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Supplemental Material to:

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Genome-wide DNA methylation in neonates exposed to maternal depression, anxiety, or SSRI medication during pregnancy

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Table S1. CpG sites significantly different between neonates exposed to non-medicated maternal depression/anxiety and controls (FDR-adj $p \leq 0.1$)

CpG Site	Gene Name	Beta Coefficient[A]	FDR-adj. p-value	Relation to CpG Island	Relation to Gene
cg11846236	COL7A1	-0.080	0.031*	Island	1stExon
cg25109393	ENC1	-0.011	0.041*	Island	1stExon; 5'UTR
cg10283969	SHANK2	0.032	0.041*	Island	Body
cg21675030	ABHD10	-0.010	0.046*	Island	1stExon
cg17913386	COL7A1	-0.087	0.046*	Island	1stExon
cg01070078	HIC1	0.009	0.046*	Island	TSS1500; 5'UTR
cg24141135	MARS	-0.009	0.046*	Island	1stExon
cg20242129	NSF	0.036	0.046*		Body
cg04556542	SLC24A4	-0.015	0.046*	Island	Body; 5'UTR
cg27180315	SRRM3	-0.008	0.046*	Island	TSS200
cg01952012	AKAP11	0.200	0.066		5'UTR
cg05444556	CBFA2T3	0.014	0.066	N_Shelf	Body
cg23760189	FAM111A	-0.009	0.066	Island	TSS200
cg04718306	GADD45A	-0.009	0.066	Island	5'UTR; 1stExon
cg05812089	LOC100126784; NAV2	-0.053	0.066	S_Shore	TSS1500; Body
cg22664157	PWWP2B	-0.007	0.066	Island	1stExon
cg15207742	RIMS4	-0.007	0.066	Island	Body
cg26740249	TNRC6C	-0.076	0.066	N_Shore	5'UTR
cg04068601	VPS16; PTPRA	0.030	0.066		TSS200; Body
cg10852662	ZNF311	-0.022	0.066		Body
cg12599700		-0.010	0.066	Island	
cg13654797		-0.056	0.066		
cg26105725		0.038	0.066		
cg21516341	NELL2	-0.014	0.070	Island	Body; TSS1500; TSS200
cg18869404		-0.022	0.070	S_Shore	
cg25923240	SOX17	-0.035	0.073	S_Shore	3'UTR
cg27516774	SPG11	-0.019	0.073	Island	1stExon
cg15105359	TNS3	-0.029	0.073	Island	5'UTR
cg17858880	TNS3	-0.078	0.077	Island	5'UTR
cg18809151	NDUFAF1	-0.004	0.082	Island	1stExon; 5'UTR
cg16223510	BCLAF1	-0.014	0.083	Isand	5'UTR
cg04115659	PIK3AP1	-0.010	0.083	Island	Body
cg02387613	TOPBP1	-0.008	0.083	Island	5'UTR
cg22223531	C1orf163	-0.008	0.085	Island	1stExon
cg21355042		-0.065	0.085		

cg16561392	ANKFY1	0.017	0.089		Body
cg13319417	C6orf222	-0.020	0.089		5'UTR
cg02571678	OCA2	-0.036	0.089	S_Shelf	Body
cg10622825	PIGC; C1orf105	-0.025	0.097	S_Shore	TSS200; Body
cg17898329		-0.023	0.097	N_Shore	
cg00443788	C10orf110; IDI2	0.022	0.098		Body; TSS1500
cg21883621	C18orf25	-0.006	0.098	Island	TSS200

[A]Beta coefficient represents difference in methylation level between neonates born to mothers with non-medicated maternal depression/anxiety relative to controls. All regression were adjusted for maternal age, maternal BMI, SES, and chip.

*P-value < 0.05.

Table S2. Gene Ontology analysis for biological pathways based on top 100 differentially methylated sites among those exposed to non-medicated maternal depression/anxiety relative to controls.

