

Supplementary tables:

Table S1. Sequenom Assays and primer design for validation.

Candidate Gene	Forward Primer (5'–3')	Reverse Primer (5'–3')	Amplicon Size (bp)	HM850 Probe name	Genomic CpG Location*	Assay CpG Identity
IL17D	TTTAGGTAGGTATTTGGGGTA TTT	CCTCATTCTATTCTAATAACACCTATA AAC	354	cg18786411	CpG13:21,295,528 hg19	IL17D CpG14
NFIC	GGGTTTTATTATGTTGGTTAGGT TG	AAATCCCCACACAAATACATTCATA	266	cg03641241	CpG19:3,392,471 hg19	NFIC CpG6
TBCD	TTGGAAATTTAGATGTTAGTGG AAT	CCCACATAACCCAAAAAATAACT	392	cg16538568	CpG17:80,823,310 hg19	TBCD CpG14

* named as CpG"chromosome number location": "genomic numerical location" "UCSC human genome build".

Table S2. HM850 probes – Beta values and differences.

Corresponding-HM850 Probes	Intervention (Beta Value)	Control (Beta Value)	Mean % Difference	Mean Difference	P-value
cg18786411 HM850 (IL17D CpG14)	0.88 (0.02)	0.90 (0.02)	-2.27	-0.02	<0.00001
cg03641241 HM850 (NFIC CpG6)	0.86 (0.04)	0.89 (0.02)	-3.02	-0.03	<0.00001
cg16538568 HM850 (TBCD CpG14)	0.85 (0.04)	0.89 (0.02)	-3.70	-0.04	<0.00001

Table S3. Sequenom Data: Total Group ($n = 60$) methylation values and differences.

Gene & CpG	Intervention	Control	Mean Difference	Mean % Difference	P-value
IL17D CpG 1&13	0.56 (0.03)	0.56 (0.04)	0.002	0.17	0.842
IL17D CpG 2	0.15 (0.04)	0.13 (0.5)	0.014	1.36	0.228
IL17D CpG 8&9	0.55 (0.04)	0.55 (0.06)	0.008	0.82	0.524
IL17D CpG 10&11	0.64 (0.04)	0.64 (0.07)	0.008	0.84	0.558
IL17D CpG 12	0.56 (0.12)	0.55 (0.10)	0.016	1.61	0.615
IL17D CpG 14	0.94 (0.01)	0.93 (0.03)	0.011	1.12	0.090
IL17D CpG 15	0.89 (0.03)	0.88 (0.04)	0.006	0.59	0.506
IL17D CpG 16	0.68 (0.13)	0.70 (0.13)	-0.016	-1.59	0.963 ^a
IL17D (mean)	0.62 (0.03)	0.61 (0.03)	0.008	0.82	0.262

NFIC CpG 1	0.90 (0.03)	0.93 (0.03)	-0.005	-0.49	0.337 ^a
NFIC CpG 2	0.88 (0.06)	0.89 (0.05)	-0.003	-0.28	0.825 ^a
NFIC CpG 3	0.87 (0.02)	0.88 (0.02)	-0.004	-0.37	0.533
NFIC CpG 4	0.87 (0.03)	0.86 (0.04)	0.007	0.68	0.648 ^a
NFIC CpG 5	0.98 (0.02)	0.99 (0.01)	-0.003	-0.35	0.829 ^a
NFIC CpG 6	0.87 (0.05)	0.86 (0.05)	0.003	0.30	0.818
NFIC (mean)	0.90 (0.03)	0.90 (0.03)	-0.001	-0.08	0.912
TBCD CpG 2&4&11&12	0.89 (0.08)	0.83 (0.08)	0.051	5.12	0.028
TBCD CpG 5&6&7	0.93 (0.03)	0.92 (0.05)	0.008	0.75	0.486
TBCD CpG 13	0.82 (0.09)	0.78 (0.12)	0.044	4.39	0.194
TBCD CpG 14	0.92 (0.03)	0.89 (0.08)	0.032	3.18	0.188 ^a
TBCD (mean)	0.89 (0.05)	0.85 (0.06)	0.040	4.03	0.020

P-values calculated using independent samples T-test unless otherwise stated. ^aMann-Whitney U test used to calculate *p*-value.

Table S4. Sequenom Data: Males only (n = 30) methylation values and differences.

