49E-04	1.99E-06
cg03917020	16	85285139	0.1358	0.1385	-1.99	5.52E-06	5.95E-06	0.1348	0.1335	1.00	3.62E-04	6.03E-04	0.1375	0.1465	-6.56	3.04E-03	4.85E-03
cg02600679	16	88832700	0.2547	0.2379	6.60	4.92E-06	4.38E-05	0.1731	0.1716	0.87	2.55E-02	2.94E-02	0.3420	0.3176	7.13	2.37E-04	2.02E-05
cg04267691	17	9862752	0.1773	0.1201	32.26	6.98E-06	3.00E-03	0.1198	0.0777	35.10	3.09E-02	1.60E-01	0.2360	0.1688	28.48	4.45E-03	2.57E-05
cg10517535	17	9929698	0.3120	0.2380	23.72	6.46E-06	1.90E-03	0.1564	0.1318	15.72	6.97E-02	1.44E-01	0.5930	0.5129	13.51	4.10E-04	3.08E-07
cg03762242	17	9940004	0.2302	0.2073	9.95	3.33E-06	8.55E-05	0.2044	0.1836	10.18	2.08E-03	6.12E-03	0.2627	0.2463	6.23	4.08E-03	4.12E-04
cg16768966	17	9940227	0.3131	0.2725	12.97	1.92E-06	1.34E-04	0.2345	0.1953	16.72	7.31E-03	3.19E-02	0.4156	0.3957	4.80	5.75E-04	3.12E-05
cg10618704	17	9940970	0.3593	0.3191	11.19	5.91E-06	1.87E-04	0.2385	0.2150	9.85	2.03E-02	4.17E-02	0.5373	0.5160	3.96	3.81E-04	1.65E-05
cg11851174	17	17712609	0.1900	0.1754	7.68	3.69E-06	3.85E-05	0.1961	0.1984	-1.19	1.07E-04	1.12E-04	0.1783	0.1247	30.07	1.02E-01	1.10E-02
cg19944656	17	76876040	0.1762	0.1666	5.45	2.62E-06	2.50E-05	0.1726	0.1659	3.88	8.06E-04	1.72E-03	0.1803	0.1675	7.08	4.84E-03	9.82E-04
cg11707084	19	48345263	0.2230	0.2342	-5.02	2.09E-07	7.99E-08	0.2269	0.2410	-6.21	3.10E-05	9.61E-06	0.2164	0.2220	-2.60	2.34E-03	1.93E-03
cg03566291	21	44831040	0.2335	0.2073	11.22	9.46E-06	1.89E-04	0.2085	0.2126	-2.00	9.57E-04	1.07E-03	0.2913	0.1928	33.82	7.14E-02	2.38E-03

**Table S3.** P-values from Wilcoxon-tests to examine whether variations in estimated putative cell type (PCT) proportions were associated with maternal smoking during pregnancy in each cohort.

<b>T-test p-values</b>	<b>PCT-1</b>	<b>PCT-2</b>	<b>PCT-3</b>	<b>PCT-4</b>	<b>PCT-5</b>
NHBCS	0.09	0.24	0.24	0.39	0.69
RICHs	0.29	0.42	0.7	0.86	NA

**Table S4.** P-values from Wilcox-tests to examine whether variations in gene-expression at our top genes were associated with maternal smoking during pregnancy in the RICHS sample.

T-test p-values	<i>TNFAIP2</i>	<i>EXOC3L4</i>	<i>GAS7</i>	<i>RORA</i>	<i>ACOT7</i>	<i>SREBF1</i>
RICHS	0.68	0.5	0.26	0.28	0.07	0.78

## **Additional File – Excel Document**

**Excel Table S1.** GO-term enrichment for the 10,000 CpG sites with greatest variation in putative cell type specific DNA methylation levels. Enrichment was performed within each cohort, then top GO-terms by ranked enrichment p-value were compared. N = number of genes represented by a GO term, DE = number of genes from that GO term that were included in our top 10,000 CpGs, FDR = FDR-adjusted p-value for enrichment, Rank = cohort-specific rank for GO-term enrichment.

**Excel Table S2.** KEGG-pathway enrichment for the 10,000 CpG sites with greatest variation in putative cell type specific DNA methylation levels. Enrichment was performed within each cohort, then top KEGG-pathway by ranked enrichment p-value were compared. N = number of genes represented by a KEGG-pathway, DE = number of genes from that KEGG-pathway that were included in our top 10,000 CpGs, FDR = FDR-adjusted p-value for enrichment, Rank = cohort-specific rank for KEGG-pathway enrichment.

**Excel Table S3.** Results from cohort-specific and meta-analyses of Cd-associated DNA methylation; adjusted for maternal smoking during pregnancy, maternal education and fetal sex.

**Excel Table S4.** Results from cohort-specific and meta-analyses of Cd-associated DNA methylation; adjusted for maternal smoking during pregnancy, maternal education, fetal sex and estimated tissue heterogeneity.

**Excel Table S5.** GO Term with a nominal enrichment p-value  $< 0.05$ , among the top 250 CpGs from the PCT adjusted meta-analyses.

**Excel Table S6.** KEGG Pathways with a nominal enrichment p-value  $< 0.05$ , among the top 250 CpGs from the PCT adjusted meta-analyses.

**Excel Table S7.** Modeling results for Cd-Sex interactions at CpGs with meta-analysis p-values  $< 1e-05$ ; adjusted for maternal smoking during pregnancy and maternal education.