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:2001295	1.48E-05	Inf	0.01	2	2	malonyl-CoA biosynthetic process
GO:0008630	0.0007	19.37	0.17	3	45	intrinsic apoptotic signaling pathway in response to DNA damage
GO:0034030	0.0010	53.27	0.05	2	12	ribonucleoside bisphosphate biosynthetic process
GO:0034033	0.0010	53.27	0.05	2	12	purine nucleoside bisphosphate biosynthetic process
GO:0015936	0.0015	40.97	0.06	2	15	coenzyme A metabolic process
GO:0009892	0.0018	2.94	5.33	13	1371	negative **regulation** of metabolic process
GO:0010558	0.0021	3.33	3.49	10	899	negative **regulation** of macromolecule biosynthetic process
GO:0045892	0.0025	3.48	2.97	9	765	negative **regulation** of transcription, DNA-dependent
GO:0031327	0.0029	3.19	3.64	10	936	negative **regulation** of cellular biosynthetic process
GO:0001828	0.0039	Inf	0.00	1	1	inner cell mass cellular morphogenesis
GO:0003142	0.0039	Inf	0.00	1	1	cardiogenic plate morphogenesis
GO:0010793	0.0039	Inf	0.00	1	1	**regulation** of mRNA export from nucleus
GO:0015700	0.0039	Inf	0.00	1	1	arsenite transport
GO:0015828	0.0039	Inf	0.00	1	1	tyrosine transport
GO:0019391	0.0039	Inf	0.00	1	1	glucuronoside catabolic process
GO:0032938	0.0039	Inf	0.00	1	1	negative **regulation** of translation in response to oxidative stress
GO:0043990	0.0039	Inf	0.00	1	1	histone H2A-S1 phosphorylation
GO:0045299	0.0039	Inf	0.00	1	1	otolith mineralization
GO:0051089	0.0039	Inf	0.00	1	1	constitutive protein ectodomain proteolysis
GO:0060807	0.0039	Inf	0.00	1	1	**regulation** of transcription from RNA polymerase II promoter involved in definitive endodermal cell fate specification
GO:1900477	0.0039	Inf	0.00	1	1	negative **regulation** of G1/S transition of mitotic cell cycle by negative **regulation** of transcription from RNA polymerase II promoter
GO:0006084	0.0042	23.14	0.10	2	25	acetyl-CoA metabolic process
GO:0046129	0.0047	9.65	0.34	3	87	purine ribonucleoside biosynthetic process
GO:0033865	0.0052	20.46	0.11	2	28	nucleoside bisphosphate metabolic process

GO:0045934	0.0058	3.03	3.38	9	870	negative **regulation** of nucleobase-containing compound metabolic process
GO:1900117	0.0064	5.86	0.74	4	191	**regulation** of execution phase of apoptosis
GO:2001235	0.0072	17.16	0.13	2	33	positive **regulation** of apoptotic signaling pathway
GO:0010212	0.0073	8.18	0.40	3	102	response to ionizing radiation
GO:0070271	0.0077	3.10	2.90	8	746	protein complex biogenesis
GO:0006431	0.0078	261.31	0.01	1	2	methionyl-tRNA aminoacylation
GO:0007493	0.0078	261.31	0.01	1	2	endodermal cell fate determination
GO:0043987	0.0078	261.31	0.01	1	2	histone H3-S10 phosphorylation
GO:0043988	0.0078	261.31	0.01	1	2	histone H3-S28 phosphorylation
GO:0048866	0.0078	261.31	0.01	1	2	stem cell fate specification
GO:1900041	0.0078	261.31	0.01	1	2	negative **regulation** of interleukin-2 secretion
GO:2000035	0.0078	261.31	0.01	1	2	**regulation** of stem cell division
GO:0006812	0.0079	3.36	2.32	7	609	cation transport
GO:0009163	0.0087	7.64	0.42	3	109	nucleoside biosynthetic process
GO:0065003	0.0091	2.81	3.63	9	933	macromolecular complex assembly

Only includes terms with p-value<0.01.

Table S3. Robust SE regressions results for selected candidate genes.

Candidate Genes	Non-medicated depression/anxiety exposure beta coefficient (95% CI)	Non-medicated depression/anxiety unadjusted p-value	SSRI exposure beta coefficient (95% CI)	SSRI exposure unadjusted p-value	Gene Region	Relation to CpG Island	Probe Type
Stress/Behavior-related							
BDNF (96 probes; p<0.0005)	--	--	--	--	--	--	
FKBP5 (34 probes; p< 0.001)							
cg18726036	0.009 (0.004, 0.01)	0.0017¥	--	--	3'UTR	--	I
cg07061368	--	--	-0.03 (-0.05, -0.01)	0.004¥	5'UTR	--	II
NR3C1 (39 probes; p<0.001)							
cg00629244	-0.006 (-0.01, -0.003)	0.0019¥	--	--	TSS200; 5'UTR	Island	I
SLC6A4 (16 probes; p<0.003)							
cg10901968	--	--	-0.007 (-0.01, -0.003)	$9.9x10^{-4}$*	TSS200	Island	I
CRHR1 (23 probes, p<0.002)							