Gene & CpG	Intervention	Control	Mean Difference	Mean % Difference	<i>P</i> -value
IL17D CpG 1&13	0.57 (0.03)	0.56 (0.04)	0.004	0.43	0.745
IL17D CpG 2	0.15 (0.03)	0.13 (0.05)	0.016	1.62	0.275
IL17D CpG 8&9	0.55 (0.03)	0.53 (0.06)	0.019	1.87	0.268
IL17D CpG 10&11	0.62 (0.02)	0.63 (0.08)	-0.004	-0.37	0.866
IL17D CpG 12	0.55 (0.13)	0.54 (0.12)	0.008	0.75	0.883
IL17D CpG 14	0.93 (0.01)	0.92 (0.04)	0.012	1.21	0.231
IL17D CpG 15	0.88 (0.03)	0.87 (0.04)	0.009	0.92	0.445
IL17D CpG 16	0.68 (0.13)	0.70 (0.14)	-0.017	-1.73	0.821 ^a
IL17D (mean)	0.62 (0.02)	0.61 (0.03)	0.013	1.32	0.144
NFIC CpG 1	0.90 (0.01)	0.90 (0.03)	0.002	0.18	0.813 ^a
NFIC CpG 2	0.90 (0.03)	0.89 (0.05)	0.018	1.75	0.334 ^a
NFIC CpG 3	0.88 (0.02)	0.88 (0.02)	-0.003	-0.27	0.732
NFIC CpG 4	0.88 (0.03)	0.85 (0.05)	0.026	2.57	0.102 ^a
NFIC CpG 5	0.99 (0.01)	0.99 (0.01)	0.005	0.51	0.298 ^a

NFIC CpG 6	0.88 (0.02)	0.85 (0.05)	0.025	2.55	0.099
NFIC (mean)	0.91 (0.02)	0.89 (0.03)	0.012	1.22	0.163
TBCD CpG 2&4&11&12	0.89 (0.07)	0.81 (0.09)	0.084	8.42	0.010
TBCD CpG 5&6&7	0.92 (0.02)	0.92 (0.06)	0.003	0.31	0.857
TBCD CpG 13	0.84 (0.08)	0.77 (0.11)	0.076	7.61	0.106
TBCD CpG 14	0.91 (0.03)	0.89 (0.07)	0.029	2.90	0.316 ^a
TBCD (mean)	0.91 (0.05)	0.84 (0.07)	0.067	6.66	0.007

P-values calculated using independent samples T-test unless otherwise stated. ^aMann-Whitney U test used to calculate *p*-value.

Table S5: Sequenom Data: Female only (n=30) descriptives and methylation values

Gene & CpG	Intervention	Control	Mean Difference	Mean % Difference	<i>P</i> -value
IL17D CpG 1&13	0.56 (0.03)	0.56 (0.02)	-0.001	-0.13	0.909
IL17D CpG 2	0.15 (0.05)	0.14 (0.04)	0.009	0.91	0.617
IL17D CpG 8&9	0.56 (0.05)	0.57 (0.04)	-0.010	-1.01	0.567
IL17D CpG 10&11	0.66 (0.04)	0.65 (0.03)	0.015	1.52	0.316
IL17D CpG 12	0.57 (0.10)	0.55 (0.06)	0.022	2.16	0.585
IL17D CpG 14	0.94 (0.01)	0.93 (0.01)	0.007	0.66	0.195
IL17D CpG 15	0.89 (0.03)	0.89 (0.03)	-0.002	-0.16	0.904
IL17D CpG 16	0.68 (0.14)	0.70 (0.12)	-0.015	-1.55	0.820 ^a
IL17D (mean)	0.63 (0.03)	0.63 (0.03)	0.000	0.01	0.991
NFIC CpG 1	0.90 (0.03)	0.91 (0.04)	-0.014	-1.37	0.274 ^a
NFIC CpG 2	0.87 (0.08)	0.89 (0.05)	-0.022	-2.25	0.595 ^a
NFIC CpG 3	0.87 (0.03)	0.88 (0.02)	-0.005	-0.50	0.605
NFIC CpG 4	0.87 (0.03)	0.88 (0.04)	-0.020	-1.96	0.160 ^a
NFIC CpG 5	0.98 (0.03)	0.99 (0.01)	-0.012	-1.24	0.527 ^a
NFIC CpG 6	0.85 (0.07)	0.88 (0.04)	-0.025	-2.46	0.289
NFIC (mean)	0.89 (0.04)	0.91 (0.03)	-0.016	-1.63	0.235
TBCD CpG 2&4&11&12	0.88 (0.09)	0.87 (0.07)	0.007	0.74	0.824
TBCD CpG 5&6&7	0.93 (0.03)	0.92 (0.03)	0.010	1.01	0.429
TBCD CpG 13	0.80 (0.09)	0.79 (0.14)	0.014	1.44	0.780