cg11731737	-0.005 (-0.01, -0.002)	0.0023¥	--	--	Body	Island	I
CRHR2 (17 probes; p<0.003)	--	--	--	--	--	--	--
NFKB1 (19 probes; p<0.003)	--	--	--	--	--	--	--
NKFB2 (27 probes; p<0.002)							
cg23606922	-0.007 (-0.01, -0.003)	0.0018*	-0.01 (-0.02, -0.01)	2.6x10-^{5}*	5'UTR;TSS200	Island	I
Epigenetic Regulator Genes							
DNMT1 (20 probes, p<0.003)	--	--	--	--	--	--	
DNMT3a (132 probes; p<0.0004)							
cg15843262	--	--	0.008 (0.004, 0.01)	8x10^{-4}¥	Body	N_Shore	I

*Indicates significantly associated sites, ¥indicates marginally significant sites, after Bonferroni adjustment for the number of probes on microarray related to each gene of interest. Beta coefficients and p-values are listed only for probes with significant associations. Beta coefficients represent change in percent methylation. All regression were adjusted for maternal age, maternal BMI, SES, and chip.

Supp. Table 4. Pyrosequencing Primer information.

Gene	Chromosome location (build hg19)	Amplicon length	Primer Type	Primer sequence (5'→3')
Col7a1	3p21.31	193 bps	Forward	GGTGTTGGGGATGAAGGT
			Reverse	5' Biotin-CCTAACTAACCACTTTTACTACCTAAAAT
	chr3:48,632,554-48,632,588		Sequencing	GGGGAGTTTTTGTTAGGAT
			Unconverted Sequence to analyze	CCCGGCGCAGAGCGCGGCCACCAGAAGCCGCAGCG
			Converted Sequence to analyze ('-' strand)	TTYGGYGTAGAGYGYGGTTATTAGAAGTYGTAGY

* Chromosomal location is based on the UCSC Human Feb. 2009 (GRCh37/hg19) Build

Supp Figures:

Figure S1. Normalization

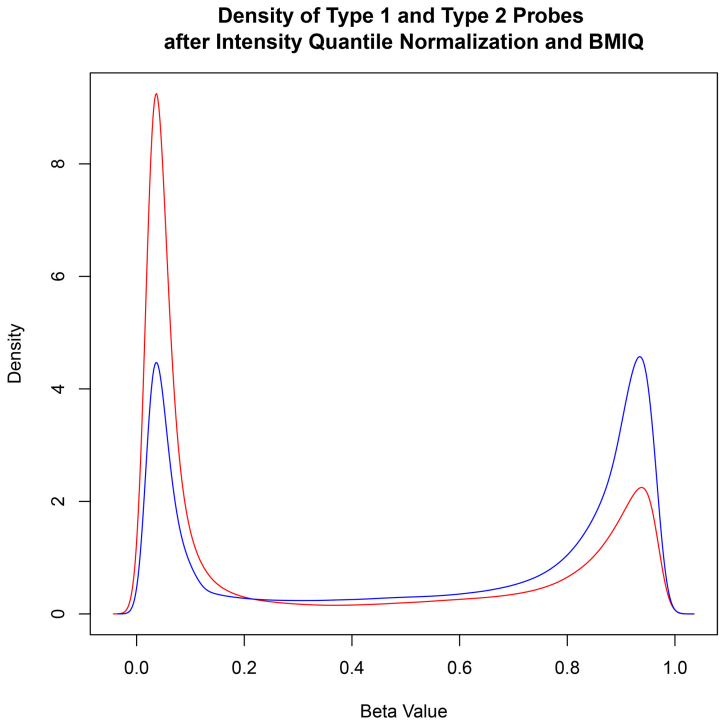


Figure S2 Correlations between replicate quality control and cord blood samples.

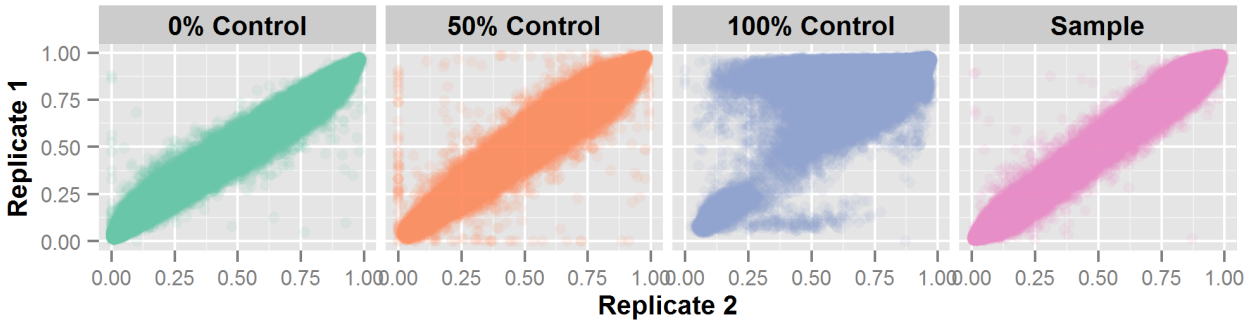


Figure S3. Density of DNA Methylation for replicate quality control samples and cord blood sample.

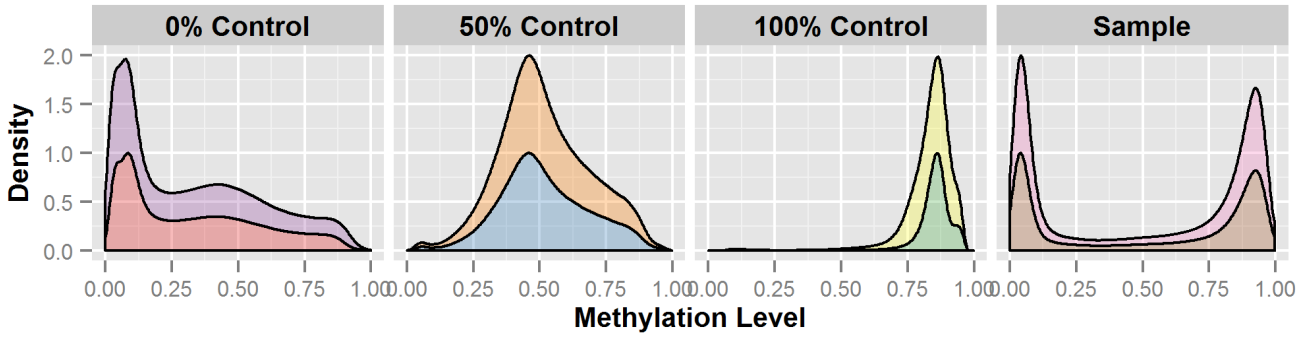
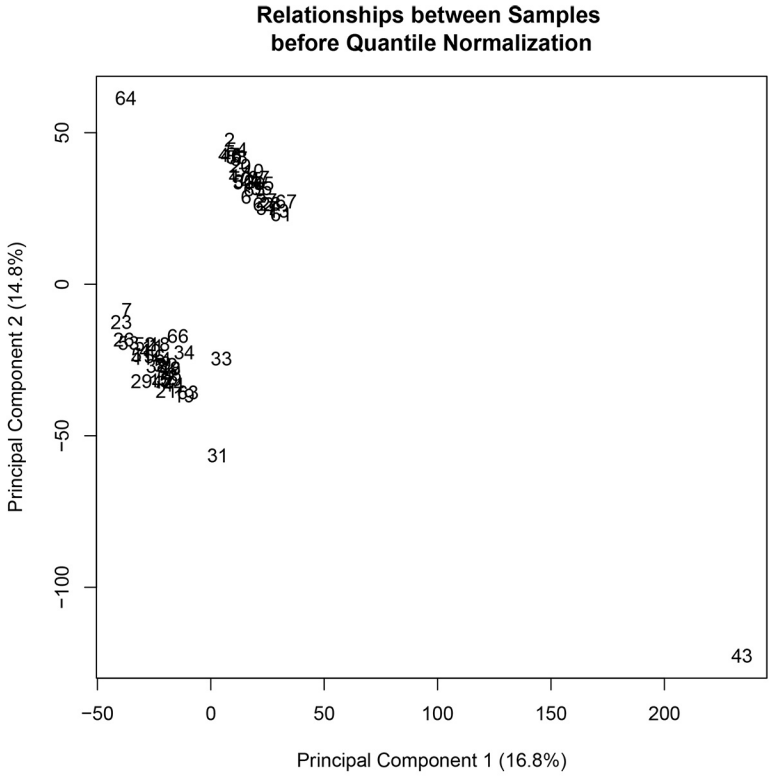