TBCD CpG 14	0.93 (0.10)	0.90 (0.10)	0.036	3.57	0.346 ^a
TBCD (mean)	0.88 (0.06)	0.87 (0.04)	0.004	0.43	0.854

P-values calculated using independent samples T-test unless otherwise stated. ^aMann-Whitney U test used to calculate *p*-value

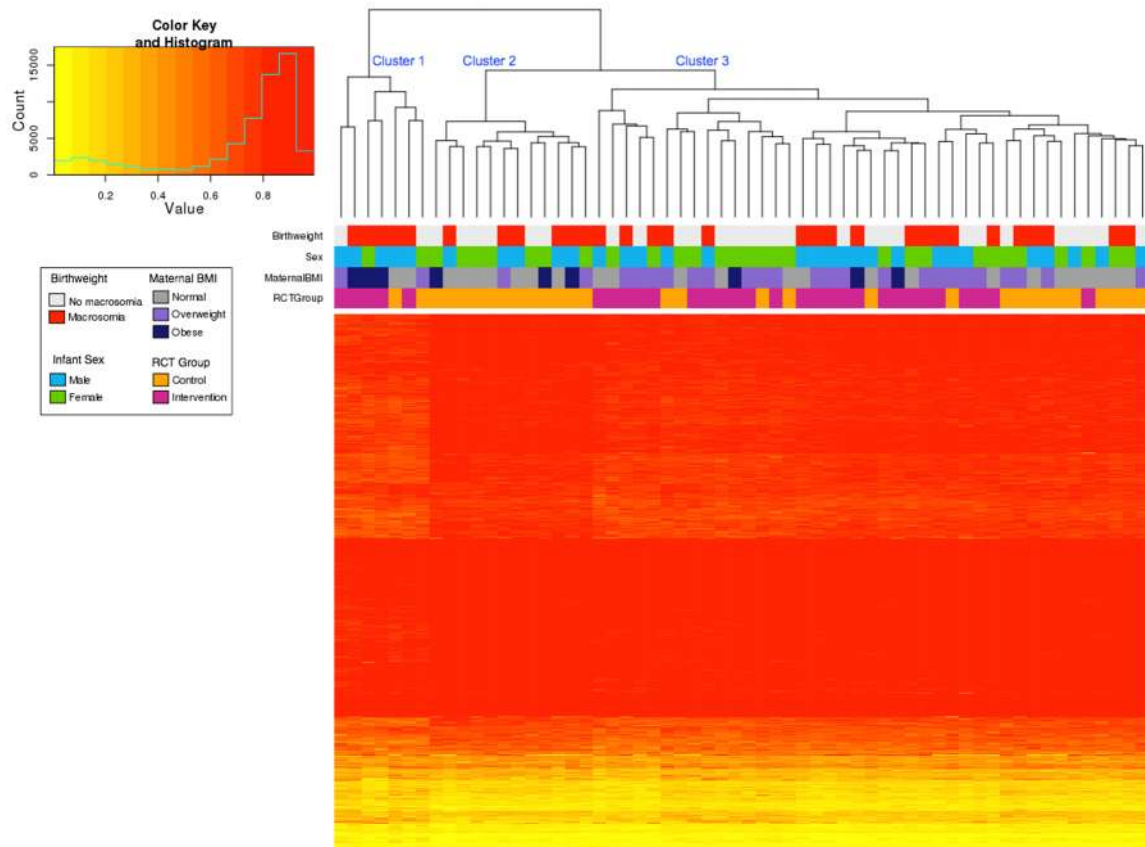


Figure S1. Hierarchical clustering and heatmap of HM850 methylation values of the top 1000 probes associated with intervention/control. Controlled for sex, gestational age, chip, chip position, and cell types (B cells, CD4T, CD8T, granulocytes, monocytes, NK cells, and nRBCs). The histogram depicts the distribution of methylation levels across all samples and probes, the beta value is plotted on the x-axis and number of probes on the y-axis. Individual cord blood samples are plotted on the x-axis, and individual probes on the y-axis. Completely unmethylated probes (beta value of 0) are represented by yellow and completely methylated probes (beta value of 1) are represented as red. The associated dendrogram indicates the relatedness of samples by methylation, with branches closer together more similar than those further apart.