Figure S4. Principle Component Plot to identify outliers



Supplemental Methods

Quality Control of Microarray Data

Data were assembled at the USC Epigenome Center by converting fluorescence intensities from methylated (M) and unmethylated (U) alleles to methylation level, ranging from 0-1, given by Beta=M/(M+U+100). For sites where signal intensity was not significantly different from background measurements (detection p value>0.01), the beta value was recorded as "NA" and excluded from all analyses. Additionally, 65 CpG sites identified by Illumina as SNPS were removed. Color bias adjustment and quantile normalization (QN) were performed on signal intensities using the "lumi" Bioconductor package.[48] Adjustment for probe-type bias was conducted on QN data using beta-mixture quantile normalization (BMIQ) on β-values using the "wateRmelon" Bioconductor package.[49, 50] The density plot of methylation values for Type 1 and Type 2 probes following quantile normalization can be seen in Figure S1. To avoid areas of high methylation on the inactive X chromosome, statistical analyses were restricted to autosomal CpGs with unique probe target sequences reducing the data set after normalization to 472,487 probes. Non-specific probe sites were identified and removed from analysis if the probe had multiple DNA target sequences with at least 40 matching bases, at least 90% identity, end-nucleotide match, and gapless sequence alignment, as identified by BLAT analysis tested against both strands of the full bisulfite converted and non-converted human genome (build 19), following methods of Chen et al.[51] This resulted in the removal of an additional 18,630 non-specific probes, resulting in a final sample size of 453,857 probes. The 0, 50, 100% methylation control samples were not

included in the inter-sample normalizing steps due to expected differences in methylation distribution. These control samples were only background corrected.

Reproducibility on the array was assessed among one pair of technical replicate cord blood samples and three sets of quality control samples (0, 50, 100% methylation) by randomly sampling 100 loci 10,000 times and calculating Pearson correlations on each set. In general, the median correlation was extremely high for the control samples ($r>0.98$), with the exception of the 100% control samples. Between cord blood technical replicates, the median correlation was very strong ($r>0.99$). Correlation plots for all sites in the replicate samples can be seen in Figure S2. The distribution of DNA methylation levels for duplicate control samples are shown in Figure S3. Possible outliers were explored by inspecting overall signal intensity, distribution of M-values, number of detected sites, and the relationship between samples after hierarchical clustering and multidimensional scaling. One extreme outlier was detected and removed from subsequent analyses (Figure S4, sample 43). The outlier did not differ in maternal age, BMI, race, or any measured characteristics from other samples in the dataset. A final quality control analysis was performed by testing if each neonate sample clustered with the expected sex, as identified in labor and delivery charts. Multiple dimensional scaling analyses, PCAs, and heatmaps were created using probes from the X and Y chromosomes (data not shown). All three analyses consistently identified one individual as clustering with the opposite of the expected sex. The finding of only one misclassified sample likely indicated an error in recording the gender, rather than sample mix-up. This individual was excluded from subsequent analyses.

Pyroverification of Microarray Results

Pyrosequencing was performed on 6 CpG sites in the *Col7a1* gene, which contained two sites identified as significant in the site-by-site analysis. Genomic DNA was bisulfite treated in duplicate on every sample using the EZ DNA Methylation Gold Kit (Zymo Research, CA), according to manufacturer's protocol. Pyrosequencing primers (Table S4) were designed using PyroMark Assay Design Software 2.0 (www.qiagen.com). Bisulfite-converted DNA was mixed with 0.2uM of each primer and amplified using the HotstarTaq plus Master Mix (Qiagen). A bisulfite conversion check was included in the assay to verify full conversion of the DNA. Methylation levels for all CpG sites were assessed using the Pyromark Q24 pyrosequencer (Qiagen). The assay was validated with a methylation scale (0%, 20%, 40%, 60%, >80%) in duplicate created from whole genome amplified DNA (representing 0% methylation), and DNA treated with CpG methyltransferase *M.SssI* (representing >80% methylation). For each sample, PCRs were performed on each of the duplicate bisulfite treatments. If the difference between two replicates exceeded two standard deviations of the variation in the entire study population, a third bisulfite treatment was tested and the average of the two closest results was used.